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(54) **DYSFUNCTIONAL ANTIGEN-SPECIFIC CD8+ T CELLS IN THE TUMOR MICROENVIRONMENT**

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C07K 16/28 (2006.01)
A61K 35/17 (2015.01)
G01N 33/569 (2006.01)

(52) **U.S. Cl.**
CPC ***C07K 16/2878*** (2013.01); ***A61K 35/17*** (2013.01); ***C07K 16/2803*** (2013.01); ***G01N 33/56972*** (2013.01); *C07K 2317/75* (2013.01); *C07K 2317/76* (2013.01)

(58) **Field of Classification Search**
CPC C07K 16/2878; C07K 16/2803; A61K 35/17; G01N 33/56972
See application file for complete search history.

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WO	WO 2015/119923	8/2015	
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(57) **ABSTRACT**

Provided herein are compositions and methods for detecting and/or targeting dysfunctional tumor antigen-specific CD8+ T cells in the tumor microenvironment for diagnostic, therapeutic and/or research applications. In particular, dysfunctional tumor antigen-specific CD8+ T cells are detected and/or targeted via their expression of cell surface receptors described herein, such as 4-1BB, LAG-3, or additional markers that correlate with 4-1BB and LAG-3 expression, such as markers differentially expressed on the surface of the T cells.

13 Claims, 51 Drawing Sheets

Specification includes a Sequence Listing.

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FIG. 1A

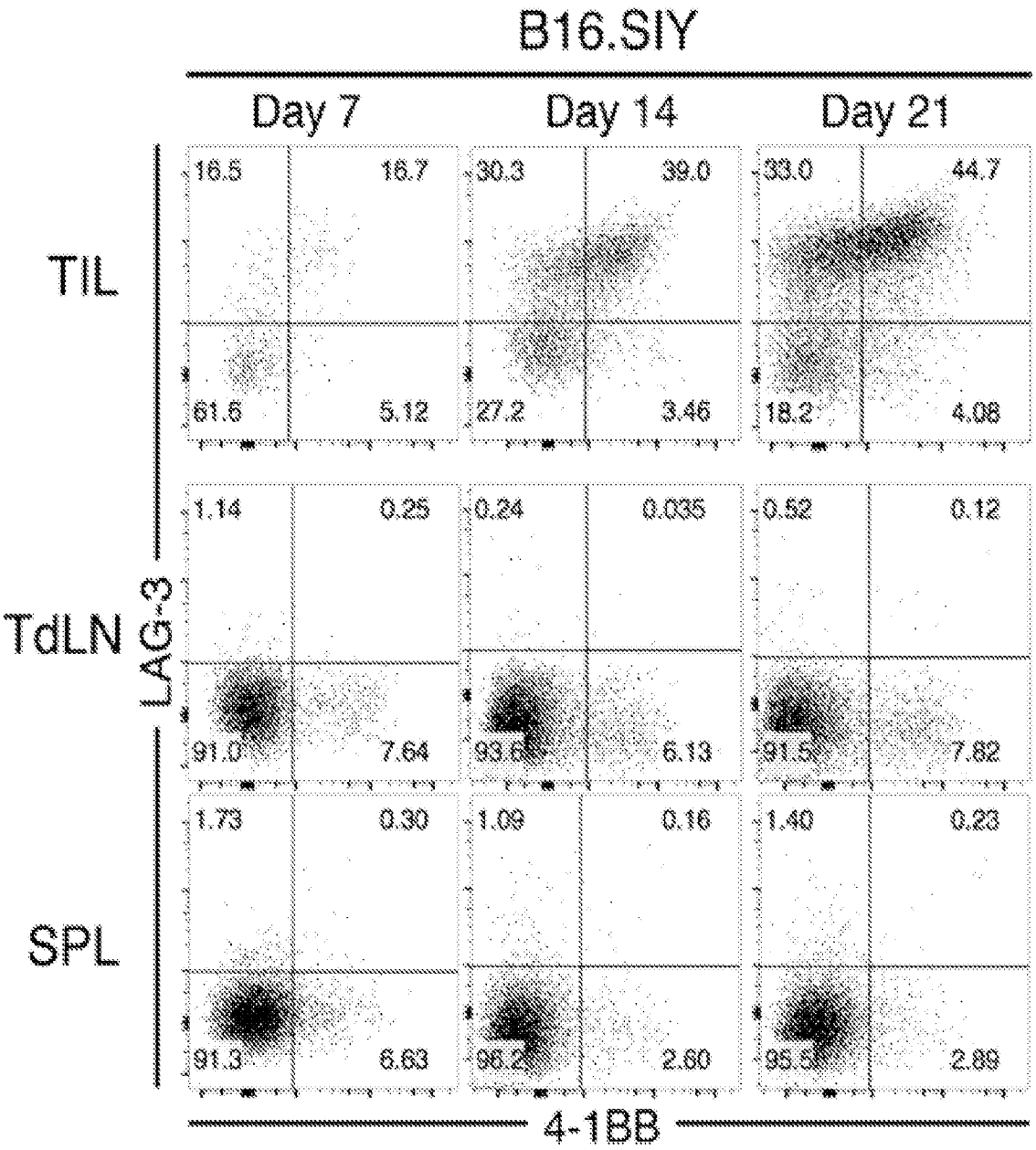

B16.SIY
Day 7
Day 14
Day 21
TIL
TdLN
SPL
LAG-3
4-1BB
16.5
16.7
61.6
5.12
30.3
39.0
27.2
3.46
33.0
44.7
18.2
4.08
1.14
0.25
91.0
7.64
0.24
0.035
93.6
6.13
0.52
0.12
91.5
7.82
1.73
0.30
91.3
6.63
1.09
0.16
96.2
2.60
1.40
0.23
95.5
2.89

FIG. 1B

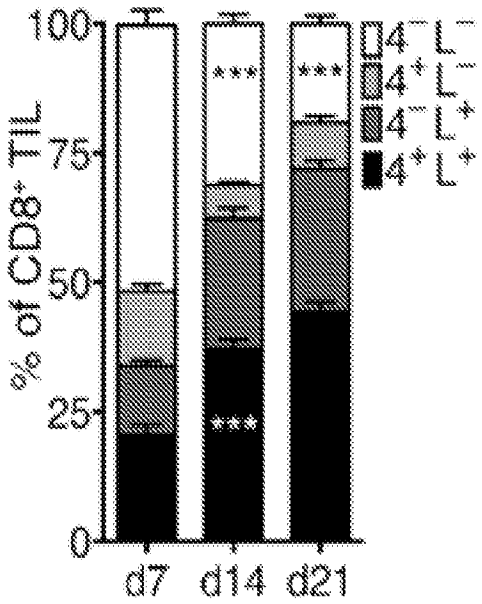

100
75
50
25
0
% of CD8+ TIL
***
***
***
4⁻L⁻
4⁺L⁻
4⁻L⁺
4⁺L⁺
d7
d14
d21

FIG. 1C

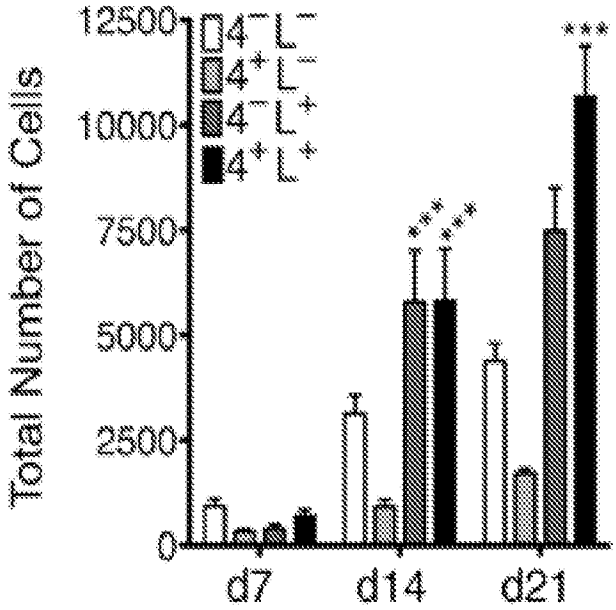

12500
10000
7500
5000
2500
0
Total Number of Cells
4⁻L⁻
4⁺L⁻
4⁻L⁺
4⁺L⁺
***
***
***
d7
d14
d21

FIG. 1D

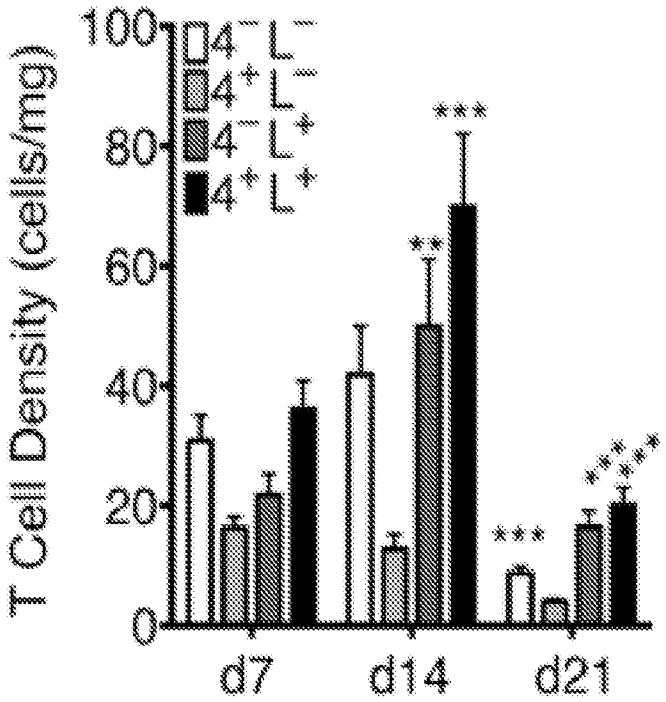

T Cell Density (cells/mg)
100
80
60
40
20
0
4⁻L⁻
4⁺L⁻
4⁻L⁺
4⁺L⁺
***
**
***
***
d7
d14
d21

FIG. 1E

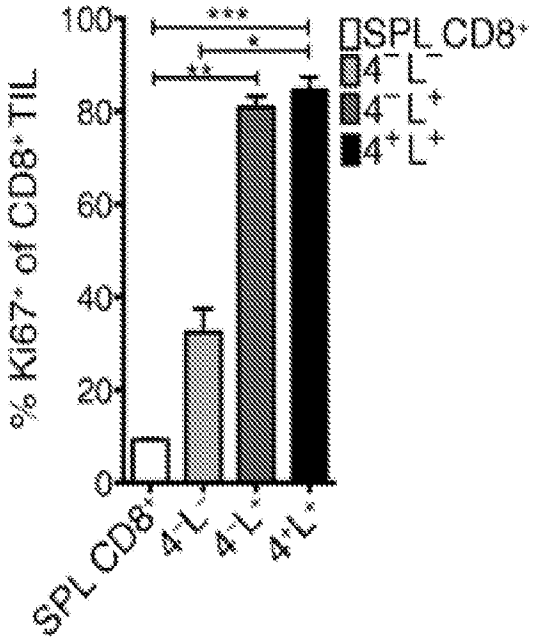

% Ki67⁺ of CD8⁺ TIL
100
80
60
40
20
0
***
*
**
SPL CD8⁺
4⁻L⁻
4⁻L⁺
4⁺L⁺
SPL CD8⁺
4⁻L⁻
4⁻L⁺
4⁺L⁺

FIG. 1F

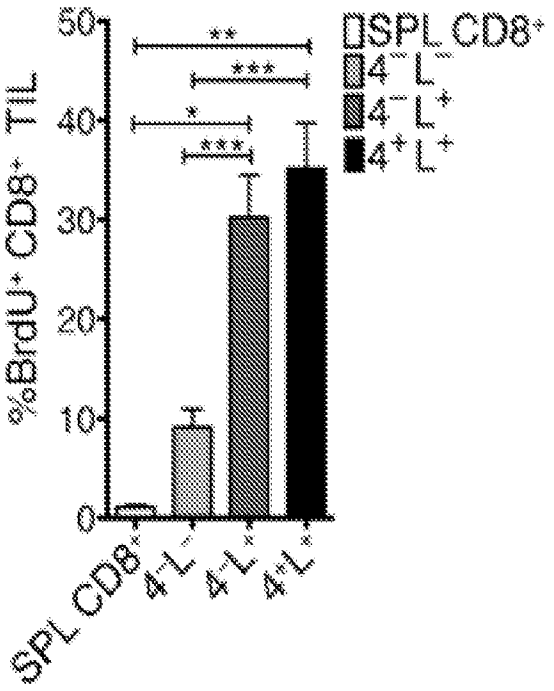

%BrdU+ CD8+ TIL
50
40
30
20
10
0
**
***
*
***
SPL CD8+
4-L-
4-L+
4+L+
SPL CD8+
4-L-
4-L+
4+L+

FIG. 1J

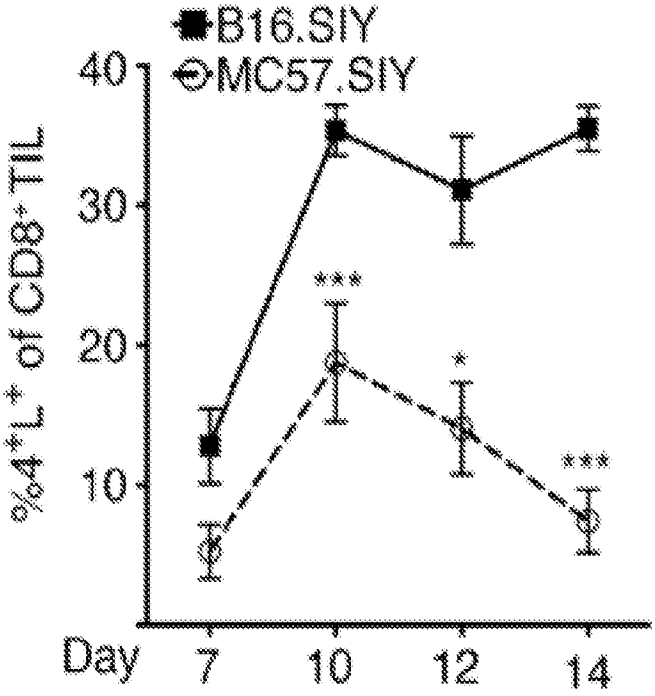

B16.SIY
MC57.SIY
%4+L+ of CD8+ TIL
40
30
20
10
***
*
***
Day
7
10
12
14

FIG. 1G

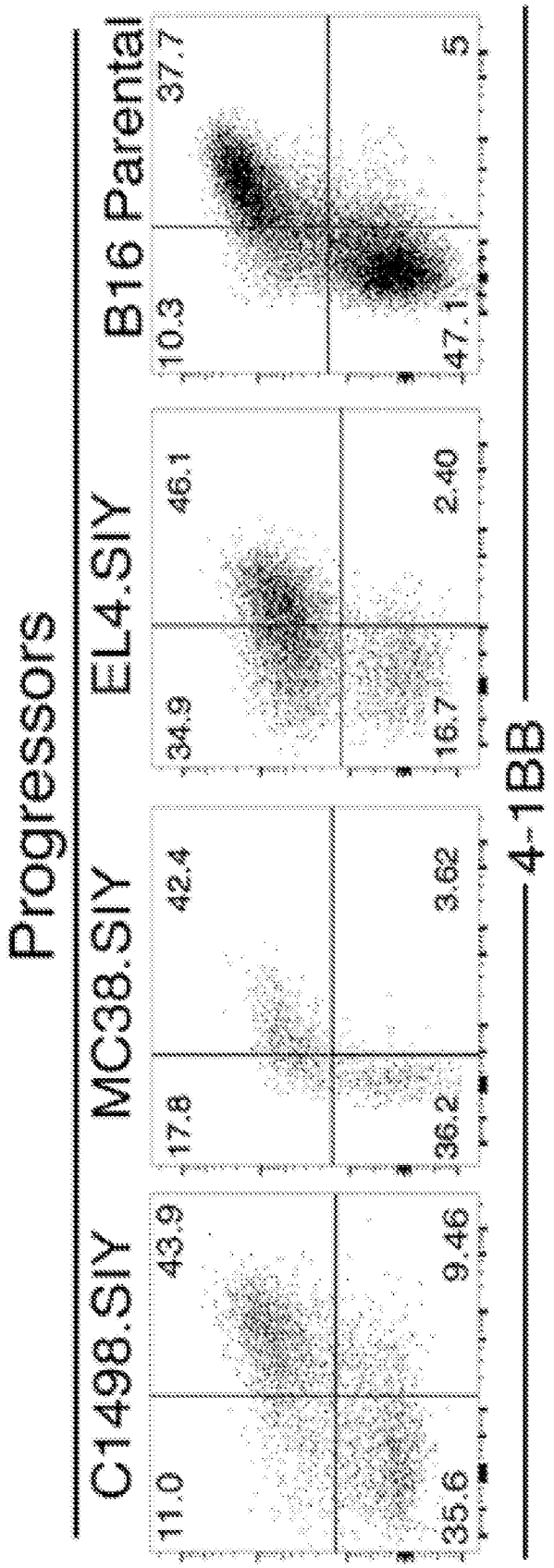

Progressors
C1498.SIY
MC38.SIY
EL4.SIY
B16 Parental
LAG-3
4-1BB
11.0
43.9
35.6
9.46
17.8
42.4
36.2
3.62
34.9
46.1
16.7
2.40
10.3
37.7
47.1
5

FIG. 1H

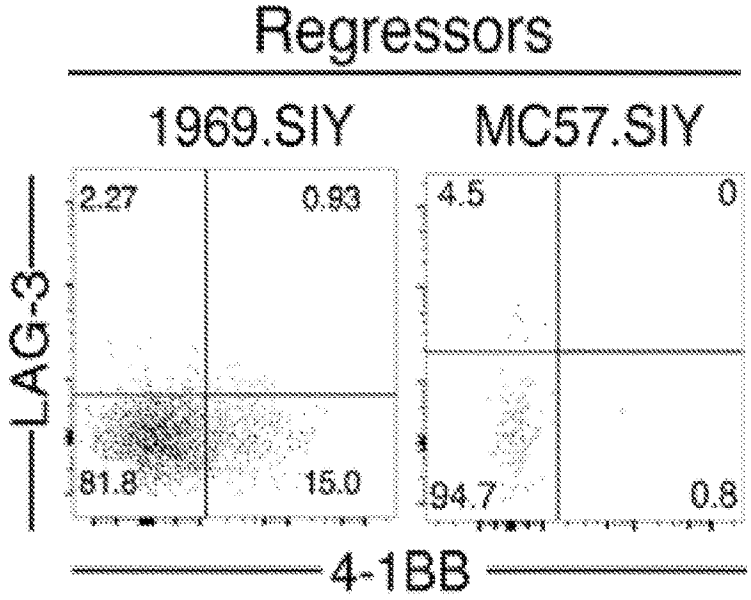

Regressors
1969.SIY
MC57.SIY
LAG-3
4-1BB
2.27
0.93
81.8
15.0
4.5
0
94.7
0.8

FIG. 1I

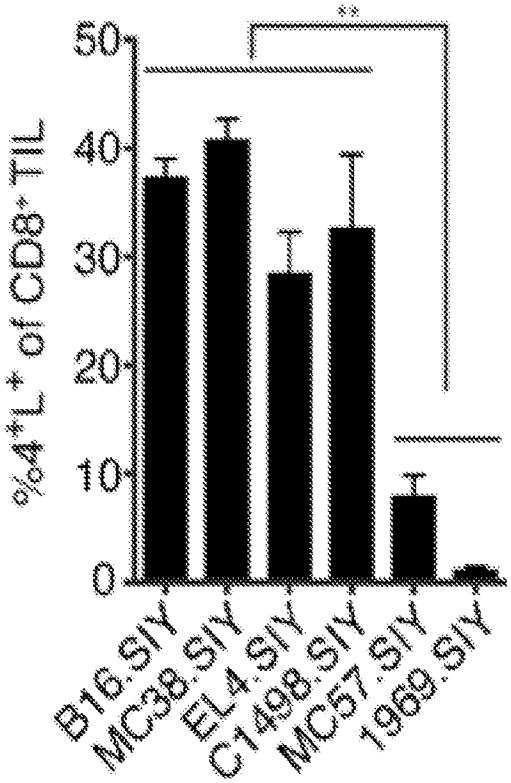

**
%4+L+ of CD8+ TIL
50
40
30
20
10
0
B16.SIY
MC38.SIY
EL4.SIY
C1498.SIY
MC57.SIY
1969.SIY

FIG. 2A

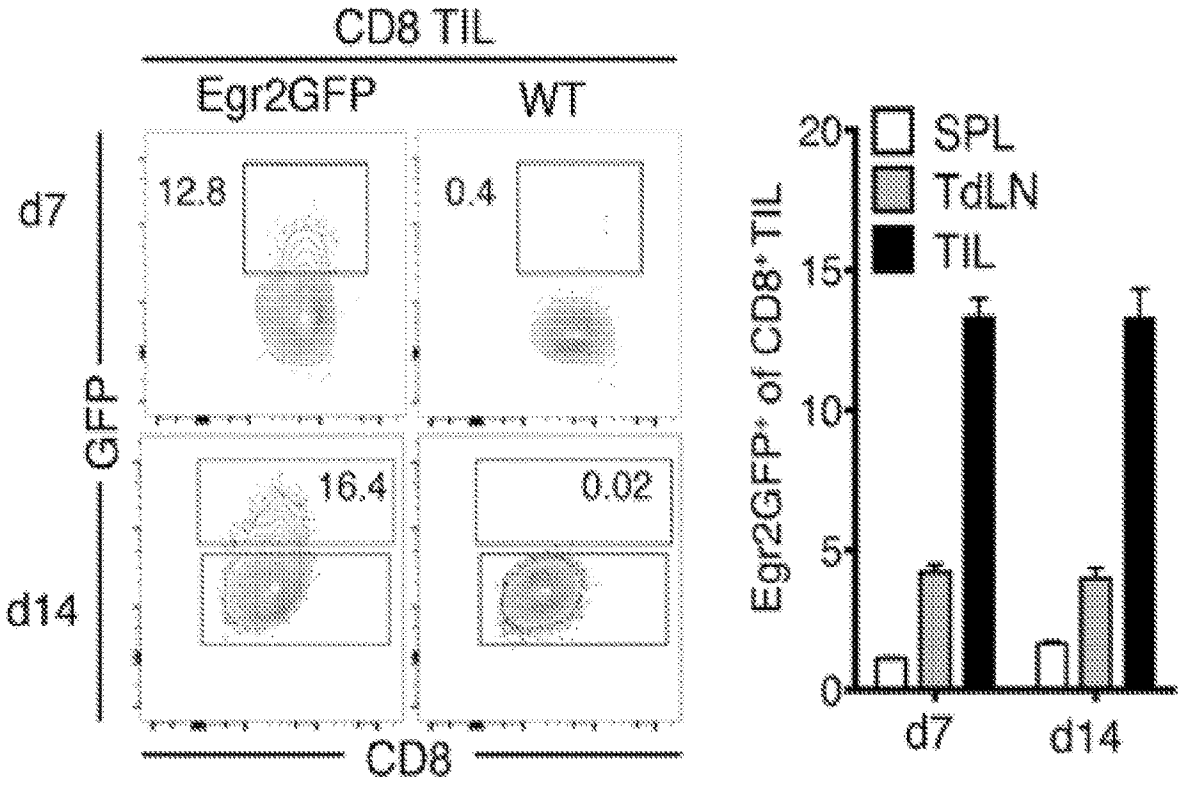

CD8 TIL
Egr2GFP
WT
d7
d14
GFP
12.8
0.4
16.4
0.02
CD8
Egr2GFP+ of CD8+ TIL
20
15
10
5
0
SPL
TdLN
TIL
d7
d14

FIG. 2B

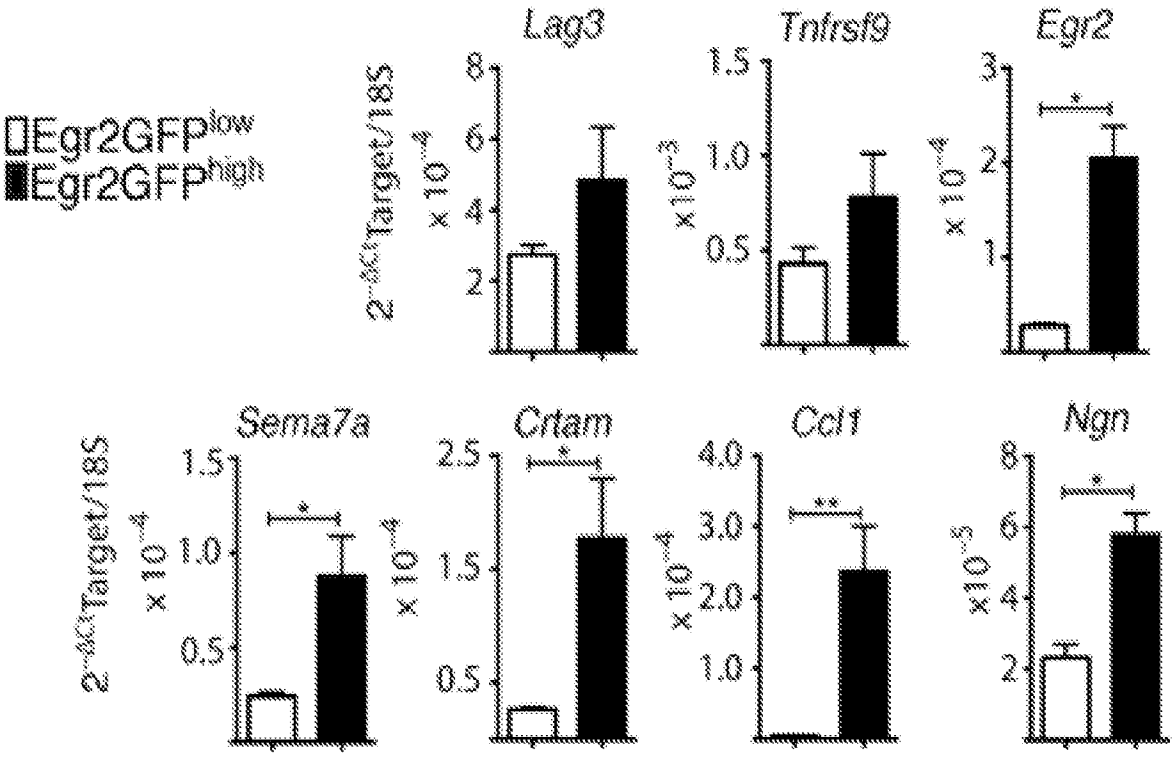

Egr2GFP^low
Egr2GFP^high
Lag3
Tnfrsf9
Egr2
Sema7a
Crtam
Ccl1
Ngn
2^-ΔCt Target/18S
x 10^-4
x 10^-3
*
**

FIG. 2C

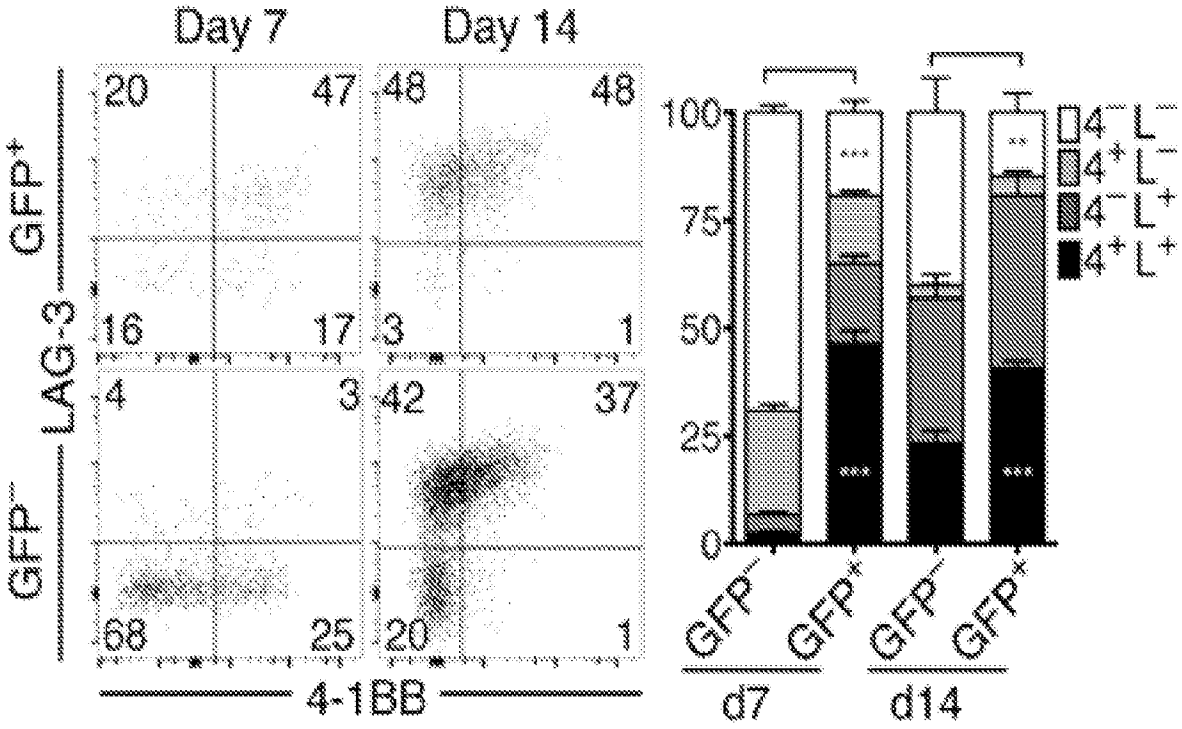

Day 7
Day 14
GFP+
GFP−
LAG-3
4-1BB
20
47
16
17
48
48
3
1
4
3
68
25
42
37
20
1
100
75
50
25
0
GFP−
GFP+
GFP−
GFP+
d7
d14
4−L−
4+L−
4−L+
4+L+

FIG. 2D

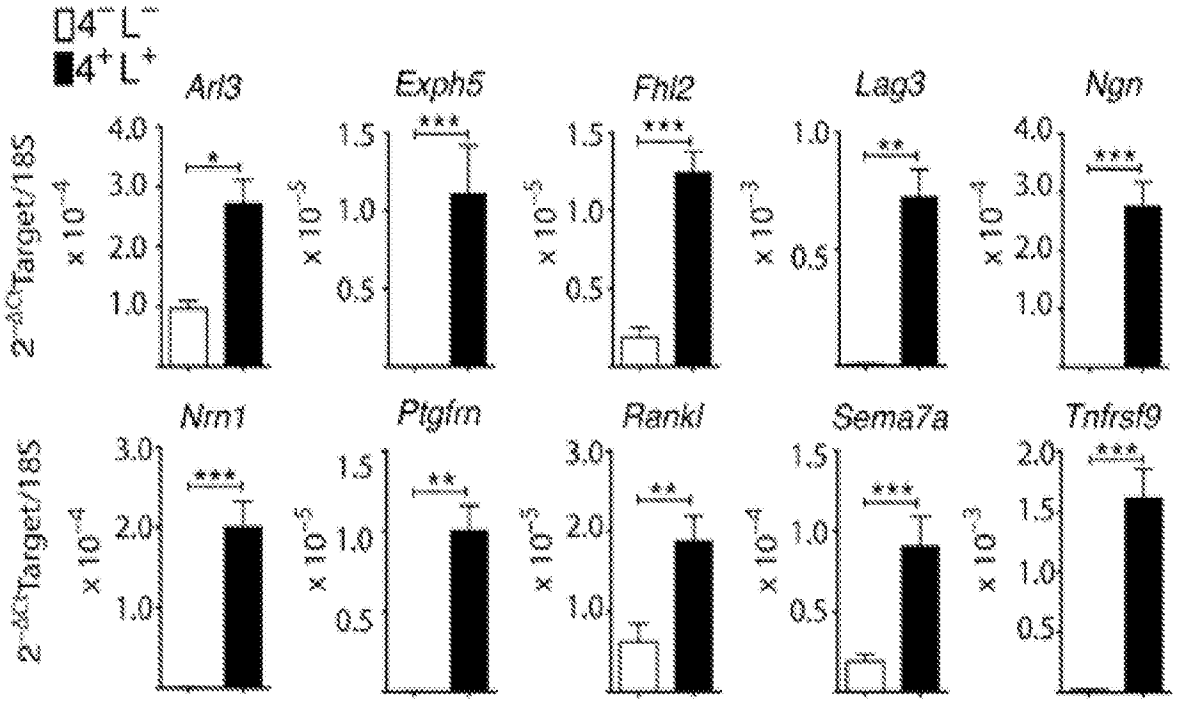

4⁻L⁻
4⁺L⁺
Arl3
Exph5
Fhl2
Lag3
Ngn
Nrn1
Ptgfrn
Rankl
Sema7a
Tnfrsf9
2^-ΔCTarget/18S
× 10⁻⁴
× 10⁻³
*
**
***

FIG. 2E

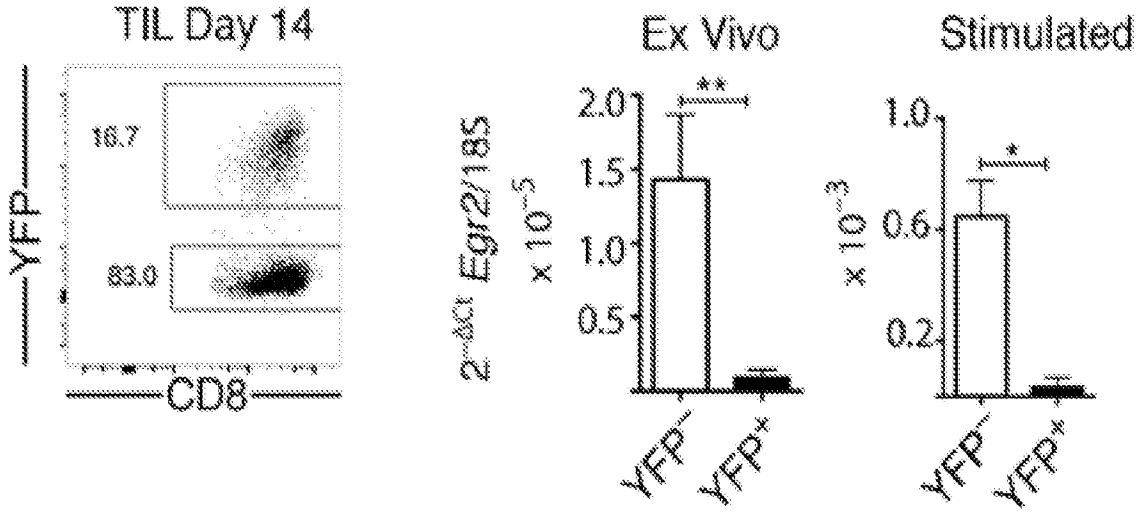
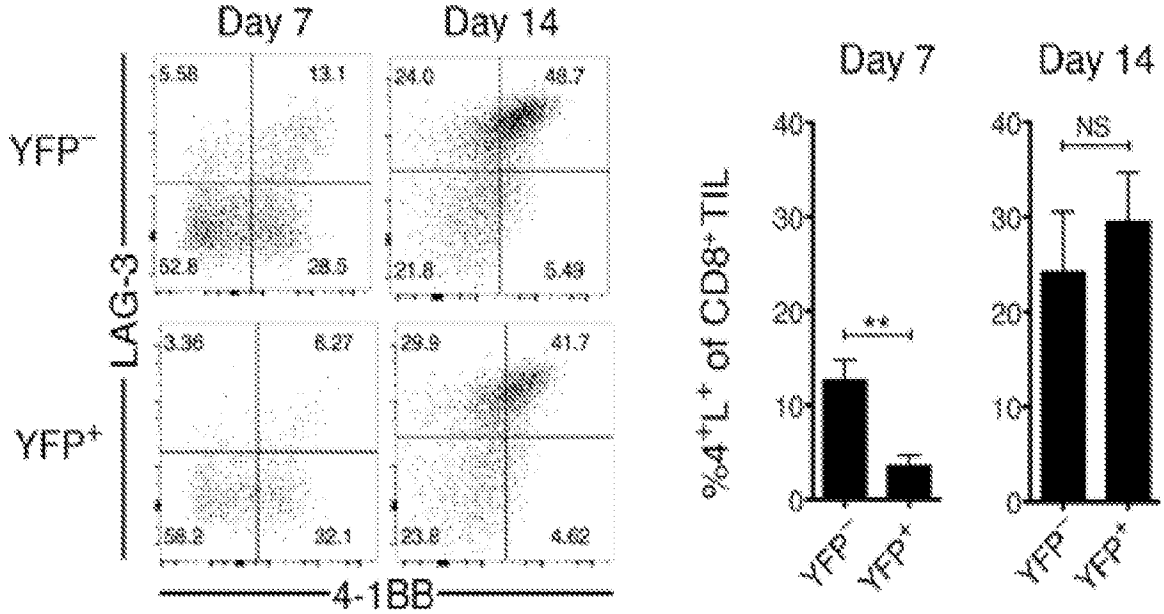

TIL Day 14
YFP
CD8
16.7
83.0
Ex Vivo
Stimulated
2^-ΔCt Egr2/18S
x 10^-5
x 10^-3
2.0
1.5
1.0
0.5
0.6
0.2
**
*
YFP-
YFP+

FIG. 2F


Day 7
Day 14
YFP-
YFP+
LAG-3
4-1BB
5.58
13.1
52.6
28.9
24.0
48.7
21.8
5.49
3.36
8.27
58.2
32.1
29.9
41.7
23.8
4.62
%4+1L+ of CD8+ TIL
40
30
20
10
0
**
NS
YFP-
YFP+

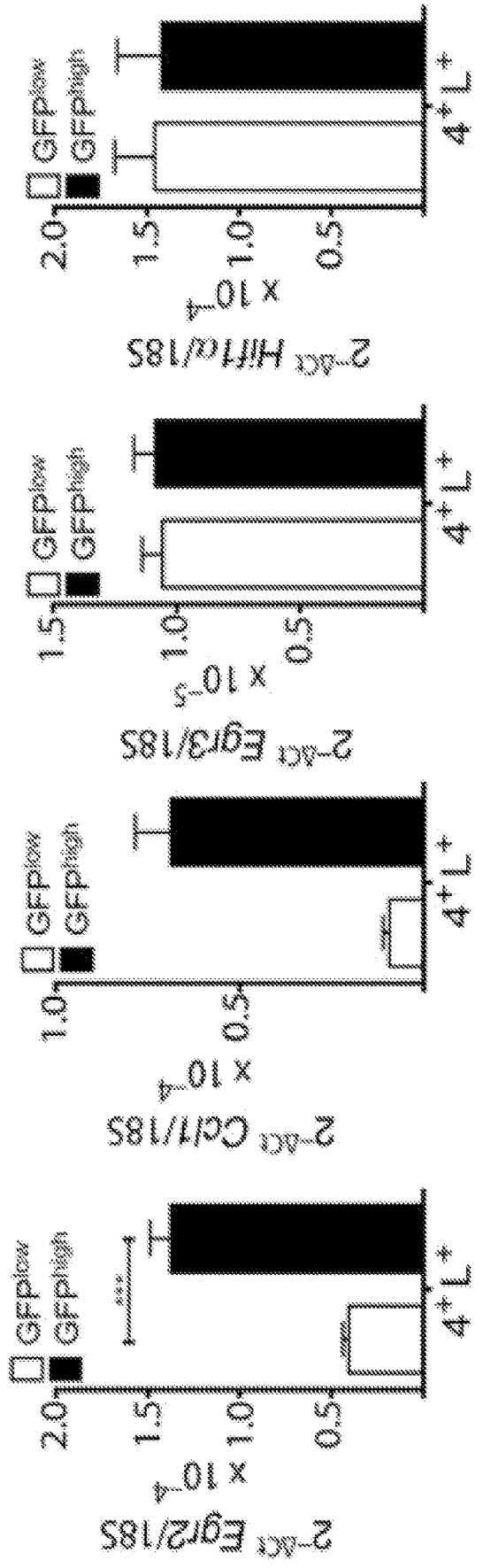

GFPlow
GFPhigh
4+L+
2.0
1.5
1.0
0.5
x 10−4
2−ΔCt Hif1α/18S
GFPlow
GFPhigh
4+L+
1.5
1.0
0.5
x 10−5
2−ΔCt Egr3/18S
GFPlow
GFPhigh
4+L+
1.0
0.5
x 10−4
2−ΔCt Cd1/18S
GFPlow
GFPhigh
4+L+
2.0
1.5
1.0
0.5
x 10−4
2−ΔCt Egr2/18S

FIG. 2G

FIG. 3A

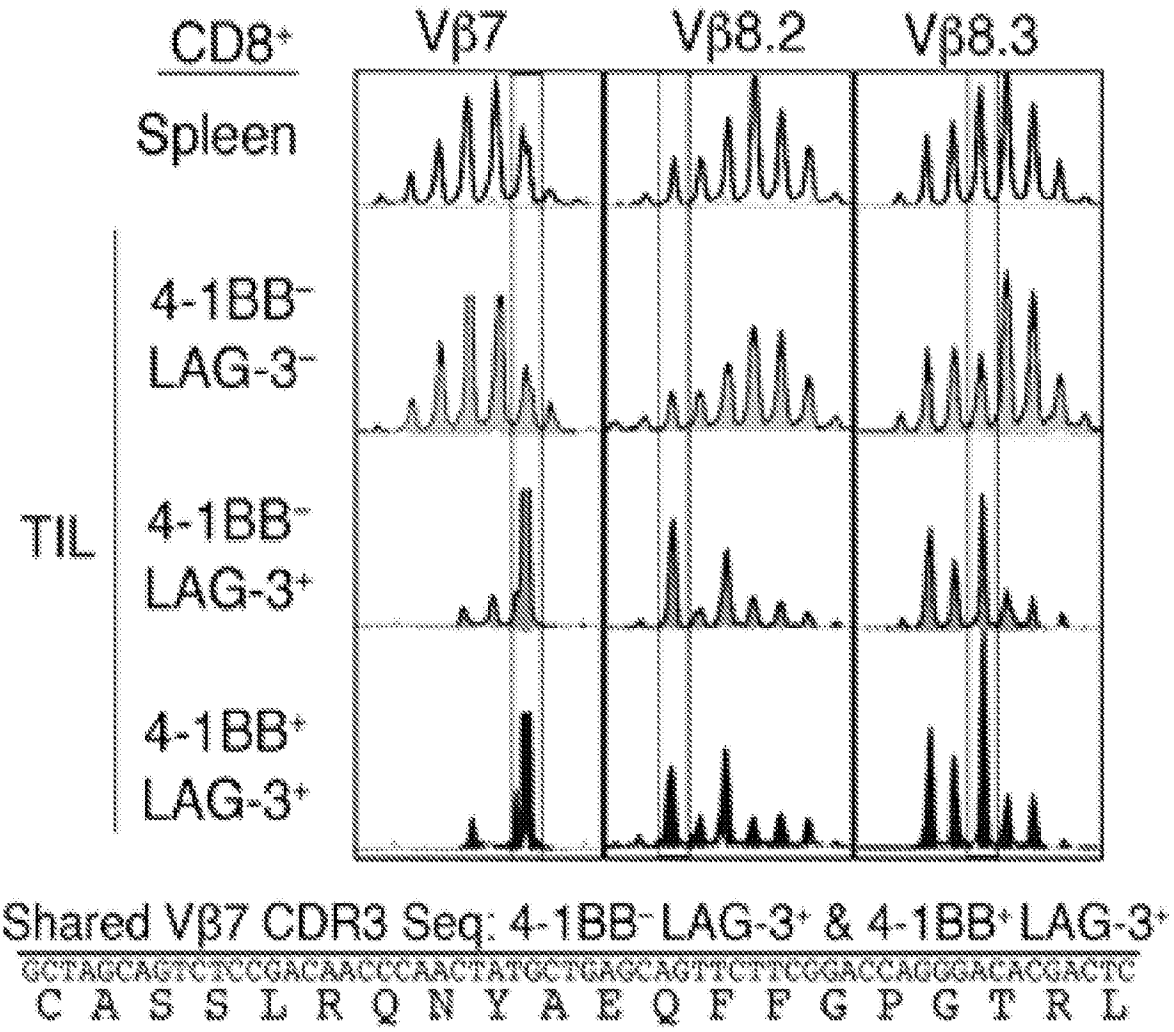

CD8+
Vβ7
Vβ8.2
Vβ8.3
Spleen
TIL
4-1BB−
LAG-3−
4-1BB−
LAG-3+
4-1BB+
LAG-3+
Shared Vβ7 CDR3 Seq: 4-1BB− LAG-3+ & 4-1BB+ LAG-3+
GCTAGCAGTCTCCGACAACCCAACTATCCTGAGCAGTTCTTCGGACCAGGGACACGACTC
C A S S L R Q N Y A E Q F F G P G T R L

FIG. 3B

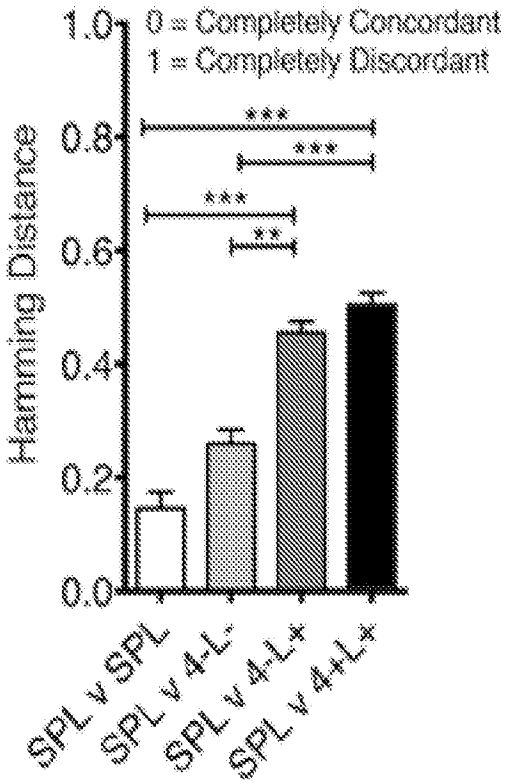

0 = Completely Concordant
1 = Completely Discordant
Hamming Distance
1.0
0.8
0.6
0.4
0.2
0.0
***
***
***
**
SPL v SPL
SPL v 4-L-
SPL v 4-L+
SPL v 4+L+

FIG. 3C

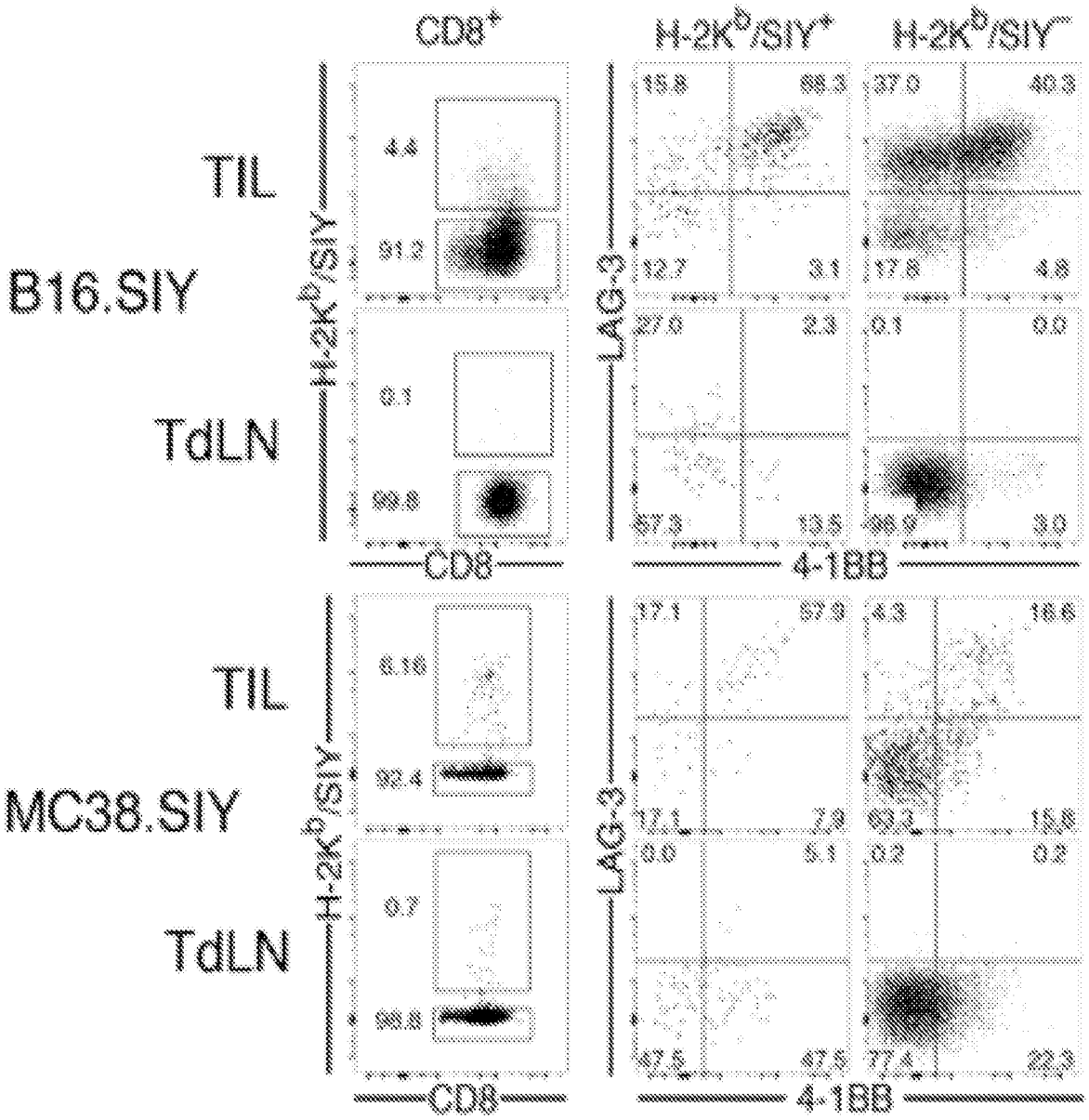

CD8+
H-2Kb/SIY+
H-2Kb/SIY−
TIL
B16.SIY
TdLN
TIL
MC38.SIY
TdLN
H-2Kb/SIY
CD8
LAG-3
4-1BB
4.4
91.2
0.1
99.8
15.8
68.3
12.7
3.1
27.0
2.3
57.9
13.5
37.0
40.3
17.8
4.8
0.1
0.0
96.9
3.0
6.16
92.4
0.7
98.8
17.1
57.9
17.1
7.9
0.0
5.1
47.5
47.5
4.3
16.6
63.1
15.8
0.2
0.2
77.4
22.3

FIG. 3D

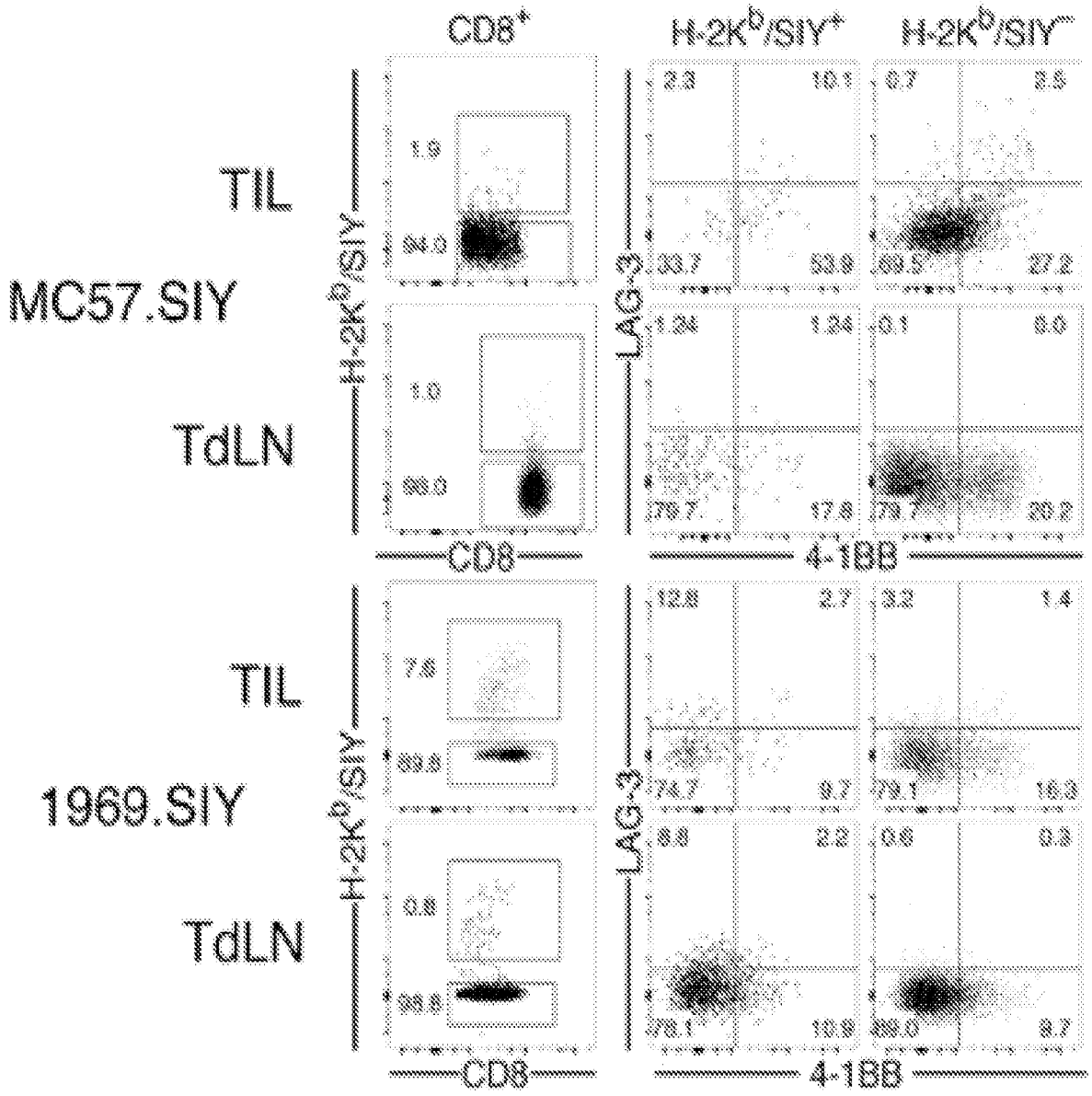

CD8+
H-2Kb/SIY+
H-2Kb/SIY−
TIL
MC57.SIY
TdLN
TIL
1969.SIY
TdLN
H-2Kb/SIY
LAG-3
CD8
4-1BB
1.9
94.0
2.3
10.1
0.7
2.6
30.7
53.9
69.5
27.2
1.0
98.0
1.24
1.24
0.1
0.0
79.7
17.8
79.7
20.2
7.6
89.8
12.8
2.7
3.2
1.4
74.7
9.7
79.1
16.3
0.8
98.6
8.8
2.2
0.6
0.3
78.1
10.9
89.0
9.7

FIG. 3E

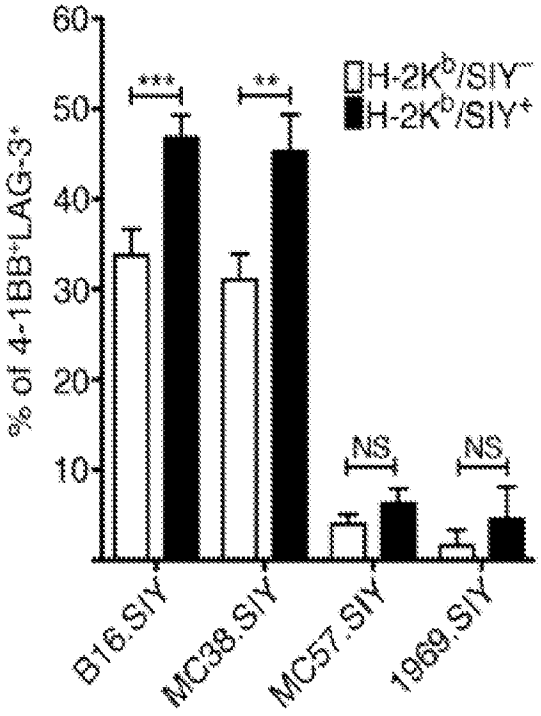

% of 4-1BB+LAG-3+
60
50
40
30
20
10
***
**
NS
NS
H-2Kb/SIY−
H-2Kb/SIY+
B16.SIY
MC38.SIY
MC57.SIY
1969.SIY

FIG. 3F

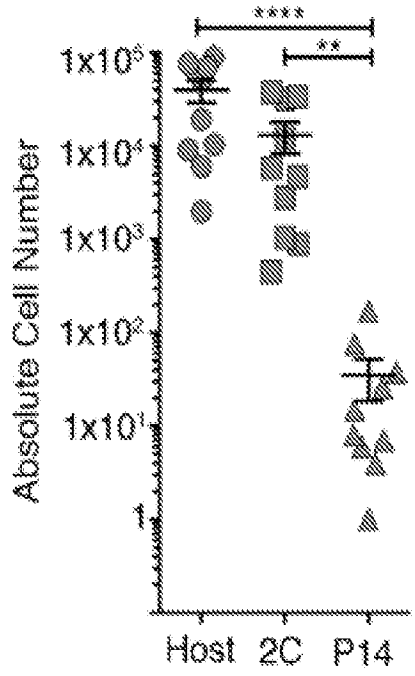

Absolute Cell Number
1x10⁵
1x10⁴
1x10³
1x10²
1x10¹
1
****
**
Host
2C
P14

FIG. 3G

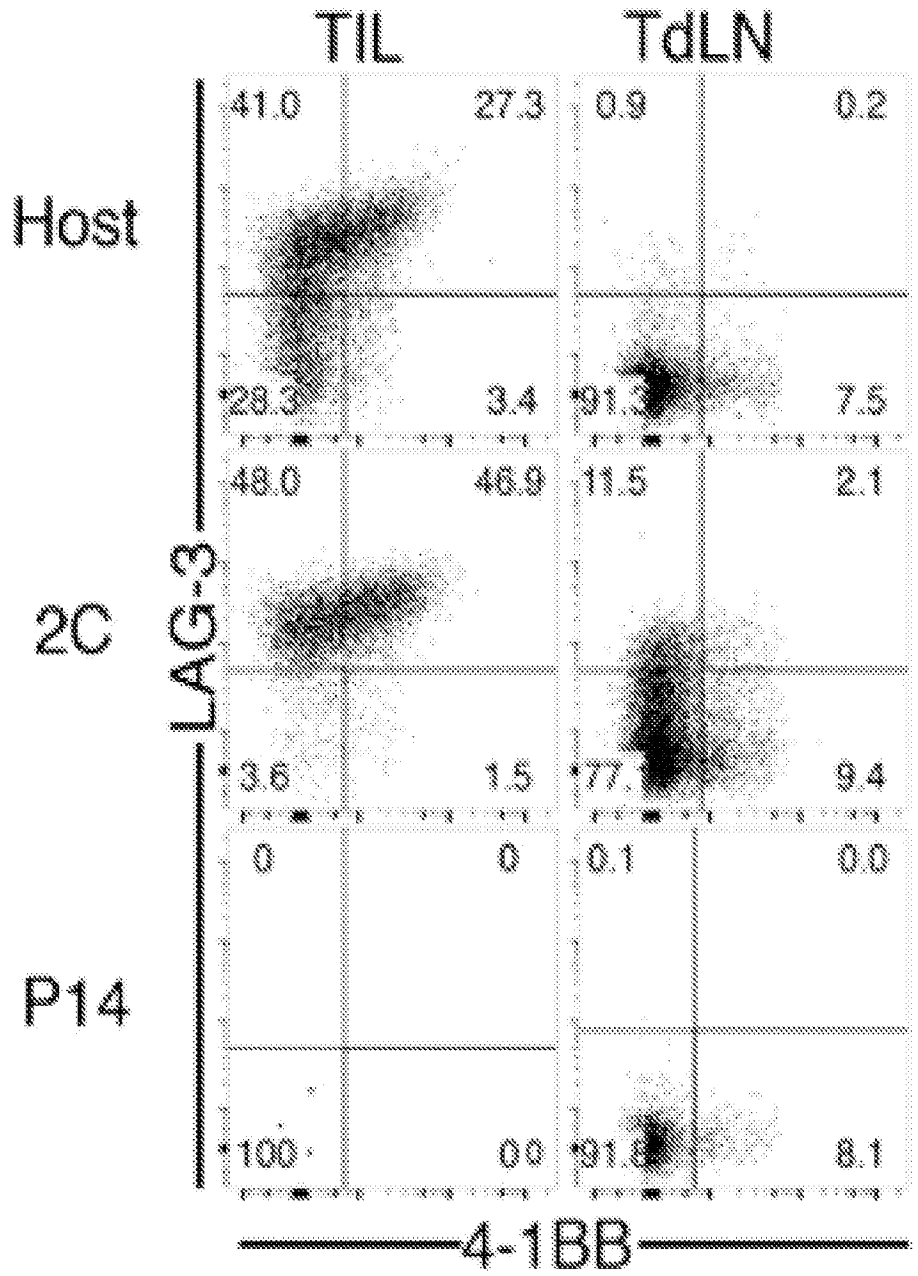

TIL
TdLN
Host
2C
P14
LAG-3
4-1BB
41.0
27.3
28.3
3.4
0.9
0.2
91.3
7.5
48.0
46.9
3.6
1.5
11.5
2.1
77.1
9.4
0
0
100
0.0
0.1
0.0
91.8
8.1

FIG. 3H

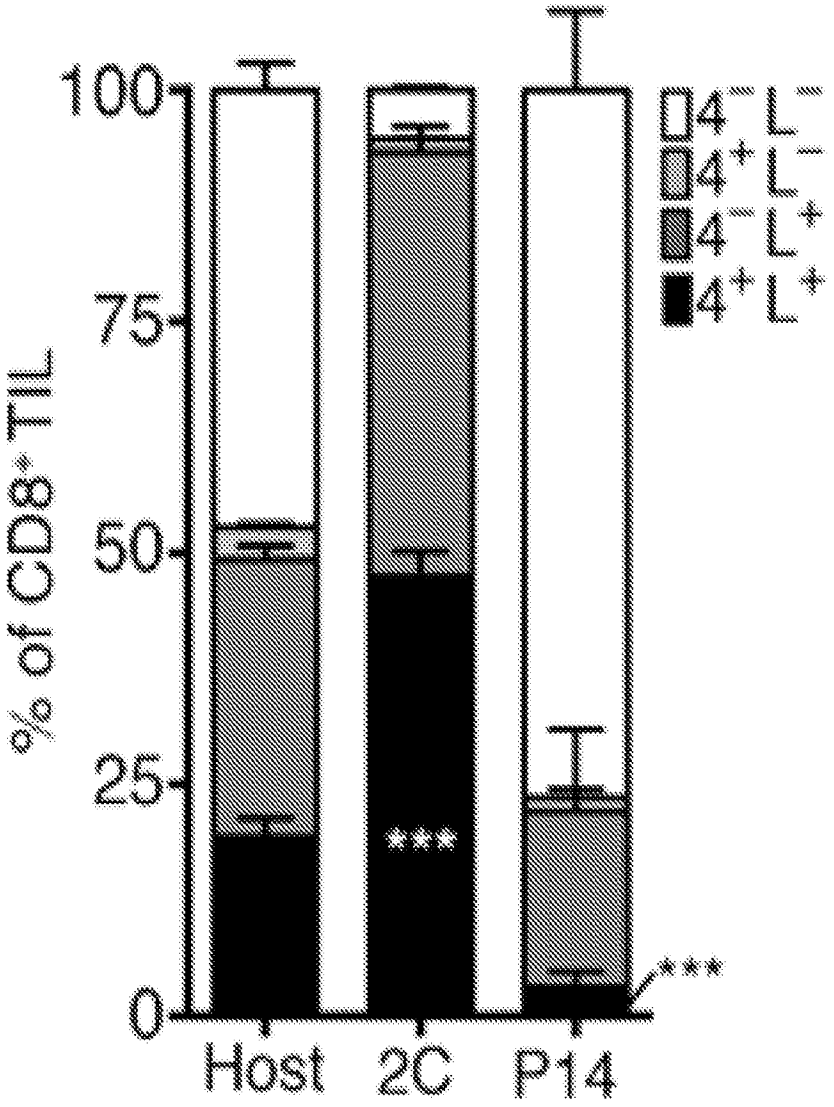


FIG. 4A

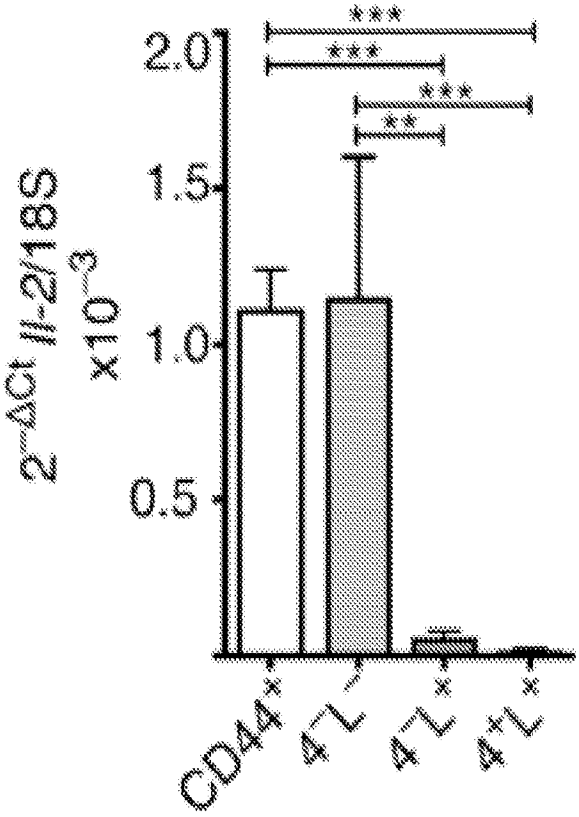

***
***
***
**
2.0
1.5
1.0
0.5
$2^{-\Delta Ct}$ Il-2/18S
x10^{-3}
CD44+
4-L-
4-L+
4+L+

FIG. 4B

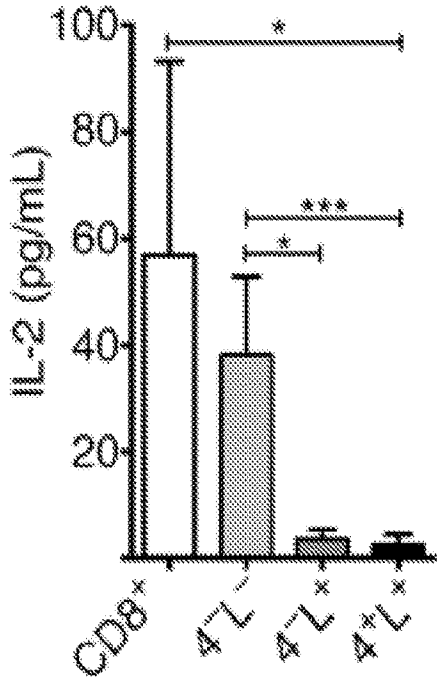

*
***
*
100
80
60
40
20
IL-2 (pg/mL)
CD8+
4-L-
4-L+
4+L+

FIG. 4C

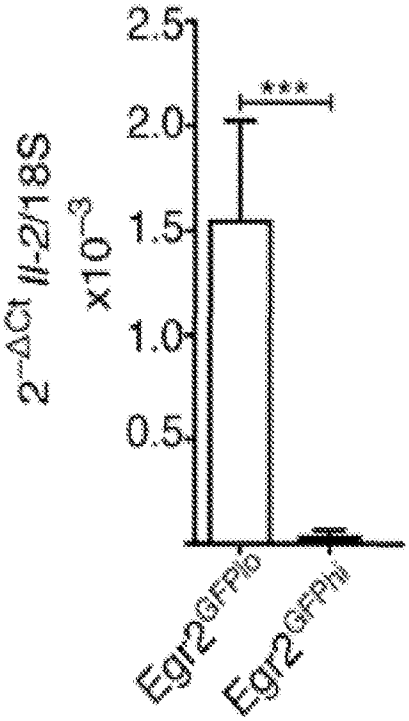

2.5
2.0
1.5
1.0
0.5
***
$2^{-\Delta Ct}$ Il-2/18S
x10^{-3}
Egr2^{GFPlo}
Egr2^{GFPhi}

FIG. 4D

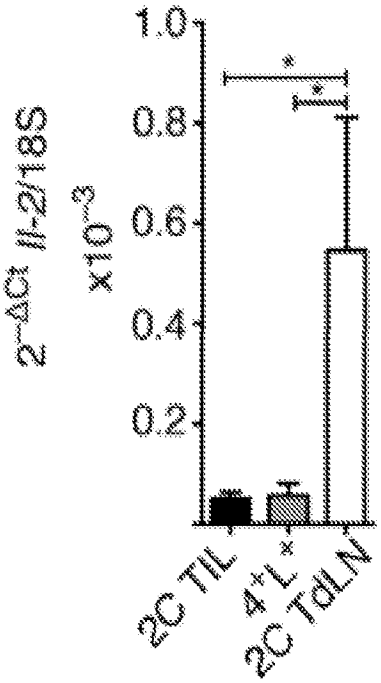

1.0
0.8
0.6
0.4
0.2
*
*
$2^{-\Delta Ct}$ Il-2/18S
x10^{-3}
2C TIL
4^{+}L^{+}
2C TdLN

FIG. 4E

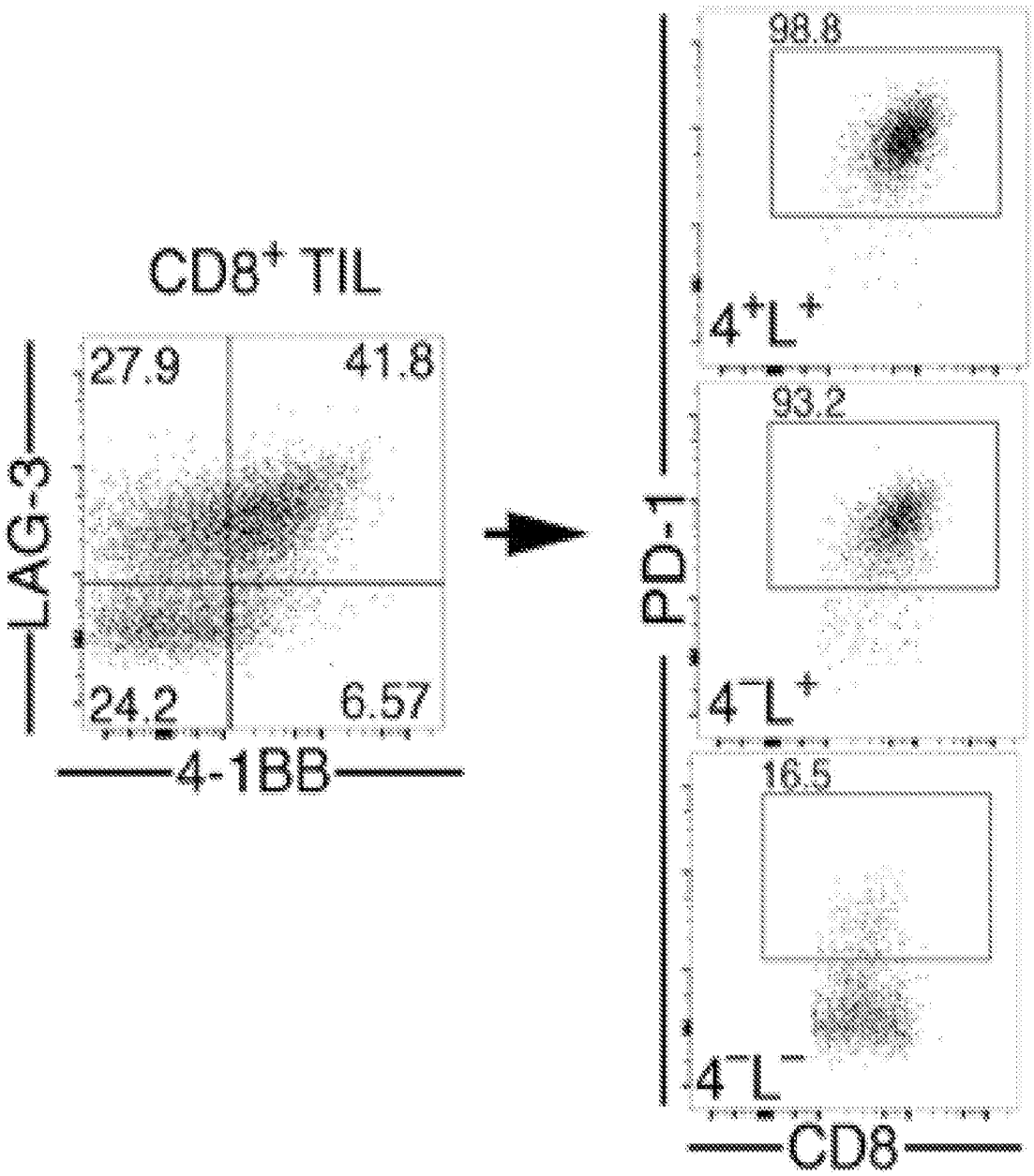


FIG. 4F

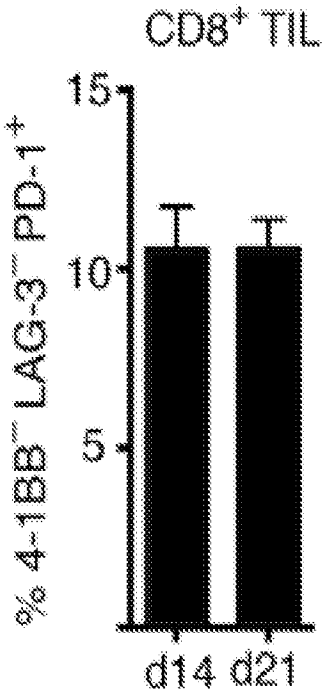

CD8+ TIL
% 4-1BB− LAG-3− PD-1+
15
10
5
d14 d21

FIG. 4G

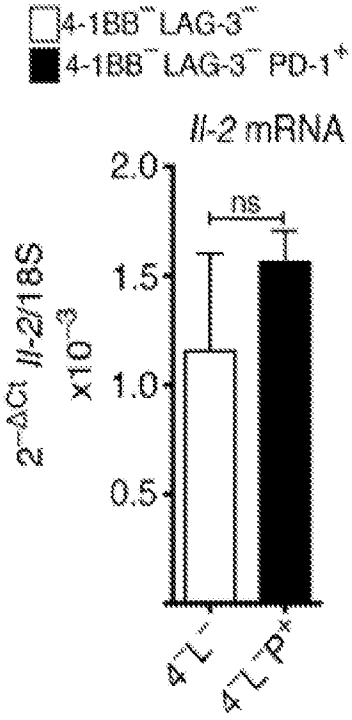
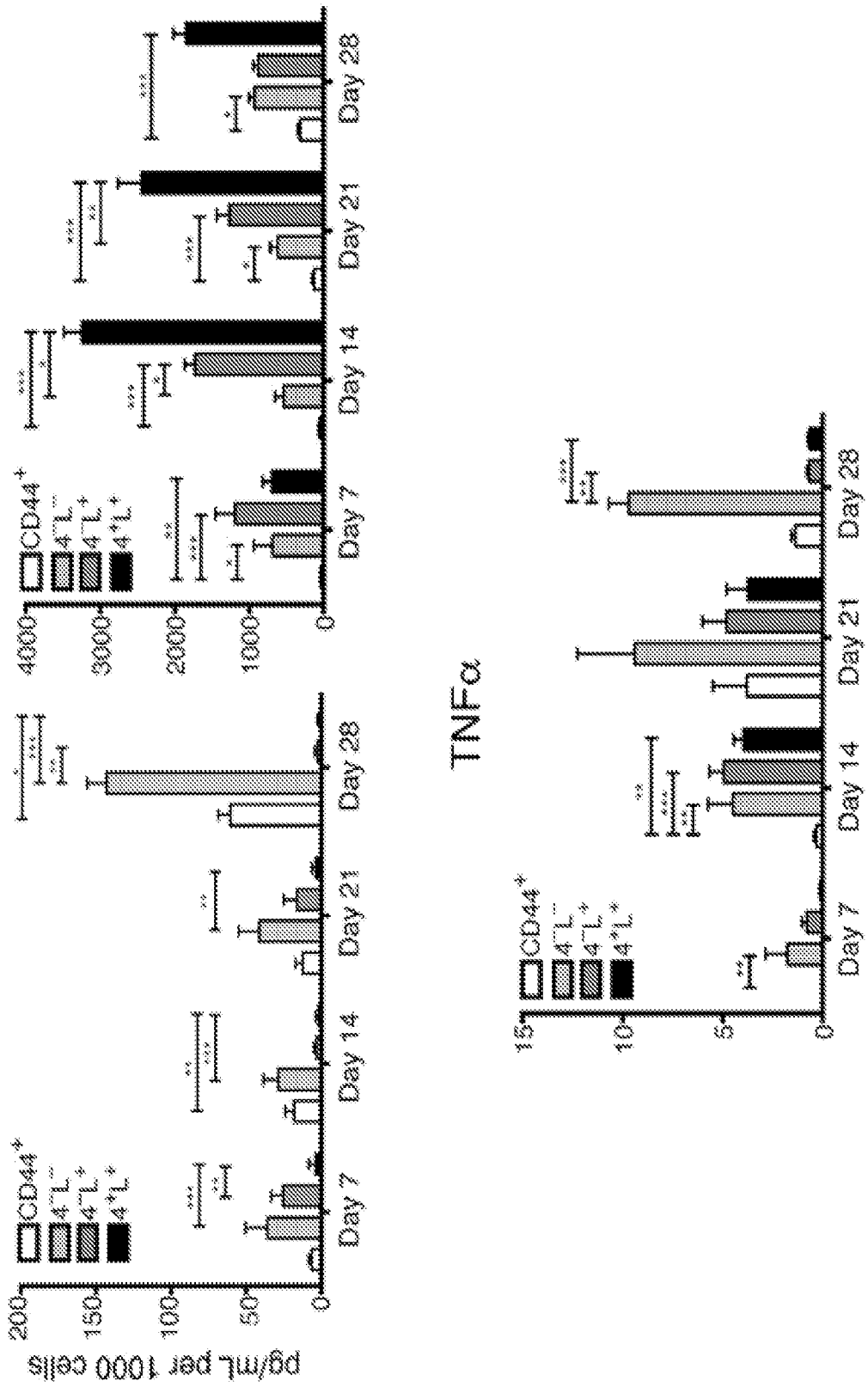

4-1BB− LAG-3−
4-1BB− LAG-3− PD-1+
Il-2 mRNA
2−ΔCt Il-2/18S x10−3
2.0
1.5
1.0
0.5
ns
4−L−
4−L−P+

FIG. 5A


IL-2
IFN-γ
TNFα
pg/mL per 1000 cells
CD44+
4+L-
4-L+
4+L+
Day 7
Day 14
Day 21
Day 28

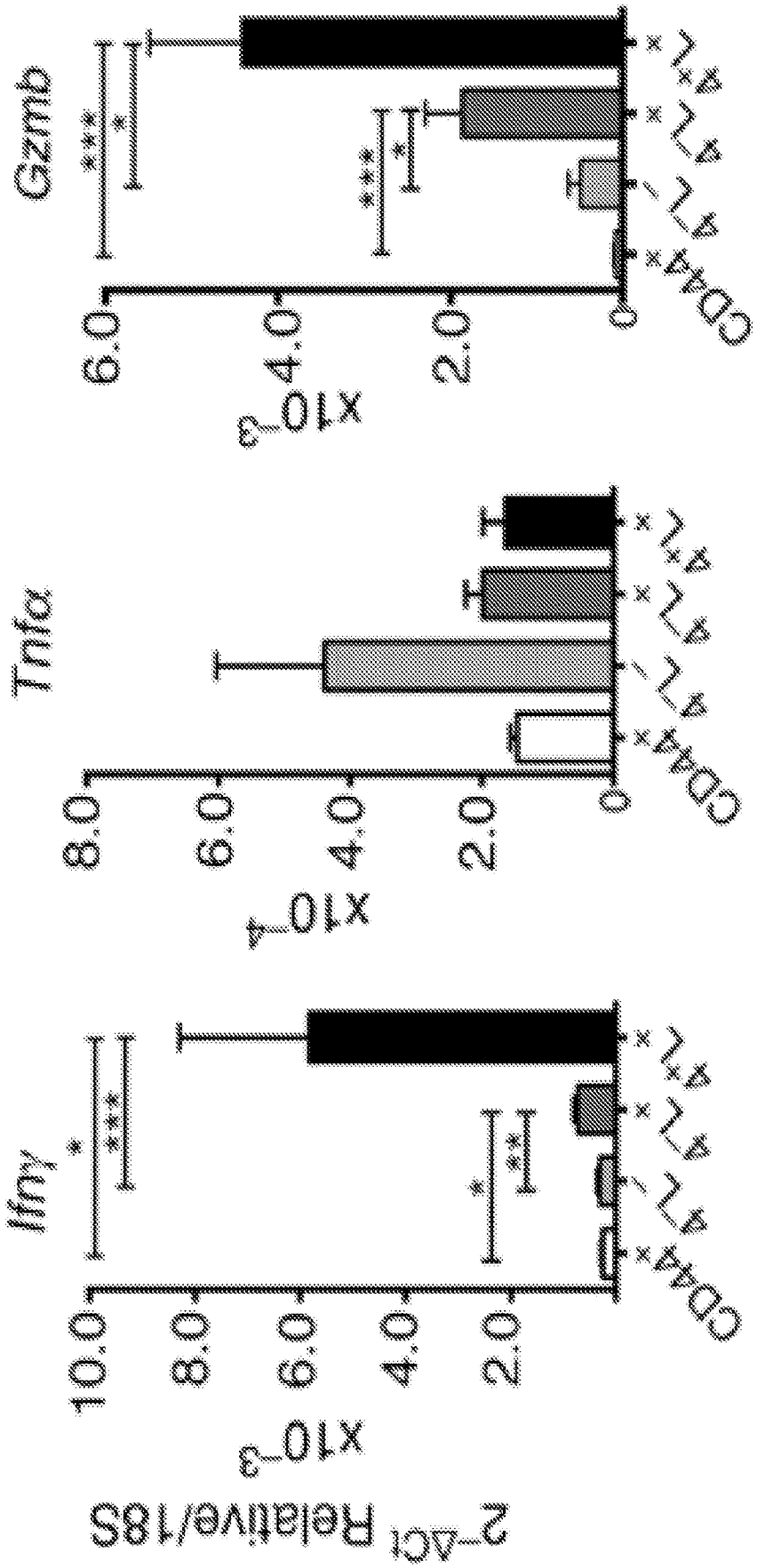

Gzmb
Tnfα
Ifnγ
2^-ΔCt Relative/18S
x10^-3
x10^-4
x10^-3
6.0
4.0
2.0
0
8.0
10.0
CD4 Δ/Δ
CD4 Δ/-
CD4 +/Δ
CD4 +/+
***
**
*

FIG. 5B

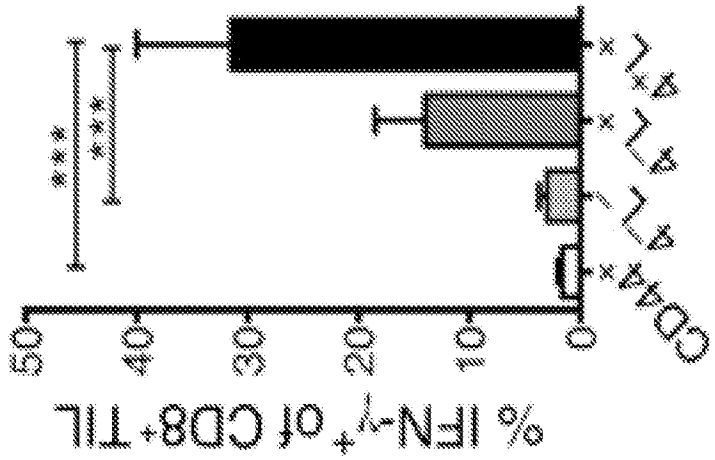

% IFN-γ+ of CD8+ TIL
60
40
30
20
10
0
CD4+4-1BB+
CD4+4-1BB-
CD4-4-1BB+
CD4-4-1BB-
***
***
***

FIG. 5C

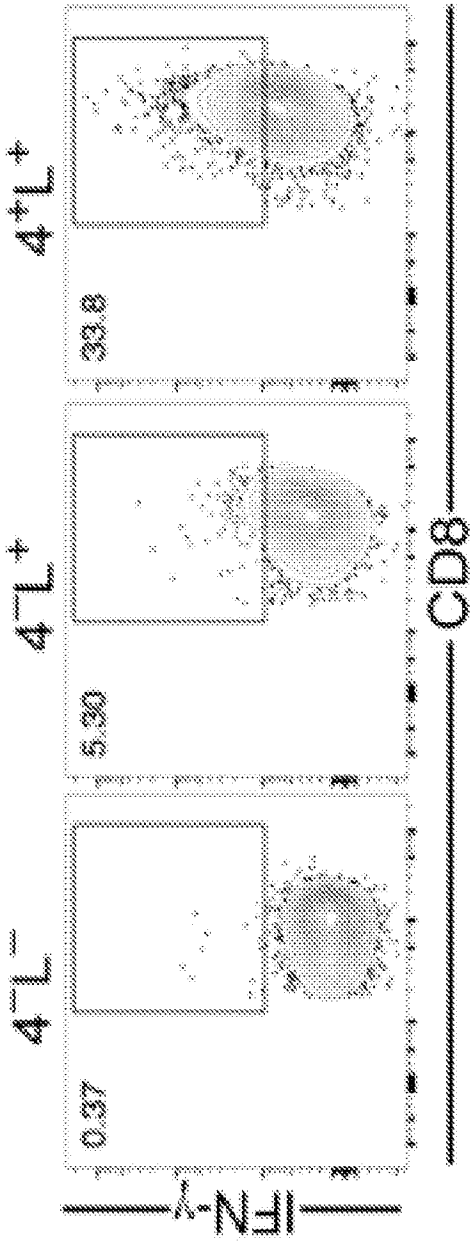
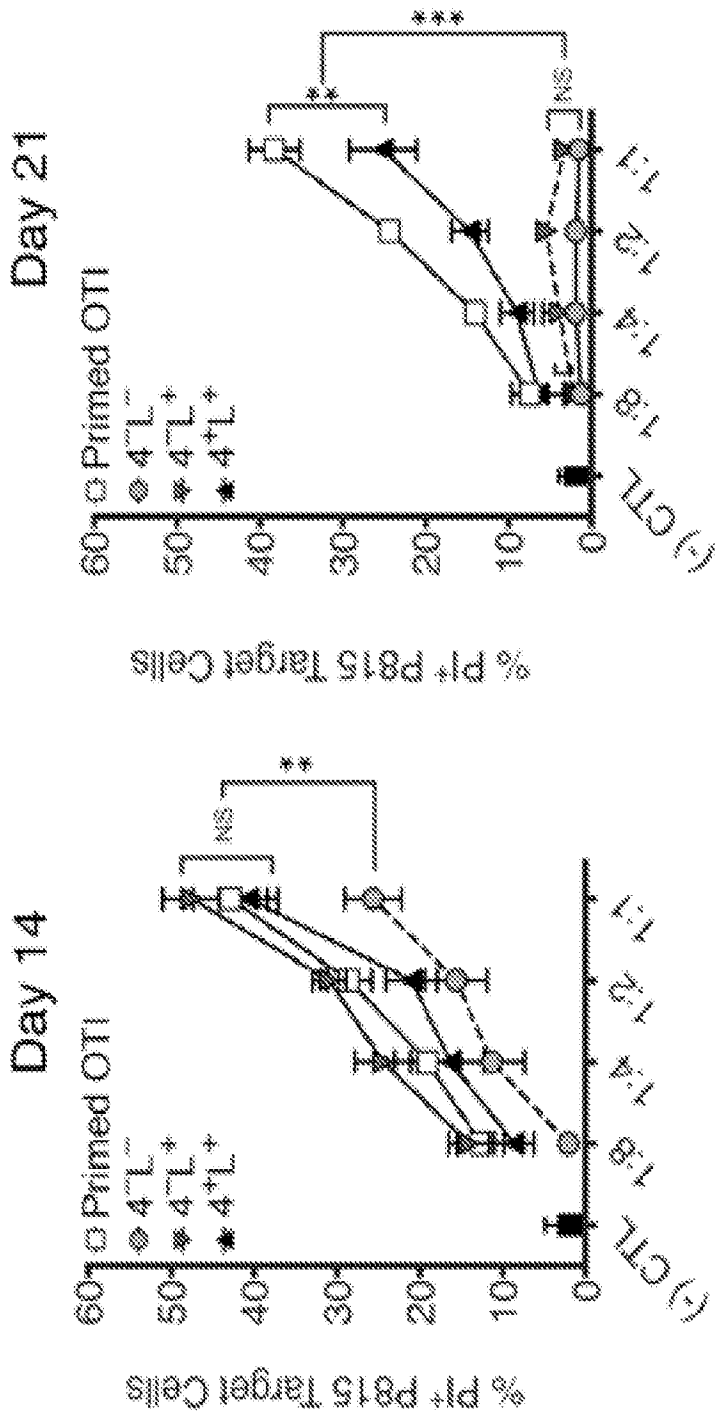

4+L+
38.8
4-L+
6.30
4-L-
0.37
CD8
IFN-γ

FIG. 5D


Day 21
Day 14
% Pr+ P815 Target Cells
CTL (-)
1:1
1:2
1:4
1:8
0
10
20
30
40
50
60
Primed OTI
4-L-
4-L+
4+L+
***
**
ns

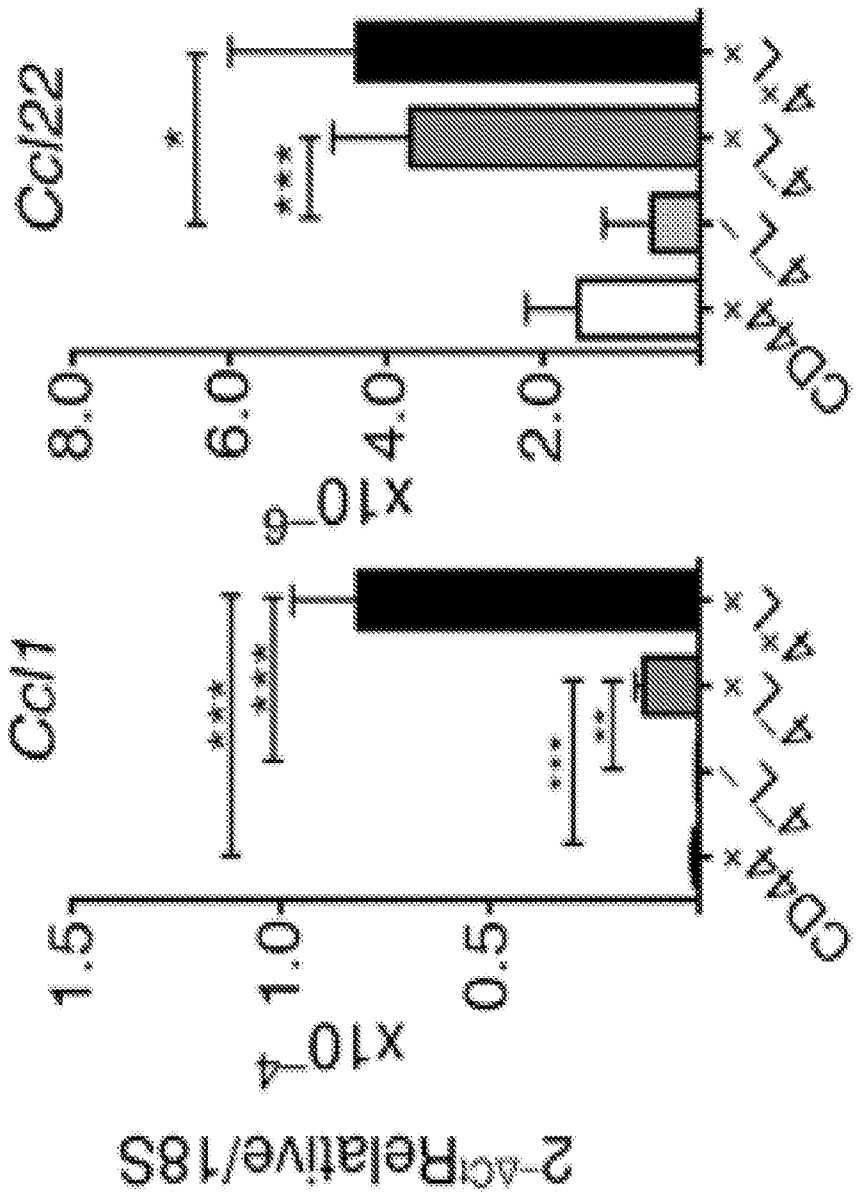

Ccl22
Ccl1
2−ΔCtRelative/18S
x10−6
x10−4
8.0
6.0
4.0
2.0
1.5
1.0
0.5
CD4+
CD4+ Vγ4−/−
CD4+ Vγ4+
CD4+ Vγ4+
*
***
***
***
***
**

FIG. 5E

FIG. 6A

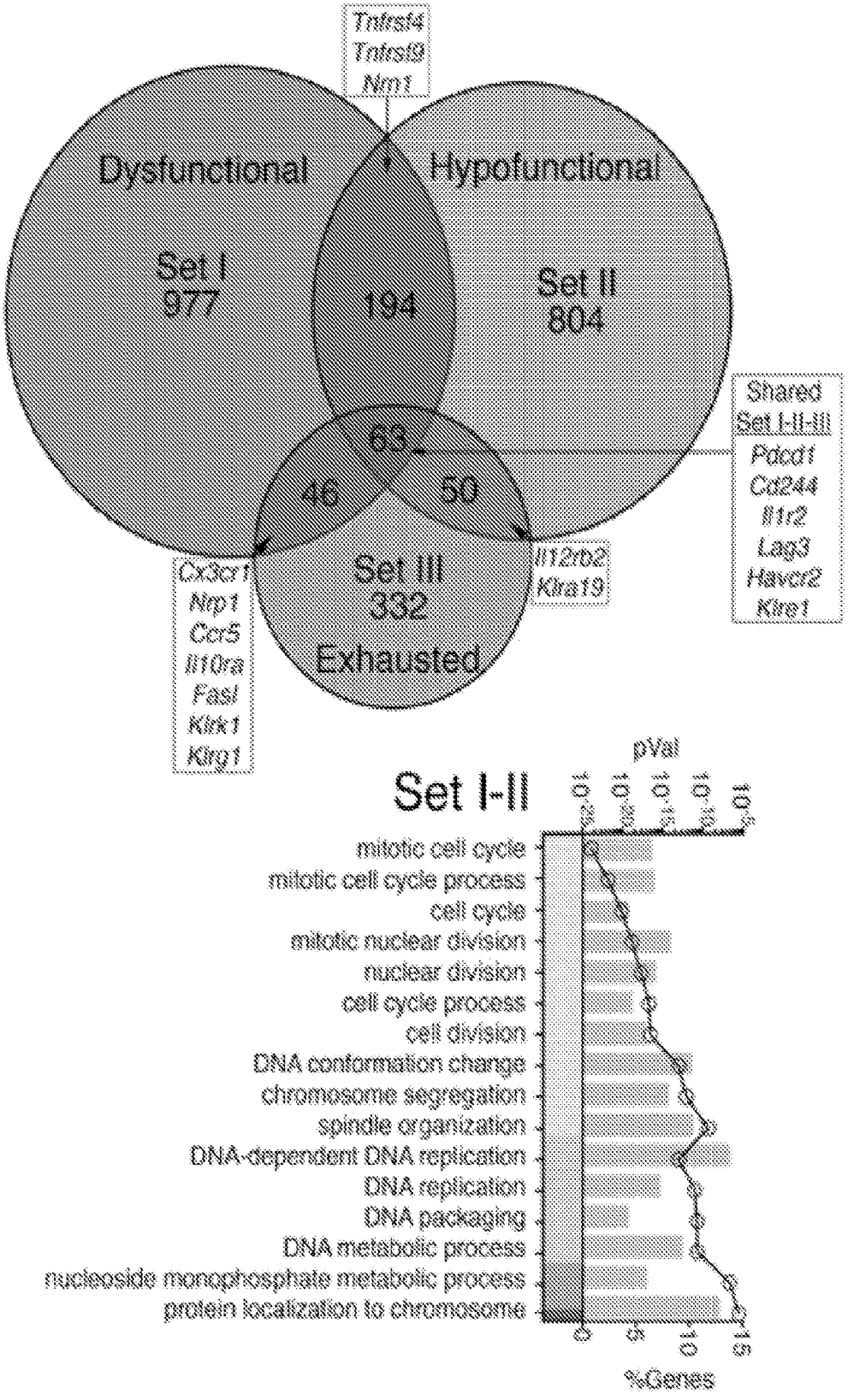
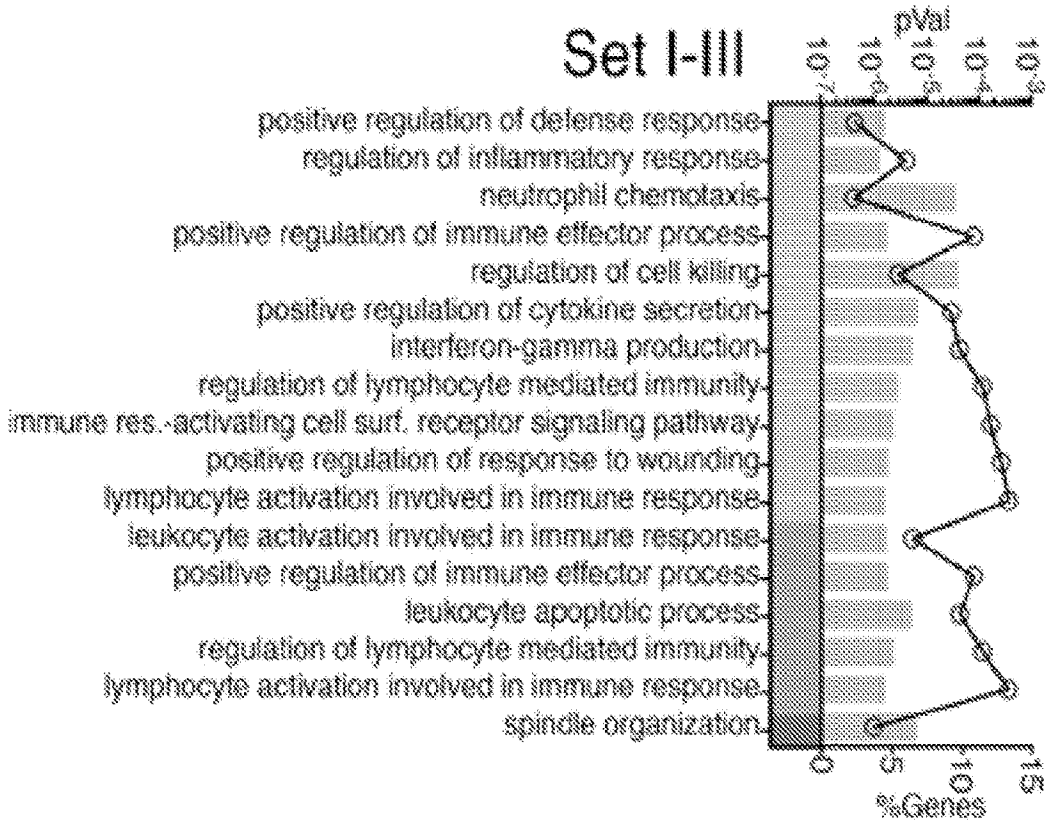

Tnfrsf4
Tnfrsf9
Nrp1
Dysfunctional
Hypofunctional
Set I
977
194
Set II
804
63
46
50
Set III
332
Exhausted
Shared
Set I-II-III
Pdcd1
Cd244
Il1r2
Lag3
Havcr2
Klre1
Il12rb2
Klra19
Cx3cr1
Nrp1
Ccr5
Il10ra
Fasl
Klrk1
Klrg1
pVal
Set I-II
mitotic cell cycle
mitotic cell cycle process
cell cycle
mitotic nuclear division
nuclear division
cell cycle process
cell division
DNA conformation change
chromosome segregation
spindle organization
DNA-dependent DNA replication
DNA replication
DNA packaging
DNA metabolic process
nucleoside monophosphate metabolic process
protein localization to chromosome
%Genes

FIG. 6A (cont.)


Set I-III
pVal
positive regulation of defense response
regulation of inflammatory response
neutrophil chemotaxis
positive regulation of immune effector process
regulation of cell killing
positive regulation of cytokine secretion
interferon-gamma production
regulation of lymphocyte mediated immunity
immune res.-activating cell surf. receptor signaling pathway
positive regulation of response to wounding
lymphocyte activation involved in immune response
leukocyte activation involved in immune response
positive regulation of immune effector process
leukocyte apoptotic process
regulation of lymphocyte mediated immunity
lymphocyte activation involved in immune response
spindle organization
%Genes

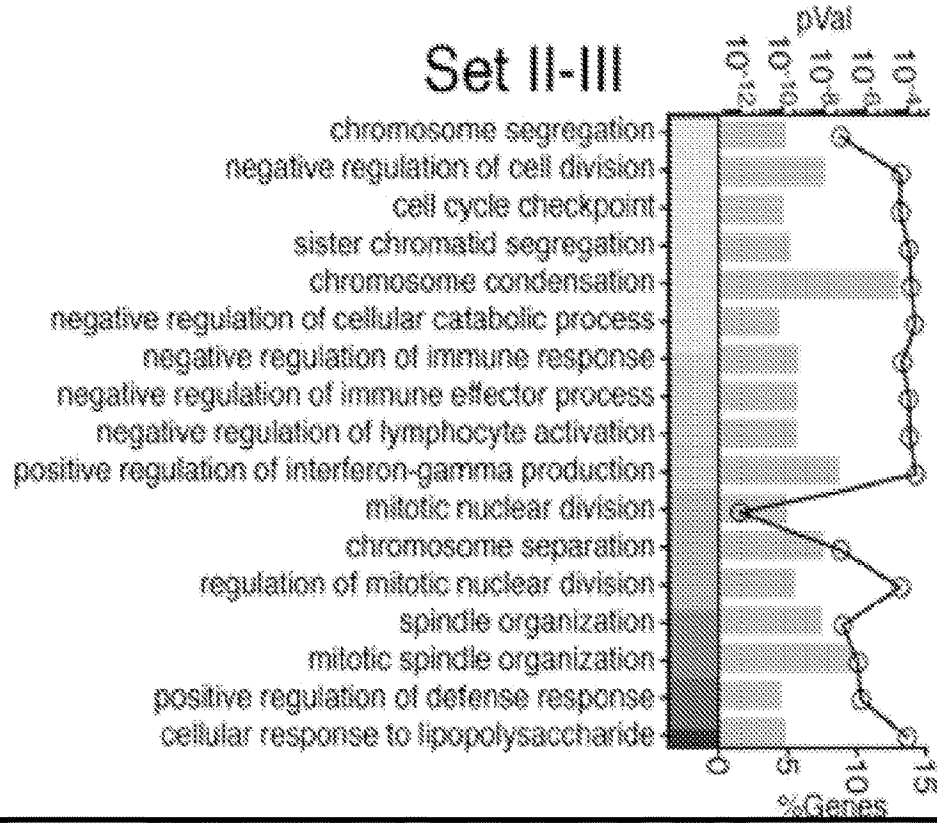

Set II-III
pVal
chromosome segregation
negative regulation of cell division
cell cycle checkpoint
sister chromatid segregation
chromosome condensation
negative regulation of cellular catabolic process
negative regulation of immune response
negative regulation of immune effector process
negative regulation of lymphocyte activation
positive regulation of interferon-gamma production
mitotic nuclear division
chromosome separation
regulation of mitotic nuclear division
spindle organization
mitotic spindle organization
positive regulation of defense response
cellular response to lipopolysaccharide
%Genes

FIG. 6B

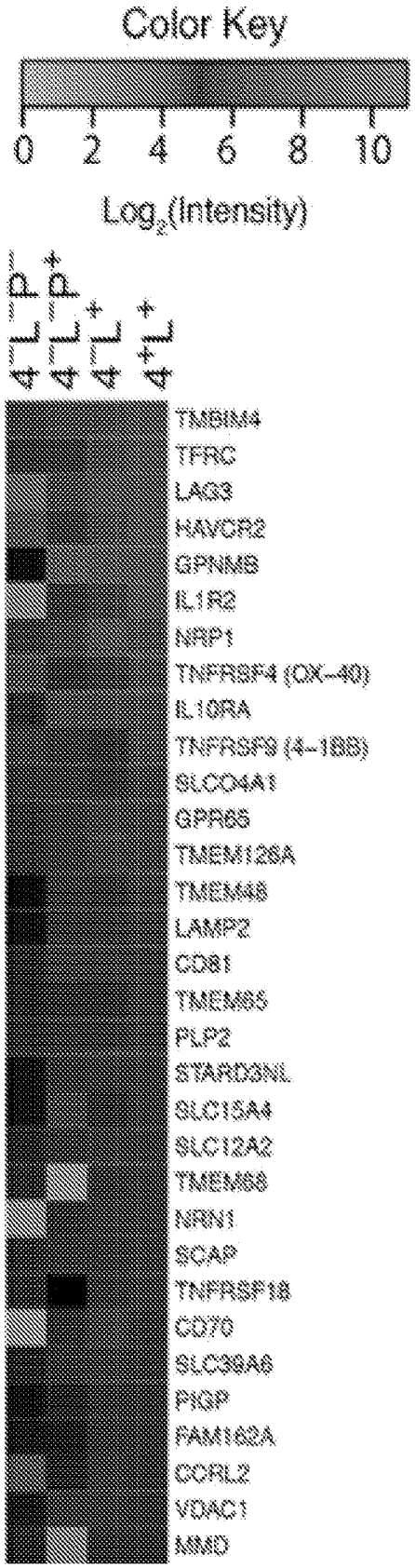

Color Key
0 2 4 6 8 10
Log2(Intensity)
p+ p+ 4+ 4+ 4+ 4+
TMBIM4
TFRC
LAG3
HAVCR2
GPNMB
IL1R2
NRP1
TNFRSF4 (OX-40)
IL10RA
TNFRSF9 (4-1BB)
SLCO4A1
GPR65
TMEM126A
TMEM48
LAMP2
CD81
TMEM65
PLP2
STARD3NL
SLC15A4
SLC12A2
TMEM68
NRN1
SCAP
TNFRSF18
CD70
SLC39A6
PIGP
FAM162A
CCRL2
VDAC1
MMD

FIG 6B (cont.)

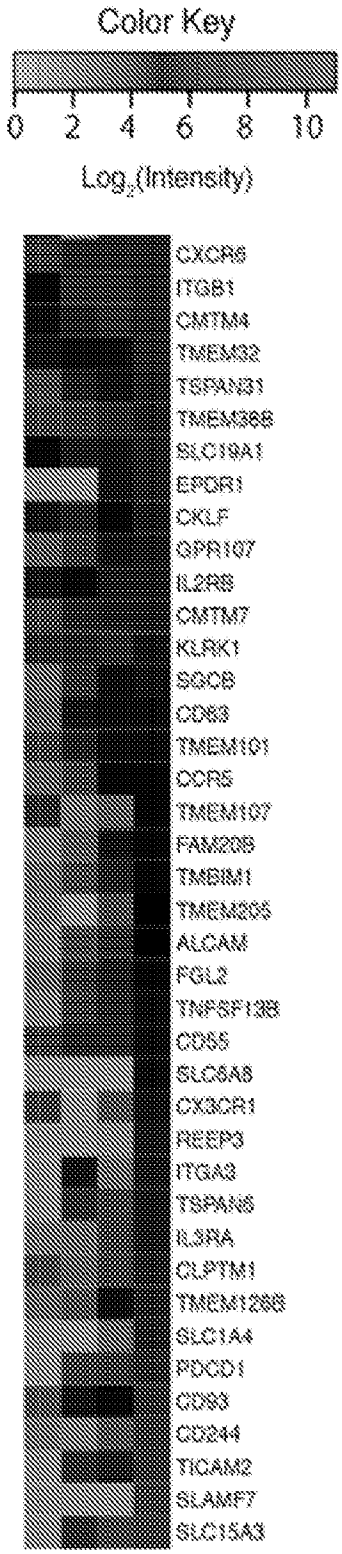

Color Key
0 2 4 6 8 10
Log2(Intensity)
CXCR6
ITGB1
CMTM4
TMEM32
TSPAN31
TMEM366
SLC19A1
EPDR1
CKLF
GPR107
IL2RB
CMTM7
KLRK1
SGCB
CD63
TMEM101
CCR5
TMEM107
FAM209
TMBIM1
TMEM205
ALCAM
FGL2
TNFSF13B
CD55
SLC6A8
CX3CR1
REEP3
ITGA3
TSPAN6
IL3RA
CLPTM1
TMEM126B
SLC1A4
PDCD1
CD93
CD244
TICAM2
SLAMF7
SLC15A3

FIG. 6C

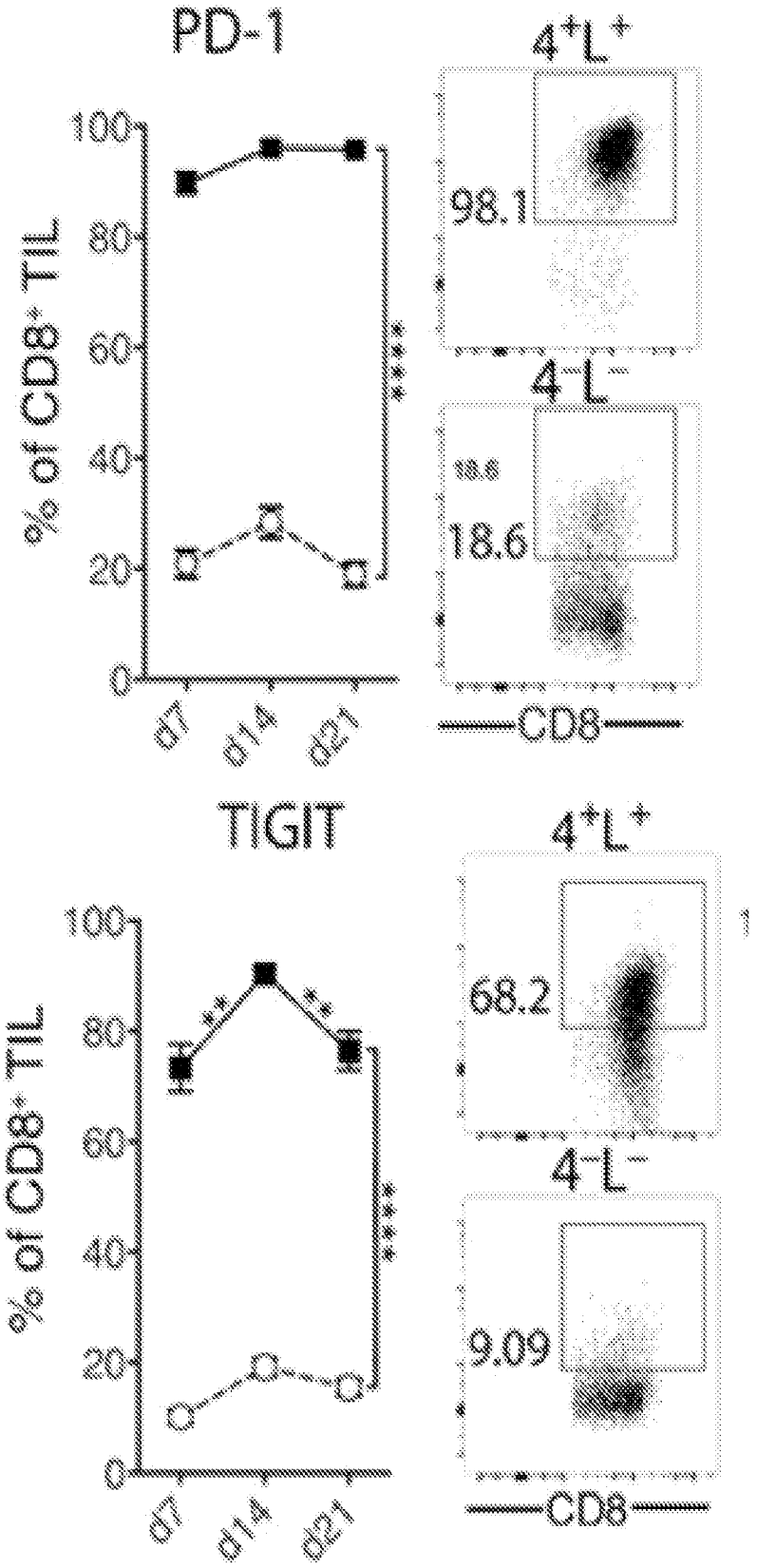

PD-1
% of CD8+ TIL
100
80
60
40
20
0
d7
d14
d21
****
4+L+
98.1
4-L-
18.6
CD8
TIGIT
% of CD8+ TIL
100
80
60
40
20
0
d7
d14
d21
**
**
****
4+L+
68.2
4-L-
9.09
CD8

FIG. 6C (cont.)

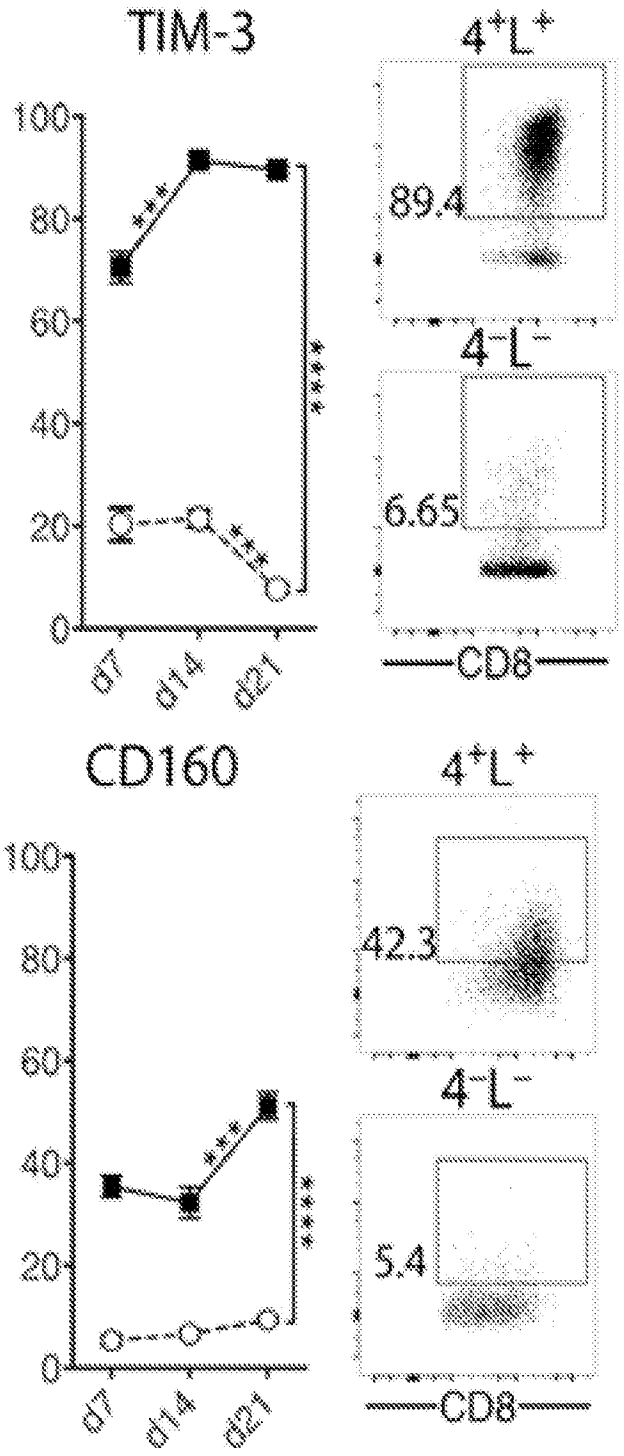

TIM-3
100
80
60
40
20
0
d7
d14
d21
4+L+
89.4
4-L-
6.65
CD8
CD160
100
80
60
40
20
0
d7
d14
d21
4+L+
42.3
4-L-
5.4
CD8

FIG. 6C (cont.)

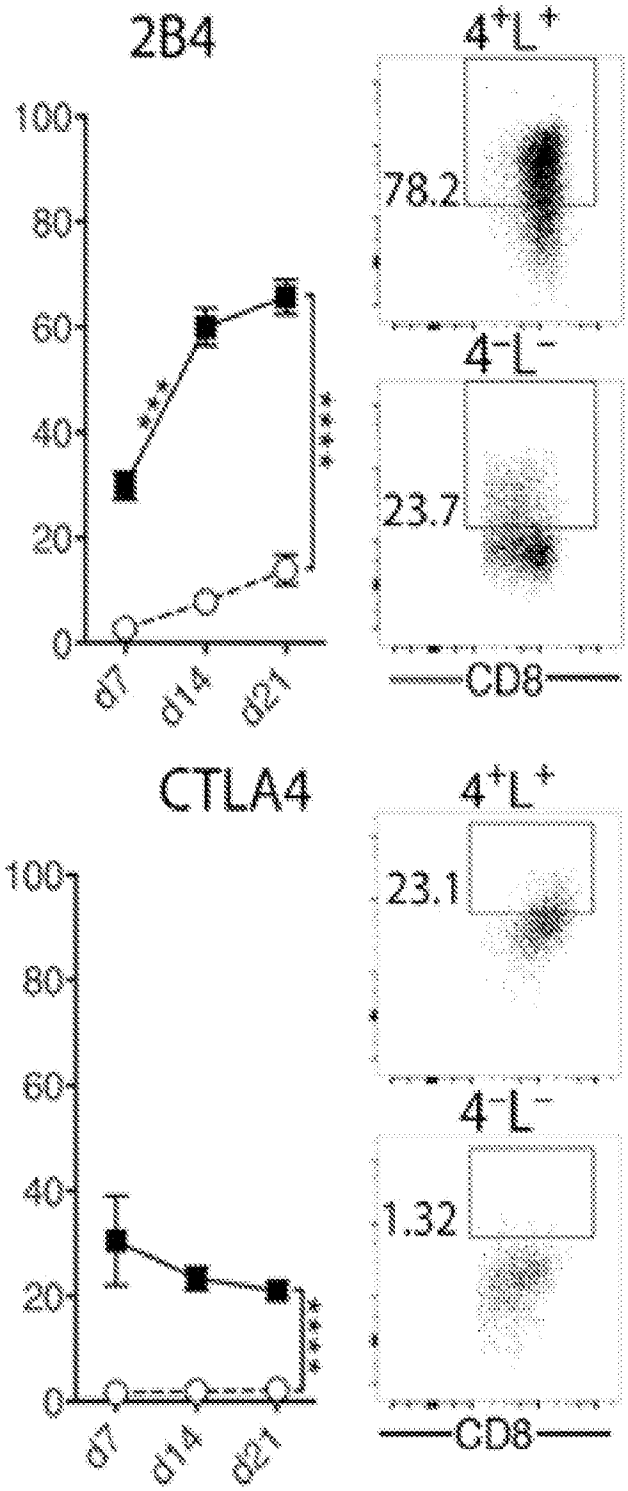

2B4
4+L+
78.2
4-L-
23.7
CD8
CTLA4
4+L+
23.1
4-L-
1.32
CD8
100
80
60
40
20
0
d7
d14
d21

FIG. 6C (cont.)

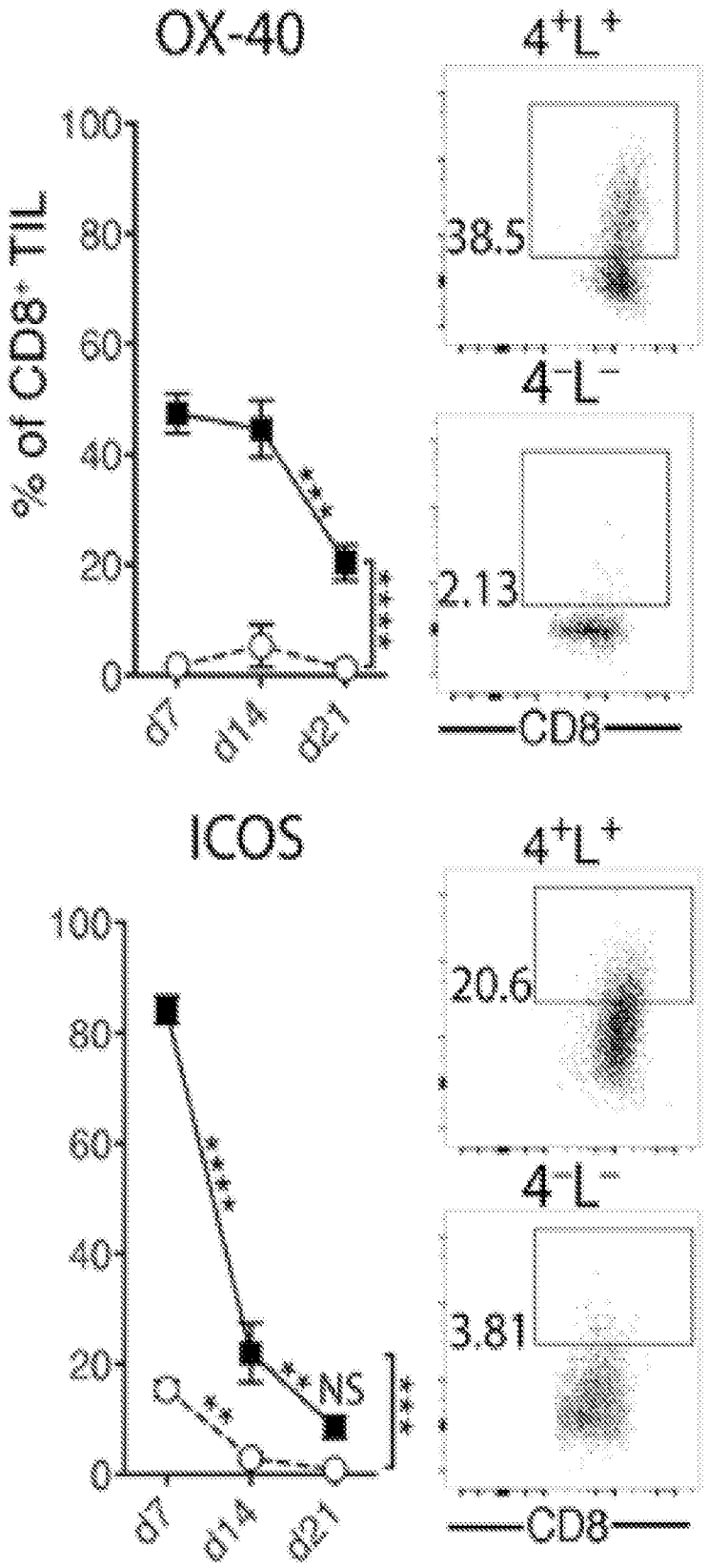

OX-40
% of CD8+ TIL
100
80
60
40
20
0
d7
d14
d21
4+L+
38.5
4-L-
2.13
CD8
ICOS
100
80
60
40
20
0
d7
d14
d21
NS
4+L+
20.6
4-L-
3.81
CD8

FIG. 6C (cont.)

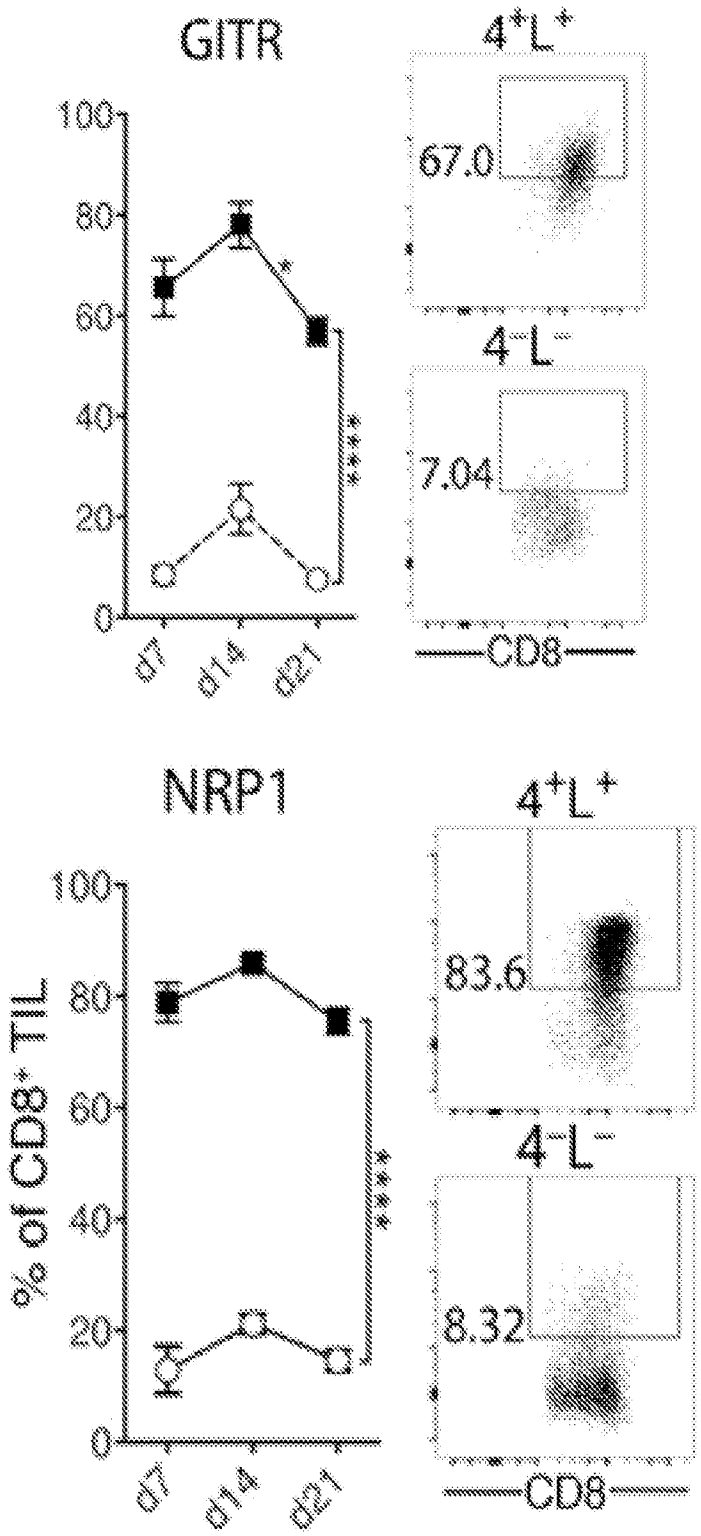

GITR
4+L+
67.0
4-L-
7.04
CD8
100
80
60
40
20
0
d7
d14
d21
NRP1
4+L+
83.6
4-L-
8.32
CD8
% of CD8+ TIL

FIG. 6D

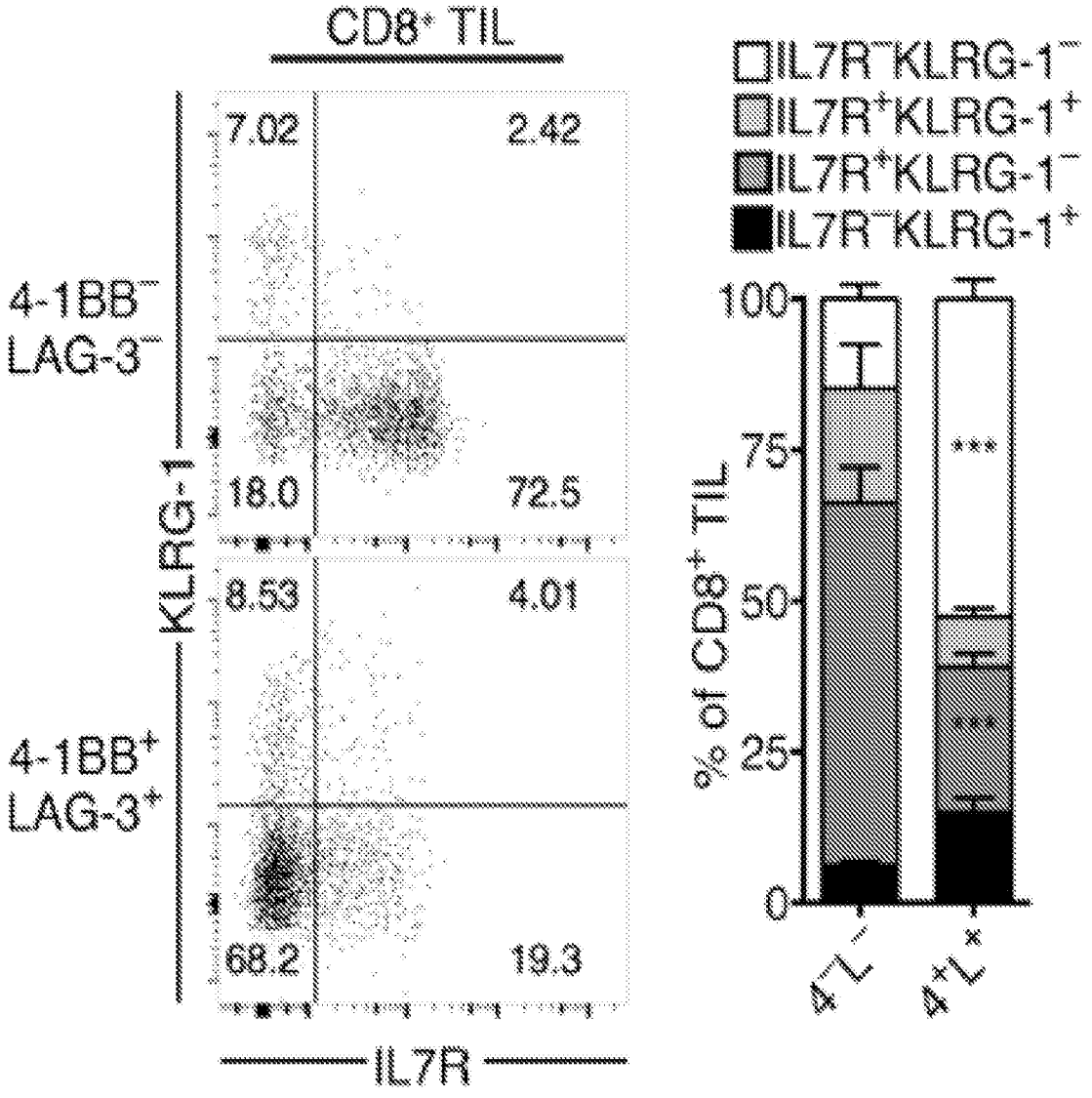

CD8+ TIL
7.02
2.42
18.0
72.5
8.53
4.01
68.2
19.3
4-1BB−
LAG-3−
4-1BB+
LAG-3+
KLRG-1
IL7R
IL7R−KLRG-1−
IL7R+KLRG-1+
IL7R+KLRG-1−
IL7R−KLRG-1+
100
75
50
25
0
% of CD8+ TIL
***
***
Δ−L−
Δ+L+

FIG. 7

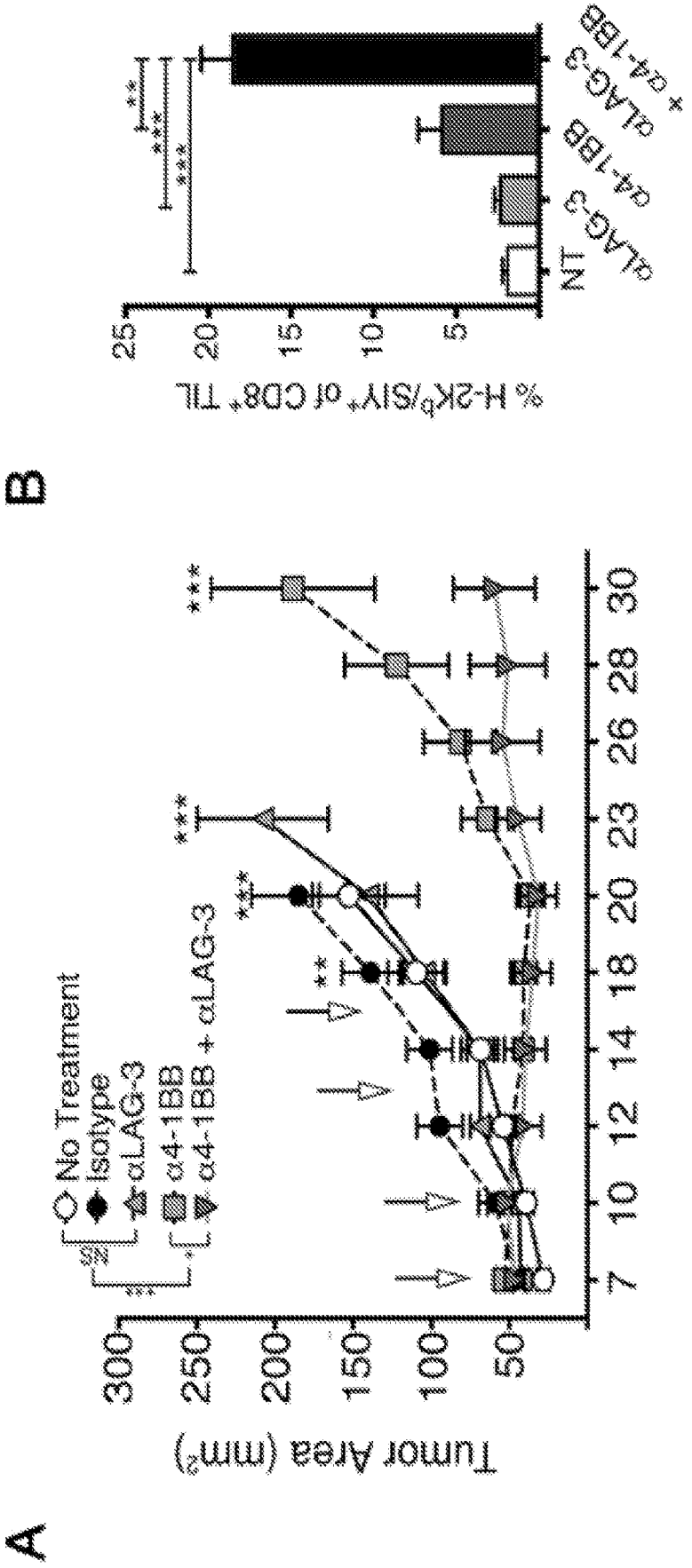

A
Tumor Area (mm²)
300
250
200
150
100
50
7
10
12
14
18
20
23
26
28
30
No Treatment
Isotype
αLAG-3
α4-1BB
α4-1BB + αLAG-3
***
**
B
% H-2Kᵇ/SIY⁺ of CD8⁺ TIL
25
20
15
10
5
NT
αLAG-3
α4-1BB
αLAG-3 + α4-1BB

FIG. 7 (cont.)

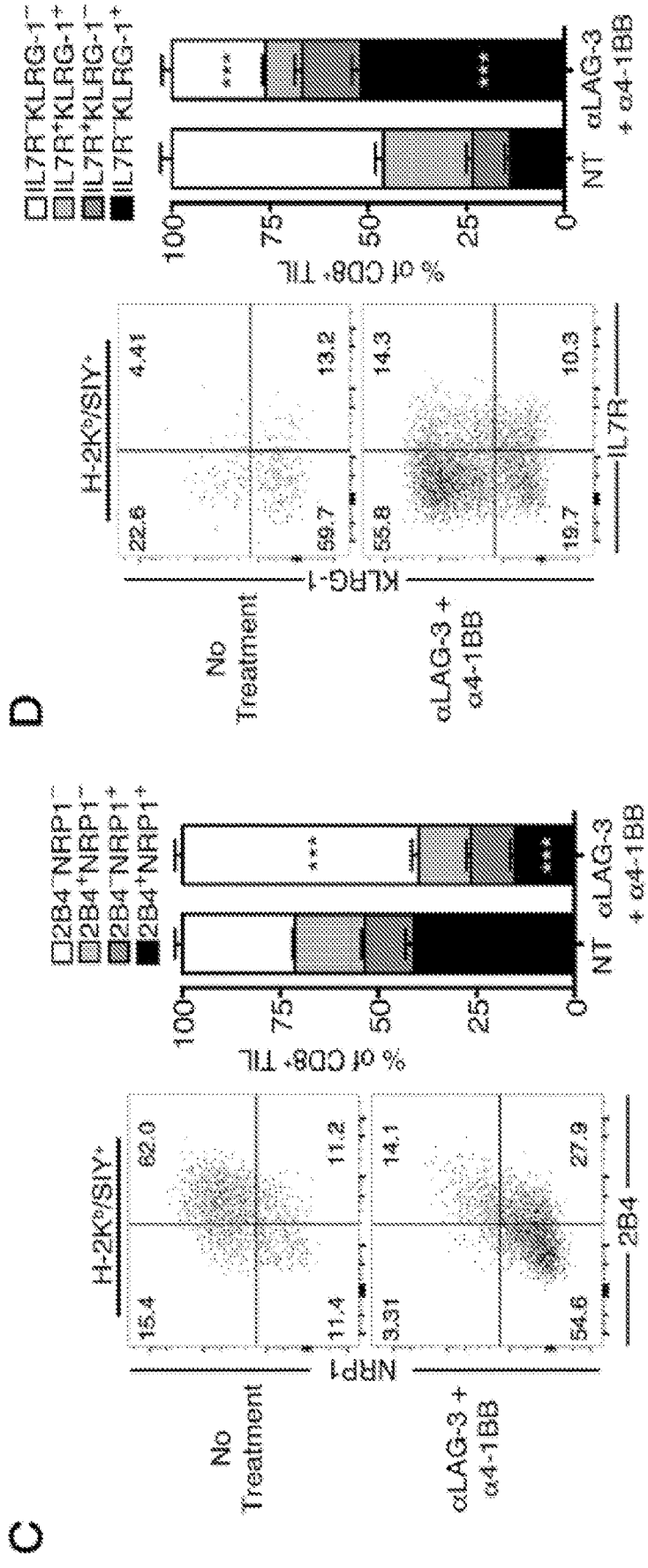

C
D
No Treatment
αLAG-3 + α4-1BB
H-2Kᵇ/SIY⁺
NRP1
2B4
KLRG-1
IL7R
22.8
59.7
13.2
4.41
55.8
14.3
19.7
10.3
15.4
11.4
11.2
62.0
9.31
14.1
54.6
27.9
% of CD8⁺ TIL
NT
αLAG-3 + α4-1BB
2B4⁻NRP1⁻
2B4⁺NRP1⁻
2B4⁻NRP1⁺
2B4⁺NRP1⁺
IL7R⁻KLRG-1⁻
IL7R⁺KLRG-1⁻
IL7R⁻KLRG-1⁺
IL7R⁺KLRG-1⁺
***

FIG. 7 (cont.)

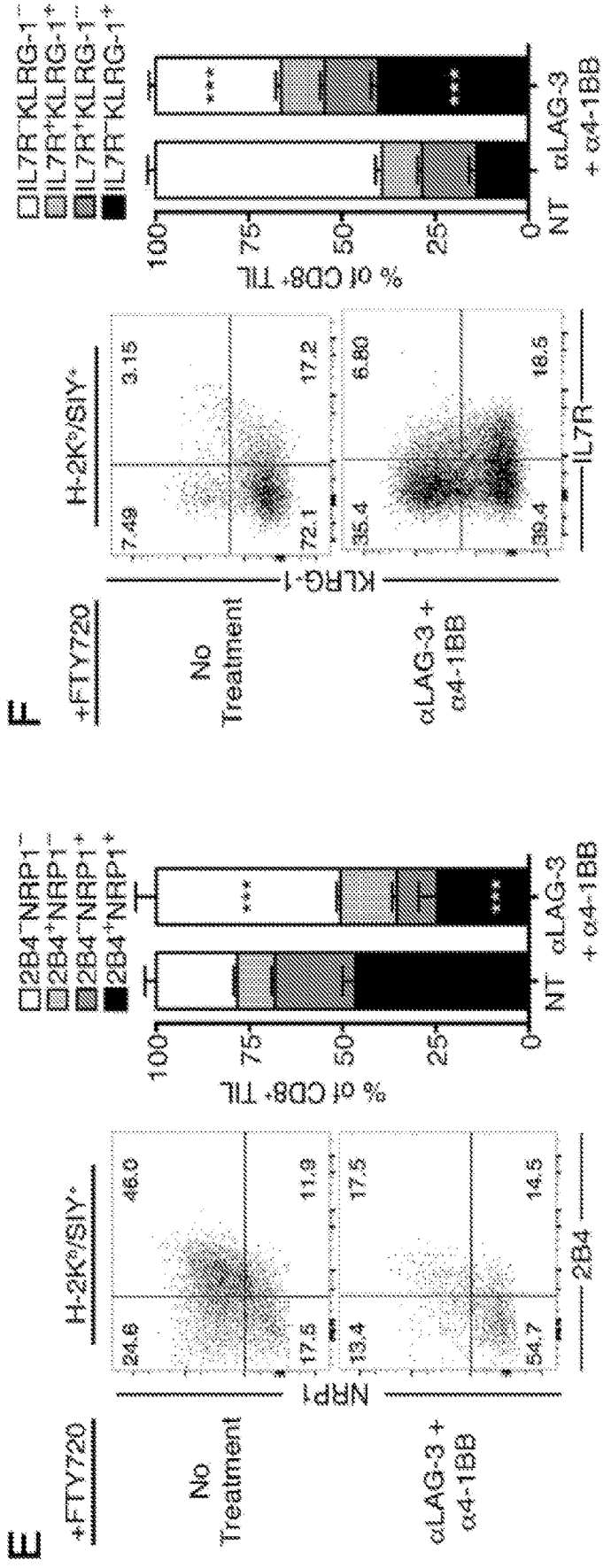

E
+FTY720
No Treatment
αLAG-3 + α4-1BB
H-2Kb/SIY+
NRP1
2B4
24.6
17.5
46.0
11.9
13.4
17.5
54.7
14.5
2B4-NRP1-
2B4+NRP1-
2B4-NRP1+
2B4+NRP1+
% of CD8+ TIL
NT
αLAG-3 + α4-1BB
***
F
+FTY720
No Treatment
αLAG-3 + α4-1BB
H-2Kb/SIY+
KLRG-1
IL7R
7.49
72.1
3.15
17.2
35.4
39.4
6.80
16.6
IL7R-KLRG-1-
IL7R+KLRG-1-
IL7R-KLRG-1+
IL7R+KLRG-1+
% of CD8+ TIL
NT
αLAG-3 + α4-1BB
***

FIG. 7 (cont.)

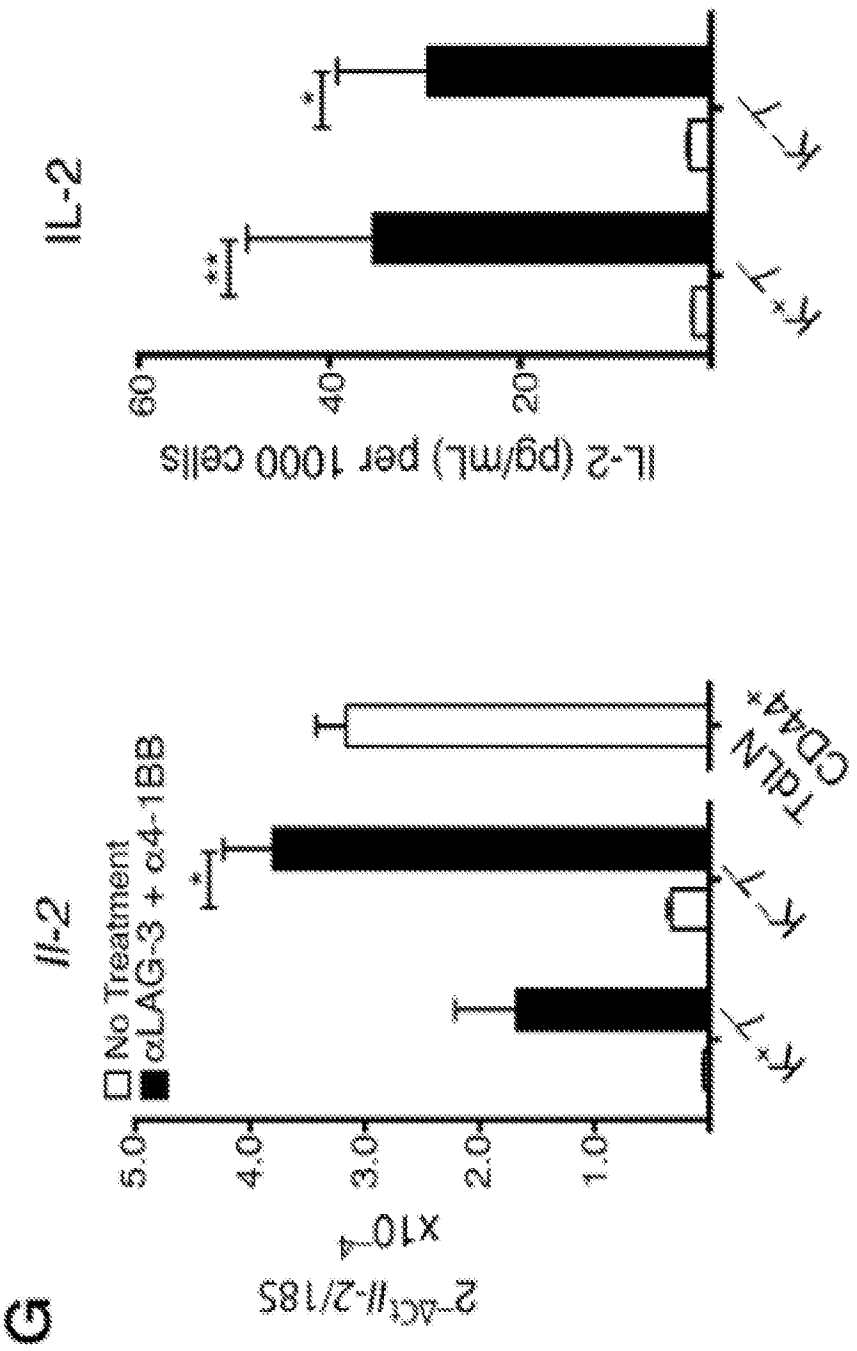

G
Il-2
2^-ΔCt Il-2/18S
x10^-4
6.0
4.0
3.0
2.0
1.0
No Treatment
αLAG-3 + α4-1BB
TdLN CD4+
Il-2
IL-2 (pg/mL) per 1000 cells
60
40
20

FIG. 8

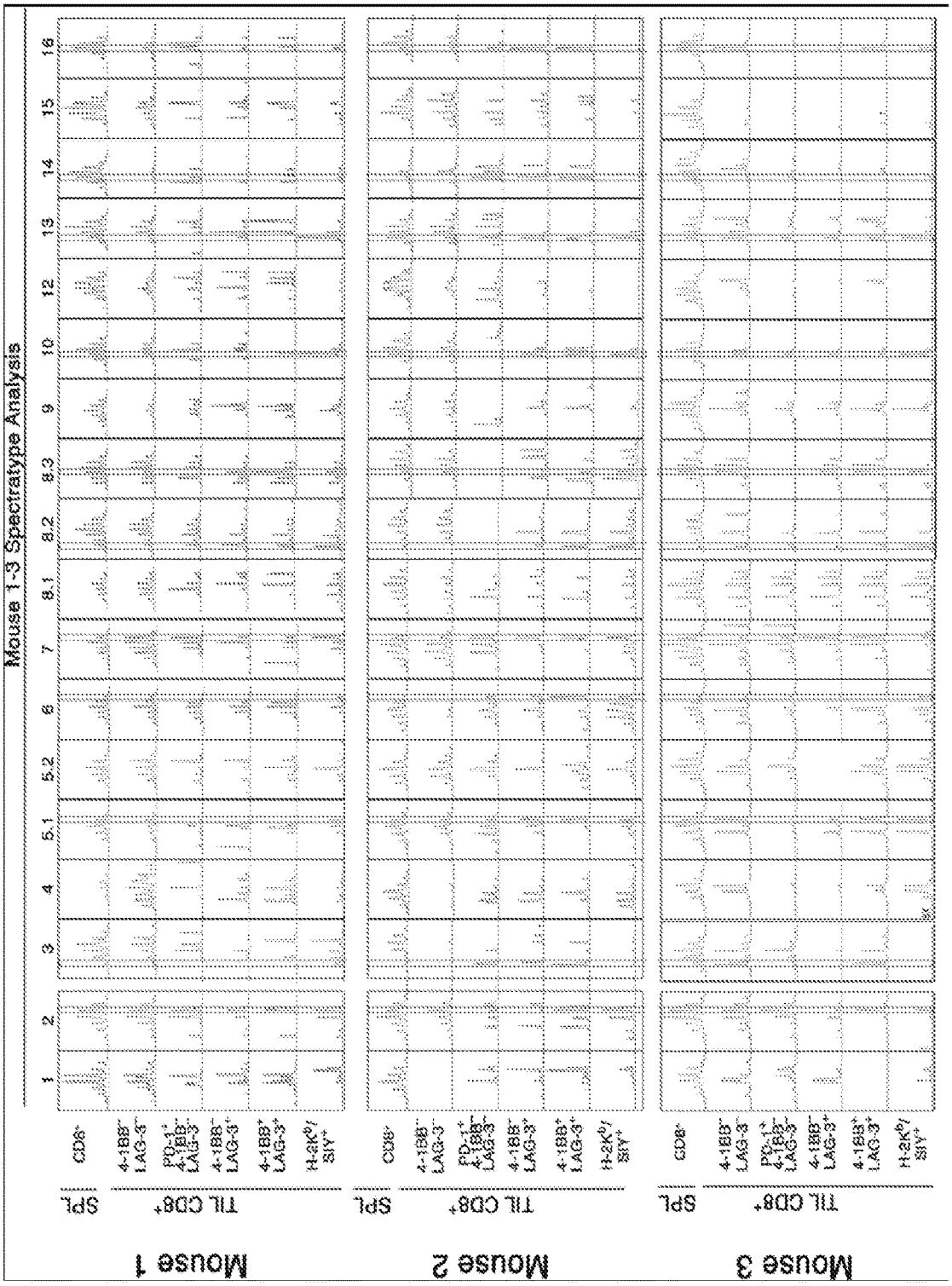

Mouse 1-3 Spectratype Analysis
Mouse 1
Mouse 2
Mouse 3
SPL
TIL CD8+
CD8+
4-1BB+ LAG-3+
PD-1+ 4-1BB+ LAG-3+
4-1BB+ LAG-3+
4-1BB+ LAG-3+
H-2Kb/ SIY+
1
2
3
4
5.1
5.2
6
7
8.1
8.2
8.3
9
10
12
13
14
15
16

FIG. 9

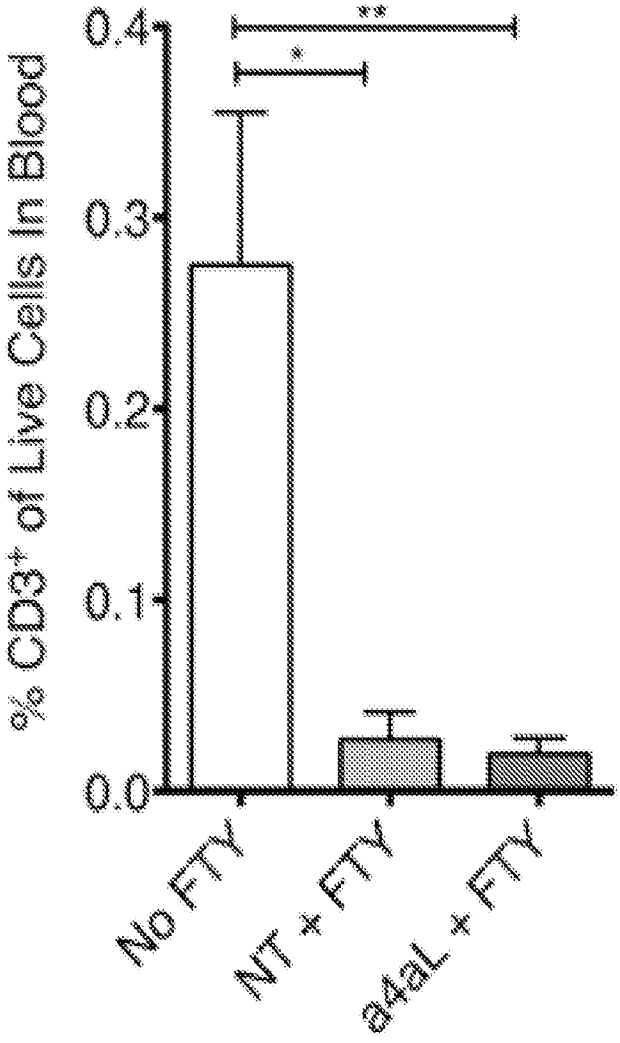


FIG. 10A

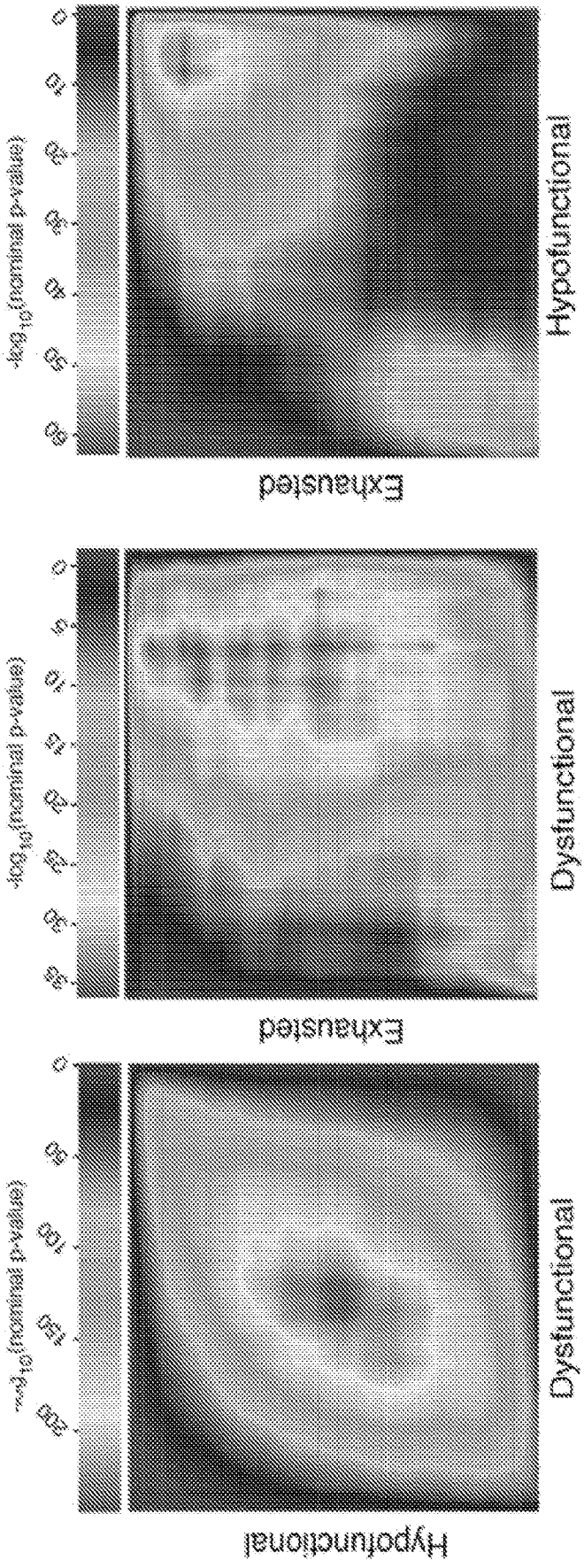
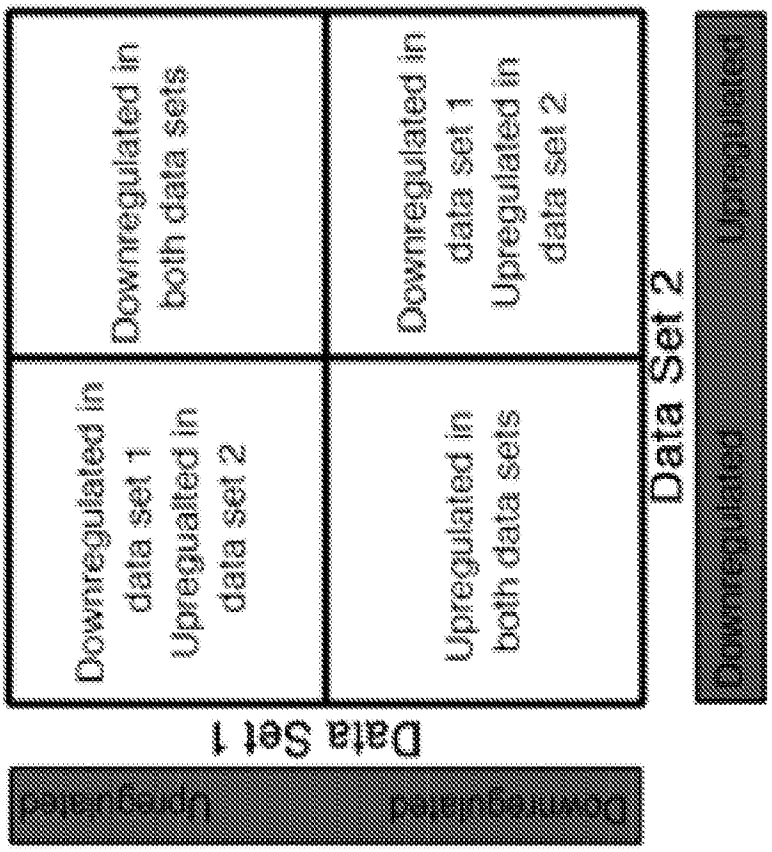

Hypofunctional
Dysfunctional
Exhausted
Hypofunctional
Dysfunctional
Exhausted
Exhausted
Dysfunctional
Hypofunctional
-log10(nominal p-value)
-log10(nominal p-value)
-log10(nominal p-value)

FIG. 10A (cont.)


Downregulated in both data sets
Downregulated in data set 1 Upregulated in data set 2
Upregulated in both data sets
Downregulated in data set 1 Upregulated in data set 2
Data Set 2
Downregulated
Upregulated
Data Set 1
Downregulated
Upregulated

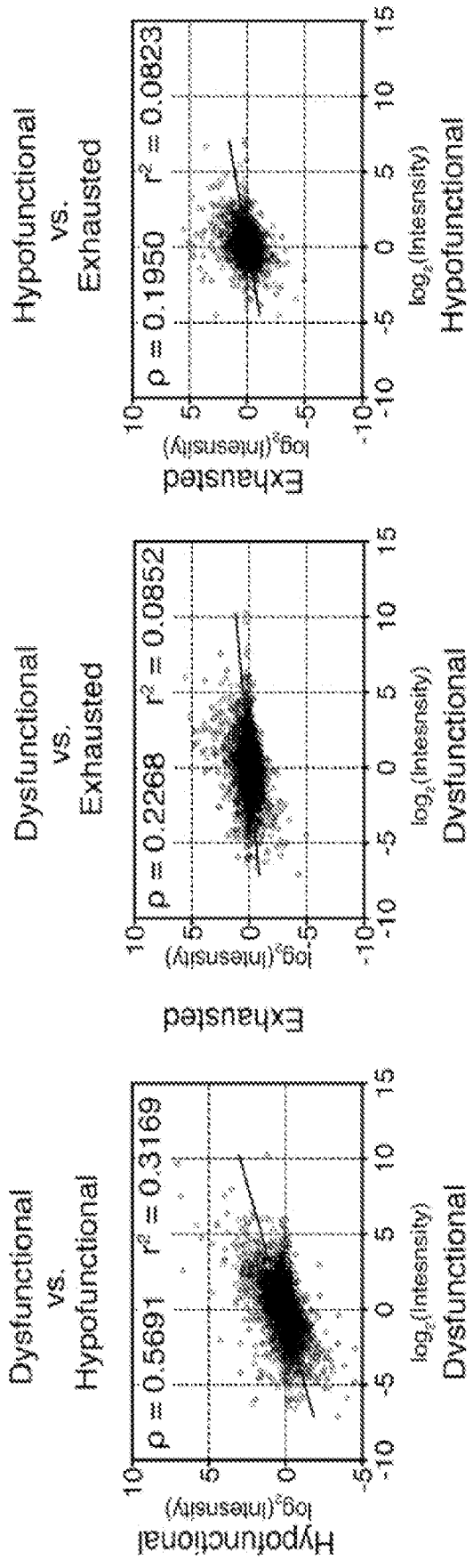

Dysfunctional
vs.
Hypofunctional
ρ = 0.5691 r² = 0.3169
Hypofunctional
log₂(Intensity)
Dysfunctional
log₂(Intensity)
Dysfunctional
vs.
Exhausted
ρ = 0.2268 r² = 0.0852
Exhausted
log₂(Intensity)
Dysfunctional
log₂(Intensity)
Hypofunctional
vs.
Exhausted
ρ = 0.1950 r² = 0.0823
Exhausted
log₂(Intensity)
Hypofunctional
log₂(Intensity)

FIG. 10B

FIG. 11

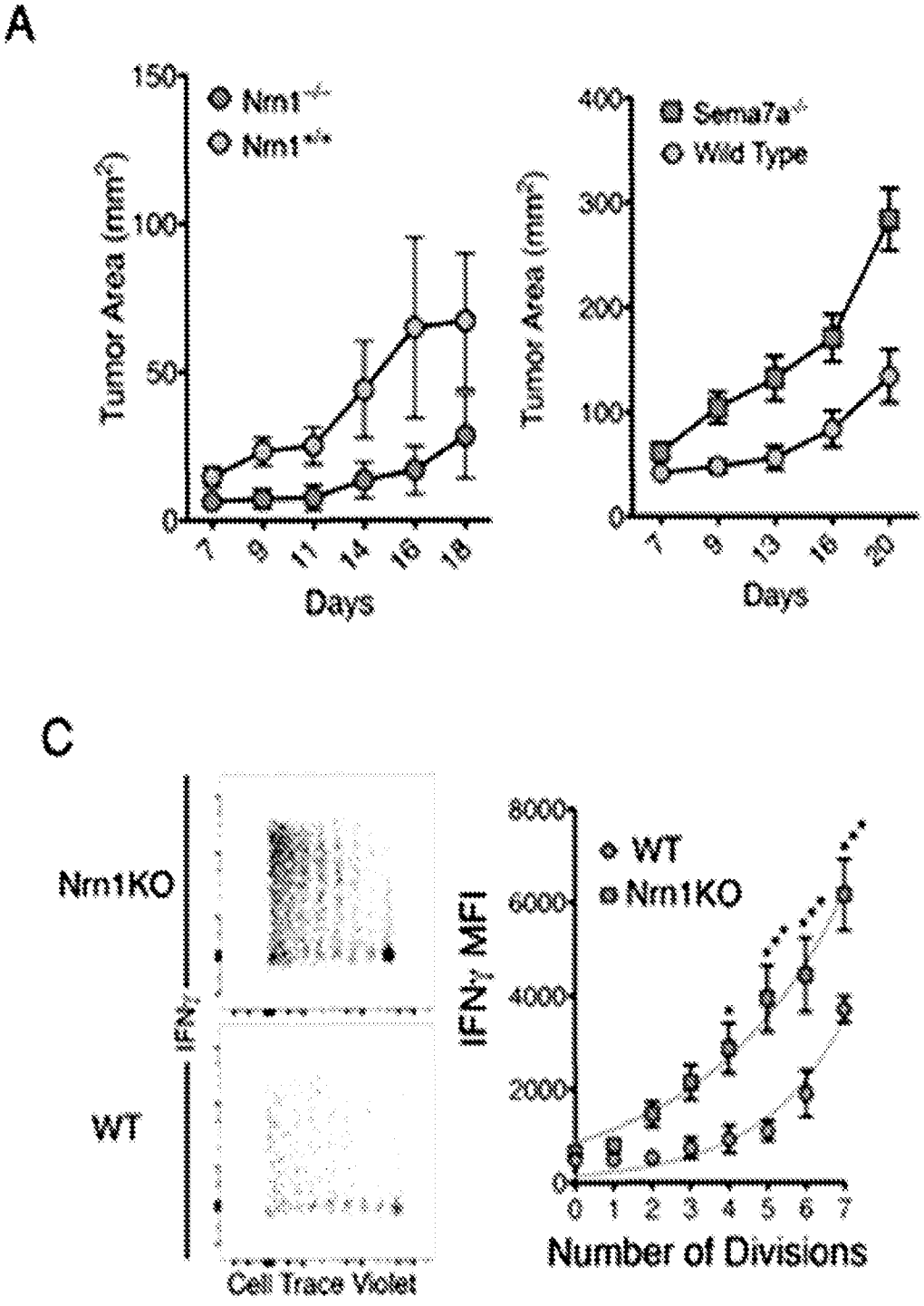

A
Nrn1-/-
Nrn1+/+
Tumor Area (mm2)
Days
Sema7a-/-
Wild Type
Tumor Area (mm2)
Days
C
Nrn1KO
WT
IFNγ
Cell Trace Violet
WT
Nrn1KO
IFNγ MFI
Number of Divisions

FIG. 11 (cont.)

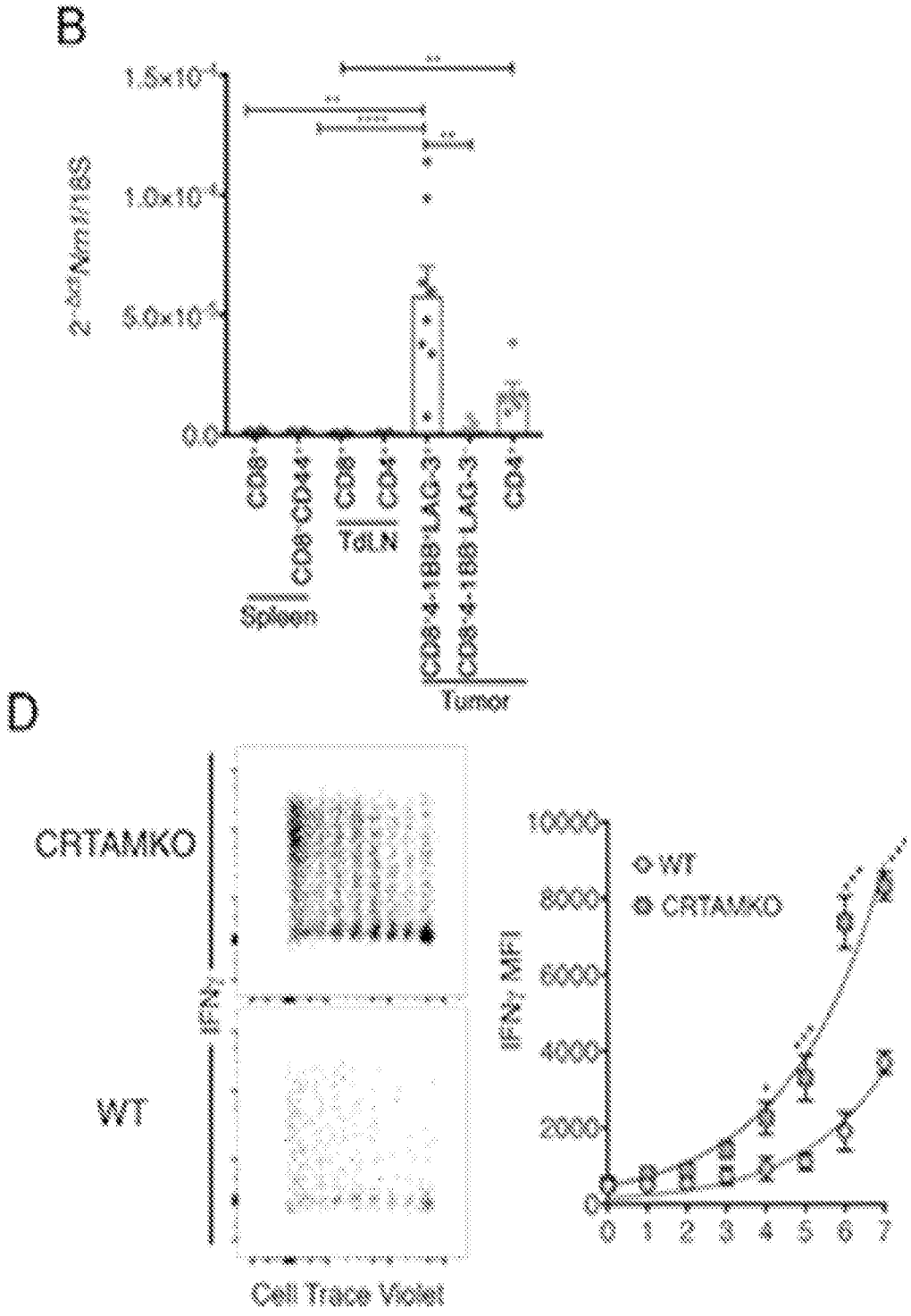

B
1.5×10⁻⁴
1.0×10⁻⁴
5.0×10⁻⁵
0.0
2^-ΔCt(Nrm1/18S
CD8
CD8 CD44
CD8
CD4
CD8 4-1BB LAG-3
CD8 4-1BB LAG-3
CD4
Spleen
TdLN
Tumor
D
CRTAMKO
WT
IFNγ
Cell Trace Violet
IFNγ MFI
10000
8000
6000
4000
2000
0
WT
CRTAMKO
0 1 2 3 4 5 6 7

FIG. 11 (cont.)

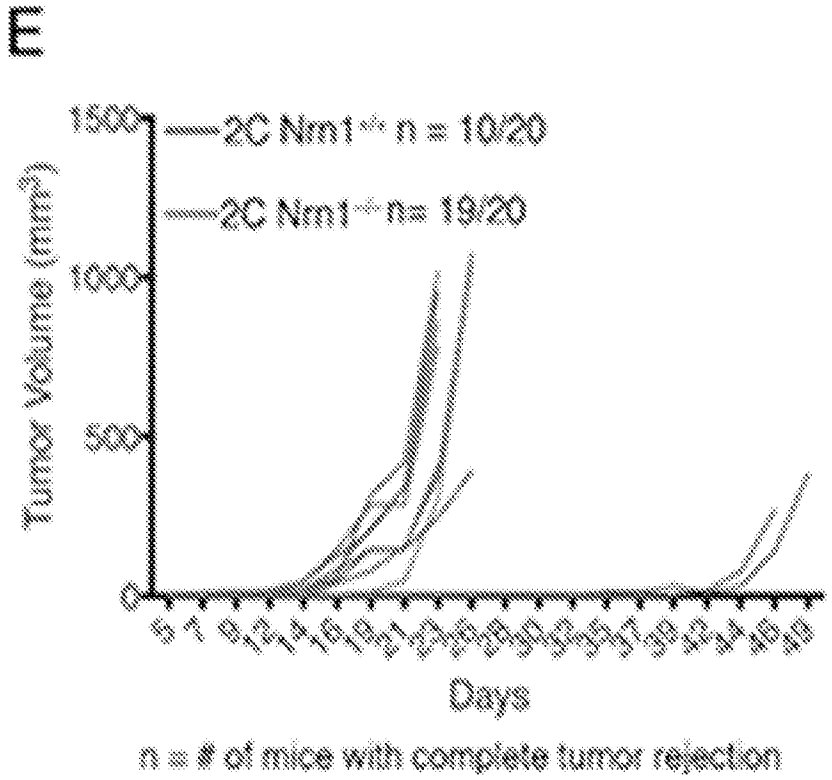
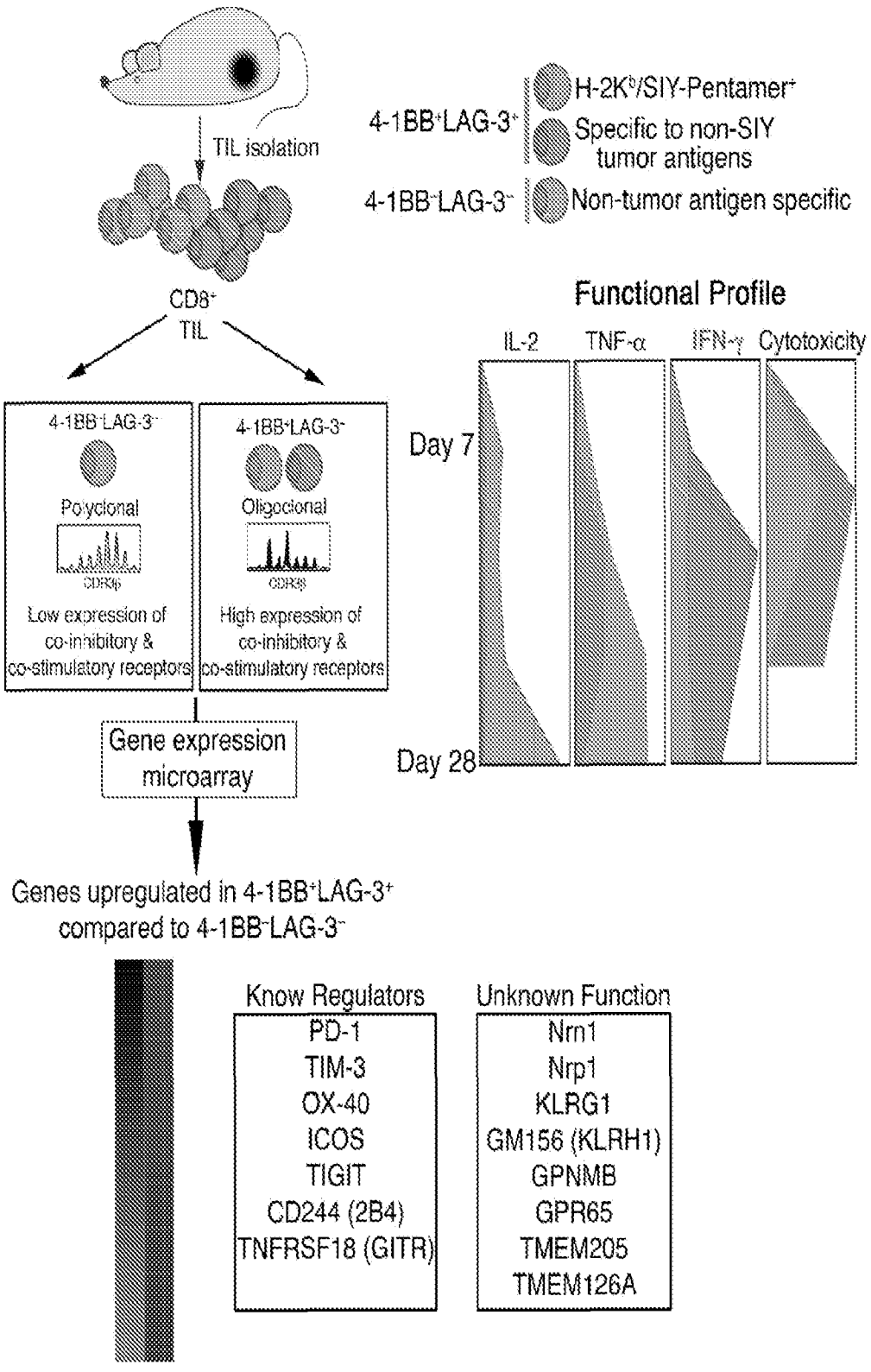

E
1500
1000
500
0
2C Nm1-/- n = 10/20
2C Nm1-/- n= 19/20
Tumor Volume (mm3)
Days
n = # of mice with complete tumor rejection

FIG. 12


TIL isolation
4-1BB+LAG-3+
H-2Kb/SIY-Pentamer+
Specific to non-SIY tumor antigens
4-1BB-LAG-3-
Non-tumor antigen specific
CD8+ TIL
Functional Profile
IL-2
TNF-α
IFN-γ
Cytotoxicity
Day 7
Day 28
4-1BB-LAG-3-
Polyclonal
CDR3β
Low expression of co-inhibitory & co-stimulatory receptors
4-1BB+LAG-3+
Oligoclonal
CDR3β
High expression of co-inhibitory & co-stimulatory receptors
Gene expression microarray
Genes upregulated in 4-1BB+LAG-3+ compared to 4-1BB-LAG-3-
Know Regulators
PD-1
TIM-3
OX-40
ICOS
TIGIT
CD244 (2B4)
TNFRSF18 (GITR)
Unknown Function
Nrm1
Nrp1
KLRG1
GM156 (KLRH1)
GPNMB
GPR65
TMEM205
TMEM126A

DYSFUNCTIONAL ANTIGEN-SPECIFIC CD8+ T CELLS IN THE TUMOR MICROENVIRONMENT

CROSS-REFERENCE TO RELATED APPLICATIONS

The present invention claims priority to U.S. Provisional Patent Application Ser. No. 62/447,199, filed Jan. 17, 2017, which is incorporated by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under Grant No. R01 CA161005 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD

Provided herein are compositions and methods for detecting and/or targeting dysfunctional tumor antigen-specific CD8+ T cells in the tumor microenvironment for diagnostic, therapeutic and/or research applications. In particular, dysfunctional tumor antigen-specific CD8+ T cells are detected and/or targeted via their expression of cell surface receptors described herein, such as 4-1BB, LAG-3, or additional markers that correlate with 4-1BB and LAG-3 expression, such as markers differentially expressed on the surface of the T cells.

BACKGROUND

The immune system plays a critical role in protecting the host from cancer (Vesely et al., 2011; incorporated by reference in its entirety). Innate sensing of tumors leads to an adaptive T cell response through the presentation of tumor-associated antigens (TAAs) derived from mutations and epigenetic changes that contribute to carcinogenesis (Gajewski et al., 2013; incorporated by reference in its entirety). Spontaneously-primed CD8+ T cells home to tumor sites in mouse tumor models (Harlin et al., 2009; Fuertes et al., 2011; incorporated by reference in their entireties) and in a subset of patients with advanced cancer (Harlin et al., 2006; incorporated by reference in its entirety). These tumor-infiltrating lymphocytes (TIL) have the ability to recognize tumor antigens and are believed to contribute to tumor control in cancer patients, based on the correlation between activated CD8+ T cell infiltration with improved prognosis and response to immunotherapy (Fridman et al., 2012; Tumeh et al., 2014; incorporated by reference in their entireties). However, without additional manipulation, this endogenous anti-tumor response is usually not sufficient to mediate complete rejection of an established tumor (Gajewski, 2007b; Pardoll, 2012; Baitsch et al., 2011; Gajewski et al., 2006; Larkin et al., 2015). Data accumulated over the past several years have indicated that tumors with spontaneous anti-tumor T cell responses have high expression of immune-inhibitory pathways that subvert the effector phase of the response. These include PD-L1/PD-1 interactions (Pardoll, 2012; incorporated by reference in its entirety), recruitment of CD4+Foxp3+ regulatory T (Treg) cells (Gajewski, 2007a; incorporated by reference in its entirety), and metabolic dysregulation by indoleamine-2,3-dioxygenase (IDO) (Spranger et al., 2013; incorporated by reference in its entirety). However, even when CD8+ T cells specific for tumor antigens are isolated from tumors, away from these extrinsic immune inhibitory factors, they still show altered functional properties ex vivo (Harlin et al., 2006; Baitsch et al., 2011; incorporated by reference in their entireties).

Expression of PD-1 has been described to identify tumor-specific exhausted T cells (Ahmadzadeh et al., 2009; Fourcade et al., 2012; Wu et al., 2014; Gros et al., 2014; incorporated by reference in their entireties). However, T cells expressing PD-1 in the context of chronic infection can still retain effector function (Wherry and Kurachi, 2015; incorporated by reference in its entirety), and PD-1 is not required for the induction of T cell exhaustion (Odorizzi et al., 2015; incorporated by reference in its entirety). In addition to PD-1, several additional co-inhibitory receptors, including CD223 (LAG-3), CD244 (2B4), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), hepatitis A virus cellular receptor 2 (TIM-3), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), are also be expressed on dysfunctional T cells and expression of a greater number of inhibitory receptors has been correlated with diminished cytokine secretion (in particular IFN-g and TNF-α) as well as proliferative capacity (Blackburn et al., 2009; incorporated by reference in its entirety). Expression of these receptors has been observed in both viral and cancer models, however, a complete analysis of both co-inhibitory and co-stimulatory receptors on the same population is lacking in the tumor setting.

SUMMARY

Provided herein are compositions and methods for detecting and/or targeting dysfunctional tumor antigen-specific CD8+ T cells in the tumor microenvironment for diagnostic, therapeutic and/or research applications. In particular, dysfunctional tumor antigen-specific CD8+ T cells are detected and/or targeted via their expression of cell surface receptors described herein, such as 4-1BB, LAG-3, or additional markers that correlate with 4-1BB and LAG-3 expression, such as markers differentially expressed on the surface of the T cells (e.g., PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, CRTAM and Sema7a).

In some embodiments, provided herein are methods of treating a subject with cancer comprising administering an agent that specifically targets dysfunctional tumor antigen-specific CD8+ T cells. In some embodiments, the subject suffers from a solid tumor cancer. In some embodiments, the tumor allows T cell infiltration, but is resistant to immunotherapies. In some embodiments, the tumor environment comprises dysfunctional tumor antigen-specific CD8+ T cells. In some embodiments, contacting the dysfunctional tumor antigen-specific CD8+ T cells with an anti-4-1BB and/or anti-LAG3 agent. In some embodiments, the anti-4-1BB and/or anti-LAG3 agent is an antibody, antibody fragment, or antibody mimetic molecule. In some embodiments, methods further comprise co-administration of an additional therapeutic agent. In some embodiments, the additional therapeutic agent is a chemotherapeutic or an immunotherapeutic agent. In some embodiments, the additional therapeutic agent is an immunotherapeutic agent selected from the list consisting of cell-based therapies, monoclonal antibody (mAb) therapy, cytokine therapy, and adjuvant treatment. In some embodiments, the immunotherapeutic agent is a mAb therapy selected from the list consisting of anti-CTLA-4 monoclonal antibodies and/or anti-PD-L1 monoclonal antibodies. In some embodiments, the immunothera-

peutic agent is a cell-based therapy selected from the list consisting of dendritic-cell therapy and T-cell therapy. In some embodiments, the additional therapeutic agent targets one of the markers/receptors listed in Table 2. In some embodiments, the additional therapeutic targets a marker/receptor expressed on the surface of the T cells. In some embodiments, the additional therapeutic targets PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, CRTAM or Sema7a. In some embodiments, the additional therapeutic agent targets Nrn1, Sema7a, or CRTAM.

In some embodiments, provided herein are methods of treating a subject with cancer comprising administering a therapeutic agent that specifically targets dysfunctional tumor antigen-specific $CD8^+$ T cells, wherein the agent targets one of the receptors listed in Table 2. In some embodiments, the therapeutic targets a marker/receptor expressed on the surface of the T cells. In some embodiments, the therapeutic targets PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, CRTAM or Sema7a. In some embodiments, the therapeutic agent targets Nrn1, Sema7a, or CRTAM. In some embodiments, the therapeutic agent is an antibody, antibody fragment, or antibody mimetic molecule that binds the target marker/receptor. In some embodiments, the therapeutic agent is an anti-Nrn antibody, antibody fragment, or antibody mimetic molecule. In some embodiments, the therapeutic agent is an anti-Sema7a antibody, antibody fragment, or antibody mimetic molecule. In some embodiments, the therapeutic agent is an anti-CRTAM antibody, antibody fragment, or antibody mimetic molecule.

In some embodiments, provided herein are compositions comprising: (a) one or more of an anti-4-1BB agent, an anti-LAG-3 agent, an anti-Nrn1 agent, an anti-Sema7a agent, and an anti-CRTAM agent; and (b) an immunotherapeutic agent, said composition formulated for therapeutic delivery to a subject. In some embodiments, the anti-4-1BB agent, anti-LAG-3 agent, anti-Nrn1 agent, anti-Sema7a agent, and/or anti-CRTAM agent is an antibody, antibody fragment, or antibody mimetic molecule.

In some embodiments, provided herein are compositions comprising: (a) an agent that targets and/or binds one of PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A; and (b) an immunotherapeutic agent, said composition formulated for therapeutic delivery to a subject.

In some embodiments, provided herein are methods comprising: (a) testing $CD8^+$ T cells from a cell population to determine whether the CD8+ T Cells co-express LAG-3 and 4-1BB; and (b) administering one or more agents that target and/or bind one of PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A. In some embodiments, the agent is an anti-Nrn1 agent, an anti-Sema7a agent, and an anti-CRTAM agent. In some embodiments, the anti-Nrn1 agent, anti-Sema7a agent, and/or anti-CRTAM agent is an antibody, antibody fragment, or antibody mimetic molecule. In some embodiments, testing is performed in vitro.

In some embodiments, provided herein are methods of identifying dysfunctional T cells by testing said cells for co-expression of 4-1BB and LAG-3. In some embodiments, provided herein are methods of identifying dysfunctional T cells by testing said cells for expression of one or more of the markers/receptors of Table 2 (e.g., a T-cell surface marker/receptor (e.g., PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, TMEM126A).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A-J. Co-expression of 4-1BB and LAG-3 identifies a significant fraction of the $CD8^+$ TIL compartment found in progressing tumors. (A) Representative analysis of 4-1BB and LAG-3 expression on $CD8^+$ T cells from B16.SIY tumors and the spleen and TdLN from tumor bearing mice on day 7, 14 and 21 after s.c. tumor inoculation. (B-D) Longitudinal summary of the composition, n=5; four to five independent experiments per time point, (C) absolute cell number, n=5; seven to nine independent experiments per time point, and (D) cellular density of the $CD8^+$4-1BB/LAG-3 TIL subpopulations, n=5; two to five independent experiments per time point. Absolute cell numbers were determined by acquiring the complete tumor sample by flow cytometery. (E) Day 14 summary of the proportion of the $CD8^+$4-1BB/LAG-3 TIL subpopulations that are $Ki67^+$. n=3-5; two independent experiments. (F) Summary of BrdU uptake on day 13 in the $CD8^+$4-1BB/LAG-3 TIL subpopulations after a 24 hour BrdU pulse. n=5; three independent experiments. (G-I) Representative flow plots (G and H) and summary (I) of the 4-1BB/LAG-3 populations in other tumor models. Mice were inoculated with 2×10^6 C1498.SIY, MC38.SIY, EL4.SIY, B16 Parental, MC57.SIY or 1969.SIY subcutaneously and analyzed for 4-1BB and LAG-3 expression on day 14 after tumor inoculation. n=3-5; two to 5 independent experiments for each time point. (J) Mice were inoculated on both flanks with 2×10^6 MC57.SIY or B16.SIY, at indicated time points tumors from each mouse were pooled and analyzed for co-expression of 4-1BB and LAG-3 in the $CD8^+$ TIL compartment. n=3-5; two independent experiments for each time point. All error bars indicate±SEM. *:$P<0.05$, **:$P<0.01$, ***:$P<0.001$. A two-way ANOVA with Bonferroni post-hoc test was used for (B, C, D, H) longitudinal studies and Kruskal-Wallis (non-parametric) test was used for (E and F) analysis at one time-point.

FIG. **2**A-G. Egr2 and a component of the Egr2-transcriptional network are enriched in 4-$1BB^+$LAG-3^+CD8^+ TILs. (A) Representative flow plot and summary of $Egr2^{EGFP}$ expression. $Egr2^{EGFP}$ mice were inoculated with 2×10^6B16.SIY tumors s.c. $CD8^+$ T cells from the tumor, TdLN and spleen were analyzed for $Egr2^{EGFP}$ expression on day 7 and day 14. n=4-5; two-independent experiments. (B) Expression of Egr2 target genes (Zheng et al., 2013). $CD8^+$ TILs from day 14 tumor bearing mice were sorted based on high or low expression of $Egr2^{EGFP}$ and analyzed directly for expression of Egr2 targets by qRT-PCR. Two tumors on opposite flanks pooled per mouse. n=3; two independent experiments. (C) Representative flow plots and summary of the 4-1BB/LAG-3 subpopulations in $CD8^+$ $Egr2GFP^{hi}$ and $Egr2GFP^{lo}$ TILs on day 7 and 14. n=4-5. Two-independent experiments per time point. (D) Expression of Egr2 targets in the 4-$1BB^+$LAG-3^+ and 4-$1BB^-$LAG-3^- subpopulations. The subpopulations were sorted and analyzed directly for the expression of targets by qRT-PCR. Two tumors on opposite flanks pooled per mouse. n=4; two-independent experiments. (E) $Egr2^{flox/flox}$×pLCKCreERT2×YFP-Rosa26 mice given 5 doses of tamoxifen by gavage and inoculated 3 days later with 2×10^6B16.SIY cells. YFP^+ or YFP^-CD8^+ TILs were sorted and analyzed for Egr2 transcript directly and after in vitro stimulation. Two tumors on opposite flanks

pooled per mouse. n=3; two independent experiments. (F) Representative flow plots and summary of 4-1BB/LAG-3 co-expression in YFP^+ or YFP^-CD8^+ TILs on day 7 and 14. n=3; two independent experiments. (G) Expression of Egr3 and Hif1α in $Egr2GFP^{hi}$ and $Egr2GFP^{lo}$ from day 7 $CD8^+$ TILs isolated from Egr2GFP mice. n=5; two-independent experiments. Error bars indicate±SEM. *:P<0.05, **:P<0.01, ***:P<0.001. A two-way ANOVA with Bonferroni post-hoc test was used for longitudinal studies (A and C) and a Mann-Whitney test was used to compute significance in (B, D, E, F and G).

FIG. **3**A-H. Co-expression of 4-1BB and LAG-3 identifies tumor antigen-specific TILs in progressing tumors. (A) Representative CDR3β distributions from the different 4-1BB/LAG-3 subpopulations and $CD8^+$ T cells isolated from the spleen. Boxed regions represent dominant peaks in the $4\text{-}1BB^+LAG\text{-}3^+CD8^+$ TIL subpopulation. (B) As a measure of skewness, the Hamming Distance (HD) for each Vβ spectratype was calculated between each TIL subpopulation and $CD8^+$ T cell spleen population within the same mouse. As a control the HDs from $CD8^+$ splenocyte populations between mice (grey bar) were calculated. n=3; one independent experiment. (C-D) Representative flow analysis of the 4-1BB/LAG-3 subpopulation in $H\text{-}2K^b/SIY^+$ and $H\text{-}2K^b/SIY^-$ $CD8^+$ TILs on day 14 after B16.SIY and MC38.SIY or (D) MC57.SIY and 1969.SIY tumor inoculation. n=3-4; three to five independent experiments. (E) Summary of the composition of $H\text{-}2K^b/SIY^+$ and $H\text{-}2K^b/SIY^-$ $CD8^+$ TILs co-expressing 4-1BB and LAG-3 comparing B16.SIY, MC38.SIY, MC57.SIY and 1969.SIY tumors on day 14 after tumor inoculation. n=5; three to four independent experiments. (F-H) On day 7 after tumor inoculation 1×10^6 P14/CD45.2 and 2C/CD45.1/2 Tg T cells were adoptively transferred, via tail vein, into CD45.1 congenic tumor bearing hosts and analyzed for the (F) total number of recovered cells in the tumor, (G and H) profile of 4-1BB and LAG-3 expression in 2C, P14 and host $CD8^+$ TILs. n=5; two-independent experiments. All error bars indicate±SEM. *:P<0.05, **P<0.01, ***:P<0.001. A Kruskal-Wallis (non-parameteric) test was used for (B) spectratype analysis and (E and F) $H\text{-}2K^b/SIY$ analysis. A two-way ANOVA with Bonferroni post-hoc test was used for (H) 2C, Host and P14 composition analysis.

FIG. **4**A-G. Co-expression of 4-1BB and LAG-3 but not PD-1 define dysfunctional $CD8^+$ TILs with diminished IL-2. (A and B) Sorted cells from day 14 B16.SIY tumor bearing mice were stimulated in vitro with anti-CD3ε and anti-CD28 for 12 hours and analyzed for (A) Il-2 transcript by qRT-PCR and (B) IL-2 protein by ELISA. Two tumors on opposite flanks pooled per mouse. n=4-5; three independent experiments. (C) $Egr2GFP^{hi}$ and $Egr2GFP^{lo}$ TILs were sorted from day 14 B16.SIY tumor bearing $Egr2^{GFP}$ mice and stimulated in vitro for 12 hours and analyzed for Il-2 transcript by qRT-PCR. Two tumors on opposite flanks pooled per mouse. n=5; two independent experiments. (D) On day 7 after tumor inoculation 1×10^6 2 C/CD45.1/2 Tg T cells were transferred into mice, 7 days later host $4\text{-}1BB^+LAG\text{-}3^+$ T cells sorted from the tumor and 2C T cells sorted from the tumor or TdLN were stimulated in vitro and analyzed for expression of Il-2 transcript by qRT-PCR. Two tumors on opposite flanks pooled per mouse. n=3; two independent experiments. (E and F) Representative flow analysis of PD-1 expression on 4-1BB/LAG-3 $CD8^+$ TIL subpopulations and (F) summary of the composition of the $4\text{-}1BB^-LAG\text{-}3^-PD\text{-}1^+$ subpopulation in the $CD8^+$ TIL compartment on day 14 and 21. n=5; three independent experiments. (G) $4\text{-}1BB^-LAG\text{-}3^-PD\text{-}1^+$ and $LAG\text{-}3^+4\text{-}1BB^+CD8^+$ TILs were sorted from day 14 tumor bearing mice, stimulated in vitro and analyzed for Il-2 transcript by qRT-PCR. Two tumors on opposite flanks pooled per mouse. n=3; two independent experiments. All error bars indicate±SEM. *:P<0.05, **:P<0.01, ***:P<0.001 ****:P<0.0001. A Kruskal-Wallis (non-parametric) test was used for analysis of multiple comparisons (A, B, and D) and a Mann-Whitney test was used for pair-wise comparisons (C and G).

FIG. **5**A-E. Dysfunctional $CD8^+$ TILs retain IFN-γ production, cytolytic capacity and produce Treg-recruiting chemokines. (A) Longitudinal analysis $CD8^+$ TIL subpopulation cytokine production capacity. $CD8^+$ TIL subpopulations were sorted and stimulated with anti-CD3ε and anti-CD28 for 10-12 hours and the concentration of IL-2, IFN-γ and TNF-α was measured. Concentration was normalized to cell number. Two tumors on opposite flanks pooled for day 7 and 14. n=4-5; two-independent experiments. (B) Ifn-γ Tnf-α and Gzmb transcript levels in the 4-1BB/LAG-3 subpopulations analyzed directly ex vivo. Two tumors on opposite flanks pooled per mouse. n=3-5; three-independent experiments. (C) Representative flow plot and summary of IFN-γ production analyzed directly ex vivo. Briefly, 100 μl of PBS containing 2 mg/mL GolgiPlug was injected intratumorally on day 14 after tumor inoculation. 8 hours later TILs were isolated. All steps were performed on ice with media containing 1 mg/mL GolgiStop until fixation. n=5; two independent experiments. (D) $CD8^+$ TIL subpopulations at indicated time points were sorted and plated with 50,000 P815 target cells and 1 μg/mL anti-CD3ε. Lysed target cells were measured by positive staining for propidium iodide and/or live/dead fixable viability dye. P815 target cells plated without CTLs were used as a negative control (black bar). Primed OTI cells were used as a positive control. Tumors from 10 mice with 2 tumors on opposite flank were pooled to obtain sufficient quantities of $CD8^+$ TILS. Data are representative of three independent experiments. (E) Ccl1 and Ccl22 transcript levels in the 4-1BB/LAG-3 subpopulations analyzed directly ex vivo by qRT-PCR. n=4; two independent experiments. *:P<0.05, **:P<0.01, ***:P<0.001, ****:P<0.0001. A Kruskal-Wallis (non-parametric) test was used for (A-C, E) cytokine/chemokine analysis and a two-way ANOVA with Bonferroni post-hoc test was used for (D) cytolytic assay.

FIG. **6**A-D. Dysfunctional $CD8^+$ TILS express a wide range of co-inhibitory and co-stimulatory receptors. (B) Gene expression profile of cell surface receptors in the 4-1BB/LAG-3 $CD8^+$ TIL subsets. Probe sets that revealed a 1.5-fold increase in the $4\text{-}1BB^+LAG\text{-}3^+$ population relative to the $4\text{-}1BB^-LAG\text{-}3^-PD\text{-}1^-$ population are displayed. Columns show the $\log_2$-transformed signal intensity. (C) Longitudinal study of selected un-regulated cell surface receptors. Flow plots are representative of the $CD8^+$ TIL subsets on day 14. n=5; two to five independent experiments for each time point. (D) Representative flow plot and summary of KLRG-1 and IL-7Rα expression among the 4-1BB/LAG-3 subpopulations on day 14 after tumor inoculation. n=5; two independent experiments. *:P<0.05, **:P<0.01, ***:P<0.001, ****:P<0.0001. A two-way ANOVA with Bonferroni post-hoc test was used for all analyses.

FIG. **7**A-G. Anti-4-1BB and anti-LAG-3 acts synergistically to control tumor outgrowth and restore TIL function. (A) Tumor outgrowth measured in mm^2. Arrows indicate on which days mice received antibody therapy. Statistical significance at indicate time points is in comparison to anti-4-1BB+anti-LAG-3 treatment. n=5; two independent experiments. (B) Composition of $H\text{-}2K^b/SIY^+CD8^+$ TILS on day 14. Mice received antibody doses (100 μg each) on days 7,

10, 13 and 16. n=5; two independent experiments. (C-F) Representative flow plot and summary of NRP1/2B4 (C and E) and KLRG-1/IL-7Rα (D and F) expression in H-2K^b/SIY$^+$CD8$^+$ TILS without FTY720 (C and D) and with FTY720 (E and F) on day 14 after tumor inoculation. Mice received antibody treatment as in (A and B) and FTY720 was administered at a dose of 25 μg/mouse by gavage starting one day before treatment and continuing one dose per day until analysis (day 6 to day 13). n=5; two-independent experiments. (G) IL-2 production after treatment. Sorted cells from treated or untreated day 14 B16.SIY tumor bearing mice were stimulated in vitro for 12 hours and analyzed for Il-2 transcript by qRT-PCR. Protein concentration was determined by the bead-based LEGENDplex immunoassay and normalized to cell number. Two tumors on opposite flanks pooled per mouse. n=2-3; two independent experiments. A two-way ANOVA with Bonferroni post-hoc test was used for all analyses. *:P<0.05, **:P<0.01, ***:P<0.001.

FIG. **8**. Spectratype graphs used in the analysis in FIG. **3**B.

FIG. **9**. CD3+ T cells on day 14 after FTY720 administration.

FIG. **10**A-B. Statistical analysis of the cross-study comparison of gene expression profiles. (A) Rank-Rank Hypergeometric plots of each pair-wise comparison. (B) Pair-wise correlation of expression values between each data set. Rho (p) is the spearman rank correlation coefficient.

FIG. **11**A-E. Nrn1, CRTAM and Sema7a are regulators of anti-tumor immunity. (A) Tumor growth measured in mm2. Nrn1$^{-/-}$ or Sema7a$^{-/-}$ and littermate control mice were engrafted with 2×106 B16.SIY cells subcutaneously. (B) Gene expression analysis of Nrn1 in T cell subsets of the spleen, TdLN and Tumor. (C) Representative flow plot and summary of IFN-g production of WT, Nrn1$^{-/-}$ or (D) CRTAM$^{-/-}$ 2C T cells on day 7. Briefly, on the same day as tumor inoculation, 1×10^6 Cell Trace Violet-labeled 2C T cells were transferred into mice by tail vein injection. On day 7, whole TdLN suspensions were restimulated with SIY peptide for 12 hours and analyzed for cell trace dilution and IFN-g production. (E) Mice that received 1×10^6 Nrn1$^{-/-}$ 2 C T cells are more likely to exhibit complete tumor control compared to mice that received the same number of WT 2C T cells. Adoptive transfer of T cells was performed the same way as in (C).

FIG. **12**. Exemplary experimental protocol and data.

DEFINITIONS

Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments described herein, some preferred methods, compositions, devices, and materials are described herein. However, before the present materials and methods are described, it is to be understood that this invention is not limited to the particular molecules, compositions, methodologies or protocols herein described, as these may vary in accordance with routine experimentation and optimization. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the embodiments described herein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. However, in case of conflict, the present specification, including definitions, will control. Accordingly, in the context of the embodiments described herein, the following definitions apply.

As used herein and in the appended claims, the singular forms "a", "an" and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

As used herein, the term "comprise" and linguistic variations thereof denote the presence of recited feature(s), element(s), method step(s), etc. without the exclusion of the presence of additional feature(s), element(s), method step(s), etc. Conversely, the term "consisting of" and linguistic variations thereof, denotes the presence of recited feature(s), element(s), method step(s), etc. and excludes any unrecited feature(s), element(s), method step(s), etc., except for ordinarily-associated impurities. The phrase "consisting essentially of" denotes the recited feature(s), element(s), method step(s), etc. and any additional feature(s), element(s), method step(s), etc. that do not materially affect the basic nature of the composition, system, or method. Many embodiments herein are described using open "comprising" language. Such embodiments encompass multiple closed "consisting of" and/or "consisting essentially of" embodiments, which may alternatively be claimed or described using such language.

As used herein, the term "subject" broadly refers to any animal, including but not limited to, human and non-human animals (e.g., dogs, cats, cows, horses, sheep, poultry, fish, crustaceans, etc.). As used herein, the term "patient" typically refers to a subject that is being treated for a disease or condition (e.g., cancer, solid tumor cancer, etc.).

As used herein, an "immune response" refers to the action of a cell of the immune system (e.g., T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells, neutrophils, etc.) and soluble macromolecules produced by any of these cells or the liver (including antibodies, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a subject of invading pathogens, cells or tissues infected with pathogens, or cancerous or other abnormal cells.

As used herein, the term "immunoregulator" refers to a substance, an agent, a signaling pathway or a component thereof that regulates an immune response. "Regulating," "modifying" or "modulating" an immune response refers to any alteration in a cell of the immune system or in the activity of such cell. Such regulation includes stimulation or suppression of the immune system which may be manifested by an increase or decrease in the number of various cell types, an increase or decrease in the activity of these cells, or any other changes which can occur within the immune system. Both inhibitory and stimulatory immunoregulators have been identified, some of which may have enhanced function in the cancer microenvironment.

As used herein, the term "immunotherapy" refers to the treatment or prevention of a disease or condition by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response.

As used herein, "potentiating an endogenous immune response" means increasing the effectiveness or potency of an existing immune response in a subject. This increase in effectiveness and potency may be achieved, for example, by overcoming mechanisms that suppress the endogenous host immune response or by stimulating mechanisms that enhance the endogenous host immune response.

As used herein, the term "antibody" refers to a whole antibody molecule or a fragment thereof (e.g., fragments such as Fab, Fab', and $F(ab')_2$), unless otherwise specified (e.g., "whole antibody," "antibody fragment"). An antibody may be a polyclonal or monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, etc.

A native antibody typically has a tetrameric structure. A tetramer typically comprises two identical pairs of polypeptide chains, each pair having one light chain (in certain embodiments, about 25 kDa) and one heavy chain (in certain embodiments, about 50-70 kDa). In a native antibody, a heavy chain comprises a variable region, V_H, and three constant regions, C_{H1}, C_{H2}, and C_{H3}. The V_H domain is at the amino-terminus of the heavy chain, and the C_{H3} domain is at the carboxy-terminus. In a native antibody, a light chain comprises a variable region, V_L, and a constant region, C_L. The variable region of the light chain is at the amino-terminus of the light chain. In a native antibody, the variable regions of each light/heavy chain pair typically form the antigen binding site. The constant regions are typically responsible for effector function.

In a native antibody, the variable regions typically exhibit the same general structure in which relatively conserved framework regions (FRs) are joined by three hypervariable regions, also called complementarity determining regions (CDRs). The CDRs from the two chains of each pair typically are aligned by the framework regions, which may enable binding to a specific epitope. From N-terminus to C-terminus, both light and heavy chain variable regions typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The CDRs on the heavy chain are referred to as H1, H2, and H3, while the CDRs on the light chain are referred to as L1, L2, and L3. Typically, CDR3 is the greatest source of molecular diversity within the antigen-binding site. H3, for example, in certain instances, can be as short as two amino acid residues or greater than 26. The assignment of amino acids to each domain is typically in accordance with the definitions of Kabat et al. (1991) Sequences of Proteins of Immunological Interest (National Institutes of Health, Publication No. 91-3242, vols. 1-3, Bethesda, Md.); Chothia, C., and Lesk, A. M. (1987) J. Mol. Biol. 196:901-917; or Chothia, C. et al. Nature 342:878-883 (1989). In the present application, the term "CDR" refers to a CDR from either the light or heavy chain, unless otherwise specified.

As used herein, the term "heavy chain" refers to a polypeptide comprising sufficient heavy chain variable region sequence to confer antigen specificity either alone or in combination with a light chain.

As used herein, the term "light chain" refers to a polypeptide comprising sufficient light chain variable region sequence to confer antigen specificity either alone or in combination with a heavy chain.

As used herein, when an antibody or other entity "specifically recognizes" or "specifically binds" an antigen or epitope, it preferentially recognizes the antigen in a complex mixture of proteins and/or macromolecules, and binds the antigen or epitope with affinity which is substantially higher than to other entities not displaying the antigen or epitope. In this regard, "affinity which is substantially higher" means affinity that is high enough to enable detection of an antigen or epitope which is distinguished from entities using a desired assay or measurement apparatus. Typically, it means binding affinity having a binding constant (K_a) of at least 10^7 M^{-1} (e.g., $>10^7$ M^{-1}, $>10^8$ M^{-1}, $>10^9$ M^{-1}, $>10^{10}$ M^{-1}, $>10^{11}$ M^{-1}, $>10^{12}$ M^{-1}, $>10^{13}$ M^{-1}, etc.). In certain such embodiments, an antibody is capable of binding different antigens so long as the different antigens comprise that particular epitope. In certain instances, for example, homologous proteins from different species may comprise the same epitope.

As used herein, the term "anti-4-1BB antibody" or "4-1BB antibody" refers to an antibody which specifically recognizes an antigen and/or epitope presented by 4-1BB. Similarly, the terms "anti-LAG-3 antibody" and "LAG-3 antibody" refer to an antibody which specifically recognizes an antigen and/or epitope presented by LAG-3, the terms "anti-Nrn1 antibody" and "Nrn1 antibody" refer to an antibody which specifically recognizes an antigen and/or epitope presented by Nrn1, the terms "anti-CRTAM antibody" and "CRTAM antibody" refer to an antibody which specifically recognizes an antigen and/or epitope presented by CRTAM, and the terms "anti-Sema7a antibody" and "Sema7a antibody" refer to an antibody which specifically recognizes an antigen and/or epitope presented by Sema7a. Antibodies that recognize epitopes on other molecular entities may be referred to according to a similar scheme (e.g., anti-CTLA-4, anti-PD-L1, etc.).

As used herein, the term "monoclonal antibody" refers to an antibody which is a member of a substantially homogeneous population of antibodies that specifically bind to the same epitope. In certain embodiments, a monoclonal antibody is secreted by a hybridoma. In certain such embodiments, a hybridoma is produced according to certain methods known to those skilled in the art. See, e.g., Kohler and Milstein (1975) Nature 256: 495-499; herein incorporated by reference in its entirety. In certain embodiments, a monoclonal antibody is produced using recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). In certain embodiments, a monoclonal antibody refers to an antibody fragment isolated from a phage display library. See, e.g., Clackson et al. (1991) Nature 352: 624-628; and Marks et al. (1991) J. Mol. Biol. 222: 581-597; herein incorporated by reference in their entireties. The modifying word "monoclonal" indicates properties of antibodies obtained from a substantially-homogeneous population of antibodies, and does not limit a method of producing antibodies to a specific method. For various other monoclonal antibody production techniques, see, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.); herein incorporated by reference in its entirety.

As used herein, the term "antibody fragment" refers to a portion of a full-length antibody, including at least a portion antigen binding region or a variable region. Antibody fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, Fv, scFv, Fd, diabodies, and other antibody fragments that retain at least a portion of the variable region of an intact antibody. See, e.g., Hudson et al. (2003) Nat. Med. 9:129-134; herein incorporated by reference in its entirety. In certain embodiments, antibody fragments are produced by enzymatic or chemical cleavage of intact antibodies (e.g., papain digestion and pepsin digestion of antibody) produced by recombinant DNA techniques, or chemical polypeptide synthesis.

For example, a "Fab" fragment comprises one light chain and the C_{H1} and variable region of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A "Fab'" fragment comprises one light chain and one heavy chain that comprises additional constant region, extending between the C_{H1} and C_{H2} domains. An interchain disulfide bond can be formed between two heavy chains of a Fab' fragment to form a "$F(ab')_2$" molecule.

An "Fv" fragment comprises the variable regions from both the heavy and light chains, but lacks the constant regions. A single-chain Fv (scFv) fragment comprises heavy and light chain variable regions connected by a flexible linker to form a single polypeptide chain with an antigen-binding region. Exemplary single chain antibodies are discussed in detail in WO 88/01649 and U.S. Pat. Nos. 4,946,778 and 5,260,203; herein incorporated by reference in their entireties. In certain instances, a single variable region (e.g., a heavy chain variable region or a light chain variable region) may have the ability to recognize and bind antigen.

Other antibody fragments will be understood by skilled artisans.

As used herein, the term "chimeric antibody" refers to an antibody made up of components from at least two different sources. In certain embodiments, a chimeric antibody comprises a portion of an antibody derived from a first species fused to another molecule, e.g., a portion of an antibody derived from a second species. In certain such embodiments, a chimeric antibody comprises a portion of an antibody derived from a non-human animal fused to a portion of an antibody derived from a human. In certain such embodiments, a chimeric antibody comprises all or a portion of a variable region of an antibody derived from a non-human animal fused to a constant region of an antibody derived from a human.

A "humanized" antibody refers to a non-human antibody that has been modified so that it more closely matches (in amino acid sequence) a human antibody. A humanized antibody is thus a type of chimeric antibody. In certain embodiments, amino acid residues outside of the antigen binding residues of the variable region of the non-human antibody are modified. In certain embodiments, a humanized antibody is constructed by replacing all or a portion of a complementarity determining region (CDR) of a human antibody with all or a portion of a CDR from another antibody, such as a non-human antibody, having the desired antigen binding specificity. In certain embodiments, a humanized antibody comprises variable regions in which all or substantially all of the CDRs correspond to CDRs of a non-human antibody and all or substantially all of the framework regions (FRs) correspond to FRs of a human antibody. In certain such embodiments, a humanized antibody further comprises a constant region (Fc) of a human antibody.

The term "human antibody" refers to a monoclonal antibody that contains human antibody sequences and does not contain antibody sequences from a non-human animal. In certain embodiments, a human antibody may contain synthetic sequences not found in native antibodies. The term is not limited by the manner in which the antibodies are made. For example, in various embodiments, a human antibody may be made in a transgenic mouse, by phage display, by human B-lymphocytes, or by recombinant methods.

As used herein, the term "natural antibody" refers to an antibody in which the heavy and light chains of the antibody have been made and paired by the immune system of a multicellular organism. For example, the antibodies produced by the antibody-producing cells isolated from a first animal immunized with an antigen are natural antibodies. Natural antibodies contain naturally-paired heavy and light chains. The term "natural human antibody" refers to an antibody in which the heavy and light chains of the antibody have been made and paired by the immune system of a human subject.

Native human light chains are typically classified as kappa and lambda light chains. Native human heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has subclasses, including, but not limited to, IgG1, IgG2, IgG3, and IgG4. IgM has subclasses including, but not limited to, IgM1 and IgM2. IgA has subclasses including, but not limited to, IgA1 and IgA2. Within native human light and heavy chains, the variable and constant regions are typically joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See, e.g., Fundamental Immunology (1989) Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y.); herein incorporated by reference in its entirety.

The term "neutralizing antibody" or "antibody that neutralizes" refers to an antibody that reduces at least one activity of a polypeptide comprising the epitope to which the antibody specifically binds. In certain embodiments, a neutralizing antibody reduces an activity in vitro and/or In vivo. In some embodiments, by neutralizing the polypeptide comprising the epitope, the neutralizing antibody inhibits the capacity of the cell displaying the epitope.

As used herein, the term "glycoengineered", as used herein, includes any manipulation of the glycosylation pattern of a naturally occurring or recombinant protein, polypeptide or a fragment thereof.

The term "antigen-binding site" refers to a portion of an antibody capable of specifically binding an antigen. In certain embodiments, an antigen-binding site is provided by one or more antibody variable regions.

The term "epitope" refers to any polypeptide determinant capable of specifically binding to an immunoglobulin or a T-cell or B-cell receptor. In certain embodiments, an epitope is a region of an antigen that is specifically bound by an antibody. In certain embodiments, an epitope may include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl groups. In certain embodiments, an epitope may have specific three dimensional structural characteristics (e.g., a "conformational" epitope) and/or specific charge characteristics.

An epitope is defined as "the same" as another epitope if a particular antibody specifically binds to both epitopes. In certain embodiments, polypeptides having different primary amino acid sequences may comprise epitopes that are the same. In certain embodiments, epitopes that are the same may have different primary amino acid sequences. Different antibodies are said to bind to the same epitope if they compete for specific binding to that epitope.

A "conservative" amino acid substitution refers to the substitution of an amino acid in a polypeptide with another amino acid having similar properties, such as size or charge. In certain embodiments, a polypeptide comprising a conservative amino acid substitution maintains at least one activity of the unsubstituted polypeptide. A conservative amino acid substitution may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include, but are not limited to, peptidomimetics and other reversed or inverted forms of amino acid moieties. Naturally occurring residues may be divided into classes based on common side chain properties, for example: hydrophobic: norleucine, Met, Ala, Val, Leu, and Ile; neutral hydrophilic: Cys, Ser, Thr, Asn, and Gln; acidic: Asp and Glu; basic: His, Lys, and Arg; residues that influence chain orientation: Gly and Pro; and aromatic: Trp, Tyr, and Phe. Non-conservative substitutions may involve the exchange of a member of one of these classes for a member

from another class; whereas conservative substitutions may involve the exchange of a member of one of these classes for another member of that same class.

As used herein, the term "sequence identity" refers to the degree to which two polymer sequences (e.g., peptide, polypeptide, nucleic acid, etc.) have the same sequential composition of monomer subunits. The term "sequence similarity" refers to the degree with which two polymer sequences (e.g., peptide, polypeptide, nucleic acid, etc.) have similar polymer sequences. For example, similar amino acids are those that share the same biophysical characteristics and can be grouped into the families (see above). The "percent sequence identity" (or "percent sequence similarity") is calculated by: (1) comparing two optimally aligned sequences over a window of comparison (e.g., the length of the longer sequence, the length of the shorter sequence, a specified window, etc.), (2) determining the number of positions containing identical (or similar) monomers (e.g., same amino acids occurs in both sequences, similar amino acid occurs in both sequences) to yield the number of matched positions, (3) dividing the number of matched positions by the total number of positions in the comparison window (e.g., the length of the longer sequence, the length of the shorter sequence, a specified window), and (4) multiplying the result by 100 to yield the percent sequence identity or percent sequence similarity. For example, if peptides A and B are both 20 amino acids in length and have identical amino acids at all but 1 position, then peptide A and peptide B have 95% sequence identity. If the amino acids at the non-identical position shared the same biophysical characteristics (e.g., both were acidic), then peptide A and peptide B would have 100% sequence similarity. As another example, if peptide C is 20 amino acids in length and peptide D is 15 amino acids in length, and 14 out of 15 amino acids in peptide D are identical to those of a portion of peptide C, then peptides C and D have 70% sequence identity, but peptide D has 93.3% sequence identity to an optimal comparison window of peptide C. For the purpose of calculating "percent sequence identity" (or "percent sequence similarity") herein, any gaps in aligned sequences are treated as mismatches at that position.

The term "effective dose" or "effective amount" refers to an amount of an agent, e.g., an antibody, that results in the reduction of symptoms in a patient or results in a desired biological outcome. In certain embodiments, an effective dose or effective amount is sufficient to treat or reduce symptoms of a disease or condition.

As used herein, the terms "administration" and "administering" refer to the act of giving a drug, prodrug, or other agent, or therapeutic to a subject or in vivo, in vitro, or ex vivo cells, tissues, and organs. Exemplary routes of administration to the human body can be through space under the arachnoid membrane of the brain or spinal cord (intrathecal), the eyes (ophthalmic), mouth (oral), skin (topical or transdermal), nose (nasal), lungs (inhalant), oral mucosa (buccal), ear, rectal, vaginal, by injection (e.g., intravenously, subcutaneously, intratumorally, intraperitoneally, etc.) and the like.

The term "treatment" encompasses both therapeutic and prophylactic/preventative measures unless otherwise indicated. Those in need of treatment include, but are not limited to, individuals already having a particular condition as well as individuals who are at risk of acquiring a particular condition or disorder (e.g., those having a genetic or epigenetic predisposition; based on age, gender, lifestyle, etc.).

The term "treating" refers to administering an agent to a subject for therapeutic and/or prophylactic/preventative purposes.

A "therapeutic agent" refers to an agent that may be administered In vivo to bring about a therapeutic and/or prophylactic/preventative effect.

A "therapeutic antibody" refers to an antibody that may be administered In vivo to bring about a therapeutic and/or prophylactic/preventative effect.

As used herein, the terms "co-administration" and "co-administering" refer to the administration of at least two agent(s) or therapies to a subject. In some embodiments, the co-administration of two or more agents or therapies is concurrent. In other embodiments, a first agent/therapy is administered prior to a second agent/therapy. Those of skill in the art understand that the formulations and/or routes of administration of the various agents or therapies used may vary. The appropriate dosage for co-administration can be readily determined by one skilled in the art. In some embodiments, when agents or therapies are co-administered, the respective agents or therapies are administered at lower dosages than appropriate for their administration alone. Thus, co-administration is especially desirable in embodiments where the co-administration of the agents or therapies lowers the requisite dosage of a potentially harmful (e.g., toxic) agent(s), and/or when co-administration of two or more agents results in sensitization of a subject to beneficial effects of one of the agents via co-administration of the other agent.

As used herein, the term pharmaceutical composition" refers to the combination of an active agent (e.g., binding agent) with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use in vitro, in vivo or ex vivo.

The terms "pharmaceutically acceptable" or "pharmacologically acceptable," as used herein, refer to compositions that do not substantially produce adverse reactions, e.g., toxic, allergic, or immunological reactions, when administered to a subject.

As used herein, the term "pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers including, but not limited to, phosphate buffered saline solution, water, emulsions (e.g., such as an oil/water or water/oil emulsions), and various types of wetting agents, any and all solvents, dispersion media, coatings, sodium lauryl sulfate, isotonic and absorption delaying agents, disintigrants (e.g., potato starch or sodium starch glycolate), and the like. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see, e.g., Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, Pa. (1975), incorporated herein by reference in its entirety.

As used herein, a "diagnostic" or "diagnostic test" includes the detection, identification, or characterization of a disease state or condition of a subject. For example, a disease or condition may be characterized to determine the likelihood that a subject with a disease or condition will respond to a particular therapy, determine the prognosis of a subject with a disease or condition (or its likely progression or regression), determine the effect of a treatment on a subject with a disease or condition, or determine a future treatment course of action.

DETAILED DESCRIPTION

Provided herein are compositions and methods for detecting and/or targeting dysfunctional tumor antigen-specific

CD8+ T cells in the tumor microenvironment for diagnostic, therapeutic and/or research applications. In particular, dysfunctional tumor antigen-specific CD8+ T cells are detected and/or targeted via their expression of cell surface receptors described herein, such as 4-1BB, LAG-3, or additional markers that correlate with 4-1BB and LAG-3 expression, such as markers differentially expressed on the surface of the T cells (e.g., PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, CRTAM and Sema7a).

Experiments conducted during development of embodiments herein identified markers/receptors that correlate and/or are responsible for tumor antigen-specific CD8+ T cell dysfunction. In some embodiments, the markers/receptors are overexpressed in dysfunctional tumor antigen-specific CD8+ T cells. In such embodiments, detecting the level (e.g., above a threshold) of such markers provides a diagnostic for detecting tumor antigen-specific CD8+ T cell dysfunction. Further, in such embodiments, targeting (e.g., inhibiting (e.g., expression and/or activity of)) such markers/receptors provides a therapeutic. In other embodiments, the markers/receptors are underexpressed in dysfunctional tumor antigen-specific CD8+ T cells. In such embodiments, detecting the level (e.g., below a threshold) of such markers provides a diagnostic for detecting tumor antigen-specific CD8+ T cell dysfunction. Further, in such embodiments, targeting (e.g., enhancing (e.g., expression and/or activity of)) such markers/receptors provides a therapeutic.

Transcription factor Egr2 is a critical regulator of the anergic state in CD4+ T cell clones manipulated in vitro (Zheng et al., 2013; 2012; incorporated by reference in their entireties). Egr2 has also been shown to be involved in negative regulation of T cell activation in several in vivo model systems (Sumitomo et al., 2013; incorporated by reference in its entirety). Egr2 contributes to upregulation of DGKa and -z which act to blunt TCR-mediated Ras pathway activation (Zha et al., 2006; incorporated by reference in its entirety). By comparing gene expression profiling of anergized cells along with Egr2 ChIP-Seq analysis multiple additional Egr2-driven gene targets were identified (Zheng et al., 2013; incorporated by reference in its entirety). These gene targets include 4-1BB (Tnfrsf9 or CD137), Lag3, Nrn1, Sema7a, Crtam, and Rankl, which encode cell surface proteins.

4-1BB is a co-stimulatory molecule transiently expressed after TCR engagement. Lag3 (lymphocyte-activation gene 3 or CD223) is a CD4 homologue and functions as an inhibitory receptor. Expression of 4-1BB and Lag3 is regulated following TCR engagement and continues throughout differentiation. In humans, 4-1BB and LAG-3 are expressed on CD8+ TILs from human melanoma tumors (Gros et al., 2014; Baitsch et al., 2012; incorporated by reference in their entireties). In both mice and humans, either molecule alone are expressed on populations of activated T cells. However, co-expression is more limited and is rarely observed in circulating T cells. The function of CD8+ TILs co-expressing these markers is unknown.

Experiments were conducted during development of embodiments herein to investigate the detailed characteristics of CD8+ TILs expressing 4-1BB and LAG-3 using mouse tumor models. It was found that the co-expression of 4-1BB and LAG-3 was sufficient to identify tumor antigen-specific dysfunctional CD8+ TILs enriched in the expression of Egr2 target genes. These CD8+ TILs failed to make IL-2 following in vitro stimulation, yet still produced IFN-g and Treg-recruiting chemokines and lysed target cells ex vivo, indicating they are not completely functionally inert. Combinatorial treatment with anti-LAG-3/anti-4-1BB restored the function of this population and promoted in situ acquisition of KLRG-1hi effector cells. Additional gene expression profiling provided a complete phenotyping of this T cell subset, which revealed expression of a broad panel of both inhibitory receptors and co-stimulatory receptors (e.g., receptors of Table 2 (e.g. Nrn1, Sema7a, CRTAM, etc.)). Inhibitory receptors and co-stimulatory receptors identified in this profiling that are displayed on the surface of T cells include PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A. These approaches have thus enabled the characterization of the population of tumor antigen-specific CD8+ T cells that arise specifically within the tumor microenvironment having altered functional properties. In some embodiments, this population is a target for immunotherapeutic approaches to restore desired functionality and promote tumor regression. In some embodiments, the receptors/markers identified herein (e.g., 4-1BB, LAG-3, receptors/markers of Table 2 (e.g., surface markers/receptors (e.g. Nrn1, Sema7a, CRTAM, etc.), etc.) etc.) are targeted (e.g., via immunotherapeutic approaches) to restore desired immunoresponsiveness, to promote tumor regression, and/or for the treatment of cancer.

Experiments conducted during development of embodiments herein applied knowledge of Egr2 targets to evaluate applicability of these markers toward understanding dysfunctional T cells within tumors in vivo. The data indeed confirm that co-expression of LAG-3 and 4-1BB is sufficient to identify the majority of tumor antigen-specific CD8+ T cells within the tumor microenvironment. Co-expression of these markers was not observed within peripheral lymphoid organs in tumor-bearing mice, indicating that a property unique to the tumor context drives 4-1BB and LAG-3 expression. In addition, acquisition of LAG-3 and 4-1BB expression was not observed within tumors that were undergoing successful rejection, indicating that the acquisition of this phenotype occurs under conditions of incomplete antigen clearance.

In some embodiments, cancer treatment methods described herein comprise administration (or co-administration with one or more additional therapies/therapeutics) of one or more anti-4-1BB and/or anti-LAG-3 agents (e.g., antibodies, antibody fragments, antibody mimetic molecules (e.g., DARPins, affibodies, aptamers, nanobodies, etc.), etc.). In some embodiments, an anti-4-1BB and/or anti-LAG-3 agents is administered to render cancer cells, tumor(s), and/or the tumor microenvironment accessible or susceptible to treatment with additional therapies/therapeutics (e.g., immunotherapeutics). Anti-4-1BB and/or anti-LAG-3 agents that find use in embodiments described herein are not limited by their mechanism of action. Agents may be small molecules, peptide, polypeptides, proteins, nucleic acids (e.g., antisense, RNAi, etc.), antibodies, antibody fragments, etc.

In some embodiments, cancer treatment methods described herein comprise enhancing the activity or expression of a marker/receptor identified herein that negatively correlates with tumor antigen-specific CD8+ T cell dysfunction.

Experiments conducted during development of embodiments herein identified receptors/markers that are differentially expressed in dysfunctional CD8+ TILs (See Table 2). Testing of targets of interest identified in that screen demonstrate that at least neuritin 1 (Nrn1), cytotoxic and regulatory t-cell molecule (CRTAM), and Semaphorin 7A

(Sema7a) are regulators of anti-tumor immunity, with Nm1 and CRTAM blockade correlating with increased tumor area, and Sema7a blockade correlating with decreased tumor area.

In some embodiments, cancer treatment methods described herein comprise administration (or co-administration with one or more additional therapies/therapeutics) of agents (e.g., antibodies, antibody fragments, antibody mimetic molecules (e.g., DARPins, affibodies, aptamers, nanobodies, etc.), etc.) that target one or more receptors/markers of Table 2 (e.g. PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, Nrn1, CRTAM, Sema7a, etc.). In some embodiments, an agent is administered to render cancer cells, tumor(s), and/or the tumor microenvironment accessible or susceptible to treatment with additional therapies/therapeutics (e.g., immunotherapeutics). Agents targeting one or more receptors/markers of Table 2 (e.g. PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, Nrn1, CRTAM, Sema7a, etc.) that find use in embodiments described herein are not limited by their mechanism of action. Agents may be small molecules, peptide, polypeptides, proteins, nucleic acids (e.g., antisense, RNAi, etc.), antibodies, antibody fragments, etc. In some embodiments, an antagonist of Nrn1 is administered. In some embodiments, an antagonist of CRTAM is administered. In some embodiments, an agonist of Sema7a is administered.

In some embodiments, antibodies, antibody fragments, antibody mimetic molecules (e.g., DARPins, affibodies, aptamers, nanobodies, etc.) targeting 4-1BB, LAG-3 and/or one or more receptors/markers of Table 2 (e.g. PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, CRTAM, Sema7a, etc.), or fragments thereof, are provided. Such agents may be naked, deriving their effect by target binding (e.g., neutralizing the target), or may be conjugated to a functional moiety (e.g., drug, toxin, effector moiety, etc.).

In some embodiments, a subject is treated with (i) one or more agents (e.g., antibodies, antibody fragments, antibody mimetic molecules (e.g., DARPins, affibodies, aptamers, nanobodies, etc.), etc.) that target 4-1BB, LAG-3 and/or one or more receptors/markers of Table 2 (e.g. PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, CRTAM, Sema7a, etc.), as well as (ii) one or more additional cancer therapies. Such therapies include chemotherapy, immunotherapy, radiation, surgery, etc. In some embodiments, agents targeting the receptors/markers described herein are co-administered with one or more additional agents for the treatment of cancer.

In some embodiments, exemplary anticancer agents suitable for use in compositions and methods described herein include, but are not limited to: 1) alkaloids, including microtubule inhibitors (e.g., vincristine, vinblastine, and vindesine, etc.), microtubule stabilizers (e.g., paclitaxel (Taxol), and docetaxel, etc.), and chromatin function inhibitors, including topoisomerase inhibitors, such as epipodophyllotoxins (e.g., etoposide (VP-16), and teniposide (VM-26), etc.), and agents that target topoisomerase I (e.g., camptothecin and isirinotecan (CPT-11), etc.); 2) covalent DNA-binding agents (alkylating agents), including nitrogen mustards (e.g., mechlorethamine, chlorambucil, cyclophosphamide, ifosphamide, and busulfan (MYLERAN), etc.), nitrosoureas (e.g., carmustine, lomustine, and semustine, etc.), and other alkylating agents (e.g., dacarbazine, hydroxymethylmelamine, thiotepa, and mitomycin, etc.); 3) noncovalent DNA-binding agents (antitumor antibiotics), including nucleic acid inhibitors (e.g., dactinomycin (actinomycin D), etc.), anthracyclines (e.g., daunorubicin (daunomycin, and cerubidine), doxorubicin (adriamycin), and idarubicin (idamycin), etc.), anthracenediones (e.g., anthracycline analogues, such as mitoxantrone, etc.), bleomycins (BLENOXANE), etc., and plicamycin (mithramycin), etc.; 4) antimetabolites, including antifolates (e.g., methotrexate, FOLEX, and MEXATE, etc.), purine antimetabolites (e.g., 6-mercaptopurine (6-MP, PURINETHOL), 6-thioguanine (6-TG), azathioprine, acyclovir, ganciclovir, chlorodeoxyadenosine, 2-chlorodeoxyadenosine (CdA), and 2'-deoxycoformycin (pentostatin), etc.), pyrimidine antagonists (e.g., fluoropyrimidines (e.g., 5-fluorouracil (ADRUCIL), 5-fluorodeoxyuridine (FdUrd) (floxuridine)) etc.), and cytosine arabinosides (e.g., CYTOSAR (ara-C) and fludarabine, etc.); 5) enzymes, including L-asparaginase, and hydroxyurea, etc.; 6) hormones, including glucocorticoids, antiestrogens (e.g., tamoxifen, etc.), nonsteroidal antiandrogens (e.g., flutamide, etc.), and aromatase inhibitors (e.g., anastrozole (ARIMIDEX), etc.); 7) platinum compounds (e.g., cisplatin and carboplatin, etc.); 8) monoclonal antibodies (e.g., conjugated with anticancer drugs, toxins, and/or radionuclides, etc.; neutralizing antibodies; etc.); 9) biological response modifiers (e.g., interferons (e.g., IFN-.alpha., etc.) and interleukins (e.g., IL-2, etc.), etc.); 10) adoptive immunotherapy; 11) hematopoietic growth factors; 12) agents that induce tumor cell differentiation (e.g., all-trans-retinoic acid, etc.); 13) gene therapy techniques; 14) antisense therapy techniques; 15) tumor vaccines; 16) therapies directed against tumor metastases (e.g., batimastat, etc.); 17) angiogenesis inhibitors; 18) proteosome inhibitors (e.g., VELCADE); 19) inhibitors of acetylation and/or methylation (e.g., HDAC inhibitors); 20) modulators of NF kappa B; 21) inhibitors of cell cycle regulation (e.g., CDK inhibitors); and 22) modulators of p53 protein function.

In some embodiments, agents targeting 4-1BB, LAG-3 and/or one or more receptors/markers of Table 2 (e.g. Nrn1, Sema7a, CRTAM, etc.) are administered to overcome immune invasion of the cancer cells, tumor, tumor microenvironment, etc. In some embodiments, one or more additional cancer immunotherapies are employed (e.g., concurrently or serially) to make use of the immune-responsiveness of the treated cells/tumor. Suitable immunotherapies may include, but are not limited to: cell-based therapies (e.g., dendritic cell or T cell therapy, etc.), monoclonal antibody (mAb) therapy (e.g., naked mAbs, conjugated mAbs), cytokine therapy (e.g., interferons, interleukins, etc.), adjuvant treatment (e.g., polysaccharide-K), etc.

In some embodiments, agents targeting 4-1BB, LAG-3 and/or one or more receptors/markers of Table 2 (e.g. PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, CRTAM, Sema7a, etc.) are co-administered with agents (e.g., small molecules, peptides, antibodies, antibody fragments, etc.) that target one or more cancer cell or tumor) markers or components. In some embodiments, such co-administration renders the cancer cells, tumor, and/or tumor microenvironment susceptible and/or accessible to the treatment with the additional agent.

In some embodiments, agents for use in the methods and compositions described herein target and/or binds a cancer or tumor cell marker or component, selected from the group including but not limited to, epidermal growth factor receptor (EGFR, EGFR1, ErbB-1, HER1). ErbB-2 (HER2/neu),

ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGFR ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family: TRK receptor family; ephrin (EPH) receptor family: AXL receptor family; leukocyte tyrosine kinase (LTK) receptor family; TIE receptor family, angiopoietin 1, 2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF-α), TGF-α receptor; Transforming growth factor-beta (TGF-β), TGF-β receptor; Interleukin β receptor alpha2 chain (IL13Ralpha2), Interleukin-6 (IL-6), 1L-6 receptor, interleukin-4, IL-4 receptor, Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/1L-10 family) receptors, tumor necrosis factor (TNF) family, TNF-α, tumor necrosis factor (TNF) receptor superfamily (TNTRSF), death receptor family, TRAIL-receptor; cancer-testis (CT) antigens, lineage-specific antigens, differentiation antigens, alpha-actinin-4, ARTC1, breakpoint cluster region-Abelson (Bcr-abl) fusion products, B-RAF, caspase-5 (CASP-5), caspase-8 (CASP-8), beta-catenin (CTNNB1), cell division cycle 27 (CDC27), cyclin-dependent kinase 4 (CDK4), CDKN2A, COA-1, dek-can fusion protein, EFTUD-2, Elongation factor 2 (ELF2), Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein, fibronectin (FN), GPNMB, low density lipid receptor/GDP-L fucose: beta-Dgalactose 2-alpha-Lfucosyltraosferase (LDLR/FUT) fusion protein, HLA-A2, MLA-A11, heat shock protein 70-2 mutated (HSP70-2M), KIAA0205, MART2, melanoma ubiquitous mutated 1, 2, 3 (MUM-1, 2, 3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class 1, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT12, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2, 3, 4, 5, GAGE-1, 2, 3, 4, 5, 6, 7, 8, GnT-V (aberrant N-acetyl glucosaminyl transferase V, MGAT5), HERV-K MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-A1 (MAGE-1). MAGE-A2, MAGE-A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10. MAGE-All, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5. MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUC1), MART-1/Melan-A (MLANA), gp100, gp100/Pme117 (S1LV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-1/LAGE-2, SAGE, Sp17. SSX-1, 2, 3, 4, TRP2-1NT2, carcino-embryonic antigen (CEA), Kallikrein 4, mammaglobin-A, OA1 prostate specific antigen (PSA), prostate specific membrane antigen, TRP-1/, 75. TRP-2 adipophilin, interferon inducible protein absent in melanoma 2 (AIM-2). BING-4, CPSF, cyclin D1, epithelial cell adhesion molecule (Ep-CAM), EpbA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUCI, p53 (TP53), PBF, PRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRCS), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WT1), SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIP1, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA66I, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHLI7, NXF2 TDRD1, TEX 15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, CD4, CD25, CD3, cancer antigen 72-4 (CA 72-4), cancer antigen 15-3 (CA 15-3), cancer antigen 27-29 (CA 27-29), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), beta-human chorionic gonadotropin, 1-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enolase, heat shock protein gp96. GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryogenic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), etc.

Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as trastuzumab (anti-HER2/neu antibody); Pertuzumab (anti-HER2 mAb); cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR); panitumumab (anti-EGFR antibody); nimotuzumab (anti-EGFR antibody); Zalutumumab (anti-EGFR mAb); Necitumumab (anti-EGFR mAb); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); Rituximab (chimeric murine/human anti-CD20 mAb); Obinutuzumab (anti-CD20 mAb); Ofatumumab (anti-CD20 mAb); Tositumumab-1131 (anti-CD20 mAb); Ibritumomab tiuxetan (anti-CD20 mAb); Bevacizumab (anti-VEGF mAb); Ramucirumab (anti-VEGFR2 mAb); Ranibizumab (anti-VEGF mAb); Aflibercept (extracellular domains of VEGFR1 and VEGFR2 fused to IgG1 Fc); AMG386 (angiopoietin-1 and -2 binding peptide fused to IgG1 Fc); Dalotuzumab (anti-IGF-1R mAb); Gemtuzumab ozogamicin (anti-CD33 mAb); Alemtuzumab (anti-Campath-1/CD52 mAb); Brentuximab vedotin (anti-CD30 mAb): Catumaxomab (bispecific mAb that targets epithelial cell adhesion molecule and CD3); Naptumomab (anti-5T4 mAb); Girentuximab (anti-Carbonic anhydrase ix); or Farletuzumab (anti-folate receptor). Other examples include antibodies such as Panorex™ (17-1A) (murine monoclonal antibody); Panorex (@(17-1A)) (chimeric murine monoclonal antibody); BEC2 (ami-idiotypic mAb, mimics the GD epitope) (with BCG); Oncolym (Lym-1 monoclonal antibody); SMART M195 Ab, humanized 13' 1 LYM-1 (Oncolym). Ovarex (B43.13, anti-idiotypic mouse mAb); 3622W94 mAb that binds to EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas; Zenapax (SMART Anti-Tac (IL-2 receptor); SMART M195 Ab, humanized Ab, humanized); NovoMAb-G2 (pancarcinoma specific Ab); TNT (chimeric mAb to histone antigens); TNT (chimeric mAb to histone antigens); Gliomab-H (Monoclonals—Humanized Abs); GNI-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized IL.L.2 antibody); and MDX-260 bispecific, targets GD-2, ANA Ab, SMART IDIO Ab, SMART ABL 364 Ab, or ImmuRAIT-CEA.

In some embodiments, an agent that finds use in embodiments herein specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-κB (RANK); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor

necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R). In some embodiments, the agent is an agonist that increases the function of the targeted molecule. In other embodiments, the agent is an antagonist that inhibits the function of the targeted molecule.

In some embodiments, an agent that finds use in embodiments herein binds a specific cytokine, cytokine receptor, co-stimulatory molecule, co-inhibitory molecule, or immunomodulatory receptor that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: tumor necrosis factor (TNF) superfamily; tumor necrosis factor-α (TNF-α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; Programmed death-1 (PD-1); PD-1 ligand I (PD-L1: B7-H1); or PD-1 ligand 2 (PD-L2; B7-DC); B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR: GITRL/AITRL; BTLA; CD70; CD27; LIGHT; HVEM: Toll-like receptor (TLR) (TLR 1, 2, 3, 4, 5, 6, 7, 8, 9, 10). In some embodiments, the agent is an agonist that increases the function of the targeted molecule. In other embodiments, the agent is an antagonist that inhibits the function of the targeted molecule.

In some embodiments, agents (e.g., immunotherapeutics) targeting 4-1BB, LAG-3 and/or one or more receptors/markers of Table 2 (e.g. PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, CRTAM, Sema7a, etc.) are co-administered (e.g., serially or sequentially) with one or more adjuvants. Suitable adjuvants include, but are not limited to, one or more of: oil emulsions (e.g., Freund's adjuvant); saponin formulations; virosomes and viral-like particles; bacterial and microbial derivatives; immunostimulatory oligonucleotides; ADP-ribosylating toxins and detoxified derivatives; alum; BCG; mineral-containing compositions (e.g., mineral salts, such as aluminium salts and calcium salts, hydroxides, phosphates, sulfates, etc.); bioadhesives and/or mucoadhesives; microparticles; liposomes; polyoxyethylene ether and polyoxyethylene ester formulations; polyphosphazene; muramyl peptides; imidazoquinolone compounds; and surface active substances (e.g. lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol).

Adjuvants may also include immunomodulators such as cytokines, interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g., interferon-.gamma.), macrophage colony stimulating factor, and tumor necrosis factor. In addition to variant B7-DC polypeptides, other co-stimulatory molecules, including other polypeptides of the B7 family, may be administered. Proteinaceous adjuvants may be provided as the full-length polypeptide or an active fragment thereof, or in the form of DNA, such as plasmid DNA.

Pharmaceutical and immunotherapeutic compositions described herein may be delivered by any suitable route of administration (e.g., oral delivery, parenteral delivery, mucous membrane delivery, pulmonary delivery, intravenous delivery, etc.). Appropriate formulations for such delivery routes are understood in the field.

Non-limiting examples of cancers that may be treated with the compositions and methods described herein include, but are not limited to: melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g. clear cell carcinoma), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), pancreatic cancer (e.g., adenocarcinoma), breast cancer, colon cancer, lung cancer (e.g. non-small cell lung cancer), esophageal cancer, squamous cell carcinoma of the head and neck, liver cancer, ovarian cancer, cervical cancer, thyroid cancer, glioblastoma, glioma, leukemia, lymphoma, and other neoplastic malignancies. In some embodiments, the cancer is a solid tumor cancer.

Some embodiments described herein are particularly useful for the treatment of tumors that do not otherwise respond to immunotherapeutic approaches. In some embodiments, provided herein is the treatment of cancers that are non-responsive (or have a reduced response) to T cells or antigen presenting cells (e.g., dendritic cells (e.g., $CD103^+$DCs, etc.), etc.). In some embodiments, provided herein is the treatment of cancers that are non-responsive to treatments, despite T cell infiltration. In some embodiments, compositions and methods described herein find use in the treatment of cancers in which T cells are not appropriately primed against tumor-associated antigens. In some embodiments, compositions and methods described herein find use in the treatment of cancers comprising tumors or cells that are defective in recruitment of dendritic cells (e.g., $CD103^+$ DCs, etc.). In some embodiments, compositions and methods described herein find use in the treatment of cancers comprising tumors or cells that are defective in production of the chemokine CCL4.

In some embodiments, the therapeutic compositions and methods herein find use with those described in, for example WO 2016/141312; incorporated by reference in its entirety.

In some embodiments, methods are provided for testing sample (e.g., cell, tissue, population of cells, tumor, blood, urine, saliva, etc.) from a subject for one or more biomarkers (e.g., biomarkers of dysfunctional tumor antigen-specific $CD8^+$ T cells). Such biomarkers may comprise nucleic acids, small molecules, proteins, peptides, etc., and may be detected using any suitable assay of technique. In some embodiments, provided herein are DNA-, RNA-, small molecule, and/or protein-based diagnostic methods that either directly or indirectly detect the biomarkers of the evasion of immune response or immunotherapy by cancer cells or tumors. The present invention also provides compositions, reagents, and kits for such diagnostic purposes.

In some embodiments, biomarkers are detected at the nucleic acid (e.g., RNA) level. For example, the presence or amount of biomarker nucleic acid (e.g., mRNA) in a sample is determined (e.g., to determine the presence or level of biomarker expression). Biomarker nucleic acid (e.g., RNA, amplified cDNA, etc.) may be detected/quantified using a variety of nucleic acid techniques known to those of ordinary skill in the art, including but not limited to nucleic acid sequencing, nucleic acid hybridization, nucleic acid amplification (e.g., by PCR, RT-PCR, qPCR, etc.), microarray, Southern and Northern blotting, sequencing, etc. Non-amplified or amplified nucleic acids can be detected by any conventional means. For example, in some embodiments, nucleic acids are detected by hybridization with a detectably labeled probe and measurement of the resulting hybrids. Nucleic acid detection reagents may be labeled (e.g., fluorescently) or unlabeled, and may by free in solution or immobilized (e.g., on a bead, well, surface, chip, etc.).

In some embodiments, biomarkers are detected at the protein level. For example, the presence or amount of biomarker protein in a sample is determined (e.g., to determine the presence or level of biomarker expression or localization). In some embodiments, reagents are provided for the detection and/or quantification of biomarker proteins. Suitable reagents include primary antibodies (e.g., that bind

to the biomarkers), secondary antibodies (e.g., that bind primary antibodies), antibody fragments, aptamers, etc. Protein detection reagents may be labeled (e.g., fluorescently) or unlabeled, and may by free in solution or immobilized (e.g., on a bead, well, surface, chip, etc.).

In some embodiments, biomarker capture reagents are provided to localize, concentrate, aggregate, etc. a biomarker. For example, in some embodiments a biomarker capture reagent that interacts with the biomarker is linked to a solid support (e.g., a bead, surface, resin, column, and the like) that allows manipulation by the user on a macroscopic scale. Often, the solid support allows the use of a mechanical means to isolate and purify the biomarker from a heterogeneous solution. For example, when linked to a bead, separation is achieved by removing the bead from the heterogeneous solution, e.g., by physical movement. In embodiments in which the bead is magnetic or paramagnetic, a magnetic field is used to achieve physical separation of the capture reagent (and thus the target) from the heterogeneous solution. Magnetic beads used to isolate targets are described in the art, e.g., as described in European Patent Application No. 87309308, incorporated herein in its entirety for all purposes.

Compositions for use in the diagnostic methods or testing steps described herein include, but are not limited to, probes, amplification oligonucleotides, and antibodies. Any of the detection and/or diagnostic reagents used in embodiments described herein may be provided alone or in combination with other compositions in the form of a kit. Kits may include any and all components necessary or sufficient for assays including, but not limited to, the detection reagents, buffers, control reagents (e.g., tissue samples, positive and negative control sample, etc.), solid supports, labels, written and/or pictorial instructions and product information, inhibitors, labeling and/or detection reagents, package environmental controls (e.g., ice, desiccants, etc.), and the like. In some embodiments, the kits provide a sub-set of the required components, wherein it is expected that the user will supply the remaining components. In some embodiments, the kits comprise two or more separate containers wherein each container houses a subset of the components to be delivered.

In some embodiments, a computer-based analysis program is used to translate the raw data generated by the detection assay (e.g., the presence, absence, or amount of expression a biomarker) into data of predictive value for a clinician. In some embodiments, computer analysis combines various data into a single score or value that is predictive and/or diagnostic. The clinician can access the predictive data using any suitable means. Thus, in some preferred embodiments, the present invention provides the further benefit that the clinician, who is not likely to be trained in genetics or molecular biology, need not understand the raw data. The data is presented directly to the clinician in its most useful form. The clinician is then able to immediately utilize the information in order to optimize the care of the subject. Contemplated herein are any methods capable of receiving, processing, and transmitting the information to and from laboratories conducting the assays, information providers, medical personal, and subjects. For example, in some embodiments of the present invention, a sample (e.g., a biopsy, cell, or blood sample) is obtained from a subject and submitted to a profiling service (e.g., clinical lab at a medical facility, third-party testing service, genomic profiling business, etc. to generate raw data. Where the sample comprises a tissue or other biological sample, the subject may visit a medical center to have the sample obtained and sent to the profiling center, or subjects may collect the sample themselves and directly send it to a profiling center. In some embodiments, a report is generated (e.g., by a clinician, by a testing center, by a computer or other automated analysis system, etc.). A report may contain test results, diagnoses, and/or treatment recommendations.

EXPERIMENTAL

Materials and Methods

Mice and Tumor Inoculation

Female C57BL/6 mice ranging from 6 to 8 weeks were purchased from Taconic Farms. CD45.1 and Rag2$^{-/-}$ mice on the C57BL/6 background were obtained from Taconic Farms and bred at the University of Chicago. 2C/Rag2$^{-/-}$ and P14/Rag2$^{-/-}$ mice have been previously described (Brown et al., 2006; incorporated by reference in its entirety). pLCK-CreERT2×ROSA-YFP mice were generated and have been described (Evaristo et al., 2016; incorporated by reference in its entirety). B16.SIY.dsRed (Kline et al., 2012; incorporated by reference in its entirety), C1498.SIY.GFP (Zhang et al., 2009; incorporated by reference in its entirety), and MC57.SIY.GFP (Spiotto et al., 2002; incorporated by reference in its entirety) tumor cells were engineered to express either dsRed or GFP in frame with the H2-K^{b}-restricted model antigen SIYRYYGL. The 1969.SIY.GFP cell line was engineered by retroviral transduction of the 1969 cell line (Diamond et al., 2011; incorporated by reference in its entirety) using the pLEGFP plasmid expressing cDNA for SIYRYYGL (Spiotto et al., 2002; incorporated by reference in its entirety). For experiments, mice 6 to 9 weeks of age and received 2×10^{6} tumor cells subcutaneously on either the left flank or both the left and right flank. All mice were maintained according to the National Institute of Health Animal Care guidelines and studied under IACUC-approved protocols.

To generate the targeting construct for the Egr2^{EGFP} knock-in reporter mice, a 12.6 kb mouse genomic DNA fragment including the egr2 gene was excised with SacII and cloned into a pEasy-Flox vector adjacent to the thymidine kinase (TK) selection marker. A cassette containing IRES2-eGFP and a LoxP-flanked neomycin selection marker was inserted into an Nhe1 site between the translation stop codon (TGA) and the polyadenylation signal of the egr2 gene. ES cell clones from 129 mice were electroporated and selected for Neomycin resistance. ES cell clones were verified for homologous insertion in the endogenous locus by PCR and southern blot with 5' and 3' probes. Mice were backcrossed to C57BL/6 for over 8 generations.

TIL Isolation

Tumors were harvested from mice at the indicated time points. Tumors were dissociated through a 50 μm filter and washed with PBS. TILs were further enriched by layering Ficoll-Hypaque beneath the cell suspension followed by centrifugation without breaks for 30 min at 400×g. The buffy-layer was isolated and washed twice with PBS before staining. For isolating specific cell populations by FACS, tumors were pooled when indicated and the cell layer was re-purified by Ficoll-Hypaque centrifugation twice. For day 28 tumors, after Ficoll-Hypaque separation, T cells were further purified by negative bead selection according to manufacturer's instructions (MAGNISORT, eBiosciences). Cells were then washed with PBS, stained at 4° C. for 15 minutes before resuspending in complete DMEM (cDMEM: 10% FBS, 100U/mL Penicillin-Streptomycin, 1% MEM Non-Essential Amino Acids, 50 μM (3-ME, 0.01M MOPS), and were sorted into either RLT lysis buffer (QIAGEN) or

cDMEM depending on the experimental assay. Cells sorted into RLT buffer were put directly on dry ice as soon as the sort was finished.

Flow Cytometry and Antibodies

Cell suspensions were washed twice in PBS before staining an FACS buffer (10% FBS, 2 mM EDTA, 0.001% NaN_3). Cells were stained for 30 min on ice and fixed in 1% PFA. Antibodies against the following molecules were used: CD3 (17A2, AX700), 2B4 (2B4, FITC), CD127 (A7R34, PE), OX-40 (OX-86, PE), 4-1BB (17B5, Biotin, APC), CD160 (7H1, PE-Cy7), LAG-3 (C9B7W, PerCPeFluor710), PD-1 (RMP1-30, PE-Cy7), NRP1 (3E12, BV421), GITR (DTA-1, FITC), ICOS (7E.17G9, BV421), KLRG-1 (2F1, eF450, BV605), TIGIT (1G9, APC), TIM-3 (RMT3-23, PE), CD4 (RM4-5, BV605), CD45.1 (A20, FITC), CD45.2 (104, PE), CD8a (53-6.7, BV711). Fixable Viability Dye 506 (eBioscience) was used for live/dead discrimination. Staining of SIY-specific T cells was performed utilizing the SIYRYYGL-Pentamer (PE) (Proimmune); a SIINFEKL-pentamer (PE) was used as a non-specific control. All flow cytometric analysis was conducted on an LSRFortessa (BD) and analyzed using FlowJo software (Tree Star).

Quantitative Real-Time PCR

Total RNA was extracted from sorted cell populations using the RNEasy Micro Kit (QIAGEN) following the manufacturer's protocol. cDNA was synthesized using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to manufacturer's instructions. Transcript levels were determined using primer-probe sets (Tables 1a and 1b) developed through the online ProbeFinder Software and the Universal Probe Library (Roche) with the exception of IL-2 (Mm00434256_m1) and 18S (Hs99999901_s1). To minimize batch effect, when possible, all samples probed for a gene were run on the same 96-well qRT-PCR plate. All primer-probe sets either contained a primer spanning an exon-exon boundary or primers spanning an intron. Expression levels of transcripts were normalized to 18S expression.

TABLE 1a

Primer Sequences

#	Wilson IMGT	IMGT	Sequence	SEQ ID NO:
0	Cβ1.1	TRBC1	CTCAAACAAGGAGACCTTGGGTGG	1
1	Vβ1	TRVB5	CAGACAGCTCCAAGCTACTTTTAC	2

TABLE 1a -continued

Primer Sequences

#	Wilson IMGT	IMGT	Sequence	SEQ ID NO:
2	Vβ2	TRVB1	ATGAGCCAGGGCAGAACCTTGTAC	3
3	Vβ3	TRVB26	GAAATTCAGTCCTCTGAGGCAGGA	4
4	Vβ4	TRVB2	CTAAAGCCTGATGACTCGGCCACA	5
5	Vβ5.1	TRVB12-2	CTTTGGAGCTAGAGGACTCTGCCG	6
6	Vβ5.2	TRVB12-1	CCTTGGAACTGGAGGACTCTGCTA	7
7	Vβ6	TRVB19	GCCCAGAAGAACGAGATGGCCGTT	8
8	Vβ7	TRVB29	GGATTCTGCTAAAACAAACCAGACATCTGT	9
9	Vβ8.1	TRVB13-3	GCTTCCCTTTCTCAGACAGCTGTA	10
10	Vβ8.2	TRVB13-2	GCTACCCCCTCTCAGACATCAGTG	11
11	Vβ8.3	TRVB13-3	GGCTTCTCCCTCTCAGACATCTT	12
12	Vβ9	TRVB17	CTCTCTCTACATTGGCTCTGCAGG	13
13	Vβ10	TRVB4	CTTCGAATCAAGTCTGTAGAGCCG	14
14	Vβ11	TRVB16	TGAAGATCCAGAGCAGCGGGCCCC	15
15	Vβ12	TRVB15	CCACTCTGAAGATTCAACCTACAGAACCC	16
16	Vβ13	TRVB14	CAAGATCCAGTCTGCAAAGCAGGG	17
17	Vβ14	TRVB31	GCACGGAGAAGCTGCTTCTCAGCC	18
18	Vβ15	TRVB20	GCATATCTTGAAGACAGAGGC	19
19	Vβ16	TRVB3	CTCTGAAAATCCAACCCACAGCACTGG	20
20	Vβ17	TRVB24	TCTGAAGAAGACGACTCAGCACTG	21
21	Vβ18	TRVB30	GCAAGGCCTGGAGACAGCAGTATC	22

TABLE 1b

Primer/Probe

Gene	SEQ ID NO:	Primer1	Primer2	SEQ ID NO:	Roche Probe #
Lag3	23	tgctttgggaagctccagt	gctgcagggaagatggac	42	79
Tnfrsf9	24	ccggtcttaagcacagacct	gaacggtactggcgtctgtc	43	108
Egr2	25	ctacccggtggaagacctc	aatgttgatcatgccatctcc	44	60
Sema7a	26	tcaatcggctgcaagatgt	cgcagacagctgagtagttcc	45	15
Crtam	27	agatccaacaacgaggagaca	tcatgcaacgcttagactgg	46	71
Ccl1	28	tcaccatgaaacccactgc	agcagcagctattggagacc	47	71

TABLE 1b -continued

Primer/Probe						
Gene	SEQ ID NO:	Primer1	Primer2	SEQ ID NO:	Roche Probe #
Ngn	29	caccctagcctaacctcaacc	tgaaaacctcctcccctctt	48	45
Arl3	30	ctggcagatccagtcctgtt	acccagttcatgccatcct	49	100
Exph5	31	atgagggaggagagcggtat	cagcttgttgtccaaatcgtc	50	67
Fhl2	32	agaaaaccatcatgccaggt	acaggtgaagcaggtctcgt	51	74
Nrn1	33	atcctcgcggtgcaaata	gcccttaaagactgcatcaca	52	108
Ptgfrn	34	ccggggagatctcatcaaa	tcgaaggccatgtcatctg	53	12
Rankl	35	tgaagacacactacctgac tcctg	cccacaatgtgttgcagttc	54	88
Tnfa	36	gctgctcactgtgaaggaagt	tggggaatgcattttaccat	55	2
Egr3	37	caatctgtaccccgaggaga	ccgatgtccatcacattctct	56	74
Tnfa	38	ctgtagcccacgtcgtagc	ttgagatccatgccgttg	57	25
Gzmb	39	gctgctcactgtgaaggaagt	tggggaatgcattttaccat	58	2
Ccl1	40	tcaccatgaaacccactgc	agcagcagctattggagacc	59	71
Ccl22	41	tcttgctgtggcaattcaga	gcagagggtgacggatgtag	60	74

In Vivo Proliferation Assay

In vivo proliferation was measured by a BrdU pulse 24 hours prior to flow cytometric analysis. Each mouse received 0.8 mg BrdU injected i.p. (intraperitoneal) on day 12 after tumor inoculation. TILs were isolated and surface stain was performed as described above. Following surface staining, cells were fixed and permeabilized using the Foxp3 staining kit (BD), according to manufacturer's protocol, and incubated with 100 μl PBS/DNase solution (300 μg/ml) for 30 minutes at 37° C. Cells were washed and incubated for 30 minutes at room temperature with anti-BrdU (FITC, Bu20a) and then washed with and resuspended in PBS.

In Vitro Stimulation Assays

Tissue culture-treated 96-well round bottom plates were coated with anti-CD3E (1 μg/ml; 2C11) in DPBS overnight at 4° C. or for 2 hours at 37° C. Cells were sorted into cold cDMEM media and put on ice as soon as the sort was finished. Cells were then pelleted, resuspended in 50 μl cDMEM and incubated with soluble anti-CD28 (2 μg/ml; PV-1) for 10-12 hours for a final volume of 100 μl. After stimulation supernatants were removed for ELISA or bead-based immunoassay (LegendPlex), and cells were washed once with DPBS and resuspended in 15 μl of RNAlater Stabilization Solution (QIAGEN) or 300 μl of RLT buffer. Cells were stored at −80° C. until RNA isolation was performed.

Protein Quantification

Measurement of protein concentration was determined either by a standard ELISA or bead-based immunoassay (LEGENDplex, BioLegend). ELISAs were performed according to manufacturer's protocol (Ready-SET-Go ELISA; eBioscience) on supernatants from in vitro stimulations. Absorbance values were obtained at 450 nm using an Emax microplate reader (Molecular Devices) and IL-2 concentration was determined by standard curve. Protein concentration values were normalized to the number of sorted cells plated. LEGENDplex assays were performed according to manufacturer's protocols. IL-2 concentration (FIG. **4**B) was confirmed by both methods in separate experiments with no significant difference in IL-2 concentration between the two methods.

Spectratype Analysis and Sequencing Mice were injected with 2×10^6 B16.SIY.dsRed tumor cells. 14 days later, tumors were harvested and specific $CD8^+$ TIL subpopulations were sorted into RLT buffer (QIAGEN) and immediately frozen. cDNA was synthesized from sorted cell populations and CDR3 regions were amplified by PCR with 21 different Vβ-5' primers paired with a FAM-Cβ1.1 primer (Table 1). Three Vβ PCR reactions did not reach significant amplification for analysis and were removed from the analysis. For sequencing, Cβ-Vβ PCR products were purified using the QIAquick PCR purification kit (QIAGEN) and sequenced at the University of Chicago Genomics Core Facility. Cβ-Vβ PCR products were analyzed by capillary electrophoresis at the University of Chicago Genomics core and CDR3 peaks were aligned using the Liz500 ladder. Spectratype graphs were displayed using the GeneiousR9 software (Kearse et al., 2012). To generate the frequency profile for each Vβ spectratype, the area under each peak was measured using peak studio. The Hamming Distance (Currier and Robinson, 2001; incorporated by reference in its entirety) was calculated between each Vβ spectratype from each $CD8^+$ spleen and TIL population within a given mouse. To determine significance between the HD from each comparison the HDs for each Vβ from mice were averaged and a One-Way ANOVA with Dunn's correction for multiple comparisons was performed.

TCR Transgenic T Cell Transfer Experiments

Cell suspensions were generated from spleens and lymph nodes from congenic 2C/$Rag2^{-/-}$/CD45.1/2 and/or P14/$Rag2^{-/-}$/CD45.2 mice and T cells were purified by $CD8^+$ negative selection (Miltenyi Biotechnologies) over magnetic columns according to the manufacturer's protocol. TCR Transgenic (Tg) T cells were washed with PBS, resuspended

at a concentration of 10×10^6/ml and 1×10^6 TCR Tg cells were adoptively transferred into CD45.1 tumor bearing mice by tail vein transfer in a volume of 0.1 mL. After indicated times, 2C T cells and corresponding host $CD8^+$ T cells were sorted and stimulated as described above.

In Vitro Cytotoxicity Assay

Per individual experiment, 10 C57BL/6 mice were injected s.c. (subcutaneous) with 2×10^6B16.SIY cells on both left and right flanks. On day 14, all 20 tumors were pooled and dissociated using the Tumor Dissociation Kit (Miltenyi Biotec) following the manufacturer's protocol. Tumor cell suspensions were washed 3-5 times with PBS and TILs were enriched for by Ficoll-Hypaque gradient centrifugation. TILs were stained, sorted and put directly on ice. TILs were titrated and added directly to a 96-well plate containing 50,000 P815 mastocytoma cells and 1 μg/mL anti-CD3. For a positive control, OT-I cells were isolated from OT-I/$Rag2^{-/-}$ mice and stimulated with plate-bound anti-CD3 (0.25 μg/mL), anti-CD28 (2 μg/mL) and 100 U/mL IL-2 for 2-3 days. For a negative control, P815 cells were cultured alone or cultured with naïve $CD8^+$ T cells isolated from lymph nodes. After 12 hours of incubation, cells were stained for Thy1, CD45, CD8α, Fixable Viability Dye 450 (eBioscience) and/or propidium Iodide.

Gene Expression Analysis

Total RNA for the $CD8^+$ TIL subpopulations was isolated following the manufacturer's protocol (RNEasy Micro Kit: QIAGEN) from sorted cells pooled from 10 mice. Samples were analyzed by the University of Chicago Genomics Facility using Illumina MouseRef8 microarray chips. Two experimental replicates were performed, and the results were $\log_2$ transformed and averaged. Probe sets that revealed a 1.5-fold difference abs($\log_2$(ratio)>1.5)) relative to $CD8^+$4-1BB^-LAG-3^-PD-1^- cells were identified and used for subsequent analysis. The microarray data are available in the Gene Expression Omnibus database under accession number GSE79919. For cross-study comparisons, log 2-fold change values were extracted using the GEO2R online software from the hypofunctional $CD8^+$ TIL data set, GSE79858 ((GSM2107353, GSM2107353 and GSM2107355) versus (GSM2107350, GSM2107351, GSM210732)) and the $CD8^+$ T cell exhausted data set, GSE41870 ((GSM1026819, GSM1026820, GSM1026821) versus (GSM1026786, GSM1026787, GSM1026788, GSM1026789)). Upregulated genes showing a 2-fold difference were used for analysis. Multiple genes names with from the GEO2R extracted data were identified and matched to gene names from the Illumina data set. The rank-rank hypergeometric overlap (RRHO) analysis (Plaisier et al., 2010; incorporated by reference in its entirety) was conducted (Rosenblatt and Stein, 2014; incorporated by reference in its entirety).

Gene Ontology Enrichment Analysis

In a pair-wise fashion, shared upregulated genes were used as the input for the ClueGO software with the Cytoscape application (Shannon et al., 2003; incorporated by reference in its entirety). Both the Biological Process and Immune System Process Gene Ontology Annotations were used for analysis. Only pathways with a Bonferroni step down correction p-value>0.01 were considered when generating pathway nodes. Non-redundant pathways with the greatest number of genes found within each node were used as examples in FIG. **6**A.

Antibody and FTY720 Treatments

Mice were treated i.p. with 100 μg/mouse of anti-4-1BB (Bio-X-Cell; LOB12.3) antibody and/or 100 μg/mouse anti-LAG-3 (Bio-X-Cell; C9B7W). For tumor outgrowth experiments, mice were treated on day 7, 10, 13 and 16 after tumor inoculation. For ex vivo functional experiments mice were treated on day 7, 10 and 13 and cells were sorted on day 14. For experiments blocking lymph node egress, 25 μg of FTY720 was given by gavage one day prior to first antibody treatment (day 6) and continued every day until endpoint on day 14.

Results

4-1BB and LAG-3 Identify a Major Population of $CD8^+$ TILs

To determine whether 4-1BB and LAG-3 could identify dysfunctional $CD8^+$ TILs, the expression pattern of LAG-3 and 4-1BB was examined using the well-characterized B16.SIY model of melanoma. On day 7 following tumor inoculation, the 4-1BB^+LAG-3^+ population comprised 15.8% of all $CD8^+$ TILs. The frequency of this population significantly increased to 44% by day 21. The frequency of 4-1BB^-LAG-3^+ (4^-L^+) population also increased 1.9-fold from day 7 to day 14 to comprise 25% of the $CD8^+$ TIL compartment. In contrast, the frequency of the 4-1BB^-LAG-3^- (4^-L^-) population decreased by 2.7-fold by day 21. There was no significant increase in the proportion or number of 4-1BB^+LAG-3^-CD8^+ TILs within the time frame of the experiment (FIGS. **1**A and B). Similar patterns were seen when analyzing absolute numbers of cell subsets (FIGS. **1**C and D). Acquisition of these phenotypes was specific for the tumor microenvironment, as they were not observed in the spleen or tumor-draining lymph node (TdLN) (FIG. **1**A). These data indicate that the tumor microenvironment preferentially supports the induced co-expression of LAG-3 and 4-1BB.

The selective increase in cell numbers and proportional shift towards the 4-1BB^- LAG-3^+ and 4-1BB^+LAG-3^+ populations during tumor progression indicated that expansion of these populations was occurring within the tumor microenvironment. $CD8^+$ TILs were stained for Ki67 at day 14 after tumor inoculation and analyzed by flow cytometry. 81% of 4-1BB^-LAG-3^+ cells and 85% of 4-1BB^+LAG-3^+ cells were $Ki67^+$ compared to only 32% of the 4-1BB^-LAG-3^- TILs (FIG. **1**E). Mice were pulsed with BrdU on day 12, and 24 hours later the $CD8^+$ TIL subpopulations were analyzed for BrdU incorporation. Indeed, the 4-1BB^-LAG-3^+ and 4-1BB^+LAG-3^+ populations incorporated more BrdU compared to the 4-1BB^-LAG-3^- population (FIG. **1**F). These data indicate that once $CD8^+$ T cells arrive at the tumor site, a fraction of TILs expands within the tumor, and that these expanding TILs are identified by increased expression of 4-1BB and LAG-3.

To determine if upregulation of LAG-3 and 4-1BB was simply a product of the B16.SIY tumor model or if it is a more general feature of $CD8^+$ T cells within tumors, T cells from three additional progressively growing tumor models, C1498.SIY, MC38.SIY, EL4.SIY and B16F10 parental were analyzed. TILs were analyzed for expression of 4-1BB and LAG-3 at day 14. A pattern of expression was found that is similar to that seen in $CD8^+$ TILs isolated from B16.SIY tumors (FIGS. **1**G and I). The results from the B16F10 parental tumor confirm that presence of SIY is not required to see co-expression of 4-1BB and LAG-3. In order to determine whether the 4-1BB^+LAG-3^+ TIL subset was generated only in progressing tumors or also in tumors that were rejected, T cell phenotypes in the 1969.SIY and MC57.SIY fibrosarcoma tumor models were analyzed, which are more immunogenic and undergo spontaneous rejection. Distinctly fewer 4-1BB^+LAG-3^+ cells were found among the $CD8^+$ TIL compartment in the 1969.SIY and MC57.SIY tumors (Figure H and I). Over time, co-expression of 4-1BB and

LAG-3 was maintained in B16.SIY tumors but not MC57.SIY tumors (FIG. 1J). These data indicate that the acquisition of the LAG-3$^+$4-1BB$^+$ TIL phenotype preferentially occurs within the tumor microenvironment and only upon conditions of tumor progression rather than regression.

CD8$^+$ 4-1BB$^+$LAG-3$^+$ TILs Express Egr2 and Multiple Egr2 Gene Targets

Experiments conducted during development of embodiments herein to determine whether Egr2 expression itself was also characteristic of T cells within the CD8$^+$ TIL compartment; an Egr2-IRES-GFP (Egr2^{GFP}) knock-in reporter mouse was utilized. Approximately 14% of all CD8$^+$ TILs were GFP$^+$ on both day 7 and day 14 (FIG. 2A). To confirm that Egr2 is faithfully reported, CD8$^+$ TILs expressing high and low levels of EGFP were sorted and screened for Egr2 and several Egr2 targets by qRT-PCR. The Egr2-GFP^{hi} population expressed greater levels of Egr2 and many Egr2-target genes previously defined using in vitro anergy models. These include Tnfrsf9, Lag3, Ngn, Sema7a, Crtam, Ccl1 and Nrn1 (FIG. 2B). Expression of 4-1BB and LAG-3 in the Egr2-GFP^{hi} CD8$^+$ TILs was confirmed by flow cytometry. The majority of Egr2-GFP^{hi} cells expressed LAG-3 and/or 4-1BB. The Egr2GFP^{lo} cells also showed expression of 4-1BB and LAG-3 on a subpopulation at day 14 (FIG. 2C). This result indicates either that CD8$^+$ TILs expressing Egr2 encompass only a subset of the TILs expressing LAG-3 and/or 4-1BB, or that Egr2 is transiently expressed and is subsequently downregulated after the induction of LAG-3 and 4-1BB.

Using Egr2 target genes from in vitro anergic CD4$^+$ T cell clones (Zheng et al., 2013; incorporated by reference in its entirety), the Egr2-driven transcriptional program was examined in sorted 4-1BB$^-$LAG-3$^-$ and 4-1BB$^+$LAG-3$^+$ cells by qRT-PCR. Of the 43 Egr2 target genes examined, 10 showed detectably increased expression in 4-1BB$^+$LAG-3$^+$ population, while expression of a similar subset of genes was increased in the 4-1BB$^-$LAG-3$^+$ population (FIG. 2D). Collectively, these data demonstrate that Egr2 is expressed in a subpopulation of CD8$^+$ TILs expressing LAG-3 and/or 4-1BB, and that a subset of known Egr2 targets was detected in these larger T cell populations as a whole.

It was next examined whether Egr2 was required for expression of LAG-3 and 4-1BB among CD8$^+$ TIL in vivo. To this end Egr2$^{flox/flox}$×pLCK-CreERT2×ROSA-YFP mice were utilized, in which oral tamoxifen administration results in a fraction of the CD8$^+$ T cells deleting Egr2 and expressing YFP (FIG. 2E). This allowed comparison of both Egr2-sufficient (YFP$^-$) and Egr2-deficient (YFP$^+$) CD8$^+$ within the same tumor. To determine that Egr2 was in fact deleted from the YFP$^+$ fraction, both YFP$^+$ and YFP$^-$CD8$^+$ TILs were sorted and Egr2 transcripts were measured directly ex vivo and upon ex vivo stimulation. The YFP$^+$CD8$^+$ TILs expressed substantially less Egr2 transcripts compared to the YFP$^-$ counterparts (FIG. 2E). To determine if Egr2 is required for 4-1BB and LAG-3 expression, CD8$^+$ TILs were analyzed at day 7 and 14 after tumor inoculation and compared the YFP$^+$ and YFP$^-$ populations to mice not treated with tamoxifen. At day 7, the YFP$^+$ fraction expressed less 4-1BB and LAG-3 compared to the YFP$^-$ population and the WT CD8$^+$ TILs. However, expression of 4-1BB and LAG-3 was not significantly different at day 14 (FIG. 2F). This indicates that other transcriptional regulators compensate and contribute to the expression of LAG-3 and 4-1BB, especially at later time points.

Egr3 has been shown to have overlapping function with Egr2 (Safford et al., 2005; incorporated by reference in its entirety) and HIF1α can contribute to 4-1BB expression (Palazón et al., 2012). To investigate whether these transcription factors may compensate for 4-1BB and/or LAG-3 expression we sorted Egr2GFP^{hi} and Egr2GFP^{lo} CD8$^+$ TILs expressing 4-1BB and LAG-3 on day 7 and analyzed expression of Egr3 and HIF1α by qRT-PCR. Egr3 and HIF1α were indeed expressed in both the Egr2GFP^{hi} and Egr2GFP^{lo} populations. It was confirmed differential expression of Egr2 and CCL1 to between the Egr2GFP^{hi} and Egr2GFP^{lo} populations to assure sort purity (FIG. 2G). Together, these data indicate that Egr2 contributes to upregulation of 4-1BB and LAG-3 expression at early time points, but that other transcriptional regulators compensate and drive expression of LAG-3 and 4-1BB as the T cell-tumor interaction progresses.

CD8$^+$ 4-1BB$^+$LAG-3$^+$ TILs are Oligoclonal and Enriched for Tumor Antigen Specificity

Not all T cells in the tumor microenvironment are specific for tumor-associated antigens, as memory T cells specific for irrelevant antigens are often found among TIL, and non-specific T cell trafficking has been documented in vivo (Harlin et al., 2006; incorporated by reference in its entirety). Experiments conducted during development of embodiments herein to determine whether 4-1BB$^+$LAG-3$^+$ CD8$^+$ TILs are tumor-antigen specific. LAG-3, 4-1BB and Egr2 are upregulated after TCR stimulation and experiments indicate that this population expands within the tumor microenvironment in situ. Three complementary techniques were employed. First, the CD8$^+$ TILs were isolated based on LAG-3 and 4-1BB expression by cell sorting and performed TCRβ spectratype analysis. Compared to the 4-1BB$^-$LAG-3$^-$ TILs and CD8$^+$ splenocytes, the 4-1BB$^+$LAG-3$^+$ TILs had a non-Gaussian distribution and shared one or two dominant peaks (FIG. 3A). Analysis of several Vβs displaying one dominant peak revealed that Vβ7 contained a single CDR3β sequence shared between the 4-1BB$^-$LAG-3$^+$ and 4-1BB$^+$LAG-3$^+$ populations, indicating a clonal relationship (FIG. 3A). To measure the oligoclonality of the CDR3β repertoires the Hamming Distance (HD) was calculated for each Vβ between the CD8$^+$ TIL subpopulations and the splenic CD8$^+$ population within three separate mice (FIG. 8). By transforming each spectratype into area under the curve frequency profiles the Hamming Distance computes the changes in frequency and reports a value of comparison between 0 and 1, with 0 indicating a completely identical frequency profile and 1 signifying a completely discordant profile. As a control, the HD of the splenic CD8$^+$ populations between different mice was calculated (FIG. 3B, black bar). Since the splenic CD8$^+$ spectratypes are largely Gaussian this value represents the HD between two similar distributions. Analysis of the HD between the CD8$^+$ TIL subpopulations revealed that the 4-1BB$^+$LAG-3$^+$ and 4-1BB$^-$LAG-3$^+$ but not the 4-1BB$^-$LAG-3$^-$CDR3β distributions are significantly different (less Gaussian) compared to the splenic CD8$^+$ population (FIG. 3B). These data indicate that the 4-1BB$^+$LAG-3$^+$ and 4-1BB$^-$LAG-3$^+$ populations are oligoclonal expanded subsets of TILs, indicating antigen specificity in these subpopulations.

As a second approach, the B16.SIY melanoma and MC38.SIY adenocarcinoma models were utilized. CD8$^+$ T cells specific for the H-2K^b-restricted SIY epitope (SIYRYYGL) were monitored. SIYRYYGL/K^b pentamer$^+$ (H-2K^b/SIY) cells were found in expanded numbers within B16.SIY and MC38.SIY tumors at day 14 after tumor inoculation (FIG. 3C). Nearly 47% of the H-2K^b/SIY$^+$ cells expressed both 4-1BB and LAG-3, in contrast to 32% of the H-2K^b/SIY$^-$ population (FIGS. 3C and E). This enrichment of antigen-specific CD8$^+$ TILs in the 4-1BB$^+$LAG-3$^+$ popu-

lations indicates that these markers identify tumor antigen-specific TILs. The H-2K^b/SIY$^-$ cells also contained significant numbers of 4-1BB$^+$LAG-3$^+$ cells, which is consistent with the notion that tumor antigens other than SIY are also recognized by subsets of CD8$^+$ TILs in vivo (FIG. **3**C). H-2K^b/SIY$^+$ cells in the spleen or TdLN did not co-express 4-1BB and LAG-3, indicating that this phenotype is acquired within the tumor microenvironment.

These features were also analyzed in the context of tumor-antigen specific CD8$^+$ TILs in two spontaneously rejected tumor models. To this end, H-2K^b/SIY-specific CD8$^+$ TILs cells were evaluated from MC57.SIY and 1969.SIY tumors. At day 14 after tumor inoculation, approximately 5% of the H-2K^b/SIY-specific CD8$^+$ TILs were found in the 4-1BB$^+$LAG-3$^+$ fraction. As with the B16.SIY tumors, no H-2K^b/SIY-specific CD8 T cells co-expressed 4-1BB and LAG-3 in the TdLN or spleen (not shown) (FIG. **3**D). Unlike the B16.SIY and MC38.SIY tumors, no significant enrichment of 4-1BB$^+$LAG-3$^+$ H-2K^b/SIY-specific CD8$^+$ TILs was observed (FIGS. **3**D and E). These data indicate that tumor antigen specificity per se does not determine dysfunctionality, and that this is a feature unique to the microenvironment of progressing tumors.

As a third measure to determine if tumor-antigen specific CD8$^+$ T cells acquire the 4-1BB$^+$LAG-3$^+$ phenotype, congenically marked 2C and P14 transgenic (Tg) T cells, isolated from 2C/Rag2$^{-/-}$ and P14/Rag2$^{-/-}$ mice, were transferred into tumor-bearing hosts. The 2C TCR is specific for the SIY model antigen expressed by B16.SIY tumor cells, while P14 is an irrelevant TCR specific for the LCMV-derived gp_{33-41} epitope; both TCRs are H-2K^b-restricted. 2C and P14 Tg CD8$^+$ T cells were transferred via tail vein 7 days after tumor inoculation. Seven days after transfer, tumors and TdLNs were extracted and the phenotypic profile of the transferred populations was analyzed. This system allowed for the analysis of two T cell populations with defined antigen specificities within the same tumor microenvironment, as well as the polyclonal host CD8$^+$ T cells. The 2C T cells were more efficiently recruited and expanded within the tumor microenvironment compared to the P14 T cells and encompassed a large fraction of the total CD8$^+$ TIL population (FIG. **3**F). Of the 2C T cells, nearly all expressed LAG-3 and or 4-1BB while this was true for only a small percentage of the P14 cells (FIGS. **3**G and H). Consistent with the SIY-K^b pentamer analysis, the co-expression of LAG-3 and 4-1BB on 2C T cells was not observed in the TdLN. Together, these results demonstrate that the 4-1BB$^+$ LAG-3$^+$ phenotype is a property of tumor antigen-specific TIL under conditions of tumor progression.

CD8$^+$ TILs Expressing LAG-3 and 4-1BB Exhibit Defective IL-2 Production Yet Produce IFN-γ and Treg-Recruiting Chemokines

Based on the characteristics of the in vitro T cell anergy model that led to the identification of Egr2 as an important regulator, experiments conducted during development of embodiments herein to determine whether the tumor-antigen specific 4-1BB$^+$LAG-3$^+$CD8$^+$ TIL population is dysfunctional in their capacity to produce IL-2. To this end each subpopulation was sorted and stimulated with anti-CD3 and anti-CD28 mAb and analyzed IL-2 production by qRT-PCR and ELISA. Since nearly all CD8$^+$ TILs displayed an activated phenotype, CD8$^+$CD44$^+$ splenocytes were used as a positive control. Indeed, the 4-1BB$^+$LAG-3$^+$ cells showed a 100-fold reduction in Il-2 mRNA and as much as a 40-fold reduction in IL-2 protein levels compared to the 4-1BB$^-$ LAG-3$^-$ population (FIGS. **4**A and **4**B). As a second approach, Egr2^{hi} TIL (which are also largely 4-1BB$^+$LAG-3$^+$) was examined by utilizing the Egr2-GFP reporter mice. Indeed, ex vivo stimulated Egr2-GFP^{hi} CD8$^+$ TILs also exhibited reduced Il-2 transcript compared to Egr2-GFP^{lo} cells (FIG. **4**C). As a final approach, congenically marked 2C T cells were adoptively transferred intravenously into tumor-bearing hosts and recovered the 2C T cells 7 days later from the tumor and TdLN. 2C T cells isolated from tumors exhibited a reduced capacity to produce Il-2 transcripts, at a level equivalent to 4-1BB$^+$LAG-3$^+$ TILs, compared to 2C CD44$^+$ T cells isolated from the TdLN (FIG. **4**D). In chronic infection models, expression of PD-1 has been suggested to identify intrinsically dysfunctional or "exhausted" CD8$^+$ T cells. To determine if PD-1 alone might be sufficient to identify cells that lack the capacity to produce IL-2, CD8$^+$ TILs that lacked expression of LAG-3 and 4-1BB were isolated and tested for the ability of the PD-1$^+$ fraction to produce IL-2. Approximately ~10% of CD8$^+$ TILs were 4-1BB$^-$LAG-3$^-$PD-1$^+$ on day 14 and 21 (FIGS. **4**E and F). Upon ex vivo stimulation, this population retained the capacity to produce Il-2 mRNA at a level comparable to the 4-1BB$^-$LAG-3$^-$ cells (FIG. **4**G). These results indicate that PD-1 expression alone is not sufficient to identify dysfunctional TIL in the tumor microenvironment.

To further examine functional alterations during tumor progression protein levels of IL-2, IFN-γ and TNF-α were tested after TCR stimulation. As the loss of the ability of CD8$^+$ TILs to produce cytokines is suggested to be a temporal process reported initiated following entry into the tumor microenvironment (Waugh et al., 2016; Schietinger et al., 2016; incorporated by reference in their entireties) or progressively after 30 days in the chronic LCMV model (Wherry et al., 2007; incorporated by reference in its entirety), cytokine production was tested on day 7, 14, 21 and 28. The 4-1BB$^+$LAG-3$^+$ population lost the capacity to produce IL-2 as early as day 7 while the 4-1BB$^-$LAG-3$^+$ population lost IL-2 production between day 7 and day 14 (FIG. **5**A). The 4-1BB$^-$LAG-3$^-$ population did not lose the ability to produce IL-2 at any time point tested (FIG. **5**A), supporting the notion that this population is not tumor antigen specific and that differentiation into the dysfunctional state is an antigen-dependent process (Schietinger et al., 2016; incorporated by reference in its entirety). The 4-1BB$^+$LAG-3$^+$ population produced more IFN-γ at all time points after day 7 compared to their negative counterparts, albeit with a slight decrease in IFN-γ production over time. While the increase in IFN-γ was maintained until later time points, TNF-α production was lost by day 28 (FIG. **5**A).

Experiments were conducted during development of embodiments herein to evaluate production of cytokines directly in the tumor without in vitro restimulation, which may more closely reflect which T cells were receiving TCR stimulation in situ. Each T cell population was sorted directly ex vivo without any culturing and mRNA levels were measured by qRT-PCR. Elevated Ifn-γ and Gzmb transcripts were observed from the 4-1BB$^+$LAG-3$^+$ subpopulation, along with a slight decrease in Tnf-α levels, compared to the 4-1BB$^-$LAG-3$^-$ cells (FIG. **5**B). Production of IFN-γ in primary TILs was confirmed by injecting tumors with Brefeldin A prior to analysis by intracellular cytokine staining. Consistent with the mRNA expression, the 4-1BB$^+$ LAG-3$^+$ population produced significantly greater amounts of IFN-γ protein (FIG. **5**C). Thus, the 4-1BB$^+$LAG-3$^+$ TIL are not completely devoid of functionality, as they continue to produce IFN-γ despite defective production of IL-2. This

phenotype is consistent with in vitro T cell anergy models (Jenkins et al., 1987; incorporated by reference in its entirety).

To test whether the 4-1BB$^+$LAG-3$^+$ population still retains cytotoxic capacity, re-directed lysis was performed by co-culturing anti-CD3 bound P815 mastocytoma target cells with the different CD8$^+$ TIL subpopulations directly after sorting. 4-1BB$^+$LAG-3$^+$CD8$^+$ TILs isolated from day 14 tumors were able to lyse target cells at a comparable efficacy to in vitro primed OT-I cells. 4-1BB$^+$LAG-3$^+$ TILs isolated from day 21 tumors were still able to lyse target cells, albeit to a lesser extent compared to primed OT-I cells (FIG. **5**D).

CD8$^+$ T cells in the tumor can be the source of the chemokine CCL22 that recruits FoxP3$^+$ regulatory T cells (Tregs) to the tumor microenvironment (Spranger et al., 2013; incorporated by reference in its entirety). In addition, the chemokine Ccl1 was an Egr2 target in anergic T cells (Zheng et al., 2013; incorporated by reference in its entirety), and it has been suggested that CCL1 also contribute to Treg recruitment in the tumor context in vivo (Hoelzinger et al., 2010; incorporated by reference in its entirety). However, whether all CD8$^+$ T cells in the tumor produce these chemokines or if they are only produced by subpopulations of T cells had not been determined. To address this the CD8$^+$ TIL phenotypic subpopulations were analyzed for Ccl1 and Ccl22 mRNA expression directly ex vivo by qRT-PCR. Indeed, the 4-1BB$^+$LAG-3$^+$ TIL population produced substantially greater Ccl1 and Ccl22 compared to their negative counterparts or to splenic CD8$^+$CD44$^+$ T cells (FIG. **4**K). As a control, expression of a distinct chemokine Ccl5 was found not to be differentially expressed.

Together, these data show that co-expression of 4-1BB and LAG-3 delineates tumor antigen-specific CD8$^+$ TIL that lack the ability to produce IL-2 yet retain the ability to produce IFN-γ, kill target cells in vitro, and secrete chemokines capable of Treg recruitment. Given the fact that IFN-γ is responsible for the upregulation of PD-L1 and IDO in the tumor microenvironment, and that chemokines produced by CD8$^+$ TIL contribute to Treg recruitment (Spranger et al., 2013; incorporated by reference in its entirety), these data indicate that the 4-1BB$^+$LAG-3$^+$ population contributes to the network of immune suppressive mechanisms within the tumor microenvironment that limit the efficacy of anti-tumor immunity.

Gene Expression Profiling Reveals that CD8$^+$ 4-1BB$^+$LAG-3$^+$ TILs Express an Extensive Array of Additional Co-Stimulatory and Co-Inhibitory Receptors

Having in hand surface markers that define tumor antigen-specific dysfunctional CD8$^+$ TILs, experiments conducted during development of embodiments herein to compare the gene expression profile of this population to other published profiles of dysfunctional CD8$^+$ T cells to determine genes that regulate or are differentially expressed in cells in this dysfunctional state. To this end, a cross-study comparison was conducted of the transcriptional profiles of the "dysfunctional" 4-1BB$^+$LAG-3$^+$CD8$^+$ TILs, "hypofunctional" CD8$^+$ TILs from a study utilizing the murine CT26 tumor model (Waugh et al., 2016; incorporated by reference in its entirety) and LCMV "exhausted" GP33 specific CD8$^+$ T cells (Doering et al., 2012; incorporated by reference in its entirety). The results are depicted in Table 2. Only genes with a 2-fold increase over controls from each study independently were considered. Over a 2-fold greater number of genes was found to be shared between the dysfunctional TIL dataset and the previously published hypofunctional CD8$^+$ TIL data, than with the exhausted T cell profile (FIG. **6**A). In addition, a rank-rank hypergeometric overlap (RRHO) analysis indicated a greater statistically significant overlap (FIG. **10**A) and a greater correlation (FIG. **10**B) between the current dysfunctional TIL and the published hypofunctional CD8$^+$ TIL gene expression profiles compared to the virally-induced exhausted CD8$^+$ T cell profile, indicating a more similar molecular program between CD8$^+$ T cells isolate from tumors compared to chronic viral infection.

TABLE 2

Differentially regulated genes in CD8$^+$4-1BB$^+$LAG-3$^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
GLDC	glycine decarboxylase	11.25109772	CRY2	cryptochrome circadian clock 2	−1.546648257
GZMD	Granzyme D	10.66720027	KCMF1	potassium channel modulatory factor 1	−1.546835341
SLC17A6	solute carrier family 17 member 6	8.946467699	RHOB	ras homolog family member B	−1.548813112
IL1R2	interleukin 1 receptor type 2	7.595353131	KRT15	keratin 15	−1.549018071
LTF	lactotransferrin	7.530211233	RRAD	RRAD, Ras related glycolysis inhibitor and calcium channel regulator	−1.549530357
NRGN	neurogranin	7.334049768	C3	complement component 3	−1.549960037
GZME	granzyme E	7.160375687	ITFG3	Description Not Found	−1.550162812
RPL6	ribosomal protein L6	7.142107057	HAAO	3-hydroxyanthranilate 3,4-dioxygenase	−1.550553207
NRN1	neuritin 1	7.087993146	RNF138	ring finger protein 138	−1.551449524
LPL	lipoprotein lipase	7.004501392	UNC93B1	unc-93 homolog B1 (*C. elegans*)	−1.551767491
CLGN	calmegin	6.933690655	ANKZF1	ankyrin repeat and zinc finger domain containing 1	−1.552214097
CD70	CD70 molecule	6.906890596	IFITM3	interferon induced transmembrane protein 3	−1.552644542
AREG	amphiregulin	6.712870868	TXNIP	thioredoxin interacting protein	−1.552785452
ZRANB3	zinc finger RANBP2-type containing 3	6.595443985	LMAN1L	lectin, mannose binding 1 like	−1.554588852

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
ASNS	asparagine synthetase (glutamine-hydrolyzing)	6.59496878	ALDH3B1	aldehyde dehydrogenase 3 family member B1	−1.554711558
FANCD2	Fanconi anemia complementation group D2	6.353146826	GIP	gastric inhibitory polypeptide	−1.555511104
GM156	predicted gene 156(Gm156)	6.293701542	COX7A2L	cytochrome c oxidase subunit 7A2 like	−1.555572553
ACAA1B	acetyl-Coenzyme A acyltransferase 1B(Acaa1b)	6.293701542	APPL2	adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 2	−1.555598704
IGF2BP3	insulin like growth factor 2 mRNA binding protein 3	6.186857067	KLHL22	kelch like family member 22	−1.555929583
GZMG	granzyme G	6.093813673	OLFR272	olfactory receptor 272(Olfr272)	−1.557482156
CIB2	calcium and integrin binding family member 2	6.007868243	LRRC29	leucine rich repeat containing 29	−1.559366716
ATG9B	autophagy related 9B	5.986410935	A630095E13RIK	Description Not Found	−1.560714954
XKR8	XK related 8	5.977279924	OLFR194	olfactory receptor 194(Olfr194)	−1.560714954
EPDR1	ependymin related 1	5.956521363	OLFR1013	olfactory receptor 1013(Olfr1013)	−1.560714954
SPP1	secreted phosphoprotein 1	5.797769502	GLRA4	glycine receptor alpha 4	−1.560714954
RGS8	regulator of G-protein signaling 8	5.753805672	P2RY6	pyrimidinergic receptor P2Y6	−1.560714954
MDFIC	MyoD family inhibitor domain containing	5.730639956	RASGEF1B	RasGEF domain family member 1B	−1.560714954
DMWD	dystrophia myotonica, WD repeat containing	5.687200695	IL22RA2	interleukin 22 receptor subunit alpha 2	−1.560714954
KIF11	kinesin family member 11	5.669593751	LIN7C	lin-7 homolog C, crumbs cell polarity complex component	−1.560714954
LGI2	leucine rich repeat LGI family member 2	5.655351829	DMRT1	doublesex and mab-3 related transcription factor 1	−1.560714954
ZFP41	ZFP41 zinc finger protein	5.615445725	TSPAN12	tetraspanin 12	−1.560714954
MLKL	mixed lineage kinase domain-like	5.605849867	PAK3	p21 (RAC1) activated kinase 3	−1.560714954
CENPH	centromere protein H	5.563768278	COL2A1	collagen type II alpha 1 chain	−1.560714954
SERPINF1	serpin family F member 1	5.5360529	SLC37A1	solute carrier family 37 member 1	−1.560714954
UNC13B	unc-13 homolog B (*C. elegans*)	5.503030646	PSD3	pleckstrin and Sec7 domain containing 3	−1.560714954
MLANA	melan-A	5.496654083	RDH5	retinol dehydrogenase 5	−1.560714954
PES1	pescadillo ribosomal biogenesis factor 1	5.484376709	ABCA3	ATP binding cassette subfamily A member 3	−1.561263453
2900026A02RIK	Description Not Found	5.477353527	PLA2G4E	phospholipase A2 group IVE	−1.561650879
OSR2	odd-skipped related transciption factor 2	5.416164165	DDIT3	DNA damage inducible transcript 3	−1.563566526
MPP6	membrane palmitoylated protein 6	5.408506442	ZFP12	zinc finger protein 12(Zfp12)	−1.564308646
HIST1H3C	histone cluster 1, H3c	5.397460726	PIGYL	phosphatidylinositol glycan anchor biosynthesis, class Y-like(Pigyl)	−1.564585219
PI4K2B	phosphatidylinositol 4-kinase type 2 beta	5.375039431	CCDC97	coiled-coil domain containing 97	−1.565355117
SH3YL1	SH3 and SYLF domain containing 1	5.375039431	OLFR1112	olfactory receptor 1112(Olfr1112)	−1.56589319
RAD51	RAD51 recombinase	5.371558863	ACTN2	actinin alpha 2	−1.566931646
ZBTB32	zinc finger and BTB domain containing 32	5.318316841	POLG	polymerase (DNA) gamma, catalytic subunit	−1.567265595
MSC	musculin	5.285402219	FBXO32	F-box protein 32	−1.567281905
TG	thyroglobulin	5.259272487	MRPL15	mitochondrial ribosomal protein L15	−1.570722678
RSPH1	radial spoke head 1 homolog	5.236492618	FCHSD2	FCH and double SH3 domains 2	−1.571821211

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
ARL11	ADP ribosylation factor like GTPase 11	5.21916852	RECQL	RecQ like helicase	−1.572889668
NUDT11	nudix hydrolase 11	5.215290306	NDUFB11	NADH: ubiquinone oxidoreductase subunit B11	−1.572889668
APBB1	amyloid beta precursor protein binding family B member 1	5.197708158	SOX8	SRY-box 8	−1.573341535
SPINK2	serine peptidase inhibitor, Kazal type 2	5.189824559	1700030J22RIK	Description Not Found	−1.57662394
HMGN3	high mobility group nucleosomal binding domain 3	5.168922782	EMB	embigin	−1.577890585
FAM20B	family with sequence similarity 20 member B	5.12722055	CELSR1	cadherin EGF LAG seven-pass G-type receptor 1	−1.578201987
CDC25C	cell division cycle 25C	5.11997861	COL1A2	collagen type I alpha 2 chain	−1.580682782
FAM20A	family with sequence similarity 20 member A	5.108524457	1700080E11RIK	Description Not Found	−1.581046002
PPP1R16B	protein phosphatase 1 regulatory subunit 16B	5.09592442	GALNT12	polypeptide N-acetylgalactosaminyltransferase 12	−1.581363645
SBNO1	strawberry notch homolog 1 (*Drosophila*)	5.050936965	RMND5B	required for meiotic nuclear division 5 homolog B	−1.583960816
ST14	suppression of tumorigenicity 14	5.026800059	LRRC28	leucine rich repeat containing 28	−1.583987499
LRRC49	leucine rich repeat containing 49	5.024704311	OLFR622	olfactory receptor 622(Olfr622)	−1.584962501
TIAM1	T-cell lymphoma invasion and metastasis 1	5.004501392	OLFR339	olfactory receptor 339(Olfr339)	−1.584962501
APLF	aprataxin and PNKP like factor	4.951867504	NEIL3	nei like DNA glycosylase 3	−1.584962501
PGPEP1	pyroglutamyl-peptidase I	4.927185358	SNX24	sorting nexin 24	−1.584962501
ALCAM	activated leukocyte cell adhesion molecule	4.909293086	SLC7A11	solute carrier family 7 member 11	−1.584962501
B9D1	B9 domain containing 1	4.906890596	FOXJ1	forkhead box J1	−1.584962501
SCIN	scinderin	4.87282876	TAF3	TATA-box binding protein associated factor 3	−1.584962501
EXOC3L	exocyst complex component 3-like(Exoc3l)	4.844013973	MATN2	matrilin 2	−1.584962501
SLC35D3	solute carrier family 35 member D3	4.840463234	ADHFE1	alcohol dehydrogenase, iron containing 1	−1.586280668
ALDOC	aldolase, fructose-bisphosphate C	4.832890014	NANOS1	nanos C2HC-type zinc finger 1	−1.586914831
TMEM205	transmembrane protein 205	4.830182468	PPP2R5B	protein phosphatase 2 regulatory subunit B'beta	−1.586914831
PLEKHA8	pleckstrin homology domain containing A8	4.820178962	USP22	ubiquitin specific peptidase 22	−1.588703598
SPC25	SPC25, NDC80 kinetochore complex component	4.817623258	DAGLB	diacylglycerol lipase beta	−1.588817933
PCYT1B	phosphate cytidylyltransferase 1, choline, beta	4.749534268	KCTD6	potassium channel tetramerization domain containing 6	−1.589690033
SLC6A8	solute carrier family 6 member 8	4.749534268	ACTL6B	actin like 6B	−1.591351555
TUBB6	tubulin beta 6 class V	4.749241128	FAM129B	family with sequence similarity 129 member B	−1.5915039
BSPRY	B-box and SPRY domain containing	4.711494907	APOE	apolipoprotein E	−1.591683393
ICA1	islet cell autoantigen 1	4.708739041	GPR18	G protein-coupled receptor 18	−1.592384168
TNFSF13B	tumor necrosis factor superfamily member 13b	4.703211467	GSTP2	glutathione S-transferase, pi 2(Gstp2)	−1.592559885
GSTCD	glutathione S-transferase C-terminal domain containing	4.700439718	GPR114	Description Not Found	−1.593829527
CCNB1	cyclin B1	4.699051844	CHUK	conserved helix-loop-helix ubiquitous kinase	−1.594823937
4930539E08RIK	Description Not Found	4.693211287	TAS1R3	taste 1 receptor member 3	−1.596595048
SRXN1	sulfiredoxin 1	4.66106548	SLC7A7	solute carrier family 7 member 7	−1.596935142

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
SERF1	small EDRK-rich factor 1(Serf1)	4.632268216	SPIB	Spi-B transcription factor	−1.597677703
CCDC77	coiled-coil domain containing 77	4.62935662	POLR3A	polymerase (RNA) III subunit A	−1.599588488
RHBDF1	rhomboid 5 homolog 1	4.626439137	OLFR952	olfactory receptor 952(Olfr952)	−1.599679175
REEP3	receptor accessory protein 3	4.599912842	1700021F05RIK	Description Not Found	−1.601623253
ITGA3	integrin subunit alpha 3	4.590961241	CCDC79	Description Not Found	−1.602195565
SCCPDH	saccharopine dehydrogenase (putative)	4.590961241	FAM134B	family with sequence similarity 134 member B	−1.602715966
MYADM	myeloid associated differentiation marker	4.587964989	SEMA3B	semaphorin 3B	−1.602884409
FAM132A	family with sequence similarity 132 member A	4.581953751	FA2H	fatty acid 2-hydroxylase	−1.604494406
FOXRED2	FAD dependent oxidoreductase domain containing 2	4.572889668	ULK1	unc-51 like autophagy activating kinase 1	−1.604653903
CENPK	centromere protein K	4.569855608	MCOLN1	mucolipin 1	−1.606242992
DCXR	dicarbonyl and L-xylulose reductase	4.562242424	BMP5	bone morphogenetic protein 5	−1.606760033
TSPAN6	tetraspanin 6	4.54225805	ANKRD50	ankyrin repeat domain 50	−1.607137028
UPP1	uridine phosphorylase 1	4.53838296	OLFR560	olfactory receptor 560(Olfr560)	−1.608809243
DOK4	docking protein 4	4.520422249	OLFR366	olfactory receptor 366(Olfr366)	−1.608809243
ELOVL4	ELOVL fatty acid elongase 4	4.501439145	OLFR273	olfactory receptor 273(Olfr273)	−1.608809243
KNDC1	kinase non-catalytic C-lobe domain containing 1	4.499790117	FHIT	fragile histidine triad	−1.608809243
KRT17	keratin 17	4.491853096	AQP11	aquaporin 11	−1.608809243
CHST2	carbohydrate sulfotransferase 2	4.487315031	TMEM176A	transmembrane protein 176A	−1.608809243
TPX2	TPX2, microtubule nucleation factor	4.475733431	ENAH	enabled homolog (*Drosophila*)	−1.608809243
DUSP14	dual specificity phosphatase 14	4.456149035	CLDN6	claudin 6	−1.608809243
BGN	biglycan	4.449561375	SP1	Sp1 transcription factor	−1.608809243
FKBP9	FK506 binding protein 9	4.442943496	SP140	SP140 nuclear body protein	−1.608809243
CAPN5	calpain 5	4.385431037	RASGRP3	RAS guanyl releasing protein 3	−1.608809243
SLC1A4	solute carrier family 1 member 4	4.375039431	HIF3A	hypoxia inducible factor 3 alpha subunit	−1.609422664
IDI2	isopentenyl-diphosphate delta isomerase 2	4.357552005	FYCO1	FYVE and coiled-coil domain containing 1	−1.611220598
AKR1E1	aldo-keto reductase family 1, member E1(Akr1e1)	4.346596388	FBXL12	F-box and leucine rich repeat protein 12	−1.6119368
GNB4	G protein subunit beta 4	4.336088936	KLRA10	killer cell lectin-like receptor subfamily A, member 10(Klra10)	−1.618484777
CPNE2	copine 2	4.318640898	ABAT	4-aminobutyrate aminotransferase	−1.62058641
FAM132B	family with sequence similarity 132, member B(Fam132b)	4.259272487	AMHR2	anti-Mullerian hormone receptor type 2	−1.62058641
SLC6A12	solute carrier family 6 member 12	4.259272487	DDX3Y	DEAD-box helicase 3, Y-linked	−1.620649859
CPLX1	complexin 1	4.240314329	LGALS4	galectin 4	−1.621550215
PDCD1	programmed cell death 1	4.221103725	SPG20	spastic paraplegia 20 (Troyer syndrome)	−1.621653602
UTF1	undifferentiated embryonic cell transcription factor 1	4.201633861	CTRL	chymotrypsin like	−1.62729369
WDR60	WD repeat domain 60	4.14974712	GREM2	gremlin 2, DAN family BMP antagonist	−1.627927342
EGFL7	EGF like domain multiple 7	4.137503524	ZMAT3	zinc finger matrin-type 3	−1.628362075
ASPM	abnormal spindle microtubule assembly	4.133399125	AP4M1	adaptor related protein complex 4 mu 1 subunit	−1.628898157
TMBIM1	transmembrane BAX inhibitor motif containing 1	4.104628811	NT5C2	5'-nucleotidase, cytosolic II	−1.63059747

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
KNTC1	kinetochore associated 1	4.093952772	TMIE	transmembrane inner ear	−1.631606148
1700019D03RIK	Description Not Found	4.087462841	OLFR556	olfactory receptor 556(Olfr556)	−1.632268216
TM4SF5	transmembrane 4 L six family member 5	4.087462841	OLFR463	olfactory receptor 463(Olfr463)	−1.632268216
BIRC5	baculoviral IAP repeat containing 5	4.027905997	CTS3	cathepsin 3(Cts3)	−1.632268216
SYNGR3	synaptogyrin 3	4.022367813	OAS1B	2'-5' oligoadenylate synthetase 1B(Oas1b)	−1.632268216
PLSCR4	phospholipid scramblase 4	4	KCNF1	potassium voltage-gated channel modifier subfamily F member 1	−1.632268216
KIF15	kinesin family member 15	3.962376898	GCGR	glucagon receptor	−1.632268216
TICAM2	toll like receptor adaptor molecule 2	3.958842675	NR1I3	nuclear receptor subfamily 1 group I member 3	−1.632268216
CENPM	centromere protein M	3.957682486	FSTL1	follistatin like 1	−1.632268216
KIF4	kinesin family member 4(Kif4)	3.956097191	ASAP3	ArfGAP with SH3 domain, ankyrin repeat and PH domain 3	−1.632268216
E2F2	E2F transcription factor 2	3.93191939	IHH	indian hedgehog	−1.632268216
MSN	moesin	3.930737338	SEMA3A	semaphorin 3A	−1.632268216
PTPRA	protein tyrosine phosphatase, receptor type A	3.928989949	RAMP1	receptor activity modifying protein 1	−1.632575446
BC026585	cDNA sequence BC026585(BC026585)	3.882643049	NFKBID	NFKB inhibitor delta	−1.633158642
IQGAP3	IQ motif containing GTPase activating protein 3	3.867896464	KLK15	kallikrein related peptidase 15	−1.633773522
CD244	CD244 molecule	3.867896464	CYP1B1	cytochrome P450 family 1 subfamily B member 1	−1.634684534
HIST1H3G	histone cluster 1, H3g	3.837943242	DNAJA1	DnaJ heat shock protein family (Hsp40) member A1	−1.635111002
SLC15A3	solute carrier family 15 member 3	3.832890014	SDSL	serine dehydratase like	−1.635807742
GIPC2	GIPC PDZ domain containing family member 2	3.817623258	CCDC137	coiled-coil domain containing 137	−1.636838653
UTP15	UTP15, small subunit processome component	3.812498225	ZSWIM4	zinc finger SWIM-type containing 4	−1.638152805
PDIA6	protein disulfide isomerase family A member 6	3.812498225	BBC3	BCL2 binding component 3	−1.638336813
JDP2	Jun dimerization protein 2	3.807354922	SOCS3	suppressor of cytokine signaling 3	−1.638876738
MESDC1	mesoderm development candidate 1	3.806723946	2900092C05RIK	Description Not Found	−1.639157339
GAS2	growth arrest specific 2	3.802193217	CSRNP2	cysteine and serine rich nuclear protein 2	−1.639383642
IL4I1	interleukin 4 induced 1	3.802193217	BLOC1S3	biogenesis of lysosomal organelles complex 1 subunit 3	−1.639585785
PHF19	PHD finger protein 19	3.802193217	ELL	elongation factor for RNA polymerase II	−1.64021945
CKAP2L	cytoskeleton associated protein 2 like	3.797012978	GTF3C4	general transcription factor IIIC subunit 4	−1.640658029
GSTT1	glutathione S-transferase theta 1	3.791814071	MYLPF	myosin light chain, phosphorylatable, fast skeletal muscle	−1.640660074
ADAM3	a disintegrin and metallopeptidase domain 3 (cyritestin)(Adam3)	3.781359714	CYP2A12	cytochrome P450, family 2, subfamily a, polypeptide 12(Cyp2a12)	−1.641947141
SLAMF7	SLAM family member 7	3.781359714	RNF139	ring finger protein 139	−1.642010395
MCPT8	mast cell protease 8(Mcpt8)	3.770829046	C78339	Description Not Found	−1.643573868
DGKG	diacylglycerol kinase gamma	3.765534746	EDEM1	ER degradation enhancing alpha-mannosidase like protein 1	−1.64385619
NLGN2	neuroligin 2	3.716990894	UBE2E1	ubiquitin conjugating enzyme E2 E1	−1.645859791
SERPINE2	serpin family E member 2	3.694880193	PALMD	palmdelphin	−1.646322067

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
IL10	interleukin 10	3.689299161	AMICA1	adhesion molecule, interacts with CXADR antigen 1(Amica1)	−1.647478619
SLC6A13	solute carrier family 6 member 13	3.689299161	KLHL11	kelch like family member 11	−1.650611828
STAU2	staufen double-stranded RNA binding protein 2	3.666756592	IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)	−1.651050175
ARHGDIG	Rho GDP dissociation inhibitor gamma	3.655351829	DECR1	2,4-dienoyl-CoA reductase 1, mitochondrial	−1.651406438
TK1	thymidine kinase 1	3.637477097	SAMD3	sterile alpha motif domain containing 3	−1.653213853
PCYT1A	phosphate cytidylyltransferase 1, choline, alpha	3.617728231	9130409I23RIK	Description Not Found	−1.655351829
LAMB3	laminin subunit beta 3	3.608809243	2010107G12RIK	Description Not Found	−1.655351829
UBE2N	ubiquitin conjugating enzyme E2 N	3.590961241	ZFP354B	zinc finger protein 354B(Zfp354b)	−1.655351829
STARD8	StAR related lipid transfer domain containing 8	3.578938713	TAS2R143	taste receptor, type 2, member 143(Tas2r143)	−1.655351829
PRR5	proline rich 5	3.578938713	OLFR65	olfactory receptor 65(Olfr65)	−1.655351829
BDH2	3-hydroxybutyrate dehydrogenase, type 2	3.554588852	NRP	neural regeneration protein(Nrp)	−1.655351829
FAM124B	family with sequence similarity 124 member B	3.548436625	DOK3	docking protein 3	−1.655351829
MGAT3	mannosyl (beta-1,4-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase	3.548436625	HIGD1A	HIG1 hypoxia inducible domain family member 1A	−1.655351829
LAG3	lymphocyte activating 3	3.542346309	CCDC13	coiled-coil domain containing 13	−1.655351829
GDPD5	glycerophosphodiester phosphodiesterase domain containing 5	3.538812733	ANGPTL2	angiopoietin like 2	−1.655351829
RNF168	ring finger protein 168	3.5360529	CNGB3	cyclic nucleotide gated channel beta 3	−1.655351829
LYPLA1	lysophospholipase I	3.529820947	HOXD4	homeobox D4	−1.655351829
TUBGCP4	tubulin gamma complex associated protein 4	3.523561956	KIFC3	kinesin family member C3	−1.655351829
PYGL	phosphorylase, glycogen, liver	3.51412226	AMACR	alpha-methylacyl-CoA racemase	−1.655351829
CCL3	C-C motif chemokine ligand 3	3.510281539	2310014L17RIK	Description Not Found	−1.655707015
BCAT1	branched chain amino acid transaminase 1	3.508163667	BRAP	BRCA1 associated protein	−1.657090723
ATP6V0A1	ATPase H+ transporting V0 subunit al	3.501439145	SLC39A1	solute carrier family 39 member 1	−1.657631089
EIF4E	eukaryotic translation initiation factor 4E	3.498250868	OLFR419	olfactory receptor 419(Olfr419)	−1.65813796
HIST1H4B	histone cluster 1, H4b	3.491853096	NHP2L1	NHP2 non-histone chromosome protein 2-like 1 (*S. cerevisiae*)(Nhp2l1)	−1.658298045
LAD1	ladinin 1	3.49085426	STOML2	stomatin like 2	−1.659357735
ITGAV	integrin subunit alpha V	3.485426827	SAMM50	SAMM50 sorting and assembly machinery component	−1.662400762
MRPL47	mitochondrial ribosomal protein L47	3.485426827	CCDC91	coiled-coil domain containing 91	−1.6632299
CAMK2N1	calcium/calmodulin dependent protein kinase II inhibitor 1	3.484460783	ATF3	activating transcription factor 3	−1.663483642
UEVLD	UEV and lactate/malate dehyrogenase domains	3.465974465	RAI1	retinoic acid induced 1	−1.663885989
SFXN4	sideroflexin 4	3.462706751	RRAS2	related RAS viral (r-ras) oncogene homolog 2	−1.665826896
2810417H13RIK	Description Not Found	3.461634298	UROS	uroporphyrinogen III synthase	−1.665923156
RAD51AP1	RAD51 associated protein 1	3.459431619	SCOC	short coiled-coil protein	−1.666272349
FUT4	fucosyltransferase 4	3.452858965	DUSP10	dual specificity phosphatase 10	−1.666485948
CTNNBIP1	catenin beta interacting protein 1	3.44625623	CYB5R4	cytochrome b5 reductase 4	−1.666756592

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
ZBTB8OS	zinc finger and BTB domain containing 8 opposite strand	3.426264755	9930104L06RIK	Description Not Found	−1.667150978
LYSMD4	LysM domain containing 4	3.42259008	ZFP579	zinc finger protein 579(Zfp579)	−1.669023741
DIAP3	Description Not Found	3.40599236	RGP1	RGP1 homolog, RAB6A GEF complex partner 1	−1.669393721
PTGIS	prostaglandin I2 (prostacyclin) synthase	3.399171094	PIAS2	protein inhibitor of activated STAT 2	−1.672137196
MOAP1	modulator of apoptosis 1	3.392317423	METTL1	methyltransferase like 1	−1.672425342
SLC27A3	solute carrier family 27 member 3	3.392317423	POU5F1	POU class 5 homeobox 1	−1.673854965
MRPL39	mitochondrial ribosomal protein L39	3.371492175	SERPINB6C	serine (or cysteine) peptidase inhibitor, clade B, member 6c(Serpinb6c)	−1.673932658
WTAP	Wilms tumor 1 associated protein	3.364572432	STXBP4	syntaxin binding protein 4	−1.675552278
RAD54L	RAD54-like (*S. cerevisiae*)	3.356589854	RIMS3	regulating synaptic membrane exocytosis 3	−1.676120648
CETN4	centrin 4(Cetn4)	3.336283388	XYLT2	xylosyltransferase 2	−1.676976793
CEP55	centrosomal protein 55	3.329123596	TAS2R107	taste receptor, type 2, member 107(Tas2r107)	−1.678071905
CYP4F39	cytochrome P450, family 4, subfamily f, polypeptide 39(Cyp4f39)	3.321928095	SKP1A	S-phase kinase-associated protein 1A(Skp1a)	−1.678071905
PTPN5	protein tyrosine phosphatase, non-receptor type 5	3.314696526	OLFR165	olfactory receptor 165(Olfr165)	−1.678071905
TUBE1	tubulin epsilon 1	3.292781749	OLFR111	olfactory receptor 111(Olfr111)	−1.678071905
TCAM1	testicular cell adhesion molecule 1(Tcam1)	3.285402219	CYP4A12A	cytochrome P450, family 4, subfamily a, polypeptide 12a(Cyp4a12a)	−1.678071905
MID1IP1	MID1 interacting protein 1	3.263034406	TLR6	toll like receptor 6	−1.678071905
ABHD6	abhydrolase domain containing 6	3.260682276	KCNS3	potassium voltage-gated channel modifier subfamily S member 3	−1.678071905
ZCCHC4	zinc finger CCHC-type containing 4	3.255500733	FARSA	phenylalanyl-tRNA synthetase alpha subunit	−1.678071905
MGST3	microsomal glutathione S-transferase 3	3.25353624	SLC2A4	solute carrier family 2 member 4	−1.678071905
BC022687	cDNA sequence BC022687(BC022687)	3.247927513	GDPD4	glycerophosphodiester phosphodiesterase domain containing 4	−1.678071905
ACSF3	acyl-CoA synthetase family member 3	3.24325855	RCAN1	regulator of calcineurin 1	−1.678071905
ADAM8	ADAM metallopeptidase domain 8	3.240314329	CCDC82	coiled-coil domain containing 82	−1.678071905
SGCB	sarcoglycan beta	3.237034772	CDYL2	chromodomain protein, Y-like 2	−1.678071905
SOCS2	suppressor of cytokine signaling 2	3.232660757	MBD5	methyl-CpG binding domain protein 5	−1.678071905
HIST1H2AG	histone cluster 1, H2ag	3.223000387	ACSL1	acyl-CoA synthetase long-chain family member 1	−1.678071905
CRMP1	collapsin response mediator protein 1	3.201633861	OTUB2	OTU deubiquitinase, ubiquitin aldehyde binding 2	−1.678071905
RPS19BP1	ribosomal protein S19 binding protein 1	3.201633861	NPPA	natriuretic peptide A	−1.678071905
1700020L24RIK	Description Not Found	3.193771743	LY96	lymphocyte antigen 96	−1.679594789
CCDC109B	coiled-coil domain containing 109B(Ccdc109b)	3.181276986	OLFR351	olfactory receptor 351(Olfr351)	−1.680730557
UBE2C	ubiquitin conjugating enzyme E2 C	3.177917792	TGFBR1	transforming growth factor beta receptor 1	−1.681068055
SLC25A16	solute carrier family 25 member 16	3.177917792	KLHL6	kelch like family member 6	−1.683531539
ARHGAP19	Rho GTPase activating protein 19	3.167705534	ELMO2	engulfment and cell motility 2	−1.683696454
TYMS-PS	thymidylate synthase, pseudogene(Tyms-ps)	3.166362514	POLR3D	polymerase (RNA) III subunit D	−1.683942043
IL3RA	interleukin 3 receptor subunit alpha	3.145793675	RALGPS1	Ral GEF with PH domain and SH3 binding motif 1	−1.685524532

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
TMEM53	transmembrane protein 53	3.141596278	ATL2	atlastin GTPase 2	−1.685731341
THNSL2	threonine synthase like 2	3.141596278	RAD52	RAD52 homolog, DNA repair protein	−1.689523672
2810408M09RIK	Description Not Found	3.129283017	GPC1	glypican 1	−1.689646894
ADAMDEC1	ADAM like decysin 1	3.121015401	ARHGAP15	Rho GTPase activating protein 15	−1.690804518
ASB2	ankyrin repeat and SOCS box containing 2	3.118792343	GPRC5B	G protein-coupled receptor class C group 5 member B	−1.693999744
SLC37A4	solute carrier family 37 member 4	3.112700133	ZBTB1	zinc finger and BTB domain containing 1	−1.694046727
NICN1	nicolin 1	3.108478268	NARFL	nuclear prelamin A recognition factor like	−1.694880193
2310067B10RIK	Description Not Found	3.087462841	SLC26A6	solute carrier family 26 member 6	−1.695252347
PIGL	phosphatidylinositol glycan anchor biosynthesis class L	3.077239787	MAPKBP1	mitogen-activated protein kinase binding protein 1	−1.695908738
1190005I06RIK	Description Not Found	3.070389328	RAB6B	RAB6B, member RAS oncogene family	−1.697541036
DHFR	dihydrofolate reductase	3.070389328	ARL2	ADP ribosylation factor like GTPase 2	−1.700349879
FABP5	fatty acid binding protein 5	3.06608919	ZFP646	zinc finger protein 646(Zfp646)	−1.700439718
POMT2	protein O-mannosyltransferase 2	3.055794286	SELENBP2	selenium binding protein 2(Selenbp2)	−1.700439718
F2RL2	coagulation factor II thrombin receptor like 2	3.053111336	ACOT3	acyl-CoA thioesterase 3(Acot3)	−1.700439718
GRB7	growth factor receptor bound protein 7	3.048852907	REG3G	regenerating family member 3 gamma	−1.700439718
SNX21	sorting nexin family member 21	3.044394119	GAB1	GRB2 associated binding protein 1	−1.700439718
SUFU	SUFU negative regulator of hedgehog signaling	3.044394119	LCN10	lipocalin 10	−1.700439718
RFC3	replication factor C subunit 3	3.029288361	MTHFD2L	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2-like	−1.700439718
CLDN12	claudin 12	3.017921908	PTCD3	pentatricopeptide repeat domain 3	−1.700439718
C1QTNF6	C1q and tumor necrosis factor related protein 6	3.014450679	NTHL1	nth-like DNA glycosylase 1	−1.700439718
PLCXD1	phosphatidylinositol specific phospholipase C X domain containing 1	2.99095486	NUDT3	nudix hydrolase 3	−1.700439718
SULT4A1	sulfotransferase family 4A member 1	2.99095486	CLEC12A	C-type lectin domain family 12 member A	−1.700439718
CTTNBP2NL	CTTNBP2 N-terminal like	2.981852653	ZBTB3	zinc finger and BTB domain containing 3	−1.700439718
SNX5	sorting nexin 5	2.977279924	AMT	aminomethyltransferase	−1.700439718
HPS5	HPS5, biogenesis of lysosomal organelles complex 2 subunit 2	2.972692654	ZDHHC14	zinc finger DHHC-type containing 14	−1.700439718
WISP1	WNT1 inducible signaling pathway protein 1	2.968090752	NKX2-5	NK2 homeobox 5	−1.700491519
PTPN9	protein tyrosine phosphatase, non-receptor type 9	2.963474124	FOXA3	forkhead box A3	−1.702815694
USP37	ubiquitin specific peptidase 37	2.95419631	WASF1	WAS protein family member 1	−1.706412734
SH3BGRL	SH3 domain binding glutamate rich protein like	2.935459748	OLFR690	olfactory receptor 690(Olfr690)	−1.707192688
NCALD	neurocalcin delta	2.935459748	ENTPD5	ectonucleoside triphosphate diphosphohydrolase 5	−1.707764551
CDC42EP4	CDC42 effector protein 4	2.916476644	PCDHGA4	protocadherin gamma subfamily A, 4	−1.709042655
IGFBP7	insulin like growth factor binding protein 7	2.910553168	TCF12	transcription factor 12	−1.710308209
ABHD4	abhydrolase domain containing 4	2.908868748	MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	−1.711494907

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
CSF1	colony stimulating factor 1	2.906890596	CDKN1C	cyclin dependent kinase inhibitor 1C	−1.711690028
COX7A1	cytochrome c oxidase subunit 7A1	2.897240426	PRICKLE1	prickle planar cell polarity protein 1	−1.713410822
TTYH2	tweety family member 2	2.892391026	ATXN7L1	ataxin 7 like 1	−1.71669984
ACO1	aconitase 1	2.87774425	SLCO3A1	solute carrier organic anion transporter family member 3A1	−1.719235762
BARD1	BRCA1 associated RING domain 1	2.867896464	TMEM110	transmembrane protein 110	−1.720046704
GPN1	GPN-loop GTPase 1	2.867896464	KLF2	Kruppel like factor 2	−1.721374729
PTTG1	pituitary tumor-transforming 1	2.867896464	FGG	fibrinogen gamma chain	−1.722466024
2810408A11RIK	Description Not Found	2.857980995	ASAH2	N-acylsphingosine amidohydrolase 2	−1.722466024
BBX	BBX, HMG-box containing	2.857980995	LAP3	leucine aminopeptidase 3	−1.722466024
LTBP3	latent transforming growth factor beta binding protein 3	2.837943242	STAB2	stabilin 2	−1.722466024
ACTG2	actin, gamma 2, smooth muscle, enteric	2.827819025	IL22RA1	interleukin 22 receptor subunit alpha 1	−1.722466024
ISLR	immunoglobulin superfamily containing leucine rich repeat	2.827819025	SERINC4	serine incorporator 4	−1.722466024
NARS2	asparaginyl-tRNA synthetase 2, mitochondrial (putative)	2.823087408	GPR180	G protein-coupled receptor 180	−1.722466024
ICAM4	intercellular adhesion molecule 4 (Landsteiner-Wiener blood group)	2.81452379	TIPARP	TCDD inducible poly(ADP-ribose) polymerase	−1.722466024
ABCB8	ATP binding cassette subfamily B member 8	2.813358991	USP11	ubiquitin specific peptidase 11	−1.722466024
IDI1	isopentenyl-diphosphate delta isomerase 1	2.811782922	TRIP6	thyroid hormone receptor interactor 6	−1.722466024
GLS2	glutaminase 2	2.797012978	KCNH2	potassium voltage-gated channel subfamily H member 2	−1.722466024
HDAC8	histone deacetylase 8	2.797012978	ESR2	estrogen receptor 2	−1.722466024
BRIP1	BRCA1 interacting protein C-terminal helicase 1	2.797012978	FGF13	fibroblast growth factor 13	−1.722639247
USP6NL	USP6 N-terminal like	2.794415866	KBTBD7	kelch repeat and BTB domain containing 7	−1.724237927
TLCD2	TLC domain containing 2	2.791814071	UHRF1BP1	UHRF1 binding protein 1	−1.725835292
GUCY1A3	guanylate cyclase 1 soluble subunit alpha	2.787502763	BCAM	basal cell adhesion molecule (Lutheran blood group)	−1.726509704
OCA2	OCA2 melanosomal transmembrane protein	2.786596362	ELOVL6	ELOVL fatty acid elongase 6	−1.726565554
VAT1	vesicle amine transport 1	2.772502543	PPM1K	protein phosphatase, Mg2+/Mn2+ dependent 1K	−1.726643643
HIST1H2AB	histone cluster 1, H2ab	2.767914142	SPATA6	spermatogenesis associated 6	−1.727673077
PIGC	phosphatidylinositol glycan anchor biosynthesis class C	2.760220946	NAV1	neuron navigator 1	−1.727920455
PARG	poly(ADP-ribose) glycohydrolase	2.756558208	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	−1.727920455
ESCO2	establishment of sister chromatid cohesion N-acetyltransferase 2	2.754887502	KCNAB1	potassium voltage-gated channel subfamily A member regulatory beta subunit 1	−1.727920455
HIPK2	homeodomain interacting protein kinase 2	2.754887502	CYP27A1	cytochrome P450 family 27 subfamily A member 1	−1.727920455
IMPA1	inositol monophosphatase 1	2.752945007	MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4	−1.729756006
COQ4	coenzyme Q4	2.744161096	ANKRD7	ankyrin repeat domain 7	−1.730646873
ZBTB7A	zinc finger and BTB domain containing 7A	2.744161096	IFRD1	interferon related developmental regulator 1	−1.732447522

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
GAMT	guanidinoacetate N-methyltransferase	2.744161096	ALX3	ALX homeobox 3	−1.733354341
BIK	BCL2 interacting killer	2.744161096	SNURF	SNRPN upstream reading frame	−1.733354341
PMS1	PMS1 homolog 1, mismatch repair system component	2.733354341	AMZ2	archaelysin family metallopeptidase 2	−1.73350053
HAVCR2	hepatitis A virus cellular receptor 2	2.729769667	ROGDI	rogdi homolog	−1.73419198
FHL2	four and a half LIM domains 2	2.727254747	DAGLA	diacylglycerol lipase alpha	−1.734471203
CHAF1A	chromatin assembly factor 1 subunit A	2.725248783	4930432K21RIK	Description Not Found	−1.736243886
2810004N23RIK	Description Not Found	2.722466024	KRCC1	lysine rich coiled-coil 1	−1.73665741
TBC1D14	TBC1 domain family member 14	2.722466024	OLFR1331	olfactory receptor 1331(Olfr1331)	−1.736826447
EHD2	EH domain containing 2	2.711494907	SLC25A25	solute carrier family 25 member 25	−1.73690749
APH1A	aph-1 homolog A, gamma-secretase subunit	2.705977902	CXCR4	C-X-C motif chemokine receptor 4	−1.737779353
TMEM2	transmembrane protein 2	2.703211467	EPB4.1L3	Description Not Found	−1.738767837
LCAT	lecithin-cholesterol acyltransferase	2.700439718	CEP164	centrosomal protein 164	−1.738795736
FBXO15	F-box protein 15	2.689299161	AGER	advanced glycosylation end product-specific receptor	−1.73961488
ADAP1	ArfGAP with dual PH domains 1	2.674391397	B3GALT5	beta-1,3-galactosyltransferase 5	−1.740215306
PPAPDC1B	Description Not Found	2.666756592	OLFR450	olfactory receptor 450(Olfr450)	−1.74228265
CD48	CD48 molecule	2.666756592	ZFP780B	zinc finger protein 780B(Zfp780b)	−1.744161096
CAMK4	calcium/calmodulin dependent protein kinase IV	2.655351829	OLFR485	olfactory receptor 485(Olfr485)	−1.744161096
SAC3D1	SAC3 domain containing 1	2.64385619	OLFR47	olfactory receptor 47(Olfr47)	−1.744161096
ECHDC2	enoyl-CoA hydratase domain containing 2	2.640725033	CYP4F18	cytochrome P450, family 4, subfamily f, polypeptide 18(Cyp4f18)	−1.744161096
INCENP	inner centromere protein	2.638460117	PLOD2	procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	−1.744161096
INTS9	integrator complex subunit 9	2.634920268	OSBPL1A	oxy sterol binding protein like 1A	−1.744161096
KLRA17	killer cell lectin-like receptor, subfamily A, member 17(Klra17)	2.632268216	CHRNA5	cholinergic receptor nicotinic alpha 5 subunit	−1.744161096
MAN2B2	mannosidase alpha class 2B member 2	2.632268216	TSSK4	testis specific serine kinase 4	−1.744161096
DOLK	dolichol kinase	2.632268216	ALKBH8	alkB homolog 8, tRNA methyltransferase	−1.744161096
SAP30BP	SAP30 binding protein	2.632268216	GPX2	glutathione peroxidase 2	−1.744161096
RTN1	reticulon 1	2.627898616	ATG4D	autophagy related 4D cysteine peptidase	−1.744161096
ADAM15	ADAM metallopeptidase domain 15	2.626439137	SCRN3	secernin 3	−1.744161096
STAG3	stromal antigen 3	2.62058641	NOTCH3	notch 3	−1.744161096
NUDT2	nudix hydrolase 2	2.610775705	OLFR113	olfactory receptor 113(Olfr113)	−1.744357436
GLT8D2	glycosyltransferase 8 domain containing 2	2.609988757	CD28	CD28 molecule	−1.744605653
CAPSL	calcyphosine like	2.608809243	SAG	S-antigen; retina and pineal gland (arrestin)	−1.745224161
CALR	calreticulin	2.608809243	AGTRAP	angiotensin II receptor associated protein	−1.749107415
CRYBG3	crystallin beta-gamma domain containing 3	2.605393551	BLK	BLK proto-oncogene, Src family tyrosine kinase	−1.749534268
DIXDC1	DIX domain containing 1	2.596940379	MGAT5	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase	−1.749534268
TACSTD2	tumor-associated calcium signal transducer 2	2.593926161	RNF2	ring finger protein 2	−1.750890228

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
TRP53RK	Description Not Found	2.588066506	COL14A1	collagen type XIV alpha 1 chain	−1.752093722
PDCD1LG2	programmed cell death 1 ligand 2	2.584962501	PLEKHG3	pleckstrin homology and RhoGEF domain containing G3	−1.752109698
SEC23IP	SEC23 interacting protein	2.584962501	ARHGEF18	Rho/Rac guanine nucleotide exchange factor 18	−1.754100479
ORM1	orosomucoid 1	2.584962501	LEF1	lymphoid enhancer binding factor 1	−1.754887502
ZFP322A	zinc finger protein 322A(Zfp322a)	2.575024164	COMMD9	COMM domain containing 9	−1.75490709
4931406C07RIK	Description Not Found	2.560714954	SLC20A1	solute carrier family 20 member 1	−1.758637847
ZFP382	zinc finger protein 382(Zfp382)	2.560714954	ACTR5	ARP5 actin-related protein 5 homolog	−1.759244091
CLIP2	CAP-Gly domain containing linker protein 2	2.560714954	UBQLN3	ubiquilin 3	−1.765109548
TNFAIP8L1	TNF alpha induced protein 8 like 1	2.560714954	ZFP770	zinc finger protein 770(Zfp770)	−1.765534746
NRCAM	neuronal cell adhesion molecule	2.560714954	PCDHB18	protocadherin beta 18(Pcdhb18)	−1.765534746
HPSE	heparanase	2.560714954	OLFR700	olfactory receptor 700(Olfr700)	−1.765534746
RTKN	rhotekin	2.558985655	FOXP4	forkhead box P4	−1.765534746
DLGAP5	DLG associated protein 5	2.550125328	CDC34	cell division cycle 34	−1.765534746
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	2.548436625	HIST1H1E	histone cluster 1, H1e	−1.765534746
GCNT1	glucosaminyl (N-acetyl) transferase 1, core 2	2.548436625	G6PC2	glucose-6-phosphatase catalytic subunit 2	−1.765534746
SASS6	SAS-6 centriolar assembly protein	2.548436625	FUT1	fucosyltransferase 1 (H blood group)	−1.765534746
AMIGO3	adhesion molecule with Ig-like domain 3	2.548436625	ZFP69	ZFP69 zinc finger protein	−1.765534746
APH1B	aph-1 homolog B, gamma-secretase subunit	2.548436625	WBSCR27	Williams Beuren syndrome chromosome region 27	−1.765534746
ABCC5	ATP binding cassette subfamily C member 5	2.547846505	METTL8	methyltransferase like 8	−1.766880868
YIPF6	Yip1 domain family member 6	2.543805176	TMEM170	transmembrane protein 170(Tmem170)	−1.767462508
FFAR1	free fatty acid receptor 1	2.5360529	TRP53INP1	transformation related protein 53 inducible nuclear protein 1(Trp53inp1)	−1.767518474
TSSK6	testis specific serine kinase 6	2.5360529	H2-Q5	histocompatibility 2, Q region locus 5(H2-Q5)	−1.769676967
ETV6	ETS variant 6	2.535385323	ADCK1	aarF domain containing kinase 1	−1.770033995
PTGDS	prostaglandin D2 synthase	2.529838423	IMPAD1	inositol monophosphatase domain containing 1	−1.771434505
SH3D19	SH3 domain containing 19	2.523561956	E4F1	E4F transcription factor 1	−1.772427885
KIF5C	kinesin family member 5C	2.518298014	ZFYVE20	Description Not Found	−1.772942676
PTGER2	prostaglandin E receptor 2	2.517275693	PNPLA6	patatin like phospholipase domain containing 6	−1.775074114
INSR	insulin receptor	2.510961919	TRIB3	tribbles pseudokinase 3	−1.775215233
MAPK6	mitogen-activated protein kinase 6	2.504620392	GM614	predicted gene 614(Gm614)	−1.776103988
OXSR1	oxidative stress responsive 1	2.502211192	D5ERTD579E	DNA segment, Chr 5, ERATO Doi 579, expressed(D5Ertd579e)	−1.776306798
EZH2	enhancer of zeste 2 polycomb repressive complex 2 subunit	2.501439145	SCAND1	SCAN domain containing 1	−1.77785827
BNIP1	BCL2 interacting protein 1	2.498250868	ASB13	ankyrin repeat and SOCS box containing 13	−1.782205107
LPCAT4	lysophosphatidylcholine acyltransferase 4	2.495285165	ARHGEF4	Rho guanine nucleotide exchange factor 4	−1.784072601
PPAP2C	Description Not Found	2.485426827	H1FNT	H1 histone family member N, testis specific	−1.78485543

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
IFNA12	interferon alpha 12(Ifna12)	2.485426827	BLOC1S1	biogenesis of lysosomal organelles complex 1 subunit 1	−1.784911393
DCLK1	doublecortin like kinase 1	2.485426827	ZFYVE27	zinc finger FYVE-type containing 27	−1.7851013
MX1	MX dynamin like GTPase 1	2.485426827	RHOX4B	reproductive homeobox 4B(Rhox4b)	−1.786596362
SMTN	smoothelin	2.485426827	OLFR1134	olfactory receptor 1134(Olfr1134)	−1.786596362
PLA2G15	phospholipase A2 group XV	2.48194563	CAR11	carbonic anhydrase 11(Carl1)	−1.786596362
OLFR192	olfactory receptor 192(Olfr192)	2.472487771	LRRIQ4	leucine rich repeats and IQ motif containing 4	−1.786596362
ITGB5	integrin subunit beta 5	2.472487771	CASP12	caspase 12 (gene/pseudogene)	−1.786596362
RAPSN	receptor associated protein of the synapse	2.465974465	ODF3L1	outer dense fiber of sperm tails 3 like 1	−1.786596362
SNX3	sorting nexin 3	2.459431619	CCDC3	coiled-coil domain containing 3	−1.786596362
FERMT2	fermitin family member 2	2.459431619	SSPN	sarcospan	−1.786596362
CCR5	C-C motif chemokine receptor 5 (gene/pseudogene)	2.444410478	KLK1	kallikrein 1	−1.786596362
UPK1A	uroplakin 1A	2.439623138	SENP7	SUMO1/sentrin specific peptidase 7	−1.786897131
BCL2L2	BCL2 like 2	2.43629512	CAML	calcium modulating ligand(Caml)	−1.787735284
2610002M06RIK	Description Not Found	2.432959407	YEATS2	YEATS domain containing 2	−1.788627083
CENPN	centromere protein N	2.432959407	SERPINF2	serpin family F member 2	−1.791814071
HBEGF	heparin binding EGF like growth factor	2.43096254	KCNMB1	potassium calcium-activated channel subfamily M regulatory beta subunit 1	−1.792597191
TYMS	thymidylate synthetase	2.427103287	FCHO2	FCH domain only 2	−1.792666489
MGA	MGA, MAX dimerization protein	2.426939834	BBS9	Bardet-Biedl syndrome 9	−1.792734984
RAI14	retinoic acid induced 14	2.426264755	OLFR323	olfactory receptor 323(Olfr323)	−1.794609131
CFI	complement factor I	2.419538892	CD247	CD247 molecule	−1.796081585
PLK4	polo like kinase 4	2.419538892	HIST2H2AA1	histone cluster 2, H2aal(Hist2h2aa1)	−1.796847743
SLC6A9	solute carrier family 6 member 9	2.419538892	PDK1	pyruvate dehydrogenase kinase 1	−1.800563818
TMED2	transmembrane p24 trafficking protein 2	2.419538892	NRARP	NOTCH-regulated ankyrin repeat protein	−1.803049246
TMEM120B	transmembrane protein 120B	2.41857423	BTBD11	BTB domain containing 11	−1.804793263
TRIM36	tripartite motif containing 36	2.417852515	CSF2RA	colony stimulating factor 2 receptor alpha subunit	−1.805089518
CCDC93	coiled-coil domain containing 93	2.416164165	DEXI	Dexi homolog	−1.806998156
SLC25A35	solute carrier family 25 member 35	2.409367225	OLFR1276	olfactory receptor 1276(Olfr1276)	−1.807354922
BNC1	basonuclin 1	2.40599236	TCSTV3	2-cell-stage, variable group, member 3(Tcstv3)	−1.807354922
FOXL2	forkhead box L2	2.40599236	SPRR2D	small proline rich protein 2D	−1.807354922
TFPI2	tissue factor pathway inhibitor 2	2.40599236	SEMA4G	semaphorin 4G	−1.807354922
NET1	neuroepithelial cell transforming 1	2.40599236	KCNK9	potassium two pore domain channel subfamily K member 9	−1.807354922
SLCO2A1	solute carrier organic anion transporter family member 2A1	2.40599236	SNAPC3	small nuclear RNA activating complex polypeptide 3	−1.807385513
A730008H23RIK	Description Not Found	2.399275037	AXIN2	axin 2	−1.808429403
CDKN2B	cyclin dependent kinase inhibitor 2B	2.397264578	PCNXL3	Description Not Found	−1.808995133
ZFP532	zinc finger protein 532(Zfp532)	2.393138801	KLHL7	kelch like family member 7	−1.809016035
GTSE1	G2 and S-phase expressed 1	2.392428431	ZFP281	zinc finger protein 281(Zfp281)	−1.811556991

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
CCDC14	coiled-coil domain containing 14	2.392317423	CHRNB2	cholinergic receptor nicotinic beta 2 subunit	−1.812498225
ADAT1	adenosine deaminase, tRNA specific 1	2.392317423	TBC1D15	TBC1 domain family member 15	−1.812909044
DGKH	diacylglycerol kinase eta	2.392317423	GALNT9	polypeptide N-acetylgalactosaminyltransferase 9	−1.813407449
ZRSR1	zinc finger CCCH-type, RNA binding motif and serine/arginine rich 1	2.392317423	DYNC1I1	dynein cytoplasmic 1 intermediate chain 1	−1.813434179
NFE2	nuclear factor, erythroid 2	2.391529377	MYH8	myosin heavy chain 8	−1.81403224
CD63	CD63 molecule	2.387853137	CEP57	centrosomal protein 57	−1.815684972
MIB1	mindbomb E3 ubiquitin protein ligase 1	2.38645559	LTK	leukocyte receptor tyrosine kinase	−1.817623258
TSN	translin	2.382349023	COMMD2	COMM domain containing 2	−1.817623258
2510003E04RIK	Description Not Found	2.378511623	MEF2C	myocyte enhancer factor 2C	−1.817623258
BC043934	cDNA sequence BC043934(BC043934)	2.378511623	LONRF2	LON peptidase N-terminal domain and ring finger 2	−1.817941412
AHCYL1	adenosylhomocysteinase like 1	2.366734247	PDCD6IP	programmed cell death 6 interacting protein	−1.820575529
OLFR731	olfactory receptor 731(Olfr731)	2.364572432	DHX16	DEAH-box helicase 16	−1.820661084
CDKN2A	cyclin dependent kinase inhibitor 2A	2.364572432	ZFYVE19	zinc finger FYVE-type containing 19	−1.825281028
SLC29A4	solute carrier family 29 member 4	2.364572432	H2-T10	histocompatibility 2, T region locus 10(H2-T10)	−1.826218639
SLC4A10	solute carrier family 4 member 10	2.364572432	ARID1A	AT-rich interaction domain 1A	−1.827043205
CYCS	cytochrome c, somatic	2.351872866	NOD1	nucleotide binding oligomerization domain containing 1	−1.827185706
COL5A1	collagen type V alpha 1	2.350497247	2610318N02RIK	Description Not Found	−1.827819025
UTRN	utrophin	2.350497247	BC048644	cDNA sequence BC048644(BC048644)	−1.827819025
AURKA	aurora kinase A	2.349678136	CDC42EP2	CDC42 effector protein 2	−1.827819025
KREMEN2	kringle containing transmembrane protein 2	2.349431709	CCL25	C-C motif chemokine ligand 25	−1.827819025
FGL2	fibrinogen like 2	2.346409407	TBX6	T-box 6	−1.827819025
NCAM1	neural cell adhesion molecule 1	2.343407822	PLEKHG4	pleckstrin homology and RhoGEF domain containing G4	−1.827819025
ALG8	ALG8, alpha-1,3-glucosyltransferase	2.343407822	RAD18	RAD18, E3 ubiquitin protein ligase	−1.830642494
OLFR703	olfactory receptor 703(Olfr703)	2.336283388	SLC12A9	solute carrier family 12 member 9	−1.830807586
SLC39A10	solute carrier family 39 member 10	2.336283388	NR1D2	nuclear receptor subfamily 1 group D member 2	−1.837943242
HIST1H2AH	histone cluster 1, H2ah	2.322141712	NLK	nemo like kinase	−1.840170811
TSGA8	testis specific gene A8(Tsga8)	2.321928095	TTC37	tetratricopeptide repeat domain 37	−1.840462743
ELOVL2	ELOVL fatty acid elongase 2	2.321928095	DLG3	discs large MAGUK scaffold protein 3	−1.841507525
MLF1	myeloid leukemia factor 1	2.321928095	PCF11	PCF11 cleavage and polyadenylation factor subunit	−1.843349827
FZD6	frizzled class receptor 6	2.321928095	HIST1H4D	histone cluster 1, H4d	−1.846386944
PLD1	phospholipase D1	2.321928095	PEX26	peroxisomal biogenesis factor 26	−1.847440096
IFRD2	interferon-related developmental regulator 2	2.321928095	CYP2B10	cytochrome P450, family 2, subfamily b, polypeptide 10(Cyp2b10)	−1.847996907
OLA1	Obg-like ATPase 1	2.321928095	GDF3	growth differentiation factor 3	−1.847996907
ASPA	aspartoacylase	2.321928095	GPR33	G protein-coupled receptor 33 (gene/pseudogene)	−1.847996907
TGFB3	transforming growth factor beta 3	2.321928095	TDG	thymine DNA glycosylase	−1.847996907
PKIG	protein kinase (cAMP-dependent, catalytic) inhibitor gamma	2.314696526	HIPK3	homeodomain interacting protein kinase 3	−1.847996907
TNFRSF4	tumor necrosis factor receptor superfamily member 4	2.308832886	PAPOLA	poly(A) polymerase alpha	−1.847996907

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
IQCB1	IQ motif containing B1	2.307984443	MAPK4	mitogen-activated protein kinase 4	−1.847996907
SLC16A11	solute carrier family 16 member 11	2.307662797	FRAT2	frequently rearranged in advanced T-cell lymphomas 2	−1.84969115
1190002N15RIK	Description Not Found	2.307428525	HEXIM1	hexamethylene bisacetamide inducible 1	−1.851035845
LCE1L	late cornified envelope 1L(Lce1l)	2.307428525	TATDN2	TatD DNase domain containing 2	−1.851433223
RGS13	regulator of G-protein signaling 13	2.307428525	KLRB1C	killer cell lectin-like receptor subfamily B member 1C(Klrb1c)	−1.854253843
FBXW8	F-box and WD repeat domain containing 8	2.299987517	SLC16A9	solute carrier family 16 member 9	−1.855083462
SNCA	synuclein alpha	2.296457407	ACBD4	acyl-CoA binding domain containing 4	−1.855739032
OSGIN1	oxidative stress induced growth inhibitor 1	2.294491702	REXO1	RNA exonuclease 1 homolog	−1.857980995
BC004004	cDNA sequence BC004004(BC004004)	2.292781749	OLFR1442	olfactory receptor 1442(Olfr1442)	−1.859286959
WNT10A	Wnt family member 10A	2.292781749	PHOSPHO1	phosphoethanolamine/phosphocholine phosphatase	−1.859747926
THG1L	tRNA-histidine guanylyltransferase 1 like	2.292781749	ITPKA	inositol-trisphosphate 3-kinase A	−1.859881803
MLH1	mutL homolog 1	2.292781749	ZFHX2	zinc finger homeobox 2	−1.860513882
RRM2	ribonucleotide reductase regulatory subunit M2	2.289435485	TOR1A	torsin family 1 member A	−1.860949348
SHISA4	shisa family member 4	2.277984747	CDKAL1	CDK5 regulatory subunit associated protein 1 like 1	−1.862794137
DDAH2	dimethylarginine dimethylaminohydrolase 2	2.277984747	SMAD1	SMAD family member 1	−1.863462947
APBA1	amyloid beta precursor protein binding family A member 1	2.269085766	ZC3H13	zinc finger CCCH-type containing 13	−1.863535399
MMAB	methylmalonic aciduria (cobalamin deficiency) cblB type	2.264911693	ZSCAN20	zinc finger and SCAN domain containing 20	−1.863962106
DIAP1	Description Not Found	2.263034406	EPB4.1L4A	Description Not Found	−1.867896464
CAR14	carbonic anhydrase 14(Car14)	2.263034406	ZFP280C	zinc finger protein 280C(Zfp280c)	−1.867896464
C2	complement component 2	2.263034406	GM1322	predicted gene 1322(Gm1322)	−1.867896464
MAG	myelin associated glycoprotein	2.263034406	OLFR472	olfactory receptor 472(Olfr472)	−1.867896464
KCNIP3	potassium voltage-gated channel interacting protein 3	2.263034406	OLFR171	olfactory receptor 171(Olfr171)	−1.867896464
CFD	complement factor D	2.263034406	OLFR1249	olfactory receptor 1249(Olfr1249)	−1.867896464
CCNE1	cyclin E1	2.262723645	PRH1	proline rich protein HaeIII subfamily 1	−1.867896464
RYR1	ryanodine receptor 1	2.261305322	ARSI	arylsulfatase family member I	−1.867896464
PROC	protein C, inactivator of coagulation factors Va and VIIIa	2.255500733	KRT7	keratin 7	−1.867896464
ZFP27	zinc finger protein 27(Zfp27)	2.247927513	PCGF3	polycomb group ring finger 3	−1.867896464
TBX1	T-box 1	2.247927513	PCTP	phosphatidylcholine transfer protein	−1.867896464
DHRS13	dehydrogenase/reductase 13	2.247927513	CALD1	caldesmon 1	−1.867896464
HSPG2	heparan sulfate proteoglycan 2	2.247927513	TREML2	triggering receptor expressed on myeloid cells like 2	−1.867896464
FRMD8	FERM domain containing 8	2.24777312	RTN4RL1	reticulon 4 receptor like 1	−1.867896464
MIOX	myo-inositol oxygenase	2.240579987	PARVA	parvin alpha	−1.868479018
LYRM1	LYR motif containing 1	2.232660757	NPCD	neuronal pentraxin chromo domain(Npcd)	−1.871902039
STAP1	signal transducing adaptor family member 1	2.232660757	RFXANK	regulatory factor X associated ankyrin containing protein	−1.87206109

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
NAT2	N-acetyltransferase 2	2.232660757	MAP3K14	mitogen-activated protein kinase kinase kinase 14	−1.872291304
SRGAP3	SLIT-ROBO Rho GTPase activating protein 3	2.232660757	KLHL9	kelch like family member 9	−1.874528943
NXT2	nuclear transport factor 2 like export factor 2	2.232660757	SESN1	sestrin 1	−1.875260951
RCOR1	REST corepressor 1	2.232660757	ADAMTS7	ADAM metallopeptidase with thrombospondin type 1 motif 7	−1.879404807
SRR	serine racemase	2.230836503	SNAPC1	small nuclear RNA activating complex polypeptide 1	−1.88488993
IKBKAP	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	2.226177109	ADAR	adenosine deaminase, RNA specific	−1.885299379
AI597479	expressed sequence AI597479(AI597479)	2.225819675	LCE1C	late cornified envelope 1C	−1.885626461
POP1	POP1 homolog, ribonuclease P/MRP subunit	2.224966365	FBXO21	F-box protein 21	−1.886155099
SLC35E4	solute carrier family 35 member E4	2.217230716	2610524H06RIK	Description Not Found	−1.887525271
XAB2	XPA binding protein 2	2.217230716	1700016K19RIK	Description Not Found	−1.887525271
MREG	melanoregulin	2.2129258	ZFP715	zinc finger protein 715(Zfp715)	−1.887525271
FKBP11	FK506 binding protein 11	2.210721954	OLFR446	olfactory receptor 446(Olfr446)	−1.887525271
IGF2BP2	insulin like growth factor 2 mRNA binding protein 2	2.207789851	PTK7	protein tyrosine kinase 7 (inactive)	−1.887525271
NUP133	nucleoporin 133	2.207447199	TMEM117	transmembrane protein 117	−1.887525271
OLFR1183	olfactory receptor 1183(Olfr1183)	2.201633861	ITIH2	inter-alpha-trypsin inhibitor heavy chain 2	−1.887525271
IL1F6	interleukin 1 family, member 6(Il1f6)	2.201633861	TAGLN3	transgelin 3	−1.887525271
OTX1	orthodenticle homeobox 1	2.201633861	IFI203	interferon activated gene 203(Ifi203)	−1.887644112
MSH3	mutS homolog 3	2.201633861	ATP1B1	ATPase Na+/K+ transporting subunit beta 1	−1.887664186
SCN4B	sodium voltage-gated channel beta subunit 4	2.201633861	BLCAP	bladder cancer associated protein	−1.888596201
CROCC	ciliary rootlet coiled-coil, rootletin	2.201633861	IGF1R	insulin like growth factor 1 receptor	−1.89024137
NSUN2	NOP2/Sun RNA methyltransferase family member 2	2.194349986	HMG20A	high mobility group 20A	−1.890579593
GAS2L1	growth arrest specific 2 like 1	2.193771743	WDR24	WD repeat domain 24	−1.891527175
3110007F17RIK	Description Not Found	2.190740399	CDX4	caudal type homeobox 4	−1.892655439
DEFB15	defensin beta 15(Defb15)	2.185866545	CLDN18	claudin 18	−1.893449375
C1QTNF2	C1q and tumor necrosis factor related protein 2	2.185866545	IL4RA	interleukin 4 receptor, alpha(Il4ra)	−1.895369594
RAP1GAP	RAP1 GTPase activating protein	2.185866545	RETNLA	resistin like alpha(Retnla)	−1.895739477
SNTB1	syntrophin beta 1	2.185866545	AA388235	expressed sequence AA388235(AA388235)	−1.895739477
FAH	fumarylacetoacetate hydrolase	2.182925576	ZC3H6	zinc finger CCCH-type containing 6	−1.896127489
AVPI1	arginine vasopressin induced 1	2.174393775	D930015E06RIK	RIKEN cDNA D930015E06 gene(D930015E06Rik)	−1.899656973
RPA2	replication protein A2	2.172751912	NPFFR2	neuropeptide FF receptor 2	−1.902073579
BRCA2	BRCA2, DNA repair associated	2.168732488	IRAK1	interleukin 1 receptor associated kinase 1	−1.90243374
RBM47	RNA binding motif protein 47	2.165911939	CWF19L2	CWF19-like 2, cell cycle control (*S. pombe*)	−1.903704505
MSL3L2	male-specific lethal 3-like 2 (*Drosophila*)(Msl312)	2.159061455	STK40	serine/threonine kinase 40	−1.903964448

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
TNFRSF9	tumor necrosis factor receptor superfamily member 9	2.156071704	MARS2	methionyl-tRNA synthetase 2, mitochondrial	−1.904571951
TRF	transferrin(Trf)	2.154588207	RAB5A	RAB5A, member RAS oncogene family	−1.906350687
ZDHHC15	zinc finger DHHC-type containing 15	2.154372546	OLFR1037	olfactory receptor 1037(Olfr1037)	−1.906890596
IGJ	Description Not Found	2.153805336	ARHGAP22	Rho GTPase activating protein 22	−1.906890596
FBXO27	F-box protein 27	2.153805336	DENND1B	DENN domain containing 1B	−1.906890596
ZDHHC24	zinc finger DHHC-type containing 24	2.153805336	EAPP	E2F associated phosphoprotein	−1.906890596
SPCS2	signal peptidase complex subunit 2	2.153805336	ANKRD13D	ankyrin repeat domain 13D	−1.906890596
UCN3	urocortin 3	2.153805336	EFCAB2	EF-hand calcium binding domain 2	−1.906890596
SLC35A1	solute carrier family 35 member A1	2.153805336	HOXC9	homeobox C9	−1.906890596
PODXL	podocalyxin like	2.153805336	SENP6	SUMO1/sentrin specific peptidase 6	−1.907956932
FAM154B	Description Not Found	2.153792145	SIDT1	SID 1 transmembrane family member 1	−1.908286674
NRP1	neuropilin 1	2.147470553	2310057J18RIK	Description Not Found	−1.916476644
ERGIC1	endoplasmic reticulum-golgi intermediate compartment 1	2.147104727	SPRYD4	SPRY domain containing 4	−1.916476644
RNF26	ring finger protein 26	2.146810011	LY6D	lymphocyte antigen 6 complex, locus D	−1.916476644
LCN3	lipocalin 3(Lcn3)	2.137503524	PPARGC1B	PPARG coactivator 1 beta	−1.917291956
FMO1	flavin containing monooxygenase 1	2.137503524	SH3TC1	SH3 domain and tetratricopeptide repeats 1	−1.917906346
RAB20	RAB20, member RAS oncogene family	2.137503524	FOXO1	forkhead box O1	−1.920209106
KATNAL1	katanin catalytic subunit A1 like 1	2.137503524	DHX40	DEAH-box helicase 40	−1.920623917
GPR107	G protein-coupled receptor 107	2.136424717	RECQL5	RecQ like helicase 5	−1.920664575
MELK	maternal embryonic leucine zipper kinase	2.133399125	RBM15	RNA binding motif protein 15	−1.922616041
KCTD9	potassium channel tetramerization domain containing 9	2.13207329	EGLN2	egl-9 family hypoxia inducible factor 2	−1.924079933
PBK	PDZ binding kinase	2.130417144	GPR112	Description Not Found	−1.925999419
ENPP5	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative)	2.124112676	OLFR829	olfactory receptor 829(Olfr829)	−1.925999419
ZDHHC16	zinc finger DHHC-type containing 16	2.12361008	OLFR684	olfactory receptor 684(Olfr684)	−1.925999419
OLFR1346	olfactory receptor 1346(Olfr1346)	2.121015401	RETN	resistin	−1.925999419
MILL1	MHC I like leukocyte 1(Mill1)	2.121015401	ST6GALNAC2	ST6N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	−1.925999419
RHCG	Rh family C glycoprotein	2.121015401	FES	FES proto-oncogene, tyrosine kinase	−1.925999419
CLDN1	claudin 1	2.121015401	KIF13A	kinesin family member 13A	−1.925999419
LHX3	LIM homeobox 3	2.121015401	TRPT1	tRNA phosphotransferase 1	−1.926457816
TUBB2A	tubulin beta 2A class IIa	2.121015401	PLCB2	phospholipase C beta 2	−1.927343833
GSG2	germ cell associated 2, haspin	2.119412265	NADSYN1	NAD synthetase 1	−1.929674394
HYAL2	hyaluronoglucosaminidase 2	2.107345942	4833420G17RIK	Description Not Found	−1.93060469
1700003F12RIK	Description Not Found	2.10433666	P2RY10	purinergic receptor P2Y10	−1.930737338
RUSC2	RUN and SH3 domain containing 2	2.10433666	PPAPDC3	Description Not Found	−1.935459748
LRRIQ3	leucine rich repeats and IQ motif containing 3	2.10433666	DIP2B	disco interacting protein 2 homolog B	−1.935459748
CHSY1	chondroitin sulfate synthase 1	2.10433666	RHAG	Rh-associated glycoprotein	−1.935459748
DUSP23	dual specificity phosphatase 23	2.10433666	EMID1	EMI domain containing 1	−1.935459748

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
RRAGB	Ras related GTP binding B	2.10433666	RNF4	ring finger protein 4	−1.938834579
KCNAB3	potassium voltage-gated channel subfamily A regulatory beta subunit 3	2.10433666	UBL5	ubiquitin like 5	−1.938952478
GRPEL2	GrpE like 2, mitochondrial	2.103129681	PROSC	proline synthetase cotranscribed homolog (bacterial)	−1.94016675
TRAF2	TNF receptor associated factor 2	2.102029095	FZD5	frizzled class receptor 5	−1.942503137
COQ7	coenzyme Q7, hydroxylase	2.100205246	UBE2D1	ubiquitin conjugating enzyme E2 D1	−1.942775467
TMEM126B	transmembrane protein 126B	2.099187297	KLRA7	killer cell lectin-like receptor, subfamily A, member 7(Klra7)	−1.943510757
SGPL1	sphingosine-1-phosphate lyase 1	2.097112667	TMEM63C	transmembrane protein 63C	−1.94425562
CAPN2	calpain 2	2.096447979	2810006K23RIK	Description Not Found	−1.944858446
CHEK2	checkpoint kinase 2	2.088457439	OLFR672	olfactory receptor 672(Olfr672)	−1.944858446
GLRP1	glutamine repeat protein 1(Glrp1)	2.087462841	OLFR1347	olfactory receptor 1347(Olfr1347)	−1.944858446
RTN4R	reticulon 4 receptor	2.087462841	MTTP	microsomal triglyceride transfer protein	−1.944858446
TRIM37	tripartite motif containing 37	2.087462841	MSX1	msh homeobox 1	−1.944858446
NUCB2	nucleobindin 2	2.087462841	BSND	barttin CLCNK type accessory beta subunit	−1.944858446
UBE2T	ubiquitin conjugating enzyme E2 T	2.073616696	MARK1	microtubule affinity regulating kinase 1	−1.944858446
CREB3L3	cAMP responsive element binding protein 3 like 3	2.070389328	CHRNB1	cholinergic receptor nicotinic beta 1 subunit	−1.944858446
CHRM4	cholinergic receptor muscarinic 4	2.070389328	CRYL1	crystallin lambda 1	−1.946419425
SLC16A13	solute carrier family 16 member 13	2.070389328	TEC	tec protein tyrosine kinase	−1.947330641
OLFML2B	olfactomedin like 2B	2.070389328	XKR6	XK related 6	−1.95031589
CSNK1G1	casein kinase 1 gamma 1	2.070389328	ARC	activity-regulated cytoskeleton-associated protein	−1.953636949
S100A14	S100 calcium binding protein A14	2.070389328	WFDC10	WAP four-disulfide core domain 10(Wfdc10)	−1.95419631
SMYD4	SET and MYND domain containing 4	2.070389328	OLFR866	olfactory receptor 866(Olfr866)	−1.959768144
CH25H	cholesterol 25-hydroxylase	2.070389328	WIPI2	WD repeat domain, phosphoinositide interacting 2	−1.960171668
TEX2	testis expressed 2	2.067875748	OLFR948	olfactory receptor 948(Olfr948)	−1.963474124
SYN1	synapsin I	2.063429187	CRTAM	cytotoxic and regulatory T-cell molecule	−1.963474124
CYP3A13	cytochrome P450, family 3, subfamily a, polypeptide 13(Cyp3a13)	2.060581758	CCDC116	coiled-coil domain containing 116	−1.963474124
CBX8	chromobox 8	2.060297534	ALAS2	5'-aminolevulinate synthase 2	−1.963474124
TOR2A	torsin family 2 member A	2.056535553	SDC4	syndecan 4	−1.963474124
E230025N22RIK	Riken cDNA E230025N22 gene(E230025N22Rik)	2.053111336	LENG1	leukocyte receptor cluster member 1	−1.963474124
OLFR963	olfactory receptor 963(Olfr963)	2.053111336	TRIM65	tripartite motif containing 65	−1.963474124
OLFR694	olfactory receptor 694(Olfr694)	2.053111336	ADRA2B	adrenoceptor alpha 2B	−1.963474124
AKR1B8	aldo-keto reductase family 1, member B8(Akr1b8)	2.053111336	CPSF4	cleavage and polyadenylation specific factor 4	−1.964016356
UGDH	UDP-glucose 6-dehydrogenase	2.053111336	LRCH1	leucine rich repeats and calponin homology domain containing 1	−1.966068313
CLPB	ClpB homolog, mitochondrial AAA ATPase chaperonin	2.053111336	CPXM1	carboxypeptidase X (M14 family), member 1	−1.96782195

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
KLHDC9	kelch domain containing 9	2.053111336	PARP6	poly(ADP-ribose) polymerase family member 6	−1.968362498
MCPH1	microcephalin 1	2.051211057	GTF3C2	general transcription factor IIIC subunit 2	−1.975687807
IL2RA	interleukin 2 receptor subunit alpha	2.049225103	NEDD4L	neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein ligase	−1.978518523
CAR9	carbonic anhydrase 9(Car9)	2.044394119	DICER1	dicer 1, ribonuclease III	−1.97959126
USP10	ubiquitin specific peptidase 10	2.044394119	GBA2	glucosylceramidase beta 2	−1.980387638
FASTKD2	FAST kinase domains 2	2.044394119	OLFR1269	olfactory receptor 1269(Olfr1269)	−1.981852653
STRA13	stimulated by retinoic acid 13	2.044394119	EAR10	eosinophil-associated, ribonuclease A family, member 10(Ear10)	−1.981852653
HIST1H2AD	histone cluster 1, H2ad	2.044111161	ADAM5	ADAM metallopeptidase domain 5 (pseudogene)	−1.981852653
PLA1A	phospholipase A1 member A	2.037157781	MED1	mediator complex subunit 1	−1.981852653
MCM3	minichromosome maintenance complex component 3	2.036462274	FGFRL1	fibroblast growth factor receptor-like 1	−1.981852653
PIF1	PIF1 5'-to-3' DNA helicase	2.036094966	EXTL1	exostosin like glycosyltransferase 1	−1.981852653
GALR1	galanin receptor 1	2.03562391	ZFHX3	zinc finger homeobox 3	−1.981852653
DLD	dihydrolipoamide dehydrogenase	2.03562391	FBXO30	F-box protein 30	−1.981852653
GGCX	gamma-glutamyl carboxylase	2.03562391	RNF112	ring finger protein 112	−1.984681148
CEP68	centrosomal protein 68	2.03562391	PARP3	poly(ADP-ribose) polymerase family member 3	−1.98599548
MMP11	matrix metallopeptidase 11	2.03562391	AIRE	autoimmune regulator	−1.986410935
STMN1	stathmin 1	2.033316653	CYB561D1	cytochrome b561 family member D1	−1.987107951
SLCO4A1	solute carrier organic anion transporter family member 4A1	2.03217627	TRAPPC5	trafficking protein particle complex 5	−1.987269174
TIAL1	TIA1 cytotoxic granule-associated RNA binding protein-like 1	2.02888965	RFTN2	raftlin family member 2	−1.98749308
0610009B22RIK	Description Not Found	2.017921908	FRAT1	frequently rearranged in advanced T-cell lymphomas 1	−1.999894159
GM1673	predicted gene 1673(Gm1673)	2.017921908	DMC1	DNA meiotic recombinase 1	−2
CCL26	C-C motif chemokine ligand 26	2.017921908	RIPK4	receptor interacting serine/threonine kinase 4	−2
ZWILCH	zwilch kinetochore protein	2.017921908	PVR	poliovirus receptor	−2
GABRA1	gamma-aminobutyric acid type A receptor alpha1 subunit	2.017921908	LPIN2	lipin 2	−2
ACP2	acid phosphatase 2, lysosomal	2.017143376	THAP2	THAP domain containing 2	−2
FAM131A	family with sequence similarity 131 member A	2.013219985	SHE	Src homology 2 domain containing E	−2
PXMP4	peroxisomal membrane protein 4	2.012497517	ARHGAP25	Rho GTPase activating protein 25	−2.005618551
CDC6	cell division cycle 6	2.011166077	CSF1R	colony stimulating factor 1 receptor	−2.006350699
AXL	AXL receptor tyrosine kinase	2.008131619	ZFP1	ZFP1 zinc finger protein	−2.007904843
RBBP7	RB binding protein 7, chromatin remodeling factor	2.006746832	SFN	stratifin	−2.008988783
PABPC4	poly(A) binding protein cytoplasmic 4	2.005260152	COL17A1	collagen type XVII alpha 1	−2.010386372

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
HIST1H2AK	histone cluster 1, H2ak	2.003307679	XKRX	XK related, X-linked	−2.0105696
MTFMT	mitochondrial methionyl-tRNA formyltransferase	2.001754595	BRD8	bromodomain containing 8	−2.01346226
ZFP449	zinc finger protein 449(Zfp449)	2	ZFP213	zinc finger protein 213(Zfp213)	−2.013532276
D930020B18RIK	RIKEN cDNA D930020B18 gene(D930020B18Rik)	2	ZFY2	zinc finger protein 2, Y-linked(Zfy2)	−2.015657249
LCE1D	late cornified envelope 1D	2	MAP3K3	mitogen-activated protein kinase kinase kinase 3	−2.01612652
UCN	urocortin	2	ZFP445	zinc finger protein 445(Zfp445)	−2.017921908
SYT4	synaptotagmin 4	2	MTAP7D3	MAP7 domain containing 3(Mtap7d3)	−2.017921908
GPR132	G protein-coupled receptor 132	2	TMPRSS11A	transmembrane protease, serine 11A	−2.017921908
SDHD	succinate dehydrogenase complex subunit D	2	OLFM2	olfactomedin 2	−2.017921908
PANK3	pantothenate kinase 3	2	GRM4	glutamate metabotropic receptor 4	−2.017921908
SBSN	suprabasin	1.99095486	ONECUT2	one cut homeobox 2	−2.017921908
WDR59	WD repeat domain 59	1.989976974	HNRNPH3	heterogeneous nuclear ribonucleoprotein H3	−2.017921908
MTMR9	myotubularin related protein 9	1.987844644	ZMYM5	zinc finger MYM-type containing 5	−2.020204421
IL15RA	interleukin 15 receptor subunit alpha	1.985628881	RAPGEF6	Rap guanine nucleotide exchange factor 6	−2.020953989
RHBDF2	rhomboid 5 homolog 2	1.984681148	CD34	CD34 molecule	−2.026714044
NHLRC2	NHL repeat containing 2	1.98375117	ACVR2B	activin A receptor type 2B	−2.026714044
NMRAL1	NmrA-like family domain containing 1	1.983370163	RILP	Rab interacting lysosomal protein	−2.026800059
OLFR120	olfactory receptor 120(Olfr120)	1.981852653	EMR1	Description Not Found	−2.031218731
OLFR1051	olfactory receptor 1051(Olfr1051)	1.981852653	DNAJA2	DnaJ heat shock protein family (Hsp40) member A2	−2.031291874
PCDHGA9	protocadherin gamma subfamily A, 9	1.981852653	SEMA4B	semaphorin 4B	−2.031985281
FST	follistatin	1.981852653	1700015E13RIK	Description Not Found	−2.03562391
RECQL4	RecQ like helicase 4	1.976611605	RHOX1	reproductive homeobox 1(Rhox1)	−2.03562391
NFKBIL1	NFKB inhibitor like 1	1.970969489	TCP11	t-complex 11	−2.03562391
TUBD1	tubulin delta 1	1.964367355	FBXW11	F-box and WD repeat domain containing 11	−2.03562391
FSD1	fibronectin type III and SPRY domain containing 1	1.963474124	ALX1	ALX homeobox 1	−2.03562391
GDF5	growth differentiation factor 5	1.963474124	BST1	bone marrow stromal cell antigen 1	−2.03562391
TREML4	triggering receptor expressed on myeloid cells like 4	1.963474124	GPR83	G protein-coupled receptor 83	−2.03562391
SORD	sorbitol dehydrogenase	1.963474124	RECK	reversion inducing cysteine rich protein with kazal motifs	−2.036112118
HEBP1	heme binding protein 1	1.963474124	ABHD14B	abhydrolase domain containing 14B	−2.040460993
KDELR2	KDEL endoplasmic reticulum protein retention receptor 2	1.96155465	GPRC6A	G protein-coupled receptor class C group 6 member A	−2.042122888
TRPV4	transient receptor potential cation channel subfamily V member 4	1.958842675	GRAMD3	GRAM domain containing 3	−2.042296131
ABHD5	abhydrolase domain containing 5	1.957389419	IMPACT	impact RWD domain protein	−2.042436285
YOD1	YOD1 deubiquitinase	1.95419631	TOP1	topoisomerase (DNA) I	−2.044394119
MAGOHB	mago homolog B, exon junction complex core component	1.952932368	NACC2	NACC family member 2	−2.044394119
TSPAN2	tetraspanin 2	1.95176103	PKNOX1	PBX/knotted 1 homeobox 1	−2.045797958
LDB3	LIM domain binding 3	1.94850842	TMEM79	transmembrane protein 79	−2.046628729
1700067P10RIK	Description Not Found	1.944858446	MYCBP2	MYC binding protein 2, E3 ubiquitin protein ligase	−2.047368853

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
9530091C08RIK	Description Not Found	1.944858446	MAS1	MAS1 proto-oncogene, G protein-coupled receptor	−2.048055651
RHOJ	ras homolog family member J	1.944858446	GEMIN6	gem nuclear organelle associated protein 6	−2.053111336
SFRP1	secreted frizzled related protein 1	1.944858446	TMEM100	transmembrane protein 100	−2.053111336
XPNPEP2	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane-bound	1.944858446	FOXI1	forkhead box I1	−2.053111336
RNASE4	ribonuclease A family member 4	1.935459748	OPLAH	5-oxoprolinase (ATP-hydrolysing)	−2.053111336
NAPSA	napsin A aspartic peptidase	1.931586931	BC094916	Description Not Found	−2.058337935
TIMM22	translocase of inner mitochondrial membrane 22 homolog (yeast)	1.931202999	GZMM	granzyme M	−2.061193332
MTCH2	mitochondrial carrier 2	1.929774464	RCOR2	REST corepressor 2	−2.06280495
ADCK4	aarF domain containing kinase 4	1.927921426	NR2E1	nuclear receptor subfamily 2 group E member 1	−2.06366268
PDSS1	prenyl (decaprenyl) diphosphate synthase, subunit 1	1.926245513	NT5DC1	5'-nucleotidase domain containing 1	−2.065994119
ZFP94	zinc finger protein 94(Zfp94)	1.925999419	SCN8A	sodium voltage-gated channel alpha subunit 8	−2.06750099
FABP9	fatty acid binding protein 9	1.925999419	CBX7	chromobox 7	−2.06750099
RNF170	ring finger protein 170	1.925999419	FHAD1	forkhead associated phosphopeptide binding domain 1	−2.068114527
TLR3	toll like receptor 3	1.925999419	KCNQ3	potassium voltage-gated channel subfamily Q member 3	−2.068885643
LIPH	lipase H	1.925999419	BC025920	zinc finger protein pseudogene(BC025920)	−2.070389328
PLEKHA7	pleckstrin homology domain containing A7	1.925999419	FCGR1	Fc receptor, IgG, high affinity I(Fcgr1)	−2.070389328
LXN	latexin	1.9244606	SYN3	synapsin III	−2.070389328
PPCS	phosphopantothenoylcysteine synthetase	1.92294738	KLHL5	kelch like family member 5	−2.070389328
BTRC	beta-transducin repeat containing E3 ubiquitin protein ligase	1.92065845	EDA2R	ectodysplasin A2 receptor	−2.070389328
APIP	APAF1 interacting protein	1.920326443	STK38	serine/threonine kinase 38	−2.070389328
ANK1	ankyrin 1	1.916476644	CDKN2D	cyclin dependent kinase inhibitor 2D	−2.072205467
TOMM70A	translocase of outer mitochondrial membrane 70 homolog A (yeast)(Tomm70a)	1.913107017	IL6ST	interleukin 6 signal transducer	−2.072660321
ABCB1B	ATP-binding cassette, sub-family B (MDR/TAP), member 1B(Abcb1b)	1.908033945	OLFR427	olfactory receptor 427(Olfr427)	−2.074318985
ACN9	Description Not Found	1.906890596	BAIAP2	BAI1 associated protein 2	−2.078951341
DLX1AS	distal-less homeobox 1, antisense(Dlx1as)	1.906890596	TIMP2	TIMP metallopeptidase inhibitor 2	−2.079805224
MRGPRD	MAS related GPR family member D	1.906890596	CDCP1	CUB domain containing protein 1	−2.083991945
WDHD1	WD repeat and HMG-box DNA binding protein 1	1.906890596	RGS14	regulator of G-protein signaling 14	−2.084198537
USP46	ubiquitin specific peptidase 46	1.906890596	VASP	vasodilator-stimulated phosphoprotein	−2.086359868
PKN3	protein kinase N3	1.906890596	ZFP318	zinc finger protein 318(Zfp318)	−2.087462841
OSCAR	osteoclast associated, immunoglobulin-like receptor	1.906890596	PSG25	pregnancy-specific glycoprotein 25(Psg25)	−2.087462841
CDK2	cyclin dependent kinase 2	1.906746727	PDZD8	PDZ domain containing 8	−2.087462841
TRIM62	tripartite motif containing 62	1.905520967	DET1	de-etiolated homolog 1 (*Arabidopsis*)	−2.087462841
SQLE	squalene epoxidase	1.903767694	CHST3	carbohydrate sulfotransferase 3	−2.087462841

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
MCM10	minichromosome maintenance 10 replication initiation factor	1.89598378	EHHADH	enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase	−2.087462841
CCDC90B	coiled-coil domain containing 90B	1.894803124	FCGRT	Fc fragment of IgG receptor and transporter	−2.090735607
SPATS1	spermatogenesis associated serine rich 1	1.892848083	CFP	complement factor properdin	−2.09437407
GPNMB	glycoprotein nmb	1.891427809	SOCS6	suppressor of cytokine signaling 6	−2.094638136
MST1	macrophage stimulating 1	1.88993148	SYT11	synaptotagmin 11	−2.09592442
LTB4R1	leukotriene B4 receptor 1(Ltb4r1)	1.887644112	MBTPS2	membrane bound transcription factor peptidase, site 2	−2.09592442
DNAJC5B	DnaJ heat shock protein family (Hsp40) member C5 beta	1.887525271	MEFV	Mediterranean fever	−2.097059135
PCDHGC4	protocadherin gamma subfamily C, 4	1.887525271	SRPK2	SRSF protein kinase 2	−2.10044313
HMX2	H6 family homeobox 2	1.887525271	DUSP16	dual specificity phosphatase 16	−2.102740277
NDUFAB1	NADH: ubiquinone oxidoreductase subunit AB1	1.887525271	SLC6A7	solute carrier family 6 member 7	−2.103129681
MGP	matrix Gla protein	1.887525271	HBB-B1	hemoglobin, beta adult major chain(Hbb-b1)	−2.10433666
ZKSCAN2	zinc finger with KRAB and SCAN domains 2	1.887525271	TNPO3	transportin 3	−2.10433666
CCDC51	coiled-coil domain containing 51	1.887525271	CSNK2B	casein kinase 2 beta	−2.10433666
CTSK	cathepsin K	1.887525271	BCAS1	breast carcinoma amplified sequence 1	−2.10433666
PRDM9	PR domain 9	1.887525271	INO80	INO80 complex subunit	−2.10433666
C8A	complement component 8 alpha subunit	1.887525271	MPG	N-methylpurine DNA glycosylase	−2.10433666
NEUROG1	neurogenin 1	1.887082413	FOXP1	forkhead box P1	−2.107557734
NUSAP1	nucleolar and spindle associated protein 1	1.886951242	USP21	ubiquitin specific peptidase 21	−2.107658353
LZIC	leucine zipper and CTNNBIP1 domain containing	1.877899051	LIMS1	LIM zinc finger domain containing 1	−2.112700133
ZFP609	zinc finger protein 609(Zfp609)	1.87774425	FXYD1	FXYD domain containing ion transport regulator 1	−2.112700133
GPR87	G protein-coupled receptor 87	1.87774425	POU3F1	POU class 3 homeobox 1	−2.113574207
GMPPB	GDP-mannose pyrophosphorylase B	1.871523637	OLFR591	olfactory receptor 591(Olfr591)	−2.114494844
TMEM115	transmembrane protein 115	1.870364796	GRAMD4	GRAM domain containing 4	−2.114673101
DSN1	DSN1 homolog, MIS12 kinetochore complex component	1.868479018	BCL2	BCL2, apoptosis regulator	−2.115878669
A530099J19RIK	Description Not Found	1.867896464	PELI3	pellino E3 ubiquitin protein ligase family member 3	−2.118915146
1700007K09RIK	Description Not Found	1.867896464	PPP1CB	protein phosphatase 1 catalytic subunit beta	−2.119236221
1810043G02RIK	Description Not Found	1.867896464	TFF2	trefoil factor 2	−2.121015401
UCHL1	ubiquitin C-terminal hydrolase L1	1.867896464	GCA	grancalcin	−2.121015401
PTCH2	patched 2	1.867896464	LYL1	LYL1, basic helix-loop-helix family member	−2.121015401
APBB3	amyloid beta precursor protein binding family B member 3	1.867896464	ATG4B	autophagy related 4B cysteine peptidase	−2.121015401
PTER	phosphotriesterase related	1.867896464	CCDC102A	coiled-coil domain containing 102A	−2.121015401
PRKCE	protein kinase C epsilon	1.867896464	ATP2A1	ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 1	−2.121015401

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
PLEKHM3	pleckstrin homology domain containing M3	1.867896464	TERF2	telomeric repeat binding factor 2	−2.123585568
HIST1H4C	histone cluster 1, H4c	1.867896464	LCN5	lipocalin 5(Lcn5)	−2.124432612
PLS3	plastin 3	1.867896464	TM6SF1	transmembrane 6 superfamily member 1	−2.124533495
DUSP4	dual specificity phosphatase 4	1.867686654	SSBP2	single stranded DNA binding protein 2	−2.129283017
SCLY	selenocysteine lyase	1.862802277	KRTAP6-2	keratin associated protein 6-2	−2.137503524
RPRD1A	regulation of nuclear pre-mRNA domain containing 1A	1.861777838	CRHBP	corticotropin releasing hormone binding protein	−2.137503524
CCRL2	C-C motif chemokine receptor like 2	1.86175579	TOPBP1	topoisomerase (DNA) II binding protein 1	−2.137503524
CCT7	chaperonin containing TCP1 subunit 7	1.861636037	SLC35A3	solute carrier family 35 member A3	−2.137503524
ZFP217	zinc finger protein 217(Zfp217)	1.861097096	CACNB4	calcium voltage-gated channel auxiliary subunit beta 4	−2.137503524
ACTN4	actinin alpha 4	1.859689938	TASP1	taspase 1	−2.137503524
KCNA3	potassium voltage-gated channel subfamily A member 3	1.859135363	HMBOX1	homeobox containing 1	−2.145313833
CUL7	cullin 7	1.858597911	ZFP62	ZFP62 zinc finger protein	−2.145677455
LRRC59	leucine rich repeat containing 59	1.857543219	PCDHB4	protocadherin beta 4	−2.148666128
PHTF2	putative homeodomain transcription factor 2	1.855602651	SLC35F3	solute carrier family 35 member F3	−2.15120644
KDELC1	KDEL motif containing 1	1.852556218	AW549877	expressed sequence AW549877(AW549877)	−2.151324826
SEC24D	SEC24 homolog D, COPII coat complex component	1.8483841	GIMAP9	GTPase, IMAP family member 9(Gimap9)	−2.152400921
OLFR222	olfactory receptor 222(Olfr222)	1.847996907	ZFP329	zinc finger protein 329(Zfp329)	−2.153805336
OLFR118	olfactory receptor 118(Olfr118)	1.847996907	KRT74	keratin 74	−2.153805336
CASKIN2	CASK interacting protein 2	1.847996907	REG3A	regenerating family member 3 alpha	−2.153805336
TPK1	thiamin pyrophosphokinase 1	1.847996907	RAB4A	RAB4A, member RAS oncogene family	−2.154308231
NOL3	nucleolar protein 3	1.847996907	CECR5	cat eye syndrome chromosome region, candidate 5	−2.155682653
UBA6	ubiquitin like modifier activating enzyme 6	1.847388943	ESM1	endothelial cell specific molecule 1	−2.157156463
RAVER1	ribonucleoprotein, PTB binding 1	1.846151947	HS6ST1	heparan sulfate 6-O-sulfotransferase 1	−2.164820712
NAT10	N-acetyltransferase 10	1.843300131	DDB2	damage specific DNA binding protein 2	−2.168338824
HIST1H3H	histone cluster 1, H3h	1.842055889	5430435G22RIK	Description Not Found	−2.169925001
SNX8	sorting nexin 8	1.840985134	ALOX12B	arachidonate 12-lipoxygenase, 12R type	−2.169925001
POLR3K	polymerase (RNA) III subunit K	1.839538616	SLC34A3	solute carrier family 34 member 3	−2.169925001
WDR55	WD repeat domain 55	1.835957408	TNS4	tensin 4	−2.169925001
WDR93	WD repeat domain 93	1.830541464	CANX	calnexin	−2.169925001
PLSCR1	phospholipid scramblase 1	1.828635636	BET1	Bet1 golgi vesicular membrane trafficking protein	−2.169925001
ARL6	ADP ribosylation factor like GTPase 6	1.827819025	BEST2	bestrophin 2	−2.169925001
NOL9	nucleolar protein 9	1.827819025	USP28	ubiquitin specific peptidase 28	−2.172998154
PNKD	paroxysmal nonkinesigenic dyskinesia	1.827819025	PDE4B	phosphodiesterase 4B	−2.173614018
TMEM139	transmembrane protein 139	1.827819025	CNOT4	CCR4-NOT transcription complex subunit 4	−2.177917792
ASPH	aspartate beta-hydroxylase	1.827819025	NECAP1	NECAP endocytosis associated 1	−2.178043245
LZTFL1	leucine zipper transcription factor like 1	1.827819025	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	−2.178565309
RHEBL1	Ras homolog enriched in brain like 1	1.827819025	SLC10A7	solute carrier family 10 member 7	−2.17990909

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
CHCHD5	coiled-coil-helix-coiled-coil-helix domain containing 5	1.82552849	IL17A	interleukin 17A	−2.181702586
GPD2	glycerol-3-phosphate dehydrogenase 2	1.824148697	ERICH1	glutamate rich 1	−2.182286216
STK39	serine/threonine kinase 39	1.823608879	HN1L	hematological and neurological expressed 1-like	−2.185866545
MAGED2	MAGE family member D2	1.820863253	SLFNL1	schlafen like 1	−2.185866545
TBC1D9B	TBC1 domain family member 9B	1.813219568	MYOD1	myogenic differentiation 1	−2.185866545
LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	1.809540228	TRIM35	tripartite motif containing 35	−2.185866545
OLFR859	olfactory receptor 859(Olfr859)	1.807354922	CHRNE	cholinergic receptor nicotinic epsilon subunit	−2.186397884
OLFR1225	olfactory receptor 1225(Olfr1225)	1.807354922	PHF21A	PHD finger protein 21A	−2.190943197
IFNA11	interferon alpha 11(Ifna11)	1.807354922	HIST1H2AE	histone cluster 1, H2ae	−2.196698179
ARG1	arginase 1	1.807354922	SATB1	SATB homeobox 1	−2.198659952
ASCL3	achaete-scute family bHLH transcription factor 3	1.807354922	LCN8	lipocalin 8	−2.201633861
AGA	aspartylglucosaminidase	1.807354922	ABCG5	ATP binding cassette subfamily G member 5	−2.201633861
MAP3K12	mitogen-activated protein kinase kinase kinase 12	1.806530545	KRBA1	KRAB-A domain containing 1	−2.202959029
COMMD10	COMM domain containing 10	1.802771724	CD274	CD274 molecule	−2.206081393
STYX	serine/threonine/tyrosine interacting protein	1.801251483	DYRK2	dual specificity tyrosine phosphorylation regulated kinase 2	−2.206730511
EPHA6	EPH receptor A6	1.797583147	ZFP292	zinc finger protein 292(Zfp292)	−2.209453366
SERPINA3F	serine (or cysteine) peptidase inhibitor, clade A, member 3F(Serpina3f)	1.794445043	PRX	periaxin	−2.209453366
PUS10	pseudouridylate synthase 10	1.791814071	SPAG1	sperm associated antigen 1	−2.209453366
RASL12	RAS like family 12	1.791652715	ASGR2	asialoglycoprotein receptor 2	−2.209784456
MRPL51	mitochondrial ribosomal protein L51	1.787631232	PTEN	phosphatase and tensin homolog	−2.215013513
OLFR1306	olfactory receptor 1306(Olfr1306)	1.786596362	IL1A	interleukin 1 alpha	−2.217230716
BCL2A1C	B cell leukemia/lymphoma 2 related protein A1c(Bcl2a1c)	1.786596362	TPCN2	two pore segment channel 2	−2.217230716
HOXD1	homeobox D1	1.786596362	IKBKB	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	−2.217230716
MEMO1	mediator of cell motility 1	1.786596362	ST6GAL1	ST6 beta-galactoside alpha-2,6-sialyltransferase 1	−2.218342351
ARCN1	archain 1	1.786596362	TMEM161A	transmembrane protein 161A	−2.232660757
NUDT10	nudix hydrolase 10	1.786596362	STK32B	serine/threonine kinase 32B	−2.232660757
SLC4A4	solute carrier family 4 member 4	1.786596362	CHST14	carbohydrate sulfotransferase 14	−2.232660757
DHRS4	dehydrogenase/reductase 4	1.786596362	AQP3	aquaporin 3 (Gill blood group)	−2.232660757
TOM1	target of myb1 membrane trafficking protein	1.786596362	RASSF3	Ras association domain family member 3	−2.233505898
TST	thiosulfate sulfurtransferase	1.786596362	OTUD7B	OTU deubiquitinase 7B	−2.242923867
RIPK2	receptor interacting serine/threonine kinase 2	1.784428584	AP3M2	adaptor related protein complex 3 mu 2 subunit	−2.247481244

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
NAIP2	NLR family, apoptosis inhibitory protein 2(Naip2)	1.780351745	PSMA6	proteasome subunit alpha 6	−2.247927513
OLFR133	olfactory receptor 133(Olfr133)	1.77946628	PRCC	papillary renal cell carcinoma (translocation-associated)	−2.247927513
NBR1	NBR1, autophagy cargo receptor	1.776995396	ZFP688	zinc finger protein 688(Zfp688)	−2.262218541
GLIS1	GLIS family zinc finger 1	1.776512203	DOCK11	dedicator of cytokinesis 11	−2.262218541
SLC35A2	solute carrier family 35 member A2	1.776232819	PLA2G4F	phospholipase A2 group IVF	−2.263034406
AU022252	expressed sequence AU022252(AU022252)	1.774559318	MYPN	myopalladin	−2.263034406
OLFR64	olfactory receptor 64(Olfr64)	1.773991786	FRS2	fibroblast growth factor receptor substrate 2	−2.263034406
PPAPDC2	Description Not Found	1.771983065	STARD6	StAR related lipid transfer domain containing 6	−2.263034406
DIS3	DIS3 homolog, exosome endoribonuclease and 3'-5' exoribonuclease	1.771375295	WSCD2	WSC domain containing 2	−2.270653766
4931440F15RIK	Description Not Found	1.770829046	TLE1	transducin like enhancer of split 1	−2.272631746
ZFP771	zinc finger protein 771(Zfp771)	1.77019569	HDHD3	haloacid dehalogenase like hydrolase domain containing 3	−2.272966802
HMBS	hydroxymethylbilane synthase	1.769676967	1700029J07RIK	Description Not Found	−2.277984747
RCC1	regulator of chromosome condensation 1	1.768267605	CLEC2D	C-type lectin domain family 2 member D	−2.277984747
SPAG5	sperm associated antigen 5	1.767980257	PPM1G	protein phosphatase, Mg2+/Mn2+ dependent 1G	−2.277984747
TSPAN31	tetraspanin 31	1.767626782	CDKN1B	cyclin dependent kinase inhibitor 1B	−2.280970508
PCDHGB8	protocadherin gamma subfamily B, 8(Pcdhgb8)	1.765534746	OASL1	2'-5' oligoadenylate synthetase-like 1(Oasl1)	−2.28169825
PRL2B1	prolactin family 2, subfamily b, member 1(Prl2b1)	1.765534746	G0S2	G0/G1 switch 2	−2.282045463
OBOX5	oocyte specific homeobox 5(Obox5)	1.765534746	TMEM17	transmembrane protein 17	−2.285402219
PIK3R3	phosphoinositide-3-kinase regulatory subunit 3	1.765534746	BLVRB	biliverdin reductase B	−2.290619427
MAP3K4	mitogen-activated protein kinase kinase kinase 4	1.765534746	GOSR1	golgi SNAP receptor complex member 1	−2.290897209
LRRC30	leucine rich repeat containing 30	1.765534746	ZFP26	zinc finger protein 26(Zfp26)	−2.292781749
EN2	engrailed homeobox 2	1.765534746	CXCL2	C-X-C motif chemokine ligand 2	−2.292781749
HOOK3	hook microtubule-tethering protein 3	1.765534746	SNX7	sorting nexin 7	−2.292781749
MYO9A	myosin IXA	1.765534746	ZDHHC23	zinc finger DHHC-type containing 23	−2.292781749
STX7	syntaxin 7	1.765060364	GALNT6	polypeptide N-acetylgalactosaminyltransferase 6	−2.292781749
ATM	ATM serine/threonine kinase	1.763504031	AMPD1	adenosine monophosphate deaminase 1	−2.297844157
KCNK6	potassium two pore domain channel subfamily K member 6	1.763385753	GIMAP5	GTPase, IMAP family member 5	−2.303246615
PQLC3	PQ loop repeat containing 3	1.759954577	ATP5F1	ATP synthase, H+ transporting, mitochondrial Fo complex subunit B1	−2.305399163
KIFAP3	kinesin associated protein 3	1.758843168	LHFPL2	lipoma HMGIC fusion partner-like 2	−2.307428525
E2F4	E2F transcription factor 4	1.757752886	KIF1B	kinesin family member 1B	−2.313231129
ETV5	ETS variant 5	1.757709335	TLE6	transducin like enhancer of split 6	−2.321928095

TABLE 2-continued

Differentially regulated genes in $CD8^+4$-$1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
GTF2E2	general transcription factor IIE subunit 2	1.75666387	SHF	Src homology 2 domain containing F	−2.330691998
GPR150	G protein-coupled receptor 150	1.75547927	NGFR	nerve growth factor receptor	−2.331438521
E130308A19RIK	RIKEN cDNA E130308A19 gene(E130308A19Rik)	1.754887502	KLRA4	killer cell lectin-like receptor, subfamily A, member 4(Klra4)	−2.334485632
DPYSL4	dihydropyrimidinase like 4	1.754887502	ITGAE	integrin subunit alpha E	−2.335948972
FNBP1	formin binding protein 1	1.75468902	PQLC2	PQ loop repeat containing 2	−2.336141568
TMOD4	tropomodulin 4	1.754064107	KLRB1A	killer cell lectin-like receptor subfamily B member 1A(Klrb1a)	−2.336283388
ERLIN1	ER lipid raft associated 1	1.751154691	IRF9	interferon regulatory factor 9	−2.336308285
ENOPH1	enolase-phosphatase 1	1.748447442	GATA3	GATA binding protein 3	−2.338971433
RAB31	RAB31, member RAS oncogene family	1.746215332	RSAD2	radical S-adenosyl methionine domain containing 2	−2.33997952
HOXA6	homeobox A6	1.745184623	RNF215	ring finger protein 215	−2.341976415
TAS2R126	taste receptor, type 2, member 126(Tas2r126)	1.744161096	IL7R	interleukin 7 receptor	−2.343395577
AGXT2	alamne-glyoxylate aminotransferase 2	1.744161096	ACP5	acid phosphatase 5, tartrate resistant	−2.345270806
STK32C	serine/threonine kinase 32C	1.744161096	STYXL1	serine/threonine/tyrosine interacting-like 1	−2.346956889
P2RY2	purinergic receptor P2Y2	1.744161096	NOXO1	NADPH oxidase organizer 1	−2.35030956
NWD1	NACHT and WD repeat domain containing 1	1.744161096	IGFALS	insulin like growth factor binding protein acid labile subunit	−2.358664554
UQCRQ	ubiquinol-cytochrome c reductase complex III subunit VII	1.744161096	STIM1	stromal interaction molecule 1	−2.359335599
PPP1R3A	protein phosphatase 1 regulatory subunit 3A	1.744161096	TMEM186	transmembrane protein 186	−2.361030771
GOLT1A	golgi transport 1A	1.744161096	OLFR1043	olfactory receptor 1043(Olfr1043)	−2.364572432
EZH1	enhancer of zeste 1 polycomb repressive complex 2 subunit	1.744161096	D8ERTD82E	DNA segment, Chr 8, ERATO Doi 82, expressed(D8Ertd82e)	−2.364572432
MTHFD2	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	1.744154314	MYOG	myogenin	−2.364572432
PGRMC1	progesterone receptor membrane component 1	1.742545062	NCLN	nicalin	−2.364572432
DNAJB12	DnaJ heat shock protein family (Hsp40) member B12	1.741863621	MTSS1	metastasis suppressor 1	−2.364572432
DNAJC11	DnaJ heat shock protein family (Hsp40) member C11	1.738767837	TRMU	tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase	−2.364572432
TOMM6	translocase of outer mitochondrial membrane 6	1.738448709	EMILIN2	elastin microfibril interfacer 2	−2.369119767
RPS6KL1	ribosomal protein S6 kinase like 1	1.738393453	MPV17L	MPV17 mitochondrial inner membrane protein like	−2.371558863
CDC73	cell division cycle 73	1.73665741	WWC2	WW and C2 domain containing 2	−2.371558863
NDC80	NDC80, kinetochore complex component	1.732078892	TMEM178	transmembrane protein 178(Tmem178)	−2.374005585
TACC3	transforming acidic coiled-coil containing protein 3	1.731372884	TPCN1	two pore segment channel 1	−2.375232208
CPSF3	cleavage and polyadenylation specific factor 3	1.727926568	LRRC45	leucine rich repeat containing 45	−2.377207351
ARID3A	AT-rich interaction domain 3A	1.726471722	1110059G10RIK	Description Not Found	−2.377915929
LLPH	LLP homolog, long-term synaptic facilitation	1.726107859	MCOLN2	mucolipin 2	−2.378511623

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
PCNA	proliferating cell nuclear antigen	1.725441599	DDX58	DEXD/H-box helicase 58	−2.378511623
GJC2	gap junction protein gamma 2	1.722978517	H2-OA	histocompatibility 2, O region alpha locus(H2-Oa)	−2.382329516
OLFR373	olfactory receptor 373(Olfr373)	1.722466024	RARG	retinoic acid receptor gamma	−2.388827772
H2-T24	histocompatibility 2, T region locus 24(H2-T24)	1.722466024	SERPINB1A	serine (or cysteine) peptidase inhibitor, clade B, member 1a(Serpinb1a)	−2.392317423
AKAP7	A-kinase anchoring protein 7	1.722466024	GHRL	ghrelin/obestatin prepropeptide	−2.392317423
NDUFB7	NADH: ubiquinone oxidoreductase subunit B7	1.722466024	ZMAT4	zinc finger matrin-type 4	−2.392317423
PRR11	proline rich 11	1.722466024	BTBD6	BTB domain containing 6	−2.392897478
TJP1	tight junction protein 1	1.722466024	KLRA16	killer cell lectin-like receptor, subfamily A, member 16(Klra16)	−2.394534969
S100A3	S100 calcium binding protein A3	1.722466024	EPS15L1	epidermal growth factor receptor pathway substrate 15 like 1	−2.397012831
KRT78	keratin 78	1.718729711	VCPIP1	valosin containing protein interacting protein 1	−2.397303585
GMDS	GDP-mannose 4,6-dehydratase	1.717904741	RRP7A	ribosomal RNA processing 7 homolog A	−2.404992223
PDGFB	platelet derived growth factor subunit B	1.714400534	IL1B	interleukin 1 beta	−2.40599236
SLC36A1	solute carrier family 36 member 1	1.714297338	NAT14	N-acetyltransferase 14 (putative)	−2.40599236
RSU1	Ras suppressor protein 1	1.712647036	SLC40A1	solute carrier family 40 member 1	−2.40599236
STX12	syntaxin 12	1.711911478	RAB37	RAB37, member RAS oncogene family	−2.40599236
SLC25A34	solute carrier family 25 member 34	1.711494907	IL17RA	interleukin 17 receptor A	−2.40599236
AFG3L2	AFG3 like matrix AAA peptidase subunit 2	1.711057	BACE1	beta-secretase 1	−2.40599236
RPL24	ribosomal protein L24	1.709193708	CTNS	cystinosin, lysosomal cystine transporter	−2.40599236
UBE3C	ubiquitin protein ligase E3C	1.708789682	IFIT3	interferon induced protein with tetratricopeptide repeats 3	−2.411404504
CAR12	carbonic anhydrase 12(Car12)	1.70867626	ZFYVE21	zinc finger FYVE-type containing 21	−2.412378292
ZFP207	zinc finger protein 207(Zfp207)	1.707603009	1700016D06RIK	Description Not Found	−2.419538892
XIST	X inactive specific transcript (non-protein coding)	1.706065607	STK25	serine/threonine kinase 25	−2.419538892
NCAPD2	non-SMC condensin I complex subunit D2	1.705012178	PLEKHJ1	pleckstrin homology domain containing J1	−2.419538892
ZSWIM2	zinc finger SWIM-type containing 2	1.704802998	TGIF2	TGFB induced factor homeobox 2	−2.419538892
CASP1	caspase 1	1.70065942	SLC25A29	solute carrier family 25 member 29	−2.419538892
OLFR701	olfactory receptor 701(Olfr701)	1.700439718	DAPL1	death associated protein like 1	−2.419661316
CBLC	Cbl proto-oncogene C	1.700439718	P2RX4	purinergic receptor P2X 4	−2.425748008
HIST1H2AC	histone cluster 1, H2ac	1.700439718	1700001O22RIK	Description Not Found	−2.426264755
EPHA10	EPH receptor A10	1.700439718	C9	complement component 9	−2.429615964
NDUFC2	NADH: ubiquinone oxidoreductase subunit C2	1.700439718	KLF13	Kruppel like factor 13	−2.430628023
DLG1	discs large MAGUK scaffold protein 1	1.700439718	GADD45A	growth arrest and DNA damage inducible alpha	−2.432591239
SCN10A	sodium voltage-gated channel alpha subunit 10	1.700439718	OLFR788	olfactory receptor 788(Olfr788)	−2.432959407
RGL3	ral guanine nucleotide dissociation stimulator like 3	1.700439718	FADS6	fatty acid desaturase 6	−2.432959407
TMCO3	transmembrane and coiled-coil domains 3	1.700439718	CHCHD2	coiled-coil-helix-coiled-coil-helix domain containing 2	−2.432959407

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
BCL2L14	BCL2 like 14	1.700439718	MPPE1	metallophosphoesterase 1	−2.432959407
THOP1	thimet oligopeptidase 1	1.700290033	CHAC1	ChaC glutathione specific gamma-glutamylcyclotransferase 1	−2.432959407
MTIF3	mitochondrial translational initiation factor 3	1.698305331	2310011J03RIK	Description Not Found	−2.435017448
XDH	xanthine dehydrogenase	1.697717724	LRSAM1	leucine rich repeat and sterile alpha motif containing 1	−2.437473925
ANXA9	annexin A9	1.697184071	SIRPA	signal regulatory protein alpha	−2.443125132
OLFR1502	olfactory receptor 1502(Olfr1502)	1.694046727	CYP24A1	cytochrome P450 family 24 subfamily A member 1	−2.44625623
HCFC2	host cell factor C2	1.693780609	NQO1	NAD(P)H quinone dehydrogenase 1	−2.44625623
DIDO1	death inducer-obliterator 1	1.693596948	HRH4	histamine receptor H4	−2.44625623
PGAM1	phosphoglycerate mutase 1	1.689846917	NUDCD1	NudC domain containing 1	−2.44625623
RASGEF1C	RasGEF domain family member 1C	1.689299161	CCND1	cyclin D1	−2.447924527
SLC25A42	solute carrier family 25 member 42	1.686774817	ADAM22	ADAM metallopeptidase domain 22	−2.452858965
CPT2	carnitine palmitoyltransferase 2	1.686364794	MDK	midkine (neurite growth-promoting factor 2)	−2.456149035
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	1.686161103	STX1A	syntaxin 1A	−2.456729828
NQO2	NAD(P)H quinone dehydrogenase 2	1.685558757	HEMK1	HemK methyltransferase family member 1	−2.459431619
HIP1R	huntingtin interacting protein 1 related	1.685473307	B4GALT7	beta-1,4-galactosyltransferase 7	−2.459431619
ALOX12E	arachidonate lipoxygenase, epidermal(Alox12e)	1.684373244	ASXL2	additional sex combs like 2, transcriptional regulator	−2.459431619
LMAN1	lectin, mannose binding 1	1.683514205	TLR7	toll like receptor 7	−2.46052038
ASB3	ankyrin repeat and SOCS box containing 3	1.680142991	TDP1	tyrosyl-DNA phosphodiesterase 1	−2.464461869
XKR5	XK related 5	1.679254438	1700025G04RIK	Description Not Found	−2.469303076
ZFP235	zinc finger protein 235(Zfp235)	1.678071905	SLC16A6	solute carrier family 16 member 6	−2.471045434
OLFR971	olfactory receptor 971(Olfr971)	1.678071905	DOXL2	diamine oxidase-like protein 2(Doxl2)	−2.472487771
OLFR374	olfactory receptor 374(Olfr374)	1.678071905	PKD1L3	polycystin 1 like 3, transient receptor potential channel interacting	−2.472487771
NOS1AP	nitric oxide synthase 1 adaptor protein	1.678071905	ZC3H11A	zinc finger CCCH-type containing 11A	−2.472487771
GALM	galactose mutarotase	1.678071905	LY6K	lymphocyte antigen 6 complex, locus K	−2.472487771
MEGF9	multiple EGF like domains 9	1.678071905	KLF7	Kruppel like factor 7	−2.474755307
CCDC66	coiled-coil domain containing 66	1.678071905	BTLA	B and T lymphocyte associated	−2.475604026
LRRC40	leucine rich repeat containing 40	1.678071905	CDON	cell adhesion associated, oncogene regulated	−2.485426827
RALA	RALA Ras like proto-oncogene A	1.678071905	DDC	dopa decarboxylase	−2.485426827
YIPF4	Yip1 domain family member 4	1.678071905	GTF2A2	general transcription factor IIA subunit 2	−2.485426827
TAL2	T-cell acute lymphocytic leukemia 2	1.678071905	DTX4	deltex E3 ubiquitin ligase 4	−2.485426827
LRRC8A	leucine rich repeat containing 8 family member A	1.678071905	GSTK1	glutathione S-transferase kappa 1	−2.486195934
APOM	apolipoprotein M	1.678071905	OLFR213	olfactory receptor 213(Olfr213)	−2.489125048
KCNG3	potassium voltage-gated channel modifier subfamily G member 3	1.678071905	PDE5A	phosphodiesterase 5A	−2.490571469
CNN1	calponin 1	1.678071905	TOB1	transducer of ERBB2, 1	−2.496763907
STAC2	SH3 and cysteine rich domain 2	1.678071905	1700109H08RIK	Description Not Found	−2.498250868

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
SFRP2	secreted frizzled related protein 2	1.678071905	LEFTY1	left-right determination factor 1	−2.498250868
SERPINB9E	serine (or cysteine) peptidase inhibitor, clade B, member 9e(Serpinb9e)	1.670169131	SNAPC4	small nuclear RNA activating complex polypeptide 4	−2.500878922
TFB1M	transcription factor B1, mitochondrial	1.668946692	RNF41	ring finger protein 41	−2.503551585
SLC25A10	solute carrier family 25 member 10	1.668856925	KLHL34	kelch like family member 34	−2.504620392
BID	BH3 interacting domain death agonist	1.667992567	SSH2	slingshot protein phosphatase 2	−2.505492762
MRPS27	mitochondrial ribosomal protein S27	1.667295766	CAMK2B	calcium/calmodulin dependent protein kinase II beta	−2.507047355
NEDD4	neural precursor cell expressed, developmentally down-regulated 4, E3 ubiquitin protein ligase	1.666756592	IRF7	interferon regulatory factor 7	−2.507590939
VANGL2	VANGL planar cell polarity protein 2	1.666756592	SCML4	sex comb on midleg-like 4 (*Drosophila*)	−2.523118672
UBE2R2	ubiquitin conjugating enzyme E2 R2	1.666641116	EPB4.1	Description Not Found	−2.523561956
KLHL30	kelch like family member 30	1.666519523	PARP12	poly(ADP-ribose) polymerase family member 12	−2.523561956
FBXO36	F-box protein 36	1.665588375	CACNB3	calcium voltage-gated channel auxiliary subunit beta 3	−2.529877218
DCT	dopachrome tautomerase	1.664016818	NRG4	neuregulin 4	−2.53318567
CCDC120	coiled-coil domain containing 120	1.663931727	OLFR1383	olfactory receptor 1383(Olfr1383)	−2.5360529
TMEM38B	transmembrane protein 38B	1.663455268	PTGR1	prostaglandin reductase 1	−2.5360529
ENDOD1	endonuclease domain containing 1	1.663327923	NFAM1	NFAT activating protein with ITAM motif 1	−2.5360529
PTPRD	protein tyrosine phosphatase, receptor type D	1.663215776	ARL4C	ADP ribosylation factor like GTPase 4C	−2.5360529
ARL3	ADP ribosylation factor like GTPase 3	1.661690196	LACE1	lactation elevated 1	−2.5360529
CDC37	cell division cycle 37	1.661567827	CDC14B	cell division cycle 14B	−2.545350645
MKKS	McKusick-Kaufman syndrome	1.66106548	GUCA1A	guanylate cyclase activator 1A	−2.548436625
CHN2	chimerin 2	1.660998764	KIF21B	kinesin family member 21B	−2.554588852
CRTAP	cartilage associated protein	1.659431912	ARID3B	AT-rich interaction domain 3B	−2.558087884
CXCR6	C-X-C motif chemokine receptor 6	1.657515938	HBA-A1	hemoglobin alpha, adult chain 1(Hba-a1)	−2.560714954
BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B	1.65691495	CSF2RB2	colony stimulating factor 2 receptor, beta 2, low-affinity (granulocyte-macrophage)(Csf2rb2)	−2.560714954
B430306N03RIK	RIKEN cDNA B430306N03 gene(B430306N03Rik)	1.655351829	ATP6V1B1	ATPase H+ transporting V1 subunit B1	−2.560714954
OLFR1262	olfactory receptor 1262(Olfr1262)	1.655351829	PCSK1N	proprotein convertase subtilisin/kexin type 1 inhibitor	−2.560714954
SLC38A5	solute carrier family 38 member 5	1.655351829	ZFP667	zinc finger protein 667(Zfp667)	−2.566670372
VAT1L	vesicle amine transport 1-like	1.655351829	SH3BP1	SH3 domain binding protein 1	−2.566734604
HOXB7	homeobox B7	1.655351829	FFAR2	free fatty acid receptor 2	−2.572889668
GAN	gigaxonin	1.655351829	EEF2K	eukaryotic elongation factor 2 kinase	−2.572889668
MMP28	matrix metallopeptidase 28	1.655351829	SLPI	secretory leukocyte peptidase inhibitor	−2.574721828
METTL10	methyltransferase like 10	1.655351829	CMA1	chymase 1	−2.584962501
SIX4	SIX homeobox 4	1.655351829	ASCL1	achaete-scute family bHLH transcription factor 1	−2.584962501
TDRD6	tudor domain containing 6	1.655351829	ACPP	acid phosphatase, prostate	−2.584962501

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
COMMD5	COMM domain containing 5	1.654604999	CLCNKB	chloride voltage-gated channel Kb	−2.596935142
PRDX4	peroxiredoxin 4	1.651923925	FBXW7	F-box and WD repeat domain containing 7	−2.596935142
HS3ST3A1	heparan sulfate-glucosamine 3-sulfotransferase 3A1	1.649298274	OLIG3	oligodendrocyte transcription factor 3	−2.596935142
CALCA	calcitonin related polypeptide alpha	1.649067786	WHRN	whirlin	−2.606789951
SLC12A2	solute carrier family 12 member 2	1.648449243	DNAJC14	DnaJ heat shock protein family (Hsp40) member C14	−2.608809243
TJP2	tight junction protein 2	1.644145647	PIGT	phosphatidylinositol glycan anchor biosynthesis class T	−2.611031218
LRRC16B	Description Not Found	1.64385619	AP1G2	adaptor related protein complex 1 gamma 2 subunit	−2.614709844
AP3S2	adaptor related protein complex 3 sigma 2 subunit	1.64385619	SAA2	serum amyloid A2	−2.62058641
PSMD9	proteasome 26S subunit, non-ATPase 9	1.64385619	USP30	ubiquitin specific peptidase 30	−2.62058641
PARD6G	par-6 family cell polarity regulator gamma	1.643379419	RPE65	retinal pigment epithelium specific protein 65	−2.632268216
CIAPIN1	cytokine induced apoptosis inhibitor 1	1.643219709	CML1	Description Not Found	−2.634891632
CKAP5	cytoskeleton associated protein 5	1.642747156	SLC6A19	solute carrier family 6 member 19	−2.640930751
E430025E21RIK	RIKEN cDNA E430025E21 gene(E430025E21Rik)	1.641902626	FGF15	fibroblast growth factor 15(Fgf15)	−2.64385619
PIAS3	protein inhibitor of activated STAT 3	1.641884484	HERC3	HECT and RLD domain containing E3 ubiquitin protein ligase 3	−2.64385619
USP1	ubiquitin specific peptidase 1	1.640233791	ADAMTSL4	ADAMTS like 4	−2.64385619
RAB3GAP2	RAB3 GTPase activating non-catalytic protein subunit 2	1.639592623	HYAL3	hyaluronoglucosaminidase 3	−2.64385619
CSRP2	cysteine and glycine rich protein 2	1.639046229	SLC15A2	solute carrier family 15 member 2	−2.648217996
MOV10	Mov10 RISC complex RNA helicase	1.638073837	UFSP1	UFM1-specific peptidase 1 (inactive)	−2.649553823
GM1965	predicted gene 1965(Gm1965)	1.637881562	6430573F11RIK	Description Not Found	−2.655351829
POMGNT1	protein O-linked mannose N-acetylglucosaminyltransferase 1 (beta 1,2-)	1.636237884	DNM3OS	DNM3 opposite strand/antisense RNA	−2.655351829
FIGNL1	fidgetin like 1	1.633950492	F2RL1	F2R like trypsin receptor 1	−2.655351829
TMEM177	transmembrane protein 177	1.633475547	SNX33	sorting nexin 33	−2.666654581
ALX4	ALX homeobox 4	1.632864872	CXCL9	C-X-C motif chemokine ligand 9	−2.666756592
OLFR533	olfactory receptor 533(Olfr533)	1.632268216	TEAD2	TEA domain transcription factor 2	−2.666756592
H2-M10.3	histocompatibility 2, M region locus 10.3(H2-M10.3)	1.632268216	QSOX1	quiescin sulfhydryl oxidase 1	−2.666756592
GPX7	glutathione peroxidase 7	1.632268216	TLR13	toll-like receptor 13(Tlr13)	−2.678071905
STXBP6	syntaxin binding protein 6	1.632268216	SCD3	stearoyl-coenzyme A desaturase 3(Scd3)	−2.678071905
RAB33A	RAB33A, member RAS oncogene family	1.632268216	SDC3	syndecan 3	−2.678071905
PDCL3	phosducin like 3	1.632268216	GRPR	gastrin releasing peptide receptor	−2.678071905
GPR20	G protein-coupled receptor 20	1.632268216	MAFK	MAF bZIP transcription factor K	−2.678071905
GSTA2	glutathione S-transferase alpha 2	1.632268216	DIRC2	disrupted in renal carcinoma 2	−2.678071905
ADCY10	adenylate cyclase 10 (soluble)	1.632268216	ZCCHC12	zinc finger CCHC-type containing 12	−2.67833354
PEX12	peroxisomal biogenesis factor 12	1.632268216	ADCY6	adenylate cyclase 6	−2.680886921

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
IQCC	IQ motif containing C	1.632268216	ECM1	extracellular matrix protein 1	−2.68345512
ENPP1	ectonucleotide pyrophosphatase/ phosphodiesterase 1	1.632086279	AFP	alpha fetoprotein	−2.689299161
ADAL	adenosine deaminase-like	1.630664126	GP5	glycoprotein V platelet	−2.689299161
SCRN2	secernin 2	1.630566247	GAB3	GRB2 associated binding protein 3	−2.691405681
CEP78	centrosomal protein 78	1.629851642	USP2	ubiquitin specific peptidase 2	−2.693334369
SLC25A15	solute carrier family 25 member 15	1.629798606	PLXNB1	plexin B1	−2.700439718
ADSSL1	adenylosuccinate synthase like 1	1.628272149	PODXL2	podocalyxin like 2	−2.700799925
TM6SF2	transmembrane 6 superfamily member 2	1.627758638	RAD9B	RAD9 checkpoint clamp component B	−2.70103836
TUBG1	tubulin gamma 1	1.624511879	AKAP10	A-kinase anchoring protein 10	−2.705977902
FASTK	Fas activated serine/threonine kinase	1.623336662	PIGW	phosphatidylinositol glycan anchor biosynthesis class W	−2.716990894
RBBP5	RB binding protein 5, histone lysine methyltransferase complex subunit	1.622163711	COL12A1	collagen type XII alpha 1 chain	−2.722466024
1700071K01RIK	Description Not Found	1.621465074	GPR137B	G protein-coupled receptor 137B	−2.733354341
SLC25A33	solute carrier family 25 member 33	1.621282718	IMMP2L	inner mitochondrial membrane peptidase subunit 2	−2.733354341
MDM4	MDM4, p53 regulator	1.62058641	PIK3CB	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta	−2.737320423
TOP2A	topoisomerase (DNA) II alpha	1.620374948	TGFBI	transforming growth factor beta induced	−2.740276443
OLFR139	olfactory receptor 139(Olfr139)	1.619731323	ZFP106	zinc finger protein 106(Zfp106)	−2.744161096
PAPLN	papilin, proteoglycan like sulfated glycoprotein	1.618762248	ARNTL	aryl hydrocarbon receptor nuclear translocator like	−2.744161096
PACSIN2	protein kinase C and casein kinase substrate in neurons 2	1.617401771	HS3ST3B1	heparan sulfate-glucosamine 3-sulfotransferase 3B1	−2.744161096
TRDMT1	tRNA aspartic acid methyltransferase 1	1.615239219	OASL2	2'-5' oligoadenylate synthetase-like 2(Oasl2)	−2.744892108
4932438H23RIK	Description Not Found	1.614681809	PRDX6	peroxiredoxin 6	−2.75084462
SPAG9	sperm associated antigen 9	1.614567709	RASA2	RAS p21 protein activator 2	−2.751203108
RPA3	replication protein A3	1.61436984	HOXB2	homeobox B2	−2.754887502
GNPTAB	N-acetylglucosamine-1-phosphate transferase alpha and beta subunits	1.613298199	TULP3	tubby like protein 3	−2.754887502
SNX9	sorting nexin 9	1.609251493	MFRP	membrane frizzled-related protein	−2.754887502
OLFR550	olfactory receptor 550(Olfr550)	1.609195813	MEN1	menin 1	−2.757556689
ZFP160	zinc finger protein 160(Zfp160)	1.608809243	C330021F23RIK	RIKEN cDNA C330021F23 gene(C330021F23Rik)	−2.762199201
TAS2R129	taste receptor, type 2, member 129(Tas2r129)	1.608809243	CSTAD	CSA-conditional, T cell activation-dependent protein(Cstad)	−2.765534746
OLFR371	olfactory receptor 371(Olfr371)	1.608809243	ALDH5A1	aldehyde dehydrogenase 5 family member A1	−2.773022439
OLFR281	olfactory receptor 281(Olfr281)	1.608809243	EPM2AIP1	EPM2A interacting protein 1	−2.773468928
OLFR195	olfactory receptor 195(Olfr195)	1.608809243	PDE8B	phosphodiesterase 8B	−2.776103988
OLFR142	olfactory receptor 142(Olfr142)	1.608809243	DMRTA1	DMRT like family A1	−2.776184379
PRSS3	protease, serine 3	1.608809243	LYPD6B	LY6/PLAUR domain containing 6B	−2.780048768
CX3CL1	C-X3-C motif chemokine ligand 1	1.608809243	CD300E	CD300e molecule	−2.786596362

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
TMPRSS6	transmembrane protease, serine 6	1.608809243	NPFF	neuropeptide FF-amide peptide precursor	−2.786596362
ALK	anaplastic lymphoma receptor tyrosine kinase	1.608809243	FASTKD1	FAST kinase domains 1	−2.793765229
ITGA9	integrin subunit alpha 9	1.608809243	OLFR802	olfactory receptor 802(Olfr802)	−2.797012978
TIMM13	translocase of inner mitochondrial membrane 13	1.608809243	HIVEP1	human immunodeficiency virus type I enhancer binding protein 1	−2.797012978
MSH5	mutS homolog 5	1.608809243	HIC1	hypermethylated in cancer 1	−2.797012978
XPO4	exportin 4	1.605818241	TRIM33	tripartite motif containing 33	−2.802009226
MED21	mediator complex subunit 21	1.603309406	SELL	selectin L	−2.803274253
CHST12	carbohydrate sulfotransferase 12	1.602612589	EPHX1	epoxide hydrolase 1	−2.803758579
6030408B16RIK	Description Not Found	1.602195565	BCL9	B-cell CLL/lymphoma 9	−2.807354922
SLU7	SLU7 homolog, splicing factor	1.601548066	STAT2	signal transducer and activator of transcription 2	−2.808521822
CDK5RAP2	CDK5 regulatory subunit associated protein 2	1.601120229	ELMO3	engulfment and cell motility 3	−2.812498225
CASP7	caspase 7	1.6002402	HDC	histidine decarboxylase	−2.815167456
KIF22	kinesin family member 22	1.599011705	AI317395	Description Not Found	−2.817623258
E2F1	E2F transcription factor 1	1.598449678	RPL14	ribosomal protein L14	−2.817623258
MXI1	MAX interactor 1, dimerization protein	1.597690116	SNAI1	snail family transcriptional repressor 1	−2.818256244
DONSON	downstream neighbor of SON	1.596935142	NUPR1	nuclear protein 1, transcriptional regulator	−2.827819025
TBX22	T-box 22	1.596935142	IGSF8	immunoglobulin superfamily member 8	−2.827819025
INPPL1	inositol polyphosphate phosphatase like 1	1.596300192	SLC12A7	solute carrier family 12 member 7	−2.827819025
CSE1L	chromosome segregation 1 like	1.59586273	RENBP	renin binding protein	−2.837431463
NDFIP2	Nedd4 family interacting protein 2	1.594709608	ZFP553	zinc finger protein 553(Zfp553)	−2.837943242
LYPD6	LY6/PLAUR domain containing 6	1.592962293	LRFN2	leucine rich repeat and fibronectin type III domain containing 2	−2.837943242
DDX49	DEAD-box helicase 49	1.592190323	HP	haptoglobin	−2.839737506
MGLL	monoglyceride lipase	1.590948822	TOMM40	translocase of outer mitochondrial membrane 40	−2.847996907
NR4A3	nuclear receptor subfamily 4 group A member 3	1.59092994	GABARAPL2	GABA type A receptor associated protein like 2	−2.847996907
LRRN3	leucine rich repeat neuronal 3	1.590360181	TMEM86A	transmembrane protein 86A	−2.855497819
PTPRK	protein tyrosine phosphatase, receptor type K	1.587927102	LRP1	LDL receptor related protein 1	−2.857980995
OLFR1212	olfactory receptor 1212(Olfr1212)	1.584962501	ATXN1	ataxin 1	−2.857980995
KLHL2	kelch like family member 2	1.584962501	FAS	Fas cell surface death receptor	−2.861524641
UBE2G2	ubiquitin conjugating enzyme E2 G2	1.584962501	ZDHHC18	zinc finger DHHC-type containing 18	−2.882740655
GRIN2A	glutamate ionotropic receptor NMDA type subunit 2A	1.584962501	LARGE	Description Not Found	−2.887525271
INHA	inhibin alpha subunit	1.584962501	SP5	Sp5 transcription factor	−2.887525271
RNPC3	RNA binding region (RNP1, RRM) containing 3	1.584962501	ATG7	autophagy related 7	−2.895440528
XKR7	XK related 7	1.584962501	DNAJC27	DnaJ heat shock protein family (Hsp40) member C27	−2.897240426
STX19	syntaxin 19	1.584962501	PCSK4	proprotein convertase subtilisin/kexin type 4	−2.900866808
SLC5A5	solute carrier family 5 member 5	1.584962501	RNF141	ring finger protein 141	−2.902073579

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
VPS37C	VPS37C, ESCRT-I subunit	1.584022655	GRAP2	GRB2-related adaptor protein 2	−2.904150467
ERMP1	endoplasmic reticulum metallopeptidase 1	1.582531434	VIPR1	vasoactive intestinal peptide receptor 1	−2.904484098
ZFP790	zinc finger protein 790(Zfp790)	1.581046002	CAR15	carbonic anhydrase 15(Car15)	−2.906890596
AA467197	expressed sequence AA467197(AA467197)	1.579947242	RELL2	RELT like 2	−2.906890596
UBE2Z	ubiquitin conjugating enzyme E2 Z	1.57976541	HECA	hdc homolog, cell cycle regulator	−2.916545968
SOAT2	sterol O-acyltransferase 2	1.577460518	DPM1	dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit	−2.933100475
ZMAT5	zinc finger matrin-type 5	1.576986214	AOC2	amine oxidase, copper containing 2	−2.936235748
CDCA3	cell division cycle associated 3	1.576323153	HIST2H2BE	histone cluster 2, H2be	−2.936320631
NEUROD2	neuronal differentiation 2	1.576266476	ACAD10	acyl-CoA dehydrogenase family member 10	−2.942514505
WDR35	WD repeat domain 35	1.576120636	NT5E	5'-nucleotidase ecto	−2.944039663
TWSG1	twisted gastrulation BMP signaling modulator 1	1.5758728	SSH1	slingshot protein phosphatase 1	−2.944858446
PPT1	palmitoyl-protein thioesterase 1	1.575321868	SEMA4F	ssemaphorin 4F	−2.948329995
IRF8	interferon regulatory factor 8	1.574489283	NKD2	naked cuticle homolog 2	−2.953960396
PLEKHG5	pleckstrin homology and RhoGEF domain containing G5	1.574066379	TCEB3	transcription elongation factor B subunit 3	−2.95419631
CDC20	cell division cycle 20	1.573718243	HDAC4	histone deacetylase 4	−2.95419631
MFI2	antigen p97 (melanoma associated) identified by monoclonal antibodies 133.2 and 96.5(Mfi2)	1.572889668	PCNX	pecanex homolog (*Drosophila*)(Pcnx)	−2.972692654
HDAC9	histone deacetylase 9	1.571625208	ARL5C	ADP ribosylation factor like GTPase 5C	−2.972692654
ASF1B	anti-silencing function 1B histone chaperone	1.570544039	1600014C10RIK	Description Not Found	−2.981852653
B3GNT1	Description Not Found	1.569171715	ANKRD23	ankyrin repeat domain 23	−2.981852653
SLC25A14	solute carrier family 25 member 14	1.569127395	CLOCK	clock circadian regulator	−2.985543793
FYN	FYN proto-oncogene, Src family tyrosine kinase	1.567462919	SFI1	SFI1 centrin binding protein	−2.986410935
SERPINB6B	serine (or cysteine) peptidase inhibitor, clade B, member 6b(Serpinb6b)	1.567348435	HEY1	hes related family bHLH transcription factor with YRPW motif 1	−2.987632559
TOP1MT	topoisomerase (DNA) I, mitochondrial	1.567180597	ATP11C	ATPase phospholipid transporting 11C	−2.99095486
CCDC50	coiled-coil domain containing 50	1.566273906	NUDCD3	NudC domain containing 3	−3
ZFP414	zinc finger protein 414(Zfp414)	1.565776574	CDC25A	cell division cycle 25 A	−3.000238201
OGFOD2	2-oxoglutarate and iron dependent oxygenase domain containing 2	1.565512016	OLFR135	olfactory receptor 135(Olfr135)	−3.017921908
CTNNAL1	catenin alpha like 1	1.563586461	RC3H1	ring finger and CCCH-type domains 1	−3.019621529
CREB3L2	cAMP responsive element binding protein 3 like 2	1.561361122	NSG2	neuron specific gene family member 2(Nsg2)	−3.020466888
OLFR492	olfactory receptor 492(Olfr492)	1.560714954	ID1	inhibitor of DNA binding 1, HLH protein	−3.026800059
OLFR1312	olfactory receptor 1312(Olfr1312)	1.560714954	CYP2D22	cytochrome P450, family 2, subfamily d, polypeptide 22(Cyp2d22)	−3.044282215
UPK2	uroplakin 2	1.560714954	H2AFJ	H2A histone family member J	−3.044297135
RESP18	regulated endocrine specific protein 18	1.560714954	TGFBR3	transforming growth factor beta receptor 3	−3.053111336
CRCT1	cysteine rich C-terminal 1	1.560714954	IRS2	insulin receptor substrate 2	−3.061776198

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
NEUROD4	neuronal differentiation 4	1.560714954	ADCY7	adenylate cyclase 7	−3.06608919
SENP1	SUMO1/sentrin specific peptidase 1	1.560714954	HYI	hydroxypyruvate isomerase (putative)	−3.072315809
MR1	major histocompatibility complex, class I-related	1.560714954	TRIP4	thyroid hormone receptor interactor 4	−3.078951341
BIVM	basic, immunoglobulin-like variable motif containing	1.560714954	D730001G18RIK	RIKEN cDNA D730001G18 gene(D730001G18Rik)	−3.087462841
KPNA2	karyopherin subunit alpha 2	1.560714954	PRR7	proline rich 7 (synaptic)	−3.087462841
BAG2	BCL2 associated athanogene 2	1.560714954	GFPT2	glutamine-fructose-6-phosphate transaminase 2	−3.09592442
SLC12A8	solute carrier family 12 member 8	1.560714954	SCMH1	sex comb on midleg homolog 1 (*Drosophila*)	−3.100136671
SCN7A	sodium voltage-gated channel alpha subunit 7	1.560714954	ANKRD12	ankyrin repeat domain 12	−3.107456458
SLC5A7	solute carrier family 5 member 7	1.560714954	PTPRV	protein tyrosine phosphatase, receptor type, V(Ptprv)	−3.112700133
ENPEP	glutamyl aminopeptidase	1.560714954	TMEM135	transmembrane protein 135	−3.112700133
ANGPTL4	angiopoietin like 4	1.56060777	AKAP3	A-kinase anchoring protein 3	−3.11460665
OSBPL3	oxysterol binding protein like 3	1.559778376	CBR2	carbonyl reductase 2(Cbr2)	−3.129283017
MCFD2	multiple coagulation factor deficiency 2	1.559617874	CXCL16	C-X-C motif chemokine ligand 16	−3.129283017
MAP2K1	mitogen-activated protein kinase kinase 1	1.558556708	MBTD1	mbt domain containing 1	−3.145677455
ING2	inhibitor of growth family member 2	1.557223521	UBE2J2	ubiquitin conjugating enzyme E2 J2	−3.161887682
CDCA5	cell division cycle associated 5	1.55643411	STK36	serine/threonine kinase 36	−3.161887682
MAP3K7	mitogen-activated protein kinase kinase kinase 7	1.554463905	SLC14A1	solute carrier family 14 member 1 (Kidd blood group)	−3.16922072
GSTT3	glutathione S-transferase, theta 3(Gstt3)	1.55048277	CTSE	cathepsin E	−3.177917792
PFN2	profilin 2	1.549690793	HSD3B7	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7	−3.177917792
HPS4	HPS4, biogenesis of lysosomal organelles complex 3 subunit 2	1.549115647	3010003L21RIK	Description Not Found	−3.179249632
CAPN8	calpain 8	1.548436625	BAI1	Description Not Found	−3.186461055
RAB11FIP5	RAB11 family interacting protein 5	1.548436625	ZFP451	zinc finger protein 451(Zfp451)	−3.187711618
CD9	CD9 molecule	1.548429184	CCDC28B	coiled-coil domain containing 28B	−3.192207249
CCR6	C-C motif chemokine receptor 6	1.548250633	MCF2L	MCF.2 cell line derived transforming sequence like	−3.199672345
ALG2	ALG2, alpha-1,3/1,6-mannosyltransferase	1.547992668	BCL6	B-cell CLL/lymphoma 6	−3.201024389
BCDIN3D	BCDIN3 domain containing RNA methyltransferase	1.546046129	PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	−3.204935584
NT5DC3	5'-nucleotidase domain containing 3	1.54522349	PROS1	protein S (alpha)	−3.209453366
DNAJC18	DnaJ heat shock protein family (Hsp40) member C18	1.544626916	CTSH	cathepsin H	−3.21628737
SH3RF1	SH3 domain containing ring finger 1	1.544156019	CRTC3	CREB regulated transcription coactivator 3	−3.217230716
RGS16	regulator of G-protein signaling 16	1.541382294	TNKS	tankyrase	−3.217230716
NCAPH	non-SMC condensin I complex subunit H	1.540788228	GRM6	glutamate metabotropic receptor 6	−3.224966365
USP14	ubiquitin specific peptidase 14	1.540333713	SPSB1	splA/ryanodine receptor domain and SOCS box containing 1	−3.255500733

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
RFT1	RFT1 homolog	1.54031759	PARP8	poly(ADP-ribose) polymerase family member 8	−3.263034406
SLC31A1	solute carrier family 31 member 1	1.540275536	KCNRG	potassium channel regulator	−3.263034406
TCTEX1D2	Tctex1 domain containing 2	1.538332378	POU6F1	POU class 6 homeobox 1	−3.268517714
TTF2	transcription termination factor 2	1.537871953	REV3L	REV3 like, DNA directed polymerase zeta catalytic subunit	−3.270528942
ZFP7	zinc finger protein 7(Zfp7)	1.5360529	TCF7	transcription factor 7 (T-cell specific, HMG-box)	−3.272419178
G6PD2	glucose-6-phosphate dehydrogenase 2(G6pd2)	1.5360529	NME4	NME/NM23 nucleoside diphosphate kinase 4	−3.283551423
DEFB14	defensin beta 14(Defb14)	1.5360529	PLAUR	plasminogen activator, urokinase receptor	−3.285402219
SLC18A3	solute carrier family 18 member A3	1.5360529	CD4	CD4 molecule	−3.285402219
AHNAK2	AHNAK nucleoprotein 2	1.5360529	ZMYND11	zinc finger MYND-type containing 11	−3.293186363
HOXC12	homeobox C12	1.5360529	ARMCX5	armadillo repeat containing, X-linked 5	−3.298404158
CEACAM16	carcinoembryonic antigen related cell adhesion molecule 16	1.5360529	LPHN1	Description Not Found	−3.300123725
MOSPD3	motile sperm domain containing 3	1.5360529	PIK3IP1	phosphoinositide-3 -kinase interacting protein 1	−3.307428525
DCTN1	dynactin subunit 1	1.5360529	ERDR1	erythroid differentiation regulator 1(Erdr1)	−3.317651188
MYB	MYB proto-oncogene, transcription factor	1.5360529	PLD4	phospholipase D family member 4	−3.328444792
GLIPR1L2	GLI pathogenesis-related 1 like 2	1.5360529	BMF	Bcl2 modifying factor	−3.336283388
ALDH1A3	aldehyde dehydrogenase 1 family member A3	1.5360529	GALNT11	polypeptide N-acetylgalactosaminyltransferase 11	−3.345118795
SLC2A8	solute carrier family 2 member 8	1.5360529	LCN2	lipocalin 2	−3.378511623
SRC	SRC proto-oncogene, non-receptor tyrosine kinase	1.5360529	PAG1	phosphoprotein membrane anchor with glycosphingolipid microdomains 1	−3.385431037
ZCCHC17	zinc finger CCHC-type containing 17	1.535618518	DTX1	deltex E3 ubiquitin ligase 1	−3.425576064
HNRNPUL1	heterogeneous nuclear ribonucleoprotein U like 1	1.534420207	RFFL	ring finger and FYVE-like domain containing E3 ubiquitin protein ligase	−3.426684082
TRIM68	tripartite motif containing 68	1.533057052	MAFF	MAF bZIP transcription factor F	−3.429615964
TPST1	tyrosylprotein sulfotransferase 1	1.53140111	TOR1AIP2	torsin 1A interacting protein 2	−3.432316325
OLFR922	olfactory receptor 922(Olfr922)	1.531260941	SNN	stannin	−3.432316325
FIG4	FIG4 phosphoinositide 5-phosphatase	1.530442167	CLEC4N	C-type lectin domain family 4, member n(Clec4n)	−3.433567144
SETMAR	SET domain and mariner transposase fusion gene	1.530442167	RREB1	ras responsive element binding protein 1	−3.443780274
GSTM5	glutathione S-transferase mu 5	1.530053218	CCDC84	coiled-coil domain containing 84	−3.445188687
TUBA3B	tubulin, alpha 3B(Tuba3b)	1.527986221	ID3	inhibitor of DNA binding 3, HLH protein	−3.46350285
PDCL	phosducin like	1.527807072	BC065397	cDNA sequence BC065397(BC065397)	−3.465974465
SMPDL3B	sphingomyelin phosphodiesterase acid like 3B	1.527243888	VRK1	vaccinia related kinase 1	−3.46760555
ABHD14A	abhydrolase domain containing 14A	1.527213882	HOXD13	homeobox D13	−3.491853096
TIPIN	TIMELESS interacting protein	1.526972991	MAPK8IP2	mitogen-activated protein kinase 8 interacting protein 2	−3.491853096
DSCC1	DNA replication and sister chromatid cohesion 1	1.525986429	HOXA5	homeobox A5	−3.517275693

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
PSMD1	proteasome 26S subunit, non-ATPase 1	1.525574957	HIST1H1A	histone cluster 1, H1a	−3.523561956
BZRAP1	benzodiazepine receptor associated protein 1(Bzrap1)	1.524166255	MAML1	mastermind like transcriptional coactivator 1	−3.523603553
ENO3	enolase 3	1.523778831	PTPDC1	protein tyrosine phosphatase domain containing 1	−3.526694846
E330034G19RIK	RIKEN cDNA E330034G19 gene(E330034G19Rik)	1.523561956	TNFRSF12A	tumor necrosis factor receptor superfamily member 12A	−3.528725998
GABRP	gamma-aminobutyric acid type A receptor pi subunit	1.523561956	TNIP2	TNFAIP3 interacting protein 2	−3.539158811
SLC14A2	solute carrier family 14 member 2	1.523561956	HIST2H4	histone cluster 2, H4(Hist2h4)	−3.540773411
YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon	1.522478712	PIM2	Pim-2 proto-oncogene, serine/threonine kinase	−3.557655155
EHBP1L1	EH domain binding protein 1 like 1	1.522282169	DOK7	docking protein 7	−3.567781854
CHGB	chromogranin B	1.51924262	TNFSF14	tumor necrosis factor superfamily member 14	−3.588895735
TXNRD2	thioredoxin reductase 2	1.519008256	TDRKH	tudor and KH domain containing	−3.590961241
NCF1	neutrophil cytosolic factor 1	1.518873761	FIBCD1	fibrinogen C domain containing 1	−3.608656121
OAF	out at first homolog	1.517431856	RBBP9	RB binding protein 9, serine hydrolase	−3.608809243
FAM110A	family with sequence similarity 110 member A	1.517263583	DERL1	derlin 1	−3.617651119
ANGEL1	angel homolog 1 (*Drosophila*)	1.515832566	LENG9	leukocyte receptor cluster member 9	−3.62058641
RTN4IP1	reticulon 4 interacting protein 1	1.515760776	TRPC2	transient receptor potential cation channel subfamily C member 2, pseudogene	−3.62058641
LAMP2	lysosomal associated membrane protein 2	1.515709038	CCDC134	coiled-coil domain containing 134	−3.632268216
KRT4	keratin 4	1.514299789	OAS2	2'-5'-oligoadenylate synthetase 2	−3.632268216
PAFAH1B3	platelet activating factor acetylhydrolase 1b catalytic subunit 3	1.5142935	2410127L17RIK	Description Not Found	−3.646738698
STT3A	STT3A, catalytic subunit of the oligosaccharyltransferase complex	1.513537695	RSAD1	radical S-adenosyl methionine domain containing 1	−3.649220471
PRKAR1B	protein kinase cAMP-dependent type I regulatory subunit beta	1.51340003	H2-DMB1	histocompatibility 2, class II, locus Mb1(H2-DMb1)	−3.649615459
HIST1H2BB	histone cluster 1, H2bb	1.512941595	IFT81	intraflagellar transport 81	−3.673839056
ZFP39	zinc finger protein 39(Zfp39)	1.511385424	MID1	midline 1	−3.683696454
PLK1	polo like kinase 1	1.511151166	DEPDC1B	DEP domain containing 1B	−3.683696454
1700028P14RIK	Description Not Found	1.510961919	SMAD3	SMAD family member 3	−3.716296166
D10BWG1379E	Description Not Found	1.510961919	UBTD1	ubiquitin domain containing 1	−3.716990894
TREM3	triggering receptor expressed on myeloid cells 3(Trem3)	1.510961919	FBXO44	F-box protein 44	−3.738767837
GM128	predicted gene 128(Gm128)	1.510961919	KCNMB4	potassium calcium-activated channel subfamily M regulatory beta subunit 4	−3.741951111
OLFR741	olfactory receptor 741(Olfr741)	1.510961919	FAIM3	Description Not Found	−3.754887502
OLFR523	olfactory receptor 523(Olfr523)	1.510961919	CCM2	CCM2 scaffolding protein	−3.754887502
DCPP1	demilune cell and parotid protein 1(Dcpp1)	1.510961919	DAG1	dystroglycan 1	−3.760220946
RPRML	reprimo like	1.510961919	FCGR3	Fc receptor, IgG, low affinity III(Fcgr3)	−3.776103988

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
CHRD	chordin	1.510961919	ZNRF1	zinc and ring finger 1, E3 ubiquitin protein ligase	−3.776103988
C5AR1	complement component 5a receptor 1	1.510961919	TLR1	toll like receptor 1	−3.786596362
APOA2	apolipoprotein A2	1.510961919	HSD17B11	hydroxysteroid 17-beta dehydrogenase 11	−3.789207575
PRG2	proteoglycan 2, pro eosinophil major basic protein	1.510961919	ZPBP	zona pellucida binding protein	−3.887525271
VCAM1	vascular cell adhesion molecule 1	1.510961919	ZSWIM3	zinc finger SWIM-type containing 3	−3.892391026
LY6G5B	lymphocyte antigen 6 complex, locus G5B	1.510961919	SOCS1	suppressor of cytokine signaling 1	−3.892391026
AIM2	absent in melanoma 2	1.510961919	KLF9	Kruppel like factor 9	−3.902021342
DMBX1	diencephalon/mesencephalon homeobox 1	1.510961919	AHSA2	AHA1, activator of heat shock 90 kDa protein ATPase homolog 2 (yeast)	−3.904760449
HCN2	hyperpolarization activated cyclic nucleotide gated potassium channel 2	1.510961919	DDHD1	DDHD domain containing 1	−3.914086097
MRGPRF	MAS related GPR family member F	1.510961919	CNKSR3	CNKSR family member 3	−3.930737338
CYTH4	cytohesin 4	1.510961919	CPEB2	cytoplasmic polyadenylation element binding protein 2	−4.017516295
ANGPTL3	angiopoietin like 3	1.510961919	TRP53BP2	transformation related protein 53 binding protein 2(Trp53bp2)	−4.021932279
DHX29	DEAH-box helicase 29	1.510667738	FAM178A	family with sequence similarity 178, member A(Fam178a)	−4.03562391
PMPCB	peptidase, mitochondrial processing beta subunit	1.509477625	RCN3	reticulocalbin 3	−4.03562391
HRH3	histamine receptor H3	1.508554002	SPTLC2	serine palmitoyltransferase long chain base subunit 2	−4.040015679
ZFP282	zinc finger protein 282(Zfp282)	1.507419453	ZFP810	zinc finger protein 810(Zfp810)	−4.070389328
TBC1D7	TBC1 domain family member 7	1.504847821	NAGA	alpha-N-acetylgalactosaminidase	−4.074676686
ARSB	arylsulfatase B	1.504845728	KLRA20	killer cell lectin-like receptor subfamily A, member 20(Klra20)	−4.078951341
RAD17	RAD17 checkpoint clamp loader component	1.504177542	STK11IP	serine/threonine kinase 11 interacting protein	−4.083213368
CMTM7	CKLF like MARVEL transmembrane domain containing 7	1.503297831	KLF4	Kruppel like factor 4	−4.084306687
NFKB2	nuclear factor kappa B subunit 2	1.500363085	INADL	Description Not Found	−4.086667018
TOP3A	topoisomerase (DNA) III alpha	−1.50007357	URM1	ubiquitin related modifier 1	−4.0907078
RAB33B	RAB33B, member RAS oncogene family	−1.50054042	PELI1	pellino E3 ubiquitin protein ligase 1	−4.093813673
LYSMD1	LysM domain containing 1	−1.500614885	FBLN1	fibulin 1	−4.098032083
POLG2	polymerase (DNA) gamma 2, accessory subunit	−1.500707646	HR	hair growth associated	−4.135452784
TGIF1	TGFB induced factor homeobox 1	−1.501196523	ASB6	ankyrin repeat and SOCS box containing 6	−4.137503524
RELL1	RELT like 1	−1.50300255	SLC27A5	solute carrier family 27 member 5	−4.141596278
CYP26B1	cytochrome P450 family 26 subfamily B member 1	−1.50439813	PPP1R3F	protein phosphatase 1 regulatory subunit 3F	−4.14974712
PTRH2	peptidyl-tRNA hydrolase 2	−1.504678598	AB124611	cDNA sequence AB124611(AB124611)	−4.173373402
ZKSCAN3	zinc finger with KRAB and SCAN domains 3	−1.504916722	CD40	CD40 molecule	−4.181897643
SP8	Sp8 transcription factor	−1.505999092	SMAD5	SMAD family member 5	−4.183883459
SAMD14	sterile alpha motif domain containing 14	−1.506272343	COL23A1	collagen type XXIII alpha 1 chain	−4.221103725
MX2	MX dynamin like GTPase 2	−1.507268463	ZFP595	zinc finger protein 595(Zfp595)	−4.228818691

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
OCRL	OCRL, inositol polyphosphate-5-phosphatase	−1.507638755	PECAM1	platelet and endothelial cell adhesion molecule 1	−4.232789973
SYNJ2BP	synaptojanin 2 binding protein	−1.507669173	TMEM138	transmembrane protein 138	−4.241228289
CPLX4	complexin 4	−1.508554002	RFX2	regulatory factor X2	−4.244125943
LGALS9	galectin 9	−1.509246723	KCTD12	potassium channel tetramerization domain containing 12	−4.247846204
TAZ	tafazzin	−1.509269953	TRIM56	tripartite motif containing 56	−4.262008929
2310002L09RIK	Description Not Found	−1.510961919	EIF4EBP2	eukaryotic translation initiation factor 4E binding protein 2	−4.263034406
ZFP97	zinc finger protein 97(Zfp97)	−1.510961919	RALGPS2	Ral GEF with PH domain and SH3 binding motif 2	−4.279842694
OLFR1494	olfactory receptor 1494(Olfr1494)	−1.510961919	TGM2	transglutaminase 2	−4.293161941
BC030867	cDNA sequence BC030867(BC030867)	−1.510961919	ENC1	ectodermal-neural cortex 1	−4.311067102
CEACAM9	carcinoembryonic antigen-related cell adhesion molecule 9(Ceacam9)	−1.510961919	LRIG1	leucine rich repeats and immunoglobulin like domains 1	−4.375039431
LRIT1	leucine rich repeat, Ig-like and transmembrane domains 1	−1.510961919	PRM1	protamine 1	−4.375039431
KLK5	kallikrein related peptidase 5	−1.510961919	DUSP7	dual specificity phosphatase 7	−4.383538076
KRT27	keratin 27	−1.510961919	SERTAD3	SERTA domain containing 3	−4.399171094
CACNG4	calcium voltage-gated channel auxiliary subunit gamma 4	−1.510961919	KCNC1	potassium voltage-gated channel subfamily C member 1	−4.409390936
IL13RA1	interleukin 13 receptor subunit alpha 1	−1.510961919	UBE2D3	ubiquitin conjugating enzyme E2 D3	−4.462706751
TMEM121	transmembrane protein 121	−1.510961919	SEPP1	selenoprotein P, plasma, 1	−4.463383458
HIST1H2AA	histone cluster 1, H2aa	−1.510961919	ADRB2	adrenoceptor beta 2	−4.463910999
MPZL3	myelin protein zero like 3	−1.510961919	PPP1R13B	protein phosphatase 1 regulatory subunit 13B	−4.471417658
TGFB2	transforming growth factor beta 2	−1.510961919	ARRDC3	arrestin domain containing 3	−4.504620392
IFT74	intraflagellar transport 74	−1.510961919	GNGT2	G protein subunit gamma transducin 2	−4.531381461
FCRL1	Fc receptor like 1	−1.510961919	SIAH1A	seven in absentia 1A(Siah1a)	−4.539158811
ADRB1	adrenoceptor beta 1	−1.510961919	XPC	XPC complex subunit, DNA damage recognition and repair factor	−4.563768278
MAGI2	membrane associated guanylate kinase, WW and PDZ domain containing 2	−1.510961919	HIPK1	homeodomain interacting protein kinase 1	−4.683696454
SCG5	secretogranin V	−1.510961919	H2-OB	histocompatibility 2, O region beta locus(H2-Ob)	−4.700439718
GCK	glucokinase	−1.510961919	BACH2	BTB domain and CNC homolog 2	−4.716990894
ASB10	ankyrin repeat and SOCS box containing 10	−1.510961919	MAP1LC3A	microtubule associated protein 1 light chain 3 alpha	−4.722466024
SELE	selectin E	−1.510961919	LRRFIP1	LRR binding FLU interacting protein 1	−4.761551232
IGFBP3	insulin like growth factor binding protein 3	−1.510961919	ATP10D	ATPase phospholipid transporting 10D (putative)	−4.766581958
TPT1	tumor protein, translationally-controlled 1	−1.510961919	IGFBP4	insulin like growth factor binding protein 4	−4.790993785
ROCK1	Rho associated coiled-coil containing protein kinase 1	−1.510961919	TMEM108	transmembrane protein 108	−4.865423978
OGFRL1	opioid growth factor receptor-like 1	−1.510961919	PTK2	protein tyrosine kinase 2	−4.875719796
TMEM38A	transmembrane protein 38A	−1.510961919	CLEC11A	C-type lectin domain family 11 member A	−4.897240426

TABLE 2-continued

Differentially regulated genes in $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
RLTPR	Description Not Found	−1.51227339	LRP12	LDL receptor related protein 12	−4.955029571
ITPKC	inositol-trisphosphate 3-kinase C	−1.512389725	GCNT2	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group)	−4.958842675
TLE4	transducin like enhancer of split 4	−1.51341989	F10	coagulation factor X	−4.965784285
PDE4D	phosphodiesterase 4D	−1.513667908	DBP	D-box binding PAR bZIP transcription factor	−4.966549451
A130010J15RIK	Description Not Found	−1.514296211	ABCG1	ATP binding cassette subfamily G member 1	−5.002252452
RNF167	ring finger protein 167	−1.514765492	WDR78	WD repeat domain 78	−5.017921908
CCBL1	Description Not Found	−1.515626494	DNAJC6	DnaJ heat shock protein family (Hsp40) member C6	−5.017921908
HSD17B1	hydroxysteroid 17-beta dehydrogenase 1	−1.516875069	AFF4	AF4/FMR2 family member 4	−5.033423002
OSM	oncostatin M	−1.517234668	TNFRSF26	tumor necrosis factor receptor superfamily, member 26(Tnfrsf26)	−5.040015679
RHPN1	rhophilin, Rho GTPase binding protein 1	−1.517275693	GFOD2	glucose-fructose oxidoreductase domain containing 2	−5.070389328
TAS2R105	taste receptor, type 2, member 105(Tas2r105)	−1.517431856	TYROBP	TYRO protein tyrosine kinase binding protein	−5.114783447
NIPBL	NIPBL, cohesin loading factor	−1.517569618	TMEM176B	transmembrane protein 176B	−5.118941073
CXCR3	C-X-C motif chemokine receptor 3	−1.519325267	ZFP710	zinc finger protein 710(Zfp710)	−5.159871337
SMURF1	SMAD specific E3 ubiquitin protein ligase 1	−1.520263252	ENPP4	ectonucleotide pyrophosphatase/ phosphodiesterase 4 (putative)	−5.181897643
RNF208	ring finger protein 208	−1.52126647	MAPK8	mitogen-activated protein kinase 8	−5.259272487
ITGA5	integrin subunit alpha 5	−1.523517983	TNFRSF25	tumor necrosis factor receptor superfamily member 25	−5.289096702
USP18	ubiquitin specific peptidase 18	−1.524814077	LCN4	lipocalin 4(Lcn4)	−5.366322214
PIP5K1A	phosphatidylinositol-4-phosphate 5-kinase type 1 alpha	−1.525074369	CRIM1	cysteine rich transmembrane BMP regulator 1	−5.369815424
STRBP	spermatid perinuclear RNA binding protein	−1.52561213	RTP4	receptor transporter protein 4	−5.444600814
GRAMD2	GRAM domain containing 2	−1.52652805	PRNP	prion protein	−5.495055528
ZFP101	zinc finger protein 101(Zfp101)	−1.526555668	ZFP747	zinc finger protein 747(Zfp747)	−5.496654083
RUNDC1	RUN domain containing 1	−1.526563287	CD7	CD7 molecule	−5.504620392
SLC13A3	solute carrier family 13 member 3	−1.528487927	ARHGAP26	Rho GTPase activating protein 26	−5.548436625
CCDC94	coiled-coil domain containing 94	−1.528487927	S100A9	S100 calcium binding protein A9	−5.557655155
MRPS14	mitochondrial ribosomal protein S14	−1.528962318	AQP9	aquaporin 9	−5.572889668
NEU4	neuraminidase 4 (sialidase)	−1.529820947	CXCR5	C-X-C motif chemokine receptor 5	−5.573647187
PCGF1	polycomb group ring finger 1	−1.53059536	CCNO	cyclin O	−5.574404309
PNPLA7	patatin like phospholipase domain containing 7	−1.53207883	LYNX1	Ly6/neurotoxin 1	−5.666756592
SPATA19	spermatogenesis associated 19	−1.533014103	CLDN10	claudin 10	−5.782015335
AP4B1	adaptor related protein complex 4 beta 1 subunit	−1.533821865	AMIGO2	adhesion molecule with Ig-like domain 2	−5.83541884
BC068281	cDNA sequence BC068281(BC068281)	−1.5360529	CD79B	CD79b molecule	−5.94016675
GK2	glycerol kinase 2	−1.5360529	USP53	ubiquitin specific peptidase 53	−5.980710829
PIGM	phosphatidylinositol glycan anchor biosynthesis class M	−1.5360529	IKBKE	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon	−6.005624549

TABLE 2-continued

Differentially regulated genes in CD8+4-1BB+LAG-3+ TILs

Gene	Gene Description	Log2-Fold Change	Gene Symbol	Gene Description	Log2-Fold Change
FKBP6	FK506 binding protein 6	−1.5360529	ALOX5AP	arachidonate 5-lipoxygenase activating protein	−6.008988783
EVI5	ecotropic viral integration site 5	−1.5360529	GGT1	gamma-glutamyltransferase 1	−6.010108453
BCL11A	B-cell CLL/lymphoma 11A	−1.5360529	CAMK2D	calcium/calmodulin dependent protein kinase II delta	−6.047669251
PER1	period circadian clock 1	−1.537278499	RAB3D	RAB3D, member RAS oncogene family	−6.156841525
BTBD9	BTB domain containing 9	−1.537451456	MAP3K8	mitogen-activated protein kinase kinase kinase 8	−6.376776572
USP38	ubiquitin specific peptidase 38	−1.537763627	NOTCH4	notch 4	−6.495055528
LRRC57	leucine rich repeat containing 57	−1.538083341	MACROD1	MACRO domain containing 1	−6.581200582
5830415F09RIK	Description Not Found	−1.53855912	RNF144A	ring finger protein 144A	−6.632268216
EGR2	early growth response 2	−1.540038325	PDE2A	phosphodiesterase 2A	−6.86913112
GMEB2	glucocorticoid modulatory element binding protein 2	−1.541122795	THA1	threonine aldolase 1(Tha1)	−6.885086225
PIK3R4	phosphoinositide-3-kinase regulatory subunit 4	−1.541975323	APP	amyloid beta precursor protein	−6.940754047
KRR1	KRR1, small subunit processome component homolog	−1.54225805	FAM109A	family with sequence similarity 109 member A	−6.968666793
COL9A1	collagen type IX alpha 1	−1.54225805	LRG1	leucine rich alpha-2-glycoprotein 1	−6.995484519
POLD4	polymerase (DNA) delta 4, accessory subunit	−1.542654605	IL11RA1	interleukin 11 receptor, alpha chain 1(Il11ra1)	−7.016251155
ACSS2	acyl-CoA synthetase short-chain family member 2	−1.544045378	CNR2	cannabinoid receptor 2	−7.213347282
PDLIM1	PDZ and LIM domain 1	−1.544785186	NUAK2	NUAK family kinase 2	−7.369815424
A430107P09RIK	Description Not Found	−1.544921568	GPR146	G protein-coupled receptor 146	−7.577806447
SLC38A11	solute carrier family 38 member 11	−1.546222547			

*Log 2Fold Change=log 2(4+L+/4−L−)

To investigate the molecular pathways between these three populations, gene ontology networks were grouped into nodes and the most significant pathways within each node were determined (FIG. **6**A). Gene ontology (GO) terms shared between our dysfunctional T cell dataset and the published hypofunctional T cell dataset were greatly enriched in cell cycle genes, consistent with the observation that the dysfunctional population is largely Ki67+. GO terms shared between dysfunctional and exhausted gene sets encompassed effector programs such as regulation of cell killing, chemotaxis, interferon-γ production. GO terms shared between hypofunctional and exhausted gene sets consisted of cell cycle pathways, negative regulation of lymphocytes, and interferon-γ production. These data indicate that while some conserved molecular programs likely exist in these dysfunctional differentiation states, many pathways may be differentially regulated between chronic viral infections and in the tumor context.

While many inhibitory receptors, including Pdcd1 (PD-1), Havcr2 (TIM-3), Cd244 (2B4), Klre1, and Lag3 were shared between all data sets; the co-stimulatory receptors Tnfrsf4 (OX-40) and Tnfrsf9 (4-1BB) were upregulated in dysfunctional and hypofunctional CD8+ TIL data sets. Therefore, to enrich in potential markers and therapeutic targets on tumor specific CD8+ TILs, the complete cell surface phenotype of the 4-1BB+LAG-3+CD8 TIL population was characterized. Comparing the different CD8+ TIL subpopulations, several additional upregulated co-stimulatory receptors were found: Tnfrsf18 (GITR), Nkg2d (KLRK1) and Cd27. The transcript for Nrp1 (neuropilin-1), which encodes for a cell surface receptor protein implicated in CD4+ Treg function (Sarris et al., 2008; incorporated by reference in its entirety), was also highly expressed. Expression of many of these molecules was confirmed by flow cytometry at day 7, 14 and 21 after tumor inoculation (FIG. **6**C). The analysis was extended to include the co-stimulatory molecules ICOS and CD160 and the inhibitory receptor T cell immunoreceptor with Ig and ITIM domains (TIGIT) because ICOS and CD160 were close to the cutoff value and no probe was present for TIGIT in the gene array. In addition, recent reports indicate that targeting these receptors can be therapeutic in murine models of cancer (Johnston et al., 2014; Fan et al., 2014; incorporated by reference in their entireties). PD-1, TIGIT, TIM-3, CD27 and NRP1 were expressed the majority of the 4-1BB+LAG-3+ TIL population and expression was maintained over time. 2B4, CD160, CTLA4, OX-40, and GITR subdivided a lesser fraction of the 4-1BB+LAG-3+ population. The expression of several inhibitory receptors, 2B4, TIM3 and CD160 increased over this 3-week time frame while expression of the co-stimulatory receptors, ICOS and OX-40, decreased (FIG. **6**C).

To address if the dysfunctional CD8+ TILs are terminally-differentiated short term effector cells or memory-like cells,

the expression of KLRG-1 and IL-7Rα (Joshi et al., 2007). Most of the $CD8^+$ TIL were negative for KLRG-1 expression and there was no difference between the 4-$1BB^+LAG$-3^+ and 4-$1BB^-LAG$-3^- populations. However, the majority of the 4-$1BB^+LAG$-3^+ TIL did not express the IL-7 receptor (IL-7Rα) compared to their negative counter parts (FIG. 6D). These results indicate that the 4-$1BB^-LAG$-3^- TIL, which are not apparently specific for antigens expressed in the tumor microenvironment, are more memory-like, yet at the same time the tumor antigen-specific LAG-3^+4-$1BB^+$ subset has not fully acquired a terminal effector phenotype.

Functional Relevance of Genes that are Differentially Regulated in $CD8^+$ 4-$1BB^+LAG$-3^+ TILs

The gene array results in Table 2 provide a list of genes characterizing $CD8^+$ 4-$1BB^+LAG$-3^+ TILs. The list includes therapeutic targets and additional markers of anti-tumor immunity. Experiments conducted during development of embodiments herein to test the functional relevance of these additional targets/markers (FIG. 11). Data indicate that the array has identified targets for immunotherapy, using knockout mice (e.g., PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, CRTAM, Sema7a, etc.). Experiments demonstrate that Nrn1 and CRTAM are negative regulators of the anti-tumor immune response, as knockout mice lacking either of these molecules showed improved immune-mediated tumor control in vivo. In contrast, Sema7a is a positive regulator of anti-tumor immune responses, as knockout mice lacking this molecule show diminished immune-mediated tumor control in vivo (FIG. 11). These experiments indicate that agonists of Sema7a signaling and antagonists of Nrn1 and/or CRTAM should be useful therapeutics for the treatment of cancer.

Targeting 4-1BB and LAG-3 Exerts Anti-Tumor Activity In Vivo and Normalizes the Function and Phenotypic Composition of $CD8^+$ TILs

Experiments were conducted during development of embodiments herein to assess whether targeting these receptors might have therapeutic utility. To this end, an agonistic anti-4-1BB mAb was administered alone or in combination with a blocking anti-LAG-3 mAb in mice bearing established B16.SIY tumors. While each antibody treatment alone had some therapeutic effect as reflected by slower tumor growth, the combination was particularly potent (FIG. 7A). Analysis of the tumor microenvironment revealed that improved tumor control with the combination therapy was accompanied by an increase in the number of $CD8^+$ TILs specific for the SIY antigen (FIG. 7B), consistent with results reported previously with anti-PD-L1+anti-CTLA-4 mAb (Spranger et al., 2014b; Twyman-Saint Victor et al., 2015; incorporated by reference in their entireties).

It was next examined whether the therapeutic effect of anti-4-1BB+anti-LAG-3 mAbs was associated with a loss of phenotypic markers defining dysfunctional T cells in the steady state. Due to concern that re-analyzing the T cells for expression of LAG-3 and 4-1BB might be problematic, as the administered Abs could theoretically modulate the target receptors from the cell surface, the coordinate expression of additional receptors as identified above by gene expression profiling was taken advantage of Preliminary analyses of the bulk TIL subpopulations revealed decreased expression of NRP1 and 2B4 following anti-LAG-3+anti-4-1BB treatment (data not shown). Co-expression of 2B4 and NRP1 on SIY-reactive $CD8^+$ TILs identified by pentamer staining was analyzed. A 2.7-fold-decrease in the co-expression of 2B4 and NRP1 was observed upon anti-4-1BB+ and anti-LAG-3 mAb treatment (FIG. 7C), indicating a loss of the surface phenotype associated with T cell dysfunction. To determine whether this change was accompanied by a shift towards an effector phenotype, expression of KLGR-1 was examined. Indeed, a marked increase in KLGR-1 expression was observed on the SIY-reactive TIL following treatment, and a 3.7-fold increase in the KLRG-1^{hi}IL-$7RA^{lo}$ population was observed (FIG. 7D).

To eliminate the possibility that treatment with anti-LAG-3+anti-4-1BB mAbs was not altering the phenotype of T cells already within the tumor but rather was supporting recruitment of newly primed functional T cells from secondary lymphoid organs, the S1PR inhibitor FTY720, which prevents T cell egress from lymph nodes (Halin et al., 2005; incorporated by reference in its entirety), was utilized. The efficacy of anti-PD-L1-based immunotherapies was preserved in the presence of FTY720, arguing for re-functionalization of TIL as the major mechanism of action (Spranger et al., 2014a; incorporated by reference in its entirety). FTY720 administration was started on day 6 after tumor inoculation, 24 hours before the start of anti-LAG-3+anti-4-1BB treatment, and continued every day until TIL analysis on day 14. Peripheral blood analyzed at the same time point revealed marked depletion of circulating T cells (FIG. 9). Despite this loss of circulating T cells, the down regulation of 2B4 and NRP1 and the shift towards the KLRG1^{hi}IL-$7RA^{lo}$ phenotype was nonetheless preserved (FIGS. 7E and F).

To examine functional restoration of the TIL, the KLRG-1^{lo}IL-$7RA^{lo}$ and KLRG-1 IL-$7RA^{lo}$ $CD8^+$ TIL populations were sorted from B16.SIY tumors on day 14 following treatment and analyzed for IL-2 after restimulation in vitro. Indeed, the KLRG-1^{lo}IL-$7RA^{lo}$ and KLRG-1^{hi}IL-$7RA^{lo}$ populations showed an increased capacity to produce IL-2 upon stimulation (FIG. 7G). The relative level of Il-2 mRNA was comparable between the two $CD8^+$ TIL populations and control $CD8^+CD44^+$ TdLN T cells. Collectively, these data indicate that anti-4-1BB/anti-LAG-3 combinatorial treatment induces significant changes in the phenotype profile and promotes functional restoration of tumor antigen-specific $CD8^+$ T cells already present within the tumor microenvironment.

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The invention claimed is:

1. A method of treating dysfunctional tumor antigen-specific CD8$^+$ T cells in a subject in need thereof with a solid tumor cancer comprising identifying dysfunctional T cells by testing said cells for expression of GPNMB; and administering to the subject an antibody or antibody fragment that specifically targets dysfunctional tumor antigen-specific CD8$^+$ T cells, wherein the antibody or antibody fragment targets GPNMB expressed on the surface of the T cells, and wherein the dysfunctional tumor antigen-specific CD8+ T cells are within a tumor microenvironment.

2. The method of claim **1**, wherein the tumor allows T cell infiltration, but is resistant to immunotherapies.

3. The method of claim **1**, further comprising contacting the dysfunctional tumor antigen-specific CD8+ T cells with an anti-4-1BB and/or anti-LAG3 agent.

4. The method of claim **3**, wherein the anti-4-1BB and/or anti-LAG3 agent is an antibody, antibody fragment, or antibody mimetic molecule.

5. The method of claim **1**, further comprising co-administration of an additional therapeutic agent.

6. The method of claim **5**, wherein the additional therapeutic agent is a chemotherapeutic or an immunotherapeutic agent.

7. The method of claim **6**, wherein the additional therapeutic agent is an immunotherapeutic agent selected from the list consisting of cell-based therapies, monoclonal antibody (mAb) therapy, cytokine therapy, and adjuvant treatment.

8. The method of claim **7**, wherein the immunotherapeutic agent is a mAb therapy selected from the list consisting of anti-CTLA-4 monoclonal antibodies and/or anti-PD-L1 monoclonal antibodies.

9. The method of claim 7, wherein the immunotherapeutic agent is a cell-based therapy selected from the list consisting of dendritic-cell therapy and T-cell therapy.

10. The method of claim 5, wherein the additional therapeutic agent targets PD-1, TIM-3, OX-40ICOS, TIGIT, CD244, TNFRSF18, Nrn1, Nrp1, KLRG1, GM156, GPNMB, GPR65, TMEM205, and TMEM126A, Nrn1, CRTAM and/or Sema7a.

11. The method of claim 1, wherein the antibody or antibody fragment is an anti-Nrn1 antibody, antibody fragment, or antibody mimetic molecule.

12. The method of claim 1, wherein the antibody or antibody fragment is an anti-Sema7a antibody, antibody fragment, or antibody mimetic molecule.

13. The method of claim 1, wherein the antibody or antibody fragment is an anti-CRTAM antibody, antibody fragment, or antibody mimetic molecule.

* * * * *