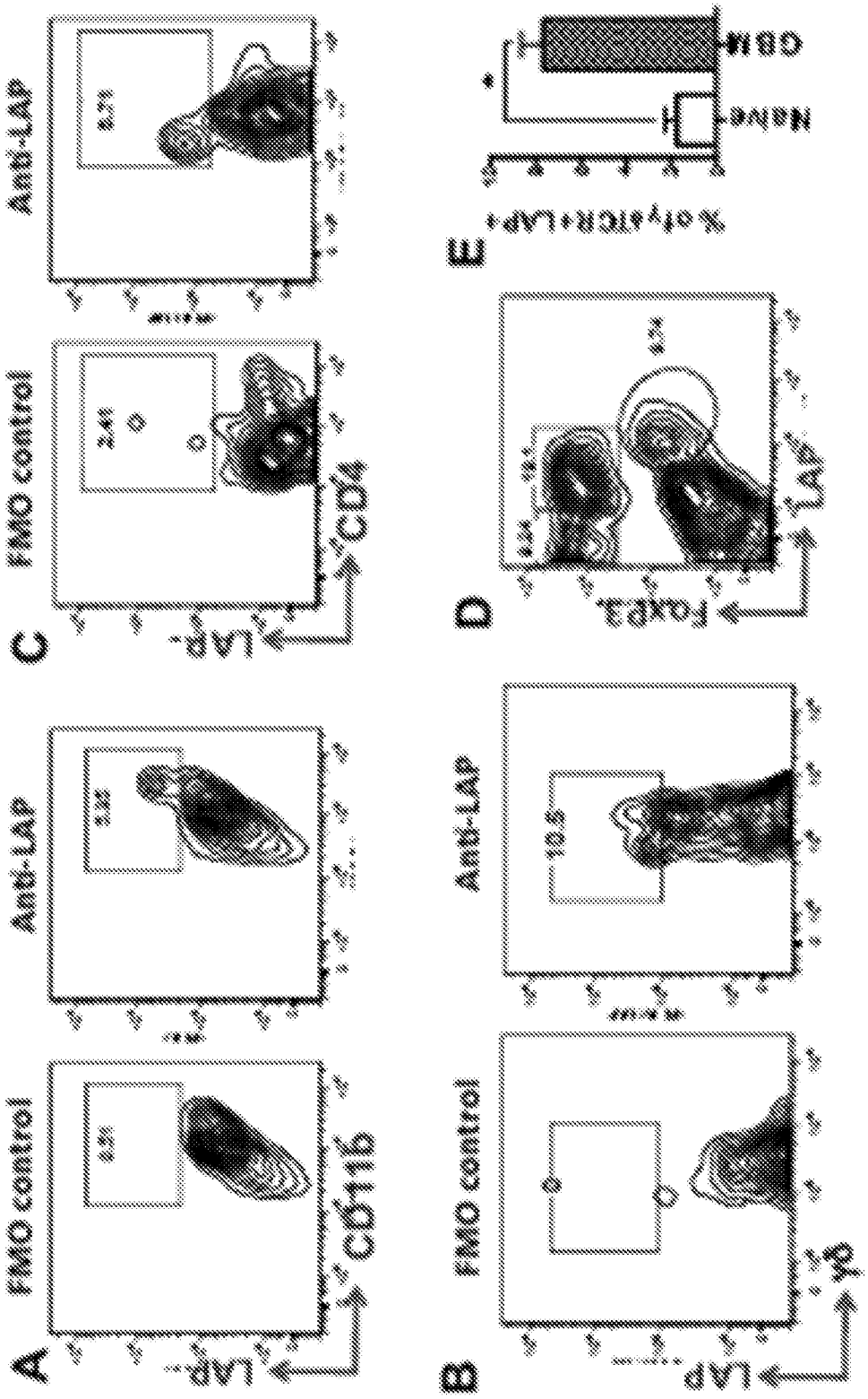
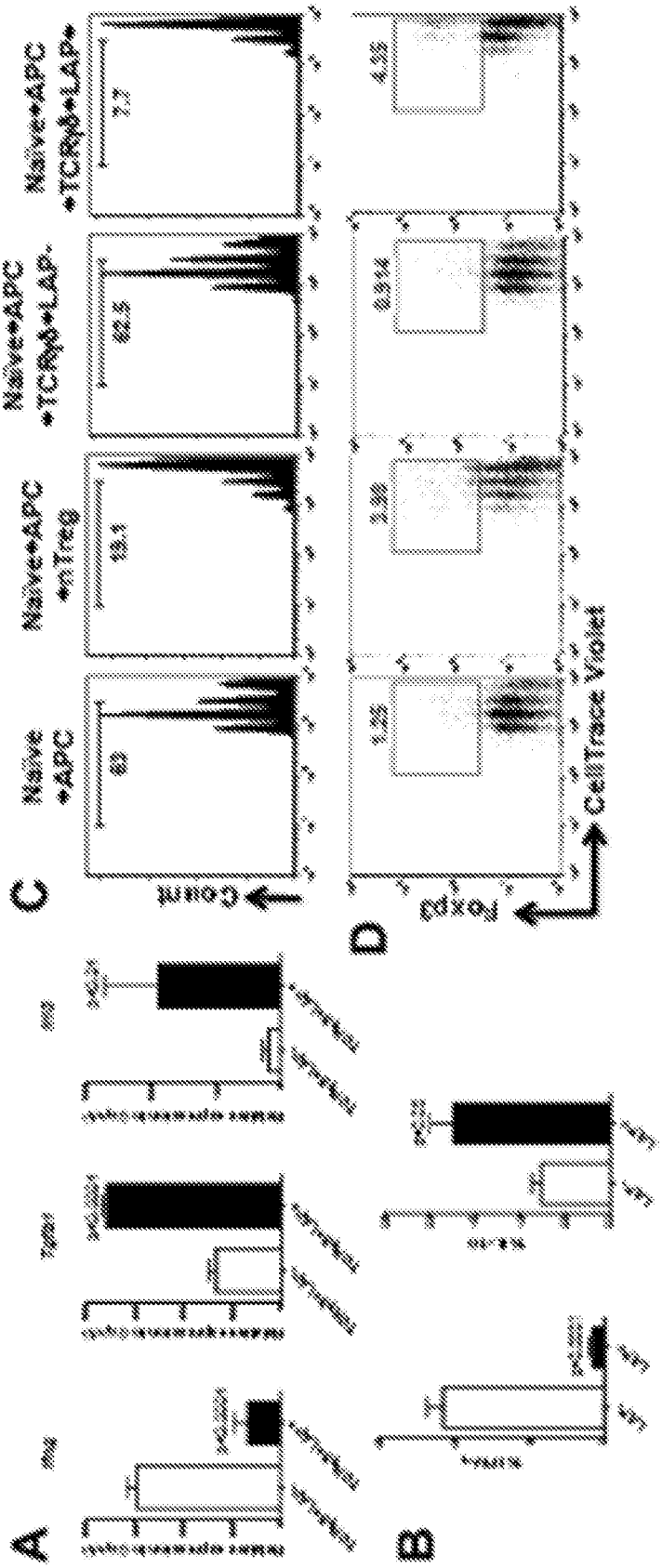
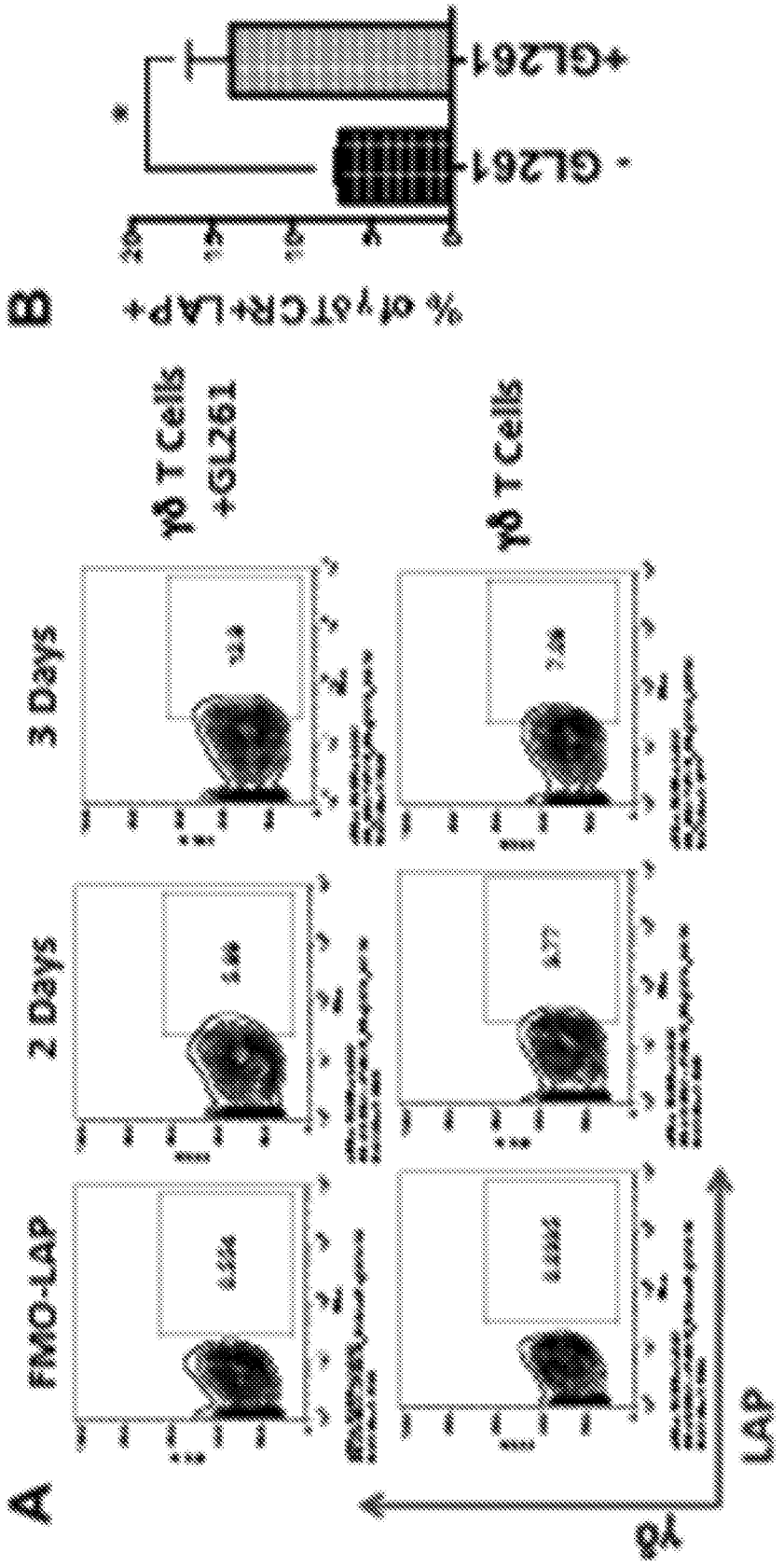
FIGS. 1A-1E


A
FMO control
Anti-LAP
LAP
CD116
B
FMO control
Anti-LAP
LAP
Foxp3
C
FMO control
Anti-LAP
LAP
CD4
D
Foxp3
LAP
E
% αIγδTCR+LAP+
Naïve
CD4

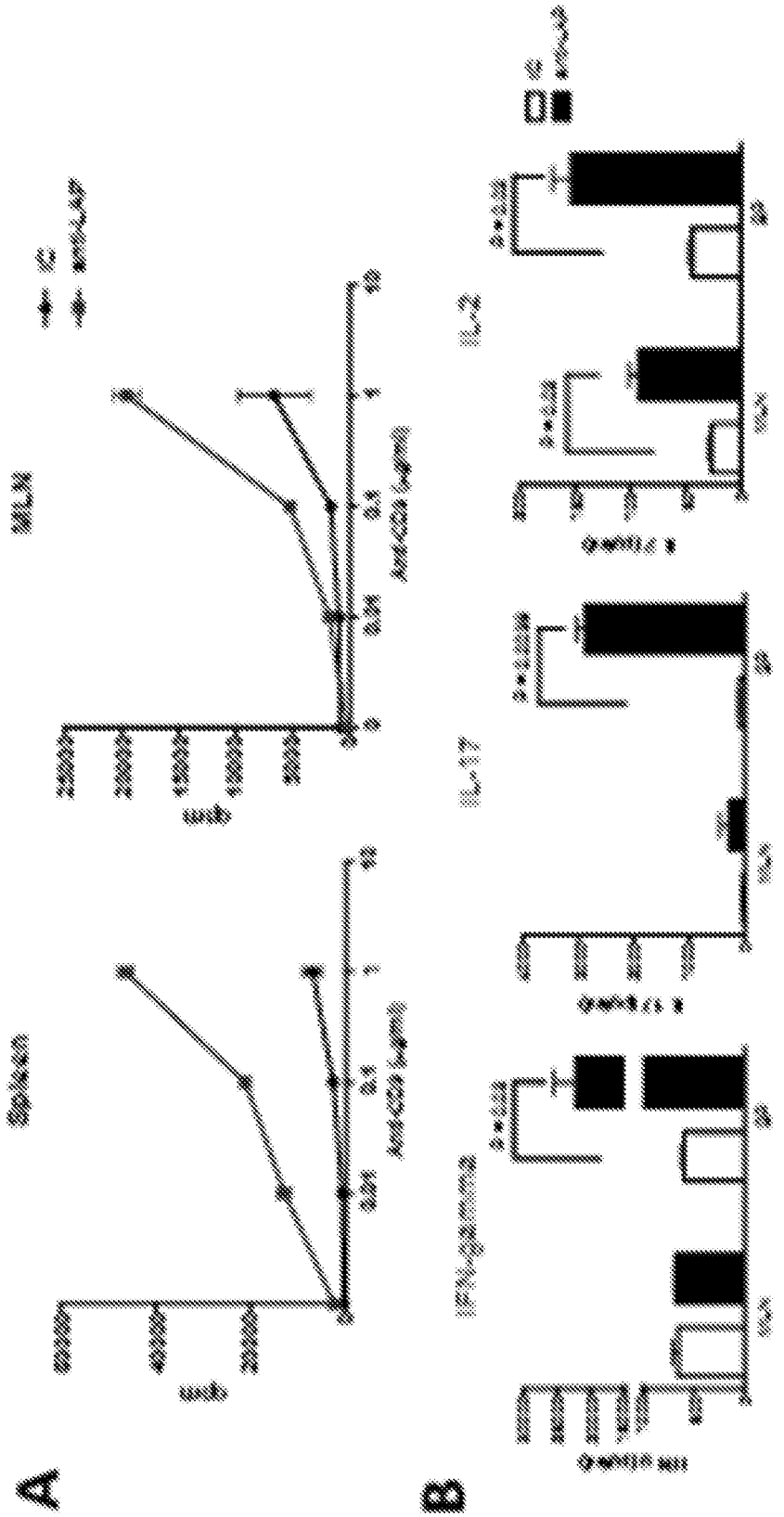
FIGS. 2A-2D


A
B
C
D
Naive
+APC
Naive+APC
+nTreg
Naive+APC
+TCRVβ+LAP-
Naive+APC
+TCRVβ+LAP+
Count
Foxp3
CellTrace Violet
0
18.1
62.3
7.7
1.25
2.36
8.314
4.55

FIGS. 3A-3B


A
FMO-LAP
2 Days
3 Days
γδ T Cells
γδ T Cells +GL261
LAP
B
% αβγδTCR+LAP+
- GL261
+GL261

FIGS. 4A-4B



FIGS. 5A-5K

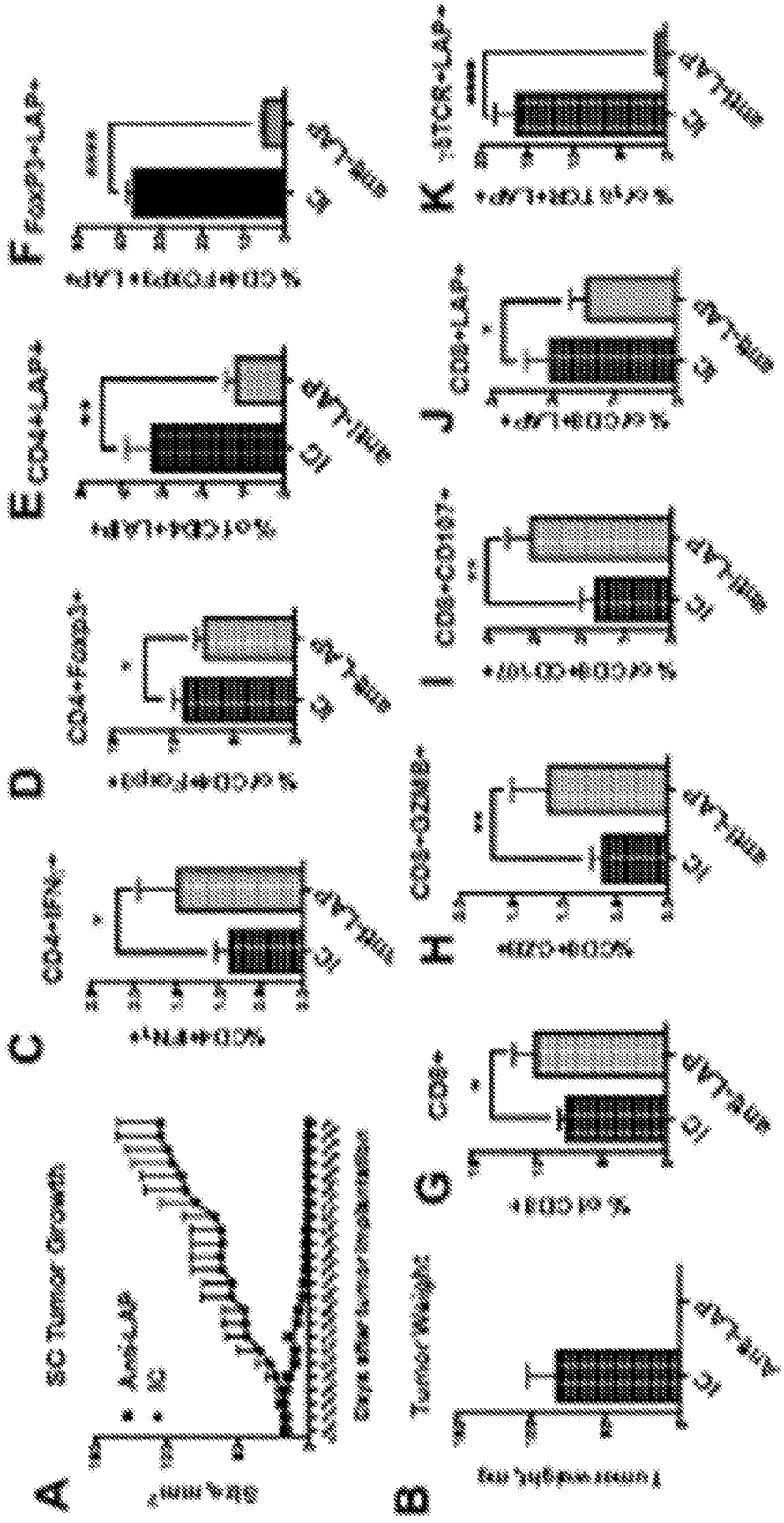
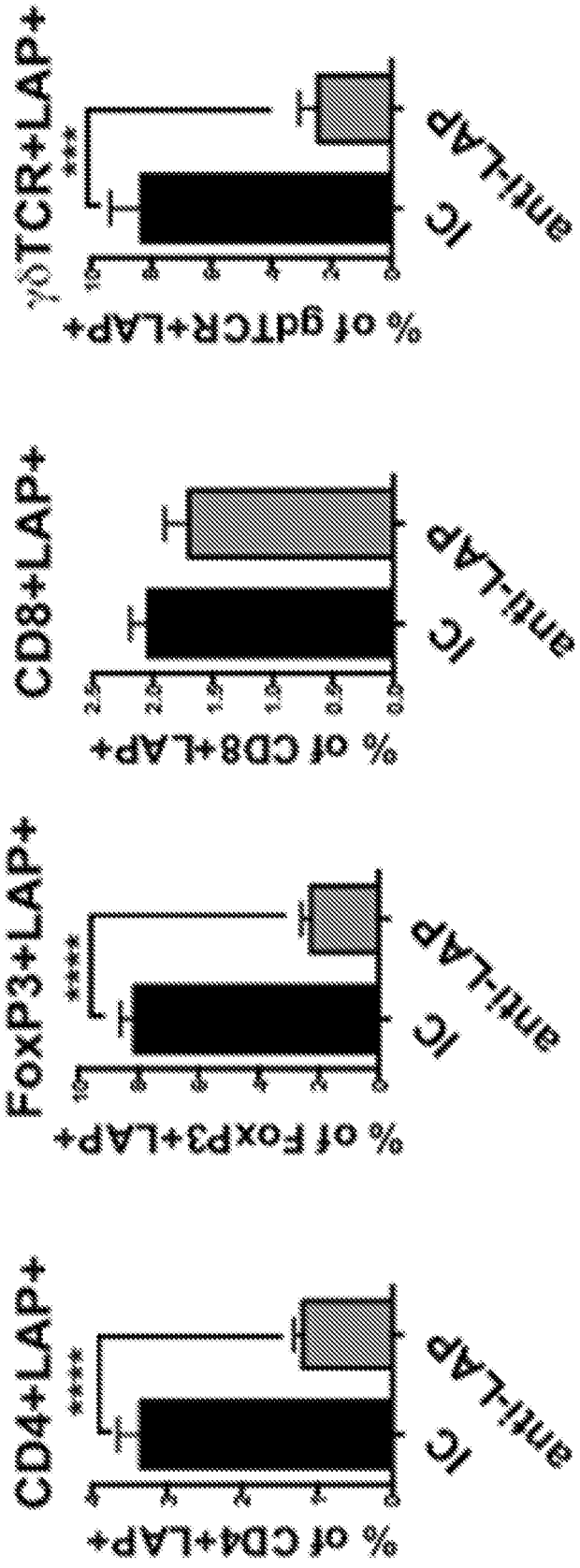

A
SC Tumor Growth
Anti-LAP
Ig
B
Tumor Weight
C
CD4+IFNγ+
D
CD4+Foxp3+
E
CD4+LAP+
F
FoxP3+LAP+
G
CD8+
H
CD8+GZMB+
I
CD8+CD107+
J
CD8+LAP+
K
γδTCR+LAP+

FIG. 6


CD4+LAP+
****
% of CD4+LAP+
IC
anti-LAP
FoxP3+LAP+
****
% of FoxP3+LAP+
IC
anti-LAP
CD8+LAP+
% of CD8+LAP+
IC
anti-LAP
γδTCR+LAP+
***
% of gdTCR+LAP+
IC
anti-LAP

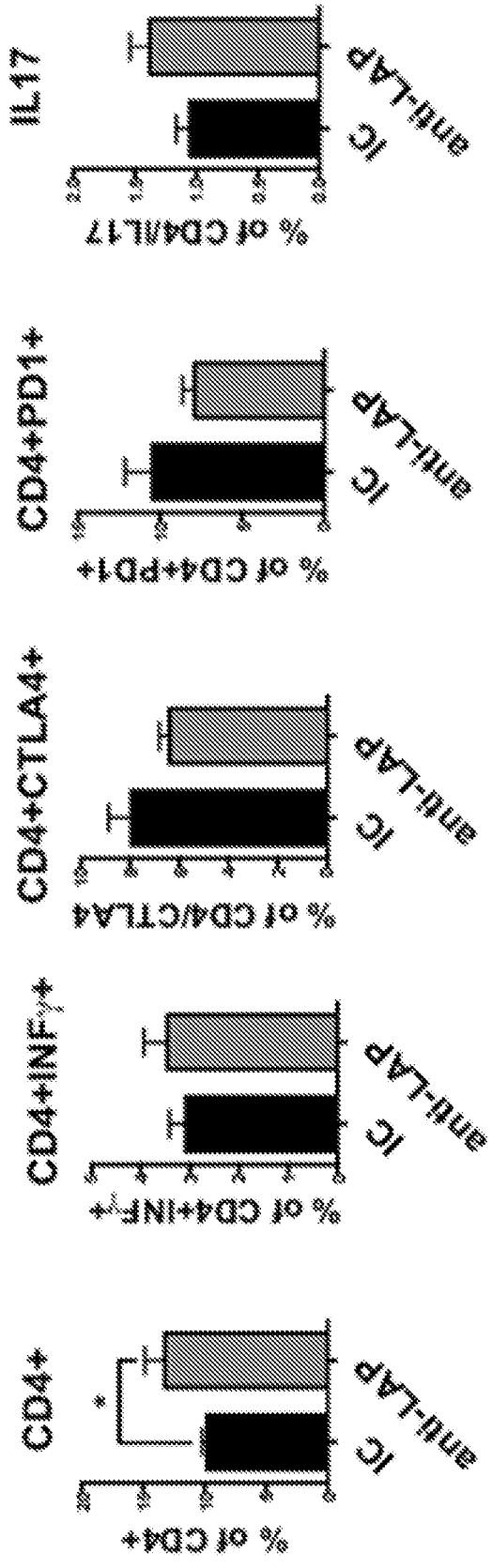

CD4+
% of CD4+
*
IC
anti-LAP
CD4+INFγ+
% of CD4+INFγ+
IC
anti-LAP
CD4+CTLA4+
% of CD4/CTLA4
IC
anti-LAP
CD4+PD1+
% of CD4+PD1+
IC
anti-LAP
IL17
% of CD4/IL17
IC
anti-LAP

FIG. 7

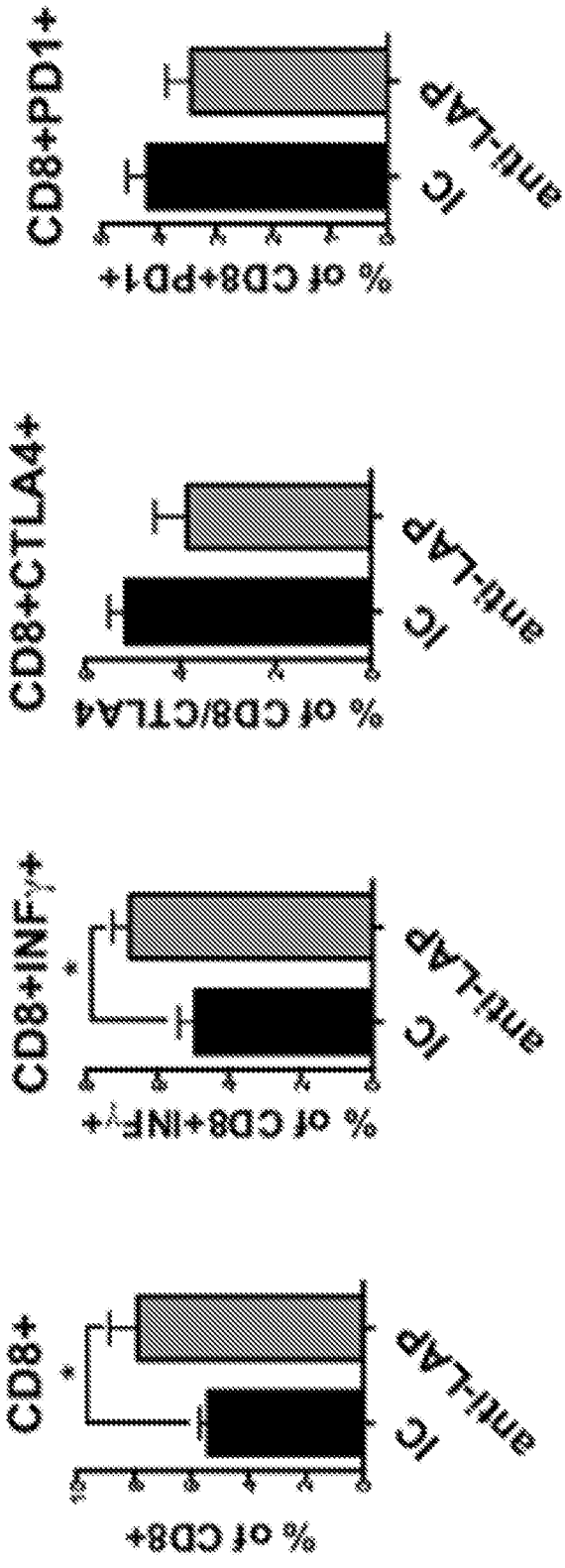

CD8+PD1+
% of CD8+PD1+
IC
anti-LAP
CD8+CTLA4+
% of CD8/CTLA4
IC
anti-LAP
CD8+INFγ+
% of CD8+INFγ+
*
IC
anti-LAP
CD8+
% of CD8+
*
IC
anti-LAP

FIG. 8

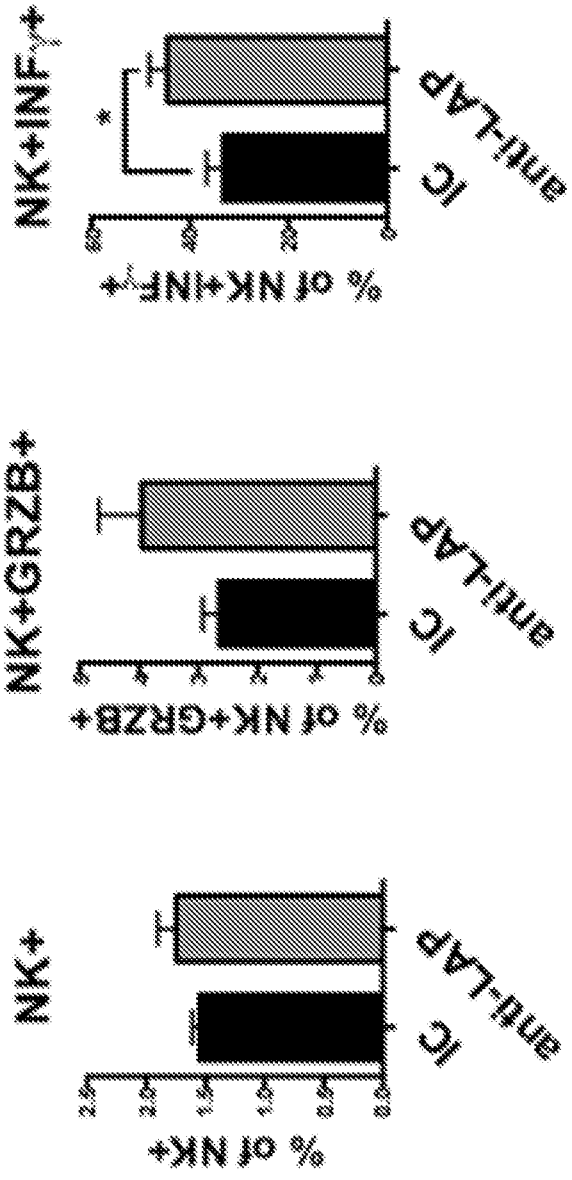

NK+
% of NK+
anti-LAP
IC
NK+GRZB+
% of NK+GRZB+
anti-LAP
IC
NK+INFγ+
% of NK+INFγ+
anti-LAP
IC
*

FIG. 9

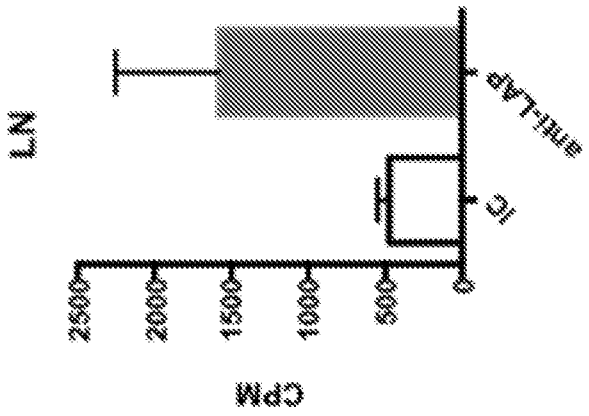


FIG. 10

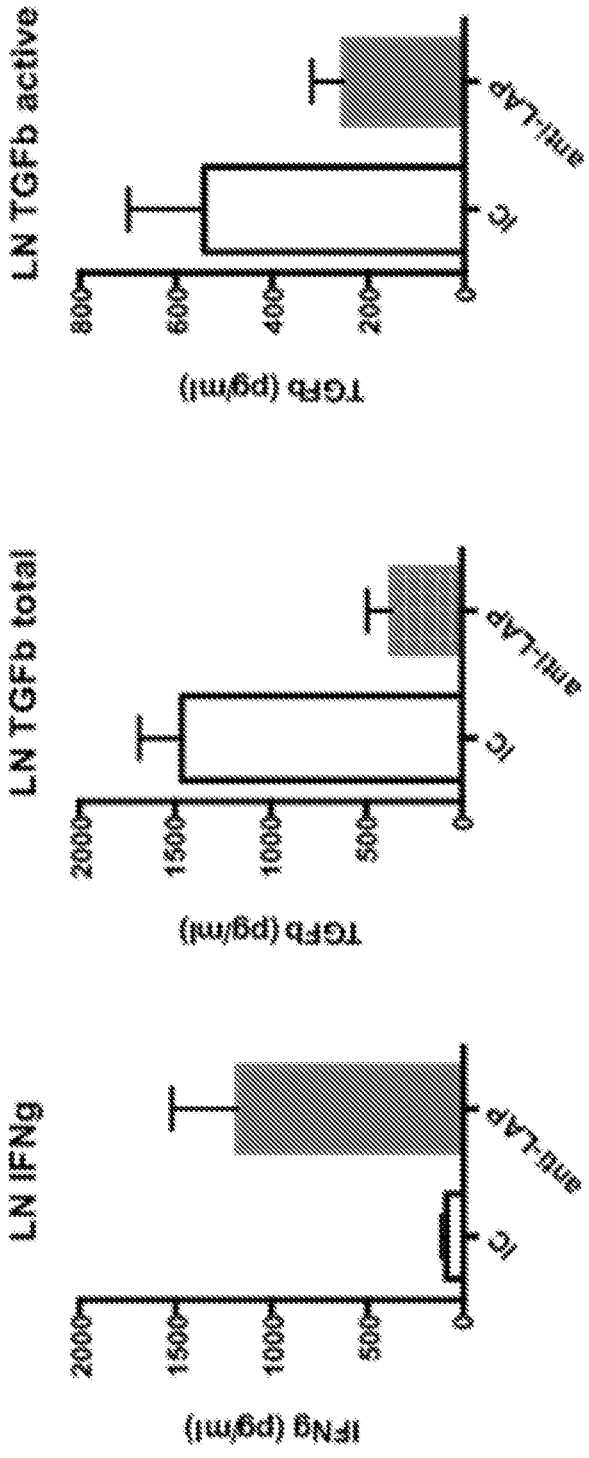

LN TGFb active
TGFb (pg/ml)
800
600
400
200
0
IC
anti-LAP
LN TGFb total
TGFb (pg/ml)
2000
1500
1000
500
0
IC
anti-LAP
LN IFNg
IFNg (pg/ml)
2000
1500
1000
500
0
IC
anti-LAP

FIG. 11

FIG. 12

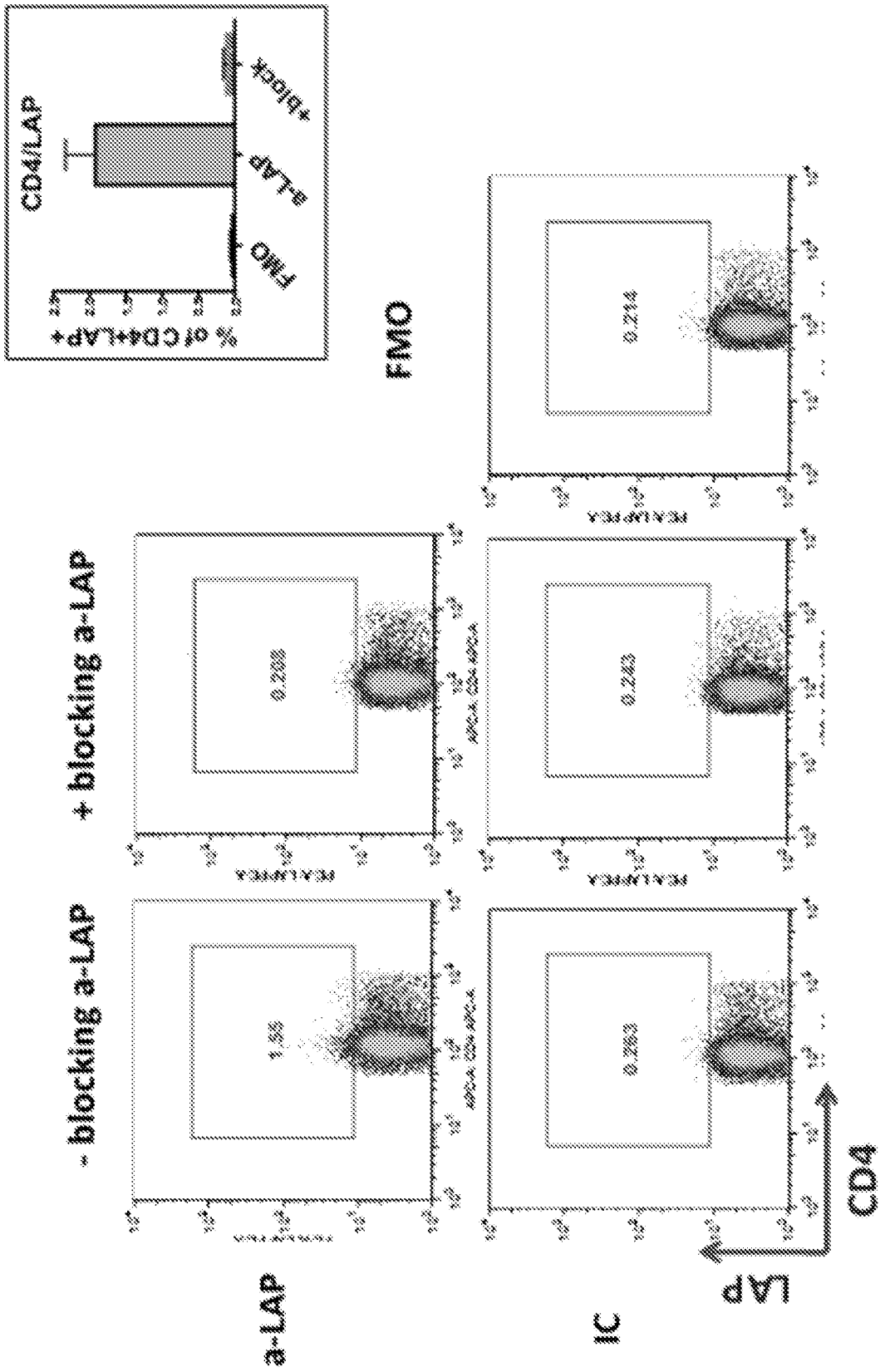
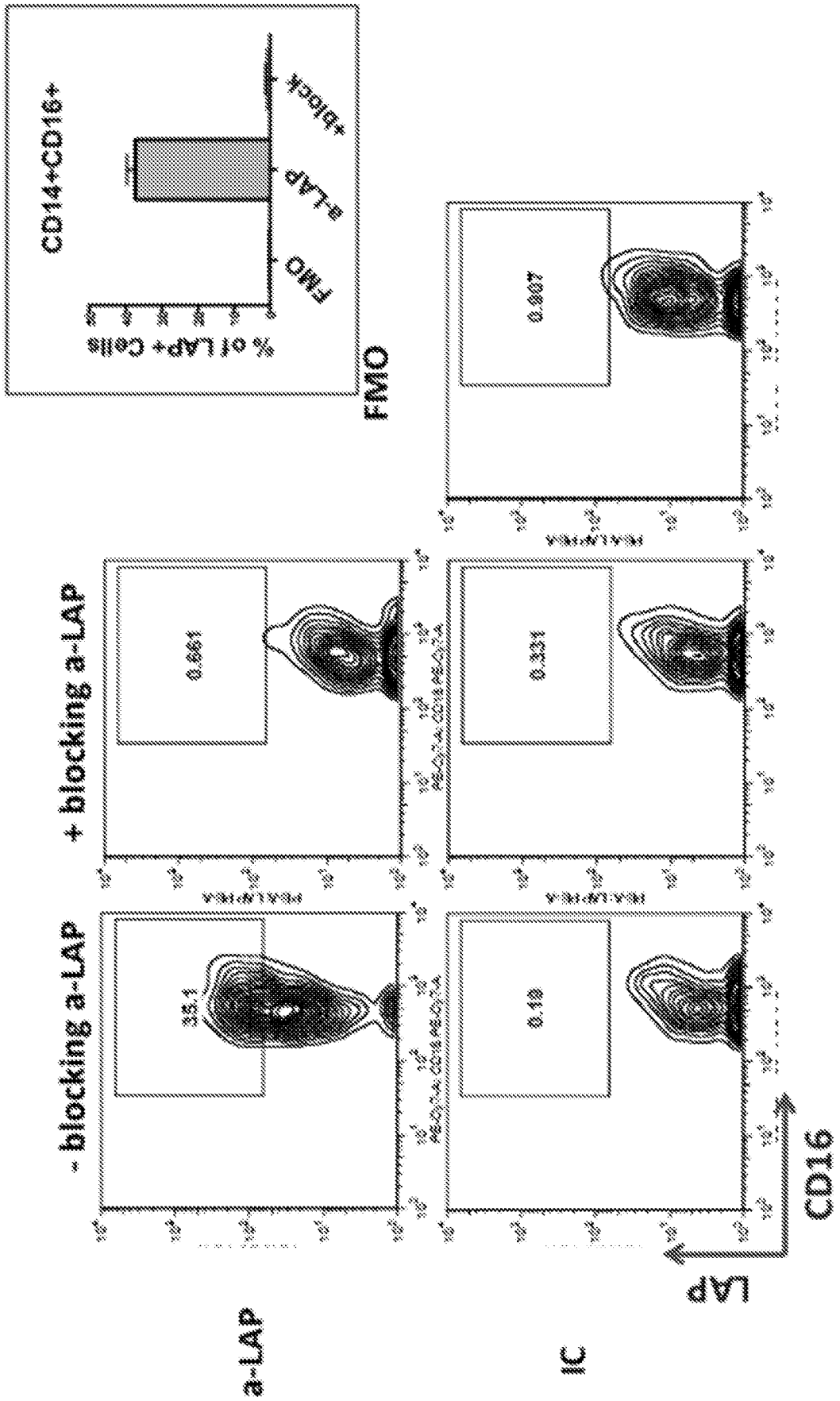

CD4/LAP
% of CD4+LAP+
FMO
a-LAP
+block
FMO
+ blocking a-LAP
- blocking a-LAP
a-LAP
IC
LAP
CD4
0.214
0.243
0.208
0.263
1.59

FIG. 13


FMO
CD14+CD16+
% of LAP+ Cells
FMO
a-LAP
+block
- blocking a-LAP
+ blocking a-LAP
35.1
0.661
0.10
0.331
0.007
a-LAP
IC
LAP
CD16

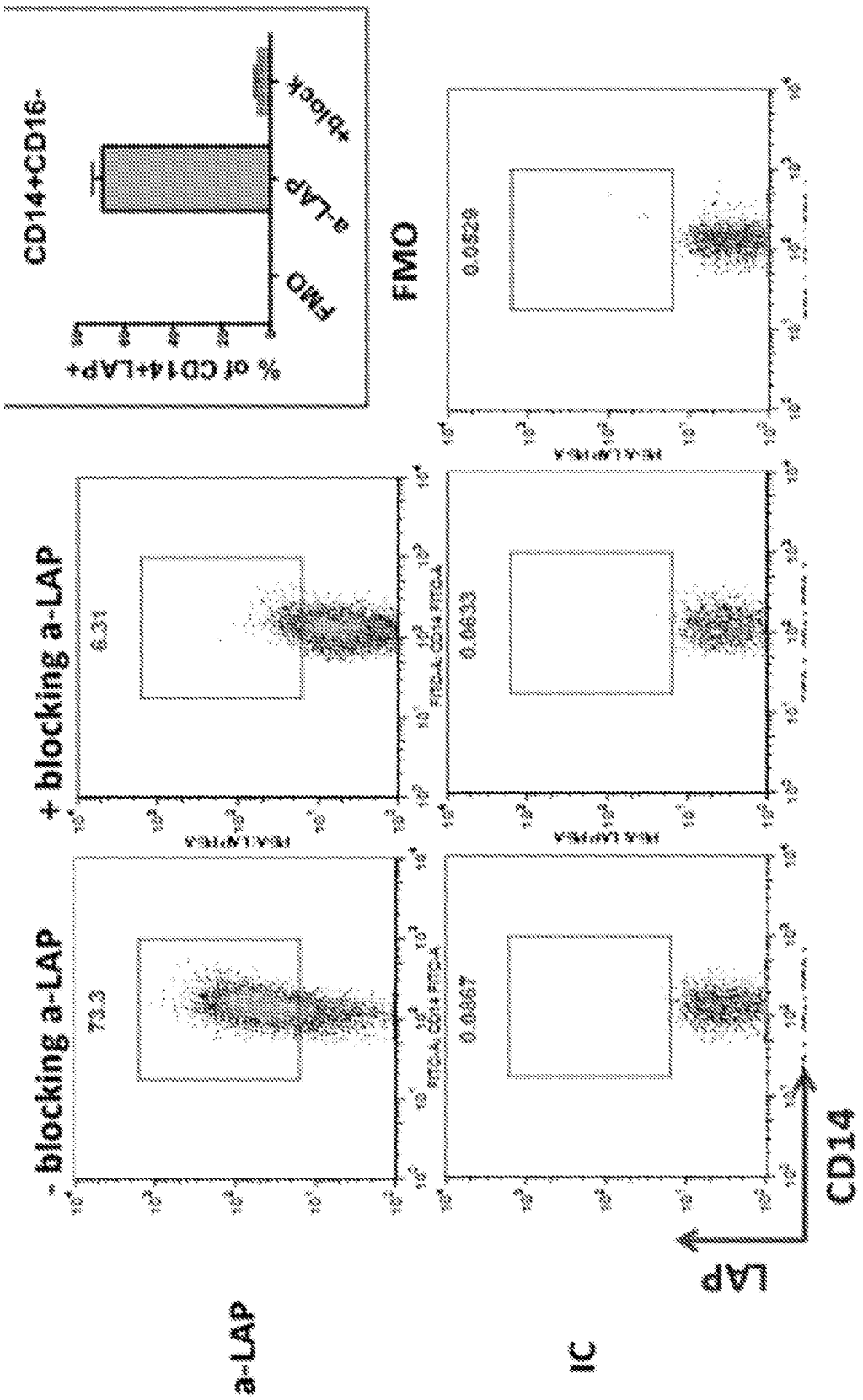

FIG. 14
a-LAP
IC
- blocking a-LAP
+ blocking a-LAP
FMO
CD14+CD16-
% of CD14+LAP+
FMO
a-LAP
+block
73.3
6.31
0.0529
0.0667
0.0633
LAP
CD14

FIG. 15

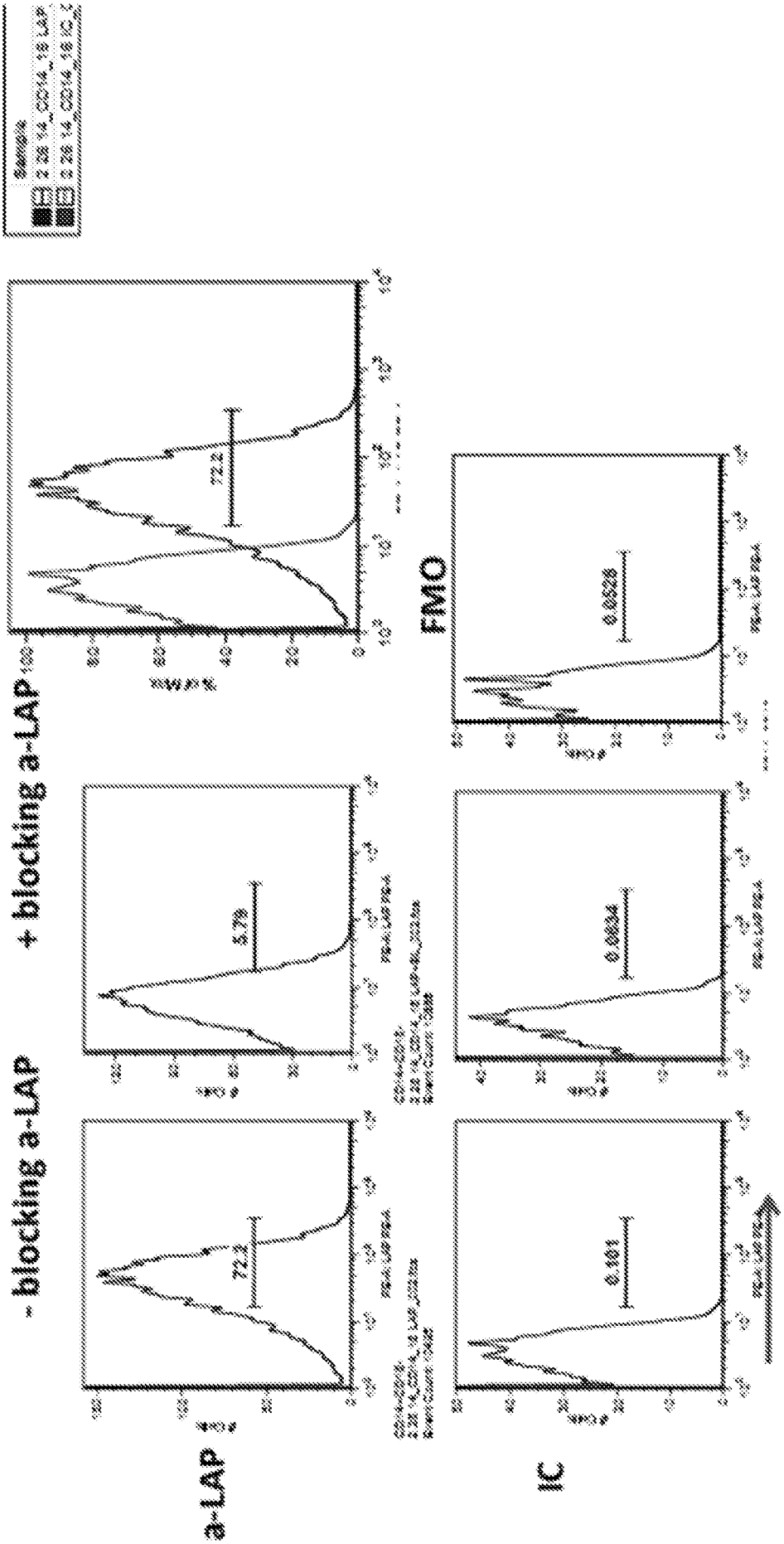

- blocking a-LAP
+ blocking a-LAP
a-LAP
IC
FMO
72.2
5.79
0.101
0.0604
0.0526

FIG. 16

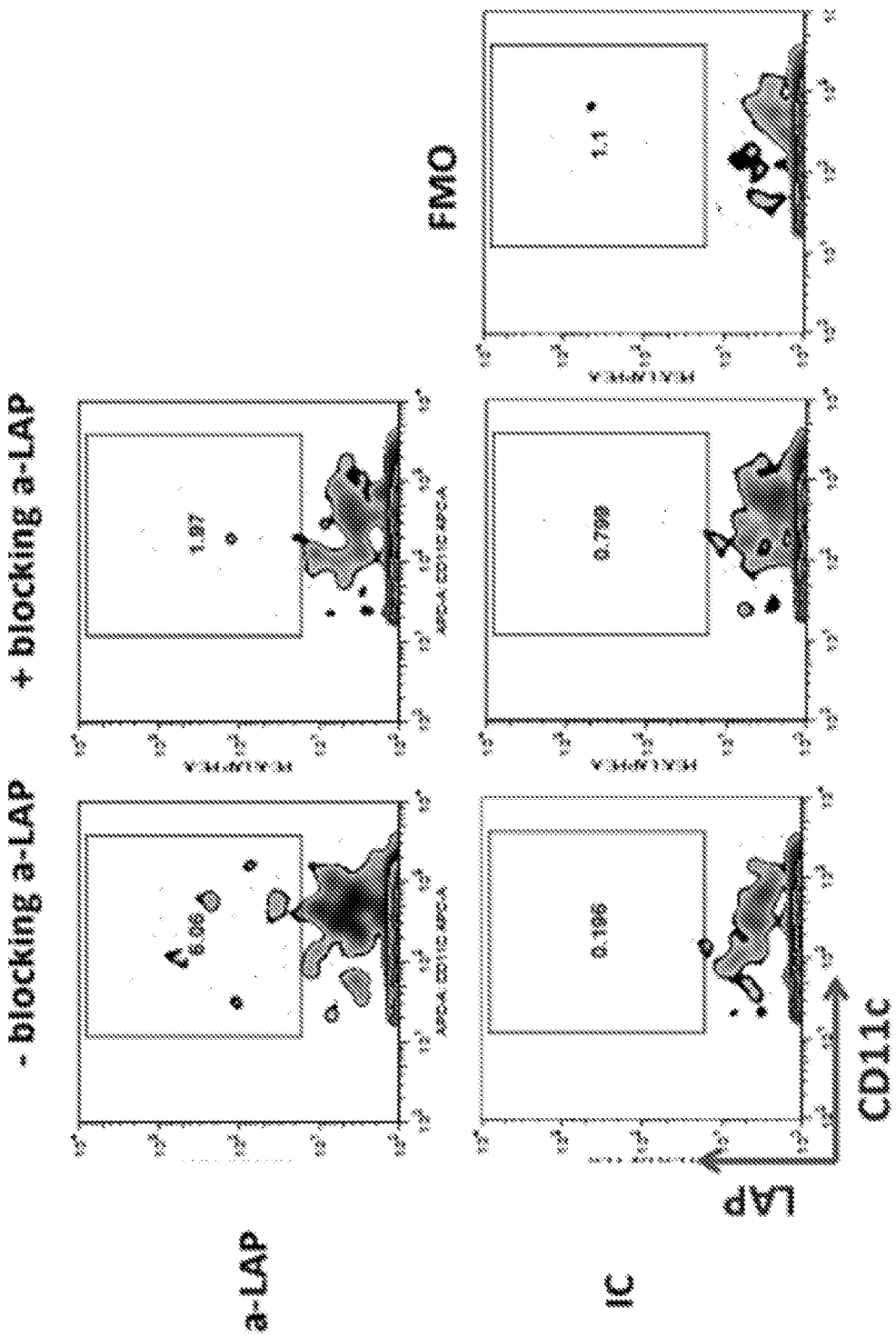

- blocking a-LAP
+ blocking a-LAP
FMO
a-LAP
IC
LAP
CD11c
6.66
1.87
0.799
0.196
1.1

FIG. 17

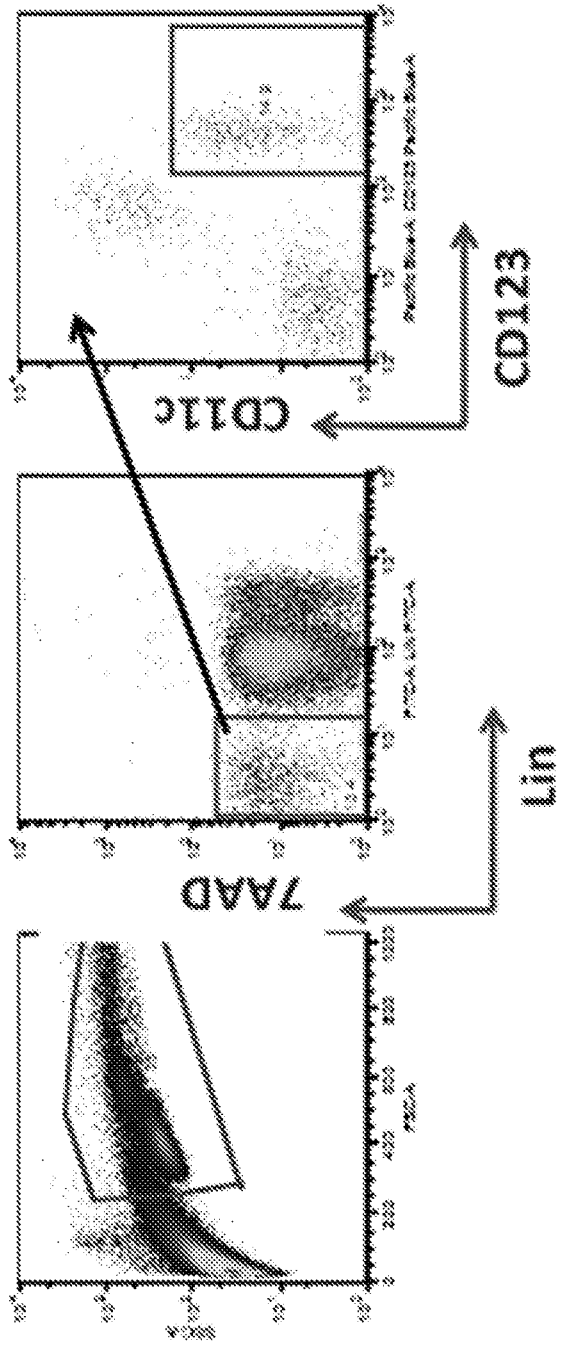


FIG. 18

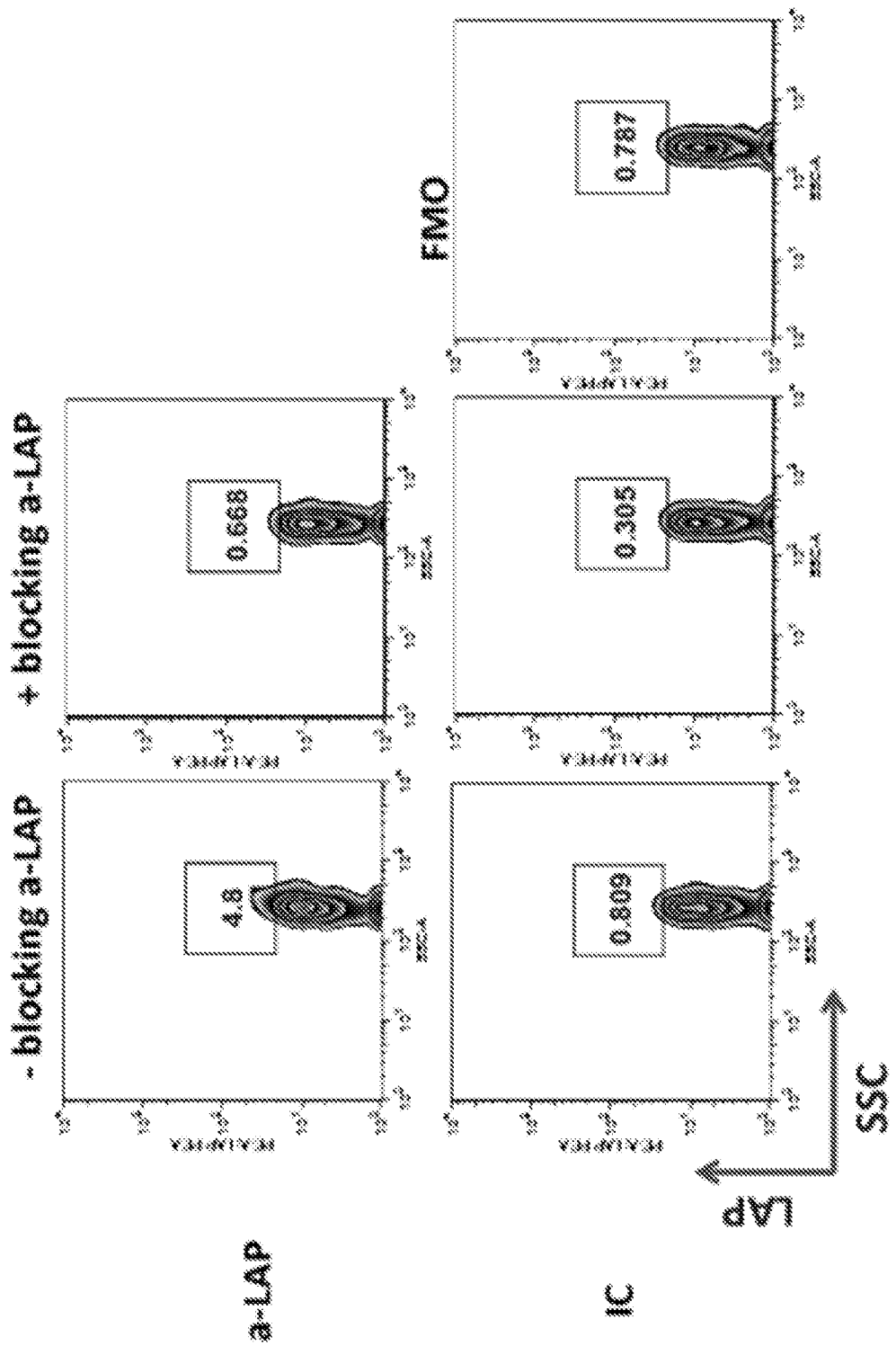
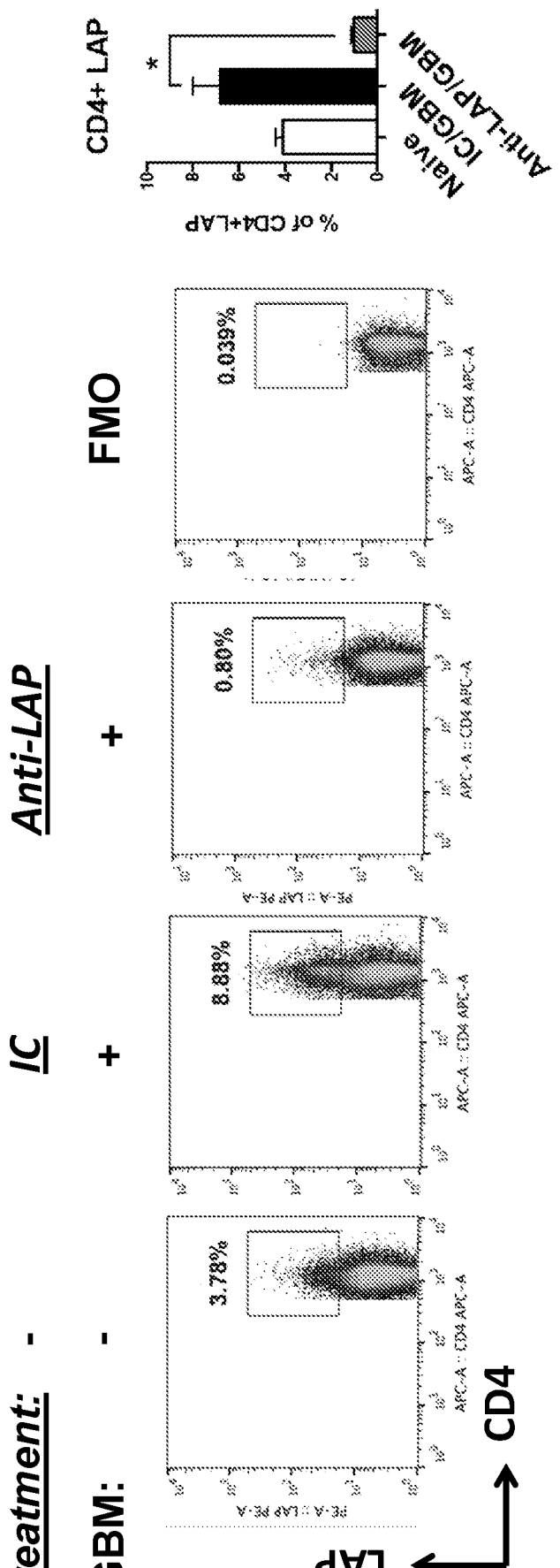

- blocking a-LAP
+ blocking a-LAP
FMO
a-LAP
IC
4.8
0.668
0.787
0.809
0.305
LAP
SSC

FIG. 19



SC GBM Model

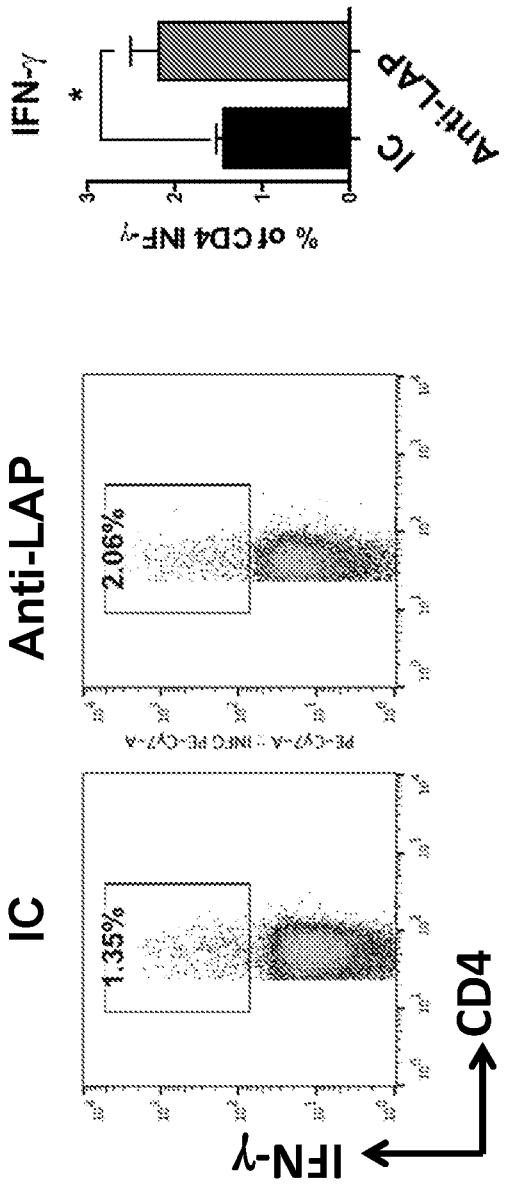
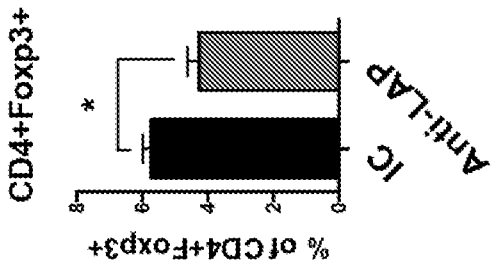


FIG. 20

SC GBM Model



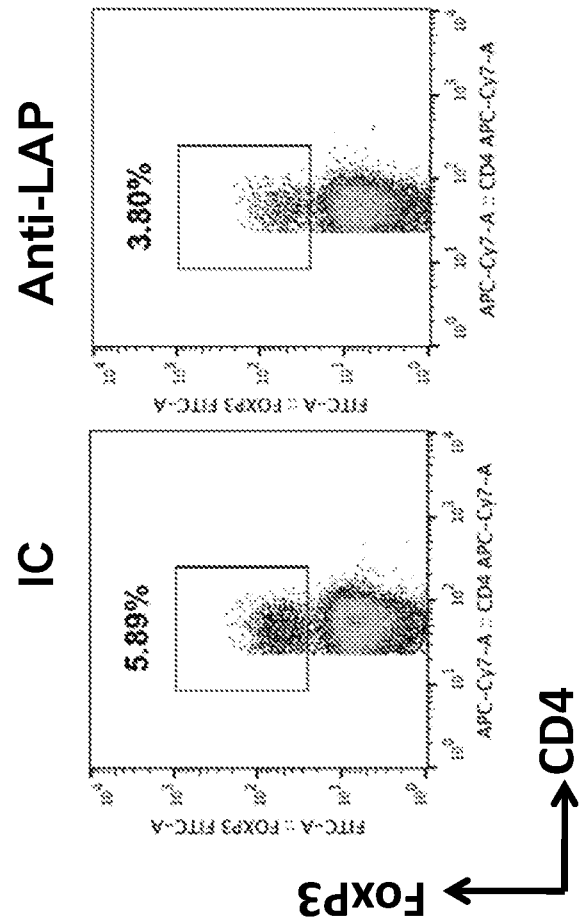


FIG. 21

SC GBM Model

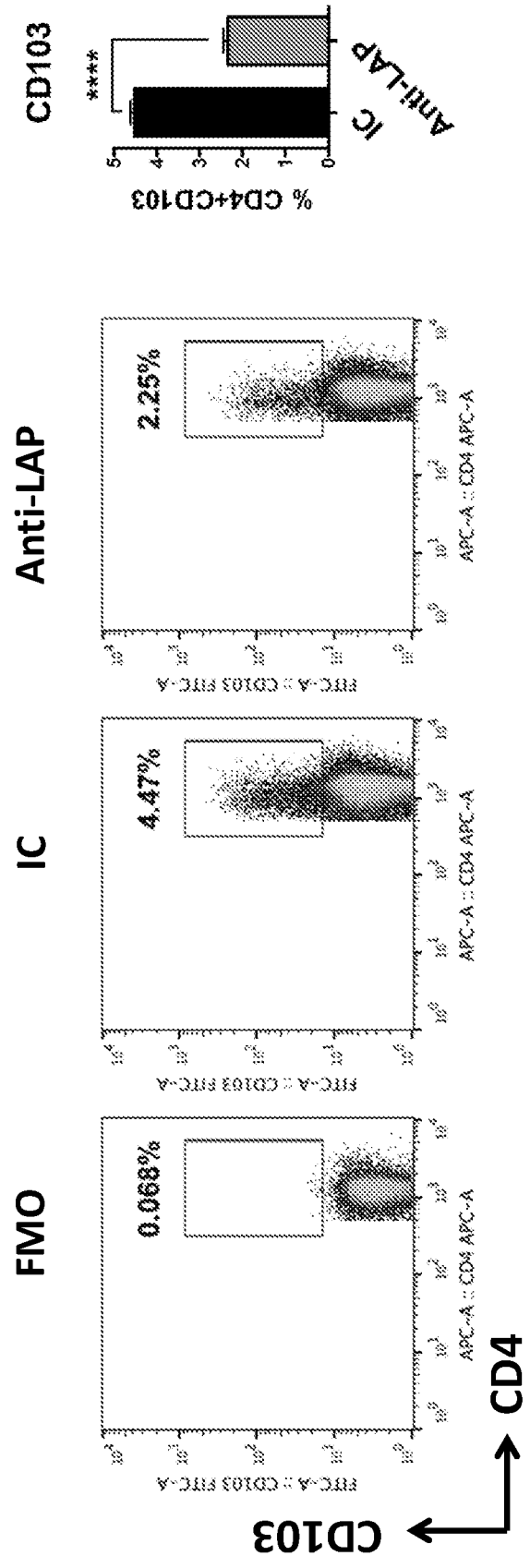

FMO
IC
Anti-LAP
0.068%
4.47%
2.25%
CD103
CD4
APC-A :: CD4 APC-A
FITC-A :: CD103 FITC-A
CD103
% CD4+CD103
****
IC
Anti-LAP
SC GBM Model

FIG. 22

FIG. 23

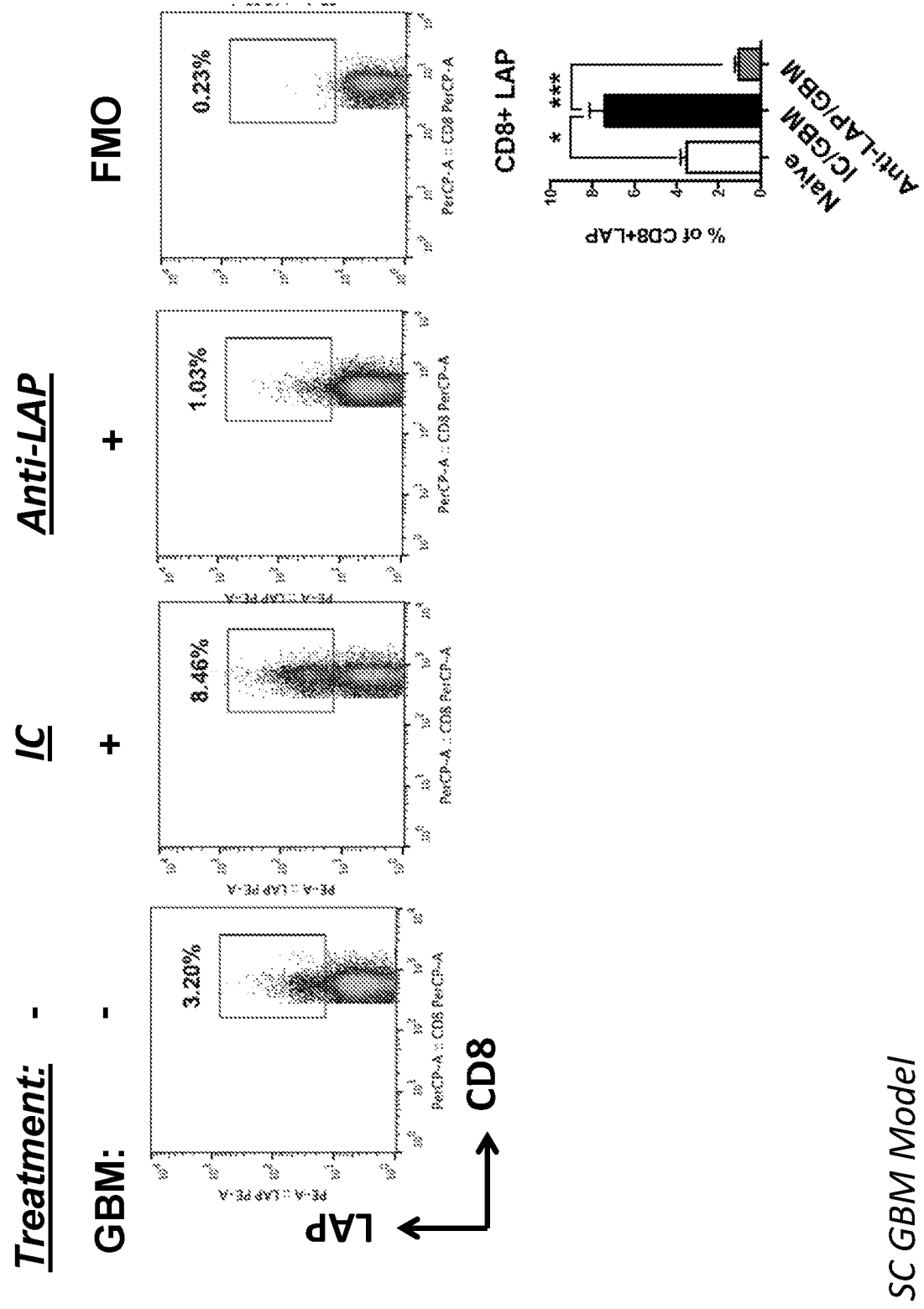

Treatment:
IC
Anti-LAP
GBM:
FMO
3.20%
8.46%
1.03%
0.23%
LAP
CD8
CD8+ LAP
% of CD8+LAP
Naive
IC/GBM
Anti-LAP/GBM
SC GBM Model

FIG. 24

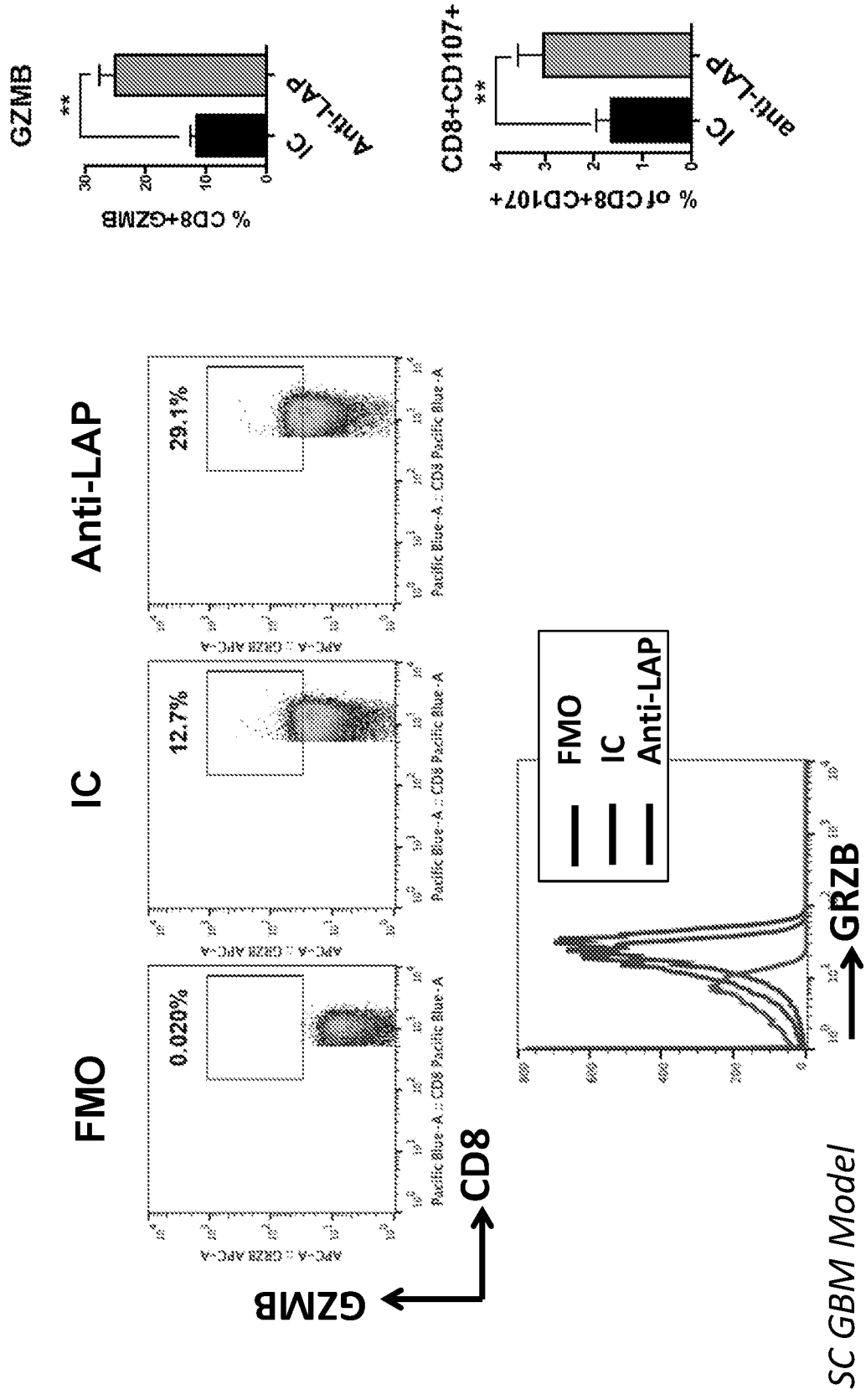
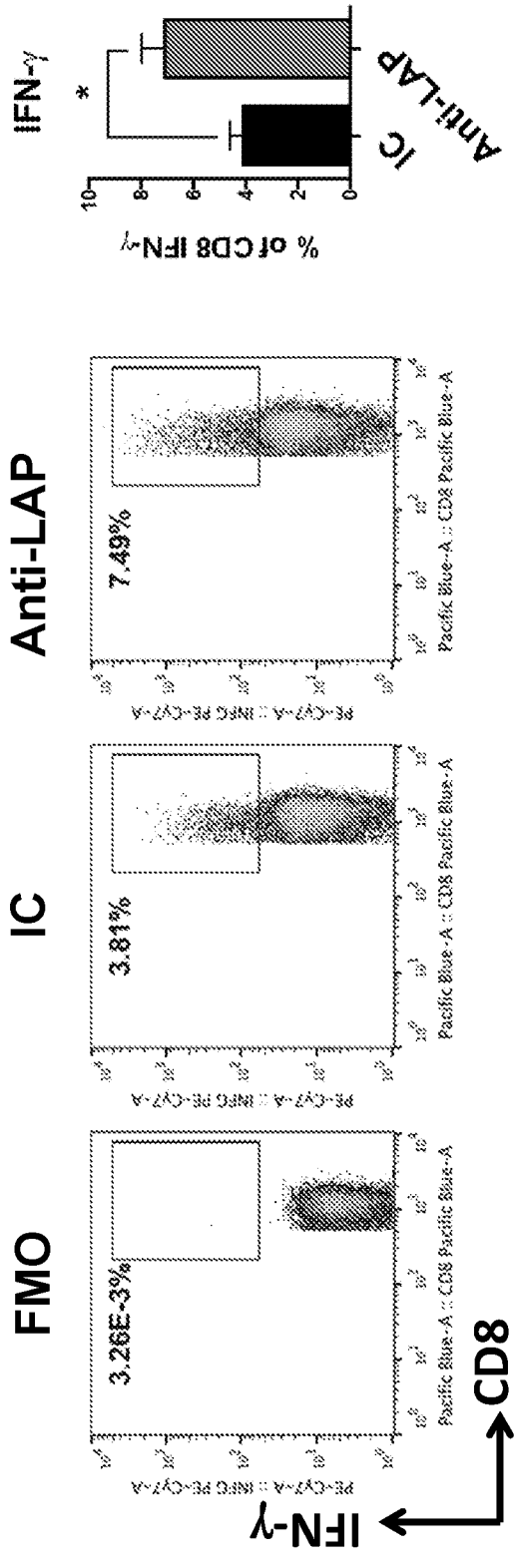

GZMB
**
IC
Anti-LAP
% CD8+GZMB
CD8+CD107+
**
IC
anti-LAP
% of CD8+CD107+
FMO
IC
Anti-LAP
0.020%
12.7%
29.1%
GZMB
CD8
FMO
IC
Anti-LAP
GRZB
SC GBM Model

FIG. 25



SC GBM Model

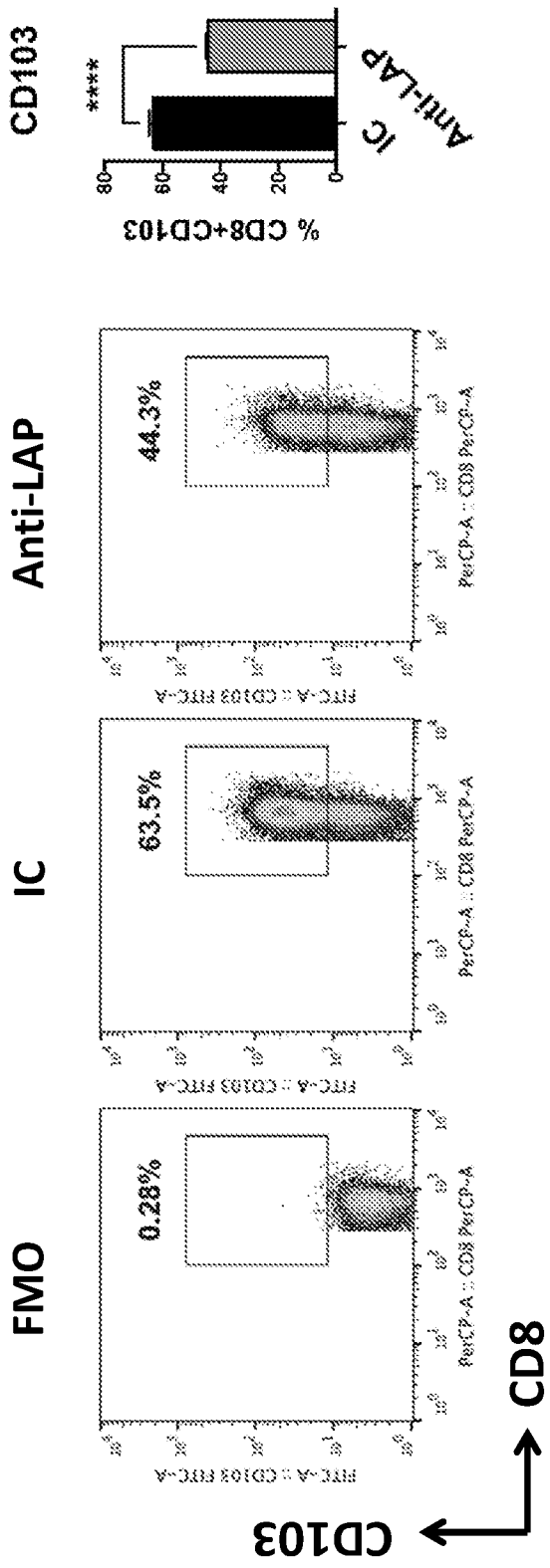

FMO
IC
Anti-LAP
0.28%
63.5%
44.3%
CD103
CD8
CD103
% CD8+CD103
IC
Anti-LAP
****

FIG. 26

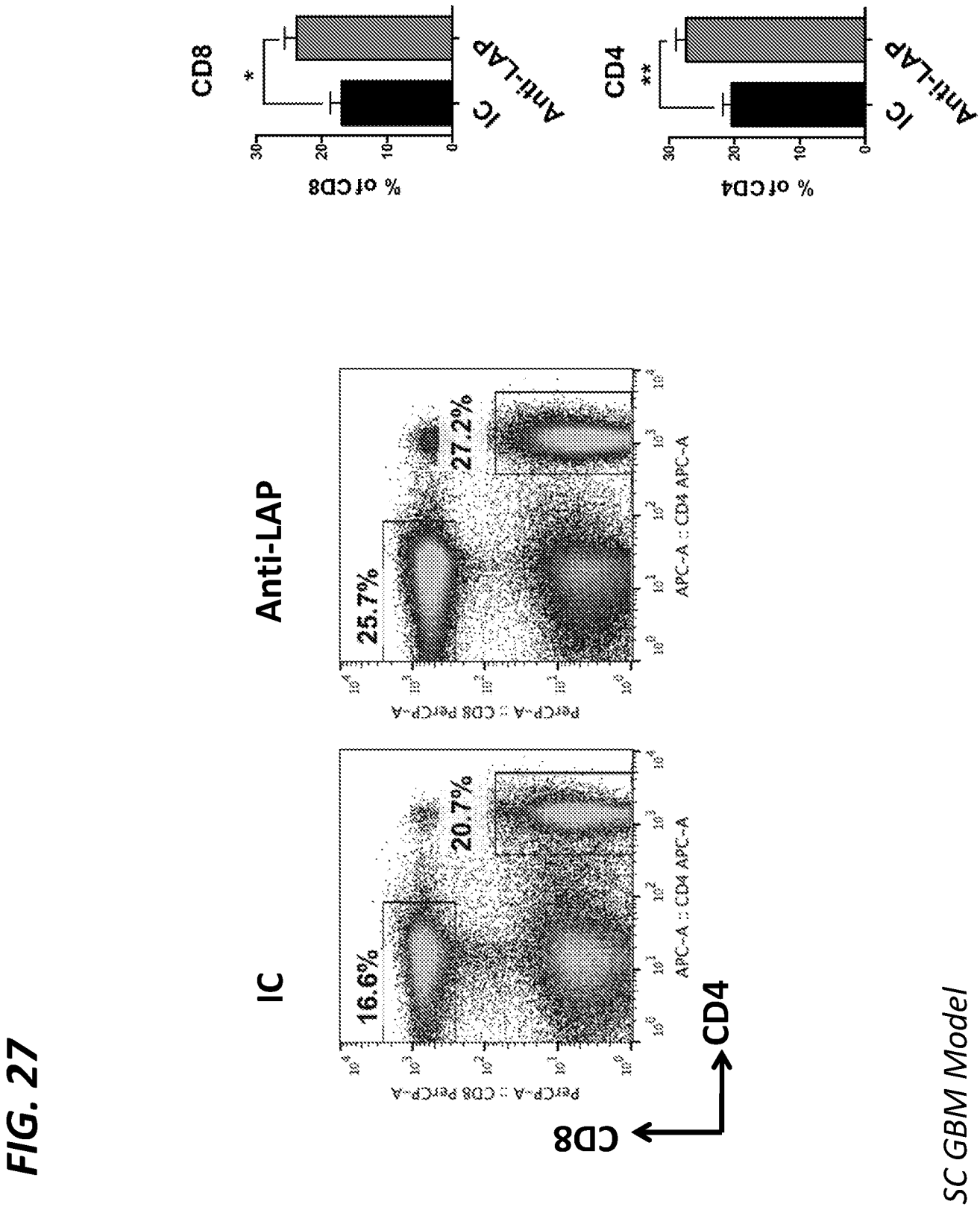

CD8
*
IC
Anti-LAP
% of CD8
CD4
**
IC
Anti-LAP
% of CD4
Anti-LAP
IC
25.7%
27.2%
16.6%
20.7%
PerCP-A :: CD8 PerCP-A
APC-A :: CD4 APC-A
CD8
CD4
SC GBM Model

FIG. 27

FIG. 28

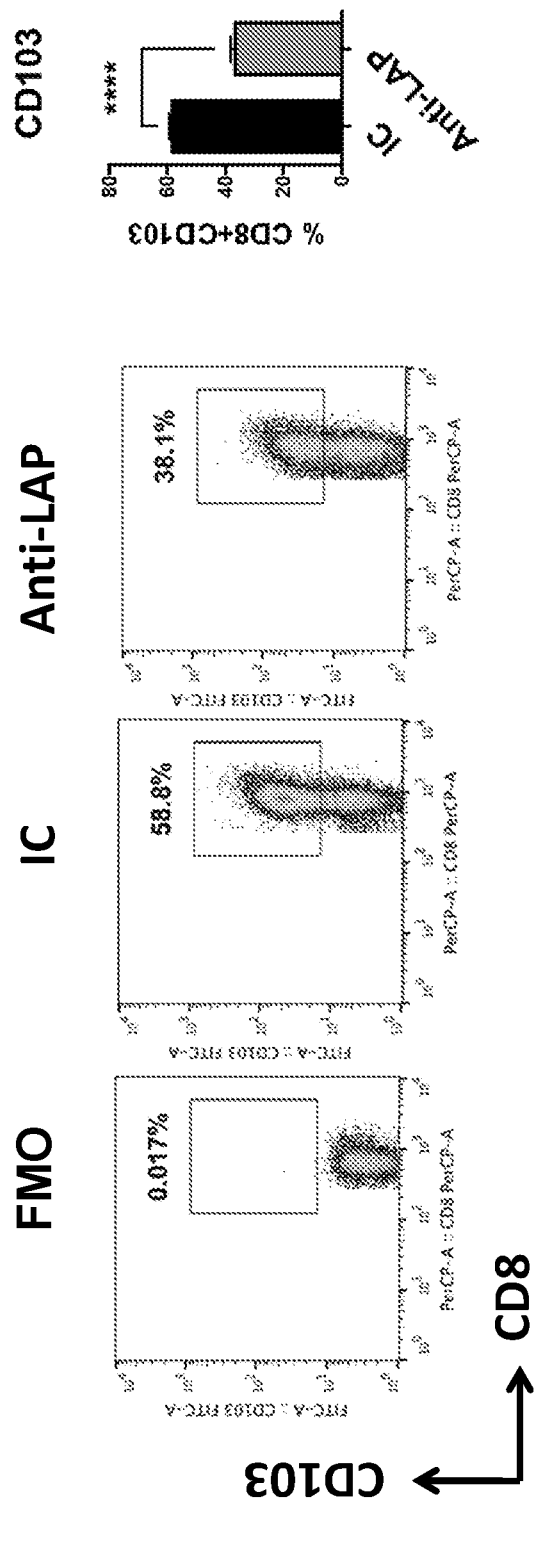

CD103
****
% CD8+CD103
80
60
40
20
0
IC
Anti-LAP
FMO
IC
Anti-LAP
0.017%
58.8%
38.1%
CD103
CD8
SC GBM Model

FIG. 29

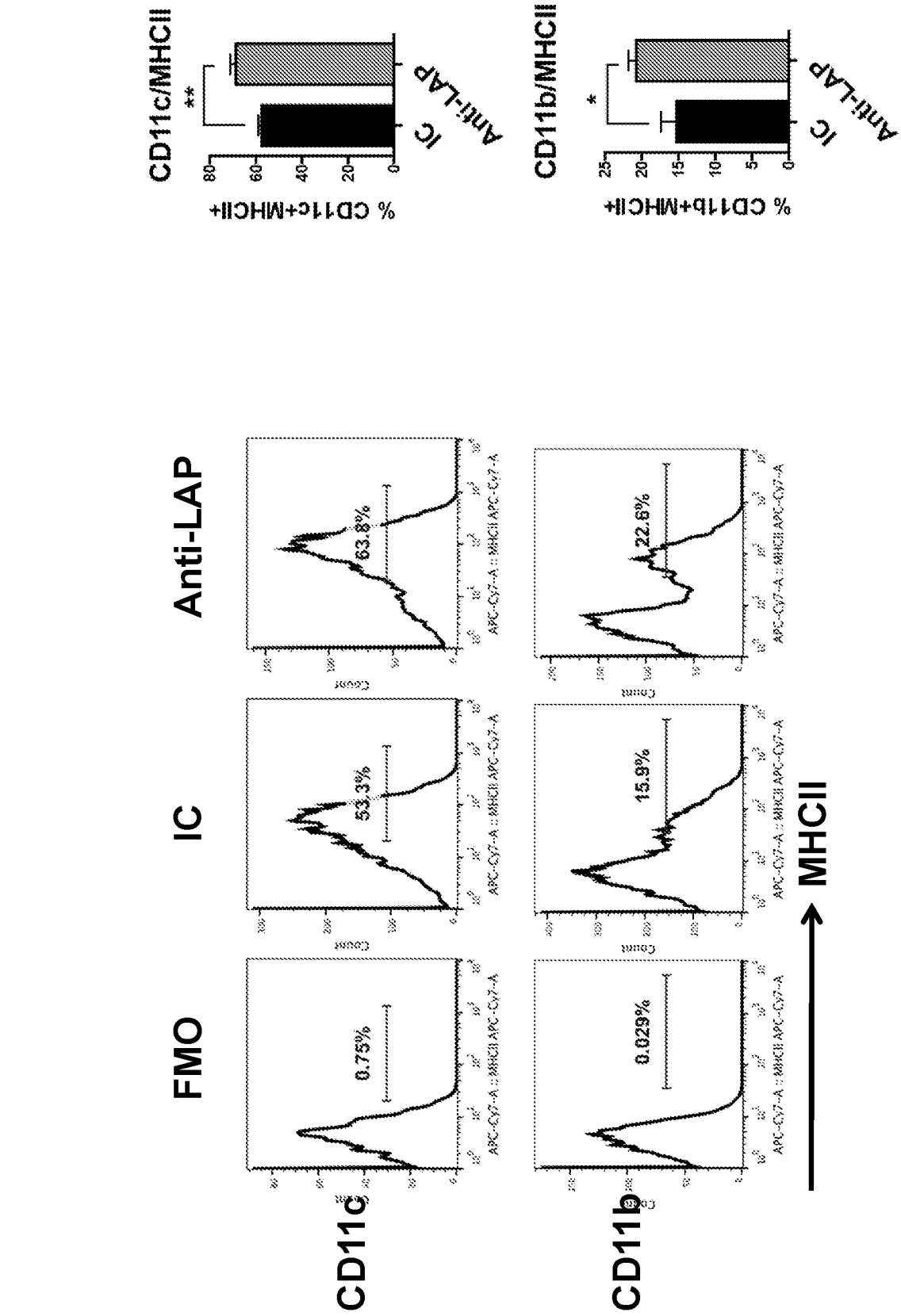
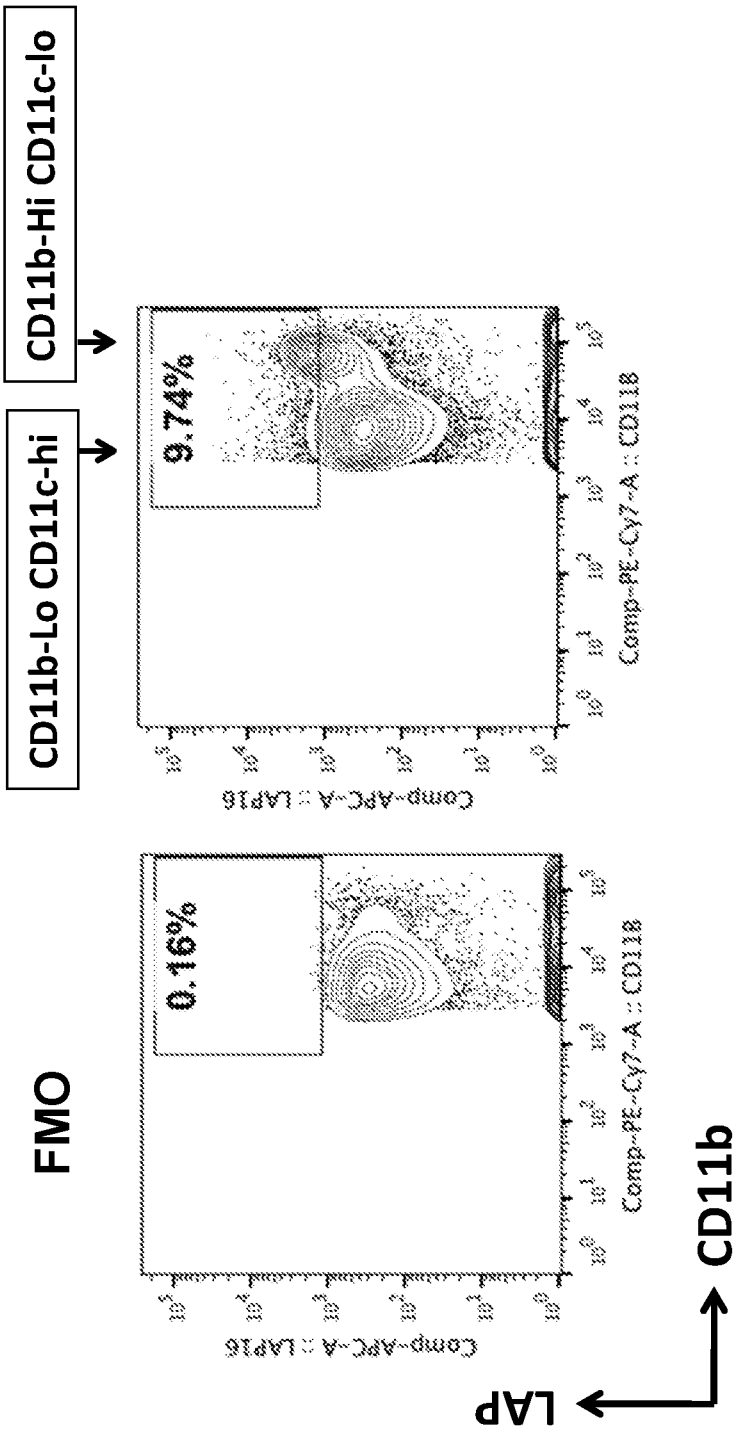

FMO
IC
Anti-LAP
CD11c
CD11b
0.75%
53.3%
63.8%
0.629%
15.9%
22.6%
APC-Cy7-A :: MHCII APC-Cy7-A
Count
MHCII
CD11c/MHCII
CD11b/MHCII
% CD11c+MHCII+
% CD11b+MHCII+
IC
Anti-LAP
**
*
SC GBM Model

FIG. 30



Naïve mice

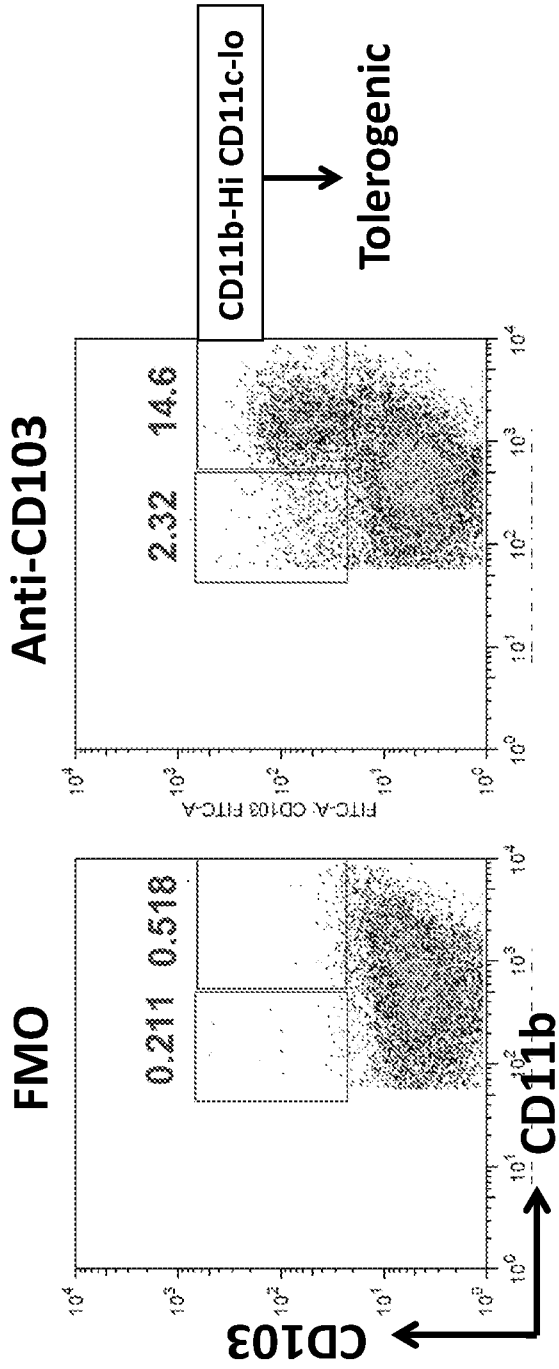
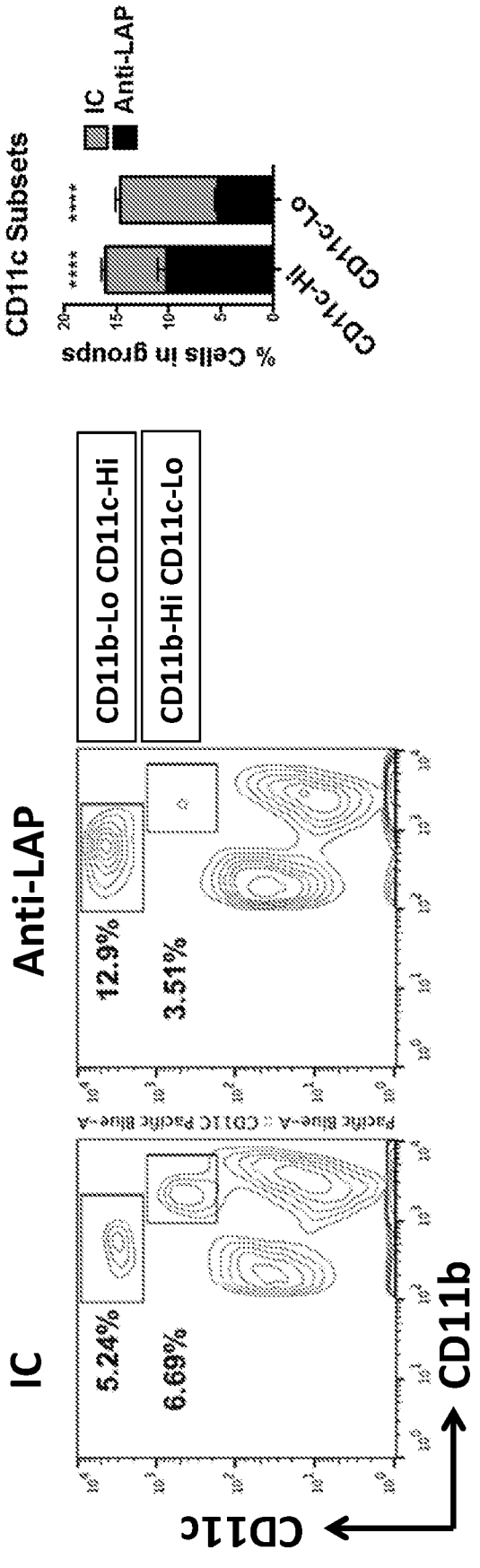

FMO
0.211 0.518
Anti-CD103
2.32 14.6
CD11b-Hi CD11c-lo
Tolerogenic
CD103
CD11b
FITC-A CD103 FITC-A
Naïve mice

FIG. 31

FIG. 32



SC GBM Model

FIG. 33

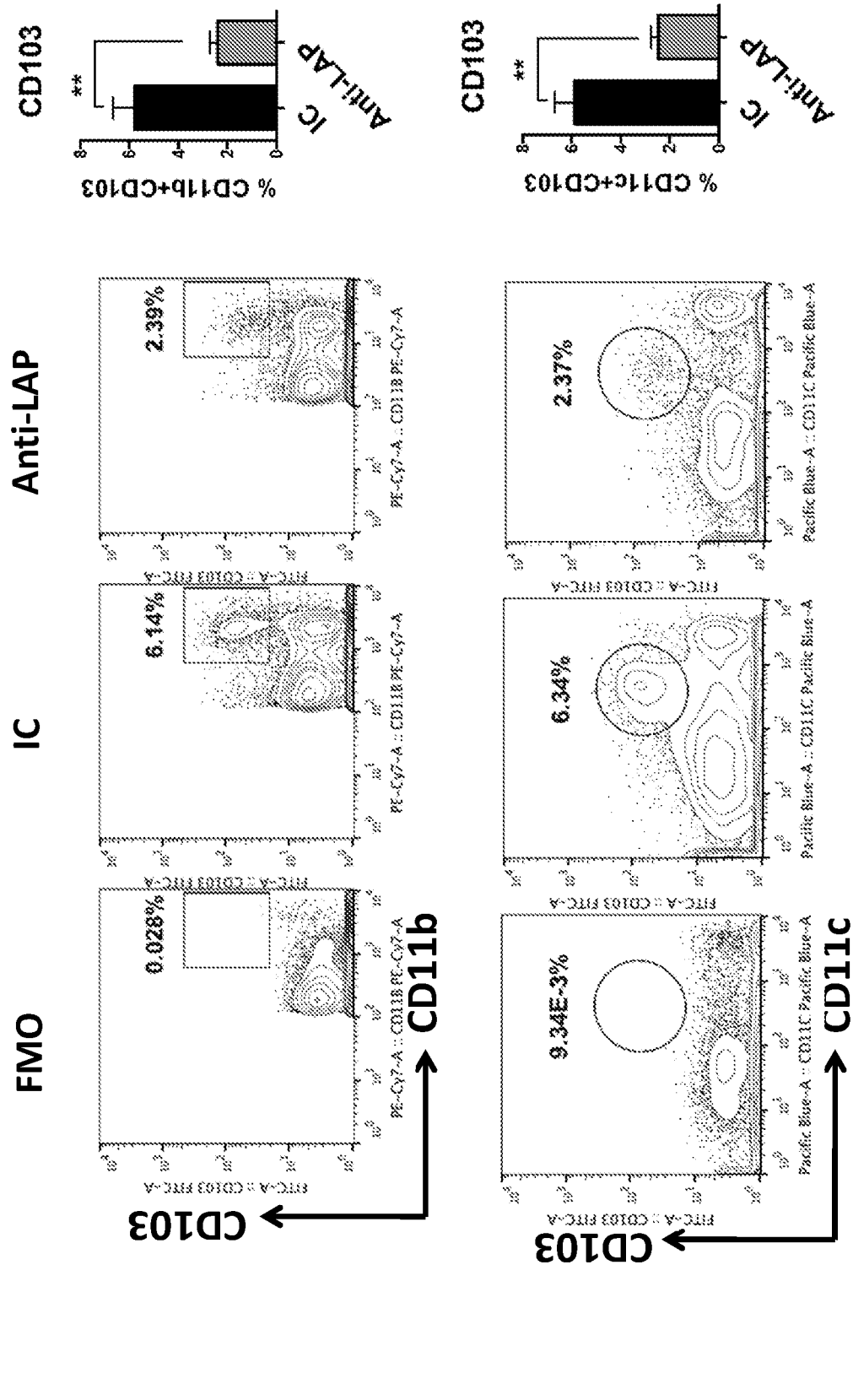

FMO
IC
Anti-LAP
CD103
CD11b
CD11c
0.028%
6.14%
2.39%
9.34E-3%
6.34%
2.37%
CD103
% CD11b+CD103
% CD11c+CD103
IC
Anti-LAP
**
SC GBM Model

FIG. 34

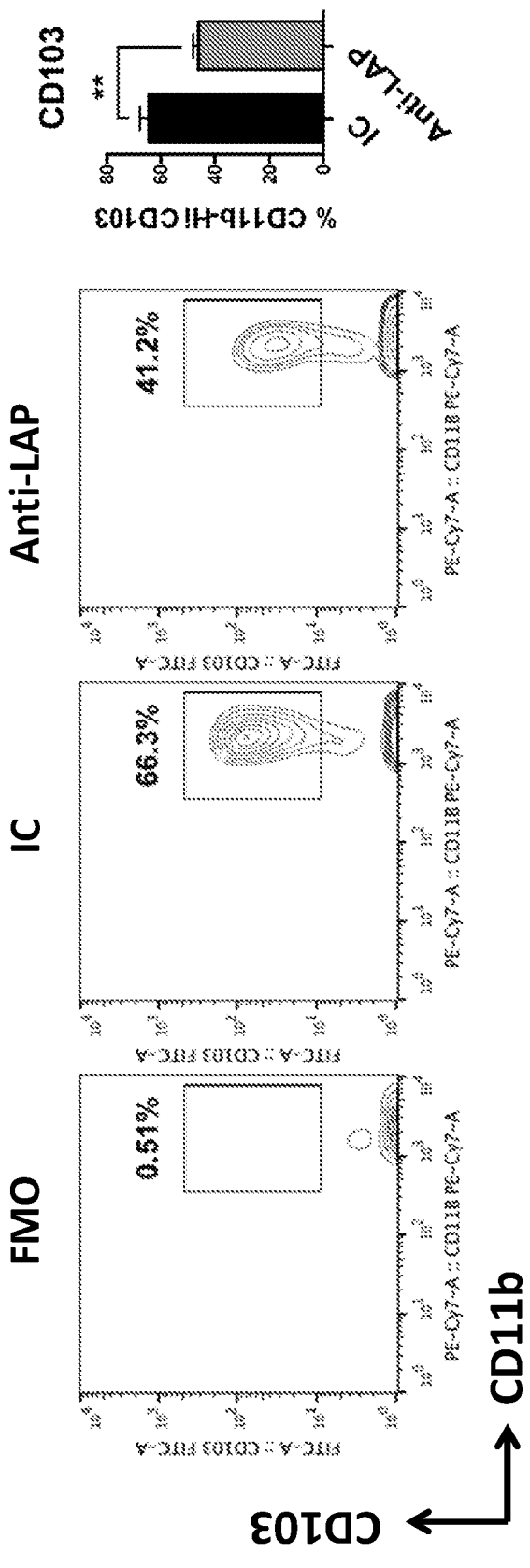

FMO
IC
Anti-LAP
0.51%
66.3%
41.2%
CD103
CD11b
SC GBM Model
CD103
% CD11b-Hi CD103
IC
Anti-LAP
**

FIG. 35

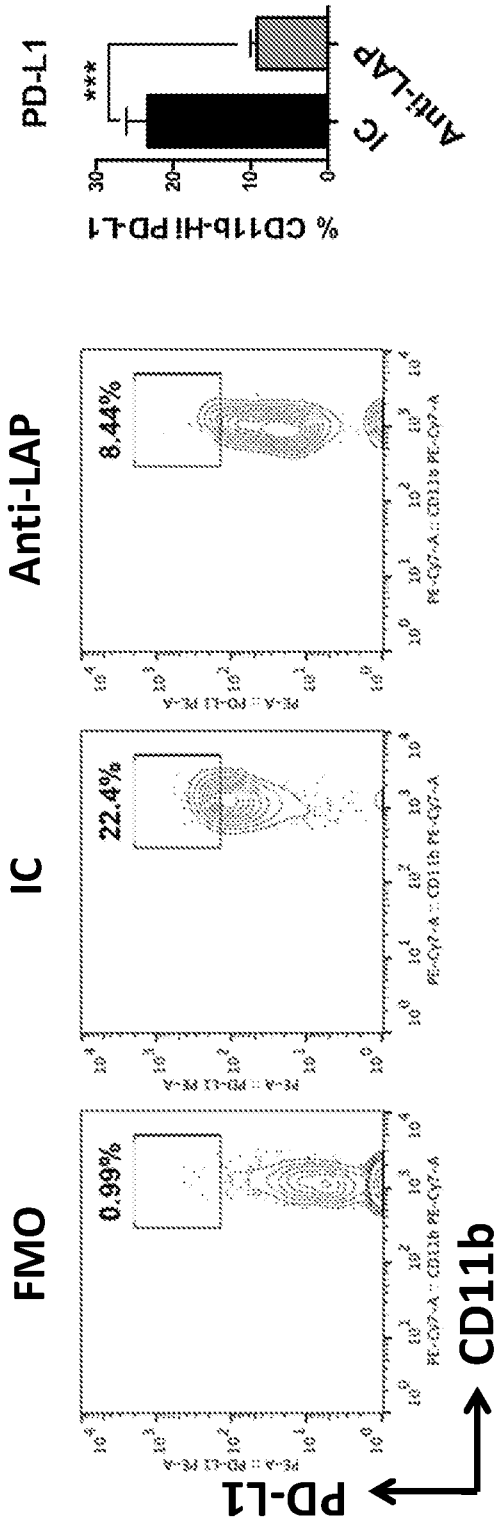
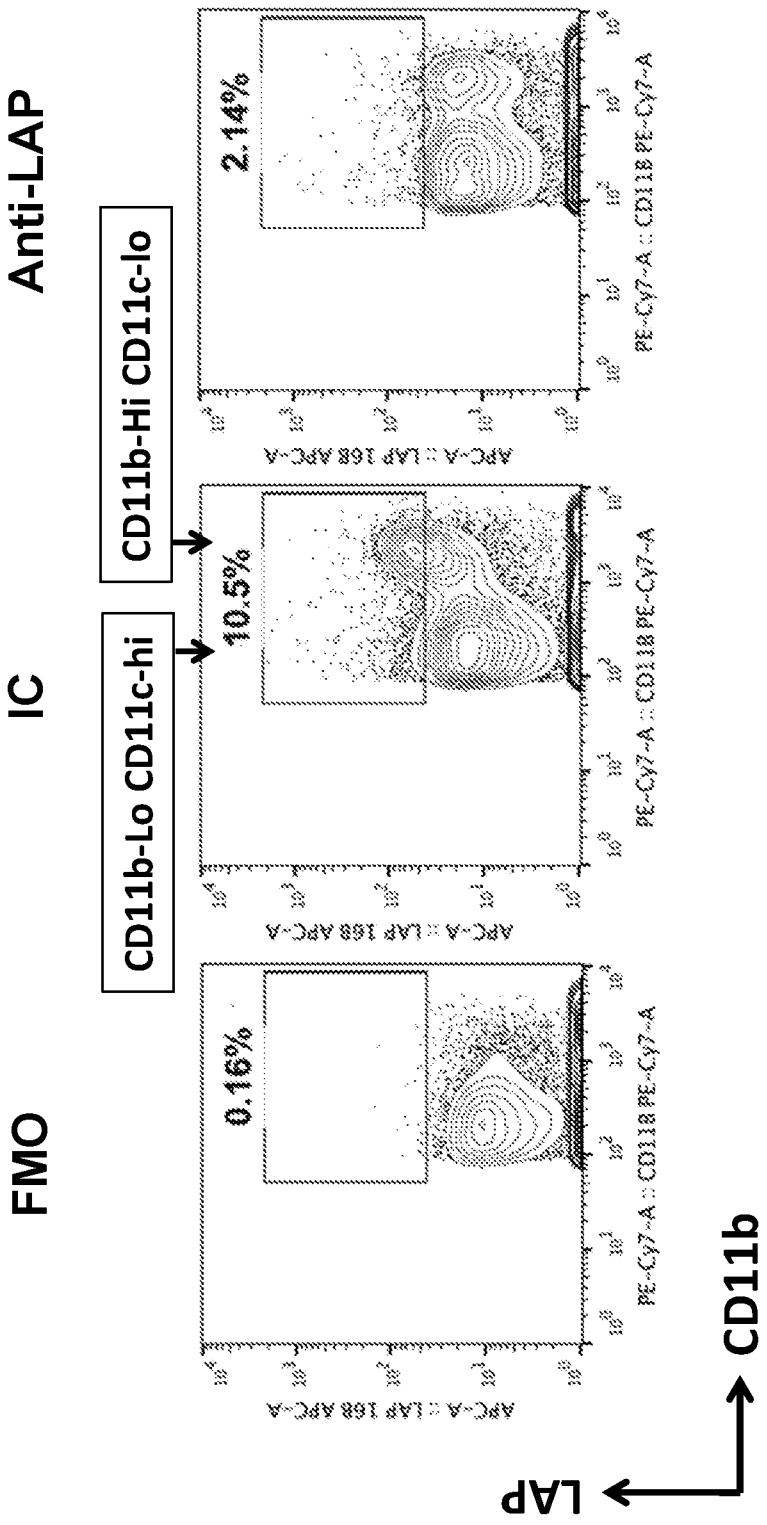

PD-L1
***
IC
Anti-LAP
% CD11b-Hi PD-L1
30
20
10
0
FMO
IC
Anti-LAP
0.99%
22.4%
8.44%
PD-L1
CD11b
SC GBM Model

FIG. 36


FMO
IC
Anti-LAP
CD11b-Lo CD11c-hi
CD11b-Hi CD11c-lo
0.16%
10.5%
2.14%
LAP
CD11b
SC GBM Model

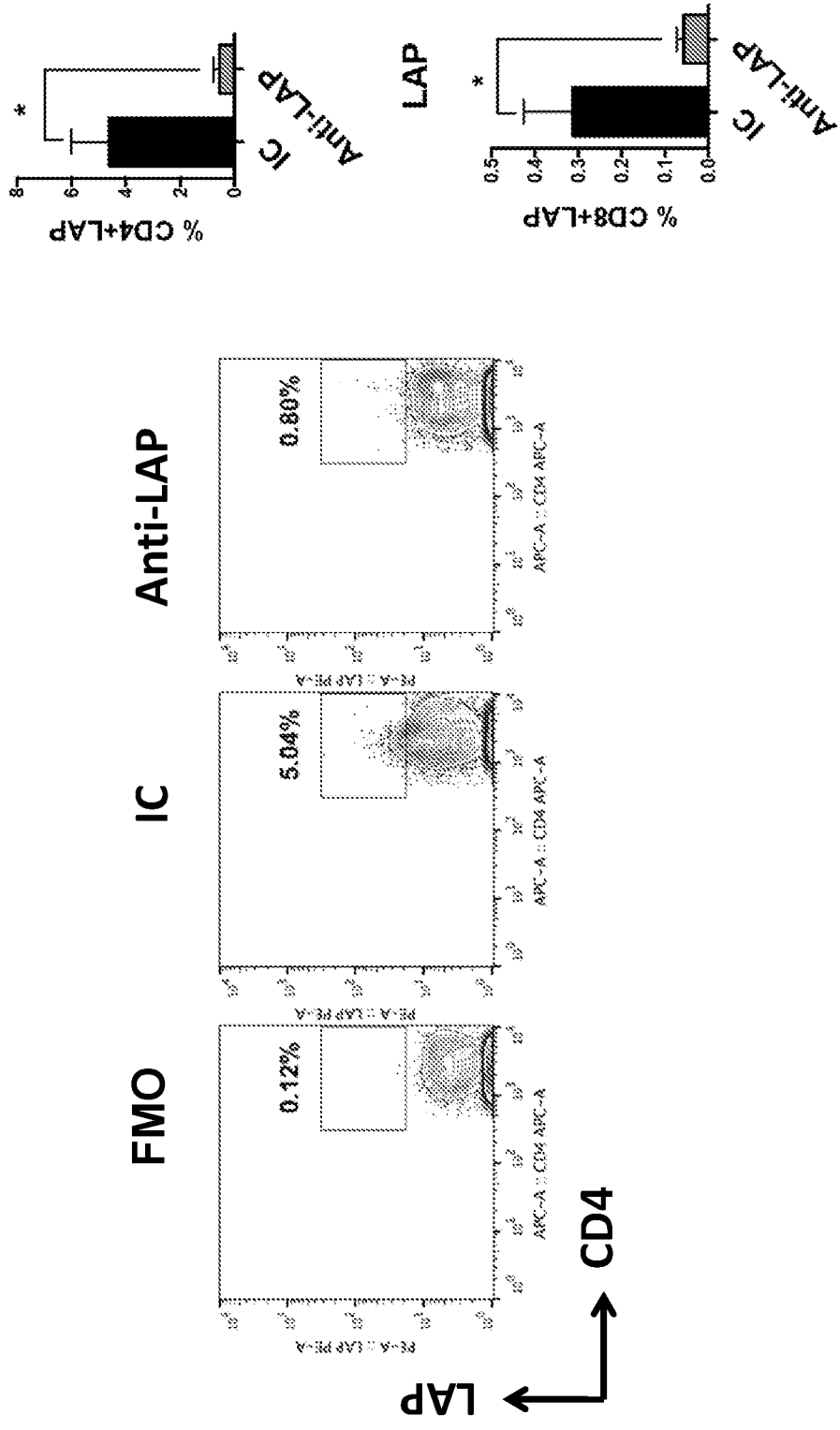

LAP
Anti-LAP
IC
% CD4+LAP
% CD8+LAP
*
FMO
IC
Anti-LAP
0.12%
5.04%
0.80%
LAP
CD4
SC GBM Model

FIG. 37

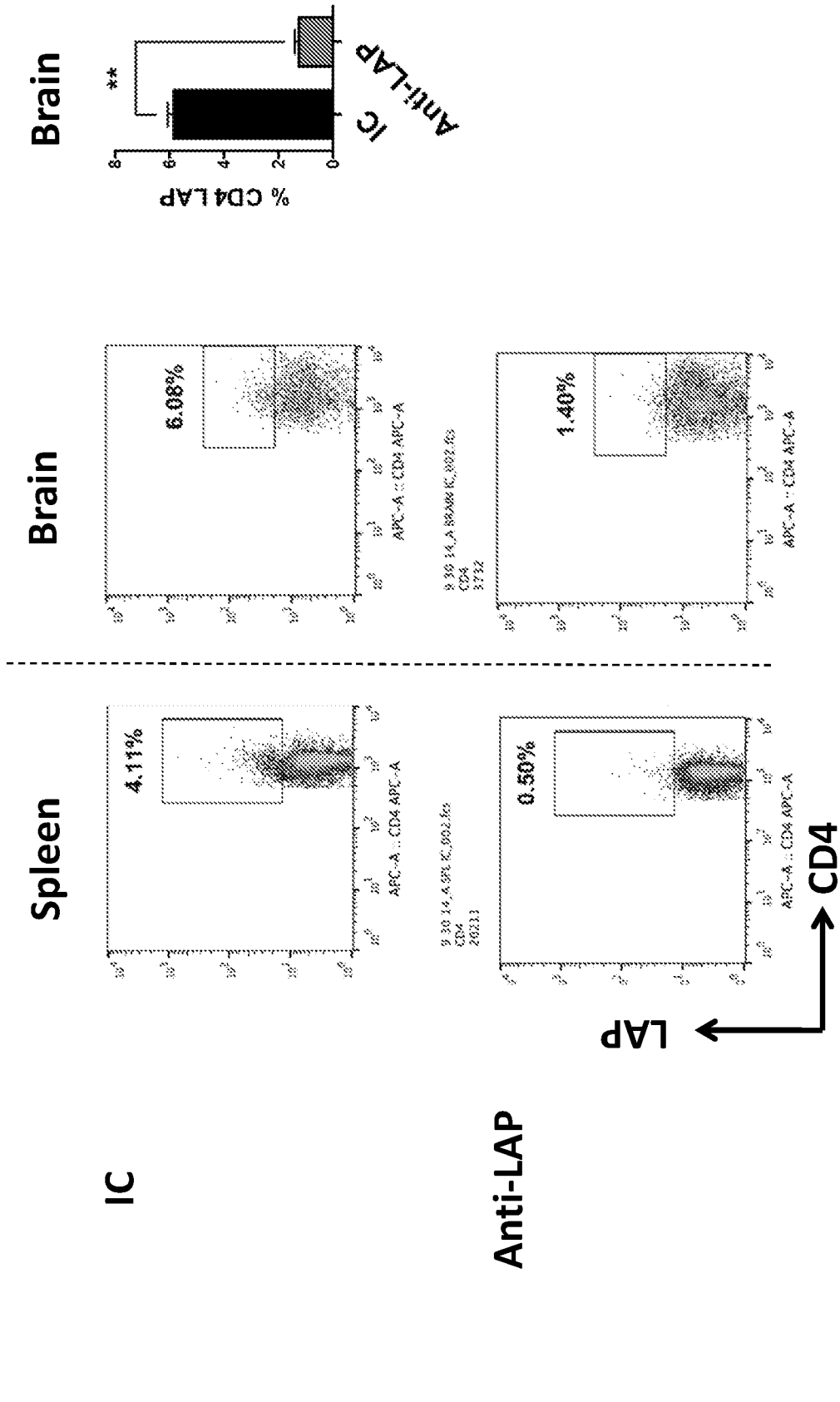
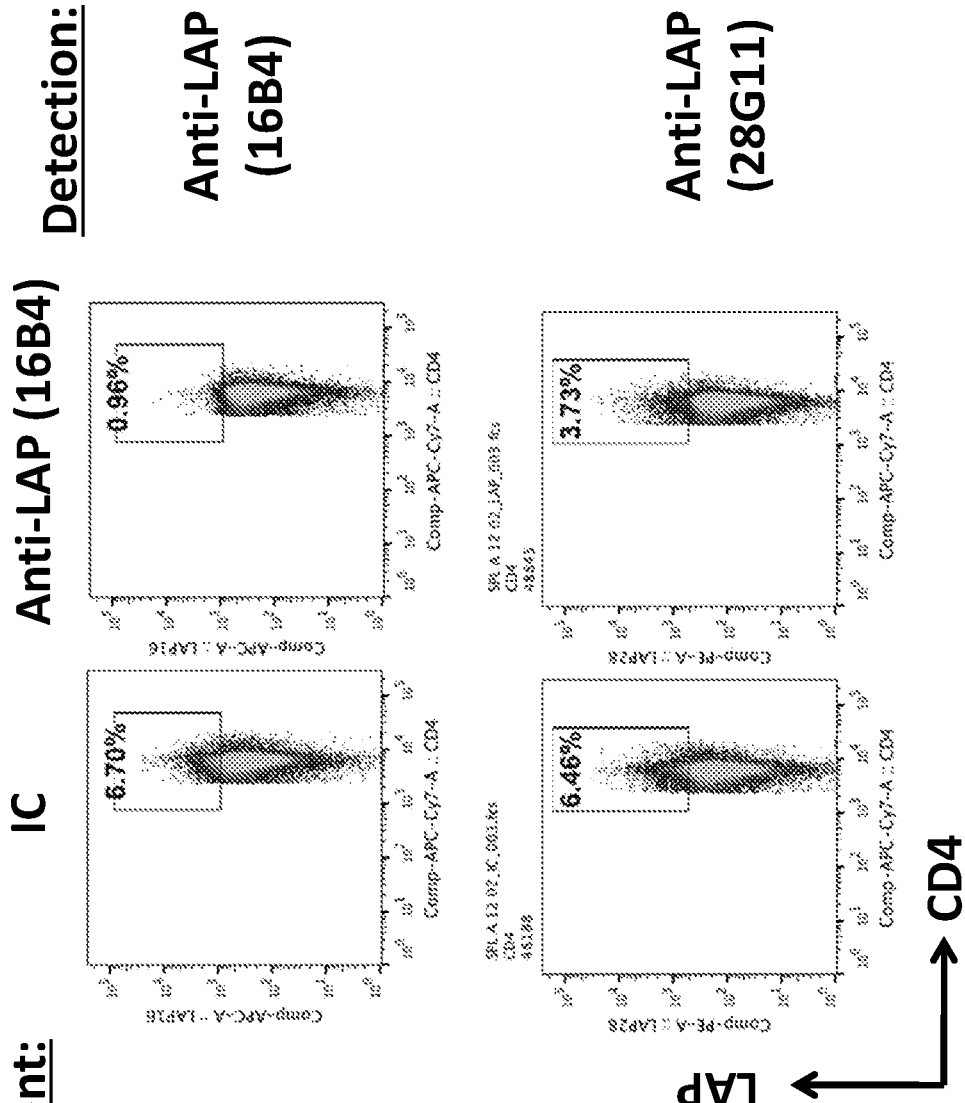

Brain
**
% CD4 LAP
IC
Anti-LAP
Brain
6.08%
1.40%
Spleen
4.11%
0.50%
IC
Anti-LAP
LAP
CD4
INC GBM Model

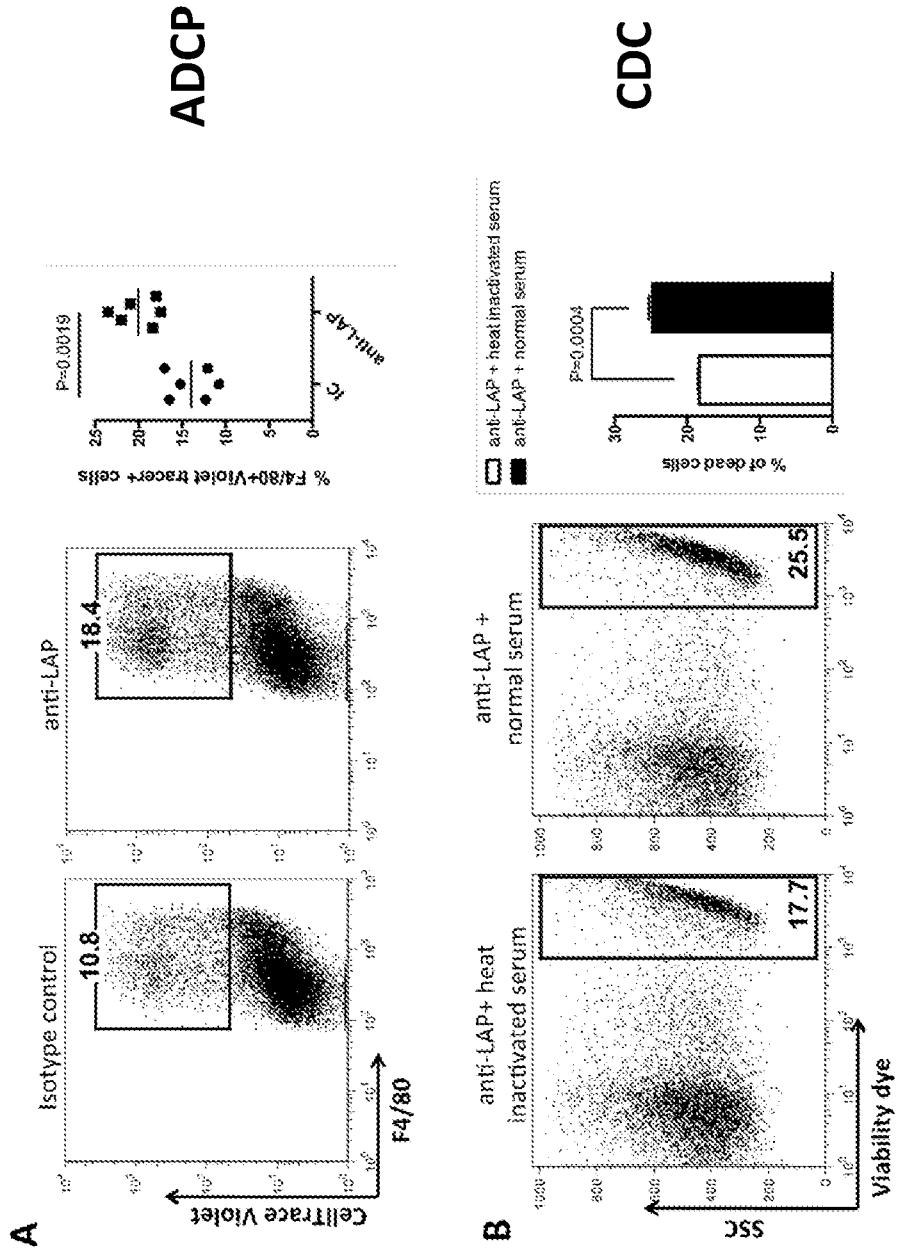
FIG. 38

FIG. 39



INC GBM Model

FIGS. 40A-40B



da Cunha AP, et al. Int Immunol, 2014

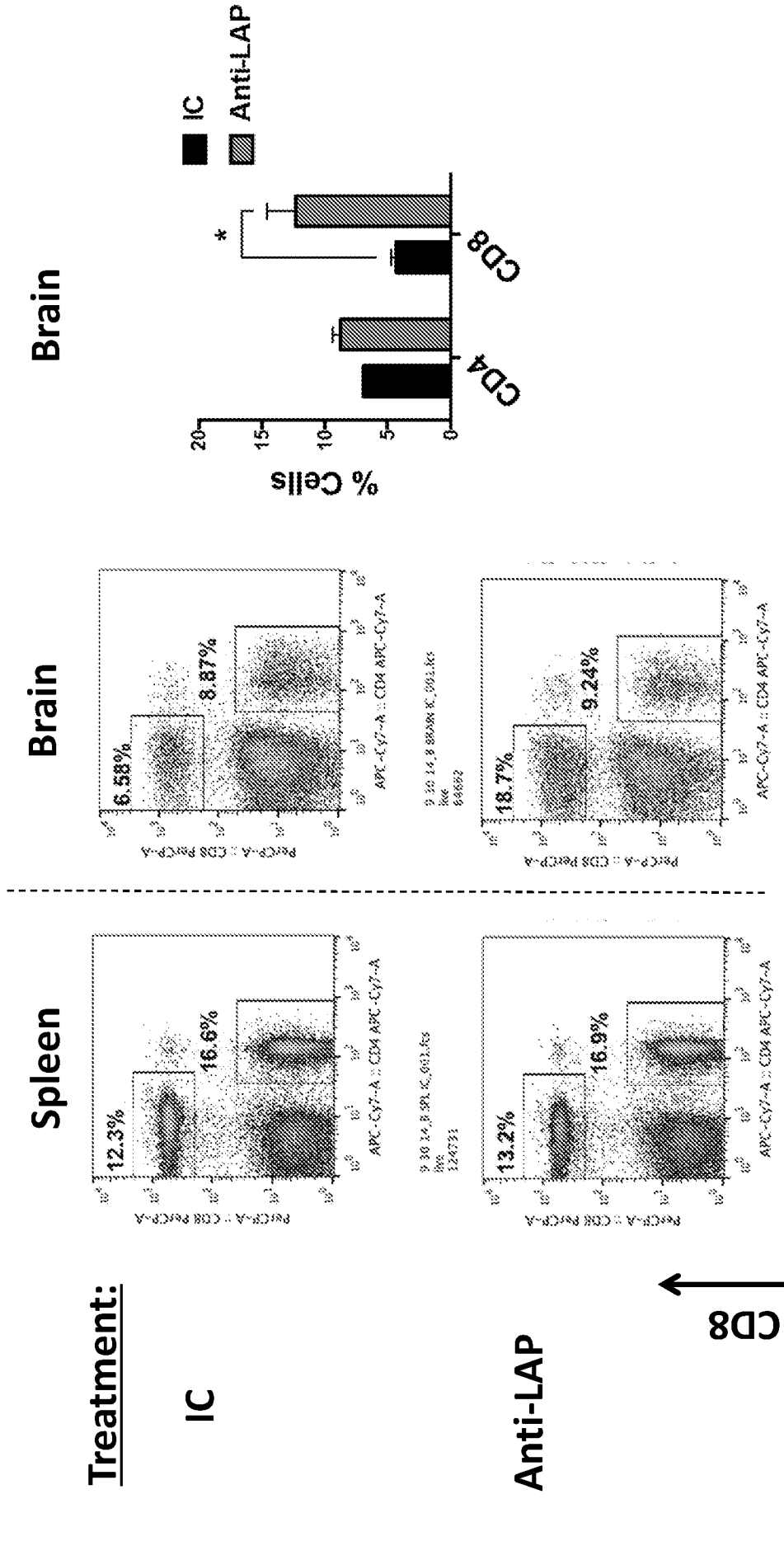

Brain
IC
Anti-LAP
% Cells
20
15
10
5
0
CD4
CD8
*
Brain
6.58%
8.87%
18.7%
9.24%
Spleen
12.3%
16.6%
13.2%
16.9%
Treatment:
IC
Anti-LAP
CD8
CD4
INC GBM Model

FIG. 41

FIG. 42

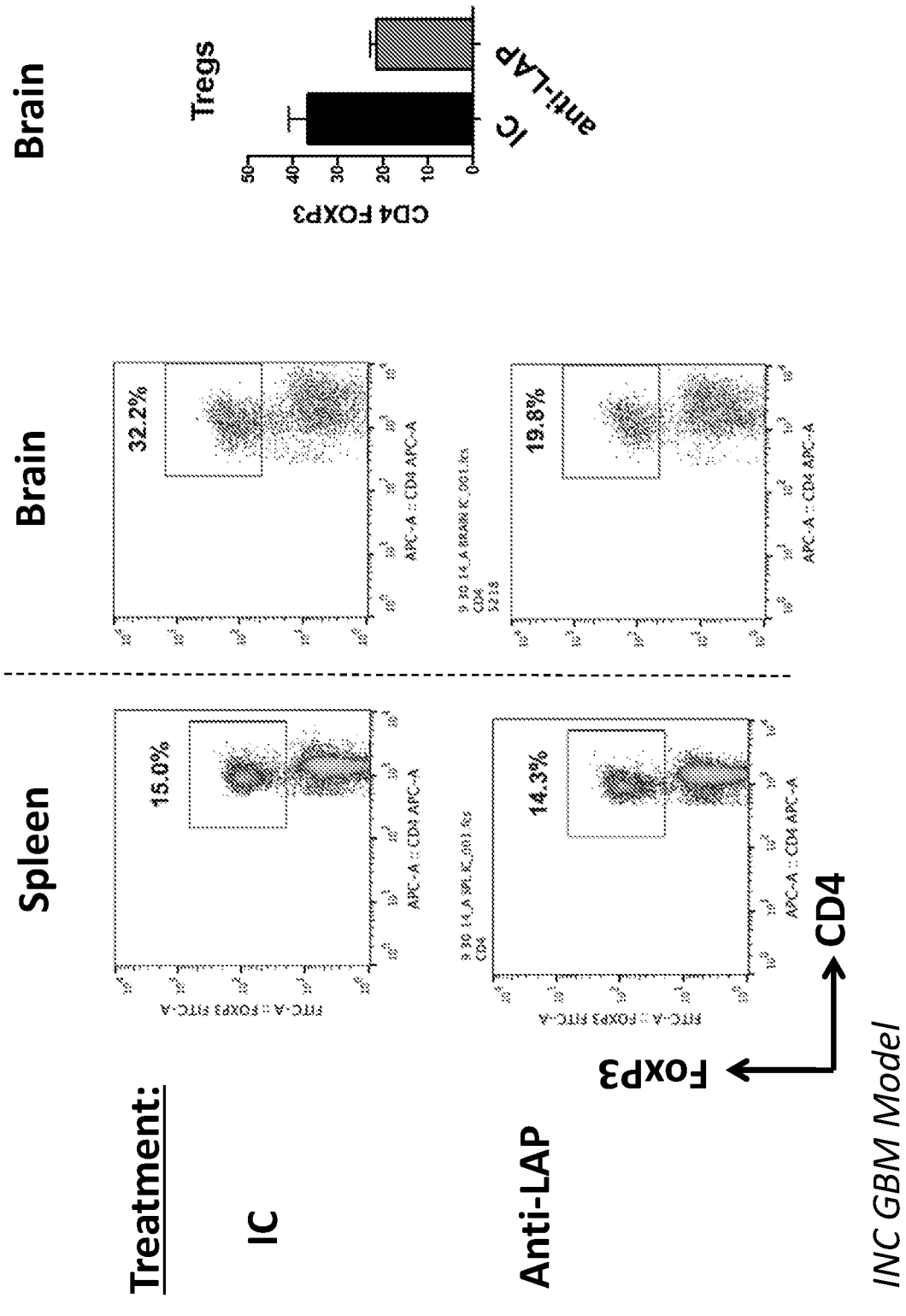

Brain
Tregs
CD4 FOXP3
IC
anti-LAP
Brain
Spleen
32.2%
19.8%
15.0%
14.3%
Treatment:
IC
Anti-LAP
FoxP3
CD4
INC GBM Model

FIG. 43

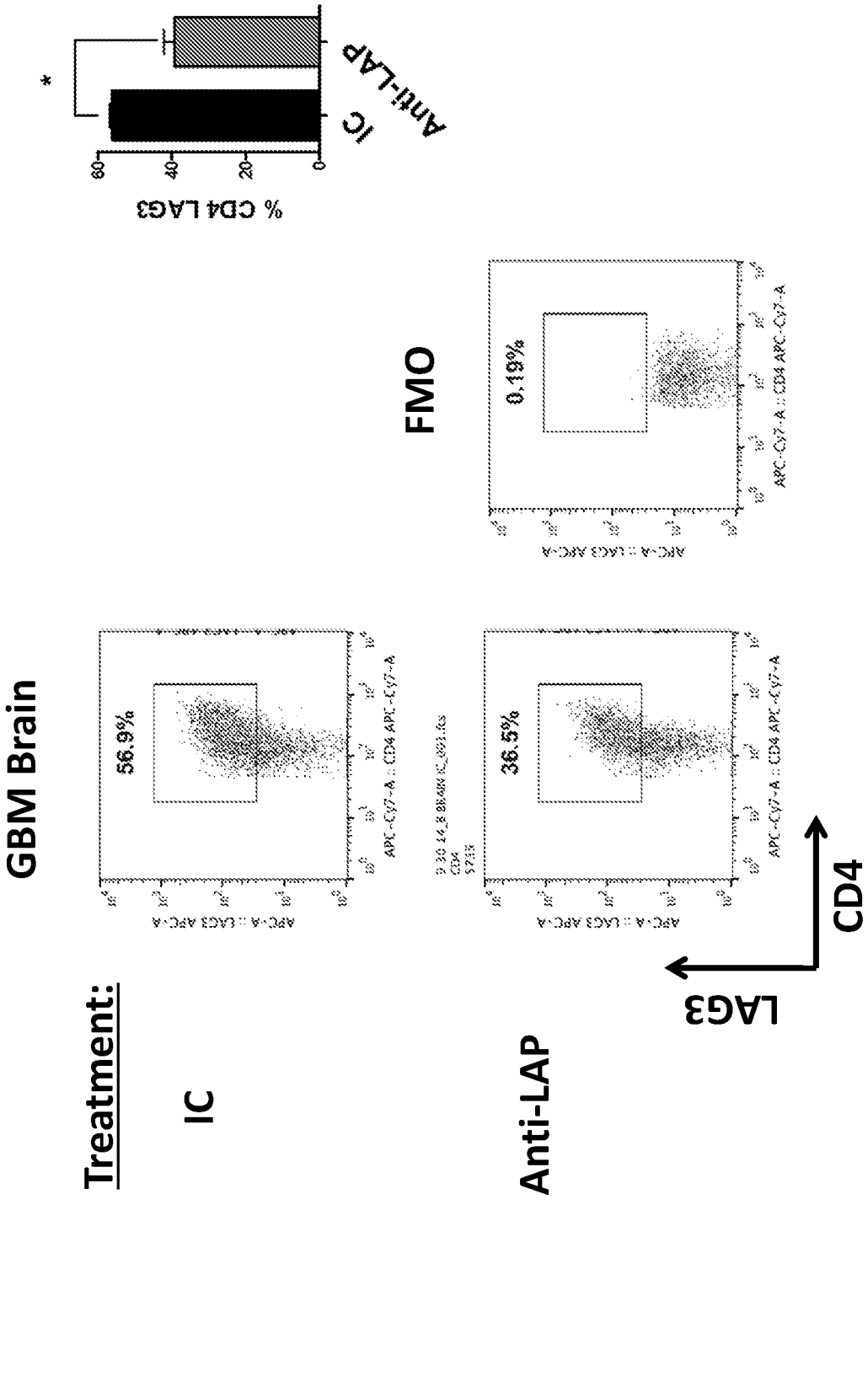

GBM Brain
Treatment:
IC
Anti-LAP
FMO
56.9%
36.5%
0.19%
LAG3
CD4
% CD4 LAG3
IC
Anti-LAP
*
INC GBM Model

FIG. 44

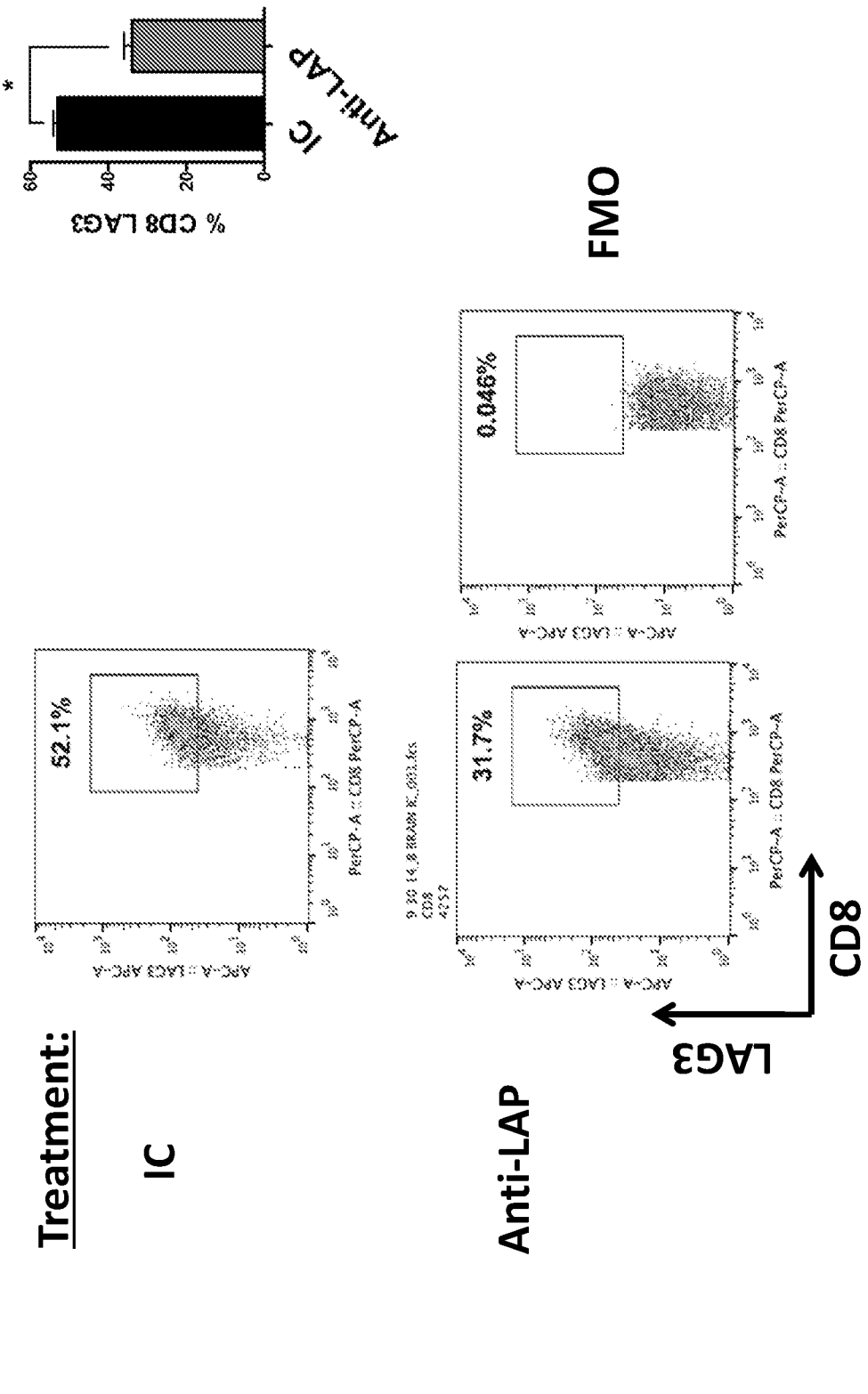
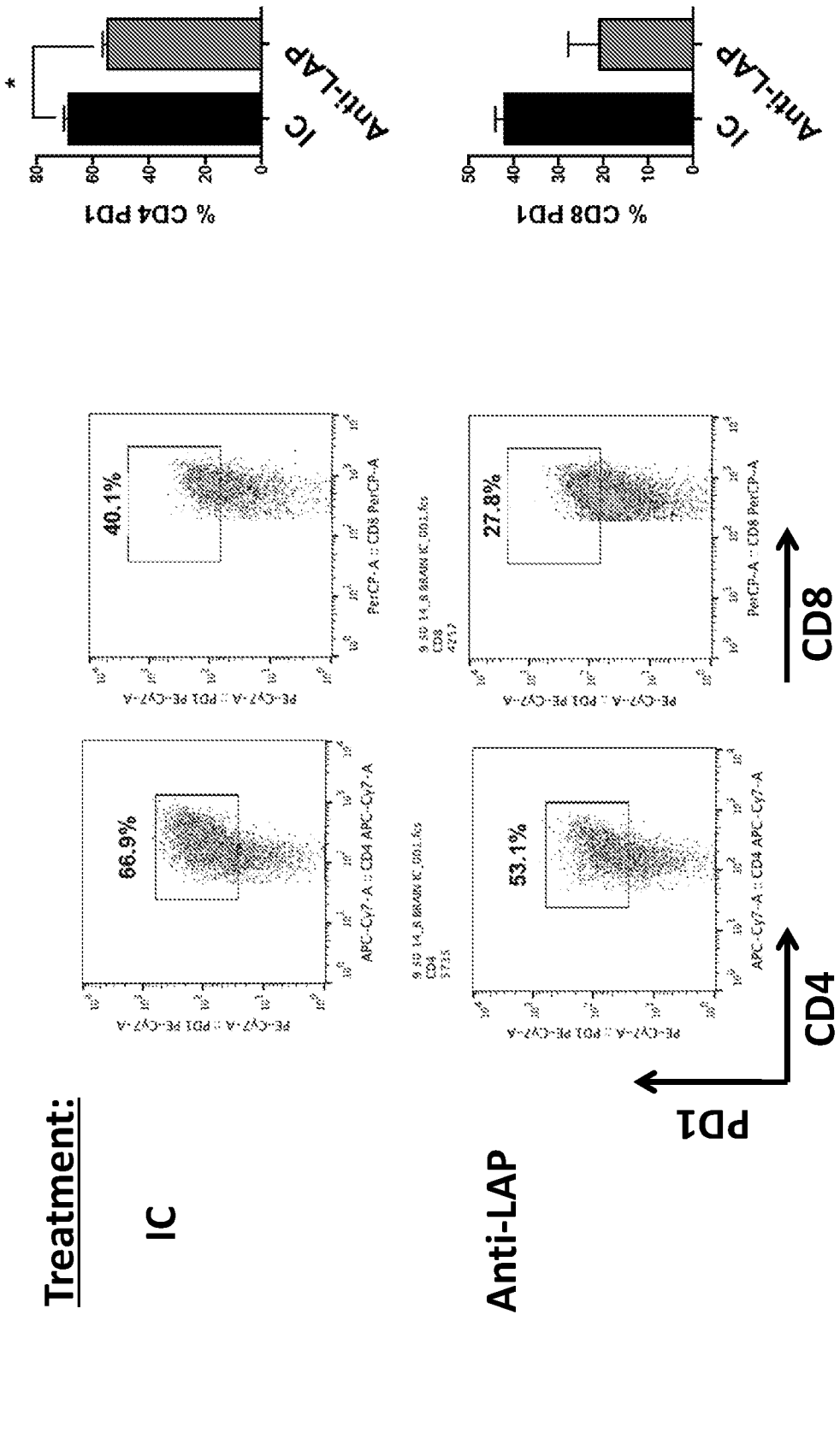

% CD8 LAG3
IC
Anti-LAP
*
Treatment:
IC
Anti-LAP
52.1%
31.7%
FMO
0.046%
LAG3
CD8
INC GBM Model

FIG. 45



INC GBM Model

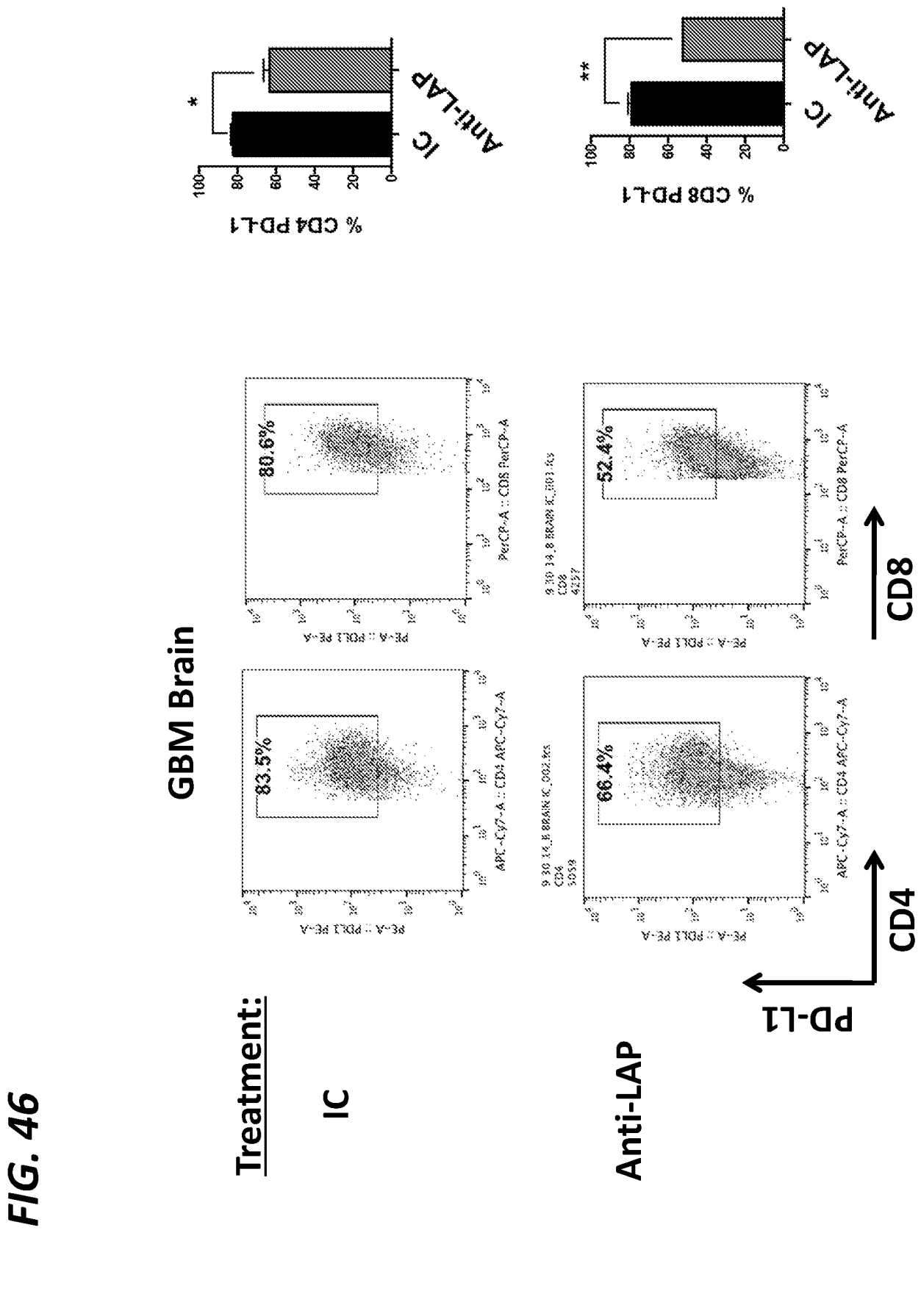

FIG. 46
GBM Brain
Treatment:
IC
Anti-LAP
80.6%
83.5%
52.4%
66.4%
PD-L1
CD4
CD8
INC GBM Model
% CD4 PD-L1
% CD8 PD-L1
IC
Anti-LAP
*
**

FIG. 47

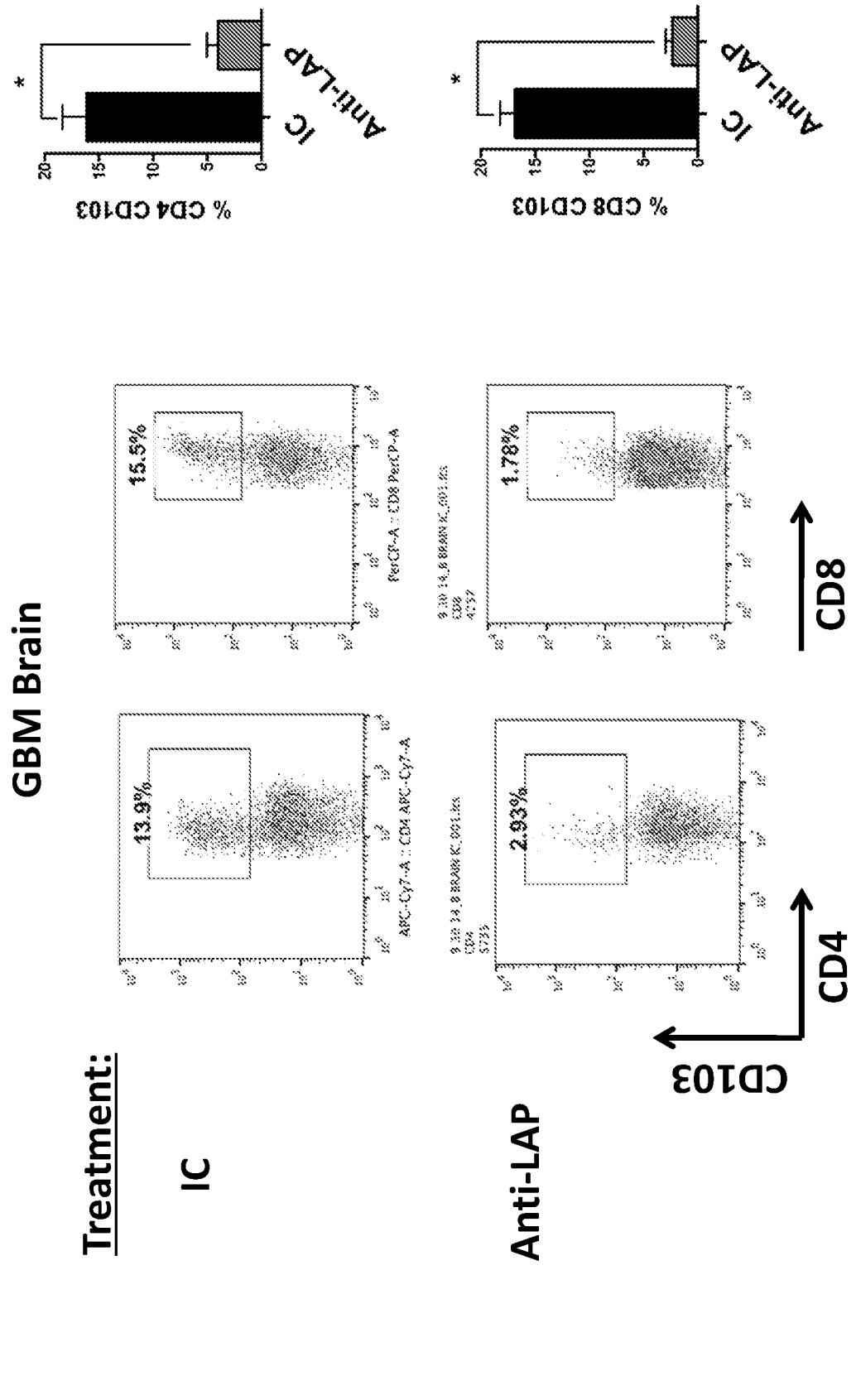
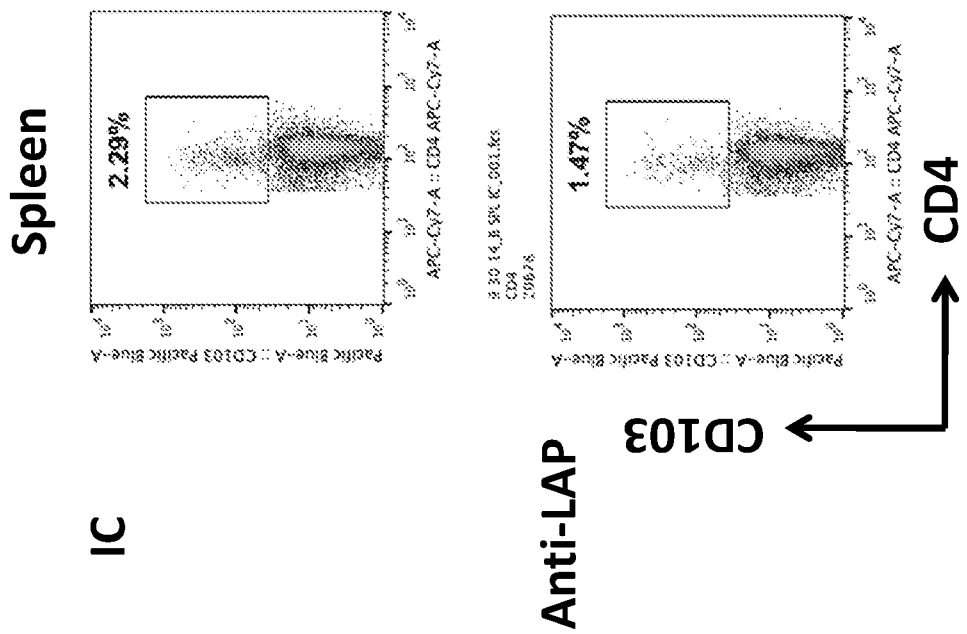

GBM Brain
Treatment:
IC
Anti-LAP
13.9%
15.5%
2.93%
1.78%
CD103
CD4
CD8
% CD4 CD103
% CD8 CD103
IC
Anti-LAP
*
*
INC GBM Model

FIG. 48



INC GBM Model

FIG. 49

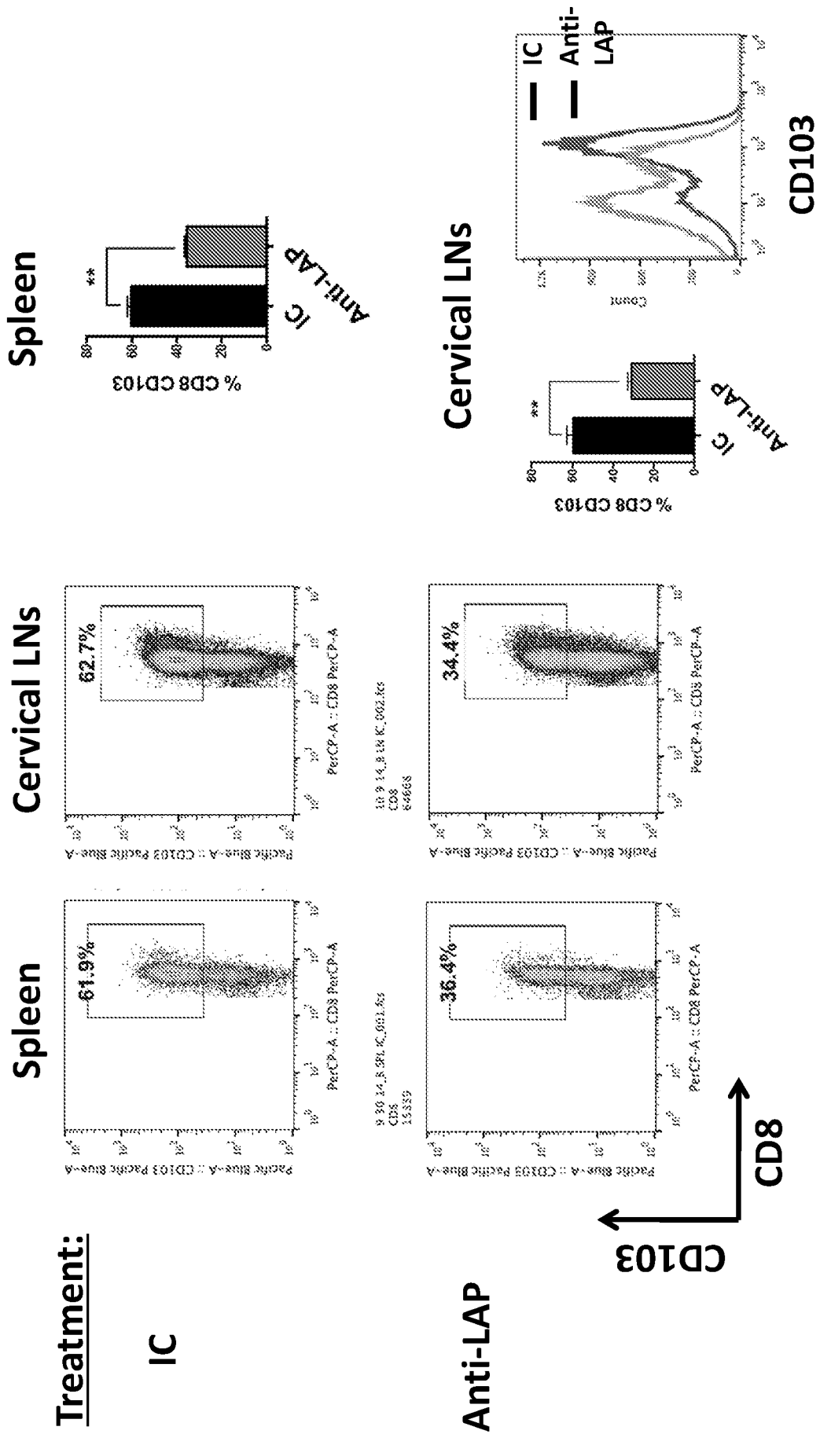

Spleen
Cervical LNs
Spleen
Cervical LNs
CD103
IC
Anti-LAP
% CD8 CD103
**
Count
Treatment:
IC
Anti-LAP
61.9%
62.7%
36.4%
34.4%
CD103
CD8
INC GBM Model

FIG. 50

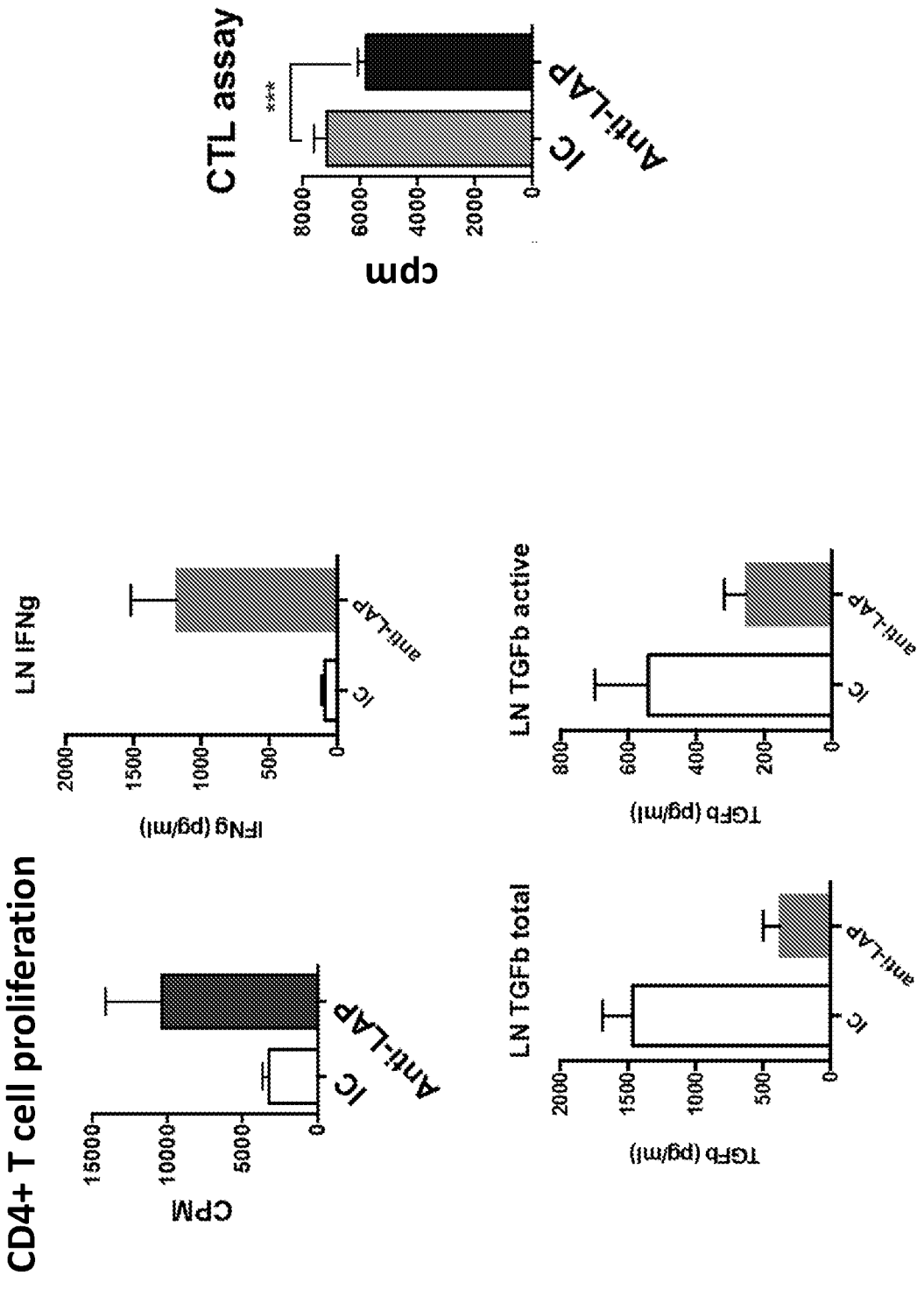

CD4+ T cell proliferation
CPM
15000
10000
5000
0
IC
Anti-LAP
LN IFNg
IFNg (pg/ml)
2000
1500
1000
500
0
IC
anti-LAP
LN TGFb total
TGFb (pg/ml)
2000
1500
1000
500
0
IC
anti-LAP
LN TGFb active
TGFb (pg/ml)
800
600
400
200
0
IC
anti-LAP
CTL assay
cpm
8000
6000
4000
2000
0
***
IC
Anti-LAP
B16-OVA Model

FIG. 51

FIG. 52

```
huLAP1  lacckeidrelvkrkrieeirgqileklrlaappaqgeevppvpgpipeevlalymatdrva
huLAP2  lacceeldmdqfsrkrieeirgqileklklteappedyrdpreevppeviaiymatdrdlq
huLAP3  lacceeldfghikkkrveeirgqileklrltcappeptcvrsrhkvppyvalymatdrele
        ********** ********** ********** ********** ********** *****

huLAP1  geeae-------ppppeedyyrekevtrvlmvetnneeivd----------------------
huLAP2  ekaarraaceredeevyekevykidmpdfipetvcpvvtcpseavgalcenqeavl-------
huLAP3  emqgereeecgcqeuceeeyyrekeihkfdmigglleehuele----------vcp-------
                                                                       

huLAP1  ----------------xfkqaekkiymffntseelrsaevpeavllaraelrlirll-----kveqkve
huLAP2  cgyldeaippfyrypyffri-vrffdveame-----knaaulvkaefrvfirlqpkarvpegrie
huLAP3  ----------------k-----gieakv-frfnveave-----knrkulfraefrvlrvpdpeekxreqrie
                                                                       

huLAP1  lvpkyeu----------nmvylaullapaedapevlafdvtgvvrqwlargeleqfrlaaqca
huLAP2  lvplkekdlteptqrvidekvvkuraeevlafdvtdavheullkkdrnlqfkielhcp
huLAP3  lfglirpdeh-laxqrvigekulpcrgeaevlafdvtdavreullrreanlqleielihcp
                                                                       

huLAP1  cdeedneiq----------ydingfeqgrzqdlaeih----------gmrrpfillmaep
huLAP2  ccfgvpeanvyiipnkeeleartaqidgtaetytaqdqkiikatrikkaeqkcqpillmlp
huLAP3  chcfgpuud-ilenlhevmeikfkgvdueddhgzgdlgrlk----kqkdhuqphlilmaip
                                                                       

huLAP1  lerapltqeerhrr----
huLAP2  eyrleeqcnrrkkr----
huLAP3  phrldnpghhhkrkkr
```

FIG. 53

```
mOLAP1  latcekkidmelvkrkrieairgqilekirlaappsqgevppgplpeavlalymsrdrva
mOLAP2  latcaeildmdqfmrkrieairgqilekirlkcappedy-pepdevppevialymsrdlla
mOLAP3  latctcldfghikkrveairgqilekirlkcappepa-v-mplvpdvvlalymsrdelle
        ****.*:**  . :*****:*********:*****.          :* *******:* :

mOLAP1  geaad-------pepqeadyyakevtrvlmvdrq----naiyekckdishmiymffncadir
mOLAP2  ekaarrnaaceremdeeyyakevykidmpshlpsemaigptfyrpyfrivr-fdvsatm-
mOLAP3  emhgerecgcrgetcseeeyyakeikfdmiqglaehmelavcpkgikekwfr-fnvaev-
          .       :  :*******: :.:  *  *  :  :  *:*:*
mOLAP1  eavpepplkraelrglrlke----gvegmvelyqkyen----nawrylgurlltptdt
mOLAP2  ---ekmanlvkaefrvfrlgnpkarvvecrielyqllkkdlcapcgrvidekvvkrrae
mOLAP3  ---ekgtnlfraefrvlrvpnpewkrtegrielfqllrpdeh-iakgrvigpkulprgt
          :  * :*****:*:  .  ***:*****  .  :  :  :  :

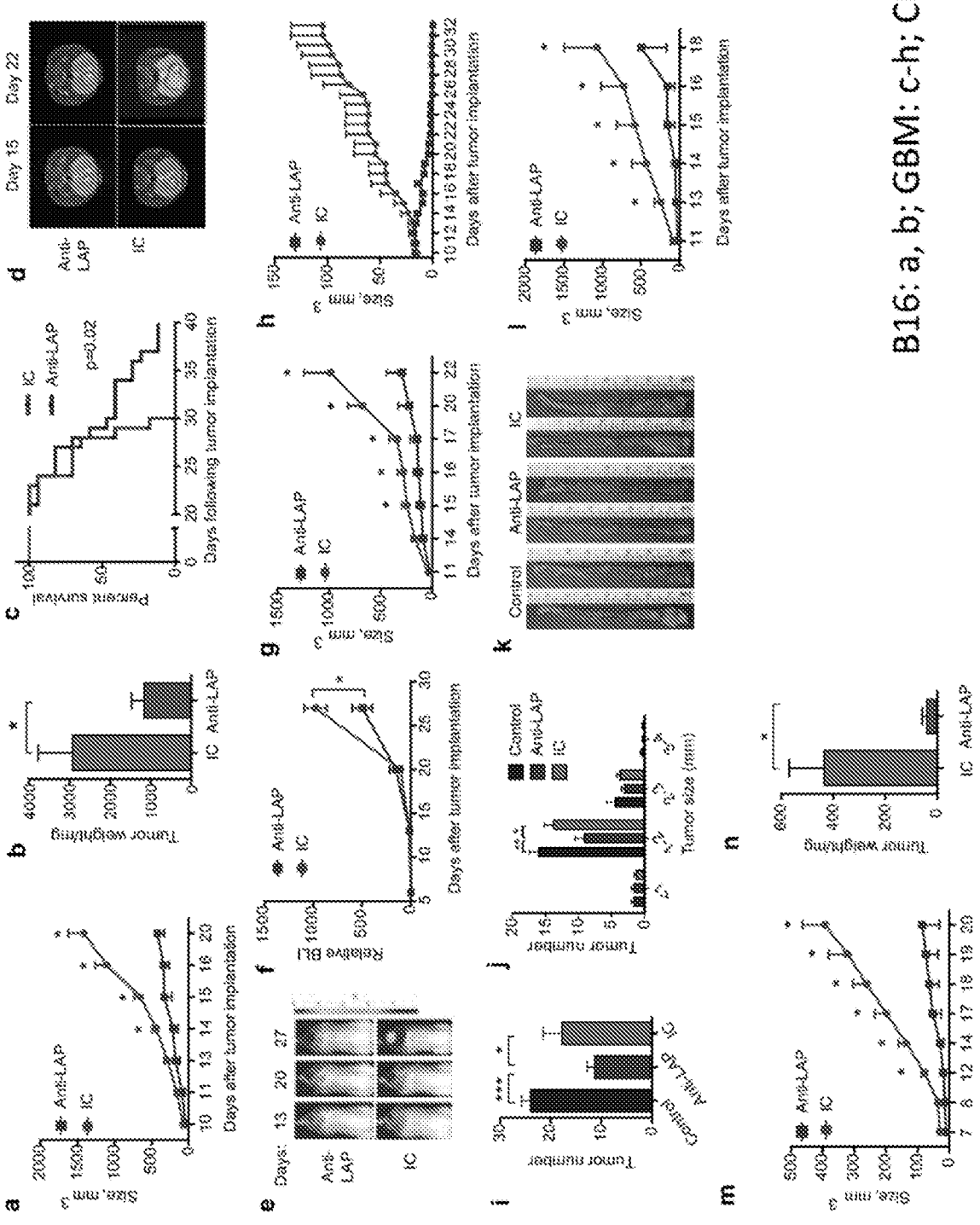
mOLAP1  pewlafdvtcgvvrqwlnqgdqlqgfrfaahcscdnkdnk----lh----veing
mOLAP2  gewlafdvtcdavqewlhhkdrnlqfkielhcpccfvpanyliprnkeeleanfagidg
mOLAP3  aewlafdvtcdkvrewllrrempleieihcpchcfqpnqd-ilenvhevmeikfkgvdn
        ********  .*:***** : :  *:*:** * *  :  .  :  :  ::

mOLAP1  iapkrrgdlgkih----dmrpfillmapleraqhlhnmrhr---
mOLAP2  tacyaeqdqkeikktrkkteqktphllmlpeyrleqqweirkkr-
mOLAP3  eddhgrgdlgrlk---kqkdhnnphllmmipphrldpqqwqrkkr
          .  **  ::    .  .  *.*:**  *  :  .  :::
```

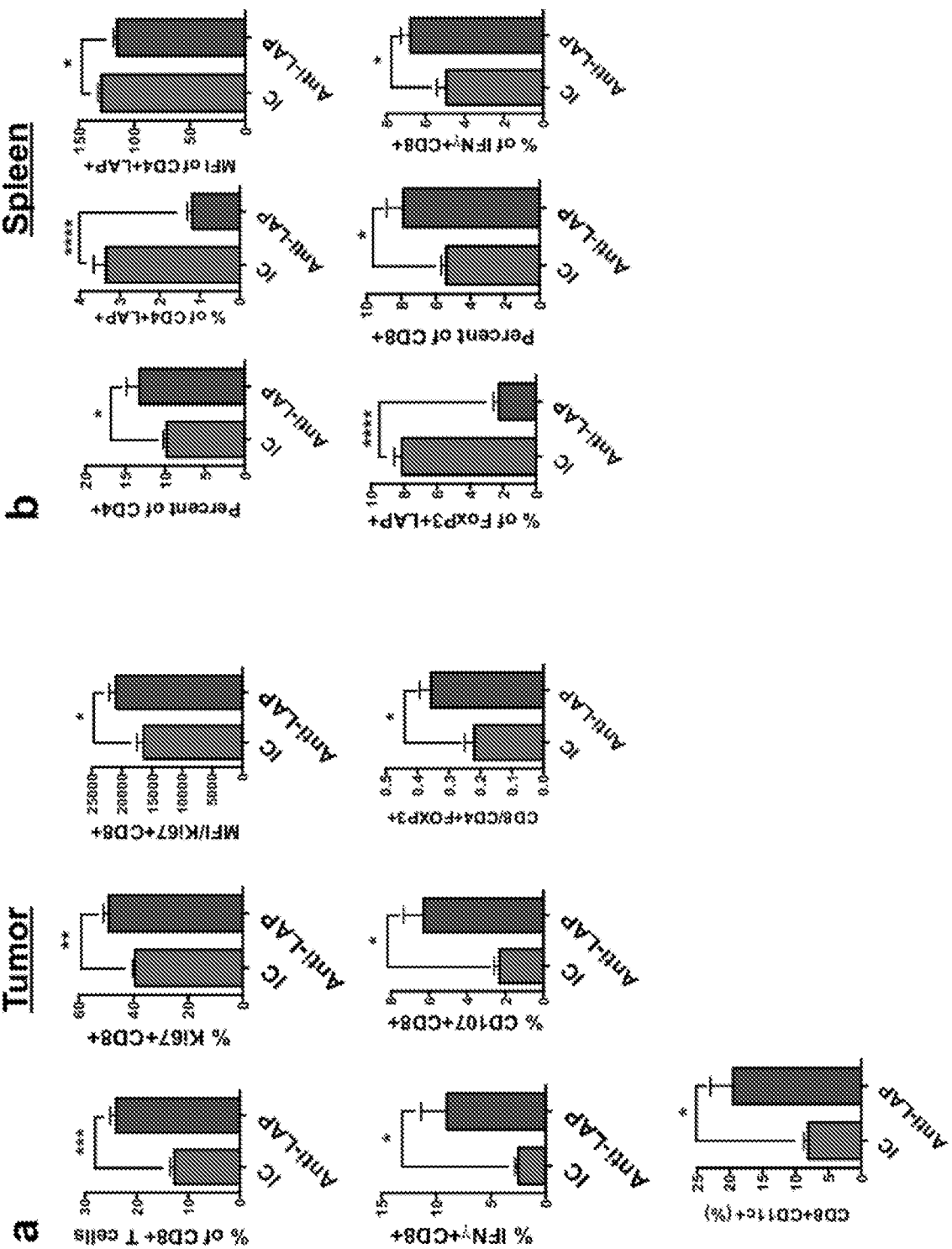
FIG. 54



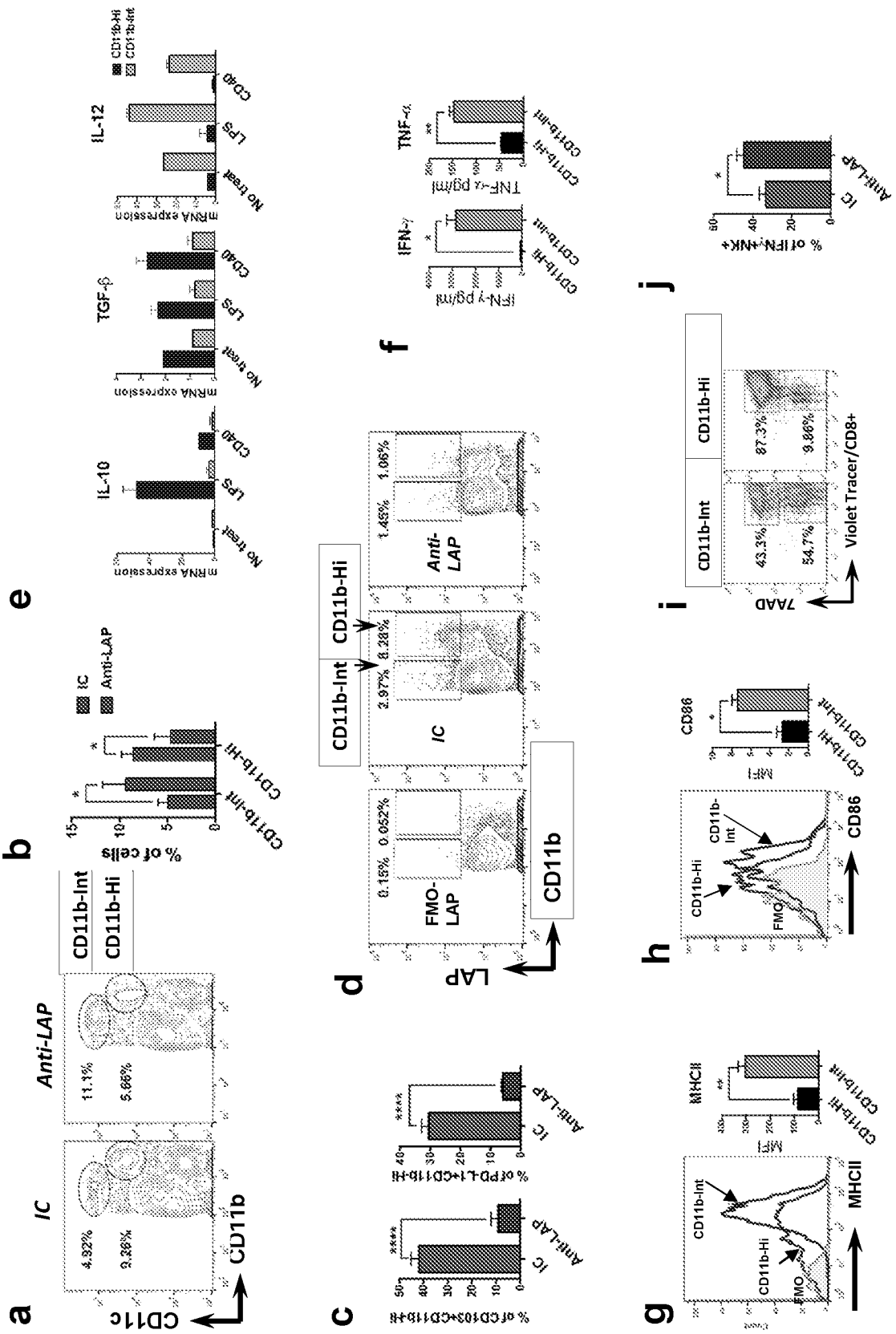
FIGS. 55A-55N


B16: a, b; GBM: c-h; CRC: i-n
Anti-LAP
IC
Control
Days after tumor implantation
Tumor weighting
Survival
Tumor number
Tumor size (mm)
Relative BLI
Size, mm 3
Day 15
Day 22

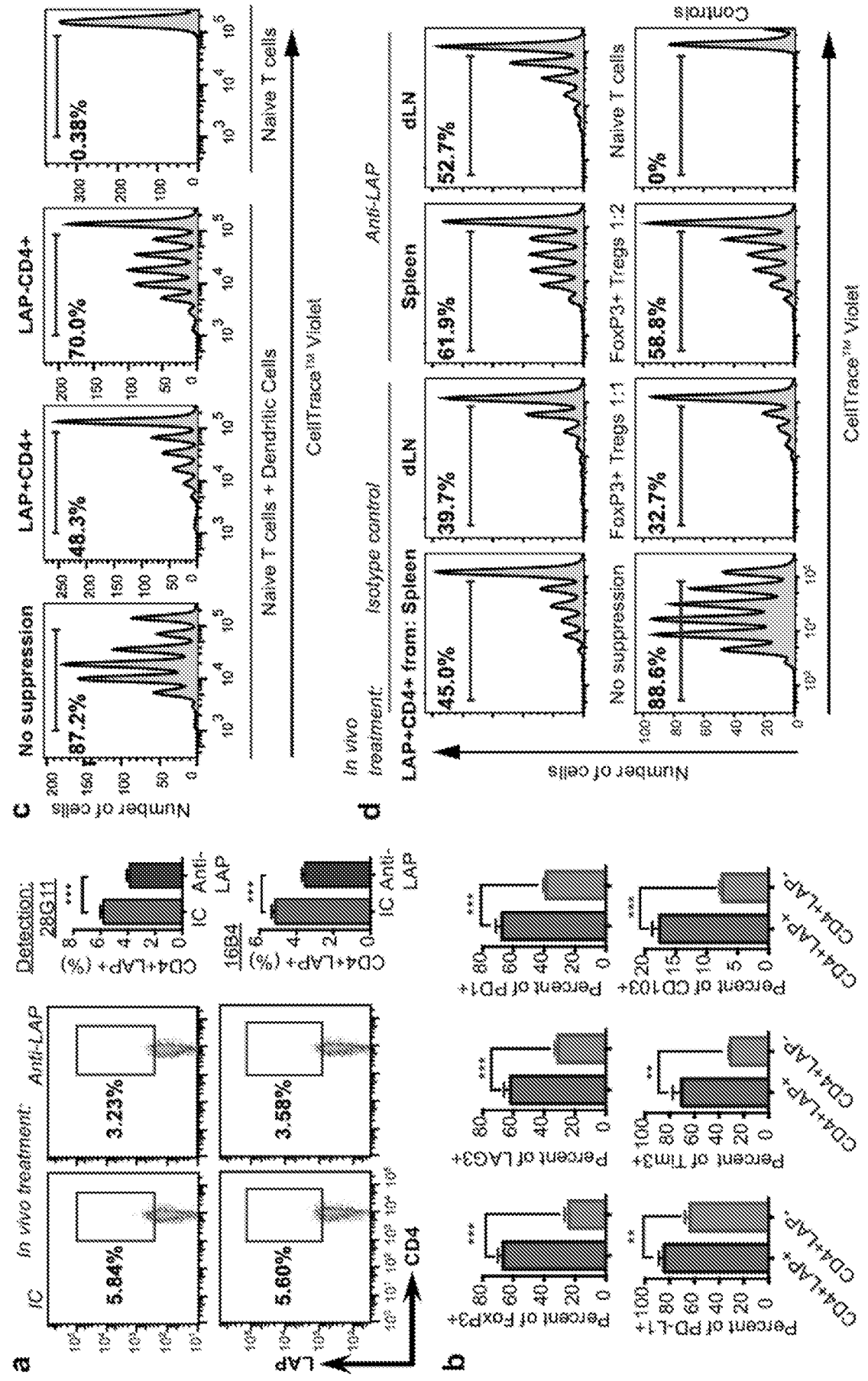
FIGS. 56A-56B


a
Tumor
% of CD8+ T cells
% Ki67+CD8+
MFI/Ki67+CD8+
% IFNγ+CD8+
% CD107+CD8+
CD8/CD4+FOXP3+
CD8+CD11c+(%)
b
Spleen
Percent of CD4+
% of CD4+LAP+
MFI of CD4+LAP+
% of FoxP3+LAP+
Percent of CD8+
% of IFNγ+CD8+
IC
Anti-LAP

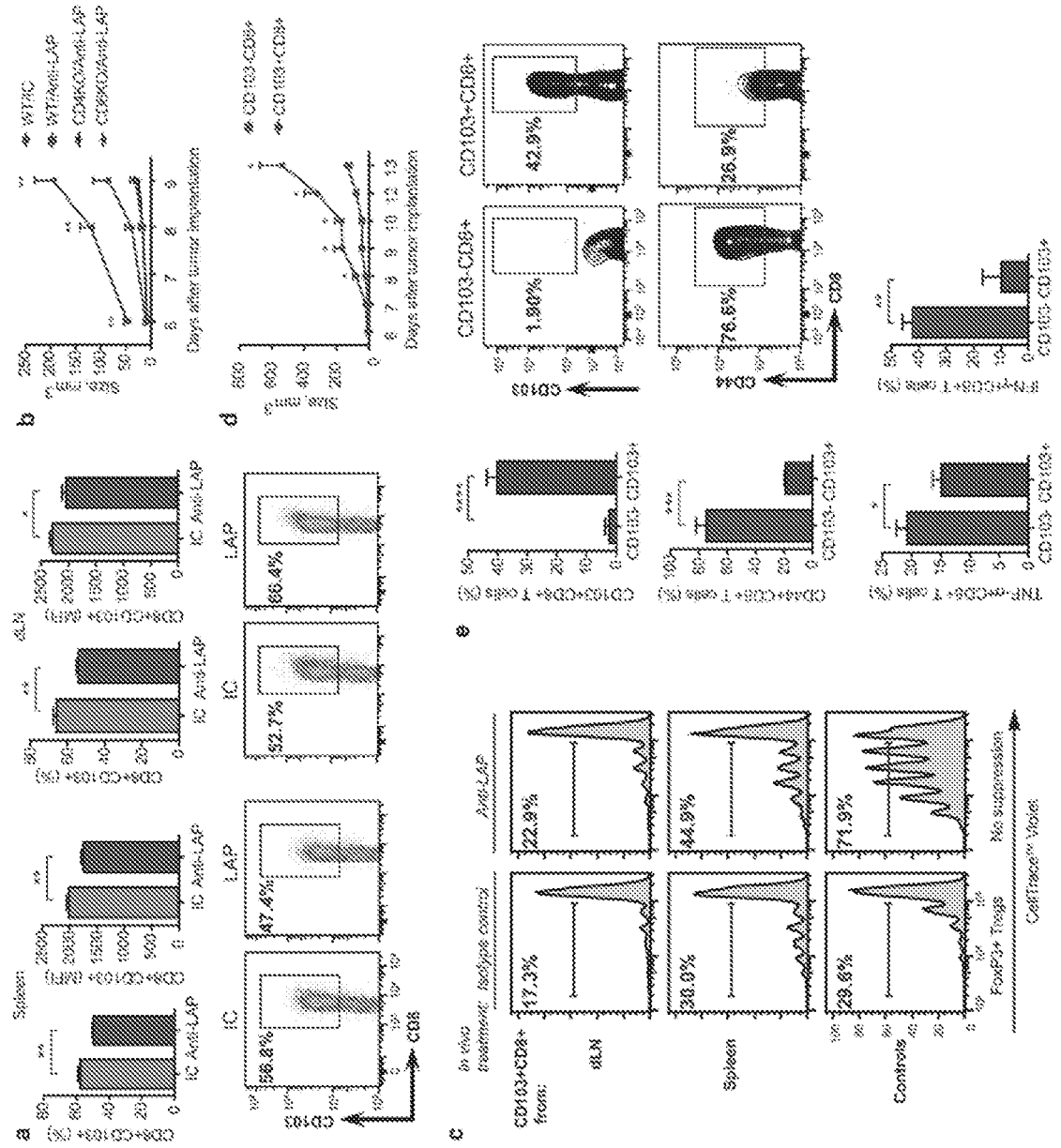
FIGS. 57A-57J


a
IC
Anti-LAP
CD11b-Int
CD11b-Hi
4.92%
9.36%
11.1%
5.66%
CD11c
CD11b
b
IC
Anti-LAP
% of cells
CD11b-Int
CD11b-Hi
e
IL-10
TGF-β
IL-12
mRNA expression
No treat
LPS
CD40
CD11b-Hi
CD11b-Int
c
% of CD103+CD11b-Hi
% of PD-L1+CD11b-Hi
IC
Anti-LAP
d
CD11b-Int
CD11b-Hi
FMO-LAP
IC
Anti-LAP
0.15%
0.052%
2.97%
8.28%
1.45%
1.06%
LAP
CD11b
f
IFN-γ
TNF-α
IFN-γ pg/ml
TNF-α pg/ml
CD11b-Hi
CD11b-Int
i
CD11b-Int
CD11b-Hi
43.3%
54.7%
87.3%
9.66%
7AAD
Violet Tracer/CD8+
j
% of IFN-γ+NK+
IC
Anti-LAP
h
CD11b-Hi
CD11b-Int
FMO
CD86
MFI
CD11b-Hi
CD11b-Int
g
CD11b-Int
CD11b-Hi
FMO
MHCII
MFI
CD11b-Hi
CD11b-Int

FIGS. 58A-58D


a
IC
In vivo treatment:
Anti-LAP
5.84%
3.23%
5.60%
3.58%
LAP
CD4
Detection:
28G11
1684
CD4+LAP+ (%)
IC
Anti-LAP
b
Percent of FoxP3+
Percent of LAG3+
Percent of PD-L1+
Percent of PD1+
Percent of Tim3+
Percent of CD103+
CD4+LAP+
CD4+LAP-
c
No suppression
LAP+CD4+
LAP-CD4+
Number of cells
87.2%
48.3%
70.0%
0.38%
Naive T cells + Dendritic Cells
Naive T cells
CellTrace™ Violet
d
In vivo treatment:
Isotype control
Anti-LAP
LAP+CD4+ from: Spleen
dLN
Spleen
45.0%
39.7%
61.9%
52.7%
No suppression
FoxP3+ Tregs 1:1
FoxP3+ Tregs 1:2
Naive T cells
88.6%
32.7%
58.8%
0%
Controls
Number of cells
CellTrace™ Violet

FIGS. 59A-59E



FIGS. 60A-60F

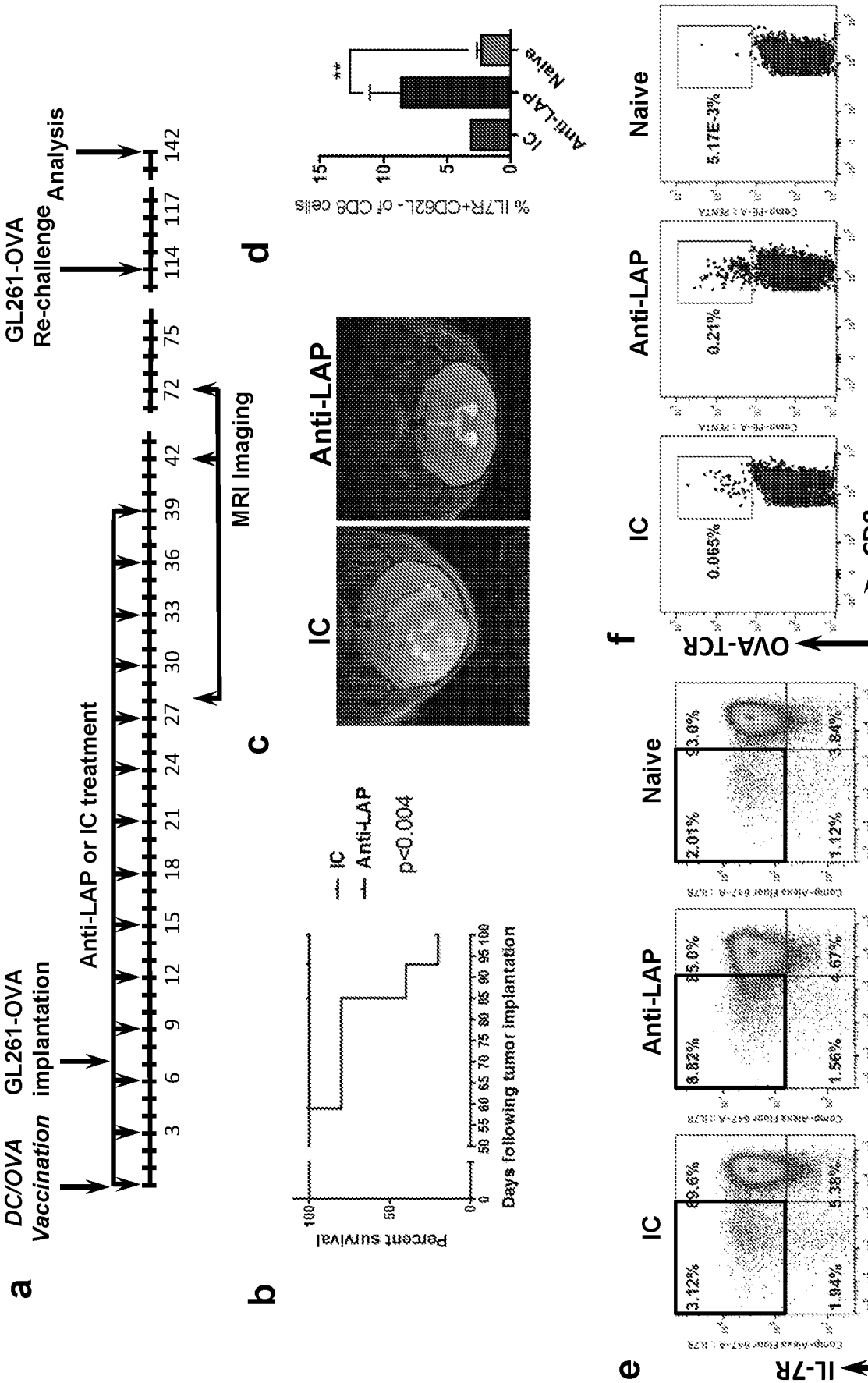
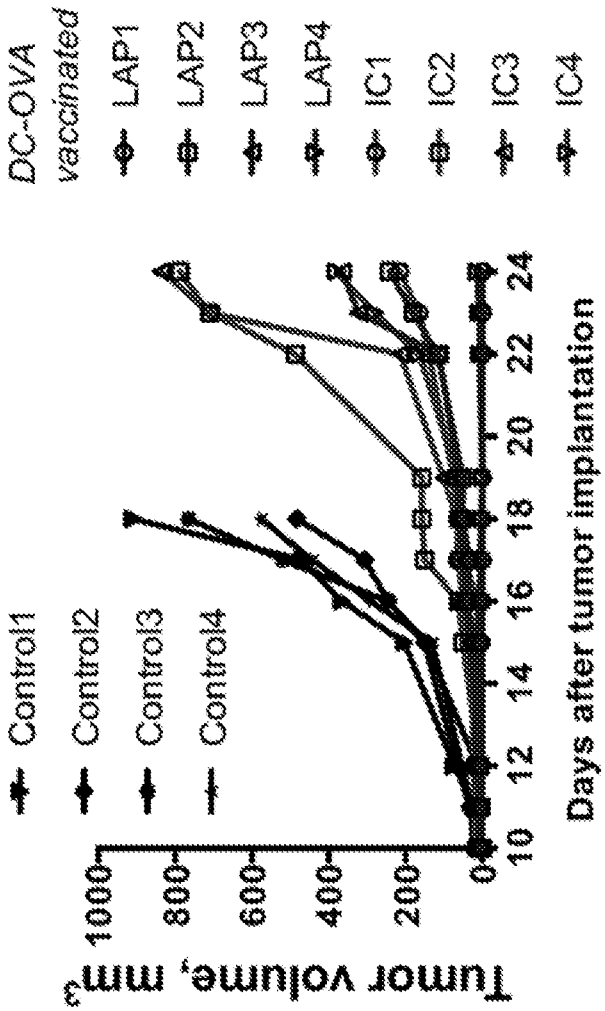

a
DC/OVA Vaccination
GL261-OVA implantation
Anti-LAP or IC treatment
GL261-OVA Re-challenge
Analysis
MRI Imaging
3 6 9 12 15 18 21 24 27 30 33 36 39 42 72 75 114 117 142
b
Percent survival
Days following tumor implantation
IC
Anti-LAP
p<0.004
c
IC
Anti-LAP
d
% IL7R+CD62L- of CD8 cells
IC
Anti-LAP
Naive
**
e
IC
Anti-LAP
Naive
IL-7R
CD62L
3.12%
69.6%
1.94%
5.38%
8.82%
85.0%
1.56%
4.67%
2.01%
93.0%
1.12%
3.84%
f
IC
Anti-LAP
Naive
OVA-TCR
CD8
0.065%
0.21%
5.17E-3%

FIG. 61



B16-OVA Model

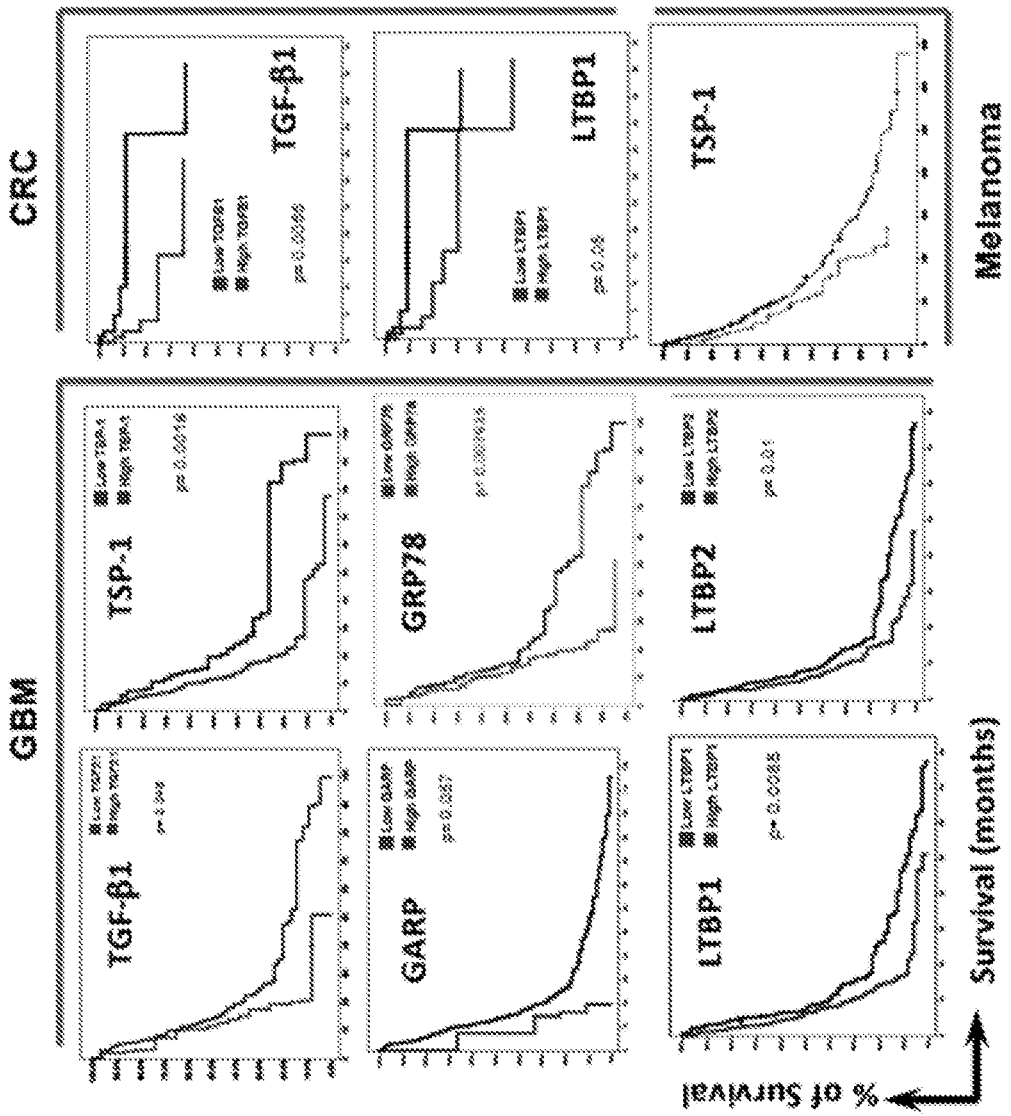

GBM
CRC
TGF-β1
TSP-1
GARP
GRP78
LTBP1
LTBP2
TSP-1
TGF-β1
LTBP1
Melanoma
% of Survival
Survival (months)

FIG. 62

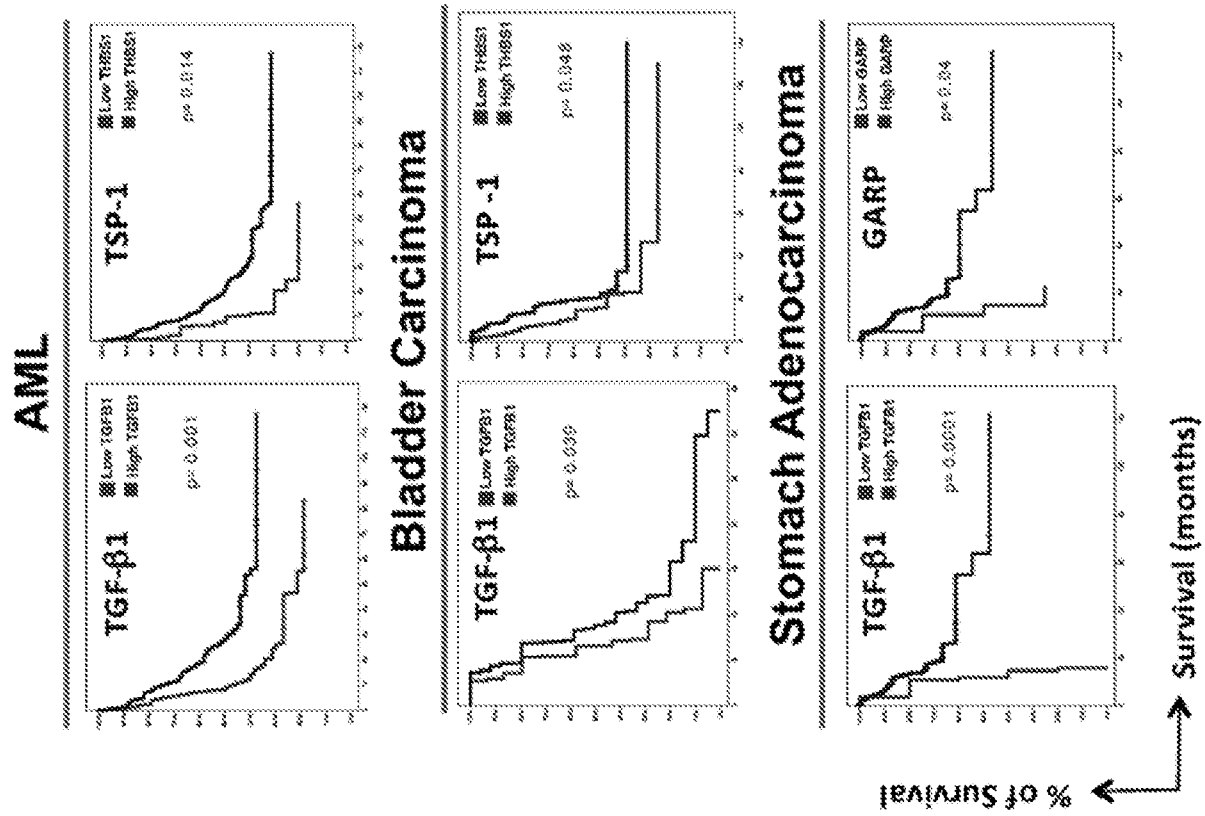

AML
TGF-β1
TSP-1
Bladder Carcinoma
TGF-β1
TSP -1
Stomach Adenocarcinoma
TGF-β1
GARP
% of Survival
Survival (months)

FIG. 63

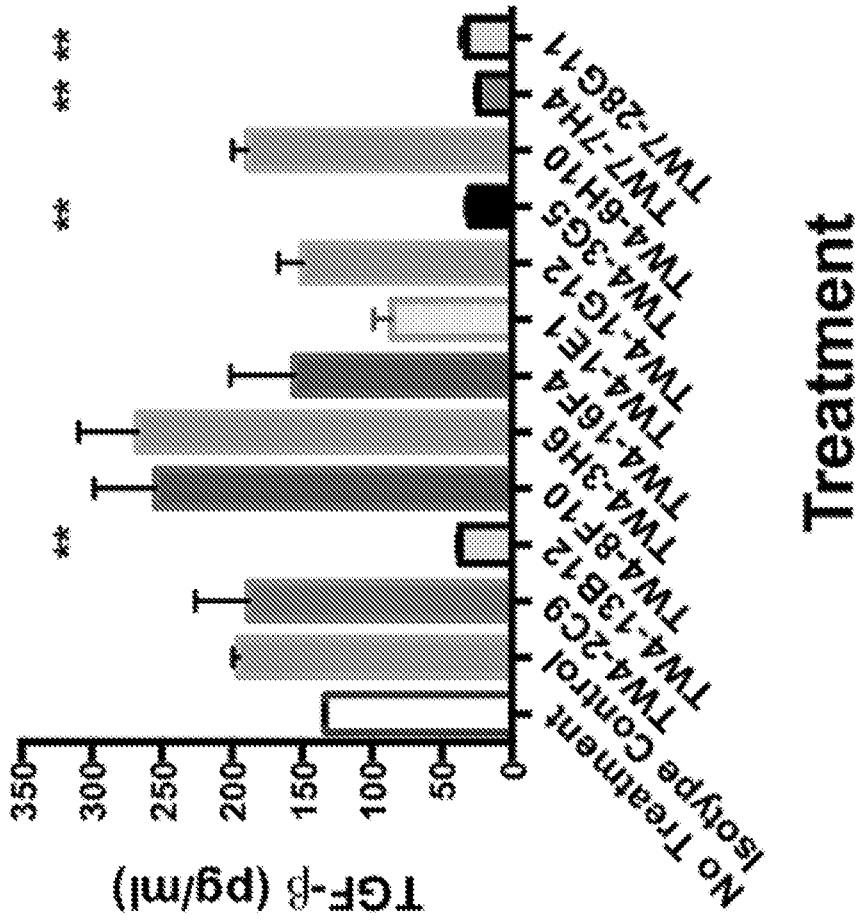

TGF-β (pg/ml)
350
300
250
200
150
100
50
0
**
** **
** **
No Treatment
Isotype Control
TWA-2C9
TWA-13B12
TWA-8F10
TWA-3H6
TWA-16F4
TWA-1E1
TWA-1G12
TWA-3G5
TWA-6H10
TWT-7H4
TWT-28G11
Treatment

FIG. 64

FIG. 65

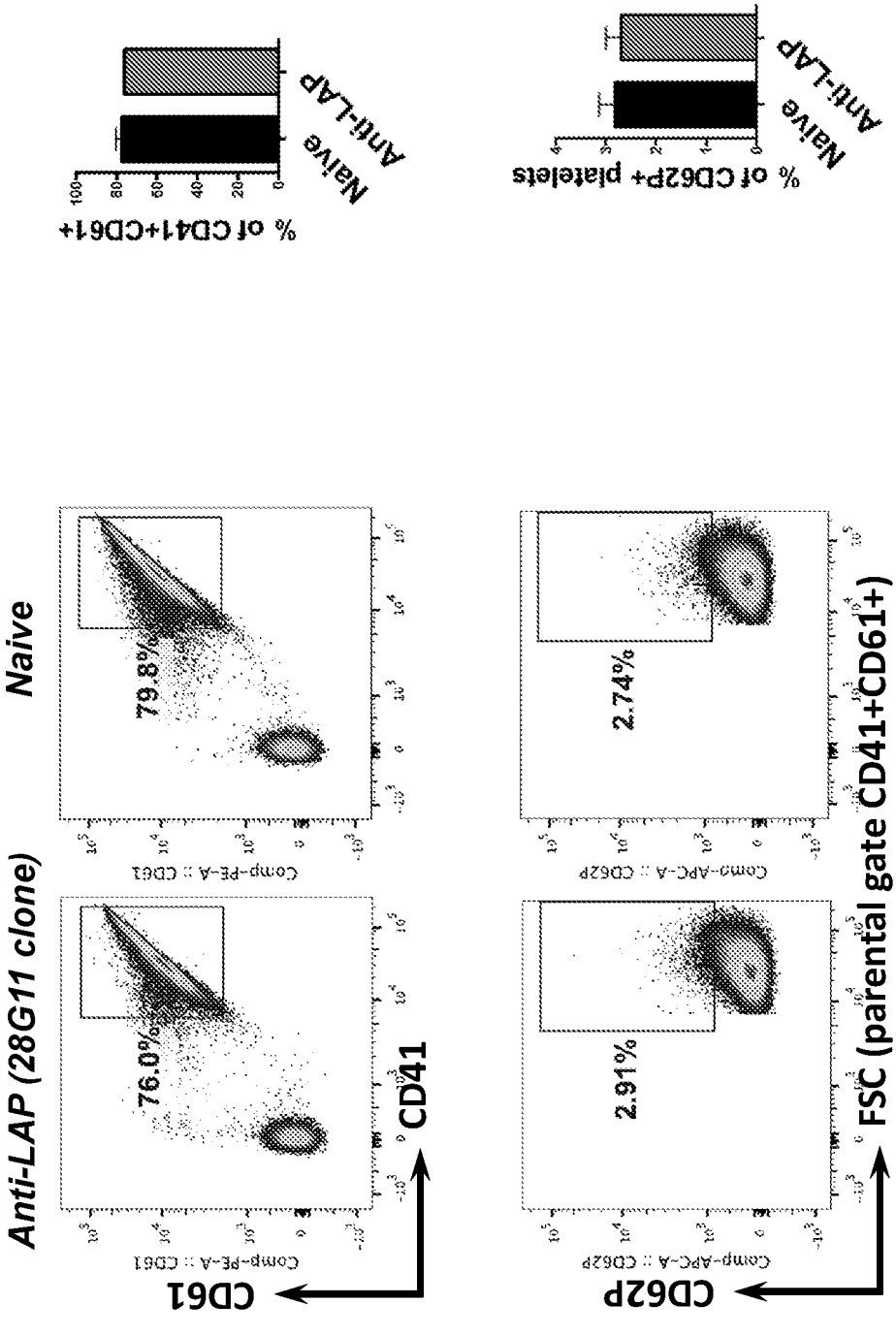
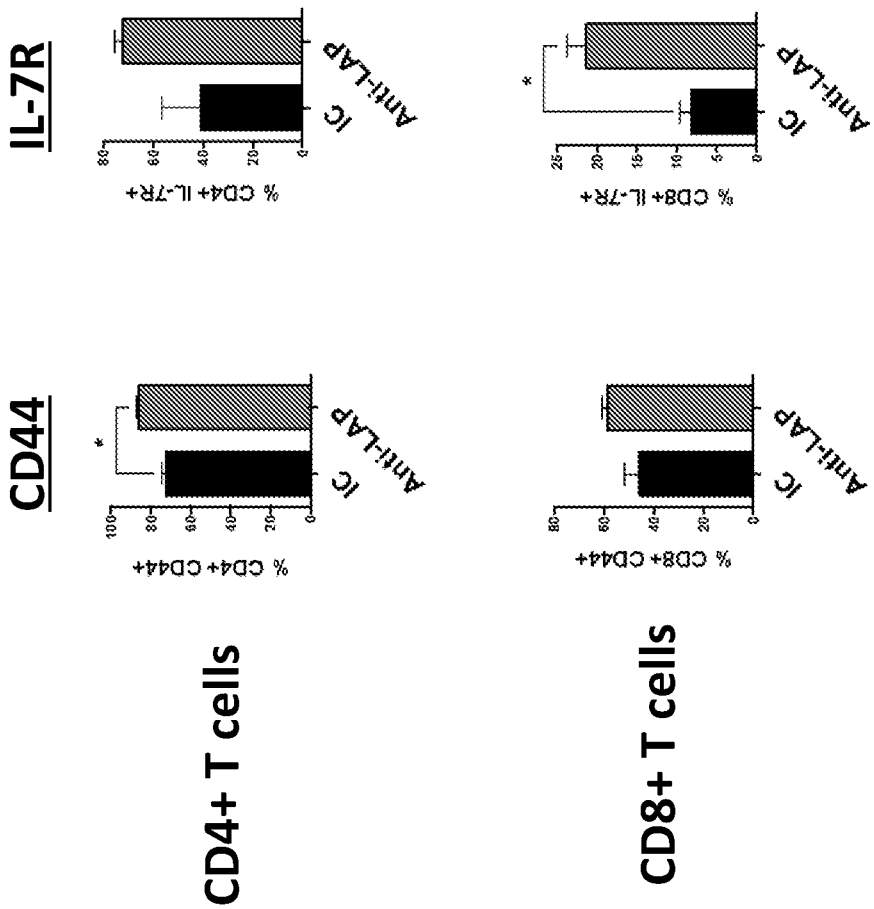

Anti-LAP (28G11 clone)
Naive
CD61
CD41
79.8%
76.0%
CD62P
FSC (parental gate CD41+CD61+)
2.74%
2.91%
% of CD41+CD61+
Naive
Anti-LAP
% of CD62P+ platelets
Naive
Anti-LAP

FIG. 66

FIG. 67


IL-7R
CD44
% CD4+ IL-7R+
% CD8+ IL-7R+
% CD4+ CD44+
% CD8+ CD44+
IC
Anti-LAP
*
CD4+ T cells
CD8+ T cells
INC GBM Model (brain)