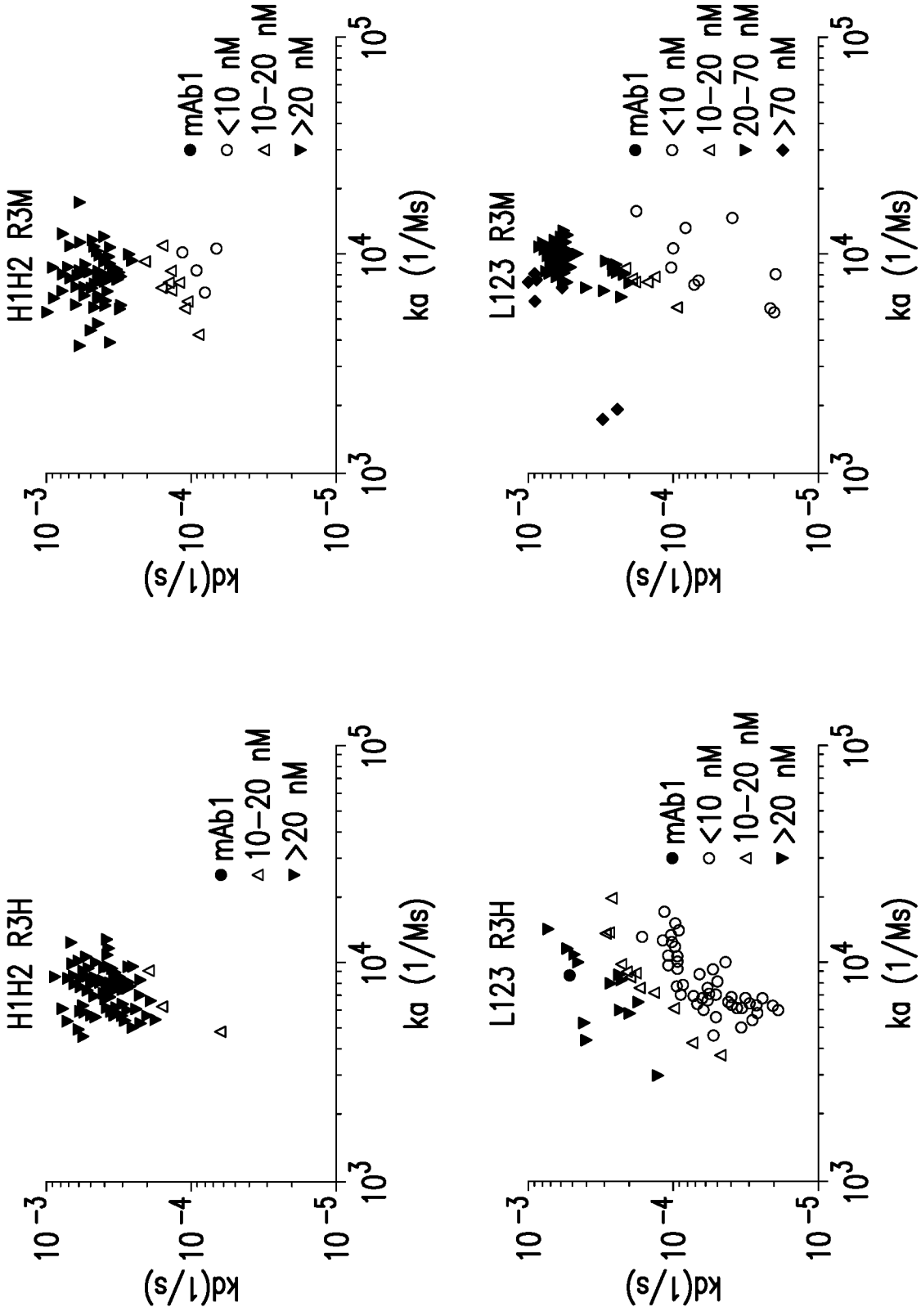
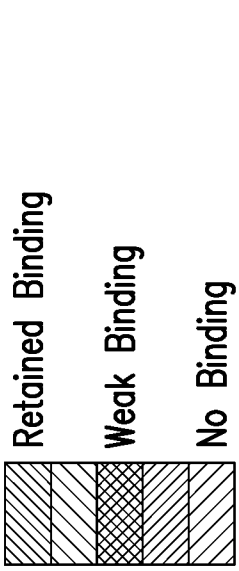

H1H2 R3M
L123 R3M
H1H2 R3H
L123 R3H
mAb1
<10 nM
10–20 nM
20–70 nM
>20 nM
>70 nM
kd(1/s)
ka (1/Ms)
10^-3
10^-4
10^-5
10^3
10^4
10^5

FIG. 1



Position	95	96	97	98	99	100	100A	100B	100C	100D	100E	100F	100G	100H	100I
Amino Acid	D	S	P	F	L	L	D	D	Y	Y	Y	Y	Y	Y	M
huCD137															
mCD137															

FIG.2

LQDPCSNCPAGTFCDNNRNQIC
SPCPPNSFSSAGGQRTCDICRQCKGVFRTRKECSSTSNAEC
DCTPGFHCLGAGCSMCEQDCKQGQELTKKGCK
DCCFGTFNDQKRGICRPWTNCSLDGKSVLVNGTKERDVVCG

mAb1

LQDPCSNCPAGTFCDNNRNQIC
SPCPPNSFSSAGGQRTCDICRQCKGVFRTRKECSSTSNAEC
DCTPGFHCLGAGCSMCEQDCKQGQELTKKGCK
DCCFGTFNDQKRGICRPWTNCSLDGKSVLVNGTKERDVVCG

mAb4

LQDPCSNCPAGTFCDNNRNQIC
SPCPPNSFSSAGGQRTCDICRQCKGVFRTRKECSSTSNAEC
DCTPGFHCLGAGCSMCEQDCKQGQELTKKGCK
DCCFGTFNDQKRGICRPWTNCSLDGKSVLVNGTKERDVVCG

mAb5

FIG.3A

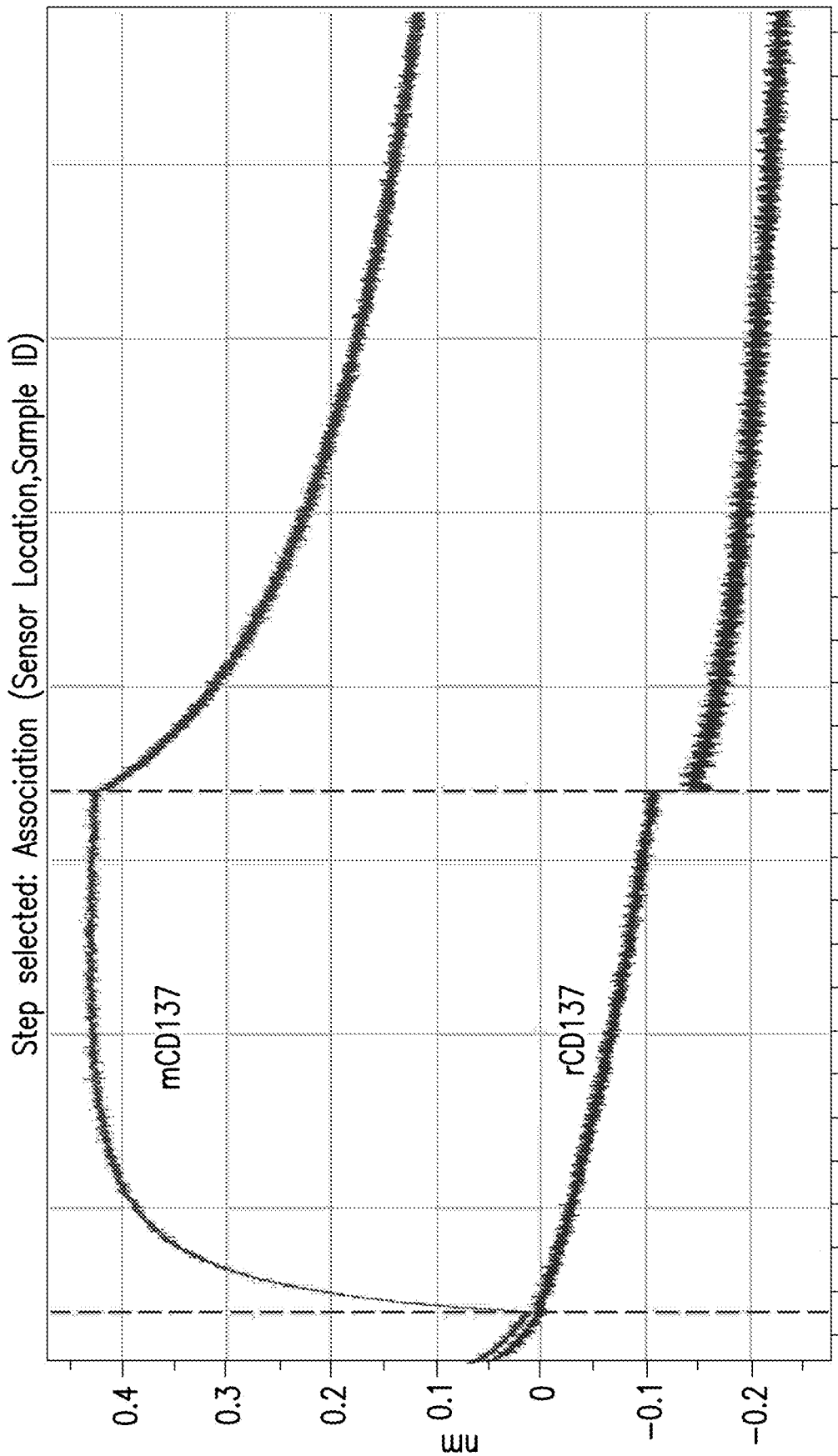

Step selected: Association (Sensor Location,Sample ID)
mCD137
rCD137
nm
0.4
0.3
0.2
0.1
0
−0.1
−0.2
1200
1400
1600
1800
2000
2200
2400
Time (s)

FIG.3B

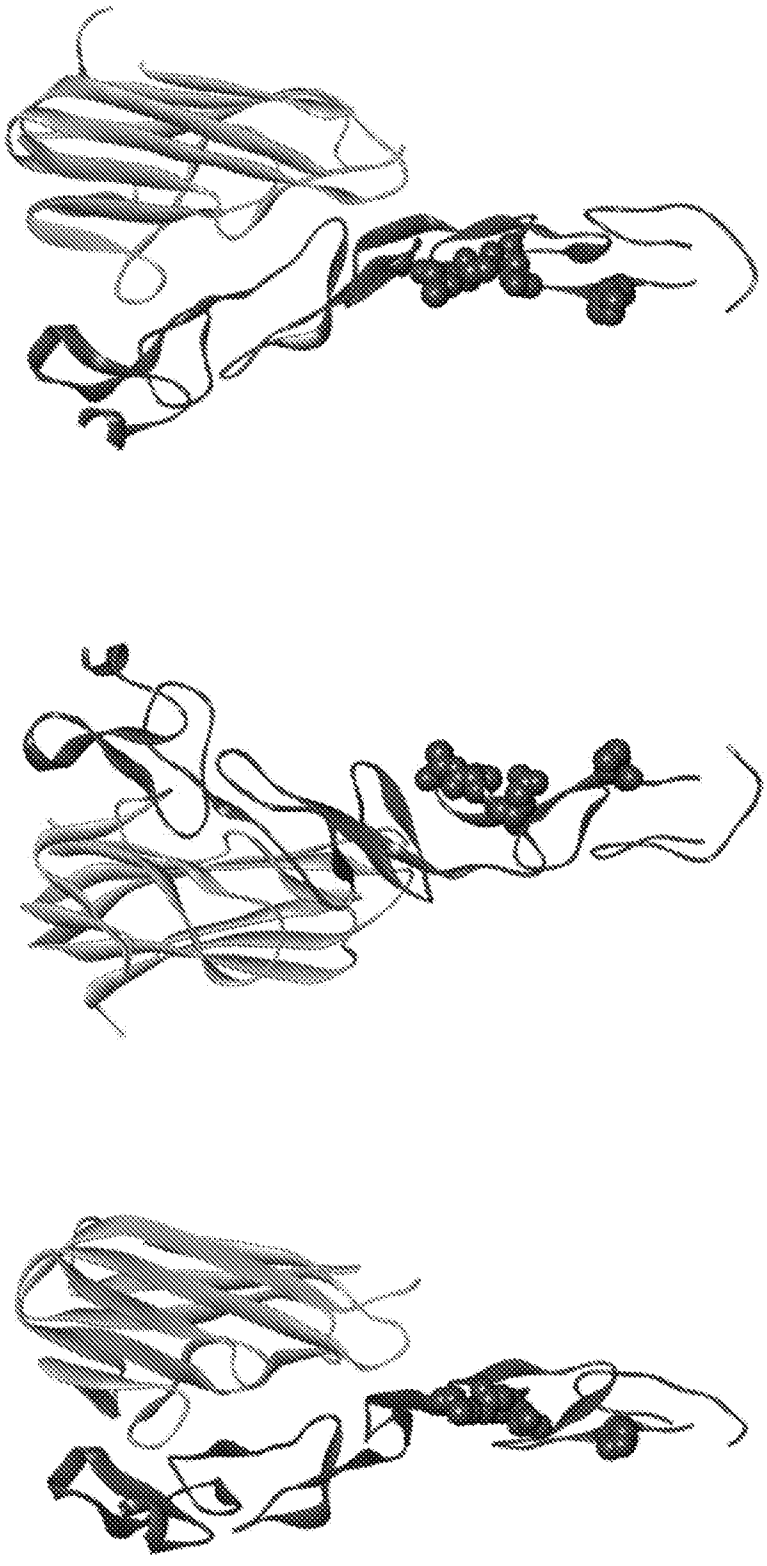


FIG.3C

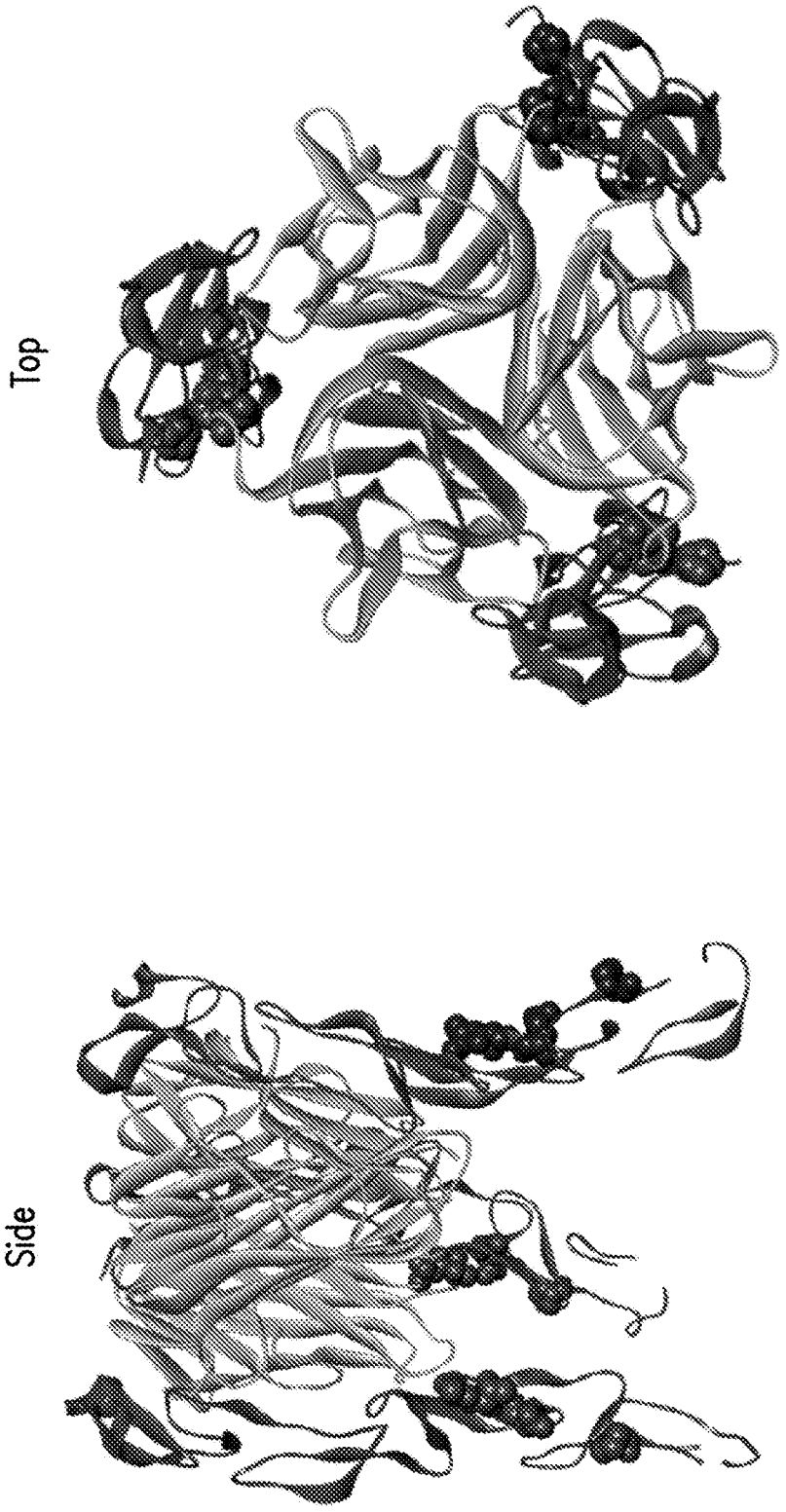


FIG.3D

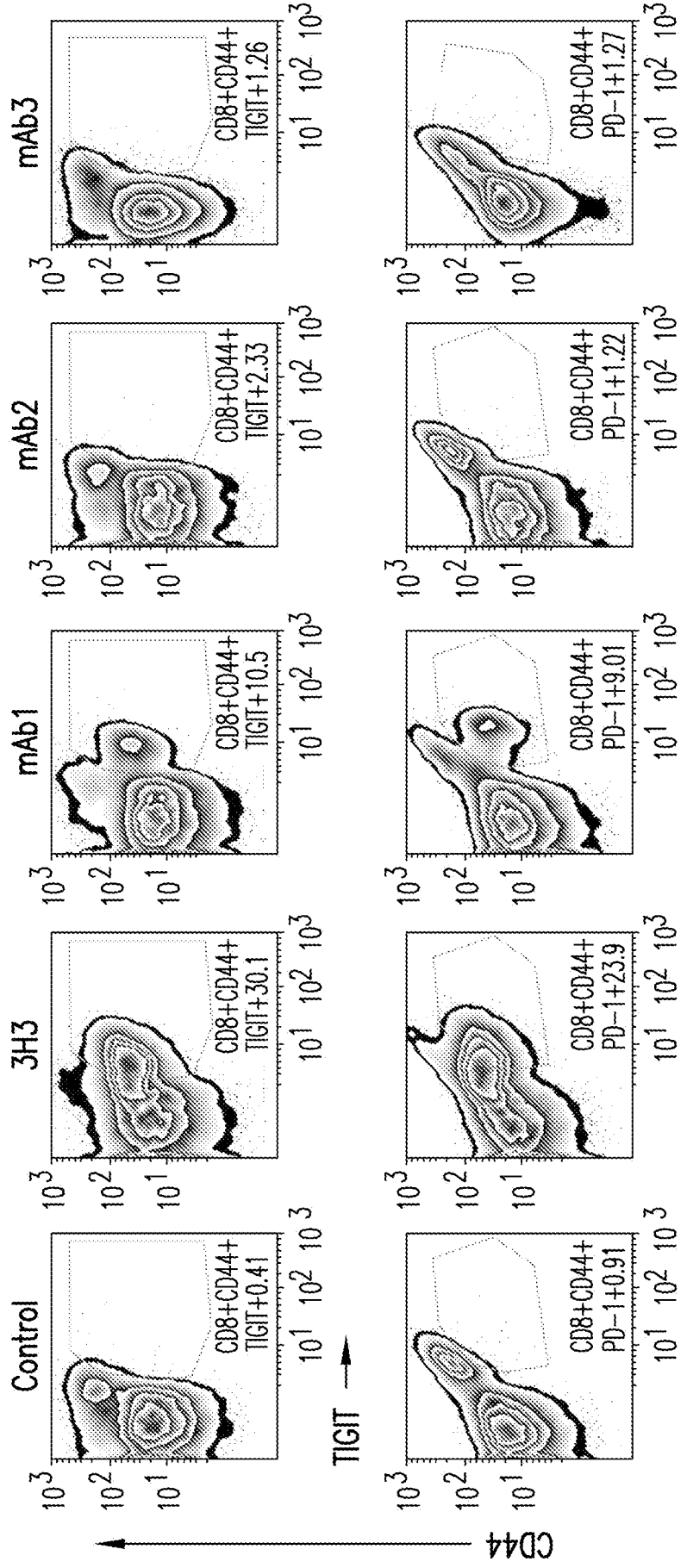

Control
3H3
mAb1
mAb2
mAb3
CD8+CD44+ TIGIT+0.41
CD8+CD44+ TIGIT+30.1
CD8+CD44+ TIGIT+10.5
CD8+CD44+ TIGIT+2.33
CD8+CD44+ TIGIT+1.26
CD8+CD44+ PD-1+0.91
CD8+CD44+ PD-1+23.9
CD8+CD44+ PD-1+9.01
CD8+CD44+ PD-1+1.22
CD8+CD44+ PD-1+1.27
CD44
TIGIT
PD-1

FIG.4A

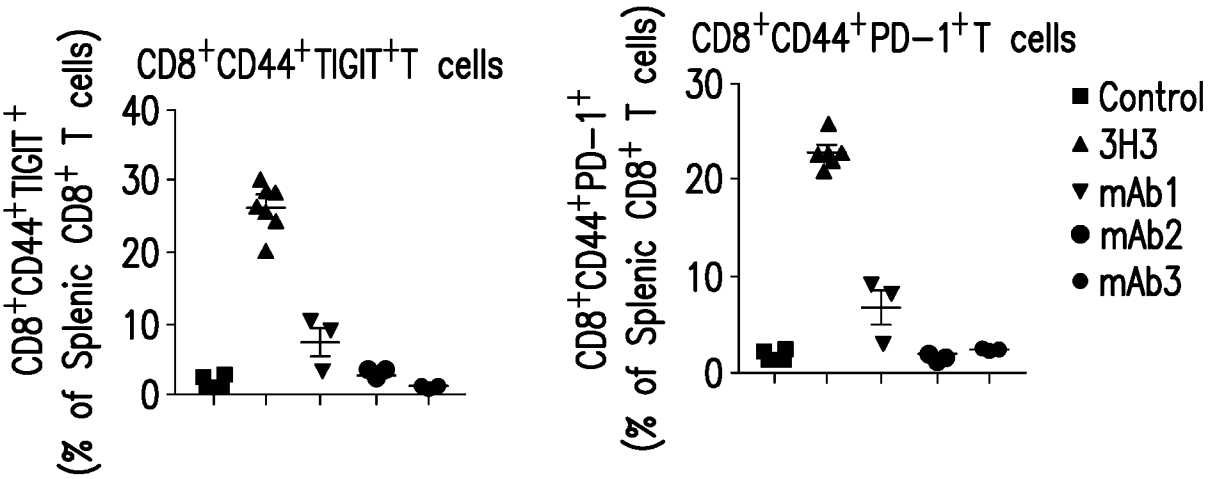

CD8+CD44+TIGIT+T cells
(% of Splenic CD8+ T cells)
CD8+CD44+TIGIT+
40
30
20
10
0
CD8+CD44+PD-1+T cells
(% of Splenic CD8+ T cells)
CD8+CD44+PD-1+
30
20
10
0
Control
3H3
mAb1
mAb2
mAb3

FIG.4B

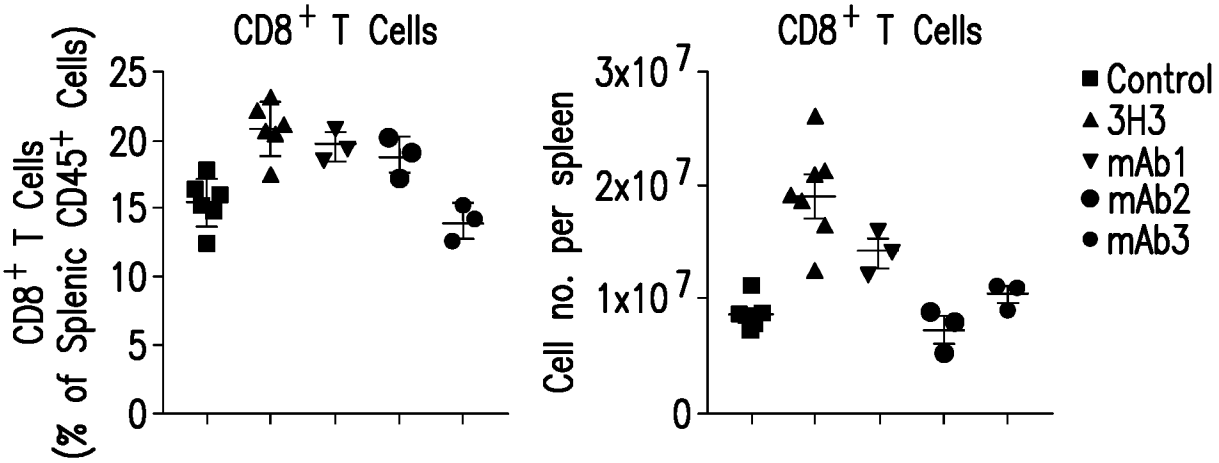

CD8+ T Cells
(% of Splenic CD45+ Cells)
CD8+ T Cells
25
20
15
10
5
0
CD8+ T Cells
Cell no. per spleen
3x10 7
2x10 7
1x10 7
0
Control
3H3
mAb1
mAb2
mAb3

FIG.4C

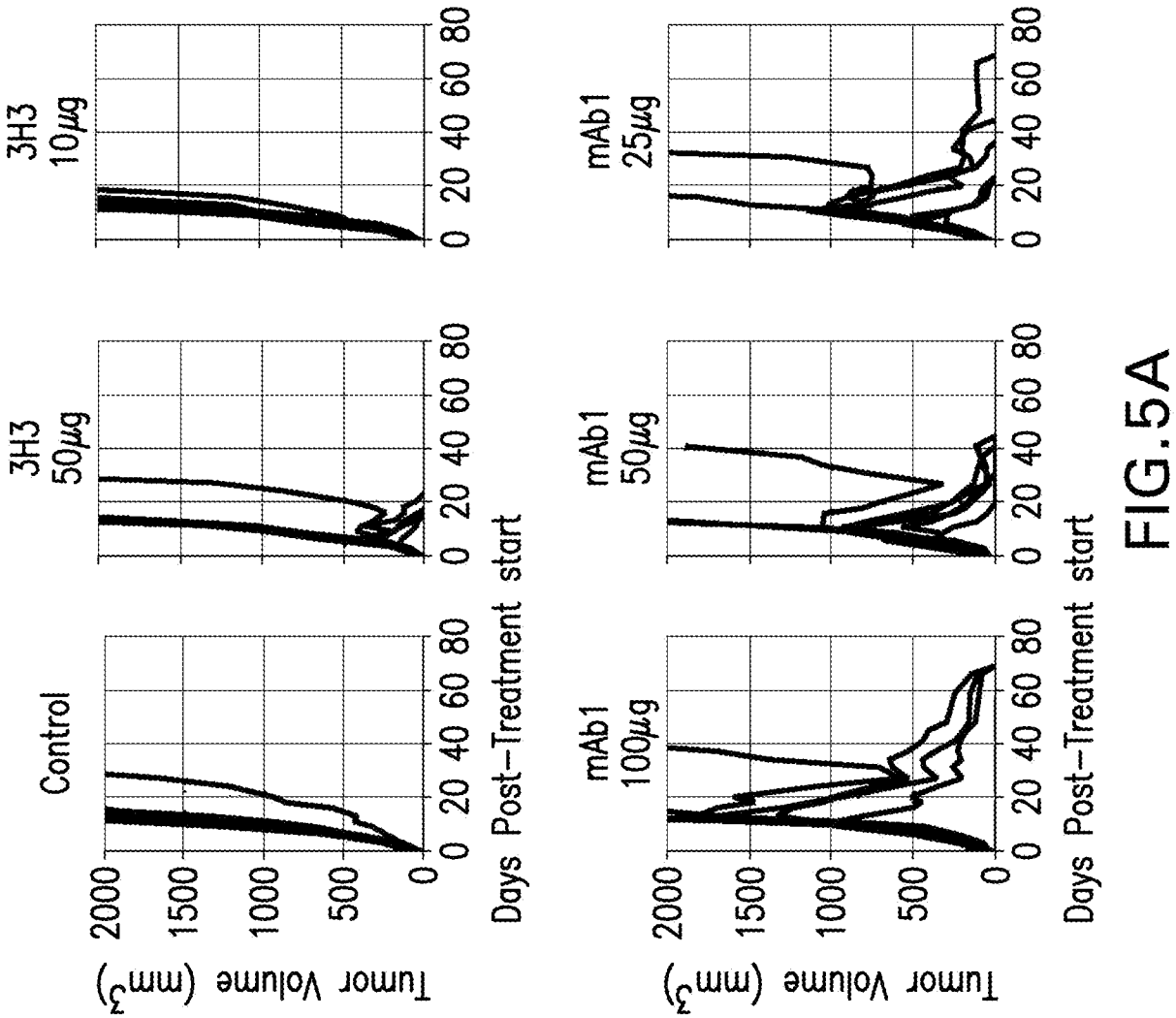

Control
3H3 50μg
3H3 10μg
mAb1 100μg
mAb1 50μg
mAb1 25μg
Tumor Volume (mm³)
2000
1500
1000
500
0
0 20 40 60 80
Days Post−Treatment start

FIG.5A

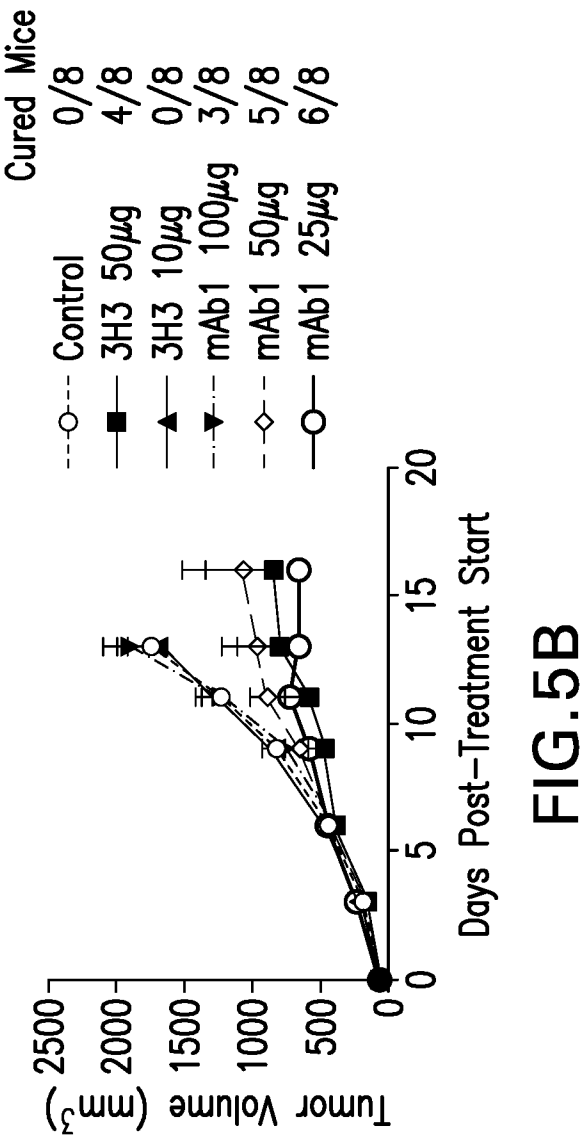


FIG.5B

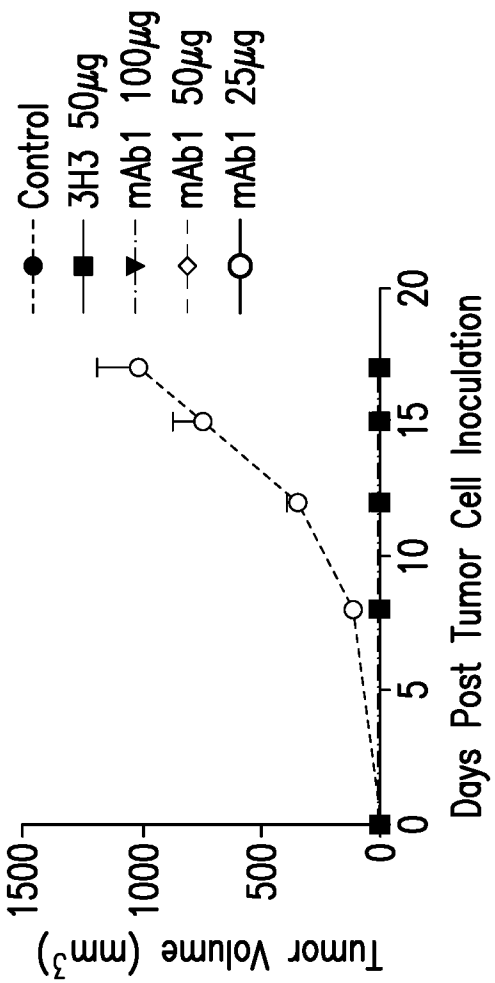


FIG.5D

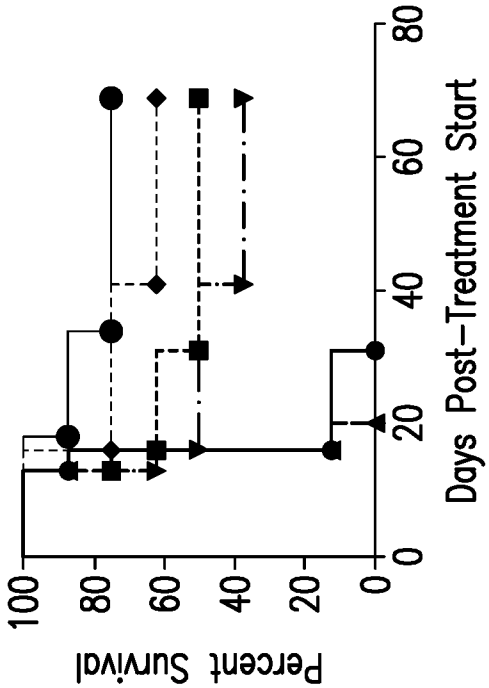


FIG.5C

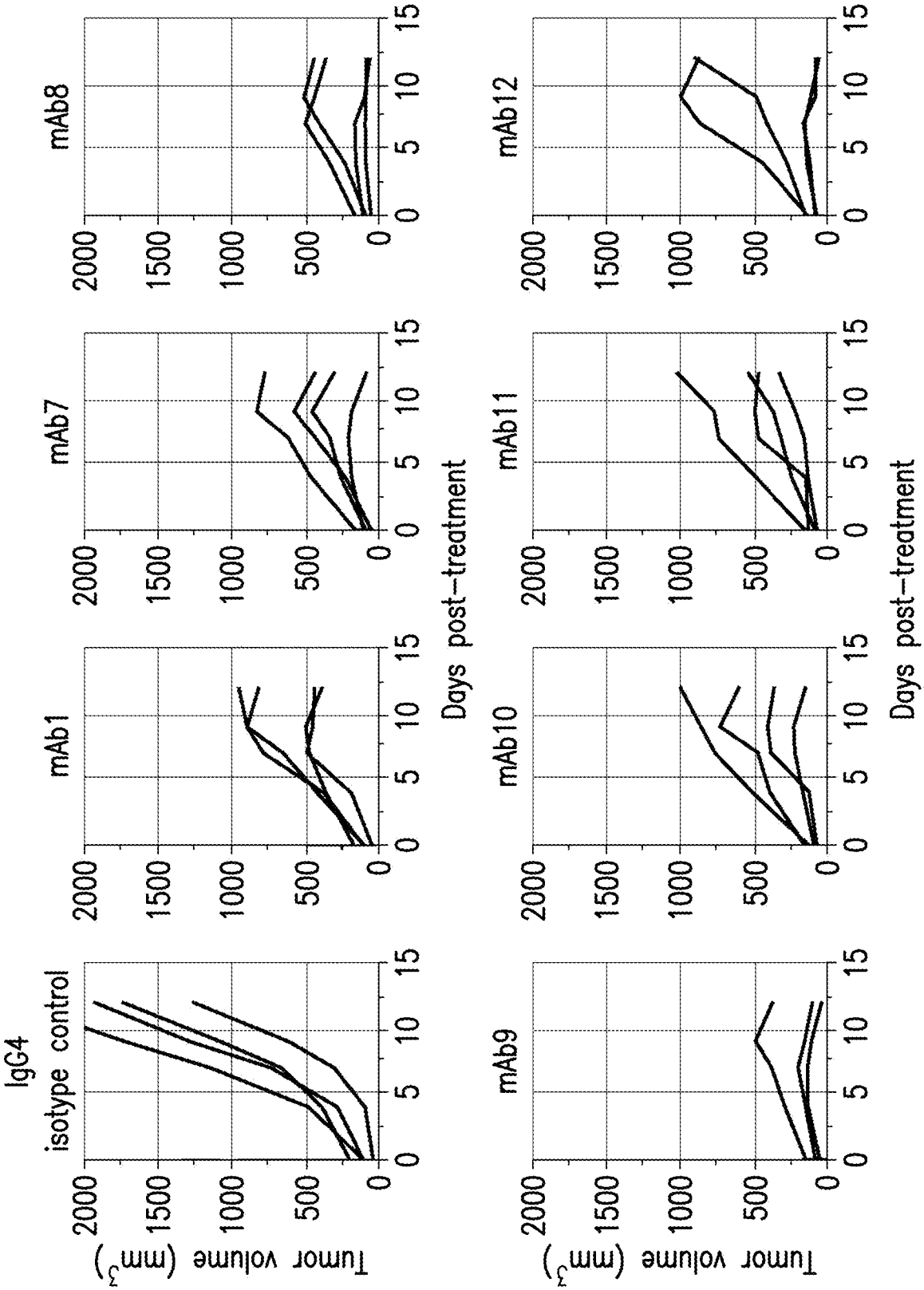

IgG4
isotype control
mAb1
mAb7
mAb8
mAb9
mAb10
mAb11
mAb12
Tumor volume (mm³)
Days post-treatment
0
500
1000
1500
2000
0
5
10
15

FIG.6A

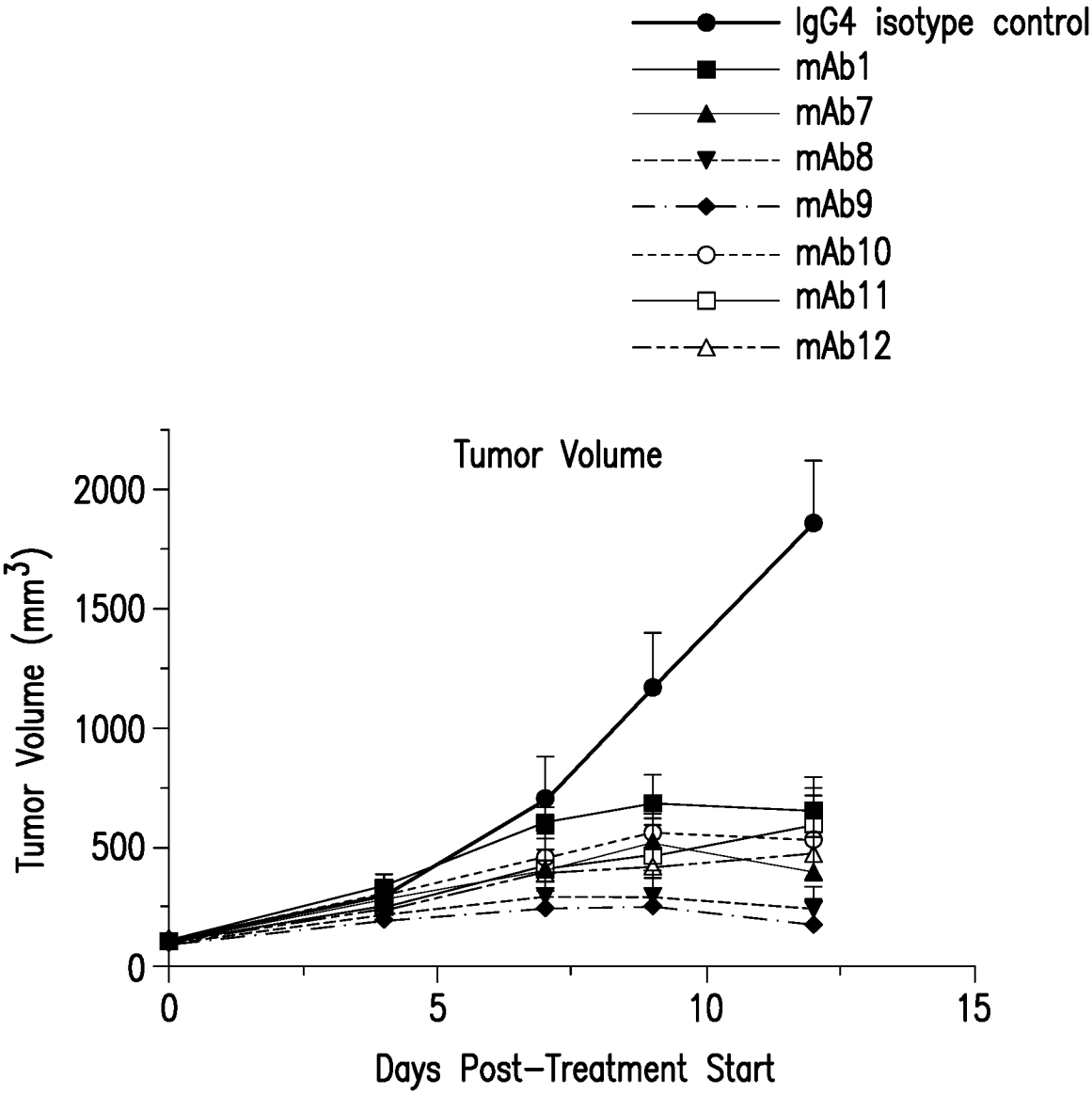

IgG4 isotype control
mAb1
mAb7
mAb8
mAb9
mAb10
mAb11
mAb12
Tumor Volume
Tumor Volume (mm3)
2000
1500
1000
500
0
0
5
10
15
Days Post-Treatment Start

FIG.6B

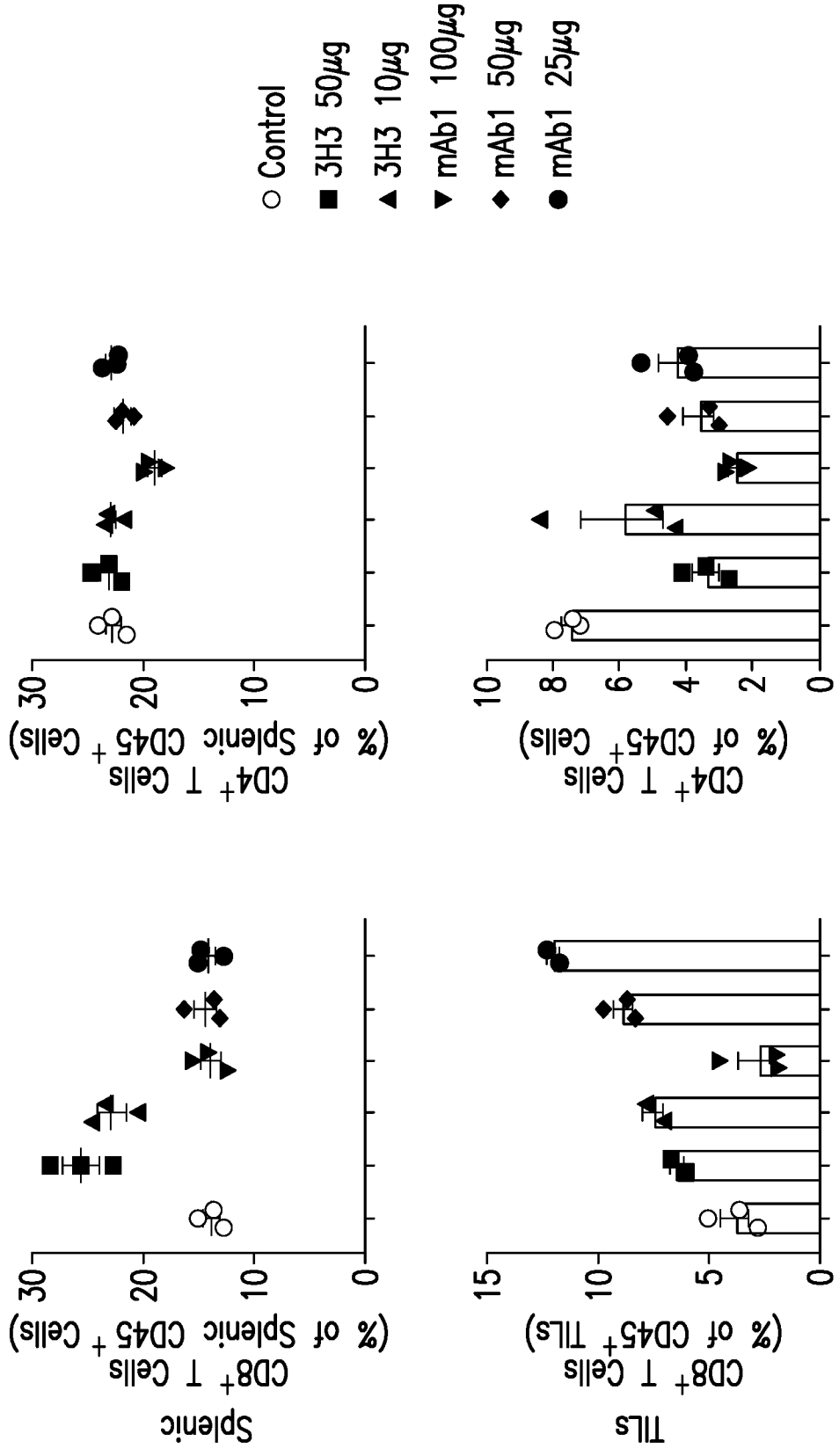

Control
3H3 50μg
3H3 10μg
mAb1 100μg
mAb1 50μg
mAb1 25μg
Splenic
CD8+ T Cells
(% of Splenic CD45+ Cells)
CD4+ T Cells
(% of Splenic CD45+ Cells)
TILs
CD8+ T Cells
(% of CD45+ TILs)
CD4+ T Cells
(% of CD45+ Cells)

FIG.7

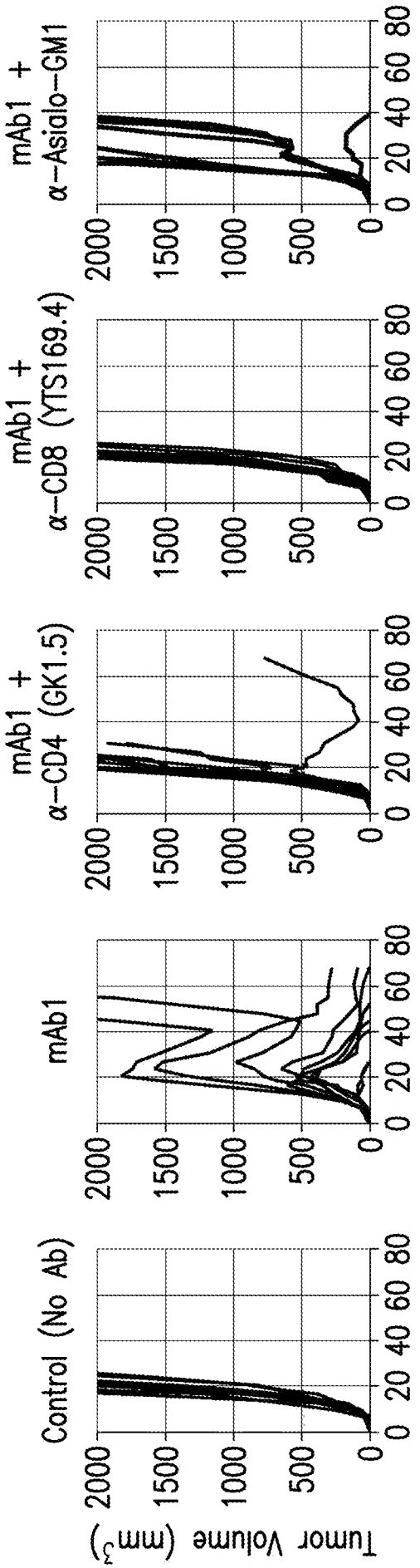

Control (No Ab)
mAb1
mAb1 + α−CD4 (GK1.5)
mAb1 + α−CD8 (YTS169.4)
mAb1 + α−Asialo−GM1
Tumor Volume (mm3)
Days Post Tumor Inoculation
0
500
1000
1500
2000
0 20 40 60 80

FIG.8

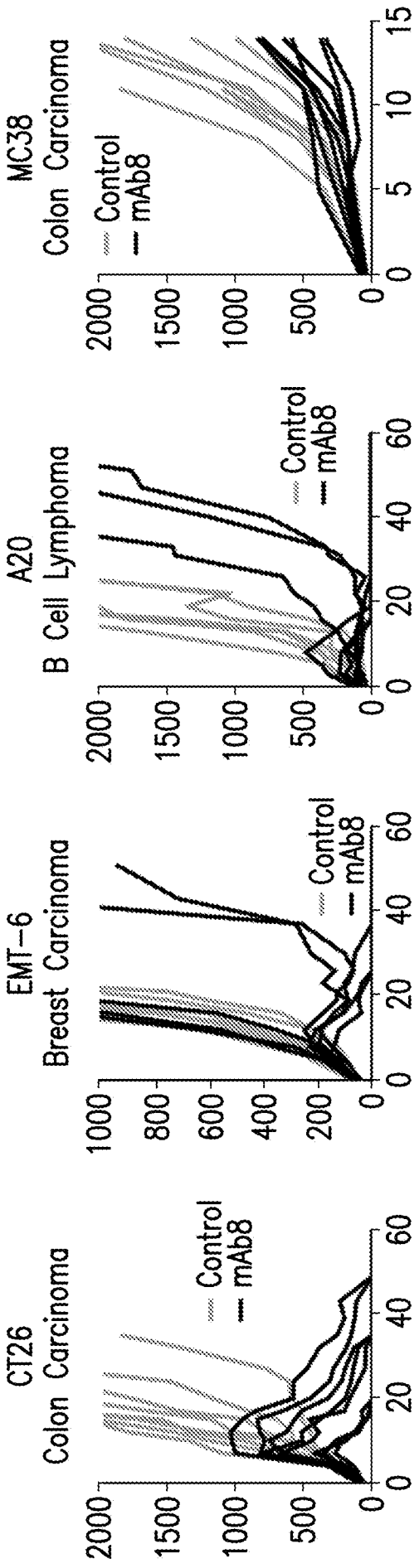

CT26
Colon Carcinoma
Control
mAb8
EMT-6
Breast Carcinoma
A20
B Cell Lymphoma
MC38
Colon Carcinoma

FIG.9

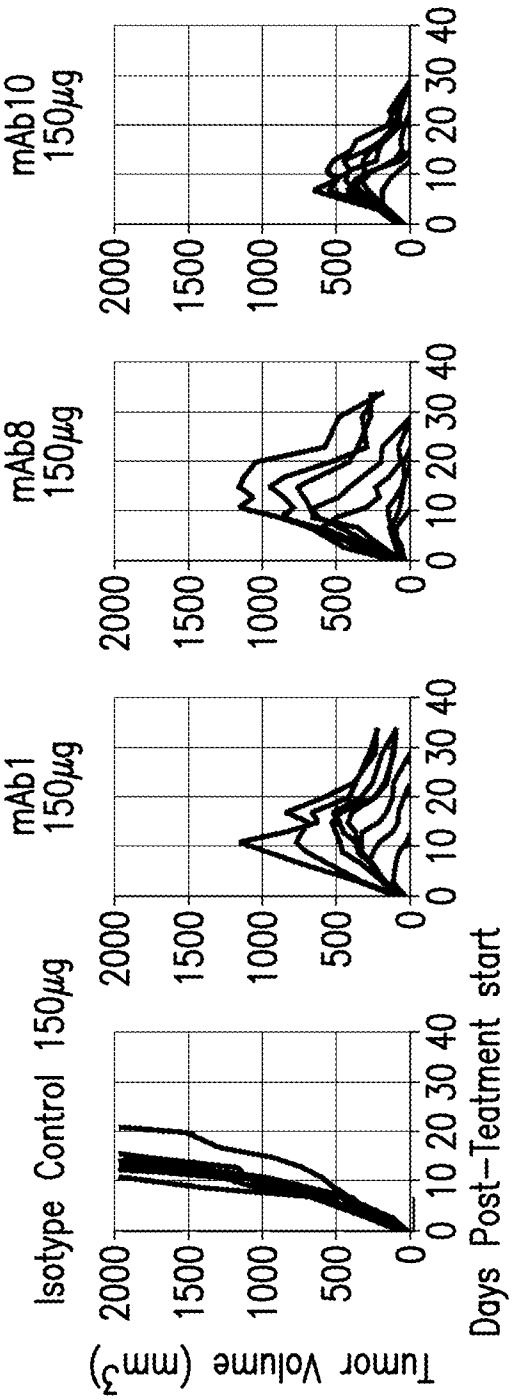


FIG.10A

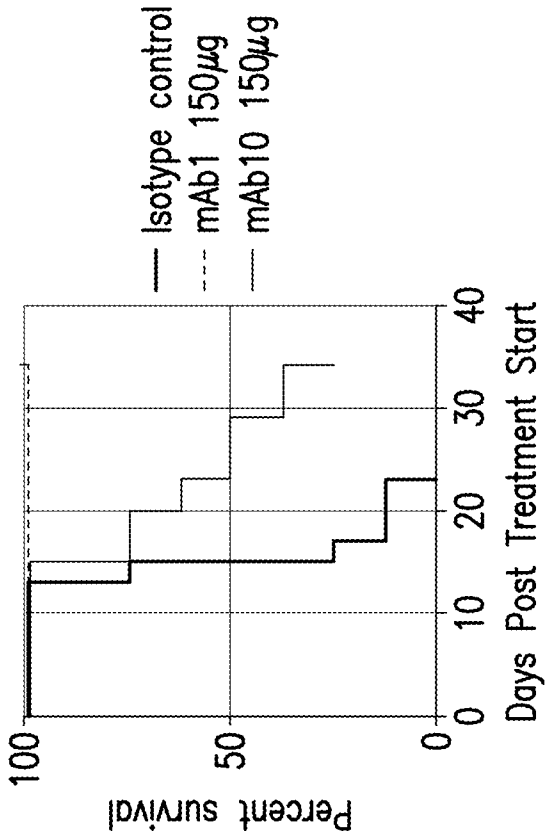


FIG.10C

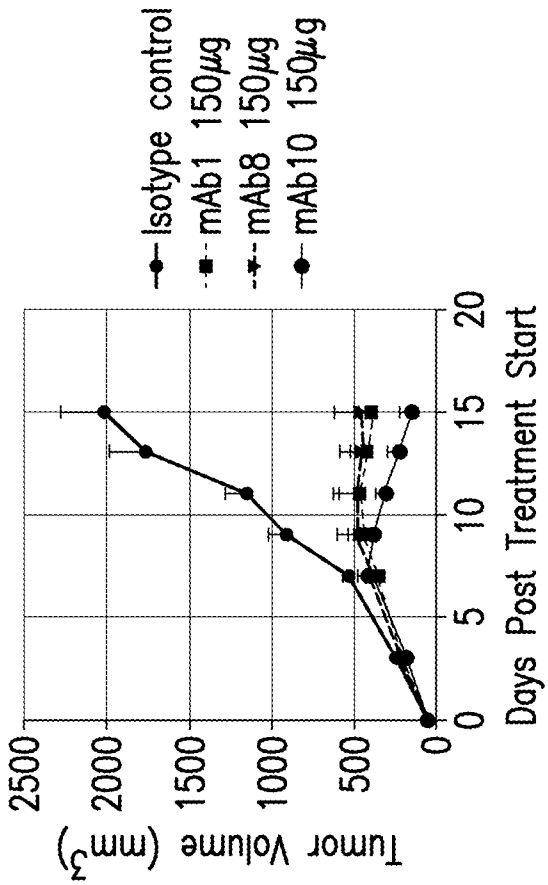


FIG.10B

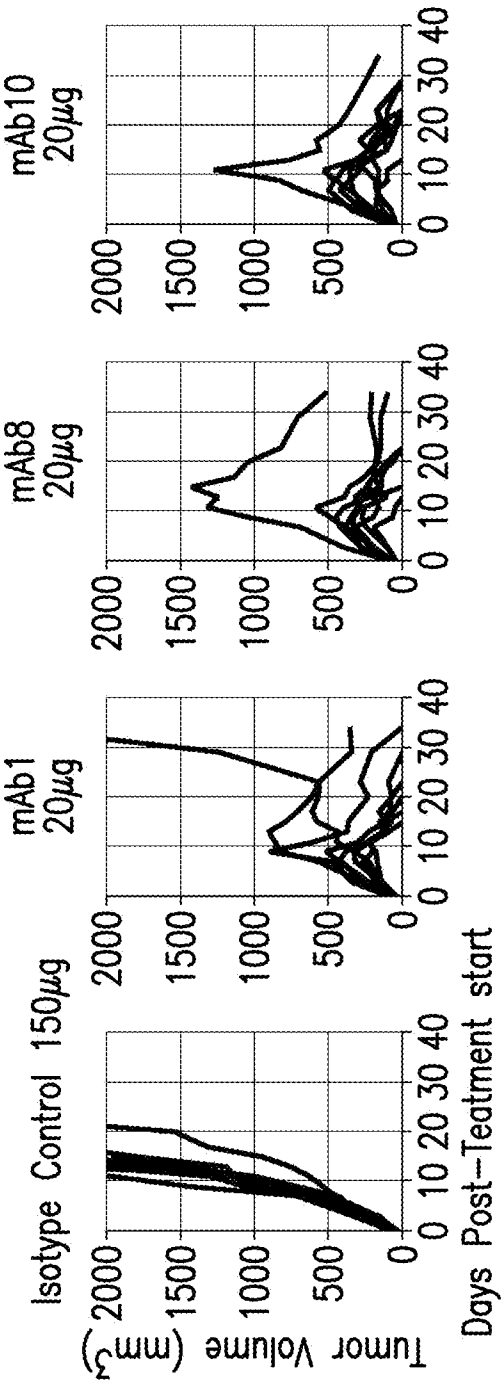


FIG. 11A

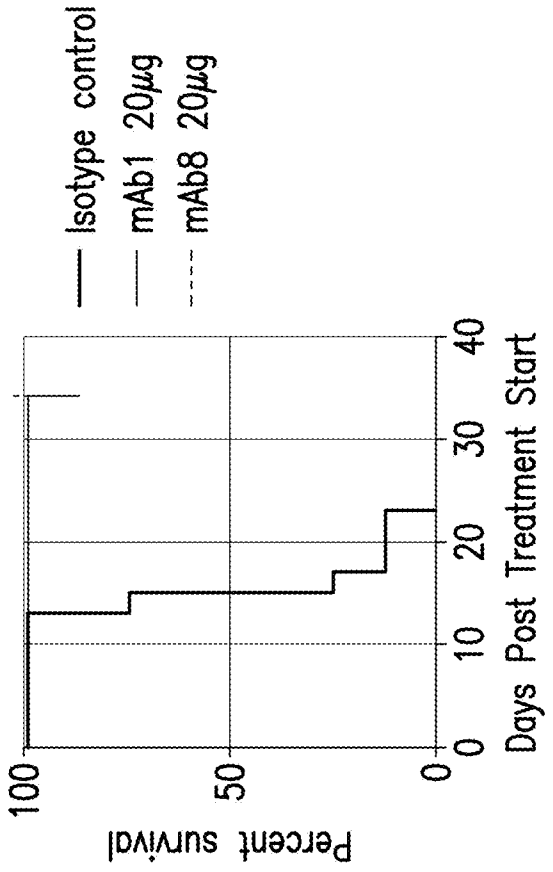


FIG. 11C

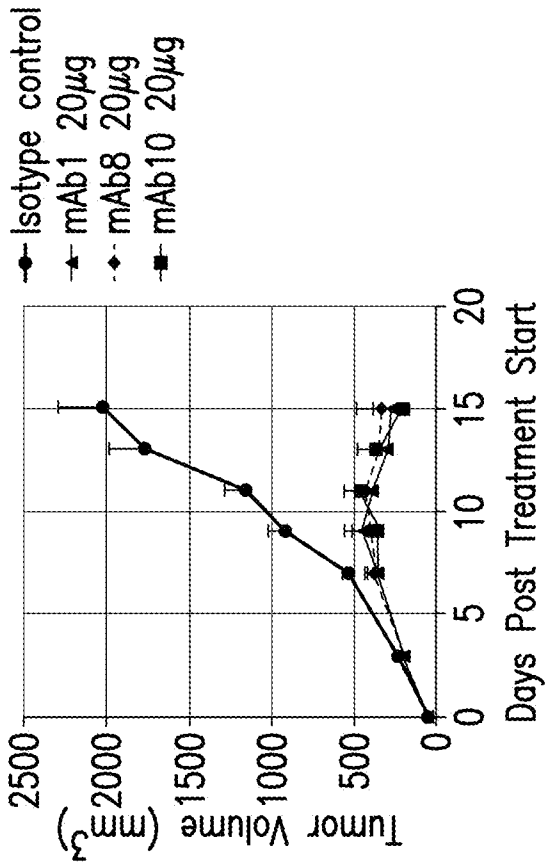


FIG. 11B

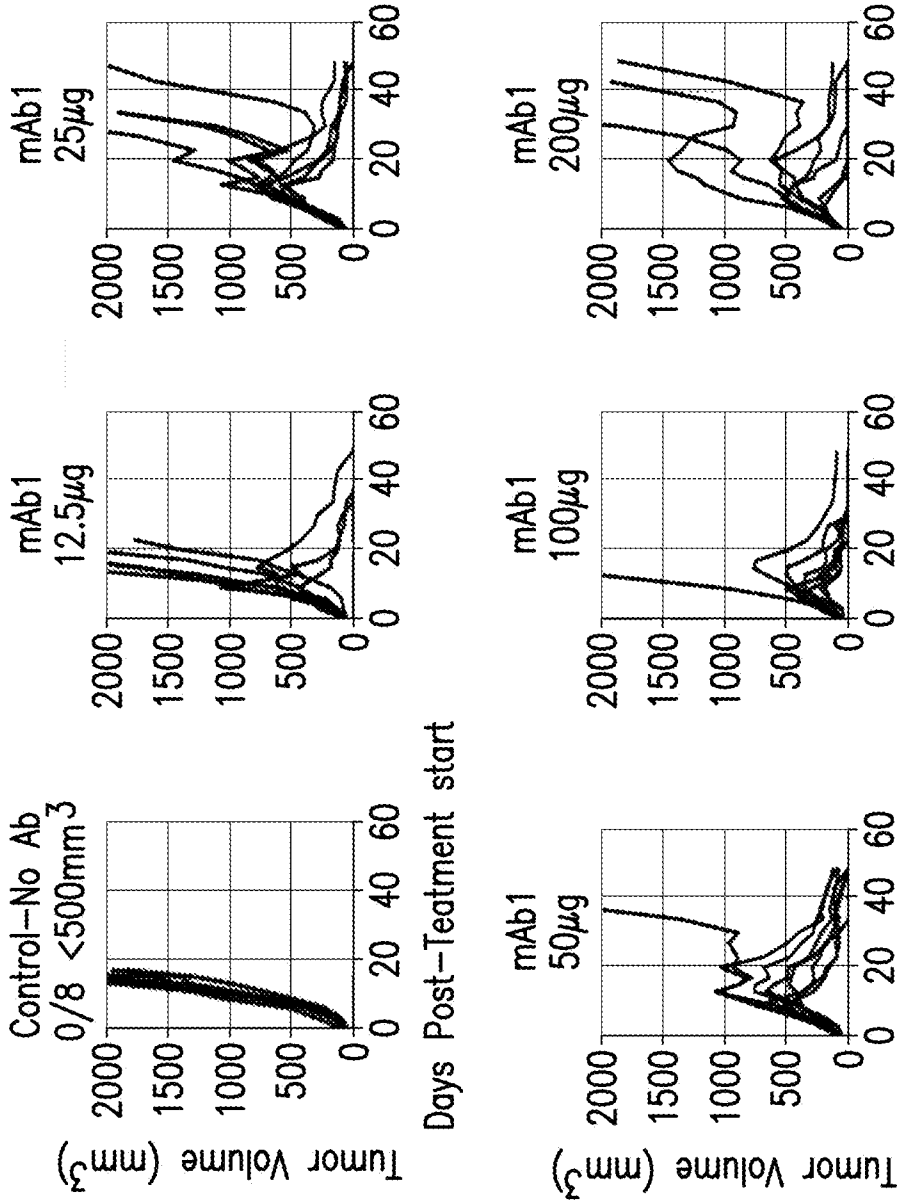

Control–No Ab
0/8 <500mm3
mAb1
12.5μg
mAb1
25μg
mAb1
50μg
mAb1
100μg
mAb1
200μg
Tumor Volume (mm3)
Days Post–Teatment start
0
500
1000
1500
2000
0
20
40
60

FIG. 12

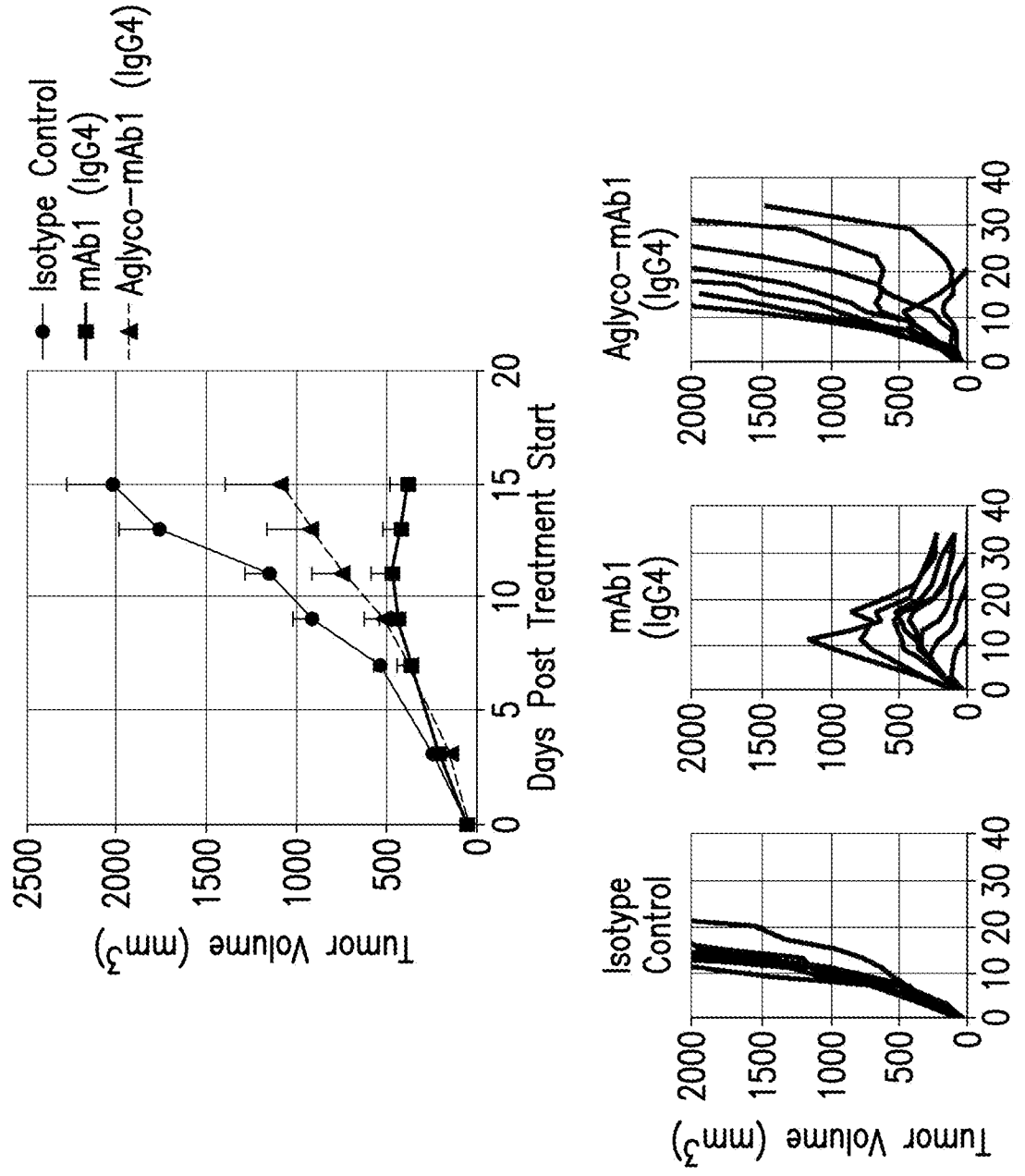

Isotype Control
mAb1 (IgG4)
Aglyco-mAb1 (IgG4)
Tumor Volume (mm3)
Days Post Treatment Start
Isotype Control
mAb1 (IgG4)
Aglyco-mAb1 (IgG4)

FIG.13A

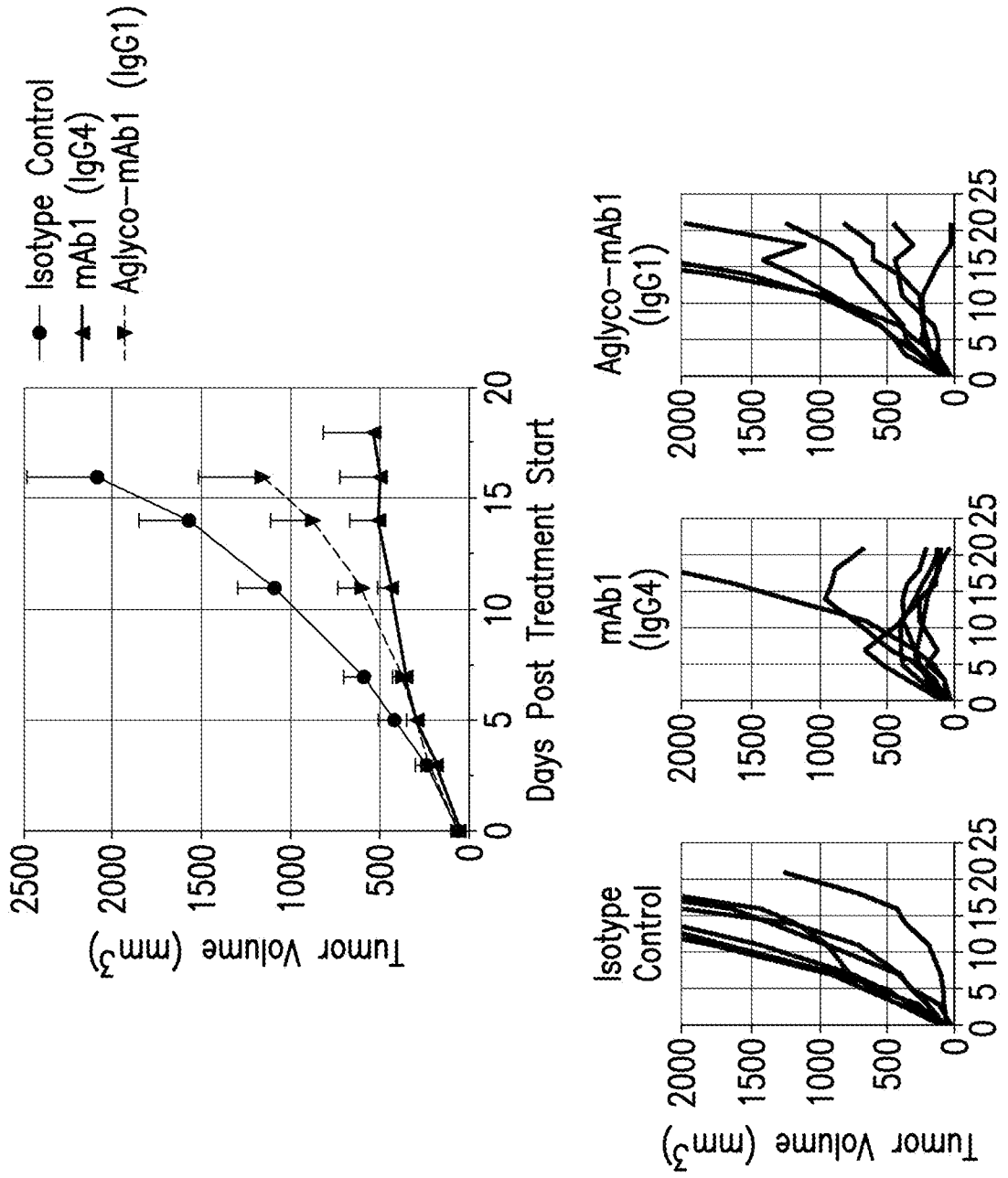

Isotype Control
mAb1 (IgG4)
Aglyco−mAb1 (IgG1)
Tumor Volume (mm3)
Days Post Treatment Start
Isotype Control
mAb1 (IgG4)
Aglyco−mAb1 (IgG1)

FIG.13B

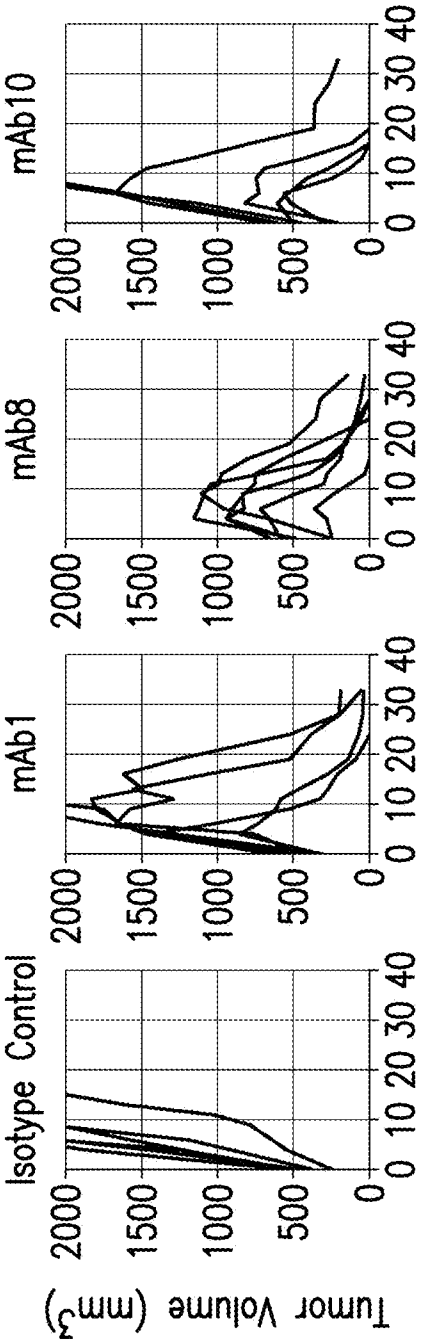


FIG.14A

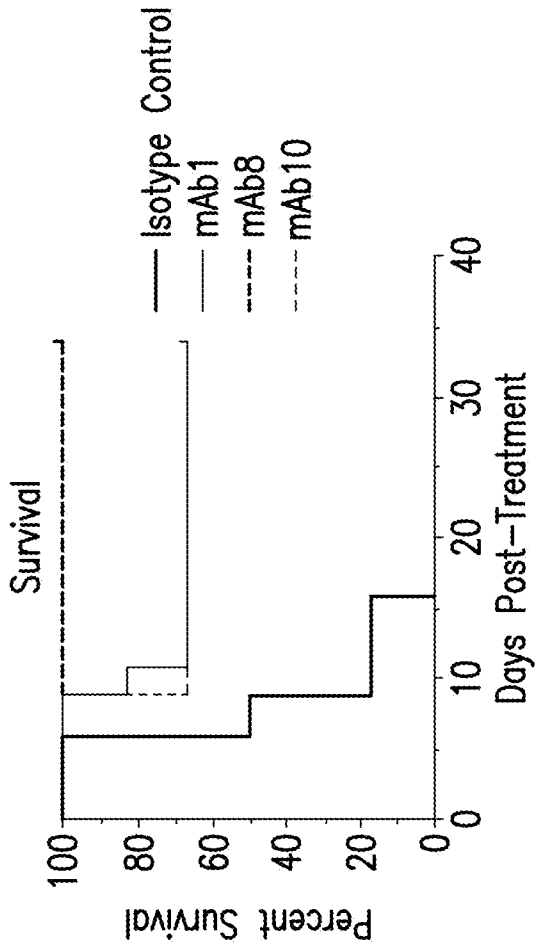


FIG.14C

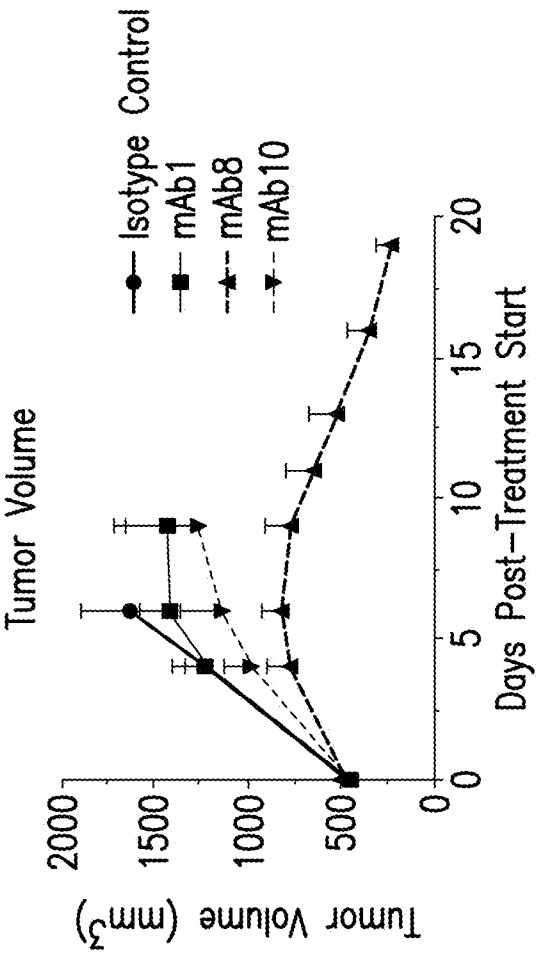


FIG.14B

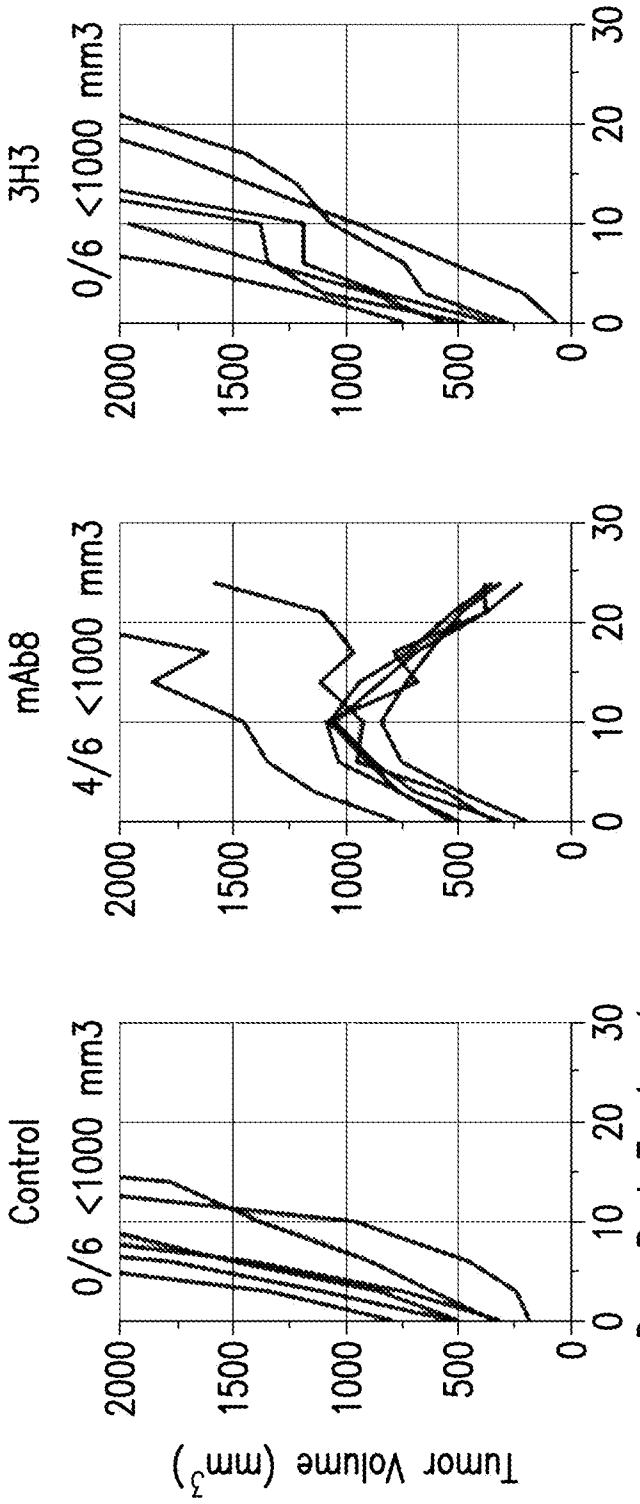

Control
0/6 <1000 mm3
mAb8
4/6 <1000 mm3
3H3
0/6 <1000 mm3
Tumor Volume (mm3)
2000
1500
1000
500
0
0
10
20
30
Days Post-Treatment

FIG.14D

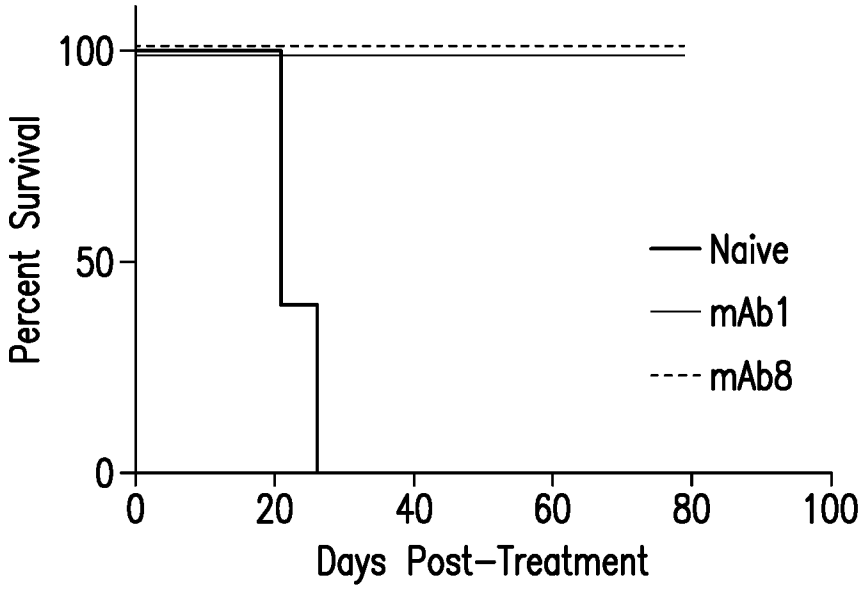

100
50
0
Percent Survival
0
20
40
60
80
100
Days Post-Treatment
Naive
mAb1
mAb8

FIG.15

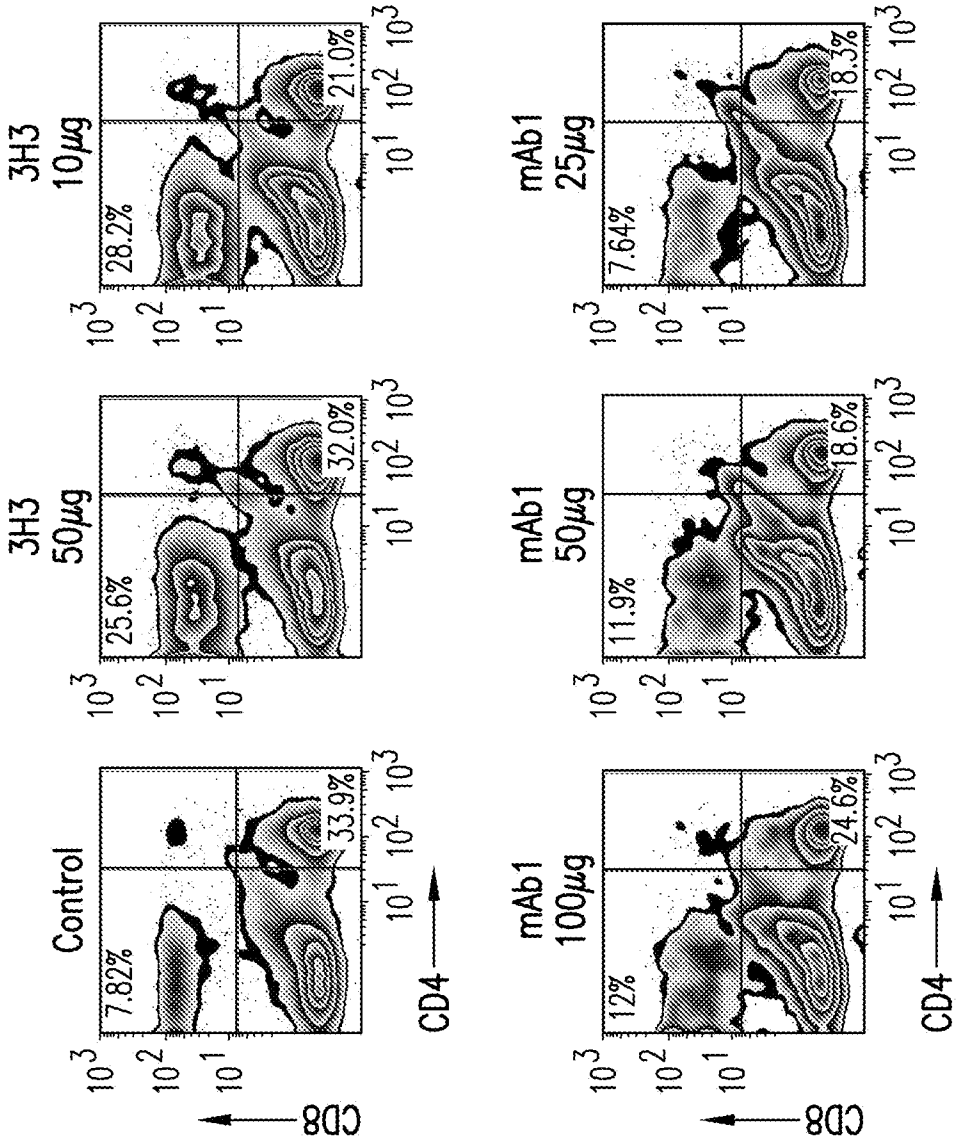

Control
7.82%
33.9%
3H3
50μg
25.6%
32.0%
3H3
10μg
28.2%
21.0%
mAb1
100μg
12%
24.6%
mAb1
50μg
11.9%
18.6%
mAb1
25μg
7.64%
18.3%
CD8
CD4

FIG.16A

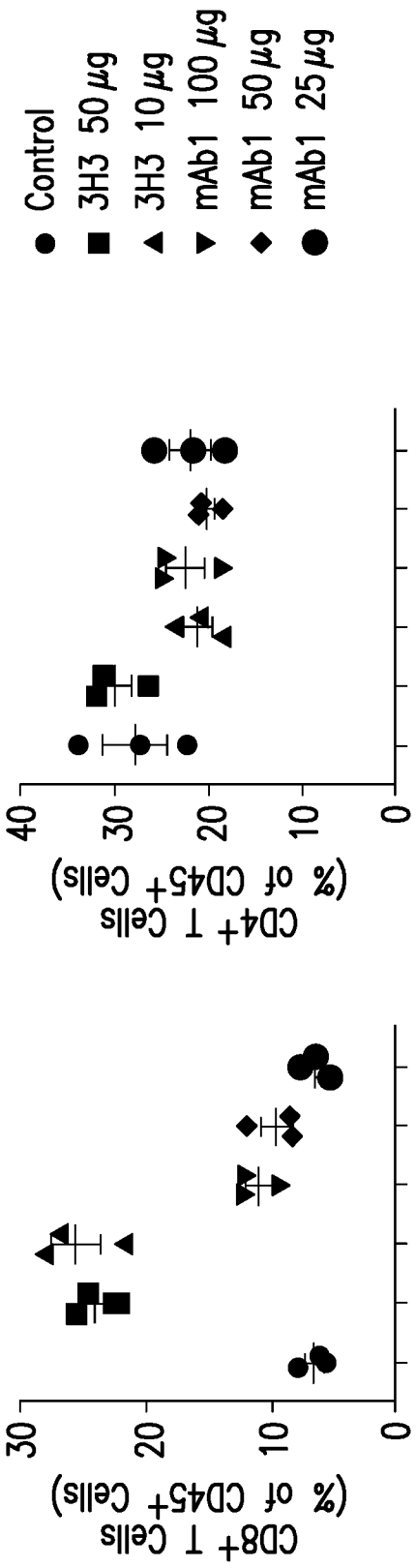

Control
3H3 50 μg
3H3 10 μg
mAb1 100 μg
mAb1 50 μg
mAb1 25 μg
CD8+ T Cells
(% of CD45+ Cells)
0
10
20
30
CD4+ T Cells
(% of CD45+ Cells)
0
10
20
30
40

FIG.16B

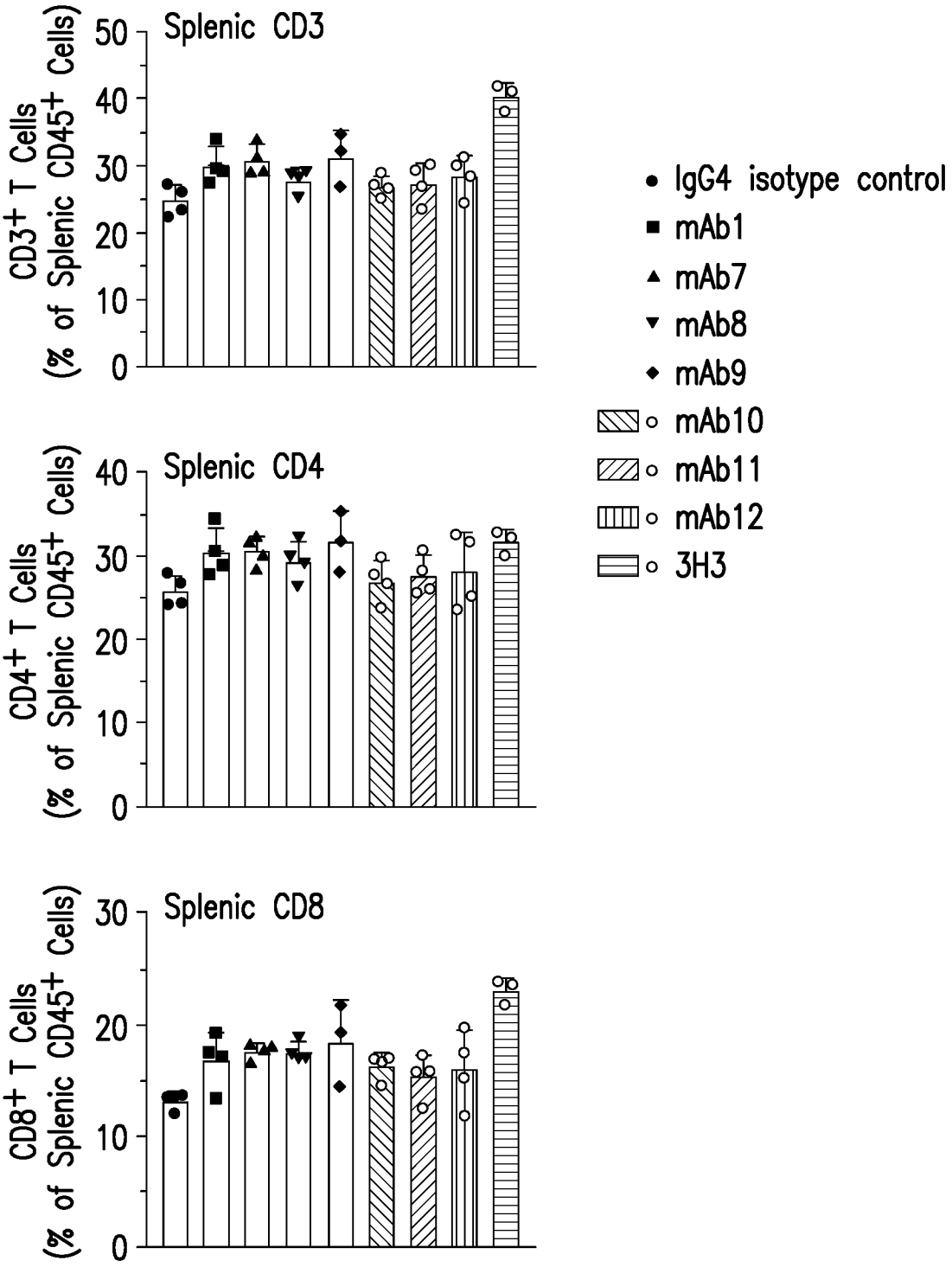

Splenic CD3
CD3+ T Cells (% of Splenic CD45+ Cells)
50
40
30
20
10
0
Splenic CD4
CD4+ T Cells (% of Splenic CD45+ Cells)
40
30
20
10
0
Splenic CD8
CD8+ T Cells (% of Splenic CD45+ Cells)
30
20
10
0
IgG4 isotype control
mAb1
mAb7
mAb8
mAb9
mAb10
mAb11
mAb12
3H3

FIG.17A

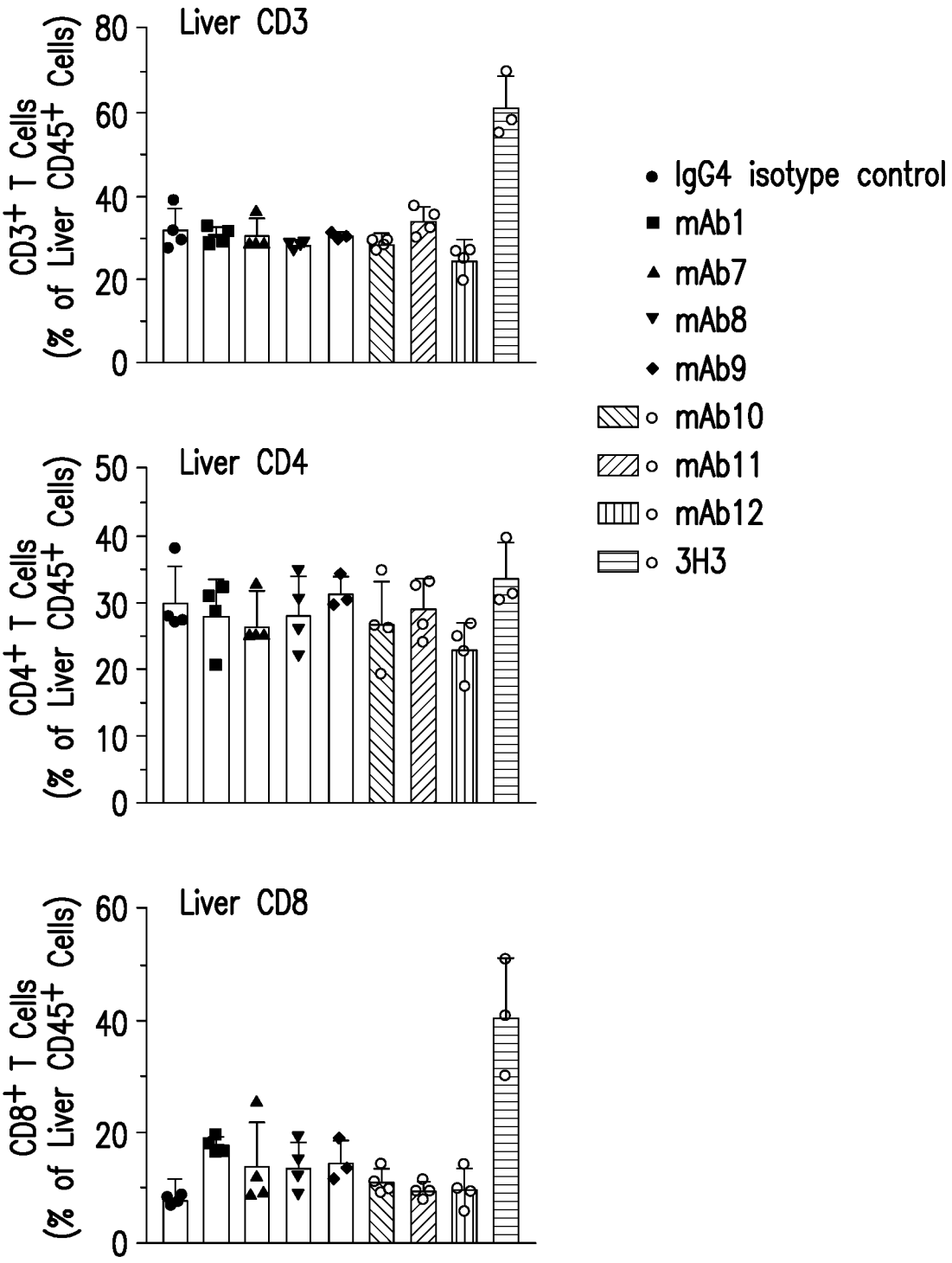

Liver CD3
CD3+ T Cells (% of Liver CD45+ Cells)
80
60
40
20
0
Liver CD4
CD4+ T Cells (% of Liver CD45+ Cells)
50
40
30
20
10
0
Liver CD8
CD8+ T Cells (% of Liver CD45+ Cells)
60
40
20
0
IgG4 isotype control
mAb1
mAb7
mAb8
mAb9
mAb10
mAb11
mAb12
3H3

FIG.17B

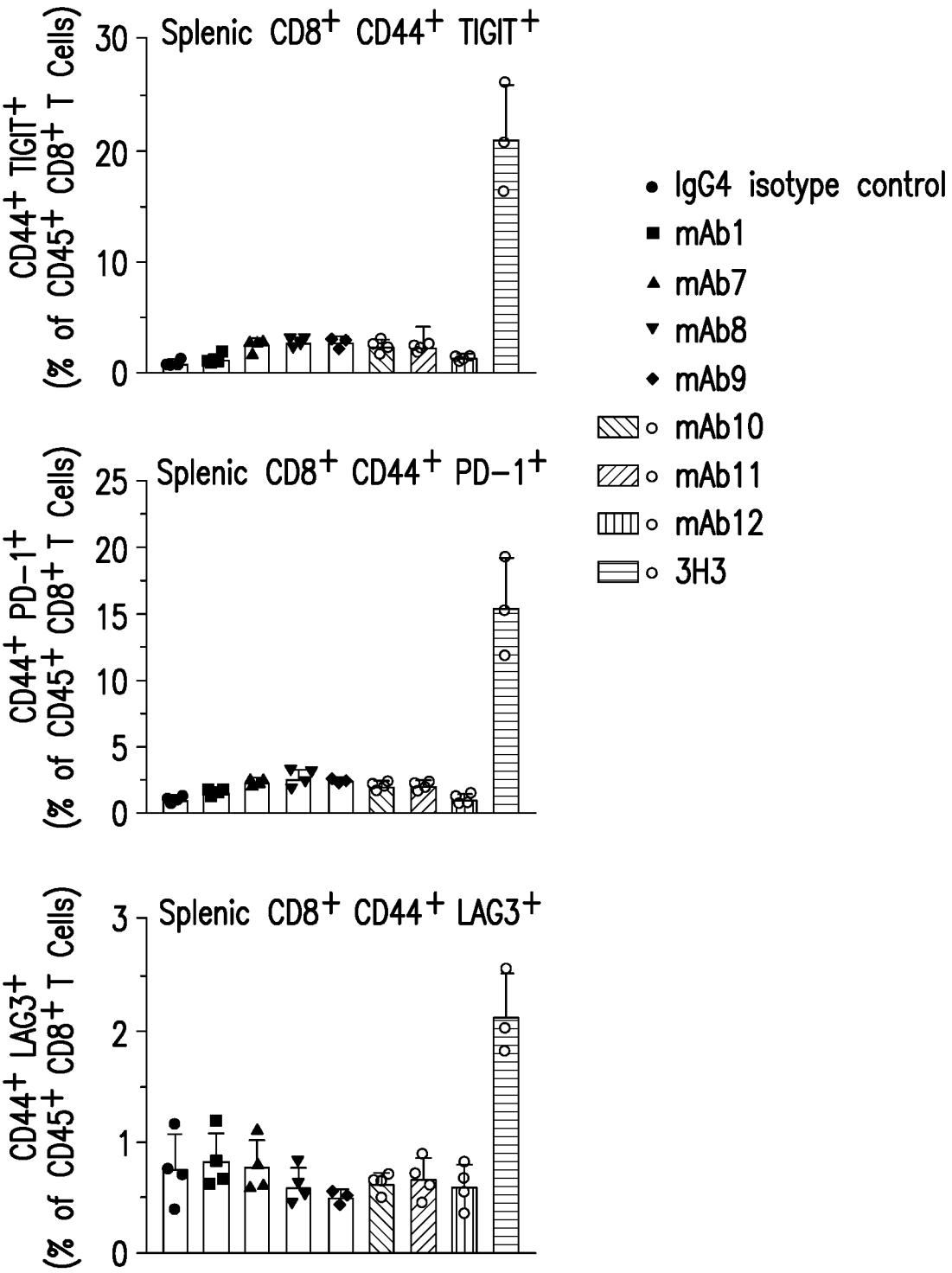

Splenic CD8+ CD44+ TIGIT+
CD44+ TIGIT+ (% of CD45+ CD8+ T Cells)
30
20
10
0
Splenic CD8+ CD44+ PD-1+
CD44+ PD-1+ (% of CD45+ CD8+ T Cells)
25
20
15
10
5
0
Splenic CD8+ CD44+ LAG3+
CD44+ LAG3+ (% of CD45+ CD8+ T Cells)
3
2
1
0
IgG4 isotype control
mAb1
mAb7
mAb8
mAb9
mAb10
mAb11
mAb12
3H3

FIG.18A

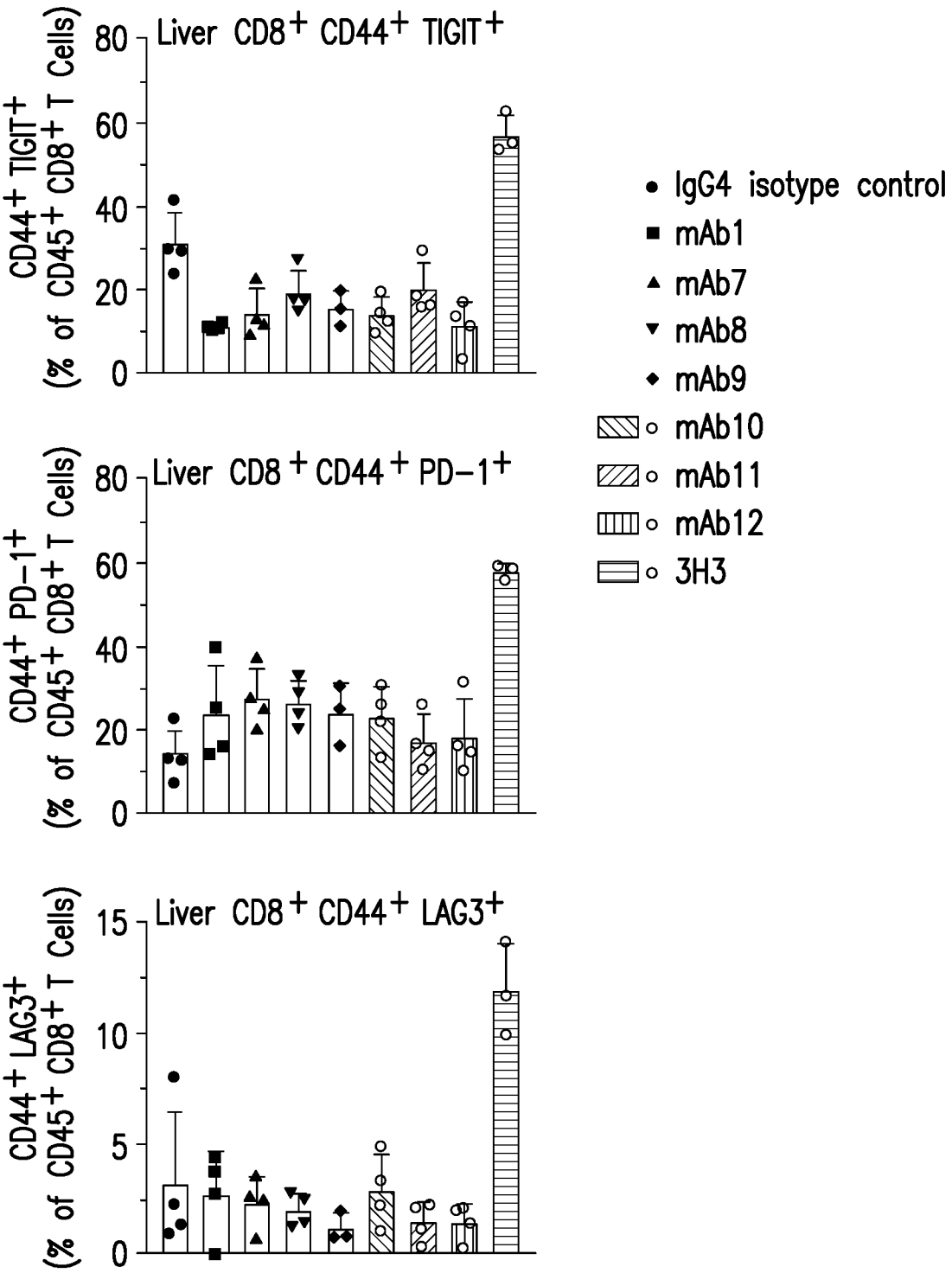

Liver CD8+ CD44+ TIGIT+
CD44+ TIGIT+ (% of CD45+ CD8+ T Cells)
80
60
40
20
0
Liver CD8+ CD44+ PD-1+
CD44+ PD-1+ (% of CD45+ CD8+ T Cells)
80
60
40
20
0
Liver CD8+ CD44+ LAG3+
CD44+ LAG3+ (% of CD45+ CD8+ T Cells)
15
10
5
0
IgG4 isotype control
mAb1
mAb7
mAb8
mAb9
mAb10
mAb11
mAb12
3H3

FIG.18B

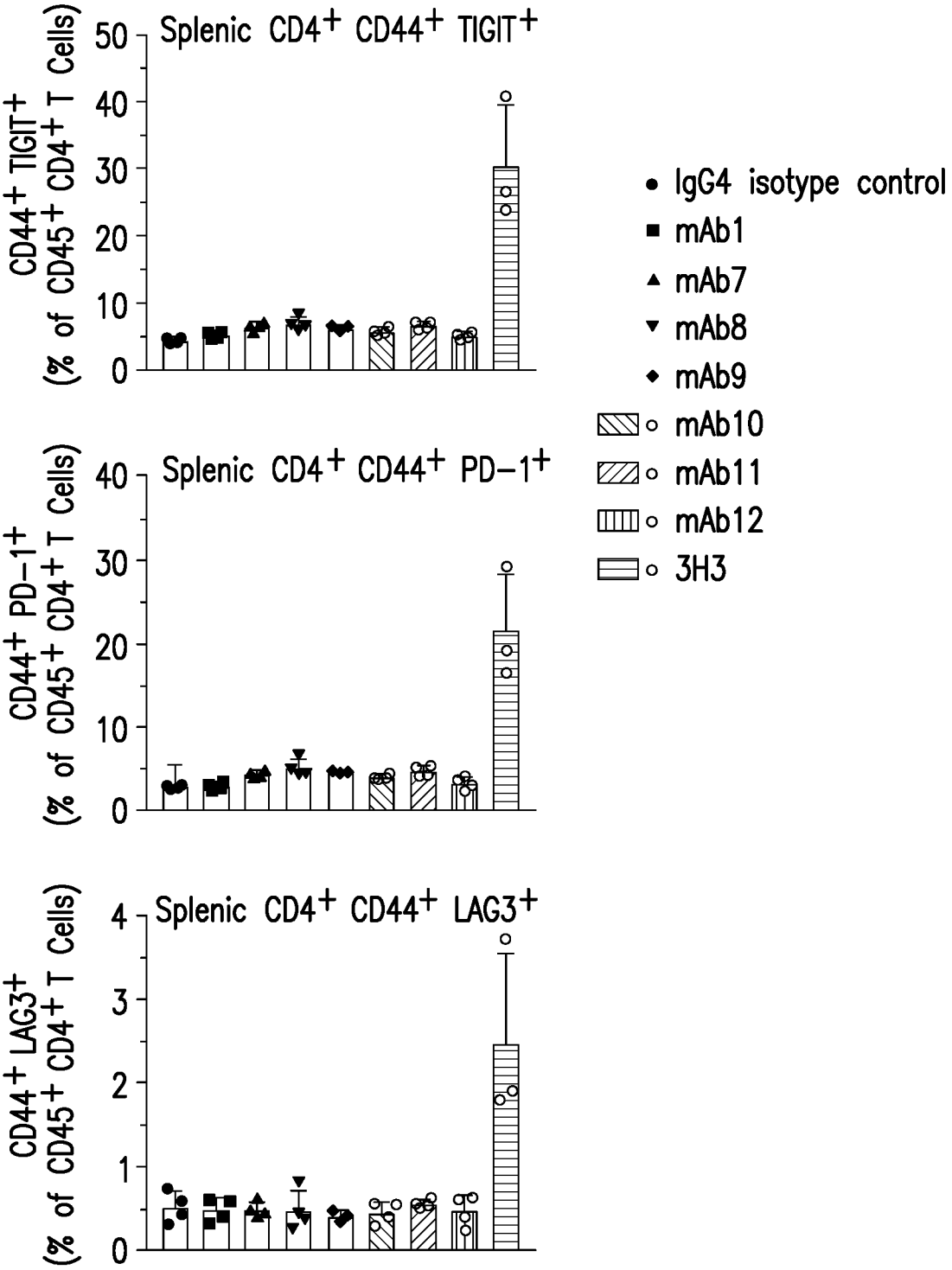

Splenic CD4+ CD44+ TIGIT+
CD44+ TIGIT+ (% of CD45+ CD4+ T Cells)
50
40
30
20
10
0
Splenic CD4+ CD44+ PD-1+
CD44+ PD-1+ (% of CD45+ CD4+ T Cells)
40
30
20
10
0
Splenic CD4+ CD44+ LAG3+
CD44+ LAG3+ (% of CD45+ CD4+ T Cells)
4
3
2
1
0
IgG4 isotype control
mAb1
mAb7
mAb8
mAb9
mAb10
mAb11
mAb12
3H3

FIG.19A

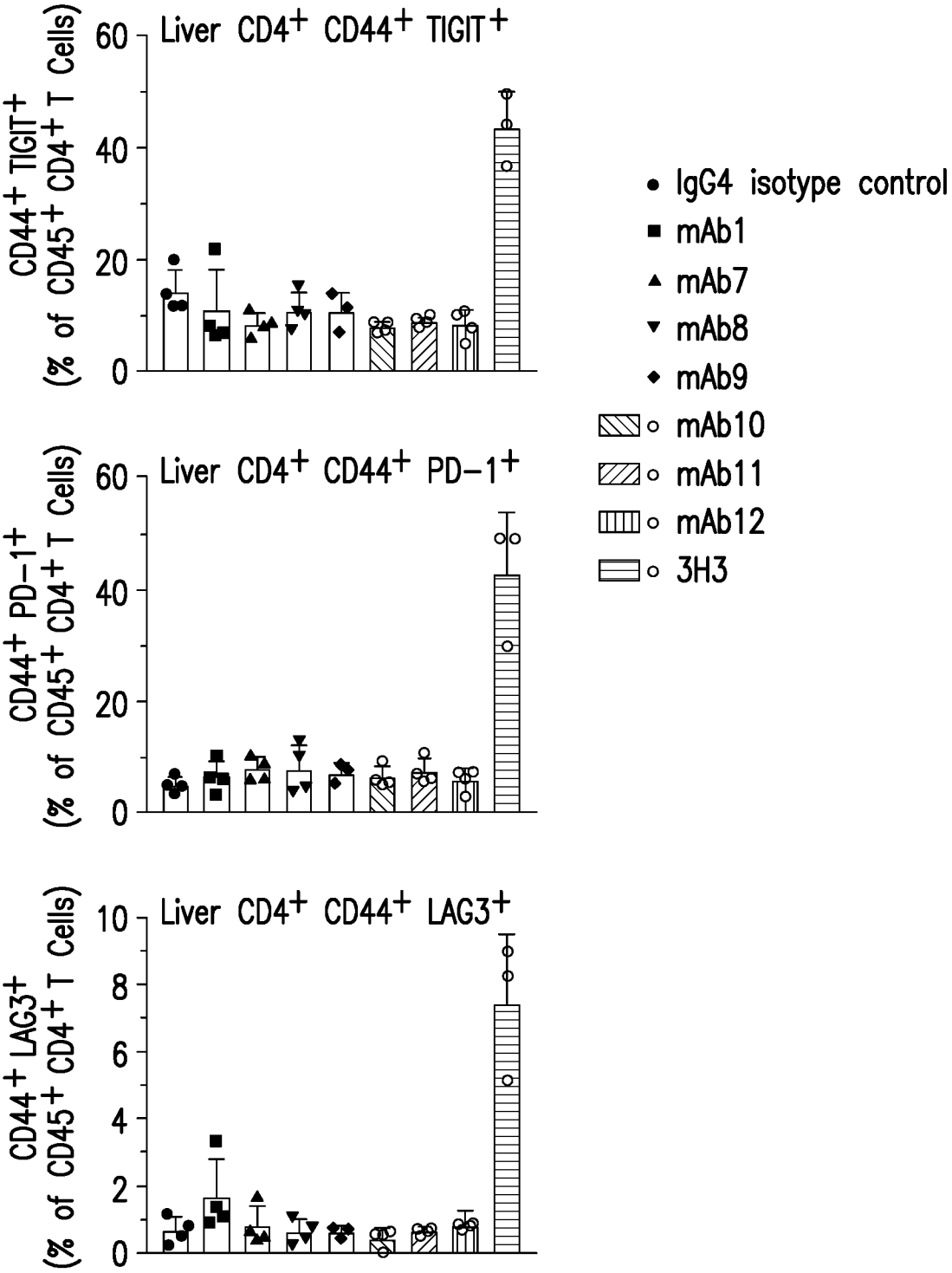

Liver CD4+ CD44+ TIGIT+
CD44+ TIGIT+ (% of CD45+ CD4+ T Cells)
60
40
20
0
Liver CD4+ CD44+ PD-1+
CD44+ PD-1+ (% of CD45+ CD4+ T Cells)
60
40
20
0
Liver CD4+ CD44+ LAG3+
CD44+ LAG3+ (% of CD45+ CD4+ T Cells)
10
8
6
4
2
0
IgG4 isotype control
mAb1
mAb7
mAb8
mAb9
mAb10
mAb11
mAb12
3H3

FIG.19B

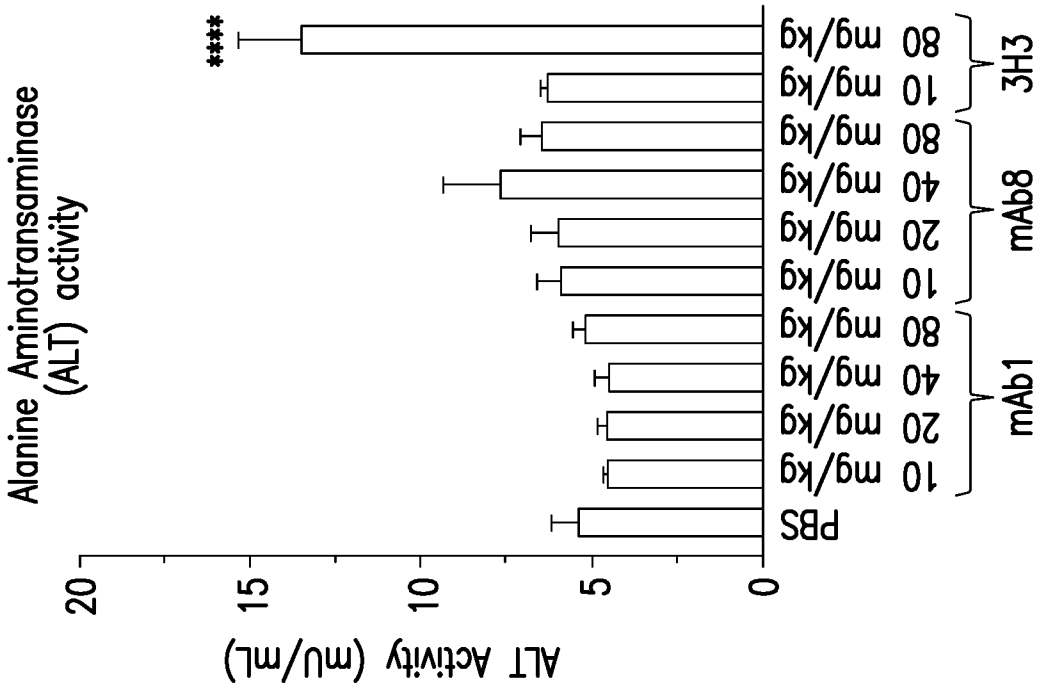


FIG.20B

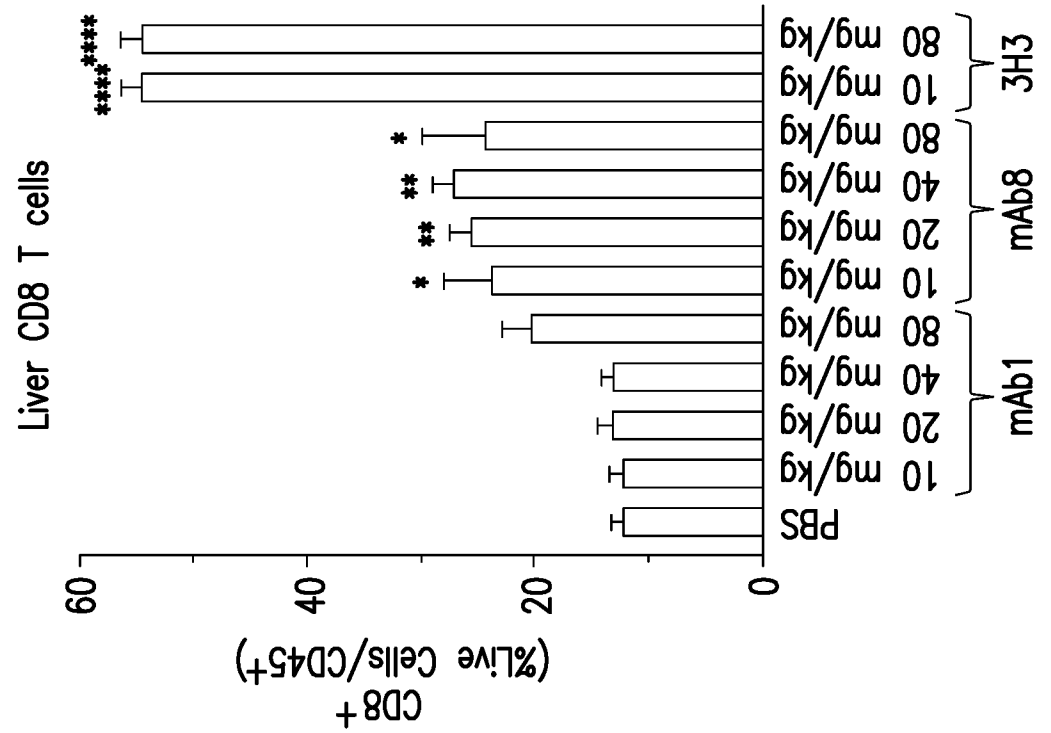


FIG.20A

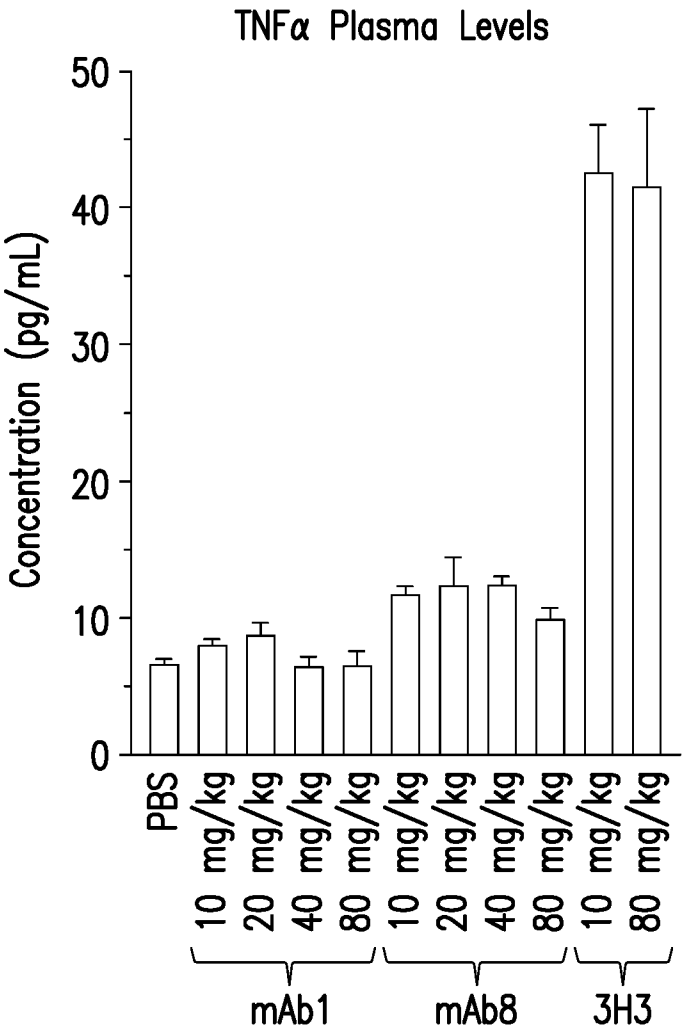

TNFα Plasma Levels
Concentration (pg/mL)
50
40
30
20
10
0
PBS
10 mg/kg
20 mg/kg
40 mg/kg
80 mg/kg
10 mg/kg
20 mg/kg
40 mg/kg
80 mg/kg
10 mg/kg
80 mg/kg
mAb1
mAb8
3H3

FIG.20C

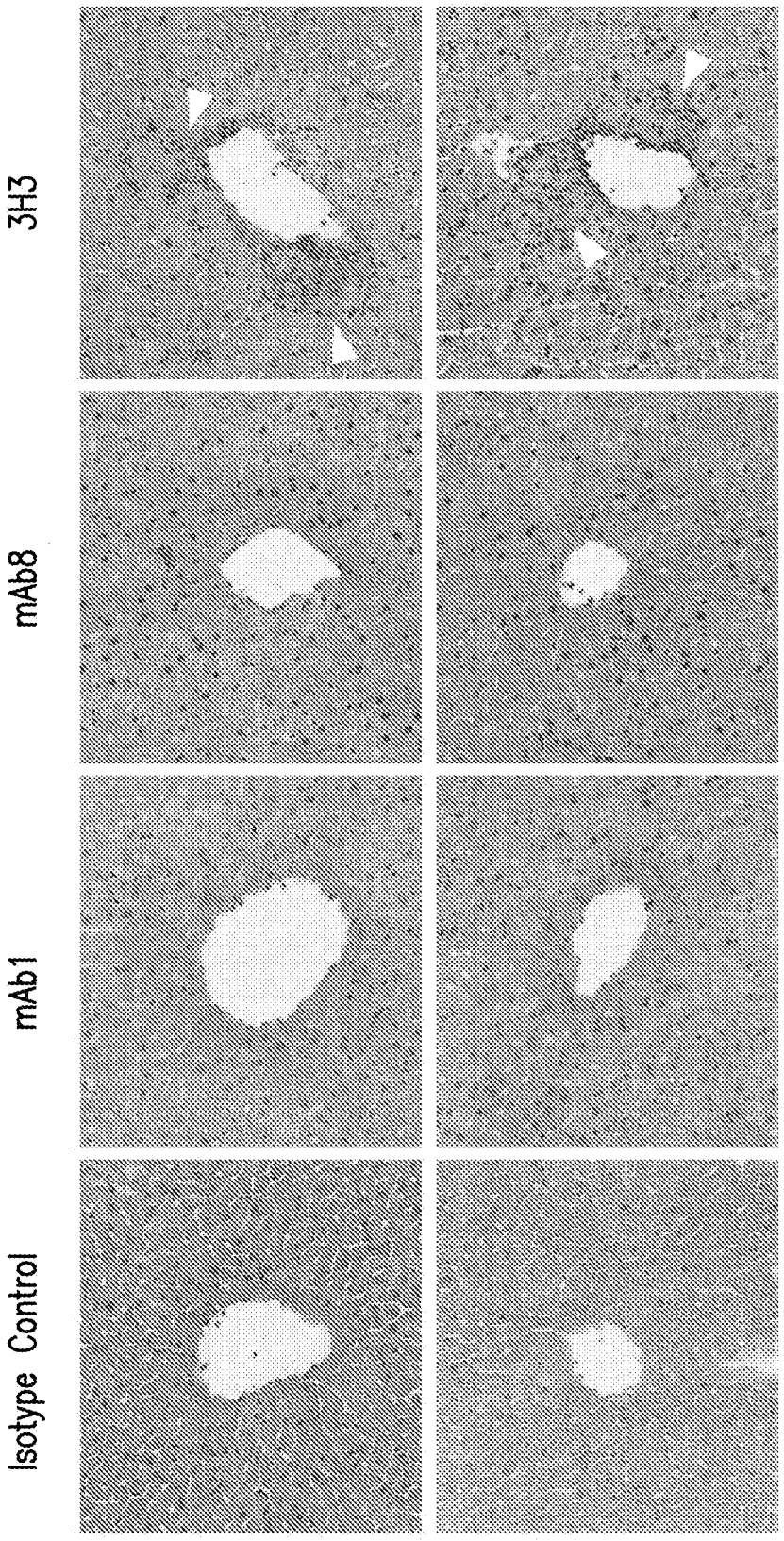


FIG.21

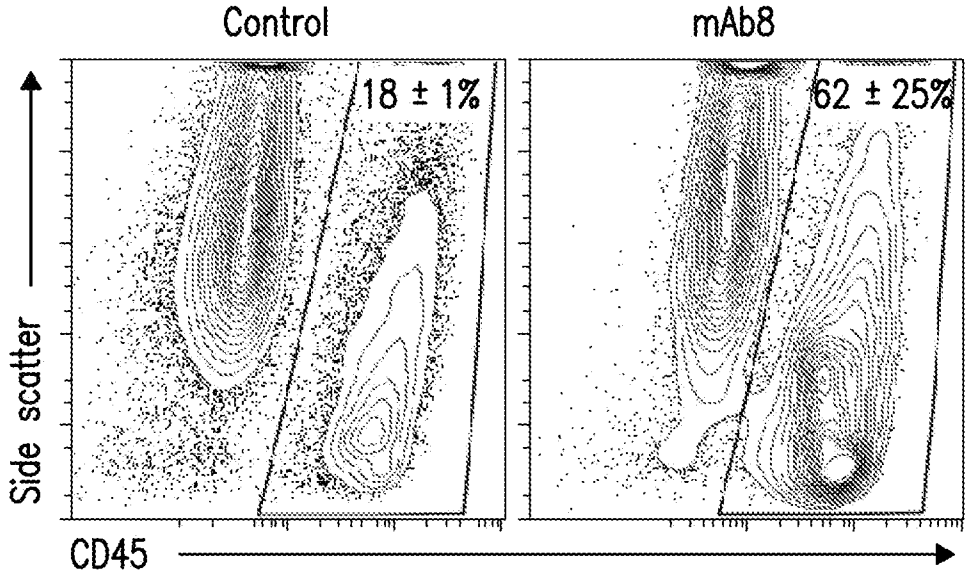


FIG.22A

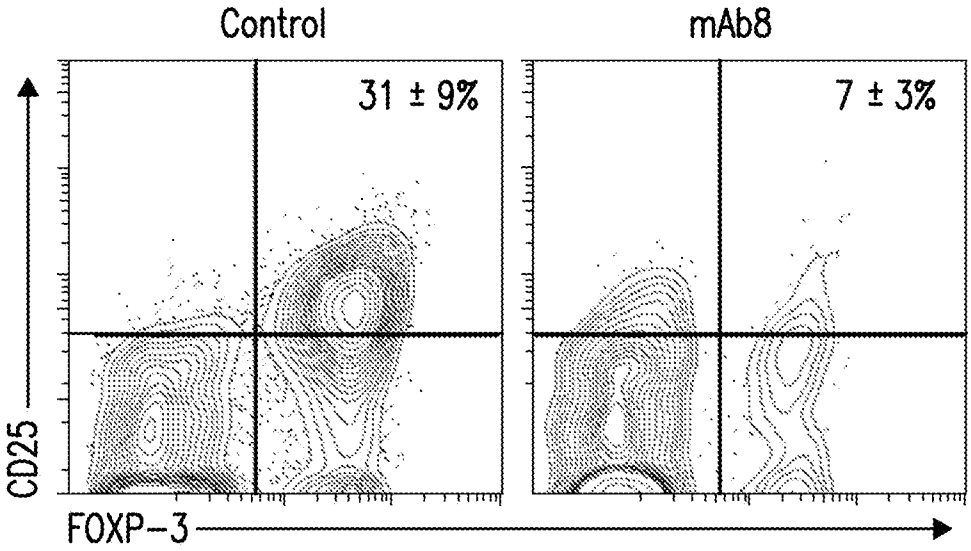


FIG.22B

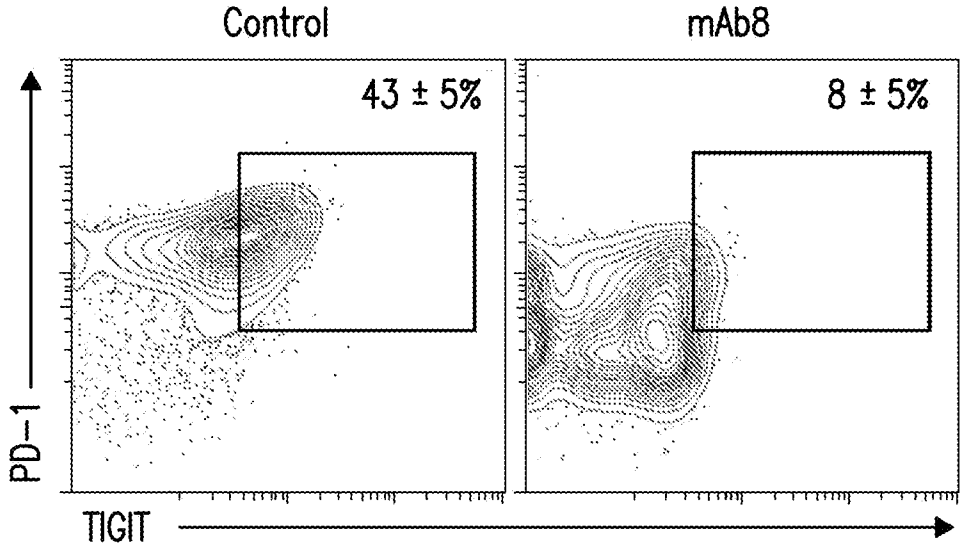


FIG.22C

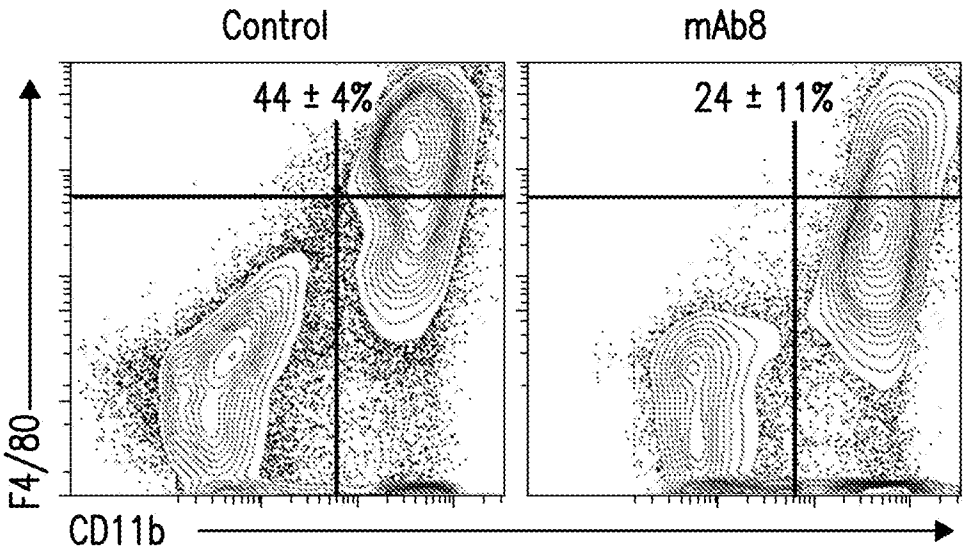


FIG.22D

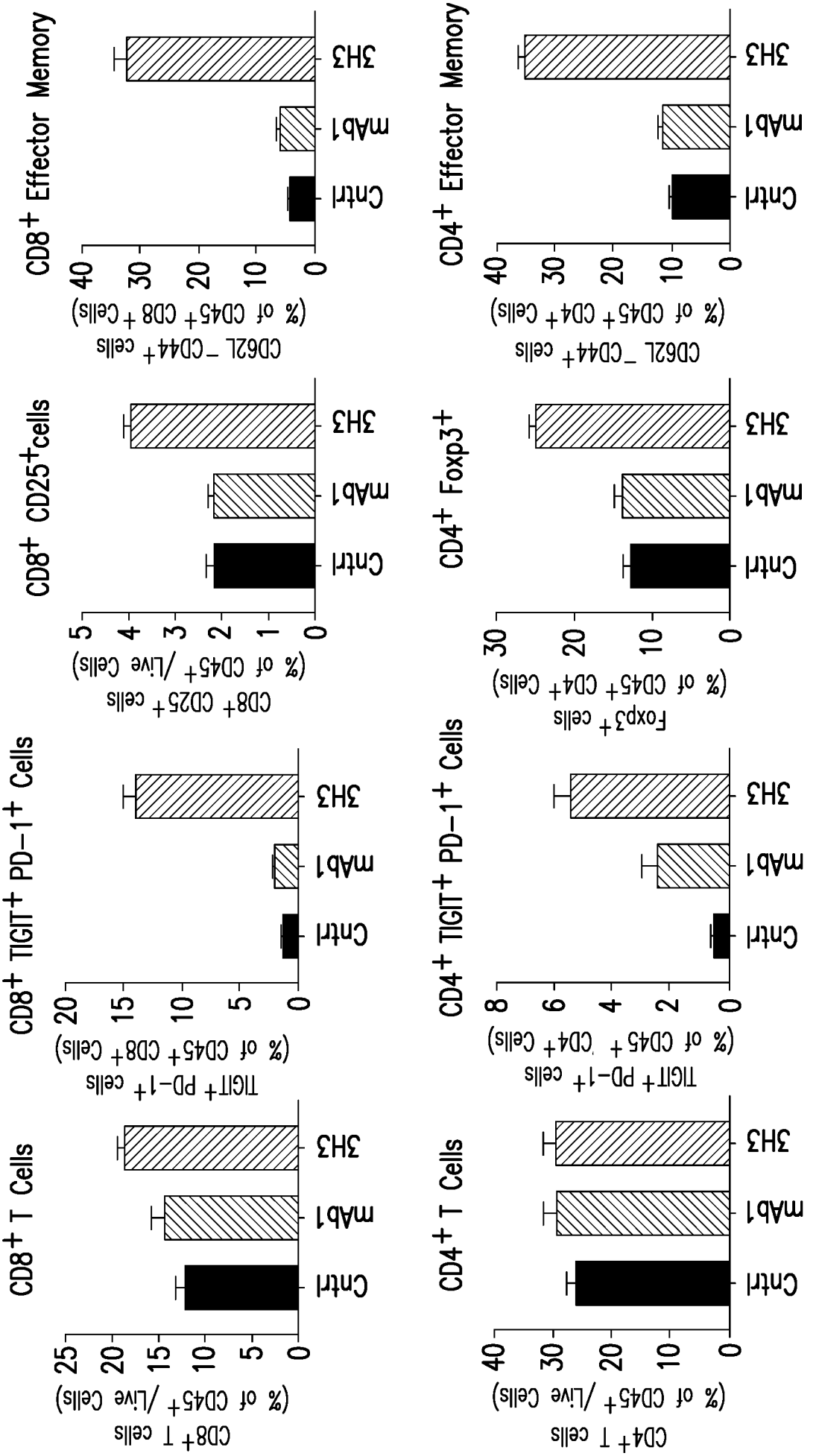

CD8+ T Cells
CD8+ T cells (% of CD45+ /Live Cells)
TIGIT+ PD-1+ cells (% of CD45+ CD8+ Cells)
CD8+ TIGIT+ PD-1+ Cells
CD8+ CD25+cells
CD8+ CD25+ cells (% of CD45+ /Live Cells)
CD62L −CD44+ cells (% of CD45+ CD8 + Cells)
CD8+ Effector Memory
CD4+ T Cells
CD4+ T cells (% of CD45+ /Live Cells)
TIGIT+ PD-1+ cells (% of CD45+ CD4+ Cells)
CD4+ TIGIT+ PD-1+ Cells
CD4+ Foxp3+
Foxp3+ cells (% of CD45+ CD4+ Cells)
CD62L −CD44+ cells (% of CD45+ CD4+ Cells)
CD4+ Effector Memory
Cntrl
mAb1
3H3

FIG.23

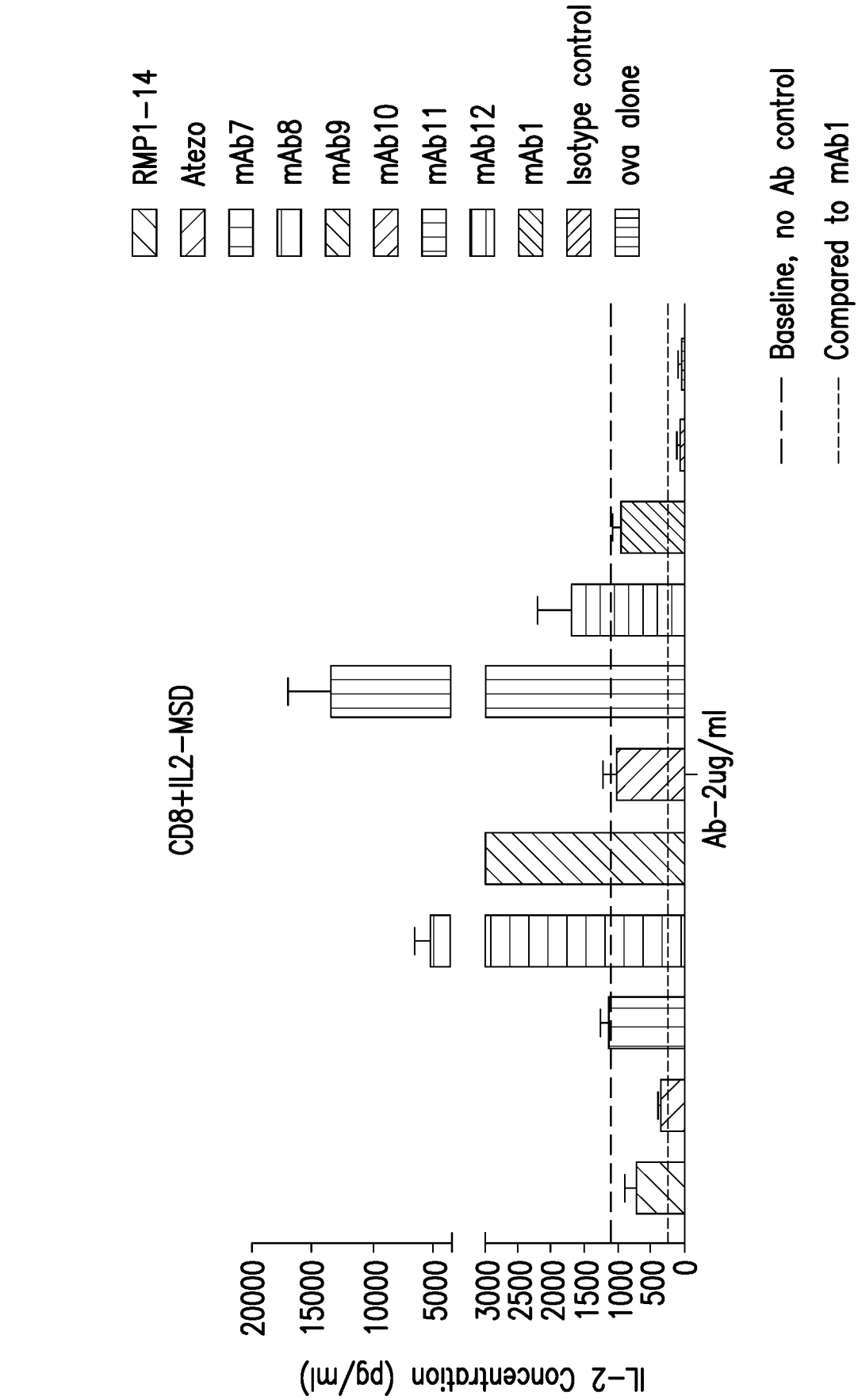

CD8+IL2-MSD
IL-2 Concentration (pg/ml)
20000
15000
10000
5000
3000
2500
2000
1500
1000
500
0
Ab-2ug/ml
RMP1-14
Atezo
mAb7
mAb8
mAb9
mAb10
mAb11
mAb12
mAb1
Isotype control
ova alone
Baseline, no Ab control
Compared to mAb1

FIG.24

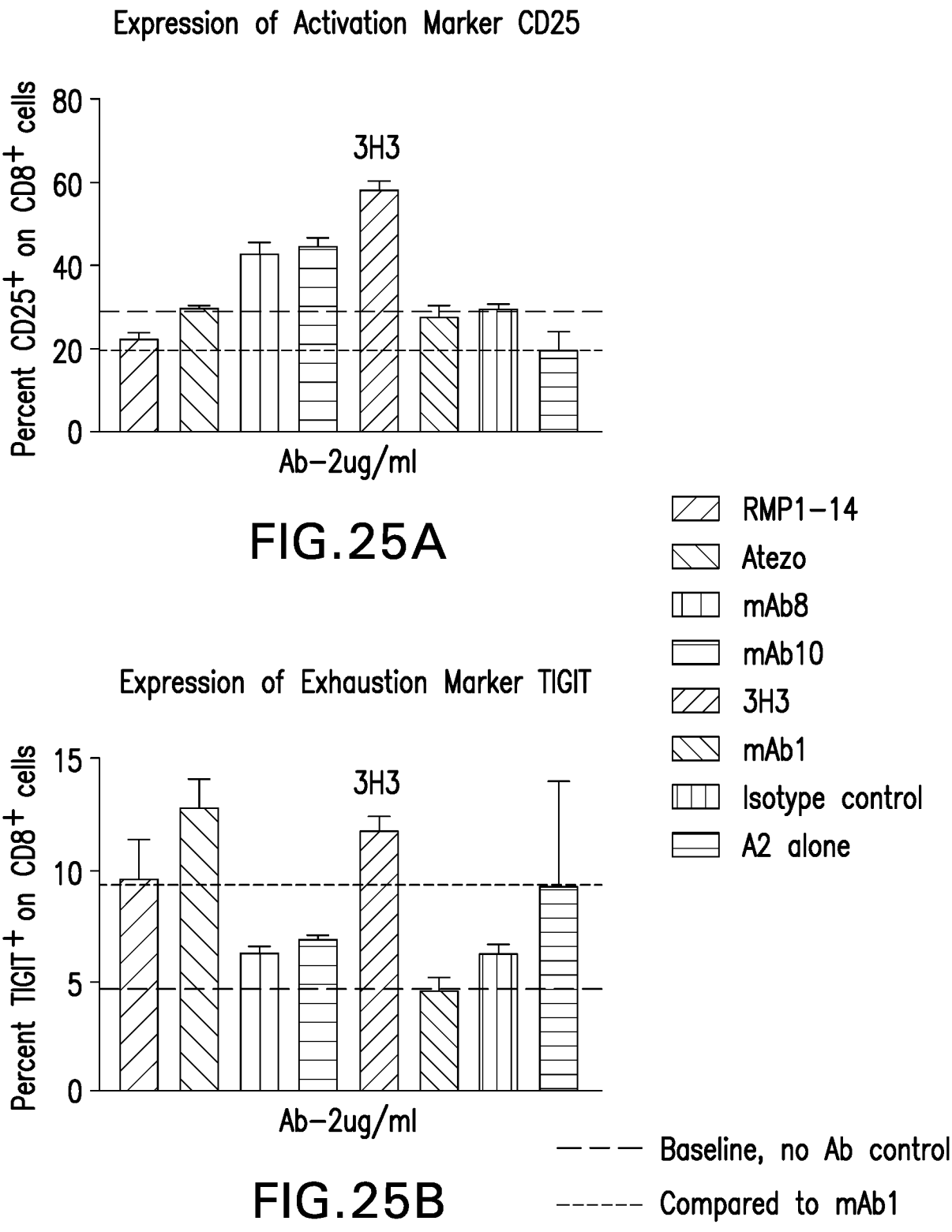


FIG.25A

FIG.25B

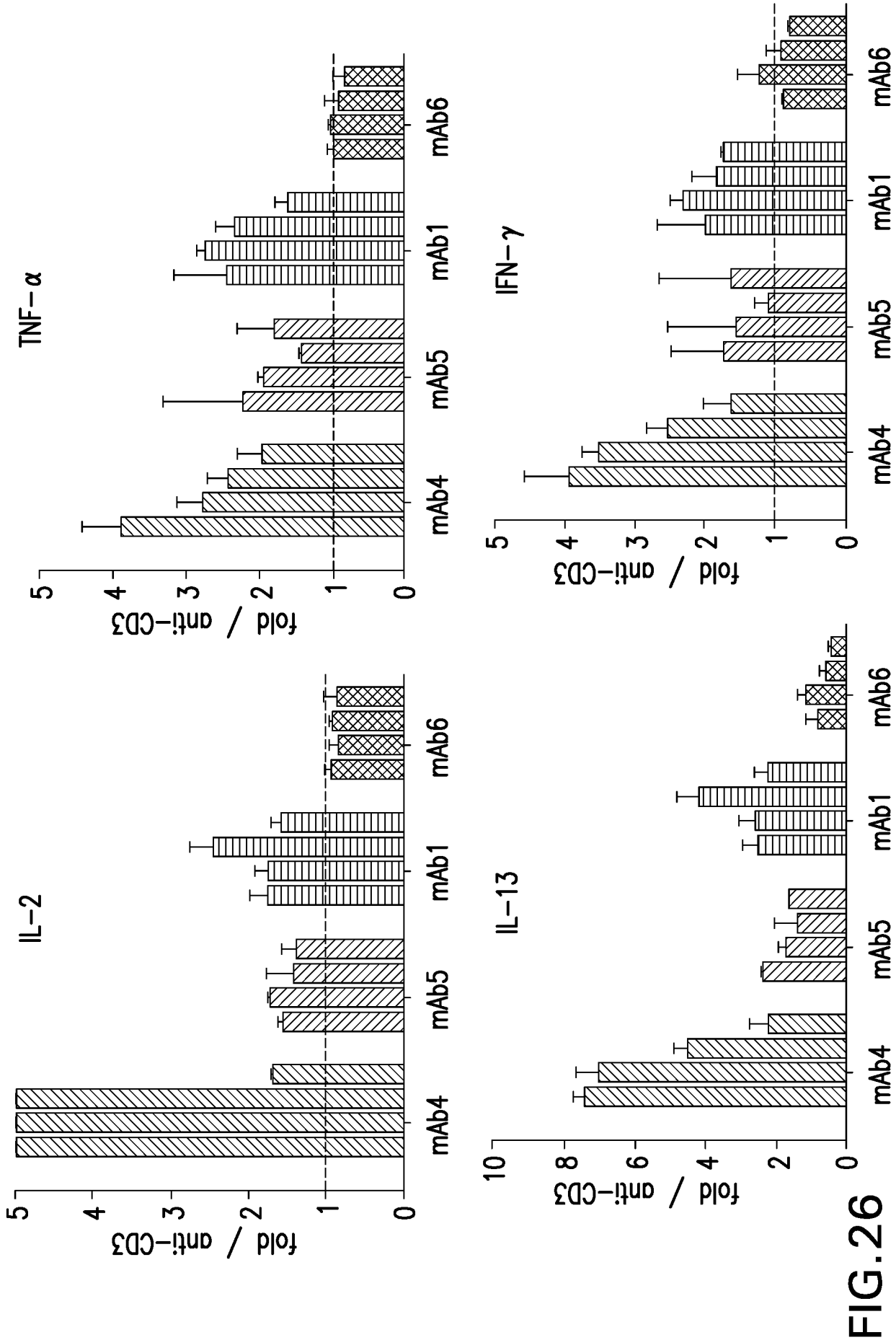
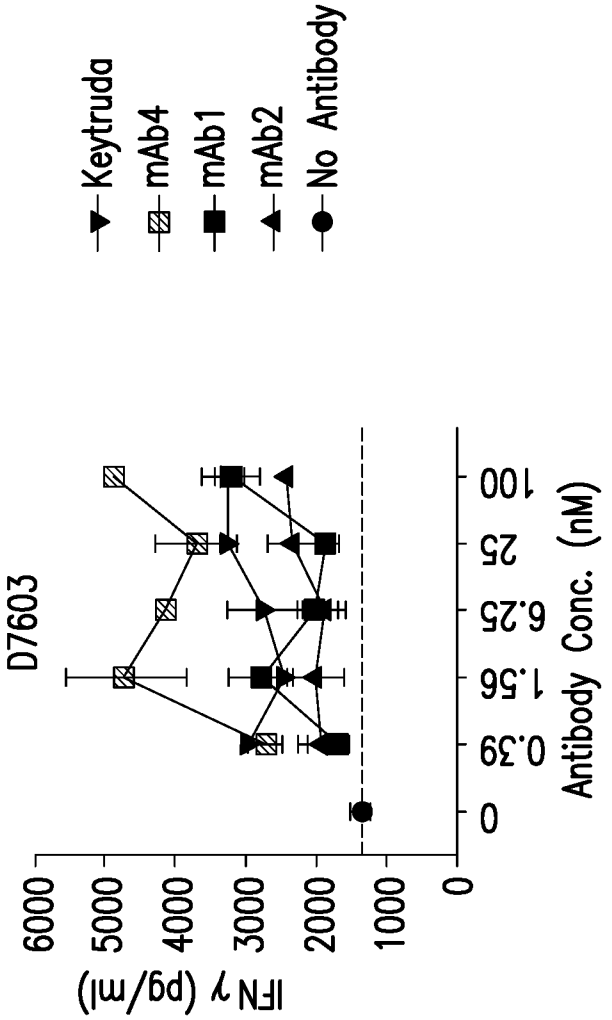

TNF-α
IFN-γ
IL-2
IL-13
fold / anti-CD3
0
1
2
3
4
5
6
8
10
mAb4
mAb5
mAb1
mAb6

FIG.26



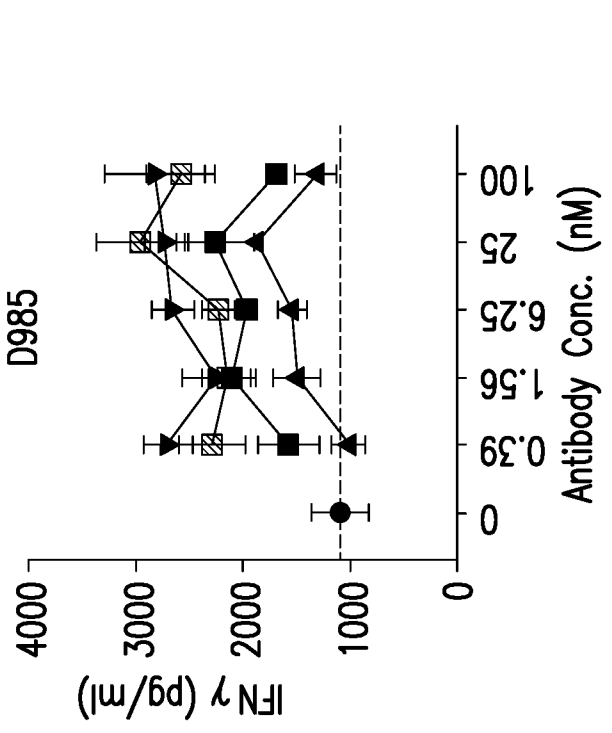
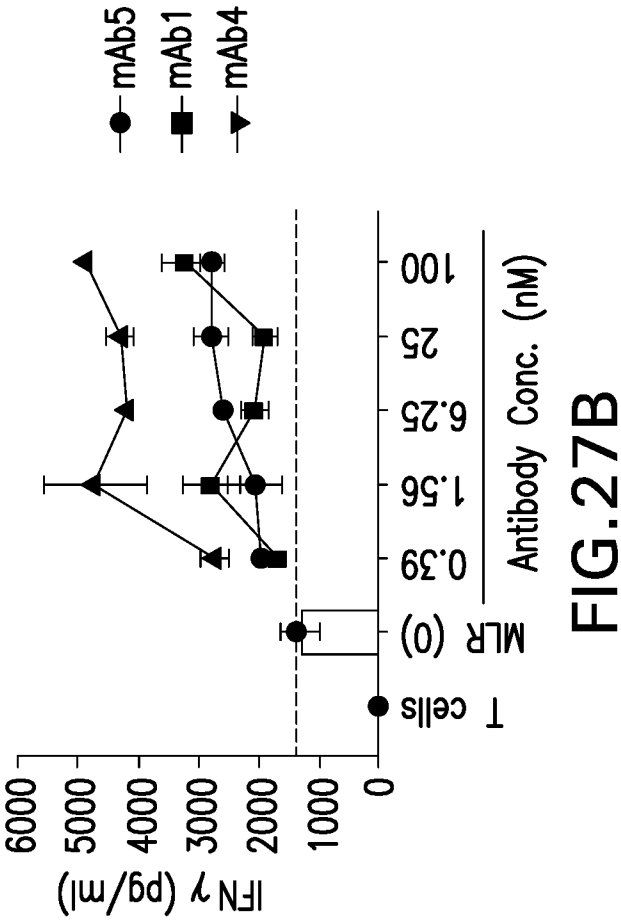


FIG.27A



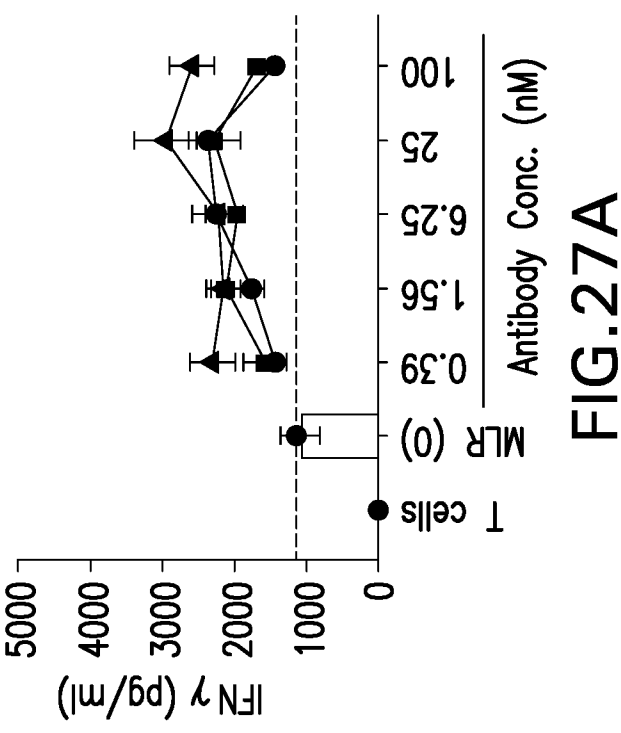


FIG.27B

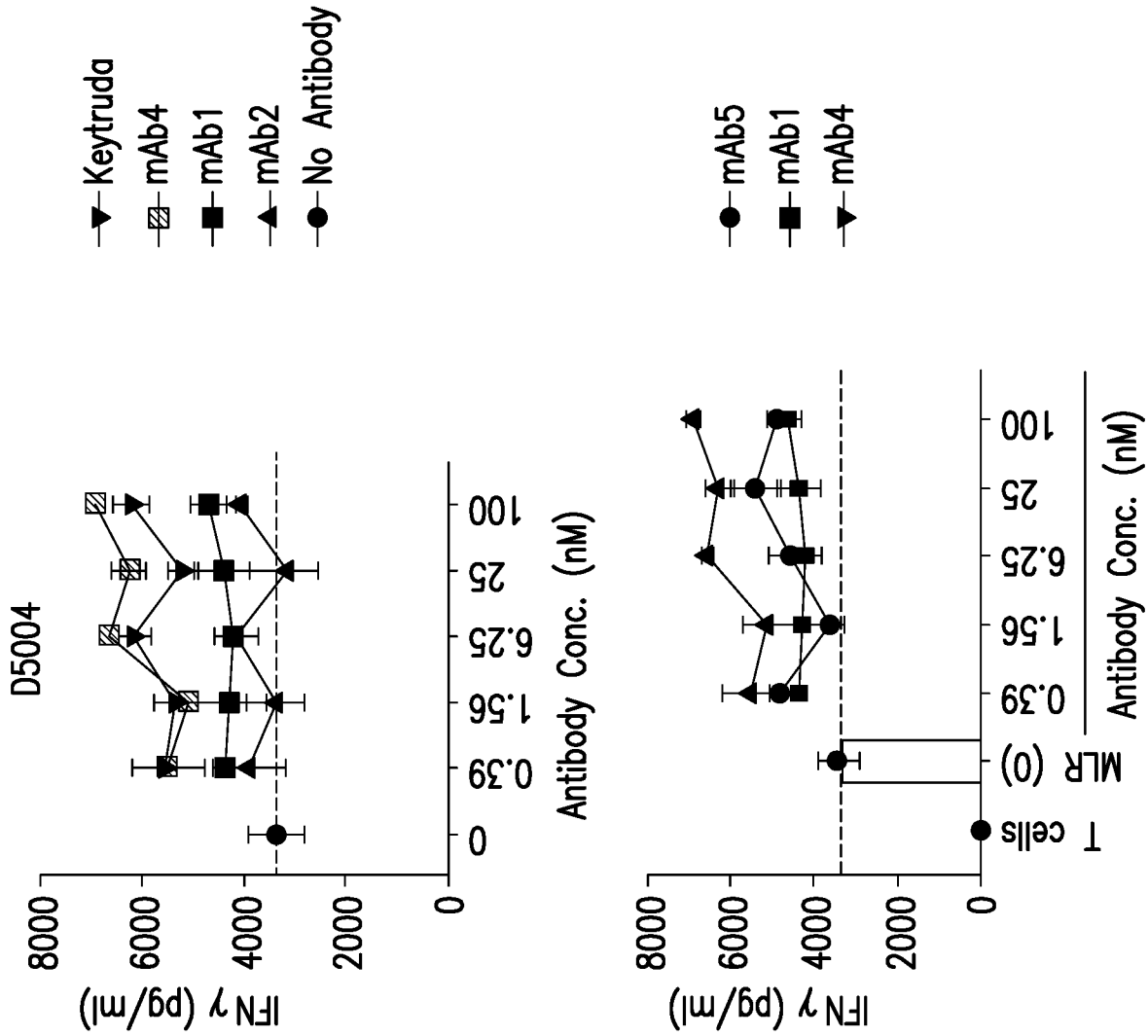

Keytruda
mAb4
mAb1
mAb2
No Antibody
D5004
IFN γ (pg/ml)
8000
6000
4000
2000
0
0
0.39
1.56
6.25
25
100
Antibody Conc. (nM)
mAb5
mAb1
mAb4
T cells
MLR (0)
0
0.39
1.56
6.25
25
100
Antibody Conc. (nM)

FIG.27C

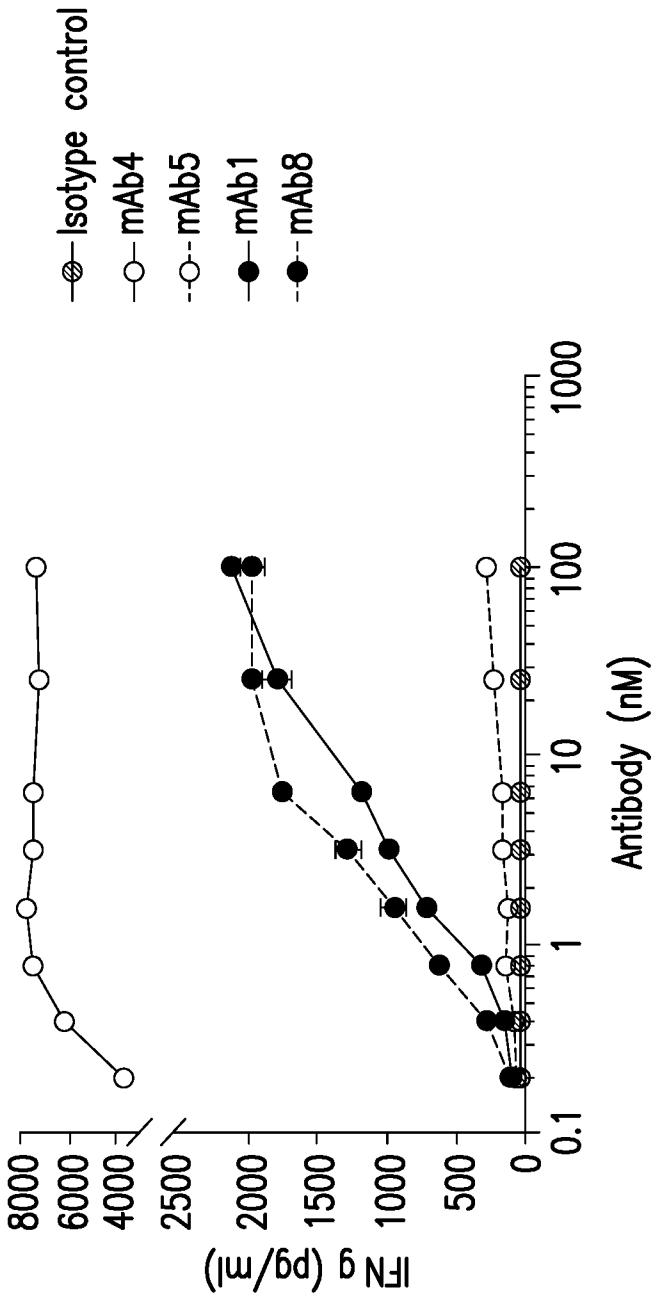

Isotype control
mAb4
mAb5
mAb1
mAb8
IFN g (pg/ml)
8000
6000
4000
2500
2000
1500
1000
500
0
0.1
1
10
100
1000
Antibody (nM)

FIG.28

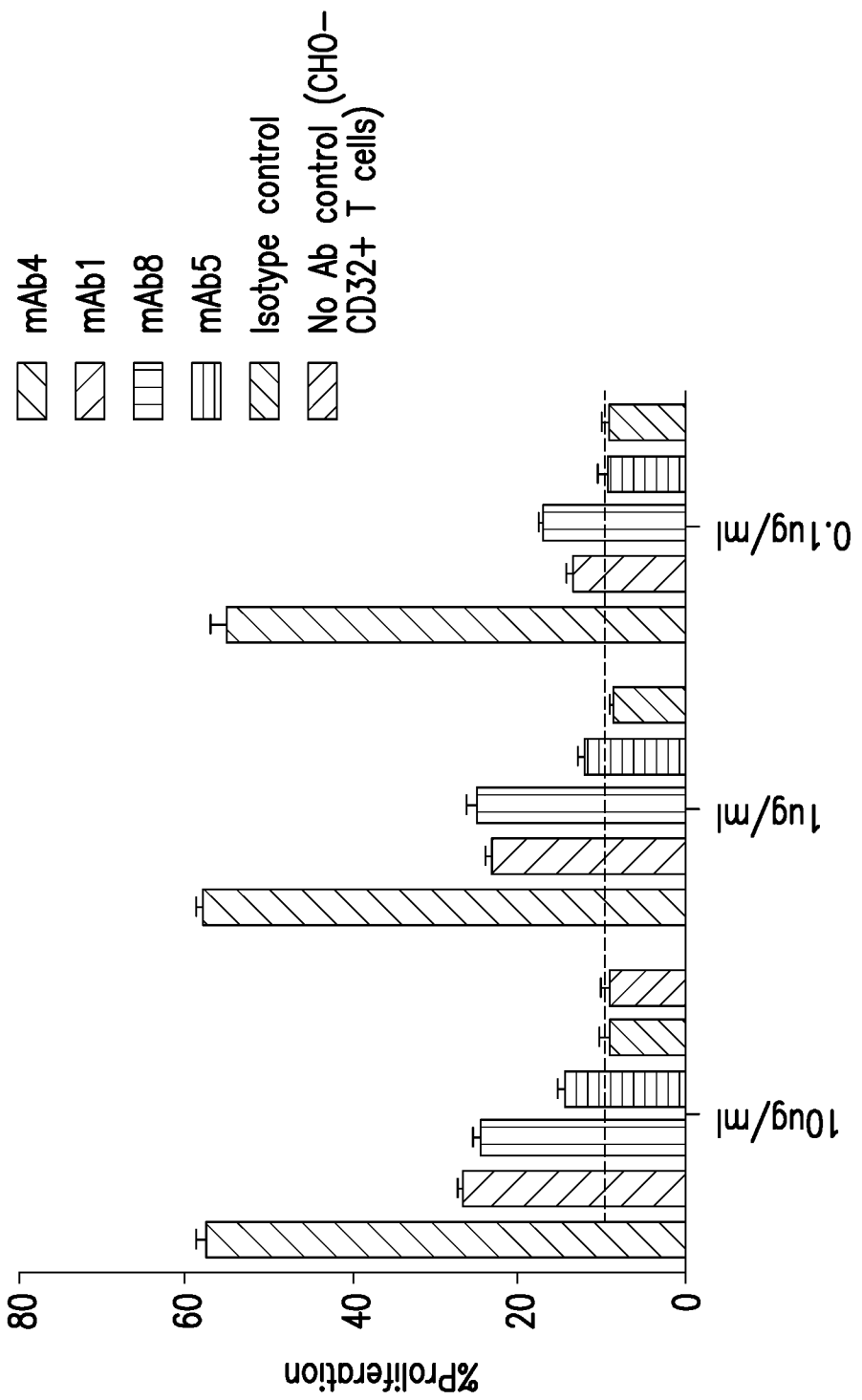

mAb4
mAb1
mAb8
mAb5
Isotype control
No Ab control (CHO-CD32+ T cells)
%Proliferation
0
20
40
60
80
10ug/ml
1ug/ml
0.1ug/ml

FIG.29

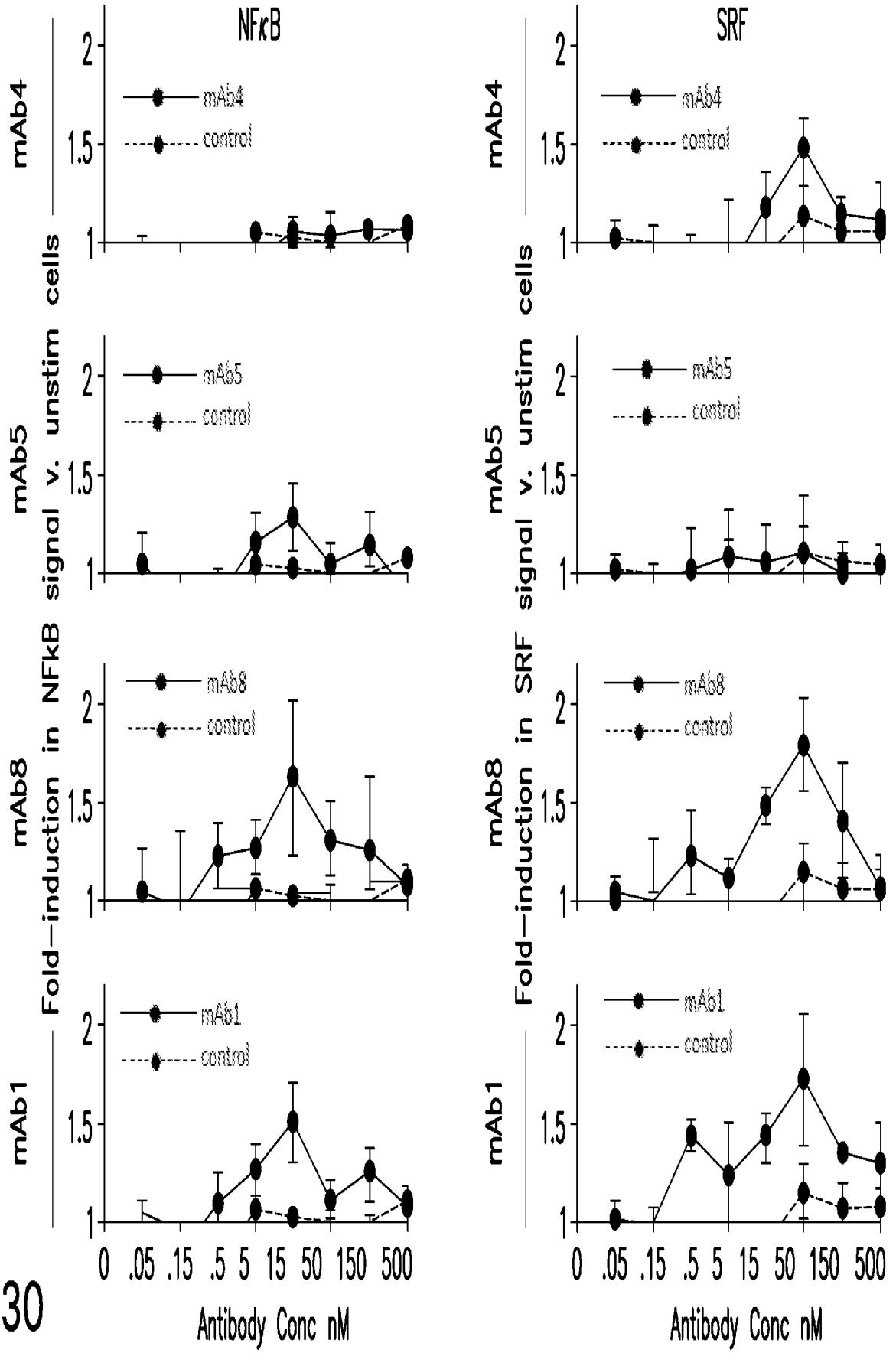

NFκB
SRF
mAb4
mAb5
mAb8
mAb1
control
Fold—induction in NFkB signal v. unstim cells
Fold—induction in SRF signal v. unstim cells
0 .05 .15 .5 5 15 50 150 500
Antibody Conc nM

FIG.30

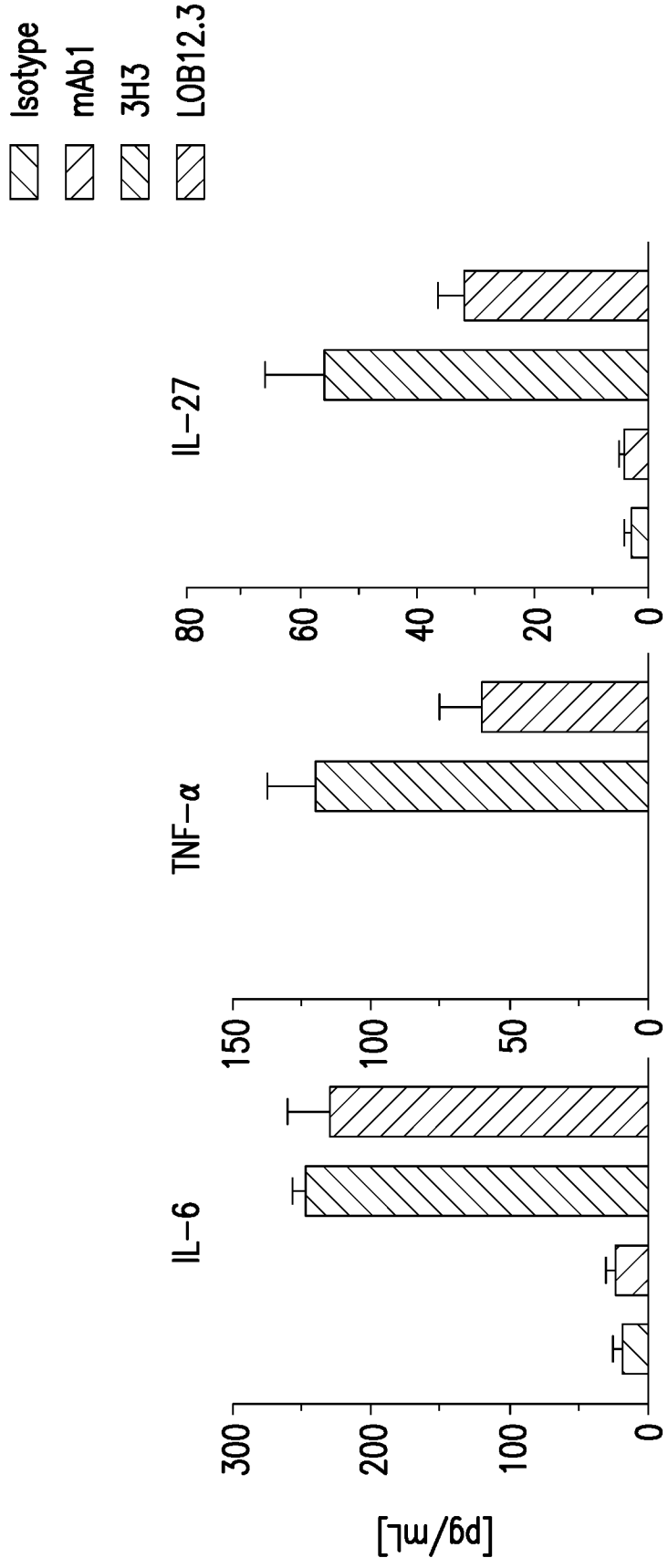

Isotype
mAb1
3H3
LOB12.3
IL-6
TNF-α
IL-27
[pg/mL]
0
100
200
300
0
50
100
150
0
20
40
60
80

FIG.31

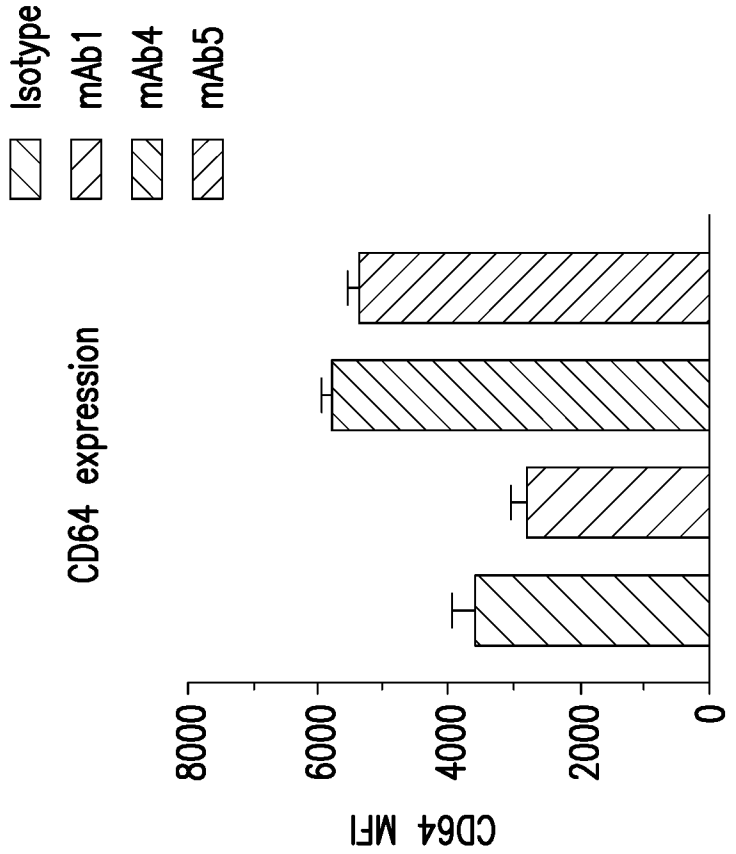

Isotype
mAb1
mAb4
mAb5
CD64 expression
CD64 MFI
8000
6000
4000
2000
0

FIG.33

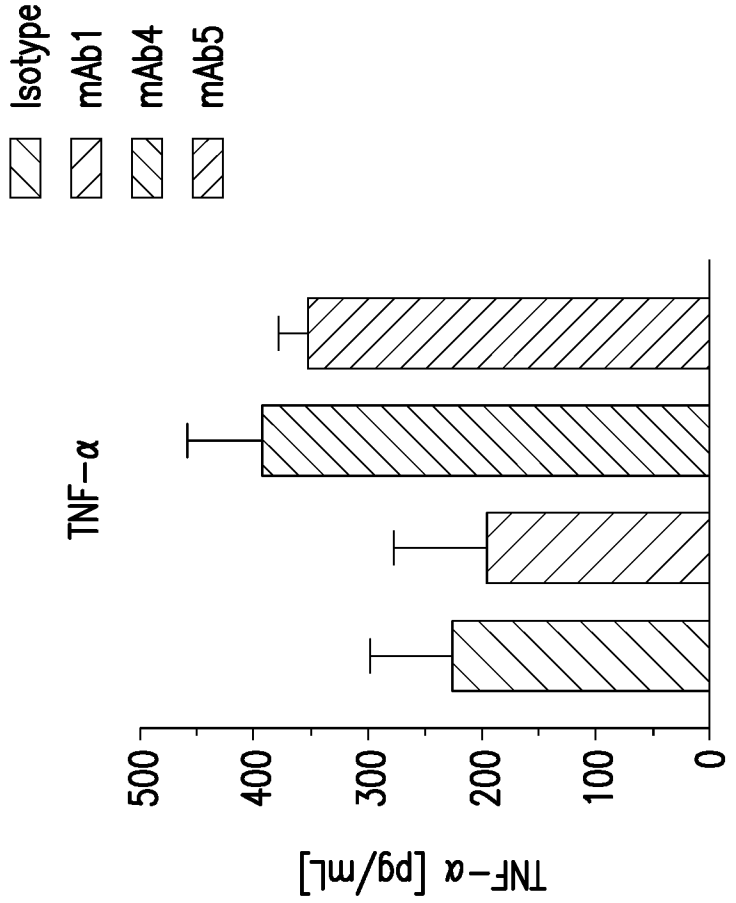

Isotype
mAb1
mAb4
mAb5
TNF-α
TNF-α [pg/mL]
500
400
300
200
100
0

FIG.32

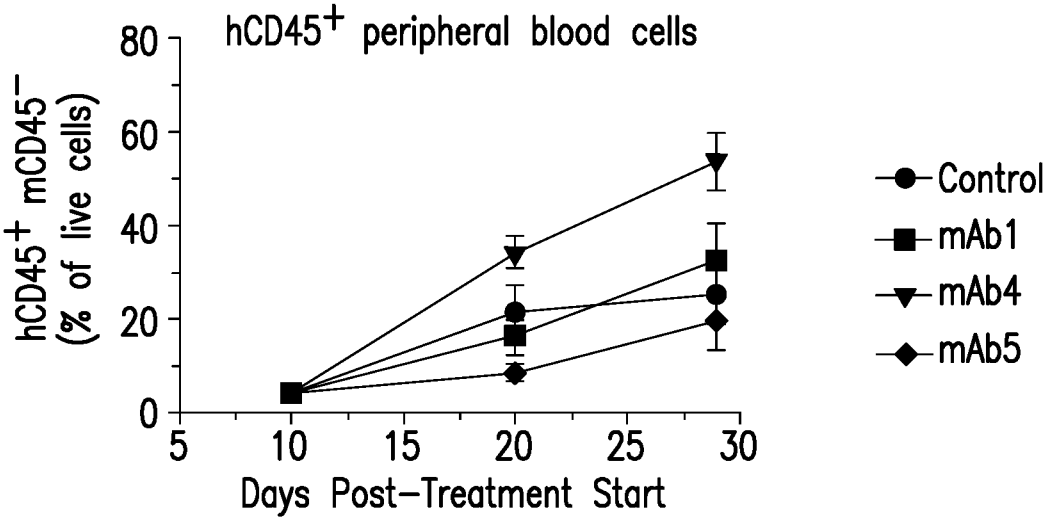


FIG.34A

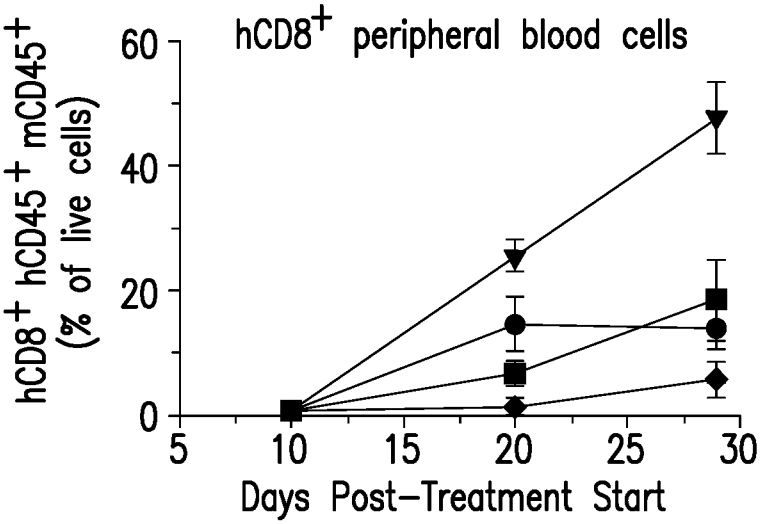


FIG.34B

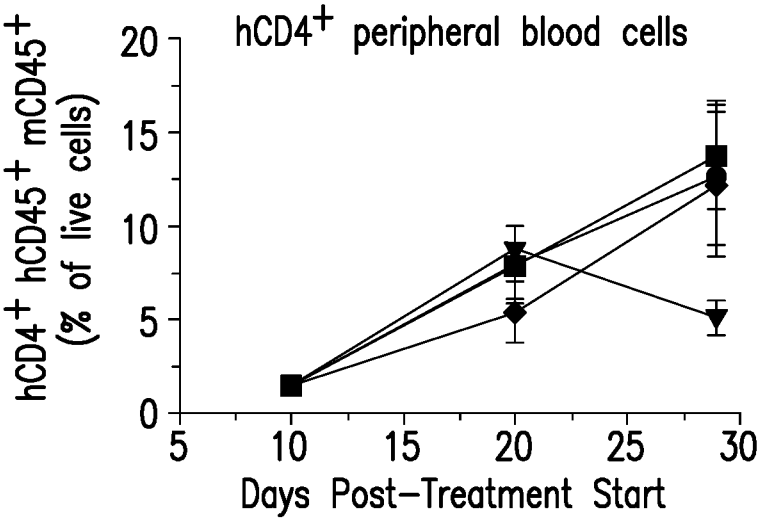


FIG.34C