FIG. 1A

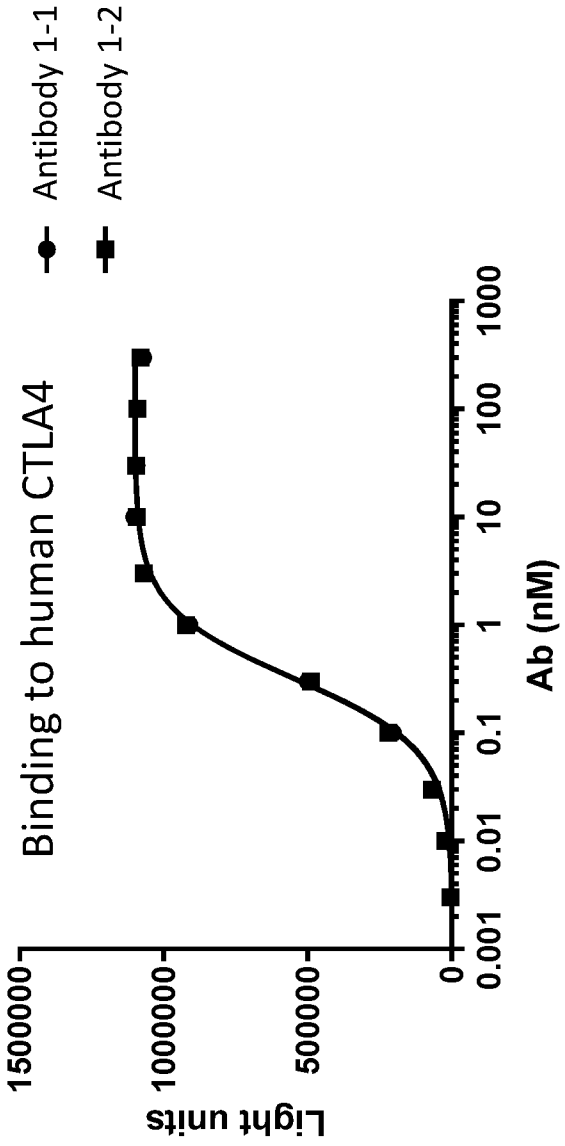

Binding to human CTLA4
Antibody 1-1
Antibody 1-2
Light units
1500000
1000000
500000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)

FIG. 1B

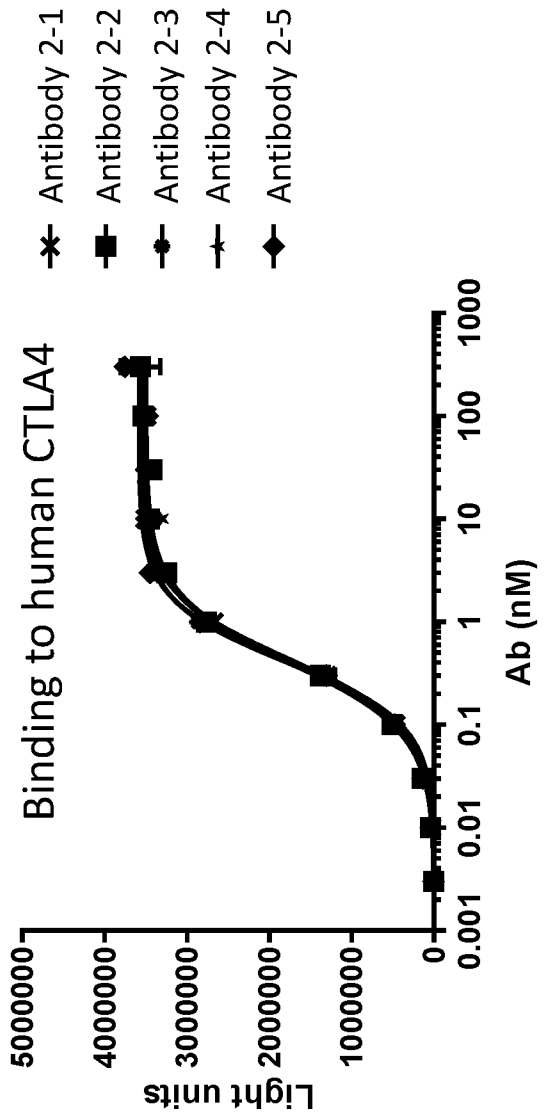

Binding to human CTLA4
Antibody 2-1
Antibody 2-2
Antibody 2-3
Antibody 2-4
Antibody 2-5
Light units
5000000
4000000
3000000
2000000
1000000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)

FIG. 1C

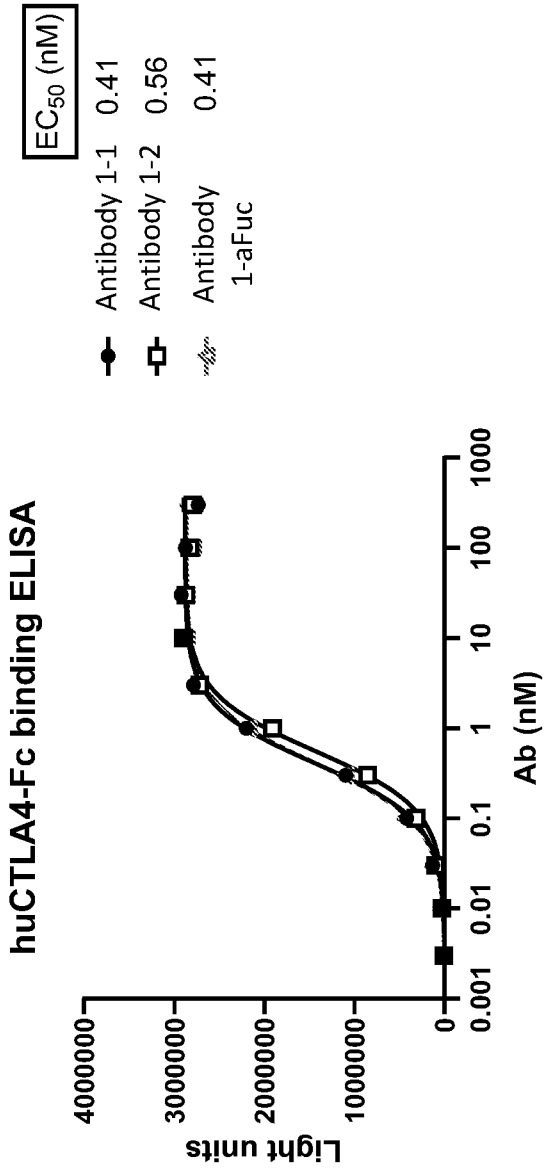

huCTLA4-Fc binding ELISA
EC50 (nM)
Antibody 1-1 0.41
Antibody 1-2 0.56
Antibody 1-aFuc 0.41
Light units
4000000
3000000
2000000
1000000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)

FIG. 1D

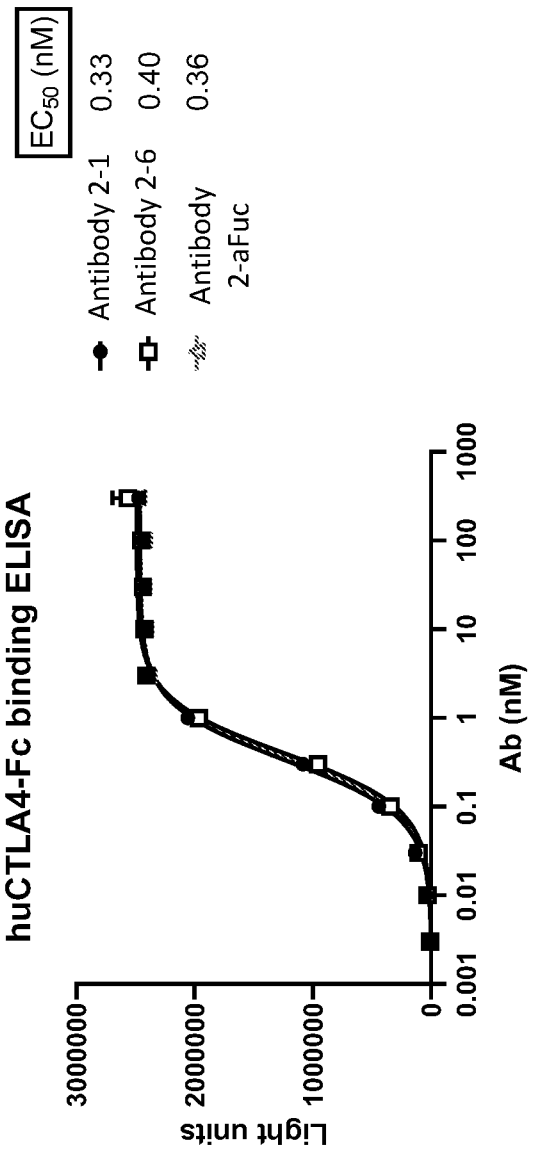

huCTLA4-Fc binding ELISA
EC50 (nM)
Antibody 2-1 0.33
Antibody 2-6 0.40
Antibody 2-aFuc 0.36
Light units
3000000
2000000
1000000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)

FIG. 1E

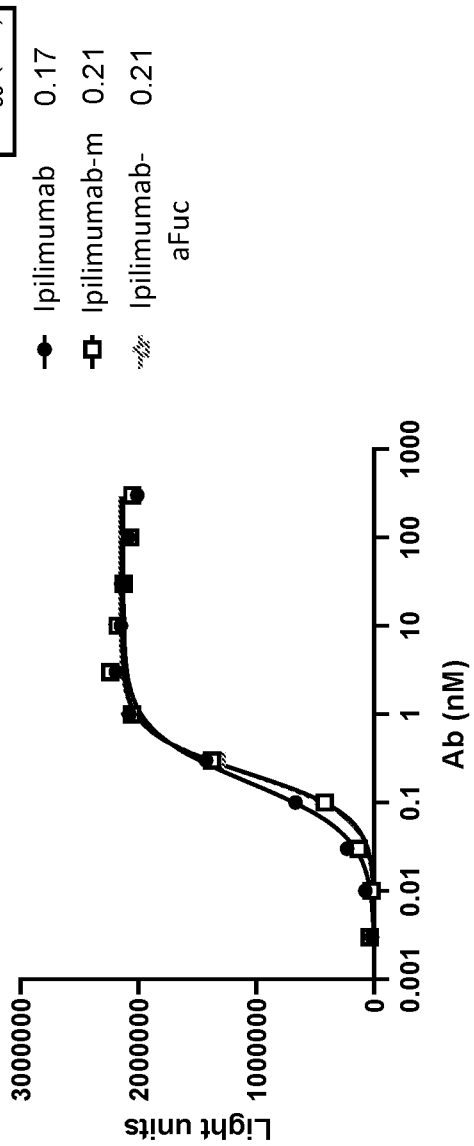

huCTLA4-Fc binding ELISA
EC50 (nM)
Ipilimumab 0.17
Ipilimumab-m 0.21
Ipilimumab-aFuc 0.21
Light units
3000000
2000000
1000000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)

FIG. 2A

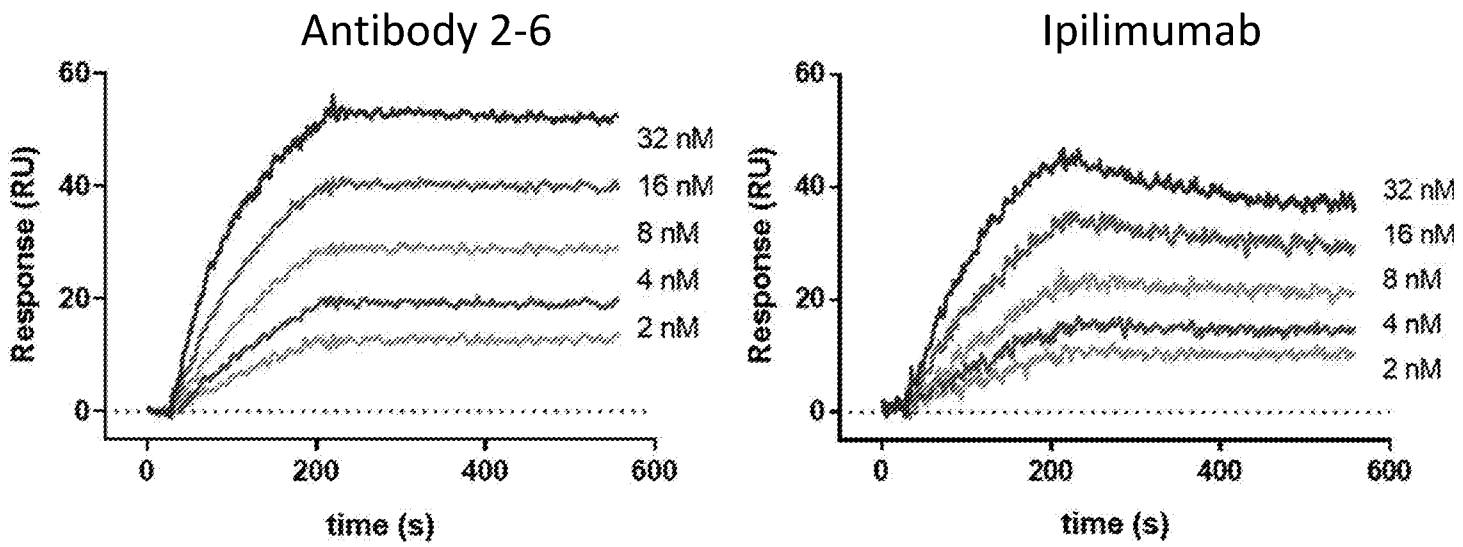


FIG. 2B

Antibody	ka (1/(M*s))	kd (1/s)	K_D
Antibody 2-6	3.66E+5	3.33E-5	91 pM
Ipilimumab	2.90E+5	4.75E-4	1640 pM
Fold difference (Antibody 2-6 vs ipilimumab)		~14x	~18x

FIG. 2C

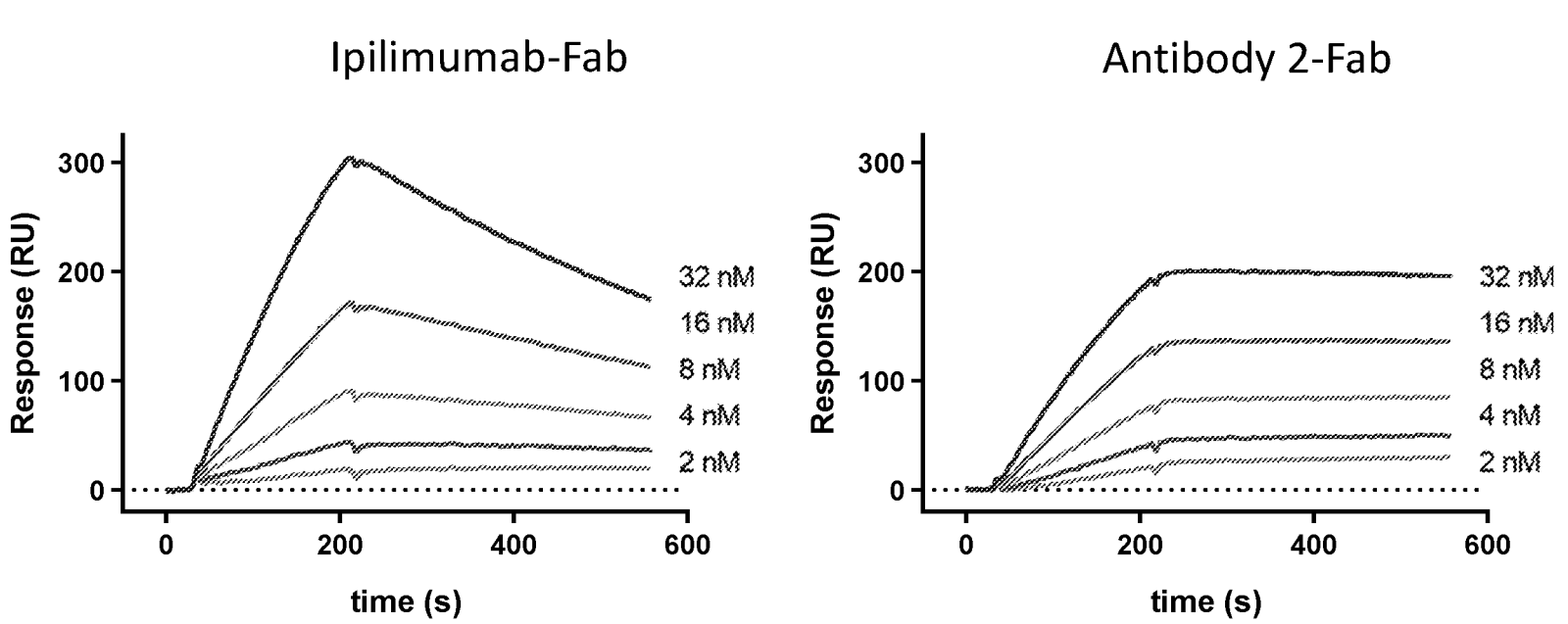


FIG. 2D

Channel	Sample	ka (1/(M*s))	kd (1/s)	K_D	Chi^2
Ch1	Ipilimumab-Fab	2.45e+5	1.40e-3	4.74 nM	1.40
Ch1	Antibody 2-Fab	3.61e+5	1.63e-6	4.53 pM	2.85

FIG. 2E

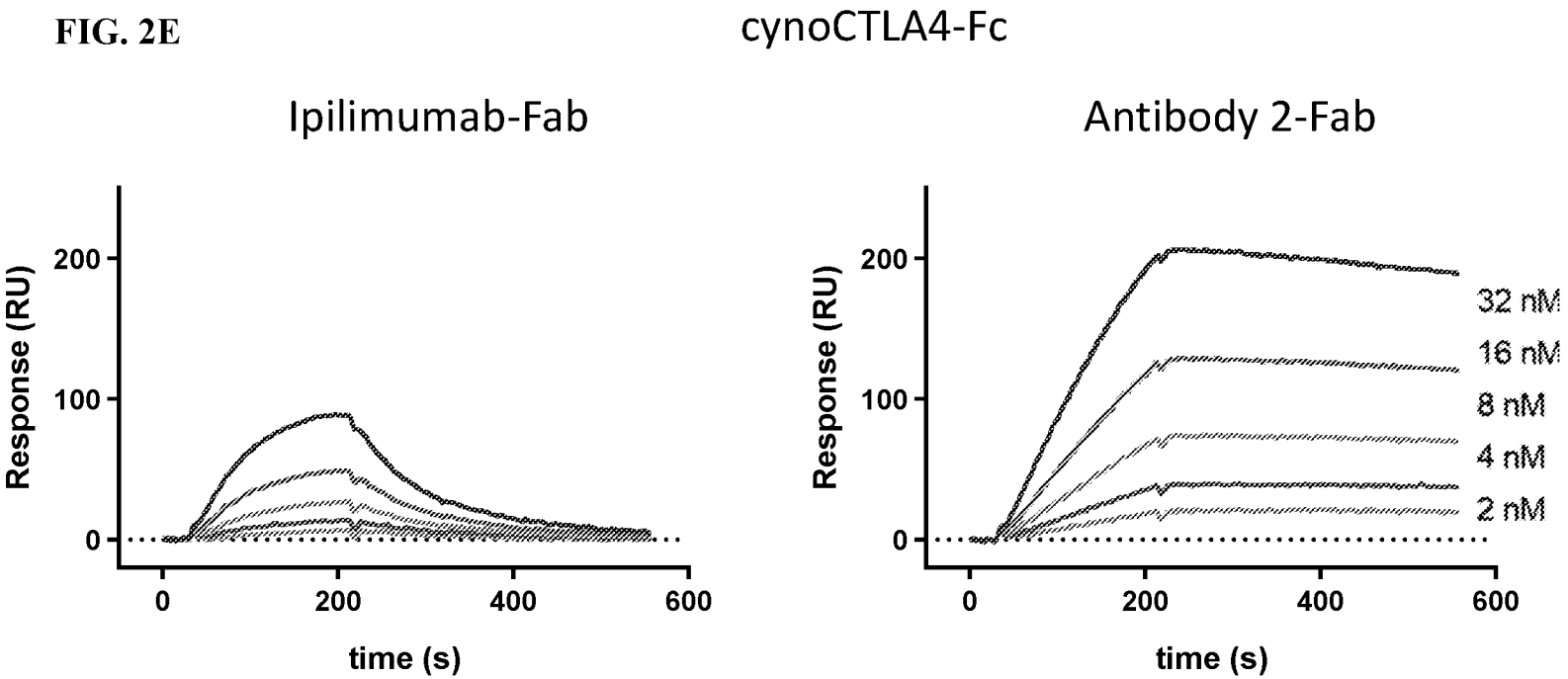


FIG. 2F

Channel	Sample	ka (1/(M*s))	kd (1/s)	K_D	Chi^2
Ch1	Ipilimumab-Fab	3.33e+5	9.51e-3	28.6 nM	1.97
Ch1	Antibody 2-Fab	3.63e+5	2.26e-4	0.623 nM	2.30

FIG. 3

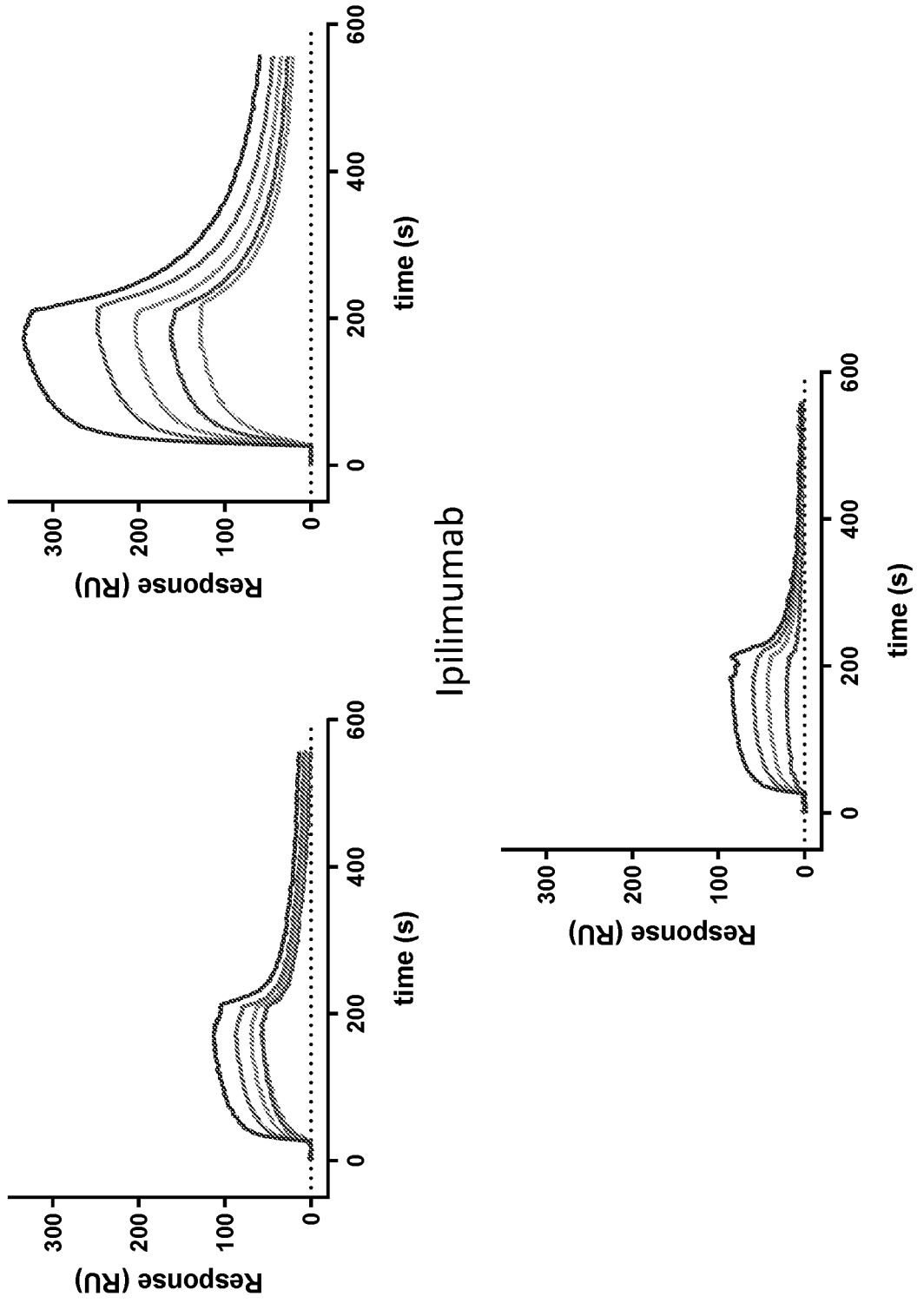

CD16a
Antibody 2-1
Antibody 2-6
Ipilimumab
Response (RU)
time (s)
0
100
200
300
400
600

FIG. 4A

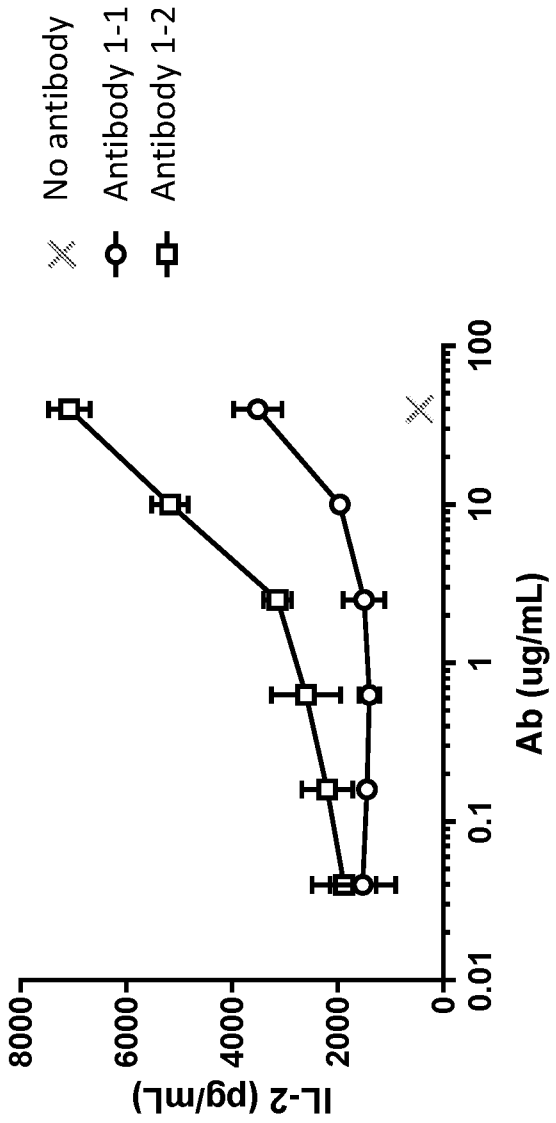

No antibody
Antibody 1-1
Antibody 1-2
IL-2 (pg/mL)
8000
6000
4000
2000
0
0.01
0.1
1
10
100
Ab (ug/mL)

FIG. 4B

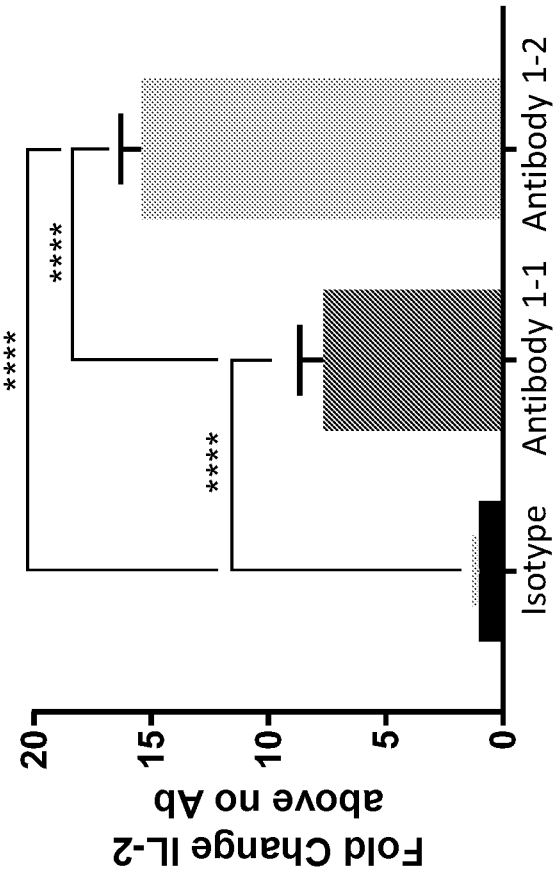

Fold Change IL-2 above no Ab
20
15
10
5
0
****
****
****
Isotype
Antibody 1-1
Antibody 1-2

FIG. 4C

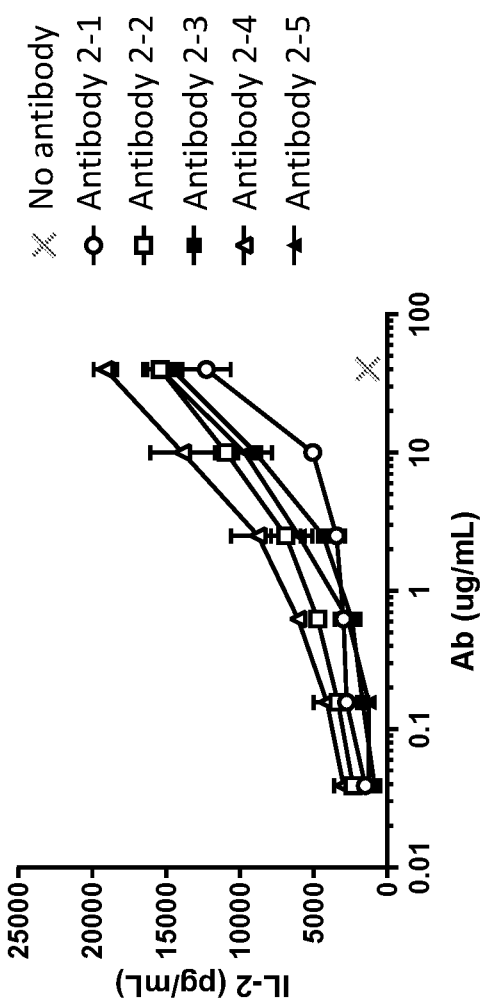

No antibody
Antibody 2-1
Antibody 2-2
Antibody 2-3
Antibody 2-4
Antibody 2-5
IL-2 (pg/mL)
25000
20000
15000
10000
5000
0
0.01
0.1
1
10
100
Ab (ug/mL)

FIG. 4D

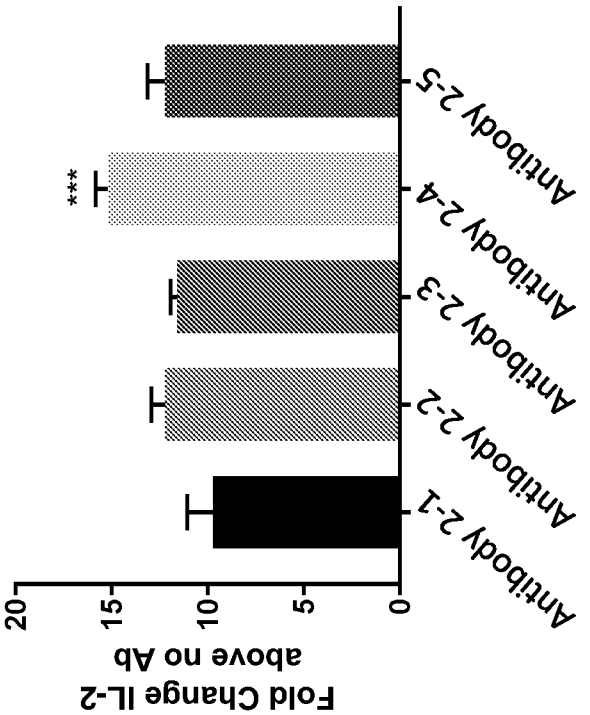

Fold Change IL-2 above no Ab
20
15
10
5
0
***
Antibody 2-1
Antibody 2-2
Antibody 2-3
Antibody 2-4
Antibody 2-5

FIG. 4E

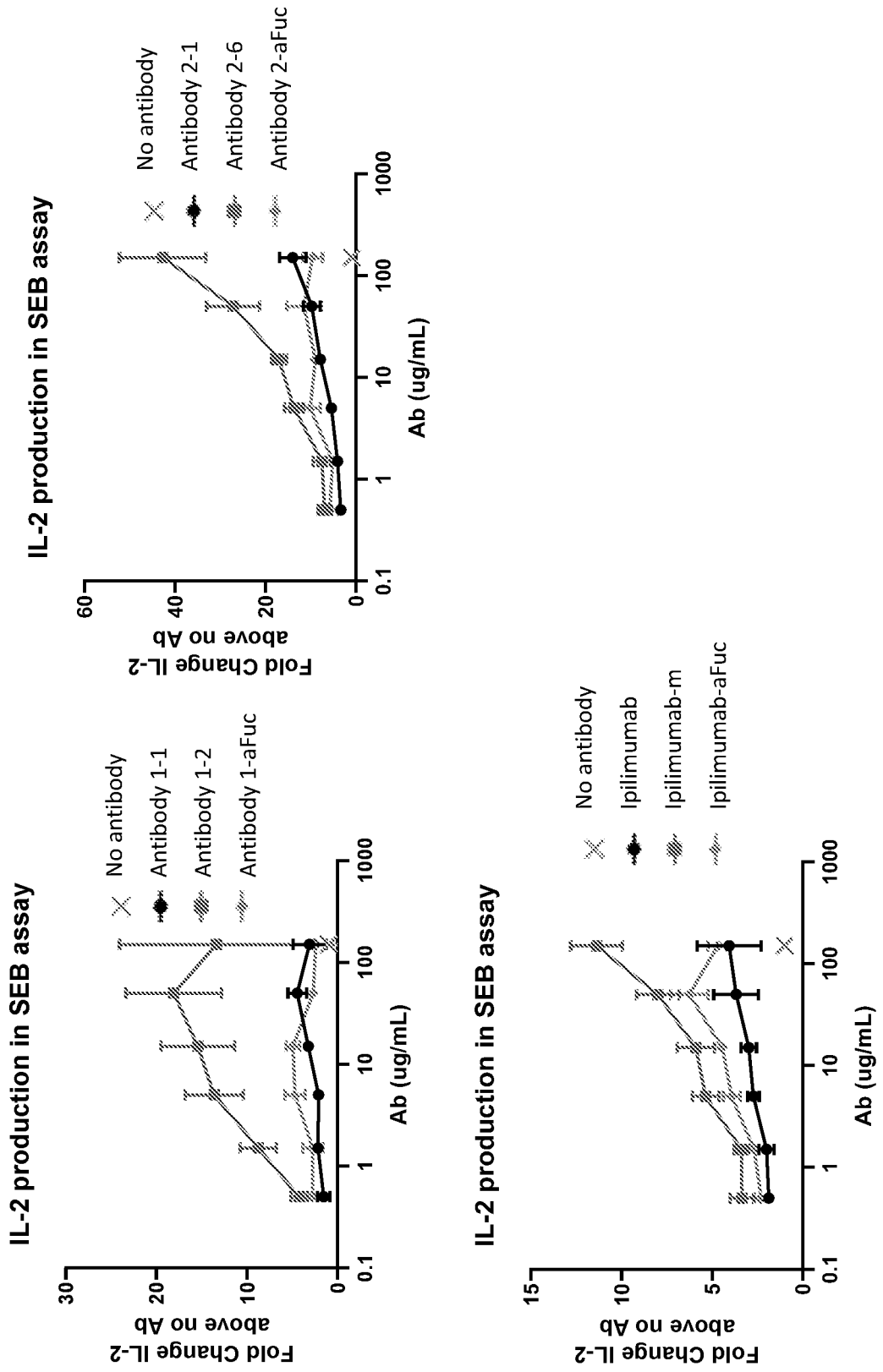

IL-2 production in SEB assay
Fold Change IL-2 above no Ab
Ab (ug/mL)
No antibody
Antibody 1-1
Antibody 1-2
Antibody 1-aFuc
IL-2 production in SEB assay
Fold Change IL-2 above no Ab
Ab (ug/mL)
No antibody
Antibody 2-1
Antibody 2-6
Antibody 2-aFuc
IL-2 production in SEB assay
Fold Change IL-2 above no Ab
Ab (ug/mL)
No antibody
Ipilimumab
Ipilimumab-m
Ipilimumab-aFuc

FIG. 5A

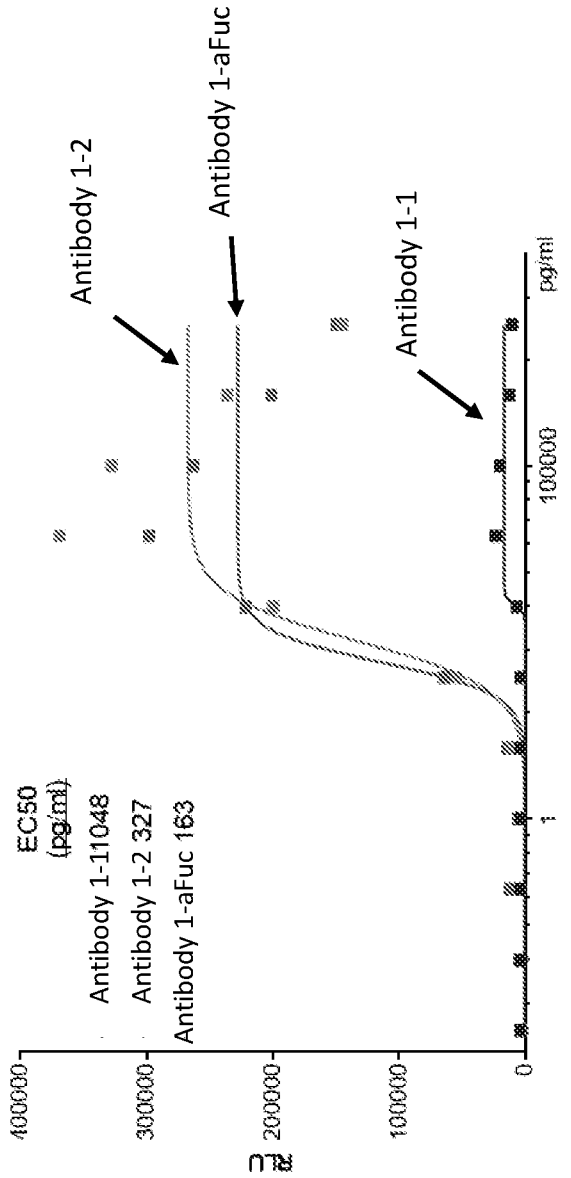

Reporter Bioassay
EC50 (pg/ml)
Antibody 1-11048
Antibody 1-2 327
Antibody 1-aFuc 163
Antibody 1-2
Antibody 1-aFuc
Antibody 1-1
RLU
400000
300000
200000
100000
0
1
100000
pg/ml

FIG. 5B

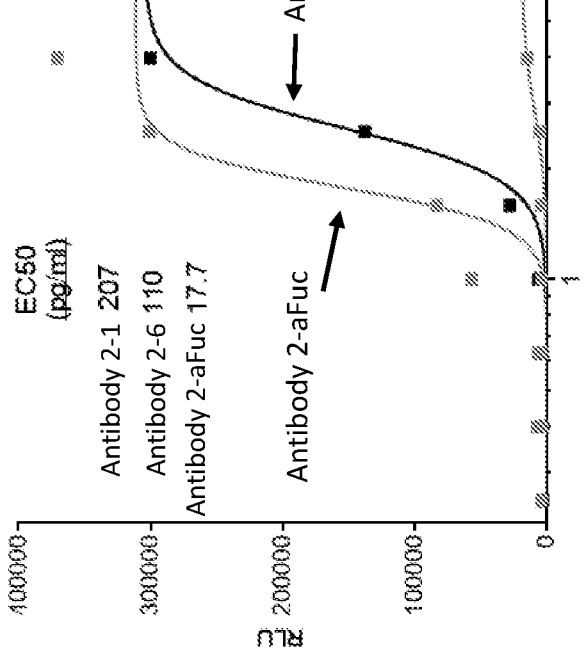

Reporter Bioassay
EC50 (pg/ml)
Antibody 2-1 207
Antibody 2-6 110
Antibody 2-aFuc 17.7
Antibody 2-aFuc
Antibody 2-6
Antibody 2-1
RLU
400000
300000
200000
100000
0
1
100000
pg/ml

FIG. 5C

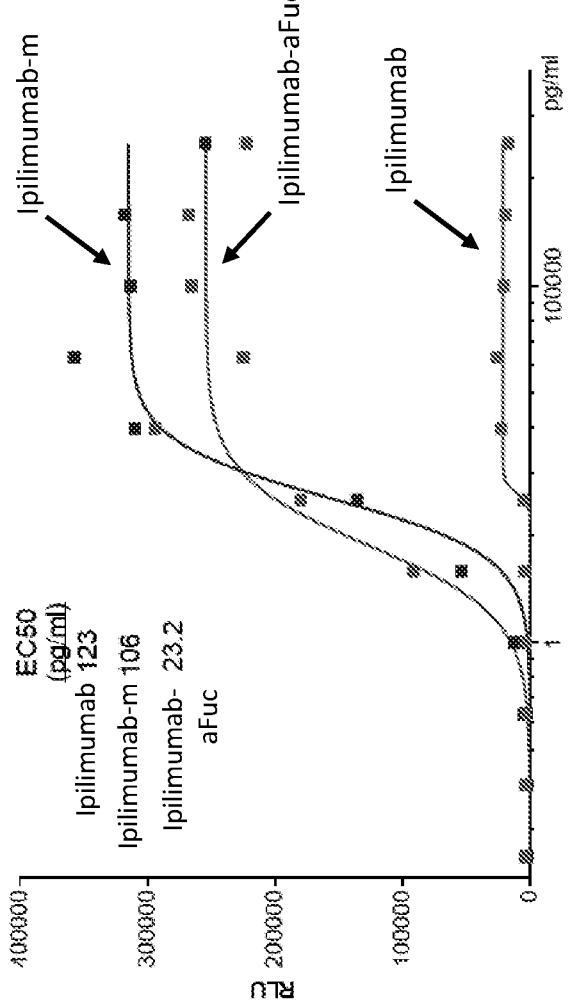

Reporter Bioassay
EC50 (pg/ml)
Ipilimumab 123
Ipilimumab-m 106
Ipilimumab- aFuc 23.2
Ipilimumab-m
Ipilimumab-aFuc
Ipilimumab
RLU
400000
300000
200000
100000
0
1
100000
pg/ml

FIG. 5D

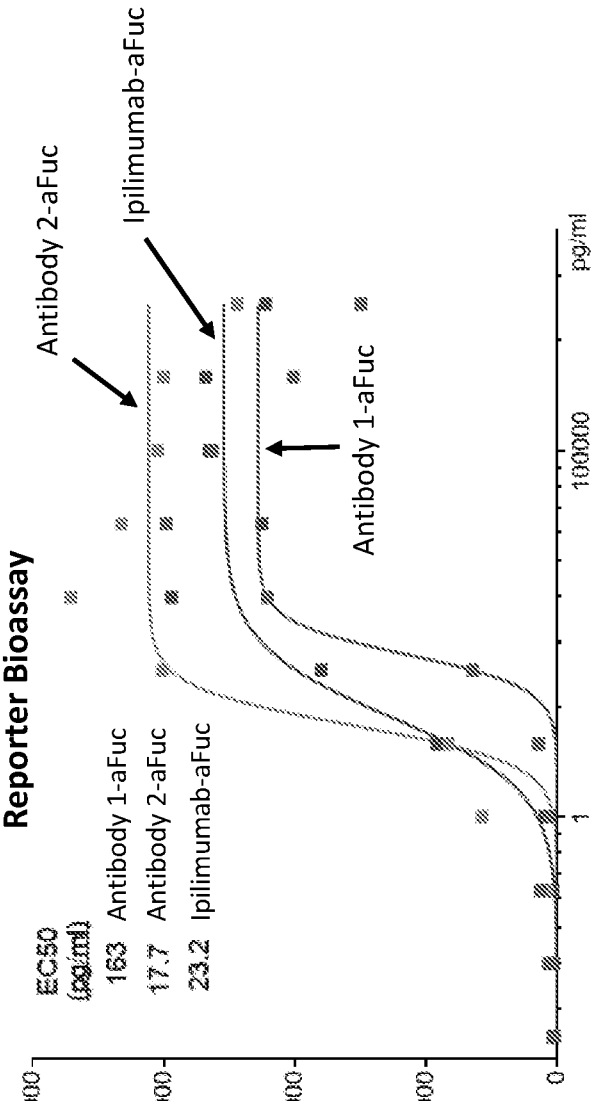

Reporter Bioassay
EC50 (pg/ml)
163 Antibody 1-aFuc
17.7 Antibody 2-aFuc
23.2 Ipilimumab-aFuc
Antibody 2-aFuc
Ipilimumab-aFuc
Antibody 1-aFuc
RLU
400000
300000
200000
100000
0
1
100000
pg/ml

FIG. 6A

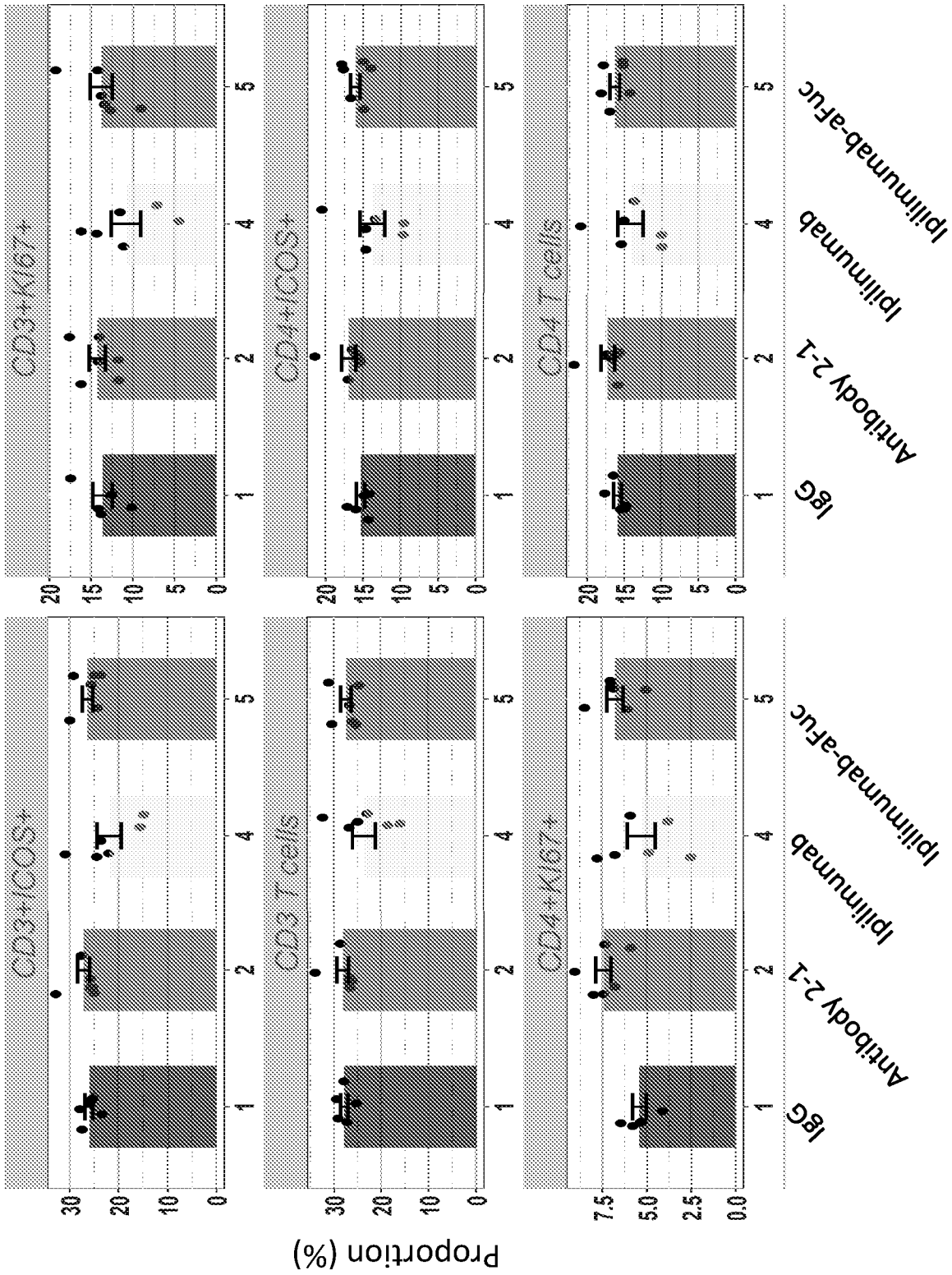

Splenic CD45+ Cells
CD3+ICOS+
CD3+KI67+
CD3 T cells
CD4+ICOS+
CD4+KI67+
CD4 T cells
Proportion (%)
IgG
Antibody 2-1
Ipilimumab
Ipilimumab-aFuc

FIG. 6B

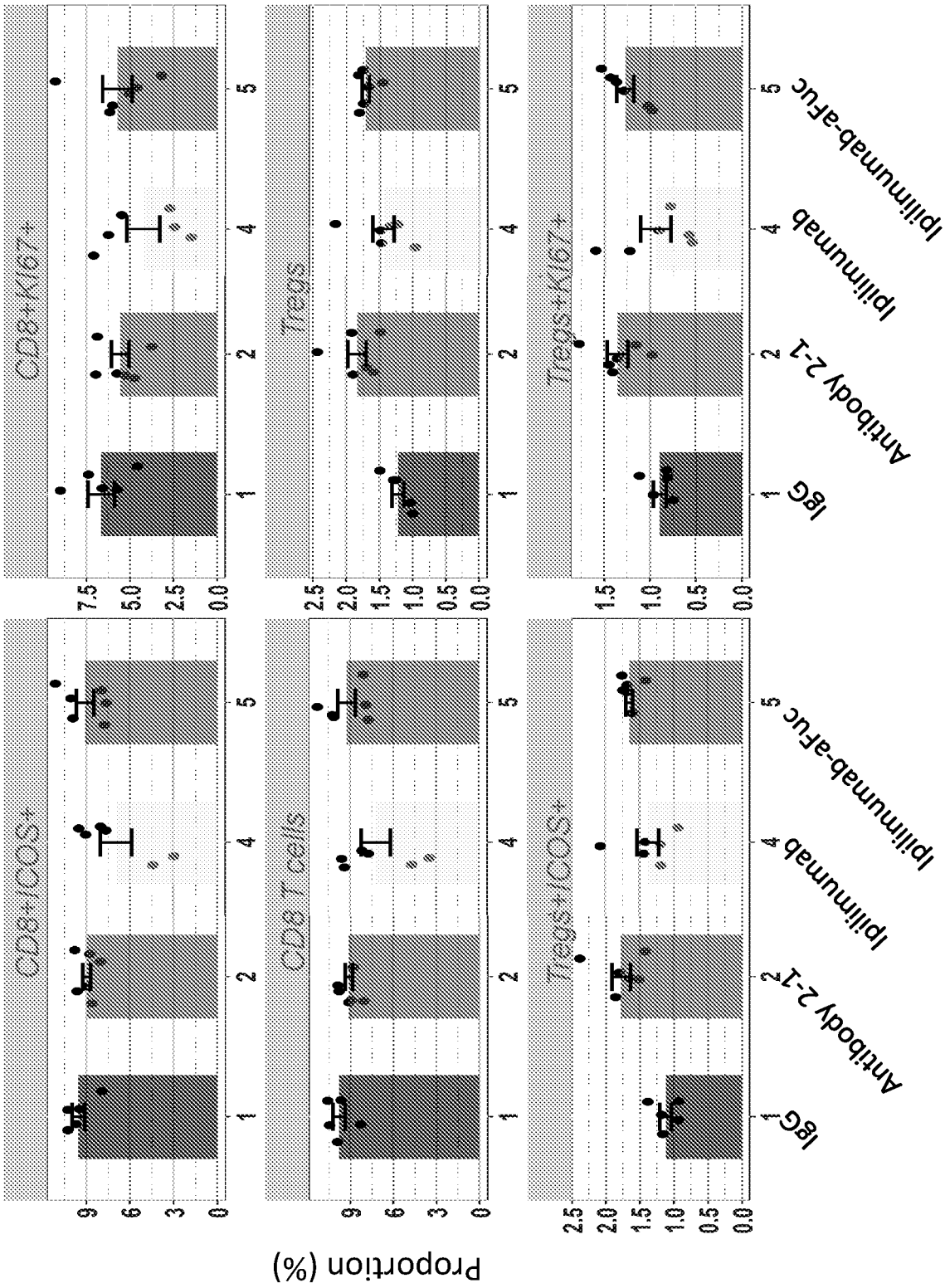

Splenic CD45+ Cells
CD8+ICOS+
CD8+KI67+
CD8 T cells
Tregs
Tregs+ICOS+
Tregs+KI67+
Proportion (%)
IgG
Antibody 2-1
Ipilimumab
Ipilimumab-aFuc

FIG. 6C

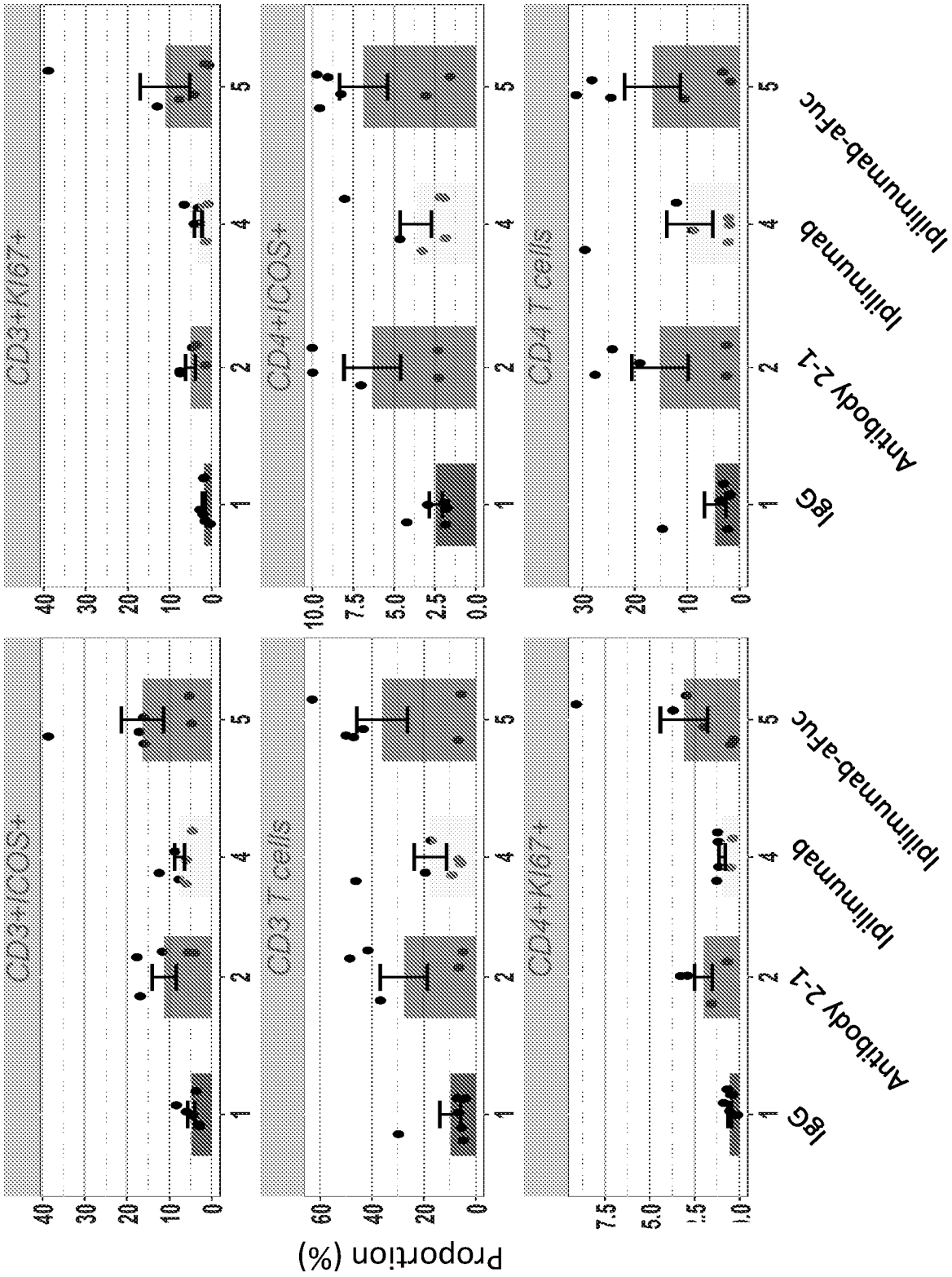

Intratumoral CD45+ Cells
CD3+ICOS+
CD3+KI67+
CD3 T cells
CD4+ICOS+
CD4+KI67+
CD4 T cells
Proportion (%)
IgG
Antibody 2-1
Ipilimumab
Ipilimumab-aFuc

FIG. 6D

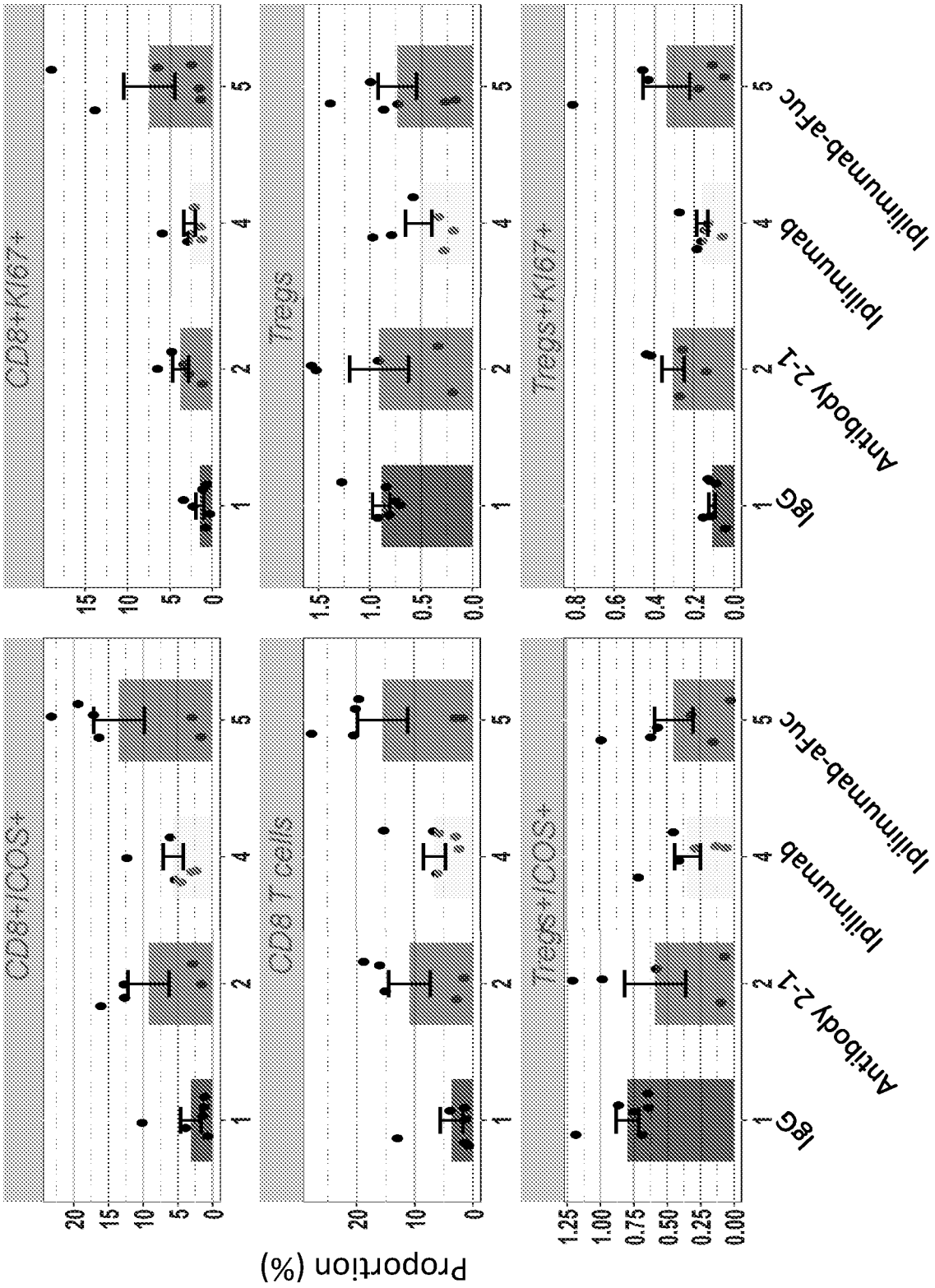

Intratumoral CD45+ Cells
CD8+KI67+
CD8+ICOS+
Tregs
CD8 T cells
Tregs+KI67+
Tregs+ICOS+
Proportion (%)
IgG
Antibody 2-1
Ipilimumab
Ipilimumab-aFuc

FIG. 7A

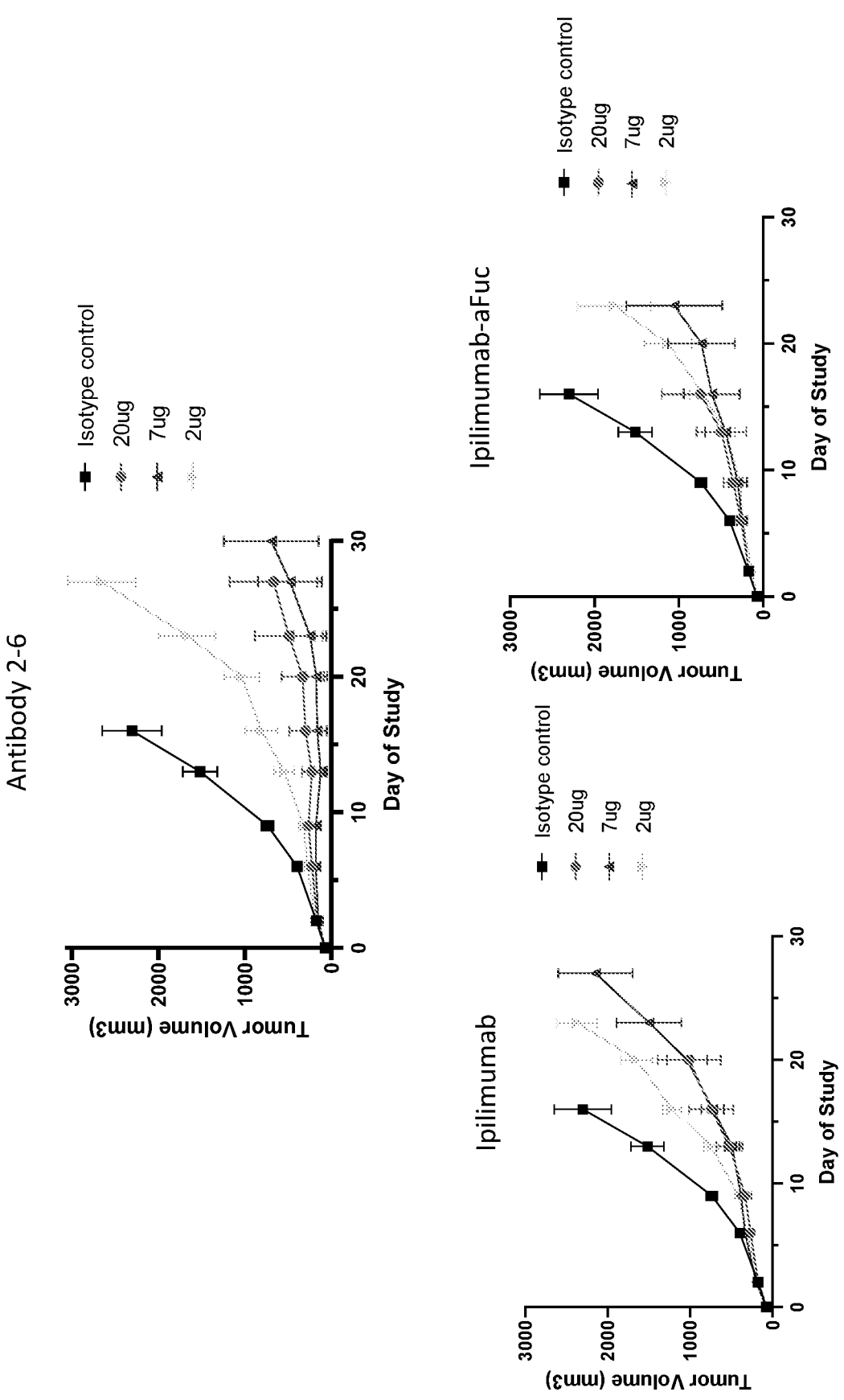

Antibody 2-6
Isotype control
20ug
7ug
2ug
Tumor Volume (mm3)
Day of Study
0
1000
2000
3000
0
10
20
30
Ipilimumab-aFuc
Isotype control
20ug
7ug
2ug
Tumor Volume (mm3)
Day of Study
Ipilimumab
Isotype control
20ug
7ug
2ug
Tumor Volume (mm3)
Day of Study

FIG. 7B

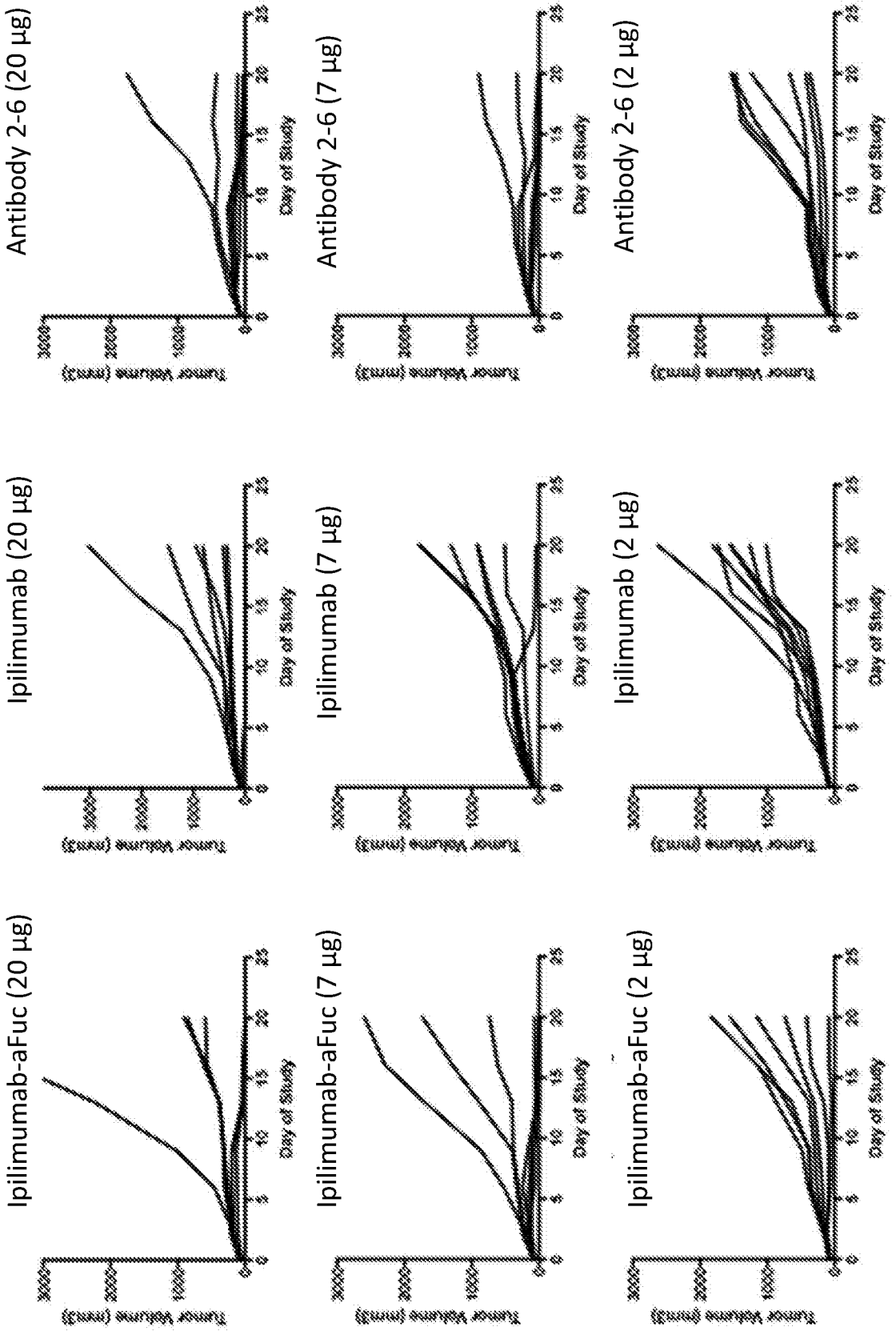

Ipilimumab-aFuc (20 µg)
Ipilimumab (20 µg)
Antibody 2-6 (20 µg)
Ipilimumab-aFuc (7 µg)
Ipilimumab (7 µg)
Antibody 2-6 (7 µg)
Ipilimumab-aFuc (2 µg)
Ipilimumab (2 µg)
Antibody 2-6 (2 µg)
Tumor Volume (mm3)
Day of Study
0
5
10
15
20
25
1000
2000
3000

FIG. 7C

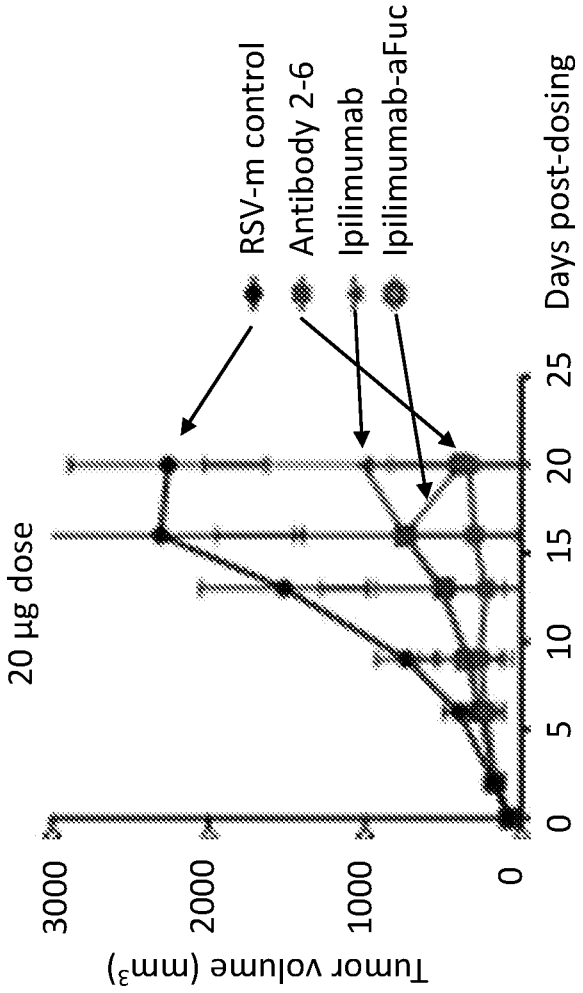
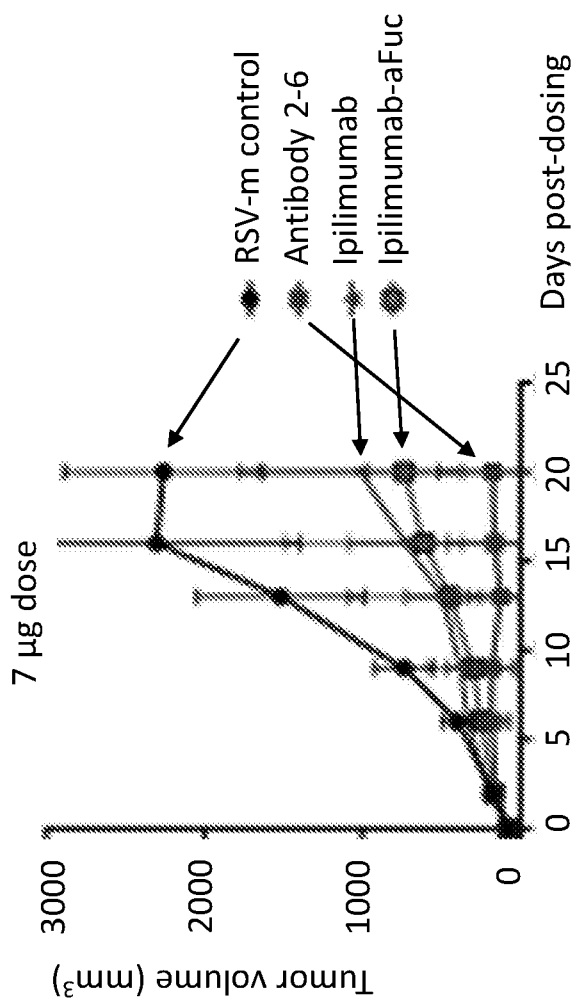

20 µg dose
Tumor volume (mm3)
3000
2000
1000
0
0
5
10
15
20
25
Days post-dosing
RSV-m control
Antibody 2-6
Ipilimumab
Ipilimumab-aFuc

FIG. 7D


7 µg dose
Tumor volume (mm3)
3000
2000
1000
0
0
5
10
15
20
25
Days post-dosing
RSV-m control
Antibody 2-6
Ipilimumab
Ipilimumab-aFuc

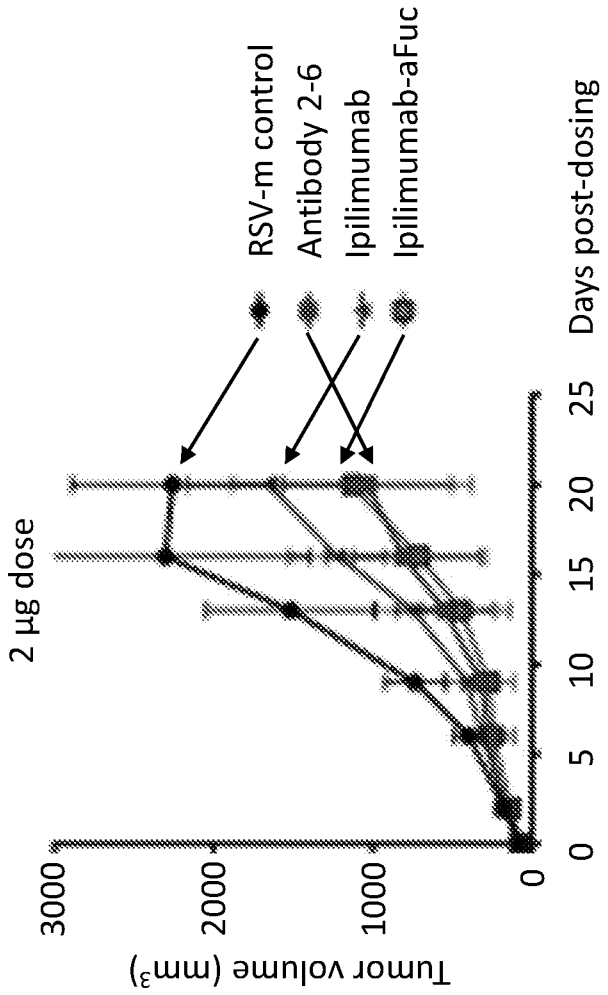

2 µg dose
Tumor volume (mm³)
3000
2000
1000
0
0
5
10
15
20
25
Days post-dosing
RSV-m control
Antibody 2-6
Ipilimumab
Ipilimumab-aFuc

FIG. 7E

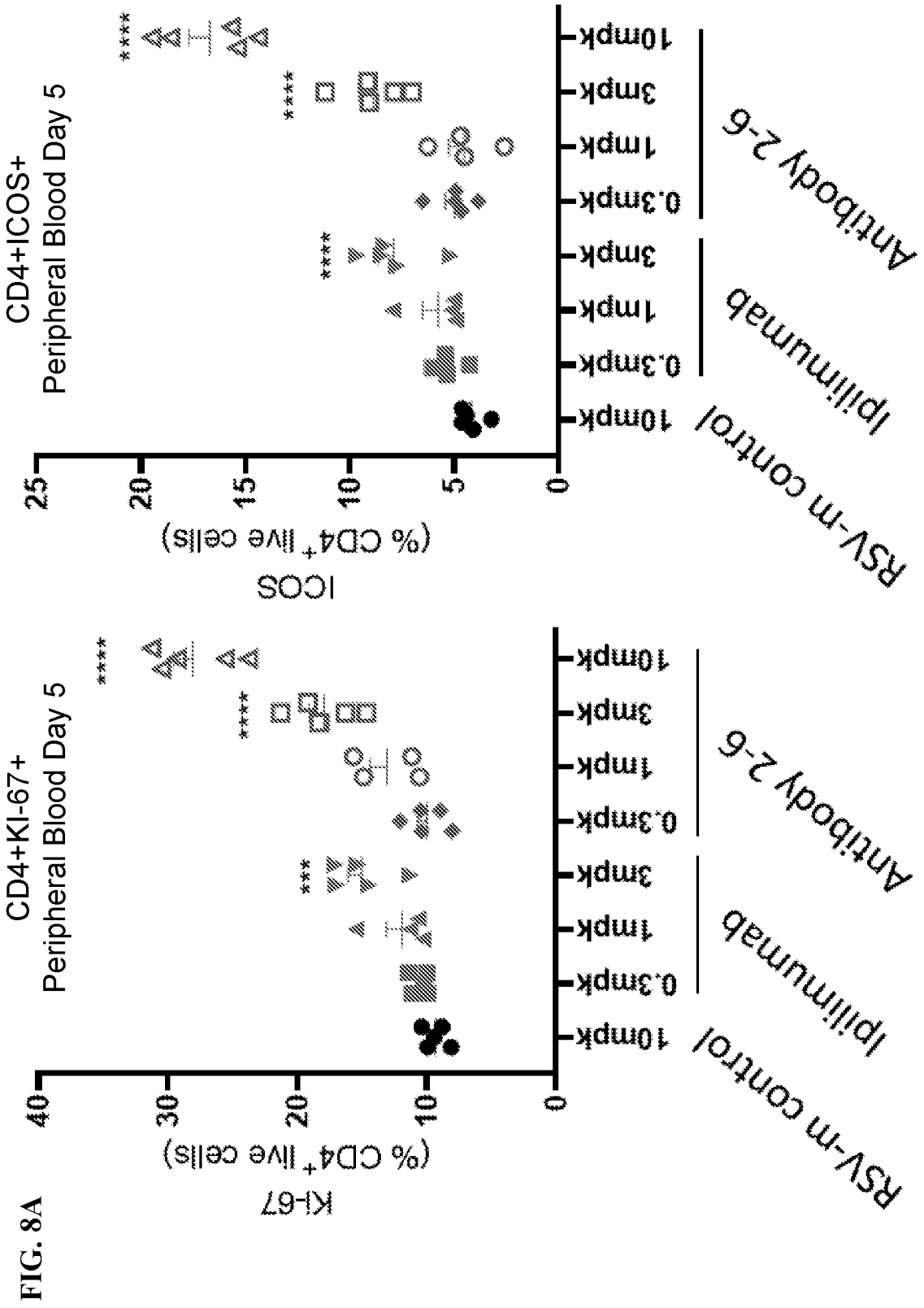
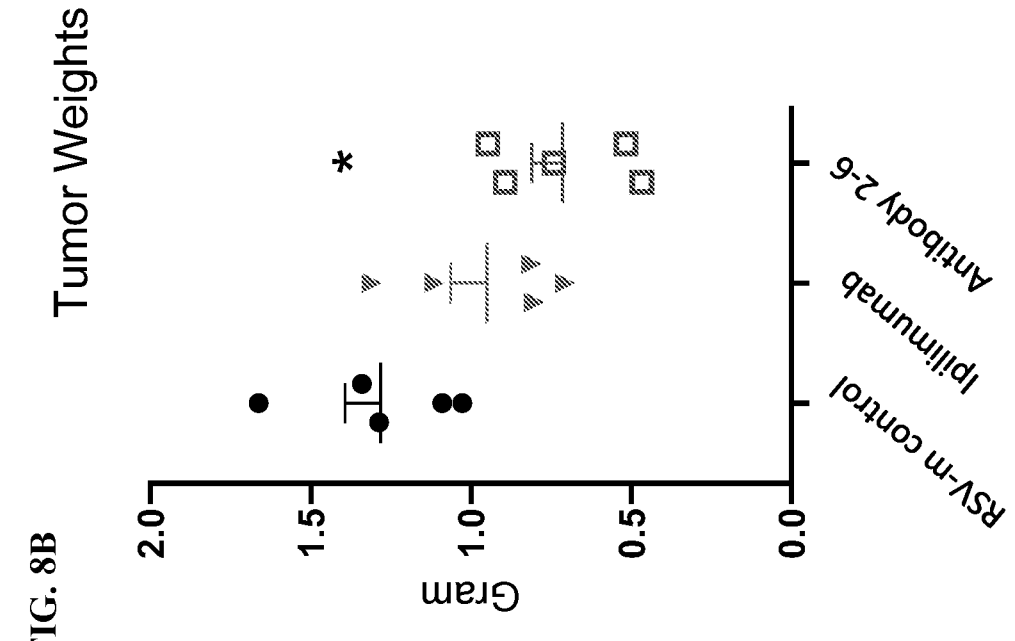
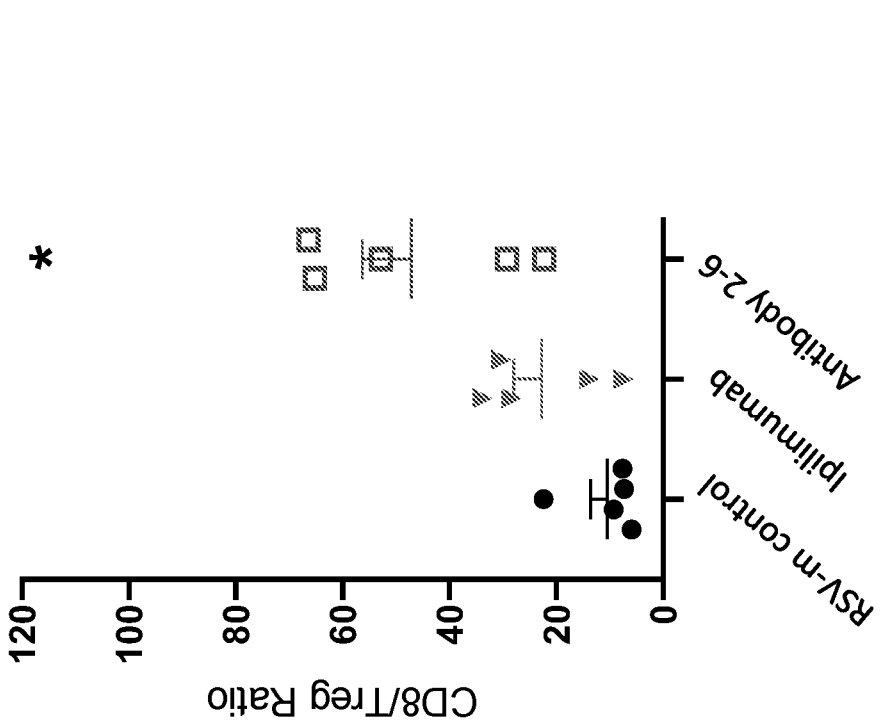

CD4+ICOS+
Peripheral Blood Day 5
ICOS
(% CD4+ live cells)
25
20
15
10
5
0
RSV-m control
Ipilimumab
Antibody 2-6
10mpk
3mpk
1mpk
0.3mpk
3mpk
1mpk
0.3mpk
10mpk
3mpk
1mpk
0.3mpk
****
****
****
CD4+KI-67+
Peripheral Blood Day 5
KI-67
(% CD4+ live cells)
40
30
20
10
0
RSV-m control
Ipilimumab
Antibody 2-6
10mpk
3mpk
1mpk
0.3mpk
3mpk
1mpk
0.3mpk
10mpk
****
****
***

FIG. 8A

FIG. 8B


Tumor Weights
Gram
2.0
1.5
1.0
0.5
0.0
*
RSV-m control
Ipilimumab
Antibody 2-6


CD8/Treg Ratio
tumor microenvironment
CD8/Treg Ratio
120
100
80
60
40
20
0
*
RSV-m control
Ipilimumab
Antibody 2-6

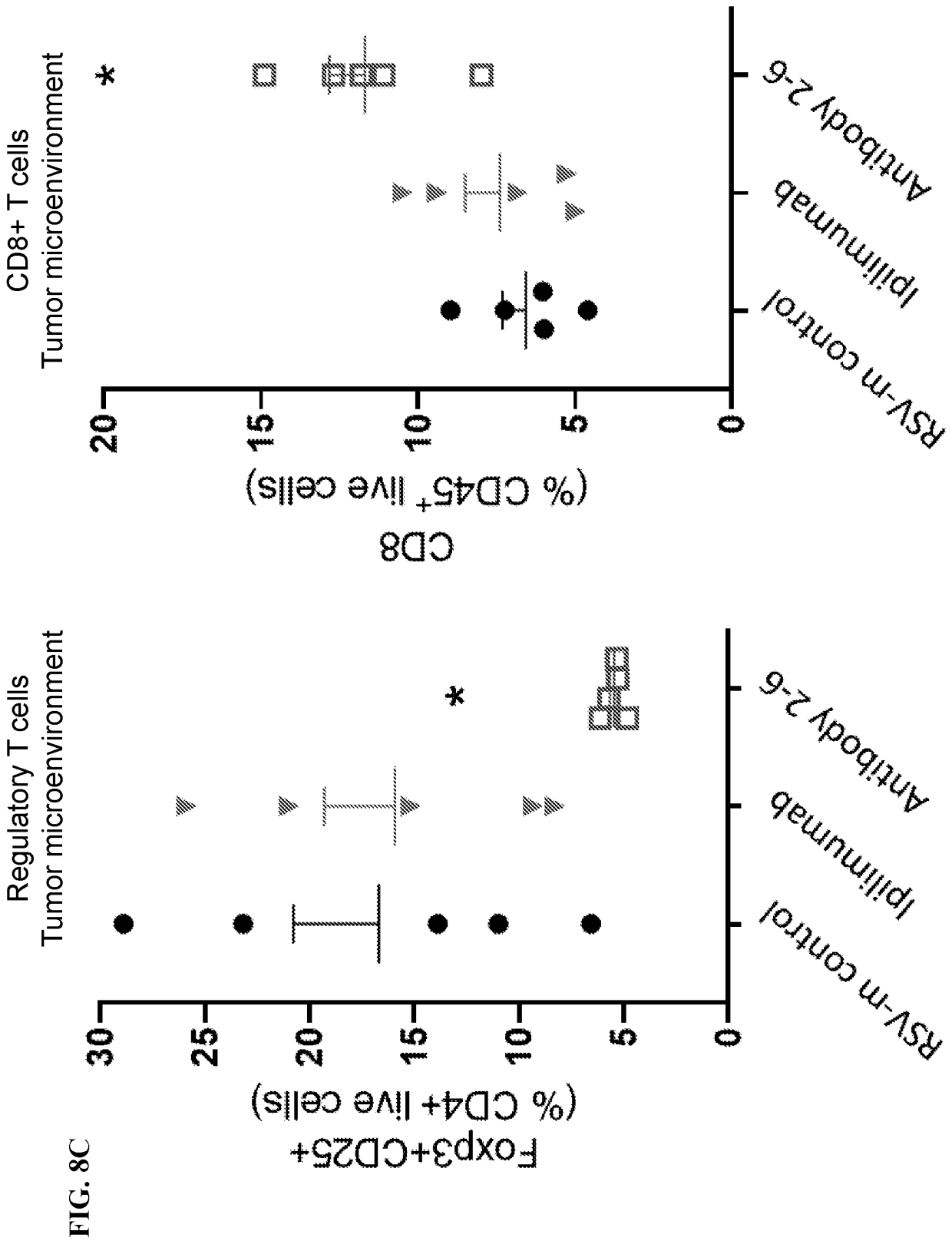

CD8+ T cells
Tumor microenvironment
CD8
(% CD45+ live cells)
20
15
10
5
0
RSV-m control
Ipilimumab
Antibody 2-6
*
Regulatory T cells
Tumor microenvironment
Foxp3+CD25+
(% CD4+ live cells)
30
25
20
15
10
5
0
RSV-m control
Ipilimumab
Antibody 2-6
*

FIG. 8C

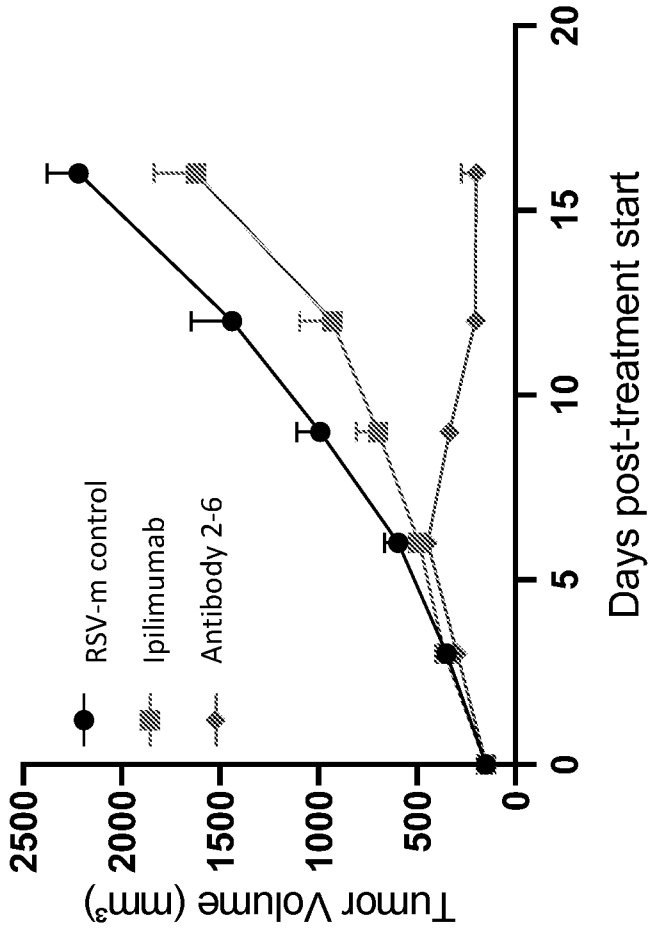

0.3mpk dose
RSV-m control
Ipilimumab
Antibody 2-6
Tumor Volume (mm³)
2500
2000
1500
1000
500
0
0
5
10
15
20
Days post-treatment start

FIG. 8D

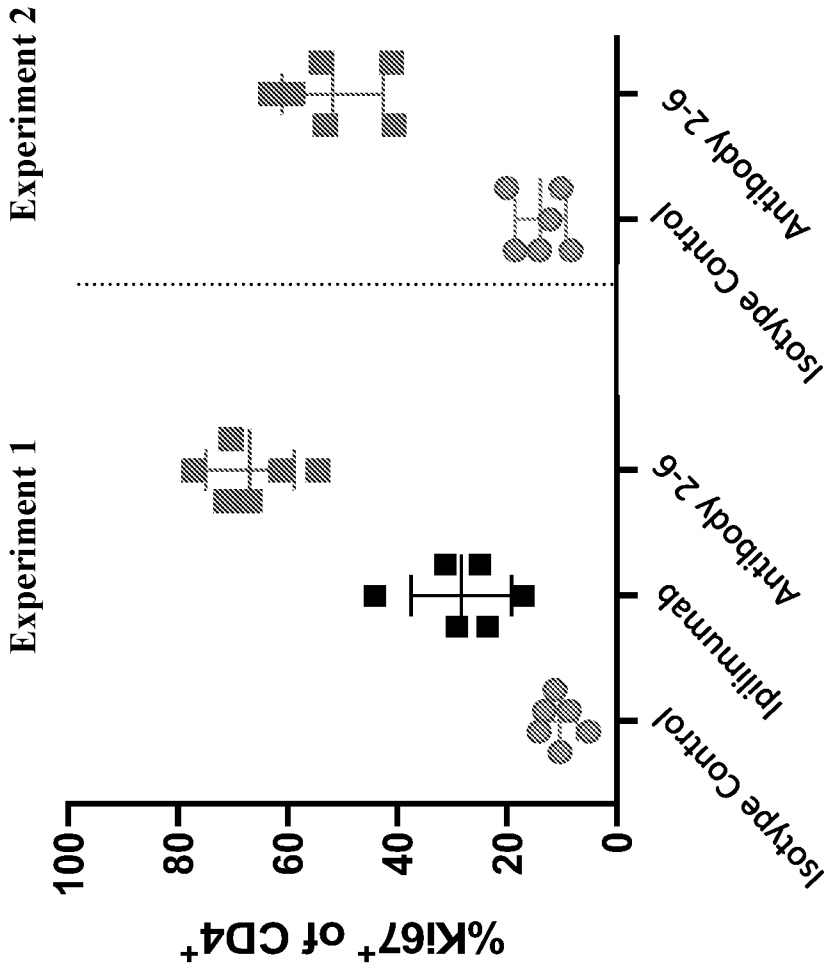

Experiment 1
Experiment 2
%Ki67+ of CD4+
100
80
60
40
20
0
Isotype Control
Ipilimumab
Antibody 2-6
Isotype Control
Antibody 2-6

FIG. 9