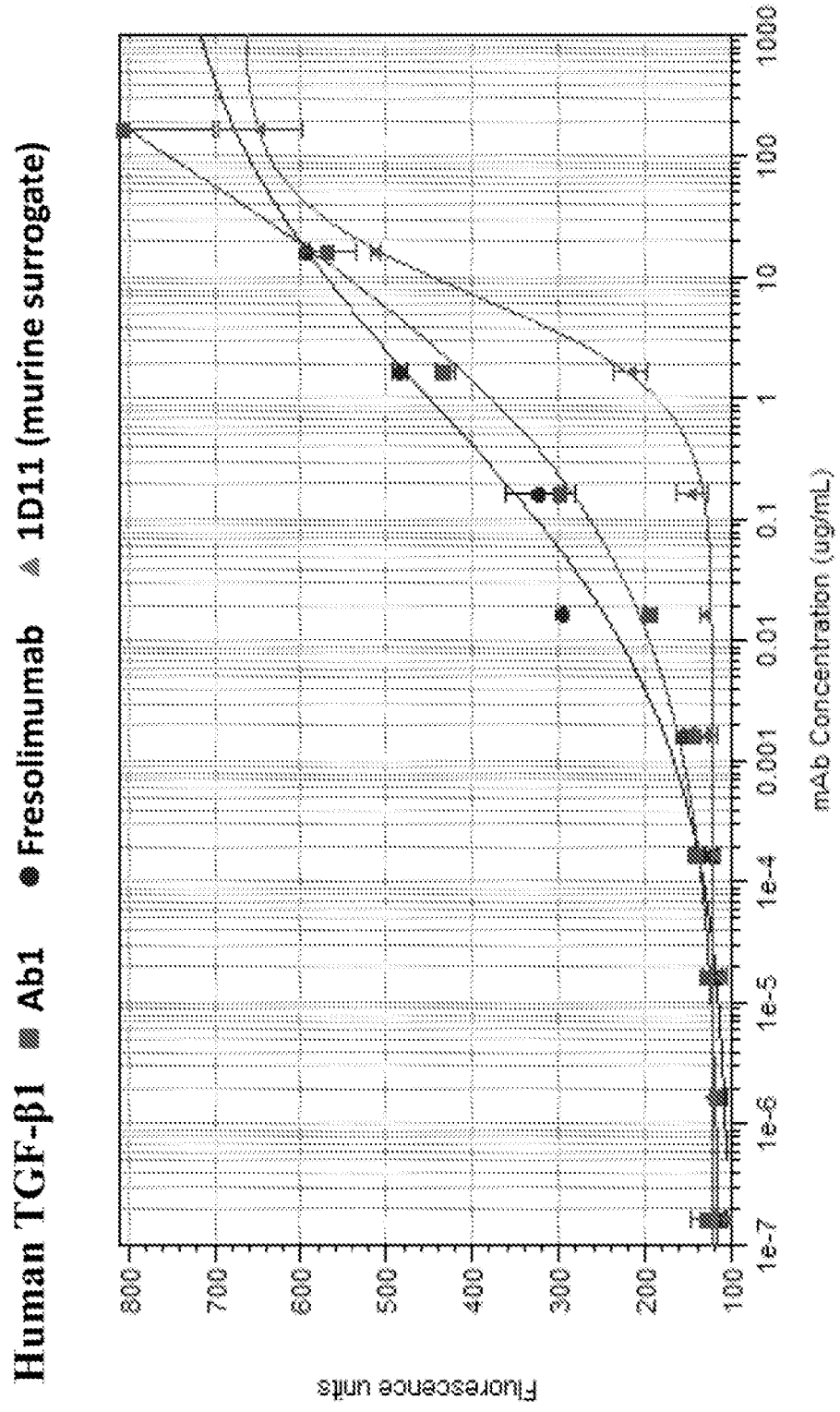
FIG. 1A


Human TGF-β1
Ab1
Fresolimumab
1D11 (murine surrogate)
Fluorescence units
mAb Concentration (ug/mL)
800
700
600
500
400
300
200
100
1e-7
1e-6
1e-5
1e-4
0.001
0.01
0.1
1
10
100
1000

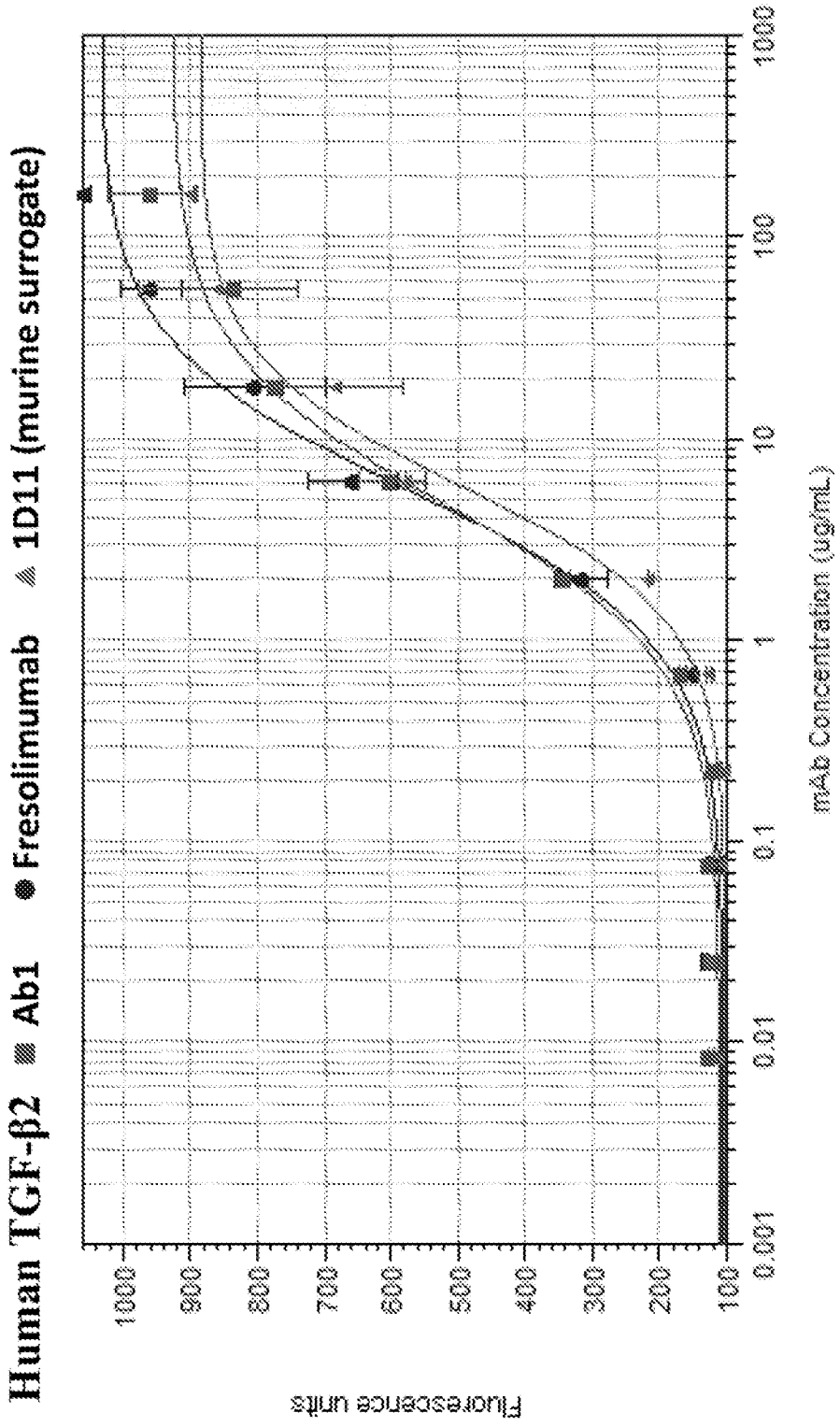
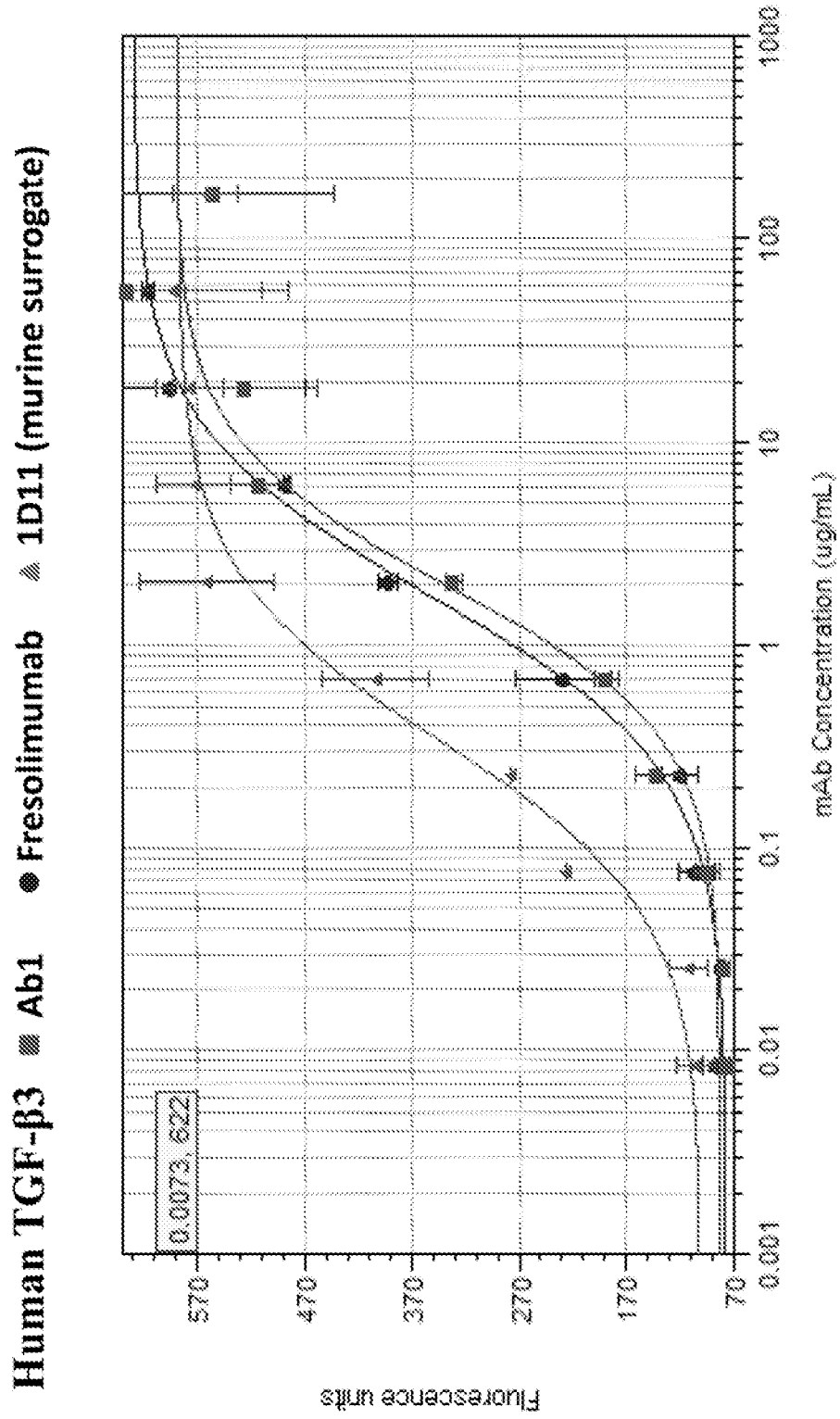

Human TGF-β2
Ab1
Fresolimumab
1D11 (murine surrogate)
Fluorescence units
mAb Concentration (ug/mL)
100
200
300
400
500
600
700
800
900
1000
0.001
0.01
0.1
1
10
100
1000

FIG. 1B

FIG. 1C


Human TGF-β3
Ab1
Fresolimumab
1D11 (murine surrogate)
0.0073, 622
570
470
370
270
170
70
Fluorescence units
0.001
0.01
0.1
1
10
100
1000
mAb Concentration (ug/mL)

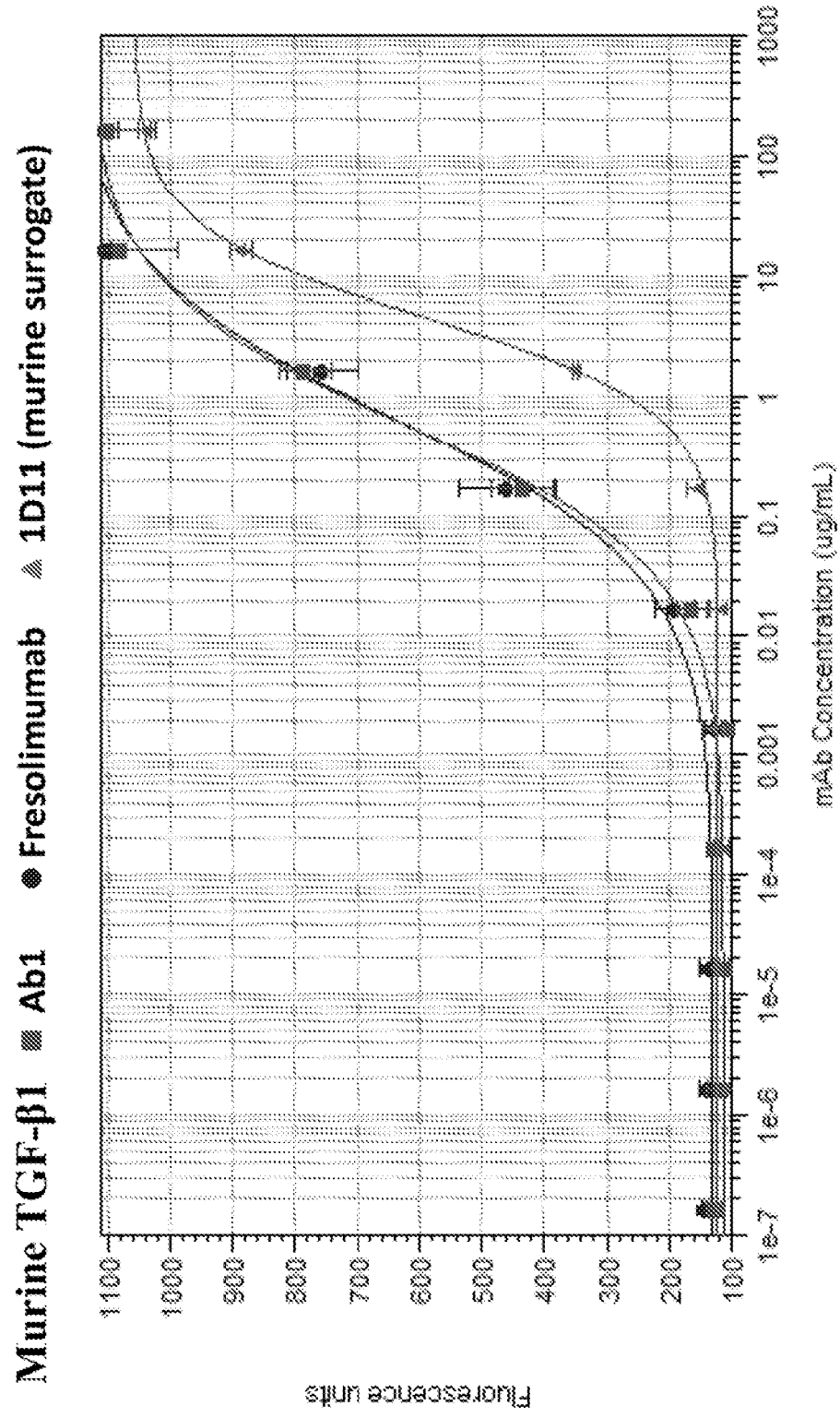

Murine TGF-β1
Ab1
Fresolimumab
1D11 (murine surrogate)
Fluorescence units
mAb Concentration (ug/mL)
1100
1000
900
800
700
600
500
400
300
200
100
1e-7
1e-6
1e-5
1e-4
0.001
0.01
0.1
1
10
100
1000

FIG. 1D

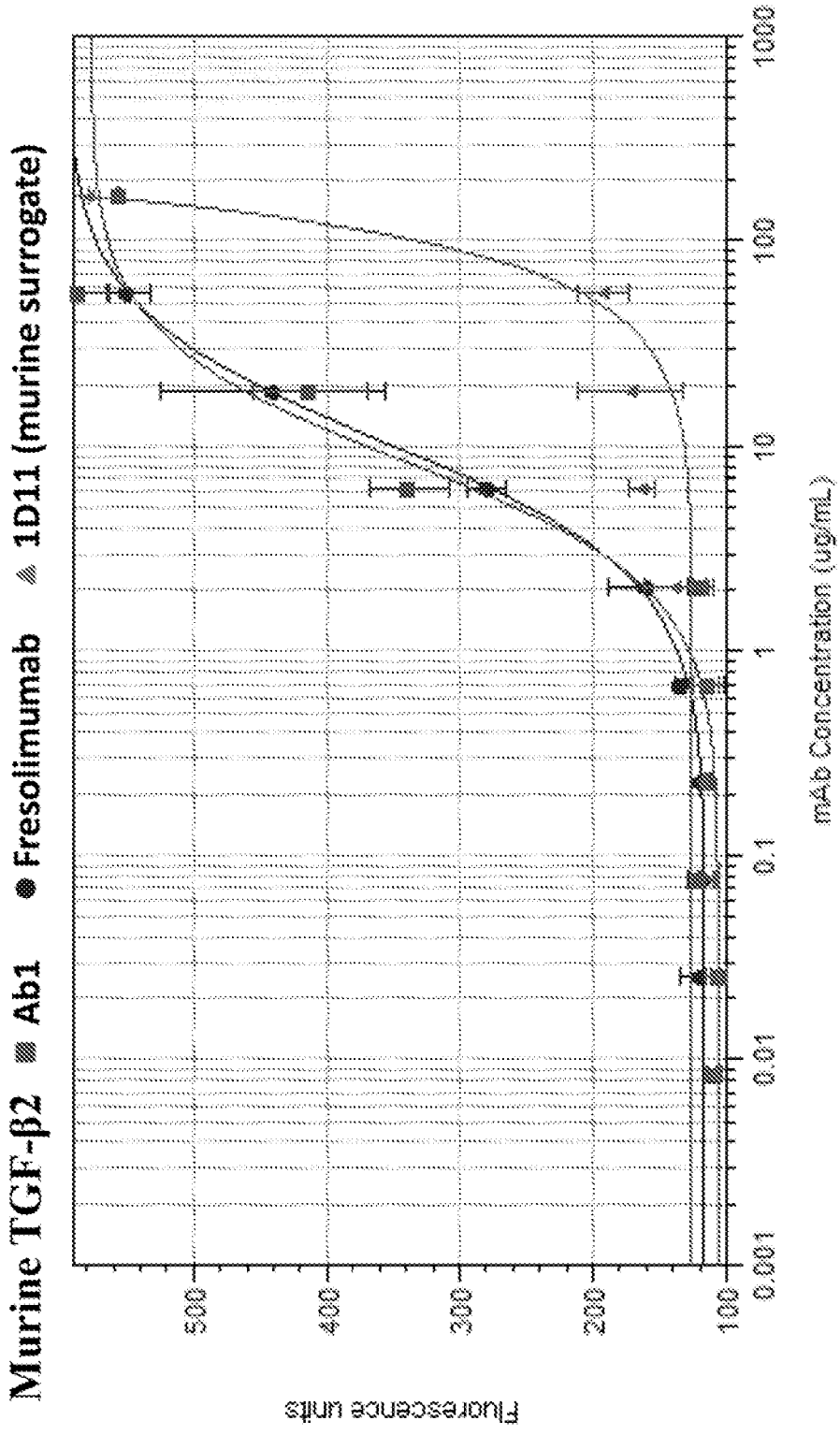

Murine TGF-β2
Ab1
Fresolimumab
1D11 (murine surrogate)
Fluorescence units
100
200
300
400
500
0.001
0.01
0.1
1
10
100
1000
mAb Concentration (ug/mL)

FIG. 1E

FIG. 2

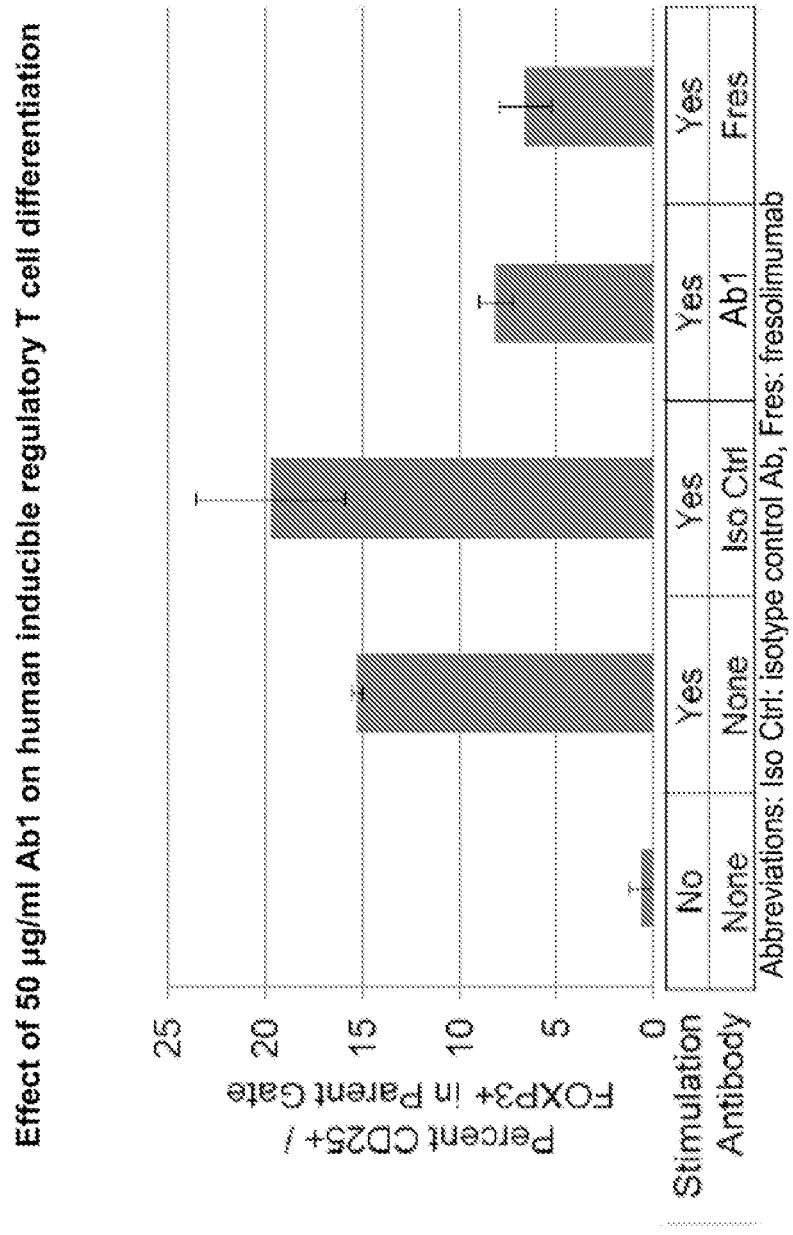
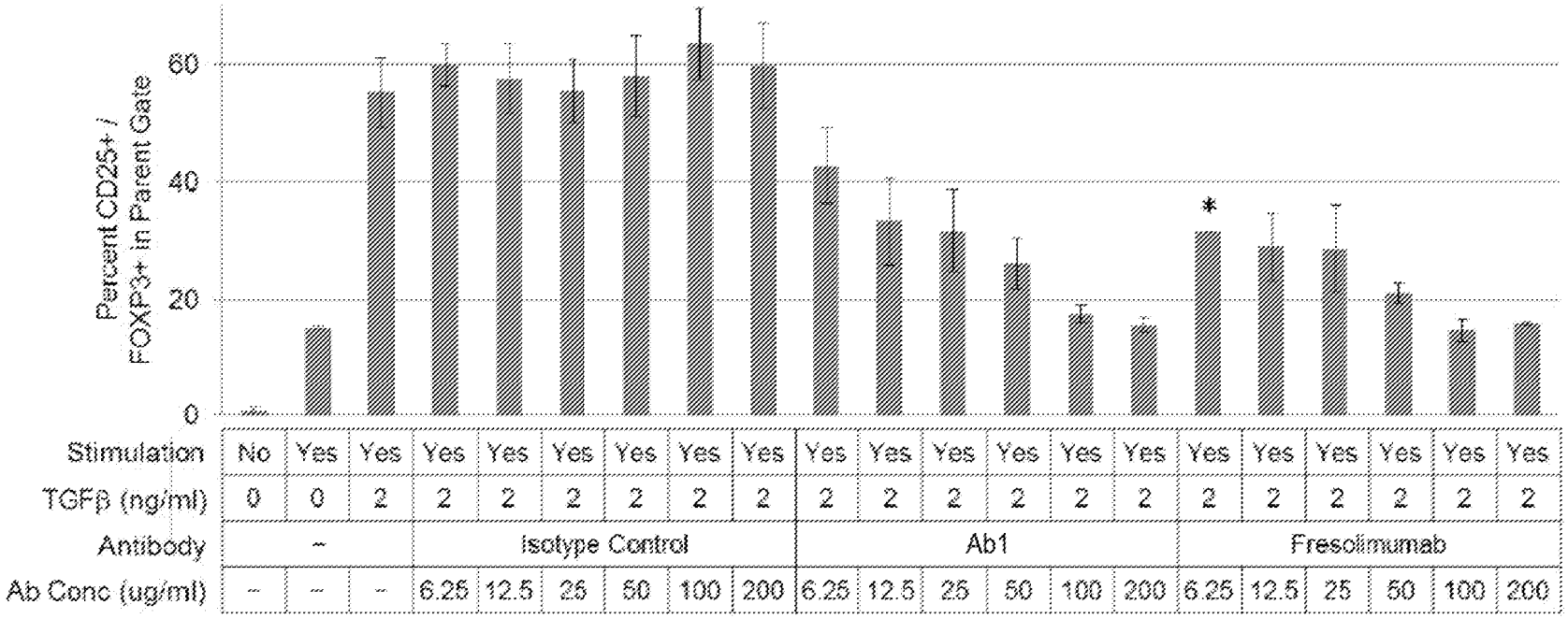

Effect of 50 µg/ml Ab1 on human inducible regulatory T cell differentiation
Percent CD25+ / FOXP3+ in Parent Gate
25
20
15
10
5
0
Stimulation
Antibody
No
None
Yes
None
Yes
Iso Ctrl
Yes
Ab1
Yes
Fres
Abbreviations: Iso Ctrl: isotype control Ab, Fres: fresolimumab

FIG. 3

Effect of Ab1 on human inducible regulatory T cell differentiation in cultures treated with 2 ng/ml TGF-β1



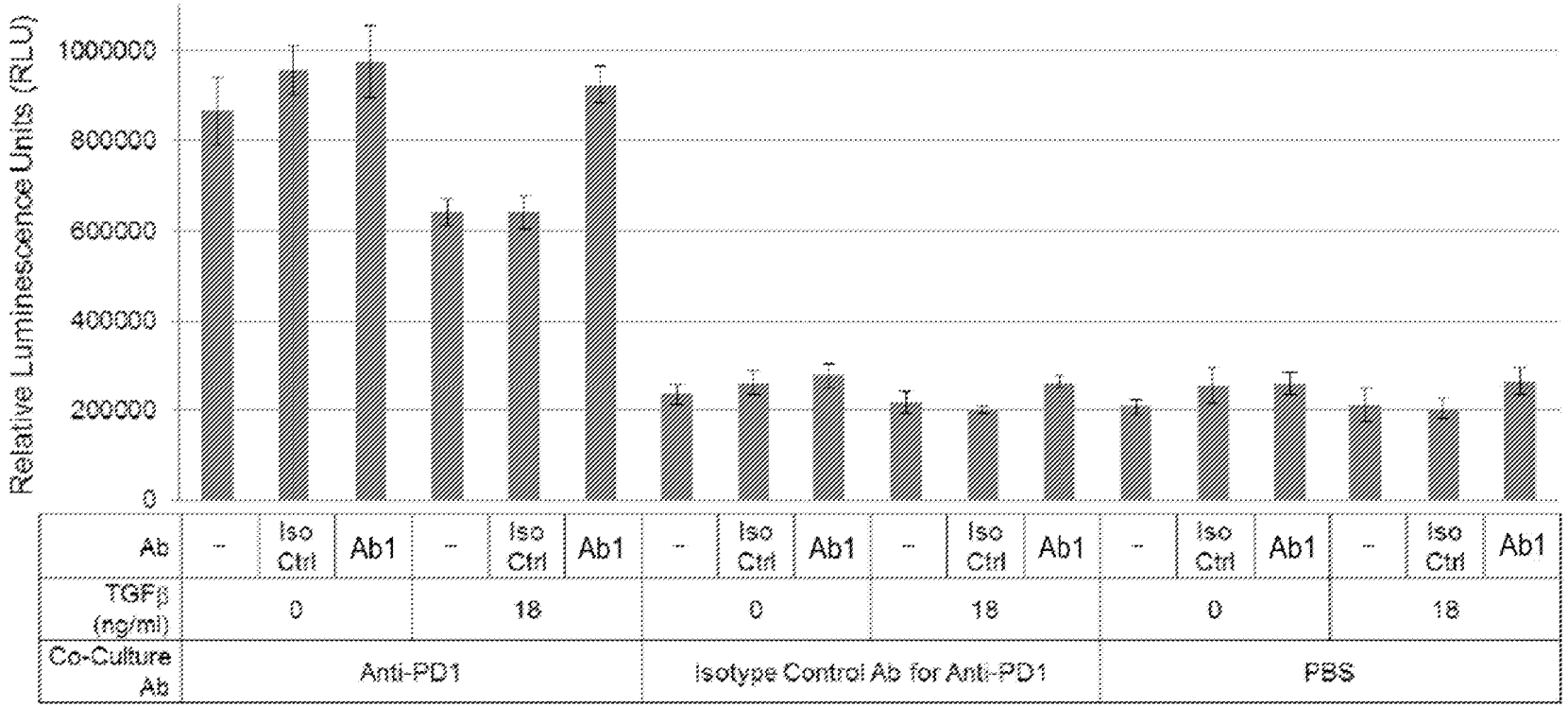
Stimulation	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
TGFβ (ng/ml)	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Antibody	–			Isotype Control						Ab1							Fresolimumab					
Ab Conc (ug/ml)	–	–	–	6.25	12.5	25	50	100	200	6.25	12.5	25	50	100	200		6.25	12.5	25	50	100	200

Abbreviation: Ab Conc: antibody concentration

*: single sample

FIG. 4

Effect of Ab1 (30 µg/ml) and TGF-β1 (18 ng/ml) on NFATc-driven luciferase expression on Jurkat T cells following T cell stimulation and anti-PD-1 treatment



Abbreviations: Ab: Antibody, Iso Ctrl: isotype control Ab

FIG. 5

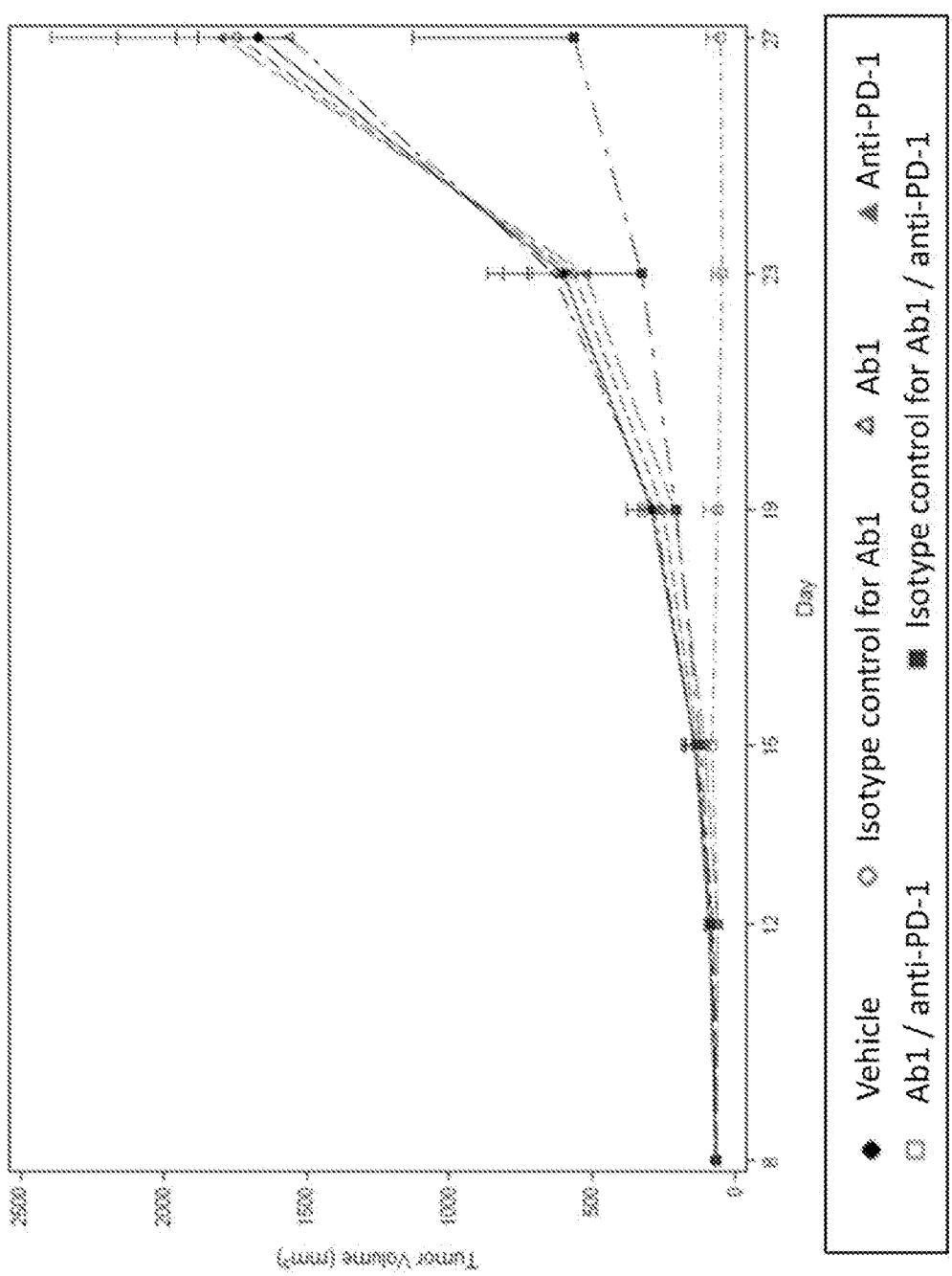
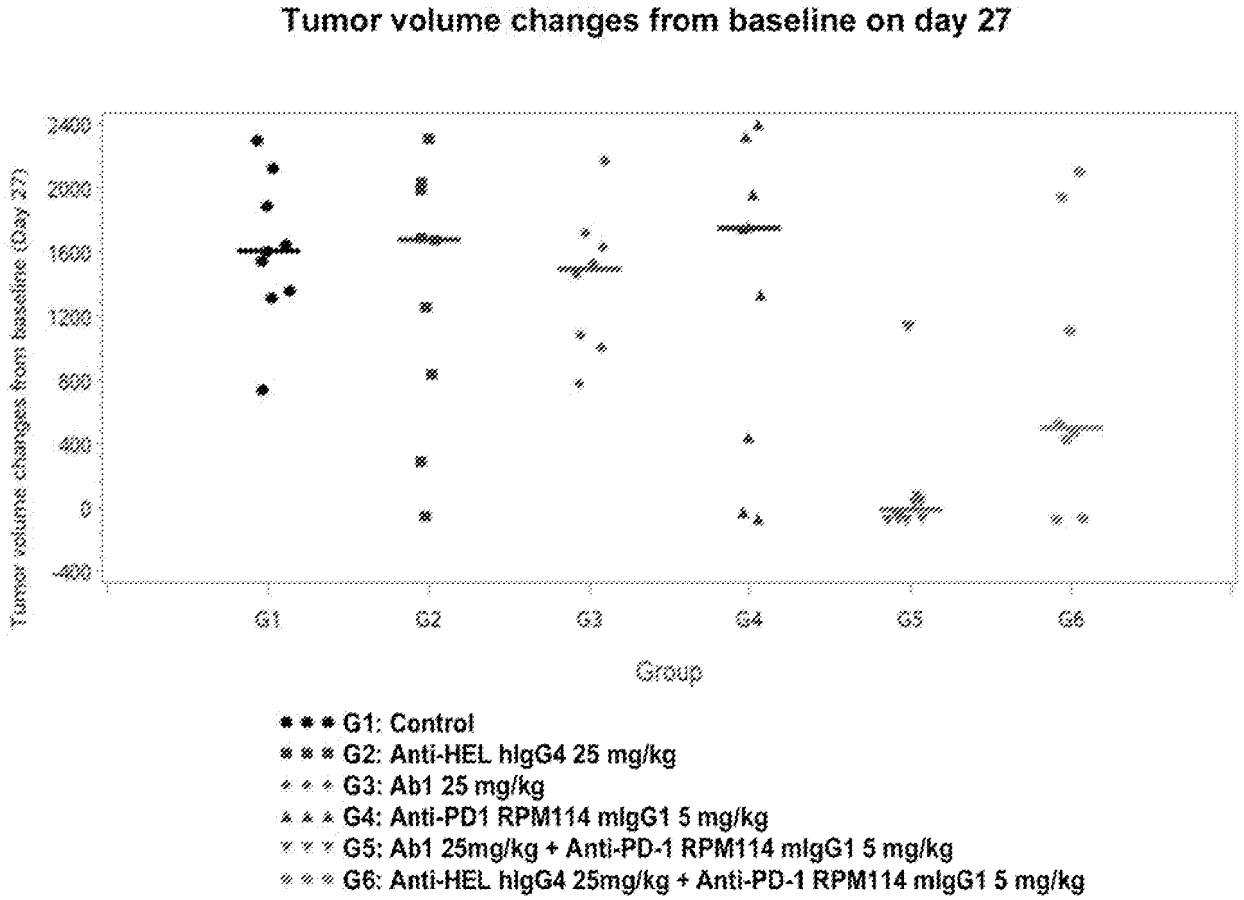

Tumor volume (mm^3) by treatment group – medians and MAD
Tumor volume (mm^3)
2500
2000
1500
1000
500
0
8
12
15
19
23
27
Day
Vehicle
Isotype control for Ab1
Ab1
Anti-PD-1
Ab1 / anti-PD-1
Isotype control for Ab1 / anti-PD-1

FIG. 6



*Points represent individual tumor volume changes from baseline on day 27, bars correspond to medians.

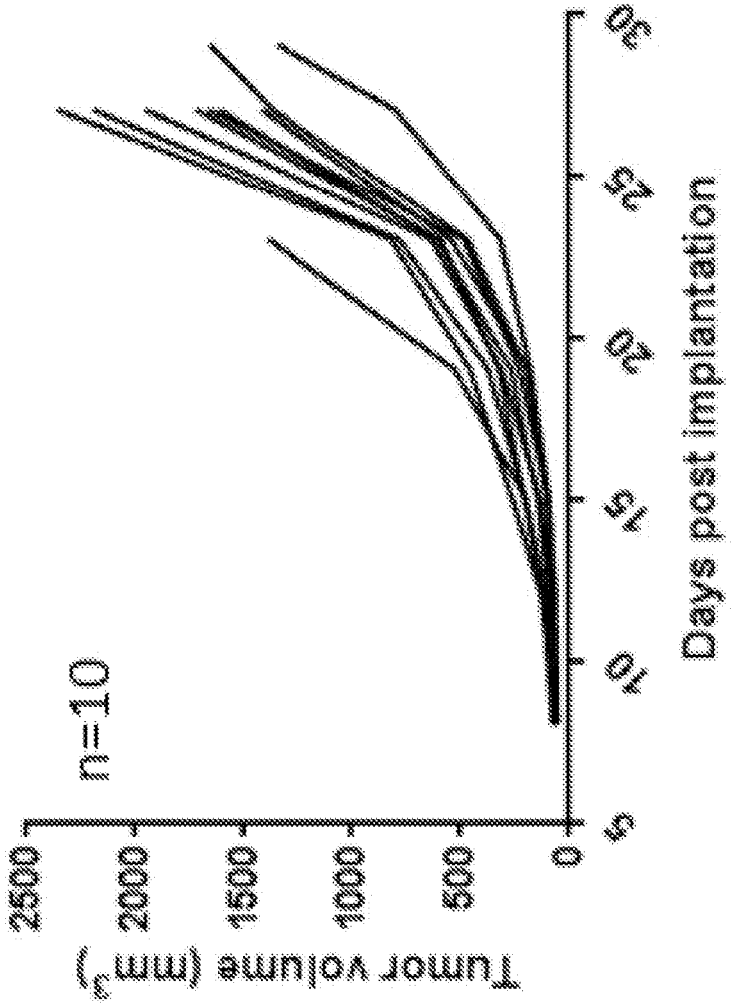

PBS
n=10
Tumor volume (mm3)
2500
2000
1500
1000
500
0
5
10
15
20
25
30
Days post implantation

FIG. 7A

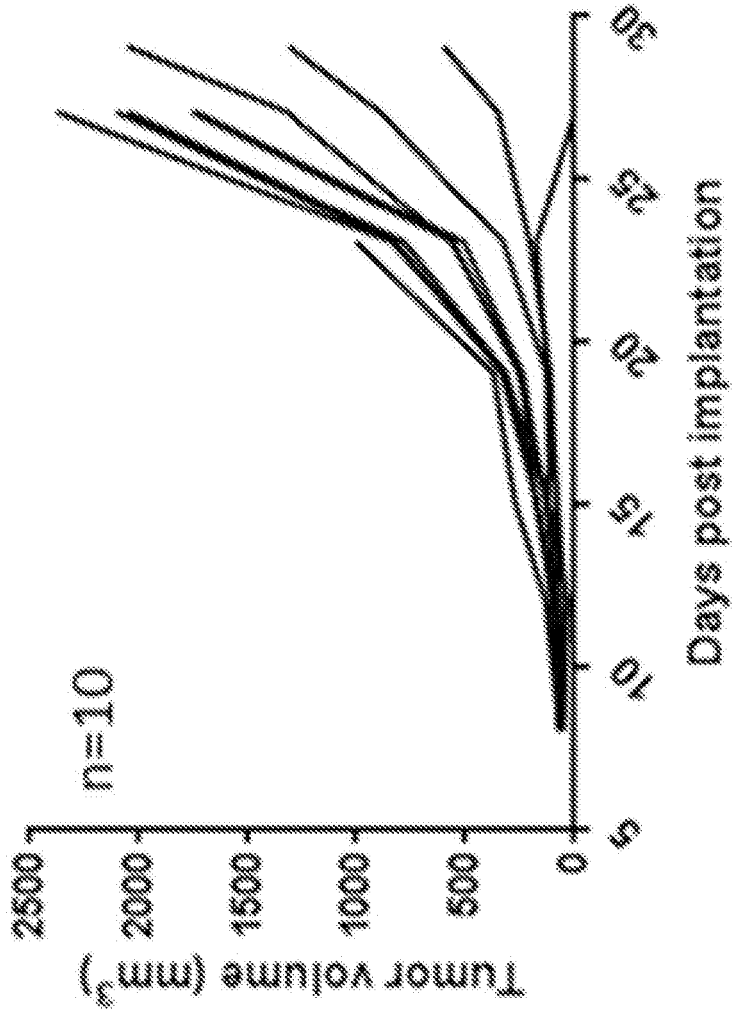

Ab1 Isotype Ctrl (25mpk)
n=10
Tumor volume (mm³)
2500
2000
1500
1000
500
0
5
10
15
20
25
30
Days post implantation

FIG. 7B

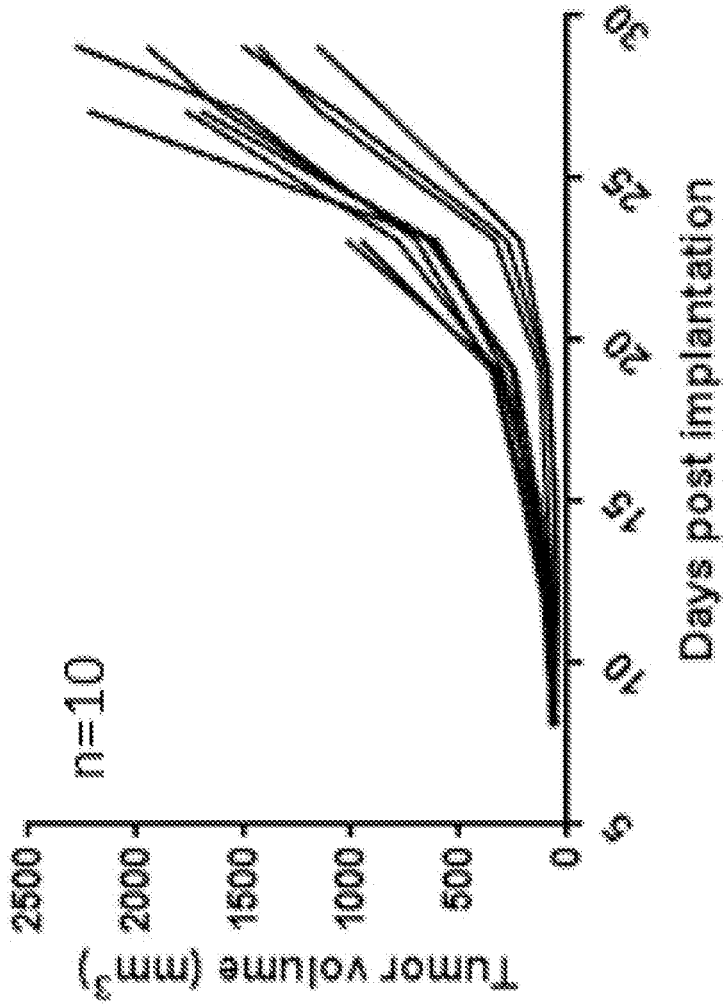

Ab1 (25mpk)
n=10
Tumor volume (mm³)
2500
2000
1500
1000
500
0
0
10
15
20
25
30
Days post implantation

FIG. 7C

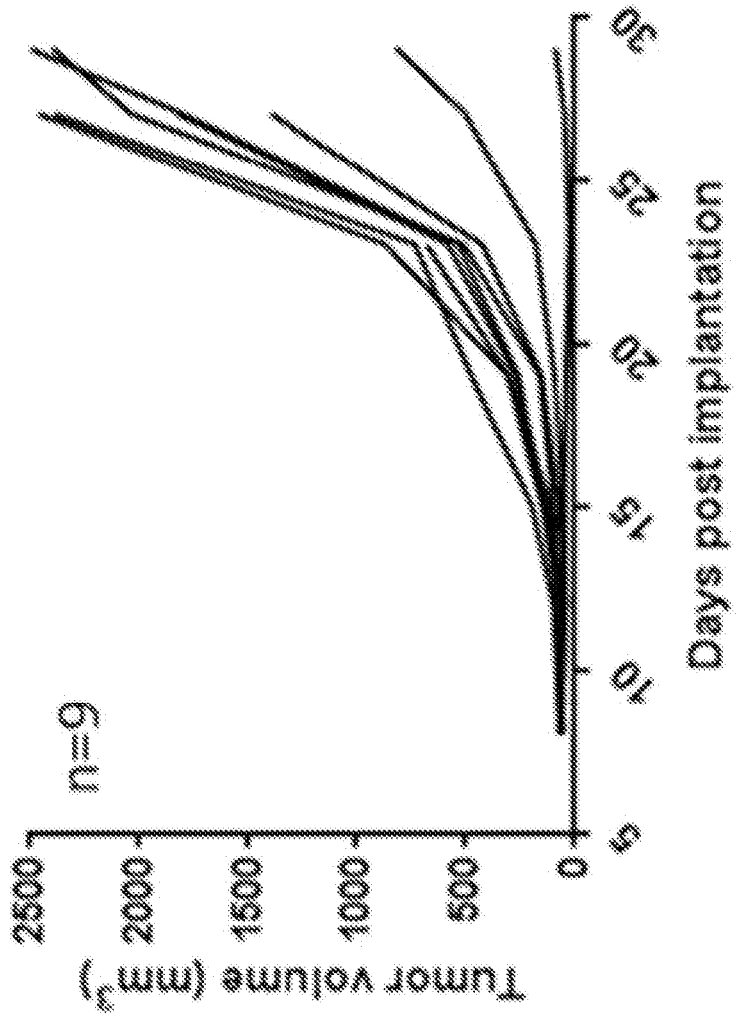

αPD1 (5mpk)
n=9
Tumor volume (mm³)
2500
2000
1500
1000
500
0
5
10
15
20
25
30
Days post implantation

FIG. 7D

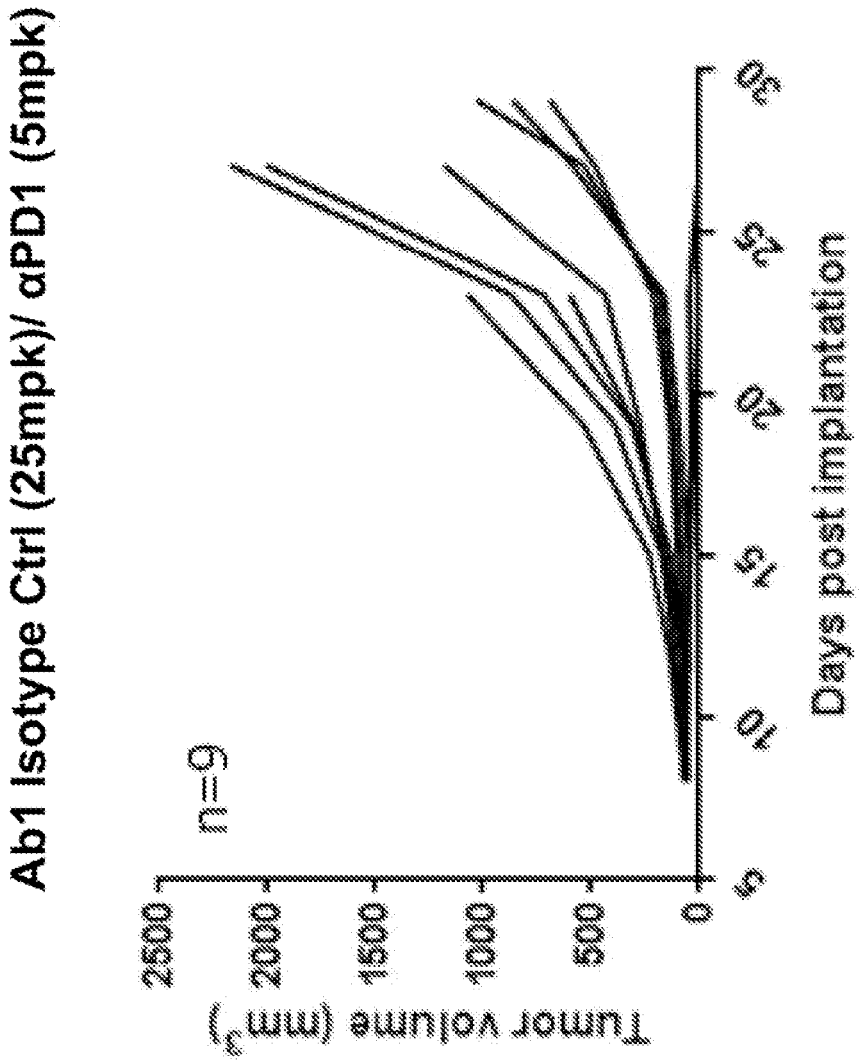

Ab1 Isotype Ctrl (25mpk)/ αPD1 (5mpk)
n=9
Tumor volume (mm³)
2500
2000
1500
1000
500
0
5
10
15
20
25
30
Days post implantation

FIG. 7E

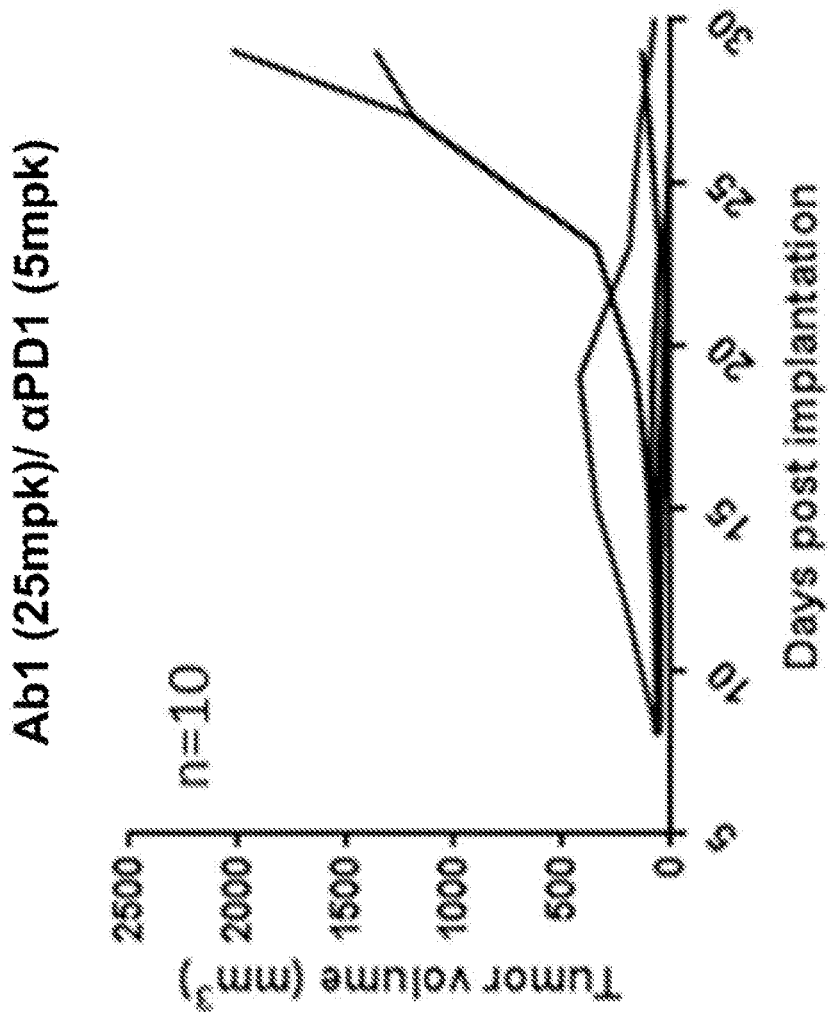

Ab1 (25mpk)/ αPD1 (5mpk)
n=10
Tumor volume (mm³)
2500
2000
1500
1000
500
0
5
10
15
20
25
30
Days post implantation

FIG. 7F

FIG. 8

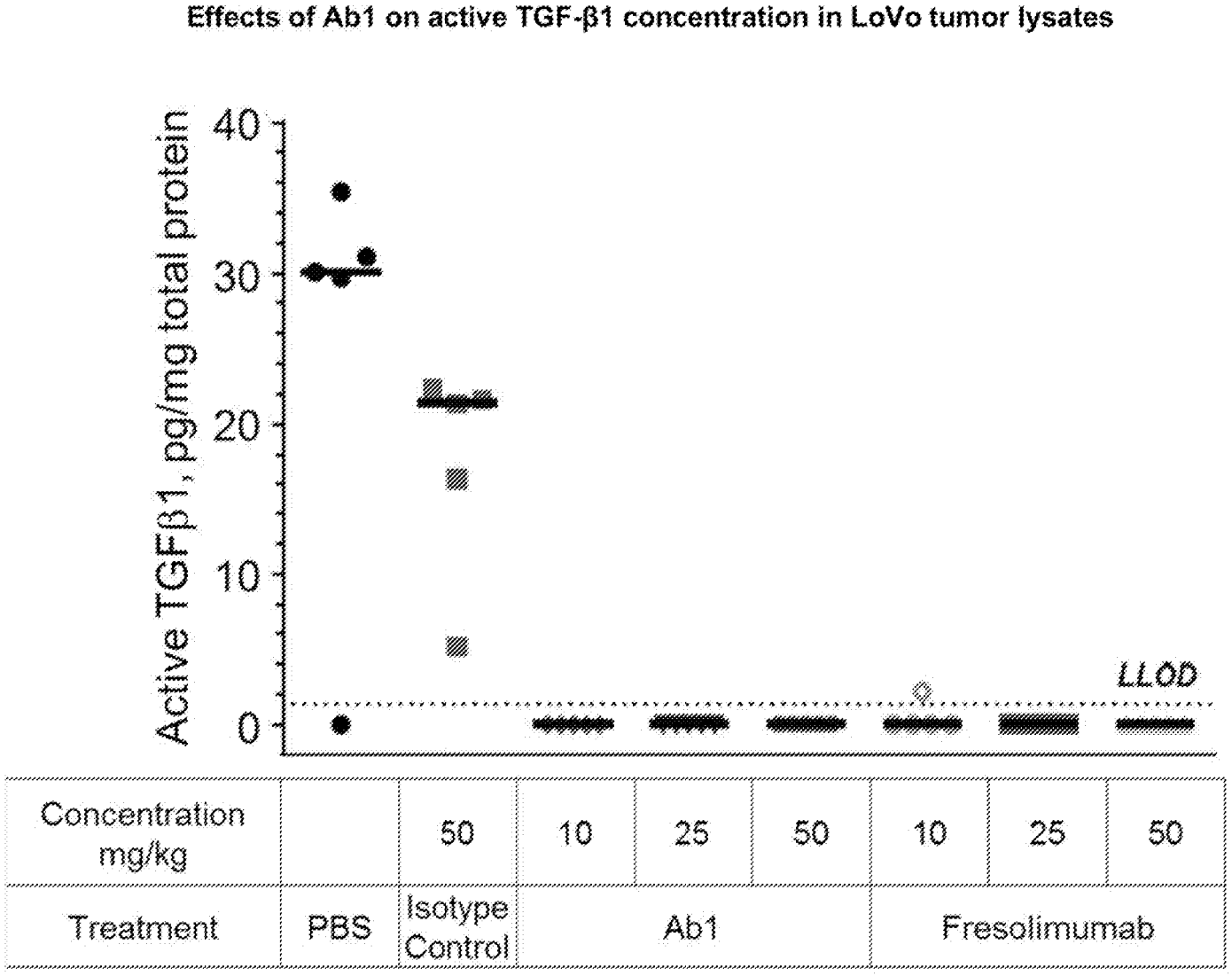

Effects of Ab1 on active TGF-β1 concentration in LoVo tumor lysates
Active TGFβ1, pg/mg total protein
40
30
20
10
0
LLOD
Concentration mg/kg
50
10
25
50
10
25
50
Treatment
PBS
Isotype Control
Ab1
Fresolimumab

FIG. 9A

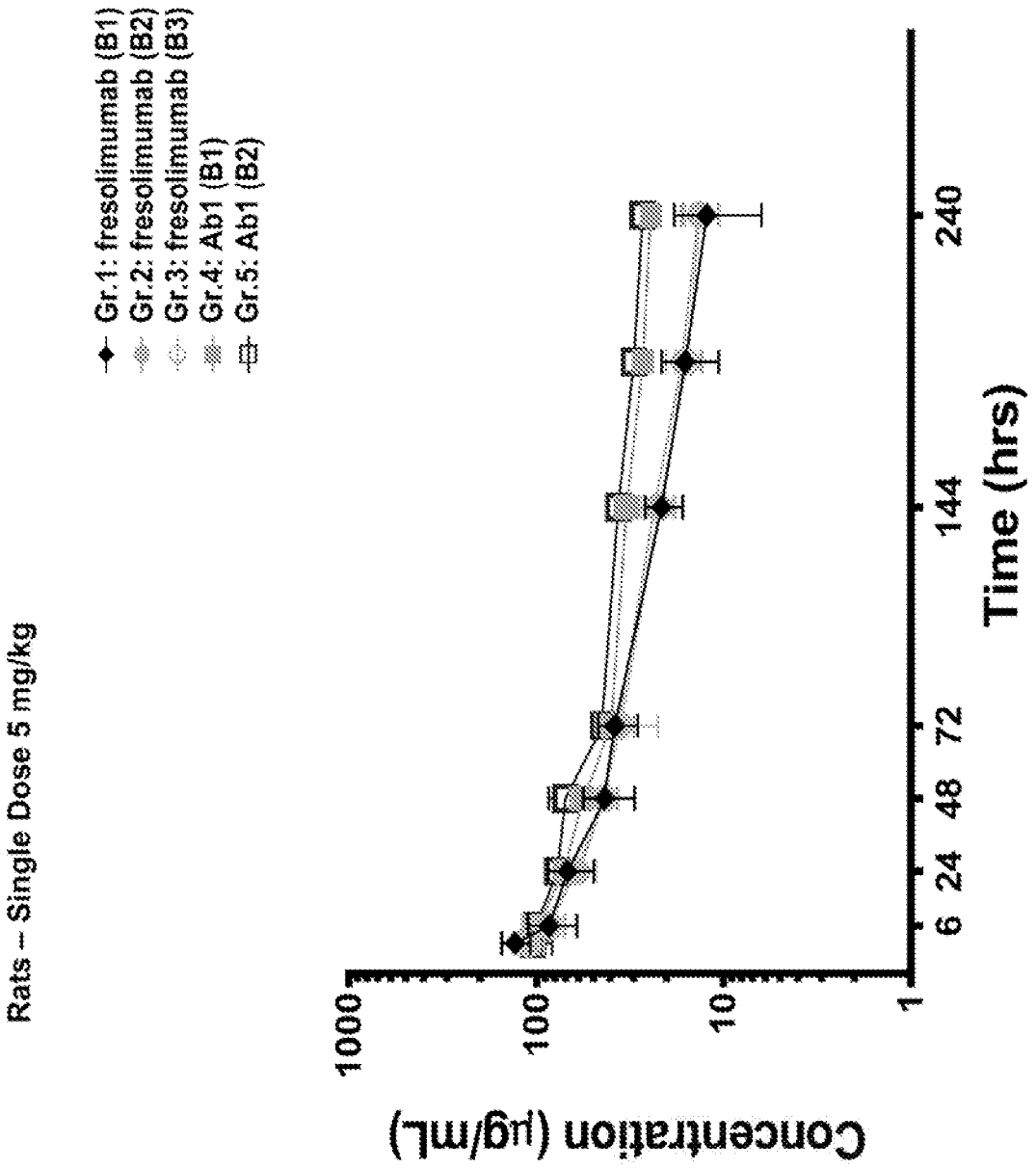

Rats – Single Dose 5 mg/kg
Gr.1: fresolimumab (B1)
Gr.2: fresolimumab (B2)
Gr.3: fresolimumab (B3)
Gr.4: Ab1 (B1)
Gr.5: Ab1 (B2)
Concentration (µg/mL)
1000
100
10
1
6
24
48
72
144
240
Time (hrs)

FIG. 9B

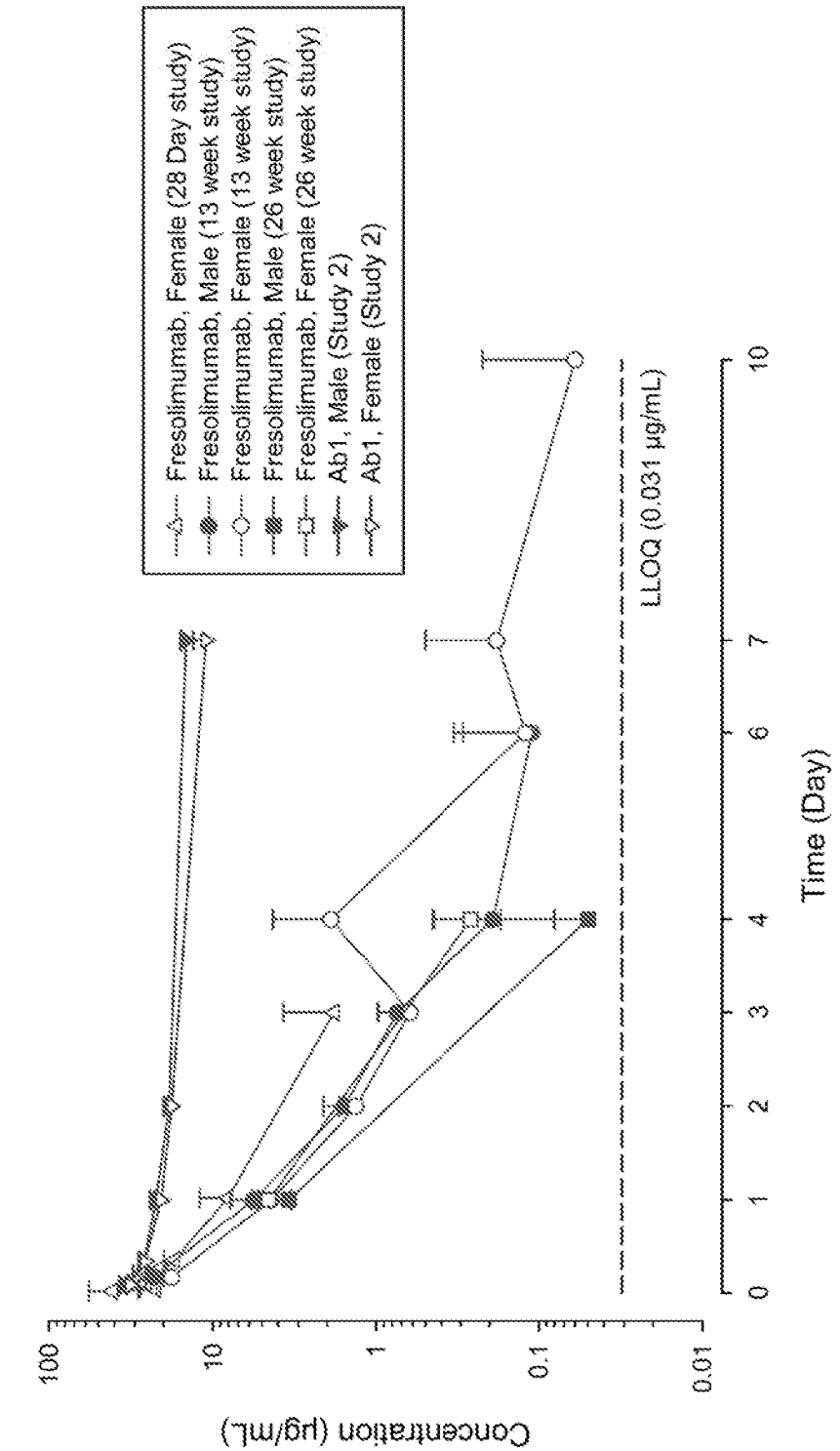
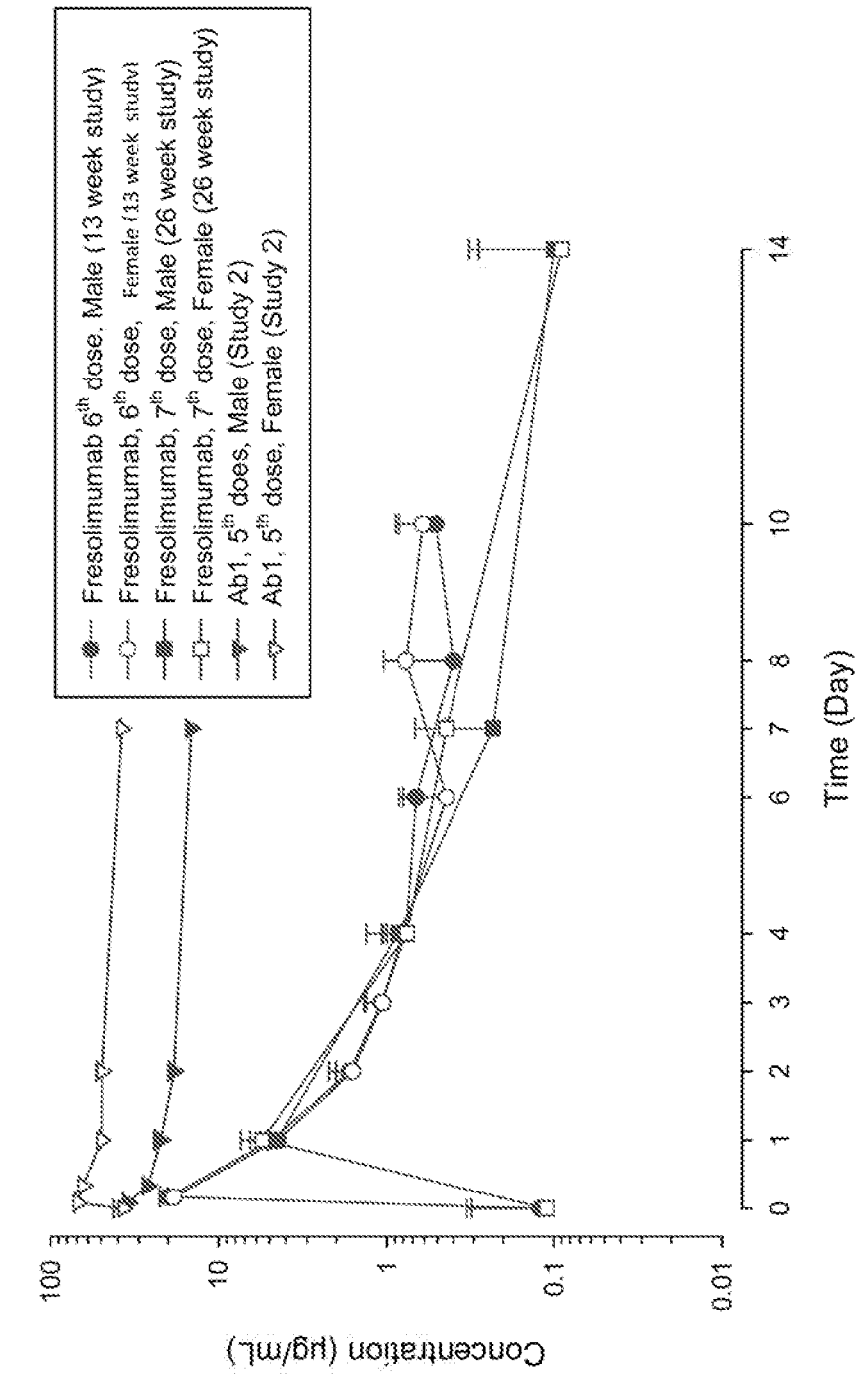

Monkey - Single dose 1 mg/kg
Fresolimumab, Female (28 Day study)
Fresolimumab, Male (13 week study)
Fresolimumab, Female (13 week study)
Fresolimumab, Male (26 week study)
Fresolimumab, Female (26 week study)
Ab1, Male (Study 2)
Ab1, Female (Study 2)
LLOQ (0.031 µg/mL)
Concentration (µg/mL)
100
10
1
0.1
0.01
0
1
2
3
4
6
7
10
Time (Day)

FIG. 9C


Monkey - repeated dose 1 mg/kg
Fresolimumab 6th dose, Male (13 week study)
Fresolimumab, 6th dose, Female (13 week study)
Fresolimumab, 7th dose, Male (26 week study)
Fresolimumab, 7th dose, Female (26 week study)
Ab1, 5th does, Male (Study 2)
Ab1, 5th dose, Female (Study 2)
Concentration (μg/mL)
100
10
1
0.1
0.01
0
1
2
3
4
6
7
8
10
14
Time (Day)

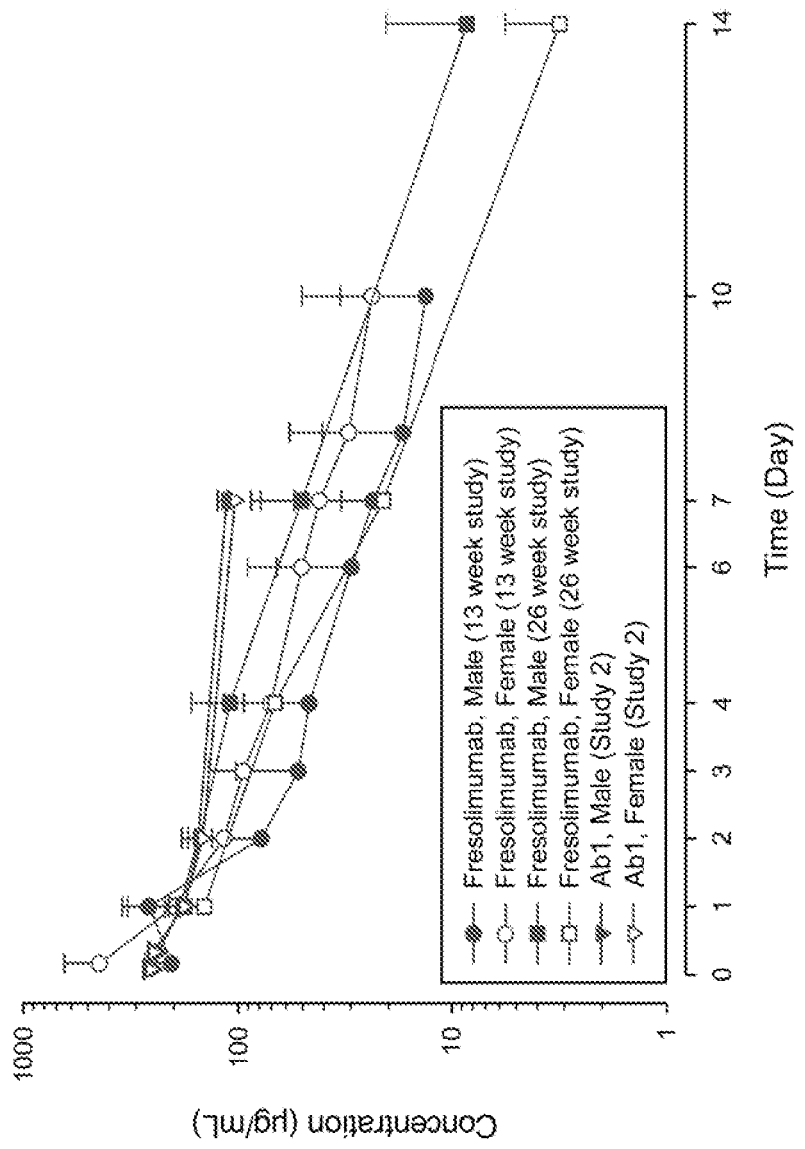

Monkey - Single dose 10 mg/kg
Concentration (µg/mL)
1000
100
10
1
0
1
2
3
4
6
7
10
14
Time (Day)
Fresolimumab, Male (13 week study)
Fresolimumab, Female (13 week study)
Fresolimumab, Male (26 week study)
Fresolimumab, Female (26 week study)
Ab1, Male (Study 2)
Ab1, Female (Study 2)

FIG. 9D

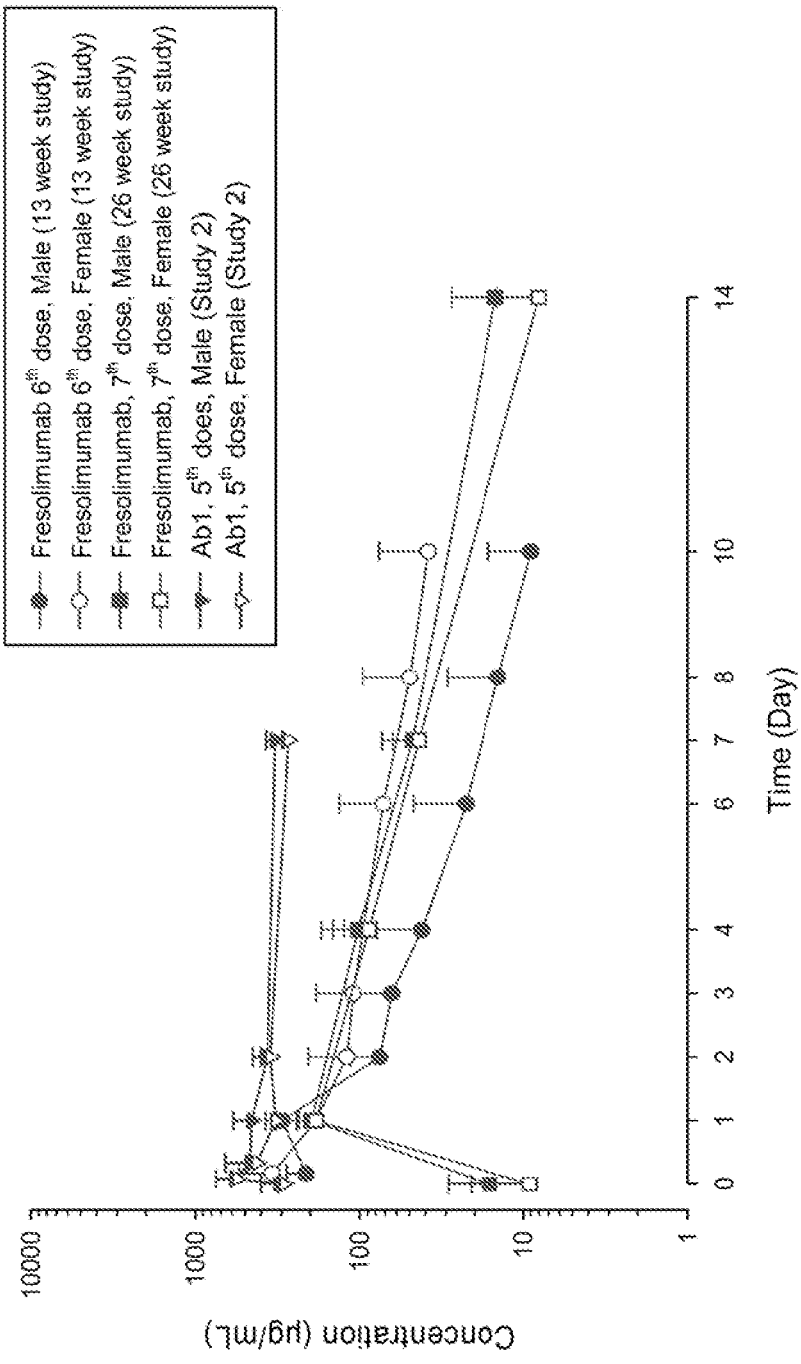

Monkey - repeated dose 10 mg/kg
Fresolimumab 6th dose, Male (13 week study)
Fresolimumab 6th dose, Female (13 week study)
Fresolimumab, 7th dose, Male (26 week study)
Fresolimumab, 7th dose, Female (26 week study)
Ab1, 5th does, Male (Study 2)
Ab1, 5th dose, Female (Study 2)
Concentration (µg/mL)
10000
1000
100
10
1
0
1
2
3
4
6
7
8
10
14
Time (Day)

FIG. 9E

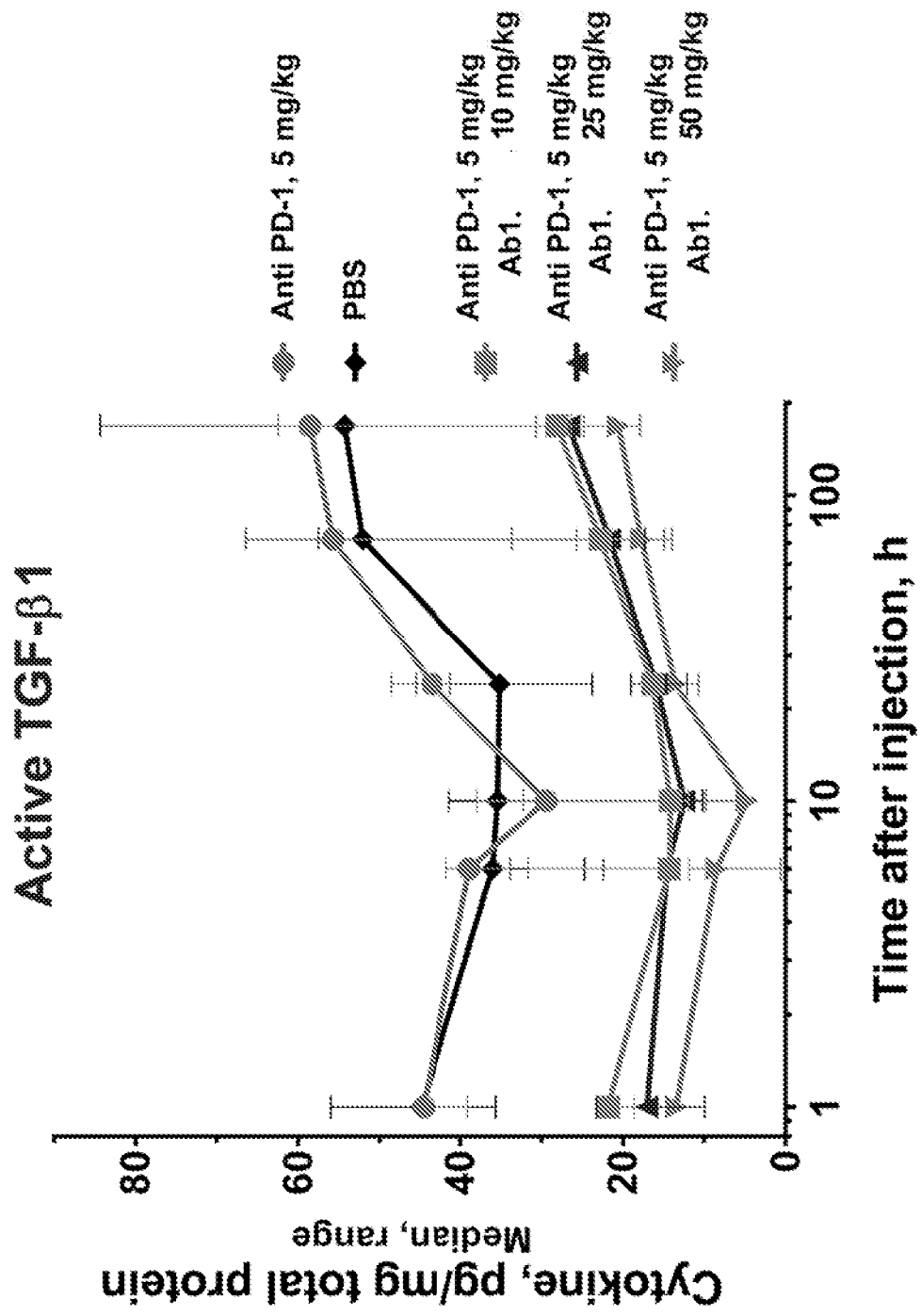

Active TGF-β1
Cytokine, pg/mg total protein
Median, range
0
20
40
60
80
1
10
100
Time after injection, h
Anti PD-1, 5 mg/kg
PBS
Anti PD-1, 5 mg/kg Ab1. 10 mg/kg
Anti PD-1, 5 mg/kg Ab1. 25 mg/kg
Anti PD-1, 5 mg/kg Ab1. 50 mg/kg

FIG. 10A

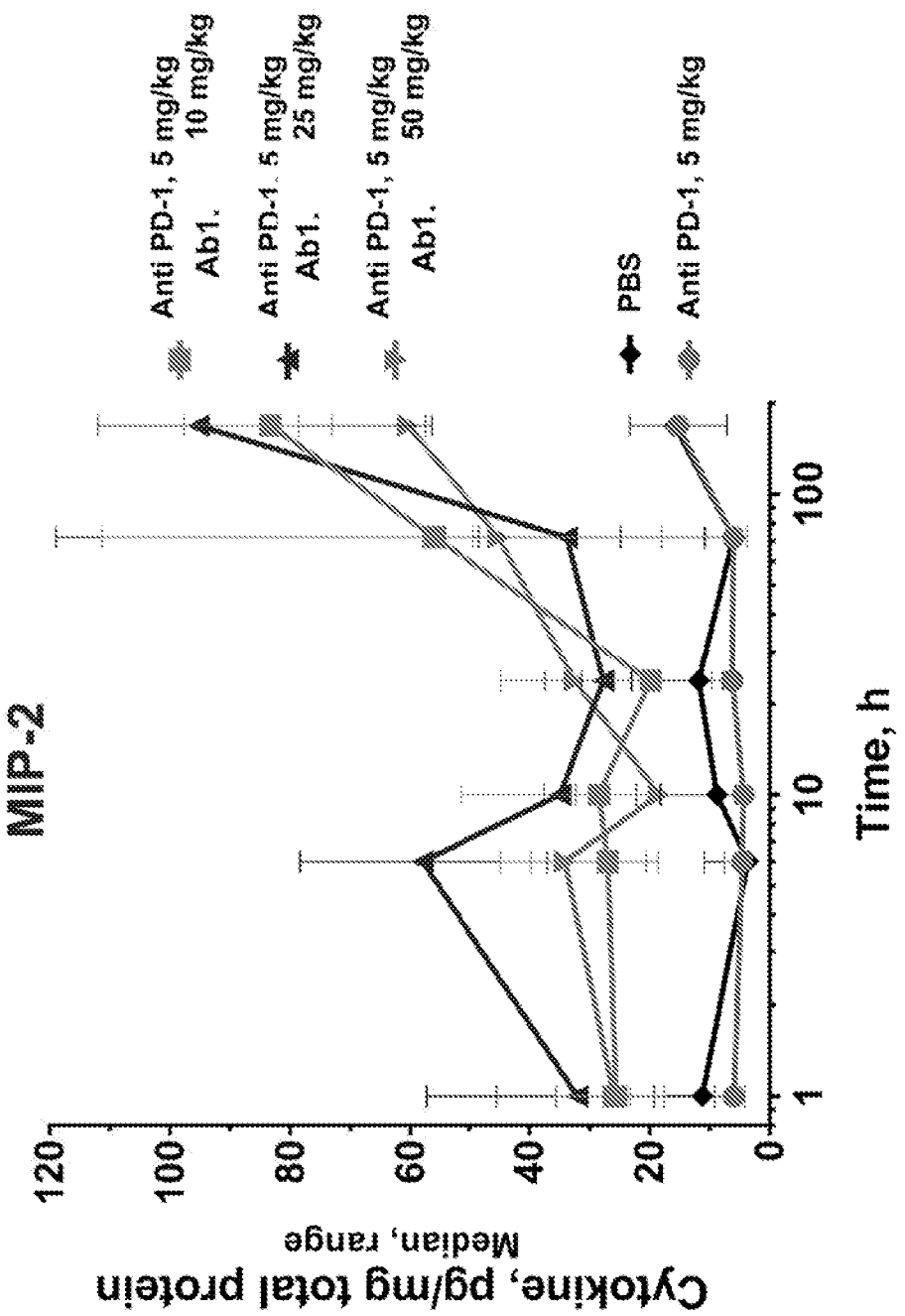

MIP-2
Cytokine, pg/mg total protein
Median, range
Time, h
0
20
40
60
80
100
120
1
10
100
Anti PD-1, 5 mg/kg
Ab1, 10 mg/kg
Anti PD-1, 5 mg/kg
Ab1, 25 mg/kg
Anti PD-1, 5 mg/kg
Ab1, 50 mg/kg
PBS
Anti PD-1, 5 mg/kg

FIG. 10B

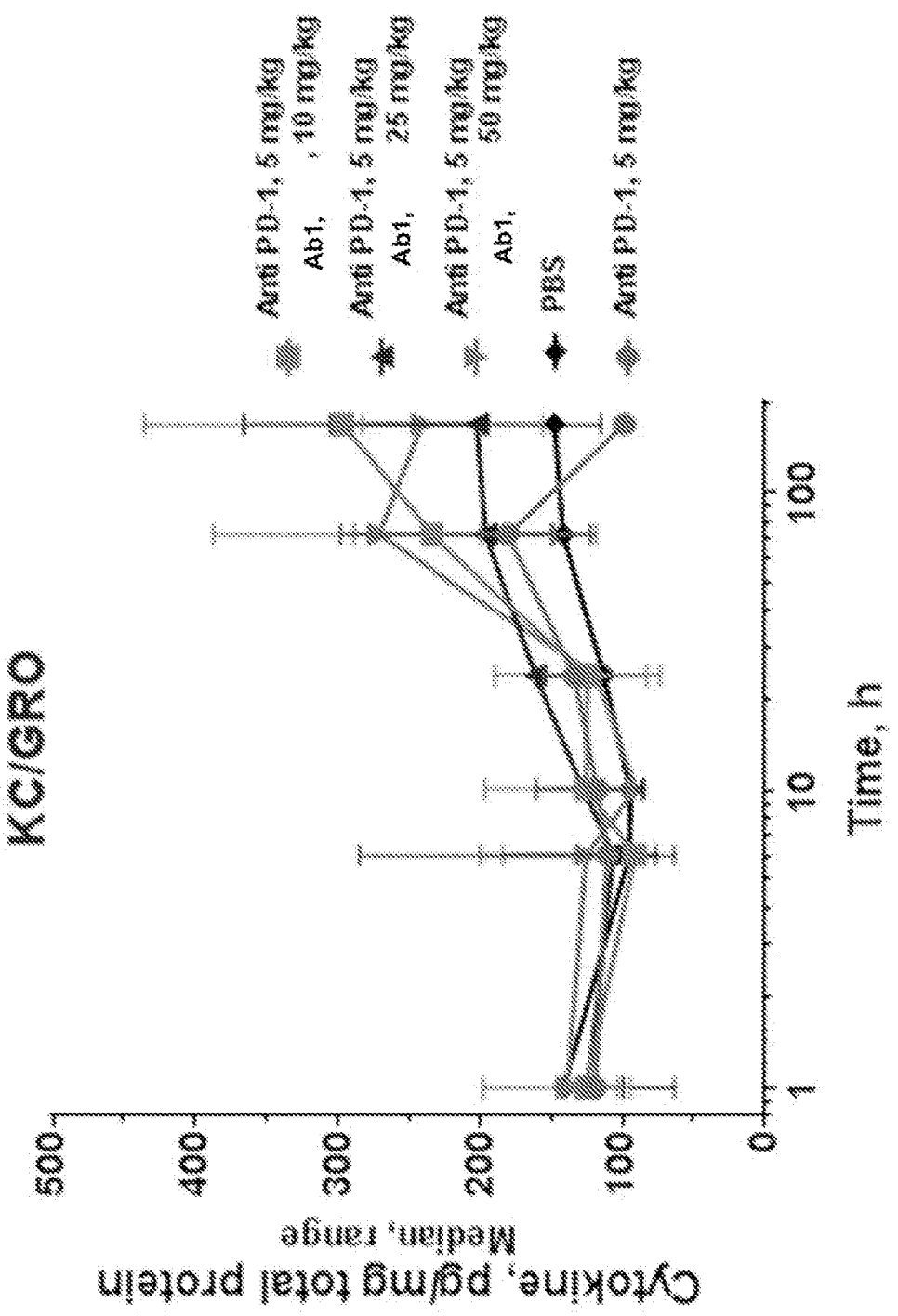
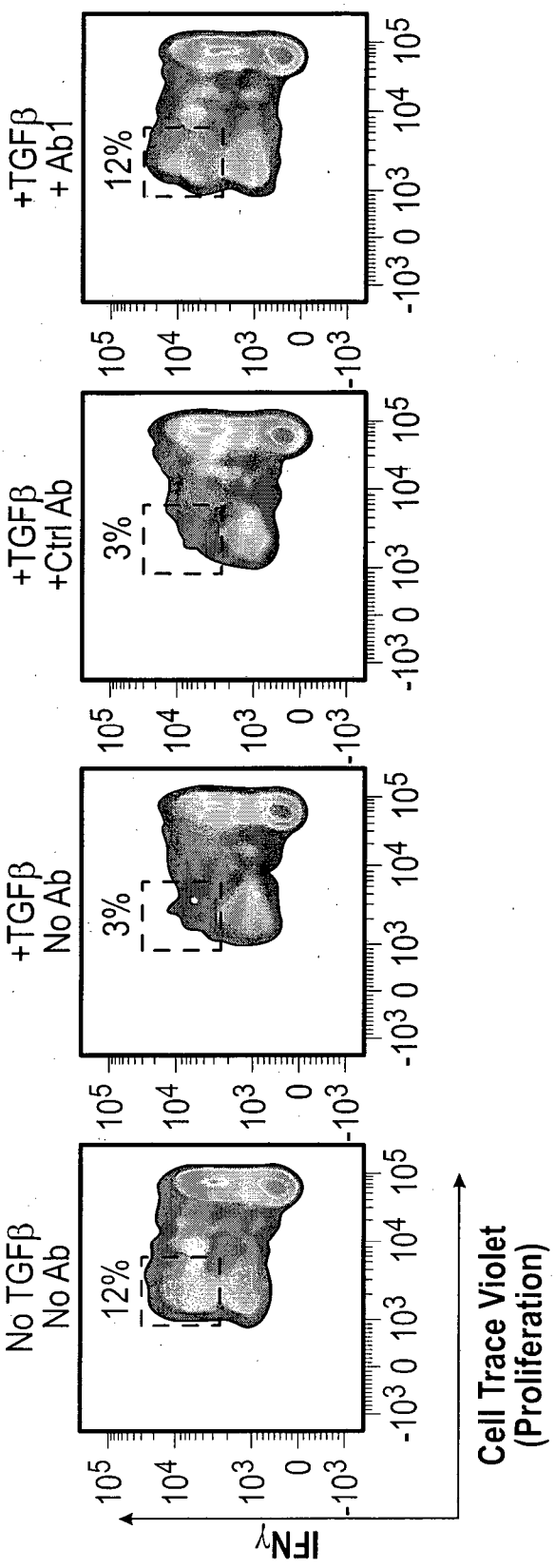

KC/GRO
Cytokine, pg/mg total protein
Median, range
500
400
300
200
100
0
1
10
100
Time, h
Anti PD-1, 5 mg/kg
Ab1, 10 mg/kg
Anti PD-1, 5 mg/kg
Ab1, 25 mg/kg
Anti PD-1, 5 mg/kg
Ab1, 50 mg/kg
PBS
Anti PD-1, 5 mg/kg

FIG. 10C

FIG. 11A


No TGFβ
No Ab
12%
+TGFβ
No Ab
3%
+TGFβ
+Ctrl Ab
3%
+TGFβ
+ Ab1
12%
IFNγ
Cell Trace Violet
(Proliferation)

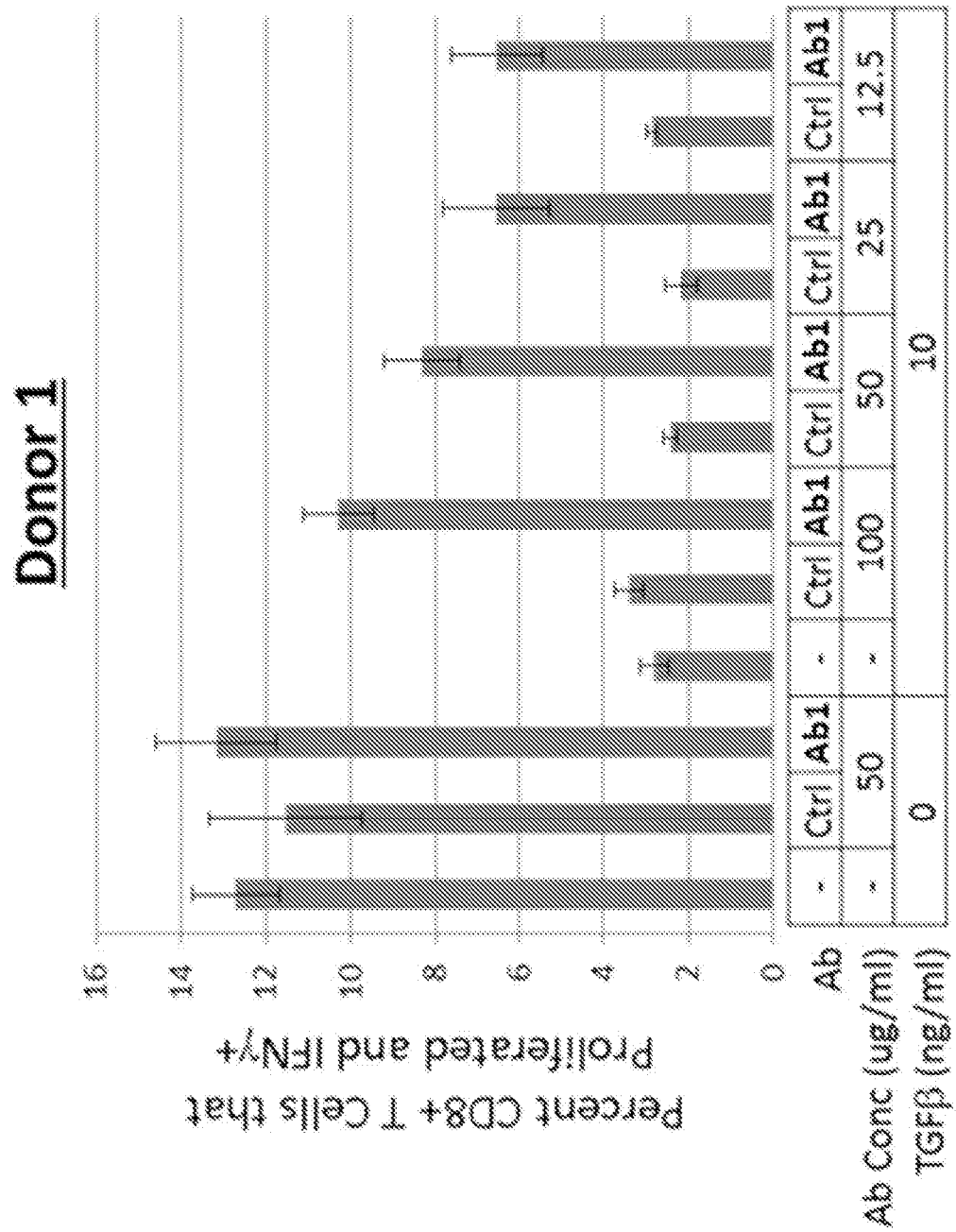

Donor 1
Percent CD8+ T Cells that Proliferated and IFNγ+
16
14
12
10
8
6
4
2
0
Ab
Ab Conc (ug/ml)
TGFβ (ng/ml)
- Ctrl Ab1 - Ctrl Ab1 Ctrl Ab1 Ctrl Ab1
- 50 - 100 50 25 12.5
0 10

FIG. 11B

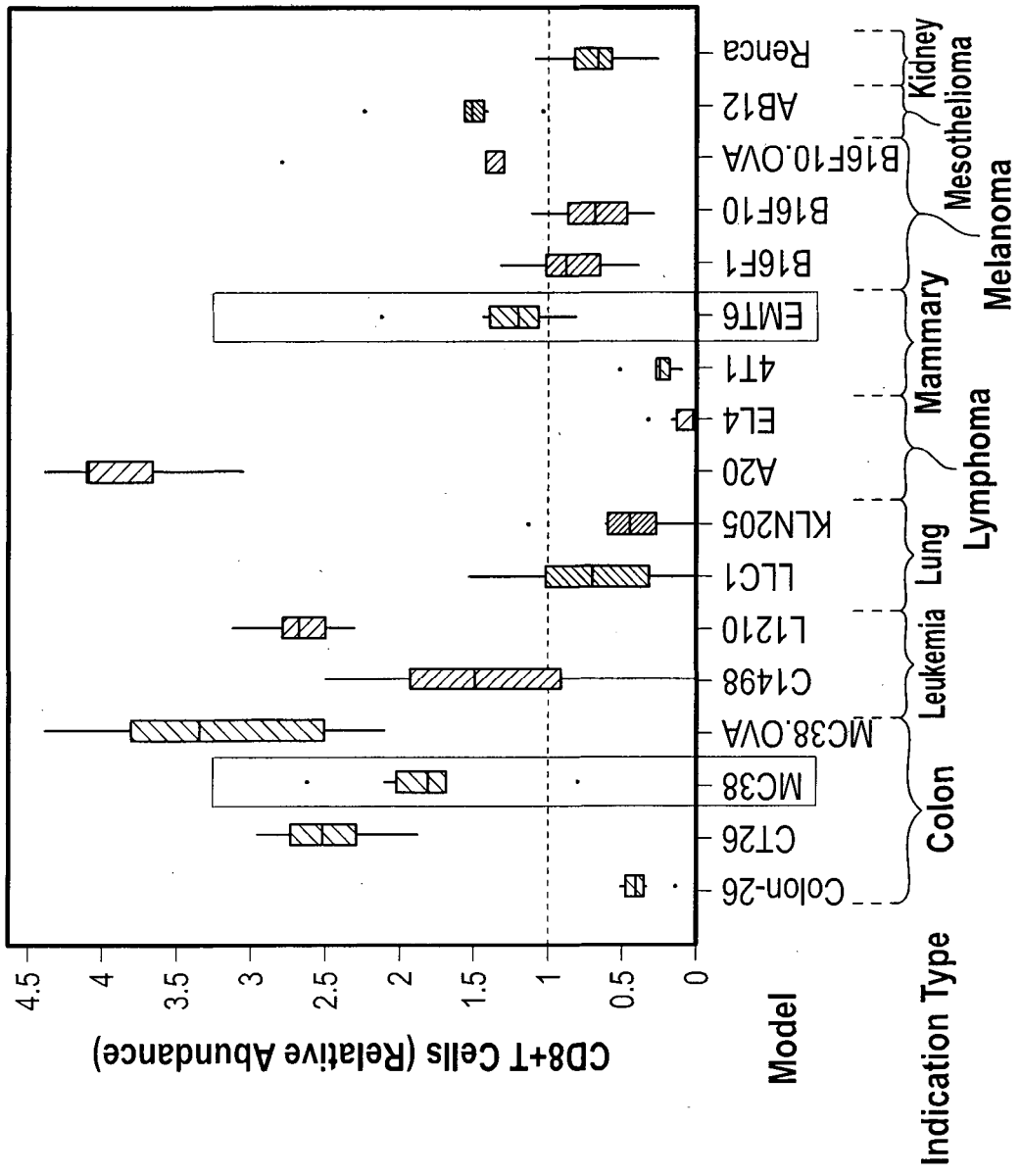
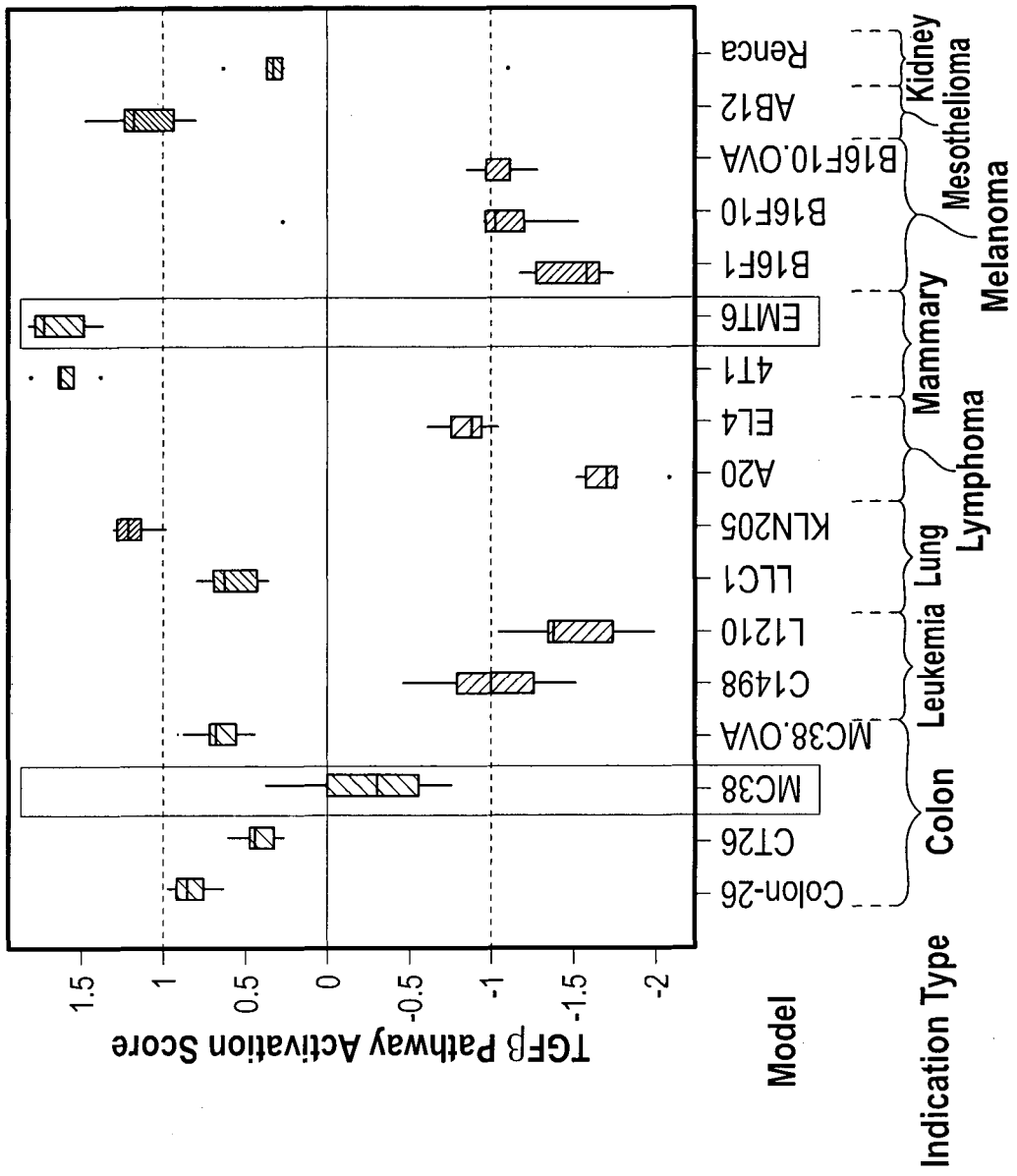

CD8+T Cells (Relative Abundance)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Model
Colon-26
CT26
MC38
MC38.OVA
C1498
L1210
LLC1
KLN205
A20
EL4
4T1
EMT6
B16F1
B16F10
B16F10.OVA
AB12
Renca
Indication Type
Colon
Leukemia
Lung
Lymphoma
Mammary
Melanoma
Mesothelioma
Kidney

FIG. 12A

FIG. 12B


TGFβ Pathway Activation Score
1.5
1
0.5
0
-0.5
-1
-1.5
-2
Model
Colon-26
CT26
MC38
MC38.OVA
C1498
L1210
LLC1
KLN205
A20
EL4
4T1
EMT6
B16F1
B16F10
B16F10.OVA
AB12
Renca
Indication Type
Colon
Leukemia
Lung
Lymphoma
Mammary
Mesothelioma
Melanoma
Kidney