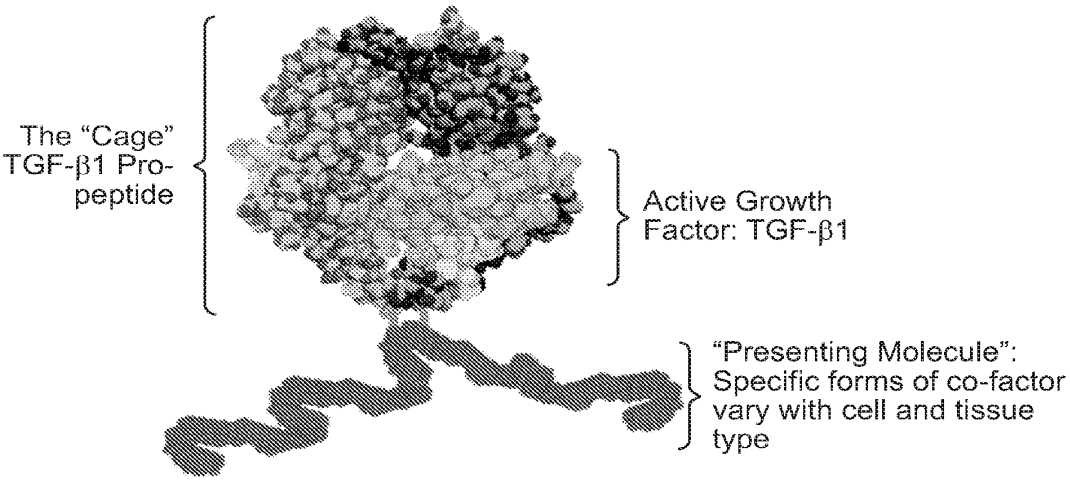

The "Cage"
TGF-β1 Pro-
peptide
Active Growth
Factor: TGF-β1
"Presenting Molecule":
Specific forms of co-factor
vary with cell and tissue
type

FIG. 1

FIG. 2B

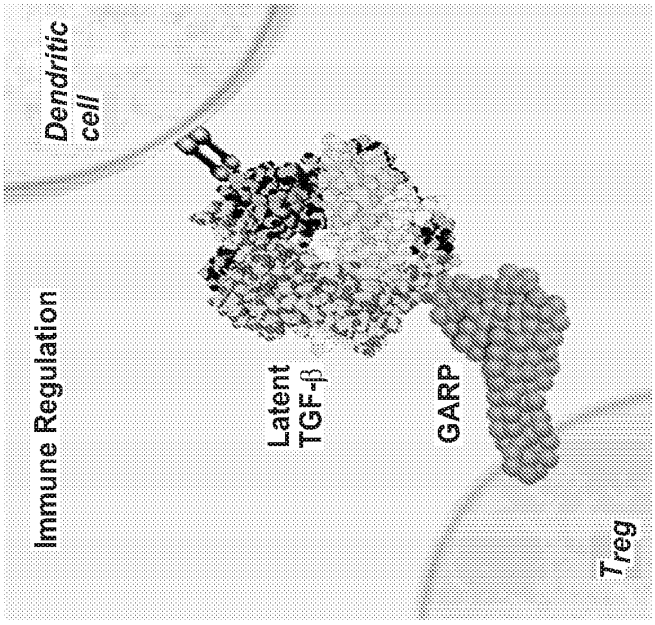

Dendritic cell
Immune Regulation
Latent TGF-β
GARP
Treg

FIG. 2A

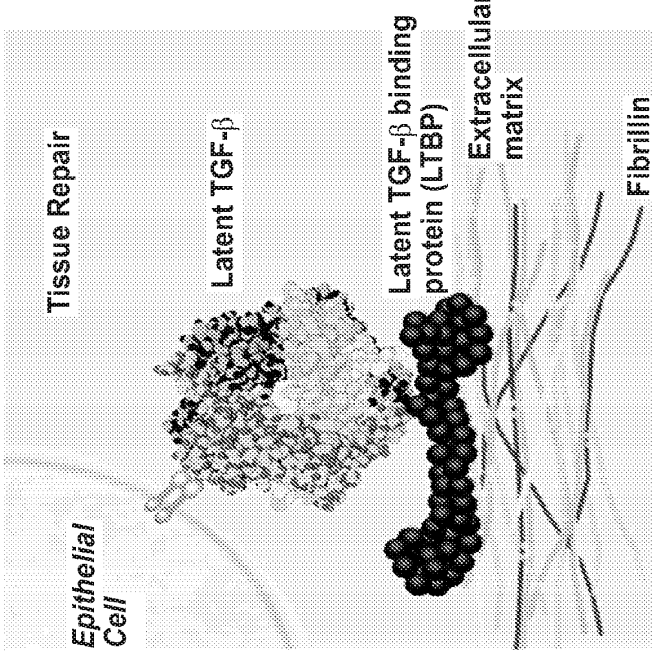

Tissue Repair
Latent TGF-β
Latent TGF-β binding protein (LTBP)
Extracellular matrix
Fibrillin
Epithelial Cell

FIG. 3

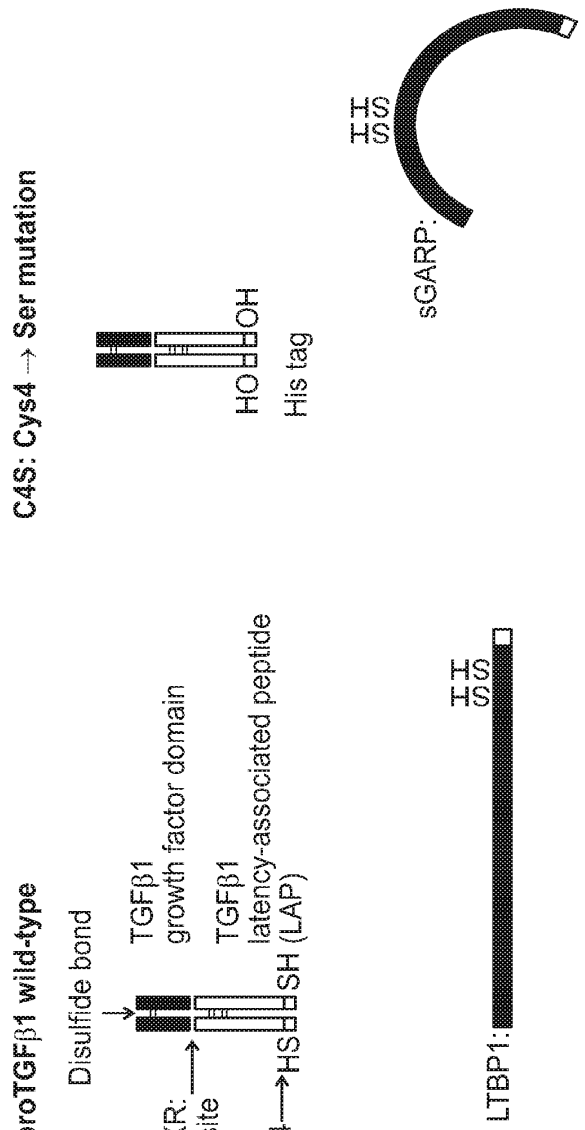
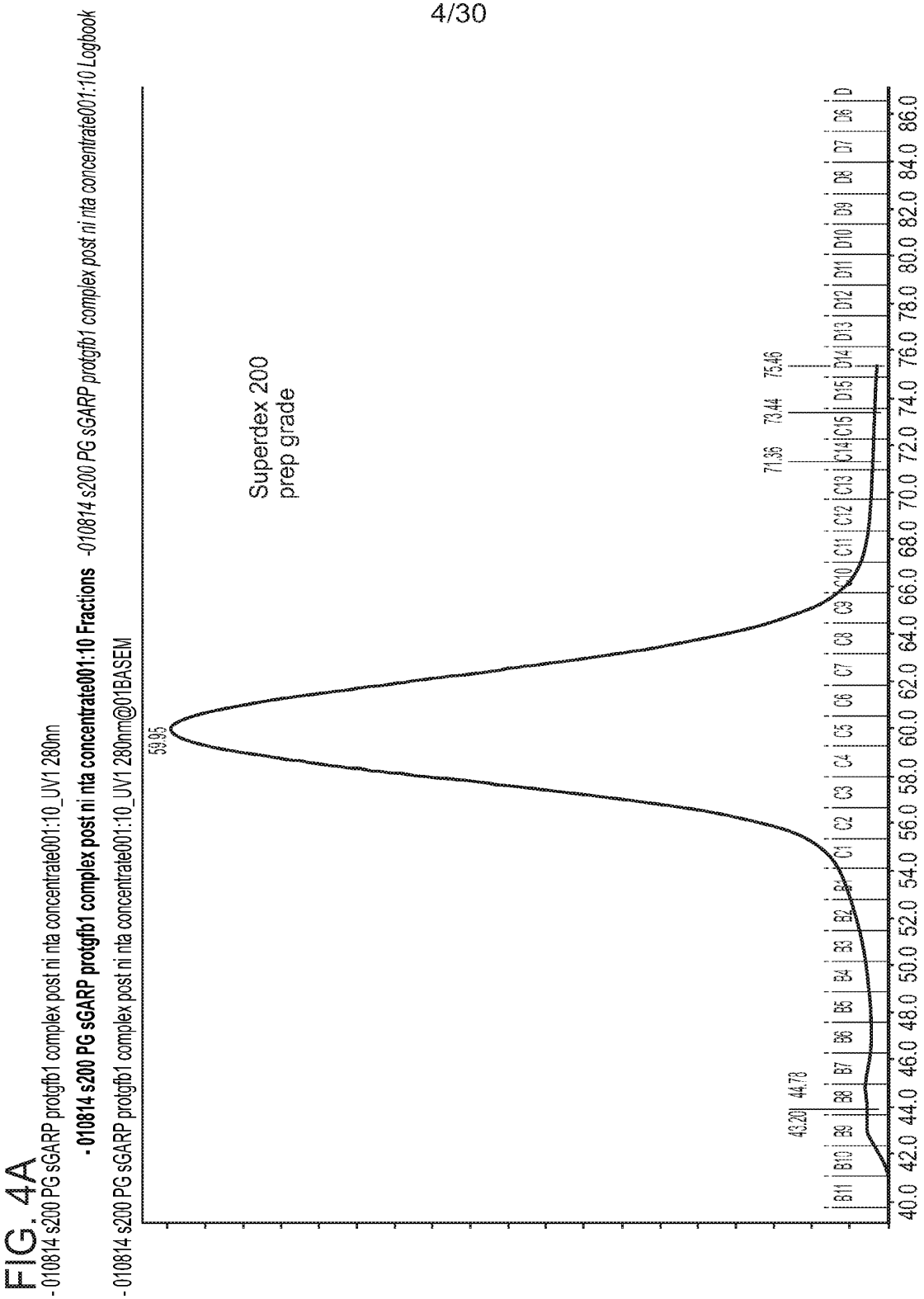

proTGFβ1 wild-type
Disulfide bond
TGFβ1
growth factor domain
TGFβ1
latency-associated peptide
RXXR:
furin cleavage site
Cys4
HS
SH (LAP)
LTBP1:
S
S
C4S: Cys4 → Ser mutation
HO
OH
His tag
sGARP:
S
S

FIG. 4A


- 010814 s200 PG sGARP protgfb1 complex post ni nta concentrate001:10_UV1 280nn
- 010814 s200 PG sGARP protgfb1 complex post ni nta concentrate001:10 Fractions -010814 s200 PG sGARP protgfb1 complex post ni nta concentrate001:10 Logbook
- 010814 s200 PG sGARP protgfb1 complex post ni nta concentrate001:10_UV1 280nm@01BASEM
Superdex 200
prep grade
59.95
46.20
44.78
71.66
73.44
75.46
B11 B10 B9 B8 B7 B6 B5 B4 B3 B2 B1 C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 C15 D15 D14 D13 D12 D11 D10 D9 D8 D7 D6 D
40.0 42.0 44.0 46.0 48.0 50.0 52.0 54.0 56.0 58.0 60.0 62.0 64.0 66.0 68.0 70.0 72.0 74.0 76.0 78.0 80.0 82.0 84.0 86.0

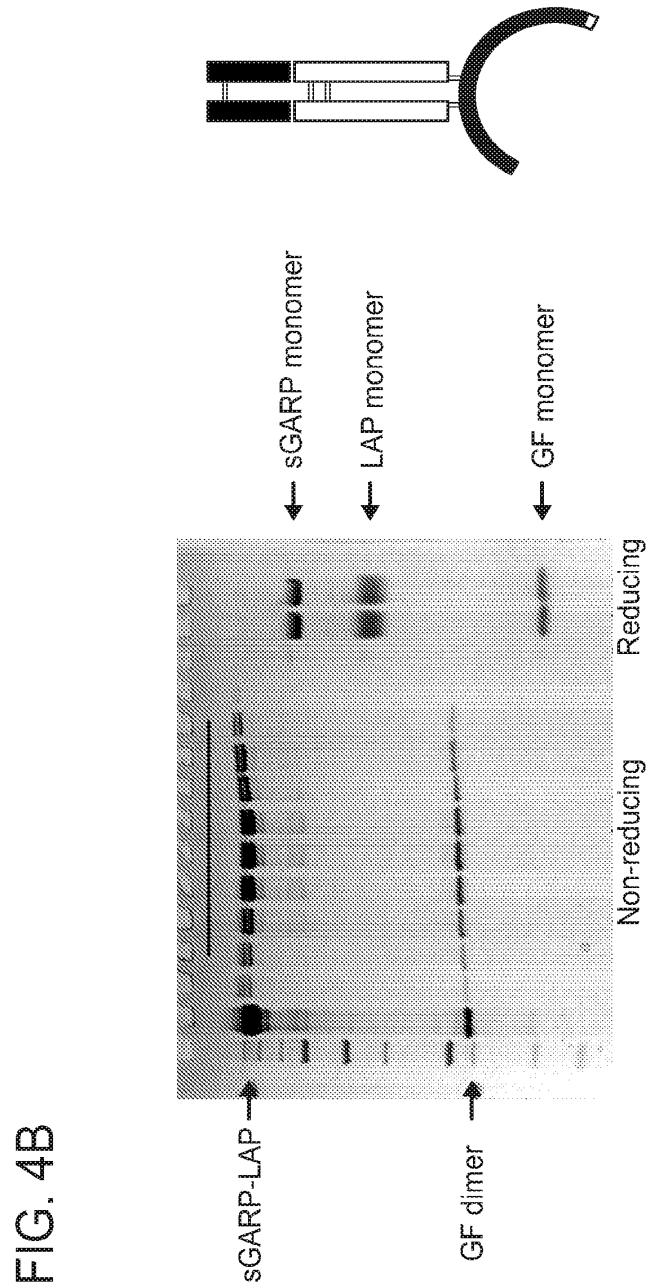

sGARP monomer
LAP monomer
GF monomer
Reducing
Non-reducing
sGARP-LAP
GF dimer

FIG. 4B

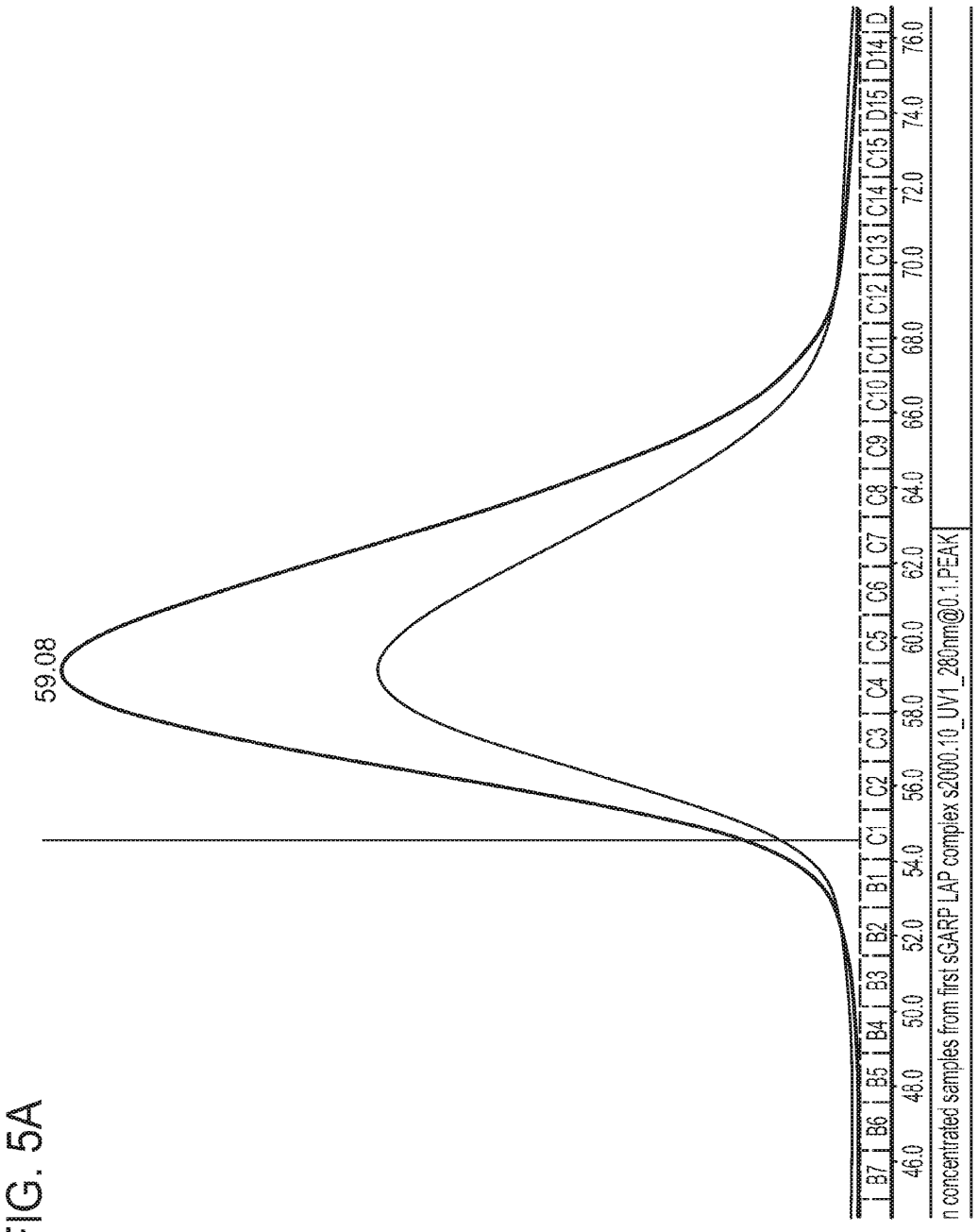

59.08
B7 B6 B5 B4 B3 B2 B1 C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 C15 D15 D14 D
46.0 48.0 50.0 52.0 54.0 56.0 58.0 60.0 62.0 64.0 66.0 68.0 70.0 72.0 74.0 76.0
n concentrated samples from first sGARP LAP complex s2000.10_UV1_280nm@0.1.PEAK

FIG. 5A

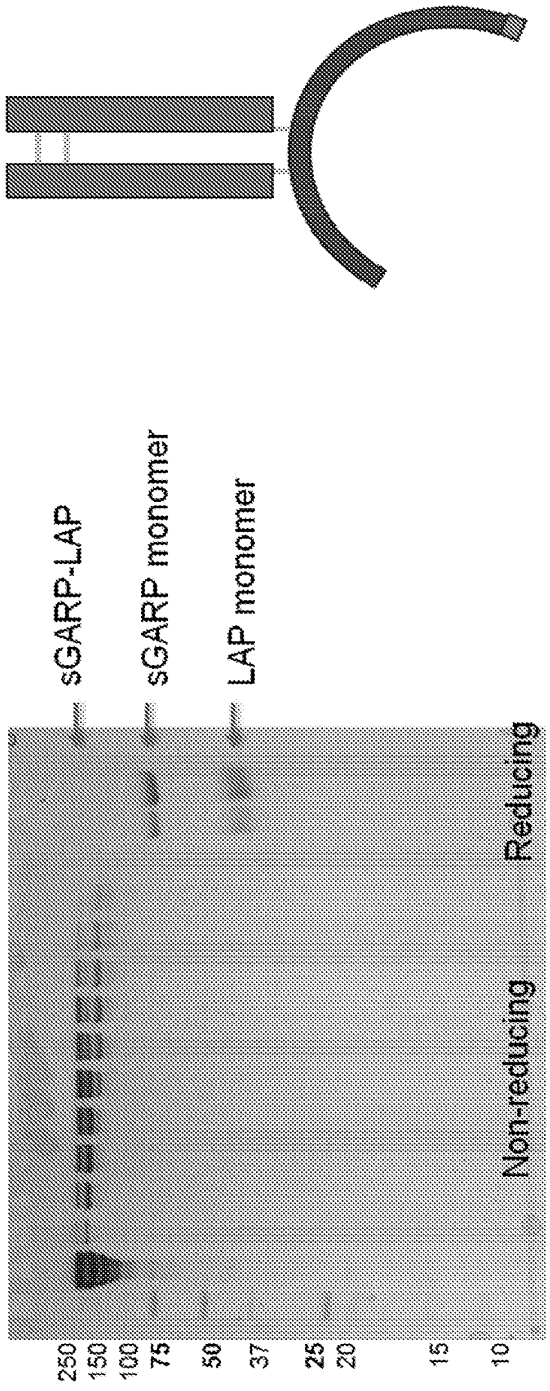

sGARP-LAP
sGARP monomer
LAP monomer
Reducing
Non-reducing
250
150
100
75
50
37
25
20
15
10

FIG. 5B

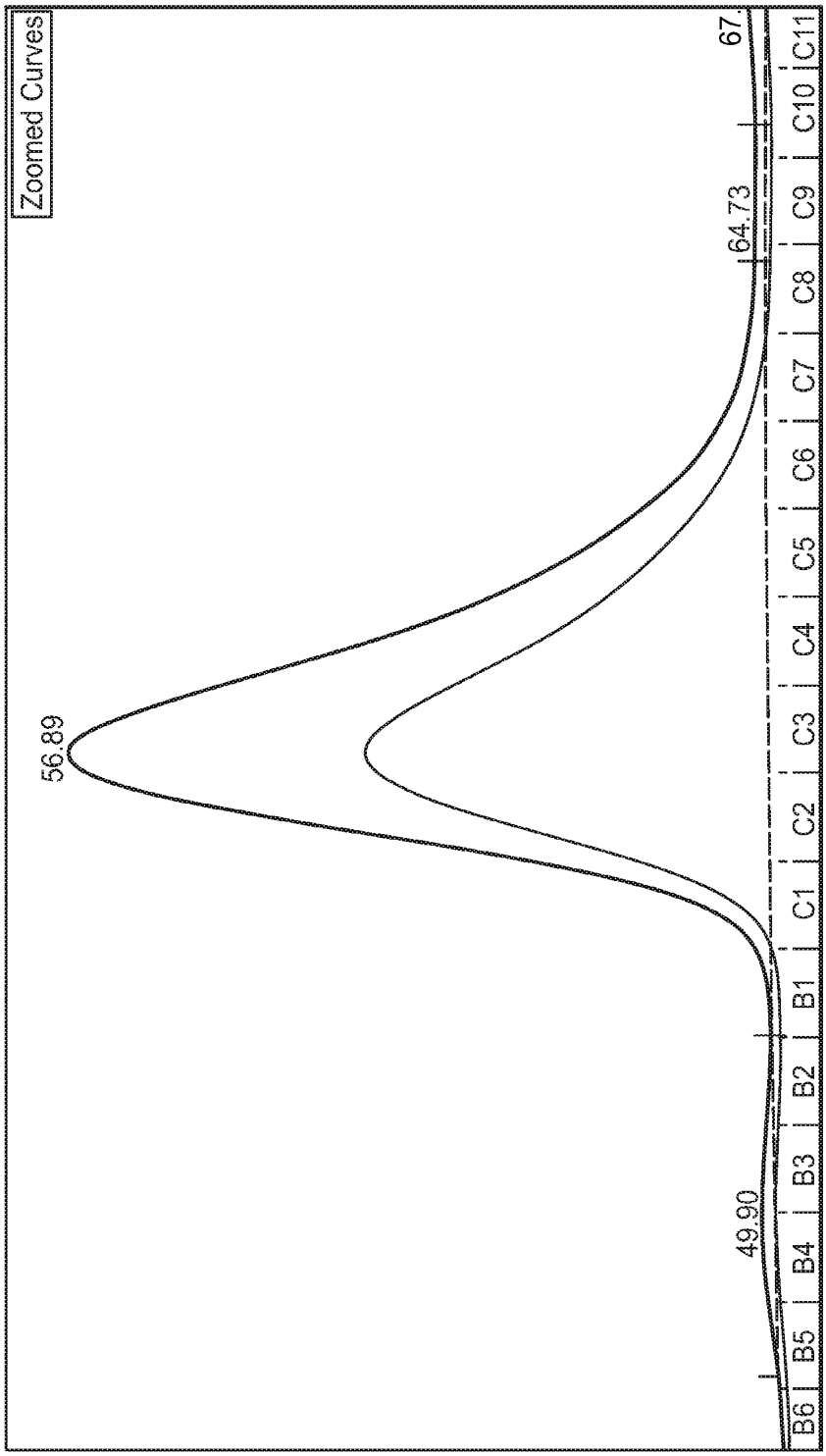

Zoomed Curves
56.89
49.90
64.73
67
B6
B5
B4
B3
B2
B1
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11

FIG. 6A

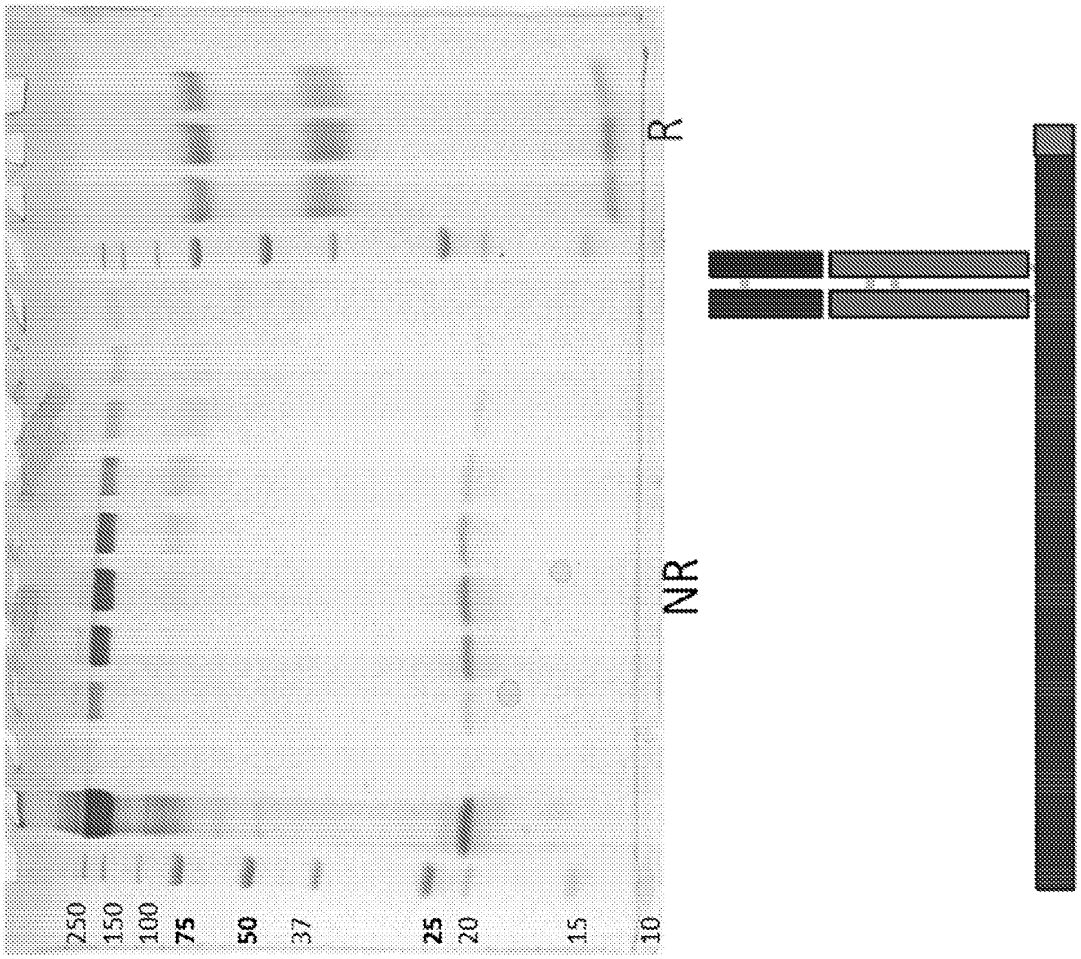


FIG. 6B

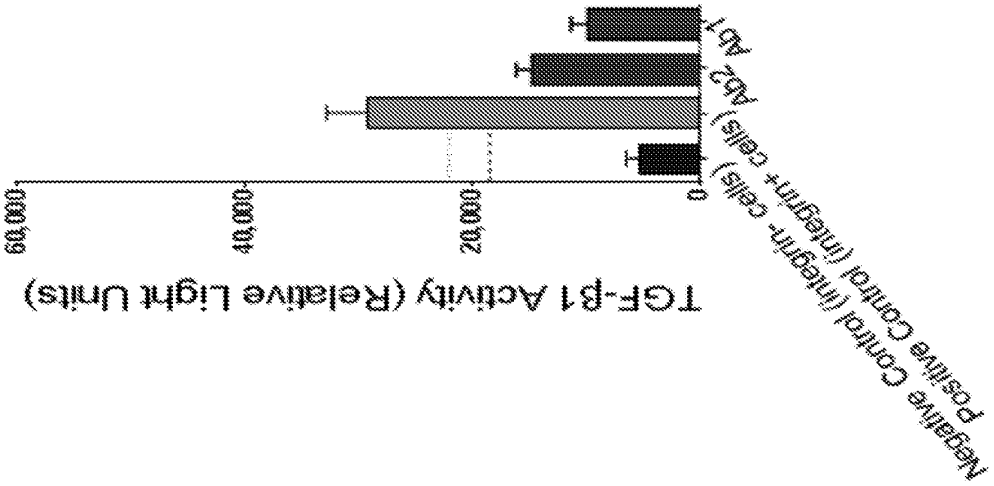

TGF-β1 Activity (Relative Light Units)
60,000
40,000
20,000
0
Negative Control (Integrin- cells)
Positive Control (Integrin+ cells)
Ab2
Ab1

FIG. 7

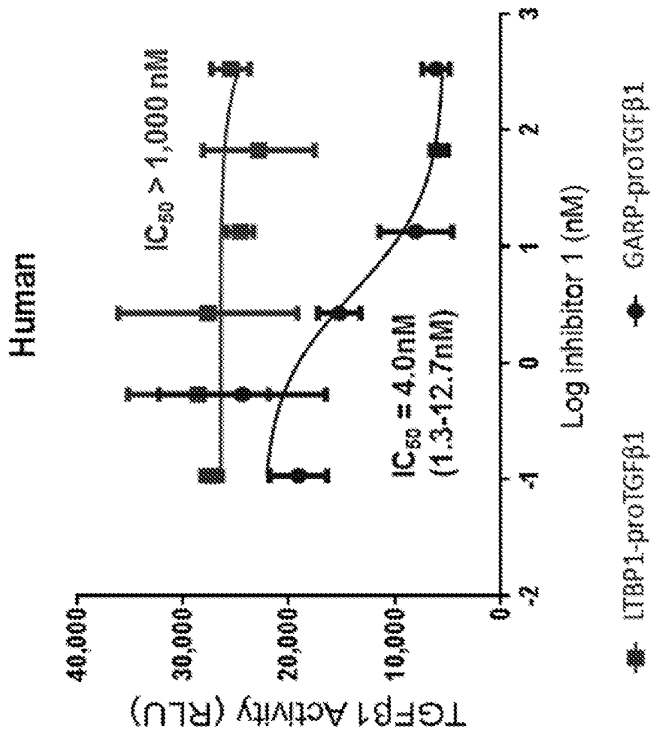

Human
IC50 > 1,000 nM
IC50 = 4.0nM
(1.3-12.7nM)
TGFβ1 Activity (RLU)
40,000
30,000
20,000
10,000
0
-2
-1
0
1
2
3
Log inhibitor 1 (nM)
LTBP1-proTGFβ1
GARP-proTGFβ1

FIG. 8

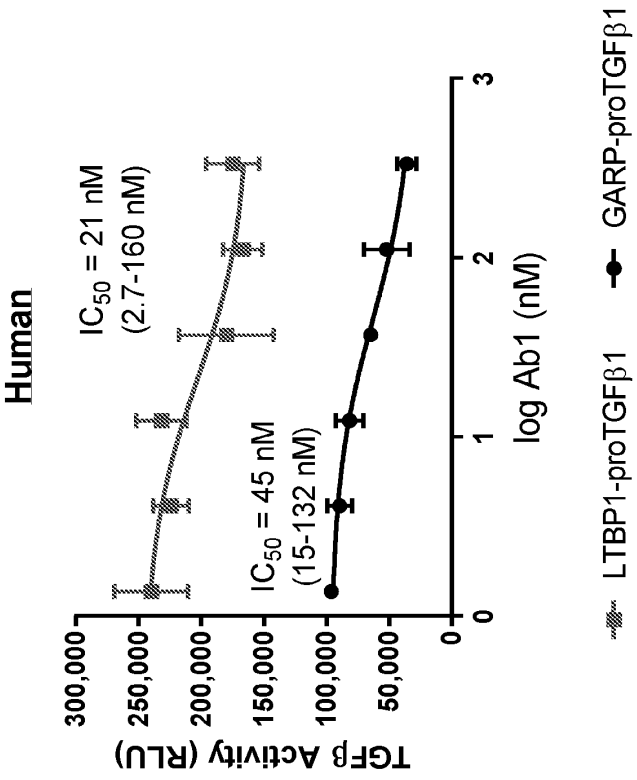

Human
IC50 = 21 nM
(2.7-160 nM)
IC50 = 45 nM
(15-132 nM)
TGFβ Activity (RLU)
300,000
250,000
200,000
150,000
100,000
50,000
0
0
1
2
3
log Ab1 (nM)
LTBP1-proTGFβ1
GARP-proTGFβ1

FIG. 9

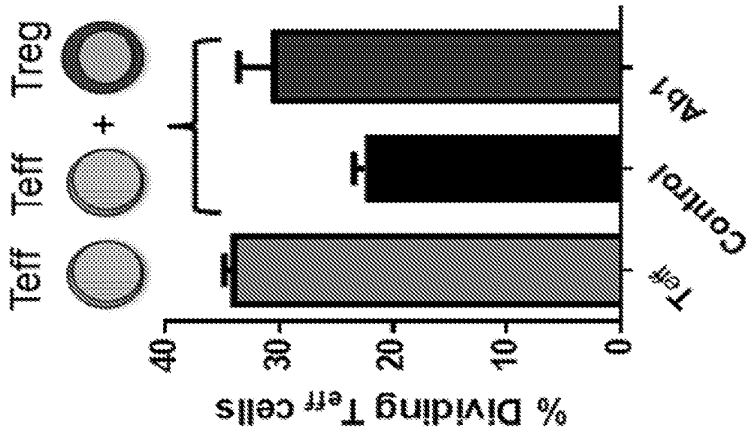

Treg
+
Teff
Teff
Teff
Control
Ab1
0
10
20
30
40
% Dividing Teff cells

FIG. 10

FIG. 11A

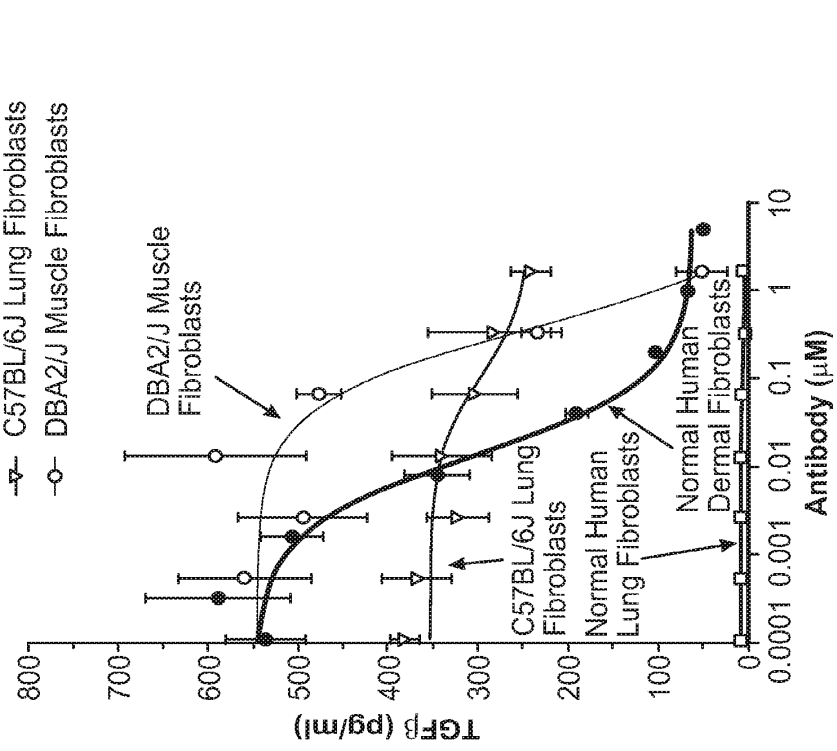
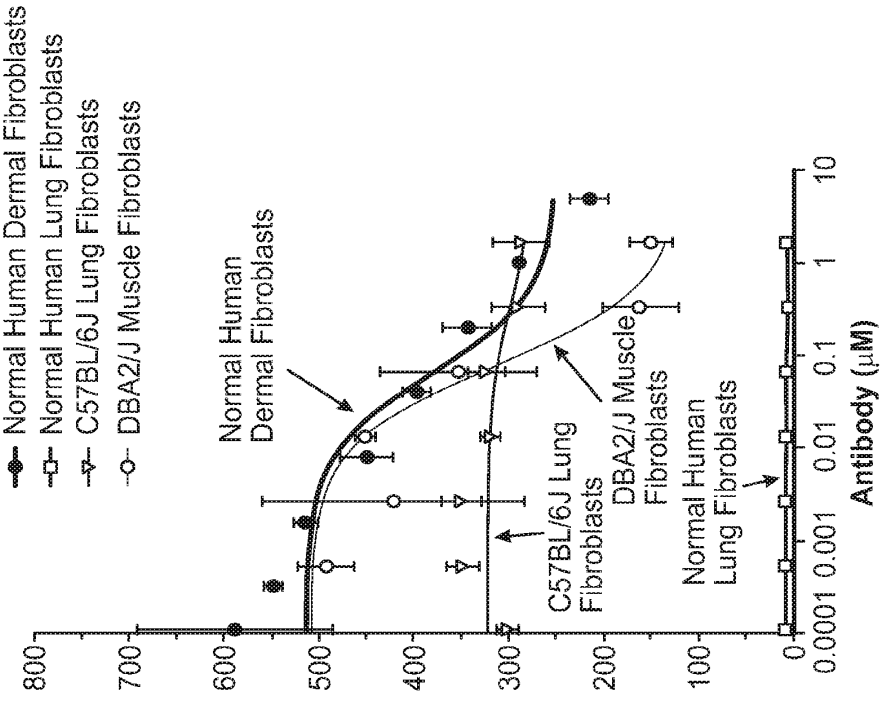


FIG. 11B



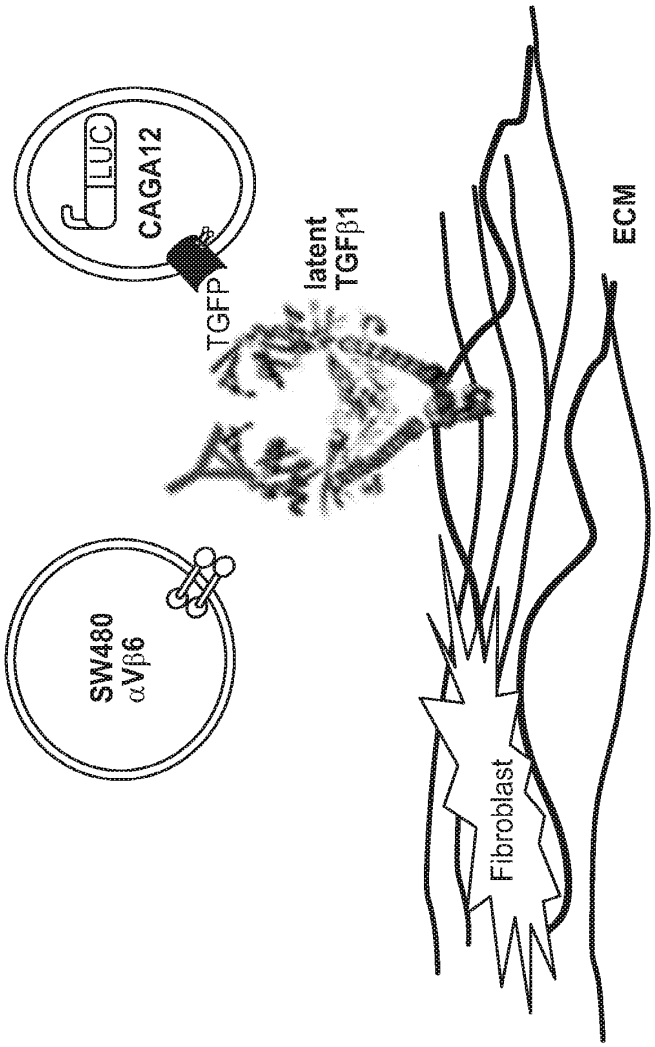


FIG. 11C

FIG. 12A

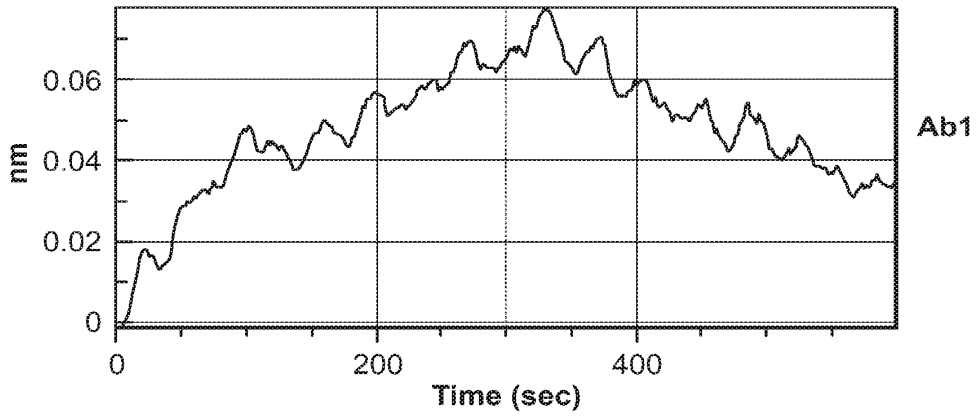
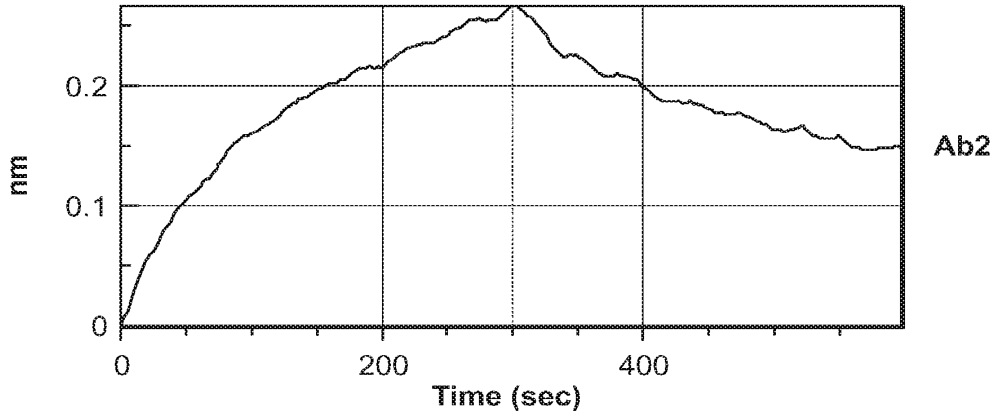


FIG. 12B



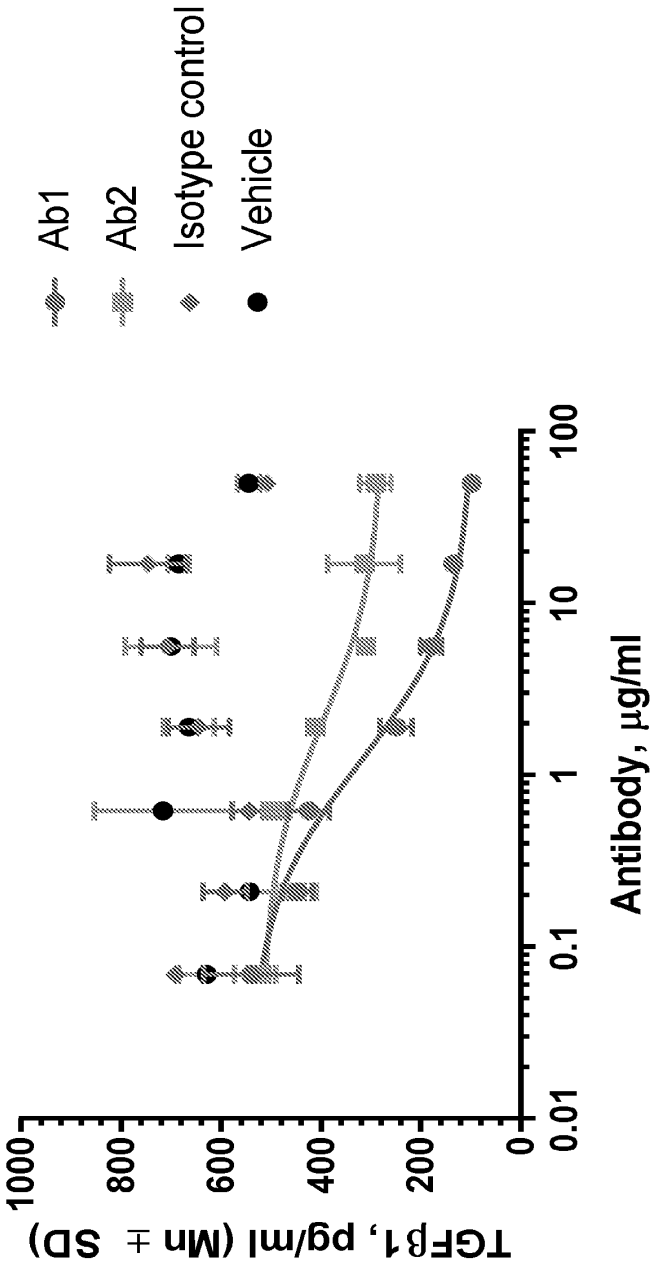

GARP complex inhibition
Ab1
Ab2
Isotype control
Vehicle
TGFβ1, pg/ml (Mn ± SD)
1000
800
600
400
200
0
0.01
0.1
1
10
100
Antibody, μg/ml

FIG. 13A

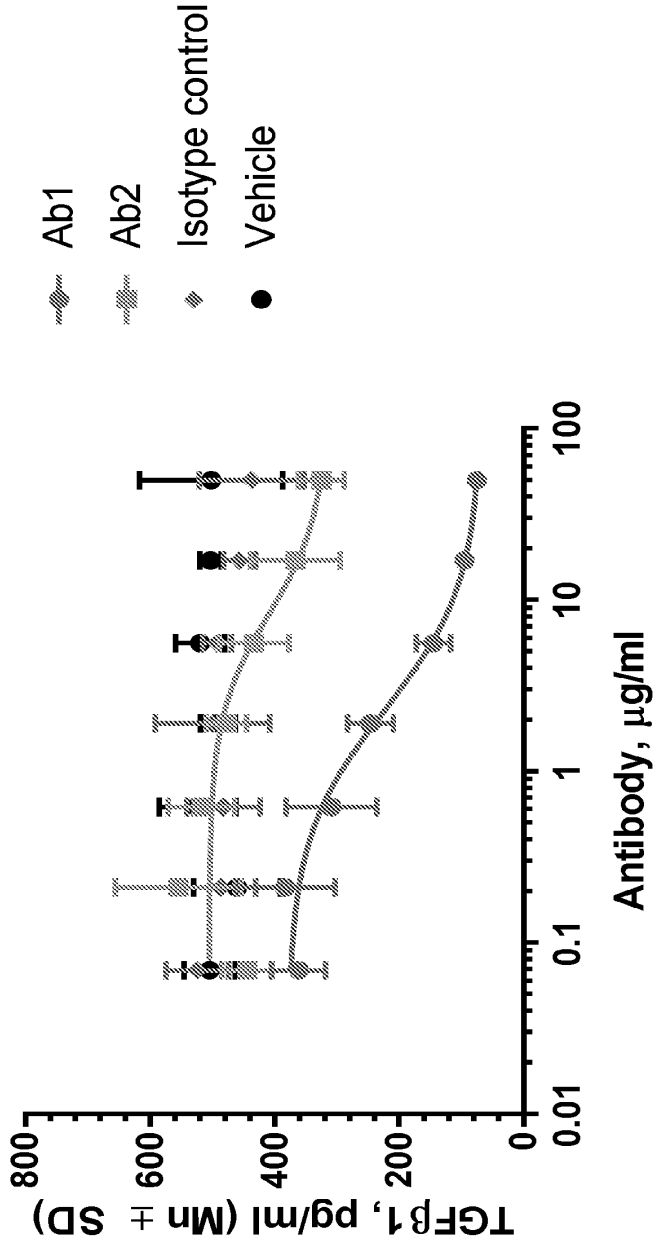

LRRC33 complex inhibition
Ab1
Ab2
Isotype control
Vehicle
TGFβ1, pg/ml (Mn ± SD)
800
600
400
200
0
0.01
0.1
1
10
100
Antibody, μg/ml

FIG. 13B

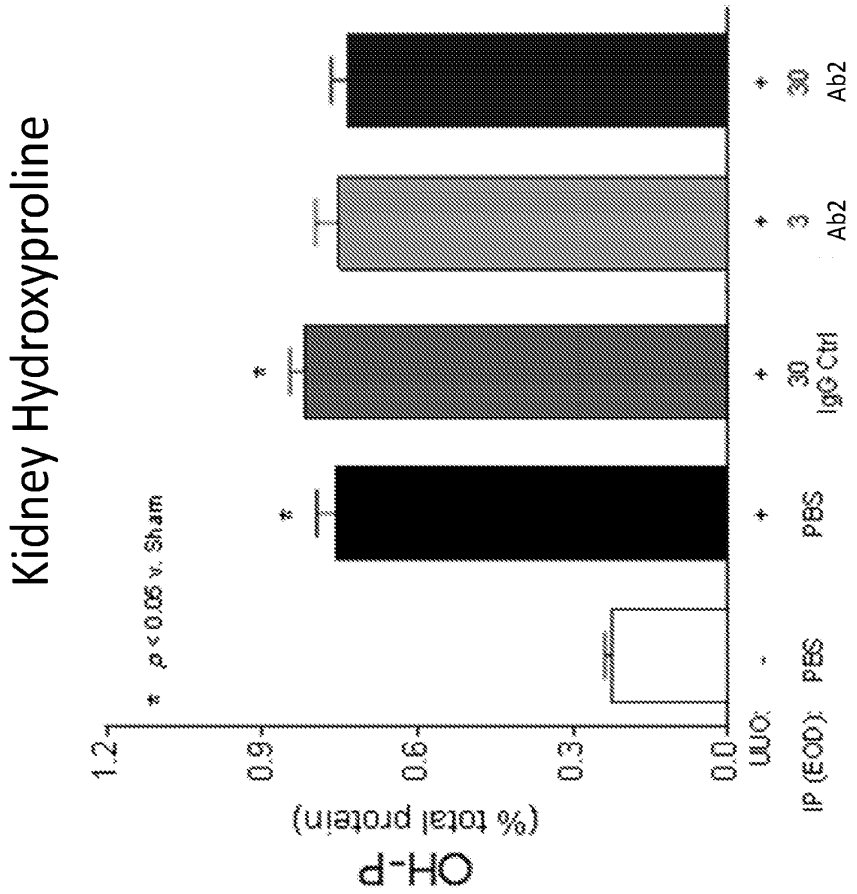

Kidney Hydroxyproline
OH-P
(% total protein)
1.2
0.9
0.6
0.3
0.0
* p < 0.05 v. Sham
UUO
-
PBS
PBS
30
IgG Ctrl
3
Ab2
30
Ab2
IP (ECD):

FIG. 14

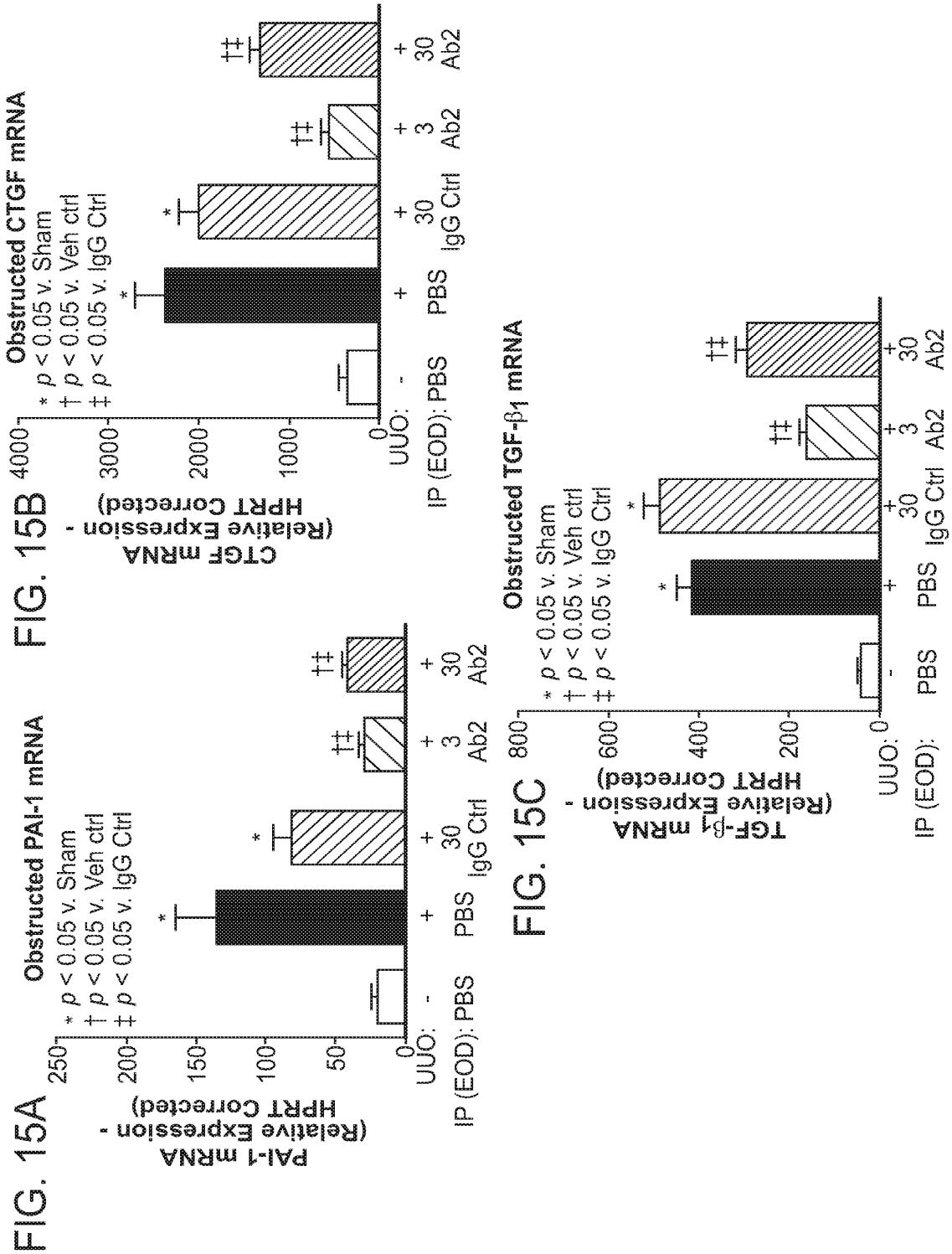


FIG. 15A
Obstructed PAI-1 mRNA
* p < 0.05 v. Sham
† p < 0.05 v. Veh ctrl
‡ p < 0.05 v. IgG Ctrl
PAI-1 mRNA (Relative Expression - HPRT Corrected)
250
200
150
100
50
0
UUO: - + + + +
IP (EOD): PBS PBS IgG Ctrl 3 Ab2 30 Ab2
FIG. 15B
Obstructed CTGF mRNA
* p < 0.05 v. Sham
† p < 0.05 v. Veh ctrl
‡ p < 0.05 v. IgG Ctrl
CTGF mRNA (Relative Expression - HPRT Corrected)
4000
3000
2000
1000
0
UUO: - + + + +
IP (EOD): PBS PBS IgG Ctrl 3 Ab2 30 Ab2
FIG. 15C
Obstructed TGF-β1 mRNA
* p < 0.05 v. Sham
† p < 0.05 v. Veh ctrl
‡ p < 0.05 v. IgG Ctrl
TGF-β1 mRNA (Relative Expression - HPRT Corrected)
800
600
400
200
0
UUO: - + + + +
IP (EOD): PBS PBS IgG Ctrl 3 Ab2 30 Ab2

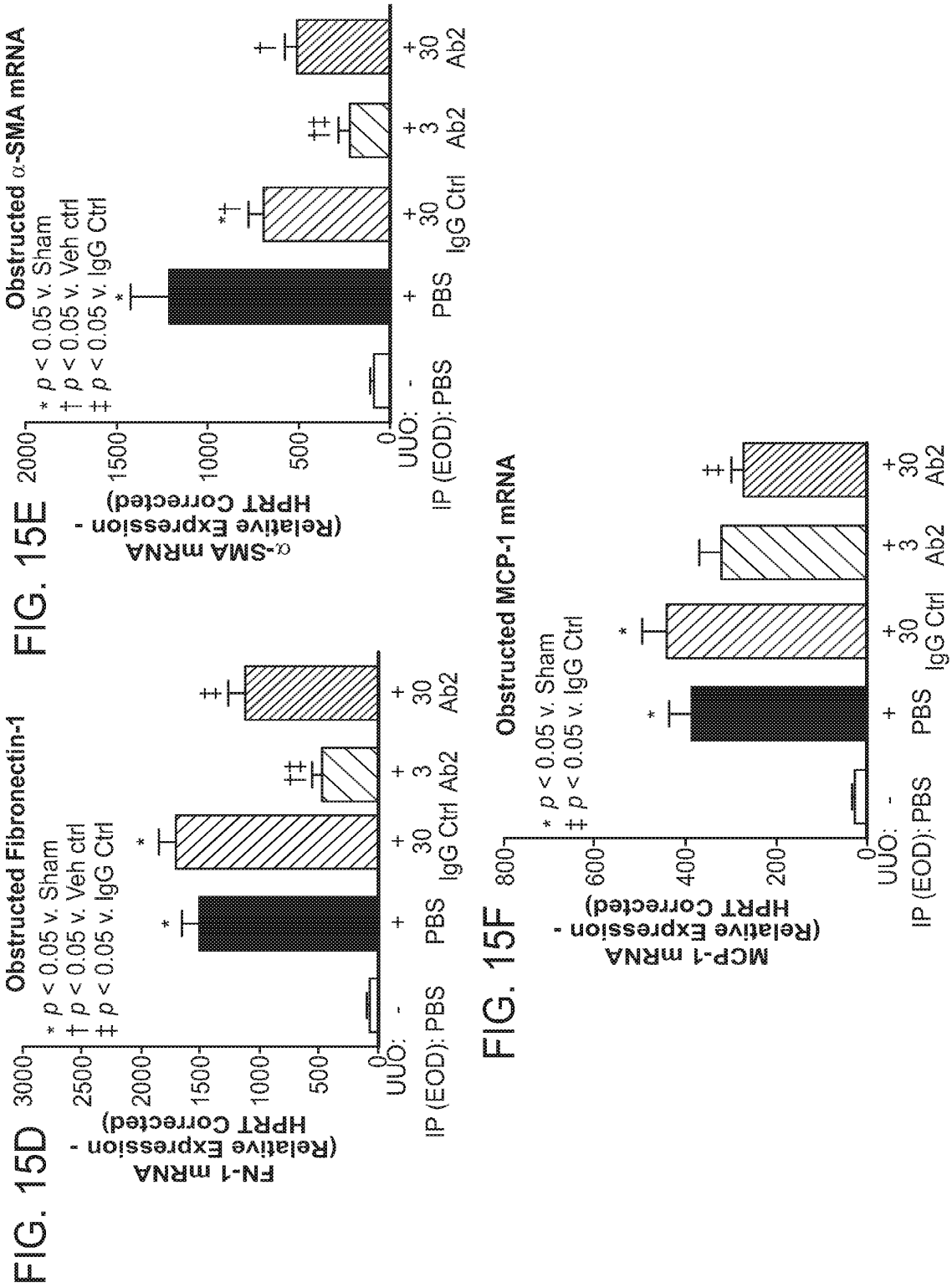


FIG. 15D
Obstructed Fibronectin-1
* p < 0.05 v. Sham
† p < 0.05 v. Veh ctrl
‡ p < 0.05 v. IgG Ctrl
FN-1 mRNA (Relative Expression - HPRT Corrected)
3000
2500
2000
1500
1000
500
0
UUO: - + + + +
IP (EOD): PBS PBS IgG Ctrl Ab2 Ab2
30 3 30
FIG. 15E
Obstructed α-SMA mRNA
* p < 0.05 v. Sham
† p < 0.05 v. Veh ctrl
‡ p < 0.05 v. IgG Ctrl
α-SMA mRNA (Relative Expression - HPRT Corrected)
2000
1500
1000
500
0
UUO: - + + + +
IP (EOD): PBS PBS IgG Ctrl Ab2 Ab2
30 3 30
FIG. 15F
Obstructed MCP-1 mRNA
* p < 0.05 v. Sham
‡ p < 0.05 v. IgG Ctrl
MCP-1 mRNA (Relative Expression - HPRT Corrected)
800
600
400
200
0
UUO: - + + + +
IP (EOD): PBS PBS IgG Ctrl Ab2 Ab2
30 3 30

FIG. 15H

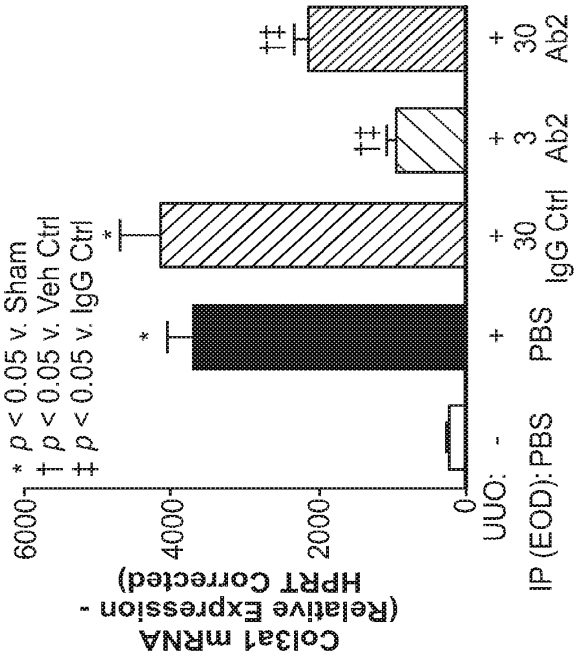


FIG. 15G

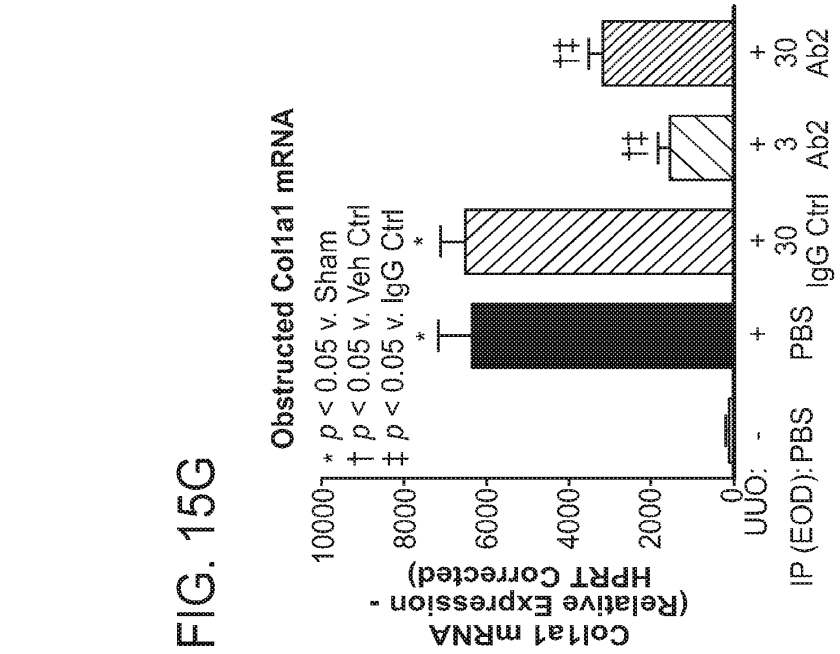
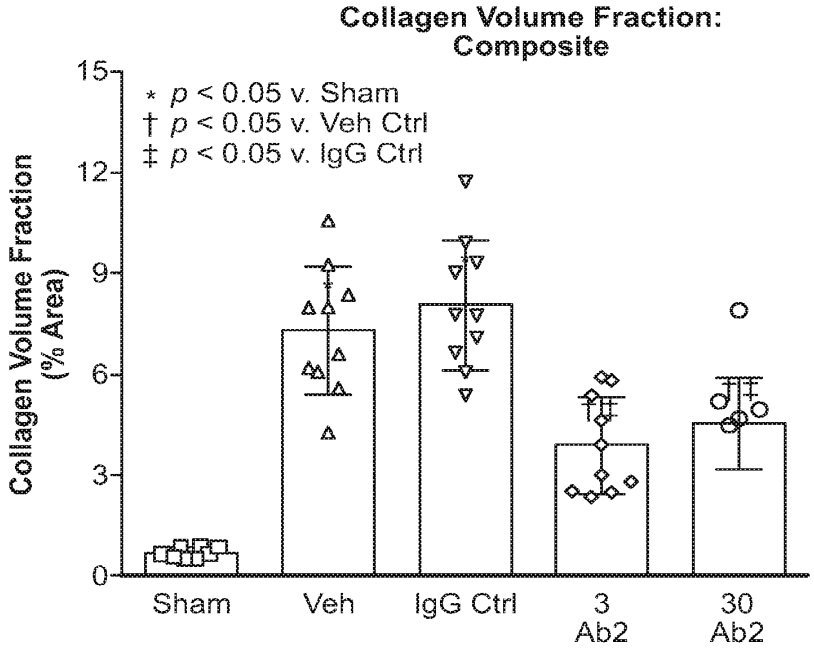


FIG. 16


Collagen Volume Fraction:
Composite
* p < 0.05 v. Sham
† p < 0.05 v. Veh Ctrl
‡ p < 0.05 v. IgG Ctrl
Collagen Volume Fraction
(% Area)
15
12
9
6
3
0
Sham
Veh
IgG Ctrl
3
Ab2
30
Ab2

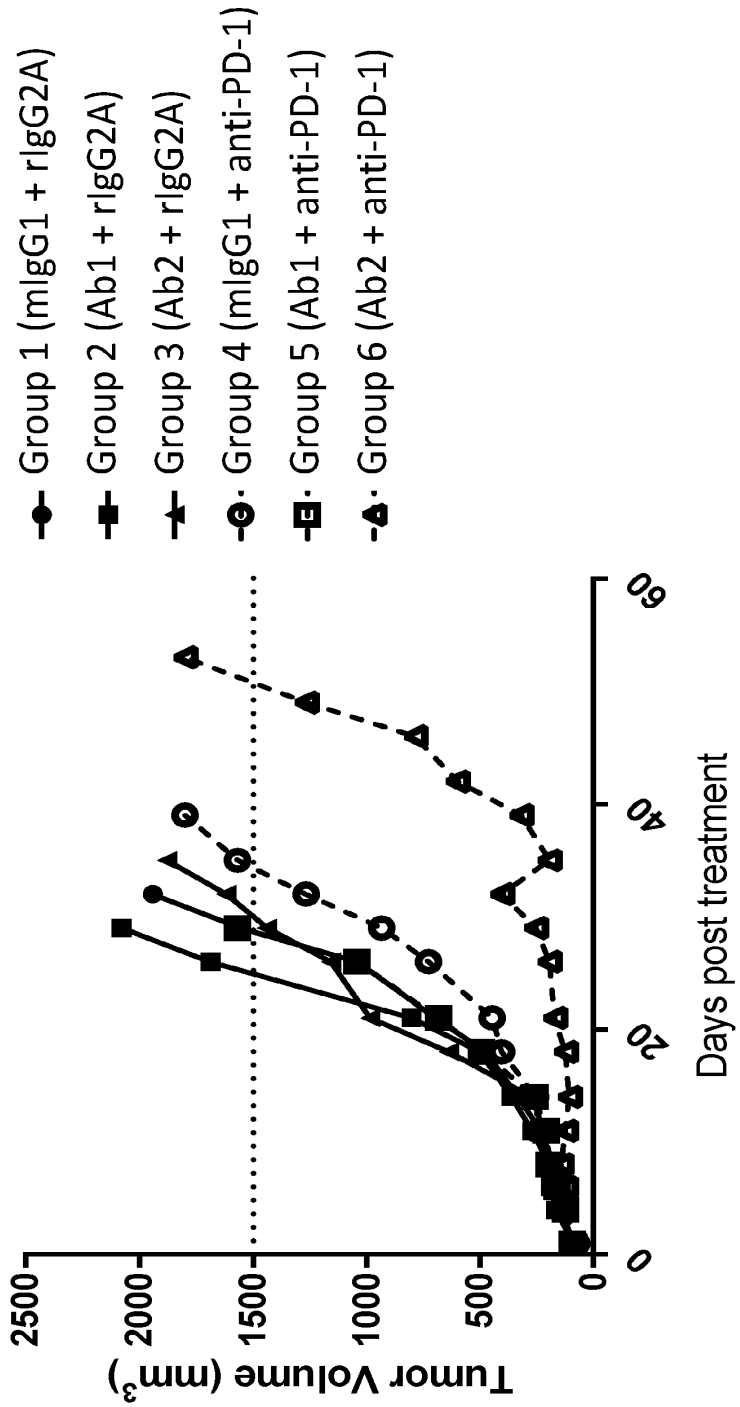

Group 1 (mIgG1 + rIgG2A)
Group 2 (Ab1 + rIgG2A)
Group 3 (Ab2 + rIgG2A)
Group 4 (mIgG1 + anti-PD-1)
Group 5 (Ab1 + anti-PD-1)
Group 6 (Ab2 + anti-PD-1)
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
20
40
60
Days post treatment

FIG. 17

FIG. 18A

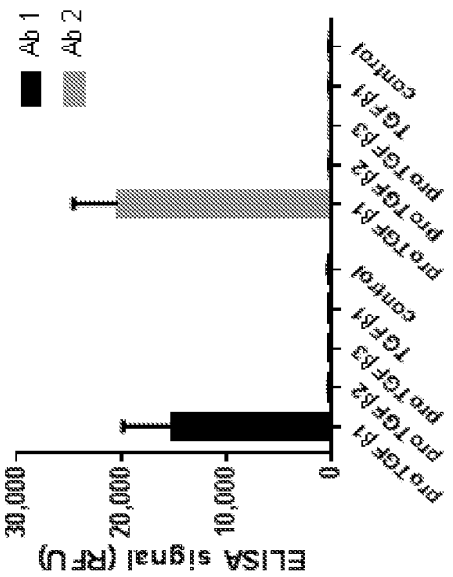


FIG. 18B

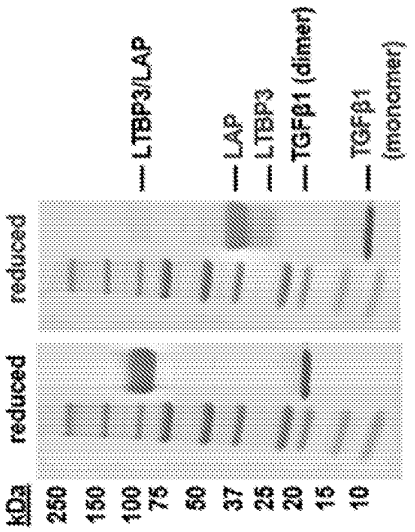
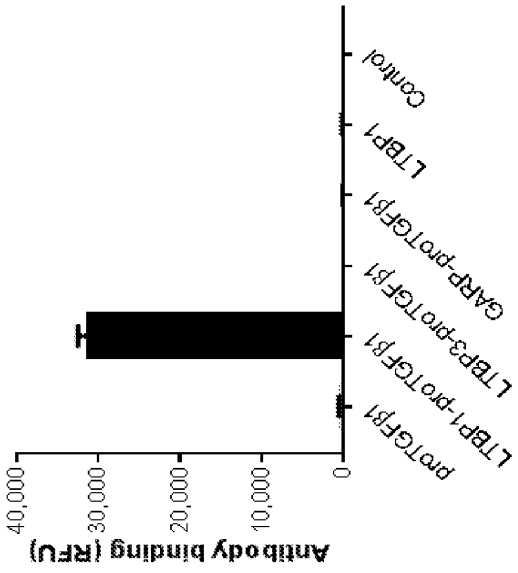


FIG. 18C



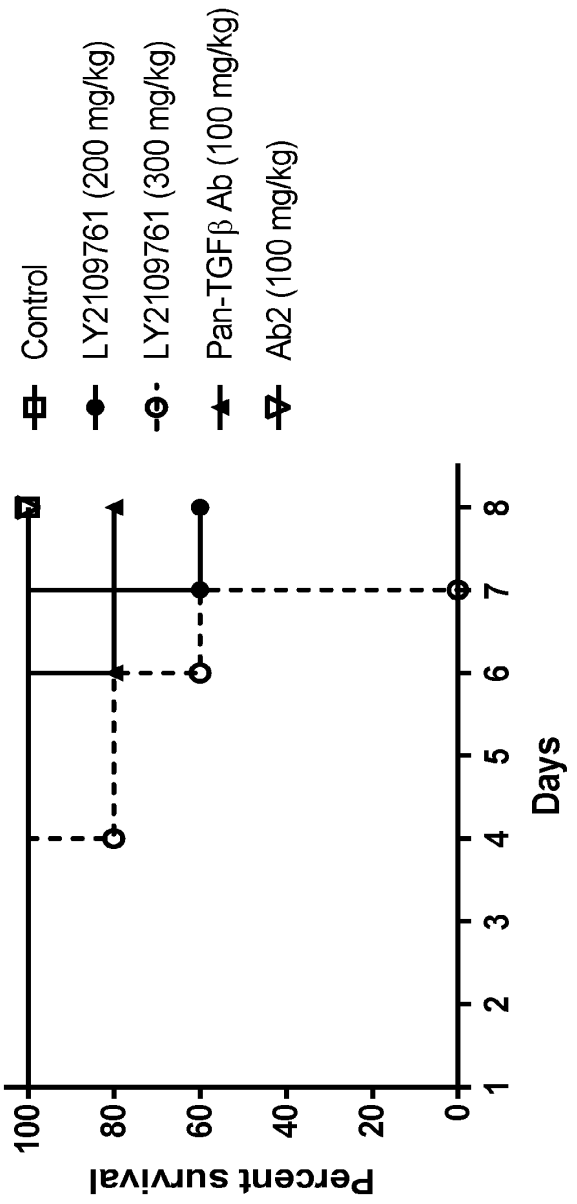

Control
LY2109761 (200 mg/kg)
LY2109761 (300 mg/kg)
Pan-TGFβ Ab (100 mg/kg)
Ab2 (100 mg/kg)
Percent survival
100
80
60
40
20
0
1
2
3
4
5
6
7
8
Days

FIG. 19

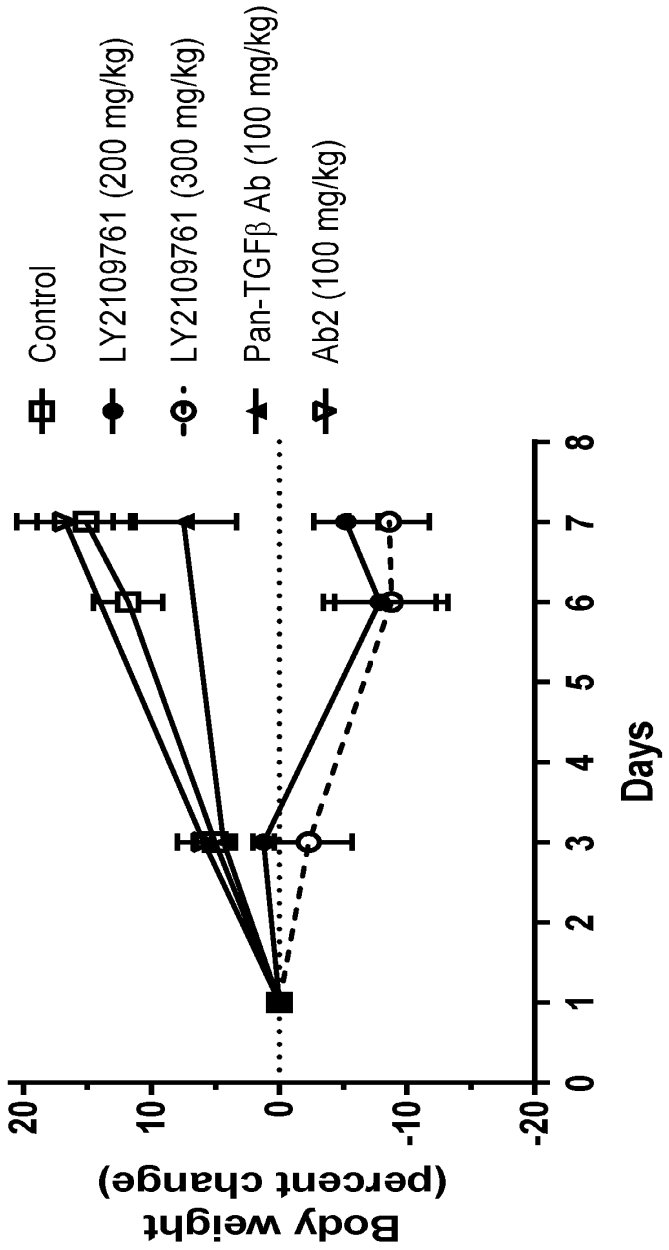

Control
LY2109761 (200 mg/kg)
LY2109761 (300 mg/kg)
Pan-TGFβ Ab (100 mg/kg)
Ab2 (100 mg/kg)
Body weight
(percent change)
20
10
0
-10
-20
0
1
2
3
4
5
6
7
8
Days

FIG. 20

FIG. 21C

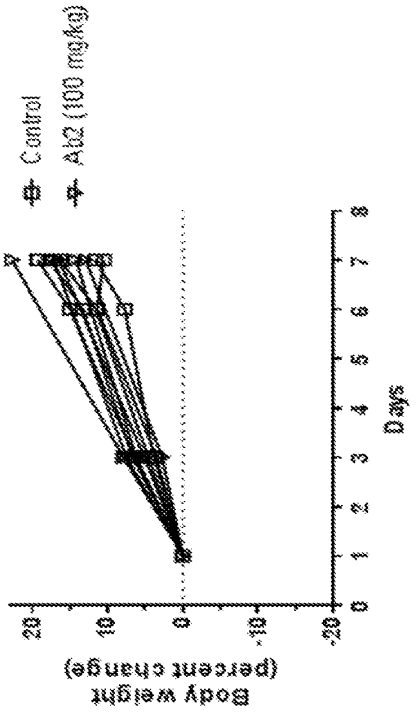


FIG. 21A

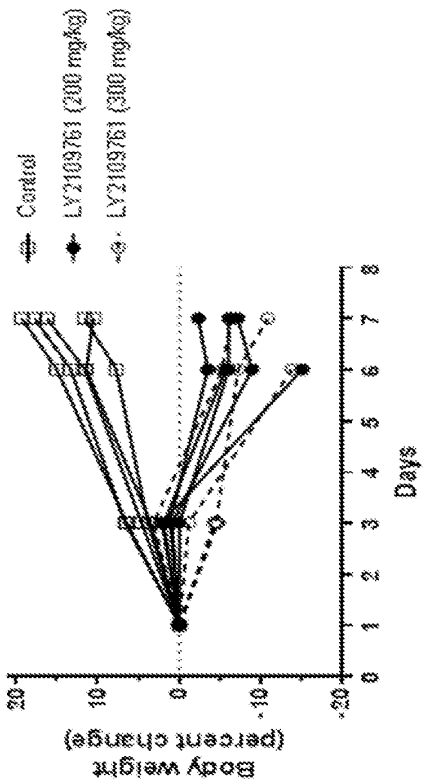


FIG. 21B

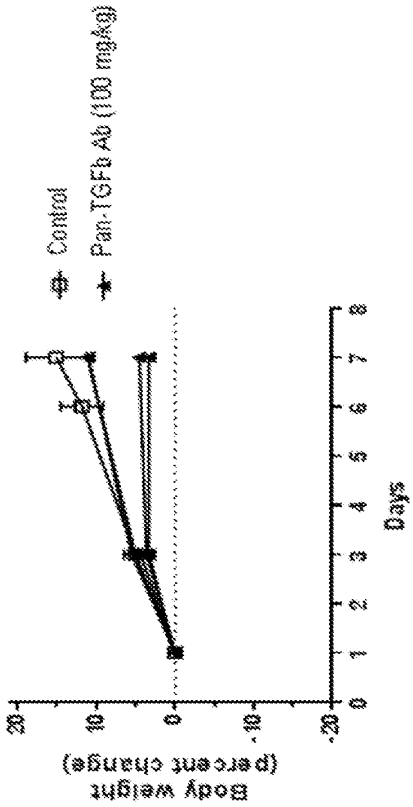


FIG. 22B

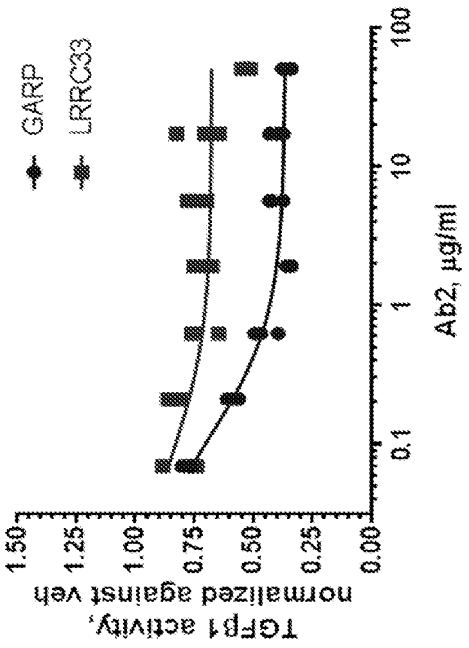


FIG. 22A

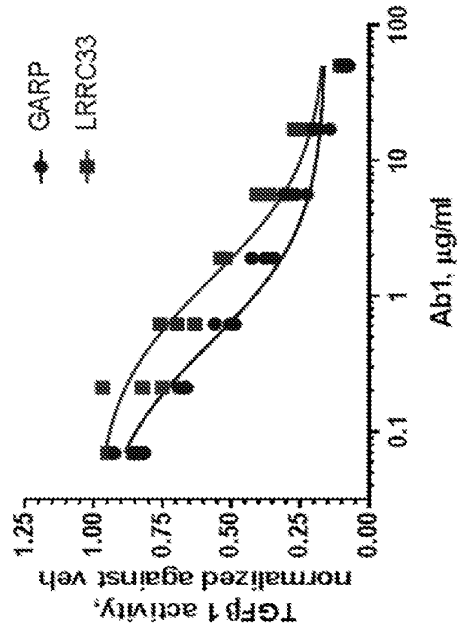
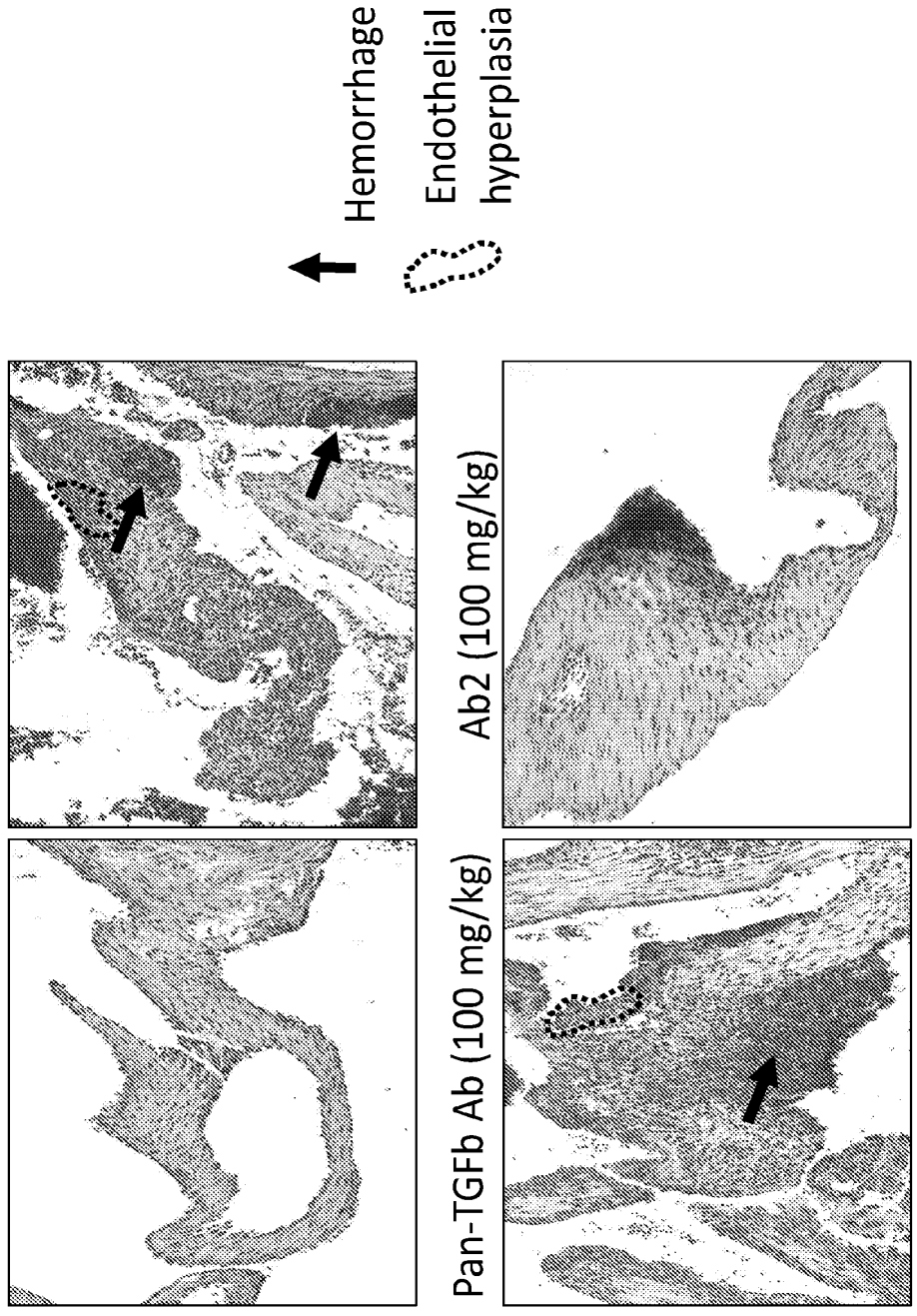


FIG. 23


Heart valve (valvulopathy)
Hemorrhage
Endothelial hyperplasia
Untreated
LY2109761 (200 mg/kg)
Pan-TGFb Ab (100 mg/kg)
Ab2 (100 mg/kg)