
mTLL2 Cleavage Site
Furin Cleavage Site
Prodomain
Mature
Disulfide bond

FIG. 1A

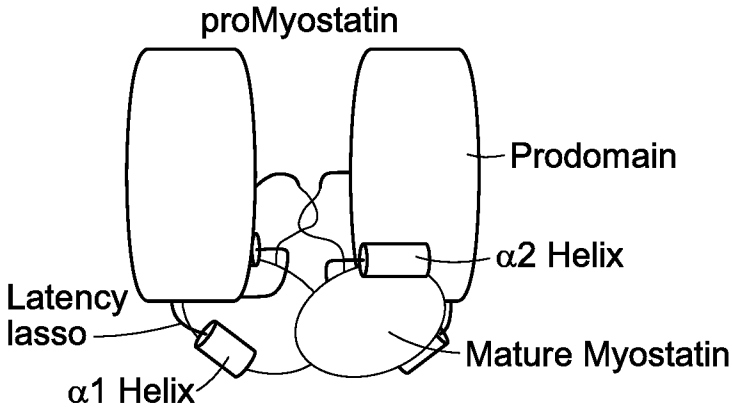

proMyostatin
Prodomain
α2 Helix
Latency lasso
Mature Myostatin
α1 Helix

FIG. 1B

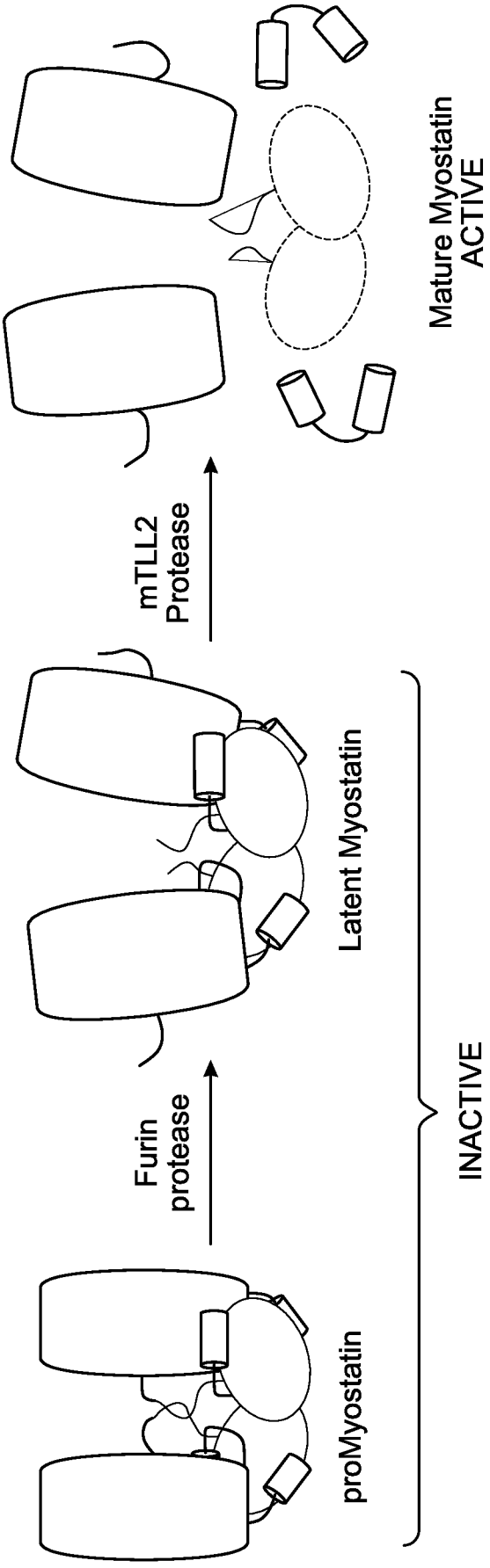

Mature Myostatin
ACTIVE
mTLL2
Protease
Latent Myostatin
Furin
protease
proMyostatin
INACTIVE

FIG. 2

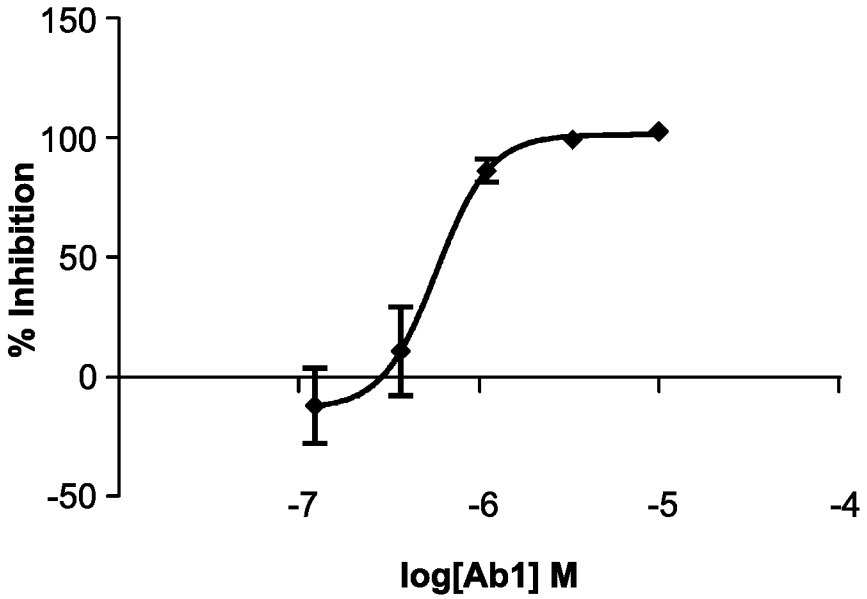

150
100
50
0
-50
% Inhibition
-7
-6
-5
-4
log[Ab1] M

FIG. 3A

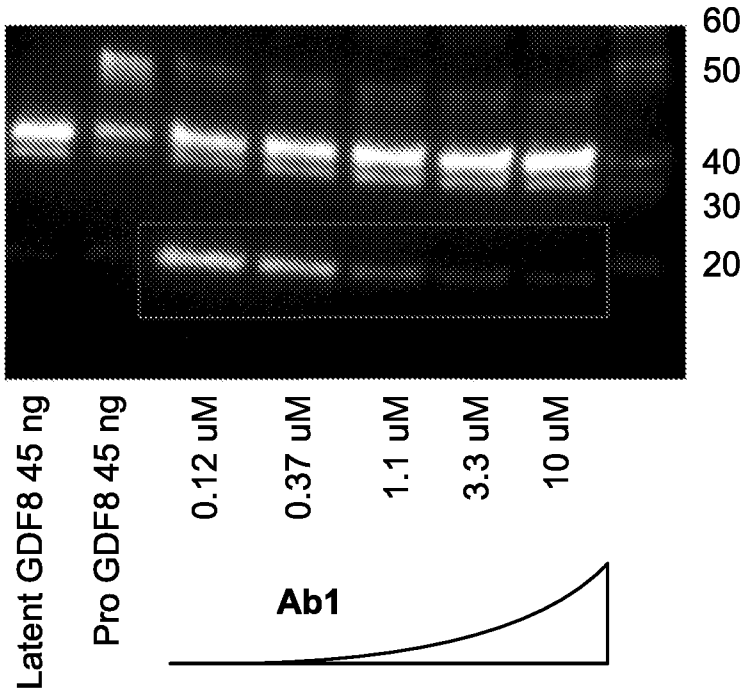

60
50
40
30
20
Latent GDF8 45 ng
Pro GDF8 45 ng
0.12 uM
0.37 uM
1.1 uM
3.3 uM
10 uM
Ab1

FIG. 3B

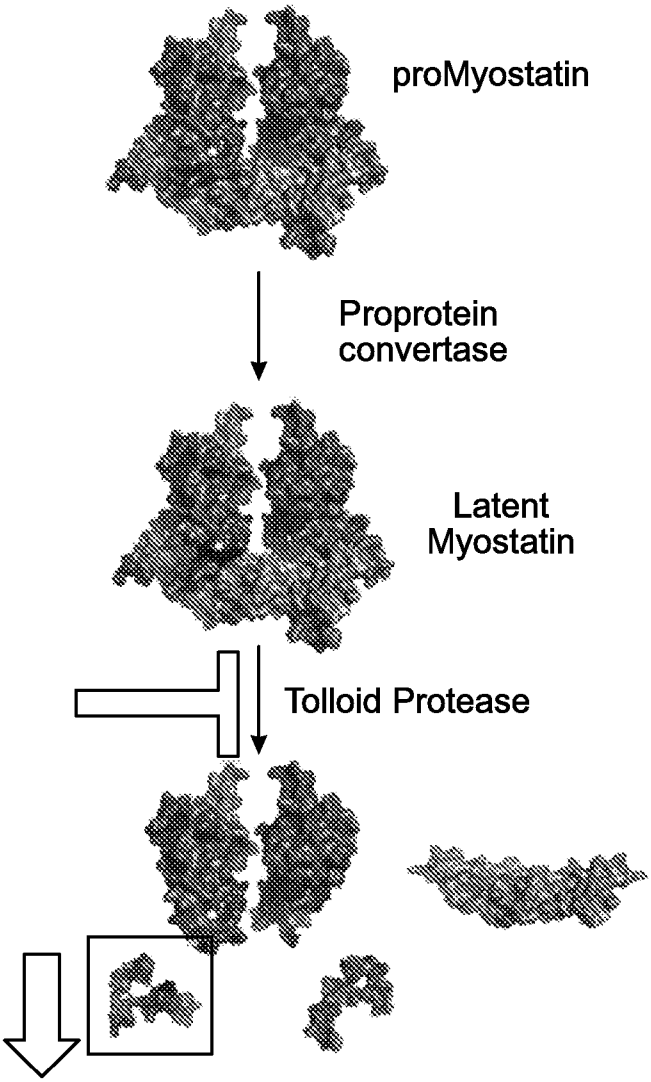

proMyostatin
Proprotein convertase
Latent Myostatin
Tolloid Protease

FIG. 3C

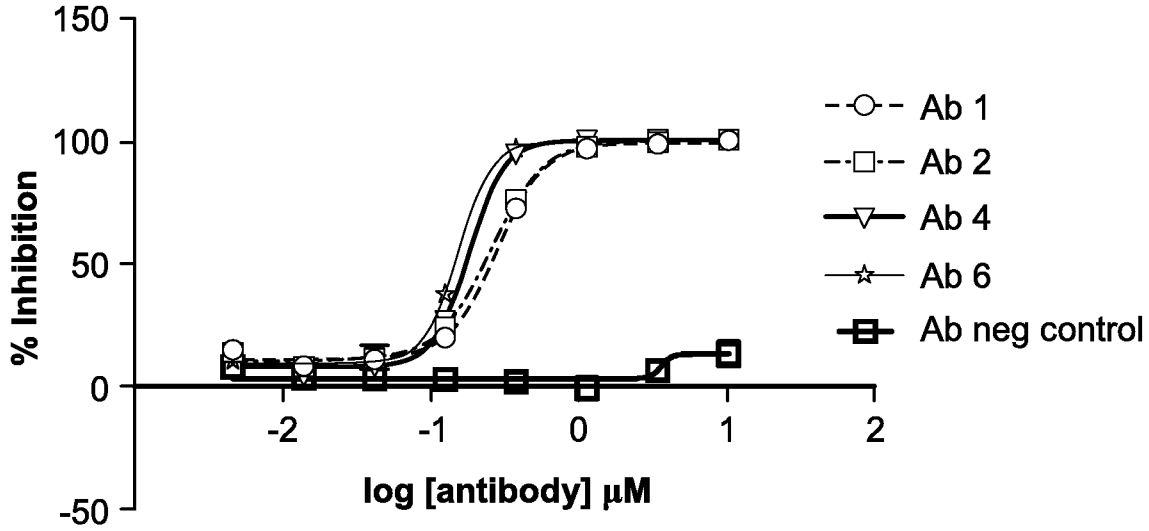

150
100
50
0
-50
% Inhibition
-2
-1
0
1
2
log [antibody] µM
Ab 1
Ab 2
Ab 4
Ab 6
Ab neg control

FIG. 4

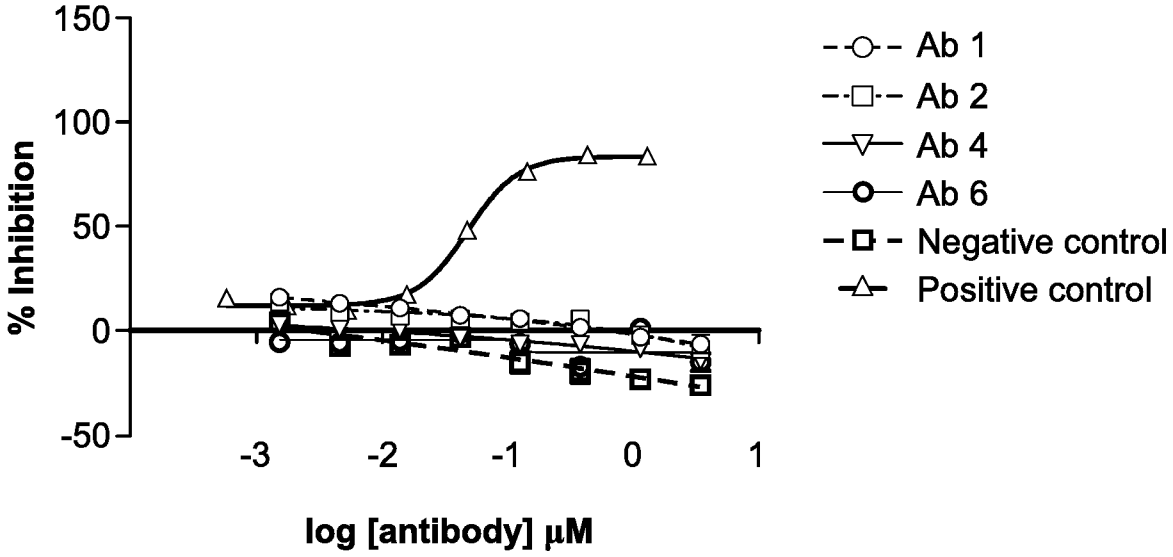

150
100
50
0
-50
% Inhibition
-3
-2
-1
0
1
log [antibody] µM
Ab 1
Ab 2
Ab 4
Ab 6
Negative control
Positive control

FIG. 5

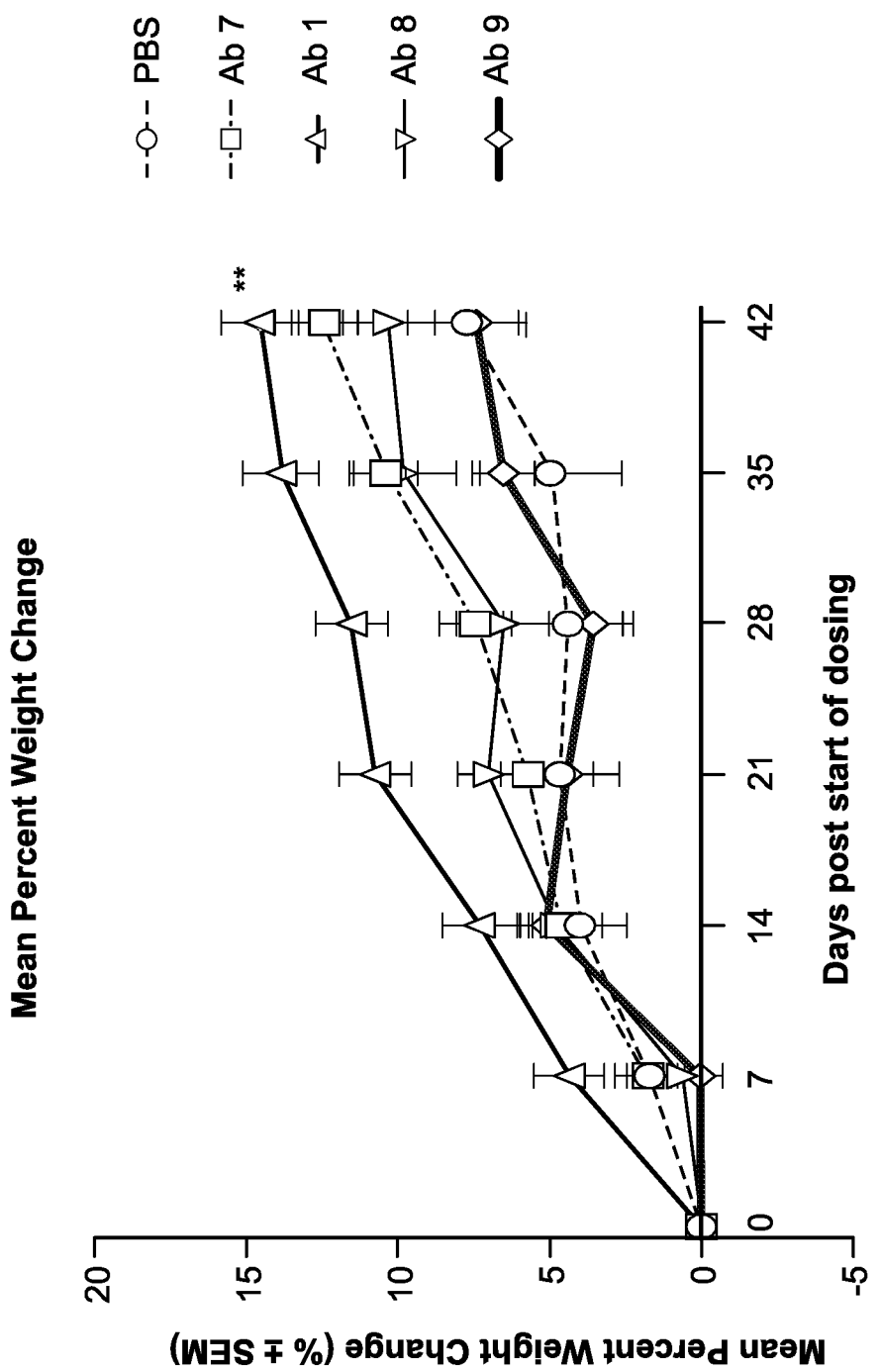

Mean Percent Weight Change
PBS
Ab 7
Ab 1
Ab 8
Ab 9
**
Mean Percent Weight Change (% ± SEM)
20
15
10
5
0
-5
0
7
14
21
28
35
42
Days post start of dosing

FIG. 6

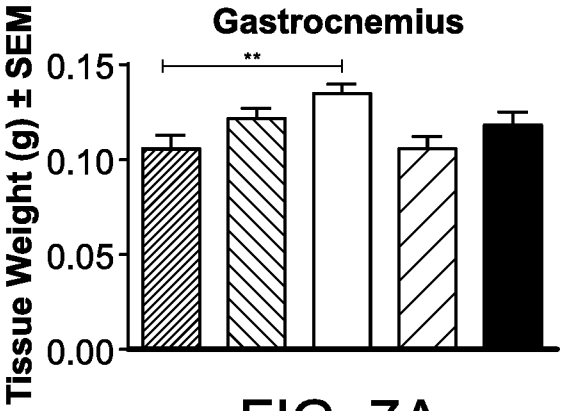


FIG. 7A

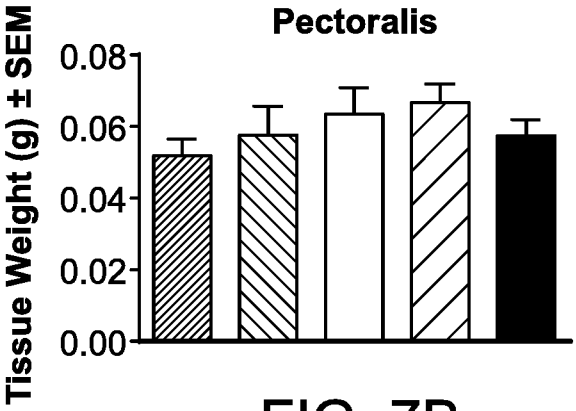


FIG. 7B

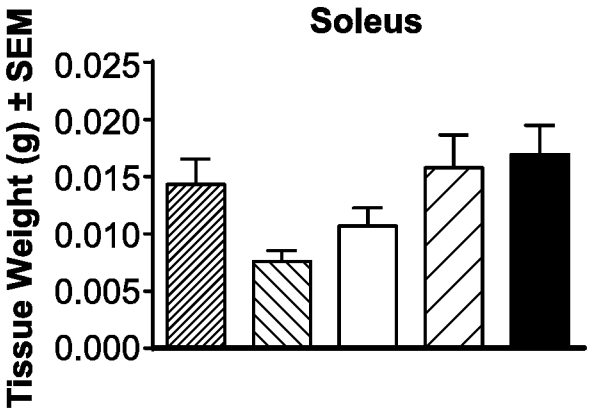


FIG. 7C

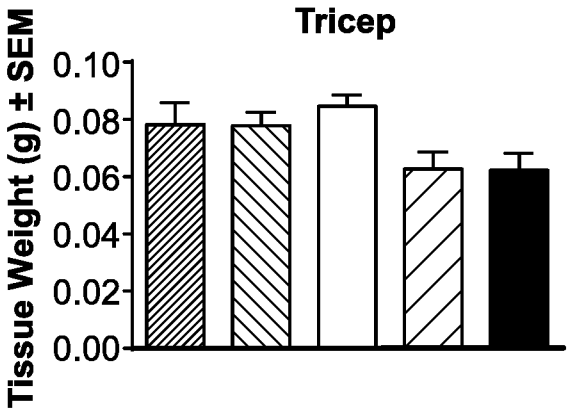


FIG. 7D

Group 1: Vehicle Control
Group 2: Ab7 (25mg/kg)
Group 3: Ab1 (25mg/kg)
Group 4: Ab8 (25mg/kg)
Group 5: Ab9 (25mg/kg)

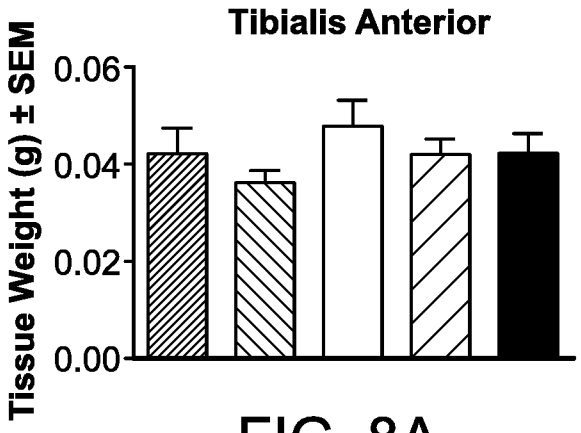


FIG. 8A

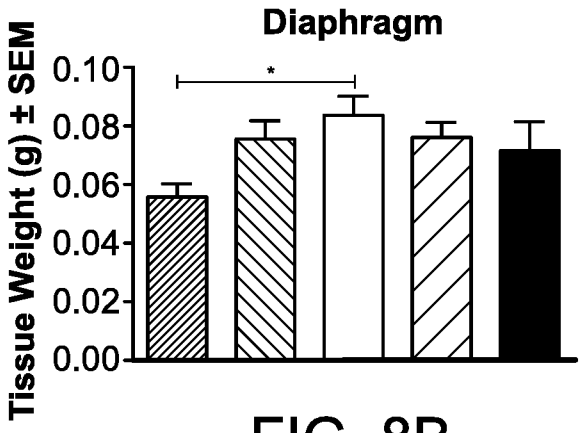


FIG. 8B

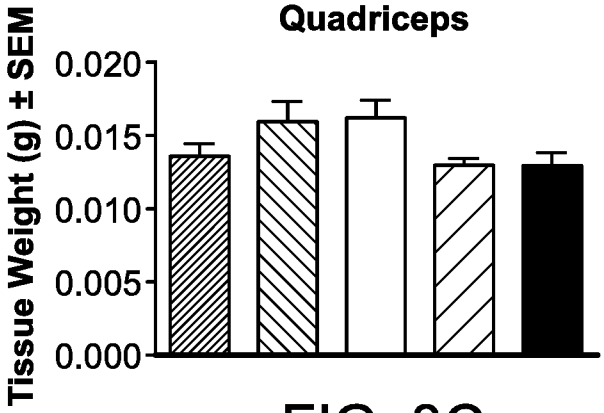


FIG. 8C

Group 1: Vehicle Control
Group 2: Ab7 (25mg/kg)
Group 3: Ab1 (25mg/kg)
Group 4: Ab8 (25mg/kg)
Group 5: Ab9 (25mg/kg)

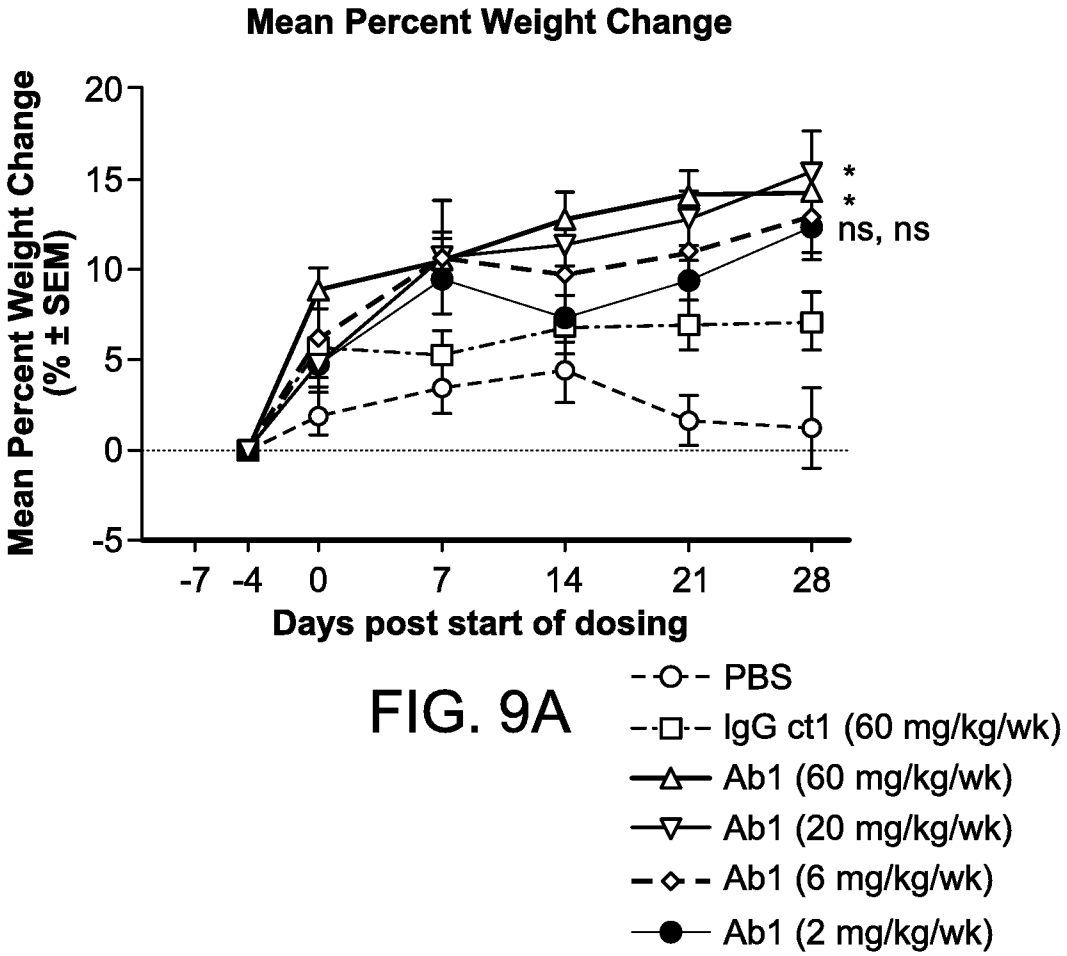

Mean Percent Weight Change
Mean Percent Weight Change (% ± SEM)
20
15
10
5
0
-5
-7 -4 0 7 14 21 28
Days post start of dosing
*
*
ns, ns
PBS
IgG ct1 (60 mg/kg/wk)
Ab1 (60 mg/kg/wk)
Ab1 (20 mg/kg/wk)
Ab1 (6 mg/kg/wk)
Ab1 (2 mg/kg/wk)

FIG. 9A

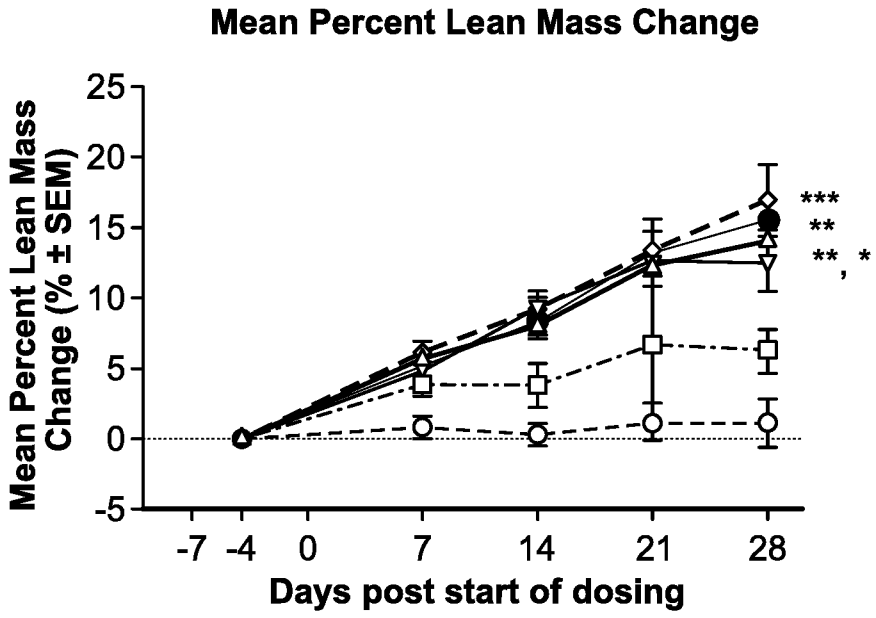

Mean Percent Lean Mass Change
Mean Percent Lean Mass Change (% ± SEM)
25
20
15
10
5
0
-5
-7 -4 0 7 14 21 28
Days post start of dosing
***
**
**, *

FIG. 9B

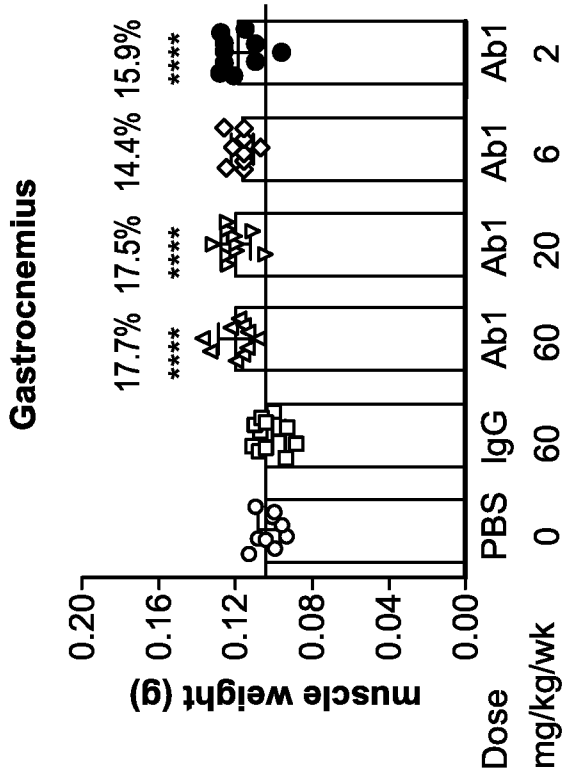


FIG. 10B

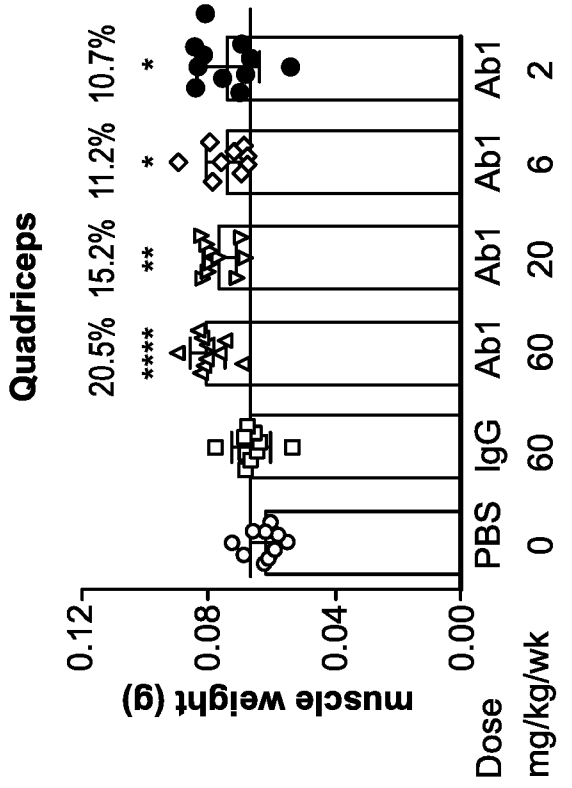


FIG. 10A

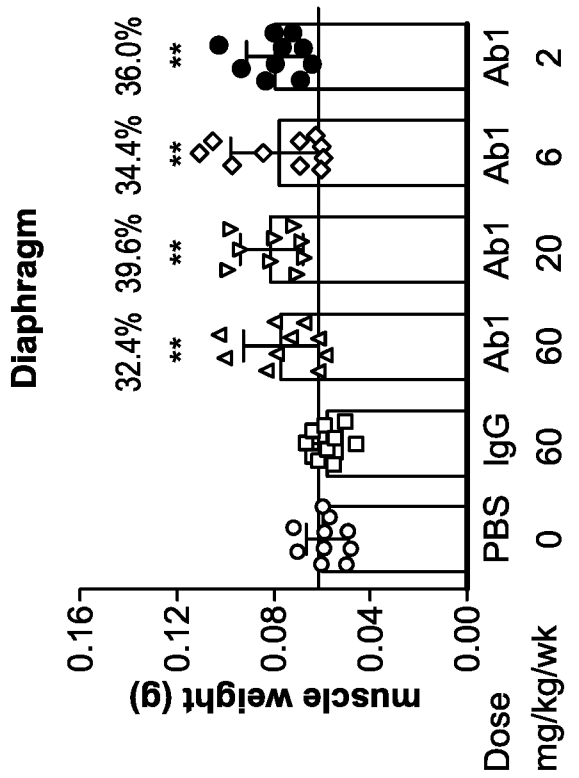


FIG. 10D

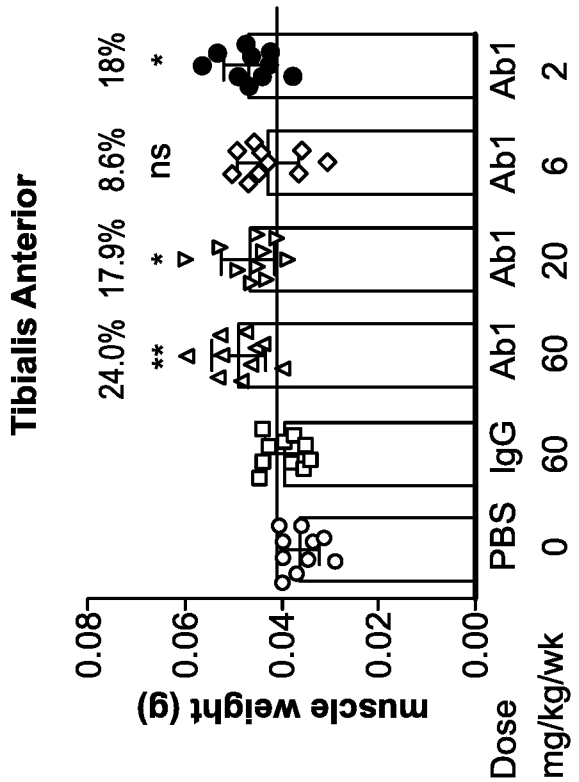


FIG. 10C

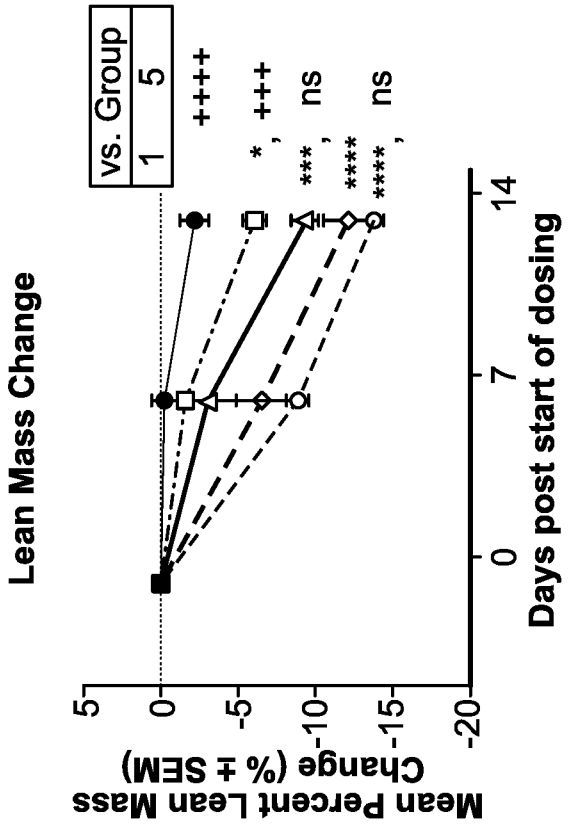


FIG. 11B

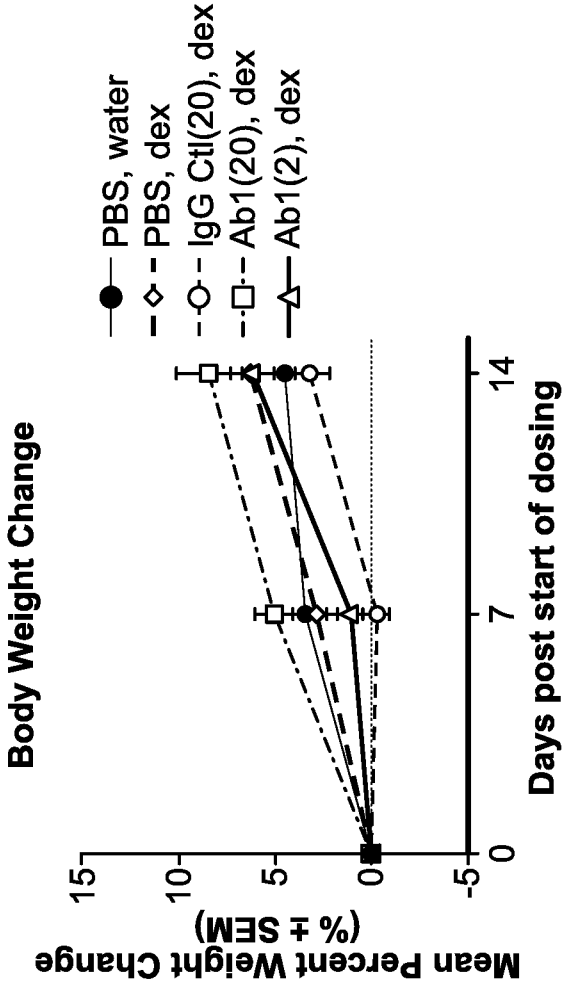


FIG. 11A

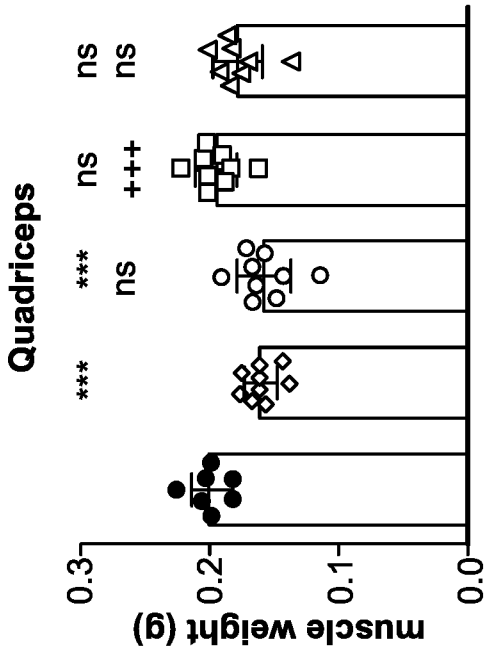


FIG. 12B

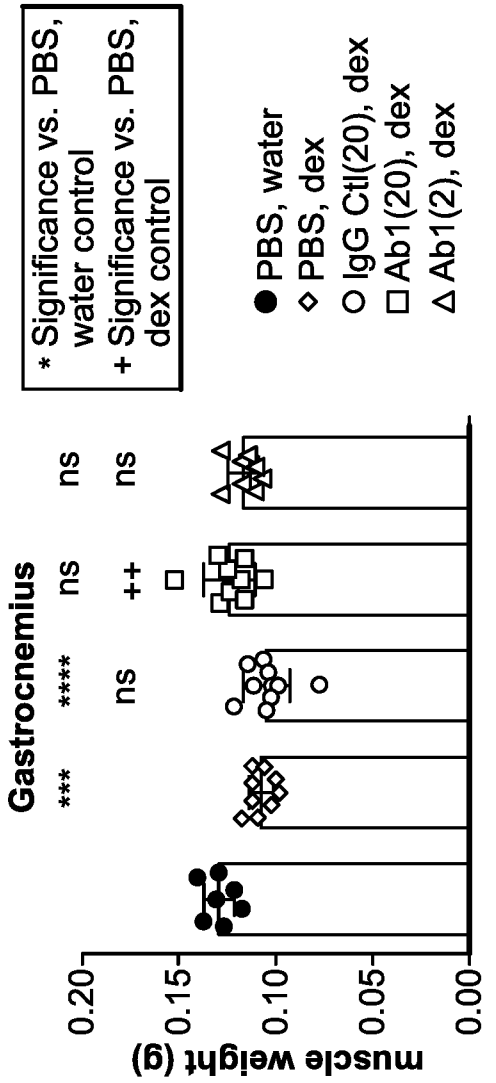


FIG. 12A

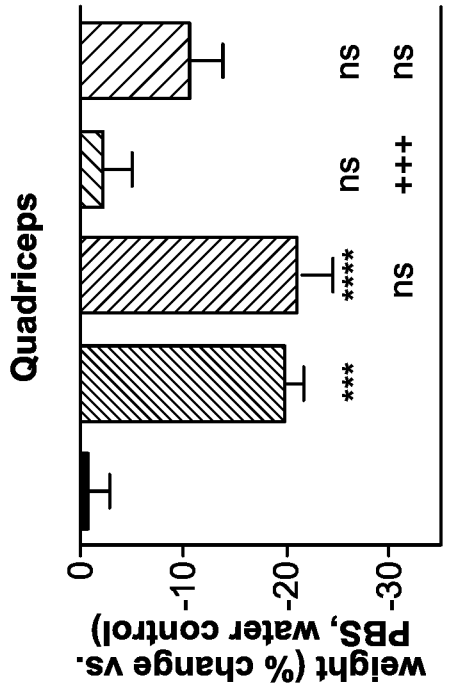


FIG. 12D

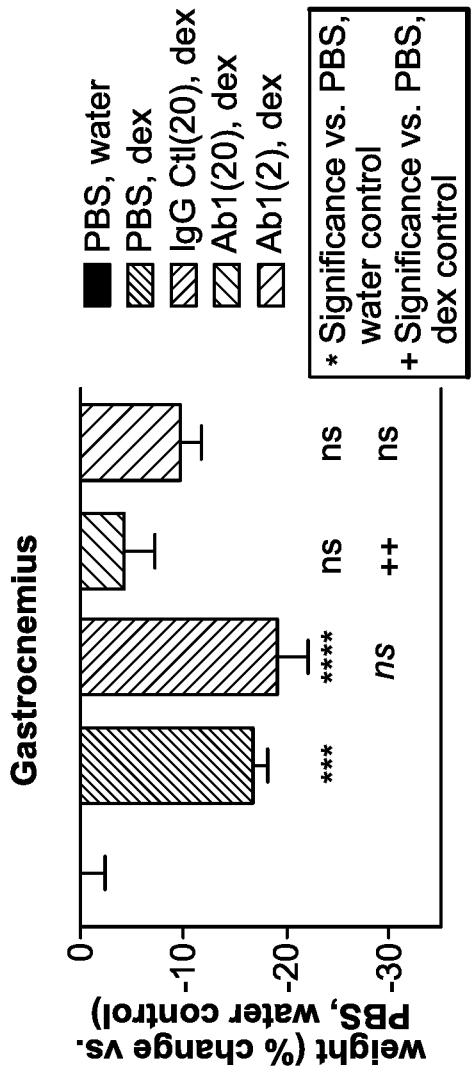


FIG. 12C

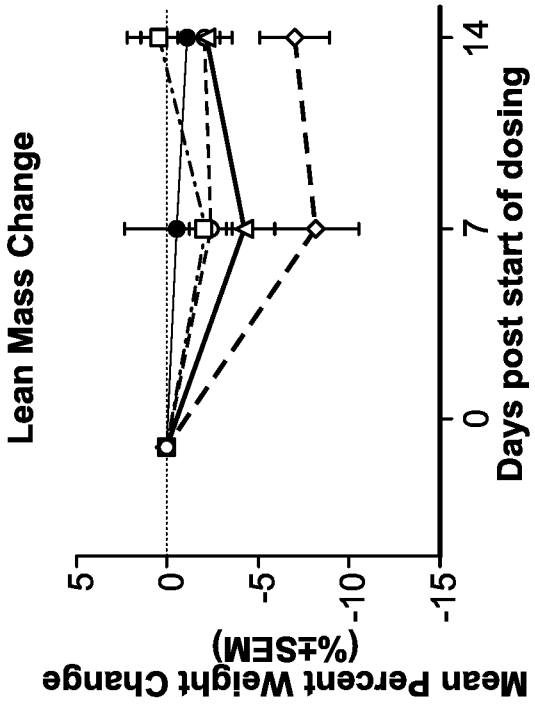


FIG. 13B

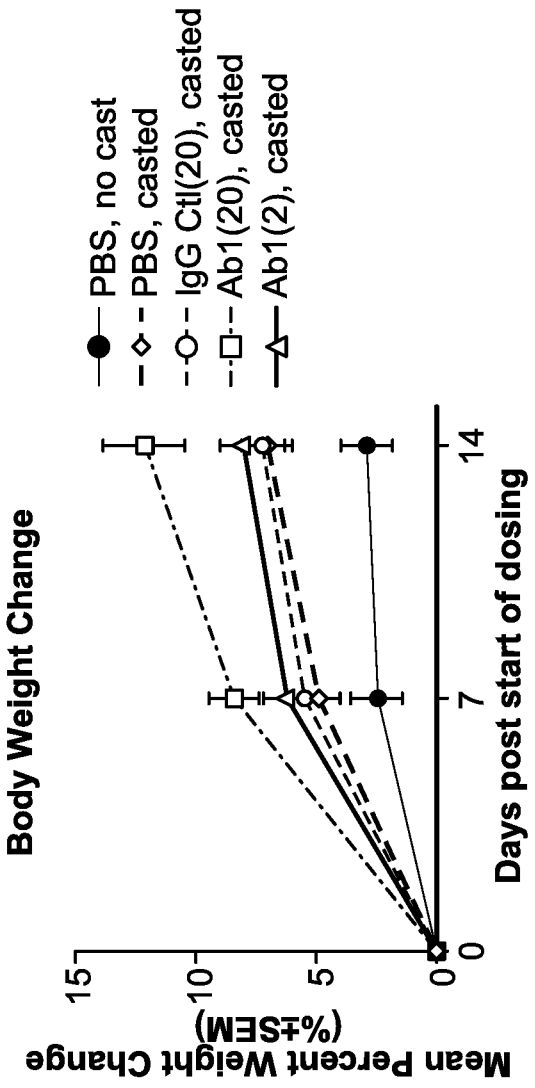


FIG. 13A

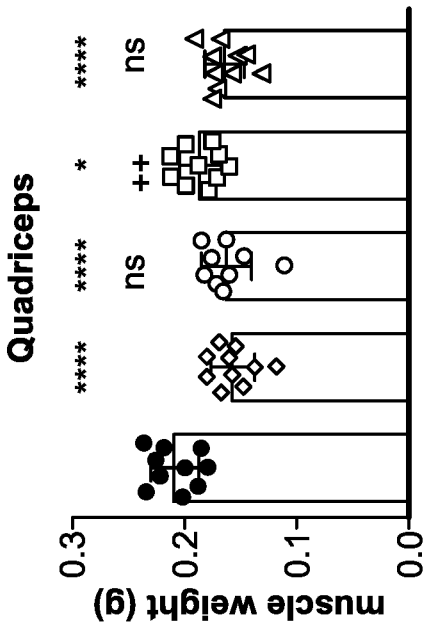


FIG. 14B

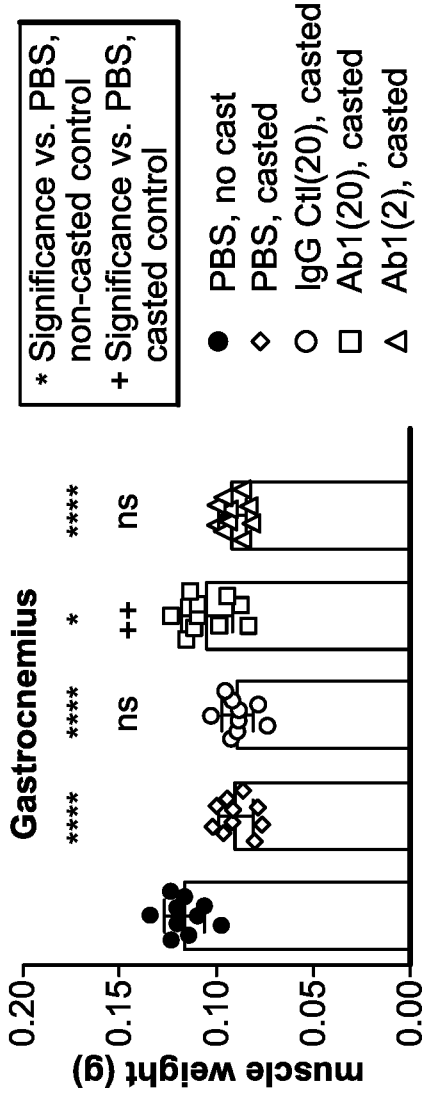


FIG. 14A

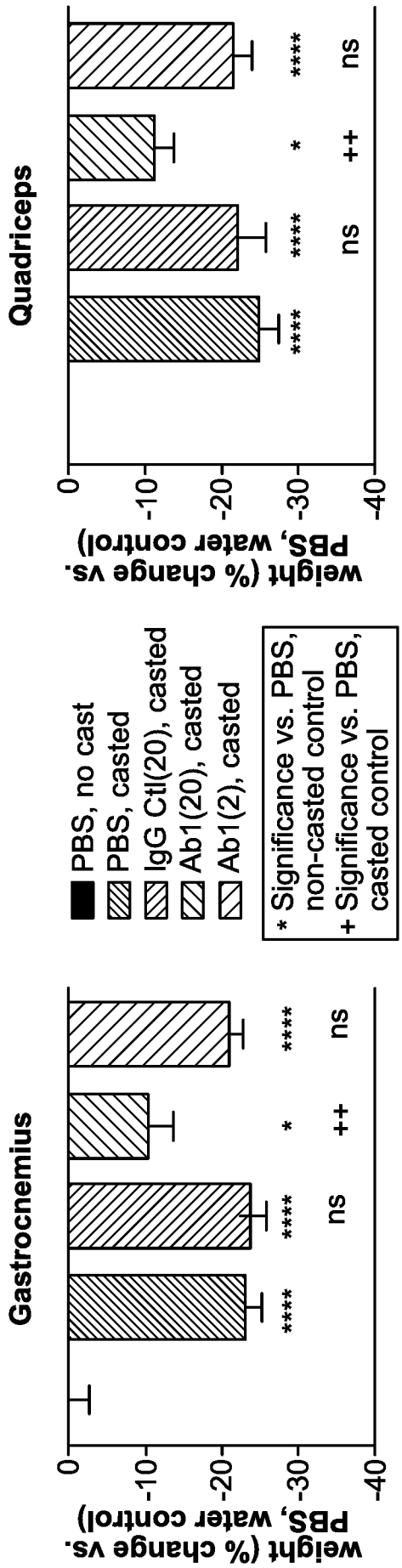


FIG. 14C

FIG. 14D

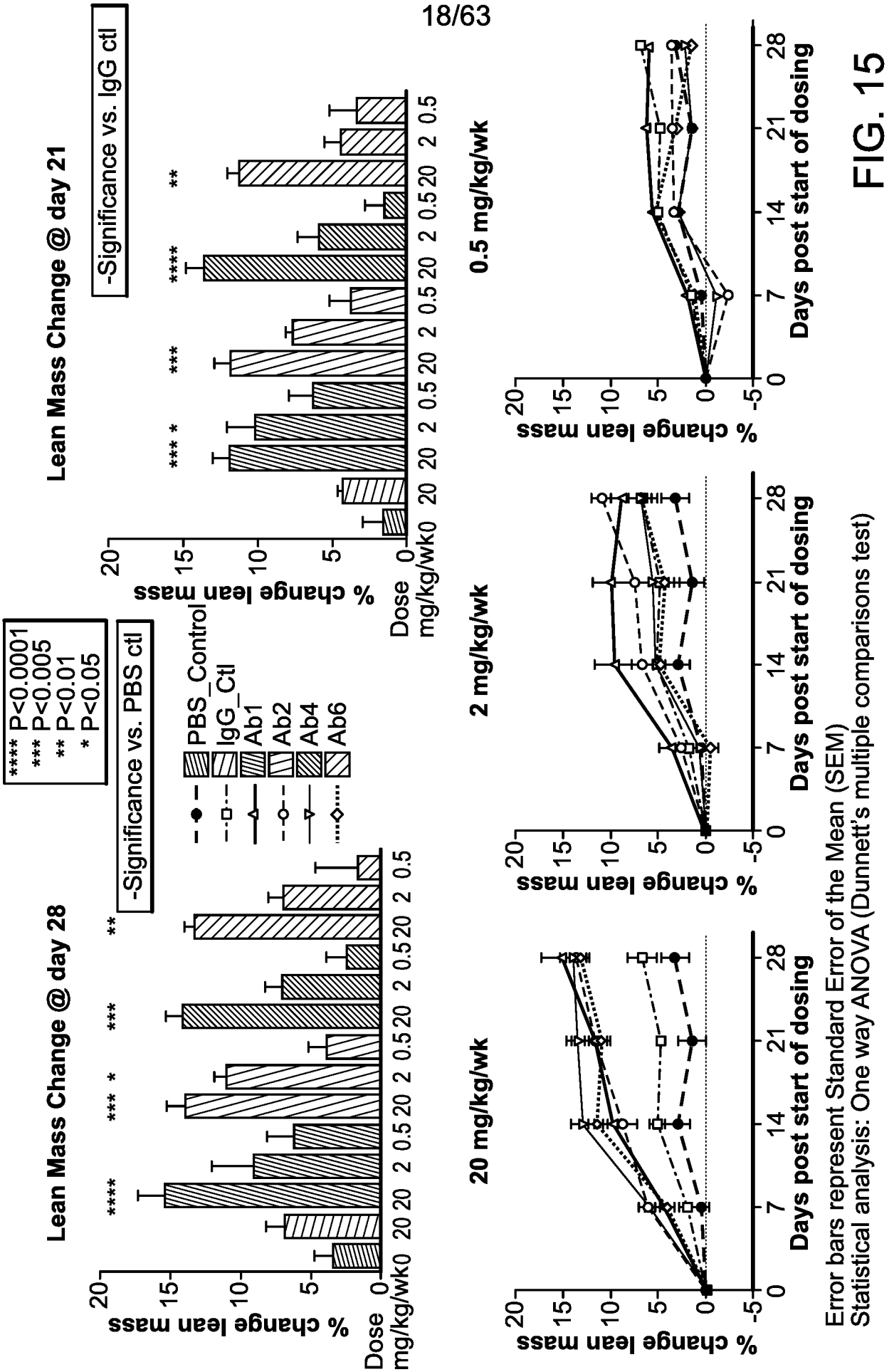

Lean Mass Change @ day 28
Lean Mass Change @ day 21
% change lean mass
Dose mg/kg/wk
0 20 20 0.5 2 0.5 20 2 0.5 20 2 0.5 20 2 0.5
**** *** * *** **
-Significance vs. PBS ctl
-Significance vs. IgG ctl
**** P<0.0001
*** P<0.005
** P<0.01
* P<0.05
PBS_Control
IgG_Ctl
Ab1
Ab2
Ab4
Ab6
20 mg/kg/wk
2 mg/kg/wk
0.5 mg/kg/wk
Days post start of dosing
Error bars represent Standard Error of the Mean (SEM)
Statistical analysis: One way ANOVA (Dunnett's multiple comparisons test)

FIG. 15

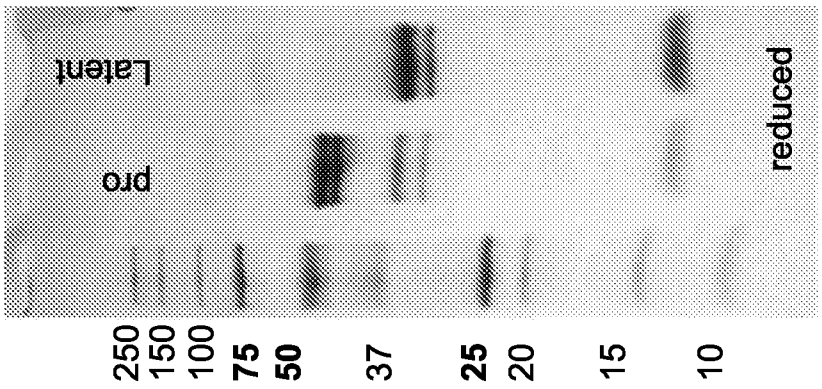


FIG. 16B

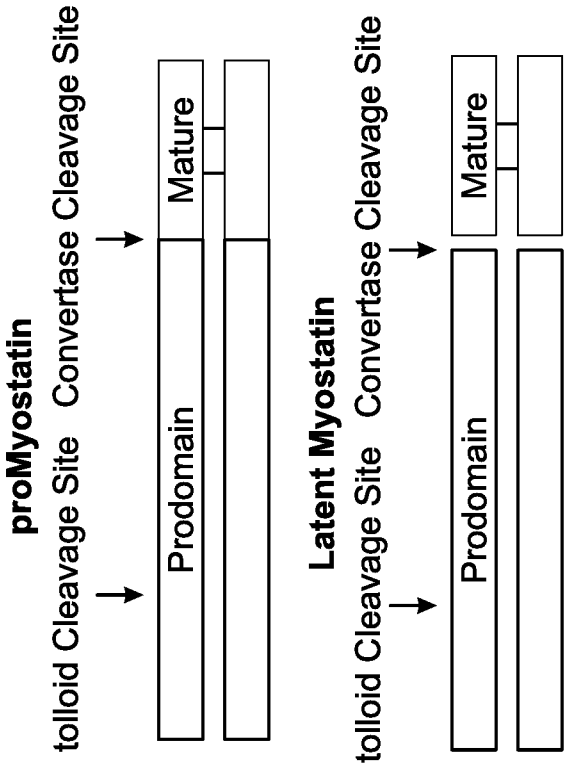


FIG. 16A

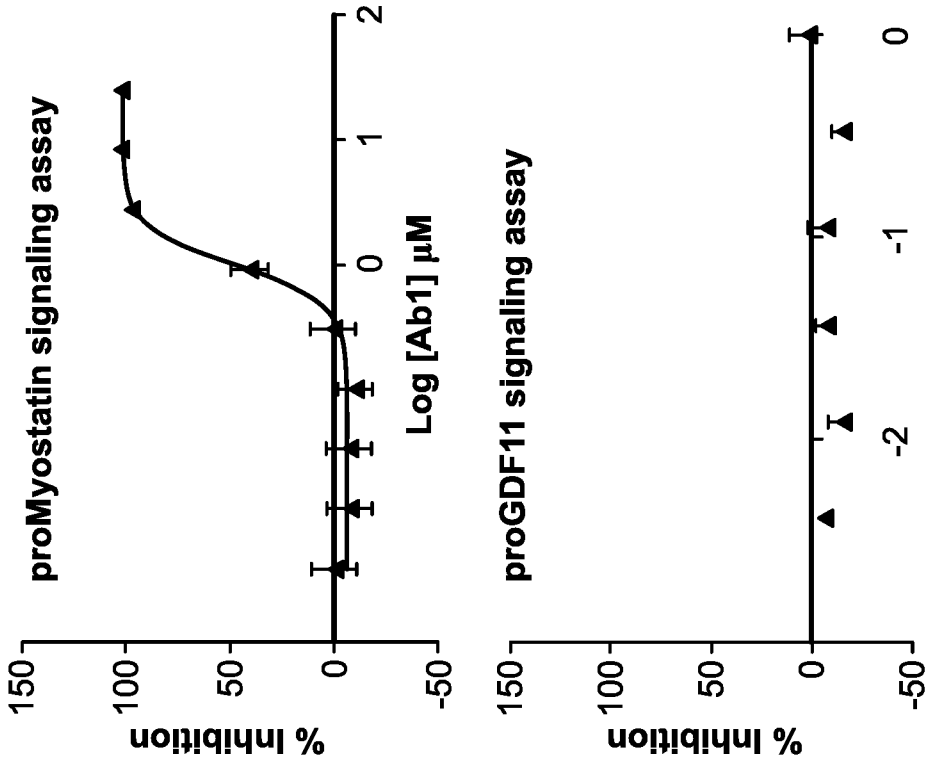


FIG. 17B

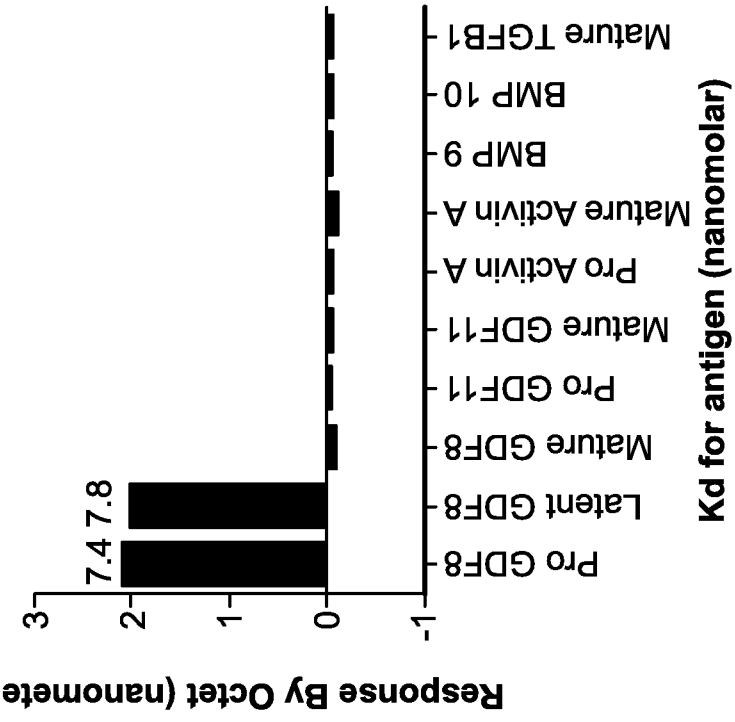


FIG. 17A

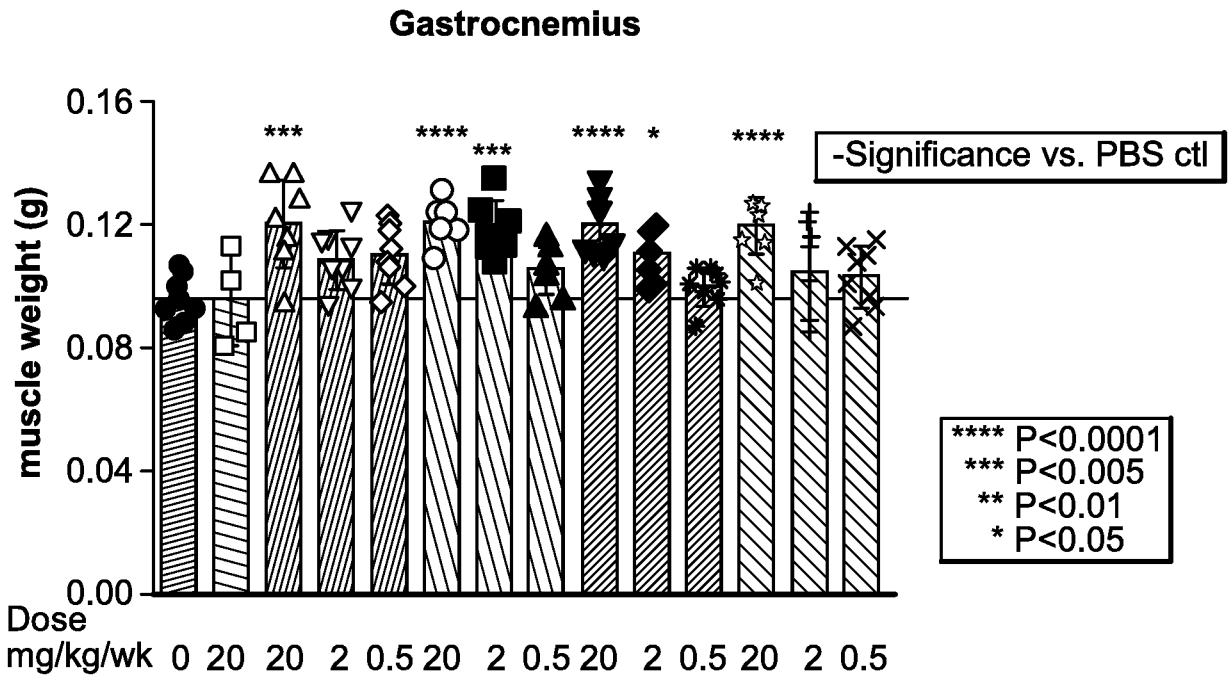
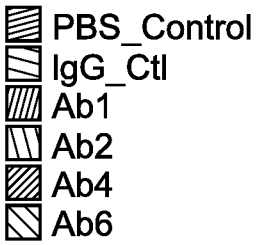


FIG. 18A



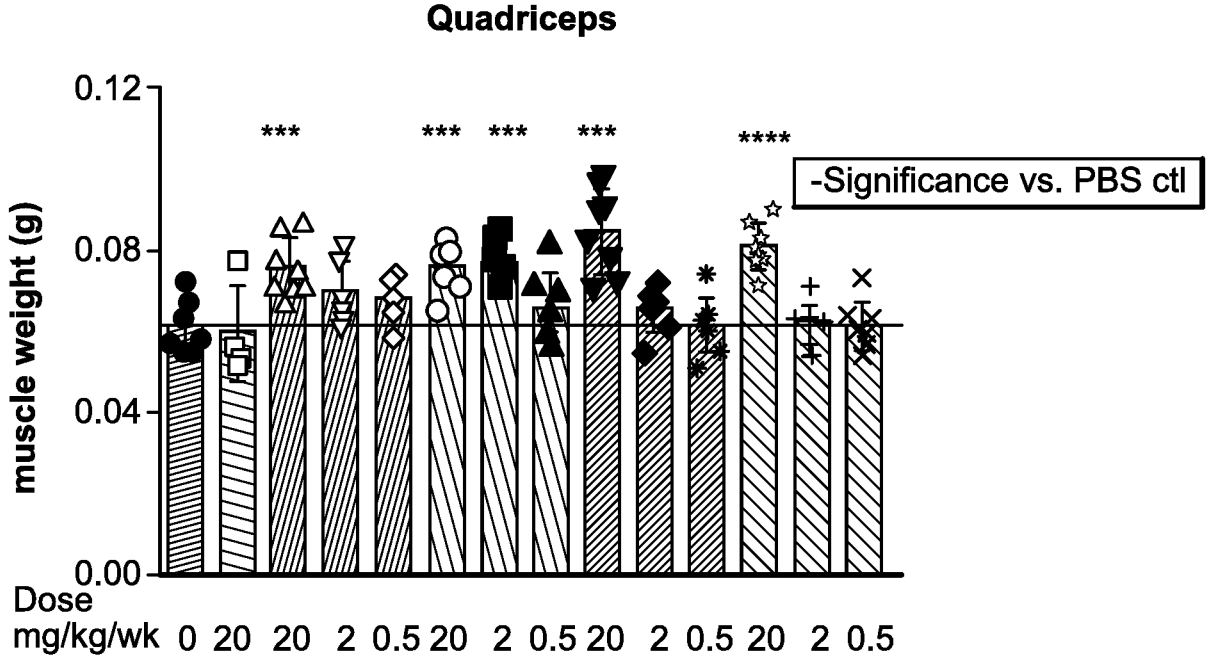


FIG. 18B

Muscle	Ab	PBS_Ctl	20 mg/kg/wk	2 mg/kg/wk	0.5 mg/kg/wk
Gastrocnemius	Ab1	0	25.1	13.0	14.9
Gastrocnemius	Ab2	0	25.6	24.0	9.8
Gastrocnemius	Ab4	0	25.0	15.0	3.1
Gastrocnemius	Ab6	0	24.8	9.3	7.4
Quadriceps	Ab1	0	23.9	13.9	10.8
Quadriceps	Ab2	0	23.9	26.1	7.6
Quadriceps	Ab4	0	37.8	6.4	0.2
Quadriceps	Ab6	0	32.0	0.4	0.2

FIG. 18C

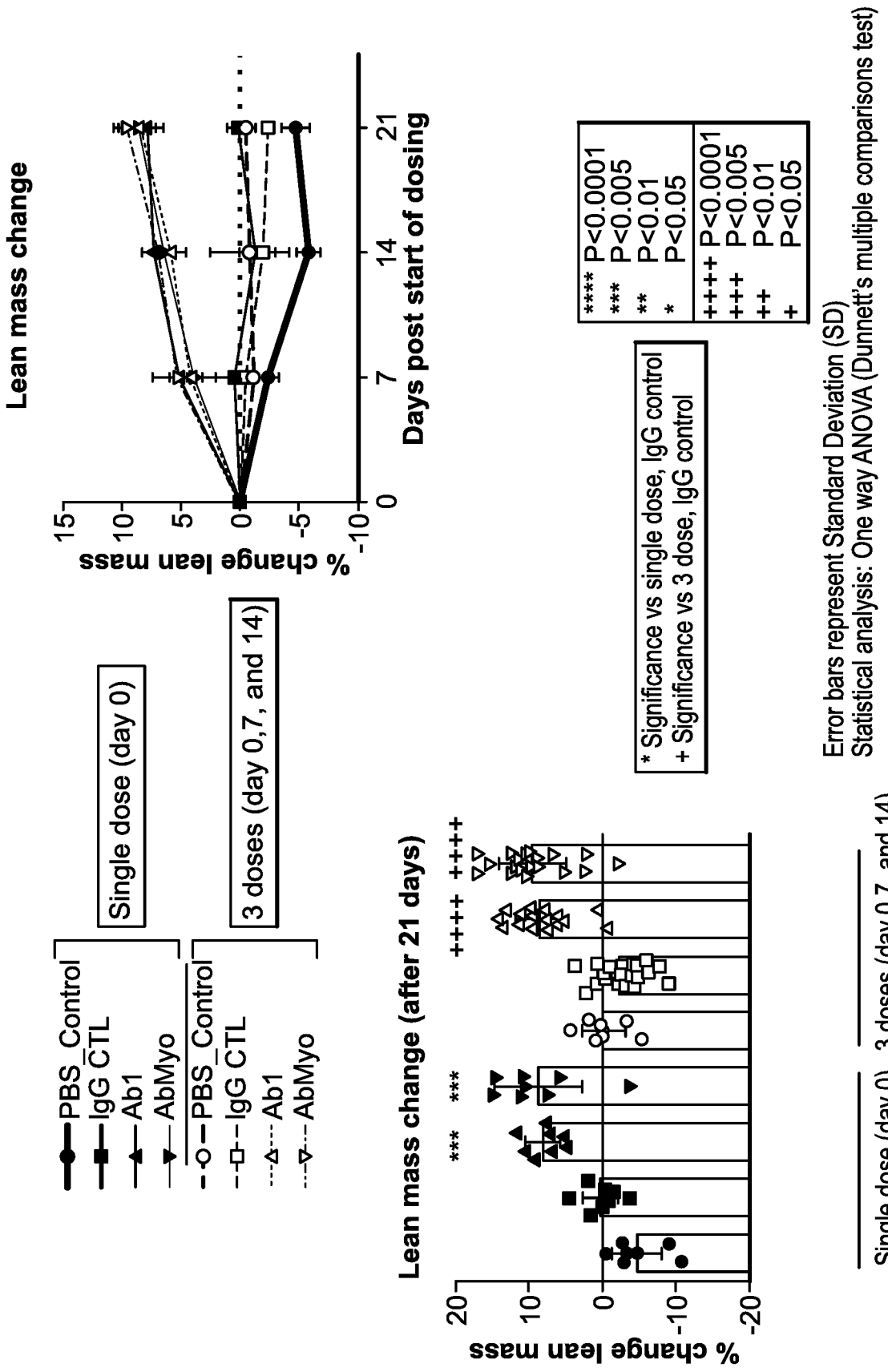

Lean mass change
% change lean mass
Days post start of dosing
PBS_Control
IgG CTL
Ab1
AbMyo
Single dose (day 0)
3 doses (day 0,7, and 14)
Lean mass change (after 21 days)
% change lean mass
* Significance vs single dose, IgG control
+ Significance vs 3 dose, IgG control
**** P<0.0001
*** P<0.005
** P<0.01
* P<0.05
++++ P<0.0001
+++ P<0.005
++ P<0.01
+ P<0.05
Error bars represent Standard Deviation (SD)
Statistical analysis: One way ANOVA (Dunnett's multiple comparisons test)
Single dose (day 0)
3 doses (day 0,7, and 14)

FIG. 19

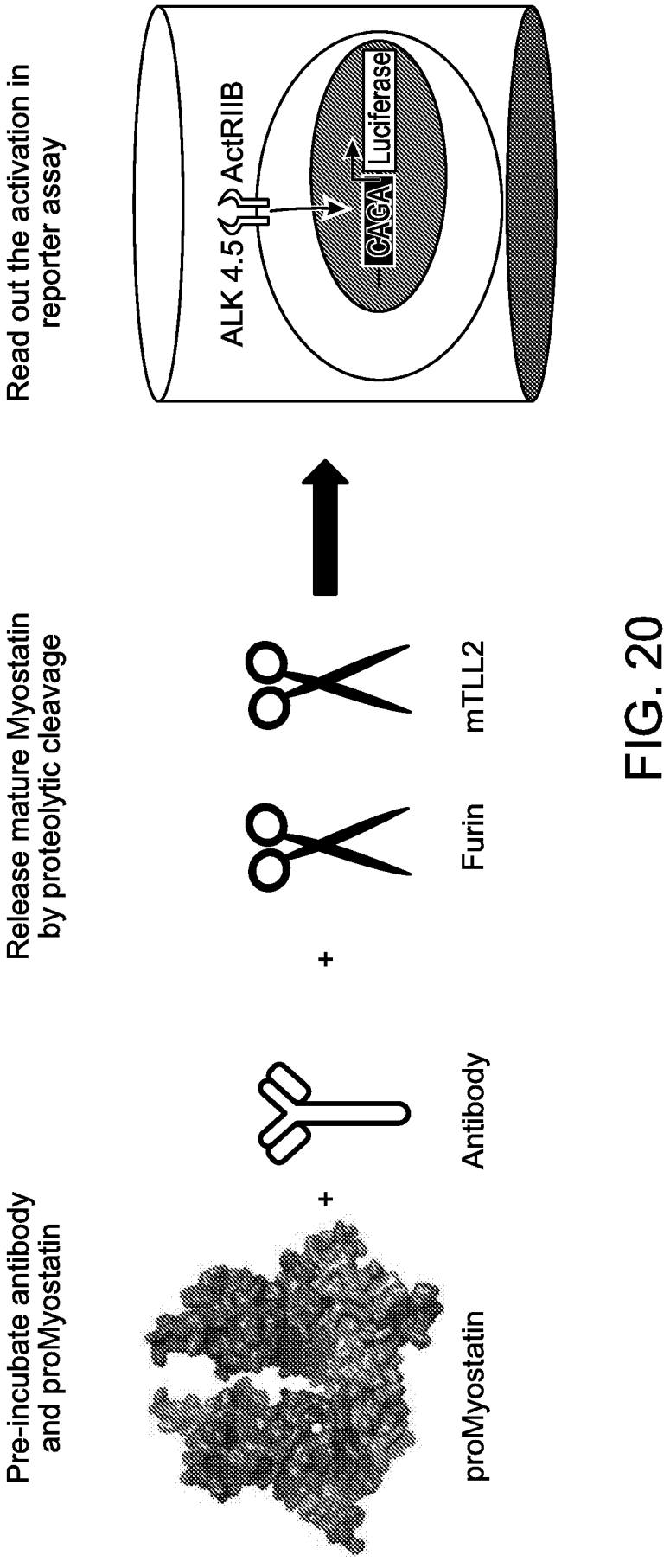

Pre-incubate antibody and proMyostatin
proMyostatin
+
Antibody
+
Release mature Myostatin by proteolytic cleavage
Furin
mTLL2
Read out the activation in reporter assay
ALK 4.5
ActRIIB
CAGA
Luciferase

FIG. 20

```
1QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYY  60
61ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDLLVRFLEWSHYYGMDVWGQGT 120
121TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP 180
181AVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF 240
241LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE 300
301QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS 360
361QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDK 420
421SRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG                               452
```

FIG. 21A

```
1QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNTVHWYQQLPGTAPKLLIYSDNQRPSGVP  60
61DRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGVFGGGTKLTVLGQPKAAPSVTL 120
121FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSY 180
181LSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS                           215
```

FIG. 21B

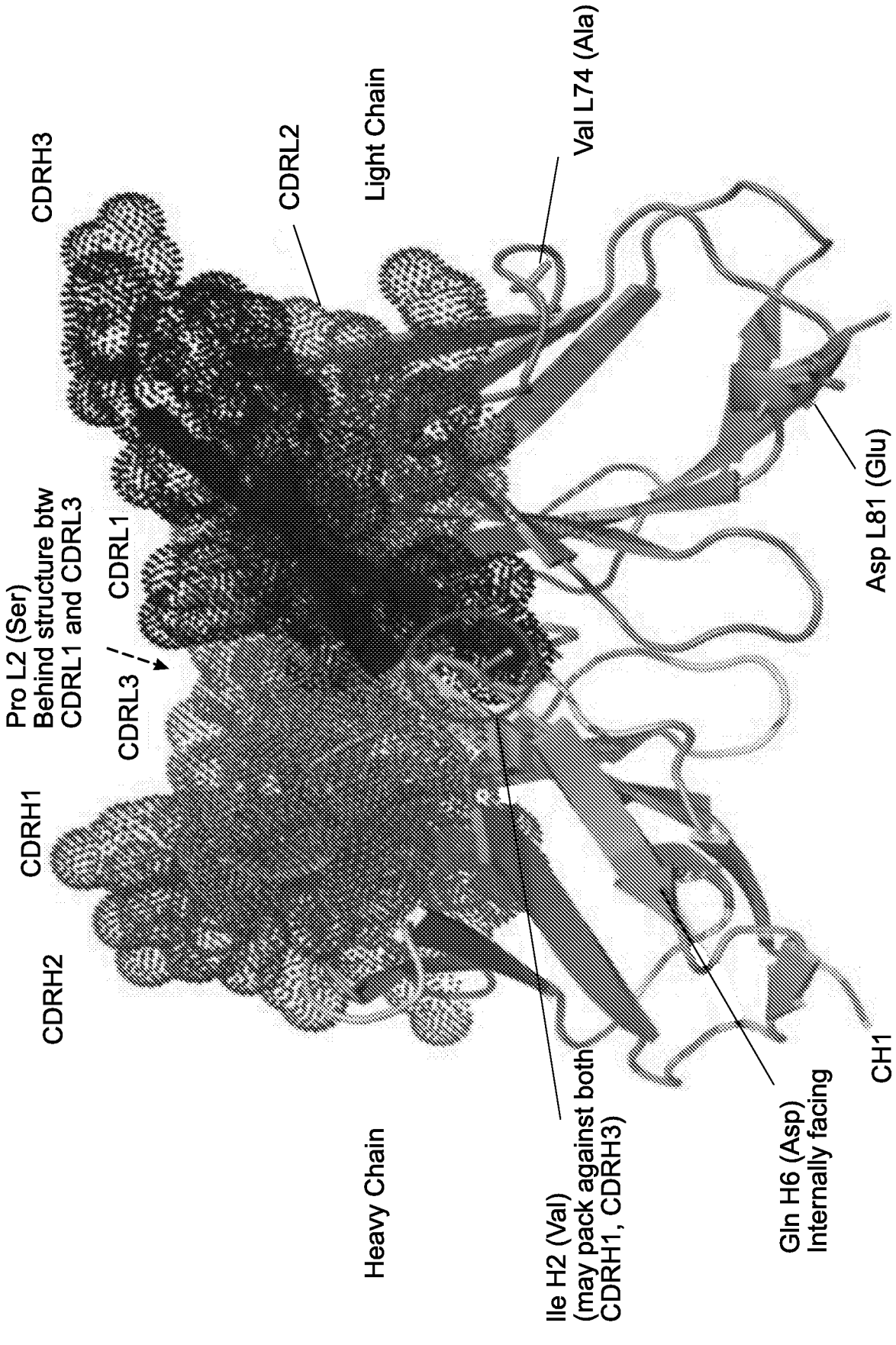

CDRH3
CDRL2
Light Chain
Val L74 (Ala)
C-Lambda
Pro L2 (Ser)
Behind structure btw
CDRL1 and CDRL3
CDRL1
CDRL3
CDRH1
CDRH2
Asp L81 (Glu)
Heavy Chain
Ile H2 (Val)
(may pack against both
CDRH1, CDRH3)
Gln H6 (Asp)
Internally facing
CH1

FIG. 22

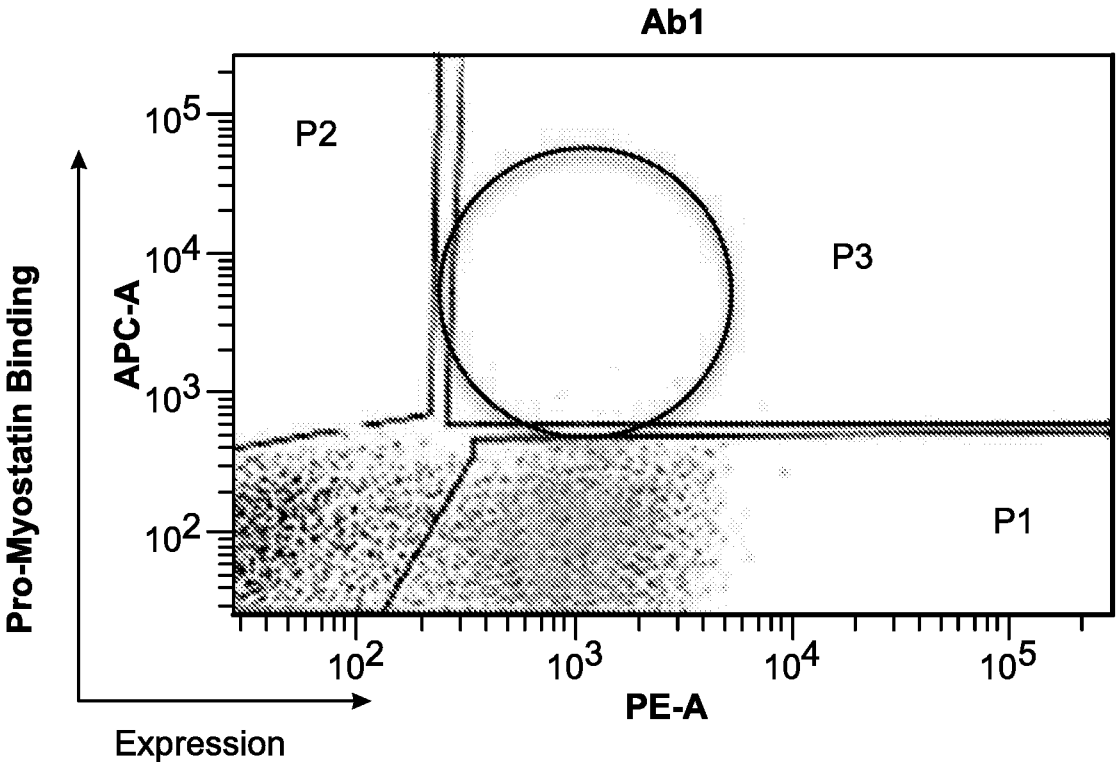


FIG. 23A

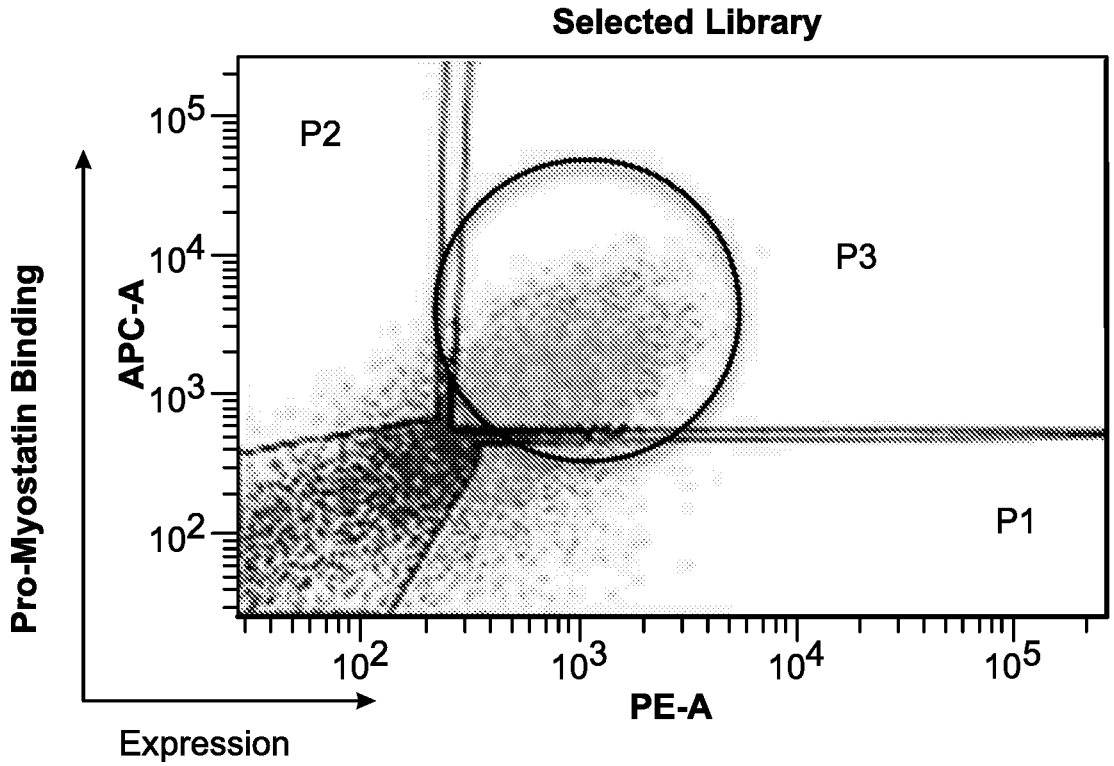
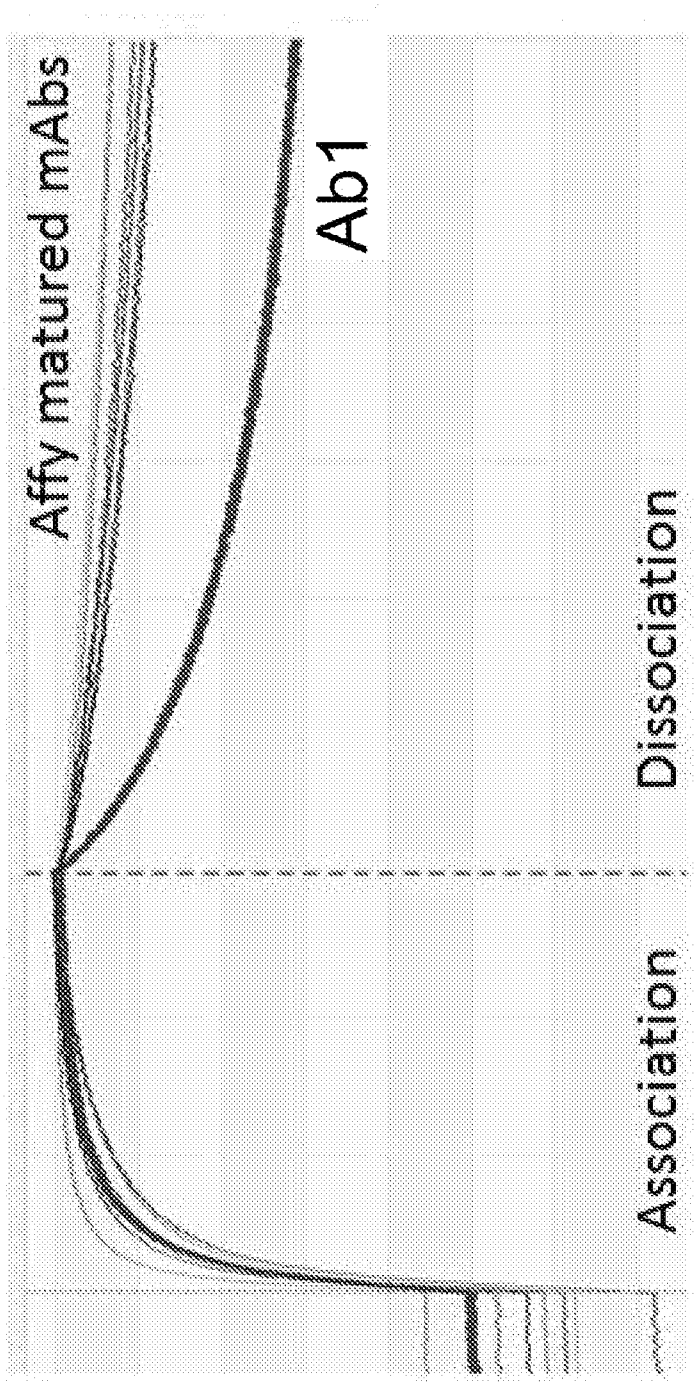


FIG. 23B



Top 12 clones selected for further characterization

Ab1:	4.8 nM		
Ab4:		0.47 nM	10-fold improved
Ab6:		0.48 nM	10-fold improved
Best affy matured mAb:		<10 pM	>480-fold improvement

FIG. 23C

```
                FRAMEWORK 1          CDR1      FRAMEWORK 2
Ab1   QIQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVA
Ab3   QIQLVQSGGGVVQPGRSLRLSCAASGFAFSSYGMHWVRQAPGKGLEWVA
Ab5   QIQLVQSGGGVVQPGRSLRLSCAASGFAFSSYGMHWVRQAPGKGLEWVA

                 CDR2              FRAMEWORK 3
Ab1   VISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
Ab3   VISYDGSIKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
Ab5   VISYDGNNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR

                  CDR3           FRAMEWORK 4
Ab1   DLLVRFLEWSHYYGMDVWGQGTTVTVSS
Ab3   DLLVRFLEWSHKYGMDVWGQGTTVTVSS
Ab5   DLLVRFLEWSHKYGMDVWGQGTTVTVSS
```

FIG. 24A

```
                FRAMEWORK 1          CDR1       FRW2
Ab1   QPVLTQPPSASGTPGQRVTISCSGSSSNIGSNTVHWYQQLPGTAPKLLIY
Ab3   QPVLTQPPSASGTPGQRVTISCSGSTSNIGSNTVHWYQQLPGTAPKLLIY
Ab5   QPVLTQPPSASGTPGQRVTISCSGSSSNIGGNTVHWYQQLPGTAPKLLIY

                 CDR2           FRAMEWORK 3
Ab1   SDNQRPSGVPDRFSGSKSGTSASLVISGLQSDDEADYYC
Ab3   SDDQRPSGVPDRFSGSKSGTSASLVISGLQSDDEADYYC
Ab5   SDDQRPSGVPDRFSGSKSGTSASLVISGLQSDDEADYYC

                 CDR3       FRAMEWORK 4
Ab1   AAWDDSLNGVFGGGTKLTVL
Ab3   AAWDESLNGVFGGGTKLTVL
Ab5   AAWDESLNGVFGGGTKLTVL
```

FIG. 24B

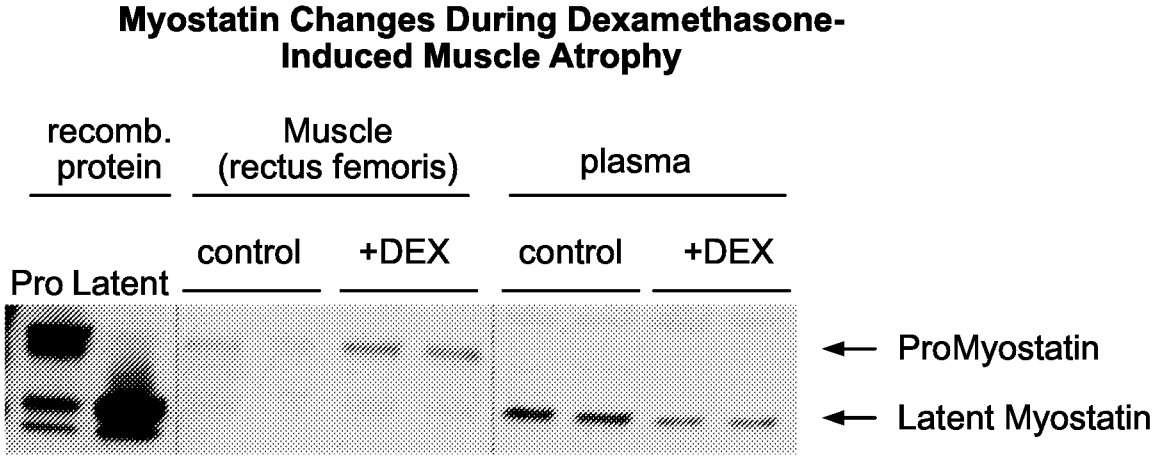


FIG. 25

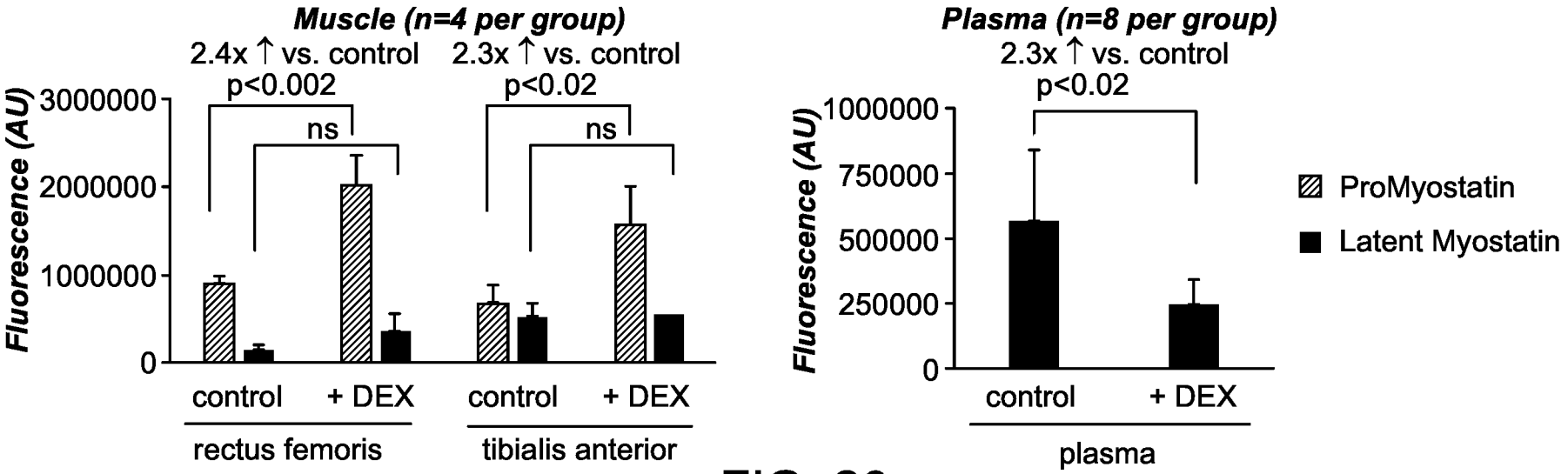


FIG. 26

SUBSTITUTE SHEET (RULE 26)

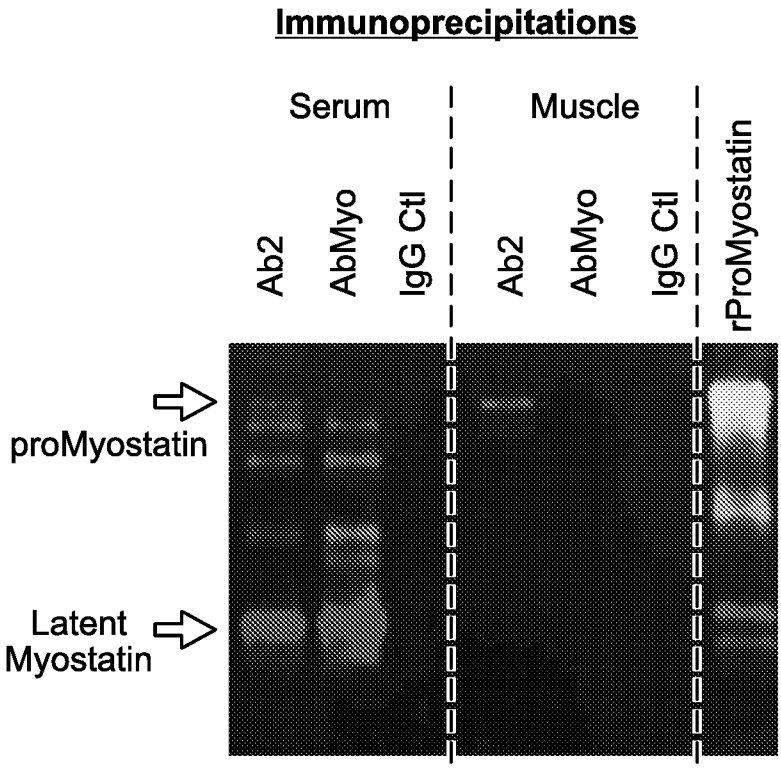

Immunoprecipitations
Serum
Muscle
Ab2
AbMyo
IgG Ctl
Ab2
AbMyo
IgG Ctl
rProMyostatin
proMyostatin
Latent
Myostatin

FIG. 27

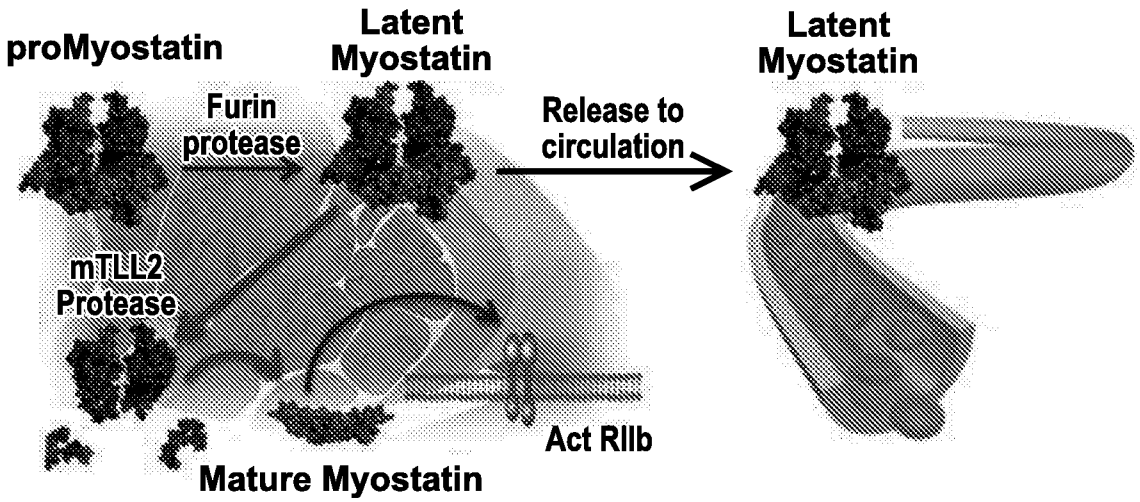


FIG. 28A

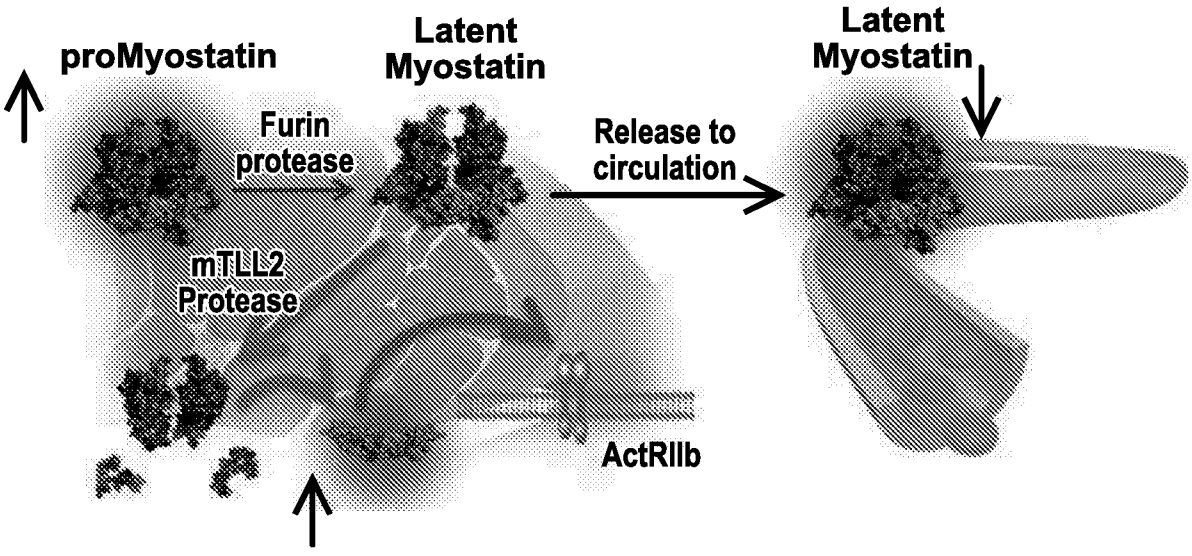


FIG. 28B

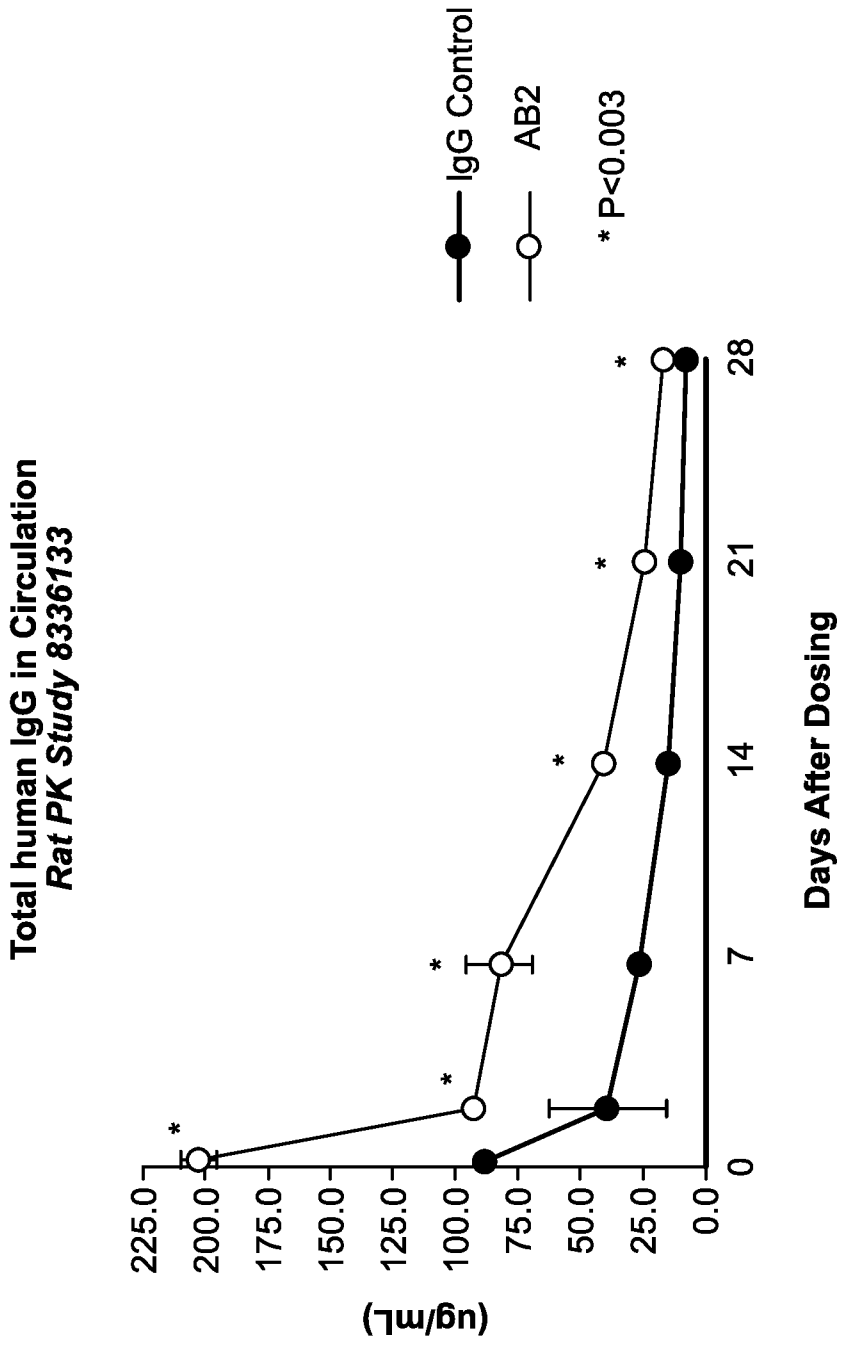

Total human IgG in Circulation
Rat PK Study 8336133
IgG Control
AB2
* P<0.003
(ug/mL)
225.0
200.0
175.0
150.0
125.0
100.0
75.0
50.0
25.0
0.0
0
7
14
21
28
Days After Dosing

FIG. 29

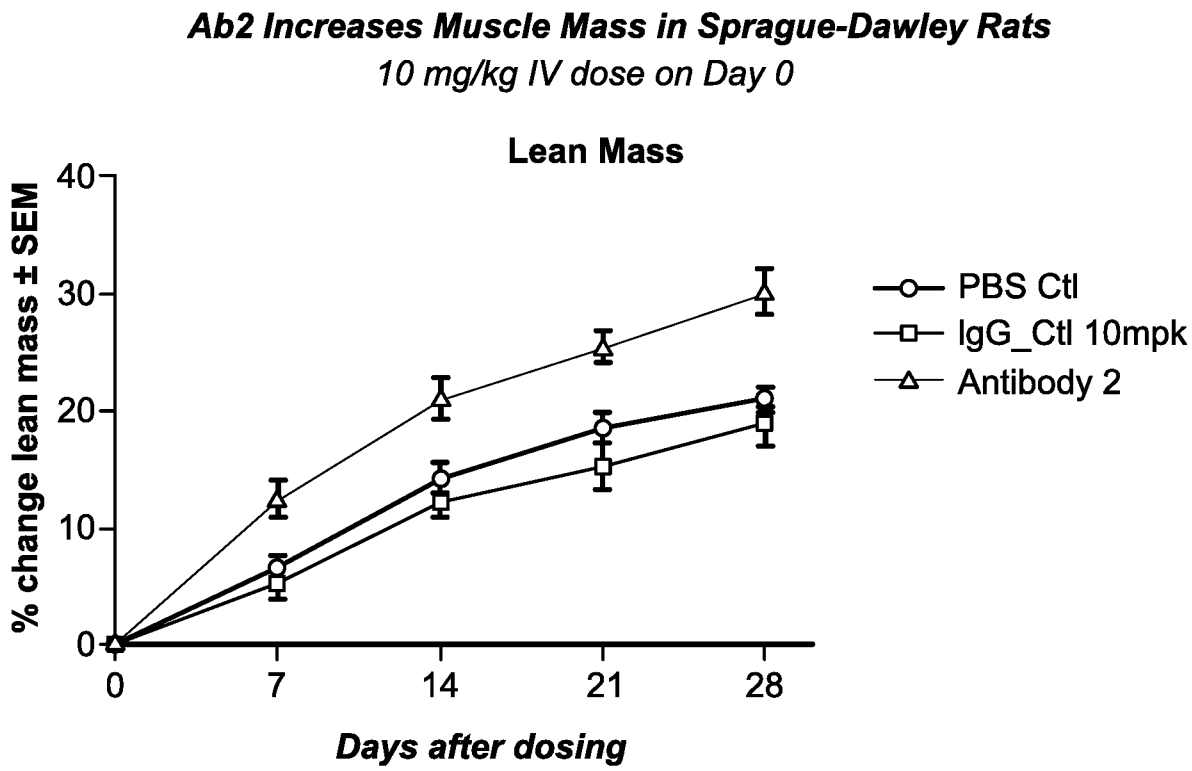


FIG. 30A

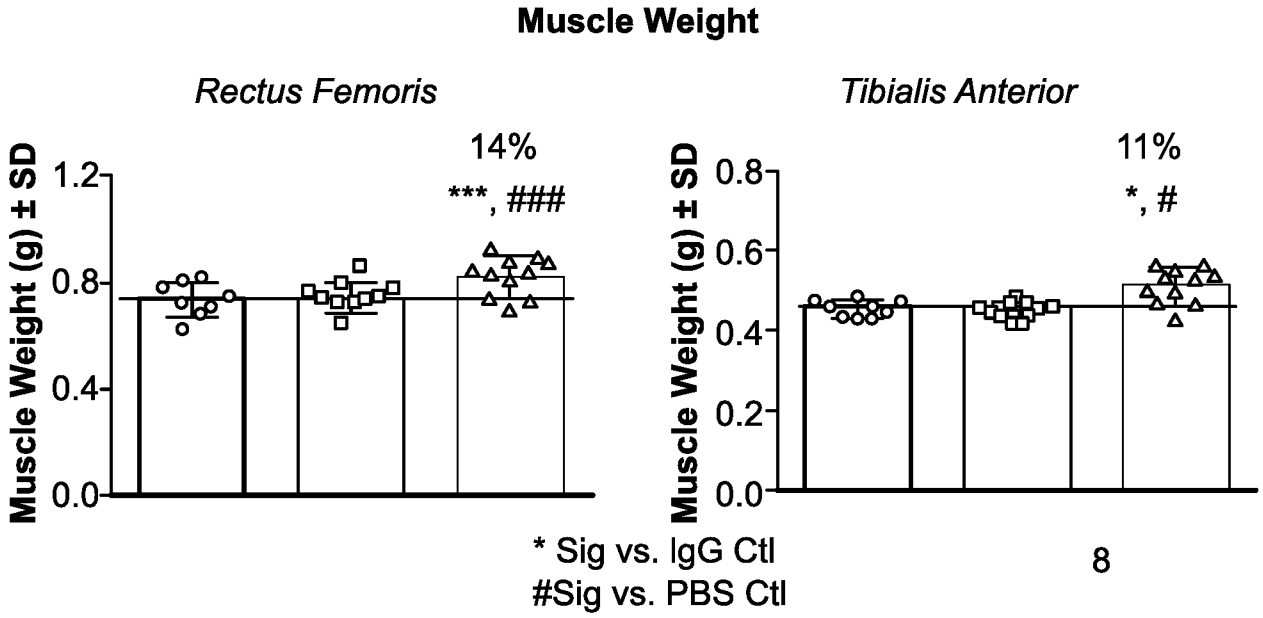


FIG. 30B

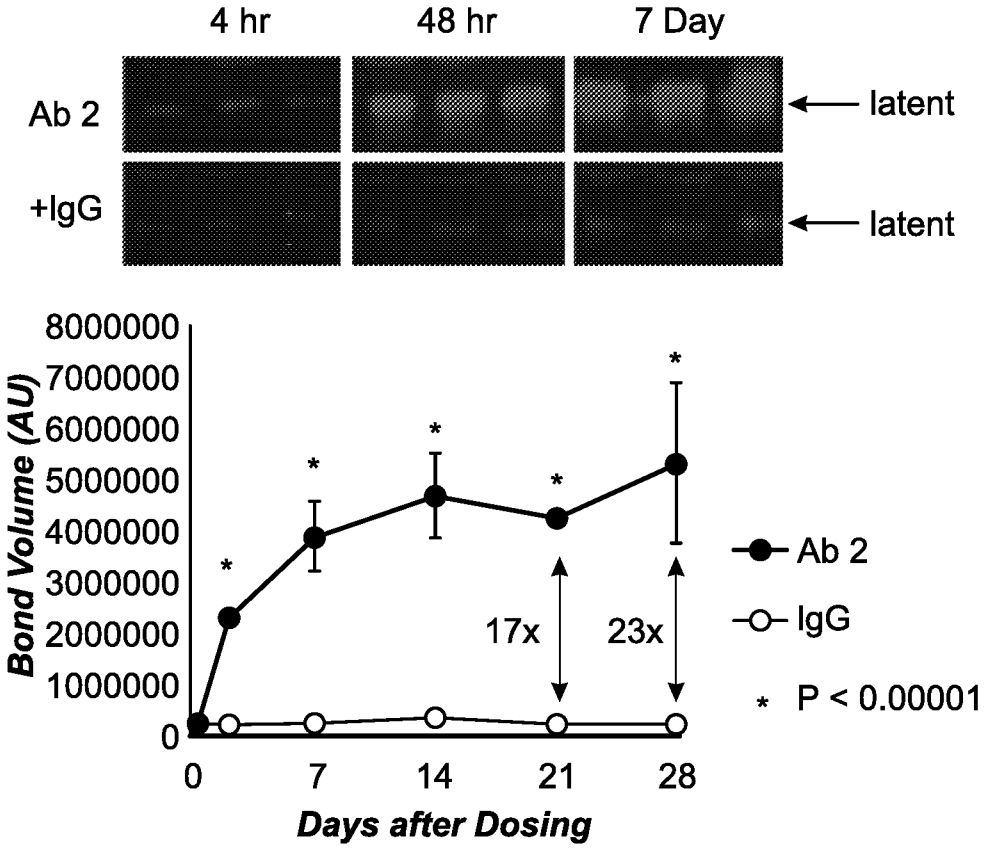


FIG. 31A

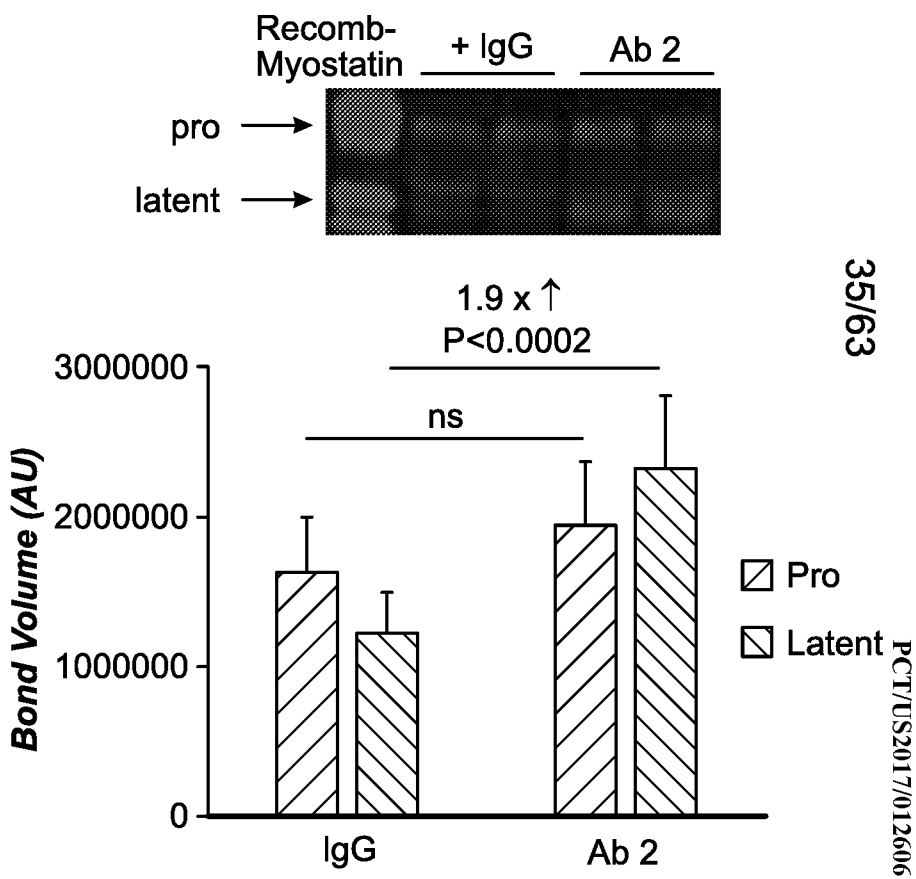


FIG. 31B

Increased Duration of Muscle Mass Gain in Mice Treated with Ab2

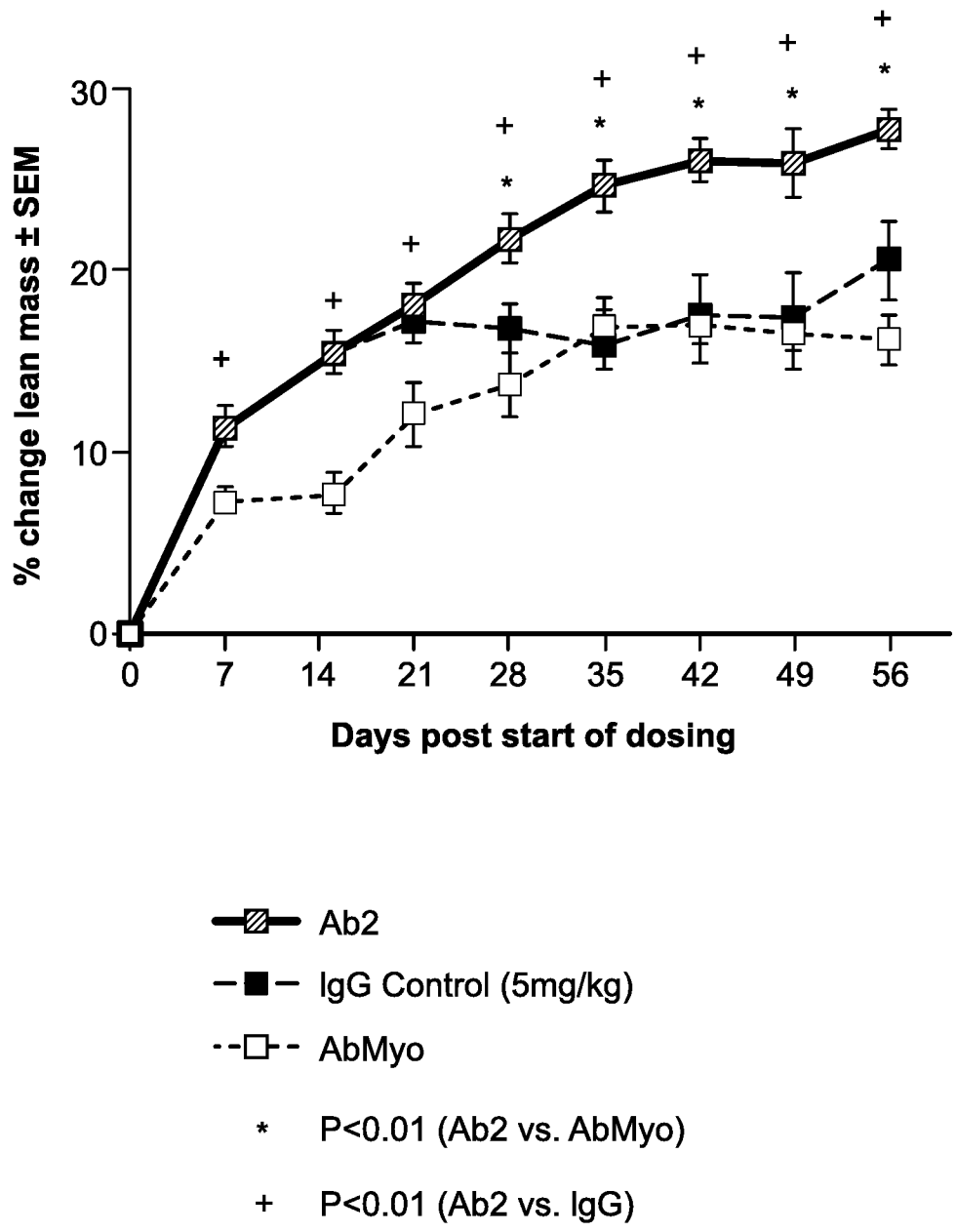
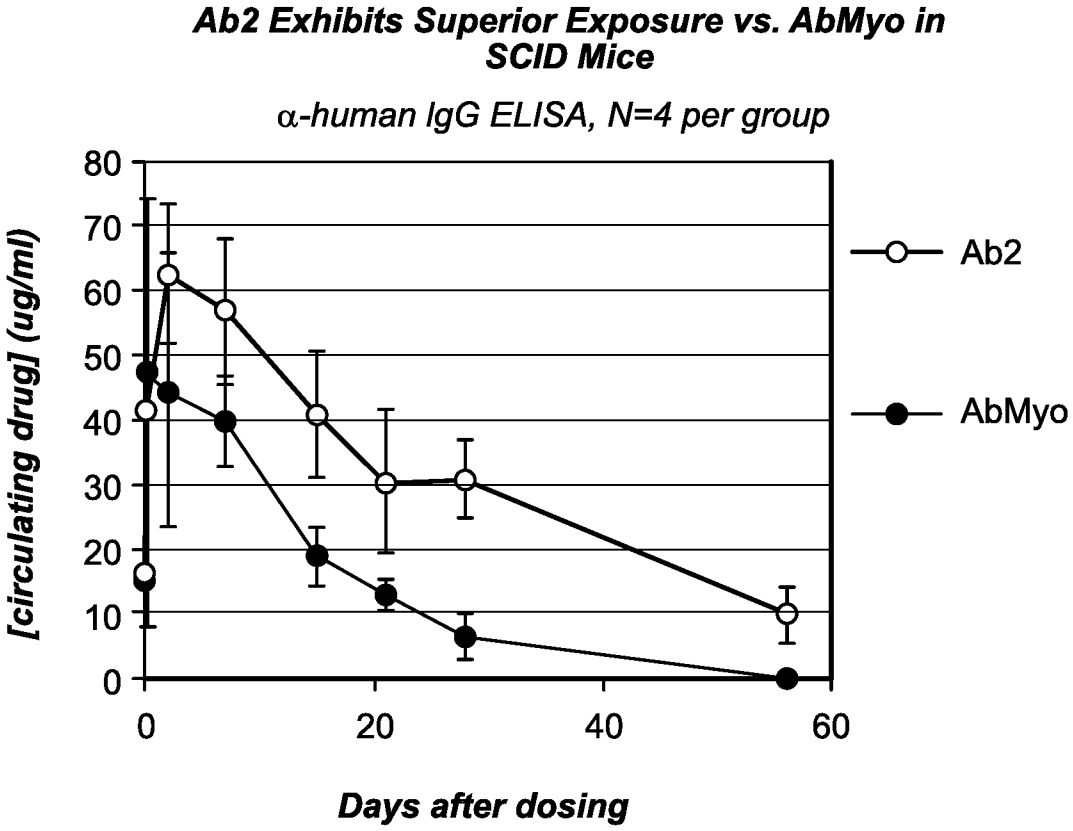


FIG. 32



	$t_{1/2}$ (day)	AUC_{INF} (day*ug/mL)
AbMyo	8.2	773
Ab2	20.3	2072

FIG. 33

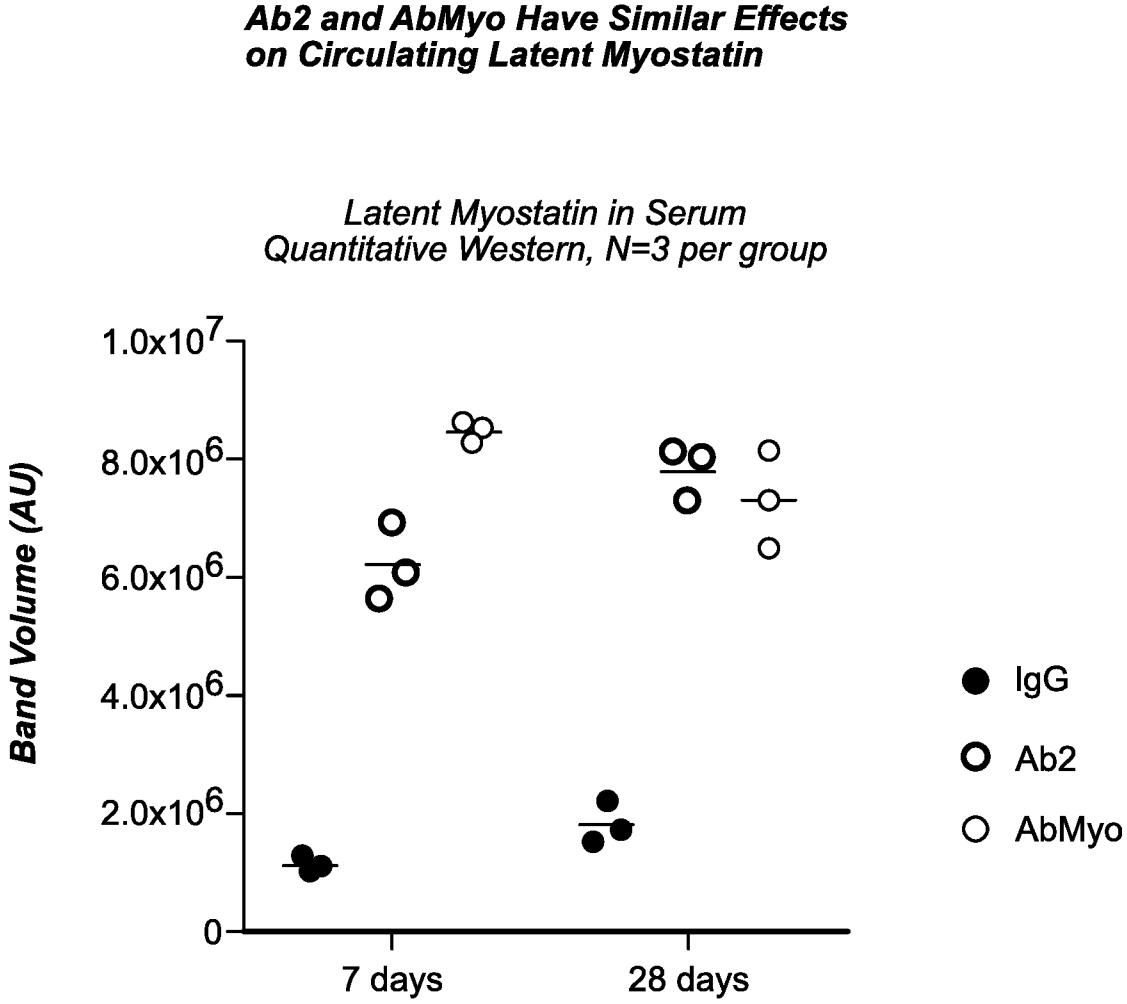

Ab2 and AbMyo Have Similar Effects on Circulating Latent Myostatin
Latent Myostatin in Serum
Quantitative Western, N=3 per group
Band Volume (AU)
1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0
7 days
28 days
IgG
Ab2
AbMyo

FIG. 34

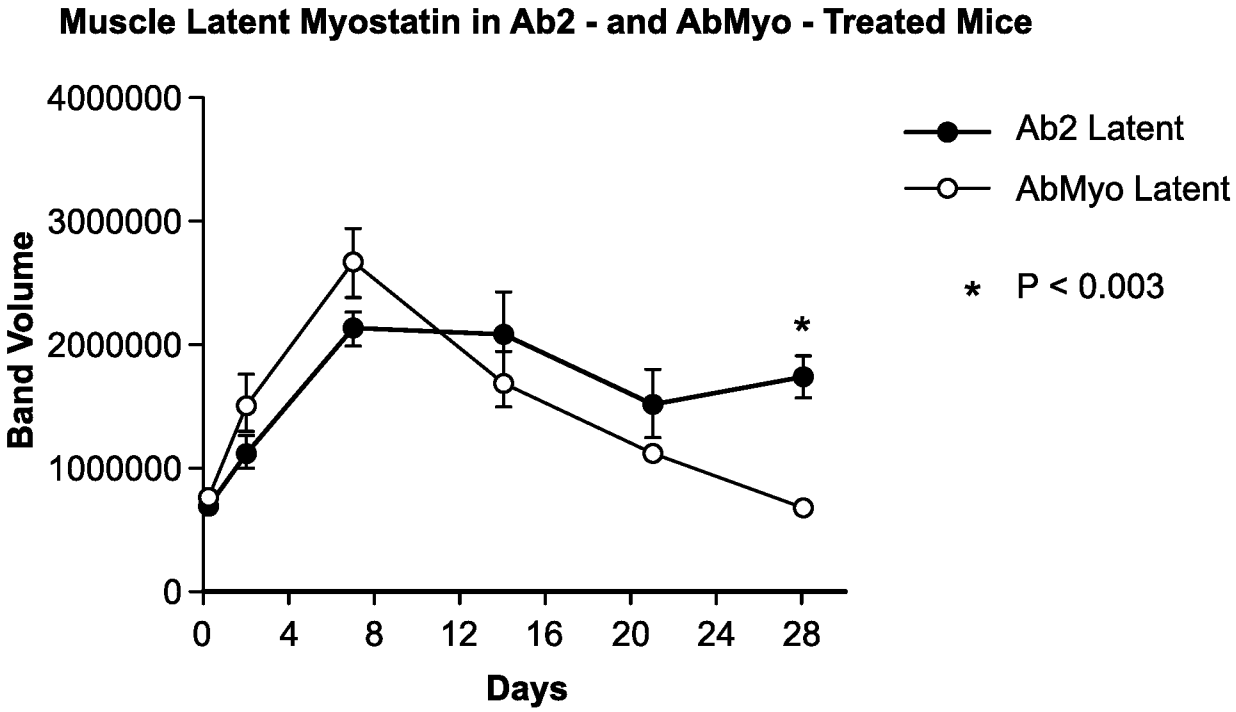


FIG. 35A

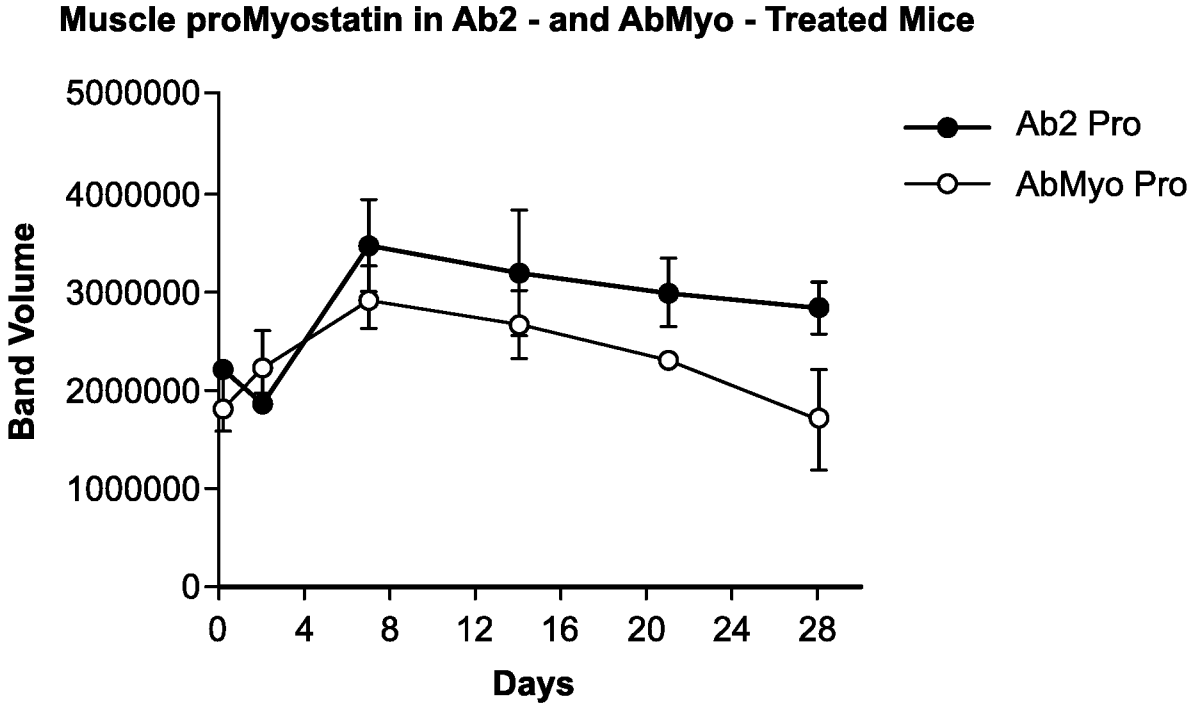
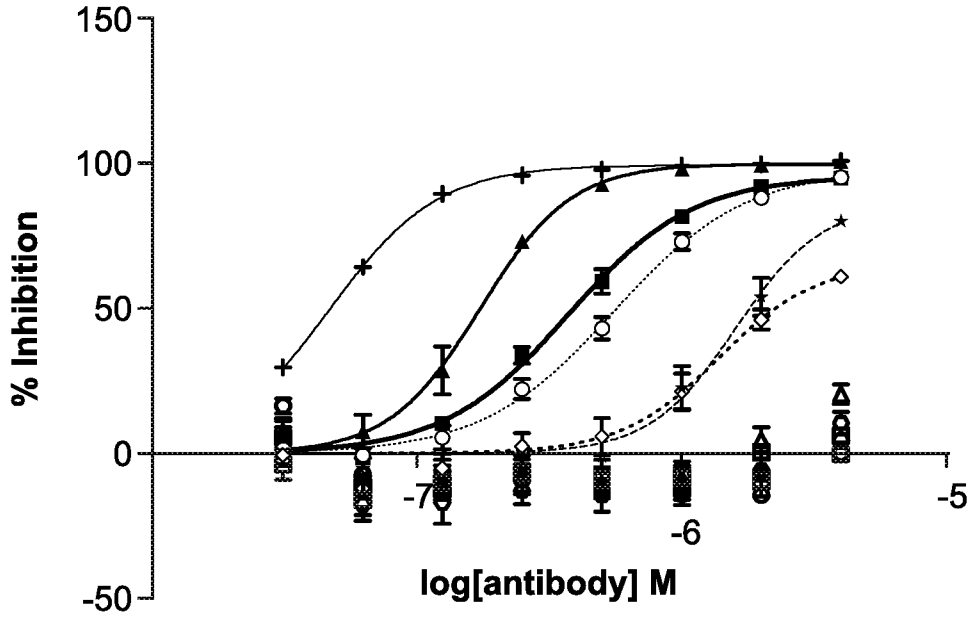


FIG. 35B



Active	No activity	Binding Specificity
Ab-C5	Ab14	**Latent/mature**
Ab2	Ab9	**Pro/Latent**
Ab10	Ab12	
Ab7		
Ab11		
Ab8	Ab13	**proMyostatin**
	Ab15	**Pro/Latent/Mature**
	IgG Control	

FIG. 36A

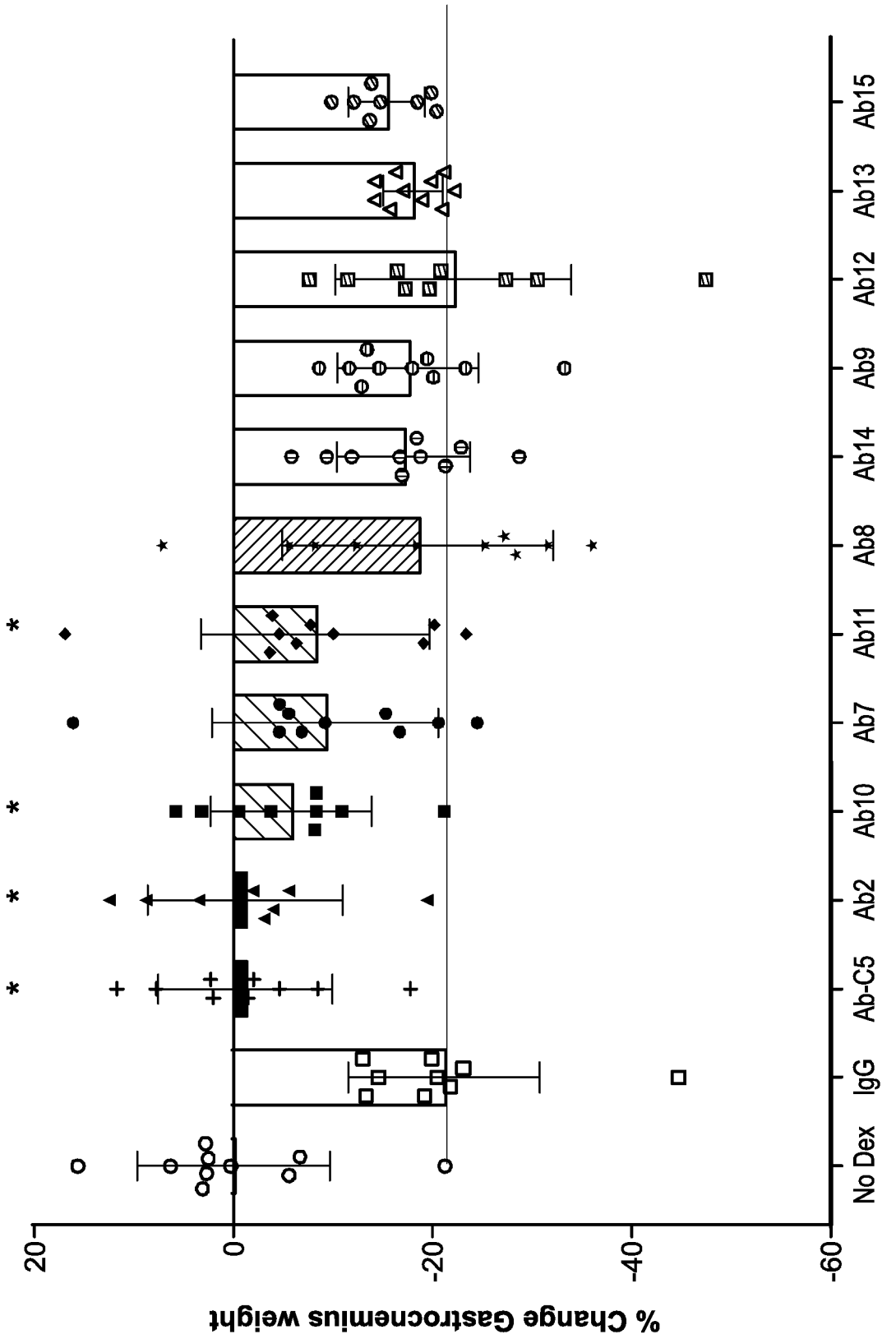

% Change Gastrocnemius weight
20
0
-20
-40
-60
No Dex
IgG
Ab-C5
Ab2
Ab10
Ab7
Ab11
Ab8
Ab14
Ab9
Ab12
Ab13
Ab15
*
*
*

FIG. 36B

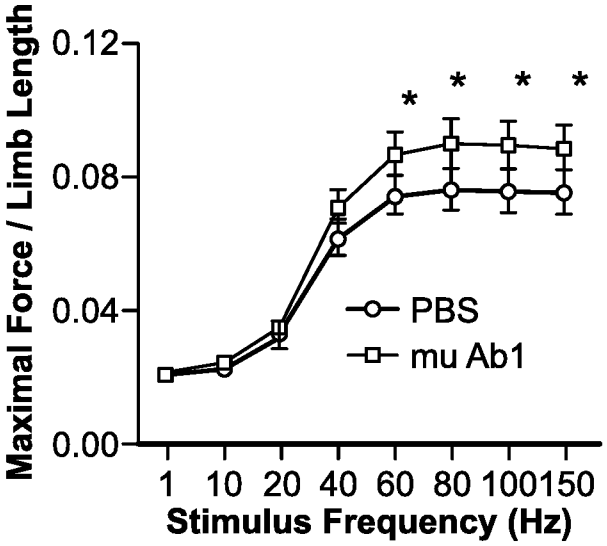


FIG. 37A

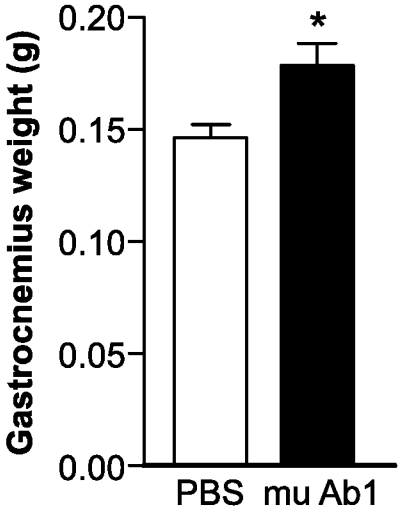


FIG. 37B

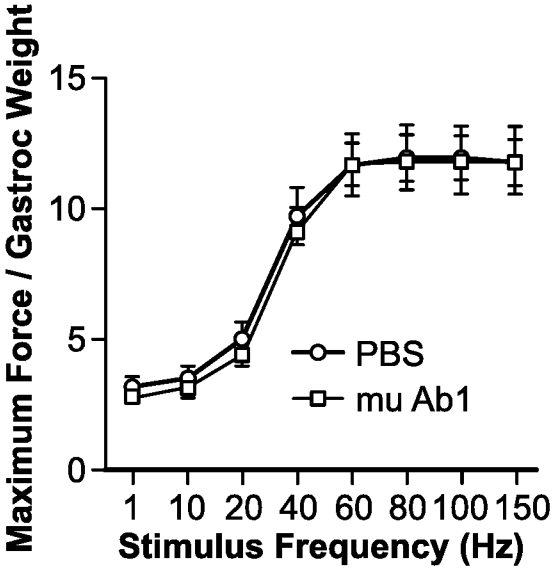


FIG. 37C

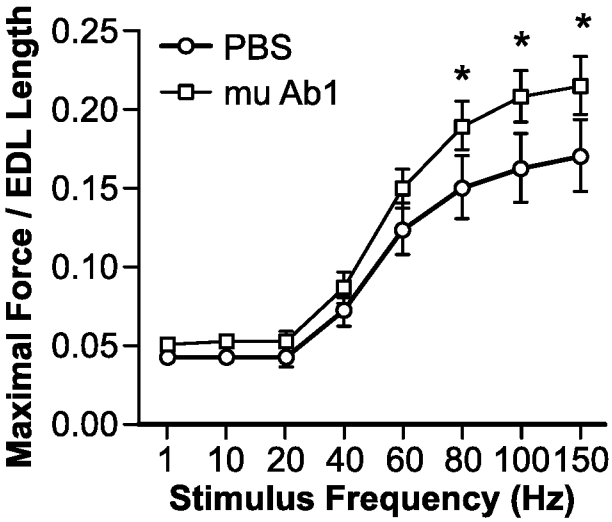


FIG. 37D

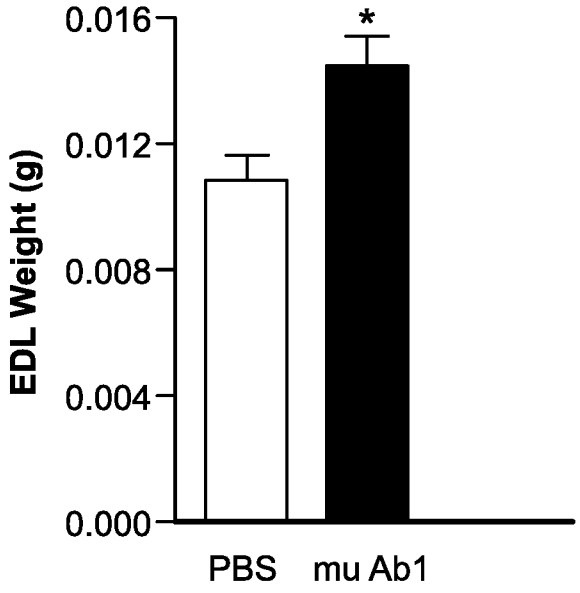


FIG. 37E

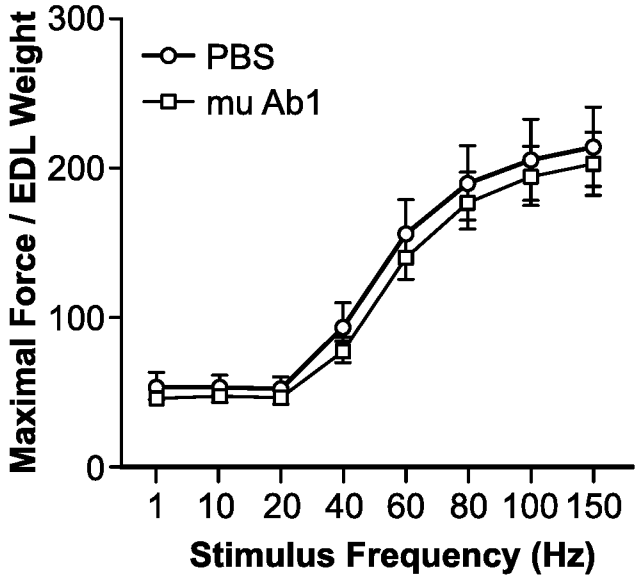


FIG. 37F

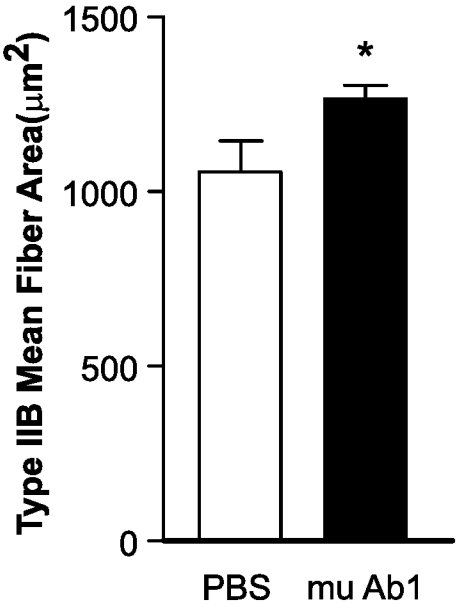


FIG. 37G

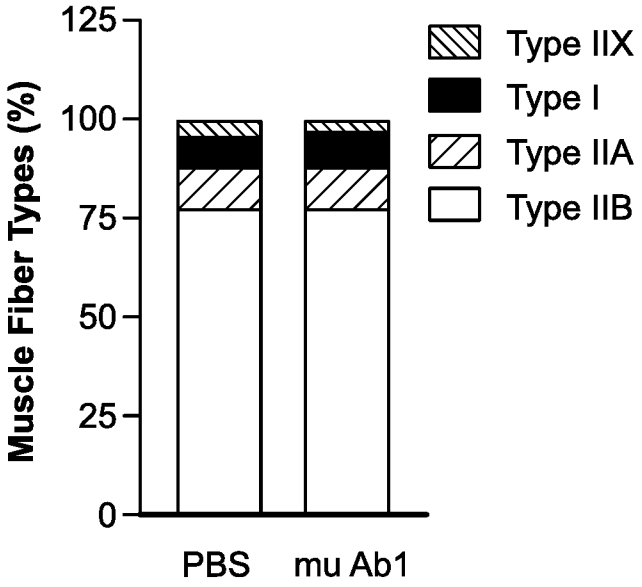


FIG. 37H

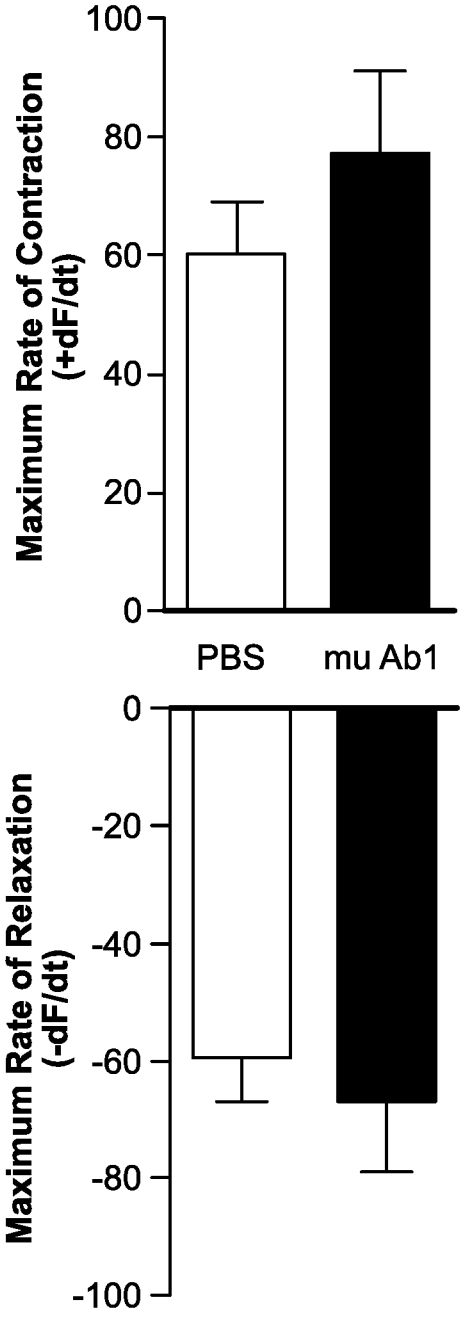


FIG. 38A

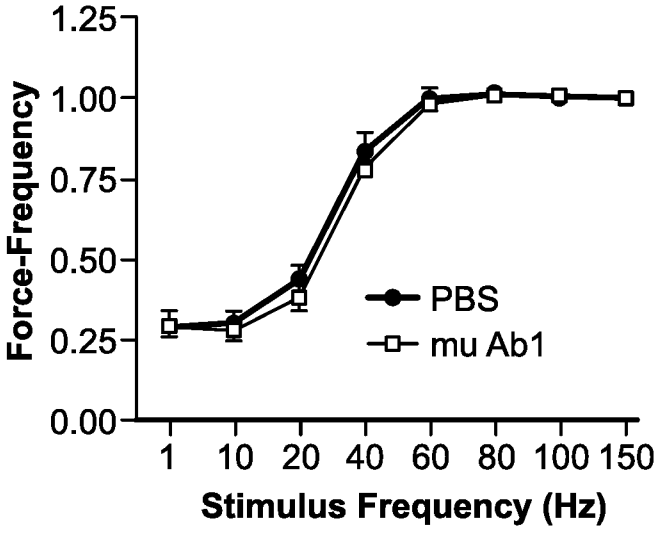


FIG. 38B

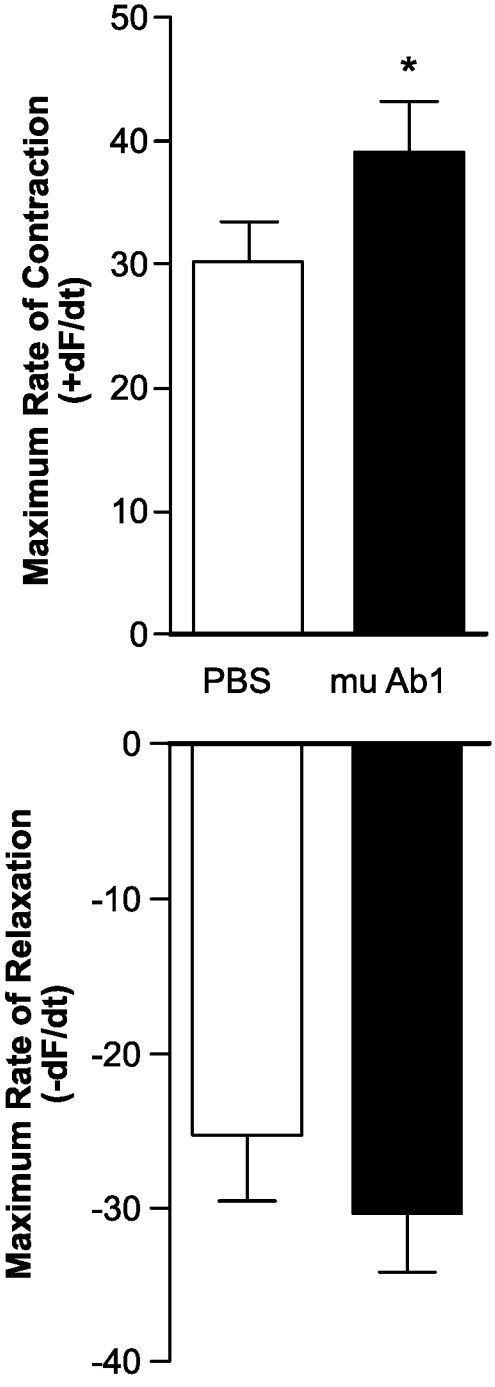


FIG. 38C

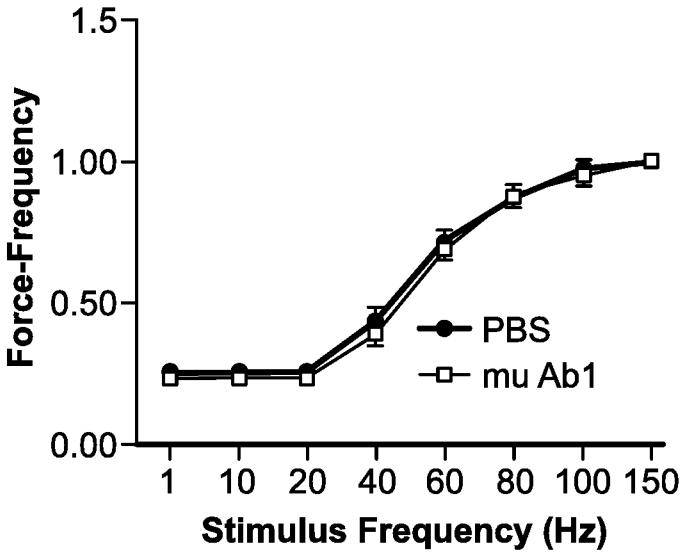


FIG. 38D

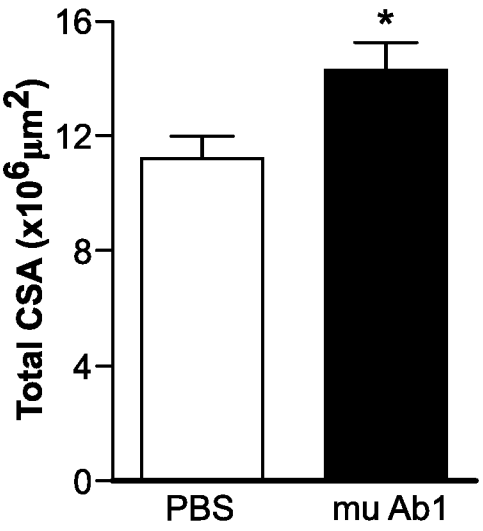


FIG. 38E

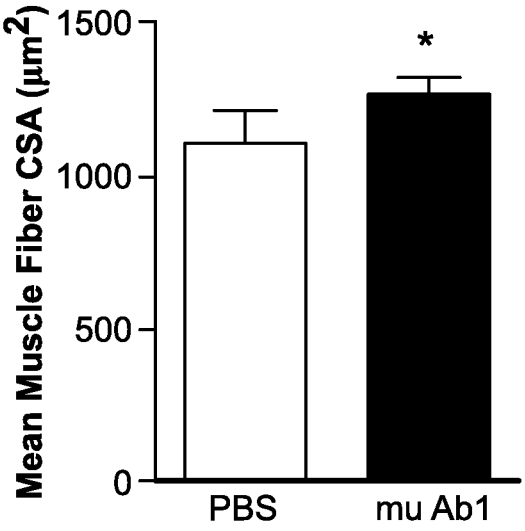


FIG. 38F

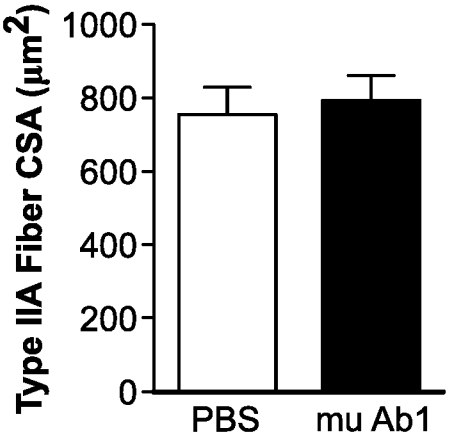


FIG. 38G

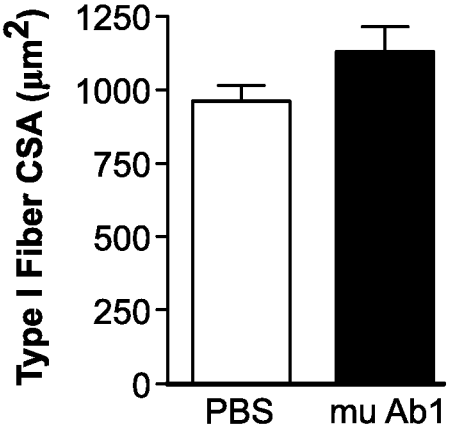


FIG. 38H

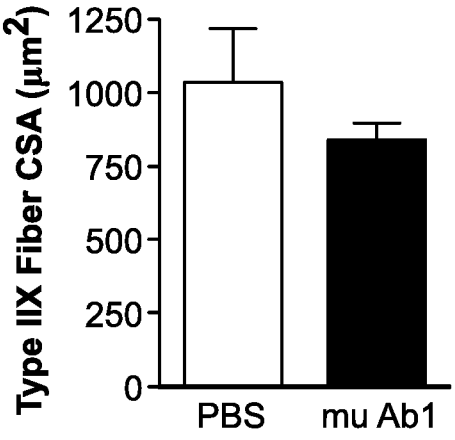


FIG. 38I

Ab10 DAPI

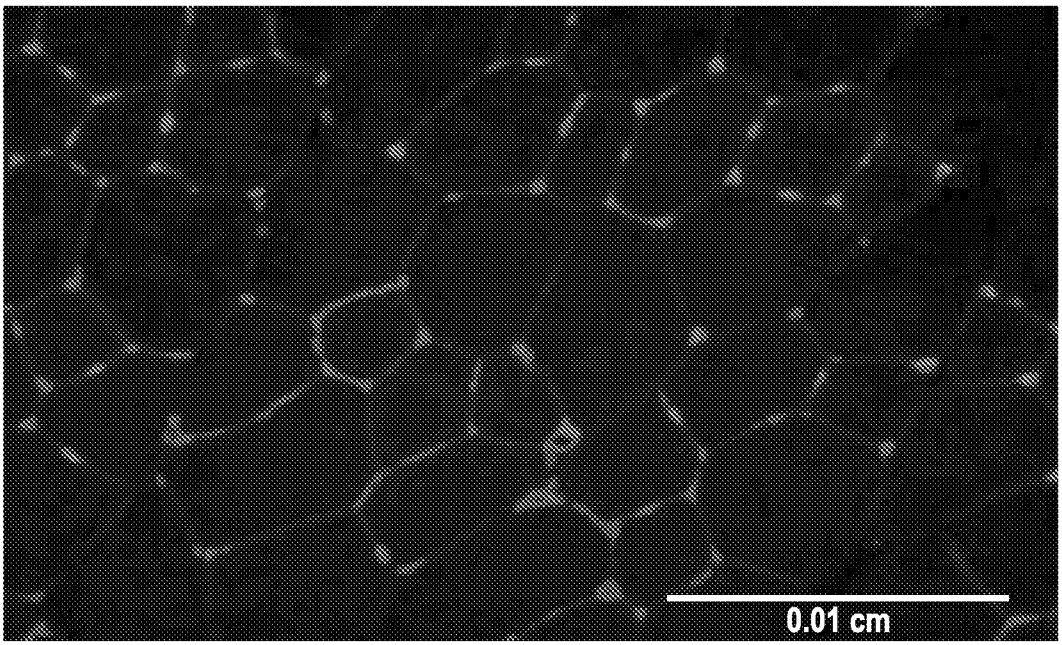


FIG. 39A

HuNeg DAPI

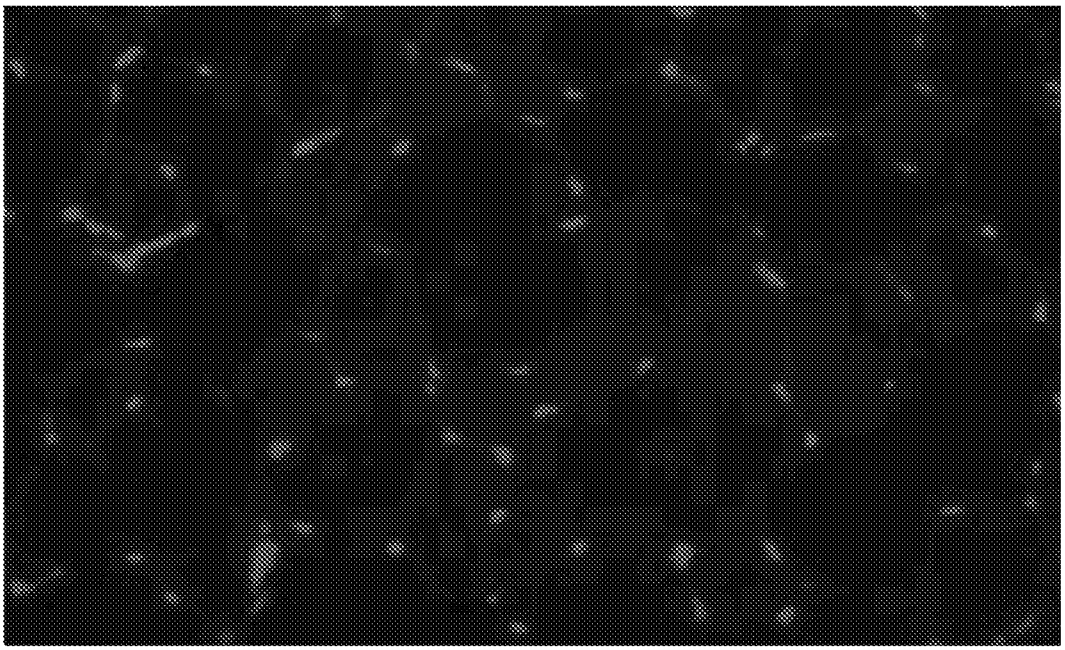


FIG. 39B

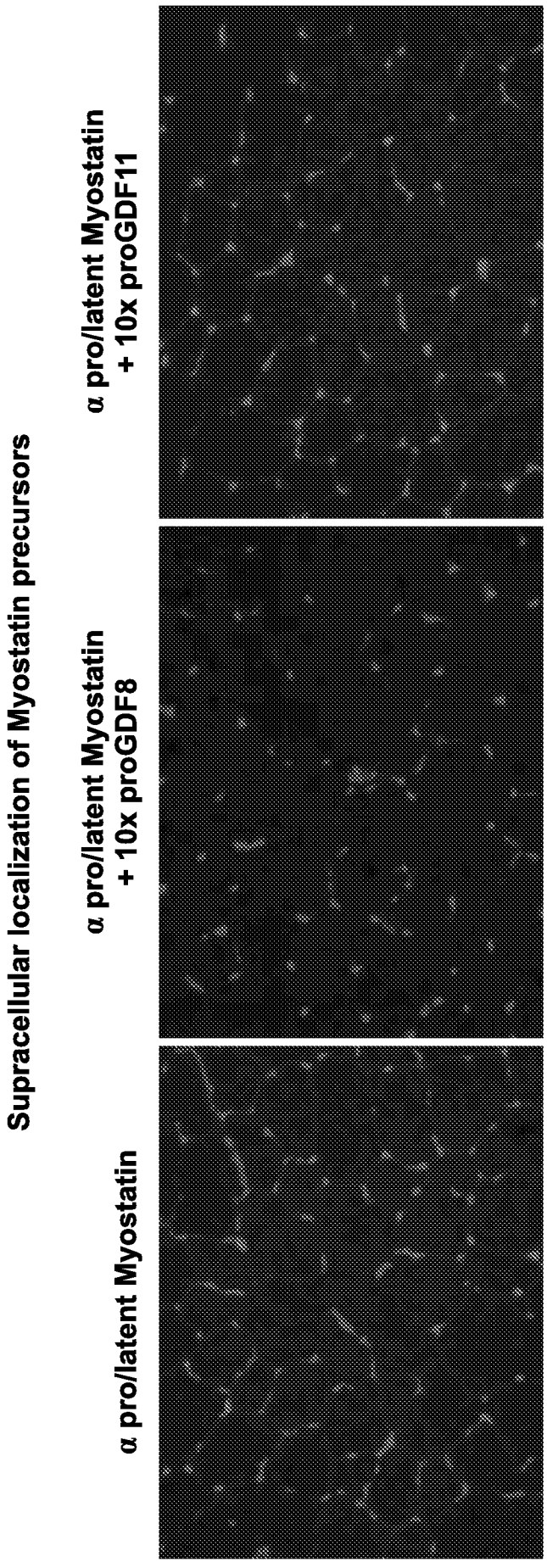


FIG. 40A

FIG. 40B

FIG. 40C

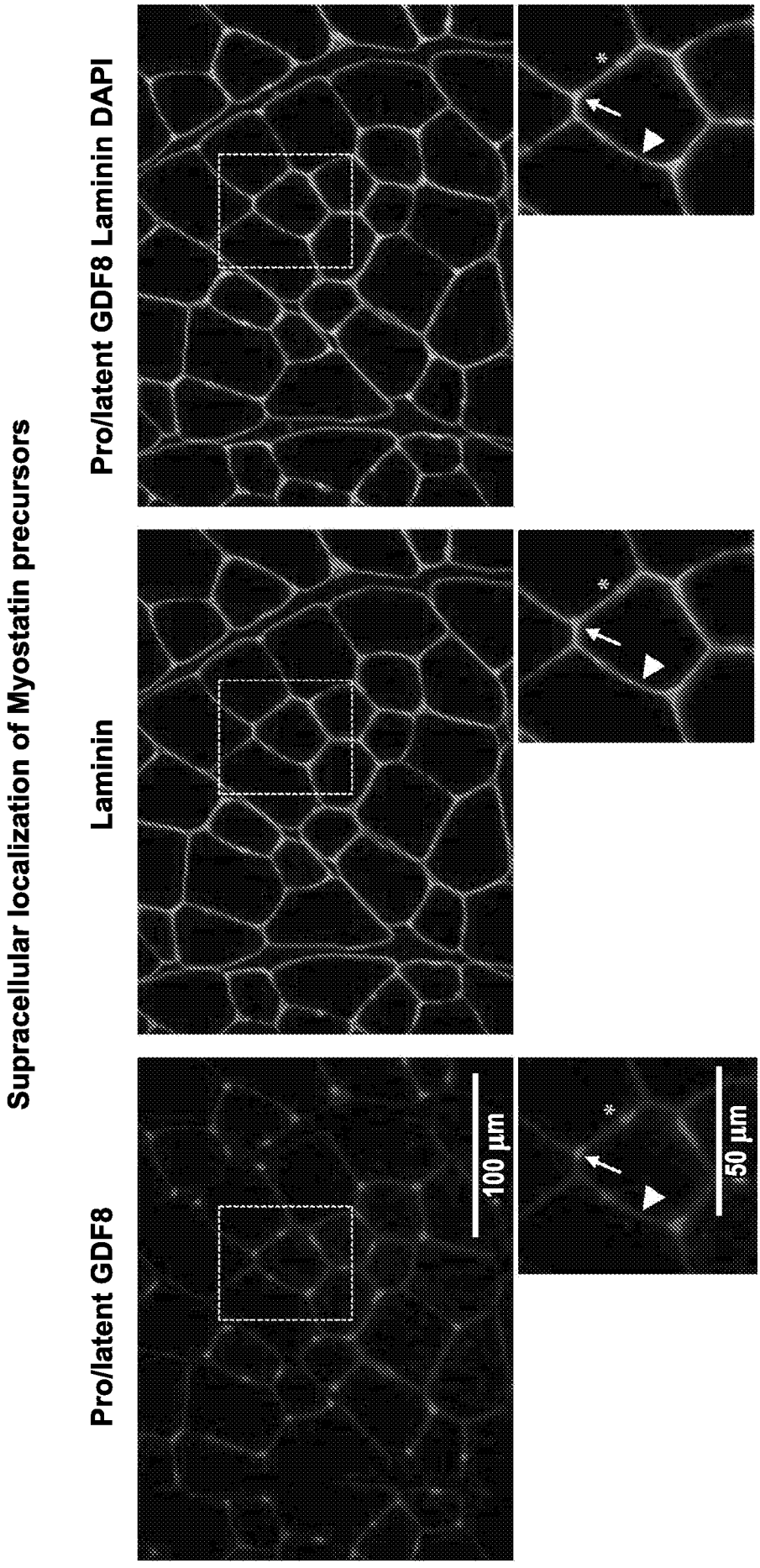


FIG. 41A

FIG. 41B

FIG. 41C

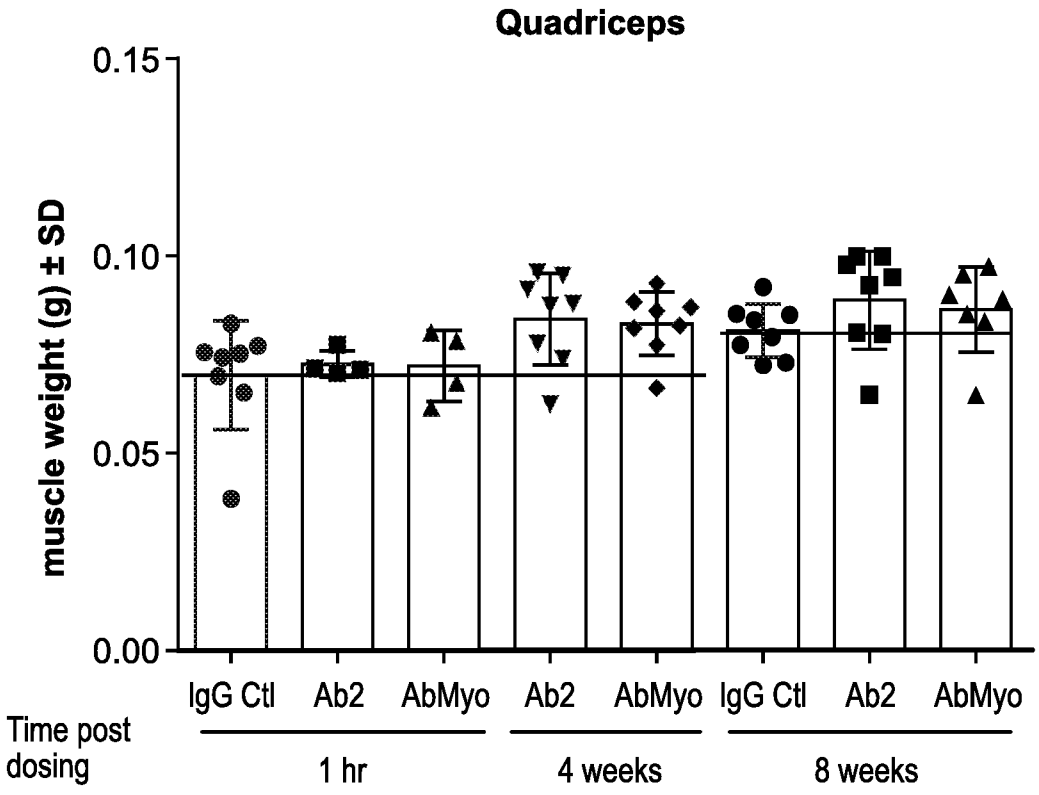


FIG. 42A

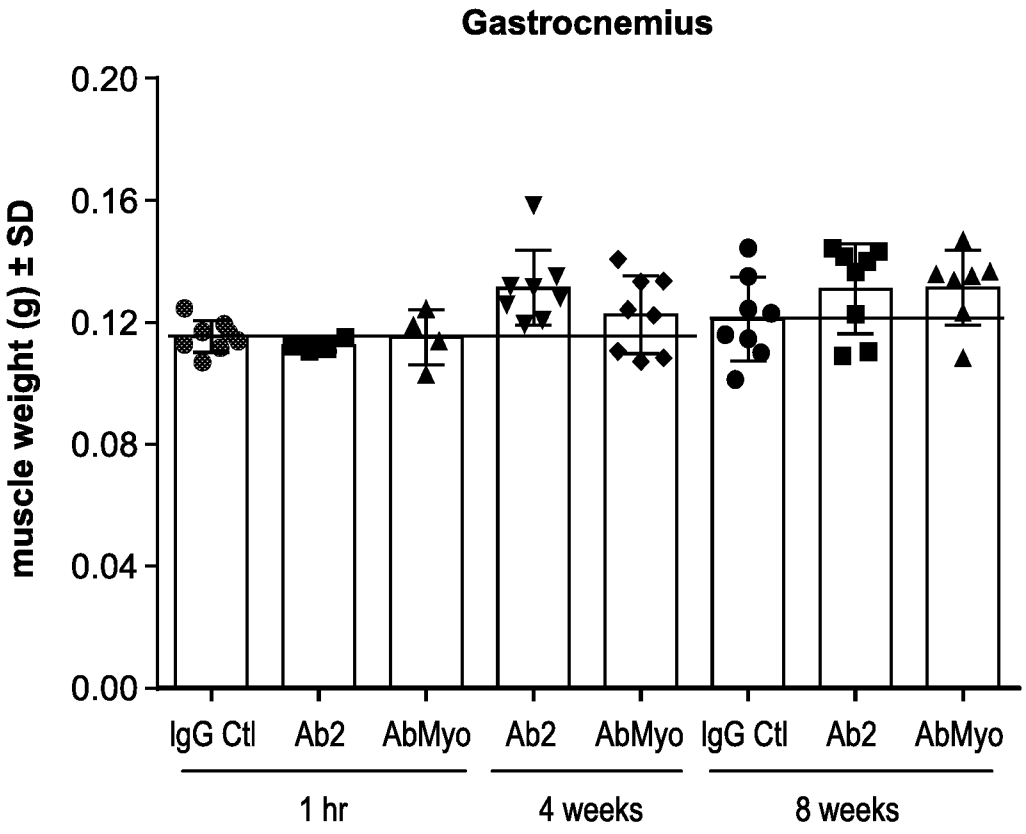


FIG. 42B

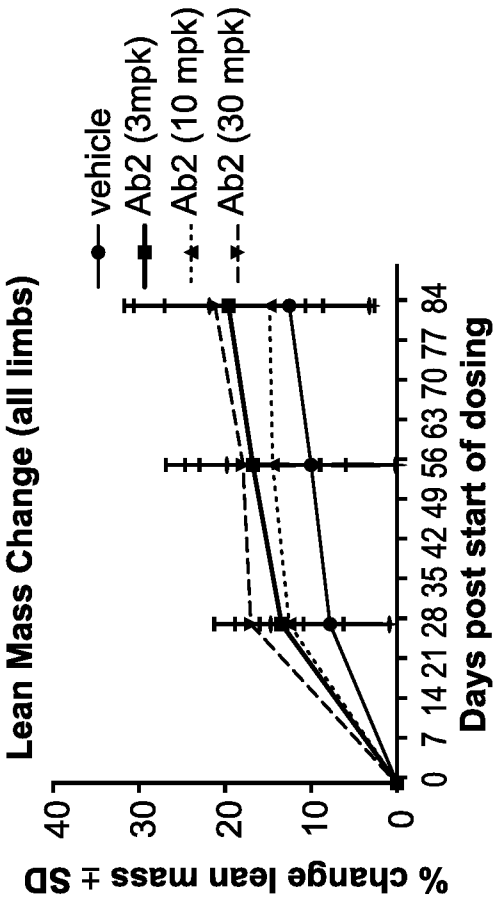


FIG. 43A

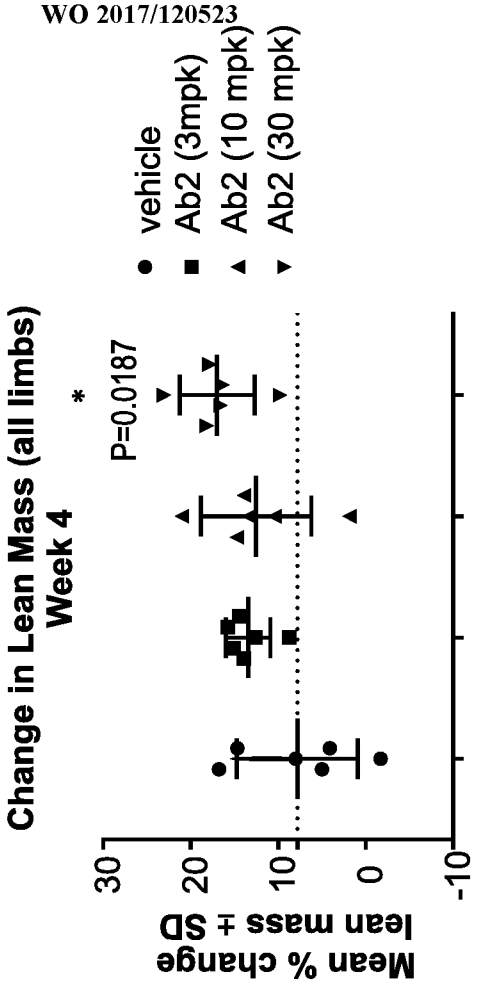


FIG. 43B

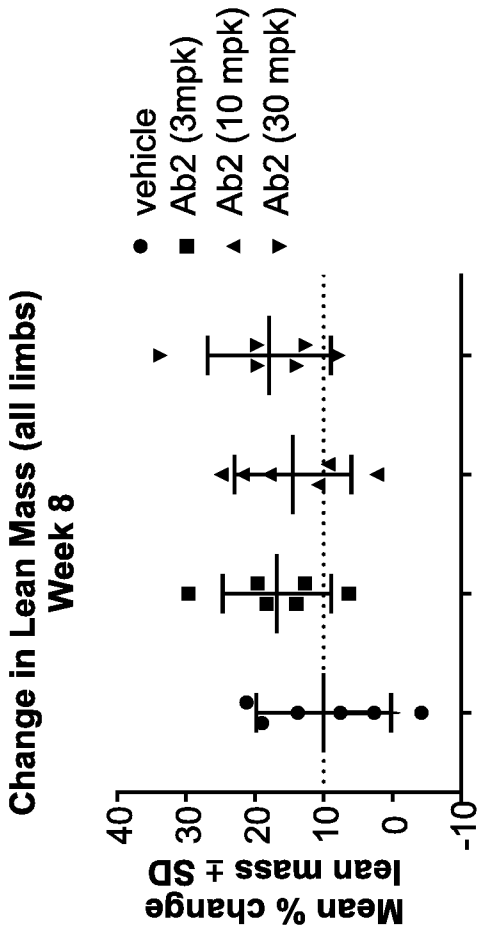


FIG. 43C

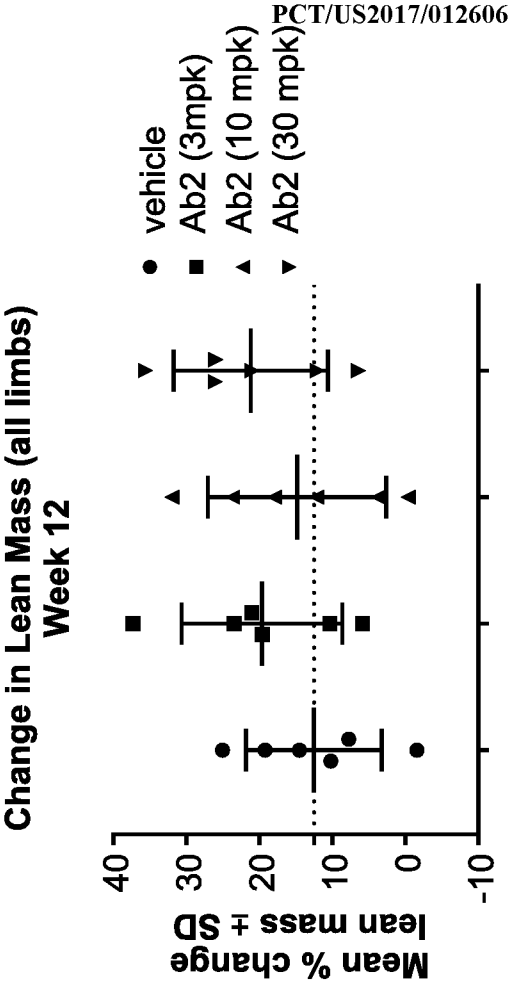


FIG. 43D

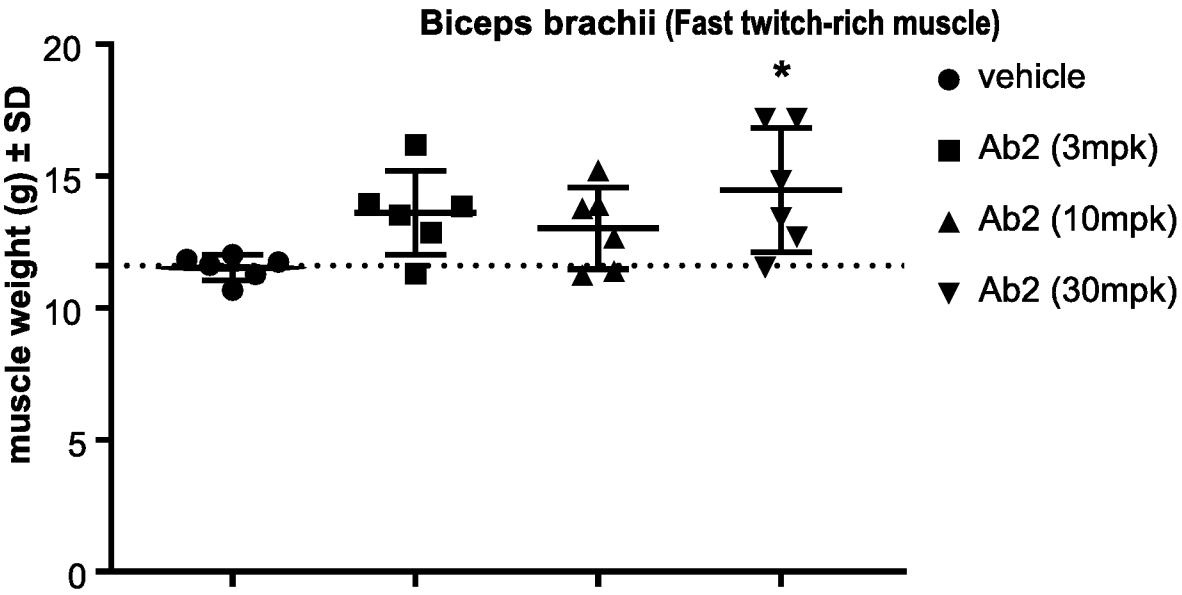


FIG. 44A

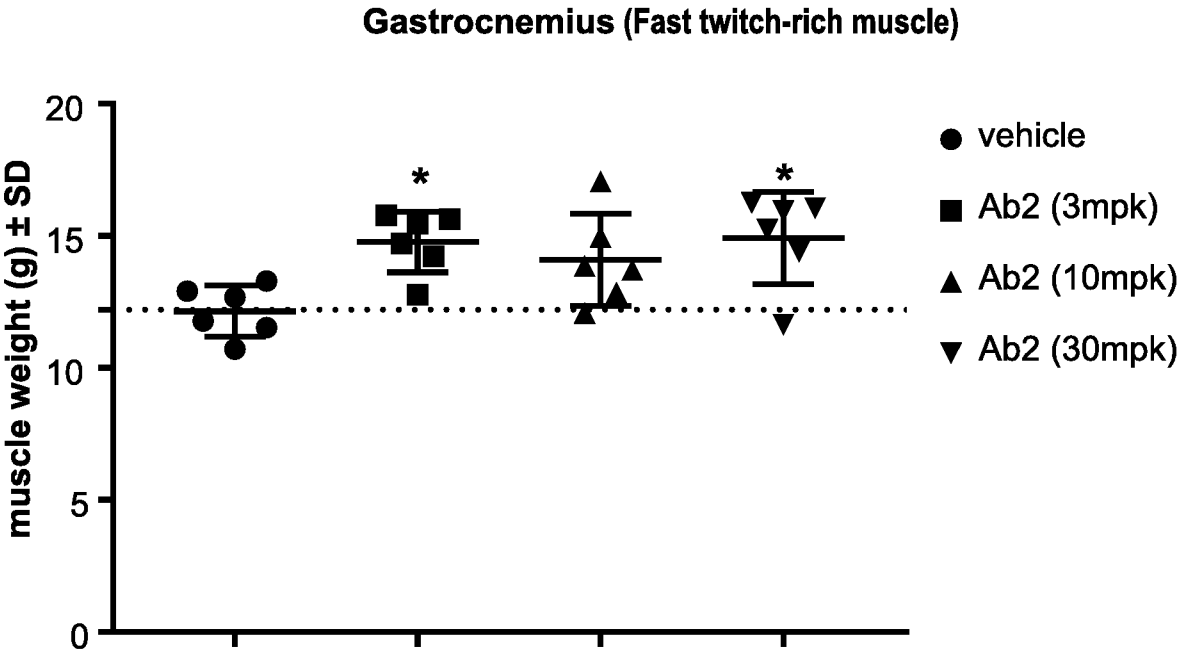


FIG. 44B

Readout	vehicle	Ab2 (3mpk)	Ab2 (10mpk)	Ab2 (30mpk)
Mean % change in lean mass @ 4 weeks	7.8	13.5	12.6	17.0
Mean % change in lean mass @ 8 weeks	10.0	16.8	14.5	17.9
Mean % change in lean mass @ 12 weeks	12.5	19.6	14.8	21.2
% difference in biceps weight vs. vehicle group	0.0	18.0	13.0	25.5
% difference in gastroc weight vs. vehicle group	0.0	21.7	16.0	22.9

FIG. 45

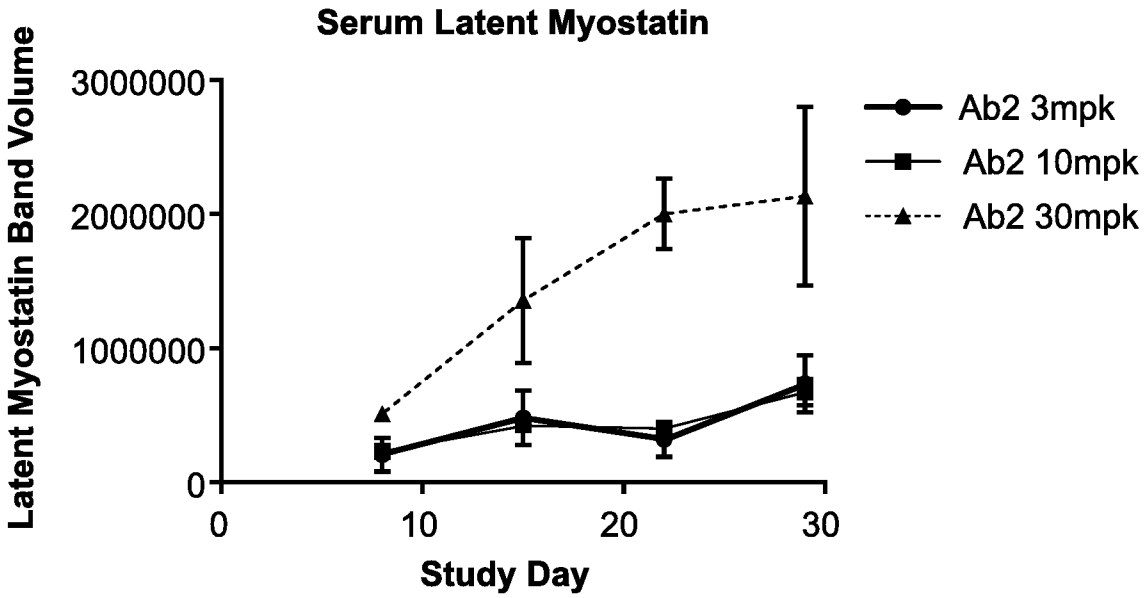


FIG. 46A

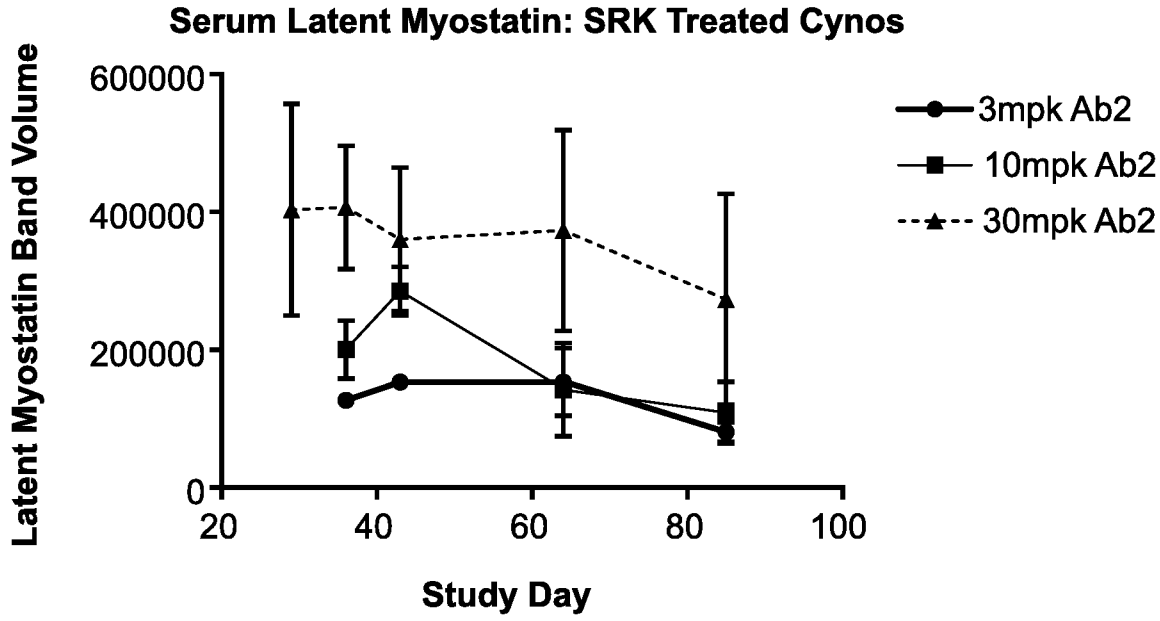


FIG. 46B

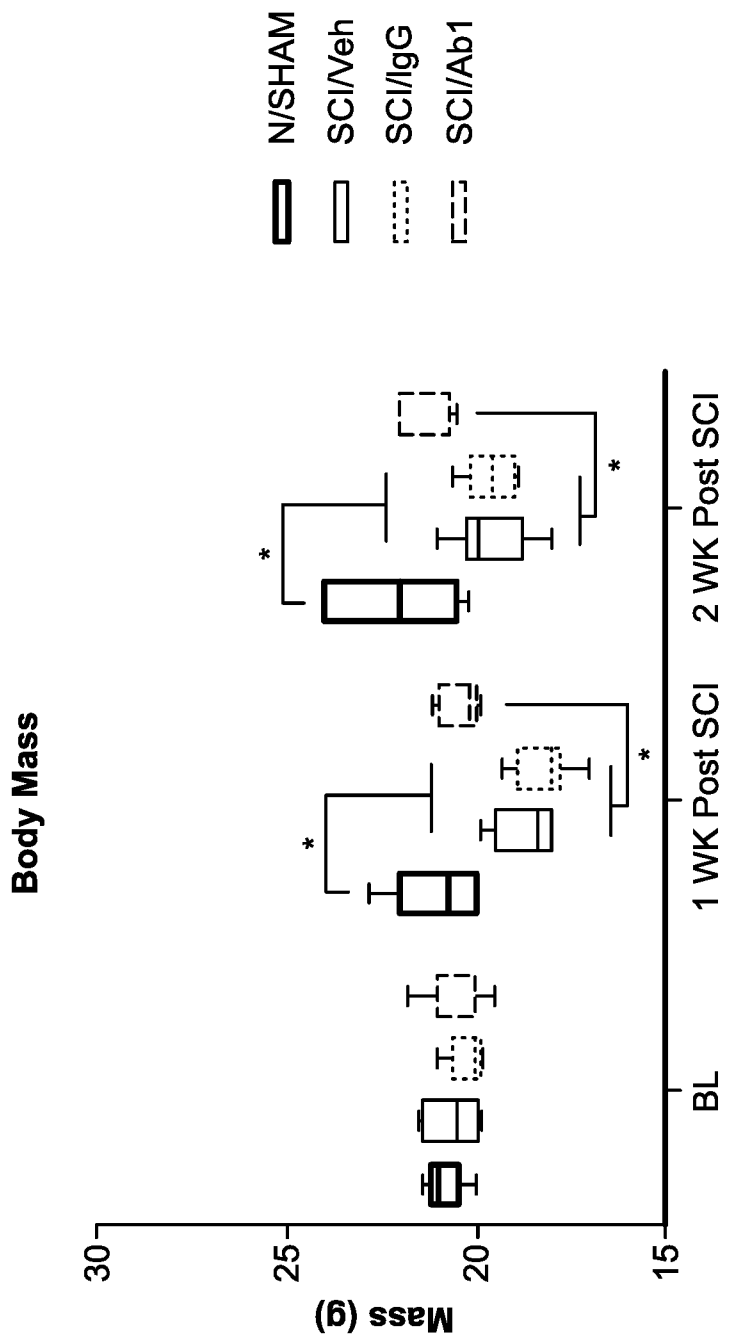

N/SHAM
SCI/Veh
SCI/IgG
SCI/Ab1
Body Mass
Mass (g)
30
25
20
15
BL
1 WK Post SCI
2 WK Post SCI
Group
*
*
*
*

FIG. 47

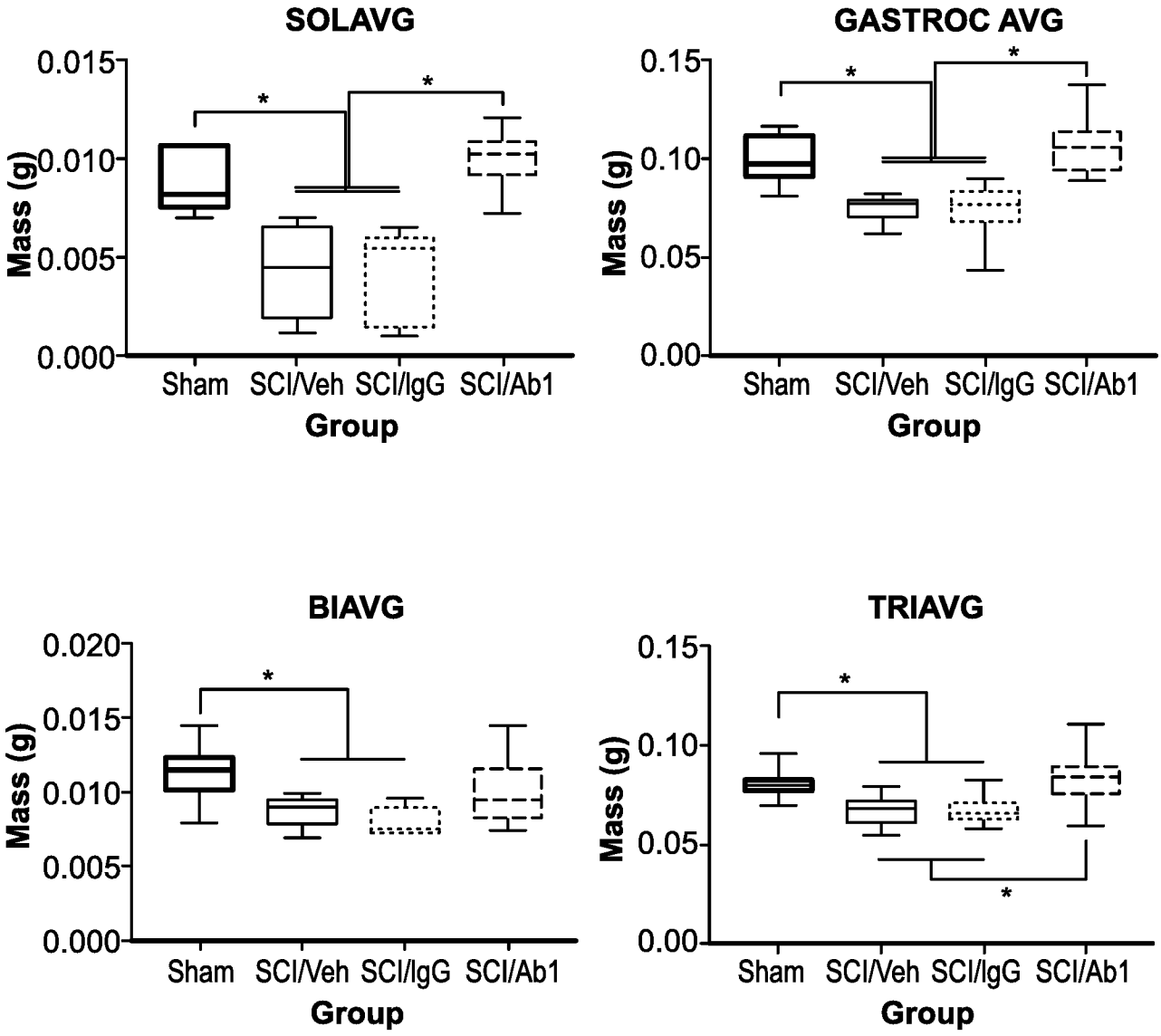

SOLAVG
Mass (g)
0.015
0.010
0.005
0.000
Sham
SCI/Veh
SCI/IgG
SCI/Ab1
Group
GASTROC AVG
Mass (g)
0.15
0.10
0.05
0.00
Sham
SCI/Veh
SCI/IgG
SCI/Ab1
Group
BIAVG
Mass (g)
0.020
0.015
0.010
0.005
0.000
Sham
SCI/Veh
SCI/IgG
SCI/Ab1
Group
TRIAVG
Mass (g)
0.15
0.10
0.05
0.00
Sham
SCI/Veh
SCI/IgG
SCI/Ab1
Group

FIG. 48

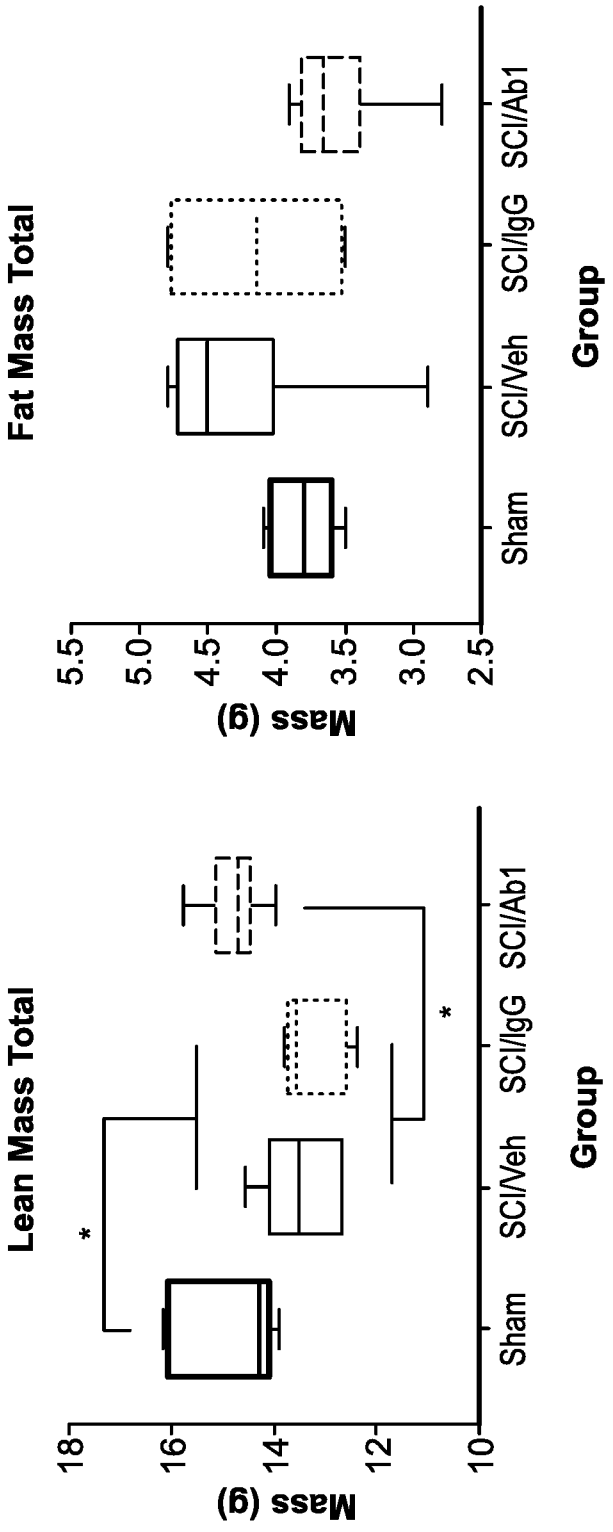

Fat Mass Total
Mass (g)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
Sham
SCI/Veh
SCI/IgG
SCI/Ab1
Group
Lean Mass Total
Mass (g)
18
16
14
12
10
*
*
Sham
SCI/Veh
SCI/IgG
SCI/Ab1
Group

FIG. 49

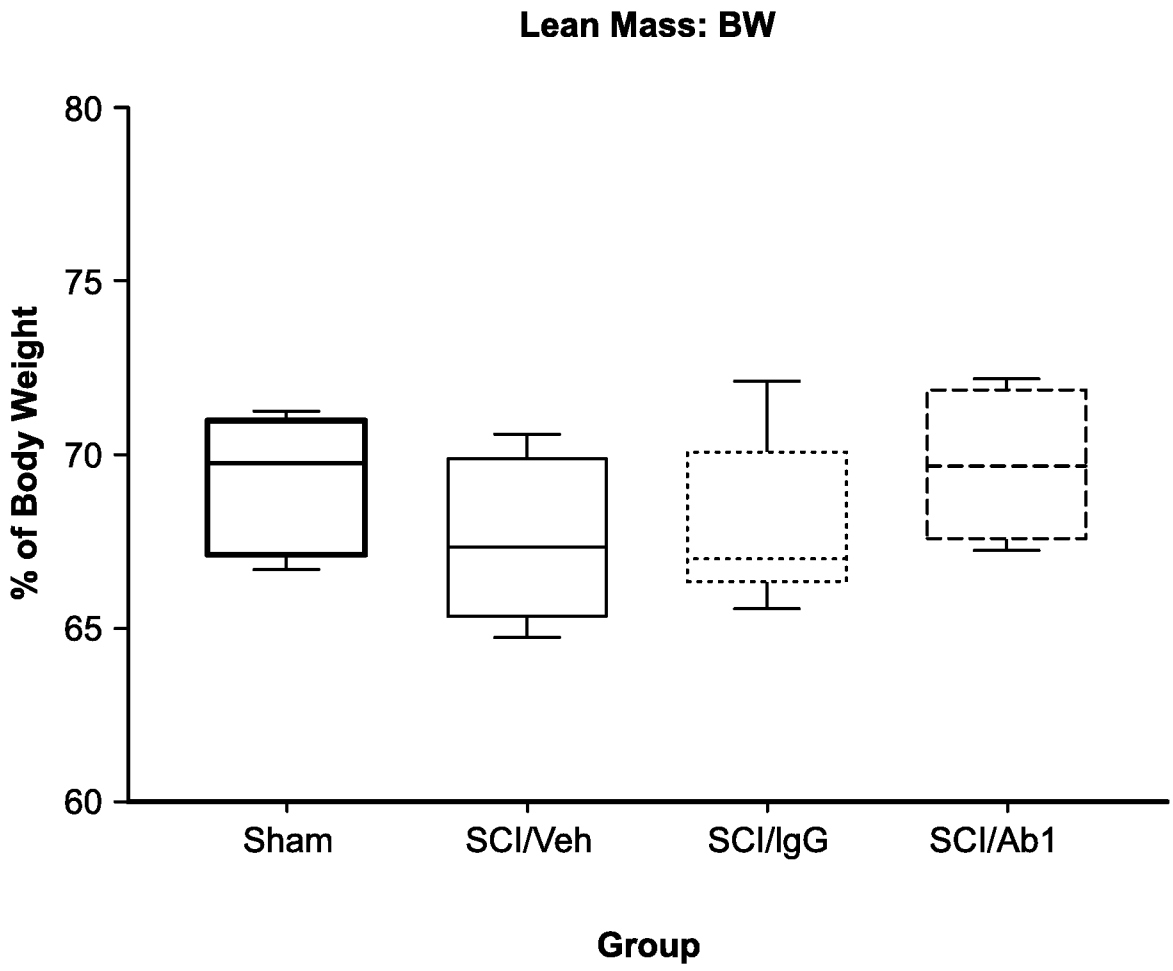

Lean Mass: BW
% of Body Weight
80
75
70
65
60
Sham
SCI/Veh
SCI/IgG
SCI/Ab1
Group

FIG. 50

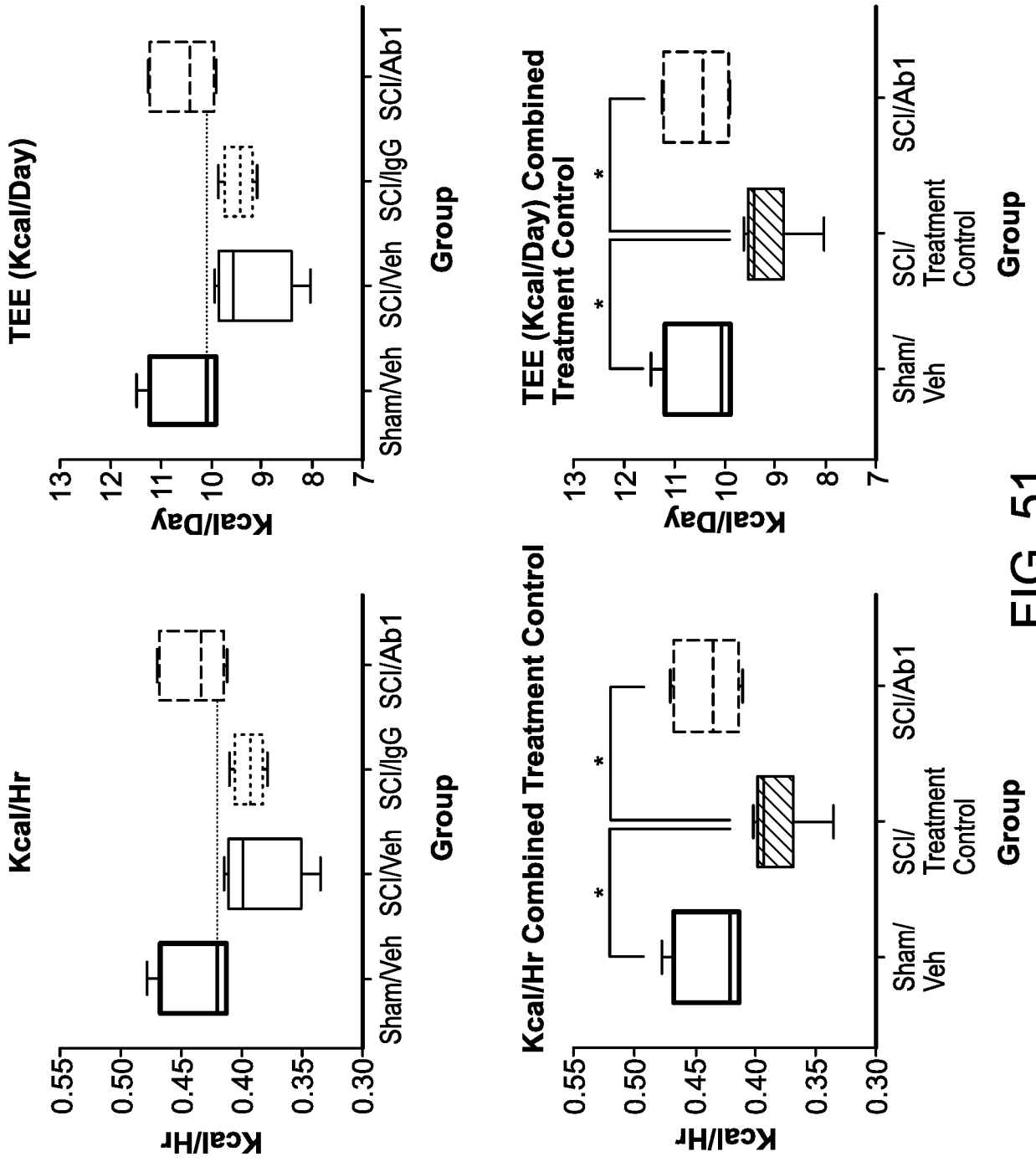

TEE (Kcal/Day)
Kcal/Day
13
12
11
10
9
8
7
Sham/Veh
SCI/Veh
SCI/IgG
SCI/Ab1
Group
TEE (Kcal/Day) Combined Treatment Control
Kcal/Day
13
12
11
10
9
8
7
*
*
Sham/ Veh
SCI/ Treatment Control
Group
Kcal/Hr
Kcal/Hr
0.55
0.50
0.45
0.40
0.35
0.30
Sham/Veh
SCI/Veh
SCI/IgG
SCI/Ab1
Group
Kcal/Hr Combined Treatment Control
Kcal/Hr
0.55
0.50
0.45
0.40
0.35
0.30
*
*
Sham/ Veh
SCI/ Treatment Control
Group

FIG. 51

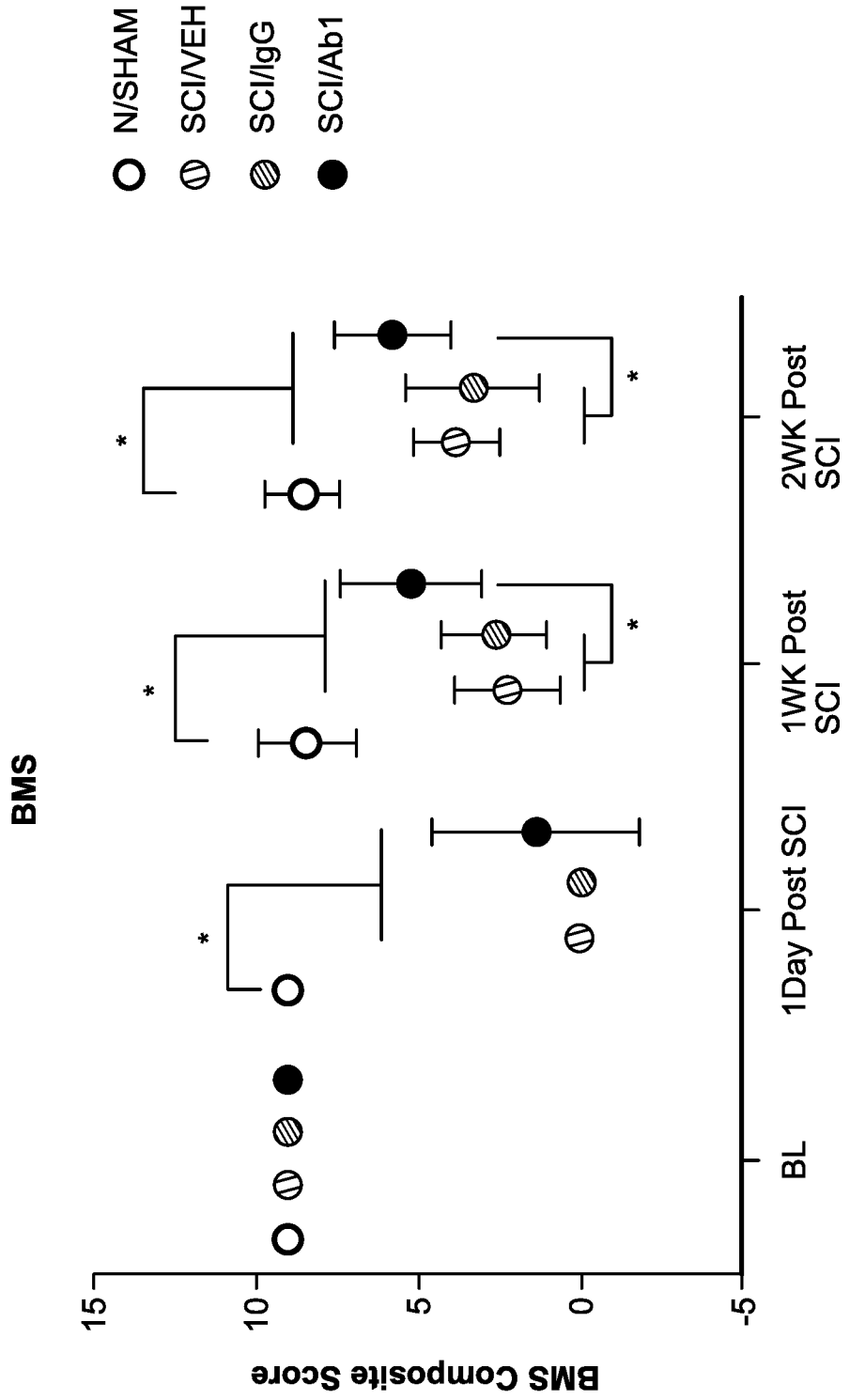

N/SHAM
SCI/VEH
SCI/IgG
SCI/Ab1
BMS
BMS Composite Score
15
10
5
0
-5
*
*
*
*
BL
1Day Post SCI
1WK Post SCI
2WK Post SCI
Testing Session

FIG. 52

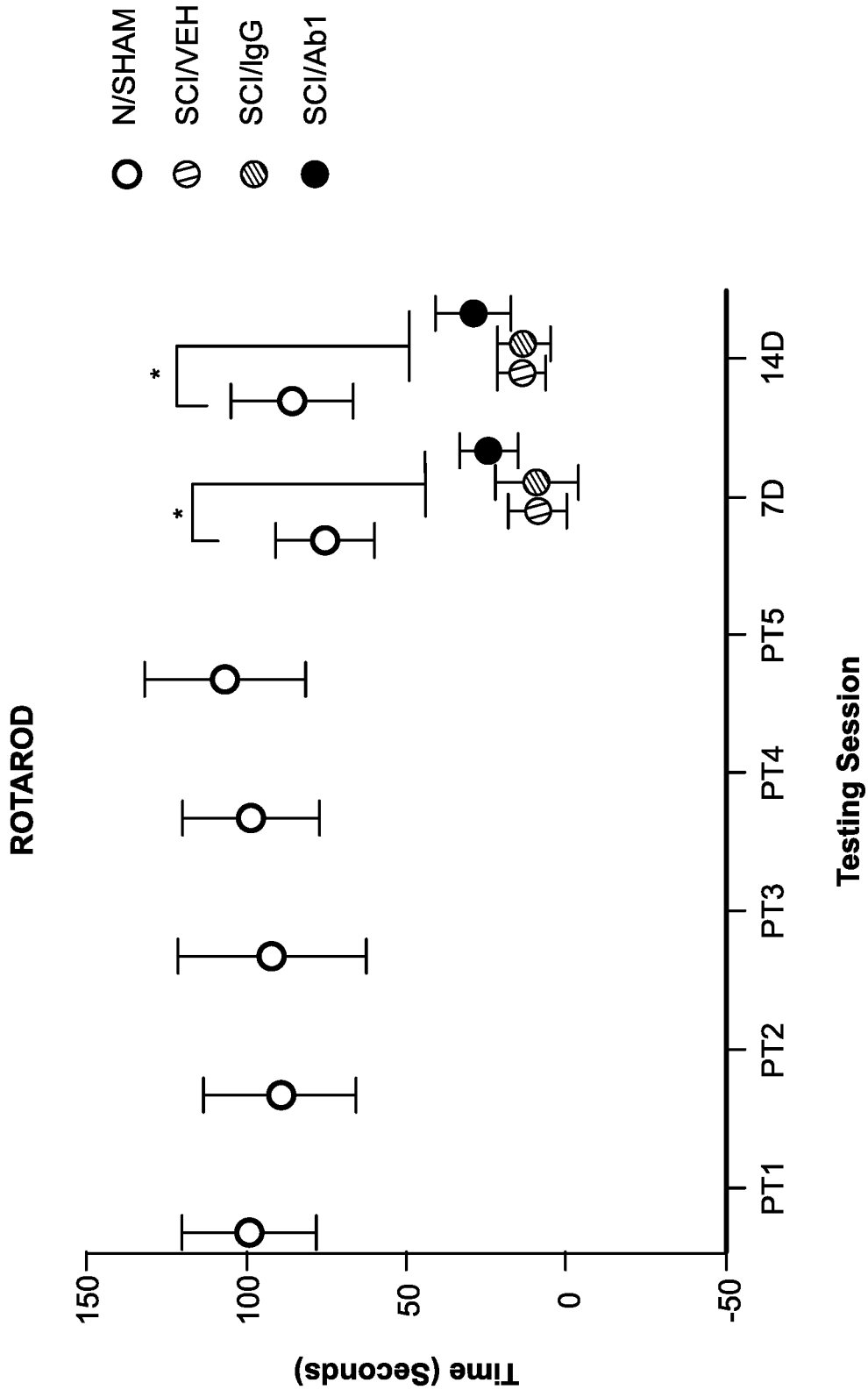

N/SHAM
SCI/VEH
SCI/IgG
SCI/Ab1
ROTAROD
Time (Seconds)
150
100
50
0
-50
PT1
PT2
PT3
PT4
PT5
7D
14D
Testing Session
*
*

FIG. 53

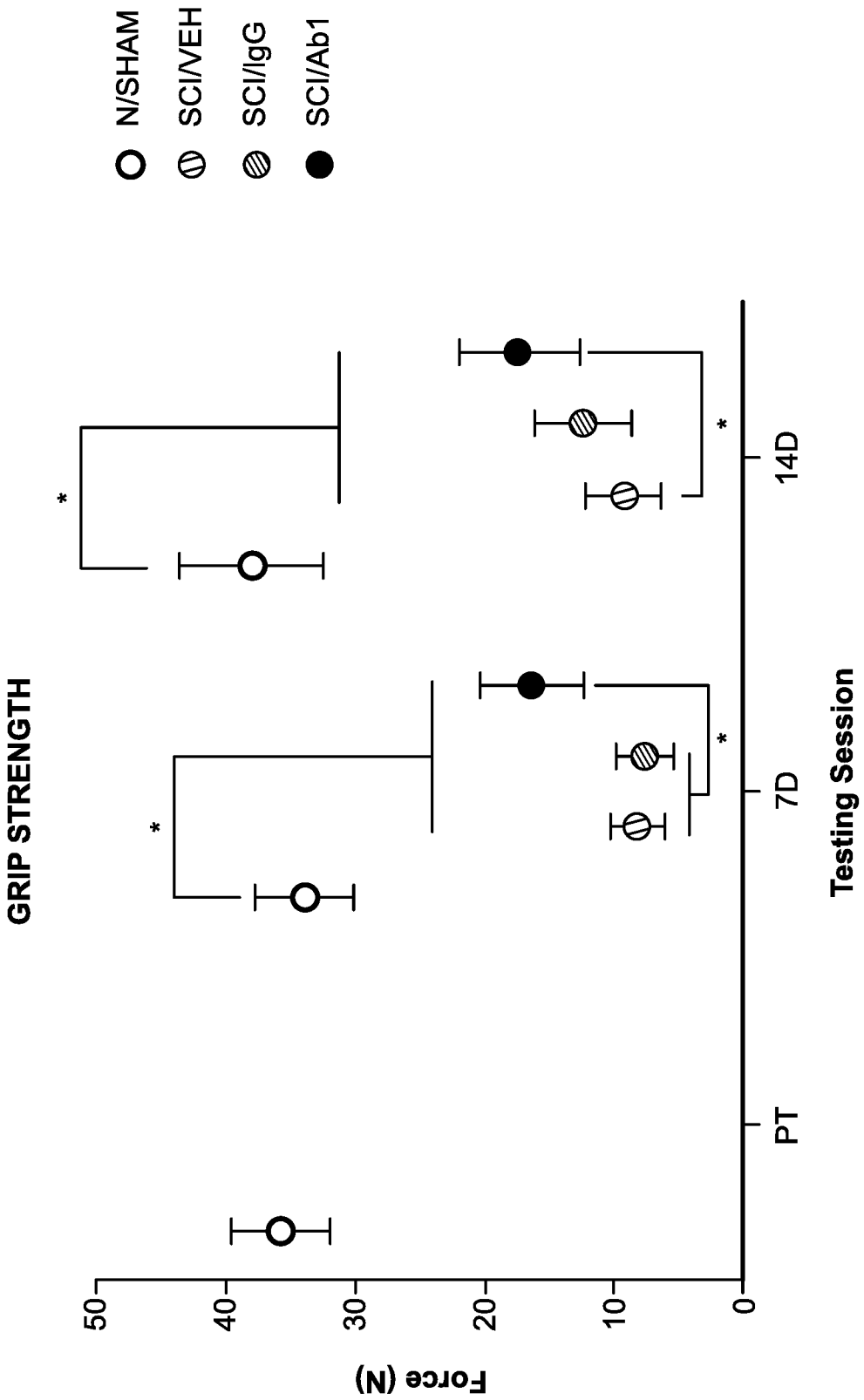

N/SHAM
SCI/VEH
SCI/IgG
SCI/Ab1
GRIP STRENGTH
Force (N)
50
40
30
20
10
0
PT
7D
14D
Testing Session
*
*
*
*

FIG. 54

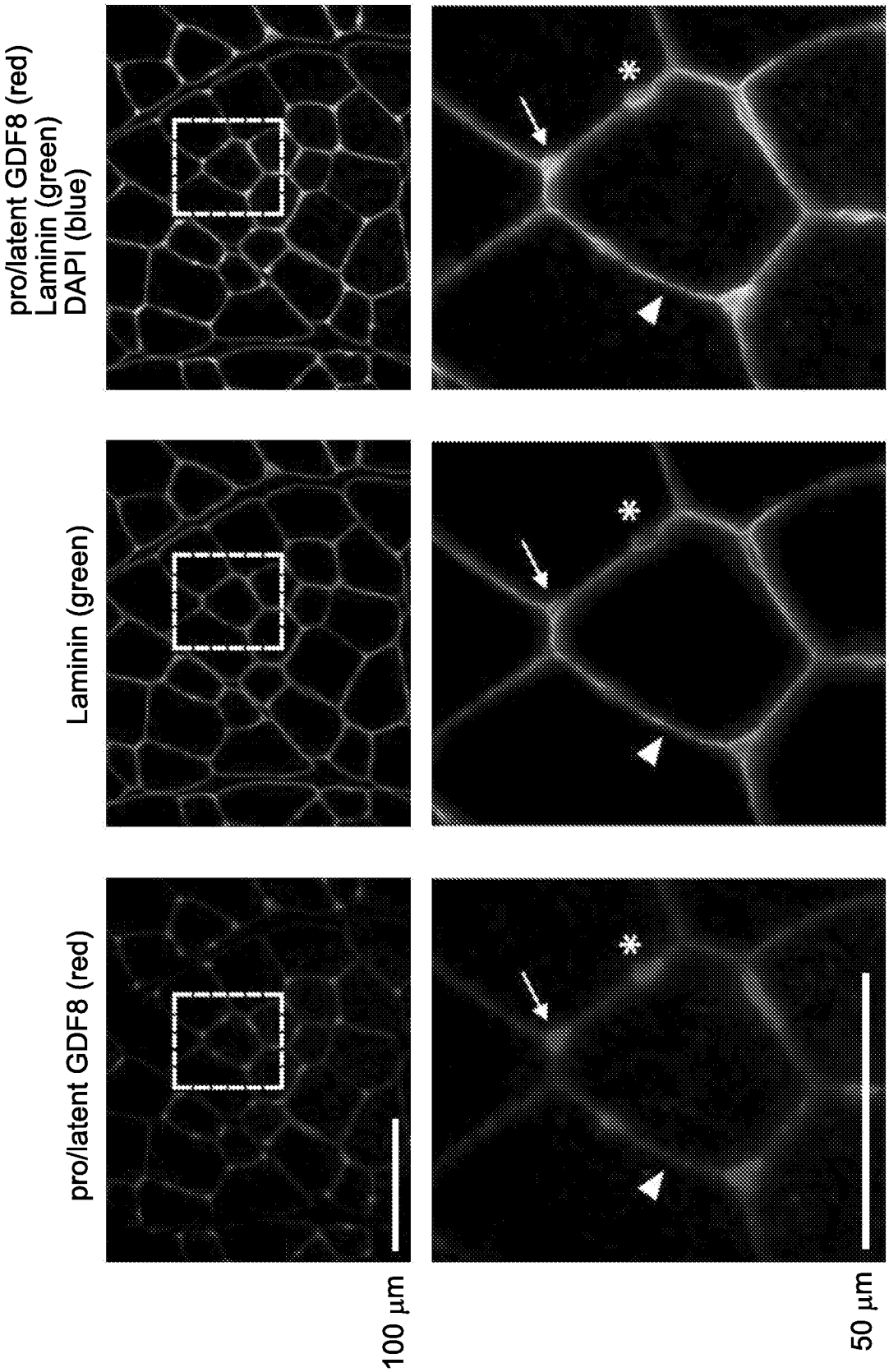

pro/latent GDF8 (red)
Laminin (green)
DAPI (blue)
Laminin (green)
pro/latent GDF8 (red)
100 µm
50 µm

FIG. 55