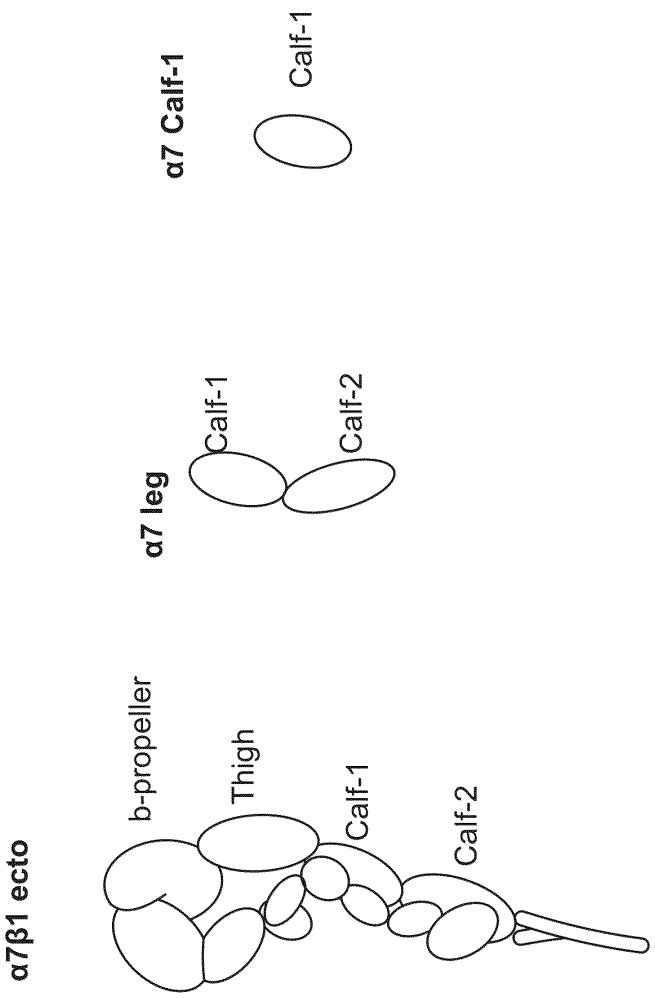

α7β1 ecto
b-propeller
Thigh
Calf-1
Calf-2
α7 leg
Calf-1
Calf-2
α7 Calf-1
Calf-1

FIG. 1A

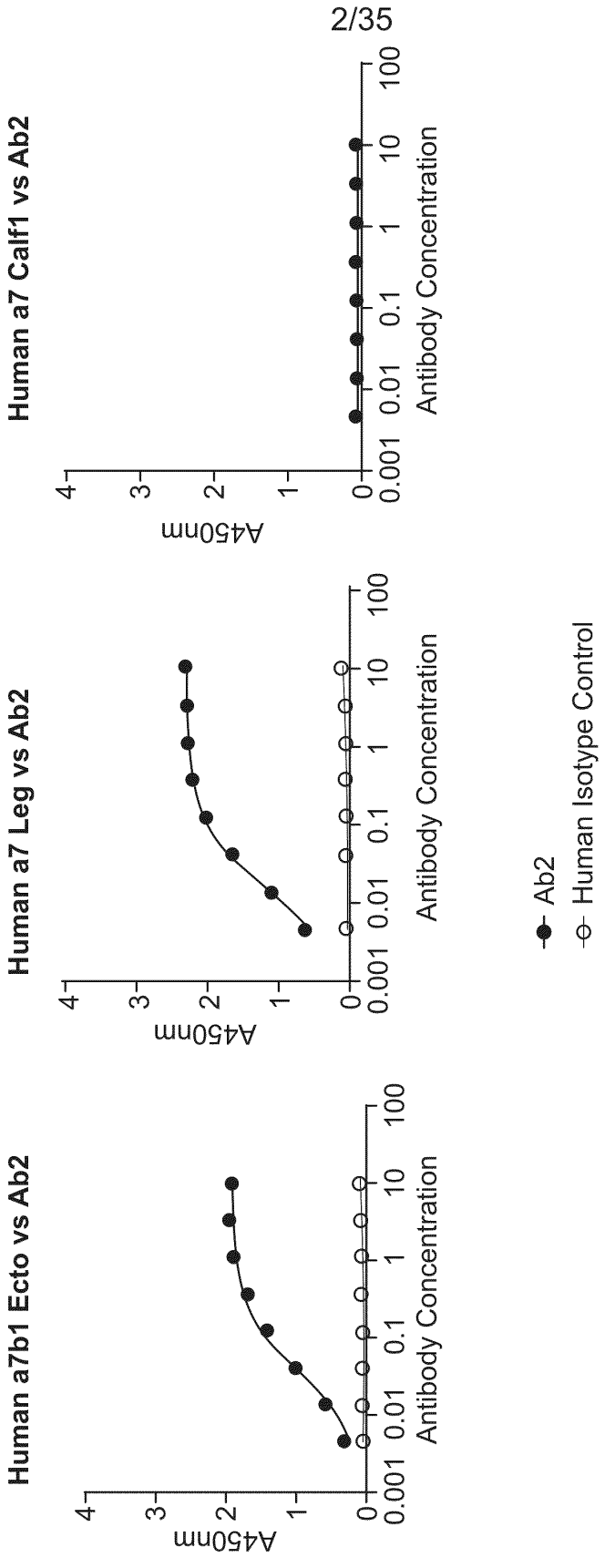

Human a7b1 Ecto vs Ab2
Human a7 Leg vs Ab2
Human a7 Calf1 vs Ab2
A450nm
0
1
2
3
4
0.001
0.01
0.1
1
10
100
Antibody Concentration
Ab2
Human Isotype Control

FIG. 1B

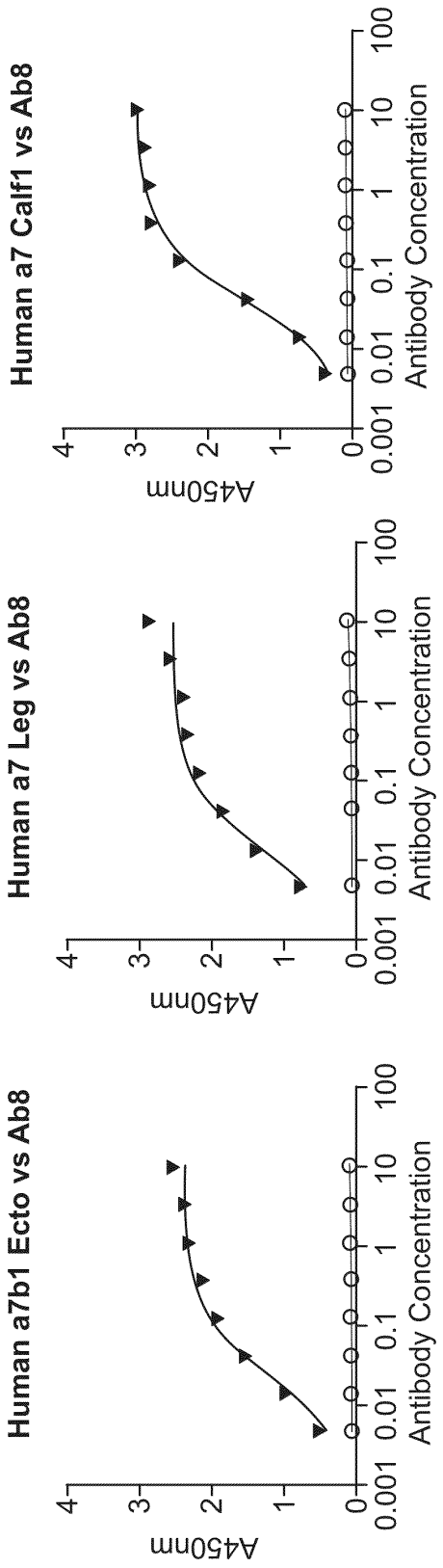

Human a7b1 Ecto vs Ab8
Human a7 Leg vs Ab8
Human a7 Calf1 vs Ab8
A450nm
0
1
2
3
4
0.001
0.01
0.1
1
10
100
Antibody Concentration
Ab8
Human Isotype Control

FIG. 1C

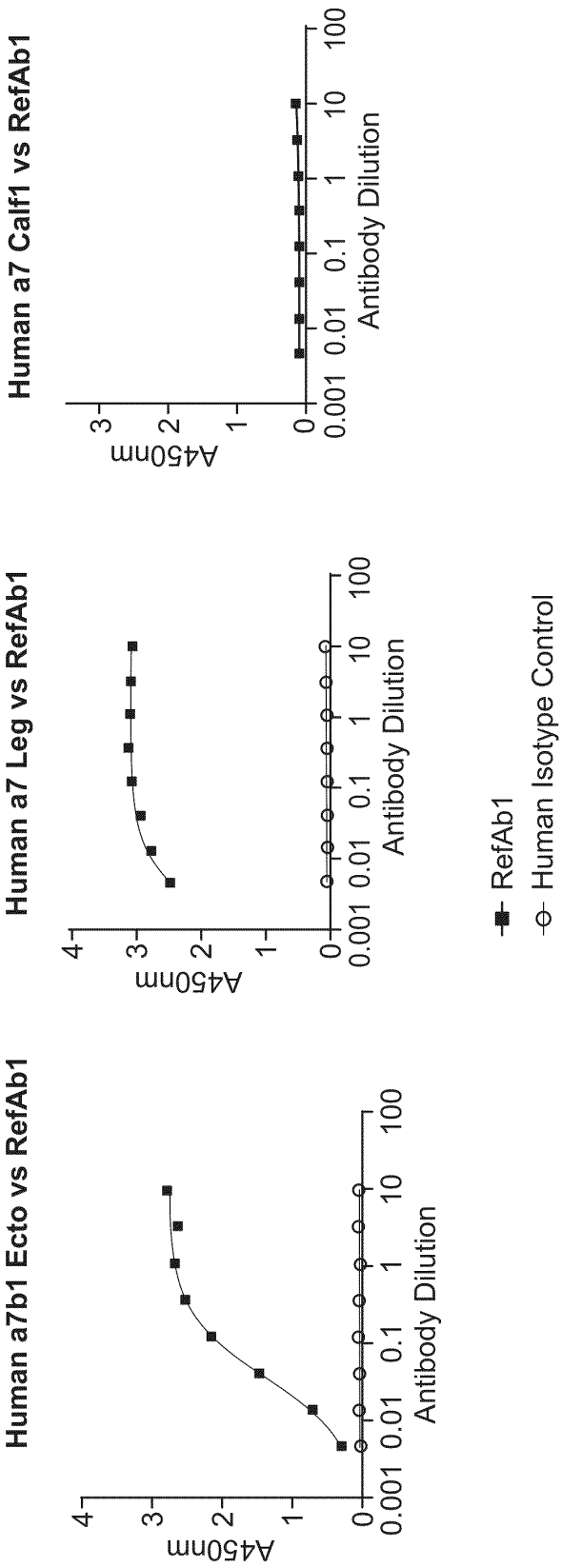

Human a7b1 Ecto vs RefAb1
Human a7 Leg vs RefAb1
Human a7 Calf1 vs RefAb1
A450nm
Antibody Dilution
0.001
0.01
0.1
1
10
100
RefAb1
Human Isotype Control

FIG. 1D

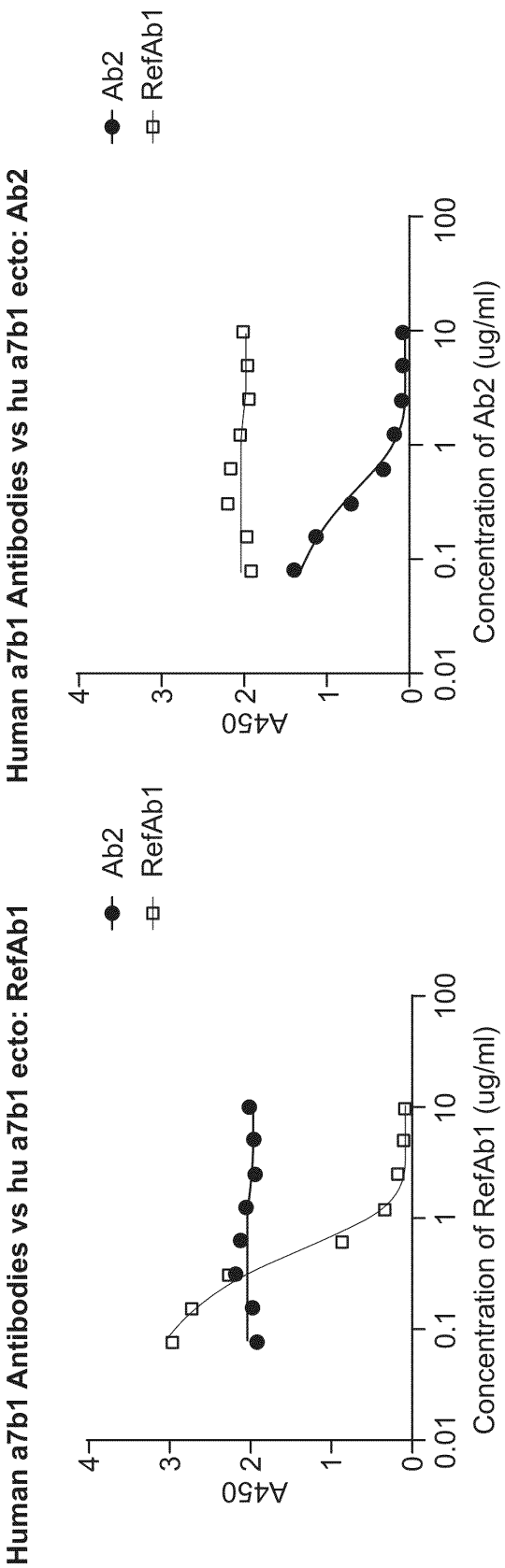

Human a7b1 Antibodies vs hu a7b1 ecto: RefAb1
Ab2
RefAb1
A450
0
1
2
3
4
0.01
0.1
1
10
100
Concentration of RefAb1 (ug/ml)
Human a7b1 Antibodies vs hu a7b1 ecto: Ab2
Ab2
RefAb1
A450
0
1
2
3
4
0.01
0.1
1
10
100
Concentration of Ab2 (ug/ml)

FIG. 2

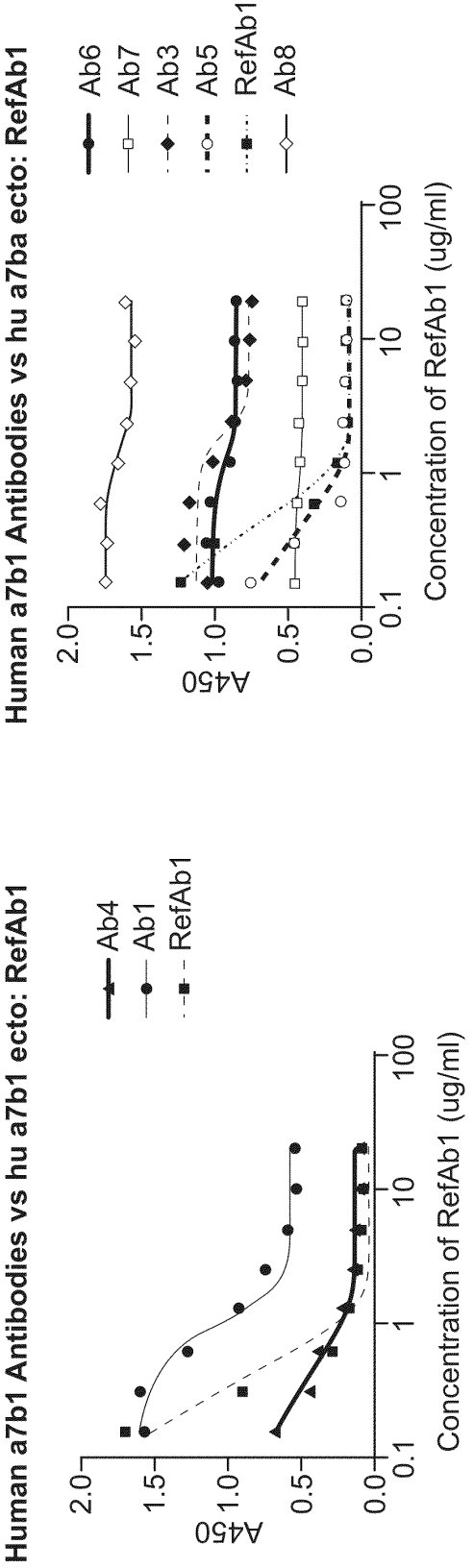

Human a7b1 Antibodies vs hu a7b1 ecto: RefAb1
Ab4
Ab1
RefAb1
A450
2.0
1.5
1.0
0.5
0.0
0.1
1
10
100
Concentration of RefAb1 (ug/ml)
Human a7b1 Antibodies vs hu a7ba ecto: RefAb1
Ab6
Ab7
Ab3
Ab5
RefAb1
Ab8
A450
2.0
1.5
1.0
0.5
0.0
0.1
1
10
100
Concentration of RefAb1 (ug/ml)

FIG. 3

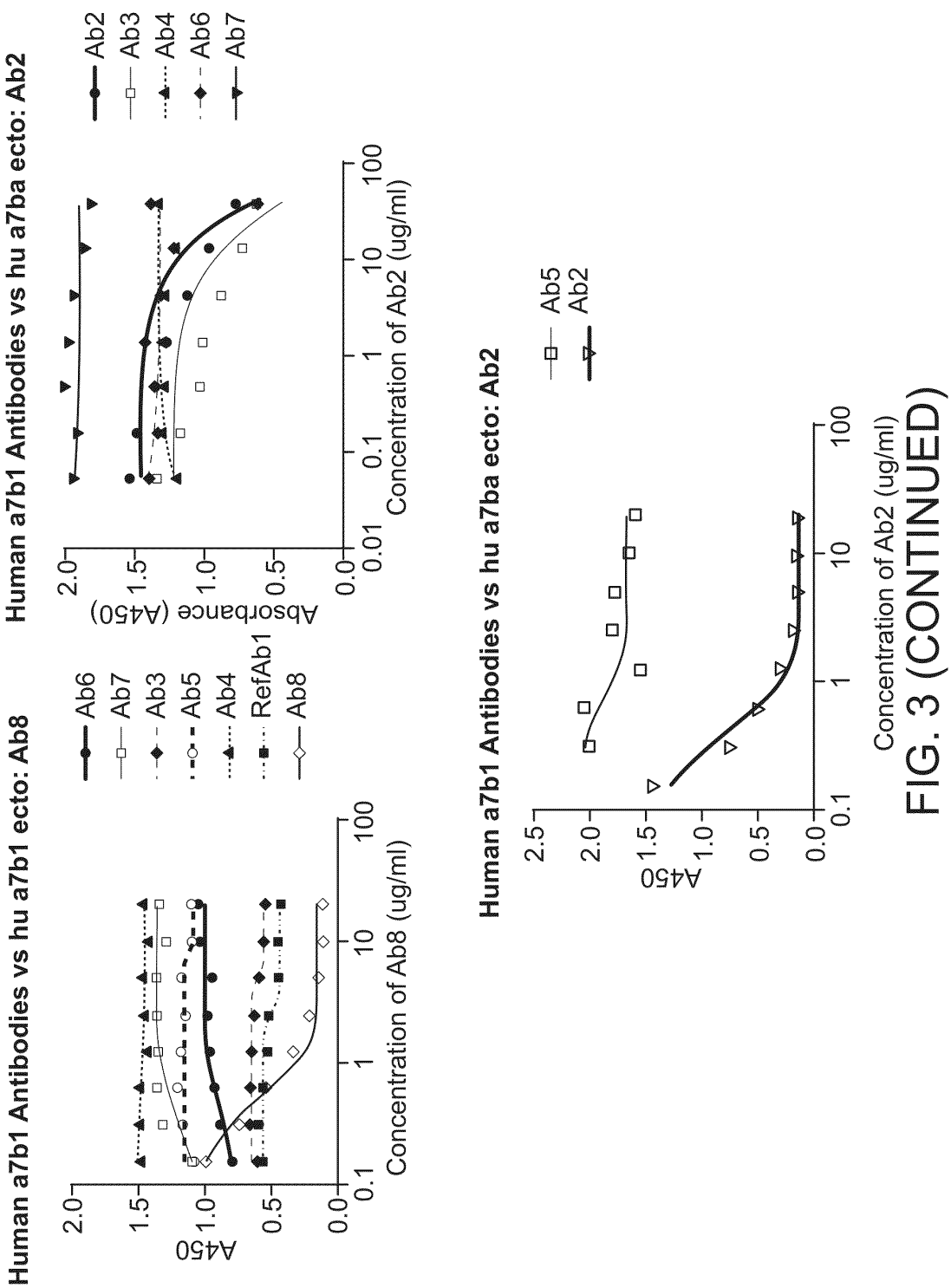


FIG. 3 (CONTINUED)

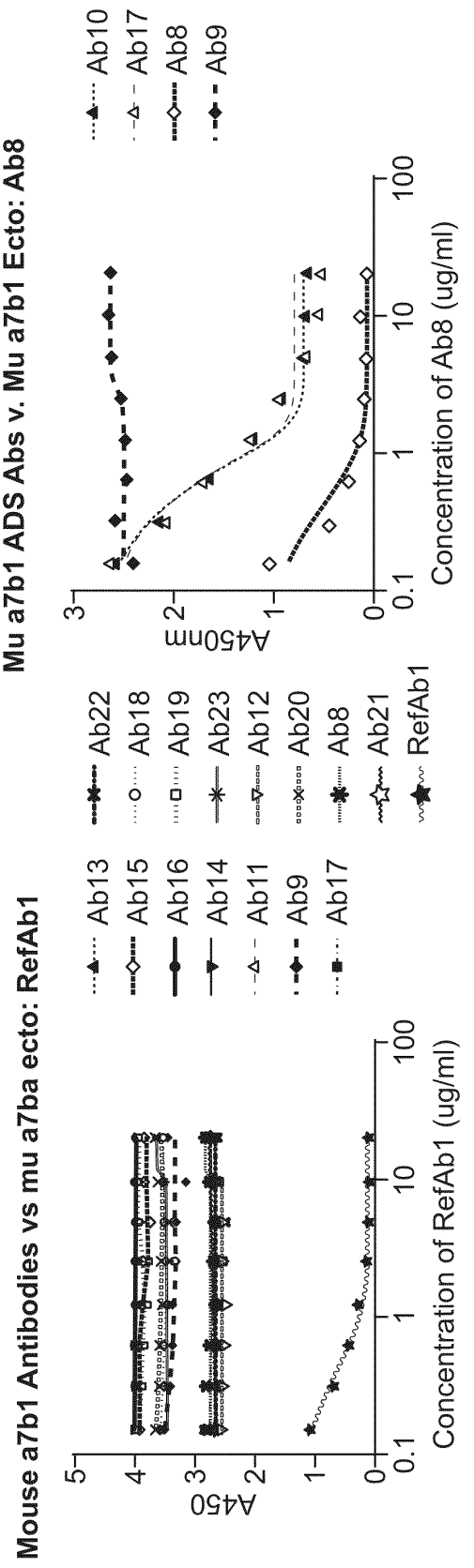

Mouse a7b1 Antibodies vs mu a7ba ecto: RefAb1
A450
Concentration of RefAb1 (ug/ml)
Ab13
Ab15
Ab16
Ab14
Ab11
Ab9
Ab17
Ab22
Ab18
Ab19
Ab23
Ab12
Ab20
Ab8
Ab21
RefAb1
Mu a7b1 ADS Abs v. Mu a7b1 Ecto: Ab8
A450nm
Concentration of Ab8 (ug/ml)
Ab10
Ab17
Ab8
Ab9

FIG. 4

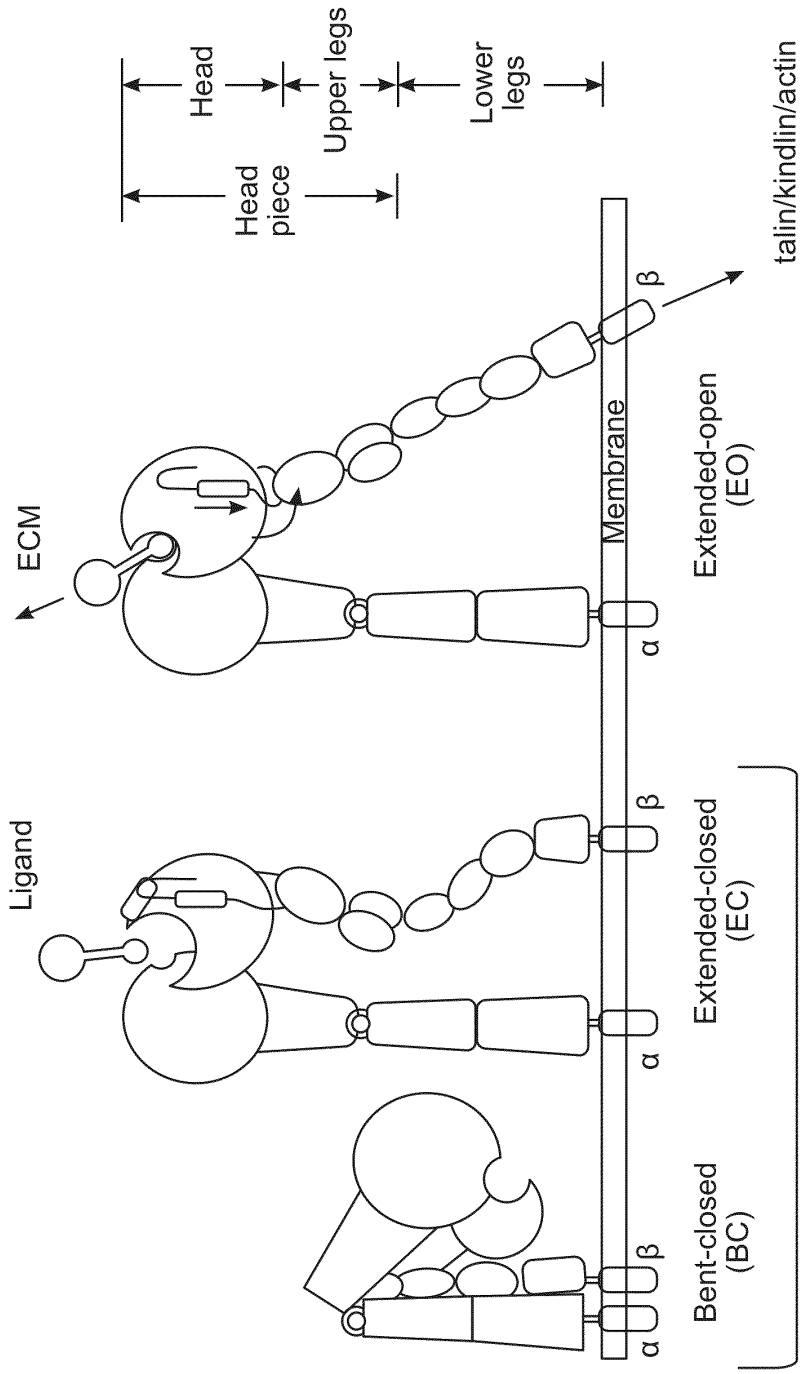


FIG. 5A FIG. 5B FIG. 5C

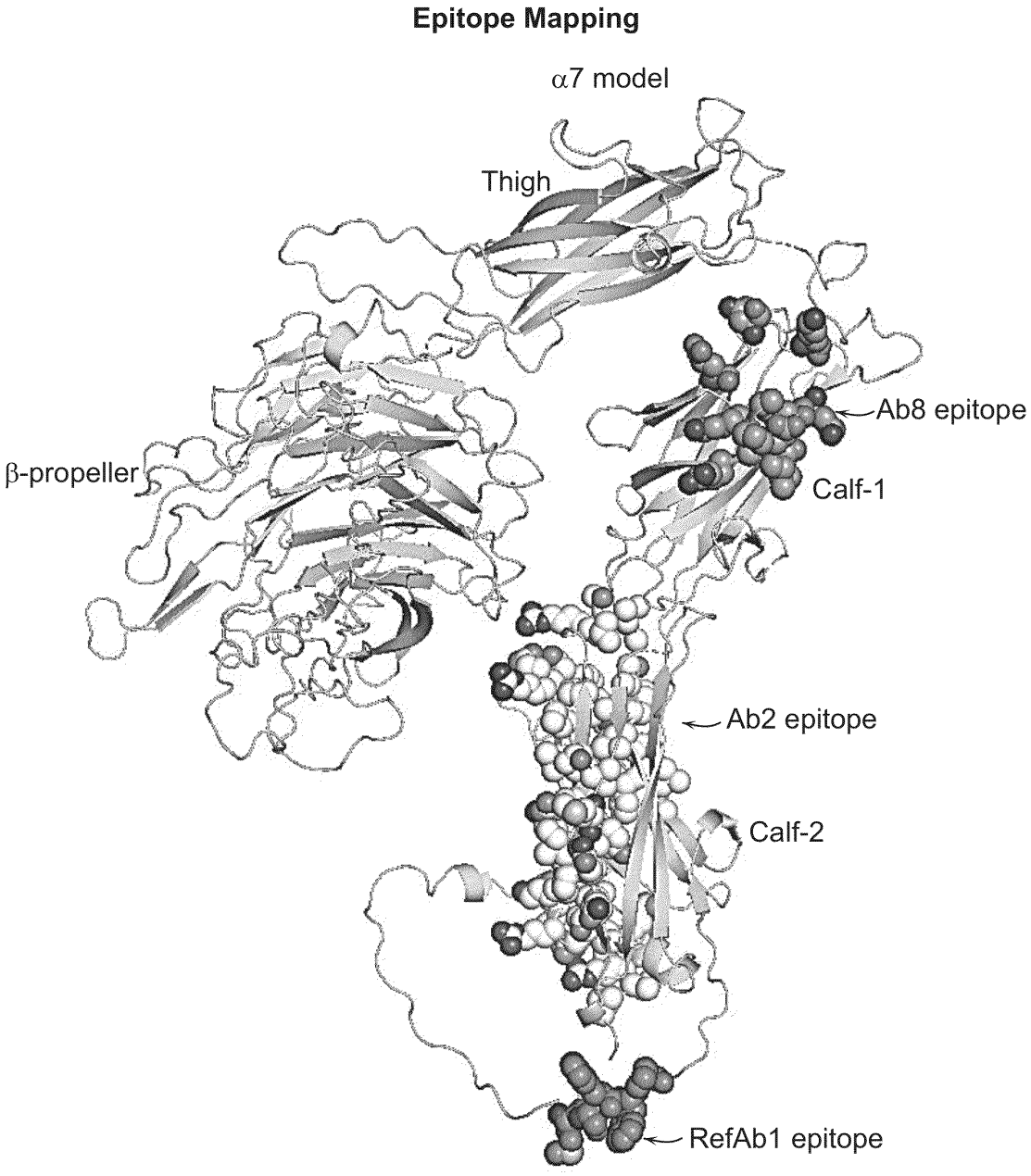

Epitope Mapping
α7 model
Thigh
β-propeller
Ab8 epitope
Calf-1
Ab2 epitope
Calf-2
RefAb1 epitope

FIG. 6

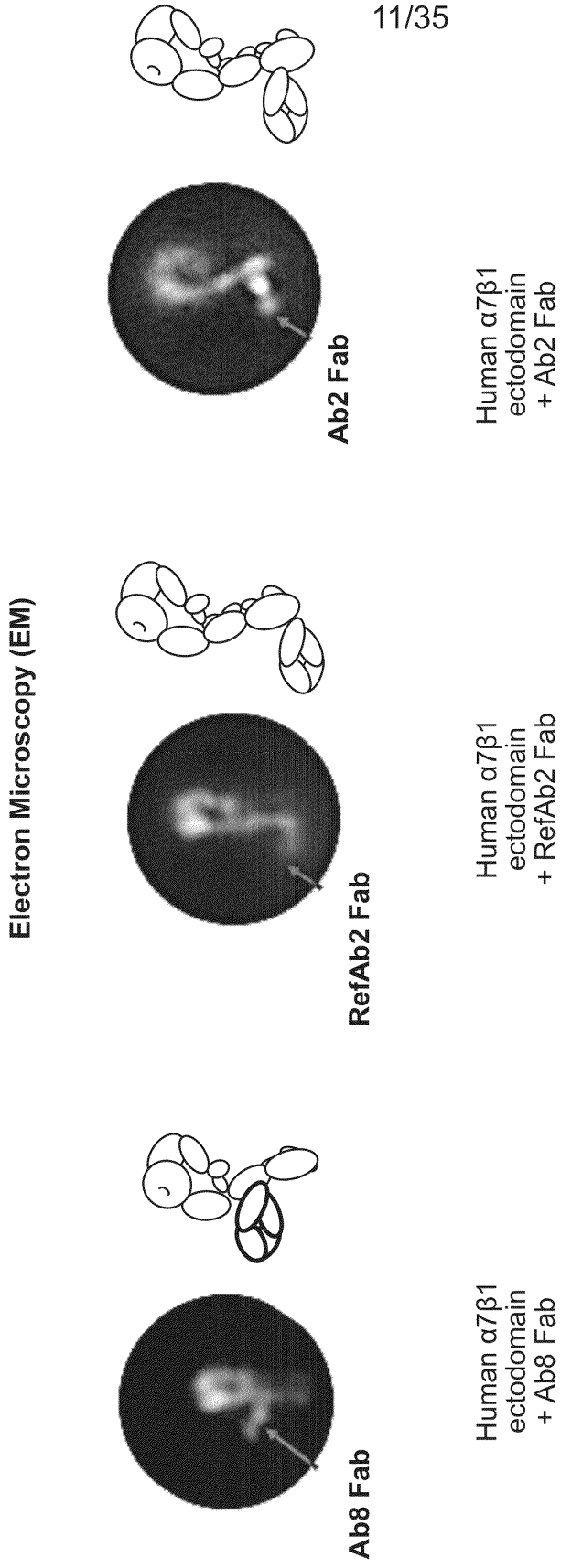

Electron Microscopy (EM)
Ab8 Fab
RefAb2 Fab
Ab2 Fab
Human α7β1 ectodomain + Ab8 Fab
Human α7β1 ectodomain + RefAb2 Fab
Human α7β1 ectodomain + Ab2 Fab

FIG. 7

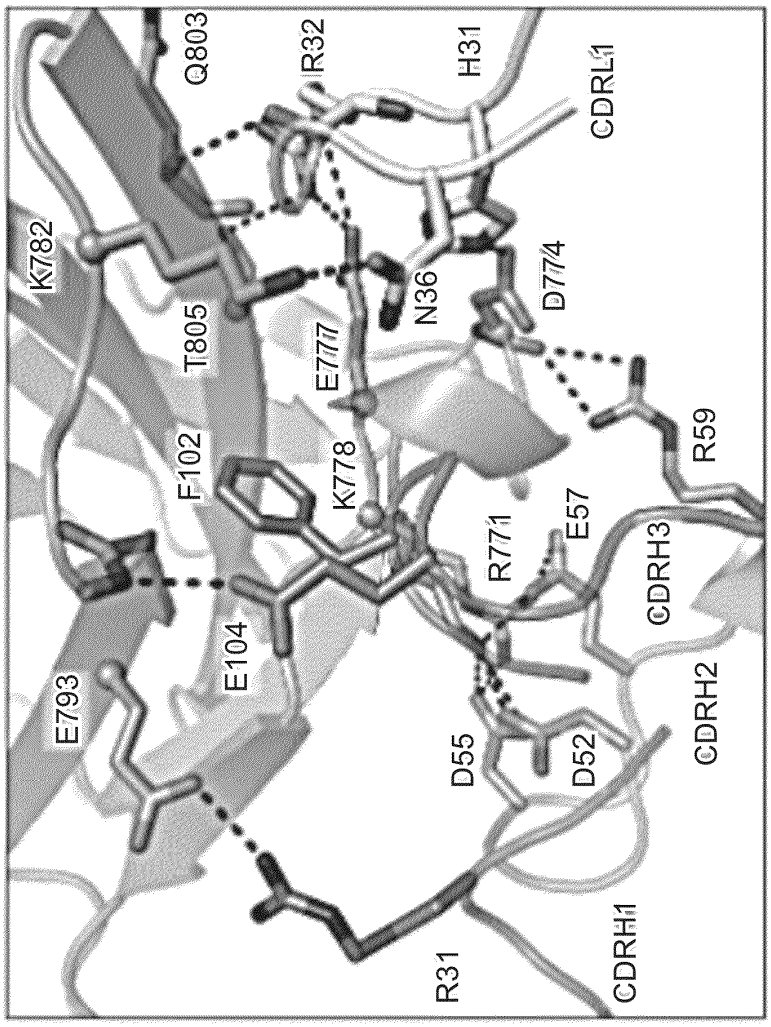

Q803
R32
H31
CDRL1
K782
N36
D774
T805
E777
F102
K778
R59
E57
R771
CDRH3
E104
E793
CDRH2
D55
D52
CDRH1
R31

FIG. 8B

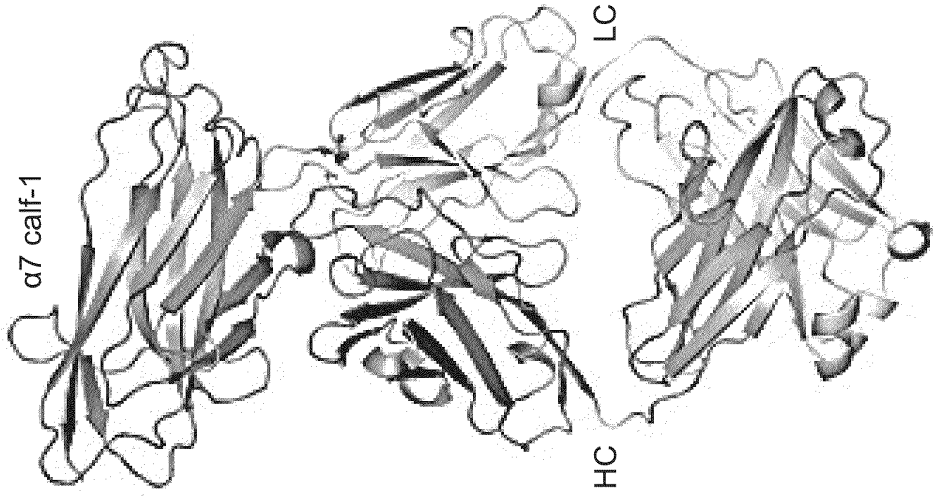

α7 calf-1
LC
HC

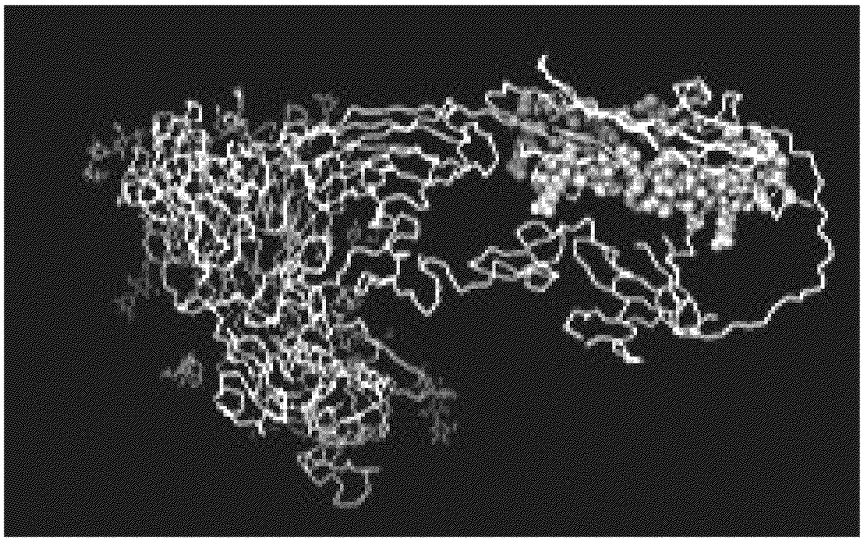
FIG. 8A

```
HUMAN  QGCGEDKICQSNLQLVRARFCTRVSDTEFQPLPMDVDGTTALFALSGQPVIGLELMVTNL 738
 CYNO  QGCGEDKICQSNLQLVRARFCARVSDTEFQPLPMDADGTTALFALSGQPVIGLELMVTNL 694
MOUSE  QGCGQDKICQSNLQLERYQFCSRISDTEFQALPMDLDGRTALFALSGQPFIGLELTVTNL 738
       ****:**********  *  :**:*:*:*******  *****  **  ********** .******  *****

HUMAN  PSDPAQPQADGDDAHEAQLLVMLPDSLHYSGVRALDPAEKPLCLSNENASHVECELGNPM 798
 CYNO  PSDPAQPQADGDDAHEAQLLVMLPDSLHYSGVRALDPAEKPLCLSNENASHVECELGNPM 754
MOUSE  PSDPSRPQADGDDAHEAQLLVTLPASLRYSGVRALDSVEKPLCLSNDSASHVECELGNPM 798
       *****::**********  **  **:********** .*********:.*********

HUMAN  KRGAQVTFYLILSTSGISIETTELEVELLLATISEQELHPVSARARVFIELP 850
 CYNO  KRGAQVTFYLILSTSGISIETTELEVELLLATISEQELHPVSARARVFIELP 806
MOUSE  KRGAQVTFYLILSTSGITIETTELEVKLLLATISEQELDPVSVRAHVFIELP 850
       ********************:**********:*********.***.**:*******
```

FIG. 8C



KQGCGEDKICQSNLQLVRARFCTRVSDTEFQPLPMDVDGTTAL
FALSGQPVIGLELMVTNLPSDPAQPQADGDDAHEAQLLVMLPD
SLHYSGVRALDPAEKPLCLSNENASHVECELGNPMKRGAQVTF
YLILSTSGISIETTELEVELLLATISEQELHPVSARARVFIEL
PLSIAGMAIPQQLFFSGVVRGERAMQSERDVGSKVKYEVTVSN
QGQSLRTLGSAFLNIMWPHEIANGKWLLYPMQVELEGGQGPGQ
KGLCSPRPNILHLDVDSRDRRRELEPPEQQEPGERQEPSMSW
WPVSSAEKKKNITLDCARGTANCVVFSCPLYSFEDRAAVLHVWG
RLWNSTFLEEYSAVKSLEVIVRANITVKSSIKNLMLRDASTVI
PVMVYLDPMAVVAEA

FIG. 9

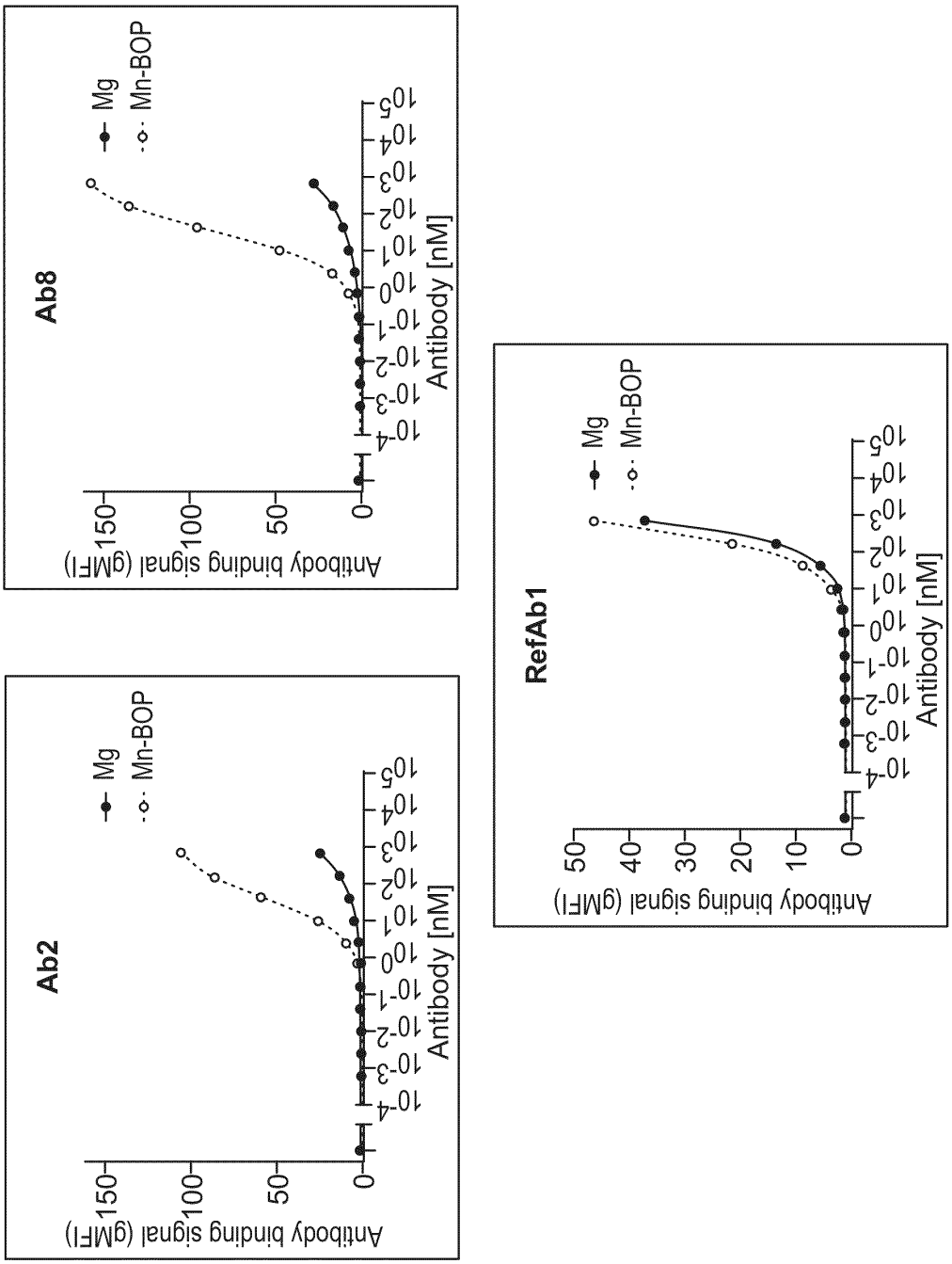

Ab8
Mg
Mn-BOP
Antibody binding signal (gMFI)
Antibody [nM]
Ab2
RefAb1

FIG. 10

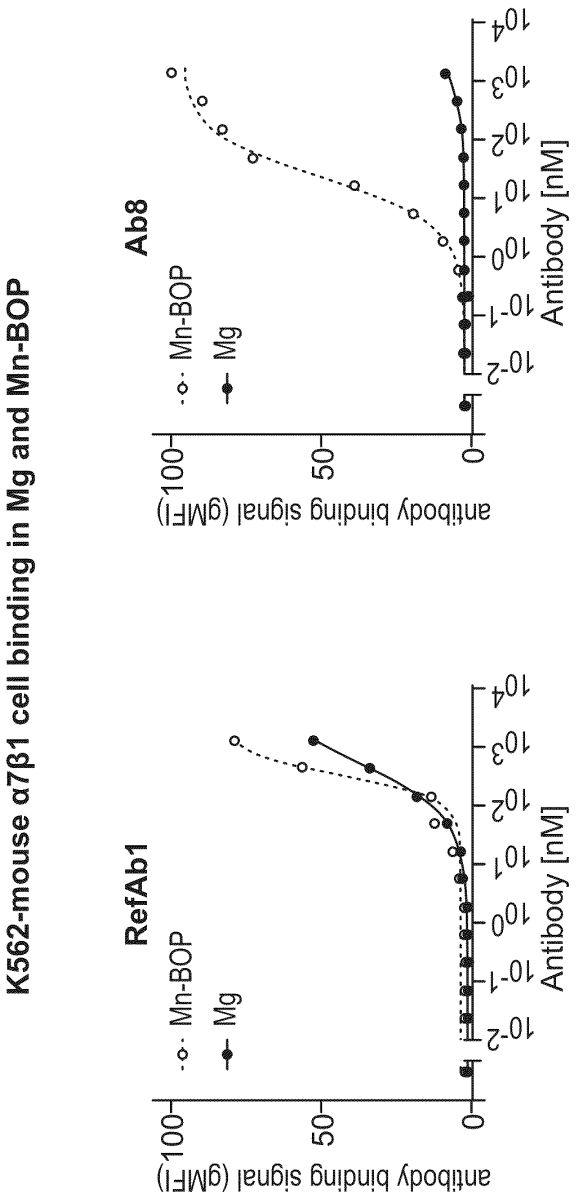

K562-mouse α7β1 cell binding in Mg and Mn-BOP
RefAb1
Ab8
Mn-BOP
Mg
antibody binding signal (gMFI)
Antibody [nM]
0
50
100
10-2
10-1
100
101
102
103
104

FIG. 11

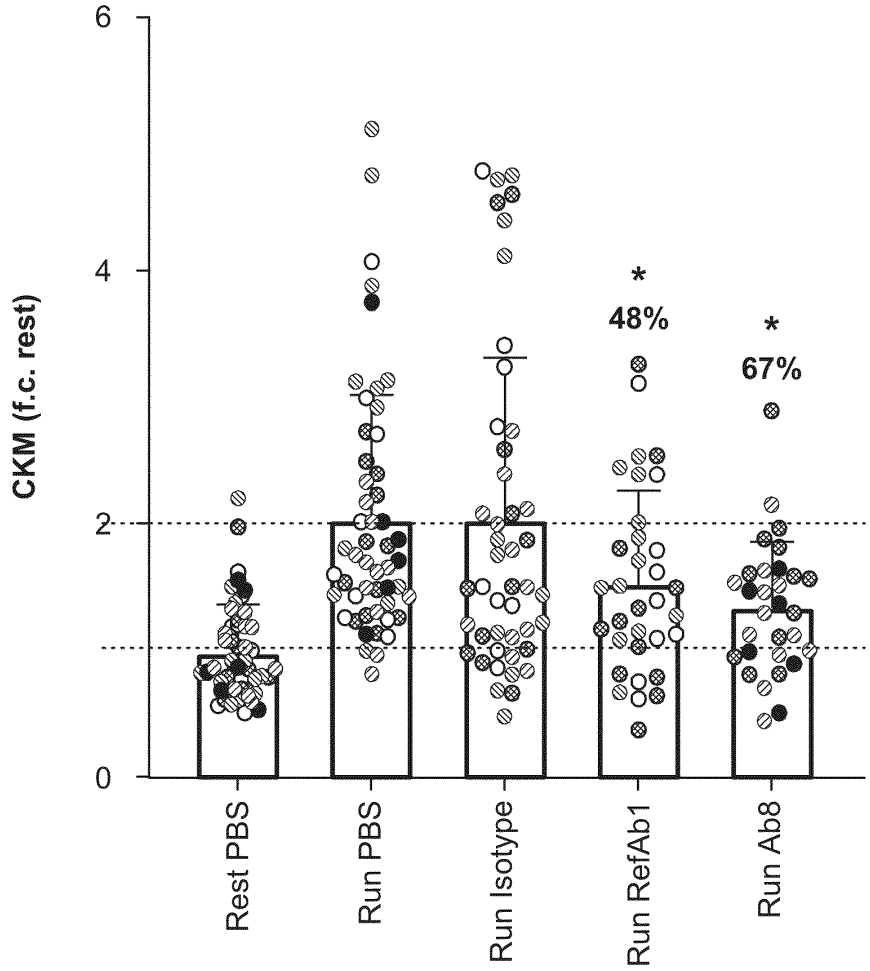

6
4
2
0
CKM (f.c. rest)
*
48%
*
67%
Rest PBS
Run PBS
Run Isotype
Run RefAb1
Run Ab8

FIG. 12

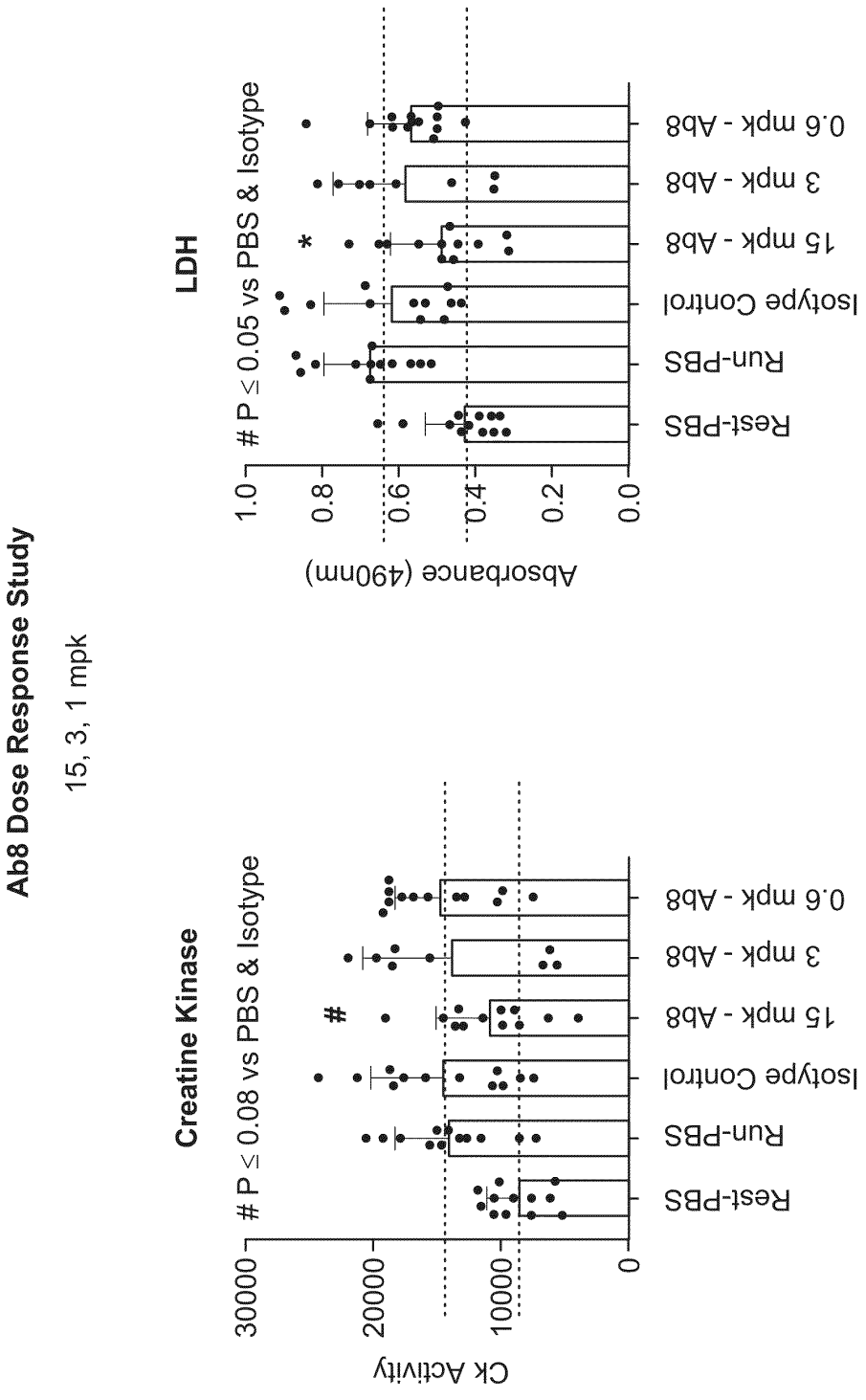

Ab8 Dose Response Study
15, 3, 1 mpk
Creatine Kinase
P ≤ 0.08 vs PBS & Isotype
CK Activity
30000
20000
10000
0
#
Rest-PBS
Run-PBS
Isotype Control
15 mpk - Ab8
3 mpk - Ab8
0.6 mpk - Ab8
LDH
P ≤ 0.05 vs PBS & Isotype
Absorbance (490nm)
1.0
0.8
0.6
0.4
0.2
0.0
*
Rest-PBS
Run-PBS
Isotype Control
15 mpk - Ab8
3 mpk - Ab8
0.6 mpk - Ab8

FIG. 13

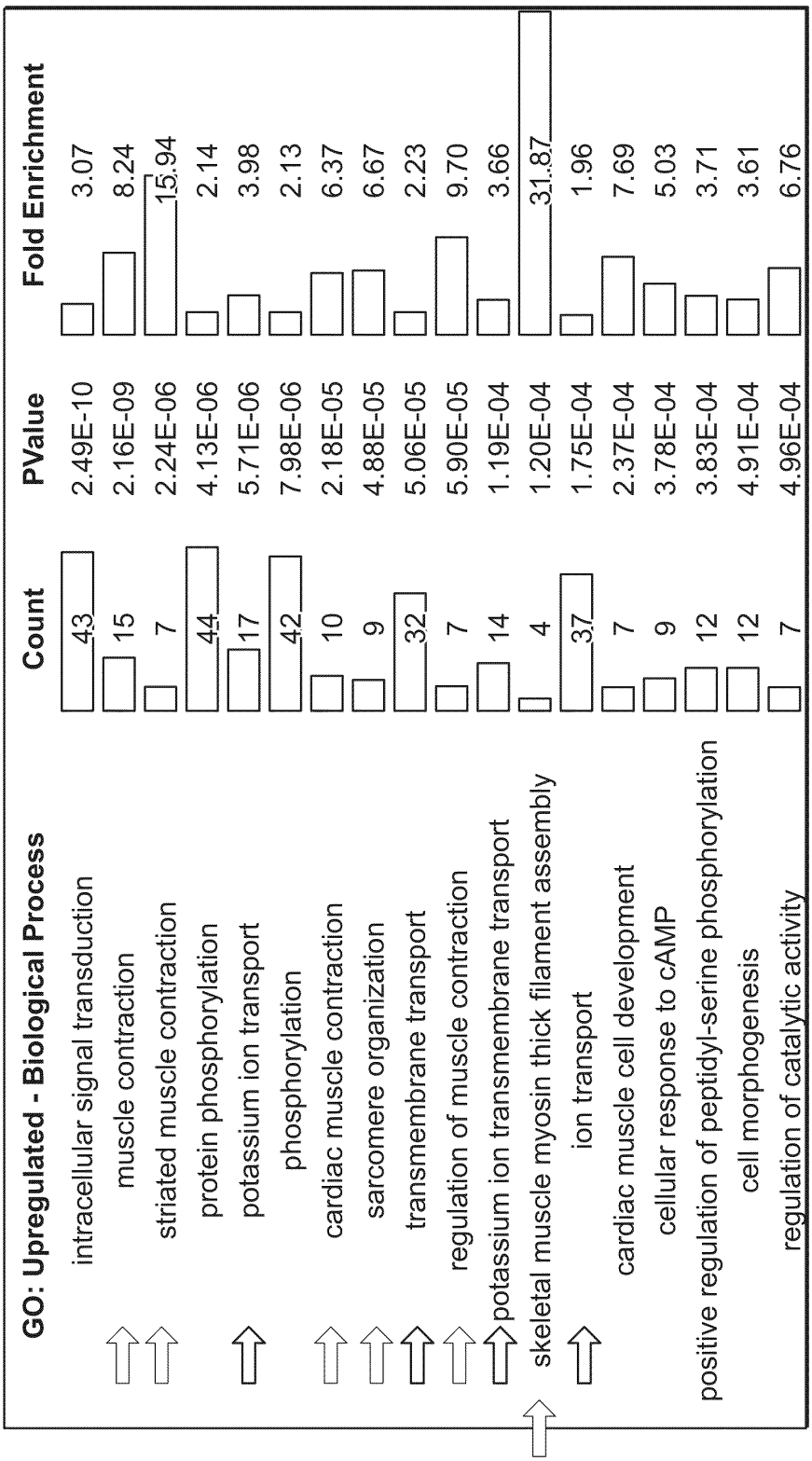

GO: Upregulated - Biological Process
Count
PValue
Fold Enrichment
intracellular signal transduction 43 2.49E-10 3.07
muscle contraction 15 2.16E-09 8.24
striated muscle contraction 7 2.24E-06 15.94
protein phosphorylation 44 4.13E-06 2.14
potassium ion transport 17 5.71E-06 3.98
phosphorylation 42 7.98E-06 2.13
cardiac muscle contraction 10 2.18E-05 6.37
sarcomere organization 9 4.88E-05 6.67
transmembrane transport 32 5.06E-05 2.23
regulation of muscle contraction 7 5.90E-05 9.70
potassium ion transmembrane transport 14 1.19E-04 3.66
skeletal muscle myosin thick filament assembly 4 1.20E-04 31.87
ion transport 37 1.75E-04 1.96
cardiac muscle cell development 7 2.37E-04 7.69
cellular response to cAMP 9 3.78E-04 5.03
positive regulation of peptidyl-serine phosphorylation 12 3.83E-04 3.71
cell morphogenesis 12 4.91E-04 3.61
regulation of catalytic activity 7 4.96E-04 6.76

FIG. 14

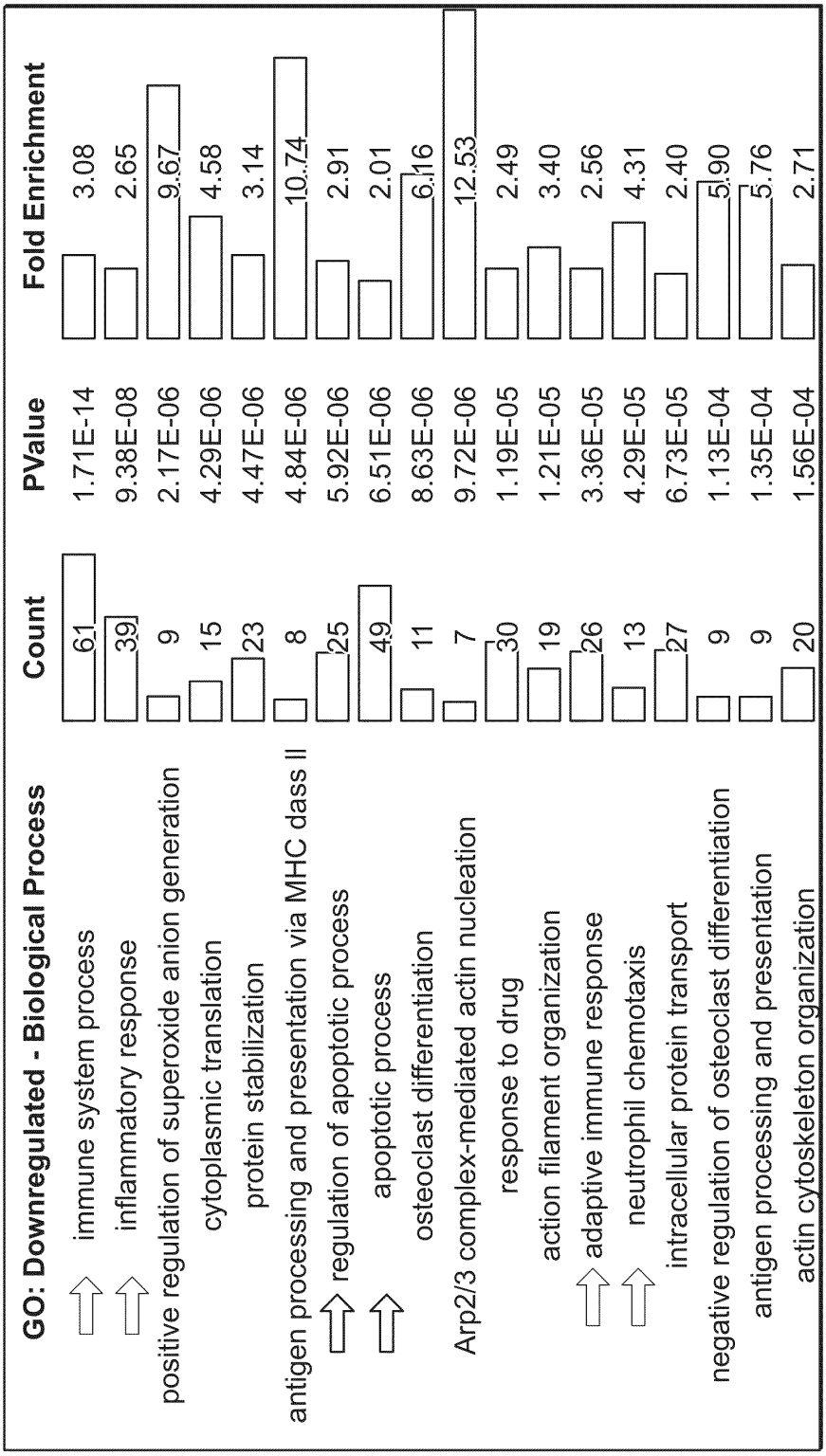

GO: Downregulated - Biological Process
Count
PValue
Fold Enrichment
immune system process
61
1.71E-14
3.08
inflammatory response
39
9.38E-08
2.65
positive regulation of superoxide anion generation
9
2.17E-06
9.67
cytoplasmic translation
15
4.29E-06
4.58
protein stabilization
23
4.47E-06
3.14
antigen processing and presentation via MHC dass II
8
4.84E-06
10.74
regulation of apoptotic process
25
5.92E-06
2.91
apoptotic process
49
6.51E-06
2.01
osteoclast differentiation
11
8.63E-06
6.16
Arp2/3 complex-mediated actin nucleation
7
9.72E-06
12.53
response to drug
30
1.19E-05
2.49
action filament organization
19
1.21E-05
3.40
adaptive immune response
26
3.36E-05
2.56
neutrophil chemotaxis
13
4.29E-05
4.31
intracellular protein transport
27
6.73E-05
2.40
negative regulation of osteoclast differentiation
9
1.13E-04
5.90
antigen processing and presentation
9
1.35E-04
5.76
actin cytoskeleton organization
20
1.56E-04
2.71

FIG. 15

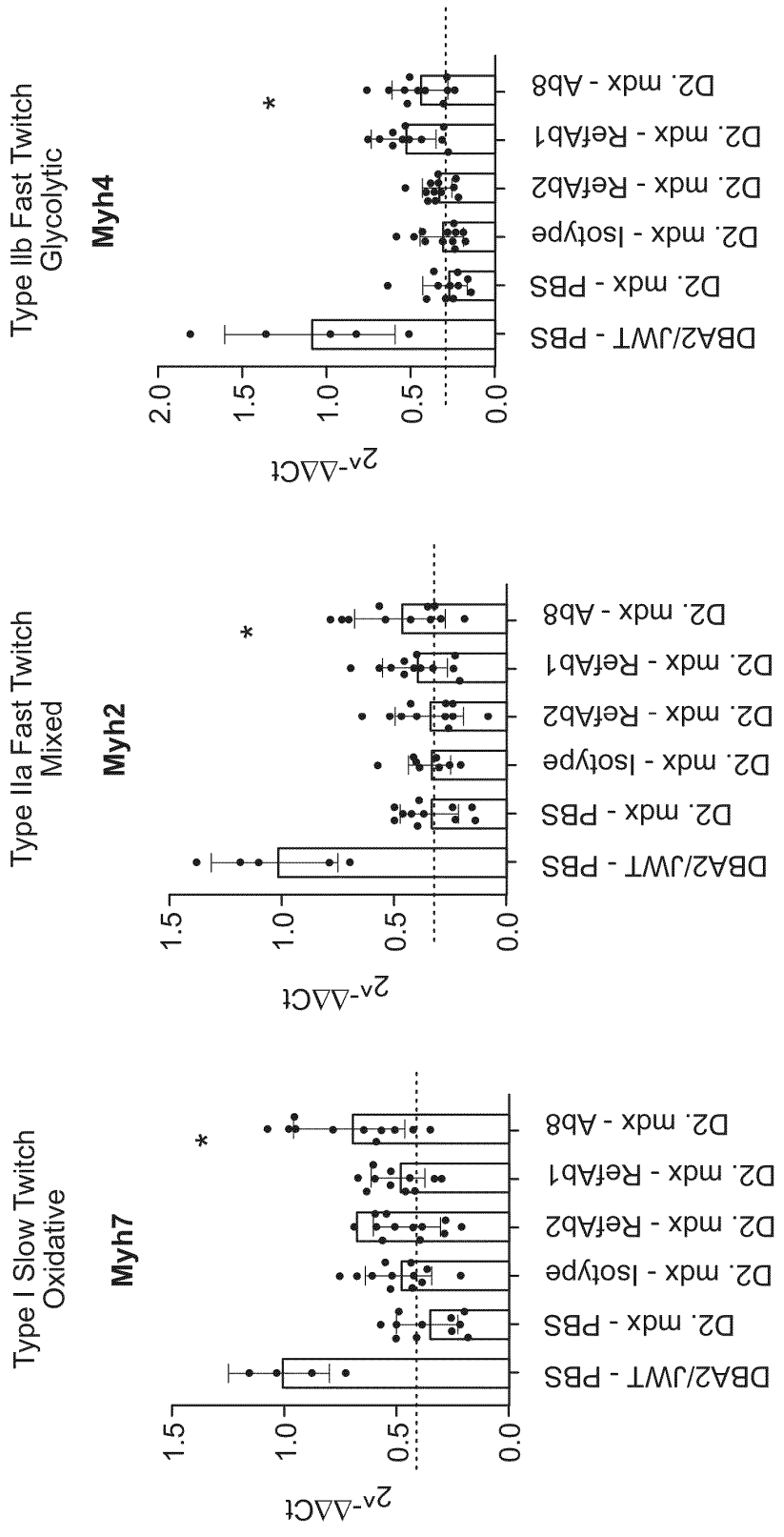

Type I Slow Twitch
Oxidative
Myh7
Type IIa Fast Twitch
Mixed
Myh2
Type IIb Fast Twitch
Glycolytic
Myh4
2^-ΔΔCt
0.0
0.5
1.0
1.5
2.0
*
DBA2/JWT - PBS
D2. mdx - PBS
D2. mdx - Isotype
D2. mdx - RefAb2
D2. mdx - RefAb1
D2. mdx - Ab8

FIG. 16A

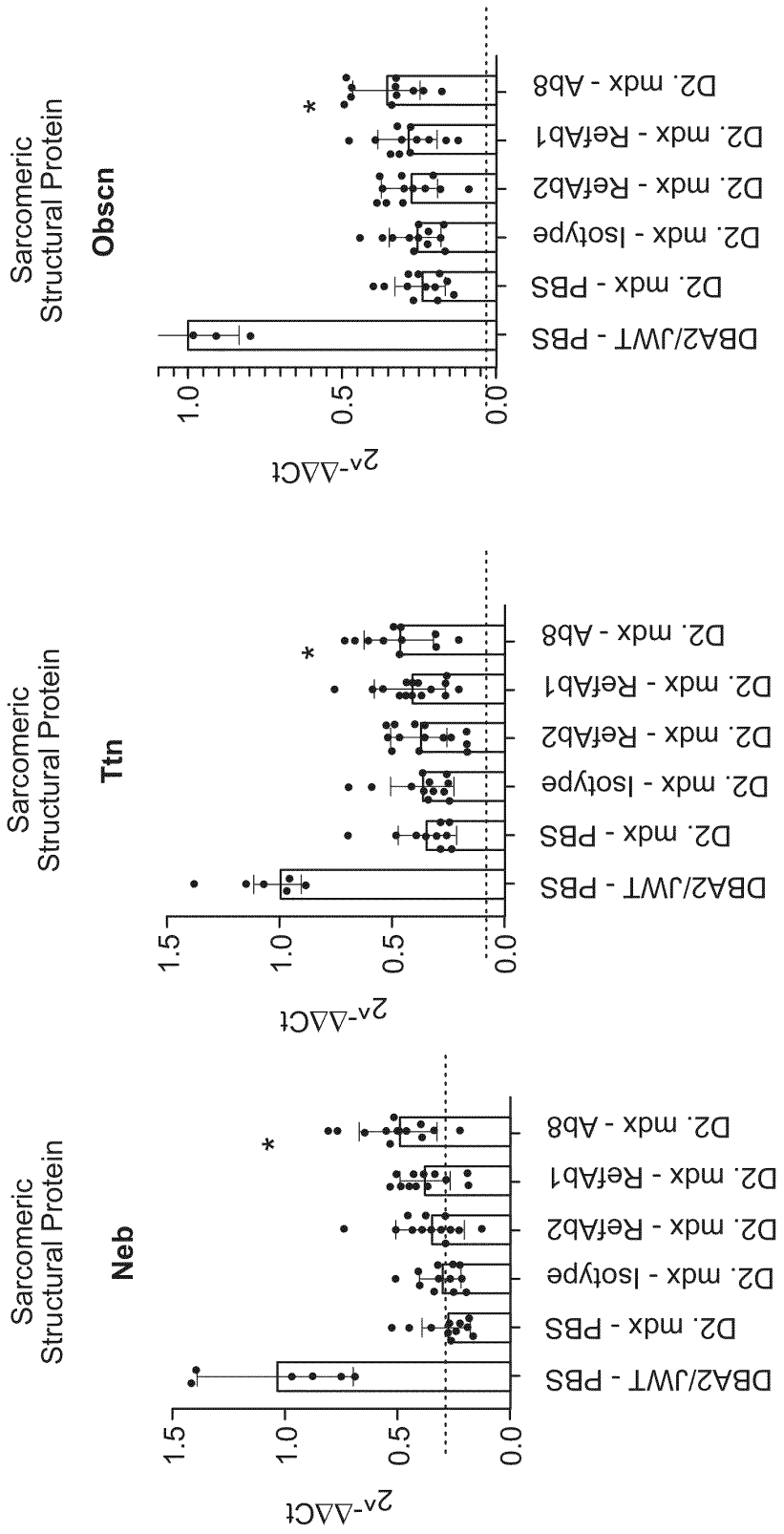
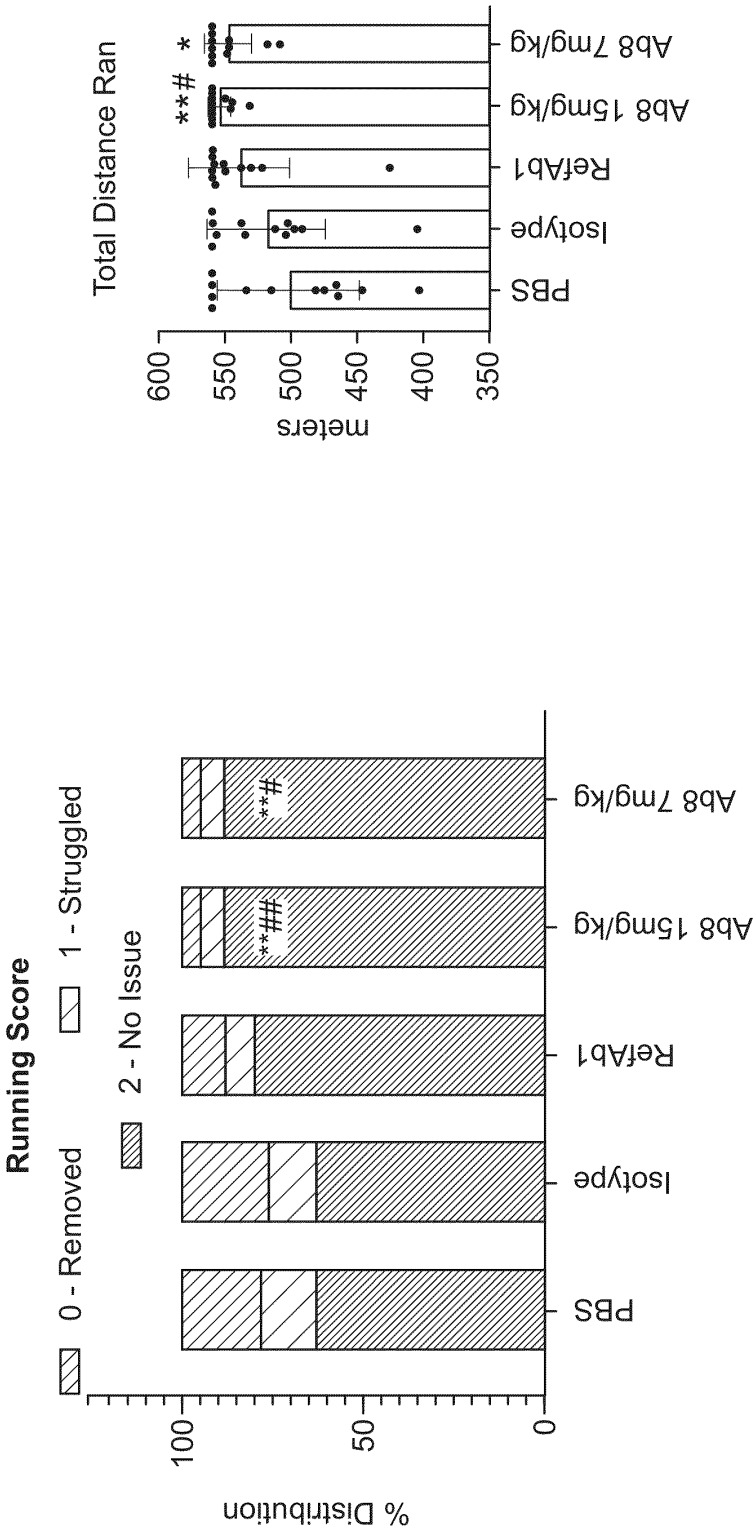
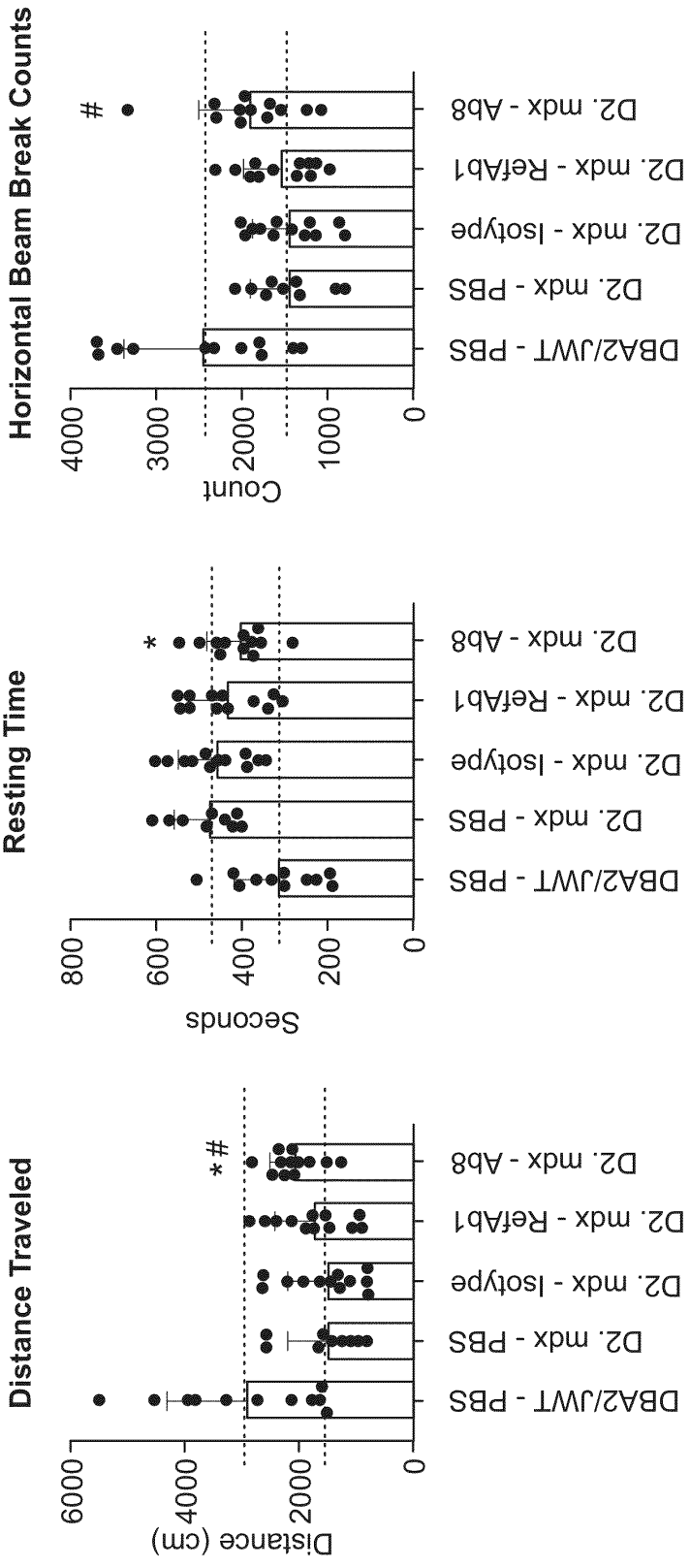

Sarcomeric Structural Protein
Neb
Ttn
Obscn
2^-ΔΔCt
1.5
1.0
0.5
0.0
*
DBA2/JWT - PBS
D2. mdx - PBS
D2. mdx - Isotype
D2. mdx - RefAb2
D2. mdx - RefAb1
D2. mdx - Ab8

FIG. 16B



*p<0.05, **p<0.005 compared to PBS-treated B10.mdx mice, #p<0.05, ##p<0.05 compared to Isotype-treated B10.mdx mice; by One-Way ANOVA followed by unpaired t-tests. All data are mean ± standard deviation.

FIG. 17



***vs.PBS**
#vs. Isotype

*p<0.05 compared to PBS-treated D2.mdx mice, #p<0.05 compared to Isotype-treated D2.mdx mice; by One-Way ANOVA followed by unpaired t-tests. All data are mean ± standard deviation.

FIG. 18

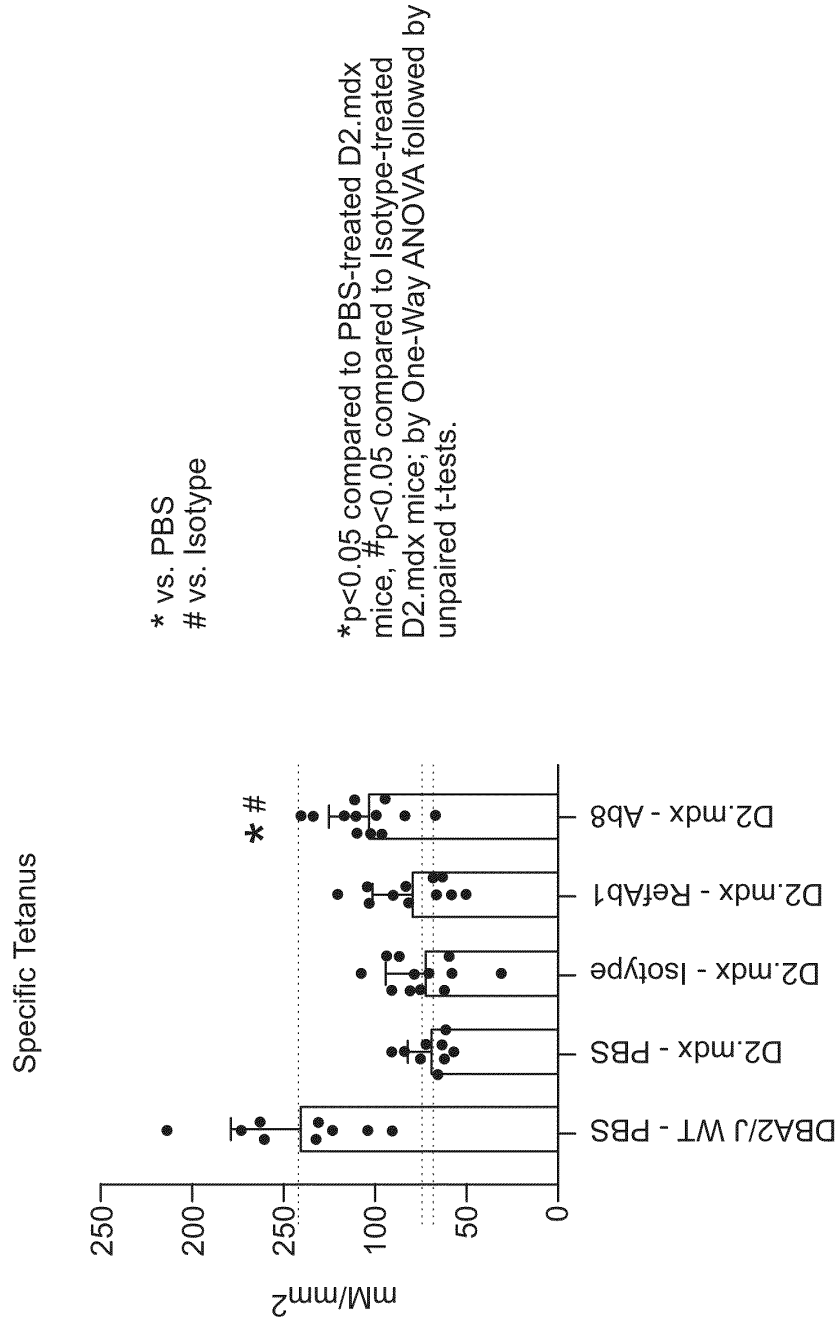

Specific Tetanus
mM/mm2
250
200
250
100
50
0
DBA2/J WT - PBS
D2.mdx - PBS
D2.mdx - Isotype
D2.mdx - RefAb1
D2.mdx - Ab8
* #
* vs. PBS
# vs. Isotype
*p<0.05 compared to PBS-treated D2.mdx mice, #p<0.05 compared to Isotype-treated D2.mdx mice; by One-Way ANOVA followed by unpaired t-tests.

FIG. 19A

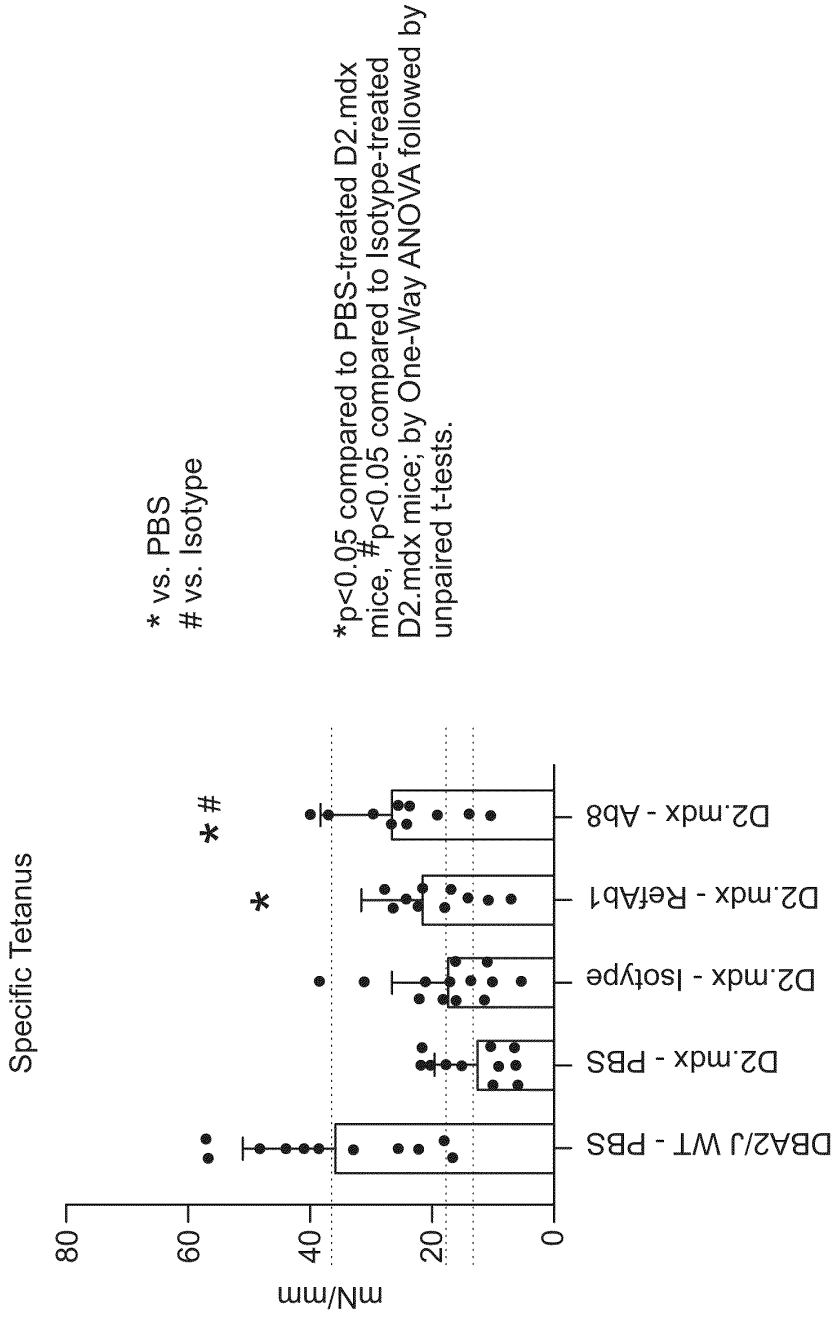

Specific Tetanus
mN/mm
0
20
40
60
80
DBA2/J WT - PBS
D2.mdx - PBS
D2.mdx - Isotype
D2.mdx - RefAb1
D2.mdx - Ab8
*
* #
* vs. PBS
# vs. Isotype
*p<0.05 compared to PBS-treated D2.mdx mice, #p<0.05 compared to Isotype-treated D2.mdx mice; by One-Way ANOVA followed by unpaired t-tests.

FIG. 19B

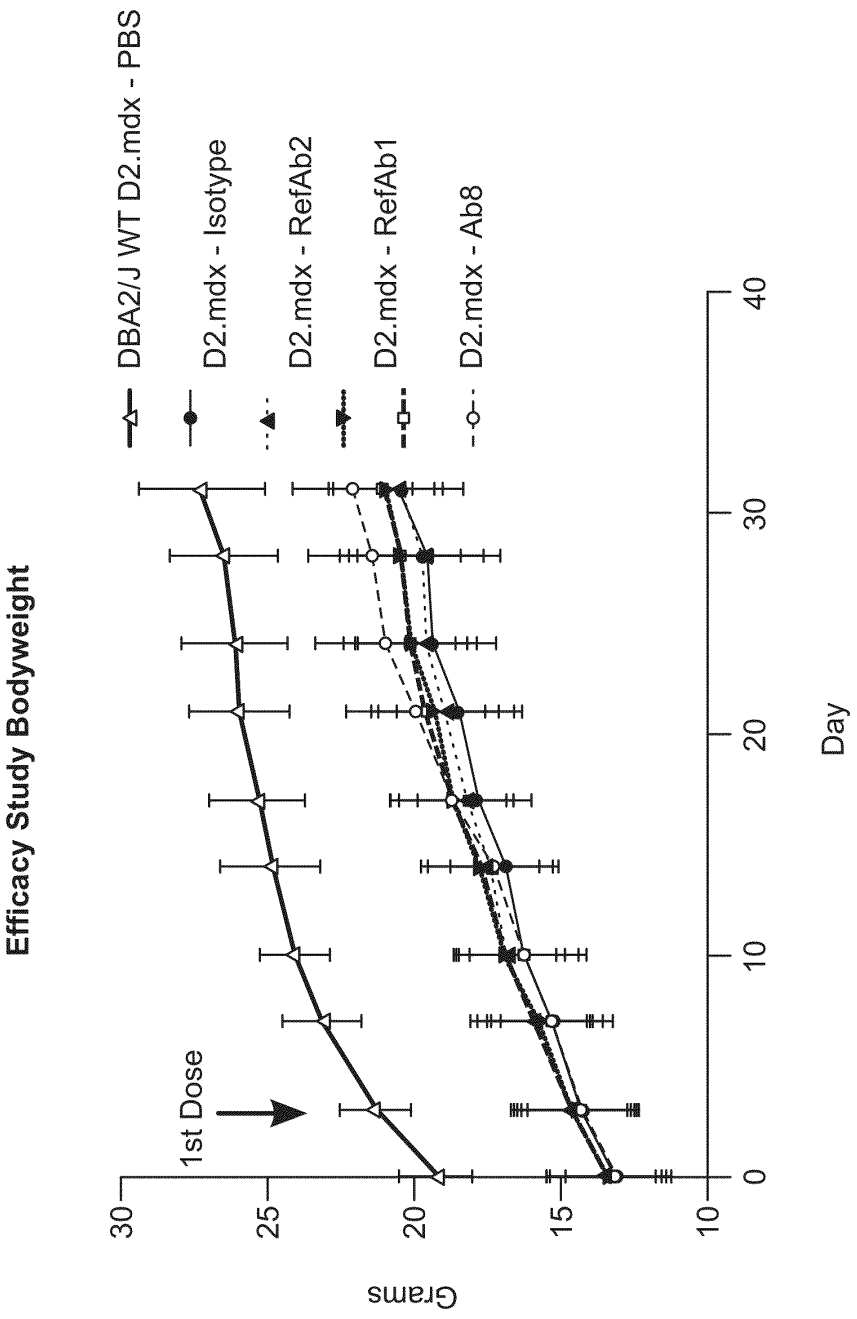

Efficacy Study Bodyweight
DBA2/J WT D2.mdx - PBS
D2.mdx - Isotype
D2.mdx - RefAb2
D2.mdx - RefAb1
D2.mdx - Ab8
1st Dose
Grams
Day
30
25
20
15
10
0
10
20
30
40

FIG. 20

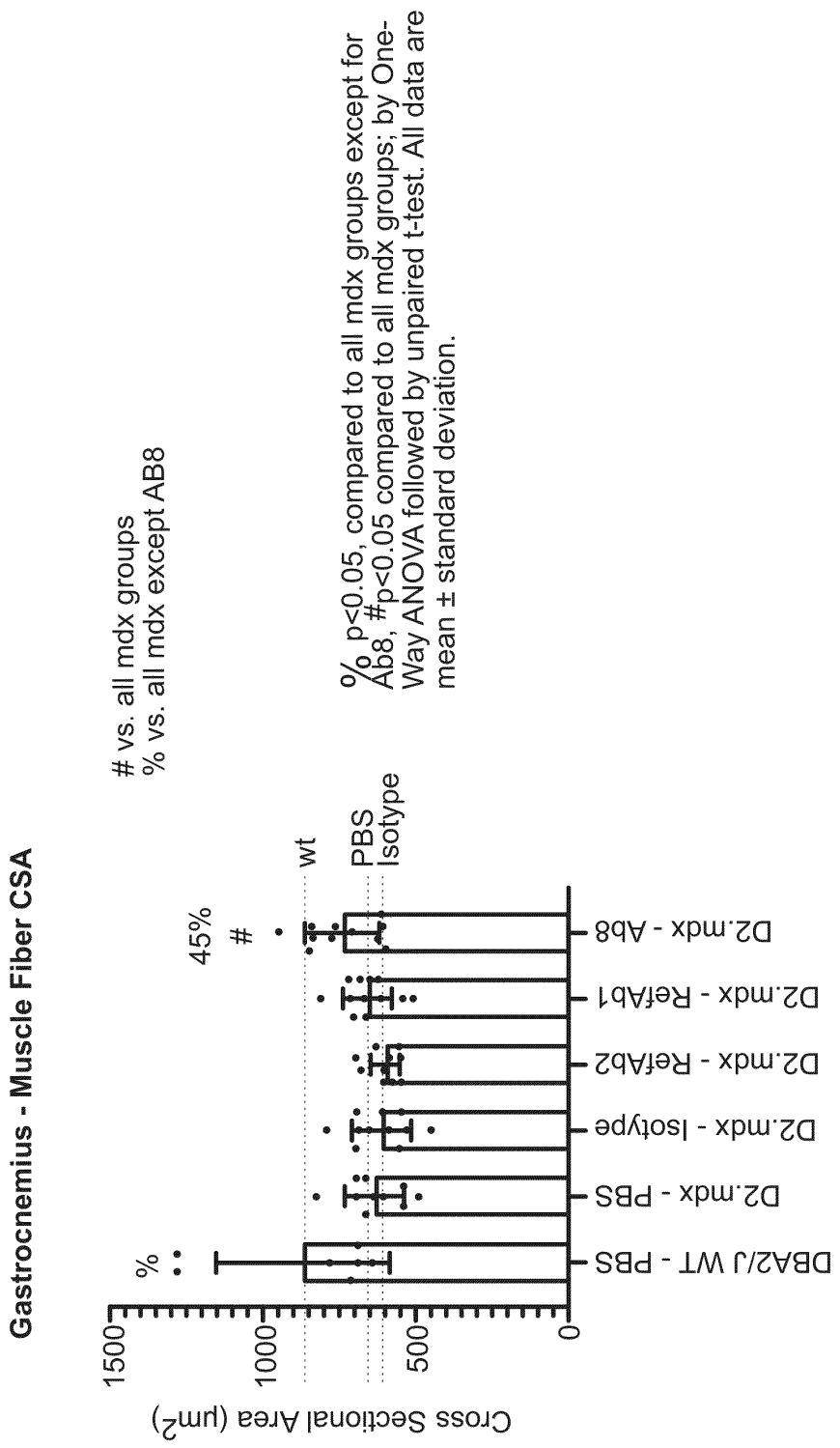

Gastrocnemius - Muscle Fiber CSA
vs. all mdx groups
% vs. all mdx except AB8
% p<0.05, compared to all mdx groups except for Ab8, #p<0.05 compared to all mdx groups; by One-Way ANOVA followed by unpaired t-test. All data are mean ± standard deviation.
Cross Sectional Area (um2)
1500
1000
500
0
45%
#
%
wt
PBS
Isotype
DBA2/J WT - PBS
D2.mdx - PBS
D2.mdx - Isotype
D2.mdx - RefAb2
D2.mdx - RefAb1
D2.mdx - Ab8

FIG. 21

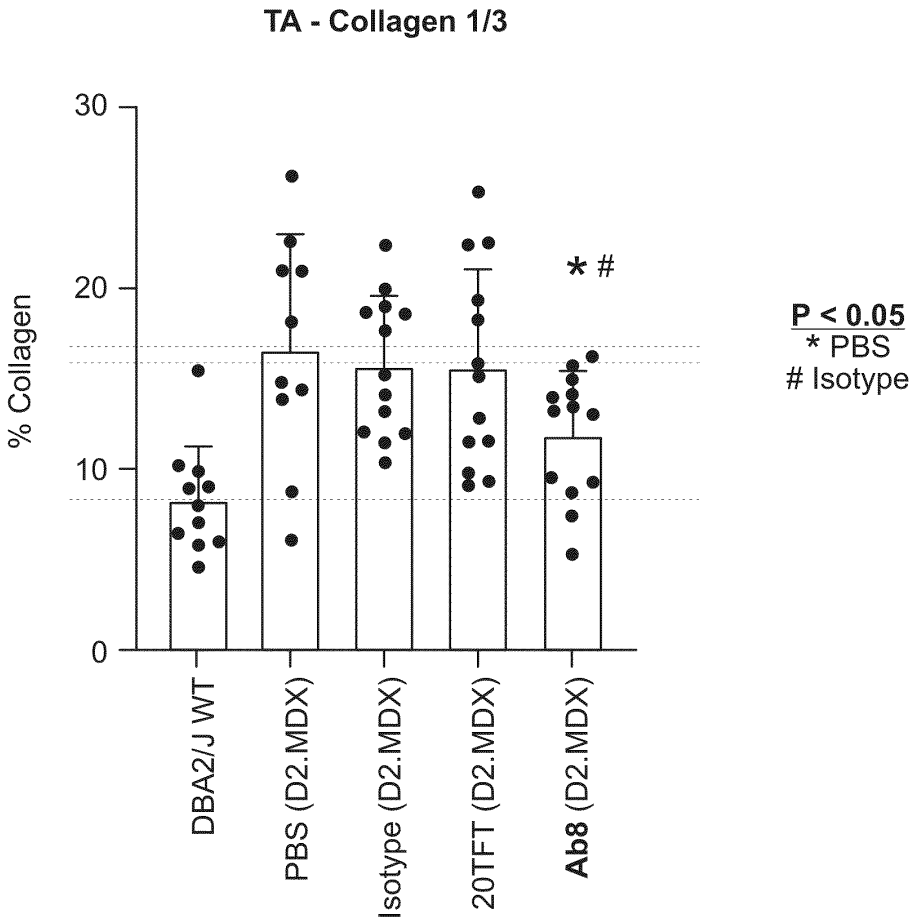

TA - Collagen 1/3
% Collagen
30
20
10
0
* #
DBA2/J WT
PBS (D2.MDX)
Isotype (D2.MDX)
20TFT (D2.MDX)
Ab8 (D2.MDX)
P < 0.05
* PBS
# Isotype

FIG. 22A

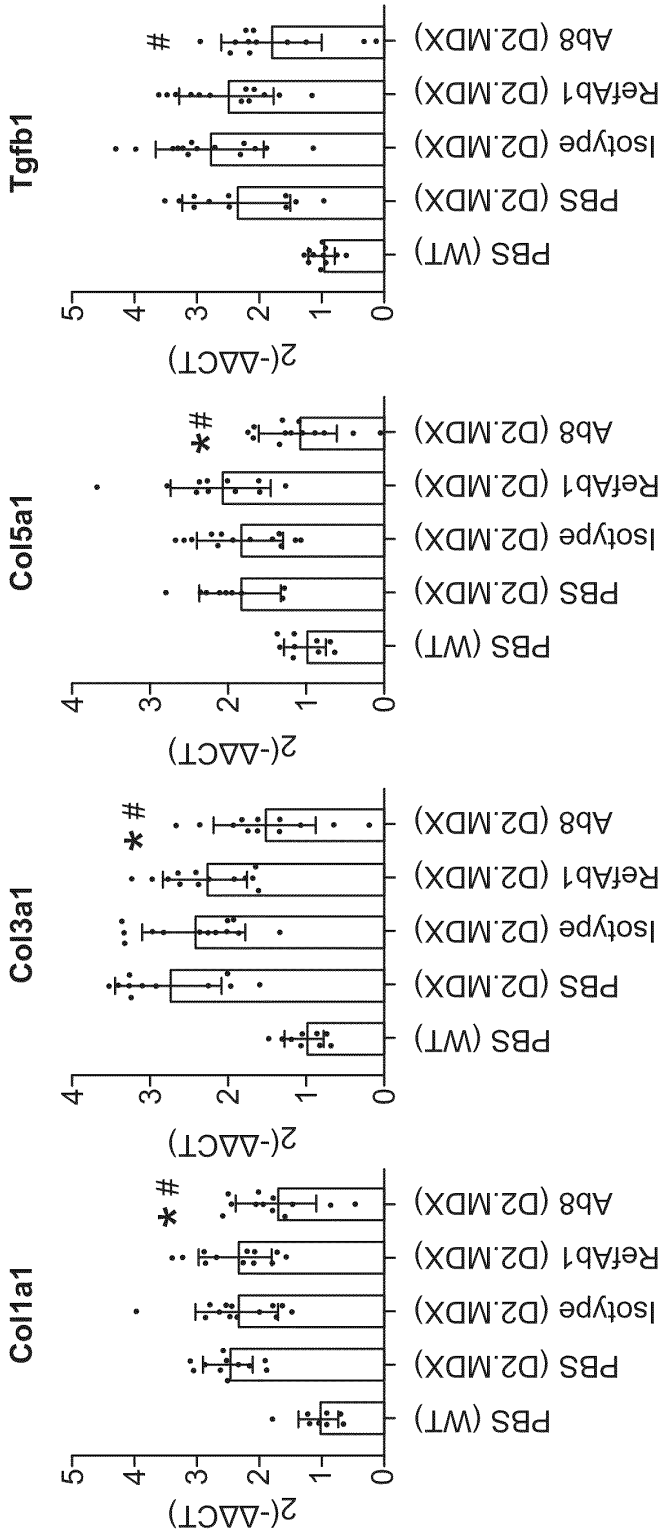


FIG. 22A (Continued)

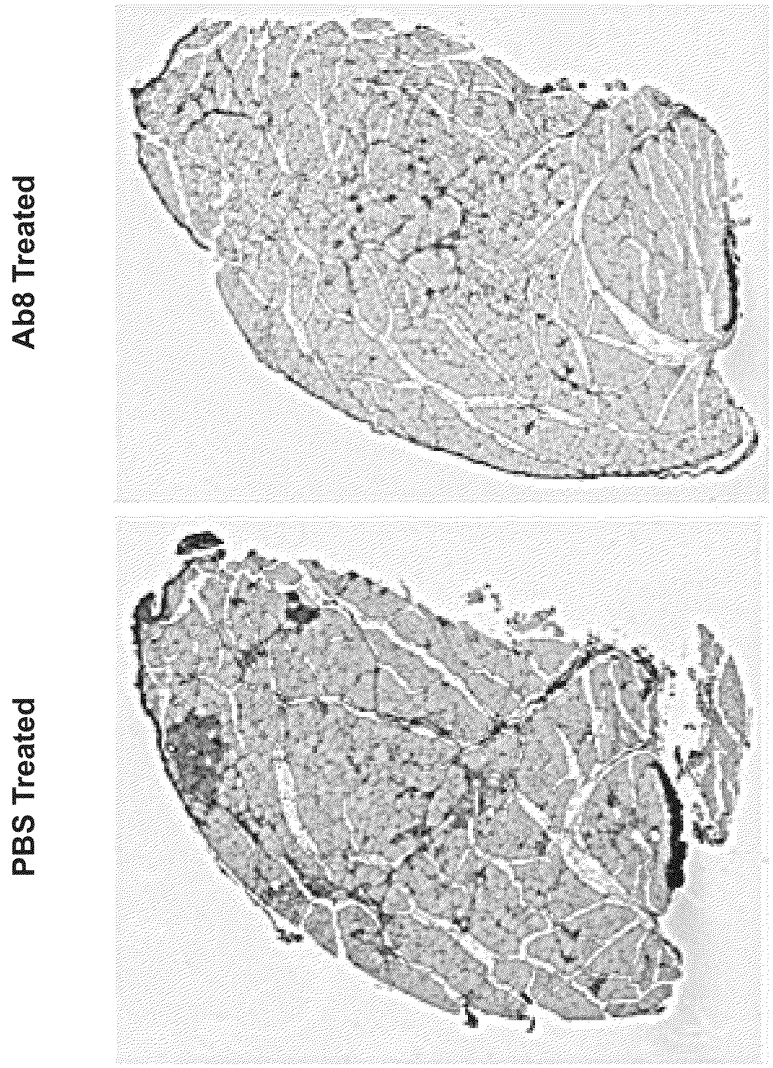


FIG. 22B

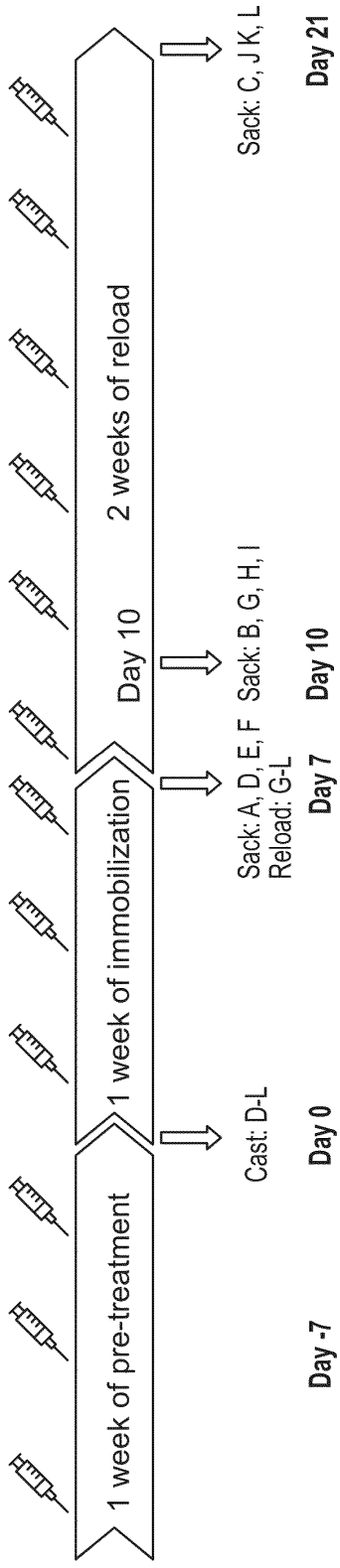

1 week of pre-treatment
1 week of immobilization
2 weeks of reload
Day 10
Cast: D-L
Day -7
Day 0
Sack: A, D, E, F
Reload: G-L
Day 7
Sack: B, G, H, I
Day 10
Sack: C, J K, L
Day 21

FIG. 23

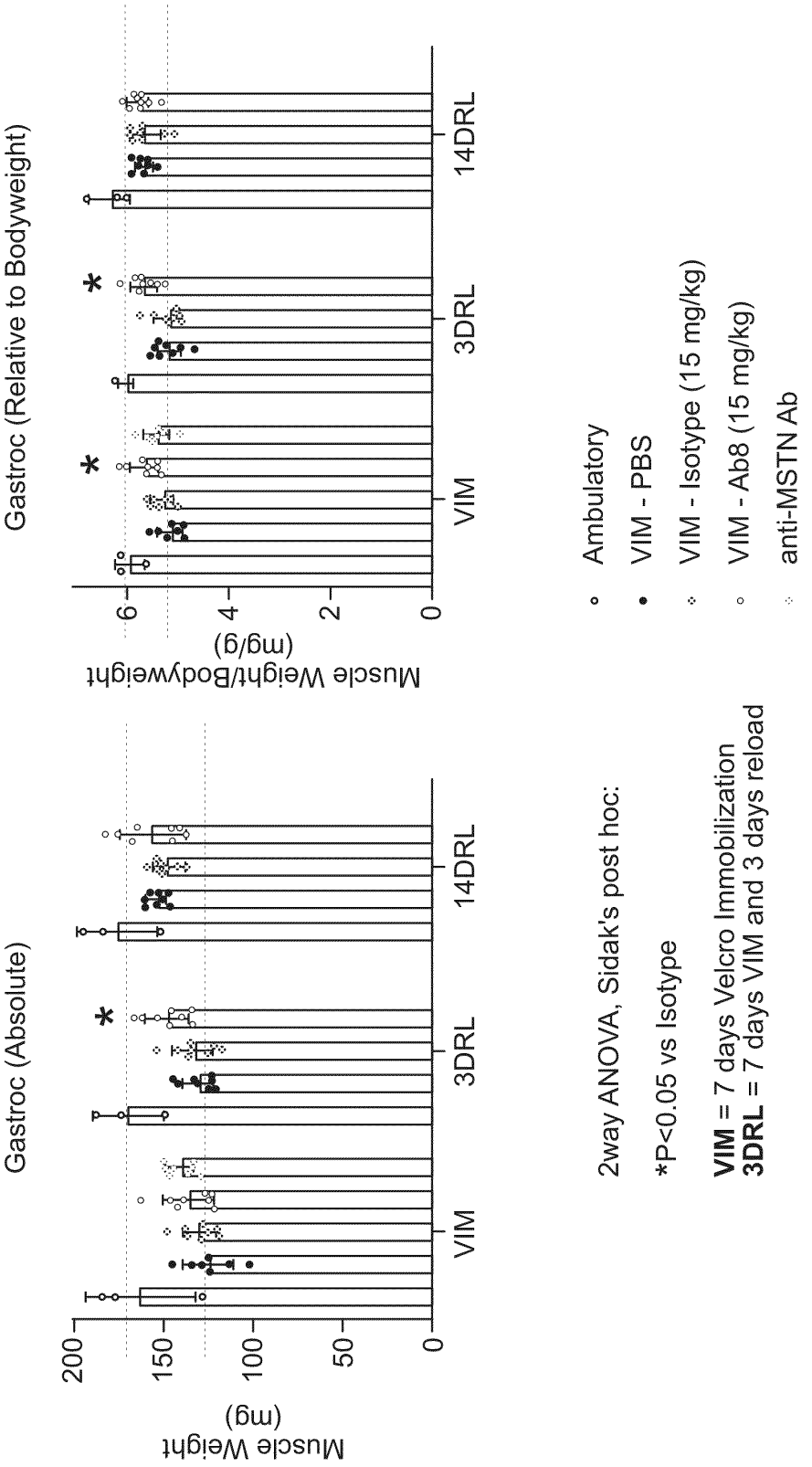

Gastroc (Absolute)
Muscle Weight (mg)
200
150
100
50
0
VIM
3DRL
14DRL
*
Gastroc (Relative to Bodyweight)
Muscle Weight/Bodyweight (mg/g)
6
4
2
0
VIM
3DRL
14DRL
*
*
Ambulatory
VIM - PBS
VIM - Isotype (15 mg/kg)
VIM - Ab8 (15 mg/kg)
anti-MSTN Ab
2way ANOVA, Sidak's post hoc:
*P<0.05 vs Isotype
VIM = 7 days Velcro Immobilization
3DRL = 7 days VIM and 3 days reload

FIG. 24

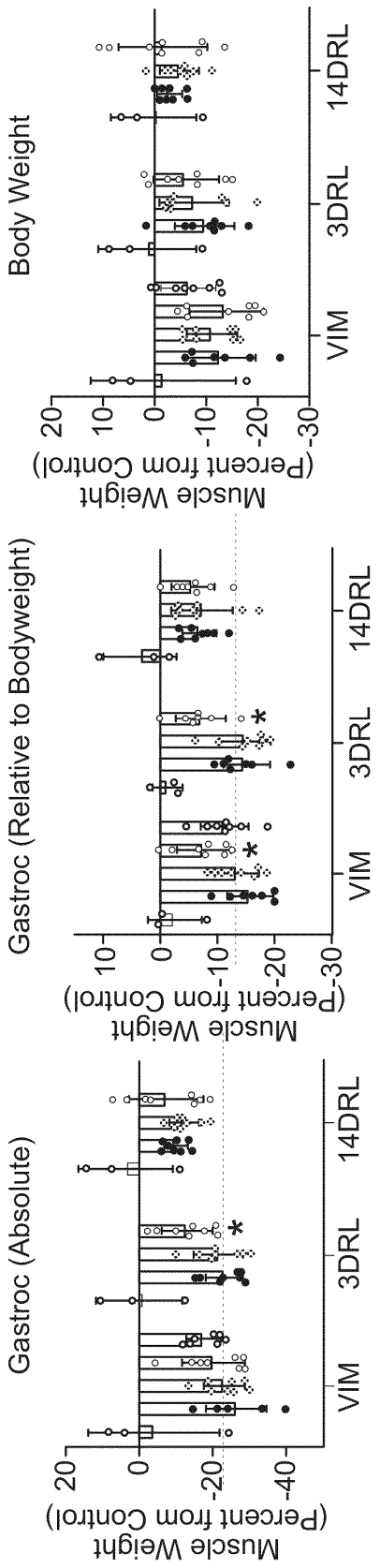


FIG. 24 (Continued)

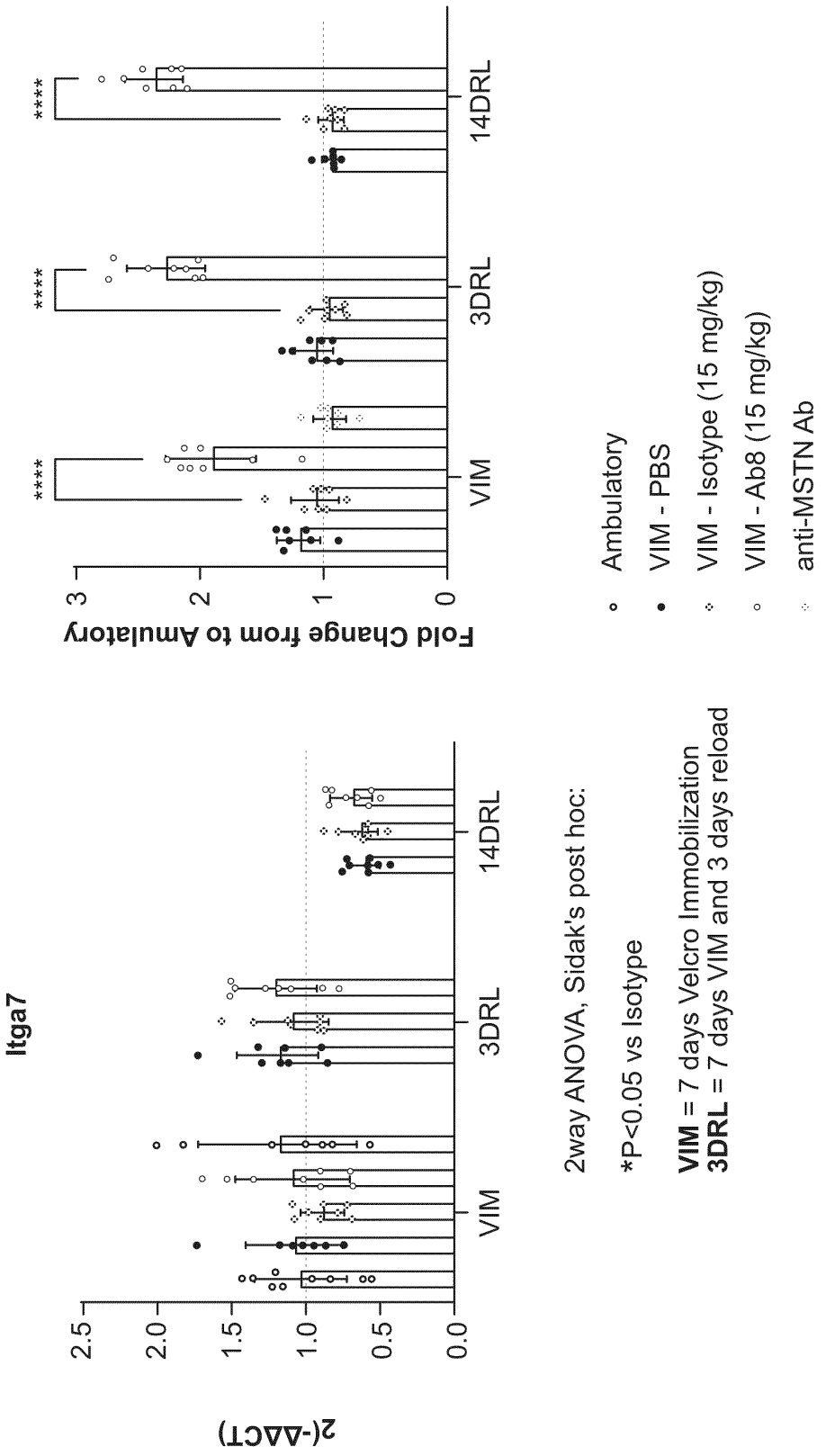

Fold Change from to Amulatory
****
****
****
VIM
3DRL
14DRL
0
1
2
3
Itga7
2(-ΔΔCT)
0.0
0.5
1.0
1.5
2.0
2.5
VIM
3DRL
14DRL
2way ANOVA, Sidak's post hoc:
*P<0.05 vs Isotype
VIM = 7 days Velcro Immobilization
3DRL = 7 days VIM and 3 days reload
Ambulatory
VIM - PBS
VIM - Isotype (15 mg/kg)
VIM - Ab8 (15 mg/kg)
anti-MSTN Ab

FIG. 25