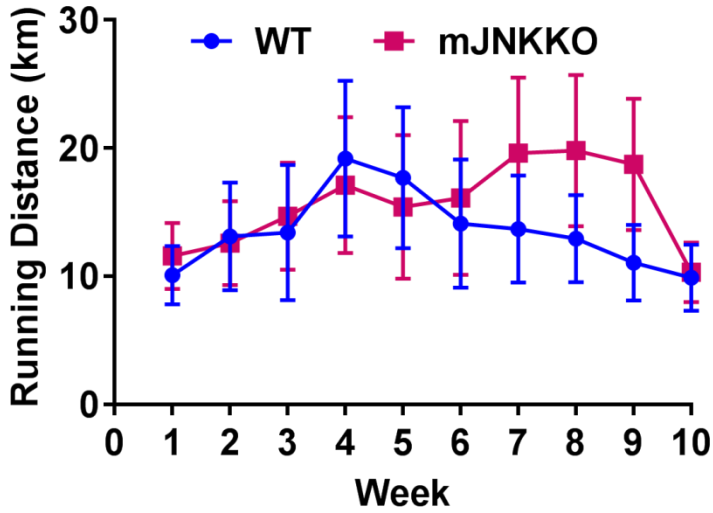


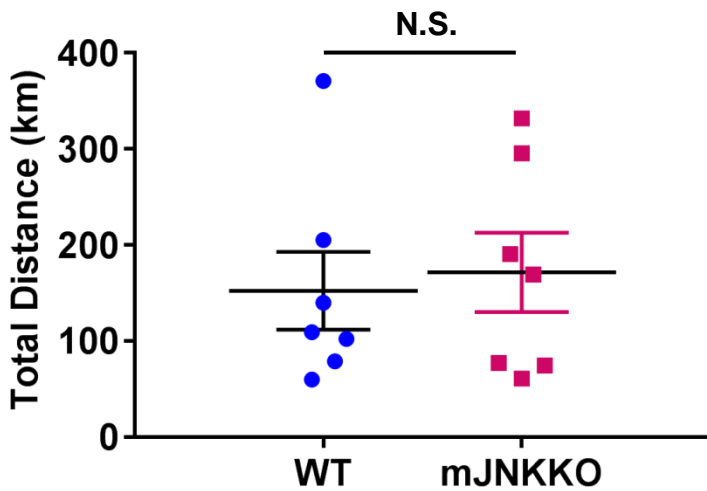
Supplementary Information File:

JNK regulates muscle remodeling via Myostatin/SMAD inhibition
Lessard et al., *Nature Communications*, 2018

A Voluntary Wheel Running

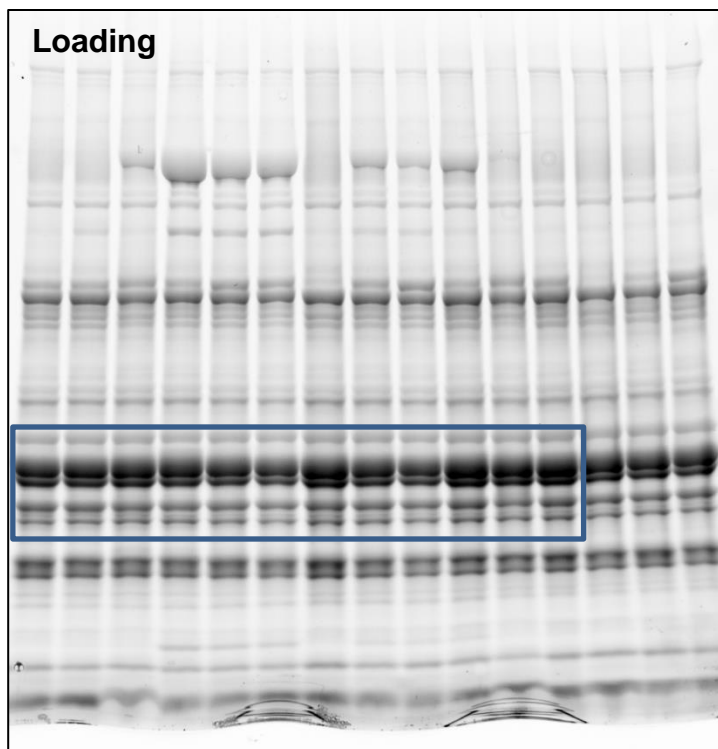
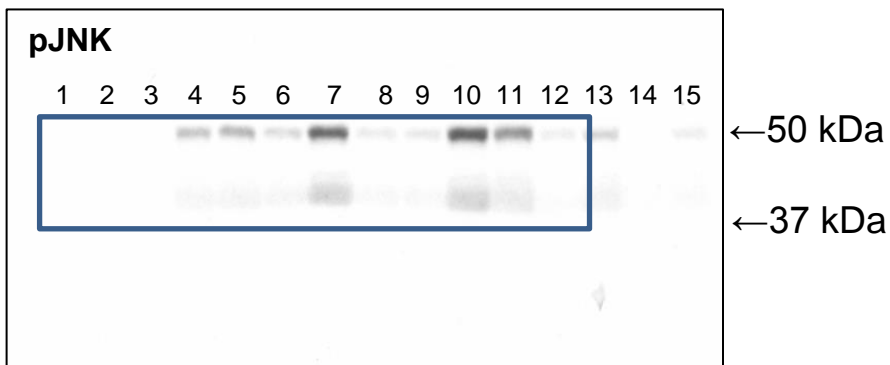
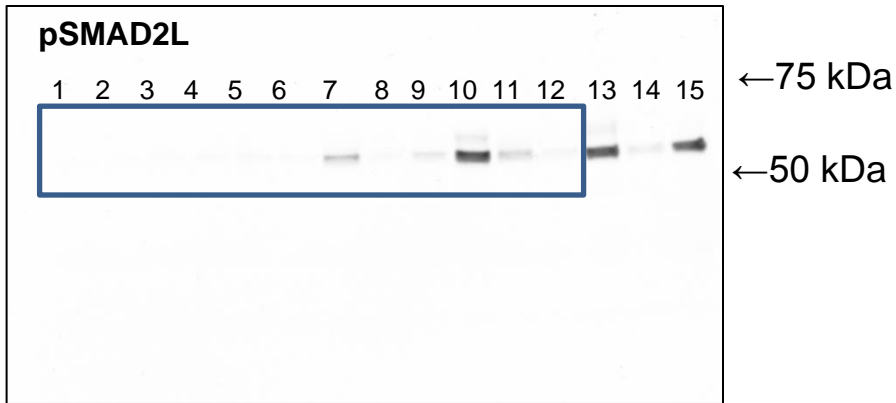


B Cumulative Running Distance



Supplementary Figure 1. Muscle-specific JNK1/2 knockout mJNKKO mice and wild-type (WT) were housed in cages with running wheels for 10 wks. A) Average weekly voluntary running distance, and B) Total running distance were calculated. N=7 animals/group. Data are presented as Mean +/- SEM. N.S., not statistically significant by unpaired T-Test.

Supplementary Figure 2
Uncropped Blots from Figure 3A



Mouse Treadmill Running

Lanes:

1-3: Rest

4-6: 15 min running

7-9: 30 min running

10-12: 60 min running

13-15: 60 min running + 30 min rest

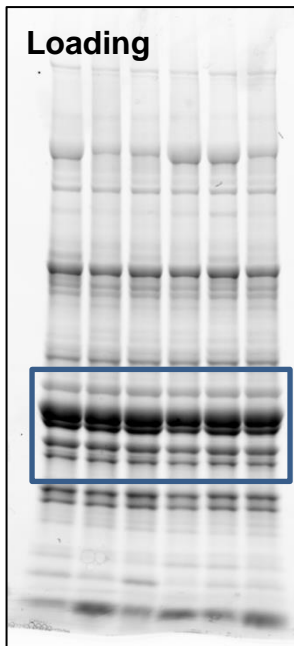
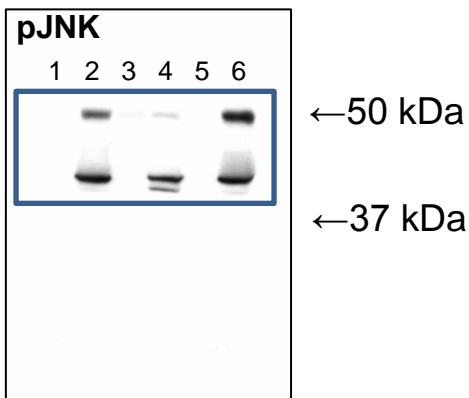
Uncropped Blots from Figure 3B



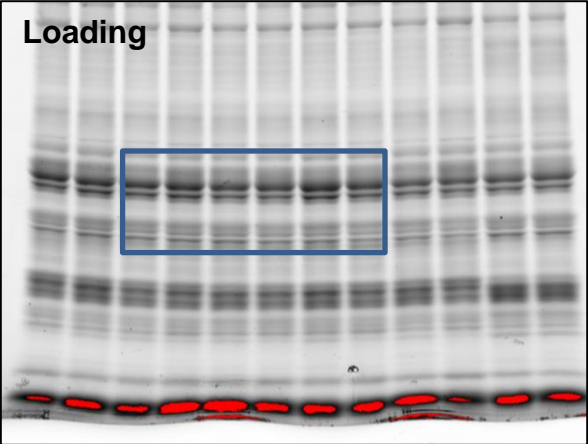
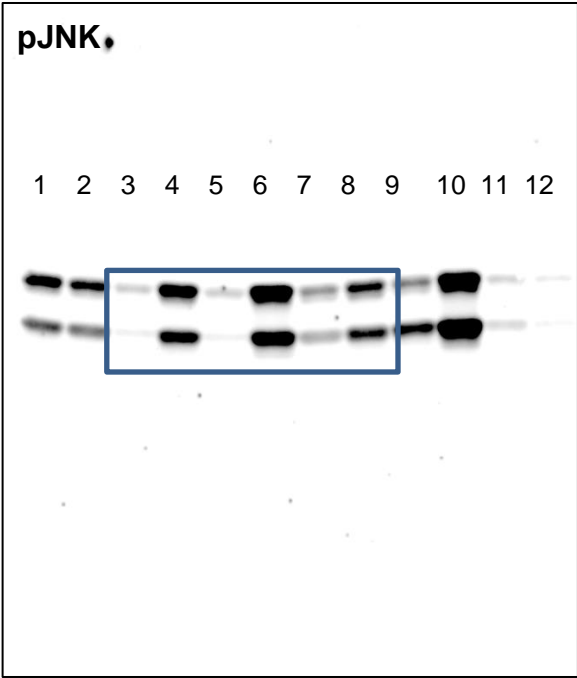
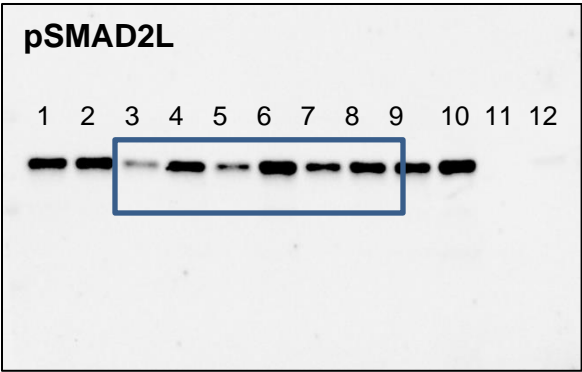
Mouse In Situ Contraction

Lane:

- 1: Mouse 1 Basal leg
- 2: Mouse 1 Contracted leg
- 3: Mouse 2 Basal leg
- 4: Mouse 2 Contracted leg
- 5: Mouse 3 Basal leg
- 6: Mouse 3 Contracted leg



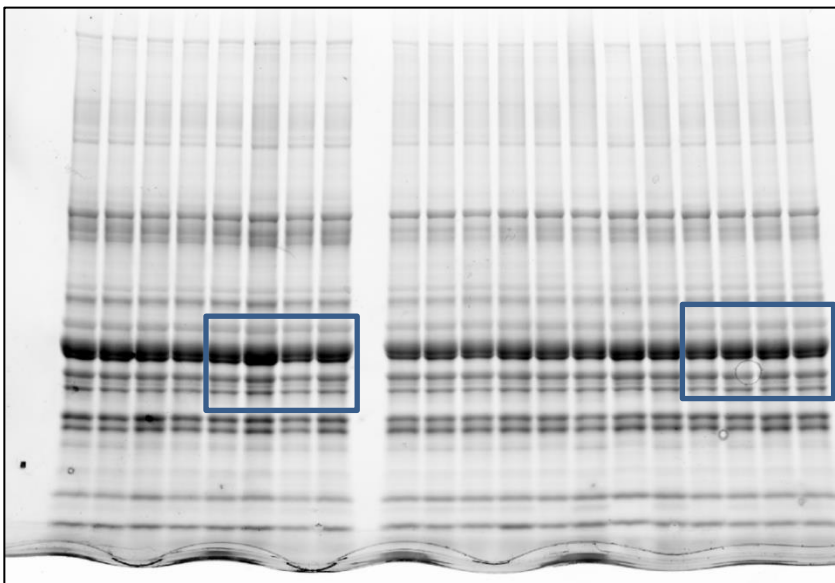
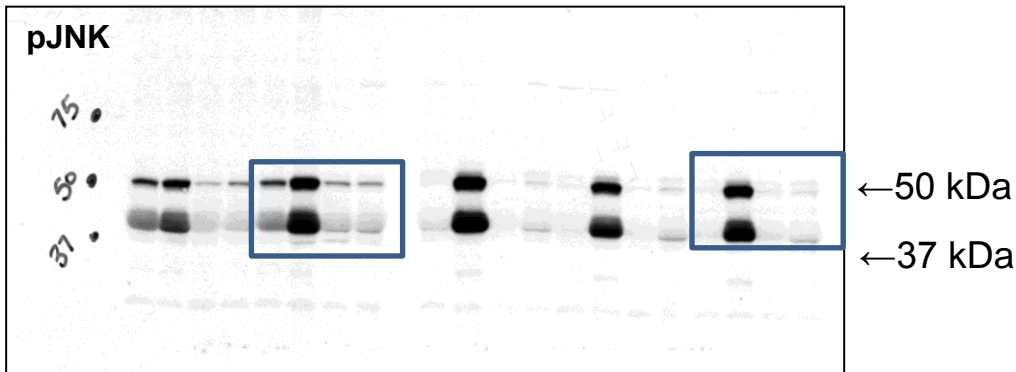
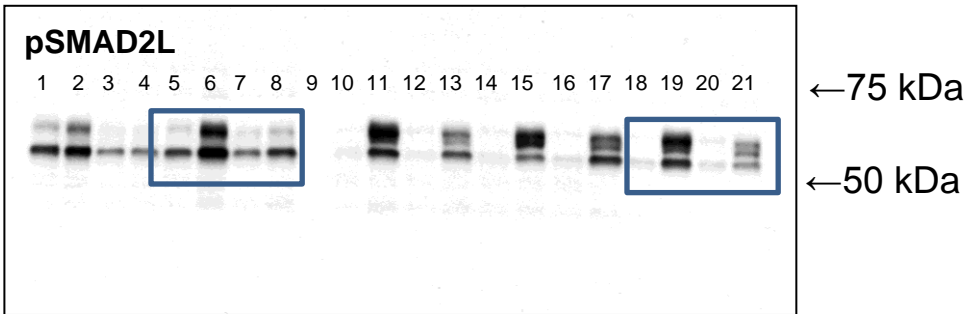
Uncropped Blots from Figure 3C



Mouse Stretch Experiments

- Lane:
- 1: Mouse 1 Basal Muscle
 - 2: Mouse 1 Stretched Muscle
 - 3: Mouse 2 Basal Muscle
 - 4: Mouse 2 Stretched Muscle
 - 5: Mouse 3 Basal Muscle
 - 6: Mouse 3 Stretched Muscle
 - 7: Mouse 4 Basal Muscle
 - 8: Mouse 4 Stretched Muscle
 - 9: Mouse 5 Basal Muscle
 - 10: Mouse 5 Stretched Muscle
 - 11: Mouse 6 Control Muscle
(flash frozen: Non-incubated)
 - 12: Mouse 6 Control Muscle
(flash frozen: Non-incubated)

Uncropped Blots from Figure 4A



Mouse In Vitro Contract Experiment

Lane:

- 1: WT 1 Basal Muscle
- 2: WT 1 Contracted Muscle
- 3: KO 2 Basal Muscle
- 4: KO 2 Contracted Muscle
- 5: WT 3 Basal Muscle
- 6: WT 3 Contracted Muscle
- 7: KO 4 Basal Muscle
- 8: KO 4 Contracted Muscle
- 9: Empty Lane

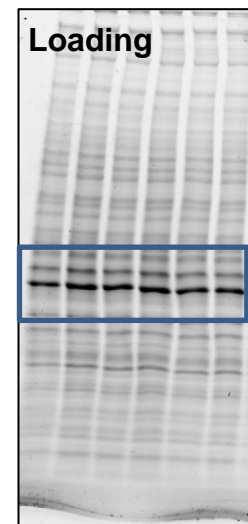
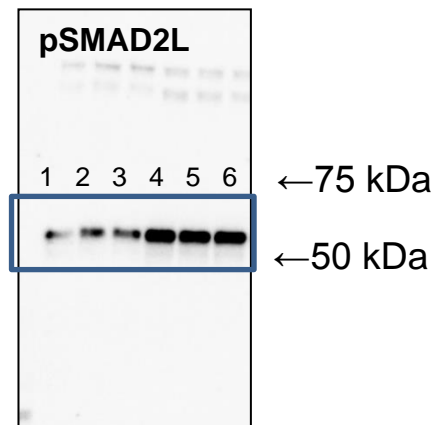
Mouse In Situ Contract Experiment

Lanes:

- 10: WT 5 Basal Muscle
- 11: WT 5 Contracted Muscle
- 12: KO 6 Basal Muscle
- 13: KO 6 Contracted Muscle
- 14: WT 7 Basal Muscle
- 15: WT 7 Contracted Muscle
- 16: KO 8 Basal Muscle
- 17: KO 8 Contracted Muscle
- 18: WT 9 Basal Muscle
- 19: WT 9 Contracted Muscle
- 20: KO 10 Basal Muscle
- 21: KO 10 Contracted Muscle

WT: Wild-type Control Mouse
KO: mJNKKO Mouse

Uncropped Blots:
Figure 4D



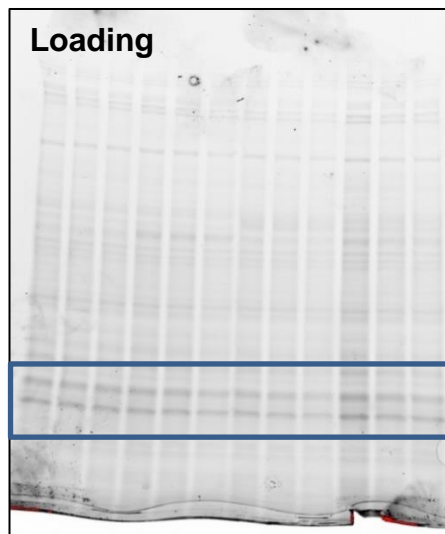
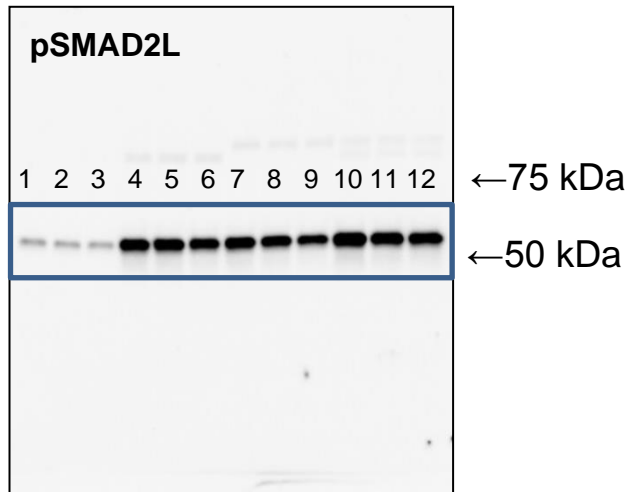
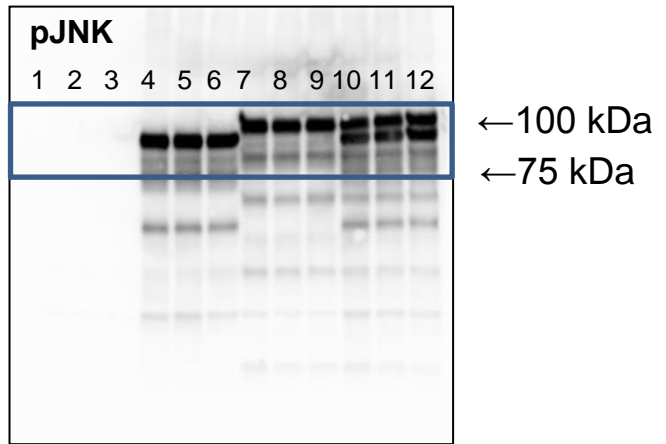
C2C12 Myoblasts

Lanes:

1-3: Vehicle

4-6: 5 μ M Anisomycin

Uncropped Blots:
Figure 4E



C2C12 Myoblasts

Lanes:

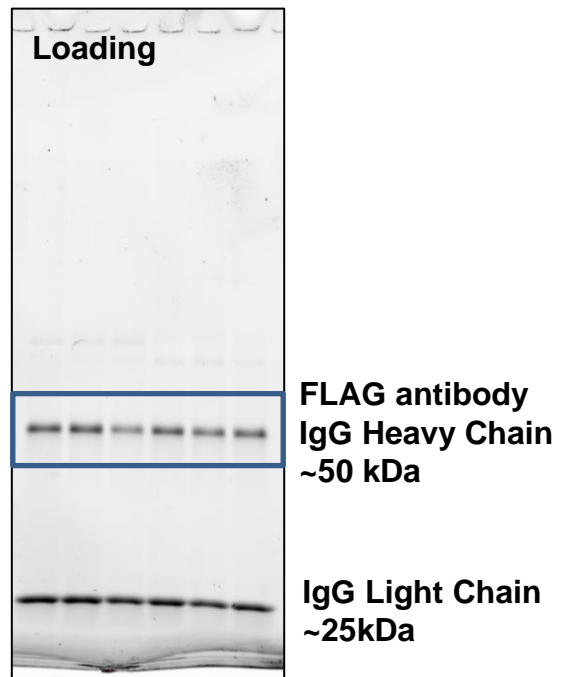
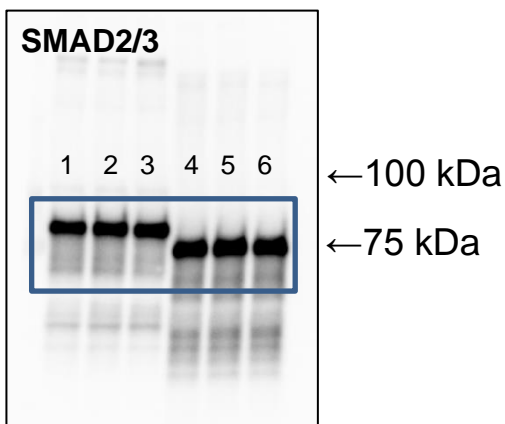
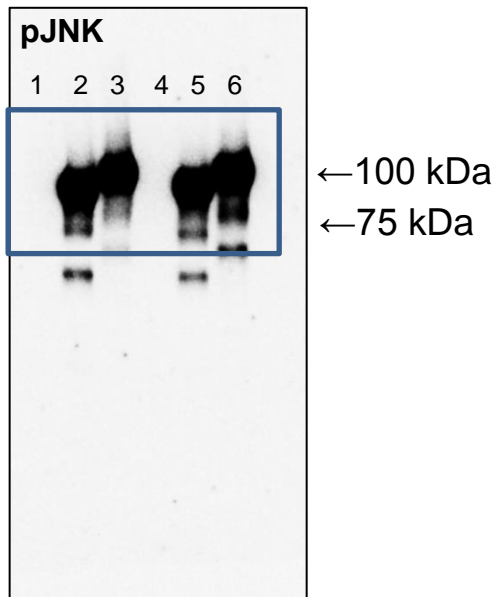
1-3: Empty Vector

4-6: Active JNK1 overexpression

7-9: Active JNK 2 overexpression

10-12: Active JNK1 and JNK2 overexpression

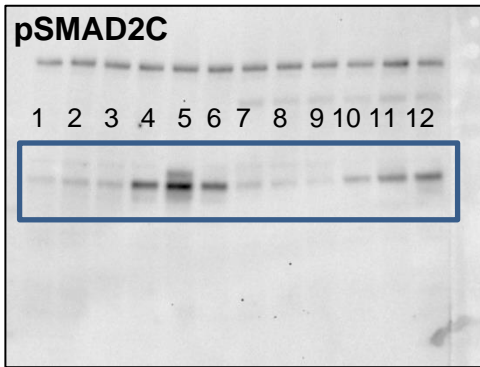
Uncropped Blots from Figure 4F



In vitro JNK activity assay experiment Lane

- 1: Empty Vector + Recombinant SMAD2
- 2: Active JNK1 + Recombinant SMAD2
- 3: Active JNK2 + Recombinant SMAD2
- 4: Empty Vector + Recombinant SMAD3
- 5: Active JNK1 + Recombinant SMAD3
- 6: Active JNK2 + Recombinant SMAD3

Uncropped Blots from Figure 5B



Active JNK + Myostatin Experiment Whole-Cell Lysates

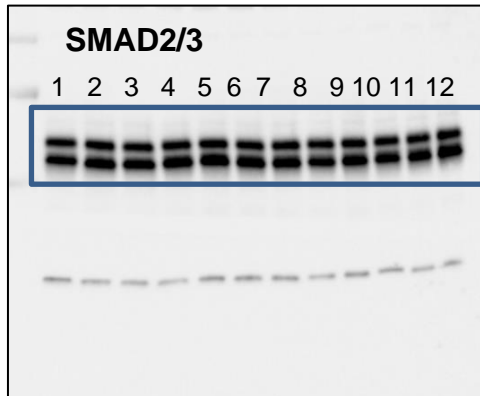
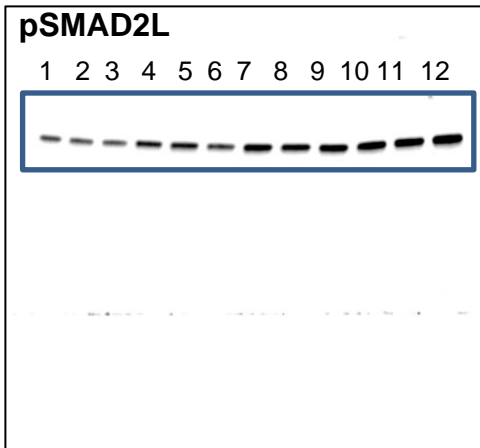
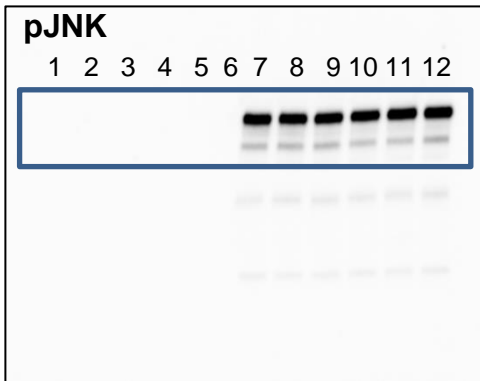
Lane

1-3: EV + Vehicle Control

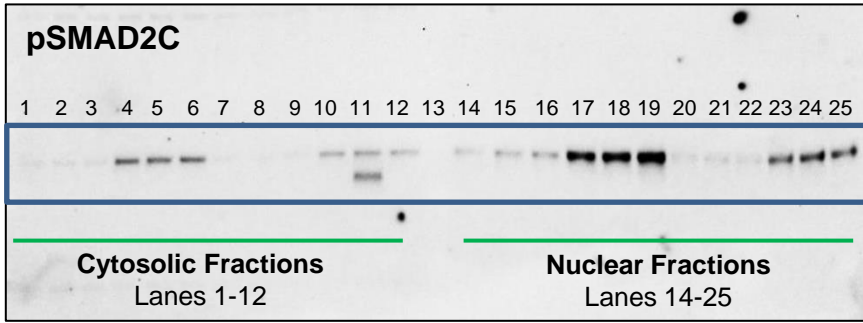
4-6: EV + 20 nM Myostatin

7-9: Active JNK + Vehicle Control

10-12: Active JNK + 20 nM Myostatin



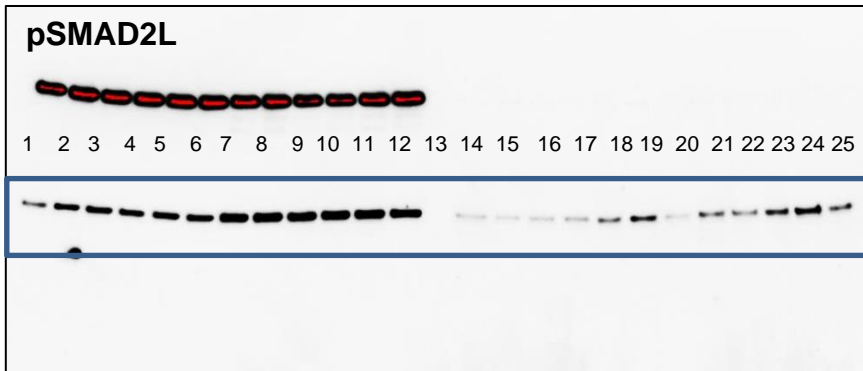
Uncropped Blots from Figure 5C



←75 kDa

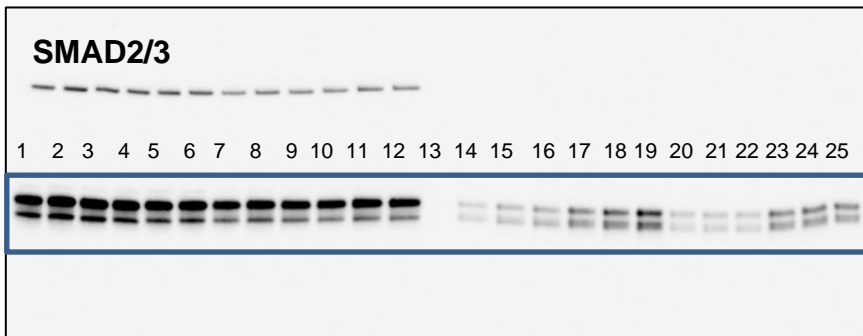
←50 kDa

*See next page for detailed lane identification



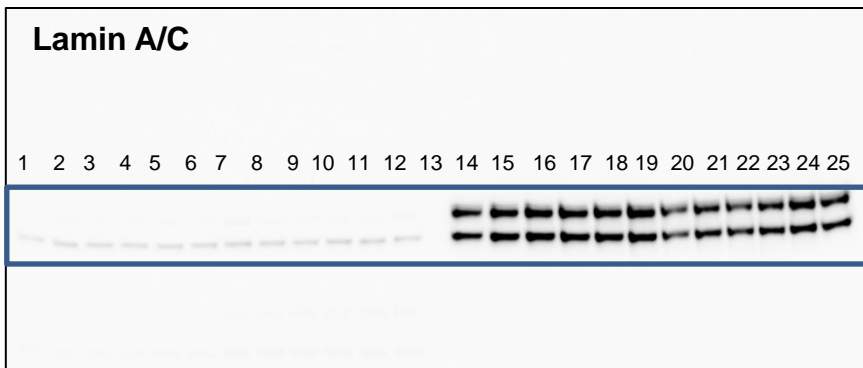
←75 kDa

←50 kDa



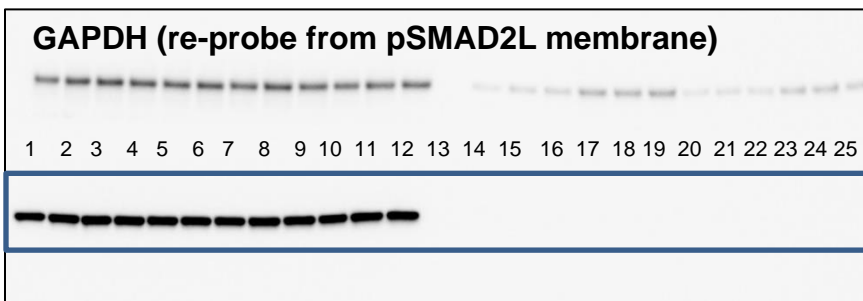
←75 kDa

←50 kDa



←75 kDa

←50 kDa



←pSMAD2L band

←37 kDa
GAPDH band

**Uncropped Blots from Figure 5C
(continued)**

**Active JNK + Myostatin Experiment in C2C12
Cytosolic Fractions**

Lane

1-3: EV + Vehicle Control

4-6: EV + 20 nM Myostatin

7-9: Active JNK + Vehicle Control

10-12: Active JNK + 20 nM Myostatin

13: Empty Lane

Nuclear Fractions

Lane

14-16: EV + Vehicle Control

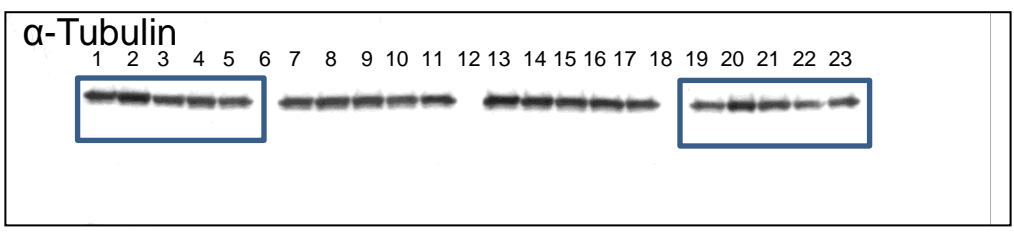
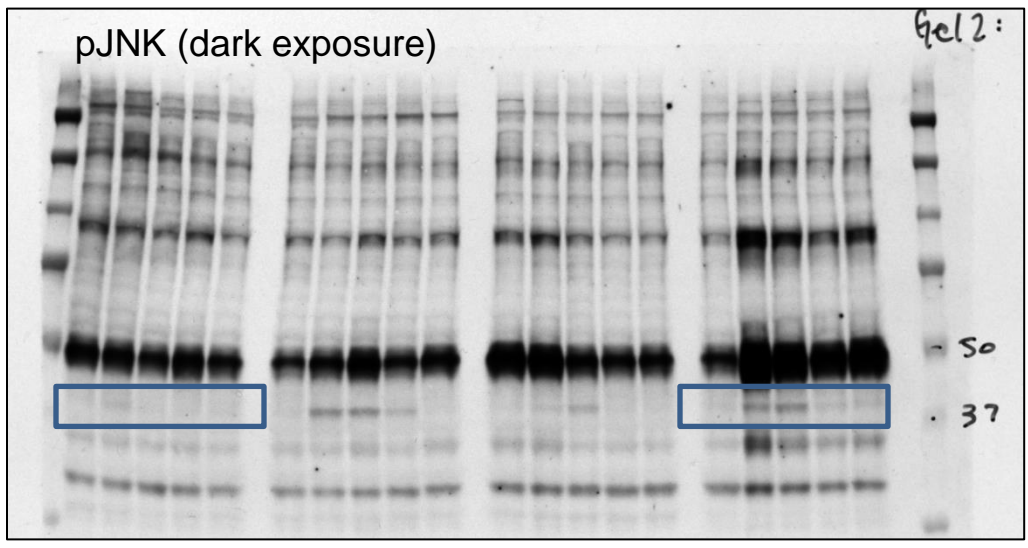
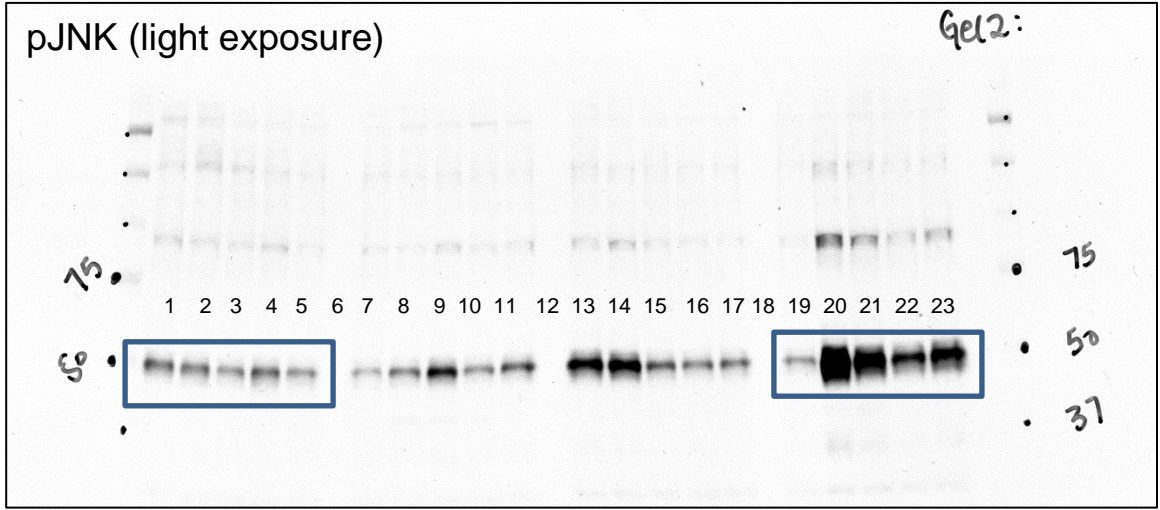
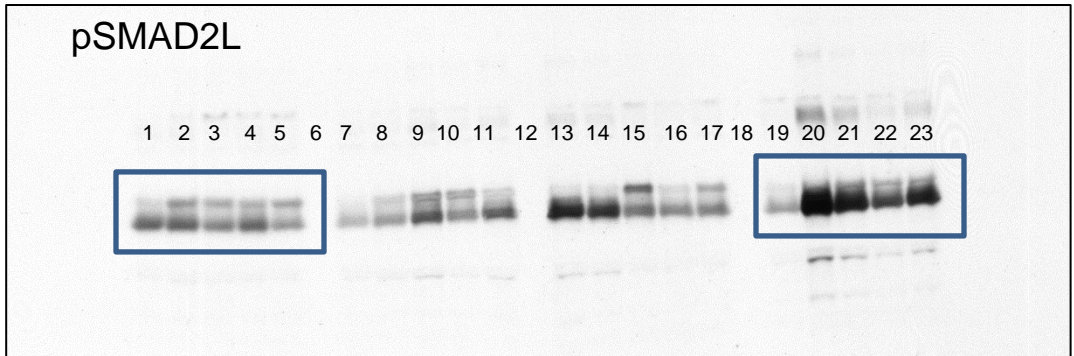
17-19: EV + 20 nM Myostatin

20-22: Active JNK + Vehicle Control

23-25: Active JNK + 20 nM Myostatin

Uncropped Blots from Figure 7A

*See next page for detailed lane identification



**Uncropped Blots from Figure 7A
(continued)**

Human Endurance and Resistance Exercise Time-Course Samples

Lane:

- 1: Subject C3 Rest
- 2: Subject C3 0 min Post-Exercise
- 3: Subject C3 15 min Post-Exercise
- 4: Subject C3 30 min Post-Exercise
- 5: Subject C3 60 min Post-Exercise
- 6: Empty Lane
- 7: Subject R3 Rest
- 8: Subject R3 0 min Post-Exercise
- 9: Subject R3 15 min Post-Exercise
- 10: Subject R3 30 min Post-Exercise
- 11: Subject R3 60 min Post-Exercise
- 12: Empty Lane
- 13: Subject C4 Rest
- 14: Subject C4 15 min Post-Exercise
- 15: Subject C4 0 min Post-Exercise
- 16: Subject C4 30 min Post-Exercise
- 17: Subject C4 60 min Post Exercise
- 18: Empty Lane
- 19: Subject R4 Rest
- 20: Subject R4 0 min Post-Exercise
- 21: Subject R4 15 min Post-Exercise
- 22: Subject R4 30 min Post-Exercise
- 23: Subject R4 60 min Post-Exercise