Supplemental Material

Matrix Metalloproteinase 13 from Satellite Cells is Required for Efficient Muscle Growth and Regeneration

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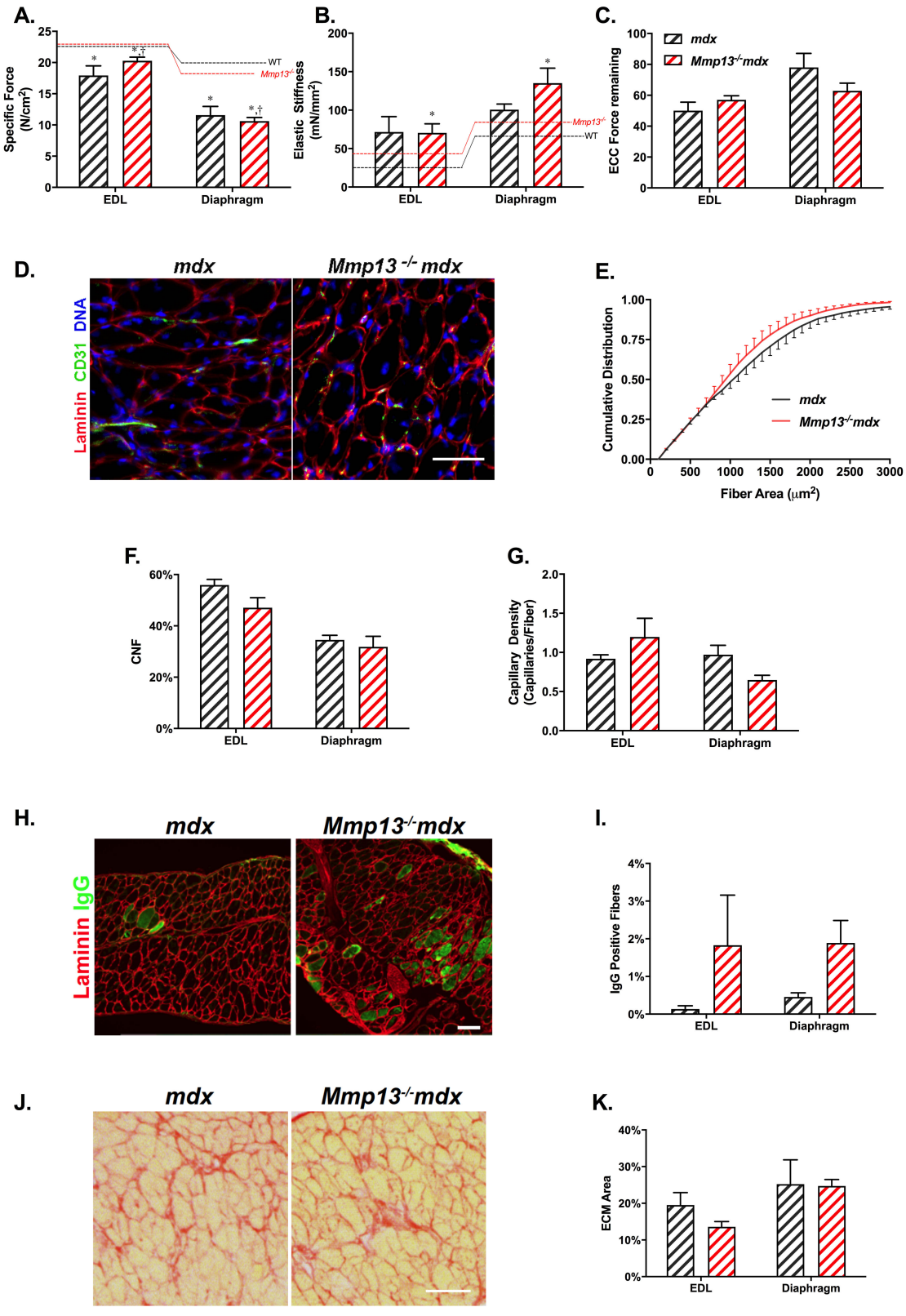
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Table 1. Oligonucleotides Used for genotyping and gene expression.

Gene	Primers (5' – 3')		
	Sense	**Antisense**	**Common**
18s	CTCTGTTCCGCCTAGTCCTG	AATGAGCCATTCGCAGTTTC	
Mmp2	ACCCTGGGAGAAGGACAAGT	ATCACTGCGACCAGTGTCTG	
Mmp13$^{-/-}$	GCCCATGAGCTTGGCCACTCC	GTTTTCTACCCAGACAAGCAG	
	CGCTGCCCCAAAGGCCTACC	GCATGAAATGGCTTTTGCCAGTG	
Mmp13 $^{fl/fl}$ JAX005710	TGATGACGTTCAAGGAATTCAGTTT	GGTGGTATGAACAAGTTTTCTGAGC	CCACACTGCTCGACATTG
Pax7 Cre JAX012476	TACCAGAGGCAACAAACAGG	TTGATGAAGACCCCACCAAG	CAAAGGTGGCTAAGGTGGAG
ROSA$^{mT/mG}$ JAX007676	AAGGGAGCTGCAGTGGAGTA	CCGAAAATCTGTGGGAAGTC	CGGGCCATTTACCGTAAGTTAT

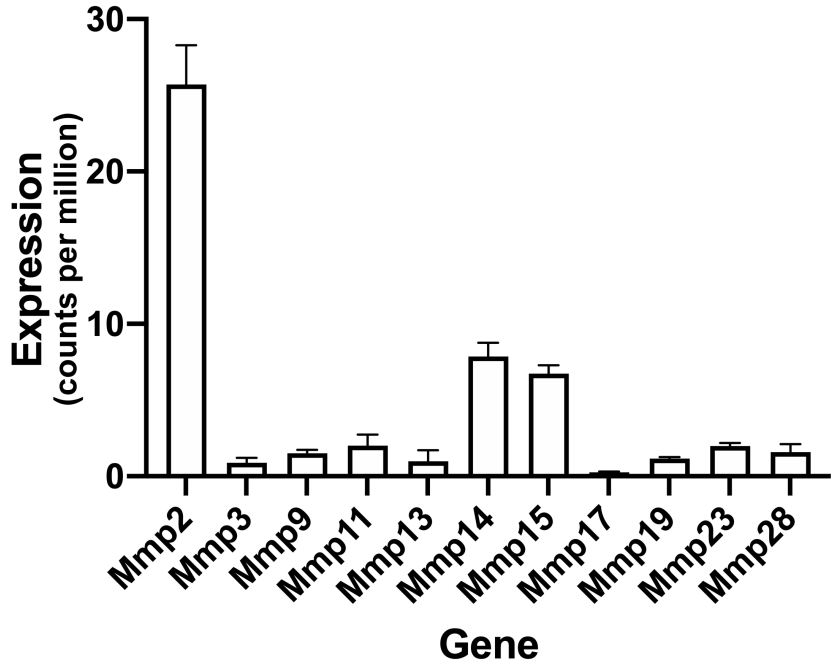
Supplemental Figure 1: *Ablation of Mmp13 does not exacerbate the phenotype of mdx muscles*

(A) The normalized force producing capacity of *Mmp13$^{-/-}$:mdx* (n=5) mice is not compromised further than that of the *mdx* (n=3) EDL and diaphragm muscles in young adult mice. Muscles from both strains are significantly weaker than muscles from WT mice and/or *Mmp13$^{-/-}$* mice. Similarly, the **(B)** elastic stiffness and the **(C)** damaged induced by eccentric contractions (ECC) are not changed in the *Mmp13$^{-/-}$:mdx* mouse compared to *mdx*. Muscles from *Mmp13$^{-/-}$:mdx* mice have greater elastic stiffness than those from WT mice. **(D)** Examples of *mdx* and *Mmp13$^{-/-}$:mdx* with staining of laminin, CD31, and DNA on diaphragm muscles. Scale bar 50 μm. **(E)** The cumulative distributions of fiber areas from *mdx* and *Mmp13$^{-/-}$:mdx* diaphragm muscles is unchanged. There are no differences between genotypes for either **(F)** CNF fraction or **(G)** capillary density. **(H)** Examples of an *mdx* and *Mmp13$^{-/-}$:mdx* diaphragm muscles with staining of laminin, IgG to label damaged or necrotic fibers, and DNA. Scale bar 50 μm. **(I)** There is a significant main effect of increased IgG+ damaged/necrotic fibers in the *Mmp13$^{-/-}$:mdx* compared to mdx across EDL and diaphragm muscles. **(J)** Sirius red staining of diaphragm muscles shows areas occupied by ECM from *mdx* and *Mmp13$^{-/-}$:mdx* muscles. Scale bar 50 μm. **(K)** The area fraction of ECM was similar for *mdx* and *Mmp13$^{-/-}$:mdx* EDL and diaphragm. Dotted lines in A and B represent means for outcome measures in WT and *Mmp13$^{-/-}$* muscles. *, $p<0.05$ for differences vs. WT; and †, $P<0.05$ for differences vs. *Mmp13$^{-/-}$*.


A.
Specific Force (N/cm²)
25
20
15
10
5
0
WT
Mmp13-/-
EDL
Diaphragm
B.
Elastic Stiffness (mN/mm²)
200
150
100
50
0
Mmp13-/-
WT
EDL
Diaphragm
C.
ECC Force remaining
100
80
60
40
20
0
mdx
Mmp13-/-mdx
EDL
Diaphragm
D.
mdx
Mmp13 -/- mdx
Laminin
CD31
DNA
E.
Cumulative Distribution
1.00
0.75
0.50
0.25
0.00
0
500
1000
1500
2000
2500
3000
mdx
Mmp13-/-mdx
Fiber Area (μm²)
F.
CNF
60%
40%
20%
0%
EDL
Diaphragm
G.
Capillary Density (Capillaries/Fiber)
2.0
1.5
1.0
0.5
0.0
EDL
Diaphragm
H.
mdx
Mmp13-/-mdx
Laminin
IgG
I.
IgG Positive Fibers
4%
3%
2%
1%
0%
EDL
Diaphragm
J.
mdx
Mmp13-/-mdx
K.
ECM Area
40%
30%
20%
10%
0%
EDL
Diaphragm

Supplemental Figure 2: *Comparison of Mmp gene expression.*

Expression levels of Mmp gene family members in control skeletal muscle. Global transcriptional analysis (RNASeq) was performed on RNA extracted from tibialis anterior (TA) samples from 16-week old male C57 mice (N=3). Analysis was completed by University of Florida Interdisciplinary Center for Biotechnology Research. Mean ± SD of counts per million are shown.

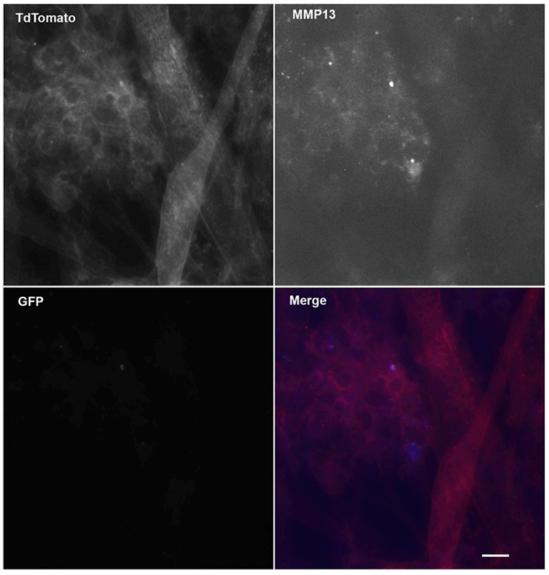


Supplemental Figure 3: *Evaluation of MMP-13 in primary cultures following tamoxifen treatment.*

Bulk primary cultures were generated from freshly dissected TA and gastrocnemius muscles as described in methods. Cells were cultures in 4-chamber slides fixed with 4% paraformaldehyde at the desired timepoint, and then subjected to immunocytochemistry to detect MMP-13 (1:100 mouse monoclonal anti-MMP13 (VIIIA2), IM78, Sigma) and secondary Antibody (1:200 Alexa Fluor 350 goat anti-mouse IgG, ThermoFisher). Visualization of the Alexa Fluor 350, and the Rosa reporter signals for Cre recombinase activity (TdTomato, negative for Cre; GFP, positive for Cre) were visualized by epifluorescence microscopy (Leica DMR). (A) Bulk primary cultures obtained from muscles of -sc*Mmp13*$^{fl/fl}$ mice (no TAM treatment) and differentiated for 72 hours show MMP-13 signal in contaminating fibroblasts and faintly in myotubes. (B) Contaminating fibroblasts in bulk primary cultures obtained from muscles of -sc*Mmp13*$^{fl/fl}$ mice after 24 hours in differentiation medium show strong MMP-13 signal. (C) and (D) Bulk primary cultures obtained from muscles of +sc*Mmp13*$^{fl/fl}$ mice (with TAM treatment) and differentiated for 72 hours show MMP-13 signal in contaminating fibroblasts which are red indicative of no Cre activity, and GFP positive myotubes indicative of Cre activity with no MMP-13 signal. Scale bar 10μm.

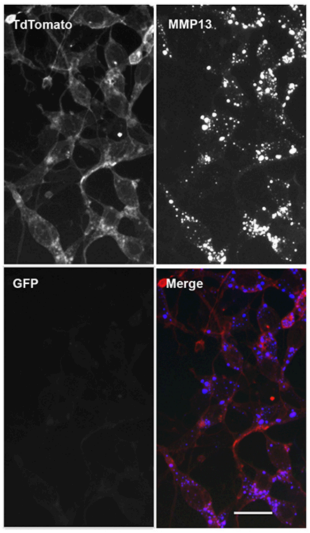
A.

Bulk Primary Cultures Differentiated 72 hours
No TAM control



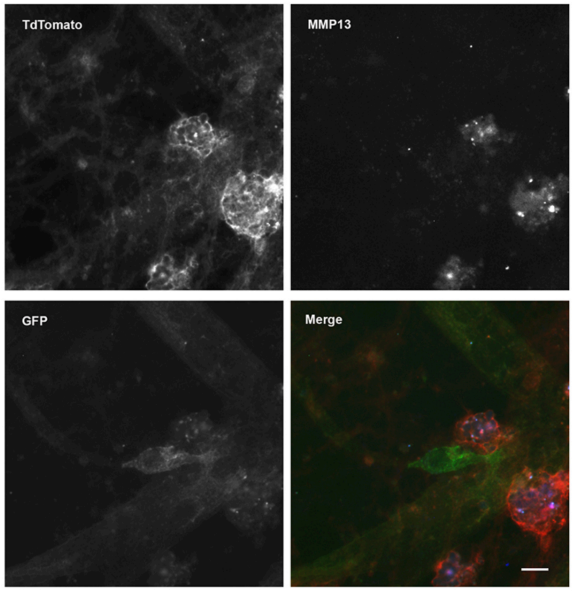
B.

Bulk Primary Culture Differentiated 24 Hours
No TAM Control fibroblasts



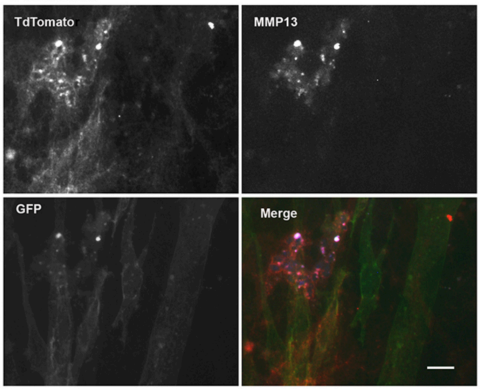
C.

Bulk Primary Culture Differentiated 72 hours
TAM treated



D.

Bulk Primary Culture Differentiated 72 hours
TAM treated



Supplemental Videos. Representative movies for live cell imaging data shown in Figures 4 and 5.

To see the videos, please use the following links.

Figure 4:

Overlay_BF-wt-20.avi, WT cultures in 20% serum.
https://www.cellphysiolbiochem.com/Articles/000223/SM/Overlay_BF-wt-20.avi

Overlay_BF-wt-2.avi, WT cultures in 2% serum.
https://www.cellphysiolbiochem.com/Articles/000223/SM/Overlay_BF-wt-2.avi

Overlay_BF-ko-20.avi, *Mmp13*$^{-/-}$ cultures in 20% serum.
https://www.cellphysiolbiochem.com/Articles/000223/SM/Overlay_BF-ko-20.avi

Overlay_BF-ko-2.avi, *Mmp13*$^{-/-}$ cultures in 2% serum.
https://www.cellphysiolbiochem.com/Articles/000223/SM/Overlay_BF-ko-2.avi

Figure 5:

Overlay_RG-Control.avi, *-scMmp13*$^{fl/fl}$ cultures in 2% serum, where cells express TdTomato.
https://www.cellphysiolbiochem.com/Articles/000223/SM/Overlay_RG-Control.avi

Overlay_RG-ko.avi, *+scMmp13*$^{fl/fl}$ cultures in 2% serum, where cells express GFP following TAM induced Cre expression.
https://www.cellphysiolbiochem.com/Articles/000223/SM/Overlay_RG-ko.avi