Supplemental Materials

Molecular Biology of the Cell

Gokhin et al.

Supplementary Figure 1. Quantitative western blot determination of amounts of Tmod1, Tmod4, and actin in myofibrils. (A-B) Western blots of increasing amounts of purified (A) Tmod1 and (B) Tmod4, which were spiked into *Tmod1*$^{-/-}$ and *Tmod4*$^{-/-}$ skeletal muscle homogenates, respectively, to equalize the effects of endogenous non-Tmod proteins on the western transfer efficiencies of endogenous vs. recombinant purified Tmods used for standard curves. (C) Coomassie blue-stained gel of increasing amounts of purified rabbit skeletal muscle actin. (D-E) Western blots of increasing volumes of TA and EDL myofibrils, probed for (D) Tmod1 or (E) Tmod4. (F) Coomassie blue-stained gel of increasing volumes of TA and EDL myofibrils. Amounts of Tmod1, Tmod4, and actin corresponding to the densitometry signals for each protein in the myofibril samples were calculated from the appropriate standard curves, based on the myofibril signals in the linear range of the standard curves. Densitometry signals outside of the range of the standard curve were excluded from analysis. Nanograms were converted to moles using the molecular weights of the proteins (42 kDa, 39 kDa, and 42 kDa for Tmod1, Tmod4, and actin, respectively).

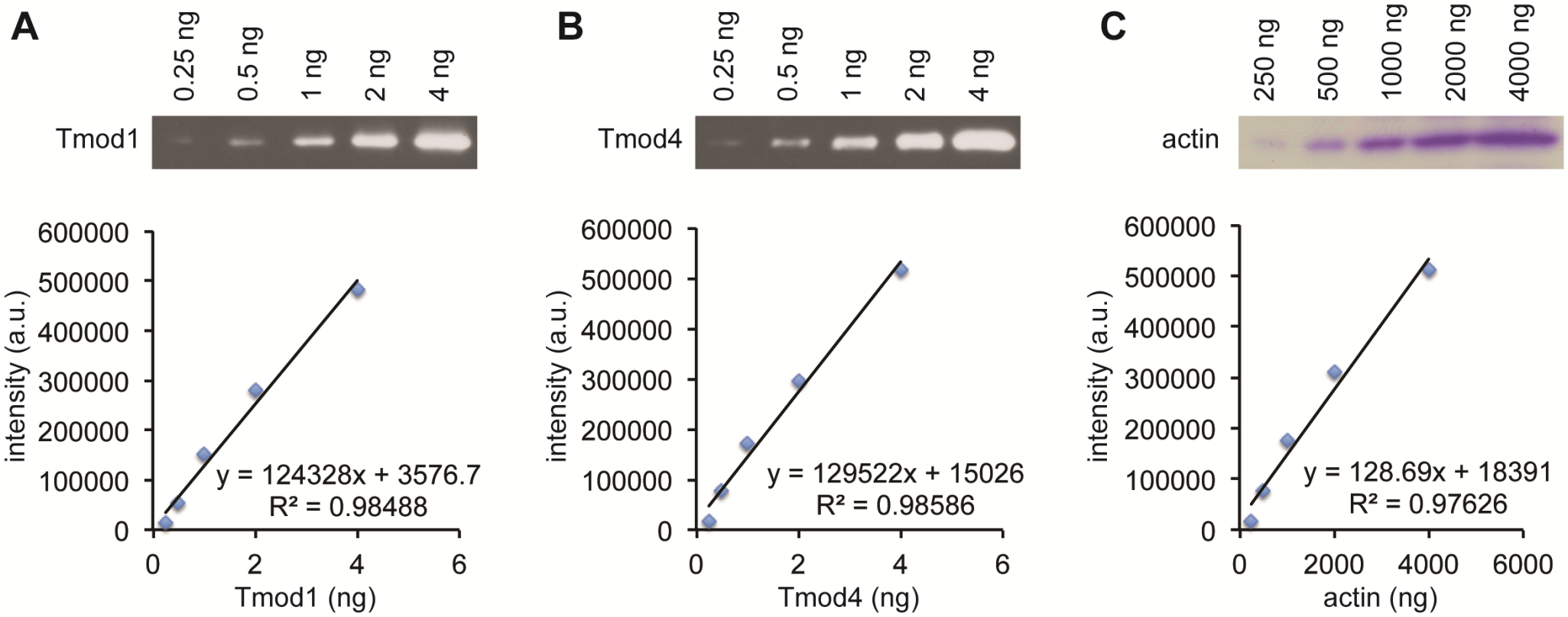
Supplementary Figure 2. Z-line-flanking and M-line localization of m-calpain in WT muscle. Longitudinal cryosections of TA and soleus muscles from 2-mo-old WT mice were immunostained for m-calpain and Tmod1 and phalloidin-stained for F-actin. M, M-line; Z, Z-line. Bars, 1 μm.

Supplementary Figure 3. Prenecrotic TA muscles from young *mdx*/mTR mice show normal localization of Tmod1. Longitudinal cryosections of TA muscles from 2-week-old WT and *mdx*/mTR mice were immunostained for Tmod1 and α-actinin and phalloidin-stained for F-actin. P, thin filament pointed ends; Z, Z-line. Bars, 1 μm.

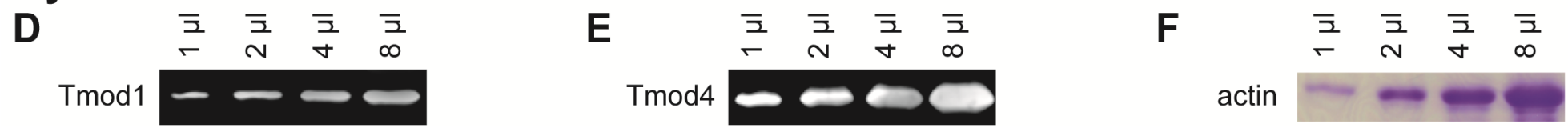
Supplementary Figure 4. Elevated Tmod3 and γ_{cyto}-actin protein levels in *mdx* muscles. (A) Western blots of homogenates of TA, EDL, and soleus muscles from 2-mo-old WT and *mdx* mice were probed using antibodies against Tmod3 and γ_{cyto}-actin. GAPDH was used as a loading control. (B) Quantification of western blots. Error bars reflect mean±SEM of n=4 lanes/genotype within a single blot. *, p<0.01.

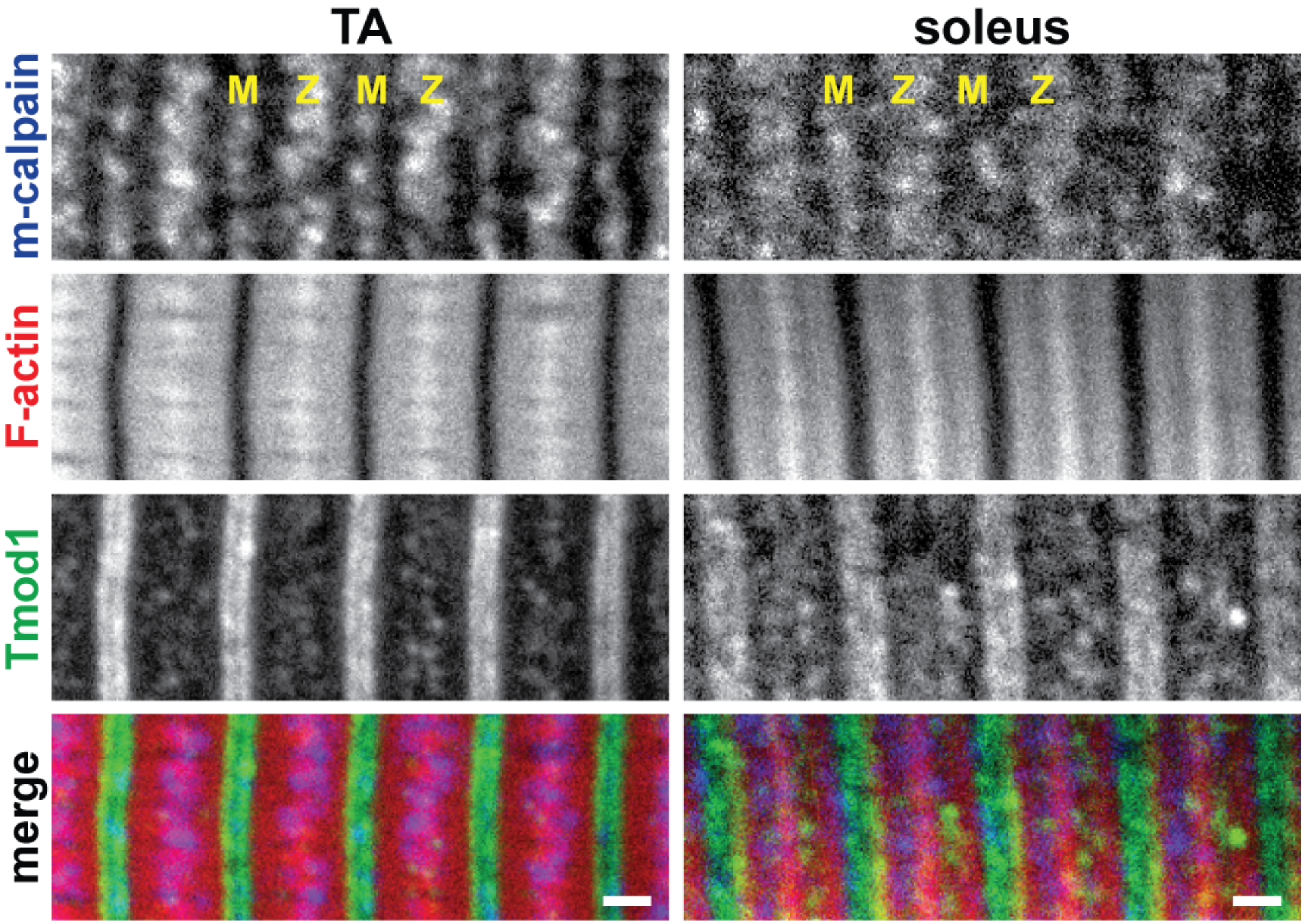
Supplementary Figure 5. Schematics indicating locations of the various fluorescently labeled proteins in Figures 4-8. Light grey rectangles indicate thick filaments. M, M-line; P, thin filament pointed ends; Z, Z-line.

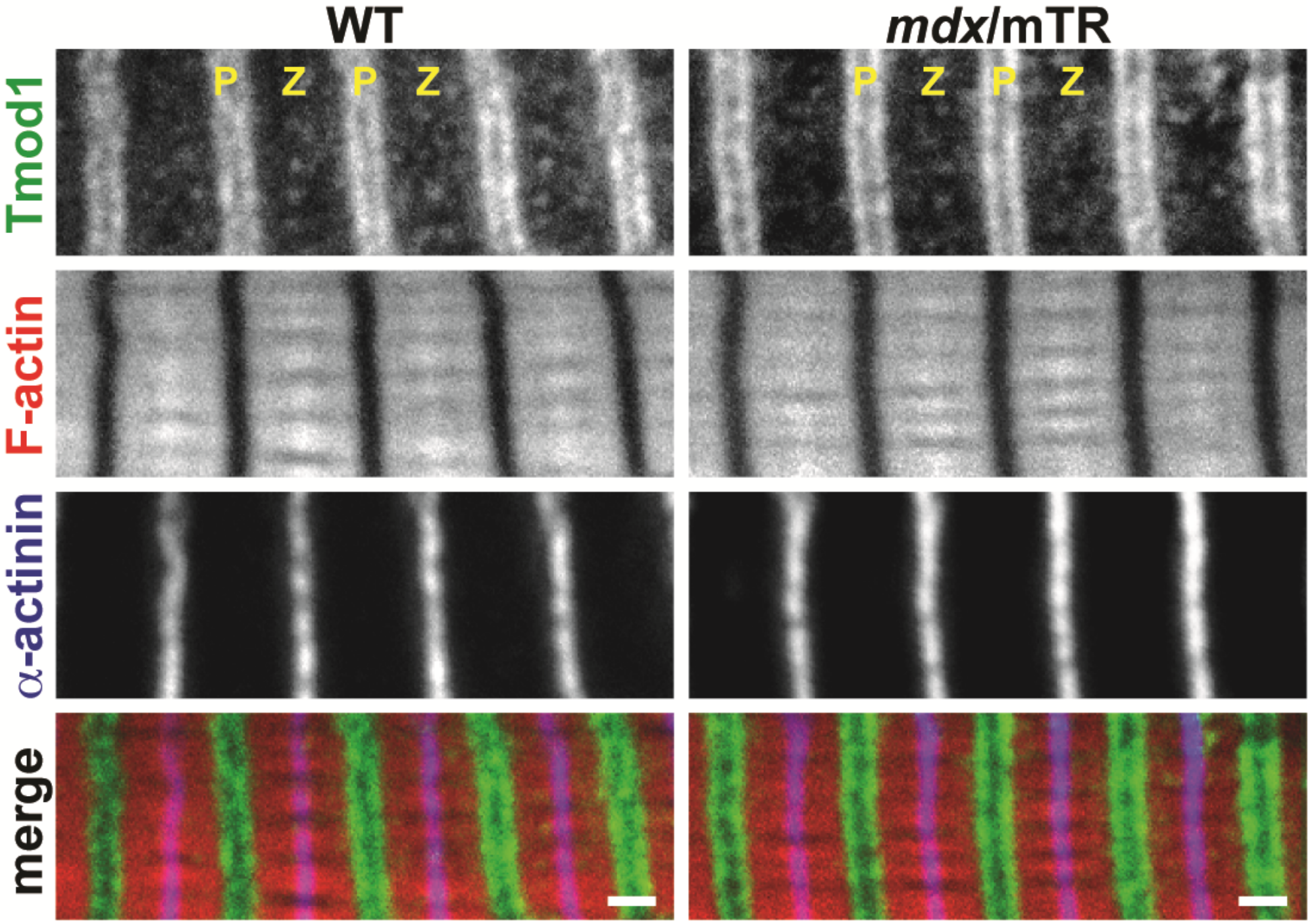
Protein standards



Myofibrils




TA
soleus
m-calpain
F-actin
Tmod1
merge
M Z M Z
M Z M Z


WT
mdx/mTR
Tmod1
F-actin
α-actinin
merge
P
Z
P
Z
P
Z
P
Z

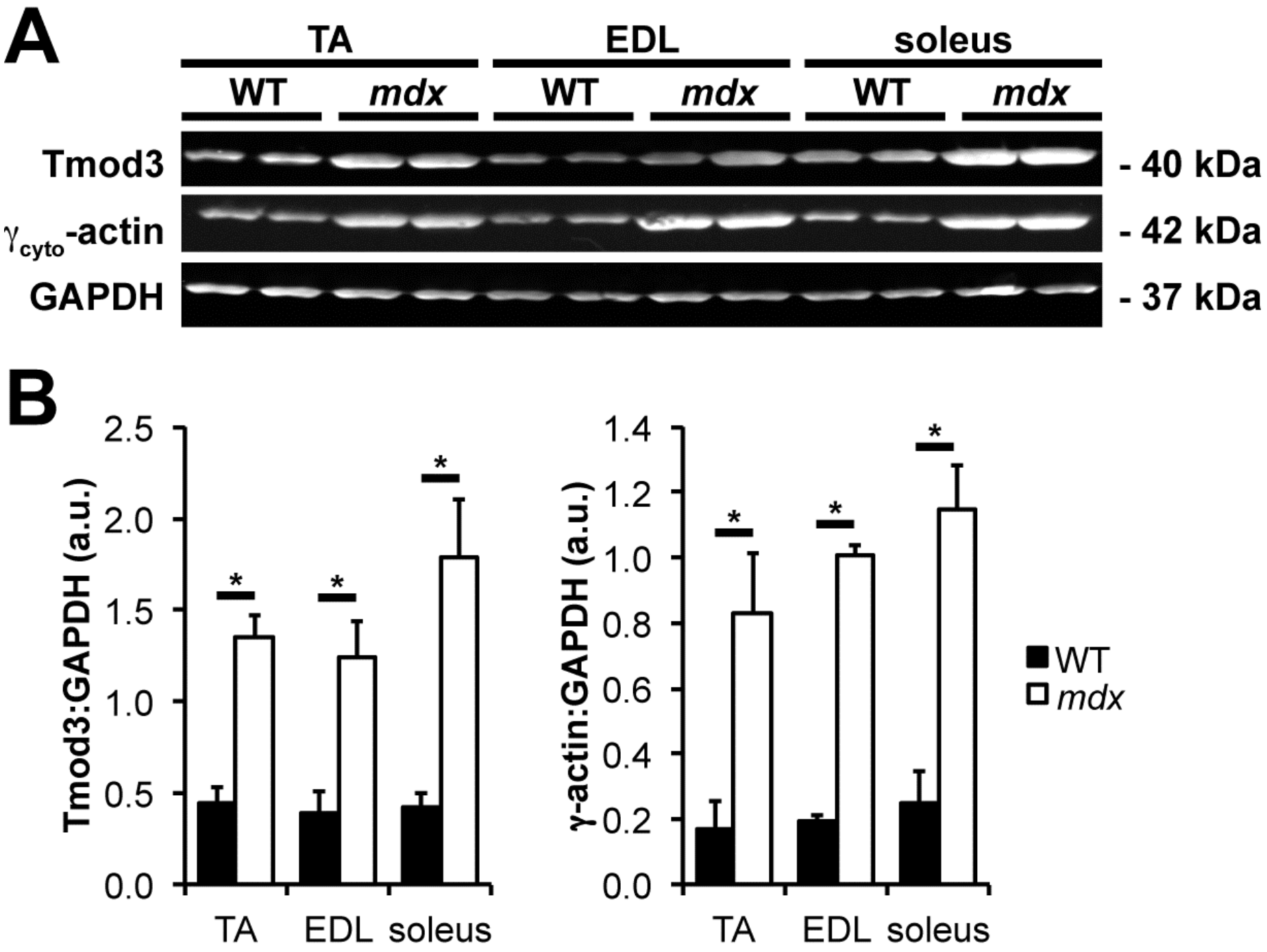

A
TA
EDL
soleus
WT
mdx
WT
mdx
WT
mdx
Tmod3
γcyto-actin
GAPDH
- 40 kDa
- 42 kDa
- 37 kDa
B
Tmod3:GAPDH (a.u.)
2.5
2.0
1.5
1.0
0.5
0.0
TA
EDL
soleus
γ-actin:GAPDH (a.u.)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
TA
EDL
soleus
WT
mdx

Figure 4: Tmod1, F-actin, α-actinin

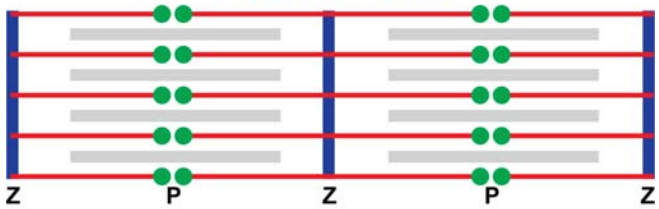


Figure 5: Tmod4, F-actin, α-actinin

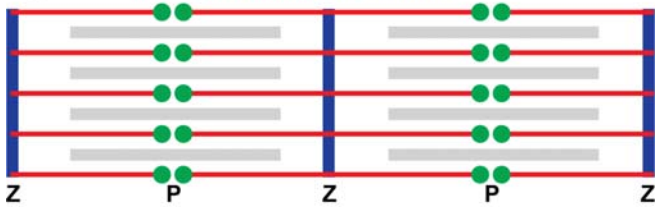


Figure 6: nebulin M1M2M3, F-actin, α-actinin

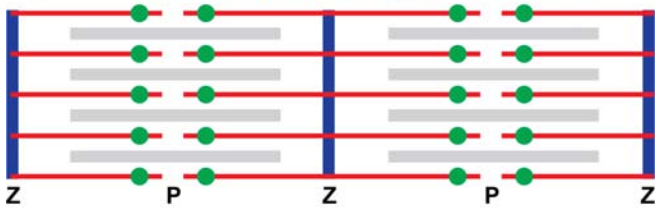


Figure 7: Tmod3, F-actin, α-actinin

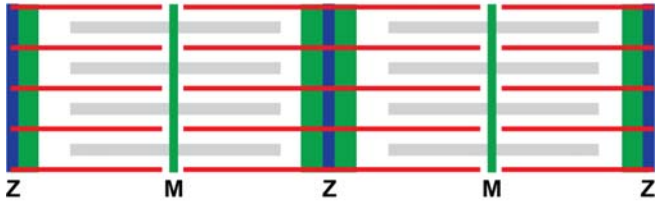
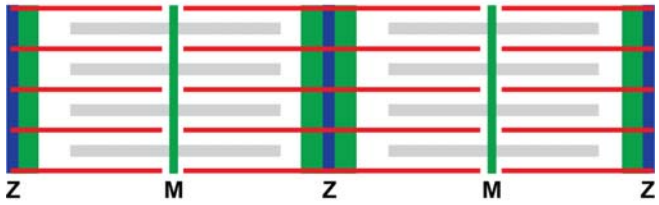


Figure 8: γ_{cyto}-actin, F-actin, α-actinin



<u>Supplementary Table 1.</u> Thin filament lengths in TA muscles from 2-week-old (prenecrotic) mice determined by DDecon analysis of fluorescence images.

		WT	*mdx*/mTR
Phalloidin	Mean±SD (µm)	1.04±0.03	1.04±0.04
	Min-max (µm)	0.95-1.14	0.94-1.18
	n	104	66
Tmod1	Mean±SD (µm)	1.10±0.06	1.11±0.07
	Min-max (µm)	1.01-1.19	1.03-1.25
	n	58	52

Parameters correspond to the breadth of phalloidin staining and the distance of Tmod1 from the Z-line. *n*, number of myofibrils.