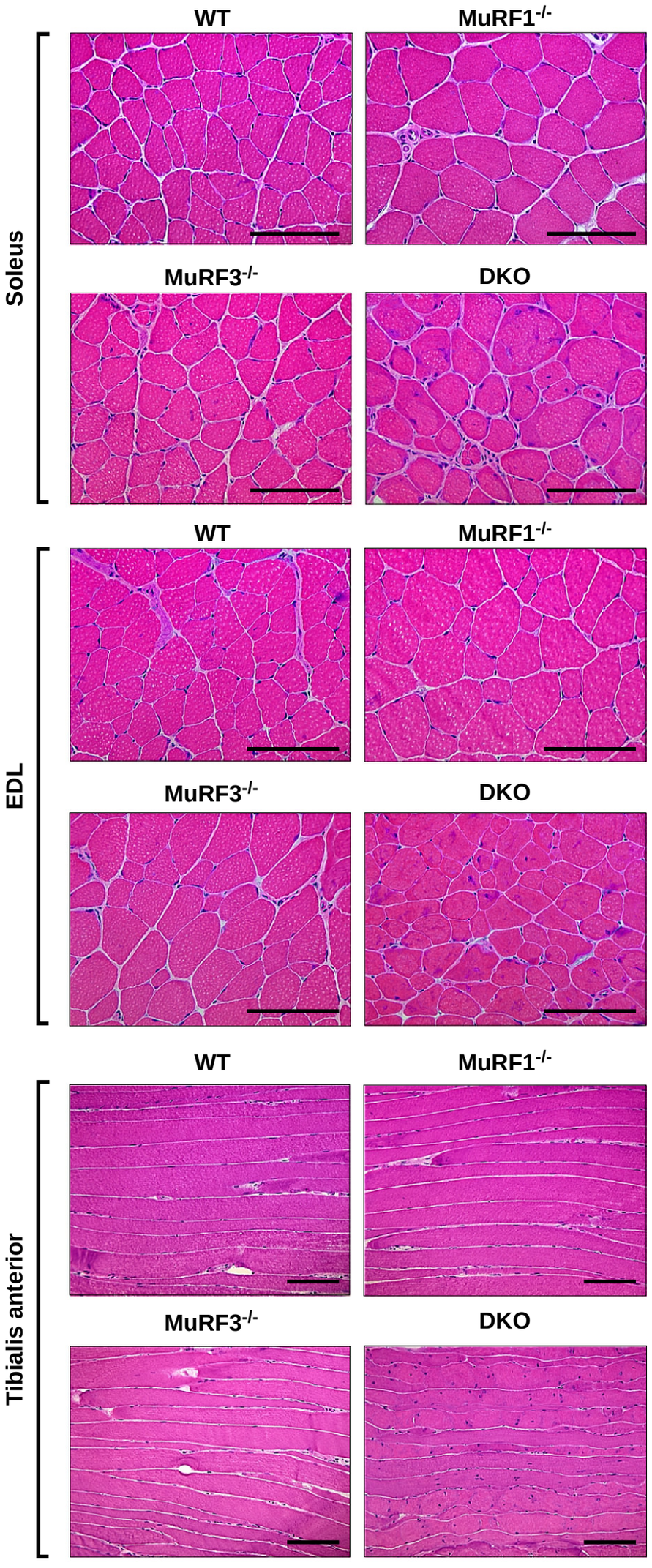
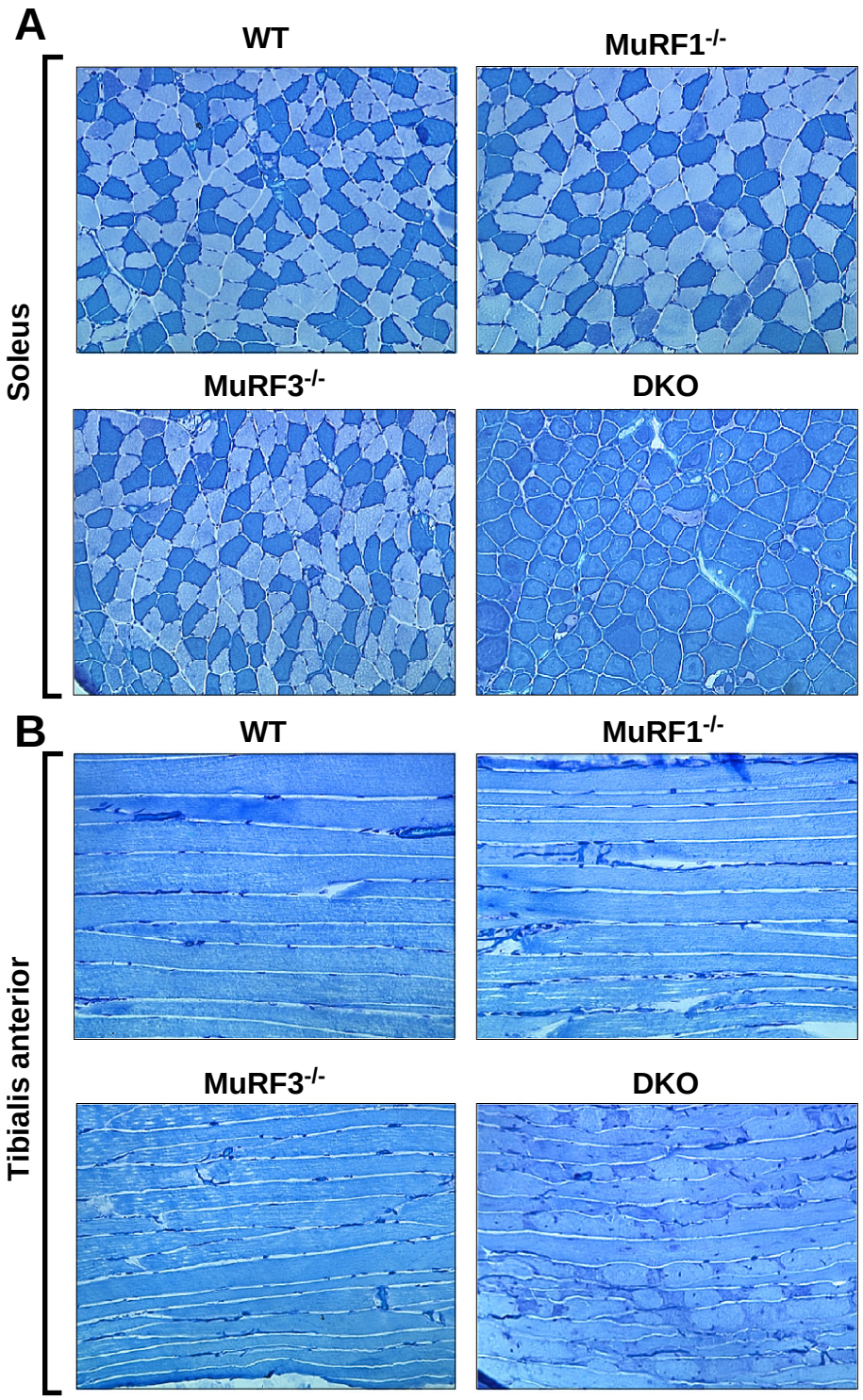
Protein Name	MW (kDa)	Sequence
Desmin	53.5	APSYGAGELLDFSLADAVNQEFLATR
Myh 4 (type IIb), skeletal muscle	222.7	TLAFLFSGGQAAEAEGGGGK
Myh 2 (type IIa), skeletal muscle	219.6	TLAYLLFSGAQTAEAEASSGGAAK
Myh 1 (type IIx) skeletal muscle	223.2	HADSVAELGEQIDNLQR
Myh 2 (type IIa)	183.0	TLAYLFSGAQTAEAEASSGGAAK
Calsequestrin 1	45.6	KLGLTEEDSVYVFK
Myh 6 (type I)	222.7	LEEAGGATSVQIEMNK
Actin, α1, skeletal muscle	42.0	DLYANNVMSGGTTMYPGIADR
Titin, isoform N2-B	2984.5	VTGLTEGLEYEFER
Actin, α1, skeletal muscle	41.8	TTGIVLDSGDGVTHNVPIYEGYALPHAIMR

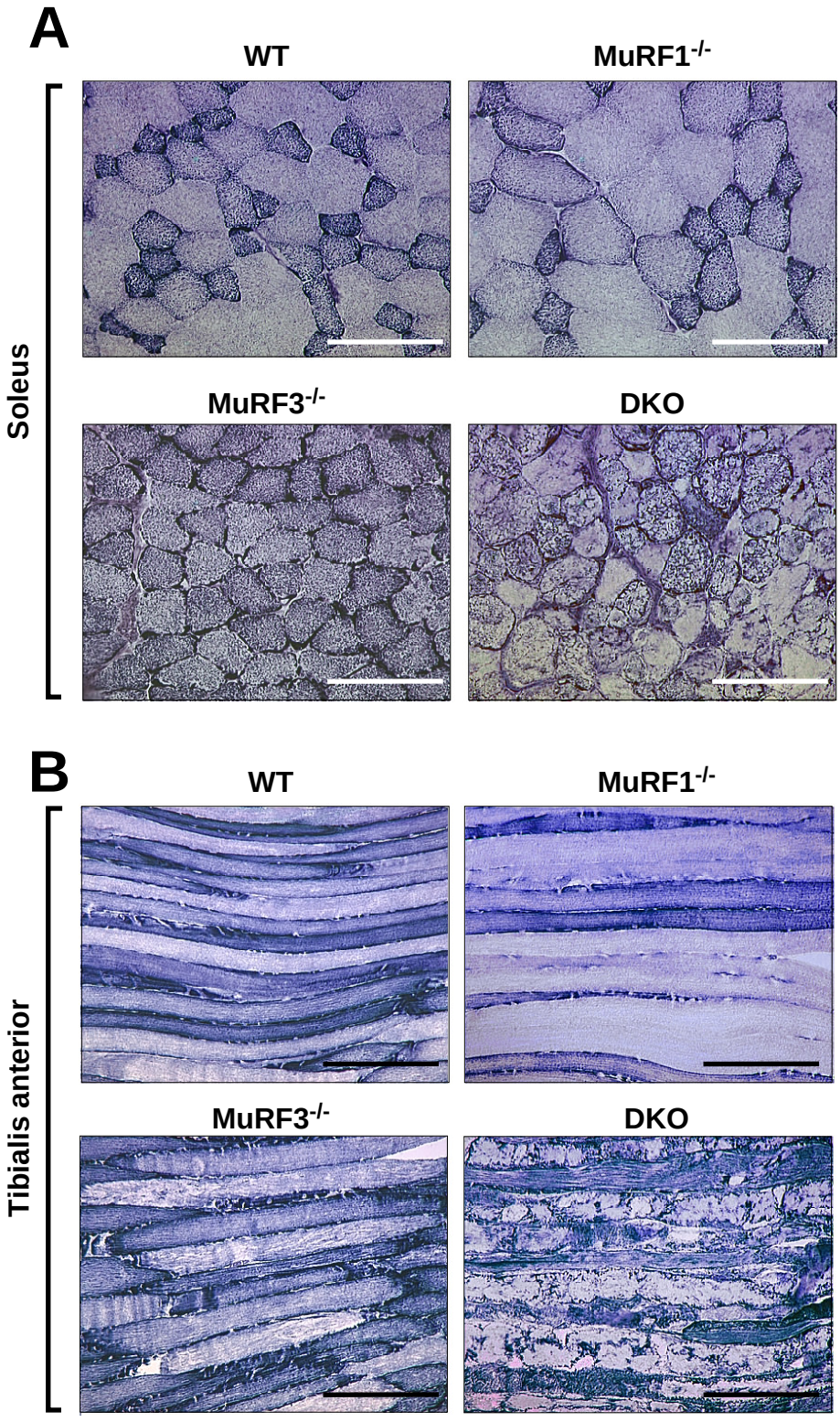
Supplemental Table 1



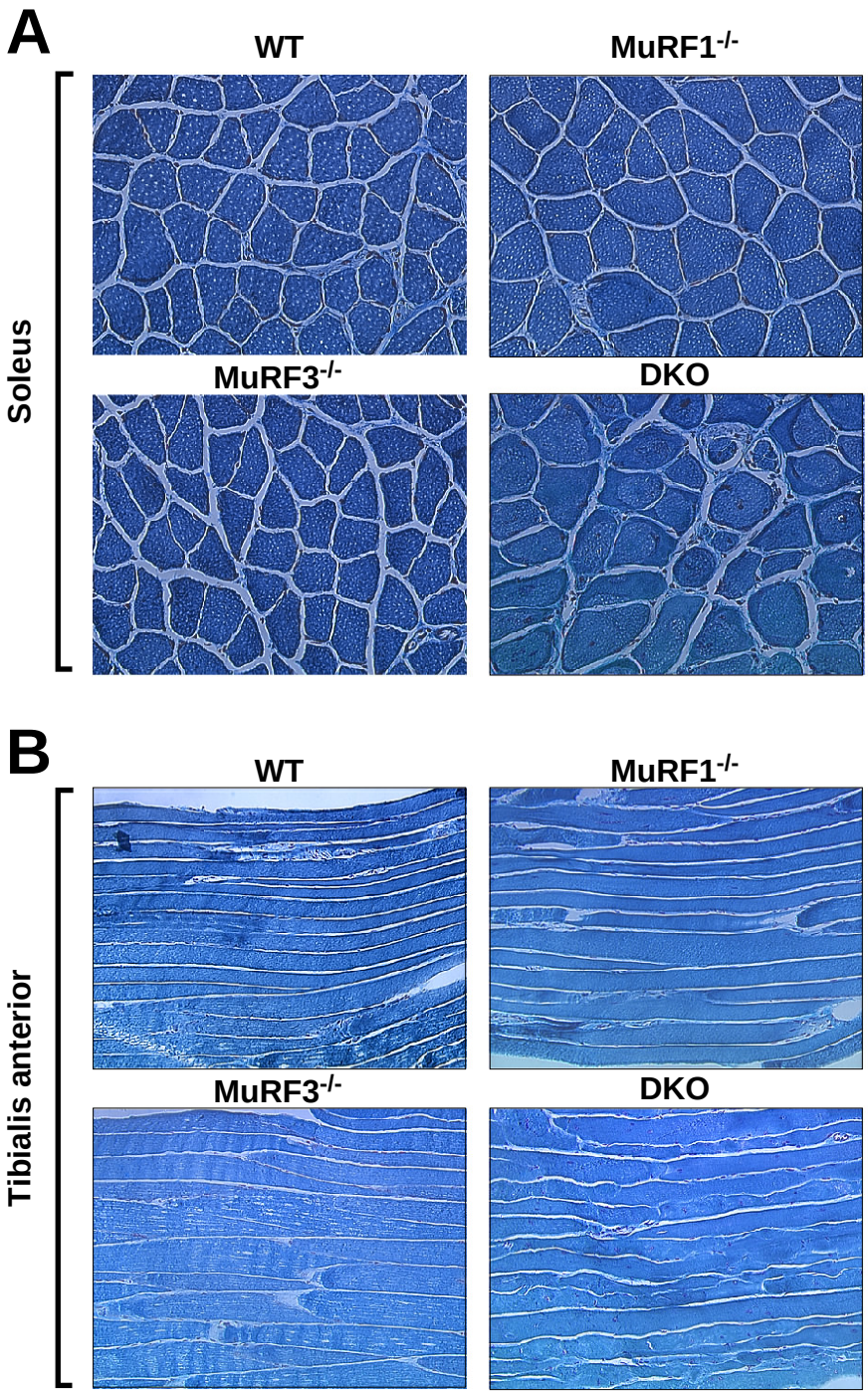
Supplemental Figure 1



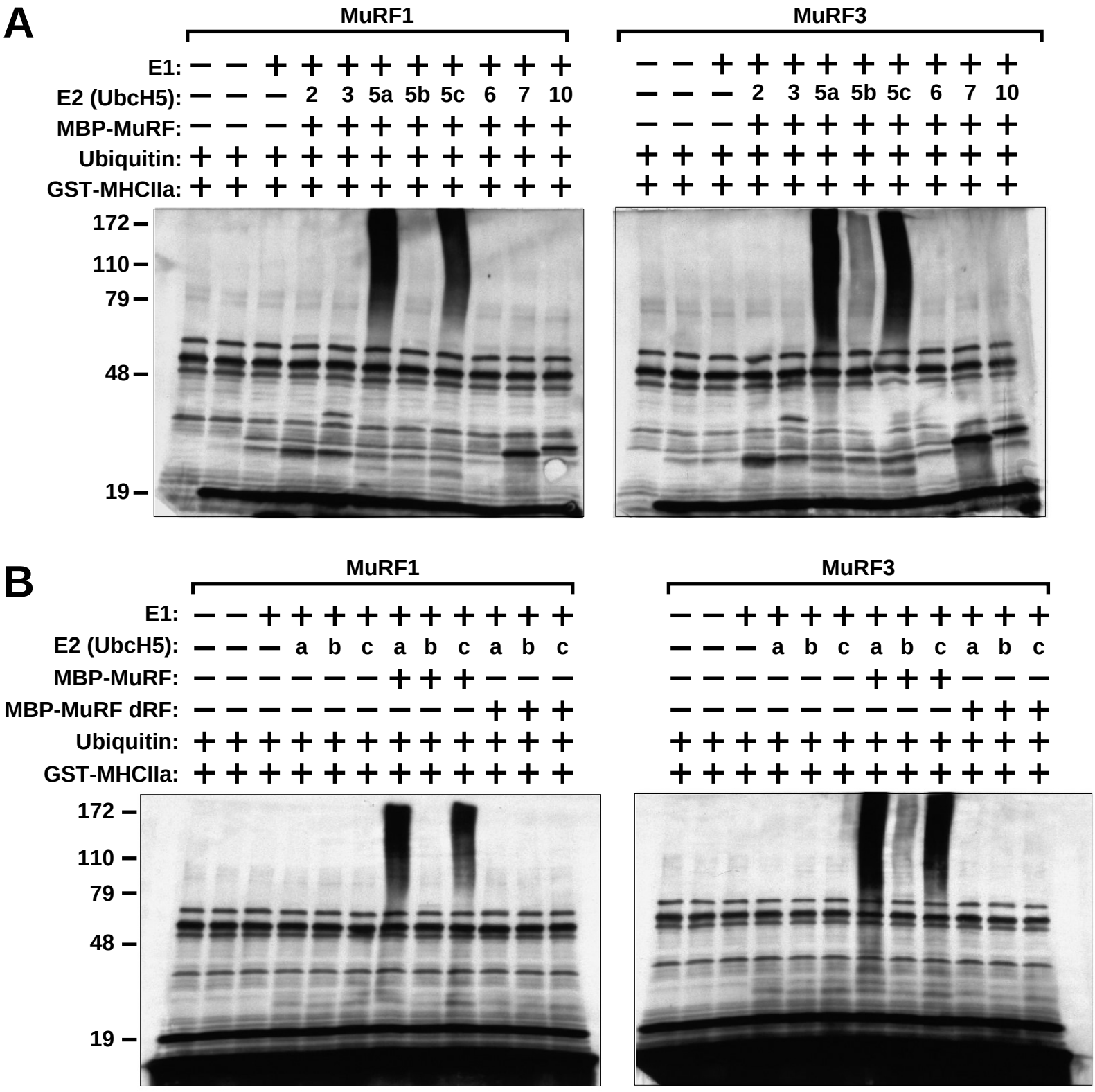
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5

Supplemental Online information

Supplemental Table 1

Mass spectroscopy of the 60kDa protein band isolated from soleus of DKO mice by SDS-PAGE shows accumulation of myosin fragments within the soleus of MuRF1 and 3 double mutant mice.

Supplemental Figure 1

H&E staining of histological sections of soleus (A, cross section), extensor digitorum longus (EDL) (cross section) and tibialis anterior (longitudinal section) from wild-type (WT), MuRF1$^{-/-}$, MuRF3$^{-/-}$ and DKO mice. Subsarcolemmal eosinophilic material accumulates along the entire length of the myofiber around a central myofiber core of DKO muscle. Abnormal heterogeneity of fiber size, centrally localized nuclei, atrophic myofibers, split fibers and more nuclei were found in DKO muscle. Bar=100 μm.

Supplemental Figure 2

Detection of myosin ATPase activity using a metachromatic dye ATPase assay in soleus (A, cross sections) and tibialis anterior (B, longitudinal sections) muscles from wild-type (WT), MuRF1$^{-/-}$, MuRF3$^{-/-}$ and DKO mice. Beta/slow MHC myofibers stain dark blue and MHC II myofibers stain light blue. Bar=100 μm.

Supplemental Figure 3

NADH stain of soleus (A, cross sections) and tibialis anterior (B, transverse sections) muscles of wild-type (WT), MuRF1$^{-/-}$, MuRF3$^{-/-}$ and DKO mice where dark and punctuate blue staining indicating mitochondria, shows mitochondria are displaced and disorganized in DKO muscle. Bar=100 μm.

Supplemental Figure 4

Modified Gomori's trichrome stain of soleus (A, cross sections) and tibialis anterior (B, transverse sections) muscles of wild-type (WT), MuRF1$^{-/-}$, MuRF3$^{-/-}$ and DKO mice. No ragged red fibers were observed in DKO muscle. Bar=100 μm.

Supplemental Figure 5

a) *In vitro* ubiquitination assays were performed with recombinant E1 ubiquitin-activating enzyme, different E2 ubiquitin-conjugating enzymes (as indicated), MBP-MuRF1 (left panel) or MBP-MuRF3 (right panel), ubiquitin (Ub), ATP and GST-MHCIIa for 2 hours. Ub-protein conjugates were resolved by SDS-PAGE and detected by anti-Ub antibody. Ubiquitination activity, as assessed by the detection of high-molecular-weight multiubiquitin chains, was detected only in reactions containing E1, UbcH2, 5a, 5b, 5c, MBP-MuRF1 or MBP-MuRF3, Ub and GST-MHCIIa. No ubiquitination activity was detected when the E2 ubiquitin-conjugating enzymes UbcH3, 6, 7 and 10 were used.

b) *In vitro* ubiquitination assays were performed with recombinant E1 ubiquitin-activating enzyme, the E2 ubiquitin-conjugating enzymes UbcH5a, 5b and 5c, ubiquitin (Ub), ATP, GST-MHCIIa and MBP-MuRF1, MBP-MuRF3 or RING-finger deletion mutants MBP-MuRF1ΔRF (left panel) and MBP-MuRF3ΔRF (right panel), respectively, for 2 hours. Ub-protein conjugates were resolved by SDS-PAGE and detected by anti-Ub antibody. Ubiquitination activity of MuRF1 and MuRF3 towards GST-MHCIIa was totally abolished when the RING-finger domain of MuRF1 and MuRF3 were delete.