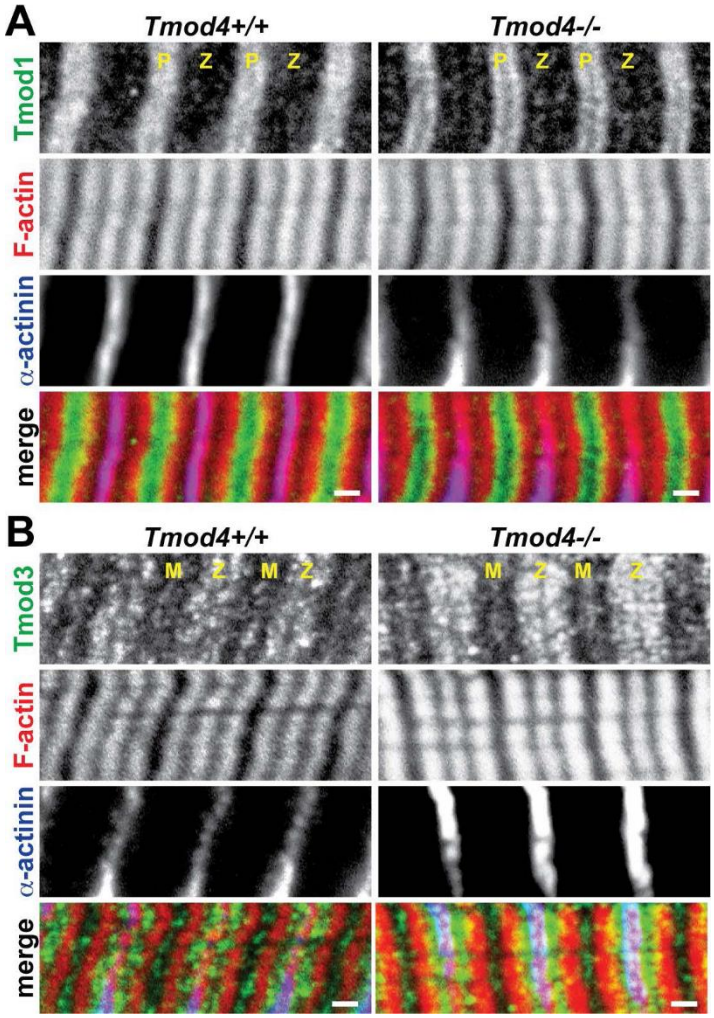
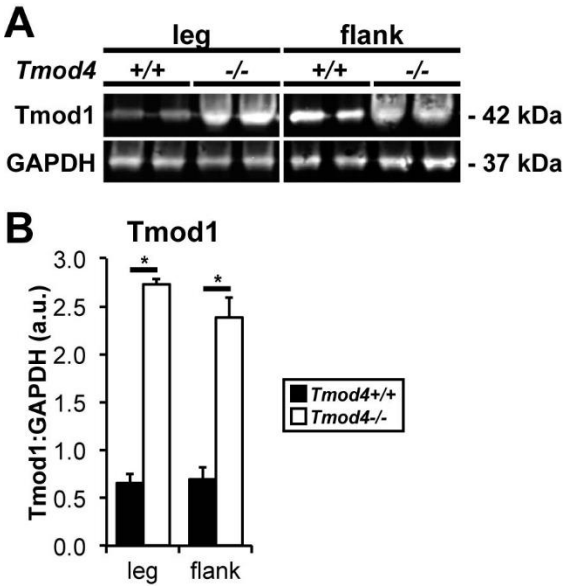
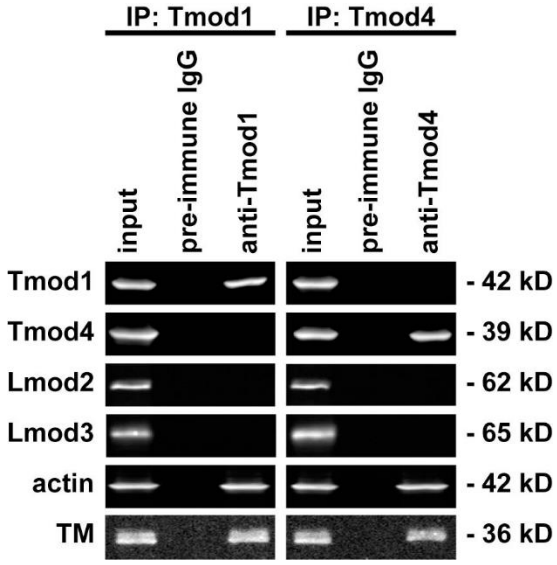
## SUPPLEMENTARY FIGURES



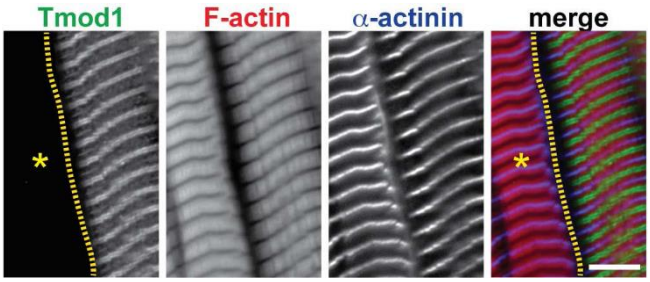

**Supplementary Figure 1. Deletion of Tmod4 does not impact myofibril organization in soleus muscle.** (A-B) Longitudinal cryosections of soleus muscles from 2-mo-old *Tmod4*$^{+/+}$ and *Tmod4*$^{-/-}$ mice were immunostained for either (A) Tmod1 or (B) Tmod3, immunostained for α-actinin, and phalloidin-stained for F-actin. Note that increased Tmod1 protein levels in *Tmod4*$^{-/-}$ soleus muscle determined by western blotting (Fig. 3E) are not consistently measurable by immunofluorescence and confocal microscopy. P, thin filament pointed ends; Z, Z-line; M, M-line. Bars, 1 μm.



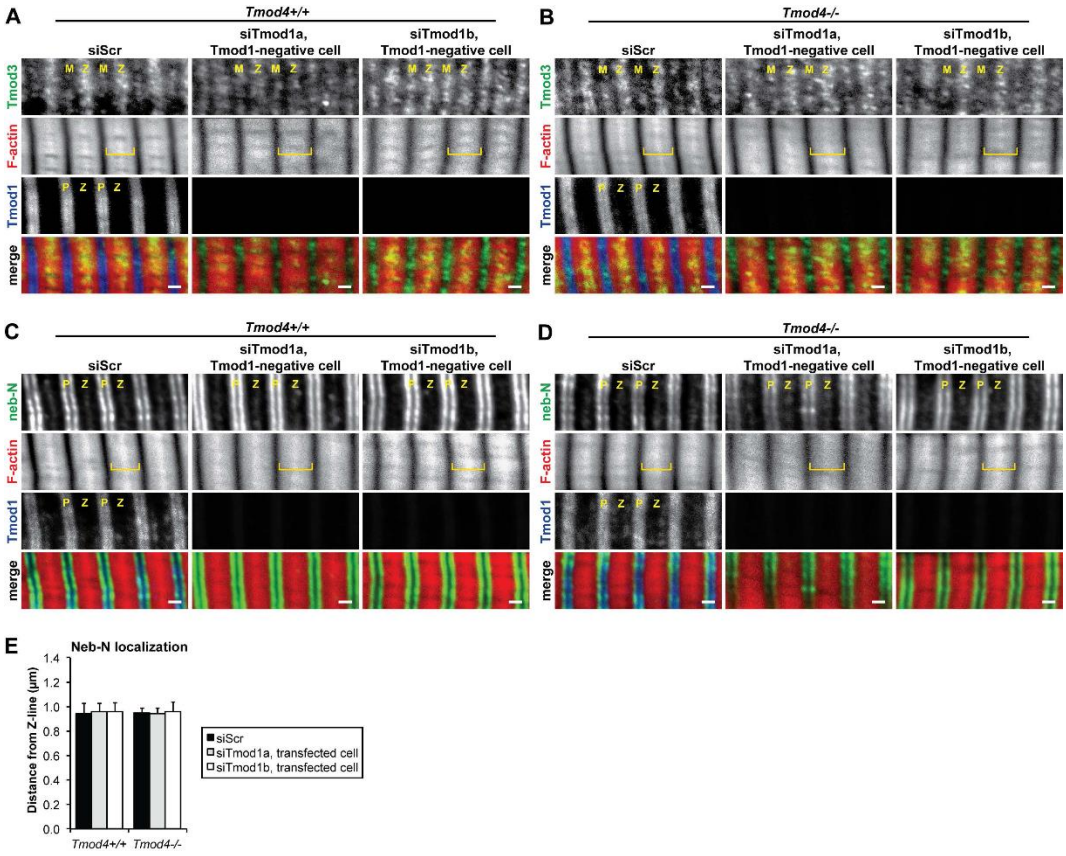

**Supplementary Figure 2. Deletion of Tmod4 induces a compensatory increase in Tmod1 protein levels in neonatal skeletal muscle.** (A) Western blots of homogenates of leg and flank muscles from newborn (P0) *Tmod4*$^{+/+}$ and *Tmod4*$^{-/-}$ mice were probed using an anti-Tmod1 antibody. GAPDH was used as a loading control. (B) Quantification of western blots. Error bars reflect mean±s.e.m. of $n$=3 lanes/genotype within a single blot.




**Supplementary Figure 3. Tmod1 and Tmod4 do not coimmunoprecipitate with Lmod2 or Lmod3.** Shown are western blots of supernatants of RIPA buffer-extracted muscle lysates coimmunoprecipitated using anti-Tmod1- or anti-Tmod4-coated beads. Input extract was used as a positive control, and beads coated with pre-immune IgG were used as a negative control. Note coimmunoprecipitation of Tmod1 and Tmod4 with actin and α/βTM but neither Lmod2 nor Lmod3.




**Supplementary Figure 4. Sample low-magnification image of Tmod1-positive and Tmod1-negative muscle fibers coexisting within an siTmod1a-injected muscle.** A TA muscle from a 2-mo-old *Tmod4*$^{+/+}$ mouse was injected with siTmod1a and excised 1 week post-injection. Shown is a longitudinal cryosection immunostained for either immunostained for Tmod1 and α-actinin, and phalloidin-stained for F-actin. Yellow asterisk indicates a Tmod1-negative fiber; dotted yellow line indicates the periphery of the Tmod1-negative fiber. Bar, 7 μm.




**Supplementary Figure 5. RNAi knockdown of Tmod1 from *Tmod4*$^{+/+}$ or *Tmod4*$^{-/-}$ muscles does not affect the localization of Tmod3 or neb-N.** (A-D) TA muscles from 2-mo-old (A,C) *Tmod4*$^{+/+}$ and (B,D) *Tmod4*$^{-/-}$ mice were injected with siScr, siTmod1a, or siTmod1b and excised 1 week post-injection. Shown are longitudinal cryosections immunostained for either (A,B) Tmod3 or (C,D) neb-N, immunostained for Tmod1, and phalloidin-stained for F-actin. Yellow brackets signify thin filament arrays (I-Z-I regions) that widen after Tmod1 knockdown. P, thin filament pointed ends; Z, Z-line; M, M-line. Bars, 1 μm. (E) Distance of neb-N from the Z-line in TA muscles from 2-mo-old *Tmod4*$^{+/+}$ and *Tmod4*$^{-/-}$ mice injected with siScr, siTmod1a, or siTmod1b, determined using DDecon analysis of fluorescence images. Error bars reflect mean±s.d. of $n$=50 myofibrils/genotype randomly selected from $n$=3-4 muscles/genotype.