Gokhin and Fowler, http://www.jcb.org/cgi/content/full/jcb.201011128/DC1

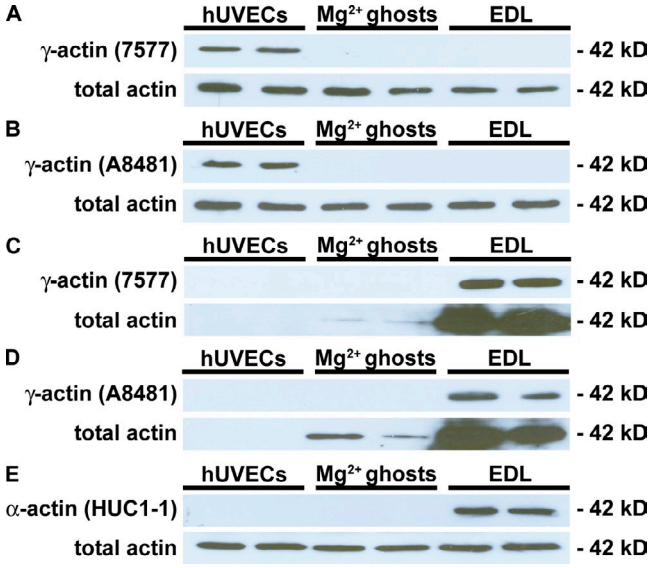

Figure S1. **Specificity of actin antibodies.** (A and B) Western blots for γ_{cyto}-actin probed with rabbit pAb *7577* (A) and mouse mAb A8481 (B) on lysates of the following cells/tissues: mouse EDL muscle, which contains α_{sk}-actin overwhelmingly and only 1/4,500 as much γ_{cyto}-actin (Hanft et al., 2006); human erythrocyte membranes (Mg^{2+} ghosts), which contain β_{cyto}-actin exclusively (Pinder et al., 1978; Pinder and Gratzer, 1983; Hoock et al., 1991); and cultured hUVECs, which contain γ_{cyto}-actin exclusively (Hoock et al., 1991; Galustian et al., 1995). When total actin levels were kept constant, both antibodies detected a γ_{cyto}-actin band in hUVECs but no γ_{cyto}-actin band in Mg^{2+} ghosts and EDL muscle, consistent with the actin isoform compositions of these samples. (C and D) A γ_{cyto}-actin band could only be detected in skeletal muscle when hUVECs were underloaded 100-fold, and EDL muscle was overloaded 100-fold before probing blots with rabbit pAb *7577* (C) or mouse mAb A8481 (D). (E) An antibody that was raised against smooth muscle α-actin but recognizes all muscle α-actins (mouse mAb HUC1-1) was able to detect an α_{sk}-actin band in EDL muscle but no band in hUVECs or Mg^{2+} ghosts.

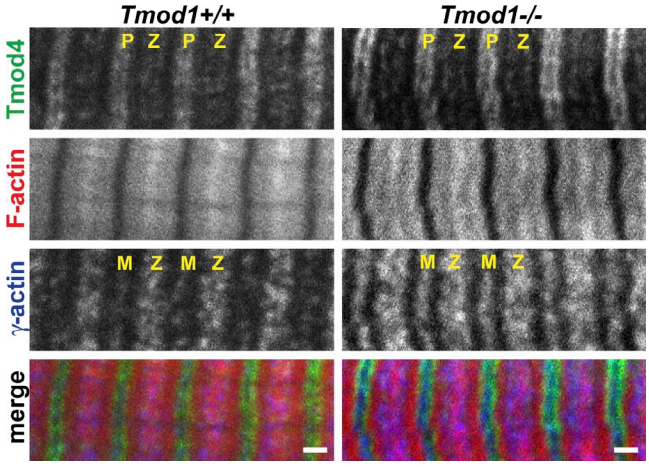

Figure S2. **Z line–flanking localization of Tmod4.** Longitudinal cryosections of 1-mo-old TA muscle fibers from *Tmod1*$^{+/+Tg+}$ and *Tmod1*$^{-/-Tg+}$ mice immunostained for Tmod4, γ_{cyto}-actin, and F-actin. M, M line; P, thin filament pointed ends; Z, Z line. Bars, 1 µm.

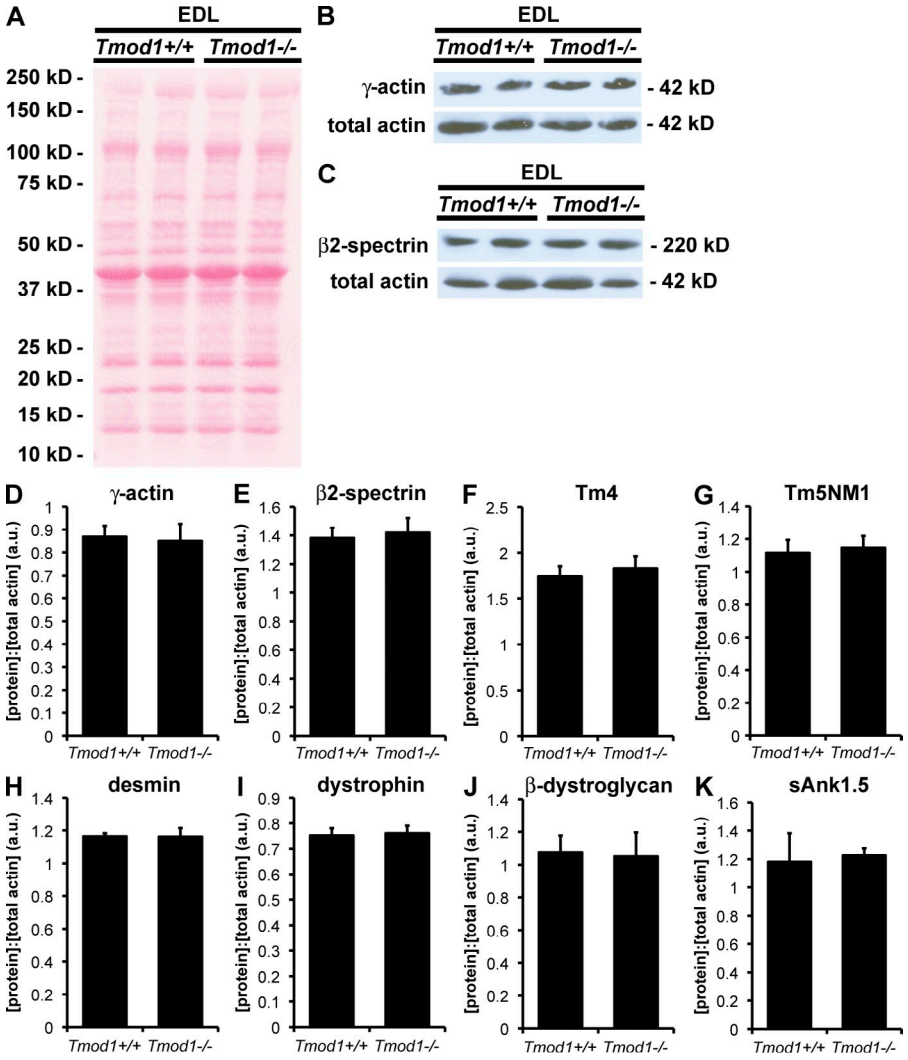

Figure S3. **Deletion of Tmod1 does not alter the levels of extrasarcomeric cytoskeletal proteins.** (A) Ponceau S–stained blots of EDL tissue lysates show no changes in protein composition in *Tmod1*$^{-/-Tg+}$ muscle. (B and C) Western blots of EDL tissue lysates show no changes in γ_{cyto}-actin (B) and β2-spectrin (C) levels in *Tmod1*$^{-/-Tg+}$ muscle. The γ_{cyto}-actin blot was probed with pAb *7577*. (D–K) Quantitation of γ_{cyto}-actin (D), β2-spectrin (E), Tm4 (F), Tm5NM1 (G), desmin (H), dystrophin (I), β-dystroglycan (J), and sAnk1.5 (K) protein bands with normalization to total actin (four muscles/genotype). No differences were observed between *Tmod1*$^{+/+Tg+}$ and *Tmod1*$^{-/-Tg+}$ muscle. Each bar reflects $n = 4$ lanes. Data are presented as mean ± SD. a.u., arbitrary unit.

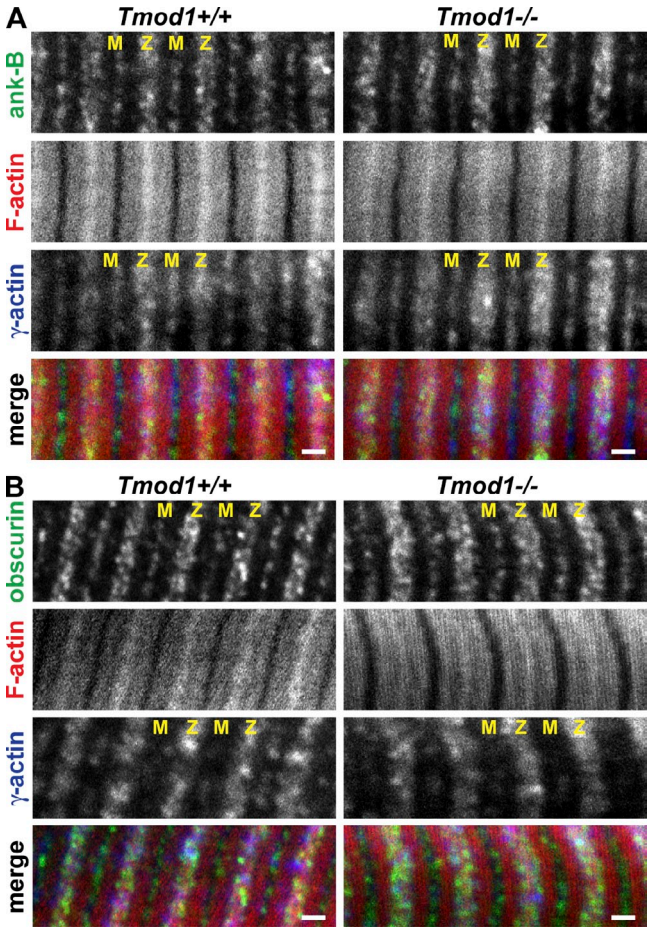

Figure S4. **Deletion of Tmod1 does not alter the localization of ankyrin-B or obscurin.** (A and B) Longitudinal cryosections of 1-mo-old TA muscle fibers from *Tmod1*$^{+/+Tg+}$ and *Tmod1*$^{-/-Tg+}$ mice immunostained for γ_{cyto}-actin, F-actin, and either ankyrin-B (A) or obscurin (B). M, M line; Z, Z line. Bars, 1 µm.

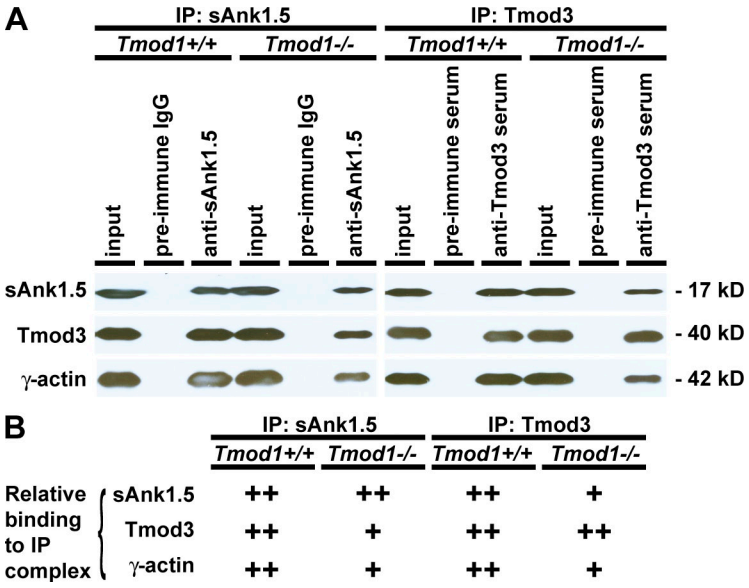

Figure S5. **Negative controls for co-IP experiments showing that the deletion of Tmod1 disrupts the Tmod3–γ_{cyto}-actin–sAnk1.5 complex.** (A) Western blots of clarified muscle extract (input) coimmunoprecipitated using antibodies against sAnk1.5 or Tmod3 or their respective preimmune negative controls. Note the reduced co-IP of Tmod3, γ_{cyto}-actin, and sAnk1.5 in *Tmod1*$^{-/-Tg+}$ muscle. (B) Semiquantitation of the co-IPs shown in A. IP, immunoprecipitated.

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