Randazzo et al., http://www.jcb.org/cgi/content/full/jcb.201205118/DC1

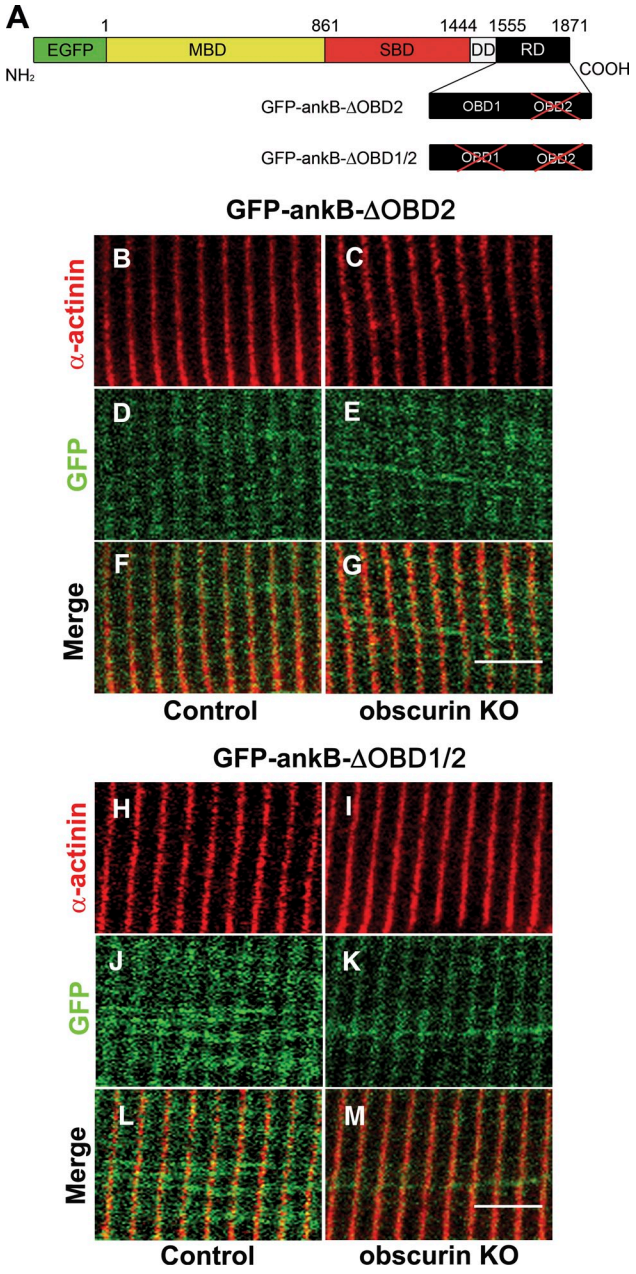

Figure S1. **Localization of GFP-ankB-ΔOBD2 and GFP-ankB-ΔOBD1/2 in in vivo FDB muscles of control and obscurin KO mice.** (A) Schematic representation of the GFP-ankB-ΔOBD2 and GFP-ankB-ΔOBD1/2 constructs. MBD, membrane-binding domain; SBD, spectrin-binding domain; DD, death domain; RD, regulatory domain containing the two OBD sites. (B–M) Immunostaining of FDB muscle of control and obscurin KO mice transfected with GFP-ankB-ΔOBD2 (B–G) and GFP-ankB-ΔOBD1/2 (H–M) constructs. Bars, 5 µm.

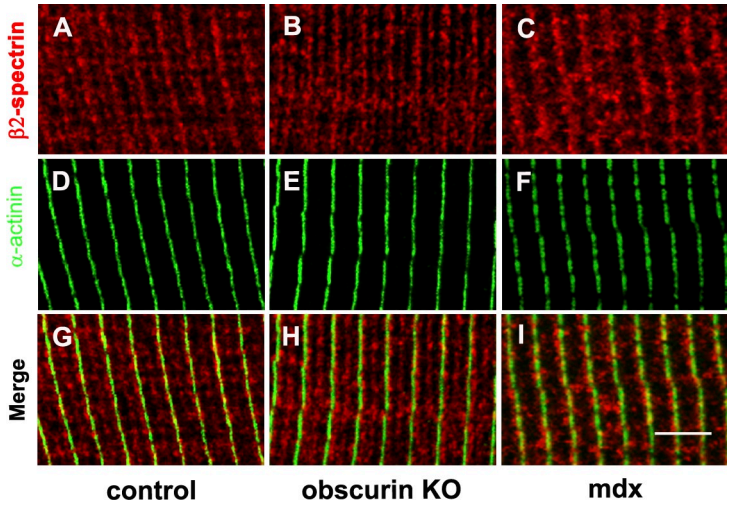

Figure S2. **Localization of β2-spectrin in control, obscurin KO, and mdx muscle fibers.** (A–I) Immunostaining of FDB muscle from control (A, D, and G), obscurin KO (B, E, and H), and mdx (C, F, and I) mice with an antibody against β2-spectrin. Bar, 5 µm.

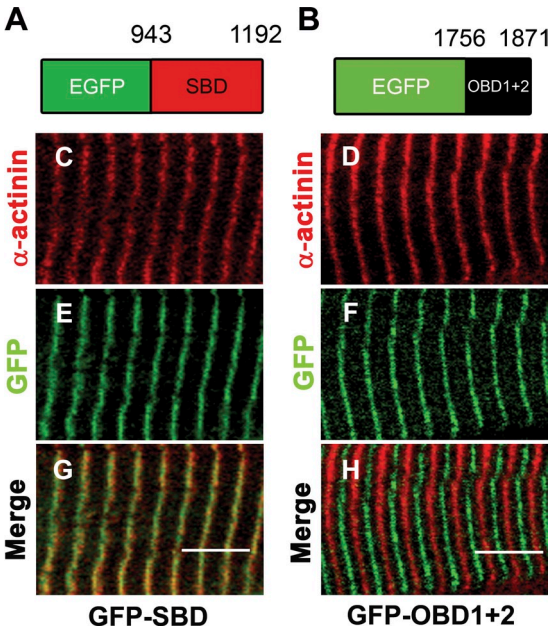

Figure S3. **Localization of GFP-SBD and GFP-OBD1+2 in FDB muscle fibers of control mice.** (A and B) Schematic representation of constructs containing the ankB spectrin binding site and OBD, GFP-SBD, and GFP-OBD1+2, respectively. (C–H) Immunostaining of FDB muscle of control mice transfected with GFP-SBD (943–1,192 aa) construct (C, E, and G) or with GFP-OBD1+2 (1,756–1,871 aa) construct (D, F, and H). Bars, 5 µm.

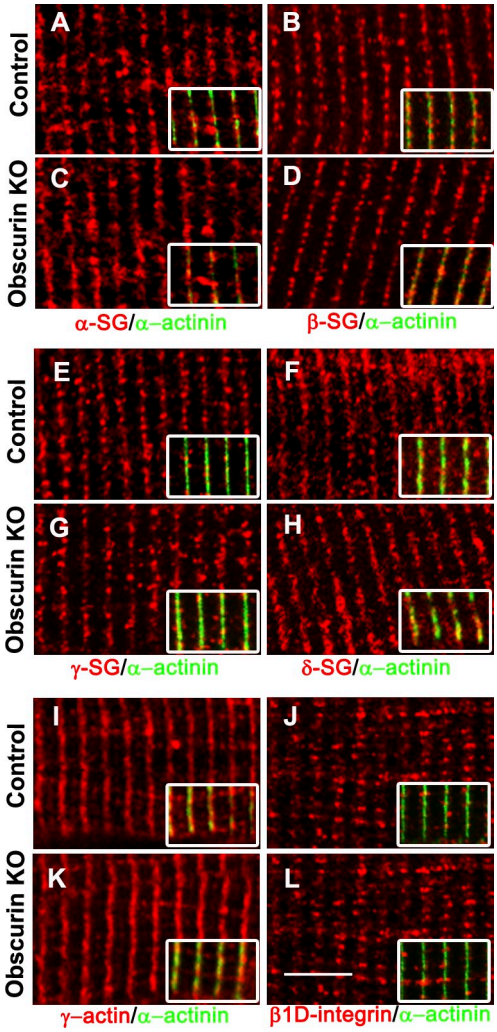

Figure S4. **Immunofluorescence analysis for α-, β-, γ-, and δ-sarcoglycans, β1D-integrin, and γ-actin in control and obscurin KO muscle fibers.** (A–H) Immunofluorescence analysis shows that the sarcoglycans (SG): α-SG (A and C), β-SG (B and D), γ-SG (E and G), and δ-SG (F and H) remain correctly localized at the Z disk/costamere region in skeletal muscle fibers from control and obscurin KO muscle. (I–L) Furthermore, both γ-actin (I and K) and β1D-integrin localization at costameres (J and L) are not affected by obscurin ablation. α-Actinin is used to mark the Z disk as shown in the merged images reported in the insets. Bar, 5 μm.

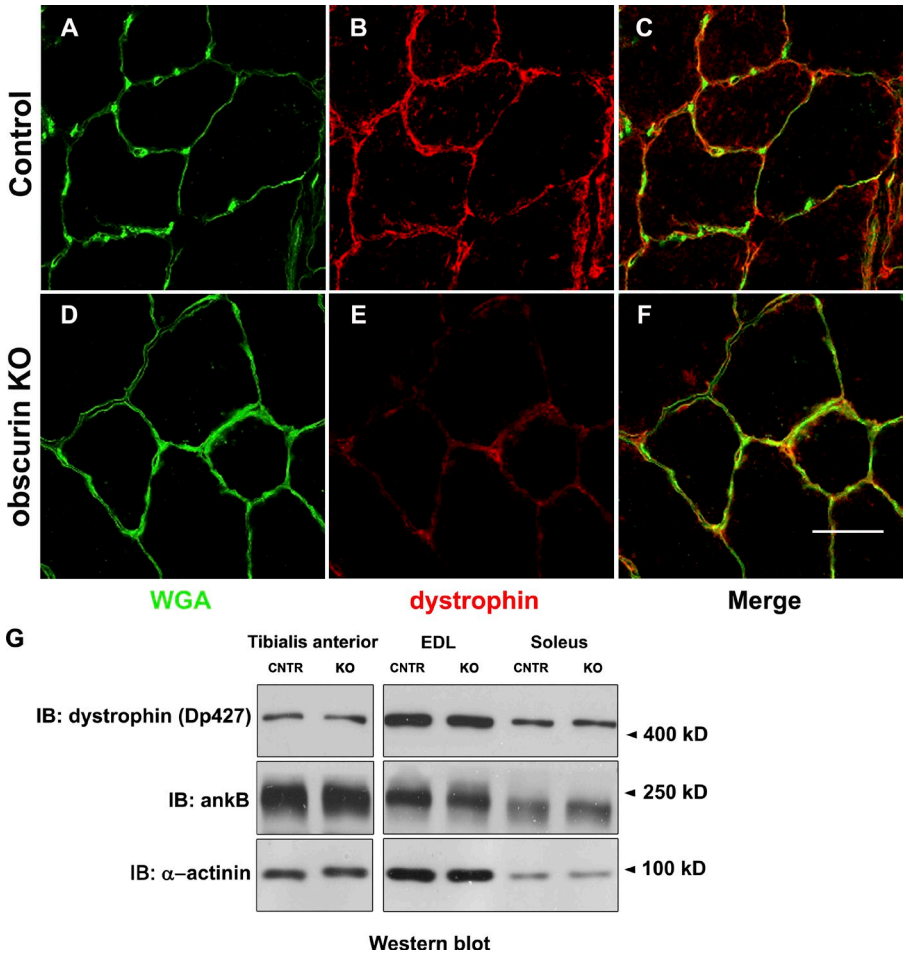

Figure S5. **Immunostaining of tibialis anterior cross sections with WGA and dystrophin (MANDRA1) antibodies and Western blot analysis of protein lysates from tibialis anterior, EDL, and soleus of control and obscurin KO mice.** (A–F) Immunofluorescence of tibialis anterior cross sections display a reduced level of dystrophin localization at the sarcolemma of obscurin KO mice (E) compared with wild-type controls (B). WGA is used to visualize the plasma membrane (A and D; green in overlay in C and F). Bar, 20 µm. (G) Western blot analysis performed on protein lysates from tibialis anterior, EDL, and soleus muscles shows no difference in ankB and dystrophin (Dp427) expression levels between control (CNTR) and obscurin KO mice. α-Actinin is used as loading control. IB, immunoblot.