Demonbreun et al., http://www.jcb.org/cgi/content/full/jcb.201512022/DC1

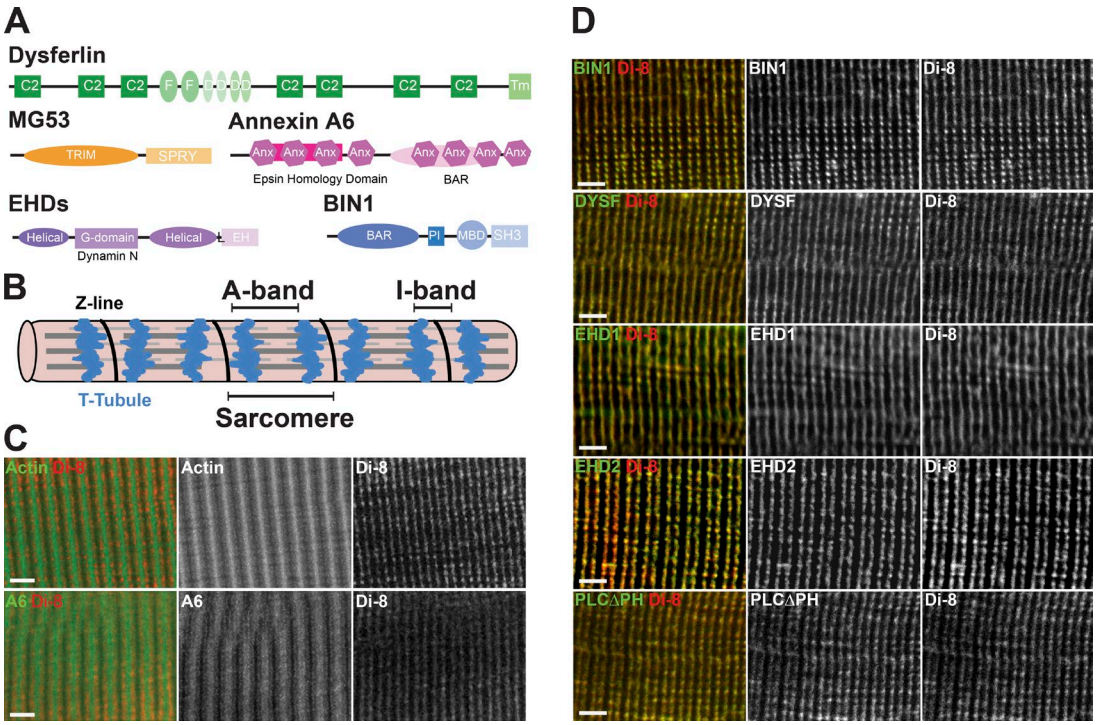


Figure S1. **Annexin A6 flanks the T-tubules in normal, uninjured skeletal muscle.** (A) Schematic diagram of proteins associated with T-tubules and implicated in membrane repair, including BIN1, EHD proteins, annexin A6, and dysferlin. The genes encoding BIN1, also known as amphiphysin II, dysferlin, and MG53, are implicated in inherited muscle disease in humans and mice (Bansal et al., 2003; Nicot et al., 2007; Cai et al., 2009). A, annexin domain; C2, C2 domain; D, Dysf domain; EH, eps homology domain; F, ferlin domain; MBD, Myc-binding domain; PI, phosphoinositide domain; SH3, Sarc homology domain; SPRY, SPla and ryanodine receptor; T, transmembrane domain; TRIM, tripartite motif-containing. (B) Myofiber schematic indicating the close approximation of T-tubules to the actin-enriched Z-disc. (C) Myofibers expressing fluorescently labeled actin (LifeAct-mTurq2) show the concentration of actin in the I-band and its position adjacent to Di-8, which marks T-tubules. Annexin A6 is found in a pattern similar to actin and is flanked by the T-tubules. (D) Live-cell imaging of myofibers electroporated with tagged plasmids and imaged with Di-8 to mark T-tubules. BIN1, DYSF, EHD1, and EHD2 colocalize to the T-tubule in live muscle. The PLCΔPH-GFP plasmid expresses a PH domain known to bind phosphatidyl inositol 4,5 biphosphate 2 (PIP2) and localizes to the T-tubule (Stauffer et al., 1998). Bars, 4 µm.

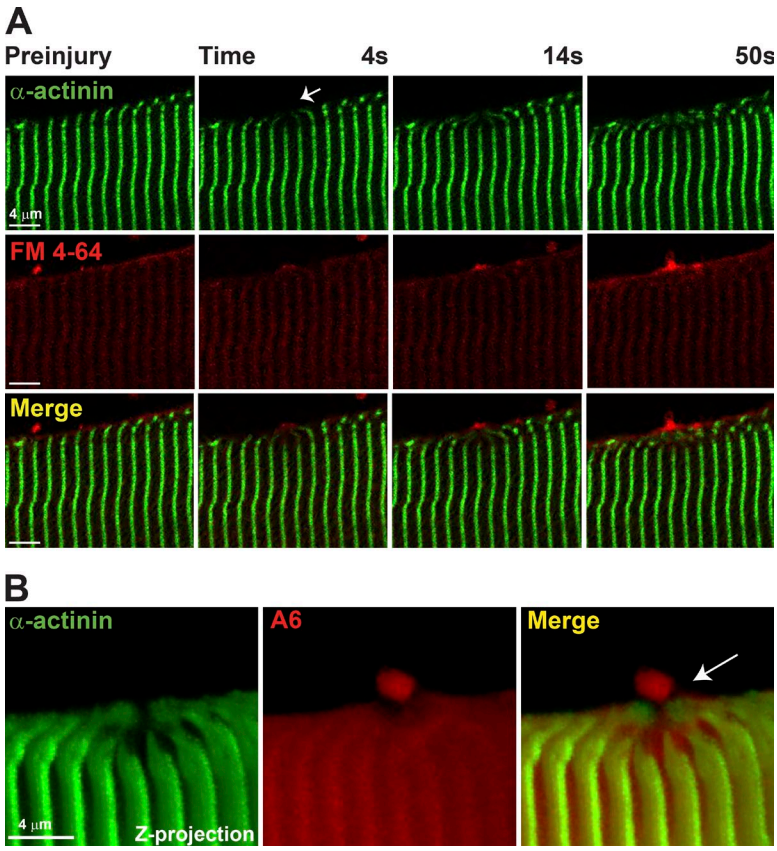


Figure S2. **Local sarcomere disruption after membrane injury.** (A) Myofibers were electroporated with α-actinin–GFP, which localizes to the z-line. Myofibers were damaged in the presence of FM4-64 (red). Sarcomere disruption was visible at the injury site (arrow), whereas large-scale contraction was absent. (B) Myofibers were coelectroporated with α-actinin (green) and annexin A6 (red) and subjected to laser-induced damage. Sarcomere disruption marked by α-actinin occurred beneath the annexin A6 cap (arrow). Bars, 4 µm. *n* = 12 myofibers from *n* = 3 mice per condition.

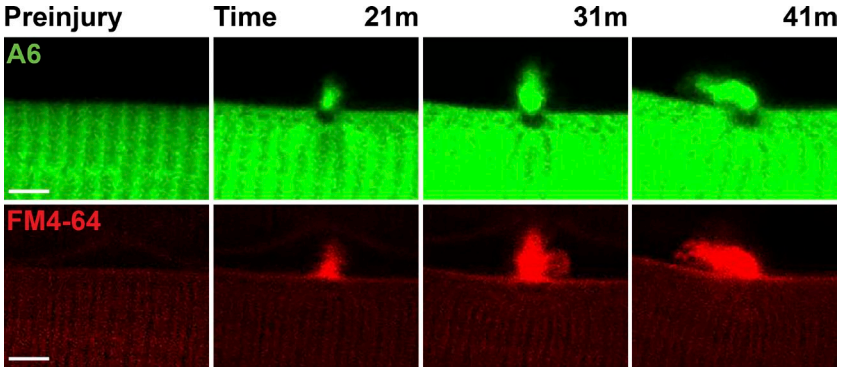
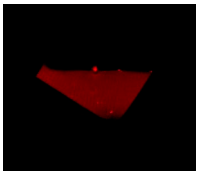
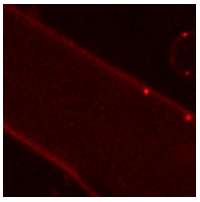


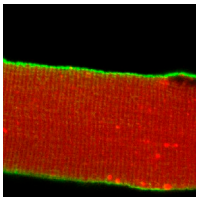
Figure S3. **Annexin A6 caps remained visible for over 40 min after damage.** Myofibers were electroporated with annexin A6–GFP and damaged in the presence of FM4-64. Myofibers remained viable and intact by the absence of global FM4-64 uptake and no evidence of hypercontraction. Annexin A6 cap formation persisted throughout the 40 min of imaging. Bars, 4 µm.



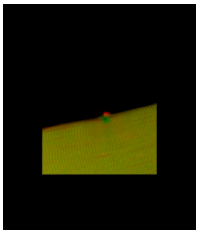
Video 1. **mCherry-labeled annexin A6 formed a repair cap positioned on the exterior surface of the myofiber.**



Video 2. **Lateral movement of FM4-64 puncta was seen moving toward the site of injury.**



Video 3. **mCherry-labeled annexin A6 along with GFP-labeled EHD2 highlighting the distinct subdomains of the repair complex.** Annexin A6 localizes to the cap and EHD2 localizes to the shoulder.



Video 4. **Shown is a z-stack image of mCherry-labeled annexin A6 expressed in conjunction with LifeAct-mTurq2, demonstrating that F-actin localized to the annexin A6 clearance zone.**

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