SUPPLEMENTAL MATERIAL

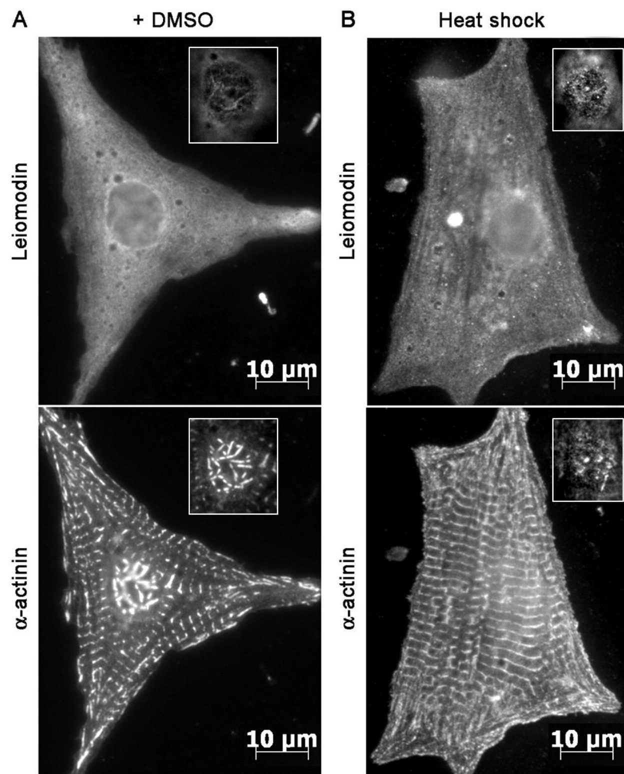
Supplemental Fig. S1. Nuclear localization of endogenous Lmod in cardiomyocytes after heat-shock and DMSO treatment. (A) Cardiomyocytes cultured for 2 days were incubated with 10% DMSO for 1 h at 37°C and fixed. **(B)** Cardiomyocytes cultured for 2 days were incubated for 1 h in a water bath at 42-43°C, followed by fixation. Hepes 20 mM was added to cells before heat-shock to maintain a constant pH. After fixation, cells were co-stained with antibodies against Lmod and α-actinin. Insets display images from an upper focal plane to highlight the presence of rod-like Lmod structures inside the nucleus after heat-shock and DMSO treatment. Bars, 10 μm.

Supplemental Fig. S2. The role of the C-terminal extension of Lmod in the latrunculin-B sensitivity of myofibril localization. (A). Examples of representative cells expressing GFP-$Lmod_{FL}$ or GFP-$Lmod_{1-342}$ after 5 min and 15 min latrunculin-B treatment. Cells were co-stained with phalloidin and an antibody against the Z-disk protein α-actinin. Bars, 10 μm. **(B.)** Analysis of GFP-Lmod and GFP-Lmod1-342 localization 5/15/30 min after treatment with 20 μM latrunculin-B (LatB). Incubation of cardiomyocytes with latrunculin-B induced rapid dissociation of GFP-$Lmod_{FL}$ from sarcomeres, whereas the localization of GFP-$Lmod_{1-342}$ was less sentitive to this actin monomer sequestering drug. For each experiment, >30 cells were analyzed.

Supplemental Fig. S3. Co-sedimentation of Lmod constructs with F-actin. (A) Co-sedimentation of $Lmod_{FL}$ with F-actin in the absence and the presence of TM. $Lmod_{FL}$ purified using two affinity tags, first a C-terminal intein and second an N-terminal His-tag, includes two C-terminal degradation bands. In the absence of F-actin, all three Lmod bands are found in the supernatant. In the presence of F-actin, only the band corresponding to full-length protein partially cosediments with F-actin, indicating that a C-terminal sequence (most likely the W domain) is involved in the interaction with F-actin. The same result was obtained in the presence of TM (used at a 7:2 actin:TM ratio), indicating that TM does not increase nor prevent the binding of Lmod to F-actin. Notice that rabbit skeletal muscle TM consists of a natural mixture of α and β isoforms present in muscle at a ratio of between 3 and 4 to 1. (B) The Tmod-like

construct $Lmod_{1-342}$ does not co-sediment significantly with F-actin in the absence or the presence of TM. This is consistent with our interpretation above that a C-terminal sequence, most likely the W domain, is responsible for the co-sidementation with F-actin. Notice that $Lmod_{1-342}$ migrates only slightly higher than actin. The experiments shown here were carried out using 4-15% SDS gradient gels.

Skwarek-Maruszewska et al., Fig. S1



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A

5 min | 5 min | 15 min

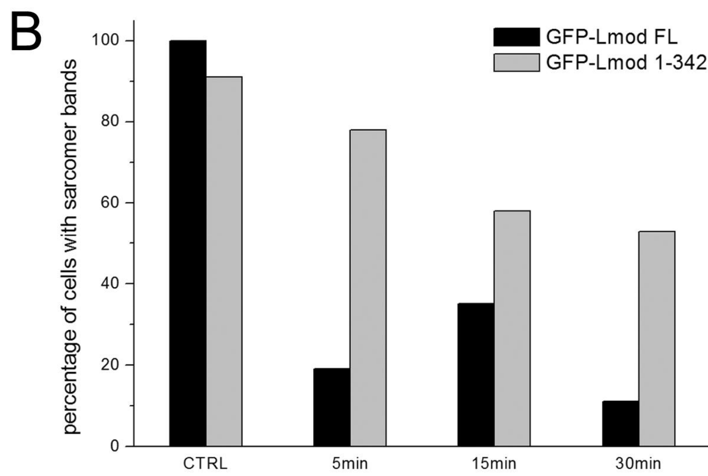
GFP-Lmod FL | GFP-Lmod 1-342

F-actin | F-actin

anti-α–actinin | anti-α–actinin

10 μm

B



Skwarek-Maruszewska et al., Fig. S3