Supplementary Material

DEV012849 Supplementary Material

Files in this Data Supplement:

Table S1.

Table S2.

Table S3.

Table S4.

Movie S1. Time-lapse movie of a contracting primary myotube in culture. The myotube expresses GFP as a result of the fusion between *Dmef2-Gal4* and *UAS-2EGFP* cells.

Movie S2. Time-lapse movie of contracting primary myotubes shown at lower magnification.

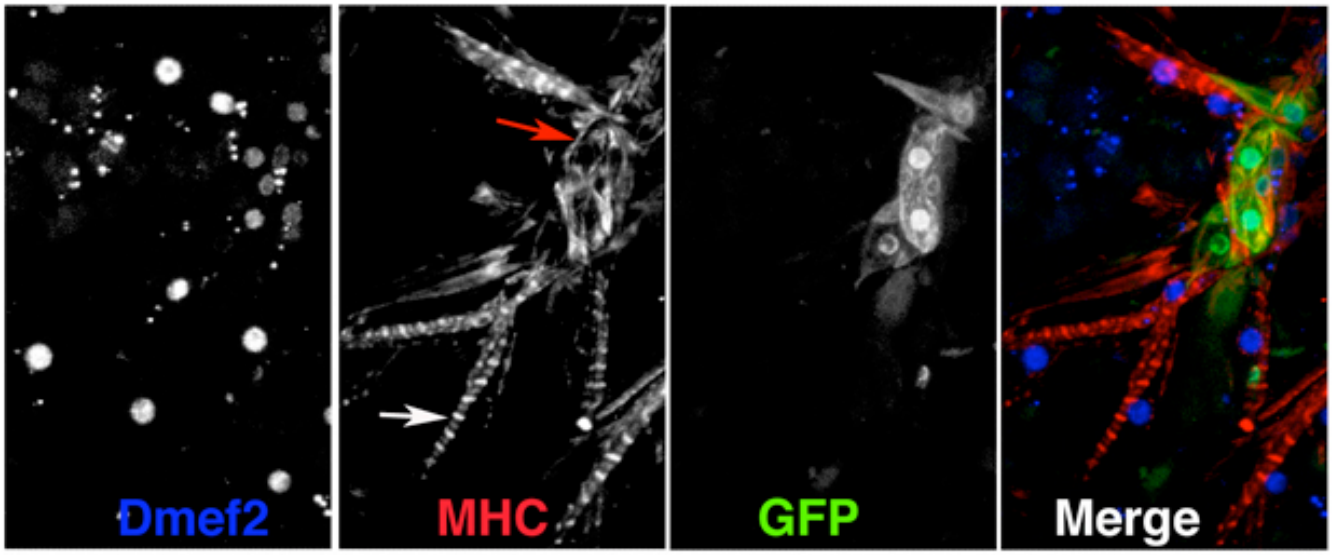

Fig. S1. GFP-expressing myotubes in primary culture derived from embryos carrying *Hand-Gal4*/*UAS-2EGFP* transgenes. Primary cells were stained for Dmef2 (blue in merge), Mhc (red in merge) and GFP (green in merge). Note that GFP-positive cells are also stained for Mhc, which shows periodic sarcomeric organization (red arrow), but myofibrils in these cells are thinner and less bundled (red arrow) compared with those in non-GFP primary muscles (white arrow). The number of nuclei per cell in the cells positive for both GFP (derived from *Hand-Gal4, UAS-2EGFP*) and Dmef2 was ~1.2±0.4, many fewer than in the GFP-negative Dmef2-positive primary myotubes presumably derived from somatic muscles (~3.7±0.2).

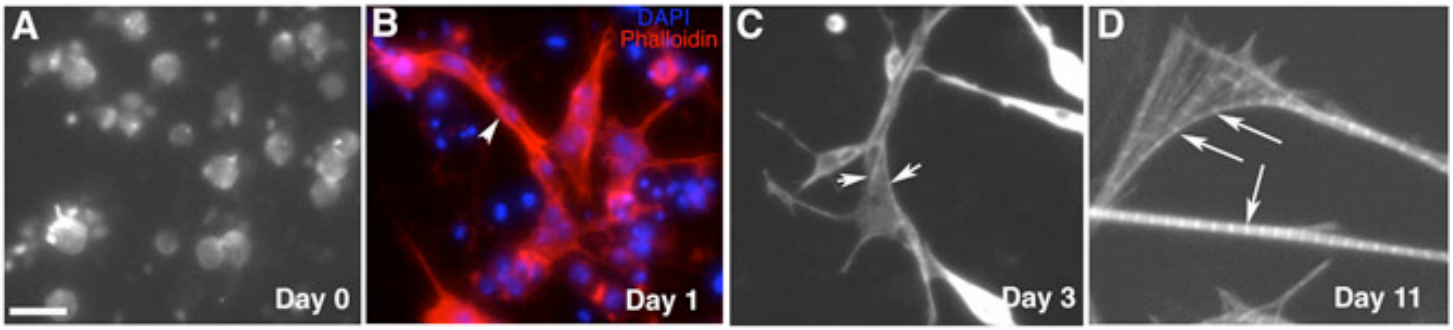

Fig. S2. Myogenesis in culture with a serum starvation step. (**A**) Freshly isolated cells were stained for Actin with phalloidin. (**B**) Primary cells were stained for Actin with phalloidin and with DAPI (blue) immediately before the addition of serum. Note that multinucleated myotubes began to form, but that the periodic pattern of Actin filaments was not obvious (arrowhead). Phalloidin stainings of primary myotubes at 2 days (**C**) and 10 days (**D**) after serum addition at 18°C in culture. Note that the periodic pattern of Actin filaments is obvious in C and D, whereas myofibrils are not bundled in C (short arrows), but are in D (long arrows). Scale bar: 40 µm.

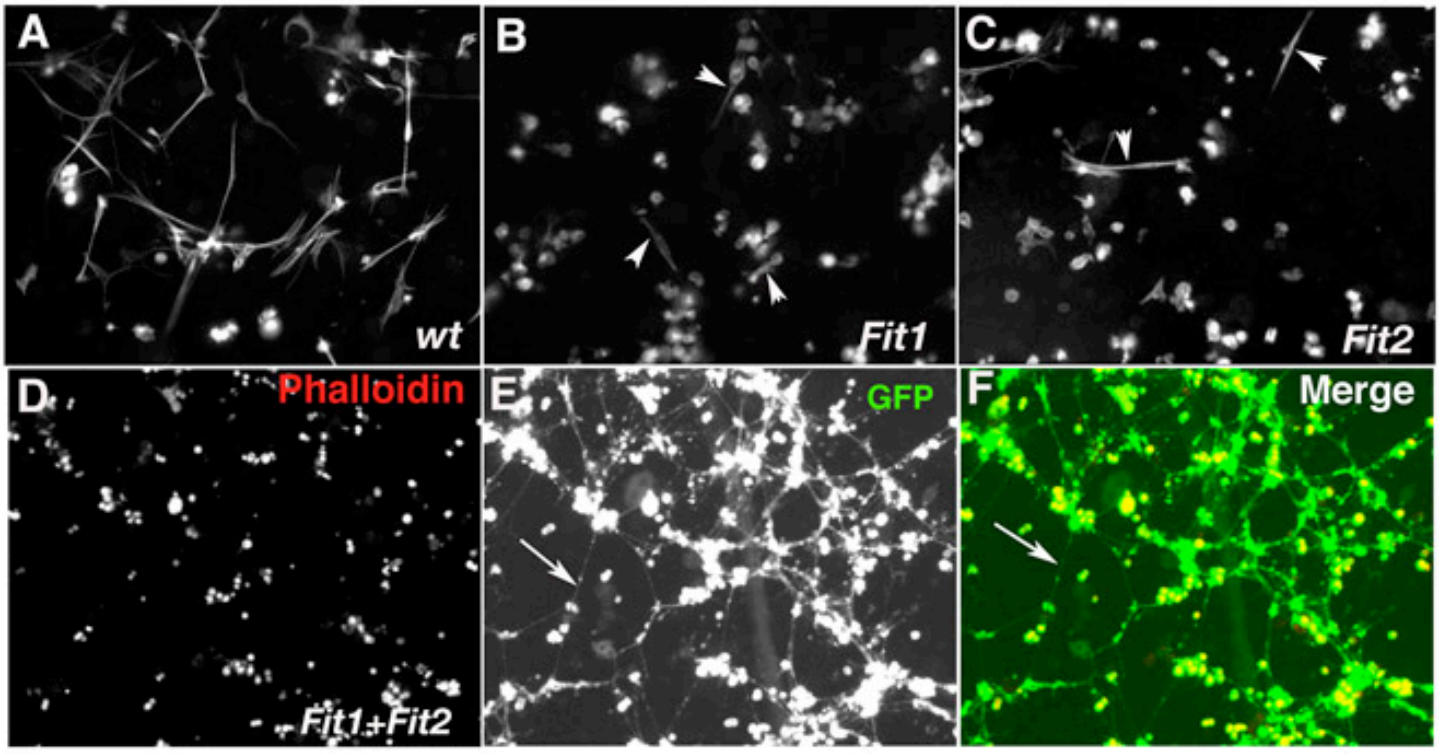

Fig. S3. RNAi phenotypes of *Fit1* and *Fit2* in primary culture. Primary cells treated with dsRNAs targeting (**A**)*lacZ*, (**B**) *Fit1* and (**C**) *Fit2* stained with phalloidin. (**D-F**) Primary cells were isolated from embryos carrying*Dmef2-Gal4, D42-Gal4, UAS-mito-GFP* and treated with dsRNAs targeting both *Fit1* and *Fit2.* Primary muscles in culture were detected by phalloidin staining for Actin (D and red in F). mito-GFP (E and green in F) revealed both neuronal axons (white arrows in E,F) and rounded-up muscles (yellow in F).