METHODS

Electroporation and protein injection into muscle

For *in vivo* electroporation, 40 μg of plasmid DNA in 0.9% NaCl was injected directly into the right *tibialis anterior* (TA) muscle of anesthetized male C57BL/6 mice (10 weeks of age). Immediately after injection of plasmid, electric stimulation was applied directly to the TA muscle using a pulse generator (ECM 830, BTX) of 100 volts for 6 pulses, with a fixed duration of 20 ms and an interval of 200 ms between the pulses equipped with 5 mm needle electrodes (BTX). Experimental and contralateral muscles were isolated and embedded in OCT-10% sucrose (Tissue-Tec), cooled with liquid nitrogen. Cross-sections (14 μm) of the muscle were obtained using a cryostat (Leica CM1850). To ensure that the same areas of the muscles were compared we cut the TA muscle in the middle of the belly and counted the sections which were used for immunostainings. Protein injections of recombinant Wnt7a protein (R&D Systems) were conducted in a similar fashion as injection of plasmid DNA. Mice were sacrificed two weeks after electroporation or protein injection. Adult (10 weeks of age) C57/Bl6 mice were obtained from Charles River. All experiments were performed following the regulations of the University of Ottawa for animal handling and animal care.

Cell culture and transfection

Mononucleated muscle derived cells were isolated from hind-limb muscles of Balb/C mice (4 weeks old), cultured and differentiated as previously described[36]. Cells were sorted by FACS and cultured as previously described[4]. C2C12 myoblasts were cultivated using standard methodology[37]. Recombinant Wnt7a, Wnt3a and Wnt5a (R&D Systems) were administered to the cells in a final concentration of 50 ng/ml. Rapamycin (NEB), LY294002 (NEB), AG1024 (EMD Bioscience), AraC (Cytosine β- D –arabinofuranoside, Sigma), suramin (Sigma) and BIO

(6-bromoindirubin-3'-oxime**,** Sigma) were applied in the following final concentrations: rapamycin (20 ng/ml), LY294002 (50 μM), AG1024 (10 μM), (AraC 50 μM), suramin (250 μM), BIO (1 μM). C2C12 cells were transfected with the expression plasmids or siRNA (Ambion) using the lipofectamine 2000 (Roche) reagent according to instructions provided by the manufacturer.

Protein and RNA analyses

Western and co-immunoprecipitation analyses were performed as described previously [38, 39]. Immunoprecipitation was conducted using Chromotek-GFP-Trap-Beads purchased from Allele Biotechnology & Pharmaceuticals according to instructions provided by the manufacturer. Immunofluorescence analyses were performed as described by [38]. Nuclei were counterstained with Hoechst as recommended by the manufacturer. For antibodies used in this study, see Table S1. RNA extraction and quantitative Real-Time PCR was performed as described previously[4]. For primers used in this study see Table S2. Tap purification and mass spectrometry were performed as described earlier[40].

Statistical analyses

A minimum of three up to five replicates were analyzed for each experiment presented. Data are presented as standard error of the mean (Microsoft Excel), we assessed statistical significance using a Student's t test (Microsoft Excel). Differences were considered as statistically significant with a p value <0.01.

Mice and animal care

Adult (10 weeks of age) C57/Bl6 mice were obtained from Charles River. All mice were maintained inside the animal care facility and experiments were performed following the regulations of the University of Ottawa for animal handling and animal care.

Electroporation and protein injection into TA muscles

For *in vivo* electroporation 40 ug of plasmid DNA in 0.9% NaCl was injected directly into the right TA muscle of anesthetised mice. Immediately after injection of the plasmid DNA electric stimulation was applied directly to the TA muscle using a pulse generator (ECM 830, BTX) of 100 volts for 6 pulses, with a fixed duration of 20 ms and an interval of 200 ms between the pulses equipped with 5 mm needle electrodes (BTX). Experimental and contralateral muscles were isolated and embedded in OCT-10%sucrose (Tissue-Tec), cooled with liquid nitrogen. Cross-sections (14 um) of the muscle were obtained using a cryostat (Leica CM1850). To ensure that the same areas of the muscles were compared we cut the TA muscle in the middle and counted the sections which were used for immunostainings. Protein injections of recombinant Wnt7a protein (2.5 ug; R&D Systems) were conducted in a similar fashion as injection of plasmid DNA. Mice were sacrificed two weeks after electroporation or protein injection.

Cell culture and transfection

Mononucleated muscle derived cells were isolated from hind-limb muscles of Balb/C mice (4 weeks old) as previously described[1]. Cells were sorted by FACS and cultured as previously described [2]. C2C12 myoblasts were cultivated and differentiated as described by [3]. Recombinant Wnt7a, Wnt3a and Wnt5a (R&D Systems) were administered to the cells in a final concentration of 50 ng/ml. Rapamycin (NEB), LY294002 (NEB), suramin (Sigma) and AG1024 (EMD

Bioscience) were applied in the following final concentrations: rapamycin (20 ng/ml), LY294002 (50 uM), AG1024 (10 uM), suramin (250 μM). C2C12 cells were transfected with the expression plasmids or siRNA (Ambion) using the lipofectamine 2000 (Roche) reagent according to instructions provided by the manufacturer.

Immunoblot analyses and immunoprecipitation

Immunoblot analyses were performed as described by [4]. The following antibodies were obtained from Cell Signaling and used in a concentration recommended by the manufacturer: pan-Akt; Ser473-Akt; pS6; pS6kinase. Antibodies directed to myogenin and MyHC were obtained from Santa Cruz, antibodies directed to tubulin were purchased from Sigma. Immunoprecipitation was conducted using Chromotek-GFP-Trap-Beads purchased from Allele Biotechnology & Pharmaceuticals according to instructions provided by the manufacturer.

Immunofluorescence analysis

Immunofluorescence analyses were performed as described by [4]. Antibodies directed to laminin were obtained from Sigma (1:1000). Nuclei were counterstained with Hoechst as recommended by the manufacturer.

Real-Time PCR analysis

RNA extraction and Real-Time PCR was performed as described by [2].

References Cited

1. Kuang, S., Kuroda, K., Le Grand, F. & Rudnicki, M.A. Asymmetric self-renewal and commitment of satellite stem cells in muscle. *Cell* **129**, 999-1010 (2007).
2. Le Grand, F., Jones, A.E., Seale, V., Scime, A. & Rudnicki, M.A. Wnt7a activates the planar cell polarity pathway to drive the symmetric expansion of satellite stem cells. *Cell stem cell* **4**, 535-547 (2009).
3. Parker, M.H., Perry, R.L., Fauteux, M.C., Berkes, C.A. & Rudnicki, M.A. MyoD synergizes with the E-protein HEB beta to induce myogenic differentiation. *Molecular and cellular biology* **26**, 5771-5783 (2006).
4. von Maltzahn, J., Euwens, C., Willecke, K. & Sohl, G. The novel mouse connexin39 gene is expressed in developing striated muscle fibers. *J Cell Sci* **117**, 5381-5392 (2004).