

# Supporting Information

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## SI Materials and Methods

**Materials.** Total RNA from skeletal muscle mouse tissue was obtained from Zyagen. nicotinic acid adenine dinucleotide phosphate-acetoxymethylester (NAADP-AM) was synthesized in house. Ned-19 was obtained from IBScreen. Ryanodine, thapsigargin, xestospongine C, and U73122 were obtained from Calbiochem; dantrolene and bafilomycin were obtained from Sigma.

The following specific primers were used for the PCR:

RyR1s: GAAGTTTCTGGACAAACACG  
RyR1as: GCTCTTGTGTAGAAATTTGCG  
RyR2s: TCCAGCGATACTGCTAAAGTG  
RyR2as: CATCGCTGAAATCTAGTGCAG  
RyR3s: GGTAACCTGAGTTCACGAC  
RyR3as: CAAGCTGATTCTGGAGACAG  
Myos: ATGGAGCTGTATGAGACATCC  
Myoas: TCAGTTGGGCATGGTTTCGT  
TPC1s: ATGGCTGTAAGTTTAGATGAC  
TPC1as: GACGAACGTGTGGAAGCCC  
TPC2s: ATGGCGGAGAAGAGCAG  
TPC2as: CCGCAGGGGCTCCTCAC  
InsP<sub>3</sub>R1s: TTAACCAAGATCCTCTTGCCA  
InsP<sub>3</sub>R1as: CAAAGGTGTGTTCTCCTCAC  
InsP<sub>3</sub>2s: ACCACCACAGACAGGAAACA  
InsP<sub>3</sub>2as: GGCTGGTCTCCAAGAGCT  
InsP<sub>3</sub>s: ACCACAGGGGGCCTGGGG  
InsP<sub>3</sub>as: CCTCTCCTCCTCCTGCAG

**Cell Culture.** C2C12 cells (cell line service, Eppelheim passage 20–30) were grown in DMEM with 1 g/L glucose (Biochrom; FG 0415) supplemented with 20% FBS until 90% to 100% confluence. C3H Primary myoblasts were isolated as described previously (1). The cells were grown on collagen I coated plates (Greiner Bio-One) in 80% Nutrient mixture F-10 Ham (Sigma; N6908), 20% FBS, and 2.5 ng/mL basic fibroblast growth factor (Roche). Cells were cultured at 37 °C in a humidified incubator containing 5% CO<sub>2</sub>. For differentiation, growth medium was replaced with DMEM supplemented with 2% horse serum. Medium was changed every 24 to 48 h.

**Calcium Imaging.** Undifferentiated C2C12 cells were loaded with the fluorescent calcium indicator fura-2 (2 μM) by incubation of the cells in HBSS (Sigma) plus fura-2-AM (fura 2 acetoxymethyl ester) for 45 min and HBSS alone for an additional 15 min to allow de-esterification of the dye. Coverslips were mounted in a 3-mL bath on the stage of a Zeiss inverted microscope equipped with a Princeton charge-coupled device camera. Fura-2 fluorescence [excitation at 340 and 380 nm (Till Photonics polychrome II), emission at 520 nm] of single cells was acquired every 5 s. All imaging was controlled by Metaflour software (Molecular Devices) and data were analyzed off line using Metaflour Analysis (Molecular Devices).

**Preparation of cDNA and PCR.** Complementary DNA was synthesized using the cDNA first-strand synthesis kit (Fermentas) and 3 μg RNA from C2C12 cells as a template. Amplification of DNA

fragments was carried out with specific primers using the following PCR schema: 35 cycles with 58 °C annealing temperature for 30 s and 1-min synthesis with dream Taq (Fermentas) and 1% cDNA.

**Cloning and Preparation of Probes.** PCR products were cloned into the pBluescript SK-vector using the BamHI and EcoRI cloning sites. Inserts were verified by sequencing. For the synthesis of antisense riboprobes, 1 μg of the vector carrying an insert was digested with EcoRI for 3 h. Transcription was carried out over night using T3 polymerase (Fermentas) and the digoxigenin RNA labeling kit (Roche). Probes were purified with the RNAsasy mini kit (Qiagen), stored at –80 °C and used in a dilution of 1:1,000 to 1:5,000.

**Transfection with TPC1 and TPC2-Specific siRNA.** Transfection with specific siRNA was carried out using predesigned, TPC1-specific siRNA (sense: 5'-GCGUCUUCUUCUAUGUAUATT-3', antisense: 5'-UAUACAAUGAAGAAGACGCAG-3') and TPC2-specific siRNA (sense: 5'-UCAUCCUGCUGGUUUCGGATT-3', antisense: 5'-UCCGAAACCAGCAGGAUGATG-3') purchased from Ambion. C2C12 cells were transfected using the transfection reagent Interferin (Polyplus Transfection) according to the manufacturer's protocol.

**Northern Blot Analysis.** Total cellular RNA was isolated using the RNAsasy mini isolation kit (Qiagen). RNA was quantified and separated on a 1% agarose gel containing 2% formaldehyde. Subsequently, RNA was transferred to a nylon membrane (Amersham) by capillary forces, cross linked, and stained with methylene blue. The methylene staining of the 18S RNA was used as loading control. The membranes were hybridized with digoxigenin labeled probes at 68 °C for at least 12 h. After washing, membranes were incubated with phosphatase-coupled anti-digoxigenine antibody (Roche) and developed with CDP-Star (Roche) as a chemoluminescent substrate. Signals were detected via exposure to x-ray films for 1 to 10 min.

**Immunofluorescence.** For staining with myosin heavy chain, cells were grown and differentiated in the presence or absence of one of the named compounds or DMSO for 4 d. Medium was removed and the cells were washed 3 times with PBS, fixed with ice cold methanol for 20 min at –20 °C, blocked with 10% FCS, and incubated with the myosin heavy-chain antibody (Acris Antibodies GmbH) in 10% FCS for 30 min. Cells were washed three times with PBS and then incubated with FITC-conjugated goat anti-mouse antibody (BD Bioscience) in 10% FCS for 25 min. Nuclei were stained with DAPI.

**Cellular Labeling.** The NAADP receptor was labeled by incubation with 100 μM Ned-19 for 1 h. Lysosomes cells were labeled by incubation with 50 nM lysotracker red for 15 min. A Zeiss LSM 510 confocal microscope was used to detect lysotracker red with excitation at 568 nm and emission collected at 590 nm with a long-pass filter. Ned-19 was monitored with excitation at 355 nm and emission collected at 415 nm with 30-nm band-pass filter.

1. Rando TA, Blau HM (1994) Primary mouse myoblast purification, characterization, and transplantation for cell-mediated gene therapy. *J Cell Biol* 125:1275–1287.