Supplementary Materials and Methods:

Genotyping

Genomic DNA was isolated from ear punches or tail biopsies of mice using the HOTSHOT method as described previously (Truett et al., 2000). The PCR conditions for genotyping: 95°C for 3 min, 95°C for 30 sec, 60°C for 30 sec, 72°C for 40 sec, repeated for 30 cycles; 72°C for 5 min. The forward primer and the reverse primers for the $Ca_V1.1$ gene were 5´ -CCTGTCTCTGTCTGGTCTTCC- 3´ and 5´ -GCCTGCTCTAAGGAAAGGAG- 3´, respectively. Expected band size for $Ca_V1.1\Delta E29$ is 344 bp and for wildtype is 373 bp.

Quantitative TaqMan RT-PCR

For expression analysis of $Ca_V1.1$ splice variants RNA was isolated from soleus (Sol), extensor digitorum longus (EDL) and diaphragm (Dia) muscle of E17, newborn, 3 weeks, 6 weeks, 16 weeks and 15-18 months old mice using the RNeasy® Fibrous Tissue Mini kit (Qiagen, Cat. No.74704, Venlo, NL). Except for the 15-18 months old mice, in which case only wildtype males were used, muscles were isolated from mice of either sex and of all genotypes. Following reverse transcription (SuperScript®II Reverse Transcriptase, Invitrogen, Carlsbad, CA, USA), the absolute number of $Ca_V1.1a$ and $Ca_V1.1e$ transcripts was assessed by quantitative TaqMan PCR (50 cycles), using a standard curve generated from PCR products of known concentrations as described previously (Schlick et al., 2010). For primers see Supplementary Table 1.

To analyze expression of other genes involved in fiber type regulation, only Sol and EDL muscles were used from 6-7 months old mice. The relative mRNA expression levels of PGC-1α and SIX1 were calculated as relative amount of specific cDNA versus HPRT1, using the ΔΔCt method ($2^{-\Delta\Delta Ct}$), where ΔCt was defined as Ct (gene) – Ct (HPRT1, housekeeping gene) and ΔΔCt as ΔCt – ΔCt (WT control). Taqman gene expression assays designed to span exon-exon boundaries (Table S1) were purchased from Applied Biosystems (Vienna, AT). Data were normalized as described previously (Schlick et al., 2010) and analyzed using the ABI PRISM 7500 sequence detector (Applied Biosystems, Vienna, AT).

Affymetrix GeneChip analysis

The whole-genome gene expression data were obtained at the Expression Profiling Unit of the Medical University Innsbruck using the Affymetrix GeneChip MoGene-1.0-ST-v1 Array. Sample

preparation was performed according to the manufacturer's protocols. In brief, RNA quantity and purity was determined by optical density measurements (OD 260/280 ratio) and by measuring the RNA integrity using the Agilent Technologies 2100 Bioanalyzer. Then, 250 ng of RNA per sample were processed to generate biotinylated hybridization targets using the Affymetrix GeneChip WT Expression kit and the Affymetrix GeneChip WT Terminal Labeling KIR. Resulting targets were hybridized to the Affymetrix GeneChip MoGene-1.0 ST v1 and stained in an Affymetrix fluidic station 450. Raw fluorescence signal intensities were recorded by an Affymetrix scanner 3000 and image analysis was performed with the Affymetrix GeneChip Command Console software (AGCC). All further analysis was performed in R (version 3.1.2) using packages from the Bioconductor project (Gentleman et al., 2004). Pre-processing of the raw microarray data was performed as described in (Bindreither et al., 2014). In brief, raw microarray data was pre-processed using the "generalgcrma" package (Rainer et al., 2012) and our custom transcript-level "CEL definition file" (CDF) that defines probe sets for each transcript of all genes in the Ensembl database version 75. After GCRMA pre-processing a representative transcript probe set was selected for each gene based on a combination of its average expression and variance of expression across all EDL or Soleus samples.

Differential gene expression analysis was performed using the limma package (Smyth, 2004). The resulting p-values were subsequently adjusted for multiple hypothesis testing using the method from Benjamini and Hochberg (Benjamini and Hochberg, 1995) for a strong control of the false discovery rate (FDR). Genes with an M-value > 1 (representing more than 2-fold regulation) at a 5% FDR (adjusted p-value < 0.05) were considered to be significantly differentially expressed.

The raw and preprocessed microarray data have been submitted to the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=shkxmwesffehzcb&acc=GSE67803).

Behavioral experiments

All behavioral experiments were done at 2 and 8 months of age.

Wire hang test: Mice were put on a wire mesh which was then turned upside down. Time was recorded until the mice fell off, or tests were ended at 60 s. Tests were repeated thrice for each mouse.

Homecage activity: To monitor the homecage activity, mice were individually placed in cages with free access to food and water, enriched with a plastic tube. Movement of mice was monitored through an infrared detection system (InfraMot, TSE Systems, Homburg, DE). Analysis was started at

5 PM for a 72 h period. Arbitrary activity counts for a period of 48 h starting from 6:46 AM were collapsed into 60 min bins to examine the hourly distribution of activity.

Rotarod test: The mice had two familiarization trials on the rotarod (Acceler Rota-Rod 7650, Ugo Basile, IT) at 4 rpm for 1 min each with an interval of 10 min between the trials. 30 min after the second familiarization trial, they were tested on an accelerating (4-40 rpm) rotarod for up to 300 s. The test was repeated after 15 min.

Treadmill: The mice were familiarized to the treadmill (Exer 3/6 open treadmill, Columbus Instruments, Ohio, USA) for 30 min at rest followed by two times for 5 min at a speed of 10 m/min at an interval of 5 min. The next day before starting the test, the mice underwent another familiarization for 5 min at 10m/min. 15 min after this familiarization the mice were tested with an accelerating speed starting at 10 m/min increasing for 2 m/min every 5 min. The cut off time was 30 min.

Voluntary activity wheel measurement: Mice from both groups were singly housed in a cage with a mouse running wheel (Campden Instruments Ltd., Loughborough, UK). Wheels were interfaced to a computer and revolutions were recorded in 20 minutes intervals, continuously for 8 days. The average and the maximal speed, the distance and the duration of running was calculated for the individual mice and then averaged by groups.

Forepaw grip test: The force of forepaw was measured as described earlier (Bodnar et al., 2014). Briefly, when the animals reliably grasped the bar of the grip test meter, they were then gently pulled away from the device. The maximal force before the animal released the bar were digitized at 2 kHz and stored by an online connected computer. For better comparison the maximal force was normalized to the body weight of the animals.

Measurement of contractile force

Muscle contractions of 3-6 month old mice were measured as described previously (Oddoux et al., 2009). In brief, EDL and Sol were placed horizontally in an experimental chamber continuously superfused (10 ml/min) with Krebs' solution (containing in mM: NaCl 135, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1, Hepes 10, glucose 10, $NaHCO_3$ 10; pH 7.2; room temperature) equilibrated with 95% O_2 plus 5% CO_2. One end of the muscle was attached to a rod, the other to a capacitive mechanoelectric force transducer. Contractions were elicited by 2 ms supramaximal electrical pulses delivered by two platinum electrodes placed adjacent to the muscle. Force responses were digitized at 2 kHz by using Digidata 1200 A/D card and acquired with Axotape software (Axon Instruments, Foster City, CA,

USA). Muscles were then stretched by adjusting the position of the transducer to a length that produced the maximal force response and allowed to equilibrate for 60 min before testing. At least 10 twitches at 2 s intervals were recorded from each muscle. The individual force transients within such a train varied by less than 3% in amplitude, thus the mean of the amplitude of all transients was used to characterize the given muscle. To elicit tetanic contractions, trains of pulses were applied with a frequency of 200 Hz for 200 ms (EDL) or 100 Hz for 500 ms (Sol). Duration of individual twitches and tetani were determined by calculating the time between the onset of the transient and the relaxation to 90% of maximal force. The time constant (Tau) of the On and Off phase of contraction was determined from a single exponential fit to the rising and falling phase of the force transient. To test muscles fatigue 150 tetani were applied with 0.5 Hz (Oddoux et al., 2009). The degree of fatigue was expressed by normalizing the amplitude of each tetanus to that of the first tetanus. The tetanic fusion frequency was tested with a series of repeated pulses stimulated at increasing frequencies starting from 10 Hz. To quantify development of complete tetanus the following equation was fitted to the maximum of the force transient in one series:

$$T = A/(1+\exp(-(F-F_{50})/k) \qquad \text{(Eqn. 1.)}$$

where T is the actual tension at frequency F, A is the amplitude of the maximal tetanus, F_{50} is the frequency at half maximal tension and k is the slope factor of the function.

Isolation of whole skeletal muscles and single muscle fibers

3-4 month old mice were anesthesied with pentobarbital (27 mg/kg), then *m. flexor digitorum brevis* (FDB) from the fore limb, and the EDL and Sol from the hind limb were dissected. Single muscle fibers were enzymatically dissociated in calcium free modified Tyrode's solution (in mM, 137 NaCl, 5.4 KCl, 0.5 MgCl2, 11.8 Hepes, pH 7.4) containing 0.2% Type I collagenase (Sigma, St. Louis, USA) at 37°C for 50-55 minutes (Csernoch et al., 2008). To release single fibers muscles were triturated gently in modified Tyrode's solution supplemented with 1.8 mM $CaCl_2$. The fibers were then mounted on laminin-coated cover slip floors of culture dishes and kept at 4°C until use.

Voltage clamp and I_{Ca} measurement

The experimental design was as described in (Sztretye et al., 2011). Briefly, isolated fibers were voltage-clamped (Axoclamp 2B, Axon Instruments, Foster City, CA, USA) and imaged using a confocal microscope (Zeiss 5 Live, Oberkochen, Germany, 20x objective). Fibers were dialyzed with the rhod-2-containing internal solution. Experimental temperature was 20-22°C and the holding potential was

-80 mV. Pipette resistance varied between 1 and 2 MΩ. Correction for linear capacitive currents was performed by analog compensation. The peak current *versus* voltage relationship for I_{Ca} was fitted with:

$$I = (V_m - V_{Ca}) * G(V_m) \quad \text{(Eqn. 2)}$$

where V_m is the transmembrane potential, V_{Ca} is the estimated equilibrium potential for Ca, and $G(V_m)$ is the voltage dependence of the conductance given as:

$$G(V_m) = G_{max} / (1 + \exp(-(V_m - V_{50})/k)) \quad \text{(Eqn. 3)}$$

where G_{max} is the maximal conductance, V_{50} is the potential where the conductance is half of G_{max}, and k is the slope factor. All currents and the maximal conductance were normalized to fiber capacitance to take the size of the individual fibers into account.

External bath solution (in mM): 140 TEA-CH3SO3, 2 $CaCl_2$, 2 $MgCl_2$, 10 Hepes, 1 4-AP, 0.001 TTX (citrate), and 0.05 BTS (N-benzyl-p-toluene sulphonamide; Sigma-Aldrich). pH was adjusted to 7.2 with TEA-OH and osmolarity was adjusted to 320 mOsm with TEA methanesulfonate. Internal (pipette) solutions (mM): 110 N-methylglucamine, 110 L-glutamic acid, 10 EGTA, 10 Tris, 10 glucose, 5 Na ATP, 5 phosphocreatine Tris, 0.1 rhod-2, 3.56 $CaCl_2$, and 7.4 mM $MgCl_2$ were added for a nominal 1 mM [Mg^{2+}] and 100 nM [Ca^{2+}]. pH was set to 7.2 with NaOH and osmolarity to 320 mOsm with N-methylglucamine. Normal Tyrode's solution (in mM): 137 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 11.8 Hepes-NaOH, 1 g/l glucose, pH 7.4).

SOCE measurement

Isolated FDB fibers loaded with the Ca^{2+} sensitive dye fluo-8 AM (4 µM, 20 min, room temperature) were imaged with a laser scanning confocal microscope (Zeiss 5 Live, Oberkochen, DE) and subjected to multiple manual solution exchanges. Changes in the fluorescence were recorded in the presence or absence of [Ca^{2+}]e following the application of a releasing cocktail and presence of a SOCE inhibitor (10 µM BTP2) and/or 1 µM nisoldipine, a potent L-type Ca^{2+} channel blocker. Following the manual delimitation of the cell border, the change of [Ca^{2+}]i was calculated as ΔF/F0, where ΔF was calculated over the cell, while F0 next to cell. "Releasing cocktail" (in mM): 0.4 4-chloro-M-cresol (4-CMC), 0.004 thapsigargin (TG), and 0.05 BTS. In some experiments, the cells were preincubated with 1 µM nisoldipine and/or 10 µM BTP2.

Elementary calcium events

Isolated intact mouse skeletal muscle fibres from the FDB were loaded with 5 µM Fluo-8 AM for 20 min at RT. This solution was then replaced by normal Tyrode's solution. Images were captured with a Zeiss LSM 510 LIVE confocal microscope (Zeiss, Oberkochen, DE) equipped with a 40x oil immersion objective (NA=1.3). Fluo-8-AM was excited with the 488-nm line of an argon laser and the emitted fluorescent light was measured at wavelengths >505 nm. 15 min following application of the recording solution, series of 200 512×512 (x,y) images captured every 67 ms were collected in each tested fibre. Test experiments were carried out in the presence of 10 µM nisoldipine in the recording solution. In some cases the recording solution contains 1.8 mM calcium.

Detection of calcium release events and their analysis were performed using methods and algorithms described previously by (Szabo et al., 2010).

Immunostaining and image processing

Muscles were isolated from 6 month old mice and embedded in Tissue Tek and freshly frozen in isopentane cooled to -80°C. Frozen muscles were stored at -80°C and transferred to -20°C one day before sectioning. Cryosections of 8 µm thickness were prepared and stored at -80°C till further use. Before immunostaining, cryosections were thawed and air dried at room temperature (RT) for 30 min. Then the muscle sections were incubated in blocking buffer consisting of 5% normal goat serum in PBS containing 1% bovine serum albumin (BSA) and 0.5% Triton X-100 (PBS/BSA/Triton) or in M.O.M blocking solution (Vector Laboratories, Burlingame, CA, USA) for 1 h at RT. Next the sections were incubated in primary antibodies (Table S2) overnight at 4°C. On the following day the sections were washed in PBS/BSA/Triton thrice at interval of 10 min and then stained with goat anti-mouse IgG-Alexa Fluor 594 or goat anti-mouse IgM-Alexa Fluor 594 (1:4000; Invitrogen) for 1 h at RT. After washing with PBS/BSA/Triton thrice at intervals of 10 min, the sections were mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA). Samples were analyzed on a confocal microscope (TCS SP5, Leica microsystems, Wetzlar, DE) using a 40X objective (1.25 NA) and 16 bit images were acquired with the LasAF acquisition software (Leica microsystems, Wetzlar, DE). Figures were arranged in Adobe Photoshop CS6, and where necessary linear adjustments were performed to correct black level and contrast.

For fiber type analysis, all fibers within the entire muscle/cross-section were characterized. Fibers stained with specific antibodies against MHCs were counted in each section. For hybrid fibers serial cross-sections were analyzed simultaneously to locate the fibers stained with more than one

antibody. Fiber counts and percentages of fiber types were performed with Metamorph software (Molecular Devices, Sunnyvale, CA, USA).

SDH staining and analysis

Frozen sections of Sol and EDL were air dried for 30 min at RT and incubated in 0.2 M phosphate buffer (pH 7.4), 0.1 M succinic acid and 1.2 mM nitroblue tetrazolium for 1 h in a humidity chamber. Following incubation the slides were washed with milliQ water for 3 min and dried in methanol (Roth, Karslruhe, DE) for 2 min. The slides were then mounted in DPX mounting medium. Preparations were analyzed on an AxioImager microscope (Carl Ziess, Oberkochen, DE) using 25X (0.8 NA) and 40X (1.25 NA) objectives. 12-bit images were acquired with the SPOT Idea 1.3 Mp Color Mosaic Camera (SPOT Imaging solutions; Diagnostic Instruments Inc., Sterling Heights, MI, USA) and Spot Idea software (Version 4.6). The images were first converted to black and white tiff images in ImageJ (U.S. National Institutes of Health, Bethesda, MD, USA). Then the staining intensity of each fiber was measured using Metamorph software (Molecular Devices, Sunnyvale, CA).

Electron Microscopy and Morphometry

Sol and EDL muscles were dissected from two matched wildtype and $Ca_V1.1\Delta E29$ mouse pairs 4 and 5 months of age, immediately fixed with 3.5% glutaraldehyde in 0.12 M Na-Cacodylate buffer and processed for transmission electron microscopy as previously described in (Hess et al., 2000).

Longitudinal sections of the muscles were systematically imaged across muscle fibers and at two levels of each fiber. Thirty to forty images of each condition were analyzed using the *Metamorph* software (Molecular Devices, Sunnyvale, CA). The persons taking the images and conducting the analysis were both “blinded” with regard to the experimental condition. In each image a region was traced along the myofibril bundles and the Z-lines. Within these regions healthy mitochondria were traced to measure the size of the mitochondria and the total area covered by healthy mitochondria. From these data the fractional content of healthy mitochondria, the percentage of damaged mitochondria, and the average mitochondrial size were calculated. To exclude a possible influence of contractile state and sectioning plane on the analysis, the sarcomere length was measured and the sarcomeres in each region were counted so that the number of good and damaged mitochondria per sarcomere could be calculated. Because these controls gave the same results as when expressed as fraction of the analyzed area, they are not shown.

Protein extraction and Western blotting

Mouse Sol and EDL were isolated from 6 month old wildtype and knockout ($Ca_V1.1\Delta E29$) mice and snap frozen in liquid nitrogen. Then the frozen muscle was ground into powder using a mortar and pestle. The powder was homogenized in RIPA buffer composed of 1% Igepal, 50 mM Tris HCl, 150 mM NaCl, 0.1% SDS, 10 mM NaF and 10% glycerol to extract all the protein. The homogenized samples were centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant containing the whole protein was collected. For separation of the cytoplasmic and nuclear fractions a different procedure was used as described previously (Dimauro et al., 2012). Protein concentrations were determined using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific Inc., Rockford, IL, USA) and measured with the NanoDrop 2000 (Thermo Fisher Scientific Inc., Rockford, IL, USA). A standard curve was determined with different concentrations of bovine serum albumin (BSA) every time protein extraction was done. 20-40 µg of protein was loaded per lane onto 6-10% Bis-Tris Gel and separated at 196 V, 40 mA for 50-60 min. The blot was performed at 25 V, 100 mA for 3 h at 4°C with a semidry-blot system (Roth, Karslruhe, DE). Primary antibodies were applied overnight at 4°C and incubation with HRP-conjugated anti-mouse or anti-rabbit secondary antibody (1:5000, Pierce) was done for 1h at RT. The primary antibodies used were as follows: Mouse antibodies against $Ca_V1.1$ (1:1000, MA3-920, Thermo Fisher Scientific), phospho-CaMKII (1:1000, #12716, Cell Signaling Technology, MA, USA), HDAC4 (1:2000, #2072, Cell Signaling Technology, MA, USA), NFATc1 (1:500, sc-13033, Santa Cruz Biotechnology, Heidelberg, DE), Histone H3 (1:2000, Cell Signaling Technology, MA, USA), GAPDH (1:100,000, SC32233, Santa Cruz Biotechnology, Heidelberg, DE) and α-tubulin (1:1000, ab7291, Abcam, Cambridge, UK). The development was performed with SuperSignal® West Pico Chemiluminiscent Substrate (Thermo Fisher Scientific Inc., Rockford, IL, USA) and ImageQuant Las 4000 (GE Healthcare Europe GmbH, Vienna, AT) was used to visualize the bands. Quantification of the bands was done with ImageJ (U.S. National Institutes of Health, Bethesda, MD, USA, imagej.nih.gov/ij) software (mean ± SEM, N=3, $p > 0.05$, * $p < 0.05$, *** $p < 0.001$).

Calcineurin activity assay

Mouse Sol and EDL tissue samples were frozen in liquid N_2 at the time of dissection, stored at -80°C and homogenized in a buffer containing 0.1 M sucrose, 46 mM KCl, 0.5 % BSA, 100 mM Tris-HCl (pH 7.4) and EDTA-free protease inhibitors (SIGMA). Protein determination were carried out as described before (Lontay et al., 2004). Calcineurin (protein phosphatase 2B; PP2B) activity was determined by the Calcineurin Phosphatase Activity Colorimetric Assay (Abcam) following the the manufacturer's instructions. Shortly, PP2B activity of the skeletal muscle lysates was measure by using RII

phosphopeptide substrate. The quantity of the free-phosphate liberated was detected by Malachite green assay at 620 nm and it was correlated with the PP2B activity. Human recombinant calcineurin was applied as a positive control. To discriminate between the contribution of other protein phosphatases lysates were incubated with 100 nM okadaic acid (OA; a specific protein phosphatase 1 and 2A inhibitor) with or without EGTA before the enzyme activity measurement. Calcineurin activity was calculated as the difference of the enzyme activities measured in the OA-treated and the OA/EGTA-treated lysates and was normalized in each case to the total protein concentration.

Statistical analysis.

A two way ANOVA with Bonferroni post hoc test was used for homecage activity and fiber type analysis (Fig. 1A and 1B). A one way ANOVA was used for the other behavioral tests. The Student's t-test was used to calculate the statistical significance for fiber type analysis in SDH staining, mitochondria analysis in electron microscopy and all tests for contractile properties. Levels of significance for t-test and ANOVA are indicated as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. The statistical analysis was performed with GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

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Supplementary Tables:

Table S1: Oligonucleotides for real time PCR

Gene	Ref. no.	Method	Forward primer (5'-3')	Reverse primer (5'-3')	Probe
CACNA1S	NM_001081023	TaqMan	gttacatgagctggatcacacag	atgagcatttcgatggtgaag	
CACNA1S-E29	NA	TaqMan	ctaatcgtcatcggcagcat	tctcatctgggtcatcgatct	attgacgtcatcctgagc
CACNA1S+E29	NA	TaqMan	ctaatcgtcatcggcagcat	ctccacccaggcaatacagt	attgacgtcatcctgagc
Ppargc1a	NM_008904	TaqMan	ctccatctgtcagtgcatca	ccaaccagtacaacaatgagc	agggcaatccgtcttcatccacg
Six 1	NM_009189	TaqMan	gagagagttgattctgcttgttg	ggtcagcaactggttaagaac	cgaggccaaggaaagggagaaca

Table S2: Antibodies for immunostaining

Fibre type	Primary Antibody*	Concentration	Secondary Antibody	Concentration
I	BA-D5	1:2000	Anti-mouse IgG Alexa 594	1:4000
IIA	SC-71	1:2000	Anti-mouse IgG Alexa 594	1:4000
IIB	BF-F3	1:2000	Anti-mouse IgG Alexa 594	1:4000
IIX	6H1	1:200	Anti-mouse IgM Alexa 594	1:4000

*All primary antibodies were purchased from Developmental Studies Hybridoma Bank, Iowa, USA.

Supplementary Figures:

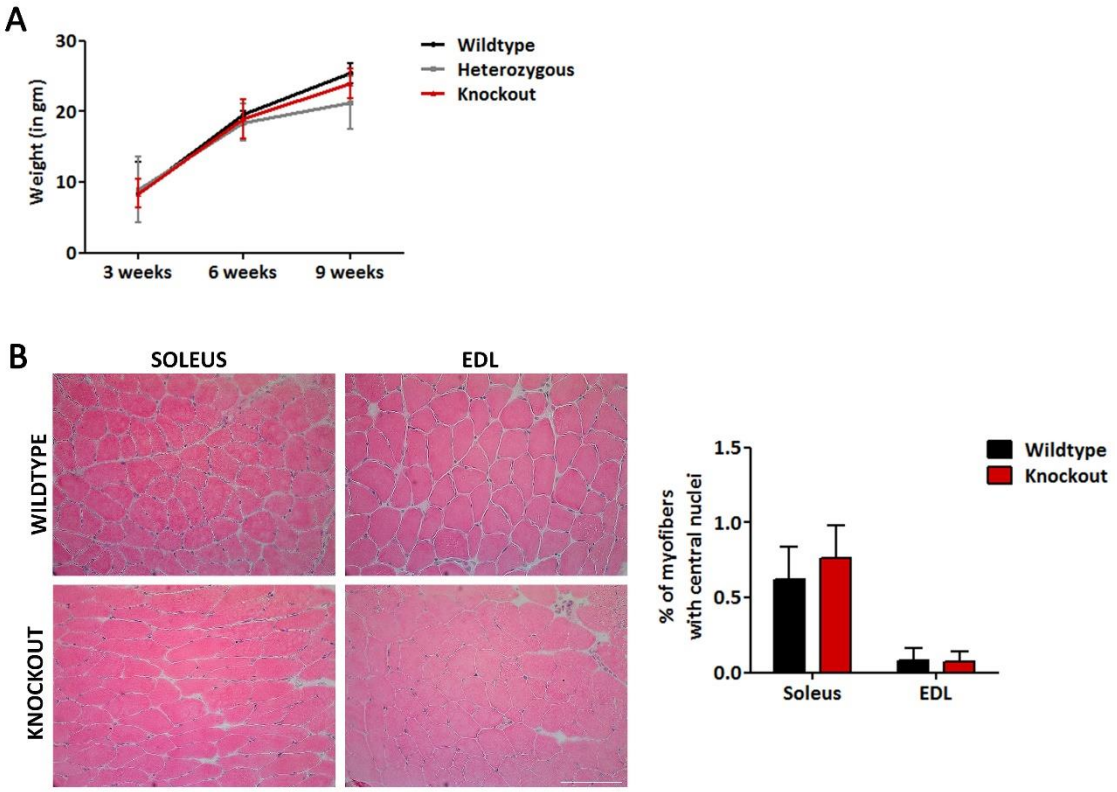

Figure S1. Phenotype of $Ca_V1.1\Delta E29$ mice: Normal weight gain and muscle histology. (**A**) The increase of body weight measured at 3, 6 and 9 weeks of age was similar in $Ca_V1.1\Delta E29$ mice compared to the wildtype and heterozygous siblings (mean ± SEM, N=15-37, $p>0.05$). (**B**) Haematoxylin and eosin stained cryosections of soleus and EDL muscles at 6 months revealed no centrally located nuclei in $Ca_V1.1\Delta E29$ muscle fibers (Scale bar: 100µm). Quantitative analysis showed no significant differences between $Ca_V1.1\Delta E29$ and wildtype mice (mean ± SEM, N=3, $p > 0.05$).

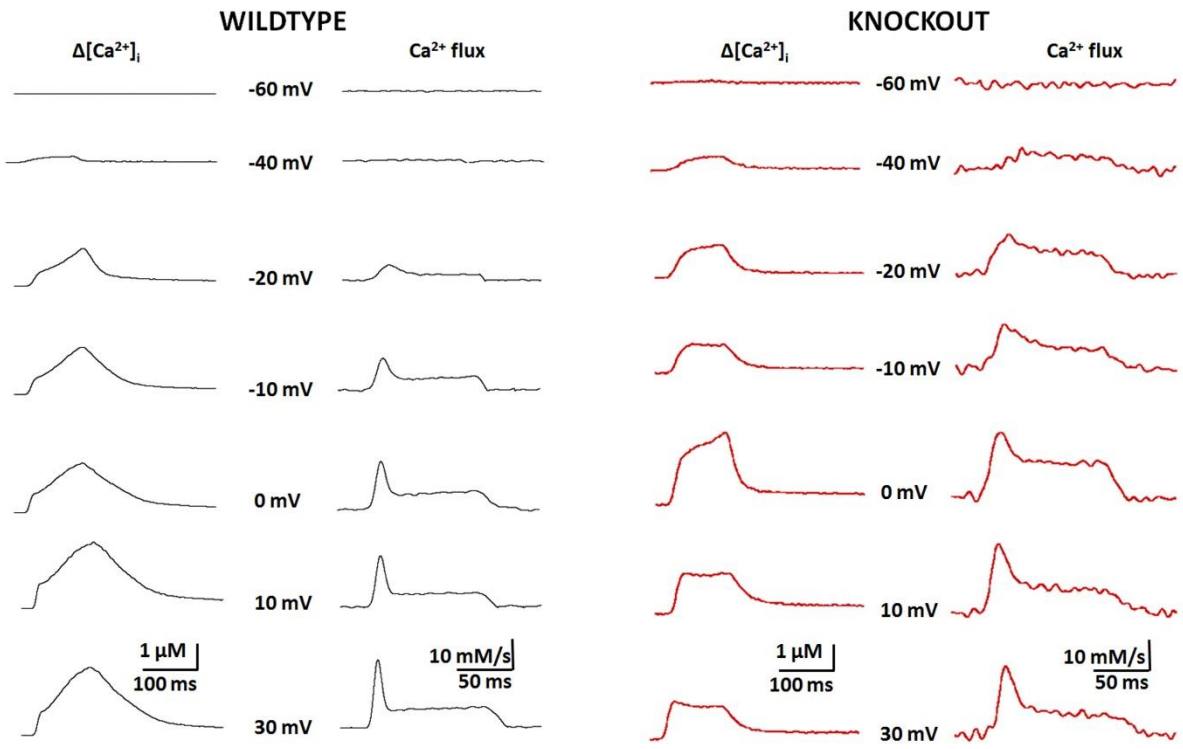

Figure S2. Voltage-dependence of calcium transients and calcium fluxes in FDB muscle fibers of wildtype and $Ca_V1.1\Delta E29$ mice. Calcium transients were recorded in voltage-clamped, Rhod-2 loaded FDB fibers and calcium fluxes were calculated as described in the extended materials and methods. At intermediate voltages the plateau flux relative to the peak is substantially higher in $Ca_V1.1\Delta E29$ than in wildtype controls, indicating a strong contribution of voltage-dependent calcium influx to the myoplasmic calcium transients.

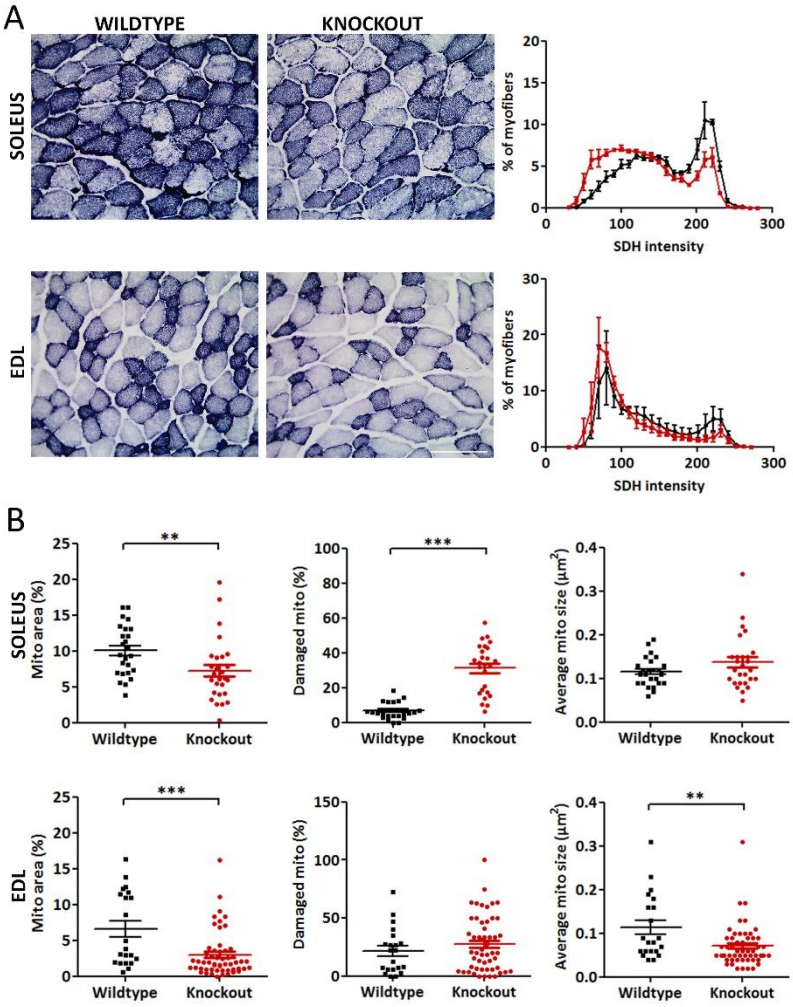

Figure S3. SDH staining and electron microscopy analysis demonstrates reduced mitochondrial function and content in $Ca_V1.1\Delta E29$ muscles. (**A**) SDH activity was analyzed in sections of 12 months old wildtype (black) and $Ca_V1.1\Delta E29$ (red) mice (Scale bar: 100 µm). Staining intensity was measured in each fiber profile and plotted in intensity distribution diagrams. SDH activity is significantly reduced in $Ca_V1.1\Delta E29$ soleus and EDL muscles visible as left shift in the distribution curves (mean ± SEM, N=3 (**B**) (Biological replicate of experiment shown in Fig. 6C and D, and corresponding to the second data set given in Results). Morphometric analysis demonstrates significantly decreased fraction of the area occupied by intact mitochondria in both soleus ($p<0.01$) and EDL ($p<0.001$) muscles of $Ca_V1.1\Delta E29$. Mitochondrial size is decreased in $Ca_V1.1\Delta E29$ EDL ($p<0.01$) and the fraction of damaged mitochondria increased ($p<0.001$) in $Ca_V1.1\Delta E29$ soleus muscles (mean ± SEM, N=2).

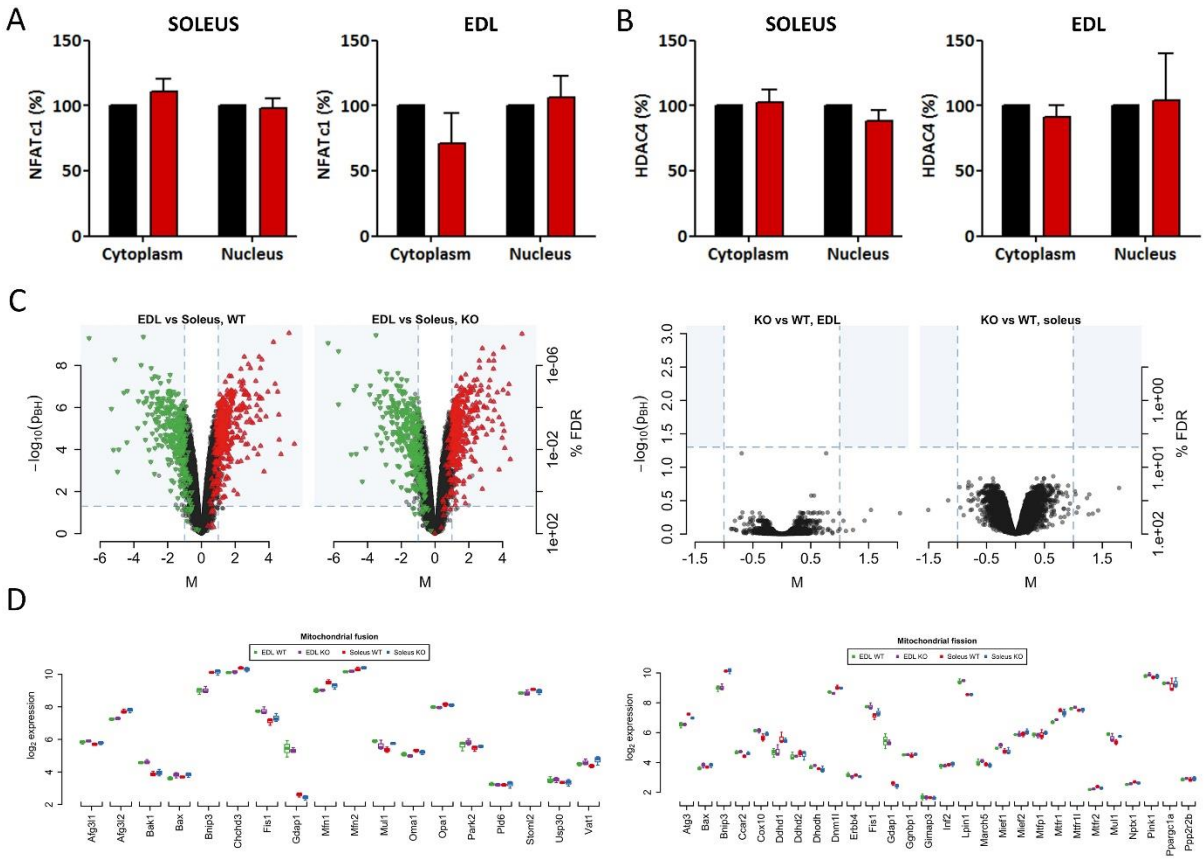

Figure S4. Expression of the transcriptional regulators NFATc1 and HDAC4 and the gene expression profile were not altered in $Ca_V1.1\Delta E29$ mice. (**A, B**) Quantitative analysis of NFATc1 and HDAC4 Western blots shown in Fig. 7 revealed no significant differences in cytoplasmic or nuclear fractions of soleus and EDL muscles of 5-6 month old $Ca_V1.1\Delta E29$ mice and wildtype controls (mean ± SEM, $p > 0.05$, N=3). (**C**) Volcano plots of *Affymetrix* gene chip analysis show differentially expressed genes in soleus and EDL muscles, but differential expression in 6 month old wildtype and $Ca_V1.1\Delta E29$ muscles did not reach significance. Both soleus and EDL muscles were pooled from three female $Ca_V1.1\Delta E29$ and wildtype mice six months of age (mean ± SEM, $p>0.05$, N=3). (**D**) Expression of genes linked to mitochondrial fusion (left) and fission (right) was not altered in $Ca_V1.1\Delta E29$ muscles.