

**Supplementary Materials****Materials and Methods****Animals**

All experimental procedures were performed in accordance with institutional guidelines for animal studies and were approved by an ethics committee (Ethics Committee MENESR: Project authorization # 17975). Adult C57/BL6 female mice were purchased from Janvier Lab and Envigo Lab.

**Human biopsies**

Four quadriceps (Q, Y6-9) and two fascia lata (FL1, 2) biopsies from healthy subjects, detailed in Table S3, were collected from Institute of Myology Myobank (ref: BB-0033-00012). One human spinal cord biopsy was obtained from AP-HP, Hôpital de la Pitié-Salpêtrière, Service de Neurologie 2, Paris, France. Muscle biopsies were obtained during surgical procedures and spinal cord post-mortem, all after informed consent in accordance with the French legislation on ethical rule.

Twenty four quadriceps biopsies were gathered by the Institute of Myology within the frame of the European project MyoAge (41) (Project ID: 223576, funded under: FP7-HEALTH).

**Plasmids and AAV production**

AAV-ShCaV $\beta$ 1E (Individual: TRC Mouse *Cacnb1* shRNA Clone Id: TRCN000006951, Dharmacon) have been generated by cloning both pALK0.1sh CaV $\beta$ 1E in pSUPER under the control of the H1 promoter, by PCR insertion of BglII and HindIII sites. The H1 cassette was then introduced into an AAV1-based vector between the two ITRs using BamHI and SalI sites of pSMD2-sh AAV2 vector backbones. pSUPER retro puro Scr ShRNA (SCRA) was a gift from John Gurdon (Addgene plasmid #30520) (54). BamHI site has been inserted by PCR and the H1-SCRA cassette has been cloned in pSMD2-sh through BamHI and SalI sites. AAV2/1

pseudotyped vectors have been prepared by the AAV production facility of the Center of Research in Myology, by transfection in 293 cells as described previously (55). AAV- CaV $\beta$ 1E, CaV $\beta$ 1D and AAV-GDF5 have been generated by direct cloning of *Cacnb1*-E ORF (NM\_001282977) *Cacnb1*-D ORF (NM\_001159320) or *Gdf5* ORF (NM\_008109.2), flanked by EcoRI and NheI sites (GeneArt string; ThermoFisher), in pSMD2 AAV2 vectors backbones, under CMV promoter. The final viral preparations were kept in PBS solution at  $-80^{\circ}\text{C}$ . The particle titer (number of viral genomes) was determined by quantitative PCR. ShCaV $\beta$ 1E, shSCRA, were also cloned in pCDNA3 (ThermoFisher) for luciferase assay. *Gdf5* promoter region has been designed using the public domain <http://epd.vital-it.ch>, getting a sequence from -312 to the TSS of *Gdf5*. This sequence flanked by EcoRI and NheI sites has been synthesized (GeneArt string; ThermoFisher) and cloned upstream Firefly Luciferase gene in HSVTK-Luc3' modified plasmid for luciferase assay.

### **Antibodies**

The following antibodies from Cell Signaling Technology were used: rabbit polyclonal antibody to phosphorylated SMAD1/5 (Ser463/465) (41D10); rabbit polyclonal antibody to SMAD5 (#9517); rabbit monoclonal antibody to SMAD4 (D3M6U). For Caveolin 3, we used a monoclonal antibody (BD Biosciences, 610421). Rabbit polyclonal antibody for Cav $\beta$ 1 C-terminus (AP16144b) was purchased from AbGent, while the rabbit polyclonal antibody against the Cav $\beta$ 1 internal region (no longer available: sc-25689) and the mouse monoclonal to GDF5 was obtained by Santa Cruz Biotechnologies (SC-373744). Mouse monoclonal antibodies to actin (A4700) and to  $\alpha$ -actinin (A7732) were purchased from Sigma Aldrich. The monoclonal antibody against RyR1 was from Thermo Scientific (MA3-925), the polyclonal antibody against Laminin was from DAKO (Z0097) and Anti-nAChR, Antibody from Biolegend (Previously Covance)

Supernatant BA-D5-s (MyHC-I) ; SC-71-s (MyHC-IIA) ; 6H1-s (MyHC-IIX) ; BF-F3-s (MyHC-IIB) were from DSHB (University of Iowa).

### **In vivo gene transfer**

Experiments were performed on adult 6-8 or 78-80-week-old C57/BL6 female mice. Anesthesia was achieved using isoflurane (3% induction, 2% maintenance), analgesia by buprenorphine (vetergesic 1mg/Kg, subcutaneous). One intramuscular injection (40  $\mu$ l/TA) was performed in both Tibialis Anterioris (TA) muscles. All AAV2/1 were used at final titer of 5E+10 vector genomes (vg)/TA, except for AAV-GDF5 used at 5E+9 in aged mice to have a more physiological GDF5 expression. As control, 6-8-week-old or 78-80-wk-old C57/BL6 mice were injected using the same procedure with SCRA AAV vector. Mice were sacrificed 10 or 12 weeks after the injection. Project agreement number (MENESR) # 2703 and 5448.

### **Denervation experiments**

Ten weeks after injection of mice with AAV, the sciatic nerve was neuroectomized (ablation of a 5-mm segment of the sciatic nerve) under general anesthesia (Isoflurane, 3% induction, 2% maintenance) with Buprenorphine (vetergesic 1mg/Kg, subcutaneous). Mice were sacrificed 1, 3, 7 or 15 days after denervation, and TA were dissected, weighed and thereafter frozen in isopentane precooled in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until histology or molecular analysis.

### **Single treadmill exercise bout**

C57/BL6 female, 12 or 78 weeks of age, were subjected to a single treadmill as described (38) (Model LE8710MTS treadmill 5 lanes for mice, with shock, Bioseb) running bout at 18 m/min and  $10^{\circ}$  incline for 20 min. Mice were sacrificed by cervical dislocation under general anesthesia with ketamine / xylazine (100mg/Kg-20mg/Kg), immediately after exercise (0h) or at 3h of

recovery. Resting mice were euthanized at the time points corresponding to 0h and 3h of recovery. Samples were stored at -80°C until histology or molecular analysis.

### **Force measurement**

The function of TA was evaluated by measuring *in vivo* muscle contraction in response to nerve stimulation, as previously described (2). Mice were anesthetized and the knee and paw were fixed in place, and the distal tendon of the muscle was attached to the lever arm of a servomotor system (305B, Dual-Mode Lever, Aurora Scientific) using a silk ligature. Data were analyzed using the PowerLab system (4SP, ADInstruments) and software (Chart 4, ADInstruments). The sciatic nerve was stimulated using supramaximal square-wave pulses of 0.1 ms in duration. Capacity for force generation was evaluated by measuring the absolute maximal force that was generated during isometric contractions in response to electrical stimulation (frequency of 75–150 Hz; train of stimulation of 500 ms). Maximal isometric force was determined at  $L_0$  (length at which maximal tension was obtained during the tetanus). Force was normalized by muscle mass as an estimate of specific force. After force measurements, mice were sacrificed by cervical dislocation under general anesthesia with xylazine/ketamine (100mg/Kg-20mg/Kg), and muscles were dissected, weighed and frozen in liquid nitrogen or in isopentane precooled in liquid nitrogen. Samples were stored at -80 °C for histological analyses. Muscles analyzed for force generation were not investigated for gene expression to avoid any possible bias due to muscle electrical stimulations and repeated contractions induced during the assay.

### **Body composition and muscle function assessment**

The assessment of lean mass percentage and muscle power of twenty two healthy volunteers, corresponding to muscle biopsies tested have been reported previously (41). Briefly, standing height was measured to the nearest millimeter and body mass was measured to the nearest

0.1 kg with shoes removed and light clothing only. Total body composition was assessed by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, version EnCore 12.30) as described (41). Details of fat mass and lean mass of the whole body were recorded.

To assess leg extension muscle power, a maximal-effort countermovement vertical jump was performed on a force platform (AMTI OR6-7). The test was repeated a further two times with a rest interval of 60 s.

### **Gene expression analysis**

Total RNA was prepared from TA cryosections, C2C12 or human biopsies using TRizol (Life Technologies) following the manufacturer's instructions. Complementary DNA was generated with Superscript II Reverse transcriptase (Life Technologies), amplified using PCR Master Mix (M7505, Promega) for RT-PCR or analyzed by real-time qPCR. Real-time qPCR was performed on StepOne Plus Real-Time PCR System (Applied Biosystems) using Power SyberGreen PCR MasterMix (Applied Biosystems). All data were analyzed using the  $\Delta\Delta CT$  method and normalized to PO (mouse or human acidic ribosomal phosphoprotein) mRNA expression. The sample reference to calculate mRNA fold change is indicated in each panel. Primers used are listed in table S4.

The investigators were blinded to allocation in using MyoAge samples (RT-qPCR) and outcome assessment. We included in the analysis also Y6-Y8 biopsies collected from Myology Institute Myobank. Two different investigators replicated RT-qPCR in two independent experiments. Reported mRNA fold changes are the average of the two experiments. No statistical methods were used to predetermine sample size and no randomization method has been applied in this study. The correspondence between qPCR values and volunteer data has been determined after the qPCR results were obtained.

## **RNA isolation and Affymetrix exon array data processing**

RNA was prepared as for gene expression analysis. RNA samples were quantified using a ND-1000 NanoDrop spectrophotometer (NanoDrop Technologies) and purity/integrity were assessed using disposable RNA chips (Agilent RNA 6000 Nano LabChip kit) and an Agilent 2100 Bioanalyzer (Agilent Technologies). mRNA library preparation were realized following manufacturer's recommendations (TruSeq® Stranded mRNA Library Prep ILLUMINA). Final pooled library preps for the 6 samples were sequenced on Nextseq 500 ILLUMINA with HighOutput cartridge (2x400Millions of 75 bases reads), corresponding to 2x66Millions of reads per sample after demultiplexing.

## **RNA-Seq data processing**

RNA-Seq data analysis was performed by GenoSplice technology ([www.genosplice.com](http://www.genosplice.com)). Sequencing, data quality, reads repartition (for example, for potential ribosomal contamination), and insert size estimation were performed using FastQC, Picard-Tools, Samtools and rseqc. Reads were mapped using STARv2.4.0. Gene expression was estimated as already described (56, 57) using Mouse FAST DB v2016\_1 annotations. In a given comparison between two experimental conditions, only genes expressed in at least one condition were further analyzed. Genes were considered as expressed if their rpkms value was greater than the background rpkms value based on intergenic regions. Analysis at the splicing level was first performed taking into account only exon reads and flanking exon-exon junction reads ("EXON" analysis) in order to potentially detect new alternative events that could be differentially regulated (for example, without taking into account known alternative events). Analysis at the splicing level was also performed by taking into account known patterns ("PATTERN" analysis) using the FAST DB splicing patterns annotation (for example, for each gene, all possible splicing patterns were defined by comparing exon content of transcripts). All types of alternative events can be

analyzed: Alternative first exons, alternative terminal exons, cassette exon, mutually exclusive exons, alternative 5' donor splice site, alternative 3' acceptor splice sites, intron retention, internal exon deletion and complex events corresponding to mix of several alternative event categories). "EXON" and "PATTERN" analyses were based on the splicing-index calculation as previously described (56, 57). Results were considered statistically significant for P-values < 0.05 and fold-changes  $\geq 2.0$ . Finally, significant results from "EXON" and "PATTERN" analyses were merged to obtain a single result list.

### **Immunoblotting**

Cryosections from frozen TA were homogenized with a dounce homogenizer in a lysis buffer containing 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.5% NP40 and Halt Protease and Phosphatase inhibitor cocktail (ThermoFisher Scientific). Samples were then centrifuged for 5 min at 1500g and denatured at room temperature for 30 min with Laemmli buffer. Protein concentration was determined by Bradford assay (ThermoFisher Scientific). Proteins were separated by electrophoresis (Nu-PAGE 4–12% Bis-Tris gel; Life Technologies) and then transferred to nitrocellulose membranes (GE Healthcare) and labeled with primary antibodies and secondary antibodies coupled to horseradish peroxidase. Signals were visualized with SuperSignal West Pico Chemiluminescent substrate (ThermoFisher Scientific). Images were acquired with camera LAS4000 (GE Healthcare). Western blot image analysis was performed with the public domain software Fiji ImageJ (analyze gel tool) (58). Blots were stripped using Restore Western Blotting Stripping Buffer (ThermoFisher Scientific) according to the manufacturer's instructions and reprobed if necessary.

### **Immunofluorescence and Histology**

For immunofluorescence procedures, sections of mouse or human tissues were performed at 10  $\mu\text{m}$  on a cryostat (Leica Biosystems), fixed on glass slides and stored at  $-80^{\circ}\text{C}$ . Slides were

rehydrated in phosphate-buffered saline (PBS), fixed with paraformaldehyde (PFA) 4% for 10 min, permeabilized with 0.5% Triton X-100 (Sigma-Aldrich) and blocked in PBS/4% bovine serum albumin/0.1% Triton X-100 for 1 h. Sections were incubated in PBS/2% BSA/0.1% Triton X-100 with a primary antibodies overnight at 4°C, washed in PBS, incubated for 1 h with secondary antibodies, thoroughly washed in PBS, incubated with 4',6'-diamidino-2-phenylindole for nuclear staining for 5 min and mounted in Fluoromount (Southern Biotech).

For H&E staining, sections were fixed in 4% PFA for 10 min, washed in PBS and then stained in haematoxylin for 5 min and eosin for 30 sec. The muscle sections were further dried in gradually increasing concentration of ethanol/water solutions and, after fixation in 100% xylene, were mounted in Vectamount (Vector Laboratories).

Sirius Red staining was performed to visualize total collagen I and III content. Muscle cryosections were fixed in PFA 4% for 10 min, washed in water and dried in 100% ethanol for 5 min. Sections were then stained in Picro-Sirius Red (0.3%) solution for 1 h while protected from light. After a washing in acidified water (5 min in acetic acid 0.5% vol/vol), sections were fixed in 100% ethanol (3 washes for 5 min) and the final dehydration was performed in xylene 100%, mounted in Vectamount and visualized using a microscope Nikon AZ100.

Confocal images were taken with Leica SPE or a Nikon Ti2 microscope equipped with a motorized stage and a Yokogawa CSU-W1 spinning disk head coupled with a Prime 95 sCMOS camera (Photometrics).

### **Morphometric analysis**

Sections of tissues were immunolabeled with laminin antibody and acquired using a Nikon Ti2 microscope. Images were first processed with a machine learning algorithm (WEKA) under Fiji for accurate segmentation using the training features Gaussian blur, Hessian, Sobel filter and



Difference of gaussians. The resulting images were analyzed using the Fiji particle analysis plugin for fibers areas between 75 and 20 000  $\mu\text{m}^2$ , with a circularity between 0.3 and 1.

### **Fluorescence intensity quantification**

Mouse muscle sections were immunolabelled with different MyHC and CaV $\beta$ 1E antibodies and images were acquired Nikon Ti2 microscope. Mean of pixel intensity relative to CaV $\beta$ 1E staining was measured for each fiber type using Image J Software. The average gray value (fluorescence intensity) was calculated by dividing the sum of the gray values of all the pixels by the number of pixels in the selection.

### **LineScan profiling**

Extensor digitorum longus (EDL) fibers were immunolabelled with CaV $\beta$ 1-central peptide or CaV $\beta$ 1E antibodies together with RyR1 or  $\alpha$ -sarcomeric actinin antibodies respectively.

LineScan profiles, to measure fluorescence intensity along a line region, were performed and analyzed using Metamorph software (Molecular Device).

### **Cell transfection and luciferase assay**

C2C12 muscle cell line was purchased from ATCC and were cultured in IMDM medium (Gibco–Life Technologies) supplemented with 20% FBS and 0.1% gentamicin. Differentiation was induced by medium replacement with IMDM supplemented with 2% Horse serum (HS) and 0.1% gentamicin (Gibco–Life Technologies). Cells were transfected using Lipofectamine 2000 (Life Technologies) according to the manufacturer’s instructions. 25 ng Gdf5 promoter-luciferase was co-transfected in C2C12 cells with 75 ng of either pCDNA3-ShCaV $\beta$ 1E or pCDNA3-sh Scra alone using lipofectamine 2000 diluted in Optimem reduced medium. The plasmid CMV-Renilla luciferase (1.5 ng) was also transfected in each condition as normalizer. Five hours post-transfection, Optimem reduced-medium was replaced with IMDM added with HS 2%. Cells were analyzed 24 and 48 hours after medium replacement. Firefly and Renilla luciferase

luminescences were quantified with Dual-Glo Luciferase Assay System (Promega, according to manufacturer's instructions) on TECAN Spark™ 10M Microplate reader. Firefly luciferase activity was normalized on Renilla luciferase activity.

### **Single fibers isolation**

EDL single fibers were isolated as described (43). Innervated or denervated EDL muscles were explanted from 12-week-old female C57/BL6 mice and then digested in DMEM containing 0.2% type I collagenase (Sigma-Aldrich) for 2 h at 37°C. Mechanical dissociation of fibers was performed using a thin pasteur pipette and followed under a transilluminating-fluorescent stereomicroscope. Isolated fibers were fixed with PFA 4% for 10 min, rinsed twice with cold PBS and kept at 4°C until immunostaining.

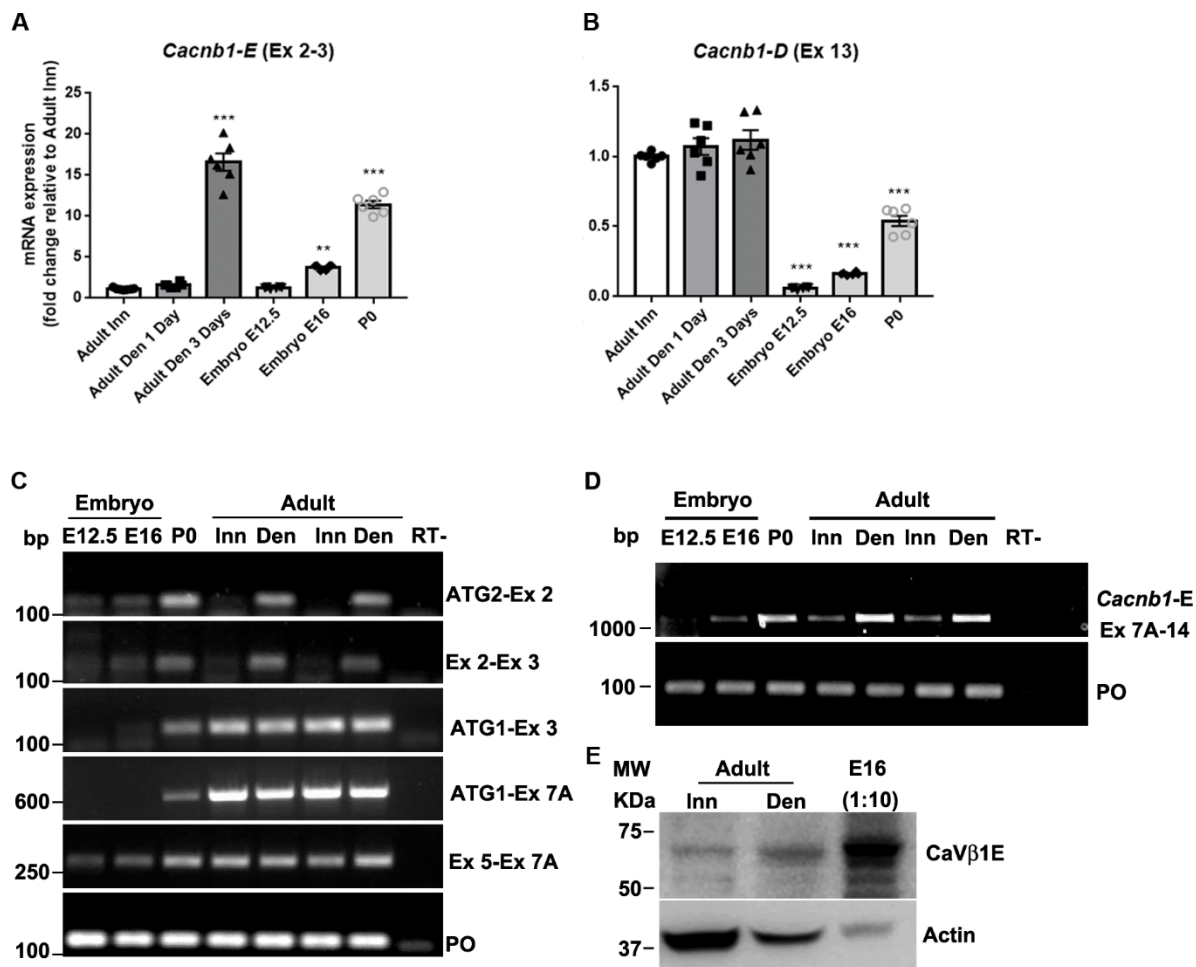


Fig S1

### Fig. S1: Expression of embryonic CaVβ1 isoform

**A, B:** RT-qPCR for (A) *Cacnb1-E* (Ex 2-3) and (B) *Cacnb1-D* (Ex 13) in adult Tibialis Anterior muscles (TAs) innervated (Inn) or denervated (Den) for 1 and 3 days, in embryonic (E12.5 and E16) and neonatal (P0) muscles.

**C:** RT-PCR for different *Cacnb1* regions in embryonic and neonatal muscles (day 12.5 (E12.5) and 16 (E16) since fertilization; post-natal (P0)) and in adult TAs Inn or Den for 15 days. Primers were designed for different exons (Ex) of the predicted coding region of *Cacnb1*. Ribosomal phosphoprotein (PO) was used as a loading control.

**D:** RT-PCR of the expression of *Cacnb1* (ex7A-14), amplifying only *Cacnb1-E* variant, in embryonic (E12.5 and E16) and neonatal (P0) muscles and in Inn or Den adult TAs.

**E:** Western blot of CaV $\beta$ 1 expression in embryonic E16 muscle and adult TAs Inn or Den for 3 days using an antibody targeting only CaV $\beta$ 1E in mouse muscle. Actin was the loading control.

**A, B:** Means  $\pm$  s.e.m (n=6) \*\* P < 0.01, \*\*\*P < 0.001, (ordinary one-way Anova - Dunnett's test).

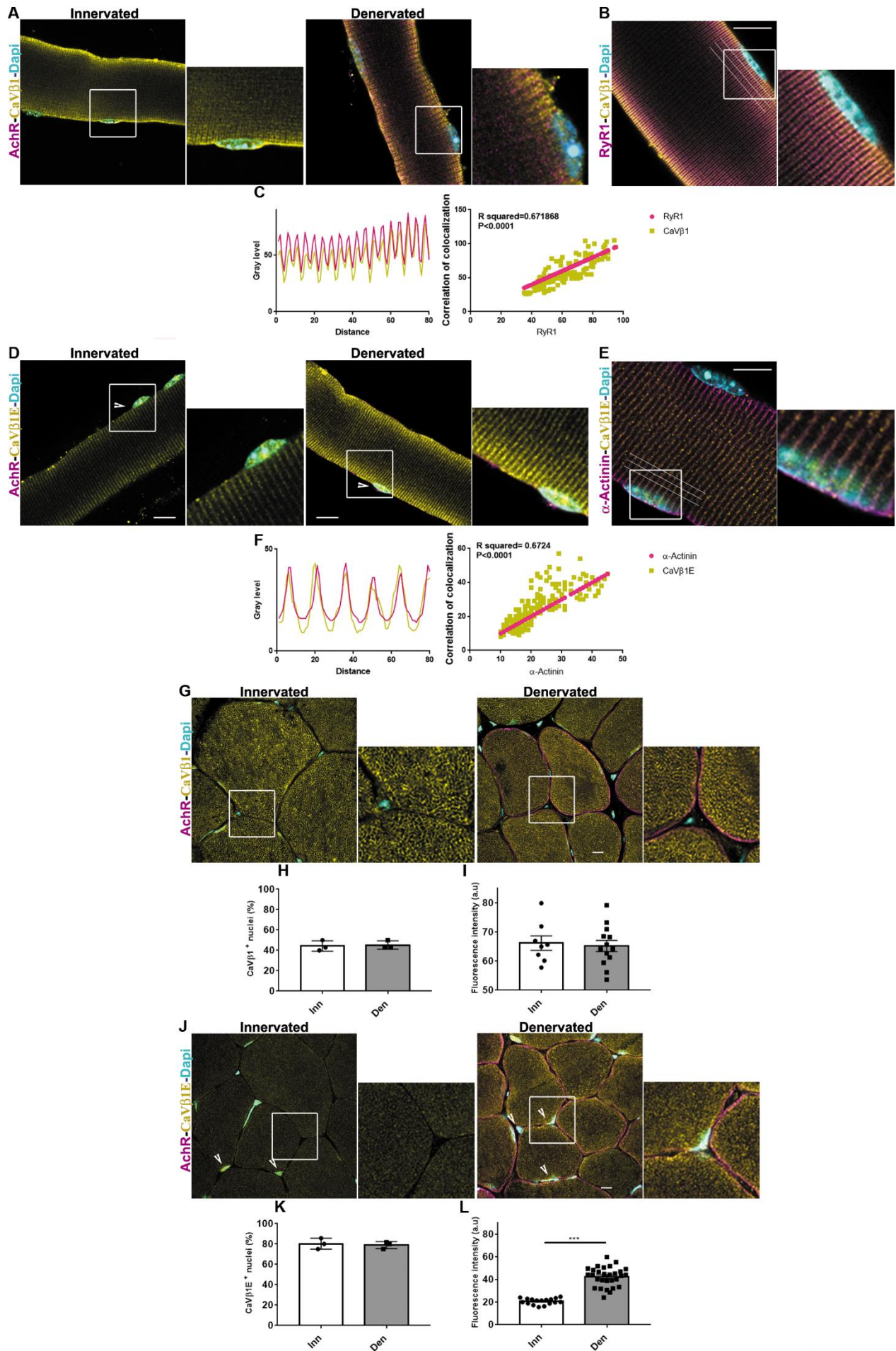


Fig S2

**Fig. S2: CaV $\beta$ 1 isoforms localization in adult skeletal muscle**

**A, B:** (A) Immunofluorescence images of EDL fibers innervated or denervated for 15 days stained with AchR (Magenta), CaV $\beta$ 1 -central peptide- (Yellow) and Dapi (Cyan); (B) immunofluorescence images of EDL fibers denervated for 15 days stained with RyR1 (Magenta), CaV $\beta$ 1 -central peptide- (Yellow) and Dapi (Cyan). Bar: 10 $\mu$ m.

**C:** LineScan representation of RyR1 (Magenta) and CaV $\beta$ 1 -central peptide- (Yellow) staining and correlation of co-localization in isolated EDL fibers. LineScan region is showed in B.

**D, E:** (D) Immunofluorescence images of EDL fibers innervated or denervated for 15 days stained with AchR (Magenta), CaV $\beta$ 1E (Yellow) and Dapi (Cyan); (E) Immunofluorescence images of isolated fibers denervated for 15 days stained with  $\alpha$ -sarcomeric actinin (Magenta), CaV $\beta$ 1E (Yellow) and Dapi (Cyan) showing the tethering of CaV $\beta$ 1E to the Z-lines, zoomed in the inset. Bar: 10 $\mu$ m.

**F:** LineScan representation of  $\alpha$ -sarcomeric actinin (Magenta) and CaV $\beta$ 1E (Yellow) staining and correlation of co-localization in isolated EDL fibers. LineScan region is showed in E.

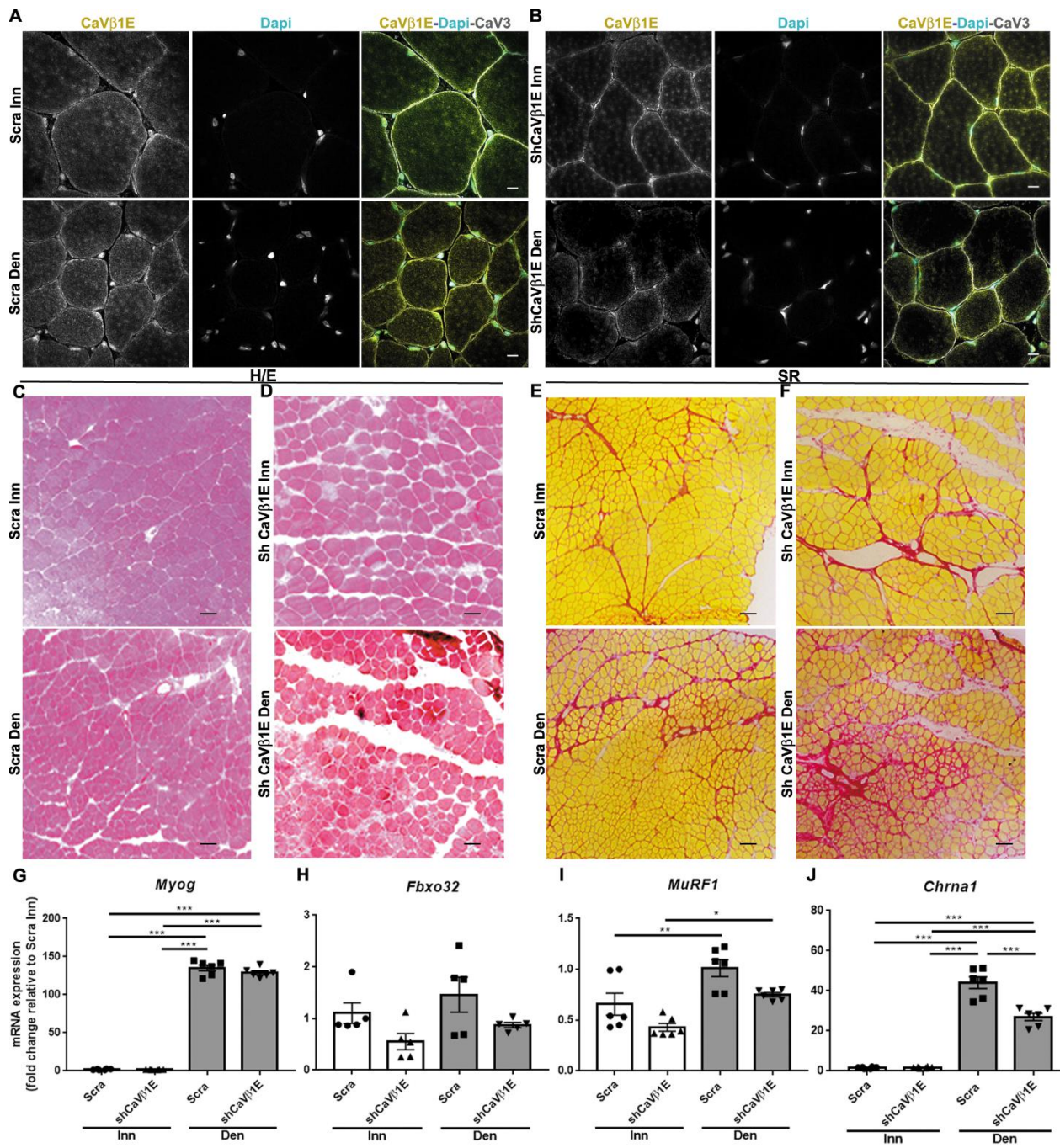
**G:** Immunofluorescence images of TAs innervated (Inn) and denervated (Den) for 15 days stained with AchR (Magenta), CaV $\beta$ 1 -central peptide- (Yellow) and Dapi (Cyan). Bar: 10 $\mu$ m

**H, I:** (H) Percentage of CaV $\beta$ 1<sup>+</sup> nuclei and (I) fluorescence intensity of CaV $\beta$ 1 -central peptide staining in TAs innervated (Inn) and denervated (Den) for 15 days.

**J:** Immunofluorescence images of TAs innervated (Inn) and denervated (Den) for 15 days stained with AchR (Magenta), CaV $\beta$ 1E (Yellow) and Dapi (Cyan). Arrows indicate the nuclear accumulation of CaV $\beta$ 1E. Bar: 10 $\mu$ m.

**K, L:** (K) Percentage of CaV $\beta$ 1E<sup>+</sup> nuclei and (L) fluorescence intensity of CaV $\beta$ 1E staining in TAs innervated (Inn) and denervated (Den) for 15 days.

**H, I, K, L:** Means  $\pm$  s.e.m. (n=3 cryosection quantified per condition) \*\*\*P < 0.001,  
(independent samples t-test (two tailed)).



**Fig S3**



**Fig. S3: Histological characterization and myogenin signaling in CaV $\beta$ 1E knock-down muscles**

**A-B:** Immunofluorescence images of TA innervated (Inn) (top) or denervated for 15 days (Den) (bottom) treated with (A) Adeno-Associated Virus (AAV) sh scrambled (Scra) or (B) AAV-ShCaV $\beta$ 1E (ShCaV $\beta$ 1E), stained with: CaV $\beta$ 1E (Yellow) Dapi (Cyan) Cav-3 (Gray). Bar: 10 $\mu$ m.

**C-D:** Haematoxylin and eosin (H/E) staining of TA innervated (Inn) (top) or denervated for 15 days (Den) (bottom) treated with (C) Scra or (D) ShCaV $\beta$ 1E. Bar 100  $\mu$ m.

**E-F:** Sirius red (SR) staining of TA Inn (top) or Den (bottom) treated with (E) Scra or (F) ShCaV $\beta$ 1E. Bar 100  $\mu$ m.

**G-J:** RT-qPCR for (G) *Myogenin*, (H) *Fbxo32*, (I) *MuRF1* and (J) *Chrna1* in adult TA Inn or Den for 3 days treated with Scra or shCaV $\beta$ 1E. Means  $\pm$  s.e.m. (n=6) \*P < 0.05, \*\* P<0.01, \*\*\*P < 0.001, (Anova-Sidak's test).

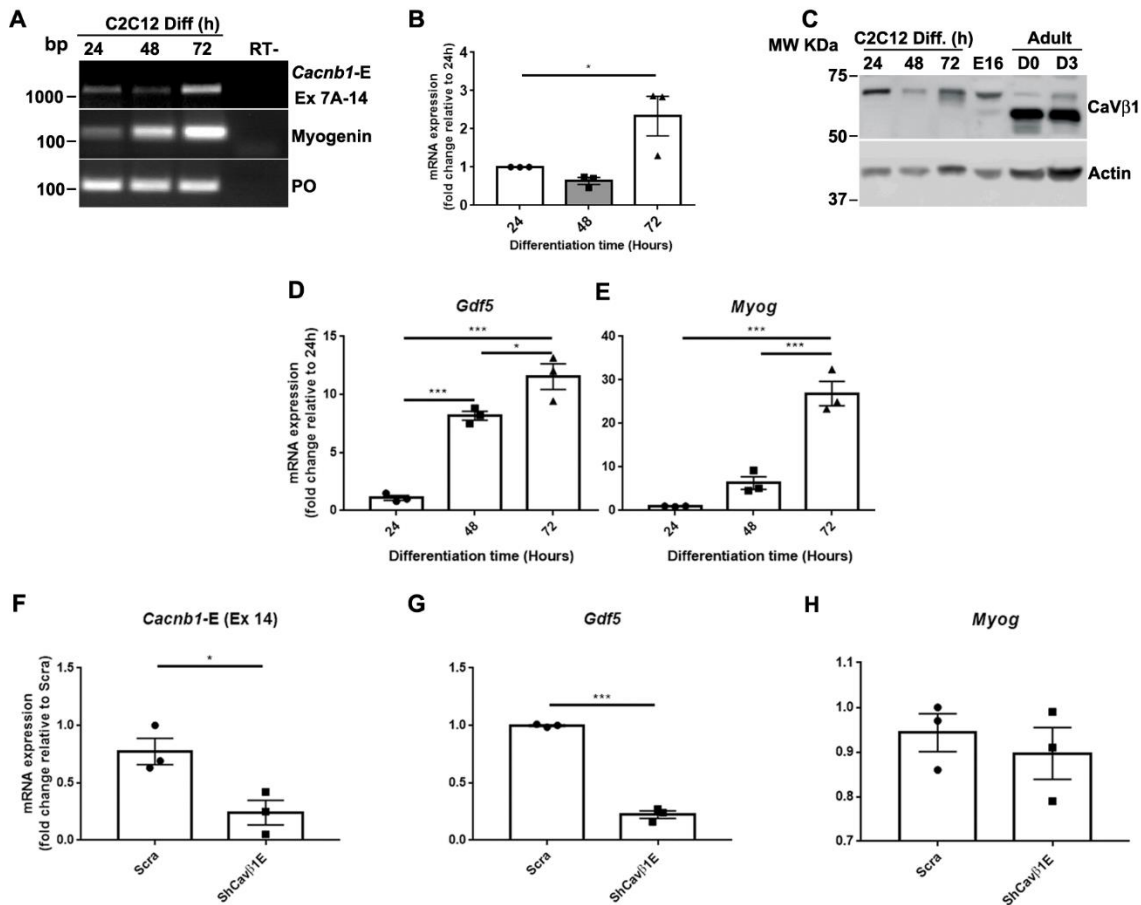


Fig S4

**Fig. S4: Expression of *Cacnb1-E* and *Gdf5* in C2C12**

**A, B:** (A) RT-PCR and (B) quantification of *Cacnb1-E* (ex7A-14), in differentiating C2C12 myotubes. Myogenin was a differentiation marker and PO a loading control.

**C:** Western blot analysis of CaVβ1 in differentiating C2C12 myotubes, in embryonic muscle (E16) and in adult TA innervated (D0) or denervated for 3 days (D3) using AbCaVβ1 (central peptide). Actin was the loading control.

**D, E:** RT-qPCR for (D) *Gdf5* and (E) *Myogenin* in 24, 48 and 72 hours differentiated C2C12.

**F-H:** RT-qPCR for (F) *Cacnb1-E* (Ex 14) and (G) *Gdf5* and (H) *Myogenin* in 48h differentiated C2C12 transfected with pCDNA3-Scrambled (Scra) or pCDNA3- ShCaVβ1E (ShCaVβ1E).

**B, D, E:** Means ± s.e.m. (n=3 independent experiments) \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001, (ordinary one-way Anova, A: Dunnett's; D, E: Tukey's test).

**F-H:** Means  $\pm$  s.e.m. (n=3 independent experiments) \*P < 0.05, \*\*\*P < 0.001, (independent samples t-test (two tailed)).

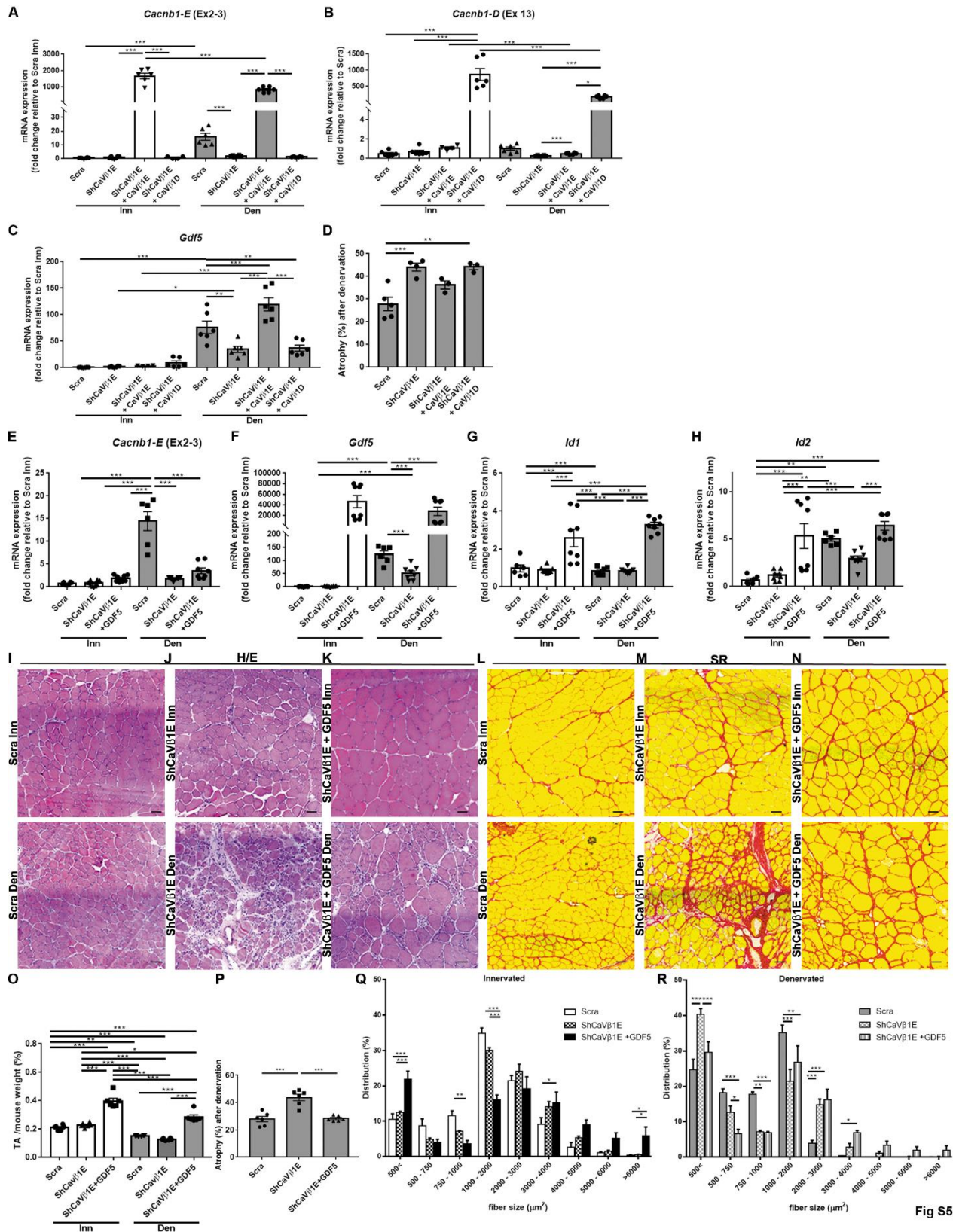


Fig S5

**Fig. S5: Effects of CaV $\beta$ 1E, CaV $\beta$ 1D and GDF5 over-expression on AAV-ShCaV $\beta$ 1E treated muscles.**

**A-C:** RT-qPCR for (A) *Cacnb1*-E (Ex 2-3) (minimum top axis: 35) (B) *Cacnb1*-D (Ex13) (minimum top axis: 5) and (C) *Gdf5* in adult TAs innervated (Inn) or denervated for 15 days (Den) treated with Scra or ShCaV $\beta$ 1E alone or in combination with AAV-CaV $\beta$ 1E (CaV $\beta$ 1E) or AAV-CaV $\beta$ 1D (CaV $\beta$ 1D).

**D:** Percentage of atrophy after denervation of adult TAs treated with Scra, ShCaV $\beta$ 1E alone or in combination with CaV $\beta$ 1E or CaV $\beta$ 1D.

**E, F:** RT-qPCR for (E) *Cacnb1*-E (Ex 2-3) and (F) *Gdf5* (minimum top axis: 200) in adult TA Inn or Den treated with Scra or ShCaV $\beta$ 1E alone or in combination with AAV-GDF5 (GDF5).

**G, H:** RT-qPCR for (G) *Id1* and (H) *Id2* in adult TA Inn or Den treated with Scra or ShCaV $\beta$ 1E alone or in combination with GDF5.

**I-K:** Haematoxylin and eosin (H/E) staining of TA Inn (top) or Den (bottom) treated with (I) Scra or (J) ShCaV $\beta$ 1E alone or (K) in combination with GDF5. Bar 100  $\mu$ m.

**L-N:** Sirius red (SR) staining of TA Inn (top) or Den (bottom) treated with (L) Scra or (M) ShCaV $\beta$ 1E alone or (N) in combination with GDF5. Bar 100  $\mu$ m.

**A, B, F:** Means  $\pm$  s.e.m. (n=6) \*P < 0.05, \*\*\*P < 0.001, \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001, calculated by two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%. Each row was analyzed individually, without assuming a consistent SD.

**D:** Means  $\pm$  s.e.m. (Scra; ShCaV $\beta$ 1E n=4, ShCaV $\beta$ 1E+CaV $\beta$ 1E: n=3; ShCaV $\beta$ 1E+CaV $\beta$ 1D: n=3) \*P < 0.05, \*\*\*P < 0.001, \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001, (ordinary one-way Anova - Dunnet's test).

**C, E-H, O, P:** Means  $\pm$  s.e.m. (n=6) \*P < 0.05, \*\*\*P < 0.001, \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001, (one-way Anova -Sidak's test).

**Q, R:** Means  $\pm$  s.e.m. (F: n=3 muscle cryosections quantified per condition), \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001, (ordinary two-way Anova -Tukey's test).

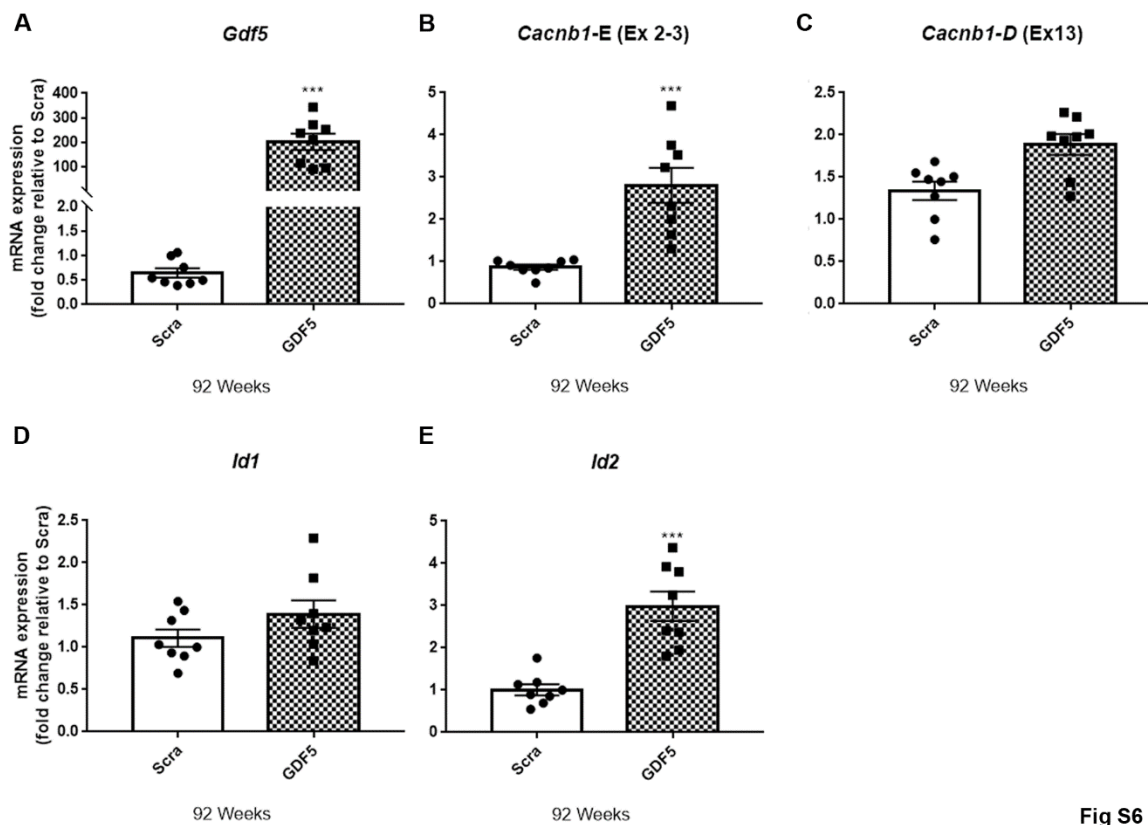
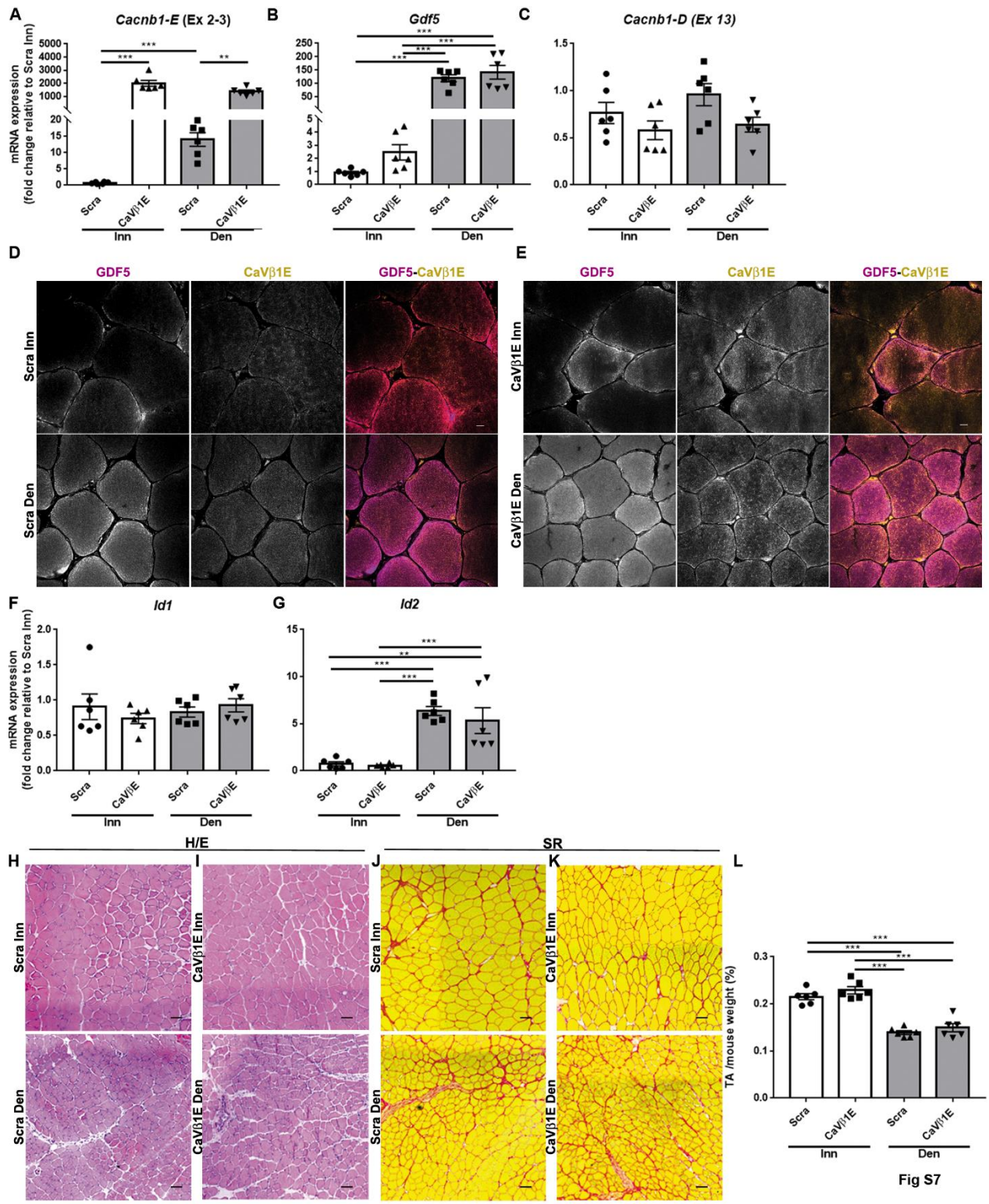


Fig S6

### Fig S6: GDF5 over-expression in aged mice muscle

**A-E:** RT-qPCR results showing the expression of (A) *Gdf5* (B) *Cacnb1-E* (ex 2-3), (C) *Cacnb1-D* (ex13), (D) *Id1* and (E) *Id2* in innervated TA from 92-week-old mice treated with AAV- sh scrambled (Scra) or AAV-GDF5 (GDF5). A: Minimum top axis= 2

**A-E:** Data are shown as mean  $\pm$  s.e.m. (n=8); \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001, calculated by independent samples t-test (two tailed).





**Fig S7: CaVβ1E over-expression in young mice muscle**

**A-C:** RT-qPCR for (A) *Cacnb1*-E (Ex2-3), (B) *Gdf5* and (C) *Cacnb1*-D in adult TA innervated (Inn) or denervated for 15 days (Den) treated with Scra, or CaVβ1E. A: minimum top axis =20;

B: minimum top axis =5.

**D, E:** Immunofluorescence images of TA Inn (top) or Den (bottom) treated with (D) Scra or (E) CaVβ1E: stained with GDF5 (Magenta), CaVβ1E (Yellow). Bar: 10 μm.

**F, G:** RT-qPCR for (F) *Id1* and (G) *Id2* in TA Inn or Den treated with Scra or CaVβ1E.

**H, I:** Haematoxylin and eosin (H/E) staining of TA Inn (top) or Den (bottom) treated with (H) Scra or (I) CaVβ1E. Bar 100 μm.

**J, K:** Sirius red (SR) staining of TA Inn (top) or Den (bottom) treated with (J) Scra or (K) CaVβ1E. Bar 100 μm.

**L:** Muscle/body-weight ratio of Inn or Den adult TAs treated with Scra or CaVβ1E.

**A:** Means ± s.e.m. (n=6) \*P < 0.05, \*\*\*P < 0.001, \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001; (two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%. Each row was analyzed individually, without assuming a consistent SD).

**B, C, F-G, L:** Means ± s.e.m. (n=6) \*P < 0.05, \*\*\*P < 0.001, \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001, (ordinary one-way Anova - Sidak's test).

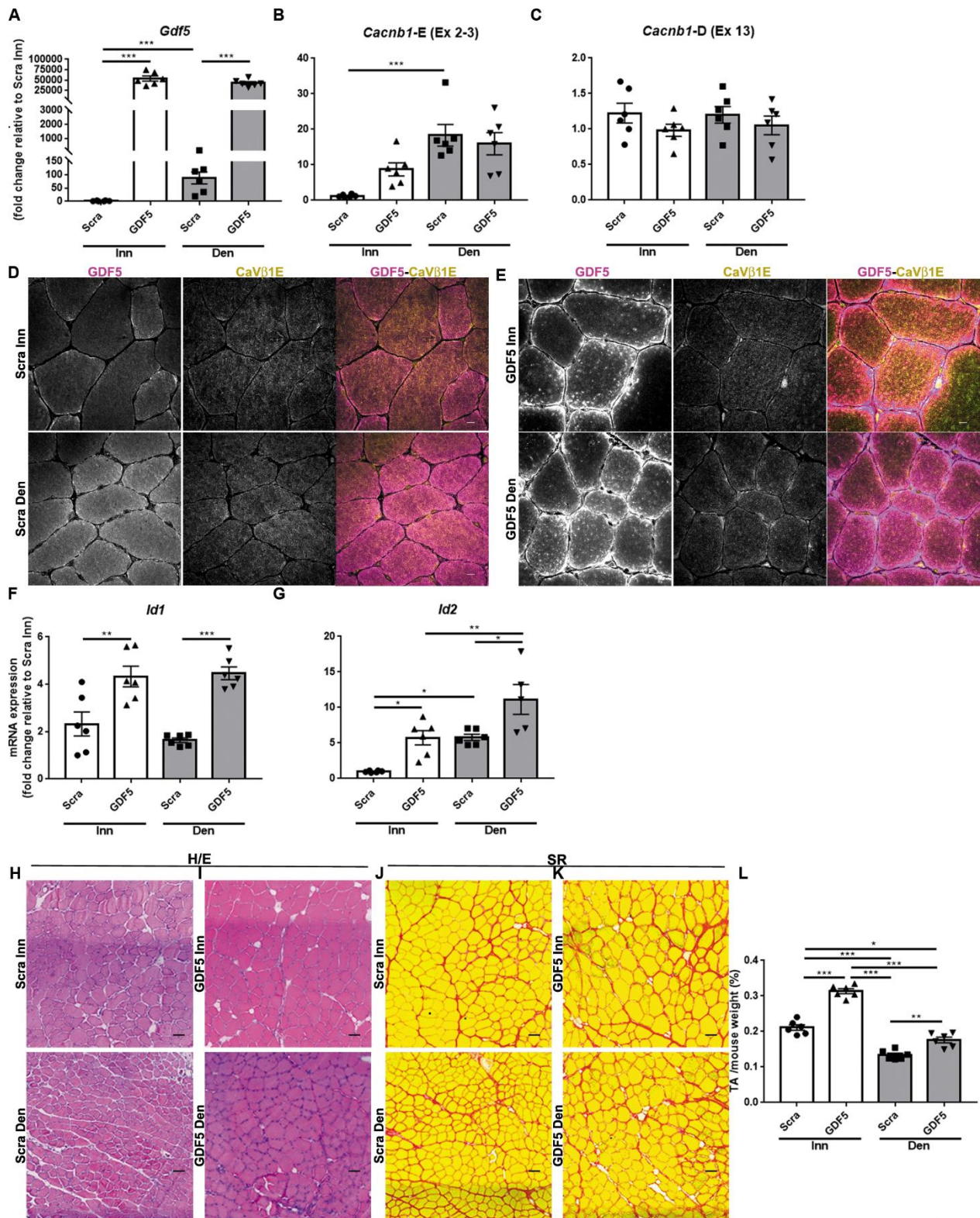


Fig. S8

**Fig S8: GDF5 over-expression in young mice muscle**

**A-C:** RT-qPCR for (A) *Gdf5*, (B) *Cacnb1*-E (Ex2-3) and (C) *Cacnb1*-D in adult TAs innervated (Inn) or denervated for 15 days (Den) treated with Scra or GDF5. A: minimum medium axis=150; minimum top axis =3000.

**D, E:** Immunofluorescence images of TA Inn (top) or Den (bottom) treated with (D) Scra or (E) GDF5, stained with GDF5 (Magenta), CaV $\beta$ 1E (Yellow). Bar: 10  $\mu$ m.

**F, G:** RT-qPCR for (F) *Id1* and (G) *Id2* in TAs Inn or Den treated with Scra or GDF5.

**H, I:** Haematoxylin and eosin (H/E) staining of TAs Inn (top) or Den (bottom) treated with (H) Scra or (I) GDF5. Bar 100  $\mu$ m.

**J, K:** Sirius red (SR) staining of TAs Inn (top) or Den (bottom) treated with (J) Scra or (K) GDF5. Bar 100  $\mu$ m.

**L:** Muscle/body-weight ratio of Inn or Den adult TAs treated with Scra or GDF5.

**A:** Means  $\pm$  s.e.m. (n=6) \*P < 0.05, \*\*\*P < 0.001, \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001; (two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%. Each row was analyzed individually, without assuming a consistent SD).

**B, C, F-G, L:** Means  $\pm$  s.e.m. (n=6) \*P < 0.05, \*\*\*P < 0.001, \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001, (ordinary one-way Anova - by Sidak's test).

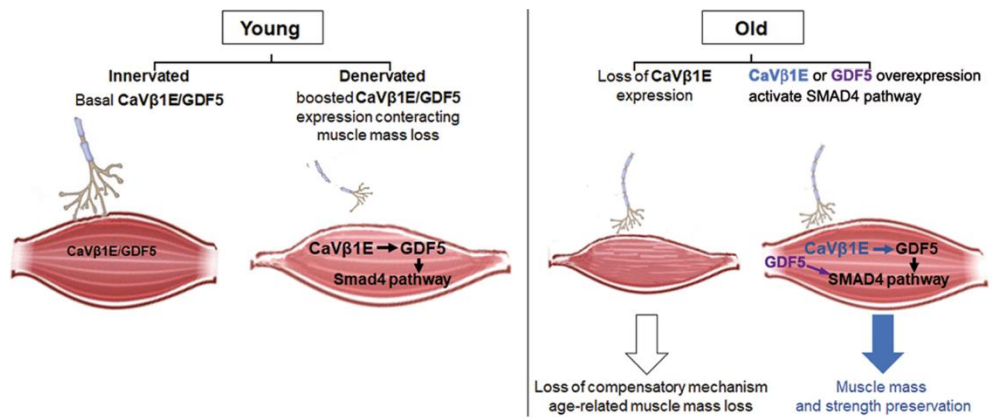


Fig S9

**Fig S9: Representation of CaVβ1E/GDF5 axis in skeletal muscle.**

In young mice CaVβ1E/GDF5 basal expression is low in innervated muscle, denervation boosts CaVβ1E expression which mediates GDF5 increase counteracting muscle mass loss. Aged skeletal muscle displays reduced basal expression of CaVβ1E leading alteration of the compensatory mechanism that might be responsible of age-related muscle mass loss. CaVβ1E or GDF5 over-expression in mouse aged muscle increases GDF5 signaling and preserves age-related muscle decline.

Table S1

Expression Data GSMG0007319	Controls			Denervated		
	TAG8	TAG9	TAG10	TAD8	TAD9	TAD10
e1.0 (1-301) (Prom2)	10.918583 1	3.4792192 4	7.7669751 1	41.328882 4	70.564966	62.094244 3
i1.0 (302-2876)	1.0382975 9	1.0263702	1.0821036 8	1.1996670 3	1.2353359 6	1.193923
e2.0 (2877-2963)	13.456805	3.4208049 6	7.7609201 2	54.539102 9	88.357732 8	78.075394 4
i2.0 (2964-4269)	1.2048651	1.0099492 8	1.0335141 4	1.4434678 9	1.6314482 5	1.2190471 3
Prom1 (4270-4370)	17.845409 6	11.705383 5	21.505688 9	4.7016939 7	9.3069813 7	6.4954670 4
iProm1.0 (4371-5800)	1.2335325 4	1.1854241 7	1.4988598 2	1.5441177 5	1.7633286 9	1.6542060 3
e3.0 (5801-5920)	56.530007 8	32.317532 2	58.744081 6	54.422048 9	88.005657 6	78.852236 8
i3.0 (5921-9708)	1.0248702 9	1.0344666 9	1.0284867 6	1.0722433 1	1.0737645	1.0609656 3
e4.0 (9709-9831)	65.799608 9	39.726804 2	66.810395 7	55.743609 1	90.857526	79.511738 9
i4.0 (9832-10321)	1.0143686 5	1.0094437 9	1.0179997 7	1.0141231 6	1.0408515 7	1.0136248 5
e5.0 (10322-10458)	50.970335 9	31.588272 5	53.892807 1	48.629176 4	80.796917 7	64.903456 1
i5.0 (10459-10700)	1.2075943 7	1.1029581 7	1.2341625 8	1.1660990 3	1.2818069 4	1.2397293 1
e6.0 (10701-10777)	80.192616	48.337876 6	86.620448 1	80.089465 5	116.22003 5	99.066124
i6.0 (10778-11145)	1.3083235 4	1.3661400 5	1.2065785 2	1.2041893 5	1.7623545 4	1.3694309
e7A.0 (11146-11300)	77.163826 3	50.611684 1	91.830671 6	82.624014 8	112.09787 5	108.22818 1
i7A.0 (11301-11996)	2.2358494 4	1.8463546 2	1.5956165 7	2.1914588 4	3.5820005 3	2.6711678 1
e7B.0 (11997-12016)	3.7645780 2	1.8077097	2.3279048 6	5.2556470 3	5.8901006 9	4.6592213 2
i7B.0 (12017-12266)	2.0250760 7	1.6132907 5	1.9820311 4	2.9902597 3	2.9951916 8	2.1076149
e8.0 (12267-12271)	63.598203 8	49.427932 6	96.236104 4	78.087431 3	90.196409 2	94.833730 2
e8.0 (12272-12347)	73.532090 4	50.127881 3	110.17296 6	85.246653 3	96.249931	101.58685 3
i8.0 (12348-12726)	2.2021629 8	1.5333952	1.8563750 8	1.6777031 7	1.8567148 4	1.6712327
e9.0 (12727-12785)	110.70005 1	76.484530 6	153.78064 9	125.58118 6	144.62921 1	156.08990 6
i9.0 (12786-12964)	1.6617753 8	1.5260609 5	1.5947627 6	1.6975634 7	1.521738	1.8042432 9
e10.0 (12965-13074)	119.82044 8	74.959405 3	131.89628 6	129.18297 6	150.53628 6	158.99352 1
i10.0 (13075-13210)	1.8986065 6	1.6085023 3	1.9865525 2	2.1599406 4	2.0956733 7	2.1987542 4
e11.0 (13211-13362)	194.10124 3	110.29333 6	173.83801 4	227.55867 4	202.38845 3	255.41187 1
i11.0 (13363-16859)	1.3389368 9	1.1202744 8	1.1270516 4	1.2885857 1	1.2768531 9	1.1495027 7
e12.0 (16860-16955)	192.43222 1	106.44010 1	132.26332 4	222.41622 2	182.83107 7	247.06902 5
i12.0 (16956-17328)	1.3397302 8	1.2911665 3	1.0847291 4	1.6662955 3	1.5214851 8	1.6350431 6
e13.0 (17329-17514)	145.41075	77.998826 1	86.248349 9	180.70555	137.48114 7	183.90718 2
ae13.0 (17515-17896)	54.272180 5	33.214470 2	49.519626 2	70.732289 7	44.064172 8	66.959990 6

i13.0 (17897-19280)	16.315111 5	11.982134 1	15.969867 5	14.865795 2	24.639612 1	23.340037 7
e14.0 (19281-21135)	25.512106 7	17.485891 9	23.631139 2	31.107097 8	27.108336 9	43.803958 3
<b>Junction Data GSMG0007319</b>	<b>TAG8</b>	<b>TAG9</b>	<b>TAG10</b>	<b>TAD8</b>	<b>TAD9</b>	<b>TAD10</b>
Feature name						
eprom1 (4354-4364)/e3.0(5801-4364)	0	2	4	0	0	0
e12.0 (16884-16894)/e12.0(16918-16894)	0	0	0	0	0	0
i3.0 (8370-8380)/ae13.0(17550-8380)	0	0	0	0	0	0
e6.0 (10767-10777)/e7B.0(11997-10777)	5	0	4	2	10	12
e4.0 (9821-9831)/e5.0(10322-9831)	379	198	306	315	684.5	492
e3.0 (5910-5920)/i3.0(6104-5920)	0	0	0	0	0	0
i13.0 (18807-18817)/i13.0(18855-18817)	0	0	0	0	0	0
e13.0 (17504-17514)/e14.0(20426-17514)	0	0	0	0	2	0
e2.0 (2953-2963)/e3.0(5801-2963)	151.5	23	65	625	1159	903
e3.0 (5910-5920)/e7A.0(11238-5920)	0	0	0	0	1	0
e7A.0 (11290-11300)/e8.0(12271-11300)	2	0	0	0	0	0
i13.0 (18327-18337)/i13.0(18882-18337)	0	0	0	0	0	0
i1.0 (1412-1422)/e2.0(2877-1422)	0	0	0	0	0	0
e10.0 (13028-13038)/e11.0(13286-13038)	0	0	1	1	2	0
e11.0 (13352-13362)/e14.0(16869-13362)	0	0	2	0	0	0
i1.0 (554-564)/e2.0(2877-564)	0	0	0	0	0	0
e10.0 (13031-13041)/e11.0(13292-13041)	2	0	7	3	0	2
e4.0 (9821-9831)/e5.0(10325-9831)	1	0	1	3	2	0
e6.0 (10767-10777)/e8.0(12271-10777)	2	0	0	0	2	1
e8.0 (12341-12351)/e9.0(12728-12351)	0	0	0	0	0	0
e12.0 (16935-16945)/e13.0(17329-16945)	0	0	0	0	0	0
i1.0 (1189-1199)/e2.0(2877-1199)	0	0	0	0	0	0
i1.0 (2243-2253)/e2.0(2877-2253)	0	0	0	0	0	4
iprom1.0 (4724-4734)/e3.0(5801-4734)	1	0	0	0	1	0
e2.0 (2953-2963)/e4.0(9709-2963)	0	0	0	0	0	0
e8.0 (12335-12345)/e9.0(12726-12345)	0	0	0	0	1	0
e5.0 (10450-10460)/e6.0(10701-10460)	0	0	0	0	0	0
e14.0 (19532-19542)/e14.0(19587-19542)	0	0	0	0	4	0
e2.0 (2954-2964)/e4.0(5803-2964)	0	0	0	1	0	0
e1.0 (291-301)/e2.0(2877-301)	83	20	68	514	825.5	644.5
i1.0 (1741-1751)/e2.0(2877-1751)	0	0	0	0	0	0
e10.0 (13034-13044)/e11.0(13292-13044)	0	0	0	0	1	0

i6.0 (11135-11145)/e8.0(12267-11145)	0	0	0	0	0	0
eprom1.0 (4360-4370)/e3.0(5801-4370)	466.5	269	520	89	224	128
e1.0 (41-51)/e1.0(186-51)	0	0	0	0	0	0
e7A.0 (11290-11300)/e8.0(12267-11300)	450	300	654	559.5	726.5	715
eprom1.0 (4360-4370)/e3.0(5811-4370)	0	0	1	0	0	0
e3.0 (4360-4370)/i3.0(4685-4370)	3	0	4	0	0	0
e6.0 (10767-10777)/e8.0(12267-10777)	0	2	4	0	4	0
ae13.0 (17522-17532)/ae13.0(17603-17532)	0	0	0	0	2	0
e14.0 (20444-20454)/e14.0(20821-20454)	0	0	0	2	0	0
e8.0 (12337-12347)/i8.0(12449-12347)	0	0	0	0	0	0
e5.0 (10448-10458)/e6.0(10705-10458)	0	0	0	0	0	0
e13.0 (17504-17514)/e14.0(19308-17514)	0	0	0	0	0	0
e10.0 (13064-13074)/e11.0(13222-13074)	0	0	0	0	0	0
e12.0 (16944-16954)/e13.0(17329-16954)	3	2	0	0	0	0
e7A.0 (11290-11300)/e9.0(12727-11300)	0	2	0	2	2	3
e6.0 (10767-10777)/e11.0(12727-10777)	0	0	0	0	0	0
e13.0 (17504-17514)/e14.0(19469-17514)	0	0	0	0	0	0
e7A.0 (11290-11300)/e7B.0(11997-11300)	1	0	0	0	0	0
e10.0 (13064-13074)/e11.0(13211-13074)	1026	565	1222	1161	1588.5	1523
e11.0 (13352-13362)/e12.0(16860-13362)	1603.5	860	1079.5	1888.5	1708	2197
i1.0 (1700-1710)/e2.0(2877-1710)	0	0	0	0	0	2
i1.0 (2064-2074)/e2.0(2877-2074)	0	0	0	0	0	0
e11.0 (13350-13360)/e12.0(16859-13360)	0	0	0	0	1	0
e8.0 (12337-12347)/i8.0(12611-12347)	0	0	0	0	0	0
e4.0 (9808-9818)/e9.0(12765-9818)	2	0	0	0	0	0
e8.0 (12337-12347)/e9.0(12727-12347)	555	367	827.666667	688	894.5	864.5
e13.0 (17522-17532)/ae13.0(17603-17532)	0	0	0	0	0	4
e6.0 (10767-10777)/e7A.0(11179-10777)	0	0	0	0	0	0
i1.0 (1932-1942)/e2.0(2877-1942)	0	0	0	0	2	0
e3.0 (5909-5919)/e4.0(9709-5919)	0	0	0	0	1	0
e10.0 (12994-13004)/e11.0(13327-13004)	0	0	0	1	0	0
e13.0 (17504-17514)/e14.0(19281-17514)	18	4	6	11	17	12
e7B (12006-12016)/e8.0(12267-12016)	7	1	4	6	23	22
e6.0 (10767-10777)/e7A(11146-10777)	899.5	486	978.5	918.5	1472.5	1190
e12.0 (16945-16955)/i12.0(17262-16955)	0	0	0	0	1	0
e5.0 (10448-10458)/e6.0(10701-10458)	548	287	481	553	959	726
e3.0 (5910-5920)/e4.0(9709-5920)	458.5	247	423	411	834	757
i8.0 (12629-12639)/e9.0(12727-12639)	0	0	0	0	1	0

e12.0 (16945-16955)/e13.0(17329-16955)	1609.5	825.5	908	2006	1733	2093.5
e13.0 (17504-17514)/i13.0(18542-17514)	1	0	0	0	0	0
e1.0 (291-301)/e4.0(9709-301)	0	0	0	0	0	0
i1.0 (1361-1371)/e2.0(2877-1371)	0	0	0	0	0	2
e9.0 (12775-12785)/e10.0(12965-12785)	1078	631	1282	1190.5	1646.5	1552.5
i3.0 (6379-6389)/e10.0(13019-6389)	0	0	0	0	0	0
e13.0 (17469-17479)/ae13.0(17560-17479)	0	0	0	0	0	0



**Table S2**

**100 Top Up-regulated Genes in Innervated Samples**

<b>GenoSplice Stable ID (2016_1)</b>	<b>Symbol or Genbank Transcript ID</b>	<b>Description</b>	<b>Coordinates (mm10)</b>	<b>Fold-Change</b>	<b>Adjusted P-Value</b>	<b># E-Box in core promoter</b>
GSMG0001037	Mpz	myelin protein zero	chr1:171150709-171161130	96.90	1.99E-09	3
GSMG0038980	Kcng4	potassium voltage-gated channel, subfamily G, member 4	chr8:119623854-119635680	91.15	2.68E-10	4
GSMG0013699	Gm4544	predicted gene, 35853	chr15:102657694-102660402	71.40	2.39E-37	3
GSMG0000095	Col9a1	collagen, type IX, alpha 1	chr1:24177610-24252738	70.13	3.44E-17	3
GSMG0004504	<i>AK085787</i>		chr10:95823977-95824412	66.17	2.17E-07	6
GSMG0027511	Perm1	PPARGC1 and ESRR induced regulator, muscle 1	chr4:156215925-156221307	57.01	2.40E-49	4
GSMG0055775	<i>DQ716315</i>		chr17:27365340-27365399	56.93	3.24E-06	8
GSMG0040627	Tecta	tectorin alpha	chr9:42329619-42399929	54.42	1.63E-09	5
GSMG0016466	Gm16758	predicted gene, 16758	chr17:29890084-29897066	52.87	1.31E-44	4
GSMG0027338	Lrrc38	leucine rich repeat containing 38	chr4:143349750-143371028	52.72	7.06E-106	6
GSMG0017912	Gm19689	predicted gene, 19689	chr17:83033592-83078226	50.86	9.11E-06	3
GSMG0002639	Gm32200	predicted gene, 32200	chr1:191436340-191465049	48.93	1.74E-24	8
GSMG0004314	Odf3l2	outer dense fiber of sperm tails 3-like 2	chr10:79639526-79645740	48.56	1.67E-08	8
GSMG0025477	A730090N16Rik	RIKEN cDNA A730090N16 gene	chr3:65324020-65349430	48.37	3.22E-12	3
GSMG0002406	Lrrc52	leucine rich repeat containing 52	chr1:167445675-167466780	43.53	1.50E-09	5
GSMG0055774	<i>DQ723921</i>		chr17:27365228-27365254	42.77	2.53E-05	9
GSMG0003439	<i>AK009289</i>		chr10:95680807-95681124	42.52	4.05E-05	4
GSMG0018737	Aqp4	aquaporin 4	chr18:15389394-15411015	41.91	2.93E-06	5

GSMG0046244	<i>DQ713904</i>		chr17:27367238-27367267	40.19	5.16E-05	8
GSMG0014613	Sec14I5	SEC14-like lipid binding 5	chr16:5147109-5183934	40.11	5.44E-61	3
GSMG0031785	Cntnap2	contactin associated protein-like 2	chr6:45060061-47301369	39.38	1.08E-12	2
GSMG0026471	Car9	carbonic anhydrase 9	chr4:43507024-43513729	37.76	1.52E-32	5
GSMG0001539	Mettl21c	methyltransferase like 21C	chr1:44009408-44020035	37.65	3.08E-35	7
GSMG0046243	<i>DQ700568</i>		chr17:27365542-27365579	37.07	8.17E-05	5
GSMG0020438	Pdzd7	PDZ domain containing 7	chr19:45026906-45045772	33.98	8.21E-33	8
GSMG0014879	Smco1	single-pass membrane protein with coiled-coil domains 1	chr16:32271467-32274779	33.22	2.20E-09	8
GSMG0003440	<i>AK133093</i>		chr10:95731226-95739353	31.81	5.34E-35	5
GSMG0001881	Asb18	ankyrin repeat and SOCS box-containing 18	chr1:89952591-90014666	31.44	1.19E-46	9
GSMG0018923	Spinkl	serine protease inhibitor, Kazal type-like	chr18:44166358-44242898	30.97	5.26E-09	4
GSMG0008774	2310016D03Rik	RIKEN cDNA 2310016D03 gene	chr12:30410559-30467359	29.61	3.94E-14	1
GSMG0000465	2310015K22Rik	RIKEN cDNA 2310015K22 gene	chr1:80225043-80228792	29.31	2.81E-18	7
GSMG0051039	Ccdc146	coiled-coil domain containing 146	chr5:21292961-21424677	29.17	4.48E-05	4
GSMG0013711	<i>AK084656</i>		chr15:103135879-103140669	28.39	3.25E-38	7
GSMG0004493	<i>AK042410</i>		chr10:95261229-95264978	27.51	5.52E-11	3
GSMG0020177	Aldh1a7	aldehyde dehydrogenase family 1, subfamily A7	chr19:20692953-20727556	27.27	1.95E-15	6
GSMG0051426	Klhl33	kelch-like 33	chr14:50891389-50893255	26.98	9.38E-27	5
GSMG0006072	<i>AK033778</i>		chr11:110520907-110523007	26.48	7.01E-32	5
GSMG0039999	Ky	kyphoscoliosis peptidase	chr9:102505747-102546244	26.20	2.58E-31	6

GSMG0030435	Fgfbp1	fibroblast growth factor binding protein 1	chr5:43978858-43981799	25.79	2.01E-32	5
GSMG0016269	4933401D09Rik	RIKEN cDNA 4933401D09 gene	chr17:15631787-15641103	25.27	2.93E-13	4
GSMG0003359	1700113H08Rik	RIKEN cDNA 1700113H08 gene	chr10:87058046-87230599	25.06	3.13E-09	3
GSMG0052027	Tmem233	transmembrane protein 233	chr5:116040532-116083246	24.69	2.76E-49	3
GSMG0037249	Lrp2bp	Lrp2 binding protein	chr8:45999302-46029476	24.27	7.63E-17	7
GSMG0000328	2310016D23Rik	RIKEN cDNA 2310016D23 gene	chr1:60778844-60803788	24.21	5.51E-18	5
GSMG0030985	<i>AK047066</i>		chr5:116038762-116040414	24.20	1.83E-42	6
GSMG0012351	<i>AK009210</i>		chr14:50888590-50890567	23.71	5.02E-28	3
GSMG0034468	Ntf5	neurotrophin 5	chr7:45413902-45414534	23.53	8.27E-05	1
GSMG0021576	A530058N18Rik	RIKEN cDNA A530058N18 gene	chr2:114013563-114068324	23.53	8.27E-24	5
GSMG0051194	Mir3058	microRNA 3058	chr10:95559231-95559321	23.47	2.81E-03	7
GSMG0037614	Irx3os	iroquois homeobox 3, opposite strand	chr8:91800413-91807539	23.47	7.34E-11	1
GSMG0024013	B230312C02Rik	RIKEN cDNA B230312C02 gene	chr2:180370491-180386453	22.55	1.02E-34	4
GSMG0015713	Casr	calcium-sensing receptor	chr16:36490585-36562134	22.47	1.15E-12	5
GSMG0011412	Gm28651	predicted gene 28651	chr14:32686558-32689247	21.52	2.53E-42	2
GSMG0018350	Kcnn2	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2	chr18:45268860-45686024	21.29	4.50E-19	5
GSMG0055773	<i>DQ547669</i>		chr17:27365160-27365187	20.79	7.70E-04	8
GSMG0028504	Ncmap	noncompact myelin associated protein	chr4:135369577-135398229	20.67	5.18E-08	4
GSMG0016734	<i>AK218500</i>		chr17:46674726-46674849	20.63	1.34E-03	3

GSMG0014662	Rmi2	RecQ mediated genome instability 2	chr16:10835058-10892966	20.61	1.58E-34	3
GSMG0000429	Plcd4	phospholipase C, delta 4	chr1:74542887-74567794	20.09	2.12E-26	9
GSMG0005742	<i>AK082227</i>		chr11:88165778-88167194	19.84	2.79E-07	5
GSMG0032499	Fgf6	fibroblast growth factor 6	chr6:127015542-127028184	19.83	7.14E-62	5
GSMG0005369	Dhrs7c	dehydrogenase/reductase (SDR family) member 7C	chr11:67798271-67816005	19.51	1.40E-59	6
GSMG0012353	A930018M24Rik	RIKEN cDNA A930018M24 gene	chr14:50895942-50897456	19.41	2.29E-21	6
GSMG0001880	<i>AK081208</i>		chr1:89950220-89951759	19.37	1.03E-09	4
GSMG0024386	Ptx3	pentraxin related gene	chr3:66219887-66225806	19.24	1.61E-34	5
GSMG0034496	Kcnc1	potassium voltage gated channel, Shaw-related subfamily, member 1	chr7:46396468-46438704	19.23	1.93E-43	2
GSMG0014846	Ostn	osteocrin	chr16:27307489-27351209	18.92	6.77E-06	2
GSMG0041691	Atp1b4	ATPase, (Na+)/K+ transporting, beta 4 polypeptide	chrX:38316122-38336784	18.77	2.98E-13	3
GSMG0006069	Map2k6	mitogen-activated protein kinase kinase 6	chr11:110399121-110525522	18.35	1.37E-31	2
GSMG0037890	Wfdc1	WAP four-disulfide core domain 1	chr8:119666363-119688222	18.27	1.18E-30	6
GSMG0002657	G0s2	G0/G1 switch gene 2	chr1:193272160-193273188	18.19	1.98E-43	3
GSMG0006287	Emid1	EMI domain containing 1	chr11:5106266-5152242	18.06	6.06E-61	5
GSMG0024399	Mlf1	myeloid leukemia factor 1	chr3:67374096-67399998	17.67	2.42E-23	6

GSMG0017337	Mdga1	MAM domain containing glycosylphosphatidylinositol anchor 1	chr17:29827958-29970087	16.84	3.95E-58	7
GSMG0025486	AK084367		chr3:66220286-66221419	16.69	3.54E-31	5
GSMG0013663	Nr4a1	nuclear receptor subfamily 4, group A, member 1	chr15:101266846-101274795	16.54	1.28E-36	1
GSMG0018815	Nrep	neuronal regeneration related protein	chr18:33437019-33464029	16.49	9.08E-72	2
GSMG0023811	AK138493		chr2:160356151-160359618	16.11	1.33E-13	4
GSMG0025745	Adamtsl4	ADAMTS-like 4	chr3:95676204-95687917	16.09	1.14E-39	8
GSMG0029690	C330018A13Rik	RIKEN cDNA C330018A13 gene	chr5:116123613-116129110	15.96	4.36E-09	1
GSMG0000571	Ramp1	receptor (calcitonin) activity modifying protein 1	chr1:91179822-91225196	15.87	5.93E-43	6
GSMG0012352	AK034241		chr14:50893641-50895537	15.54	1.05E-15	7
GSMG0019704	Golga7b	golgi autoantigen, golgin subfamily a, 7B	chr19:42247573-42270348	15.37	2.54E-13	5
GSMG0006232	Slc16a3	solute carrier family 16 (monocarboxylic acid transporters), member 3	chr11:120948484-120960868	15.37	8.26E-38	4
GSMG0001542	Mettl21e	methyltransferase like 21E	chr1:44204070-44218932	15.18	5.67E-22	6
GSMG0027819	Gm12514	predicted gene 12514	chr4:55129708-55279483	14.91	3.23E-18	3
GSMG0034467	Ntf5	neurotrophin 5	chr7:45413695-45417178	14.91	3.53E-22	2
GSMG0039455	Scn4b	sodium channel, type IV, beta	chr9:45138751-45154155	14.88	3.92E-08	2
GSMG0011730	Stmn4	stathmin-like 4	chr14:66344296-66361679	14.88	1.05E-08	5
GSMG0037381	Gdf1	growth differentiation factor 1	chr8:70315773-70331592	14.84	1.26E-58	4

GSMG0010596	Gm33489	predicted gene, 33489	chr13:44010592-44018524	14.65	2.10E-05	2
GSMG0038765	Ces1d	carboxylesterase 1D	chr8:93166072-93197804	14.64	8.98E-06	6
GSMG0002946	1700027J07Rik	RIKEN cDNA 1700027J07 gene	chr10:43746157-43765836	14.14	1.94E-09	8
GSMG0060166	<i>KF703534</i>		chr1:181256317-181257313	14.04	1.43E-04	6
GSMG0027446	<i>AK132360</i>		chr4:153603838-153606492	14.00	5.93E-13	8
GSMG0034469	Lhb	luteinizing hormone beta	chr7:45418353-45421854	13.73	5.17E-16	4
GSMG0055771	<i>DQ723385</i>		chr17:27364259-27364287	13.67	1.57E-02	6
GSMG0034863	Gm45012	predicted gene 45012	chr7:99202365-99227080	13.65	1.85E-10	5
GSMG0055772	<i>DQ540404</i>		chr17:27364595-27364623	13.65	1.78E-02	6
GSMG0020389	Frat2	frequently rearranged in advanced T cell lymphomas 2	chr19:41845978-41848134	13.62	4.36E-46	3

### 100 Up-regulated Genes in Denervated Samples

GenoSplice Stable ID (2016_1)	Symbol or Genbank Transcript ID	Description	Coordinates (mm10)	Fold-Change	Adjusted P-Value	# E-Box in core promoter
GSMG0026637	Orm3	orosomucoid 3	chr4:63356162-63359511	311.87	8.62E-18	8
GSMG0040825	Cplx3	complexin 3	chr9:57599992-57606281	272.43	4.89E-19	6
GSMG0042491	Mbnl3	muscleblind-like 3 (Drosophila)	chrX:51113494-51205997	182.50	4.68E-16	5
GSMG0006564	Adra1b	adrenergic receptor, alpha 1b	chr11:43774605-43901237	177.85	1.20E-63	4
GSMG0059989	<i>AB344123</i>		chr16:94358979-94359022	173.24	2.71E-11	5
GSMG0031189	Cldn4	claudin 4	chr5:134945126-134946935	172.18	4.65E-17	4
GSMG0008034	Nrcam	neuronal cell adhesion molecule	chr12:44328885-44601964	157.76	1.71E-130	1
GSMG0052404	Gm6524	predicted gene 6524	chr8:11692982-11694514	119.52	3.24E-13	10
GSMG0026638	Orm2	orosomucoid 2	chr4:63362449-63365878	115.38	7.89E-07	5
GSMG0039504	Plet1	placenta expressed transcript 1	chr9:50491035-50505639	110.54	2.11E-40	4
GSMG0055538	<i>AB344132</i>		chr16:94359158-94359178	109.71	6.03E-09	7

GSMG0016088	<i>AK042189</i>		chr16:92691406-92694925	106.62	3.13E-44	1
GSMG0060036	<i>AB344128</i>		chr16:94359039-94359058	103.70	6.29E-08	6
GSMG0041439	Scn5a	sodium channel, voltage-gated, type V, alpha	chr9:119483408-119579016	96.76	1.37E-51	8
GSMG0015322	<i>AK040682</i>		chr16:92698176-92700539	85.41	3.46E-14	2
GSMG0025814	Cd101	CD101 antigen	chr3:100993530-101029495	85.23	8.67E-26	4
GSMG0051427	Ang2	angiogenin, ribonuclease A family, member 2	chr14:51195486-51195923	75.33	2.01E-08	2
GSMG0022012	Bpifb1	BPI fold containing family B, member 1	chr2:154190818-154220343	75.29	1.14E-08	5
GSMG0028484	Gm7534	predicted gene 7534	chr4:134190804-134203004	74.71	9.38E-37	6
GSMG0026518	Stra6l	STRA6-like	chr4:45848814-45887010	73.73	9.31E-10	8
GSMG0011044	<i>AK037785</i>		chr13:101515246-101518270	63.72	9.64E-15	3
GSMG0027329	Agmat	agmatine ureohydrolase (agmatinase)	chr4:141746675-141759263	62.59	1.71E-17	7
GSMG0024005	Hrh3	histamine receptor H3	chr2:180099466-180104408	60.19	4.86E-15	3
GSMG0029510	Spp1	secreted phosphoprotein 1	chr5:104435111-104441053	59.36	2.91E-80	4
GSMG0015618	Atp13a4	ATPase type 13A4	chr16:29396091-29544866	56.85	1.52E-06	7
GSMG0023761	Gdf5	growth differentiation factor 5	chr2:155941023-155945364	51.85	5.01E-17	3
GSMG0040150	Prss46	protease, serine 46	chr9:110844506-110856522	48.84	1.67E-11	4
GSMG0022339	Meig1	meiosis expressed gene 1	chr2:3409043-3422648	48.79	1.35E-06	5
GSMG0014549	Krt8	keratin 8	chr15:101996698-102004342	48.65	1.34E-09	2
GSMG0014526	Krt80	keratin 80	chr15:101347444-101370164	46.38	5.74E-51	8
GSMG0027027	Mycl	v-myc avian myelocytomatosis viral oncogene lung carcinoma derived	chr4:122995931-123002478	41.97	2.05E-29	5
GSMG0040826	Lman1l	lectin, mannose-binding 1 like	chr9:57607033-57620774	41.68	7.95E-33	6
GSMG0003914	<i>AK136640</i>		chr10:25697993-25699481	40.15	6.15E-25	3

GSMG0001855	Htr2b	5-hydroxytryptamine (serotonin) receptor 2B	chr1:86099037-86111971	38.80	1.99E-16	4
GSMG0026636	Orm1	orosomuroid 1	chr4:63344556-63348163	37.11	2.45E-21	8
GSMG0022046	<i>AK028903</i>		chr2:155842574-155863035	36.49	1.41E-19	9
GSMG0033167	4933431G14Rik	RIKEN cDNA 4933431G14 gene	chr6:72120213-72123198	34.89	1.14E-12	3
GSMG0000524	Chrnd	cholinergic receptor, nicotinic, delta polypeptide	chr1:87190597-87200069	33.81	2.91E-63	11
GSMG0033888	Gm32914	predicted gene, 32914	chr6:145999543-146001763	32.46	2.00E-33	5
GSMG0010208	Ankrd55	ankyrin repeat domain 55	chr13:112288450-112383979	31.41	9.63E-30	8
GSMG0029270	Slc10a4	solute carrier family 10 (sodium/bile acid cotransporter family), member 4	chr5:73006903-73014683	30.40	3.42E-24	5
GSMG0037184	Dusp4	dual specificity phosphatase 4	chr8:34807295-34819894	28.31	1.86E-64	1
GSMG0000791	Chil1	chitinase-like 1	chr1:134182404-134190029	27.77	7.34E-25	7
GSMG0064962	Gm14486	predicted gene 14486	chr2:30658189-30678011	27.62	1.12E-08	4
GSMG0024551	Kcnn3	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3	chr3:89520162-89672494	26.91	1.69E-51	5
GSMG0040364	<i>AK018026</i>		chr9:14586096-14592612	26.83	1.11E-03	7
GSMG0016148	Tiam2	T cell lymphoma invasion and metastasis 2	chr17:3326573-3531344	26.54	1.09E-66	3
GSMG0013677	Krt18	keratin 18	chr15:102028216-102032026	24.94	2.23E-06	4
GSMG0001792	<i>AK017575</i>		chr1:78595031-78609509	24.46	3.62E-23	2
GSMG0008245	Plekhh1	pleckstrin homology domain containing, family H (with MYTH4 domain) member 1	chr12:79029163-79081653	24.42	2.36E-25	7
GSMG0029232	Chrna9	cholinergic receptor, nicotinic, alpha polypeptide 9	chr5:65967124-65977486	23.33	2.87E-05	6



GSMG0024589	S100a8	S100 calcium binding protein A8 (calgranulin A)	chr3:90669070-90670034	22.65	1.92E-16	4
GSMG0029428	Shroom3	shroom family member 3	chr5:92683435-92965759	22.65	1.72E-29	2
GSMG0027202	E130218I03Rik	RIKEN cDNA E130218I03 gene	chr4:134243762-134245873	22.41	3.32E-20	4
GSMG0016087	Runx1	runt related transcription factor 1	chr16:92601467-92826076	21.92	4.52E-08	7
GSMG0020331	Ankrd1	ankyrin repeat domain 1 (cardiac muscle)	chr19:36111963-36119885	21.89	3.90E-10	8
GSMG0041841	Dusp9	dual specificity phosphatase 9	chrX:73639417-73643512	21.41	1.57E-17	7
GSMG0026602	Musk	muscle, skeletal, receptor tyrosine kinase	chr4:58285960-58374303	20.73	3.13E-49	7
GSMG0029692	Srrm4os	serine/arginine repetitive matrix 4, opposite strand	chr5:116438719-116465487	20.72	1.16E-11	3
GSMG0000794	Myog	myogenin	chr1:134289989-134292549	20.71	9.43E-12	4
GSMG0040459	Epor	erythropoietin receptor	chr9:21958898-21963576	20.18	2.04E-09	13
GSMG0016492	Gm30571	predicted gene, 30571	chr17:31912665-31913320	19.97	1.99E-13	7
GSMG0022979	Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	chr2:73563281-73580338	19.85	6.52E-42	6
GSMG0043880	Lincmd1	long intergenic non-coding RNA muscle differentiation 1	chr1:20669882-20682958	18.90	4.53E-38	7
GSMG0000525	Chrng	cholinergic receptor, nicotinic, gamma polypeptide	chr1:87204657-87212694	18.75	1.31E-07	5
GSMG0002996	Pln	phospholamban	chr10:53337686-53345999	18.28	1.95E-14	5
GSMG0019961	Tmem151a	transmembrane protein 151A	chr19:5079330-5085532	18.05	5.80E-28	6
GSMG0008516	Hhip1	hedgehog interacting protein-like 1	chr12:108306270-108329625	17.83	1.42E-29	4
GSMG0034638	Gm30459	predicted gene, 30459	chr7:74765986-74771243	17.75	8.16E-18	4
GSMG0037185	<i>AK012530</i>		chr8:34820584-34821951	17.32	3.11E-71	3

GSMG0016992	Plekhh2	pleckstrin homology domain containing, family H (with MyTH4 domain) member 2	chr17:84511892-84622142	17.26	1.19E-22	6
GSMG0023915	1200007C13Rik	RIKEN cDNA 1200007C13 gene	chr2:167827693-167833647	17.24	1.51E-10	3
GSMG0016904	Lpin2	lipin 2	chr17:71182558-71249818	16.77	1.10E-30	4
GSMG0038829	Rrad	Ras-related associated with diabetes	chr8:104628066-104631321	16.60	8.14E-06	1
GSMG0046004	Snora2b	small nucleolar RNA, H/ACA box 2B	chr15:98526348-98526459	16.44	4.30E-04	4
GSMG0023204	Cd82	CD82 antigen	chr2:93419102-93462950	15.94	2.22E-22	5
GSMG0020376	Blnk	B cell linker	chr19:40928924-40994535	15.91	5.75E-37	6
GSMG0013892	Gm35019	predicted gene, 35019	chr15:37893003-37899717	15.85	1.99E-12	10
GSMG0027862	Susd1	sushi domain containing 1	chr4:59314683-59438633	15.72	7.83E-21	4
GSMG0024714	Zfp697	zinc finger protein 697	chr3:98382479-98431951	15.45	1.40E-51	3
GSMG0034691	Prc1	protein regulator of cytokinesis 1	chr7:80294451-80316258	15.42	1.72E-23	4
GSMG0012081	Kcnk5	potassium channel, subfamily K, member 5	chr14:20140058-20181810	15.30	3.15E-18	4
GSMG0039920	Slc9a9	solute carrier family 9 (sodium/hydrogen exchanger), member 9	chr9:94669892-95230452	14.88	3.52E-17	6
GSMG0033944	Ttyh1	tweety family member 1	chr7:4119529-4135407	14.53	9.66E-20	6
GSMG0035933	<i>AK015435</i>		chr7:46345771-46349055	14.46	9.19E-06	6
GSMG0029802	Kntc1	kinetochore associated 1	chr5:123749726-123821593	14.25	7.69E-24	4
GSMG0033107	Gadd45a	growth arrest and DNA-damage-inducible 45 alpha	chr6:67035096-67037407	14.18	4.36E-26	4
GSMG0042710	Awat2	acyl-CoA wax alcohol acyltransferase 2	chrX:100402224-100442719	13.98	1.00E-13	3
GSMG0042701	Eda2r	ectodysplasin A2 receptor	chrX:97333837-97377216	13.96	5.57E-14	3
GSMG0028082	Gm830	predicted gene 830	chr4:95464724-95487395	13.81	6.06E-31	3

GSMG0037841	Vat1l	vesicle amine transport protein 1 like	chr8:114205630-114374070	13.70	5.94E-14	1
GSMG0009110	Pnma1	paraneoplastic antigen MA1	chr12:84146131-84148492	13.61	3.10E-10	5
GSMG0015588	Gm38456	predicted gene, 38456	chr16:24122699-24289838	13.48	7.77E-26	6
GSMG0020910	Aif1l	allograft inflammatory factor 1-like	chr2:31950273-31973441	13.14	6.42E-16	4
GSMG0013667	Gm35853	predicted gene, 35853	chr15:101322886-101405834	13.00	5.04E-13	4
GSMG0039010	Slc7a5	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	chr8:121881146-121907688	12.97	6.06E-31	4
GSMG0006183	Card14	caspase recruitment domain family, member 14	chr11:119307767-119345375	12.95	1.28E-19	5
GSMG0035028	Ampd3	adenosine monophosphate deaminase 3	chr7:110768206-110812409	12.81	3.66E-29	5
GSMG0017276	Arhgdig	Rho GDP dissociation inhibitor (GDI) gamma	chr17:26199183-26201350	12.69	2.34E-18	9
GSMG0041664	Lonrf3	LON peptidase N-terminal domain and ring finger 3	chrX:36328351-36366856	12.44	4.38E-15	2

**Table S3: List of human samples**

Myobank Institute of Myology (ref:BB-0033-00012)		
Age (Years)	Gender	Muscle type
37	M	Quadriceps (Y6)
38	M	Quadriceps (Y7)
42	M	Quadriceps (Y8)
38	M	Quadriceps (Q)
40	M	Fascia Lata (FL1)
70	F	Fascia Lata (FL2)
Neuropathology Lab, R. Escorolle-APHP-Paris		
ND	ND	Cervical tract spinal cord (Post-mortem)

**Table S4: Primer list**

Gene Name	Primer Sequence	Specie	Annotations	Use
<i>Myogenin</i> fw	AGTGAATGCAACTCCCAGAG	mouse		qPCR/RT-PCR
<i>Myogenin</i> rv	AGCATGGACGTAAGGGAGTG	mouse		
<i>Fbxo32</i> fw	GCCTTCAAAGGCCTCACG	mouse		qPCR
<i>Fbxo32</i> rv	CTGAGCACATGCAGGTCTGGG	mouse		
<i>MuRF 1</i> fw	CGACCGAGTGCAGACGATCATCTC	mouse		qPCR
<i>MuRF 1</i> rv	GTGTCAAACCTTCTGACTCAGC	mouse		
<i>Chrna1</i> fw	AAGCTACTGTGAGATCATCGTCAC	mouse		qPCR
<i>Chrna1</i> rv	TGACGAAGTGGTAGGTGATGTCCA	mouse		
<i>Chrng</i> fw	GCTCAGCTGCAAGTTGATCTC	mouse		qPCR
<i>Chrng</i> rv	CCTCCTGCTCCATCTCTGTC	mouse		
<i>Chrne</i> fw	GCTGTGTGGATGCTGTGAAC	mouse		qPCR
<i>Chrne</i> rv	GCTGCCCAAAAACAGACATT	mouse		
<i>Musk</i> fw	CCCTGCAAGTGAAGATGAAA	mouse		qPCR
<i>Musk</i> rv	TTCAAGAAGTGCATTCTGG	mouse		
<i>PO</i> fw	CTCCAAGCAGATGCAGCAGA	mouse		qPCR/RT-PCR
<i>PO</i> rv	ATAGCCTTGCGCATCATGGT	mouse		
<i>Gdf8</i> fw	AGTAAAAGCCCAACTGTGGATA	mouse		qPCR
<i>Gdf8</i> rv	TCATGTCAAGTTTCAGAGATCG	mouse		
<i>Bmp7</i> fw	GGCTGGCAGGACTGGATCAT	mouse		qPCR
<i>Bmp7</i> rv	GGCGCACAGCAGGGCTTGG	mouse		
<i>Id-2</i> fw	CTCCAAGCTCAAGGAAGTGG	mouse		qPCR
<i>Id-2</i> rv	ATTCAGATGCCTGCAAGGAC	mouse		
<i>Id-1</i> fw	AGTGAGCAAGGTGGAGATCC	mouse		qPCR
<i>Id-1</i> rv	GATCGTCGGCTGGAACAC	mouse		
<i>Gdf5</i> fw	ATGCTGACAGAAAGGGAGGTAA	mouse		qPCR
<i>Gdf5</i> rv	GCACTGATGTCAAACACGTACC	mouse		
GDF5 fw	ATGCTGACAGAAAGGGAGG	<b>human</b>		qPCR
GDF5 rv	GGCTTCTTCCGCAAGATCCG	<b>human</b>		
<i>MyHC-I</i> fw	CCAAGGGCCTGAATGAGG-AG	mouse		qPCR
<i>MyHC-I</i> rv	GCAAAGGCTCCAGGTCTGAG	mouse		
<i>MyHC-IIA</i> fw	AAGCGAAGAGTAAGGCTGTC	mouse		qPCR
<i>MyHC-IIA</i> rv	GTGATTGCTTGCAAAGGAAC	mouse		
<i>MyHC-IIB</i> fw	CGAAGGCGGAGCTACGGTCA	mouse		qPCR
<i>MyHC-IIB</i> rv	CGGCAGCCACTTGTAGGGGT	mouse		
<i>MyHC-IIX</i> fw	GCCAGGGTCCGTGAACTTGAAG	mouse		qPCR
<i>MyHC-IIX</i> rv	CCTCCGCTTCCCTCAGCTTGTCT	mouse		

PO fw	GGCGACCTGGAAGTCCAAC	human		qPCR/RT-PCR
PO rv	CCATCAGCACCACAGCCTTC	human		qPCR/RT-PCR
<b>Cacnb1 gene</b>	<b>Primer Sequence</b>	<b>Specie</b>	<b>Annotations</b>	<b>Use</b>
Ex5 fw	GACAGCCTTCGCCTGCTGCAG	human	Ex5-Ex9 CACNB1-A/E band 380 bp, CACNB1-B band 245 pb	RT-PCR
Ex9 rv	ATGTCTGTAACCTCGTAGCCC			
Ex13 (end) fw	CAGGTACAGGTGCTCACCTC	human	Ex13 CACNB1-A 105 bp	qPCR/RT-PCR
Ex13 (end) rv	CATGGCATGTTCTGCTCCTG			
Ex14 fw	CAGGGACCCTACCTTGCTTC	human	Ex14 CACNB1-B/E band 465 bp	RT-PCR
Ex14 rv	GCGAATGTAGACGCCTCGTC			
ATG2 fw	GACCGTGGTGGTGGGCTCA	human	ATG2-Ex7A CACNB1-E band 624 bp	RT-PCR
Ex 7A rv	TTCTTAAAGAAGGGGATGCG			
Ex14 fw	AGGAAGAGCTGACCGACAAC	human	Ex14 CACNB1-E	qPCR
Ex14 rv	TCAGCGAATGTAGACGCCTC	human		
Ex1 fw	ATGGTCCAGAAGAGCGGCATG	mouse	Ex1-Ex2 all splicing isoforms (except Cacnb1-D) band 154 pb	RT-PCR
Ex2 rv	TGGATGTTGTATCCGAGGACG	mouse		
Ex2 fw	GGCAAGTACAGCAAGAGGAAAG	mouse	Ex2-3 all splicing isoforms (except Cacnb1-D) band 160 pb	RT-PCR
Ex3 rv	TTAAGGCTTCCCGGTCTCTCC	mouse		
ATG1 (Intronic region 2-3) fw	CAGCCGGACCCTGGTAGTG	mouse	ATG1-Ex3 Cacnb1-D band 146 pb	RT-PCR
Ex3 rv	GTTTGGTCTTGGCTTTCTCG	mouse		
ATG1 (Intronic region 2-3) fw	CAGCCGGACCCTGGTAGTG	mouse	ATG1-Ex7A Cacnb1-D band 622 pb	RT-PCR
Ex7A rv	GAAGGGGATGCGCTTGCCGT	mouse		
Ex 5 fw	GACAGCCTTCGTCTGCTGCAG	mouse	Ex5-7A all splicing isoforms (except Cacnb1-B/C/F) band 279 pb	RT-PCR
Ex 7A rv	GAAGGGGATGCGCTTGCCGT	mouse		
Ex 14 fw	CAGGGACCCTACCTTGCTTC	mouse	Ex14 Cacnb1-B/E band 462 pb	RT-PCR
Ex 14 rv	GCGGATGTAGACGCCTTGTC	mouse		
Ex 5 fw	GACAGCCTTCGTCTGCTGCAG	mouse	Ex5-Ex8/9 Cacnb1-B band 246 pb, Cacnb1-D/E band 381 pb	RT-PCR
Ex 8/9 rv	CATGTCTGTCACCTCATAGCC	mouse		
Ex 2 fw	GTTCAAAAGGTCAGACGGG	mouse	Ex2-Ex3 Cacnb1-E First exon splicing 101pb	qPCR
Ex 3 rv	CAGAGTCTGATGGTCGGCTCGTG	mouse		
Ex 13 (end) fw	CAGGTACAGGTGCTCACCT	mouse		qPCR

Ex 13 (end) rv	CATGGCGTGCTCCTGAGGCTG	mouse	Ex13 (end) Cacnb1-D band 108 pb	
Ex 14 fw	CAGGGACCCTACCTTGCTTC	mouse	Ex14 Cacnb1-E	qPCR
Ex 14 rv	CATCAAAGGTGTCTTGCGG	mouse		