SUPPLEMENTARY MATERIALS

Supplementary Experimental Procedures

Generation of (Neo+/LoxP+) ES cells - A genomic fragment containing exon 9 and part of intron 9 of mouse *Col4a3bp* was amplified by PCR using primers Exon9-F, 5'-GATGCTGTTGAAGCTGCTCTTGACAG-3', and Exon9-R, 5'-CTCAAACCATCCCTGTCTTGGCC-3', and DNA from 129 SvEv embryonic stem cells (129 SvEv ES cells) as a template. The identity of the PCR product was confirmed by direct nucleotide sequencing and further labeled with [α-^{32}P]dCTP, 3000 Ci/mmol, 10 mCi/ml, (Amersham Pharmacia Biotech) using Prime-it II Random Priming Labeling Kit (Stratagene), following the manufacturer's recommendations. The [α-^{32}P]-DNA was used to probe a 129 SvEv ES cell line genomic library [RPCI-22 (129S6/SvEvTac), CHORI BAC-pack, 6 filters, plates 289-576, 109039 clones] and six clones specifically hybridizing with labeled DNA were identified (RP22-420C12, RP22-495J16, RP22-347E1, RP22-397A17, RP22-303M17 and RP22-350E17). All six clones were characterized by nuclease restriction and Southern blot analysis, and were demonstrated to contain the region of interest (data not shown).

Clone RP22-420C12 was digested with *Hind*III and run on a 0.8% agarose gel. A 10-kb fragment representing the region of interest (Fig. S1A), was purified with a gel extraction kit (Qiagen) and cloned into a modified pBluescript SK(-) vector (Stratagene) in which the *Xho*I site in the polylinker region had been eliminated. The resulting construct (pBcHind3) was subjected to restriction nuclease digestion and partial nucleotide sequencing for diagnostic purposes and further digested with *Xho*I, and an internal 5-kb fragment removed to generate the pBcHind3-Xho1 construct.

To generate *Col4a3bp* knockout mice which still expressed GPBP-2, a targeting vector harboring a LoxP site between exons 10 and 11 as well as a floxed neomycin resistance cassette between exons 11 and 12, was constructed (Fig. S1B). The LoxP site between exons 10 and 11 was inserted using a PCR strategy. Briefly, two genomic sequences were amplified by PCR using pBcHind3 as template: the first one with oligonucleotide (ON)-Xho-F, 5'-GGAGATTGAGATGATTTGACCAGGG-3', and ON-LoxPM-R, 5'-ATAACTTCGTATAATGTATGCTATACGAACGGTACTCTATCCTCACCCCTCCTCTTCACC-3', and the second one with ON-LoxPM-F, 5'-TACCGTTCGTATAGCATACATTATACGAAGTTATGCTAGGGAGTGGGGAGTGGGAAATAG-3', and ON-Hinc-R, 5'-ACGCTTACCCAGATGACACACACAGC-3'. The two PCR products were purified and combined, and the mixture was used as template in a third PCR with primers ON-Xho-F and ON-Hinc-R. The resulting product was digested with *Xho*I and *Hinc*II and cloned into pBC SK(-) (Stratagene) to yield pBcXLH. The insertion of the LoxP site was verified by restriction nuclease digestion and direct sequencing.

The neomycin resistance cassette was inserted into the targeting construct using pBcXLH vector. First, a genomic fragment was amplified using pBcHind3 as template using primers ON-LoxPM-F and ON-Bgl-R, 5'-CCACTGGTGTAATTGAACATAGAG-3'. The resulting PCR product was cloned into the *Hinc*II site of pBluescript SK(-) to obtain pBcLB, that was digested with *Hinc*II (a site localized within the insert) and *Hind*III (a site localized within the vector), and the released *Hinc*II/*Hind*III fragment was cloned into pBcXLH vector previously digested with *Hinc*II and *Hind*III, to yield pBcXLB. The neomycin resistance cassette (PGKneobpA) flanked by LoxP sites (floxed PGKneobpA), was released from pBluescript KS(-) vector by enzymatic digestion with *Sal*I and *BamH*I, and subsequently blunt-ended with DNA polymerase I Klenow fragment. The neomycin cassette was then inserted into the *Hinc*II site of pBcXLB construct to render pBcXLNeoB. The presence of the insert and the proper orientation of the LoxP sites and floxed PGKneobpA were confirmed by restriction nuclease digestion and direct sequencing.

The pBcXLNeoB construct was digested with *Xho*I and *Bgl*II, and the insert containing the LoxP site and the PGKneobpA cassette was inserted into pBcHind3-Xho1 in substitution of the wild type *Xho*I/*Bgl* II fragment to yield pBcHind3Neo-Xho.

The final replacement vector was constructed by inserting the 5-kb *Xho*I fragment coming from pBcHind3 (see above), into pBcHind3Neo-Xho to obtain pBcHind3Neo. The correct orientation of the inserted fragment was

confirmed by restriction nuclease digestion and partial direct sequencing. The replacement vector was linearized with *Sal*I and electroporated into mouse 129 SvEv ES cells, which were grown on neomycin-containing culture medium and resistant clones were screened for presence of homologous recombination. For this purpose, a 0.6-kb probe was generated from DNA of 129 SvEv ES cells by PCR with ON-BGL5F, 5'-CCAGACTGCACCCTTACTGTCATGAC-3', and ON-E75R, 5'-GAATTCCTATCGTGTCTTTTTTGCCAGCT CTC-3'. DNA from neomycin resistant clones was extracted, digested with *Bgl*II, and subjected to Southern blot with the 0.6-kb probe. Homologous recombinants (Neo+ clones) yielded a 12-kb DNA fragment which was recognized by the probe (not shown), containing the PGKNeobpA cassette. The presence of the first LoxP site between exons 10 and 11 was also confirmed by PCR with primers ON-LOX71F, 5'-CTCTTCTCAGGAATTTAGGTAGGAAGGG-3', and ON-LOX71R, 5'-GCAGCAAGTGCTGGACTCCACGCACC-3' (LoxP+).

The resulting (Neo+/LoxP+) ES cells were used to obtain (Neo+/LoxP+) and *gpbp1*$^{-/-}$ mice following procedures described in the Main Manuscript. The genomic structure of *gpbp1*$^{-/-}$ mice in the region of interest is depicted in Fig. S1C.

Mice genotyping- Tails from individual mice were subjected to DNA extraction with High Pure PCR Template Preparation Kit (Roche). Subsequently, mouse DNA was subjected to two different PCRs: 1) PCR-1 with primers ON-LOX71F and ON-LOX71R and 2) PCR-2 with primers ON-LOX71F and ON-KOE11R, 5'-TACTATAATCCCAGTGAAGGGTCAGG-3' (see primer mapping in Fig. S1D). PCRs were performed with a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems) using AmpliTaq GOLD polymerase (Applied Biosystems) with the following program: 3 minutes at 94 ºC, followed by 40 cycles of 45 seconds at 94 ºC, 15 seconds at 60 ºC, and 20 seconds at 72 ºC, and a final 5-minute extension period at 72 ºC. Products were separated on a 2% agarose gel and visualized with ethidium bromide. PCR-1 was designed to yield a product in WT and heterozygous *gpbp-1*$^{+/-}$ mice, and PCR-2 in *gpbp-1*$^{-/-}$ and heterozygous *gpbp-1*$^{+/-}$ mice. A typical analysis is depicted in (Fig. S1E).

Morphological studies- Phase contrast images of cell cultures were obtained with an Olympus CKX41 inverted microscope using an Olympus CAMEDIA C-7070 camera. For immunofluorescence microscopy analysis, ten thousand cells were seeded on poly-Lys-coated crystal slides and cultured during 48 h at 37º C. Then, cells were subjected to differentiation in low serum conditions and processed for observation. Briefly, cells were washed once with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde in PBS for 30 min, and permeabilized with 0.2 % Triton X-100 in PBS for 5 min. Cells were washed with PBS, blocked with 3% BSA in PBS (blocking buffer) for 30 min, and incubated with primary antibodies diluted in blocking buffer for 1.5 h. Cells were washed three times with PBS and incubated with secondary antibodies diluted in blocking buffer for 1 h. Finally, cells were washed three times with PBS and mounted for observation using 1 μg/ml DAPI for nuclei visualization. All steps were performed at room temperature except incubation with primary antibodies, which was carried out at 37 ºC in humid chamber. All PBS washes were 5-min long. Cell preparations were observed either by conventional or by confocal microscopy.

For morphological studies of transfected myoblasts, ten thousand cells were seeded on poly-Lys-coated crystal slides in a 24-well plate and cultured overnight at 37 ºC in growth medium. The following day, cells were transfected with 1 μg of the appropriate construct and further cultured for 48 additional hours in growth medium. Subsequent differentiation and processing for observation were performed as above.

For confocal microscopy analysis of tissue samples, mouse quadriceps were immersed in OCT and frozen in liquid nitrogen. Seven-μm sections were cut with a Leica CM1900 cryostat and mounted on poly-lysine-coated glass slides. Samples were fixed with chilled acetone for 10 min, blocked with 3% BSA in PBS for 30 min at room temperature and processed as above.

Confocal microscopy analysis of isolated myofibrils was performed essentially as described (1) using suitable specific antibodies. We used a Zeiss AXIOSKOP 2 and a Leica TCS SP2 AOBS for conventional

immunofluorescence and confocal microscopy, respectively.

For electron microscopy (EM) studies, mice were perfused with 2% paraformaldehyde, 2.5 % glutaraldehyde in 0.1 M phosphate buffer pH 7.4 (PB). Subsequently, muscles were excised, immersed in the same fixative solution for 16 h at 4 ºC, and extensively washed with PB to remove the fixative agents. Alternatively, muscles of mice sacrificed in CO_2 atmosphere were excised and fixed by immersion in 2% paraformaldehyde, 2.5 % glutaraldehyde in PB for 24 h at 4 ºC, and washed with PB to remove the fixative agents. In all cases, fixed muscles were finally conserved in PB containing 0.1% NaN_3 at 4 ºC until use. Sections of 200 µm were cut with a vibratome (Leica VT-1000) and post-fixed with 2% osmium tetroxide in PB for 1.5 h. Sections were rinsed with chilled water and dehydrated with sequential 5-10 min washes with chilled ethanol solutions of increasing concentrations (30%, 50%, and 70%). Sections were then washed with 2% uranile acetate in 70% ethanol for 2.5 h at 4º C, and further dehydrated with 5-10 min washes in increasing ethanol concentrations (70%, 96% and 100%), and a single 10 min-long wash in dried 100% ethanol. Finally, sections were subjected to 2 x 10 min washes with propylene oxide and embedded in araldite (Durcupan, Fluka). Semi-thin sections (1.5 µm) were cut with a diamond knife and lightly stained with 1% toluidine blue. Ultrathin sections (0.08 µm) were cut with a diamond knife, stained with lead citrate (Reynolds solution) and examined under a FEI Tecnai G2 Spirit transmission electron microscope. For similar analysis, mouse myoblasts were seeded on 4-well Permanox® slides (Nunc), cultured in DMEM supplemented with 10% fetal cal serum for 24 h, subjected to differentiation conditions in DMEM supplemented with 2% horse serum during 5 days, and processed for EM observation as previously described (2).

For histochemical and immunohistochemical purposes, 2 µm sections of paraffin-embedded mouse muscles were either subjected to hematoxylin-eosin staining, or stained by the ABC-peroxidase method (3) using suitable antibodies. An Olympus BX41 light microscope and an Olympus CAMEDIA C-7070 camera were used for image acquisition.

Additional materials- Mouse mAb were used for detection of actinin (Sigma) and mitochondrial complex II 70-kDa subunit (Invitrogen). Rabbit polyclonal antibodies were used for detection of GRP78 (Abcam) and actin (Sigma), and goat polyclonal antibodies for detection of VAP A (Santa Cruz Biotechnology). Purified VAP-A, bovine serum albumin and actin used in far-Western procedures were from GenWay Biotech, Sigma and Cytoskeleton Inc, respectively.

Yeast two-hybrid studies- We used *Saccharomyces cerevisiae* HF7c cells (Clontech) to perform a two-hybrid screening of a human skeletal muscle cDNA library (Matchmaker Cat no. HL4010AB, Clontech) with pGBT9-GPBP-1 and we isolated pGAD10-I20. Interaction between GPBP-1 and GIP90 or GIP130 was confirmed in SFY526 cells (Clontech) following manufacturer's instructions.

cDNA cloning - The cDNA in pGAD10-I20 (I20) was ^{32}P-labeled and used to search for overlapping clones in the cDNA library above by standard colony hybridization under highly stringent conditions. This resulted in the identification and isolation of overlapping clones extending the 5' and 3' ends. Because the overlapping sequence predicted an ORF containing a Met for translation initiation but not an in-frame stop codon, we further screened the library using ^{32}P-labeled ON-I20-4m, 5'-GAAGGTATGCTAATGAACGAG-3', a synthetic oligonucleotide representing the 3'-end sequence and isolated an additional clone containing additional ORF, an in-frame stop codon and a 3' UTR ending with a poly (A^+) tail. The overlapping clones were joined by recombinant DNA procedures to yield GIP90 cDNA (GenBank accession AF329092). The sequence of the original I20 clone corresponds to nucleotides 728-1522 of AF329092. Based on sequence of GIP90, we designed RT-PCR primers and using human skeletal muscle RNA amplified GIP130, a continuous cDNA (GenBank accession AF514867) containing an 1135-residue-long ORF. Briefly, 5 µg of total RNA were subjected to reverse transcription (RT) with ON-GIP-EX6c, 5'-TAGAAGAAAACGATTTTTTAATAAA-3', and nested PCR amplifications with ON-GIP-10m, 5'-CTGAGAGGGTGTCCAAAAAGAGAAG-3', and ON-GIP-EX6c, 5'-TAGAAGAAAACGATTTTTTAATAAA-3',

and with ON-GIP-12m, 5'-GGAATTACCAGGTGTGATCAACAC-3', and ON-GIP-8c, 5'–GACTGGGATTTGGAATGCC-3' to obtain GIP130 cDNA. GIP130 nucleotide sequence fully matched genomic sequence whereas GIP90 cDNA contained a single adenosine insertion that caused a frame shift and a premature stop codon resulting in shorter GIP isoform of 90-kDa (data not shown). To obtain the cDNA of FILIP1L-102 isoform (GenBank Accession BC027860), purified plasmid from human skeletal muscle cDNA library was subjected to PCR using ON-DOC1m, 5'-AAAGGATCCATGGTGGTGGATGAACAGC-3' and ON-DOCSc, 5'-AAAAAAGTCGACCAGTATGGACTGGATGAG-3'.

To obtain mouse GIP130 cDNA (GenBank accession EU704258), 4 μg of RNA from three-day differentiated C2C12 cells were subjected to RT with ON-XRm-1c, 5'-CTGCATAGTCACATCAAGTGT-3' and nested PCRs with ON-XRm-1m, 5'-GTGCTCAGGCTCCACTGCTG-3' and ON-XRm-1c, and with ON-XRm-2m, 5'-GAGAACGGCCAGGCGTGATG-3', and ON-XRm-2c, 5'-CCAGATACTCTTGTTCAGTC-3'. The mouse orthologue of FILIP1L-102 cDNA (GenBank accession EU623548) was similarly obtained using ON-XRm-1c for RT, and ON-mDOC-mod, 5'-GGCGCCCGCAGCTCAGA-3'/ON-XRm-1c, and ON-mDOC 1m, 5'-GCCCGCAGCTCAGATTAAAGAA-3'/ ON-XRm-2c for nested PCR.

All the cDNAs were characterized by direct nucleotide sequencing.

Vectors and constructs- For the yeast two-hybrid screening, the nucleotides 358-2389 in GenBank accession AF136450 were cloned in-frame downstream of GAL4 binding domain of pGBT9 vector (Clontech) to obtain pGBT9-GPBP-1, a construct expressing the GAL4 binding domain fused to the entire ORF of GPBP-1.

We cloned GIP90 cDNA into Bluescribe vector (Stratagene) to yield BS-GIP90 construct, which was used as template for PCR purposes.

GIP90 ORF was cloned downstream and in-frame of a FLAG-tag cDNA in pGBT9 to obtain pGBT9-FLAG-GIP90. For these purposes, ON-GPBP-54m and ON-I20-F, 5'-AAACTGAGCCCTCGGTATCACTGCCTCTGGAACGCATGGATCCCTTGTCATCGTCG -3' were used following procedures similar to those described elsewhere (4) to insert FLAG-tag sequence in-frame between the GAL4 binding or activation domain and the ORF of GIP90. The pGBT9-FLAG-GIP90 construct was used to generated pGBT9-FLAG-GIP130 and pGBT9-FLAG-FILIP1L-102 using standard recombinant DNA procedures. Briefly, the GIP90 construct contains unique *Bam*HI site downstream of FLAG sequence and *Sal*I site downstream of GIP90 cDNA which allowed GIP90 cDNA excision and subsequent in-frame insertion of the ORFs of GIP130 and FILIP1L-102, obtained by PCR amplification with suitable primers.

For bacterial expression, FLAG-GIP90 cDNA was cloned in-frame downstream of the glutathione S-transferase (GST) in pGEX-5x-1 (GE Healthcare) to generate pGEX-FLAG-GIP90 which expressed GST-FLAG-GIP90.

For preparative yeast expression, the cDNA encoding FLAG-GIP130 was cloned into pHIL-D2 vector (Invitrogen) upstream in-frame of a cDNA encoding for a polyHis tag to obtain pHIL-FLAG-GIP130-polyHis construct, expressing FLAG-GIP130-polyHis. The production of pHIL-FLAG-n4' expressing FLAG-GPBP-1 has been previously reported (4, 5).

For mammalian expression, GIP130 and FILIP1L-102 cDNAs were cloned downstream in-frame of c-Myc tag in pRK5 (a gift of Jacques Camonis) to generate pRK5-c-Myc-GIP130 and pRK5-c-Myc-FILIP1L-102. Briefly, inserts from pGBT9-FLAG-GIP130 and pGBT9-FLAG-FILIP1L-102 were excised with *Bam*HI /*Sal*I and cloned into homologous sites of pRK5-c-Myc vector. For similar applications, enhanced green fluorescence protein (EGFP) cDNA was amplified by PCR from pEGFP-N1 (Clontech) and cloned downstream in-frame of GIP130 cDNA in pRK5-c-Myc-GIP130 to yield pRK5-c-Myc-GIP130-EGFP. For controls, the cDNA of GIP130 was excised from pRK5-c-Myc-GIP130-EGFP to obtain pRK5-c-Myc-EGFP. For inducible recombinant expression of GPBP-1 in HEK 293 cells, n4' cDNA (GenBank accession AF136450) representing the entire mRNA of GPBP-1 was cloned into pTRE2hyg vector (Clontech) to yield pTRE2hyg-n4'. When indicated, previously reported pc-FLAG-n4' (4) and pc-FLAG-GPBPΔ26 (6) constructs were

used for constitutive recombinant expression or PCR.

For silencing mGIP130, ON-sh-mGIP-F2, 5'-GATCCGAAGAGTGATGAATTCATATTCAAGAGATATGAATTCATCACTCTTCTTTTTTGGAAA-3' and ON-sh-mGIP-R2, 5'-AGCTTTTCCAAAAAAGAAGAGTGATGAATTCATATCTCTTGAATATGAATTCATCACTCTTCG-3' were annealed and inserted into pSilencer 2.1 U6-hygro vector (Ambion) according to manufacturers' indications to yield pSi-U6-mGIP-2.

All the constructs were characterized by nucleotide sequencing.

PCR studies- To obtain cDNA from cells and tissues, total RNA was extracted with RNAspin Mini (GE Healthcare) or TRI-REAGENT (Sigma) following manufacturer's instructions and subjected to RT with Ready-To-Go™ You-Prime First-Strand Beads (GE Healthcare) using Random Hexamers (Applied Biosystems). For PCR assessment of mGPBP-1, 2 and 3 mRNAs levels, the cDNA was amplified with AmpliTaq GOLD (Applied Biosystems) using ON-GPBP-26-1F, 5'-GCTGTTGAAGCTGCTCTTGACA-3' and ON-2c-RAT, 5'-TGTGGTTCTGTACCATTTCTTCAA-3'. For PCR assessment of mGIP130 and mFILIP1L-102 expression we used nested PCR described above (cDNA cloning). For control in PCR studies, we amplified pc-FLAG-n4' (4) or pc-FLAG-GPBPΔ26 (6) with ON-GPBP-26-1F and ON-GPBP-62c, 5'-GTGGTTCTGCACCATCTCTTCAA-3'.

Real-time PCR studies were performed with SYBR Green PCR Master Mix and a SDS 5700 apparatus (Applied Biosystems), using ON GPBP-26-1F and ON-GPBPe26-1R, 5'-CCTGGGAGCTGAATCTGTGAA-3' or ON GPBP-26-1F and ON-GPBP-26-1R, 5'-TCTCTTCAACCTTTTGGACA-3', for mGPBP-1 and 3 or mGPBP-2, respectively. To assess the mRNA levels of mGIP130 in sh-mGIP130 clones, ON-mGIP-9m, 5'-AGCGAGAGGGTGCCCAAAA-3' and ON-mGIP-9c, 5'-ATCACGCCTGGCCGTTCT-3', were used. For normalization, GAPDH levels were determined with ON-GAPDH-F, 5'-GGGAAGCCCATCACCATCT-3' and ON-GAPDH-R, 5'-CGACATACTCAGCACCGGC-3' using the ΔΔCt method.

Nucleic acid hybridization studies- I20 cDNA was amplified by PCR with primers ON-GAL4ACM, 5'-CCAAACCCAAAAAAAGAGATC-3', and ON-GAL4ACC, 5'-TTCAGTATCTACGATTCATAG-3'. The cDNA exclusive for GIP proteins was obtained by PCR amplification of BS-GIP90 with T7 promoter primer and ON-PHBR-4c, 5'-AAACTGCAGCGGCCGCTTATCTTTCACATTCCTGTTCTAG-3'. Amplified cDNAs were purified with GenElute™ Agarose Spin Columns (Sigma) and labeled with [^{32}P-α]dCTP and the *redi*prime™ system (GE Healthcare) following manufacturer's instructions, and used to probe pre-made Northern Blots (Clontech) or for colony hybridization assays. When indicated, synthetic oligonucleotides were labeled with [^{32}P-γ] ATP using T4 polynucleotide kinase (USB) and used for colony hybridization assays.

Expression and purification of recombinant proteins- Expression and purification of GST and GST-FLAG-GIP90 was essentially performed as elsewhere described (7) using *E. coli* DH5α transformed with pGEX-5x-1 or pGEX-FLAG-GIP90.

Pichia pastoris GS115 cells (Invitrogen) were transformed with pHIL-FLAG-GIP130-polyHis using Electroporator II (Invitrogen), and clones displaying the highest recombinant expression were used for fermentation purposes with Biostat A fermentor (B. Braun Biotech) according to manufacturer's instructions. After fermentation, cells were collected by centrifugation and stored at -70ºC until needed. For purification purposes, cells from 300 ml of culture were disrupted with glass beads with 300 ml of denaturing breaking buffer (8 M urea, 1% Triton X-100, 0.5 % SDS, 40 mM Tris-HCl pH 8.0, 100 mM NaCl, 5% glycerol, 1 mM PMSF). Cell debris was removed by consecutive centrifugation at 4000 x g for 10 min and at 45000 x g for 20 min and the final supernatant was incubated with 1 ml Talon™ Metal Affinity Resin (BD Biosciences) with gentle rocking for 1 h. The beads were washed five times with 30 ml of washing buffer (8 M urea, 20 mM Tris-HCl pH 8.0, 100 mM NaCl) and bound material recovered with three column volumes of elution buffer (8 M urea, 20 mM Tris-HCl pH 8.0, 100 mM NaCl, 100 mM imidazol). Affinity-purified FLAG-GIP130 was dialyzed against decreasing concentrations of urea (6, 4, 2 and 0 M) in Tris-buffered saline. Dialysis in several steps was critical to prevent protein aggregation and precipitation. All purification steps were at 4ºC.

The expression and purification of FLAG-GPBP-1 was previously described (4, 5).

Mitochondria isolation and assessment of cytochrome c oxidase activity- Freshly excised heart or quadriceps from mice (100 mg) were used for mitochondria isolation using Mitochondria Isolation Kit (Sigma), following manufacturer's recommendation. The protein concentration of mitochondria preparations was determined using Bio-Rad Protein Assays (Bio-Rad), and the cytochrome c oxidase activity of 40 μg measured using Cytochrome c Oxidase Assay Kit (Sigma).

Functional studies- Grip strength measurements were performed with a Bioseb GS3 grip strength meter (Bioseb), following manufacturer's instructions. Three consecutive tests spaced by 1-min intervals were performed, and the highest score was considered for subsequent statistical calculations. Results were normalized using the weight (g) of the mice.

Fatigue trials were performed with a LE8700 treadmill (Panlab) of adjustable slope, equipped with a shock grid and a recorder of the distance travelled in individual trials. Mice were first adapted to the treadmill with three sequential 5-min runs at 10 m/min performed in three consecutive days. The slope of the treadmill was adjusted to 0 º, 5 º and 10 º on the first, second and third training day, respectively. After the training period, in a first fatigue trial mice were forced to run up a 10 º slope at an initial speed of 40 m/min. Speed was progressively increased at a rate of 1 m/min every 30 sec till 50 m/min, and was maintained at that intensity until exhaustion. The day after, mice were subjected to additional modified fatigue trials carried out in three consecutive days. In the additional trials, slope was maintained at 10 º, speed was increased from 40 to 60 m/min at a rate of 1 m/min every 30 sec, and maintained at 60 m/min until exhaustion of the animal. In each trial, animals were considered to be exhausted when they remained on the shock grid, without being able to move back onto the treadmill, for at least 10 sec. The work developed in each trial was calculated with the following formula: Work (J) = Weight (Kg) x Gravity (9.81 m/s^2) x Distance (m) x sin 10 º, where 10 º is the angle of inclination of the treadmill belt, and [Distance travelled (m) x sin 10 º] is the vertical distance travelled (m).

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Deletion of Exon 11 in *Col4a3bp* to generate *gbpb-1*$^{-/-}$ mice. The scheme depicts the exon (red bars), intron (solid lines) distributions and restriction sites of interest (black bars) in the 10-kb region of mouse *Col4a3bp* targeted in deletion procedures, before (A) and after (B) insertion of the Lox P sites and the neomycin resistance cassette (PGKNeo), and after deletion of exon 11 and neomycin resistance cassette by Cre recombinase (C). In (D), depicted are the annealing sites in

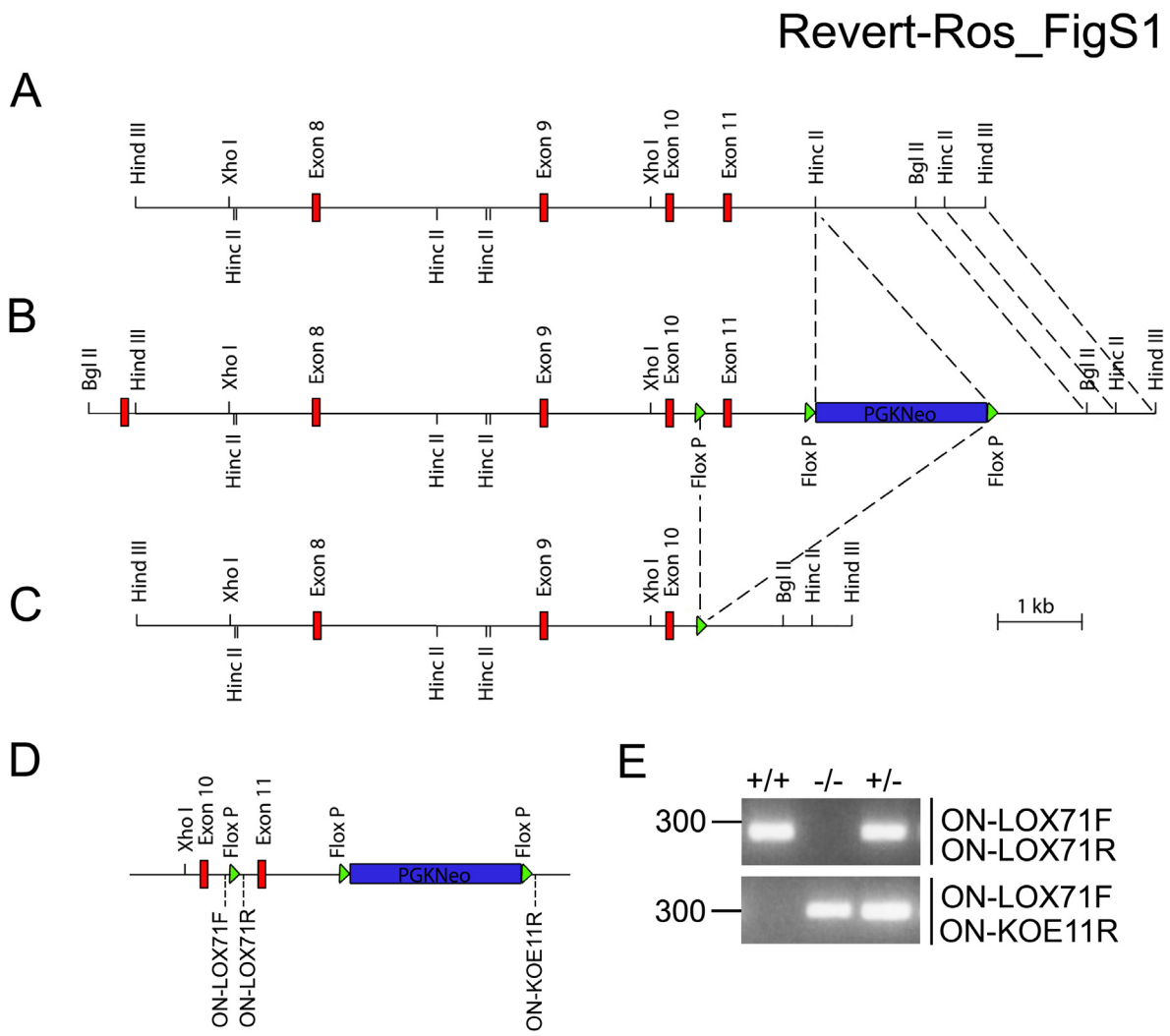
(Neo+/LoxP+) DNA of the primers used for mice genotyping. After Cre digestion, the DNA between the first LoxP site (placed between exons 10 and 11) and the third LoxP site (placed 3' respect to the PGKneobpA cassette) is excised, the annealing site for ON-LOX71R is eliminated, and the annealing sites for ON-LOX71F and ON-KOE11R come into close proximity. As a consequence, DNA from *gbpb-1*$^{-/-}$ mice does not yield a PCR product with ON-LOX71F and ON-LOX71R, while ON-LOX71F and ON-KOE11R yield a 300-bp band. In contrast, DNA from *gbpb-1*$^{+/+}$ mice yields product in PCRs with ON-LOX71F and ON-LOX71R, but not with ON-LOX71F and ON KOE11R, since they anneal too far away to generate a product in the PCR conditions used in genotyping. In (E), DNA from *gbpb-1*$^{+/+}$ (+/+), *gbpb-1*$^{-/-}$ (-/-) or *gbpb-1*$^{+/-}$ (+/-) mice were subjected to PCR with the indicated primers, mixtures were analyzed by electrophoresis on a 2% agarose gel and stained with ethidium bromide. Note that *gbpb-1*$^{+/+}$ and *gbpb-1*$^{-/-}$ mice yield a single product with ON-LOX71F and ON-LOX71R, and with ON-LOX71F and ON KOE11R, respectively, whereas *gbpb-1*$^{+/-}$ mice yield a band from each pair of these primers.

Figure S2. *Col4a3bp* expression in mouse striated muscle. (A) Fifty micrograms of protein extract from mouse hind limb muscles were analyzed by Western blot with mAb 14. (B) PCR was performed using the cDNAs of human GPBP-1 (lane 1), GPBP–2 (lane 2), and gastrocnemius (lane 3), soleus (lane 4) or quadriceps (lane 5) of an adult mouse with primers representing the flanking regions of exon 11 characteristic of GPBP-1 and 3. The corresponding PCR mixtures were further analyzed by agarose gel electrophoresis and stained with ethidium bromide. The *Col4a3bp* products are indicated by arrows. In A, B bars and numbers denote the position and size (kDa in A and base-pair in B) of the standards used in the studies. (C) Paraffin-embedded mouse hind limb muscle was stained with mAb 14 by peroxidase-based ABC method. Original magnification: x 400. (D) Myofibrils isolated from mouse hind legs were subjected to confocal microscopy analysis. *Col4a3bp* products, actinin and MyoHC (Myosin) were detected with chicken polyclonal, mouse monoclonal and rabbit polyclonal antibodies, respectively. Graphs represent the distribution of fluorescence intensity in the region indicated by the arrow in the merged image. Bars: 5 μm. Biological materials in A-D were obtained from C57BL/6 mice (n=2). (E) At left, protein extracts from *gpbp-1*$^{+/+}$ (+/+) and *gpbp-1*$^{-/-}$ (-/-) hind limb muscles were analyzed by Western blot with the indicated antibodies for assessment of *Col4a3bp* protein product expression (n=2). At right, total RNA from hind limb muscles of *gpbp-1*$^{+/+}$ (+/+) or *gpbp-1*$^{-/-}$ (-/-) mice was extracted and subjected to conventional RT-PCR to analyze *Col4a3bp* expression as in B. (F) Hematoxylin-eosin staining of paraffin-embedded sections of quadriceps (top) or soleus (bottom) from *gbpb-1*$^{+/+}$ (+/+) and *gbpb-1*$^{-/-}$ (-/-) mice. Original magnification: x 100. (G) EM micrographs of sarcomeres (top) and mitochondria (bottom) of soleus from *gbpb-1*$^{+/+}$ (+/+) and *gbpb-1*$^{-/-}$ (-/-) mice. In F and G, comparative studies were performed with four independent pairs of littermates (n=8) (H) Myofibrils isolated from *gbpb-1*$^{+/+}$ (+/+) and *gbpb-1*$^{-/-}$ (-/-) hind legs were analyzed by confocal microscopy with specific rabbit and chicken antibodies for detection of MyoHC (Myosin) and *Col4a3bp* products (mGPBP-1 in *gbpb-1*$^{+/+}$ and mGPBP-2 in *gbpb-1*$^{-/-}$), respectively (n=4). Bars: 5 μm.

Figure S3. During myogenesis GPBP-1 undergoes phosphorylation and aligns with VAP A at the M line of band A. (A) Mouse quadriceps was analyzed by confocal microscopy with chicken and goat polyclonal antibodies for detection of mGPBP-1 and VAP A, respectively. Bars: 5 μm. Graphs represent the distribution of fluorescence intensity in the region indicated by the arrow in the merged image. Arrowheads denote co-localization of the indicated proteins at the M lines of A bands. (B) Purified rGPBP-1 (200 ng), BSA (600 ng) and actin (400 ng) were subjected to SDS-PAGE and either Coomassie-stained (Coomassie) or transferred to a PDVF membrane and probed with purified VAP A (1μg/ml) and anti-VAP A antibodies (far Western), or with anti-VAP A antibodies (control). (C) Fifty micrograms of protein extracts from undifferentiated (U) or five-day differentiated (D) *gbpb-1* $^{+/+}$ (+/+) or *gbpb-1*$^{-/-}$ (-/-) myoblast cultures were subjected to Western blot analysis to assess the expression of MyoHC (Myosin), *Col4a3bp* (mAb 14) or GAPDH. (D) Differentiated cells in C were untreated (-) or treated (+) with λ-phosphatase (λ-PPase) and analyzed by Western blot with mAb 14 antibodies.

Figure S4. Tg-hGPBP-1 mice displayed no evident muscular phenotype. (A) On the left composite, the indicated protein extracts (50 μg) from hind limb muscles of WT and Tg-hGPBP-1 (Tg) mice were analyzed by Western blot for detection of both recombinant human and native murine GPBP-1 (mAb N27), actinin (ACTN) and GAPDH (n=2). Bent arrows pinpoint reactive polypeptides representing the indicated proteins. On the right composite, myofibrillar protein extracts were further analyzed by FLAG-precipitation (IP) and Western blot (WB) analysis of precipitates for detection of FLAG-tagged human recombinant GPBP-1 (arrowhead) using the indicated antibodies. (B) Hematoxylin-eosin staining of paraffin-embedded sections of quadriceps from control (WT) and Tg-hGPBP-1 mice. Original magnification: x 100. (C) Isolated myofibrils from WT and Tg-hGPBP-1 hind legs were analyzed by confocal microscopy with rabbit polyclonal and mouse monoclonal antibodies for detection of MyoHC (Myosin) and actinin, respectively. In B and C, comparative studies were performed with control (n=4) and Tg-hGPBP-1 (n=4) littermate mice. Bars: 5 μm. (D) EM micrographs of sarcomeres (top) and mitochondria (bottom) of soleus from control (n=2) and Tg-hGPBP-1 (n=2) littermate mice.

Figure S5. The *gpbp-1*$^{-/-}$ mice displayed no muscular functional phenotype while Tg-hGPBP-1 mice displayed reduced performance in exhaustion trials. (A) Outcome of grip strength trials performed with *gbpb-1*$^{+/+}$ (+/+, n=7) and *gbpb-1*$^{-/-}$ (-/-, n= 9) mice. Results are shown as mean ± SEM of the Force developed referred to mouse weight and expressed in Newton/g of mouse weight x 100. (B) The mice assayed in A were subjected to a treadmill exhaustion trial (left) and subsequently, additional treadmill exhaustion trials (right) were performed in three consecutive days under more stringent conditions. Results are expressed as means ± SEM of the Work developed expressed in Joules. (C) Outcome of grip strength trials performed with WT (n=8) and Tg-hGPBP-1 (n=12) mice. Values are expressed as in A. (D) The mice in C were subjected to a treadmill exhaustion trial (left) and then to additional exhaustion trials in three consecutive days under more demanding conditions (right). Results are expressed as in B (** $p<0.01$). (E) The heart mitochondria from mice in D were used for cytochrome c oxidase activity assessment. Results are given as means ± SEM in the indicated units (n=2). (F) Protein extracts (50 μg) from hind limb muscles of the mice in D were analyzed by Western blot with antibodies specific for GRP78 (an ER stress marker), mitochondrial complex II 70-kDa subunit (MCII-70) or actin (n=2). (G) EM micrographs of gastrocnemius muscles from WT and Tg-hGPBP-1 mice not subjected (top) or subjected (bottom) to the treadmill exhaustion trials performed in D. Shown is a typical tubular aggregate found in gastrocnemius muscles of Tg-hGPBP-1 mice after exhaustion trials. Bars: 1 μm.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A
B
C
D
E
Hind III
Xho I
Exon 8
Exon 9
Exon 10
Exon 11
Hinc II
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+/+
-/-
+/-
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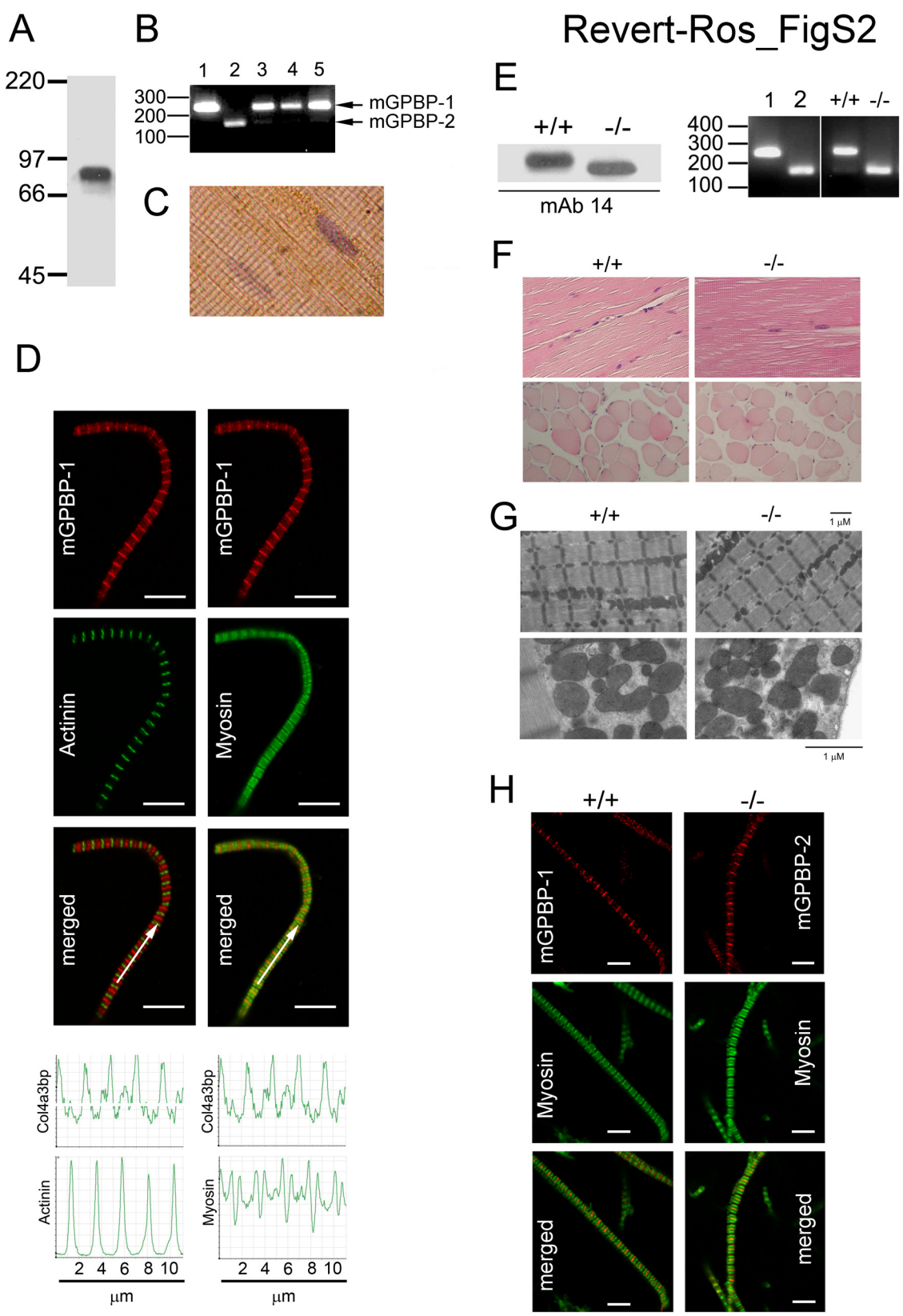
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C
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mGPBP-1
Actinin
Myosin
merged
merged
Col4a3bp
Actinin
Col4a3bp
Myosin
2 4 6 8 10
µm
2 4 6 8 10
µm
E
+/+ -/-
mAb 14
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400
300
200
100
F
+/+
-/-
G
+/+
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Myosin
Myosin
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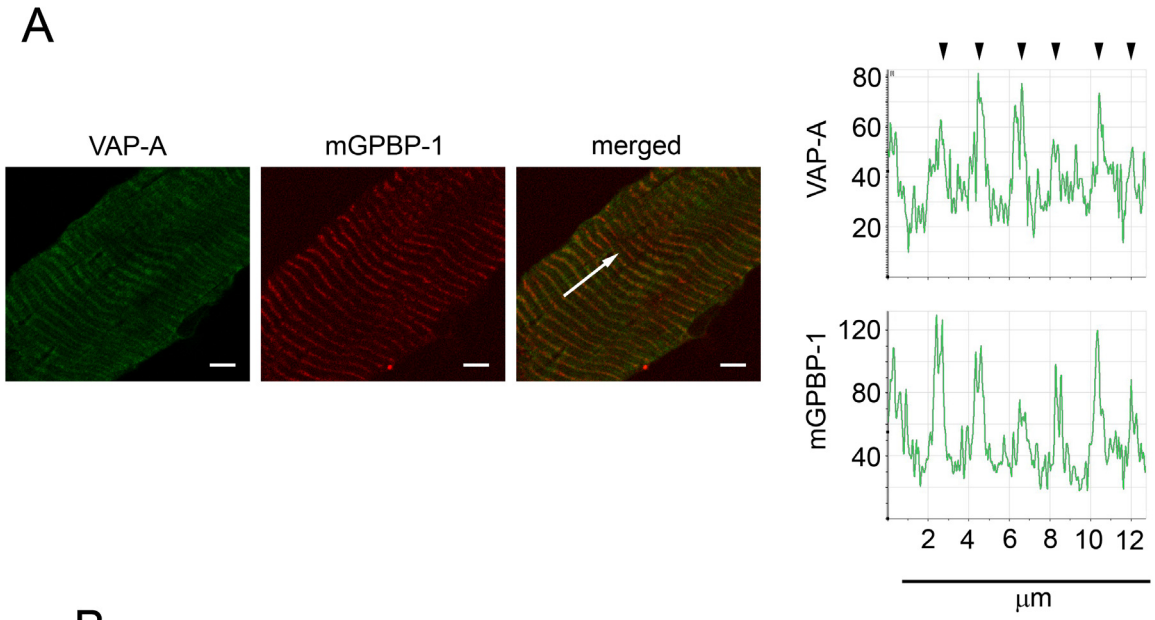
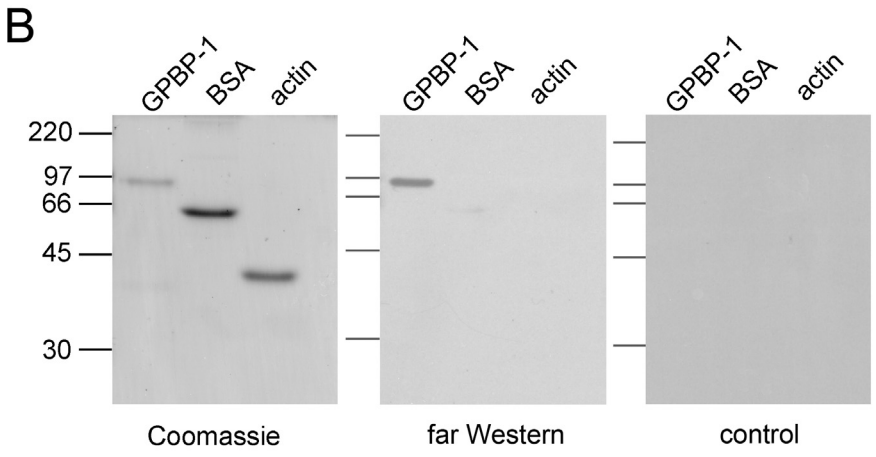
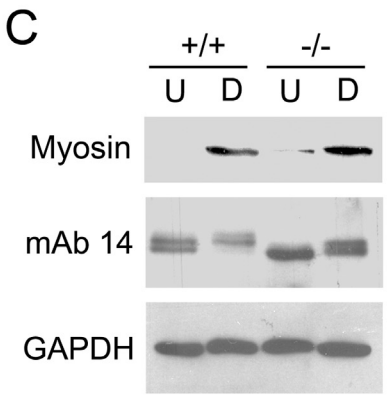
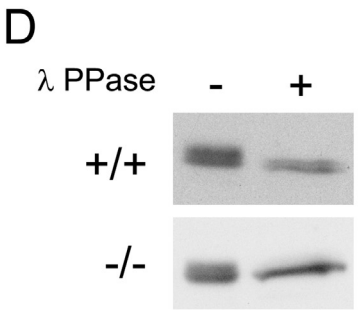
merged

Revert-Ros_FigS3









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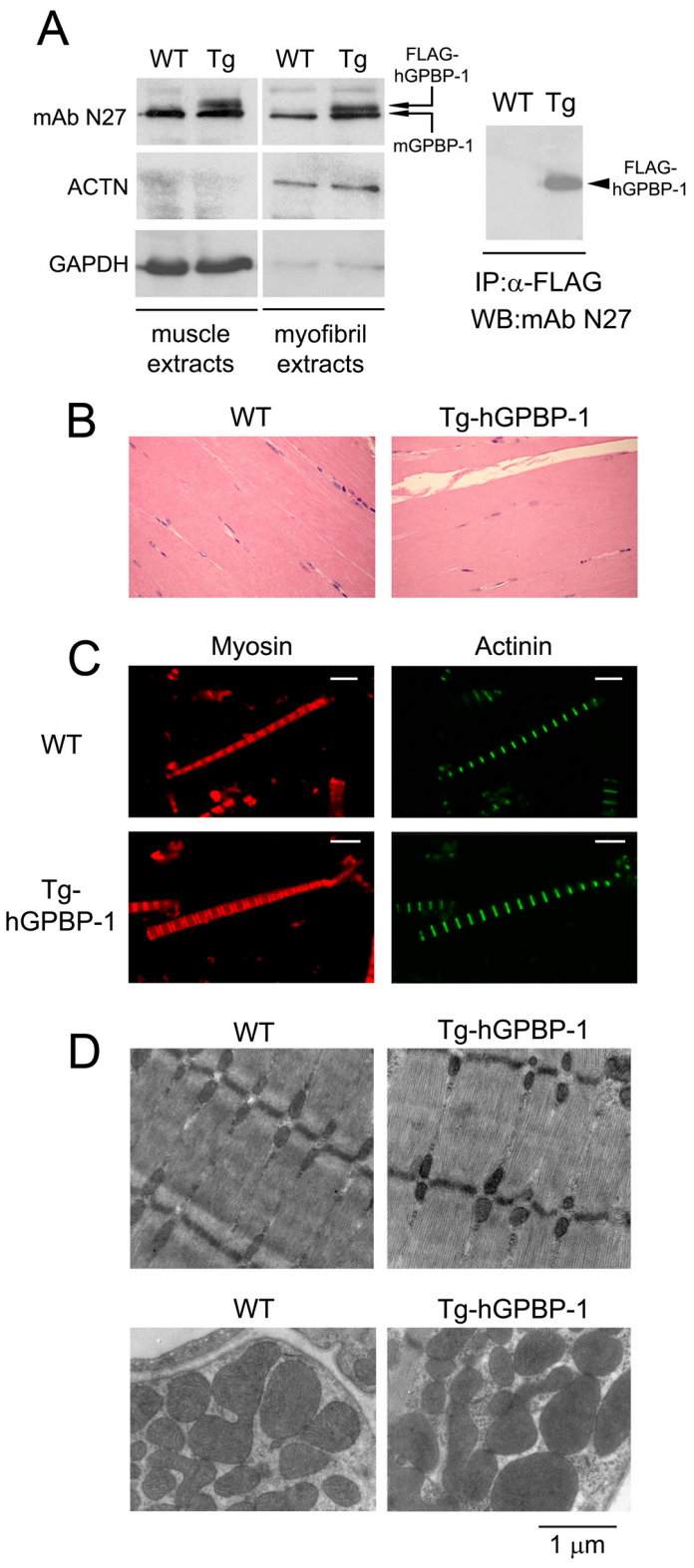

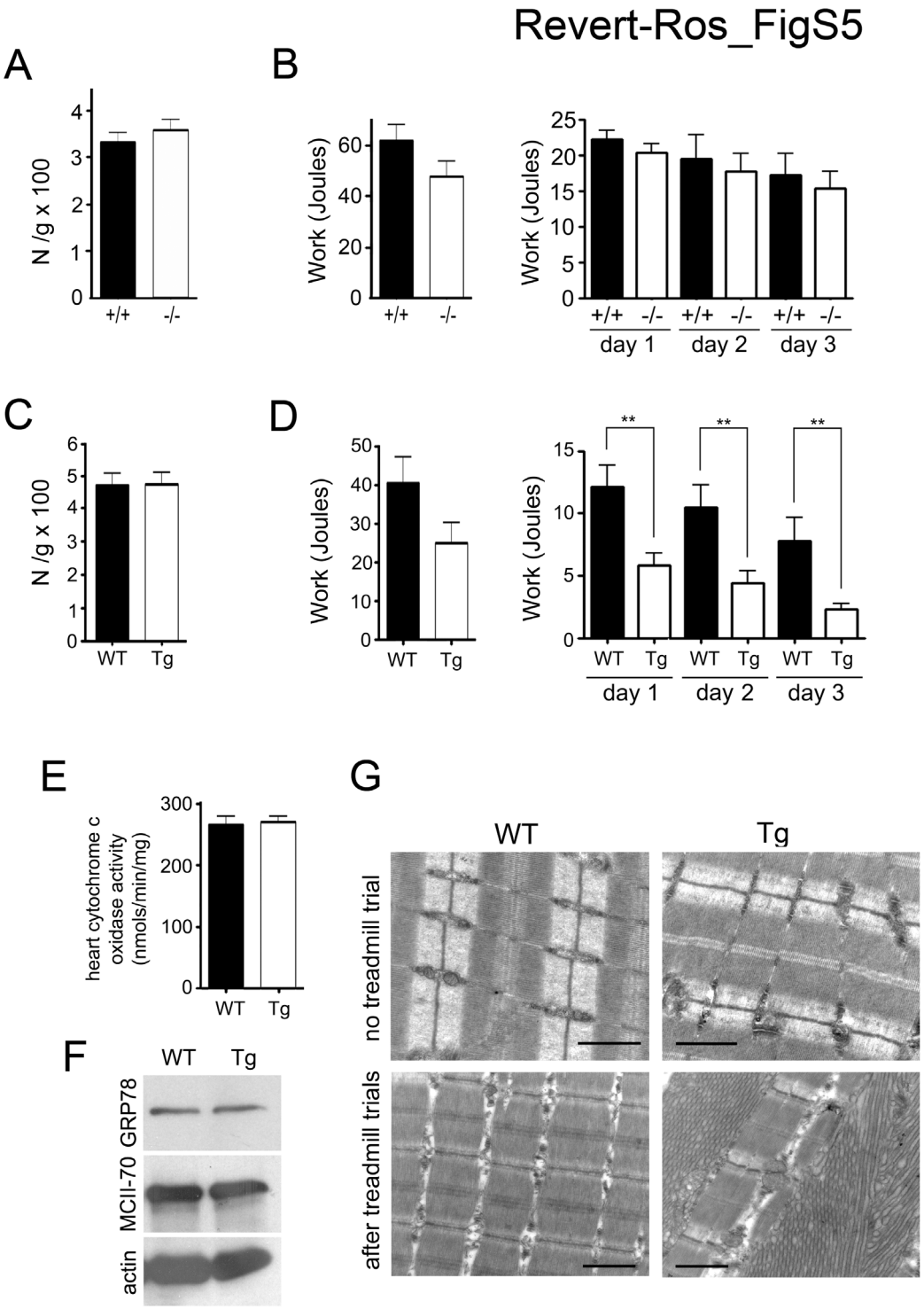
Revert-Ros_FigS5
A
N /g x 100
+/+
-/-
B
Work (Joules)
day 1
day 2
day 3
C
WT
Tg
D
**
E
heart cytochrome c oxidase activity (nmols/min/mg)
F
MCII-70
GRP78
actin
G
no treadmill trial
after treadmill trials