Supplemental Material

Supplementary figures:

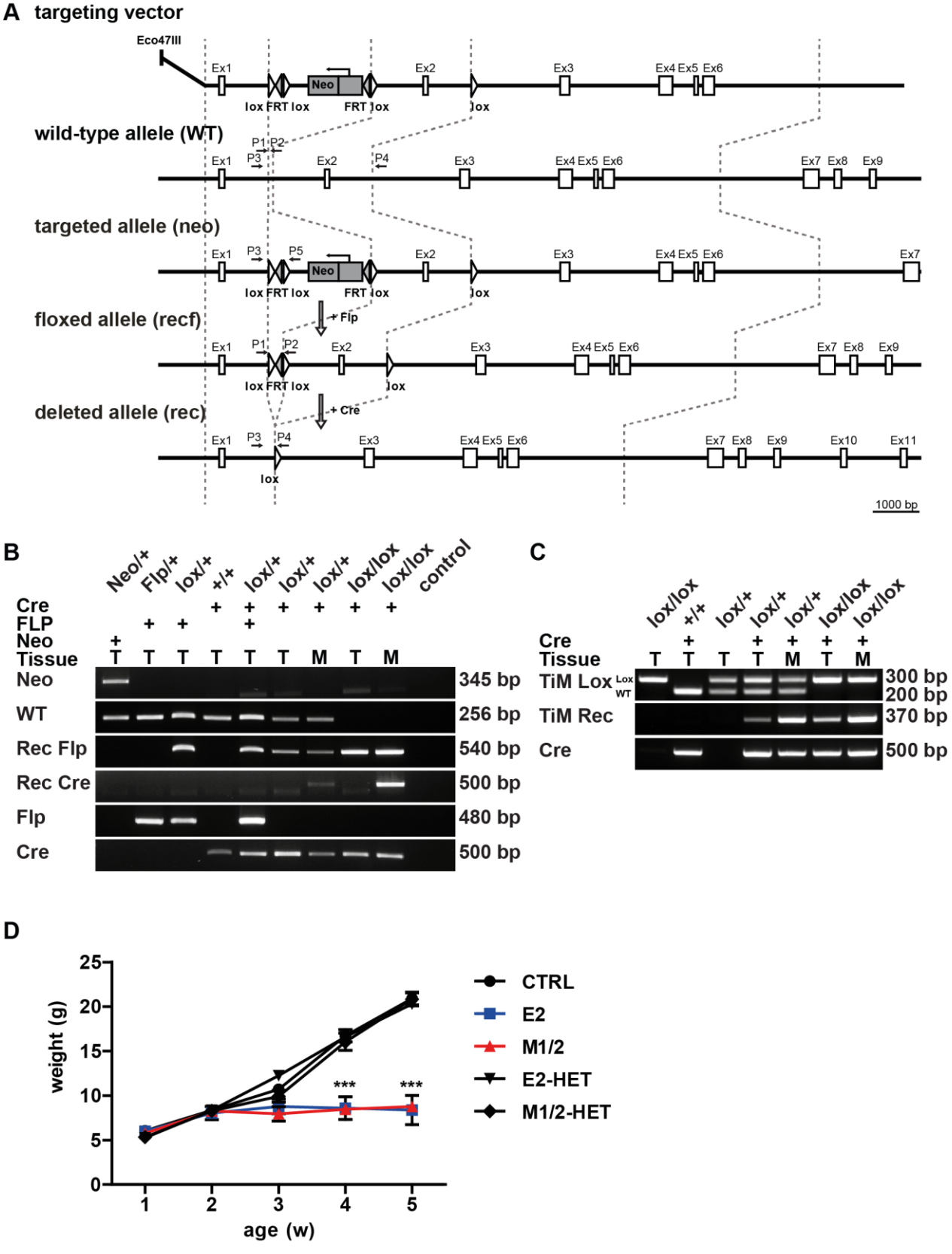

Figure S1. Generation and genotyping of the titin exon 2 knockout.

A) Targeting strategy to generate the titin E2-KO. A neomycin resistance cassette (Neo) for selection of homologous recombination in ES cell culture was inserted into intron 1. The FLP recombinase was used to remove the Neomycin resistance-cassette (Neo). Cre-mediated recombination of LoxP sites flanking exon 2 generates the deleted allele. FRT sites are depicted as grey arrowheads, loxP sited as white arrowheads, primer- binding sites for PCR genotyping are indicated as black arrows. **B)** PCR genotyping of the E2-KO mice to detect allele variations. Fragments of the expected sizes reflect the four alleles and the presence of Flp or Cre recombinase. **C)** PCR genotyping of the M1/2-KO identifies the floxed, wildtype, and recombined alleles. T, tail; M, muscle. The detailed targeting strategy of M1/2-KO has been published.[1] Additional details are provided in the supplemental table S1 and S2. **D)** Body weight of heterozygote animals (E2-HET and M1/2-HET), which carry the MCKcre transgene and one floxed titin allele, does not differ from control mice (CTRL). Body weight of E2- and M1/2-KO knockouts does not increase from week two after birth and stays below 10 g. Repeated measure 2-way ANOVA with Bonferroni post-test, *** $p < 0.001$; n = 12 CTRL, n = 10 E2-HET, n = 8 M1/2-HET, n=6 per homozygote knockout.

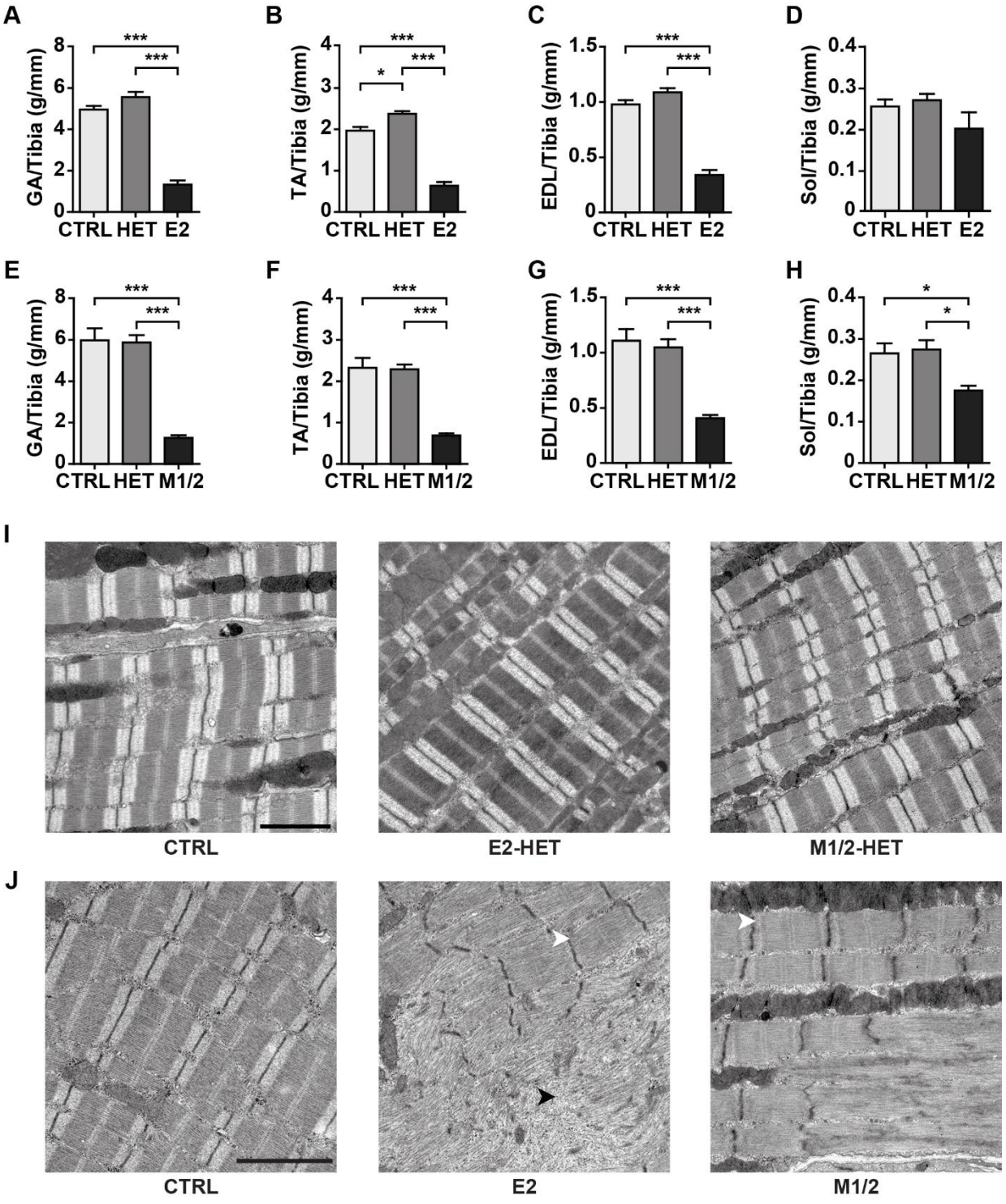

Figure S2. Heterozygote deficient titin E2 and M1/2 animals maintain muscle mass and sarcomere structure.

A-H) Skeletal muscle weight (GA, gastrocnemius muscle; TA, tibalis anterior; EDL, extensor digitorum longus; Sol, soleus) is decreased in E2- and M1/2-KO mice for all muscle types analyzed, except for soleus in E2-KO mice (D). Heterozygotes are not affected. Muscle weight was normalized to tibia length. One-way ANOVA with Bonferroni post-test, * $p < 0.05$, *** $p < 0.001$; n = 6 per genotype. **I)**

Ultrastructure does not differ between quadriceps muscle of control, E2-HET, or M1/2-HET mice. Size bar = 2µm. **J)** In E2 or M1/2-KO quadriceps sarcomeres disassemble (sarcomere disassembly – dark arrowheads; intact sarcomere structure – white arrowheads). Size bar = 2 µm.

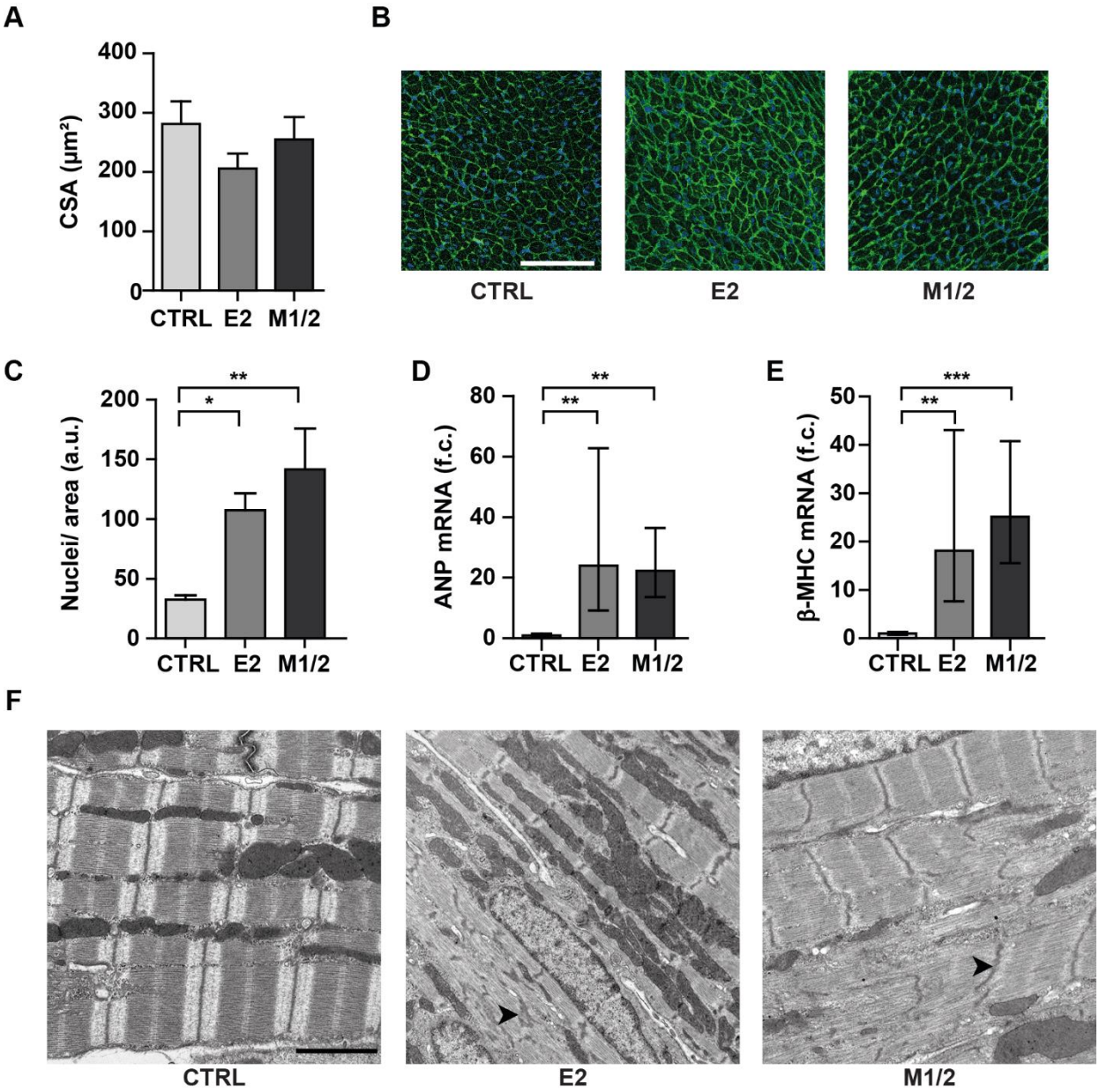

Figure S3. Signs of cardiomyopathy in titin deficient animals.

A, B) The cross-sectional area (CSA) of cardiomyocytes derived from wildtype, E2- or M1/2-KO mice is not significantly different as determined by quantification of heart sections stained for cell membrane proteins (~550 cells; n = 3 to 4 animals per group). **B)** Cell sections are less homogenous in E2-KO mice, consistent with the DCM phenotype. **C)** The number of nuclei is increased in E2- and M1/2-KO-mice. One-way ANOVA with Tukey post-test, * $p < 0.05$; ** $p < 0.01$; n = 6 slides for control, n = 3 per knockout. **D, E)** Upregulation of ANP and beta-MHC in both E2- and M1/2-KO mice as determined by RT-qPCR analysis. One-way ANOVA with Tukey post-test, *** $p < 0.001$; n = 10 for control, n = 5 per knockout. **F)** Sarcomere disassembly in hearts of CTRL, E2- and M1/2-KO hearts. In E2-KO sarcomere assembly, Z-discs are less well maintained as compared to M1/2-KO (dark arrowheads). Size bar = 2 µm.

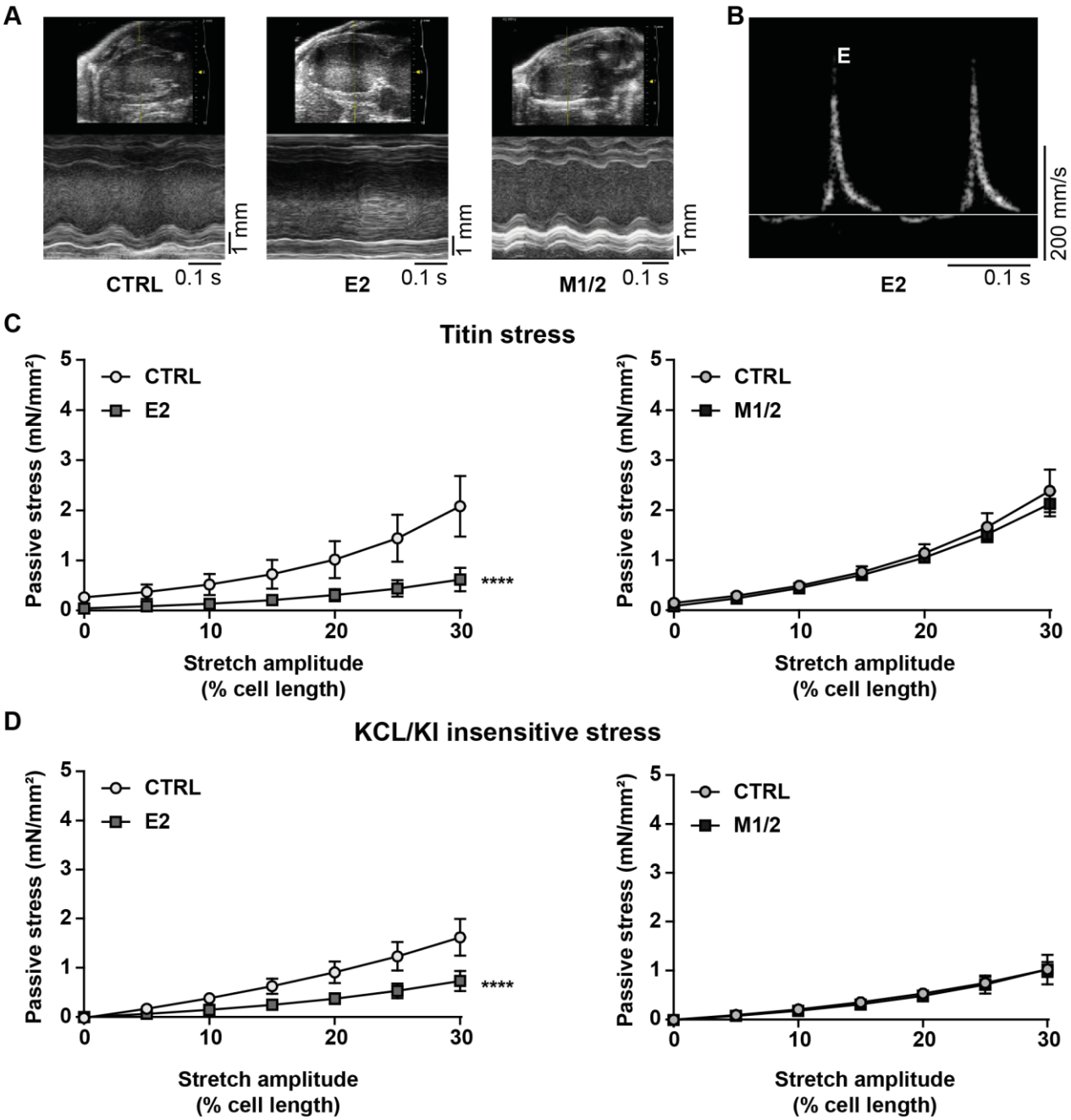

Figure S4. Increased ventricle size, reduced contraction and passive properties in E2 knockout.

A) M-mode analysis of E2 and M1/2 KO mice: increased size and reduced cardiac function in the E2 KO mice versus decreased ventricle size and restored function in the M1/2 KO mice. **B)** Doppler trace of an E2-KO mouse lacking an A wave. **C)** Reduced titin based passive properties of cardiomyocytes in the E2 KO, but not in the M1/2 KO. **D)** Stress contributed by the extracellular matrix (KCl/KI insensitive) is reduced in E2- but not in the M1/2-KO**;** n = 11-14 cardiomyocytes from 6-7 animals per group. B and C were analyzed for significance with a mono-exponential curve fit and an extra sum of squares F-test, **** $p < 0.0001$.

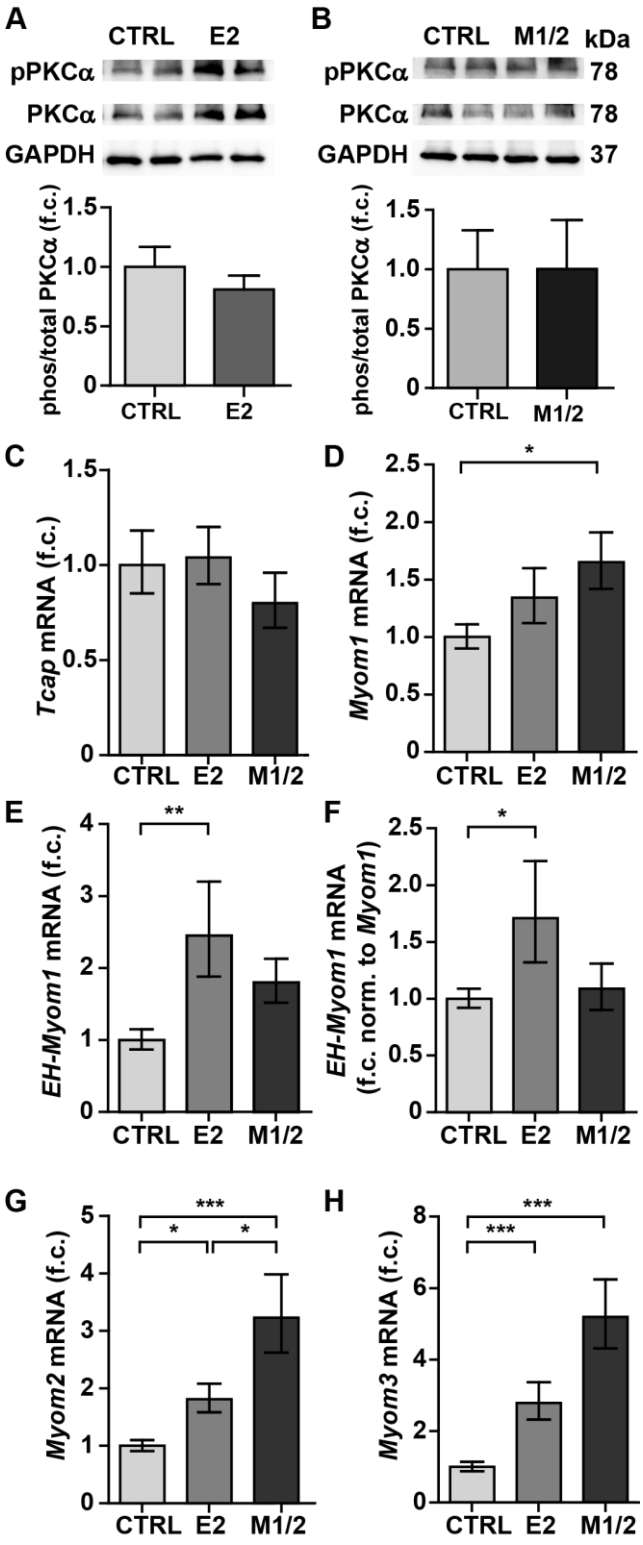

Figure S5. Titin kinase phosphorylation and transcription of titin anchor proteins.

Western blot analysis of heart lysate from control and E2-KO mice (left) and control and M1/2-KO mice (right) at 4 weeks of age. **A, B)** Posphorylation of the titin kinase PKCα is unchanged between genotypes.

No statistical significance was found by using Student's T-test, n = 8-11 animals per group. RT- qPCR on cDNA derived from left ventricle. **C)** *Tcap* is expressed at similar levels between all genotypes; CTRL n = 10; E2 n = 5; M1/2 n = 5. **D)** Increased expression of *myomesin* (*Myom1*) in M1/2-KO mice. **E)** The expression of the embryonic *EH-Myom1* isoform is only upregulated in E2-KO mice. **F)** Normalization of the *EH-Myom1* isoform to total *Myom1* expression. **G)** Increased expression of *M-protein* (*Myom2*) and **H)** *myomesin 3* (*Myom3*) in E2-KO- and stronger upregulation in M1/2-KO mice. 1-way ANOVA with Tukey post test; * $p < 0.05$; ** $p < 0.01$; CTRL n = 16; E2 n = 8; M1/2-KO n = 8. f.c. fold change.

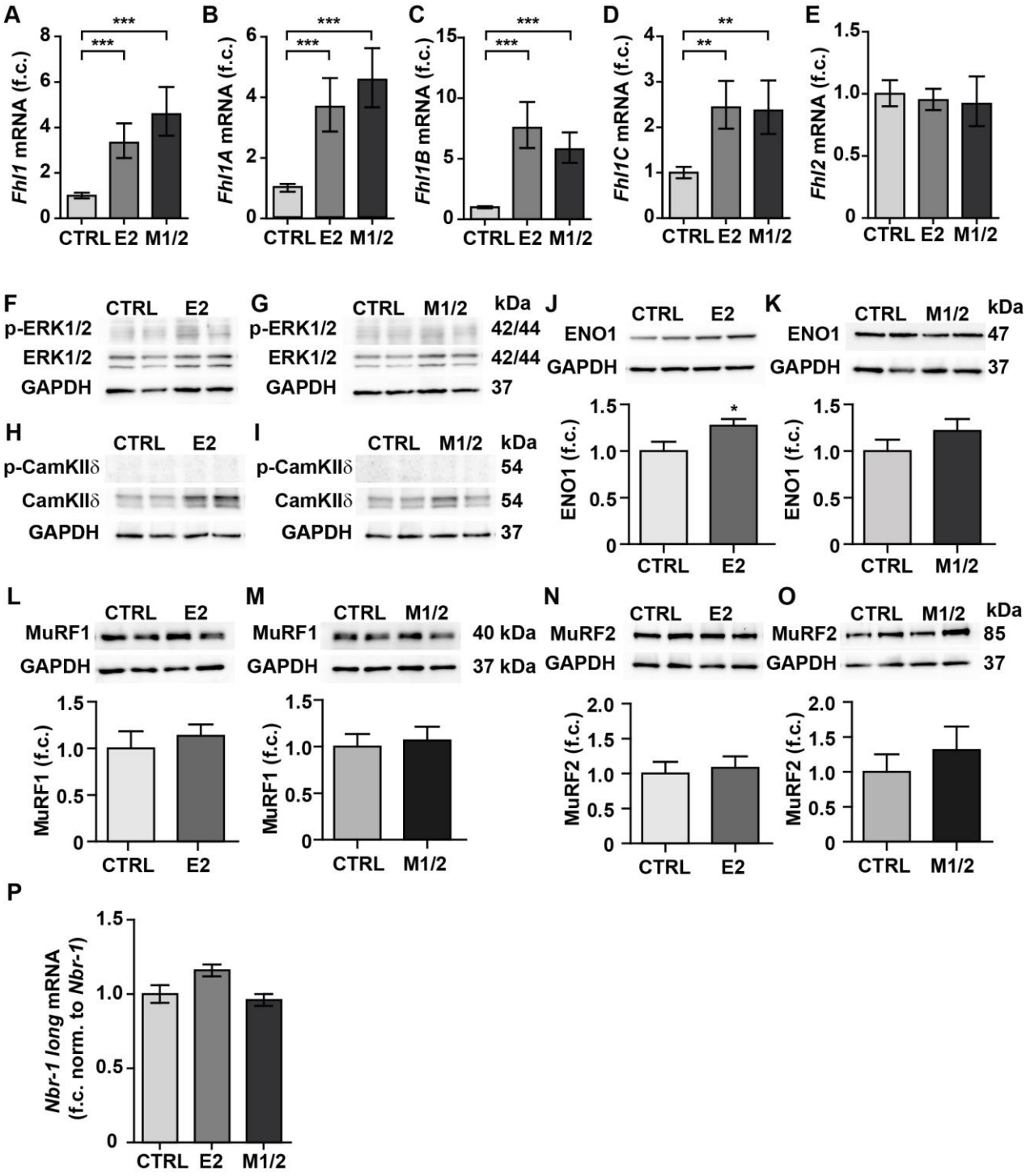

Figure S6. Expression of titin binding proteins.

A) Total *Fhl1* is upregulated on the transcriptional level in E2 and M1/2 KO mice. **B-C)** All *Fhl1* isoforms are affected. **E)** *Fhl*2 transcript levels are unchanged between E2, M1/2 and CTRL. **F-I)** Phosphorylation of ERK1/2 and CamKIId were not changed between KO and CTRL. **J-K)** Upregulation of ENO1 is significant only in E2-KO hearts. **L-O)** Expression of MuRF1 and MuRF2 was not different between genotypes. **P)** Nbr-1 transcript levels of long isoform normalized to short isoform are not significant

different. (A-E, P) One-way ANOVA with Tukey post-test, ** $p < 0.01$, *** $p < 0.001$, CTRL n = 16; E2 n = 8; M1/2 n = 8. (J-O) Students T-test, * $p < 0.05$. (F-O) n = 8-11 animals per genotype.

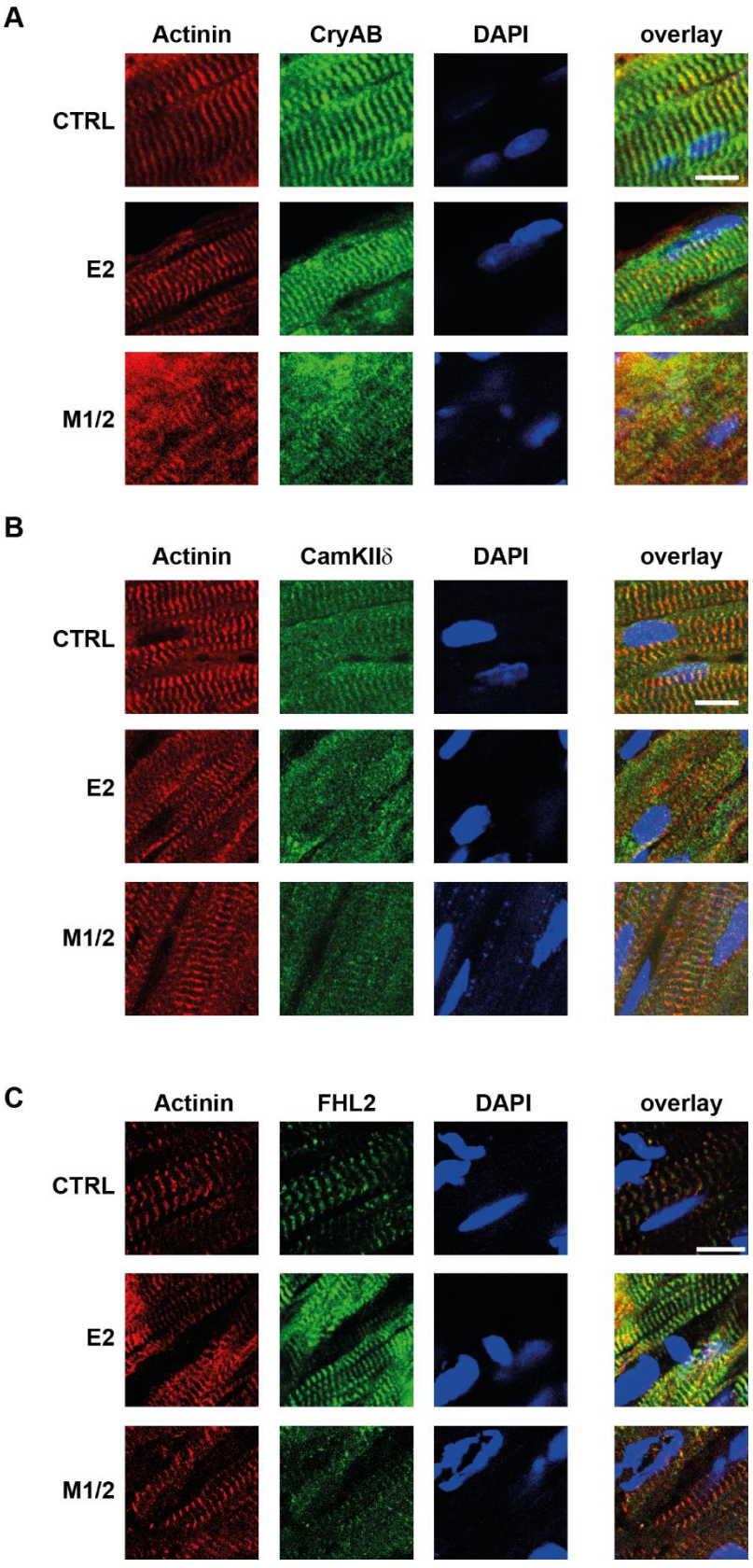

Figure S7. Localization of titin binding proteins.

Immunofluorescence staining of cardiac sections with antibodies against titin binding proteins. **A)** Actinin marks the Z-disc. CryAB localizes at the I-band independent of the genotype. **B)** CamKIIδ localizes at the I-band in the wildtype. The striated I-band staining is partially dissolved in the M1/2-KO and more prominently in the E2 knockout. **C)** FHL2 localizes to the I-band with stronger signal intensity in the E2 knockout, but no nuclear localization in WT, E2 or M1/2 hearts. Size bar = 10 µm.

Supplementary Materials and Methods

Genotyping of knockout mice

Genotyping PCRs were performed using the Taq DNA Polymerase Kit as well as the 100 mM dNTP set from Invitrogen. 50 ng/µl of genomic DNA were used as template for the PCR reactions. Primers and the expected PCR products sizes are listed in Table S1 and S2.

Table S1: Primers for genotyping

Primer	Sequence
3'neoflox-rev	5'-TCGACTAGAGGATCAGCTTGGGCTG-3'
NBfP1Ex2-for	5'-CACTGGCTTACAGACAGGAAAA-3'
NBrP2Ex2-rev	5'-CATTAAAGGGCAGGCTCTGA-3'
MRZE2 5'-for	5'-ACTTTGATTCCCTATCTTCCTGG-3'
MRZE2 3'-rev	5'-GGGGATACATCCTATAATCAGCC-3'
MG Ti-SL1-for	5'-GTGTCTGGCACTGCTTCCTTGGAAGTG-3'
MG Ti-SL2-rev	5'-ACCGCTCCCATGCCTTCGAGAGTCTTG-3'
Ti-FRTr2-rev	5'-AAGTTCGCTATACAACTGAGGCTAAG-3'
MG-FLP1-for	5'-GTCACTGCAGTTTAAATACAAGACG-3'
MG-FLP-rev	5'-GTTGCGCTAAAGAAGTATATGTGCC-3'
Cre800-for	5'-GCTGCCACGACCAAGTGACAGCAATG-3'
Cre1200-rev	5'-GTAGTTATTCGGATCATCAGCTACAC-3'

for: forward; rev: reverse.

Table S2: Genotyping and PCR products

Mouse	PCR	Analysis	Forward-Primer	Reverse-Primer	Product size
E2	Neo	Integration	MRZE2 5'-for	3'neoflox-rev	345bp
	RecFlp	Genotype	NBfP1Ex2-for	NBrP2Ex2-rev	256 bp WT
					540 bp RECf
	RecCre	Recombination	MRZE2 5'-for	MRZE2 3'-rev	500 bp
	Flp	Flp	MG-FLP1-for	MG-FLP2-rev	400 bp
	Cre	Cre	Cre800-for	Cre1200-rev	480 bp
M1/2	TiMlox	Genotype	MG Ti-SL1-for	MG Ti-SL2-rev	200 bp WT
					300 bp RECf
	TiMrec	Recombination	MG Ti-SL1-for	Ti-FRTr2-rev	370 bp
	Cre	Cre	Cre800-for	Cre1200-rev	480 bp

WT: wild-type; KO: knockout; RecCre or TiMrec: deleted allele; RecFlp or TiMlox: floxed allele; Flp: flippase recombination enzyme; Cre: Cre recombinase enzyme; for: forward; rev: reverse.

Analysis of survival

The male offspring of titin E2 and M1/2 breedings were observed over 7 weeks. Pups were weaned at 21 days of age. Premature death was recorded and a tail biopsy of the corresponding animal was used for genotyping. The surviving animals were tailed and genotyped after 5-7 weeks.

Weight analysis

Titin deficient E2 and M1/2, heterozygote and control male littermates were observed over a time range of 7 weeks. The pups were genotyped with an age of 15 days but stayed by the dam until they were weaned with an age of 21 days. The weight was determined every 7 days.

Muscle force measurements (grid holding)

The same animals described for the weight analysis were used for the four limb wire grid holding test [2]. The force performance was determined every 7 days. Therefor a box of 27 x 27 x 30 cm, filled with wood chip bedding up to 3 cm was used. The wire grid used had a netting of 0.25 cm^2. The animals were set on the grid and accumulated to the environment for a few seconds before the experiments started. The grid was flipped, positioned over the box and the time the mice are able to hold their own body weight was measured. Each animal was tested 3 times with a break of 10 min between trials.

Tissue preparation

The hearts and the skeletal muscles of the lower extremities were dissected and the weights were determined. The tissues were collected and snap frozen in liquid nitrogen for protein preparation and DNA or RNA isolation. Furthermore the tibia bone was dissected and the length was measured to determine the weight per tibia length ratio. Animals for paraffin and electron microscopy sections were sacrificed by cervical dislocation and perfused by fixation buffers. The hearts and the quadriceps muscle were dissected and processed accordingly to the specific protocols. Tissues for immunofluorescence staining were covered with OCT and snap frozen in N2 cooled Isopentane.

Immunofluorescence staining of frozen tissue

Tissue sections at 8-10 µm thickness were generated on a Microm HM 560 (Thermo Fischer Scientific). Slides were dried at room temperature for 10 min and fixed with 4% paraformaldehyde in PBS for 30 min. After 5 times washing in PBS slided were incubated in 200µl blocking solution (goat block) at room temperature for 2 hours. First antibodies in blocking solution were incubated over night in 4°C. After 5 times washing in PBS the slides were incubated with the 2.nd antibody, diluted in PBS and DAPI for 2 h at room temperature. After 5 times washing with PBS and 2 times washing in ddH2O the slides were coverd with mounting medium (Dako) and cover glass. Confocal fluorescence pictures were taken at 40x or 63x magnification on SPE Microscope (Leica). WGA staining was performed with WGA 488 Conjugate (Thermo Fisher Scientific) at 5µg/ml following the manufacturer's instructions. Confocal fluorescence pictures were taken at 20x magnification on a Leica SP5 Microscope and analyzed using ImageJ software.

Isolation of RNA

Tissue was dissected and snap frozen in liquid nitrogen. The samples were stored at -80 °C. The frozen tissue was grinded with mortar and pestle. During this procedure the tissue and the material were cooled by liquid nitrogen and dry ice to guarantee the stability of the RNA. 50 mg of the powder as well as a

ceramic ball were transferred to a 2 ml reaction tube and 1 ml TriZol™ was added. The further solubilization was done in the TissueLyzer (TissueLyzer II, QIAGEN) using a frequency of 20 hits per second for 2 min. Subsequently the reaction was centrifuged for 10 min at 12.000 g and 4 °C. The supernatant was taken and dislocated with 200 µl phenol/chloroform/isoamyl alcohol. After 2 min of inverting the reaction incubated 3 min at RT followed by centrifugation at 12.000 g and 4 °C for 15 min. The RNA containing phase was transferred to a new 1.5 ml reaction tube and incubated with 500 µl of isopropanol for 10 min at room temperature. The additional centrifugation at 12.000 g and 4 °C for 10 min precipitated the RNA which was washed with 75% ethanol and centrifuged for 10 min at 7.500 g and at 4 °C. The supernatant was discarded and the RNA pellet was dried at room temperature and resuspended in 89 µl DEPC-H_2O. RNA clean up was done with DNase I (QIAGEN) for 10 min at room temperature. Continuously the RNA was purified with the RNeasy Mini Kit (QIAGEN) following the manufacturer's instructions. RNA was stored at -80 °C. RNA concentrations were determined at 260 nm with the NanoDrop ND-1000 spectrophotometer (Thermo Scientific).

cDNA synthesis

The cDNA synthesis was required for the implementation of a quantitative real-time Polymerase chain reaction (RT-qPCR). Therefor 3 µg of the RNA was transcribed using the RNA-to-cDNA Kit (Applied Biosystems) following the manufacturer's protocol. The cDNA synthesis was performed in a thermocycler for 1 h at 37 °C. The inactivation of the reverse transcriptase occurred at 95 °C for 5 min and the cDNA was stored at -80 °C.

Echocardiography

For echocardiography 4 week old mice were anesthetized with an intermixture of oxygen and 2.5% isoflurane. The fur was removed using a hair removal cream and a shaver. Contact gel was used for an optimal imaging of the transducer. Vital parameters as cardiac and breathing frequency were measured by electrodes at the paws. The ultrasonic probe MS-400 was used to determine the cardiac parameters. LV Mass was calculatedas $1.053 * ((LVID;d + LVPW;d + IVS;d)^3 – LVID;d^3)$.

Cardiomyocyte studies

Mouse LV tissues were flash-frozen and stored at -80 °C. Before the day of experiment, frozen LV tissues were placed in 50% (v/v%) glycerol relaxing solution ([in mmol/L] 40 BES, 10 EGTA, 6.56 MgCl2, 5.88 Na-ATP, 1.0 DTT, 46.35 K-propionate, 15 creatine phosphate, pH 7.0) at -20 °C overnight. Then LV tissues were removed from 50% glycerol relaxing solution at -20 °C and washed in plain relaxing solution at 4 °C. Cardiomyocytes were mechanically isolated from frozen LV tissue in cold relaxing solution by Bio-Gen PRO200 homogenizer 5,000 rpm for 30 sec. Mouse cells, isolated as explained above, were skinned for 10 min in relaxing solution with protease inhibitors ([in mmol/L] 0.4 leupeptin, 0.1 E64 , and 0.5 PMSF) and 0.3% Triton X-100 (Ultrapure; Thermo Fisher Scientific). Cells were washed extensively with relaxing solution pCa 9 and stored on ice. Skinned myocytes were used for mechanic studies within 48 h after time of cell isolation. Myocyte suspension was added to a temperature regulated chamber mounted on the stage of an inverted microscope (Diaphot 200; Nikon). Skinned myocytes were glued at one end to a force transducer (Model 406A, Aurora Scientific). The other end was bent with a pulled glass pipette attached to micromanipulator so that the myocyte axis aligned with the microscope optical axis and cross sectional area (CSA) was measured directly. The cross sectional images of skinned cells were analyzed using ImageJ Fiji software (National Institutes of Health) and were used to convert measured

force to stress. Then, the free end of the cell was glued to a servomotor (Model 315C-I, Aurora Scientific) that imposes controlled stretches. Sarcomere length (SL) was measured with a Video sarcomere length software (VSL 900B, Aurora Scientific) attached to a computer. Passive stress was measured in relaxing solution (pCa 9) with protease inhibitors at 15 °C. Cells were stretched from base length at a speed of 1.0 base length/sec with different stretch amplitude based on % cell length followed by a 20 sec hold and then a release back to the original length. Recovery time of at least 7 min in between stretches was utilized to prevent memory-effects in subsequent measurements. Data were collected using a real-time muscle data acquisition and analysis software (600A, Aurora Scientific) at a sample rate of 2 kHz. Measured forces were converted to stress (force/unit undeformed CSA). The stress during the 1 base length/sec stretch was plotted against the stretch amplitude and fitted with an exponential curve to derive stress-SL relationships. To prevent protein degradation, all solutions contained protease inhibitors ([in mmol/L] 0.01 leupeptin, 0.04 E64, and 0.5 PMSF). After the passive stress was obtained, cells were extracted using a high KCl concentration (0.6 mol/L) relaxing buffer to depolymerize the thick filaments and high KI concentration (1.0 mol/L) buffer to depolymerize the thin filaments. Protocols were then repeated to determine ECM based stiffness.

Quantitative real-time PCR

The RT-qPCR was performed with the TaqMan probe based chemistry as well as the Sequence Detection System 7900 HT from Applied Biosystems. The reaction mix was prepared following the manufacturer's instructions with adaptation to a reaction volume of 10 µl. Data was collected and analyzed with the Sequence Detection System 2.3 software (Applied Biosystems). The comparative CT Method ($\Delta\Delta C_T$ Method) was used as described in the User Bulletin 2: ABI PRISM 7700 Sequence Detection System. Values were normalized to 18S RNA.

Table S3: Primers and probes for quantitative real-time PCR (RT-qPCR)

Primer and probe	Sequence
CP-Ex2/3 for	5'-CGTTACAAAGCGTTGTGGTACT-3'
CP-Ex2/3-rev	5'-GGCAGAGTGGAAGTTGAAATC-3'
CP-Ex2/3-probe	6-FAM-TCACGTTAGTGGTTCCCCAGTTCCTG-TAMRA
NB fExon 357- 358 for	5'-CCGATGGACTCAAGTACAGGATT-3'
NB fExon 357- 358 rev	5'-CCCATGCCTTCGAGAGTCTT-3'
NB fExon 357- 358 probe	6-FAM-TCCTTGGAAGTGGAAGTTCCAGCTAAGATACAC-TAMRA
Mouse-ANP-for	5'-CATCACCCTGGGCTTCTTCCT-3'
Mouse-ANP-rev	5'-TGGGCTCCAATCCTGTCAATC-3'
Mouse-ANP-probe	6-FAM-ATTTCAAGAACCTGCTAGACCACCTGGA-TAMRA
Mouse-BNP-for	5'-AGCTGCTGGAGCTGATAAGAGAA-3'
Mouse-BNP-rev	5'-GTGAGGCCTTGGTCCTTCAA-3'
Mouse-BNP-probe	6-FAM-AGTCAGAGGAAATGGCCCAGAGACAGCTA-TAMRA
Mouse-ß-MHC-for	5'-ATGTGCCGGACCTTGGAA-3'
Mouse-ß-MHC-rev	5'-CCTCGGGTTAGCTGAGAGATCA-3'

Mouse-ß-MHC-probe	6-FAM-CAGCGTTCTGTCAATGACCTCACCAG-TAMRA
FHL1 C for	5'-TGACTTGCCATGAGACCAAG-3'
FHL1 C rev	5'-CCTTCATAGGCCACCACACT-3'
FHL1 C probe	6-FAM-TGCAACAAGGGTTTGGTAAAGGC-BHQ1

For: forward; rev: reverse; 6-FAM: 6-carboxyfluorescein; TAMRA: 6-carboxytetramethylrhodamine.

Sequence of FHL1 C for and FHL1 C probe from [3]

Table S4: Amplicons for quantitative real-time PCR (RT-qPCR)

Set	Order number	Manufacturer
MURF1 (TRIM63)	Mm01185221_m1	Applied Biosystems
CamKIIδ	Mm00499266_m1	Applied Biosystems
FHL1	Mm04204611_g1	Applied Biosystems
FHL1 A	Mm04204613_mH	Applied Biosystems
FHL1 B	Mm00938359_g1	Applied Biosystems
FHL2	Mm00515781_m1	Applied Biosystems
Myomesin	Mm00440394_m1	Applied Biosystems
Myom1 EH- Isoform	Mm00440402_m1	Applied Biosystems
T-cap	Mm00495557_g1	Applied Biosystems
Myomesin 2 (M-protein)	Mm00500665_m1	Applied Biosystems
Myomesin 3	Mm01193371_m1	Applied Biosystems
Nbr-1 short Isoform	Mm00476296_m1	Applied Biosystems
Nbr-1 long Isoform	Mm01249798_m1	Applied Biosystems

Vertical SDS-agarose gel electrophoresis (VAGE)

The heart and the quadriceps muscles of 5 weeks old titin E2, M1/2 and control animals were dissected and snap frozen in liquid nitrogen. The samples were stored at -80 °C. Sample preparation was done as described by Warren et. al. [4]. With light modifications, cooled mortar and pestles were used to prepare a tissue powder which was balanced and lysed in 20 volumes of VAGE sample buffer (8 M urea, 2 M thiourea, 3% SDS, 0.03% bromphenol blue, 0.05 M Tris-HCl, 75 mM DTT, pH 6.8). The suspension was placed into a Dounce homogenizer (Wheaton) and processed for 5 min at 60 °C. Subsequently 50% glycerol buffer (50 ml H2O, 50 ml Ultrapure Glycerol, 1 Tablet protease inhibitor cocktail (Roche)) was added (final concentration 12%) and the samples were processed for another 5 min at RT. After a cooling period of 5 min on ice a centrifugation for 5 min at 13.000 rpm occurred. The supernatant was taken and stored at -80°C. Protein concentration was determined by the colorimetric amido black method and the samples were analyzed using VAGE. The gel was fixed for 1 h in 50% methanol, 12% acetic acid 5% glycerol, ddH_2O and dried overnight. The gel was rehedryted in H_2O and stained with Coomassie. Analysis and quantification was performed with AIDA software.

Protein preparation for SDS polyacrylamide gel electrophoresis (PAGE)

The hearts and skeletal muscles of 5 weeks old titin E2, M1/2 and control animals were dissected and snap frozen in liquid nitrogen. The samples were stored at -80 °C. Tissue powder was prepared, using a mortar and pestles cooled by liquid nitrogen and dry ice. 50 mg of the powder as well as a ceramic ball were transferred to a 2 ml reaction tube and 500 ml PIPA buffer (50 mM Tris pH 8; 150 mM NaCl; 1% IGPAL; 0.1% Na-DOC; 5 mM EDTA; 0.1% SDS, protease inhibitor complex (Invitrogen)) was added. The decomposition occurs in the Tissuelyzer (Tissuelyzer II, QIAGEN) at a frequency of 20 for 30 seconds. After an incubation of 30 min on ice the samples were centrifuged at 4 °C for 15 min and 12.000 g. The supernatant was transferred into a new reaction tube and the protein concentration was determined by the colorimetric amido black method[5].

Western blotting

After the SDS-PAGE the proteins were transferred to a PVDF-membrane (GE-Healthcare). Therefor the Mini-PROTEAN Tetra Cell (BioRad) was used. Blotting was performed at 200 mA for 3 h. followed by a separate probing with antibodies (Table S5). The analysis of densitometry was done with AIDA software.

Table S5: Antibody for Western blot

Antibody	Species	Dilution	Manufacturer
CAMKIIδ	rabbit	1:200	Abcam
FHL1	mouse	1:200	Abcam
FHL2	mouse	1:1000	MBL
GAPDH	mouse	1:8000	Calbiochem
Eno1	rabbit	1:1000	Abcam
CryAB	rabbit	1:1000	Merck
MuRF1	rabbit	1:500	Gift from T.Sommer
MuRF2	goat	1:1000	Abcam
MYOM1	goat	1:250	Abcam
Nbr1	rabbit	1:800	Novous Biological
P42/P44 (ERK1/2)	rabbit	1:1000	Cell signaling
p62	mouse	1:1000	Abcam
PKCα	rabbit	1:1000	Abcam
T-CAP	rabbit	1:1000	Santa Cruz
Anti-goat-HRP	donkey	1:5000	Santa Cruz
Anti-mouse-HRP	sheep	1:5000	GE Healthcare
Anti-rabbit-HRP	donkey	1:5000	GE Healthcare

References

1. Gotthardt M, Hammer RE, Hubner N, Monti J, Witt CC, McNabb M, Richardson JA, Granzier H, Labeit S, Herz J. Conditional expression of mutant M-line titins results in cardiomyopathy with altered sarcomere structure. *J Biol Chem*. 2003;278:6059–6065.

2. Carlson CG, Rutter J, Bledsoe C, Singh R, Hoff H, Bruemmer K, Sesti J, Gatti F, Berge J, McCarthy L. A simple protocol for assessing inter-trial and inter-examiner reliability for two noninvasive measures of limb muscle strength. *J Neurosci Methods*. 2010;186:226–230.

3. Domenighetti AA, Chu P-H, Wu T, Sheikh F, Gokhin DS, Guo LT, Cui Z, Peter AK, Christodoulou DC, Parfenov MG, Gorham JM, Li DY, Banerjee I, Lai X, Witzmann FA, Seidman CE, Seidman JG, Gomes AV, Shelton GD, Lieber RL, Chen J. Loss of FHL1 induces an age-dependent skeletal muscle myopathy associated with myofibrillar and intermyofibrillar disorganization in mice. *Hum Mol Genet*. 2014;23:209–225.

4. Warren CM, Krzesinski PR, Greaser ML. Vertical agarose gel electrophoresis and electroblotting of high-molecular-weight proteins. *Electrophoresis*. 2003;24:1695–1702.

5. Schaffner W, Weissmann C. A rapid, sensitive, and specific method for the determination of protein in dilute solution. *AnalBiochem*. 1973;56:502–514.