

## Supplemental material

### Methods

#### *Generation of heterozygous $Mypn^{WT/Q526X}$ knock-in mice*

To generate a  $Mypn^{WT/Q526X}$  knock-in mouse, exon 10 of the mouse *Mypn* gene was targeted using the homologous recombination in *E. coli* EL350 cells. Briefly, an MC1 retrieval vector was created with AB and YZ arms for homologous recombination corresponding to 548 bp and 526 bp sequences within intron 6 and 9, respectively. Primers used to create the recombination arms included: A-NotI-cagagacgcacagaggagtcag, B-HindIII-cagagacggaataacctggaccg, Y-HindIII-cctcccaccaagcctggctgact, and Z-SpeI-ctactggactatctcctggctcc. The 11.3 kb genomic DNA fragment was obtained through homologous recombination by transforming the HindIII-linearized MC1 construct in EL350 cells containing a RPL24 library BAC (BACPAC, Oakland CA) harboring the genomic region of interest. A PL452 mini-targeting vector was constructed containing the *Mypn*-Q526X mutation in the CD-arm with 417 bp of homology and an EF arm with 502 bp of homology to intron 9. In between the CD and EF arms a 2.5 kb Neomycin selection cassette under the control of the phosphoglycerate kinase (Pgk) promoter is present, flanked by two loxP sites. Primers used for the creation of the mini-targeting vector are C-SalI-gtgtctagcatcgcatagttagacg, D-EcoRI-ccctggaagtggagttacagatg, E-BamHI-gtccatcaccctcttctggc, and F-NotI-tgtatcagaggctgaccttgag. The mini-targeting cassette was excised by SalI and NotI digestion and transformed into recombination-competent EL350 cells containing MC1-11.3 kb *Mypn* plasmid. Restriction mapping and direct sequencing were used to confirm correct recombination and insertion of the mini-targeting cassette. After NotI digestion to linearize the complete targeting construct, 40  $\mu$ g of DNA was electroporated into 6 million Embryomax 129/SVEV embryonic stem cells (Millipore, Billerica MA USA). Cells resistant to G418 were selected by culturing for 9 days in medium containing 0.26 mM G418 and 2  $\mu$ M gancyclovir. Recombined embryonic stem cell clones were identified using PCR-based screening. Clones that were confirmed to be correct using direct sequencing were injected into C57BL/6 blastocysts at

the Transgenic Mouse Core facility of Cincinnati Children's Hospital Medical Center (Cincinnati, OH). Mouse colonies were maintained under barrier conditions. Mice were genotyped by PCR. Primers used for identification of the mutant allele were GenoF- TGCCTGCTTGCCGAATATCATGGTGG and GenoR- GGATGTGGGTATGATTCCTTGAAC, yielding a 1.5 kb fragment. Primer SeqF- GTTAGTCATTGCTGAGGTGTTTTTC in combination with GenoR gave a 1.5 kb wild-type allele fragment and a 3.4 kb mutant allele. All genotyping PCR was performed using LongAmp PCR mastermix (NEB). Chimeric animals were bred with C57/Bl6 mice (Charles River) or backcrossed to obtain heterozygote  $Mypn^{WT/Q526X}$  and homozygote  $Mypn^{Q526X}$  mice. After confirmation of the truncated Mypn protein expression in mouse hearts using Western blot analysis (Supplemental Figure 1B), phenotype screening using echocardiography and cardiac magnetic resonance (CMR) imaging was performed.

### ***Echocardiographic evaluation of heart function***

Experiments were conducted at 6 and 12 weeks of age on male heterozygote  $Mypn^{WT/Q526X}$  and homozygote  $Mypn^{Q526X}$  mutants and wild-type (WT) littermates (at least 12 animals per group) were used as controls. Cardiac function was assessed by cross-sectional, two-dimensional and Doppler transthoracic echocardiography under 1-2% oxygenated isoflurane anesthesia using a Vevo 2100 Micro-Imaging System (VisualSonics Inc., Toronto, Canada) with a 30-mHz transducer. The chests of the mice were treated with a chemical hair remover to reduce ultrasound attenuation. Normothermic mouse core temperatures were maintained using a heated platform. Images were stored in the ultrasound system hard drive and transferred to an image server for off-line analysis. Measurements including left ventricular (LV) and left atrial (LA) dimensions as well as ventricular wall thickness were performed by echocardiography investigators blinded to genotype. To assess diastolic function, spectral Doppler of antegrade flow across the mitral and

tricuspid valve was recorded and measured off line. Initial echocardiograms were performed at 6 weeks of age and repeated on the same cohort at age of 12 weeks.

### ***Electrocardiograms (ECG)***

Mice were anesthetized by oxygenated 1.5% isoflurane and core temperatures were maintained using a heated platform set at 37°C. Subcutaneous needle electrodes were placed in both front and rear left legs in all mice in similar positions to obtain consistent recordings across all mice. ECGs were recorded for 30 seconds at a sampling rate of 200 Hz on a BIOPAC (Goleta, CA USA) ECG system using AcqKnowledge 3.9.2 software.

### ***Cardiac magnetic resonance (CMR) imaging***

CMR imaging was performed in male 12-week-old animals when mice showed signs of diastolic dysfunction by echocardiography. CMR imaging was performed using a Bruker 7T scanner on 4 animals per group according to the previously described protocol (1). Image acquisition was prospectively ECG-gated using pediatric ECG probes attached to the paws. A bolus of Gd-DTPA (0.3–0.6 mmol/kg) was given intra-peritoneally while the mouse was placed in the scanner bore. Delayed enhancement MRI was performed using a T1 weighted cine sequence. Cine imaging was performed in the short axis using a segmented FLASH sequence. Slice thickness=1.0 mm, matrix size=256×256, inplane resolution=117×117  $\mu\text{m}^2$ . TE/TR=3/5.2ms, flip angle=20°, segments=1. Approximately 15–20 cine frames were acquired during the cardiac cycle with a temporal resolution of TR ms. Tagged images were acquired in the middle, basal and apical planes of the left ventricle. The LV maximum circumferential strain (Ecc) was calculated using HARP (Diagnosoft Plus, Diagnosoft Inc., CA, USA). Atrial and ventricular end diastolic volumes, LV and RV ejection fractions (LVEF, RVEF) were calculated using freely available software Segment (<http://segment.heiberg.se>) (2). Diastolic function was quantified as the rate of change of

Ecc in diastole,  $\frac{d(Ecc)}{dt}$  using the mid ventricular tagged images. The tagged images were also used to quantify maximum apical twist,  $T$ , rate of change of  $T$  in diastole,  $\frac{d(T)}{dt}$  and as defined in literature (3). The LV sphericity was defined as the ratio of LV maximum width (approximately at the midlevel) to the LV base-to-apex length at end diastole in the four chamber view.

### ***Histopathology, immunohistochemistry and electron microscopy***

Following echocardiography and CMR, the mice were sacrificed; the hearts were removed and perfused with cardioplegic solution or alternatively snap-frozen in liquid nitrogen. Perfused hearts were fixed in 10% formalin. After 48 hours in formalin, hearts were embedded in paraffin. Frozen heart tissues were cryo-sectioned and stained with primary antibodies against myopalladin, palladin and nebulette (gifts from Dr. S. Labeit, Heidelberg, Germany), CARP,  $\alpha$ -actinin (Abcam), connexin 43 (Sigma Chemical), N- and C-dystrophin (Vector), and desmoplakin2 (Fitzgerald). Then, samples were incubated with Alexa Fluor conjugated secondary antibodies (Molecular Probes, 1:500 dilution) for 30 minutes, counterstained with phalloidin (Molecular Probes, 1:400) and DAPI (2.5  $\mu$ g/ml, Roche Diagnostics), and subsequently, mounted with Vectashield mounting media (Vector Laboratories). Alternatively, peroxidase-conjugated secondary antibodies (Histofine) were applied for 30 minutes, the sections incubated with simple stain AEC (Histofine) and hematoxyline then mounted with an aqueous mounting media (Sigma). All slides were examined in a blinded fashion with an Olympus BX53 fluorescent microscope or a Nikon C2<sup>+</sup> confocal microscope system. 6 animals per group were used.

Ultrastructural analysis was performed on glutaraldehyde perfused hearts by transmission electron microscopy (TEM). Briefly, hearts were fixed by perfusion with 3.5% glutaraldehyde in cardioplegic buffer for 2 minutes, followed by 3.5% glutaraldehyde in 100 mM cacodylate buffer (pH 7.3) for 2 minutes. Hearts were dissected into fragments and fixed in 1% glutaraldehyde/2% paraformaldehyde overnight at 4°C. Tissue fragments were post-fixed on ice

in 1%  $O_3O_4$  in cacodylate buffer, dehydrated in acetone, and embedded in a Poly/Bed 812 resin mixture. Sections were counterstained with uranyl acetate and lead citrate and examined on a Zeiss 912 transmission electron microscope at an accelerating voltage of 100 kV.

#### ***Cytoplasmic and nuclear protein extraction and Western blotting of heart tissues***

Ventricular tissues were homogenized with beads (Precellys 24, Bertin Technologies) in RIPA lysis buffer and total protein quantified via the Bradford assay. Equal amounts (15 $\mu$ g) of proteins were separated on 15% SDS-polyacrylamide gels in a minigel apparatus (Mini-PROTEAN II, Bio-Rad Laboratories Inc) and transferred electrophoretically to PDVF membranes. The membranes were blocked with 5% non-fat milk in Tris-buffered saline or BSA 5% before overnight incubation at 4°C with anti-CARP (1:1000), anti-Desmin (1:1000), anti-N-cadherin (N-Cadh, 1:1000), anti-p-AKT (1:1000), anti-MEK1/2 (1:1000), anti-p-Erk1/2 (1:1000), ERK1/2 (1:1000), and anti-GAPDH (1:2000) antibodies. Membranes were then incubated for one hour with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000) and bands were detected with ECL kit (GE Healthcare). Photographic films were developed and band intensity quantified with ImageJ software. 3 animals per group were used.

#### ***Quantitative real-time PCR of human and mouse myocardium***

After isolation of total RNA from ventricular tissues with TRIzol (Invitrogen), 1 $\mu$ g of purified RNA was used to synthesize cDNA using qScript Supermix (Quanta Biosciences). Real-time PCR (QPCR) amplification was performed with a 7500 Fast Real-Time PCR system (Applied Biosystems) using Power SYBR Green PCR Master Mix (Life Technologies). All samples were assayed in triplicate, and the average value was used for quantification. The data were analyzed using the comparative  $\Delta$ Ct method ( $\Delta\Delta$ Ct method) and the results were expressed in fold changes compared to WT. GAPDH was used as the housekeeping gene. Six animals per group were used.

### ***Immuno and co-immunoprecipitation in HEK293***

Mixtures of equal amount of human GFP-MYPN<sup>WT</sup> and GFP-MYPN<sup>Q529X</sup> cDNAs (GFP-MYPN<sup>WT/Q529X</sup>) were used to resemble the heterozygous pattern of MYPN expression. HEK293 cells were transfected or co-transfected transiently with GFP-MYPN<sup>WT</sup>, GFP-MYPN<sup>Q529X</sup>, and GFP-MYPN<sup>WT/Q529X</sup> with CARP-V5 as follows: 1, control; 2, GFP-MYPN<sup>WT</sup>; 3, GFP-MYPN<sup>Q529X</sup>; 4, GFP-MYPN<sup>WT/Q529X</sup>; 5, CARP-V5; 6, CARP-V5 + GFP-MYPN<sup>WT</sup>; 7, CARP-V5 + GFP-MYPN<sup>Q529X</sup>; 8, CARP-V5 + GFP-MYPN<sup>WT/Q529X</sup>. Expressed or co-expressed proteins were pulled with anti-GFP or anti-V5 (Sigma) antibody at 1:100 dilution for 2 h at room temperature. Protein A/G Plus Agarose (Santa Cruz Biotechnology) was used to capture antigen and bound proteins. Protein was detected using anti-GFP (Sigma) or anti-V5 antibodies by Western blot analysis.

### **Supplemental Figure Legends**

**Supplemental Figure 1.** Strategy of creating and genotyping *Mypn* knock-in mutant mice.

**A.** Gene targeting construct for the generation of *Mypn* knock-in mice. Neo = neomycin cassette; HSV-TK = Herpes Simplex Virus-thymidine kinase; LoxP = locus of X-over P1 site. Exon 10 contains the murine nonsense *Mypn* p.Q526X (c.1576C to T) mutation. **B.** Quantification of MYPN protein expression in mouse hearts. Presence of murine 151-kDa *Mypn* protein (human MYPN protein is 145 kDa) in WT and *Mypn*<sup>WT/Q526X</sup> (lines 1 and 2) mice is indicated. Mutant truncated 65 kDa peptide was detectable in Lines 2 and 3 of *Mypn*<sup>WT/Q526X</sup>. Although no WT MYPN protein was evident in Line 3, this line had the highest expression of the truncated *Mypn*<sup>Q526X</sup> protein. Therefore, this line was utilized for further breeding and experiments. Relative density (RD) units are calculated relative to MYPN levels in WT mice. **C.** Direct sequencing of genomic DNA obtained from mouse tails to confirm heterozygous inheritance of the *Mypn-Q526X* mutation in *Mypn*<sup>WT/Q526X</sup> mice. **D.** Quantitative RT-PCR of *Mypn* mRNA in the heart (H), skeletal muscle (M), lung (L) and kidney (K) in mice. As expected, no *Mypn* mRNA transcription was evident in non-striated muscle tissues such as lung and kidney among

all groups. The levels of *Mypn* mRNA are comparable in the hearts from *Mypn*<sup>WT/Q526X</sup> and WT mice, while *Mypn*<sup>Q526X</sup> homozygotes demonstrate reduced levels of *Mypn* mRNA transcription.

**E.** Quantification of *Mypn* mRNA transcription by QPCR in mouse hearts using specific primers at the N-terminus (white column), C-terminus (grey column), and exons 9-11 (black column).

Transcription of *Mypn* mRNA was noticeably reduced in *Mypn*<sup>WT/Q526X</sup> compared to WT, while no transcription is seen in *Mypn*<sup>Q526X</sup> homozygote hearts.

**Supplemental Figure 2.** Immunohistochemical and Western blot analysis of heart tissues. **A.** No difference in dystrophin staining in green was observed between WT (a), *Mypn*<sup>WT/Q526X</sup> (b) and *Mypn*<sup>Q526X</sup> mice (c). Bar = 10  $\mu$ m. **B.** Palladin (PALLD) staining in green was used to visualize expression of this isomer in the heart. PALLD was expressed in the intercalated disks of WT (a) and homozygote (c) mouse hearts, presumably, in adherence junctions. In mutant heterozygote *Mypn*<sup>WT/Q526X</sup> mice (b), expression of PALLD is extended to the Z-disks adjacent to the cell-cell junction (arrows). **C.** Expression of structural cytoskeletal (TUBA1), sarcomeric (cTnI and p-cTnI), gap junction (Cx43), adherens junction (N-CADH, VLC), and desmosomal (DSP2) proteins. No significant alterations were observed in any groups. **D.** Expression of MAPK-dependent signaling proteins including p-53, JNK, p38, FAK, STAT3, NF- $\kappa$ B, ERB4 and TGF- $\beta$ 1. No difference in levels of expression was seen in any of these proteins.

#### References:

1. Wansapura JP, Millay DP, Dunn RS, Molkentin JD, Benson DW. Magnetic resonance imaging assessment of cardiac dysfunction in delta-sarcoglycan null mice. *Neuromuscul Disord* 2011;21:68-73.
2. Heiberg E, Sjogren J, Ugander M, Carlsson M, Engblom H, Arheden H. Design and validation of Segment--freely available software for cardiovascular image analysis. *BMC medical imaging* 2010;10:1.
3. Maiellaro-Rafferty K, Wansapura JP, Mendsaikhan U et al. Altered regional cardiac wall mechanics are associated with differential cardiomyocyte calcium

handling due to nebulin mutations in preclinical inherited dilated cardiomyopathy. *J Mol Cell Cardiol* 2013;60C:151-160.



**Supplemental Table 1.** Echocardiographic assessment of cardiac function in Mypn mice at 12 weeks of age

<b>Genotype</b>	<b>WT</b> (n = 27)	<b>Mypn<sup>WT/Q526X</sup></b> (n = 25)
<b>EF</b>	50 ± 2	51 ± 2
<b>FS</b>	26 ± 1	27 ± 1
<b>HR [beat/min]</b>	317 ± 10	328 ± 7
<b>LV Mass [μg]</b>	105 ± 4	96 ± 2
<b>LV Volume;d [μl]</b>	99.8 ± 4.6	<b>89.4 ± 2.3*</b>
<b>LV Volume;s [μl]</b>	52.5 ± 2.0	<b>44.4 ± 2.4*</b>
<b>LV ID;d [mm]</b>	4.62 ± 0.09	<b>4.42 ± 0.05*</b>
<b>LV ID;s [mm]</b>	3.44 ± 0.08	3.26 ± 0.07
<b>IVS;d [mm]</b>	0.81 ± 0.01	0.80 ± 0.02
<b>LV PW;d [mm]</b>	0.62 ± 0.02	0.62 ± 0.01
<b>MV E [mm/s]</b>	504 ± 32	560 ± 18
<b>MV A [mm/s]</b>	383 ± 20	<b>323 ± 37*</b>
<b>MV E/A ratio</b>	1.5 ± 0.1	<b>2.0 ± 0.2*</b>
<b>TV E [mm/s]</b>	285 ± 35	288 ± 23
<b>TV A [mm/s]</b>	336 ± 46	202 ± 48
<b>TV E/A ratio</b>	0.7 ± 0.1	<b>1.6 ± 0.6*</b>

**Comments:** EF = ejection fraction; FS = fractional shortening; HR = heart rate; IVS = interventricular septum; d = diastole; s = systole; LV = left ventricular; ID = internal diameter; PW = posterior wall; MV = mitral valve; TV = tricuspid valve. \* = indicates a significant (p<0.05) change from WT littermates. Heterozygous Mypn<sup>WT/Q526X</sup> mice have decreased LV volumes at diastole and systole and abolished ID in diastole with increase in E/A ratios on MV and TV, reminiscent of diastolic dysfunction with preserved systolic function in humans.

**Supplemental Table 2.** Assessment of cardiac function by cardiac magnetic resonance imaging in Mypn mice at 12 weeks of age

Genotype	WT (n =5)	Mypn <sup>WT/Q526X</sup> (n = 5)
Max strain [%]	-16.07±2.47	-15.91±2.29
Max diastolic strain rate [%/time frame]	4.31±1.37	4.49±1.59
Max torsion [degrees]	17.63±3.19	19.18±4.36
Max diastolic torsion rate (degrees/time frame)	4.38±0.98	5.36±0.72
Base to apex length [cm]	0.53±0.05	0.56±0.05
Base diameter average [cm]	0.41±0.03	0.39±0.02
Apex diameter average [cm]	0.32±0.02	0.27±0.04
Twist	12.23±3.08	11.19±2.6
Max diastolic twist rate	3.03±0.83	3.17±0.71
LV EF [%]	59.49±4.98	62.83±7.45
Stroke Volume [ul]	27.12±9.23	18.21±6.32
RV EF [%]	57.91±12.11	62.54±9.57
LV Mass [ug]	76.23±14.14	61.25±6.34
LV Volume;d [ul]	66.21±14.27	<b>48.43±7.34*</b>
RV Volume;d [ul]	36.45±10.24	27.27±9.37
EDL [mm]	7.48±0.23	7.63±0.84
EDD [mm]	4.19±0.19	3.87±0.31
ASW [mm]	0.85±0.31	0.96±0.09
PLW [mm]	0.88±0.26	0.93±0.08
Wall thickness [mm]	0.86±0.28	0.94±0.07
Sphericity	0.562±0.032	<b>0.509±0.025*</b>
LA area [cm <sup>2</sup> ]	0.035±0.006	0.038±0.005

**Comments:** EF = ejection fraction; LV = left ventricle; RV = right ventricle; Volume;d = end-diastolic volume; EDL = end-diastolic length; EDD = end-diastolic diameter; ASW = apical systolic wall; PLW = posteriolateral wall; \* = indicates a significant (p<0.05) change from WT. Comparative analysis of cardiac function showed significant increase in LV end-diastolic volume in heterozygote Mypn<sup>WT/Q526X</sup> compared to WT littermates. Mutant heart sphericity was reduced compared to WT.

**Supplemental Table 3.** Electrocardiography in mice at 12 weeks of age

		<b>WT (n = 16)</b>	<b>Mypn<sup>WT/Q526X</sup> (n = 37)</b>
<b>Duration (ms)</b>	<b>P</b>	28.33±5	31.18±6.74
	<b>PR</b>	47.22±15.43	46.18±11.53
	<b>RS</b>	33.33±6.61	34.44±6.84
	<b>T</b>	42.22±12.01	<b>34.12±7.75*</b>
	<b>RR</b>	412±66	424.79±48.64
<b>Voltage (mm)</b>	<b>P(+)</b>	2.99±0.69	2.81±0.81
	<b>P(-)</b>	1.41±0.51	1.21±0.76
	<b>R</b>	16.55±9.47	17.43±5.63
	<b>P(+)/R</b>	0.21±0.09	0.17±0.06
<b>Arrhythmias (%)</b>		1 (6.7%)	8 (27.6%)
<b>PAC (%)</b>		1 (6.7%)	6 (20.7%)
<b>PVC (%)</b>		0	1 (3.4%)
<b>AV block (%)</b>		0	1 (3.4%)

**Comment:** The only significant difference in ECG parameters observed was decreased T wave duration in Mypn<sup>WT/Q526X</sup> mice compared to WT littermates. Arrhythmias were recorded in 8 (27.6%) of 37 Mypn<sup>WT/Q526X</sup> mice, where premature atrial contraction (PAC) was seen in 6 (20.7%), premature ventricular contraction (PCV) was recorded in one, and atrioventricular (AV) block was recorded in one mouse.

**Supplemental Table 4.** Evaluation of transcription levels in mouse hearts using QPCR

Gene	WT (n = 6)	Mybn <sup>WT/Q526X</sup> (n = 6)	P value
<i>Nppa/Anp</i>	<b>1.00 (0.79 - 1.27)</b>	<b>4.53 (3.64 - 5.63)</b>	<b>0.002**</b>
<i>Nppb/Bnp</i>	<b>1.00 (0.88 - 1.14)</b>	<b>2.88 (2.37 - 3.51)</b>	<b>0.001**</b>
<i>Myh6</i>	<b>1.00 (0.85 - 1.17)</b>	<b>2.02 (1.8 - 2.28)</b>	<b>0.008**</b>
<i>Myh7</i>	1.00 (0.68 - 1.48)	2.76 (1.53 - 4.96)	0.172
<i>Myh7/Myh6</i>	1.00 (0.97 - 1.03)	1.09 (1.04 - 1.14)	0.151
<i><math>\alpha</math>Sma</i>	<b>1.00 (0.89 - 1.13)</b>	<b>4.53 (3.43 - 5.99)</b>	<b>&lt;0.001***</b>
<i>Tgfb1</i>	<b>1.00 (0.93 - 1.08)</b>	<b>1.62 (1.51 - 1.74)</b>	<b>0.003**</b>
<i>Postn</i>	<b>1.00 (0.87 - 1.16)</b>	<b>7.87 (6.56 - 9.46)</b>	<b>&lt;0.001***</b>
<i>Opn</i>	<b>1.00 (0.66 - 1.51)</b>	<b>9.52 (5.85 - 15.49)</b>	<b>0.006**</b>
<i>Vcam</i>	<b>1.00 (0.86 - 1.16)</b>	<b>3.21 (2.21 - 4.66)</b>	<b>0.013*</b>
<i>Icam</i>	<b>1.00 (0.82 - 1.22)</b>	<b>2.97 (2.05 - 4.32)</b>	<b>0.024*</b>
<i>Il-1b</i>	<b>1.00 (0.81 - 1.24)</b>	<b>2.13 (1.87 - 2.43)</b>	<b>0.016*</b>
<i>Il-6</i>	1.00 (0.76 - 1.31)	3.59 (1.62 - 7.97)	0.204
<i>Il-17</i>	1.00 (0.54 - 1.84)	7.61 (3.16 - 18.31)	0.084
<i>Bcl-2</i>	<b>1.00 (0.77 - 1.30)</b>	<b>12.54 (6.78 - 23.22)</b>	<b>0.003**</b>
<i>Tnf-a</i>	1.00 (0.69 - 1.44)	3.03 (1.62 - 5.65)	0.144
<i>Bax</i>	1.00 (0.74 - 1.34)	0.84 (0.73 - 0.97)	0.637
<i>Coll1a1</i>	1.00 (0.86 - 1.16)	1.52 (1.29 - 1.78)	0.089
<i>Col3a1</i>	1.00 (0.83 - 1.21)	0.87 (0.66 - 1.13)	0.661
<i>Col4a1</i>	1.00 (0.81 - 1.23)	1.2 (0.78 - 1.86)	0.681
<i>Ctgf</i>	1.00 (0.72 - 1.39)	1.42 (1.06 - 1.9)	0.450
<i>Mpeg</i>	1.00 (0.75 - 1.34)	0.98 (0.86 - 1.11)	0.954

**Comments:** Significant (\* = p<0.05, \*\* = p<0.01 and \*\*\* = p<0.005) increase in heart failure (*Nppa*, *Nppb*, *Myh6*), fibrotic ( *$\alpha$ -Sma*, *Tgfb1*, *Postn*, *Opn*), inflammatory (*Vcam*, *Icam*, *Il1 $\beta$* ), and anti-apoptotic (*Bcl-2*) in Mybn<sup>WT/Q526X</sup> compared to WT mice are shown. Results are expressed in fold changes to WT as a reference. *Gapdh* was used as the housekeeping gene.



