# Supplemental Material

## Supplemental Methods

### Sequences of synthetic microRNAs

miR-FYCO1 #1: 5'-TGC TGT TCA CTA AGT GTT CCA GGC TAG TTT TGG CCA CTG ACT GAC TAG CCT GGC ACT TAG TGA A-3'

miR-FYCO1 #2: 5'-TGC TGT TCA TTG GCT GTC TTT AGT CTG TTT TGG CCA CTG ACT GAC AGA CTA AAC AGC CAA TGA A-3'

miR-control: 5'-GAA ATG TAC TGC GCG TGG AGA CGT TTT GGC CAC TGA CTG ACG TCT CCA CGC AGT ACA TTT-3'.

### RNA isolation, purification and quantitative real time PCR

Total RNA from excised left ventricles and from cultivated cells was isolated using QIAzol (Qiagen) according to the manufacturer's protocol. Purification of mRNAs and DNase I digestion was carried out by using the RNeasy system (Qiagen). MicroRNAs were purified by using the miRNeasy kit (Qiagen).

Reverse transcription of mRNA was performed using the SuperScript III first strand synthesis system (Life technologies). For quantitative real-time PCR we used the Platinum SYBR Green qPCR SuperMix-UDG system (Life technologies) in a CFX96 real time PCR detection system (Bio-Rad). Rpl32 served as an internal control.

For reverse transcription of microRNAs the miScript II RT Kit (Qiagen) was used. Real-time PCR was performed with the miScript SYBR Green PCR kit (Qiagen) and miScript primer assays (Qiagen). RNU6B served as an internal control.

**Gene expression analysis**

RT² Profiler PCR arrays for genes involved in autophagy were performed according to manufacturer's protocol (SABiosciences/Qiagen).

For *FYCO1* gene expression in human samples RNA was isolated from powdered human ventricular samples using the Promega RNA Isolation Kit in accordance with suppliers' instructions. All materials from patients and from individuals who had no cardiac disease but died of another cause (non-failing samples, NF) were taken with informed consent and with approval of the local ethical boards and according to the Declaration of Helsinki. RNA concentration and purity was determined photometrically using the Nanodrop ND-1000. For expression analysis with the nanoString nCounter® Elements technology a total amount of 50 ng of pooled RNAs from patients with either NF, dilated cardiomyopathy (DCM) or ischemic cardiomyopathy (ICM) was hybridized with a customized nanoString Gene Expression CodeSet and analyzed using the nCounter® Sprint Profiler. mRNA levels were normalized to 5 housekeeping genes (*ABCF1, CLTC, GAPDH, PGK1, TUBB*) and *FYCO1* was expressed as fold change in DCM and ICM over NF.

**Tissue processing, immunochemistry**

For histological staining whole mouse hearts or heart sections were embedded in Tissue-Tek O.C.T. Compound (Sakura) and frozen at –80°C until cryosectioning. Serial transversal and longitudinal 7 µm thick cryosections were cut with a refrigerated microtome (CryoStar NX70, Thermo Fisher Scientific), mounted on Superfrost Plus slides (Thermo Fisher Scientific) and stored at -80°C. To assess myocyte cross-sectional area (MCSA) transversal cryosections were dried over night at 37°C, rehydrated with PBS for 5 min and stained with 5 µg/ml fluorescein isothiocyanate (FITC) conjugated lectin (Sigma-Aldrich) for 120 min at room temperature. After that samples were covered with FluorPreserve mounting medium (Calbiochem).

For all other stainings cryosections were briefly thawed, washed with PBS and then fixed in 4% (w/v) paraformaldehyde (PFA, Sigma-Aldrich) in PBS for 10 min at room temperature. After two washing steps the tissue sections were permeabilized with methanol at -20°C for 10 min, washed three additional times and then blocked with 12% (w/v) BSA in PBS for 2 h at room temperature. Incubation with the primary antibodies diluted in 2.5% (w/v) BSA in PBS was performed overnight at 4°C. Fluorophore conjugated secondary antibodies anti-rabbit IgG-Cy3 (1:200, Dianova) and anti-mouse IgG-FITC (1:200, Dianova) diluted in 2.5% (w/v) BSA in PBS were subsequently used for 2 h at room temperature. To visualize cell nuclei 4',6-diamidino-2-phenylindole (DAPI, 1:5000, Sigma-Aldrich) was mixed with the secondary antibodies before incubation. FluorPreserve mounting medium (Calbiochem) was used for mounting.

Neonatal rat cardiomyocytes were infected with an adenovirus encoding for a GFP-tagged LC3 (25 moi), that served as reporter, as well as with the indicated adenoviruses. C2C12 cells were maintained in DMEM containing 10% FBS, 2 mM l-glutamine, and penicillin/streptomycin. C2C12 myoblasts were transfected with 1 µg of a FYCO1 expression plasmid and 2 µg of a ptflc3 expression plasmid using Lipofectamine 2000 (Invitrogen). PtfLC3 was a gift from Tamotsu Yoshimori (Addgene plasmid # 21074). C2C12 cells were harvested 3 days after transfection. Bafilomycin A1 dissolved in DMSO (50 nmol/L final concentration) was applied for 4 h. DMSO served as a control. Cells were fixed in 4% paraformaldehyde (Sigma-Aldrich) in phosphate-buffered saline (PBS), permeabilized with 0.3% Triton X-100 (Sigma-Aldrich), and blocked for 1 h with 2.5% bovine serum albumin (Sigma-Aldrich) in PBS. The antibody against FYCO1 was applied overnight. The secondary antibody (Cy3-coupled anti-rabbit antibody, Dianova) was used at 1:200 for 1 h. Nuclei were labeled by DAPI. Please see Supplemental Table 1 for a complete antibody list.

All images were captured at room temperature using the BZ-9000E HS all-in-one fluorescence microscope (Keyence) with the BZ-II Viewer software (version 2.1, Keyence). Bright-field macro images of whole heart sections were taken in the Z-sync and AutoRangePhoto mode with a

20% overlap between neighboring pictures and merged automatically by the BZ-II Analyzer software (version 2.1, Keyence).

The MCSA was measured in fluorescence micrographs captured of the heart sections stained with lectin. For that purpose the outlines of 100-200 circular to oval shaped myocytes were traced in three different regions on each section using ImageJ software (version 1.46, http://rsb.info.nih.gov/ij). Altogether four individual hearts were analyzed per genotype.

**Animal experiments**

Transverse aortic constriction (TAC) was performed in female knockout mice and their wildtype littermates (11-13 weeks old) for 1 and 2 weeks. TAC was performed in male transgenic mice and their wildtype littermates for 2 weeks. The animals received analgesia before surgery (buprenorphine 0.1 mg/kg body weight). Mice were anesthetized with isoflurane (1.5% v/v). The mice were orally intubated with 22 G tube and ventilated (Harvard Apparatus) at 120 breaths per minute (0.2 ml tidal volume). The aortic constriction was created via a lateral thoracotomy through the second intercostal space. A suture (Prolene 6-0) was placed around the transverse aorta between the brachiocephalic and left carotid artery. The suture was ligated against a 26 G needle. The needle was removed leaving a discrete stenosis. The chest was closed. Sham operated animals underwent the same procedure except ligation. We examined the animals 1 or 2 weeks after surgery.

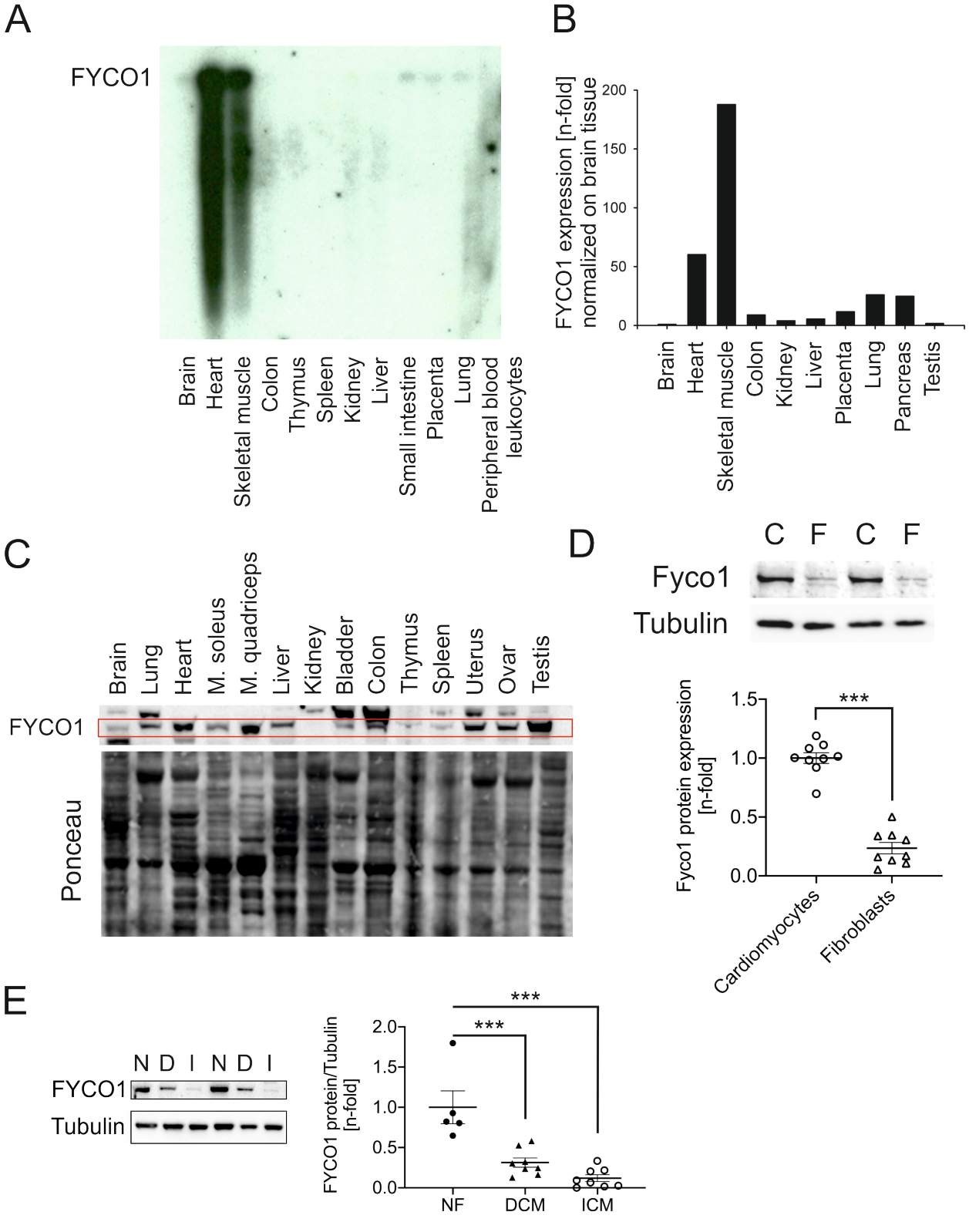
Echocardiography was performed using a Vivid 7 system (GE Healthcare) with an I13L transducer (14.1 MHz, GE Healthcare) or the Vevo 1100 imaging system with an MS400 transducer (18-38 MHz, VisualSonics). Mice were anesthetized by isoflurane (1.5% v/v). Three independent M-mode measurements per animal were obtained by a genotype-blinded examiner in a short axis view on the level of the papillary muscles. The ejection fraction was measured in the parasternal long axis by tracing the endocardium during diastole and systole.

**Human Myocardial Tissue**

Failing hearts were obtained from patients undergoing heart transplantation due to terminal heart failure (Dilated cardiomyopathy, DCM, and ischemic cardiomyopathy, ICM). LV ejection fraction was 16-25%, cardiac index 1.7 – 2.7 l/min x $m^2$. Most patients received ACE inhibitors, diuretics and cardiac glycosides, 7 received calcium channel blockers, 13 nitrates and 3 antiarrhythmic drugs in addition. No patient received b-blockers. Non-failing donor hearts (NF) that could not be transplanted for technical reasons were used for comparison. Donor patient histories or echocardiography revealed no signs of heart disease. The study conforms with the principles outlined in the Declaration of Helsinki and was reviewed and approved by the Ethical Committee of the University Hospital Hamburg (Az. 532/116/9.7.1991).

**Supplemental Figures and Figure Legends**

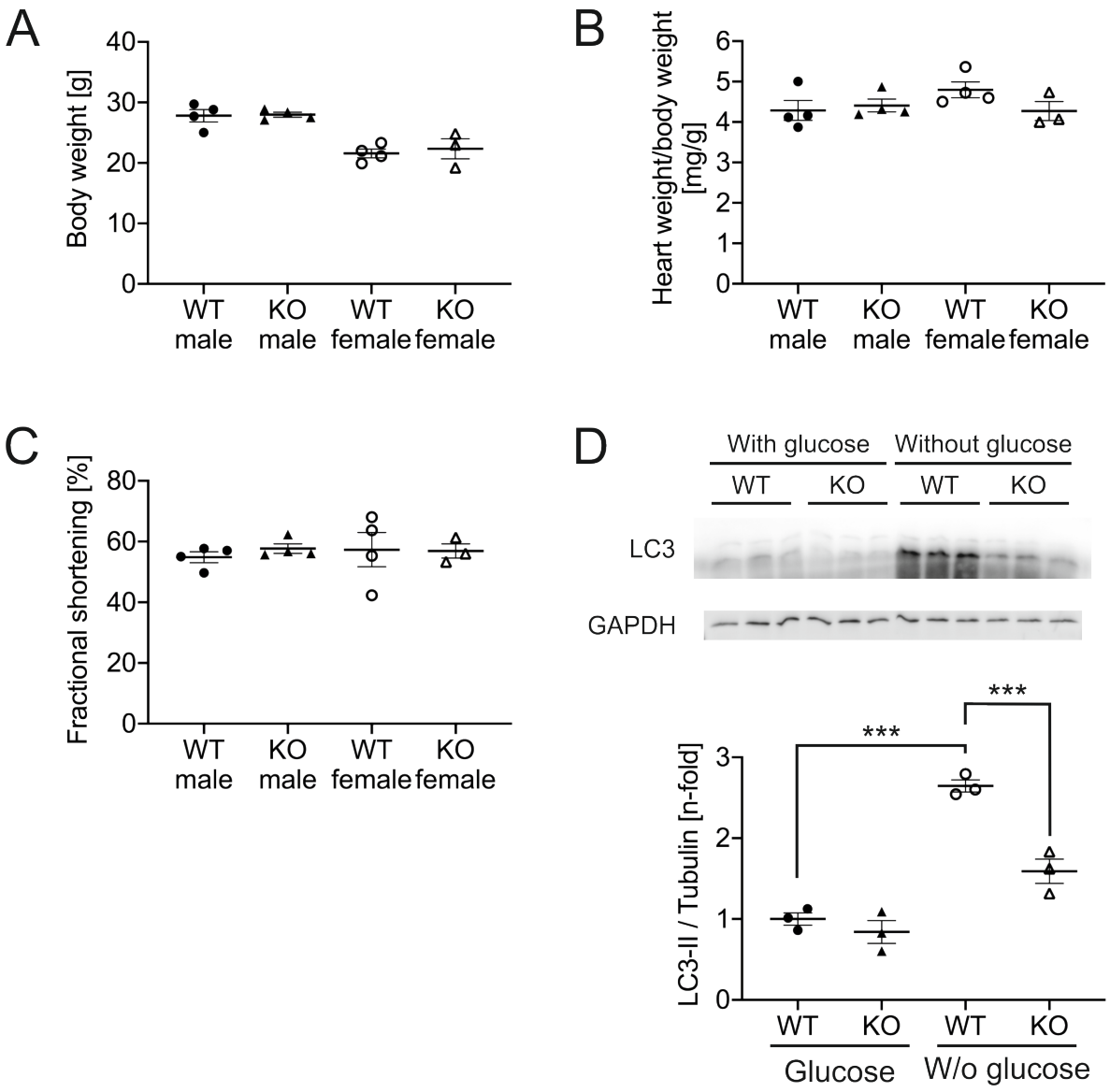
# Supplemental Figure 1



**Supplemental Figure 1. FYCO1 is enriched is striated muscle.**

(A) FYCO1 is specifically enriched in human skeletal and heart muscle as indicated by Northern hybridization. (B) FYCO1 mRNA expression in different human tissues determined by a real time PCR assay. Data are normalized on brain tissue. HPRT served as an internal control. (C) Immunoblot for FYCO1 in different organs. (D) Western blot and quantification of FYCO1 in cardiomyocytes and fibroblasts. C: cardiomyocytes, F: fibroblasts. Data are presented as dot plots with mean ± SEM (Two sided Student's t-test). (E) Western blot and quantification of human FYCO1 in non-failing hearts (N), dilated cardiomyopathy (D) and ischemic cardiomyopathy (I). Data are represented as dot plots with mean ± SEM (One-way ANOVA followed by Student-Newman-Keuls post-hoc tests). *** $p<0.001$.

# Supplemental Figure 2

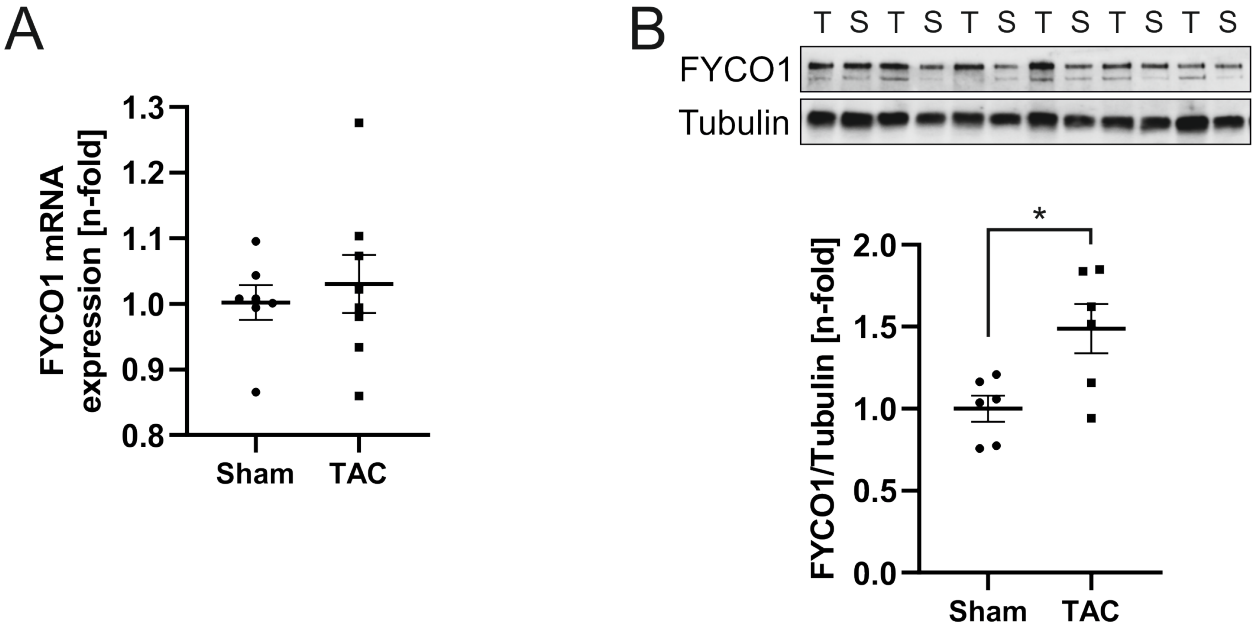



**Supplemental Figure 2. Phenotype of adult FYCO1 knockout mice and starvation of neonatal mouse cardiomyocytes isolated from *Fyco1* knockout mice.**

At the age of 4 months there are no overt differences between female and male wildtype (WT, n=4) as well as female and male FYCO1 knockout mice (KO, n=4/3) with respect to body weight (A), ratio of heart weight vs. body weight (B) and fractional shortening (C). (D) Cardiomyocytes were isolated from neonatal wildtype (WT) and *Fyco1* knockout mice (KO). Cells were treated with either supplemented DMEM or glucose free DMEM. Glucose deprivation caused a

2.65±0.08-fold induction of LC3-II in WT cells. However, induction was blunted in KO cardiomyocytes (1.59±0.15-fold, n=3). Data are represented as dot plots with mean ± SEM (Two-way ANOVA followed by Student-Newman-Keuls post-hoc tests). *** $p<0.001$.

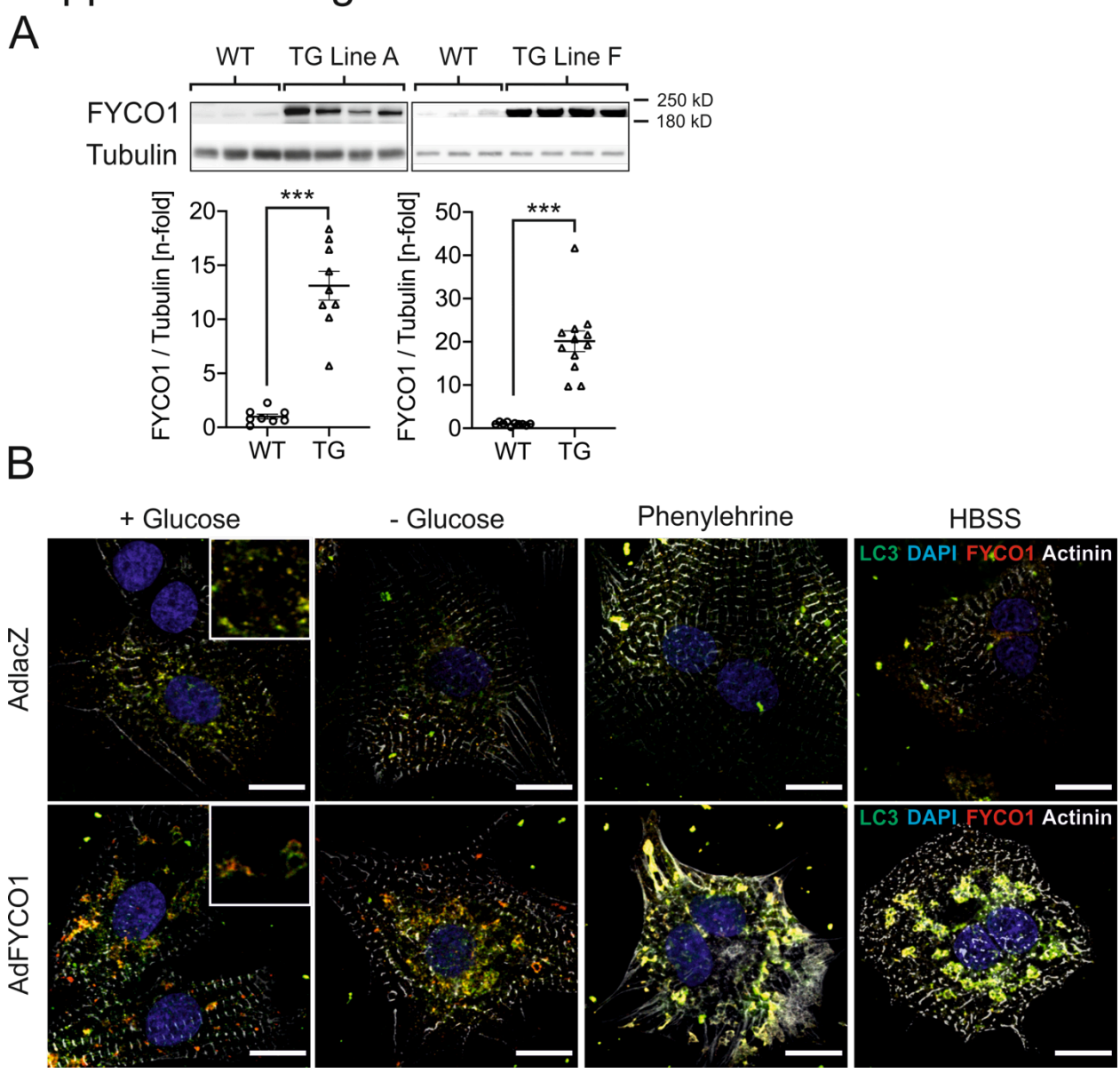
# Supplemental Figure 3




**Supplemental Figure 3. FYCO1 protein is induced by transverse aortic constriction (TAC).**

(A) qPCR of FYCO1 mRNA in sham and TAC operated mice. (B) Immunoblot and FYCO1 protein quantification in sham and TAC operated mice. T: TAC, S: Sham. Data are presented as dot plots with mean ± SEM (Two sided Student's t-test). * $p<0.05$.

# Supplemental Figure 4

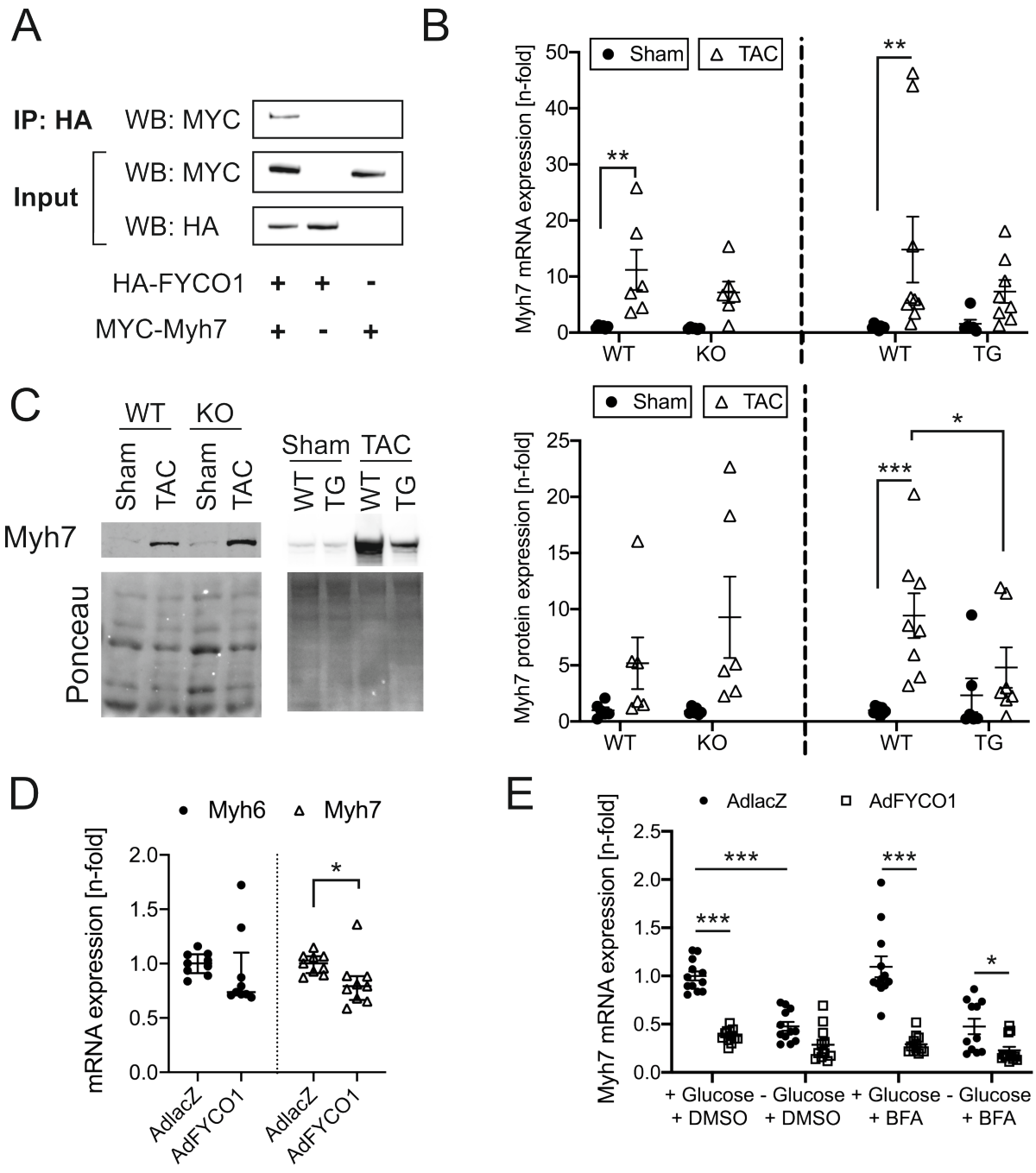



**Supplemental Figure 4. FYCO1 overexpression causes accumulation of autophagosomes independent of applied stimuli.**

(A) Cardiac specific overexpression of FYCO1 in 3 months old male mice (TG) results in 13.1±1.3-fold higher protein level of Fyco1 than in wildtype (WT) littermates (Line A, WT n=8, TG n=9). Another transgenic mouse line reveals an even higher overexpression (Line F, 20.1±2.4-fold, WT n=10, TG n=12). The analysis of these 2 transgenic mouse lines shows a similar phenotype under basal conditions. Further findings are presented for line F in the main

text. Data are represented as dot plots with mean ± SEM (Two sided Student's t-tests). (B) Immunostainings of FYCO1, α-Actinin and GFP-LC3 in neonatal rat cardiomyocytes after FYCO1 overexpression and starvation by depletion of glucose for 24 h or treatment with HBSS for 4 h or 100 µM phenylephrine for 24 h. *** $p<0.001$. Scale bar 10 µm.

# Supplemental Figure 5




**Supplemental Figure 5. FYCO1 interacts with β-MHC/Myh7 and influences its expression.**

(A) To examine the underlying mechanism that is responsible for the contractile phenotype of and transgenic mice (TG) after transverse aortic constriction (TAC) we performed a yeast two-hybrid screen. We identified β-MHC/Myh7 as potential interacting protein. Co-

immunoprecipitation of overexpressed, MYC-tagged Myh7 with overexpressed, HA-tagged FYCO1 confirms the protein interaction. (B) TAC causes an induction of Myh7 mRNA in wildtype mice after 1 week, while there is no difference between sham operated and banded KO animals (WT sham: 1.00±0.11-fold; KO sham: 0.74±0.06-fold; WT TAC: 11.18±3.59-fold; KO TAC: 7.20±1.91-fold, n=6 per group). Similar effects are obvious in WT and TG mice after 2 weeks of TAC (WT sham: 1.00±0.14-fold, n=8; TG sham: 1.55±0.75-fold, n=6; WT TAC: 14.81±5.88-fold, n=9; TG TAC: 7.33±2.05-fold, n=8). (C) Analysis of Myh7 protein levels does not reveal statistically significant differences between sham operated or banded WT and KO cohorts due to high standard deviations (WT sham: 1.00±0.27-fold; KO sham: 0.93±0.12-fold; WT TAC: 5.19±2.30-fold; KO TAC: 9.27±3.62-fold, n=6 per group). In TAC TG mice Myh7 protein is significantly lower compared to banded WT (WT sham: 1.00±0.11-fold, n=8; TG sham: 2.34±1.50-fold, n=6; WT TAC: 9.42±1.99-fold, n=8; TG TAC: 4.80±1.80-fold, n=7). Data are represented as dot plots with mean ± SEM (Two-way ANOVA followed by Student-Newman-Keuls post-hoc tests). (D) Overexpression of FYCO1 by adenoviral gene transfer (AdFYCO1, 50 moi) in isolated neonatal rat cardiomyocytes (NRCMs) results in lower Myh7 mRNA expression (0.82±0.08-fold, n=9) than in control cells (AdlacZ 1.00±0.03-fold, n=9). FYCO1 overexpression does not change Myh6 expression (Data are presented as dot plots with median with 25$^{th}$ and 75$^{th}$ percentiles, Mann-Whitney rank sum test). (E) To analyze the impact of the autophagic flux on Myh7 by FYCO1 overexpression NRCMs are infected by AdlacZ or AdFYCO1 and starved by glucose deprivation for 24 h and treated with bafilomycin A1 (BFA) 4 h before harvesting these cells. Overexpression of FYCO1 causes reduction of Myh7 mRNA expression in fed cardiomyocytes treated with vehicle DMSO compared to control NRCMs (AdFYCO1 + glucose + DMSO: 0.38±0.02-fold). While glucose deprivation itself leads to suppression of Myh7 mRNA in control cardiomyocytes, the overexpression of FYCO1 results in lower levels of Myh7 in BFA treated, fed and starved cardiomyocytes (AdlacZ - glucose + DMSO: 0.48±0.05-fold; AdlacZ - glucose + BFA: 0.48±0.08-fold, n=11; AdFYCO1 - glucose + DMSO: 0.29±0.05-fold; AdFYCO1 -

glucose + BFA: 0.23±0.04-fold). N=12 per group. Data are represented as dot plots with mean ± SEM (Two-way ANOVA followed by Student-Newman-Keuls post-hoc tests). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

## Supplemental Tables

### Supplemental Table 1: Primary and secondary antibodies

| **Target protein** | **Company** | **Catalog Number** | **Application** |
|---|---|---|---|
| α-Actinin | Sigma-Aldrich | A7811 | IF |
| Atg5 | Cell Signaling | 8540 | WB |
| Beclin-1 | Cell Signaling | 3495 | WB |
| FYCO1 | Novus Biologicals | NBP1-47266 | IF, WB |
| GAPDH | Sigma-Aldrich | G8795 | WB |
| LC3 | Cell Signaling | 2775 | WB |
| Rab7 | Sigma-Aldrich | R4779 | WB |
| SQSTM1/p62 | Origene | EPR4844 | WB |
| α-Tubulin | Sigma-Aldrich | 078K4781 | WB |
| **Secondary target** | **Company** | **Catalog Number** | **Application** |
| Donkey anti-Rabbit IgG-HRP | Santa Cruz | sc2305 | WB |
| Goat anti-Mouse IgG-HRP | Santa Cruz | sc2302 | WB |
| Goat IgG anti-Rabbit IgG (H+L)-Cy3 | Dianova | 111-165-003 | IF |
| Sheep IgG anti-Mouse IgG (H+L)-Cy3 | Dianova | 515-165-003 | WB |
| Sheep IgG anti-Mouse IgG (H+L)-FITC | Dianova | 515-095-062 | IF |

**Supplemental Table 2: Echocardiography of *Fyco1* KO mice at the age of 1 year.**

| | **WT** n=5 | **Fyco1 KO** n=8 | **P value** |
|---|---|---|---|
| Fractional shortening [%] | 42.1±1.8 | 37.6±2.2 | 0.18 |
| LVIDd [mm] | 3.88±0.07 | 3.93±0.10 | 0.72 |
| IVSd [mm] | 0.73±0.02 | 0.77±0.03 | 0.33 |
| LVPWd [mm] | 0.79±0.03 | 0.79±0.03 | 0.99 |
| LVIDs [mm] | 2.25±0.05 | 2.46±0.14 | 0.27 |
| IVSs [mm] | 1.43±0.05 | 1.34±0.04 | 0.21 |
| LVPWs [mm] | 1.33±0.07 | 1.29±0.03 | 0.49 |
| Heart rate [bpm] | 597.1±14.2 | 576.5±18.7 | 0.45 |

LVID Left ventricular internal diameter, IVS Interventricular septum, LVPW Left ventricular posterior wall, d diastolic, s systolic. Data are presented as mean ± SEM.

**Supplemental Table 3: Echocardiography of *Fyco1* KO mice after 48h of starvation.**

| | **WT fed** n=5 | **KO fed** n=4 | **WT starved** n=5 | **KO starved** n=5 | **P value** WT fed vs. WT starved | **P value** WT starved vs. KO starved |
|---|---|---|---|---|---|---|
| Fractional shortening [%] | 50.2±2.9 | 52.3±2.0 | 49.7±0.8 | 37.9±3.0 | 0.89 | 0.003 |
| LVIDd [mm] | 2.83±0.10 | 2.80±0.07 | 2.94±0.16 | 2.92±0.17 | 0.59 | 0.94 |
| IVSd [mm] | 0.71±0.01 | 0.77 ±0.03 | 0.78±0.02 | 0.76±0.03 | 0.041 | 0.54 |
| LVPWd [mm] | 0.64±0.01 | 0.68±0.04 | 0.62±0.04 | 0.66±0.04 | 0.81 | 0.51 |
| LVIDs [mm] | 1.42±0.12 | 1.34±0.09 | 1.47±0.07 | 1.85±0.17 | 0.77 | 0.036 |
| IVSs [mm] | 1.42±0.05 | 1.52±0.04 | 1.41±0.09 | 1.31±0.03 | 0.92 | 0.24 |
| LVPWs [mm] | 1.32±0.06 | 1.28±0.03 | 1.36±0.03 | 1.08±0.09 | 0.68 | 0.007 |
| Heart rate [bpm] | 543.3±62.1 | 630±20.0 | 546.4±37.6 | 471.5±31.8 | 0.96 | 0.22 |

LVID Left ventricular internal diameter, IVS Interventricular septum, LVPW Left ventricular posterior wall, d diastolic, s systolic. Data are presented as mean ± SEM.

**Supplemental Table 4: Echocardiography of *Fyco1* KO mice after 1 week of TAC.**

| | **WT sham** n=6 | **KO sham** n=7 | **WT TAC** n=7 | **KO TAC** n=10 | **P value** WT sham vs. WT TAC | **P value** KO sham vs. KO TAC | **P value** WT TAC vs. KO TAC |
|---|---|---|---|---|---|---|---|
| Fractional shortening [%] | 46.1±2.2 | 50.2±0.8 | 34.2±6.1 | 23.9±3.2 | 0.044 | <0.001 | 0.049 |
| LVIDd [mm] | 2.90±0.07 | 2.95±0.13 | 3.49±0.20 | 3.51±0.15 | 0.019 | 0.013 | 0.92 |
| IVSd [mm] | 0.86±0.04 | 0.80±0.02 | 0.97±0.04 | 1.04±0.03 | 0.047 | <0.001 | 0.11 |
| LVPWd [mm] | 0.77±0.02 | 0.77±0.03 | 0.90±0.03 | 0.93±0.03 | 0.008 | <0.001 | 0.47 |
| LVIDs [mm] | 1.56±0.06 | 1.47±0.08 | 2.37±0.35 | 2.71±0.21 | 0.023 | <0.001 | 0.26 |
| IVSs [mm] | 1.50±0.03 | 1.49±0.03 | 1.49±0.07 | 1.42±0.07 | 0.93 | 0.37 | 0.39 |
| LVPWs [mm] | 1.31±0.03 | 1.36±0.03 | 1.29±0.08 | 1.14±0.07 | 0.84 | 0.014 | 0.07 |
| Heart rate [bpm] | 622.8±17.5 | 608.3±8.9 | 676.4±17.6 | 644.9±19.2 | 0.05 | 0.13 | 0.19 |

LVID Left ventricular internal diameter, IVS Interventricular septum, LVPW Left ventricular posterior wall, d diastolic, s systolic. Data are presented as mean ± SEM.

**Supplemental Table 5: Echocardiography of *Fyco1* KO mice after 2 weeks of TAC.**

| | **WT sham** n=6 | **KO sham** n=6 | **WT TAC** n=8 | **KO TAC** n=9 | **P value** WT sham vs. WT TAC | **P value** KO sham vs. KO TAC | **P value** WT TAC vs. KO TAC |
|---|---|---|---|---|---|---|---|
| Fractional shortening [%] | 45.4±1.3 | 45.9±0.7 | 26.0±2.8 | 19.7±1.9 | <0.001 | <0.001 | 0.028 |
| LVIDd [mm] | 3.38±0.06 | 3.29±0.06 | 3.76±0.09 | 3.97±0.12 | 0.009 | <0.001 | 0.12 |
| IVSd [mm] | 0.77±0.02 | 0.83±0.03 | 1.04±0.04 | 0.91±0.05 | <0.001 | 0.15 | 0.018 |
| LVPWd [mm] | 0.82±0.03 | 0.90±0.04 | 1.05±0.02 | 0.90±0.03 | <0.001 | 0.88 | 0.002 |
| LVIDs [mm] | 1.85±0.06 | 1.79±0.04 | 2.81±0.14 | 3.20±0.16 | <0.001 | <0.001 | 0.034 |
| IVSs [mm] | 1.39±0.03 | 1.45±0.03 | 1.46±0.07 | 1.29±0.04 | 0.33 | 0.036 | 0.017 |
| LVPWs [mm] | 1.32±0.05 | 1.41±0.06 | 1.29±0.05 | 1.13±0.05 | 0.74 | 0.001 | 0.028 |
| Heart rate [bpm] | 623.7±12.3 | 599.1±15.7 | 610.4±22.1 | 599.4±16.6 | 0.62 | 0.99 | 0.64 |

LVID Left ventricular internal diameter, IVS Interventricular septum, LVPW Left ventricular posterior wall, d diastolic, s systolic. Data are presented as mean ± SEM.

**Supplemental Table 6: Morphometric and echocardiography of FYCO1 transgenic (TG) mice with moderate (Line A) overexpression of FYCO1 under baseline conditions at the age 3 months.**

| **Line A** | **WT** n=8 | **FYCO1 TG** n=9 | **P value** |
|---|---|---|---|
| Body weight [g] | 25.89±0.65 | 26.08±0.41 | 0.80 |
| Heart vs. body weight [mg/g] | 4.40±0.10 | 4.85±0.05 | <0.001 |
| Lung weight [mg] | 151.2±4.91 | 150.5±5.82 | 0.93 |
| Fractional shortening [%] | 38.5±1.9 | 38.6±2.2 | 0.98 |
| LVIDd [mm] | 3.81±0.08 | 4.04±0.07 | 0.046 |
| IVSd [mm] | 0.77±0.02 | 0.73±0.02 | 0.11 |
| LVPWd [mm] | 0.82±0.04 | 0.78±0.03 | 0.44 |
| LVIDs [mm] | 2.35±0.10 | 2.50±0.12 | 0.37 |
| IVSs [mm] | 1.35±0.03 | 1.39±0.05 | 0.55 |
| LVPWs [mm] | 1.36±0.06 | 1.38±0.03 | 0.78 |

LVID Left ventricular internal diameter, IVS Interventricular septum, LVPW Left ventricular posterior wall, d diastolic, s systolic. Data are presented as mean ± SEM.

**Supplemental Table 7: Echocardiography of FYCO1 transgenic (TG) mice with high (Line F) overexpression of FYCO1 under baseline conditions at the age 3 months.**

| Line F | WT n=10 | FYCO1 TG n=9 | P value |
|---|---|---|---|
| Fractional shortening [%] | 41.1±2.1 | 44.0±2.3 | 0.21 |
| LVIDd [mm] | 3.90±0.11 | 3.64±0.07 | 0.07 |
| IVSd [mm] | 0.77±0.01 | 0.87±0.03 | 0.011 |
| LVPWd [mm] | 0.73±0.02 | 0.85±0.02 | <0.001 |
| LVIDs [mm] | 2.31±0.15 | 2.06±0.12 | 0.14 |
| IVSs [mm] | 1.42±0.05 | 1.57±0.06 | 0.07 |
| LVPWs [mm] | 1.34±0.03 | 1.47±0.04 | 0.029 |

LVID Left ventricular internal diameter, IVS Interventricular septum, LVPW Left ventricular posterior wall, d diastolic, s systolic. Data are presented as mean ± SEM.

**Supplemental Table 8: Echocardiography of FYCO1 transgenic (TG) mice after 2 weeks of TAC.**

| | **WT sham** n=8 | **TG sham** n=6 | **WT TAC** n=9 | **TG TAC** n=8 | **P value** WT sham vs. WT TAC | **P value** TG sham vs. TG TAC | **P value** WT TAC vs. TG TAC |
|---|---|---|---|---|---|---|---|
| Ejection fraction [%] | 49.7±2.5 | 45.7±3.2 | 35.6±2.2 | 45.4±3.3 | <0.001 | 0.96 | 0.014 |
| Fractional shortening [%] | 36.4±4.6 | 32.8±3.2 | 24.7±2.6 | 31.5±2.5 | 0.020 | 0.80 | 0.16 |
| LVIDd [mm] | 3.95±0.12 | 3.66±0.17 | 4.16±0.35 | 3.97±0.18 | 0.53 | 0.40 | 0.56 |
| IVSd [mm] | 0.73±0.03 | 0.74±0.06 | 0.96±0.09 | 0.97±0.05 | 0.011 | 0.035 | 0.90 |
| LVPWd [mm] | 0.91±0.03 | 0.88±0.09 | 0.96±0.06 | 1.07±0.06 | 0.51 | 0.06 | 0.17 |
| LVIDs [mm] | 2.55±0.25 | 2.48±0.23 | 3.12±0.32 | 2.72±0.15 | 0.11 | 0.54 | 0.25 |
| IVSs [mm] | 1.03±0.07 | 0.98±0.06 | 1.13±0.05 | 1.23±0.06 | 0.22 | 0.018 | 0.19 |
| LVPWs [mm] | 1.13±0.06 | 1.04±0.09 | 1.11±0.06 | 1.25±0.05 | 0.82 | 0.05 | 0.10 |
| Heart rate [bpm] | 457.5±25.2 | 443.5±19.9 | 423.6±32.6 | 481.9±24.3 | 0.37 | 0.39 | 0.13 |

LVID Left ventricular internal diameter, IVS Interventricular septum, LVPW Left ventricular posterior wall, d diastolic, s systolic. Ejection fraction was measured in parasternal long axis view by tracing of the left ventricular endocardium during diastole and systole. All other parameters were measured in parasternal short axis view. Data are presented as mean ± SEM.