Detailed Methods

Generation of the \(\text{PLM}^{\text{3SA}}\) mouse

Transgenic \(\text{PLM}^{\text{3SA}}\) animals were generated by genOway. PLM is coded by the \(\text{FXYD}-1\) gene resident on mouse chromosome 7. The \(\text{PLM}^{\text{3SA}}\) point mutation knock-in was generated by homologous recombination in embryonic stem cells (ES) using a targeting vector that also inserted a LoxP-flanked neomycin selection cassette between exons 5 and 6. The targeting alters the murine \(\text{Fxyd}-1\) gene in the codons encoding S63 in exon 6, and S68 and S69 in exon 7, replacing them with alanes, resulting in insertion of S63A, S68A and S69A point mutations and the expression of a constitutively unphosphorylatable \(\text{FXYD}-1\) (PLM) protein. After electroporation of the targeting vector into 129Sv ES cells, G418 resistant ES cell clones were screened by PCR and Southern Blot for the homologous recombination event. Recombined ES cell clones were thereafter injected into C57BL/6J derived blastocysts to generate chimeric mice. Germline transmission of the mutated \(\text{Fxyd}-1\) allele and \text{in vivo} deletion of the loxP-neomycin-loxP selection cassette were then assessed after breeding of chimeras with \(\text{Cre}\)-expressing deleter C57BL/6J mice and selecting according to coat colour and PCR genotyping respectively. The mutated \(\text{Fxyd}-1\) gene is expressed under the control of the endogenous \(\text{FXYD}-1\) promoter. Knock-in heterozygous mice were characterized by PCR and Southern Blot. For PCR reaction were used primers with sequence 5'-3': TCTGCGTGCTAAGATGATCACAGATGC and CTTGACTTTGTTGGGAGAGGGACGG. Heterozygous breeding pairs were used to generate PLM\(^{\text{3SA}}\) and WT littermates. All mice received humane care in accordance with "Guidance on the Operation of the Animals (Scientific Procedures) Act of 1986" published by HM Stationery Office, London UK and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Hypertrophy Model

Myocardial hypertrophy was induced by pressure overload following suprarenal aortic constriction\(^1\) in 6-week-old male C57BL/6J mice (20-22 g), PLM\(^{\text{3SA}}\) knock-in mice or their WT littermates. Mice were anesthetized with an isoflurane/O\(_2\) mixture (2/98%) and aortic constriction was performed by placing a suture around the abdominal aorta and a 28-gauge blunted needle stent, which was subsequently removed. To standardise the extent of aortic constriction between animals the aortic diameter was assessed by echocardiography and, post-mortem, the cross-sectional area of each ligature ring was measured. Residual Luminal Area (RLA) was then calculated as the ligature area expressed as a percentage of the aortic cross-sectional area (assuming the aorta to be circular in cross section). RLA was strongly correlated with the morphometric data and this allowed exclusion criteria to be established. Any animal having a RLA>22% was excluded from subsequent analysis. RLA was similar in all experimental groups: C57/Bl6 mice, PLM\(^{\text{3SA}}\) mice or their WT littermates. Sham surgery comprised an identical procedure with the exception of band placement. Animals were studied up to 8 weeks post surgery. Acute post-operative analgesia was a single buprenorphine (Vetgesic 0.3 mg/ml solution) injected i.p. at dose 20µg/kg.

Assessment of cardiac function

\textit{Echocardiography:} Cardiac function was measured using 2D echocardiography in control mice at 14-16 weeks or in sham and banded animals 4 and 8 weeks after surgery. Briefly, mice were anesthetized with an isoflurane/O\(_2\) mixture (2/98%) and imaged using a Visualsonics Vevo 770 ultrasound system with a 30-MHz linear signal transducer. Averaged left ventricle (LV) M-mode measures from parasternal long-axis and short-axis images were recorded. Interventricular septal (IVS), LV anterior wall (LVAW) and LV posterior wall (LVPW) dimensions were taken in diastole and systole, in addition to LV internal dimensions (LVIDd and LVIDs, respectively). Fractional shortening (FS) was calculated as (LVIDd – LVIDs/LVIDd) × 100 and ejection fraction (EF) as (LVIDd3– LVIDs3)/LVIDd3 × 100. Blood
flow in the aortic arch was measured by pulse-wave Doppler. Body temperature, heart rate and ECG were controlled during imaging and all experiments were performed under physiological conditions.

**Invasive Pressure-volume analysis**
Real-time pressure volume loops were obtained using the ADVantage™ system (Scisense Inc, Canada) which uses a miniaturized 1.2 Fr admittance catheter. In our experiments measurements of LV function were performed in the more physiological closed chest mode when a catheter was placed in LV by retrograde approach. Briefly, 15 weeks old or 9 weeks post-operative mice were anesthetized, right internal carotid artery (ICA) was exposed, and catheterized. Then 1.2 Fr catheter was advanced towards heart and inserted into the LV cavity via the aortic valve.

**Langendorff perfusion**
Mice at 5 or 9 weeks after abdominal aorta constriction were anæsthetized with intraperitoneal pentobarbital (Pentoject 300 mg/kg) and heparin (150 U). Hearts were rapidly isolated and perfused in Langendorff-mode, as described previously. A fluid-filled balloon within the left ventricular (LV) cavity was used to monitor contractile function and heart rate. During stabilization hearts were paced at 550bpm via a silver/silver chloride wire touching the apex of the LV and referenced to the steel aortic cannula. After 25 min stabilization, a force-frequency relationship (FFR) was constructed as previously described. At the end of perfusion hearts were snap-frozen for biochemical analysis.

**Electrophysiological measurements of Na/K pump activity**
Measurements of Na/K pump current were performed in ventricular myocytes. Cells were isolated from the hearts of sham and banded mice (from 5 to 9 weeks after surgery) or from non-operated 14-16 weeks old mice by collagenase-based enzymatic digestion, using an adaptation of the method described previously. Isolated myocytes were pelleted by brief centrifugation at 50 g and washed at room temperature with modified M199 culture medium (Invitrogen, Carlsbad, CA, USA) containing 100 IU/ml penicillin, 2 mmol/L creatine, 2 mmol/L carnitine and 5 mmol/L taurine. Following further centrifugation at 50 g, myocytes were again re-suspended in modified M199 culture medium. Cells were then left for 120 min in an incubator (37°C, 5% CO₂) prior to being used for electrophysiological experiments. Mouse ventricular myocytes were voltage-clamped and Na/K pump current (Ip) was recorded at 35°C using the whole-cell ruptured-patch technique as described previously. Briefly, myocytes were studied under whole-cell voltage-clamp using electrodes which had resistances of 1-2 MΩ when filled with (in mmol/L) 95 CsCH₃O₃S, 25 NaCH₃O₃, 20 CsCl, 1 MgCl₂, 1.5 CaCl₂, 10 HEPES, 5 EGTA, 5 MgATP, 5 creatine phosphate pH 7.2. Free intracellular Ca in this solution was estimated to be approximately 100nmoles/L (CaBuf Software, G. Droogmans, KU Leuven). Note: this is essential as buffering Ca to lower levels abrogates both the response to kinase activation and to PLM mutation. Isolated myocytes were placed in Ca²⁺ free Tyrode on laminin-coated coverslips and incubated with 10 μmole/L sodium-binding benzofuran isophthalate/acetoxymethyl ester (SBFI-AM) in the presence of Pluronic F-127 (0.05% wt/vol), for 90-120 minutes at room temperature. External dye was washed and SBFI-AM allowed to de-esterify for 20 minutes in normal Tyrode’s solution containing (in mmol/L): 140 NaCl, 4 KCl, 1 MgCl₂, 1 CaCl₂, 10 HEPES, and 10 glucose (pH 7.4). SBFI was excited alternately at 340 and 380 nm with a DeltaRAM X™ monochromator (PTI Inc., New Jersey, US) and emission fluorescence
collected at 535 nm. The SBFI fluorescence ratio (at 340 and 380 nm; F340 and F380) F340/F380 was calculated after background subtraction and converted to [Na] by calibration at the end of each experiment (using divalent-free solutions with 0, 10, or 20 mmol/L extracellular [Na]). Calibration of SBFI was accomplished by exposing the myocytes to various extracellular [Na] ([Na]ex) in the presence of 10 μmol/L gramicidin D and 100 μmol/L strophanthidin. The solutions with various [Na]ex were prepared by mixing, in different proportions, two solutions of equal ionic strength. One solution contained 145 mmol/L Na (30 mmol/L NaCl, 115 mmol/L sodium gluconate) and no K, while the other one had 145 mmol/L K (30 mmol/L KCl, 115 mmol/L potassium gluconate) and was Na free. Both calibration buffers also contained 10 mmol/L Hepes, 10 mmol/L glucose and 2 mmol/L EGTA and the pH was adjusted to 7.2 with Tris base. All the measurements in this study were at room temperature.

**NCX activity measurement in isolated cardiomyocytes**

Isolated myocytes were incubated with 10 μmoles/L FURA-2AM in the presence of pluronic acid (F-127, 0.05% wt/vol), for 30-45 minutes at room temperature. Extracellular dye was washed off and FURA-2 allowed to de-esterify for 20 minutes in normal Tyrode’s solution containing (in mmol/L): 140 NaCl, 4 KCl, 1 MgCl₂, 2 CaCl₂, 10 HEPES, and 10 glucose (pH 7.4). Cells were plated in Tyrode on laminin-coated coverslips and FURA-2 was excited alternately at 340 and 380 nm with a DeltaRAM X™ monochromator (PTI Inc., New Jersey, US) and emission fluorescence collected at 510 nm. In order to measure NCX activity, cells were paced at 0.5 Hz for 2-5 min, the pacing stimulus was then stopped and one second later, the SR was rapidly emptied by continuous application of 10 mmol/L of caffeine using a rapid switcher (Warner Instruments, Fast Step SF-77B). Caffeine was maintained until the Ca decline was complete. The time constant (τ) of Ca decline was calculated from a mono-exponential function fitted to the fluorescence trace. While accepting that a small component of Ca recovery under these conditions is due to the activity of the plasma membrane Ca ATPase, τ is assumed to be inversely related to NCX activity. Control experiments were performed whereby caffeine-induced Ca decline was abolished by substituting Na⁺ with N-Methyl-D-glucamine. All the measurements in this study were at room temperature.

**Quantitative immunoblotting**

Hearts were harvested from banded and sham operated animals after 4 and 8 weeks after aortic banding surgery. After harvesting hearts were dissected and LV were frozen and stored at -80°C. LV homogenates (10% w/v) were size-fractionated on gradient (7.5% - 15%) SDS-PAGE gels. Proteins were transferred to PVDF membranes (0.45μm; GE Healthcare, UK). Immunoblots were blocked with 5% non-fat milk in PBS-Tween overnight at 4°C. Blots then were incubated 1-1.5 hours at room temperature with primary antibody: isoform specific of Na/K pump α-1 (1:15000; Millipore) and α-2 (1:1000; Millipore); custom made anti-PLM antibodies, anti-phospholamban total (1:2000; Millipore), anti-phospho (Ser 16) phospholamban (1:15000; Badrilla), anti-calsequestrin (1:2500; Pierce Antibody), anti-SERCA2a ATPase (1:1000, Pierce Antibody) and anti-Na/Ca exchanger (1:1000, Swant). After incubation with HRP-labeled secondary antibodies, blots were developed using enhanced chemiluminescence (Amersham Pharmacia Biotech). Calsequestrin was used as a loading control. Signals from hypertrophic samples were normalized to sham samples signals on the same gels.
RESULTS

Figure 1: Cardiac function and morphometry assessed by echocardiography in sham and banded C57BL/6J mice. (A): Representative echocardiographic images (M-mode) 8 weeks after aorta constriction. (B): Quantitative measures of cardiac function and hypertrophy in sham and banded hearts. LVw/TL is left ventricular weight as a function of tibia length. (Data are mean±SEM, *P<0.05; n=8 for sham and banded).
Figure 2: Protein expression measured 8 weeks after aortic banding in WT and PLM<sup>3SA</sup> mice. Representative Western blots for Na/K pump α subunits and PLM expression (A). Quantitative changes in protein expression (B). (Alpha1/2 = Na/K ATPase α1/2 subunits, PLM = phospholemman). Note: WT and PLM<sup>3SA</sup> samples were run on the same gels. In Panel A each row represents images cropped from different gels probed with different antibodies as shown. CSQ was used as a loading control. (Data are mean±SEM, *P<0.05; n=4-6 (WT and PLM<sup>3SA</sup>))
Table 1: Echocardiographic measurements 4 weeks and 8 weeks after aorta banding in C57BL/6J mice.

|                          | 4 weeks            | 8 weeks            |
|--------------------------|--------------------|--------------------|
| **Sham (n=8)**           | **Banded (n=8)**   | **Sham (n=8)**     | **Banded (n=8)**   |
| Heart Rate, bpm          | 568 ± 3.10         | 552 ± 4.54         | p<0.01             | 562 ± 2.56         | 550 ± 8.35         | ns                  |
| Body weight, g           | 24.2 ± 0.56        | 24.3 ± 0.58        | ns                  | 26.8 ± 0.69        | 25.0 ± 0.68        | ns                  |
| Body temperature, C      | 36.8 ± 0.13        | 36.7 ± 0.18        | ns                  | 37.2 ± 0.18        | 36.7 ± 0.13        | ns                  |
| Intraventricular septum diastolic thickness, mm | 0.93 ± 0.02       | 1.21 ± 0.03        | p<0.001             | 0.97 ± 0.02        | 1.18 ± 0.02        | p<0.001             |
| LV diastolic dimension, mm | 3.57 ± 0.05      | 4.53 ± 0.15        | p<0.001             | 3.68 ± 0.09        | 5.09 ± 0.19        | p<0.001             |
| Posterior wall diastolic thickness, mm | 0.94 ± 0.03      | 1.17 ± 0.04        | p<0.001             | 0.93 ± 0.01        | 1.15 ± 0.04        | p<0.001             |
| Anterior wall diastolic thickness, mm | 0.95 ± 0.01     | 1.19 ± 0.03        | p<0.001             | 0.94 ± 0.02        | 1.18 ± 0.03        | p<0.001             |
| Intraventricular septum systolic thickness, mm | 1.55 ± 0.04      | 1.64 ± 0.06        | ns                  | 1.60 ± 0.04        | 1.48 ± 0.07        | ns                  |
| LV systolic dimension, mm | 2.10 ± 0.08       | 3.67 ± 0.23        | p<0.001             | 2.21 ± 0.09        | 4.43 ± 0.28        | p<0.001             |
| Posterior wall systolic thickness, mm | 1.53 ± 0.06      | 1.43 ± 0.04        | ns                  | 1.51 ± 0.03        | 1.34 ± 0.03        | p<0.001             |
| Anterior wall systolic thickness, mm | 1.57 ± 0.03     | 1.61 ± 0.05        | ns                  | 1.60 ± 0.03        | 1.51 ± 0.06        | ns                  |
| LV systolic volume, µL   | 13.8 ±1.45        | 59.2 ± 7.89        | p<0.001             | 15.7 ±1.88         | 93.1 ± 11.95       | p<0.001             |
| LV diastolic volume, µL  | 51.3 ±1.97        | 92.8 ± 7.34        | p<0.001             | 55.6 ± 3.76        | 123.3 ± 10.59      | p<0.001             |
| LV mass, mg              | 98.3 ±2.22        | 199.5 ± 13.99      | p<0.001             | 101.8 ± 2.95       | 237.2 ± 20.14      | p<0.001             |
| Stroke volume, µL        | 37.5 ±0.82        | 33.6 ± 1.32        | p<0.05              | 39.9 ± 2.01        | 30.3 ± 1.98        | p<0.01              |
| Ejection fraction, %     | 73.5 ±1.81        | 38.7 ± 4.79        | p<0.001             | 72.4 ± 1.61        | 27.2 ± 4.86        | p<0.001             |
| Fraction shortening, %   | 41.8 ±1.51        | 19.0 ± 2.84        | p<0.001             | 40.8 ± 1.33        | 13.0 ± 2.65        | p<0.001             |
| Cardiac output, mL/min   | 21.3 ±0.45        | 18.6 ± 0.76        | p<0.01              | 22.5 ± 1.06        | 16.7 ± 1.09        | p<0.01              |
| Endocardial fractional area change, % | 44.1 ± 2.05 | 20.6 ± 1.62 | p<0.001             | 40.6 ± 2.13        | 13.1 ± 3.70        | p<0.001             |
| Peak velocity, mm/s      | 1611.5 ±58.34     | 1847.1 ± 88.08     | p<0.05              | 1569.5 ± 48.47     | 1807.5 ± 60.25     | p<0.01              |
| Peak gradient, mmHg      | 10.5 ±0.78        | 13.9 ± 1.21        | p<0.05              | 9.9 ± 0.60         | 13.2 ± 0.86        | p<0.01              |
| Velocity time integral, cm | 5.1 ±0.20        | 4.8 ± 0.21         | ns                  | 5.1 ± 0.18         | 4.3 ± 0.19         | p<0.05              |
**Table 2:** Cardiac phenotype of WT and PLM$^{3SA}$ mice.

| Echocardiography measurements                  | WT  | 3SA |
|-----------------------------------------------|-----|-----|
| **n=15**                                      |     |     |
| Age, days                                     | 88.5 ± 1.00 | 91.5 ± 1.99 | ns |
| Heart Rate, bpm                               | 522 ± 2.42  | 522 ± 2.80  | ns |
| Body weight, g                                | 29.3 ± 0.59 | 30.7 ± 0.90 | ns |
| Body temperature, C                           | 37.5 ± 0.08 | 37.4 ± 0.12 | ns |
| Intraventricular septum diastolic thickness, mm | 1.00 ± 0.01 | 1.00 ± 0.02 | ns |
| LV diastolic dimension, mm                    | 3.87 ± 0.08 | 3.82 ± 0.08 | ns |
| Posterior wall diastolic thickness, mm        | 0.96 ± 0.02 | 0.99 ± 0.02 | ns |
| Anterior wall diastolic thickness, mm         | 1.01 ± 0.01 | 0.97 ± 0.02 | ns |
| Intraventricular septum systolic thickness, mm | 1.68 ± 0.02 | 1.70 ± 0.02 | ns |
| LV systolic dimension, mm                     | 2.30 ± 0.09 | 2.17 ± 0.08 | ns |
| Posterior wall systolic thickness, mm         | 1.49 ± 0.03 | 1.62 ± 0.04 | p<0.05 |
| Anterior wall systolic thickness, mm          | 1.69 ± 0.03 | 1.69 ± 0.02 | ns |
| Stroke volume, µL                             | 44.6 ± 1.95 | 44.7 ± 2.11 | ns |
| Ejection fraction, %                          | 72.4 ± 1.40 | 75.3 ± 1.08 | ns |
| Fraction shortening, %                        | 41.1 ± 1.16 | 43.5 ± 0.92 | ns |
| Cardiac output, mL/min                        | 23.0 ± 1.01 | 23.2 ± 1.06 | ns |
| Peak velocity, mm/s                           | 1706.7 ± 54.90 | 1968.7 ± 135.00 | ns |
| Peak gradient, mmHg                           | 11.1 ± 0.71 | 15.2 ± 1.96 | p<0.05 |
| Velocity time integral, cm                    | 5.4 ± 0.18  | 6.0 ± 0.36  | ns |
| **Pressure-Volume measurements**              |     |     |
| **n=12**                                      |     |     |
| End diastolic pressure, mmHg                  | 6.1 ± 0.89  | 5.9 ± 0.43  | ns |
| LV developed pressure, mmHg                   | 104.9 ± 2.63 | 107.8 ± 2.45 | ns |
| Stroke work, mmHg,µL                          | 1671.7 ± 74.18 | 1784.9 ± 87.80 | ns |
| dP/dt max, mmHg/s                             | 9236.6 ± 309.29 | 10031.0 ± 322.21 | ns |
| dP/dt min, mmHg/s                             | 10484.3 ± 425.85 | 10514.5 ± 470.22 | ns |
| Tau (Weiss), ms                               | 5.5 ± 0.22  | 5.4 ± 0.25  | ns |
| **Gravimetric measurements**                  |     |     |
| **n=16**                                      |     |     |
| LV weight : body weight ratio, mg/g           | 3.5 ± 0.15  | 3.6 ± 0.11  | ns |
| LV weight : tibia length ratio, mg/mm         | 6.2 ± 0.24  | 6.5 ± 0.27  | ns |
| Heart weight : body weight ratio, mg/g        | 4.6 ± 0.19  | 4.7 ± 0.27  | ns |
| Heart weight : tibia length ratio, mg/mm      | 8.1 ± 0.30  | 8.4 ± 0.32  | ns |
Table 3. Assessment of LV function and structure after 8 weeks of pressure-overload in WT and PLM<sup>3SA</sup> mice

|                            | WT                           | 3SA                          |
|---------------------------|------------------------------|------------------------------|
| **Echocardiography measures** | **sham, n=10** vs WT sham sham, n=6 vs 3SA sham | **banded, n=9** vs WT banded banded, n=7 vs WT banded |
| Heart Rate, bpm           | 518 ± 2.79                   | 516 ± 4.65                   | 524 ± 4.79                   | 517 ± 6.84                   |
| Body weight, g            | 29.3 ± 0.71                  | 27.7 ± 0.82                  | 27.8 ± 0.61                  | 29.0 ± 1.72                  |
| Body temperature, °C      | 37.2 ± 0.07                  | 37.4 ± 0.16                  | 37.4 ± 0.17                  | 37.6 ± 0.21                  |
| Intraventricular septum diastolic thickness, mm | 1.03 ± 0.02                  | 1.16 ± 0.05                  | p<0.01                      | 0.98 ± 0.03                  | 1.28 ± 0.03 | p<0.001 | ns |
| LV diastolic dimension, mm | 3.79 ± 0.05                  | 4.38 ± 0.19                  | p<0.01                      | 3.67 ± 0.09                  | 4.96 ± 0.32 | p<0.01 | ns |
| Posterior wall diastolic thickness, mm | 0.97 ± 0.01                  | 1.10 ± 0.04                  | p<0.01                      | 0.93 ± 0.02                  | 1.27 ± 0.04 | p<0.01 | ns |
| Anterior wall diastolic thickness, mm | 1.04 ± 0.01                  | 1.17 ± 0.04                  | p<0.01                      | 0.98 ± 0.03                  | 1.28 ± 0.03 | p<0.01 | ns |
| Intraventricular septum systolic thickness, mm | 1.72 ± 0.03                  | 1.73 ± 0.07                  | ns                          | 1.69 ± 0.06                  | 1.73 ± 0.06 | ns | ns |
| LV systolic dimension, mm | 2.20 ± 0.05                  | 3.12 ± 0.24                  | p<0.001                     | 2.01 ± 0.09                  | 4.07 ± 0.38 | p<0.001 | p<0.05 |
| Posterior wall systolic thickness, mm | 1.53 ± 0.04                  | 1.51 ± 0.06                  | ns                          | 1.53 ± 0.05                  | 1.54 ± 0.05 | ns | ns |
| Anterior wall systolic thickness, mm | 1.75 ± 0.02                  | 1.72 ± 0.05                  | ns                          | 1.71 ± 0.07                  | 1.69 ± 0.05 | ns | ns |
| LV systolic volume, µL    | 15.9 ± 0.93                  | 41.6 ± 8.86                  | p<0.01                      | 12.8 ± 1.65                  | 77.7 ± 17.80 | p<0.01 | ns |
| LV diastolic volume, µL   | 57.1 ± 2.40                  | 85.5 ± 9.80                  | p<0.01                      | 55.2 ± 3.43                  | 118.8 ± 19.11 | p<0.01 | ns |
| LV mass, mg               | 119.3 ± 2.08                 | 176.3 ± 16.60                | p<0.01                      | 104.3 ± 2.29                 | 260.2 ± 26.22 | p<0.001 | p<0.05 |
| LV weight : body weight ratio, mg/g | 4.1 ± 0.06                  | 6.4 ± 0.65                   | p<0.01                      | 3.8 ± 0.12                   | 9.2 ± 0.18 | p<0.001 | p<0.05 |
| Stroke volume, µL         | 41.3 ± 1.74                  | 44.0 ± 2.02                  | ns                          | 42.3 ± 2.07                  | 41.1 ± 4.06 | ns | ns |
| Ejection fraction, %      | 72.3 ± 1.05                  | 54.7 ± 3.91                  | p<0.001                     | 77.1 ± 1.57                  | 37.9 ± 4.81 | p<0.001 | p<0.05 |
| Fraction shortening, %    | 40.8 ± 0.90                  | 28.5 ± 2.44                  | p<0.001                     | 45.0 ± 1.42                  | 18.6 ± 2.57 | p<0.001 | p<0.05 |
| Cardiac output, mL/min    | 21.5 ± 0.90                  | 22.8 ± 1.15                  | ns                          | 22.1 ± 1.02                  | 21.3 ± 2.14 | ns | ns |
| Endocardial fractional area change, % | 38.6 ± 3.06                  | 29.6 ± 3.47                  | ns                          | 47.3 ± 2.19                  | 17.8 ± 4.29 | p<0.001 | p<0.05 |
| Peak velocity, mm/s       | 1793.5 ± 58.10               | 2022.3 ± 118.42              | ns                          | 2184.1 ± 236.13              | 1929.5 ± 219.16 | ns | ns |
| Peak gradient, mmHg       | 12.2 ± 0.74                  | 15.9 ± 1.87                  | ns                          | 18.6 ± 4.36                  | 14.6 ± 3.29 | ns | ns |
| Velocity time integral, cm | 5.9 ± 0.19                  | 5.3 ± 0.17                   | ns                          | 6.7 ± 0.47                   | 4.6 ± 0.61 | p<0.05 | ns |
| **Pressure-volume measurements** | **sham, n=6** vs 3SA sham sham, n=4 vs WT sham | **banded, n=5** vs 3SA banded banded, n=4 vs WT banded |
| Heart Rate, bpm           | 602 ± 20.54                  | 588 ± 14.97                  | ns                          | 578 ± 10.12                  | 569 ± 18.74 | ns | ns |
| Body temperature, °C      | 37.3 ± 0.04                  | 37.4 ± 0.10                  | ns                          | 37.3 ± 0.05                  | 37.3 ± 0.03 | ns | ns |
| End diastolic pressure, mmHg | 5.8 ± 0.81                  | 8.1 ± 2.18                   | ns                          | 7.2 ± 3.59                   | 15.6 ± 3.03 | ns | ns |
| End systolic pressure, mmHg | 98.9 ± 4.24                  | 128.0 ± 6.65                 | p<0.01                      | 105.8 ± 4.64                 | 140.0 ± 7.01 | p<0.01 | ns |
| LV developed pressure, mmHg | 105.9 ± 3.21                 | 128.1 ± 7.75                 | p<0.01                      | 108.3 ± 4.48                 | 134.2 ± 9.96 | p<0.05 | ns |
| End systolic volume, µL   | 18.2 ± 5.73                  | 23.4 ± 5.15                  | ns                          | 19.1 ± 4.44                  | 32.5 ± 5.85 | ns | ns |
| End diastolic volume, µL  | 31.7 ± 3.93                  | 36.9 ± 4.37                  | ns                          | 33.1 ± 2.59                  | 45.8 ± 1.54 | p<0.01 | ns |
| dP/dt max, mmHg/s         | 10187.6 ± 490.23             | 9303.5 ± 389.96              | ns                          | 10063.4 ± 277.60             | 9397.3 ± 651.07 | ns | ns |
| dP/dt min, mmHg/s         | -11493.3 ± 933.99            | -9108.8 ± 532.90             | ns                          | -11201.8 ± 1403.49           | -6711.7 ± 525.13 | p<0.05 | p<0.01 |
| Tau (Weiss), ms           | 5.4 ± 0.44                   | 6.1 ± 0.36                   | ns                          | 5.6 ± 0.70                   | 9.7 ± 0.91 | p<0.05 | p<0.01 |
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