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# Supplemental Material

## Supplemental Methods

### Echocardiography

Echocardiographic measurements were made using a 15-MHz transducer and a Sonos 5500 echocardiograph (Agilent Technologies, Andover, MA). Three to six beats were averaged for each measurement. LV dimension and wall thickness were made at end-diastole and end-systole using the American Society of Echocardiography criteria (21). Mean wall thickness was calculated as the average of the interventricular septal wall thickness (IVS) and left ventricular posterior wall thickness (LVPW). Relative wall thickness (RWTH) was calculated as the mean LV wall thickness divided by the LV internal dimension at end-diastole (LVIDd). LV mass was calculated using the formula: LV Mass = $1.005*[(IVSd+LVPWd+LVIDd)^3–(LVID)^3]$ (21). LV mass was normalized to body weight (LV/BW ratio) and tibial length (LV/TL ratio). LV end diastolic volume (LVEDV) and end systolic volume (LVESV) were determined using the Simpson's method of discs (21). Ejection fraction (EF, %) was calculated as: EF = 100* (LVEDV-LVESV)/LVEDV. The aortic pressure gradient created by the TAC was measured using the modified Bernoulli equation: Pressure gradient = $4(V_{peak})^2$, where $V_{peak}$ is the peak Doppler velocity across the TAC site (21).

**Transverse Aortic Constriction (TAC)**

Mice were initially anesthetized using inhalation of isoflurane (in a chamber containing 5% isoflurane vapors). Once a surgical anesthetic plane was established, the surgical area was treated with NAIR hair removal gel, sterilized with alcohol and then betadine and the mouse was placed on a thermostatically controlled heating pad under a dissecting microscope. A continuous flow of isoflurane (2%) was provided to the mouse through a modified mask assembly. Electrodes for a surface ECG were inserted subcutaneously and the ECG signal (PONEMAH, LDS Gould) monitored. Pedal reflex and heart rate were continuously monitored to assess the depth of anesthesia. Using aseptic techniques, a small (5 mm) skin incision was made in the neck region and the trachea visualized. The mouse was orotracheally intubated and the correct placement of the endotracheal tube confirmed visually. The mouse was connected to a rodent ventilator (MiniVent 845, Hugo-Sachs, 250 breaths per minute, tidal volume 250 μL, with 2% isoflurane). Buprenorphine (0.05 mg/kg sc) was injected for analgesia. A horizontal incision of 5 mm was made at the level of the suprasternal notch. Blunt dissection was done using a surgical microscope to expose the transverse aorta. Transverse arch banding was performed by placing a 7.0 silk suture over a 30-gauge needle, causing complete occlusion of the aorta. The needle was then removed, restoring the aortic lumen but leaving a severely stenotic aortic orifice. A drainage tube was placed in the incision, air was evacuated via the drainage tube, the drainage tube was then removed and the incision was closed, the mouse extubated, and the animal was recovered under veterinary care on a warming blanket in a container supplemented with oxygen.

**Papillary Muscle Preparation and Myocardial Function Measurements**

Previously, our laboratory performed isolated papillary muscle studies in normal and pressure-overload cats and rats (22-25). The methods used in these studies were modified in order to study murine papillary muscles (26). Murine papillary muscles were isolated from the left ventricle and placed in an isolated muscle chamber with continuously circulating oxygenated Krebs-Henseleit buffer at 29-30$^{o}$C. The muscle was attached to a force/length servo-system (model 300B, Aurora Scientific Inc., ASI, Aurora, Canada) and controlled by custom-made software (Dynamic Muscle Control, ASI Aurora, Canada). Using parallel platinum electrodes the muscle was stimulated at a rate of 0.5 Hz with a square wave pulse of 5 ms duration, and voltage 10% above the threshold. Each muscle was allowed to contract at a light isotonic preload of 0.1 g for 30 minutes until it reached an equilibrium (baseline) state. During this preconditioning period, at 10 min intervals, each muscle was subjected to sets of three isometric contractions and isotonic contractions. After the preconditioning period, isotonic and isometric contractions were performed to derive measurements of the percent and rate of shortening, and the extent and rate of force development. Passive diastolic stiffness was examined two ways: 1) define rest stress at Lmax and 2) perform a muscle stretch at a very slow stretch rate (1 mm/min) beginning from near slack length (very lightly preloaded muscle at 0.1 g) to a muscle length of 15% greater then that at slack length (equivalent to Lmax preload). The myocardial stress vs. strain relationship during this muscle stretch were used to calculate the passive stiffness constant, β, as $Stress=Ae^{(\beta Strain)}+C$. Myocardial stress was calculated from muscle force divided by muscle cross-sectional area and strain was calculated as $L-L_0 / L_0$ where L = muscle length during stretch and $L_0$ = muscle length at 0.1 g preload.

**Cardiomyocyte Isolation and Function Measurements**

Cardiomyocyte isolation was performed using collagenase digestion methods (33). Mice were anesthetized with ketamine (50 mg/kg IP) and xylazine (2.5 mg/kg IP), heparinized (200 IU IP), intubated, and placed on a respirator. The hearts were rapidly excised, cannulated via the aorta, and perfused using a Langendorff preparation at 80 cm$H_2O$ pressure and 37°C. Hearts were initially perfused for 5 min with 1.8 mM $Ca^{2+}$ Tyrode solution containing (in mM) 137 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 10 HEPES, and 10 glucose at pH 7.4 and then in $Ca^{2+}$-free buffer containing 0.05% collagenase D (Boehringer-Mannheim, Indianapolis, IN) and 0.01% protease XIV (Sigma, St. Louis, MO) for 6-8 minutes. After the hearts were palpably flaccid, the collagenase digestion solution was washed out with $Ca^{2+}$-free Tyrode solution for 30 seconds. The hearts were removed from the cannula, and the LV including septum was separated, minced, and gently agitated, allowing cardiomyocytes to be dispersed in the $Ca^{2+}$-free Tyrode solution. After 15 minutes, the cardiomyocytes were resuspended in Tyrode buffer with gradually increasing $Ca^{2+}$ concentration in steps from 0.06 to 0.24 to 0.60 to 1.20 mM $Ca^{2+}$ at room temperature. They were maintained thereafter and studied at 32°C in 1.2 mM $Ca^{2+}$ oxygenated Krebs-Henseleit buffer at pH 7.4. Cardiomyocyte isolation was performed in 6 WT and 6 SPARC Null control mice. Using methods described below, 20 cardiomyocytes from each mouse underwent study to examine cardiomyocytes systolic and diastolic properties using the IonOptix system and 10 cardiomyocytes from each mouse underwent study to examine cardiomyocytes passive stiffness using our custom stretch system.

**Cardiomyocyte Systolic and Diastolic Relaxation Properties**

Cardiomyocyte systolic and diastolic relaxation properties were measured in isolated cardiomyocytes using the IonOptix system (IonOptix, Milton, MA) (34, 35). Briefly, cardiomyocytes were viewed with an inverted microscope; the cell image, collected by an X40 objective lens, was diverted to the side port of the microscope and transmitted to a MyoCam video CCD camera. Cardiomyocyte length and contraction amplitude were recorded in real time with a video edge detector and specialized data acquisition software (SoftEdge and IonWizard; IonOptix). The CCD camera was adapted to acquire images at a 240-Hz frame rate and a cell length measurement time resolution of 4.2 ms. The signal-to-noise ratios were significantly improved by averaging 10 sequential runs. From these studies the following parameters were measured: shortening extent, percent and rate, and lengthening rate.

**Cardiomyocyte Passive Stiffness Properties**

Cardiomyocyte passive stiffness properties of isolated cardiomyocytes were determined using a passive stretch method (36, 37). Briefly, cardiomyocytes were embedded in a 2% agarose gel containing HEPES-Krebs buffer and laminin. This gel was then cast in a shape that allowed it to be placed in a custom made stretching device. The ends of the gel were held between a movable roller and fixed plates that acted as mandibles. The distance between the right and left mandibles was increased using a stepper motor system. The force applied to the gel was measured by a force transducer. The resultant cardiomyocyte strain was determined by imaging the cells with an inverted microscope, capturing the images with a CCD camera, and measuring cardiomyocyte length changes. Cardiomyocyte stress was derived from the gel stress by means of finite-element analysis. Cardiomyocyte stress vs. strain were used to determine cardiomyocyte stiffness, $\beta$ [$Stress=Aexp(\beta*Strain)+C$].

**Specificity of PSR Birefringent Staining**

PSR birefringent staining was used to identify and quantify mature fully processed cross-linked insoluble fibrillar collagen within the myocardium. Previous studies have suggested that PSR birefringent staining is specific for this fibrillar collagen within the myocardium (28, 29). The birefringence of the collagen fibers stained with PSR occurs because of lateral association of collagen molecules stabilized by covalent cross-links. It is believed that unprocessed or incompletely processed procollagen does not exhibit PSR birefringent staining. To prove this, LV sections from WT control mice were stained with antibodies generated against the C-terminal propeptide of collagen I. The majority of procollagen within a myocardial sample is located within the fibroblast. Once procollagen is secreted by the fibroblast into the extracellular space of the interstitium, it is very rapidly processed or degraded. LV sections stained with the antibody against the C-terminal propeptide were compared to those without the primary antibody. Control sections without the primary antibody exhibited no staining of fibroblast cell bodies. By contrast, sections stained with antibodies against the C-terminal propeptide exhibited immunoreactivity localized to the fibroblast cell bodies and not to the extracellular interstitial space. When the same areas of the LV myocardium were stained with PSR, no PSR staining was associated with fibroblast cell bodies, all the staining occurred within the extracellular interstitial space. Therefore, these studies showed that there were two distinct and non-overlapping staining patterns: the C-terminal propeptide antibody staining pattern for procollagen was restricted to the fibroblast; and the PSR staining of mature fully processed cross-linked fibrillar collagen was restricted to the extracellular space. Thus, these data suggest that procollagen molecules are not stained by PSR.

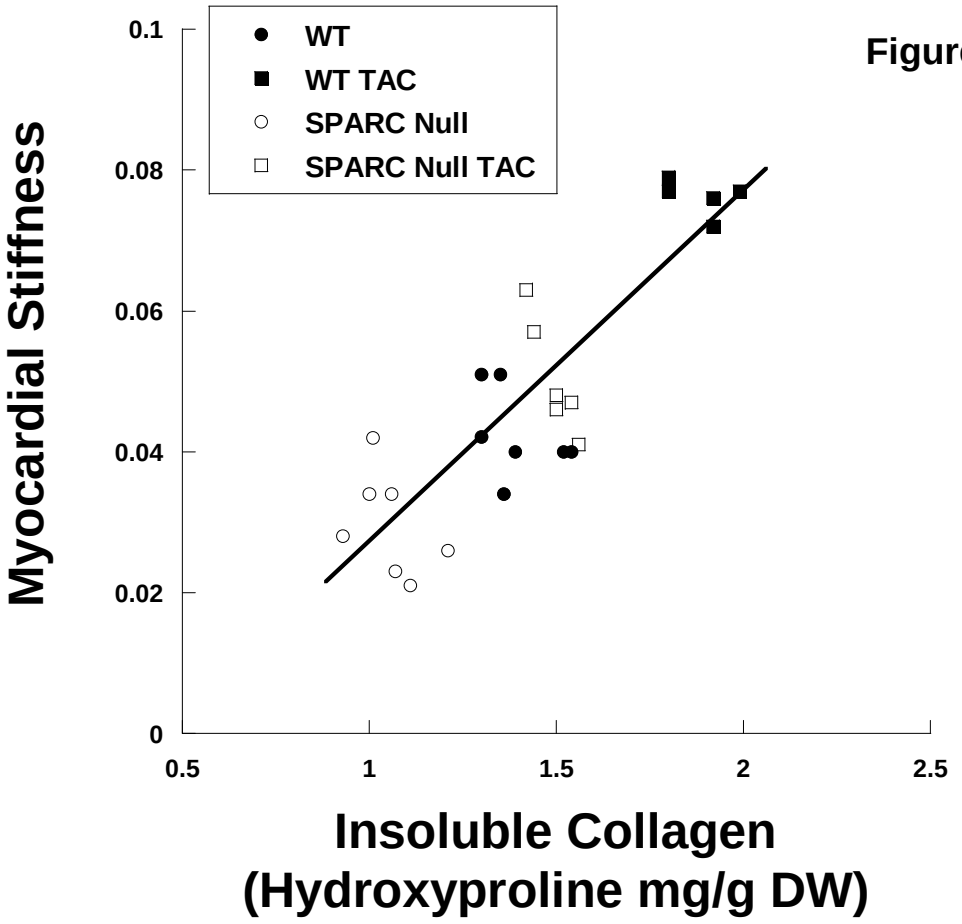


**Figure 1S:** Relationship between myocardial stiffness and insoluble collagen content. SPARC = Secreted Protein Acidic and Rich in Cysteine, TAC = transverse aortic constriction, WT = wild-type littermate mice C57Bl6/SV129, SPARC Null = mouse created using targeted gene deletion to abrogate expression of SPARC.