SUPPLEMENTAL MATERIAL

Supplemental Methods

Experimental mice groups. Mice were maintained on standard rodent chow and 1.0% sodium chloride drinking water for 4 weeks[1, 2]. The 4 groups studied were: (a) WT mice infused with saline (WT-saline, n = 7); (b) APNTG mice infused with saline (APNTG-saline, n = 7); (c) WT mice infused with *d*-aldosterone (WT-aldosterone, n = 15); (d) APNTG mice infused with *d*-aldosterone (APNTG-aldosterone, n = 15).

Another group of WT mice underwent the same surgical procedures as outlined above; however 14 days after surgery, mice were treated with adenoviral vectors expressing either APN (Ad-APN) or β-galactosidase (Ad-βgal) as a control after aldosterone or saline infusion. $2X10^8$ plaque-forming units (pfu) of Ad-APN or Ad-βgal were injected into the jugular vein of WT mice. (a) WT-saline plus Ad-βgal, n=3; (b) WT-aldosterone plus Ad-βgal, n=4; (c) WT-saline plus Ad-APN, n=3; (d) WT-aldosterone plus Ad-APN, n=4.

Physiological Measurements. Heart rate and blood pressure were measured weekly using a noninvasive tail-cuff blood pressure analyzer, BP-2000 Blood Pressure Analysis System (Visitech Systems, Inc., Apex, NC)[1, 2].

Echocardiography.

Diastolic function measurements: To assess diastolic function, mice were anesthetized with isoflurane (0.5% for induction followed by 0.5 to 1.5% for maintenance) and maintained at a heart rate (HR) of ~350 beats per minute (bpm) since diastolic function is sensitive to HR and loading conditions. The maximum dose of isoflurane 1.5% has minimal effects on diastolic function[2, 3]. Pulse wave and tissue Doppler measurements were recorded.

LV structure and function: Interventricular septum wall thickness (IVST), LV posterior wall thickness (LVPWT), LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), and LV ejection fraction (LVEF) were obtained. Total wall thickness (TWT) was derived from an average of the IVST and LVPWT. LV mass was calculated using the formula LV mass= $1.05[(\text{LV EDD} + \text{IVST} + \text{PWT})^3 - (\text{LV EDD})^3]$ as described by Kiatchoosakun S et al [4].

Biomarker, organ weight and tissue analysis. After 4 weeks mice were sacrificed, and blood was obtained to determine serum adiponectin (B-Bridge International, Inc., Cupertino, CA) and aldosterone levels (Alpha Diagnostic Intl. Inc., San Antonio, TX). Body weights and heart weights were determined. Hearts were either arrested in diastole by KCl (30mmol/l), weighed, perfused with 10% buffered formalin and sliced horizontally for histology, or snap-frozen in liquid nitrogen. To measure fibrosis, Masson trichrome-stained sections (5μm) were visualized by using Olympus BX41 Clinical Microscope (Olympus America Inc., Center Valley, PA). The ratio of the fibrotic area to the entire heart area was calculated using ImageJ (National Institutes of Health, Bethesda, MD).

LV cardiomyocyte cross-sectional (C/S) area was assessed. For each section, 100 cardiomyocytes, showing a central nucleus, were randomly selected and C/S areas were measured (Area=πr^2) using ImageJ (National Institutes of Health, Bethesda, MD). The wet-to-dry lung ratio, as an indicator of pulmonary congestion and HF was determined[2, 5].

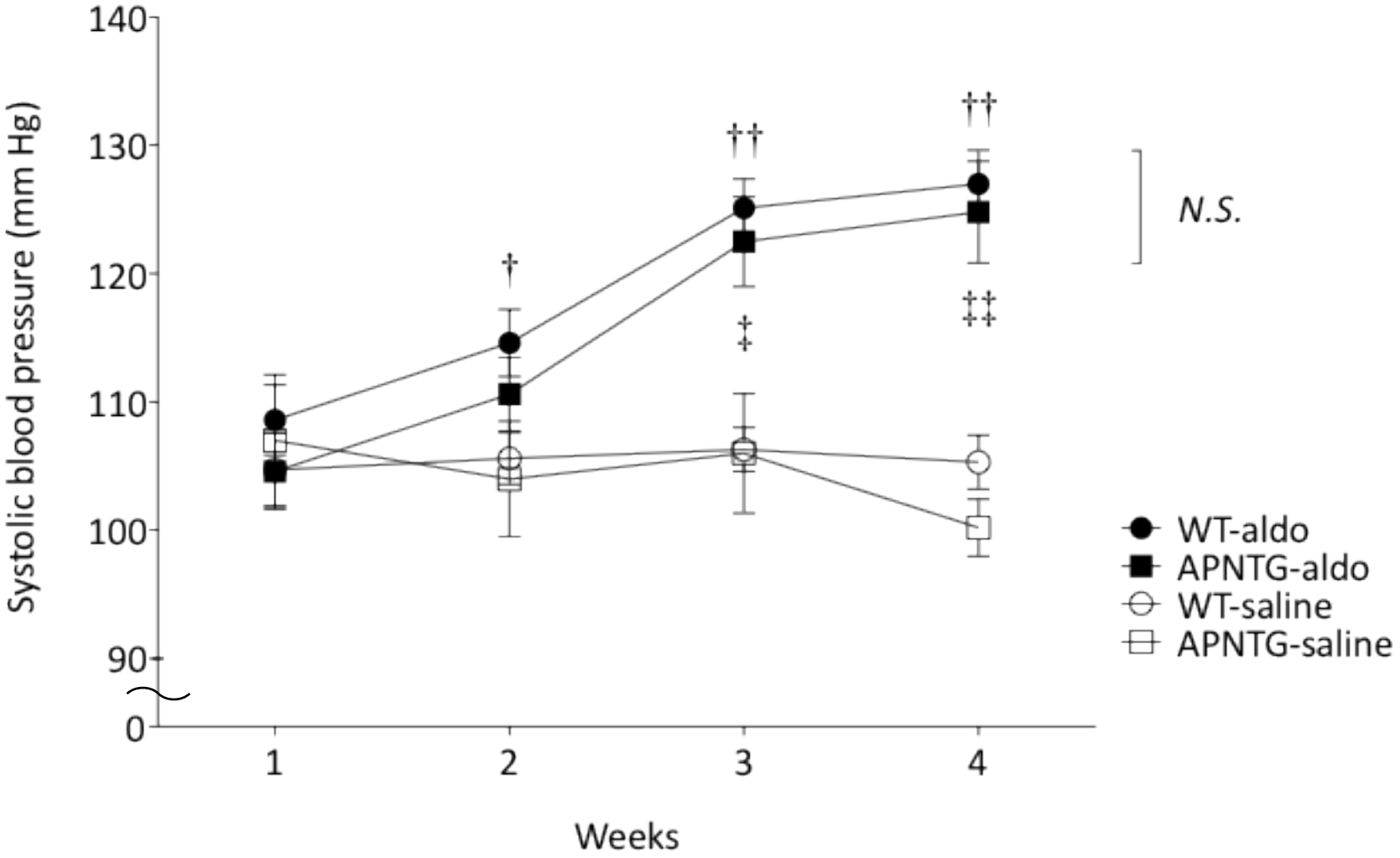
Assessment of myocardial oxidative stress. Myocardial specimens were stained with 3-nitrotyrosine staining as described previously[6]. Briefly, sections were treated with 10 mmol/L citric acid (pH 6.0) and heated with a microwave (2 minutes, 3 times at 700W) to recover antigenicity. Nonspecific binding was blocked with 10% normal goat serum in phosphate-buffered saline (PBS) (pH 7.4) for 30 minutes before incubation with polyclonal anti–3-nitrotyrosine antibody (1 μg/mL) (Millipore, Billerica, MA) in PBS with 1% bovine serum albumin overnight at 4°C. Tissue sections were then incubated for 30 minutes at room temperature with a biotinylated anti-rabbit IgG (1:800) secondary antibody by using the Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA). Vector Red alkaline phosphatase substrate (Vector Laboratories, Inc., Burlingame, CA) was used to visualize 3-nitrotyrosine. Semiquantitative analysis of tissue immunoreactivity for nitrotyrosine was done by estimating the degree of staining with the use of an arbitrary grading system from 1 to 4 as described previously[7].

Western Blot Analysis. Protein kinase A (PKA) C-α, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), phospho-CaMKII at Thr286, sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2a), phospholamban (PLN), phospho-PLN at Ser16, and phospho-PLN at Thr17 protein expression in the heart were determined by western blot analysis. Aliquots of cardiac tissue lysates (5-30 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidine fluoride (PVDF) membranes (GE Healthcare UK Ltd., Little Chalfont, Buckinghamshire, England). The membranes were immunoblotted with the following primary antibodies: anti-PKA C-α (Cell Signaling Technology, Inc., Danvers, MA), anti-CaMKII (Cell Signaling Technology, Inc., Danvers, MA), anti-phospho-CaMKII at Thr286 (Cell Signaling Technology, Inc., Danvers, MA), anti-SERCA2a (Thermo Fisher Scientific Inc., Waltham, MA), anti-PLN (Thermo Fisher Scientific Inc., Waltham, MA), anti-phospho-PLN at Ser16 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-phospho-PLN at Thr17 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and anit-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Abcam plc., Cambridge, MA), followed by the horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Immunoblots were detected by ECL or ECL plus Western Blotting Detection Reagents (GE Healthcare UK Ltd., Little Chalfont, Buckinghamshire, England). The chemiluminescence intensities were quantified by ImageJ (National Institutes of Health, Bethesda, MD) and normalized to those of GAPDH or Coomassie Brilliant Blue (Sigma-Aldrich Co., St. Louis, MO) staining of the PVDF membranes.

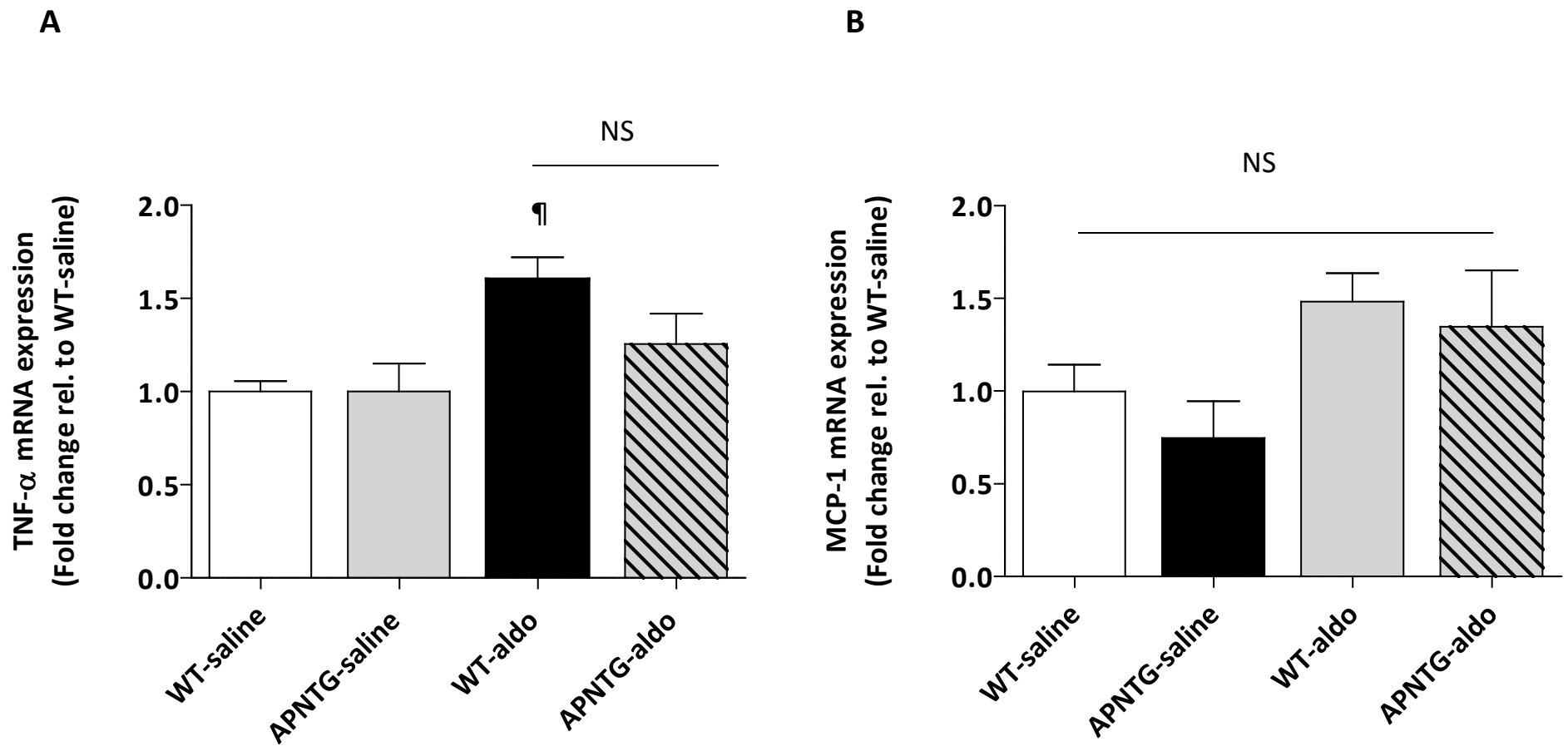
Quantitative real-time polymerase chain reaction (qRT-PCR). Atrial natriuretic peptide (ANP) mRNA expression was determined by qRT-PCR. Total RNA was extracted by using RNeasy Fibrous Tissue Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's protocol. Complementary DNA (cDNA) from 1000 ng of

total RNA was synthesized by using a ThermoScript™ Reverse Transcriptase (RT)-PCR System (Life Technologies Corporation, Carlsbad, CA) according to the manufacturer's protocol. qRT-PCR was performed on the StepOne™ Real-Time PCR System (Life Technologies Corporation, Carlsbad, CA) using SYBR Green PCR Master Mix (Life Technologies Corporation, Carlsbad, CA). The primer sequences were as follows: 5'-ATCTGCCCTCTTGAAAAGCA-3' and 5'-AAGCTGTTGCAGCCTAGTCC-3' for mouse **ANP**; 5'-CCAAGGTCATCCATGACAACT-3' and 5'-GGGCCATCCACAGTCTTCT -3' for mouse **GAPDH**. The expression levels of examined transcripts were compared to those of GAPDH and normalized to the mean value of controls.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure Legends

Supplemental Figure 1. Tail cuff systolic blood pressure in WT and APNTG mice. There was a significant and progressive rise in tail-cuff blood pressure in APNTG-aldosterone. †P<0.01 vs. WT-saline, ††P<0.01 vs. WT-saline, ‡P<0.05 vs. APNTG-saline, ‡‡P<0.01 vs. APNTG-saline

Supplemental Figure 2. (**A**) Aldosterone infusion significantly TNF-α mRNA expression in the hearts of WT-aldosterone vs. WT-saline mice (¶ P<0.05) but not in APNTG-aldosterone (P=NS vs. APNTG-saline mice and WT-aldosterone mice). (**B**) There was no significant difference in myocardial MCP-1 mRNA expression between saline and aldosterone-infused WT and APNTG mice.

Supplemental References

(1) Wilson RM, De Silva DS, Sato K, Izumiya Y, Sam F. Effects of fixed-dose isosorbide dinitrate/hydralazine on diastolic function and exercise capacity in hypertension-induced diastolic heart failure. *Hypertension* 2009;54:583-90.

(2) Sam F, Duhaney TA, Sato K, Wilson RM, Ohashi K, Sono-Romanelli S, Higuchi A, De Silva DS, Qin F, Walsh K, Ouchi N. Adiponectin deficiency, diastolic dysfunction, and diastolic heart failure. *Endocrinology* 2010;151:322-31.

(3) Zhou YQ, Foster FS, Parkes R, Adamson SL. Developmental changes in left and right ventricular diastolic filling patterns in mice. *Am J Physiol Heart Circ Physiol* 2003;285:H1563-H1575.

(4) Kiatchoosakun S, Restivo J, Kirkpatrick D, Hoit BD. Assessment of left ventricular mass in mice: comparison between two-dimensional and m-mode echocardiography. *Echocardiography* 2002;19:199-205.

(5) Sam F, Sawyer DB, Xie Z, Chang DL, Ngoy S, Brenner DA, Siwik DA, Singh K, Apstein CS, Colucci WS. Mice lacking inducible nitric oxide synthase have improved left ventricular contractile function and reduced apoptotic cell death late after myocardial infarction. *Circ Res* 2001;89:351-6.

(6) Kuster GM, Kotlyar E, Rude MK, Siwik DA, Liao R, Colucci WS, Sam F. Mineralocorticoid receptor inhibition ameliorates the transition to myocardial failure and decreases oxidative stress and inflammation in mice with chronic pressure overload. *Circulation* 2005;111:420-7.

(7) Matsui R, Xu S, Maitland KA, Hayes A, Leopold JA, Handy DE, Loscalzo J, Cohen RA. Glucose-6 Phosphate Dehydrogenase Deficiency Decreases the Vascular Response to Angiotensin II. *Circulation* 2005;112:257-63.