SUPPLEMENTAL MATERIAL

Cell culture. HEK 293T cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, 50 U/ml penicillin, and 50 μg/ml streptomycin. A murine atrial cardiomyocyte cell line, HL-1[1], was kindly provided by Dr. William C. Claycomb (Louisiana State University Medical Center, New Orleans) and cultured in Claycomb's medium with 10% FBS, 100 μM norepinephrine, and 4 mM L-glutamine on 0.02% gelatin/fibronectin-coated flasks or plates.

Transfection, Immunoblotting and Immuno-staining. Fugene6 (Roche) was used for transfection. For immunoblotting, immuno-reactive rat BNPs were detected using rabbit anti-rat BNP1-45 antibody (AssayPro) and HRP-conjugated anti-rabbit IgG antibody. Immunostaining of immuno-reactive rat BNP was performed using the same anti-rat BNP1-45 antibody and FITC-conjugated anti-rabbit IgG antibody.

IVIS imaging. The cardiac luciferase expression was monitored by Xenogen IVIS biophotonic imaging machine. Upon luciferin administration through IP, anesthetized rats were euthanized and the organs were harvested immediately. Harvested tissues were placed on the 10cm plates on the imaging chamber anda background photo of the tissues and a color overlay of the emitted photon data were obtained.

Toxicological and pharmacological tests. For toxicological and pharmacological tests, hematological parameters (VetScan HM2 Hematology

System; 50 µl blood in EDTA for WBC counts, WBC histogram, Hb, Hct, MCV, MCH, MCHC, RDW, graphic RBC histogram, PLT count, MPV, PCT, PDW and Graphic platelet histogram) and chemistry (VetScan Classic; 100µl blood in lithium heparin; ALB, ALP, ALT, AMY, BUN, CA++, CRE, GLOB, GLU, K+, Na+, PHOS, TBIL, TP) were measured.

Echocardiography (ECHO) for non-invasive assessment of ventricular function and structure. To evaluate cardiac function and structure we performed both standard ECHO and Two-Dimensional Speckle-Derived Strain ECHO (2DSE) examinations at four and nine months post injections in the BNP-treated and the untreated SHR. We also performed standard ECHO and 2DSE in normal Wistar rats at 4 weeks after AAV9 injections. All ECHO examinations were performed by skilled sonographer (E.A.O.) blinded to the treatment. Detailed protocols for ECHO examinations were described in Supplemental Materials. Standard ECHO: After removing chest hair, ultrasonic scans was performed in all rats in supine position using a Vivid 7 system (GE Healthcare, Milwaukee, WI) equipped with a 10S ultrasound probe (11.5 MHz) with ECG monitoring. M-mode images and gray scale 2D images (300-350 frames /sec) of the parasternal long-axis and mid-LV was recorded for off-line analysis. LV end-diastolic (LVDd) and end-systolic (LVDs) dimensions, septal diastolic (SWTd) and posterior wall diastolic (PWTd) and systolic (PWTs) thicknesses were measured from M-mode images. LV mass was calculated according to uncorrected cube assumptions as LV mass = 1.055 x $[(LVDd+SWTd+PWTd)^3-(LVDd)^3]$, where 1.055 is the specific gravity of myocardium. LV mass was

corrected for body weight (LVMi) for analysis. End-systolic (ESV), end-diastolic and stroke volumes (SV), and ejection fraction (EF) was calculated using the Teichholz formula: LV volume = 7 x $[(LVDd)^3/(2.4+LVDd)]$. Relative wall thickness (RWT) was calculated as RWT = (SWTd + PWTd)/LVDd. All parameters represented the average of three beats. 2DSE: Using EchoPAC software (EchoPAC PC – 2D strain, BTO 6.0.0, GE Healthcare, Milwaukee, WI), which included a high resolution speckle tracking analysis library for off-line analysis, endocardial border was carefully manually traced at end-systole in LV short-axis views at the middle level (i.e. at the level of papillary muscles). Ideal width of circular region of interest was chosen in order to include the entire myocardial wall. Speckle tracking was performed by the software and global strain and circumferential strain rate parameters were measured computing the mean of the six middle LV segments. The analysis included peak circumferential systolic strains (sS) and strain rates (sSR) for evaluation of myocardial systolic function and peak early circumferential strain rates (dSR-E) for evaluation of myocardial diastolic function. All parameters represented the average of three beats. Relevant to this proposal, using standard ECHO and 2DSE, we were able to detect significant improvement in both systolic and diastolic function in a rat model of cardiac dysfunction when compared to the untreated SHR.

1. Claycomb WC, Lanson NA, Jr., Stallworth BS, Egeland DB, Delcarpio JB, Bahinski A, Izzo NJ, Jr. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. *Proceedings of the National Academy of Sciences of the United States of America.* 1998;95(6):2979-2984.