BNP DELETION LEADS TO PROGRESSIVE HYPERTENSION, ASSOCIATED ORGAN DAMAGE, AND REDUCED SURVIVAL: A NOVEL MODEL FOR HUMAN HYPERTENSION

Sara J. Holditch[1], Claire A. Schreiber[1], Ryan Nini[1], Jason M. Tonne[1], Kah-Whye Peng[1], Aron Geurts[2], Howard J. Jacob[2], John C. Burnett[3], Alessandro Cataliotti[3,4], Yasuhiro Ikeda[1]

[1]Department of Molecular Medicine, Mayo Clinic, College of Medicine, Rochester, MN, USA, [2]Department of Physiology, Cardiovascular Center, Medical College of Wisconsin, Milwaukee, WI, USA, [3]Cardiorenal Research Laboratory, Division of Cardiovascular Diseases, Departments of Medicine and Physiology, Mayo Clinic, College of Medicine, Rochester, MN, USA, [4]Institute for Experimental Medical Research, Oslo University Hospital, University of Oslo, Oslo, Norway

Corresponding author:

Yasuhiro Ikeda, PhD

Department of Molecular Medicine

Mayo Clinic, 200 First St. SW,

Rochester MN 55905, USA

Fax: 507-266-2111,

Phone: 507-538-0153

E-mail: Ikeda.yasuhiro@mayo.edu

SUPPLEMENTAL INFORMATION

METHODS

Animals.

Nppb -/- rats were generated on Dahl Salt Sensitive (Dss) backgrounds as described previously [1]. Briefly, strains were generated by microinjection of CompoZr ZFNs (Sigma) into one-cell SS/JrHsdMcwi (S) rat embryos and then implanted into pseudopregnant Sprague Dawley females[2]. A total of 22 Nppb -/- and 12 of Nppb +/+ male rats were used for this study. A separate group of Nppb -/- (n=4) and Nppb +/+ (n=4) male rats underwent three weeks of high salt diet beginning at 10 weeks of age. For reversal studies, neonates were generated from breed pairs, and littermates were randomly assigned to two groups: (group 1) received an intraparenteral injection with proBNP-expressing adeno-associated virus serotype 9 (AAV9) vector (1×10^{11} genome copies/rat), while littermates were injected with either PBS, or a luciferase expressing AAV9 vector (group 2). The effects of sustained proBNP expression were analyzed three, six, and nine months post injection.

Tissue harvesting, RNA extraction.

For tissue studies (qPCR, Histology, Immunohistochemistry), rats were anesthetized with isoflurane, jugular vein bled, and then euthanized with carbon dioxide. Organs were removed, and either frozen in 2mg pieces in 1.5mL SC Micro Tube, Sarstedt (Numbrecht, Germany) and stored at -80°C for later mRNA extraction, or frozen in Tissue-Tec O.T.C Compound (Miles, Elkhart, IN) freezing media, and stored at -80°C until use. For histology, organs were brought to -20°C overnight, and cross-sectioned in a cryostat at -20°C, in 7-12 µm thick sections, then subjected to either Masson's trichrome staining, or hematoxylin and eosin staining (American Master Tech, Lodi, CA) as per manufacturer's instructions. For RNA extraction, 1mg of frozen tissue was homogenized in TRIzol (500ul; GIBCO BRL, Gaithersburg, MD) and then subsequent phenol-chloroform extraction according to manufacturer's instructions. 2 µg of total RNA was then reverse transcribed into cDNA by RNA to cDNA EchoDry Premix (Clontech Laboratories, Mountain View, CA) and the resulting cDNA was mixed with gene-specific primers and assayed by quantitative-pcr (qPCR). 1 µl of cDNA were added to 2.5 µl of a 10x Primer assay (Supplemental Table 1) and 12.5 µl of 2x Master Mix FastSYBR® Green (Applied Biosystems, CITY and STATES), and 9 µl of sterile water was added to reach a final volume of 25 µl. The amplification reactions were performed in a 7300 Real Time PCR System (Applied Biosystems) following the conditions of standard protocol for FastSYBR Green® (same for all genes): After denaturation of the cDNA and enzyme activation at 95°C for 5 minutes, 40 cycles were performed in 2 steps (95°C for 10 seconds and 60°C for 30 seconds). The expression of the ribosomal RNA (18s RNR) was employed as a housekeeping gene, and expression levels are presented as relative transcripts to 18s RNR expression.

Hematology and urinalysis.

Plasma BNP concentrations were determined using commercially available EIA (Assay pro, St. Charles, MO). Rats were placed in metabolic cages that provided free access to tap water and food pellets. Overnight urine volume and urinary sodium, potassium,

albumin, creatinine and protein excretion were measured. For toxicological and pharmacological tests, hematological parameters (VetScan HM2 Hematology System; 100 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; 100ul blood in lithium heparin; ALB, ALP, ALT, AMY, BUN, CA++, CRE, GLOB, GLU, K+, Na+, PHOS, TBIL, TP) were measured (Figure S5).

Non-invasive BP measurement.
Blood pressure is measured by tail-cuff using the CODA high throughput non-invasive BP system (Kent Scientific), detailed previously[3, 4]. Briefly, the animal is placed in the holder at least 10 minutes prior to obtaining pressure measurements. Acclimatization is accomplished through training sessions. A minimum of 10 readings were taken, and the mean of acceptable readings (as determined by the software instrument) were recorded per animal, at each observation time.

Telemetry Surgical Procedure:
Surgical procedures were carried out as detailed by StellarTelemetry. Briefly, rats were anesthetized first in an induction chamber, and then maintained at 1.5-2% Isoflurane via a nose cone. Rats are then shaved, and incised through the midline linea alba. The intestines cephalad and lateral were displaced exposing the posterior abdominal wall and the aorta. Blunt dissection revealed the aorta, and a 28 gauge needle was used to accomplish the arteriotomy. The pressure sensor was implanted and secured using 4-0 silk suture and the arteriotomy was closed by tissue adhesive. The muscle layer of the abdomen was then closed with the implant inside using 4-0 prolene suture, and electrocardiographic leads were then sutured to muscle using 4-0 silk. Skin incisions were closed using skin staples, and removed 7-10 days post-surgery. Although a total of 12 rats were instrumented with telemetry in each group, due to a manufacture defect, we were able to assess recorded parameters in 2 Nppb -/- and in 2 Nppb +/+ rats at 3 months, in 3 Nppb -/- and in 5 Nppb +/+ rats at 6 months, and 3 Nppb -/- and 3 Nppb +/+ rats at 9 months .

Echocardiography (ECHO) for non-invasive assessment of cardiac function and structure.
Standard ECHO examinations were performed at 3, 6 and 9 months in Nppb-/- and control rats. All ECHO examinations, ECHO analysis, and speckle tracking were performed by a skilled sonographer (S.H) blinded to treatment, as previously described[4, 5]. Briefly; a Vivid 7 ultrasound machine (General Electric, Waukesha, WI) with a GE Vivid 7 *10S transducer were used to collect ECHO data*. Images were taken, at a depth of 2-2.5cm, of the parasternal short-axis left ventricular view, using the papillary muscles as anatomical landmarks. Images were then exported to a separate workstation for analysis using EchoPAC with Q-analysis software (General Electric, Waukesha WI). Radial and circumferential strain and strain rates were analyzed as previously published [6, 7]. Briefly, the left ventricle (LV) was divided into 6 segments, the endocardial border was traced, and the outer border was adjusted to fit the epicardial contour. Q-analysis software then automatically selected speckles within the

myocardium, tracked, and computed strain and strain rate in radial and circumferential directions over the segments of the left ventricle through a cardiac cycle. The average of individual segment strain and strain rates were calculated for peak systolic, early-, and late-diastole. LV studies were measured using anatomic M-mode of the 2D B-mode images.

REFERENCES

1. Geurts AM, Cost GJ, Remy S, Cui X, Tesson L, Usal C, Menoret S, Jacob HJ, Anegon I, Buelow R. Generation of gene-specific mutated rats using zinc-finger nucleases. *Methods in molecular biology*. 2010;597:211-225.
2. Flister MJ, Tsaih SW, O'Meara CC, Endres B, Hoffman MJ, Geurts AM, Dwinell MR, Lazar J, Jacob HJ, Moreno C. Identifying multiple causative genes at a single gwas locus. *Genome research*. 2013;23:1996-2002.
3. Cataliotti A, Chen HH, Redfield MM, Burnett JC, Jr. Natriuretic peptides as regulators of myocardial structure and function: Pathophysiologic and therapeutic implications. *Heart failure clinics*. 2006;2:269-276.
4. Tonne JM, Holditch SJ, Oehler EA, Schreiber CA, Ikeda Y, Cataliotti A. Cardiac bnp gene delivery prolongs survival in aged spontaneously hypertensive rats with overt hypertensive heart disease. *Aging*. 2014;6:311-319.
5. Cataliotti A, Tonne JM, Bellavia D, Martin FL, Oehler EA, Harders GE, Campbell JM, Peng KW, Russell SJ, Malatino LS, Burnett JC, Jr., Ikeda Y. Long-term cardiac pro-b-type natriuretic peptide gene delivery prevents the development of hypertensive heart disease in spontaneously hypertensive rats. *Circulation*. 2011;123:1297-1305.
6. Teske AJ, De Boeck BW, Melman PG, Sieswerda GT, Doevendans PA, Cramer MJ. Echocardiographic quantification of myocardial function using tissue deformation imaging, a guide to image acquisition and analysis using tissue doppler and speckle tracking. *Cardiovasc Ultrasound*. 2007;5:27.
7. Migrino RQ, Zhu X, Pajewski N, Brahmbhatt T, Hoffmann R, Zhao M. Assessment of segmental myocardial viability using regional 2-dimensional strain echocardiography. *J Am Soc Echocardiogr*. 2007;20:342-351.

Gene Name	Gene Abbreviation	Species	Cat No.	Primer Assay
Natriuretic peptide receptor 1	Npr1	Rat	QT00178339	Rn_Npr1_1_SG QuantiTect Primer Assay (200)
Natriuretic peptide receptor 2	Npr2	Rat	QT00187432	Rn_Npr2_1_SG QuantiTect Primer Assay (200)
Natriuretic peptide receptor 3	Npr3	Rat	QT00181251	Rn_Npr3_1_SG QuantiTect Primer Assay
Atrial Natriuretic Peptide	Nppa	Rat	QT00366170	Rn_RGD:3193_1_SG QuantiTect Primer Assay
B-Type Natriuretic Peptide	Nppb	Rat	QT00183225	Rn_Nppb_1_SG QuantiTect Primer Assay
C-Type Natriuretic Peptide	Nppc	Rat	QT00182455	Rn_RGD:620850_1_SG QuantiTect Primer Assay
Vascular endothelial growth factor A	Vegfa	Rat	QT00198954	Rn_RGD:619991_1_SG QuantiTect Primer Assay
Collagen type 1 alpha 1	Col1a1	Rat	QT01081059	Rn_Col1a1_1_SG QuantiTect Primer Assay (200)
Transforming growth factor beta	Tgfb	Rat	QT00187796	Rn_Tgfb1_1_SG QuantiTect Primer Assay (200)
Fibronectin 1	Fn1	Rat	QT00179333	Rn_Fn1_1_SG QuantiTect Primer Assay (200)
Tissue inhibitor metalloprotease 1	Timp1	Rat	QT00185304	Rn_Timp1_1_SG QuantiTect Primer Assay (200)
Actc1	Actc1	Rat	QT01081185	Rn_Actc1_1_SG QuantiTect Primer Assay (200)
tropomyosin 1 (alpha)	Tpm1	Rat	QT00194264	Rn_Tpm1_1_SG QuantiTect Primer Assay (200)
Ttr1	Ttr1	Rat	QT01081304	Rn_Ttr_1_SG QuantiTect Primer Assay (200)
Myosin heavy chain 7	Myh7	Rat	QT00189504	Rn_Myh7_1_SG QuantiTect Primer Assay (200)
Apolipoprotein 1	Apoe1	Rat	QT00408373	Rn_Apoe_1_SG QuantiTect Primer Assay (200)
Nphs1	Nphs1	Rat	QT00189805	Rn_Nphs1_1_SG QuantiTect Primer Assay (200)
Glucose 6 phosphate	G6pc	Rat	QT00185948	Rn_G6pc_1_SG QuantiTect Primer Assay (200)

Table S1. Quantitative Real Time PCR Primer List

	Category	p values	Molecules
1	Cardiac Hypertrophy	9.2E-11-4.47E-01	CRYAB,CTGF,BMP4,F2R,RAB2A,TNNC1,HSPB2,NOS3,ATP2A2,EEF1D,SOD2,FHL2,GAA,CAV1,PNKD,PLCB1,SERPINE1,CASQ2,PLN,GUCY1A3,CACNA1C,MYH7,STAT3,IER3,GLRX3,DES,SLC2A4,AKAP13,ARAF,IRX4,CREM,HPRT1,ADAM17,GNA11,MYLK3,HSPB8,BMPR2,KCNQ1,EP300,AKT1,JUN,IGF1,FABP3,CTNNB1,PPP3CA,EGFR,PLAT,CKMT2,RGS2,GRB2,MAPK8,MDM2,IGF2R,MEF2D,SLC6A4,CSRP3,AKAP1,MAPK1,PBX1,GPX3,IKBKB,CAMK2D,MYOM1,ABCC8,POSTN,CYBB,GSK3B,ACTG2,CAV3,TAB1,ELN,MIF,CPT1A,RRAD,MYH14,IL6R,FXYD1,RGS4,TRIM54,PFKM,IL18,ANKRD1,PTPN11,DUSP1,NPR1,CPT2,AGTR1,IL6ST,MYH6,ANGPT2,NTF3,RAB4A,KCNJ11,NFKBIA,PPP3CB,MAP3K7,HCK,GATA6,MAP2K1,ACE,TIMP3,SLC25A4,RNLS,CKM,FLT1,RYR2,SMAD7,SIRT3,PNPLA2,PARP1,CTF1,GNAI2,HOPX,MAPK14,LAMA4,Cxcl12,KLF5,EDNRA,NPPA,Rcan2,CTSC,GATA4,MYBPC3
2	Cardiac Necrosis/Cell Death	1.01E-07-5.46E-01	CRYAB,MAPK1,NOS3,SGCA,IKBKB,STK4,CAMK2D,SOD2,BAG1,ADCY5,PLA2G5,CYBB,GSK3B,CAV3,DSP,HADHA,TBX5,CALCRL,CASP3,RRAD,NMNAT1,TRIM54,STAT3,THBD,DAXX,NOL3,CBL,PTPN11,CREM,THBS2,ZYX,AGTR1,PVRL2,IL6ST,MYH6,PTN,MYLK3,HSPB8,GAPDH,IQGAP1,FSTL1,AKT1,IGF1,MAP3K7,Manf,HSPE1,PRKAA2,CACNB2,NAMPT,MAP2K1,HSPB6,MFN2,PPP3CA,AIFM1,PLAT,S100A1,PPIF,CXCR4,CSK,MAP3K1,MAPK8,MDM2,HSPD1,KIFAP3,PNPLA2,PARP1,CTF1,IVNS1ABP,GNAI2,NKX2-5,MAPK14,BNIP3L,CYP2J2,NPPA,GATA4,MYBPC3,MAOA
3	Cardiac Fibrosis	1.55E-06-6.27E-01	NOS3,ATP2A2,SGCA,TREX1,STK4,SOD2,POSTN,CYBB,PNKD,CAV1,GSK3B,SERPINE1,CAV3,DSP,ETS1,SMAD2,PLN,SGCG,SNAI1,CACNA1C,SGCB,STAT3,DES,SLC2A4,ACADL,PRDX3,NOL3,NPR1,CREM,THBS2,AGTR1,PVRL2,KLF15,ANGPT2,MYH6,FN1,GPX1,MYLK3,Nppb,AR,BMPR1A,IGF1R,XIRP1,ACADM,PPP3CA,PLAT,TIMP3,NPY1R,GRB2,TRDN,MAPK8,PNPLA2,HOPX,MAPK14,LAMA4,MEF2D,KLF5,SLC6A4,CSRP3,ACSL1,MYBPC3
4	Cardiac Arrythmia	6.99E-06-4.89E-01	Kcnip2,KCND3,PPP2CA,SNTA1,ATP2A2,SCN4B,ATP1A1,ANK2,CAV3,ADRA1B,CASQ2,SCN5A,KCNJ12,TBX5,PLN,KCNK2,RGS4,CACNA1C,KCNG2,RGS6,SCN1B,KCNJ5,CACNA2D1,CREM,P2RY1,TFPI,AGTR1,LCP2,COL3A1,PTGFR,MYH6,KCNE3,RANGRF,KCNQ1,KCNJ11,TGM2,KCNA4,AR,KCNA5,CACNB2,VCL,ABCC9,ACE,PTGS1,RYR2,DGAT2,GJA5,GNAI2,KITLG,LAMA4,GPD1L,PRKAG2,NPPA,DGAT1,CHI3L1,ADORA2A,AKAP9,MAOA
5	Cardiac Proliferation	2.94E-05-1.79E-01	ADAM17,KRAS,SMARCA4,AKT1,NFAT5,IGF1,CRTC2,BMPR1A,RARA,GATA6,GSK3B,CTNNB1,MAP2K1,EGFR,TBX5,FGF16,MYCN,GJA1,CALCRL,H19,FOXP1,FGF9,RGS4,FGF1,CTF1,IGF2,MAPK14,FOXO1,DUSP1,GATA4,PRKAR1A
6	Congenital Heart Anomaly	3.61E-05-5.46E-01	MYH6,NTF3,GATA5,PKD2,PBX1,KCNQ1,GPC3,NOS3,ATP2A2,COL1A2,STK4,JUN,FLNA,RARA,DGCR8,TGFB2,GATA6,ECE1,MGAT1,TEAD2,DSP,CITED2,SOX4,TBX5,GJA1,CCNE2,FOXP1,PKP2,RYR2,SMAD7,GJA5,DES,PLXND1,LTBP1,NKX2-5,KAT6A,EDNRA,INVS,P2RY1,HHEX,BAZ1B,JAG1,EYA1,DSG2,GATA4,ACVR2A,NRP1
7	Cardiac Infarction	2.3E-04-5.58E-01	F2R,MAPK1,EEF1A2,NOS3,SCARB1,PPP3R1,PLA2G5,CAV1,CYBB,POSTN,GSK3B,SERPINE1,ACTA1,GUCY1B3,ADRA1B,ATP5J,PSMA6,GUCY1A3,CACNA1C,PPP3CC,THBD,NOL3,NPR1,PECAM1,CD14,P2RY1,TFPI,AGTR1,COL3A1,DUSP6,App,TGM2,FSTL1,AR,PPP3CB,ACE,PPP3CA,PLAT,GSTM1,RNLS,PPIF,CXCR4,TNFRSF1A,PTGS1,VWF,CSF1R,MAPK14,Cxcl12,SLC6A4,CYP2J2,NPPA,ADORA2A

Figure S1: Top toxicity pathways augmented in *Nppb*$^{-/-}$. *Nppb*$^{+/+}$ (n=3) and *Nppb*$^{-/-}$ (n=3). Top toxicity functions (IPA INGENUITY analysis of heart transcriptome) are shown.

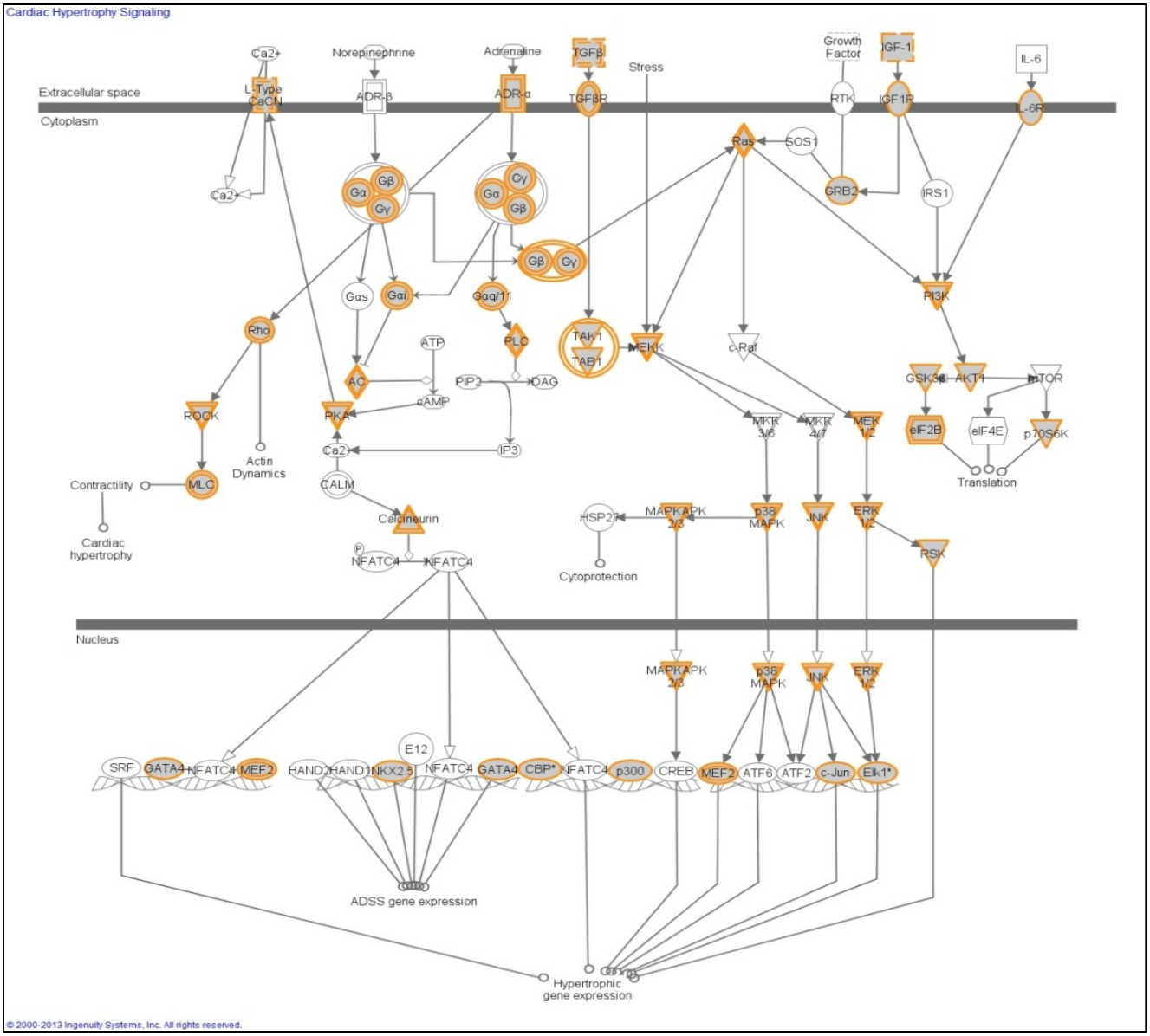


Figure S2: Transcriptome analysis of cardiac hypertrophy signaling pathways augmented in *Nppb*$^{-/-}$.

A

Cardiac hypertrophy signaling genes

Gene Symbol	Fold change	Gene Symbol	Fold change
Adcy4	0.09*	Mapk8	0.89
Adcy5	0.99	Mapk14	2.91*
Adra1b	11.6*	Mapkapk3	2.22*
Cacna1c	1.01	Mef2c	0.38*
Eif2b2	5.62*	Mef2d	2.01*
Eif2b3	3.93*	Myl3	9.13*
Eif2b4	3.14*	Nkx2-5	8.37*
Eif2b5	1.05	Pdia3	0.44*
Elk1	4.20*	Pik3c2a	0.21*
Ep300	0.30*	Pik3c2g	19.1*
Fnbp1	0.37*	Pik3r6	0.33*
Gata4	2.22*	Plcb1	0.29*
Gna11	0.47*	Plcb4	3.10*
Gnai2	0.29*	Plcg1	0.49*
Gnao1	2.71*	Plcl1	0.33*
Gnb1	0.49*	Ppp3ca	0.40*
Gnb3	3.24*	Ppp3cb	2.04*
Gng2	5.02*	Ppp3cc	2.18*
Gng5	2.41*	Ppp3r1	9.73*
Gng11	0.43*	Prkaca	3.32*
Grb2	1.00	Prkag1	2.10*
Gsk3b	0.34*	Prkag2	2.94*
Igf1	0.43*	Prkar1a	5.87*
Igf1r	0.17*	Rhob	0.46*
Il6r	2.60*	Rhoj	0.47*
Jun	0.41*	Rhog	0.65*
Kras	0.37*	Rhot2	2.36*
Map2k1	2.66*	Rnd3	3.58*
Map2k2	2.26*	Rock2	0.32*
Map3k1	0.15*	Rps6ka1	0.29*
Map3k3	0.38*	Rps6kb1	3.33*
Map3k7	2.00*	Rras2	4.14*
Map3k10	0.48*	Tab1	2.15*
Map3k11	0.30*	Tgfb2	0.23*
Mapk1	0.63	Tgfbr1	0.41*

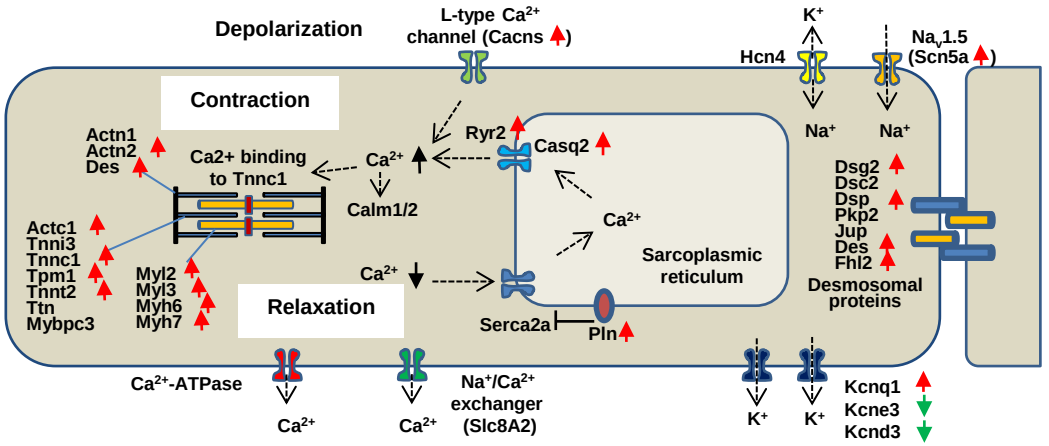
B

Genes associated with cardiac toxicity (hypertrophy, cell death, fibrosis and/or arrhythmia)

Gene Symbol	Fold change
Bmp4	0.46*
F2r	0.33*
Hspb2	40.9*
Nos3 (eNos)	0.11*
Atp2a2 (Serca2)	2.88*
Acta1	11.9*
Ryr2	7.54*
Sod2	5.66*
Fhl2	21.3*
Cav1	0.33*
Serpine1	6.04*
Pln	9.19*
Gucy1a3	0.33*
Cacna1c	3.32*
Stat3	0.41*
Ier3	5.41*
Des	9.97*
Cryab	4.83*
Akap13	0.19*
Hprt1	3.81*
Adam17	0.28*
Mylk3	3.09*
Hspb8	64.7*
Bmpr2	0.28*
Kcnq1	5.02*
Fabp3	7.98*
Ctnnb1	0.44*
Plat	0.22*
Ckmt2	4.86*
Csrp3	9.50*
Akap1	3.44*
Mapk1	2.35*
Camk2d	4.44*
Myom1	8.16*
Abcc8	2.22*
Cav3	24.7*
Rrad	12.3*
Fxyd1	6.63*
Il18	3.24*
Ankrd1	5.47*
Ptpn11	0.28*
Dusp1	3.08*
Cpt2	15.6*
Angpt2	0.14*
Ntf3	0.42*
Kcnj11	2.03*
Gata6	3.67*
Ace	0.25*
Timp3	0.38*
Ckm	11.1*
Flt1	0.31*
Smad7	0.29*
Gata4	2.22*
Scn5a	5.12*
Gpd1l	4.09*
Abcc9	0.47*
Akt1	0.97
Egfr	1.00

Figure S3: A. Transcriptome analysis of cardiac hypertrophy genes, and **B.** markers of cardiac toxicity (hypertrophy, cell death, fibrosis and/or arrhythmia) upregulated in *Nppb*$^{-/-}$. *Nppb*$^{+/+}$ (n=3) and *Nppb*$^{-/-}$ (n=3) are shown. *p<0.05, vs. *Nppb*$^{+/+}$ rats.

A



B

	Gene Symbol	Fold change
Sodium channels	Scn5a	5.12*
	Scn1b	7.90*
	Scn3b	0.97
	Scn4b	30.7*
Intracellular calcium homeostasis	Ryr2	7.54*
	Casq2	20.5*
	Trdn	2.02*
	Calm1/2	0.89
	Jph2	7.86*
	Pln	9.19*
	Psen1	1.42
	Psen2	0.76
	Calr3	1.04
Cytoskeletal	Cryab	4.83*
	Dmd	0.99
	Des	9.97*
	Pdlim3	2.35*
	Fkrp	0.82
	Fktn	0.63
	Fxn	9.31*
	Sgcg	7.00*
Other channels	Trpm4	0.84
	Hcn4	1.01

	Gene Symbol	Fold change
L-type calcium channels	Cacna1c	3.17*
	Cacnb2	11.3*
	Cacna2d1	2.83*
Potassium channels	Kcne3	0.22*
	Kcnd3	0.26*
	Kcnq1	5.02*
	Kcnh2	1.28
	Kcne1	1.01
	Kcne2	0.99
	Kcnj2	0.98
	Kcnj5	0.98
	Kcnj8	1.12
	Abcc9	0.47*
Channel-interacting proteins	Dpp6	1.00
	Gpd1l	4.10*
	Rangrf	9.53*
	Snta1	2.59*
	Cav3	24.7*
	Slmap	5.54*
	Ank2	1.03
	Akap9	0.36*

	Gene Symbol	Fold change
Sarcomeric	Ttn (CMH9)	0.95
	Myh7 (CMH1)	24.6*
	Myh6	4.51*
	Myl2 (CMH10)	4.32
	Myl3 (CMH8)	9.13*
	Mybpc3 (CMH4)	2.22*
	Tnnt2 (CMH2)	3.24
	Tnni3 (CMH7)	2.56
	Tpm1 (CMH3)	8.89*
	Actc1 (CMH11)	25.3*
	Tnnc1	9.10*
Z-disc	Ldb3	1.32
	Vcl	1.72
	Csrp3 (CMH12)	9.50*
	Ankrd1	19.9*
	Mypn	18.5*
	Actn2	9.86*
	Myoz2	6.71*
	Nexn	1.68
Membrane	Hfe	0.99
	Tmem43	0.60*

	Gene Symbol	Fold change
Cellular enzymes	Prkag2 (CMH6)	4.33*
	Ptpn11	2.97*
	Raf1	1.79*
	Mylk2	1.24
Desmosomal	Dsp	9.05*
	Fhl1	2.83*
	Fhl2	21.3*
	Jup	0.54*
	Pkp2	2.13*
	Dsg2	4.21*
	Dsc2	1.98*
Mito-chondrial	Taz	3.82*
	Sdha	6.97*
RNA processing	Rbm20	4.94*
	Lmna	1.37
	Tmpo	0.34*
	Gatad1	1.18
	Dolk	1.96*
Lysosomal	Lamp2	0.42*
	Gla	1.22

Figure S4: A. Depiction of transcriptome analysis and modulated gene expression (red arrows, increased, green arrows, decreased) specific to cardiomyocyte contraction and relaxation of *Nppb*$^{-/-}$ rats. **B.** Table details genes associated with ion channel flux, contractility, and relaxation pathways modulated in the *Nppb*$^{-/-}$ rat. *Nppb*$^{+/+}$ (n=3) and *Nppb*$^{-/-}$ (n=3). *p<0.05, vs. *Nppb*$^{+/+}$ rats.

CBC	Nppb+/+ (n=6)	Nppb-/- (n=6)
WBC	8.1 (±0.91)	7.85 (±0.63)
RBC	10.12 (±0.28)	**9.71* (±0.23)**
HGB	16.42 (±0.24)	16.3 (±0.51)
HCT	52.3 (±1.8)	50.6 (±1.3)
PLT	673.2 (±63.5)	628.2 (±103.5)
ALB	1.43 (±0.08)	**1.2* (±0.08)**
ALP	137.7 (±5.7)	150.3 (±28.2)
ALT	60.7(±8.1)	61.7(±9.1)
TBIL	0.27(±0.1)	0.3 (±0.0)
BUN	19.5 (±3.5)	21.2 (±1.1)
CRE	0.3 (±0.1)	0.4 (±0.1)
GLU	183.2 (±37.8)	160.3 (±16.4)
TP	7.1 (±0.2)	7.0 (±0.2)

Figure S5: Complete blood count (CBC), and blood chemistry for *Nppb*$^{+/+}$ (n=6) and *Nppb*$^{-/-}$ (n=8) at 9 months. White blood cell, WBC; red blood cell, RBC; Hemoglobin, HGB; hematocrit, HCT; platelets, PLT; serum-albumin, ALB; serum-alkaline phosphate, ALP; serum-alanine aminotransferase, ALT; serum-total bilirubin, TBIL; blood-urea-nitrogen, BUN; serum-creatinine, CRE; glucose, GLU; serum-total protein, TP. *p<0.05, vs. *Nppb*$^{+/+}$ rats.

A

Top toxicity functions (IPA INGENUITY analysis of kidney transcriptome)

	Category	p values	Molecules
1	Renal Damage	6.33E-06-2.24E-01	ADM,ST6GAL1,CP,FCGR1A,SLC22A6,OCLN,COL5A1,HAVCR1,KL,CYP2E1,SLC22A1,Fasn,FABP4,IGFBP1,GC,CTNNB1,FUT4,ITGB1,RAC1,REN,G6PD,ST6GALNAC3,C5,IGFBP5,SLC13A1,SLC38A3,TXNRD1,GSR,POU2F1,DUSP1,CLDN1,Mbl1,IGFBP3,CYP2J2,ITGB6,ADORA2A,S100G,AGTR1,HADH
2	Renal Necrosis/ Cell Death	7.1E-06-5.7E-01	CRYAB,CA4,BCL6,PKN2,MYC,YWHAQ,ATP1A1,PAK1,FGFR4,PPP3R1,SLC22A1,CAV1,PLCB1,PPM1A,HIPK2,CALB1,WT1,CUL4B,TJP2,ATF3,SLC2A1,RAC1,FGD1,IER3,PTGDS,PFKM,DUSP1,TMX1,IGFBP3,NEIL1,TFRC,VDAC1,AGTR1,CDK2,LDHA,APOE,TCF4,PIK3CA,GSTM5,TFAP2B,Nrg1,GAPDH,DDN,JAK2,NPHS1,AQP3,NFAT5,AR,KL,IGF1R,NAMPT,CTNNB1,CASP8,NFE2L2,ATP13A2,ATN1,PRNP,DNASE1,ITGB1,PAK2,BGN,MAP3K1,AQP11,HIP1,F3,SULT2B1,GRB10,STRA6,COX11,SLC7A9,PTH1R,PLAU,PTGR1,CASP7,PSEN1
3	Renal Tubule Injury	1.3E-05-1.87E-01	ST6GAL1,CP,SLC22A6,OCLN,COL5A1,HAVCR1,KL,CYP2E1,SLC22A1,Fasn,FABP4,IGFBP1,FUT4,G6PD,ST6GALNAC3,IGFBP5,SLC13A1,TXNRD1,SLC38A3,GSR,POU2F1,CLDN1,IGFBP3,S100G,HADH
4	Renal Hydronephrosis	7.52E-05-7.52E-05	TIMP3,DLG1,ID2,NTF3,SFRP2,REN,LZTS2,GPC3,SLC7A9,NFAT5,LGMN,RARA,GFRA1,KCNJ1,IGF1R,PSAP,ITGB4,AGTR1,HSPA4L
5	Kidney Failure	7.69E-05-3.97E-01	ADM,F2R,PDE7A,SLC9A3,XDH,MMP14,FKBP1A,NPHS1,ATP1A1,AR,LGMN,HAVCR1,PDE3B,PPP3R1,HEXB,GUCY1B3,DNASE1,WT1,PODXL,SLC34A1,ATP1B1,ATF3,GUCY1A3,PTGS1,AQP11,REN,PTGDS,STC1,SERPINC1,DUSP1,P2RY1,ADORA2A,CDK2,AGTR1,PRKCB

B

Top 30 differentially expressed genes (Renal)

Down-regulated		Up-regulated	
Gene Symbol	Fold change	Gene Symbol	Fold change
Snhg11	0.091	Slc5a2	167.9
Fst	0.094	Slc4a4	124.7
Acox2	0.097	Slc5a12	116.0
Slc14a2	0.131	G6pc	86.5
LOC307495	0.133	Gldc	81.9
Car7	0.143	Fam151a	55.8
Fa2h	0.144	Epb41l3	51.3
Zbtb8os-ps1	0.144	Slc34a3	50.1
Smoc2	0.149	Stra6	49.2
Ppp1r1b	0.151	Slc22a8	46.0
Pou3f1	0.158	Car14	43.2
Lrrc8a	0.162	Apcs	40.8
Slfn3	0.163	Tnnc1	40.0
Xpnpep3	0.166	Slc7a7	37.6
Alx1	0.168	Folh1	35.3
LOC680097	0.176	Slc16a1	33.3
Cfh	0.176	Spp2	30.7
P2rx3	0.178	Ptpro	29.1
LOC498222	0.178	Serpinc1	28.4
Hip1	0.179	Nphs1	26.9
Tle2	0.182	Ptgds	25.0
Mall	0.185	Slc31a1	22.5
Kif1b	0.191	Slc30a2	21.5
Klhdc8a	0.191	Apoe	20.0
Wfdc10	0.199	Pdpn	19.7
Sult2b1	0.199	Klotho	19.5
Mras	0.200	Klk1c7	18.9
LOC689926	0.201	Slc12a3	18.5
Capg	0.208	Dnah7	18.0
LOC100361122	0.210	Slc22a23	17.5

Nppb-/- vs. Nppb+/+ (n=3; p<0.05)

C

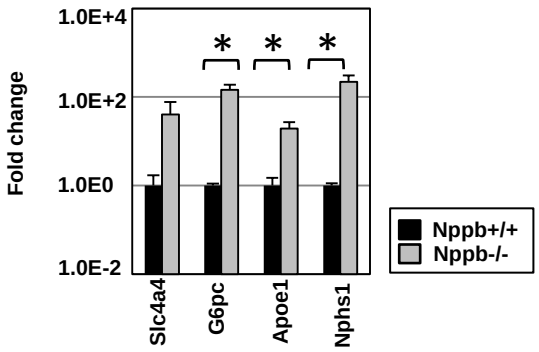


Figure S6: A. IPA INGENUITY analysis of renal total RNA transcriptome; Top 5 toxicity pathways augmented in *Nppb*$^{-/-}$. **B.** Top 30 differentially expressed genes in the kidney are shown (vs. *Nppb*$^{+/+}$ rats). **C.** RT-QPCR confirmed augmented transcripts of SLC4a4, G6pc, Nphs1, Apoe1 in *Nppb*$^{-/-}$ (n=6), *Nppb*$^{+/+}$ (n=6). *, p<0.05, **, p<0.01, ***, p<0.001, vs. *Nppb*$^{+/+}$ rats.

A **Vector mediated Reversal of Cardiac parameters in Nppb-/-**

Echocardiographic Parameters, Age/Strain:	[a]3 months Nppb -/-	[a]3 months Nppb -/- +AAV BNP	[b]6 months Nppb -/-	[b]6 months Nppb -/- +AAV BNP	[c]9 months Nppb -/-	[c]9 months Nppb -/- +AAV BNP
Weight (g)	378 (±20)*	347 (±28)!,#	425 (±22)	397 (±18)!,#	452 (±21)	446 (±20)
IVSd (mm)	2.01 (±0.08)	1.42 (±0.08)!,#	1.92 (±0.12)	1.90 (±0.20)	2.26 (±0.23)	2.36 (±0.20)
LVIDd (mm)	7.52 (±0.32)	8.33 (±0.40)!,#	8.68 (±0.33)	8.33 (±0.64)	7.99 (±0.62)	8.52 (±0.51)
LVPWd (mm)	2.03 (±0.06)*	1.54 (±0.17)!,#	1.89 (±0.16)	1.88 (±0.14)	2.17 (±0.17)	1.94 (±0.20)
IVSs (mm)	3.19 (±0.20)*	2.69 (±0.06)!,#	3.24 (±0.21)	3.18 (±0.34)	3.48 (±0.26)	3.52 (±0.21)
LVIDs (mm)	3.98 (±0.45)	4.64 (±0.36)!,#	4.83 (±0.35)	4.54 (±0.91)	4.67 (±0.74)	5.18 (±0.51)
LVPWs (mm)	3.29 (±0.19)*	2.62 (±0.20)!,#	3.17 (±0.17)	3.23 (±0.43)	3.41 (±0.50)	3.13 (±0.43)!
EFteich	83.3 (±4.3)	80.4 (±2.8)	80.2 (±3.4)	81.0 (±7.1)	77.4 (±6.0)	74.5 (±6.4)
%FS	47.3 (±4.1)	44.3 (±2.9)	44.3 (±2.8)	46.0 (±8.3)	41.6 (±5.6)	39.2 (±5.8)
LVd Mass (g)	1.53 (±0.08)*	1.32 (±0.04)#	1.67 (±0.06)*	1.59 (±0.05)#	1.78 (±0.04)	1.84 (±0.08)
HR (bpm)	382 (±45)*	361 (±11)	349 (±20)	348 (±19)	327 (±19)	342.5 (±20)

B **Vector mediated Reversal of elevated blood pressure in Nppb-/-**

Blood Pressure (mm Hg)	[a]3 months Nppb -/-	[a]3 months Nppb -/- +AAV BNP	[b]6 months Nppb -/-	[b]6 months Nppb -/- +AAV BNP	[c]9 months Nppb -/-	[c]9 months Nppb -/- +AAV BNP
Diastolic	128 (±22)	94(±11)!,#	142 (±28)	118 (±13)#	145 (±31)	149 (±30)!
Systolic	172 (±13)	141 (±12)!,#	195 (±30)	175 (±8)#	197 (±26)	201 (±17)!
Mean	142 (±12)	109 (±11)!,#	158 (±27)	137(±11)#	162 (±29)	167 (±25)!

C **Vector mediated Reversal of polyuria in Nppb-/-**

Urine Parameters	[a]3 months Nppb -/-	[a]3 months Nppb -/- +AAV BNP	[b]6 months Nppb -/-	[b]6 months Nppb -/- +AAV BNP	[c]9 months Nppb -/-	[c]9 months Nppb -/- +AAV BNP
Urine Output (mL/24)	14.7(±2.6)	13.4 (±2.6)	21.1(±7.6)*	14.0 (±6.7)	19.5 (±4.6)*	13.8 (±3.3)#

Figure S7: (A) Neonate *Nppb-/-* rats were treated by AAV9-BNP vectors for long-term systemic BNP over-expression. At 3, 6, and 9 months, ECHO was performed on control and AAV vector-treated *Nppb-/-* littermates. **(B)** Noninvasive blood pressure was monitored at 3, 6, and 9 months of AAV vector-treated *Nppb-/-* and littermates. **(C)** Influence of systemic BNP over-expression on urine volumes at 3, 6, and 9 months in *Nppb-/-* and vector treated littermates. For all experiments, the numbers of AAV vector-treated rats were n=7, n=7 and n=6 at 3, 6 and 9 months. Data from untreated litter mates, previously presented in Table 1, are included here for comparison. #p<0.05, comparison between $Nppb^{-/-}$ and $Nppb^{-/-}$ +AAV-BNP; !p<0.05, comparison between $Nppb^{+/+}$ and $Nppb^{-/-}$ +AAV-BNP rats (*Nppb+/+* data set previously shown).