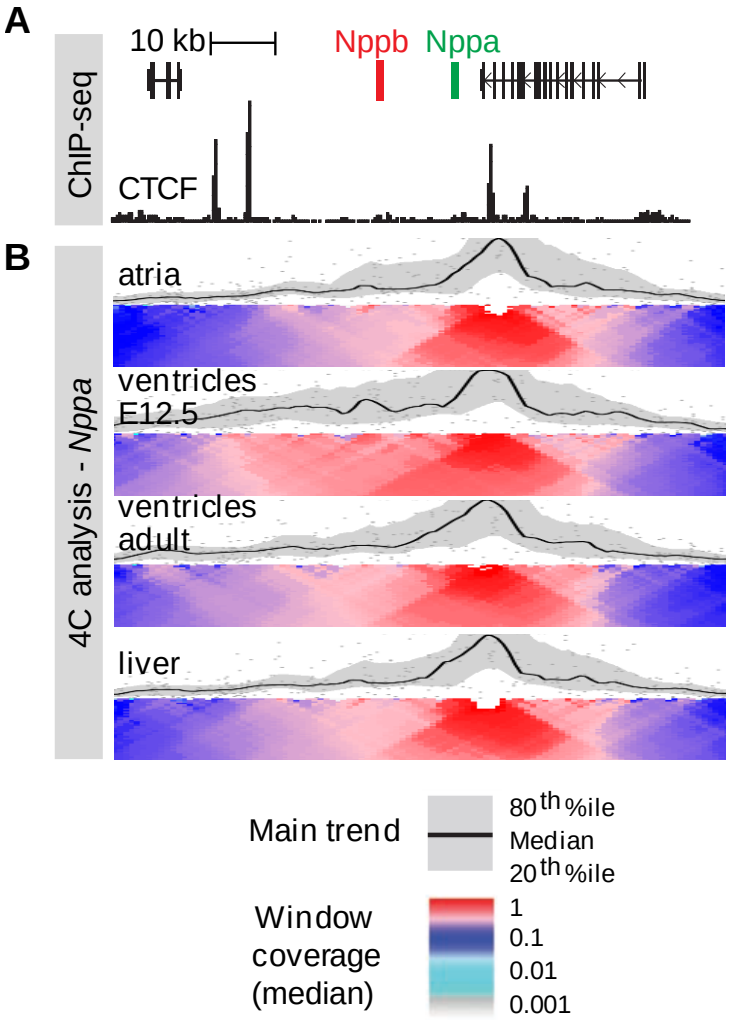

Fig. S1. Contact profiles of *Nppa* are similar during heart development and stress. (A) UCSC genome browser view of ChIP-seq data of the CTCF binding sites (Stamatoyannopoulos et al., 2012). (B) Integrated contact profile for the *Nppa* viewpoint is similar between different tissues. The top panel represents normalized contact intensities (gray dots), their running median (black line) analyzed with 4 kb sliding window, and the 20-80% percentile for these windows (gray band). In the bottom panel, contact intensities are computed using linearly increasing sliding windows (scaled 2 (top) - 50 kb (bottom)) and are displayed as a color-coded heatmap of positive 4C signals (maximum of interaction set to 1) (Werken et al., 2012b). Local color changes are log-scaled to indicate changes of statistical enrichment of captured sequences, corresponding to the DNA interaction.

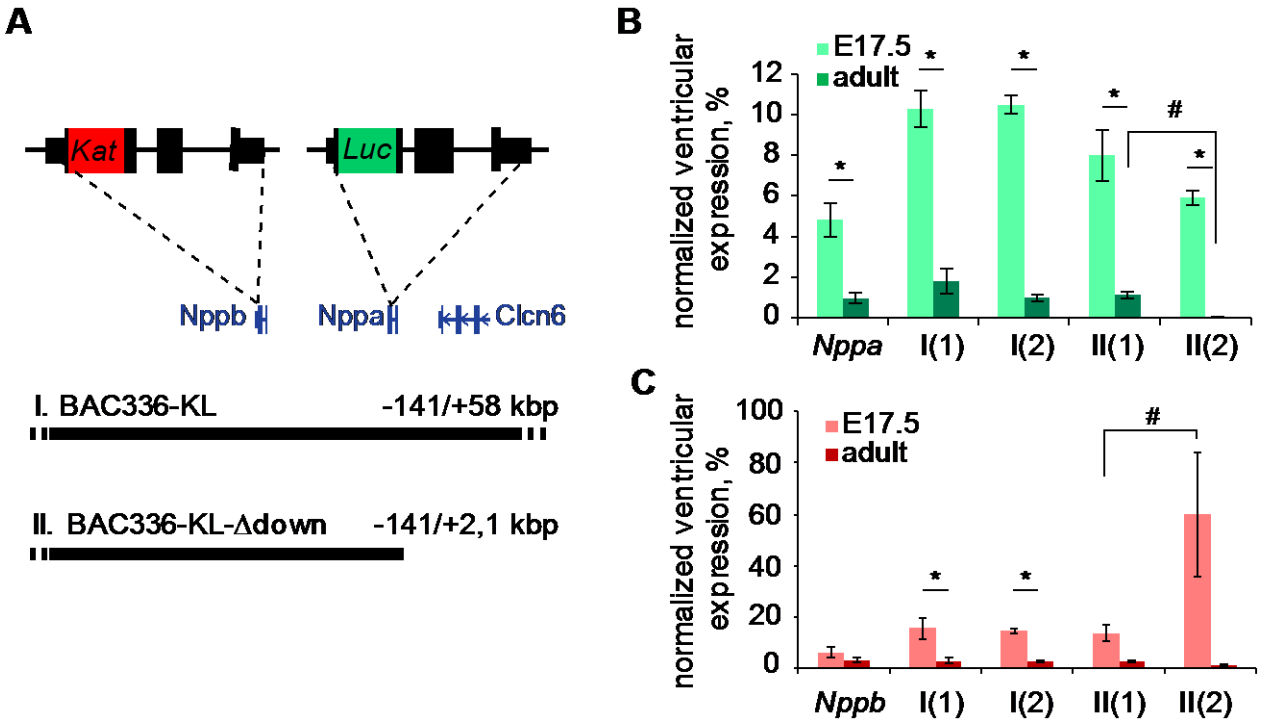

Fig. S2. Two independent founder lines exhibit similar developmental behavior of the reporter genes. (A) Schematic representation of BAC336-KL(I) and BAC336-KL-Δdown(II) constructs. BAC reporter constructs were modified with Katushka and Luciferase (KL) genes inserted into *Nppb* and *Nppa* translation start sites, respectively. (B,C) Normalized fetal and adult ventricular activity of *Luciferase* (B) and *Katushka* (C) measured with qPCR (n=3-6). *Luciferase* and *Katushka* were downregulated in the ventricles after birth, following significant and moderate downregulation of *Nppa* and *Nppb,* respectively. Although adult ventricular activities of *Luciferase* was lower and the fetal ventricular activity of *Katushka* was higher in line 2 of BAC336-KL-Δdown(II), the developmental behavior of the reporter genes remained comparable. The efficiency of BAC transgenesis after oocyte injection is usually lower than that of small plasmid-based constructs (Van Keuren et al., 2009). In this study, only one or two lines per BAC construct have been analyzed. However, some of the BAC constructs are complementary to each other in their coverage of the *Nppa-Nppb* locus (Figure 3, BAC336-KL-Δdown(II) and RP23-139J21-L(VI)). The data obtained from these mice validate each other's results, thus compensating for the lack of multiple independent lines per BAC. Student's two-tailed t-test was used in (B,C); *p<0.05 E17.5 vs. adult, $^{\#}$p<0.05 line (1) vs line (2). Error bars represent s.e.m.

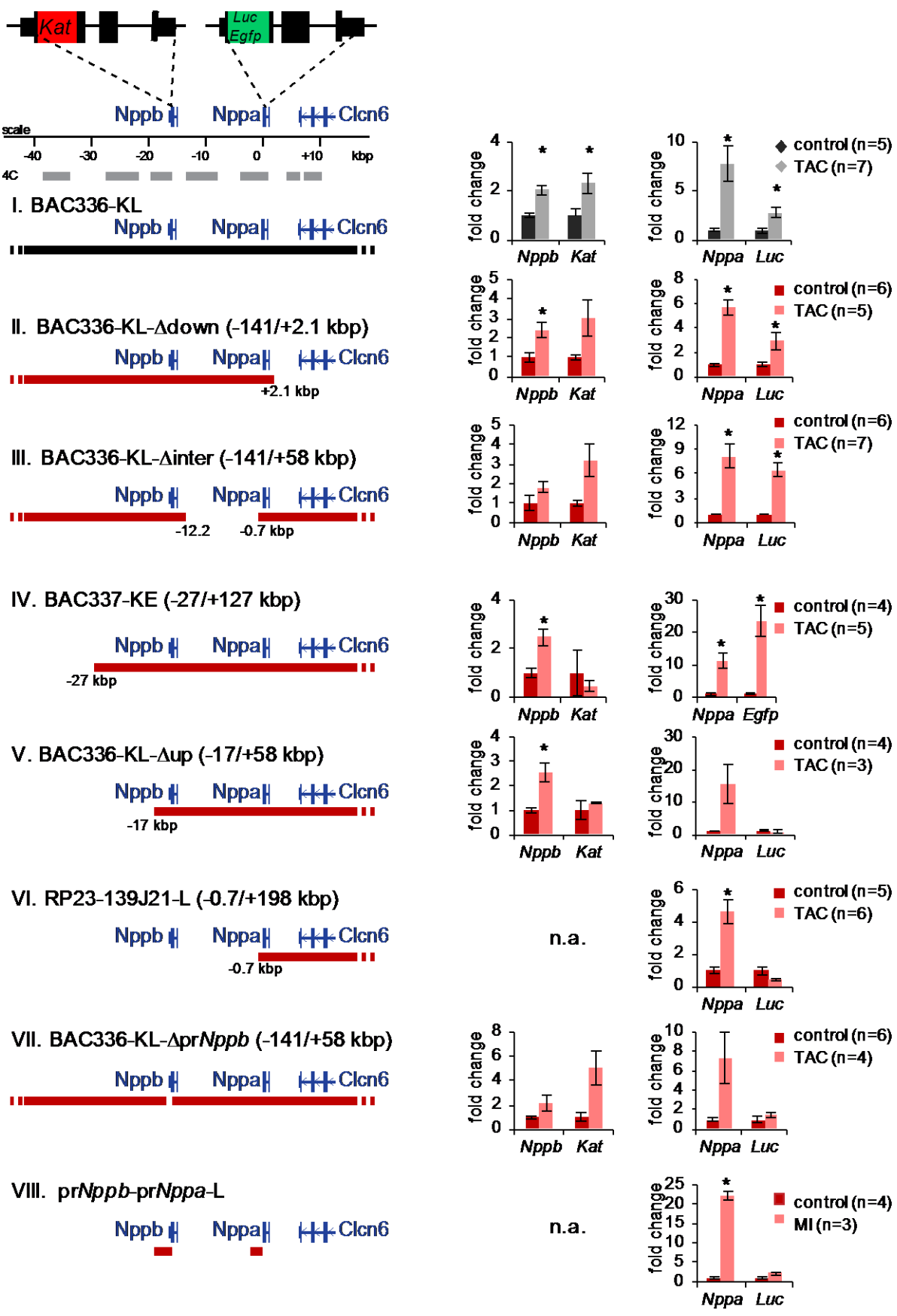

Fig. S3. Overview of the activity of regulatory *Nppa* and *Nppb* sequences during stress. BAC336-KL (black line), overlapping BACs and BAC deletion clones (red lines) with inserted *Katushka* and *Luciferase/EGFP*

reporters, and the schematic representation of interacting regions (gray lines) as identified by 4C-seq are presented in the left panel. Changes in mRNA levels of *Nppb, Katushka, Nppa* and *Luciferase/EGFP* are shown in the right panel. mRNA levels of the genes were measured with qPCR 2 weeks (I), 2-4 weeks (II-VII) after TAC surgery or 1 week after MI (VIII). *p<0.05 vs. control (Student's two-tailed t-test). Error bars represent s.e.m.

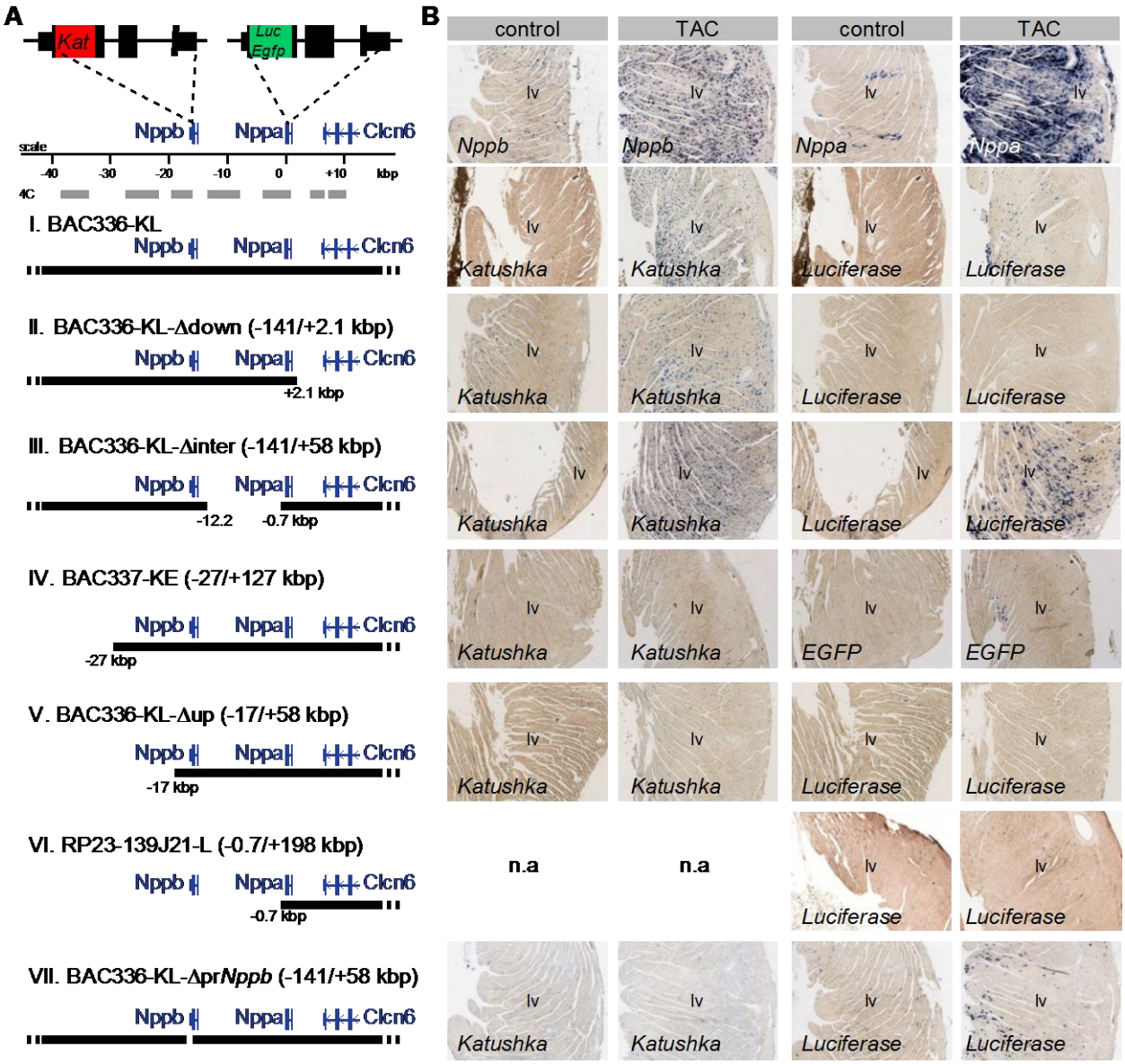

Fig. S4. Overview of the activity of regulatory *Nppa* and *Nppb* sequences during stress. (A) Overview of the overlapping BACs and BAC deletion clones (black lines) with the schematic representation of interacting region (gray lines) as analyzed by 4C-seq. BAC reporter constructs were modified with Katushka and Luciferase/EGFP (KL/E) genes inserted into *Nppb* and *Nppa* translation start sites, respectively (if applicable). (B) Upregulation of *Nppb* and *Nppa* (top raw), and the corresponding *Katushka* and *Luciferase*/*EGFP* genes in the left ventricular myocardium was studied with *in situ* hybridization in the representative TAC hearts compared with the controls.

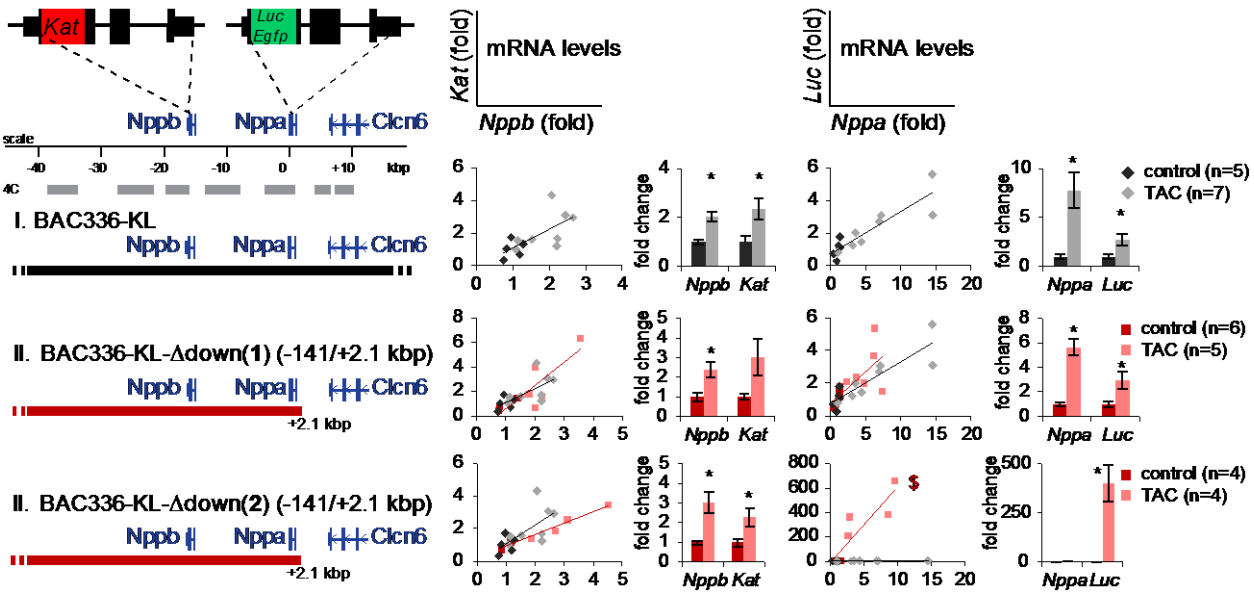

Fig. S5. Two independent founder lines exhibit similar stress-induced activation of the reporter gene expression. BAC336-KL(I) (black line) and BAC336-KL-Δdown(II) (red lines) constructs, and the schematic representation of interacting region (gray lines) as analyzed by 4C-seq are presented in the left panel. BAC reporter constructs were modified with Katushka and Luciferase (KL) genes inserted into *Nppb* and *Nppa* translation start sites, respectively. Correlation of *Nppb* and *Katushka* mRNA levels, and *Nppa* and *Luciferase* mRNA levels in the ventricular myocardium of the control and TAC mice are shown in the 2nd and the 4th columns. Correlation of each pair of genes in the BAC336-KLΔdown (represented in red) was compared with those in BAC336-KL(I) (represented in black), which contains all necessary information for the stress response. Changes in mRNA levels of *Nppb*, *Katushka*, *Nppa* and *Luciferase* are shown in the 3rd and the 5th columns. mRNA levels of the genes were measured by qPCR 2-4 weeks after TAC surgery. *p<0.05 vs. control (Student's two-tailed t-test), § significant vs. BAC336-KL(I) (t-test between the slopes of the regression lines). Error bars represent s.e.m.

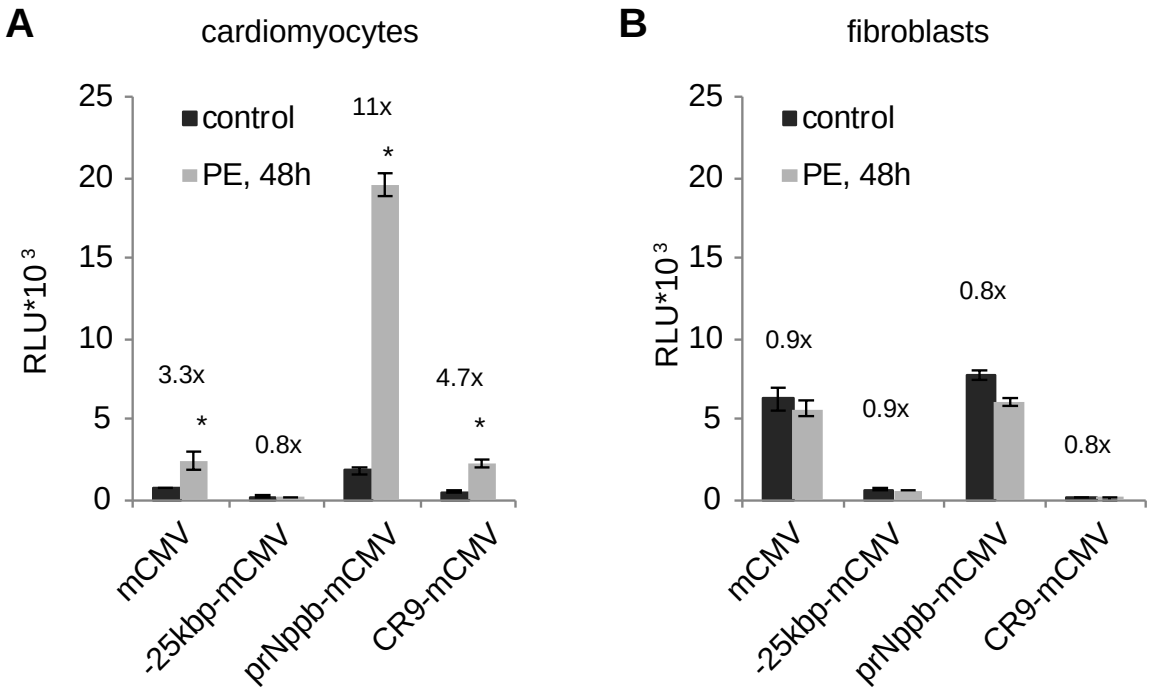

Fig. S6. Identification of enhancer activity of the *Nppb* promoter in response to an α1-adrenergic receptor agonist. Rat neonatal cardiomyocytes (A) and cardiac fibroblasts (B) were treated with PE (100 uM) for 48h. We measured luciferase reporter activities of the minimal CMV promoter (mCMV), the *Nppb* promoter (pr*Nppb*-mCMV), similar size fragment at -25 kbp relative to *Nppa* (-25kbp-mCMV) and the stress-response enhancer CR9 at -37 kbp relative to *Nppa* (CR9-mCMV) used as a positive control (Matsuoka et al., 2014). Numbers above the graphs represent fold activation of Luciferase activity in PE-treated cells compared to control, showing enhancer activity of the *Nppb* promoter and CR9 enhancer specifically in cardiomyocytes. Error bars represent s.e.m. (n = 6 wells in two independent experiments). *P < 0.05 vs. control, Student's two-tailed *t*-test.

construct		Scatter plot Nppb/Kat		Bar charts		Scatter plot Nppa/Lic		Bar charts	
# BAC	Name	Gene correla-tion	2 slopes are different	Nppb	Katush-ka	Gene correla-tion	2 slopes are different	Nppa	Lucife-rase
I(1)	BAC336_KL(TAC)	0.009	n.a	0.002	0.036	4.5E-05	n.a	0.010	0.032
I(2)	BAC336_KL(TAC)	0.023	0.999	0.076	0.102	0.574	0.137	0.095	0.266
I(1)	BAC336_KL(MI)	0.036	n.a	0.157	0.099	0.007	n.a	0.141	0.137
II(1)	BAC336_KL_Δdown	0.009	0.216	0.038	0.095	0.010	0.840	0.001	0.044
II(2)	BAC336_KL_Δdown	4.63E-05	0.221	0.033	0.036	0.002	8.221	0.072	0.023
III	BAC336_KL_ Δinter	4.29E-04	0.059	0.188	0.063	4.68E-04	0.056	0.008	0.005
IV	BAC337_KE	0.823	0.094	0.011	0.618	2.54E-04	4.974	0.011	0.009
V	BAC336_KL_ Δup	0.464	0.065	0.008	0.534	0.336	6.890	0.142	0.856
VI	RP23-139J21_L	n.a.	n.a	n.a.	n.a	0.201	0.001	0.002	0.121
VII	BAC336_KL_ Δ *prNppb*	0.001	0.080	0.190	0.055	0.602	0.004	0.096	0.404
VIII	prNppb-prNppa_L	0.338	0.108	0.304	0.831	0.086	0.048	0.001	0.125

Table S1. P values of the statistical analysis of reporter gene expression in the transgenic mouse lines 2-4 weeks after TAC operation (I-VII) and 1 week after MI (I, VIII), represented in Fig. 4. P values of *Nppa-Luciferase*/*EGFP* and *Nppb-Katushka* correlations on a scatter plot represent the ability of a reporter gene to follow upregulation of *Nppa* or *Nppb,* respectively. Significant slope difference shows that the response of the reporter gene in a given BAC transgenic line is different from that in BAC336-KL(I) mice. P values in a bar chart represent significance of upregulation of *Nppa*, *Nppb* and the reporter gene expression in the ventricles.

Supplementary Materials and Methods

Animals

Animal care and experiments conform to the Directive 2010/63/EU of the European Parliament. All animal work was approved by the Animal Experimental Committee of the Academic Medical Center, Amsterdam (DAE101977, DAE101892, DAE102292, DAE102821), and was carried out in compliance with the Dutch government guidelines. Transgenic mice were generated and bred on the FVB/N background. Genotype of the founder lines and their offspring was analyzed by PCR with tissue-derived (ear, tail, toe, embryo) DNA, and *EGFP* specific (5'Fw- ATCTTCTTCAAGGACGACGG; 5'Rv- AGTTGTACTCCAGCTTGTGC) or *Luciferase* specific primers (5'Fw- ATGTCCGTTCGGTTGGCAGA; 5'Rv- CTGAAATCCCTGGTAATCCGTT) were used.

4C experiment

Preparation of 4C templates was described previously (Simonis et al., 2009). In short, adult mouse hearts and liver were isolated in ice-cold PBS with 10% FCS. Heart tissue was dissociated with IKA Ultra Turrax T5 FU, followed by dounce homogenization to obtain single cell suspension. Liver tissue was homogenized with a douncer only. Chromatin was cross-linked with 2% formaldehyde in 40ml PBS with 10% FCS for 10 min at room temperature, nuclei were isolated in 25ml cold lysis buffer (50 mM Tris-HCl, pH 8.0; 150 mM NaCl; 5 mM EDTA, pH 8.0; 0.5% NP-40; 1% Triton X-100; 1× Protease Inhibitor Cocktail (Roche)) for 1h, and cross-linked DNA was digested with DpnII, recognizing 4-bp restriction site. Digestion was followed by proximity ligation, removal of cross-links, a secondary restriction digestion with Csp6I and a second proximity ligation. For all experiments, 200 ng of the resulting 4C template was used for the subsequent PCR reaction, of which 16 or 8 (total: 3.2 or 1.6 ug of 4C template) were bar-coded, pooled and purified for next generation sequencing. The PCR products were purified using 2 columns per sample of the High Pure PCR Product Purification Kit (Roche), which separates the PCR products larger than 120 bp from the adaptor-containing primers (~75 and ~40 nt).

4C-seq primer design

Detailed rules for 4C primer design were described previously (Werken et al., 2012b). In short, the size of the view point was at least 500 bp to allow efficient cross-linking to other DNA fragments. The region between the primary and secondary restriction enzymes was at least 300 bp to allow efficient circularization during the second ligation step. The reading primer, 20 nt in size, always hybridizes to, and ends at, the 3' side of the first restriction recognition site. The nonreading primers, 18-20 nt in size, were designed at a distance ≤100

bp from the secondary restriction site. 4C primer pairs carry additional 5' overhangs composed of the adapter sequences (obtained from Illumina technical support). The strategy therefore produces sequencing reads (36-mers) composed of the 4C primer sequence (20 nt, specific to a given view point) followed by 16 nt that identify a capture sequence. Primers used in this study are listed below.

primer name	5'-adaptor-**primer**-3'
CTCF left_DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCT CTTCCGATCT**AAAAGCTCAGAGTGGAGATC**
CTCF left_Csp6I	CAAGCAGAAGACGGCATACGA**ATTCTACTGATGCTGCATGG**
*Nppb*_DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCT CTTCCGATCT**CAGCTCTCTCTTAGCTGATC**
*Nppb*_Csp6I	CAAGCAGAAGACGGCATACGA**GTGAGCCACATAGCTCCTTC**
*Nppa*_DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCT CTTCCGATCT**GAGACAGCAAACATCAGATC**
*Nppa*_Csp6I	CAAGCAGAAGACGGCATACGA**TGTCAGGGGCTCCAAATA**
CTCF right_DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCT CTTCCGATCT**TTCAGTGAGTTCTGGGGATC**
CTCF right_Csp6I	CAAGCAGAAGACGGCATACGA**GTCCTTGCAACAAACAGAAG**

4C-seq data analysis and statistics

4C templates were mixed and sequenced simultaneously in 1 Illumina HiSeq 2000 lane. The sequence tags generated by the procedure were prefixed by the 4C reading primer, which includes the DpnII restriction site sequence (see 4C-seq primer design). The 4C reading primer sequences were separated from multiplexed 4C-seq libraries, and the suffixes were extracted for further processing. Mapping and filtering of the sequence reads was done as previously described (Werken et al., 2012b). The algorithm constructs a background model for remote intra- and interchromosomal contacts to correct for systematic biases that can occur during the 4C-seq experimental protocol. The algorithm is designed to use controls for sequencing errors and nonunique sequences while considering the high coverage (100×–100,000×) of fragment ends that are proximal to the viewpoint fragment. To normalize the interactions in close proximity to the viewpoint, the algorithm was used to calculate the median of normalized coverage for running windows of size 4 kb and sliding windows of 2–50 kb of linearly increasing size. All median values represent enrichment relative to the maximum attainable 4-kb median value, whereas sliding windows represent enrichment relative to the maximum attainable 12-kb median value. The 20th and 80th percentiles were

also computed and depicted as the gray area around the 4-kb running windows. The 4C-seq contact profile comparison plots (Figure 1 C and 2C) were generated combining the mapping (Werken et al., 2012a) and normalization (Werken et al., 2012b) strategies. Within samples the distribution of the blind fragment-end reads and the distribution of the non-blind fragment-end reads were quantile normalized after linear interpolation of the center of the fragment-end using R's limma package (Smyth, 2005). The reads between samples were normalized based on library size within the locus. Subsequently, a running trimmed (10%) mean was calculated on 21 fragment-ends to smoothen the 4C-seq data. The R statistical package version 3.1.0 was used for the statistical calculations and for generating the 4C-Seq plots (R Core Team, 2013).

Chromatin immunoprecipitation

Left ventricles of the control and TAC hearts were dissected in ice-cold PBS, ground in liquid nitrogen and cross-linked in 1% formaldehyde for 10 min at room temperature. Cross-linking was quenched by addition of 0.125M glycine. Tissues were further dissociated by IKA Ultra Turrax T5 FU, pelleted, and resuspended in cold lysis buffer (50 mM Tris-HCl, pH 8.1; 10 mM EDTA, pH 8.0; 1% SDS; 1× Protease Inhibitor Cocktail (Roche); and phosphatase inhibitors: 100 mM PMSF, 54 mM Na-ortho-vanadate, 0.5M Sodium Fluoride, 0.5M β-glycero-phosphate). Nuclei were obtained by use of a tight glass dounce homogenizer. Cross-linked nuclei were sonicated under conditions established to yield an average fragment size of approximately 300 bp. Antibodies were anti-H3K27ac (2 µg, Abcam, ab4729) and anti-Pol II (2 ug, Santa Cruz Biotech, sc-899X). Immunoprecipitation, washing, elution, and reverse cross-linking were performed as previously described (Boogaard et al., 2013). Quality of the ChIP was assessed using the primers on locations of known cardiac enhancers (Boogaard et al., 2012). ChIP-qPCR was performed on a Roche LightCycler 480 System using Sybr Green detection. The ratio of ChIPed DNA normalized for input DNA was compared with that of a negative control region within *Hprt* and is presented as fold enrichment. Control and TAC samples were compared using Student's t-test. Statistical analysis of differences between the region of interest and the negative control region was done with Wilcoxon one-sample test, separately for control and TAC condition. Tested locations were chosen within the *Nppa-Nppb* locus. Primer sequences used in the experiment are listed below.

number	coordinates (mm9)	5'-primer	3'-primer
1	chr4:147327740+147327907	GCTTGAATGTGAATGCCCTC	GGGCTCACATCTCAAACTGC
2	chr4:147331341+147331469	TTGTGACCATTGACCCAGTG	ATACTCTGGATGGGAGATCG
3	chr4:147334441+147334533	GAATGCTGCTACTCTATCTCC	TGTTCAGTGTCATCCAGAGG

4	chr4:147337055+147337151	TCTCTCAGATCCGTCACTTC	AGGTCATAGCCCAAACTCTC
5	chr4:147337649+147337760	ATGATGGCTGGAGGAGGTCT	TTTCGGTGTGGCAAGTTCTG
6	chr4:147337840+147337987	AGGGATAGGAGTGAAACCAG	CGCTTCCTGGGGTCACATT
7	chr4:147339938+147340027	AGCACTTTGCCCGTTCTTTC	ACGTGGAACCCAGAGGAGC
8	chr4:147343583+147343704	AGAACACAGCAGTATGGACG	AACTCTGTCACGTTAGCAGC
9	chr4:147350069+147350184	GGTGTGTGTGTGATGATTAGG	AGCCATTCAAACCCTTGTGC
10	chr4:147353113+147353189	GGGTCACTCTCACTATTCTG	TGAATGTGAGTGACCCCTCC
11	chr4:147353881+147353969	ATTCCGATGTCATTGCTCTCC	CAGAGATGCTGAGTGGCTTAC
12	chr4:147356406+147356568	GTTCAAGCCCAGCCACTAACT	ATAGGCGTGCAGAAATAAGGC
13	chr4:147358867+147359005	TTCTCCATCTCTGCCTTAACC	GAGGAAGTGAAGCTGAGGCAT
14	chr4:147359847+147359958	TGAATACTCCACACAGGCAC	CAGACCTTTTCACGGAGTAG
15	chr4:147360613+147360731	GTTTGGGCTGTAACGGTGAG	GCAGGAGTATGCCAGATAGC
16	chr4:147362077+147362196	CACAATCTCTTCCAAGCCAG	CAGGTGATGGCTCAAATGTG
17	chr4:147363823+147363995	TGCAAGTCAACCTGGTGGAG	ACAACGCTTATGATGGGTGTC
18	chr4:147367710+147367874	CTCAGCAGTCTTTGGTTTCG	AAGGCTTAGTCACTTGGGAG
19	chr4:147373820+147373972	TGTTAAAGGTGGACTGCCTG	GGGACACATACACCATTCTC
20	chr4:147374352+147374444	TAGCTCCAGCATGTGTACTCC	TGTCAGGGGCTCCAAATAAGG
21	chr4:147374890+147374962	AACCAGAGTGGGCAGAGACAG	TGATGGAGAAGGAGCCCATGC
22	chr4:147377093+147377173	ATGTGCGTGTGCCTGTGTA	TACTACTCGGGTTATCCTCC
23	chr4:147378646+147378742	CACCAAGGTCTGCATCAAAC	TGAGATGTCTGCATGTGACC
24	chr4:147382732+147382894	CAGATGAGGGACACTACTTC	CCTTCACCATTTACCGTCTC
25	chr4:147397438+147397598	CCAGACACACTGAACAGCAC	TGGCAGCAGGTTCTGGAATC
Neg(Hprt)	chrX:50361898+50362108	CAACCACTTACTTAGAGGTACT	TTAGCAATATGGACTGTGAGGG
Positive*	chr9:119378854+119379067	TTTGCAGAGGAGGCATGGTG	TCCTCCCTGCAGAAGGGCCT

* Positive control – Scn5a enhancer F9 (Boogaard et al., 2012).

BAC and vector constructs

Generation of BAC336-Katushka-Luciferase (BAC336-KL(I)) and BAC337-EGFP has been described previously (Horsthuis et al., 2008; Sergeeva et al., 2014). In short, BAC modification protocol (Gong et al., 2002) was used to replace the sequences cggcATGgatc and cccacgccagcATGggc at the translation start sites of *Nppb* and *Nppa* (ATG region) with *Katushka* and *Luciferase/EGFP,* respectively. In present study, BAC337EGFP was additionally modified with Katushka (*Nppb*). Using the same protocol (Gong et al., 2002), RP23-139J21(VI) harboring the sequence from -0.65 to +179 kb relative to the transcription start site of *Nppa* was modified with Luciferase (*Nppa*). Also, several truncated BAC336-KL constructs were generated: BAC336-KL-Δdown(II), BAC336-KL-Δpr*Nppb*(VII), BAC336-KL-Δinter(III), BAC336-KL-Δup(V) and BAC336-KL-Δ(-22). In short, two homologous arms (hom) around the targeted regions were amplified with the primers listed below, cloned into the pLD53.SC shuttle vector and used for two consecutive homologous recombination steps in Pir2^{+} bacteria. After both cointegration and resolution, correct recombination was verified by PCR screen using one primer outside a homology arm and another within the

Luciferase/Katushka/EGFP gene. The insertion of the reporter genes and the integrity of the recombinant BAC were confirmed by restriction digest. BAC336-KL(I), RP23-139J21(VI), BAC336-KL-Δ(-22), BAC336-KL-Δdown(II), BAC336-KL-Δpr*Nppb*(VII) DNA was purified using CsCl gradient (Gong et al., 2002). DNA was dialyzed against the injection buffer (10 mM Tris-HCl, pH7.5, 0.1 mM EDTA, 100 mM NaCl, 30 uM Spermine (Sigma), 70 uM Spermidine (Sigma)). BAC337-KE(IV), BAC336-KL-Δinter(III), BAC336-KL-Δup(V) DNA was purified using Nucleobond PC20 Kit (Macherey-Nagel) from the overnight culture. DNA was dialyzed against PBS. An aliquot of purified BAC was checked for degradation via digestion with SpeI and overnight electrophoresis, after which DNA was submitted for pronuclear injection. One or two lines per construct were generated and characterized for fetal and adult expression of the reporter genes.

homol. arm	deleted sequence (chr4)	Fw primer	Rv primer
A(*Nppa*)	cccacgccagcATGggc	CCTGACAGCTGAGCAGCAAG	TCGGGGCACGATCTGATGTT
B(*Nppa*)		TCCTTCTCCATCACCCTG	CCAATCCTGTCAATCCTACC
C(*Nppb*)	cggcATGgatc	TGGTGATGGTGGGTGTTGTTT	CAGAAGCGATGGGCCAGGCA
D(*Nppb*)		TCCTGAAGGTGCTGTCCCAGAT	AAGTGGCAAGGTAGGTGCTCAC
E(Δ(-22))	147,352,566-147,357,560	AGGAGTTTGGGATCAGTGCT	TCAGACTGGTGTCCTTGTGT
F(Δ(-22))		GCCTGTGTCTGTTCTGCCTA	GTGGCTTGGAACTCACTTTG
M(Δinter)	147,362,658-147,374,150	CATAGACACTCTAACCCACTC	CTGTGTAGCCTTGACTATCC
N(Δinter)		TGCTCTTCTAACATCCCTTGG	TCACATTCTTGCTGATTTGCC
O(Δdown)	147,376,982-147,417,481	TTCTGGTTAGAGCCTGGGTG	AGCCCTGAATGTGTTCTCTG
P(Δdown)		TCGGCCATTCTGCCAGAGTT	AAGCCCATTGCAGTTGGTCC
J(Δup)	147,234,647-147,357,560	GTTTCTCTGTGTAGCCCTGG	CCTGGTGTCCTAAACTCATCG
F(Δup)		GCCTGTGTCTGTTCTGCCTA	GTGGCTTGGAACTCACTTTG

To generate EYFP reporter vectors, 5 kb fragments from the *Nppa-Nppb* locus located at -22 kbp and -11 kbp position relative to *Nppa* were amplified from BAC336 (129 SvJ BAC library, Incyte, St Louis, Mo) and cloned into the pGL3basic reporter vector (Promega) modified to contain −638/+70-bp rNppa promoter and EYFP reporter. Primers and genomic coordinates of the putative enhancer regions were:

fragment	Coordinates (mm9)	primers
-22 kbp	chr4:147,352,574-147,357,566	Fw: AGCGGACACCCACATTTATC
		Rv: ACAGGCTGTTAGGTCAGTTG
-11 kbp	chr4:147,362,068-147,366,438	Fw: GTTCTGACCCAGGTTCTC
		Rv: GTCGTGTGATACTTTCCTGAA

Mouse -1.8/+0.28 *Nppb* promoter region (chr4:147358026-147360180) coupled to the bovine growth hormone gene (BGH) poly(A) signal were cloned in reverse orientation

into the pGL3basic-0.7kb*Nppa*-Luciferase reporter downstream Luciferase-polyA sequence. Linearized vector-free constructs were injected in the pronuclei of fertilized eggs, which were subsequently implanted into the oviducts of the FVB preudopregnant foster females to generate transgenic mouse lines.

Transverse aortic constriction

8-12 week-old wild-type or transgenic FVB/N male mice (littermates) were subjected for transverse aortic constriction (TAC) as described (Deel et al., 2011). For 95% confidence interval and the power 0.8, we used 8 animals per TAC group and 4 animals per control group. All mice were weighed, sedated with 4% isoflurane, intubated and connected to a pressure-controlled ventilator (Hugo Sachs Electronic, Harvard Apparatus). A gas mixture of O_2/N_2 (v/v = 1/2) containing 2.5% isoflurane was used to maintain anesthesia. Body temperature was kept at 37˚C and buprenorphine (50 µg/kg) was injected s.c. for postsurgical analgesia. Thoracotomy was performed through the first intercostal space and the aorta was constricted with a 7-0 silk suture, between the truncus brachiocephalicus and the arteria carotis communis sinistra. A 27 G needle was used to induce severe TAC. Age-matched unbanded animals were used as controls. After 4 weeks or if discomfort (dyspnoe, decreasing mobility) was diagnosed earlier, mice were anaesthetized in a CO_2/O_2 mixture, subsequently killed by cervical dislocation and heart tissue was collected. HW/TL ratio was calculated to confirm hypertrophic growth of the hearts. Ventricular apex was used for qPCR and the left ventricle was fixed for 4C and ChIP experiments. Ventricular tissue was dissected and apportioned for qPCR and ISH.

Myocardial infarction

Infarction was created in BAC336KL and pr*Nppb*-pr*Nppa*-Luc mice by permanent ligation of the left anterior descending coronary artery (LAD) according to the protocol described previously (De Celle et al., 2004) with slight modifications. For 95% confidence interval and the power 0.8, we used 12 animals per MI group and 4 animals per control group (taking into account a 65% rate of non-successful experiment, as defined from the experience in the laboratory). Male mice (littermates) mice were sedated with 4% isoflurane, shaved, analgesized subcutaneously with buprenorphine (0.068 mg/kg) and intubated on a heating mat to maintain body temperature. Anesthesia was maintained via ventilation by a Minivent volume controlled Mouse Ventilator (Hugo Sachs Electronic, Harvard Apparatus) with 2% isoflurane in O_2 (1L/min flow rate). Left thoracotomy was performed between the fourth and fifth intercostal space. Infarction was created by permanent ligation of the LAD by surpassing a BV-4 5mm taper point needle with a 9-0 Polyamid wire under the LAD, tied with a double knot. The thoracotomy and skin were closed with a C-1 12mm cutting needle with a 6-0

silicone coated brained silk wire. After 1 week or if discomfort (decreasing mobility) was diagnosed earlier, mice were anaesthetized in a CO_2/O_2 mixture, subsequently killed by cervical dislocation and heart tissue was collected. The border zone of the operated mice and a piece of left ventricles of the control mice were dissected and used for qPCR analysis.

RNA isolation and RT-qPCR

Ventricular tissue of control adult and TAC/MI operated mice was dissected and snap frozen in liquid nitrogen and stored at -80°C. Total RNA was isolated using Trizol Reagent according to the manufacturer's protocol (Invitrogen). 300 ng (fetal tissue) or 500 ng (adult tissue) of total RNA was used for reverse transcription with the Superscript II system (Invitrogen) and Oligo-dT as primers. Expression of different genes was assayed with quantitative real-time PCR using the LightCycler Real-Time PCR system (Roche Diagnostics, Almere, The Netherlands). Target quantities were determined with LinRegPCR and values were normalized to *Gapdh* and *Hprt* expression levels. Primers used:

gene	5'-Fw	5'-Rv
Nppa	TTCCTCGTCTTGGCCTTTTG	CCTCATCTTCTACCGGCATC
Nppb	GTCCAGCAGAGACCTCAAAA	AGGCAGAGTCAGAAACTGGA
Gapdh	GTCAGCAATGCATCCTGCA	CCGTTCAGCTCTGGGATGA
Hprt	TCCTCCTCAGACCGCTTT	CCTGGTTCATCATCGCTAATC
Miip	GAGCAGCATCAGGGTCAAAC	TCTTTTGTCCTGTGAGCTGG
Fv1	CCGCCACAGGATTGAAGTTG	AGTGCCCTCACCTTATCTGC
Mfn2	CTGCTTCAGGATCACCAGTA	GAGAGCAGGAGAGCATGTAG
Plod1	CTGCTTCAGGATCACCAGTA	GAGAGCAGGAGAGCATGTAG
2510039O18Rik	CATCACCCTCTTCCACCTCT	GCTGGGTGTCTTGTGCAAAC
Clcn6	TTCCAGCTCCAGGTCACATC	TCCTGGGAGTTGAAGAAGAG
Mthrf	TCTGGTGACAAGTGGTTCTC	GCCAGGTAACATCTACGAAG
Agtrap	CTTCTCTTGCTGCCTCGTCT	GCAGCGTCTGATGATGAGTC
Nppb in BAC-TG*	AGGGAGAACACGGCATCATTG	TCTCTTATCAGCTCCAGCAG
Luciferase	AGGTGGACATCACTTACGCT	CACTGCATACGACGATTCTG
Katushka	CTTCGACATCCTGGCTACC	TCGTATGTGGTGATCCTCTC
EG/YFP	ATCTTCTTCAAGGACGACGG	AGTTGTACTCCAGCTTGTGC

* Katushka cDNA was inserted in the BAC without its own polyA signal. To prevent miscalculation of the endogenous *Nppb* transcript due to the presence of additional copies of BAC DNA, we used the primers designed in the 5'-UTR and the 2nd exon of *Nppb*.

ISH

Non-radioactive *in situ* hybridization on sections was performed as described previously (Moorman et al., 2001). Embryos and hearts were fixed in 4% formaldehyde, embedded in paraplast and sectioned at 10 µm. The cDNA probes used were *Nppa* (Zeller et al., 1987), *Nppb* (Houweling et al., 2005), *Luciferase*, *Katushka and EYFP.* DIG-labeled *Luciferase* probe was generated from the HindIII linearized pSP-luc+NF vector (Promega) using T7 polymerase. Katushka cDNA was subcloned from pTurboFP-635C (Evrogen) into *pBluescript SK* vector, linearized with EcoRV and labeled with T3 RNA. Images were acquired with the Zeiss Axiophot microscope.

Lentiviral enhancer assay

Lentivirus development

Three fragments were PCR amplified from the mouse BAC336 clone containing the *Nppa* and *Nppb* loci (129 SvJ BAC library, Incyte, St Louis, Mo). The PCR fragments were subcloned into the lentiviral vector encoding the firefly luciferase reporter (pGreenFire Transcriptional Reporter Lentivector; System Biosciences, Mountain View, CA, USA). The lentiviral particles were produced by transfection of 293T cells with the 3 lentiviral packaging plasmids (i.e., pMDLg/pRRE, pRSV-Rev, and pMD2.VSV.G) using polyethylenimine 25 kDa (PEI, Brunschwik) at 1:3 ratio (DNA:PEI). The supernatant from 293T cells containing the lentiviral particles was collected 48 and 72 h after transfection and concentrated by centrifugation in Amicon Ultra-15 Centrifugal Filter Unit (Millipore, UFC910008).

Cardiac myocytes, fibroblasts and luciferase assay

Rat neonatal ventricular myocytes (NRVM) and fibroblasts were isolated from 1-2-day-old Lewis rats, sacrificed by decapitation. Hearts were carefully taken out, atria removed and ventricles cut into small pieces in 1x Hank's balanced salt solution (HBSS, H4641). Ventricular tissue was enzymatically digested using 0.1% trypsin (Affymetrix, 22720) in 1x HBSS over night at 4˚C followed by using 0.1% collagenase I (Invitrogen, 17100-017) in 1x HBSS at 37˚C. Cells were centrifuged, passed through 70 µm cell strainer and pre-plated for 2h in Tung medium (Medium 199 (Invitrogen, 15630-056) supplemented with 10% fetal calf serum (Invitrogen, 26010-074), 10 mM HEPES (Invitrogen. 15630-056), 1x NEAA (Invitrogen 11140-035), 1:100 L-glutamine (Invitrogen, 25030-024), 0,35 g/L D-glucose, 2µg/ml vitamin B12 (Sigma V2876), 1:1000 gentamycin (Invitrogen, 15750-037), 1:100 penicillin/streptomycin (Invitrogen, 15070-063)) to separate myocytes from fibroblasts. After 1 h, cardiomyocytes were collected, counted, and plated at a density of 2,5*105 cells per well in 24-well plates coated with 1% fibronectin (CORNING, 356008). Overnight, NRVM were grown in Tung medium supplemented with 10% fetal calf serum and antibiotics. Fibroblasts were plated at a density $3,3*10^4$ cells per well of in 24-well plates in DMEM

(Invitrogen, 41965-039) supplemented with 10% fetal calf serum (Invitrogen, catalog no. 10270-106) and antibiotics. After 24h, the cardiomyocyte medium was changed to Tung medium without serum (M199 (Invitrogen, 15630-056), 10 mM HEPES (Invitrogen. 15630-056), 1x NEAA (Invitrogen 11140-035), 1:100 L-glutamine (Invitrogen, 25030-024), 0,35 g/L D-glucose, 2ug/ml vitamin B12 (Sigma V2876) and antibiotics). Cardiomyocytes and fibroblast were infected with the lentiviral vectors and the cells were incubated for 16h. Subsequently, the cells were exposed to 100 µM phenylephrine (Sigma, P6126-10G) for 48h. Cell extracts and luciferase assay were performed using *Renilla* Luciferase Assay System according to the protocol (Promega) in the Glomax-Multi detection system.

Statistical analysis of *in vivo* experiments

Direct comparison of *Luciferase* and *Katushka* mRNA levels between different BAC transgenic lines is not possible because the levels of expression in each line depend on the copy-number and the site of integration of the transgenic construct. Therefore, we normalized both fetal and adult ventricular *Luciferase*/*EGFP* and *Katushka* expression levels to that of fetal atrial *Luciferase*/*EGFP* (Figure 2C-D). Fetal atrial expression of *Luciferase/EGFP* was used as a reference because it is provided by the *Nppa* proximal promoter (Habets et al., 2002) present in all the BACs studied. All data in bar charts were represented as mean value ± SEM. The two-tailed Student's t-test was used to determine statistical significance ($p<0.05$) of the differences between the fetal (E17.5) and adult ventricular activity of the reporter genes, and the differences in normalized ventricular activity between different BAC transgenic lines and those of BAC336KL(I).

In TAC and MI experiments, factor correction (Ruijter et al., 2006) was used to remove variability between experiments performed in several sessions. The two-tailed Student's t-test was used to determine statistical significance ($p<0.05$) of genes upregulation. The upregulation of gene expression in the ventricles of TAC mice varied strongly, because of the variable severity of myocardial stress in response to aortic banding. Therefore, we used scatter plots and regression analysis to assess correlations between the levels of endogenous *Nppb* and *Nppa* and their reporters *Katushka* and *Luciferase*/*EGFP*, respectively. Correlation coefficient was used to determine the relationship between the levels of *Nppb* and *Katushka* mRNA, and the levels of *Nppa* and *Luciferase*/*EGFP* mRNA, which represents the response of the reporter genes to stress. T-test between the slopes of the regression lines was used to estimate the difference between the response of the reporter genes in two transgenic lines (Armitage et al., 2008).

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