Supplementary Methods and Results

Immunoblotting

Hearts were rapidly excised and snap-frozen in liquid nitrogen. Samples were homogenized in protein extraction buffer (50 mmol/L Tris-HCl; pH 7.5, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% (w/v) Triton X-100, 0.1 % β-mercaptoethanol, 1 mmol/L sodium orthovanadate, 50 mmol/L sodium fluoride, 5 mmol/L sodium pyrophosphate and protease inhibitor tablets (Complete®, Rosh Applied Science), 1 Tab/50 ml of buffer. One millilitre of extraction buffer was used per 100 mg of frozen tissue. The homogenates were centrifuged at 4 °C for 10 min at 13,000 g to remove insoluble fraction and the supernatant was used for electrophoresis. Protein content was estimated using a Bio-Rad Protein Assay Kit. Twenty micrograms of protein were electrophoresed on a 10 % polyacrylamide gel and then blotted onto a polyvinylidene difluoride membrane. After blocking with 5 % non-fat milk, membranes were incubated with primary antibodies and then appropriate secondary antibodies. Primary antibodies used were against phospho-GSK-3α/β (Ser21/9) (#9331), total-Akt (#9272), phospho-Akt (Ser473) (#9271) (all from Cell Signalling), total-GSK-3α/β (#05-903) (Upstate), total-glycogen synthase (#MAB3106) (Chemicon) and phospho-glycogen synthase (Ser 641 and 645) (#44-1092G) (BIOSOURCE) at dilutions of 1:1000. Proteins were visualized using an ECL kit (Amersham).

Real-time RT-PCR

The following primers were used (5'-3'):

β-actin forward CGTGAAAAGATGACCCAGATCA, reverse TGGTACGACCAGAGGCAT-ACAG; ANF forward ATTGGAGCCCAGAGTGGACTA, reverse CCTTTTCCTCCTTGGC-TGTTATC; α-skeletal actin forward TGACCACAGCTGAACGTGAGAT, reverse CAGGGA-GGAGGAAGAGGCAG; pro-collagen IαI forward CCTCAGGGTATTGCTGGACAAC, reverse TTGATCCAGAAGGACCTTGTTTG; pro-collagen IIIαI forward AGGAGCCAGTG-GCCATAATG, reverse TGACCATCTGATCCAGGGTTTC; fibronectin forward CCGGTG-GC TGTCAGTCAGA, reverse CCGTTCCCACTGCTGATTTATC;

Invasive Pressure-volume analysis

In vivo measurements were performed using a 1.4F micromanometer-tipped pressure-volume catheter (SPR-839, Millar Instruments) inserted into the cavity of the left ventricle. Mice were anesthetized at day 14 post-pump insertion with 2% isoflurane/oxygen inhalation and ventilated by tracheal intubation. The apex of the left ventricle was exposed via a trans-diaphragmatic approach and cannulated. Pressure-volume (PV) loops were acquired, as previously described.[1, 2] Pre-load pressure-volume relationship was determined by transient occlusion of the inferior vena cava.

Cardiac cine-MRI

Imaging was performed on a 3T Philips Achieva MR scanner using a dedicated cardiac software package (R2.5.3) and clinical gradient system (30mT/m, 200mT/m/ms). Mice were anesthetized and maintained under inhalational anesthesia via nose-cone (2% Isoflurane/oxygen). Rectal temperature was monitored and body temperature maintained at 37°C by warm air flow (using an adapted MRI-compatible heater system, SA Instruments Inc., Stony Brook, NY). Cine MRI was performed with prospective ECG triggering and respiratory gating using a spoiled gradient echo technique. For localization of the heart, a low-resolution gradient echo scout scan was performed in the coronal and transverse orientation. High-resolution (0.2 x 0.2 x 1mm) short axis, two and four chamber (biplane) views were then acquired using the following imaging parameters: FOV = 35mm, matrix = 160, slice thickness = 1mm, TR/TE = 11/5.3ms, flip angle = 20°, 1 line / RR interval, temporal resolution = 11ms, NSA = 2. Segment v1.702 software (http://segment.heiberg.se) was used for off-line analysis;

manual endocardial and epicardial mapping was performed for each 1mm slice at end-diastole and end-systole, from which ventricular volumes, ejection fraction and ventricular mass were determined. Ventricular wall thickness was measured at the mid papillary level in end-diastole and end-systole.

Reference List

(1) Burkhoff D, Mirsky I, Suga H. Assessment of systolic and diastolic ventricular properties via pressure-volume analysis: a guide for clinical, translational, and basic researchers. Am J Physiol Heart Circ Physiol (2005) **289(2)**:H501-H512.

(2) Tam CW, Husmann K, Clark NC, Clark JE, Lazar Z, Ittner LM *et al*. Enhanced vascular responses to adrenomedullin in mice overexpressing receptor-activity-modifying protein 2. Circ Res (2006) **98(2)**:262-270.

Supplement Table 1 Haemodynamic parameters in isolated Langendorff-perfused hearts of WT and KI mice subjected to acute isoproterenol perfusion. Peak (max), trough (min) and logEC_{50} values are given for heart rate (HR), left ventricular developed pressure (LVDP) and contractile performance (dP/dt). Mean values ± SEM, n= n=6/group, ns between groups in all categories.

	HR (bpm)				**LVDP (mmHg)**				**dP/dt (mmHg/s)**		
	Min	**Max**	**LogEC_{50}**		**Min**	**Max**	**LogEC_{50}**		**Min**	**Max**	**LogEC_{50}**
WT	387 ± 32	595 ± 13	-7.7 ± 0.7		68.1 ± 4.5	110.5 ± 12.3	-7.7 ± 0.7		2905 ± 400	7909 ± 714	-7.7 ± 0.7
KI	455 ± 33	552 ± 23	-7.7 ± 1.0		68.4 ± 3.5	104.3 ± 8.7	-7.7 ± 0.7		2923 ± 309	7363 ± 915	-7.7 ± 0.7

Supplement Table 2: Cardiac MRI. Structural and functional changes with chronic ISO/vehicle control at 2 weeks (Day 14) and in parallel experiments at a further 2 weeks following pump removal (Day 28). Respiratory- and ECG-gated images were acquired under isoflurane anesthesia. LVVI=left ventricular volume index, LVMI=left ventricular mass index, EDVI=end diastolic volume index, ESVI=end systolic volume index, SVI=stroke volume index, HR=heart rate, CI=cardiac index, EF=ejection fraction, LVd/s= mean left ventricular wall thickness diastole/systole, SWT=systolic wall thickening. n=6/group, *$p<0.05$ vs CON within genotype; †$p<0.05$ vs genotype at each time point.

	GSK-3 WT		**GSK-3 KI**		**GSK-3 WT**		**GSK-3 KI**	
	2 weeks Treatment (Day 14)				**2 weeks Recovery (Day 28)**			
	CON	**ISO**	**CON**	**ISO**	**CON**	**ISO**	**CON**	**ISO**
LVVI (ml/g)	2.8 ± 0.1	3.5 ± 0.2*	2.7 ± 0.1	3.1 ± 0.1	2.7 ± 0.1	2.9 ± 0.2	2.8 ± 0.3	3.1 ± 0.4
LVMI (mg/g)	2.9 ± 0.1	3.6 ± 0.2*	2.9 ± 0.1	3.2 ± 0.1	2.8 ± 0.1	3.0 ± 0.2	3.0 ± 0.4	3.3 ± 0.4
EDVI (µl/g)	1.6 ± 0.1	1.6 ± 0.2	1.7 ± 0.1	3.1 ± 0.2*†	1.4 ± 0.04	1.3 ± 0.1	1.7 ± 0.2	1.5 ± 0.3
ESVI (µl/g)	0.7 ± 0.1	1.3 ± 0.2*	0.8 ± 0.1	1.7 ± 0.2*	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.2
SVI (µl/g)	0.9 ± 0.04	0.3 ± 0.1*	0.9 ± 0.1	1.4 ± 0.1*†	0.8 ± 0.1	0.5 ± 0.1*	0.9 ± 0.1	0.8 ± 0.1
HR	511 ± 10	514 ± 8	517 ± 10	529 ± 7	516 ± 12	422 ± 8*†	508 ± 8	506 ± 8
CI (µl/min.g)	483 ± 21	164 ± 29*	458 ± 38	713 ± 61*†	406 ± 48	213 ± 27*†	440 ± 29	404 ± 51
EF (%)	58 ± 2	20 ± 4*†	53 ± 2	44 ± 4	57 ± 6	39 ± 5	53 ± 4	51 ± 4
LVd (mm)	0.9 ± 0.04	1.2 ± 0.1*†	0.9 ± 0.04	0.9 ± 0.03	1.0 ± 0.02	1.0 ± 0.04	0.9 ± 0.1	1.0 ± 0.03
LVs (mm)	1.4 ± 0.04	1.4 ± 0.1	1.4 ± 0.03	1.3 ± 0.1	1.5 ± 0.1	1.3 ± 0.04*	1.3 ± 0.1	1.5 ± 0.1
SWT (%)	58 ± 5	16 ± 2*	53 ± 8	44 ± 9	53 ± 4	29 ± 4*†	54 ± 8	53 ± 6