Supplementary Methods

Mice

The transgenic mice were generated by pronuclear injection of a ~6 kb cDNA fragment consisting of the α-myosin heavy chain (α-MHC) promoter, the rat full-length I-1 cDNA, and the simian virus 40 polyadenylation site. The F_0 generation was screened for integration of the transgene by Southern blot analysis with a 1.8 kb radio-labelled external probe including the C-terminal part of the α-MHC promoter and the I-1 cDNA after EcoRI digestion or by PCR: sense primer - 5′CGG CAC TCT TAG CAA ACC TC3′, located in the α-MHC promoter and a sense/antisense primer, 5′CCC TTG TTT CTG TTG CCC TA3′, located in the I-1 non-coding sequence (intron 4) and an antisense primer, 5′-TAA CCT CAT CAG CAC AGC TCA-3′, located in the I-1 coding sequence (exon 5). These primers amplified a ~550 bp product from the α-MHC-I-1 transgene and a ~400 bp product from WT I-1 as an internal control.

I-1-deficient mice (C57BL/6J) were a kind gift of Dr. A. Greengard.[1] Genotyping was performed by PCR with following primers: Sense - 5'CCC ACG GAA GAT CCA GTT TA3', located in the targeted exon 1, antisense, 5'CAC TTA GCC GGG AAA CTC TG3, located in the I-1 non-coding sequence (Intron 1), and antisense, 5'–TAA AGC GCA TGC TCC AGA CT-3', located in the neo-casette. Primers generated 380 and 280 bp fragments for the WT and the I-1 knockout gene, respectively.

Echocardiographic studies

A Vevo 660 system (Visual Sonics Inc., Toronto, Canada) with a 30 MHz single crystal mechanical transducer was used for transthoracic ultrasound biomicroscopy. Animals were kept under light temperature- and ECG-controlled anesthesia (isoflurane,

1.5 Vol%). 2-dimensional echocardiography images were obtained in a modified parasternal long and a short axis view at midpapillary muscle level at a frame rate of 60 Hz. Echocardiographic studies were performed in unstressed mice and after intraperitoneal injection of a maximally effective dose of the β-agonist dobutamine (20 µg/g) before and after chronic isoprenaline infusion. The thickness of the anterior and posterior wall and the epicardial and endocardial area of the left ventricular cavity were obtained in a short axis view in diastole and systole. Area shortening (AS) was calculated by: %AS = (LV end-diastolic area – LV end-systolic area) / LV end-diastolic area. The left ventricular mass (LVM) was calculated by the 2D-area length method: LVM = 1.05 • [5/6 • EPIs • (L + (AWThs + PWThs) / 2)] – [5/6 • ENDOs • L], where 1.05 is the specific gravity of muscle, EPIs is the epicardial area in systole, L is the parasternal long axis length, AWThs and PWThs are the anterior and posterior wall thicknesses in systole, respectively, and ENDOs is the endocardial area in systole.

Cardiac catheter and pressure-volume measurements

Mice were anesthetized by isoflurane inhalation and intubated. Anesthesia was maintained by continuous isoflurane inhalation (1.5% vol.). The mice were ventilated with a volume-controlled ventilator (Minivent, Harvard Apparatus, Holliston, MA, USA) with a tidal volume of 150 µl and a frequency of 150 breaths/minute. The end-exspiratory pressure (PEEP) was kept constant at 1 cm H_2O by immersion of the expiration tube into water. Body temperature was measured with a rectal probe and kept constant at 37 °C by a heating pad and an infrared bulb with feedback regulation (TKM-0903, FMI GmbH, Seeheim, Germany). The right carotid artery was accessed via a mid-cervical incision. After distal ligation and proximal clamping the artery was cut open and the catheter was inserted. After releasing the proximal clamp the catheter was secured by a ligation around the artery and advanced into the left ventricle. To assure

adequate preload, fluid supplementation was provided by intraperitoneal injection of physiological saline (30ml/kg). Baseline hemodynamic parameters were measured at least 10 min after saline injection when a stable reading of pressure and volume was obtained. After the hemodynamic measurements the offset of the volume signal (parallel volume, V_P) was determined by injection of 10 µl hypertonic saline (5%) via a 30G catheter into the left jugular vein. For vena cava occlusions the abdomen was opened by a midline incision (1 cm) and the V. cava was compressed with a cotton bud between the liver and the diaphragm. At the end of the experiment the catheter was withdrawn from the carotid artery, the mouse was killed by cervical dislocation and the blood was sampled in a heparinized syringe for determination of the blood conductance by use of the cuvettes provided by the manufacturer.

Western blot analysis, real-time RT-PCR, protein phosphatase assay and radioligand binding experiments

Western blotting was performed as described previously[2] with primary antibodies against inhibitor-1 (I-1, custom-made, Eurogentec, Brussels), Thr35 phosphorylated I-1 (Cell Signaling), Ser67 phosphorylated I-1 (custom-made, Eurogentec, Brussels), phosphatase type 1 (PP1c, 1/500, Upstate, Lake Placid, NY, USA), calsequestrin (Dianova, Hamburg, Germany), total phospholamban (PLB), Thr17-phosphorylated PLB and Ser16-phosphorylated PLB (PhosphoProtein Research, Bardsey, UK), total myosin binding protein-C (MyBP-C, kind gift from Wolfgang Linke, University of Münster, Germany), and Ser282 phosphorylated MyBP-C (Eurogentec, Brussels), troponin-I (Tn-I, Chemicon, Dundee, Scotland), Ser23/24 phosphorylated Tn-I (Cell Signaling Technology, Boston, MA, USA), phospho-glycogen synthase kinase 3β (GSK3β, Cell Signaling Technology, Boston, MA, USA), phospho-Akt, phospho-p44/42 MAPK and phospho-S6 ribosomal protein and eIF4E

(PathScan® Multiplex Western Cocktail Cell Signaling Technology). Total ryanodine receptor (RYR2), Ser2809 and Ser2815 phosphorylated RYR2 (kind gifts from Dr. A. Marks, Columbia University, NY). Real-time RT-PCR was performed with primers and probes specific for I-1 and ANP with the TaqMan system (ABIPrism 7900, Applied Biosystems). I-1 protein was enriched by an optimized trichloroacetic acid (TCA) extraction procedure as described before.[3]

Protein phosphatase activity was determined as described previously with [^{32}P]phosphorylase a as substrate.[2]

For radioligand binding assay frozen tissue from a whole mouse heart was powdered in liquid nitrogen and homogenized in 1600 µl β-adrenoceptor assay buffer (100 mM NaCl, 5 mM MgCl2, 50 mM Tris-HCl pH 7.4, 0.5 mM EDTA) by polytron homogenizer at maximal speed for 3x 20 sec. After low speed (15 min, 4°C, 2000 rpm) and subsequent high speed (20 min, 4°C, 14000 rpm) centrifugation the pellet was properly resuspended in 400 µl assay buffer and protein concentration was determined by Bradford assay. Total ß-adrenoceptor density was determined in crude membrane fraction as previously described. We incubated 100 µg crude membrane protein per assay with 3 nM of the non-specific β_1/β_2 antagonist 3H-CGP 12177 (4-[3-tertiarybutylamino-2-hydroxypropoxy]-benzimidazole-2-on) for 90 min at 37 °C. Nonspecific binding was determined with 10 µmol/l of the nonselective β-adrenoceptor antagonist nadolol. Bovine serum albumine was used as standard.

1. Allen PB, Hvalby O, Jensen V, Errington ML, Ramsay M, Chaudhry FA, Bliss TV, Storm-Mathisen J, Morris RG, Andersen P, Greengard P. Protein phosphatase-1 regulation in the induction of long-term potentiation: heterogeneous molecular mechanisms. *J Neurosci*. 2000;20:3537-43.

2. El-Armouche A, Rau T, Zolk O, Ditz D, Pamminger T, Zimmermann WH, Jackel E, Harding SE, Boknik P, Neumann J, Eschenhagen T. Evidence for protein phosphatase inhibitor-1 playing an amplifier role in beta-adrenergic signaling in cardiac myocytes. *Faseb J*. 2003;17:437-9.

3. El-Armouche A, Pamminger T, Ditz D, Zolk O, Eschenhagen T. Decreased protein and phosphorylation level of the protein phosphatase inhibitor-1 in failing human hearts. *Cardiovasc Res*. 2004;61:87-93.

Online table 1:

Cardiac catheterization and pressure-volume loop measurements.

	WT (n)	**KO (n)**
Heart rate (min^{-1})	611 ± 16 (7)	639 ± 11 (7)
LVEDD (µl)	17.6 ± 2.0 (7)	17.7 ± 1.9 (7)
LVESV (µl)	8.5 ± 1.6 (7)	8.2 ± 1.3 (7)
LVESP (mmHg)	85.2 ± 3.7 (7)	81.3 ± 2.9 (7)
LVEDP (mmHg)	5.9 ± 1.5 (7)	3.6 ± 0.4 (7)
Ejection fraction (%)	61.1 ± 3.6 (7)	60.3 ± 4.6 (7)
Cardiac output ($\mu l*min^{-1}$)	7361 ± 670 (7)	7447 ± 799 (7)
dp/dt_{max} ($mmHg*s^{-1}$)	10841 ± 747 (7)	10710 ± 702 (7)
dp/dt_{min} ($mmHg*s^{-1}$)	-9351 ± 507 (7)	-9936 ± 236 (7)
ESPVR ($mmHg*\mu l^{-1}$)	7.25 ± 1.4 (7)	5.47 ± 1.1 (6)
EDPVR ($mmHg*\ \mu l^{-1}$)	0.32 ± 0.04 (7)	0.42 ± 0.06 (6)
E_{max} ($mmHg*\mu l^{-1}$)	20.3 ± 4.1 (7)	17.6 ± 4.7 (6)
PRSW (mmHg)	94.0 ± 10.3 (7)	103.7 ± 8.6 (6)
dp/dt_{max}-EDV ($mmHg*s^{-1}*\mu l^{-1}$)	457.3 ± 63.3 (7)	478.3 ± 41.0 (6)

LVEDD	LV end diastolic volume
LVESV	LV end systolic volume
LVESP	LV end systolic pressure
LVEDP	LV end diastolic pressure
ESPVR	End systolic pressure volume relation
EDPVR	End diastolic pressure volume relation
PRSW	Preload-recruitable stroke work
E_{max}	maximal ventricular elastance

Online table 2:

Heart weight (HW in mg), body weight (BW in g) and Heart-to-body weight ratio (HW/BWin mg/g) in WT and I-1-KO mice treated for 7 days either with NaCl or isoprenaline (Iso).

	WT						I-1-KO					
	NaCl			Iso			NaCl			Iso		
	HW	BW	HW/BW	HW	BW	HW/BW	HW	BW	HW/BW	HW	BW	HW/BW
	128.0	25.6	5.0	160.0	27.1	5.9	141	23.3	6.1	163.0	30.5	5.3
	126.0	24.3	5.2	180.0	26.2	6.9	123.0	28.1	4.4	171.0	30.6	5.6
	153.0	27.6	5.6	176.0	26.5	6.6	126.0	29.8	4.2	167.0	30.3	5.5
	136.0	26.5	5.1	172.0	28.2	6.1	157.0	29.3	5.4	156.0	31.1	5.0
	139.0	23.7	5.9	194.0	30.2	6.4	131.0	28.8	4.5	174.0	30.6	5.7
	143.0	30.5	4.7	179.0	27.7	6.5	133.0	26.3	5.1	125.0	30.6	4.1
	134.0	26.5	5.1	197.0	33.3	5.9	121.0	26.6	4.5	154.0	27.6	5.6
	129.0	24.6	5.3	175.0	26.1	6.7	127.0	26.9	4.7	171.0	30.4	5.6
	142.0	25.0	5.7	158.0	27.1	5.8	170.0	28.3	6.0	160.0	27.0	5.9
	142.0	29.5	4.8	170.0	30.4	5.6	156.0	29.5	5.3	175.0	30.4	5.8
	139.0	29.9	4.6	167.0	29.1	5.7	120.0	26.7	4.5	160.0	28.8	5.6
	131.0	27.9	4.7	180.0	32.5	5.5	158.0	28.9	5.5	194.0	30.6	6.3
	123.0	27.6	4.5	174.0	27.6	6.3	140.0	29.4	4.8	175.0	31.7	5.5
	155.0	26.6	5.8	170.0	29.8	5.7				147.0	28.4	5.2
	125.0	28.0	4.5	161.0	30.7	5.2				178.0	31.9	5.6
	119.0	25.4	4.7	166.0	30.2	5.5				162.0	32.0	5.1
	148.0	29.8	5.0	145.0	31.7	4.6						
				180.0	33.2	5.4						
				188.0	33.5	5.6						
M	**136**	**27.0**	**5.1**	**173**	**29.5**	**5.9**	**138.7**	**27.8**	**5.0**	**164.5**	**30.1**	**5.5**
N	**17**	**17**	**17**	**19**	**19**	**19**	**13**	**13**	**13**	**16**	**16**	**16**
SEM	**2.5**	**0.5**	**0.1**	**2.9**	**0.6**	**0.1**	**4.6**	**0.5**	**0.2**	**3.8**	**0.4**	**0.1**

Online table 3:

Western blot quantification of abundance and/or phosphorylation state (P) of Ca^{2+} homeostasis and myofibrillar proteins in inhibitor-1-deficient (KO) and littermate wild type (WT) mice.

	Ctr	
	WT (n≥5)	KO (n≥5)
CSQ	1.00 ± 0.1	0.99 ± 0.1
NCX1/CSQ	1.00 ± 0.3	0.79 ± 0.2
MyBPC/CSQ	1.00 ± 0.1	1.24 ± 0.2
P-MyBPC/MyBPC	1.00 ± 0.1	0.72 ± 0.1
TnI/CSQ	1.00 ± 0.1	0.86 ± 0.1
P-TnI/TnI	1.00 ± 0.1	1.05 ± 0.1

CSQ, calsequestrin; NCX1, Na^{+}/Ca^{2+}-exchanger; Serca2a, SR Ca^{2+}-ATPase; MyBP-C, myosin binding protein-C; TnI, troponin-I; *P<0.05 vs. WT Ctr.

Online table 4:

Western blot quantification of the phosphorylation state (P) of nodal points of hypertrophic pathways in inhibitor-1-deficient (KO) and littermate wild type (WT) mice.

	Ctr	
	WT (n≥5)	KO (n≥5)
CSQ	1.00 ± 0.1	0.86 ± 0.1
P-GSK3β/CSQ	1.00 ± 0.1	1.33 ± 0.2
elF4E	1.00 ± 0.3	0.94 ± 0.2
P-Akt/elF4E	1.00 ± 0.1	0.79 ± 0.0
P-ERK 1/elF4E	1.00 ± 0.2	0.67 ± 0.1
P-ERK 2/elF4E	1.00 ± 0.2	0.77 ± 0.1
P-S6/elF4E	1.00 ± 0.1	1.23 ± 0.1

CSQ, calsequestrin; GSK3β, glycogen synthase kinase 3β, eIF4E (internal control), Akt, protein kinase B, ERK 1/2 p44/42 MAPK, mitogen activated kinases; S6, S6 ribosomal protein kinase.