Fig-S1

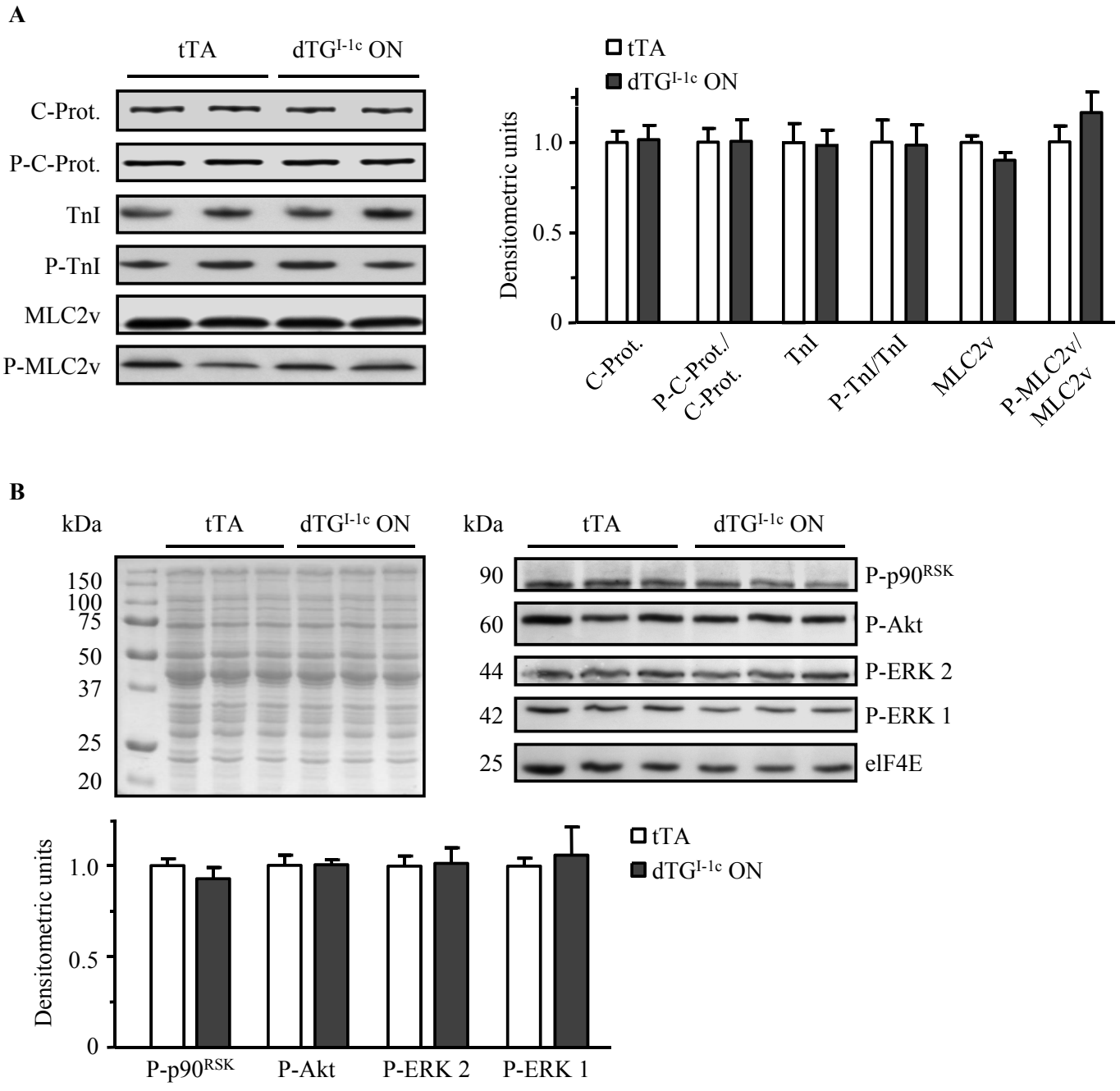
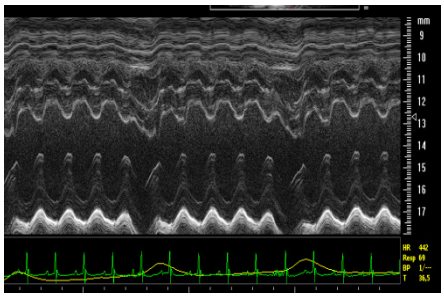
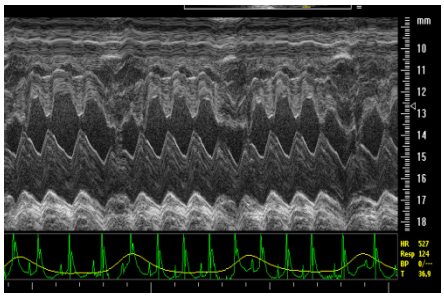


Figure S1 Western blot and quantification in dTG$^{I\text{-}1c}$ ON and tTA mice of (**A**) abundance and phosphorylation state (P) of myofibrillar proteins (n≥6 each). C-Prot.: myosin binding protein-C, TnI: troponin-I, MLC2v: ventricular regulatory myosin light chain (samples were normalized to CSQ) and (**B**) the phosphorylation state (P) of nodal points of hypertrophic pathways normalized to Ponceau staining (left panel, n=6 each). p90^{RSK}: p90 ribosomal S6 kinase, Akt: protein kinase B, ERK 1/2: p44 and p42 MAP kinases, elF4E (loading control): eukaryotic translation initiation factor 4E).

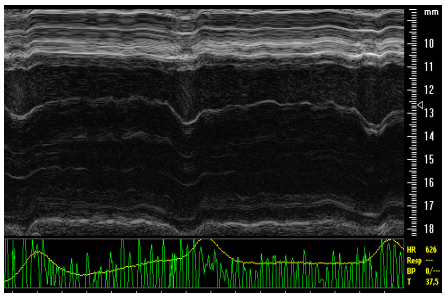
Fig-S2



Baseline



1. Iso (2 µg/g): Irregular sinus rhythm



2. Iso (2 µg/g): Sustained VTs

Figure S2 Echocardiographical detection of sustained (>12 sec) lethal cardiac arrhythmia in one dTG$^{I\text{-}1c}$ ON mouse after two times isoprenaline (Iso) injection (2 µg/g, i.p.). Panels depict M-Mode views and simultaneous ECGs.

Fig-S3

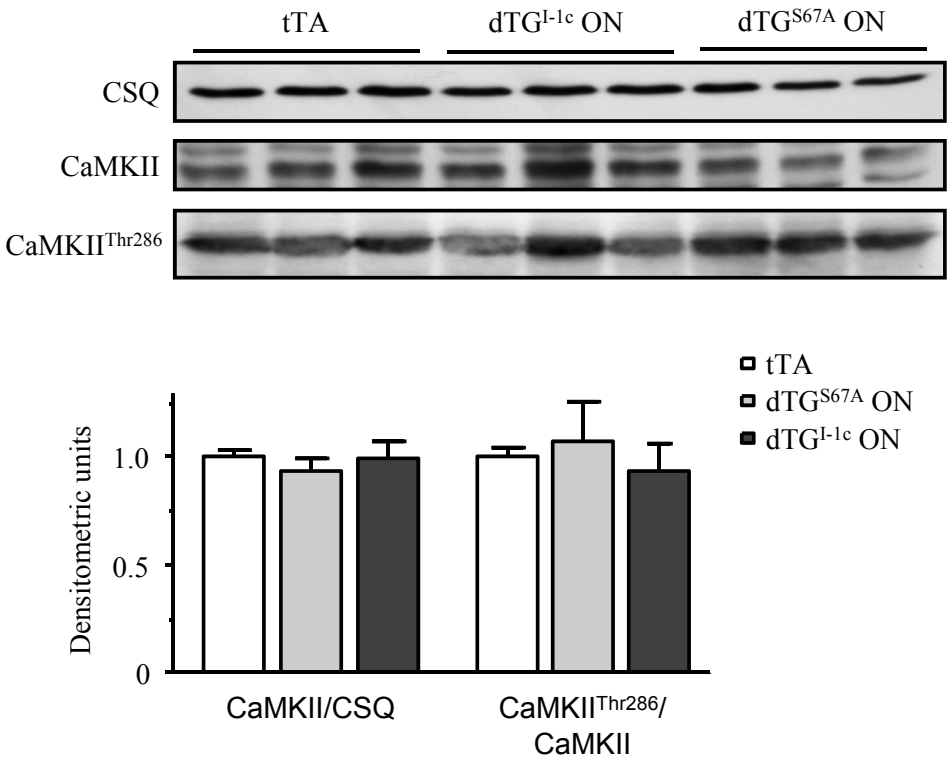


Figure S3 Neither I-1^{S67A} nor I-1c do affect autophosphorylation of CaMKII at Thr286. Abundance and statistical analysis of CaMKII protein and phosphorylation (P) at Thr286 determined by Western blotting in dTG^{S67A} ON /dTG^{I-1c} ON and tTA mice (n=8 for each group).

Fig-S4

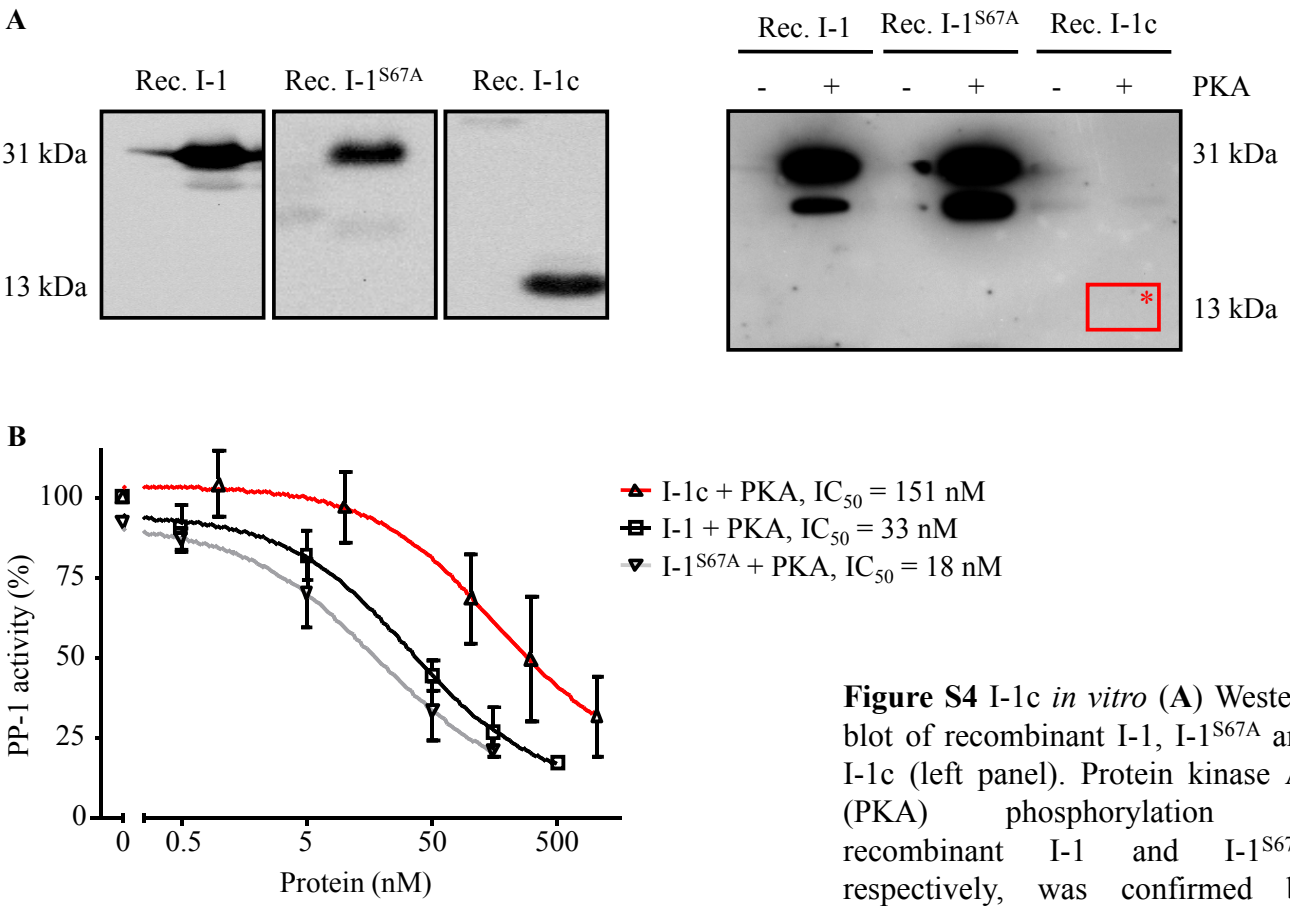


Figure S4 I-1c *in vitro* (**A**) Western blot of recombinant I-1, I-1^{S67A} and I-1c (left panel). Protein kinase A-(PKA) phosphorylation of recombinant I-1 and I-1^{S67A}, respectively, was confirmed by Western blot with an I-1 Thr35-phospho-specific antibody. (**B**) Phosphatase-1 (PP-1) activity was determined with ^{32}P-phosphorylase-A as a substrate in the presence of increasing concentrations of I-1/I-1^{S67A}/I-1c. (**C**) Western blot of recombinant I-1, I-1^{S67A} and I-1c in transfected HEK cell lysates. HEK cells were either single transfected with the responder construct or cotransfected with a vector expressing tTA (pTet-Off) in the presence or absence of doxycycline (Dox). I-1, I-1^{S67A} and I-1c were only detectable in the presence of tTA and absence of doxycycline. I-1^{S67A} was not detectable with the I-1 Ser67-phosphospecific antibody, whereas I-1c was neither detectable with the I-1 Thr35- nor Ser67-phosphospecific antibody (*). β-Actin was used as loading control.

Supplemental methods

Mice

The transgenic mice were generated by pronucleus injection of a ~0.8 kb (I-1c) or ~1.1 kb (I-1^{S67A}) cDNA fragment consisting of the TetO7/CMV_{min} promoter, mouse I-1c and I-1^{S67A} cDNA, respectively, and the simian virus 40-polyadenylation site (Figure 1A). The F0 generation was screened for integration of the transgene by Southern blot analyses with a 0.6 kb (I-1c) and 0.75 kb (I-1^{S67A}) [^{32}P]-dCTP-labelled probe, respectively, including the promoter and N-terminal part of the I-1c/I-1^{S67A} cDNA (nucleotide 1-600/754) after *Hind*III digestion or by PCR: (i) I-1c: sense-primer: 5'-ggtaccgccatggagcccgacaac-3', located in the promoter and exon1, an antisense-primer: 5'-gcggccgctcaattcagatcttcgctaataagcttttgctctgacagtggacttgag-3', located in the exon4 and in the c-myc-tag and as an internal control, a sense-primer: 5'-taacctcatcagcacagctca-3', located in the wild-type I-1 non-coding sequence (intron4) and an antisense-primer: 5'-cccttgtttctgttgcccta-3', located in exon5. These primers amplified a ~248 bp product from the I-1c transgene and a ~395 bp product from the WT I-1 as an internal control and (ii) I-1^{S67A}: the same sense-primer, as used for I-1c transgene, an antisense-primer: 5'- cccttgtttctgttgcccta -3', located in the exon5 and as internal control, the same primer pair was used as for I-1c. These primers amplified a ~304 bp product I-1^{S67A} and again a ~395 bp product from the WT I-1. I-1-deficient mice (C57BL/6J) were a kind gift of Dr. A. Greengard[1]. Genotyping of the I-1c and I-1^{S67A} Responder lines and the α-MHC-TetActivator line after crossing with I-1 KO mice for I-1 KO was performed by PCR as described previously[2]. The α-MHC TetActivator mice[3] were genotyped for the tTA by PCR: sense-primer: 5'-cgctgtggggcattttactttag-3', antisense-primer: 5'-catgtccagatcgaaatcgtc-3' and as an internal control, sense-primer: 5'-

caaatgttgcttgtctggtg-3' and antisense-primer: 5'-gtcagtcgagtgcacagttt-3'. Primers generated a 450 and 200 bp fragment for the tTA and as an internal control, respectively.

Isoprenaline administration

Isoprenaline (Sigma, Deisenhofen, Germany) was delivered to mice by subcutaneously implanted osmotic minipumps (Alzet, model 2002, Sulzfeld, Germay) that released isoprenaline in 0.9% NaCl for 14 days at a dose of 30 µg/g*day. Anaesthesia was performed with isoflurane (1.5 vol%). The pumps were immersed in 0.9% NaCl overnight at 37 °C to achieve a constant pumping rate from the beginning. Littermate mice of 9-12 weeks of age (male/female: ≥7/≥6 each group) were used in this protocol.

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

Western blotting was performed as described previously[4] 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 (PP-1, Upstate, Lake Placid, NY, USA), c-myc (Sigma, Saint Louis, Missouri, USA), calsequestrin (Dianova, Hamburg, Germany), total phospholamban (PLB), Ser16-phosphorylated PLB (Badrilla, Leeds, 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), Ser22/23-phosphorylated Tn-I (Cell Signaling Technology, Boston, MA, USA), CaMKII (BD Transduction Laboratories, USA) and Thr286-autophosphorylated CaMKII (Dianova, Germany), β-Actin (Sigma, Saint Louis, Missouri, USA), SR Ca^{2+} ATPase (SERCA2a, Santa

Cruz, California, USA), Na^+/Ca^{2+}-exchanger (NCX, Abcam, Cambridge, UK), Junctin (JCN, provided by Uwe Kirchhefer, University of Münster, Germany, origin[5]), ventricular regulatory myosin light chain (MLC2v, provided by the University of Texas Southwestern Medical Center at Dallas, TX, USA), Ser21/22-phosphorylated MLC2v (Axxora Deutschland GmbH, Germany, origin[6]), phospho-Akt, phospho-p44/42 MAP kinases and phospho-p90 ribosomal S6 kinase and eIF4E (PathScan® Multiplex Western Cocktail, Cell Signaling Technology, Boston, MA, USA). Total ryanodine receptor (RYR2, Acris, Hiddenhausen, Germany), Ser2809-phosphorylated RYR2 (Badrilla, Leeds, UK) and Ser2815-phosphorylated RYR2 (kind gift from Dr. A. Marks and Dr. Xander Wehrens, respectively, Columbia University, NY, USA). Antibody binding was detected by horseradish peroxidase-conjugated and alkaline-phosphatase-conjugated (RyR2-total) antibodies.

For the quantitative analysis of cardiac I-1, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), RNA from pulverized ventricular tissue was isolated using the SV Total RNA Isolation System (Promega, Madison, USA). 1 µg of RNA was transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, California, USA). Real-time PCR was performed with primers and probes specific for I-1, ANP and BNP with the TaqMan system (ABIPrism 7900, AppliedBiosystems, Foster City, USA). I-1, ANP and BNP and transcripts were normalized to GAPDH. Primers and probes were exon-spanning designed to eliminate genomic DNA amplification (Table 1). I-1 protein was enriched by an optimized trichloroacetic acid (TCA) extraction procedure as described before[7].

Protein phosphatase activity was determined as described previously with ^{32}P-phosphorylase-A as a substrate[4].

For radioligand binding assay 100 mg frozen ventricle tissue was powdered in liquid nitrogen and homogenized in 1600 µl β-adrenoceptor assay buffer (100 mM NaCl, 5 mM $MgCl_2$, 50 mM Tris-HCl pH 7.4, 0.5 mM EDTA) by polytron homogenizer at maximal speed for 3 x 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. Bovine serum albumine was used as standard. Total β-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 [^{3}H]-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.

Table 1 Primer and probes

Gene	Sense-primer 5'-3'	Antisense-primer 5'-3'	Probe 5'-3'
I-1c/I-1^{S67A}	ccacggaagatccagtttacg	gactgatcactggtcagcacaa	aggcggcggagcagattcggag
ANP	gtgcggtgtccaacacagat	gcttcctcagtctgctcactca	tgatggatttcaagaacctgctaga ccacctg
BNP	ccagtctccagagcaattcaa	agctgtctctgggccatttc	tgcagaagctgctggagctgataagaga
GAPDH	atgttccagtatgactccact cacg	gaagacaccagtagactccac gaca	aagcccatcaccatcttccagga gcgaga

Echocardiographic studies

A Vevo 770® 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 under basal conditions, before, in between and after chronic isoprenaline infusion, respectively. The thickness of the anterior and posterior wall, the left ventricular diameter and the epicardial and endocardial area of the left ventricular cavity were obtained in a short axis view in diastole and systole. Fractional area shortening (FAS) was calculated by: %FAS = (LV end-diastolic area – LV end-systolic area) / LV end-diastolic area • 100. The left ventricular mass (LVM) was calculated by the 2D-area length method: LVM = 1.05 • [5/6 • EPId • (L + (AwThd + PwThd) / 2)] – [5/6 • ENDOd • L], where 1.05 is the specific gravity of muscle, EPIs is the epicardial area in dystole, L is the parasternal long axis length, AwThd and PwThd are the anterior and posterior wall thicknesses in dystole, respectively, and ENDOs is the endocardial area in dystole. The left ventricular end-diastolic volume was calculated as LVEDV = 5/6 • (ENDOd • Ld). Ejection fraction was calculated by: %EF = (LVEDV – LVESV) / LVEDV • 100.

Histological analysis

Cross sections from the mid-papillary level of the heart (~2 mm thick) were fixed with 4% paraformaldehyde, dehydrated with graded concentrations of isopropylalcohol, embedded in paraffin, and cut into 4 µm slices. Sections were stained with hematoxylin/eosin or sirius red reagent. Microscopic images were taken at x 20 magnification (Zeiss IM-35, Jena, Germany)

for the determination of degree of fibrosis or cell area, which was determined by evaluation of nucleated transversely cut muscle fibers.

Isolation of cardiomyocytes

Mice were anesthetized in a gas chamber with isoflurane, after death by cervical dislocation, hearts were quickly excised, mounted on a Langendorff perfusion apparatus and retrogradely perfused with a nominally Ca^{2+}-free Tyrodes' solution containing (in mM) 113 NaCl, 4.7 KCl, 0.6 KH_2PO_4, 0.6 Na_2HPO_4 x $2H_2O$, 1.2 $MgSO_4$ x $7H_2O$, 12 $NaHCO_3$, 10 $KHCO_3$, 10 HEPES, 30 Taurine, 10 BDM, 5.5 glucose, 0.032 phenol-red for 4 min at 37 °C (pH 7.4). Then, 7.5 mg/ml liberase 1 (Roche diagnostics, Mannheim, Germany), trypsin 0.6% and 0.125 mM $CaCl_2$ was added to the perfusion solution. Perfusion was continued for about 3 min until the heart became flaccid and pale. The atria were cut off and ventricular tissue was collected in perfusion buffer supplemented with 5% bovine calf serum. It was then cut into small pieces and dispersed by repeated pipetting until no solid cardiac tissue was left. Ca^{2+} reintroduction was performed carefully via sedimentation steps in solution with stepwise increasing [Ca^{2+}] from 0.1 to 0.8 mM. For measurements, cells were freshly plated onto superfusion chambers, the glass bottoms of which had been treated with laminin to allow cell adhesion, which occurred within 15 min of plating. Cell yields were usually high with >75% viable cells. All investigations conformed to the "Guide for the Care and Use of Laboratory Animals" published by the US NIH (Publication No. 85-23, revised 1996).

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, Wittköpper K, Degenhardt F, Weinberger F, Didiè M, Melnychenko I, Grimm M, Peeck M, Zimmermann WH, Unsöld B, Hasenfuss G, Dobrev D, Eschenhagen T. Phosphatase inhibitor-1-deficient mice are protected from catecholamine-induced arrhythmias and myocardial hypertrophy. *Cardiovasc Res.* 2008;80:396-406.

3. Passman RS, Fishman GI. Regulated expression of foreign genes in vivo after germline transfer. *J Clin Invest.* 1994;94:2421-5.

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

5. Jones LR, Zhang L, Sanborn K, Jorgensen AO, Kelley J. Purification, primary structure, and immunological characterization of the 26-kDa calsequestrin binding protein (junctin) from cardiac junctional sarcoplasmic reticulum. *J Biol Chem.* 1995;270:30787-96.

6. Grimm M, Mahnecke N, Soja F, El-Armouche A, Haas P, Treede H, Reichenspurner H, Eschenhagen T. The MLCKmediated alpha1-adrenergic inotropic effect in atrial myocardium is negatively modulated by PKCepsilon signaling. *Br J Pharmacol.* 2006;148:991–1000.

7. 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.

8. Koop A, Goldmann P, Chen SR, Thieleczek R, Varsányi M.. ARVC-related mutations in divergent region 3 alter functional properties of the cardiac ryanodine receptor. *Biophys J.* 2008;94:4668-77.

9 Kong H, Wang R, Chen W, Zhang L, Chen K, Shimoni Y, Duff HJ, Chen WSR. Skeletal and cardiac ryanodine receptors exhibit different responses to Ca2+ overload and luminal ca2+. *Biophys J.* 2007;92:2757-70.

Table 1 Echocardiographical data from $dTG^{I\text{-}1c}$ ON and tTA mice (n=13 each), $^{\#}p=0.05$.

Parameters	tTA	$dTG^{I\text{-}1c}$ ON
BW (g)	26.0±0.6	27.6±1.0
HR (bpm)	487.8±8.4	480.6±5.7
AwThd (mm)	0.7±0.01	0.7±0.01
PwThd (mm)	0.7±0.01	0.7±0.01
LVEDD (mm)	4.7±0.1	4.6±0.1
EF (%)	52.6±1.2	$56.3\pm1.4^{\#}$
LVM (mg)	124.2±8.7	120.8±4.1
LVM/BW (mg/g)	4.4±0.2	4.4±0.1
LVEDV (µl)	112.4±6.0	108.1±3.6
LVEDV/BW (µl/g)	4.0±0.1	3.9±0.1

BW:	Body weight
HR:	Heart rate
AwThd:	Anterior wall thickness in diastole
PwThd:	Posterior wall thickness in diastole
LVEDD:	Left ventricular end-diastolic diameter
EF:	Ejection fraction
LVM:	Left ventricular mass
LVEDV	Left ventricular end-diastolic volume

Table 2 Echocardiographical data from dTG^{I-1c} ON and tTA mice before (day 0), after 4 and 14 days isoprenaline infusion with or without doxycycline (Dox) application; $^{\S}p<0.05$ day 14 vs. day 0, $^{\#}p<0.05$ vs. $tTA^{+/-10d\ Dox}$, $^{*}p<0.05$ vs. dTG^{I-1c} 10d OFF, $^{\&}p=0.05$ vs. $tTA^{+/-10d\ Dox}$.

Parameter	Day	tTA^{-Dox} (n=6)	$tTA^{+10d\ Dox}$ (n=7)	$tTA^{+/-10d\ Dox}$ (n=13)	dTG^{I-1c} 10d OFF (n=6)	dTG^{I-1c} ON (n=6)
BW (g)	0	--------	--------	28.0±1.1	--------	27.7±0.9
	4	--------	--------	28.6±1.0	--------	27.8±0.8
	14	29.9±1.7§	31.3±1.3§	30.7±1.0§	29.4±1.1§	30.6±1.4§
HR (bpm)	0	--------	--------	487.8±8.4	--------	480.6±5.7
	4	--------	--------	531.2±13.9	--------	527.0±10.2
	14	576.2±14.0§	622.9±14.8§	601.3±11.9§	591.5±14.8§	580.0±13.2§
LVM/TL (mg/mm)	0	--------	--------	7.7±0.4	--------	7.1±0.2
	4	--------	--------	9.3±0.5	--------	9.2±0.4
	14	10.8±1.1§	10.9±0.7§	10.9±0.6§	9.7±0.3§	9.4±0.8§
LVEDV/TL (µl/mm)	0	--------	--------	6.6±0.3	--------	6.4±0.2
	4	--------	--------	6.8±0.4	--------	6.5±0.2
	14	8.9±1.0§	8.1±0.6§	8.5±0.5§	7.7±0.3§	9.1±0.6§
AwThd (mm)	0	--------	--------	0.7±0.01	--------	0.7±0.01
	4	--------	--------	0.9±0.03	--------	0.9±0.02
	14	0.8±0.03§	0.9±0.03§	0.9±0.02§	0.9±0.02§	0.7±0.03$^{*/\#}$
PwThd (mm)	0	--------	--------	0.7±0.01	--------	0.7±0.01
	4	--------	--------	0.9±0.03	--------	0.9±0.02
	14	0.8±0.03§	0.9±0.03§	0.9±0.02§	0.9±0.02§	0.7±0.03$^{*/\#}$
EF (%)	0	--------	--------	52.6±1.2	--------	56.3±1.4$^{\&}$
	4	--------	--------	47.6±1.1	--------	43.5±1.3
	14	42.3±3.2§	44.0±2.4§	43.2±1.9§	45.3±3.0§	38.5±3.4§

TL: Tibia length

For abbr. see Table 1

Table 3 Different parameters in tTA mice after 14 days isoprenaline infusion with or without doxycycline (Dox) application from day 4.

Parameters	tTA^{-Dox} (n)	$tTA^{+10d\ Dox}$ (n)
Lung weight/TL (mg/mm)	10.2±0.2 (6)	10.5±0.6 (7)
Interstitial fibrosis (%)	11.2±1.9 (20/4)	15.0±2.3 (20/4)
Cross-sectional area (μm^2)	296.7±6.7 (140/4)	285.7±6.3 (149/4)
BNP/GAPDH mRNA	1.0±0.2 (6)	0.7±0.1 (7)

For abbr. see Table 2

Table 4 Echocardiographical data from aged dTG^{I-1c} ON and tTA mice (20 months, n=5), *p<0.05 vs. tTA.

Parameters	tTA	dTG^{I-1c} ON
BW (g)	36.3±1.3	46.6±3.7*
HR (bpm)	446.6±26.6	472.8±8.8
AwThd (mm)	0.9±0.1	0.9±0.1
PwThd (mm)	0.9±0.1	0.9±0.1
LVEDs (mm)	3.7±0.1	4.4±0.2*
EF (%)	45.7±1.8	35.4±4.4*
LVM (mg)	171.4±16.2	191.4±20.2
LVEDV (µl)	126.8±8.6	147.5±16.2

LVEDs: Left ventricular end-systolic diameter

For abbr. see Table 1

Table 5 Echocardiographical data from dTG^{S67A} ON and tTA mice at the age of 2 months (n=12 each).

Parameters	tTA	dTG^{S67A} ON
BW (g)	27.9±0.9	26.4±0.9
HR (bpm)	499.3±9.0	503.2±13.1
AwThd (mm)	0.7±0.02	0.7±0.02
PwThd (mm)	0.7±0.02	0.7±0.02
LVEDD (mm)	4.8±0.1	4.8±0.2
FAS (%)	46.8±1.4	47.1±2.3
EF (%)	52.1±1.8	52.8±2.3
LVM (mg)	136.0±5.7	133.6±10.4
LVM/BW (mg/g)	4.9±0.2	5.0±0.3
LVEDV (µl)	121.2±4.8	122.7±10.5
LVEDV/BW (µl/g)	4.3±0.1	4.6±0.3

FAS: Fractional area shortening

For abbr. see Table 1

Table 6 Cardiac catheterization measurements from dTG^{S67A} ON and tTA mice at the age of 3 months (n=6 each), $^{\#}$p=0.05.

Parameters	tTA	dTG^{S67A} ON
HR (bpm)	590.5±20.2	580.3±11.5
LVESP (mmHg)	93.4±2.2	85.0±3.0$^{\#}$
LVEDP (mmHg)	6.0±0.9	4.0±0.9
CO (µl/min)	6976.7±485.9	8263.0±478.3
dP/dt_{min} (mmHg*s^{-1})	-9861.2±652.4	-10024.3±416.3
Ea (mmHg/ml)	8.1±0.5	6.1±0.6$^{\#}$

LVESP: Left ventricular end-systolic pressure
LVEDP: Left ventricular end-diastolic pressure
CO: Cardiac output
dP/dt_{min}: Minimal rate of rise of left ventricular pressure
Ea: Arterial elastance
For abbr. see Table 1

Table 7 Ca^{2+} spark-characteristics from dTG^{S67A} ON and tTA mice, *p<0.05 vs. tTA.

Parameters	tTA (n=34/6)	dTG^{S67A} ON (n=39/6)
Spark-amplitude (F/F_0)	1.8±0.1	1.6±0.1
Spark-width (µm)	2.9±0.1	2.7±0.1*
Duration; $RT_{50\%}$ (%)	11.5±0.7	19.4±1.6*
SR Ca^{2+} leak (mF/F_0)	0.7±0.1	1.2±0.1*

Caffeine-induced SR Ca^{2+} release (F/F_0)	7.4±0.5 (20)	7.1±0.4 (22)

Table 8 Echocardiographical data from dTG^{S67A} ON and tTA mice before (day 0), after 4 and 14 days isoprenaline infusion with or without doxycycline (Dox) application; $^{\S}p<0.05$ day 14 vs. day 0, $^{\#}p<0.05$ vs. $tTA^{+/-10d\ Dox}$, $^{*}p<0.05$ vs. dTG^{S67A} 10d OFF, $^{\&}p=0.05$ vs. $tTA^{+/-10d\ Dox}$.

Parameter	Day	tTA^{-Dox} (n=4)	$tTA^{+10d\ Dox}$ (n=8)	$tTA^{+/-10d\ Dox}$ (n=12)	dTG^{S67A} 10d OFF (n=8)	dTG^{S67A} ON (n=7)
BW (g)	0	--------	--------	30.0±1.0	--------	30.7±1.0
	4	--------	--------	28.2±0.8	--------	30.1±0.9
	14	31.0±1.6	32.2±1.3§	31.8±1.0§	33.0±1.4§	32.8±1.8
HR (bpm)	0	--------	--------	489.8±7.9	--------	509.9±7.4
	4	--------	--------	479.9±49.3	--------	524.5±25.3
	14	613.0±10.3§	576.4±10.6§	588.6±9.2§	485.4±70.4	590.1±11.4§
LVEDD (mm)	0	--------	--------	4.5±0.1	--------	4.6±0.1
	4	--------	--------	4.5±0.1	--------	4.7±0.1$^{\#}$
	14	4.8±0.1§	4.9±0.1§	4.8±0.1§	5.1±0.1$^{\S/\#}$	5.2±0.2$^{\S/\&}$
LVM/TL (mg/mm)	0	--------	--------	7.0±0.3	--------	7.6±0.3
	4	--------	--------	8.6±0.4	--------	8.9±0.4
	14	8.9±0.1§	10.5±0.6§	10.0±0.7§	11.1±0.7§	10.1±0.7§
LVEDV/TL (µl/mm)	0	--------	--------	5.9±0.3	--------	6.6±0.4
	4	--------	--------	5.9±0.2	--------	6.7±0.3$^{\#}$
	14	7.6±0.5§	7.6±0.3§	7.7±0.3§	8.7±0.5§	8.2±0.7§
AwThd (mm)	0	--------	--------	0.7±0.02	--------	0.8±0.02
	4	--------	--------	0.9±0.03	--------	0.9±0.02
	14	0.8±0.02§	0.9±0.02§	0.9±0.02§	0.9±0.04	0.8±0.02$^{\S/\#}$
PwThd (mm)	0	--------	--------	0.7±0.02	--------	0.8±0.02
	4	--------	--------	0.9±0.03	--------	0.9±0.02
	14	0.8±0.02§	0.9±0.02§	0.9±0.02§	0.9±0.04	0.8±0.02$^{\S/\#}$
EF (%)	0	--------	--------	54.2±1.0	--------	51.5±1.0
	4	--------	--------	47.7±1.1	--------	41.8±1.8$^{\#}$
	14	43.9±5.4	46.3±2.4§	45.5±2.3§	43.2±2.2§	33.9±3.4$^{\S/\#/*}$

For abbr. see Table 1 and 2

Table 9 Echocardiographical data from aged dTG^{S67A} ON and tTA mice (15 months), *p<0.05 vs. tTA.

Parameters	tTA(n=6)	dTG^{S67A} ON (n=5)
BW (g)	35.9±2.0	35.2±1.9
HR (bpm)	446.7±12.2	457.0±10.4
AwThd (mm)	0.8±0.02	0.8±0.03
PwThd (mm)	0.8±0.02	0.8±0.03
LVEDs (mm)	3.3±0.1	3.9±0.2*
EF (%)	52.5±2.2	37.7±3.4*
LVM (mg)	117.9±4.8	140.3±13.5
LVEDV (µl)	96.0±3.4	112.2±0.2

For abbr. see Table 1