SUPPLEMENTAL MATERIAL

β-Arrestin-Biased AT_1 Agonist, TRV027 Causes a Neonatal-Specific Sustained Positive Inotropic Effect without Increasing Heart Rate

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DETAILED METHODS

Ethical Approval

All mice used in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. All experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of the Shinshu University and were approved by the Committee for Animal Experimentation (Approval number: 280035, 019034, 020044, and 17-009). All mice were provided free access to water and a standard diet throughout the experiments and were maintained in a temperature- (21–26°C) and humidity (50%–60%)-controlled room, under a 12 h photophase. All efforts were made to minimize animal suffering. Prior to surgery or euthanasia, mice were deeply anesthetized with subcutaneous administration of medetomidine (0.3 mg/kg), midazolam (4.0 mg/kg), and butorphanol (5.0 mg/kg). For UCG or ECG recording, neonatal or adult mice were anesthetized, respectively, with intraperitoneal or subcutaneous administration of 2,2,2-tribromoethanol (250 mg/kg), which has a minimum suppressing effect on cardiac contraction. (1)

Both male and female pups at P0–5 (n = 211) and adult male mice (8-week old) (n = 12) of C57BL/6 strain used for analysis were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Knock-in mice bearing a point mutation (ΔK210) in cTnT causing human congenital DCM with a BALB/c background were produced as previously described. (2,3) *cTnTΔK210* (n = 8) and WT mice were (n = 10) obtained by crossing the heterozygous mutant mice and used in the study. Experimental mice were genotyped at P3-4 using the sense primer: 5′-CCTAAGCCCCAGACCTATGC-3′ and antisense primer:

5′-GGTTCCTCCCCGTCCC-3′ with EmeraldAmp® PCR Master Mix (Takara Bio Inc., Kusatsu, Japan) for amplification.

Solutions

Modified Tyrode solution contained (in mmol/L): 136.5 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.53 $MgCl_2$, 5.5 HEPES, and 5.5 glucose (pH adjusted to 7.4 with NaOH). EDTA buffer contained (in mmol/L): 130 NaCl, 5 KCl, 0.5 NaH_2PO_4, 10 HEPES, 5 EDTA, and 10 glucose (pH adjusted to 7.8 with NaOH). Perfusion buffer contained (in mmol/L): 130 NaCl, 5 KCl, 0.5 NaH_2PO_4, 10 HEPES, 1 $MgCl_2$ and 10 Glucose (pH was adjusted to 7.8 with NaOH). Enzyme solution contained (in mg/mL): 0.8 collagenase type I, 0.065 protease, 1.2 hyaluronidase, 0.5 BSA, and 0.15 DNase I in the perfusion buffer. The pipette solution for Na^+-Ca^{2+} exchanger currents measurement with the patch-clamp method contained (in mmol/L): 120 D-glutamate, 10 NaCl, 0.1 EGTA, 5 $MgCl_2$, 5 HEPES, 10 KCl, and 3 ATP (pH adjusted to 7.4 with KOH)

UCG Recording

In order to assess the effect of vehicle (saline), TRV027 (3 mg/kg), or AngII (3 mg/kg) on cardiac contraction, the drugs were intraperitoneally injected into neonatal (P3-5) or adult (8-week old) mice 2 h before UCG recording unless otherwise indicated. UCG recording in *cTnTΔK210* or wild-type mice was performed at P4-6. In some experiments (Figs. 1 & Supple. Figs. 1 & 2), Atr (1 mg/kg) plus Prop (1 mg/kg) (plus Prazosin (Praz) only in Supplement Fig. 2) was added intraperitoneally. The UCG recording was repeated 10 min later. Additionally, in some experiments (Fig. 1), Cand (3 mg/kg) was intraperitoneally injected, and the recording was repeated 20 min later. Mice were placed

in the supine position on a heated stage (37 °C) and anesthetized with subcutaneous injection of 2,2,2-tribromoethanol. Transthoracic UCG was performed with a Vevo2100 linear array imaging system (FUIJIFILM VisualSonics Inc., Toronto, ON, Canada) equipped with a MicroScan transducer (MS-400 with 30 MHz for adult mice or MS-700 with 50 MHz for neonatal mice). The heart was viewed at the level of the anterior papillary muscle in the LV. In M-mode images, the following parameters were measured in 3 consecutive heart beats and averaged: interventricular septum end-diastolic thickness (IVSEDT), interventricular septum end-systolic thickness (IVSEST), LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVEDS), LV posterior wall end-diastolic thickness (LVPEDW), LV posterior wall end-systolic thickness (LVPESW), ejection fraction (EF), and percent fractional shortening (%FS). EF and %FS were calculated using the echocardiogram system.

ECG Recording

ECG (lead II) was recorded in anesthetized mice using a dual-channel bioelectric amplifier (Nihon Kohden, Tokyo, Japan) and Power Lab/4SP (ADInstruments, Sydney, Australia). After 2,2,2-tribromoethanol administration, P3-5 pups were placed in a supine position on a heating gel pad for small animals (SpaceGel^{TM}, BEX Co. Ltd., Tokyo, Japan). The cathode and anode were attached to their left and right arms, respectively, and their right pow was grounded. A mouse was covered with a small plastic box to maintain an ambient temperature of ~33 °C. ECG signals were obtained and analyzed using the LabChart7 software (ADInstruments, Tokyo, Japan). Several hundred heart beats were continuously measured and the average was determined. Then, different parameters were visually measured. QTc was calculated as follows: $QTc = QT/(RR/100)^{1/3}$, where QT and

RR are the QT and RR intervals in ms. (4,5)

Cell Culture and Preparation

Mouse NVCMs were isolated as previously reported, with some modifications. (6) Briefly, mice were anesthetized with subcutaneous injection of the aforementioned three agents plus heparin (5000 U/kg) and the chest was opened to expose the heart. After the descending aorta was cut off, the prewarmed EDTA buffer was injected into the right ventricular chamber with a syringe equipped with a 30-gauge needle (NIPRO CORPORATION, Osaka, Japan). The volume of injection was 2–10 mL depending on the age of the mice. The ascending aorta was then clamped, and the heart was placed in a 60 mm plastic dish with EDTA buffer. Afterwards, 2-10 mL of the EDTA buffer, 1–3 mL of the perfusion buffer, and 2–10 mL of the enzyme solution was sequentially injected into the LV chamber. All solutions were prewarmed to 37 °C before administration. The digested heart was dissected with fine scissors in the enzyme solution and suspended in a BSA solution (1 mg/mL BSA in perfusion buffer) at room temperature. After most CMs were precipitated, the upper half of the suspension was discarded and replaced with the same volume of the modified Tyrode solution. By repeating this procedure three times with a 5–10 min interval, the Ca^{2+} concentration in the solution gradually returned to 1.8 mM. Afterward, isolated CMs were treated with vehicle (saline) or TRV027 (3 μmol/L) for 2 h at 37 °C, and then subjected to the following analyses.

To measure mitochondrial respiration, mouse NVCMs were isolated from P0-2 mice using the Pierce™ Primary Cardiomyocyte Isolation Kit according to the manufacturer' s protocol. Isolated CMs were maintained in complete DMEM containing cardiomyocyte growth supplement for primary cell isolation for a few days depending on

subsequent experiments.

Human iPSC-CMs were generated from 253G1 iPSCs (7) by previously reported cardiac differentiation protocol with some modification. (8-10) Briefly, undifferentiated iPS cells were cultured on Matrigel-coated dishes in Essential 8 (E8) medium. When the cells reached 90% confluency, E8 medium was supplied with 1 μM CHIR99201. The next day (day 0), E8 medium was changed to cardiac differentiation medium (RPMI 1640 plus B27 supplement minus insulin plus L-glutamine with activin A). On day 1, bone morphologic protein 4 (BMP4; 10 ng/mL) and 1 μM CHIR99201 were added, followed by the addition of 1 μM XAV939 on day 3. On day 5, the differentiation medium was replaced without XAV939. On day 7, the medium was changed to RPMI 1640 with B27 supplement and replaced every other day. On day 17, the iPSC-CMs were harvested and re-plated on Matrigel-coated glass bottom dishes (Matsunami, Osaka, Japan) and maintained with RPMI 1640 plus B27 supplement. Twenty-four hours later, the Ca^{2+} transient was assessed in individual colonies of the cells.

Ca^{2+} Imaging

Twitch Ca^{2+} transients were measured as previously described. (11) Briefly, isolated mouse NVCMs were incubated in serum-free DMEM containing Fluo 4-AM (6 μmol/L), cremophor EL (0.01%), and BSA (0.02%) for 45 min at room temperature. NVCMs were transferred to a modified Tyrode solution in a 35-mm glass bottom dish coated with laminin. It was placed on the stage of an LSM 7 LIVE laser-scanning microscope with an Objective Plan-Apochromat 20×/0.8 (Carl Zeiss, Jena, Germany). Afterward, they were stimulated with field stimulation (i.e., 1 ms pulse of 50 V applied at 0.2 Hz). Human iPSC-CMs were labeled with Cal-520 (5 μmol/L) with cremophor EL

(0.04%) for 45 min. Field stimulation was conducted with a 10 ms pulse of 50 V applied at 0.3 Hz. Each image was taken with 128 × 128 pixels and digitized at 144 and 60 Hz for NVCMs and human iPSC-CMs, respectively. The time course of Ca^{2+} transients was assessed with a change in fluorescence from an ROI set in individual CMs, and an increment in fluorescence intensity from a baseline (ΔF) was normalized to the baseline intensity (F_0) ($\Delta F/F_0$). The time from simulation to the peak $\Delta F/F_0$ (time-to-peak) and the time from the peak to the half-maximum ($T_{1/2}$) were analyzed with a house-made program written with LabView ™ (National Instruments, Tokyo, Japan). The SR Ca^{2+} content was estimated as previously described (12): briefly, APs were elicited 10 times at 1 Hz with the pipette solution and modified Tyrode solution in current-clamp mode of the patch-clamp method. Myocytes were then promptly voltage-clamped at -70 mV and rapidly exposed to 10 mmol/L caffeine. The caffeine-induced inward Na^+-Ca^{2+} exchanger currents were integrated and converted to Ca^{2+} concentration (μmol/L cytosol) with the whole-cell capacitance and a value of 13 pF/pL.

Mitochondria Stress Test

The OCR of mitochondria was assessed using an Extracellular Flux Analyzer XFp (Agilent Technologies, Tokyo, Japan). Forty thousand mouse NVCMs suspended in complete DMEM with 10% fetal bovine serum for primary cell isolation were seeded onto a Seahorse XFp Cell Culture Miniplate (Agilent Technologies) a day before the assay. The following day, the cells were maintained in serum-free DMEM containing saline or TRV027 (3 μmol/L) for 1 h at 37 °C with 5 % CO_2, followed by further incubation with Seahorse XF base medium containing glucose (5.5 mmol/L), sodium pyruvate (2 mmol/L) and L-glutamine (1 mmol/L), and saline or TRV027 (3 μmol/L) for another hour

without CO_2. In some experiments, nifedipine (10 μmol/L) was added to the cells 30 min before the assay. Seahorse XFp Extracellular Flux Cartridge (Agilent Technologies) was hydrated with water overnight and with Seahorse XF Calibrant solution for 45-60 min at 37 °C without CO_2. A 10× concentrated solution (oligomycin A [20 μmol/L]), FCCP (20 μmol/L), and a mixture of antimycin A (10 μmol/L) and rotenone (10 μmol/L) in assay medium) was prepared and applied to the appropriate ports of the cartridge. After calibration in the cartridge, a prepared cell plate was loaded into the XFp analyzer and the assay was run to measure OCR. To assess the short-term (≤ 35 min) effects of TRV027, saline or TRV027 (30 μmol/L, 10 × concentrated solution) was applied to a port prior to oligomycin A, FCCP, and antimycin A/rotenone sequential injections. The OCR level was normalized to the total amount of protein in each well measured with the Pierce™ BCA Protein Assay Kit. The basal and maximum respiration rates were calculated by subtracting OCR after antimycin A and rotenone from that before oligomycin A or after FCCP, respectively.

Assessment of ROS Production in the Heart

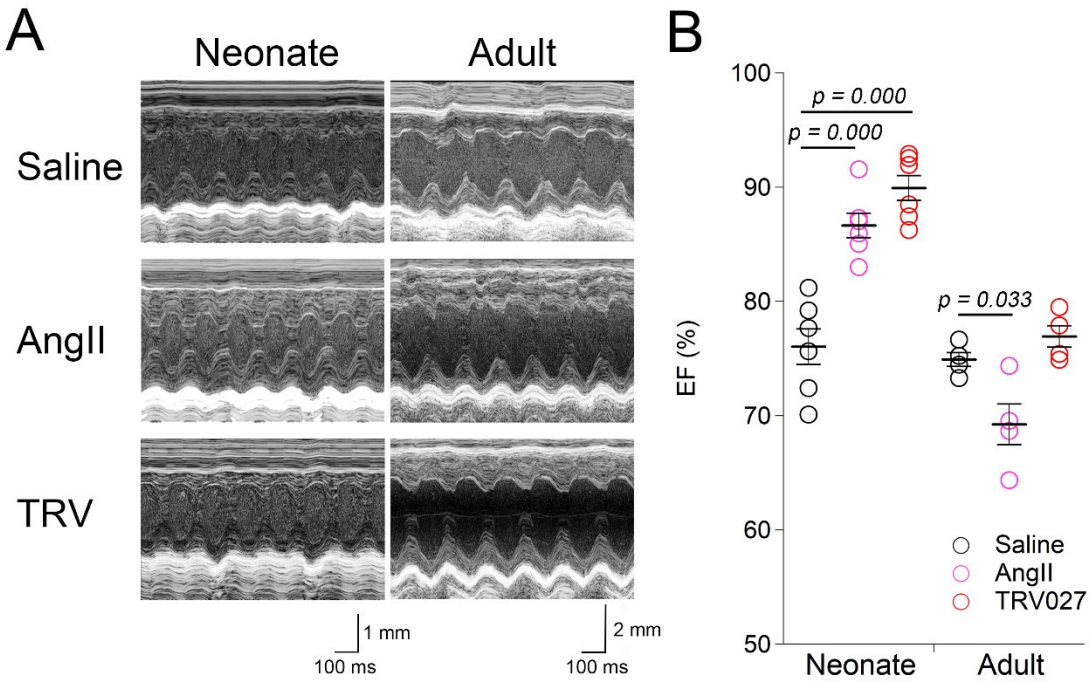
P1 pups were administered saline, AngII (3 mg/kg), or TRV027 (3 mg/kg) for 2 h or isoproterenol (30 mg/kg) for 1 h. Then, these pups were sacrificed, and their hearts were embedded in OCT compound and snap frozen in liquid nitrogen-cooled isopentane. DHE staining was conducted as described previously. (13) Briefly, cryosections (10-μm thick) on glass slides were stained with DHE (5 μmol/L) for 30 min at room temperature. After washing, coverslips were mounted on the slides with ProLong Diamond antifade mountant with 4',6-diamidino-2-phenylindole (DAPI). DHE and DAPI fluorescence was imaged by fluorescent microscopy (Zeiss AxioObserverZ1) with Cy5 and DAPI-specific

filter cubes with a 20× magnification lens (Carl Zeiss, Jena, Germany). DHE fluorescence was quantified in the region of interest of nuclei based on DAPI fluorescence with ImageJ (National Institutes of Health, Bethesda, MD).

Measurement of Plasma Catecholamine Levels and Serum Aldosterone Levels

P3-5 neonatal C57BL/6 mice were sacrificed 2 or 8 h after injection of saline or TRV027 (3 mg/kg). To measure adrenaline and noradrenaline levels in plasma, whole blood was collected from decapitated mice and transferred into a tube containing EDTA (final concentration 5 mM) to prevent clot formation. After centrifugation at 1,000 × g for 15 min, plasma was collected in sample tubes. Plasma adrenaline and noradrenaline levels were quantified using HPLC at the Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd. (Shizuoka, Japan). For measurement of aldosterone levels in serum, whole blood was collected by decapitation, stored at room temperature for 30 min, and centrifuged at 1,000 × g for 15 min. Serum was transferred to sample tubes. Plasma and Serum were stored at -80 °C until the procedure was performed. Measurement of aldosterone was performed using the Accuraseed Aldosterone Chemiluminescent Enzyme Immunoassay Kit (Fujifilm Wako Pure Chemical, Co. Ltd.) at the Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Japan.

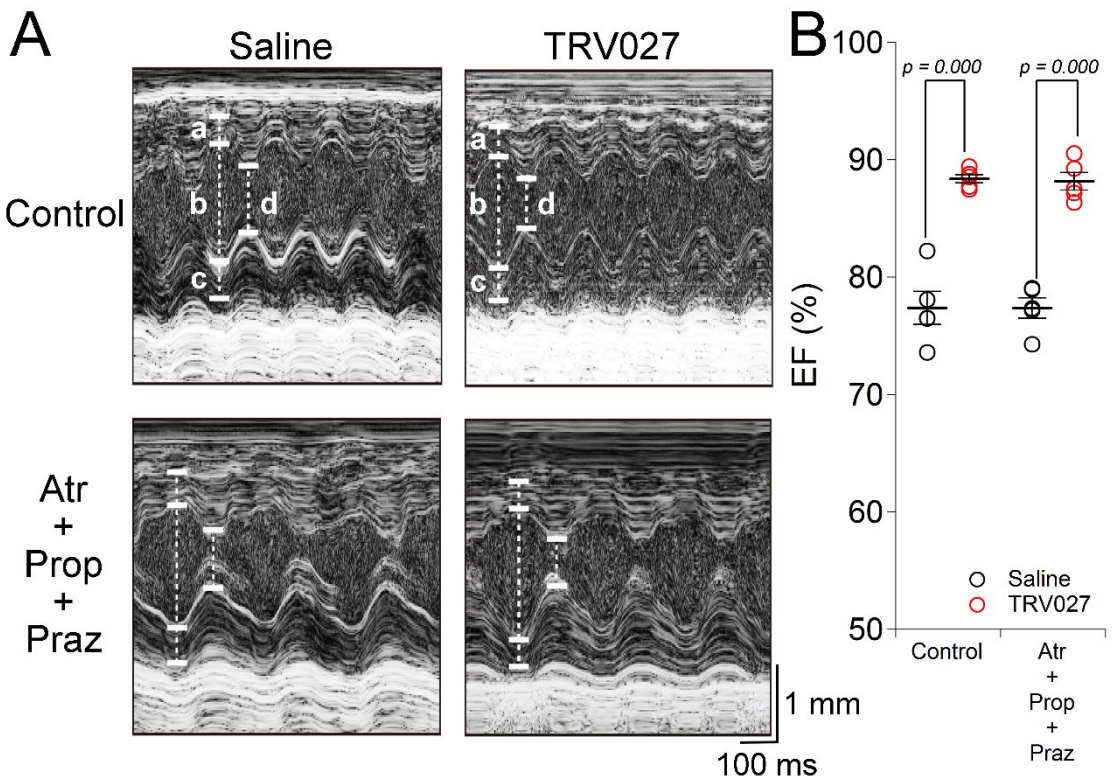
SUPPLEMENTAL FIGURE 1



Supplemental Figure 1: AT_1R/β-arrestin pathway-mediated positive inotropic effect of AngII on the neonatal but not adult murine heart

A. Representative M-mode ultrasound cardiogram (UCG) recorded from neonatal mice (left-hand column) and adult mice (right-hand column). UCG was recorded 2 h after injection of saline (top row), AngII (3 mg/kg) (middle row), or TRV027 (3 mg/kg) (bottom row). **B.** Ejection fraction (EF) values measured under each condition. Symbols indicate each data. Statistical significance was analyzed with one-way ANOVA followed by Dunnett's test. n = 4-6 in each group.

SUPPLEMENTAL FIGURE2



Supplemental Figure 2: The effect of TRV027 on the neonatal murine heart was not inhibited by Atr + Prop + Prazosin (Praz)

A. Representative M-mode ultrasound cardiogram (UCG) recorded of the neonatal murine heart. UCG in the upper panels was recorded 3 h after administration of saline (left-hand column) or TRV027 (3 mg/kg) (right-hand column). UCG was once again recorded 10 min after injection Atr + Prop + Praz (1 mg/kg each) (bottom panel). **B.** Ejection fraction (EF) values measured under each condition. Symbols indicate each data. Statistical significance was analyzed with one-way ANOVA followed by Tukey's test. n = 5 in each group.

SUPPLEMENTAL TABLES

Supplemental Table 1. Effect of angiotensin II and TRV027 on ultrasound cardiogram parameters in neonatal and adult mice

	Neonate			Adult		
	Saline	AngII	TRV027	Saline	AngII	TRV027
n	6	6	6	4	4	4
Body weight (g)	2.19 ± 0.27	2.38 ± 0.24	2.16 ± 0.26	22.2 ± 1.3	21.7 ± 0.1	23.6 ± 0.2
LV mass (mg)	8.70 ± 0.87	10.19 ± 1.90	9.45 ± 1.16	90.9 ± 8.2	79.3 ± 5.7	81.7 ± 5.2
IVSEDT (mm)	0.32 ± 0.02	0.38 ± 0.03	0.35 ± 0.02	0.71 ± 0.05	0.74 ± 0.01	0.72 ± 0.04
IVSEST (mm)	0.55 ± 0.02	0.69 ± 0.0.05	0.68 ± 0.04	1.23 ± 0.05	1.12 ± 0.05	1.21 ± 0.07
LVEDD (mm)	1.60 ± 0.07	1.50 ± 0.11	1.54 ± 0.06	3.30 ± 0.05	3.18 ± 0.08	3.36 ± 0.02
LVEDS (mm)	0.93 ± 0.04	0.71 ± 0.06**	0.65 ± 0.02**	1.89 ± 0.04	1.98 ± 0.08	1.86 ± 0.02
LVPEDW (mm)	0.38 ± 0.02	0.44 ± 0.04	0.42 ± 0.02	0.89 ± 0.04	0.85 ± 0.04	0.79 ± 0.04
LVPESW (mm)	0.62 ± 0.03	0.75 ± 0.04	0.76 ± 0.04	1.43 ± 0.06	1.23 ± 0.06	1.30 ± 0.04
EF (%)	76.0 ± 1.6	86.7 ± 1.1***	89.9 ± 1.1***	74.9 ± 0.6	69.2 ± 1.8*	76.9 ± 0.9
%FS (%)	41.7 ± 1.4	52.8 ± 1.4**	57.6 ± 1.7***	42.6 ± 0.5	37.8 ± 1.4*	44.6 ± 0.9

These parameters were measured 2 h after administration of saline, AngII (3 mg/kg), or TRV027 (3 mg/kg). Statistical significance was analyzed with one-way ANOVA followed by Dunnett's test. * and ***: $P < 0.05$ or 0.001 vs saline, respectively.

Abbreviations: LV, left ventricle; IVSEDT, interventricular septum end-diastolic thickness; IVSEST, interventricular septum end-diastolic thickness; LVEDD, LV end-diastolic diameter; LVEDS, LV end-systolic diameter; LVPEDW, LV posterior wall end-diastolic thickness; LVPESW, LV posterior wall end-systolic thickness; EF, ejection fraction; and %FS, percent fractional shortening.

Supplemental Table 2. Effect of atropine, propranolol, and candesartan on TRV027 effect on neonatal mouse heart

	Saline			TRV027		
	Control	A + P	A + P + C	Control	A + P	A + P + C
Body weight (g)		2.19 ± 0.17			2.22 ± 0.18	
LV mass (mg)		10.90 ± 0.94			9.93 ± 0.60	
n	5	5	5	5	5	5
IVSEDT (mm)	0.39 ± 0.02	0.39 ± 0.02	0.41 ± 0.01	0.37 ± 0.01	0.36 ± 0.01	0.38 ± 0.01
IVSEST (mm)	0.60 ± 0.03	0.60 ± 0.02	0.60 ± 0.03	0.70 ± 0.02	0.70 ± 0.03	0.61± 0.03
LVEDD (mm)	1.53 ± 0.03	1.64 ± 0.03	1.64 ± 0.03	1.50 ± 0.04	1.57 ± 0.03	1.53 ± 0.05*
LVEDS (mm)	0.88 ± 0.03	0.97 ± 0.03	1.03 ± 0.03	0.64 ± 0.02**	0.70 ± 0.01**	0.92 ± 0.02*
LVPEDW (mm)	0.47 ± 0.02	0.48 ± 0.01	0.49 ± 0.01	0.46 ± 0.02	0.48 ± 0.01	0.47 ± 0.02
LVPESW (mm)	0.73 ± 0.03	0.72 ± 0.03	0.71 ± 0.01	0.83 ± 0.02	0.83 ± 0.03	0.73 ± 0.03
EF (%)	77.7 ± 0.9	75.3 ± 1.1	70.9 ± 1.4	90.0 ± 0.4**	88.5 ± 0.6**	74.1 ± 1.0
%FS (%)	43.0 ± 0.8	41.1 ± 1.0	37.4 ± 1.1	57.3 ± 0.6**	55.3 ± 0.9**	39.8 ± 0.9

These parameters were measured 2 h after administration of saline or TRV027 (3 mg/kg) (Control). Then, atropine (1 mg/kg) (A) plus propranolol (1 mg/kg) (P) was added, and the measurements were repeated 10 min later (A + P). Furthermore, candesartan (3 mg/kg) (C) was added, and the measurements were repeated 20 min later (A + P + C). Statistical significance was analyzed with unpaired Student's t-test. * and **: $P < 0.05$ or 0.01 vs Saline, respectively.

Supplemental Table 3. Effect of TRV027 on ECG parameters

Time (h)		0	0.5	2	4	8
n	Saline			5		
	A + P			5		
HR	Saline	480.5 ± 24.2	445.3 ± 32.8	507.7 ± 10.0	502.7 ± 14.1	472.3 ± 16.1
	A + P	333.1 ± 42.2	343.1 ± 30.6	364.8 ± 28.6	299.3 ± 21.6	255.5 ± 0.8
Rhythm	Saline	NSR	NSR	NSR	NSR	NSR
	A + P	NSR	NSR	NSR	NSR	NSR
RR	Saline	126.2 ± 6.7	137.7 ± 10.0	118.4 ± 2.4	119.8 ± 3.6	127.6 ± 4.4
	A + P	190.6 ± 21.2	180.5 ± 15.8	168.1 ± 11.7	204.1 ± 12.4	234.9 ± 0.7
P	Saline	11.9 ± 1.1	11.1 ± 0.2	10.3 ± 0.6	11.0 ± 0.7	10.5 ± 0.4
	A + P	11.5 ± 0.6	12.0 ± 0.6	12.2 ± 1.4	13.4 ± 0.9	13.2 ± 0.5
PQ	Saline	45.3 ± 4.9	41.2 ± 3.5	41.0 ± 1.6	36.1 ± 0.6	36.6 ± 1.6
	A + P	40.6 ± 1.6	45.3 ± 4.0	45.8 ± 2.7	44.2 ± 1.5	45.0 ± 1.9
QRS	Saline	10.1 ± 0.2	10.8 ± 0.9	10.6 ± 0.5	9.6 ± 0.2	10.3 ± 0.5
	A + P	11.4 ± 0.6	11.5 ± 0.8	11.4 ± 0.8	11.0 ± 0.6	11.8 ± 0.6
QT	Saline	30.9 ± 0.3	31.0 ± 1.5	33.3 ± 1.3	27.8 ± 0.7	31.3 ± 1.5
	A + P	33.5 ± 1.8	35.3 ± 4.2	34.3 ± 2.1	35.1 ± 0.7	41.1 ± 3.1
QTc	Saline	27.6 ± 0.9	26.5 ± 0.6	30.6 ± 1.2	25.4 ± 0.6	27.8 ± 1.5
	A + P	24.5 ± 0.9	26.2 ± 2.2	26.5 ±1.5	24.7 ± 0.7	26.8 ± 2.0

At the indicated time after administration of saline or TRV027 (3 mg/kg) to neonatal mice, ECG (lead II) was recorded. The HR unit is bpm, whereas the unit for each duration is ms. Abbreviations: ECG, electrocardiogram; A + P, data obtained 10 min after administration of atropine (1 mg/kg) plus propranolol (1 mg/kg); HR, heart rate, NSR, normal sinus rhythm; and P, P wave duration. One-way ANOVA followed by Dunnett's test indicated no significant time-dependent changes in parameters. Two-way ANOVA

indicated that significant effect of A+P on HR ($p = 0.000$), RR ($p = 0.000$), P ($p = 0.004$), and PQ ($p = 0.020$) and significant interaction between Time and Drugs in RR ($p = 0.032$).

Supplemental Table 4. Effect of TRV027 on ultrasound cardiogram parameters of WT and *cTnTΔK210* mice

	WT		*cTnTΔK210*	
	Saline	TRV027	Saline	TRV027
Body weight (g)	4.29 ± 0.24	4.06 ± 0.22	3.49 ± 0.20	3.12 ± 0.19†
LV mass (mg)	9.64 ± 1.50	9.77 ± 1.52	10.54 ± 1.52	10.51 ± 0.72
n	5	5	5	5
IVSEDT (mm)	0.33 ± 0.03	0.31 ± 0.01	0.32 ± 0.03	0.30 ± 0.01
IVSEST (mm)	0.51 ± 0.03	0.56 ± 0.04	0.46 ± 0.04	0.51 ± 0.02
LVEDD (mm)	1.70 ± 0.06	1.66 ± 0.10	1.85 ± 0.11	1.87 ± 0.06
LVEDS (mm)	0.80 ± 0.02	0.65 ± 0.04	1.25 ± 0.08††	1.16 ± 0.04†††
LVPEDW (mm)	0.42 ± 0.03	0.41 ± 0.02	0.41 ± 0.03	0.38 ± 0.02
LVPESW (mm)	0.58 ± 0.03	0.69 ± 0.05	0.53 ± 0.03	0.56 ± 0.02
EF (%)	86.26 ± 0.8	92.1 ± 0.9***	64.6 ± 1.0†††	71.0 ± 0.6***†††
%FS (%)	52.5 ± 1.0	61.1 ± 1.6**	32.8 ± 0.6†††	37.7 ± 0.5***†††

These parameters were measured 2 h after administration of saline or TRV027 (3 mg/kg) to neonatal WT and *cTnTΔK210*. Statistical significance was analyzed one-way ANOVA followed by Tukey's test. *, **, and ***: $p < 0.05$, 0.01, and 0.001 vs. Saline, respectively. †, ††, and †††: $p < 0.05$, 0.01, and 0.001 vs. WT, respectively.

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