In Vivo Suppression of MiR-24 Prevents the Transition toward Decompensated Hypertrophy in Aortic-constricted Mice

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ABSTRACT

**Rationale:** During the transition from compensated hypertrophy to heart failure, the signaling between L-type Ca\(^{2+}\) channels (LCCs) in the cell membrane/T-tubules (TTs) and ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR) becomes defective, partially due to the decreased expression of a TT-SR anchoring protein, junctophilin-2 (JP2). MiR-24, a JP2 suppressing microRNA, is up-regulated in hypertrophied and failing cardiomyocytes.

**Objective:** To test whether miR-24 suppression can protect the structural and functional integrity of LCC-RyR signaling in hypertrophied cardiomyocytes.

**Methods and Results:** *In vivo* silencing of miR-24 by a specific antagonir in an aorta-constricted mouse model effectively prevented the degradation of heart contraction but not ventricular hypertrophy. Electrophysiology and confocal imaging studies showed that antagonir treatment prevented the decreases in LCC-RyR signaling fidelity/efficiency and whole-cell Ca\(^{2+}\) transients. Further studies showed that antagonir treatment stabilized JP2 expression and protected the ultrastructure of TT-SR junctions from disruption.

**Conclusions:** MiR-24 suppression prevented the transition from compensated hypertrophy to decompensated hypertrophy, providing a potential strategy for early treatment against heart failure.

**Keywords:** Hypertrophy, remodeling heart failure, myocardial contraction, Ca\(^{2+}\) signaling, hypertrophic cardiomyopathy

**Non-standard Abbreviations:**

- E-C: excitation-contraction
- \(I_{Ca}\): whole-cell Ca\(^{2+}\) current through L-type Ca\(^{2+}\) channels
- LCC: L-type Ca\(^{2+}\) channel
- NC: negative control
- NFAT: nuclear factor of activated T-cells
- RyR: ryanodine receptor
- SR: sarcoplasmic reticulum
- TAC: transverse aortic constriction
- TT: transverse tubule
INTRODUCTION

Transition from compensated hypertrophy to decompensated hypertrophy represents a key step in the development of heart failure. One of the hallmarks of this transition is the decreased strength of cardiac contraction. In heart cells, the contraction is initiated by periodic transient increases in intracellular Ca\(^{2+}\). During each Ca\(^{2+}\) transient, the Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels (LCCs) in the cell membrane and transverse tubules (TTs) triggers Ca\(^{2+}\) release from ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR). The structural integrity of the LCC-RyR signaling apparatus relies on a TT-SR linker protein, known as junctophilin-2 (JP2), which is down-regulated in all tested animal models and human specimens of decompensated hypertrophy and heart failure. Recently, we found that miR-24, a microRNA that suppresses JP2 expression, is up-regulated in hypertrophy/heart failure. Since over-expression of miR-24 suppresses both JP2 expression and E-C coupling efficiency, we hypothesized that miR-24 up-regulation is a key factor in the transition from compensated hypertrophy to heart failure.

In the present study, we tested this hypothesis by treating aorta-constricted mouse models of hypertrophy with a specific antagomir against miR-24. We found that in vivo silencing of miR-24 indeed protected the E-C coupling from structural and functional remodeling, preventing the transition from compensated hypertrophy to decompensated hypertrophy.

METHODS

We created a chronic mouse model of pressure-overload hypertrophy by transverse aortic constriction (TAC) surgery as described. In one of the TAC groups, we suppressed the expression of miR-24 by periodic injection (Online Figure I) of a chemically modified antisense oligonucleotide antagomir specific for miR-24. An oligonucleotide with mismatches to miR-24 was injected into another TAC group for negative control (NC). Single cardiomyocytes were isolated around 30 weeks after surgery for structural and functional analysis using electron microscopy, electrophysiology and confocal Ca\(^{2+}\) imaging as described. The methods are detailed in the online supplemental materials.

RESULTS

MiR-24 suppression prevented decompensation but not hypertrophy.

Compared with that in the sham-operated group, the miR-24 level in isolated ventricular myocytes exhibited a ~2.5-fold increase in the NC group, but not in the antagomir group (Fig. 1A), indicating that the up-regulation of miR-24 associated with TAC-induced hypertrophy was successfully suppressed by the antagomir treatment.

Echocardiographic measurements (Fig. 1B) showed that left ventricle hypertrophy developed 4 weeks after TAC surgery in our models (Fig. 1C). Around 15 weeks later, the fractional shortening became decreased (Fig. 1D), indicating a transition from compensated to decompensated hypertrophy. Notably, although in vivo antagomir treatment did not interfere with the development of hypertrophy (Fig. 1C), it did prevent the reduction of fractional shortening (Fig. 1D), indicating that the transition toward decompensated hypertrophy was effectively prevented by miR-24 suppression.
In vivo miR-24 suppression protected E-C coupling in cardiomyocytes.

To examine whether miR-24 suppression protected E-C coupling at the cellular level, we recorded the Ca\(^{2+}\) transient evoked by whole-cell LCC Ca\(^{2+}\) current (\(I_{Ca}\)) (Fig. 2A) under a condition (resting cardiomyocytes equilibrated in 2 mM extracellular Ca\(^{2+}\)) where the SR Ca\(^{2+}\) load was comparable among all groups (Online Figure II). In the NC group, TAC induced a significant reduction in Ca\(^{2+}\) transient amplitude without altering \(I_{Ca}\) density (Fig. 2B), leading to a decreased gain of E-C coupling (Fig. 2C) and reduced fraction of cell contraction (Fig. 2D). In contrast, the Ca\(^{2+}\) transient amplitude (Fig. 2B), the E-C coupling gain (Fig. 2C) and the fractional shortening (Fig. 2D) were well maintained after TAC in the antagomir group, indicating that miR-24 suppression protected the integrity of E-C coupling in hypertrophied cardiomyocytes.

Ca\(^{2+}\) transients are composed of numerous Ca\(^{2+}\) sparks evoked by LCC openings. Using unique loose-patch confocal imaging technology,\(^7,12\) we investigated the effect of the antagomir on LCC-RyR intermolecular Ca\(^{2+}\) signaling. To visualize single LCC activity, in the form of Ca\(^{2+}\) sparklets,\(^7\) we included in the pipette solution 20 mM Ca\(^{2+}\) and 10 \(\mu\)M FPL64176, an LCC agonist. Depolarization of on-cell patches evoked two distinct populations of local Ca\(^{2+}\) events (Fig. 3A): steep, ryanodine-sensitive Ca\(^{2+}\) sparks from RyRs; and flat, ryanodine-resistant but nifedipine-sensitive Ca\(^{2+}\) sparklets from individual LCCs.\(^7\) With comparable Ca\(^{2+}\) release duration (time-to-peak), the amplitude of Ca\(^{2+}\) sparks was significantly lower in the NC group but not in the TAC antagomir group (Fig. 3B), indicating that the TAC-induced decrease of local Ca\(^{2+}\) release flux was prevented by antagomir treatment. To quantify the fidelity of LCC-RyR coupling, we measured the percentage of the first detectable Ca\(^{2+}\) sparklets that successfully triggered Ca\(^{2+}\) sparks during the depolarization. The fidelity was decreased significantly in the NC group but unchanged in the antagonist group (Fig. 3C, upper). Also, the percentage of depolarization pulses that failed to trigger a Ca\(^{2+}\) spark (“miss index”) was increased in the NC group but not in the antagomir group (Fig. 3C, lower). We also quantified LCC-RyR coupling kinetics by the latency from the onset of a Ca\(^{2+}\) sparklet to the takeoff of a triggered Ca\(^{2+}\) spark (Fig. 3D). Exponential fitting of the coupling latency (Fig. 3E) showed that the time constant for LCC-RyR coupling was prolonged in the NC group but unchanged in the antagomir group (Fig. 3F). These results indicated that miR-24 suppression effectively prevented the decreased efficiency and slowed kinetics of LCC-RyR signaling in failing heart cells.\(^12,18\)

MiR-24 suppression prevented structural remodeling of E-C coupling apparatus.

Next, we checked the ultrastructural basis of LCC-RyR communication using transmission electron microscopy. Stereological analysis (Online Figure III) showed that the volume density and the surface area of TTs apparently coupled to SRs were dramatically decreased in the NC group but not in the antagomir group (Fig. 4A). The increase of bald TTs and decrease of junctional SRs were also suppressed by the antagonist. In failing heart cells, TT-SR junctions were displaced from the Z-line area, exhibiting increased junction-Z distance (Fig. 4B and C).\(^10\) The increased junction-Z distance was not observed in the antagonist group (Fig. 4C). The spatial span of individual TT-SR junctions is one of the determinants of LCC-RyR signaling efficiency.\(^10\) We found that the antagomir prevented the shrinkage of individual junction size (Fig. 4D). These data indicated that the defects of TT-SR junctions in failing cardiomyocytes were prevented by miR-24 suppression.

JP2 is a structural protein maintaining the morphology of TT-SR junctions and efficiency of LCC-RyR signaling.\(^8-10\) We found that the levels of both JP2 mRNA and protein, which were significantly decreased in the NC group, were unchanged in the antagonist group (Fig. 4E).

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DISCUSSION

E-C coupling becomes defective during the chronic transition from compensated hypertrophy to heart failure.\textsuperscript{12,20} In the present study, we show that \textit{in vivo} silencing of miR-24 in an aortic-constricted mouse model effectively protects cardiomyocytes from structural/functional disruption of E-C coupling and prevents the transition toward decompensated hypertrophy.

MiR-24 is expressed in cardiomyocytes and many other cell types and regulates multiple target proteins.\textsuperscript{19-22} We have recently shown that over-expression of miR-24, as observed in heart failure/hypertrophy models, suppresses JP2 expression and leads to defective E-C coupling in cardiomyocytes.\textsuperscript{15} In the present study, we show that the JP2 down-regulation is prevented by the miR-24 antagomir in TAC mice. As our bioinformatic analysis was not able to identify other miR-24 targets with known function related to E-C coupling, the stabilization of JP2 at least partially explains the protective effects of miR-24 suppression on TT-SR junctions and E-C coupling. Besides E-C coupling, whether other histological/molecular hallmarks of decompensation, such as fibrosis, are altered by miR-24 modulation still needs further in-depth studies.

The pathogenesis of hypertrophy and heart failure involves a variety of intracellular signaling cascades, including the calcineurin-nuclear factor of activated T-cells (NFAT) pathway, the calmodulin-dependent protein kinase pathway, and pathways involving other protein kinases.\textsuperscript{23,24} The calcineurin-NFATc3 pathway controls the microRNA cluster miR-23a–27a–24-2, which is up-regulated in hypertrophy.\textsuperscript{21,22,25} In this cluster, miR-23, but not miR-24 and miR-27, is found essential in the isoproterenol/aldosterone-induced cardiomyocyte hypertrophy.\textsuperscript{25} Agreeing with this report, our present study shows that miR-24 suppression \textit{in vivo} does not prevent TAC-induced hypertrophy. Excitingly, miR-24 suppression does prevent the structural and functional degradation of E-C coupling, indicating that miR-24 up-regulation is important in the transition from compensated hypertrophy to heart failure.

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DISCLOSURES
None

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FIGURE LEGENDS

Figure 1. In vivo miR-24 silencing in mouse hypertrophy models. A, Real-time PCR assay of miR-24 expression in sham (n = 4), NC (n = 3) and antagomir (n = 3) groups. B, Representative echocardiograms before and 25 weeks after TAC surgery in NC and antagomir groups. C, Left ventricle wall thickness (PWD, upper) and, D, fractional shortening (FS, lower) measured by echocardiography. *P <0.05 and **P <0.01 vs. sham; #P <0.05 and ##P <0.01 vs. NC.

Figure 2. The effect of miR-24 silencing on E-C coupling. A, Whole-cell patch-clamp and confocal imaging were used to measure I_{Ca} density (upper), Ca^{2+} transients (middle) and cell shortening (lower). B, I_{Ca} density and amplitude of Ca^{2+} transients were compared among sham (14 cells), NC (19 cells) and antagomir (18 cells) groups. C, Gain of E-C coupling calculated as the amplitude of Ca^{2+} transient per unit I_{Ca} density. D, Fractional shortening of cardiomyocytes measured by cell edge-detection of Ca^{2+} transients at 0 mV. *P <0.05 and **P <0.01 vs. sham; #P <0.05 vs. NC.

Figure 3. The effect of miR-24 silencing on LCC-RyR communications. A, Representative loose-patch confocal images (middle) and their time profiles (lower) in NC and antagomir groups, showing that LCC Ca^{2+} sparklets (blue arrows) triggered RyR Ca^{2+} sparks (red arrows) in a probabilistic manner during 70-mV depolarizations from resting potential (RP+70, upper). B, Amplitude (upper) and time-to-peak (lower) of triggered Ca^{2+} sparks in sham (187 events), NC (150 events) and antagomir (185 events) groups. C, LCC-RyR coupling fidelity (upper) was indexed by the percentage of the first apparent Ca^{2+} sparklet that successfully activated a Ca^{2+} spark during a patch depolarization. The miss index (lower) was defined as the percentage of depolarizing pulses that failed to trigger any Ca^{2+} spark. The percentages were first determined for each cell, and then averaged in the sham (59 cells), NC (52 cells) and antagomir (62 cells) groups. D, Example of a confocal image (upper) and its time profile (lower) from the antagomir group, illustrating the measurement of LCC-RyR coupling latency from the onset of a Ca^{2+} sparklet (blue arrow) to the takeoff of the triggered Ca^{2+} spark (red arrow). E, The distributions (bars) and their exponential fits (curves) of coupling latency in sham (109 events), NC (105 events) and antagomir (123 events) groups. F, Comparison of time constants (τ_L) of the LCC-RyR coupling latency among groups. *P <0.05 and **P <0.01 vs. sham; ##P <0.01 vs. NC.

Figure 4. Effect of miR-24 silencing on the structure of TT-SR junctions. A, Results of stereological analysis of volume density (upper) and surface area per unit volume (lower) of TTs coupled with SRs, bald TTs and junctional SRs (JSRs) in sham (183 images), NC (154 images) and antagomir (169 images) groups. B, Typical images showing the measurement of junction-Z distance (red double arrow) between the center of a junction cleft (red line) and its adjacent Z-line (blue line). C, Comparison of junction-Z distance (left) and its distribution (right) among sham (183 images), NC (154 images) and antagomir (169 images) groups. D, TT-SR junction length was measured as the curvilinear length of the junctional cleft (marked in yellow in B). E, Comparison of JP2 mRNA (left) and protein (right) expression levels among sham (n = 4), NC (n = 3) and antagomir (n = 3) groups. *P < 0.05 and ** P < 0.01 vs. sham; #P <0.05 and ##P <0.01 vs. NC.
Novelty and Significance

What Is Known?

- Cardiac excitation-contraction (E-C) coupling becomes defective during the transition from compensated hypertrophy to heart failure.

- The defective E-C coupling in cardiac myocytes of failing hearts could be partially attributed to the physical uncoupling between T-tubules and sarcoplasmic reticulum (SR) associated with the down-regulation of junctophilin-2 (JP2).

- MiR-24, a microRNA that suppresses JP2 expression, is up-regulated in hypertrophied/failing cardiomyocytes.

What New Information Does This Article Contribute?

- In vivo suppression of miR-24 does not interfere with transverse aortic constriction (TAC)-induced hypertrophy, but prevents the progressive decrease in the contraction of the left ventricle.

- MiR-24 suppression protects cardiomyocytes from TAC-induced defects in L-type calcium channel-ryanodine receptor Ca\(^{2+}\) signaling.

- Suppression of miR-24 prevents TAC-induced de-stabilization of TT-SR junctions in cardiac myocytes, presumably by maintaining JP2 levels.

During the transition from compensated hypertrophy to heart failure, cardiac E-C coupling becomes defective, partially due to the down-regulation of T-tubule SR anchoring protein - JP2. Because miR-24, which suppresses JP2, is up-regulated in failing cardiomyocytes, we tested whether suppression of miR-24 protects the integrity of E-C coupling. We found that in vivo silencing of miR-24 blocks the transition to decompensate hypertrophy while allowing compensated hypertrophy to persist in mice subjected to TAC. Cellular studies showed that miR-24 antagonir treatment protects cardiac myocytes from structural and functional remodeling of E-C coupling apparatus. These findings suggest that miR-24 may be a potential target in the treatment of heart failure.
Fig. 1

A

- Sham
- NC
- Antagomir

mR expression (mR-24:06)

0 2 4 6

* #

B

NC Antagomir

Before TAC

Week 25

C

PWD (mm)

0.0 0.3 0.6 0.9 1.2

D

FS %

0 10 20 30 40

Time (weeks after TAC)
Fig. 2

Panel A: Graph showing membrane potential changes with time and voltage.

Panel B: Comparison of current (I_{Ca}) profiles under different conditions (Sham, NC, Antagomir).

Panel C: Voltage-dependent gain (Gain) analysis showing statistical significance (*).

Panel D: FS (%) and cell length comparison under different conditions (Sham, NC, Antagomir) with statistical significance (#, *).
Fig. 4

A. Graph showing volume density with different treatments.
   - Sham
   - NC
   - Antagomir

B. Images showing junctions and junctional SR.

C. Histograms showing junction-Z distance.

D. Histograms showing junction length.

E. Bar graphs showing mRNA and protein expression levels.
   - mRNA expression (JP2/GAPDH)
   - Protein expression (JP2/GAPDH)
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**Online Methods**

**TAC surgery**
TAC surgery was performed on male C57BL/6 mice (8 weeks old) as described.¹ All experimental protocols were approved by the Peking University Institutional Committee for Animal Care and Use. Briefly, mice were anesthetized with a ketamine-xyalazine mixture (5:3, 1.32 mg/kg ip). A longitudinal cut was made in the proximal portion of the sternum. A 7-0 silk suture was tied around a 26-gauge needle and the aorta between the right innominate artery and left common carotid artery. After ligation, the needle was promptly removed. The sham procedure was identical except that the aorta was not ligated. To characterize the models, echocardiography was performed before and every two weeks after the surgery using a Vevo 770 ultrasound system (VisualSonics Inc., Toronto, ON, Canada) as reported.² The left ventricular fractional shortening was calculated as $FS = (LVD_d - LVD_s) / LVD_d$.

**Oligonucleotide Administration and Echocardiographic measurements**
A chemically-modified antisense oligonucleotide (antagomir)³ specific for miR-24 and a non-specific control oligonucleotide were synthesized by RiboBio Co., Ltd (Guangzhou, China). The sequence of the antagomir against microRNA-24 is: 5'-mC(s)mU(s)mGmUmUmCmCmUmGmCmUmGmAmAmCmUmGmAmG(s)mC(s)mC(s)mA(s)-Chol-3', where m is a 2'-OMe-modified nucleotide, (s) is a phosphorothiate linkage, and Chol is a cholesterol group linked through a hydroxyprolinol linkage. After ~2 weeks of recovery from the TAC surgery, the mice were treated with the oligonucleotides (diluted in 0.2 ml saline) at 80 mg/kg body weight through tail vein injection for 3 consecutive days. The 3-day treatment was repeated every 6-8 weeks (Online Figure II) according to the manufacturer’s suggestion. Saline was injected into sham-operated mice and a group of TAC mice for control purposes.

**MicroRNA and mRNA expression assays**
Total RNA and total microRNAs were extracted from cardiac tissues and cell samples using Trizol reagent (Invitrogen) and a microRNA isolation kit (mirVana, Ambion), respectively, according to the manufacturer’s instructions. The first strand cDNA was first synthesized by microRNA-specific reverse-transcription primers (RiboBio Co., Ltd) (for miR-24 ) or oligodT15 (for JP2) using SuperScript III Reverse Transcriptase (Invitrogen Crop). 10 ng of cDNA was applied for real-time PCR amplification using Brilliant II SYBR Green QPCR master mix (Stratagene), and the fluorescent signals were monitored by an Mx3000p Real-Time PCR System (Stratagene). The thermo-cycling program was as follows: 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s, and finally an additional dissociation step to ensure the specificity of amplification. The primers for microRNA sample amplification were provided by RiboBio Co., Ltd, and the primers for mouse JP2 and GAPDH were the following: mouse JP2 (forward: 5’-AGG CCG GTG CCA AGA AGA AG-3’; reverse: 5’-CGA TGT TCA GCA AGA TCA CCA-3’); mouse GAPDH (forward: 5’-ATC AAG AAG GTG GTG AAG CA -3’; reverse: 5’-AAG GTG GAA GAG TGG GAG TTG -3’). The small nuclear RNA U6 was used as a control for microRNA samples and GAPDH was used as a control for JP2 mRNA quantification.
**MicroRNA target prediction**

Putative targets of miR-24 in mouse were predicted by the TargetScan software by searching for target sites within the 3′ UTR of genes. Genes with at least one miR-24 target site which is conserved across mouse, rat and human were selected as miR-24 target candidates.

**Western blot**

Total proteins were extracted from isolated cells using lysis buffer containing 1% sodium deoxycholate, 10 mM Na₂HPO₄, 1% Triton X-100, 10% glycerol, 150 mM NaCl, 5 mM EDTA-Na₂, 20 mM Tris (pH 7.4), 0.1% SDS, 50 mM NaF, 1 mM Na₃VO₄, 1 mM PMSF, and protease inhibitor cocktail (Roche). The sample lysate was separated on 10% SDS-PAGE and then transferred to PVDF membrane. The membrane was incubated with a self-made rabbit polyclonal antibody against JP2 (1 µg/ml), which specifically recognized the rat JP2 p434-p447 peptide (QEILENSENSELLEPR). A horseradish peroxidase-conjugated GAPDH antibody (KangChen Bio-tech Inc., China) was used to measure the GAPDH content as a loading control.

**Whole-cell and loose-seal patch clamp**

Myocytes were bathed in an extracellular solution containing (in mM) 137 NaCl, 4 KCl, 2 CaCl₂, 1 MgCl₂, 1.2 NaH₂PO₄, 10 glucose, 0.02 tetrodotoxin and 10 HEPES, pH 7.35 adjusted with NaOH. The pipette electrode was filled with a solution containing (in mM) 110 CsCl, 6 MgCl₂, 5 Na₂ATP, 15 TEA-Cl, 10 HEPES and 0.2 fluo-4 pentapotassium, pH 7.2 adjusted with CsOH. I_{Ca} was activated at 10-s intervals using an EPC7 amplifier (List Medical Electronic, Germany). For loose-seal patch clamping, glass pipettes of 3-5 MΩ were filled with (in mM) 120 TEA-Cl, 20 CaCl₂, 10 HEPES, 0.01 tetrodotoxin and 10 µM FPL64176, pH 7.2 adjusted with TEA-OH. The membrane potential (V_m) was determined by proportionally dividing the test voltages between the pipette resistance and the seal resistance (15-20 MΩ). All experiments were performed at room temperature (25°C).

**Ca²⁺ imaging**

Intracellular Ca²⁺ dynamics were recorded using inverted confocal microscopes (LSM-510 or LSM-710, Carl Zeiss, Germany). Line-scan images were acquired at 3.84 ms/line for whole-cell recording and 0.47 ms/line for local Ca²⁺ detection. The Ca²⁺ concentration was either reported as the fluorescence normalized to the resting level (R = F/F₀), or calculated by \([\text{Ca}^{2+}] = k_d \cdot R/(k_d/C_0 + 1 – R)\), assuming a resting Ca²⁺ concentration C₀ = 100 nM and a dissociation constant k_d = 1.1 µM. The change in cell length was derived from edge-detection of the fluorescence.

**TEM and stereological measurement**

Cell samples were first fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M PBS buffer (pH 7.4). To specifically stain membrane, the samples were post-fixed in a mixture of 0.8% potassium ferrocyanide and 2% osmium tetroxide in 0.1 M sodium cacodylate buffer for 30 min. After dehydration in a graded series of alcohol, the samples were embedded in Spurr resin and sectioned with a glass knife on a Leica Ultracut R cutter. Thin sections were stained with uranyl acetate and lead citrate, then observed and randomly imaged under an FEI Tecnai G² 20 Twin
system. For stereological measurement of the volume density and surface area of TTs and JSRs, we followed Mobley’s stereological method.6

Statistical analysis
Results are expressed as mean ± SE. Statistical analysis was performed, where appropriate, using Student’s t-test, the Mann-Whitney rank sum test and two-way ANOVA with repeated measures. A value of $P < 0.05$ was considered significant.

References
Online Figures

Online Figure I. Design of *in vivo* experiment testing the effect of antagomir-24 on a TAC mouse model of hypertrophy and heart failure.

Online Figure II. Comparison of SR Ca$^{2+}$ load among sham, NC and antagomir groups. A, Typical images showing that the SR Ca$^{2+}$ load was measured as the amplitude ($\Delta F/F_0$) of 10 mM caffeine-induced Ca$^{2+}$ transients. B, Statistical results of SR Ca$^{2+}$ load from sham, NC and antagomir groups. Data from 59, 55 and 65 cells in 4 sham, 3 NC and 3 antagomir mice, respectively. Resting ventricular myocytes were equilibrated with 2 mM extracellular Ca$^{2+}$. Under this condition, moderate differences in SERCA activity would not make differences in steady-state SR Ca$^{2+}$ load.
Online Figure III. Stereological analysis of TT-SR junctions in human heart failure. The representative TEM images illustrate the stereological analysis of myocytes. The grid lines were spaced 0.167 μm apart. The closed and open circles denote examples of point counts for volume density and intersection counts for surface area per unit volume, respectively.