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Circ Res. Author manuscript; available in PMC 2014 February 15.

Published in final edited form as:
Circ Res. 2013 February 15; 112(4): 601–605. doi:10.1161/CIRCRESAHA.112.300806.

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 antagomir in an aorta-constricted mouse model effectively prevented the degradation of heart contraction but not ventricular hypertrophy. Electrophysiology and confocal imaging studies showed that antagomir treatment prevented the decreases in LCC-RyR signaling fidelity/efficiency and whole-cell Ca^{2+} transients. Further studies showed that antagomir 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

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DISCLOSURES
None

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INTRODUCTION

Transition from compensated hypertrophy to decompensated hypertrophy represents a key step in the development of heart failure.[1,2] One of the hallmarks of this transition is the decreased strength of cardiac contraction.[1,3] 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).[4–7] The structural integrity of the LCC-RyR signaling apparatus relies on a TT-SR linker protein, known as junctophilin-2 (JP2),[8–10] which is down-regulated in all tested animal models and human specimens of decompensated hypertrophy and heart failure.[10–14] Recently, we found that miR-24, a microRNA that suppresses JP2 expression, is up-regulated in hypertrophy/heart failure.[15] Since over-expression of miR-24 suppresses both JP2 expression and E-C coupling efficiency,[15] 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[16] 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.[17] 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[16] 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,[10] electrophysiology[12] and confocal Ca^{2+} imaging[12] 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

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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 μM 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 antagomir 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 antagomir. 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 antagomir 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 antagomir group (Fig. 4E).

DISCUSSION

E-C coupling becomes defective during the chronic transition from compensated hypertrophy to heart failure.[12,20] In the present study, we show that *in vivo* silencing of miR-24 in an aortic-constricted mouse model effectively protects cardiomyocytes from

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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.[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 carrdiomyocytes.[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.[23,24] The calcineurin-NFATc3 pathway controls the microRNA cluster miR-23a~27a~24-2, which is up-regulated in hypertrophy.[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.[25] Agreeing with this report, our present study shows that miR-24 suppression *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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Drs. Xue-Mei Hao and Ying-Chun Hu for professional technical support.

SOURCES OF FUNDING

This study was supported by the 973 Program of China (2011CB809101), the National Natural Science Foundation of China (81070196, 81030001, 81121061), the Strategic Priority Research Program of Chinese Academy of Sciences (XDA01020105), the Program for New Century Excellent Talents in University, the Beijing Talents Foundation, and the NIH, USA (R01 TW007269).

Non-standard Abbreviations

E-C	excitation-contraction
I_{Ca}	whole-cell Ca^{2+} current through L-type Ca^{2+} channels
JP2	junctophilin-2
LCC	L-type Ca^{2+} channel
NC	negative control
NFAT	nuclear factor of activated T-cells
RyR	ryanodine receptor
SR	sarcoplasmic reticulum.

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TAC	transverse aortic constriction
TT	transverse tubule

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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 ventricule.
- 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 antagomir 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.

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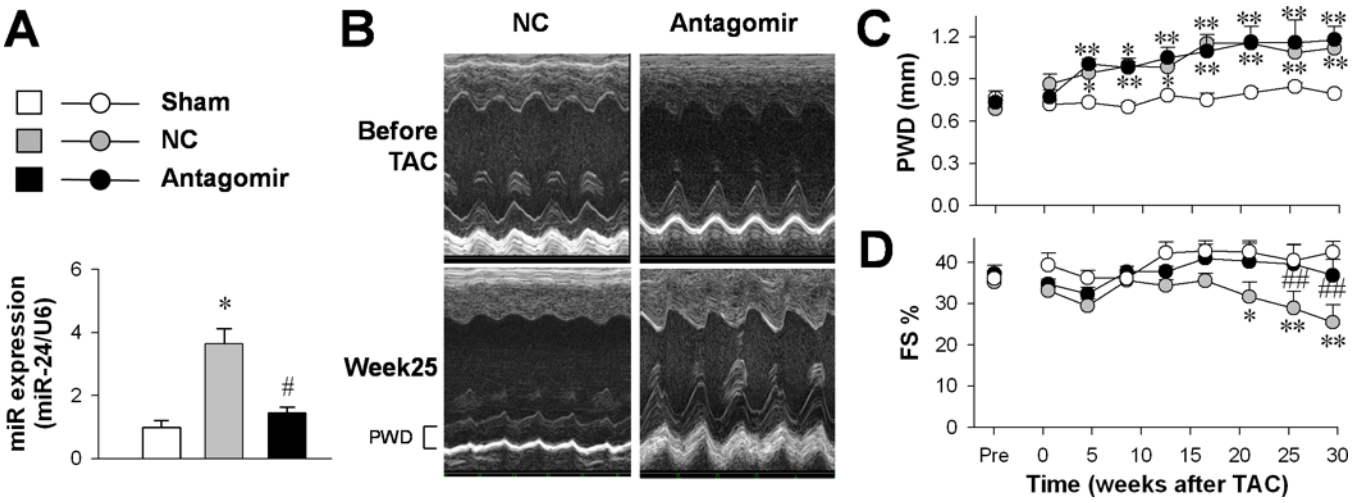

Figure 1. ***In vivo*** **miR-24 silencing in mouse hypertrophy models**

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.

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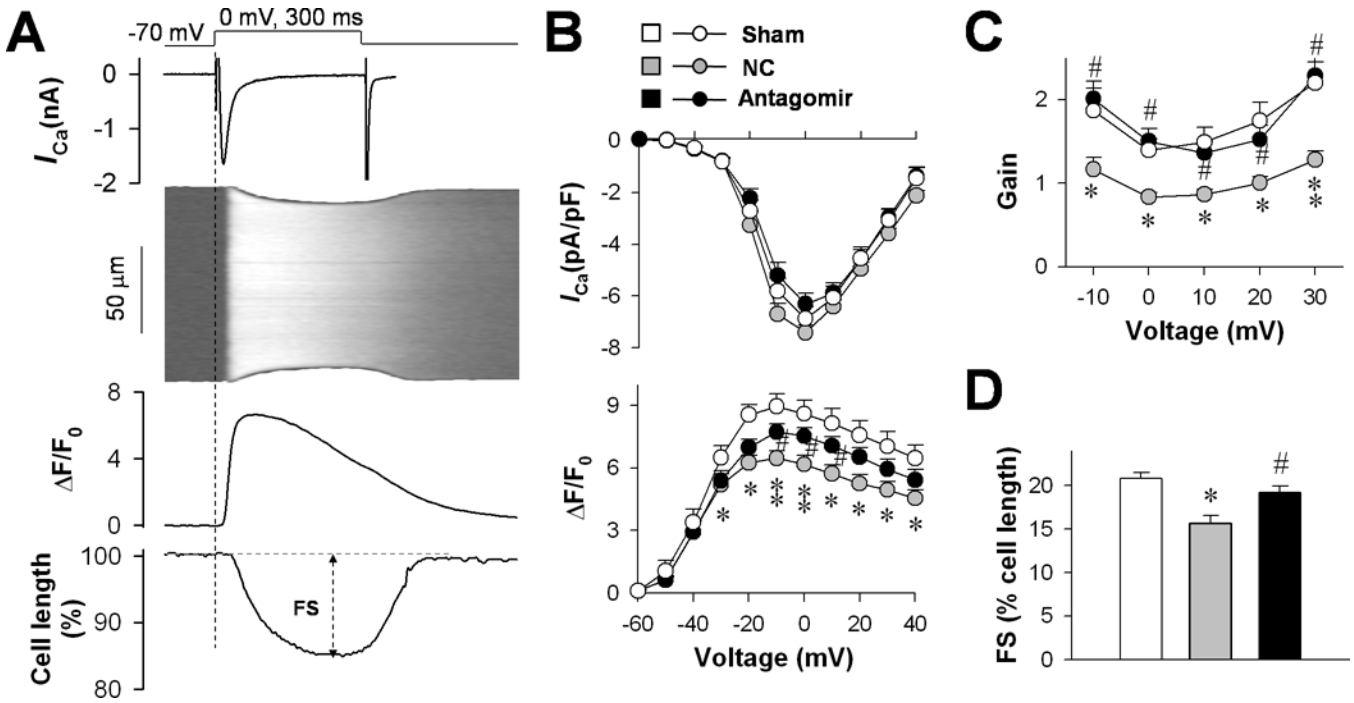

Figure 2. The effect of miR-24 silencing on E-C coupling

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.

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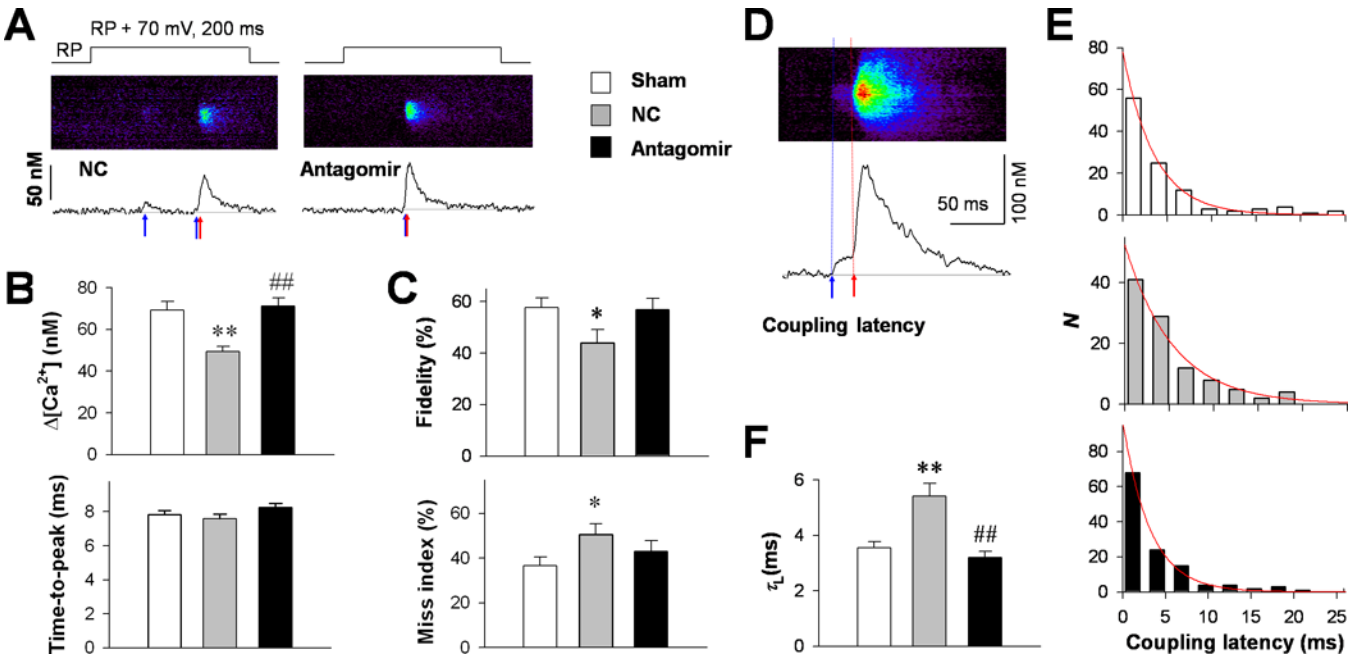

Figure 3. The effect of miR-24 silencing on LCC-RyR communications

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.

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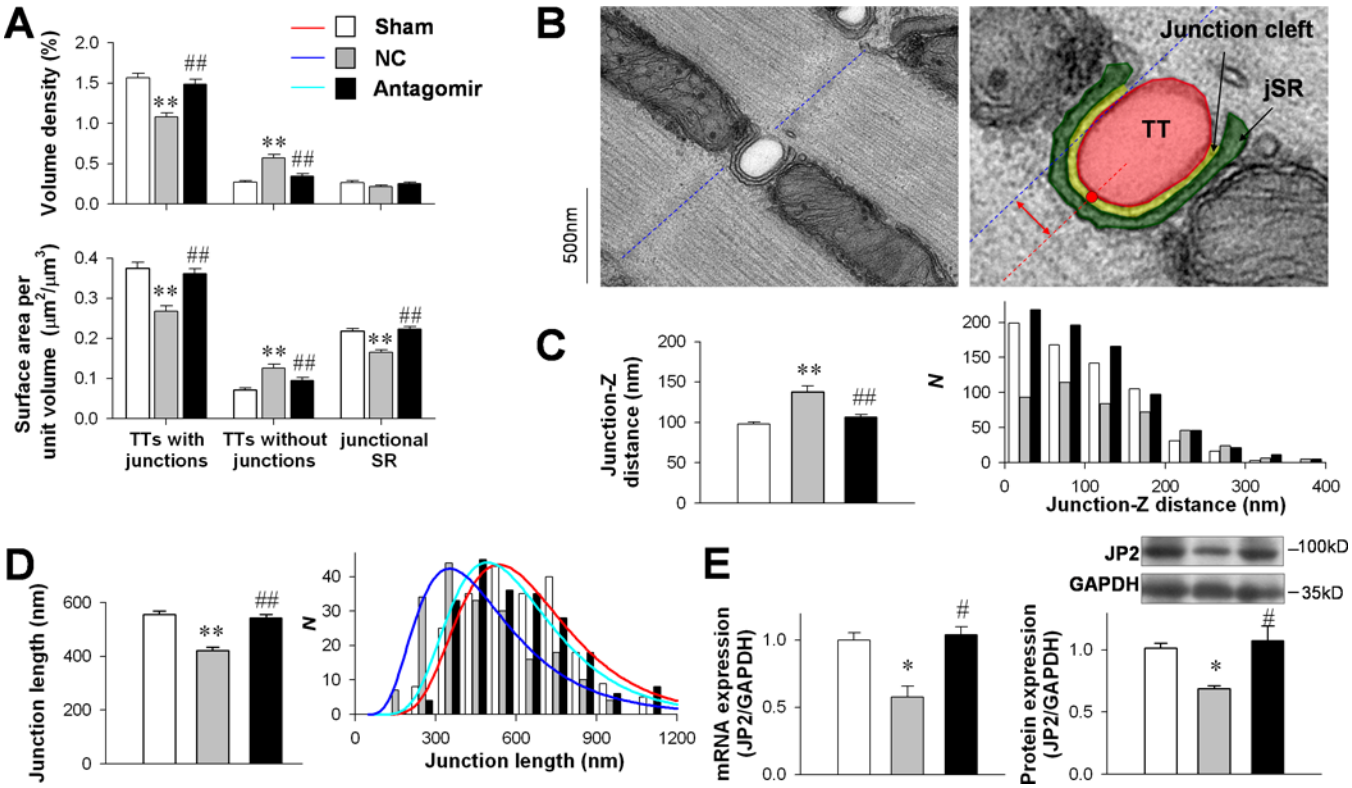

Figure 4. Effect of miR-24 silencing on the structure of TT-SR junctions

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.