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-xylazine 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 = (LVDd - LVDs) / LVDd.

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)mGmUmUmCmCmUmGm CmUmGmAmAmCmUmGmAmG(s)mC(s)mC(s)mA(s)-Chol-3', where m is a 2'-*O*Me-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 follwing: 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 $Na_4P_2O_7$, 1% Triton X-100, 10% glycerol, 150 mM NaCl, 5 mM EDTA·Na_2, 20 mM Tris (pH 7.4), 0.1% SDS, 50 mM NaF, 1 mM Na_3VO_4, 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 (QEILENSESLLEPR). 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 $CaCl_2$, 1 $MgCl_2$, 1.2 NaH_2PO_4, 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 $MgCl_2$, 5 Na_2ATP, 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 $CaCl_2$, 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).

Ca^{2+} imaging

Intracellular Ca^{2+} 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 Ca^{2+} detection. The Ca^{2+} concentration was either reported as the fluorescence normalized to the resting level ($R = F/F_0$), or calculated by $[Ca^{2+}] = k_d \cdot R/(k_d/C_0+1-R)$, assuming a resting Ca^{2+} concentration $C_0 = 100$ 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).[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[5]. 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 G^2 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

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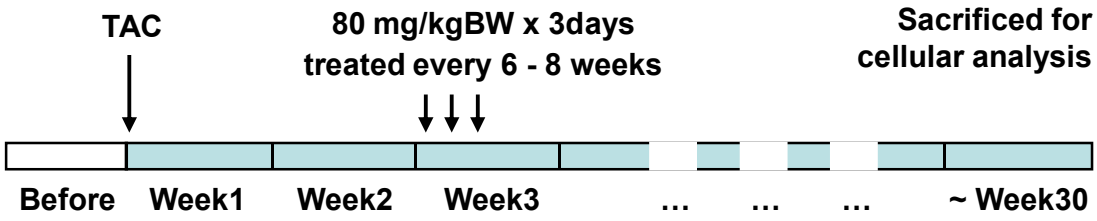
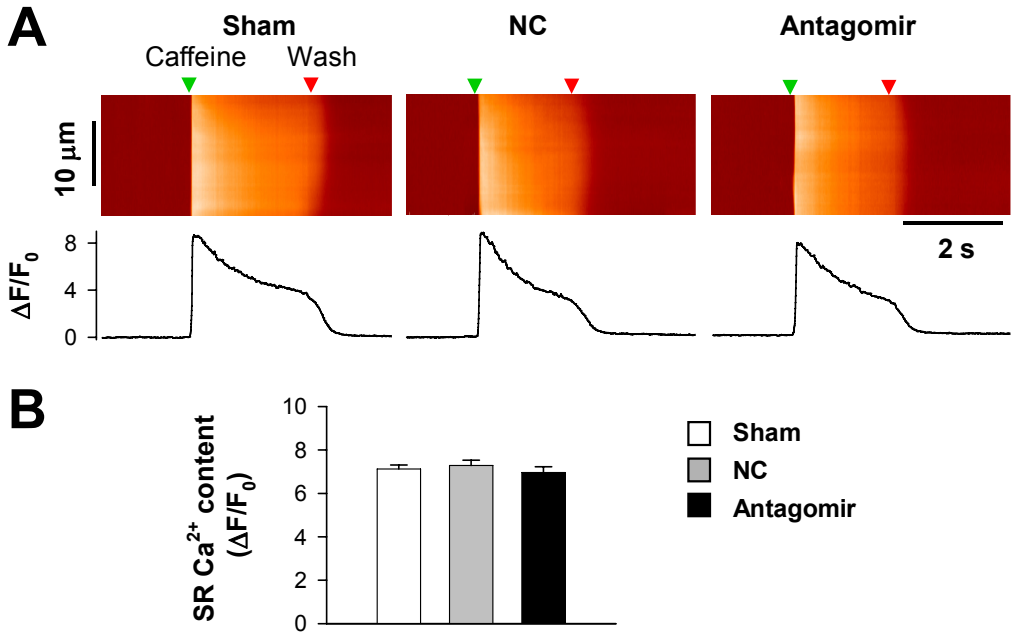
3. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. Silencing of microRNAs in vivo with 'antagomirs'. *Nature.* 2005;438:685-689.
4. Wu HD, Xu M, Li RC, Guo L, Lai YS, Xu SM, Li SF, Lu QL, Li LL, Zhang HB, Zhang YY, Zhang CM, Wang SQ. Ultrastructural remodelling of Ca(2+) signalling apparatus in failing heart cells. *Cardiovasc Res.* 2012;95:430-438.
5. Forbes MS, Plantholt BA, Sperelakis N. Cytochemical staining procedures selective for sarcotubular systems of muscle: modifications and applications. *J Ultrastruct Res.* 1977;60:306-327.
6. Mobley BA, Eisenberg BR. Sizes of components in frog skeletal muscle measured by methods of stereology. *J Gen Physiol.* 1975;66:31-45.

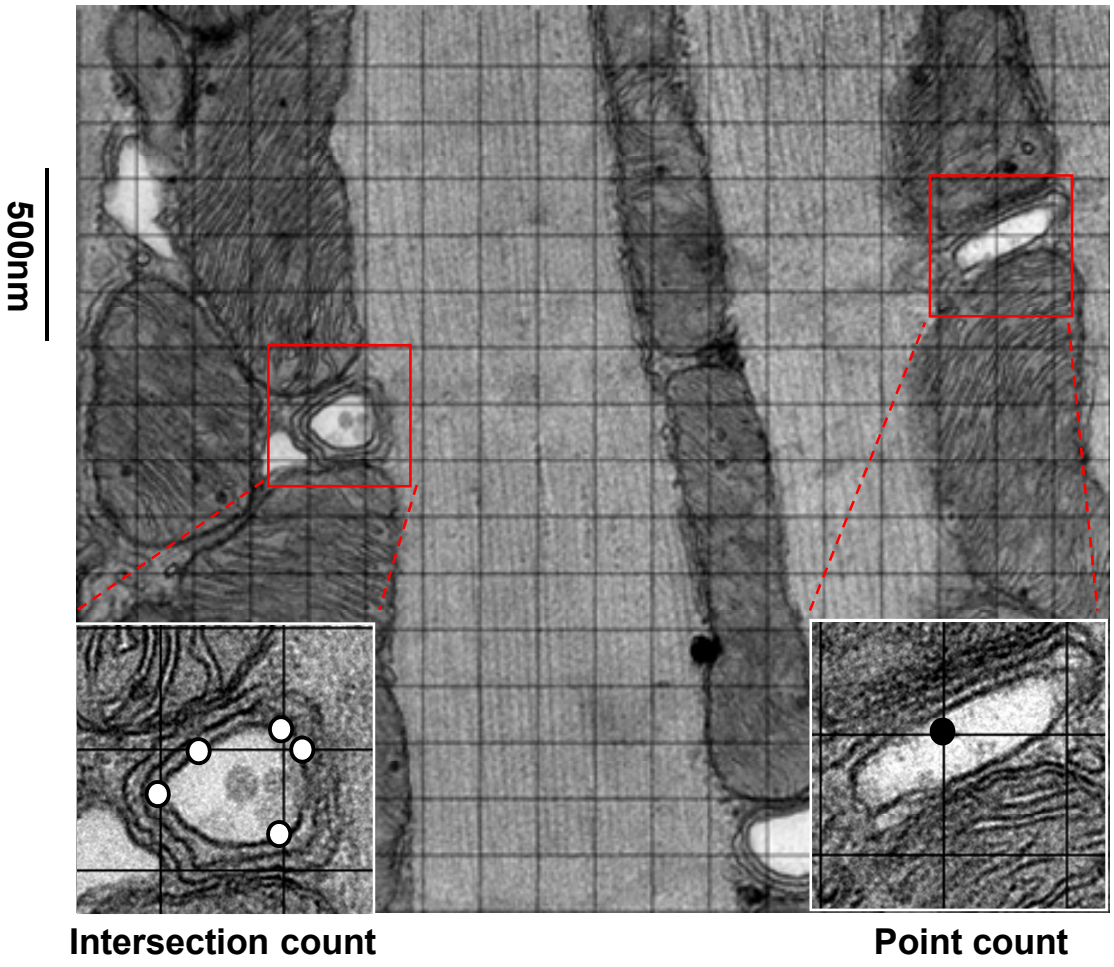
Online Figures



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 (ΔF/F0) 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.