Supplemental Data

Methods

Plasmid Constructs

The plasmids were designed as follow: The *STARS* promoter fragments were inserted upstream of a lacZ gene construct using Hind III / Xho I or Kpn I / Xho I sites to generate *STARS* promoter-lacZ reporter constructs. To generate *STARS* promoter-luciferase constructs (-1581STARS-luc and -1043STARS-luc), 5'-upstream fragments of the *STARS* gene spanning from -1581 to +53 and -1043 to +53 were inserted into Hind III / Xho I sites of pBluescript II KS+. The Sac I-Xho I fragments containing the 5'-upstream regions of the *STARS* gene were isolated and inserted upstream of a luciferase gene in the pGL3 vector using Sac I/Xho I sites (Promega). To generate –164, -138, -115. -94, –77 and -20STARS-luc plasmids, fragments from –164, -138, -115. -94 –77 or –20 bp were inserted upstream of a luciferase gene in pGL3 using Kpn I / Xho I sites. For -735, -502 and -55STARS-luc, DNA fragments were inserted upstream of a luciferase gene in pGL3 using Sac I/Xho I sites.

The following PCR primers were used to generate DNA fragments from the 5' flanking region of the *STARS* gene; -1581 Hind III sense primer:5-GGACATT CCC AAG CTT CAG TGG CAA AGA A A AA CAA AGG TC-3, -1043Hiind III sense primer: GGA CATT CCC AAG CTT CGA TAA AAG ATA AAA CTG AAC TAA A-3, -164 Kpn I sense primer: 5-ACG GGG TAC CTC TGC TGT GAT GAA ATG GGA CTT GA-3. -77 Kpn I sense primer: 5-CAC GGG GTA CCA TAG TT CCA CCG TCA CAG GCT-3. Xho I STARS promoter antisense primer: TCT GAC TAC CGC TCG AGG CTA CCT GTT TCT TCT CTG CTG-3.

The following sense primers and previously described Xho I STARS promoter anti-sense primer were used; -1452 Sac I sense primer: CAT TCC GAG CTC TTC TAT TAA AAA TAC TTA TTT AC-3, -735 Sac I sense primer: CAT TCC GAG CTC TAA GAG ATT ATT TA GTC AT-3, -502 Sac I sense primer: CAT TCC GAG CTC TTG TTA TAG ATT AAA AGG AAG C-3, -55 Sac I sense primer: CAT TCC GAG CT CTC TCC TTC CCA CTC ACA CC-3.

The following primer were used: -138 Kpn I sence primer: 5-CAC GGG GTA CCA GGG TTT AAA AAC AGA ACA-3. -115 Kpn I sence primer: 5-CAC GGG GTA CGT CAG GGC CAT GGC ACC ACA-3. -94 Kpn I sence primer: 5-CAC GGG GTA CTT GGC AAG AAA TAC TTT GCA-3. -20 Kpn I sence primer: 5-CAC GGG GTA CTC CCA GGC ATC TCT CTC A-3.

Mutations were introduced into the STARS 5' flanking region using the following primers; mut M1 sense: 5-GGG TTT AAG GGC AGA ACA CCG GGT TTA AGG GCA GAA CAC C -3, mut M1 antisense: 5- GGT GTT CTG CCC TTA AAC CCG GTG TTC TGC CCT TAA ACC C-3, mut M2 sense: 5- CAT CTT GGC AAG GGG TAC TTT GCA TCT TGG CAA GGG GTA CTT TG-3, mut M2 antisense: 5-CAA AGT ACC CCT TGC CAA GAT GCA AAG TAC CCC TTG CCA AGA TG -3.

PCR-based mutagenesis in the two CArG sequences of the ANP promoter was performed using the following primers; mut distal CArG sense: 5-TCT CCA CCC TGC GGG TGA GGC CCT GA -3, mut distal CArG antisense: 5- TCA GGG CCT CAC CCG CAG GGT GGA GA-3, mut proximal CArG sense: 5- CGGGAT GAT AAC TTG CCC CTG GCA TCT CCT GCT G-3, mut proximal CArG antisense: 5-CAG CAG GAG ATG CCA GGG GCA AGT TAT CAT CCC G -3.

Adenovirus Infection and Immunocytocheistry

For adenovirus infection, cardiomyocytes grown on cover slips in 6-well dishes were infected 36 hours after plating with recombinant adenovirus at a multiplicity of infection of 5 for 6 hours and subsequently maintained in serum-containing medium for 36 hours. After the medium was changed to serum-free medium, cells were further incubated for 12 hours and then fixed with 4% formaldehyde in PBS.

For immunocytochemistry, cells infected with recombinant adenovirus expressing STARS and MRTF-A and grown on gelatin-coated cover slips were rinsed with PBS, fixed with 4% formaldehyde for 10min and permeablized with 0.1% Triton X-100 in PBS for 5 minutes. Cells were blocked for 30 minutes at room temperature in 3% BSA in PBS and then incubated with primary antibody diluted in 3% BSA in PBS for 60 minutes at room temperature. Anti-FLAG polyclonal antibody (1:200; Sigma-Aldrich), and anti-α-actinin monoclonal antibody (clone EA-53) (1:400; Sigma-Aldrich) were used. For control experiments, adenovirus expressing *lacZ* was used. Secondary antibodies were anti-mouse/rabbit IgG fluorescein isothiocyanate or Texas Red (Vector Lab) used at 1:200 dilution. Vectashield® with DAPI (Vector) was used for mounting and detection of nuclei.

Electrophoretic Mobility Shift Assay

Double-stranded oligonucleotides corresponding to consensus MEF2 or STARS MEF2-like sequences are as follows: MEF2 consensus: 5'-GCT ATA AAT AGA GCT ATA AAT AGA GCT ATA AAT AGA-3', STARS M1: 5'-GGG TTT AAA AAC AGA ACA

CCG GGT TTA AAA ACA GAA CAC C-3', STARS M2: 5'-CAT CTT GGC AAG AAA TAC TTT GCA TCT TGG CAA GAA ATA CTT TG -3'. For gel mobility shift assays utilizing MEF2C tagged with a myc epitope, 2 μl of a coupled in vitro translation reaction (TNT kit, Promega) was incubated with the indicated ^{32}P-labeled oligonucleotides probe in the presence of 1 μl of poly dIdC (1.5 μg/μl) for 20 min at room temperature, followed by nondenaturing electrophoresis. Unlabeled competitor oligonucleotides were added at a 100-fold molar excess and 2 μl of anti-myc antibody (Santa-Cruz) was added for supershift experiments.

RNA Interference Assay

For RNA interference (RNAi) analysis of MRTF-A, a siGENOME SMART pool® reagent against rat MRTF-A (M-081405-00-0010) with guaranteed minimum 75% mRNA knockdown was purchased from Dharmacon. BLOCK-iT Fluorescent Oligo® (Invitrogen) was used as a non-specific control. For luciferase assays, transfections using 100 pmol of siRNAs and 500 ng of luciferase reporter plasmids were performed in neonatal rat ventricular myocytes in DMEM with 10% fetal bovine serum using Fugene. A RSV-lacZ expression plasmid was included in all transfections as an internal control. Luciferase assay was performed 48 hours after transfection. To verify the efficiency of siRNA-mediated knock down of MRTF-A expression, rat smooth muscle cells maintained in 6-well dishes were transfected with 200 pmol of siRNAs. Cells were harvested 48 hours after transfection for Real-Time RT-PCR analysis. Using rat MRTF-A siRNA, we observed an 88% reduction in expression of endogenous MRTF-A mRNA in rat smooth muscle cells compared to control siRNA.

Thoracic Aortic Banding, Echocardiography and Histology Analysis

Six- to 8-week-old male mice either underwent a sham operation or were subjected to pressure overload induced by thoracic aorta banding (TAB). A constriction with a 27-gauge needle is placed in the transverse aorta between the innominate and left carotid arteries. We have shown previously that constriction to a 27-gauge stenosis induces moderate hypertrophy without clinical signs of heart failure or malignant ventricular arrhythmia (42). The mice were sacrificed 3 weeks after aortic banding. We confirmed the integrity of aortic banding by inspection of the surgical constriction and by visualization of marked differences in caliber of the right and left carotid arteries.

Cardiac function was evaluated by echocardiography on conscious mice using a Hewlett Packard Sonos 5500 Ultrasound system with a 12 MHz transducer. Views were taken in planes that approximated the parasternal short-axis view (chordal level) and the apical long-axis view. Left ventricle internal diameters and wall thicknesses were measured (at least 3 cardiac cycles) at end systole and end diastole.

For histological analysis, excised hearts were rinsed in PBS and incubated in Krebs-Henseleit solution lacking Ca^{2+}. Hearts were fixed in 4% paraformaldehyde for 12 h at room temperature. Samples were dehydrated with ethanol, mounted in paraffin, and sectioned at 5μm thickness. Sections were then stained with hematoxylin and eosin (H&E) to visualize tissue architecture.

Real Time RT-PCR

The primers and probes for detection of mouse ANP, and SERCA2 transcripts by real time PCR were as follows; mouse ANP forward primer:5'-GCC ATA TTG GAG CAA ATC CT-3', reverse primer; 5-GCA GGT TCT TGA AAT CCA TCA-3', probe; 5'-FAM-TGT ACA GTG CGG TGT CCA ACA CAG AT-TAMRA-3'. Mouse SERCA2 forward primer; 5'-CAT CTG CTT GTC CAT GTC ACT T-3', reverse primer; 5'-CGG TGT GAT CTG GAA AAT GAG-3', Probe; 5'-FAM-TCT TGA TCC TCT ACG TGG AAC CTT TGC-TAMRA-3'. The primers and probes for detection of mouse and human STARS transcripts by real time PCR (Mm00615375_m1 and Hs00373623_m1) were purchased from ABI.