**SUPPLEMENTAL MATERIAL**

## Detailed Methods

*Myocardial infarction*

All experiments utilizing animals were previously approved by the Institutional Animal Care and Use Committee at UT Southwestern Medical Center. Myocardial infarctions were generated using male MRTF-A$^{-/-}$ and WT mice at 12 weeks of age (25-30g) by surgical ligation of the left anterior descending (LAD) coronary artery. Mice were sedated using a self-designed coaxial mask supplying continuous isoflurane (2.0%) and oxygen (98.0%) under positive pressure using a Harvard small animal respirator. Following anterolateral thoracotomy, through the fourth intercostals space, myocardial ischemia was induced by ligation of the proximal left anterior descending (LAD) coronary artery using 7-0 Maxon polyglyconate suture equipped with a CV-1 (3/8 9mm) taper needle. Confirmation of myocardial ischemia is evident by immediate blanching of the ischemic myocardial segments. Sham operated mice underwent the same procedure without occlusion of the LAD.

*Cell culture and transfection*

Cardiac fibroblasts (CFs) were collecting from the pre-plating fraction of the myocyte isolation and used after passage 2 to 3. CFs were plated on uncoated multi-well plates for RNA and protein isolation, or laminin coated coverslips for immunocytochemistry (BD Biosciences). Twenty-four hours after plating, or when cells reach confluence, CFs were cultured for twenty-four hours in serum free media, followed by infection with flag-MRTF-A adenovirus, or a β-gal control adenovirus (10 MOI). CFs were then treated with recombinant TGFβ-1 (10ng/ml, R&D) and/or the ROCK inhibitor, Y-27632 (10 μm, Calbiochem) and harvested after an additional 24-48 hrs of culture, as noted in text.

For luciferase assays, CFs or COS were cultured in DMEM with 10% FBS, L-glutamine (2mmol), and penicillin-steptomycin in 24 well plates were transfected with a total of 300ng of plasmid DNA using FuGENE6 (Roche). 20ng of pCMV-lacZ was used as an internal control and total plasmid amount was kept constant using empty pcDNA3.1. 20ng of pCMV-lacZ was used as an internal control and total plasmid amount was kept constant using empty pcDNA3.1. 48 hrs after transfection, cell lysates were harvested in 150 μl of Passive Lysis Buffer (Promega) and luciferase and β-galactosidase assays were carried using a luciferase assay kit (Promega).

*RNA extraction and RT-PCR*

Total RNA from myocardial infarct border zone (BZ), remote region or sham LV tissue was isolated using Trizol (Invitrogen). 2 μg RNA from each sample was used to generate cDNA using Super Script III reverse transcriptase using the manufacturer's protocol (Invitrogen). Real time PCR was cycled between 95°C/30 s and 60°C / 30 s for 40 cycles, following initial 95°C denaturation step for 3 min. Amplification products were quantified using the relative Ct method, where the amount of target normalized to the amount of endogenous control (18S) and relative to the control sample is given by $2^{-\Delta\Delta Ct}$. Taqman primer and probesets were used for mouse Col1a1, Col1a2, Col3a1, Eln, SM22, SMA, ANF, SM22, or SMA, TnC,

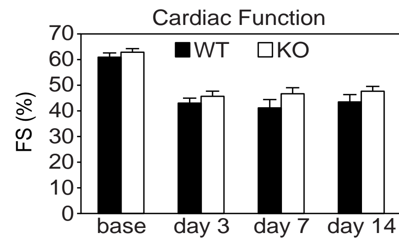
MRTF-A and rat Col1a2, SM22 and SMA (Applied Biosystems). Gene expression was normalized to 18s ribosomal RNA or GAPDH and calculated as relative change to WT samples or between experimental groups.

*Electrophoretic mobility shift assay*
Annealed oligonucleotides were radiolabeled with [$^{32}$P]dCTP using the Klenow fragment of DNA polymerase and purified using G50 spin columns (Roche). Nuclear cell extracts were isolated from COS cells transfected with pcDNA3.1 flag-SRF or empty pcDNA3.1 for lysate. Unlabeled oligonucleotides used as competitors were annealed and added to the reactions at 1000-fold molar excess. DNA-protein complexes were resolved on 5% polyacrylamide native gels and exposed to BioMax X-Ray film (Kodak). Oligonucleotide probe sequence is as follows:
Col1a2 CArG: 5'- CTAAAGTGCTTCCAAACTTGGCAAGGGCGA -3';
Col1a2 mut CArG: 5'- CTAAAGTGCTTACACACGTGGCAAGGGCGA -3'.

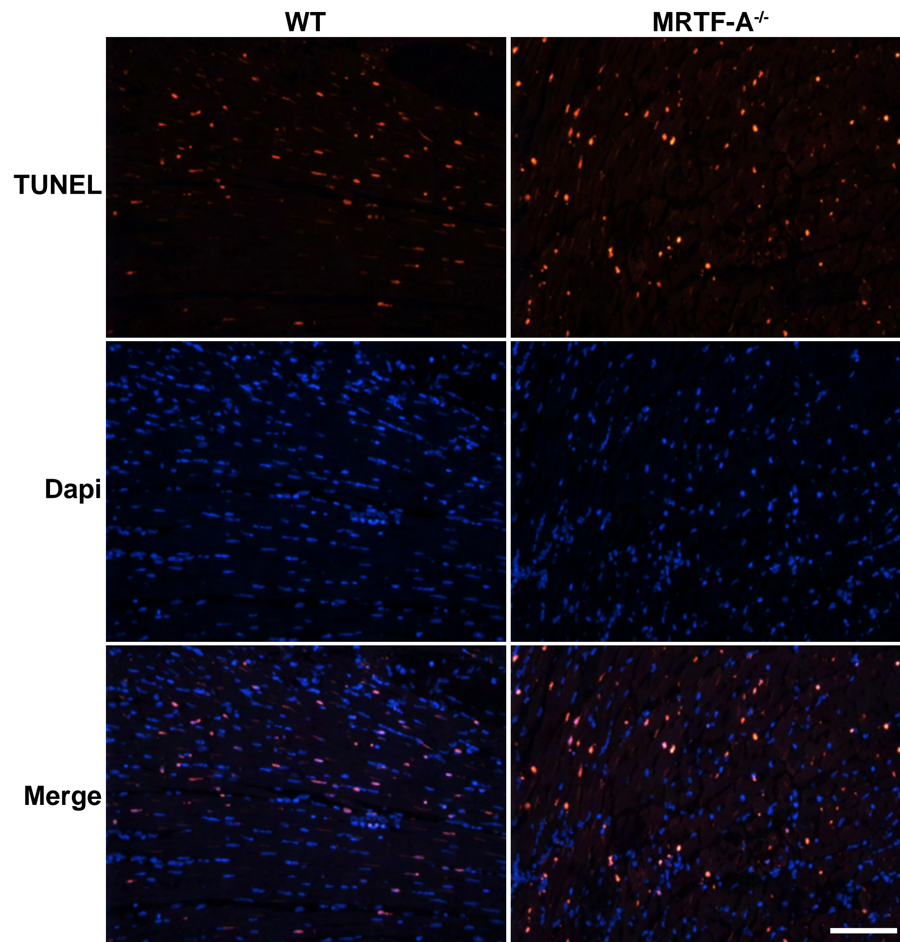
*Chromatin immunoprecipitation assay*
Chromatin immunoprecipitation was performed on native chromatin from 10T1/2 cells cultured in SF media or media containing TGFβ1 (10μg/ml) for 24 hrs using the EZ ChIP kit (Millipore). Protein-chromatin complexes were crosslinked for 10 min at room temperature with 1.32 ml of 16% formaldehyde in 20ml growth media in a 15 cm plate of 10T1/2 cells (1% final concentration). 2ml of 10x Glycine was then added to the media to quench formaldehyde for 5 min. at RT. Cells were scraped into 2 ml cold PBS containing 1x Protease Inhibitor Cocktail II (Millipore) and centrifuged at 700g for 5 min. at 4°C. Cell pellet was lysed in 1 ml SDS Lysis buffer and sonicated in 300 μl aliquots for 15 minutes of 30 sec of high intensity / 30 sec of rest in a bioruptor (Diagenode). Insoluble material was removed by centrifugation at 15,000g for 10 minutes at 4°C and 100 μl of supernatant was diluted to 1 ml with Dilution buffer. Chromatin was precleared with 60 μl of Protein G beads for 1 hr at 4°C. 10 μl (1%) of supernatant was saved for input and the remaining fraction was incubated with 2 μg of an immunopreciptating antibody overnight at 4°C. Antibodies directed against SRF (Santa Cruz) or PolII (Millipore) or mouse IgG were used. The antibody/antigen/DNA complex was collected with Protein G beads for 1 hr at 4°C followed by washing of the pellet with subsequent Low Salt / High Salt / LiCL/ and TE Buffers. DNA-protein complexes were eluted from the Protein G beads with elution buffer at room temperature for 15 min. The protein-DNA complexes were reverse crosslinked in 5 M NaCl at 65°C for 5 hrs, followed by RNase A incubation at 37°C for 30 minutes and Proteinase K treatment at 45°C for 2 hrs. DNA was then purified using spin columns and the Col1a2 promoter region was PCR amplified to reveal SRF / DNA complex using the following primers and PCR program: Col1a2 forward 5' – AAAGTGAAGCAGGACTGGACA – 3', Col1a2 reverse 5' – GACGTGGCTACAGGGCTTCTT – 3'. The following GAPDH primers were used as a positive control for PolII immunoprecipitation and a negative control for SRF immunoprecipitation: GAPDH forward 5' – CCTCTGCGCCCTTGAGCTAGGA - 3', GAPDH reverse 5' –

CACAAGAAGATGCGGCCGTCTC – 3'. Initial denaturation at 94°C for 3 minutes followed by 35 cycles of 94°C for 20 sec / 56°C for 30 sec / 72°C for 30 sec. and a final 72°C extension for 2 min.

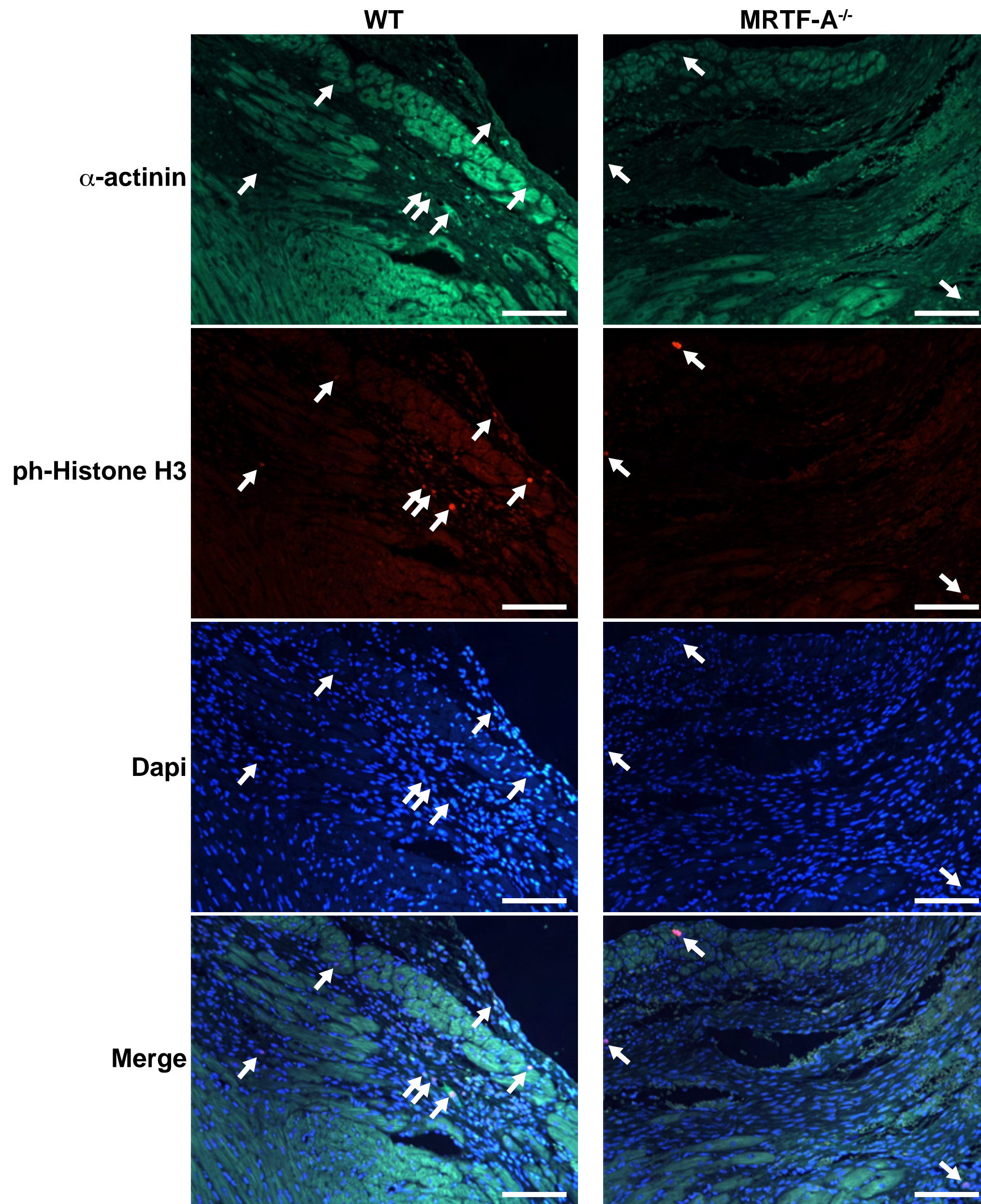
**Supplemental Figures and Figure Legends**



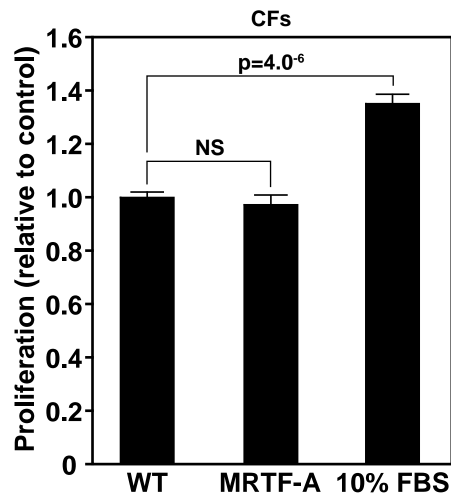

**Online Figure I. Post-MI cardiac function.**
Cardiac function (FS%) in WT and MRTF-A$^{-/-}$ mice at baseline and 3, 7 and 14 days post-MI. $p = 0.226$ ($n = 13$ for WT mice and 14 MRTF-A$^{-/-}$).



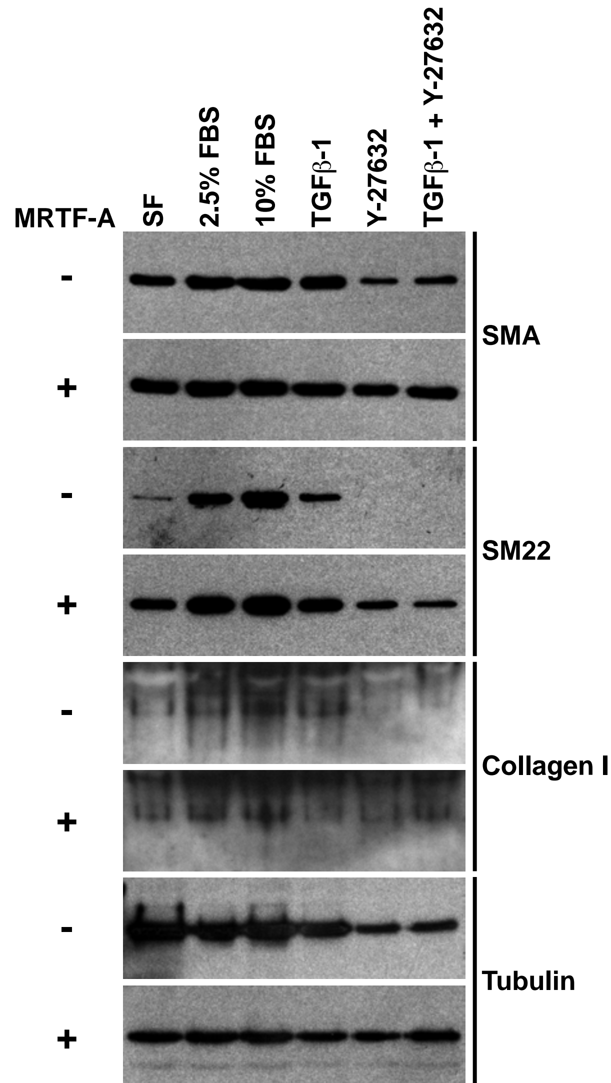

**Online Figure II. Effect of MRTF-A on post-MI cell death.**
Cell death was detected by TUNEL staining histological sections of WT and MRTF-A$^{-/-}$ hearts 24 hours post-MI. Nuclei were visualized using Dapi. Scale bar = 40 μm.




**Online Figure III. Effect of MRTF-A on cell proliferation in the BZ 14 days post-MI.**
Co-immunohistochemistry on heart sections of WT and MRTF-A$^{-/-}$ mice 14 days post-MI to detect phospho-histone H3 (red), α-actinin (green) and Dapi (blue) in the BZ. Arrows mark proliferating cells as determined by phospho-Histone H3 positive nuclei. Scale bar = 200 μm.




**Online Figure IV. Effect of MRTF-A overexpression on cultured CF proliferation.**
Proliferation of CFs infected with 10MOI of β-gal control or MRTF-A expressing adenovirus was measured using colorimetric detection. 2.5% FBS was added to the media as a control for proliferation. Data are represented as absorbance relative to control cells and error bars represent SEM.




**Online Figure V. MRTF-A stimulates production of Col1a2 protein.**
Detection of endogenous SMA, SM22 and Col1a2 protein from CFs grown in serum free media or treated with serum (2.5% FBS), TGFβ-1 (10ng/ml), Y-27632 (10 μM) or TGFβ-1 + Y-27632, and infected with10MOI of β-gal control or MRTF-A expressing adenovirus by Western blot. GAPDH was detected as control for protein loading.