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Myocardin-related transcription factors are required for cardiac development and function

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Abstract

Myocardin-Related Transcription Factors A and B (MRTF-A and MRTF-B) are highly homologous proteins that function as powerful coactivators of serum response factor (SRF), a ubiquitously expressed transcription factor essential for cardiac development. The SRF/MRTF complex binds to CArG boxes found in the control regions of genes that regulate cytoskeletal dynamics and muscle contraction, among other processes. While SRF is required for heart development and function, the role of MRTFs in the developing or adult heart has not been explored. Through cardiac-specific deletion of *MRTF* alleles in mice, we show that either MRTF-A or MRTF-B is dispensable for cardiac development and function, whereas deletion of both MRTF-A and MRTF-B causes a spectrum of structural and functional cardiac abnormalities. Defects observed in *MRTF-A/B* null mice ranged from reduced cardiac contractility and adult onset heart failure to neonatal lethality accompanied by sarcomere disarray. RNA-seq analysis on neonatal hearts identified the most altered pathways in MRTF double knockout hearts as being involved in cytoskeletal organization. Together, these findings demonstrate redundant but essential roles of the MRTFs in maintenance of cardiac structure and function and as indispensible links in cardiac cytoskeletal gene regulatory networks.

Keywords

cardiac morphogenesis; heart function; sarcomere arrangement; MRTF-A/MKL1; MRTF-B/MKL2; cytoskeletal dynamics

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Introduction

Development of the heart and maintenance of cardiac function require precise regulation of many genes involved in cardiac morphogenesis, contractility, and energy homeostasis (Olson, 2006). Perturbations in cardiac gene regulation can result in congenital heart defects, one of the most common birth defects in the United States (Reller et al., 2008).

Members of the myocardin family of transcription factors act as transcriptional co-activators for serum response factor (SRF), a ubiquitously expressed MADS-box transcription factor, to potently stimulate SRF-dependent gene expression (D. Wang et al., 2001; D.-Z. Wang et al., 2002). SRF binds as a homodimer to CArG boxes (consensus sequence: [$CC(A/T)_6GG$]) found in the control regions of many genes that regulate actin dynamics and muscle contractility (Miano et al., 2007; Treisman, 1987). Myocardin and Myocardin-Related Transcription Factors A and B (MRTF-A/MAL/MKL1 and MRTF-B/MKL2) are essential for SRF function and enhance SRF activity (Olson and Nordheim, 2010). The MRTFs are thought to act in part by relaying information about the state of actin polymerization from the cytoplasm to the nucleus (Miralles et al., 2003; Vartiainen et al., 2007). In contrast, the N-terminus of myocardin is thought to lack the RPEL domains necessary to mediate interaction with actin, suggesting that myocardin and the MRTFs might have unique biological functions despite their strong homology (Kuwahara et al., 2005; Olson and Nordheim, 2010; D. Wang et al., 2001). Consistent with this notion, while myocardin is expressed specifically in cardiac and smooth muscle cells (D. Wang et al., 2001), MRTF-A and MRTF-B are expressed in a broad range of embryonic and adult tissues (D.-Z. Wang et al., 2002). MRTFs are sequestered in the cytoplasm as a result of their interaction with G-actin monomers. Upon stimulation by actin polymerization, MRTFs are liberated from their interaction with G-actin and subsequently translocate into the nucleus where they act with SRF to activate genes involved in cytoskeletal dynamics (Miralles et al., 2003; Pipes et al., 2006; Vartiainen et al., 2007).

Cardiac deletion of SRF in the developing mouse heart results in embryonic lethality, indicating its essential role in heart development (Miano et al., 2004; Niu et al., 2005). Similarly, conditional deletion of SRF in the adult heart results in dilated cardiomyopathy and lethality within 10 weeks of inactivation, confirming a critical role for SRF in cardiac function (Parlakian et al., 2005). *Myocardin*-null mice die during early embryogenesis due to a failure in vascular smooth muscle development (S. Li et al., 2003) while cardiac-specific loss of myocardin during early cardiac development using a floxed *Myocardin* allele together with an Nkx2.5-Cre transgene led to defects in cardiac growth and maturation; this defect was mediated at least in part via inactivation of *Bmp10* (Huang et al., 2012). In contrast, cardiac-specific deletion of myocardin with an αMHC-Cre transgene, which becomes active slightly later in development, causes adult onset heart failure accompanied by sarcomere disruption, but no overt cardiac morphological defects (Huang et al., 2009), though a fraction of these animals succumb to early postnatal lethality. In addition to its role in postnatal cardiac function, myocardin is also essential for vascular homeostasis; deletion of myocardin in smooth muscle cells in adult animals led to lethality within 6 months, accompanied by defects in arterial structure and visceral smooth muscle maintenance (Huang et al., 2015).

MRTF-A$^{-/-}$ mice are viable and display no cardiac abnormalities (S. Li et al., 2006; Sun et al., 2006) while *MRTF-B*$^{-/-}$ mice die during mid-gestation from defects in branchial arch neural crest development and smooth muscle cell differentiation without overt cardiac abnormalities (J. Li et al., 2005; Oh et al., 2005). Thus, it remains unclear whether the MRTFs are essential for heart development. In other contexts, however, including neural (Mokalled et al., 2010), hematopoietic (Costello et al., 2015) and angiogenic (Weinl et al., 2013) development, a redundant role for the MRTFs has been demonstrated.

To explore the functions of MRTFs in heart development, we generated mice lacking combinations of *MRTF-A* and *MRTF-B* alleles through cardiac-specific gene deletion. Here, we show that cardiac deletion of MRTF-B in an MRTF-A null background results in a range of cardiac defects from neonatal lethality with abnormalities in sarcomere organization to adult-onset heart failure. We identify a broad collection of cardiac genes that are dysregulated in response to MRTF depletion in the heart. Our findings reveal a requirement for MRTF-A and MRTF-B in cardiac function that reflects the essential roles of MRTFs in cardiac cytoskeletal gene regulatory networks.

Materials and methods

Mouse lines

The *MRTF-A*$^{-/-}$ mice and *MRTF-B*$^{fl/fl}$ conditional mice have been previously described (S. Li et al., 2006; Mokalled et al., 2010). *MRTF-A*$^{-/-}$, *MRTF-B*$^{fl/fl}$, and αMHC-Cre mice were crossed to generate *MRTF-A*$^{-/-}$; *MRTF-B*$^{fl/fl}$; αMHC-Cre animals, which we refer to as MRTF cardiac double knockout (*MRTF*^{cdKO}) mice. Littermates were used as control animals. Controls were *MRTF-A*$^{-/-}$,*MRTF-B*$^{f/f}$ or *MRTF-A*$^{+/-}$;*MRTF-B*$^{f/f}$. All animal experiments were approved by the UT Southwestern Institutional Animal Care and Use Committee.

Histology and electron microscopy

Control and *MRTF*^{cdKO} mice were sacrificed at the indicated time points, hearts were fixed in 4% paraformaldehyde for 48 hours, embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome using standard procedures. For transmission electron microscopy, heart tissue was isolated and prepped according to standard protocols. Electron microscopy protocols are described in detail in the Supplementary Material and Methods.

Quantitative RT-PCR analysis

Total RNA was purified from tissues using TRIzol reagent according to the manufacturer's instructions (Invitrogen). For RT–PCR, total RNA was used as a template for RT using random hexamer primers. qPCR was performed using primers located within the deleted exons of the *MRTF-A* and *MRTF-B* genes.

RNA isolation and RNA-seq analysis

Hearts were harvested from P0 pups of both wild type and *MRTF*^{cdKO} mice and were immediately frozen in liquid nitrogen. Heart tissues were resuspended in 1 ml of Trizol and

homogenized using 20g needles. Following chloroform extraction, the supernatant was mixed with equal volume of 75% ethanol and loaded on RNeasy mini-columns (Qiagen). Total RNA was isolated according to manufacturer's instructions (Qiagen). RNA quality was verified by the Agilent 2100 Bioanalyzer and RNA-seq was performed using Illumina HiSeq 2500 by the Next-Generation Sequencing Core at the McDermott Center at UT Southwestern Medical Center (GEO accession number GSE69726). Bioinformatics analysis methods are described in Supplementary Materials and Methods.

Transthoracic echocardiography

Cardiac function and heart dimensions were evaluated by 2-dimensional echocardiography on conscious 7–8 week-old mice using a Visual SonicsVevo 2100 system equipped with a 35MHz transducer. Analysis information is found in Supplementary Materials and Methods.

Statistical analysis

Results are expressed as mean ± SEM. Unpaired 2-tailed Student *t*-test with Welch correction was performed to determine statistical significance. *P* values of <0.05 were considered significant.

Results

Generation of mice lacking MRTF-A and MRTF-B in the heart

Because *MRTF-A*$^{-/-}$ mice are viable and display no overt cardiac abnormalities (S. Li et al., 2006), whereas *MRTF-B*$^{-/-}$ mice die embryonically from vascular defects (Oh et al., 2005), we first investigated the potential role of MRTF-B in heart development by cardiac-specific deletion of a conditional floxed *MRTF-B* allele (Mokalled et al., 2010), using an αMHC-Cre transgene, which mediates gene recombination and deletion in cardiomyocytes (Agah et al., 1997). Mice with cardiac deletion of MRTF-B (*MRTF-B*^{cKO} mice) were viable and showed no abnormalities for up to a year of age (data not shown). As neither MRTF-A nor MRTF-B is individually required for heart function, we hypothesized that they might possess partially redundant functions. To assess this, we generated mice lacking both MRTF-A and MRTF-B in the heart by breeding *MRTF-B*^{cKO} mice to *MRTF-A*$^{-/-}$ mice (Fig. S1A). We refer to these mice as MRTF cardiac double knockout (cdKO) mice. *MRTF*^{cdKO} mice were born at approximately Mendelian ratios, suggesting that MRTF activity is not required for early cardiovascular development. However, we observed that ~75% of *MRTF*^{cdKO} mice died within the first two weeks of birth with the remaining 25% dying between 4 and 12 weeks of age (Fig. 1A), indicating that MRTFs play a critical role in cardiac function.

To confirm efficient deletion of the *MRTF* genes, we performed quantitative real-time PCR (qPCR) on isolated M*RTF*^{cdKO} hearts at postnatal day 0 (P0) using primers located within the deleted exons of the *MRTF-A* and *MRTF-B* genes (Fig. S1B). We observed an almost complete absence of *MRTF-A* expression while *MRTF-B* levels were reduced by ~50%, likely reflecting its residual expression in non-cardiomyocyte cell populations in which the αMHC-Cre transgene is inactive. Alternatively, it has been reported that deletion of *Gata4*$^{fl/fl}$ alleles using the αMHC-Cre transgene only results in loss of about 70% of the GATA4 protein by 8 weeks of age (Oka et al., 2006), suggesting that part of the residual

MRTF-B expression may be due to incomplete takeout of the *MRTF-B*$^{fl/fl}$ alleles. While *MRTF-A*$^{-/-}$ mice and *MRTF*-B^{cKO} mice in a mixed background were viable, ~20% of *MRTF*-A$^{+/-}$;*MRTF-B*^{cKO} and *MRTF-A*$^{-/-}$;*MRTF-B*$^{+/-}$ mice, which each retain one functional *MRTF* allele in the heart, died between weeks 2 and 4 of age (Fig. 1A), with the remaining 75% surviving until at least 12 months. The premature lethality we noted in mice with only one functional *MRTF* allele indicates that MRTF-A and MRTF-B act redundantly in the heart and that a threshold of MRTF activity is required for normal cardiac function.

Cardiac abnormalities resulting from MRTF deletion

Interestingly, at P0, *MRTF*^{cdKO} hearts appeared normal both in overall appearance and at a histological level by H&E staining (Fig. 1B). However, *MRTF*^{cdKO} animals that survived to 10 days of age exhibited dilation of the left ventricle as well as fibrosis, as assessed by Masson's trichrome stain, suggesting poor cardiac function (Fig. 1C and Fig. S1C). While most *MRTF*^{cdKO} mice died within 2 weeks of birth, those that survived to 3 weeks of age showed extreme cardiac dilation and regions of fibrosis, together indicative of heart failure (Fig. 1C and Fig. S1C). To characterize the cardiac defects present in mice lacking three of the four *MRTF* alleles, we performed histological analysis of hearts by H&E staining; this revealed a range of cellular and structural cardiac abnormalities (Fig. 2A). Approximately 75% of *MRTF-A*$^{+/-}$;*MRTF-B*^{cKO} hearts at 3 weeks of age appeared normal and indistinguishable from control hearts, whereas the other 25% were dilated with thin ventricular walls. Similar results were obtained for *MRTF-A*$^{-/-}$; *MRTF-B*$^{+/-}$ animals, suggesting a minimal level of MRTF activity is required for proper cardiac function.

To assess cardiac function in *MRTF*^{cdKO} animals, we performed echocardiography at 7–8 weeks of age; these studies revealed a slight reduction in fractional shortening in the hearts of adult *MRTF-A*$^{+/-}$;*MRTF-B*^{cKO} and *MRTF-A*$^{-/-}$;*MRTF-B*$^{+/-}$ mice (Fig. 2B), suggesting a modest decrease in cardiac capacity and function. The few *MRTF*^{cdKO} mice that survived to adulthood showed the most severe cardiac dysfunction, with extreme ventricular dilation and ~50% reduction in cardiac function compared to control hearts (Fig. 2B and Fig. S1D), indicating severe deficits in cardiac output.

MRTF^{cdKO} hearts display sarcomere disarray

While we observed the most penetrant lethality of *MRTF*^{cdKO} mice shortly after birth, the hearts of these animals appeared grossly normal and indistinguishable from control hearts at P0 (Fig. 1B). Given the known role of the MRTFs in relaying information about the state of the cytoskeleton to the nucleus (Olson and Nordheim, 2010), we sought to examine sarcomere formation in the ventricles of P0 hearts. Strikingly, *MRTF*^{cdKO} hearts displayed extensive regions of severely disrupted sarcomeres as observed by electron microscopy (Fig. 3A). While control hearts displayed the expected organized myofibrils of actin and myosin filaments, *MRTF*^{cdKO} hearts showed areas of thickened and disorganized Z-bands and an obvious decrease in the presence of M-bands. Additionally, we observed that mitochondrial structure and number were compromised in *MRTF*^{cdKO} hearts at P0 (Fig. 3A). While we observed varying degrees of sarcomere disruption in the *MRTF*^{cdKO} animals, all hearts examined showed extensive defects in sarcomere formation, suggesting sarcomeric abnormalities as a primary cause of the increased lethality. Similarly, electron microscopy

on the left ventricle of a 7-week *MRTF*^{cdKO} animal revealed sarcomeric defects. While some regions of the cdKO heart displayed the expected sarcomere arrangement and cytoskeletal organization, other areas showed complete disarray, with minimal M-bands and only a few disorganized Z-bands (Fig. 3B), explaining the observed reduction in cardiac contractility by echocardiography (Fig. 2B). Together, these results indicate that *MRTF*^{cdKO} hearts display defects in sarcomeric structure that impede cardiac function.

RNA-seq analysis reveals changes in sarcomeric and cytoskeleton genes

To determine the genetic and molecular mechanisms underlying the enhanced lethality in *MRTF*^{cdKO} mice, we performed RNA-seq analysis on hearts isolated at P0 and validated the results using RT-qPCR (Fig. S3). By bioinformatics analysis, we identified 605 genes with differential expression in *MRTF*^{cdKO} hearts compared to wild type; within this set, 317 were down-regulated while 288 showed an increase in expression (Fig. 4A and Table S1). Using Ingenuity Pathway Analysis (IPA), we identified multiple pathways that were significantly altered in *MRTF*^{cdKO} hearts (Fig. 4B). The top 5 pathways identified as dysregulated all related to cytoskeletal organization, confirming the essential role of the MRTFs in cytoskeleton maintenance and function (Olson and Nordheim, 2010). Sixteen out of 21 genes in these pathways were down-regulated (Fig. 4C and Table S2). While we did observe that 5 genes related to the cytoskeleton were upregulated in *MRTF*^{cdKO} hearts, it remains to be elucidated if these changes are compensatory alterations due to sarcomere disruption or if these genes represent true MRTF targets genes in the heart. We also utilized DAVID to identify enriched Gene Ontology (GO) terms among the genes differentially regulated in *MRTF*^{cdKO} hearts (Fig. S2A). In addition to several metabolism-related categories, GO analysis identified 37 genes as implicated in cell death (Fig. S2B and Table S3), suggesting a critical role for the MRTFs in cardiac cell survival. Importantly, myocardin has also been identified as critical for cardiomyocyte survival (Huang et al., 2009), suggesting the MRTF family is essential for cardiomyocyte maintenance.

As MRTF is thought to act primarily as a transcriptional activator through its interaction with SRF (Olson and Nordheim, 2010), we focused on the 317 genes that displayed decreased expression in *MRTF*^{cdKO} hearts. DAVID analysis of the down-regulated genes identified a variety of enriched GO terms related to muscle and the cytoskeleton, including actomyosin structure organization, myofibril assembly, striated muscle development, and muscle thin filament assembly, confirming the critical role of MRTF in muscle function. In addition, GO identified other categories as significantly altered in *MRTF*^{cdKO} hearts, including translation and DNA metabolism, replication, and repair, suggesting the MRTFs could play a role in these processes as well (Fig. 4D).

As the MRTFs are known to signal with SRF via binding of the SRF-MRTF complex to CArG boxes, we performed a bioinformatics search for conserved CArG boxes associated with genes that were differentially expressed as measured by RNA-seq. Of these differentially expressed genes, we found that 212 (~35%) had at least one CArG box that was conserved (Table S4) and located within 5kb of the transcriptional start site (TSS), suggesting that MRTF exerts at least part of its effects on cardiac development through an interaction with SRF and the binding of CArG boxes. A recent analysis of SRF binding sites

in the genome of fibroblasts suggested that ~70% of SRF binding sites were found within 2kb of the TSS (Esnault et al., 2014); however, some binding sites far from the TSS were also reported, making it possible that we are underestimating the number of regulated genes that contain CArG boxes by focusing our analysis only within 5kb of the TSS. In order to compare the genes we identified as regulated by MRTF in the heart with known SRF binding sites, we utilized publicly available ChIP-Seq data that identified locations of SRF binding in the genome of HL-1 immortalized cardiomyocyte-like cells (Schlesinger et al., 2011). Of the 605 differentially expressed genes in *MRTF*^{cdKO} hearts, we identified 53 (8.7%) that were also locations of SRF binding in HL-1 cells (Table S5), suggesting that they could be direct targets of MRTF-SRF regulation. Among these genes were multiple regulators of the cytoskeleton and muscle function including *Actc1*, *Actb*, *Myl9*, *Tpm2*, *Cnn1*, *Cnn2*, *Actg1*, and *Acta1*. The multitude of cytoskeletal genes in this small subset of regulated genes suggests that the defects in sarcomere formation and cardiomyocyte function we observed are likely a result of direct regulation of key SRF target genes by MRTF binding. Together, our RNA-seq data confirm that the MRTFs are critical regulators of cardiac development and suggest that alterations in the cytoskeleton are the predominant cause of the lethality in *MRTF*^{cdKO} animals.

Discussion

The results of this study demonstrate that the MRTFs are required for cardiac development and function. Ablation of all four *MRTF* alleles causes postnatal lethality with sarcomeric disarray whereas deletion of three *MRTF* alleles results in dilated cardiomyopathy and mild adult onset heart failure. The dysregulation of genes controlled by SRF and the similarities between the phenotypes of *MRTF* and *SRF* mutant mice (Miano et al., 2004; Niu et al., 2005; Parlakian et al., 2005; 2004) point to MRTFs as critical mediators of SRF activity in the heart *in vivo*.

Global SRF deletion in mice results in lethality at gastrulation due to a gross failure in mesoderm formation, preventing analysis of the role of SRF in cardiac development or function (Arsenian et al., 1998). However, deletion of a conditional SRF allele in the embryonic heart, using an αMHC-Cre transgene, results in cardiac insufficiency and lethality by E12.5 (Niu et al., 2005) while deletion of SRF in the adult heart yields dilated cardiomyopathy and heart failure, indicating that SRF remains essential for adult cardiac function (Parlakian et al., 2005). The abnormalities observed with the *MRTF*^{cdKO} mice phenocopy multiple aspects of the defects observed with cardiac SRF deletion, including cardiac dilation and sarcomere disarray, and support the conclusion that MRTFs are obligatory regulators of SRF activity in the heart. However, unlike mice in which SRF was deleted with αMHC-Cre, which die embryonically by E12.5 (Niu et al., 2005), *MRTF*^{cdKO} mice survive until after birth, and occasionally into adulthood. We speculate that myocardin, which is highly expressed in the embryonic heart, partially compensates for the absence of MRTF-A and –B and allows cardiac development to proceed beyond the point in which cardiac deletion of SRF causes lethality. Generation of *myocardin*/*MRTF-A/B* triple knockout animals will be required to test this prediction.

The phenotype of *MRTF*^{cdKO} mice is reminiscent of animals in which αMHC-Cre was used to delete a floxed *Myocardin* allele. These mice showed enhanced lethality, beginning shortly after birth, with all mice dying before 1 year of age, accompanied by cardiac dilation, decreased heart function, and sarcomere disarray (Huang et al., 2009). The similarity between the phenotypes of mice in which MRTF-A and MRTF-B or myocardin were deleted with αMHC-Cre suggests that they may play similar roles in cardiac development and function. In addition to their cardiomyocyte-specific functions, MRTFs have been shown to play other roles in the heart, including activation of the cardiac fibrotic gene program (Small et al., 2010) and coronary vascular development and maturation (Trembley et al., 2015), while functions for myocardin and MRTFs in smooth muscle differentiation and development have been extensively investigated (S. Li et al., 2006; 2003; Oh et al., 2005; Z. Wang et al., 2004), indicating that MRTFs are critical regulators of multiple aspects of cardiac development and function.

Consistent with the role of the MRTF/SRF pathway as a critical regulator of heart development and homeostasis, our RNA-seq analysis on P0 *MRTF*^{cdKO} hearts confirmed that the MRTFs play an essential role in cardiac function, largely through regulation of the cytoskeleton and associated proteins. Interestingly, while the MRTF/SRF complex is considered a transcriptional activator, we observed that nearly half of the regulated genes in *MRTF*^{cdKO} hearts showed increased expression. Whether up-regulation of these genes reflects indirect effects of the loss of the MRTFs in the heart or whether the MRTFs have some other function outside their role as SRF coactivators remains to be elucidated. One notable observation in our RNA-seq analysis was that several of the upregulated genes in *MRTF*^{cdKO} hearts are also predicted to be sites of SRF binding (Table S5). It is unclear if these are valid SRF target genes. Additionally, it is conceivable that the MRTFs and/or SRF could have other binding partners that remain to be discovered. Consistent with this possibility, several instances of the MRTFs acting partially independent of SRF to alter gene expression have been described (Asparuhova et al., 2011; Hayashi et al., 2015), suggesting the MRTFs could have SRF-independent roles in the heart; future work will explore this possibility further. However, the significant down-regulation of known SRF target genes that encode essential regulators of cytoskeleton maintenance and function in *MRTF*^{cdKO} hearts underscores the critical role of the MRTFs as SRF coactivators and suggests that regulation of SRF activity is the primary cause of the lethality observed after cardiac MRTF deletion.

Together, our results show that loss of MRTF-A and MRTF-B in cardiomyocytes results in defects in cardiac contractility and heart failure and point to the MRTF/SRF pathway as a potential mediator of cardiomyopathy in humans. Numerous human mutations causing cardiomyopathy have been identified in components of the sarcomere and nuclear matrix that influence actin dynamics, confirming that these pathways play critical roles in the development of heart failure (McNally and Dellefave, 2009; Morita et al., 2010). Of particular interest is the discovery that caspase-3 cleavage of SRF in failing human hearts yields a dominant negative SRF protein that blocks SRF-dependent gene expression (Chang et al., 2003). Such a dominant negative SRF mutant would be expected to evoke a phenotype similar to that of *MRTF*^{cdKO} mice, resulting in a loss of SRF activity. Given the

pivotal role of MRTFs in modulating gene expression in response to actin signaling and cytoskeletal dynamics, it is reasonable to speculate that perturbation of MRTF activity in response to such mutations may contribute to pathological changes in gene expression associated with these disorders. In the future, it will be of interest to investigate whether pharmacologic or genetic stimulation of MRTF activity provides benefit in settings of sarcomere dysfunction and heart failure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Deletion of MRTFs in the heart results in cardiac abnormalities
- Hearts lacking MRTFs display sarcomere disarray
- MRTFs are essential for maintenance of cardiac structure and function
- MRTFs are critical mediators of SRF activity in the heart
- Deletion of MRTFs in the heart alters pathways related to cytoskeletal organization

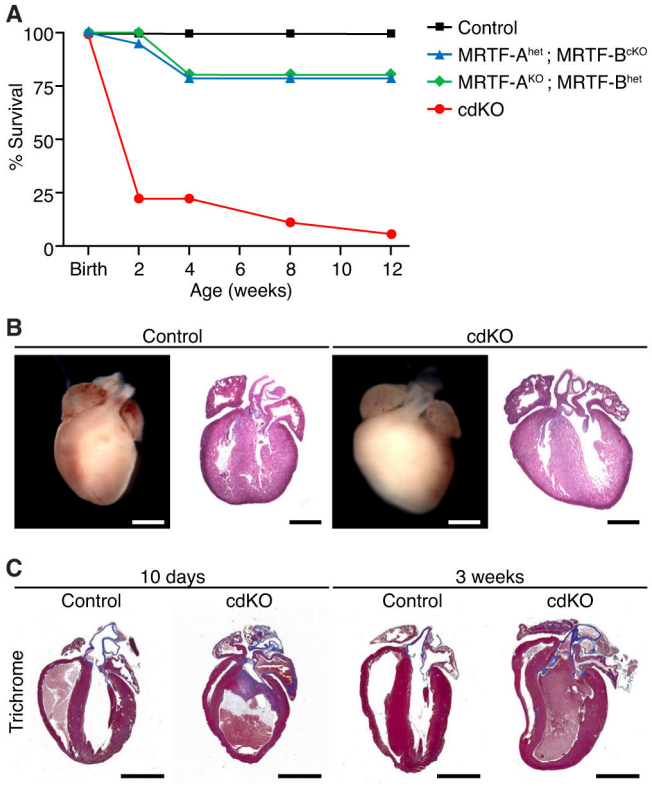

Figure 1. *MRTF*^{cdKO} mice display postnatal lethality

(A) Shown is the percent survival of control and *MRTF*^{cdKO} mice (n=20/genotype). More than 75% of *MRTF*^{cdKO} mice died within 2 weeks of birth while ~25% of mice lacking 3/4 *MRTF* alleles died between 2 and 4 weeks of age. (B) Whole mount and H&E-stained heart sections from control and *MRTF*^{cdKO} P0 mice are shown. *MRTF*^{cdKO} hearts showed no obvious cardiac defects. Scale bar: 1mm. (C) Masson's trichrome staining revealed the presence of left ventricular dilation and fibrosis in *MRTF*^{cdKO} hearts at both 10 days and 3 weeks after birth, indicative of cardiomyopathy and heart failure. Scale bar: 2mm.

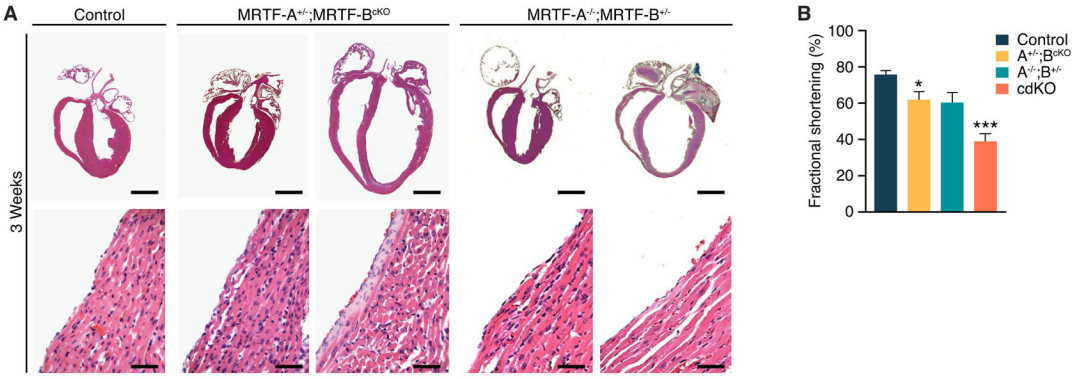

Figure 2. *MRTF*^{cdKO} animals show defects in cardiac function

(A) H&E staining of heart sections from control, *MRTF-A*$^{+/-}$;*MRTF-B*^{cKO}, and *MRTF-A*$^{-/-}$;*MRTF-B*$^{+/-}$ mice at 3 weeks of age are shown at low and high magnification in the upper and lower panels, respectively. Scale bars: 2mm for upper panels; 40μm for lower panels. Approximately 25% of *MRTF-A*$^{+/-}$;*MRTF-B*^{cKO}, and *MRTF-A*$^{-/-}$;*MRTF-B*$^{+/-}$ mice showed cardiac dilation and cardiomyocyte disorganization. (B) *MRTF-A*$^{+/-}$;*MRTF-B*^{cKO} and *MRTF-A*$^{-/-}$;*MRTF-B/*$^{+-}$ showed a slight reduction in fractional shortening (FS) compared to control animals at 7–8 weeks of age by echocardiography while surviving *MRTF*^{cdKO} animals showed an ~50% reduction in FS (n=4/genotype).

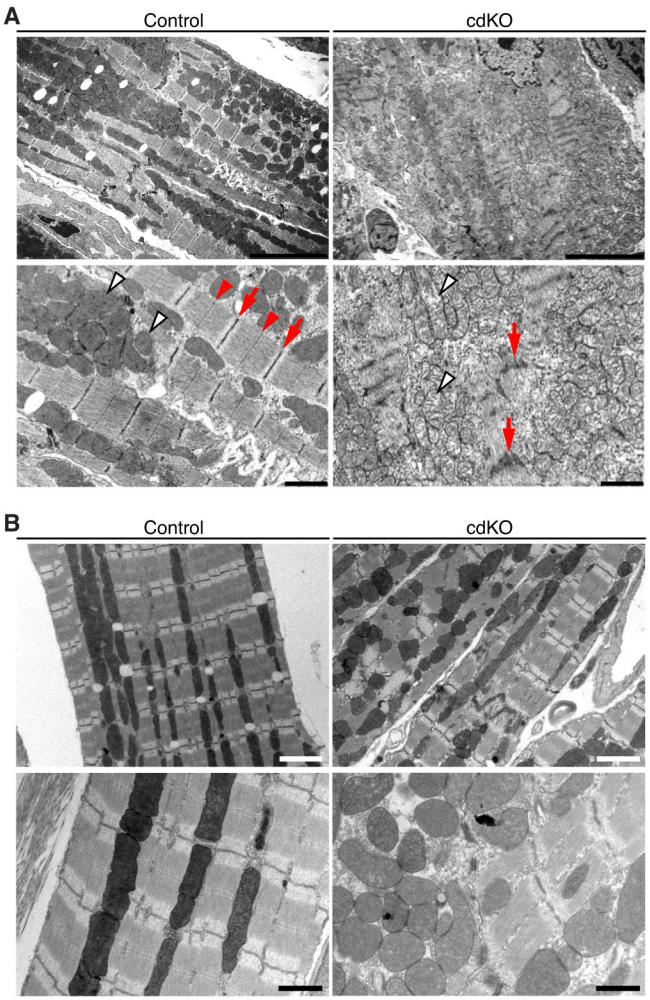

Figure 3. Defects in sarcomere arrangement in *MRTF*^{cdKO} hearts

(A) Electron micrographs of control and *MRTF*^{cdKO} hearts at P0 reveal severe sarcomere disarray with disorganized Z-bands (arrows) and absent M-bands (black arrowheads) as well as mitochondrial abnormalities (white arrowheads). Scale bars: 4μm for the upper panels and 1μm for the lower panels; n=4/genotype. (B) Electron micrographs of control and *MRTF*^{cdKO} left ventricle at 7 weeks of age showed regions of sarcomere disarray, as well as regions that looked relatively normal. Scale bars: 2μm for the upper panels and 1μm for the lower panels.

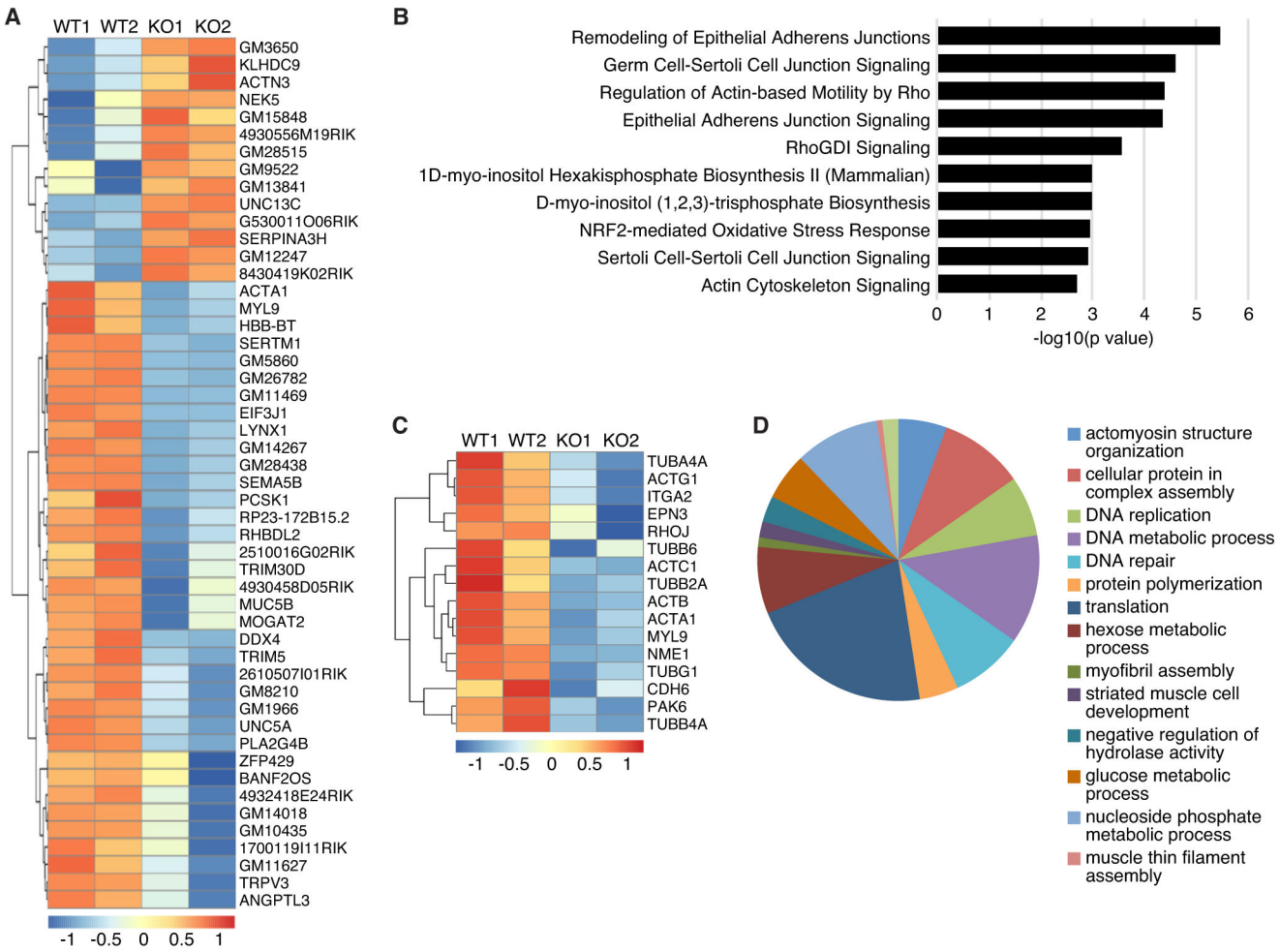

Figure 4. Molecular analysis of *MRTF*^{cdKO} hearts

(A) Heat map of the top 50 genes whose expression was altered in *MRTF*^{cdKO} hearts at P0. (B) Pathway analysis of differentially expressed genes in *MRTF*^{cdKO} hearts revealed regulation of many pathways related to the cytoskeleton. (C) Heat map showing 16 genes related to actin and cytoskeletal signaling have decreased levels of expression in *MRTF*^{cdKO} hearts. (D) GO analysis identified multiple enriched terms related to genes down-regulated in *MRTF*^{cdKO} hearts.