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Loss of microRNA-155 Protects the Heart from Pathological Cardiac Hypertrophy

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Abstract

Rationale—In response to mechanical and/or pathological stress, adult mammalian hearts often undergo mal-remodeling, a process commonly characterized as pathological hypertrophy, which is associated with upregulation of fetal genes, increased fibrosis, and reduction of cardiac dysfunction. The molecular pathways that regulate this process are not fully understood.

Objective—To explore the function of microRNA-155 (miR-155) in cardiac hypertrophy and remodeling.

Methods and Results—Our previous work identified miR-155 as a critical microRNA that repressed the expression and function of the myocyte enhancer factor 2A (MEF2A). In this study, we found that miR-155 is expressed in cardiomyocytes and that its expression is reduced in pressure overload-induced hypertrophic hearts. In mouse models of cardiac hypertrophy, miR-155 null hearts suppressed cardiac hypertrophy and cardiac remodeling in response to two independent pathological stressors – transverse aortic restriction (TAC) and an activated calcineurin (CnA) transgene. Most importantly, loss of miR-155 prevents the progress of heart failure and substantially extends the survival of CnA transgenic mice. The function of miR-155 in hypertrophy is confirmed in isolated cardiomyocytes. We identified Jarid2/jumonji as a miR-155 target in the heart. miR-155 directly represses Jarid2, whose expression is increased in miR-155 null hearts. Inhibition of endogenous Jarid2 partially rescues the effect of miR-155 loss in isolated cardiomyocytes.

Conclusions—Our studies uncover miR-155 as an inducer of pathological cardiomyocyte hypertrophy and suggest that inhibition of endogenous miR-155 might have clinical potential to suppress cardiac hypertrophy and heart failure.

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DISCLOSURES
The authors have declared that no competing interests exist.

Keywords

miR-155; heart; cardiac hypertrophy; cardiomyocyte; Jarid2; MEF2A; post-transcriptional regulation; cardiomyocyte hypertrophy; cardiac remodeling

INTRODUCTION

The adult heart is primarily composed of terminally differentiated, mature cardiomyocytes that express signature genes related to contraction. In response to physiological, mechanical or pathological stress, the heart undergoes hypertrophic growth, anatomically defined as an increase in the size of cardiomyocytes without an increase in cell number. [1,2] Pressure overload-induced cardiac hypertrophy is initially an adaptive response to maintain cardiac output. However, prolonged hypertrophic growth is associated with adverse consequences that often lead to heart failure and sudden death. [3,2,1,4]

MicroRNAs (miRNAs) are a class of small non-coding RNAs that modulate gene expression at the post-transcriptional level. Recent functional studies using both gain- and loss-of-function approaches in mice have started to uncover the important roles of miRNAs in cardiac hypertrophy and remodeling [5–7]. We showed that miR-22 protects the heart from stress-induced cardiac remodeling, [8] whereas the miR-17-92 cluster is a potent regulator of cardiomyocyte proliferation and cardiac regeneration [9]. miR-155 is encoded and co-expressed from the non-coding RNA (ncRNA) gene BIC, and it is highly expressed in activated B and T cells and in monocytes/macrophages [10]. Genetic studies in mouse models have demonstrated that miR-155 plays a vital role in hematopoiesis, lymphocyte homeostasis and tolerance. [11,12,10] In the cardiovascular system, miR-155 was reported to be expressed in atherosclerotic plaques and proinflammatory macrophages. miR-155 knockout mice displayed reduced plaque size after partial carotid ligation in atherosclerotic ($Apoe^{-/-}$) mice, suggesting a role of miR-155 in atherosclerosis [13]. In another study, overexpression of miR-155 in human cardiomyocyte progenitor cells was linked to protection from necrotic cell death in vitro [14]. Furthermore, it was reported that inhibition of endogenous miR-155 attenuated cardiac infiltration by monocyte-macrophages [15]. Most recently, the in vivo function of miR-155 in cardiomyocyte hypertrophy was reported [16].

Previously, we examined the post-transcriptional regulation of the myocyte enhancer factor 2A (MEF2A) gene and reported that miR-155 represses the expression and function of MEF2A in myocytes [17]. Here, we study the function of miR-155 in the heart, and we report that miR-155 is expressed in cardiomyocytes and its expression is dynamically regulated during cardiac hypertrophy. We show that genetic deletion of miR-155 prevents cardiac hypertrophy induced by pressure overload and the calcineurin transgene. Furthermore, inhibition of miR-155 in isolated cardiomyocytes diminishes agonist-induced cardiomyocyte hypertrophy. We identified Jarid2 as a miR-155 target in the hypertrophy pathway.

METHODS

Cell culture, quantitative RT-PCR (qPCR), Western blot analyses and immunochemistry were performed according to routine protocols. miR-155 mutant mice and calcineurin

transgenic mice (Tg(Myh6-Ppp3ca)37Eno), which were originally generated by Drs. Molkentin and Olson, were obtained from the Jackson lab. The transverse aortic constriction (TAC) operation and measurement of cardiac function by echocardiography are described as described [9].

Statistics

Values are reported as means±SEM unless indicated otherwise. The 2-tailed Mann-Whitney U test was used for comparing 2 means (Prism, GraphPad). Values of $P<0.05$ were considered statistically significant.

RESULTS

miR-155 is expressed in cardiomyocytes and its expression is reduced in hypertrophic hearts

In a prior study, we identified miR-155 as a key miRNA that repressed the expression of MEF2A, and we reported that miR-155 repressed C2C12 skeletal muscle myoblast differentiation, at least in part, by repressing MEF2A [17]. Numerous studies have documented that miR-155 is ubiquitously expressed in adult mouse tissues and enriched in lymphocytes and macrophages [10, 12, 15, 16]. We examined the expression of miR-155 in the hearts of fetal, postnatal and adult mice. qPCR analyses showed that the expression of miR-155 was relatively low in embryonic and neonatal hearts. However, miR-155 expression was increased in postnatal day 7 and adult hearts, with the highest miR-155 expression detected in the hearts of 15 month old mice (Fig. 1A). These data suggest that miR-155 may play an important role in the adult heart and cardiac remodeling.

Next, we examined the distribution of miR-155 expression in cardiomyocyte (CM) and non-cardiomyocyte (Non-CM) fractions of the adult heart. We found that miR-155 expression is enriched in cardiomyocytes of adult hearts (Fig. 1B). For positive controls, we showed that expression of the cardiomyocyte-specific genes cardiac troponin T (cTNT) and miR-1 [8] is enriched in the CM fraction. In contrast, expression of periostin (Postn), which marks cardiac epithelial cells [18], was restricted to the Non-CM fraction (Fig. 1B).

We next asked whether the expression of miR-155 is altered in cardiac hypertrophy. miR-155 expression was reduced in cardiac hypertrophy induced by pressure overload via transverse aortic constriction (TAC) at 2 and 4 weeks[8, 9] (Fig. 1C), consistent with prior report [19]. However, miR-155 expression was not altered in the hypertrophic heart of calcineurin (CnA) transgenic mice (Fig. 1C). Together, these data demonstrate that miR-155 is expressed in cardiomyocytes of adult hearts and that its expression is regulated in hypertrophic hearts.

miR-155 is required for pressure overload induced cardiac hypertrophy and remodeling

In order to study the function of miR-155 in the heart, we examined miR-155 knockout mice. miR-155 null mice were viable and fertile as previously reported [11, 20]. We verified that no miR-155 expression was detectable in tissues of miR-155 mutant mice using sensitive qPCR assays (data not shown). The gross morphology and the heart weight/body

weight ratio (HW/BW) of miR-155 mutant mice did not differ from that of wild-type littermate controls. Histologic examination and Sirius Red/Fast Green staining did not reveal abnormal cardiac morphology or fibrosis in miR-155 mutant mice. Echocardiographic measures of left ventricular size and cardiac function (as documented as fractional shortening) did not reveal any differences between miR-155 mutant mice and their littermate controls (Online Tables I and II). Together, these studies indicate that miR-155 is dispensable for normal mouse development and cardiac function under physiological conditions.

Next, we tested whether miR-155 plays a role in stress-dependent cardiac response and remodeling. We tested the functional involvement of miR-155 in cardiac hypertrophy after pressure overload induced by transverse aortic constriction (TAC), a widely used animal model for cardiac hypertrophy [2]. TAC induced massive cardiac hypertrophy in wild-type mice, evidenced by an increase in heart size. However, heart size of miR-155 null mice was substantially smaller than that of the control wild-type mice after TAC (Fig. 2A). The development of cardiac hypertrophy in the hearts of wild-type mice and the repression of hypertrophic growth in miR-155 null mice under the TAC condition is supported by calculating HW/BW ratio (Fig. 2B). Histological analysis further confirmed the lack of increase in ventricle wall thickness in miR-155 null mice in response to TAC, whereas TAC-induced hypertrophy was obvious in ventricles of control mice (Fig. 2C). At the cellular level, TAC significantly increased the size of cardiomyocytes in wild-type control hearts. Loss of miR-155 substantially eliminated the increase in cardiomyocyte size (Fig. 2D, E). TAC also increased cardiac fibrosis at 4 weeks, consistent with prior report [21]. However, cardiac fibrosis was substantially suppressed in the hearts of miR-155-KO mice (Fig. 2F).

We performed echocardiography measurements to document cardiac function at different time points (two and four weeks) after TAC. There was a dramatic increase in the thickness of the left ventricular posterior wall (LVPW) and the interventricular septum (IVS) in wild-type control mice after TAC surgery. This hypertrophic response was significantly reduced in miR-155 mutant hearts (Online Tables I and II), indicating that loss of miR-155 reduced TAC-induced cardiac hypertrophy. Furthermore, increases in LV internal dimension (LVID) and volume (LV Vol) were substantially mitigated in miR-155 null hearts after TAC (Online Tables I and II), indicating that loss of miR-155 suppressed the development of cardiac hypertrophy and the progression of dilated cardiomyopathy. These observations are consistent with the results of gross cardiac morphology and histology. Functionally, we found that miR-155-KO hearts preserve ventricular systolic function under pressure overload, as evidenced by the substantially higher fractional shortening (FS) in hearts of miR-155-KO mice compared with wild-type controls (Fig. 2G, Online Tables I and II).

We examined the expression of hypertrophic markers in miR-155-KO and control mice after TAC. TAC-mediated induction of BNP and β-MHC expression, as measured by qPCR assays, was suppressed in the hearts of miR-155 mutant mice (Fig. 2H). In addition, we confirmed that expression of β-MHC protein, induced by TAC in the hearts of control wild-type mice, was markedly reduced in the hearts of miR-155 mutant mice (Fig. 2I). Together,

these data demonstrate that miR-155 loss of function protects the heart from developing pathological cardiac hypertrophy in the face of cardiac stress.

Loss of miR-155 suppresses calcineurin-induced cardiac hypertrophy and heart failure

Calcineurin (CnA) is a calcium-regulated phosphatase, and previous studies have shown that cardiac-specific overexpression of CnA in α-MHC-CnA transgenic mice induces striking cardiac hypertrophy [22]. To test whether miR-155 is involved in CnA-induced cardiac hypertrophy, we bred miR-155 null mice with α-MHC-CnA transgenic mice. As expected, α-MHC-CnA transgenic mice underwent dramatic cardiac hypertrophy, with hearts substantially larger than control hearts. However, the hearts of CnA/miR-155-KO mice were much smaller than those of the α-MHC-CnA transgenic mice (Fig. 3A). The HW/BW ratio was smaller in the CnA/miR-155-KO compound mice than in the α-MHC-CnA transgenic mice (Fig. 3B). Histological analyses verify the reduction of ventricle wall thickness in CnA/miR-155-KO hearts compared with α-MHC-CnA hearts (Fig. 3C). Cardiomyocyte hypertrophy, as evidenced by an increase in cross sectional area (CSA) in CnA transgenic hearts, was drastically reduced in CnA/miR-155-KO hearts (Fig. 3D, E), suggesting that miR-155 is required for CnA transgene-induced cardiomyocyte hypertrophy. We also observed that loss of miR-155 reduced the development of fibrosis, which is induced by the CnA transgene and associated with cardiac hypertrophy and remodeling (Fig. 3F).

Echocardiography analyses indicated that loss of miR-155 reduced cardiac-specific CnA transgene-induced thickening of the left ventricular free wall (Fig. 3G and Online Table III). In addition, the left ventricle end systolic internal dimension (LVID;s) was reduced in CnA/miR-155-KO hearts when compared with α-MHC-CnA hearts (Online Table III). As a consequence, cardiac function, measured as fractional shortening (FS), was markedly improved in the CnA/miR-155-KO mice when compared with α-MHC-CnA mice (Fig. 3G). Most importantly, loss of miR-155 substantially increased the survival rate of CnA transgenic mice (Fig. 3H), highlighting the critical role of miR-155 in the regulation of pathological cardiac hypertrophy and heart failure.

Next, we examined the expression of hypertrophic markers BNP and β-MHC, which was dramatically induced in the hearts of CnA transgenic mice. We found that the expression levels of both transcripts and the β-MHC protein were repressed in the hearts of the CnA/miR-155-KO compound mice when compared with controls (Fig. 3I, J). These results indicate that miR-155 participates in the calcineurin-dependent cardiac hypertrophy pathway.

Inhibition of endogenous miR-155 suppresses cardiomyocyte hypertrophy in isolated cardiomyocytes

The above results, generated from miR-155-KO mice, strongly suggest that miR-155 plays a vital role in the regulation of cardiac hypertrophy in vivo. However, miR-155 is expressed in multiple cell and tissue types in addition to cardiomyocytes, so the global loss of function strategy used above cannot fully rule out the possibility that miR-155 acts in non-cardiomyocytes to regulate cardiac hypertrophy. To overcome this limitation and more specifically define the function of miR-155 in cardiomyocytes, we isolated and cultured

neonatal cardiomyocytes from wild-type and miR-155-KO hearts. Isolated cardiomyocytes were treated with phenylephrin (PE) to induce hypertrophy. Whereas cardiomyocytes isolated from wild-type control hearts developed massive hypertrophy after PE treatment, as evidenced by an increase in cell size and organized sarcomere structure, hypertrophic growth was markedly suppressed in miR-155-KO cardiomyocytes (Fig. 4A, high magnification images in Online Figure I). Quantitative measurement of cardiomyocyte size confirmed this observation (Fig. 4B). We examined the expression of hypertrophy-induced fetal genes, including ANP and β-MHC. We found that the expression of these fetal genes was substantially induced by PE treatment in wild-type control neonatal cardiomyocytes. This PE-induced increase is suppressed in miR-155-KO cardiomyocytes (Fig. 4C).

In order to independently verify the above observation, we isolated neonatal cardiomyocytes from rats and transfected them with miR-155 inhibitors, as previously reported [23, 24]. Cultured cardiomyocytes were then treated with PE to induce hypertrophy. As expected, inhibition of endogenous miR-155 in cardiomyocytes significantly repressed PE-induced hypertrophy, suggesting that miR-155 is required for the development of hypertrophy in cardiomyocytes (Fig. 4D, E, high magnification images in Online Figure II). We examined the cardiac hypertrophy markers ANP and β-MHC and found that their expression is induced by PE treatment in wild-type cardiomyocytes. This PE-induced expression was reduced in miR-155 inhibitor-treated cardiomyocytes, as compared to controls (Fig. 4F, G). Together, these data support the view that miR-155 is required for cardiomyocyte hypertrophy. Furthermore, our results suggest that miR-155 could mediate agonist-induced hypertrophic growth.

Regulation of miR-155 target genes

Individual miRNAs can repress the expression of many targets simultaneously, while a protein-coding target transcript can also be repressed by multiple miRNAs at the same time. Many miR-155 target genes have been identified previously, primarily in cancer cells and the immune system [25, 26]. In a recent study, transcriptome-wide screening for miR-155 targets was performed in activated CD4+ T-cells. Using differential high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (dCLIP) to identify miR-155 targets from wild-type and miR-155 null T-cells, the investigators identified 191 miR-155 canonical and noncanonical targets [27].

We reasoned that many of the miR-155 targets identified in T-cells are also expressed in the heart and that their expression should be up-regulated in miR-155-KO hearts. We first compared the 191 miR-155 targets identified from T-cells with the list of 257 putative miR-155 targets predicted by the TargetScan algorithm. We identified 35 of them as common, including the Mef2a gene, which we have previously identified [17] (Online Table IV). Next, we tested whether the expression of miR-155 targets was increased in the hearts of miR-155-KO mice. qPCR analyses showed that the expression of several predicted targets was upregulated in miR-155-KO hearts. These include Jarid2/jumonji and MAFb (Fig. 5A). Jarid2 is a histone demethylase, and previous studies have demonstrated that it plays a central role in cardiac development and hypertrophy [28–30]. We therefore focused on Jarid2 and examined its expression in hypertrophic hearts in more detail. We found that

Jarid2 expression was increased in neonatal cardiomyocytes isolated from miR-155-KO mice (Fig. 5B). We further determined that the expression of this gene was increased in the heart of CnA/miR-155-KO mice when compared with that in α-MHC-CnA controls (Fig. 5C). To further confirm that miR-155 represses the expression of Jarid2 transcripts in cardiomyocytes, neonatal rat cardiomyocytes were transfected with miR-155 inhibitors or control inhibitors, and the expression level of Jarid2 transcripts was determined using qPCR. Indeed, Jarid2 expression level was increased when endogenous miR-155 was antagonized by miR-155 inhibitors (Fig. 5D), suggesting that Jarid2 is a canonical miR-155 target in cardiomyocytes.

We performed luciferase reporter assays to examine the direct repression of Jarid2 by miR-155. We built luciferase reporters containing the 3' UTRs of the mouse Jarid2 gene and tested their repression by miR-155. Indeed, miR-155 potently repressed the expression of the Jarid2 3'UTR luciferase reporter. A miR-155 sensor reporter was used as a positive control (Fig. 5E). When the two miR-155 targeting sites were mutated, miR-155 mediated repression was lost (Fig. 5E), demonstrating the specificity of this repres.

Jarid2 mediates the function of miR-155 in cardiomyocytes

Next, we investigated whether Jarid2 mediates the function of miR-155 in the regulation of cardiomyocyte hypertrophy. We hypothesized that if the function of miR-155 is mediated by Jarid2, which is increased in miR-155-KO hearts, then inhibition of Jarid2 should, at least in part, rescue the loss-of miR-155 phenotype in cardiomyocytes. We designed several independent siRNAs to knockdown endogenous Jarid2 in neonatal rat cardiomyocytes (Online Figure III). Neonatal mouse cardiomyocytes were isolated from miR-155-KO and control hearts. Cultured cardiomyocytes were then treated with PE to induce hypertrophy. Whereas PE-induced hypertrophic growth was markedly suppressed in miR-155-KO cardiomyocytes, as shown by reduced cell size and loss of organized sarcomere structure and consistent with previous observation, Jarid2 knockdown de-represses this loss-of miR-155 phenotype (Fig. 6A, high magnification images in Online Figure IV). Quantification of cardiomyocyte cell size confirms this observation (Fig. 6B). Analyses of the expression of hypertrophic marker genes ANF and β-MHC show that inhibition of Jarid2 partially restores the expression of these genes, further supporting the view that Jarid2 mediates miR-155-dependent hypertrophic growth (Fig. 6C).

We decided to perform independent experiments to confirm the above observations. We isolated neonatal rat cardiomyocytes and inhibited endogenous miR-155 by specific inhibitors. We found that inhibition of Jarid2 partially rescues hypertrophic growth, which is inhibited by the loss of miR-155 (Fig. 6D, high magnification images in Online Figure V), consistent with what we observed in miR-155-KO mouse cardiomyocytes. Quantification of cardiomyocyte cell size and the expression of hypertrophic marker genes demonstrates that the inhibition of Jarid2 reverses the repression of cardiomyocyte hypertrophy resulting from inhibition of miR-155 (Fig. 6E, F). Together, these data indicate that the function of miR-155 in cardiomyocyte hypertrophy is partially mediated by its target Jarid2.

Our previous studies showed that miR-155 directly targets MEF2A in skeletal muscle cells [17]. Though the mRNA level of *Mef2a* was not altered in the hearts of miR-155

knockout mice (Fig. 5A), we asked whether miR-155 could decrease the MEF2A protein level. As expected, the expression of endogenous MEF2A protein was elevated in the hearts of miR-155 knockout mice (Online Figure VI), suggesting that miR-155 represses MEF2A expression at the translational step.

DISCUSSION

In this study, we explored the in vivo function of miR-155 in the heart and found that miR-155 plays a critical role in the regulation of cardiomyocyte hypertrophy. We demonstrated that cardiomyocyte hypertrophy, induced by pressure overload or a calcineurin transgene, was attenuated in miR-155-KO hearts. Genetic deletion of miR-155 prevented progression to dilated cardiomyopathy and heart failure and substantially extended lifespan in CnA-Tg mice, indicating that inhibition of miR-155 could become an effective therapeutic approach to prevent or minimize cardiac hypertrophy and heart failure.

While our current investigation was under preparation, a recent study reported that targeted deletion of miR-155 suppressed cardiac hypertrophy in response to stress. The authors suggested that macrophage-expressed miR-155 is responsible for the induction of cardiac hypertrophy [16]. Our studies demonstrate that miR-155 also acts in cardiomyocytes to directly regulate hypertrophy. We provided multiple lines of evidence to support this conclusion. A) miR-155-KO/CnA-Tg compound mice exhibit decreased cardiac hypertrophy when compared with CnA-Tg mice. The cardiac hypertrophy exhibited in the CnA-Tg heart is directly induced by cardiomyocyte-specific overexpression of CnA, driven by the cardiomyocyte-specific α-MHC promoter. Therefore, the observation that loss of miR-155 in miR-155-KO mice suppresses the CnA-Tg hypertrophic phenotype strongly suggests that cardiomyocyte-expressed miR-155 is directly responsible for the development of hypertrophy. B) Inhibition of endogenous miR-155 represses agonist-induced hypertrophy in isolated neonatal rat cardiomyocytes. C) Similarly, isolated neonatal mouse cardiomyocytes from miR-155-KO hearts failed to develop cardiomyocyte hypertrophy in response to PE stimulation. In the future, it will be necessary to generate cardiomyocyte-specific miR-155 knockout mice in order to more precisely define the in vivo function of miR-155 in cardiomyocytes. We predict that cardiomyocyte-specific deletion of miR-155 will, at least in part, suppress pathomechanically induced cardiac hypertrophy in vivo. Together, previously published studies and results from the current investigation establish a critical role of miR-155 in cardiac hypertrophy and remodeling. It is evident that miR-155 regulates cardiomyocyte hypertrophy autocrinally via myocyte-expressed miR-155 or paracrinally through macrophage-expressed miR-155.

Among many miR-155 targets, we found that the expression of Jarid2 was significantly increased in the hearts of miR-155-KO mice. Furthermore, we demonstrated that Jarid2 expression was elevated in isolated cardiomyocytes when endogenous miR-155 was inhibited. Jarid2 was previously shown to be a key transcriptional regulator of cardiac development and function [28, 29]. Genetic deletion of Jarid2 resulted in embryonic lethality. There was an increase in cardiomyocyte proliferation in Jarid2 null hearts, at least in part due to the derepression of cyclin D expression [29]. Jarid2 was previously shown to repress the expression of ANF, a hallmark of cardiac hypertrophy [31, 32]. In light of its role in ANF

repression and inhibition of cardiac hypertrophy, our finding that Jarid2 was substantially increased in the hearts of miR-155-KO mice under stress strongly suggests that Jarid2 is a key miR-155 target that mediates its function in cardiac hypertrophy and remodeling. Interestingly, while we found that inhibition of endogenous Jarid2 in cardiomyocytes could partially rescue the effect of miR-155 loss, we noticed that inhibition of Jarid2 by itself did not lead to hypertrophy. As a matter of fact, inhibition of Jarid2 slightly reduces PE-induced hypertrophy in neonatal cardiomyocytes. These observations indicate that Jarid2 may play distinct roles during the development of hypertrophy. Evidently, the identification of additional miR-155 targets in the heart and the determination of how each target mediates the function of miR-155 will remain a challenging task for future investigation. Nevertheless, it is conceivable that the expression and function of miR-155 is associated with human cardiovascular disease and that miR-155 is a putative therapeutic target for cardiac defects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Nonstandard Abbreviations and Acronyms

ANP	atrial natriuretic peptide
BNP	brain natriuretic peptide
BW	body weight
CM	cardiomyocyte
CnA	calcineurin
CSA	cross sectional area
cTNT	cardiac troponin T
FS	fractional shortening
H&E	haematoxylin and eosin
HW	heart weight
MEF2A	myocyte enhancer factor 2A
βMHC	beta myosin heavy chain

PE	phenylephrine
qPCR	quantitative polymerase chain reaction
TAC	transverse aortic constriction

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Novelty and Significance

What Is Known?

- The adult heart remodels in response to pathological and physiological conditions.
- microRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and function.
- miR-155 plays a key role in the immune system.

What New Information Does This Article Contribute?

- miR-155 is required for the development of cardiac hypertrophy in response to stress.
- Inhibition of miR-155 protects cardiac function in a mouse model of cardiac hypertrophy.
- miR-155 could be a therapeutic target for the treatment of pathological cardiac hypertrophy.

miR-155 has been implicated in a variety of biological processes and diseases, including immune disorders and cancer. However, the expression and function of this miRNA in the cardiovascular system has not been fully established. In this study, we found that miR-155 plays a key role in regulating cardiac hypertrophy, both in vivo in intact hearts and in vitro in isolated cardiomyocytes. We identified Jarid2 as a direct miR-155 target that mediates its function in cardiomyocytes. These findings suggest that miR-155 may be a potential therapeutic target in the prevention or treatment of cardiac hypertrophy.

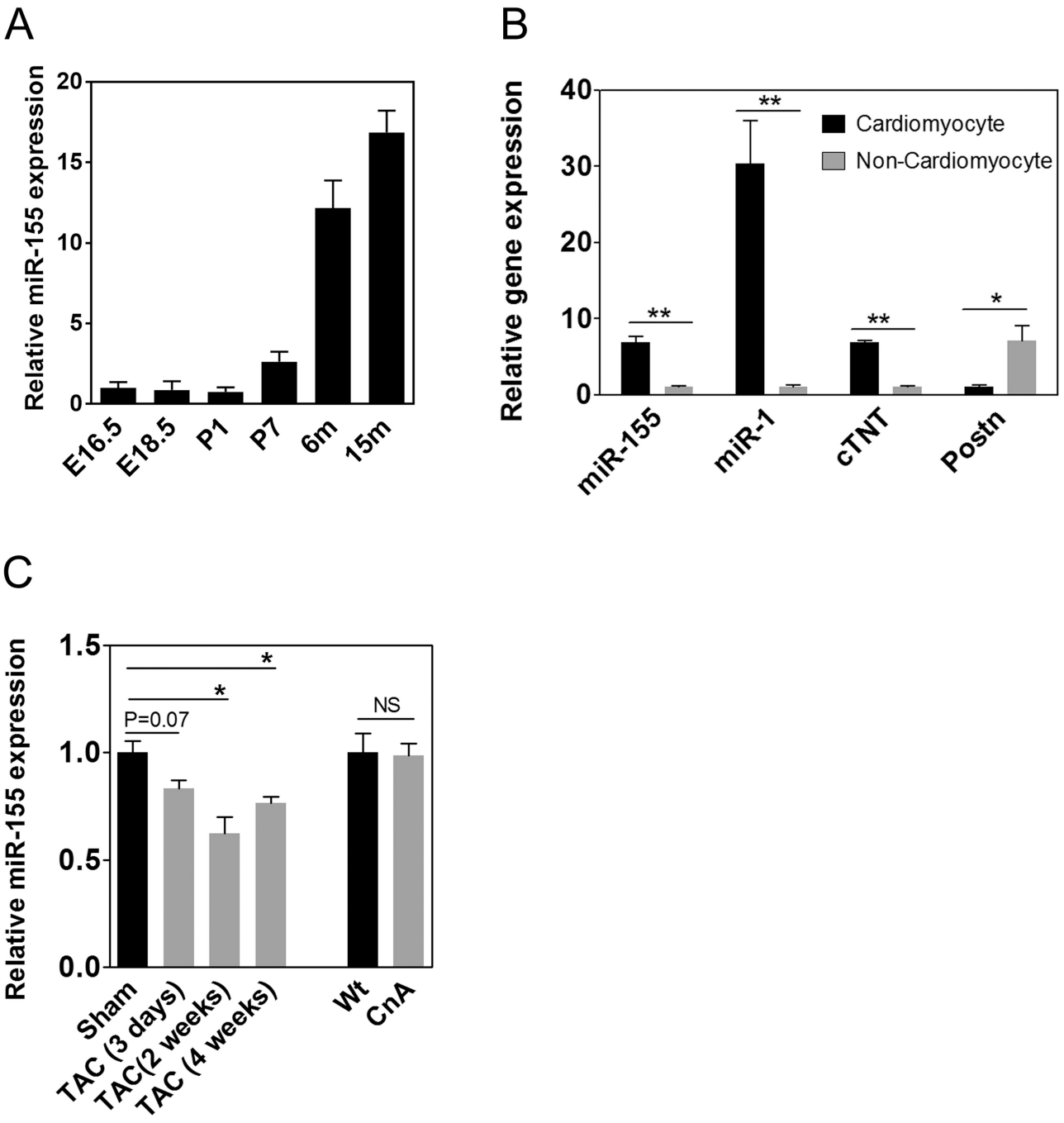

Figure 1. Altered miR-155 expression in hypertrophic hearts

(A) Expression of miR-155 in embryonic, postnatal and adult hearts, as determined by qPCR assays. (N=3 for each embryo or animal group).

(B) Expression of miR-155 in cardiomyocytes (CM) or non-cardiomyocytes (Non-CM) of adult mouse hearts, as determined by qPCR assays. miR-1 and cTNT serve as positive controls for CM. Periostin (Postn) marks cardiac fibroblasts.

(C) Expression of miR-155 in TAC-induced hypertrophic hearts at 3 days, 2 weeks and 4 weeks, as determined by qPCR assays. (N=3 for each group). Expression of miR-155 in control (WT) and calcineurin (CnA) transgenic hearts (right panel).

Data represent the mean ± SEM from at least three independent experiments. *: $P<0.05$; **: $P < 0.01$.

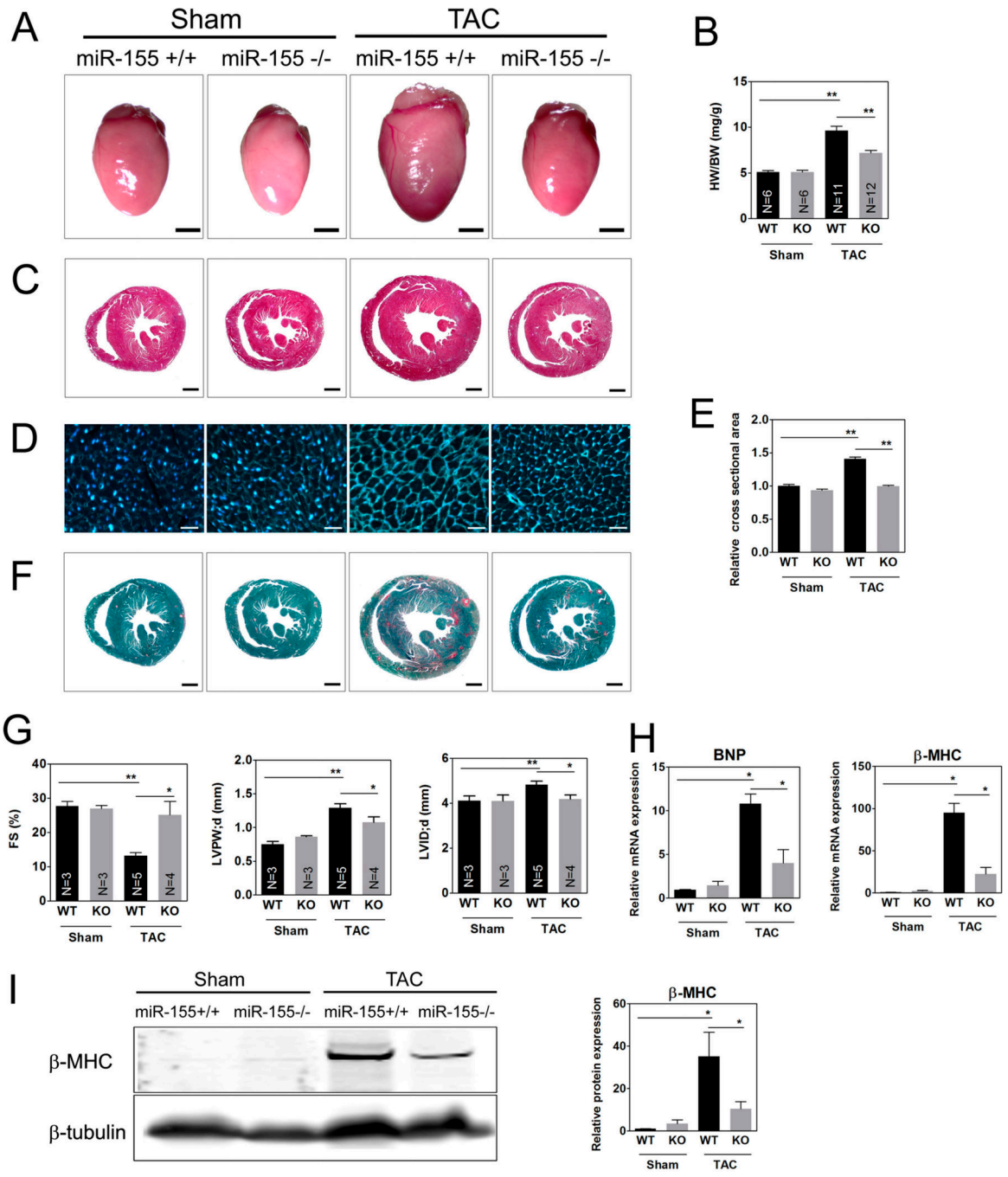

Figure 2. Loss of miR-155 suppresses pressure overload-induced cardiac hypertrophy

(A) Gross morphology of adult hearts from miR-155-KO mice and their control littermates 4 weeks after TAC. Sham was used as control. Bars = 2 mm.

(B) Heart weight (HW) to body weight (BW) ratios of adult wild type (WT) and miR-155-KO mice (KO) 4 weeks after TAC (or Sham) (N of each group was indicated).

(C) H&E staining of adult hearts from miR-155-KO mice and their control littermates 4 weeks after TAC. Bars = 1 mm.

(D) Wheat germ agglutinin (WGA) staining of transverse sections of adult hearts from miR-155-KO mice and their control littermates 4 weeks after TAC. Bars = 32 μm.
(E) Quantification of the size of cardiomyocytes by measurement of cross sectional area. More than 300 cells from three different hearts were analyzed per group.
(F) Fast Green/Sirius Red staining of adult hearts from miR-155-KO mice and their control littermates 4 weeks after TAC. Bars = 1 mm.
(G) Echocardiography analyses of cardiac function of adult miR-155-KO mice and their control littermates 4 weeks after TAC. Sham was used as control. FS, fractional shortening; LVPW;d, left ventricular posterior wall at diastole; LVID;d, left ventricular internal dimension at diastole. (N of each group was indicated).
(H) qPCR analyses of relative expression of BNP and β-MHC genes.
(I) Western blot analyses of protein expression of β-MHC. β-tubulin was used as a loading control. The intensity of the Western blot signal was quantified and is shown as relative protein expression after normalization to β-tubulin (right).
Data represent the mean ± SEM from at least three independent experiments. *: $P<0.05$; **: $P < 0.01$.

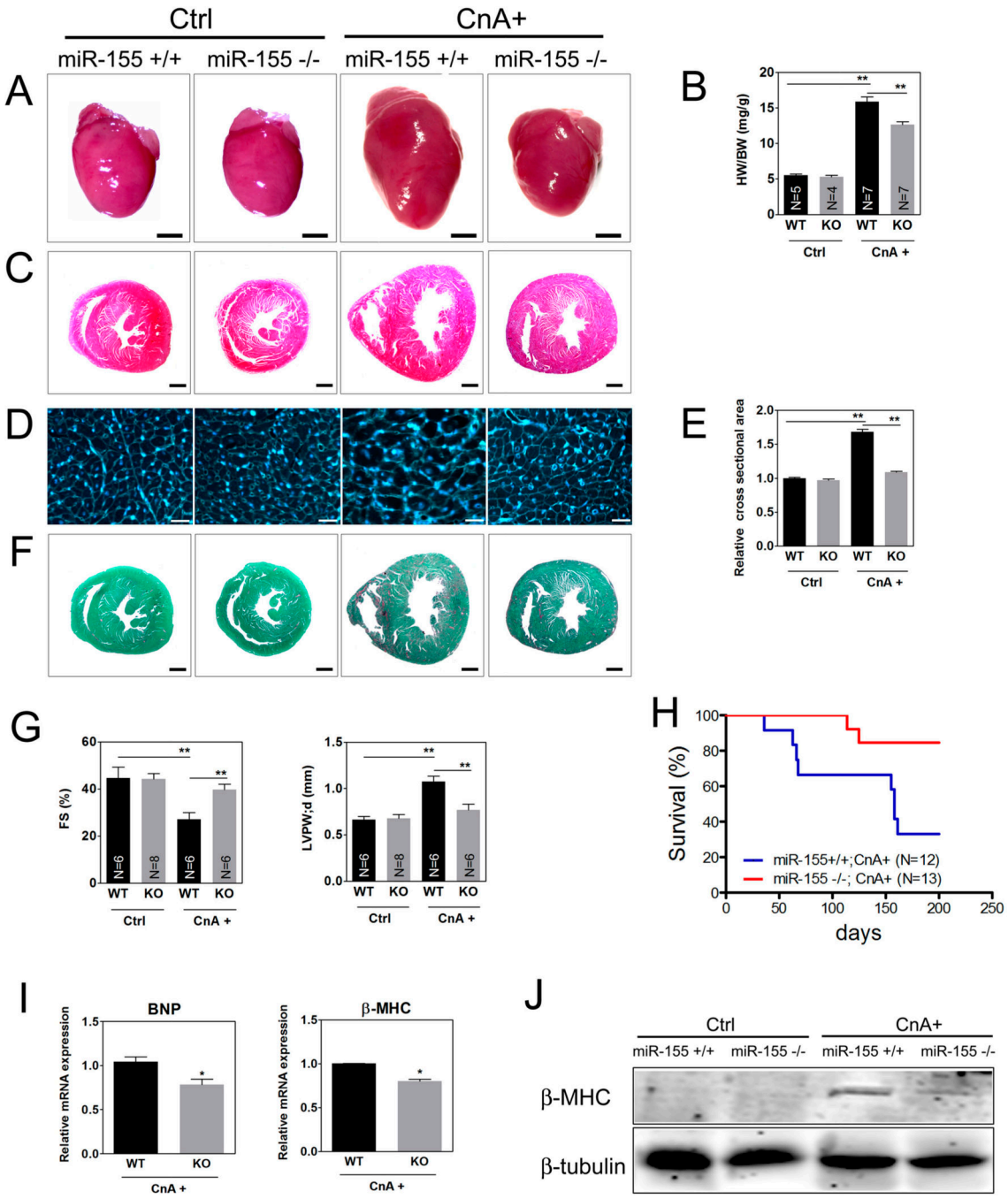

Figure 3. miR-155 participates in the calcineurin pathway to regulate cardiac hypertrophy

(A) Gross morphology of adult hearts from adult wild type (+/+) and miR-155 null mice (−/−) intercrossed with cardiac-specific calcineurin transgenic mice (CnA+). Bars = 2 mm.

(B) Heart weight (HW) to body weight (BW) ratios of adult wild type (WT) and miR-155-null mice (KO) intercrossed with calcineurin transgenic mice (CnA+). (N of each group was indicated).

(C) H&E staining of hearts from adult wild type (+/+) and miR-155 null mice (−/−) intercrossed with calcineurin transgenic mice (CnA+). Bars = 1 mm.

(D) Wheat germ agglutinin (WGA) staining of transverse sections of hearts from adult wild type (+/+) and miR-155 null mice (−/−) intercrossed with calcineurin transgenic mice (CnA +). Bars = 32 μm.

(E) Quantification of the size of cardiomyocytes of adult wild type (WT) and miR-155-null mice (KO) intercrossed with calcineurin transgenic mice (CnA+). More than 300 individual cells from three different hearts were analyzed per group.

(F) Fast Green/Sirius Red staining of hearts from adult wild type (+/+) and miR-155 null mice (−/−) intercrossed with calcineurin transgenic mice (CnA+). Bars = 1 mm.

(G) Echocardiography analyses of cardiac function of adult wild type (+/+) and miR-155 null mice (−/−) intercrossed with calcineurin transgenic mice (CnA+).

(H) Survival curve of adult wild type (+/+) and miR-155 null mice (−/−) intercrossed with calcineurin transgenic mice (CnA+).

(I) qPCR measurement of relative expression of BNP and β-MHC genes.

(J) Western blot analyses of protein expression of β-MHC. β-tubulin was used as a loading control.

Data represent the mean ± SEM from at least three independent experiments. *: $P<0.05$; **: $P < 0.01$.

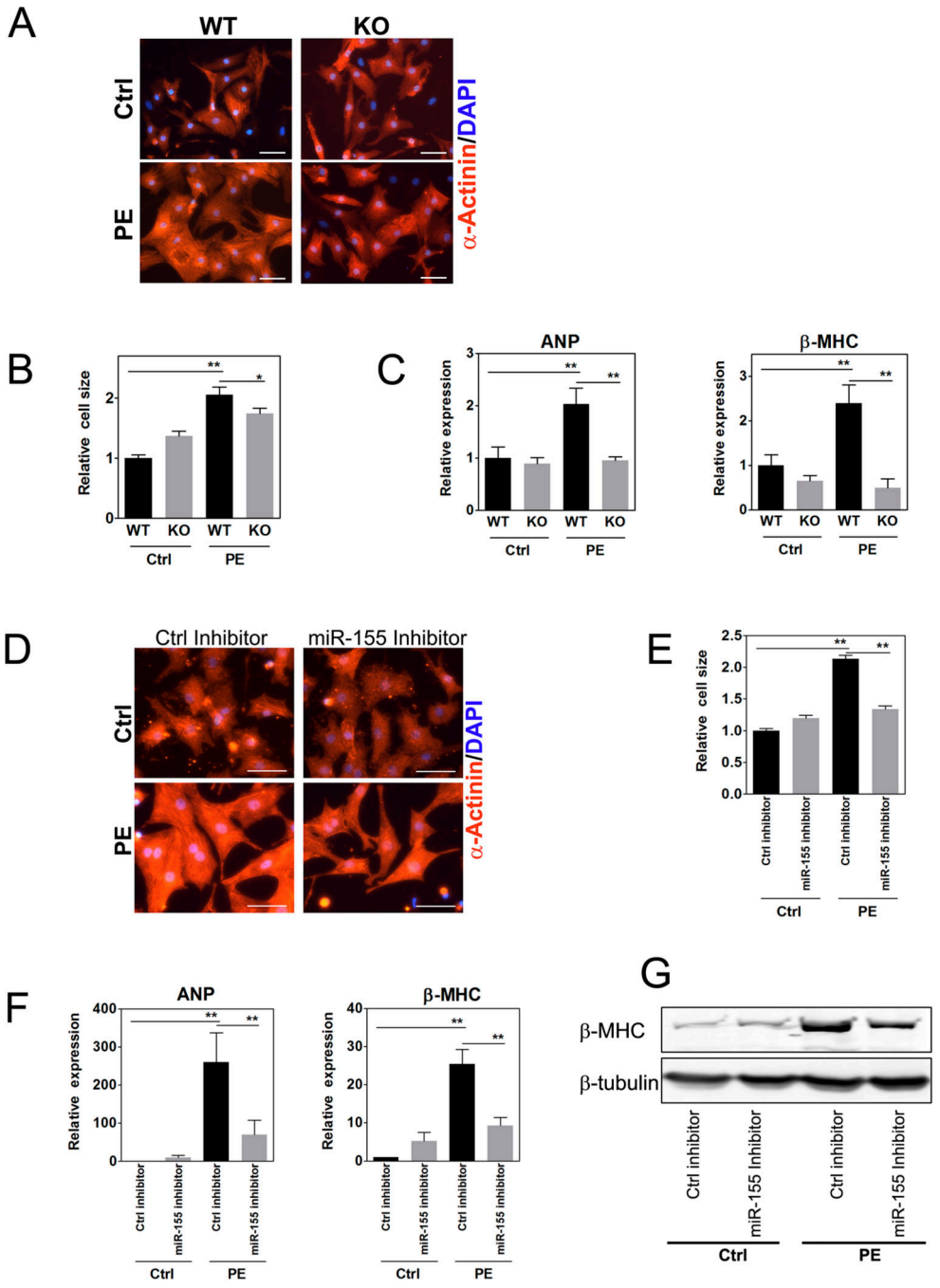

Figure 4. miR-155 is required for agonist-induced cardiomyocyte hypertrophy in vitro

(A) Neonatal mouse cardiomyocytes were isolated from wild type (+/+) and miR-155 null (−/−) hearts and stained for α-actinin after treatment with PE or without treatment (Ctrl). Bars = 16 μm.

(B) Mean cell size of α-actinin positive neonatal mouse cardiomyocytes. One hundred individual cells were analyzed per group.

(C) qPCR measurement of relative expression of ANP and β-MHC genes.

(D) Isolated neonatal (P1) rat cardiomyocytes were transfected with miR-155 inhibitors or control inhibitor. Cells were stained for α-actinin after treatment with PE or without treatment (Ctrl). Bars = 16 μm.

(E) Mean cell size of α-actinin positive neonatal rat cardiomyocytes. One hundred individual cells were analyzed per group.

(F) qPCR measurement of relative expression of ANP and β-MHC genes.

(G) Western blotting analyses of β-MHC protein expression. β-tubulin was used as a loading control.

Data represent the mean ± SEM from at least three independent experiments. *: $P<0.05$; **: $P < 0.01$.

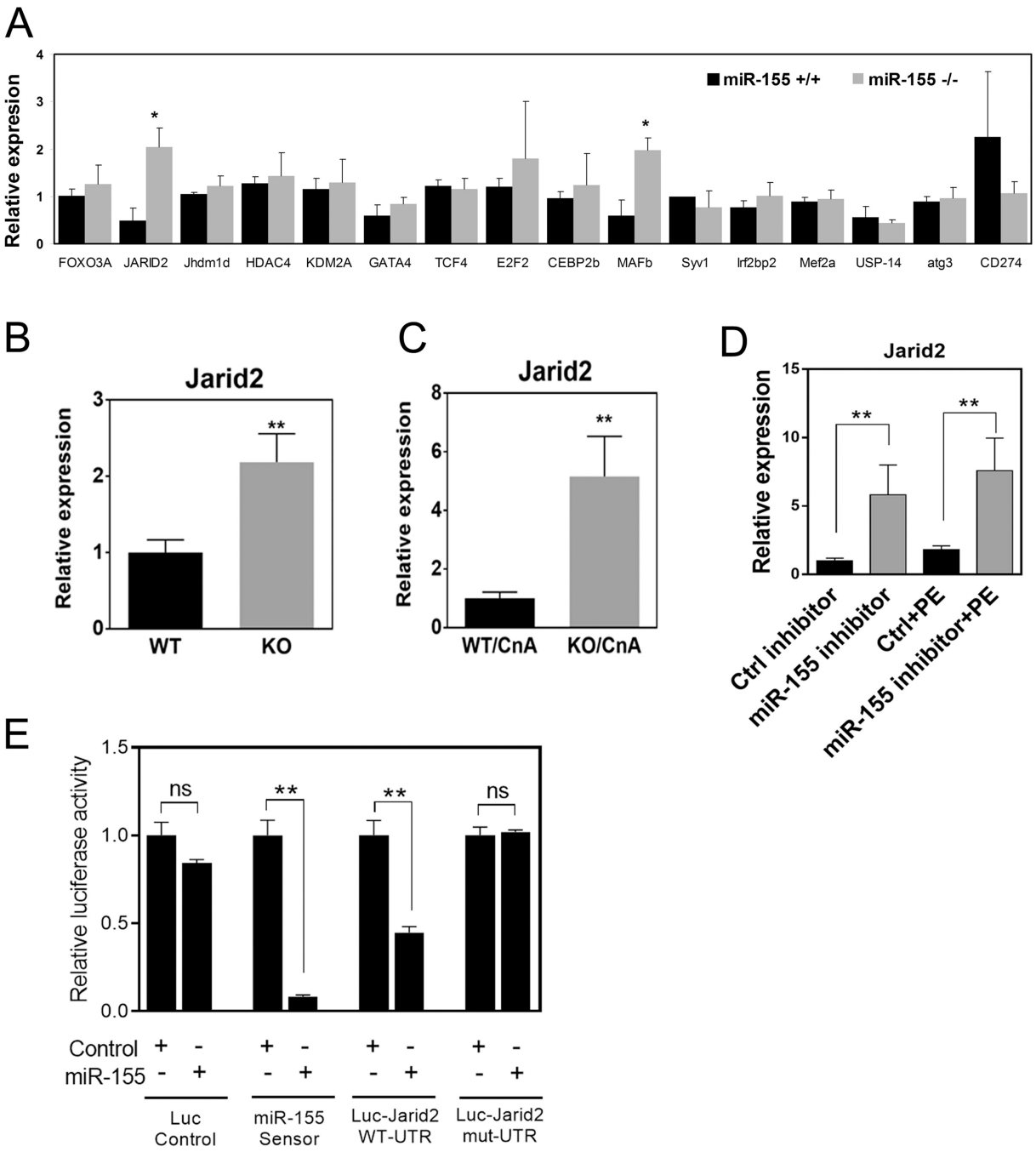

Figure 5. miR-155 represses its targets in cardiomyocytes

(A) qPCR analyses of the expression of putative miR-155 targets in adult hearts of miR-155 mutant (−/−) and control (+/+) mice.

(B) qPCR analyses of relative expression of Jarid2 in isolated neonatal cardiomyocytes of wild type (WT) and miR-155-KO mice (KO). N=3 each group.

(C) qPCR analyses of relative expression of Jarid2 in hearts of adult wild type (WT/CnA) and miR-155 null mice (KO/CnA) intercrossed with cardiac-specific calcineurin transgenic mice. N=3 for each group.

(D) qPCR analyses of the expression of Jarid2 transcripts in isolated neonatal rat cardiomyocytes transfected with miR-155 inhibitors or control inhibitor. Cells were treated with PE or without treatment.

(E) Luciferase reporters with empty luciferase reporter (Luc Control), a putative miR-155 binding site (miR-155 Sensor), wild-type or mutant Jarid2-UTR (Luc-Jarid2-WT-UTR or Luc-Jarid2-mut-UTR respectively) were co-transfected with miR-155 or control expression plasmids and luciferase activity determined. Values are presented as relative luciferase activity ± SD relative to the luciferase activity of reporters co-transfected with controls. (N=3).

Data represent the mean ± SEM from at least three independent experiments. *: $P<0.05$; **: $P < 0.01$.

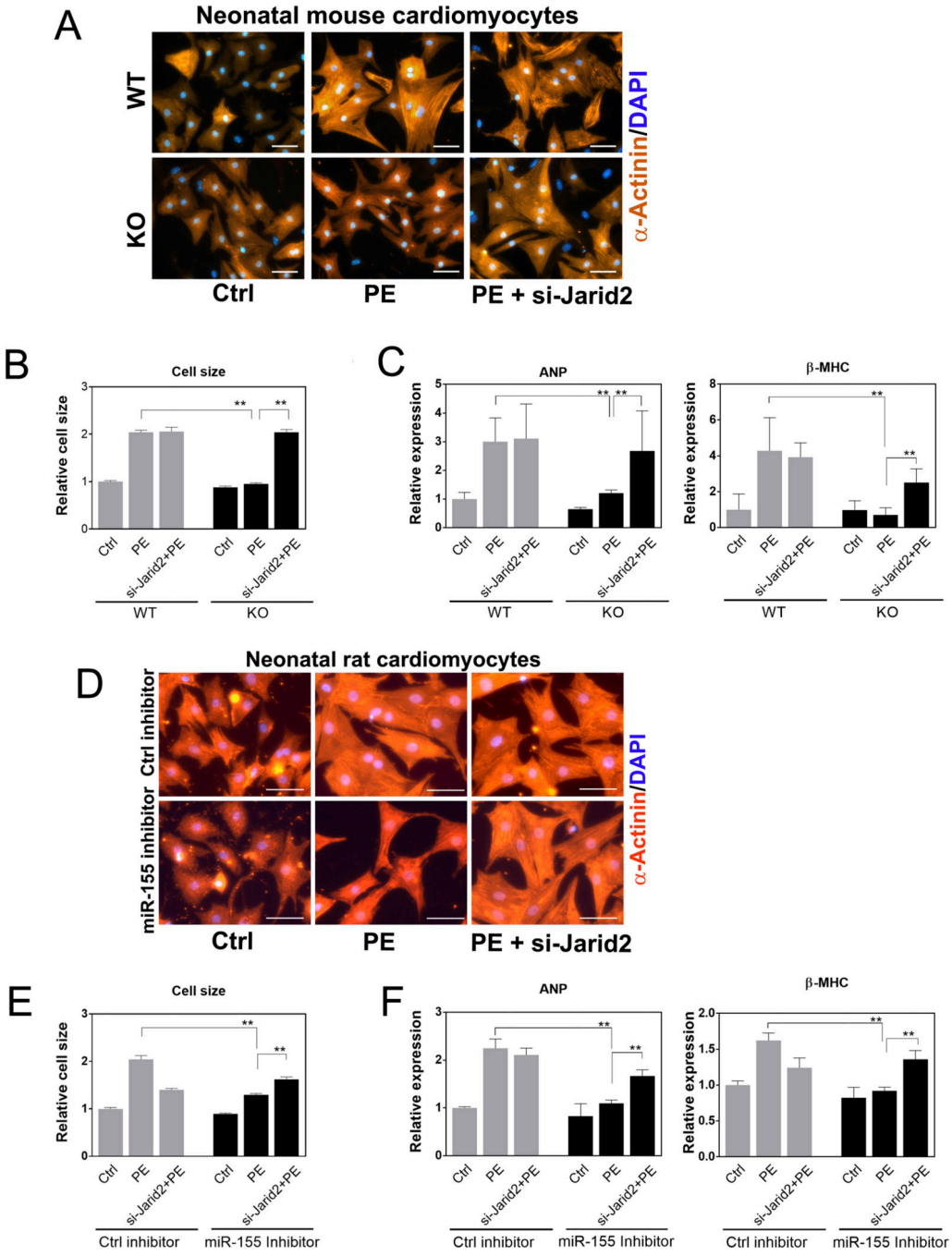

Figure 6. Jarid2 mediates the function of miR-155 in cardiomyocytes

(A) Neonatal mouse cardiomyocytes were isolated from wild type (WT) and miR-155 null (KO) hearts and stained for α-actinin after treatment with PE or PE plus si-Jarid2. Non-treated samples serve as controls (Ctrl). Bars = 16 μm.

(B) Mean cell size of α-actinin positive neonatal mouse cardiomyocytes. One hundred individual cells were analyzed per group.

(C) qPCR measurement of relative expression of ANP and β-MHC genes.

(D) Isolated neonatal rat cardiomyocytes were transfected with miR-155 inhibitors or control inhibitor. Cells were stained for α-actinin after treatment with PE or PE plus si-Jarid2. Non-treated samples serve as controls (Ctrl). Bars = 16 μm.

(E) Mean cell size of α-actinin positive neonatal rat cardiomyocytes. One hundred individual cells were analyzed per group.

(F) qPCR measurement of relative expression of ANP and β-MHC genes.

Data represent the mean ± SEM from at least three independent experiments. *: $P<0.05$; **: $P < 0.01$.