A long noncoding RNA protects the heart from pathological hypertrophy

Pei Han1,2, Wei Li1,2*, Chieh-Yu Lin2*, Jin Yang1, Ching Shang2, Sylvia T. Nurnberg2, Kevin Kai Jin3, Weihong Xu3, Chieh-Yu Lin2, Chien-Jung Lin2, Yi-Tsün Xiong2, Huan-Chieh Chien2, Bin Zhou4, Euan Ashley2, Daniel Bernstein5, Peng-Sheng Chen6, Huei-Sheng Vincent Chen6, Chiou-Hong Lin6,7, Thomas Quertermous6 and Ching-Pin Chang7,8

The role of long noncoding RNA (lncRNA) in adult hearts is unknown; also unclear is how lncRNA modulates nucleosome remodelling. An estimated 70% of mouse genes undergo antisense transcription1, including myosin heavy chain 7 (Myh7), which encodes molecular motor proteins for heart contraction2. Here we identify a cluster of lncRNA transcripts from Myh7 loci and demonstrate a new lncRNA–chromatin mechanism for heart failure. In mice, these transcripts, which we named myosin heavy-chain-associated RNA transcripts (Myheart, or Mhrt), are cardiac-specific and abundant in adult hearts. Pathological stress activates the Brg1–Hdac–Parp chromatin remodeler complex3 to inhibit Mhrt transcription in the heart. This stress-induced Mhrt repression is essential for cardiomyopathy to develop: restoring Mhrt to the pre-stress level protects the heart from hypertrophy and failure. Mhrt antagonizes the function of Brg1, a chromatin-remodelling factor that is activated by stress to trigger aberrant gene expression and cardiac myopathy4. Mhrt prevents Brg1 from recognizing its genomic DNA targets, thus inhibiting chromatin targeting and gene regulation by Brg1. It does so by binding to the helicase domain of Brg1, a domain that is crucial for tethering Brg1 to chromatinized DNA targets. Brg1 helicase has dual nucleic-acid-binding specificities: it is capable of binding lncRNA (Mhrt) and chromatinized—but not naked—DNA. This dual-binding feature of helicase enables a competitive inhibition mechanism by which Mhrt sequesters Brg1 from its genomic DNA targets to prevent chromatin remodelling. A Mhrt–Brg1 feedback circuit is thus crucial for heart function. Human Mhrt also originates from MYH7 loci and is repressed in various types of myocardial hearts, suggesting a conserved lncRNA mechanism in human cardiomyopathy. Our studies identify a cardioprotective lncRNA, define a new targeting mechanism for ATP-dependent chromatin-remodelling factors, and establish a new paradigm for lncRNA–chromatin interaction.

By 5' and 3' rapid amplification of complementary DNA ends, we discovered an alternative splicing of Myh7 antisense transcription into a cluster of RNAs of 709 to 1,147 nucleotides (Mhrt RNAs), containing partial sequences of Myh7 introns and exons (Fig. 1a and Supplementary Note). Mhrt RNAs were cardiac-specific (Fig. 1b), present at low levels in fetal hearts, with increasing abundance as the hearts matured and Myh7/Myh6/Myh2 ratio increased (Fig. 1c). RNA in situ analysis showed that Mhrt RNAs resided in the myocardium but not endocardium or epicardium (Fig. 1d and Extended Data Fig. 1a). Quantification of nuclear/cytoplasmic RNA in heart extracts revealed that Mhrt transcripts were primarily nuclear RNAs (Fig. 1e). Coding substitution frequencies4,5 of Mhrt RNAs predicted a negative/low protein-coding potential, in vitro translation of Mhrt RNAs yielded no proteins, and ribosome profiling6 revealed no/minimal ribosomes on Mhrt (Fig. 1f, Extended Data Fig. 1b–f and Supplementary Note). Consequently, Mhrt RNAs are non-coding RNAs in cardiomyocyte nuclei.

Mhrt RNAs were downregulated by 46–68% in hearts pressure-overloaded by transaortic constriction (TAC)3, beginning by 2 days and lasting for ~42 days after TAC (Fig. 2a). Such Mhrt reduction coincided with the TAC-induced Myh6 to Myh7 isoform switch characteristic of cardiomyopathy4–8 (Extended Data Fig. 2a). To define Mhrt function, we focused on Mhrt779, the most abundant Mhrt species, with 779 nucleotides (Fig. 2b, c and Extended Data Fig. 2b–e). We generated a transgenic mouse line to restore Mhrt779 level in stressed hearts. This transgenic line, driven by tetracycline response element (Tet-Mhrt779), was crossed to a cardiac-specific driver line (Tmn2-rtTA)3 that employs troponin promoter (Tmtn2) to direct expression of reverse tetracycline-dependent transactivator (rtTA). The resulting Tmn2-rtTA;Tet-Mhrt779 line (abbreviated as Tg779) enabled the use of doxycycline to induce Mhrt expression in cardiomyocytes. Within 7–14 days of doxycycline treatment, Mhrt779 increased by ~1.5-fold in left ventricles of Tg779 mice; this offset Mhrt779 suppression in TAC-stressed hearts to maintain Mhrt779 at the pre-stress level (Fig. 2d). Six weeks after TAC, doxycycline-treated control mice (Tnnt2-Mhrt779, Tmn2-rtTA or wild type) developed severe cardiac hypertrophy and fibrosis with left ventricular dilatation and reduced fractional shortening. Conversely, doxycycline-treated Tg779 hearts—with Mhrt779 maintained at the pre-stress level—developed much less pathology, with a 45.7% reduction in the ventricle/body-weight ratio (Fig. 2e) and a 61.3% reduction in cardiomyocyte size (Fig. 2f and Extended Data Fig. 3a), minimal/absent cardiac fibrosis (Fig. 2g), a 45.5% improvement of fractional shortening (Fig. 2h and Extended Data Fig. 3b), normalized left ventricular size (Fig. 2i), and reduced pathological changes of Anf (also known as Nppa), Bnp (also known as Nppb), Serca2 (also known as Atp2a2), Tgfb1 and Oprn (also known as Spp1) expression10–13 (Extended Data Figs 3c and 6e). To further test the cardioprotective effects of Mhrt, we induced Mhrt779 after 1–2 weeks of TAC when hypertrophy had begun. This approach reduced hypertrophy by 23% and improved fractional shortening by 33% in 8 weeks after TAC (Extended Data Fig. 3d–f). The efficacy of late Mhrt779 introduction suggests that a sustained repression of Mhrt in stressed hearts is essential for continued decline of cardiac function.

To study Mhrt regulation, we examined the 5' upstream region of the Mhrt genomic site (~2329 to +143) (Extended Data Fig. 4a) for signatures of a lncRNA promoter: RNA polymerase II (Pol II), histone H3 trimethylated lysine 4 (H3K4me3) and histone H3 trimethylated lysine 36 (H3K36me3)14,15. By chromatin immunoprecipitation (ChIP) of left ventricles, we found that this putative promoter contained four evolutionarily conserved elements (a1 to a4)16 that were enriched with Pol II

1Kraemer Institute of Cardiology and Division of Cardiology, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA. 2Division of Cardiovascular Medicine, Cardiovascular Institute, Stanford University School of Medicine, Stanford, California 94305, USA. 3Stanford Genome Technology Center, Stanford University School of Medicine, Stanford, California 94305, USA. 4Department of Genetics, Pediatrics, and Medicine (Cardiology), Albert Einstein College of Medicine of Yeshiva University, 1301 Morris Park Avenue, Price Center 420, Bronx, New York 10461, USA. 5Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305, USA. 6Del E. Webb Neuroscience & Stem Cell Research Center, Sanford/Burnham Medical Research Institute, La Jolla, California 92037, USA. 7Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA. 8Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA. *These authors contributed equally to this work.

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Figure 1 | Profile of the noncoding RNA Mhrt. a, Schematic illustration of Mhrt RNAs originating from the intergenic region between Myh6 and Myh7 and transcribed into Myh7. Myh7 exons and introns are indicated. m, mid region of the RNAs. F1 and R1, targeting the 5' and 3' Mhrt common sequences, are the primers used for subsequent polymerase chain reaction (PCR). b, Quantitative PCR with reverse transcription (RT-qPCR) of Mhrt RNAs using primers targeting common regions of Mhrt RNAs in tissues from 2-month-old mice. c, RT-qPCR of Mhrt, Myh6 and Myh7 in mouse hearts at different ages. Mhrt and Myh6/Myh7 ratio of embryonic day (E)11 hearts are set as 1. P, postnatal day. d, RNA in situ analysis of Mhrt (blue) in adult hearts.

(a1 to a4), H3K4me3 (a1 and a4) and H3K36me3 (ref. 16–18) (a1 to a3/a4) (Extended Data Fig. 4a). Conversely, no Pol II, H3K4me3 or H3K36me3 enrichment was found in control Slk and Vegfa promoters or in thymus and lungs that did not express Mhrt RNAs (Extended Data Fig. 4b–d). These results reveal an active, cardiac-specific IncRNA promoter controlling Mhrt expression.

We then asked how Mhrt was repressed in stressed hearts. We postulated that cardiac stress activated Brg1, leading it to occupy the a1–a4 promoter and to repress Myh6 (ref. 3) and Mhrt in opposite transcription directions (Extended Data Fig. 4a). Indeed, Mhrt repression required Brg1: TAC suppressed Mhrt RNAs in control but not Brg1-null hearts (Tmut2-rTA;Tre-Cre;Brg1+/−) (Extended Data Fig. 4e). To test Brg1 activity on the Mhrt promoter, we cloned the a1–a4 promoter in the Mhrt transcription direction (−2329 to +143) into an episomal luciferase reporter, pREP4, that allows promoter chromatinization16. Brg1 was then transfected into Brg1-deficient SW13 cells16 to reconstitute the Brg1/BAF complex for reporter assays. Brg1 transfection caused a ~50% reduction of Mhrt promoter activity (P < 0.0001), and such Mhrt repression was virtually abolished by Hdac inhibition with trichostatin-A or Parp inhibition.

Figure 2 | Mhrt inhibits cardiac hypertrophy and failure. a, Quantification of cardiac Mhrt RNAs 2–42 days (d) after TAC operation. b, RT-PCR of Mhrt RNAs in adult heart ventricles. Primers (F1 and R1; Fig. 1a) target Mhrt common regions. Size controls 779, 826 and 709 are PCR products of recombinant Mhrt779, Mhrt826 and Mhrt709, respectively. c, Northern blot of Mhrt RNAs in adult heart ventricles. The probe targets common regions of Mhrt RNAs. Negative: control RNA from 293T cells. Size control 826 is recombinant Mhrt826, 643 (not a distinct Mhrt species) contains the 5' common region of Mhrt. d, Quantification of Mhrt779 in control or Tg779 mice with or without doxycycline (Dox) or TAC operation. Mhrt779-specific PCR primers were used. Ctrl, control mice. e, Ventricular/body-weight ratio of hearts 6 weeks (wk) after TAC. Scale bars = 1 mm. f, Quantification of cardiomyocyte size in control and Tg779 mice 6 weeks after TAC by wheatgerm agglutinin staining. g, Trichrome staining in control and Tg779 hearts 6 weeks after TAC. Red indicates cardiomyocytes; blue indicates fibrosis. Scale bars = 20 μm. h, i, Echocardiographic measurement of left ventricular fractional shortening (FS), h and internal dimensions at end-diastole (LVIDd) and end-systole (LVIDs) (i) 6 weeks after TAC. P values: Student’s t-test. Error bars show s.e.m.
Myh6 and Mhrt were both regulated by the a1–a4 promoter, we hypothesized that a1–a4 contained two elements to regulate Myh6 and Mhrt—with the a1 element controlling Myh6 and the a3/4 element controlling Mhrt (Extended Data Fig. 4a). On a1 and a3/4 (but not a2), we found cardiac-specific enrichment of Brg1 (ref. 3), H3K4me3 and H3K36me3 (Extended Data Fig. 4c–d), and DNasel genomic footprints (Fig. 3a)22. To test a3/4 for Mhrt regulation, we conducted deletional analysis of the a1–a4 promoter in the Mhrt transcription direction. In reporter assays, a3/4 was necessary and sufficient for Mhrt promoter activity and for Brg1-dependent Mhrt repression, whereas a1 was not essential for either (Extended Data Fig. 4h). Conversely, a1 is necessary and sufficient for Brg1 to repress the Myh6 promoter4, but a3/4 is not required5. Therefore, a1 and a3/4 are two functionally distinct elements for Brg1 to separately control Myh6 and Mhrt.

In stressed hearts, Brg1 represses Myh6 and activates Myh7 (ref. 3), causing a pathological switch of Myh6/7 expression, contributing to cardiomyopathy23. This stress/Brg1-dependent Myh switch was largely eliminated by Mhrt779 (Fig. 3b), and the inhibition of the Myh switch by Mhrt did not involve RNA–RNA sequence interference between Mhrt and Myh (Extended Data Fig. 5a–j and Supplementary Note). Instead, it required a physical interaction between Mhrt RNA and Brg1. RNA immunoprecipitation of TAC-stressed adult hearts or Brg1-expressing neonatal hearts showed that Brg1 co-immunoprecipitated with Mhrt779 but not control RNAs, and that Mhrt779 complexed with Brg1 but not with the polycomb proteins Ezh2 or Suz12 (Fig. 3c and Extended Data Figs 5a–j and Supplementary Note). Instead, a1–a4 contained two elements to regulate Mhrt repression, whereas a1 was not required6. We then hypothesized that a1–a4 could directly co-immunoprecipitate without in-...
Figure 4 | Mhrt inhibits chromatin targeting and gene regulation by Brg1. a, Gel electrophoresis and quantification of nucleosomal 5S rDNA, the Myh6 promoter and Neo DNA. Arrowheads indicate the DNA–histone complex; arrows indicate naked DNA. Nucleosomal assembly efficiency is defined as the fraction of DNA bound to histones (arrowheads). b–d, Quantification of amylose pull-down of MBP–D1D2 (D1D2) with nucleosomal and naked Myh6 promoter DNA (b), with nucleosomal Myh6 promoter, Neo and 5S rDNA (c), or with nucleosomal Myh6 promoter in the presence of Mhrt779 (d). e, Amylose pull-down of MBP–D1D2 and histone H3. Anti-histone H3 and anti-MBP antibodies were used for western blot analysis. f, ChIP analysis of Brg1 on chromatinized and naked Myh6 promoter in rat ventricular cardiomyocytes. GFP, green fluorescent protein control. g, h, Luciferase reporter activity of Brg1 on naked Myh6 promoter (g) or of helicase-deficient Brg1 on chromatinized Myh6 promoter (h) in rat ventricular cardiomyocytes. ΔD1, Brg1 lacking amino acid 774–913; ΔD2, Brg1 lacking 1086–1246. ChIP: H-10 antibody recognizing N terminus, non-disrupted region of Brg1. i, j, ChIP analysis in SW13 cells of chromatinized Myh6 promoter in the presence of Mhrt779 (i) or helicase-deficient Brg1 (j). Mhrt, pAdd2-Mhrt779; Vector, pAdd2 empty vector. k, Schematic illustration and PCR of human MHR. MHR originates from MYH7 and is transcribed into MYH7. MYH7 exons and introns are indicated. R1 and R2 are strand-specific primers; F1 and R1 target MHR and MYH7; F2 and R2 are specific for MHR. l, Quantification of MHR in human heart tissues. Ctrl, control; ICM, ischaemic cardiomyopathy; IDCM, idiopathic dilated cardiomyopathy; LVH, left ventricular hypertrophy. m, Working model of a Brg1–Mhrt feedback circuit in the heart. Brg1 represses Mhrt transcription, whereas Mhrt prevents Brg1 from recognizing its chromatin targets. Brg1 functions through two distinct promoter elements to bidirectionally repress Myh6 and Mhrt expression. n, Molecular model of how Brg1 binds to its genomic DNA targets. Brg1 helicase (D1D2) binds chromatinized DNA, C-terminal extension (CTE) binds histone H3, and bromodomain binds acetylated histone H3 or H4. P values: Student’s t-test. Error bars show s.e.m.

protein–RNA complex that was competitively displaced by unlabelled Mhrt779 (Fig. 3f). Brg1, which belongs to the SW1/SNF family of chromatin-remodelling factors, contains a helicase/ATPase core that is split by an insertion into two ReA-like domains: DEAD-like helicase superfamily C-terminal domain, D1 (DEEx-c) and helicase superfamily C-terminal domain, D2 (HELIC-c)24,25 with signature motifs of DEAD-box, superfamily 2 RNA helicase26–28 (Fig. 3g and Extended Data Fig. 8). SW1/SNF proteins although conserved with RNA helicases, were observed to bind DNA22 and mediate DNA structural changes and repair19. The binding properties of Brg1 remained undefined. To test whether Mhrt could bind to Brg1 helicase, we generated maltose-binding protein (MBP)-tagged recombinant proteins that contained the Brg1 DEEx-c domain (MBP–D1, amino acids 774–913), the HELIC-c domain with C-terminal extension (MBP–D2, 1086–1310), or the entire helicase (MBP–D1D2, 774–1310) (Extended Data Fig. 9a). D1D2 showed the highest Mhrt binding affinity (dissociation constant (Kd) = 0.76 μM); D1 showed moderate affinity (Kd = 1.8 μM); D2 modest affinity (Kd > 150 μM); and MBP did not bind at all (Fig. 3h, i). Therefore, Brg1 helicase binds Mhrt with high affinity.

Contrary to its potent RNA binding, Brg1 helicase showed no detectable binding to the naked DNA of the Myh6 promoter (596 bp, –426 to +170, critical for the control of Myh6 by Brg1 (ref. 3)) (Extended Data Fig. 9b). To test whether Brg1 helicase could bind chromatinized DNA, we generated nucleosomal DNA in vitro by assembling histone octamers (histones H2A, H2B, H3 and H4)29 on Myh6 promoter DNA, as well as on control neomycin phosphotransferase gene (Neo) and 5S ribosomal (r)DNA (5S rDNA). We achieved 50–65% efficiency of nucleosome assembly, comparable between Myh6, Neo and 5S rDNA (Fig. 4a). Because there is the large nucleosome size precluded a clear EMSA resolution, we used amylose to pull down MBP-tagged D1D2 proteins. We found that D1D2 pulled down nucleosomal Myh6 promoter DNA but not the naked one (Fig. 4b). The pull-down efficiency of nucleosomal Myh6 was ~3–6-fold that of Neo or 5S rDNA (Fig. 4c), and Mhrt779 was capable of disrupting D1D2–Myh6 pull-down (Fig. 4d). Although D1D2 bound to histone H3 (Fig. 4e), histone binding was insufficient to anchor D1D2 to nucleosomal DNA, as D1D2 bound poorly to nucleosomal Neo and 5S rDNA that also contained histones (Fig. 4c). Therefore, chromatinized DNA targets are biochemically recognized by Brg1 helicase, and this process is inhibited by Mhrt.

To test the ability of Brg1 to distinguish chromatinized from naked DNA promoters in cells, we cloned Myh6 promoter into the luciferase reporter plasmid pREP4 (allowing promoter chromatinization15) and pGL3 (containing naked, non-chromatinized promoter). In rat ventricular cardiomyocytes and SW13 cells, ChIP and luciferase analyses showed that Brg1 bound and repressed chromatinized but not naked Myh6 promoter (Fig. 4g, f and Extended Data Fig. 9c, d). However, without D1/D2 domain or in the presence of Mhrt, Brg1 was unable to bind or repress chromatinized Myh6 promoter (Fig. 4h–j and Extended Data Fig. 9e), indicating the necessity of D1D2 for the interaction between Brg1, chromatin and Mhrt. Consistently, all our genetic, biochemical and cellular
studies show that Brg1 requires the helicase domain to bind to chromatinized DNA targets, and Mhrt seizes the helicase to disrupt Brg1–chromatin binding. We then asked how Brg1 surpassed its basal suppression by Mhrt to control Myh, Mhrt, Otn, or other genes to trigger cardiomyopathy (Supplementary Note). Amylose pull-down experiments showed that Brg1 dose-dependently escaped from Mhrt inhibition to occupy Mhrt promoter (Extended Data Fig. 10). Brg1 protein, which increases under stress conditions\(^2\), could therefore outrun Mhrt and gain control over the Mhrt promoter to repress Mhrt expression and tip the balance towards Brg1. Contrary to the endogenous Mhrt that was repressible by Brg1, the Mhrt transgene (Tg779)—driven by Tmnt2/Tre promoters—was not subject to repression by Brg1 and was thus able to keep Mhrt at pre-stress levels to inhibit Brg1 and reduce hypertrophy. This further demonstrates the necessity of Mhrt repression for myopathy to develop.

Human MYH7 loci encoded RNA that resembled Mhrt in primary sequence and secondary structure, as predicted by minimal free energy\(^2\) (Fig. 4k and Extended Data Fig. 11a, b). Human MhRT was also repressed in stressed hearts, with 82.8%, 72.8% and 65.9% reduction of MhRT in hypertrophic, ischaemic or idiopathic cardiomyopathy tissues, respectively (Fig. 4l and Extended Data Fig. 11c). This suggests a conserved MhRT mechanism of cardiac myopathy. Mhrt is the first example, to our knowledge, of an lncRNA that inhibits myopathy and chromatin remodelers. Reciprocal Mhrt–Brg1 inhibition constitutes a feedback circuit critical for maintaining cardiac function (Fig. 4m).

The helicase core of Brg1, combined with the histone-binding domains of the Brg1/BAF complex, adds a new layer of specificity control to Brg1/BAF targeting and chromatin remodelling (Fig. 4n). The Mhrt–helicase interaction also exemplifies a new mechanism by which lncRNA controls chromatin structure. To further elucidate chromatin regulation, it will be essential to define helicase domain function in all ATP-dependent chromatin-remodelling factors and to identify new members of lncRNA that act through this domain to control chromatin. The cardioprotective Mhrt may have translational value, given that RNA can be chemically modified and delivered as a therapeutic drug. This aspect of lncRNA–chromatin regulation may also inspire new therapies for human disease.

**METHODS SUMMARY**

**Tg779 mouse generation**, rapid amplification of cDNA ends (RACE), RNA in situ hybridization, RT–qPCR, codon substitution frequencies (CSF), echocardiography, northern blot, EMSA, ChIP, RNA immunoprecipitation, reporter assay, nucleosome assembly, and the amylose pull-down assay were performed as described\(^{19,20}\).

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Author Contributions** C.-P.C. and P.H. were responsible for the original concepts, design and manuscript preparation. W.L. and C.-H.L. contributed equally to the work. P.H. conducted most experiments; W.L. and J.Y. assisted with TAC, echo and reporter analyses; C.-H.L. assisted with protein purification; S.T.N. assisted with ribosome data analysis; K.K.J. assisted with protein sequence and motif analysis; C.S. assisted with western blot studies; W.J. assisted with CSF scoring; Y.X. assisted with RNA/protein staining; C.-P.C. by NIH (HL109512); H.-S.V.C. by CIRM (RB2-01512, RB4-06276) and NIH (HL105194); P.-S.C. by NIH (HL79831, HL711140); B.Z. by NIH (HL116997, HL111770).

**Supplementary Information** is available in the online version of the paper.

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METHODS
Mice, animal sample size, and randomization. For the generation of Tg779 mice, Mhrt779 was cloned into the pTRE2 backbone (Clontech). A DNA fragment containing the Tre promoter and Mhrt779 were injected into the pronucleus of fertilized oocytes (B6C3H/F1). Embryos were implanted into a pseudogestant CD-1 mouse. The Tre-Mhrt779 transgene was identified by PCR genotyping using primers MCGCTGACGACGACCTACG and TGTCTTCAAGGTCATCCTC. Tre-Mhrt779 mice with 3–5 copies of the transgene were backcrossed with Tmnt2-rtTA mice as described previously26,27 to generate Tmnt2-rtTA;Tre-Mhrt779 (Tg779) mice. The number of animals used (n) is denoted in each test in the figures, including technical replicates when applicable. We routinely used mouse littermates to control and perform our experiments. Each subgroup of experiments had n = 3 to 14 biological replicates, many of which had technical replicates of three. Assignment to each experimental subgroup was based on genotypes. Littermate mice with the same genotypes regardless of gender were randomly selected from the cage and assigned to different control and experimental subgroups. Major procedures were blinded. The use of mice for studies was in compliance with the regulations of Indiana University, Stanford University and the National Institutes of Health.

RACE and cloning of full length of Mhrt transcripts. The 3’ and 5’ RACE were performed using the FirstChoice RLM-RACE kit (Ambion) following the manufacturer’s instruction. RNA was extracted from adult heart ventricles. Primers used for 3’ and 5’ RACE were designed based on the known sequence information: TCATTTTGCAACCAGACAT (first-round Mhrt 3’-prime specific) and GAGCA TGGTGGATGTTAT (second-round Mhrt 3’-prime specific); CCAACTTTCCATTTTTCT (second-round Mhrt 3’-prime specific) and TCTGCTTCA TGGGTCTT (second-round Mhrt 5’-prime specific). Once we reached the 5’ and 3’ cDNA ends, we used primers F1 (1a: AAGACCGCTACAGTCTG ATGAAACAGA) and R1 (1a: CACCTCCACACACATTATTTATG) to amplify the full-length Mhrt transcripts and cloned them into pDrive TA cloning vector (Qiagen) for sequencing. Mhrt RNAs were further cloned into shuttle vector pAdd2 (ref. 31, 32) for expression in cells.

Northern blot and in situ hybridization. We obtained 5 μg of total RNA using Quick-RNA Mini Kit (Zymo Research). RNA blot was performed using Northern-Max Kit (Ambion) following the manufacturer’s protocol. Single-stranded RNA probe was generated in vitro transcription with MaxiScript SP6/T7 kit (Ambion) with ATP [-32P] (PerkinElmer) using full-length Mhrt779, Myh6 and Myh7 as the template and followed by digestion with DNase I (Ambion). Hybridization was performed at 65 °C. The blot was washed and imaged by Phosphor storage scanning by Typhoon 8600 Imager (GE Healthcare). In situ hybridization experiments were performed as previously described.

RNA fractionation. To isolate cytosolic and nuclear RNAs from adult heart tissues, we used a PARIS kit (Ambion) and followed the manufacturer’s instruction. Ten milligrams of tissue were homogenized in cell fractionation buffer thoroughly before centrifuging for 5 min at 500 g. Supernatant was collected as the cytosolic fraction, while the nuclear pellet was washed and lysed by cell disruption buffer. Such samples were further mixed with 2X lysis/binding solution before extracting RNA using the manufacturer’s protocol.

Codon substitution frequency predication. To measure the coding potential of Mhrt, we used the previously described codon substitution frequencies (CSF) method10 to evaluate the evolutionary characteristics in their alignments with orthologous regions in six other sequenced mammalian genomes (rat, human, hamster, rhesus monkey, cat, and dog). CSF generates a likelihood score for a given sequence considering all codon substitutions observed within its alignment across multiple species, which was based on the relative frequency of similar substitutions occurring in known coding and noncoding regions. CSF compares two empirical codon models; one generated from alignments of known coding regions and the other according to noncoding regions, producing a likelihood ratio. The ratio reflects whether the protein-coding model better explains the alignment.

Ribosome profiling and RNA deep sequencing. For ribosome profiling,4,5 overnight culture of the mid papillary muscle level. Left ventricular chamber size and wall thickness were measured in at least three beats from each projection and averaged. Left ventricular internal dimensions at diastole and systole (LVIDd and LVIDd, respectively) were measured. The fractional shortening (FS) of the left ventricle was defined as 100% × (1 − LVIDd/LVIDd), representing the relative change of left ventricular diameters during the cardiac cycle. The mean FS of the left ventricle was determined by the average of FS measurements of the left ventricular contraction over five beats. P values were calculated by Student’s t-test. Error bars indicate s.e.m.

Histology, trichrome staining and morphometric analysis of cardiomyocytes. Histology and trichrome staining were performed as described24. Trichrome stain (Picosirius Red) was used to stain collagen fibers of the myocardium. For morphometric analysis of cardiomyocytes, paraffin sections of the heart were immunostained with a fluorescein isothiocyanate-conjugated wheat germ agglutinin (WGA) antibody (F49, Biomeda) that highlighted the cell membrane of cardiomyocytes. Cardiomyocytes were counted using NIH Image software (National Institutes of Health). For quantification of slices per kilobase of exon per million fragments mapped (FPKM) values, cuffdiff as part of the tophat suite v.2.0.6 beta5 was run on a merged bam file containing the human and the Mhrt reads using a custom gtf file comprising the human hg19 Genomes and the Mhrt transcripts. To generate scatter plots of the genes, cuffdiff files were used for visualization with cumbervud v.2.3.1 (ref. 37).

In vitro translation and biotin labelling. TNT Quick Coupled Translation/ Transcription System (Promega) was used for in vitro translation. Briefly, 1 μg plasmids of coxs6, luciferase and various Mhrt transcripts inserted into forward vector were added to 40 μl rabbit reticulocyte lysates containing 35S-methionine. After 1 h of incubation, the reactions were analysed on 10–20% Tris–Tricine gel. The gel was dried and visualized by the Typhoon 8600 Imager (GE Healthcare). Biotin-NTP was added to the in vitro translation reaction. Total RNAs were extracted and the biotin-labelled RNAs were detected subsequently by IRDye 680 Streptavidin (Li-COR, 926-68079) using an Odyssey Infrared Imaging System.

TAC. The TAC surgery was performed as described on adult mice of 8–10 weeks of age and between 20 and 25 g in weight. Mice were fed with dry chow mixed with 6 mg doxycycline per kg of food. Polymer 7–14 days before the TAC operation. Mice were anesthetized with isoflurane (2–3%), inhalation) in an induction chamber and then intubated with a 20-gauge intravenous catheter and ventilated with a mouse ventilator (Minivent, Harvard Apparatus). Anaesthesia was maintained with inhaled isoflurane (1–2%). A longitudinal 5 mm incision of the skin was made with scissors at the midline of sternum. The chest cavity was opened by a small incision at the level of the second intercostal space 2–3 mm from the left sternal border. While opening the chest wall, the chest retractor was gently inserted to spread the wound 4–5 mm in width. The transverse portion of the aorta was bluntly dissected with a curved forceps. Then, 6.0 silk was brought underneath the transverse aorta between the left common carotid artery and the brachiocephalic trunk. One 27-gauge needle was placed directly above and parallel to the aorta. The loop was then tied around the aorta and needle and secured with a second knot. The needle was immediately removed to create a lumen with a fixed stenotic diameter. The chest cavity was closed by 6-0 silk suture. Sham-operated mice underwent similar surgical procedures, including isolation of the aorta and looping of the aorta, but without tying of the suture. The pressure load caused by TAC was verified by the pressure gradient across the aortic constriction measured by echocardiography. The flow of isoflurane (inhalational) was adjusted to anesthetize the mice while maintaining their heart rates at 450–550 beats per minute. The peak aortic pressure gradient was measured by continuous-wave Doppler across the aortic constriction. Left ventricular function was assessed by M-mode scanning of the left ventricular chamber, standardized by two-dimensional, short-axis views of the left ventricle at the mid papillary muscle level. Left ventricular chamber size and wall thickness were measured in at least three beats from each projection and averaged. Left ventricular internal dimensions at diastole and systole (LVIDd and LVIDd, respectively) were measured. The fractional shortening (FS) of the left ventricle was defined as 100% × (1 − LVIDd/LVIDd), representing the relative change of left ventricular diameters during the cardiac cycle. The mean FS of the left ventricle was determined by the average of FS measurements of the left ventricular contraction over five beats. P values were calculated by Student’s t-test. Error bars indicate s.e.m.

RT–qPCR and strand-specific reverse transcription PCR analysis. RT–qPCR analyses were performed as described. The following primer sequences (listed in Table 2) were used. For directional RT–qPCR analysis, human tRNAs were also further cloned into shuttle vector pAdd2 (refs 31, 32) for expression in cells.
Anf-construct as the template promoter. With some modifications. Briefly, P1 hearts, sham hearts with GraphPad Prism (GraphPad). The software facil-

binding buffer (10 mM HEPES-KOH, pH 7.3, 10 mM NaCl, 1 mM MgCl

promoter-F, promoter-R, GAAGCAGTGAGGTTGGTGG; mouse ChIP-

promoter DNA were added for incubation at 4

transcription with MAXIscript SP6/T7 kit (Ambion) with biotin labelling

values based

For the

through cloning the PCR-

promoter, various regions of the promoter were deleted from

calculation.

and

protease inhibitor) for tissues or lysis buffer (10 mM

lysis buffer (50 mM Tris-HCl pH 8.1, 150 mM NaCl, 0.1% NP-40, 1 mM DTT,

(TAAAGCACGAGGAAGCGGTC; TCGACCC

promoter-F, promoter-R, GAAGCAGTGAGGTTGGTGG; mouse ChIP-

promoter DNA were done according to the manufacturer's protocol (Magna ChIP Protein G

Brg1 H-10 antibody (Santa Cruz Biotechnology, against 115–149 amino acids of N

Mini Kit with the on-column DNase I digest (ZymoResearch). RT and qPCR were

sodium deoxycholate) with 5

rDNA, and thus serve as controls. Primers

PCR primers for RT–qPCR of miRNA were as follows. Mouse Tfiib-F, CTTCG

TGGCGGCAGCAGCTATTT, mouse

TfIIb-F (common), GAGCATTTGG

the 1

binding buffer (10 mM HEpes-KOH, pH 7.3, 10 mM NaCl, 1 mM MgCl

Tnnt2-R, GCCACAGTGGCAATGTGACCAA; mouse

TfIIb-R, GCCACAGTGGCAATGTGACCAA; human

Hprt1-F, CTGGAGCTGGGACAGGTCAGCA) and R1 were used. Mouse ChIP-

PCR primers (listed later) were designed to amplify the promoter regions of mouse Myh6 (−426, −320), mouse Myh7 (−102, +58), mouse Shh (−7142, −6991), mouse Vegfa (+1, +150) human GAPDH (−45, +121). The DNA positions are denoted relative to the transcrptional start site (+1).

PCR primers for ChIP–qPCR are as follows. Mouse ChIP-Myh6 promoter-F, GCAGATGCGAGGGAGTCAA, mouse ChIP-Myh6 promoter-R, TGGTGAAGGGCTAGCTTCCGTCAT, mouse ChIP-Myh7 promoter-F, GTGACACCAGGCAGCAGAC, human TFIIB-F (common), A CGGGACGAGACACTGCTTCTCTTCAAT (human (goat) IgG), human TFIIB-R, GCCACAGTGGCAATGTGACCAA; human TFIIB-R (2), GGGGCTGAAGAGTGAGCCTT were designed on the fitting curve. The errors and

N-terminal MBP fusion proteins of mouse Brg1 helicase domains, the DExx-box domain (D1) and the entire helicase domain (D1D2) proteins using a previ-

Protein expression and purification of Brg1 helicase domains. To generate MBP fusion proteins of mouse Brg1 helicase domains, the DExx-box domain (D1) (amino acids 774–913 of Brg1), helicase-C domain (D2) together with C-terminal extension (CTE) (amino acids 1086–1310 of Brg1), as well as the entire helicase region (D1D2) (774–1310) were amplified by PCR and cloned into pMAL vector. MBP fusion proteins were induced by isoprpyl-β-D-thiogalactoside (IPTG) and purified by anion exchange resin (E80215, NEB).

Nucleosome assembly and amylose pull-down. Nucleosome assembly was performed with purified Nucleosome Assembly and amylose pull-down experiment as assembly Kit (E353508, NEB) following the manufacturer's instruction. In brief, recombinant human core histone octamer, which consists of the 2:1 mix of histone H2A/H2B dimer and histone H3.1/H4 tetramer, were mixed with purified 55 rDNA (208 bp; N12025, NEB), Neo (512 bp, amplified from pST18 Neo; 1175025, Roche), Myh6 core promoter (596 bp, −426 to +170) and Mhtrt1 core promoter (a3/a4, 986 bp, −2290 to −1775) DNA at 2 μg/ml. PCR primers to amplify Neo are CGATTGCCTGGAAATCTGGCAGA and CCTGTA AGCGGGAAAGGACT. The salt concentration was gradually lowered to allow the formation of nucleosomes. The EMSA assay was used to assess the efficiency of nucleosome assembly. For amylase pull-down assay, the amylase resin (E80215, NEB) was washed thoroughly and equilibrated with binding buffer (10 mM Tris-HCL, pH 7.5, 150 mM NaCl) before incubation with purified MBP- or MBP–D1/D2 proteins for 2 h. Nucleosome mixture or naked DNA mixture of 55 rDNA, Neo and Myh6 promoter DNA were added for incubation at 4 °C for overnight. The resin was then washed excessively by washing buffer (20 mM Tris-HCL, pH 8.1, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS) before decross-linking and extraction of the DNA with phenol/chloroform/isomyl alcohol. For competition assays, in vitro transcribed Mhtrt779 was incubated with MBP–D1/D2 in binding buffer (10 mM HEpes-KOH, pH 7.3, 10 mM NaCl 1 mM MgCl2, 1 mM DTT) with ribonuclease inhibitor at room temperature for 30 min before adding nucleosomal DNA. The subsequent incubation, wash and DNA purification were performed as regular amylase pull-down assays. The qPCR signal of individual pull-down reaction was standardized to its own input RT–qPCR signal. qPCR primers were designed to amplify the SS DNA reporter (CAAGCAAGAGCCTTCACTCAGA; ATTC GTTGGAAATTCCTCGGG), Neo (TAAGACAGCAAGGGAGCTTG; TCAGACC AAGCGGAACAT), Myh6 promoter (CGGATATCCAGGGGTTAGCAAGA; TGGG TAAAGCGAGGGTTAGCAAC) and Mhtrt1 promoter (ATGCGAAATGGTGGTTGCTTCT; GAGCTTGAAGACCGGACT).
Cloning of Brg1 truncation constructs. For cloning of truncated Brg1 with deletion of amino acids 774–913 (ΔD1) or 1086–1246 (ΔD2), primers with an NheI restriction digestion site, which complement the downstream and upstream sequences of the truncated region (ΔD1: CCCGGGGCTAGCTCGACGAAACA AGCTACGGAGGT and CCCGGGGCTAGCCAGTGGTGTGTGACAGG GACA; ΔD2: CCCGGGGCTAGCATAGAAATTTCCCGGAAAAGAAAGGCAGAACGAGCACGAGCAGC) were used to amplify from pActin-Brg1-IRE5-eGFP by KOD Xtreme Hot Start DNA Polymerase (Novagen). After digestion with NheI, the linearized fragment was subject to ligation and transformation. The truncation constructs were sequenced to confirm the fidelity of the cloning. Western blot was further performed to assess the expression of the constructs. Monoclonal H-10 antibodies (Santa Cruz Biotech, sc-374197), which were raised against Brg1 N-terminal amino acids, were used in the experiments involving truncated Brg1.

**Protein sequence analysis.** Brg1 core helicase domain (774–1202) was applied for secondary structure prediction using the Fold & Function Assignment System (FFAS) server (http://ffas.burnham.org/ffas-cgi/cgi/ffas.pl). The output revealed that Brg1 core helicase domain is a structural homologues of SF2 helicases: Vasa
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(zebrafish, Protein Data Bank (PDB) accession number 1Z3I), Sulfolobus solfataricus PDB accession 1Z63) and Chd1 (ref. 46) (yeast, PDB accession 3MWY). Those proteins, together with Brg1, were further employed for multiple sequence alignment with T-Coffee, which is a program allowing combination of the results obtained with several alignment methods (http://www.ebi.ac.uk/Tools/msa/tcoffee/).

**RNA secondary structural prediction.** To predict the secondary structure and transformation for mouse Mhrt and human MHRT, the single-stranded sequence of Mhrt779 and human MHRT were analysed on the Vienna RNAfold web server (http://rna.tbi.uni-koeln.de). The output revealed that Brg1 core helicase domain is a structural homologues of SF2 helicases: Vasa
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Extended Data Figure 1 | Mhrt RNAs have no coding potential. a, RNA in situ analysis of Mhrt (blue) in a mouse E12 heart. The RNA probe targets all Mhrt species. Red: nuclear fast red. Black arrowheads indicate nuclei of endothelial, endocardial or epicardial cells. Inset shows magnified region from the boxed area. endo, endocardium; epi, epicardium; IVS, interventricular septum; LV, left ventricle; RA and RV, right atrium and ventricle, respectively. Scale bars = 100 μm. b, Codon substitution frequency (CSF) scores of TfIIb and Hprt1 mRNA, as well as full-length Mhrt species. c, In vitro translation of control Mhrt species (709, 779, 826, 828, 857, 1147) and luciferase (Luc). Arrow points to the protein product of luciferase. d, Biotin-labelling of Mhrt species (709, 779, 826, 828, 857, 1147) and luciferase in the in vitro translation reactions. Arrow points to the RNA product of luciferase. e, Ribosome profiling relative to whole transcriptome RNA sequencing. x-axis: genomic position at the human GAPDH and the murine Myh7 loci. y-axis: mapped reads. f, Scatter plot of RNA in fragments per kilobase per million reads (FPKM). Noncoding RNAs (purple) cluster towards the x-axis; coding RNAs (orange) towards the y-axis. Mhrt779 is found below both the identity line (dashed, slope = 1, intercept = 0) and the smooth-fit regression line (in blue). RNA examples are endogenous except that HOTAIR was co-transfected with Mhrt779.
Extended Data Figure 2 | Quantification of Myh6/Myh7, northern blot, and Mhrt779 characterization. 

a, Quantification of cardiac Myh6/Myh7 ratio 2–42 days after sham or TAC operation. 

b, Northern blot analysis of Mhrt, Myh6 and Myh7. Negative: control RNA from 293T cells. Size control: 826 is recombinant Mhrt826; 643 (not a distinct Mhrt species) contains the 5' common region of Mhrt. Heart: adult heart ventricles. 

c, Un-cropped northern blots of Mhrt, Myh6 and Myh7. 

d, RNA in situ hybridization of Mhrt779 of adult heart ventricles. White arrowheads indicate nuclei of myocardial cells. 

Black arrowheads indicate nuclei of endothelial, endocardial or epicardial cells. Blue: Mhrt779; Red: nuclear fast red. Epi, epicardium. The dashed line separates the epicardium from myocardium. Scale bars = 50 μm. 

e, Quantification of TfIIb, Hprt1, 28S rRNA, Neat1 and Mhrt779 in the nuclear and cytoplasmic fraction of adult heart ventricle extracts. The nuclear/ cytoplasmic ratio of TfIIb is set as 1. P values: Student’s t-test. Error bars show s.e.m.
Extended Data Figure 3 | Wheat germ agglutinin staining, time course and molecular marker studies of the stressed Tg779 mice. a, Wheat germ agglutinin (WGA) immunostaining 6 weeks after the sham or TAC operation. Green: WGA stain, outlining cell borders of cardiomyocytes. Blue: 4’,6-diamidino-2-phenylindole (DAPI). Ctrl, control mice. Scale bars = 50 μm. b, Time course of fractional shortening (FS) in control and Tg779 mice. c, Quantification of Anf, Bnp, Serca2 and Tgfb1 in control and Tg779 mice 2 weeks after sham or TAC operation. d, Experimental design for treatment study and time course of left ventricular fractional shortening changes. e, Fractional shortening of the left ventricle (LV) 8 weeks after the operation. f, Ventricular weight/body weight ratio of hearts harvested 8 weeks after sham or TAC operation. P values: Student’s t-test. Error bars show s.e.m.
Extended Data Figure 4  | Regulation of the Mhrt promoter. a, Sequence alignment of Mhrt promoter loci from mouse, human and rat. Peak heights indicate degree of sequence homology. Black boxes (a1–a4) are sequences of high homology, which were used for further ChIP analysis. Green box region between Myh6 and Mhrt is the putative Mhrt promoter. Red, promoter regions; salmon, introns; yellow, untranslated regions. b–d, ChIP–qPCR analysis of Mhrt promoter using antibodies against Pol II (b), H3K4me3 (c), and H3K36me3 (d) in tissues of adult mice. e, RT–qPCR quantification of Mhrt in control and Brg1-null hearts after 7 days of TAC. Ctrl, control. Brg1-null, Tnnt2-rtTA;Tre-Cre;Brg1<sup>fl/fl</sup>. f, Luciferase reporter assay of Mhrt promoter in SW13 cells. Ctrl: dimethylsulphoxide (DMSO). PJ-34, PARP inhibitor; TSA, trichostatin (HDAC inhibitor). g, ChIP analysis of BRG1, HDAC2, HDAC9 and PARP1 in SW13 cells. The cells were transfected with episomal Mhrt promoter cloned in pREP4. h, Deletional analyses of the Mhrt promoter in luciferase reporter assays in SW13 cells. Luciferase activity of full-length Mhrt promoter was set up as 1. P values: Student’s t-test. Error bars show s.e.m.
Extended Data Figure 5 | Mhrt does not affect Myh expression by direct RNA sequence interference. a, qPCR analysis of Mhrt779, Myh6 and Myh7 in mice without TAC operation. Expression levels were normalized to TfIIb, and the control is set as 1. Ctrl, control mice. b, c, RNA quantification of Mhrt (b) and HOTAIR (c) in SW13 cells transfected with Vector (pAdd2), HOTAIR (pAdd2-HOTAIR) or Mhrt (pAdd2-Mhrt779). Expression in vector-transfected cells is set as 1. Constructs containing Myh6 or Myh7 were co-transfected into SW13 cells used for Fig. 2b–i. d, e, RNA quantification of Myh6 (d) and Myh7 (e) in SW13 cells relative to GAPDH. f, g, Western blot analysis of Myh6 (f) and Myh7 (g) in SW13 cells. Constructs containing Myh6- and Myh7-coding sequences were tagged with Flag and co-transfected with vector, HOTAIR or Mhrt779. GAPDH was used as the loading control. Flag-D1 was used as a positive control for the Flag antibody. h, i, Protein quantification of Myh6 (h) and Myh7 (i) in control and transfected SW13 cells relative to GAPDH. Signals of Myh6 and Myh7 from major bands or the entire lanes were quantified. WB, western blot. j, Luciferase reporter assay of Myh6 and Myh7 promoters in SW13 cells transfected with vector (pAdd2) or Mhrt (pAdd2-Mhrt779). P values: Student’s t-test. Error bars show s.e.m.
Extended Data Figure 6 | RNA-IP controls; Opn is another target gene of Brg1 in stressed hearts. a, Immunostaining of Brg1 in P1 heart. Red: Brg1. Green: WGA. Blue: DAPI. Ctrl, control. Scale bar = 50 μm. b, RNA-IP of Mhrt in P1 hearts using antibodies against Ezh2 and Suz12. Right panels show immunostaining of Ezh2 and Suz12 in P1 hearts. PRC2, polycomb repressor complex 2. Red: Ezh2 or Suz12. Green: WGA. Blue: DAPI. Scale bars = 50 μm.

c, Quantification of Opn mRNA in control and Brg1-null (Tnnt2-rtTA;Treact-CreBrg1<sup>fl/fl</sup>) mice after sham or TAC operation. d, ChIP of Brg1 on Opn proximal promoter in control and transgenic (Tg779) mice after sham or TAC operation. e, Quantification of Opn in control and transgenic (Tg779) mice after sham or TAC operation. P values: Student’s t-test. Error bars show s.e.m.
Extended Data Figure 7 | Induction of Mhrt779 is insufficient to change Brg1 mRNA or protein level. a, qPCR analysis of Brg1 expression in hearts without TAC operation. Ctrl: control mice. b–e, Immunostaining of Brg1 (red) in adult heart ventricles 2 weeks after sham or TAC operation. Green: WGA. Blue: DAPI. Scale bars = 50 μm. f, Western blot analysis of Brg1 and Coo massie staining of total proteins in control or Tg779 hearts after 2 weeks of sham or TAC operation. g, Quantification of Myh6 and Myh7 in control (Ctrl) and Tg779 hearts after 2 weeks of sham or TAC operation. P values: Student’s t-test. Error bars show s.e.m.
Extended Data Figure 8  | Brg1 sequence alignment and motif analysis.

Schematics of the architecture of mouse Brg1 and the sequence alignment of Brg1, Vasa (fruit fly), Rad54 (zebrafish, *Sulfolobus solfataricus*) and Chd1 (yeast). The motifs were outlined by blue boxes (D1 domain) and purple boxes (D2 domain).
Extended Data Figure 9 | Purification of Brg1 helicase core domains, EMSA of naked Myh6 promoter, ChIP and reporter studies in SW13 cells.

a, Coomassie blue staining of purified MBP-tagged Brg1 helicase domains. Bovine serum albumin (BSA) was loaded as a control. b, EMSA assay of naked Myh6 promoter (−426 to +170) with helicase domains of Brg1. Probe: biotin-labelled Myh6 promoter. 50 μM of MBP, MBP–D1, MBP–D2 and MBP–D1D2 proteins were used for EMSA.  c, d, ChIP (c) and luciferase reporter (d) analysis of Brg1 on chromatinized (episomal) and naked Myh6 promoter in SW13 cells. GFP, green fluorescent protein control. e, The luciferase reporter of helicase-deficient Brg1 on chromatinized (episomal) Myh6 promoter in SW13 cells. ΔD1: Brg1 lacking amino acids 774–913. ΔD2: Brg1 lacking amino acids 1086–1246. ChIP: H-10 antibody recognizing N terminus, non-disrupted region of Brg1. P values: Student’s t-test. Error bars show s.e.m.
**Extended Data Figure 10 | Brg1 outruns Mhrt to bind to its target Mhrt promoter.**

**a.** Assembly of nucleosomes on the Mhrt promoter (a3/4).

**b.** Amylose pull-down assay: amylose was used to pull down the chromatinized Mhrt promoter that was incubated with various doses of MBP and MBP–Brg1 D1D2. DNA precipitated by amylose was further quantified by qPCR. 

*P* values: Student’s *t*-test. Error bars show s.e.m.
Extended Data Figure 11 | Sequence alignment and secondary structure prediction of human and mouse MyHEART, and demography of heart transplantation donors. a, Sequence alignment of human MHRT and mouse Mhrt779. b, Predicted secondary structure of mouse Mhrt779 and human MHRT, using minimal free energy (MFE) calculation of RNAfold WebServer. c, Demography of human subjects whose tissues were used for RT-qPCR analysis (Fig. 4l). ICM, ischaemic cardiomyopathy; IDCM, idiopathic cardiomyopathy; LVH, left ventricular hypertrophy.

Demography of human subjects

| Control individuals with normal hearts | Patients with cardiomyopathy |
|---------------------------------------|-----------------------------|
| **Age** | **Gender** | **Clinical diagnosis** | **Age** | **Gender** | **Clinical diagnosis** |
| 38 | M | Normal LV thickness and function | 49 | M | LVH* |
| 49 | M | Normal LV thickness and function | 46 | M | LVH |
| 42 | F | Normal LV thickness and function | 28 | F | LVH |
| 31 | M | Normal LV thickness and function | 48 | M | LVH |
| 39 | M | LVH | 59 | M | ICM* |
| 59 | M | ICM# |
| 58 | M | ICM |
| 57 | M | ICM |
| 59 | F | IDCM# |
| 59 | M | IDCM |
| 56 | M | IDCM |
| 51 | F | IDCM |
| 35 | F | IDCM |
| 54 | F | IDCM |

*LVH: left ventricular hypertrophy.
*ICM: ischemic cardiomyopathy.
#IDCM: idiopathic dilated cardiomyopathy.