Heart Failure

Dissociation of Structural and Functional Phenotypes in Cardiac Myosin-Binding Protein C Conditional Knockout Mice

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Background—Cardiac myosin-binding protein C (cMyBP-C) is a sarcomeric protein that dynamically regulates thick-filament structure and function. In constitutive cMyBP-C knockout (cMyBP-C<sup>-/-</sup>) mice, loss of cMyBP-C has been linked to left ventricular dilation, cardiac hypertrophy, and systolic and diastolic dysfunction, although the pathogenesis of these phenotypes remains unclear.

Methods and Results—We generated cMyBP-C conditional knockout (cMyBP-C-cKO) mice expressing floxed cMyBP-C alleles and a tamoxifen-inducible Cre-recombinase fused to 2 mutated estrogen receptors to study the onset and progression of structural and functional phenotypes caused by the loss of cMyBP-C. In adult cMyBP-C-cKO mice, knockdown of cMyBP-C over a 2-month period resulted in a corresponding impairment of diastolic function and a concomitant abbreviation of systolic ejection, although contractile function was largely preserved. No significant changes in cardiac structure or morphology were immediately evident; however, mild hypertrophy developed after near-complete knockdown of cMyBP-C. In response to pressure overload induced by transaortic constriction, cMyBP-C-cKO mice treated with tamoxifen also developed greater cardiac hypertrophy, left ventricular dilation, and reduced contractile function.

Conclusions—These results indicate that myocardial dysfunction is largely caused by the removal of cMyBP-C and occurs before the onset of cytoarchitectural remodeling in tamoxifen-treated cMyBP-C-cKO myocardium. Moreover, near ablation of cMyBP-C in adult myocardium primarily leads to the development of hypertrophic cardiomyopathy in contrast to the dilated phenotype evident in cMyBP-C<sup>-/-</sup> mice, which highlights the importance of additional factors such as loading stress in determining the expression and progression of cMyBP-C-associated cardiomyopathy. (Circulation. 2012;126:1194-1205.)

Key Words: cardiomyopathy ■ heart failure ■ hypertrophy ■ myocardial contraction ■ remodeling

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease of the myocardium characterized by left ventricular (LV) hypertrophy, myocyte disarray, interstitial fibrosis, and diastolic dysfunction in the absence of known precipitating factors (eg, hypertension, aortic stenosis). In addition to being the leading cause of sudden cardiac death in young athletes, HCM is among the most common genetic diseases of the heart, affecting ∼1 in 500 people. Consequently, systematic investigations into the pathogenesis and pathophysiology of HCM are of clinical importance, particularly because phenotypic expression of HCM can lead to significant morbidity and mortality in a wide range of patient age groups.

Clinical Perspective on p 1205

Among the most common targets of HCM mutations are the gene that encodes the cardiac isoform of myosin-binding protein C (cMyBP-C), a thick-filament accessory protein found in the A bands in myocardial sarcomeres. Unlike other HCM-associated genes, the majority of cMyBP-C gene mutations are predicted to encode truncated proteins that lack various C-terminal–binding domains involved in targeting cMyBP-C to the thick filament. Consequently, truncation mutants of cMyBP-C are likely unable to incorporate properly into the sarcomere and are rapidly degraded by the ubiquitin-proteasome system of the cell. Consistent with this idea, detectable levels of truncated cMyBP-C have yet to be reported in human myectomy samples harboring heterozygous truncation mutations, although several studies have reported a reduction in the levels of endogenous cMyBP-C. These findings lend credence to the hypothesis that cMyBP-C insufficiency contributes to the pathogenesis of HCM. Accordingly, greater losses of cMyBP-C may account for the enhanced phenotypes observed in patients harboring homozy-
ous, or compound heterozygous cMyBP-C gene mutations, as well as the clinical finding that a subset of patients with HCM also later develop LV dilation and systolic dysfunction pathognomonic for dilated cardiomyopathy (DCM), otherwise known as burn-out phase HCM, end-stage HCM, or HCM-associated dilation. Still other patients with cMyBP-C gene mutations bypass the clinical expression of HCM altogether and are initially diagnosed as having DCM. The mechanisms whereby loss of cMyBP-C results in the clinical expression of HCM, HCM-associated dilation, or DCM, however, remain to be elucidated and require further understanding of the roles of cMyBP-C in regulating cardiac structure and function in vivo.

Over the past decade, several unique mouse models have been generated to determine whether elimination of the expression of cMyBP-C (cMyBP-C−/−) or expression of very low amounts of truncated cMyBP-C (cMyBP-Ct/t) is sufficient to cause cardiomyopathies and how the loss of cMyBP-C leads to the pathophysiology of these diseases. Initial studies in cMyBP-C−/− mice and cMyBP-Ct/t mice have demonstrated marked LV dilation, myocardial hypertrophy, impaired relaxation, and depressed systolic contractility, which leads to the conclusion that complete or near-complete loss of cMyBP-C is sufficient to cause the development of HCM-associated dilation and DCM. Mechanical measurements on cMyBP-C−/− skinned myocardium have also demonstrated changes in contractility at the sarcomeric level which raises the possibility that functional derangements associated with removal of cMyBP-C underlie the development of cardiac dysfunction in cMyBP-C−/− and cMyBP-Ct/t mice. A critical gap, however, exists with respect to our understanding of the extent to which loss of cMyBP-C directly contributes to the pathogenesis of cardiac dysfunction. Because characterization studies of cMyBP-C−/− and cMyBP-Ct/t mice hitherto have largely elucidated the end-stage phenotypes of cMyBP-C ablation, relatively little is known regarding the early changes that occur specifically in response to loss of cMyBP-C. Thus, it is unclear whether functional phenotypes in these mice reflect primary responses of the heart caused by removal of cMyBP-C or secondary responses of the heart caused by activation of compensatory mechanisms after the removal of cMyBP-C. Given the prominent effects of constitutive cMyBP-C ablation (or near ablation) on LV architecture and geometry, it is important to determine whether the direct loss of cMyBP-C predominately mediates the functional derangements observed in cMyBP-C−/− and cMyBP-Ct/t mice or whether the development of cardiac hypertrophy and LV dilation drives the expression of diastolic and systolic dysfunction. In the present study, we developed a tamoxifen-inducible cMyBP-C conditional knockout (cMyBP-C-cKO) mouse model using Cre/loxP technology to dissociate the primary effects of removing cMyBP-C from the secondary effects of cardiac hypertrophy and LV dilation on cardiac function in vivo. Because cardiac dysfunction had previously been reported to precede the onset of LV remodeling in other mouse models of HCM, we hypothesized that conditional knockdown of cMyBP-C would allow us to isolate the early consequences of removing cMyBP-C before the development of compensatory remodeling, thereby enabling us to unequivocally determine the functional roles of cMyBP-C in the intact heart. Using transthoracic echocardiography, we studied the onset and progression of structural and functional phenotypes caused by the near-complete knockout of cMyBP-C in adult tamoxifen-treated cMyBP-C-cKO mice and compared these findings with age-matched cMyBP-C+/− mice previously generated in our laboratory. Adult cMyBP-C-cKO mice treated with tamoxifen were also subjected to mechanical pressure overload induced by transaortic constriction (TAC) to determine the consequences of removing cMyBP-C in the context of hemodynamic stress.

Methods

A detailed description of the materials and methods used in the present study can be found in the online-only Data Supplement.

Experimental Animals

The strategy of Liu et al was used to construct the conditional knockout (cKO) targeting vector for the cMyBP-C gene (Mybp3). Mice homozygous for the targeted Mybp3 allele (Mybp3fl/fl-NEO) were crossed with FLPeR deleter mice (The Jackson Laboratory, Bar Harbor, ME) to remove the neomycin (NEO) cassette. Mice homozygous for the floxed Mybp3 allele (Mybp3fl/fl) were then bred to α-MHC-MerCreMer+/− mice (The Jackson Laboratory) and bred back to Mybp3fl/fl to produce Mybp3fl/fl-α-MHC-MerCreMer+/− mice, ie, cMyBP-C-cKO mice. All procedures involving animal care and handling were approved by the University of Wisconsin School of Medicine and Public Health Animal Care and Use Committee.

Knockdown of cMyBP-C

Tamoxifen (40 mg/kg; Sigma-Aldrich) was administered to 12-week-old cMyBP-C-cKO mice of either sex by intraperitoneal injection for 7 days to induce knockdown of cMyBP-C. Hearts from tamoxifen- and vehicle-treated cMyBP-C-cKO mice were collected subsequently at 2-week intervals to determine the extent of cMyBP-C knockdown.

Protein Analysis

Myofibrillar proteins were isolated from frozen ventricles, separated via SDS-PAGE, and visualized with silver staining. Western blotting was performed with primary antibodies to cMyBP-C (1:2000) and β-actin (1:400) and secondary antibodies conjugated to Alexa Fluor 488 and Alexa Fluor 647 (Invitrogen), respectively.

RNA Analysis

Total RNA was isolated from frozen ventricles with TRIzol reagent (Invitrogen). Reverse transcription of total RNA was performed with oligo(dT) primers and Superscript Reverse Transcriptase III (Invitrogen) to generate total cDNA. Gene expression was assessed with commercial TaqMan assays (Applied Bio-Systems) for atrial natriuretic peptide (Nppa), brain natriuretic peptide (Nppb), β-myosin heavy chain (Myh7), and β-actin (Actb) and analyzed according to the ∆∆Ct method.

Histology and Immunohistochemistry

Expianted hearts were fixed with 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 μm in the coronal plane, and stained with either hematoxylin and eosin or Masson’s trichrome. Immunohistochemical labeling of cMyBP-C was performed with polyclonal antibodies against cMyBP-C (1:400 dilution) and secondary antibodies conjugated to Alexa Fluor 488 and Alexa Fluor 647 (Invitrogen).

In Vivo Analysis

Transcardiac echocardiography was performed on anesthetized mice as described previously with a Vevo770 high-resolution imaging system (VisualSonics) equipped with a 30-MHz transducer.
at 2- or 4-week intervals. TAC was performed on anesthetized mice as described previously.32

**Statistical Analysis**

All data are presented as mean±SEM. Statistical analyses were performed by 2-way ANOVA followed by the Holm-Sidak method for multiple comparisons versus wild-type (WT) controls at baseline (time: 1 week). For data collected serially with the same experimental animals, statistical analyses were performed with 2-way repeated-measures ANOVA followed by the Holm-Sidak method for multiple comparisons versus WT controls at baseline. Normality was confirmed with the Shapiro-Wilk test. A value of \( P<0.05 \) was considered significant.

**Results**

**Generation of Tamoxifen-Inducible cMyBP-C-cKO Mice**

Figure 1A illustrates the conditional gene–targeting strategy we used to introduce loxP sites and an FRT-flanked NEO selection cassette into Mybp3.33 Southern blot of genomic DNA from targeted and untargeted embryonic stem (ES) cells. EcoRI-digested DNA was probed with a \( ^{32} \text{P} \)-labeled 5’ probe. The wild-type (WT) allele is detected as a 9.2-kb fragment and the targeted allele as a 2.4-kb fragment. Correct targeting was detected as a 7.5-kb fragment in addition to the native 9.2 EcoRI band. B, Polymerase chain reaction genotyping strategy used to generate cMyBP-C-cKO mice. Shown are polymerase chain reaction products amplified from genomic DNA isolated from Mybp3\(^{1196}\) NEO/NEO, Mybp3\(^{1196} \) FLPeR\(^{1196} \), Mybp3\(^{1196} \), Mybp3\(^{1196} \) α-MyHC-MerCreMer\(^{1196} \), and Mybp3\(^{1196} \) α-MyHC-MerCreMer\(^{1196} \) mice.

Figure 1. Generation of cardiac isoform of myosin-binding protein C conditional knockout (cMyBP-C-cKO) mice. A, Mybp3 conditional knockout gene-targeting strategy, illustrating (I) mini-targeting vector, (II) retrieval vector, (III) targeting vector, and (IV) exons 2 to 6 of Mybp3. Homologous recombination (dotted lines) between the endogenous Mybp3 locus and the targeting vector resulted in a floxed chromosome carrying loxP flanked exons 3 to 5 and an FRT flanked neomycin (NEO) cassette (V). The floxed allele after FLPeR excision of NEO is shown in (VI). Exons are represented by black boxes. Open boxes represent positive (NEO) and negative (herpes simplex virus thymidine kinase [HSV-TK]) selection cassettes. Open triangles represent loxP sites. Open circles represent FRT sites. B, Southern blot of genomic DNA from targeted and untargeted embryonic stem (ES) cells. EcoRI-digested DNA was probed with a \( ^{32} \text{P} \)-labeled 5’ probe. The wild-type (WT) allele is detected as a 9.2-kb fragment and the targeted allele as a 2.4-kb fragment. Correct targeting was detected as a 7.5-kb fragment in addition to the native 9.2 EcoRI band. C, Polymerase chain reaction genotyping strategy used to generate cMyBP-C-cKO mice. Shown are polymerase chain reaction products amplified from genomic DNA isolated from Mybp3\(^{1196}\) NEO/NEO, Mybp3\(^{1196} \) FLPeR\(^{1196} \), Mybp3\(^{1196} \), Mybp3\(^{1196} \) α-MyHC-MerCreMer\(^{1196} \), and Mybp3\(^{1196} \) α-MyHC-MerCreMer\(^{1196} \) mice.
homologous recombination at the 5′ and 3′ ends, detected by the appearance of a 2.4- and 7.5-kb EcoRI band, respectively. Of these, 5 clones were karyotyped to confirm normal chromosome complement, 2 of which were microinjected into C57BL/6 blastocysts to produce highly chimeric founders. Subsequent mating of male chimeras with C57BL/6 females produced black and agouti F1 generation mice, the latter of which were genotyped to identify germline transmission of the targeted Mybp3 allele.

Figure 1C shows the polymerase chain reaction genotyping strategy used to generate cMyBP-C-cKO mice (Mybp3f0/fl-α-MyHC-MerCreMer−/+). F2 generation Mybp3f0/fl-NEO-NEO mice were crossed with FLPeR+/mice expressing an enhanced version of FLP recombinase (FLPeR) to remove the FRT-flanked NEO selection cassette in vivo, which yielded a floxed Mybp3 transgene that was distinguishable from WT Mybp3 by ∼100 bp (corresponding to the 2loxP sequences flanking exons 3–5 and the residual FRT sequence in intron 2). Appropriate breeding of Mybp3f0/fl-FLPeR−/+ mice generated Mybp3f0/fl mice lacking FLPeR recombinase, the latter of which were crossed with α-MyHC-MerCreMer−/+ mice31 to confer temporal and tissue-specific regulation of the floxed Mybp3 allele. The resulting Mybp3f0/fl-α-MyHC-MerCreMer−/+ mice were later bred back to Mybp3f0/fl mice to produce Mybp3f0/fl-α-MyHC-MerCreMer−/+ mice, ie, cMyBP-C-cKO mice. These mice appeared healthy, produced normal-sized litters, and were indistinguishable from cMyBP-C-cKO mice were later bred back to Mybp3f0/fl mice to produce Mybp3f0/fl-α-MyHC-MerCreMer−/+ littermates.

Conditional Knockdown of cMyBP-C in Adult Murine Myocardium
cMyBP-C-cKO mice were injected with tamoxifen to activate MerCreMer recombinase activity to induce knockdown of cMyBP-C. Figure 2A shows a representative agarose gel demonstrating tamoxifen-dependent site-specific recombination of exons 3 to 5 from the floxed Mybp3 transgene. In the absence of tamoxifen, a single ∼1-kb polymerase chain reaction product corresponding to the unrecombined floxed Mybp3 transgene was amplified from cMyBP-C-cKO myocardium treated with vehicle only, which indicates little or no leakage of MerCreMer activity. In contrast, a prominent ∼200-bp polymerase chain reaction product was amplified from tamoxifen-treated cMyBP-C-cKO myocardium corresponding to the removal of exons 3 to 5. Consistent with previously published studies,31 a faint ∼1-kb band was still detected in tamoxifen-treated cMyBP-C-cKO myocardium.

To assess the expression of cMyBP-C at the myofilament level after tamoxifen-dependent activation of MerCreMer activity in cMyBP-C-cKO mice, standard SDS-PAGE and Western blot techniques were used to quantify the loss of cMyBP-C. Figure 2C shows a representative silver-stained SDS-PAGE gel of myofibrillar proteins isolated from WT, cMyBP-C−/−, and cMyBP-C-cKO myocardium treated with either vehicle or tamoxifen. cMyBP-C expression in noninduced cMyBP-C-cKO myocardium did not differ significantly from WT myocardium, in agreement with the tamoxifen-dependent activity of MerCreMer recombinase shown in Figure 2A. In contrast, cMyBP-C levels in cMyBP-C-cKO myocardium decreased ∼50% in the first 2 weeks after tamoxifen treatment and continued to decline thereafter until <10% of total cMyBP-C remained after 8 weeks postinduction. This was confirmed by Western blot analysis (Figure 2C) and fluorescent immunohistochemical labeling of cMyBP-C in cardiac tissue sections collected 8 weeks after tamoxifen treatment, which demonstrates near-complete knockdown of cMyBP-C in tamoxifen-treated cMyBP-C-cKO myocardium (Figure 2B). No detectable levels of cMyBP-C were observed in cMyBP-C−/− myocardium by either SDS-PAGE (Figure 2C), Western blot (Figure 2C), or immunohistochemistry (Figure 2B), as previously published.19

LV Function in Response to Loss of cMyBP-C in Adult cMyBP-C-cKO Mice
To assess the early changes in cardiac function that occur in vivo after the removal of cMyBP-C, serial transthoracic echocardiograms were collected in cMyBP-C-cKO mice before and after tamoxifen treatment and compared with age-matched WT, cMyBP-C−/−, vehicle-treated cMyBP-C-cKO, and tamoxifen-treated Mybp3f0/fl and α-MyHC-MerCreMer−/+ controls. Summary data for all echocardiographic measurements are listed in online-only Data Supplement Tables I through V. Before tamoxifen administration (t−1 week), LV function in cMyBP-C-cKO mice was not statistically different from WT mice. Importantly, heart rates and body weights were not significantly different among the genotypes studied. Consistent with previously published results,19,32 ejection time (ET; Figure 3A) and endocardial fractional shortening (EnFS; Figure 4) were significantly reduced in cMyBP-C−/− mice compared with WT mice, whereas isovolumic relaxation time (IVRT; Figure 3B) was significantly longer.

In tamoxifen-treated cMyBP-C-cKO mice, ET shortened concurrent with the loss of cMyBP-C (Figure 3A). This abbreviation of ET became progressively shorter until after 8 weeks, when little or no cMyBP-C remained in the sarcomere. In a similar manner, IVRT increased concomitantly in cMyBP-C-cKO mice after tamoxifen treatment (Figure 3B), although IVRT continued to increase 8 weeks postinduction, albeit to a lesser degree. Neither ET nor IVRT in tamoxifen-treated cMyBP-C-cKO mice reached the values reported in cMyBP-C−/− mice. Interestingly, EnFS was largely preserved in tamoxifen-treated cMyBP-C-cKO mice compared with WT mice despite near-complete knockout of cMyBP-C (Figure 4). No statistically significant effects of vehicle were observed in cMyBP-C-cKO mice. Similarly, no statistically significant effects of tamoxifen were observed in Mybp3f0/fl or α-MyHC-MerCreMer−/+ controls compared withagematched WT mice (online-only Data Supplement Figures I and II).

Loss of cMyBP-C in Adult Murine Myocardium and Cardiac Remodeling
To study the development and progression of structural and morphological phenotypes caused by the loss of cMyBP-C, gross and histological analyses were performed on formalin-fixed hearts obtained from tamoxifen-treated cMyBP-C-cKO mice (Figure 5). Interestingly, despite near-complete ablation of cMyBP-C, no significant difference in gross cardiac morphology was evident 8 weeks after tamoxifen treatment in cMyBP-C-cKO mice compared with WT mice. Consistent
with these results, ventricle weight-to-body weight ratios were not statistically different among WT and cMyBP-C-cKO mice treated with either vehicle or tamoxifen up to 8 weeks after treatment (Figure 6A). However, by 20 weeks after induction, myocyte hypertrophy was discernible via histology (Figure 5), and a significant increase in ventricle weight-to-body weight ratio was measured in tamoxifen-treated cMyBP-C-cKO mice (Figure 6A). In addition, histopathological analysis of cardiac tissue sections stained with Masson’s trichrome revealed a slightly increased incidence of focal and diffuse interstitial fibrosis in cMyBP-C-cKO myocardium 20 weeks after tamoxifen treatment (Figure 5), although the extent and location of fibrosis were highly variable. In contrast to WT and cMyBP-C-cKO mice, cMyBP-C−/− mice demonstrated marked dilation of the LV, which resulted in a globular morphology on gross examination (Figure 5). Moreover, coronal sections of cMyBP-C−/− hearts stained with hematoxylin and eosin demonstrated LV hypertrophy and
chamber dilation, whereas staining with Masson’s trichrome showed similar areas of diffuse and focal interstitial fibrosis in agreement with previous results.19

Changes in cardiac structure after the removal of cMyBP-C in tamoxifen-treated cMyBP-C-cKO mice were also assessed via M-mode echocardiography to measure and quantify differences in LV chamber and wall dimensions. Consistent with morphometric data presented above, no statistically significant differences in LV posterior wall thickness in diastole or LV internal dimension in diastole were measured in tamoxifen-treated cMyBP-C-cKO mice up to 8 weeks after induction compared with WT mice (Figure 3C and 3D);

Figure 3. Analysis of left ventricular (LV) structure and function. Time course analysis of ejection time (ET; A), isovolumic relaxation time (IVRT; B), LV posterior wall thickness in diastole (C), and LV internal dimension in diastole (D) collected from the same experimental animals in wild-type mice (WT; n=5), mice deficient in the cardiac isoform of myosin-binding protein C (cMyBP-C−/−; n=5), and mice with conditional knockout of cMyBP (cMyBP-cKO) treated with either vehicle (n=6) or tamoxifen (n=8). ET and IVRT were measured from pulsed-wave Doppler tracings of aortic outflow and mitral inflow collected in the suprasternal and apical 4-chamber views on 2-dimensional transthoracic echocardiography. Tamoxifen treatment designated by the horizontal bar. Values are mean±SEM.

Figure 4. Analysis of systolic contractile function. Endocardial fractional shortening (EnFS) was assessed longitudinally in the same experimental animals in wild-type mice (WT; n=5), mice deficient in the cardiac isoform of myosin-binding protein C (cMyBP-C−/−; n=5), and mice with conditional knockout of cMyBP (cMyBP-cKO) treated with either vehicle (n=6) or tamoxifen (n=8). EnFS was calculated with the following equation: EnFS=(LVIDd−LVIDs)/LVIDd×100, where LVIDd and LVIDs are left ventricular internal dimension at diastole and systole, respectively. Echocardiographic measurements of LVIDd and LVIDs were collected at the level of the papillary muscle in M-mode. Values are mean±SEM. *Statistical significance compared with WT mice at baseline. P<0.05.
however, LV posterior wall thickness in diastole was significantly greater after 20 weeks in tamoxifen-treated cMyBP-C-cKO mice (Figure 3C). Interestingly, no significant changes in LV internal dimension in diastole were measured in tamoxifen-treated cMyBP-C-cKO mice 20 weeks after tamoxifen treatment (Figure 3D). In contrast, LV internal dimension in diastole and LV posterior wall thickness in diastole were significantly increased in cMyBP-C mice at all time points, in agreement with previous results.19,32

**Molecular Markers of Hypertrophy in Tamoxifen-Treated cMyBP-C-cKO Myocardium**

To determine whether the expression of hypertrophy-associated genes in tamoxifen-treated cMyBP-C-cKO myocardium was evident even in the absence of cardiac hypertrophy 8 weeks after tamoxifen treatment, mRNA levels of *Nppa*, *Nppb*, and *Myh7* were quantified by real-time quantitative polymerase chain reaction and compared with WT, cMyBP-C−/−, and vehicle-treated cMyBP-C-cKO mice (Figure 6B). No significant differences were observed between WT and cMyBP-C-cKO myocardium treated with either vehicle or tamoxifen; however, an increasing trend in the mRNA expression of *Nppa* and *Myh7* was noted in tamoxifen-treated cMyBP-C-cKO myocardium. In contrast, *Nppa*, *Nppb*, and *Myh7* mRNA levels were all dramatically elevated in cMyBP-C−/− myocardium, as reported previously.19 By 20 weeks after tamoxifen induction, however, mRNA levels of *Nppa*, *Nppb*, and *Myh7* were all statistically elevated in tamoxifen-treated cMyBP-C-cKO mice compared with WT mice, consistent with the initial development of myocardial hypertrophy.

**Effects of Pressure Overload in Tamoxifen-Treated cMyBP-C-cKO Mice**

To investigate the phenotypic effects of cMyBP-C knockdown in the context of hemodynamic stress, tamoxifen- and

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**Figure 5.** Cardiac morphometry and histology in wild-type mice (WT), mice deficient in the cardiac isoform of myosin-binding protein C (cMyBP-C−/−; *n* = 5), and mice with conditional knockout of cMyBP-C (cMyBP-C-cKO) treated with either vehicle or tamoxifen (8 or 20 weeks postinduction). First row shows representative images (magnification ×10) of formalin-fixed hearts. Second row shows representative hematoxylin-and-eosin–stained coronal sections (magnification ×10) of paraffin-embedded cardiac tissue. Third row shows representative high-magnification (magnification ×400) photomicrographs of hematoxylin and eosin–stained sections. Fourth row shows high-magnification (magnification ×400) photomicrographs of Masson’s trichrome–stained sections, demonstrating a focus of fibrosis in cMyBP-C−/− and tamoxifen-treated cMyBP-C-cKO mice 20 weeks postinduction.
vehicle-treated cMyBP-C-cKO mice were subjected to pressure overload induced by TAC for a period of up to 12 weeks. Both tamoxifen- and vehicle-treated cMyBP-C-cKO mice developed LV hypertrophy comparable to that measured in cMyBP-C−/− mice after 4 weeks of TAC (Figure 7A); however, LV chamber dilation only developed in tamoxifen-treated cMyBP-C-cKO mice after 8 weeks of TAC (Figure 7B). With respect to cardiac function, EnFS was largely preserved in vehicle-treated cMyBP-C-cKO mice after TAC (Figure 7C). In contrast, EnFS progressively declined in tamoxifen-treated cMyBP-C-cKO mice after TAC until LV performance was comparable to cMyBP-C−/− mice after 12 weeks. Importantly, tamoxifen-treated cMyBP-C-cKO mice subjected to TAC also exhibited prolonged IVRT and abbreviated ET compared with WT mice. No statistically significant differences in ET or IVRT were detected in Mybp3fl/fl or α-MyHC-MerCreMer+/− experimental controls treated with tamoxifen and subjected to TAC compared with WT mice (online-only Data Supplement Figure III).

With respect to histopathology, cMyBP-C-cKO mice treated with vehicle and tamoxifen both developed myocyte hypertrophy in response to TAC. Likewise, Mybp3fl/fl and α-MyHC-MerCreMer+/− experimental controls treated with tamoxifen and subjected to TAC also developed myocyte hypertrophy (online-only Data Supplement Figure IV). However, only tamoxifen-treated cMyBP-C-cKO mice developed myocyte disarray and myocardial fibrosis 12 weeks after TAC.

Discussion
In the present study, we developed a cMyBP-C-cKO mouse model expressing floxed Mybp3 alleles and a tamoxifen-dependent MerCreMer recombinase under the transcriptional control of the α-MyHC promoter. Using a conditional knockout approach to acutely terminate the expression of cMyBP-C in adult myocardium, we determined and interrelated the time course of cMyBP-C knockdown with the onset and development of structural and functional phenotypes,
thereby allowing us to differentiate the primary effects of removing cMyBP-C from the secondary effects of compensatory mechanisms (eg, ventricular remodeling) on cardiac function in vivo. On the basis of gross morphometric, histological, and echocardiographic data, we found that cardiac dysfunction preceded the development of LV hypertrophy in cMyBP-C-cKO mice after the removal of cMyBP-C. More specifically, loss of cMyBP-C resulted in concurrent shortening of ET and prolongation of IVRT before the onset of cardiac remodeling that was commensurate with the degree of cMyBP-C knockdown, which implies a prominent role for cMyBP-C in regulation of the period of cardiac systole and diastole. Interestingly, subsequent development of LV hypertrophy after near-complete ablation of cMyBP-C in cMyBP-C-cKO mice did not have an additional effect on ET, whereas IVRT continued to increase in parallel with the development of cardiac hypertrophy. These results indicate that truncation of ET and prolongation of IVRT in cMyBP-C-deficient mice occurs primarily in response to the removal of cMyBP-C, although IVRT increases further in response to LV hypertrophy.

Our finding that cardiac dysfunction preceded the onset of cardiac remodeling in the present study is consistent with other mouse models of HCM that harbor either α-MyHC27 or cardiac troponin T mutations.28 Interestingly, this dissociation of structural and functional phenotypes in response to the removal of cMyBP-C in cMyBP-C-cKO mice continued until cMyBP-C knockout was nearly complete, which indicates that adult murine myocardium tolerates considerable reductions in the expression of cMyBP-C without immediately undergoing significant cardiac remodeling. Despite near-complete ablation of cMyBP-C in tamoxifen-treated cMyBP-C-cKO mice, however, only mild cardiac hypertrophy developed, in contrast to the marked LV hypertrophy and chamber dilation present in cMyBP-C−/−19,20 and cMyBP-C+/− mice.21 Moreover, near ablation of cMyBP-C in tamoxifen-treated cMyBP-C-cKO mice did not recapitulate the severe systolic dysfunction previously demonstrated in cMyBP-C−/−19,20,32 and cMyBP-C+/− mice,21 ie, EnFS was largely preserved in tamoxifen-treated cMyBP-C-cKO even when <10% of total cMyBP-C remained. This apparent lack of effect of cMyBP-C knockdown on EnFS is perhaps surprising given the significant shortening of ET in these mice, which in turn might be predicted to limit EnFS. A concomitant acceleration of contraction kinetics caused by the loss of cMyBP-C, however, could potentially account for the preservation of EnFS in tamoxifen-treated cMyBP-C-
cKO mice. Indeed, considerable evidence from mechanical measurements performed in cMyBP-C−/− skinned myocardium suggests that cross-bridge cycling kinetics are accelerated in the absence of cMyBP-C,22,23 which would in turn speed the rate of pressure development in the intact heart. Thus, loss of systolic contractile function in cMyBP-C−/− and cMyBP-CΔα mice most likely involves modifying factors activated subsequent to the ablation of cMyBP-C. Together, these results demonstrate that acute loss of cMyBP-C in adult mice per se is insufficient to cause the expression of DCM or HCM-associated dilation previously reported in cMyBP-CΔα mice19,21 and cMyBP-C−/− mice,19,20 respectively.

To investigate whether additional stressors could induce the expression of HCM-associated dilation in tamoxifen-treated cMyBP-C-cKO mice, TAC was introduced to increase hemodynamic workload. Interestingly, loss of systolic contractile function was precipitated in tamoxifen-treated cMyBP-C-cKO mice subjected to TAC. In addition, these mice developed progressive LV chamber dilation and further hypertrophy of the LV wall, recapitulating the dilated HCM phenotype previously reported in cMyBP-C−/− mice.19,20 Importantly, these phenotypes were largely absent in vehicle-treated cMyBP-C-cKO mice subjected to TAC (with the exception of hypertrophy). Together, these results indicate that both knockout of cMyBP-C and additional precipitating factors are needed to drive the expression of HCM-associated dilation in tamoxifen-treated cMyBP-C-cKO mice. Consistent with this idea, loss of systolic contractile function in tamoxifen-treated cMyBP-C-cKO mice subjected to TAC was not statistically significant until knockout of cMyBP-C was nearly complete. Interestingly, this effect of cMyBP-C knockdown on contractile function in tamoxifen-treated cMyBP-C-cKO mice coincided with the development of LV dilation, which suggests that increases in LV chamber dilation may exacerbate the functional effects of cMyBP-C ablation on systolic function. Indeed, increases in the diameter of the LV would be expected to increase wall stress according to the law of Laplace, which could in turn limit EnFS.28 Although increased afterload induced by TAC might also be expected to diminish EnFS, this consideration is not applicable in cMyBP-C−/− mice, because hemodynamic load in these mice is not significantly different from WT mice. Taken together, these results suggest that the development of LV dilation in cMyBP-C−/− mice contributes to the pathogenesis of depressed cardiac function (beyond effects caused by ablation of cMyBP-C).

The mechanisms underlying the development of LV dilation in cMyBP-C−/− and cMyBP-CΔα mice, however, remain largely unknown. Previous studies have suggested a gene-dosage effect,19–21,34 because cMyBP-C−/+ and cMyBP-Cα/+ mice exhibited milder or unaltered phenotypes compared with their respective homozygous counterparts. Results presented in this study suggest that additional disease-modifying factors are required to precipitate the expression of HCM-associated dilation or DCM, because near-to-complete loss of cMyBP-C in adult murine myocardium was insufficient to cause ventricular dilation and systolic dysfunction per se. An important difference between the cMyBP-C-cKO mice in the present study and the cMyBP-C−/− and cMyBP-CΔα mice of previous studies was the timing of cMyBP-C knockout, i.e., adult versus embryonic knockout. This distinction may provide valuable insights into potential mechanisms underlying the development of LV dilation and systolic dysfunction in cMyBP-C−/− and cMyBP-CΔα mice versus the development of HCM in cMyBP-C-cKO mice. There are several fundamental differences between mature myocardium and developing myocardium in utero, which in turn may modulate the effects that loss of cMyBP-C may have on cardiac structure and function. For example, the predominant isoform of MyHC expressed during murine cardiac development is β-MyHC, which later switches to the α-MyHC isoform during early neonatal development and becomes the predominant isoform in adult myocardium.35 Replacement of α-MyHC with predominantly β-MyHC in cMyBP-CΔα mice previously has been shown to exacerbate the expression of the morphological phenotype associated with cMyBP-CΔα mice,21 which suggests that the functional effects of removing cMyBP-C are more severe on a β-MyHC background than on a predominantly α-MyHC background. There is increasing evidence to suggest that the introduction of HCM mutations onto an α-MyHC or β-MyHC background may have disparate effects on contractile function.37 Indeed, the slower turnover kinetics of β-MyHC38 could result in a greater reduction in peak twitch force production after ablation of cMyBP-C. These studies raise the possibility in mice that loss of cMyBP-C requires a predominantly β-MyHC background (at least initially) to lead to the development of HCM-associated dilation or DCM. Along this same line of reasoning, expression of the α-MyHC isoform may be cardioprotective with respect to the deleterious functional effects of removing cMyBP-C in murine myocardium.

In summary, tamoxifen-induced loss of cMyBP-C in cMyBP-C-cKO mice causes functional derangements in ET and IVRT that occur before the onset of compensatory cardiac remodeling, which supports the idea that cMyBP-C plays important roles in regulating normal systolic and diastolic function. In addition, we provide novel evidence suggesting that LV dilation may contribute to the depression in cardiac function previously demonstrated in cMyBP-C−/−19,20 and cMyBP-CΔα mice, whereas LV hypertrophy only contributes to the prolongation of IVRT. These results underscore the role of additional factors or stressors in governing the clinical expression of cMyBP-C–associated cardiomyopathy and predict the clinical utility of interventions that prevent or delay the onset of cardiac remodeling or reverse the progression of LV hypertrophy to LV chamber dilation.

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We thank Dr Timothy Hacker (UW Cardiovascular Research Center) for echocardiography services; Dr Ruth Sullivan and Beth Gray (UW Research Animal Resource Center) for histology services; Andy Peper, Maggie Maes, Becky Plutz, Anne Dronen, Jasmine Giles, Jennifer Wachholz, and Dr Matthew Locher for technical assistance; and Dr Willem (Toy) Delange for fruitful discussions.

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Disclosures

None.

References


23. Kotte FS, McDonald KS, Harris SP, Moss RL. Load shortening, power output, and rate of force redevelopment are increased with knockout of cardiac myosin-binding protein-C. Circ Res. 2003;93:752–758.


Mutations in the gene encoding cardiac myosin-binding protein-C (cMyBP-C) are common causes of familial hypertrophic cardiomyopathy (HCM) and can also lead to HCM-associated dilation. This primary disease of the myocardium affects a wide range of patient age groups, accounts for the majority of cardiac genetic diseases, and leads to significant morbidity and mortality associated with the development of heart failure, ventricular and atrial arrhythmias, myocardial infarction, and sudden cardiac death. Nevertheless, little is known regarding the mechanisms underlying the development of cMyBP-C–associated HCM, although deficiencies in cMyBP-C are thought to contribute to the pathogenesis of this disease in many cases. Herein, we investigate the onset, progression, and saturation of structural and functional phenotypes caused by the removal of cMyBP-C and assess whether loss of cMyBP-C per se is sufficient to cause the development of HCM-associated dilation. Using adult cMyBP-C conditional knockout mice, we show that acute loss of cMyBP-C affects the duration of cardiac systole and diastole and contributes to the development of diastolic dysfunction, all of which occurs before the onset of cardiac hypertrophy. Moreover, we show that near-complete ablation of cMyBP-C is insufficient to cause HCM-associated dilation in adult mice, whereas the imposition of additional stresses such as increased afterload is sufficient to recapitulate the phenotype. These results help define the gradual changes that precede and progress with the clinical expression of cMyBP-C–associated cardiomyopathy and identify potential avenues in which to prevent, delay, or reverse the progression of this disease.
Dissociation of Structural and Functional Phenotypes in Cardiac Myosin-Binding Protein C Conditional Knockout Mice

Peter P. Chen, Jitandrakumar R. Patel, Patricia A. Powers, Daniel P. Fitzsimons and Richard L. Moss

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SUPPLEMENTAL MATERIALS

DISSOCIATION OF STRUCTURAL AND FUNCTIONAL PHENOTYPES IN CARDIAC MYOSIN BINDING PROTEIN-C CONDITIONAL KNOCK-OUT MICE

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SUPPLEMENTAL METHODS

Conditional gene targeting

The recombineering strategy of Liu et al. 1 was used to construct the conditional knock-out (cKO) targeting vector for the cMyBP-C gene (Mybp3). A P1 clone containing the entire murine cardiac Mybp3 sequence obtained from a 129/SvJ embryonic stem (ES) cell library (Genome Systems) was used as the source of genomic DNA. A 6 kb Sac I genomic fragment containing Mybp3 exons 3, 4, and 5 was isolated from the Mybp3 P1 clone and introduced into the plasmid pL253 containing the MC1-TK selection cassette. A mini-targeting vector was constructed by cloning the following 3 PCR fragments into the vector pL452 containing a loxP site and the FRT-flanked pGK promoter/EM7 promoter-NEO-pGHpA cassette: (a) a 700 bp sequence immediately upstream of exon 3; (b) a loxP site and a 800 bp sequence containing exons 3, 4, and 5; and (c) a 900 bp sequence immediately downstream of exon 5. The mini-targeting cassette was excised by Acc65 I and Sac II digestion and transformed into recombination-competent DY380 bacteria cells previously transformed with the 6 kb Sac I – MCI-TK plasmid (6S-TK). Recombinants that integrated the pGK promoter/EM7 promoter - NEO-pGHpA cassette into 6S-TK were selected on kanamycin plates. Restriction mapping and DNA sequencing were performed to confirm the homologous integration of the mini-targeting cassette into the 6 kb Sac I plasmid.

The targeting vector was linearized by digestion with Asc I and introduced by electroporation into murine SV/129 R1 ES cells 2. ES cells that integrated the targeting vector were selected by growth on geneticin and gancyclovir containing media, replicated, and expanded. DNA isolated from replica ES clones were digested with Eco RI and screened by
Southern Blot to confirm the proper targeting of the vector due to homologous recombination. DNA sequence analysis confirmed the integrity of the loxP sites and the presence of the floxed exons 3, 4, and 5. Properly targeted, karyotypically normal ES clones were then microinjected into C57BL/6 blastocysts to produce chimeric founders, which were mated subsequently to C57BL/6 females. Germ-line transmission of the targeted *Mybp3* allele was confirmed by PCR analysis of genomic DNA isolated from tail biopsies. Mice homozygous for the targeted *Mybp3* allele (*Mybp3*^{fl-NEO/fl-NEO}) were crossed with FLPeR deleter (*FLPeR^{+/+}* ) mice ³ (Jackson Laboratory) to remove the neomycin (NEO) cassette. Subsequent breeding generated mice homozygous for the floxed *Mybp3* allele (*Mybp3*^{fl/fl}).

**Experimental animals**

To generate functional cMyBP-C-cKO mice, *Mybp3*^{fl/fl} mice were crossed with transgenic mice expressing a tamoxifen-inducible Cre recombinase fused to two mutated estrogen receptors under the transcriptional control of the α-myosin heavy chain (MyHC) promoter (α-*MyHC-MerCreMer^{+/+}* )⁴ (Jackson Laboratory). The generation of cMyBP-C- mice has previously been reported ⁵. 129 SvE wild-type (WT) mice controls were purchased from Taconic Farms, Inc. All procedures involving animal care and handling were reviewed and approved by the UW School of Medicine and Public Health Animal Care and Use Committee.

**Knock-down of cMyBP-C**

Conditional expression of the *Mybp3* null allele and subsequent knock-down of cMyBP-C was induced by administering tamoxifen (Sigma-Aldrich) to 12-week-old cMyBP-C-cKO mice of either sex. 10 mg/mL solutions of tamoxifen were prepared by dissolving 100 mg of
tamoxifen in 10 mL of vehicle containing 1 mL of ethanol and 9 mL of peanut oil. To effect maximum knock-down of cMyBP-C, cMyBP-C-cKO mice were given 100-200 μL of tamoxifen solution once a day for 7 consecutive days via intraperitoneal injection. Vehicle-treated cMyBP-C-cKO mice served as negative controls. Cardiac ventricles from tamoxifen- and vehicle-treated cMyBP-C-cKO mice were collected at 2 weeks intervals (up to 8 weeks) following completion of the 7-day treatment regimen to determine the extent and rate of cMyBP-C knock-down.

**Myofibrillar protein isolation**

Frozen ventricles from WT, cMyBP-C<sup>−/−</sup>, and tamoxifen- or vehicle-treated cMyBP-C-cKO mice (either sex, 12 weeks old) were isolated as previously described<sup>6</sup>, pulverized under liquid nitrogen, and homogenized in ice-cold relaxing solution (100 mM KCl, 20 mM immidazole, 7 mM MgCl<sub>2</sub>, 2 mM EGTA, 4 mM ATP, pH 7.0) containing 1:100 dilution of protease (P8340, Sigma-Aldrich) and phosphatase (P-0044, Sigma-Aldrich) inhibitor cocktails. Myofibrillar proteins were collected by centrifugation, washed with fresh ice-cold relaxing solution, and solubilized in sample buffer (8M Urea, 50 mM Tris, 3% SDS, pH 6.8). Protein concentrations were determined using the Bio-Rad DC Protein Assay.

**SDS-PAGE analysis**

To determine the relative expression of cMyBP-C, solubilized myofibrillar proteins extracted from WT, cMyBP-C<sup>−/−</sup>, and tamoxifen-treated or vehicle-treated cMyBP-C-cKO cardiac ventricles were reduced with 75 mM DTT, loaded onto 7.5% Tris-HCl Criterion gels (Bio-Rad) along with bromophenol blue dye, and subjected to SDS-PAGE. Silver staining was
performed to visualize myofibrillar proteins as previously described. Stained SDS-PAGE gels were imaged using a UVP BioImaging System (UVP, Inc.). Densitometric analysis was performed using LaserPix Software (Bio-Rad), and the ratio of the integrated optical density (IOD) of the cMyBP-C band to the IOD of the corresponding α-actinin band was calculated to correct for loading and to permit comparison between samples. All cMyBP-C IOD/α-actinin IOD ratios were further normalized to WT values.

**Western blot analysis**

Myofibrillar proteins extracted from WT, cMyBP-C+/−, and tamoxifen-treated or vehicle-treated cMyBP-C-cKO cardiac ventricles were resolved on 10% Tris-HCl Criterion gels (Bio-Rad) and transferred overnight at 4°C onto nitrocellulose membranes. After blocking with 5% nonfat dry milk for one hour, membranes were incubated with rabbit polyclonal anti-cMyBP-C (1:2000) and mouse monoclonal anti-β-actin (1:400) antibodies overnight at 4°C followed by multiple washes in TBST buffer. Membranes were then incubated with Alexa Fluor 488 goat anti-rabbit IgG (1:1000, Invitrogen) and Alexa Fluor 647 goat anti-mouse IgG (1:1000, Invitrogen) secondary antibodies to detect cMyBP-C and β-actin, respectively, followed by a final series of washes in TBST buffer. Western blot images were obtained using a ChemiDoc MP System (Bio-Rad) and analyzed with Image Lab software, v4.0 (Bio-Rad).

**RNA transcript analysis**

Total RNA was isolated from frozen WT, cMyBP-C+/−, and tamoxifen- or vehicle-treated cMyBP-C-cKO ventricles using Trizol reagent (Invitrogen) according to the manufacturer’s protocol, purified with RNeasy Mini Kit (Qiagen), and quantified spectrophotometrically.
Reverse transcription of total RNA was performed using oligo-d(T) primers and Superscript Reverse Transcriptase III (Invitrogen) to generate total cDNA. Gene expression was assessed using commercial Taqman assays (Applied Bio-Systems) for atrial natriuretic peptide (Nppa), brain natriuretic peptide (Nppb), β-myosin heavy chain (Myh7), and β-actin (Actb) and run on a Stratagene Mx3000 qPCR system. Analysis of Nppa, Nppb, Myh7, and Actb expression was performed using MxPro real-time qPCR software. The relative levels of mRNA expression normalized to Actb expression were calculated according to the ΔΔC_T method.

**Morphometry and histology**

Excised WT, cMyBP-C<sup>−/−</sup>, and tamoxifen- or vehicle-treated cMyBP-C-cKO hearts were cannulated and perfused with Ca<sup>2+</sup>-free Ringer’s solution (120 mM NaCl, 5 mM KCl, 1.2 mM MgSO<sub>4</sub>, 19 mM NaHCO<sub>3</sub>, 1.2 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM glucose, equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 37°C) containing 30 mM 2,3 butanedione monoxime on a Langendorff perfusion setup and fixed with 10% neutral buffered formalin. Whole heart images were captured on an Olympus SZ-STS Stereoscope fitted with an 18 megapixel Canon EOS Rebel XTi Digital SLR camera. Cross-sectional views along the coronal plane of the hearts were obtained from paraffin embedded samples sectioned at 5 μm and stained with hemotoxylin and eosin (H&E). Evidence of interstitial fibrosis was visualized using Masson’s Trichrome staining. High magnification light micrographs were taken using a Zeiss Axioplan 2 Imaging photomicroscope provided by the Cellular and Molecular Neuroscience Core at the University of Wisconsin-Madison Waisman Center.

**Immunohistochemistry**
5 μm sections from WT, cMyBP-C<sup>−</sup>, and tamoxifen- or vehicle-treated cMyBP-C-cKO hearts were deparaffinized, rehydrated, and incubated with rabbit polyclonal antibodies against cMyBP-C (1:400 dilution) overnight at 4°C in a humidified chamber. Immunofluorescent detection was carried out using goat anti-rabbit secondary antibodies conjugated to Alexa 647 (Invitrogen). All slides were mounted with SloFade Gold antifade reagent containing DAPI counterstain (Invitrogen) and imaged on a Nikon C1 laser scanning confocal microscope provided by the Cellular and Molecular Neuroscience Core at the University of Wisconsin-Madison Waisman Center.

**Transthoracic echocardiography**

Serial echocardiograms were collected from cMyBP-C-cKO mice before and after tamoxifen or vehicle treatment at 2 week intervals for the first 8 weeks and then at 4 week intervals up to 20 weeks. Age-matched WT, cMyBP-C<sup>−</sup>, and tamoxifen-treated Mybp<sup>3fl/fl</sup> and α-MyHC-MerCreMer<sup>+/−</sup> mice were also subject to echocardiographic analysis and served as additional controls. Transthoracic echocardiography was performed and analyzed blinded to genotype and treatment using a VisualSonics Vevo770 high resolution imaging system equipped with a 30 MHz probe as previously described. Briefly, mice were lightly anesthetized with 1% inhaled isoflurane and maintained on a heated platform. Two-dimensionally guided M-mode images of the left ventricle (LV) and Doppler studies were acquired at the tip of the papillary muscles. LV internal dimension in systole (LVIDs) and diastole (LVIDd), thickness of the LV posterior wall in systole (LVPWs) and diastole (LVPWd), and isovolumic relaxation time (IVRT) were recorded. Endocardial fractional shortening (EnFS) was calculated as (LVIDd-LVIDs)/LVIDd x 100. Systolic ejection time (ET) and heart rate were measured from pulse-
wave Doppler tracings of the LV outflow tract recorded in the suprasternal view. All parameters were measured over at least three consecutive cycles.

**Transaortic constriction**

Transaortic constriction (TAC) was performed on tamoxifen- or vehicle-treated cMyBP-C-cKO mice and tamoxifen-treated Mybp30flo and α-MyHC-MerCreMer+/− mice as previously described. Briefly, mice were anesthetized with 1% inhaled isoflurane and intubated, and the chest cavity was entered in the second intercostal space at the left upper sternal border through a small incision. TAC was performed by tying a 7-0 nylon suture ligature against a 0.012-in. stainless steel wire to yield a narrowing 0.4 mm in diameter when the needle was removed. Mice were given subcutaneous buprenorphine (0.8 mg/kg) for pain relief, allowed to recover in a heated chamber with 100% oxygen, and maintained for up to 12 weeks. Serial transthoracic echocardiograms were collected at 4 week intervals following TAC.

**Statistics**

All data are presented as means ± SEM. Statistical analyses were carried out using two-way ANOVA followed by the Holm-Sidak method for multiple comparisons versus WT controls at baseline (time = -1 week). For data collected serially using the same experimental animals, statistical analyses were carried out using two-way repeated measures ANOVA followed by the Holm-Sidak method for multiple comparisons versus WT controls at baseline. Normality was confirmed using the Shapiro-Wilk test. A value of $P < 0.05$ was considered significant.
SUPPLEMENTAL TABLES

TABLE S1. Echocardiographic summary data in WT and cMyBP-C<sup>−/−</sup> mice.

<table>
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<tr>
<th>Time (wks)</th>
<th>-1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
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<td><strong>WT (n = 5, 3 male and 2 female)</strong></td>
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<tr>
<td>Heart Rate (bpm)</td>
<td>480 ± 16</td>
<td>497 ± 34</td>
<td>483 ± 30</td>
<td>458 ± 22</td>
<td>468 ± 22</td>
<td>460 ± 13</td>
<td>459 ± 20</td>
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<td>Body Weight (g)</td>
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<td>26.59 ± 1.84</td>
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<td>28.18 ± 3.03</td>
<td>27.12 ± 3.54</td>
<td>28.00 ± 2.27</td>
<td>27.95 ± 2.93</td>
<td>28.75 ± 1.89</td>
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<td>LVIDd (mm)</td>
<td>3.72 ± 0.11</td>
<td>3.97 ± 0.06</td>
<td>3.74 ± 0.10</td>
<td>3.74 ± 0.13</td>
<td>3.90 ± 0.12</td>
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<td>LVPWd (mm)</td>
<td>0.76 ± 0.03</td>
<td>0.77 ± 0.03</td>
<td>0.84 ± 0.03</td>
<td>0.80 ± 0.03</td>
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<td>LVIDs (mm)</td>
<td>2.22 ± 0.16</td>
<td>2.52 ± 0.15</td>
<td>2.22 ± 0.16</td>
<td>2.51 ± 0.11</td>
<td>2.64 ± 0.17</td>
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<td>LVPWs (mm)</td>
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<td>1.17 ± 0.06</td>
<td>1.26 ± 0.05</td>
<td>1.29 ± 0.05</td>
<td>1.29 ± 0.05</td>
<td>1.18 ± 0.04</td>
<td>1.17 ± 0.04</td>
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<td>EnFS (%)</td>
<td>40.42 ± 3.16</td>
<td>36.84 ± 3.05</td>
<td>39.54 ± 3.09</td>
<td>35.37 ± 2.42</td>
<td>37.94 ± 2.08</td>
<td>32.45 ± 2.36</td>
<td>27.81 ± 1.65</td>
<td>29.69 ± 1.88</td>
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<td>IVRT (ms)</td>
<td>15.58 ± 0.24</td>
<td>15.33 ± 0.50</td>
<td>15.02 ± 0.46</td>
<td>16.72 ± 0.79</td>
<td>16.95 ± 0.54</td>
<td>17.49 ± 0.67</td>
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<td>Ejection Time (ms)</td>
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<th><strong>cMyBP-C&lt;sup&gt;−/−&lt;/sup&gt; (n = 5, 3 male and 2 female)</strong></th>
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<td>4.74 ± 0.16</td>
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<td>4.80 ± 0.27</td>
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<td>5.47 ± 0.46</td>
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<tr>
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<td>1.22 ± 0.08</td>
<td>1.19 ± 0.04</td>
<td>1.16 ± 0.11</td>
<td>1.24 ± 0.06</td>
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<td>1.15 ± 0.08</td>
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<td>LVIDs (mm)</td>
<td>3.76 ± 0.22</td>
<td>3.96 ± 0.18</td>
<td>4.06 ± 0.18</td>
<td>3.98 ± 0.21</td>
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<td>4.20 ± 0.25</td>
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<td>LVPWs (mm)</td>
<td>1.46 ± 0.05</td>
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<td>1.42 ± 0.13</td>
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<td>EnFS (%)</td>
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<td>16.33 ± 1.23</td>
<td>14.78 ± 1.38</td>
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<td>11.52 ± 1.53</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>28.85 ± 0.49</td>
<td>29.35 ± 0.92</td>
<td>28.58 ± 1.43</td>
<td>30.86 ± 1.27</td>
<td>29.43 ± 0.81</td>
<td>29.24 ± 0.47</td>
<td>28.24 ± 1.00</td>
<td>30.46 ± 0.74</td>
</tr>
<tr>
<td>Ejection Time (ms)</td>
<td>30.42 ± 0.38</td>
<td>30.82 ± 1.05</td>
<td>30.33 ± 1.05</td>
<td>28.83 ± 0.47</td>
<td>30.00 ± 0.86</td>
<td>29.63 ± 0.75</td>
<td>31.90 ± 0.90</td>
<td>30.46 ± 0.59</td>
</tr>
</tbody>
</table>

Values are means ± SEM. LVIDd, left ventricular internal dimension diastole; LVPWd, left ventricular posterior wall in diastole; LVIDs, left ventricular internal dimension in systole; LVPWs, left ventricular posterior wall in systole; EnFS, endocardial fractional shortening; IVRT, isovolumic relaxation time. * indicates statistical significance compared to WT mice at baseline. \( P < 0.05 \).
**TABLE S2.** Echocardiographic summary data in cMyBP-C-cKO mice treated with vehicle or tamoxifen.

<table>
<thead>
<tr>
<th>Time (wks)</th>
<th>-1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cMyBP-C-cKO + vehicle (n = 6, 3 male and 3 female)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>23.66 ± 1.35</td>
<td>24.98 ± 1.11</td>
<td>26.42 ± 1.37</td>
<td>27.20 ± 1.64</td>
<td>26.86 ± 1.81</td>
<td>30.00 ± 2.68</td>
<td>31.00 ± 2.97</td>
<td>33.25 ± 3.59</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>3.65 ± 0.13</td>
<td>3.89 ± 0.12</td>
<td>3.88 ± 0.13</td>
<td>3.82 ± 0.14</td>
<td>4.09 ± 0.14</td>
<td>4.15 ± 0.11</td>
<td>4.20 ± 0.06</td>
<td>3.97 ± 0.09</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>0.81 ± 0.03</td>
<td>0.77 ± 0.02</td>
<td>0.74 ± 0.06</td>
<td>0.78 ± 0.04</td>
<td>0.81 ± 0.04</td>
<td>0.78 ± 0.03</td>
<td>0.73 ± 0.02</td>
<td>0.79 ± 0.02</td>
</tr>
<tr>
<td>LVIDs (mm)</td>
<td>2.29 ± 0.29</td>
<td>2.45 ± 0.17</td>
<td>2.44 ± 0.16</td>
<td>2.41 ± 0.25</td>
<td>2.51 ± 0.18</td>
<td>2.47 ± 0.10</td>
<td>2.80 ± 0.09</td>
<td>2.68 ± 0.04</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>1.30 ± 0.05</td>
<td>1.21 ± 0.04</td>
<td>1.15 ± 0.03</td>
<td>1.19 ± 0.07</td>
<td>1.17 ± 0.05</td>
<td>1.28 ± 0.05</td>
<td>1.10 ± 0.06</td>
<td>1.17 ± 0.06</td>
</tr>
<tr>
<td>EnFS (%)</td>
<td>41.93 ± 2.64</td>
<td>37.25 ± 2.62</td>
<td>38.87 ± 2.73</td>
<td>37.53 ± 4.41</td>
<td>36.55 ± 3.75</td>
<td>38.94 ± 2.70</td>
<td>33.58 ± 2.62</td>
<td>31.86 ± 1.59</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>15.56 ± 0.63</td>
<td>15.74 ± 0.40</td>
<td>16.07 ± 0.88</td>
<td>16.02 ± 0.44</td>
<td>16.62 ± 0.94</td>
<td>14.91 ± 0.76</td>
<td>16.40 ± 1.26</td>
<td>17.05 ± 0.60</td>
</tr>
<tr>
<td>Ejection Time (ms)</td>
<td>46.03 ± 2.23</td>
<td>47.82 ± 1.53</td>
<td>44.95 ± 1.88</td>
<td>47.68 ± 0.47</td>
<td>44.19 ± 2.74</td>
<td>43.33 ± 0.99</td>
<td>49.79 ± 2.47</td>
<td>46.67 ± 2.99</td>
</tr>
<tr>
<td></td>
<td>cMyBP-C-cKO + tamoxifen (n = 8, 4 male and 4 female)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>458 ± 10</td>
<td>499 ± 15</td>
<td>474 ± 23</td>
<td>458 ± 8</td>
<td>488 ± 11</td>
<td>514 ± 29</td>
<td>446 ± 9</td>
<td>461 ± 10</td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>24.15 ± 1.25</td>
<td>26.64 ± 0.91</td>
<td>27.36 ± 1.05</td>
<td>27.77 ± 1.20</td>
<td>27.94 ± 1.15</td>
<td>30.17 ± 1.62</td>
<td>30.83 ± 1.78</td>
<td>33.17 ± 2.33</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>3.81 ± 0.11</td>
<td>3.94 ± 0.10</td>
<td>3.97 ± 0.12</td>
<td>3.96 ± 0.12</td>
<td>4.00 ± 0.1</td>
<td>3.95 ± 0.14</td>
<td>4.12 ± 0.14</td>
<td>3.98 ± 0.12</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>0.73 ± 0.03</td>
<td>0.79 ± 0.03</td>
<td>0.78 ± 0.03</td>
<td>0.77 ± 0.03</td>
<td>0.80 ± 0.02</td>
<td>0.82 ± 0.02</td>
<td>0.90 ± 0.06</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>LVIDs (mm)</td>
<td>2.38 ± 0.12</td>
<td>2.46 ± 0.15</td>
<td>2.50 ± 0.15</td>
<td>2.52 ± 0.14</td>
<td>2.56 ± 0.09</td>
<td>2.59 ± 0.18</td>
<td>2.63 ± 0.18</td>
<td>2.71 ± 0.16</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>1.16 ± 0.04</td>
<td>1.28 ± 0.07</td>
<td>1.16 ± 0.05</td>
<td>1.14 ± 0.07</td>
<td>1.18 ± 0.03</td>
<td>1.13 ± 0.06</td>
<td>1.24 ± 0.06</td>
<td>1.35 ± 0.05</td>
</tr>
<tr>
<td>EnFS (%)</td>
<td>38.49 ± 1.59</td>
<td>37.85 ± 2.97</td>
<td>34.34 ± 2.46</td>
<td>31.48 ± 2.12</td>
<td>31.94 ± 2.00</td>
<td>33.82 ± 2.70</td>
<td>29.21 ± 2.55</td>
<td>28.81 ± 2.33</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>16.69 ± 0.42</td>
<td>21.12 ± 0.79</td>
<td>22.72 ± 0.51</td>
<td>22.25 ± 0.58</td>
<td>22.58 ± 0.47</td>
<td>23.73 ± 1.27</td>
<td>24.48 ± 1.70</td>
<td>24.55 ± 0.89</td>
</tr>
<tr>
<td>Ejection Time (ms)</td>
<td>46.49 ± 0.95</td>
<td>38.18 ± 0.67</td>
<td>37.04 ± 1.22</td>
<td>37.33 ± 0.62</td>
<td>33.71 ± 0.51</td>
<td>33.74 ± 1.12</td>
<td>35.45 ± 0.61</td>
<td>35.37 ± 0.54</td>
</tr>
</tbody>
</table>

Values are means ± SEM. LVIDd, left ventricular internal dimension diastole; LVPWd, left ventricular posterior wall diastole; LVIDs, left ventricular internal dimension systole; LVPWs, left ventricular posterior wall systole, EnFS, endocardial fractional shortening; IVRT, isovolumic relaxation time. * indicates statistical significance compared to WT mice at baseline. P < 0.05.
TABLE S3. Echocardiographic summary data in *Mybp3*<sup>fl/fl</sup> and *α-MyHC-MerCreMer<sup>+/−</sup></sup> mice treated with tamoxifen.

<table>
<thead>
<tr>
<th>Time (wks)</th>
<th>-1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mybp3</em>&lt;sup&gt;fl/fl&lt;/sup&gt; + tamoxifen (n = 4, 2 male and 2 female)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>511 ± 12</td>
<td>441 ± 27</td>
<td>462 ± 24</td>
<td>501 ± 28</td>
<td>469 ± 18</td>
<td>508 ± 23</td>
<td>459 ± 21</td>
<td>461 ± 15</td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>25.22 ± 1.66</td>
<td>27.24 ± 1.63</td>
<td>27.57 ± 2.82</td>
<td>28.06 ± 1.73</td>
<td>28.19 ± 2.20</td>
<td>29.25 ± 2.32</td>
<td>31.00 ± 2.80</td>
<td>33.05 ± 4.04</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>3.86 ± 0.08</td>
<td>3.86 ± 0.08</td>
<td>4.00 ± 0.04</td>
<td>4.10 ± 0.18</td>
<td>3.90 ± 0.10</td>
<td>3.90 ± 0.09</td>
<td>3.82 ± 0.13</td>
<td>3.98 ± 0.05</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>0.75 ± 0.02</td>
<td>0.77 ± 0.02</td>
<td>0.78 ± 0.04</td>
<td>0.78 ± 0.03</td>
<td>0.81 ± 0.03</td>
<td>0.78 ± 0.03</td>
<td>0.79 ± 0.01</td>
<td>0.84 ± 0.03</td>
</tr>
<tr>
<td>LVIDs (mm)</td>
<td>2.38 ± 0.16</td>
<td>2.37 ± 0.10</td>
<td>2.50 ± 0.06</td>
<td>2.62 ± 0.35</td>
<td>2.60 ± 0.13</td>
<td>2.32 ± 0.17</td>
<td>2.62 ± 0.15</td>
<td>2.53 ± 0.11</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>1.25 ± 0.17</td>
<td>1.23 ± 0.05</td>
<td>1.19 ± 0.08</td>
<td>1.22 ± 0.03</td>
<td>1.21 ± 0.10</td>
<td>1.26 ± 0.07</td>
<td>1.26 ± 0.11</td>
<td>1.30 ± 0.03</td>
</tr>
<tr>
<td>EnFS (%)</td>
<td>40.61 ± 3.10</td>
<td>38.44 ± 0.44</td>
<td>37.55 ± 1.19</td>
<td>38.23 ± 4.61</td>
<td>37.99 ± 3.33</td>
<td>35.94 ± 3.56</td>
<td>30.12 ± 5.43</td>
<td>32.07 ± 3.28</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>15.25 ± 0.48</td>
<td>16.01 ± 0.30</td>
<td>15.11 ± 1.13</td>
<td>15.92 ± 0.98</td>
<td>16.47 ± 0.61</td>
<td>14.75 ± 0.85</td>
<td>15.38 ± 3.08</td>
<td>16.71 ± 0.21</td>
</tr>
<tr>
<td>Ejection Time (ms)</td>
<td>44.00 ± 0.49</td>
<td>49.38 ± 1.07</td>
<td>47.01 ± 1.09</td>
<td>44.50 ± 1.88</td>
<td>45.96 ± 1.81</td>
<td>45.34 ± 1.24</td>
<td>47.46 ± 1.35</td>
<td>46.61 ± 1.02</td>
</tr>
</tbody>
</table>

| α-MyHC-MerCreMer<sup>+/−</sup> + tamoxifen (n = 4, 2 male and 2 female) |
| Heart Rate (bpm) | 493 ± 18 | 481 ± 16 | 495 ± 20 | 492 ± 14 | 489 ± 19 | 466 ± 18 | 504 ± 16 | 491 ± 34 |
| Body Weight (g) | 25.66 ± 2.24 | 25.05 ± 1.08 | 26.07 ± 1.93 | 26.12 ± 2.20 | 26.59 ± 2.59 | 27.25 ± 3.25 | 30.50 ± 4.17 | 32.00 ± 4.85 |
| LVIDd (mm) | 3.38 ± 0.14 | 3.37 ± 0.07 | 3.59 ± 0.06 | 3.77 ± 0.06 | 3.73 ± 0.05 | 3.64 ± 0.05 | 3.63 ± 0.06 | 3.59 ± 0.08 |
| LVPWd (mm) | 0.82 ± 0.05 | 0.84 ± 0.03 | 0.80 ± 0.03 | 0.83 ± 0.04 | 0.80 ± 0.02 | 0.85 ± 0.03 | 0.83 ± 0.05 | 0.87 ± 0.02 |
| LVIDs (mm) | 2.16 ± 0.16 | 2.49 ± 0.03 | 2.34 ± 0.13 | 2.49 ± 0.07 | 2.48 ± 0.07 | 2.43 ± 0.09 | 2.54 ± 0.15 | 2.75 ± 0.14 |
| LVPWs (mm) | 1.24 ± 0.04 | 1.27 ± 0.04 | 1.17 ± 0.01 | 1.20 ± 0.04 | 1.19 ± 0.02 | 1.25 ± 0.03 | 1.26 ± 0.06 | 1.37 ± 0.03 |
| EnFS (%) | 38.55 ± 2.29 | 34.36 ± 1.35 | 35.13 ± 2.39 | 33.92 ± 0.96 | 35.63 ± 1.25 | 33.16 ± 1.88 | 31.47 ± 3.26 | 32.34 ± 2.36 |
| IVRT (ms) | 16.70 ± 0.67 | 16.00 ± 0.49 | 14.69 ± 0.72 | 15.47 ± 0.33 | 16.23 ± 1.17 | 16.08 ± 0.39 | 16.95 ± 0.66 | 17.81 ± 0.65 |
| Ejection Time (ms) | 44.23 ± 1.54 | 47.33 ± 1.27 | 43.69 ± 3.40 | 46.00 ± 1.49 | 46.98 ± 1.55 | 46.58 ± 0.78 | 45.68 ± 1.71 | 46.56 ± 1.59 |

Values are means ± SEM. LVIDd, left ventricular internal dimension diastole; LVPWd, left ventricular posterior wall diastole; LVIDs, left ventricular internal dimension systole; LVPWs, left ventricular posterior wall systole, EnFS, endocardial fractional shortening; IVRT, isovolumic relaxation time. * indicates statistical significance compared to WT mice at baseline. $P < 0.05$. 
**TABLE S4.** Echocardiographic summary data in cMyBP-C-cKO mice subjected to TAC and treated with vehicle or tamoxifen.

<table>
<thead>
<tr>
<th>Time (wks)</th>
<th>cMyBP-C-cKO + vehicle + TAC (n = 6, 3 male and 3 female)</th>
<th>cMyBP-C-cKO + tamoxifen + TAC (n = 12, 6 male and 6 female)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart Rate (bpm)</td>
<td>463 ± 14</td>
</tr>
<tr>
<td></td>
<td>Body Weight (g)</td>
<td>24.80 ± 1.16</td>
</tr>
<tr>
<td></td>
<td>LVIDd (mm)</td>
<td>3.60 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>LVPWd (mm)</td>
<td>0.81 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>LVIDs (mm)</td>
<td>2.31 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>LVPWs (mm)</td>
<td>1.27 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>EnFS (%)</td>
<td>40.95 ± 4.33</td>
</tr>
<tr>
<td></td>
<td>IVRT (ms)</td>
<td>15.53 ± 0.38</td>
</tr>
<tr>
<td>Ejection Time (ms)</td>
<td>47.28 ± 1.85</td>
<td>47.25 ± 3.46</td>
</tr>
</tbody>
</table>

Values are means ± SEM. LVIDd, left ventricular internal dimension diastole; LVPWd, left ventricular posterior wall diastole; LVIDs, left ventricular internal dimension systole; LVPWs, left ventricular posterior wall systole, EnFS, endocardial fractional shortening; IVRT, isovolumic relaxation time; TAC, transaortic constriction. * indicates statistical significance compared to WT mice at baseline. P < 0.05.
TABLE S5. Echocardiographic summary data in *Mybp3*<sup>fl/fl</sup> and *α-MyHC-MerCreMer<sup>+/−</sup>* mice subjected to TAC and treated with tamoxifen.

<table>
<thead>
<tr>
<th>Time (wks)</th>
<th>1</th>
<th>4</th>
<th>8</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>Mybp3</em>&lt;sup&gt;fl/fl&lt;/sup&gt; + tamoxifen + TAC (n = 4, 2 male and 2 female)</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>477 ± 19</td>
<td>469 ± 20</td>
<td>489 ± 29</td>
<td>485 ± 15</td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>25.09 ± 1.41</td>
<td>27.38 ± 1.75</td>
<td>27.50 ± 1.65</td>
<td>29.29 ± 1.08</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>3.67 ± 0.19</td>
<td>3.35 ± 0.32</td>
<td>3.23 ± 0.25</td>
<td>3.10 ± 0.33&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>0.80 ± 0.04</td>
<td>1.30 ± 0.06&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>1.16 ± 0.08&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>1.17 ± 0.04&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVIDs (mm)</td>
<td>2.28 ± 0.14</td>
<td>2.33 ± 0.31</td>
<td>2.54 ± 0.24</td>
<td>2.20 ± 0.18&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>1.17 ± 0.07</td>
<td>1.58 ± 0.11&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>1.49 ± 0.07&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>1.46 ± 0.09&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>EnFS (%)</td>
<td>39.95 ± 4.31</td>
<td>40.01 ± 7.31</td>
<td>35.16 ± 2.64</td>
<td>30.47 ± 4.62</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>15.88 ± 0.62</td>
<td>16.45 ± 0.59</td>
<td>17.05 ± 1.01</td>
<td>16.22 ± 0.79</td>
</tr>
<tr>
<td>Ejection Time (ms)</td>
<td>44.78 ± 1.25</td>
<td>46.05 ± 3.80</td>
<td>45.03 ± 2.15</td>
<td>47.19 ± 3.47</td>
</tr>
</tbody>
</table>

| α-MyHC-MerCreMer<sup>+/−</sup> + tamoxifen + TAC (n = 4, 2 male and 2 female) |         |         |         |         |
| Heart Rate (bpm) | 462 ± 13 | 483 ± 24 | 505 ± 8 | 484 ± 21 |
| Body Weight (g) | 24.08 ± 1.11 | 26.21 ± 0.77 | 28.24 ± 1.18 | 30.34 ± 2.79 |
| LVIDd (mm) | 3.57 ± 0.08 | 3.56 ± 0.23 | 3.84 ± 0.30 | 4.12 ± 0.15 |
| LVPWd (mm) | 0.89 ± 0.08 | 1.05 ± 0.10<sup>∗</sup> | 1.12 ± 0.04<sup>∗</sup> | 1.05 ± 0.08<sup>∗</sup> |
| LVIDs (mm) | 2.49 ± 0.17 | 2.44 ± 0.18 | 2.37 ± 0.21 | 2.48 ± 0.19 |
| LVPWs (mm) | 1.29 ± 0.05 | 1.51 ± 0.07<sup>∗</sup> | 1.50 ± 0.09<sup>∗</sup> | 1.48 ± 0.10<sup>∗</sup> |
| EnFS (%) | 41.79 ± 4.31 | 34.86 ± 4.93 | 31.17 ± 5.91 | 29.21 ± 3.18 |
| IVRT (ms) | 15.03 ± 0.46 | 15.29 ± 0.91 | 17.02 ± 1.10 | 17.24 ± 1.83 |
| Ejection Time (ms) | 45.52 ± 0.98 | 44.69 ± 1.22 | 46.38 ± 1.08 | 45.07 ± 2.56 |

Values are means ± SEM. LVIDd, left ventricular internal dimension diastole; LVPWd, left ventricular posterior wall diastole; LVIDs, left ventricular internal dimension systole; LVPWs, left ventricular posterior wall systole, EnFS, endocardial fractional shortening; IVRT, isovolumic relaxation time; TAC, transaortic constriction. * indicates statistical significance compared to WT mice at baseline. <sup>P < 0.05</sup>.
SUPPLEMENTAL FIGURES

FIGURE S1

A

B

C

D

Isovolumic Relaxation Time (ms)

Ejection Time (ms)

LV Posterior Wall Thickness (mm)

LV Internal Dimension (mm)

0.00
10.00
20.00
30.00
40.00
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

0.00
10.00
20.00
30.00
40.00
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

0.00
16.00
32.00
48.00
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

0.00
4.00
8.00
12.00
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Vehicle/Tamoxifen
Vehicle/Tamoxifen
Vehicle/Tamoxifen
Vehicle/Tamoxifen

MyBP-C KO
MyBP3fl/fl + Tamoxifen
α-MyHC-MerCreMer +/- + Tamoxifen

WT cMyBP-C/-
Mybp3fl/fl + Tamoxifen
α-MyHC-MerCreMer +/- + Tamoxifen

Time (wks)
Time (wks)
Time (wks)
Time (wks)
FIGURE S2

[Graph showing Endocardial Fractional Shortening (%)]

- WT
- cMyBP-C<sup>−/−</sup>
- Myh3<sup>flo/flo</sup> + Tamoxifen
- α-MyHC-MerCreMer<sup>+/−</sup> + Tamoxifen

Time (wks): 0, 1, 2, 3, 5, 7, 9, 11, 13, 15, 17, 19, 20

Endocardial Fractional Shortening (%): 0.00, 20.00, 40.00, 60.00

Vehicle / Tamoxifen
FIGURE S3

(A) LV Posterior Wall Thickness (mm) vs. Time (wks)

(B) LV Internal Dimension (mm) vs. Time (wks)

(C) Endocardial Fractional Shortening (%) vs. Time (wks)

[Graphs and data indicating changes over time with comparison of different treatments]
FIGURE S4

cMyBP-C-cKO  
+ Vehicle + TAC

Mybp3^{fl/fl}  
+ Tamoxifen + TAC

α-MyHC-MerCreMer^{+/−}  
+ Tamoxifen + TAC

cMyBP-C-cKO  
+ Tamoxifen + TAC

Mybp3^{fl/fl}  
+ Tamoxifen + TAC

α-MyHC-MerCreMer^{+/−}  
+ Tamoxifen + TAC

cMyBP-C-cKO  
+ Tamoxifen + TAC

500 μm  
500 μm  
500 μm  
500 μm
SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Analysis of LV structure and function. Time course analysis of ejection time (A), isovolumic relaxation time (B), LV posterior wall thickness in diastole (C), and LV internal dimension in diastole (D) collected in the same experimental animals in WT (n = 5), cMyBP-C\textsuperscript{-/-} (n = 5), and tamoxifen-treated Mybp\textsuperscript{3\textit{fl/fl}} (n = 4) and \textit{α-MyHC-MerCreMer}\textsuperscript{+/+} mice (n = 4). Ejection time and isovolumic relaxation time were measured from pulsed-wave Doppler tracings of aortic outflow and mitral inflow collected in the suprasternal and apical 4-chamber views on 2-dimensional transthoracic echocardiography. Intraperitoneal injections of tamoxifen were administered between t = -1 and 0 weeks in Mybp\textsuperscript{3\textit{fl/fl}} and \textit{α-MyHC-MerCreMer}\textsuperscript{+/+} mice (designated by the horizontal bar). Values are means ± SEM.

Figure S2. Analysis of systolic contractile function. Endocardial fractional shortening (EnFS) was assessed longitudinally in the same experimental animals in WT (n = 5), cMyBP-C\textsuperscript{-/-} (n = 5), and tamoxifen-treated Mybp\textsuperscript{3\textit{fl/fl}} (n = 4) and \textit{α-MyHC-MerCreMer}\textsuperscript{+/+} (n = 4) mice. EnFS was calculated using the following equation: EnFS = (LVIDd - LVIDs)/LVIDd x 100, where LVIDd and LVIDs are left ventricle internal dimension in diastole and systole, respectively. Echocardiographic measurements of LVIDd and LVIDs were collected at the level of the papillary muscle in M-mode 2D-transthoracic echocardiography. Intraperitoneal injections of tamoxifen were administered between t = -1 and 0 weeks in Mybp\textsuperscript{3\textit{fl/fl}} and \textit{α-MyHC-MerCreMer}\textsuperscript{+/+} mice (designated by the horizontal bar). Values are means ± SEM. * indicates statistical significance compared to WT mice at baseline. \( P < 0.05 \).
**Figure S3.** Effect of transaortic constriction (TAC) on cardiac structure and function. Time course analysis of LV posterior wall thickness in diastole (A), LV internal dimension in diastole (B), and LV EnFS (C) collected from the same experimental animal in WT (n = 5), cMyBP-C−/− (n = 5), and Mybp3fl/fl (n = 4) and α-MyHC-MerCreMer+/− (n = 4) mice subjected to TAC in addition to tamoxifen treatment. EnFS was calculated using the following equation: EnFS = (LVIDd - LVIDs)/LVIDd x 100, where LVIDd and LVIDs are LV internal dimension in diastole and systole, respectively. Echocardiographic measurements of LVIDd and LVIDs were collected at the level of the papillary muscle in M-mode 2D-transthoracic echocardiography. Intraperitoneal injections of tamoxifen were administered between t = -1 and 0 weeks in Mybp3fl/fl and α-MyHC-MerCreMer+/− mice (designated by the horizontal bar). TAC was performed after tamoxifen treatment and sustained for 12 weeks (designated by dashed horizontal bar). Values are means ± SEM. * indicates statistical significance compared to WT mice at baseline. \( P < 0.05. \)

**Figure S4.** Cardiac histology in Mybp3fl/fl, α-MyHC-MerCreMer+/−, and cMyBP-C-cKO mice subjected to transaortic constriction (TAC) and treated with either vehicle or tamoxifen. Top row shows high magnification (400X) photomicrographs of H&E-stained cardiac sections. Bottom row shows high magnification (400X) photomicrographs of Masson’s Trichrome-stained cardiac sections, demonstrating fibrosis in tamoxifen-treated cMyBP-C-cKO mice subjected to TAC.
SUPPLEMENTAL REFERENCES


6. Chen PP, Patel JR, Rybakova IN, Walker JW, Moss RL. Protein kinase A-induced myofilament desensitization to Ca$^{2+}$ as a result of phosphorylation of cardiac myosin-binding protein C. *J Gen Physiol.* 2010;136:615-627