Published in final edited form as:

Cardiovasc Pathol. 2016 ; 25(3): 258–269. doi:10.1016/j.carpath.2016.02.004.

BRG1 and BRM SWI/SNF ATPases Redundantly Maintain Cardiomyocyte Homeostasis by Regulating Cardiomyocyte Mitophagy and Mitochondrial Dynamics *In Vivo*

Scott J. Bultman, PhD^{1,2}, Darcy Holley, BS¹, Gustaaf de Ridder, MD, PhD³, Salvatore Pizzo, MD, PhD³, Tatiana N. Sidorova, PhD⁴, Katherine T. Murray, MD⁴, Brian C. Jensen, MD⁵, Zhongjing Wang, PhD⁵, Ariana Bevilacqua, BS¹⁰, Xin Chen, BS⁶, Megan T. Quintana, MD⁷, Manasi Tannu, BS⁸, Gary B. Rosson, PhD¹, Kumar Pandya, PhD⁹, and Monte S. Willis, MD, PhD^{5,10,11,*}

¹Department of Genetics, University of North Carolina, Chapel Hill, NC 27599, USA

²Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599, USA

³Department of Pathology, Duke University, Durham, NC 27710, USA

⁴Departments of Medicine and Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

⁵McAllister Heart Institute, University of North Carolina, Chapel Hill, NC USA

⁶Department of Neurosurgery, Shandong Provincial Hospital affiliated to Shandong University, 250021, Jinan, PR China

⁷Department of Surgery, University of North Carolina, Chapel Hill, NC 27599, USA

⁸School of Medicine, University of North Carolina, Chapel Hill, NC 27599, USA

⁹Transviragen, Chapel Hill, NC 27599, USA

¹⁰Department of Pathology & Laboratory Medicine, University of North Carolina, Chapel Hill, NC 27599, USA

¹¹Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599, USA

Abstract

*Corresponding author: Monte S. Willis, MD, PhD, Department of Pathology and Laboratory Medicine, Medical Biomolecular Research Building, Room 2336, Campus Box #7527, Chapel Hill, NC 27599, USA, monte_willis@med.unc.edu.

Disclosures

The authors declare no conflicts of interest.

Compliance with Ethical Standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

There has been an increasing recognition that mitochondrial perturbations play a central role in human heart failure. Discovery of mitochondrial networks, whose function is to maintain the regulation of mitochondrial biogenesis, autophagy ('mitophagy') and mitochondrial fusion/fission, are new potential therapeutic targets. Yet our understanding of how the molecular underpinning of these processes is just emerging. We recently identified a role of the SWI/SNF ATP-dependent chromatin remodeling complexes in the metabolic homeostasis of the adult cardiomyocyte using cardiomyocyte-specific and inducible deletion of the SWI/SNF ATPases BRG1 and BRM in adult mice (Brg1/Brm double mutant mice). To build upon these observations in early altered metabolism, the present study looks at the subsequent alterations in mitochondrial quality control mechanisms in the impaired adult cardiomyocyte. We identified that *Brg1/Brm* double-mutant mice exhibited an increased mitochondrial biogenesis, increases in 'mitophagy', and alterations in mitochondrial fission and fusion that led to small, fragmented mitochondria. Mechanistically, increases in the autophagy and mitophagy-regulated proteins Beclin1 and Bnip3 were identified, paralleling changes seen in human heart failure. Cardiac mitochondrial dynamics were perturbed including decreased mitochondria size, reduced number, and altered expression of genes regulating fusion (Mfn1, Opa1) and fission (Drp1). We also identified cardiac protein amyloid accumulation (aggregated fibrils) during disease progression along with an increase in pre-amyloid oligomers and an upregulated unfolded protein response including increased GRP78, CHOP, and IRE-1 signaling. Together, these findings described a role for BRG1 and BRM in mitochondrial quality control, by regulating mitochondrial number, mitophagy, and mitochondrial dynamics not previously recognized in the adult cardiomyocyte. As epigenetic mechanisms are critical to the pathogenesis of heart failure, these novel pathways identified indicate that SWI/SNF chromatin remodeling functions are closely linked to mitochondrial quality control mechanisms.

Keywords

BRG1; BRM; SWI/SNF complex; cardiomyocyte; autophagy; mitochondrial dynamics; mitophagy; unfolded protein response; GRP78; IRE-1; CHOP

Introduction

As heart failure treatment has evolved, increasing recognition mitochondria's role in the pathogenesis of disease has emerged¹. New techniques allow the discovery of mitochondrial networks, whose function is maintained by three processes, including 1) mitochondrial biogenesis (increase in mitochondrial number); 2) mitophagy; and 3) continuous mitochondrial fission (division) and fusion^{2, 3}. Impaired mitochondrial biogenesis is a feature of myocardial hypertrophy and end-stage ischemic heart failure in humans⁴, while autophagy has been central to defects in human heart failure^{5, 6}. Evidence of altered mitochondrial dynamics (fission and fusion) in human heart failure, specifically in idiopathic dilated cardiomyopathy, has recently been reported⁷.

Changes in the mitochondrial network influence the production of ROS and ATP production, most notably offset by activating mitochondrial biogenesis and mitophagy in exercise and other therapeutic modalities. Complementing mitochondrial biogenesis is the ongoing process of "mitophagy" (mitochondrial autophagy), where damaged/dysfunctional

mitochondria are targeted for destruction via lysosomal degradation. Here mitochondrial proteins such as BNIP3L and BNIP3 are abundant in the heart and act as targeting molecules that attract autophagosome to mitochondria and are critical to maintaining cardiac function³. Lastly, regulation of mitochondrial dynamics (fission and fusion) involve the redistribution of mitochondrial content⁸. With the enormous continuous ATP demand of the cardiomyocyte, the importance of maintaining mitochondrial quality control is of particular significance in cardiac disease, including heart failure, ischemic heart disease/myocardial infarction, and diabetes^{9–11}.

Epigenetic regulation of heart failure occurs by several key mechanisms, including ATP-dependent chromatin remodeling¹². One ATP-dependent chromatin remodeling complex in cardiomyocytes are the SWI/SNF (SWItch/Sucrose Non-Fermentable) complexes, comprised of 9–12 subunits, including one of two ATPases critical to their function 1) BRG1 (Brahma-related gene 1) or 2) BRM (Brahma)^{13, 14}. Recent studies demonstrate a role of BRG1 and BRM in maintaining homeostasis in the adult cardiomyocyte. These studies identified that cardiomyocyte-specific and inducible deletion of BRG1 and BRM in adult mice (*Brg1*/*Brm* double mutant mice), alterations in glucose-6-phosphate, fructose-6-phosphate, and myoinositol at day 10 post-induction occurred before severe cardiomyopathy is seen¹⁵. The identification of these early metabolic changes led to the current study to identify the underlying mechanisms by which BRG1 and BRM deletion affect mitochondrial quality control mechanisms in the impaired adult cardiomyocyte.

In the present study, we build upon our findings that *Brg1*/*Brm* double mutant hearts have early metabolic changes at day ten (10) by investigating the changes occurring 15 days after inducing *Brg1* deletion, just before the development of a severe cardiomyopathy. We identified alterations in mitochondrial biogenesis, mitochondrial autophagy (mitophagy), and mitochondrial fission and fusion just before the death that occurred in all mice by day 22. We demonstrate a previously undescribed link between the chromatin remodeling SWI/SNF components BRG1 and BRM with mitochondrial homeostasis. On a more practical note, the present study should give those develop BRG1 and BRM inhibitors for cancer pause¹⁶.

Methods

Animal creation and experimental design

All mouse experiments were approved by the Institutional Animal Care and Use Committees (IACUC) review board at the University of North Carolina at Chapel Hill. The α MHC-Cre-ERT mice were obtained from The Jackson Laboratory (#005657, Bar Harbor, ME); the *Brg1* conditional mutant mouse line and *Brm* constitutive mutant mouse line have been described previously^{17–19}. Genotyping of the α MHC-MerCreMer, *Brg1* floxed, and floxed alleles and the *Brm* mutation were genotyped by PCR as previously described^{17–20}.

Histology, transmission electron microscopy (TEM), and image analysis

Immunohistochemistry (IHC) was performed on fixed histological section using an anti-BRG1 rabbit polyclonal antibody (Millipore #07-478, Temecula, CA), anti-GRP78 mouse

monoclonal antibody²¹, anti-CHOP (GADD153), or phospho-eIF2, as previously described^{22, 23}. Hearts analyzed by TEM were viewed on a LEO EM910 transmission electron microscope operating at 80 kV (LEO Electron Microscopy, Thornwood, NY). An average of 2,500 mitochondria were analyzed from 30 fields from multiple levels from three hearts per mouse cohort.

Analysis of autophagic flux *in vivo*

Autophagic flux was determined *in vivo* from the titration of bafilomycin A1 as previously described²⁴. Antibodies for LC3B isoforms (Sigma-Aldrich #L7543) and beclin were used for western immunoblot analysis of markers of autophagy (Cell Signaling #3495P, Beverly, MA).

Quantitative analysis of cardiac amyloid (protein aggregation) and pre-amyloid oligomers (PAOs)

Protein aggregation was assessed using an Enzo Proteostat Protein Aggregation Assay (#ENZ-51023, Farmingdale, NY) in conjunction with their pre-formulated aggregation standards (#ENZ-51039) and pre-amyloid oligomer staining was performed as described in detail²⁵.

RNA isolation and RT-qPCR analysis

Total RNA was isolated from cardiac tissue homogenized with a TissueLyser LT (Qiagen N.V. #69980, Venlo, The Netherlands) using

Trizol (Life Technologies #15596-026, Carlsbad, CA) according to the manufacturer's instructions. TaqMan gene expression assays (Life Technologies) were performed using universal TaqMan master mix (Life Technologies #4304437) commercial probes, as described previously²⁴.

Analysis of mitochondrial DNA by qPCR

Mitochondrial number was quantified by qPCR on DNA prepared from whole-heart homogenates using the DNAeasy Blood and Tissue Kit (Qiagen #69506). SYBR Green and primers for the mitochondrial DNA included mtNd1, mtCO1, and mtCytb1; H19 was run in parallel as a nuclear DNA control as previously detailed²⁶.

Statistics

SigmaPlot (Systat Software, Inc., San Jose, CA) was used to determine significant statistical difference by One-way ANOVA followed by post-hoc analysis using the Holm-Sidak method or a Student's t-test, as indicated. A p value < 0.05 was considered significant.

Additional materials and methods detail may be found in the online supplement.

Results

A Mouse Model Reveals SWI/SNF Complexes are Essential in Adult Cardiomyocytes

To investigate the combined role of BRG1 and BRM in adult cardiomyocytes, we crossed mice carrying an inducible, cardiomyocyte-specific *α MHC-Cre-ERT* transgene to *Brg1^{fl/fl}*, *Brm^{-/-}* mice. Adult *Brg1^{fl/fl}*, *α MHC-Cre-ERT^{+/0}*, *Brm^{-/-}* mice were then fed tamoxifen-fortified chow for 7 days to induce the *Brg1* floxed-to- floxed excision event, which was confirmed by both PCR and IHC (Supplemental Fig. 1A, B). These mice (herein referred to as *Brg1/Brm* double mutants) were monitored by echocardiography until they died, which occurred within 22 days of initiating the tamoxifen diet (Supplemental Fig. 1C). A progressive heart failure occurred prior to death in *Brg1/Brm* double-mutant but not in control mice (Table 1). Six groups of control mice were analyzed in parallel (listed in Supplemental Fig. 1C) to ensure that we were observing a *Brg1/Brm* double-mutant phenotype rather than an artifact, such as tamoxifen itself or tamoxifen-induced Cre dysfunction, which has been previously described²⁷.

Brg1/Brm Double-Mutant Cardiomyocytes Undergo Mitophagy and Altered Mitochondrial Dynamics

Considering the severity of the cardiac phenotype and the rapid lethality of mice lacking *Brg1* and *Brm* in their cardiomyocytes, the histopathology was surprisingly mild. Analysis of H&E-and Mason's Trichrome-stained sections revealed that the *Brg1/Brm* double-mutant phenotype ranged from relatively normal to moderate vacuolization (Fig. 1A) with no evidence of lipid accumulation by oil red O staining (Supplemental Fig. 2) and with fibrosis increased only in rare individuals (Fig. 1B, Supplemental Fig. 3). Next, we assessed fetal cardiomyocyte gene expression because it is a typical pathological hypertrophic response that occurs during heart failure. Skeletal muscle actin was significantly decreased at both time points in the *Brg1/Brm* double-mutant hearts compared with controls (Fig. 1C). *β MHC* fetal gene expression was significantly elevated at day 10 post-tamoxifen induction (early time point) but not at 1-day pre-mortem (late time point) (Fig. 1C and 1D). In contrast, *Bnp* and *Anf* mRNA were not changed significantly at either time point. These data are not representative of a typical pathologic hypertrophy/heart failure response.

Ultrastructural analysis of the *Brg1/Brm* double-mutant heart by TEM was distinct by the presence of alterations limited to the interfibrillar areas (mitochondrial compartment). In all of the *Brg1/Brm* double-mutant hearts, we identified mitochondria degeneration (Fig. 2A and 2B) and an increase in double-membrane bound vacuoles with mitochondrial remnants within (Fig. 2B) that were absent in all of the parallel control hearts (Fig. 2C).

Ultrastructurally, these vacuoles appeared similar to those found to be increased in the process of autophagy, specifically autophagosomes. Autophagy is a lysosomal degradation pathway for cytoplasmic material and organelles, starting at the endoplasmic reticulum-mitochondria interface²⁸. The conserved autophagic process shuttles cytoplasmic components to fuse with lysosomes. This stepwise engulfment of cytoplasmic material by the phagophore, maturing into a double-membrane-bound vesicle, forms the autophagosome. To confirm that *Brg1/Brm* double-mutant hearts had quantitative alterations in autophagy, we next analyzed autophagic flux in vivo.

The role of autophagy in cardiovascular biology has proven to be context dependent, widely characterized in cardiomyocytes, and critical to the maintenance of cardiovascular homeostasis and function²⁹. Depending on context, there is a window of optimal autophagic activity, whereby increases or decreases may be protective depending upon the cardiac stress disease state^{30, 31}. *Brg1/Brm* double-mutant hearts exhibited a significant increase in autophagic flux, as illustrated by the LC3II:LC3I ratio by western immunoblot after bafilomycin A1 treatment, compared with control mice run in parallel (Fig. 3A). In parallel with this evidence of increased autophagic flux, we identified increased Beclin 1 by western immunoblot analysis (Fig. 3B). With evidence of increased autophagy, we next investigated mechanisms responsible for upregulating autophagy.

Central to the regulation of autophagy in heart disease, the Beclin 1 protein functions as a scaffolding protein assembling Beclin 1 interactome to regulate Class II PI3K/VPS34 activity, which tightly controls autophagy at multiple stages³². The *Brg1/Brm* double-mutant mice had significantly increased Beclin 1 protein expression compared with control group mice (Fig. 3B). Autophagy is also regulated at the transcriptional level, with increased *Atg5*, *Atg7*, *Atg12*, *Bnip3*, and *Vps34* mRNA to enhance autophagy³³. RT-qPCR analysis of these genes demonstrated the *Brg1/Brm* double-mutant hearts had a significant increase in *Bnip3* mRNA levels, while *Atg12* and *Vps34* were significantly decreased compared with control hearts at day 15 (Fig. 3C). At the earlier day 9 time point, *Brg1/Brm* double-mutant hearts had increased *Vps34* mRNA and decreased *Bnip3* mRNA (Supplemental Fig. 4), illustrating the dynamic expression of autophagy regulators. The transcriptional and epigenetic network regulating autophagy is emerging (as recently reviewed³³) that underscore the diverse ways in which autophagy can be regulated depending on the stimulus. To test whether BRG1 and BRM occupy the *Bnip3* promoter, we performed quantitative ChIP assays. Significant enrichment of BRG1 and BRM was detected in cardiac tissue (Fig. 3D), which strongly suggests that BRG1- and BRM-catalyzed SWI/SNF complexes directly regulate *Bnip3*. BNIP3, primarily located in the mitochondria as an integrated protein³⁴, perturbs outer membrane integrity³⁵, induces permeabilization of the inner mitochondrial membrane³⁶, and can stimulate mitophagy directly by triggering depolarization and Parkin recruitment³⁷. Taken together, these findings illustrate a novel role for BRG1 and BRM in the maintenance of cardiomyocyte autophagic flux, specifically related to the mitophagy as illustrated by the TEM analysis and the direct regulation of *Bnip3*.

BNIP3 alters cellular mitochondrial dynamics, a continuous process by which mitochondria fuse together (fusion) and subsequently divide (fission) to maintain their functional balance³⁸. Specifically, increasing BNIP3 expression can induce mitochondrial fragmentation (fission) in cells^{34, 39}. Understanding this link led us to evaluate further the *Brg1/Brm* double-mutant heart mitochondrial phenotype in our TEM studies. Here TEM revealed an apparent increase in *Brg1/Brm* double-mutant heart mitochondrial fragmentation compared with controls (Fig. 4A). Analysis of mitochondria over multiple LV regions revealed that the number of small mitochondria identified in the *Brg1/Brm* double-mutant hearts was skewed with a marked increase in small mitochondria with significantly smaller areas (Fig. 4B and 4C). To put in context the apparent increase in small mitochondria, we determined mitochondrial number by qPCR of three mitochondrial encoded genes normalized to the nuclear-encoded H19 and found a significant decrease in the number of

mitochondria (Fig. 5A). The fusion and fission of mitochondria is a dynamic process that cells use for responding to mitophagy and maintaining protein quality. The regulation of mitochondrial dynamics by fusion and fission is regulated by four dynamin-related GTPases, including DRP1 and FIS1 (fission)^{40, 41} and mitofusins (e.g. MFN1) in the outer membrane and OPA1 in the inner membrane (fusion)^{42, 43}. RT-qPCR analysis of these GTPases in the *Brg1/Brm* double-mutant hearts demonstrated significant decreases in *Mfn1*, *Opa1*, and *Drp1* in late disease (Fig. 5B and 5C) but not at an earlier stage (Supplemental Fig. 5). These findings demonstrate the interesting juxtaposition of increased mitophagy and mitochondrial fission, which has been shown to allow the selective elimination of damaged mitochondria by autophagy^{44, 45}

Activation of the UPR in *Brg1/Brm* Double-Mutant Cardiomyocytes

Histological analysis of the *Brg1/Brm* double-mutant hearts stained with H&E revealed stereotypical pink accumulation inside of the cardiomyocytes, resembling the unfolded proteins that accumulate in cardiac amyloidosis (Supplemental Fig. 3E). Since the accumulation of unfolded proteins in mitochondria has been shown to induce mitophagy in neurons⁴⁶, we next investigated the presence of unfolded proteins using a colorimetric assay that has been used to identify amyloid and other unfolded proteins in experimental disease processes^{47–51}. At an early time point, *Brg1/Brm* double-mutant hearts did not have an increase in unfolded proteins (Fig. 6A, left). However, at later time points when mitophagy and altered fission and fusion were present (Figs. 2–5), a significant increase in unfolded proteins was present with a ~3-fold increase compared with control hearts (Fig. 6A, right). The pathogenic effects of the accumulation of amyloid fibrils, independent of soluble precursors, have been described in the heart⁵². Recent studies have identified a critical role of pre-amyloid oligomers (PAOs) that form before amyloid/insoluble fibrils in mediating heart failure directly, paralleling the pathogenesis of common neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Huntington's disease⁵³.

Using the A11 antibody, we investigated the presence of PAOs in early and late *Brg1/Brm* double-mutant heart samples (Fig. 6B). The detection of low levels of PAO by the A11 antibody (green) present in cardiomyocytes (red) detected by an antibody against the heavy chain myosin II using immunofluorescence indicates that soluble PAOs were increasing at day 15 in *Brg1/Brm* double-mutant hearts (Fig. 6B). While not reaching significant levels, these findings suggest that the protein aggregates detected in the heart at day 15 (Fig. 6A) go through a PAO state very quickly to accumulate amyloid in significant amounts when *Brg1* and *Brm* have been ablated in adult cardiomyocyte *in vivo*.

In response to stress, such as that induced by oxidized proteins, the heart induces the unfolded protein response (UPR), which includes the endoplasmic reticulum (ER) chaperone protein GRP78⁵⁴. GRP78 is found in the endoplasmic/sarcoplasmic reticulum bound to PERK, IRE1, and ATF6 receptors to prevent their activation of the UPR⁵⁵. In the presence of unfolded proteins, GRP78 preferentially shifts to these proteins to stabilize their conformation, leaving PERK, IRE1, and ATF6 to signal survival and apoptotic signaling downstream⁵⁵. Having established the presence of increased amyloid in these hearts, we next investigated cardiac expression of GRP78 by IHC and identified that *Brg1/Brm* double-

mutant mice had markedly increased GRP78 expression compared with controls (Fig. 7A) at the late time point where PAOs and amyloid were increased.

The link between the UPR and cardiovascular disease is strengthening⁵⁶, but UPRs are diverse and include signaling by IRE-1, PERK, and ATF6^{57, 58}. Identification of the spliced *Xbp-1* by RT-qPCR is one way in which IRE-1 activity is measured⁵⁸. We identified that *Brg1/Brm* double-mutant hearts had a significant increase in the spliced *Xbp-1* mRNA compared with control hearts (Fig. 7B). Downstream of the IRE1-XBP-1 pathway, XBP1 binds the promoter of the C/EBP α (*Cebpa*) gene to increase its expression⁵⁹. *Brg1/Brm* double-mutant mice had significantly more *Cebpa* mRNA expression compared with controls (Fig. 7B). The significant increase in *Brg1/Brm* double-mutant heart *Xbp-1* splice variant mRNA and *Cebpa* mRNA demonstrate the increased activity *via* IRE-1 signaling. The relationship between XBP1 and C/EBP β (*Cebpb*) is a bit more complex, with evidence that C/EBP β induces expression of XBP1 protein⁵⁹. *Brg1/Brm* double-mutant hearts had diminished *Cebpb* mRNA levels that were not significant (Fig. 7B).

The stress caused in the ER by the presence of unfolded proteins can also cause signaling through PERK, whereby PERK proteins dimerize and undergo autophosphorylation⁶⁰. This autophosphorylation leads to downstream phosphorylation of eIF2, which we investigated by IHC (Fig. 7A, column 2). Here we found evidence that the *Brg1/Brm* double-mutant mice had a higher cardiac eIF2 expression histologically, and next looked at downstream ATF4 and CHOP expression by RT qPCR⁵⁵. Significant increases in both *Chop* and *Atf3* mRNA were identified by RT qPCR in the *Brg1/Brm* double-mutant mice (Fig. 7B), with increases in CHOP protein also seen by IHC in the *Brg1/Brm* double-mutant mice (Fig. 7A, column 3). Activation of ATF4 increases target gene expression, including the transcription factor CHOP (CCAAT/-enhancer-binding protein homologous protein)⁶¹, which itself induces the expression of *Atf3* mRNA⁶². While the lack of reliable reagents prevented us from directly measuring ATF6 signaling⁶¹, we determined the mRNA levels of other reported genes involved in the UPR. We found that *Brg1/Brm* double-mutant hearts had either significant down-regulation of *Ire-1a*, *Atf6a*, and *Grp78* reflecting compensatory responses to the clear GRP78 and IRE-1 signaling present, or no change in genes not as clearly related to the UPR in cardiomyocytes⁶¹ (Supplemental Fig. 6).

Discussion

This study identifies a novel role of the SWI/SNF components BRG1 and BRM in the homeostasis of the adult cardiomyocyte regulating mitochondrial biogenesis, mitophagy, and mitochondrial fission and fusion. While there have been suggestions that SWI/SNF complexes may be related to mitochondrial biogenesis⁶³ and autophagy⁶⁴ in mammalian cells, the present study is the first to link them additionally to mitochondrial fission and fusion. While these findings parallel changes seen in heart failure phenotypes, BRG1 and BRM have previously been shown to have a role in the development of pathological cardiac hypertrophy. BRG1 interactions with the DNA-binding protein PARP1 have been implicated in the prototypical shift in cardiac MHC isoform expression in pathological cardiac hypertrophy⁶⁵, and may explain the decreases (instead of increases) of β MHC seen with the heart failure in the present study (Fig. 1B). In the present study, we describe the redundant

role of BRG1 and BRM to maintain the adult cardiomyocyte *in vivo*. Notably, multiple distinct alterations have been identified in this model, with early metabolomics changes previously identified¹⁵, accompanying the upregulation of autophagy/mitophagy, altered mitochondrial dynamics, accumulation of PAOs/aggregated protein, and activation of the UPR that culminated in severe bradycardia and death (reported here).

The increased autophagic flux present in *Brg1/Brm* double-mutant hearts was characterized morphologically by increases in mitochondria in what look like autophagosomes (Fig. 2) and by increased Beclin 1 protein and *Bnip3* mRNA (Fig. 3). While the process of autophagy has largely been described in terms of transcriptional regulation, the epigenetic control of autophagic flux is gaining ground³³. Multiple transcription factors and epigenetic regulators have been shown to be involved³³. We demonstrate that BRG1 and BRM occupy the *Bnip3* promoter and regulate its expression as well as autophagic flux. The transcriptional activation of *Bnip3*, as seen in the *Brg1/Brm* double-mutant hearts, has been reported to be by C/EBP β , E2F1, FOXO1/3, HIF1, and STAT3³³. The enhanced Beclin 1, also seen in the *Brg1/Brm* double-mutant hearts, has been reported to be regulated by FOXO1/3, NF- κ B, p63, and Stat³³. BNIP3 is a receptor, like PINK1 and Parkin, regulates mitochondrial autophagy (also known as mitophagy)^{66, 67}, consistent with the morphological findings in *Brg1/Brm* double-mutant hearts. Increases in BNIP3 expression occur after myocardial infarction and in response to chronic pressure overload hypertrophy and may be a mechanism by which mitochondrial pruning is mediated to get rid of damaged mitochondria during increased stress⁶⁸. BRG1 and BRM may be linked to the regulation of HIF1, as the SWI/SNF complex regulates HIF-1-dependent mitophagy (regulated through BNIP3 and Beclin1) as a metabolic adaptation to stress in cancer cells^{69, 70}.

The regulation of mitochondrial autophagy and mitochondrial dynamics is logically linked to mitochondrial quality control. Mitochondria are damaged by many environmental stressors, and repair is dependent upon the mitochondrial fission and fusion (broadly known as mitochondrial dynamics) and autophagy⁷¹. In fact, mitochondrial fusion and fission are processes essential for the preservation of normal mitochondrial function. In heart failure, OPA1 is important for maintaining normal cristae structure and function, while preserving the inner membrane structure and for protection against apoptosis. Confocal and TEM analysis have demonstrated that failing hearts have small, fragmented mitochondria, consistent with decreased fusion. Reducing OPA1 or MFN1 in cardiomyocytes results in increased mitochondrial fragmentation^{72, 73}. The transcriptional regulation of genes involved with mitochondrial fusion in cardiomyocytes (i.e. *OPA1* and *MFN1*) as well as fission (e.g. *DRP1*) has been recently shown to be under the control of PGC-1 α by coactivating the estrogen-related receptor α ⁷⁴. While the SWI/SNF complex component BAF60a supports PGC-1 α co-activation⁶³, the regulation of mitochondrial fission by *Brg1/Brm* demonstrated here has not been described previously.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Dr. Marco Sandri for his ideas and input in interpreting the relationship between autophagy and mitochondrial dynamics and Dr. Joe Hill for his guidance in measuring autophagic flux. We thank Janice Weaver in the UNC Animal Histopathology Laboratory for assistance in preparing histological specimens and Vicky Madden at the UNC Microscopy Services Laboratory for assistance with transmission electron microscopy.

Sources of Funding

This work was supported by funding from the National Institutes of Health (RO1HL104129 to M.W. and CA125237 to S.B.), a Jefferson-Pilot Corporation (fellowship to M.W.), and the Leducq Foundation (to M.W.).

Non-standard abbreviations

AL	light chain
ANF	atrial natriuretic factor
βMHC	beta myosin heavy chain
BNP	brain natriuretic peptide
BRM	Brahma (aka SMARCA2)
BRG1	Brahma-related gene 1 (aka SMARCA4)
CHOP	CCAAT/-enhancer-binding protein homologous protein
ER	endoplasmic reticulum
HDAC	histone deacetylase
HF	heart failure
PAO	pre-amyloid oligomer
SWI/SNF	SWItch/Sucrose Non-Fermentable
TAC	Trans-aortic constriction
TEM	transmission electron microscopy
UPR	unfolded protein response
VEC	vascular endothelial cells

References

1. Knowlton AA, Chen L, Malik ZA. Heart failure and mitochondrial dysfunction: the role of mitochondrial fission/fusion abnormalities and new therapeutic strategies. *J Cardiovasc Pharmacol.* 2014; 63:196–206. [PubMed: 23884159]
2. Romanello V, Sandri M. Mitochondrial Quality Control and Muscle Mass Maintenance. *Front Physiol.* 2015; 6:422. [PubMed: 26793123]
3. Dorn GW 2nd. Parkin-dependent mitophagy in the heart. *J Mol Cell Cardiol.* 2015
4. Pisano A, Cerbelli B, Perli E, Pelullo M, Bargelli V, Preziuso C, Mancini M, He L, Bates MG, Lucena JR, Della Monica PL, Familiari G, Petrozza V, Nediani C, Taylor RW, d'Amati G, Giordano C. Impaired mitochondrial biogenesis is a common feature to myocardial hypertrophy and end-stage ischemic heart failure. *Cardiovascular pathology : the official journal of the Society for Cardiovascular Pathology.* 2015; 25:103–12. [PubMed: 26764143]

5. Fuller MY, Wolf DA, Buja LM. Sudden death in a 15-year-old with diffuse cardiac rhabdomyomatosis: an autopsy case report. *Cardiovascular pathology : the official journal of the Society for Cardiovascular Pathology*. 2014; 23:351–3. [PubMed: 25130767]
6. Buja LM, Weerasinghe P. Unresolved issues in myocardial reperfusion injury. *Cardiovascular pathology : the official journal of the Society for Cardiovascular Pathology*. 2010; 19:29–35. [PubMed: 19026571]
7. Ikeda Y, Inomata T, Fujita T, Iida Y, Nabeta T, Naruke T, Koitabashi T, Takeuchi I, Kitamura T, Miyaji K, Ako J. Morphological changes in mitochondria during mechanical unloading observed on electron microscopy: a case report of a bridge to complete recovery in a patient with idiopathic dilated cardiomyopathy. *Cardiovascular pathology : the official journal of the Society for Cardiovascular Pathology*. 2015; 24:128–31. [PubMed: 25453728]
8. Youle RJ, van der Blik AM. Mitochondrial fission, fusion, and stress. *Science*. 2012; 337:1062–5. [PubMed: 22936770]
9. Liang Q, Kobayashi S. Mitochondrial quality control in the diabetic heart. *J Mol Cell Cardiol*. 2015
10. Bartz RR, Suliman HB, Piantadosi CA. Redox mechanisms of cardiomyocyte mitochondrial protection. *Front Physiol*. 2015; 6:291. [PubMed: 26578967]
11. Dorn GW 2nd, Vega RB, Kelly DP. Mitochondrial biogenesis and dynamics in the developing and diseased heart. *Genes & development*. 2015; 29:1981–91. [PubMed: 26443844]
12. Yang J, Xu WW, Hu SJ. Heart failure: advanced development in genetics and epigenetics. *Biomed Res Int*. 2015; 2015:352734. [PubMed: 25949994]
13. Masliah-Planchon J, Bieche I, Guinebretiere JM, Bourdeaut F, Delattre O. SWI/SNF chromatin remodeling and human malignancies. *Annu Rev Pathol*. 2015; 10:145–71. [PubMed: 25387058]
14. Shi J, Whyte WA, Zepeda-Mendoza CJ, Milazzo JP, Shen C, Roe JS, Minder JL, Mercan F, Wang E, Eckersley-Maslin MA, Campbell AE, Kawaoka S, Shareef S, Zhu Z, Kendall J, Muhar M, Haslinger C, Yu M, Roeder RG, Wigler MH, Blobel GA, Zuber J, Spector DL, Young RA, Vakoc CR. Role of SWI/SNF in acute leukemia maintenance and enhancer-mediated Myc regulation. *Genes & development*. 2013; 27:2648–62. [PubMed: 24285714]
15. Banerjee R, Bultman SJ, Holley D, Hillhouse C, Bain JR, Newgard CB, Muehlbauer MJ, Willis MS. Non-targeted metabolomics of double-mutant cardiomyocytes reveals a novel role for SWI/SNF complexes in metabolic homeostasis. *Metabolomics*. 2015; 11:1287–301. [PubMed: 26392817]
16. Hoffman GR, Rahal R, Buxton F, Xiang K, McAllister G, Frias E, Bagdasarian L, Huber J, Lindeman A, Chen D, Romero R, Ramadan N, Phadke T, Haas K, Jaskelioff M, Wilson BG, Meyer MJ, Saenz-Vash V, Zhai H, Myer VE, Porter JA, Keen N, McLaughlin ME, Mickanin C, Roberts CW, Stegmeier F, Jagani Z. Functional epigenetics approach identifies BRM/SMARCA2 as a critical synthetic lethal target in BRG1-deficient cancers. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111:3128–33. [PubMed: 24520176]
17. Bultman S, Gebuhr T, Yee D, La Mantia C, Nicholson J, Gilliam A, Randazzo F, Metzger D, Chambon P, Crabtree G, Magnuson T. A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Molecular cell*. 2000; 6:1287–95. [PubMed: 11163203]
18. Reyes JC, Barra J, Muchardt C, Camus A, Babinet C, Yaniv M. Altered control of cellular proliferation in the absence of mammalian brahma (SNF2alpha). *The EMBO journal*. 1998; 17:6979–91. [PubMed: 9843504]
19. Sumi-Ichinose C, Ichinose H, Metzger D, Chambon P. SNF2beta-BRG1 is essential for the viability of F9 murine embryonal carcinoma cells. *Molecular and cellular biology*. 1997; 17:5976–86. [PubMed: 9315656]
20. Sohail DS, Nghiem M, Crackower MA, Witt SA, Kimball TR, Tymitz KM, Penninger JM, Molkentin JD. Temporally regulated and tissue-specific gene manipulations in the adult and embryonic heart using a tamoxifen-inducible Cre protein. *Circulation research*. 2001; 89:20–5. [PubMed: 11440973]
21. de Ridder GG, Ray R, Pizzo SV. A murine monoclonal antibody directed against the carboxyl-terminal domain of GRP78 suppresses melanoma growth in mice. *Melanoma Res*. 2012; 22:225–35. [PubMed: 22495669]

22. Misra UK, Pizzo SV. Modulation of the unfolded protein response in prostate cancer cells by antibody-directed against the carboxyl-terminal domain of GRP78. *Apoptosis*. 2010; 15:173–82. [PubMed: 20091233]
23. Misra UK, Pizzo SV. Up-regulation of GRP78 and antiapoptotic signaling in murine peritoneal macrophages exposed to insulin. *J Leukoc Biol*. 2005; 78:187–94. [PubMed: 15845644]
24. Willis MS, Min JN, Wang S, McDonough H, Lockyer P, Wadosky KM, Patterson C. Carboxyl terminus of Hsp70-interacting protein (CHIP) is required to modulate cardiac hypertrophy and attenuate autophagy during exercise. *Cell biochemistry and function*. 2013; 31:724–35. [PubMed: 23553918]
25. Sidorova TN, Mace LC, Wells KS, Yermalitskaya LV, Su PF, Shyr Y, Byrne JG, Petracek MR, Greulich JP, Hoff SJ, Ball SK, Glabe CG, Brown NJ, Barnett JV, Murray KT. Quantitative Imaging of Preamyloid Oligomers, a Novel Structural Abnormality, in Human Atrial Samples. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*. 2014; 62:479–87. [PubMed: 24789805]
26. Hayashi M, Imanaka-Yoshida K, Yoshida T, Wood M, Fearn C, Tatake RJ, Lee JD. A crucial role of mitochondrial Hsp40 in preventing dilated cardiomyopathy. *Nat Med*. 2006; 12:128–32. [PubMed: 16327803]
27. Koitabashi N, Bedja D, Zaiman AL, Pinto YM, Zhang M, Gabrielson KL, Takimoto E, Kass DA. Avoidance of transient cardiomyopathy in cardiomyocyte-targeted tamoxifen-induced MerCreMer gene deletion models. *Circulation research*. 2009; 105:12–5. [PubMed: 19520971]
28. Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N, Oomori H, Noda T, Haraguchi T, Hiraoka Y, Amano A, Yoshimori T. Autophagosomes form at ER-mitochondria contact sites. *Nature*. 2013; 495:389–93. [PubMed: 23455425]
29. Lavandro S, Chiong M, Rothermel BA, Hill JA. Autophagy in cardiovascular biology. *J Clin Invest*. 2015; 125:55–64. [PubMed: 25654551]
30. Nemchenko A, Chiong M, Turer A, Lavandro S, Hill JA. Autophagy as a therapeutic target in cardiovascular disease. *J Mol Cell Cardiol*. 2011; 51:584–93. [PubMed: 21723289]
31. Xie M, Morales CR, Lavandro S, Hill JA. Tuning flux: autophagy as a target of heart disease therapy. *Current opinion in cardiology*. 2011; 26:216–22. [PubMed: 21415729]
32. Zhu H, He L. Beclin 1 biology and its role in heart disease. *Curr Cardiol Rev*. 2015; 11:229–37. [PubMed: 25373623]
33. Fullgrabe J, Klionsky DJ, Joseph B. The return of the nucleus: transcriptional and epigenetic control of autophagy. *Nature reviews Molecular cell biology*. 2014; 15:65–74.
34. Warr MR, Binnewies M, Flach J, Reynaud D, Garg T, Malhotra R, Debnath J, Passegue E. FOXO3A directs a protective autophagy program in haematopoietic stem cells. *Nature*. 2013; 494:323–7. [PubMed: 23389440]
35. Xu P, Das M, Reilly J, Davis RJ. JNK regulates FoxO-dependent autophagy in neurons. *Genes & development*. 2011; 25:310–22. [PubMed: 21325132]
36. Vidal RL, Hetz C. Unspliced XBP1 controls autophagy through FoxO1. *Cell research*. 2013; 23:463–4. [PubMed: 23337584]
37. Lee Y, Lee HY, Hanna RA, Gustafsson AB. Mitochondrial autophagy by Bnip3 involves Drp1-mediated mitochondrial fission and recruitment of Parkin in cardiac myocytes. *American journal of physiology Heart and circulatory physiology*. 2011; 301:H1924–31. [PubMed: 21890690]
38. Archer SL. Mitochondrial dynamics--mitochondrial fission and fusion in human diseases. *N Engl J Med*. 2013; 369:2236–51. [PubMed: 24304053]
39. Tracy K, Dibling BC, Spike BT, Knabb JR, Schumacker P, Macleod KF. BNIP3 is an RB/E2F target gene required for hypoxia-induced autophagy. *Molecular and cellular biology*. 2007; 27:6229–42. [PubMed: 17576813]
40. Yoon Y, Krueger EW, Oswald BJ, McNiven MA. The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. *Molecular and cellular biology*. 2003; 23:5409–20. [PubMed: 12861026]
41. Smirnova E, Griparic L, Shurland DL, van der Bliek AM. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Molecular biology of the cell*. 2001; 12:2245–56. [PubMed: 11514614]

42. Cipolat S, Martins de Brito O, Dal Zilio B, Scorrano L. OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:15927–32. [PubMed: 15509649]
43. Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, Chan DC. Structural basis of mitochondrial tethering by mitofusin complexes. *Science*. 2004; 305:858–62. [PubMed: 15297672]
44. Rubinsztein DC, Marino G, Kroemer G. Autophagy and aging. *Cell*. 2011; 146:682–95. [PubMed: 21884931]
45. Wang K, Klionsky DJ. Mitochondria removal by autophagy. *Autophagy*. 2011; 7:297–300. [PubMed: 21252623]
46. Jin SM, Youle RJ. The accumulation of misfolded proteins in the mitochondrial matrix is sensed by PINK1 to induce PARK2/Parkin-mediated mitophagy of polarized mitochondria. *Autophagy*. 2013; 9:1750–7. [PubMed: 24149988]
47. Navarro S, Ventura S. ProteoStat to detect and discriminate intracellular amyloid-like aggregates in *Escherichia coli*. *Biotechnology journal*. 2014
48. Cook NP, Kilpatrick K, Segatori L, Marti AA. Detection of alpha-synuclein amyloidogenic aggregates in vitro and in cells using light-switching dipyrrophenazine ruthenium(II) complexes. *Journal of the American Chemical Society*. 2012; 134:20776–82. [PubMed: 23237404]
49. Bershtein S, Mu W, Serohijos AW, Zhou J, Shakhnovich EI. Protein quality control acts on folding intermediates to shape the effects of mutations on organismal fitness. *Molecular cell*. 2013; 49:133–44. [PubMed: 23219534]
50. Rabeh WM, Bossard F, Xu H, Okiyonedo T, Bagdany M, Mulvihill CM, Du K, di Bernardo S, Liu Y, Konermann L, Roldan A, Lukacs GL. Correction of both NBD1 energetics and domain interface is required to restore DeltaF508 CFTR folding and function. *Cell*. 2012; 148:150–63. [PubMed: 22265408]
51. Pihlasalo S, Kirjavainen J, Hanninen P, Harma H. High sensitivity luminescence nanoparticle assay for the detection of protein aggregation. *Analytical chemistry*. 2011; 83:1163–6. [PubMed: 21247196]
52. Parry TL, Melehan JH, Ranek MJ, Willis MS. Functional amyloid signaling via the inflammasome, necrosome, and signalosome: new therapeutic targets in heart failure. *Front Cardiovasc Med*. 2015
53. Willis MS, Patterson C. Proteotoxicity and cardiac dysfunction--Alzheimer's disease of the heart? *N Engl J Med*. 2013; 368:455–64. [PubMed: 23363499]
54. Jain K, Suryakumar G, Prasad R, Singh SN, Ganju L. Myocardial ER chaperone activation and protein degradation occurs due to synergistic, not individual, cold and hypoxic stress. *Biochimie*. 2013; 95:1897–908. [PubMed: 23816873]
55. Wang M, Wey S, Zhang Y, Ye R, Lee AS. Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. *Antioxid Redox Signal*. 2009; 11:2307–16. [PubMed: 19309259]
56. Willis MS, Townley-Tilson WH, Kang EY, Homeister JW, Patterson C. Sent to destroy: the ubiquitin proteasome system regulates cell signaling and protein quality control in cardiovascular development and disease. *Circulation research*. 2010; 106:463–78. [PubMed: 20167943]
57. Dufey E, Sepulveda D, Rojas-Rivera D, Hetz C. Cellular Mechanisms of Endoplasmic Reticulum Stress Signaling in Health and Disease. 1. ER stress signaling mechanisms: an overview. *American journal of physiology Cell physiology*. 2014
58. Kitamura M. Endoplasmic reticulum stress and unfolded protein response in renal pathophysiology: Janus faces. *American journal of physiology Renal physiology*. 2008; 295:F323–34. [PubMed: 18367660]
59. Sha H, He Y, Chen H, Wang C, Zenno A, Shi H, Yang X, Zhang X, Qi L. The IRE1alpha-XBP1 pathway of the unfolded protein response is required for adipogenesis. *Cell metabolism*. 2009; 9:556–64. [PubMed: 19490910]
60. Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature*. 1999; 397:271–4. [PubMed: 9930704]

61. Hiramatsu N, Joseph VT, Lin JH. Monitoring and manipulating mammalian unfolded protein response. *Methods in enzymology*. 2011; 491:183–98. [PubMed: 21329801]
62. Seimon TA, Kim MJ, Blumenthal A, Koo J, Ehrt S, Wainwright H, Bekker LG, Kaplan G, Nathan C, Tabas I, Russell DG. Induction of ER stress in macrophages of tuberculosis granulomas. *PloS one*. 2010; 5:e12772. [PubMed: 20856677]
63. Li S, Liu C, Li N, Hao T, Han T, Hill DE, Vidal M, Lin JD. Genome-wide coactivation analysis of PGC-1alpha identifies BAF60a as a regulator of hepatic lipid metabolism. *Cell metabolism*. 2008; 8:105–17. [PubMed: 18680712]
64. Kenneth NS, Mudie S, van Uden P, Rocha S. SWI/SNF regulates the cellular response to hypoxia. *The Journal of biological chemistry*. 2009; 284:4123–31. [PubMed: 19097995]
65. Hang CT, Yang J, Han P, Cheng HL, Shang C, Ashley E, Zhou B, Chang CP. Chromatin regulation by Brg1 underlies heart muscle development and disease. *Nature*. 2010; 466:62–7. [PubMed: 20596014]
66. Zhang J, Ney PA. Role of BNIP3 and NIX in cell death, autophagy, and mitophagy. *Cell Death Differ*. 2009; 16:939–46. [PubMed: 19229244]
67. Novak I. Mitophagy: a complex mechanism of mitochondrial removal. *Antioxid Redox Signal*. 2012; 17:794–802. [PubMed: 22077334]
68. Dorn GW 2nd. Mitochondrial pruning by Nix and BNip3: an essential function for cardiac-expressed death factors. *Journal of cardiovascular translational research*. 2010; 3:374–83. [PubMed: 20559783]
69. Melvin A, Mudie S, Rocha S. The chromatin remodeler ISWI regulates the cellular response to hypoxia: role of FIH. *Molecular biology of the cell*. 2011; 22:4171–81. [PubMed: 21900490]
70. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *The Journal of biological chemistry*. 2008; 283:10892–903. [PubMed: 18281291]
71. Meyer JN, Bess AS. Involvement of autophagy and mitochondrial dynamics in determining the fate and effects of irreparable mitochondrial DNA damage. *Autophagy*. 2012; 8:1822–23. [PubMed: 22929123]
72. Papanicolaou KN, Ngoh GA, Dabkowski ER, O'Connell KA, Ribeiro RF Jr, Stanley WC, Walsh K. Cardiomyocyte deletion of mitofusin-1 leads to mitochondrial fragmentation and improves tolerance to ROS-induced mitochondrial dysfunction and cell death. *American journal of physiology Heart and circulatory physiology*. 2012; 302:H167–79. [PubMed: 22037195]
73. Chen L, Gong Q, Stice JP, Knowlton AA. Mitochondrial OPA1, apoptosis, and heart failure. *Cardiovasc Res*. 2009; 84:91–9. [PubMed: 19493956]
74. Martin OJ, Lai L, Soundarapandian MM, Leone TC, Zorzano A, Keller MP, Attie AD, Muoio DM, Kelly DP. A role for peroxisome proliferator-activated receptor gamma coactivator-1 in the control of mitochondrial dynamics during postnatal cardiac growth. *Circulation research*. 2014; 114:626–36. [PubMed: 24366168]

Fig. 1. Cardiomyocyte vacuolization and induction of α MHC in *Brg1/Brm* double-mutant hearts. **A**. Representative H&E- and Mason's Trichrome-stained heart sections from *Brg1/Brm* double-mutant and control mice as indicated. **B**. Quantitative analysis of fibrosis in *Brg1/Brm* double-mutant hearts by logarithmic non-biased analysis of collagen (N=3 per group). **C**. RT-qPCR analysis of fetal genes that are induced during cardiac hypertrophy at early (N=3 per control Group 3, 7 per *Brg1/Brm* Double Mutant group 4) and **D**, late time points (N=4 per group). Results are presented as means $\pm$ SEM. A Student's t-test was used to compare *Brg1/Brm* double-mutant hearts to controls (*, p < 0.05; **, p < 0.01).

Fig. 2.

Ultrastructural analysis of *Brg1/Brm* double-mutant hearts by transmission electron microscopy (TEM). **A.** Representative TEM images of double-mutant hearts at day 14 (post-tamoxifen induction) with **B.** degenerating mitochondria (white arrows) and autophagic vesicles (blue arrows) indicated. **C.** Representative TEM images of control hearts at day 14 (post-tamoxifen induction), which lack evidence of autophagy.

Fig. 3. *Brg1/Brm* double-mutant hearts have significantly increased autophagic flux. **A.** Top, representative western blot of LC3B isoforms in heart tissue from bafilomycin-treated double-mutant and control mice at day 15 post-tamoxifen induction. Bottom, quantification of the LC3BII/LC3BI ratio based on densitometry of above immunoblot. **B.** Representative western blot of Beclin1 in same heart samples from panel (C) with Tubulin serving as a loading control. **C.** RT-qPCR analysis of autophagy genes at day 15 post-tamoxifen induction. Data are normalized to *Gapdh* and represent means $\pm$ SEM based on 5 independent experiments run in duplicate with significant differences indicated (*, $p < 0.01$). **D.** Quantitative ChIP assays demonstrating BRG1/BRM occupancy at the *Bnip3* promoter in wild-type mouse heart tissues. As a negative control, the same heart tissues were immunoprecipitated with IgG and processed identically. Histograms show the relative enrichment by comparing each ChIP sample to input by qPCR (means $\pm$ SEM for three independent samples).

Fig. 4.

Altered mitochondrial dynamics in *Brg1/Brm* double-mutant hearts. **A.** Representative TEM images of double-mutant and control heart sections. **B.** Quantification of mitochondrial area and size based on measurements from TEM images. Data were obtained from 3 control mice and 3 double-mutant mice with >6 fields from each animal. A total of 1,529 control mitochondria and 2,380 double-mutant mitochondria were scored. **C.** Average mitochondria area and number $\pm$ SEM based on data from panel (B). (*, $p < 0.05$).

Fig. 5.

Quantitative analysis of mitochondrial number and fusion and fission genes. **A.** qPCR of 3 mitochondria-encoded loci normalized to nuclear H19 locus in control and double-mutant hearts at day 14 post-tamoxifen induction. Data are presented as means $\pm$ SEM based on 5 independent experiments with significant differences indicated (*, $p < 0.01$). **B.** RT qPCR analysis of *Mfn1* and *Opa1* fusion genes normalized to BestKeeper Index in control and double-mutant hearts at day 15 post-tamoxifen. Data are presented as means $\pm$ SEM based on 5 independent experiments with significant differences indicated (*, $p < 0.01$). **C.** RT qPCR analysis of *Fis1* and *Drp1* fission genes normalized to BestKeeper Index in control and double-mutant hearts at day 15 post-tamoxifen induction. Data are presented as means $\pm$ SEM based on 5 independent experiments with significant differences indicated (*, $p < 0.01$).

Fig. 6.

Brg1/Brm double-mutant hearts have increased protein aggregation by quantitative analysis of aggregated (amyloid) protein and Pre-Amyloid Oligomers (PAO) recognized by the A11 antibody. **A.** Quantification of unfolded protein accumulation in control and double-mutant hearts at two time points (days 9 and 15 post-tamoxifen induction). Data represent 5 animals per group with significant differences indicated (*, $p < 0.01$). **B.** Quantification of pre-amyloid oligomers in control and double-mutant hearts at day 15 post-tamoxifen induction. Five *Brg1/Brm* double-mutant hearts and three control hearts run in triplicate are represented. n.s., not significant. Representative IHC image of GRP78 staining in control and double-mutant hearts are shown at day 15 post-tamoxifen induction. Data are presented as means $\pm$ SEM.

Fig. 7.

Immunohistochemistry and RT-qPCR analysis of the unfolded protein response in *Brg1/Brm* double-mutant hearts. **A.** Immunohistochemical analysis of GRP78, phospho-eIF2 (p-eIF2), and CHOP (GADD153). **B.** RT-qPCR analysis of genes involved in the unfolded protein response normalized to *Gapdh* in day 15 hearts from control and double-mutant mice. **C.** RT-qPCR analysis of genes involved in the unfolded protein response normalized to *Gapdh* in heart failure tissues from patients with conduction defects and control healthy hearts (patients detailed in Fig. 4). Data are presented as means $\pm$ SEM based on 5 independent experiments with significant differences indicated (*, $p < 0.05$).

High-resolution transthoracic echocardiography performed on conscious mice in Groups 1–5 at baseline and after initiating tamoxifen/chow or chow diet for 7 days. Data represent means $\pm$ SEM. A One Way Analysis of Variance was performed, followed by an all pairwise multiple comparison procedure (Holm-Sidak method), when overall significance <0.05 . $^{\$}p<0.001$ vs. all other groups.

Table 1

	Group 1 Brm-/- flx/flx No Tg Chow Diet Baseline N=3	Group 2 Brm-/- flx/flx No Tg +TAM Baseline N=14	Group 3 Brm-/- flx/flx Brg1 Tg+ Chow Baseline N=5	Group 5 Brm-/- flx/- Brg1 Tg+ Baseline N=7	Group 4 Brm-/- flx/flx Brg1 Tg+ +TAM Baseline N=30	Groups 1-3, 5 (CONTROLS) Day Matched Pre-Mortem N=28	Group 4 Brm-/- flx/flx Brg1 Tg+ +TAM 1 Day Pre-Mortem (Day 11.6 $\pm$ 1.5) N=27
AWTS (mm)	1.73 $\pm$ 0.10	1.77 $\pm$ 0.04	1.92 $\pm$ 0.13	1.62 $\pm$ 0.04	1.84 $\pm$ 0.04	1.83 $\pm$ 0.04	1.37 $\pm$ 0.10 $^{\$}$
LVESD (mm)	1.28 $\pm$ 0.04	1.28 $\pm$ 0.04	1.24 $\pm$ 0.01	1.16 $\pm$ 0.08	1.29 $\pm$ 0.03	1.22 $\pm$ 0.03	3.16 $\pm$ 0.24 $^{\$}$
PWTD (mm)	1.12 $\pm$ 0.03	1.09 $\pm$ 0.02	1.18 $\pm$ 0.07	0.97 $\pm$ 0.05	1.06 $\pm$ 0.02	1.10 $\pm$ 0.02	1.01 $\pm$ 0.06
PWTS (mm)	1.91 $\pm$ 0.06	1.78 $\pm$ 0.02	1.78 $\pm$ 0.06	1.60 $\pm$ 0.06	1.68 $\pm$ 0.03	1.77 $\pm$ 0.03	1.27 $\pm$ 0.07 $^{\$}$
LV Mass (mg)	110.9 $\pm$ 4.9	114.1 $\pm$ 3.7	126.9 $\pm$ 11.1	87.9 $\pm$ 7.1	117.8 $\pm$ 5.3	111.9 $\pm$ 4.0	156.0 $\pm$ 9.1 $^{\$}$
LV Mass/BW (mg/g)	3.1 $\pm$ 0.2	3.9 $\pm$ 0.1	4.1 $\pm$ 0.4	3.9 $\pm$ 0.2	4.2 $\pm$ 0.1	3.9 $\pm$ 0.1	6.2 $\pm$ 0.3 $^{\$}$
BW	36.1 $\pm$ 0.6	29.7 $\pm$ 1.2	31.2 $\pm$ 0.7	22.5 $\pm$ 0.9	27.7 $\pm$ 1.0	28.8 $\pm$ 0.9	25.4 $\pm$ 0.9

HR, heart rate; ExLVD, external left ventricular diameter; bpm, heart beats per minute; AWTD, anterior wall thickness in diastole; AWTS, anterior wall thickness in systole; PWTD, posterior wall thickness in diastole; PWTS, posterior wall thickness in systole; LVESD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; FS, fractional shortening, calculated as (LVESD - LVESD)/LVESD x 100; EF%, ejection fraction calculated as (end Simpson's systolic volume - end Simpson's diastolic volume) / end Simpson's diastolic volume * 100, ND, not determined.