ABSTRACT 

Heart failure is associated with induction of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR). The serine/threonine protein kinase/endoribonuclease IRE1α is a key protein in ER stress signal transduction. IRE1α activity can induce both protective UPR and apoptotic downstream signaling events, but the specific role for IRE1α activity in the heart is unknown. A major aim of this study was to characterize the specific contribution of IRE1α in cardiac physiology and pathogenesis. We used both cultured myocytes and a transgenic mouse line with inducible and cardiomyocyte-specific IRE1α overexpression as experimental models to achieve targeted IRE1α activation. IRE1α expression induced a potent but transient ER stress response in cardiomyocytes and did not cause significant effects in the intact heart under normal physiological condition. Furthermore, the IRE1α-activated transgenic heart responding to pressure overload exhibited preserved function and reduced fibrotic area, associated with increased adaptive UPR signaling and with blunted inflammatory and pathological gene expression. Therefore, we conclude that IRE1α induces transient ER stress signaling and confers a protective effect against pressure overload–induced pathological remodeling in the heart. To our knowledge, this report provides first direct evidence of a specific and protective role for IRE1α in the heart and reveals an interaction between ER stress signaling and inflammatory regulation in the pathologically stressed heart.

INTRODUCTION 

Cardiovascular diseases are number one causes of mortality in the United States and worldwide (1). Treatments for heart failure remain elusive due to the complexity of etiology and our limited understanding of the underlying mechanisms. Protein homeostasis is critical to cellular health and defects in protein synthesis, maturation and turnover are implicated in many human diseases(2-5) including heart failure(6). Protein homeostasis in the ER lumen is monitored and maintained by highly conserved quality control mechanisms(7). Disruptions to ER homeostasis including altered redox...
status, calcium flux, protein aggregation or accumulation of client proteins cause ER stress and activation of a highly conserved Unfolded Protein Response (UPR)(also known as ER stress response)(8-10). The UPR conveys stress from the ER to the nucleus (11,12), culminating in activation of transcription factors to upregulate shaperone molecules and to suppress protein synthesis, leading to restored ER homeostasis (13). There are three major ER stress sensors and signal transducers involved in UPR, including Activating Transcription Factor 6 (ATF6), Protein kinase-like ER Kinase (PERK) and Inositol requiring 1 (IRE1). They act in concert to restore protein folding in the ER lumen by reducing the client protein folding load, while increasing protein folding capacities and enhancing protein degradation for misfolded peptides, through coordinated induction of chaperone genes and inhibition of protein synthesis (14-18).

In heart, ER stress signaling is activated in response to a broad spectrum of myocardial injuries including ischemia, ischemia/reperfusion, hypoxia and mechanical overload (6,8,19-24). ER stress signaling is also implicated in metabolic remodeling in diseased heart(25). However, it remains unclear whether ER stress signaling is protective or detrimental to heart. Some studies suggest ER stress contributes to myocyte apoptosis and heart failure (21,22,26,27) while other reports indicate it is cardioprotective(24,28,29). Different ER stress pathways appear to have specific roles in heart. ATF6 mediated signaling has been identified as a highly protective pathway against ischemia/reperfusion injury (24) by inducing BiP and GRP94. Similarly, XBP1, downstream of IRE1α activation, was found to be protective against hypoxia and myocardial infarction also by inducing BiP(30) and coupling with hexosamine biosynthetic pathway(25). BiP expression can inhibit apoptotic signal CHOP and reduces apoptosis in cardiomyocytes (31). However, ER stress can also activate cell death signaling pathways (32-34). In particular, IRE1α binds to TRAF2 and activates downstream ASK1 resulting in JNK and p38 MAPK activation and apoptotic cell death(14,35,36). In contrast, other reports indicate interactions between IRE1α and TRAF2 and JNK activate autophagy to promote cell survival during ER stress(37). PERK can also contribute to apoptotic signaling by promoting expression of ATF4 (17,38,39), a transcriptional activator for CHOP (40,41). Angiotensin II, along with tunicamycin and thapsigargin, can induce both adaptive protein folding chaperones and apoptotic signal CHOP (6). ER stress can also induce hypertrophy gene expression, including Atrial Natriuretic factor (ANF) and Brain Natriuretic factor (BNF), suggesting that ER stress contributes to both adaptive and pathological remodeling. Finally, ER stress is implicated in cancer therapy Imatinib induced cardiomyopathy (26). Although evidence is clear about the importance of ER stress regulation in cardiac pathogenesis, the specific contribution of IRE1 mediated signaling in heart has not yet been directly investigated.

In the current study, we investigated the direct impact of IRE1α activation in heart. In cardiomyocytes in culture, IRE1α overexpression induced significant IRE1α auto-phosphorylation and XBP-1 activation as expected.
However, there was no evidence of induction in cell death, stress signaling or hypertrophy upon prolonged IRE1α expression. Using an animal model with heart specific, tamoxifen inducible IRE1α overexpression, we investigated the direct impact of IRE1α expression in vivo. Overexpression of IRE1α did not lead to any pathological phenotype at baseline. Following pressure-overload induced by trans-aortic constriction, IRE1α expression in transgenic hearts showed better preserved function, blunted pathologic marker gene induction, and better preserved ER stress signaling. More interestingly, the expression of inflammatory cytokines TNFα and IL-6 was significantly blunted by IRE1α expression, suggesting IRE1α mediated ER stress signaling inhibits inflammatory cytokines induction in heart. Thus, IRE1α activity has a direct protective effect in heart against pressure-overload induced cardiac pathology, involving suppression of pro-inflammatory gene expression.

RESULTS:
IRE1α induces adaptive and transient UPR in cardiac myocytes

To investigate the functional impact of IRE1α activity, we generated adenoviral vectors expressing wildtype IRE1α. To validate the functionality of this expression vector, we tested the adenoviral vector in INS-1 cells. As shown in Figure 1, IRE1α expression in INS-1 cells led to significant cell death 2 days after transfection (Figure 1A). Xbp-1 splicing was induced in a dose-dependent manner by IRE1α expression similar to tunicamycin (TM) treatment (Figure 1B). In addition, IRE1α autophosphorylation, BiP expression, phosphor-EIF2α (Figure 1C) and JNK kinase activity (Figure 1D) were also induced in a dose-dependent fashion by IRE1α expression in INS-1 cells. These data is consistent with previous literature where IRE1α expression promotes cell death, associated with elevated RNase and stress-kinase signaling via oligomerization in INS-1 cells (42) (43). In contrast, NRVM with IRE1α overexpression did not show significant difference in morphology or cell sizes (Figure 2A) even though autophosphorylation and downstream Xbp-1 activation were detected as in INS-1 cells (Figure 2B, C). Other ER stress effectors such as Bip expression was also significantly induced by IRE1α overexpression while pro-apoptotic ER stress gene CHOP was not (p=0.09) (Figure 2D). As expected, expression of a kinase dead IRE1α-KA mutant did not affect any of the ER stress molecules (Figure 2E). These results revealed an unexpected myocyte specific response to IRE1α expression where only a subset of cytoprotective ER stress response was triggered in primary cardiomyocytes without activating the pro-apoptotic downstream pathway. Remarkably, prolonged IRE-1α expression (5 days) failed to sustain downstream Xbp-1 activation or other UPR gene expression even though IRE1α autophosphorylation remained elevated (Figure 2F-H). These observations have revealed two specific features in IRE1α function in cardiomyocytes, one is a selective induction of IRE1α mediated cyto-protective response vs. stress-signaling and another involves the transient nature of the IRE1α induced downstream Xbp-1 activation, most likely due to a yet to be identified negative feedback inhibition.
**Heart specific and inducible expression of IRE1α in mice**

In order to elucidate IRE1α function in intact heart, we generated an animal model where IRE1α expression was both inducible and heart specific using a Cre-loxP mediated gene switch strategy (Figure 3A) (44). Cre-dependent expression of IRE1α was demonstrated in 293 cells, validating the efficacy of the construct (Figure 3B). As shown *in vitro*, the Flox-GFP-IRE1α transgenic mice showed wide-spread expression of GFP but not the IRE1α transgene. After cross-breeding with αMHC-Mer-Cre-Mer mice (45-47), the transgenic gene IRE1α was induced only in ventricular tissue only after tamoxifen treatment (Figure 3C-E). Four weeks after transgene induction, no abnormal phenotype was observed in the IRE1α expressing hearts comparing to the littermate controls, including non- or single-transgenic mice, based on morphometric, histological and functional analysis (Figure 4). Furthermore, mRNA levels of sXbp1, Bip, and CHOP (Figure 5A) were not affected by IRE1α expression, consistent with our in vitro observation after long-term IRE1α expression. The molecular profile of ANF, β-MHC and TNFα were all unchanged following IRE1α overexpression (Figure 5B). Together, these observations suggest that IRE1α expression was not sufficient to sustain ER stress response and did not exert any detrimental effect in adult mouse heart.

**IRE1α preserves heart function after TAC**

In order to test the effect of IRE1α expression on pathological stress response in heart, IRE1α transgenic mice were subjected to pressure-overload implemented by transverse-aortic constriction (TAC) (48,49). Pressure-overload led to significant loss of cardiac function over time in the Control mice. In contrast, the IRE1α transgenic mice demonstrated preserved function even at 4 weeks post-TAC (Figure 6A), associated with reduced chamber dilation at systole (Figure 6B) based on echocardiograph measurements. Furthermore, the induction of pathological marker genes, including ANF and βMHC, was significantly blunted in response to TAC in the IRE1α transgenic heart vs. the Controls (Figure 6C). However, cardiac hypertrophy as measured from tissue weight and histology was induced by TAC to the same levels in the Control vs. the IRE1α transgenic hearts (Figure 6D and 6E). Interestingly, Fibrotic induction in myocardium was also significantly blunted in response to TAC in the IRE1α transgenic heart vs. the Controls (Figure 6E and 6F).

To investigate the potential mechanism, we analyzed the expression of UPR genes and stress-signaling molecules in post-TAC hearts. Although there was a trend in lower BiP expression in the post-TAC hearts, the IRE1α heart showed a modestly but significantly higher level of BiP expression than Controls after TAC (Figure 7A). In contrast, the mRNA level of CHOP was not affected by IRE1α expression while there was trend for lower expression of ATF4 in post-TAC IRE1α transgenic (38) (Figure 7A). Therefore, IRE1α expression in heart leads to better preserved heart function following TAC only modest impact on ER stress downstream molecules. ER stress signaling by IRE1α and TRAF2 has been reported to activate inflammatory signals (50). As shown in Figure 7B, IRE1α transgenic hearts showed significantly
reduced TAC induced mRNA expression for both TNFα and IL-6 cytokines after comparing with the Controls. Furthermore, while NFκB induction was not affected by IRE1α expression in the post-TAC hearts, the IκB kinase β induction was significantly blunted in the IRE1α transgenic hearts, suggesting that attenuated NFκB signaling may in part contribute to reduced TNFα/IL6 induction in IRE1α TAC heart. In summary, IRE1α expression in heart protects cardiac dysfunction associated with blunted induction of pro-inflammatory genes.

**DISCUSSION**

This study provided first comprehensive characterization for the specific function of IRE1α as one of the three major ER stress signaling branches in pressure-overload induced cardiac remodeling and dysfunction. In cultured cardiomyocytes, we observed transient induction of canonical Xbp-1 activation following IRE1α expression without significant effects on cell death and hypertrophy. In intact heart, IRE1α expression did not affect pressure overload induced cardiac hypertrophy but significantly attenuated cardiac dysfunction and pathological marker gene expression. Interestingly, IRE1α expression markedly blunted the induction of pro-inflammatory cytokine gene expression following TAC. Therefore, our results provide direct in vitro and in vivo evidence that IRE1α mediated signaling is cardio-protective, at least in the setting of pressure-overload induced cardiac dysfunction.

ER stress signaling pathways and the ensuing Unfolded Protein Response are important cellular responses to various insults and have been found to be activated in human congestive heart failure (6), ischemic heart disease (21) and heart failure in response to the cancer drug imatinib(26). Experimental models have also recapitulated these observations (6,24) but it is unclear whether ER stress signaling plays protective (24,30,31) or pathological roles in heart (22,26,51,52). Expression of a constitutively active ATF6 shows a strong protection against ischemia reperfusion injury in mice (24). Chop inhibition in NRVM was also protective against apoptosis in the setting of ER stress by proteasome inhibition (31) and Chop inactivation in vivo protected mouse hearts against apoptosis following ischemia/ reperfusion injury (52). Among the three major UPR pathways, i.e. IRE1, PERK and ATF6, IRE1 is unique in terms of diverse downstream outcome. IRE1 activation is capable of both protective and apoptotic signaling through XBP1 or TRAF2 and stress-activated MAPK signaling cascades, respectively as demonstrated previously (14,18,42,43,53,54) (Figure 1). However, the molecular nature of IRE1α function and regulation appear to be highly cell type specific. While constitutive expression of IRE1α led to cell death in non-myocytes, such as INS-1 cells (42), as well as other cell types(54), similar expression in cardiomyocytes achieved only transient induction of Xbp-1 but without cell death induction. More remarkably, prolonged expression led to sustained autophosphorylation of IRE1α in cardiomyocytes, but only transient induction of Xbp-1, suggesting the presence of a negative inhibitory feedback mechanism for IRE1 signaling in cardiomyocytes. It is well established that the RNase activity of IRE1 is induced by oligomerization and trans-molecular auto-phosphorylation (55). However, its
inhibition is poorly characterized, but may involve Bax inhibitor 1 (56), Hsp90/Cdc37 complex (57) or an ER specific protein phosphatase (PP2Ce) (58). In cardiomyocytes, Xbp1 splicing activity is attenuated over time while IRE1α phosphorylation level remains elevated, suggesting that the underlying molecular mechanism may involve uncoupling of its autophosphorylation status and the Xbp1 splicing activity. The molecular basis of such negative feedback regulation should be further studied.

We observed that IRE1α transgenic mice had preserved heart function in response to pressure overload. The underlying mechanism is yet to be uncovered. It is known that inflammatory cytokines are induced in stressed myocardium, contributing to cardiac dysfunction and pathological remodeling. Indeed, both TNFα and IL6 expressions were markedly reduced in the post-TAC IRE1α transgenic hearts. It is reported that both TNFα and IL-6 are induced by stress-signaling, such as p38 MAP Kinase (59,60) and NFκB pathway. Although we did not observe any effects on p38 activation, IKKβ induction was significantly attenuated by IRE1α expression. The data is consistent with the notion that IRE1α expression in cardiomyocytes promote cyto-protective ER stress signaling but not downstream stress-signaling, and offers a potential mechanistic link between ER stress signaling and anti-inflammatory gene induction in pathologically stressed myocardium. Establishing the cardioprotective role of IRE1α in intact heart illustrates the potential contribution of ER stress signaling to the pathogenesis of heart failure, as well as the feasibility of targeted manipulation of ER stress signaling for treating the disease.

**EXPERIMENTAL PROCEDURES**

**Animal models and surgical procedures**

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). All procedures were performed in accordance with the University of California, Los Angeles animal welfare guidelines and approved by UCLA institutional animal care and use committee (IACUC). IRE1α was cloned into a vector for generation of transgenic animals with cre-regulated expression of the transgene of interest(61,62). Transgenic animals were generated in C57/Bl6 background through collaboration with the UCLA Molecular Genetics Technology Center. Founder animals were identified by PCR with transgene specific primers.

Animals with heart-specific, inducible IRE1α overexpression were generated by crossing transgenic founder animals with previously established ßMHC-Mer-Cre-Mer (MCM) transgenic mice (47) (45). IRE1α transgene overexpression was induced by intraperitoneal injection of Tamoxifen Citrate Salt (Sigma) 20mg / kg body weight/ day for five days(45). Wild-type and floxed single transgenic littermate animals treated with tamoxifen or double transgenic flox-GFP/ CRE animals treated with vehicle were also used as controls. Both male and female mice age 12-16 weeks were included in this study.

Transverse Aortic Constriction (TAC) was performed as previously described with modifications(49). Mice were anesthetized with ketamine (80mg/
kg) / xylazine (20mg/kg) by i.p. injection. Respiration was provided by mechanical ventilation with 95% O₂ (tidal volume 0.5 mL, 130 breaths per minute). Left parasternal thoracotomy was performed to access the transverse aorta, which was tied with 5-0 nylon suture on a 27 gauge needle. The needle was removed, leaving in place a 65-70% constriction of the aortic lumen. Constriction of the aorta was confirmed by measuring differential blood flow through the right and left carotid arteries one week after surgery.

Animals were continuously anesthetized with 1.5% isoflurane and 95% oxygen. VisualSonics Vevo 770 and Vevo 2100 imaging systems and 30mHz scanhead (Toronto, Ontario, Canada) was used to collect short and long axis B-Mode and M-Mode views. Reported values refer to short axis measurements and calculations.

**Histology**

Hearts were perfused and fixed in 10% formalin prior to embedding in paraffin. All short axis sections were prepared from mid-ventricle. Sections of 4 μm heart were deparaffinized and rehydrated prior to staining by hematoxylin and eosin (H&E) or Masson trichrome and Verhoff’s Van Gieson. Stained tissue sections were recorded as digital images by Aperio XT whole slide scanning system and snapshot images were taken using the ImageScope software (Vista, CA).

**Cell culture**

293 cells were maintained in DMEM supplemented with 10% RBS and 1% pen/strep. Lipofectamine reagent (Life Technologies) was used according to the manufacturer’s protocol to achieve overexpression of the flox-GFP-IRE1α construct. INS-1 cells were cultured in RPMI1640 according to published methods(57). Neonatal Rat Ventricular Myocytes (NRVM) were harvested from 1-3 day old Sprague-Dawley rat pups as described previously(63) and cultured in serum-free DMEM supplemented with 1% pen/strep and ITS. NRVM were infected with adenovirus (MOI 10) and incubated for two days before additional treatment with 5g/mL Tunicamycin (TM) for 4 hours. Experiments with prolonged IRE1α expression were incubated for five days before RNA or protein analysis.

**Western blot**

Cells were harvested for protein analysis with standard lysis buffer containing 1% Triton-X 100, 1mM β-glycerophosphate, 2.5mM Na₄P₂O₇, 20mM NaF, 1mM Na₃VO₄, 1mM PMSF and protease inhibitor cocktail (Roche). Proteins were boiled for 5 minutes in LDS loading buffer containing 0.1% β-mercaptoethanol and separated on a 4-12% Bis-Tris SDS-PAGE (Life Technologies). Specific proteins were detected with antibodies directed against p-IRE1α (Novus Bio), IRE1α, Actin, (Santa Cruz Biotechnology), BiP/Grp78 (Stressgen), p-p38, p38, p-JNK, JNK, p-IEF2α, eIF2α, GFP, and CHOP (Cell Signaling Technologies) as enlisted below:

| Antibodies | Vender         | Cat#     | Dilution |
|------------|---------------|----------|----------|
| p-IRE1a (S724) | Novus Bio   | NB100-2323 | 1:1000   |
| IRE1a      | Santa Cruz   | SC-20790 | 1:500    |
| Actin      | Santa Cruz   | SC-1616 | 1:1000   |
| CHOP       | Santa Cruz   | SC-7351 | 1:500    |
| Bip/Grp78  | Santa Cruz   | SC-1050 | 1:500    |
| p-p38      | Cell Signaling | 9211 | 1:1000  |
| p38        | Cell Signaling | 9212 | 1:1000  |
| p-JNK      | Cell Signaling | 9251 | 1:1000  |
| JNK        | Cell Signaling | 9252 | 1:1000  |
| p-eIF2a    | Cell Signaling | 9721 | 1:1000  |
| eIF2a      | Cell Signaling | 9722 | 1:1000  |
| GFP        | Cell Signaling | 2956 | 1:1000  |

**RNA and RT-PCR analysis**
Total RNA was isolated from heart or cells with TRIzol (Life Technologies). For animal studies, cDNA was prepared using iScript Reverse Transcription Supermix and amplified with SsoFast EvaGreen Supermix on a CVX96 thermal cycler (all Bio-Rad). For cell studies, cDNA was prepared using Superscript II (Invitrogen) and amplified with SYBR green supermix on a MyIQ system (Bio-Rad). Melt curves were generated for each primer set during each experiment and analysis was performed using the ΔΔCT method.

Xbp1 activation

IRE1α RNase activity toward Xbp1 mRNA was monitored by semiquantitative PCR. Both unspliced and spliced Xbp1 mRNA was amplified with primers targeting the region surrounding the IRE1α splicing site (Forward 5’GGTCCAGAGGTGAGCCCA3’, Reverse 5’CATGACAGGGTCCAATGTG3’). Products were amplified with the cycling protocol of 95°C for 0:30, 60°C for 0:30 and 72°C for 0:25 followed by 72°C for 10:00. PCR products were separated on 4% agarose gel.

Statistical analysis

Data are presented as mean ± 1 standard deviation. Means of two groups were compared by two-tailed Student t-test. Means of more than two groups were compared by ANOVA and Turkey post hoc test. Differences between groups were considered statistically significant when p<0.05.

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The authors declare that they have no conflicts of interest with the contents of this article.
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**FIGURE LEGEND:**

**Figure 1. The functional impact of IRE1α activity in cells.** (A) Representative images of Adv-GFP and Adv-IRE1α treated INS-1 cells. (B) RT-PCR detection of spliced Xbp-1 (sXbp1) levels in adv-IRE1α treated INS-1 cells and quantification of the signal intensity as labeled. Tunicamycin (TM) treated cells is used as a positive control. (C) Immunoblots for the expression and autophosphorylation of IRE1α as well as ER stress related proteins in adv-IRE1α treated INS-1 cells and quantification of the signal intensity normalized by actin as labeled. (D) Immunoblots for stress activated proteins p38 MAPK and JNK in adv-IRE1α treated INS-1 cells and quantification of the signal intensity normalized by actin as labeled.

**Figure 2. The functional impact of IRE1α activity on the cardiomyocyte.** (A) Representative images of mock and adv-IRE1α treated NRVMs and cell surface are. (B) Immunoblots of phosphor-IRE1α, total IRE1α, Myc, and actin in NRVMs treated with mock, adv-IRE1α and adv-IRE1αKA vectors 2 days post transfection. (C) RT-PCR
detection of sXbp1 vs. uXbp1 levels in adv-IRE1α treated NRVMs at 2 days post transfection. (D) Quantitative measurements of mRNA for IRE1α, Bip, and CHOP in NRVMs treated with adv-IRE1α for 2 days. n=3. * p < 0.05. (E) Quantitative measurements of mRNA for IRE1α, Bip, and CHOP in NRVMs treated with adv-IRE1αKA for 2 days. n=3. * p < 0.05. (F) Immunoblots for phosphor-IRE1α in NRVMs treated with adv-IRE1α or adv-IRE1αKA for 5 days. (G) RT-PCR detection of sXbp1 levels in adv-IRE1α or adv-IRE1αKA treated NRVMs for 5 days. (H) mRNA expression of IRE1α, Bip, and CHOP in NRVMs treated with adv-IRE1α for 5 days. n=3.

**Figure 3. Establishment of IRE1α transgenic mouse line.** (A) Schematic diagram for cardiac-specific inducible IRE1α transgenic construct and expected Cre/Loxp mediated deletion. (B) Immunoblots of phosphor-IRE1α, total IRE1α, GFP, and actin in HEK293 cells transfected with flox-GFP-IREα vector. (C) Representative RT-PCR detection of IRE1α expression in different transgenic ventricular tissues from control (Cre-/GFP-IREα-) and IRE1α transgenic (Cre+/GFP-IREα+) hearts, with or without tamoxifen (TMX) treatment. (D) Quantification of IRE1α mRNA expression in heart tissue from the same cohorts as in C. (E) Immunoblots of total IRE1α and GAPDH in ventricle, lung, liver, kidney, and skeletal muscle from control and IRE1α transgenic mouse. C and I indicate control and IRE1α transgenic mouse, respectively.

**Figure 4. Characterization of IRE1α transgenic mouse heart at basal condition.** (A) Echocardiogram analysis of ejection fraction (EF) and fractional shortening (FS) in the IRE1α transgenic and control mouse. (B) Echocardiogram analysis of diastolic/systolic diameter in the LV (LVID) and diastolic/systolic posterior wall thickness in the LV (LVPW). (C) Representative image of H&E stained hearts from IRE1α transgenic and control mouse. (D) Heart weight/Body weight ratio in IRE1α transgenic and control mouse. (E) Body weights 4 weeks after vehicle or tamoxifen injection in both male and female mice.

**Figure 5. ER stress, hypertrophic, and inflammatory gene expression in IRE1α transgenic mouse heart at basal.** (A). mRNA levels of ER stress related genes BiP, sXbp1, CHOP expression in IRE1α transgenic vs. control mouse heart at basal. (B). Hypertrophic marker ANF and βMHC, and inflammatory gene TNFα expression in IRE1α transgenic vs. control mouse heart at basal, n=3.

**Figure 6. Preservation of cardiac function in IRE1α transgenic heart in response to pressure overload.** (A) Echocardiogram analysis of EF and FS in the IRE1α transgenic vs control mouse after TAC. p value *p<0.05. (B) Echocardiogram analysis of diastolic/systolic LVID and diastolic/systolic LVPW 4weeks post-TAC. p value *p<0.05. (C) Hypertrophic marker ANF and βMHC expression after 4 weeks post-TAC. p value *p<0.05. n=3-4. (D) Heart weight/body weight in IRE1α transgenic and control mouse 4
weeks post-TAC. p value ***p<0.001. (E) Representative image of H&E staining and trichrome staining in IRE1α transgenic and control mouse 4 weeks post-TAC. (F) Quantification results of fibrotic area in IRE1α transgenic and control mouse 4 weeks post-TAC. p value *p<0.05. n=4-6.

Figure 7. ER stress and inflammatory gene expression in IRE1α transgenic mouse heart in response to pressure overload. (A) ER stress markers BiP, CHOP, αTF4 mRNA expression. *p<0.05. n=3-4. (B) mRNA levels of inflammatory genes TNFα and IL6 and NFkB signaling genes NFkB and IkB kinase β in control and IRE1α transgenic hearts following Sham or pressure-overload. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05. n=3-4.
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The serine/threonine-protein kinase/endoribonuclease IRE1α protects the heart against pressure overload-induced heart failure
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