Involvement of Endoplasmic Reticulum Stress Mediated C/EBP Homologous Protein Activation in Coxsackievirus B3-Induced Acute Viral Myocarditis

Cai et al: ER Stress/CHOP Signaling Promotes AVMC

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Abstract

Background—The current study tested the hypothesis whether endoplasmic reticulum (ER) stress/ C/EBP homologous protein (CHOP) signaling is linked with Coxsackievirus B3 (CVB3) induced acute viral myocarditis (AVMC) in vivo.

Methods and Results—AVMC was induced by intraperitoneal injection of 1000 TCID$_{50}$ of CVB3 virus in mice. In mice AVMC hearts ($n=11$), ER stress and CHOP were significantly activated, and were linked to the induction of pro-apoptotic signaling including reduction of Bcl-2, activation of Bax and caspase 3, compared with the controls ($n=10$), while these could be markedly blocked by ER stress inhibitor tauroursodeoxycholic acid (TUDCA) administration ($n=11$). Moreover, chemical inhibition of ER stress significantly attenuated cardiomyocytes apoptosis, and prevented cardiac troponin I elevation, ameliorated cardiac dysfunction assessed by both hemodynamic and echocardiographic analysis, reduced viral replication, and increased survival rate after CVB3 inoculation. We further discovered that genetic ablation of CHOP ($n=10$) suppressed cardiac Bcl-2/Bax ratio reduction and caspase 3 activation, and prevented cardiomyocytes apoptosis in vivo, compared with wildtype receiving CVB3 inoculation ($n=10$). Strikingly, CHOP deficiency exhibited dramatic protective effects on cardiac damage, cardiac dysfunction, viral replication, and promoted survival in CVB3 caused AVMC.

Conclusions—Our data imply the involvement of ER stress/CHOP signaling in CVB3 induced AVMC via proapoptotic pathways, and provides a novel strategy for AVMC treatment.

Key Words: coxsackieviruses; endoplasmic reticulum stress; C/EBP homologous protein; acute viral myocarditis; apoptosis
Acute viral myocarditis (AVMC) is characterized by myocardial necrosis, apoptosis, and intense inflammation. Strong evidence showed that AVMC could cause acute heart failure in young patients, and may finally progress to chronic dilated cardiomyopathy (DCM). Among the various infectious agents, coxsackievirus B3 (CVB3) is considered the dominant cause of acute viral myocarditis in humans. Studies indicated that CVB3 viral load, replication, and persistence are directly linked with cardiac injury and progression of the disease. Currently, AVMC is treated nonspecifically, and no antiviral or immunosuppressive therapy available could benefit patients from the disease.

The endoplasmic reticulum (ER) is a crucial cellular organelle for protein synthesis, folding, and transportation. Perturbation of ER function causes accumulation of unfolded/misfolded proteins in the ER lumen, a condition collectively termed ER stress. When ER stress occurs, it triggers activation of transcription factor 6 (ATF6), PKR-like endoplasmic reticulum kinase (PERK) and inositol-requiring enzyme 1α (IRE-1α) signaling, and induces a complex cytoprotective signal network to restore ER homeostasis and promote cell survival, known as unfolded protein response (UPR). In conditions of prolonged stress, however, the UPR aims towards apoptosis, including induction of C/EBP homologous protein (CHOP) proapoptotic pathways. So far, studies have indicated that excessive activation of ER stress/CHOP signaling is associated with various cardiovascular diseases, including atherosclerosis, vascular and valvular calcification, myocardial reperfusion injury and heart failure.

Previous studies have demonstrated the important role of ER stress in viral infection. Viruses can induce ER stress either through overwhelming protein production, or directly...
disrupting ER stress signaling to facilitate virus replication and pathogenesis. Several recent studies showed that CVB3 infection could also trigger ER stress, resulting in its downstream CHOP activation and promoting apoptosis in HeLa cells and HL-1 cells. Thus, it appears that CVB3 infection may also induce cardiac ER stress, active CHOP, lead to cardiomyocytes apoptosis, and finally promote AVMC progression.

To test our hypothesis, we generated CVB3-induced AVMC in vivo, and investigated whether and how ER stress and CHOP-mediated pathways contributed to AVMC development using both pharmacological ER stress inhibitor tauroursodeoxycholic acid (TUDCA), and genetically CHOP deficient mice.

**Methods**

**Ethics statement**

All experiments were approved by the Animal Care and Use Committee of Zhejiang University (zju201308-1-01-085) and meet the Guide for the Care and Use of Laboratory Animals of the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996). All efforts were made to minimize the number of animals used and their suffering.

**Mice**

Male CHOP−/− mice with C57BL/6 background and their littermate controls were purchased from Model Animal Research Center of Nanjing University (Nanjing, China).

**Virus**

The CVB3 (3m strain), a mutant of Coxsackie virus B3 (Nancy) strain, was preserved in Institute of Hypertension and Department of Internal Medicine, Tongji Hospital, Tongji
Medical College, Huazhong University of Science and Technology. CVB3 virus was maintained by passage through Hela cells. The virus titer was determined by 50% tissue culture infectious dose (TCID$_{50}$) assay of HeLa cell monolayer according to Reed & Muench method.$^{20}$

**Animal grouping and virus inoculation**

All animal protocols were approved by the Institutional Animal Care and Use Committee of Zhejiang University, China. AVMC was induced by intraperitoneal injection of 1000 TCID$_{50}$ of CVB3 virus, while all other groups received equal amount of vehicle (DMEM). For assaying the effect of TUDCA on AVMC, 4-week old male C57BL/6 mice were randomly divided into three groups: control group (n=10); CVB3 group (n=11) receiving CBV3 injection; CVB3+TUDCA group (n=11) receiving CVB3 injection supplemented with 0.5g/kg/d TUDCA by oral gavage. Animals without TUDCA treatment received equal amount of vehicle (water) by oral gavage. For determining the involvement of CHOP in AVMC, animals were randomly assigned into three groups: WT group (n=10 of 4-week old male C57BL/6 mice); WT + CVB3 group (n=10 of 4-week old male C57BL/6 mice) receiving CVB3 injection; CHOP$^{-/-}$ + CVB3 group (n=10 of 4-week old male CHOP$^{-/-}$ mice) receiving CVB3 injection. To indicate the activation of ER stress and serve as the positive control, WT mice (4-week old male C57BL/6 mice, n=5) received subcutaneous infusion of isoproterenol (Iso, Sigma-Aldrich, St. Louis, MO, 30 µg/g/day) with mini-osmotic pumps for 14 days to induce heart failure, which were identified by both echocardiography and hemodynamic analysis.
**Echocardiography and hemodynamic measurement**

Transthoracic echocardiography was performed at day 7 after CVB3 inoculation. Mice were anesthetized by isoflurane inhalation. A comprehensive echocardiographic study was performed, including two-dimensional imaging and M-mode imaging using the Vevo 2100 system (VisualSonics, Toronto, Canada)\(^\text{21}\). For hemodynamic measurement, a 1.4 F pressure catheter (SPR 671, Millar Instruments) was inserted into the LV through the right common carotid artery after anesthesia. Left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), dP/dt max and dP/dt min were measured as described previously\(^\text{21}\). Subsequent data calculations were performed by operators blinded to the treatment assignment. Mice were then sacrificed for further analysis.

**Histopathology and immunohistochemistry**

Mice hearts were harvested and fixed in 10% phosphate-buffered formalin, then trimmed and embedded routinely in paraffin. Four μm sections were subsequently stained with hematoxylin and eosin (HE). The severity of myocarditis was assessed by a previously described using 0–4 scale, in which 0=no inflammation; 1=one to five distinct mononuclear inflammatory foci with involvement of 5% or less of the cross-sectional area; 2=more than five distinct mononuclear inflammatory foci, or involvement of between 5% and 20% of the cross-sectional area; 3=diffuse mononuclear inflammation involving over 20% of the area, without necrosis; and 4=diffuse inflammation with necrosis\(^\text{22}\). Immunohistochemical staining were performed using the following antibodies: F4/80 (1:50 dilution, Santa Cruz, Santa Cruz, CA), KDEL (1:200 dilution, Santa Cruz), CHOP (1:50 dilution, Santa Cruz), CVB3 viral
protein (VP1, 1:200, Novocastra, Buffalo Grove, IL). Image-Pro Plus (Media Cybernetics, Warrendale, PA) was applied to determine quantitative results (positive staining area/total area) 23. Investigators performing the analyses were blinded to the study groups.

**Determination of serum cardiac troponin I**

Serum cardiac troponin I (cTnI) was determined using a commercial mouse cardiac troponin-I ELISA kit (Life Diagnostics, West Chester, PA), according to the manufacturer’s instructions.

**Quantification of virus titers in mice hearts**

The CVB3 titers in hearts, liver and pancreas were determined and expressed as TCID50 as described previously 24. Briefly, tissues were partially homogenized in 2 mL Dulbecco’s modified Eagle medium (DMEM). After centrifugation of samples, the supernatants were sequentially tenfold diluted, added to 96-well microtiter plates containing HeLa cells for TCID50 determination, calculated by the method of Reed & Muench 20, and displayed after log calibration.

**Western blot analysis**

Tissue samples and were homogenized and subsequently performed for western blot as described previously 25. The following antibodies were applied: p-IRE1α (1:2000 dilution) from Pierce, Rockford, IL; p-PERK (1:500 dilution), ATF6 (1:500 dilution), CHOP (1:500 dilution), atrial natriuretic peptide (ANP, 1:500 dilution) from Santa Cruz; Bcl-2 (1:1000 dilution), Bax (1:1000 dilution), cleaved caspase 3 (1:1000 dilution) from Cell Signaling, Danvers, MA. β-actin was used as normalization for total protein. Bands were quantified by densitometry using Quantity One Software (Bio-Rad, Hercules, CA).
Statistical analysis

All values are presented as mean±SE. After confirming that all variables were normally distributed by the Kolmogorov-Smirnov test followed by Q-Q plots analysis, statistical differences were determined by ANOVA followed by Bonferroni’s multiple comparison test. Kaplan-Meier curve was applied to determine the survival data. \( P<0.05 \) was accepted as statistically significant. All statistical calculations were carried out using SPSS version 17.0 (SPSS Inc, Chicago, IL, USA).

Results

ER stress is activated in CVB3-induced AVMC

We speculated that CVB3 replication may trigger ER stress in heart. As expected, KDEL, an ER stress marker \(^{11}\), was exclusively overexpressed in the myocardium of infected mice (Figure 1A and 1B). In parallel with the increased expression of the heart failure marker, ANP, western blot analysis further confirmed the induction of ER stress by assessing the activation of three branches of UPR, PERK, IRE1a, and ATF6 \(^{26/7}\) days \( pi \) (Figure 1C-1G).

TUDCA administration inhibits CVB3 infection-induced cardiac ER stress \( in \, vivo \)

TUDCA is a classic chemical chaperone to alleviate ER stress by improving ER folding capacity both \( in \, vitro \) and \( in \, vivo \) \(^{19}\). We determined whether TUDCA administration could also suppress CVB3 infection-induced cardiac ER stress activation \( in \, vivo \). As shown in Figure 1, TUDCA markedly attenuated the ER stress response in hearts of CVB3 infected mice, indicating that TUDCA could effectively suppress CVB3 infection-induced cardiac ER stress activation \( in \, vivo \).
Inhibition of ER stress prevents CVB3-induced cardiomyocyte damage, CVB3 replication, and cardiac dysfunction

Previous reports indicated that inhibition of ER stress is protective against cardiac dysfunction in vivo, but whether it could be beneficial in CVB3-induced AVMC remains unclear. To confirm the aberrant cardiac ER stress is not secondary to CVB3 infection, we further determined whether inhibition of ER stress by TUDCA administration could attenuate CVB3 infection-induced AVMC development.

We detected that TUDCA attenuated cardiac pathological score, inflammatory cells infiltration (Figure 2), inflammatory cytokines mRNA expression (Figure S1), and reduced CVB3 titer (Figure S2).

Serum cTnI level is a sensitive indicator of myocardial injury, we used cTnI as a marker of cardiomyocyte damage during the active proliferative phase. Paralleled with reduced cardiac ER stress response, we found serum cTnI levels increased dramatically 7 days pi, while TUDCA administration significantly suppressed this effect (Figure 3A).

Significant left ventricular dysfunction occurs in CVB3-induced AVMC in mice. We further assayed the potential of TUDCA to inhibit CVB3-mediated cardiac dysfunction. We found that TUDCA effectively prevented cardiac ANP induction and heart weight/body weight ratio (HW/BW) increase (Figure 1C and 1D, Figure 3B). Cardiac function was determined by both Millar catheter and echocardiography. As Figure 3C-3F, Table S1 and S2 depicted, CVB3 infection led to significant reduced cardiac contractility and impaired diastolic function, while TUDCA administration could reverse cardiac function decline. Furthermore, we observed TUDCA significantly increased the survival rate in CVB3-infected...
mice (Figure 3G). Together, these findings suggest that ER stress plays an important role in CVB3 induced cardiac damage and dysfunction in mice.

**TUDCA suppresses CVB3 infection-induced cardiac CHOP expression and cardiomyocytes apoptosis in vivo**

It has been suggested that cardiomyocyte apoptosis contributes to development of CVB3-induced AVMC. Given ER stress could directly initiate proapoptotic signaling by activating its downstream CHOP, we hypothesized CHOP-mediated proapoptotic pathways may be involved in CVB3-induced AVMC. Heart of CVB3 infected mice exhibited similar positive staining area of CVB3 VP1 and CHOP (Figure S3). Both immunohistochemical staining and western blot analysis confirmed markedly elevated CHOP expression in heart of AVMC mice, which could be suppressed by TUDCA treatment (Figure 4A–4D).

We further assayed CHOP-mediated proapoptotic pathways in AVMC. In agreement with previous studies, we found cardiomyocyte apoptosis in CVB3-infected mice was significantly increased by TUNEL assay (Figure 4F and Figure S4), as well as an enhanced expression of cleaved caspase 3 in the infected mice hearts (Figure 4C and 4E). CHOP decreases Bcl-2 expression and leads to proapoptotic Bax activation. Indeed, Bax expression was increased while Bcl-2 expression was decreased in the AVMC hearts, leading to a reduction of the Bcl2/Bax ratio (Figure 5). These effects could all be blocked by TUDCA treatment (Figure 4 and 5).

**CHOP deficiency reduces CVB3-induced cardiomyocytes apoptosis in vivo**

To further understand the role of CHOP in CVB3-induced cardiomyocytes apoptosis, we analyzed cardiomyocytes apoptosis in CVB3-infected CHOP deficiency mice. We performed
TUNEL staining to identify apoptotic cardiomyocytes and found that CHOP deficiency markedly prevented CVB3-induced cardiomyocytes apoptosis 7 days *pi* (Figure 6A and Figure S5). Western blot analysis further confirmed that CHOP deficiency significantly attenuated markers of apoptosis pathway in heart, including cleaved caspase 3 (Figure 6B and 6D), and prevented Bcl-2 reduction and Bax induction in AVMC hearts (Figure 6E and 6F). Thus, these data support the notion that CHOP, a transcription factor involved in ER stress-initiated apoptotic signaling, participates in CVB3 induced cardiomyocytes apoptosis *in vivo*.

**Ablation of CHOP suppresses CVB3-induced AVMC *in vivo***

Encouraged by our preliminary data, the functional significance of CHOP in CVB3 infection-induced AVMC was investigated. As shown in Figure 7 and Supplementary Figure S6 and S7, CHOP deficiency significantly limited pathological score, macrophage infiltration, inflammatory cytokines mRNA expression, and viral replication 7 days *pi*. In addition, deficiency of CHOP effectively suppressed CVB3-induced serum cTnI elevation (Figure 8A).

We determined parameters of heart failure. Similar with previous reports, CHOP deficiency did not alter cardiac function (Tables S3)\(^{12,33}\). Compared with WT CVB3 infected mice, CHOP deficiency markedly reduced ANP expression and HW/BW ratio (Figure 6B and 6C, Figure 8B). Echocardiography and hemodynamic analysis were further performed. As Figure 8C-8F, Table S4 and S5 illustrated, CHOP deficiency prevented mice from reduced EF, FS, dP/dt max, dP/dt min and LVSP, as well as increased LVEDP, indicating its protective role in CVB3 induced-cardiac dysfunction. Additionally, CHOP deficiency mice
showed a significantly reduced mortality after infection (Figure 8G).

**Discussion**

Our study demonstrated that ER stress and its downstream activator CHOP are important factors in CVB3-induced AMVC development and cardiomyocytes apoptosis *in vivo*. Such evidence first came from experiment that ER stress was activated in heart of CVB3-induced AVMC mice, and was associated with enhanced cardiomyocytes apoptosis and cardiac CHOP expression. It is further supported from the results that ER stress inhibition markedly suppressed these effects, attenuated viral replication and cardiac injury, prevented cardiac dysfunction, and increased survival. We further demonstrated that genetically deficient of CHOP could suppress cardiomyocytes apoptosis, impede AVMC development and increase survival rate in CVB3-infected mice. These results indicate that inhibition of ER stress and CHOP could point to be a potential therapeutic strategy for AVMC treatment.

ER is one of the organelles that are highly susceptible to stimuli, including viral infection. Viruses utilize ER as the site of envelope protein synthesis and viral particle assembly, and some viruses develop to interfere UPR signaling for enhancement of infection and replication. CVB3 is a nonenveloped positive-strand RNA virus, and it requires ER in host cells for its replication. Recent reports indicated that CVB3 could trigger ER stress in HeLa and HL-1 cells, thus it is not surprising that ER stress is evoked in CVB3-induced AVMC hearts. Indeed, our data firstly demonstrated that all three branches of ER stress were activated in hearts of CVB3-infected AVMC mice, which was consistent with previous data in HeLa and HL-1 cells.
To cope with ER stress condition, UPR is activated to resist perturbations and restore ER functions. Despite the beneficial effect of UPR during transient ER stress, prolonged ER stress could activate CHOP, lead to cell apoptosis, and promote disease development. Reports already documented that cardiomyocytes apoptosis occurs and contributes to development and severity of CVB3-induced AVMC. Regarding the link between ER stress/CHOP signaling and apoptosis, it suggests that ER stress and its downstream activator CHOP may also be emerged in AVMC development induced by CVB3. Our observation truly confirmed this hypothesis by identifying CHOP activation accompanied with increased cardiomyocytes apoptosis in AVMC hearts.

Studies have documented that CHOP initiated proapoptotic pathways in several mechanisms, such as downregulation of the anti-apoptotic protein Bcl-2, and upregulation of the proapoptotic proteins Bax and Bim. In addition, CHOP activation leads to translocation of Bax protein to the mitochondria, and finally results in activation of caspase-3. Our finding indicates the reduction of Bcl-2/Bax ratio in CVB3-induced AVMC, further supporting that CHOP-mediated apoptosis plays a role in AVMC, which is mainly through a mitochondria-dependent pathway. However, the precise apoptosis cascade downstream of CHOP still remains unclear and requires further investigation.

While all of the results discussed above provided support to the hypothesis that ER stress/CHOP signaling plays a role in the pathogenesis of CVB3 infection-induced AVMC, they also raised a crucial question that whether altering ER stress/CHOP signaling could be utilized as a treatment strategy for CVB3-induced AVMC. TUDCA, a chemical chaperone, has been well demonstrated to be a classic ER stress inhibitor by improving ER folding.
capacity. It has been proved to be protective in various diseases, such as diabetes mellitus, hypertension, calcification, and even cardiac dysfunction via prevention of ER stress. We also observed that TUDCA administration markedly suppressed cardiac ER stress and CHOP induction, prevented cardiomyocytes apoptosis, cardiac inflammation and injury, cardiac dysfunction, and increased survival rate in our CVB3 inoculation-induced AVMC model. This further indicates ER stress in AVMC is not merely a secondary consequence.

Recent studies showed the protective effects of CHOP deficiency in various diseases, including myocardial ischemia/reperfusion injury and pressure overload-induced cardiac dysfunction. CVB3 infection induced cardiac injury and dysfunction, and caused mortality in mice, whereas these changes were significantly attenuated in CHOP deficient mice. In addition, we showed that these protective effects were associated with reduced cardiomyocytes apoptosis in vivo, possibly via restoring Bcl-2/Bax ratio and preventing caspase 3 activation. These findings also suggest that CHOP plays a crucial role in CVB3-induced AVMC progression.

The link between ER stress and viral replication is complicated. Some reports indicate that ER stress signaling promotes certain virus replication. It is interesting that TUDCA treatment or CHOP ablation also reduced CVB3 replication in vivo. This might be caused by reduced apoptosis that limits virus spreads and infects nearby cells. However, detailed mechanisms need to be explored.

The present study used TUNEL to determine the apoptotic cardiomyocytes. It should be noted that TUNEL staining may not only labeled late-stage apoptotic cell, but may also label DNA undergoing necrotic cell death.
To sum up, the ER stress–induced CHOP-mediated pathway is involved in the pathogenesis of CVB3-induced AVMC via promoting cardiomyocytes apoptosis. Inhibition of ER stress or genetic ablation of CHOP leads to reduction of cardiomyocytes apoptosis, prevention of cardiac injury, amelioration of cardiac function, and increase of survival rate in CVB3-induced AVMC. Our findings suggest the translational potentiality of ER stress/CHOP signaling in AVMC management.

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Disclosures

None.

References


17. Zhang HM, Ye X, Su Y, Yuan J, Liu Z, Stein DA and Yang D. Coxsackievirus B3...


42. Ceylan-Isik AF, Sreejayan N and Ren J. Endoplasmic reticulum chaperon tauroursodeoxycholic acid alleviates obesity-induced myocardial contractile dysfunction. *J Mol Cell Cardiol*. 2011;50:107-16.


44. Grasl-Kraupp B, Ruttkay-Nedecky B, Koudelka H, Bukowska K, Burch W and
Figure Legends

Figure 1. Endoplasmic reticulum (ER) stress inhibitor tauroursodeoxycholic acid (TUDCA) suppresses coxsackievirus B3 (CVB3) infection induced cardiac ER stress activation. (A) Representative ER stress marker KDEL staining in hearts of indicated intervention. Scale bar: 20μm. (B) TUDCA reduced CVB3 induced cardiac KDEL expression. (C) Western blot analysis of ER stress sensors activation and atrial natriuretic peptide (ANP) expression in hearts of different treatment. TUDCA reduced heart failure marker ANP (D), ER stress markers phosphorylated-PERK (p-PERK) (E), phosphorylated-IRE1α (p-IRE1α) (F) and ATF6 (G) expression induced by CVB3 inoculation. (Control group: n=10; CVB3 group: n=6; CVB3+TUDCA: n=8; **P<0.01 vs. control; ###P<0.01 vs. CVB3)

Figure 2. Histopathology and macrophage infiltration in hearts. (A) Representative histological (left panel) and macrophage marker F4/80 staining (right panel) of hearts with indicated intervention. Scale bar: 50μm. Endoplasmic reticulum (ER) stress inhibitor tauroursodeoxycholic acid (TUDCA) significantly reduced pathologic score (B) and macrophage infiltration (C) induced by coxsackievirus B3 (CVB3) infection. (Control group: n=10; CVB3 group: n=6; CVB3+TUDCA: n=8; **P<0.01 vs. control; ###P<0.01 vs. CVB3)

Figure 3. Inhibition of ER stress reduces cardiac damage, prevents cardiac dysfunction and promotes survival after coxsackievirus B3 (CVB3) infection. Tauroursodeoxycholic acid (TUDCA) administration markedly reduced cardiac troponin I (A) induction and heart weight/body weight (HW/BW) ratio (B) induced by CVB3 infection. (C) TUDCA prevented...
reduction of dP/dt max and dP/dt min caused by CVB3 infection. (D) Representative M-mode images of hearts with indicated treatment. TUDCA reversed ejection fraction (EF) (E) and fractional shortening (FS) (F) decrease induced by CVB3 infection. (G) TUDCA treatment increased survival rate after CVB3 infection. (Control group: n=10; CVB3 group: n=6; CVB3+TUDCA: n=8; **P<0.01 vs. control; ##P<0.01 vs. CVB3)

Figure 4. Tauroursodeoxycholic acid (TUDCA) prevents coxsackievirus B3 (CVB3) induced cardiac C/EBP homologous protein (CHOP) activation and cardiomyocytes apoptosis in vivo. (A) Representative images of CHOP staining in hearts of indicated treatment. Scale bar: 20μm. (B) TUDCA significantly reduced CHOP staining induced by CVB3 infection. Scale bar: 20μm. (C) Cardiomyocytes apoptosis was indicated by TUNEL staining. CVB3 infection caused increased cardiomyocytes apoptosis, while TUDCA administration prevented this effect. (D) Western blot analysis of cardiac CHOP and cleaved caspase 3. (E and F) CVB3 infection led to significantly induction of CHOP and cleaved caspase 3 expression in hearts, while TUDCA treatment markedly prevented these effects. (Control group: n=10; CVB3 group: n=6; CVB3+TUDCA: n=8; **P<0.01 vs. control; ##P<0.01 vs. CVB3)

Figure 5. Inhibition of endoplasmic reticulum (ER) stress restores cardiac Bcl-2/Bax ratio reduction induced by coxsackievirus B3 (CVB3) inoculation. (A) Western blot analysis of cardiac Bcl-2 and Bax expression. (B and C) CVB3 infection significantly reduced Bcl-2 expression and elevated Bax expression in hearts, while tauroursodeoxycholic
acid (TUDCA) markedly blocked these effects. (Control group: n=10; CVB3 group: n=6; CVB3+TUDCA: n=8; **P<0.01 vs. control; ##P<0.01 vs. CVB3)

Figure 6. C/EBP homologues protein (CHOP) deficiency attenuates cardiomyocytes apoptosis induced by coxsackievirus B3 (CVB3) infection in vivo. (A) CHOP deficiency reduced cardiomyocytes apoptosis after CVB3 infection as indicated by TUNEL staining. (B) Western blot analysis of cardiac cleaved caspase 3 and atrial natriuretic peptide (ANP) expression. Compared with wild type (WT) mice, CHOP deficiency significantly reduced ANP (C) and cleaved caspase 3 (D) expression induced by CVB3 infection. (E) Western blot analysis of cardiac Bcl-2 and Bax expression. (F) CHOP deficiency significantly prevented cardiac Bcl-2 reduction and Bax induction caused by CVB3 infection. (WT group: n=10; WT + CVB3 group: n=5; CHOP−/− + CVB3 group: n=9; **P<0.01 vs. WT; ##P<0.01 vs. WT + CVB3)

Figure 7. Genetic knockout of C/EBP homologues protein (CHOP) reduces acute viral myocarditis severity and macrophage infiltration after coxsackievirus B3 (CVB3) infection. (A) Representative histological (left panel) and macrophage marker F4/80 staining (right panel) of hearts with indicated intervention. Scale bar: 50μm. Compared with wild type (WT) mice, ablation of CHOP significantly reduced pathologic score (B) and macrophage infiltration (C) induced by coxsackievirus B3 (CVB3) infection. (WT group: n=10; WT + CVB3 group: n=5; CHOP−/− + CVB3 group: n=9; **P<0.01 vs. WT; ##P<0.01 vs. WT + CVB3)
Figure 8. Deficiency of C/EBP homologues protein (CHOP) reduces cardiac damage, prevents cardiac dysfunction and promotes survival after coxsackievirus B3 (CVB3) infection. Compared with wild type (WT) mice, CHOP deficiency markedly reduced cardiac troponin I (A) induction and heart weight/body weight (HW/BW) ratio (B) induced by CVB3 infection. (C) CHOP ablation prevented reduction of dP/dt max and dP/dt min caused by CVB3 infection. (D) Representative M-mode images of hearts with indicated treatment. CHOP deficiency reversed ejection fraction (EF) (E) and fractional shortening (FS) (F) decrease induced by CVB3 infection. (G) CHOP deficiency promoted survival after CVB3 infection. (WT group: n=10; WT + CVB3 group: n=5; CHOP−/− + CVB3 group: n=9).

**P<0.01 vs. WT; ##P<0.01 vs. WT + CVB3)**
A

WT

HE

F4/80

WT

CHOP−/−

CVB3

B

CVB3

Pathologic score

2.5

2.0

1.5

1.0

0.5

0.0

WT

WT

CHOP−/−

C

CVB3

F4/80 staining area (%)

10

8

6

4

2

0

WT

WT

CHOP−/−

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Figure S1. Endoplasmic reticulum (ER) stress inhibitor tauroursodeoxycholic acid (TUDCA) administration significantly reduced TNF-α, IL-6 and IL-8 mRNA expression in heart (A) and liver (B) 7 days after coxsackievirus B3 (CVB3) infection. (Control group: n=10; CVB3 group: n=6; CVB3+TUDCA: n=8; **P<0.01 vs. Control; *P<0.01 vs. CVB3)
Figure S2. Endoplasmic reticulum (ER) stress inhibitor tauroursodeoxycholic acid (TUDCA) administration significantly reduced heart, liver and pancreas viral titer 7 days after coxsackievirus B3 (CVB3) infection. (Control group: n=10; CVB3 group: n=6; CVB3+TUDCA: n=8; **P<0.01 vs. CVB3)
Figure S3. Representative immunohistochemistry staining of coxsackievirus B3 viral protein 1 (CVB3 VP1) and C/EBP homologues protein (CHOP) in continuous sections of CVB3 infected mice hearts. CHOP exhibited similar positive staining regions as CVB3 VP1.
Figure S4. Representative TUNEL staining of mice with different treatment.

CVB3: coxsackievirus B3; TUDCA: tauroursodeoxycholic acid.
Figure S5. Representative TUNEL staining of different genotype mice with indicated treatment. CVB3: coxsackievirus B3; CHOP: C/EBP Homologous Protein.
Figure S6. Compared with wild type (WT) mice, C/EBP homologues protein (CHOP) significantly reduced TNF-α, IL-6 and IL-8 mRNA expression in heart (A) and liver (B) 7 days after coxsackievirus B3 (CVB3) infection. (WT group: n=10; WT + CVB3 group: n=5; CHOP−/− + CVB3 group: n=9; **P<0.01 vs. WT; ###P<0.01 vs. WT + CVB3)
Compared with wild type (WT) mice, C/EBP homologues protein (CHOP) significantly reduced cardiac viral titer 7 days after CVB3 infection. (WT group: n=10; WT + CVB3 group: n=5; CHOP−/− + CVB3 group: n=9; *P<0.05 vs. WT + CVB3; **P<0.01 vs. WT + CVB3)
### Table S1. Echocardiographic Parameters of Mice with Indicated Treatment.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CVB3</th>
<th>CVB3+TUDCA</th>
<th>Control</th>
<th>CVB3</th>
<th>CVB3+TUDCA</th>
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<td>Day 3</td>
<td>Day 5</td>
<td>Day 7</td>
<td>Day 3</td>
<td>Day 5</td>
<td>Day 7</td>
</tr>
<tr>
<td>LVID-d(mm)</td>
<td>1.93±0.08</td>
<td>2.03±0.10</td>
<td>1.97±0.11</td>
<td>1.97±0.09</td>
<td>2.31±0.13**</td>
<td>2.13±0.12</td>
<td>1.95±0.07</td>
<td>2.47±0.11**</td>
<td>2.20±0.10#</td>
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<tr>
<td>LVID-s(mm)</td>
<td>0.99±0.10</td>
<td>1.09±0.08</td>
<td>1.04±0.09</td>
<td>0.97±0.09</td>
<td>1.37±0.10**</td>
<td>1.14±0.09#</td>
<td>0.98±0.09</td>
<td>1.47±0.09**</td>
<td>1.21±0.10#</td>
</tr>
<tr>
<td>LVPW-d(mm)</td>
<td>0.98±0.08</td>
<td>0.88±0.09</td>
<td>0.92±0.07</td>
<td>0.98±0.08</td>
<td>0.84±0.08</td>
<td>0.88±0.07</td>
<td>0.97±0.08</td>
<td>0.74±0.12*</td>
<td>0.85±0.11</td>
</tr>
<tr>
<td>LVPW-s(mm)</td>
<td>1.63±0.10</td>
<td>1.52±0.11</td>
<td>1.57±0.05</td>
<td>1.62±0.05</td>
<td>1.38±0.10**</td>
<td>1.40±0.08</td>
<td>1.60±0.07</td>
<td>1.28±0.08**</td>
<td>1.45±0.06#</td>
</tr>
<tr>
<td>IVS-d(mm)</td>
<td>0.99±0.08</td>
<td>0.93±0.08</td>
<td>0.83±0.08</td>
<td>0.94±0.08</td>
<td>0.78±0.08*</td>
<td>0.87±0.09</td>
<td>0.96±0.07</td>
<td>0.76±0.07*</td>
<td>0.83±0.08</td>
</tr>
<tr>
<td>IVS-s(mm)</td>
<td>1.49±0.10</td>
<td>1.40±0.07</td>
<td>1.49±0.08</td>
<td>1.54±0.11</td>
<td>1.39±0.11</td>
<td>1.46±0.10</td>
<td>1.58±0.09</td>
<td>1.23±0.10**</td>
<td>1.35±0.09</td>
</tr>
<tr>
<td>EF(%)</td>
<td>86.66±1.35</td>
<td>80.74±4.65</td>
<td>83.33±3.76</td>
<td>83.81±3.03</td>
<td>71.74±3.48**</td>
<td>76.99±2.01#</td>
<td>84.54±1.65</td>
<td>59.74±2.65**</td>
<td>67.33±2.45##</td>
</tr>
<tr>
<td>FS(%)</td>
<td>52.67±1.12</td>
<td>49.62±3.08</td>
<td>50.98±3.11</td>
<td>55.88±1.04</td>
<td>38.40±4.21**</td>
<td>44.01±2.13#</td>
<td>54.89±0.97</td>
<td>30.62±1.75**</td>
<td>39.01±1.92##</td>
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</table>

CVB3: coxsackievirus B3; TUDCA: tauroursodeoxycholic acid

n=10 for control; n=11 for CVB3; n=11 for CVB3+TUDCA

Data present as mean±SE. *P<0.05 vs. control; **P<0.01 vs. control; #P<0.05 vs. CVB3; ##P<0.01 vs. CVB3
**Table S2.** Hemodynamic Parameters of Mice with Indicated Treatment.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CVB3</th>
<th>CVB3+TUDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
<td></td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>103.86±1.09</td>
<td>67.27±2.31**</td>
<td>78.14±2.97##</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.25±1.24</td>
<td>14.77±2.14**</td>
<td>11.47±1.73##</td>
</tr>
<tr>
<td>LVDP (mmHg)</td>
<td>100.41±1.31</td>
<td>54.12±3.09**</td>
<td>66.14±2.78##</td>
</tr>
<tr>
<td>dP/dt max (mmHg/s)</td>
<td>9128±632</td>
<td>4512±376**</td>
<td>6173±489#</td>
</tr>
<tr>
<td>dP/dt min (mmHg/s)</td>
<td>-5377±579</td>
<td>-2853±338**</td>
<td>-3924±396#</td>
</tr>
</tbody>
</table>

CVB3: coxsackievirus B3; TUDCA: tauroursodeoxycholic acid

n=10 for control; n=11 for CVB3; n=11 for CVB3+TUDCA

Data present as mean±SE. *P<0.05 vs. control; **P<0.01 vs. control; #P<0.05 vs. CVB3; ##P<0.01 vs. CVB3
Table S3. Baseline analysis of mice with indicated genotypes.

<table>
<thead>
<tr>
<th>Metric</th>
<th>WT</th>
<th>CHOP⁻/⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVID-d (mm)</td>
<td>1.93±0.07</td>
<td>1.91±0.09</td>
</tr>
<tr>
<td>LVID-s (mm)</td>
<td>1.01±0.08</td>
<td>0.98±0.11</td>
</tr>
<tr>
<td>LVPW-d (mm)</td>
<td>0.97±0.06</td>
<td>0.95±0.09</td>
</tr>
<tr>
<td>LVPW-s (mm)</td>
<td>1.55±0.08</td>
<td>1.58±0.07</td>
</tr>
<tr>
<td>IVS-d (mm)</td>
<td>0.95±0.07</td>
<td>0.99±0.08</td>
</tr>
<tr>
<td>IVS-s (mm)</td>
<td>1.51±0.06</td>
<td>1.57±0.07</td>
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<tr>
<td>EF (%)</td>
<td>85.02±4.71</td>
<td>86.72±2.89</td>
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<tr>
<td>FS (%)</td>
<td>53.12±1.06</td>
<td>54.72±0.93</td>
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<tr>
<td>LVSP (mmHg)</td>
<td>103.23±1.56</td>
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</tr>
<tr>
<td>LVEDP (mmHg)</td>
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<tr>
<td>LVDP (mmHg)</td>
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<td>dP/dt max (mmHg/s)</td>
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<td>dP/dt min (mmHg/s)</td>
<td>-5737±385</td>
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CHOP: C/EBP Homologous Protein

n=5 for WT; n=5 for CHOP⁻/⁻
Table S4. Echocardiographic Parameters of Different Genotype Mice with Indicated Treatment.

<table>
<thead>
<tr>
<th></th>
<th>CVB3</th>
<th>CVB3</th>
<th>CVB3</th>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>WT</td>
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<tr>
<td></td>
<td>Day 3</td>
<td>Day 5</td>
<td>Day 7</td>
</tr>
<tr>
<td>LVID-d(mm)</td>
<td>1.88±0.05</td>
<td>1.97±0.11</td>
<td>1.93±0.09</td>
</tr>
<tr>
<td>LVID-s(mm)</td>
<td>1.01±0.06</td>
<td>1.09±0.15</td>
<td>1.05±0.08</td>
</tr>
<tr>
<td>LVPW-d(mm)</td>
<td>0.93±0.09</td>
<td>0.88±0.11</td>
<td>0.92±0.07</td>
</tr>
<tr>
<td>LVPW-s(mm)</td>
<td>1.55±0.05</td>
<td>1.46±0.09</td>
<td>1.53±0.09</td>
</tr>
<tr>
<td>IVS-d(mm)</td>
<td>0.99±0.08</td>
<td>0.84±0.07*</td>
<td>0.93±0.06</td>
</tr>
<tr>
<td>IVS-s(mm)</td>
<td>1.58±0.09</td>
<td>1.50±0.08</td>
<td>1.56±0.08</td>
</tr>
<tr>
<td>EF(%)</td>
<td>85.33±1.64</td>
<td>80.30±2.32</td>
<td>82.31±4.24</td>
</tr>
<tr>
<td>FS(%)</td>
<td>55.32±0.79</td>
<td>47.44±5.53*</td>
<td>51.55±3.82</td>
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</tbody>
</table>

CVB3: coxsackievirus B3; CHOP: C/EBP Homologous Protein

n=10 for WT; n=10 for WT+CVB3; n=10 for CHOP<sup>-/-</sup>+CVB3

Data present as mean±SE. *P<0.05 vs. WT; **P<0.01 vs. WT; #P<0.05 vs. WT + CVB3; ##P<0.01 vs. WT + CVB3
Table S5. Hemodynamic Parameters of Different Genotype Mice with Indicated Treatment.

<table>
<thead>
<tr>
<th></th>
<th>CVB3</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>WT</td>
<td>CHOP⁻⁻⁻⁻</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>107.12±1.27</td>
<td>59.07±3.58**</td>
<td>80.95±3.36##</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.44±1.52</td>
<td>15.46±2.35**</td>
<td>8.02±2.12##</td>
</tr>
<tr>
<td>LVDP (mmHg)</td>
<td>103.66±1.49</td>
<td>43.52±2.89**</td>
<td>73.37±2.97##</td>
</tr>
<tr>
<td>dP/dt max (mmHg/s)</td>
<td>9392±494</td>
<td>4852±303**</td>
<td>7253±457##</td>
</tr>
<tr>
<td>dP/dt min (mmHg/s)</td>
<td>-5548±435</td>
<td>-2709±287**</td>
<td>-4392±348##</td>
</tr>
</tbody>
</table>

CVB3: coxsackievirus B3; CHOP: C/EBP Homologous Protein

n=10 for WT; n=10 for WT+CVB3; n=10 for CHOP⁻⁻⁻⁻+CVB3

Data present as mean±SE. *P<0.05 vs. WT; **P<0.01 vs. WT; #P<0.05 vs. WT + CVB3; ##P<0.01 vs. WT + CVB3
Figure S1. Endoplasmic reticulum (ER) stress inhibitor tauroursodeoxycholic acid (TUDCA) administration significantly reduced TNF-α, IL-6 and IL-8 mRNA expression in heart (A) and liver (B) 7 days after coxsackievirus B3 (CVB3) infection. (Control group: n=10; CVB3 group: n=6; CVB3+TUDCA: n=8; **P<0.01 vs. Control; *P<0.01 vs. CVB3)
Figure S2. Endoplasmic reticulum (ER) stress inhibitor tauroursodeoxycholic acid (TUDCA) administration significantly reduced heart, liver and pancreas viral titer 7 days after coxsackievirus B3 (CVB3) infection. (Control group: n=10; CVB3 group: n=6; CVB3+TUDCA: n=8; **P<0.01 vs. CVB3)
Figure S3. Representative immunohistochemistry staining of coxsackievirus B3 viral protein 1 (CVB3 VP1) and C/EBP homologues protein (CHOP) in continuous sections of CVB3 infected mice hearts. CHOP exhibited similar positive staining regions as CVB3 VP1.
Figure S4. Representative TUNEL staining of mice with different treatment.

CVB3: coxsackievirus B3; TUDCA: tauroursodeoxycholic acid.
Figure S5. Representative TUNEL staining of different genotype mice with indicated treatment. CVB3: coxsackievirus B3; CHOP: C/EBP Homologous Protein.
Figure S6. Compared with wild type (WT) mice, C/EBP homologues protein (CHOP) significantly reduced TNF-α, IL-6 and IL-8 mRNA expression in heart (A) and liver (B) 7 days after coxsackievirus B3 (CVB3) infection. (WT group: n=10; WT + CVB3 group: n=5; CHOP<sup>−/−</sup> + CVB3 group: n=9; **P<0.01 vs. WT; ###P<0.01 vs. WT + CVB3)
Figure S7. Compared with wild type (WT) mice, C/EBP homologues protein (CHOP) significantly reduced cardiac viral titer 7 days after CVB3 infection.

(WT group: n=10; WT + CVB3 group: n=5; CHOP−/− + CVB3 group: n=9; *P<0.05 vs. WT + CVB3; **P<0.01 vs. WT + CVB3)
**Table S1.** Echocardiographic Parameters of Mice with Indicated Treatment.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CVB3</th>
<th>CVB3+TUDCA</th>
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<th>CVB3</th>
<th>CVB3+TUDCA</th>
<th>Control</th>
<th>CVB3</th>
<th>CVB3+TUDCA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
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<td>Day 7</td>
<td>Day 3</td>
<td>Day 5</td>
<td>Day 7</td>
<td>Day 3</td>
<td>Day 5</td>
<td>Day 7</td>
</tr>
<tr>
<td>LVID-d (mm)</td>
<td>1.93±0.08</td>
<td>2.03±0.10</td>
<td>1.97±0.11</td>
<td>1.97±0.09</td>
<td>2.31±0.13**</td>
<td>2.13±0.12</td>
<td>1.95±0.07</td>
<td>2.47±0.11**</td>
<td>2.20±0.10#</td>
</tr>
<tr>
<td>LVID-s (mm)</td>
<td>0.99±0.10</td>
<td>1.09±0.08</td>
<td>1.04±0.09</td>
<td>0.97±0.09</td>
<td>1.37±0.10**</td>
<td>1.14±0.09#</td>
<td>0.98±0.09</td>
<td>1.47±0.09**</td>
<td>1.21±0.10#</td>
</tr>
<tr>
<td>LVPW-d (mm)</td>
<td>0.98±0.08</td>
<td>0.88±0.09</td>
<td>0.92±0.07</td>
<td>0.98±0.08</td>
<td>0.84±0.08</td>
<td>0.88±0.07</td>
<td>0.97±0.08</td>
<td>0.74±0.12*</td>
<td>0.85±0.11</td>
</tr>
<tr>
<td>LVPW-s (mm)</td>
<td>1.63±0.10</td>
<td>1.52±0.11</td>
<td>1.57±0.05</td>
<td>1.62±0.05</td>
<td>1.38±0.10**</td>
<td>1.40±0.08</td>
<td>1.60±0.07</td>
<td>1.28±0.08**</td>
<td>1.45±0.06#</td>
</tr>
<tr>
<td>IVS-d (mm)</td>
<td>0.99±0.08</td>
<td>0.93±0.08</td>
<td>0.83±0.08</td>
<td>0.94±0.08</td>
<td>0.78±0.08*</td>
<td>0.87±0.09</td>
<td>0.96±0.07</td>
<td>0.76±0.07*</td>
<td>0.83±0.08</td>
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<tr>
<td>IVS-s (mm)</td>
<td>1.49±0.10</td>
<td>1.40±0.07</td>
<td>1.49±0.08</td>
<td>1.54±0.11</td>
<td>1.39±0.11</td>
<td>1.46±0.10</td>
<td>1.58±0.09</td>
<td>1.23±0.10**</td>
<td>1.35±0.09</td>
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<tr>
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<td>80.74±4.65</td>
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<td>83.81±3.03</td>
<td>71.74±3.48**</td>
<td>76.99±2.01#</td>
<td>84.54±1.65</td>
<td>59.74±2.65**</td>
<td>67.33±2.45##</td>
</tr>
<tr>
<td>FS (%)</td>
<td>52.67±1.12</td>
<td>49.62±3.08</td>
<td>50.98±3.11</td>
<td>55.88±1.04</td>
<td>38.40±4.21**</td>
<td>44.01±2.13#</td>
<td>54.89±0.97</td>
<td>30.62±1.75**</td>
<td>39.01±1.92##</td>
</tr>
</tbody>
</table>

CVB3: coxsackievirus B3; TUDCA: tauroursodeoxycholic acid

n=10 for control; n=11 for CVB3; n=11 for CVB3+TUDCA

Data present as mean±SE. *P<0.05 vs. control; **P<0.01 vs. control; #P<0.05 vs. CVB3; ##P<0.01 vs. CVB3
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CVB3</th>
<th>CVB3+TUDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 7</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>103.86±1.09</td>
<td>67.27±2.31**</td>
<td>78.14±2.97##</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
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<td>dP/dt min (mmHg/s)</td>
<td>-5377±579</td>
<td>-2853±338**</td>
<td>-3924±396#</td>
</tr>
</tbody>
</table>

CVB3: coxsackievirus B3; TUDCA: tauroursodeoxycholic acid

n=10 for control; n=11 for CVB3; n=11 for CVB3+TUDCA

Data present as mean±SE. *P<0.05 vs. control; **P<0.01 vs. control; #P<0.05 vs. CVB3; ###P<0.01 vs. CVB3
Table S3. Baseline analysis of mice with indicated genotypes.

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>CHOP&lt;sup&gt;−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVID-d(mm)</td>
<td>1.93±0.07</td>
<td>1.91±0.09</td>
</tr>
<tr>
<td>LVID-s(mm)</td>
<td>1.01±0.08</td>
<td>0.98±0.11</td>
</tr>
<tr>
<td>LVPW-d(mm)</td>
<td>0.97±0.06</td>
<td>0.95±0.09</td>
</tr>
<tr>
<td>LVPW-s(mm)</td>
<td>1.55±0.08</td>
<td>1.58±0.07</td>
</tr>
<tr>
<td>IVS-d(mm)</td>
<td>0.95±0.07</td>
<td>0.99±0.08</td>
</tr>
<tr>
<td>IVS-s(mm)</td>
<td>1.51±0.06</td>
<td>1.57±0.07</td>
</tr>
<tr>
<td>EF(%)</td>
<td>85.02±4.71</td>
<td>86.72±2.89</td>
</tr>
<tr>
<td>FS(%)</td>
<td>53.12±1.06</td>
<td>54.72±0.93</td>
</tr>
<tr>
<td>LVSP(mmHg)</td>
<td>103.23±1.56</td>
<td>104.76±2.18</td>
</tr>
<tr>
<td>LVEDP(mmHg)</td>
<td>4.20±2.06</td>
<td>3.67±1.37</td>
</tr>
<tr>
<td>LVDP(mmHg)</td>
<td>105.48±1.66</td>
<td>106.03±2.02</td>
</tr>
<tr>
<td>dP/dt max(mmHg/s)</td>
<td>9545±416</td>
<td>9347±532</td>
</tr>
<tr>
<td>dP/dt min(mmHg/s)</td>
<td>-5737±385</td>
<td>-5632±493</td>
</tr>
</tbody>
</table>

CHOP: C/EBP Homologous Protein

n=5 for WT; n=5 for CHOP<sup>−/−</sup>
Table S4. Echocardiographic Parameters of Different Genotype Mice with Indicated Treatment.

<table>
<thead>
<tr>
<th></th>
<th>CVB3</th>
<th></th>
<th>CVB3</th>
<th></th>
<th>CVB3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>CHOP&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>WT</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 5</td>
<td>Day 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVID-d (mm)</td>
<td>1.88±0.05</td>
<td>1.97±0.11</td>
<td>1.93±0.09</td>
<td>1.90±0.07</td>
<td>2.32±0.13&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVID-s (mm)</td>
<td>1.01±0.06</td>
<td>1.09±0.15</td>
<td>1.05±0.08</td>
<td>0.99±0.07</td>
<td>1.39±0.09&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVPW-d (mm)</td>
<td>0.93±0.09</td>
<td>0.88±0.11</td>
<td>0.92±0.07</td>
<td>0.95±0.07</td>
<td>0.83±0.08</td>
</tr>
<tr>
<td>LVPW-s (mm)</td>
<td>1.55±0.05</td>
<td>1.46±0.09</td>
<td>1.53±0.09</td>
<td>1.57±0.09</td>
<td>1.40±0.05&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>IVS-d (mm)</td>
<td>0.99±0.08</td>
<td>0.84±0.07&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.93±0.06</td>
<td>0.95±0.06</td>
<td>0.77±0.12&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>IVS-s (mm)</td>
<td>1.58±0.09</td>
<td>1.50±0.08</td>
<td>1.56±0.08</td>
<td>1.54±0.06</td>
<td>1.37±0.11&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>EF(%)</td>
<td>85.33±1.64</td>
<td>80.30±2.32</td>
<td>82.31±4.24</td>
<td>87.88±1.42</td>
<td>68.30±5.01&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>FS(%)</td>
<td>55.32±0.79</td>
<td>47.44±5.53&lt;sup&gt;*&lt;/sup&gt;</td>
<td>51.55±3.82</td>
<td>54.18±0.92</td>
<td>36.44±6.23&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

CVB3: coxsackievirus B3; CHOP: C/EBP Homologous Protein

n=10 for WT; n=10 for WT+CVB3; n=10 for CHOP<sup>−/−</sup>+CVB3

Data present as mean±SE. *P<0.05 vs. WT; **P<0.01 vs. WT; #P<0.05 vs. WT + CVB3; ##P<0.01 vs. WT + CVB3
Table S5. Hemodynamic Parameters of Different Genotype Mice with Indicated Treatment.

<table>
<thead>
<tr>
<th></th>
<th>CVB3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>107.12±1.27</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.44±1.52</td>
</tr>
<tr>
<td>LVDP (mmHg)</td>
<td>103.66±1.49</td>
</tr>
<tr>
<td>dP/dt max (mmHg/s)</td>
<td>9392±494</td>
</tr>
<tr>
<td>dP/dt min (mmHg/s)</td>
<td>-5548±435</td>
</tr>
</tbody>
</table>

CVB3: coxsackievirus B3; CHOP: C/EBP Homologous Protein

n=10 for WT; n=10 for WT+CVB3; n=10 for CHOP⁻/⁻+CVB3

Data present as mean±SE. *P<0.05 vs. WT; **P<0.01 vs. WT; #P<0.05 vs. WT + CVB3; ##P<0.01 vs. WT + CVB3