CYLD exaggerates pressure overload-induced cardiomyopathy via suppressing autolysosome efflux in cardiomyocytes

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Running Title: CYLD in autolysosome efflux

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I. Full Description of Methods

Animals

All animals were kept on a 12-hour light/dark cycle in a temperature-controlled room with ad libitum access to food and water. All animals were treated in compliance with the USA National Institute of Health Guideline for Care and Use of Laboratory Animals. The use of animals and all animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Shandong University, China, and the University of South Carolina, USA.

Cardiomyocyte-restricted (CR) Cyld transgenic (Tg) (CR-Cyld Tg) mice in a FVB/NJ genetic background were generated as we previously described [1, 2]. Briefly, full-length human Cyld cDNA was cloned into a vector containing the alpha-myosin heavy chain (α-MHC) promoter (Figure S1A and B) and was used for microinjection at Medical University of South Carolina (MUSC) Transgenic Core. CR-Cyld Tg mice were screened by PCR of tail DNA using a two-primer reaction that identified a ~700 bp product from the transgene. The forward primer was 5'-AGA AGC CTA GCC CAC ACC AGA AAT-3' (corresponding to the α-MHC promoter). The reverse primer was 5'-CTT GTC CAA TGC AAC AAA CAC GCC-3' (corresponding to the transgene of human Cyld). Representative genotyping results are shown in the upper panel of Fig. S1C. Two lines with moderately overexpression levels of CYLD were maintained (Figure S1C lower panel). Western blot analyses confirmed that CYLD was highly overexpressed in the heart and no overexpression of CYLD was observed in the other organs, with the exception of the lung, which showed a slight increase in CYLD protein expression compared with the nontransgenic control (Fig. S1E) as we observed previously [1]. The littermates of wild type (WT), CR-tTA Tg, CR-Atg7 Tg, and CR-Atg7×tTA Tg mice were generated by crossing WT with CR-Atg7×tTA Tg mice in a FVB/NJ genetic background as we previously described [3]. The genotyping for Atg7 was carried out by PCR of tail DNA using a two-primer (one pair) reaction that identified a 359 bp product from the transgene. The genotyping for tTA was carried out by PCR of tail DNA using a four-primer (one pair for tTA and another pair for WT) reaction that identified a ~500 bp product from the transgene and a ~270 bp product from wildtype mice. The littermates of WT, CR-tTA Tg, CR-Atg7 Tg, CR-Cyld×tTA Tg, CR-Atg7×tTA Tg, and CR-Cyld×Atg7×tTA Tg mice were generated by crossbreeding between CR-Cyld Tg and CR-Atg7×tTA Tg mice. Littermates of WT and Cyld knockout (KO) mice were generated using heterozygote breeding pairs as previously described [4]. The genotyping for Cyld KO was carried out by PCR of tail DNA using a three-primer (two pairs) reaction that identified a 220 bp product from the knockout and a 194 bp product from WT mice. Genotyping information is provided in supplementary Table S1.

Transverse Aortic Arch Constriction (TAC)

Male and female littermates of mice with indicated genotypes at ages of 10 weeks were subject to sham or TAC operation for 2, 4 or 8 weeks. The sham or TAC operation in mice was performed under deep anesthesia as previously described [5]. Briefly, mice were anesthetized with 3% isoflurane and maintained with 1.5% isoflurane in room air supplemented with 100% O_2. The use of a horizontal incision at the level of the suprasternal notch allows direct visualization of the transverse aorta without entering the

pleural space and thus obviates the need for mechanical ventilation. The transverse aorta was banded between the right innominate and left carotid arteries to a 27-gauge needle using a 6-0 silk suture. Sham operation on mice were similar but without actual aortic banding and these mice served as a control group for all experimental groups. Cardiac hypertrophy was determined by heart weight–to–tibial length (HW/TIBIA) ratio, heart weight–to–body weight (HW/BW) ratio and expression levels of cardiac hypertrophy marker genes including atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), alpha-myosin heavy chain (α-MHC), beta-myosin heavy chain (β-MHC), sarco-endoplasmic reticulum calcium ATPase2a (SERCA2a).

Echocardiographic Analysis

Echocardiography was performed on anesthetized mice using the Vevo 3100 High-Resolution Imaging System (VisualSonics Inc, Toronto, Canada) with a 30-MHz high-frequency linear transducer as previously described [6]. Briefly, mice were anesthetized with 3% isoflurane and maintained with 1.5% isoflurane in room air supplemented with 100% O_2. After the anterior chest was naired, the animals were placed on a warming pad to maintain normothermia. The echocardiographic gel was warmed before use to avoid hypothermia. Care was taken to avoid excessive pressure on the thorax, which can induce bradycardia. Two-dimensional (2D) long axis images of the left ventricle (LV) were obtained at the plane of the aortic and mitral valves where the LV cavity is largest and visualization of the LV apex is adequate, and a short-axis image was recorded at the level of the papillary muscles. A 2D guided M-mode echocardiogram was recorded through the anterior and posterior LV walls at 21 frames/sec. Images were obtained at the level of the papillary muscle tips, and measurements were then performed to obtain the LV internal dimension (LVID; in mm), interventricular septum thickness (IVS; in mm), and LV posterior wall thickness (LVPW; in mm). LV percent fractional shortening FS (%) was calculated via VisualSonics Measurement Software. The distal transvers aortic flow velocity (distal to constriction in TAC mice) was measured by pulsed wave (PW) Doppler to assess the presence of TAC with the pressure gradient estimated using the modified Bernoulli equation (pressure gradient (mmHg) = 4*velocity (m/s) ^{2})

Pathology

Mice were anesthetized and perfused via the LV apex with saline (0.9% NaCl) to wash out the blood in the heart. Then, the hearts were harvested, dried on gauze, weighed, dissected, and frozen. Lungs and tibias were also dissected. Lungs were dried on gauze and weighed. The length of the tibia from the condyles to the tip of the medial malleolus was measured by micrometer calipers.

Evens Blue Labelling

Evans blue dye becomes intensely red fluorescent when conjugated to albumin in the circulation. The dye/albumin complexes are excluded from cells with intact plasma membranes while accumulating in damaged myofibers when the muscle cell membrane is broken, thus providing a dye-exclusion viability test. The red auto-fluorescence accumulated in myocardium has been used as a histopathological sign of cardiomyocyte necrosis. Briefly, mice were subject to a single intraperitoneal injection of Evans blue (100 mg/kg, Cat#: E2129, Sigma-Aldrich, USA) 18 h prior to harvesting tissues. Harvested

hearts were fixed in 4% paraformaldehyde and then embedded in paraffin. Paraffin sections were prepared (5 μm, Reichert-Jung 2030 Paraffin Rotary Microtome, Holly, MI, USA) and stored at room temperature until staining. Myocardial cellular membranes were stained with Wheat Germ Agglutinin (WGA), Alexa Fluor® 488 Conjugate (Cat#: W11261, Invitrogen Corp., Carlsbad, CA, USA). Evan blue dye positive areas (red) indicate myocardial necrosis. Sections were observed using a fluorescence microscope (Nikon Eclipse 80i; Nikon Instruments Inc. Tokyo, Japan) at 200 × magnification. Eight fields of each section were randomly photographed using NIS-Elements F 4.0 imaging software (Nikon Instruments Inc. Tokyo, Japan) and the percentage of Evans blue positive areas (red) were measured using Image-Pro Plus software (Media Cybernetics, Inc., Bethesda, MD, USA). At least two section of each heart were analyzed.

Histological and Immunochemical Analysis

Hearts were cannulated via the LV apex, cleared by perfusion with saline (0.9% NaCl) at 90 mmHg, arrested in diastole with 10% KCl (w/v in ddH_2O), fixed by perfusion with 4% paraformaldehyde, and embedded in paraffin. Paraffin sections were prepared (5 μm, Reichert-Jung 2030 Paraffin Rotary Microtome, Holly, MI, USA) and stored at room temperature until staining.

For LV cardiomyocyte cross-sectional area, coronal sections were deparaffinized and the cardiomyocyte membranes were stained with Alexa Fluor 488 conjugated wheat germ agglutinin (WGA) (Cat#: W11261, Invitrogen Corp., Carlsbad, CA, USA) and observed using a fluorescence microscope (Nikon E600 Widefield Epifluorescence and Darkfield Microscopy System; Nikon Instruments Inc. Tokyo, Japan) at 400× magnification. At least 8-10 fields of each section were randomly photographed for each LV and cross-sectional areas of 400-500 circular cardiomyocytes per section was measured using Image J software. For myocardial fibrosis, coronal sections were stained for collagen with a Masson's Trichrome Kit (Cat#: k037, Poly Scientific, Bay Shore, NY, USA) according to the protocol provided by the manufacturer. Sections were observed under a light microscope (EVOS FL Auto Cell Imaging System, Thermo fisher scientific, Waltham, MA, USA). At least 8-10 fields were randomly photographed for each section. The percentage of fibrosis [the blue stained area/(blue stained area + red stained area) ×100%] was measured by Image-Pro Plus software (Media Cybernetics, Inc., Bethesda, MD, USA). At least two sections of each heart were analyzed for the measurements of cardiomyocyte cross sectional areas and cardiac fibrosis. Apoptosis was measured by TUNEL assays on tissue sections using In Situ Cell Death Detection Kit, TMR red (Cat#: 12156792910, Roche Applied Science, Indianapolis, IN, USA) according to the protocol provided by the manufacturer. Briefly, the sections were deparaffinized, rehydrated, microwaved in 0.01 M citrate buffer for 20 mins, incubated in 0.1 M Tris-HCl (pH 7.5) containing 3% BSA and 20% normal bovine serum for 30 min at room temperature (RT). They were then incubated with 50 μl of TUNEL Reaction Mixture at 37ºC for 1 h. After washing with PBS, the sections were further stained with 4',6'-diamidino-2-phenylindole (DAPI) (Cat#: D1306, Invitrogen Corp., Carlsbad, CA, USA) and mounted with Prolong Gold Antifade Reagent and DAPI (Cat#: D1306, Invitrogen Corp., Carlsbad, CA, USA). A positive control section was digested with DNase I (2000 U/ml in 50 mM Tris-HCl (pH7.5), 1 mg/ml BSA) for 10 min at RT and a negative control section was only incubated with labeling solution (without enzyme solution). The apoptotic nuclei were labeled with

TUNEL (red) and all nuclei were counterstained with DAPI (blue). Sections were observed under a light microscope (EVOS FL Auto Cell Imaging System, Thermo fisher scientific, Waltham, MA, USA). At least 8-10 fields of each section were randomly photographed for each section. TUNEL-positive cells were quantified in all nuclei of each field. At least two sections from each heart were analyzed.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Quantitative Real Time (qPCR)

The total RNA from the LV was extracted using TRizol reagent (Cat#: 15596018, Ambion, Austin, TX, USA), and the reverse transcription reaction was performed with 0.5 μg of total RNA using an iScript™ cDNA Synthesis Kit (Cat#: 1708891, Bio-Rad Laboratories Inc, Berkeley, CA, USA). qPCR was carried out using the Bio-Red CFX96™ Real-Time system (BioRad CFX Connect rtPCR, Bio-Red Laboratories Inc. Hercules, CA, USA). Expression levels of target genes were normalized by concurrent measurement of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels. Primers that were used for qPCR are summarized in Table S1.

Western Blot Analysis

LV tissues or cells were lysed in a homogenization buffer (RIPA) contained 150 mM NaCl, 1% NP-40, 50 mM Tris (pH 8.0), 0.5% sodium deoxycholate ($C_{24}H_{39}NaO_4$), 0.1% sodium dodecyl sulfate (SDS), 1 mM sodium orthovanadate (Na_3VO_4), proteinase inhibitor cocktail (Cat#: P8340, Sigma-Aldrich, St. Louis, MO, USA) and phosphatase inhibitor cocktail (Cat#: P0044, Sigma-Aldrich, St. Louis, MO, USA).

For the assay of accumulation of ubiquitinated proteins in soluble and insoluble fractions, tissue proteins were separated into detergent-soluble and -insoluble fractions with the 2% Triton X-100 buffer [50 mM Tris (pH 8.0), 150 mM NaCl, 1 mM EDTA, 10% glycerol, 2% Triton X-100, protease inhibitor cocktail and phosphatase inhibitor cocktail]. The insoluble fractions were solubilized in 1% SDS lysis buffer [50 mM Tris (pH8.0), 10 mM EDTA, 1% SDS, protease inhibitor cocktail and phosphatase inhibitor cocktail]. Immunoblotting was conducted as described elsewhere [5, 6]. The antibodies that were used for Western blot analysis are summarized in Table S2.

Autophagic Flux Assay

A cohort of female WT and CR-Cyld Tg mice at ages of 10 weeks were subject to sham or TAC operations for 2 weeks. Mice were injected intraperitoneally with bafilomycin A1 (BafA1; 3 μmol/kg, Cat#: S1413, Selleckchem, Houston, TX, USA). Heart tissues were collected 1 hour after the injection for Western blot analysis of LC3 levels [7].

Mouse embryonic fibroblasts (MEF) stably infected with lentivirus of control Sv-40 or Cyld shRNA were seeded onto 6-well plates at $2x10^5$ cells per well. After 36 hours of culture, the cells reached a 100% confluent state. Then, the cells were cultured with the full growth medium containing 100 nM BafA1 (Cat#: B1793, Sigma-Aldrich, St. Louis, MO, USA) or 50 μM chloroquine (CQ, Cat#: C6628, Sigma-Aldrich, At. Louis, MO, USA) for 4 hours or 10 nM BafA1 or 10 μM CQ for 24 hours. At the end of culture, the cells were subject to Western blot analysis of LC3.

Autophagic flux is defined as the amount of BafA1 or CQ-induced accumulation of LC3-II, which is calculated by a formula of BafA1 or CQ-induced LC3-II density – Vehicle-

induced LC3-II density.

Autophagic Flux and Autolysosome Efflux Assessments

Rat H9C2 cells (a myoblast cell line that is derived from embryonic rat hearts that maintains some features of cardiac myocytes) were grown onto coverslips and transfected with mCherry-GFP-LC3 plasmid (Cat#: 123230, Addgene, Cambridge, MA, USA) and Flag-HA-Cyld plasmids (Cat#: 22544, Addgene, Cambridge, MA, USA) by a Lipofactamine 3000 transfection kit (Cat#: L3000-015, Invitrogen Corp., Carlsbad, CA, USA) according to the protocol provided by the manufacturer. After transfection, the medium was switched to the full growth medium with vehicle (DMSO), 1 μM Rapamycin (Cat#: R0395, Sigma-Aldrich, St. Louis, MO, USA) or 1 μM Rapamycin plus 10 nM BafA1 for 24 hours. The cells were then fixed by 4% paraformaldehyde and stained with DAPI for photograph using a light microscope (EVOS FL Auto Cell Imaging System, Thermo fisher scientific, Waltham, MA, USA). The number of autophagosomes (AP, green and red double positive, i.e. yellow+ puncta) and autolysosomes (AL, red+ alone puncta) in 50 cells for each group were counted.

Transmission Electron Microscopy

Hearts were cannulated via the LV apex, cleared of blood by perfusion with normal saline at 90 mmHg, arrested in diastole with 10% KCl (w/v in ddH_2O), fixed by perfusion with 4% paraformaldehyde. The left ventricle was dissected and fixed in mixture of 2% glutaraldehyde and 2% paraformaldehyde in PBS for 3 days. Several 1 mm thick sections were removed from the fixed left ventricles and thoroughly rinsed with PBS and then post-fixed in 1% OsO_4 in PBS/1.5% potassium ferricyanide in PBS for 1 hour and then rinsed with water. The sections were dehydrated in increasing concentrations of ethanol 70%, 95% and 100%, 2x each, followed by 1:1 ethanol:acetonitrile and then pure acetonitrile. The sections were then infiltrated with 2:1 (1 hour) and 1:2 (1 hour) acetonitrile:PolyBed 812 before placing in pure PolyBed 812 for 3 hours under vacuum. The PolyBed 812 was replaced and the samples were allowed to rotate overnight. The sections were embedded in flat molds and cured at 60°C for 2 days. Embedded samples were sectioned on a Leica Ultracut R at approx. 90 nm and collected on copper grids. The sections were stained with 1% uranyl acetate (aq), 40 minutes at 37°C, and 4 minutes in Hanaichi lead stain. Sections were imaged on a transmission electron microscope (JEOL 1400 Plus TEM system, JEOL Inc., Peabody, MA, USA) at 120kV. At least 6~8 fields around the nuclei were randomly chosen from each tissue section for analyzing autophagic vacuoles. Two mice of each experimental group were randomly selected for transmission electron microscopic analysis.

Cell Death Assay

MEF stably infected with lentivirus of control Sv-40 or Cyld shRNA were seeded onto 12-well plates at 1×10^5 cells per well and cultured in full growth medium for overnight and then treated with or with 50 nM BafA1 in full growth medium for 24 hours (n=3). The cells were stained with 2 μg/ml propidium iodide (Cat#: AS-83215, Anaspec Inc., Fremont, CA, USA) in fresh full growth medium at 37°C for 30 minutes and 8-10 randomly selected fields were photographed for each group using light microscope (EVOS FL Auto Cell Imaging System, Thermo Fisher Scientific, Waltham, MA, USA). PI positive cells (red),

which indicate cell death, were counted by Image J software in all cells of each field. Totally more than $3x10^4$ cells for vehicle group and $1x10^4$ cells for BafA1 group were counted. Cell death was quantified by the percentage of PI positive (+) cells in each group: PI (+) % = (PI+ cell numbers/total cell numbers)x100%.

H9C2 cells stably infected with lentivirus of Gfp (Lenti-Gfp) or lenti-Cyld were seeded onto 24-well plates at 4×10^4 cells per well. The cells were cultured overnight in full growth medium and then exposed to 0.1 μM MG132 (Cat#: S2619, Selleck Chemicals LLC., Houston, TX, USA) and/or 10 nM BafA1 in serum free medium for 24 hours (n=6). The cells were washed once with serum free medium before stimulation. Lactate dehydrogenase (LDH) activity in 50 μl supernatant was measured by an LDH cytotoxicity detection kit (Cat#: MK401, Takara Bio Inc., Kusatsu, Shiga, Japan) according to the protocol provided by the manufacturer.

Statistics

Data are shown as mean ± SD. Differences between 2 groups were evaluated for statistical significance using the Student t test. Differences among > 3 groups were compared by one-way ANOVA with Bonferroni test for multiple comparisons. Survival rates between experimental groups after TAC were analyzed using Kaplan Meier test. Differences were considered significant at $p < 0.05$.

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II. Supplementary Figures and Tables

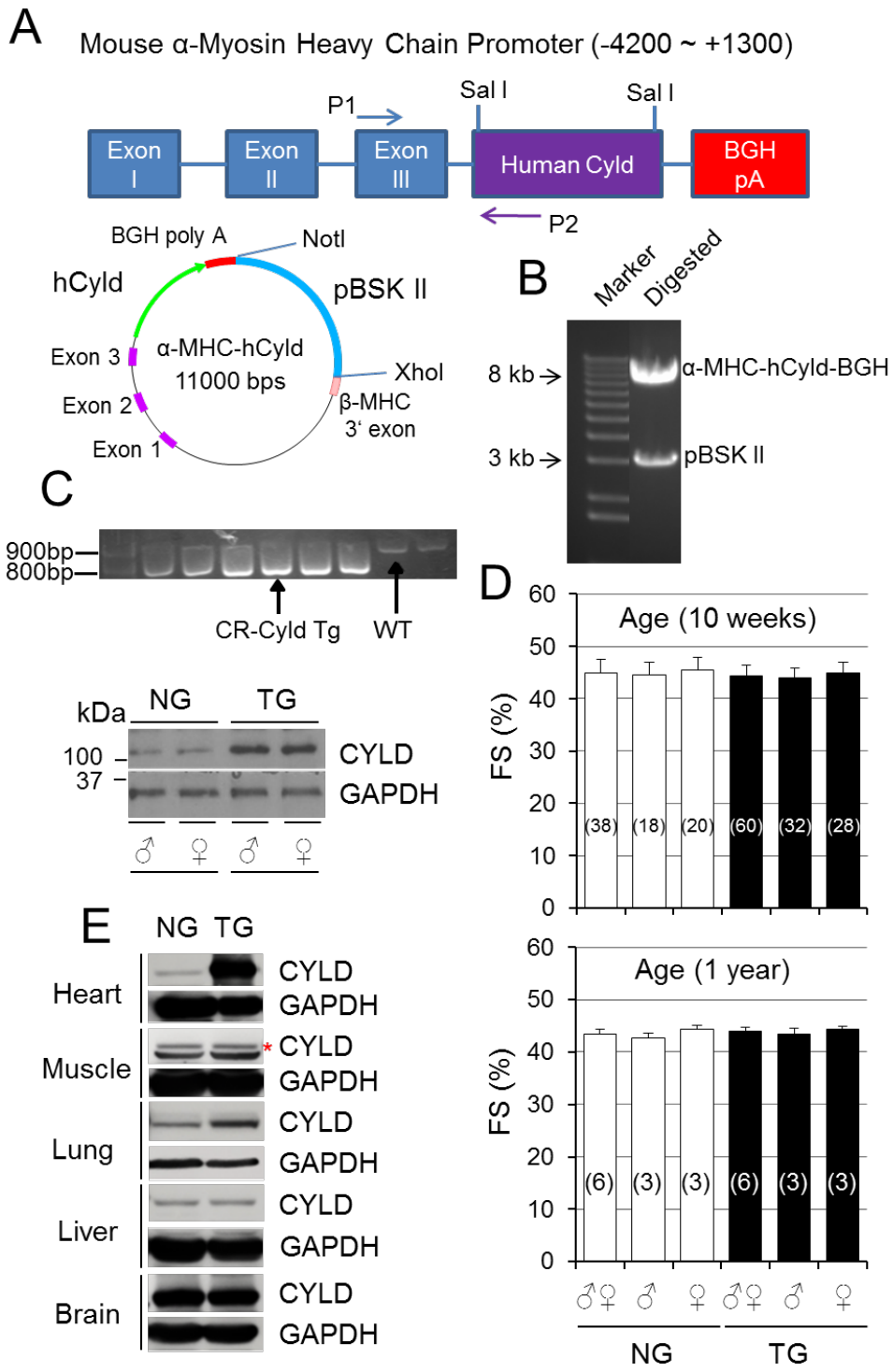

Fig. S1. Baseline characterization of cardiomyocyte-restricted Cyld transgenic (CR-Cyld Tg) mice. (A) Schematic diagram of alpha myosin heavy chain (α-MHC)-human Cyld (hCyld) transgene structure. **(B)** Enzymatic digestion of transgene cassette. The generated transgene vectors were digested with restricted enzymes of Not1 and Xho1,

revealing the size of the backbone vector and inserted genes. **(C)** Representative image of genotyping (upper panel) and Western blot analysis of CYLD expression in littermates of wild type (NG) and CR-Cyld Tg (TG) mice (lower panel). Adult male (♂) and female (♀) mice were randomly chosen for the analyses. **(D**) Echocardiography of 10-week (upper panel) and 1-year old (lower panel) littermates of WT and CR-Cyld Tg mice. Animal numbers (n) for each group are indicated in the parenthesis. **(E)** Representative Western blot analyses of CYLD expression in different tissues of 1-year old littermates of NG and TG mice. The * indicates the location of CYLD bands.

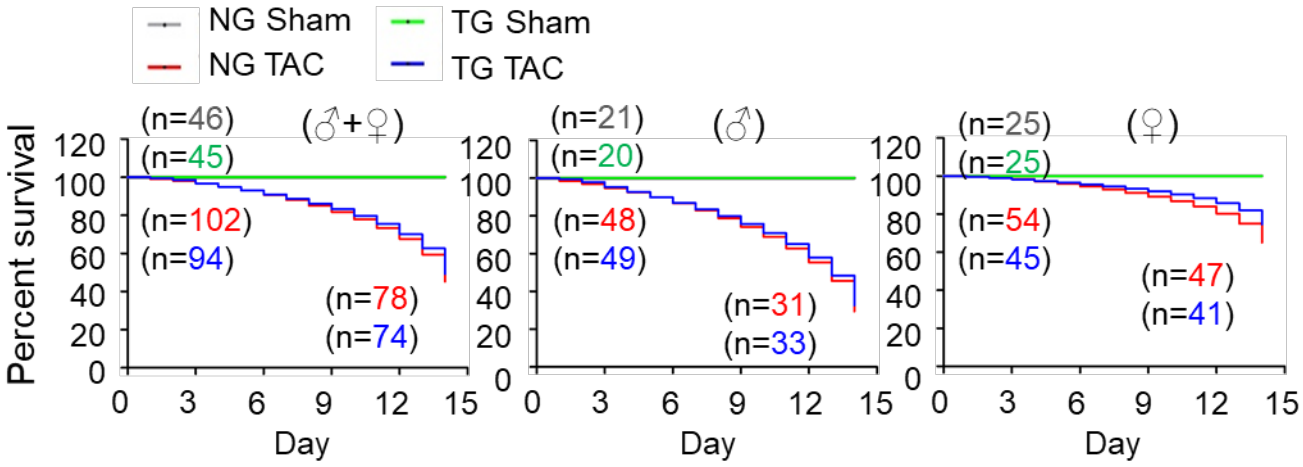

Fig. S2. The impact of CR-Cyld overexpression on TAC-induced death in mice. Littermates of male (♂) and female (♀) non-transgenic wild type (NG) and CR-Cyld Tg (TG) mice at age of 10 weeks were subject to sham or TAC operations for 2 weeks. Survived mice were counted daily. Survival rates of each experimental groups were analyzed by Kaplan Meier test. No difference is found between NG and TG groups.

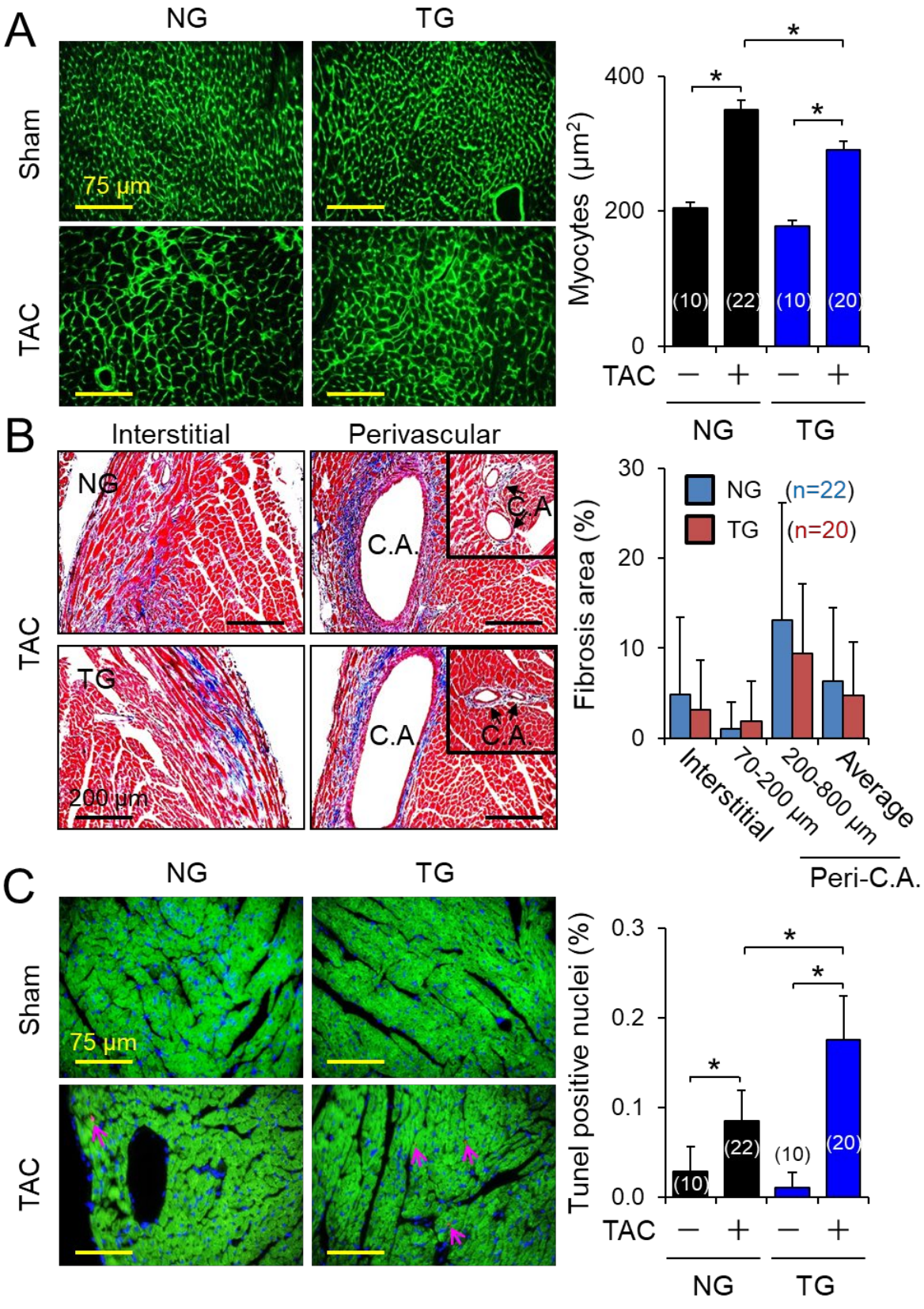

Fig. S3. TAC-induced cardiac remodeling in littermates of nontransgenic wild type

(NG) and CR-Cyld Tg (TG) mice. Littermates of NG and TG mice with mixed genders at age of 10 weeks were subject to sham or TAC for 2 weeks. **(A)** Cardiomyocyte sizes of NG and TG mice at 2 weeks after Sham or TAC. *, p<0.05 between indicated groups. **(B)** Myocardial collagen deposition of NG and TG mice at 2 weeks after Sham or TAC. **(C)** Myocardial apoptosis ratio of NG and TG mice at 2 weeks after Sham or TAC. *, p<0.05 between indicated groups. Animal numbers (n) for each group are indicated in the parenthesis. Cardiomyocytes (green) were marked using Alexa Fluor™ 488 Phalloidin (Cat#: A12379, Invitrogen Corp., Carlsbad, CA, USA) binds F-actin. CA, coronary artery.

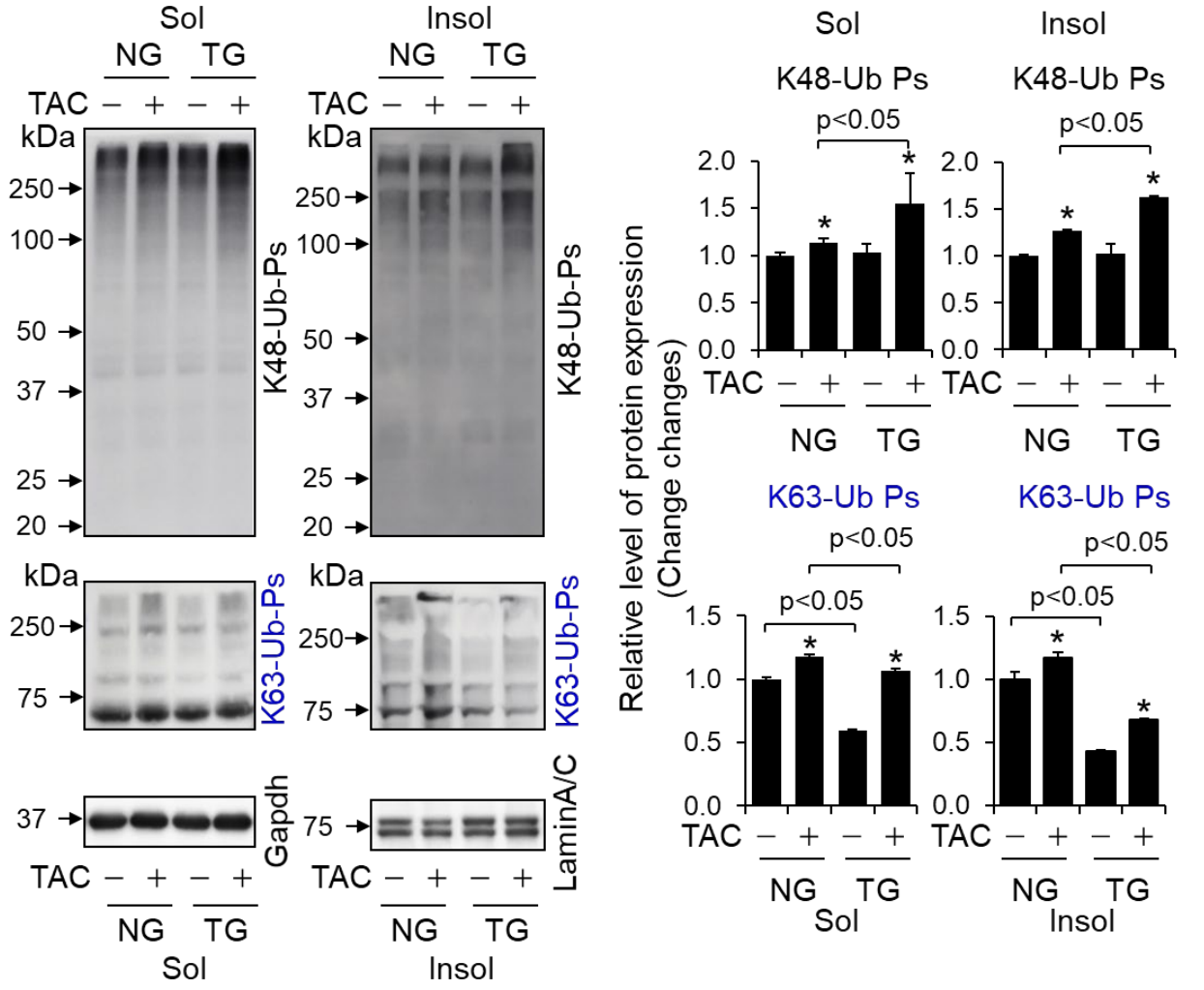

Fig. S4. The impact of CR-Cyld overexpression on PO-induced accumulation of poly-ubiquitinated proteins marked by K48-linked ubiquitin (Ub) or K63-linked Ub chains in the heart at 2 weeks after TAC. Littermates of male non-transgenic wild type (NG) and CR-Cyld Tg (TG) mice at age of 10 weeks were subject to sham or TAC for 2 weeks and then randomly assigned for Western blot analyses: *Left panels*: Representive immunoblots of poly-ubiquitinated proteins marked by K48-linked or K63-linked Ub chains (K48-Ub-Ps or K63-Ub-Ps) in left ventricles (LVs). Sol, soluble or Insol, insoluble fractions

of LV lysates. *Right panels*: Semi-quantified results (n=4~6). *, p<0.05 vs. NG in the same groups in groups.

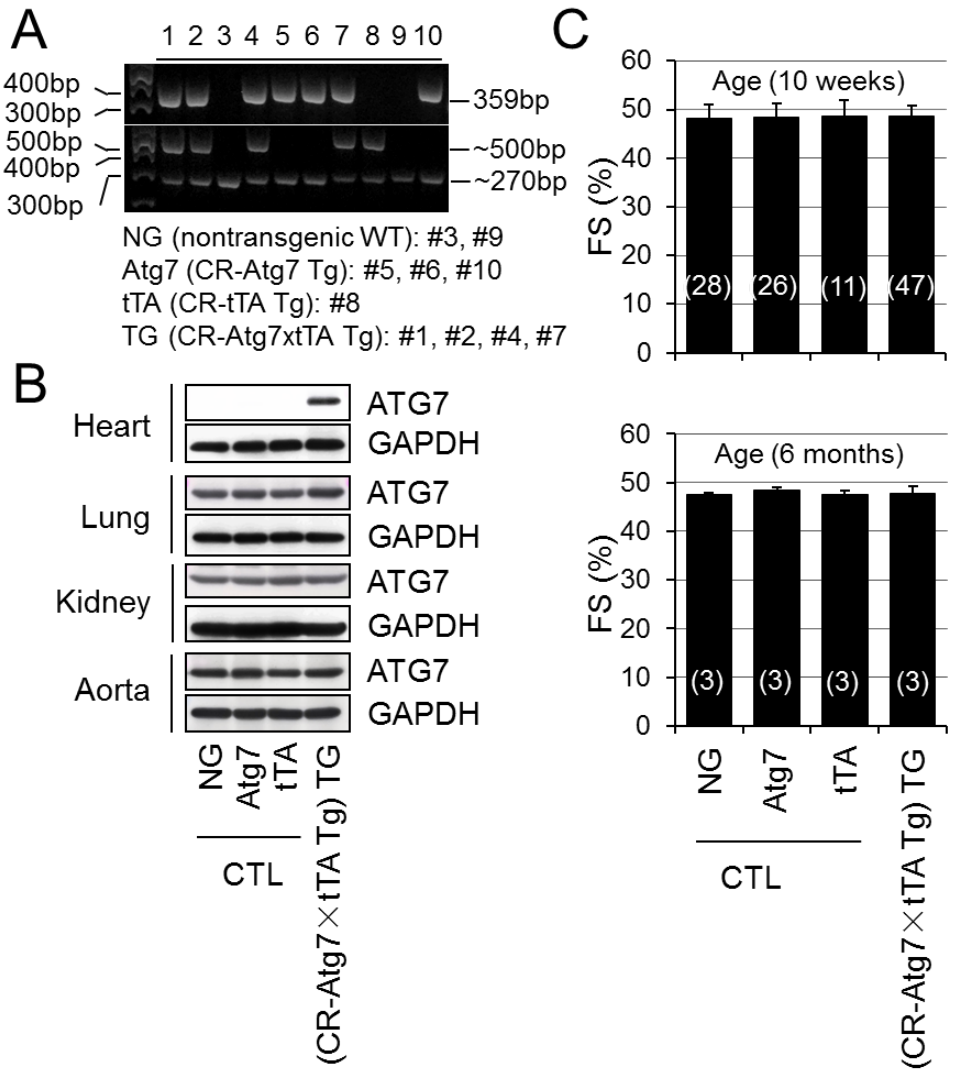

Fig. S5. Baseline characterization of cardiomyocyte-restricted Atg7 transgenic (CR-Atg7×tTA Tg) mice. (**A**) Representative image of genotyping. NG, nontransgenic wild type (WT) control (CTL) mice; Atg7, CR-Atg7 without tTA Tg CTL mice; tTA, CR-tTA Tg CTL mice, and TG, CR-Atg7xtTA Tg mice. (**B**) Western blot analysis of ATG7 expression in different tissues of 6-months-old littermates of female CTL NG, Atg7, and tTA mice as well as TG mice. (**C**) Echocardiography of 10 week- (upper panel) male (♂) and female (♀) as well as 6 month-old female (♀) (lower panel) littermates of NG, Atg7,tTA and TG mice.

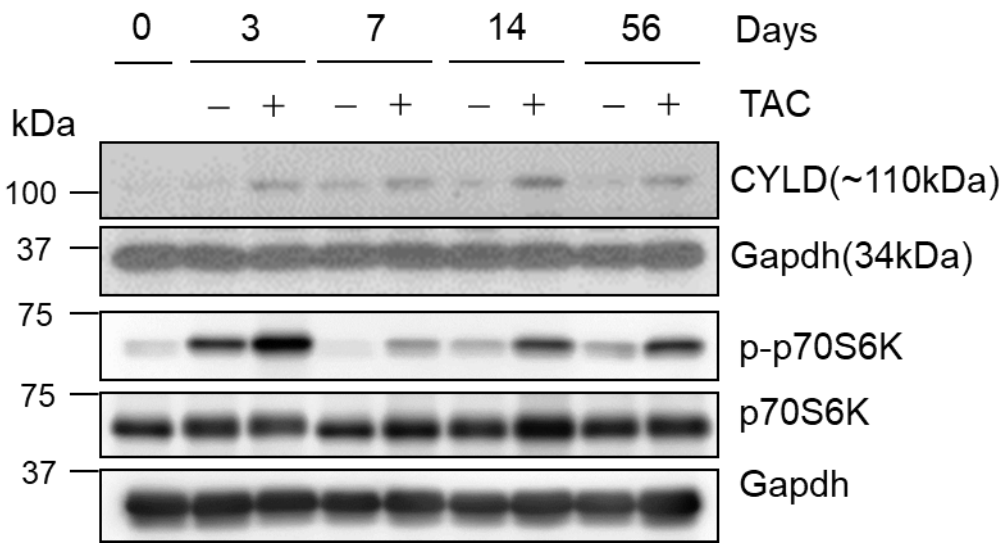

Fig. S6. A time course study of CYLD expression and mTORC1 activity in PO-hearts. Wild type male mice in FVB/N genetic background at age of 10 weeks were subject to sham and TAC operations for 8 weeks. The hearts of these mice were harvested at 0, 3, 7, 14 and 56 days after operations. LV lysates of these mice were subject to Western blot analysis. One set of representative results is shown.

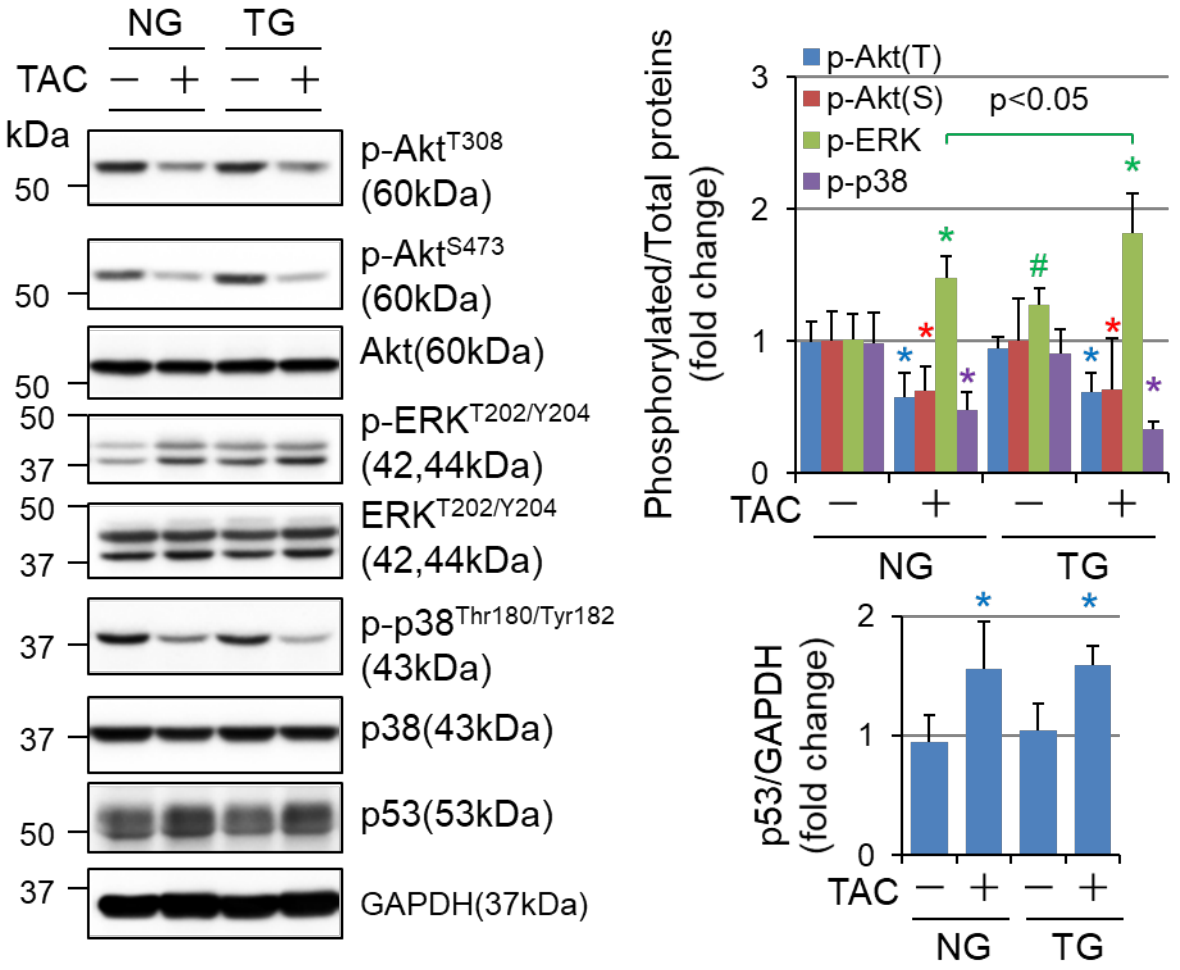

Fig. S7. The impact of CR-Cyld overexpression on the phosphorylation of Akt, ERK and p38, and the expression of p53 in PO-hearts. Littermates of male nontransgenic wild type (NG) and CR-Cyld Tg (TG) mice at age of 10 weeks were subject to sham or TAC operations for 2 weeks. LV lysates of these mice were subject to Western blot analysis. Left panel shows the representative results from 4 separated experiments; right panel shows the quantified data. *, p<0.05 vs. TAC (-) in the same groups; #, p<0.05 vs. NG TAC (-).

Table S1. Primers for qPCR and genotyping

Primers	Gene access #	Forward (5'—3')	Reverse (5'—3')	Product
qPCR				
ANF	NM_008725.2	CATCACCCTGGGCTTCTTCCT	TGGGCTCCAATCCTGTCAATC	405
BNP	NM_008726.4	GCGGCATGGATCTCCTGAAGG	CCCAGGCAGAGTCAGAAACTG	418
α-MHC	NM_010856.3	CCAATGAGTACCGCGTGAA	ACAGTCATGCCGGGATGAT	254
β-MHC	NM_080728.2	ATGTGCCGGACCTTGGAA	CCTCGGGTTAGCTGAGAGATCA	170
SERCA2a	NM_009722.3	CCATCTGCTTGTCCATGTCACT	CAAATGGTTTAGGAAGCGGTTACT	213
GAPDH	XM_001479322	ATGTTCCAGTATGACTCCACTCACG	GAAGACACCAGTAGACTCCACGACA	171
Genotyping				
CR-Cyld Tg	Cyld	AGAAGCCTAGCCCACACCAGA AAT	CTTGTCCAATGCAACAAACACGCC	~700
CR-Atg7 Tg	Atg7	GGCAGTTTCCAGTCCGTTGAAGTCCTCT	CAGCCCATCAGTTCCTAGCCACATTAC	359
CR-tTA Tg	tTA	AGCGCATTAGAGCTGCTTAATGAGGTC	GTCGTAATAATGGCGGCATACTATC	~500
	ELC1v (WT)	ATCGAGTTCACACCTGAACAGATTG	CCAGGACACGGAGCACCTCTG	~270
Cyld KO	WT	CTGTCTTTTTACAACATGGATGCCAGGTTGC	CTAAAGCGCATGCTCCAGACTGCCTTGG	194
	Cyld KO	CTGTCTTTTTACAACATGGATGCCAGGTTGC	TCCGTTCTTCCCAGTAGGGTGAAGTAAC	220

ANF, atrial natriuretic factor; BNP, brain natriuretic peptide; α-MHC, alpha-myosin heavy chain; β-MHC, beta-myosin heavy chain; SERCA2a, sarcoplasmic reticulum calcium ATPase2a; Atg7, Autophagy related 7; tTA, Tet-controlled trans-activator; ELC1v, essential myosin light chain 1v.

Table S2. Antibody information for Western blot analysis

Name	MW(kDa)	Characteristics	Source
Anti-Atg7	~75	Rabbit polyclonal antibody	Sigma-Aldrich, Cat#: A2856
Anti-Akt	60	Rabbit polyclonal antibody	Cell signaling, Cat#: 9272
Anti-p-Akt^{T308}	60	Rabbit polyclonal antibody	Cell signaling, Cat#: 9275
Anti-p-Akt^{S473}	60	Rabbit polyclonal antibody	Cell signaling, Cat#: 9271
Anti-CYLD	~110	Rabbit polyclonal antibody	Sigma-Aldrich, Cat#: SAB4200061
Anti-ERK	42, 44	Rabbit polyclonal antibody	Cell signaling, Cat#: 9102
Anti-p-ERK$^{T202/Y204}$	42, 44	Rabbit polyclonal antibody	Cell signaling, Cat#: 9101
Anti-LaminA/C	62, 69	Mouse polyclonal antibody	Santa Cruz, Cat#: sc-20681
Anti-Lamp1	120	Rabbit polyclonal antibody	Abcam, Cat#: ab24170
Anti-Lamp2	110	Rabbit polyclonal antibody	Sigma-Aldrich, Cat#: L0668
Anti-LC3	14, 16	Rabbit polyclonal antibody	Sigma-Aldrich, Cat#: L7543
Anti-p38	43	Rabbit polyclonal antibody	Cell signaling, Cat#: 9212
Anti-p-p38$^{T180/Y182}$	43	Rabbit polyclonal antibody	Cell signaling, Cat#: 9211
Anti-p62	62	Rabbit polyclonal antibody	Sigma-Aldrich, Cat#: P0067
Anti-p53	53	Mouse monoclonal antibody	Cell signaling, Cat#: 2524
Anti-mTOR	289	Rabbit monoclonal antibody	Cell signaling, Cat#: 2983

Anti-p-mTOR^{S2448}	289	Rabbit monoclonal antibody	Cell signaling, Cat#: 5536
Anti-p-p70S6K^{T389}	70, 85	Rabbit monoclonal antibody	Cell signaling, Cat#: 9234
Anti-p70S6K	70, 85	Rabbit monoclonal antibody	Cell signaling, Cat#: 2708
Anti-4EBP1	15-20	Rabbit monoclonal antibody	Cell signaling, Cat#: 9644
Anti-p-4EBP1$^{T37/46}$	15-20	Rabbit monoclonal antibody	Cell signaling, Cat#: 2855
Anti-Ub	N/A	Mouse monoclonal antibody	Santa Cruz, Cat#: sc-8017
Anti-Atg1/ULK1	~150	Rabbit polyclonal antibody	Sigma-Aldrich, Cat#: A7481
Anti-p-ULK1^{S757}	140-150	Rabbit polyclonal antibody	Cell signaling, Cat#: 6888
Anti-α-Actin	43	Mouse monoclonal antibody	Santa Cruz, Cat#: sc-58670
Anti-β-Actin	43	Mouse monoclonal antibody	Sigma-Aldrich, Cat#: A1978
Anti-GAPDH	37	Mouse monoclonal antibody	Sigma-Aldrich, Cat#: G8795

Table S3. Echocardiography analyses of adult littermates of male (♂) and female (♀) nontransgenic (NG) and CR-Cyld Tg (TG) mice at 0, 2, 4 and 8 weeks after sham or TAC operation. Littermates of male and female NG and TG mice at age of 10 weeks were subject to sham and TAC operations for 8 weeks. PG, pressure gradient; LVID; in mm, the LV internal dimension; IVS; in mm, interventricular septum thickness; LVPW, in mm, LV posterior wall thickness; FS (%), LV percent fractional shortening; EF (%), ejection fraction; SV (μL), stroke volume; CO (ml/min), cardiac output; LV mass (mg) and LV volume (LV vol; in μL). Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. NG Sham; $^{B}p<0.05$ vs. TG Sham; $^{C}p<0.05$ vs. NG TAC.

	Time (**0** Week)			
Mixed gender (♂+♀)	NG		TG	
	Sham	TAC	Sham	TAC
Echocardiography (n)	(18)	(40)	(21)	(35)
PG (mmHg)	3.36±0.40	3.35±0.68	3.36±0.52	3.35±0.58
Heart rate (BPM)	450±5	450±6	450±6	450±6
IVS;d (mm)	1.14±0.08	1.14±0.07	1.14±0.08	1.14±0.08
IVS;s (mm)	1.73±0.08	1.73±0.10	1.73±0.09	1.73±0.10
LVID;d (mm)	3.57±0.15	3.57±0.12	3.57±0.15	3.57±0.15
LVID;s (mm)	1.97±0.14	1.97±0.15	1.97±0.11	1.97±0.12
LVPW;d (mm)	0.95±0.12	0.95±0.11	0.95±0.11	0.95±0.10
LVPW;s (mm)	1.44±0.11	1.44±0.11	1.44±0.12	1.44±0.14
FS%	45.48±1.80	45.54±1.76	45.31±1.96	45.32±1.78
EF%	76.42±2.83	76.49±2.68	76.54±2.51	76.54±2.42
SV (μL)	43.38±3.55	43.45±4.71	43.49±4.62	43.47±4.47
CO (mL/min)	19.66±2.19	19.30±1.85	19.24±2.39	19.24±2.33
LV mass (mg)	145.02±7.56	145.57±6.29	145.16±11.82	145.27±12.14
LV Vol;d (μL)	55.32±4.88	55.40±7.65	55.35±6.35	55.34±6.17
LV Vol;s (μL)	12.51±2.33	12.68±2.65	12.78±2.23	12.62±2.32
	Time (**2** Weeks)			
Echocardiography (n)	(18)	(30)	(21)	(27)
PG (mmHg)	3.31±0.73	60.30±12.95^{A}	3.33±0.76	60.34±14.70^{B}
Heart rate (BPM)	450±5	450±6	450±6	450±3
IVS;d (mm)	1.15±0.07	1.46±0.12^{A}	1.15±0.07	1.35±0.12^{B,C}
IVS;s (mm)	1.73±0.10	1.94±0.14^{A}	1.73±0.08	1.80±0.10^{B,C}
LVID;d (mm)	3.58±0.11	3.30±0.17^{A}	3.58±0.13	3.57±0.17^{C}
LVID;s (mm)	1.97±0.17	2.15±0.15^{A}	1.97±0.13	2.35±0.15^{B,C}
LVPW;d (mm)	0.95±0.11	1.20±0.15^{A}	0.95±0.10	1.20±0.14^{B}
LVPW;s (mm)	1.45±0.08	1.60±0.15^{A}	1.44±0.12	1.60±0.13^{B}

FS%	45.48±1.47	40.16±2.72[A]	45.36±1.56	35.18±2.48[B,C]
EF%	76.56±1.62	72.32±2.77[A]	76.57±2.35	64.55±4.25[B,C]
SV (µL)	43.67±4.12	33.42±2.52[A]	43.39±5.70	33.22±3.65[B]
CO (mL/min)	19.57±2.54	15.59±1.96[A]	19.51±2.55	15.62±2.09[B]
LV mass (mg)	145.42±12.93	181.93±17.9[A]	145.22±13.88	207.4±27.2[B,C]
LV Vol;d (µL)	55.17±6.12	47.32±6.84[A]	55.50±7.32	55.28±5.54[C]
LV Vol;s (µL)	12.67±2.80	15.48±3.05[A]	12.69±2.60	18.62±3.43[B,C]
	Time (**4** Weeks)			
Echocardiography (n)	18	29	21	26
PG (mmHg)	3.33±0.51	60.64±9.88[A]	3.33±0.52	60.69±8.75[B]
Heart rate (BPM)	450±6	450±4	450±4	450±5
IVS;d (mm)	1.15±0.04	1.54±0.10[A]	1.15±0.04	1.39±0.10[B,C]
IVS;s (mm)	1.74±0.07	1.99±0.10[A]	1.74±0.07	1.85±0.12[B,C]
LVID;d (mm)	3.57±0.15	3.57±0.16	3.57±0.15	3.57±0.15
LVID;s (mm)	1.97±0.17	2.28±0.18[A]	1.97±0.15	2.51±0.19[B,C]
LVPW;d (mm)	0.95±0.10	1.29±0.13[A]	0.95±0.11	1.29±0.14[B]
LVPW;s (mm)	1.44±0.08	1.69±0.14[A]	1.44±0.09	1.69±0.11[B]
FS%	45.35±1.64	36.21±3.27[A]	45.55±1.31	32.84±3.56[B,C]
EF%	76.46±1.65	66.56±5.30[A]	76.70±1.54	60.26±5.21[B,C]
SV (µL)	43.50±4.79	33.44±4.87[A]	43.43±5.83	33.27±4.36[B]
CO (mL/min)	19.40±3.00	15.56±1.49[A]	19.45±2.86	15.59±2.19[B]
LV mass (mg)	145.34±10.48	213.6±26.44[A]	145.32±11.44	234.5±25.4[B,C]
LV Vol;d (µL)	55.29±7.77	55.43±7.07	55.59±8.00	55.25±6.85
LV Vol;s (µL)	12.78±2.77	18.54±3.90[A]	12.86±2.60	23.01±4.17[B,C]
	Time (**8** Weeks)			
Echocardiography (n)	18	26	21	24
PG (mmHg)	3.39±0.73	60.35±11.80[A]	3.39±0.28	60.44±9.45[B]
Heart rate (BPM)	451±6	451±2	451±4	451±5
IVS;d (mm)	1.15±0.06	1.55±0.10[A]	1.15±0.07	1.42±0.10[B,C]
IVS;s (mm)	1.73±0.07	2.05±0.14[A]	1.73±0.11	1.91±0.12[B,C]
LVID;d (mm)	3.57±0.15	3.73±0.16[A]	3.56±0.16	3.89±0.12[B,C]
LVID;s (mm)	1.97±0.09	2.39±0.17[A]	1.97±0.12	2.69±0.14[B,C]
LVPW;d (mm)	0.95±0.07	1.38±0.17[A]	0.95±0.07	1.38±0.21[B]
LVPW;s (mm)	1.44±0.07	1.76±0.17[A]	1.44±0.10	1.76±0.17[B]

FS%	45.50±2.07	33.37±3.20^{A}	45.40±2.04	28.00±3.16^{B,C}
EF%	76.58±2.50	62.56±6.69^{A}	76.66±1.35	55.57±5.73^{B,C}
SV (μL)	43.28±5.58	33.53±4.57^{A}	43.37±5.11	33.71±4.69^{B}
CO (ml/min)	19.39±2.72	15.62±1.72^{A}	19.68±2.73	15.57±2.57^{B}
LV mass (mg)	146.89±14.90	232.25±27.6^{A}	146.12±18.39	261.7±26.0^{B,C}
LV Vol;d (μL)	55.40±5.04	59.69±4.66^{A}	55.60±7.15	63.93±4.66^{B,C}
LV Vol;s (μL)	12.64±2.65	21.14±5.18^{A}	12.72±2.04	27.45±6.02^{B,C}

Table S3. ended.

Table S4. Echocardiography analyses of adult littermates of male (♂) nontransgenic (NG) and CR-Cyld Tg (TG) mice at 0, 2, 4 and 8 weeks after sham or TAC operation (data from Table S3). Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. NG Sham; $^{B}p<0.05$ vs. TG Sham; $^{C}p<0.05$ vs. NG TAC.

	Time (**0** Week)			
Male (♂)	NG		TG	
	Sham	TAC	Sham	TAC
Echocardiography (n)	(8)	(20)	(10)	(19)
PG (mmHg)	3.55±0.74	3.54±0.66	3.55±0.98	3.53±0.40
Heart rate (BPM)	452±8	451±5	452±7	451±6
IVS;d (mm)	1.16±0.13	1.15±0.10	1.16±0.12	1.15±0.09
IVS;s (mm)	1.76±0.14	1.75±0.12	1.74±0.08	1.74±0.08
LVID;d (mm)	3.61±0.13	3.61±0.18	3.61±0.18	3.61±0.17
LVID;s (mm)	2.07±0.21	2.07±0.17	2.07±0.24	2.07±0.17
LVPW;d (mm)	0.96±0.16	0.96±0.14	0.96±0.11	0.96±0.11
LVPW;s (mm)	1.43±0.17	1.43±0.15	1.43±0.16	1.43±0.14
FS%	44.71±2.58	44.82±2.30	44.64±2.40	44.64±1.48
EF%	76.13±4.42	76.65±2.40	76.39±1.57	76.48±3.02
SV (μL)	48.41±5.60	48.90±3.65	48.09±3.71	48.67±5.02
CO (mL/min)	21.16±2.00	21.49±2.39	21.25±2.29	21.11±2.29
LV mass (mg)	153.51±15.08	153.62±9.53	153.27±15.83	153.39±15.79
LV Vol;d (μL)	61.12±6.80	61.43±6.45	61.32±8.28	61.22±7.03
LV Vol;s (μL)	14.36±2.86	14.60±2.98	14.37±2.44	14.47±2.66
	Time (**2** Weeks)			
Echocardiography (n)	(8)	(12)	(10)	(13)
PG (mmHg)	3.53±0.51	60.39±13.00^{A}	3.54±0.71	59.10±17.15^{B}
Heart rate (BPM)	450±10	450±6	452±6	449±4
IVS;d (mm)	1.15±0.10	1.46±0.11^{A}	1.15±0.06	1.33±0.12^{B,C}

IVS;s (mm)	1.75±0.13	1.94±0.19[A]	1.75±0.07	1.80±0.07[B,C]
LVID;d (mm)	3.60±0.26	3.34±0.26[A]	3.60±0.31	3.61±0.18[C]
LVID;s (mm)	2.07±0.13	2.26±0.21[A]	2.06±0.25	2.44±0.08[B,C]
LVPW;d (mm)	0.96±0.04	1.17±0.22[A]	0.96±0.06	1.17±0.15[B]
LVPW;s (mm)	1.43±0.10	1.57±0.16[A]	1.43±0.14	1.57±0.12[B]
FS%	44.93±1.82	39.92±2.64[A]	44.12±2.40	35.76±2.72[B,C]
EF%	75.77±3.57	70.06±5.61[A]	75.63±2.95	64.05±4.55[B,C]
SV (µL)	48.78±1.07	36.42±2.73[A]	48.91±7.52	36.79±1.83[B]
CO (mL/min)	21.68±2.27	17.28±2.64[A]	21.68±3.70	17.29±1.10[B]
LV mass (mg)	153.33±19.84	180.66±8.63[A]	153.63±12.20	206.7±23.5[B,C]
LV Vol;d (µL)	61.86±4.81	51.11±7.70[A]	61.99±10.32	61.21±3.88[C]
LV Vol;s (µL)	14.81±2.41	17.43±1.63[A]	14.95±3.35	20.64±3.23[B,C]
	Time (**4** Weeks)			
Echocardiography (n)	(8)	(12)	(10)	(12)
PG (mmHg)	3.88±0.83	59.74±10.39[A]	3.85±0.49	59.78±6.74[B]
Heart rate (BPM)	456±7	456±5	456±7	454±4
IVS;d (mm)	1.15±0.02	1.52±0.14[A]	1.15±0.08	1.39±0.04[B,C]
IVS;s (mm)	1.76±0.05	1.99±0.12[A]	1.76±0.05	1.86±0.07[B,C]
LVID;d (mm)	3.74±0.18	3.74±0.16	3.75±0.26	3.75±0.20
LVID;s (mm)	2.09±0.03	2.35±0.20[A]	2.08±0.15	2.56±0.19[B,C]
LVPW;d (mm)	0.96±0.12	1.28±0.22[A]	0.97±0.15	1.28±0.26[B]
LVPW;s (mm)	1.43±0.08	1.68±0.14[A]	1.43±0.20	1.68±0.11[B]
FS%	44.40±0.33	36.93±3.19[A]	45.11±0.91	32.80±3.20[B,C]
EF%	75.40±1.43	65.85±6.26[A]	75.60±1.62	59.18±4.31[B,C]
SV (µL)	48.18±5.70	36.46±4.11[A]	48.67±7.79	36.58±4.86[B]
CO (mL/min)	21.50±3.08	17.89±2.76[A]	21.43±3.30	17.76±2.46[B]
LV mass (mg)	153.72±8.66	214.03±27.7[A]	153.61±14.13	244.4±39.4[B,C]
LV Vol;d (µL)	61.27±7.20	61.82±6.48	61.77±7.79	61.49±7.07
LV Vol;s (µL)	14.64±1.18	19.53±3.50[A]	14.87±2.58	24.12±3.54[B,C]
	Time (**8** Weeks)			
Echocardiography (n)	(8)	(10)	(10)	(11)
PG (mmHg)	4.01±0.89	60.36±12.37[A]	4.06±0.71	59.13±8.21[B]
Heart rate (BPM)	456±3	455±10	454±5	455±6
IVS;d (mm)	1.15±0.08	1.53±0.10[A]	1.15±0.06	1.41±0.10[B,C]

IVS;s (mm)	1.77±0.12	2.08±0.18^{A}	1.77±0.11	1.94±0.14^{B,C}
LVID;d (mm)	3.77±0.12	3.85±0.14^{A}	3.80±0.04	3.95±0.08^{B,C}
LVID;s (mm)	2.06±0.20	2.49±0.35^{A}	2.08±0.09	2.78±0.21^{B,C}
LVPW;d (mm)	0.96±0.06	1.37±0.16^{A}	0.97±0.12	1.37±0.31^{B}
LVPW;s (mm)	1.43±0.08	1.74±0.15^{A}	1.43±0.11	1.75±0.20^{B}
FS%	45.53±2.53	33.70±4.44^{A}	45.53±1.48	28.44±4.12^{B,C}
EF%	75.81±3.02	62.24±6.08^{A}	75.72±1.94	55.35±5.92^{B,C}
SV (μL)	48.74±8.66	36.82±6.25^{A}	48.84±4.24	36.76±4.28^{B}
CO (mL/min)	21.39±4.49	17.63±3.05^{A}	21.90±2.20	17.38±2.09^{B}
LV mass (mg)	152.55±15.82	252.7±20.54^{A}	156.68±9.17	272.8±30.9^{B,C}
LV Vol;d (μL)	61.32±2.44	65.85±4.52^{A}	61.89±2.85	69.19±2.70^{B,C}
LV Vol;s (μL)	14.32±1.38	65.85±4.52^{A}	14.14±1.62	30.00±6.20^{B,C}

Table S4. ended.

Table S5. Echocardiography analyses of adult littermates of female (♀) nontransgenic (NG) and CR-Cyld Tg (TG) mice at 0, 2, 4 and 8 weeks after sham or TAC operation (data from Table S3). Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. NG Sham; $^{B}p<0.05$ vs. TG Sham; $^{C}p<0.05$ vs. NG TAC.

	Time (**0** Week)			
Female (♀)	NG		TG	
	Sham	TAC	Sham	TAC
Echocardiography (n)	(10)	(20)	(11)	(16)
PG (mmHg)	3.08±0.61	3.07±0.55	3.08±0.62	3.03±0.47
Heart rate (BPM)	450±3	450±7	450±8	450±4
IVS;d (mm)	1.15±0.12	1.15±0.11	1.13±0.08	1.14±0.13
IVS;s (mm)	1.72±0.14	1.72±0.16	1.72±0.09	1.72±0.12
LVID;d (mm)	3.53±0.13	3.53±0.09	3.53±0.14	3.53±0.15
LVID;s (mm)	1.83±0.11	1.83±0.20	1.83±0.12	1.83±0.19
LVPW;d (mm)	0.94±0.11	0.94±0.16	0.94±0.14	0.94±0.11
LVPW;s (mm)	1.44±0.12	1.43±0.18	1.44±0.15	1.44±0.11
FS%	45.28±1.80	45.48±3.45	45.35±3.69	45.40±2.81
EF%	78.64±2.50	78.61±4.03	78.30±3.31	78.27±3.08
SV (μL)	38.54±2.70	38.62±3.08	38.71±3.76	38.63±3.95
CO (mL/min)	17.63±0.99	17.53±1.50	17.59±1.90	17.55±1.39
LV mass (mg)	138.69±11.22	138.62±8.81	138.32±14.48	138.70±9.87
LV Vol;d (μL)	50.52±5.30	50.54±3.38	50.45±5.83	50.44±7.33
LV Vol;s (μL)	11.43±2.34	11.48±2.39	11.40±2.39	11.44±2.82

	Time (**2** Weeks)			
Echocardiography (n)	(10)	(18)	(11)	(14)
PG (mmHg)	3.04±0.75	60.53±11.36[A]	3.01±0.66	60.77±10.97[B]
Heart rate (BPM)	450±5	450±4	450±5	450±3
IVS;d (mm)	1.15±0.08	1.47±0.12[A]	1.13±0.07	1.36±0.10[B,C]
IVS;s (mm)	1.73±0.07	1.94±0.14[A]	1.72±0.07	1.80±0.07[B,C]
LVID;d (mm)	3.53±0.08	3.25±0.16[A]	3.53±0.13	3.53±0.21[C]
LVID;s (mm)	1.83±0.06	2.05±0.17[A]	1.83±0.10	2.26±0.31[B,C]
LVPW;d (mm)	0.94±0.12	1.22±0.15[A]	0.94±0.14	1.22±0.23[B]
LVPW;s (mm)	1.43±0.09	1.65±0.18[A]	1.44±0.13	1.65±0.25[B]
FS%	45.77±0.80	40.05±2.07[A]	45.87±2.60	36.01±3.64[B,C]
EF%	78.65±0.87	73.05±2.90[A]	78.60±2.41	65.66±3.78[B,C]
SV (µL)	38.73±4.76	30.82±3.97[A]	38.61±2.39	30.52±2.61[B]
CO (mL/min)	17.68±1.95	13.66±1.97[A]	17.56±1.31	13.53±1.12[B]
LV mass (mg)	138.77±5.91	183.12±14.0[A]	138.68±11.82	207.2±26.3[B,C]
LV Vol;d (µL)	50.12±2.90	43.47±4.31[A]	50.29±3.71	50.19±5.21[C]
LV Vol;s (µL)	11.14±0.14	13.45±2.57[A]	11.53±2.20	17.03±2.61[B,C]
	Time (**4** Weeks)			
Echocardiography (n)	(10)	(17)	(11)	(14)
PG (mmHg)	3.03±0.22	60.12±7.35[A]	3.02±0.35	60.17±9.79[B]
Heart rate (BPM)	450±7	451±8	451±9	450±8
IVS;d (mm)	1.15±0.06	1.54±0.09[A]	1.13±0.10	1.39±0.09[B,C]
IVS;s (mm)	1.73±0.18	1.99±0.10[A]	1.73±0.06	1.84±0.09[B,C]
LVID;d (mm)	3.53±0.20	3.53±0.28	3.55±0.22	3.53±0.20
LVID;s (mm)	1.82±0.20	2.20±0.20[A]	1.82±0.11	2.45±0.31[B,C]
LVPW;d (mm)	0.94±0.05	1.30±0.15[A]	0.94±0.11	1.30±0.22[B]
LVPW;s (mm)	1.43±0.14	1.71±0.17[A]	1.43±0.05	1.71±0.18[B]
FS%	45.47±4.97	36.41±3.97[A]	45.47±0.89	32.21±3.25[B,C]
EF%	78.40±2.92	67.82±4.60[A]	78.44±1.01	61.21±6.02[B,C]
SV (µL)	38.16±3.86	30.88±2.63[A]	38.88±4.01	30.63±2.37[B]
CO (mL/min)	17.55±2.01	13.63±1.81[A]	17.51±1.78	13.61±1.27[B]
LV mass (mg)	138.79±12.56	208.38±13.8[A]	138.12±11.90	225.9±15.8[B,C]
LV Vol;d (µL)	50.89±6.23	50.55±5.71	50.86±5.81	50.20±5.43
LV Vol;s (µL)	11.55±2.91	18.32±3.75[A]	11.44±1.92	22.95±3.72[B,C]
	Time (**8** Weeks)			

Echocardiography (n)	(10)	(16)	(11)	(13)
PG (mmHg)	3.05±0.91	60.71±14.54[A]	3.03±0.41	59.96±12.47[B]
Heart rate (BPM)	451±8	451±3	451±9	450±9
IVS;d (mm)	1.15±0.06	1.55±0.16[A]	1.13±0.06	1.42±0.07[B,C]
IVS;s (mm)	1.72±0.06	2.02±0.06[A]	1.72±0.12	1.89±0.12[B,C]
LVID;d (mm)	3.52±0.10	3.64±0.10[A]	3.52±0.15	3.79±0.14[B,C]
LVID;s (mm)	1.83±0.17	2.30±0.28[A]	1.83±0.03	2.58±0.36[B,C]
LVPW;d (mm)	0.94±0.08	1.38±0.26[A]	0.94±0.08	1.39±0.37[B]
LVPW;s (mm)	1.44±0.06	1.77±0.24[A]	1.44±0.15	1.77±0.28[B]
FS%	45.09±2.74	33.36±3.46[A]	45.01±0.82	27.49±6.24[B,C]
EF%	78.41±3.36	62.94±4.74[A]	78.52±1.50	55.83±5.44[B,C]
SV (µL)	38.66±4.58	30.80±2.33[A]	38.78±2.92	30.77±4.74[B]
CO (mL/min)	17.57±1.51	13.87±1.24[A]	17.47±1.06	13.74±2.22[B]
LV mass (mg)	138.89±9.96	212.5±35.08[A]	138.91±15.87	249.6±30.8[B,C]
LV Vol;d (µL)	50.31±3.40	53.06±3.94[A]	50.75±2.06	57.00±4.25[B,C]
LV Vol;s (µL)	11.54±1.65	18.51±4.33[A]	11.44±0.97	25.70±8.95[B,C]

Table S5. ended.

Table S6. Echocardiography analyses of adult littermates of male (♂) and female (♀) nontransgenic (NG) and CR-Cyld Tg (TG) mice at 2 weeks after sham or TAC operation. Littermates of male and female NG and TG mice at age of 10 weeks were subject to sham and TAC operations for 2 weeks. PG, pressure gradient; LVID; in mm, the LV internal dimension; IVS; in mm, interventricular septum thickness; LVPW, in mm, LV posterior wall thickness; FS (%), LV percent fractional shortening; EF (%), ejection fraction; SV (μL), stroke volume; CO (ml/min), cardiac output; LV mass (mg) and LV volume (LV vol; in μL). Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. NG Sham; $^{B}p<0.05$ vs. TG Sham; $^{C}p<0.05$ vs. NG TAC.

Mixed gender (♂+♀)	NG		TG	
	Sham	TAC	Sham	TAC
Echocardiography (n)	(46)	(78)	(45)	(74)
PG (mmHg)	3.44±0.87	68.37±10.19^{A}	3.42±0.94	68.20±10.23^{B}
Heart Rate (BPM)	451±5	450±4	451±6	450±5
IVS;d (mm)	1.12±0.09	1.45±0.12^{A}	1.13±0.08	1.33±0.12^{B,C}
IVS;s (mm)	1.72±0.09	1.95±0.10^{A}	1.72±0.10	1.78±0.10^{B,C}
LVID;d (mm)	3.62±0.16	3.35±0.17^{A}	3.62±0.16	3.61±0.19^{C}
LVID;s (mm)	1.89±0.18	2.03±0.18^{A}	1.91±0.19	2.36±0.21^{B,C}
LVPW;d (mm)	0.96±0.12	1.23±0.13^{A}	0.96±0.11	1.24±0.14^{B}
LVPW;s (mm)	1.47±0.11	1.67±0.13^{A}	1.47±0.12	1.66±0.13^{B}
EF (%)	78.18±2.62	71.75±3.34^{A}	78.06±2.75	62.54±4.04^{B,C}
FS (%)	47.36±3.43	39.96±2.54^{A}	46.67±3.34	32.94±2.76^{B,C}
SV (μL)	42.89±4.51	32.08±3.79^{A}	43.07±4.22	32.26±3.41^{B}
CO (mL/min)	19.49±2.72	14.58±2.29^{A}	19.45±2.68	14.58±2.11^{B}
LV Mass (mg)	140.58±10.59	189.16±14.83^{A}	140.11±14.67	207.97±16.00^{B,C}
LV Vol;d (μL)	54.62±5.63	46.61±5.61^{A}	54.79±5.58	54.36±6.11^{C}
LV Vol;s (μL)	11.04±2.39	13.31±3.08^{A}	11.05±2.21	20.24±4.29^{B,C}

Table S7. Echocardiography analyses of adult littermates of male (♂) NG and TG mice at 2 weeks after sham or TAC operation. The data of male mice in Table S6 was analyzed. PG, pressure gradient; LVID; in mm, the LV internal dimension; IVS; in mm, interventricular septum thickness; LVPW, in mm, LV posterior wall thickness; FS (%), LV percent fractional shortening; EF (%), ejection fraction; SV (μL), stroke volume; CO (ml/min), cardiac output; LV mass (mg) and LV volume (LV vol; in μL). Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. NG Sham; $^{B}p<0.05$ vs. TG Sham; $^{C}p<0.05$ vs. NG TAC.

Male (♂)	NG		TG	
	Sham	TAC	Sham	TAC
Echocardiography (n)	(21)	(31)	(20)	(33)
PG (mmHg)	3.66±0.88	68.13±11.86^{A}	3.63±0.84	68.23±9.54^{B}
Heart Rate (BPM)	452±6	450±5	452±5	450±4
IVS;d (mm)	1.12±0.10	1.42±0.13^{A}	1.12±0.09	1.33±0.12^{B,C}
IVS;s (mm)	1.72±0.09	1.93±0.12^{A}	1.72±0.12	1.79±0.11^{C}
LVID;d (mm)	3.70±0.14	3.46±0.16^{A}	3.71±0.15	3.70±0.20^{C}
LVID;s (mm)	2.01±0.16	2.13±0.17^{A}	2.02±0.13	2.51±0.16^{B,C}
LVPW;d (mm)	0.93±0.11	1.23±0.16^{A}	0.93±0.10	1.24±0.16^{B}
LVPW;s (mm)	1.47±0.11	1.67±0.15^{A}	1.47±0.12	1.66±0.14^{B}
EF (%)	77.80±2.93	69.65±3.21^{A}	77.42±3.14	59.83±3.67^{B,C}
FS (%)	45.72±1.64	38.85±2.46^{A}	45.42±2.94	32.04±2.83^{B,C}
SV μL	45.51±3.63	33.98±3.45^{A}	45.53±3.70	34.75±3.15^{B}
CO (mL/min)	20.59±2.91	15.56±2.65^{A}	20.63±2.95	15.61±2.46^{B}
LV Mass (mg)	141.78±9.75	195.84±10.22^{A}	145.98±9.22	209.72±18.42^{B,C}
LV Vol;d (μL)	57.84±5.00	49.60±5.93^{A}	58.66±4.57	58.10±5.77^{C}
LV Vol;s (μL)	12.06±2.55	15.18±2.99^{A}	12.40±1.81	23.86±2.93^{B,C}

Table S8. Echocardiography analyses of adult littermates of female (♀) NG and TG mice at 2 weeks after sham or TAC operation. The data of female mice in Table S6 was analyzed. PG, pressure gradient; LVID; in mm, the LV internal dimension; IVS; in mm, interventricular septum thickness; LVPW, in mm, LV posterior wall thickness; FS (%), LV percent fractional shortening; EF (%), ejection fraction; SV (μL), stroke volume; CO (ml/min), cardiac output; LV mass (mg) and LV volume (LV vol; in μL). Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. NG Sham; $^{B}p<0.05$ vs. TG Sham; $^{C}p<0.05$ vs. NG TAC.

Female (♀)	NG		TG	
	Sham	TAC	Sham	TAC
Echocardiography (n)	(25)	(47)	(25)	(41)
PG (mmHg)	3.24±0.84	68.57±8.83^{A}	3.22±0.99	68.18±10.96^{B}
Heart Rate (BPM)	451±5	450±3	450±7	450±5
IVS;d (mm)	1.13±0.06	1.47±0.12^{A}	1.13±0.06	1.33±0.12^{B,C}
IVS;s (mm)	1.73±0.10	1.97±0.09^{A}	1.72±0.07	1.77±0.09^{C}
LVID;d (mm)	3.50±0.11	3.26±0.11^{A}	3.51±0.07	3.50±0.10^{C}
LVID;s (mm)	1.76±0.10	1.92±0.13^{A}	1.76±0.15	2.21±0.14^{B,C}
LVPW;d (mm)	1.00±0.12	1.23±0.09^{A}	1.00±0.11	1.23±0.12^{B}
LVPW;s (mm)	1.47±0.12	1.67±0.11^{A}	1.47±0.12	1.67±0.12^{B}
EF (%)	78.85±2.38	73.06±2.72^{A}	78.42±2.58	64.61±2.98^{B,C}
FS (%)	48.36±3.86	40.63±2.37^{A}	48.46±3.13	33.71±2.50^{B,C}
SV μL	40.10±3.65	30.80±3.50^{A}	40.89±3.42	30.53±2.38^{B}
CO (mL/min)	18.05±1.58	13.77±1.57^{A}	18.21±1.67	13.59±1.00^{B}
LV Mass (mg)	138.44±12.59	185.71±15.77^{A}	138.06±17.62	206.34±13.83^{B,C}
LV Vol;d (μL)	50.41±3.07	43.86±3.58^{A}	50.65±2.96	50.05±2.75^{C}
LV Vol;s (μL)	9.83±1.89	11.98±2.41^{A}	9.89±2.04	17.07±2.30^{B,C}

Table S9. Pathological analyses of adult littermates of male (♂) and female (♀) NG and TG mice at 2 weeks after sham or TAC operation. Littermates of male and female NG and TG mice at age of 10 weeks were subject to sham and TAC operations for 2 weeks. Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. NG Sham; $^{B}p<0.05$ vs. TG Sham; $^{C}p<0.05$ vs. NG TAC.

Mixed gender (♂+♀)	NG		TG	
	Sham	TAC	Sham	TAC
Pathology (n)	(29)	(48)	(26)	(49)
BW (g)	24.96±2.92	24.54±2.90	24.99±2.95	24.51±3.15
HW (mg)	158.44±38.14	210.33±35.88^{A}	159.64±37.64	236.30±36.72^{B,C}
LW (mg)	192.93±36.95	227.52±46.08^{A}	193.14±41.86	274.59±59.26^{B,C}
TIBIA (mm)	17.42±0.38	17.50±0.42	17.40±0.33	17.38±0.36
HW/TIBIA (mg/mm)	8.83±1.58	11.82±1.90^{A}	8.71±1.64	13.50±2.56^{B,C}
HW/BW (mg/g)	6.39±1.41	8.56±1.15^{A}	6.31±1.37	10.05±1.59^{B,C}
LW/TIBIA (mg/mm)	10.84±1.61	12.99±2.30^{A}	10.61±1.78	16.89±3.55^{B,C}
LW/BW (mg/g)	7.77±1.35	9.36±2.16^{A}	7.84±1.64	11.89±2.75^{B,C}
LV remodeling (n)	(10)	(22)	(10)	(20)
Myocytes (μm^2)	204.80±27.49	350.23±66.47^{A}	178.09±25.97	291.14±52.13^{B,C}
Fibrosis (%)				
Interstitial	0	4.80±8.68^{A}	0	3.14±5.50^{B}
Perivascular (diameter; 70-200μm)	0	1.02±2.97^{A}	0	1.86±4.43^{B}
Perivascular (diameter; 200-800μm)	0	13.13±13.05^{A}	0	9.37±7.76^{B}
Total (average)	0	6.31±8.23^{A}	0	4.79±5.90^{B}
Apoptosis (%)	0.03±0.09	0.09±0.16^{A}	0.01±0.06	0.18±0.21^{B,C}
qPCR (n)	(5)	(5)	(5)	(5)
ANF	0.97±0.06	30.91±7.70^{A}	1.19±0.73	30.37±6.20^{B}
BNP	1.06±0.13	3.82±0.43^{A}	0.90±0.37	4.03±0.80^{B}
αMHC	1.01±0.05	0.47±0.09^{A}	1.03±0.15	0.48±0.13^{B}
βMHC	1.03±0.12	11.35±4.73^{A}	1.07±0.09	11.36±4.49^{B}
SERCA2α	1.04±0.10	0.49±0.17^{A}	1.03±0.16	0.45±0.11^{B}

Table S10. Pathological analyses of adult littermates of male (♂) NG and TG mice at 2 weeks after sham or TAC operation. The data of male mice in Table S9 was analyzed. Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. NG Sham; $^{B}p<0.05$ vs. TG Sham; $^{C}p<0.05$ vs. NG TAC.

Male (♂)	NG		TG	
	Sham	TAC	Sham	TAC
Pathology (n)	(14)	(19)	(12)	(22)
BW (g)	27.88±1.99	27.12±2.87	27.32±1.20	27.01±2.75
HW (mg)	173.46±46.83	231.29±38.66^{A}	175.58±43.39	264.33±33.28^{B,C}
LW (mg)	198.62±38.31	245.20±56.17^{A}	200.56±50.32	306.83±56.51^{B,C}
TIBIA (mm)	17.50±0.51	17.60±0.48	17.58±0.34	17.54±0.35
HW/TIBIA (mg/mm)	9.27±1.79	12.84±1.81^{A}	9.40±1.65	15.45±2.42^{B,C}
HW/BW (mg/g)	6.38±1.72	8.50±1.21^{A}	6.45±1.65	10.38±2.19^{B,C}
LW/TIBIA (mg/mm)	10.97±2.02	13.58±3.25^{A}	10.65±2.67	18.61±3.63^{B,C}
LW/BW (mg/g)	7.27±1.25	9.50±1.98^{A}	7.33±1.92	12.25±3.43^{B,C}
LV remodeling (n)	(5)	(10)	(5)	(9)
Myocytes (μm^2)	217.69±26.83	365.86±78.05^{A}	170.21±27.60	295.23±58.83^{B,C}
Fibrosis (%)				
Interstitial	0	6.04±11.36^{A}	0	2.82±5.41^{B}
Perivascular (diameter; 70-200μm)	0	0.74±1.46^{A}	0	1.27±2.70^{B}
Perivascular (diameter; 200-800μm)	0	12.85±11.37^{A}	0	9.82±6.50^{B}
Total (average)	0	6.54±8.06^{A}		4.64±4.87^{B}
Apoptosis (%)	0.04±0.11	0.09±0.18^{A}	0.01±0.05	0.14±0.19^{B,C}

Table S11. Pathological analyses of adult littermates of female (♀) NG and TG mice at 2 weeks after sham or TAC operation. The data of female mice in Table S9 was analyzed. Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. NG Sham; $^{B}p<0.05$ vs. TG Sham; $^{C}p<0.05$ vs. NG TAC.

Female (♀)	NG		TG	
	Sham	TAC	Sham	TAC
Pathology (n)	(15)	(29)	(14)	(27)
BW (g)	22.83±1.31	22.82±1.11	22.85±2.39	22.49±1.63
HW (mg)	144.50±21.24	198.60±28.79^{A}	144.92±24.90	222.84±30.54^{B,C}
LW (mg)	188.00±36.32	219.83±39.92^{A}	187.58±35.58	251.82±51.19^{B,C}
TIBIA (mm)	17.35±0.20	17.44±0.36	17.24±0.22	17.26±0.32
HW/TIBIA (mg/mm)	8.54±1.41	11.37±1.80^{A}	8.27±1.52	12.55±2.08^{B,C}
HW/BW (mg/g)	6.40±1.06	8.61±1.13^{A}	6.18±1.09	9.83±1.02^{B,C}
LW/TIBIA (mg/mm)	10.68±0.92	12.56±1.20^{A}	10.59±0.94	14.61±1.76^{B,C}
LW/BW (mg/g)	8.18±1.35	9.52±1.13^{A}	8.30±1.26	11.48±1.71^{B,C}
LV remodeling (n)	(5)	(12)	(5)	(11)
Myocytes (μm^2)	191.92±21.71	337.20±51.92^{A}	185.95±21.85	284.00±44.97^{B,C}
Fibrosis (%)				
Interstitial	0	3.56±6.00^{A}	0	3.46±5.58^{B}
Perivascular (diameter; 70-200μm)	0	1.29±4.47^{A}	0	2.45±6.16^{B}
Perivascular (diameter; 200-800μm)	0	13.40±14.72^{A}	0	8.92±9.01^{B}
Total (average)	0	6.08±8.40^{A}	0	4.94±6.92^{B}
Apoptosis (%)	0.02±0.07	0.09±0.14^{A}	0.02±0.06	0.20±0.22^{B,C}

Table S12. Echocardiography analyses of adult littermates of nontransgenic (Ng), CR-tTA Tg (tTA), CR-Atg7 Tg (Atg7), and CR-Atg7×tTA Tg (CR-Atg7) mice at 2 weeks after sham or TAC operation. Littermates of male and female mice with indicated genotypes at age of 10 weeks were subject to sham or TAC operations for 2 weeks. PG, pressure gradient; LVID; in mm, the LV internal dimension; IVS; in mm, interventricular septum thickness; LVPW, in mm, LV posterior wall thickness; FS (%), LV percent fractional shortening; EF (%), ejection fraction; SV (μL), stroke volume; CO (ml/min), cardiac output; LV mass (mg) and LV volume (LV vol; in μL). Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. Sham group with same genotype; $^{B}p<0.05$ vs. Ng TAC. All abbreviations and units for each parameter are the same with Table S3.

Mixed gender (♂+♀)	Ng		Atg7		tTA		CR-Atg7	
	Sham	TAC	Sham	TAC	Sham	TAC	Sham	TAC
(n)	(8)	(14)	(15)	(24)	(4)	(6)	(15)	(24)
PG	3.7±0.4	70.2±13.8^{A}	3.6±1.0	70.8±6.3^{A}	3.7±1.0	71.7±6.9^{A}	3.7±0.8	70.7±4.5^{A}
HR	452±3	449±3	451±7	449±5	449±5	448±6	450±5	450±5
IVS;d	1.04±0.18	1.34±0.06^{A}	1.04±0.10	1.35±0.11^{A}	1.08±0.13	1.34±0.13^{A}	1.06±0.07	1.34±0.13^{A}
IVS;s	1.71±0.17	1.91±0.03^{A}	1.71±0.09	1.90±0.13^{A}	1.73±0.15	1.94±0.19^{A}	1.71±0.05	1.91±0.12^{A}
LVID;d	3.76±0.28	3.42±0.23^{A}	3.72±0.25	3.44±0.23^{A}	3.75±0.27	3.42±0.04^{A}	3.74±0.33	3.44±0.09^{A}
LVID;s	1.91±0.21	2.15±0.21^{A}	1.92±0.24	2.16±0.23^{A}	1.95±0.15	2.17±0.14^{A}	1.93±0.22	1.99±0.1^{A,B}
LVPW;d	1.03±0.03	1.38±0.13^{A}	1.03±0.10	1.38±0.16^{A}	1.02±0.10	1.40±0.26^{A}	1.03±0.14	1.37±0.18^{A}
LVPW;s	1.53±0.10	1.80±0.07^{A}	1.53±0.04	1.79±0.10^{A}	1.56±0.07	1.86±0.21^{A}	1.53±0.06	1.80±0.11^{A}
EF (%)	79.4±2.5	67.6±3.1^{A}	80.2±2.9	68.0±4.2^{A}	80.2±1.5	67.7±3.1^{A}	80.4±1.0	74.9±3.0^{A,B}
FS (%)	48.2±2.1	36.2±3.4^{A}	48.0±2.8	36.9±2.5^{A}	48.8±1.8	36.8±2.4^{A}	48.8±1.9	43.2±2.7^{A,B}
SV μL	45.7±9.6	34.9±5.3^{A}	45.2±7.7	34.9±3.2^{A}	45.2±8.6	35.6±4.0^{A}	45.6±7.0	40.3±3.2^{A,B}
CO	20.7±3.2	15.8±1.8^{A}	20.5±3.6	15.0±2.1^{A}	20.2±4.1	15.3±0.9^{A}	20.5±3.2	18.1±1.8^{A,B}
LV Mass	154.4±19.1	201.3±18^{A}	151.6±23	204.6±17^{A}	152.3±30	218.0±40^{A}	153.2±15	202.8±21^{A}
LV Vol;d	60.9±10.8	50.6±7.8^{A}	60.6±8.8	50.1±5.6^{A}	60.6±8.7	50.5±5.1^{A}	60.2±12.6	50.8±4.3^{A}
LV Vol;s	12.4±3.6	17.9±2.8^{A}	12.3±3.5	17.3±2.9^{A}	12.1±2.6	17.5±1.7^{A}	12.3±3.3	14.6±2.4^{A,B}

Table S13. Echocardiography analyses of adult littermates of nontransgenic (Ng), CR-tTA Tg (tTA), CR-Atg7 Tg (Atg7), and CR-Atg7×tTA Tg (CR-Atg7) mice at 4 weeks after sham or TAC operation. Littermates of male and female mice with indicated genotypes at age of 10 weeks were subject to sham or TAC operations for 4 weeks. It is the cohorts shown in Table S12. PG, pressure gradient; LVID; in mm, the LV internal dimension; IVS; in mm, interventricular septum thickness; LVPW, in mm, LV posterior wall thickness; FS (%), LV percent fractional shortening; EF (%), ejection fraction; SV (μL), stroke volume; CO (ml/min), cardiac output; LV mass (mg) and LV volume (LV vol; in μL). Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. Sham group with same genotype; $^{B}p<0.05$ vs. Ng TAC. All abbreviations and units for each parameter are the same with Table S3.

Mixed gender (♂+♀)	Ng		Atg7		tTA		CR-Atg7	
	Sham	TAC	Sham	TAC	Sham	TAC	Sham	TAC
(n)	(8)	(14)	(15)	(23)	(4)	(6)	(15)	(22)
PG	3.2±0.4	72.3±6.3^{A}	3.5±1.0	71.8±13.0^{A}	3.8±0.3	71.4±16.2^{A}	3.6±0.7	72.3±10.3^{A}
HR	453±7	450±11	452±6	449±4	448±18	450±2	452±5	452±4
IVS;d	1.11±0.11	1.46±0.11^{A}	1.10±0.07	1.46±0.14^{A}	1.09±0.20	1.44±0.12^{A}	1.10±0.06	1.45±0.11^{A}
IVS;s	1.76±0.16	1.97±0.13^{A}	1.77±0.13	1.96±0.15^{A}	1.73±0.17	1.99±0.10^{A}	1.77±0.06	1.96±0.11^{A}
LVID;d	3.69±0.32	3.64±0.28	3.70±0.23	3.66±0.17	3.57±0.05	3.64±0.17	3.68±0.18	3.69±0.11
LVID;s	1.85±0.17	2.43±0.32^{A}	1.85±0.10	2.47±0.22^{A}	1.82±0.06	2.43±0.39^{A}	1.85±0.08	2.2±0.2^{A,B}
LVPW;d	1.02±0.13	1.38±0.07^{A}	1.02±0.12	1.38±0.13^{A}	1.12±0.15	1.41±0.09^{A}	1.02±0.09	1.39±0.14^{A}
LVPW;s	1.62±0.06	1.76±0.06^{A}	1.61±0.09	1.76±0.10^{A}	1.68±0.13	1.79±0.07^{A}	1.60±0.10	1.79±0.15^{A}
EF (%)	80.7±2.0	63.2±6.8^{A}	79.8±1.5	63.4±6.5^{A}	81.1±1.4	63.213.5^{A}	80.6±2.7	71.6±4.4^{A}
FS (%)	48.5±1.9	33.7±4.5^{A}	47.9±1.4	33.8±4.7^{A}	48.9±1.4	33.5±2.2^{A}	48.5±2.6	40.4±3.6^{A,B}
SV	48.4±8.5	33.9±4.8^{A}	47.8±6.8	33.8±3.8^{A}	43.0±1.2	33.6±6.2^{A}	47.9±5.6	43.0±3.5^{A,B}
CO	21.2±4.1	15.3±2.1^{A}	21.3±2.8	15.2±1.7^{A}	19.4±0.2	15.8±3.2^{A}	21.8±2.7	19.4±1.5^{A,B}
LV Mass	154.6±28	232.1±25^{A}	156.6±15	232.1±23^{A}	160.3±36	236.7±34^{A}	157.3±18	233.2±26^{A}
LV Vol;d	58.2±12.2	57.3±10.2	58.26±8.6	57.51±6.3	53.5±1.7	54.99±11.6	57.45±6.6	58.96±5.0
LV Vol;s	11.5±3.4	21.4±7.5^{A}	11.7±2.3	21.9±5.1^{A}	10.3±0.6	21.5±6.5^{A}	11.7±2.6	16.7±3.1^{A,B}

Table S14. Pathological analyses of adult littermates of nontransgenic (Ng), CR-tTA Tg (tTA), CR-Atg7 Tg (Atg7), and CR-Atg7×tTA Tg (CR-Atg7) mice at 4 weeks after sham or TAC operation. Littermates of male (♂) and female (♀) with indicated genotypes at age of 10 weeks were subject to sham or TAC operations for 4 weeks. It is the cohorts shown in Table S12. Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. Sham group with same genotype; $^{B}p<0.05$ vs. Ng TAC. BW, body weight; HW, heart weight; LW, lung weight; TL, tibia length.

Mixed gender (♂+♀)	Ng		Atg7		tTA		CR-Atg7	
	Sham	TAC	Sham	TAC	Sham	TAC	Sham	TAC
(n)	8	13	15	23	4	6	15	22
BW (g)	25.59±3.94	25.25±2.37	25.46±3.15	25.53±2.69	25.41±2.99	25.67±3.01	25.66±3.34	25.63±1.91
HW (mg)	200±48	273±34^{A}	198±42	273±39^{A}	220±35	270±53^{A}	213±55	271±19^{A}
LW(mg)	216±49	344±121^{A}	214±44	348±104^{A}	210±61	344±58^{A}	215±49	261±61^{A,B}
TL (mm)	17.54±0.63	17.62±0.30	17.51±0.43	17.67±0.30	17.53±0.55	17.45±0.42	17.41±0.44	17.63±0.37
HW/BW (mg/g)	8.3±1.2	10.4±1.3^{A}	8.1±0.9	10.4±1.1^{A}	8.6±0.7	10.2±1.3^{A}	8.2±1.8	10.1±1.2^{A}
HW/TL (mg/mm)	11.5±2.3	15.2±2.1^{A}	11.5±2.0	15.3±1.8^{A}	11.7±0.1	15.8±3.1^{A}	11.9±2.9	15.8±2.1^{A}
LW/BW (mg/g)	8.8±2.3	14.5±5.3^{A}	8.7±1.8	14.3±4.2^{A}	8.6±2.1	12.9±1.4^{A}	8.9±1.8	10.4±1.6^{A,B}
LW/TL (mg/mm)	12.3±2.9	20.5±6.4^{A}	12.8±2.5	20.4±5.6^{A}	12.0±3.1	20.5±2.9^{A}	12.6±2.8	14.7±2.6^{A,B}

Table S15. Echocardiography analyses of adult littermates of nontransgenic (Ng), CR-Atg7×tTA Tg (CR-Atg7 Tg), CR-Cyld Tg and double CR-Atg7 and Cyld Tg (Duo-Tg) mice at 2 weeks after sham or TAC operation. Littermates of male (♂) and female (♀) mice with indicated genotypes at age of 10 weeks were subject to sham or TAC operations for 2 weeks. Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. Ng TAC; $^{B}p<0.05$ vs. CR-Cyld Tg TAC. All abbreviations and units for each parameter are the same with Table S3.

Mixed gender (♂+♀)	Ng	CR-Atg7 Tg	CR-Cyld Tg	Duo-Tg
	TAC	TAC	TAC	TAC
Echocardiography (n)	8	17	13	21
PG (mmHg)	73.49±15.74	74.50±7.87	74.21±13.23	74.04±12.42
Heart Rate (BPM)	449±9	451±5	450±5	449±6
IVS;d (mm)	1.48±0.17	1.48±0.11	$1.26±0.13^{A}$	$1.26±0.07^{A}$
IVS;s (mm)	2.03±0.21	2.01±0.11	$1.73±0.14^{A}$	$1.73±0.06^{A}$
LVID;d (mm)	3.31±0.18	3.37±0.12	$3.68±0.18^{A}$	$3.98±0.29^{A,B}$
LVID;s (mm)	1.96±0.13	1.97±0.21	$2.23±0.23^{A}$	$2.63±0.30^{A,B}$
LVPW;d (mm)	1.36±0.14	1.37±0.17	1.35±0.20	1.39±0.15
LVPW;s (mm)	1.74±0.11	1.75±0.16	1.73±0.09	1.73±0.08
EF (%)	72.75±1.25	$77.98±2.76^{A}$	$67.31±3.95^{A}$	$60.66±5.59^{A,B}$
FS (%)	40.74±0.96	$45.83±2.55^{A}$	$36.60±2.81^{A}$	$31.92±3.67^{A,B}$
SV μL	33.54±3.61	$42.65±3.73^{A}$	32.87±3.07	34.05±4.36
CO (mL/min)	15.07±1.69	$19.26±1.83^{A}$	14.87±1.31	15.45±1.83
LV Mass (mg)	193.73±24.25	199.45±28.42	$223.47±20.63^{A}$	$258.32±29.53^{A,B}$
LV Vol;d (μL)	44.52±5.87	44.51±3.91	$53.79±8.09^{A}$	$64.22±11.05^{A,B}$
LV Vol;s (μL)	12.18±2.01	12.06±2.91	$17.45±4.36^{A}$	$24.72±8.70^{A,B}$

Table S16. Pathological analyses of adult littermates of nontransgenic (Ng), CR-Atg7×tTA Tg (CR-Atg7 Tg), CR-Cyld Tg and double CR-Atg7 and Cyld Tg (Duo-Tg) mice at 2 weeks after sham or TAC operation. Littermates of male (♂) and female (♀) mice with indicated genotypes at age of 10 weeks were subject to sham or TAC operations for 2 weeks. Animal numbers (n) of each group are indicated in parentheses. $^{A}p<0.05$ vs. Ng TAC; $^{B}p<0.05$ vs. CR-Cyld Tg TAC. BW, body weight; LW, lung weight; TL, tibia length.

Mixed gender (♂+♀)	Ng	CR-Atg7 Tg	CR-Cyld Tg	Duo-Tg
	TAC	TAC	TAC	TAC
Pathology (n)	8	17	13	21
Body Weight (g)	25.81±2.80	26.05±3.19	24.80±2.16	25.46±2.45
Heart Weight (mg)	254.29±25.07	253.85±26.63	286.00±15.17^{A}	342.50±51.90^{A,B}
Lung Weight (mg)	245.71±55.33	242.86±44.24	351.43±73.35^{A}	434.54±80.42^{A,B}
TIBIA (mm)	17.65±0.55	17.75±0.29	17.48±0.29	17.62±0.34
HW/BW (mg/g)	10.02±0.32	10.00±0.42	11.30±1.00^{A}	12.90±1.45^{A,B}
HW/TL (mg/mm)	13.97±0.85	13.94±1.37	15.25±0.80^{A}	19.10±3.47^{A,B}
LW/BW (mg/g)	11.48±3.32	8.39±2.11^{A}	15.51±2.84^{A}	18.59±2.39^{A,B}
LW/TL (mg/mm)	13.95±2.70	11.13±2.22^{A}	19.01±4.77^{A}	23.56±2.86^{A,B}