

Original Article

Icariin ameliorates hypertension-induced cardiomyocyte senescence by promoting PTEN-dependent autophagy

Yuting Bi¹, Zhenhao Cai¹, Lanlan Wang², Shengnan Li², Haipeng Jie², Yunbo Xie³, Xinxin Xu³, Chao Yang³, Ying Wang³, Yalin Yu⁴, Guohua Song³, Bo Dong^{1,2}

¹First Clinical Medical College, Shandong University of Traditional Chinese Medicine, Jinan 250013, Shandong, China; ²Department of Cardiology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250021, Shandong, China; ³Clinical and Basic Medical Sciences, Shandong First Medical University and Shandong Academy of Medical Science, Jinan 250117, Shandong, China; ⁴Jinan Hospital of Integrated Traditional Chinese and Western Medicine, Jinan 271100, Shandong, China

Received June 16, 2026; Accepted September 22, 2026; Epub October 15, 2026; Published October 30, 2026

Abstract: Background: Age-related cardiovascular decline contributes to the development and progression of hypertension. Aims: This study aimed to investigate the protective effects of icariin (ICA) on hypertension-induced cardiomyocyte senescence and to explore the specific role of phosphatase and tensin homolog (PTEN). Methods: C57BL/6 mice were fed a high-salt diet to induce hypertension and subsequently treated with ICA for 8 weeks. Blood pressure and cardiac function were monitored to evaluate the therapeutic efficacy of ICA. *In vitro*, H9C2 cells that were treated with angiotensin II were used to mimic hypertension-associated stress. Cardiomyocyte senescence was assessed using senescence-associated β -galactosidase (SA- β -Gal) staining, reactive oxygen species (ROS) detection, and western blotting (WB) for senescence markers (p53, p21, and p16). The involvement of the PTEN/phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway and autophagy was examined using molecular docking, WB, PTEN siRNA knockdown, and pharmacologic inhibition of autophagy. Results: Compared to the model group, ICA treatment significantly lowered blood pressure and ameliorated left ventricular dysfunction. In both *in vivo* and *in vitro* experiments, ICA markedly reduced cardiomyocyte senescence, as evidenced by reductions in SA- β -Gal activity, ROS accumulation, and the expression of p53, p21, and p16. Mechanistically, ICA enhanced PTEN activity by reducing its phosphorylation, thereby inhibiting the PI3K/AKT pathway and promoting autophagy. Notably, the anti-senescence effects of ICA were abolished by PTEN knockdown or autophagy inhibition, indicating that ICA functions through a PTEN-dependent autophagic mechanism. Conclusions: Icariin ameliorates hypertension-induced cardiomyocyte senescence by enhancing PTEN activity, inhibiting the PI3K/AKT signaling pathway, and restoring autophagy. These findings suggest that ICA may be a promising therapeutic candidate for delaying hypertension-associated cardiac aging.

Keywords: Hypertension, phosphatase and tensin homolog, cardiomyocyte senescence, icariin, autophagy

Introduction

Hypertension, characterized by chronically high blood pressure (BP), remains a substantial global health challenge and is a leading contributor to ischemic heart disease, stroke, and chronic kidney disease [1, 2]. Despite advances in antihypertensive pharmacotherapy, the prevalence of hypertension continues to rise, partly due to aging populations and unhealthy lifestyles [3, 4]. Pathologically, hypertension is a hemodynamic disorder closely associated with accelerated cardiovascular aging [5-7].

Senescent cardiomyocytes undergo irreversible cell-cycle arrest and acquire a senescence-associated secretory phenotype (SASP), a key driver of hypertensive cardiac remodeling and heart failure [8]. During hypertension, sustained pathologic stress accelerates the aging process of myocardial cells, as characterized by the accumulation of cellular senescence markers and the progressive loss of physiological function, thereby contributing to hypertensive myocardial remodeling [9]. A previous study demonstrated a vicious cycle between hypertension and cardiovascular aging, with each

accelerating and aggravating the other [6]. Therefore, targeting the molecular mechanisms driving cardiomyocyte senescence is a promising therapeutic strategy for hypertensive heart disease [10].

As an evolutionarily conserved process, autophagy degrades damaged organelles and proteins and recycles their components to preserve cellular homeostasis [11]. Impaired autophagic flux is a hallmark of biological aging and contributes to the accumulation of cellular damage in cardiovascular diseases [12, 13]. Recent studies suggest that restoring autophagy can delay vascular and cardiac senescence [13-16]. Phosphatase and tensin homolog (PTEN) exerts its tumor-suppressive effects [17] by inhibiting the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway [18, 19]. By dephosphorylating phosphatidylinositol-3,4,5-trisphosphate (PIP3), PTEN inhibits AKT activation, which serves as a key molecular switch for autophagy induction [20, 21]. Recent reports have shown that PTEN plays an important role in delaying hypertension-associated vascular aging [22]. However, the contribution of PTEN-mediated autophagy to hypertensive cardiomyocyte senescence remains unclear.

Current antihypertensive therapies have several limitations, including adverse effects and incomplete protection against end-organ damage [23, 24]. Consequently, natural compounds from Traditional Chinese Medicine (TCM) have attracted increasing attention because of their multi-target pharmacologic activities [25]. Icariin (ICA), a flavonoid and the major bioactive component of *Epimedium Folium* (Herba Epimedium) [26], has traditionally been used to “tonify the kidney” and treat age-related conditions [27, 28]. Contemporary pharmacologic evidence indicates that ICA possesses potent antioxidant, anti-inflammatory, and cardioprotective properties [29-34]. Although ICA has been shown to protect against cardiovascular injury [26], the precise molecular mechanisms by which it modulates cardiomyocyte senescence, particularly whether it acts through the PTEN/autophagy axis, remain unclear.

In this study, we hypothesized that ICA ameliorates hypertension-induced cardiomyocyte senescence by promoting PTEN-dependent autophagy. To test this, we established an *in vivo*

mouse model of hypertension using a high-salt (HS) diet. Given that a HS diet reportedly activates the cardiac renin-angiotensin system (RAS) and enhances angiotensin II (Ang II) availability in the heart [35, 36], Ang II-treated H9C2 cells were used to mimic this local Ang II-rich environment *in vitro*. By integrating network pharmacology, molecular docking, and experimental validation, we investigated the therapeutic effects of ICA and its underlying mechanisms involving the PTEN/PI3K/AKT signaling pathway.

Materials and methods

Animals and experimental design

ICA (purity $\geq 98\%$, CAS 489-32-7) were purchased from Shanghai TOPSCIENCE Biotechnology. Male C57BL/6 mice (6-8 weeks old, $n = 27$) and experimental diets were obtained from Beijing Huafukang Biological Technology. The mice were maintained at $22 \pm 1^\circ\text{C}$ under a 12-h light/dark cycle, with food and water provided *ad libitum*. They were randomly divided into three groups ($n = 9/\text{group}$): Control group, fed a normal-salt diet containing 0.5% sodium chloride (NaCl) for 12 weeks; Model group, fed a HS diet containing 8% NaCl for 12 weeks; and HS + ICA group, fed the HS diet for 4 weeks to establish hypertension, followed by daily intraperitoneal (i.p.) injection of ICA (40 mg/kg, dissolved in vehicle) for 8 weeks while maintaining the HS diet. Body weight, food intake, and water consumption were measured weekly.

At the end of the 12-week experimental period, mice were anesthetized with 2% sodium pentobarbital, followed by blood collection through enucleation of the eyeball, and then immediately euthanized by cervical dislocation. Subsequently, plasma, liver, adipose tissue, heart, and skeletal muscle were sequentially harvested. Hearts were rapidly excised and processed for histologic and molecular analyses. The mice in each experimental group ($n = 9$) were randomly allocated into three subsets ($n = 3/\text{subset}$) for paraffin sectioning, frozen sectioning, and protein extraction, respectively.

Ethics statement: All animal procedures were conducted following the *Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care and Use Committee of Shandong First Medical University (Approval No. W202502210022).

Blood pressure (BP) measurement and echocardiography

BP was measured using a noninvasive tail-cuff system (Softron Biotechnology, Beijing, China). Conscious mice were placed in a restrainer on a warming pad. Systolic BP (SBP), diastolic BP (DBP), and mean BP (MBP) were calculated as the averages of three consecutive stable measurements. Cardiac function was evaluated under 1.5% isoflurane anesthesia using a Vevo-2100 high-resolution imaging system (Visual-Sonics, Toronto, Canada). From parasternal short-axis views at the papillary muscle level, we acquired M-mode images and then calculated left ventricular ejection fraction (LVEF), fractional shortening (LVFS), LV internal diameters (LVIDs/LVIDd), and posterior wall thickness (LVPWs/LVPWd). Echocardiographic assessment was performed in five randomly selected mice from each experimental group. This subset-based strategy was adopted because the ultrasound facility was located on a separate campus, and transporting the entire cohort could have induced stress-induced hemodynamic alterations and increased the risk of animal mortality, compromising data integrity and animal welfare. Random selection was used to minimize selection bias.

Histologic analysis

Heart tissues were fixed in 4% paraformaldehyde for 24 h, embedded in paraffin, and cut into 5- μ m-thick sections. Senescence-Associated β -galactosidase (SA- β -Gal) staining (Solarbio, Beijing, China) was performed on frozen sections according to the manufacturer's instructions to assess cardiomyocyte senescence. Masson's trichrome (Solarbio, Beijing, China) staining was performed on paraffin-embedded sections according to the manufacturer's instructions to evaluate myocardial collagen deposition.

Myocardial fibrosis was quantified in Masson's trichrome-stained sections using ImageJ software. Following color deconvolution and thresholding, the fibrotic area was calculated as the percentage of the blue-stained area relative to the total myocardial tissue area in each field.

Cell culture and treatments

The H9C2 rat cardiomyoblast cell line (CL-0089, Procell) was maintained in Dulbecco's

modified Eagle medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. Ang II was obtained from GLP BIO (GP10023, Shanghai, China). To mimic hypertension-associated stress *in vitro*, H9C2 cells were treated with Ang II (800 nM). Based on the results of Cell Counting Kit-8 (CCK-8) assays, an ICA concentration of 15 μ M was selected for subsequent experiments. To investigate the underlying mechanism, cells were pre-treated with the autophagy inhibitor chloroquine (CQ, 20 μ M; Macklin, Shanghai, China) or transfected with PTEN-siRNA (Sangon, Shanghai, China) before to Ang II/ICA stimulation.

Cell viability assay and transfection

H9C2 cells were seeded in 96-well plates and subjected to the indicated treatments for 48 h. Cell viability was subsequently assessed using a CCK-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. Absorbance at 450 nm was measured after incubation with the CCK-8 reagent using a microplate reader.

For PTEN silencing, H9C2 cells were transfected with siPTEN or control siRNA using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions.

Assessment of reactive oxygen species (ROS) and cellular senescence

Intracellular ROS levels were detected using the fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; Dojindo, Kumamoto, Japan). Post-treatment, cells were exposed to DCFH-DA for 30 min at 37°C, washed with Hanks' balanced salt solution (HBSS), and counterstained with Hoechst 33342. Fluorescence images were acquired using a fluorescence microscope.

SA- β -gal Staining Cellular senescence was assessed using a SA- β -gal staining kit. Frozen heart sections or cultured cells were fixed for 15 min, washed, and incubated with the staining solution at 37°C in a CO₂-free environment for 2 weeks. Senescent cells (stained blue) were visualized and quantified under a light microscope.

Western blot analysis

Heart tissues or H9C2 cells were lysed in radio-immunoprecipitation assay (RIPA) buffer sup-

plemented with protease and phosphatase inhibitors (Solarbio, Beijing, China) for total protein extraction. After BCA quantification, equal amounts of protein were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% nonfat milk and incubated overnight at 4°C with primary antibodies against p53, p21, p16, p62, microtubule-associated protein 1 light chain 3 beta (LC3B) (Zen-Bioscience), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Proteintech), PTEN, p-PTEN (Ser380), PI3K, p-PI3K, AKT, and p-AKT (Zen-Bioscience/Abcam). After washing, the membranes were incubated with horseradish peroxidase (HRP) - conjugated secondary antibodies (ZEN-Bioscience/Yeasen). Protein bands were visualized using an enhanced chemiluminescence detection kit (Yeasten). The list of antibodies used in this study is presented in [Supplementary Table 1](#).

Band intensities were quantified by densitometry using ImageJ software. The signals for phosphorylated and total proteins were independently normalized to GAPDH. Relative phosphorylation levels were then calculated as the ratio of the normalized phosphorylated protein level to the corresponding normalized total protein level: (phosphorylated protein/GAPDH)/(total protein/GAPDH). Statistical comparisons were performed using these normalized phosphorylation ratios.

Network pharmacology and molecular docking target prediction

ICA targets were predicted using SwissTarget-Prediction and PharmMapper, with an initial score threshold > 0. Hypertension-associated genes were retrieved from the Online Mendelian Inheritance in Man (OMIM) and GeneCards databases using the keyword “hypertension”. The initial searches identified 563 candidate genes in GeneCards and 987 candidate genes from OMIM. To obtain a high-confidence and clinically relevant target set, further filtering was performed. For GeneCards, only genes with relevance scores above the 90th percentile of the retrieved candidate genes were retained; for OMIM, only genes with documented causal variants or confirmed hypertension-associated entries were included. The filtered gene sets were merged, and duplicate entries were removed, resulting in a final set of 289

unique hypertension-related genes. The overlapping targets between ICA and hypertension were identified by intersecting the two target sets and visualized using Venny 2.1 diagrams.

PPI network: The overlapping targets were imported into the STRING database to identify potential interactions among their encoded proteins. STRING assigns interaction confidence scores, which are commonly classified as low (< 0.4), medium (0.4-0.7), or high (> 0.7). The organism was set to “Homo sapiens”, and a minimum required interaction score of 0.4 was applied to construct the protein-protein interaction (PPI) network. The acquired results were imported into Cytoscape 3.9.1 for visualization and further analysis. An ICA-target-hypertension network was subsequently constructed to illustrate the relationships among ICA, its potential targets, and hypertension-associated proteins.

Molecular docking: PTEN protein structure (Protein Data Bank [PDB] ID: 1D5R) was obtained from the RCSB Protein Data Bank (<https://www.rcsb.org/>) and prepared using the Protein Preparation Wizard in the Schrödinger Maestro platform (version 11.9). This procedure included the removal of crystallographic water molecules, addition of missing hydrogen atoms, assignment of bond orders, and reconstruction of missing peptide segments. The protein structure was then subjected to restrained energy minimization and geometry optimization using the OPLS3e force field. Ligands were prepared using the LigPrep module with the default settings. For receptor-grid generation, a 15 Å × 15 Å × 15 Å box was centered on the centroid of the co-crystallized ligand (coordinates: x = 34.95, y = -87.75, z = 24.95). Molecular docking and virtual screening were performed using the standard precision (SP) mode of the Glide module. The predicted binding potential was evaluated based on the docking score and interaction types (e.g., hydrogen bonds, π-π stacking).

Cellular thermal shift assay (CETSA)

H9C2 cells were treated with ICA (100 μM, 1 h) or an equivalent volume of vehicle (DMSO, final concentration ≤ 0.1%) for 1 h. The cells were then harvested, resuspended in HBSS, and divided into equal aliquots. The aliquots were heated at the indicated temperatures ranging from 30 to 50°C for 3 min and, then cooled.

After three freeze-thaw cycles, the samples were centrifuged to separate the soluble protein fractions. PTEN levels in the soluble fractions were determined by western blotting to evaluate ICA-induced changes in PTEN thermal stability.

Statistical analysis

Results were expressed as mean $\pm$ standard deviation (SD). GraphPad Prism software (version 9.0) was used for the statistical analyses. Student's *t*-test was used for two-group comparisons. Multiple group comparisons were performed using one-way analysis of variance followed by Tukey's (equal variances) or Tamhane's T2 (unequal variances). Statistical significance was set at $P < 0.05$.

Results

ICA ameliorated elevated BP and cardiac dysfunction in HS diet-induced hypertensive mice

To evaluate the therapeutic effects of ICA on hypertension, we established a mouse model of hypertension using an HS diet. Compared to the controls, HS-fed mice exhibited substantially higher SBP, DBP, and MBP ($P < 0.01$). Notably, ICA significantly attenuated these increases in BP (**Figure 1A, 1B**), whereas heart rate (HR) remained unaffected across all groups (**Figure 1C**). No significant differences in body weight or food intake were observed among the groups (**Figure 1D, 1E**). However, the increased water intake in HS-fed mice was mitigated by ICA treatment (**Figure 1D**). Additionally, HS-fed mice exhibited an elevated heart weight (HW)-to-body weight (BW) ratio, indicating cardiac hypertrophy, whereas ICA treatment reduced this ratio (**Figure 1F**). Echocardiographic assessment further revealed that ICA ameliorated HS-induced cardiac remodeling and dysfunction. Specifically, ICA treatment significantly reduced LVPWd, IVSd, and LVESD and restored the HS diet-induced alterations in LVEF and LVIDd (**Figure 1G, 1H**). These findings indicate that ICA effectively lowers BP and protects against hypertension-associated cardiac remodeling and dysfunction.

ICA attenuated cardiomyocyte senescence in vitro and in vivo

To assess histopathologic changes in the myocardium, heart tissues from HS-fed mice were

examined. Western blotting revealed that ICA reduced the cardiac expression of the senescence-associated proteins p53, p21, and p16 *in vivo* (**Figure 2A, 2B**). Supporting these findings, ICA administration visibly decreased the senescence burden in the hearts of hypertensive mouse, as revealed by SA- β -Gal staining (**Figure 2C**). In addition, compared to the model mice, the hearts in ICA-treated mice exhibited less collagen deposition on Masson staining (**Figure 2D**).

CCK-8 assays were then performed to select the optimal ICA concentration for *in vitro* experiments. Ang II significantly reduced cell viability at 800 nM, whereas ICA exerted its optimal protective effects at 15 μ M; therefore, these concentrations were selected for subsequent experiments (**Figure 3A**). Given the important role of oxidative stress in cellular senescence, intracellular ROS levels were measured to evaluate the antioxidant effect of ICA. ICA markedly suppressed Ang II-induced ROS accumulation in H9C2 cells (**Figure 3B, 3C**). Furthermore, SA- β -Gal staining indicated that ICA markedly reduced cellular senescence compared to that in the Ang II-treated model group (**Figure 3D**). Consistent with the *in vivo* findings, ICA reversed the Ang II-induced increases in p53, p21, and p16 expression (**Figure 3E**).

Network pharmacology identified the PTEN/PI3K/AKT pathway and autophagy as potential mechanistic targets

To elucidate the potential molecular mechanisms underlying the effects of ICA, we performed network pharmacology analyses. A total of 12,366 potential ICA targets and 289 hypertension-associated targets were identified, of which 247 were shared between the two target sets (**Figure 4A**). A PPI network was subsequently constructed (**Figure 4B**), and the top 10 hub genes were identified using the maximal clique centrality (MCC) algorithm, including *BCL2*, *MMP9*, *AKT1*, and *PTEN* (**Figure 4C**).

Gene ontology (GO) enrichment analysis indicated that these overlapping targets were involved in biological processes associated with signal transduction, BP modulation, and positive regulation of autophagy (**Figure 4D**). Furthermore, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

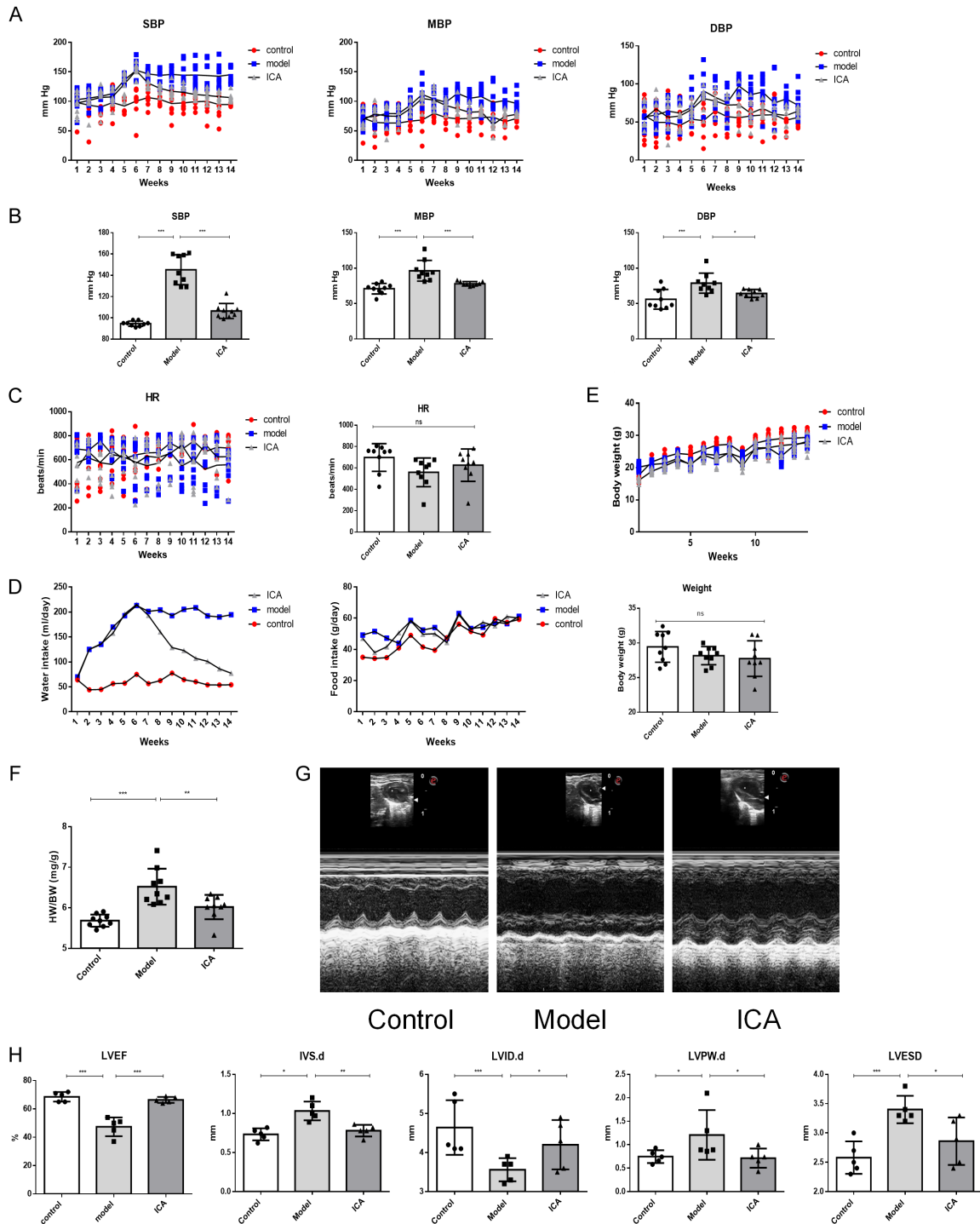


Figure 1. ICA ameliorated hemodynamic measurements and cardiac dysfunction in HS diet-fed mice. A, B. Comparison of SBP, DBP, and MBP among groups; C. Comparison of HR among groups; D. Average daily water intake and food intake; E. Changes in body weight over time; F. HW/BW ratio; G. Representative M-mode echocardiographic images of the left ventricle; H. Quantitative analysis of echocardiographic parameters, including LVPWd, IVSd, LVESD, LVEF, and LVIDd. Notes: BP, blood pressure; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HW, heart weight; BW, body weight; LVPWd, left ventricular posterior wall thickness at end-diastole; IVSd, interventricular septal thickness at end-diastole; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diameter at end-diastole. A-F. Data are presented as mean $\pm$ SD ($n = 9$ mice/group). G, H. Data are presented as mean $\pm$ SD ($n = 5$ mice/group). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus the designated control or model group.

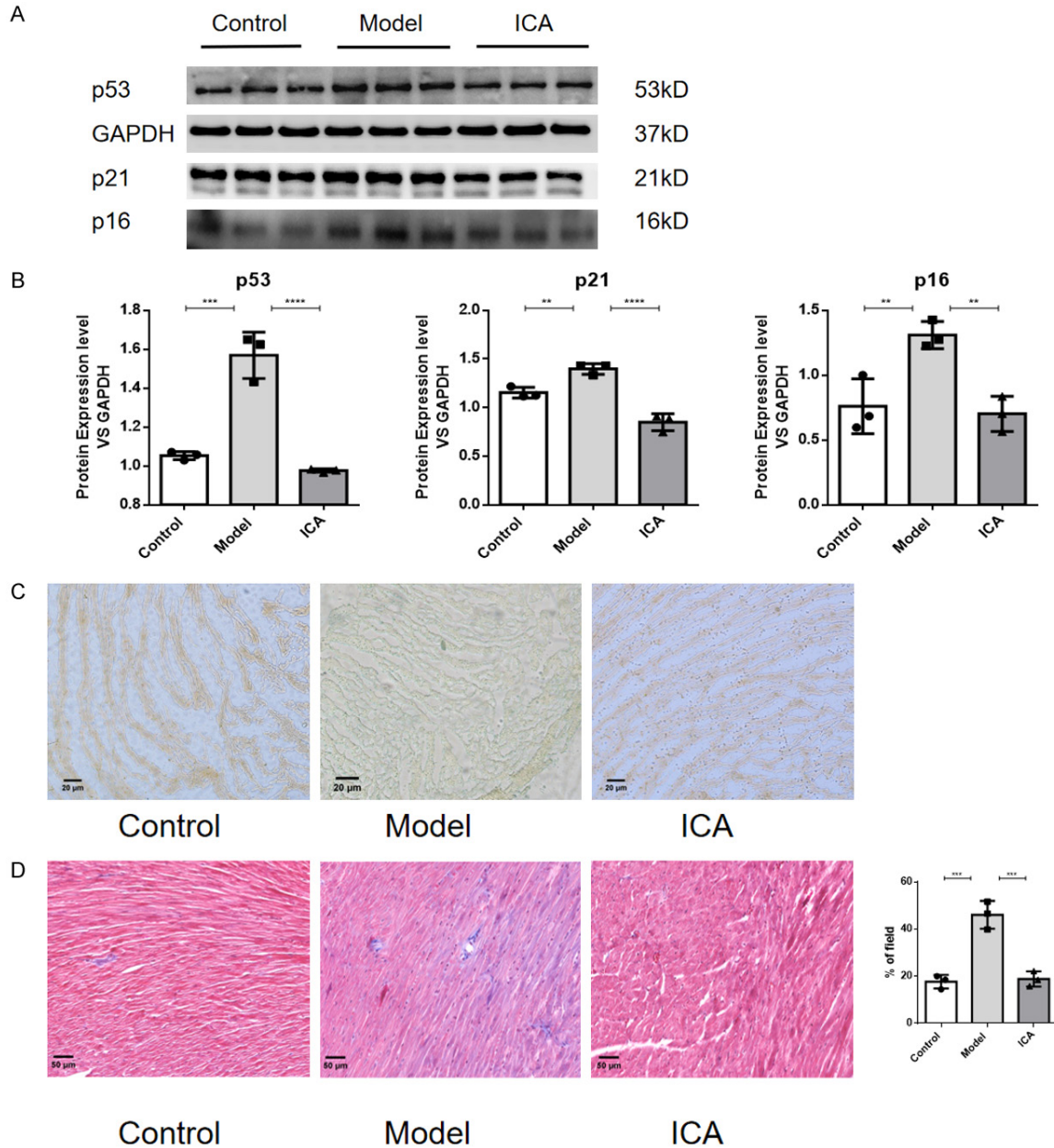


Figure 2. ICA attenuated cardiac cellular senescence and myocardial fibrosis in hypertensive mice. A, B. Representative western blots and quantitative analysis of senescence-associated proteins p53, p21, and p16 in heart tissue lysates from the three groups; C. Representative images of SA-β-Gal staining in heart tissue sections (×10 magnification; Scale bar = 20 μm); D. Representative images and quantitative analysis of Masson's staining in heart tissue sections (×20 magnification; Scale bar = 50 μm). Notes: Data are presented as mean ± SD (n = 3). **P < 0.01, ***P < 0.001 and ****P < 0.0001.

further identified the PI3K-AKT signaling pathway and cellular senescence among the significantly enriched pathways (**Figure 4D**). Collectively, these bioinformatic findings suggest that ICA may alleviate hypertension-induced cellular senescence by modulating autophagy through the PTEN/PI3K/AKT axis.

Target engagement between ICA and PTEN

Based on the network pharmacology predictions, we hypothesized that PTEN may be a direct molecular target of ICA. To investigate the potential molecular interactions between ICA and PTEN, a docking model was estab-

Icariin alleviates cardiomyocyte senescence

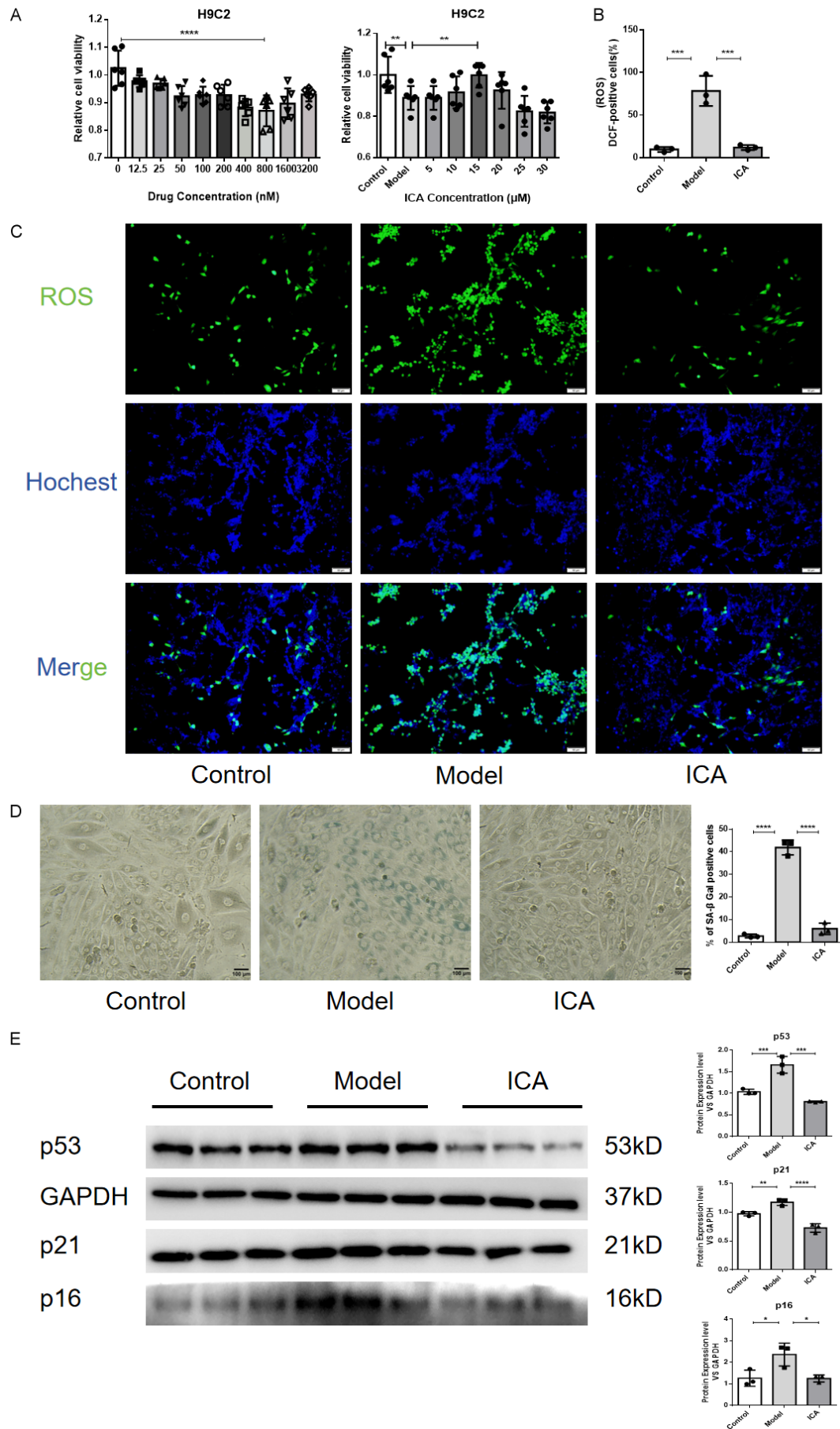


Figure 3. ICA attenuated Ang II-induced cardiomyocyte senescence in H9C2 cells. A. CCK-8 assays used to determine the experimental concentrations of Ang II and ICA. H9C2 cells were treated with varying concentrations of Ang II or ICA for 48 h; B, C. Representative fluorescence images of ROS production detected using the DCFH-DA probe (green) and quantitative analysis of ROS levels. Nuclei were stained with Hoechst 33342 (blue) ($\times 10$ magnification; Scale bar = 50 μm); D. Representative images and quantitative analysis of SA- β -Gal staining ($\times 10$ magnification; Scale bar = 20 μm); E. Representative western blotting and quantitative analysis of senescence markers p53, p21, and p16. Notes: ROS, reactive oxygen species; Data are presented as mean $\pm$ SD ($n = 3$ independent experiments). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$.

lished using the crystal structure of PTEN (PDB ID: 1D5R). The binding pocket was defined by the centroid of the co-crystallized ligand, with the grid box centered at $x = 34.95$, $y = -87.75$, $z = 24.95$. The interactions between ICA and key residues within the predicted PTEN-binding pocket, including hydrogen bonds, π - π stacking, and hydrophobic contacts, and the binding potential was evaluated based on the docking score. Molecular docking analysis revealed that ICA fitted well into the predicted binding pocket of PTEN, with a high binding affinity (binding energy < -6 kcal/mol) (**Table 1**). Visualization of the docked complex indicated that ICA formed stable hydrogen bonds with key amino acid residues, including LYS-330, HIS-272, ASP-92, and ASN-94, and forms a π - π interaction with HIS-93 (**Figure 5A-D**). To experimentally validate this physical interaction, we performed a CETSA. ICA treatment markedly enhanced PTEN thermal stability compared to the control treatment, supporting an interaction between ICA and PTEN (**Figure 5E, 5F**).

ICA attenuated cardiomyocyte senescence by promoting autophagy

To determine whether autophagy is required for the anti-senescence effects of ICA, cells were treated with chloroquine (CQ), an autophagy inhibitor. As shown in **Figure 6A**, ICA restored cell viability in Ang II-treated cells, and this protective effect was abolished by CQ cotreatment. Similarly, SA- β -Gal staining revealed that CQ reversed the ICA-mediated reduction in the proportion of senescent cells (**Figure 6B, 6C**). Western blotting showed that ICA treatment restored autophagic flux, as indicated by decreased p62 expression and increased LC3B-II/I ratio, as well as reduced expression of senescence markers (p53, p21, and p16). However, CQ suppressed p62 degradation and promoted LC3B-II accumulation, while preventing the ICA-mediated reduction in senescence-associated protein expression (**Figure 6D**). These findings suggest that the anti-senescence effects of ICA

in H9C2 cells are dependent, at least in part, on enhanced autophagic activity.

ICA modulated the PTEN/PI3K/AKT signaling pathway

Given that PTEN suppresses PI3K/AKT signaling, we investigated whether ICA affected this signaling axis. In the model group, we observed increased phosphorylation of PTEN (indicative of inactivation), accompanied by increased phosphorylation of PI3K and AKT. ICA treatment significantly reduced the levels of p-PTEN, p-PI3K, and p-AKT to levels comparable to those in the control group (**Figure 7**). These changes in PTEN/PI3K/AKT signaling were accompanied by alterations in autophagy-related proteins. Specifically, compared to the control group, the LC3B-II/I ratio was markedly decreased in the model group, whereas ICA treatment significantly increased this ratio to a level comparable to that of the control group. Conversely, p62 expression exhibited the opposite trend. These findings suggest that ICA reduces PTEN phosphorylation at Ser380 and suppresses PI3K/AKT signaling, thereby enhancing autophagy.

PTEN knockdown abolished the protective effects of ICA

To determine whether PTEN mediates ICA-induced protection, H9C2 cells were transfected with siPTEN. PTEN knockdown significantly exacerbated Ang II-induced cell viability loss and senescence (SA- β -Gal-positive cells) compared to the model group (**Figure 8A, 8B**). Notably, ICA failed to restore cell viability or reduce the proportion of senescent cells following PTEN knockdown (**Figure 8A, 8B**). Furthermore, western blotting data indicated that PTEN knockdown was associated with sustained activation of PI3K/AKT signaling, as indicated by increased p-PI3K and p-AKT levels. PTEN knockdown was also associated with reduced autophagic activity (evidenced by increased p62 expression and

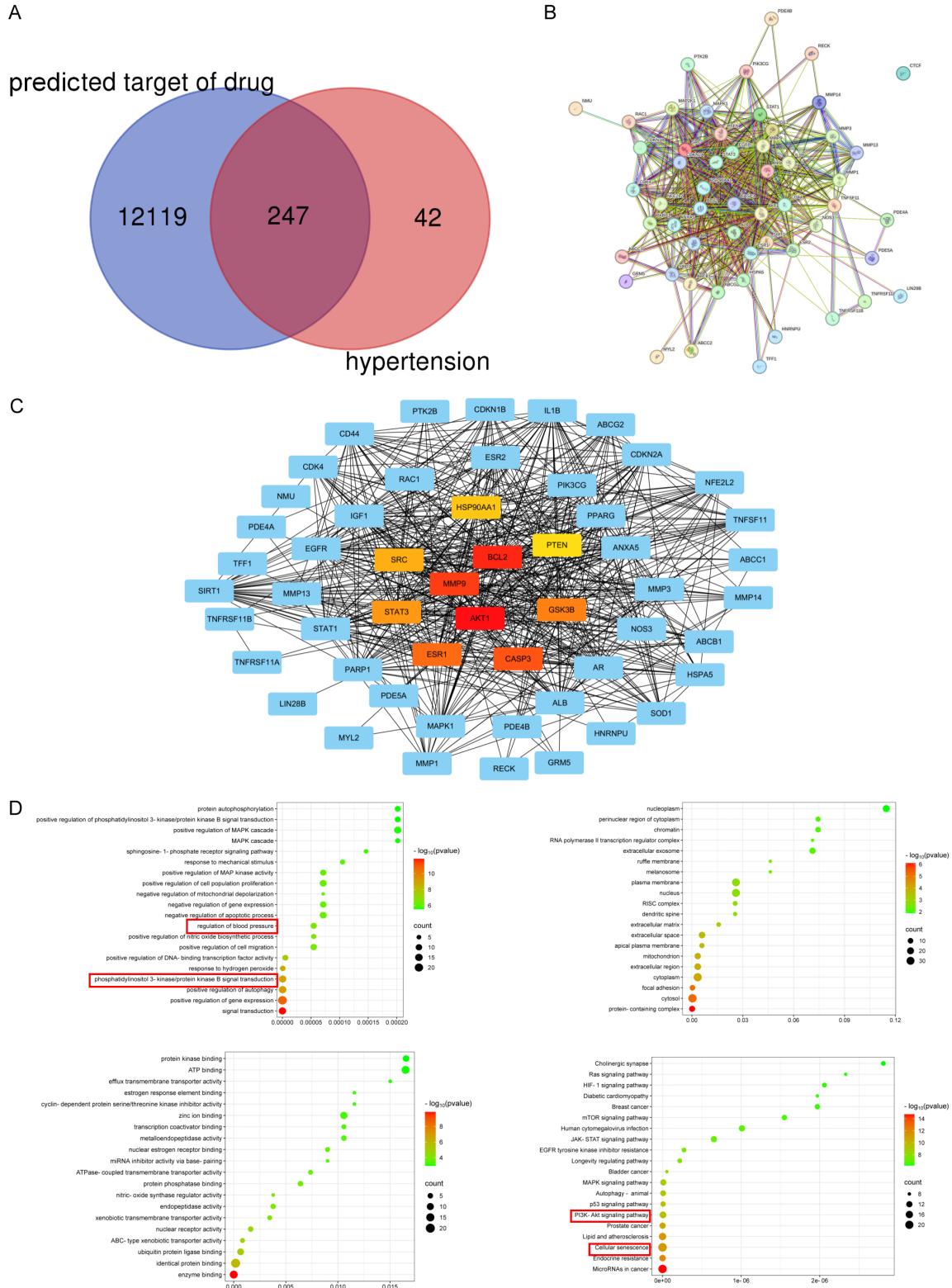
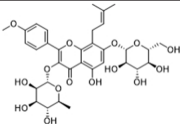


Figure 4. Network pharmacology analysis of ICA targets in hypertension. A. Venn diagram showing the overlap between the predicted targets of ICA and hypertension-related targets; B. PPI network of the overlapping targets; C. Top 10 hub genes identified from the PPI network using the MCC algorithm; D. Bubble plots revealing the top 20 enriched GO biological processes (left) and KEGG pathways (right). Notes: PPI, protein-protein interaction; MCC, maximal clique centrality; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 1. Target protein docking results for the compound

Target	Compound	Compound Structure	Binding Energy (kcal/mol)	Combination Type
PTEN	Icariin		-7.160	Hydrogen bond, Hydrophobic interaction, π -stacking

Note: Binding energy function. $\Delta G_{bind} = Clipo-lipo \sum f(r/r) + Chbond-neut-neut \sum g(\Delta r)h(\Delta \alpha) + Chbond-neut-charged \sum g(\Delta r)h(\Delta \alpha) + Cmax-metal-ion \sum f(r/r) + CrotbHrotb + Cpolar-phobVpolar-phob + CcoulEcoul + CvdwEvdW + solvationterms$.

decreased LC3B-II/I ratio), together with elevated p53, p21, and p16 levels (**Figure 8C**). Crucially, ICA treatment did not reverse these signaling alterations or reduce the expression of senescence-associated proteins in PTEN-silenced cells. Collectively, these findings indicate that the effects of ICA on cell senescence and autophagic activity are dependent on the PTEN/PI3K/AKT axis.

Discussion

In this study, we provide evidence that ICA ameliorates hypertension-induced cardiomyocyte senescence through a mechanism involving PTEN-dependent autophagy. By integrating *in vivo* and *in vitro* experiments, as well as bioinformatic tools, we demonstrated that ICA interacts with PTEN and reduces its phosphorylation, thereby suppressing aberrant PI3K/AKT signaling, restoring autophagic flux, and ultimately alleviating cardiac senescence and dysfunction.

Hypertension is a leading cause of heart failure [37]. Although hemodynamic overload is a primary driver of hypertensive cardiac injury, accumulating evidence suggests that accelerated biological aging of the myocardium also plays a fundamental role in hypertensive cardiac remodeling [8]. A previous study showed that cardiac remodeling represents an adaptive response to hypertension-induced myocardial stress but is characterized by hypertrophy, fibrosis, inflammation, mitochondrial injury, impaired autophagy, and increased apoptosis as the disease progresses [5]. These pathologic changes, including left ventricular wall thickening, collagen accumulation, and fibrosis, overlap with features of cardiac aging [6]. Although conventional antihypertensive drugs effectively lower BP, they may not fully arrest the underlying process of cellular senescence [23, 24]. Hence, interventions targeting cellular

aging have emerged as an attractive approach for treating hypertension and reversing the associated myocardial remodeling [22].

Previous studies have indicated that resveratrol attenuates doxorubicin-induced premature cardiomyocyte senescence [38, 39]. Furthermore, Cui et al. reported that Pinggan-Qianyang decoction delayed vascular aging in spontaneously hypertensive rats through regulation of the Sirtuin 1 (SIRT1)-PTEN pathway. ICA, an abundant flavonoid derived from *Epimedium Folium*, has been used in TCM to treat age-related diseases [40]. Although its antioxidant and anti-inflammatory properties have been well documented [41], the effects of ICA on cardiomyocyte senescence in hypertension have remained unclear. Our findings confirm that ICA lowers BP in HS-fed mice and preserves cardiac function and structure. Mechanistically, we postulate that attenuated polydipsia in ICA-treated mice reflects restored systemic osmotic homeostasis, potentially mediated by enhanced renal sodium excretion and a subsequent reduction in plasma osmolality. Additionally, improvements in renal concentrating ability, possibly by regulation of aquaporin-2 expression, cannot be excluded. Nevertheless, whether these effects are secondary to hemodynamic changes or direct actions on the central thirst-regulatory requires dedicated electrophysiology or pharmacologic investigation in future studies.

The ability of ICA to suppress the p53/p21/p16 senescence signaling was a major finding of our study. In our *in vivo* model, an HS diet was used because it has been reported to activate the local tissue cardiac RAS, despite suppressing systemic renin levels [35, 36]. The resulting local increase in Ang II may stimulate NADPH oxidase (NOX)-dependent ROS production, leading to DNA damage and premature cellular senescence [42]. This justifies our use of Ang

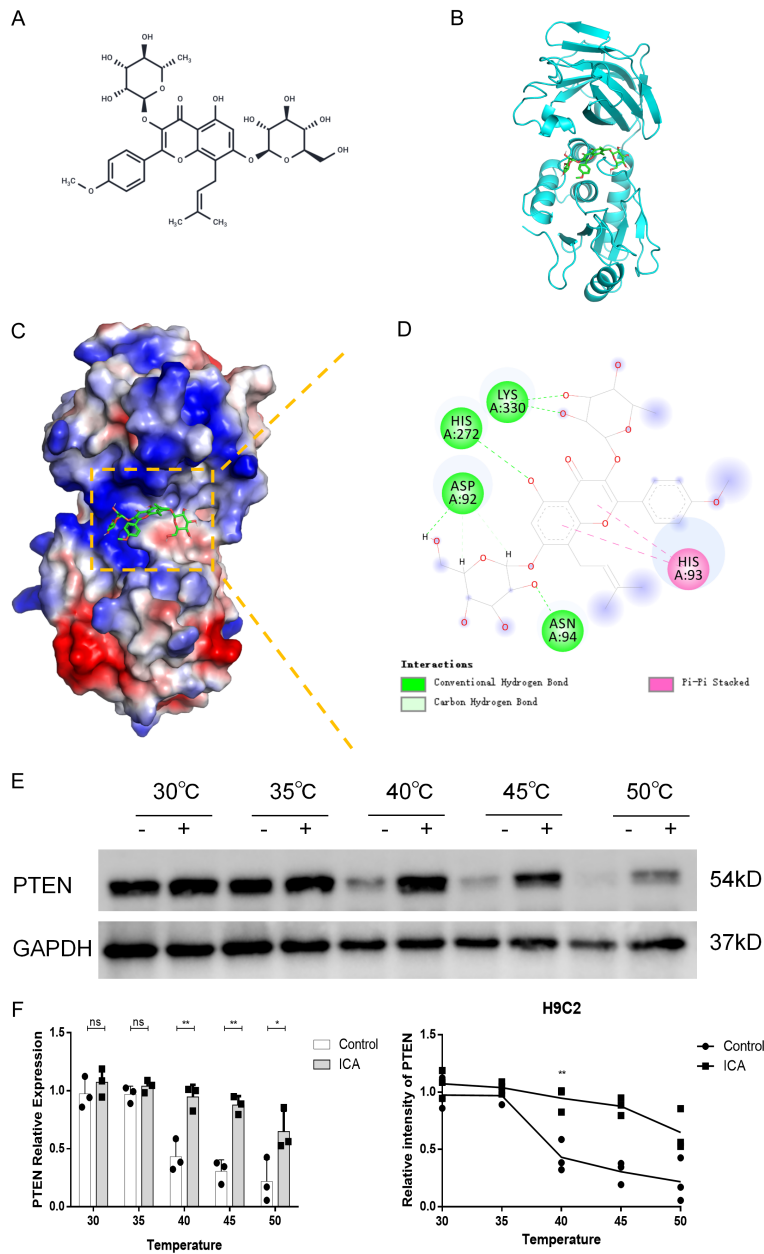


Figure 5. Molecular docking and cellular target engagement between ICA and PTEN. A. Chemical structure of ICA; B. 3D structure of PTEN used for molecular docking; C. Electrostatic surface potential of PTEN; D. Close-up view of the predicted binding mode between ICA and PTEN. The PTEN backbone is shown as slate-colored ribbons, and ICA is shown as green sticks. Hydrogen bonds and π - π interactions involving amino acid residues (LYS-330, HIS-272, ASP-92, ASN-94, and HIS-93) are highlighted; E. Representative Western blots showing soluble PTEN levels at the indicated temperatures; F. PTEN thermal melting curves and quantitative analysis exhibiting increased PTEN thermal stability following ICA treatment. Notes: Data are presented as mean $\pm$ SD ($n = 3$). * $P < 0.05$ and ** $P < 0.01$.

II-treated H9C2 cells as an *in vitro* mimic of the local Ang II-rich cardiac environment associated with HS intake. ICA significantly inhibited

ROS production and downregulated p53, p21, and p16 expression. Given that p16 is a well-established marker of persistent cell-cycle arrest, whereas p21 participates in the acute responses to DNA damage [43, 44], the ability of ICA to suppress both p26 and p21 suggests that it may act at multiple stages of the senescence response and potentially limit the progression from oxidative stress to persistent cellular senescence [45, 46].

Autophagy is an essential cellular quality-control system. Aging and hypertension typically compromise autophagic activity, resulting in the accumulation of damaged mitochondria and protein aggregates [47]. Consistent with this established mechanism, we observed autophagy-related protein expression in the model group, as evidenced by p62 accumulation and a reduced LC3B-II/LC3B-I ratio. Such impairment establishes a vicious cycle in which dysfunctional mitochondria generate additional ROS, further accelerating senescence [12, 48]. ICA treatment restored autophagic flux. Moreover, CQ, an autophagy inhibitor, attenuated the protective effects of ICA, supporting the involvement of autophagic degradation in the effects of ICA. By clearing damaged cellular components, ICA may interrupt the ROS-induced-senescence feedback loop.

A key mechanistic finding of this study was the involvement of PTEN, a negative regulator of the PI3K/AKT signaling pathway [18, 19]. Although PI3K/AKT signaling generally supports cell survival, its persistent activation can

Icariin alleviates cardiomyocyte senescence

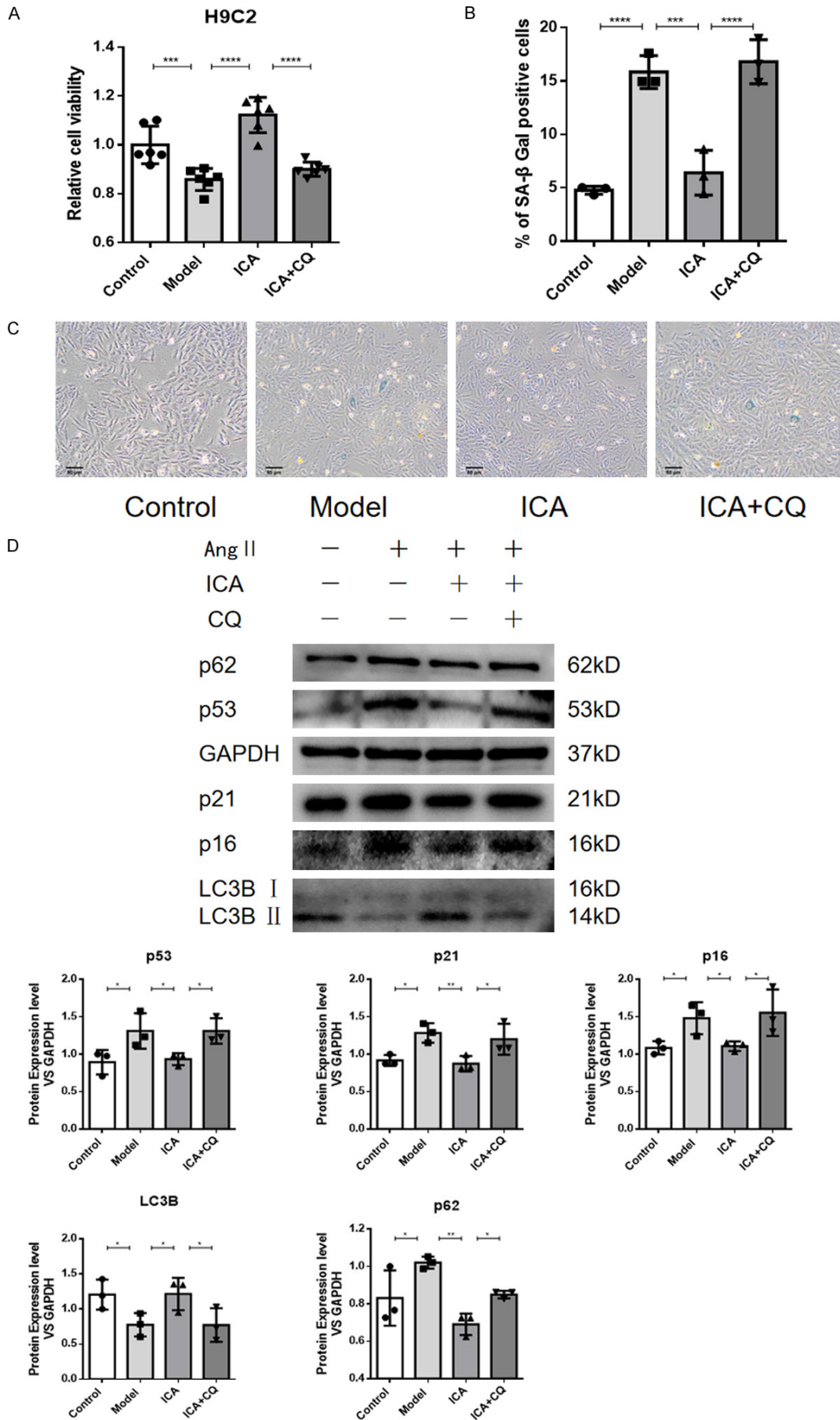


Figure 6. ICA ameliorated cardiomyocyte senescence by promoting autophagy (CQ blockage experiment). H9C2 cells were treated with Ang II (800 nM) and ICA (15 μ M) in the presence or absence of the chloroquine (CQ; 20 μ M). A. Cell viability measured by CCK-8 assay; B, C. Representative SA- β -Gal staining images and quantification, revealing that CQ attenuated the anti-senescence effects of ICA ($\times 10$ magnification; Scale bar = 50 μ m); D. Representative western blots and quantitative analysis of the senescence-associated proteins (p53, p21, and p16) and the autophagy-related markers (p62, LC3B). Notes: Data are presented as mean $\pm$ SD (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

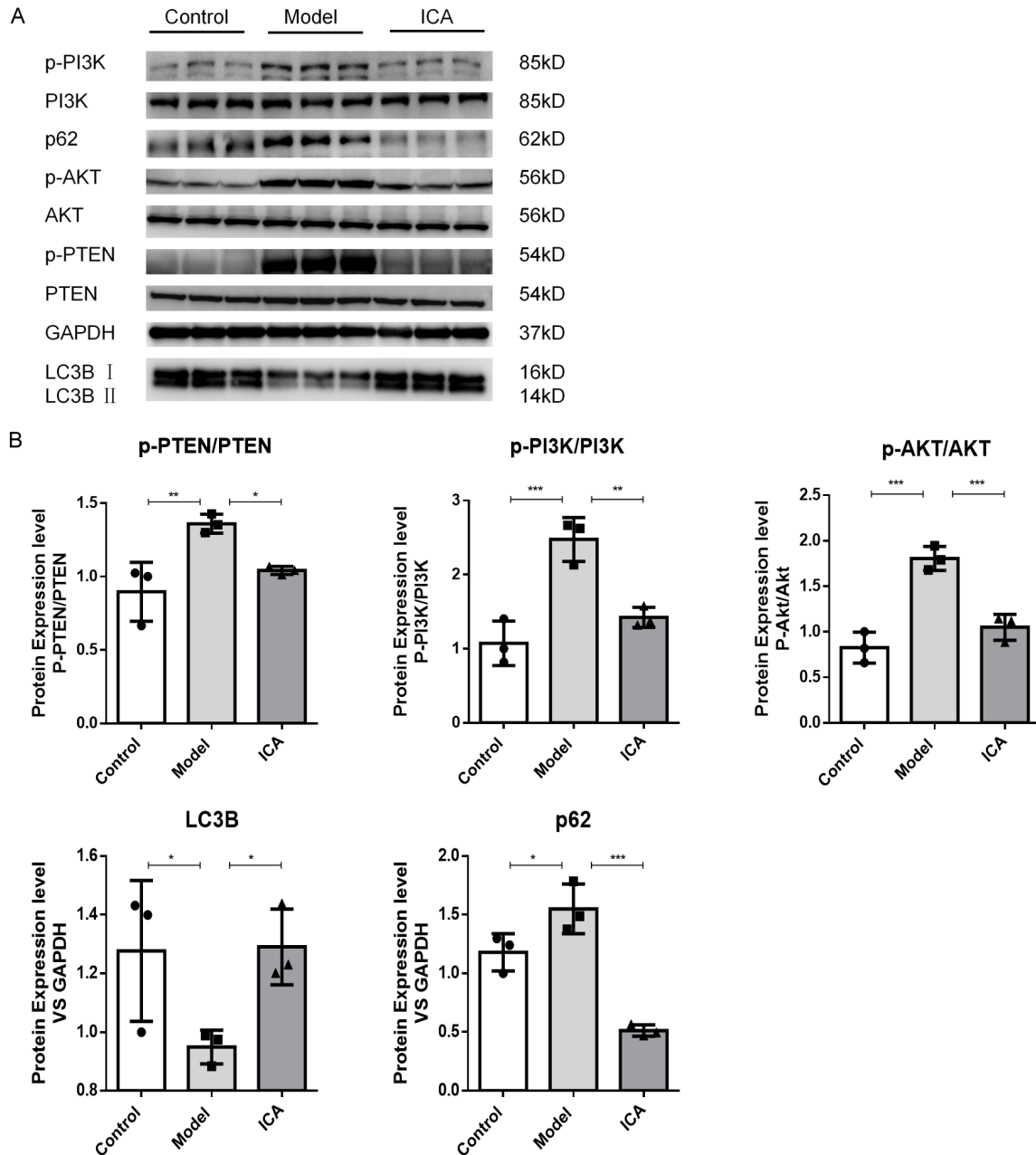


Figure 7. ICA modulated the PTEN/PI3K/AKT signaling pathway and autophagy-related protein expression in H9C2 cells. A. Representative western blots of PTEN/PI3K/AKT pathway proteins (p-PTEN, PTEN, p-PI3K, PI3K, p-AKT, and AKT) and autophagy-related proteins (p62 and LC3B) in H9C2 cells; B. Quantitative analysis of protein expression levels and phosphorylation levels. Notes: Data are presented as mean $\pm$ SD (n = 3). *P < 0.05, **P < 0.01, and ***P < 0.001.

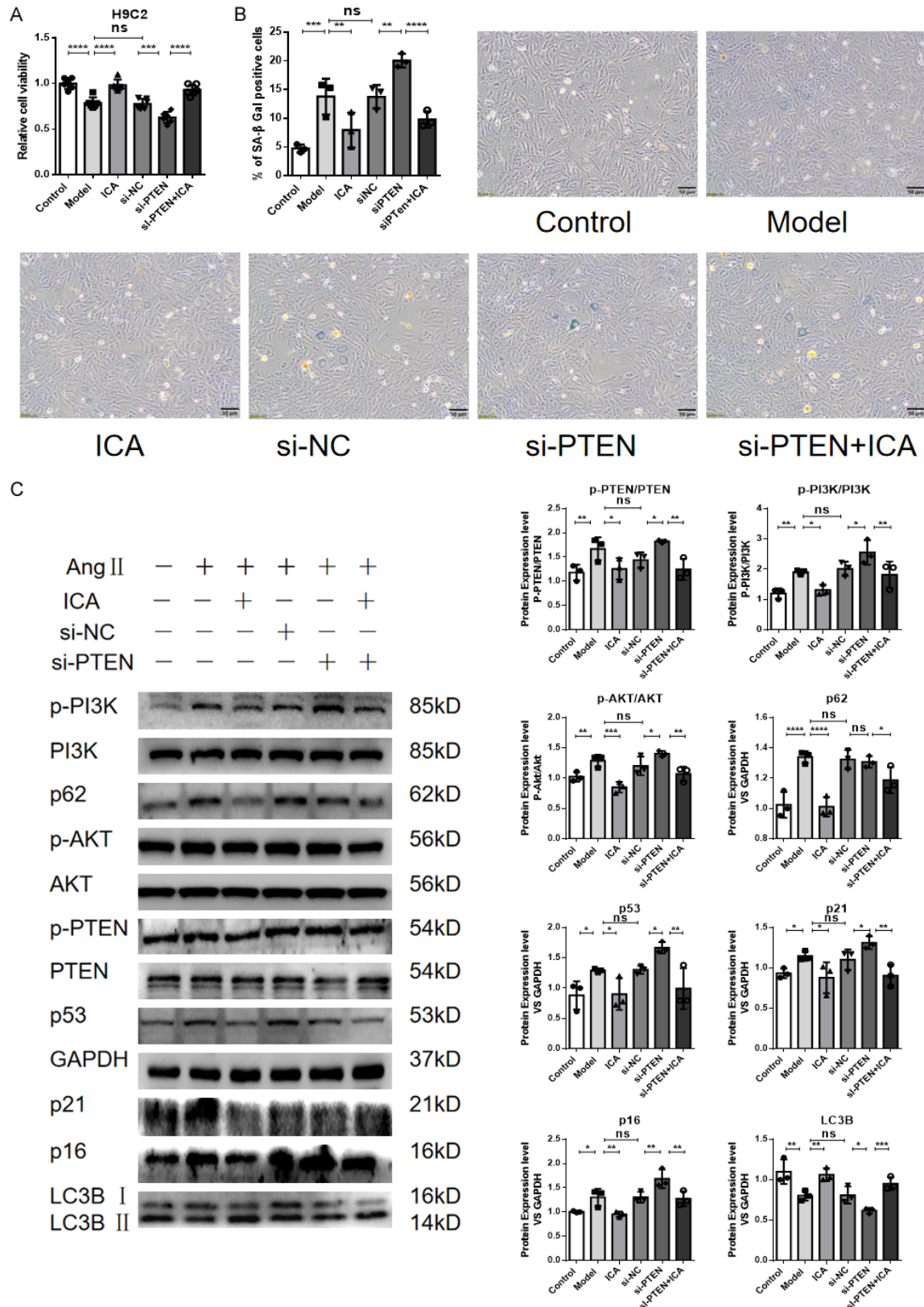


Figure 8. PTEN knockdown attenuated the protective effects of ICA in Ang II-treated H9C2 cells. H9C2 cells were transfected with PTEN-siRNA or negative control siRNA before Ang II and ICA treatment. A. Cell viability measured

by CCK-8 assay, showing that PTEN knockdown attenuated the protective effect of ICA; B. Representative SA- β -gal staining images and quantification ($\times 10$ magnification; Scale bar = 50 μ m); C. Representative western blotting and quantitative analysis of senescence markers, autophagy markers, and PI3K/AKT signaling pathway components. Phosphorylation levels were quantified as the ratio of phosphorylated protein to total protein, with both signals first normalized to GAPDH. Notes: Data are presented as mean $\pm$ SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

stimulate mTOR activation and promote “gero-conversion”, a process of cellular senescence [17, 20]. In the context of metabolic stress, we observed increased PTEN phosphorylation, accompanied by enhanced PI3K and AKT phosphorylation. Molecular docking and CETSA results provide compelling evidence that ICA interacts with a binding pocket in PTEN. Structurally, this binding likely stabilizes PTEN in an active conformation or prevents its inhibitory phosphorylation. ICA treatment subsequently decreased p-PTEN levels (restoring its phosphatase activity) and suppressed PI3K/AKT signaling. PTEN knockdown attenuated the effects of ICA on PI3K/AKT signaling, autophagy-related proteins, and senescence-associated markers, indicating that PTEN is an important mediator of ICA activity. These observations are consistent with recent studies revealing that PTEN-mediated signaling promotes autophagy and supports metabolic homeostasis [49, 50].

Nevertheless, this study had several limitations. First, H9C2 cells are cardiomyoblasts and do not fully recapitulate the electrophysiological and contractile properties of adult cardiomyocytes. Future studies using primary adult cardiomyocytes or human induced pluripotent stem cell-derived cardiomyocytes would strengthen the translational value. Second, although our results support PTEN as a potential target of ICA, the detailed structural basis of the ICA-PTEN interaction warrant further investigation using X-ray crystallography. Third, although the *in vivo* effects of the HS diet were interpreted partly in relation to elevated local cardiac Ang II levels, we did not directly measure Ang II concentration in the myocardial interstitium of HS diet-fed mice. Although previous studies support the dissociation between local and systemic RAS activity under HS conditions, the lack of direct measurement in our model remains a limitation. Future studies employing cardiac microdialysis or tissue mass spectrometry are warranted to confirm local cardiac Ang II levels.

Conclusion

Our study suggested that ICA ameliorates hypertension-induced cardiomyocyte senescence by modulating PTEN, suppressing the PI3K/AKT signaling pathway, and enhancing autophagic activity. These findings provide new insight into the cardioprotective and anti-senescence effects of ICA and highlight PTEN-mediated autophagy as a potential therapeutic mechanism in hypertensive heart disease.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 823-70446).

Disclosure of conflict of interest

None.

Abbreviations

ICA, Icariin; PTEN, Phosphatase and Tensin Homolog; HS, High-salt; Ang II, Angiotensin II; SA- β -Gal, Senescence-Associated β -galactosidase; PI3K, Phosphoinositide 3-kinase; AKT, protein kinase B; BP, Blood pressure; SASP, Senescence-associated secretory phenotype; TCM, Traditional Chinese Medicine; RAS, Renin-angiotensin system; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; MBP, Mean Blood Pressure; LVEF, Left ventricular ejection fraction; LVFS, Left ventricular fractional shortening; FBS, Fetal bovine serum; CQ, Chloroquine; CCK-8, Cell Counting Kit-8; ROS, Reactive Oxygen Species; PPI, Protein-Protein Interaction; CETSA, Cellular Thermal Shift Assay; SD, Standard deviation; HW/BW, Heart weight-to-body weight; HR, Heart Rate.

Address correspondence to: Yalin Yu, Jinan Hospital of Integrated Traditional Chinese and Western Medicine, Jinan 271100, Shandong, China. Tel: +86-0531-76233235; E-mail: 17862969068@163.com; Guohua Song, Clinical and Basic Medical Sciences, Shandong First Medical University and

Shandong Academy of Medical Science, Jinan 250117, Shandong, China. Tel: +86-0531-5955-6066; E-mail: ghsong@sdfmu.edu.cn; Bo Dong, First Clinical Medical College, Shandong University of Traditional Chinese Medicine, No. 324, Jingwu Weiqi Road, Jinan 250013, Shandong, China. Tel: +86-0531-68773121; E-mail: dbsh702@163.com; bodong@sdu.edu.cn

References

- [1] Kario K, Okura A, Hoshida S and Mogi M. The WHO Global report 2023 on hypertension warning the emerging hypertension burden in globe and its treatment strategy. *Hypertens Res* 2024; 47: 1099-1102.
- [2] Carey RM, Muntner P, Bosworth HB and Whelton PK. Prevention and control of hypertension. *J Am Coll Cardiol* 2018; 72: 1278-1293.
- [3] Qadi WSM, Fadzeli NT, Mediani A, Sarian MN, Tong X, Han R, Jam FA, Bakar ZHA and Hamezah HS. An insight review on the potential anti-hypertensive effects of *Moringa oleifera*. *Biomed Pharmacother* 2025; 191: 118500.
- [4] NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. *Lancet* 2021; 398: 957-980.
- [5] Wang LP, Han RM, Wu B, Luo MY, Deng YH, Wang W, Huang C, Xie X and Luo J. Mst1 silencing alleviates hypertensive myocardial injury associated with the augmentation of microvascular endothelial cell autophagy. *Int J Mol Med* 2022; 50: 146.
- [6] Koutouroushis C and Sarkar O. Role of autophagy in cardiovascular disease and aging. *Cureus* 2021; 13: e20042.
- [7] Zheng Y, Fang Y, Luo Y, Fan Y and Fang Z. Qianyang yuyin granule ameliorates mitochondrial dysfunction of hypertensive myocardial remodeling. *J Ethnopharmacol* 2024; 335: 118610.
- [8] Chang D, Wang J, Zhu S, Shentu Y, Wang Y, Zhu P and Ono M. Cellular senescence and cell therapy in cardiovascular diseases. *Stem Cell Res Ther* 2025; 16: 613.
- [9] Osanai T, Tanaka M, Mikami K, Kitajima M, Tomisawa T, Magota K, Tomita H and Okumura K. Novel anti-aging gene NM_026333 contributes to proton-induced aging via NCX1-pathway. *J Mol Cell Cardiol* 2018; 125: 174-184.
- [10] Abdellatif M, Schmid ST, Fuerlinger A and Kroemer G. Anti-ageing interventions for the treatment of cardiovascular disease. *Cardiovasc Res* 2025; 121: 1524-1536.
- [11] Mizushima N and Komatsu M. Autophagy: renovation of cells and tissues. *Cell* 2011; 147: 728-741.
- [12] Cuervo AM. Autophagy and aging: keeping that old broom working. *Trends Genet* 2008; 24: 604-612.
- [13] Mizushima N, Levine B, Cuervo AM and Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature* 2008; 451: 1069-1075.
- [14] Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M and Kenyon C. A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. *PLoS Genet* 2008; 4: e24.
- [15] Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A and Partridge L. Mechanisms of life span extension by rapamycin in the fruit fly *drosophila melanogaster*. *Cell Metab* 2010; 11: 35-46.
- [16] Alvers AL, Wood MS, Hu D, Kaywell AC, Dunn WA Jr and Aris JP. Autophagy is required for extension of yeast chronological life. *Autophagy* 2009; 5: 847-849.
- [17] Kotelevets L, Scott MGH and Chastre E. Targeting PTEN in colorectal cancers. *Adv Exp Med Biol* 2018; 1110: 55-73.
- [18] Chow LM and Baker SJ. PTEN function in normal and neoplastic growth. *Cancer Lett* 2006; 241: 184-196.
- [19] Endicott SJ and Miller RA. PTEN activates chaperone-mediated autophagy to regulate metabolism. *Autophagy* 2024; 20: 216-217.
- [20] Nussinov R, Zhang M, Tsai CJ and Jang H. Phosphorylation and driver mutations in PI3K α and PTEN autoinhibition. *Mol Cancer Res* 2021; 19: 543-548.
- [21] Francis JC, Capper A, Rust AG, Ferro K, Ning J, Yuan W, de Bono J, Pettitt SJ and Swain A. Identification of genes that promote PI3K pathway activation and prostate tumour formation. *Oncogene* 2024; 43: 1824-1835.
- [22] Cui Z, Jiamei Y, Yushu Y, Xia F, Haiyan Y, Zhang D, Qiong C and Guangwei Z. Effect of the traditional Chinese medicine Pinggan-Qianyang decoction on SIRT1-PTEN signaling in vascular aging in spontaneously hypertensive rats. *Hypertens Res* 2021; 44: 1087-1098.
- [23] Li Y, Yu X, Liu Y, Miao S, Liu X, Wang Z and Zhou H. Pharmacodynamic components and molecular mechanism of *Gastrodia elata* Blume in treating hypertension: absorbed components, network pharmacology analysis, molecular docking and in vivo experimental verification. *J Ethnopharmacol* 2025; 345: 119583.
- [24] Chalmers J, Mourad JJ, Brzozowska-Villatte R, De Champvallins M and Mancina G. Benefit of treatment based on indapamide mostly combined with perindopril on mortality and cardiovascular outcomes: a pooled analysis of four trials. *J Hypertens* 2023; 41: 508-515.
- [25] Wang J and Xiong X. Evidence-based Chinese medicine for hypertension. *Evid Based Complement Alternat Med* 2013; 2013: 978398.

- [26] Xia J, Hu JN, Zhang RB, Liu W, Zhang H, Wang Z, Jiang S, Wang YP and Li W. Icariin exhibits protective effects on cisplatin-induced cardiotoxicity via ROS-mediated oxidative stress injury in vivo and in vitro. *Phytomedicine* 2022; 104: 154331.
- [27] Wang K, Zheng X, Pan Z, Yao W, Gao X, Wang X and Ding X. Icariin prevents extracellular matrix accumulation and ameliorates experimental diabetic kidney disease by inhibiting oxidative stress via GPER mediated p62-dependent keap1 degradation and Nrf2 activation. *Front Cell Dev Biol* 2020; 8: 559.
- [28] Wang M, Wang L, Zhou Y, Feng X, Ye C and Wang C. Icariin attenuates renal fibrosis in chronic kidney disease by inhibiting interleukin-1 β /transforming growth factor- β -mediated activation of renal fibroblasts. *Phytother Res* 2021; 35: 6204-6215.
- [29] Xu CQ, Liu BJ, Wu JF, Xu YC, Duan XH, Cao YX and Dong JC. Icariin attenuates LPS-induced acute inflammatory responses: involvement of PI3K/Akt and NF- κ B signaling pathway. *Eur J Pharmacol* 2010; 642: 146-153.
- [30] Yoon JW, Lee SE, Park YG, Kim WJ, Park HJ, Park CO, Kim SH, Oh SH, Lee DG, Pyeon DB, Kim EY and Park SP. The antioxidant icariin protects porcine oocytes from age-related damage in vitro. *Anim Biosci* 2021; 34: 546-557.
- [31] Tian M, Yang S and Yan X. Icariin reduces human colon carcinoma cell growth and metastasis by enhancing p53 activities. *Braz J Med Biol Res* 2018; 51: e7151.
- [32] Shen R and Wang JH. The effect of icariin on immunity and its potential application. *Am J Clin Exp Immunol* 2018; 7: 50-56.
- [33] Hu Y, Liu K, Yan M, Zhang Y, Wang Y and Ren L. Effects and mechanisms of icariin on atherosclerosis. *Int J Clin Exp Med* 2015; 8: 3585-3589.
- [34] Zeng Y, Xiong Y, Yang T, Wang Y, Zeng J, Zhou S, Luo Y and Li L. Icariin and its metabolites as potential protective phytochemicals against cardiovascular disease: From effects to molecular mechanisms. *Biomed Pharmacother* 2022; 147: 112642.
- [35] Wang DH, Du Y and Yao A. Regulation of the gene-encoding angiotensin II receptor in vascular tissue. *Microcirculation* 1996; 3: 237-239.
- [36] Johansson ME, Bernberg E, Andersson IJ, Bie P, Skött O, Gan LM and Bergström G. High-salt diet combined with elevated angiotensin II accelerates atherosclerosis in apolipoprotein E-deficient mice. *J Hypertens* 2009; 27: 41-47.
- [37] Whitworth JA; World Health Organization, International Society of Hypertension Writing Group. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens* 2003; 21: 1983-1992.
- [38] Sin TK, Tam BT, Yung BY, Yip SP, Chan LW, Wong CS, Ying M, Rudd JA and Siu PM. Resveratrol protects against doxorubicin-induced cardiotoxicity in aged hearts through the SIRT1-USP7 axis. *J Physiol* 2015; 593: 1887-1899.
- [39] Ghosh AK. Acetyltransferase p300 is a putative epigenetic target for amelioration of cellular aging-related cardiovascular disease. *Cells* 2021; 10: 2839.
- [40] Sze SC, Tong Y, Ng TB, Cheng CL and Cheung HP. Herba epimedii: anti-oxidative properties and its medical implications. *Molecules* 2010; 15: 7861-7870.
- [41] Zhang X, Sun H, Su Q, Lin T, Zhang H, Zhang J, Dang S and Zhu Z. Antidepressant-like activity of icariin mediated by group I mGluRs in prenatally stressed offspring. *Brain Dev* 2017; 39: 593-600.
- [42] Dong H, Ming S, Fang J, Li Y and Liu L. Icariin ameliorates angiotensin II-induced cerebrovascular remodeling by inhibiting Nox2-containing NADPH oxidase activation. *Hum Cell* 2019; 32: 22-30.
- [43] Shen W, Huang Y, Yang X, Zhang Y, Pan Y, Xiao Y, Wang J, Wang C, Mao W and Zhao A. Senescent macrophages in tumor: phenotypes, roles, and interventions. *Cell Death Dis* 2025; 16: 677.
- [44] Gabai Y, Assouline B and Ben-Porath I. Senescent stromal cells: roles in the tumor microenvironment. *Trends Cancer* 2023; 9: 28-41.
- [45] Chen K and Sun Z. Activation of DNA demethylases attenuates aging-associated arterial stiffening and hypertension. *Aging Cell* 2018; 17: e12762.
- [46] Lee YY, Choi YS, Kim DW, Cheong JY, Song KY, Ryu MS and Lim IK. Mitochondrial nucleoid remodeling and biogenesis are regulated by the p53-p21(WAF1)-PKC ζ pathway in p16(INK4a)-silenced cells. *Aging (Albany NY)* 2020; 12: 6700-6732.
- [47] Jin S. Autophagy, mitochondrial quality control, and oncogenesis. *Autophagy* 2006; 2: 80-84.
- [48] Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T, Mizushima N, Iwata J, Ezaki J, Murata S, Hamazaki J, Nishito Y, Iemura S, Natsume T, Yanagawa T, Uwayama J, Warabi E, Yoshida H, Ishii T, Kobayashi A, Yamamoto M, Yue Z, Uchiyama Y, Kominami E and Tanaka K. Homeostatic levels of p62 control cytoplasmic in-

- clusion body formation in autophagy-deficient mice. *Cell* 2007; 131: 1149-1163.
- [49] De D, Ghosh G and Karmakar P. Sumoylation and phosphorylation of PTEN boosts and curtails autophagy respectively by influencing cell membrane localisation. *Exp Cell Res* 2024; 434: 113872.
- [50] Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P and Ogier-Denis E. The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. *J Biol Chem* 2001; 276: 35243-35246.

Icariin alleviates cardiomyocyte senescence

Supplementary Table 1. List of antibodies used in this study

Target Protein	Host Species	Dilution	Catalog Number	Manufacturer
Senescence Markers				
p53	Rabbit	1:2000	10442-1-AP	Proteintech, China
p21	Rabbit	1:2000	28248-1-AP	Proteintech, China
p16	Rabbit	1:2000	R23896	Zen-Bioscience, China
Autophagy Markers				
p62/SQSTM1	Rabbit	1:2000	380612	Zen-Bioscience, China
LC3B	Rabbit	1:2000	350140	Zen-Bioscience, China
Signaling Pathway				
PTEN	Rabbit	1:2000	ab32199	Abcam, UK
p-PTEN (Ser380)	Rabbit	1:2000	381402	Zen-Bioscience, China
PI3K	Mouse	1:2000	251221	Zen-Bioscience, China
p-PI3K (Tyr467/Tyr199)	Rabbit	1:2000	310164	Zen-Bioscience, China
AKT (Pan)	Rabbit	1:2000	23412	Zen-Bioscience, China
p-AKT (Ser473)	Rabbit	1:2000	381555	Zen-Bioscience, China
Loading Control				
GAPDH	Rabbit	1:2000	10494-1-AP	Proteintech, China
Secondary Antibodies				
HRP-conjugated Goat Anti-Rabbit IgG H&L	Goat	1:5000	511203	Zen-Bioscience, China
HRP-conjugated Goat Anti-Mouse IgG H&L	Goat	1:5000	34851ES60	YEASEN, China