

Baicalin Attenuates Cardiac Hypertrophy and Fibrosis through Inhibition of the PI3K p85 α Subunit

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ABSTRACT

Heart failure (HF) represents a major challenge in public health. Baicalin constitutes a primary active component derived from the traditional Chinese herb Huang Qin (*Scutellaria baicalensis*), commonly employed in managing patients experiencing chest pain or cardiac-related unease. Nevertheless, the precise mechanisms responsible for baicalin's protective actions on the heart remain incompletely elucidated. Experimental models of heart failure were developed using isoprenaline administration or transverse aortic constriction in animals, along with isoprenaline or angiotensin II stimulation in cellular systems. Baicalin was given in vivo at doses of 15 mg/kg/day or 25 mg/kg/day, while 10 μ M baicalin was applied in vitro. Network pharmacology approaches were utilized across multiple databases to pinpoint potential targets of baicalin and heart failure-associated genes, pointing toward involvement of the PI3K–Akt pathway. Molecular docking simulations were performed to examine baicalin's interaction with p85 α . Baicalin exhibited pronounced anti-hypertrophic and anti-fibrotic properties in both in vivo and in vitro settings. Cardiomyocyte cross-sectional area improved from an average of 390 μ m² in the HF group to 195 μ m² following baicalin treatment. Fibrotic area decreased from 2.8-fold elevation in the HF group to 1.62-fold in the baicalin-treated group. Baicalin exerted notable cardioprotection by suppressing the PI3K signaling cascade through direct interaction with five specific amino acid residues on the p85 α regulatory subunit of PI3K. Combining baicalin with a PI3K p110 inhibitor yielded enhanced protective outcomes. Mean ejection fraction rose from 54% in the baicalin-only group to 67% with the combined regimen. This study establishes baicalin as an effective bioactive compound from herbal sources capable of countering isoprenaline-induced cardiac impairment and identifies p85 α as a key pharmacological target. The results highlight the promising therapeutic value of baicalin paired with PI3K p110 inhibition in addressing heart failure, underscoring the need for further clinical investigations.

Keywords: Heart failure, Baicalin, PI3K, Isoprenaline, Transverse aortic constriction

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Introduction

Heart failure (HF) constitutes an escalating global health issue impacting 3–5% of the global population [1]. Numerous cardiac conditions, such as hypertension, coronary artery disease, and myocarditis, can impair heart function and eventually lead to HF [2]. A hallmark of HF pathophysiology involves heightened sympathetic nervous system activity, which elevates circulating catecholamines like isoprenaline. Consequently, isoprenaline is routinely applied to generate animal models of HF, provoking cardiac remodeling, hypertrophy, and impaired systolic performance [3]. Complementing this, the transverse aortic constriction (TAC) model serves as a standard approach for HF research [4]. By constricting the transverse aorta, this technique simulates pressure overload, imposing greater workload on the left ventricle and mirroring clinical scenarios like aortic stenosis or

hypertension, thereby inducing ventricular hypertrophy, diastolic and systolic impairments, and progression to heart failure.

In HF-affected cardiac tissue, prominent cardiomyocyte hypertrophy and interstitial fibrosis are evident [5]. As terminally differentiated cells, cardiomyocytes respond to stress primarily through enlargement rather than division, which raises metabolic demands and oxygen needs, often culminating in decompensation and cell loss in a self-perpetuating cycle. Conversely, cardiac fibroblasts proliferate readily and deposit excess extracellular matrix (ECM), intensifying fibrosis and myocardial rigidity [6]. Such fibrosis hinders effective cardiac contraction and relaxation, rendering the attenuation of hypertrophy and fibrosis central therapeutic goals in HF management. Present-day HF therapy encompasses pharmacological agents, implantable devices, and surgical options in severe cases. Standard drug therapies include angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers, mineralocorticoid receptor antagonists, and angiotensin receptor-neprilysin inhibitors, which help slow disease advancement [7]. Diuretics address fluid overload symptoms. Although these interventions improve quality of life and prognosis by modulating hemodynamics and neurohormonal activation, they fall short in comprehensively reversing the maladaptive structural changes, including cardiomyocyte hypertrophy and fibrosis. Baicalin ranks among the chief bioactive constituents of Huang Qin (*Scutellaria baicalensis*) [8]. This herb has longstanding clinical application for alleviating chest pain and cardiac symptoms. Analytical techniques such as liquid chromatography and mass spectrometry have characterized the chemical profile of Huang Qin, identifying baicalin and baicalein as its predominant pharmacologically relevant compounds [9]. Baicalin arises as a glucuronidated derivative of baicalein. Prior studies have documented baicalein's benefits against ischemia/reperfusion damage [10] and hypertension [11], lending credence to Huang Qin's cardiac benefits. While baicalin was traditionally viewed as a less active metabolite, emerging data demonstrate its capacity to mitigate pressure overload-related HF [12] and doxorubicin-triggered cardiotoxicity [13]. Nonetheless, its efficacy against isoprenaline-induced HF has yet to be determined, and the detailed molecular pathways underlying its cardioprotective actions require further clarification.

The present investigation aimed to clarify baicalin's mechanisms in HF therapy and assess strategies for improved efficacy. We demonstrated that baicalin effectively counters HF in both living organisms and cultured cells by diminishing cardiomyocyte hypertrophy and cardiac fibrosis. Through network pharmacology and molecular docking analyses, we established that baicalin's benefits rely on interaction with the p85 regulatory subunit of PI3K, and that pairing baicalin with PI3K p110 inhibition amplifies cardioprotection. Overall, our findings position baicalin as a valuable herbal-derived cardioprotectant acting via p85 of PI3K in HF contexts and advocate for its potential clinical translation in HF patients.

Materials and Methods

Animal experimental design

All animal procedures received approval from the Experimental Animal Centre of Huazhong University of Science and Technology (approval number: No. 2019005) and adhered to ethical guidelines for animal welfare. Male C57BL/6J mice were maintained in a specific pathogen-free (SPF) facility with controlled environmental parameters (temperature 22–26 °C, humidity 40–60%) to ensure well-being. Animals had unrestricted access to standard chow and fresh water and followed a 12-hour light/dark cycle.

Isoprenaline model experiment: The protocol followed established methods [12, 14]. Eight-week-old mice (n=40) were randomly allocated to five groups (n=8 each): PBS, ISO, ISO+Bai-L, ISO+Bai-H, and ISO+Pro. Following a one-week acclimation period, 15 mg/kg isoprenaline or equivalent PBS was administered intraperitoneally daily for three weeks to induce HF. Baicalin, prepared in 10% DMSO + 40% PEG300 + 5% Tween-80 + 45% saline, was delivered by oral gavage at 15 mg/kg or 25 mg/kg daily. Propranolol (5 mg/kg/day) served as the positive control. At the end of three weeks, echocardiography was conducted, after which animals were euthanized via anesthetic overdose. Group definitions: PBS (PBS injections); ISO (15 mg/kg isoprenaline); ISO+Bai-L (15 mg/kg isoprenaline + 15 mg/kg baicalin); ISO+Bai-H (15 mg/kg isoprenaline + 25 mg/kg baicalin); ISO+Pro (15 mg/kg isoprenaline + 5 mg/kg propranolol).

Transverse aortic constriction (TAC) model experiment: Procedures were based on prior descriptions [12, 15]. Eight-week-old mice (n=40) were randomized into five groups (n=8 each): sham, TAC, TAC+Bai-L, TAC+Bai-H, and TAC+Pro. TAC surgery involved anesthetizing mice with isoflurane, performing a midline incision, exposing the aortic arch, and placing a 6-0 suture around it with a 26G needle to achieve ~65–70% stenosis (0.4

mm lumen). The sham group underwent identical procedures without aortic ligation. Baicalin was gavaged at 15 or 25 mg/kg daily, and propranolol at 5 mg/kg/day. After eight weeks, echocardiography was performed prior to euthanasia. Group definitions: sham (sham surgery); TAC (transverse aortic constriction); TAC+Bai-L (TAC + 15 mg/kg baicalin); TAC+Bai-H (TAC + 25 mg/kg baicalin); TAC+Pro (TAC + 5 mg/kg propranolol).

Baicalin combined with LY294002 treatment model experiment: Eight-week-old mice (n=32) were divided into four groups (n=8 each): PBS, ISO, ISO+Bai, and ISO+Bai+LY294002. Isoprenaline and baicalin dosing matched the isoprenaline model. LY294002 was dissolved in the same vehicle and injected intraperitoneally at 25 mg/kg daily [16]. After eight weeks, echocardiography was conducted before euthanasia. Group definitions: PBS (PBS injections); ISO (15 mg/kg isoprenaline); ISO+Bai (15 mg/kg isoprenaline + 25 mg/kg baicalin); ISO+Bai+LY294002 (15 mg/kg isoprenaline + 25 mg/kg baicalin + 25 mg/kg LY294002).

Primary neonatal rat cardiomyocytes (NRCMs) or cardiac fibroblasts (NRCFs) culture

NRCMs and NRCFs were isolated following established enzymatic digestion protocols [17] utilizing type II collagenase, trypsin, and benzonase. Hearts from 1–4-day-old neonatal rats were excised, ventricles minced, and digested at 37 °C for 40 minutes with agitation. The resulting suspension was filtered, centrifuged, and subjected to red blood cell lysis. Cells were resuspended in DMEM/F12 medium containing 1% penicillin/streptomycin and 10% fetal bovine serum. Differential adhesion separated fibroblasts from cardiomyocytes, with the latter's medium additionally containing 5-BrdU to limit fibroblast contamination.

Antibodies and agents

Antibodies employed included: PI3Kphos607tyr (Absin, AP11, Shanghai, China), PI3K (ABclonal, A4992, Wuhan, China), Akt phos473ser (CST, 9271, Danvers, MA, USA), Vimentin (Boster, BM1035, Wuhan, China), α SMA (Boster, BM0002, Wuhan, China), NPPA (ABclonal, A22075, Wuhan, China), NPPB (ABclonal, A23996, Wuhan, China), MYH7 (ABclonal, A24664, Wuhan, China), fibronectin (ABclonal, A16678, Wuhan, China), and HRP-conjugated goat anti-rabbit IgG (ABclonal, AS014, Wuhan, China). Agents used were: baicalin (Aladdin, B110211, Shanghai, China), isoprenaline (Sigma-Aldrich, I5627, St. Louis, MO, USA), type II collagenase (Sigma-Aldrich, LS004176, St. Louis, MO, USA), Angiotensin II (MCE, HY-13948, Monmouth Junction, NJ, USA), trypsin (BioFROXX, 1004GR025, Offenbach am Main, Germany), antibody dilution buffer (Abbkine, BMU103, Wuhan, China), supersensitive ECL kit (Abbkine, BMU102, Wuhan, China), 740-Y-P (MCE, HY-0175, Monmouth Junction, NJ, USA), LY294002 (MCE, HY-10108, Monmouth Junction, NJ, USA), propranolol (MCE, HY-B0573B, Monmouth Junction, NJ, USA), red-blood-cell lysis (Servicebio, G2015, Wuhan, China), penicillin/streptomycin (Servicebio, G4003, Wuhan, China), fetal bovine serum (Yeasten, 40131ES76, Shanghai, China), DMEM/F12 (Servicebio, G4610, Wuhan, China), DAB kit (Servicebio, G1212, Wuhan, China), protease and phosphatase inhibitor cocktails (Yeasten, 20123ES and 20109ES, Shanghai, China), BCA assay kit (Yeasten, 20201ES, Shanghai, China), and skim milk (Servicebio, GC310001, Wuhan, China).

Echocardiography examination

Cardiac function was assessed using a Vevo®1100 system (Fujifilm VisualSonics, Toronto, ON, Canada) as previously outlined [18]. Mice were anesthetized with isoflurane while heart rate was maintained at 550–600 bpm via ECG monitoring. Parasternal long-axis views were obtained, with M-mode measurements taken at the papillary muscle level. Ejection fraction (EF%), ventricular wall thickness, and left ventricular mass were derived using the system's LV analysis software.

Hematoxylin–Eosin (H&E) staining and Sirius Red staining

The staining protocols followed established approaches documented earlier [19]. Ready-to-use commercial kits from Servicebio (G1076 for H&E and G1078 for Sirius Red, Wuhan, China) were applied strictly per the manufacturer's instructions. In short, freshly collected tissues underwent fixation in 4% paraformaldehyde solution for no less than 24 hours. After trimming, samples were loaded into properly labeled cassettes for processing. Dehydration occurred stepwise through increasing concentrations of alcohol, succeeded by xylene treatment for transparency and subsequent paraffin infiltration. Once embedded, 4 μ m sections were prepared on a microtome, floated in warm water at 40 °C for flattening, transferred to slides, and oven-dried at 60 °C to eliminate any remaining moisture or paraffin residue before storage.

Staining commenced with deparaffinization in xylene and stepwise rehydration in decreasing ethanol concentrations. H&E procedure involved nuclear staining via hematoxylin, rinsing, and cytoplasmic/extracellular matrix visualization with eosin. Sirius Red application entailed exposure to buffer A for 2 minutes and buffer B for 30 minutes, enabling selective collagen fiber detection. Post-staining, sections were dehydrated, cleared once more in xylene, and sealed with neutral resin for observation under the microscope. Careful execution at every stage helped safeguard tissue morphology and yield reliable staining outcomes. Fibrosis severity was assessed quantitatively by determining the proportion of collagen-stained regions compared to the overall tissue area. To capture representative histology, three separate microscopic fields were imaged per staining method for each section. Histological evaluation focused exclusively on cardiac samples from each mouse. Groups contained $n = 6$ animals each, and all sections were handled identically throughout preparation and staining to guarantee consistency.

Immunohistochemistry and immunofluorescence staining

These assays adhered to methodologies outlined in previous studies [18, 20]. Paraffin-embedded specimens were prepared by fixing fresh tissues in 4% paraformaldehyde for 24 hours, followed by progressive ethanol dehydration, xylene clearing, and paraffin embedding. Blocks were cut into 4 μm slices, flattened on 40 °C water, mounted on slides, and baked at 60 °C.

Immunohistochemistry (IHC) began with xylene deparaffinization and ethanol rehydration. Sections received blocking treatment with 5% BSA plus 10% goat serum in PBS for 30 minutes at room temperature to prevent non-specific interactions. Overnight incubation at 4 °C with appropriately diluted primary antibodies followed. After thorough PBS washing (three times), HRP-linked secondary antibodies (diluted 1:200) were applied for 2 hours at room temperature. Color development used the DAB kit (Servicebio, G1212, Wuhan, China) as specified. Final steps included dehydration, xylene clearing, and resin mounting for bright-field microscopy.

Immunofluorescence involved fixation of cells or tissues in 4% paraformaldehyde and permeabilization with 0.1% saponin in PBS. Primary antibodies at recommended dilutions were incubated overnight at 4 °C. After PBS washes, Cy3- or Alexa Fluor 488-tagged secondary antibodies were added for 1 hour in darkness at room temperature. Unbound reagents were washed away, and images were recorded on a Nikon confocal system (Nikon, Tokyo, Japan) or Zeiss microscope (Zeiss, Jena, Germany).

Three fields per section and staining type were photographed for representative coverage. Only heart tissue was examined histologically per animal. Each group had $n = 6$ mice, with uniform processing applied to all samples.

Protein extraction and western blot

Proteins were isolated from cultured cells or tissue samples using RIPA buffer enriched with protease and phosphatase inhibitor mixes. Samples rested on ice for 15 minutes, with periodic vortexing. Tissue homogenization employed a mechanical lyser fitted with a 3 mm steel ball maintained at 4 °C. Centrifugation at $16,000 \times g$ for 15 minutes separated the soluble fraction. Concentrations were measured by BCA assay. Normalized lysates were combined with sample buffer and heated. Subsequent immunoblotting followed standard procedures [21]. Transfer occurred onto PVDF membranes, which were blocked in 5% skim milk for 1 hour. Incubation with primary antibodies (1:1000) preceded secondary antibody treatment (1:5000). Detection relied on enhanced chemiluminescence, with band intensities analyzed via ImageJ.

Network pharmacology analysis, target identification, and molecular docking

Baicalin's SMILES notation was obtained from PubChem and input into PharmMapper [22], SuperPred, and STITCH. Its three-dimensional structure was docked onto PI3K p85 (PDB code 8DCP) using Schrödinger-2022-2. Target genes underwent standardization to UniProt identifiers, followed by duplicate removal. GeneCards was searched with terms "myocardial injury" and "fibrosis" to compile related genes, which were converted to UniProt IDs and deduplicated. Overlaps between baicalin targets and disease-related genes were visualized in a Venn diagram. Protein-protein interaction networks were generated in STRING 11.0 (Homo sapiens, confidence score 0.900). Cytoscape 3.7 rendered the network, adjusting node properties based on connectivity degree. GO and KEGG analyses were performed through DAVID (<https://david.ncicrf.gov/>, accessed 30 May 2023) [23], covering biological processes (BP), cellular components (CC), and molecular functions (MF) [24]. Enriched pathways were further visualized on the Bioinformatics platform (<http://www.bioinformatics.com.cn/>, accessed 30 May 2023).

Statistical analysis

Analyses utilized GraphPad Prism 8.0.2 (GraphPad Software, Inc., La Jolla, CA, USA). Normality was checked via Shapiro–Wilk test. Parametric data underwent unpaired t-tests (two groups) or one-way ANOVA with Tukey’s multiple comparison test. Non-normal data received non-parametric evaluation. Test specifics, p-values, and replicate numbers (n) appear in Figure legends. Significance thresholds were set at * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, encompassing all relevant group comparisons.

Results and Discussion

Baicalin can reverse cardiomyocyte hypertrophy and heart failure

The cardiac benefits of baicalin (Bai), (**Figure 1a**) were examined by creating an isoprenaline (ISO)-driven heart failure model in male C57BL/6 mice. Propranolol (Pro) served as a benchmark β -blocker control. As expected from existing literature [3], ISO exposure led to substantial decline in left ventricular contractile performance alongside hypertrophic growth, reflected in lowered ejection fraction (**Figure 1b**), higher heart weight-to-body weight ratios (**Figure 1c**), and elevated left ventricular mass (**Figure 1d**). Treatment with baicalin notably rescued ventricular function and limited hypertrophic changes, matching the improvements seen with propranolol (**Figures 1b and 1d**).

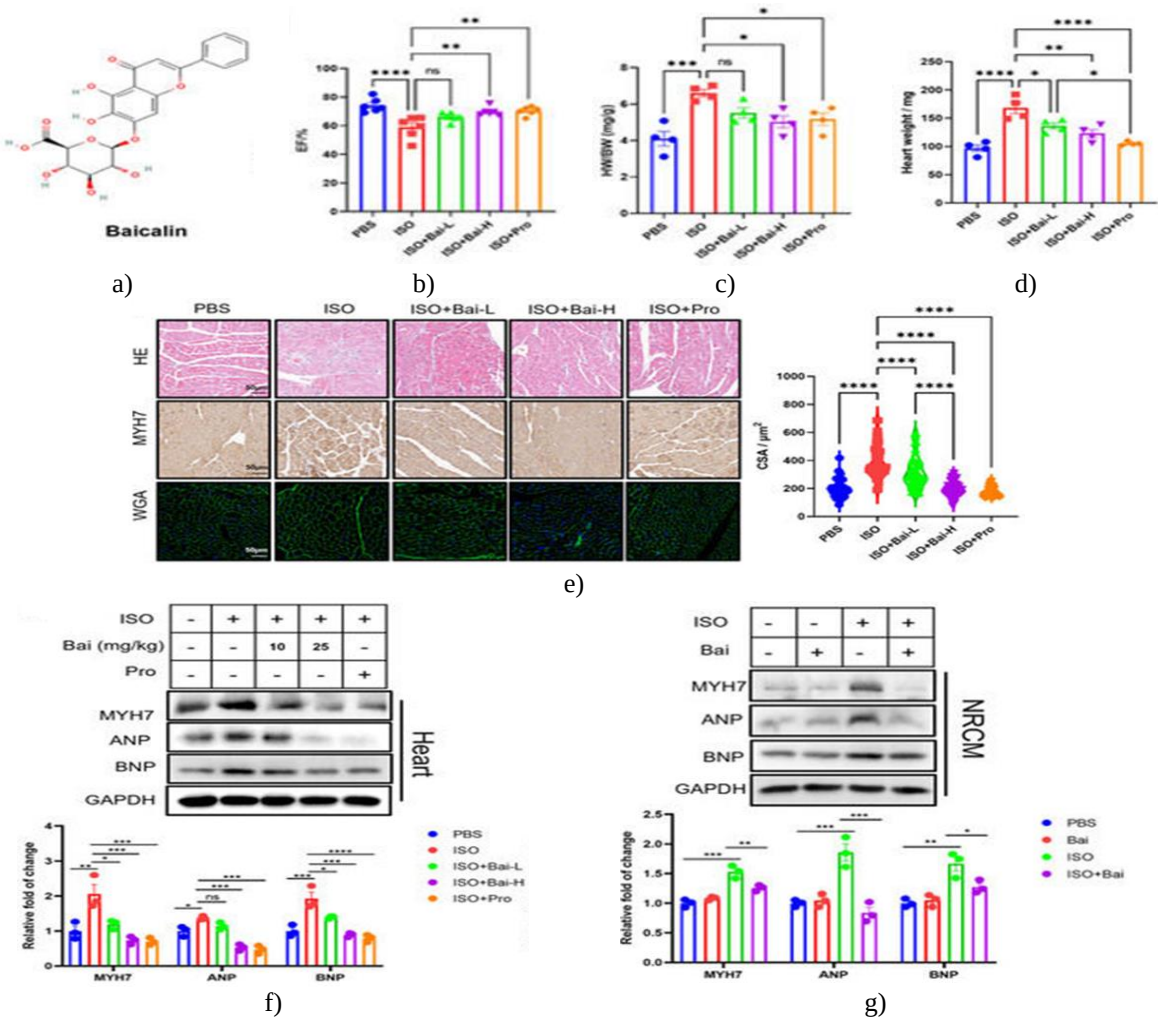


Figure 1. Baicalin improves isoprenaline-triggered heart failure and attenuates cardiomyocyte enlargement. (a) Structural formula of baicalin. (b) Left ventricular ejection fraction measurements across the five mouse groups (n = 6). (c) Heart weight-to-body weight ratios in the five mouse groups (n = 4). (d) Heart weights recorded for the five mouse groups (n = 4). (e) Typical micrographs showing H&E staining, MYH7 immunohistochemistry, and WGA staining on fixed heart sections (n = 50). (f) Sample western blot results for hypertrophy indicators (MYH7, ANP, BNP) along with GAPDH loading control from heart tissues of the

five groups ($n = 3$). (g) Sample western blot results for hypertrophy indicators (MYH7, ANP, BNP) and GAPDH in neonatal rat cardiomyocytes exposed to isoprenaline with or without baicalin ($n = 3$). ISO: isoprenaline; Bai-L: low-dose baicalin; Bai-H: high-dose baicalin; Pro: propranolol; Bai: baicalin. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference; ANOVA was applied for all statistically significant comparisons).

Additional examination of cardiac morphology in the mice demonstrated that isoprenaline provoked marked infiltration by inflammatory cells, as detected through H&E staining, and elevated expression of Myosin Heavy Chain 7 (MYH7), an indicator of myocardial hypertrophy. Wheat germ agglutinin (WGA) labeling further showed expanded cardiomyocyte cross-sectional areas (**Figure 1e**). Baicalin administration successfully counteracted all of these isoprenaline-evoked abnormalities (**Figure 1e**). Protein expression analysis of key hypertrophy and heart failure markers—MYH7, atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP)—revealed substantial upregulation in diseased heart tissue, which baicalin treatment effectively normalized (**Figure 1f**). Collectively, these observations indicate that baicalin provides robust protection against isoprenaline-induced heart failure in living animals.

Subsequent experiments assessed baicalin's direct actions on isolated cells. The compound similarly prevented isoprenaline-driven hypertrophic responses in primary neonatal rat cardiomyocytes (NRCMs) (**Figure 1g**) and restored normal levels of associated molecular markers. Overall, the findings demonstrate baicalin's ability to protect against isoprenaline-mediated cardiomyocyte damage in both animal and cell-based systems.

The transverse aortic constriction procedure replicates hypertension-related heart failure by increasing left ventricular afterload. In line with results from the isoprenaline model, baicalin exerted clear beneficial effects in the TAC setting as well (**Figure 2**).

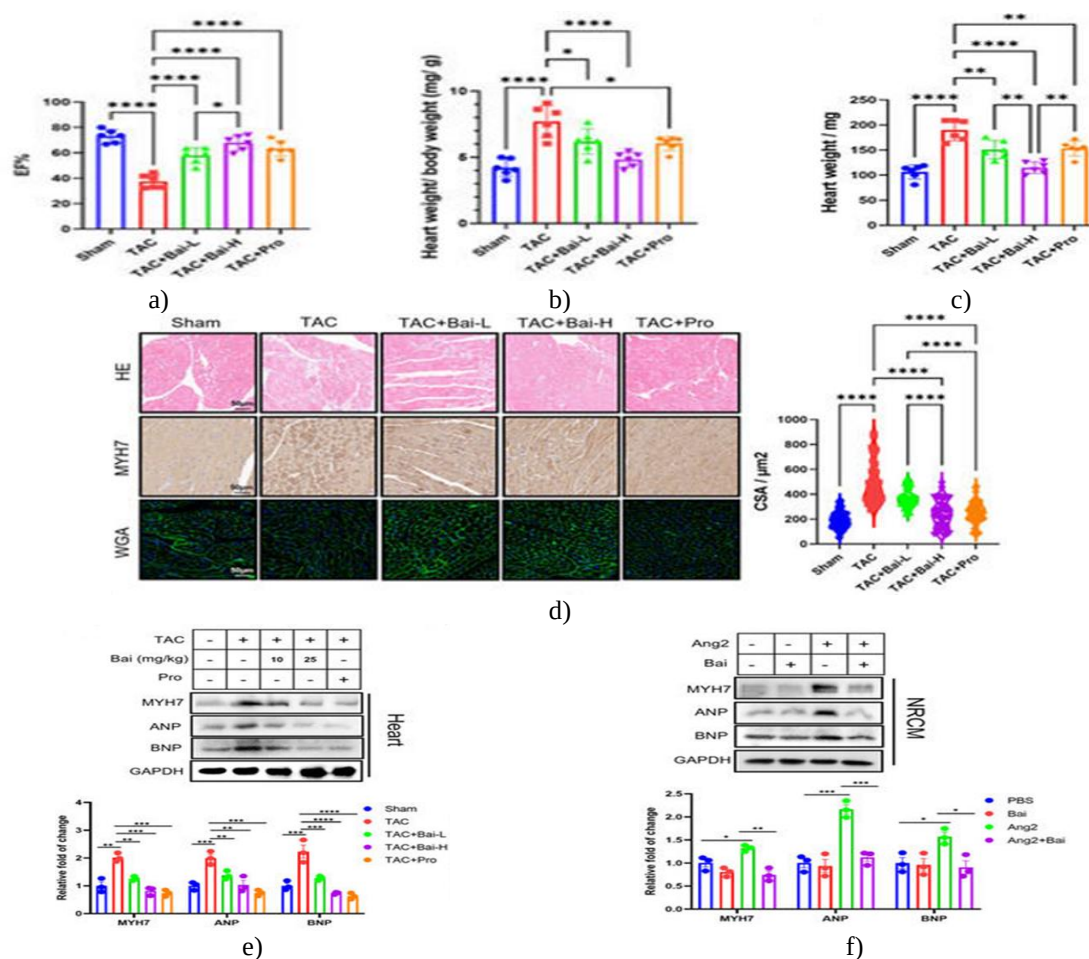


Figure 2. Baicalin counters transverse aortic constriction-triggered heart failure while limiting cardiomyocyte growth. (a) Left ventricular ejection fraction values recorded from mice belonging to the four

study groups (n = 6). (b) Heart-to-body weight ratio data obtained from the four mouse groups (n = 6). (c)

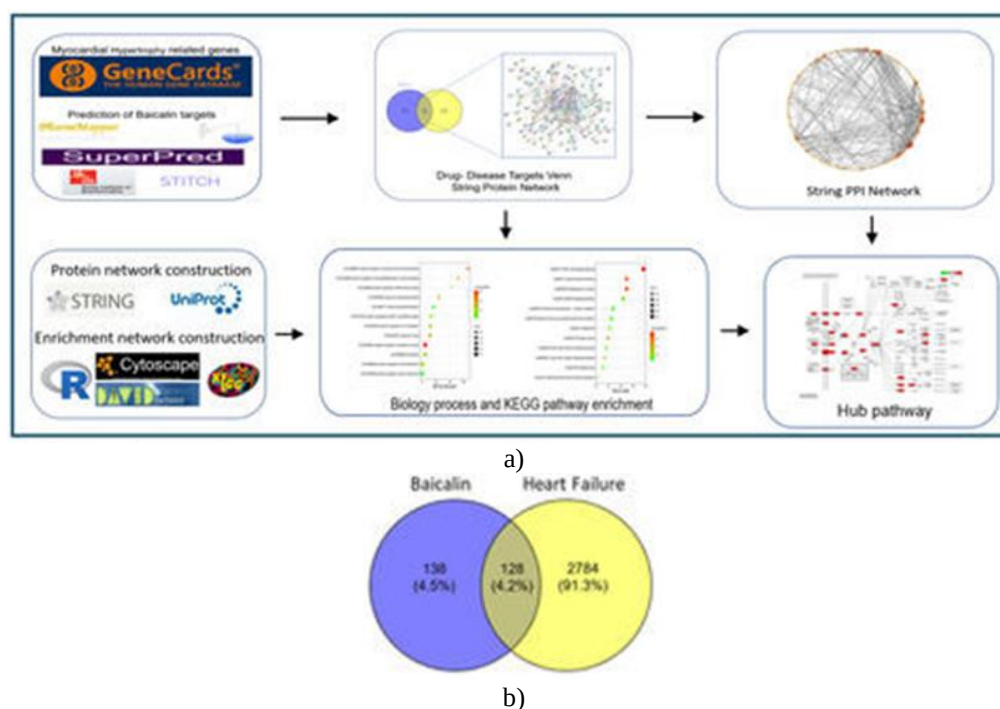
Total heart weight measurements taken across the four groups of mice (n = 6). (d) Characteristic photomicrographs illustrating H&E histology, MYH7 immunolabeling via immunohistochemistry, and WGA-based membrane staining on paraformaldehyde-fixed heart samples (n = 50). (e) Typical western blot bands showing expression of hypertrophy-associated factors (MYH7, ANP, BNP) relative to GAPDH loading control in cardiac tissue samples from the five experimental groups (n = 3). (f) Representative western blot results for the same hypertrophy proteins (MYH7, ANP, BNP) and GAPDH in primary neonatal rat cardiomyocytes stimulated with Angiotensin II in the presence or absence of baicalin treatment (n = 3).

Key terms: transverse aortic constriction (TAC), low-dose baicalin (Bai-L), high-dose baicalin (Bai-H), propranolol (Pro), baicalin (Bai), and primary neonatal rat cardiomyocytes (NRCMs). Statistical notations: * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001. All significance testing relied on ANOVA.

Baicalin engages the PI3K Signaling cascade based on multidatabase network pharmacology insights

Pinpointing the specific molecular sites of action for pharmacologically active substances from traditional Chinese medicine has long been a difficult task. Thanks to the emergence of dedicated TCM and bioinformatics platforms, it has become feasible to systematically forecast potential targets for these compounds. Applying an integrative approach across several such resources (**Figure 3a**), we detected 128 common genes situated at the intersection of baicalin's predicted binding partners (**Figure 3b**), (shown as the blue circle) and genes implicated in heart failure progression (**Figure 3b**), (shown as the yellow circle). Construction of the associated protein–protein interaction (PPI) network was achieved through STRING (**Figure 3c**) and further rendered using Cytoscape (**Figure 3d**). Within this network, prominent central nodes included PIK3CG, Akt, HSP90AA, ITGB, and Src—genes with well-established roles in the heart failure literature. Subsequent Gene Ontology (GO) categorization together with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway mapping of the overlapping gene set disclosed strong enrichment for the PI3K–Akt route (**Figures 3e and 3f**). These computational findings therefore positioned the PI3K–Akt pathway as a leading candidate for baicalin's therapeutic influence.

Empirical testing confirmed the bioinformatics predictions. Exposure to isoprenaline led to robust upregulation of PI3K–Akt signaling activity, an effect that baicalin administration potently reversed under both in vivo conditions (**Figure 4a**) and in cultured cell systems (**Figure 4b**).



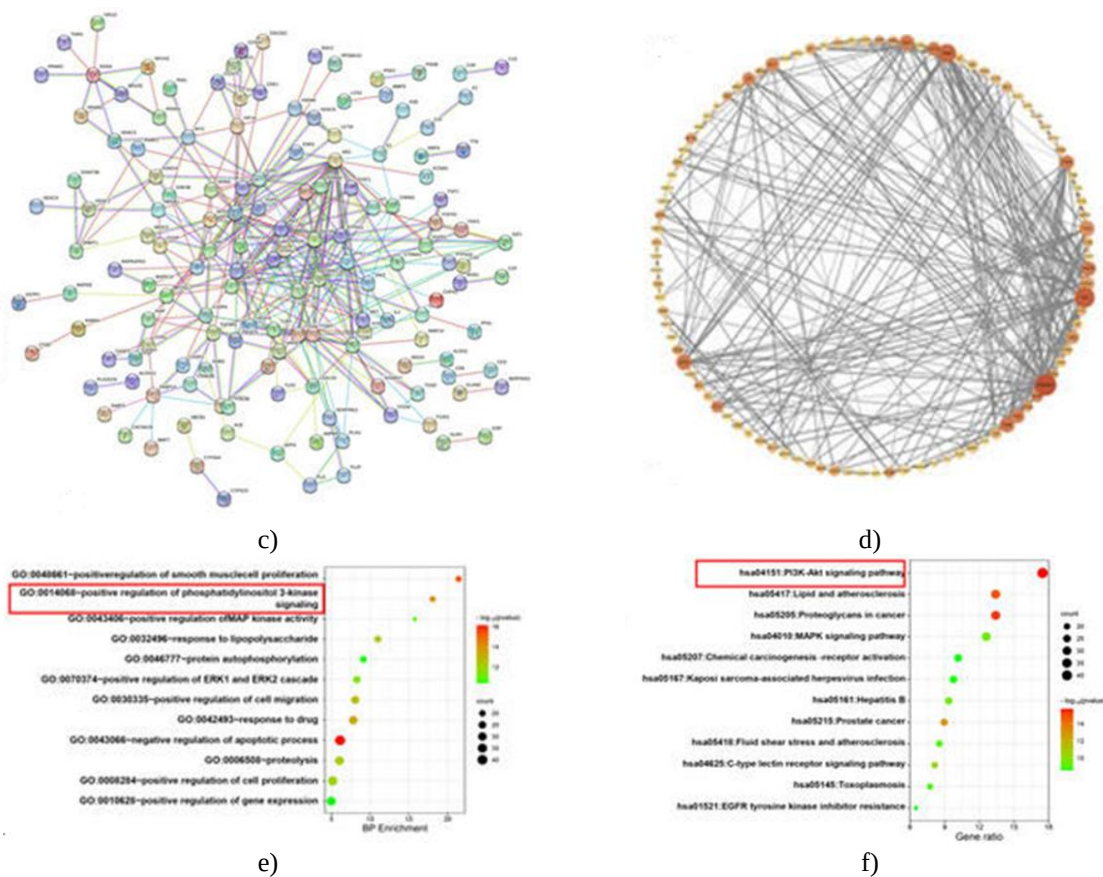
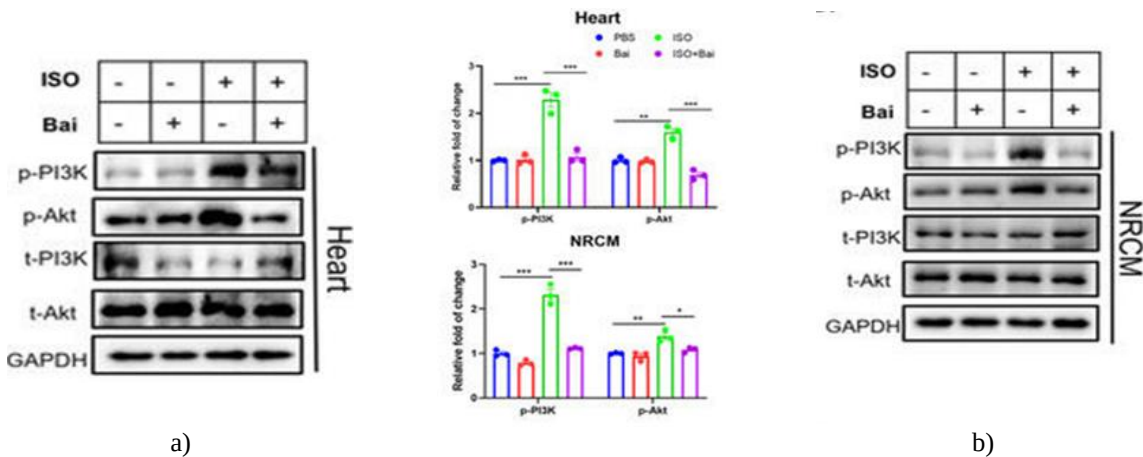


Figure 3. Baicalin interacts with the PI3K signaling route. (a) Schematic overview depicting the multistep integrated network pharmacology workflow. (b) Intersection of predicted baicalin target genes with those connected to heart failure development. (c) Protein-protein interaction map generated from the 128 shared genes identified in panel B using the STRING platform. (d) Visualization and further analysis of the protein-protein interactions performed with Cytoscape. (e,f) Enrichment results for Gene Ontology (GO) categories (e) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (f) derived from the common genes shared between baicalin targets and heart failure-associated factors.



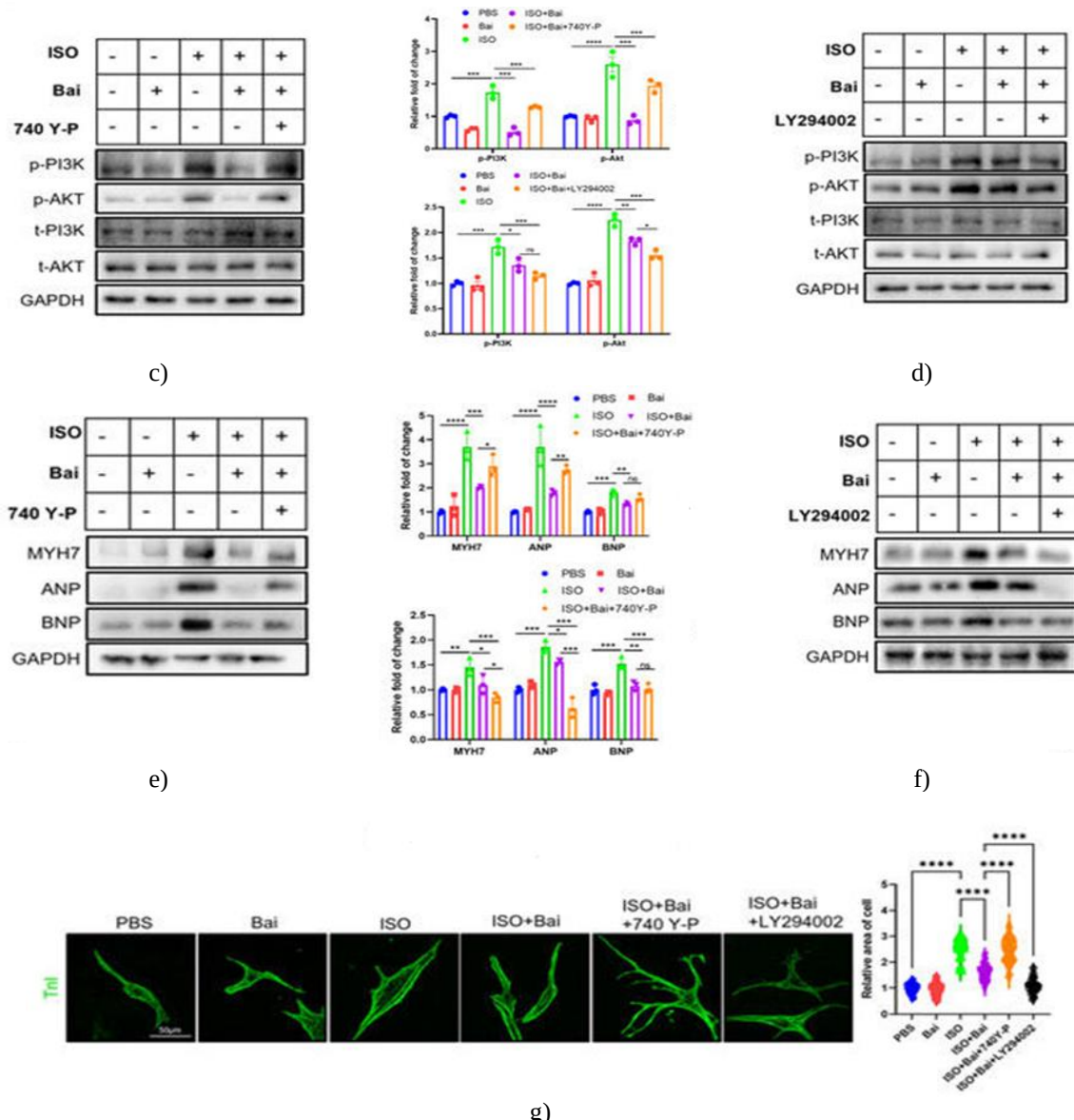


Figure 4. The ability of baicalin to suppress hypertrophy relies on modulation of PI3K function. (a) Typical western blot panels displaying components of the PI3K–Akt cascade along with GAPDH in cardiac tissues from the four experimental groups (n = 3). (b) Typical western blot panel showing PI3K–Akt pathway components and GAPDH in neonatal rat cardiomyocytes exposed to isoprenaline plus baicalin (n = 3). (c,d) Typical western blot panels of the PI3K–Akt pathway in neonatal rat cardiomyocytes treated with isoprenaline combined with baicalin or the PI3K activator 740-Y-P (c), and with the PI3K inhibitor LY294002 (d) (n = 3). (e,f) Typical western blot panels of hypertrophy indicators (MYH7, ANP, BNP) in neonatal rat cardiomyocytes under isoprenaline stimulation with baicalin or the PI3K activator 740-Y-P (e), and with the PI3K inhibitor LY294002 (f) (n = 3). (g) Representative confocal microscopy images of neonatal rat cardiomyocytes following exposure to baicalin, isoprenaline, or PI3K modulator compounds, with troponin I labeling (n = 50). Abbreviations: isoprenaline (ISO), baicalin (Bai), primary neonatal rat cardiomyocytes (NRCMs). (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, ns: no significant difference; ANOVA applied for all significant comparisons).

To solidly establish that PI3K function is essential for baicalin's heart-protective actions, both a PI3K activator and inhibitor were introduced (**Figures 4c and 4g**). The PI3K agonist 740-Y-P [25] notably weakened baicalin's anti-hypertrophic benefits, as shown by sustained high levels of MYH7, ANP, and BNP proteins following co-treatment (**Figure 4e**). Conversely, the PI3K inhibitor LY294002 [26] produced protective outcomes comparable to baicalin alone (**Figure 4f**). Immunofluorescence assessment of cardiomyocyte surface area provided results

consistent with the protein expression data (**Figure 4g**). Taken together, these observations indicate that baicalin exerts its cardiomyocyte-protective influence by suppressing PI3K activity through interference with the p85 subunit.

Baicalin attenuates cardiac fibrosis

Cardiac fibrosis represents a major pathological hallmark of heart failure that increases myocardial rigidity and impairs diastolic performance. Sirius Red staining offers a reliable approach for evaluating fibrosis levels because it selectively highlights collagen fibers, with staining intensity directly corresponding to collagen deposition. In the present study, isoprenaline exposure induced pronounced cardiac fibrosis, manifested as intense red staining (**Figure 5a**), aligning with earlier observations [27]. Treatment with baicalin markedly reduced this isoprenaline-driven fibrotic response (**Figure 5a**). Supporting molecular evidence came from analysis of fibrosis indicators including fibronectin, vimentin, and α -SMA. Baicalin significantly lowered the expression of these markers in both animal heart tissues (**Figure 5b**) and cultured cells (**Figure 5c**).

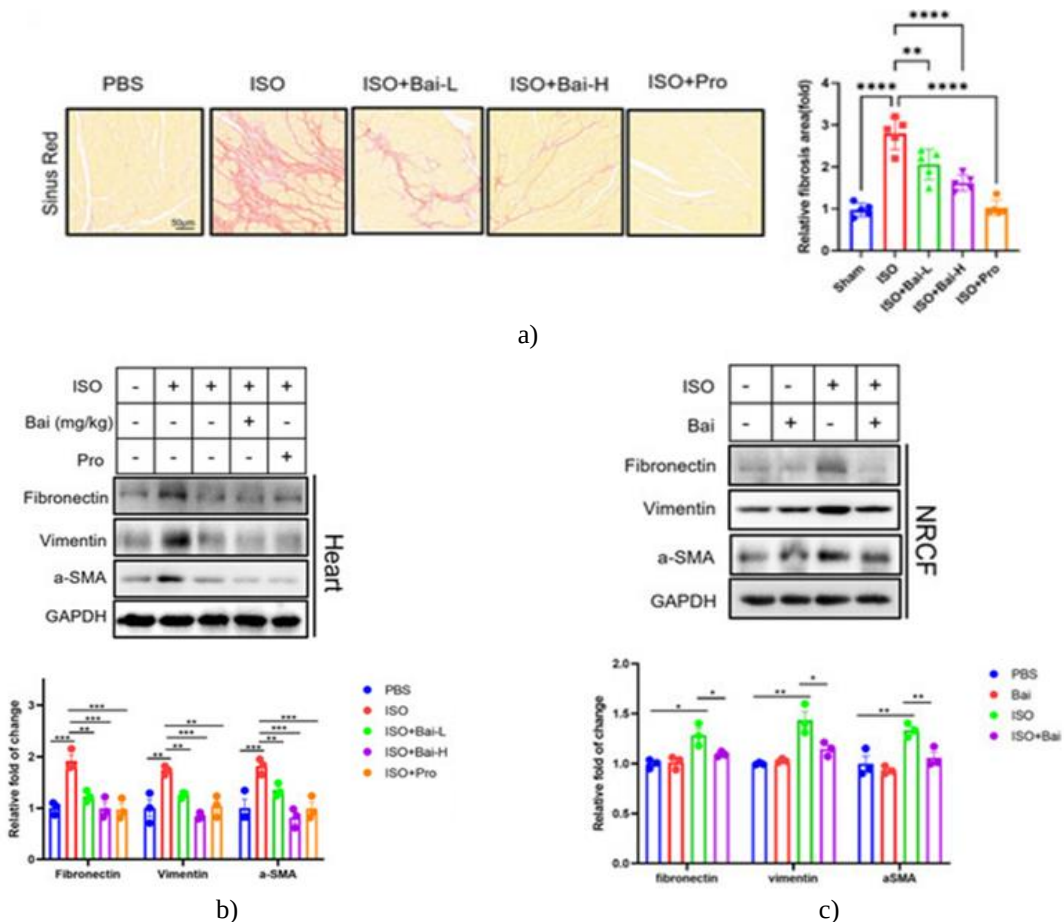


Figure 5. Baicalin's capacity to suppress fibrosis depends on its influence over PI3K function. (a) Typical micrographs of Sirius Red staining performed on paraformaldehyde-fixed heart sections ($n = 5$). (b) Typical western blot panels for fibrosis-related proteins (fibronectin, vimentin, and α -SMA) together with GAPDH in cardiac tissues from the five study groups ($n = 3$). (c) Typical western blot panel for the same fibrosis markers (fibronectin, vimentin, and α -SMA) along with GAPDH in primary neonatal rat cardiac fibroblasts exposed to isoprenaline, baicalin, and propranolol ($n = 3$). Abbreviations: isoprenaline (ISO), low-dose baicalin (Bai-L), high-dose baicalin (Bai-H), propranolol (Pro), baicalin (Bai), primary neonatal rat cardiac fibroblasts (NRCFs). (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ANOVA was utilized for all statistically meaningful comparisons).

In parallel with baicalin's protective actions on cardiomyocytes, its ability to limit fibrosis in neonatal rat cardiac fibroblasts (NRCFs) likewise involved suppression of PI3K signaling (**Figures 6a and 6c**). This was demonstrated by reduced levels of fibrotic marker proteins upon addition of the PI3K inhibitor (**figure 6a**) and

elevated marker expression when the PI3K activator was applied (**Figure 6b**). Immunofluorescence staining for vimentin produced outcomes consistent with the western blot findings (**Figure 5c**).

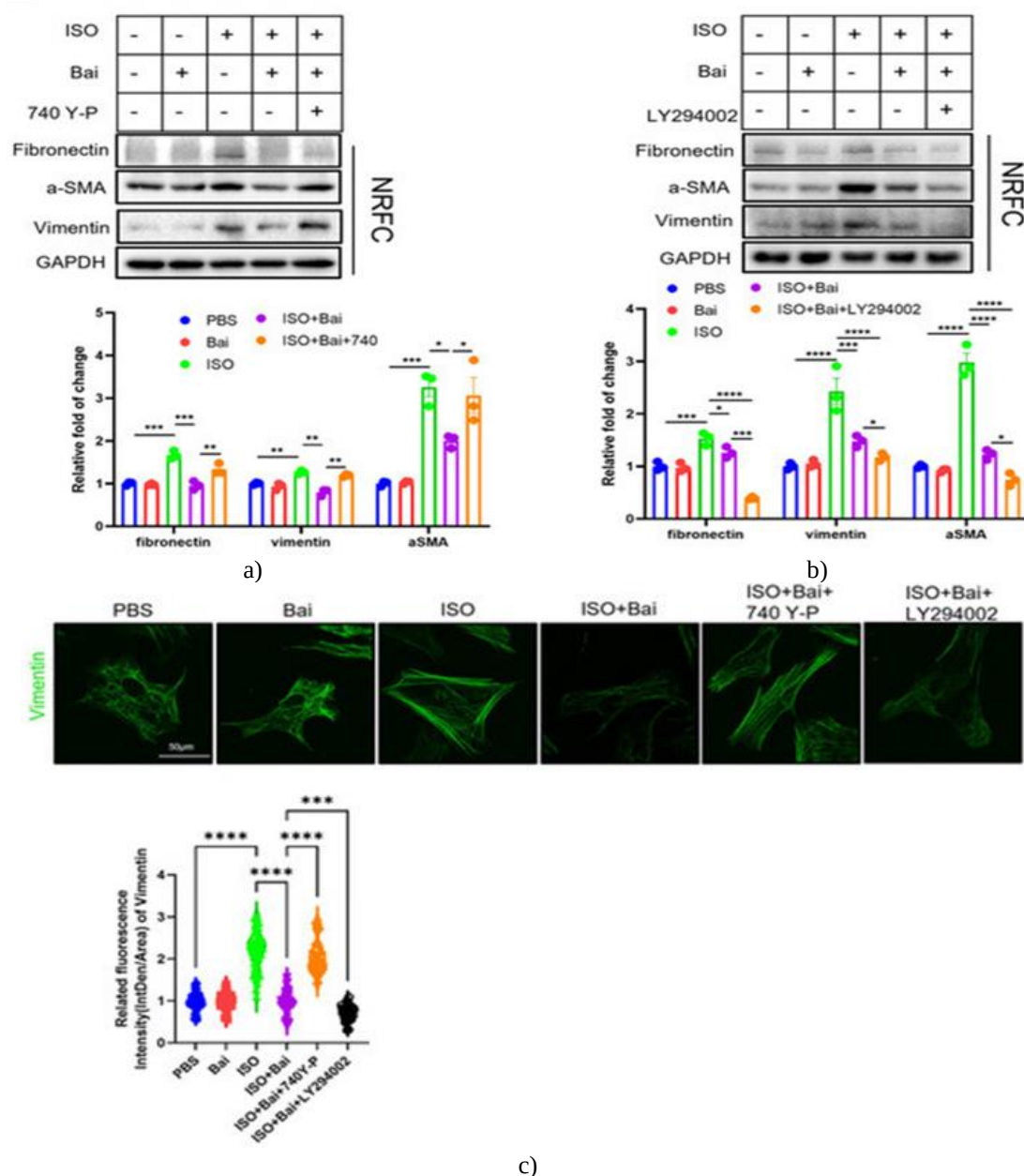


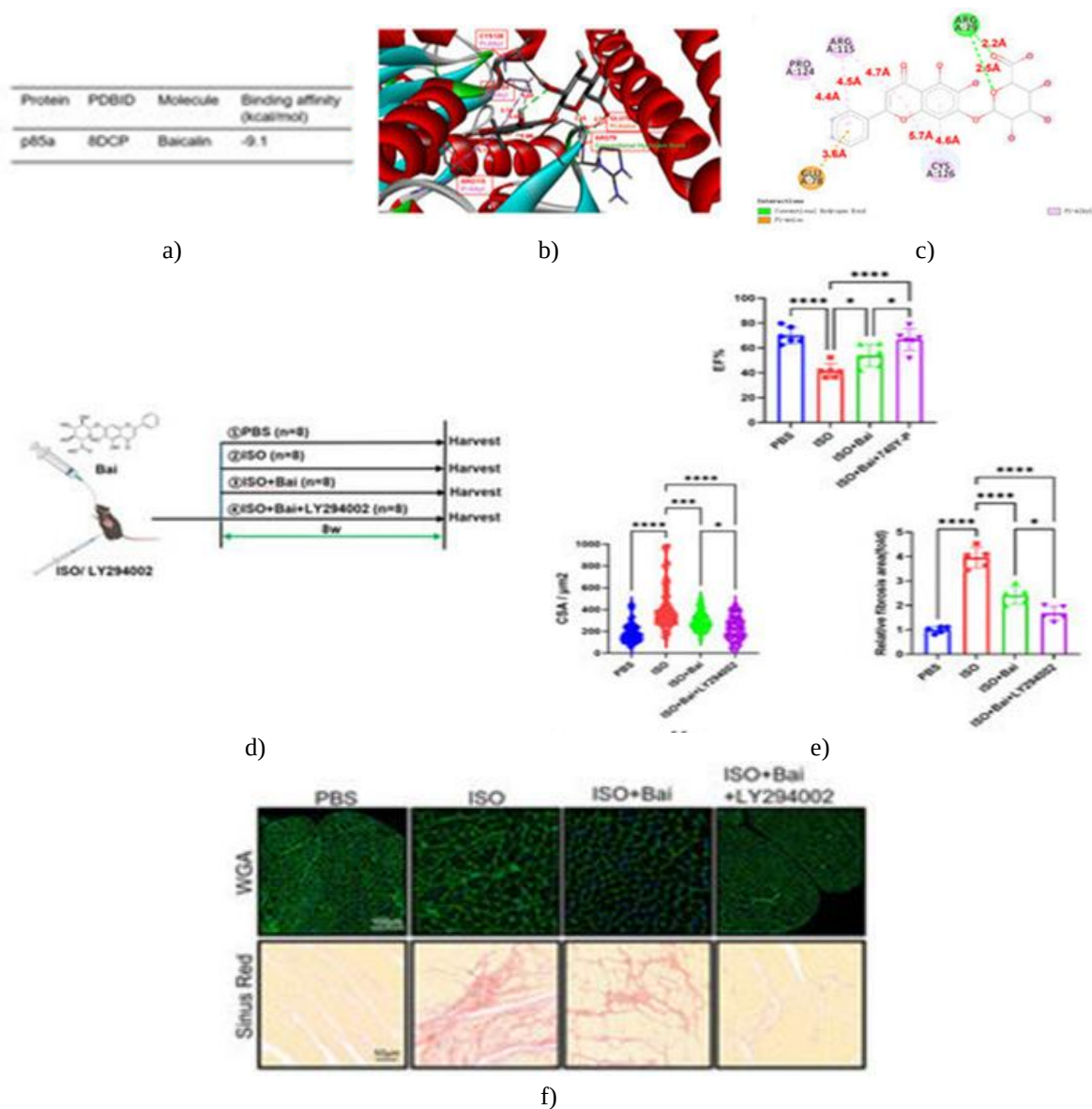
Figure 6. Baicalin's ability to inhibit fibrosis depends on modulation of PI3K activity. (a,b) Typical western blot results for fibrosis indicators (fibronectin, vimentin, and α -SMA) in primary neonatal rat cardiac fibroblasts exposed to isoprenaline and baicalin combined with the PI3K activator 740-Y-P (a) or the PI3K inhibitor LY294002 (b) ($n = 3$). (c) Representative confocal microscopy images of primary neonatal rat cardiac fibroblasts following treatment with baicalin, isoprenaline, or PI3K modulators ($n = 50$). Cells were labeled with vimentin and DAPI. Abbreviations: isoprenaline (ISO), baicalin (Bai), primary neonatal rat cardiac fibroblasts (NRCFs). (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ANOVA applied for significance testing).

These results support the conclusion that baicalin exerts antifibrotic benefits in the setting of cardiac fibrosis through its inhibitory influence on the PI3K pathway.

Combined administration of baicalin and a PI3K p110 inhibitor produces superior cardioprotective outcomes PI3K exists as a multi-subunit enzyme complex that includes four catalytic subunits and two regulatory subunits [28]. Given the network pharmacology evidence pointing to PI3K as a key target of baicalin, the three-

dimensional structures of its various subunits were retrieved from the Protein Data Bank and subjected to virtual docking simulations with baicalin using Schrödinger software. Among all subunits examined, the p85 α regulatory subunit displayed the strongest binding affinity (**Figure 7a**). Detailed interaction analysis revealed that baicalin forms contacts with specific residues on p85 α —namely Glu78, Arg79, Arg115, Pro124, and Cys126 (**Figures 7b and 7c**).

Because baicalin appears to achieve its heart-protective actions primarily by targeting the p85 α subunit, we investigated whether pairing it with a compound that inhibits the p110 catalytic subunit might yield additive or synergistic benefits. In the isoprenaline-induced heart failure model, LY294002—a selective PI3K inhibitor—was co-administered alongside baicalin (**Figure 7d**). This dual treatment led to a further elevation in ejection fraction compared with baicalin alone (**Figure 7e**). Histological assessments confirmed enhanced improvements, including greater reductions in Sirius Red-positive fibrotic areas and cardiomyocyte cross-sectional sizes (**Figure 7f**). Western blot experiments similarly demonstrated that the combination more potently lowered expression levels of cardiomyocyte hypertrophy markers (**Figure 7g**) as well as myocardial fibrosis indicators (**Figure 7h**) than baicalin monotherapy.



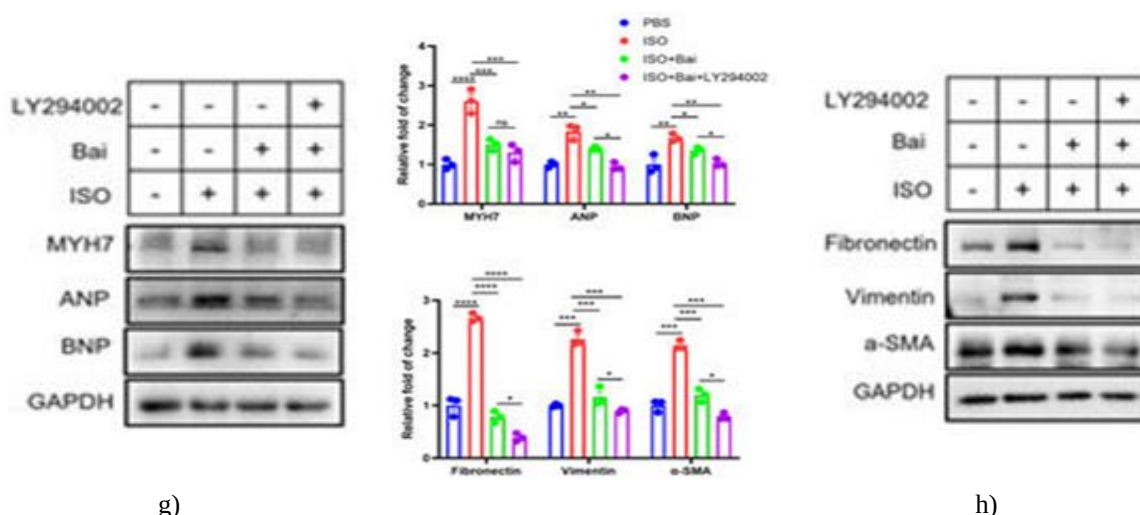


Figure 7. Combined therapy using baicalin together with a PI3K p110 inhibitor yields stronger heart protection. (a) Docking scores reflecting binding strength between baicalin and the p85 α subunit. (b) Three-dimensional representation of baicalin interacting with five specific amino acid residues on p85 α . (c) Two-dimensional diagram illustrating the molecular contacts between baicalin and the key amino acid residues. (d) Schematic of the animal experiment involving baicalin co-administered with LY294002 ($n = 3$). (e) Left ventricular ejection fraction values measured in the mice ($n = 6$). (f) Typical images from Sirius Red and WGA staining of fixed cardiac sections ($n = 50$ for WGA, $n = 5$ for Sirius Red). (g) Representative western blot results for hypertrophy proteins (MYH7, ANP, BNP) and GAPDH from heart tissues of the four groups ($n = 3$). (h) Representative western blot results for fibrosis proteins (fibronectin, vimentin, α -SMA) and GAPDH in heart tissues of the four groups ($n = 3$). Abbreviations: isoprenaline (ISO), baicalin (Bai). (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference; ANOVA was used for all significant analyses).

The current investigation provides both *in vivo* and *in vitro* support showing that baicalin effectively counters two central hallmarks of heart failure—cardiomyocyte hypertrophy and cardiac fibrosis—primarily by dampening PI3K signaling. Integrated network pharmacology approaches identified the PI3K pathway as a major responsive element to baicalin, while molecular docking studies confirmed direct interaction with the p85 α subunit. Experiments employing specific PI3K activators and inhibitors alongside baicalin further verified that suppression of PI3K activity mediates baicalin's benefits, as re-activation of the pathway restored elevated levels of hypertrophy and fibrosis markers in NRCMs and NRCFs. Moreover, pairing baicalin with the PI3K p110 inhibitor LY294002 produced superior protection in the isoprenaline-induced heart failure model. These observations suggest that the baicalin plus LY294002 regimen could represent an improved therapeutic option. As a principal bioactive constituent of the traditional Chinese herb Huang Qin, baicalin has garnered considerable interest in natural product research. It has demonstrated therapeutic potential across multiple conditions, including diverse cancers [29], inflammatory disorders [30], autoimmune pathologies [31], and cardiovascular ailments [32], highlighting its relevance for both fundamental pharmacological studies and possible clinical applications. Within cardiovascular research, prior work on baicalin has largely centered on pressure overload models of heart failure [12, 33], ischemia/reperfusion damage [34, 35], and drug-induced cardiotoxicity [13, 36]. However, relatively few studies have addressed its role in isoprenaline-induced heart failure, a widely used experimental system for exploring HF mechanisms. The present study therefore fills this gap by documenting baicalin's protective actions in this context. Notably, baicalin's efficacy was comparable to that of the standard beta-blocker propranolol. Isoprenaline challenge is known to trigger extensive fibrosis, cardiomyocyte apoptosis, and hypertrophy [37-39], all of which were significantly alleviated by baicalin treatment, underscoring the need for further exploration of its clinical potential.

The PI3K pathway ranks among the most critical signaling networks in eukaryotic cells, governing processes such as cell proliferation [40], differentiation [41], apoptosis [42], and glucose transport [43]. Class I PI3Ks, the best-characterized isoform, function as heterodimers consisting of regulatory and catalytic subunits [44]. Network pharmacology in this study highlighted PI3K as baicalin's primary target. Docking analyses comparing different PI3K subunits revealed the strongest interaction with p85 α . These results align with earlier publications

demonstrating baicalin's inhibitory effects on PI3K in contexts such as osteosarcoma [45], glaucoma [46], spinal cord injury [47], neuroinflammation [48], and pulmonary hypertension [49]. The present work extends this understanding to the cardiovascular domain. Docking simulations specifically identified interactions between baicalin and amino acid positions 78, 79, 115, 124, and 126 on p85 α . Notably, the SH3 domain spans residues 3–79, raising the possibility that baicalin disrupts SH3 domain conformation, thereby inhibiting p85 α function.

Downstream of PI3K, Akt phosphorylation promotes cardiomyocyte hypertrophy. Consistent with pathway activation, isoprenaline increased Akt phosphorylation, which baicalin effectively reversed. This matches established evidence linking excessive Akt activity to maladaptive hypertrophy and heart failure progression [50]. Reducing hypertrophic burden represents a key strategy to lower myocardial energy demands and prevent metabolic dysregulation in failing hearts. Supporting this concept, clinical metabolomics data indicate that hearts from HF patients exhibit decreased long-chain acylcarnitines and elevated ketones [51], reflecting a shift from fatty acid to ketone or glucose utilization [52]. In our experiments, baicalin reduced cardiomyocyte hypertrophy in a dose-dependent manner and helped normalize heart weight-to-body weight ratios, implying decreased energy expenditure and improved cardiac performance.

Insulin resistance is recognized as a contributor to diabetic cardiomyopathy [53, 54]. Chronic insulin resistance promotes metabolic imbalance, excess fatty acid oxidation, ectopic lipid deposition, and inflammatory responses that impair cardiac function [55]. In this state, PI3K–Akt signaling is frequently compromised, resulting in defective glucose handling, lipid accumulation, and contractile dysfunction [56, 57]. By modulating the PI3K–Akt axis, baicalin may help normalize insulin sensitivity, enhance glucose uptake, limit lipid buildup, and dampen inflammation within the myocardium. Such effects could slow heart failure advancement in diabetic contexts and position baicalin as a candidate therapy for diabetic cardiomyopathy.

We also anticipated and confirmed baicalin's strong antifibrotic activity under isoprenaline stress. Excessive sympathetic drive is a potent inducer of cardiac fibrosis [58, 59], paralleling clinical conditions like Takotsubo syndrome where prolonged sympathetic overactivation leads to persistent scarring [60]. Fibroblasts are the primary drivers of fibrotic remodeling, and baicalin suppressed fibroblast marker expression in both animal and cell models. The reversal of this antifibrotic action by a PI3K agonist further links isoprenaline-induced fibrosis to PI3K signaling. These observations are consistent with prior reports showing baicalin's ability to reduce pressure overload-induced fibrosis [61] and the antifibrotic properties of baicalin-containing formulations in hypertensive models [33]. Collectively, the data reinforce baicalin's potential as an antifibrotic agent.

LY294002 selectively inhibits PI3K α , PI3K δ , and PI3K β isoforms. Dual targeting of both p85 α (by baicalin) and p110 (by LY294002) produced more pronounced cardioprotection than baicalin monotherapy. Benefits included higher ejection fraction, reduced cardiomyocyte size, and lower levels of injury and fibrosis markers. These results highlight the promise of this combination for heart failure management. Nevertheless, given the known side effects of broad PI3K inhibition by LY294002 [62, 63], additional dose-optimization and safety studies will be required before clinical translation.

Several limitations should be acknowledged. The study relied on pharmacological agonists and antagonists rather than genetic approaches such as siRNA or overexpression in NRCM and NRCF models, leaving open the possibility of off-target drug effects. PI3K knockout mice exhibit perinatal lethality, precluding direct genetic validation *in vivo*. Future work should include long-term clinical trials to establish safe and effective dosing for the baicalin–LY294002 combination. Despite these constraints, the data clearly demonstrate baicalin's efficacy in mitigating isoprenaline-induced cardiac dysfunction and associated pathological changes, supporting its potential as an adjunct therapy for heart failure with reduced ejection fraction.

Conclusion

In conclusion, this study establishes that baicalin improves cardiac function and reverses key pathological features of heart failure, notably cardiomyocyte hypertrophy and fibrosis. Its protective mechanism involves inhibition of the p85 α subunit, likely through interaction with the SH3 domain.

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