



Basic Study

Lysine acetyltransferase 2A-mediated succinylation of adenosine monophosphate-activated protein kinase suppresses gallstone formation by inhibiting inflammation and pyroptosis

Xin-Xing Wang, Ming-Ze Ma, Li-Chao Zhu, Long-Fei Dai, Chuan Qin, Shuai Shao, Xian-Wen Xu, Ru-Xin Gao, Zhen-Hai Zhang

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Xin-Xing Wang, Long-Fei Dai, Shuai Shao, Xian-Wen Xu, Ru-Xin Gao, Department of Hepatobiliary Surgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250021, Shandong Province, China

Ming-Ze Ma, Department of Infectious Diseases, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250021, Shandong Province, China

Li-Chao Zhu, Department of Pediatric Surgery, Shandong Provincial Hospital, Jinan 250021, Shandong Province, China

Chuan Qin, Department of Hepatobiliary Surgery, Shandong Provincial Hospital, Jinan 250021, Shandong Province, China

Zhen-Hai Zhang, Department of Hepatobiliary Surgery, Shandong Provincial Hospital, Shandong University, Jinan 250021, Shandong Province, China

Co-first authors: Xin-Xing Wang and Ming-Ze Ma.

Corresponding author: Zhen-Hai Zhang, Department of Hepatobiliary Surgery, Shandong Provincial Hospital, Shandong University, No. 324 Jingwu Road, Huaiyin District, Jinan 250021, Shandong Province, China. drzhangzhenhai@163.com

Abstract

BACKGROUND

Cholelithiasis is a prevalent biliary tract disorder primarily characterized by gallbladder or biliary stone formation. Although succinylation has been extensively studied as a protein post-translational modification, its role in cholelithiasis remains unexplored.

AIM

To investigate the functional role of succinylation in cholelithiasis and determine its underlying molecular mechanisms.

METHODS

A murine cholelithiasis model was established through high-fat diet feeding,

followed by isolation of mouse gallbladder mucosal epithelial cells (GMECs) for *in vitro* analysis. Gallbladder tissues and serum samples were collected for subsequent analysis. Inflammatory cytokine production was quantified using enzyme-linked immunosorbent assay. Pyroptosis was analyzed by flow cytometry, while succinylation- and pyroptosis-related protein expression was detected *via* western blot.

RESULTS

Our findings demonstrated that lysine acetyltransferase 2A (KAT2A)-mediated succinylation regulated gallstone formation. KAT2A overexpression inhibited the pyroptosis, inflammatory responses, and promoted the activation of the adenosine monophosphate-activated protein kinase (AMPK)/silent information regulator 1 (SIRT1) signaling pathway in GMECs. Mechanistically, AMPK exhibited succinylation at lysine 170 (K170). Notably, AMPK inhibition significantly increased pyroptosis rates, inflammatory responses, and pyroptosis-related protein expression in GMECs. Furthermore, *in vivo* experiments revealed that KAT2A overexpression suppressed both inflammation and gallstone formation.

CONCLUSION

KAT2A-mediated succinylation of AMPK inhibited cholelithiasis progression by modulating the AMPK/SIRT1 signaling pathway, offering potential therapeutic strategies for this condition.

Key Words: Cholelithiasis; Succinylation; Lysine acetyltransferase 2A; Inflammation; Gallstone formation; Adenosine monophosphate-activated protein kinase/silent information regulator 1

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Core Tip: Cholelithiasis is a common biliary disorder with complex mechanisms. Lysine succinylation, a protein post-translational modification, remains unstudied in this condition. This study reveals that lysine acetyltransferase 2A-mediated succinylation of adenosine monophosphate-activated protein kinase (AMPK) inhibits cholelithiasis by regulating inflammation *via* the AMPK/sirtuin 1 pathway. This study provides a groundbreaking perspective on cholelithiasis mechanisms and position succinylation as a potential therapeutic target, addressing a significant gap in the field.

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INTRODUCTION

Cholelithiasis is a prevalent digestive tract disease in clinical practice, characterized by the formation of stones in the gallbladder or biliary tract. Its clinical manifestations are diverse and may lead to complications such as cholecystitis, cholangitis, and pancreatitis[1]. The global prevalence of cholelithiasis is rising, affecting approximately 10% to 20% of adults. Regional disparities exist, with higher incidence rates observed in Europeans and Americans (10% to 15%), Asians (3% to 15%), and less than 5% in Africans[2]. In China, the prevalence has exceeded 10% and continues to increase due to an aging population and evolving dietary patterns[3]. Cholelithiasis disproportionately affects adult women, particularly middle-aged and elderly individuals. Established risk factors include obesity, unhealthy diets, rapid weight loss, pregnancy, and genetic predisposition[4]. Pathogenesis involves disrupted cholesterol homeostasis and abnormal bilirubin metabolism in the biliary tract[5,6]. Despite these insights, the mechanisms underlying cholelithiasis remain complex, primarily influenced by lifestyle, genetic, and physical factors. Postoperative stone recurrence further complicates comprehensive investigation, underscoring the need for deeper mechanistic understanding.

Post-translational modification (PTM) is a critical regulator of protein function, bridging metabolic processes with physiological and pathological pathways[7]. Lysine succinylation, a widespread PTM in eukaryotes, participates in essential processes such as cell differentiation and metabolism[8]. Lysine acetyltransferase 2A (KAT2A) acts as a succinyltransferase by transferring a succinyl group from succinyl-CoA to lysine residues on histone proteins[9]. This modification modulates gene expression and chromatin structure by altering DNA accessibility to transcription factors and regulatory proteins. Beyond histone regulation, KAT2A-mediated succinylation influences cellular processes including proliferation, differentiation, and apoptosis[10,11]. Emerging evidence highlights its role in gastrointestinal diseases, such as gastric, hepatocellular, and pancreatic cancers[12-14]. However, the involvement of succinylation in cholelithiasis remains unexplored.

Adenosine monophosphate-activated protein kinase (AMPK) serves as a central energy sensor and metabolic regulator, governing cell growth, survival, and systemic energy homeostasis[15]. Sirtuin 1 (SIRT1), an NAD⁺-dependent deacetylase, regulates diverse biological processes, including cell cycle progression, inflammation, autophagy, and aging,

while mitigating disease risks[16,17]. SIRT1 modulates AMPK phosphorylation levels[18], and dysregulation of this axis exacerbates inflammation, as seen in obese mice with suppressed AMPK activity[19]. Numerous studies demonstrate that AMPK/SIRT1 signaling suppresses inflammation and pyroptosis in various diseases[20,21]. Notably, activation of this pathway reduces inflammatory damage to gallbladder mucosal epithelial cells, thereby protecting against gallbladder injury and gallstone formation in murine models[22].

This study investigated the role of succinylation in cholelithiasis and its underlying molecular mechanisms. Our findings revealed that KAT2A-mediated succinylation of AMPK inhibited cholelithiasis progression by modulating inflammation and gallstone formation, offering potential therapeutic insights for this condition. This study is the first to investigate the role of lysine succinylation in cholelithiasis pathogenesis, uncovering a novel link between post-translational metabolic regulation and gallstone formation. These findings provide a groundbreaking perspective on cholelithiasis mechanisms and position succinylation as a potential therapeutic target, addressing a significant gap in the field.

MATERIALS AND METHODS

Establishment of animal model

The animal study was approved by the Experimental Animal Welfare Ethics Committee of MDKN Biotechnology Co., Ltd (Approval No. MDKN-2024-129). A total of 24 male C57BL/6J mice (20 ± 5 g) were obtained from Aniphe Biolaboratory Inc. (Nanjing, China) and housed in a controlled environment at 24 °C with a 12-hour light/dark cycle, free access to food and water. Mice were randomly assigned to two initial groups: A normal control group ($n = 6$) and a model group ($n = 18$). After a 1-week acclimatization period, the normal group was fed a standard diet, while the model group received a high-fat diet (HFD). The commercial HFD composition (containing 40% kcal fat) included 15% butterfat, 1.25% cholesterol, 0.5% sodium cholate, 2% corn oil, 50% sucrose, 20% casein, and essential minerals and vitamins (Dyets Biotech Co., Ltd., Wuxi, China). The model group was further subdivided into three subgroups: Model ($n = 6$), model + lentivirus (Lv)-NC ($n = 6$), and model + Lv-KAT2A ($n = 6$). For four weeks, mice in the Lv-NC and Lv-KAT2A groups were intravenously administered 5×10^7 TU of Lv-NC or Lv-KAT2A in 200 μ L phosphate buffer solution (PBS), three times per week. Control mice in the standard diet group received the same volume of normal saline *via* intravenous injection under identical conditions. After eight weeks of dietary intervention, blood samples were collected *via* the inferior vena cava and centrifuged at 5000 rpm for 10 minutes to isolate the supernatant, which was stored at -20 °C for subsequent analysis. Mice were weighed and euthanized using 5% isoflurane (Sigma-Aldrich, St. Louis, MO, United States) inhalation. Fresh gallbladder bile was collected and assessed for microscopic and macroscopic gallstone formation according to criteria established by Zhao *et al*[23]. Gallbladder and liver tissues were then harvested and stored at -80 °C for further experiments.

Cell culture, treatment, and transfection

Primary gallbladder mucosal epithelial cells (GMECs) were isolated and characterized following a previously established protocol[22]. Cells were cultured in the ICell Primary Epithelial Cell Culture System (PriMed-iCell-001; Cellverse Bioscience Technology Co. Ltd., Shanghai, China), supplemented with 2% fetal bovine serum and 1% double antibiotics. Cultures were maintained at 37 °C in a 5% CO₂ humidified incubator, with medium replaced every 3 days. To induce an inflammatory injury model *in vitro*, GMECs were stimulated with lipopolysaccharide (LPS; ST1470; Beyotime Biotech Co., Ltd., Shanghai, China) at a concentration of 200 μ g/mL for 24 hours, as described previously[22]. Additionally, GMECs were treated with the AMPK inhibitor dorsomorphin (DOR; purity > 99%; ab120843; 10 μ mol/L; Abcam) for 24 hours to assess AMPK signaling involvement.

The empty pcDNA3.1 vector, pcDNA3.1-KAT2A overexpression vector, short hairpin RNA targeting KAT2A (sh-KAT2A), and sh-RNA negative control (sh-NC) were synthesized by HAN Biotech Co., Ltd. (Shanghai, China). Flag-WT, Flag-AMPK-K154E, Flag-AMPK-K154R, and KAT2A-Y645A constructs were designed by Genscript Biotechnology Co., Ltd. (Nanjing, China). GMECs (1×10^6 cells/well) were seeded in six-well plates. Transfection was performed using Lipofectamine 3000 transfection reagent (L3000150; Thermo Fisher Scientific, Waltham, MA, United States) after cells reached 80% confluence. Transfection was carried out for 48 hours. Hepatic total cholesterol (TC), biliary bile cholesterol saturation index (CSI), and total bile acids (TBA) were quantified following a previously established protocol[24].

Enzyme-linked immuno sorbent assay

The concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), TC, total triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), tumor necrosis factor (TNF)- α , interleukin (IL)-18, and IL-1 β in mouse serum or GMECs were quantified using commercial enzyme-linked immuno sorbent assay (ELISA) kits (Abcam, Cambridge, MA, United States) according to the manufacturer's instructions. Absorbance values for each well were measured using a microplate reader (Thermo Fisher Scientific). To enable inter-sample comparisons, the results were normalized to the total protein concentration in each sample.

Reverse transcription-quantitative polymerase chain reaction

Total RNA from tissues or GMECs was extracted using TRIzol reagent (R0016; Beyotime). The extracted RNA was reverse-transcribed into cDNA using a reverse transcription kit (BL699A; Biosharp Biotech Co. Ltd., Hefei, China). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis was performed using the BeyoFast™ SYBR Green One-Step RT-qPCR Kit (D7268S; Beyotime) under the manufacturer-specified reaction conditions. Gene

expression levels were quantified using the $2^{-\Delta\Delta CT}$ method. The used primers were synthesized by Tsingke Biotech Co. Ltd., (Beijing, China) and listed below: KAT2A, forward, 5'-GCAAGGCCAATGAAACCTGTA-3' and reverse, 5'-TCCAAGTGGGATACGTGGTCA-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-TGTGGGCATCAATG-GATTGG-3' and reverse, 5'-ACACCATGTATTCCGGGTCAAT-3'.

RNA-seq

Total RNA was extracted from the gallbladder tissues of normal and model group mice using TRIzol reagent (Beyotime) according to the manufacturer's instructions. RNA quality and concentration were evaluated using a NanoDrop spectrophotometer (Thermo Fisher Scientific) and an Agilent 2100 Bioanalyzer (Agilent Technologies, United States). Only RNA samples with an RNA Integrity Number (RIN) > 7.0 were selected for library construction. RNA-seq libraries were prepared using the NEBNext Ultra II RNA Library Prep Kit (New England Biolabs, United States) following the manufacturer's protocol. Briefly, mRNA was enriched *via* poly-A selection, fragmented, and reverse-transcribed into cDNA. The cDNA fragments were end-repaired, adenylated, and ligated with sequencing adapters. Libraries were amplified by PCR and quantified using a Qubit Fluorometer (Thermo Fisher Scientific). Sequencing was performed on the Illumina NovaSeq 6000 platform (Illumina, United States), generating 150-bp paired-end reads. Raw sequencing reads were quality-checked using FastQC and aligned to the mouse reference genome (GRCm38) using STAR. Differential gene expression analysis was conducted using DESeq2, with genes exhibiting a fold change > 2 and an adjusted *P* value < 0.05 designated as significantly differentially expressed. Data visualization was performed using R (version 4.2.0) and the ggplot2 package.

Bioinformatics analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG; <https://www.genome.jp/kegg/>) and Gene Ontology (GO; <https://www.geneontology.org/>) databases were utilized to identify signaling pathways and biological processes enriched in differentially expressed genes (DEGs).

Hematoxylin and eosin staining

Gallbladder specimens were fixed in 4% neutral-buffered formaldehyde overnight. Following fixation, tissues were embedded in paraffin and sectioned into 4-μm-thick slices. The sections were then stained with Hematoxylin and eosin (H&E). After dewaxing, staining, dehydration, and clearing, the slides were mounted and sealed with a coverslip. Histological examination was performed under a light microscope (Leica, Germany).

Western blot

Gallbladder tissues and GMECs were lysed using Radio Immunoprecipitation Assay lysis buffer (P0013B; Beyotime) for 10 minutes. The homogenate was further homogenized on ice for 30 minutes and centrifuged at 12000 rpm for 20 minutes at 4 °C to isolate the supernatant. Protein concentration was quantified using the bicinchoninic acid (BCA) assay kit (P0010S; Beyotime). Equal amounts of protein samples were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride membranes (FFP39; Beyotime). The membranes were blocked with QuickBlock™ blocking buffer (P0252; Beyotime) for 10 minutes at room temperature. Subsequently, the membranes were incubated with primary antibodies [KAT2A (ab217876; Abcam; 1:1000), KAT3B (ab275378; Abcam; 1:1000), carnitine palmitoyltransferase 1A (CPT1A; ab220789; Abcam; 1:1000), sirtuin 5 (SIRT5) (ab259967; Abcam; 1:1000), SIRT7 (ab259968; Abcam; 1:1000), Caspase-1 (ab207802; Abcam; 1:1000), gasdermin D (GSDMD; ab219800; Abcam; 1:2000), GSDMD-N (ab215203; Abcam; 1/1000), phospho-AMPK (p-AMPK) (T172) (ab314032; Abcam; 1:1000), SIRT1 (ab189494; Abcam; 1:1000), AMPK (ab214425; Abcam; 1:1000), succinylation (PTM-401; PTM Biotech Co. Ltd., Hangzhou, China; 1:1000), β-actin (ab8227; Abcam; 1:1000)] overnight at 4 °C. After three washes (10 minutes each) with Tris-buffered saline Tween (TBS-T; pH 7.4; BL315B; Biosharp), the membranes were incubated with a secondary antibody (goat anti-rabbit; ab6721; Abcam; 1:2000) at 37 °C for 2 hours. Protein signals were detected using SuperSignal™ West Pico PLUS enhanced chemiluminescence solution (34580; Thermo Fisher Scientific).

Flow cytometry

Pyroptosis in GMECs was assessed using a flow cytometry assay. Briefly, a commercial FAM-fluorochrome-labeled inhibitors of caspases (FLICA) *in vitro* Caspase Detection Kit (ImmunoChemistry Technologies, LLC, United States) was used following the manufacturer's protocol. Samples were incubated with FLICA and propidium iodide (PI) at 37 °C for 1 hours in the dark. After washing twice with wash buffer, cells were analyzed using a flow cytometer (BD Biosciences, United States).

PI staining

PI staining was performed to analyze the cell death in GMECs cells using a commercial PI kit (P4170, Sigma). First, the 40 μg/mL of PI working solution was prepared. Cells were resuspended in 500 μL of binding buffer at a concentration of 1×10^6 cells/mL. Next, add 100 μL dye working solution to the cells, gently shake the dye to completely cover the cells, and incubate for 15 minutes at 37 °C. Finally, fluorescence microscopy was used to observe, and the number of PI-positive cells (red fluorescence) was counted using the image analysis software (ImageJ).

Phospho-kinase array

A phospho-kinase array was performed to quantify the relative phosphorylation levels of kinase sites. The Human Phospho-Kinase Array Kit was obtained from R&D Systems (Minneapolis, United States). Cells were lysed in lysis buffer

at a concentration of 1×10^7 cells/mL and incubated on a rotating platform at 4 °C for 30 minutes. After centrifugation at $14000 \times g$ for 5 minutes, the total protein concentration in the supernatants was quantified. Cell lysates were mixed with biotinylated detection antibodies and incubated overnight with membranes pre-coated with capture and control antibodies. The membranes were then washed, and streptavidin-horseradish peroxidase conjugate and chemiluminescent detection reagents were applied. A chemiluminescent signal was generated at each capture spot, proportional to the amount of phosphorylated protein bound to the corresponding antibody.

Co-immunoprecipitation and IP

Co-immunoprecipitation (Co-IP) was used to detect the binding between liver kinase B1 (LKB1) and AMPK in GMECs. The procedure was conducted according to a previous study[25]. An IP assay combined with western blot was used to assess the succinylation level of AMPK or LKB1 in GMECs. Briefly, GMEC cell lysates were obtained and immunoprecipitated with anti-AMPK or anti-LKB1 antibodies and protein A/G agarose (Novex, Oslo, Norway), followed by western blot analysis for succinylation.

Site prediction and mutation

Global Prediction of Generic and Species-specific Succinylation Sites (GPSuc; <http://kurata14.bio.kyutech.ac.jp/GPSuc/index.php>) was used for site prediction of AMPK. To determine the specific sites at which AMPK was succinylated, Genescript Co. Ltd. was commissioned to generate site-directed mutagenesis of lysine residues (K179, K170, and K204) to arginine (K179R, K170R, and K204R). These mutated plasmids were then transfected into GMECs.

Determination of protein stability

An evaluation of protein stability was conducted to assess the stability of AMPK following overexpression of KAT2A in GMECs. The GMECs were treated with cycloheximide (CHX; #HY-12320; purity: 99.86%; 100 µg/mL; MedChem Express, Monmouth Junction, NJ, United States) at 0, 3, 6, and 9 hours. Subsequently, the protein concentration of AMPK was analyzed *via* western blot.

Immunofluorescence

Immunofluorescence (IF) assay was performed to determine the distribution of AMPK and LKB1 in GMECs according to a previous study[26]. Antibodies used included anti-AMPK (ab314032; Abcam; 1:200) and anti-LKB1 (PA5-96062; Thermo Fisher; 1:200).

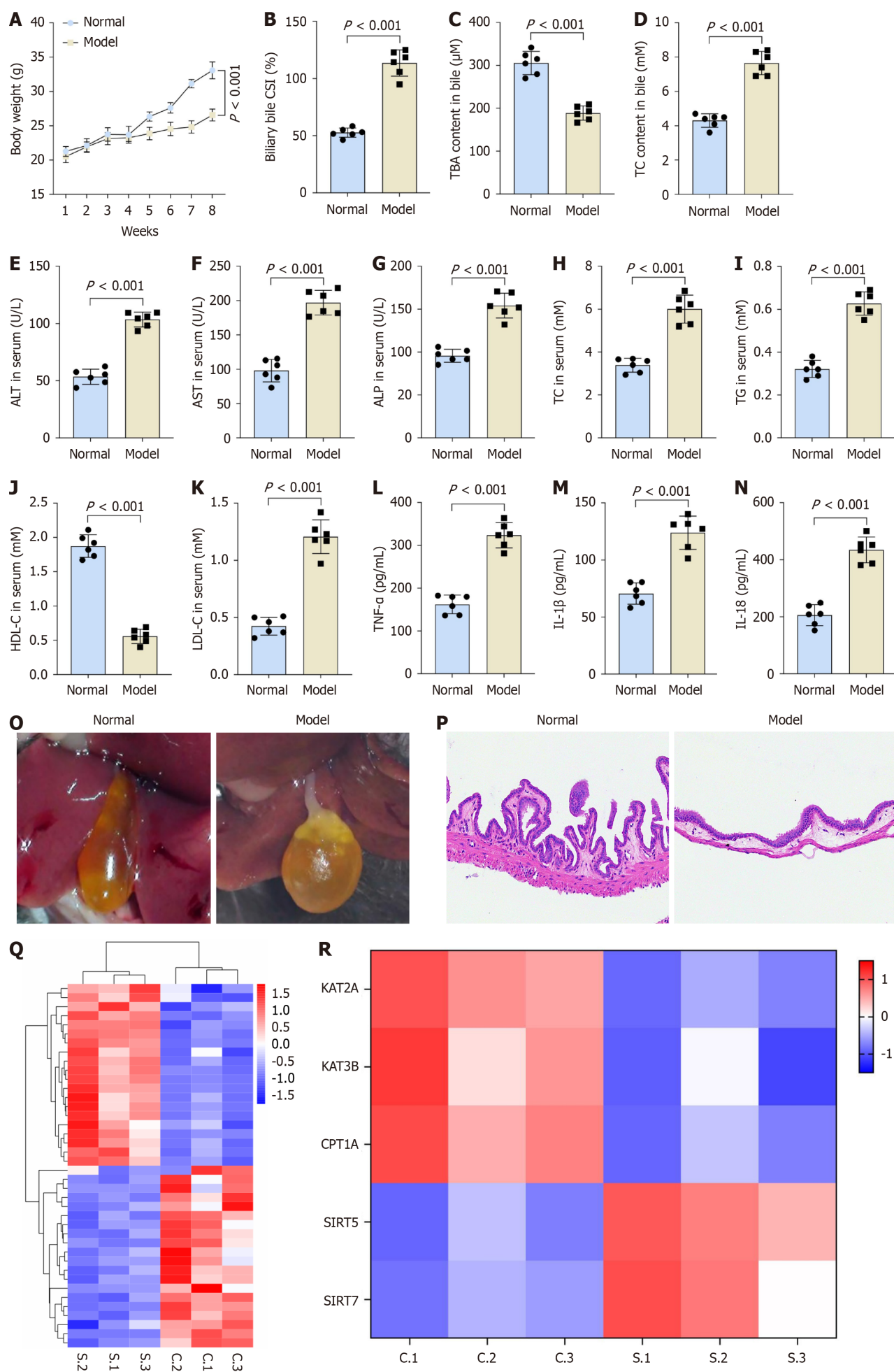
Statistical analysis

SPSS 21.0 software was used to analyze the data. Data are presented as mean $\pm$ SD. Student's *t*-test was employed for pairwise comparisons between two groups, while one-way analysis of variance (ANOVA) was utilized for comparisons among multiple groups with Tukey's *post hoc* analysis. Statistical analyses were performed using GraphPad Prism software (v8.0.1; GraphPad Software Inc., San Diego, CA, United States). A *P* value < 0.05 was considered statistically significant.

RESULTS

KAT2A-mediated succinylation was involved in the gallstone formation

A cholelithiasis mouse model was established in this study. Compared to the normal group, model group mice exhibited decreased body weight (Figure 1A). Furthermore, the model group demonstrated increased biliary bile CSI and TC content, alongside reduced TBA content in bile (Figure 1B-D). ELISA results indicated elevated levels of ALT, AST, ALP, TC, TG, LDH-C, TNF- α , IL-1 β , and IL-18 as well as decreased HDL-C level in the model group mice relative to the normal group (Figure 1E-N). Additionally, gallbladder bile from the model group appeared dark yellow with granular stones, and microscopic examination revealed classical cholesterol crystals (Figure 1O). H&E staining confirmed gallbladder inflammation in the model group, characterized by gallbladder wall thickening, inflammatory cell infiltration in the stromal layer, and submucosal vasodilation, compared to the normal group (Figure 1P). RNA-seq analysis was performed to identify DEGs between the normal and model groups (Figure 1Q). Succinylation has been implicated in the pathogenesis of digestive disorders such as colitis and hepatitis[27,28]. Despite the prevalence of cholelithiasis, no prior studies have reported its association with succinylation. We observed that multiple succinyltransferase enzymes (including KAT2A, KAT3B, CPT1A, SIRT5, and SIRT7) were present in the DEGs between the normal and model groups (Figure 1R). Based on this finding, we hypothesized that succinylation modification might be involved in gallstone formation. Subsequent GO and KEGG enrichment analyses revealed that DEGs included several succinylation-related genes, which were enriched in multiple signaling pathways and metabolic processes, including protein digestion and absorption, protein stability, positive regulation of protein binding, and protein deacetylation (Figure 1S and T). Western blot analysis demonstrated reduced global succinylation levels in the model group compared to the normal group (Figure 1U). To investigate the role of succinylation in cholelithiasis, protein levels of representative succinylases and desuccinylases were analyzed. The results revealed decreased KAT2A expression in gallbladder tissues of model group mice relative to the normal group. Conversely, no significant differences in the protein levels of KAT3B, CPT1A, SIRT5, or SIRT7 were observed between the two groups (Figure 1V and W). These findings demonstrated that KAT2A-mediated succinylation contributes to the regulation of cholelithiasis.



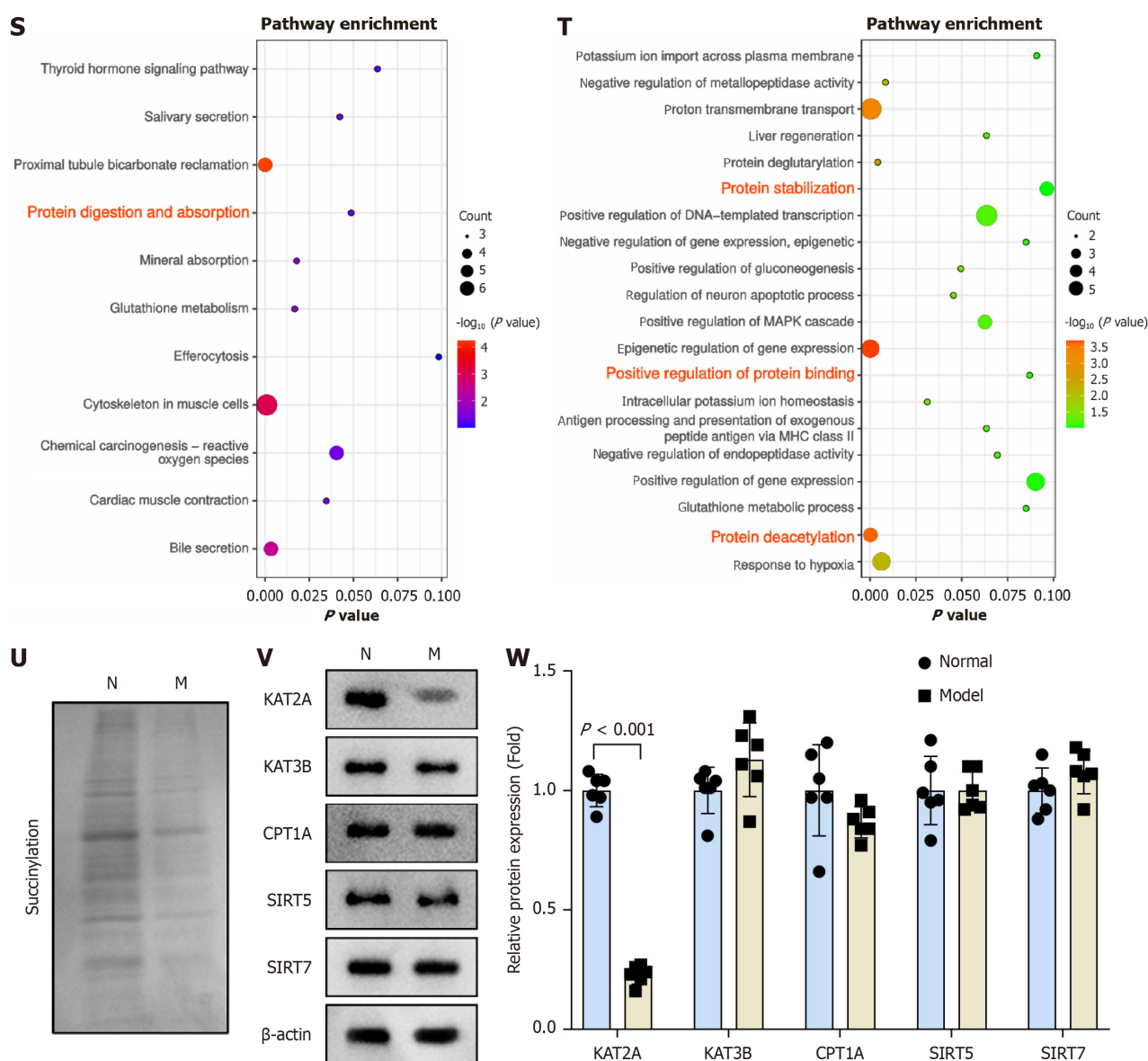


Figure 1 Lysine acetyltransferase 2A-mediated succinylation was involved in the regulation of cholelithiasis. A: Body weight of the normal and the model groups ($n = 6$); B: Biliary bile cholesterol saturation index; C: Total bile acids content; D: Total cholesterol (TC) content in bile were measured ($n = 6$); E: Enzyme-linked immunosorbent assay analysis of the concentrations of alanine aminotransferase; F: Aspartate aminotransferase; G: Alkaline phosphatase; H: TC; I: Total triglycerides; J: High density lipoprotein cholesterol; K: Low density lipoprotein cholesterol; L: Tumor necrosis factor- α ; M: Interleukin (IL)-1 β ; N: IL-18 ($n = 6$); O: Representative image of gallbladder stones of each group mice; P: Histological examination of the gallbladder by hematoxylin and eosin staining; Q: RNA-seq was performed to show differentially expressed genes between the normal and the model groups; R: Succinyltransferase enzymes were present in the differentially expressed genes between the normal and model groups; S: Gene Ontology; T: Kyoto Encyclopedia of Genes and Genomes databases were used to analyze the signaling pathways and metabolic processes enriched by differentially expressed genes; U-W: Western blot was performed to assess the protein levels of succinylation and enzymes associated with succinylation ($n = 6$). KAT2A: Lysine acetyltransferase 2A; KAT3B: Lysine acetyltransferase 3B; CPT1A: Carnitine palmitoyltransferase 1A; SIRT1: Sirtuin 1.

KAT2A overexpression suppressed pyroptosis and inflammation in GMECs

To further investigate the role of KAT2A in cholelithiasis, GMECs were isolated from gallbladder tissues for *in vitro* experiments. Following transfection of the KAT2A overexpression vector into GMECs, mRNA expression of KAT2A was significantly upregulated (Figure 2A). Additionally, treatment with LPS increased pyroptosis rate, PI positive cells, and inflammatory markers (TNF- α , IL-1 β , and IL-18) compared to the control group. Furthermore, KAT2A overexpression reduced pyroptosis rate, PI positive cells, and pro-inflammatory markers (TNF- α , IL-1 β , and IL-18) in LPS-treated GMECs (Figure 2B-H). Caspase-1 and GSDMD-N are representative pyroptosis-related proteins. After LPS treatment, protein levels of caspase-1 and GSDMD-N were elevated compared to the control group; however, KAT2A overexpression reversed these increases in the LPS + vector group (Figure 2I and J).

KAT2A overexpression activated the AMPK/SIRT1 signaling pathway in GMECs

Phospho-kinase array results showed that overexpression of KAT2A did not significantly affect most kinases on the chip, except for the specific upregulation of AMPK alpha2 (T172) phosphorylation (Figure 3A). Consequently, we further

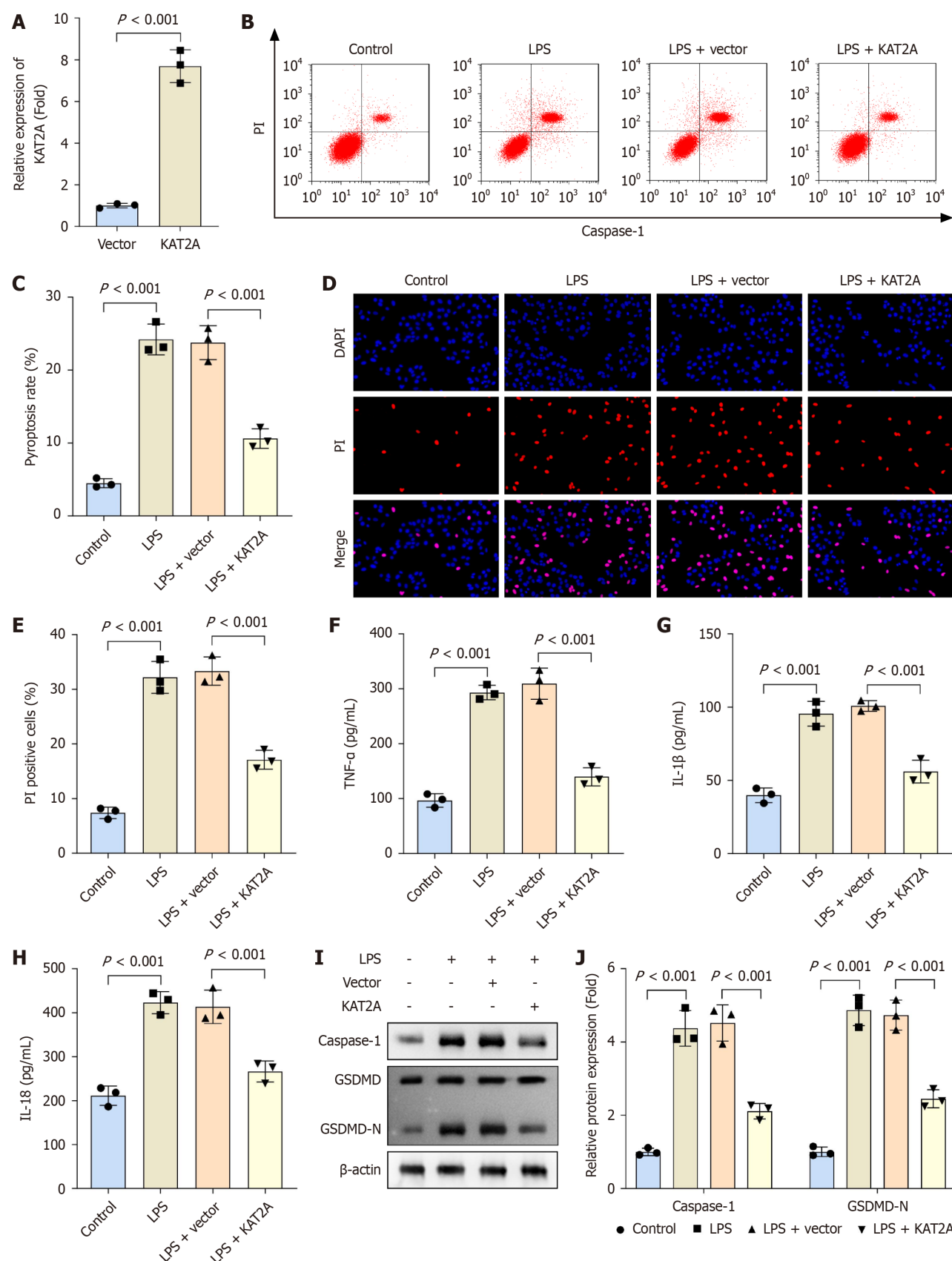


Figure 2 Lysine acetyltransferase 2A overexpression suppressed pyroptosis and inflammation in gallbladder mucosal epithelial cells. **A:** The mRNA expression of lysine acetyltransferase 2A (KAT2A) was analyzed by reverse transcription-quantitative polymerase chain reaction when KAT2A was overexpressed in gallbladder mucosal epithelial cells (GMECs) ($n = 3$); **B** and **C:** Flow cytometry was used to detect the pyroptosis rate in GMECs in each group ($n = 3$); **D** and **E:** Propidium iodide staining was used to analyze cell death in each group ($n = 3$); **F:** Enzyme-linked immunosorbent assay was performed to determine the concentrations of tumor necrosis factor- α ; **G:** Interleukin (IL)-1 β ; **H:** IL-18 in each group ($n = 3$); **I** and **J:** Western blot was performed to assess the protein levels of caspase-1 and gasdermin D-N in each group ($n = 3$). LPS: Lipopolysaccharide.

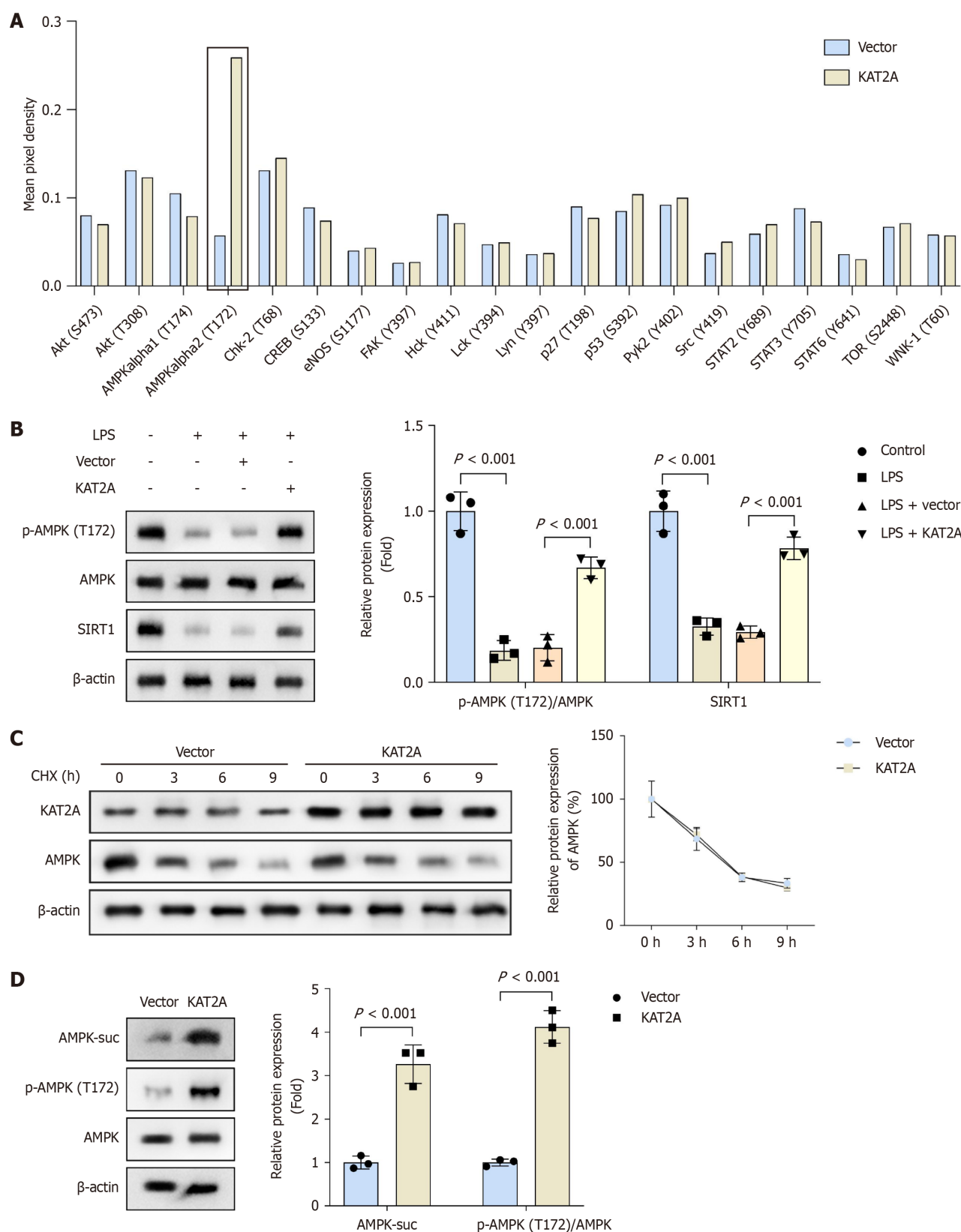


Figure 3 Lysine acetyltransferase 2A overexpression activated the adenosine monophosphate-activated protein kinase/silent information regulator 1 signaling pathway in gallbladder mucosal epithelial cells. A: The screening results of changes in phospho-protein activation in vector and lysine acetyltransferase 2A overexpression groups were obtained using the proteome profiler phospho-kinase array ($n = 3$); B: The protein levels of phospho-adenosine monophosphate-activated protein kinase (T172), adenosine monophosphate-activated protein kinase, and sirtuin 1 in each group were measured by western blot ($n = 3$); C: The protein stability of adenosine monophosphate-activated protein kinase in each group after KAT2A overexpression were measured by western blot ($n = 3$); D: The protein levels of phospho-adenosine monophosphate-activated protein kinase (T172), adenosine monophosphate-activated protein kinase, and adenosine monophosphate-activated protein kinase succinylation in each group were measured by western blot ($n = 3$). GMECs: Gallbladder mucosal epithelial cells; LPS: Lipopolysaccharide; CHX: Cycloheximide.

analyzed the protein levels of phosphorylated AMPK (p-AMPK), total AMPK, and SIRT1. Our findings revealed that LPS treatment reduced the protein levels of p-AMPK/AMPK and SIRT1 compared to the control group. Additionally, compared to the vector group, KAT2A overexpression increased the protein levels of p-AMPK/AMPK and SIRT1 (Figure 3B). Furthermore, protein stability assay results indicated that KAT2A overexpression did not alter the total AMPK protein level (Figure 3C). Notably, following KAT2A overexpression, the protein levels of succinylated AMPK (AMPK-suc) and p-AMPK/AMPK were upregulated (Figure 3D). These results suggested that KAT2A overexpression activates the AMPK/SIRT1 signaling pathway.

AMPK was succinylated at K170 site in GMECs

Three lysine residues (K78, K154, and K141) were predicted by GPSuc to be potential succinylation sites (Figure 4A). To determine the specific site(s) at which AMPK is succinylated, plasmids encoding the K78R, K154R, and K141R mutations were transfected into GMECs. Western blot analysis revealed that transfection with K154R significantly reduced the protein levels of AMPK-suc and p-AMPK/AMPK compared to K141R and K78R transfections, indicating that K154 is the critical succinylation site (Figure 4B). Notably, K154 is highly conserved across multiple species, including human, mouse, rat, pig, and *Pongo abelii* (Figure 4C). To further investigate the functional role of K154 succinylation, mutant constructs encoding AMPK-K154R (a non-succinylatable variant) and AMPK-K154E (a mimic of AMPK-suc) were generated. The K154R mutation blocks succinylation, while the K154E mutation enhances it. Results showed that FLAG-tagged K154E significantly upregulated AMPK-suc and p-AMPK/AMPK levels compared to FLAG-tagged WT AMPK, whereas FLAG-tagged K154R downregulated these levels (Figure 4D). To validate the role of KAT2A in AMPK succinylation, a KAT2A-Y645A mutant plasmid (which disrupts KAT2A's succinylase activity) was constructed. Compared to the KAT2A-WT group, the KAT2A-Y645A group exhibited reduced AMPK-suc and p-AMPK/AMPK levels in GMECs (Figure 4E). As LKB1 encodes a serine/threonine kinase that directly phosphorylates and activates AMPK, functioning as a key metabolic sensor[29], we hypothesized that KAT2A might regulate AMPK phosphorylation *via* LKB1. Western blot analysis demonstrated that neither overexpression nor silencing of KAT2A altered the expression levels of LKB1-suc or total LKB1 in GMECs (Figure 4F). However, Co-IP experiments revealed that AMPK mutation suppressed the interaction between LKB1 and AMPK; moreover, when KAT2A was overexpressed along with the AMPK site mutation, the binding was also attenuated (Figure 4G). Furthermore, IF imaging confirmed that KAT2A overexpression promoted LKB1-AMPK binding in GMECs (Figure 4H). These findings demonstrated that KAT2A promotes AMPK succinylation, thereby strengthening the LKB1-AMPK interaction and ultimately enhancing AMPK phosphorylation.

DOR treatment increased pyroptosis and inflammation in GMECs

To inhibit AMPK expression, GMECs were treated with DOR. Western blot results showed that after DOR treatment, the relative protein level of p-AMPK (T172)/AMPK was decreased (Figure 5A and B). In addition, DOR group exhibited increased pyroptosis rates, PI positive cells, pro-inflammatory markers (TNF- α , IL-1 β , and IL-18), and elevated protein levels of caspase-1 and GSDMD-N compared to the LPS + KAT2A group (Figure 5C-K).

KAT2A overexpression inhibited inflammation and gallstone formation

In the *in vivo* study, overexpression of KAT2A resulted in increased body weight compared to the Lv-NC group (Figure 6A). Furthermore, the KAT2A overexpression group exhibited decreased biliary bile CSI and TC content, alongside increased TBA content in bile compared to the Lv-NC group (Figure 6B-D). Additionally, ELISA results revealed reduced levels of ALT, AST, ALP, TC, TG, LDL-C, TNF- α , IL-1 β , and IL-18 and increased HDL-C level in the Lv-KAT2A group mice compared to the Lv-NC group (Figure 6E-N). Moreover, gallstone formation was alleviated in the Lv-KAT2A group compared to the Lv-NC group (Figure 6O). H&E staining demonstrated reduced gallbladder inflammation in the Lv-KAT2A group, characterized by lessened wall thickening, inflammatory cell infiltration, and submucosal vasodilation, compared to the Lv-NC group (Figure 6P).

The molecular mechanism diagram

Down-regulation of KAT2A reduces AMPK succinylation. Reduced AMPK succinylation inhibits the interaction between AMPK and LKB1, thereby suppressing AMPK phosphorylation. Inhibition of AMPK phosphorylation promotes pyroptosis and inflammation in GMECs, ultimately leading to gallstone formation (Figure 7).

DISCUSSION

Cholelithiasis is primarily categorized into two types: Gallbladder stones and bile duct stones. Stones in the gallbladder or bile duct stimulate the mucosa, causing inflammatory reactions and potentially leading to secondary infections. These conditions significantly impact patients' quality of life and health, often resulting in severe abdominal pain[22]. Consequently, patients with cholelithiasis frequently exhibit elevated inflammatory markers. In our study, we established a cholelithiasis mouse model by feeding mice a HFD and observed that the model group exhibited increased inflammation and gallstone formation in gallbladder tissues. Similar findings have been reported in studies showing elevated inflammation and apoptosis in patients with cholelithiasis, acute cholecystitis, or gallbladder adenomyomatosis[30-32]. Furthermore, chronic, unresolved inflammation is a primary carcinogenic mechanism in gallbladder cancer[30]. Therefore, controlling inflammation is a critical therapeutic strategy for managing cholelithiasis.

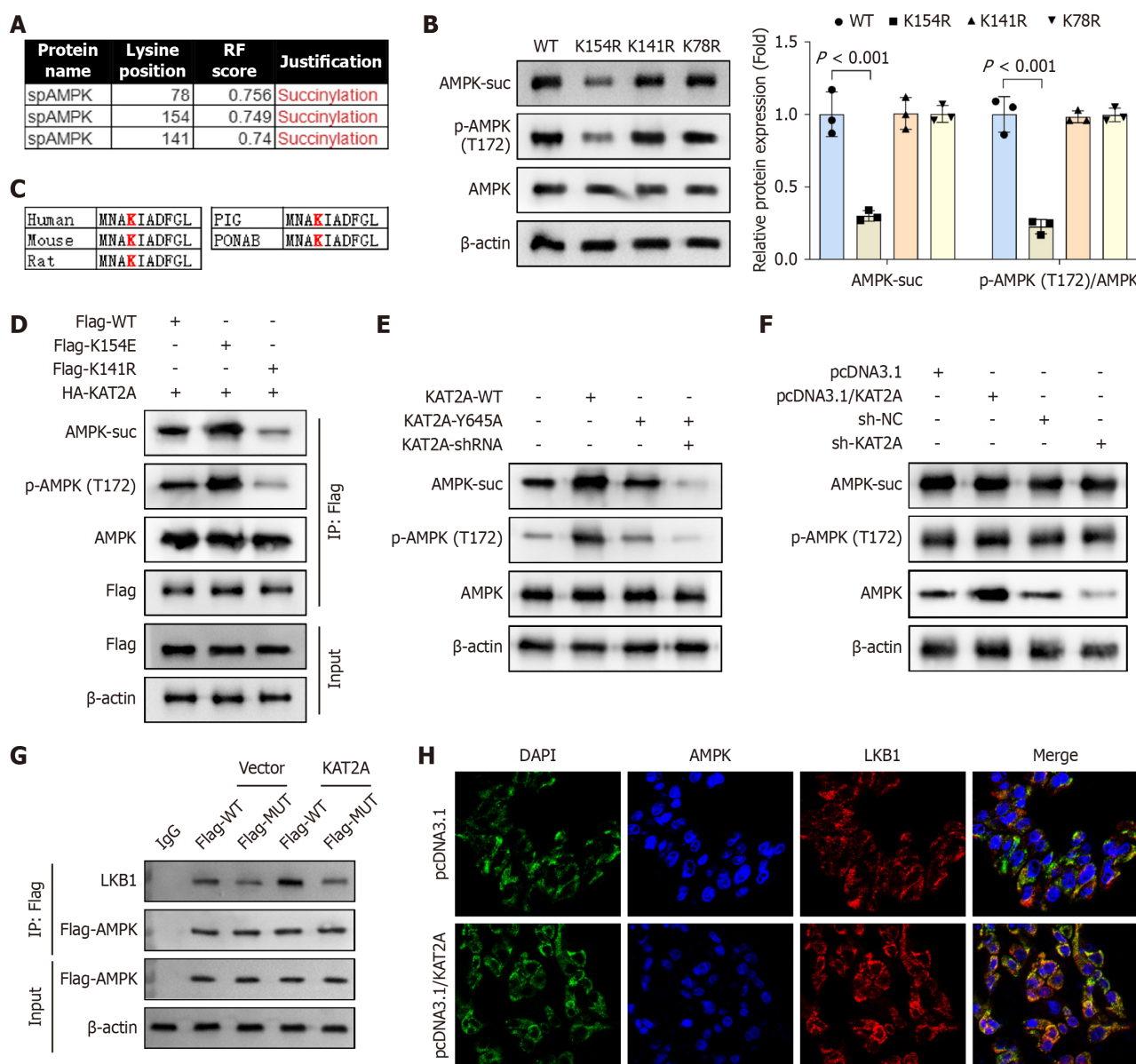
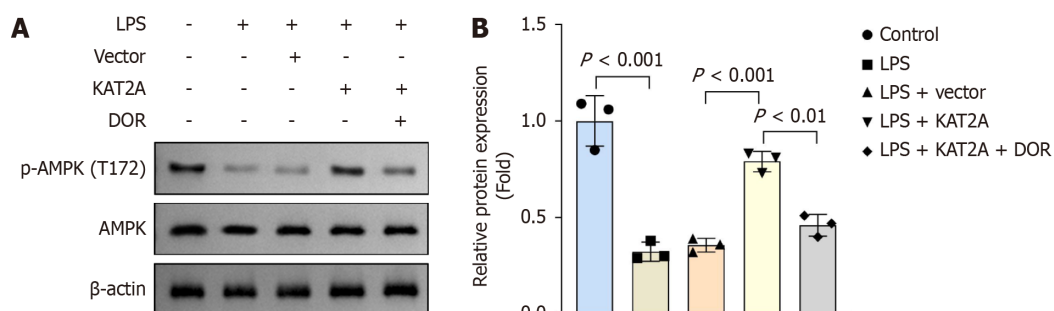


Figure 4 Adenosine monophosphate-activated protein kinase was succinylated at K170 site in gallbladder mucosal epithelial cells. **A:** The protein levels of adenosine monophosphate-activated protein kinase (AMPK) succinylation (AMPK-suc), phospho-AMPK (p-AMPK), and AMPK after lysine acetyltransferase 2A (KAT2A) overexpression were detected by immunoprecipitation (IP) and western blot assays ($n = 3$); **B:** After mutating K179, K170, and K204 to K179R, K170R, and K204R, IP and western blot were performed to analyze the protein levels of AMPK-suc, p-AMPK, and AMPK ($n = 3$); **C:** K154 was highly conserved in different species, including human, mouse, rat, pig, and pongo abelii; **D** and **E:** Gallbladder mucosal epithelial cells (GMECs) were transfected with flag-WT, flag-K154E, flag-K154R, HA-KAT2A, KAT2A-WT, KAT2A-Y645A, and KAT2A-shRNA plasmids, and the protein levels of AMPK-suc and p-AMPK/AMPK was analyzed by IP and western blot ($n = 3$); **F:** After transfecting pcDNA3.1, pcDNA3.1/KAT2A, sh-KAT2A, and sh-NC plasmids, the protein levels of liver kinase B1 (LKB1)-suc, LKB1, and KAT2A were analyzed by IP and western blot ($n = 3$); **G:** Co-immunoprecipitation (Co-IP) was performed to detect the interaction between LKB1 and AMPK in GMECs ($n = 3$); **H:** Immunofluorescence (IF) was performed to analyze the binding of LKB1 and AMPK in GMECs ($n = 3$). DAPI: 4',6-diamidino-2-phenylindole.



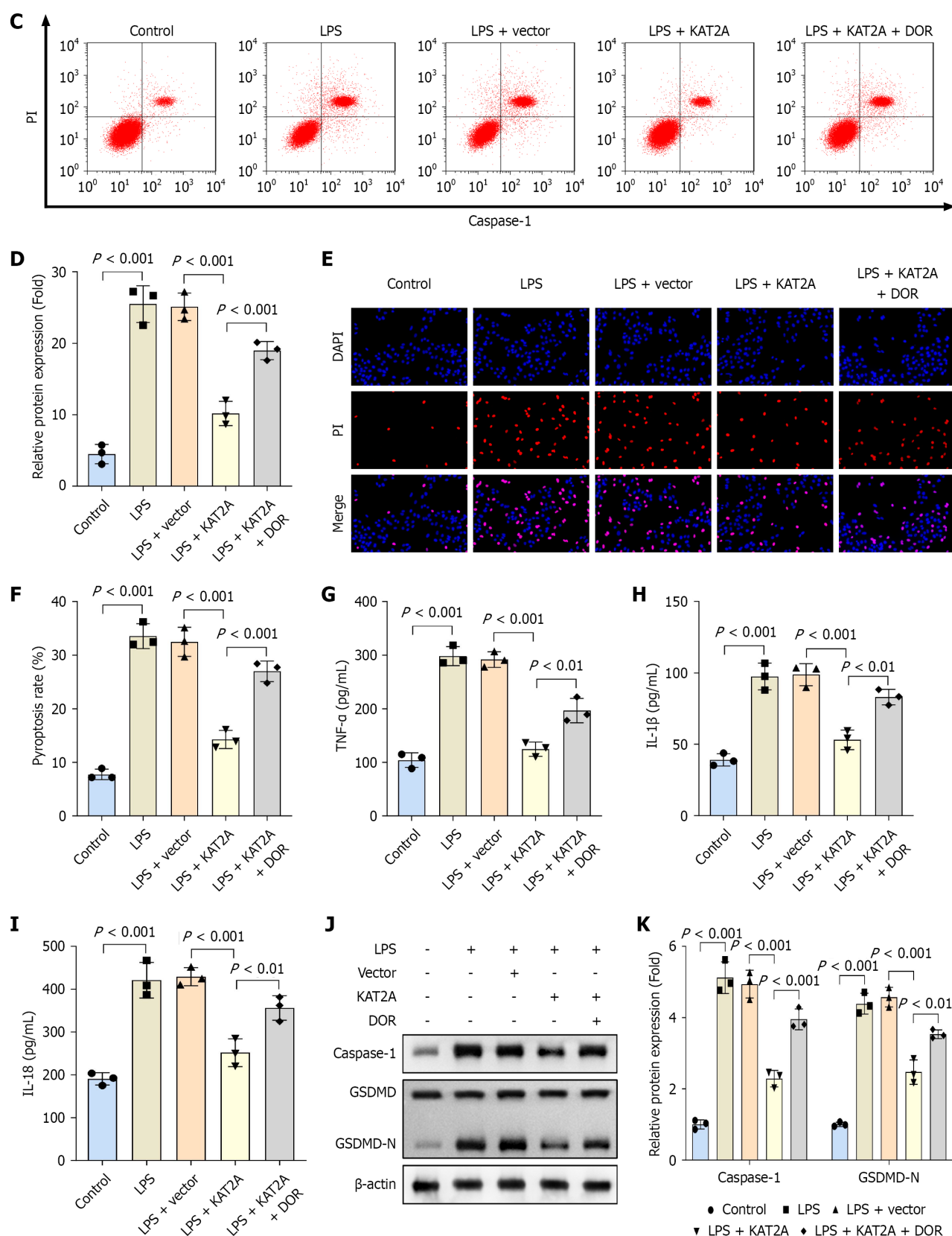
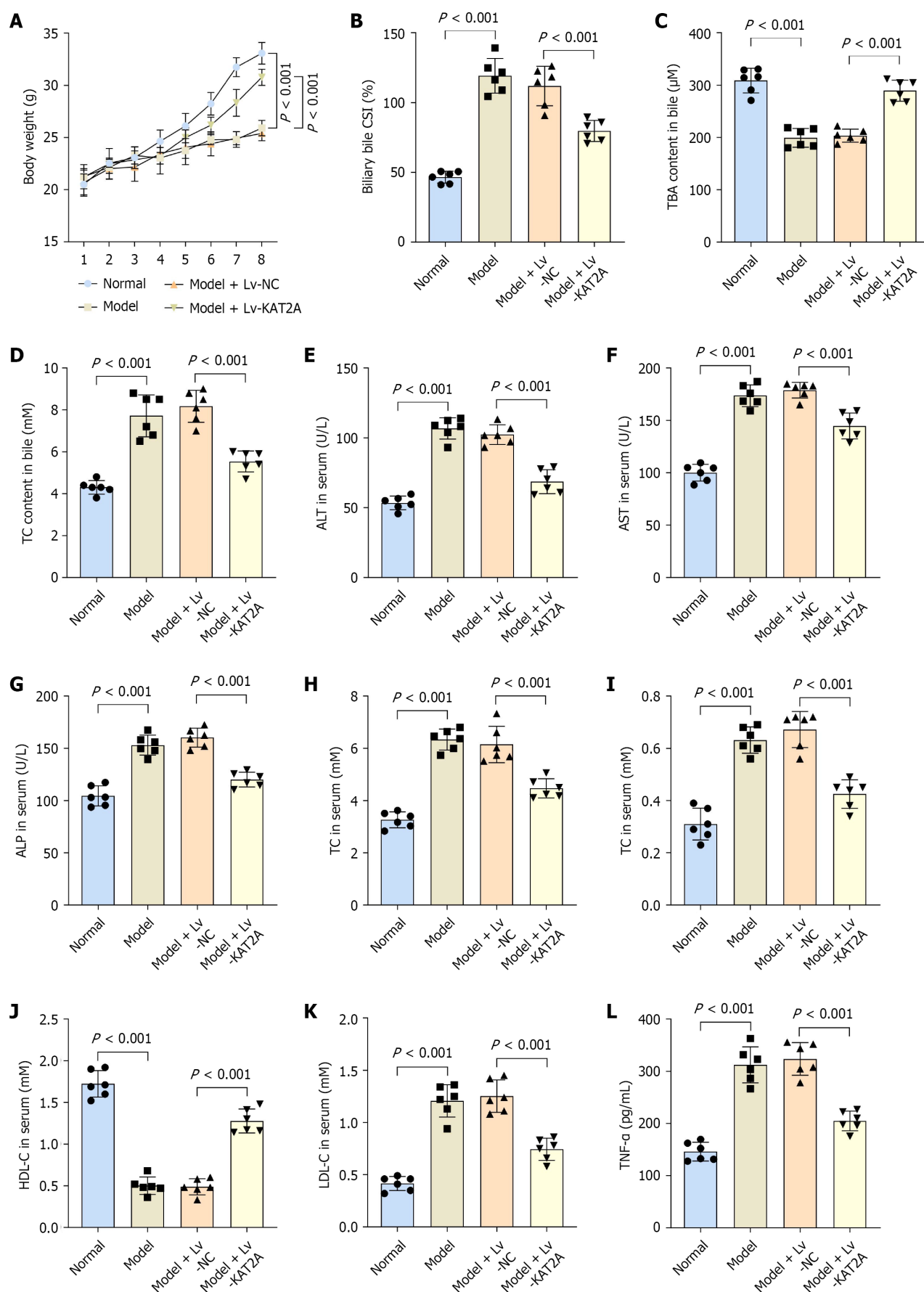


Figure 5 Dorsomorphin treatment increased pyroptosis and inflammation in gallbladder mucosal epithelial cells. A: Western blot was performed to analyze the protein level of phospho-adenosine monophosphate-activated protein kinase (AMPK) (p-AMPK) (T172)/AMPK in gallbladder mucosal epithelial cells (GMECs) ($n = 3$); B: Quantification of relative protein level of p-AMPK (T172)/AMPK in each group ($n = 3$); C and D: Flow cytometry was performed to assess the pyroptosis rate in GMECs in each group ($n = 3$); E and F: Propidium iodide staining was used to analyze cell death in each group ($n = 3$); G: Enzyme-linked immunosorbent assay was used to measure the concentrations of tumor necrosis factor- α ; H: Interleukin (IL)-1 β ; I: IL-18 in each group ($n = 3$); J and K: Western blot was performed to assess the protein levels of caspase-1 and gasdermin D-N in each group ($n = 3$). LPS: Lipopolysaccharide; DOR: Dorsomorphin.



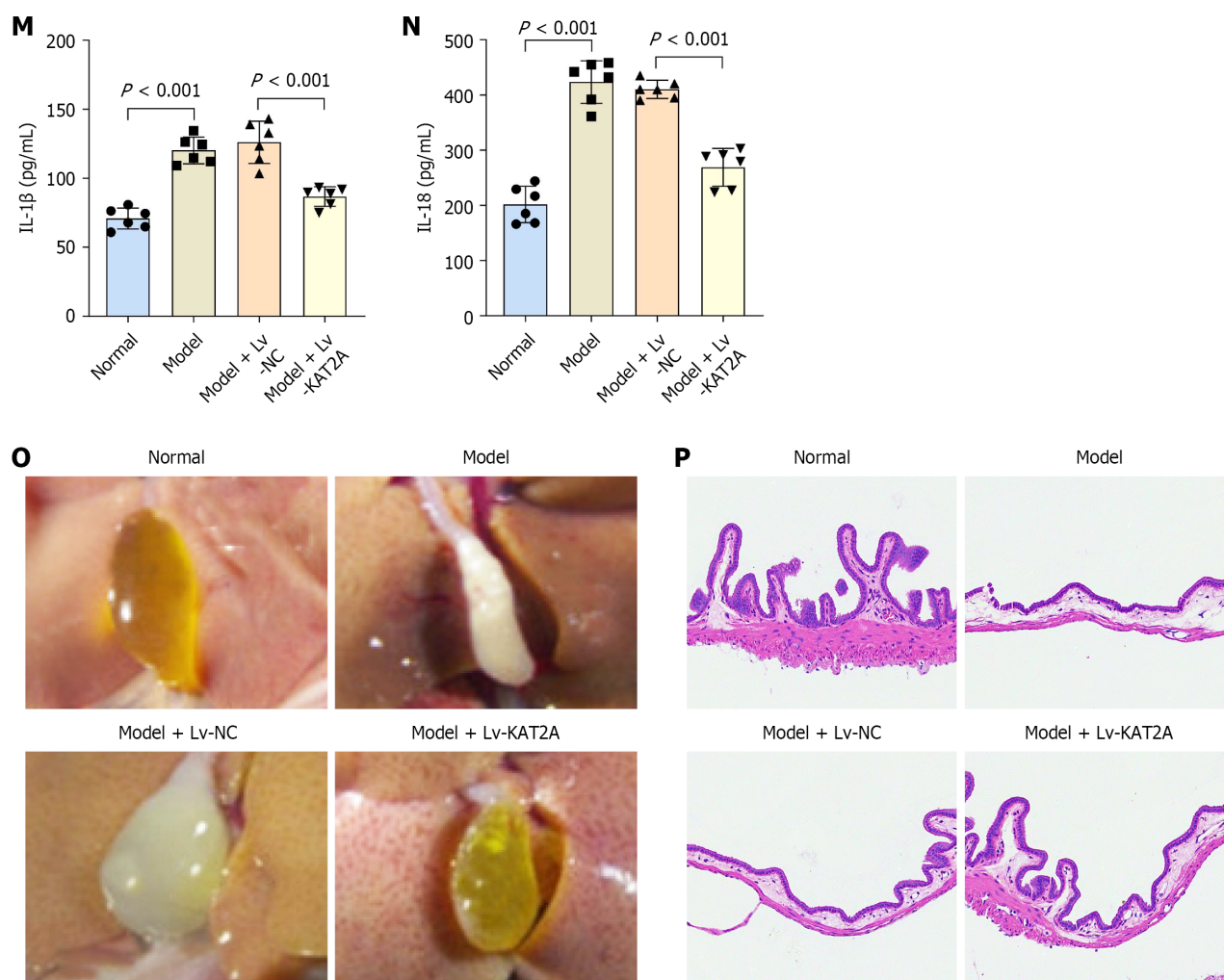


Figure 6 Lysine acetyltransferase 2A overexpression inhibited the apoptosis and the inflammation in gallbladder mucosal epithelial cells.

A: Body weight of each group ($n = 6$); B: Biliary bile cholesterol saturation index; C: Total bile acids content; D: Total cholesterol (TC) content in bile of each group were measured ($n = 6$); E: Enzyme-linked immunosorbent assay analysis of the concentrations alanine aminotransferase; F: Aspartate aminotransferase; G: Alkaline phosphatase; H: TC; I: Total triglycerides; J: High density lipoprotein cholesterol; K: Low density lipoprotein cholesterol; L: Tumor necrosis factor- α ; M: Interleukin (IL)-1 β ; N: IL-18 ($n = 6$); O: Representative image of gallbladder stones of each group mice; P: Histological examination of each group gallbladder by hematoxylin and eosin staining. KAT2A: Lysine acetyltransferase 2A.

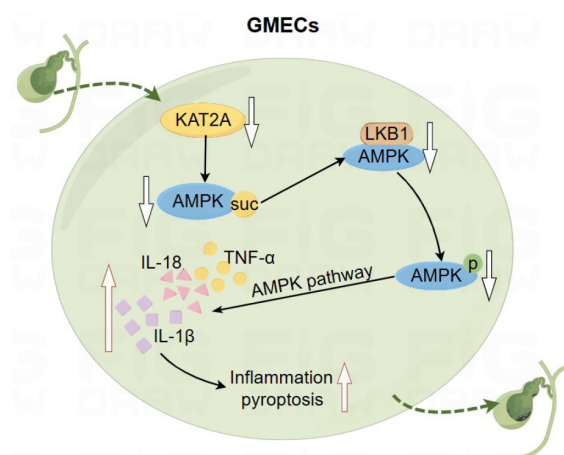


Figure 7 The molecular mechanism diagram. Down-regulation of lysine acetyltransferase 2A reduces adenosine monophosphate-activated protein kinase (AMPK) succinylation. Reduced AMPK succinylation inhibits the interaction between AMPK and liver kinase B1, thereby suppressing AMPK phosphorylation. Inhibition of AMPK phosphorylation promotes pyroptosis and inflammation in gallbladder mucosal epithelial cells, ultimately leading to gallstone formation. TNF- α : Tumor necrosis factor; IL: Interleukin; suc: Succinylation; GMECs: Gallbladder mucosal epithelial cells.

Succinylation has been implicated in various digestive tract tumors and non-neoplastic diseases. For instance, Zhang *et al*[12] indicate that KAT2A enhances the succinylation of pyruvate kinase M2 (PKM2), thereby suppressing its enzymatic activity and promoting glycolysis in gastric cancer cells. However, its role in cholelithiasis remains unexplored. Our RNA-seq analysis revealed that DEGs between the model and control groups encompass multiple succinyltransferases. KAT2A is a pivotal enzyme that mediates lysine succinylation, a PTM with profound implications for metabolic homeostasis. Emerging evidence underscores its functional significance in the pathogenesis of gastrointestinal disorders [12,13]. In addition, we observed, for the first time, the suppression of KAT2A-mediated succinylation in gallbladder tissues of the model mice. Furthermore, *in vitro* experiments demonstrated that KAT2A overexpression significantly inhibited pyroptosis and inflammation in GMECs. Our findings suggested that KAT2A exerts a protective effect by suppressing pyroptotic and inflammatory damage in GMECs, highlighting its potential as a therapeutic target for cholelithiasis treatment. Pyroptosis is an inflammatory form of programmed cell death mediated by the gasdermin (GSDM) protein family[33]. In the canonical pathway, caspase-1 cleaves GSDMD into N- and C-terminal fragments, which drives membrane pore formation and subsequent cell lysis[34]. Although KAT2A's inflammatory regulation in cholelithiasis remains unreported, its inflammatory regulation effects are documented in other digestive system diseases. For instance, previous research indicates that KAT2A participates in *Enterotoxigenic Bacteroides fragilis*-induced intestinal inflammation and colorectal carcinogenesis[35]. The regulation of cell pyroptosis by KAT2A has only been discovered in one study on neuropathic pain, which demonstrated that KAT2A promotes microglial pyroptosis in chemotherapy-induced neuropathic pain by mediating p38 pathway activation and succinylation at the K295 site[36]. Moreover, previous research has highlighted the involvement of KAT2A in regulating other forms of cell death, such as autophagy and apoptosis[37,38].

The AMPK/SIRT1 signaling pathway is widely involved in the progression of gastrointestinal diseases, such as colon neoplasia[39]. In this study, we observed that KAT2A overexpression enhanced AMPK/SIRT1 signaling activation in GMECs. Furthermore, KAT2A overexpression increased the succinylation level of AMPK at the K170 site, suggesting that KAT2A-mediated AMPK succinylation may inhibit cholelithiasis progression through modulation of the AMPK/SIRT1 pathway. Subsequent functional rescue experiments revealed that DOR treatment elevated pyroptosis rates, inflammatory responses, and the protein levels of caspase-1 and GSDMD-N, which corroborated these findings. Similarly, a previous study demonstrates that activation of the AMPK/SIRT1 signaling pathway inhibits gallbladder damage and gallstone formation[22]. Additionally, SIRT1 regulates the phosphorylation level of AMPK[18]. The role of KAT2A in AMPK/SIRT1 signaling has not been discovered before. However, other succinyltransferases have been found to regulate the activation of the AMPK signaling pathway. Zhang *et al*[40] reveal that SIRT5 knockout protects against transverse aortic constriction-induced cardiac remodeling by modulating cardiac energy metabolism and enhancing AMPK signaling.

The current study has several limitations: (1) It lacks validation using samples from patients with cholelithiasis, which restricts the direct translation of our findings to the clinical setting of human cholelithiasis; (2) This study mainly focuses on KAT2A-mediated succinylation. It is not yet clear whether there are other PTMs in cholelithiasis; and (3) Although we observed that KAT2A overexpression influences the AMPK-LKB1 interaction, we did not investigate the precise conformational mechanisms by which succinylation drives this change. We aim to address these limitations in our future research.

CONCLUSION

This study first demonstrates that KAT2A-mediated succinylation of AMPK inhibits cholelithiasis progression by modulating the AMPK/SIRT1 signaling pathway, which might offer novel insights into the molecular mechanisms underlying cholelithiasis.

FOOTNOTES

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; Wang XX and Ma MZ drafted the work and revised it critically for important intellectual content; Zhu LC, Dai LF, Qin C, Shao S, Xu XW and Gao RX were responsible for the acquisition, analysis and interpretation of data for the work; Wang XX, Ma MZ and Zhang ZH made substantial contributions to the conception or design of the work; All authors read and approved the final manuscript.

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Country of origin: China

ORCID number: Zhen-Hai Zhang [0009-0004-4699-882X](https://orcid.org/0009-0004-4699-882X).

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REFERENCES

- 1 Wilkins T, Agabin E, Varghese J, Talukder A. Gallbladder Dysfunction: Cholecystitis, Choledocholithiasis, Cholangitis, and Biliary Dyskinesia. *Prim Care* 2017; **44**: 575-597 [RCA] [PMID: 29132521 DOI: 10.1016/j.pop.2017.07.002] [FullText]
- 2 E S, Srikanth MS, Shreyas A, Desai S, Mehdi S, Gangadharappa HV, Suman, Krishna KL. Recent advances, novel targets and treatments for cholelithiasis; a narrative review. *Eur J Pharmacol* 2021; **908**: 174376 [RCA] [PMID: 34303667 DOI: 10.1016/j.ejphar.2021.174376] [FullText]
- 3 Dan WY, Yang YS, Peng LH, Sun G, Wang ZK. Gastrointestinal microbiome and cholelithiasis: Current status and perspectives. *World J Gastroenterol* 2023; **29**: 1589-1601 [RCA] [PMID: 36970590 DOI: 10.3748/wjg.v29.i10.1589] [FullText] [Full Text(PDF)]
- 4 Pak M, Lindseth G. Risk Factors for Cholelithiasis. *Gastroenterol Nurs* 2016; **39**: 297-309 [RCA] [PMID: 27467059 DOI: 10.1097/SGA.0000000000000235] [FullText]
- 5 Dutt MK, Murphy GM, Thompson RP. Unconjugated bilirubin in human bile: the nucleating factor in cholesterol cholelithiasis? *J Clin Pathol* 2003; **56**: 596-598 [RCA] [PMID: 12890809 DOI: 10.1136/jcp.56.8.596] [FullText]
- 6 Hyogo H, Tazuma S, Cohen DE. Cholesterol gallstones. *Curr Opin Gastroenterol* 2002; **18**: 366-371 [RCA] [PMID: 17033308 DOI: 10.1097/00001574-200205000-00011] [FullText]
- 7 Han ZJ, Feng YH, Gu BH, Li YM, Chen H. The post-translational modification, SUMOylation, and cancer (Review). *Int J Oncol* 2018; **52**: 1081-1094 [RCA] [PMID: 29484374 DOI: 10.3892/ijo.2018.4280] [FullText] [Full Text(PDF)]
- 8 Zhao G, Zhen J, Liu X, Guo J, Li D, Xie J, Xie L. Protein post-translational modification by lysine succinylation: Biochemistry, biological implications, and therapeutic opportunities. *Genes Dis* 2023; **10**: 1242-1262 [RCA] [PMID: 37397549 DOI: 10.1016/j.gendis.2022.03.009] [FullText] [Full Text(PDF)]
- 9 Wang Y, Guo YR, Liu K, Yin Z, Liu R, Xia Y, Tan L, Yang P, Lee JH, Li XJ, Hawke D, Zheng Y, Qian X, Lyu J, He J, Xing D, Tao YJ, Lu Z. KAT2A coupled with the α -KGDH complex acts as a histone H3 succinyltransferase. *Nature* 2017; **552**: 273-277 [RCA] [PMID: 29211711 DOI: 10.1038/nature25003] [FullText]
- 10 Ye L, Yu Z, He L, Yuan J, Zhang X, Li L, Huang X, Ma Y, Zhang L. KAT2A-mediated succinylation modification of notch1 promotes the proliferation and differentiation of dental pulp stem cells by activating notch pathway. *BMC Oral Health* 2024; **24**: 407 [RCA] [PMID: 38556862 DOI: 10.1186/s12903-024-03951-1] [FullText]
- 11 Zeng K, Yin H. KAT2A changes the function of endometrial stromal cells via regulating the succinylation of ENO1. *Open Life Sci* 2024; **19**: 20220785 [RCA] [PMID: 38585644 DOI: 10.1515/biol-2022-0785] [FullText]
- 12 Zhang C, Huang Z. KAT2A Promotes the Succinylation of PKM2 to Inhibit its Activity and Accelerate Glycolysis of Gastric Cancer. *Mol Biotechnol* 2024; **66**: 1446-1457 [RCA] [PMID: 37294531 DOI: 10.1007/s12033-023-00778-z] [FullText]
- 13 Tong Y, Guo D, Yan D, Ma C, Shao F, Wang Y, Luo S, Lin L, Tao J, Jiang Y, Lu Z, Xing D. KAT2A succinyltransferase activity-mediated 14-3-3 ζ upregulation promotes β -catenin stabilization-dependent glycolysis and proliferation of pancreatic carcinoma cells. *Cancer Lett* 2020; **469**: 1-10 [RCA] [PMID: 31610265 DOI: 10.1016/j.canlet.2019.09.015] [FullText]
- 14 Zhu Y, Lu F. Astragaloside IV inhibits cell viability and glycolysis of hepatocellular carcinoma by regulating KAT2A-mediated succinylation of PGAM1. *BMC Cancer* 2024; **24**: 682 [RCA] [PMID: 38835015 DOI: 10.1186/s12885-024-12438-9] [FullText]
- 15 Herzig S, Shaw RJ. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol* 2018; **19**: 121-135 [RCA] [PMID: 28974774 DOI: 10.1038/nrm.2017.95] [FullText]
- 16 Yang Y, Liu Y, Wang Y, Chao Y, Zhang J, Jia Y, Tie J, Hu D. Regulation of SIRT1 and Its Roles in Inflammation. *Front Immunol* 2022; **13**: 831168 [RCA] [PMID: 35359990 DOI: 10.3389/fimmu.2022.831168] [FullText] [Full Text(PDF)]
- 17 Lu C, Zhao H, Liu Y, Yang Z, Yao H, Liu T, Gou T, Wang L, Zhang J, Tian Y, Yang Y, Zhang H. Novel Role of the SIRT1 in Endocrine and Metabolic Diseases. *Int J Biol Sci* 2023; **19**: 484-501 [RCA] [PMID: 36632457 DOI: 10.7150/ijbs.78654] [FullText] [Full Text(PDF)]
- 18 Liou CJ, Lee YK, Ting NC, Chen YL, Shen SC, Wu SJ, Huang WC. Protective Effects of Licochalcone A Ameliorates Obesity and Non-Alcoholic Fatty Liver Disease Via Promotion of the Sirt-1/AMPK Pathway in Mice Fed a High-Fat Diet. *Cells* 2019; **8**: 447 [RCA] [PMID: 31083505 DOI: 10.3390/cells8050447] [FullText] [Full Text(PDF)]
- 19 Ren FF, Xie ZY, Jiang YN, Guan X, Chen QY, Lai TF, Li L. Dapagliflozin attenuates pressure overload-induced myocardial remodeling in mice via activating SIRT1 and inhibiting endoplasmic reticulum stress. *Acta Pharmacol Sin* 2022; **43**: 1721-1732 [RCA] [PMID: 34853445 DOI: 10.1038/s41401-021-00805-2] [FullText]
- 20 Yang M, Lv H, Liu Q, Zhang L, Zhang R, Huang X, Wang X, Han B, Hou S, Liu D, Wang G, Hou J, Yu B. Colchicine Alleviates Cholesterol Crystal-Induced Endothelial Cell Pyroptosis through Activating AMPK/SIRT1 Pathway. *Oxid Med Cell Longev* 2020; **2020**: 9173530 [RCA] [PMID: 32733639 DOI: 10.1155/2020/9173530] [FullText] [Full Text(PDF)]

- 21 Li F, Chen Y, Li Y, Huang M, Zhao W. Geniposide alleviates diabetic nephropathy of mice through AMPK/SIRT1/NF- κ B pathway. *Eur J Pharmacol* 2020; **886**: 173449 [RCA] [PMID: 32758570 DOI: 10.1016/j.ejphar.2020.173449] [FullText]
- 22 Wang G, Zhang H, Zhou Z, Jin W, Zhang X, Ma Z, Wang X. AQP3-mediated activation of the AMPK/SIRT1 signaling pathway curtails gallstone formation in mice by inhibiting inflammatory injury of gallbladder mucosal epithelial cells. *Mol Med* 2023; **29**: 116 [RCA] [PMID: 37641009 DOI: 10.1186/s10020-023-00712-8] [FullText]
- 23 Zhao F, Ma S, Zhou Y, Wei B, Hao Z, Cui X, Xing L, Liu G, Jin L, Ma T, Shi L. miRNA-223 Suppresses Mouse Gallstone Formation by Targeting Key Transporters in Hepatobiliary Cholesterol Secretion Pathway. *Int J Biol Sci* 2021; **17**: 4459-4473 [RCA] [PMID: 34803510 DOI: 10.7150/ijbs.65485] [FullText] [Full Text(PDF)]
- 24 Yang B, Cao P, Bao G, Wu M, Chen W, Wu S, Luo D, Bi P. Inhibiting miRNA-146a suppresses mouse gallstone formation by regulating LXR/megalin/cubilin-media cholesterol absorption. *Heliyon* 2024; **10**: e36679 [RCA] [PMID: 39296173 DOI: 10.1016/j.heliyon.2024.e36679] [FullText] [Full Text(PDF)]
- 25 Chen Y, Wu S, Han Y, Shi H, Yuan J, Cui W. LncRNA SH3PXD2A-AS1 facilitates cisplatin resistance in non-small cell lung cancer by regulating FOXM1 succinylation. *BMC Cancer* 2024; **24**: 848 [RCA] [PMID: 39020302 DOI: 10.1186/s12885-024-12624-9] [FullText]
- 26 Wu Y, Chen W, Miao H, Xu T. SIRT7 promotes the proliferation and migration of anaplastic thyroid cancer cells by regulating the desuccinylation of KIF23. *BMC Cancer* 2024; **24**: 210 [RCA] [PMID: 38360598 DOI: 10.1186/s12885-024-11965-9] [FullText]
- 27 Wang F, Wang K, Xu W, Zhao S, Ye D, Wang Y, Xu Y, Zhou L, Chu Y, Zhang C, Qin X, Yang P, Yu H. SIRT5 Desuccinylates and Activates Pyruvate Kinase M2 to Block Macrophage IL-1 β Production and to Prevent DSS-Induced Colitis in Mice. *Cell Rep* 2017; **19**: 2331-2344 [RCA] [PMID: 28614718 DOI: 10.1016/j.celrep.2017.05.065] [FullText]
- 28 Qin YP, Yu HB, Yuan SY, Yang Z, Ren F, Wang Q, Li F, Ren JH, Cheng ST, Zhou YJ, He X, Zhou HZ, Zhang Y, Tan M, Yang ML, Zhang DP, Wen X, Dong ML, Zhang H, Liu J, Li ZH, Chen Y, Huang AL, Chen WX, Chen J. KAT2A Promotes Hepatitis B Virus Transcription and Replication Through Epigenetic Regulation of cccDNA Minichromosome. *Front Microbiol* 2021; **12**: 795388 [RCA] [PMID: 35140694 DOI: 10.3389/fmicb.2021.795388] [FullText] [Full Text(PDF)]
- 29 Shackelford DB, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat Rev Cancer* 2009; **9**: 563-575 [RCA] [PMID: 19629071 DOI: 10.1038/nrc2676] [FullText] [Full Text(PDF)]
- 30 Espinoza JA, Bizama C, García P, Ferreccio C, Javle M, Miquel JF, Koshiol J, Roa JC. The inflammatory inception of gallbladder cancer. *Biochim Biophys Acta* 2016; **1865**: 245-254 [RCA] [PMID: 26980625 DOI: 10.1016/j.bbcan.2016.03.004] [FullText]
- 31 Hong K, Yang Q, Liu G, Qiu H, Yu B. Cyclophilin D Regulates Oxidative Stress and Apoptosis via Mitochondrial Permeability Transition Pore in Acute Acalculous Cholecystitis. *Curr Mol Med* 2023; **23**: 971-980 [RCA] [PMID: 36089783 DOI: 10.2174/1566524023666220908112922] [FullText]
- 32 Lee HJ, Chung WS, Kim JY, An JH, Park S. Chronic inflammation-related radiological findings of gallbladder adenomyomatosis. *Jpn J Radiol* 2020; **38**: 463-471 [RCA] [PMID: 32072463 DOI: 10.1007/s11604-020-00931-7] [FullText]
- 33 Yang F, Bettadapura SN, Smeltzer MS, Zhu H, Wang S. Pyroptosis and pyroptosis-inducing cancer drugs. *Acta Pharmacol Sin* 2022; **43**: 2462-2473 [RCA] [PMID: 35288674 DOI: 10.1038/s41401-022-00887-6] [FullText]
- 34 Burdette BE, Esparza AN, Zhu H, Wang S. Gasdermin D in pyroptosis. *Acta Pharm Sin B* 2021; **11**: 2768-2782 [RCA] [PMID: 34589396 DOI: 10.1016/j.apsb.2021.02.006] [FullText] [Full Text(PDF)]
- 35 Cao Y, Wang Z, Yan Y, Ji L, He J, Xuan B, Shen C, Ma Y, Jiang S, Ma D, Tong T, Zhang X, Gao Z, Zhu X, Fang JY, Chen H, Hong J. Enterotoxigenic Bacteroides fragilis Promotes Intestinal Inflammation and Malignancy by Inhibiting Exosome-Packaged miR-149-3p. *Gastroenterology* 2021; **161**: 1552-1566.e12 [RCA] [PMID: 34371001 DOI: 10.1053/j.gastro.2021.08.003] [FullText]
- 36 Chen R, Hu J, Zhang Y, Liu Y, Zhu J, Pan Z, Yang H, Wang Q, Chen Y, Tang S, Min B. Total glucosides of paeony ameliorates chemotherapy-induced neuropathic pain by suppressing microglia pyroptosis through the inhibition of KAT2A-mediated p38 pathway activation and succinylation. *Sci Rep* 2024; **14**: 31875 [RCA] [PMID: 39738348 DOI: 10.1038/s41598-024-83207-8] [FullText]
- 37 Ouyang C, Mu J, Lu Q, Li J, Zhu H, Wang Q, Zou MH, Xie Z. Autophagic degradation of KAT2A/GCN5 promotes directional migration of vascular smooth muscle cells by reducing TUBA/ α -tubulin acetylation. *Autophagy* 2020; **16**: 1753-1770 [RCA] [PMID: 31878840 DOI: 10.1080/15548627.2019.1707488] [FullText]
- 38 Dong Z, He W, Lin G, Chen X, Cao S, Guan T, Sun Y, Zhang Y, Qi M, Guo B, Zhou Z, Zhuo R, Wu R, Liu M, Liu Y. Histone acetyltransferase KAT2A modulates neural stem cell differentiation and proliferation by inducing degradation of the transcription factor PAX6. *J Biol Chem* 2023; **299**: 103020 [RCA] [PMID: 36791914 DOI: 10.1016/j.jbc.2023.103020] [FullText]
- 39 Talero E, Alcaide A, Ávila-Román J, García-Mauriño S, Vendramini-Costa D, Motilva V. Expression patterns of sirtuin 1-AMPK-autophagy pathway in chronic colitis and inflammation-associated colon neoplasia in IL-10-deficient mice. *Int Immunopharmacol* 2016; **35**: 248-256 [RCA] [PMID: 27085036 DOI: 10.1016/j.intimp.2016.03.046] [FullText]
- 40 Zhang M, Wu J, Sun R, Tao X, Wang X, Kang Q, Wang H, Zhang L, Liu P, Zhang J, Xia Y, Zhao Y, Yang Y, Xiong Y, Guan KL, Zou Y, Ye D. SIRT5 deficiency suppresses mitochondrial ATP production and promotes AMPK activation in response to energy stress. *PLoS One* 2019; **14**: e0211796 [RCA] [PMID: 30759120 DOI: 10.1371/journal.pone.0211796] [FullText] [Full Text(PDF)]



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