Supplementary files

1. Supplementary methods

1.1 Materials

Acteoside (ACT) (61276-17-3; purity ≥98%) was purchased from Yuanye Bio-Tech (Shanghai, China) and AnaeroPack-Anaero (c-1) was obtained from Mitsubishi Group (Tokyo, Japan). Other materials for cell culture and flow cytometry were from Sigma-Aldrich (St. Louis, USA). Purified CMPK2 protein (CSB-YP688686HU) and IRF1 protein (CSB-BP011814HU) were purchased from Ycusabio (Wuhan, China). Additional information of used antibodies was listed in **Table S1**.

1.2 Hematoxylin and eosin (H & E) staining

Immediately after obtaining mouse liver tissues, tissue samples were fixed in 4% formaldehyde for 7 days. Following dehydration, clearing and paraffin embedding, the sliced sections (5 μm) were further dewaxed and stained with H&E staining reagent, and images were captured using the Aperio Versa microscope (Leica, Wetzlar, Germany).

1.3 Biochemical analysis

The alanine aminotransferase (ALT) kit (Nanjing Jiancheng, China, C010-2-1) and aspartate aminotransferase (AST) kit (Nanjing Jiancheng, China, C009-2-1) were used to detect the changes of blood transaminase levels in mice. The malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) content in liver tissues or cells were separately determined by MDA assay kit (Nanjing Jiancheng, China, A003-1), GSH assay kit (Nanjing Jiancheng, China, A006-2-1) and SOD assay kit (Nanjing Jiancheng, China, A001-3-2). Mitochondrial membrane potential was indicated by TMRE assay kit (Beyotime, China, C2001S) and JC-1 Mitochondrial Membrane Potential assay kit (MedChemExpress, China, HY-K0601). Cell apoptosis was detected by TUNEL cell kit (Beyotime, China, C1090). Reactive oxygen species (ROS) assay kit (Beyotime, China, S0033S) was used to assay the content of hyperoxidation production. The free fatty acid (FFA) content was determined using NEFA assay kit (Nanjing Jiancheng, China, A042-2-

1). The contents of mitochondrial respiratory chain complex I, complex IV and ATP in cells were determined using the micro mitochondrial respiratory chain complex I activity assay kit (Solarbio, China, BC0515), micro mitochondrial respiratory chain complex IV activity assay kit (Solarbio, China, BC0945) and ATP assay kit, respectively (Beyotime, China, S0026). The fluorescence change of calcein-AM was measured using mPTP assay Kit (Beyotime, China, C2009S). MtDNA were extracted from animal serum and cell culture media by the DNA blood mini kit (QIAGEN, Germany, 51104). Mitochondrial content was measured using the Mito-Tracker Red CMXRos (Beyotime, China, C1049-50μg). Lysosomal fluorescence was labeled with Lyso-Tracker Red to indicate its content (Beyotime, China, C1046).

1.4 Immunofluorescence staining

After fixation and paraffin embedding, mouse liver tissues were sectioned into 5 μm slices for immunofluorescence staining. Following deparaffinization in xylene and dehydration in ethanol, the sections were blocked with a solution containing 0.2% Triton X-100, 2.5% BSA, 1× PBS, and 10% goat serum. The cells were washed 3 times with sterile PBS buffer and then fixed in 4% paraformaldehyde for 30 min after treatment and then permeabilization and blocking with PBS containing 0.1% Triton X-100 and 1% BSA for 1 h. Afterwards, mouse liver tissues and cells were incubated with relative primary antibodies overnight at 4 °C. Subsequently, stained sample were treated with secondary antibodies for 45 min at 37 °C in the dark and then were stained with DAPI. Immunofluorescence images were captured using an Olympus FV3000 confocal laser scanning microscope (Tokyo, Japan).

1.5 Immunohistochemistry analysis

The paraffin sections were dewaxed at 55°C and subsequently soaked in various reagents for hydration, followed by heating with an antigen retrieval solution. Tissues were then outlined using a specialized immunohistochemistry marker pen and endogenous peroxidases were inhibited using 3% H_2O_2. Following this, the sections were blocked in

PBS containing 0.2% Triton-X100 and 2.5% BSA, incubated with the primary antibodies overnight at 4°C, followed by the secondary antibody at 37°C for 20 min, and finally stained with DAPI solution for nuclear visualization. Images were later captured by Aperio Versa (Leica, Wetzlar, Germany).

1.6 Quantitative real-time PCR (qPCR)

The total RNA was isolated by Trizol reagent followed by chloroform/isopropanol extraction and were reverse transcribed to cDNA by HiScript III RT SuperMix kit (R323-01, Vazyme, Nanjing, China). The expressions of the target genes were detected by SYBR Green quantitative real-time polymerase chain reaction and *Hprt1* was used as reference gene for normalization. Primers sequences can be gained from the corresponding author if requested.

1.7 Western blot assay

Proteins in cells and livers were extracted, homogenized with RIPA buffer and centrifuged at 8,000 g for 10 min. The protein content in supernatant was determined by BCA kit (Biorigin, Beijing, BN27109), and the absorbance was measured at 562 nm to calculate the protein concentration. Then, proteins were separated by SDS-PAGE gel and transferred to a polyvinylidene fluoride (PVDF) membrane. Subsequently, bands were incubated with relative primary antibodies at 4°C overnight and corresponding secondary antibody for 1 h. After washing with TBST for three times, images were captured using the ChemiDocTM Touch Imaging System (Bio-Rad, USA) and assessed by Image J software.

1.8 Flow cytometry analysis

After different treatments, cells were washed with PBS, centrifuged at 500 g for 5 min and incubated with staining solution of calcium ion or JC-1 assay kit in a dark environment at 37°C. After co-incubation, cells were washed and resuspended with PBS for the following flow cytometry determination. Flow signals were detected using the CytoFLEX flow cytometer (Beckman Coulter, Pasadena, CA).

1.9 Molecular docking

By consulting the UniProt database, the three-dimensional structural data of the IRF1 (ID: Q3U5Q7) and CMPK2 (ID: P10914) proteins were obtained. Additionally, chemical structure of the ACT active moiety the MOL file of the active chemical moiety of ACT was downloaded from the PubChem database. Next, molecular docking of both proteins with the ACT was performed by using Autodock and the results were visualized through PyMOL software.

1.10 Protein stability assay

AML12 cells were cultured in six-well plates and treated with ACT (50 μM) and cycloheximide (CHX, 200 μg/ml), a protein synthesis inhibitor, for varying time points to check the stability of IRF1 and CMPK2 proteins under ACT treatment. After different treatments, total proteins were extracted by RIPA buffer and subsequently analyzed *via* western blot analysis.

1.11 RNA stability assay

To assess the mRNA stability of IRF1 and CMPK2 under ACT treatment, AML12 cells were treated with ACT (50 μM) and actinomycin D (5 μg/ml) for varying time points. At the end of different treatments, total RNA from relative groups was subsequently extracted and analyzed using qPCR experiments.