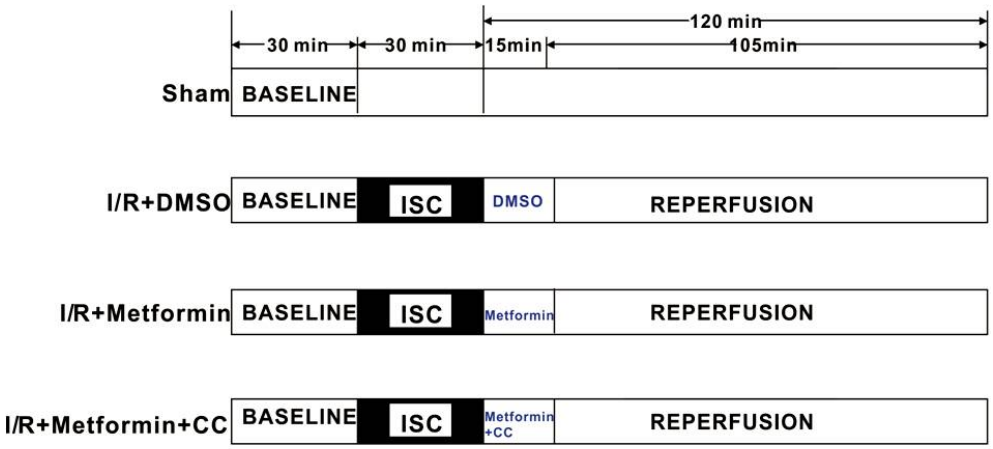

Fig. S1. Schematic illustration of the animal experimental protocol. Except the Sham group, rat hearts were subjected to 30 min of global ischemia followed by 120 min of reperfusion. IR+DMSO group received 0.1% DMSO, IR+ Metformin group was treated with 50 μM metformin for the first 15 min of reperfusion. In IR+Metformin+CC group, 10 uM compound C was co-administered with 50 μM metformin for 15 min at the onset of reperfusion.

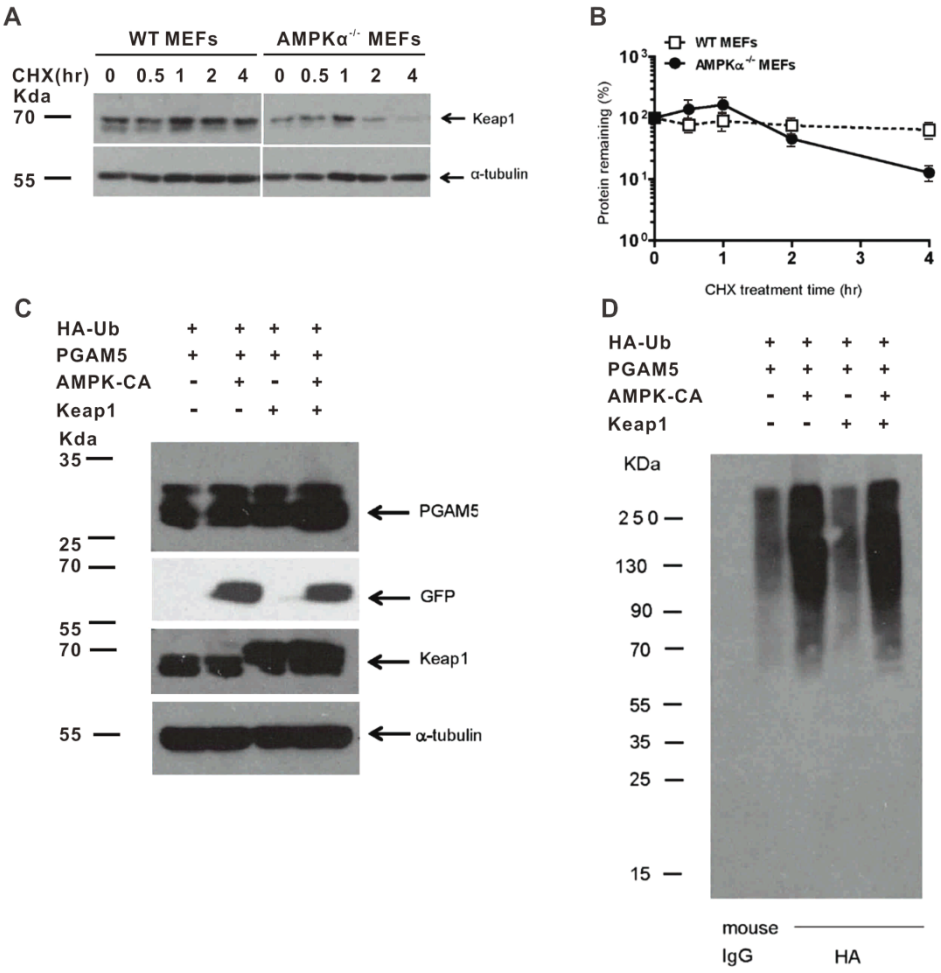

Fig. S2. AMPKα increases Keap1-mediated ubiquitination of PGAM5. (A, B) WT MEFs and AMPK-null MEFs were treated with 10 μg/ml CHX. The levels of Keap1 were determined by immunoblotting at the indicated time points after the addition of CHX. (C, D) 293T cells were transfected with HA-ubiquitin, PGAM5 in the presence or absence of GFP-AMPKα2-CA construct for 48 h. Cell lysates were collected and incubated with rabbit IgG control or PGAM5 Ab, followed by immunoprecipitation with protein A/G magnetic beads. Immunoblots were performed to determine PGAM5, GFP (AMPKα2-CA), and Keap1 in the total lysates (C) and ubiquitination in the immunoprecipitates (D), respectively.

Supplementary methods for animal experiments

The animal protocols were approved by the Committee for Experimental Animals at Jilin University in accordance with the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 8th Edition, 2011). Adult, healthy male Sprague-Dawley rats, weighing 180–230g and of healthy grade were approved by the Committee for the experiments. All animals received humane care and that study protocols comply with the institution's guidelines.

1. Langendorff-perfused rat heart model preparation

Langendorff-perfused rat heart model was built as previously reported [1,2]. Briefly, rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and heparinized (1000 IU/kg). Rat hearts were quickly excised and mounted onto a Langendorff apparatus via aorta cannulation for retrograde perfusion at constant pressure (80 mmHg) with oxygenated Krebs-Henseleit (K-H) buffer (pH 7.40 ± 0.05, 37.0 ± 0.5 ℃, 118.0 mM NaCl, 4.8 mM KCl, 1.2 mM KH2PSO4, 25.0 mM NaHCO3, 1.2 mM MgSO4, 2.5 mM CaCl2, 11.0 mM glucose). A small latex balloon was inserted into the left ventricle to monitor the heart function (heart rate = HR, left ventricular developed pressure = LVDP, and left ventricular end-diastolic pressure = LVEDP). The indicators were recorded at 30 min of equilibration (T0), 30 min (T1), 60 min (T2), 90 min (T3), and 120 min (T4) after reperfusion by Med Lab 6.0 software. Rats with refractory ventricular fibrillation, frequent arrhythmia, LVSP＜75 mmHg or HR＜180 beats/min were excluded.

2. Experimental protocol

As illustrated in Figure S1, rat hearts were randomly divided into four groups: (1) Sham group: rat hearts underwent the same operation. (2) IR + DMSO group: rat hearts received 0.1%

DMSO (vehicle control) alone for the first 15 min of reperfusion. (3) IR + Metformin group: rat hearts were administered with 50 μM metformin for the first 15 min of the reperfusion. (4) IR + Metformin + CC group: 10 μM compound C was co-administered with 50 μM metformin for 15 min at onset of reperfusion. Compound C, an AMPK inhibitor, was dissolved in DMSO.

3. Myocardial infarction size determination

Myocardial infarction size was determined by 2,3,5-triphenyltetrazolium chloride triazole (TTC) staining. At the end of reperfusion, the rat hearts were removed rapidly and frozen at -20 ℃ for 2 h. Left ventricular was cut into 5 pieces in cross section and incubated in 1% TTC in 0.1 M PBS at 37 ℃ for 10 min, and fixed overnight in 10% formalin. Subsequently, myocardial infarct area (white color) could be differentiated from the area at risk (AAR, red color). The infarct size was calculated digitally by Alpha Ease FC Imaging System.

4. LDH and CK-MB measurement

Lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) levels were measured from the effluents of Langendorff-perfused rat heart model at the end of reperfusion in each heart with commercial kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer's instructions. The activities of the enzymes were expressed as U/L.

5. Histology

Hematoxylin-eosin (H&E) staining was used to evaluate pathological changes at the end of reperfusion. 5 μm sections of cardiac tissues were dried in air, washed with PBS, and immersed in hematoxylin solution. The sections were then treated with 1% hydrochloric acid and dehydrated in gradient alcohol. Finally, sections were made transparent in xylene and mounted with neutral balsam.

6. Evans blue staining

The degree of myocardial IR injury was assessed using Evans blue staining as previously described [3]. Briefly, anesthetized rats were given intraperitoneal injections of Evans blue dye (final concentration of 1% volume to body weight) 8 h prior to sacrifice. All isolated hearts were taken and cut into 5 μm cryosections after reperfusion, in order to assess the amount of Evans blue dye uptake (red autofluorescence) by fluorescence microscopy (×200). EBD-positive areas are expressed as the percent area of the myocardium with red fluorescence, and the data were determined with Scion Image software.

References

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