1. Supplementary Methods

1.1 Quantitative PCR (qPCR) analysis

The cultured cardiomyocytes were collected by centrifugation and lysed in TRIZOL reagent (Invitrogen). RNA was isolated by phase separation and precipitation. RNA yield and quality were assessed by ultraviolet absorbance (NanoDrop ND-1000) and denaturing agarose gel electrophoresis. Complementary DNA was synthetized, and qPCR was performed. Finally, the data were analyzed by the ΔΔCt method.

1.2 Western blot analysis

Proteins extracted from the cultured cardiomyocytes were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 12% gel and were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore). After blocking with 5% bovine serum albumin, the blocked membrane was incubated with rabbit polyclonal hypoxia-inducible factor 1α (HIF-1α) antibody (Abcam, ab179483, 1:1,000), rabbit polyclonal voltage-dependent anion channel (VDAC) antibody (Cell Signaling Technology, 4661, 1:1,000), rabbit monoclonal glucose transporter 1 (GLUT1) antibody (Abcam, ab115730, 1:5,000), rabbit monoclonal pyruvate kinase 1 (PKM1) antibody (Cell Signaling Technology, 7067, 1:1,000), rabbit monoclonal 6-phosphofructo-2-kinase/fructose -2,6-biphosphatase 3 (PFKFB3) antibody (Cell Signaling Technology, 13123, 1:1,000), rabbit monoclonal Hexokinase II (HK2) antibody (Cell Signaling Technology, 2867, 1:1,000), rabbit polyclonal lactate dehydrogenase A (LDHA) antibody (Cell Signaling Technology, 2012, 1:1,000) rabbit monoclonal β-hydroxybutyrate dehydrogenase 1 (BDH1) antibody (Abcam, ab193156, 1:1,000), 3-oxoacid CoA-transferase 1 (OXCT1) antibody (Abcam, ab105320, 1:2,000), oxoacid CoA-transferase 2 (OXCT2) antibody (Abclonal, a14920, 1:1,000), solute carrier family 16 member 1 (SLC16A1) antibody (Sigma, HPA003324, 1:1,000), rabbit monoclonal prolyl hydroxylase-2 (PHD2) antibody (Cell Signaling Technology, 4835, 1:1,000), rabbit polyclonal to Von Hippel Lindau (VHL) antibody (Abcam, ab83307, 1:1,000) and rabbit monoclonal to Actin (β-actin) antibody (Abcam, ab179467, 1:5,000). Then, an enhanced chemiluminescence detection reagent (Thermo Scientific™ SuperSignal™ West Pico PLUS, Cat# 34577)was used for imaging.

1.3 Immunofluorescence

Sections were incubated with rabbit anti-mouse polyclonal HIF-1α antibody or rabbit anti-mouse monoclonal GLUT1 antibody. An Alexa Fluor 549 donkey anti-rabbit secondary antibody (Thermo-Fisher Scientific) was used for fluorescence imaging. After counterstaining with DAPI, the sections were photographed with a fluorescence confocal microscope (Nikon TE2000).

1.4 Ketone body assay

The β-OHB levels in cultured cardiomyocytes were determined using the High Sensitivity β-Hydroxybutyrate Assay Kit (Sigma-Aldrich, MAK272), according to the

manufacturer's directions. The assay was performed in triplicate, and the supplied standard was used for comparisons. Prior to addition to the reaction, samples were deproteinized by filtration using a 10-kDa molecular weight cut-off (MWCO) spin filter. Next, 25 μL of each sample was added to the wells of a 96-well plate. Samples were brought to a final volume of 50 μL with the β-OHB Assay Buffer. Then, 50 μL of the Master Reaction Mix was added to each of the wells containing the samples or standard controls. The plate was incubated for 30 min at room temperature in the dark, and then the fluorescence intensity was measured (λ_{ex}=535/λ_{em}=587 nm). We calculated β-OHB concentrations according to the standard curve. Ketone concentrations in plasma samples were normalized by volume whereas the protein concentration was measured by bicinchoninic acid assay (BCA) to determine the protein-corrected ketone concentrations in myocardium.

1.5 Intracellular ATP

Intracellular ATP was determined using the Enhanced ATP Assay Kit (Beyotime, S0027), according to the manufacturer's protocol.

1.6 Live/dead cell staining and imaging

Staining of the cultured cardiomyocytes was performed using the LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells (Thermo Scientific), according to the manufacturer's instructions. Briefly, Hoechst 33342 (Thermo Scientific), calcein, and ethidium dyes were diluted to the final concentrations of 10 μg/mL, 1 μmol/L, and 0.5 μmol/L, respectively, in culture medium made using phenol red-free M199 (Thermo Scientific). The cardiomyocytes were incubated in this medium for 30 min at room temperature and were then washed gently with fresh phenol red-free culture medium and imaged using a standard fluorescence microscope.

1.7 Lactate dehydrogenase (LDH) and Cell Counting Kit-8 (CCK-8) assays

LDH assay kits (Beyotime Biotechnology) were used to measure LDH released into the culture medium by cardiomyocytes. The CCK-8 assay (Beyotime Biotechnology) was used to detect cell viability.

1.8 Echocardiography

Mice were anesthetized with 2% isoflurane and were then subjected to transthoracic echocardiography using a VeVo 2100 Imaging System (VisualSonics) to assess cardiac structure and function at 1 day as well as 4 weeks after MI surgery. Body temperature was maintained between 36.9–37.3°C, and the heart rate was maintained between 400–500 bpm. Echocardiographic M-mode tracings were recorded, and echocardiographic parameters, including LVEF, fractional shortening (FS), left ventricular end systolic diameter (LVESD), left ventricular end diastolic diameter (LVEDD), and stroke volume (SV), were calculated.

1.9 2,3,5-triphenyltetrazolium chloride (TTC) staining

At both 24 h and 3 days after MI surgery, the hearts of the mice were harvested and cut into 1-mm slices. The slices were then incubated with 1% w/v TTC at 37°C for 15

min, followed by fixation with 10% formalin for 20 min.

1.10 Plasmid construction and transfection

HIF-1α was cloned into the GTP-C-3Flag-H vector containing a C-terminal Flag tag. The primers and restriction enzyme sites used for the plasmid construction are listed in Table S3. Lipofectamine 3000 (Invitrogen) was used for plasmid transfections, according to the manufacturer's instructions. DMEM (500 μL) was added to two clean Eppendorf tubes, and 3 μL of Lipofectamine 3000 was added to one of the tubes and mixed for 5 min. Plasmid was added to the other tube, and its contents were then added to the medium containing Lipofectamine 3000, mixed, and allowed to stand for 20 min before being added to the cardiomyocyte culture medium for transfection. The cell culture medium was replaced with serum-free medium because serum interferes with the Lipofectamine 3000 transfection efficiency. After 6 h incubation with the transfection reagents, the medium was replaced with fresh, normal medium. After 24 h, the cardiomyocytes were subjected to hypoxia for 12 h and then collected for further analyses.

1.11 RNA interference

Small interfering RNA (siRNA) was used to achieve stable knockdown in this study. Briefly, double-stranded siRNA targeting HIF-1α was purchased from ZORIN and transfected into cells using RNAiMax (Invitrogen), according to the manufacturer's instructions. The siRNA sequences are listed in Supplemental Table S3. The knockdown efficiency was verified by qPCR and Western blot.

2. Supplementary Tables

2.1 Baseline characteristics between healthy volunteers and patients with AMI

	Healthy volunteers	AMI	P
N	32	45	-
Male, n (%)	32 (100%)	45 (100%)	1
Age, years	57.25±7.06	61.60±11.84	0.0627
Diabetes diagnosis, %	0	0	1
Hypertension diagnosis, %	-	16 (35.6%)	-
Percutaneous transluminal coronary intervention, %	-	28 (62.2%)	-

2.2 Body weight, blood β-OHB and blood glucose determined after 4 weeks of diet (n=12 in each group).

	CD	KD

Body weight	21.88±0.14	17.88±2.47*
Blood β-OHB (mM)	0.27±0.08	2.71±1.12*
Blood glucose (mM)	7.57±1.04	5.34±1.66*
Blood total cholesterol (mMol/L)	2.32±0.86	3.69±1.09*
Blood triglyceride (mMol/L)	1.23±0.35	1.04±0.24
LDL-C (mmol/L)	2.11±0.39	3.94±1.21*
ALT	56.78±7.01	71.56±17.01*

2.3 Sequences of primers used for siRNA and qPCR analyses used in this study.

HIF-1α (human) siRNA-1 sense	GGAAAUGAGAGAAAUGCUUTT
HIF-1α (human) siRNA-1 antisense	AAGCAUUUCUCUCAUUUCCTC
HIF-1α (human) siRNA-2 sense	GCUGGAGACACAAUCAUAUTT
HIF-1α (human) siRNA-2 antisense	AUAUGAUUGUGUCUCCAGCGG
HIF-1α (human) siRNA-3 sense	CCGGUUGAAUCUUCAGAUATT
HIF-1α (human) siRNA-3 antisense	UAUCUGAAGAUUCAACCGGTT
HIF-1α (human) Plasmids Construction forward	TAGAGCTAGCGAATTCATGGAGGGCGCCGGCGGC
HIF-1α (human) Plasmids Construction forward	CTTTGTAGTCGGATCCGTTAACTTGATCCAAAGCTCTGAG
Aldoa Forward	AGAAGGTCCTGGCGGCTGTC
Aldoa Reverse	TGTGCGACGAAGTGCTGTGAC

Pgk1 Forward	AATGGAGCCAAGTCCGTTGTCC
Pgk1 Reverse	TGGCACAGGCATTCTCGACTTC
Pkm Forward	TGGTGACGGAGGTGGAGAATGG
Pkm Reverse	GTCGGCTGCCTTGCGGATG
Pfkl Forward	GCATCAAGCAGTCAGCCTCAGG
Pfkl Reverse	AGCCAGGTAGCCACAGTAGCC
HK-1 Forward	CCTCCGTCAAGATGCTGCCAAC
HK-1 Reverse	CCGCCGAGATCCAGTGCAATG
Hk2 Forward	GTGTGGAACTGGTGGACGGAGA
Hk2 Reverse	GTCATCCAGGCAGCCGTTGTC
Slc2a1 Forward	GCAGTTCGGCTATAACACTGG
Slc2a1 Reverse	GCGGTGGTTCCATGTTTGATTG
Hif-1a Forward	CTGCCACTGCCACCACAACTG
Hif-1a Reverse	TGCCACTGTATGCTGATGCCTTAG
Pfkp Forward	CAGAGCCACCAGAGGACCTTCG
Pfkp Reverse	CAGTCGGCACCGCAAGTCAAG
beta-actin Forward	GTGACGTTGACATCCGTAAAGA
beta-actin Reverse	GCCGGACTCATCGTACTCC