

Supplementary Materials

JOSD2 promotes cardiomyocyte apoptosis and exacerbates cardiac injury after myocardial infarction via LKB1-AMPK suppression

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Supplementary Data. 1**Table 1. Primers for quantitative RT-PCR**

Primer	Sequence (5'→3')
H- <i>ACTB</i> -F	ACTCTTCCACCTTCGATGCC
H- <i>ACTB</i> -R	TGGGATAGGGCCTCTCTTGC
H- <i>JOSD2</i> -F	CCCACCGTGTACCACGAAC
H- <i>JOSD2</i> -R	CTCCTGGCTAAAGAGCTGCTG
H- <i>KLHL40</i> -F	ACGTCAGCCTGGTTACCAAG
H- <i>KLHL40</i> -R	ACTTCGGCAGAACTGGTCAG
H- <i>MYH7</i> -F	ACTGCCGAGACCGAGTATG
H- <i>MYH7</i> -R	GCGATCCTTGAGGTTGTAGAGC
H- <i>NPPA</i> -F	CCCATGTACAATGCCGTGTC
H- <i>NPPA</i> -R	GGCAGATCGATCAGAGGAGT
H- <i>NPPB</i> -F	TGGAAACGTCCGGGTTACAGGA
H- <i>NPPB</i> -R	TCCGGTCCATCTTCCTCCCAA
H- <i>RNF185</i> -F	GTGTTTACATCAGTGGTTGGAGA
H- <i>RNF185</i> -R	GTGCTGCCCTTCCATAGAG
H- <i>UBE2L3</i> -F	CAACTTTCAGCAGAGTACCC
H- <i>UBE2L3</i> -R	TGCCCTTTTCGTCGATGTTT
H- <i>ZBTB34</i> -F	GCAGGGGAAACTATGTGACATC
H- <i>ZBTB34</i> -R	CCCGGAAATATGGGGAGCTG
M- <i>Actb</i> -F	GGCTGTATTCCCCTCCATCG
M- <i>Actb</i> -R	CCAGTTGGTAACAATGCCATGT
M- <i>Josd2</i> -F	CTGATCTTGAACCTACCCTCTCC
M- <i>Josd2</i> -R	GTCCTGACTCCATCCTCGTC
M- <i>Klhl40</i> -F	ACGTCAGCCTGGTTACCAAG
M- <i>Klhl40</i> -R	ACTTCGGCAGAACTGGTCAG
M- <i>Nppa</i> -F	GTGCGGTGTCCAACACAGAT
M- <i>Nppa</i> -R	TCCAATCCTGTCAATCCTACCC
M- <i>Nppb</i> -F	GAGGTCACCTATCCTCTGG
M- <i>Nppb</i> -R	GCCATTTCTCCGACTTTTCTC

Primer	Sequence (5'→3')
M- <i>Rnf185</i> -F	GCAGACCTATCAATCAACGCC
M- <i>Rnf185</i> -R	CGAAGGCCCTTTACTTGCCAT
M- <i>Ube2l3</i> -F	GAGATCCGCAAATGTGGAATGA
M- <i>Ube2l3</i> -R	AATAAGCCCTTGCCAAGTCAAT
M- <i>Zbtb34</i> -F	TCAAGATTACGTTTCGTGTCGG
M- <i>Zbtb34</i> -R	TGAATGGTCCCGGAAATACGG
R- <i>Actb</i> -F	CCGCGAGTACAACCTTCTTG
R- <i>Actb</i> -R	TGACCCATACCCACCATCAC
R- <i>Josd2</i> -F	CGGAAGCGAGACCAAGATCA
R- <i>Josd2</i> -R	TCACCACCAATAGCACCTCG
R- <i>Nppa</i> -F	CCCAGGCCATATTGGAGCAA
R- <i>Nppa</i> -R	CTTCGGTACCGGAAGCTGTT
R- <i>Nppb</i> -F	GTGCTGCCCCAGATGATTCT
R- <i>Nppb</i> -R	TCCGGTCTATCTTCTGCCCA

Supplementary Data. 2

Table 2. The relevant information of the primary antibodies for western blot.

Name	Species	Manufacturer	Article Number	Dilution
Rabbit anti-JOSD2				
Polyclonal Antibody(N-term)	Rabbit	Absin, China	abs100732	1:1000
Beta Actin				
Monoclonal antibody	Mouse	Proteintech, USA	66009-1-Ig	1:50000
BAX Polyclonal antibody	Rabbit	Proteintech, USA	50599-2-Ig	1:50000

Bcl2 Polyclonal antibody	Rabbit	Proteintech, USA	26593-1-AP	1:1000
Cleaved Caspase-3 (Asp175) (5A1E) Rabbit Monoclonal Antibody	Rabbit	Cell Signaling Technology, USA	9664T	1:1000
Cleaved Caspase-9 (Asp353) Antibody	Rabbit	Cell Signaling Technology, USA	9509T	1:1000
AMPKa1/AMPKa2 Rabbit pAb	Rabbit	ABclonal, China	A12718	1:10000
Phospho-AMPK alpha (Thr172) (40H9) Rabbit Monoclonal Antibody	Rabbit	Cell Signaling Technology, USA	2535s	1:1000
LKB1 (D60C5) Rabbit Monoclonal Antibody	Rabbit	Cell Signaling Technology, USA	3047s	1:1000
Phospho-LKB1 (Ser428) (C67A3) Rabbit Monoclonal Antibody	Rabbit	Cell Signaling Technology, USA	3482s	1:1000
NPPA Rabbit pAb	Rabbit	ABclonal, China	A14755	1:10000
α -Smooth Muscle Actin (ACTA2) Rabbit mAb	Rabbit	ABclonal, China	A17910	1:20000
Anti-Myc-tag mAb	Mouse	MBL, Japan	M192-3	1:10000
Recombinant Anti-HA Tag antibody (HRP Conjugated)	-	Servicebio, China	ZB151253-HRP	1:1000

Recombinant Anti-

Flag Tag antibody

- Servicebio, China

ZB15939-HRP

1:2000

(HRP Conjugated)

Supplementary Data. 3

Figure. S1

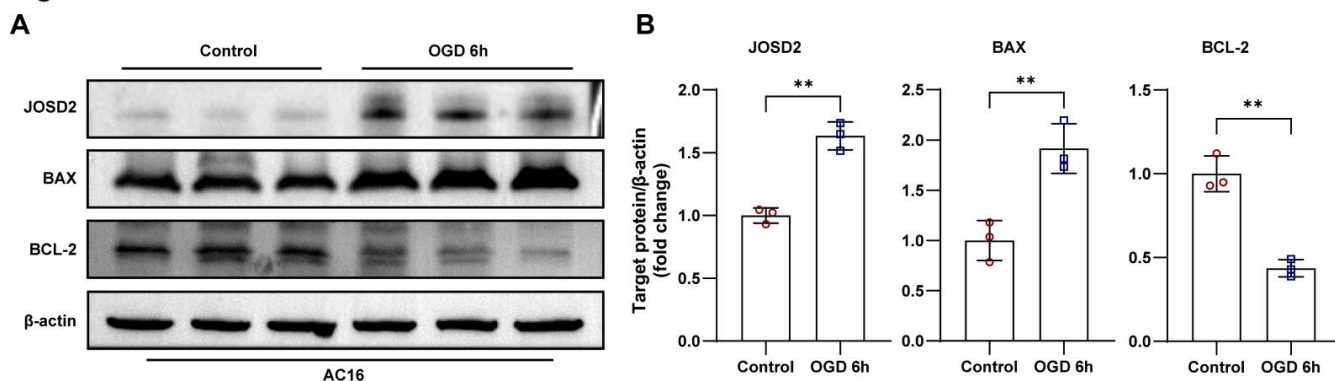


Figure S1. OGD alters JOSD2 and apoptosis-associated proteins in AC16 cells. (A) Western blots showing JOSD2, BAX, and BCL-2 protein levels in AC16 cells following OGD versus control conditions. (B) Densitometric quantification of immunoblots (n=3). Data are presented as mean \pm SD. Statistical analysis for panel (B) was performed using an unpaired two-tailed Student's t-test. **P<0.01.

Figure. S2

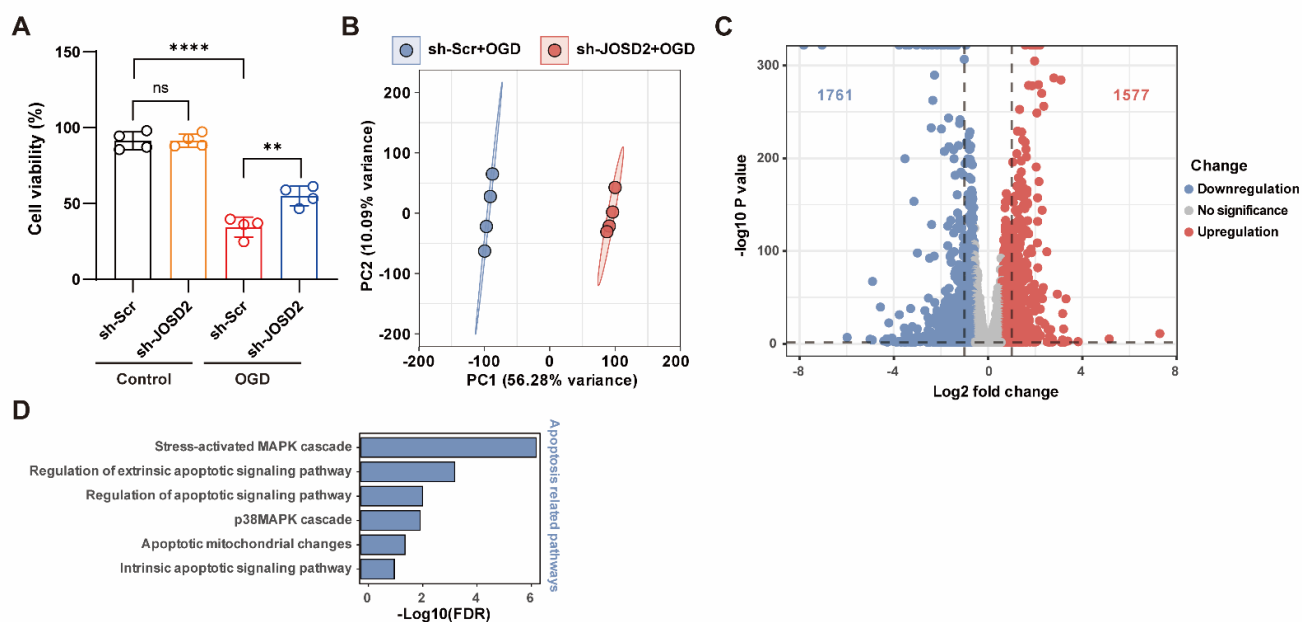


Figure S2. CCK-8 experiment and bioinformatics analysis of transcriptome sequencing data. (A) CCK-8 assay assessing cellular viability capacity in shScr and sh-JOSD2 AC16 cells under OGD stress (n=4). (B) PCA of bulk RNA-seq data comparing sh-Scr+OGD and sh-JOSD2+OGD groups (n=4 per group). (C)

Volcano plot showing differentially expressed genes between sh-Scr+OGD and sh-JOSD2+OGD. (D) Gene Ontology enrichment analysis of differentially expressed genes, highlighting enrichment of apoptosis-related pathways. Data are presented as mean \pm SD. Statistical analysis was performed using two-way ANOVA with Tukey's post hoc test. ns, not significant; * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.

Figure. S3

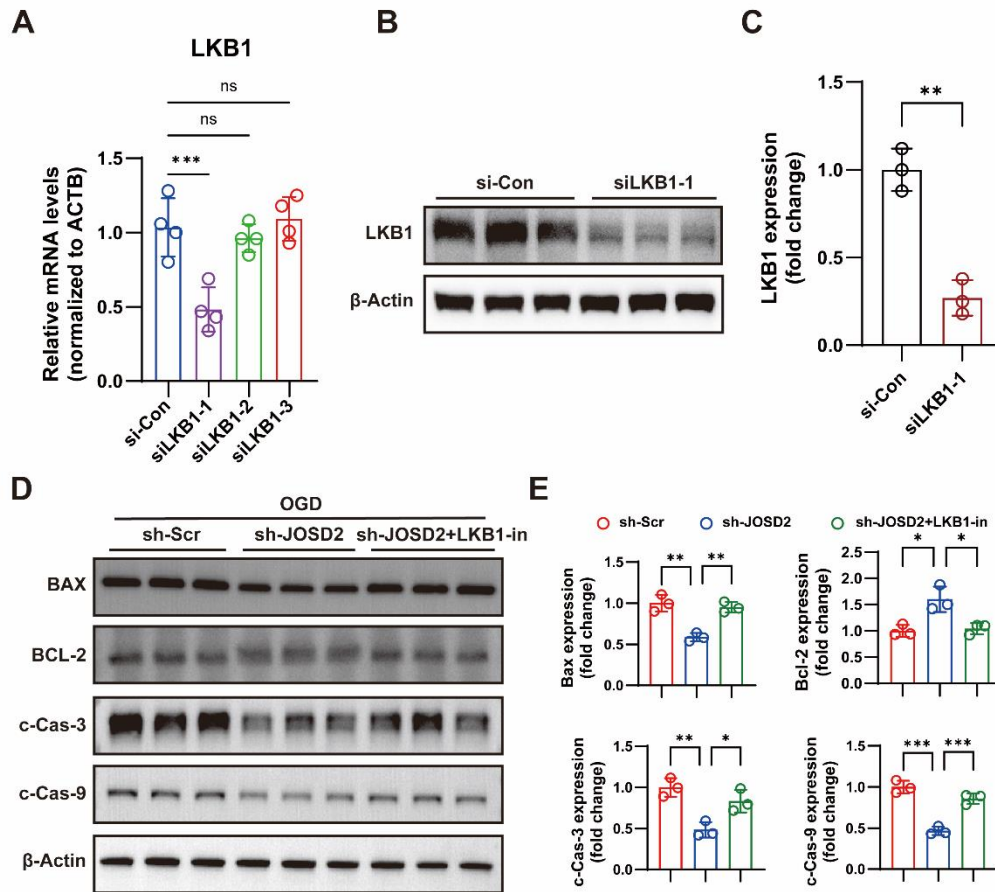


Figure S3. Validation of LKB1 silencing and LKB1-in-mediated LKB1 inhibition under OGD. (A) qPCR analysis of LKB1 mRNA levels in AC16 cells transfected with non-targeting control siRNA (si-Con) or three independent siRNAs targeting LKB1 ($n = 4$ per group). (B) Western blot analysis of LKB1 protein in AC16 cells transfected with si-Con or siLKB1-1 ($n = 3$ per group). (C) Densitometric quantification of LKB1 protein levels ($n = 3$). (D) Western blots showing apoptosis-associated proteins (BAX, BCL-2, cleaved caspase-3, cleaved caspase-9) in sh-Scr, sh-JOSD2, and sh-JOSD2 + LKB1-in AC16 cells under OGD. (E) Densitometric quantification of apoptosis-associated proteins ($n=3$). Data are presented as mean \pm SD. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test for panels (A) and (E), and an unpaired two-tailed Student's t-test for panel (C). ns, not significant; * $P < 0.05$, ** $P < 0.01$, **** $P < 0.001$.

Figure. S4

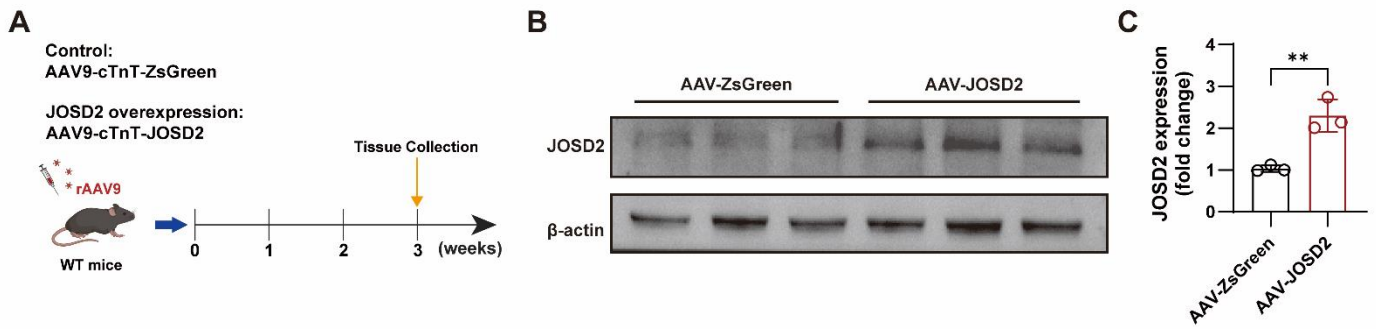


Figure S4. Validation of AAV9-mediated cardiomyocyte-specific JOSD2 overexpression *in vivo*. (A) Schematic of tail-vein injection of AAV9-cTnT-ZsGreen (control) or AAV9-cTnT-JOSD2 (JOSD2 overexpression) to drive cardiomyocyte-specific transgene expression. (B) Western blots showing cardiac JOSD2 expression in AAV-ZsGreen and AAV-JOSD2 mice at 3 weeks after AAV injection; β -actin served as a loading control. (C) Densitometric quantification of cardiac JOSD2 protein levels (n=3). Statistical analysis for panel (C) was performed using an unpaired two-tailed Student's t-test. Data are presented as mean \pm SD; **P<0.01.