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Supplementary Information

1. Materials and methods

1.1 Sampling

At 24 hours post-injection, one piglet from each pen (six piglets per treatment) was randomly selected for sample collection. Ten milliliters of blood were drawn from the anterior vena cava and collected in chilled heparinized vacutainers. After collection, the blood was centrifuged at 3,000 *g* for 20 minutes at 4°C to obtain plasma for subsequent biochemical analyses. Following which, piglets were euthanized by exsanguination after electrical stunning and the liver was immediately collected. Liver tissues from the left lobe was immediately cryopreserved in optimal cutting temperature medium (#W4583; Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China) or fixed in 4% paraformaldehyde solution (#G1101; Wuhan Servicebio Technology Co., Ltd.) for subsequent histological observation. A portion of the fresh liver was used to isolate mitochondria for biochemistry assay and to extract nuclear proteins for immunoblotting analysis. The remainder of liver samples was immediately snap-frozen and stored in −80°C freezers for subsequent analysis.

1.2 Immunofluorescence

Superoxide production was detected with a florescent dye, dihydroethidium (DHE), based on the methods described by Yueh et al.[1] DHE is an oxidant-sensitive fluorescent probe that can interact with intracellular superoxide anion and then produce a fluorescent signal.[2,3] Briefly, liver cryosections with 5-μm thickness were incubated with DHE (10 μM in phosphate-buffered saline; #309800; Sigma–Aldrich,

23 St. Louis, MO, USA) in a dark location at 37°C for 30 minutes. Cells staining positive
24 for the oxidized dye were observed using a fluorescence microscope (Nikon Eclipse
25 C1; Nikon, Tokyo, Japan). Ten random fields per section were quantified using the
26 Image J software (NIH, Baltimore, MD, USA).

27 An *in situ* detection of DNA fragmentation was performed on the paraffin-
28 embedded liver sections to measure the number of apoptotic cells in the liver using a
29 one-step terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) kit
30 (#A113-03; Vazyme, Nanjing, Jiangsu, China). After deparaffinization, the sections
31 were incubated with 20 μg/mL proteinase K in phosphate-buffered saline at 25°C for
32 20 minutes, then washed twice with phosphate-buffered saline, and incubated with the
33 TUNEL mixed reagents in a dark location at 37°C for 1 hour. After that, 4'-6-
34 diamidino-2-phenylindole (DAPI) in anti-fade mounting medium (#P0131; Beyotime
35 Institute of Biotechnology, Haimen, Jiangsu, China) was used to label the nuclei. The
36 labeled cells were visualized by a fluorescent microscope (Nikon Eclipse C1) and
37 were defined by a red color with the DAPI label. The TUNEL-positive cells were
38 quantified in fifteen randomly fields per section.

39 After deparaffinization, microwave antigen retrieval, and blocking endogenous
40 peroxidase activity, liver slices were incubated overnight with a rabbit anti-sirtuin 1
41 (SIRT1) primary antibody (1:400 dilution; #NBP2-27205; Novus Biologicals,
42 Littleton, CO, USA). Then, the sections were washed twice and incubated with a
43 CoraLite488-conjugated goat anti-rabbit secondary antibody (diluted 1:500;
44 #SA00013-2; Proteintech, Chicago, IL, USA) at room temperature for 30 minutes.

45 The slides were mounted in anti-fade mounting medium with DAPI. The images were
46 captured using a fluorescent microscope (Nikon Eclipse C1) and then quantified in ten
47 randomly fields per section.

48 **1.3 Transmission electron microscopy**

49 Liver samples were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer
50 overnight at 4°C and then post-fixed with 1% osmium tetroxide in 0.2 M phosphate-
51 buffered saline for 2 hours at 4°C. Following which, the liver sections were
52 dehydrated with a graded series of ethanol solutions, rinsed in propylene oxide, and
53 embedded in epoxy resin. Thin sections cut by the Reichert Ultracut microtome
54 (Leica, Deerfield, IL, USA) were detected and photographed using a transmission
55 electron microscope (Hitachi H-7650, Tokyo, Japan).

56 **1.4 Assessment of mitochondrial swelling**

57 Mitochondria were isolated from fresh liver samples using the Tissue Mitochondria
58 Isolation Kit (#C3606) from Beyotime Institute of Biotechnology. After quantifying
59 the total protein of each sample with the Enhanced Bicinchoninic Acid Protein Assay
60 Kit (#P0010; Beyotime Institute of Biotechnology), the mitochondrial suspension
61 (100 μg/mL) was incubated with 1 mL of swelling assay buffer containing 150 mM
62 KCl, 5 mM Hepes, 2 mM K_2HPO_4, and 5 mM glutamate, according to the method of
63 Du et al.[4] Mitochondrial swelling was triggered by the addition of calcium (500
64 nmol/mg of protein) and immediately monitored by the decrease in absorbance at 540
65 nm using the Multiskan GO Microplate Spectrophotometer (Thermo Fischer
66 Scientific, Vantaa, Finland) at 37°C for 600 seconds. Measures were taken every 60

67 seconds with 20 seconds strong shaking in the interval to avoid mitochondria
68 deposition at the bottom of the well.

69 **1.5 Measurement of hepatic mitochondrial DNA (mtDNA) copy number**

70 Total DNA was isolated from snap-frozen liver samples using the TIANamp Genomic
71 DNA kit (#DP304-03; Tiangen, Beijing, China). The copy number of mtDNA in the
72 total DNA samples was measured by co-amplifying mitochondrial D-loop (mtD-loop)
73 and beta actin (ACTB) gene by real-time quantitative PCR (qPCR) using the
74 ChamQ™ SYBR® qPCR Master Mix Kit (#Q311-02; Vazyme). Primer sequences
75 were used as follows: mtD-loop sense primer
76 GATCGTACATAGCACATATCATGTC and anti-sense primer
77 GGTCCTGAAGTAAGAACCAGATG and ACTB sense primer
78 CCCCTCCTCTCTTGCCTCTC and anti-sense primer
79 AAAAGTCCTAGGAAAATGGCAGAAG. Based on the manufacturer's protocol
80 (#Q311-02; Vazyme), amplification reactions were performed on a QuantStudio 5
81 Real-time PCR System (Applied Biosystems, Life Technologies, CA, USA). The 2^{-}
82 $^{\Delta\Delta Ct}$ method was used to determine the copy number of mtDNA.[5]

83 **1.6 Total RNA extraction and RT-qPCR analysis**

84 Approximately 50 mg of frozen liver samples were used for total RNA isolation with
85 the Total RNA Extraction Reagent (#R401-01; Vazyme). The yields and purity of the
86 total RNA samples were determined by detecting the absorbance at 260 and 280 nm
87 (NanoDrop ND-1000UV spectrophotometer; NanoDrop Technologies). The isolated
88 RNA was separated on 1.5% agarose gel with Ultra GelRed™ (diluted 1:10,000;

89 #GR501-01; Vazyme) to check for RNA integrity. After that, 1 μg of RNA was used
90 as a template to synthesize complementary DNA with the PrimeScript™ RT Master
91 Mix (#RR036A; TaKaRa Biotechnology, Dalian, Liaoning, China). Amplification
92 reactions were performed on a QuantStudio 5 Real-time PCR System (Applied
93 Biosystems) with the ChamQ™ SYBR® qPCR Master Mix Kit (#Q311-02; Vazyme),
94 as recommended in the manufacturer's guidelines. The PCR process involved a 30-
95 second pre-run at 95°C, 40 cycles of denaturation at 95°C for 5 seconds, and a 60°C
96 annealing step for 30 seconds. For the melting curve conditions, one denaturation
97 cycle was performed at 95°C for 10 seconds; the temperature was then increased from
98 65 to 95°C at a rate of 0.5°C per second. Table 1 includes details of the primer
99 sequences for the following target and reference genes: NAD(P)H quinone
100 dehydrogenase 1 (NQO1), heme oxygenase 1 (HO1), copper/zinc superoxide
101 dismutase (Cu/Zn-SOD), manganese superoxide dismutase (Mn-SOD), glutamate-
102 cysteine ligase catalytic subunit (GCLC), glutamate-cysteine ligase modifier subunit
103 (GCLM), peroxisome proliferators-activated receptor gamma coactivator 1 alpha
104 (PGC-1α), nuclear respiratory factor 1 (NRF1), estrogen-related receptor α (ERRα),
105 mitochondrial transcription factor A (TFAM), glyceraldehyde-3-phosphate
106 dehydrogenase, and ACTB. Fold change in the expression of specific mRNA was
107 calculated using the $2^{-\Delta\Delta Ct}$ method.[5]

108 **1.7 Immunoblotting analysis**

109 Total protein and nucleoprotein were isolated from liver tissues using protein
110 extraction reagents following the manufacturer's instructions (#P0013B for total

111 protein and #P0028B for nucleoprotein; Beyotime Institute of Biotechnology). After
112 quantification, proteins were mixed with a 5 × solution of sample buffer (#P0015;
113 Beyotime Institute of Biotechnology) and heated at 100°C for 5 minutes. Thereafter,
114 equal concentration of the extracted protein was separated by electrophoresis in a
115 mini-gel apparatus (Bio-Rad Laboratories, Hercules, CA, USA) on a 10% sodium
116 dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto
117 Immobilon-P (#IPVH00010; Millipore, Bedford, MA, USA). Blots were blocked with
118 5% skimmed dry milk in Tris-buffered saline combined with 0.2% Tween-20 (TBST)
119 at room temperature for 2 hours. After three washes in TBST, the membranes were
120 incubated at 4°C overnight with rabbit antibodies against NRF2 (#ab92946; Abcam,
121 Cambridge, MA, USA; 1:1,000 dilution), histone H3 (#5192S; CST, Danvers, MA,
122 USA; 1:1,000 dilution), and ACTB (#4970S; CST; 1:1,000 dilution) and a
123 mouse antibody against Mn-SOD (#66474-1-Ig; Proteintech; 1:5,000 dilution). After
124 further washing in TBST, the membranes were incubated with the corresponding
125 secondary antibody at room temperature for 2 hours. Subsequently, the signals were
126 detected by an enhanced chemiluminescence HRP substrate (#WBKLS0100;
127 Millipore, Bedford, MA, USA) on a Tanon 5200 Multi-Imaging System (Tanon
128 Science & Technology Inc., Shanghai, China) and quantified using the Gel-Pro
129 Analyzer program (Media Cybernetics, Silver Spring, MD, USA).
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147 real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, *Methods*, 2001,
148 **25**, 402–408.

149 **Table 1.** Primer sequences used for real-time PCR assay.

Name	Genbank[a]	Sequence, 5'→3'[b]	Length, bp
NQO1	NM_001159613.1	CATGGCGGTCAGAAAAGCAC	135
		ATGGCATACAGGTCCGACAC	
HO1	NM_001004027.1	TGATGGCGTCCTTGTACCAC	71
		GACCGGGTTCTCCTTGTTGT	
Cu/Zn-SOD	NM_001190422.1	AAGGCCGTGTGTGTGCTGAA	118
		GATCACCTTCAGCCAGTCCTTT	
Mn-SOD	NM_214127.2	GGCCTACGTGAACAACCTGA	126
		TGATTGATGTGGCCTCCACC	
GCLC	XM_003482164.3	CTAGTGGGTAGGCGGACTGG	81
		CGGTGTCGTGCTCTAGCTTC	
GCLM	XM_001926378.3	GGACAAAACCCAGTTGGAGC	86
		TCACACAGCAAGAGGCAAGA	
PGC-1α	NM213963.2	TCTGAAAGGGCCAAGCAGAG	111
		GTCCCTCAGTTCTGTCCGTG	
NRF1	XM005657993.1	GCCTTGTGGTGGGAGGAATG	82
		TCACAGTATGCTGGCTGACC	
ERRα	NM_001170521.1	ACGAGTGCGAGATCACCAAG	130
		TTGTACTTCTGTCGCCCACC	
TFAM	NM001130211.1	AGCGAGGTCTGAAGAGTTGC	114
		TTGCACCCGTAGACAAAGCA	

GAPDH	NM_001206359.1	CCAAGGAGTAAGAGCCCCTG AAGTCAGGAGATGCTCGGTG	125
ACTB	XM_003124280.5	TGGAACGGTGAAGGTGACAG CTTTTGGGAAGGCAGGGACT	176

ACTB, beta actin; Cu/Zn-SOD, copper/zinc superoxide dismutase; ERRα, estrogen-related receptor alpha; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase modifier subunit; HO1, heme oxygenase 1; Mn-SOD, manganese superoxide dismutase; NQO1, NAD(P)H quinone dehydrogenase 1; NRF1, nuclear respiratory factor 1; PGC-1α, peroxisome proliferation activated receptor gamma coactivator-1 alpha, TFAM, mitochondrial transcription factor A.

[a]GenBank Accession Number.

[b]Shown as the forward primer followed by the reverse primer.