Supplementary Data

Supplementary Methods

Preparation of tissue lysates for determination of hepatic lipid content

Liver lysates were prepared by homogenization of 50 mg of frozen liver in 400 μL 0.9% NaCl and addition of 1000 μL of hexane/isopropanol (3:2 vol/vol, HIp). Samples were incubated for 45 min in the dark at room temperature and centrifuged for 15 min (4000 $\times g$, 15°C). The upper organic phase was collected, and lysates were again treated with 800 μL of HIp as indicated above. The two organic elution samples were pooled, evaporated using a SpeedVac (RC 10-10, Jouan, Fisher Scientific, Schwerte, Germany), and resuspended in 200 μL of HIp. For the analysis, 10 μL of the final sample was evaporated, resuspended in 5 μL of Triton (5%)/phosphate-buffered saline (PBS), and mixed with 500 μL of the kit reagent, as indicated in the manufacturer's instruction.

Preparation of tissue lysates for hepatic protein expression (western blot)

One hundred milligrams of fresh liver tissue was homogenized in 500 μL of homogenization buffer (pH 7.9) containing proteinase (PIC; 1:100, Sigma Aldrich, Steinheim, Germany) and phosphatase inhibitors (PhosSTOP; 1:10, Roche Diagnostics, Mannheim, Germany) for 2 min (Miccra D-1 homogenizer, ART, Müllheim, Germany) and incubated for 30 min on ice. Afterward, homogenates were centrifuged (4000 $\times g$, 1 min, 4°C), and the supernatant (cytosolic fraction) was stored at -80°C until further analysis. For whole-cell lysates, 30 mg of frozen hepatic tissue was homogenized in 400 μL of RIPA buffer (pH 7.2) containing proteinase and phosphatase inhibitors for 2 $\times$ 2 min at 25 Hz using the TissueLyser II (Qiagen, Hilden, Germany). Homogenates were incubated on ice for 30 min, and supernatant after centrifugation (12,000 $\times g$, 30 min, and 4°C) was stored at -80°C until further analysis. The protein concentration of the lysates was quantified using the Pierce™ BCA Protein Assay (Thermo Scientific, Waltham, MA).

Supplementary Table S1. Primer Sequences Used for qRT-PCR Analyses in Liver RNA

Gene symbol	*Gene name*	*Forward 5′-3′*	*Reverse 3′-5′*
Cat	Catalase	GGAGCAGGTGCTTTTGGATA	CTGACTCTCCAGCGACTGTG
Eef2	Eukaryotic translation elongation factor 2	GCGTGCCAAGAAAGTAGAGG	AAGATGGGGTCCAGGATGAG
Fads1	Fatty acid desaturase 1	CATGCCATACAACCATCAGC	CATCCAGGCCAAGTCCAC
Fasn	Fatty acid synthase	GATGGAAGGCTGGGCTCTAT	TGCCTCTGAACCACTCACAC
Gclc	Glutamate-cysteine ligase, catalytic subunit	GTGGAGGCCAATATGAGGAA	GGGTGCTTGTTTATGGCTTC
Gclm	Glutamate-cysteine ligase, modifier subunit	TCCCATGCAGTGGAGAAGAT	AGCTGTGCAACTCCAAGGAC
Gpx1	Glutathione peroxidase 1	CGGGACTACACCGAGATGAA	ACCAGGTCGGACGTACTTGA
Gpx4	Glutathione peroxidase 4	ATGAAAGTCCAGCCCAAGG	CGGCAGGTCCTTCTCTATCA
G6pc	Glucose-6-phosphatase, catalytic subunit	TCGGAGACTGGTTCAACCTC	TCACAGGTGACAGGGAACTG
Hspa1b	Heat shock protein 1B	TGCACTTGATAGCTGCTTGG	CAGTGCTGCTCCCAACATTA
Hspa5	Heat shock protein 5	GGCGTATTTGGGAAAGAAGG	CAGCTGCTGTAGGCTCATTG
Hspa8	Heat shock protein 8	CTCGGAAAGACCGTTACCAA	CACATCAAAAGTGCCACCTC
Nqo1	NAD(P)H dehydrogenase, quinone 1	TTCTCTGGCCGATTCAGAGT	TCCAGACGTTTCTTCCATCC
Pck1	Phosphoenolpyruvate carboxykinase 1, cytosolic	AGCCTTTGGTCAACAACTGG	TGCCTTCGGGGTTAGTTATG
Pdia3	Protein disulfide isomerase associated 3	AGCCAATGATGTGCCTTCTC	ATTCACGGCCACCTTCATAC
Scd1	Stearoyl-coenzyme A desaturase 1	CCTGCGGATCTTCCTTATCA	CAGTTTTCCGCC CTTCTCTT
Sod1	Superoxide dismutase 1, soluble	GGGTTCCACGTCCATCAGTA	CAGGTCTCCAACATGCCTCT
Rn18S	18S ribosomal RNA	GGTAACCCGTTGAACCCCAT	CAACGCAAGCTTATGACCCG

Supplementary Table S2. Composition of Buffers Used for Western Blot and Proteasome Activity Analyses

Analyses	*Buffer*	*Ingredients*
Western blot	Homogenization buffer	1.5 mM $MgCl_2$
		10 mM KCl
		0.5 mM dithiothreitol
		10 mM Hepes
		0.1% Nonidet P-40
	RIPA buffer	0.5 M Tris-HCl
		1.5 M NaCl
		5% Sodium deoxycholate
		1% Sodium dodecyl sulfate
		10% Nonidet P-40
		2 mM EDTA
	Loading buffer	0.5 M Tris-HCl
		8 % Glycerol
		1.6% Sodium dodecyl sulfate
		0.001% Bromophenol blue
		5% β-Mercapto ethanol
Proteasome activity	Lysis buffer	20 mM Tris-HCl
		10% Glycerol
		0.5 mM EDTA
		0.5% Nonidet P-40
		5 mM $MgCl_2$
		1 mM Dithiothreitol
		1 mM Adenosine triphosphate
	Reaction buffer	20 mM Tris-HCl
		5 mM $MgCl_2$
		1 mM Dithiothreitol
		1 mM Adenosine triphosphate

Supplementary Table S3. Primary Antibodies Used in the Western Blot Analyses of Liver Lysates

Name	*Manufacturer information*	*Dilution*
Akt (pan) (C67E7)	Cat. no. 4691, Cell Signaling	1:1000
Phospho-Akt (Ser473) XP®	Cat. no. 4060, Cell Signaling	1:2000
AMPK	sc-25792, Santa Cruz Biotechnology	1:100
Phospho-AMPK (Thr172)	Cat. no. 2535, Cell Signaling	1:1000
BiP/Grp78	Cat. no. 3183, Cell Signaling	1:1000
Lamp2a	ab18528, Abcam	1:1000
LC3	NB100-2220, Novus Biologicals	1:400
mTOR	Cat. no. 2972, Cell Signaling	1:1000
Phospho-mTOR (Ser2448) XP®	Cat. no. 5536, Cell Signaling	1:1000
p70 S6 Kinase	Cat. no. 2708, Cell Signaling	1:1000
Phospho-p70 S6 Kinase (Thr389)	Cat. no. 9234, Cell Signaling	1:500
Mup	sc-21856, Santa Cruz Biotechnology	1:500