DJ-1 Deficiency Protects Hepatic Steatosis by Enhancing Fatty Acid Oxidation in mice

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Supplementary figure 1 (A) Body weight of WT and DJ-1−/− mice fed on NCD for 40 weeks. (B, C) Cumulative (dark, 17:00 to 8:00; light, 8:00 to 17:00) daily food intake per mouse of WT or DJ-1−/− mice treated with NCD for 24 weeks.
Supplementary figure 2 The concentrations of (A) ALT and (B) AST in serum of WT and DJ-1−/− mice after NCD or HFD administration for 24 weeks.
Supplementary figure 3  (A) Liver weight of WT and DJ-1−/− mice after NCD administration for 40 weeks. (B) A representative image of H&E (top) and ORO (bottom) staining of liver sections after NCD treatment for 40 weeks.
Supplementary figure 4  (A) The mRNA expression of Dj-1 in liver. 
(B) Representative western blot (left) and quantification (right) for the expression of DJ-1 in livers from the WT mice after NCD or HFD administration for 24 weeks. β-actin was used as the loading control.
Supplementary figure 5 The intracellular TG content in mouse primary hepatocytes after treatment with (A) PA and (B) OA or vehicle for 48h.
Supplementary figure 6 (A) The percent of body weight loss related to body weight and (B) the absolute value of body weight loss of the indicated mice at the end of 24th week of NCD and HFD feeding.
Table S1. Primers for quantitative real-time PCR detection.

| Gene  | Sequence 5'---3' F | Sequence 5'---3' R |
|-------|-------------------|-------------------|
| β-actin | GTGACGTTGACATCCGTAAGA | GCCGGACTCATCGTACTCC |
| Dj-1 | GCTTCAAAAA GAGCTCTGGTC | ACATCAGGGCTACACTG |
| Srebp-1c | CACTTCTGGAGACATCGCAAAC | ATGGTAGACAAACAGCCGAC |
| Acly | CCAAAGCAATTTCAGAGCAGA | CAGGAGAGATTGACCCCGAC |
| Accα | GGCCAGTGCTATGCTGAGAT | AGGTTGAAGCTGTGCTCCA |
| Fasn | CTGCGGAAACTTCAAGAATG | GGTTGGGAATGCTATCCAGG |
| Pparγ | ATTCTGGCCCAACACCTCCG | TGGAAGGGCTATGCTTATCCCC |
| Gck | AGACGAAACACAGATAATTCC | GAAGCCCTTGGTCCAGTTGAG |
| Pfk1 | GAACTACGCACTTGACCACAT | CTCCAAAACAAAGGTCTCTGG |
| Pklr | GAACATTGCACGACTCAACTTC | CAGTGCGTATCTGGGACC |
| Ucp2 | GCTGGTGGTGTTGGTGAGATA | ACTGGGCAAGCGAGATTT |
| Ppara | TATTCGGCTGAAGGCTTGATAC | CTGGCATTTTGCTCCGTTT |
| Acox1 | GTCTCCGCTGATGAATGACG | TGCGATGCCAAATTCCCTGA |
| Mcad | AACATGCGCGAGCAAGTCTCTC | AGGCGACTACTTGGGCTTCC |
| Cpt1α | AGGACCCCTGAGGAGCATCTTTT | ATGACCTCCTGGCATTTCC |
| Pdk4 | TTCACACCTTACCACATGC | AAAGGGCGGTTTTCTTGTAG |
| Cs | GGCAATTATTCTCAGAATCTGC | TCGTTTCATCTCCCTGCTAAT |
| Idh1 | ATGCAAGGAAGATGAAACTGCACG | GCATCAGATTCTCTTGCTAAA |
| Idh2 | ATCAAGGAGAAGCTCATCCTGC | TCTGTGGCCTTGACTGGTCTG |
Materials and Methods

Mice and animal models.

DJ-1 knockout (DJ-1<sup>−/−</sup>) mice on a C57BL/6 background (stock#006577) were purchased from the Jackson Laboratory (Bar Harbor, Maine, USA). Wild-type (WT) mice were purchased from SLAC laboratory (Shanghai, China). 4- to 6-week-old male mice (14-16g) were housed in a standard environment at 22 to 24°C with a 12:12 h light-dark cycle and ad libitum access to food and water. The WT mice and DJ-1<sup>−/−</sup> mice were fed with HFD (18.1% protein, 61.6% fat and 20.3% carbohydrates; D12492, Research Diets, New Brunswick, NJ) for 24 weeks to establish a mouse model of NAFLD. Mice fed with normal chow diet (NCD) were served as controls. All animal protocols were approved by the criteria outlined in Guide for the Care and Use of Laboratory Animals, which was approved by Bioethics Committee School of Medicine, Shanghai Jiao Tong University.

Mouse hepatic and serum lipid contents analysis.

Liver and blood specimens were collected from mice after fed NCD or HFD for 24 weeks, and stored at -80°C until use. The lipid contents of liver and serum were measured by using commercial kits (E1003 for serum TG, E1013 for liver TG, E1005 for serum TC, E1015 for liver TC; Applagin Technologies Inc., Beijing, China; A113-1 for serum low-density lipoprotein cholesterol (LDL-C), A112-1 for serum high-density lipoprotein cholesterol (HDL-C); Nanjing
Histopathologic analysis.

The liver sections were embedded in paraffin and cut into 5-μm-thick sections. Liver tissue sections were stained with hematoxylin-eosin (H&E) and periodic acid–Schiff (PAS) using standard procedures. Oil red O (ORO)(O0625; Sigma-Aldrich, St Louis, MO) staining of frozen liver sections were performed according to previously described protocol [14].

Glycogen contents assay.

The levels of glycogen in the liver were measured using a commercially available glycogen Assay Kit (MAK016, Sigma-Aldrich, St Louis, MO) according to the manufacturer's instructions.

qPCR and western blot analysis.

Total RNA from liver tissue was isolated with the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Reverse transcription was performed using random primers using the PrimeScript RT reagent Kit (Takara). SYBR-green based Quantitative real time-PCR (Takara) was applied to detect the relative expression levels of genes. The mRNA expression levels of target genes were normalized to β-actin. Primer
sequences are shown in Table S1. Liver tissues or cell samples were homogenized with RIPA buffer (Thermo Scientific, Rockford, IL) containing a protease inhibitor cocktail (Caliche, Raleigh, NC) and phosphatase inhibitor cocktail (HY-K0021; Med Chen Express). The Pierce BCA Protein Assay Kit (#23227, Thermo Fisher Scientific) was used to measure total protein concentrations. Protein samples (40 µg) were separated on a 10% SDS-PAGE gel. The gel was transferred to a nitrocellulose membrane. The NC membrane was blocked with 5% non-fat milk for one hour at room temperature, and then incubated with primary antibodies against indicated antigens at 4 °C overnight under shaking conditions. The membrane incubation with the corresponding secondary antibodies coupled with horseradish peroxidase for one hour at room temperature then. The Cheviot XRS+ System with Image Lath Software (Bio-Rad) was used for blotting signal detection and quantification. Primary antibodies were rabbit anti-DJ-1 (Abcam, Cambridge, MA), rabbit anti-SREBP-1 (Santa Cruz, CA), rabbit anit-ACLY (Proteintech, Wuhan, China), IRS1(Proteintech, Wuhan, China), p-IRS1(Tyr608)(Millipore, Billerica, MA), p-AKT (Ser473) (Cell Signaling Technology, Beverly, MA), AKT (Cell Signaling Technology, Beverly, MA), anti-β-actin (Sigma-Aldrich, St Louis, MO).

Glucose tolerance tests and Insulin tolerance tests.

The mice used for the glucose tolerance tests had been fasted for 16 h
(17:00-09:00) with ad libitum access to water. The fasting blood glucose levels were measured, and then each mouse was intraperitoneally injected with 2.0 g per kg body weight (g kg\(^{-1}\)) glucose (Sigma-Aldrich, St Louis, MO, USA). We measured the levels of blood glucose 15, 30, 60 and 120 min after glucose injection using a glucometer. For the insulin tolerance tests, mice were fasted for 4 h (09:00-13:00) and then intraperitoneally injected 0.75U kg\(^{-1}\) human insulin (Novolin R, Novo Nordisk, Bisgaard, Denmark). Blood glucose levels were measured 0, 15, 30, 60 and 120 min after insulin injection.

**In vitro lipid synthesis assays.**

Mouse primary hepatocyte were isolated from 6- to 8-weeks WT or DJ-1\(^{-/}\) mice similarly to a previously published procedure[8]. Isolated primary hepatocytes were seed into 6-well plates with DMEM medium treated with 0.25 mM palmitate (P9767, Sigma-Aldrich) for 48h, as previously reported [15]. The intracellular TG levels were measured using commercial kits (E1013, Applagin Technologies Inc.) according to the manufacturer's instructions.

**Statistical analysis.**

All results were presented as mean ± SEM from at least 3 independent biological replicated experiments. Unpaired or paired Student’s t-tests were used in this study. P<0.05 was considered statistically significant (*P<0.05; **P<0.01; ***P<0.001).
