

Supplementary Methods

Oil Red O staining

Liver tissues were frozen in Tissue-Tek O.C.T. compound and cut into 10- μ m sections. Slices were fixed with ice-cold 10% formalin. The slices were washed with 60% isopropanol and then stained with Oil Red O solution for 5 minutes (Sigma, St. Louis, MO, USA; Cat. #O0625). Following another 60% isopropanol wash, nuclei were stained with hematoxylin, and slices were mounted. Oil Red O positive area was calculated from at least three adjacent sections using ImageJ software (V1.8.0; National Institutes of Health, Bethesda, MD, USA).

Liver TG detection

Fresh liver samples were weighed and homogenized in phosphate buffered saline (PBS) on ice. The samples were heated to 80°C in a water bath for 5 minutes and then cooled down to room temperature. The soluble fraction was collected following centrifugation (10,000 rpm, 5 minutes), and the protein was detected with a bicinchoninic acid (BCA) assay using bovine serum albumin as a reference. Samples were diluted with PBS to achieve equal protein concentrations, and triglyceride (TG) was quantified with the Triglycerides Assay Kit (Nanjing Jiancheng Bio. Co., Nanjing, China; Cat. #F001-1-1). In brief, 2.5 μ L of the sample was incubated with 200 μ L of the working solution for 10 minutes at 37°C, and the absorbance at 510 nm was determined. TG concentration was determined via comparison to a standard curve.

ELISA

Human serum MPO-DNA (Boster, Pleasanton, CA, USA; Cat.

#EK0850) and serum interleukin (I)L-6 (Elabscience, Wuhan, China; Cat. #E-EL-M0044c) and mouse liver MPO-DNA (Boster; Cat. #EK0943) levels were detected by the ELISA kit according to the manufacturer's instructions. Briefly, liver samples were weighed and homogenized in the cell extraction buffer with protease inhibitors on ice. After the samples were vortexed for 30 minutes, the supernatant was collected with centrifugation at 5,000 rpm, and the protein content was quantified with a BCA assay using bovine serum albumin as a reference. The samples were diluted with PBS to achieve equal protein concentrations. First, 96-well plates were coated with the coating solution, and the plates were then washed with washing buffer. Next, the wells were blocked with blocking buffer, and the standards and samples (100 μ L) were added into the wells. After incubation, the wells were washed, and the detection antibody solution was added, followed by the addition of streptavidin-horseradish peroxidase solution. Next, 3,3',5,5'-tetramethylbenzidine substrate solution was added, and the stop solution was added after 30-minute incubation. Absorbances were read at 450 nm. Concentrations were determined based on a standard curve.

Determination of serum alanine aminotransferase (ALT) activity

Human and mouse sera, diluted in 1:20 ratio, were assayed for ALT using an ALT Activity Assay Kit (Elabscience; Cat. #E-BC-K235-M) according to the manufacturer's instructions. In brief, samples of the diluted sera (20 μ L) were incubated with 100 μ L of working solution for 5 minutes. ALT was quantified by measuring the resulting fluorescence (λ_{ex} =535 nm; λ_{em} =587 nm).