Appendix 1

Biochemical assay

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and triglyceride (TG) were analyzed with an automatic biochemical analyzer (Hitachi, Tokyo, Japan) in the clinical laboratory center of Capital Medical University. The hepatic TG content was detected with a Triglyceride Quantification Colorimetric/Fluorometric Kit (BioVision, San Francisco, CA, USA). Hepatic ATP was measured with a CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA). Hepatic malondialdehyde (MDA), reduced glutathione (GSH), oxidized glutathione (GSSG) levels and superoxide dismutase (SOD) activity were determined with the relevant assay kits (BioVision).

Appendix 2

Immunohistological staining

Immunohistochemical staining was performed as described previously (26). Primary antibodies directed against Flag (Sigma-Aldrich, Saint Louis, MO, USA), cleaved caspase-3, Proliferating Cell Nuclear Antigen (PCNA) (Cell Signaling, Danvers, MA, USA) and 4-hydroxynonenal (4-HNE; BIOSS, Beijing, China),were used. Positively stained areas were carefully analyzed with the Image-Pro Plus software (Media Cybernetics, Bethesda, MD, USA). For the apoptotic assay, terminal-deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) was performed with the In Situ Cell Death Detection Kit (Roche, Basel, Switzerland).

Western blotting

Proteins were extracted in RIPA lysis buffer. Equivalent amounts of 80 μg total proteins were separated by sodium dodecyl sulfate-polyacrylamide gels electrophoresis and transferred to PVDF membranes. Specific antibodies against Flag (Sigma-Aldrich), ALR (Proteintech, Rosemont, IL, USA), Bax (Santa Cruz Biotechnology, Santa Cruz, CA, USA), Bcl-2, cleaved caspase-3 and MnSOD (Cell Signaling) were used to detect the immunoreactive proteins and were revealed by enhanced chemiluminescence reagents. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or β actin expression served as internal controls. The images representative of at least 3 experiments

are shown.

Supplementary Tables

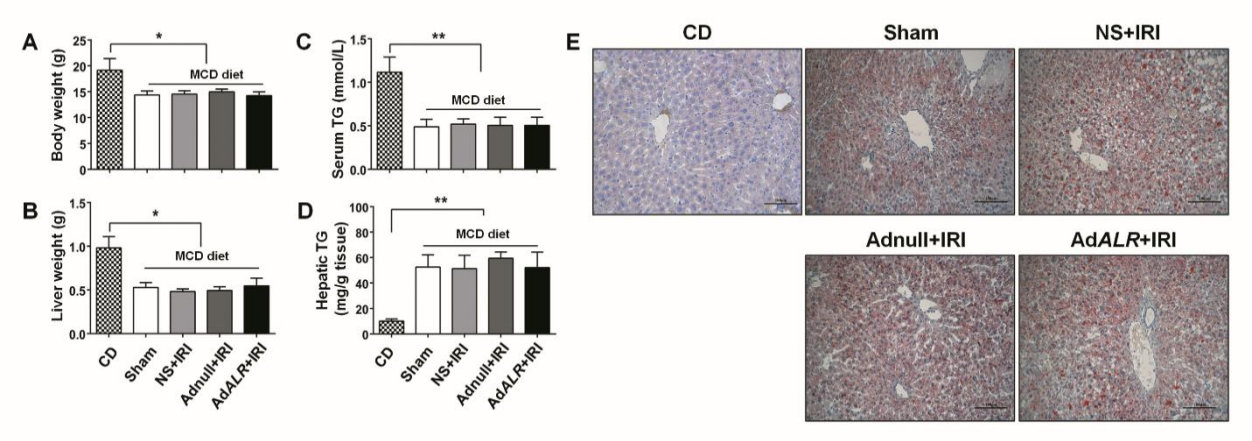
Table S1: Adenovirus transfection did not impair liver function and renal function.

Parameters	Normal Saline	Adnull	p value
ALT (U/L)	348.40 ± 17.70	344.00 ± 29.40	0.78
AST (U/L)	372.75 ± 30.85	398.00 ± 36.68	0.31
LDH (U/L)	1328.68 ± 245.84	1378.92 ± 274.55	0.76
BUN (mmol/L)	8.92 ± 1.35	8.31 ± 1.06	0.50
Crea (μmol/L)	17.14 ± 2.04	15.52 ± 3.20	0.37

Mice fed with MCD for 2 weeks were randomly divided into 2 groups. Adnull group, in which mice was injected adenovirus (1×10^{10} pfu) via tail vein and the Normal Saline group, in which mice were given equal amount of normal saline solution. Three days later, liver and renal function indexes were measured. The function of liver (ALT, ASL and LDH) and renal (BUN, Crea) remained unchanged in mice with Adnull group as compared with mice in Normal Saline group.

Supplementary Figures

Figure S1: ALR gene transfection had no significant effect on the MCD diet-induced liver steatosis. C57BL/6 mice were fed the MCD diet for 2 weeks. (A) Body weight, (B) Liver weight, and (C) Serum TG were analyzed. ALR gene transfection did not alleviate liver steatosis as shown by (D) hepatic TG contents and (E) Oil Red O staining. Scale bars indicate 100 μm. $^{*}p < 0.05$, $^{**}p$



< 0.01. Mean ± SD. (Animals in each group = 6).

Figure S2: Hepatocyte steatosis was established in HepG2 cells. HepG2 cells stably transfected either with ALR or vector-plasmid (as the control) were treated with 0.3mM OA/PA (ratio 2:1) for 6h. (A) Western blotting analysis of ALR expression in the cells. (B) TG levels in the cells. (C) Lipid droplets were tested by Oil Red O. $^{*}p < 0.05$, $^{**}p < 0.01$.

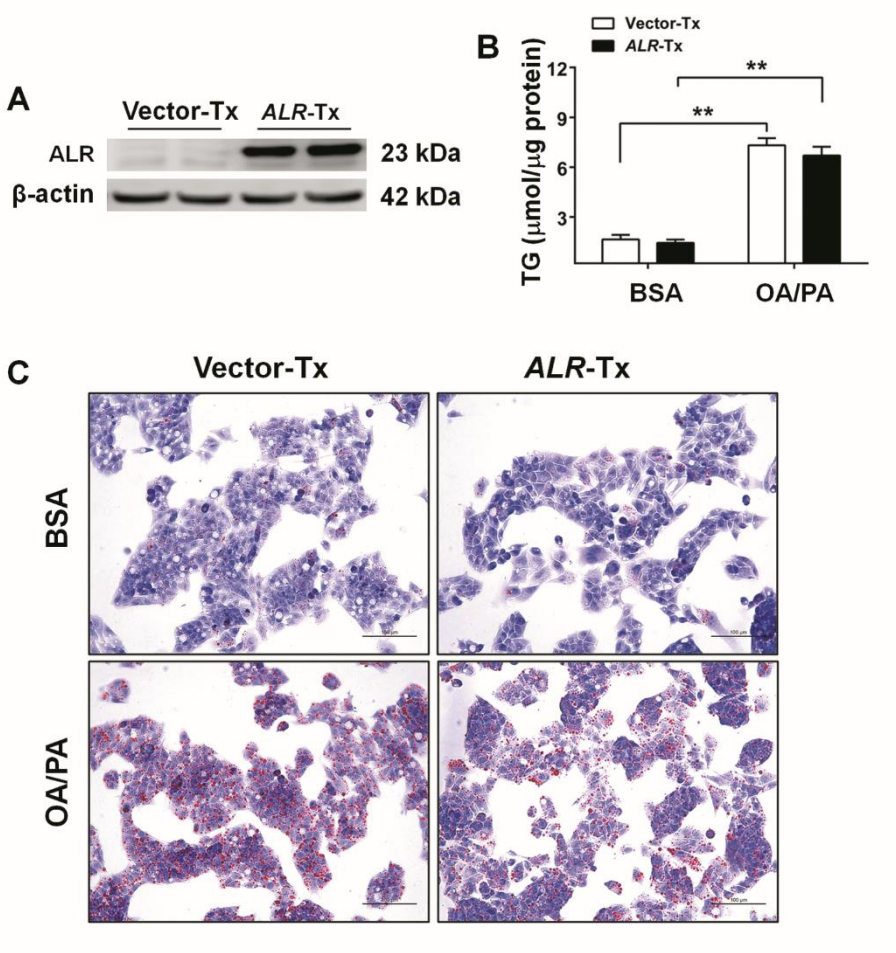


Figure S3: Dose-response protective effects of AdALR in steatosis liver underwent IRI. Three days before IRI, AdALR in a dose of 1×10^4, 1×10^6, 1×10^8, 1×10^{10} pfu respectively were injected intravenously. The expression of exogenous ALR in the liver (A), the liver function (B, C, D) and TUNEL-positive cells (E) were detected. The protective effects of ALR exhibited at 1×10^8 pfu, and the maximal protective effects occurred at 1×10^{10} pfu.