Rev-erbα Mediates Steatosis in Alcoholic Fatty Liver Through Regulating Autophagy

xue Qing
anhui medical university

Lei Xu
Anhui Yike Daxue: Anhui Medical University

fei mei Wu
Anhui Yike Daxue: Anhui Medical University

Wen yi Zhou
Anhui Yike Daxue: Anhui Medical University

fa jun Yang
Anhui Yike Daxue: Anhui Medical University

cheng huang
Anhui Yike Daxue: Anhui Medical University

Tao Xu
Anhui Yike Daxue: Anhui Medical University

jun Li
Anhui Yike Daxue: Anhui Medical University

Lei Zhang (zhanglei-1@ahmu.edu.cn)
School of Pharmacy, Anhui Institute of Innovative Drugs, Anhui Medical University, Hefei 230032, China
https://orcid.org/0000-0002-9778-5726

Research

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Abstract

AFL is a liver disease caused by long-term excessive drinking, it is characterized by steatosis. Understanding the regulatory mechanism of steatosis is crucial for the treatment of AFL. Rev-erbα has been implicated in regulation of lipid metabolism. However, the role and the underlying mechanisms of Rev-erbα in AFL remains unknown. In this study, the antagonists or agonists of Rev-erbα as well as Rev-erbα shRNA were applied in vitro and vivo. Triglyceride and lipid droplets accumulation were measured by using TG kit and ORO staining. Lipid synthesis related factor Srebp1c and lipid β-oxidation regulatory factor Pparα were measured by using Western blot, qRT-PCR and immunohistochemistry analysis. Autophagy activity was measured by western blot and electron microscope, and lysosomal probe was used to labeled lysosomal acidity. We observed that the expression of Rev-erbα was significantly increased in vivo and vitro, and Rev-erbα activation mediated steatosis in L-02 cells. then, inhibition/down-expression of Rev-erbα improved the triglyceride and lipid droplets accumulation and the abnormal expression of Pparα and Srebp1c through enhancing the autophagy activity. Furthermore, down-expression of Rev-erbα up-regulated the nuclear expression of Bmal1, which regulated the autophagy activity in vitro. Collectively, these findings indicate that Rev-erbα induces liver steatosis and leads to the progression of AFL. Our study reveals a novel steatosis regulatory

mechanism in AFL and suggest that Rev-erbα might be a potential therapeutic target for AFL.

Full Text

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Figures

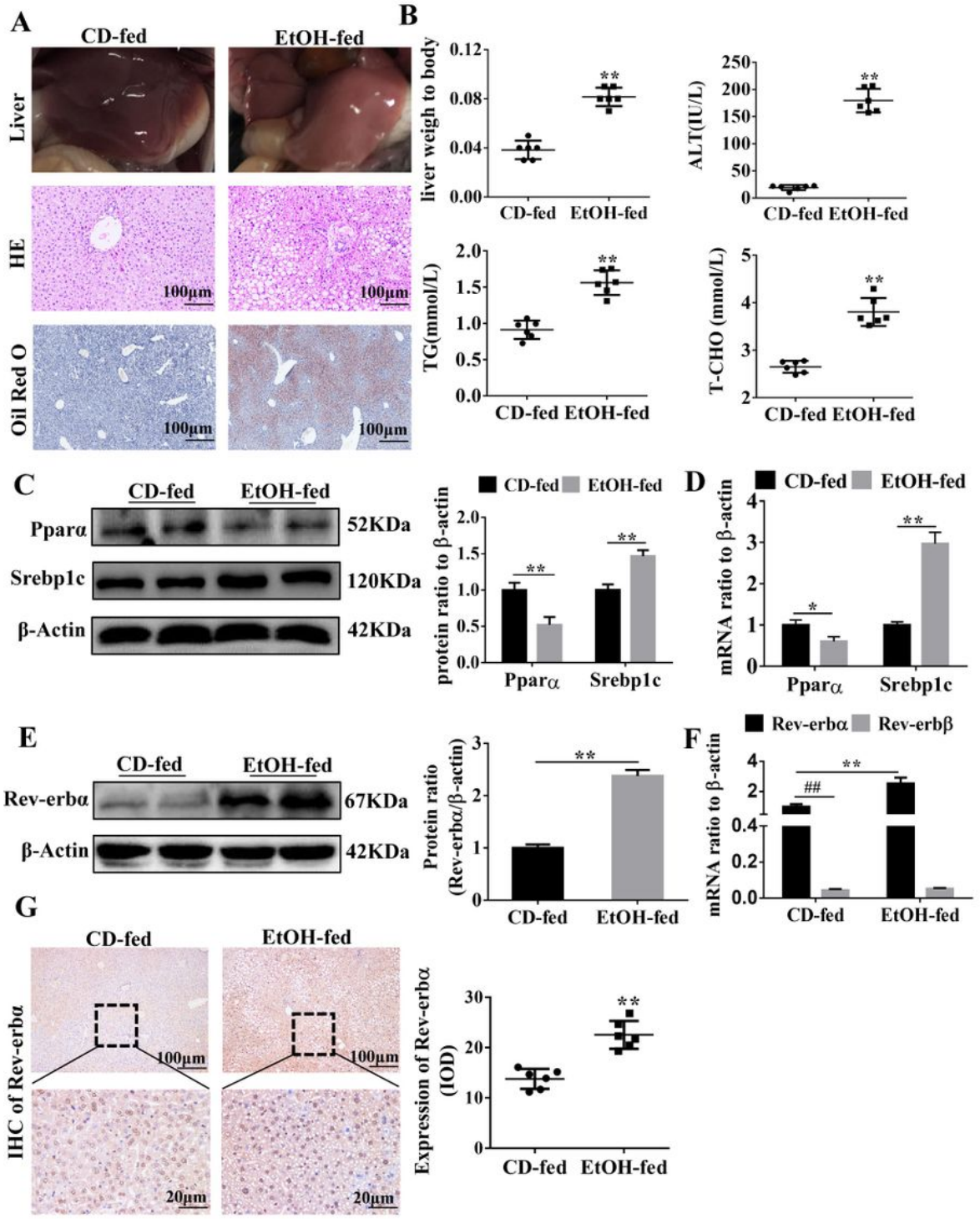

Figure 1

Rev-erbα was up-regulated in the liver of EtOH-fed mice in vivo. C57 male mice weigh 20 kg or more were fed EtOH liquid diet or the Control diet for sixteen days. (A) Representative image of liver and histological assessment of hepatic pathologic alterations by H&E and ORO staining in alcoholic fatty liver mice (scale bar =100 μm). (B) The liver weight ratio to body of mice, serum ALT, TG and TC levels in CD-fed mice and EtOH-fed mice. (C) (D) Western blot and qRT-PCR analysis of Pparα and Srebp1c in CD-fed mice

and EtOH-fed mice. (E) Western blot analysis of Rev-erbα in CD-fed mice and EtOH-fed mice. (F) qRT-PCR analysis of Rev-erbα and Rev-erbβ in CD-fed mice and EtOH-fed mice. (G) HIC shows the expression of Rev-erbα in CD-fed mice and EtOH-fed mice (scale bar =100 μm or 20 μm). (n=6). Bar represents the mean ± S.E.M.. Significance * P < 0.05, ** P < 0.01 vs. CD-fed group. # P < 0.05, ## P < 0.01 vs. Rev-erbα in CD-fed group.

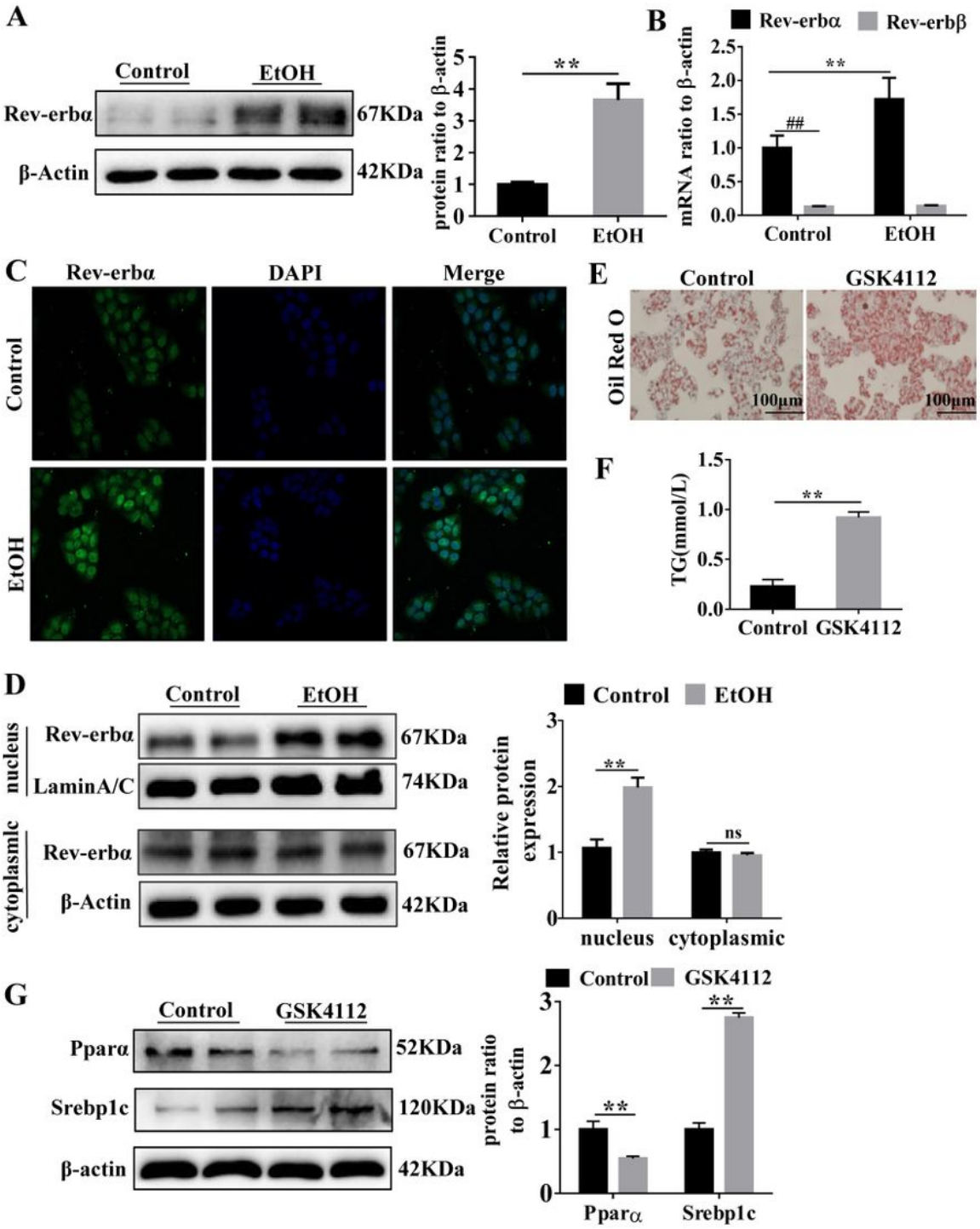

Figure 2

Rev-erbα was up-regulated in EtOH-treated L-02 cells and mediated liver steatosis in vitro. L-02 cells were treated with 150 mM EtOH for 48 h. (A) Western blot analysis of Rev-erbα expression in Control and EtOH group. (B) qRT-PCR analysis of Rev-erbα and Rev-erbβ in Control and EtOH group. (C) Immunofluorescence analysis of nuclear localization of Rev-erbα in Control and EtOH group (scale bar =40 μm). (D) Western blot analysis of Rev-erbα in nucleus and cytoplasm in Control and EtOH group. L-02 cells were treated with or without 10 μM GSK4112 for 24 h. (E) (F) ORO staining and TG content analysis in Control and GSK4112 group (scale bar =100 μm). (G) Western blot analysis of Pparα and Srebp1c in Control and GSK4112 group. (n=3). Bar represents the mean ± S.E.M.. Significance * $P < 0.05$, ** $P < 0.01$ vs. Control group , # $P < 0.05$, ## $P < 0.01$ vs. Rev-erbα in Control group.

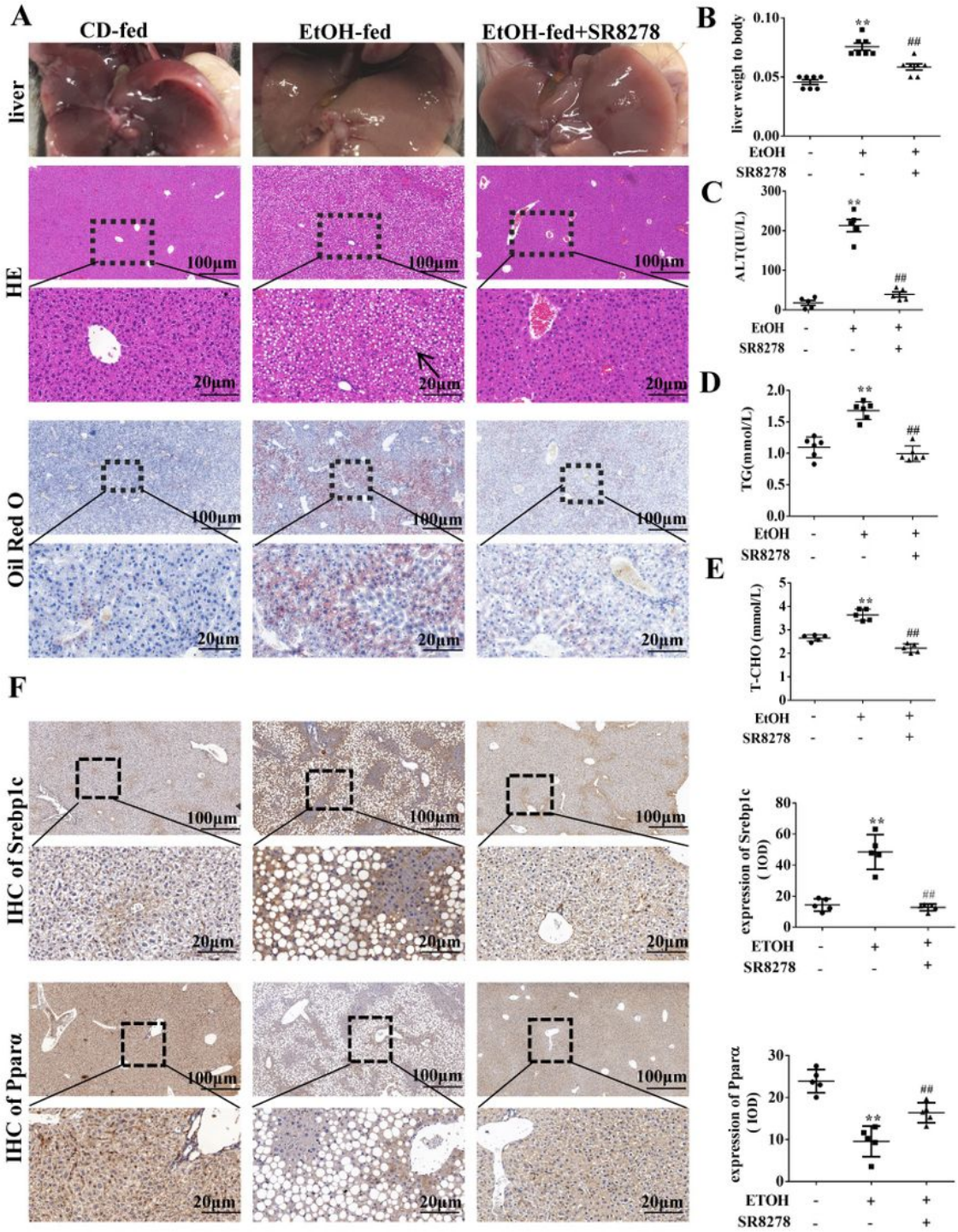

Figure 3

SR8278 (Rev-erbα antagonist) attenuates steatosis in the liver of EtOH-fed mice and EtOH-treated L-02 cells. Mice fed EtOH liquid were tail vein injection with SR8278 (2 mg/kg) for 3 days. (A) Representative image of liver and histological assessment of hepatic pathologic alterations by H&E and ORO staining in the liver of mice with or without SR8278 injection (scale bar =100 μm or 20 μm). (B-E) The liver weight ratio to body of mice, serum ALT, TG level and TC levels in the liver of mice. (F) The

immunohistochemistry staining of Pparα and Srebp1c in the liver of mice (Scale bar =100 μm or 20 μm). (n=6). Bar represents the mean ± S.E.M.. Significance * P < 0.05, ** P < 0.01 vs. CD-fed group. # P < 0.05, ## P < 0.01 vs. EtOH-fed group.

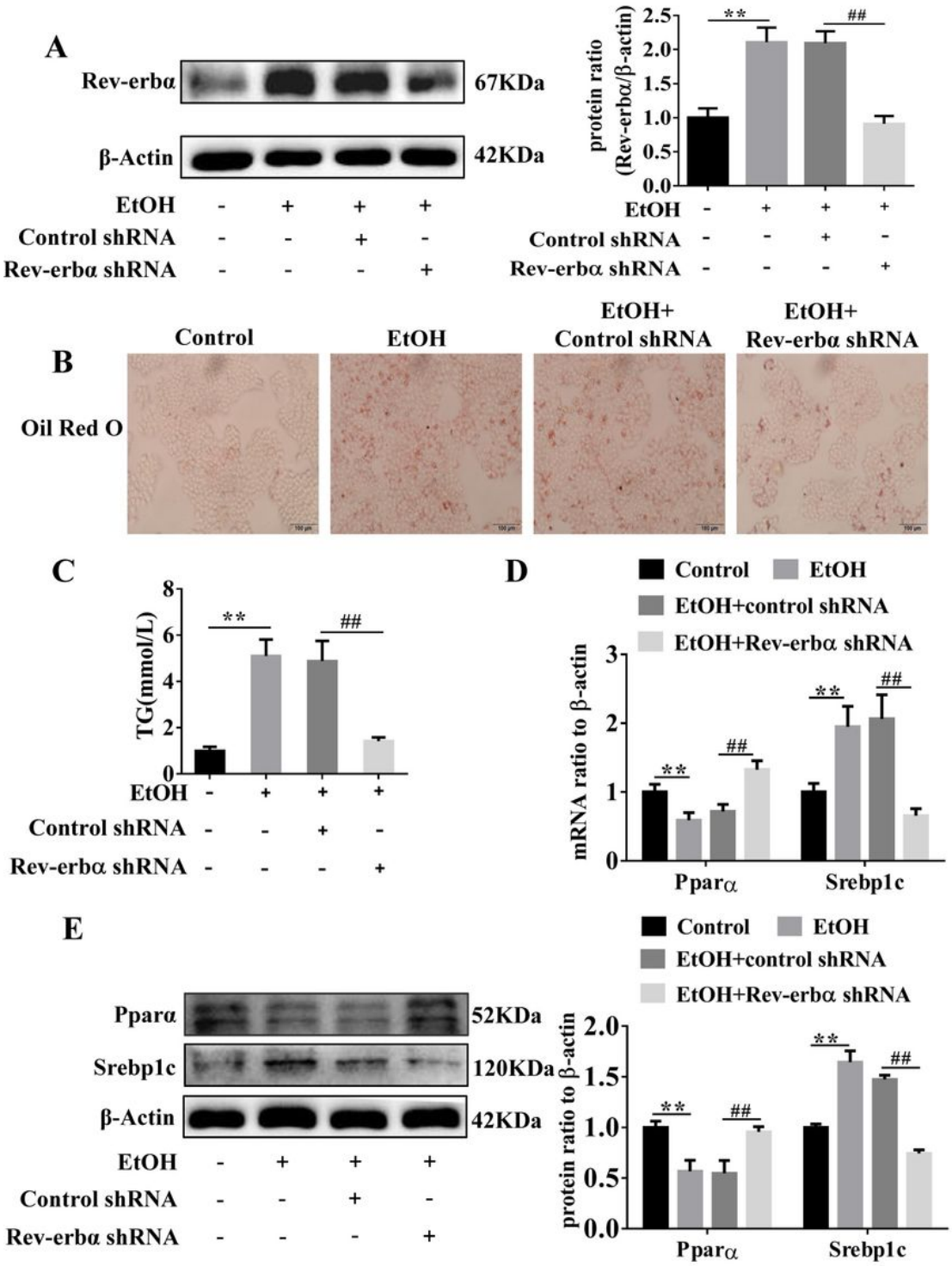

Figure 4

Down-regulated Rev-erbα attenuates steatosis in the EtOH-treated L-02 cells. (A) Western blot analysis of Rev-erbα in EtOH-treated L-02 cells transfected with Rev-erbα shRNA. (B) (C) ORO staining and TG content

analysis in EtOH-treated L-02 cells after Rev-erbα knockout (magnification x 200, Scale bar =100 μm). (D) (E)Western blot and qRT-PCR analysis of Pparα and Srebp1c in EtOH-treated L-02 cells transfected with Rev-erbα shRNA. (n=3). Bar represents the mean ± S.E.M.. Significance * P < 0.05, ** P < 0.01 vs. Control group. # P < 0.05, ## P < 0.01 vs.control shRNA group.

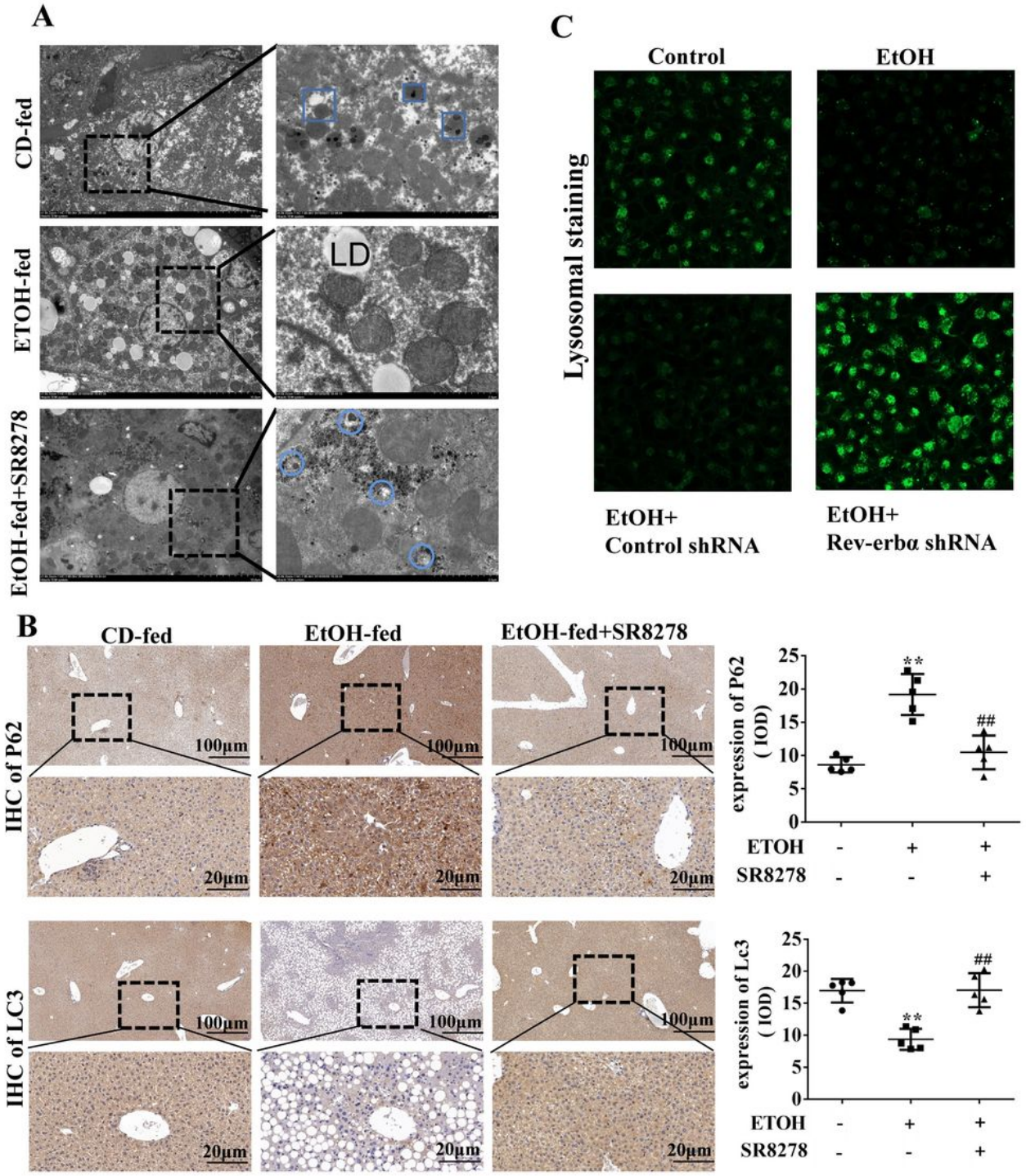

Figure 5

Rev-erbα regulated lipid metabolism by enhancing autophagy activity in vivo and vitro (A) Electron microscopy of liver tissues of EtOH fed mice injected with or without SR8278 at 10 μm and at 2 μm. (N: nucleus; M: mitochondria; LD: lipid droplet; triangle refers to autophagy; arrow refers to lysosome). (n≥6). (B) Histopathological analysis of P62 and Lc3 by immunohistochemistry in the liver of EtOH-fed mice injected with or without SR8278 (scale bar =100 μm or 20 μm). Bar represents the mean ± S.E.M.. Significance * P < 0.05, ** P < 0.01 vs. CD-fed group. # P < 0.05, ## P < 0.01 vs. EtOH-fed group. (C) Lysosome staining with Lyso Tracter Green DND-26 in EtOH-treated L-02 cells transfected with Rev-erbα shRNA (scale bars =40 μm). (n ≥3). Bar represents the mean ± S.E.M.. Significance * P < 0.05, ** P < 0.01 vs. Control group. # P < 0.05, ## P < 0.01 vs. control shRNA group.

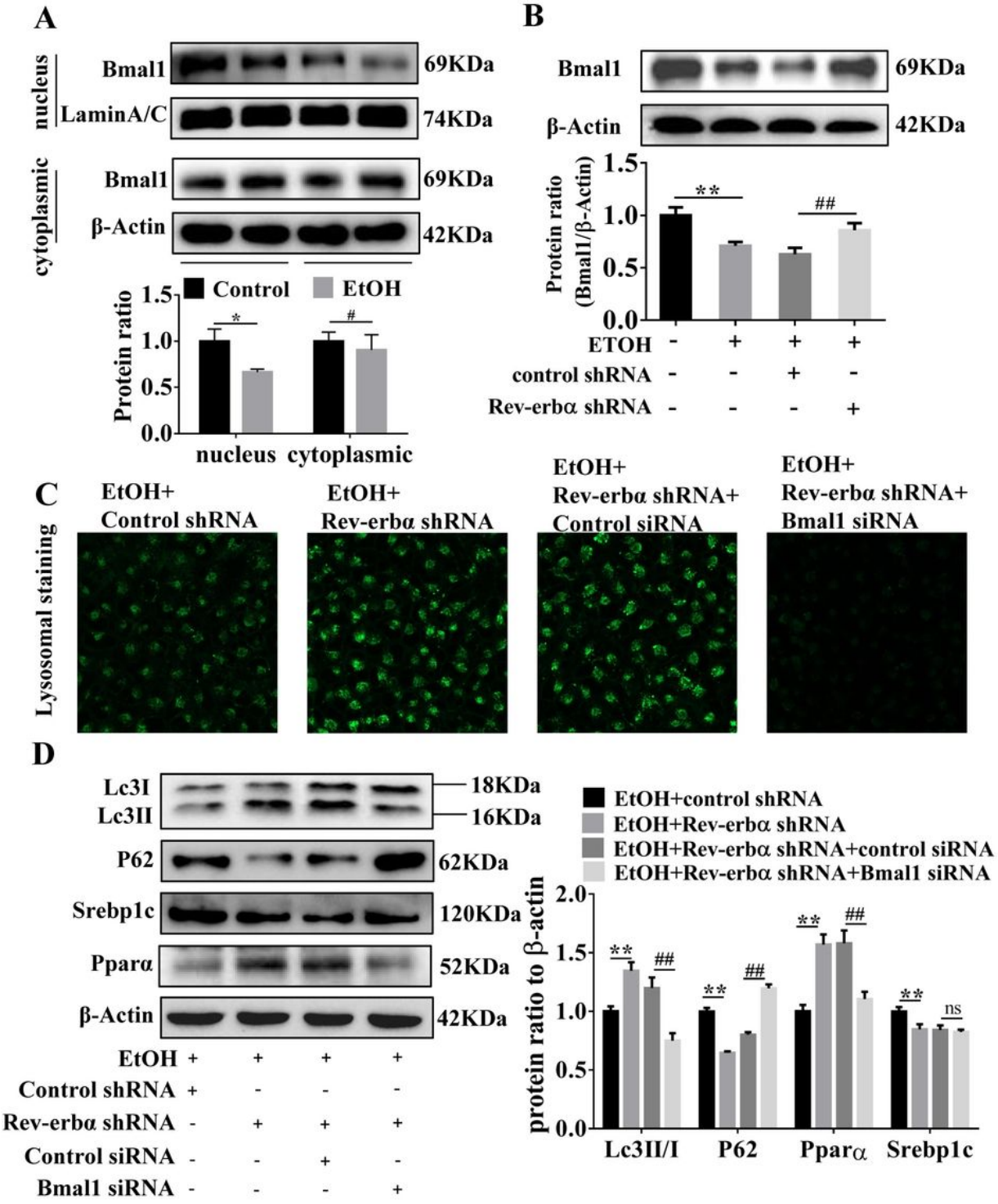

Figure 6

Rev-erbα inhibits the activity of autophagy through regulating Bmal1. (A)Western blot analysis of Bmal1 in nucleus and cytoplasm in EtOH-treated L-02 cells. Bar represents the mean ± S.E.M.. Significance * P < 0.05, ** P < 0.01 vs. EtOH-fed group or EtOH group. (B) Western blot analysis of Bmal1 in EtOH-treated L-02 cells transfected with Rev-erbα shRNA. Bar represents the mean ± S.E.M.. Significance * P < 0.05, ** P < 0.01 vs. Control group, # P < 0.05, ## P < 0.01 vs. Control shRNA group. (C) Lysosome staining by using

Lyso Tracter Green DND-26 (50 nM) in EtOH-treated L-02 cells transfected with Bmal1 siRNA and Rev-erbα shRNA (n=3) (scale bars = 40 μm). (D) Western blot analysis of Lc3II/I, P62, Pparα and Srebp1c in EtOH-treated L-02 cells transfected with Bmal1 siRNA and Rev-erbα shRNA. Bar represents the mean ± S.E.M.. Significance * P < 0.05, ** P < 0.01 vs. EtOH+Control shRNA group, # P < 0.05, ## P < 0.01 vs. EtOH+Rev-erbα shRNA+Control siRNA group.

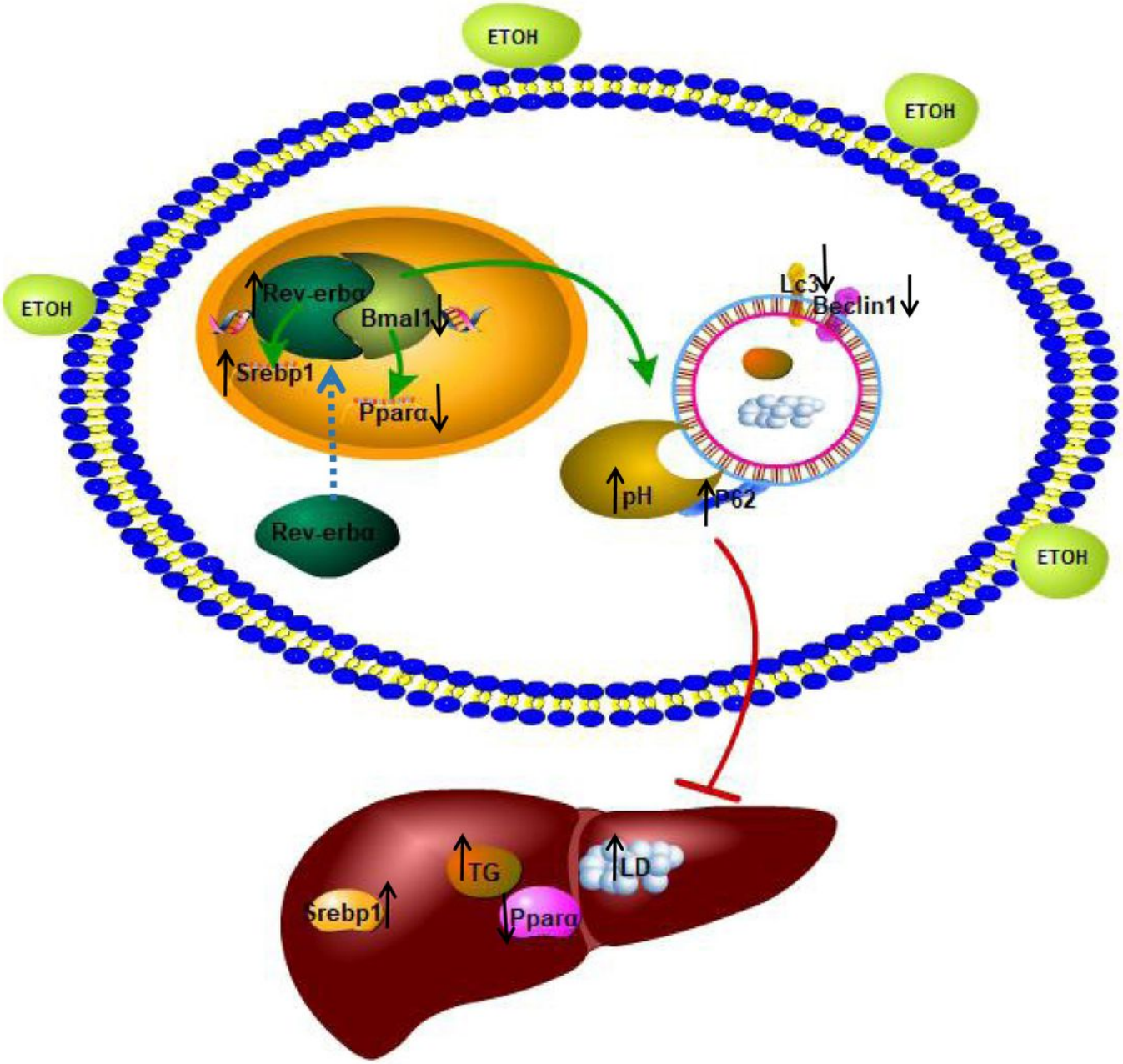

Figure 7

Schematic diagram of the molecular mechanism of Rev-erbα-induced lipid steatosis in the liver. Rev-erbα was enhanced by EtOH and bound to the Bmal1 promoter to regulated the activity of autophagy to regulated lipid metabolism.

Supplementary Files

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- table1.pdf
- supplementfigures.pdf