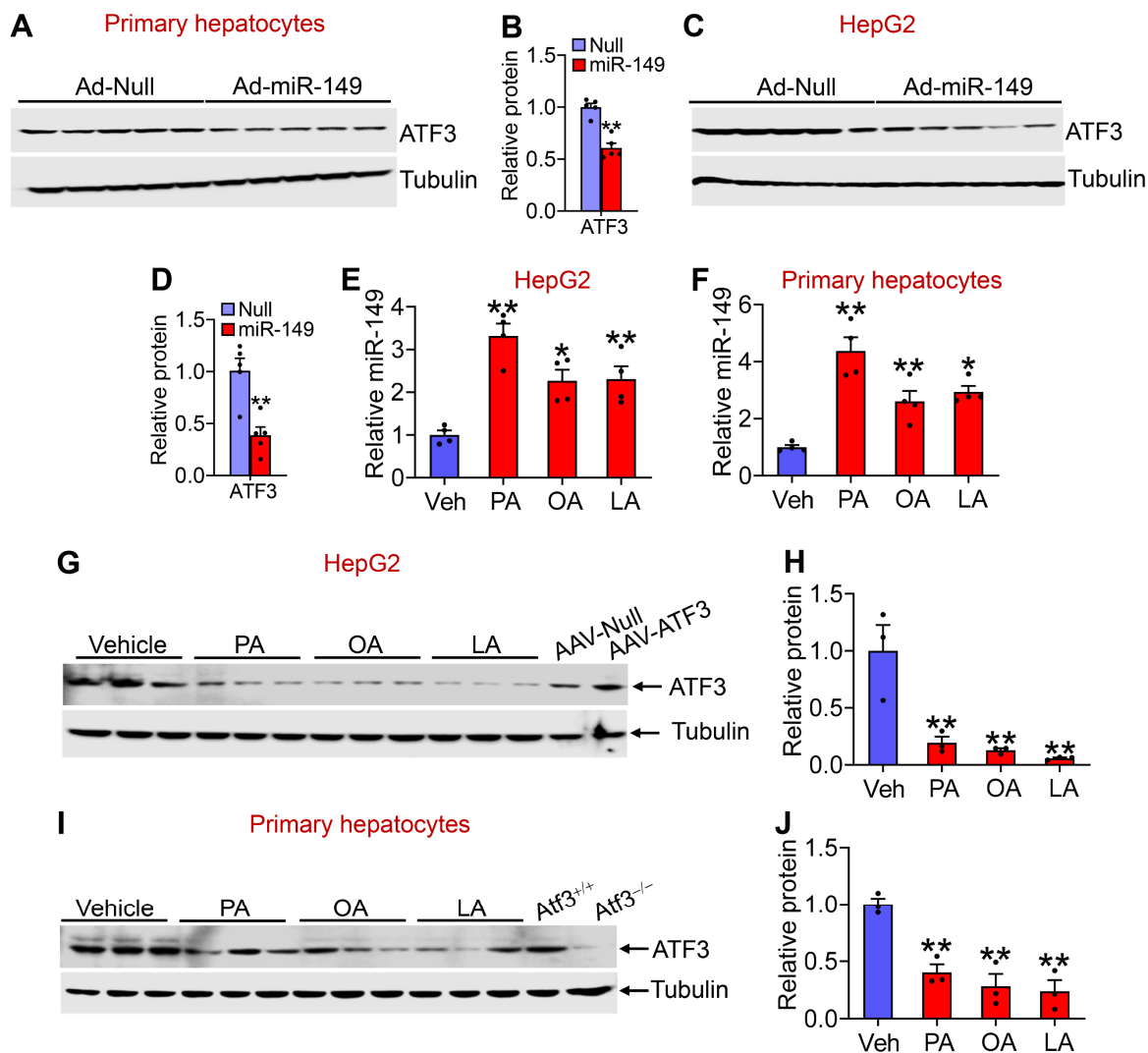**Supplementary Information**

Hepatocytic activating transcription factor 3 protects against steatohepatitis via hepatocyte nuclear factor 4α

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Figure S1. MiR-149 inhibits ATF3 expression and is induced by free fatty acids

A-D: Mouse primary hepatocytes (A, B) or HepG2 cells (C, D) were infected with Ad-Empty or Ad-miR-149 for 24 h. Western blot assays (A, C) were performed and data were quantified (B, D) (n=5).

E and **F:** HepG2 cells (E) or mouse primary hepatocytes (F) were treated with either vehicle or 300 μM palmitic acid (PA), oleic acid (OA) or linoleic acid (LA) for 24 h (n=4), and miR-149 levels were determined.

G-J: HepG2 cells (G, H) or mouse primary hepatocytes (I, J) were treated with either vehicle or 300 μM PA, OA or LA for 24 h (n=3). Western blot assays were performed (G, I) and data were quantified (H, J). In (G), liver lysates from mice infected with AAV8-ALB-Null or AAV8-ALB-hATF3 were used for positive controls. In (I), liver lysates from $Atf3^{+/+}$ or $Atf3^{-/-}$ mice were used for a positive control.

All values are expressed as mean±SEM. *P<0.05, **P<0.01 versus the control or vehicle group
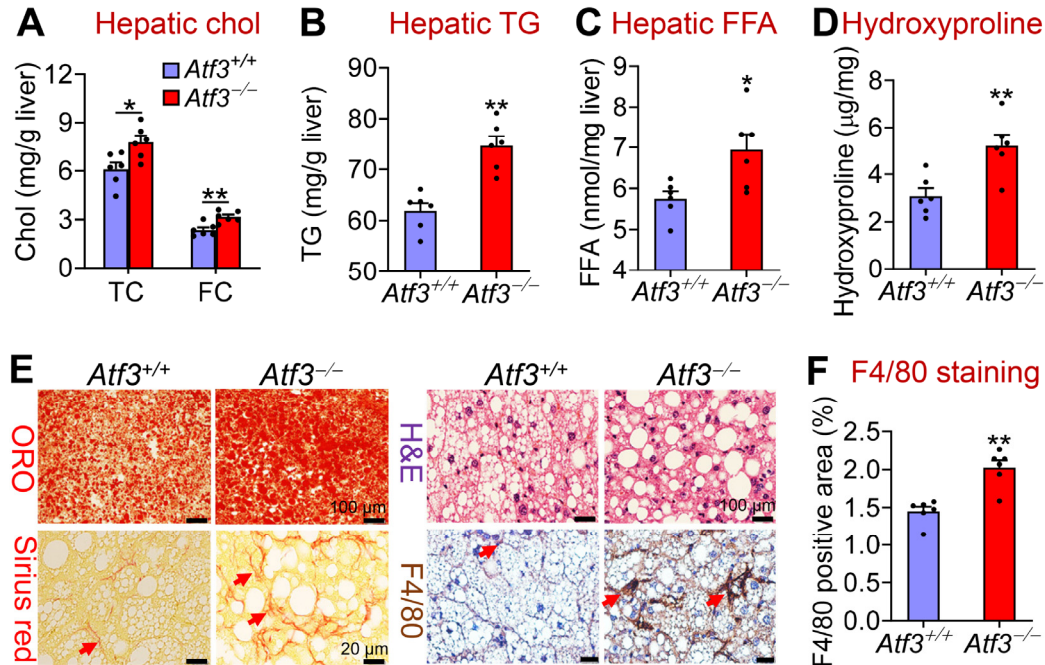
Figure S2. HFCF diet-fed *Atf3⁻/⁻* mice have increased inflammation in the liver
A-F: *Atf3⁺/⁺* mice and *Atf3⁻/⁻* mice were fed an HFCF diet for 20 weeks (n=6). Hepatic levels of total cholesterol (TC), free cholesterol (FC) (A), triglycerides (TG) (B), free fatty acids (FFAs) (C), and hydroxyproline (D) were quantified. Liver sections were stained with Oil Red O (ORO) (E, left top panel), hematoxylin and eosin (H&E) (E, right top panel) or picrosirius red (E, left bottom panel), or immunohistochemically stained with an F4/80 antibody (E, right bottom panel). F4/80 staining-positive areas were quantified (F). In (E), arrows point to fibrosis (left bottom panel) or macrophages (right bottom panel).
All values are expressed as mean±SEM. *$P<0.05$, **$P<0.01$

**A** Body, liver weight **B** Body, liver weight **C** F4/80 staining
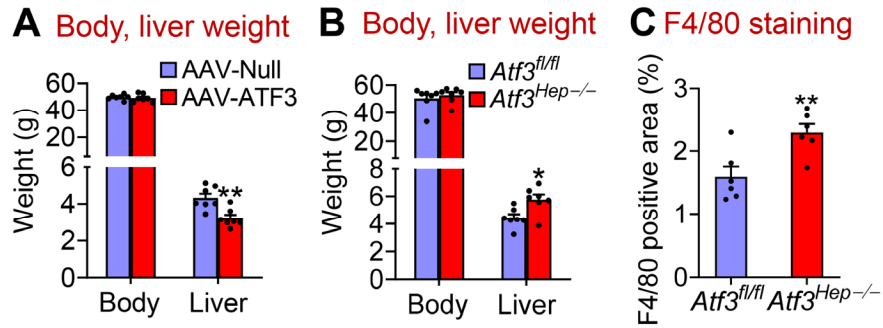
Figure S3. Hepatocytic ATF3 regulates liver weight and inflammation in HFCF diet-fed mice

A: C57BL/6J mice were i.v. injected with AAV8-ALB-Null or AAV8-ALB-ATF3 and then fed an HFCF diet for 16 weeks (n=7). Body weight and liver weight were measured.

B and **C**: $Atf3^{fl/fl}$ mice and $Atf3^{Hep-/-}$ mice were fed an HFCF diet for 16 weeks (n=7). Liver and body weight was measured (B). F4/80 staining-positive areas (%) of liver sections were quantified (C).

All values are expressed as mean±SEM. *P<0.05, **P<0.01
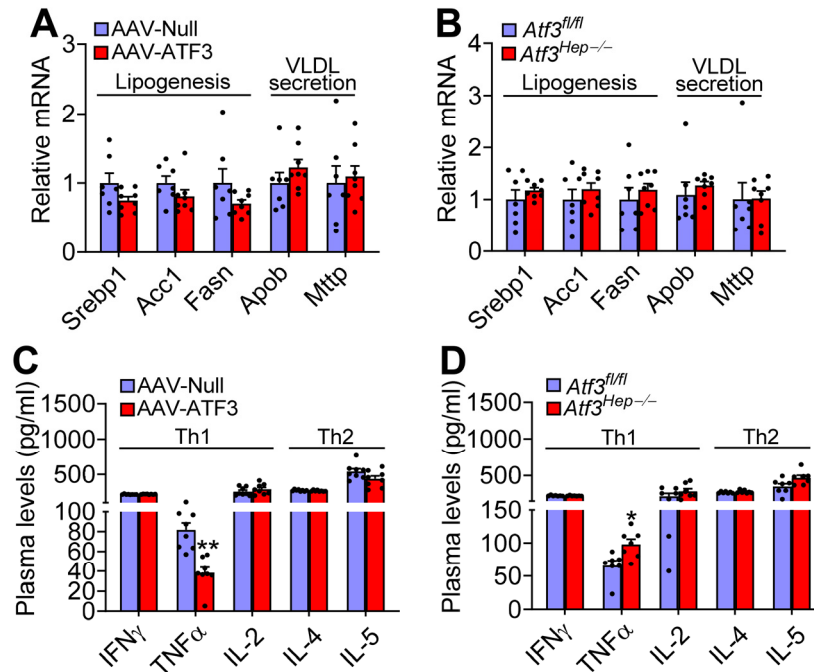
Figure S4. Effect of over-expression or loss of hepatocytic ATF3 on hepatic gene expression or plasma cytokine levels

A and **C:** C57BL/6J mice were i.v. injected with either AAV8-ALB-Null or AAV8-ALB-ATF3 and then fed an HFCF diet for 16 weeks (n=7). Hepatic mRNA levels were determined (A) and plasma Th1- or Th2-type cytokine levels were quantified (C).

B and **D:** *Atf3^fl/fl* mice and *Atf3^Hep−/−* mice were fed an HFCF diet for 16 weeks (n=7). Hepatic mRNA levels were determined (B) and plasma Th1- or Th2-type cytokine levels were quantified (D).

Acc1, acetyl-coA carboxylase 1. *Apob*, apolipoprotein b. *Fasn*, fatty acid synthase. IFNγ, interferon γ. IL-2, interleukin 2. *Mttp*, microsomal triglyceride transfer protein. *Srebp1*, sterol regulatory element-binding protein 1. TNFα, tumor necrosis factor α.

All values are expressed as mean±SEM. *P<0.05, **P<0.01