Supplementary Information

Hepatic Forkhead Box Protein A3 regulates ApoA-I expression, cholesterol efflux and atherogenesis

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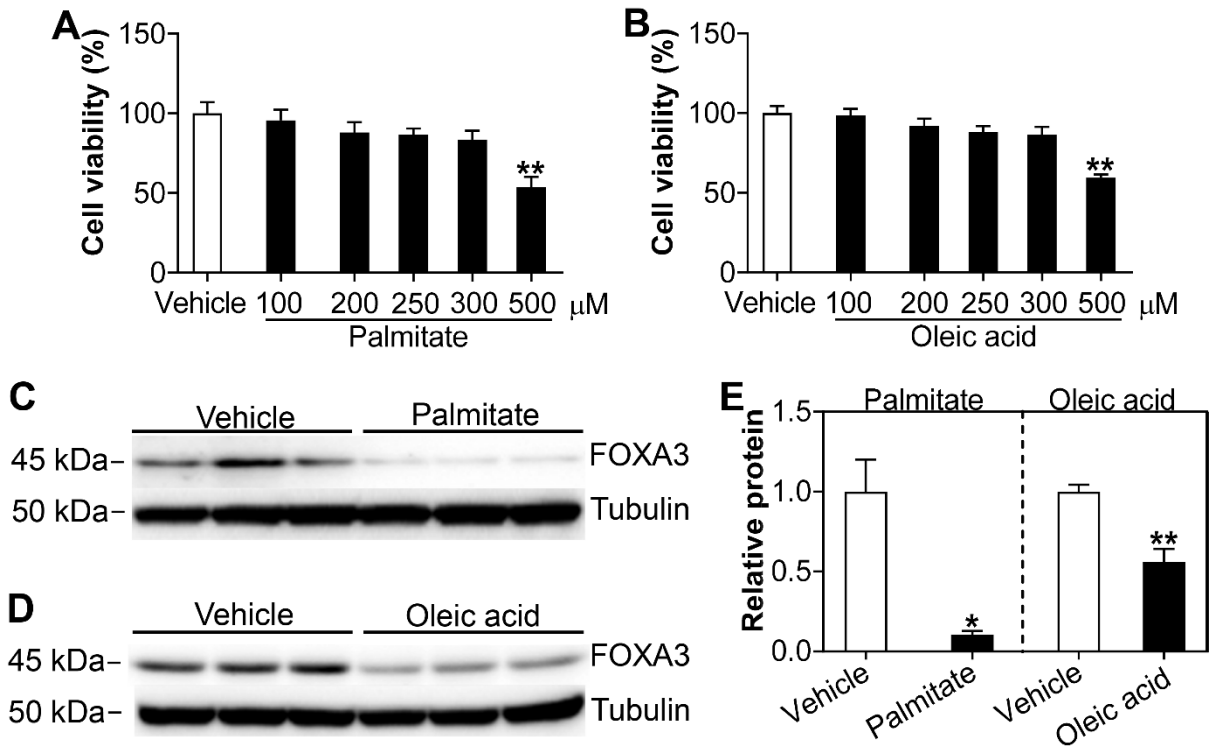
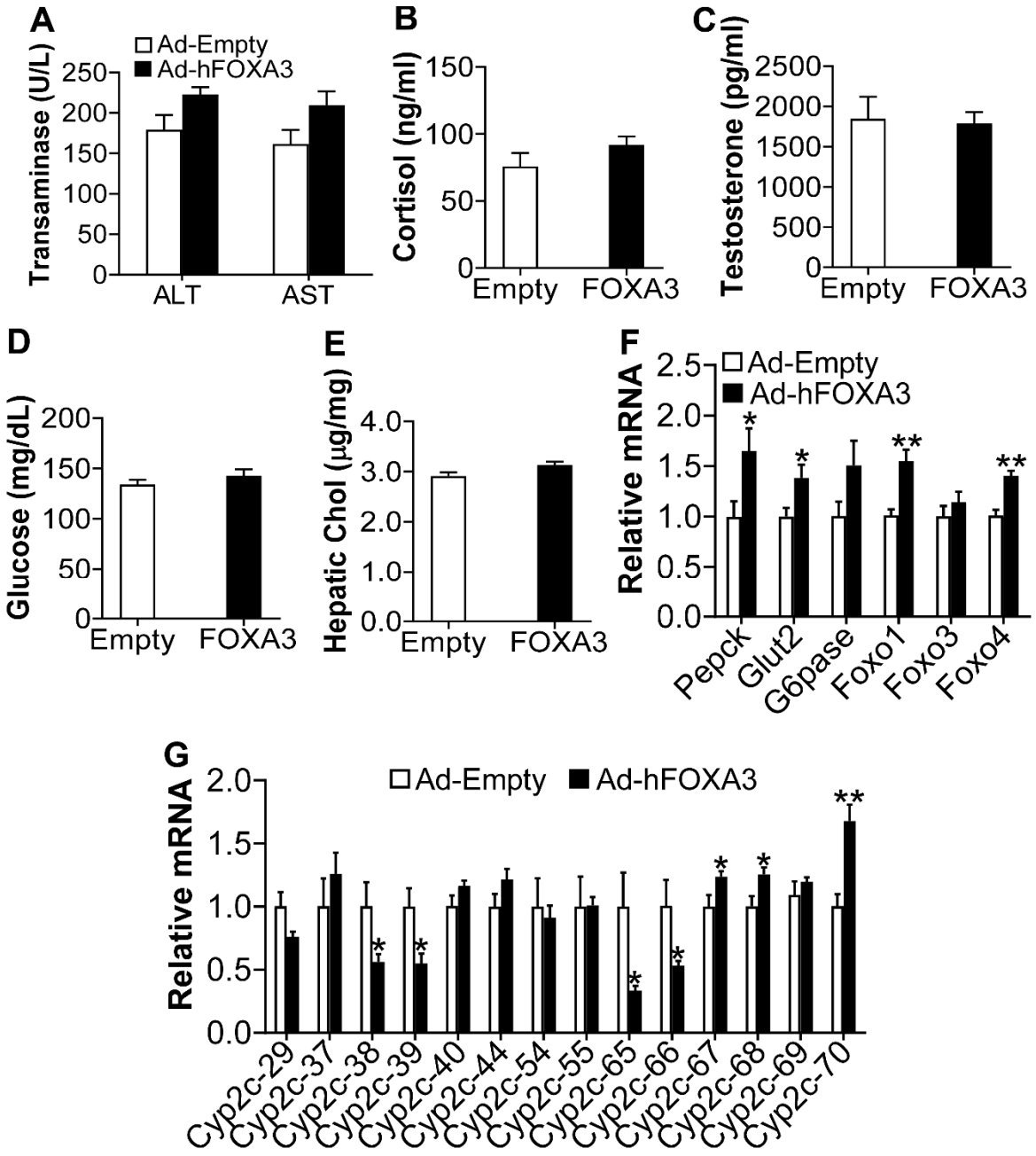
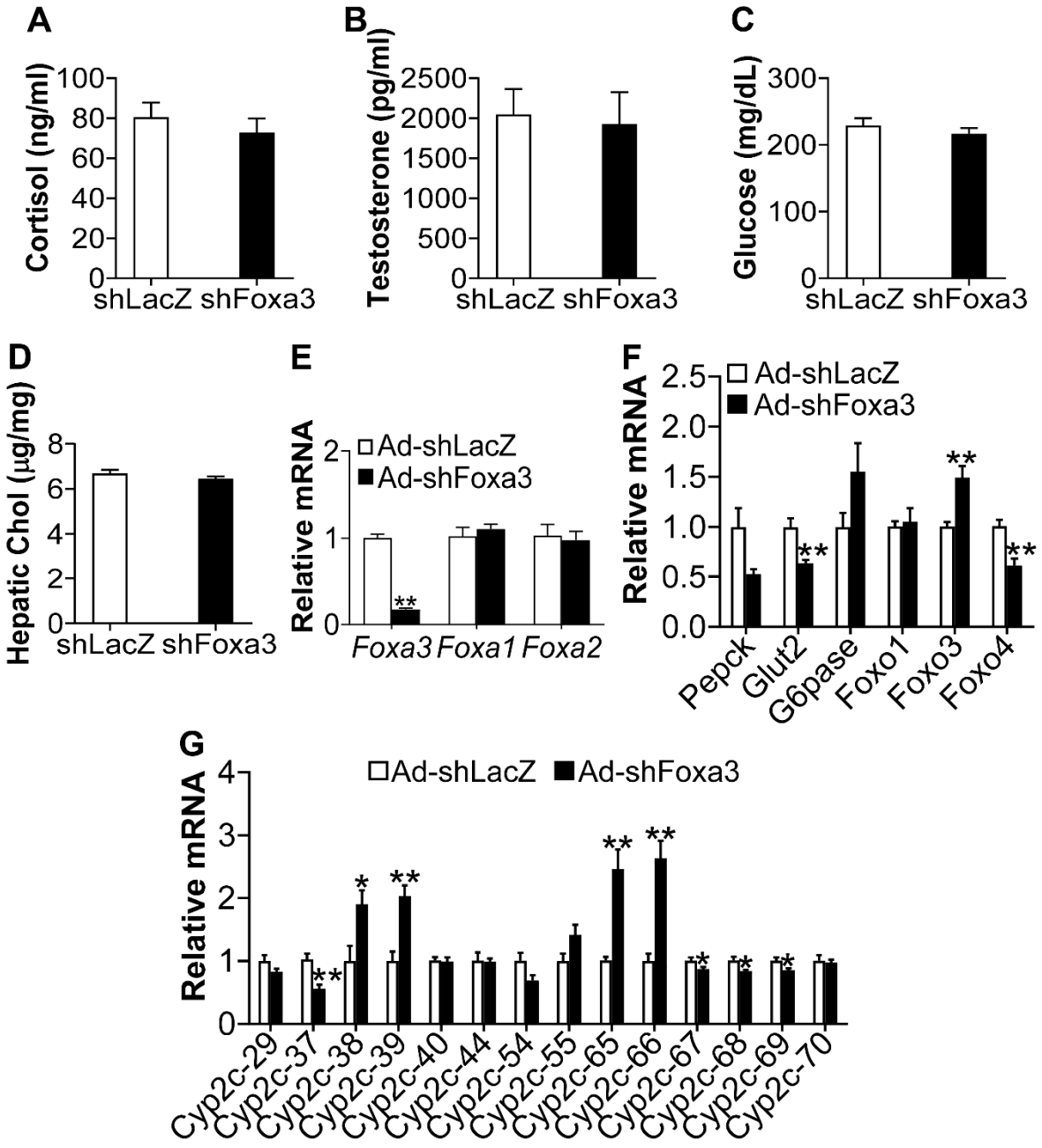


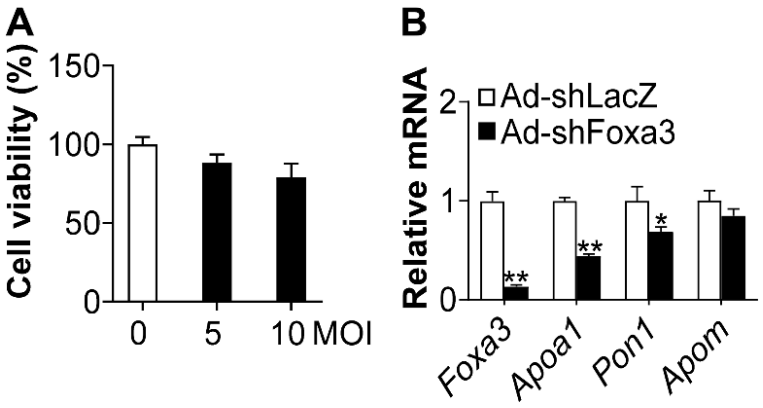
Supplementary Figure I. Fatty acids reduce FOXA3 protein expression in primary hepatocytes. **A** and **B**, Mouse primary hepatocytes were treated with various concentrations of palmitate (**A**) or oleic acid (**B**) for 24 h. MTT assays were performed to determine cell viability. **C** and **D**, Mouse primary hepatocytes were treated with 250 μM palmitate acid (**C**) or oleic acid (**D**) for 16 hours, followed by western blot analysis. **E**, Proteins levels in **C** or **D** were quantified. * $P<0.05$, ** $P<0.01$



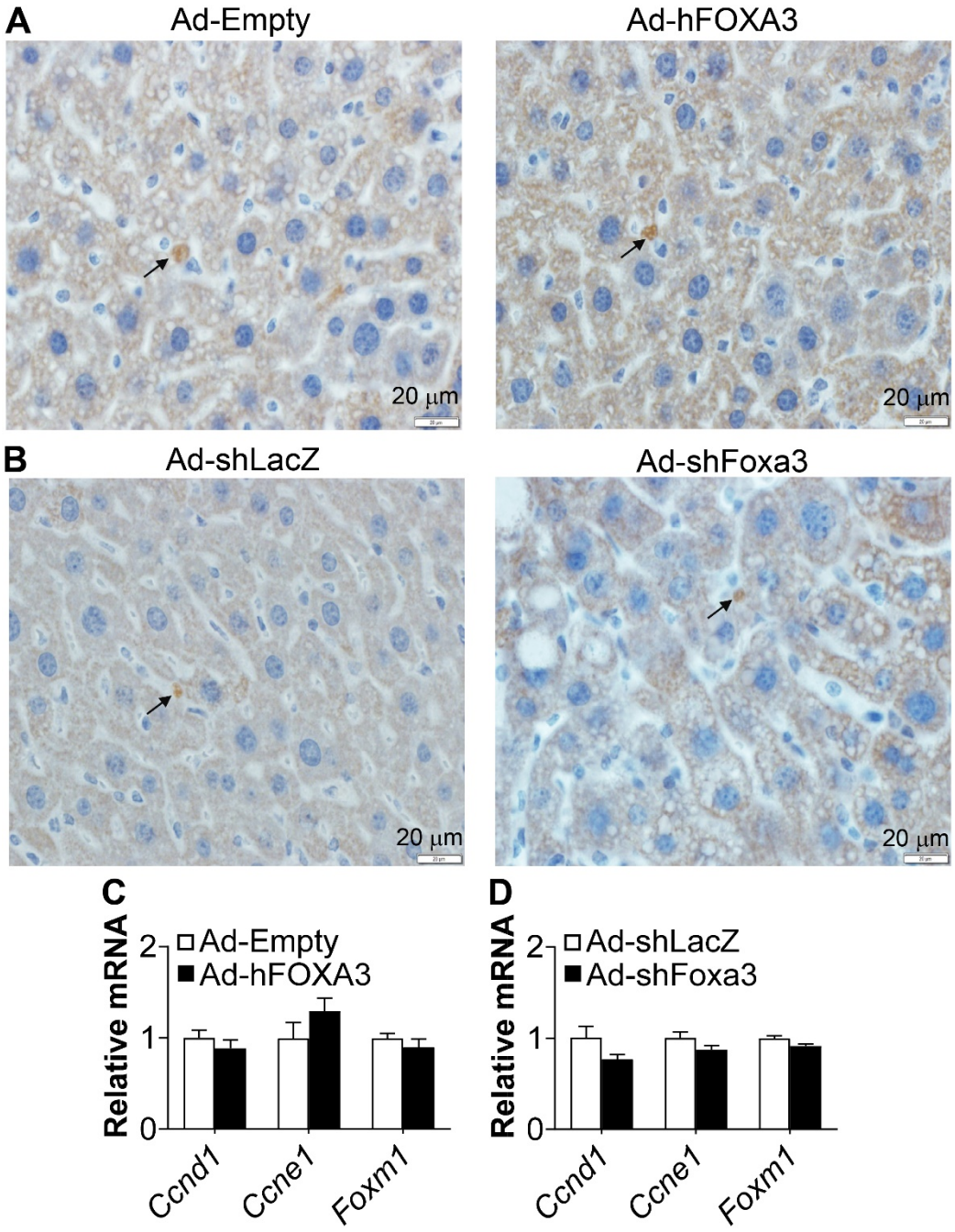
Supplementary Figure II. Plasma biochemistry, hepatic cholesterol levels, and hepatic mRNA levels in mice over-expressing FOXA3. C57BL/6J mice were i.v. injected with Ad-Empty or Ad-hFOXA3. Mice were sacrificed 7 days later (n=8 per group). **A,** Plasma ALT and AST levels. **B,** Plasma cortisol levels. **C,** Plasma testosterone levels. **D**, Plasma glucose levels. **E,** Hepatic total cholesterol levels. **F** and **G**, Hepatic mRNA levels. * $P<0.05$, ** $P<0.01$



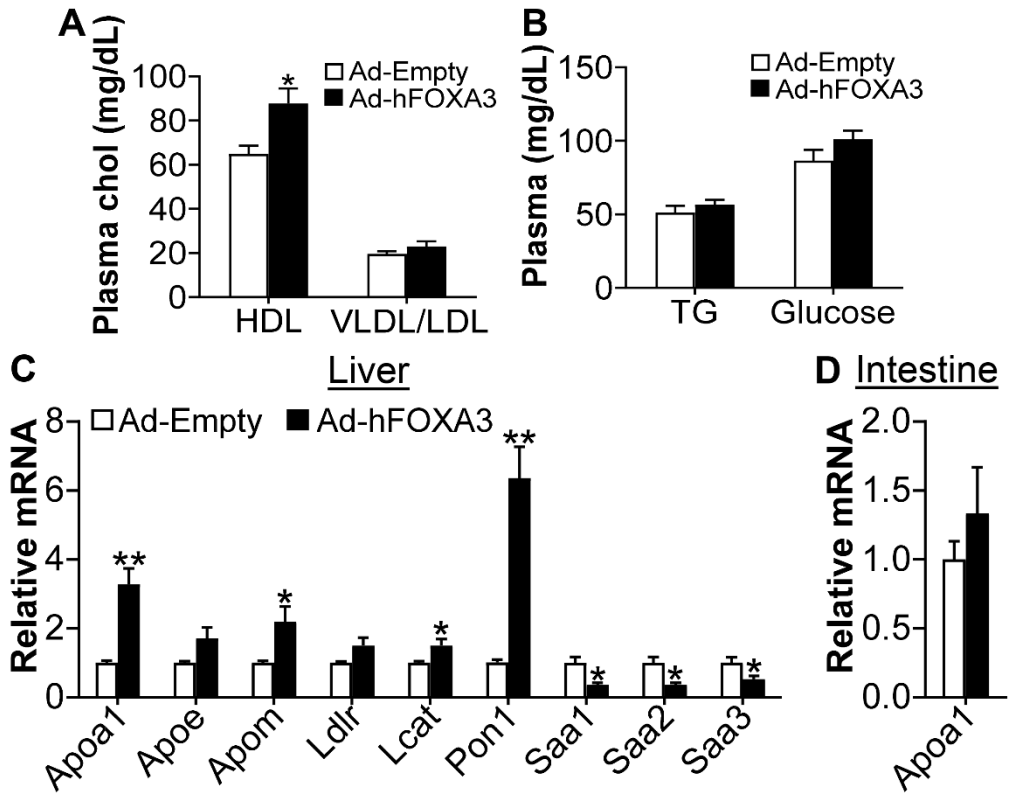
Supplementary Figure III. Plasma biochemistry, hepatic cholesterol levels, and hepatic mRNA levels in mice with deficiency in hepatic Foxa3. C57BL/6J mice were i.v. injected with Ad-shLacZ or Ad-shFoxa3 (n=8 per group). Mice were sacrificed 7 days later. **A**, Plasma cortisol levels. **B**, Plasma testosterone levels. **C**, Plasma glucose levels. **D**, Hepatic cholesterol levels. **E-G**, Hepatic mRNA levels. * $P<0.05$. ** $P < 0.01$



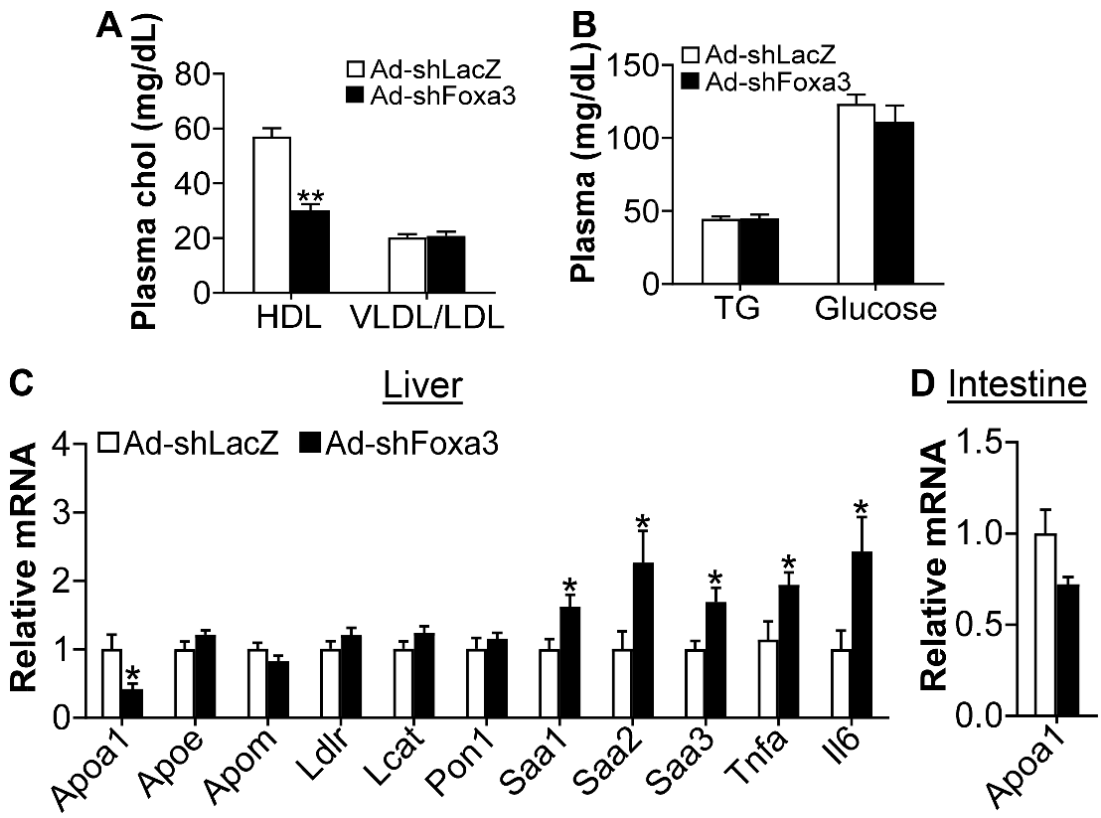
Supplementary Figure IV. Knockdown of *Foxa3* reduces *Apoa1* and *Pon1* expression in primary hepatocytes. Mouse primary hepatocytes were infected with Ad-shLacZ or Ad-shFoxa3 for 24 h. **A**, Cell viability was determined by MTT assays when primary hepatocytes were infected with adenoviruses at an MOI of 0, 5 or 10 (n=7). **B**, mRNA levels were determined in primary hepatocytes infected with adenoviruses at an MOI of 10. * $P<0.05$. ** $P < 0.01$



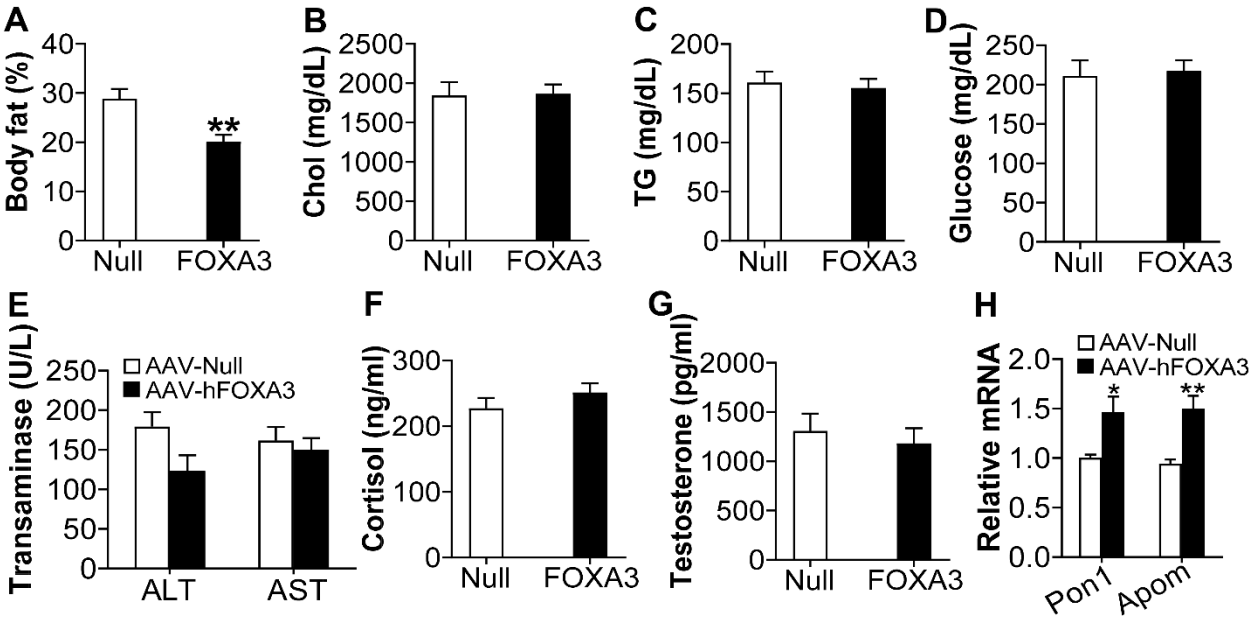
Supplementary Figure V. FOXA3 over-expression or knockdown does not affect liver proliferation in adult mice. A-D, C57BL/6J mice were i.v. injected with Ad-Empty, Ad-hFOXA3 (**A**, **C**), Ad-shLacZ or Ad-shFoxa3 (**B**, **D**) (n=8 per group). After 7 days, livers were collected. Liver sections were immunostained with a Ki-67 antibody (**A**, **B**). Hepatic mRNA levels were determined (**C**, **D**). Arrows indicate Ki-67 staining-positive cells.



Supplementary Figure VI. Effect of hepatic FOXA3 over-expression in female mice. C57BL/6J mice were i.v. injected with Ad-Empty or Ad-hFOXA3 (n=6 per group). After 7 days, mice were euthanized. **A**, Plasma cholesterol levels. **B**, Plasma TG and glucose levels. Glucose levels were determined after a 12-h fast. **C**, Hepatic mRNA levels. **D**, Intestinal *Apoa1* mRNA levels. * $P<0.05$. ** $P<0.01$



Supplementary Figure VII. Effect of hepatic FOXA3 knockdown in female mice. C57BL/6J mice were i.v. injected with Ad-shLacZ or Ad-shFoxa3 (n=6 per group). After 7 days, mice were euthanized. **A**, Plasma cholesterol levels. **B**, Plasma TG and glucose levels. Glucose levels were determined after a 12-h fast. **C**, Hepatic mRNA levels. **D**, Intestinal *Apoa1* mRNA levels. * $P<0.05$. ** $P<0.01$



Supplementary Figure VIII. Hepatocyte-specific over-expression of FOXA3 lowers body fat but does not affect plasma total cholesterol or triglyceride levels. *Apoe*$^{-/-}$ mice were i.v. injected with AAV8-ALB-Null or AAV8-ALB-FOXA3 and then fed a Western diet for 3 months (n=8 per group). **A**, Body fat content was determined by Echo-MRI. No change in food intake was observed. **B**, Plasma total cholesterol (chol) levels. **C**, Plasma triglyceride (TG) levels. **D**, Plasma glucose levels. **E**, Plasma ALT and AST levels. **F**, Plasma cortisol levels. **G**, Plasma testosterone levels. **H**, Hepatic mRNA levels. * P<0.05, ** P<0.01