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Supplemental information

Aquaporin-8 ameliorates hepatic steatosis through farnesoid X receptor in obese mice

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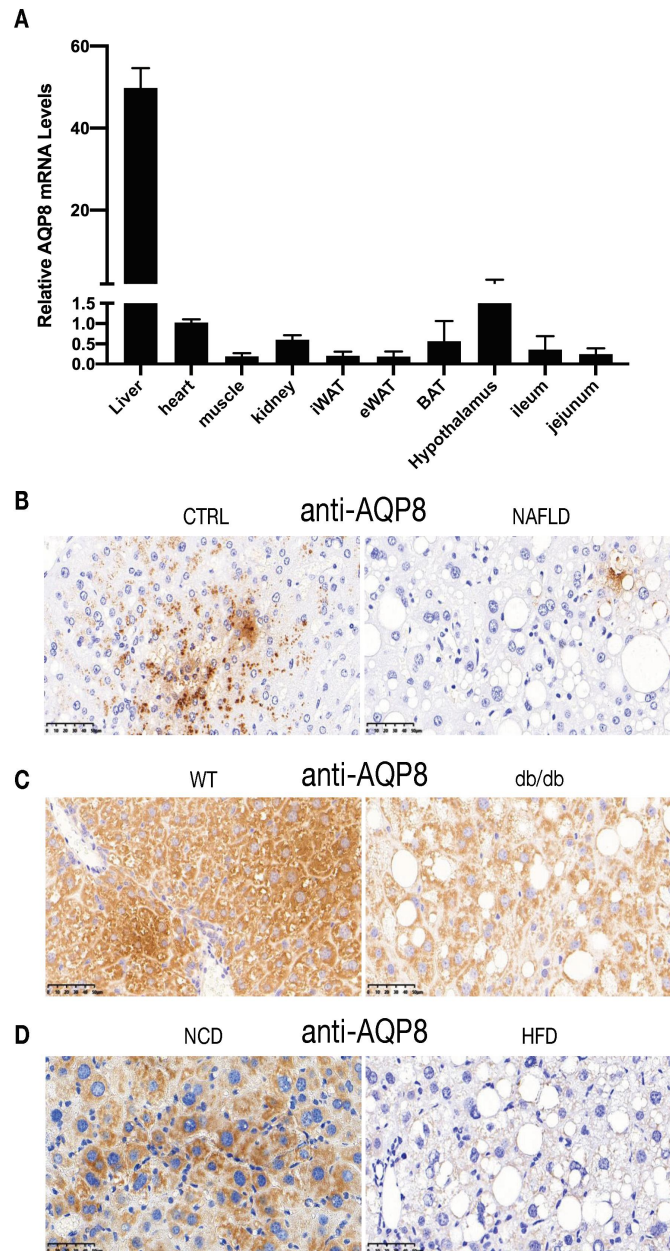


Figure S1. AQP8 is highly expressed in the liver and its expression is downregulated in the states of hepatic steatosis, related to Figure 1.

A, Normal wild type mice was sacrificed and mouse tissue distribution of AQP8 mRNA determined by RT-PCR. The results were expressed as relative expression levels after standardization by 36B4. The AQP8 mRNA levels were normalized as the average of heart mRNA levels =1. iWAT, inguinal white adipose tissue; eWAT, epididymal white adipose tissue; BAT, brown adipose tissue. n=6-8. The data represent the mean \pm SEM values. AQP8 expression was decreased in the livers of NAFLD patients and obese mice. AQP8 immunohistochemistry in the livers of NAFLD patients(B), db/db mice(C), HFD mice(D). magnification: 400 \times , scale bar, 50 μ m.

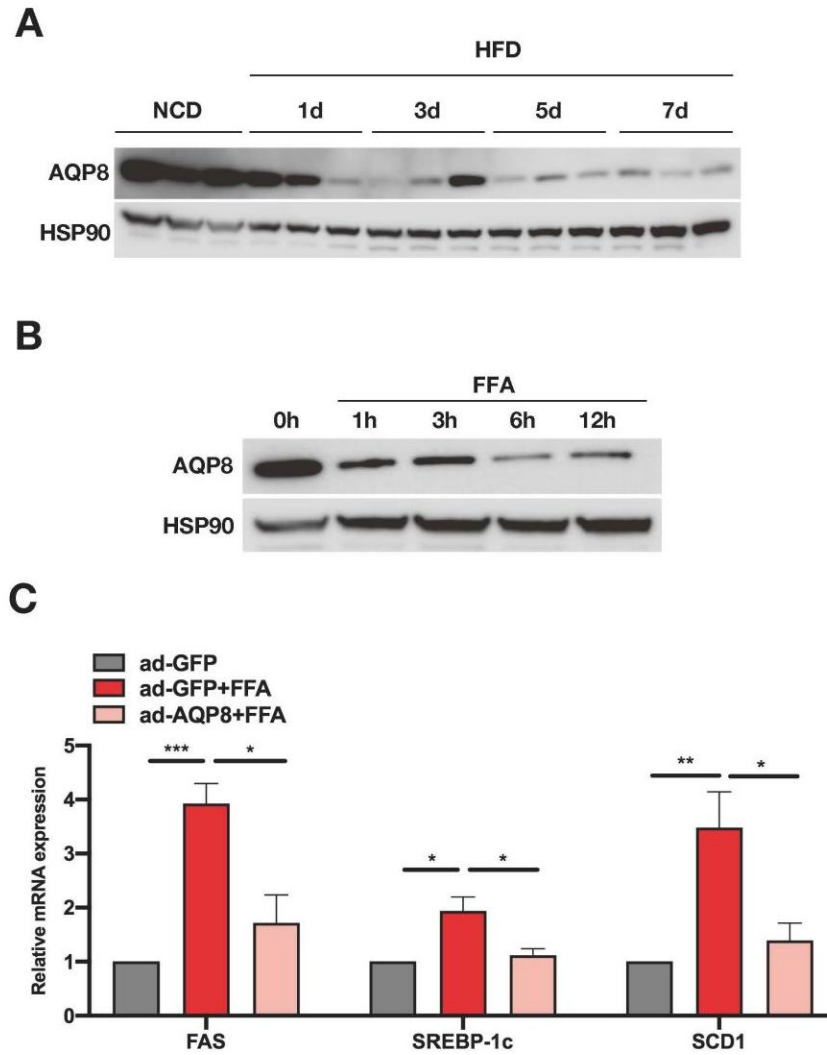


Figure S2. Downregulation of AQP8 in the livers of obese mice and in vitro NAFLD model, related to Figure 1.

A, short-term HFD mice (n=5 per group), B, HepG2 cells without or with FFA (OA:PA=2:1) treatment for the indicated time. C, Primary mouse hepatocytes (PMH) infected with either ad-GFP or ad-AQP8 with or without FFA treatment. qPCR analysis of lipogenic genes. The data represent the mean±SEM values. *p<0.05 **p<0.01 ***p<0.001

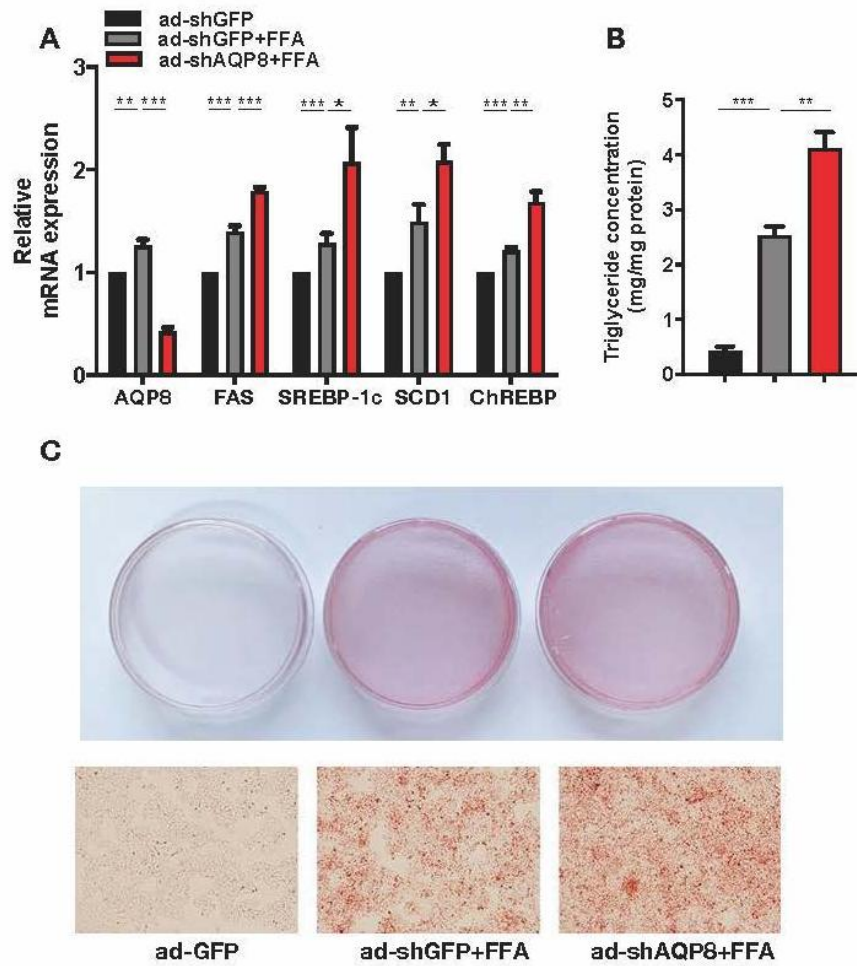


Figure S3. Knockdown of AQP8 in hepatocytes exacerbates TG accumulation and promotes hepatic lipid synthesis, related to Figure 1.

PMH and HepG2 cells were transfected with either ad-GFP or ad-shAQP8 with or without FFA treatment. A, qPCR analysis of PMH on mRNA expression related to AQP8 and lipogenesis. B, Quantification of TG contents of PMH. C, oil red O staining (200 X) of HepG2 cells. The data represent the mean \pm SEM values and three independent experiments in triplicate. * p <0.05 ** p <0.01 *** p <0.001

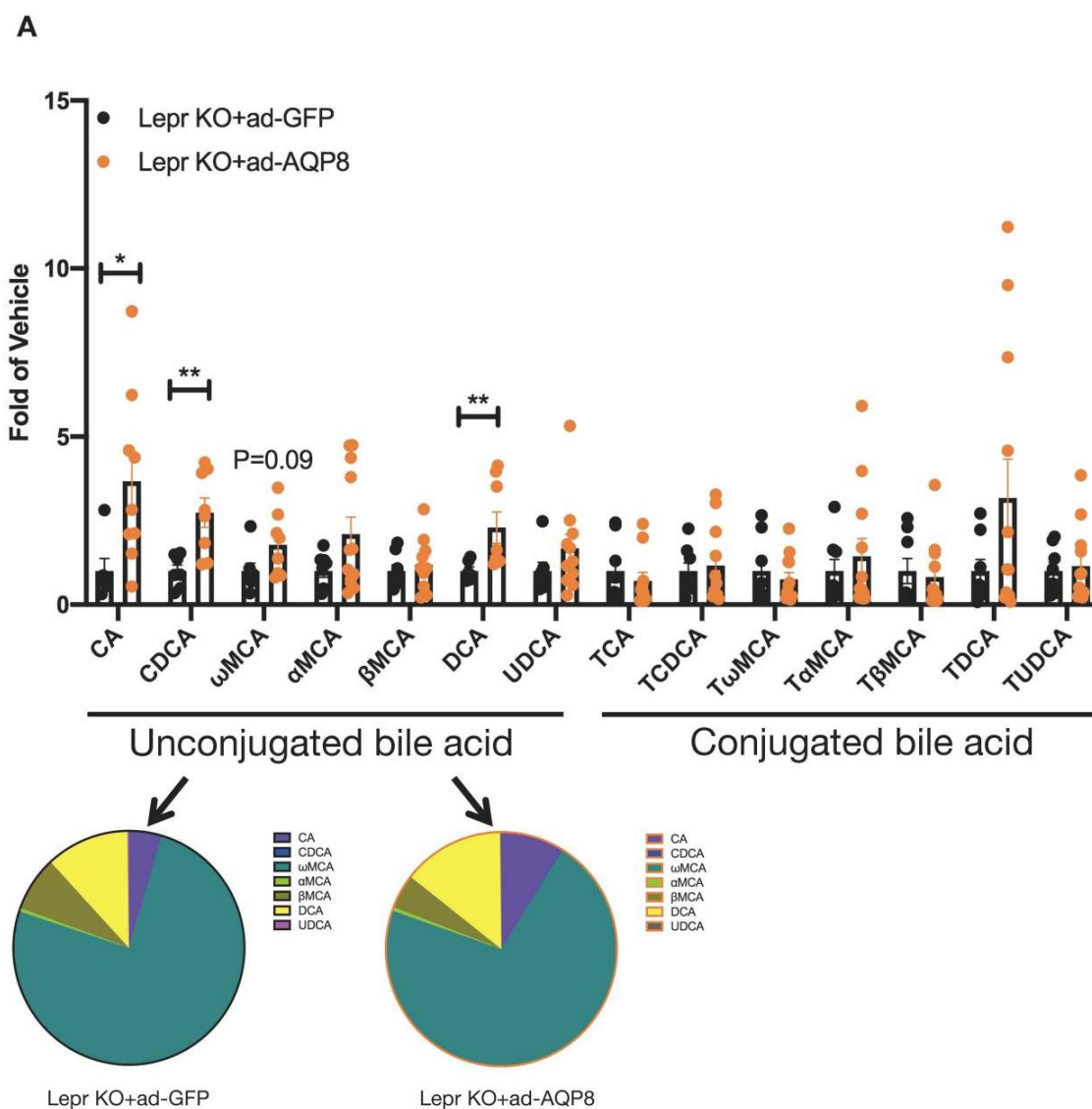


Figure S4. Hepatic overexpression of AQP8 in the livers of db/db mice alters serum bile acid composition, related to Figure 2.

A. Serum bile acids were measured by ultra-high performance liquid chromatography–tandem mass spectrometry (CA, cholic acid; CDCA, chenodeoxycholic acid; MCA, muricholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TMCA, tauromuricholic acid; TDCA, taurodeoxycholic acid; TUDCA, tauroursodeoxycholic acid). Data are presented as the mean \pm SEM. n = 8–12 mice per group.

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Figure S5. FXR Element(FXRE) sites and FXRE mutation in the human AQP8 promoter, related to Figure 6.

A, Putative FXRE motifs(marked Yellow)(<https://jaspar.genereg.net/matrix/MA1110.1/>) in nucleotide sequence of the human AQP8 gene promoter from nt-1500 to 150, which was cloned into pGL4.14-luciferase reporter plasmid. B, Mutation(site-directed mutagenesis) of FXRE motifs(marked Red) within the construct pGL4.14-AQP8.