TITLE PAGE

Title: Berberine prevents progression from hepatic steatosis to steatohepatitis and fibrosis by reducing endoplasmic reticulum stress

Short title: BBR treats NAFLD/NASH by suppressing ER stress

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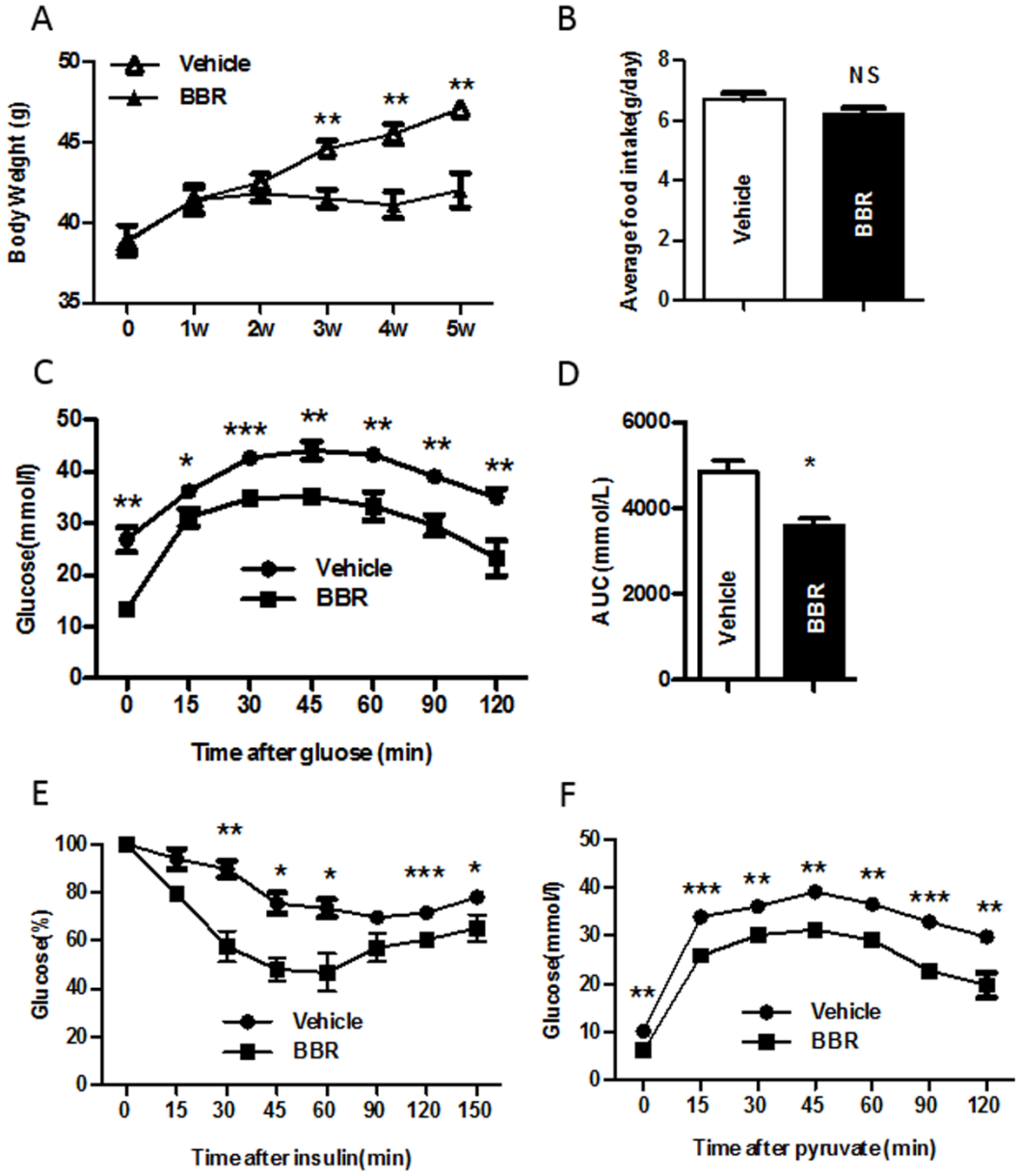
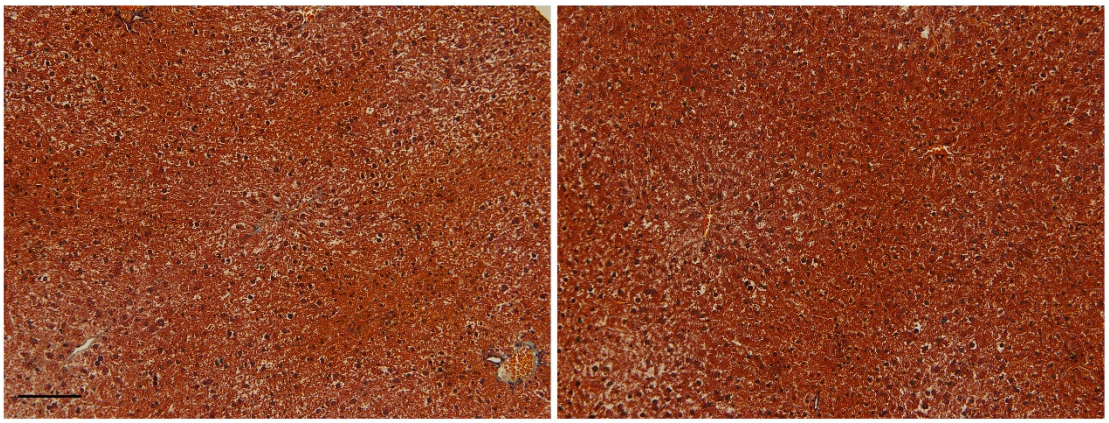


Supplementary figure legends:

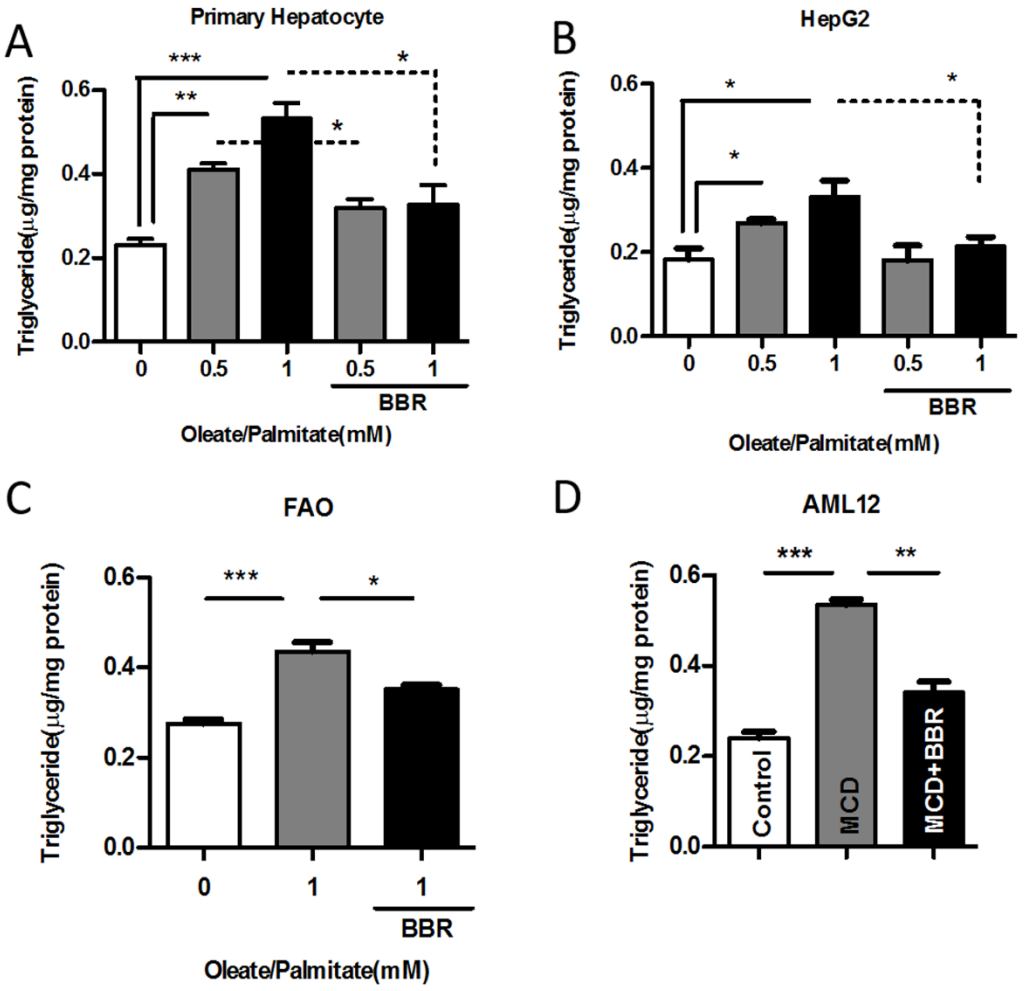
Supplementary Figure 1. BBR reduces obesity and improves insulin sensitivity.

A: Body weight evaluation of db/db mice treated with vehicle or BBR every 3 day (g) (n = 8). B: average food intake (n = 8). C-D: Intraperitoneal glucose tolerance test on db/db mice treated as in (A) and injected with 0.75 g glucose/kg after overnight fasting (n = 6-8) (C). The adjacent bar graph (D) represents the average area under the curve (n =7- 8). E: Insulin tolerance test was evaluated through and injected with 1 U

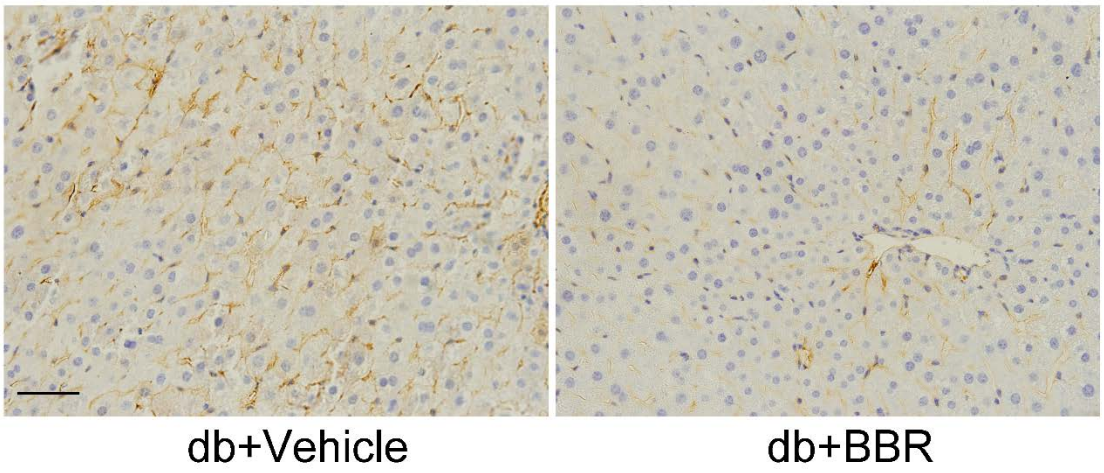
insulin/kg after 6 hr of fasting (n = 7-8). F: Pyruvate tolerance test was evaluated on db/db mice treated as in (A) and injected with 1 g pyruvate/kg after overnight fasting (n = 7-8). Values represent means ± SEM. Error bars represent SEM, and significant differences compared to vehicle controls are indicated by *P<0.05, **P<0.01, ***P < 0.001



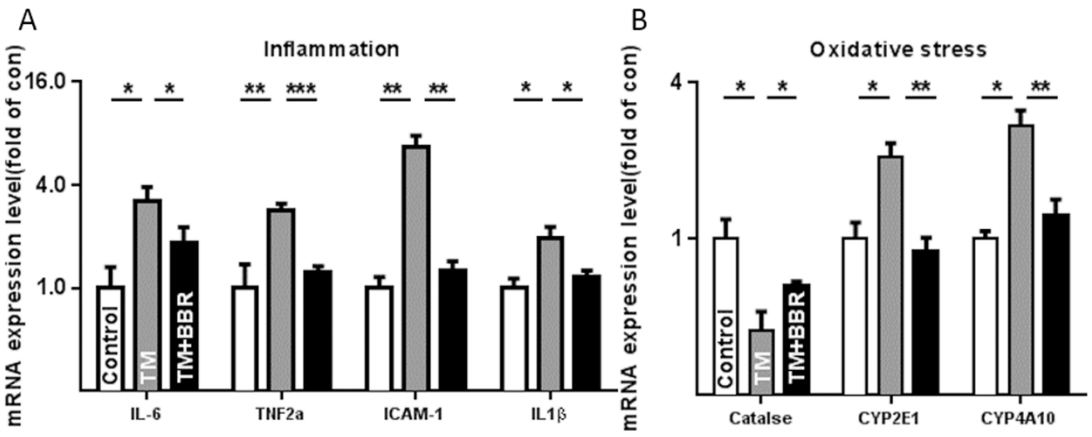
Supplementary Figure 2. collagen deposition evaluated by Masson's trichrome staining (X200), scale bar, 100 μ m.



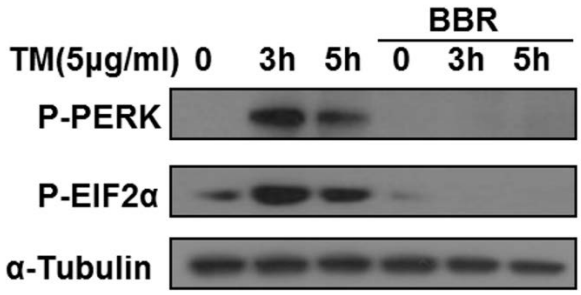
Supplementary Figure 3. BBR decreased cellular TG accumulation in different hepatocytes. A-C: Lipid accumulation levels in OA/PA induced steatosis in primary hepatocytes, HepG2, and FAO cell lines. D: Lipid accumulation level in MCD medium incubation-induced steatosis in AML12 cells. Data are presented in mean ± SEM that represent three independent experiments in triplicate. *P < 0.05, **P < 0.01, ***P<0.001.



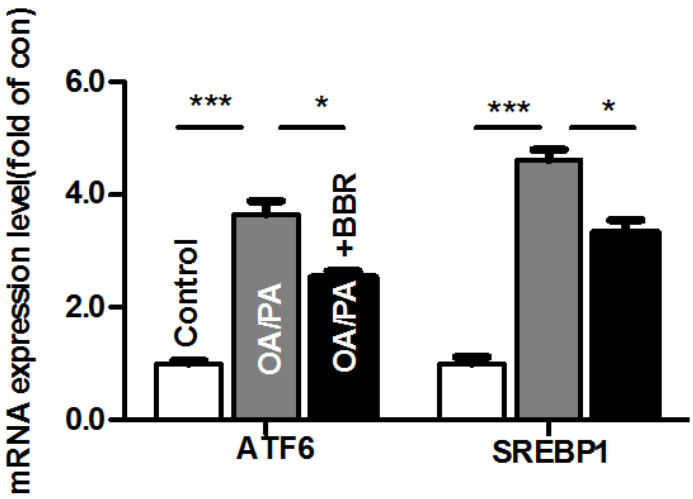
Supplementary Figure 4. Immunohistochemistry for CD68 (brown stain) in liver sections of db/db mice (X400), scale bar, 50 μ m.



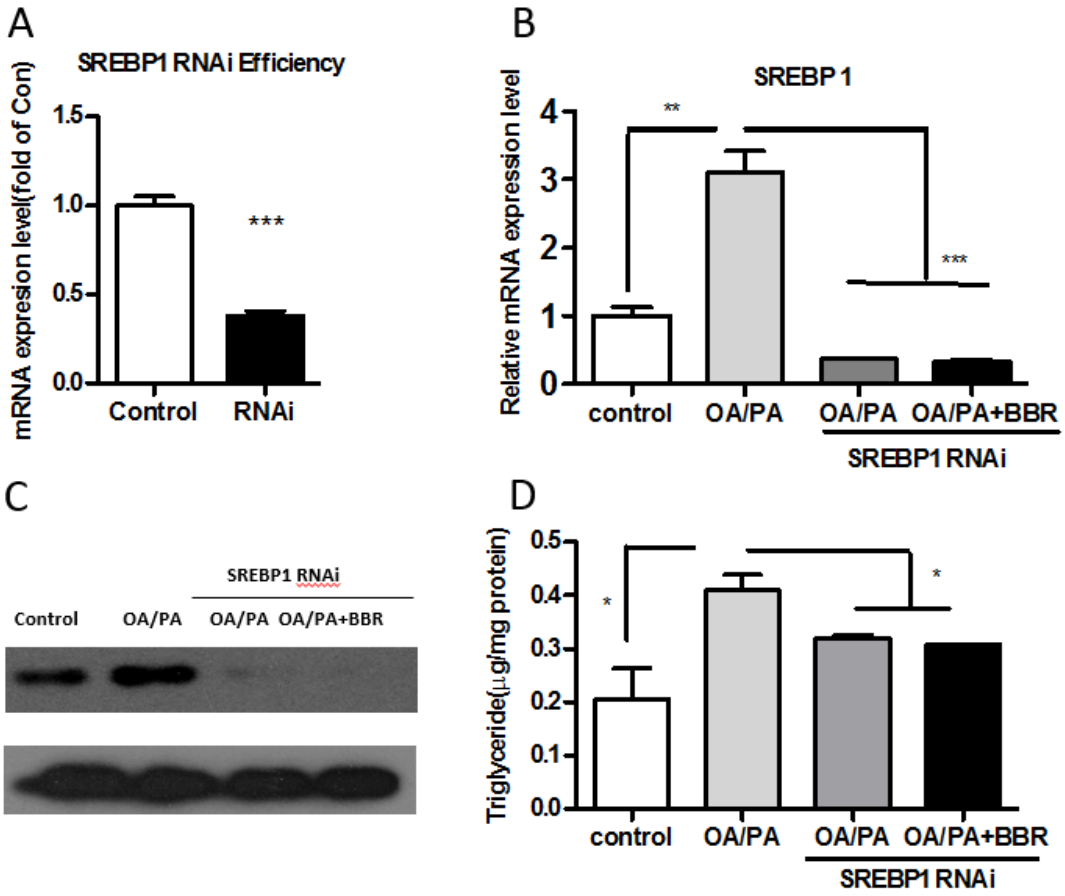
Supplementary Figure 5. BBR could function as a chemical chaperone and block TM challenge-induced ER stress and NAFLD. C57BL/6J mice were treated with vehicle and BBR for 3 days and then gave I.P. TM. After 24 hours, the liver tissues were analyzed. A-B: Analysis of mRNA on hepatic inflammation (A) and oxidative stress (B) related genes, β-actin was used as internal control. Data are presented in mean ± SEM that represent three independent experiments in triplicate.*P < 0.05, **P < 0.01, ***P<0.001, n=6-8.



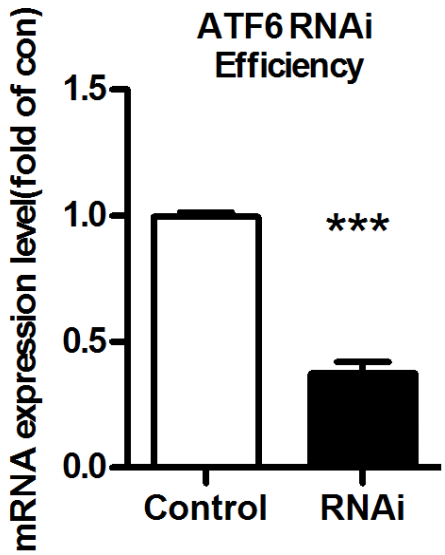
Supplementary Figure 6. Western blot of P-PERK, P-EIF2α in HepG2 cell line stimulated with TM for a different time in the presence or absence of BBR, α-Tubulin as internal control.



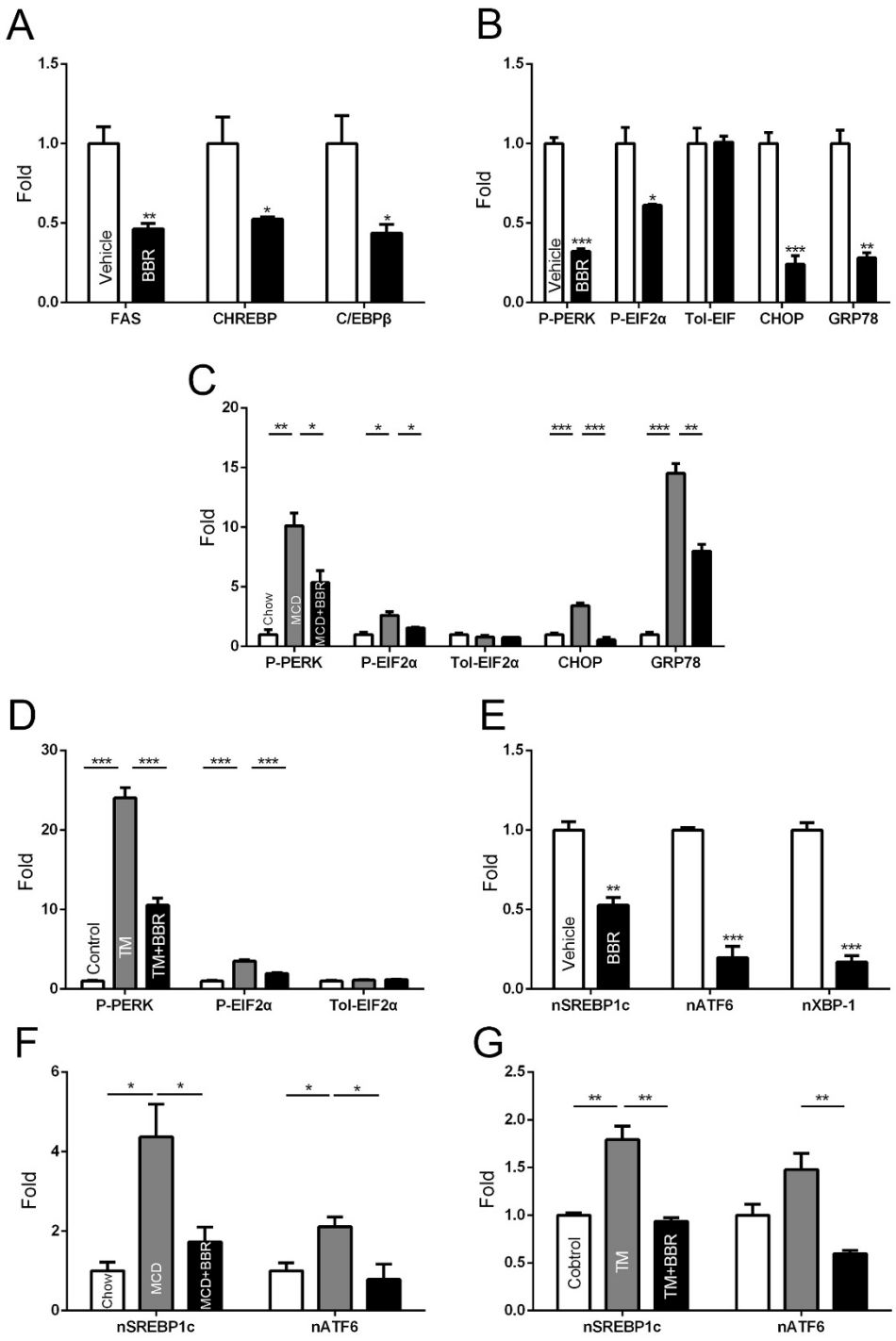
Supplementary Figure 7. ATF6 and SREBP1 mRNA expression level by incubation of 1mM OA/PA in primary hepatocytes with or without BBR treatment. Data are presented in mean ± SEM that represent three independent experiments in triplicate.*P <0.05, **P < 0.01, ***P<0.001.



Supplementary Figure 8. SREBP-1c knockdown blocked the TG reduction by BBR treatment in HepG2 cells A-B: HepG2 cells were transfected with SREBP1 or control RNAi, and were stimulated by OA/PA with or without BBR treatment. Efficiency of SREBP-1c RNAi-mediated knockdown in HepG2 cells was verified by qPCR (A), the collected samples were analyzed for SREBP-1c mRNA (B) and protein levels (C), as well as cellular TG content (D) as previously described. Data are presented in mean ± SEM that represent three independent experiments in triplicate.*P <0.05, **P < 0.01, ***P<0.001.



Supplementary Figure 9. Efficiency of ATF6 RNAi-mediated ATF6 knockdown in HepG2 cells. Data are presented in mean ± SEM that represent three independent experiments in triplicate.*P <0.05, **P < 0.01, ***P<0.001.



Supplementary Figure 10. Results of Western blots were quantified by Image J software. A. quantified result of Fig. 2.B; B. quantified result of Fig. 2E; C. quantified result of Fig. 4B; D. quantified result of Fig. 5B; E. quantified result of Fig. 6A; F. quantified result of Fig. 6B; G. quantified result of Fig. 6C.

Supplementary Table1

General parameters of BBR treatment for 5 weeks in db/db mice

Parameters	Vehicle	BBR
Serum TG(mmol/L)	4.82 ± 0.18	3.55 ± 0.15 a
Serum TC(mmol/L)	4.36 ± 0.21	3.78 ± 0.14 a
Serum HDL(mmol/L)	2.69 ± 0.12	3.08 ± 0.09 a
Serum LDL(mmol/L)	0.75 ± 0.045	0.56 ± 0.059 a
Serum BUN(mmol/L)	9.463 ± 0.42	8.10 ± 0.45
Serum Cr(μmol/L)	19.92 ± 0.37	17.16 ± 0.62 a
Serum FFA(mEq/L)	1.691 ± 0.063	1.435 ± 0.091 a

Data are presented as mean ± SEM (n = 8).

TG, total triglyceride; TC, total cholesterol; HDL, high-density lipoprotein;

LDL, low-density lipoprotein; BUN, blood urea nitrogen;

Cr, cretinine; FFA, free fatty acid.