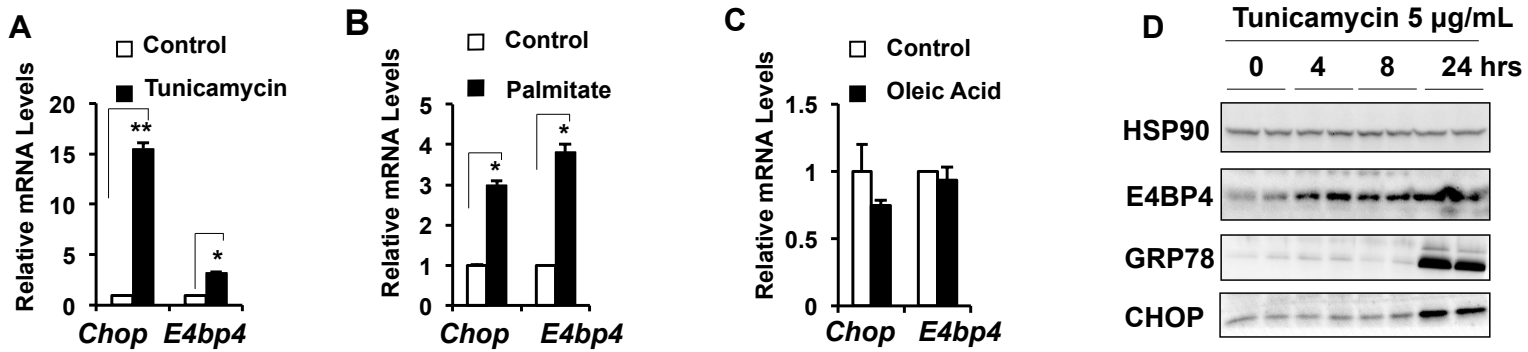
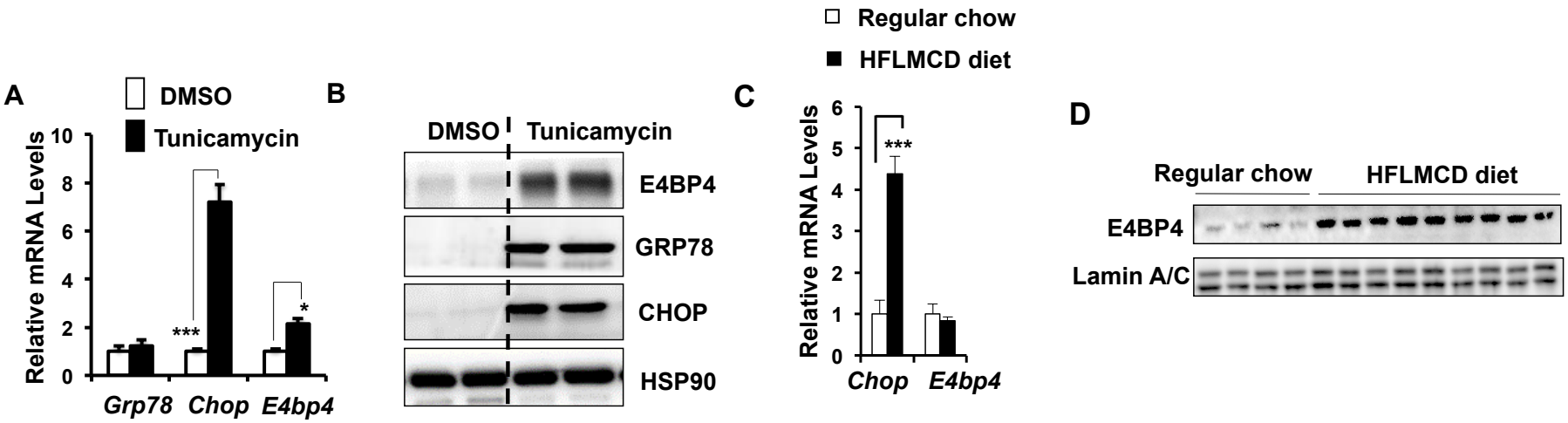
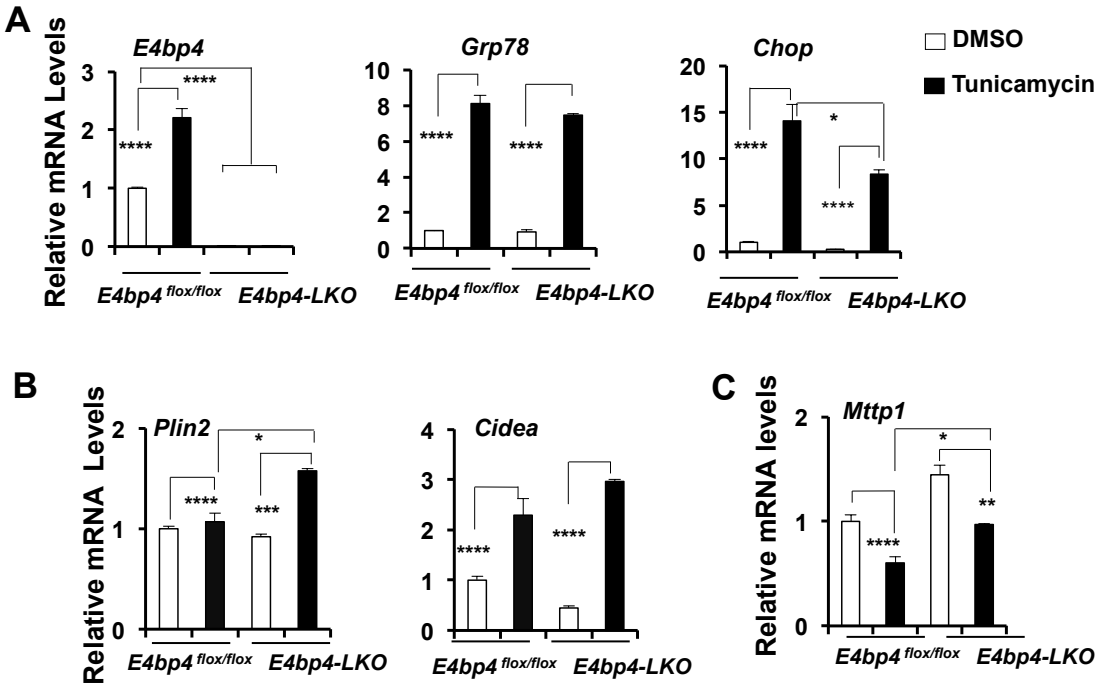


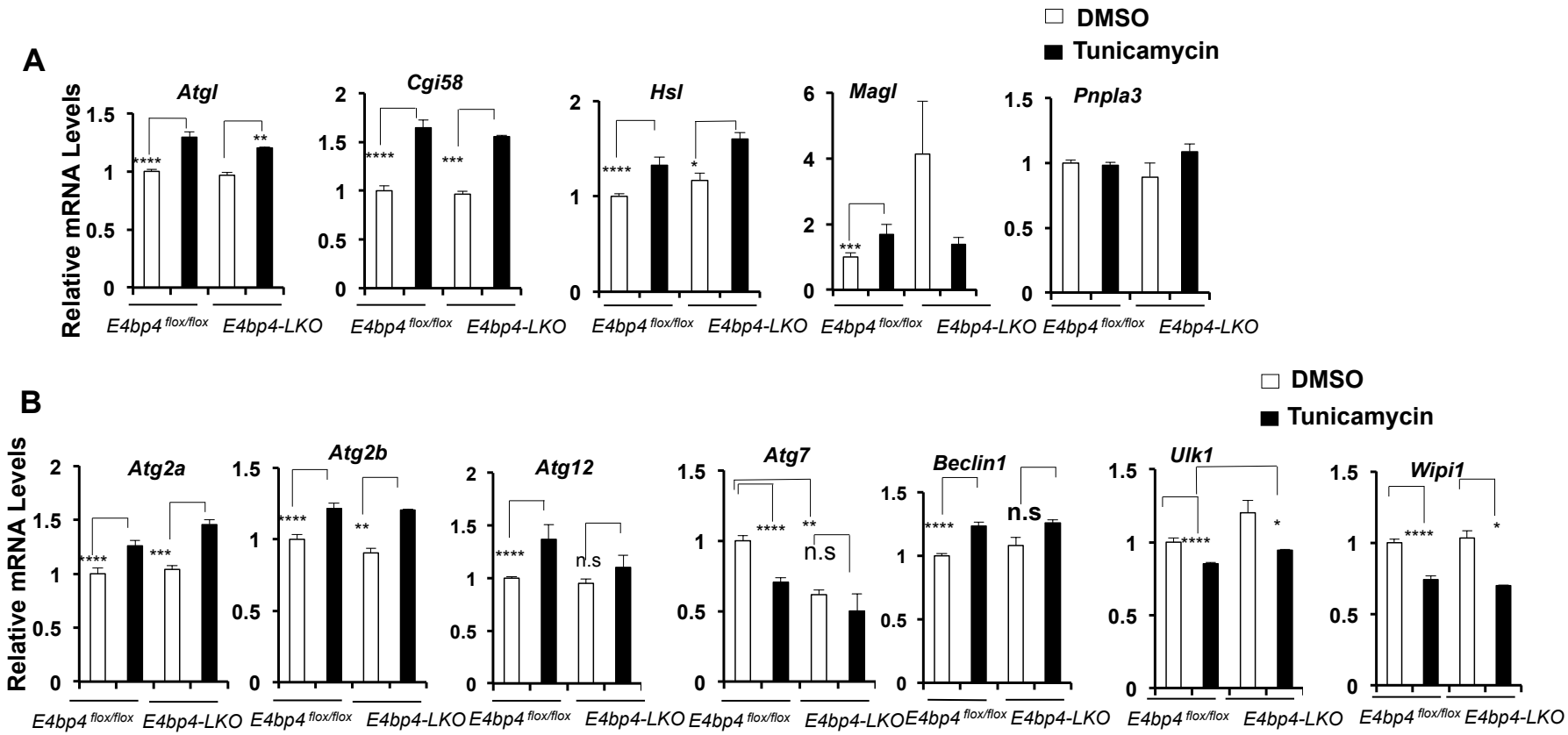
Supplementary Figure 1. Tunicamycin induces *E4bp4* in Hepa1c1c7 cells. The mouse hepatoma Hepa1c1c7 cells were treated with either DMSO or tunicamycin (5 µg/ml) (**A** & **D**) or palmitate (400 µM) (**B**) or oleic acid (300 µM) (**C**) for 16 hrs before harvest. Both the mRNA and protein levels were analyzed. The data were presented as Mean ± SEM. *$p < 0.05$ and **$p < 0.01$ by the Student *t*-test. The experiments were repeated at least three times with similar results.



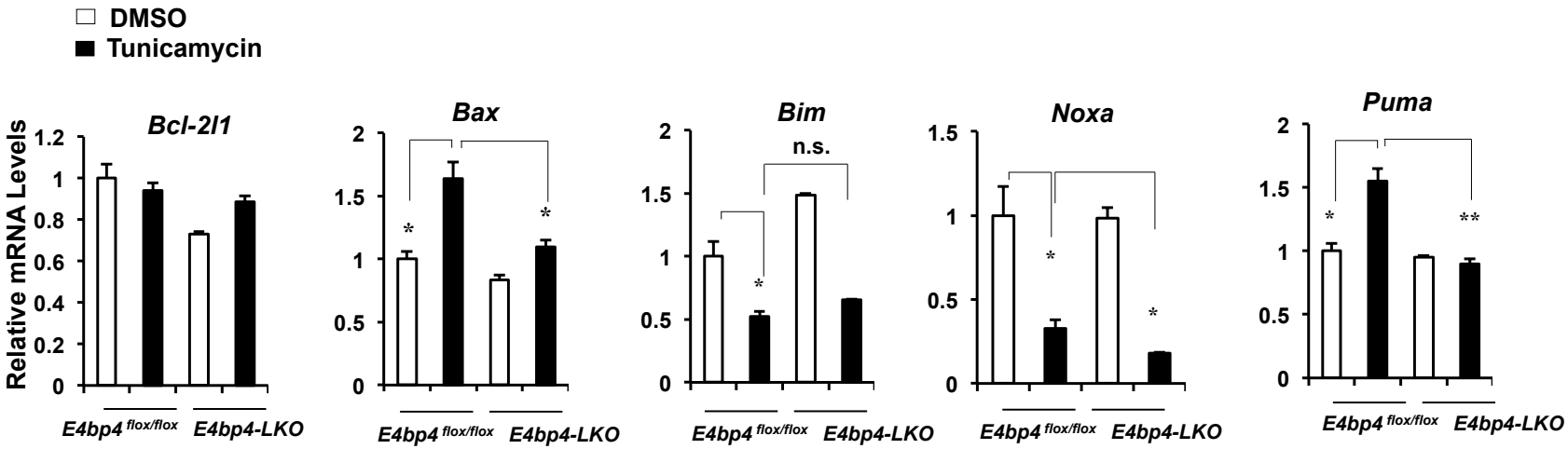
Supplementary Figure 2. Tunicamycin induces *E4bp4* in mouse macrophage RAW cells (A&B). The RAW cells were treated with either DMSO or tunicamycin (5 μg/ml) for 16 hrs before harvest. Both mRNA and protein levels were analyzed. The data were presented as Mean ± SEM. ***p* <0.01 and ****p* <0.001 by the Student *t*-test. The experiments were repeated at least three times with similar results. **Liver E4BP4 expression in WT male mice after 10-wk HFLMCD feeding (C&D).** The liver mRNA levels of *Chop* and *E4bp4* were measured by RT-qPCR (**C**) and the nuclear E4BP4 protein was detected by immunoblotting (**D**).



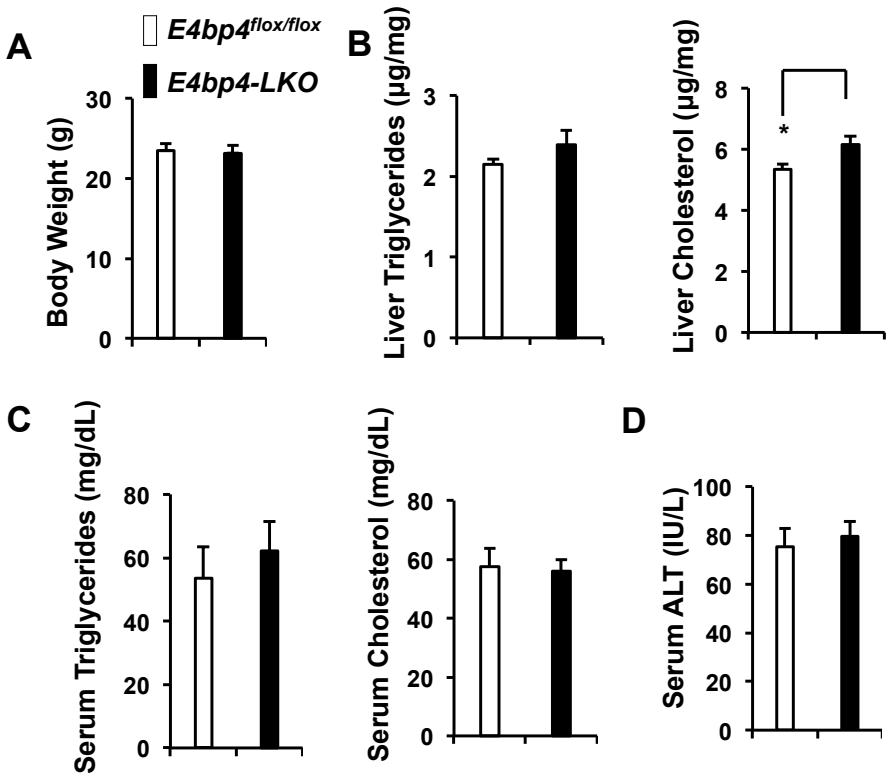
Supplementary Figure 3. Gene expression profile of lipid metabolism in tunicamycin-treated *E4bp4-LKO vs*. *E4bp4*$^{flox/flox}$ PMHs. (A) *E4bp4* and ER stress markers (*Grp78* and *Chop*), (**B**) lipid droplet genes (*Plin2* and *Cidea*); and (**C**) lipid uptake and secretion (*CD36* and *Mttp1*), **p* <0.05, ***p* <0.01, and ****p* < 0.001 by the Student's *t*-test.



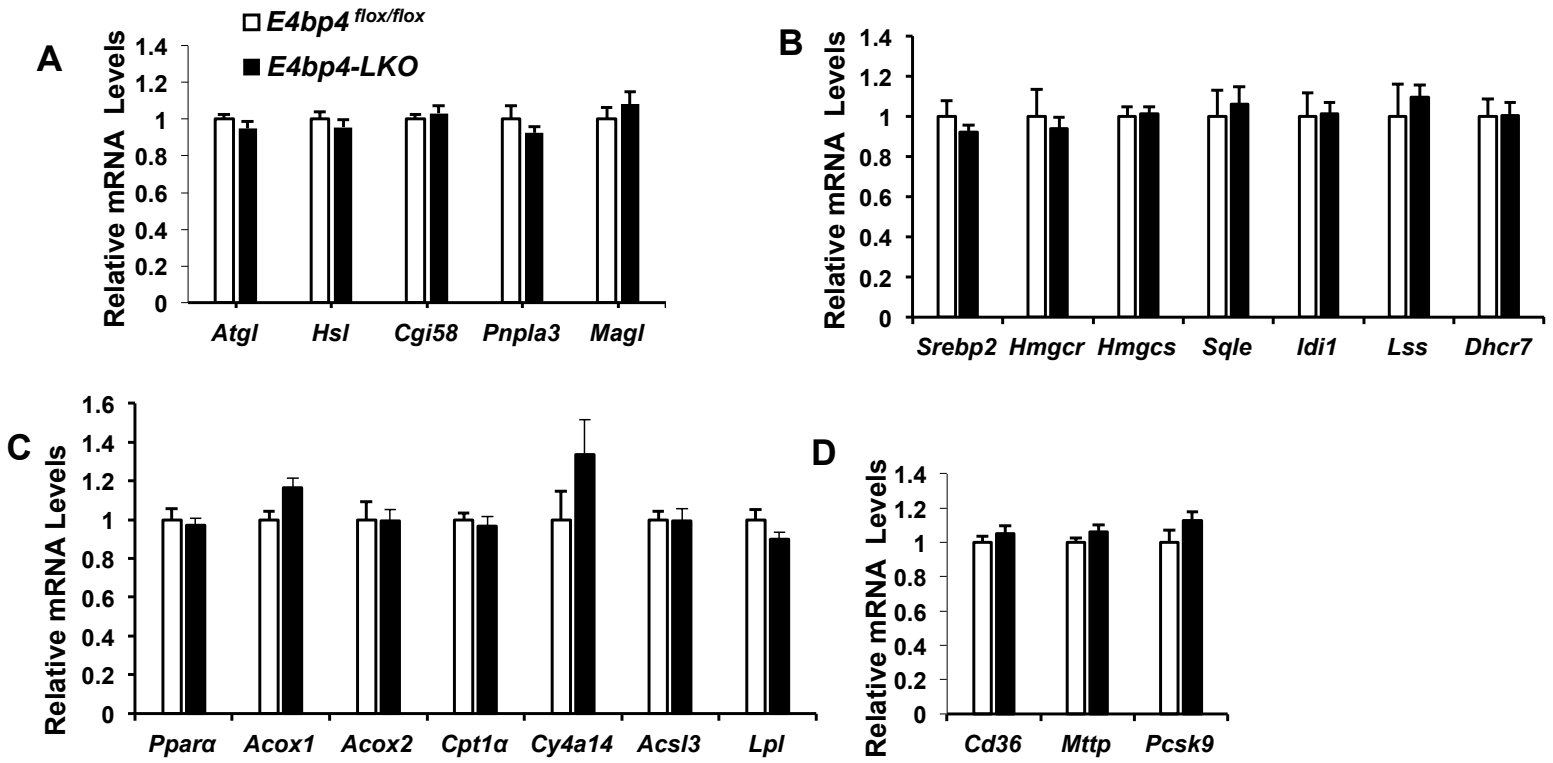
Supplementary Figure 4. Gene expression analysis of hepatic lipid metabolism in tunicamycin-treated *E4bp4 flox/flox* vs. *E4bp4-LKO* PMHs. (A) The mRNA levels of lipolysis genes (*Atgl, Cgi58, Hsl, Magl,* and *Pnpla3*); (**B**) The mRNA levels of autophagy genes.



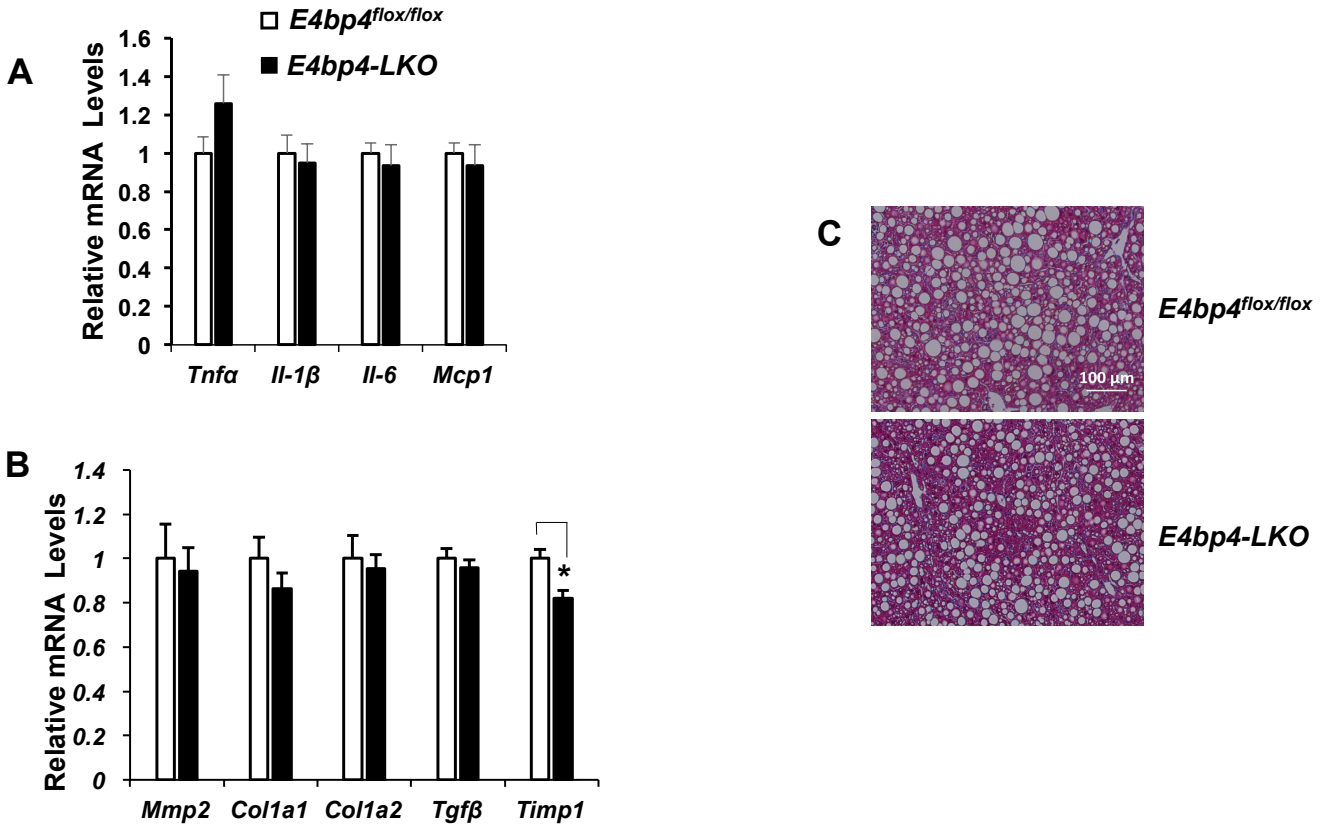
Supplementary Figure 5. The expression of apoptotic genes in tunicamycin-treated *E4bp4-LKO vs*. *E4bp4*$^{flox/flox}$ PMHs. The mRNA levels of apoptotic genes (*Bax, Noxa, Puma,* and *Bcl2l2*) in PMHs were measured by RT-qPCR. **p* <0.05, ***p* <0.01, and ****p* < 0.001 by the Student's *t*-test. n.s.: not significant



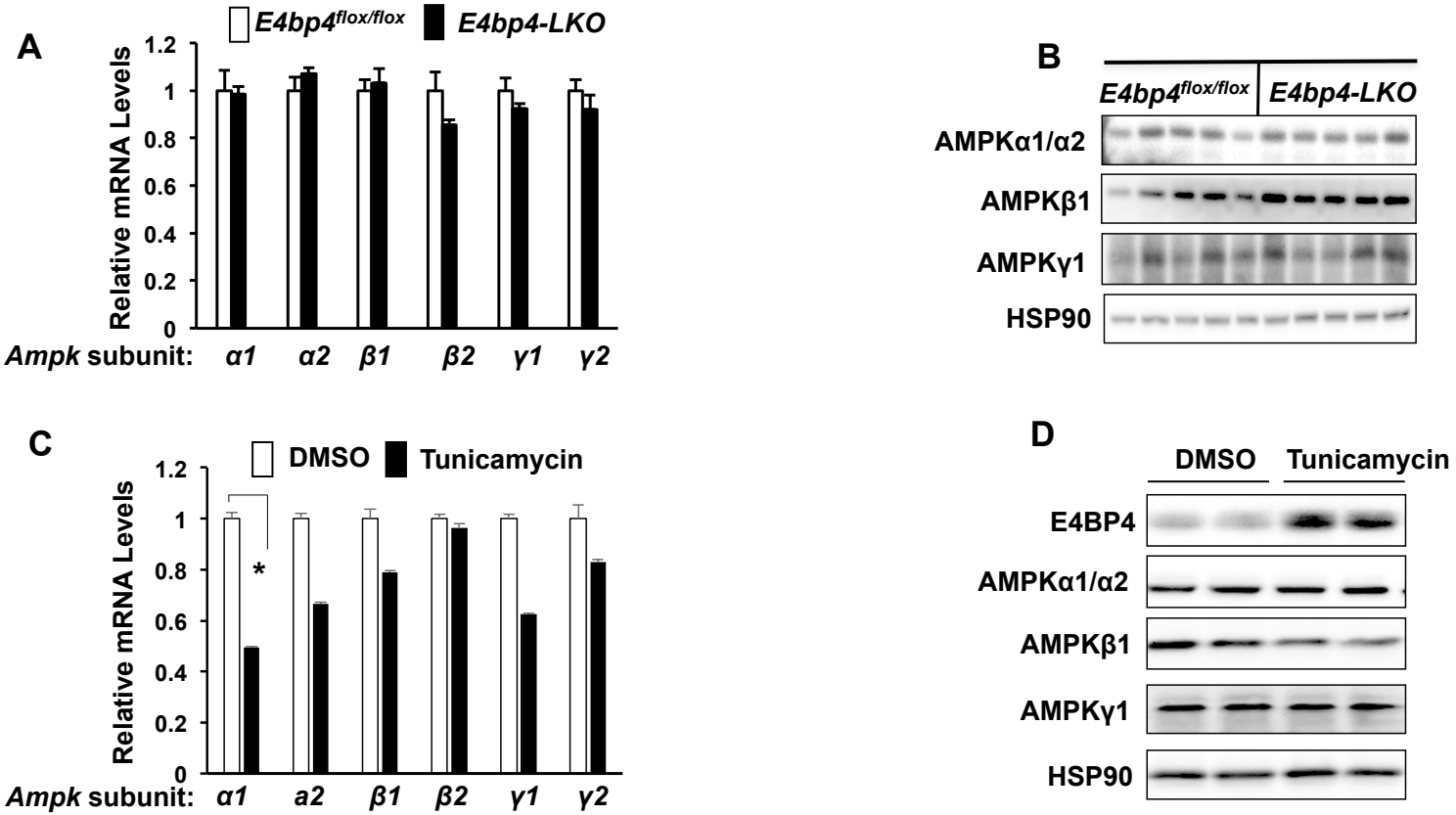
Supplementary Figure 6. Metabolic phenotype of *E4bp4-LKO vs*. *E4bp4*$^{flox/flox}$ mice on regular chow. Two-month male mice on regular chow was dissected and metabolic assays were performed as follows: **(A)** body weight, **(B)** liver triglycerides and cholesterol, **(C)** serum triglycerides and cholesterol**,** and **(D)** serum ALT.



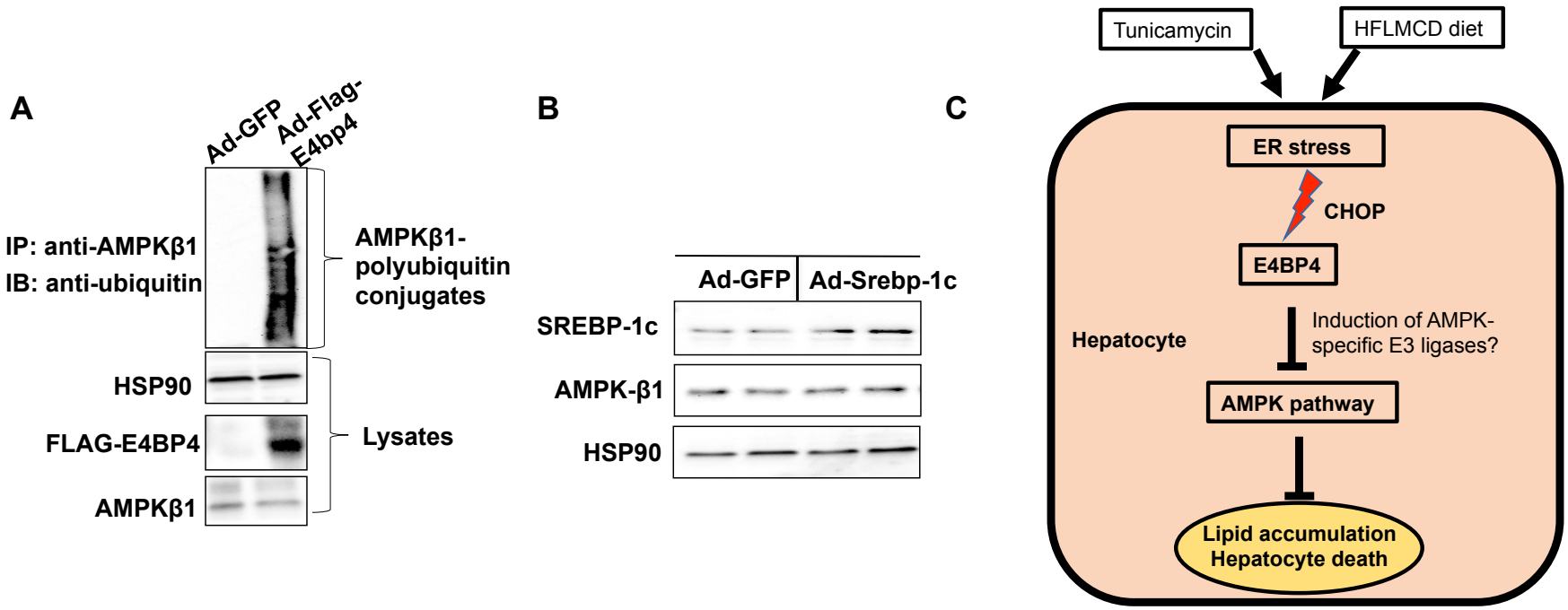
Supplementary Figure 7. Gene expression analysis of lipid metabolism in the liver of HFLMCD-fed *E4bp4-LKO vs. E4bp4*$^{flox/flox}$ mice: (**A**) lipolysis, (**B**) cholesterol biosynthesis, (**C**) fatty acid oxidation, and (**D**) fatty acid uptake.



Supplementary Figure 8. Gene analysis of hepatic inflammation and fibrosis as well as fibrosis staining of the liver of HFLMCD-fed *E4bp4*$^{flox/flox}$ *vs. E4bp4-LKO* mice. (A) mRNA levels of inflammatory genes, (**B**) mRNA levels of fibrotic genes, and **(C)** Masson's Trichrome staining of liver section of *E4bp4*$^{flox/flox}$ *vs*. *E4bp4-LKO* mice.



Supplementary Figure 9. The protein and mRNA expression of AMPK subunits in liver tissues from both *E4bp4 flox/flox* and *E4bp4-LKO* mice (A&B). The liver tissues isolated from either *E4bp4*$^{flox/flox}$ mice (n=10) or *E4bp4-LKO* (n=8) mice on regular chow were used to examine the mRNA (**A**) and protein (**B**) expression of AMPK subunits. **The protein and mRNA expression of AMPK subunits in tunicamycin-treated PMHs (C&D).** PMHs were treated with either DMSO or tunicamycin (2.5 μg/ml) overnight. (**C**) The mRNA levels of *Ampk* subunits; (**D**) The protein levels of AMPK subunits.



Supplementary Figure 10. (A) E4BP4 promotes ubiquitination of AMPK-β1 in mouse hepatocytes. PMHs were transduced with either Ad-GFP or Ad-E4BP4 for 36 hrs and and then treated with MG132 for 6 hrs before harvest. The protein lysates were used in denaturing immunoprecipitation with anti-AMPKβ1. The AMPKβ1-polyubiquitin conjugates were detected by anti-ubiquitin. **(B) Overexpression of SREBP-1c does not affect the protein abundance of AMPKβ1 in hepatocytes.** Hepa1c1c7 were transduced with either Ad-GFP or Ad-Srebp-1c and harvested 36 hrs later. The protein levels of SREBP-1c and AMPK-β1 were examined by immunoblotting. **(C)The working model.** Tunicamycin and HFLMCD diet potently induce E4BP4 in hepatocytes and the mouse liver. The ER stress transcription factor CHOP partially contributes to the induction of E4BP4 in these conditions. ER stress-induced E4BP4 may up-regulate AMPK-specific ubiquitin E3 ligases to inhibit the AMPK activity, leading to lipid accumulation and hepatocyte death in the liver.