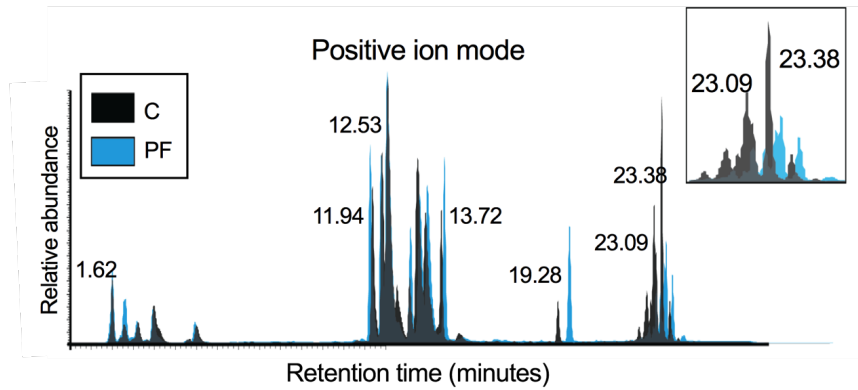
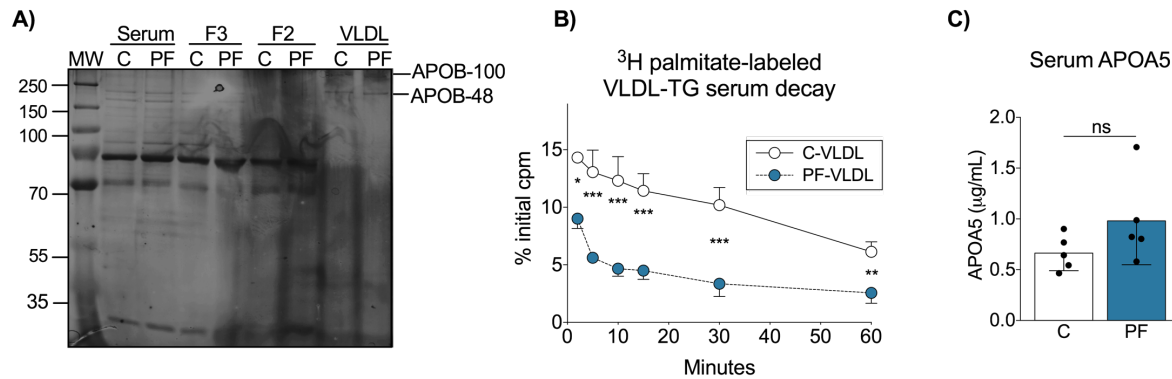


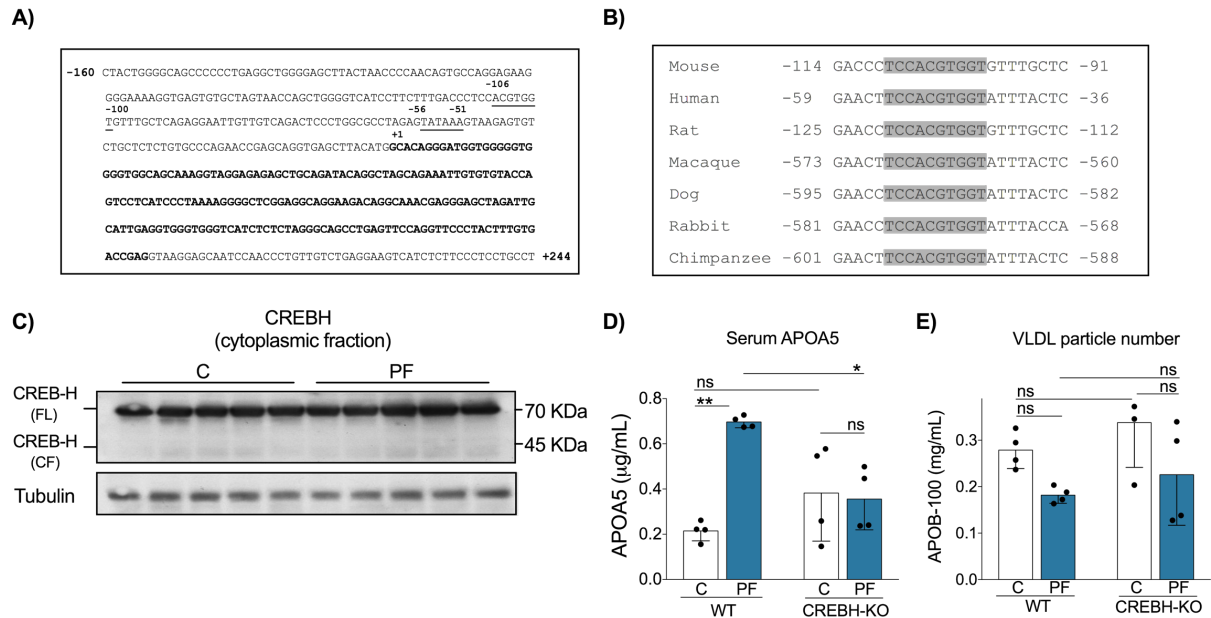
Supplemental Figures



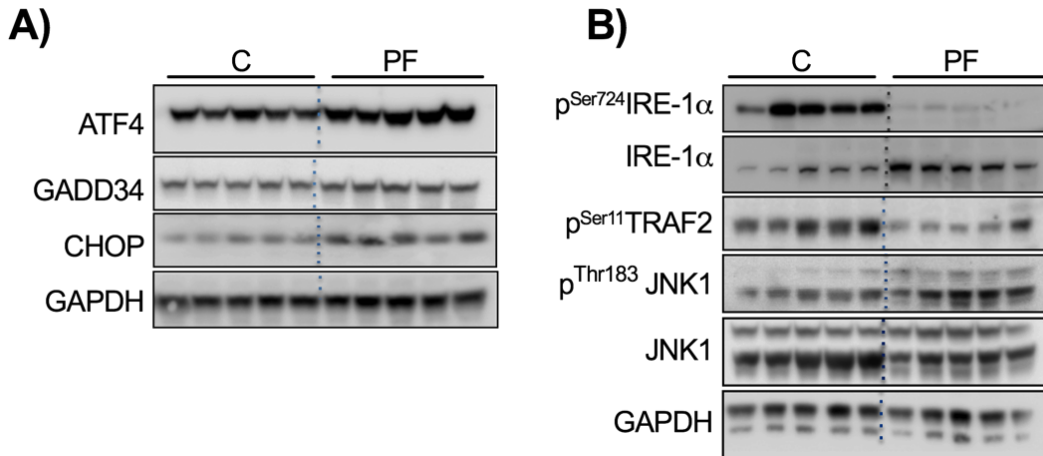
Supplemental Figure 1. Unbiased lipidomic analysis. Serum from B6D2F1 mice fed the indicated complete (C) or protein free (PF) diet for 1 week was subjected to liquid chromatography coupled to positive mode mass spectrometry (LC/MS) analysis. Insert shows a close-up of the chromatographic peaks with a retention time around 23 minutes containing most individual TGs species.



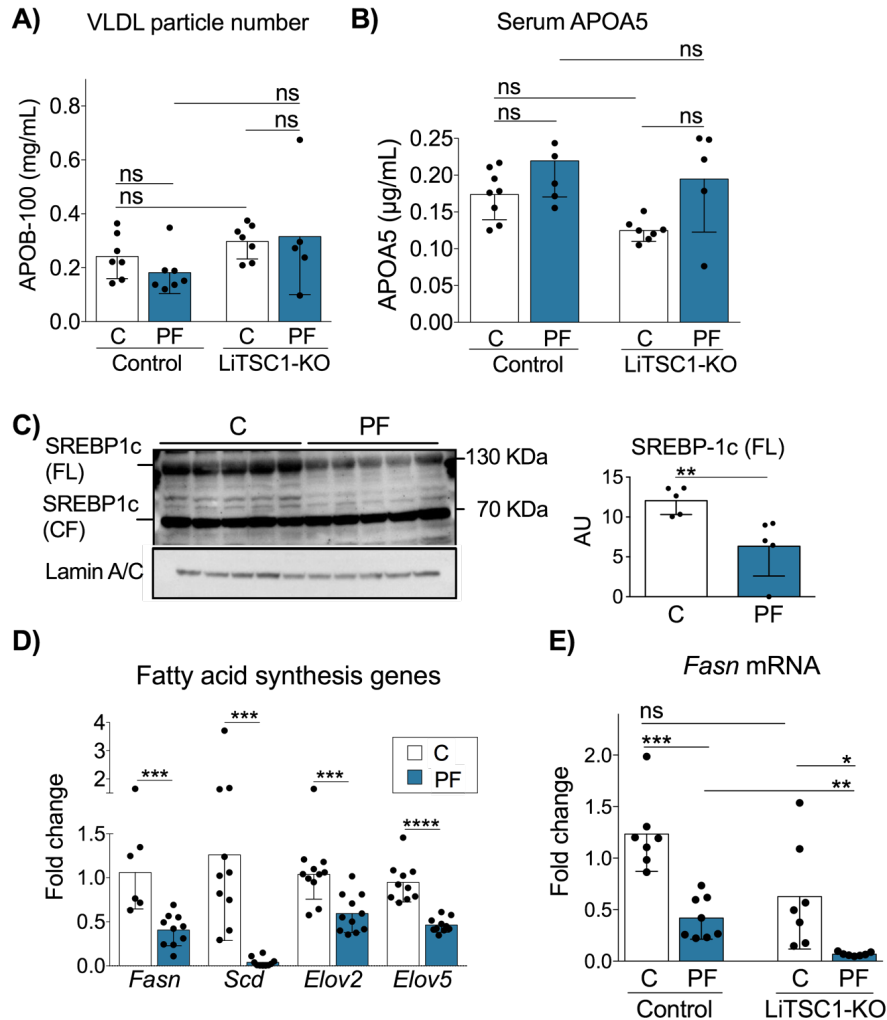
Supplemental Figure 2: Protein restriction alters VLDL-TG consumption via changes in hepatic apolipoprotein expression. (A) Silver stained-SDS polyacrylamide gel of serum from mice fed the indicated complete (C) or protein free (PF) diet for 1 week and then fractionated by density centrifugation into the following: unfractionated serum (serum); VLDL fraction (top layer); fraction 2 (F2, second layer); and fraction 3 (F3, third layer). (B) Time-dependent clearance from circulation of radiolabeled TG from [^3H]-palmitate-labeled VLDL particles purified from mice on C or PF diets, injected into mice on a complete diet (n=4/group), and expressed as a percentage of total injected radioactivity; multiple two-tailed T-tests between diet groups with Holm-Sidak post-test. (C) Serum APOA5 concentration measured by ELISA in mice on the indicated diet for 1 week; n=5/group; two-tailed Student's T-test $P=0.16$). Data expressed as mean \pm SD; * $P<0.05$, ** $P<0.01$, *** $P<0.001$.



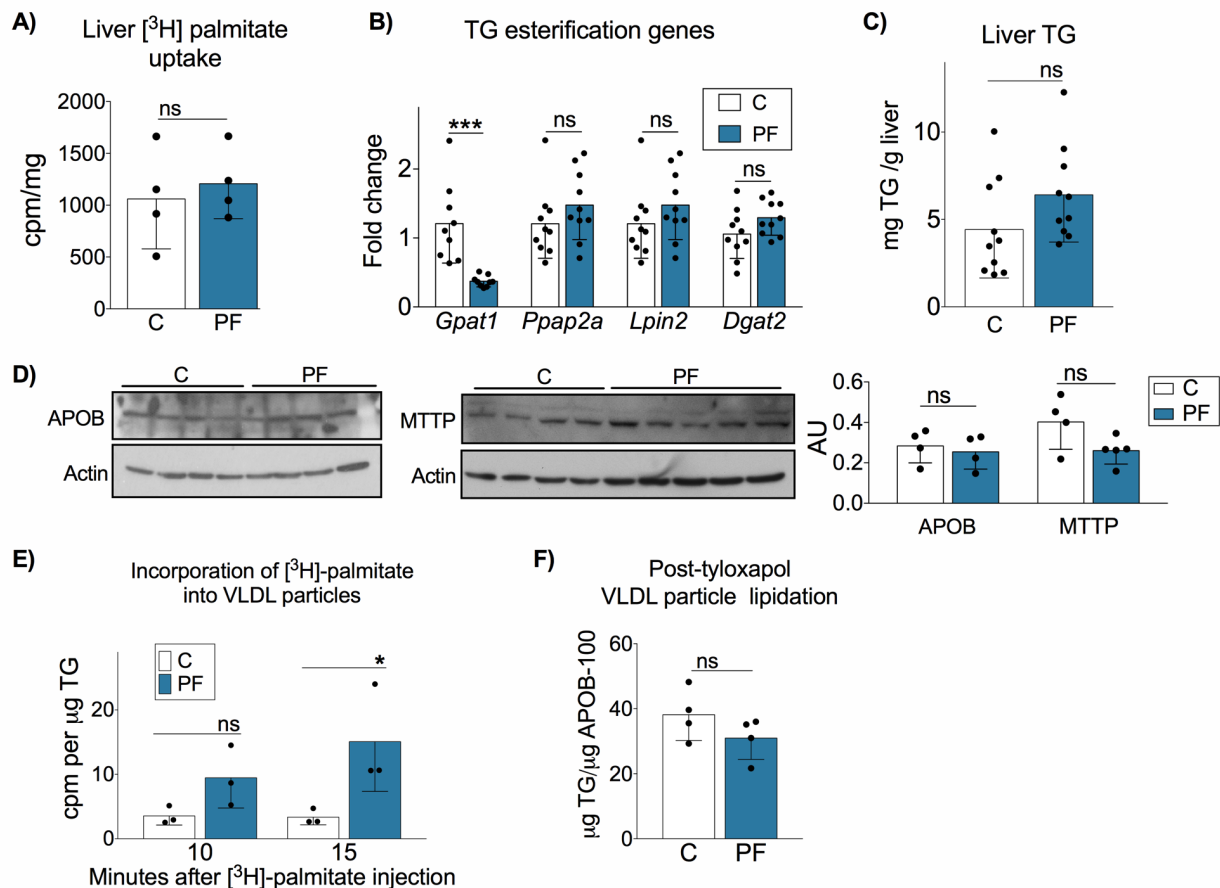
Supplemental Figure 3. CREBH regulates *Apoa5* expression upon protein restriction. (A) Sequence of the mouse *Apoa5* promoter region showing the CREBH consensus-binding sequence located 106 bp upstream of the transcriptional start site and the TATA box at -56 (both underlined). (B) Genomic alignment of the *Apoa5* promoter from different mammalian species showing conservation of the CREBH-consensus binding sequence in gray. (C) Immunoblot of CREBH full length (FL) and cleaved fragment (CF) in the cytosolic fraction of liver extracts of mice on the indicated complete (C) or protein free (PF) diet for 1 week (n=5/group). (D) Serum APOA5 in WT and CREBH-KO mice on the indicated diet (n=4/group; 2-way ANOVA with Sidak post-test between the indicated groups; interaction $P=0.002/37\%$ variation; genotype $P=0.004/29\%$ variation; diet ns). (E) Serum APOB-100 concentration representative of circulating VLDL particle number in WT and CREBH-KO mice on the indicated diet (n=4/group; 2-way ANOVA with Sidak post-test between the indicated groups; interaction ns; diet ns; genotype $P=0.01/35\%$ variation). Data expressed as mean \pm SD; * $P<0.05$; ** $P<0.01$; ns, not significant.



Supplemental Figure 4. Protein restriction does not induce endoplasmic reticulum stress in the liver. Immunoblots of ATF4, GADD34 and CHOP (**A**) and phosphorylated and total IRE1, phosphorylated TRAF2, and phosphorylated and total JNK1 (**B**) in liver extracts of mice fed the indicated complete (C) or protein free (PF) diet for 1 week.



Supplemental Figure 5. Constitutive mTORC1 signaling inhibits CREBH activation. (A, B) Liver-specific TSC1-KO (LiTSC1-KO; $TSC1^{flx/flx}|Albumin-Cre^{+/-}$) mice with constitutive mTORC1 signaling in hepatocytes and littermate controls ($TSC1^{flx/flx}|Albumin-Cre^{-/-}$) were placed on a complete (C) or protein free (PF) diet for 1 week as indicated. (A) Serum APOB-100 concentration representative of circulating VLDL particle number (n=6-7/group; 2-way ANOVA with Sidak post-test between the indicated groups; genotype $P=0.04/16\%$ variation; interaction and diet ns). (B) Serum APOA5 levels (n=5-8/group; 2-way ANOVA with Sidak post-test between the indicated groups; interaction ns; genotype $P=0.04/11\%$ variation; diet $P=0.002/28\%$ variation). (C) Immunoblot of full length (FL) and cleaved form (CF) of SREBP1c in liver extracts of B6D2F1 mice on the indicated diet. Right, quantitation of FL SREBP1c normalized to lamin A/C and expressed in arbitrary units (AU; n=5/group; two-tailed Student's T test). (D) Hepatic mRNA expression of genes involved in fatty acid synthesis, including *Fasn*, fatty acid synthetase; *Scd*, stearoyl CoA desaturase; and *Elov*, long chain fatty acid elongase (n=5-10/group; two-tailed Student's T test). (E) Hepatic *Fasn* mRNA expression (n=7-8/group; 2-way ANOVA with Sidak post-test between the indicated groups; interaction ns; genotype $P=0.0006/21\%$ variation; diet $P=0.0001/44\%$ variation). Data expressed as mean \pm SD; * $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$; ns, not significant.



Supplemental Figure 6. Reduced hepatic VLDL particle secretion rate contributes to TG lowering upon protein restriction independent of CREBH-APOA5 activation. (A) Hepatic free fatty acid uptake in B6D2F1 mice on the indicated complete (C) or protein free (PF) diet. Mice were injected intravenously with albumin-conjugated [³H]-palmitate and livers analyzed for tracer uptake 15 minutes later (n=4/group; two-tailed Student's T-test). (B) Hepatic mRNA expression of genes involved in *de novo* lipogenesis (n=9-10/group; two-tailed Student's T test between diets). (C) Liver TG levels from mice on the indicated diet 3 hours after a tyloxapol challenge (n=10/group; two-tailed Student's T test). (D) Immunoblot of microsomal triglyceride transfer protein (MTTP) and APOB-100 in liver extracts of mice on the indicated diet. Below, quantitation normalized to actin and expressed in arbitrary units (AU) (n=4-5/group; two-tailed Student's T test between diets). (E) Hepatic VLDL lipidation capacity. Incorporation of [³H]-palmitate injected into the portal vein of tyloxapol-treated mice into VLDL particles at the indicated time point, expressed per mg of TG in purified VLDL particles (n=3/group; 2-way repeated measures ANOVA with Sidak post-test between the indicated groups). (F) TG content in VLDL particles isolated from tyloxapol-treated mice (n=4/group; two-tailed Student's T test). Data expressed as mean ±SD; *P<0.05; ***P<0.001; ns, not significant.