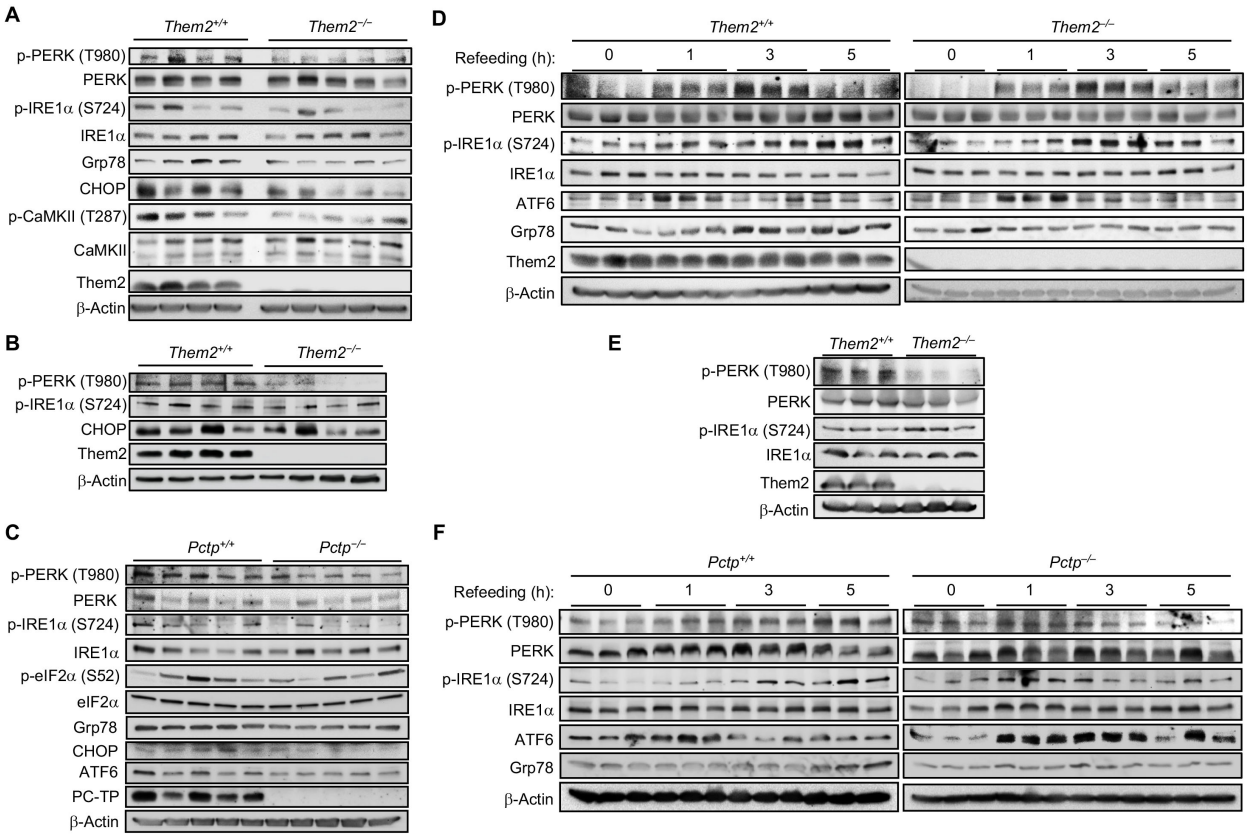
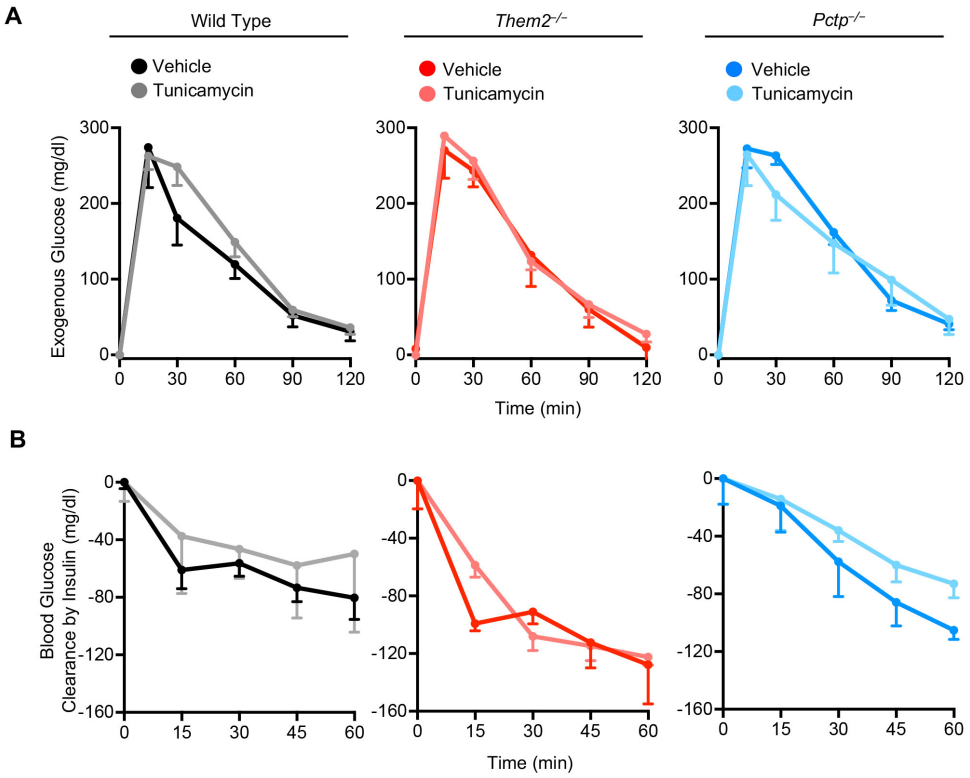
SUPPLEMENTAL FIGURES AND LEGENDS

Supplemental Figure 1



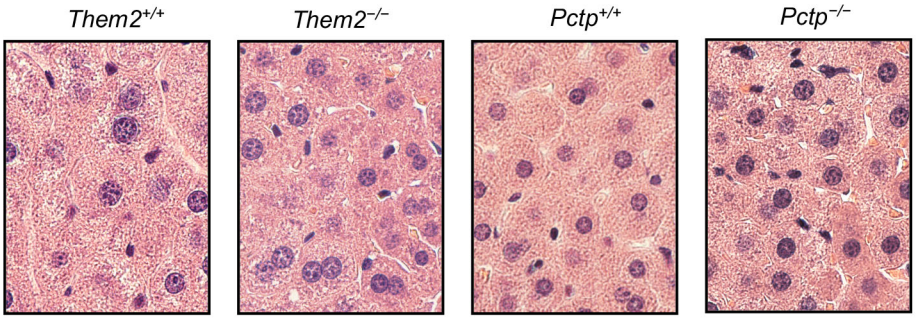
Supplemental Figure 1. Them2 and PC-TP regulate ER stress in the livers of mice. (A-C) 4 w old mice were fed high fat diet for 8 w. Liver homogenates from (A, B) *Them2*$^{+/+}$ (n = 8) and *Them2*$^{-/-}$ (n = 9) mice and (C) *Pctp*$^{+/+}$ (n = 5) and *Pctp*$^{-/-}$ (n = 5) mice were subjected to immunoblot analyses. (D-F) ER stress was induced in 8 w old chow fed mice by overnight fasting, followed by refeeding. Liver homogenates were subjected to immunoblot analyses. (E) Liver homogenates from mice that were refed for 3 h in panel (D) were subjected to immunoblot analysis for side by side comparison.

Supplemental Figure 2



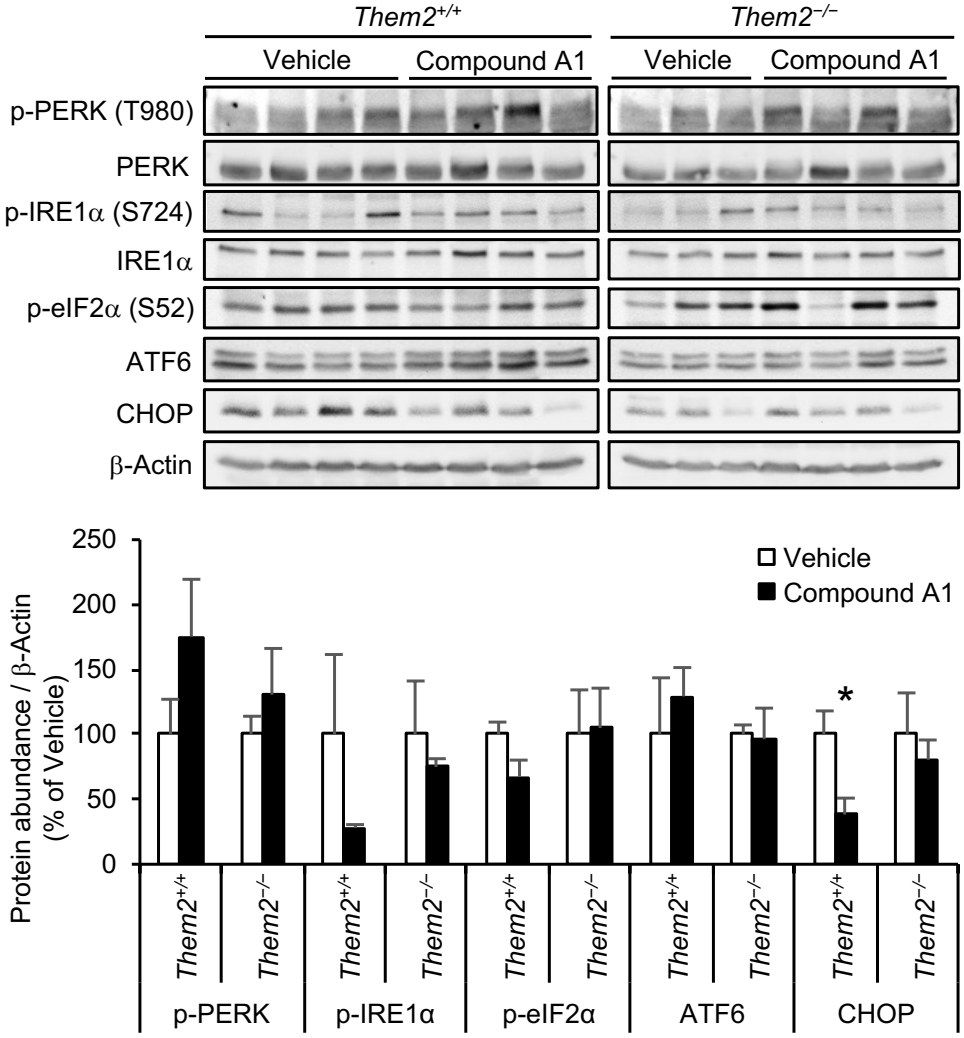
Supplemental Figure 2. Glucose and insulin tolerance tests in tunicamycin-treated *Them2*$^{-/-}$ and *Pctp*$^{-/-}$ mice and their WT littermate controls. 8 w old chow fed mice were injected I.P. with tunicamycin (0.25 mg / kg body weight) or vehicle (DMSO, 0.25% v/v) for 2 consecutive days. After 6 h of food withdrawal, *WT* (*n* = 4-5), *Them2*$^{-/-}$ (*n* = 3-4) and *Pctp*$^{-/-}$ (n = 3-5) mice were subjected to glucose tolerance tests (A) and insulin tolerance tests (B). Statistical significance was determined by Student's *t*-test.

Supplemental Figure 3



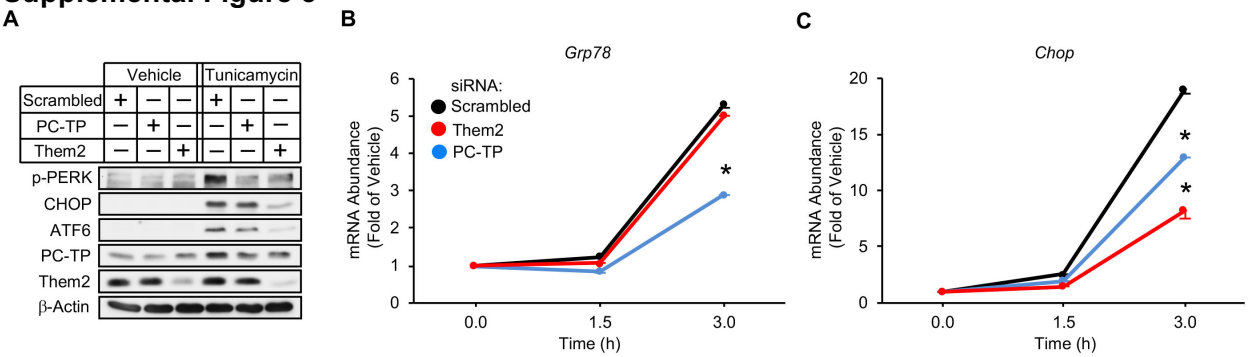
Supplemental Figure 3. Liver histology of *Them2*$^{-/-}$ and *Pctp*$^{-/-}$ mice and their WT littermate controls. 8 w old chow fed mice (n=3 per image) were injected with vehicle (DMSO, 0.25% v/v) for 2 consecutive days. Livers were harvested 6 h following food restriction and subjected to hematoxylin and eosin (H&E) staining. Figures are representative of three mice per condition.

Supplemental Figure 4.



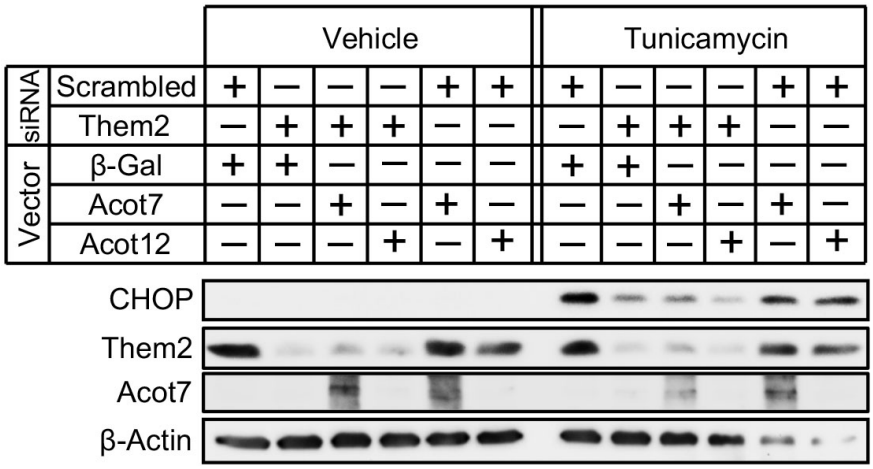
Supplemental Figure 4. Regulation of ER stress by PC-TP is mediated by interactions with Them2. 8 w old chow fed *Them2*$^{+/+}$ and *Them2*$^{-/-}$ mice were injected IP with Compound A1 (5 mg / kg body weight) or vehicle (4% DMSO and 96% of 6% hydroxypropyl-β-cyclodextrin v/v) for 5 d. ER stress was induced by administration of tunicamycin (0.25 mg / kg body weight) by IP injection on d 4 and 5 of compound A1 treatment. Liver homogenates were harvested 24 h following the final tunicamycin injection and subjected to immunoblot analyses. Bar graph represents the densitometric quantification of immunoblots normalized to β-actin as loading control. Error bars represent SEM. Statistical significance was determined by Student's *t*-test. **P* < 0.05 compared to vehicle.

Supplemental Figure 5



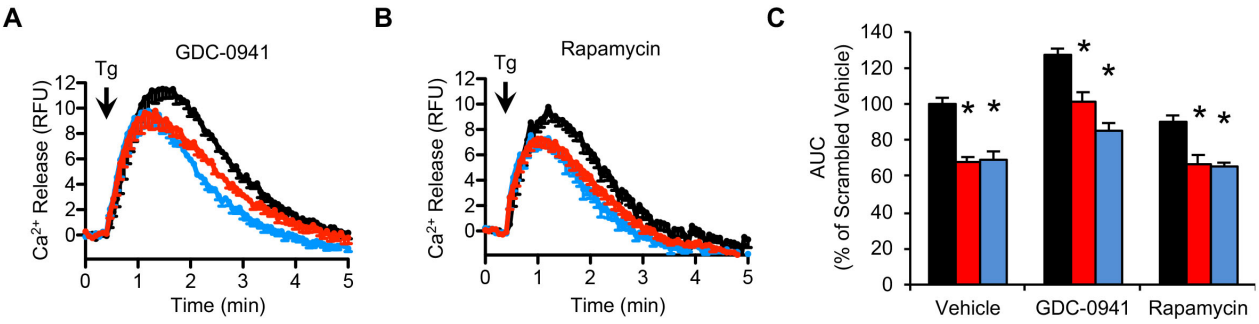
Supplemental Figure 5. Knockdown of Them2 or PC-TP reduces ER stress. siRNA-mediated knockdown of Them2 and PC-TP in Hepa1-6 cells was achieved by transfection with scrambled siRNA as control. (A) Cells were treated with vehicle (DMSO, 0.2% v/v) or tunicamycin (1 μg/ml) for 5 h prior to immunoblot analysis. (B, C) Cells were treated with vehicle (DMSO, 0.1% v/v) or tunicamycin (1 μg/ml) for 1.5 and 3 h prior to qPCR analysis for (B) *Grp78* and (C) *Chop*. Tbp mRNA was used as internal control reference. Data are representative of three independent experiments. Statistical significance was determined by Student's *t*-test adjusted by Bonferroni correction. **P* < 0.025 compared to scrambled.

Supplemental Figure 6



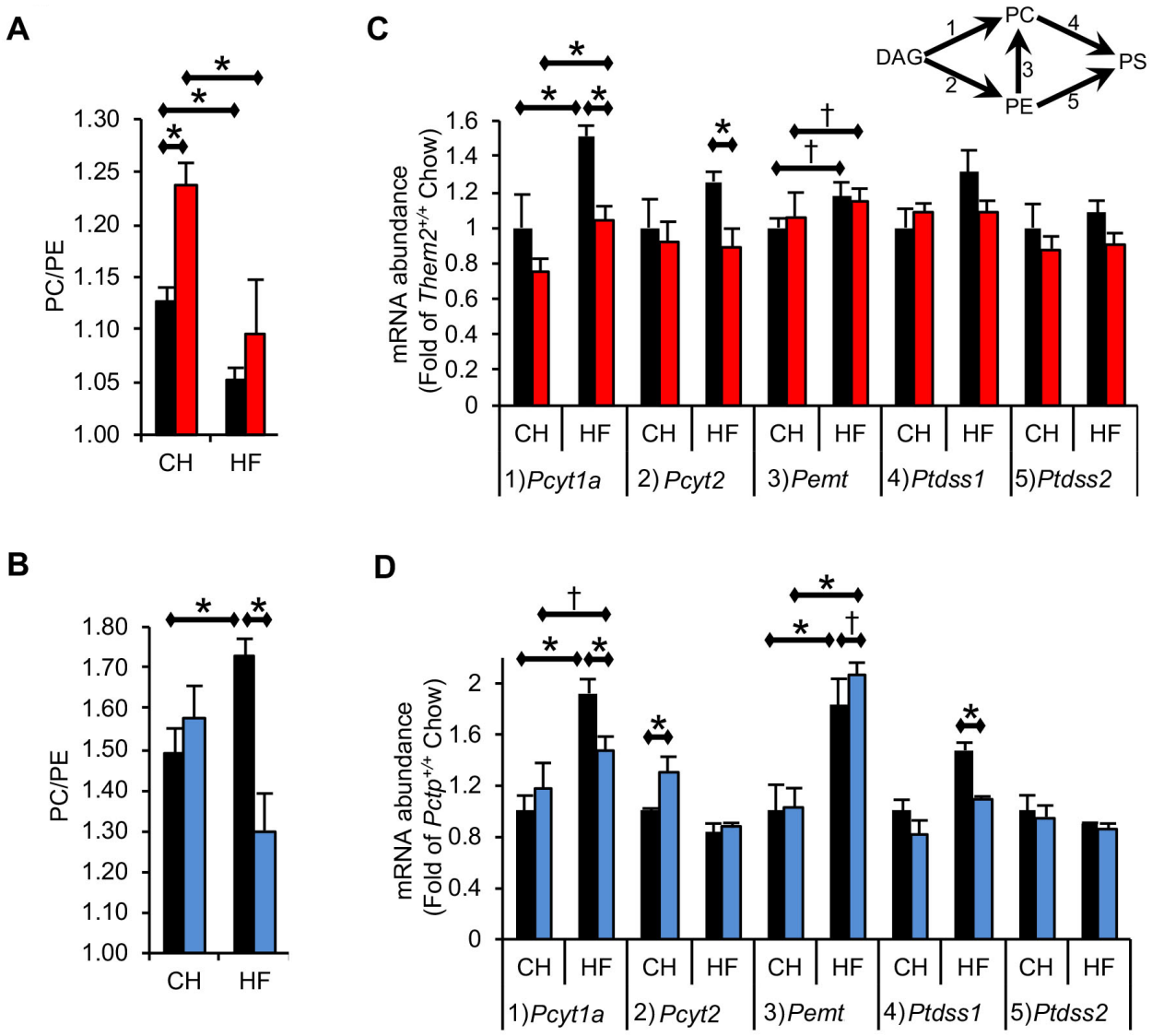
Supplemental Figure 6. ER stress is regulated by the acyl-CoA thioesterase Them2 but not Acot7 and Acot12. HEK 293E cells were co-transfected with Them2 siRNA or scrambled control along with the indicated expression vectors with β-Galactosidase (β-Gal) as control. ER stress was induced by treating cells with tunicamycin (1 μg/ml) or vehicle (DMSO, 0.1% v/v) for 5 h, and detected by the expression of CHOP in immunoblot analyses. Immunoblots represent three independent experiments.

Supplemental Figure 7



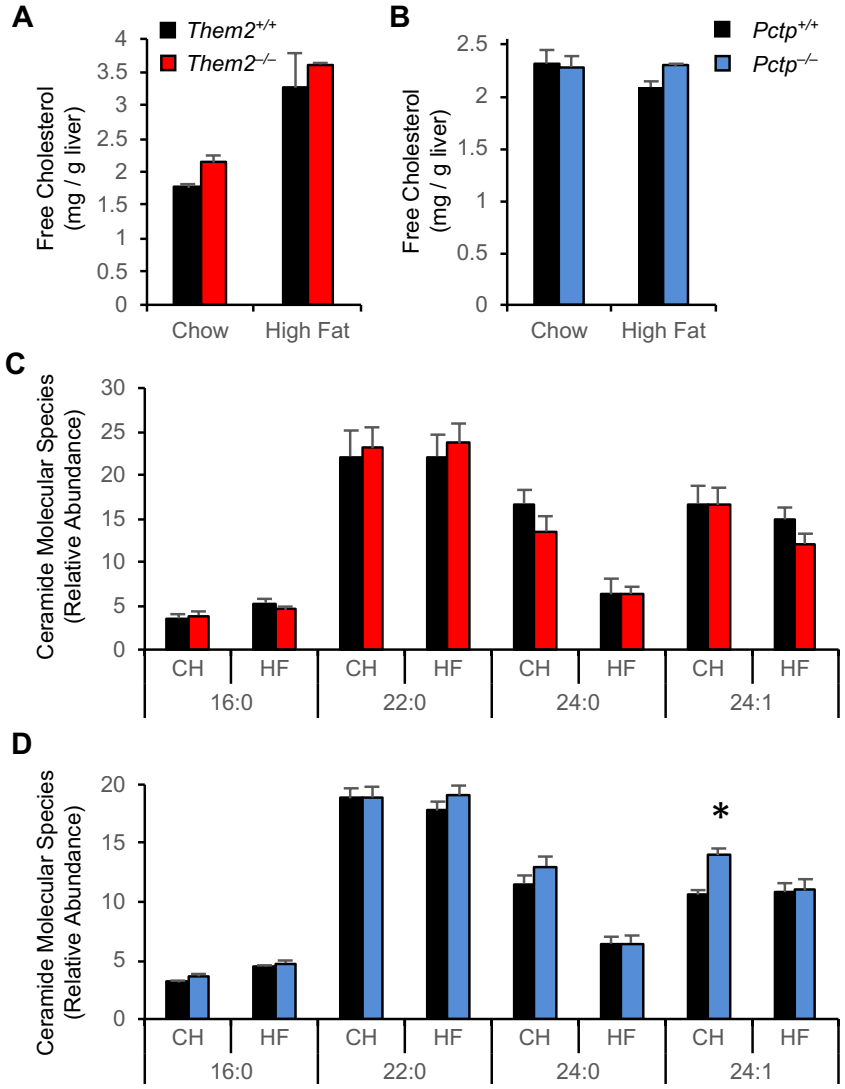
Supplemental Figure 7. Regulation of ER calcium release by Them2 and PC-TP is independent from Akt and mTOR. Thapsigargin- (Tg-) induced calcium release from ER following knockdown of Them2 or PC-TP in serum-starved HEK 293E cells. (A, B) Cells were pre-incubated with (A) GDC-0941 (500 nM), (B) rapamycin (20nM) or vehicle (0.1 % v/v DMSO) for 1 h. (C) Area under the curve (AUC) as calculated from panels (A) and (B). AUC for vehicle treatment is as demonstrated in Figure 3B. Error bars represent SEM for 6 independent measurements for GDC-0941 and rapamycin treatments and 18 independent measurements for vehicle treatment. Statistical significance was determined by Student's *t*-test adjusted by Bonferroni correction. **P* < 0.025 compared to scrambled.

Supplemental Figure 8



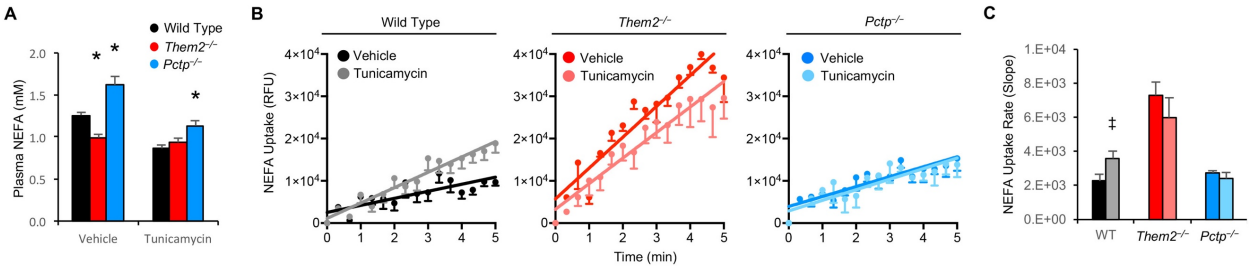
Supplemental Figure 8. Them2 and PC-TP influence hepatic ER membrane PC/PE ratio. 4 w old mice were fed chow or high fat diet for 8 w, and livers were harvested following 6 h food restriction. (A, B) ER membrane PC/PE ratio was calculated from the relative abundance of PC and PE molecules using the mass spectrometry analysis of ER microsomes that were purified from the livers of (A) *Them2*$^{+/+}$ (n = 4) and *Them2*$^{-/-}$ (n = 4) mice and (B) *Pctp*$^{+/+}$ (n = 4) and *Pctp*$^{-/-}$ (n = 4) mice. (C, D) mRNA abundance for *Pcyt1a*, *Pcyt2*, *Pemt*, *Ptdss1* and *Ptdss2* was determined by q-PCR analysis. Trb mRNA was used as internal control. Error bars represent SEM. Statistical significance was determined by Student's *t*-test. **P* < 0.05 compared to WT mice. †Not significant.

Supplemental Figure 9



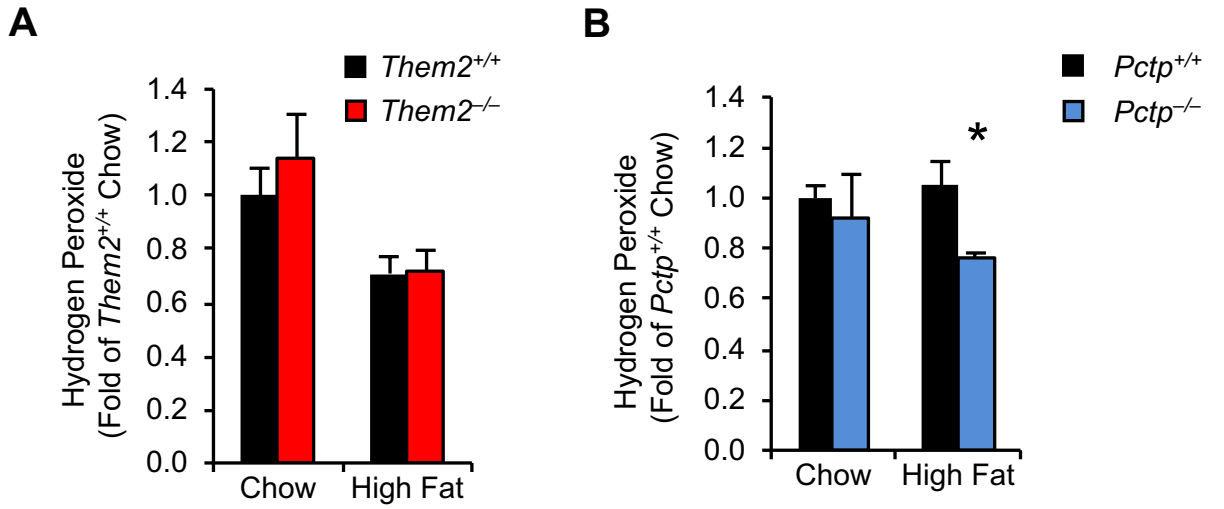
Supplemental Figure 9. Them2 and PC-TP do not influence hepatic concentrations of free cholesterol or ceramides. 5 w old *Them2*$^{+/+}$ (n = 4), *Them2*$^{-/-}$ (n = 4), *Pctp*$^{+/+}$ (n = 4) and *Pctp*$^{-/-}$ (n = 4) mice were fed chow or high fat diet for 9 w, and livers were harvested following 6 h food restriction. (A, B) Influence of (A) Them2 and (B) PC-TP on free cholesterol. (C, D) Influence of (C) Them2 and (D) PC-TP on ceramide abundance. Lipids were extracted and subjected to mass spectrometry analysis for ceramide molecular species. Error bars represent SEM. Statistical significance was determined by Student's *t*-test. **P* < 0.05 compared to WT mice.

Supplemental Figure 10



Supplemental Figure 10. Them2 and PC-TP do not regulate the hepatic uptake of NEFA in response to tunicamycin. (A) 8 w old chow fed *Them2*$^{-/-}$ (n = 5), *Pctp*$^{-/-}$ (n = 5) and WT mice (n = 5) were injected I.P. with tunicamycin (0.25 mg / kg body weight) or vehicle (DMSO, 0.25% v/v) for 2 consecutive days. Plasma was analyzed for NEFA concentrations. (B) NEFA uptake was measured in mouse primary hepatocytes that were harvested from *Them2*$^{-/-}$, *Pctp*$^{-/-}$ and WT mice and treated with tunicamcyin (1 µg/ml) of vehicle (DMSO, 0.1% v/v) for 5 h. (C) The slopes of time dependent NEFA uptake from panel B represent the NEFA uptake rates (B). Error bars represent SEM for 4-5 independent measurements. Statistical significance was determined by Student's *t*-test adjusted by Bonferroni correction. **P* < 0.025 compared to scrambled and ‡*P* = 0.063 compared to vehicle.

Supplemental Figure 11



Supplemental Figure 11. Contributions of Them2 and PC-TP to reactive oxygen species in mouse livers. 5 w old (A) *Them2*$^{+/+}$ (n = 4) and *Them2*$^{-/-}$ (n = 4) and (B) *Pctp*$^{+/+}$ (n = 4) and *Pctp*$^{-/-}$ (n = 4) mice were fed chow or high fat diets for 9 w, and livers were harvested following 6 h food restriction. (A, B) Influence of Them2 and PC-TP on hydrogen peroxide concentrations were determined in liver homogenates. Error bars represent SEM. Statistical significance was determined by Student's *t*-test. *$P < 0.05$ compared to WT mice.