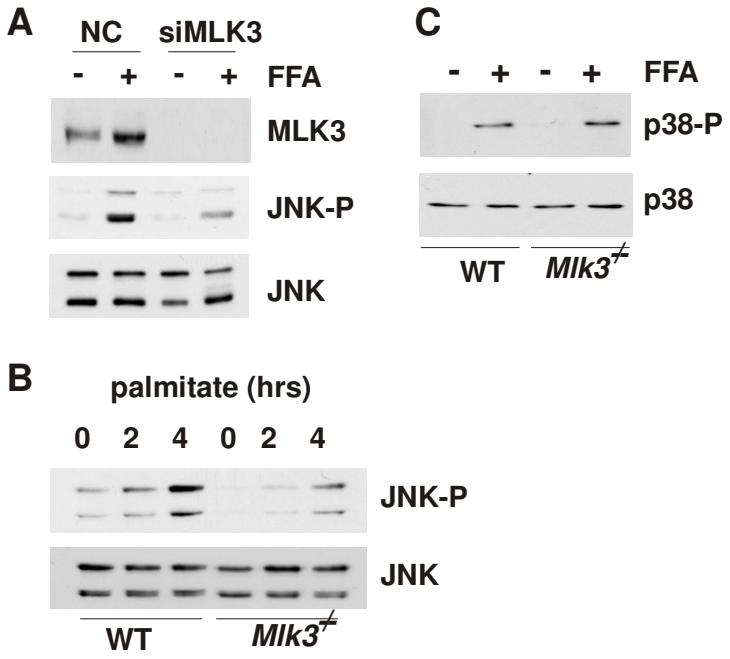
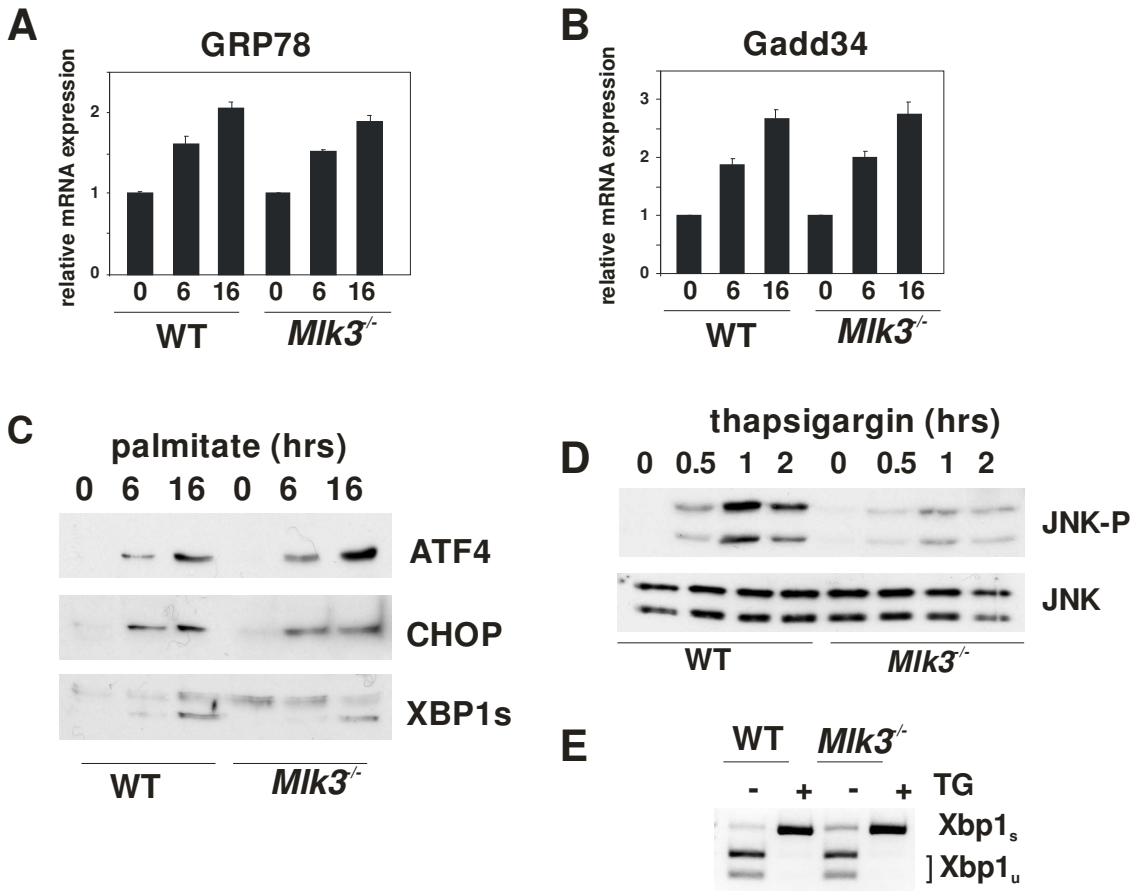
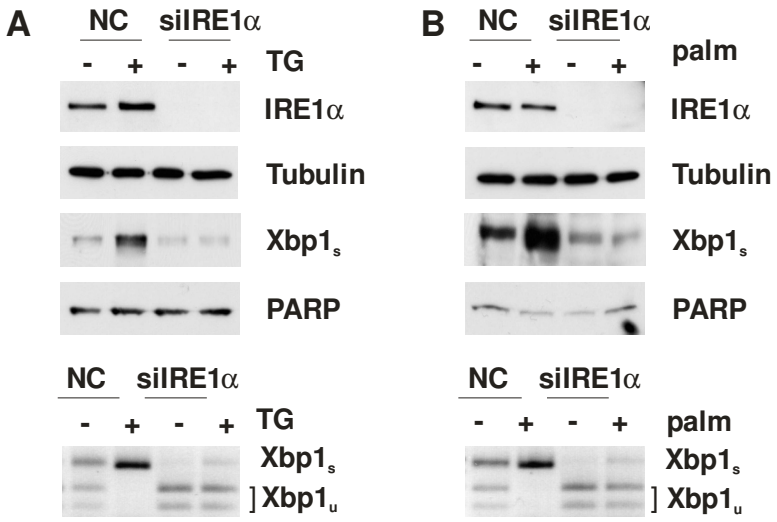


Supplemental Fig. 1: Effects of MLK3 depletion on SFA-induced JNK activation. (A) Hepa1c1c7 cells were transfected with non-silencing control or siRNA specific for MLK3 and incubated with 0.5 mM palmitic acid for 6 h. The efficacy of MLK3 depletion and the expression and phosphorylation of JNK was examined by immunoblot analysis. (B) Primary mouse hepatocytes were incubated with 0.5 mM palmitate for the indicated time points and the expression and phosphorylation of JNK was examined by immunoblot analysis. (C) Primary mouse hepatocytes were incubated with 0.5 mM palmitate for 6 h. The expression and phosphorylation of p38 MAPK was examined by immunoblot analysis.



Supplemental Fig. 2: SFA induce ER stress in hepatocytes. Primary mouse WT and KO hepatocytes were incubated with palmitate for the indicated times. mRNA levels of GRP78 (A) and Gadd 34 (B) were examined by qPCR. (C) Primary mouse hepatocytes were incubated with for the indicated times. Nuclear extracts were prepared and expression of ATF4, CHOP and spliced Xbp1 was determined by immunoblot analysis. Primary mouse WT and KO hepatocytes were incubated with thapsigargin for the indicated time points and the expression and phosphorylation of JNK was examined by immunoblot analysis (D). Spliced Xbp1 was detected by RT-PCR and PstI-digest (E).



Supplemental Fig. 3: Effects of IRE1α depletion on Xbp1 splicing. Hepa1c1c7 cells were transfected with non-silencing control or siRNA specific for IRE1α and incubated with 1 μM thapsigargin for 1 h (A) or 0.5 mM palmitate for 6 h (B) before fractionation into nuclear and cytoplasmic fractions. Efficacy of IRE1α depletion was determined by immunoblotting of cytoplasmic extracts. Spliced Xbp1 was detected by immunoblot analysis of nuclear lysates (upper panel) or by RT-PCR and PstI-digest (lower panel). Expression of Tubulin and PARP was used as loading controls.