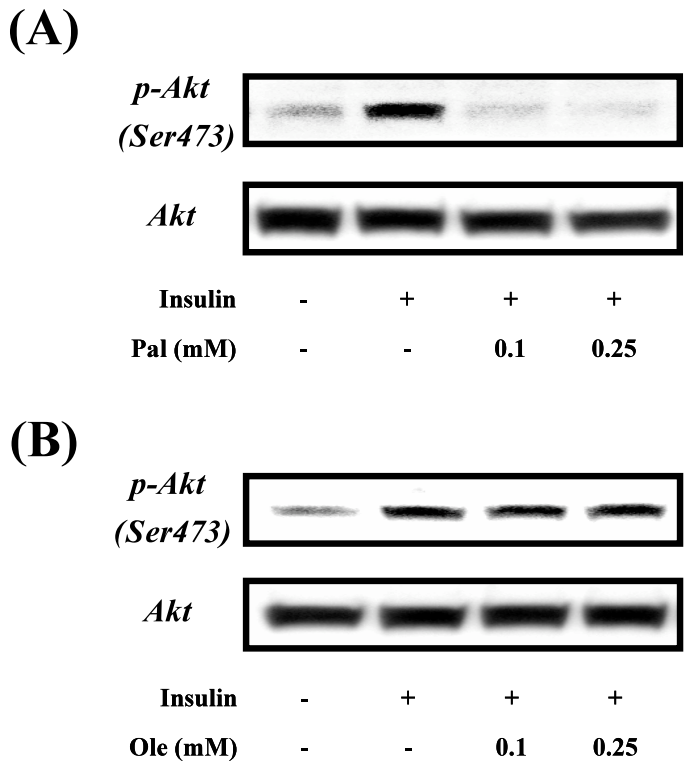
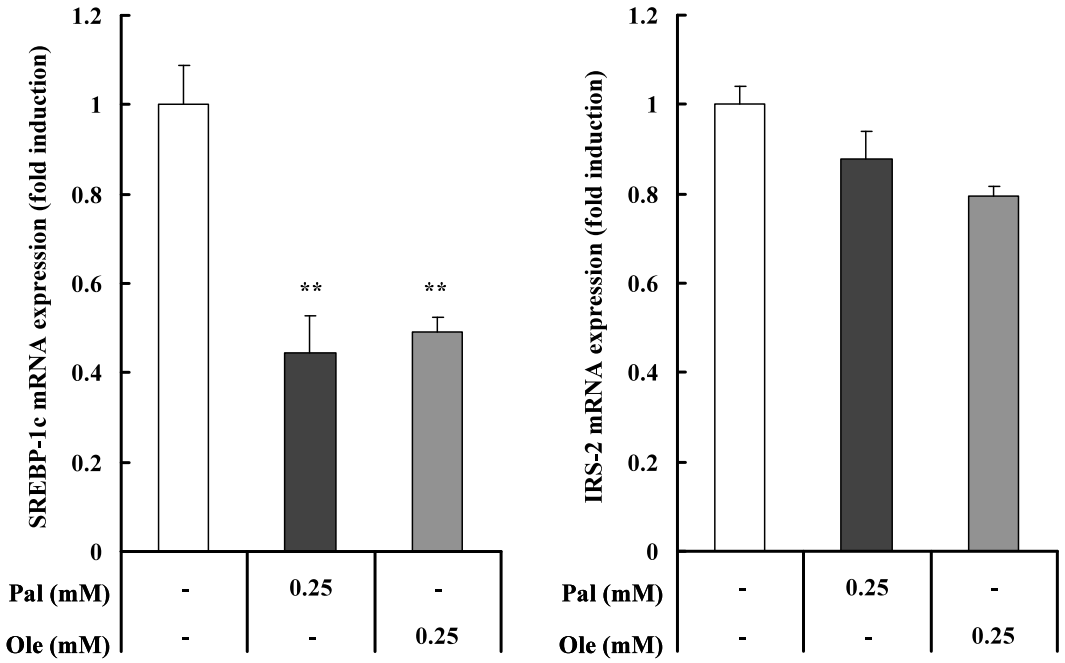
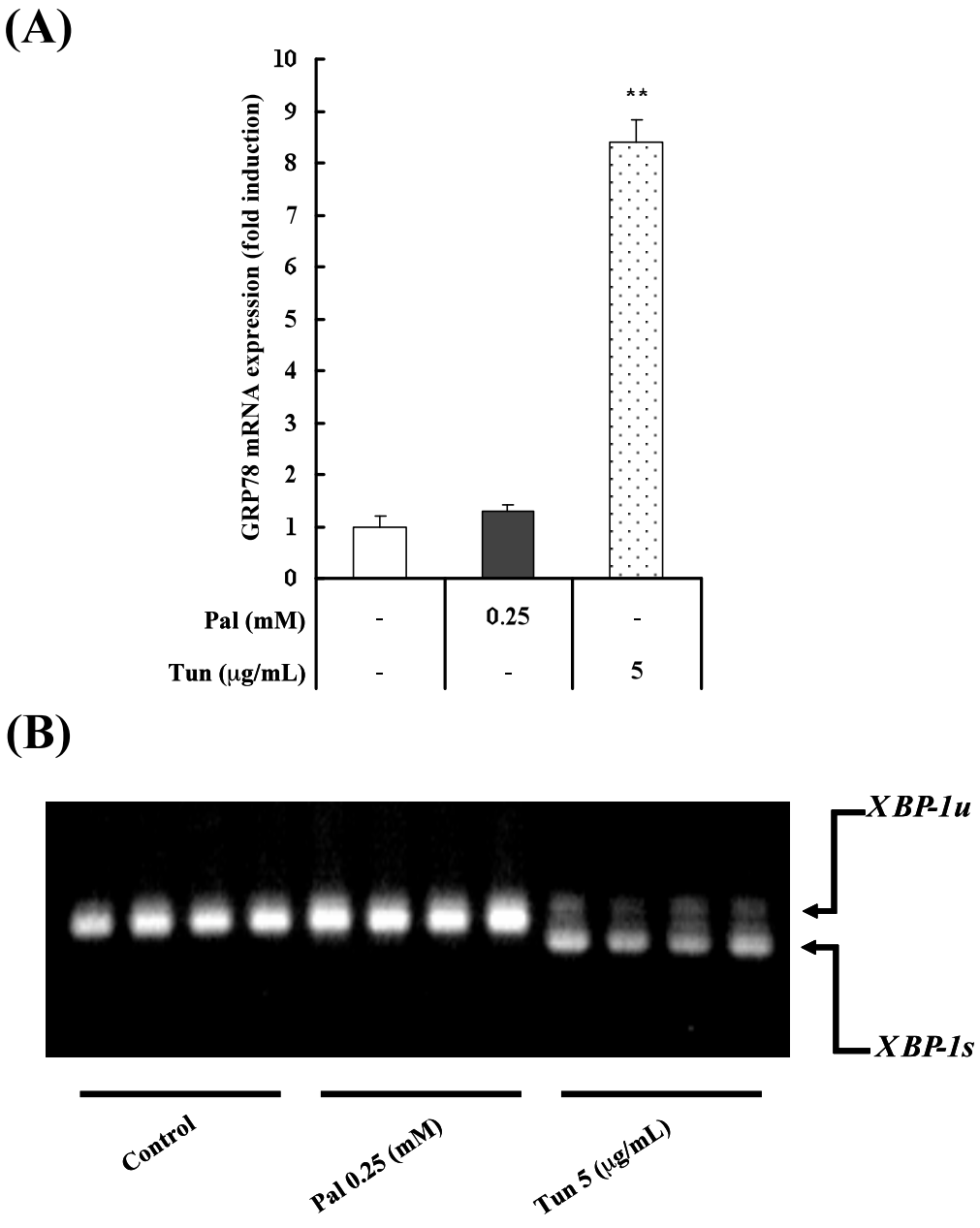
Supplemental Fig. 1. Effects of palmitate and oleate on insulin-stimulated serine phosphorylation of Akt in HepG2 cells. HepG2 cells were incubated in the presence or absence of FFAs *A*, palmitate (Pal); *B*, oleate (Ole)] for 16 h prior to stimulation with insulin (1 ng/mL, 15 min). Total cell lysates were resolved by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with the indicated antibodies. Detection was by enhanced chemiluminescence. Representative blots are shown.



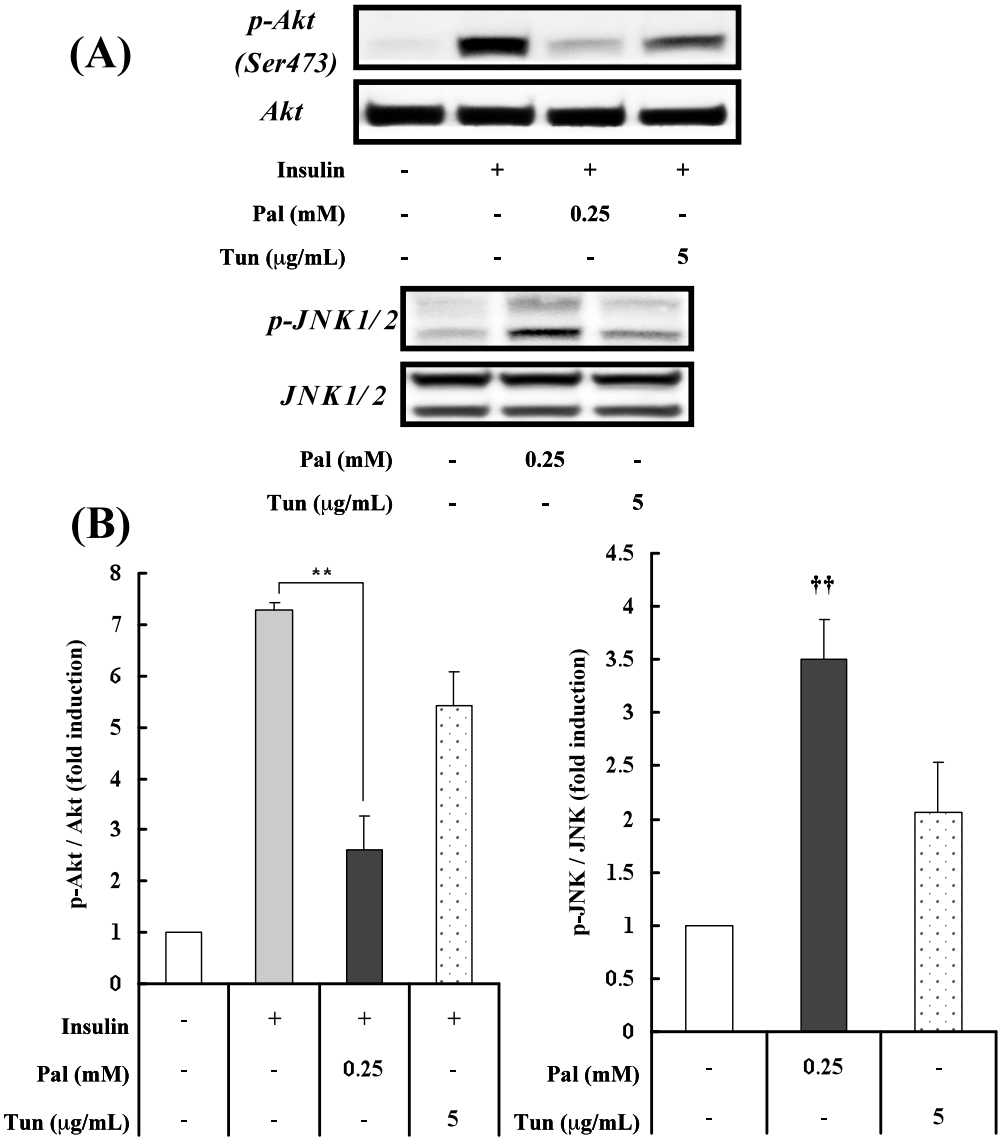
Supplemental Fig. 2. Effects of palmitate and oleate on expression levels of the SREBP-1c and IRS-2 genes in H4IIEC3 hepatocytes. H4IIEC3 cells were incubated in the presence or absence of palmitate (Pal) or oleate (Ole) for 16 h. Total RNA was extracted and subjected to reverse transcription. Using the cDNA as a template, the amounts of SREBP-1c and IRS-2 mRNA were detected by real-time PCR. The values are normalized to the level of 18S ribosomal RNA and expressed as mean fold increase over control ± S.E. (n = 3). ** $p < 0.01$, versus control.



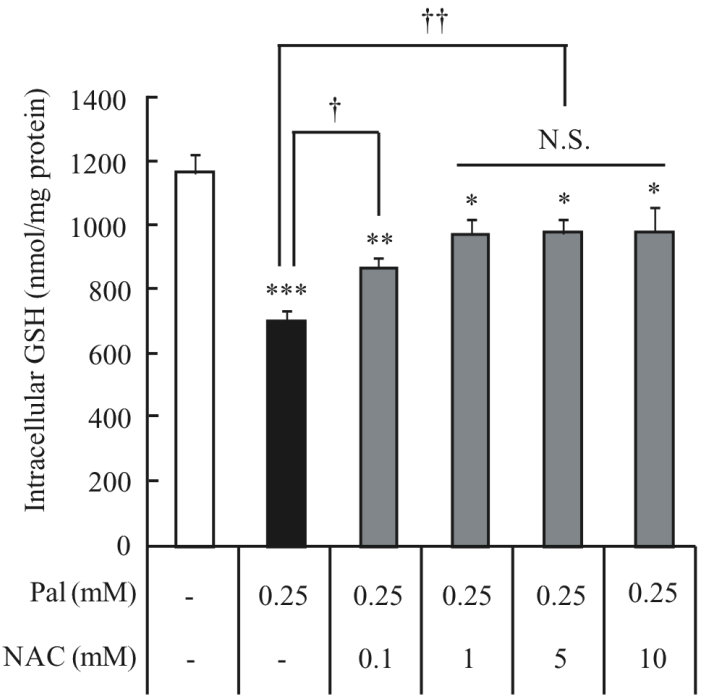
Supplemental Fig. 3. Effect of palmitate on ER stress in H4IIEC3 hepatocytes. H4IIEC3 cells were incubated in the presence or absence of palmitate (Pal) or the ER stress inducer tunicamycin (Tun) for 16 h. Total RNA was extracted and subjected to reverse transcription. Using the cDNA as a template, the amounts of GRP78 mRNA (A) or XBP-1 mRNA splicing (B) were detected by real-time PCR or reverse transcription PCR, respectively. (A) The values were normalized to the level of 18S ribosomal RNA and expressed as mean fold increase over control ± S.E. (n = 3). XBP-1u, unspliced XBP-1; XBP-1s, spliced XBP-1. ** $p < 0.01$, versus control.



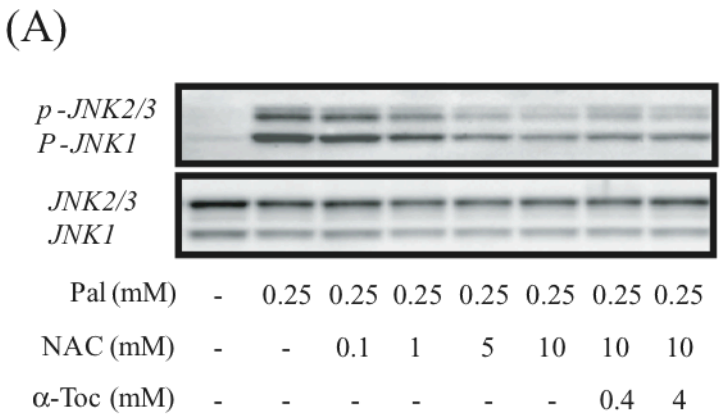
Supplemental Fig. 4. Comparison of insulin signaling with palmitate treatment and with the ER stress inducer tunicamycin in H4IIEC3 hepatocytes. (A) H4IIEC3 cells were incubated in the presence or absence of palmitate (Pal) or the ER stress inducer tunicamycin (Tun) for 16 h prior to stimulation with insulin (1 ng/mL, 15 min). Total cell lysates were resolved by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with the indicated antibodies. Detection was by enhanced chemiluminescence. Representative blots are shown. (B) The values from densitometry of four (p-Akt) or three (p-JNK) independent experiments are normalized to the level of total Akt or JNK protein, respectively, and expressed as the mean fold increase over control ± S.E. ** $p < 0.01$, versus insulin treatment alone. †† $p < 0.01$, versus control.

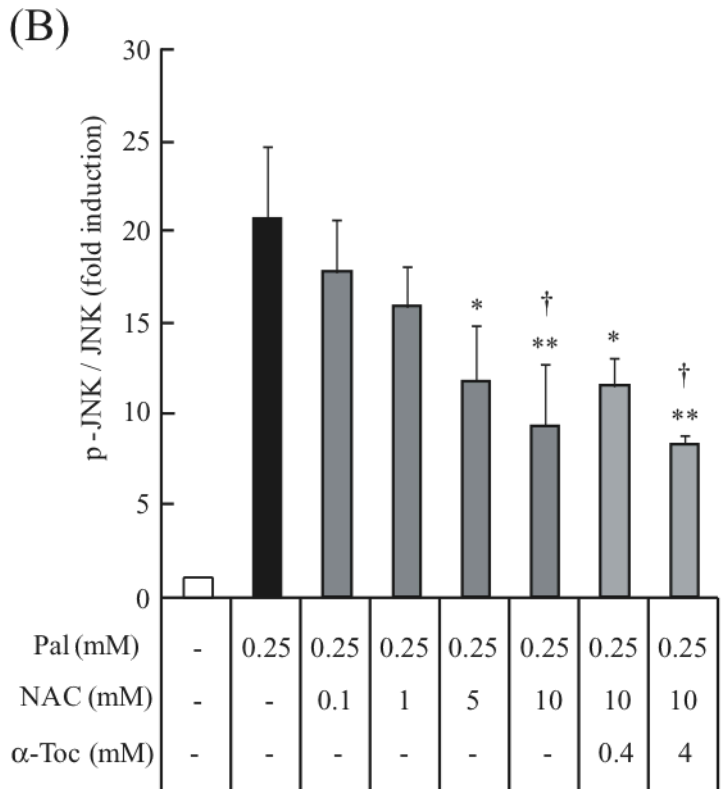


Supplemental Fig. 5. Restoration of intracellular GSH levels by N-acetyl-cysteine (NAC) in palmitate-treated H4IIEC hepatocytes. H4IIEC cells were incubated in the absence or presence of palmitate (Pal) and NAC for 16 h. Intracellular GSH levels were determined using a commercial kit (Total Glutathione Quantification Kit, DOJINDO). The values from three independent experiments were normalized to the level of total protein, respectively, and expressed as means ± S.E. (n = 3). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control. † $P < 0.05$, †† $P < 0.01$ versus 0.25 mM Pal.

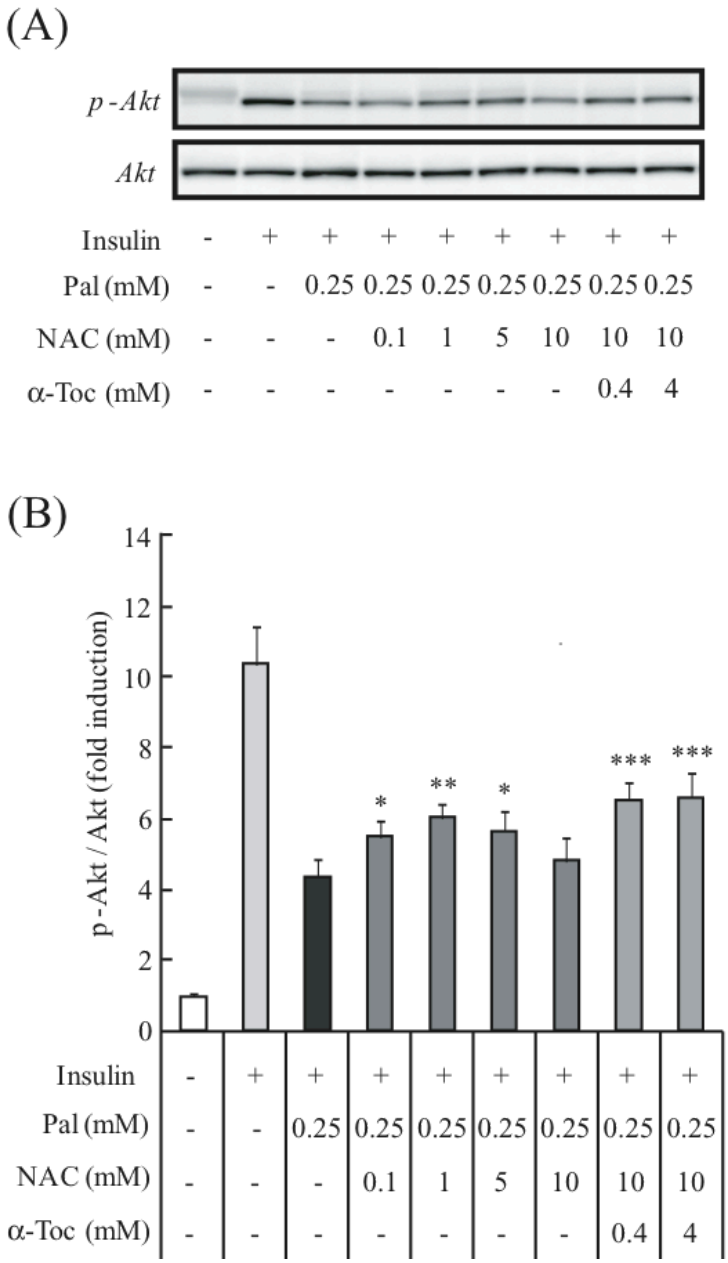


Supplemental Fig. 6. N-acetyl-cysteine (NAC) and α-tocopherol (α-Toc) decrease palmitate-induced JNK activation in H4IIEC hepatocytes. (A) H4IIEC cells were incubated in the absence or presence of palmitate (Pal) and NAC and α-Toc for 16 prior to stimulation with insulin (1 ng/mL, 15 min). Total cell lysates were resolved by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with the indicated antibodies. Representative blots are shown. (B) The densitometric values from three independent p-JNK experiments were normalized to the level of total JNK and expressed as the mean fold increase over control ± S.E. * $P < 0.05$, ** $P < 0.01$ versus 0.25 mM Pal treatment. † $P < 0.05$ versus 0.25 mM Pal/0.1 mM NAC treatment.

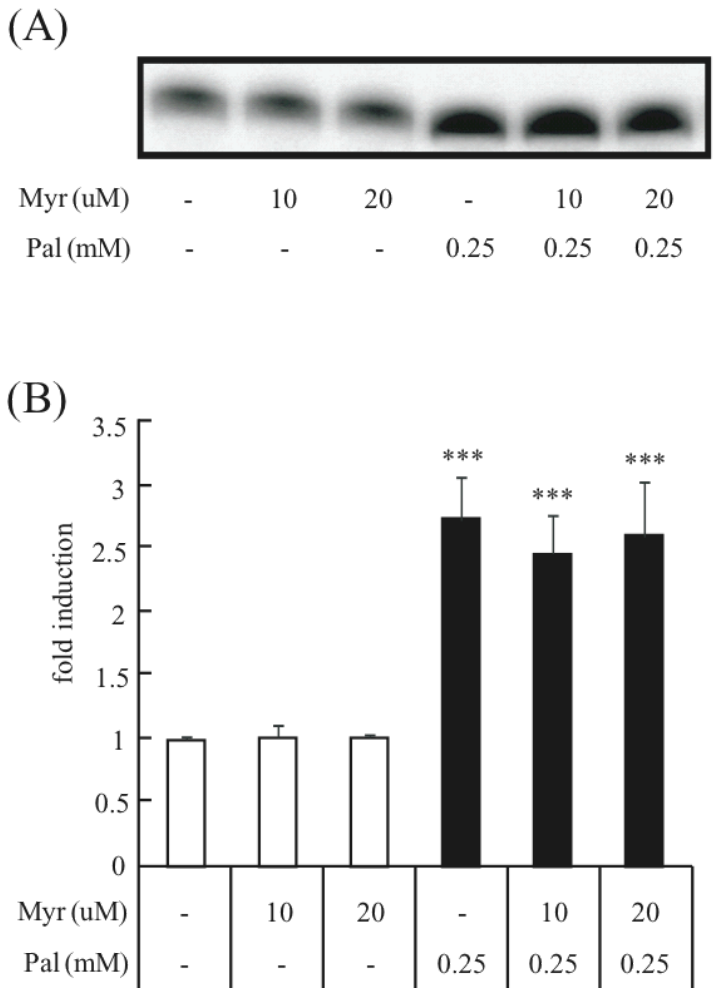




Supplemental Fig. 7. N-acetyl-cysteine (NAC) and α-tocopherol (α-Toc) reverse palmitate-induced changes in insulin-stimulated Akt phosphorylation in H4IIEC hepatocytes. (A) H4IIEC cells were incubated in the absence or presence of palmitate (Pal) and NAC and α-Toc for 16 h prior to stimulation with insulin (1 ng/mL, 15 min). Proteins in total cell lysates were resolved by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with the indicated antibodies. Representative blots are shown. (B) The densitometric values from three independent p-Akt experiments were normalized to total Akt protein and expressed as the mean fold increase over control ± S.E. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus 0.25 mM Pal treatment.



Supplemental Fig. 8. Effect of myriocin (Myr) on ceramide synthesis in H4IIEC hepatocytes. (A) H4IIEC cells were incubated in the absence or presence of palmitate (Pal) and Myr for 16 h. Ceramide was measured according to an established method (Araki et al., PNAS 94:11946, 1997). Signals were detected using a Typhoon 9400 Imager (GE Healthcare Bio-Sciences). Representative blots are shown. (B) The densitometric values from three independent experiments were normalized to the level of total protein and expressed as the mean fold increase over control ± S.E. * $P < 0.05$, *** $P < 0.001$ versus control. † $P < 0.05$ versus 0.25 mM Pal.



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