Supplemental data

DECREASE IN MEMBRANE PHOSPHOLIPID UNSATURATION INDUCES UNFOLDED PROTEIN RESPONSE

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Running head: Phosphologic unsaturation and unfolded protein response

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Supplemental experimental procedures

Materials- [1-^{14}C]Palmitoyl-CoA (55 mCi/mmol) [1-^{14}C]Stearoyl-CoA (55 mCi/mmol) [1-^{14}C]Oleoyl-CoA (55 mCi/mmol) [1-^{14}C]Linoleoyl-CoA (55 mCi/mmol) [1-^{14}C]Arachidonoyl-CoA (55 mCi/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO). Lysophosphatidylcholine (LPC) from egg yolk was obtained from Avanti Polar Lipids (Alabaster, AL).

Acyl-CoA:lysophosphatidylcholine acyltransferase assay- HeLa cells were harvested, washed with ice-cold PBS, then sonicated three times on ice for 3 s in 50 mM potassium phosphate buffer (pH 7.0) containing 0.15 M KCl, 0.25 M sucrose, 1 m EDTA, 1 mM dithiothreitol and 5 µg/mL pepstatin, leupeptin and aprotinin (homogenizing buffer). After centrifugations at 1000 × *g* for 5 min, the supernatant was collected and centrifuged at 100 000 × *g* for 1 h. The resulting pellet was resuspended in homogenizing buffer (without EDTA, dithiothreitol, and protease inhibitor cocktail) and immediately used for the enzyme assay described below. Reaction mixtures contained the indicated concentrations of an acyl-CoA and an egg yolk lysophosphatidylcholine, and 0.08 mg of microsomal protein in a total volume of 0.8 mL assay buffer [0.15 M KCl, 0.25 M sucrose, 50 mM potassium phosphate buffer (pH 6.8)]. After incubation at 37 °C for 5 min, reactions were stopped by the addition of 2 mL of methanol. Total lipid was extracted by the method of Bligh and Dyer, and separated by one-dimensional TLC on silica gel 60 plates (Merck) in chloroform/ethanol/water/triethylamine (30/35/7/35, v/v). The radioactivities of separated bands on TLC were quantified using a BAS 1500 bio-imaging analyzer.

Supplemental Figure legends

Supplemental Fig. S1. Effect of SCD knockdown on phospholipid SFA/MUFA ratio in HeLa cells.
A. HeLa cells were transfected with siControl or siSCD5. After 48 h, total RNAs were prepared. The mRNA expression level of SCD5 was normalized to the GAPDH gene and is represented as the fold change over siControl. *B*. SFA/MUFA ratios in phospholipid fraction were determined by GC-MS. The data represents the mean ± SEM of three experiments. * indicates a significant difference compared with siControl-transfected cells ($p < 0.05$)

Supplemental Fig. S2. Supplemented unsaturated fatty acids were incorporated into the phospholipid fraction.
HeLa cells were transfected with the indicated siRNA. At 48 h after transfection, cells were further

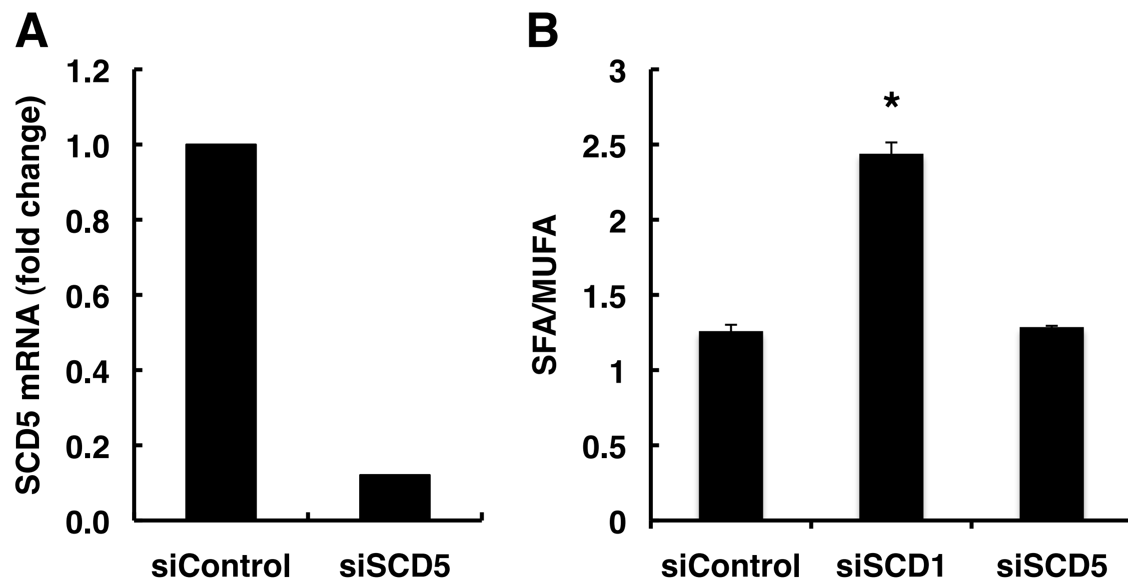
incubated for 24 h in media supplemented with the indicated fatty acid (50 μM) and then harvested. The amount of 18:1n-9 (A), 18:2 (B), 20:4 (C) and 20:5 (D) in the phospholipid fraction was measured by GC-MS and is represented as the fold change over siControl. The data represents the mean ± SEM of three experiments. * indicate significant differences compared with siControl-transfected cells ($p < 0.01$) and # indicate significant differences compared with siSCD1-transfected cells which were untreated ($p < 0.01$).

Supplemental Fig. S3. Knockdown of lysophospholipid acyltransferases in HeLa cells.
HeLa cells were transfected with the indicated siRNA. At 66 h after transfection, total RNAs were prepared. The expression level of each gene was normalized to the GAPDH gene and is represented as the fold change over siControl.

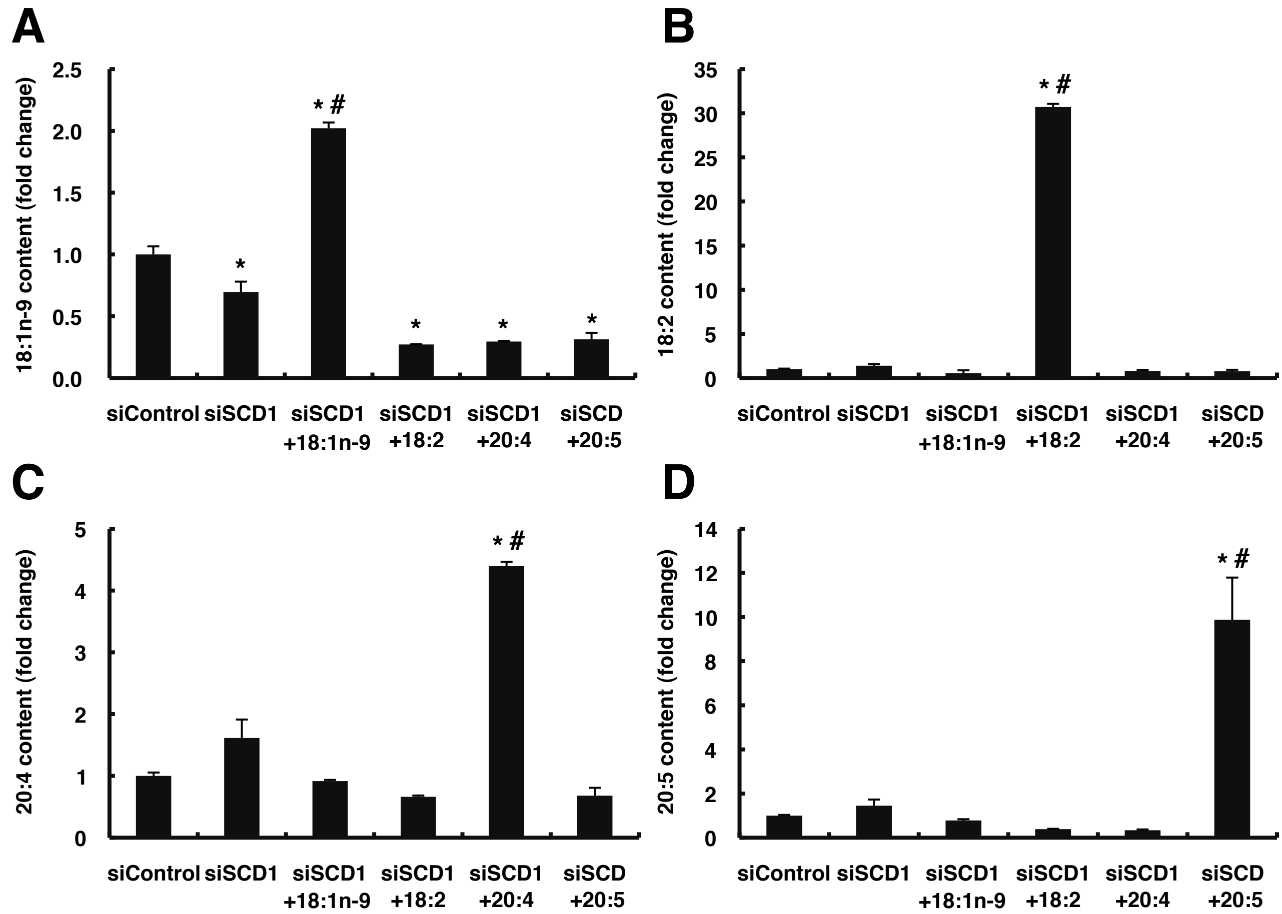
Supplemental Fig. S4. Supplementation of unsaturated fatty acid mixture canceled the effect of LPCAT3 knockdown on CHOP expression in SCD1 knockdown cells.
HeLa cells were transfected with the indicated siRNA. At 50 h after transfection, cells were further incubated for 16 h in media supplemented with a 0.5 μM PUFA mixture (25 nM 18:2, 380 nM 20:4 and 95 nM 20:5) and then harvested. *A*. PUFA content in the phospholipid fraction was determined by GC-MS and is represented as the fold change over siControl. * indicate significant differences compared with siControl-transfected cells ($p < 0.05$) and # indicates a significant difference compared with siSCD1-transfected cells ($p < 0.05$). *B*. Expression of CHOP mRNA detected by quantitative real-time PCR. The expression level was normalized to expression of the GAPDH gene and is represented as fold induction over siControl. * indicate significant differences compared with siControl-transfected cells ($p < 0.01$) and # indicates a significant difference compared with siSCD1-transfected cells ($p < 0.01$).

Supplemental Fig. S5. Effect of LPCAT3 knockdown on LPCAT activities toward stearoyl-, oleoyl-, linoleoyl- and arachidonoyl-CoA in HeLa cells.
Lysophosphatidylcholine acyltransferase activity in the membrane fraction of siControl and siLPCAT3-transfected cells with [^{14}C]stearoyl-CoA (A), [^{14}C]oleoyl-CoA (B), [^{14}C]linoleoyl-CoA (C) and [^{14}C]arachidonoyl-CoA (D). 80 μM lysophosphatidylcholine and 80 μg of membrane fractions with increasing concentrations of [^{14}C]acyl-CoA were used.

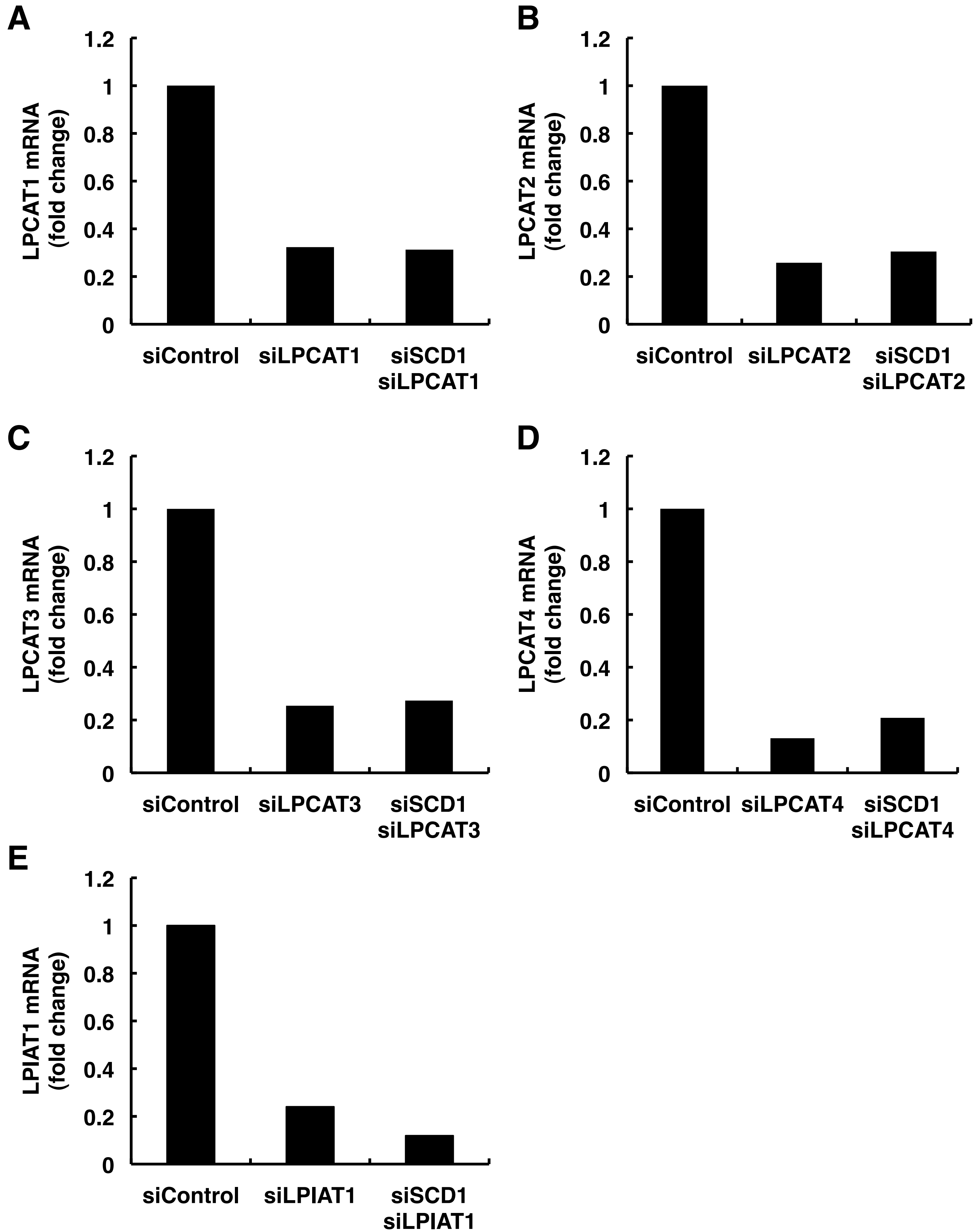
Supplemental Fig. S1



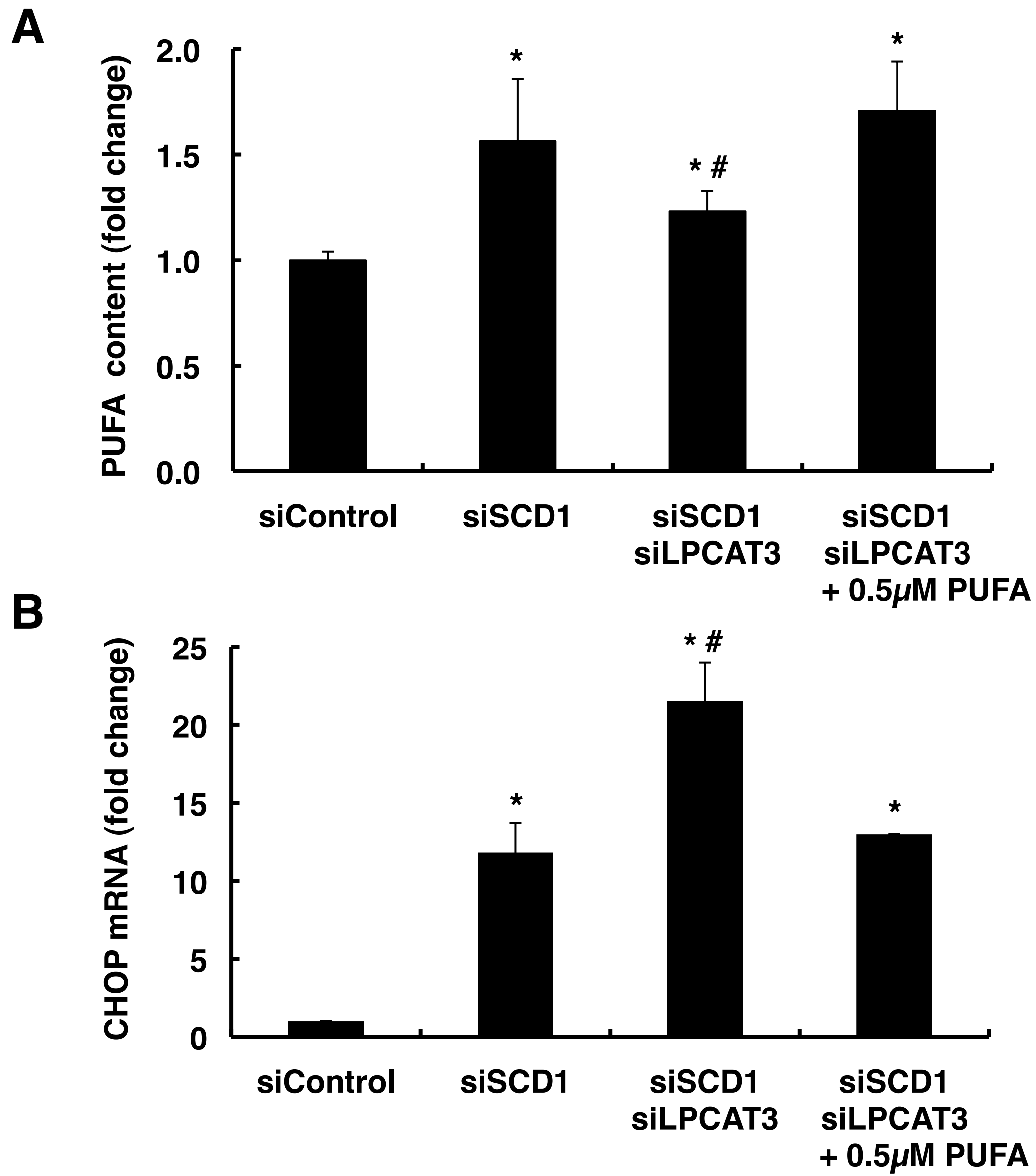
Supplemental Fig. S2



Supplemental Fig. S3



Supplemental Fig. S4



Supplemental Fig. S5