Supporting Information

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SI Materials and Methods

Materials. Lipofectamine 2000 reagent, G418, and the V5 and calnexin antibodies were obtained from Invitrogen. Culture medium and PBS (PBS) were purchased from Mediatech and FCS was obtained from Atlanta Biologicals. Complete EDTA-free protease inhibitors were from Roche, ^{35}S-labeled Met/Cys was purchased from PerkinElmer, MG-132 was obtained from EMD Biosciences, and HisPur Cobalt Resin was from ThermoScientific. The anti-ubiquitin antibody was obtained from Santa Cruz Biotechnology. Fatty acids, triacsin C, and conjugated anti-V5 and anti-FLAG antibody were from Sigma-Aldrich, as were all other chemicals unless otherwise indicated.

Real-Time PCR Assays of mRNA Abundance. Oligonucleotides specific for each gene were used to amplify by PCR in 2× SYBR Green PCR Master Mix (Applied Biosystems) in a volume of 66 μL according to manufacturer's instructions. The tissue expressing the highest level (liver for PNPLA3) was used as a reference and assigned an arbitrary expression level of 1. The mean of the 3 CT measurements for each tissue was expressed as a fraction of the level observed in the liver.

ChIP-Seq. DNA sequences (36-bp reads) were produced using an Illumina Solexa genome analyzer and mapped to a reference genome using efficient large-scale alignment of nucleotide database (ELAND). The distribution of identified binding sites was determined with reference to RefSeq genes downloaded from the UCSC Genome Browser Database. The qPCR oligonucleotide pairs used for gene-specfic ChIP included: *Pnpla3* (site 1), forward 5′- CACAGCCAGCAGGGAGACTTTGGC and reverse 5′-AATGGAGGGCTCTGAGCCCAA-3′; *Pnpla3* (site 2), forward 5′-AGTGGCAGGCAGGTGACTCT-3′ and reverse 5′- GGTAGCACCTGTCCACCAACT-3′; and *L32*, forward 5′-ACATTTGCCCTGAATGTGGT-3′ and reverse 5′-ATCCTCTTGCCCTGACC TT-3′.

EMSA were performed with recombinant SREBP-1 protein (amino acids 1–490). Briefly, labeled probe and unlabeled competitor DNAs were combined together on ice at the indicated molar equivalents before recombinant SREBP-1 protein was added. After 30 min on ice, free DNA and DNA-protein complexes were resolved by native PAGE. Where indicated, *Pnpla3* [WT or mutated (M)] or *Ldlr* (WT or M) oligonucleotides were added as competitors. Oligonucleotide sequences for EMSA were as follows: *Pnpla3*, 5′-CGGCCAGAGGCCACTCTCACTGCCCTTTACT-3′ and *Mutated Pnpla3*, 5′-CGGACCAGAGGCCTTATTCTAATGTTCTTTAC-3′.

Preparation of BSA-Bound Fatty Acids. Linoleic, linolenic, or arachidonic acid were bound to BSA by mixing 800 μL of ethanol with each fatty acid (0.1 mM) before adding 40 μL of 5 M NaOH. The ethanol was evaporated and 4 mL of sterile PBS was added to suspend the fatty acids. Five milliliters of 24% (w:v) ice-cold fatty acid-free BSA was added. PBS was added to final concentration of BSA-bound fatty acid of 10 mM. Aliquots of the solution were overlaid with nitrogen gas and stored at −80 °C. Palmitate, oleate, and eicosapentaenoate were purchased as sodium salts and dissolved in PBS at a concentration of 10 mM. An equal volume of 24% (w:v) ice-cold fatty acid-free BSA was added so the final concentration of BSA-bound fatty acid was 5 mM.

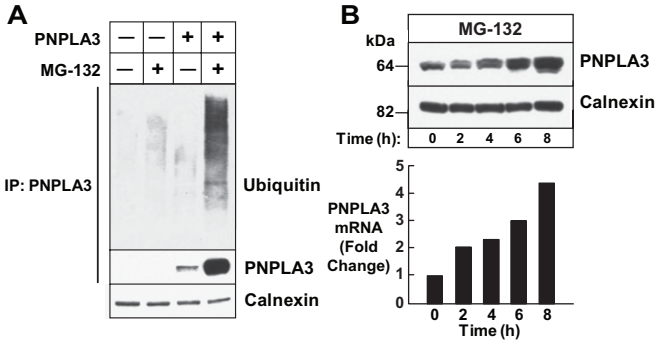

Fig. S1. PNPLA3 undergoes ubiqitination. (*A*) pCDNA3.1-PNPLA3-FLAG or control plasmid pCDNA3.1 were transfected into cultured hepatocytes (HuH7) cells. The cells were treated with DMSO or MG-132 for 8 h and then lysed. Cell lysates were subjected to immunoprecipitation using anti-FLAG antibody-conjugated beads. Bound proteins were eluted by FLAG peptide and analyzed by immunoblotting using antibodies against ubiquitin and FLAG (PNPLA3). The cell lysates were subjected to immunoblotting using the antibody against calnexin. (*B*) Cultured hepatocytes stably expressing PNPLA3 were incubated with MG132 for the times indicated. Cells were lysed and PNPLA3 protein was assayed by immunoblottting. PNPLA3 mRNA levels were determined by real-time PCR as described in *Materials and Methods*.