SUPPLEMENTARY DATA

Materials
Dulbecco's Modified Eagle's Medium (DMEM), Ham's nutrient mixture F-12 and fetal bovine serum (FBS) were purchased from Life Technologies (Gibco BRL, Grand Island, NY, USA). Palmitic acid was prepared as described previously (1). TO901317 (T2320) and Adenosine, periodate oxidized (adox; A7154) were obtained from Sigma-Aldrich (St. Louis, MO, USA). PPARγ antibody (sc-7196), ChREBP antibody (sc-33764), PEPCK antibody (sc-32879), β-actin antibody (sc-1616) and Lamin B antibody (sc-6216) were obtained from Santa Cruz Biotechnology (CA, USA). LXRα antibody (ab41902), SREBP1c antibody (ab63991), HA antibodies (ab9110, ab18181), FAS antibody (ab22759) and ABCG1 antibody (ab36969) were obtained from Abcam (Cambridge, UK). PRMT3 serum antibody and purified PRMT3 antibody were kindly provided by Mark T. Bedford (University of Texas, M.D. Anderson Cancer Center, Smithville, TX). All reagents were of the highest purity commercially available.

Cell culture
HepG2, HEK293 and AML-12 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). PRMT3 wild-type (WT) and knock-out (KO) mouse embryonic fibroblast (MEF) cells were kindly provided Mark T. Bedford (University of Texas, M.D. Anderson Cancer Center, Smithville, TX). The culture medium for HepG2 cells, HEK293 cells and PRMT3 MEF cells was DMEM supplemented with 10% FBS. Cells were grown to confluence in 60 mm dishes in DMEM with 15 mM HEPES buffer, 10% FBS, 25 mM glucose, 0.35% additional sodium bicarbonate, 2.5 mM L-glutamine, streptomycin (100 μg/ml) and penicillin-streptomycin (100 U/ml) at 37 °C in 5 % CO2. The culture medium for AML-12 cells was DMEM (5.5 mM glucose) and Ham's F12 1:1 medium with 0.005 mg/ml insulin, 0.005 mg/ml transferrin, 5 ng/ml selenium, and 40 ng/ml dexamethasone, supplemented with 10 % FBS, streptomycin (100 μg/ml) and penicillin-streptomycin (100 U/ml) at 37 °C in 5 % CO2. The media were changed every other day. Passaged cells were plated to yield near-confluent cultures at the end of the experiments (60~70% confluence in case of HepG2 cells).

Protein extraction and Western blot analysis
The medium was removed and cells were washed twice with ice-cold phosphate buffered saline (PBS), scraped, and then harvested by microcentrifugation (13000 rpm for 10 min) and removed supernatant. Pellet was resuspended in M-PER Mammalian Protein Extraction Reagent (Thermo, IL, USA; 78501, NE-PER [Thermo; 78835] was used for nuclear protein extraction) containing protease inhibitor cocktail (Sigma, Missouri, USA) and phosphatase inhibitor cocktail I + II. (Sigma, Missouri, USA) The resuspended cells were lysed mechanically on ice by vortex. The protein level was quantified using the Bradford procedure. The whole cell (30 μg of protein) were separated by SDS-polyacrylamide gel electrophoresis and transferred to an enhanced nitrocellulose membrane. The blots were then washed with TBST (10 mM Tris--HCl, pH 7.6, 150 mM NaCl, 0.05 % Tween-20), blocked with 5% skim milk powder in TBST for 1h and incubated for 15 h at 4 °C with the primary antibody at the dilutions recommended by the supplier. The membrane was then washed with TBST, and the secondary antibodies conjugated to horseradish peroxidase were incubated for 1 h at room temperature. The bands were visualized with Luminescent image analyzer (ImageQuant LAS 4000, GE Healthcare, UK) using Amersham ECL™ Western Blotting Detection Reagents (GE Healthcare, UK).
mRNA extraction and Quantitative RT-PCR

Total RNA was extracted from the cells using TRIzol, which is a monophasic solution of phenol and guanidine isothiocyanate purchased from (Invitrogen, Carlsbad, CA, USA). Reverse transcription was carried out with 1 μg RNA using an RT Premix reverse transcription system kit (AccuPower, Seoul, Korea) with oligo-dT18 primers. 0.5 μl of the RT products were amplified with 100 nM specific primers using a Power SYBR Green (Applied Biosystems, Warrington, UK). The primers used were 5′-CACGTGAGATCTGCAAAAA-3′ (sense), 5′-ATCCCATAATGCCCGTATGA-3′ (antisense) for human PRMT3, 5′-CACCACACGCCGCTCTCAG-3′ (sense), 5′-GGCTGGGGACAAGGACATT-3′ (antisense) for human ABCA1, 5′-GCCTTACCCCTTGCTTGAA-3′ (sense), 5′-CAGTGGATAACCGAGAAGG-3′ (antisense) for human ABCG5, 5′-GTGGACCTGACCAGCATTGA-3′ (sense), 5′-GAAACAGGGCTGCGAGTGA-3′ (antisense) for human ABCG8 and 5′-AGGCCAGGGCTGCGAGTGA-3′ (sense), 5′-TCAACATGATCTGGGTATC-3′ (antisense) for human β-actin. β-actin was used as a control to confirm the quantity of the RNA.

Oil Red O staining

HepG2 cells were fixed with 4% paraformaldehyde in PBS for 10 min then washed with 60% isopropanol. The cells were stained for 10 min with 0.2% Oil Red O (sigma) dissolved in 60% isopropanol and washed four times with distilled water. Cellular nuclei were counterstained with Harris Hematoxylin solution (sigma; HHS32). Stained cells were detected with a microscope (Olympus BX40) and imaged with digital camera (eXcope X3, DIXI Optics).

Plasmid constructions and transient transfection

GST, GST-PRMT3, GFP, GFP-PRMT3 and Flag-PRMT3 were kindly provided by Mark T. Bedford (University of Texas, M.D. Anderson Cancer Center, Smithville, TX). HA and HA-PRMT3 were kindly provided by Dr. Fukamizu A (Life Science Center of Tsukuba Advanced Research Alliance, University of Tsukuba, Japan). LXRE-Luc, HA-LXRα and HA-RXRα were kindly provided by Dr. Choi HS (Hormone Research Center, School of Biological Sciences and Technology, Chonnam National University, Korea). SRE-Luc, native SREBP1c promoter-Luc (up to 0.9kb) and Flag-SREBP1c plasmids were kindly provided by Dr. Jeon TI (College of Agriculture & Life Science, Chonnam National University, Korea). ChORE-Luc, Flag-ChREBP and HA-Mlx plasmids were described previously (2). Transient transfection was performed with LipofectamineTM 2000 (Invitrogen), as instructed by the manufacturers.

siRNA transfection

siRNA for human PRMT3, mouse LXRα (Santa Cruz Biotechnology; sc-41071 and sc-38829) and scrambled siRNA (Qiagen; 1027281) were used for silencing the endogenous PRMT3 and LXRα expression. 20 nM of each siRNA was transfected using Lipofectamine™ RNAiMAX reagent (Invitrogen, USA) following the reverse transfection method as instructed by the manufacturers.

FFA, TG and Cholesterol assay

Free fatty acid assay kit (EFFA-100) was purchased BioAssay Systems (CA, USA). Triglyceride quantification kit (#K622-100) and Cholesterol quantification kit (#K603-100) were obtained from BioVision (CA, USA). All samples were prepared following the colorimetric methods as instructed by the manufacturers and measured absorbance at 570 nm.
GST-pull down assay

GST and GST-PRMT3 proteins were overexpressed in *E. coli* BL21 cells by adding a 0.2 mM of isopropyl β-D-thiogalactopyranoside (Sigma; I6758) for 4 h in 30 °C. The cells were harvested then washed twice with PBS. Resuspended cells in PBS were broken by sonication then centrifuged for 10 min at 13000 rpm at 4 °C. Supernatant was incubated with Glutathione Sepharose™ 4B (GE Healthcare; 27-4574-01) on the rocker at 4 °C for overnight. After wash with PBS, GST and GST-PRMT3 bound to glutathione beads were incubated with HEK293 cell lysate which was overexpressed with HA-LXRα for overnight at 4 °C. The beads were washed three times with PBS and boiled in loading buffer to elute bound proteins. Eluted proteins were analyzed by western blotting and proved with LXRα antibody.

Co-immunoprecipitation assay

HEK293 cells were transiently transfected with Flag-PRMT3 and HA/HALXRα. Cells were harvested 24 h after transfection and whole cell extracts were prepared in Co-IP lysis buffer (25 mM Tris-HCl [pH 7.4], 250 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol). The lysates were incubated with Flag (M2) or HA (ab9110) antibody for overnight at 4 °C, followed by incubation with Protein G sepharose (sigma; F3296) for 4 h. The beads were washed three times with lysis buffer and boiled in loading buffer to elute bound proteins. Eluted proteins were analyzed by western blotting and proved with HA or Flag antibodies.

In vitro methylation assay

HEK293 cells were transiently transfected with HA-rpS2 or HA-LXRα. Cell pellets were resuspended in M-PER Mammalian Protein Extraction Reagent and immunoprecipitated with HA antibody for overnight at 4 °C, followed by incubation with Protein G sepharose for 4 h. After wash with lysis buffer, HA-rpS2 or HA-LXRα bound to anti-HA beads were incubated with eluted GST-PRMT3 protein (elution buffer 1 ml: 100 mM Tris-HCl pH 7.4, 120 mM NaCl, L-glutathione reduced [Sigma; G4251] 0.01 g) and Adenosyl-L-Methionine, S-[Methyl-3H] (PerkinElmer Life Science; NET155H250UC) for 1 h at 30 °C. Methylated proteins were separated by SDS-PAGE and transferred to a PVDF membrane then treated with EN³HANCE™ (PerkinElmer Life Science; 6NE970C) and exposed film for 3 days in deep freezer.

Immunofluorescence assay and confocal microscope

Transfected cells were washed twice in PBS and fixed for 10 min with 4% paraformaldehyde in PBS. After three washes in PBS, the cells were mounted on slides and the nuclei were visualized with 4′,6-diamidino-2-phenylindole (DAPI) present in the ProLong Gold Antifade Mounting Medium (Invitrogen, Carlsbad, CA). Immunofluorescence imaging was performed on a Leica TCS SP5 AOBS laser scanning confocal microscope (Leica Microsystems, Heidelberg, Germany) using a Leica 63× (N.A. 1.4) oil objective located at Korea Basic Science Institute Gwangju Center. Digital images were captured optical sections of 512 × 512 pixels, and were averaged four times to reduce noise. The images of cells were obtained separately with the following fluorescence excitation and emission settings: Excitation at 488 and 561 nm and emission between 495–510 and 570–610 nm for GPF- and TRITC-conjugated constructs, respectively; Excitation at 496 and 405 nm and emission between 500–535 and 449–461 nm for FITC-conjugated construct and DAPI, respectively. For all the experiments, exposure time was kept the same for all samples.
Animal experiments and LXRα KO mice
Twelve male C57BL/6J mice and LXRα KO mice, described previously (3), were separated two or three groups (C57BL/6J mice: normal diet; n=7, high fat diet; n=7, LXRα mice: WT + normal diet; n=5, WT + high fat diet; n=5, KO + high fat diet; n=5). Normal diet group was challenged with 10% Kcal fat containing feed (Research Diets, NJ, USA; D12450B) and high fat diet group was challenged 60% Kcal fat containing feed (Research Diets, NJ, USA; D12492) for 12 weeks. After 12 weeks, all mice were sacrificed and liver was extracted. Experiments were performed in accordance with National Institutes of Health animal research standards. And protocols were approved by the Chonnam National University Laboratory Animal Research Center.

Immunohistochemistry
To make tissue slide, fixed kidneys in 10% NBF were embedded in paraffin and cut at a 5 μm thickness using a microtome. Liver sections were deparaffinized with Histochoice (Amresco, OH, USA; H103) and then rehydrated with serial diluted ethanol (100% -> 70%). Antigen was retrieved with citrate-based solution at 95 °C for 30 min (10 mM citrate, 0.05 % NP-40 pH 6.0). After cooling in room temperature, immunostaining was performed according to the Vectastain ABC kit (Vector Labs; PK-6101). Briefly, endogenous peroxidase activity was quenched by 0.3 % H2O2 treatment for 30 min and then sections were blocked with goat serum for 20 min and then probed with PRMT3 antibody (diluted 1:300) for 30 min. After wash with PBS, sections were incubated with biotinylated secondary antibody for 30 min and then incubated with ABC reagent for 30 min. DAB (Vector Labs; SK-4100) was used for peroxidase substrate solution. Hematoxylin staining was performed for counter-staining. The immunohistochemistry-stained sections were observed using a BX-40 apparatus (Olympus, Tokyo, Japan) with an eXcope X3 digital camera (DIXI Optics, Daejeon, South Korea).

1. Lim JC, Lim SK, Han HJ, Park SH. Cannabinoid receptor 1 mediates palmitic acid-induced apoptosis via endoplasmic reticulum stress in human renal proximal tubular cells. J Cell Physiol 2010;225:654-663
**Supplementary Figure 1.** A: HepG2 cells were treated with 750 μM PA for the indicated time intervals. Total RNA extracted from cells and the level of PRMT3 mRNA were measured by qRT-PCR analysis and then normalized to the β-actin level. Data are the means±SEM of three independent experiments. *p < 0.05 vs. 0 h. B: HepG2 cells were treated with 750 μM PA for the indicated time intervals. Cell extracts were subjected to western blot analysis with the indicated antibodies. Representative immunoblots were from at least three independent experiments. C: After pretreatment with 30 nM Adox for 30 min, HepG2 cells were treated with 750 μM PA for 24 h. Cell extracts were subjected to western blot analysis with the indicated antibodies. Representative immunoblots were from at least three independent experiments.
Supplementary Figure 2. A: HEK293 cells were transfected with HA or HA-PRMT3 plasmids. Twenty-four hours later, cultured cells were harvested and cell extracts were subjected to western blot analysis with the indicated antibodies. Representative immunoblots were from at least three independent experiments. B: WT and KO PRMT3 MEF cells were transfected with HA or HA-PRMT3 plasmids. Twenty-four hours later, cultured cells were harvested and cell extracts were subjected to western blot analysis with the indicated antibodies. Representative immunoblots were from at least three independent experiments. C: WT and KO PRMT3 MEF cells were treated with 750 μM PA for 12 and 24 h. Cell extracts were subjected to western blot analysis with the indicated antibodies. Representative immunoblots were from at least three independent experiments. D: HepG2 cells were transfected with scramble siRNA or PRMT3 siRNA following the reverse transfection method. Twenty-four hours later, cells were treated with 750 μM PA for 12 and 24 h. The cell extracts were then subjected to western blot analysis with the indicated antibodies. Representative immunoblots were from at least three independent experiments. E: HepG2 cells were transfected with scramble siRNA or PRMT3 siRNA following the reverse transfection method. Twenty-four hours later, the cells were treated with 750 μM PA for 24 h. Cultured cells were subjected to Oil Red O staining without counterstaining. F: HepG2 cells were transfected with scramble siRNA or PRMT3 siRNA following the reverse transfection method. Twenty-four hours later, the cells were treated with 750 μM PA for 12 h. Total RNA extracted from cells and the level of indicated mRNA were measured by qRT-PCR analysis and then normalized to the β-actin level. Data are the means±SEM of three independent experiments. *p < 0.05 vs. scramble. n.s. = non-specific. G and H: HepG2 cells were transfected with scramble siRNA or PRMT3 siRNA following the reverse transfection method. Twenty-four hours later, the cells were treated with 750 μM PA for 24 h. G: The cell extracts were then subjected to western blot analysis with the indicated antibodies. Representative immunoblots were from at least three independent experiments. H: Cultured cells were subjected to Cholesterol assay. Presented data are representative of three independent experiments, and experiments were performed in triplication. Data represent the means ± SEM. n.s. = non-specific. I: Relative quantification of Fig. 2G. Data are the means±SEM of three independent experiments. *p < 0.05 vs. HA + scramble. †p < 0.05 vs. HA-PRMT3 + scramble. J: AML-12 cells were transfected with scramble siRNA or LXRα siRNA following the reverse transfection method. Twenty-four hours later, the cells were transfected with HA or HA-PRMT3 plasmids. Twenty-four hours later, cultured cells were subjected to oil red O staining then counterstained with hematoxylin. Representative images were from at least three independent experiments.
SUPPLEMENTARY DATA

A

HA + -
HA-PRMT3 - +
PPARγ
ChREBP
p-ACC
PEPCK
β-actin

B

WT KO
PRMT3
LXRα
c7-SREBP1c
FAS
ACC
β-actin

C

Pal (750 µM) 0 12 24 0 12 24 (h)
ChREBP
PEPCK
β-actin

D

HepG2 scramble siPRMT3
Pal (750 µM) 0 12 24 0 12 24 (h)
ChREBP
PEPCK
β-actin

E

scramble scramble + PA siPRMT3 + PA

F

Relative mRNA expression

abc11
abcg5
abcg8

G

Pal (750 µM) - + + scramble + + - siPRMT3 - - +

H

β-D-glucose (mg/dL)

ser ser siPRMT3 / palmitic acid

I

Relative Optical Density

c7-SREBP1c
FAS
ACC

J

HA + scramble HA-PRMT3 + scramble HA-PRMT3 + siLXRα

SUPPLEMENTARY DATA

Supplementary Figure 3. A: HEK293 cells were cotransfected with HA/HA-LXRα and HA-RXRα, GFP/GFP-PRMT3, LXRE-Luc and β-galactosidase plasmids. Twenty-four hours later, after pretreatment with 30 nM Adox or 750 μM PA for 30 min, 10 μM TO901317 was added for 6 h. Luciferase activity was measured and normalized to β-galactosidase activity. Presented data are representative of three independent experiments, and experiments were performed in triplication. Data represent the means ± SEM. *p < 0.05 vs. lane 1. †p < 0.05 vs. lane 2. ‡p < 0.05 vs. lane 4. §p < 0.05 vs. lane 3. ||p < 0.05 vs. lane 5. ¶p < 0.05 vs. lane 6. n.s. = non-specific. B: HepG2 cells were cotransfected with Flag/Flag-SREBP1c, HA/HA-PRMT3, SRE-Luc and β-galactosidase plasmids. Twenty-four hours later, 750 μM PA was added for 6 h. Luciferase activity was measured and normalized to β-galactosidase activity. Presented data are representative of three independent experiments, and experiments were performed in triplication. Data represent the means ± SEM. *p < 0.05 vs. lane 1. n.s. = non-specific. C: HepG2 cells were cotransfected with Flag and HA/Flag-ChREBP and HA-Mlx, HA/HA-PRMT3, ChORE-Luc and β-galactosidase plasmids. Twenty-four hours later, 750 μM PA was added for 6 h. Luciferase activity was measured and normalized to β-galactosidase activity. Presented data are representative of three independent experiments, and experiments were performed in triplication. Data represent the means ± SEM. *p < 0.05 vs. lane 1. n.s. = non-specific.

Supplementary Figure 4. A: HEK293 cells were transfected with HA-PRMT3 plasmid. Twenty-four hours later, cells were treated with 750 μM PA for 12 h, then fixed, immunostained with HA antibody, and evaluated. Green: HA-PRMT3, Blue: DAPI. B: HepG2 cells were treated with 750 μM PA or 10 μM TO901317 for 12 h, then fixed, immunostained with PRMT3 antibody, and evaluated. Green: endogenous PRMT3, Blue: DAPI.