SUPPLEMENTARY METHODS

Cell viability

The MTT assay was used to measure the viability of alpha mouse liver 12 (AML12) and LX-2 cells. Briefly, the cells were plated at 0.5×10<sup>5</sup> cells/well in 12-well plates and were stabilized in culture media. The stabilized cells were stimulated with indicated concentrations of DWN12088, halofuginone (HF), or palmitic acid (PA) for 24 to 48 hours. MTT/phosphate-buffered saline (PBS) solution (1 mg/mL) was added to the wells, and the plate was incubated for 2 hours at 37°C in a 5% CO<sub>2</sub> humidified incubator. The reaction was stopped by removing the supernatant, followed by dissolving the formazan product in isopropanol. The absorbance of the resulting product was measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) leader (Molecular Devices, San Jose, CA, USA).

RNA isolation and real-time quantitative reverse transcription polymerase chain reaction

Total RNA was isolated from liver tissues or cultured cells using a TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed into cDNA using a M-MLV Reverse Transcriptase (Promega, Madison, WI, USA) according to manufacturers’ protocols. mRNA expression levels were quantitated using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) with the real-time PCR cycler Rotor-Gene Q (Qiagen, Hilden, Germany) using target gene-specific primers (Supplementary Table 1). The relative mRNA expression levels were normalized to the housekeeping gene (glyceraldehyde-3-phosphate dehydrogenase [Gapdh]). All samples were performed in triplicates, and data were analyzed according to the 2<sup>-ΔΔCT</sup> method.

Antibodies

Antibodies for caspase-3 (p17, sc-22139), β-actin (sc-47778), collagen I α1 (COL1A; sc-59772), fibronectin (sc-271098), α-smooth muscle actin (α-SMA; sc-53142), Rho-kinase 1 (ROCK1; sc-365628), sterol regulatory element-binding protein-1c (SREBP-1c; sc-365513), fatty acid synthase (FAS; sc-8009), adipophilin (ADRP; sc-377429), acetyl-CoA carboxylase α (ACCo; sc-137014), peroxisome proliferator-activated receptor α (PPARα; sc-398394), peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α; sc-518025), protein kinase R-like endoplasmic reticulum kinase (PERK; sc-377400), p-PERK (sc-32577), a subunit of eukaryotic initiation factor 2 (eIF2α; sc-133132), C/EBP-homologous protein (CHOP; sc-7351), activating transcription factor 4 (ATF4; sc-390063), B-cell lymphoma 2 (Bcl2; sc-7382), BCL2 associated X, apoptosis regulator (Bax; sc-7480), poly(ADP-ribose) polymerase (PARP; sc-56197), phosphorylated-nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (p-IκBα; sc-7977), IκBα (sc-1643), nuclear factor-κB (NF-κB) p65 (F-6, sc-8008), p-p65 (Ser536, sc-33020-R), and F4/80 (sc-377009) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Antibodies against p-p65 (Ser276, #3257), p-eIF2α (#9721), phosphorylated-signal transducer and activator of transcription 6 (p-STAT6; #9361), STAT6 (#9362), phosphorylated-AMP-activated protein kinase α (p-AMPKα; #2531), AMPKα (#5831), p-SMAD2 (#3108), p-SMAD3 (#9520), and SMAD2/3 (#8685) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibody against glutamyl-prolyl-tRNA synthetase (EPRS; NMS-01-0004) was purchased from Curebio (Suwon, Korea).

Immunocytochemistry

Cells were grown until they reached 60% to 70% confluence on glass slides in 12-well plates and treated as indicated in specific experiments. The treated cells were fixed with 3.7% formaldehyde and permeabilized with 0.1% saponin solution. The slides were rinsed twice with PBS and blocked with 3% bovine serum albumin (BSA) for 2 hours. To evaluate the cytotoxic effects of DWN12088 and HF on AML12 and LX-2 cells, the fixed and blocked slides were incubated with mouse monoclonal anti-active caspase 3 (1:500; sc-21139; Santa Cruz Biotechnology) overnight at 4°C and labeled with anti-mouse immunoglobulin G (IgG)-Alexa Fluor 647 (1:1,000; #4410; Cell Signaling) at 4°C for 2 hours. To assess the anti-fibrotic effects of DWN12088 on LX-2 cells, the fixed and blocked slides of the cells were incubated with mouse monoclonal anti-COL1A (1:500; sc-59772; Santa Cruz Biotechnology) or mouse monoclonal anti-fibronectin (1:500; sc-271098; Santa Cruz Biotechnology) overnight at 4°C and labeled with...
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anti-mouse IgG-Alexa Fluor 488 (1:1,000; #4408; Cell Signaling) at 4°C for 2 hours. To determine the anti-inflammatory effects of DWN12088 on RAW264.7 cells, the fixed and blocked slides were incubated with mouse monoclonal anti-p65 (1:500; sc-8008; Santa Cruz Biotechnology) overnight at 4°C and labeled with anti-mouse IgG-Alexa Fluor 647 (1:1,000; #4410; Cell Signaling) at 4°C for 2 hours. After washing twice with PBS, the slides were incubated with 1 µg/mL 4’,6-diamidino-2-phenylindole (DAPI) for 15 minutes at room temperature. All the slides were observed using a Zeiss LSM710 confocal microscope (Carl ZEISS, Berlin, Germany) at 400× magnification. ImageJ software (National Institutes of Health, Bethesda, DM, USA) was used for quantification of the fluorescence intensity.

Immunohistological examination
Liver tissues were fixed in 3.7% formaldehyde for 24 to 48 hours at room temperature, embedded in paraffin, and sectioned at a thickness of 5 µm, and the section slides were rehydrated for antigen retrieval. To evaluate the infiltration level of M1 macrophages, liver sections were incubated overnight with mouse monoclonal anti-F4/80 (1:300; sc-377009; Santa Cruz Biotechnology) at 4°C. After washing twice with Tris-buffer saline (TBS), the section slides were labeled with anti-mouse IgG-Alexa Fluor 488 (1:500; #4408; Cell Signaling) at 4°C for 2 hours. For analyzing caspase-3 activation, slides of tissue sections were incubated overnight with mouse monoclonal anti-active caspase 3 (1:300; sc-21139; Santa Cruz Biotechnology) at 4°C. The slides were rinsed twice with TBS and labeled with anti-mouse IgG-Alexa Fluor 488 (1:500; #4408; Cell Signaling) at 4°C for 2 hours. After incubation, the slides were washed twice with TBS and stained with 1 µg/mL DAPI for 15 minutes. All the sample slides were analyzed using a confocal microscope (Carl ZEISS) at 400× magnification. The percentage of infiltrating macrophages was assessed by counting the F4/80-positive cells versus the total DAPI-stained cells, and the percentage of apoptotic hepatocytes was determined by counting the active caspase-3-positive cells versus the total number of DAPI-stained cells. The measurements were carried out in four fields per tissue slide section using the ImageJ software.

Luciferase reporter assay
Mouse peritoneal macrophages (1×10^6 cells) were transfected with 0.5 µg of NF-κB reporter plasmid (p5×NF-κB, Stratagene, San Diego, CA, USA) along with 0.5 µg of β-gal expression vector (pCMV-LacZ, Clontech, Palo Alto, CA, USA) using a Lipofectamine 3000 reagent (Invitrogen). At 6 hours after transfection, the media were replaced with complete growth media (Dulbecco’s Modified Eagle’s Medium [DMEM] containing 10% fetal bovine serum) and stabilized for 24 hours, followed by treatment with or without 250 µM PA in the presence or absence of DWN12088 (10 µM) for 24 hours. Luciferase activity was measured using a luciferase reporter assay system (Promega) according to the manufacturer’s instructions, and β-galactosidase activity was analyzed using ortho-nitrophenyl-β-galactoside. Luciferase reporter activity was normalized relative to the β-galactosidase activity.

Hematoxylin and eosin, Masson’s trichrome, and Oil red O staining
Paraffin-embedded liver section slides were stained with hematoxylin and eosin as well as Masson’s trichrome to assess the level of liver damage and fibrotic lesion formation. For Oil red O staining, the liver cryosection slides were washed with 60% isopropanol and dried under a hood for 10 minutes. Then, the slides were stained with Oil red O solution (Sigma Aldrich, St. Louis, MO, USA) for 2 hours and rinsed with water. AML12 cells (1×10^5) seeded on glass slides placed in 12-wall plates were pretreated with indicated concentrations of DWN12088 for 2 hours, followed by treatment with or without 250 µM of PA for 24 hours. The cells were fixed with 3.7% formaldehyde and rinsed twice with PBS. Oil red O staining was performed similar to the staining procedure of liver tissues. All the slides were mounted with mounting medium. Images of all samples were captured using an optical microscope (Olympus, Tokyo, Japan).
BODIPY staining
Liver cryosection slides were fixed with 3.7% formaldehyde for 10 minutes and incubated with pH 6.0 sodium citrate buffer containing 0.05% Tween-20 for 10 minutes. The slides were washed twice with PBS, blocked with 3% BSA for 1 hour, and incubated with 10 μg/mL C11-boron-dipyrromethene (BODIPY) for 30 minutes. After washing twice with PBS, the slides were incubated with 1 μg/mL DAPI for 15 minutes. The AML12 cells seeded on glass slides placed in a 12-well plate at a density of 1×10⁵ were stimulated with specific concentrations of DWN12088 for 2 hours, followed by treatment with or without 250 μM of PA for 24 hours. The cells were stained in a process similar to the BODIPY staining of the liver sections. All the slides were mounted with mounting medium and observed using a Zeiss LSM710 confocal microscope (Carl ZEISS) at 400× magnification. BODIPY intensity was measured in four fields per slide section using the ImageJ software.