SUPPLEMENTARY METHODS

Confocal imaging of lipid droplet

Cover glasses (14 to 18 pi) are washed with 1 N NaOH solution, rotated in cold room for overnight. Then, cover glasses are rinsed with distilled water for three times. A single cover glass is placed on 12 well plate and 0.01% collagen type I (Sigma-Aldrich, St. Louis, MO, USA) is applied on the cover glass. The 2.5×10^5 cells are plated and incubated 4 to 12 hours for attachments. Cells are treated with 200 mM oleic acid and 200 mM palmitic acid for 4 hours with or without 1 μM of diacylglycerol O-acyltransferase 2 (DGAT2) inhibitor. Then, the samples are washed and fixed with neutral formalin for 5 minutes. Fixed samples are rinsed and performed staining process via BODIPY and 4′,6-diamidino-2-phenylindole (DAPI). Stained samples are mounted on slide glass using mounting medium, the final samples are detected with LSM700 microscopy (Carl Zeiss, Oberkochen, Germany).

Tetramethylrhodamine, ethyl ester assay: mitochondrial potential assay

HepG2 cell lines (HepG2 shCTR and shDGAT2) are subcultured on black 96 well plate, 1.0×10^5 cell/well. On the next day, half of the wells are treated with 20 μM carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) for 10 minutes to remove mitochondrial potential. Then, 250 nM tetramethylrhodamine, ethyl ester (TMRE) are treated on the plate for 30 minutes. The TMRE solution is rinsed with 0.2% bovine serum albumin treated phosphate buffered saline (PBS) solution for twice and the fluorescence level are measured with fluorometer Varioskan Flash 3001 (Thermofisher scientific, Waltham, MA, USA).

mtDNA ratio assay

Genomic DNA (gDNA) is extracted from cell line via gDNA extraction kit (BIONEER, Daejeon, Korea). gDNA is performed quantitative polymerase chain reaction (PCR) to calculate ratio of mtDNA and reference gene, mitochondrially encoded NADH dehydrogenase 1 (mt-ND1) and pyruvate kinase (PK). Primers used are mt-ND1 F: 5′-CCCTAAAACCCGCCACATCT-3′ and R: 5′-GGCCTAGGTTGAGGTTGACC-3′; PK F: 5′-ATAACGCCTACATGGAAAAGTGT-3′ and R: 5′-TAAGCCCATCATCCACGTAGA-3.′

Western blot

Cell lines are rinsed with PBS for twice and lysed with whole cell lysis buffer (60 mM Tris-Cl, 1% sodium dodecyl sulfate [SDS]). The samples are boiled for 5 minutes, and concentration is measured via bicinchoninic acid assay kit. Equal volume of proteins is loaded to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membrane, which is blocked with everyblot, and labeled with appropriate primary antibodies for overnight in a cold room. After rinsing primary antibody, appropriate secondary antibodies with horseradish peroxidase conjugated are treated and blotted with LAS-3000 luminescent image analyzer (Fujifilm). Primary antibodies used are fission, mitochondrial 1 (FIS1; sc-376447, Santa Cruz Biotechnology, Santa Cruz, CA, USA); phospho-dynamin-related protein 1 (pDRP1) S616 (4867S, Cell Signaling Technology, Beverly, MA, USA); DRP1 (5391S, Cell Signaling Technology); pDRP1 s637 (3455S, Cell Signaling Technology); mitofusin 1 (MFN1; sc-166644, Santa Cruz Biotechnology); β-actin (sc-47778, Santa Cruz Biotechnology); cyclin-dependent kinase 2 (CDK2; BD-610145, BD Bioscience, San Jose, CA, USA); cyclin A2 (CCNA2; sc-751, Santa Cruz Biotechnology); cyclin B1 (CCNB1; sc-752, Santa Cruz Biotechnology); cyclin E1 (CCNE1; sc-481, Santa Cruz Biotechnology); estrogen-related receptor alpha (ESRRA; E1G1J, Cell Signaling Technology); prospero homeobox 1 (PROX1; 925202, Biolegend, San Diego, CA, USA); Lamin a/c (sc-376248, Santa Cruz Biotechnology); a tubulin (2144S, Cell Signaling Technology); PPARG coactivator 1 alpha (PPARGC1A; ab54481, Abcam, Cambridge, UK).

RNA extraction and quantitative PCR analysis

RNA is isolated from cell using TRIzol reagent (Invitrogen, Waltham, MA, USA) with manufacturer's protocol. Briefly, after media removal, cell is lysed with TRIzol. 1-Bromo-3-chloropropane (BCP) is mixed and incubated for 10 minutes in room temperature (RT). Then 10,000 ×g centrifugation for 15 minutes for phase differentiation. The upper clear supernatants, composing RNA, is carefully moved to 1.5 mL microcentrifuge tube and 1 mL of 100% isopropanol (Merck, Rahway, NJ, USA) is added to precipitate RNA after centrifugation for 10 minutes. RNA is then pelleted and washed with 70% ethanol with diethyl pyrocarbonate (DEPC) treated water for twice. Pellet is now rinsed and resuspended in DEPC treated water. Which is processed into reverse transcription with SuperScript reverse transcriptase III (Invitrogen) and random hexamer. cDNA is further analyzed with quantitative PCR. SYBR Green PCR Master Mix (Applied Biosystems, Waltham, MA, USA) is used to detect DNA synthe-

sis and delta delta-Ct method was used to quantify the original DNA level. Data were normalized with 18S ribosomal RNA expression level. The primers used for qPCR is DGAT2-S: 5′-GAA TGG GAG TGG CAA TGC TAT-3′; DGAT2-AS: 5′-CCT CGA AGA TCA CCT GCT TG-3′; 18S-S: 5′-CTA CCA CAT CCA AGG AAG GCA-3′; 18S-AS: 5′-TTT TTC GTC ACT ACC TCC CC-3′.

Adenosine triphosphate assay kit

Cellular adenosine triphosphate (ATP) level is measured with ATP assay kit (Abcam) following the manufacturer's protocol. Briefly, cell is homogenized in ATP assay buffer and supernatants are collected after centrifugation. Samples and ATP standards are incubated with reaction mixture for 30 minutes. The reaction products are measured via colorimeter (opical density [OD] 570 nm) via Multiskan GO (Sigma-Aldrich).

Cell cycle assay propidium iodide

Cell lines are plated on 100 pi cell culture dishes. After cell attachment, media is changed into 0.2% fetal bovine serum (FBS) media for cell cycle synchronization for 8 hours. After synchronization, media is changed into complete media of 10% FBS and cultured for 48 hours. Cells are rinsed with detached with Trypsin-ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich) and fixed with ice cold 100% ethanol for 4 hours on ice. Ethanol is then rinsed with PBS and 20 mg/mL propidium iodide is treated with 1 mg/mL RNaseA (Roche, Basel, Swiss). The samples are filtered with 70 μm strainer and measured with flow cytometry SA3800 (Sony, Tokyo, Japan). The following process are performed by Avison Biomedical Research Center, electron microscopy team (Seoul, Korea).

Wound healing assay

The 1×10^6 cells are plated on 35 pi dishes and incubated for 3 days until 100% confluent. Use a 200 μL tip to create linear wound on cell and let cell to close the wound. The microscopic photos are taken every day to track wound closure.

Lactate assay kit

Cellular lactate level is measured with EZ-Lactate Assay kit (DoGenBio, Seoul, Korea). Cell lysate is prepared with 2×10^6 cells homogenized in assay buffer. Supernatants are collected from centrifuged sample. The 50 μL of samples and lactate standards are incubated with enzyme mixture for 30 minutes in RT and measured colorimetric absorbance on A570 with Multiskan GO.

Subcellular fraction

The 1×10^6 cells are rinsed with PBS and scraped with subcellular fraction buffer (20 mM Hepes pH 7.4, 10 mM potassium chloride, 2 mM magnesium chloride, 1 mM EDTA, and 1 mM ethylene glycol-bis [2-aminoethylether]-N,N,N′, N′-tetraacetic acid). By passing through 27 G syringe needle 10 times, nucleus is isolated from cytosol. Then centrifuge the cell lysate with 720 ×g for 10 minutes. The pellets are nucleus fraction, and the supernatants are cytosol fraction reference [1].

Luciferase assay

p3x-estrogen-related response element (p3xERRE)-firefly luciferase plasmid (Addgene, Watertown, MA, USA) and renilla luciferase plasmids are transfected to HepG2 cell line via lipofectamine 3000. After a day passed, dual-luciferase reporter assay system (Progmega, Madison, WI, USA) is used to calculate activity of luciferases translated according to manufacturer's protocol. Briefly, cell lysate is prepared with passive lysis buffer. Then using Centro XS3 LB960 luminometer (EG&G Berthold, Bad Wildbad, Germany) to add luciferin and stop solution to detect the activity of firefly luciferase and renilla luciferase separately for normalization.

Cholesterol assay kit

Cellular total cholesterol levels are measured with cholesterol assay kit (Abcam). Cellular lysates of lipids are prepared via Folch extraction. The experiments are performed as manufacturer's protocol. Briefly, Folch extracted samples and cholesterol standards are added to 96 well plate and added with master reaction solution; then, the reaction is held in 37°C, light protected for 1 hour. The final product is measured in colorimeter with OD570 nm.

Immunoprecipitation

Immunoprecipitation via anti-ESRRA was performed in nucleus fraction. A 500 μg of protein is mixed with 20 μg of agarose plus A/G bead (Santa Cruz Biotechnology) for 1 hour, rotated in the cold room for cleaning. The beads are removed using centrifuge and 1 μg of ESRRA-antibody and immunoglobulin G-antibody for overnight, rotated in the cold room. Then, 40 μL of agarose beads are added and incubated to pulldown antibody-protein complexes. Then supernatants are removed and washed three times, the pellet with the beads are boiled 5 min-

utes with laemmli buffer and performed Western blot.

Spatial analysis

Spatial dataset was available from GSE192741 (healthy=2, steatosis=3) and from supplementary data of reference [2]. The dataset was analyzed with R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria) and Seurat_4.4.0 package following the 'Analysis of Image-based Spatial Data in Seurat' from reference [3]. Briefly, dataset and high-resolution image files were loaded to R and normalized with sctransform_0.4.1. Normalized data were integrated and performed standard workflow of single cell RNA sequencings; ScaleData, RunPCA, RunUMAP. Sctransform normalized data was presented via VlnPlot and SpatialFeaturePlot.

SUPPLEMENTARY REFERENCES

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