

# Supplemental Material

## Material and Methods:

***Immunocytochemistry:*** Cells grown on a sterile glass coverslip for overnight were incubated without or with palmitate (0.75 mM) and hypoxia (1% O<sub>2</sub>) condition for 16h. On termination of incubations, cells were washed in PBS and fixed with ice-cold methanol for 5 min. For intracellular staining, cells were permeabilized with 0.25% TritonX-100 in PBS for 10 min at room temperature. Cells were blocked with 1% BSA in PBS containing 0.1% Tween-20 for 30 min at room temperature and incubated with primary antibodies for 1 h in room temperature. Cells were then washed with ice-cold PBS thrice for 5 min each, followed by the incubation with fluorescence-conjugated secondary antibodies for 1h room temperature in the dark. Before mounting on a glass slide, cells were washed thrice for 5 min each with ice-cold PBS. Coverslips were mounted onto glass slides using anti-fade mounting medium with DAPI. Cellular images were captured by an inverted fluorescent microscope (Leica DMI8, Germany) and image analysis was performed using LAS X software. Fluorescence intensity was quantified using ImageJ software (1.48v, NIH, USA).

***Immunofluorescence:*** Adipose tissue samples collected from human subjects and mice models immediately washed in sterile saline and then placed in Neutral buffer formalin (10%) for overnight fixation at 4°C. After fixation, adipose tissue were embedded in OCT (optimal cutting temperature compound, Sigma) and frozen in -30°C to -60°C followed by cryosections using Cryotome (Leica CM 1860, Leica Biosystem, Wetzlar, Germany). Immunostaining was performed on tissue cryosections using specific antibodies. Briefly, tissue cryosections (10 µm) were

placed in gelatin coated glass slides, fixed in ice-cold methanol for 5 min, blocked with 5% BSA containing blocking buffer, and incubated with specific primary antibodies for 1h in room temperature. After washing, signal was visualized by subsequent incubation with fluorescence-conjugated appropriate secondary antibodies and counter-stained with anti-fade mounting medium containing DAPI. Images were captured by a fluorescence microscope (Leica DMI8, Germany) and analysis was performed using LASX software.

***Oil Red O staining:*** Fresh frozen adipose tissues (collected from patients and mice) were cryo-sectioned into 10µM thick slices and placed in membrane coated glass slides. The sections were air dried for 45 min and fixed in chilled formalin for 10 mins. Then fixed samples were air dried for 30 min in room temperature and gently rinsed with distilled water. The sections were air dried and absolute propylene glycol added for 5 mins and stained with prewarmed oil red o solution for 10 min in 60°C oven. Then the section containing slides were differentiated in 85% propylene glycol for 3 mins. The slides were rinsed 2 times with distilled water and stained with haematoxylin for 30 sec. Then running tap water was used to wash the stained sections and mounted using glycerine. Images were captured by a fluorescence microscope (Leica DMI8, Germany) and analysis was performed using LASX software.

***RNA extraction and Quantitative PCR:*** Total RNA was extracted from the cells by TRIzol (Invitrogen) and quantified using NanoDrop™ OneC spectrophotometer (NanoDrop Technologies, Thermo Scientific, Waltham, MA USA). RNA (100 ng) was then treated with DNase I and reverse transcribed using the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, California, USA). We used PowerUp™ SYBR™ Green Master Mix (Applied Biosystems) to perform real time quantitative PCR in QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Waltham, Massachusetts, USA) using

gene specific primers. microRNA was isolated from the cells using *mirVana*<sup>TM</sup> miRNA Isolation Kit (Ambion, Thermo Scientific) following manufacturer's protocol and microRNA specific cDNA synthesis was performed using TaqMan<sup>TM</sup> MicroRNA Reverse Transcription Kit (Applied Biosystems). TaqMan<sup>TM</sup> Multiplex Master Mix (Applied Biosystems) was used to perform real-time quantitative PCR for miRNA with specific miRNA primers of miR-210-3p and U6 sn RNA (Applied Biosystems). mRNA and miRNA expression were normalized to  $\beta$ -actin and U6 snRNA, respectively, following the  $\Delta\Delta CT$  method. Mean  $\Delta Ct$  value was transformed to relative expression or fold change by  $2^{\Delta\Delta CT}$  and average fold change value was calculated.

**Immunoblotting:** Control and treated cells were lysed in NP40 cell lysis buffer (Invitrogen) supplemented with the Halt protease and phosphatase inhibitor cocktail (Thermo Scientific), centrifuged at 13,000 rpm for 10 min at 4°C. Protein concentrations of cell lysates were determined by the BCA Protein Assay Kit (Pierce) following manufactures' guideline. Cell lysates (50  $\mu$ g of protein) were resolved on 10% SDS-PAGE and transferred on to PVDF membranes (GE Healthcare Biosciences) with the help of Turbo Blotting System (Bio-Rad Laboratories). Membranes were first blocked with 5% BSA in TBS (Tris-buffered saline) buffer for 1h followed by the overnight incubation with primary antibodies in a rotating shaker at 4°C. The membranes were then washed three times with TBST (TBS containing 0.1% Tween 20) buffer for 10 min intervals and incubated with peroxidise conjugated specific secondary antibodies for 2 h at room temperature. Membranes were then washed three times with TBST for 10 min intervals and subjected to Clarity<sup>TM</sup> Western ECL Substrate (Bio-Rad, Hercules, California, USA) incubation for 5 min at room temperature. Protein bands were visualized in Chemidoc XRS+ System (Bio-Rad Laboratories, Hercules, California, USA) using Image Lab Software.

**Enzyme-linked immunosorbent assay (ELISA):** We measured IL6 and TNF- $\alpha$  cytokine levels in the cell culture medium of control and treated cells using mouse IL-6 (cat. no. #431307) and TNF- $\alpha$  (cat no. #430907) ELISA kits (BioLegend, San Diego, CA) following manufacturer's instructions.

**Fluorescence-activated cell sorting (FACS):** Visceral adipose tissues collected from human and mice were rinsed in sterile PBS, chopped into small pieces and then digested in Hanks' Balanced Salt Solution containing collagenase type II (2 mg/ml), glucose (5.5 mM), and 4% BSA (fatty acid-free) for 45 min at 37°C water bath shaker. The enzymatic activity was then neutralized by the addition of serum and the digestion mixture was passed through a cell strainer (pore size: 70  $\mu$ m, HiMedia, India). The isolated cell suspension was subjected to centrifugation at 2000 rpm for 10 min. Cell pellet was washed twice with ice-cold PBS and the cells were sorted for F4/80+ (anti-mouse) or CD68+ (anti-human) antibody for the collection of adipose tissue macrophages (ATMs) to perform different experiments.

**Statistical analysis:** All data analyses were performed using GraphPad Prism software (v.8.0; GraphPad Software, Inc., La Jolla, CA). Data represented as mean  $\pm$  S.D. Student's *t*-test determined statistical significance, and *p* value indicated significance. A level of *p*<0.05 was considered significant. One way and two way ANOVA testing used for the comparisons among multiple groups. *p*<0.05 was considered as statistically significant.