RNA preparation and qRT-PCR

Total RNA from tissues and cells was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The quality of total RNA was detected at an A260/A280 ratio using 1% agarose gel electrophoresis. The GoScript Reverse Transcription System (Promega, Madison, Wis) was used to generate combinational DNA. The cDNA template was amplified by real-time RT-PCR using the SYBR Premix Dimmer Eraser kit (TaKaRa). Gene expression in each sample was normalized to β-Actin expression. Real-time RT-PCR reactions were performed by the ABI7500 system (Applied Biosystems, Carlsbad, CA, USA). The real-time PCRs were performed in triplicate. The relative expression fold change of mRNAs was calculated by the $2^{-\Delta\Delta Ct}$ method. Primers used in this study are listed in Supplementary Table S1.

The PCR array containing the gene involved in the lipid metabolism (Supplemental Table 3) were designed by Ribobio (Guangzhou, China). The expression levels of genes were detected by PCR array (Guangzhou, China).

Western blot analysis

The harvested cells were centrifuged at 6,000 rpm for 1 min. The total cellular proteins were prepared using RIPA cell lysis buffer (Cell Signaling Technology) supplemented with protease inhibitors. The lysates were then collected and subjected to ultrasonication and centrifugation. The supernatants were collected, and protein content was determined by Bradford assay. Equal amounts (30-50 μg) of proteins

were applied to an 8-12% SDS-polyacrylamide separating gel and transferred to a PVDF Immobilon-P membrane (Millipore). The membrane was blocked with 5% skim milk in TBST and then probed with indicated primary antibodies with gentle shaking at 4°C overnight. The membranes were washed with TBST (3×10 min), incubated in secondary antibodies conjugated to horseradish peroxidase at room temperature for 1 hour. Antibody-bound proteins were detected by ECL (enhanced chemiluminescence) Western Blotting Substrate (Pierce, Rockford, IL). The band intensity of western blotting and the normalization were analyzed using the Image J program (National Institutes of Health, Bethesda, MD). The antibodies used were listed in Supplementary Table S2.

Chromatin immunoprecipitation

Monocytes were serum-starved overnight. Chromatin was cross-linked with 1% formaldehyde for 10 min. After cell lysis, the chromatin was sonicated with a Bioruptor (Diagenode) in a cold room using the following parameters: H- high setting, pulse interval- 30 sec ON and 45 sec OFF, cycle time- 15 min each. Change ice in water bath chamber after each cycle. After about 9 cycles, a DNA smear with an average size of 500 bp was obtained. After centrifugation, the supernatants were subjected to immunoprecipitation overnight with antibodies at 4°C, or with isotype rabbit IgG at 4°C overnight. Chromatin-antibody complexes were isolated using Protein A/G PLUS Agarose (Santa Cruz). The crosslinks for the enriched and the input DNA were reversed and the DNA was cleaned by RNase A (0.2 mg/mL) and proteinase K (2 mg/mL) before phenol/chloroform-purification. PCR was employed to analyzed the specific sequences from immunoprecipitated and input DNA. The results are representative of at least three independent experiments.

RNA interference

The siRNA against WDR5 or MAT2A and their scrambled constructs were purchased from GenePharma (Shanghai, P.R. China). $CD14^+$ cells (1×106 cells/wells) were seeded into 6-well plates overnight before transfection. $CD14^+$ cells were left untreated or transfected with 300 nM negative control siRNA, or WDR5 or MAT2A−specific siRNA using P3 primary cell 4D-Nucleofector X kit (V4XP-3024, Lonza). All siRNA duplexes were purchased from GenePharma (Shanghai, P.R. China).

Luciferase reporter assay

The RIP1 promoter constructions were generated by PCR. The sequences of the primers were: RIP1 PF:5’-GCCGAGCTCGAGGGAGTGGACGCTGGAGCAA -3’, RIP1 PR: 5’-GCCGCTAGCTCGTCCCGTCACCCTCCTCT-3’. In brief, cycling conditions included: denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 2 min, and extension at 72°C for 1 min with a final-extension step for 10 min at 72°C. PCR products were verified by using dissociation curves and gel electrophoresis. The RIP1 promoter region was amplified by PCR and the PCR products were then sub-cloned into the expression region of vector PGL3-Basic (Promega, USA). In general, 1×10^6 cells were plated in 500 μl of OPTI-MEM (Invitrogen) with 10% FBS in 24-well plates. Firefly luciferase reporter constructs (5 μg) and pRL-thymidine kinase control vector (pRL-TK; Promega; 0.25 μg) were mixed with 5 μl of Lipofectamine 6000 (Invitrogen) to form a complex. Then, the cells were harvested after 48 hours for firefly/Renilla luciferase assays using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activities were normalized to the cotransfected pRL-TK plasmid (mean ± SD).

UPLC–MS analysis

Untargeted LC–MS analysis of polar metabolites was performed by using a UPLC system (ACQUITY, Waters Corp) interfaced with a mass spectrometer (LTQ-Orbitrap, Thermo Scientific). Electrospray ionization (ESI) in the mass spectrometer was conducted in both positive and negative mode and auxiliary gas flow were set at 40 and 15 (arbitrary units), respectively, with a capillary temperature of 400 °C. The ESI source and capillary voltages were 4.5 kV and 40 V, respectively, for positive ESI mode and 2.8 kV and −15 V for negative ESI mode. Mass calibration was performed with standard LTQ-Orbitrap calibration solution (Thermo Scientific) before sample injection. A pooled quality-control mixture comprising equal aliquots of all samples was run at regular intervals throughout each analytical batch. Samples were randomized for each analytical batch and triplicate injections were performed for each sample.

MRM transitions and MS parameters were optimized by using analytical-grade standards.

All chromatographic separations were performed on an ACQUITY UPLC HSS T3 1.7 μm, 50 × 2.1 mm i.d. column (Waters Corp). The column and autosampler temperatures were maintained at 30 oC and 4 oC, respectively. The elution condition and Optimized compound-dependent MS parameters are indicated in Supplementary Table 3,4. The injection volume was 4 μl.

Raw UPLC–MS data were preprocessed and analyzed with the XCMS peakfinding

algorithm (version 1.30.3)65. The pooled quality-control mixture was used for signal correction between and within each batch analysis. Samples were normalized on the basis of their cell counts. The identities of marker metabolites were verified by comparison of their retention time and mass spectra with those of commercially available standards.

Cell viability assay

Cell viability was assessed by the Cell Counting Kit 8 (CCK-8, Donjindo). Briefly, monocytes/macrophages were seeded into 96-well plates at an initial density of 10000 cells per 100μl. After 0, 24, and 48 hours of cultivation, CCK-8 solution (10μl per 100μl of medium in each well) was added to each well and incubated for 2 h. The absorbance was measured by scanning with a microplate reader (MRX; Dynex Technologies, West Sussex, United Kingdom) at 450 nm. Data were expressed as the percentage of viable cells as follows: relative viability (%)=($A450_{treated}$-$A450_{blank}$)/ ($A450_{control}$ - $A450_{blank}$)×100%. Three replicates for each group.

Quantitative histone methylation proteomics

Nuclei were isolated using gentle detergent treatment (0.3% NP-40 in NIB-250 buffer) of cells and centrifugation at 600g and washed with NIB-250 buffer. Histones were acid-extracted and derivatized with propionic anhydride both before and following trypsin. Propionylated histone peptides were resuspended in 50 μl water with 1% TFA, and 3 μl were injected in 3 technical replicates on nanoLC/triple quadrupole MS

which consisted of a Dionex UltiMate 3000 coupled to a ThermoFisher Scientific TSQ Quantum triple quadrupole mass spectrometer. Buffer A was 100% LC-MS grade water with 0.1% formic acid and buffer B was 100% ACN. The propionylated peptides were

loaded onto an in-house packed C18 trapping column (4 cm × 150 μm; Magic AQ C18, 3 μm, 200 Å Michrom) for 10 min at a flow-rate of 2.5 μl min−1 in 0.1% TFA loading buffer. The peptides were separated by a gradient from 1 to 35% buffer B from 5 to 45 min. The analytical column was a 10 cm × 75-μm PicoChip (1PCH7515-105H253-NV New Objective) consisting of the same C18 material as the trapping column. The triple quadrupole settings were as follows: collision gas pressure of 1.5 mTorr; Q1 peak width of 0.7 (full width at half maximum); cycle time of 3 s; skimmer offset of 10 V; electrospray voltage of 2.5 kV. Data were analysed using Skyline software (v.3.5; MacCoss Laboratory, University of Washington) with Savitzky–Golay smoothing of peaks. Automatic peak assignment and retention times were checked manually.

RNA stability assay

To measure RNA stability, 0.5 mg/ml Actinomycin D (Sigma aldrich, USA) was added to cells to inhibit transcription and then incubated for different time points as indicated. At each time point, RNA was harvested followed by qRT-PCR as previously described. Transcript levels were plotted by appropriate nonlinear regression curves using a one-phase decay equation. RNA decay rate constant (k) was

quantified by fitting an exponential curve to the data points ($y = a*e^{-kt}$; y is the relative amount of RNA, and t is time). The half-life was then estimated according to the equation $t_{1/2} = ln(2)/k$. The normalizer transcript 18S rRNA that does not decay over the course of this experiment was detected as control.