Supplementary information, Data S1 Materials and methods

Chemicals

Compounds for screening and validation were purchased from Chengdu Pufei De Biotech Co.Ltd. Compounds were stored in -20℃, avoiding from light. Other compounds used in experiments were purchase from Sigma.

High-throughput screen methods

3T3-L1 maintenance, differentiation and treatment

One thousand 3T3-L1 cells were seeded onto each well of 96-well plate and allowed to proliferate in DMEM medium (Gibco) containing 10% new calf serum (TBD) for 3-4 days until reaching confluence. Two days after contact inhibition (designated as day 0), fresh DMEM medium containing 10% fetus bovine serum (FBS, Gibco) and induction cocktail (1 μg/ml insulin, 1 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methyl-xanthine for 48hr, and then 1 μg/ml insulin for another 48hr) were added. Meanwhile, various compounds (stored in DMSO at a concentration of 10 mM) were added into each well at a final concentration of 10 μM. After induction, medium was changed every other day with corresponding compounds in it. Eight days after induction cocktail was added (designated as day 8), cells were collected for further investigation.

Oil Red O quantification

Differentiated 3T3-L1 (day 8) were fixed with 4% Poly formaldehyde, stained with Oil Red

O , washed once with ddH_2O and then dried at room temperature (RT). Thereafter, 50 μl isopropanol was added into each well. The plate was shaked gently until Oil Red O was absolutely eluted into isopropanol. The OD value was detected under 560 nm.

One step PCR

Differentiated 3T3-L1 (day 8) were washed once with PBS and then lysed with in-house lysis buffer (2% Triton X-100, 2%NP40, 4% RNAsecure and 1Unit/ml RQ1 DNAse in ddH_2O). Cytolysis was collected into PCR tubes and then proceeded to real-time qPCR as templates using TransScript Green One-Step qRT-PCR SuperMix (Transgene) and specific primers from PrimerBank (http:// pga.mgh.harvard. edu/primer bank/). This protocol was modified based on previous report to adapt to high-throughput screening.

Real-time qPCR

Total RNA were extracted by Trizol (Life Technologies) and stored in DEPC H_2O in -20℃. cDNA synthesized from total RNA with RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). Real-time qPCR was performed using Power SYBR Green qPCR Master Mix (Life technologies) in 7500 real time qPCR system (ABI). The relative abundance of mRNAs was calculated with 18S mRNA as the invariant control. The primers were from PrimerBank (http://pga.mgh.harvard.edu/primerbank/).

C3H10T1/2 maintenance, differentiation and treatment

C3H10T1/2 was maintained in DMEM medium supplemented with 10% new calf serum

(TBD) and pen/strep and was cultured in a 10% CO_2 atmosphere. After seeded onto plates for 24 hr, recombined human BMP4 (final concentration as 20 ng/ml, R&D) or BMP7 (final concentration as 100 ng/ml, ProSepc) was added into medium and treated cells for 3 days. After BMP4 treatment (designated as day 0), fresh DMEM supplemented with 10% FBS and penicillin-streptomycin containing induction cocktail (1μg/ml insulin, 1μM dexamethasone, 0.5mM 3-isobutyl-1-methyl-xanthine, 0.125mM indometacin and 1 nM T3) was added. After 48 hr, medium was changed into DMEM supplemented with 10% FBS containing 1μg/ml insulin and 1nM T3. The medium was freshened every other day. Eight days after induction (designated as day 8), cells was harvested for following analysis. ATM was stored in DMSO at a concentration of 20 mM was added at day 0 at referred concentration and sustained through 8 days until cells were collected. Dihydroartemisinin (DHA) was stored in DMSO at a concentration of 10 mM and was added at day 0 at referred concentration until day 8.

SVF isolation, differentiation and treatment

Inguinal SVF were obtained from 8-week-old male mice. Freshly isolated cells were plated onto plates in DMEM/F12 (1:1) medium (Gibco) supplemented with glutamax, pen/strep and 10% FBS (Gibco) and maintained in a 5% CO2 atmosphere. Cells were allowed to grow to confluence and were then held at confluence for 2 days without changing of the media prior to exposure to the differentiation cocktail (5 μg/ml insulin, 1μM dexamethasone, and 0.5mM 3-isobutyl-1-methyl-xanthine, 1μM rosiglitazone for 48 hr, and then 5 μg/ml insulin, 1 μM rosiglitazone for 48hr) in fresh media every other day. After 4 days of exposure to the differentiation cocktail, cells were maintained in DMEM/F12 (1:1) with 10% FBS until day 8

for further evaluation. ATM treatment started at day 0 and lasted through the whole process of adipogenesis.

Western blotting

Cultured cells or tissues were prepared with the T-PER tissue protein extraction reagent (2% SDS and 60 mM Tris·HCl-pH6.8) with the cocktail of proteinase inhibitors (Roche) in it. The total protein Tissue or cultured cells were homogenized in lysis buffer containing and were loaded onto the gel (20-40 μg) or electrophoresis. Proteins then were transferred onto 10% SDS-PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis) and were then transferred to PVDF membranes (Bio-Rad). The electroblotted membranes were blocked by TBS containing 5% non-fat milk (Santa Cruz) and were probed with primary antibodies overnight at 4°C and immunoblotted with specific antibodies.

MitoTracker Green Stain

Cultured cells (day 8) were incubated in PBS with MitoTracker green (final concentration 200 nM, Life technologies) in it for 10 min in 37℃ incubator. The cells were observed and photographed under an Axioskop 2 microscope (Carl Zeiss) with a DP70 charged-coupled device (CCD) system (Olympus).

Animal experiments

Male C57BL/6J mice (SLRC laboratory animals) of 8-week old were fed with normal chow diet (SLRC) or high fat diet (HFD, Research Diets). For subcutaneous (sub.) administration,

15 μM ATM in PBS was injected into inguinal fat pad twice a week for 8 week. Equal volume of PBS was injected into the same position of control mice. For intraperitoneal (i.p.) treatment, 6 mg/ml ATM was stored in coconut oil avoiding from light in 4℃. ATM was injected twice a week for 8 weeks at concentration of 20 mg/kg body weight. All studies were approved by Animal Care and Use Committee of the Fudan University Shanghai Medical College and followed the NIH guidelines on the care and use of animals.

Glucose and Insulin Tolerance Tests

For the glucose tolerance test (GTT), mice were injected i.p. with D-glucose (2 mg/g body weight) after an overnight fast, and tail blood glucose levels were monitored by one-touch glucometer (LifeScan). For the insulin tolerance test (ITT), mice were injected i.p. with human insulin (0.75 mU/g body weight, Eli Lily) around 2:00 p.m., and tail blood glucose levels were monitored in the same way as GTTs.

Cold exposure and rectal temperature measure

Mice were exposed to 4 ℃ environment deprived of water and food for 6 hr. Rectal temperature was monitored by rectal thermometer (PhysiTemp) every other hour.