SUPPLEMENTARY METHODS

Western blotting

3T3-L1 cells were lysed with lysis buffer containing protease inhibitor cocktail (Roche, Basel, Switzerland) and phosphatase inhibitors (2 mM sodium vanadate, 1% sodium pyrophosphate, 10 mM sodium fluoride, and 10 mM phenylmethylsulfonyl fluoride). Equal amounts of proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene fluoride membranes (Millipore, Burlington, MA, USA). The blots were blocked with 5% skimmed milk or bovine serum albumin and probed with appropriate antibodies. Band intensities were quantified using Image-J program (http://imagej.nih.gov).

RNA isolation and quantitative reverse transcriptase polymerase chain reaction

RNA was extracted from 3T3-L1 cells using QIAzol reagent (QIAGEN, Germantown, MD, USA). We synthesized cDNA using iScript cDNA synthesis kit (Bio-rad, Hercules, CA, USA). For quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), power SYBR Greren PCR Master Mix (Applied Biosystems, Waltham, MA, USA) was used. Results were normalized to gyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primers used in this study are listed (Supplementary Table 1).

Cell fractionation

3T3-L1 cells seeded in 100 mm dishes were differentiated into adipocytes. The cells were treated with 20 μg/mL of vimentin for 24 hours. Briefly, cells were homogenized using a homogenizing buffer mix and separated at 700 ×g for 10 minutes at 4°C. The supernatants were transferred to new tubes and centrifuged at 10,000 ×g for 30 minutes at 4°C. The supernatant (cytosol fraction) was collected and stored at –80°C. The pellets containing total membrane proteins were resuspended using upper phase solution and lower phase solution. The samples were separated at 4,000 rpm for 5 minutes at 4°C. The upper phase was carefully collected and diluted in five times volume of sterile water. And then, the samples were spinned down at 14,000 ×g for 10 minutes at 4°C. The supernatant was discarded and the pellet was dissolved in 0.5% Triton X-100 in phosphate buffer saline.

Oil-Red O staining

3T3-L1-derived adipocytes plated in a 6-well plate were incubated with or without vimentin (20 μg/mL) for 24 hours. Then the cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature. Cells were washed with Dulbecco's phosphate-buffered saline (DPBS) twice and incubated with Oil-Red O solution for 30 minutes. After rinsing with sterile water, cells were observed under a microscope. To extract Oil-Red O solution, cells were incubated in isopropanol (500 μL) for 5 minutes, and the Oil-Red O extract was measured using spectrophotometry (absorbance at 510 nm).

L-Lactate assay

3T3-L1-derived adipocytes were treated with vimentin (20 μg/mL) for 24 hours. After that, cell culture media were harvested. Following the instruction of L-Lactate assay kit (Abcam, Cambridge, UK), the media were mixed with reaction mix (1:1) for 30 minutes, and the absorbance of the mix was measured on a microplate reader at optical density (OD) 450 nm.

Adenosine triphosphate assay

3T3-L1-derived adipocytes were treated with vimentin (20 μg/mL) for 24 hours. After that, cells were washed with cold DPBS and transferred into a 1.5 mL eppendorf tube. To remove enzymes, Deproteinizing Sample Preparation Kit was used following the instruction. And then, equal volume of adenosine triphosphate reaction mix was added to the samples and the absorbance of the mix was measured on a microplate reader at OD 570 nm.

Triglyceride assay

3T3-L1-derived adipocytes were treated with recombinant vimentin (20 μg/mL) for 24 hours. After that, cells were washed with cold DPBS and transferred to 1.5 mL eppendorf tube. Cells were homogenized using 5% NP-40 and heated until the NP-40 solution becomes cloudy. And then, cells were centrifuged and the supernatant was used for the experiment. Lipase was added to the supernatant. After adding triglyceride reaction mix, the mixture was incubated at room temperature for 60 minutes away from light. The output was measured on a microplate reader at OD 570 nm.