SUPPLEMENTARY DATA

Histology and hepatic TG content

Mice liver and adipose tissues were prepared into paraffin-embedded sections and stained with H&E using standard protocol. The average adipocyte areas in adipose tissue sections were measured using Image-Pro Plus 6.0. The total lipid was extracted from liver homogenate, and hepatic TG content was determined with commercial TG test kit and normalized to liver wet weight.

Culture of adipose tissue explants

Epididymal adipose tissues were minced into small pieces. The explants were dispersed in DMEM containing 10% FBS at 0.3 g/mL and incubated at 37 °C, 5% CO_2 for 24 h. The supernatants were collected for detection of TNF-α and IL-10.

ELISA

Culture supernatants from macrophages or adipose tissue explants were collected for detection of TNF-α, IL-12 (Dakewe Biotech, Beijing, China) and IL-10 (eBioscience) using commercial kits according to the manufacturer's instructions.

qPCR

Total RNA was extracted using RNAfast200 (Fastagen, Shanghai, China) or Trizol (TIANGEN Biotech, Beijing, China), and reversely transcripted with ReverTra Ace qPCR RT Kit (TOYOBO Life Science, Osaka, Japan). qPCR was carried out using SYBR Green Master Mix (CWbiotech, Beijing, China). The relative mRNA levels of genes were calculated by $2^{-\Delta\Delta Ct}$ method, using *18s* rRNA or *gapdh* as internal controls. Primers for indicated genes were listed in Supplementary Table 2.

Western-blot

Equal amount of proteins from exosomes, cells or tissues lysates were separated on SDS-PAGE and transferred to PVDF membranes. After blocking with 3-5% bovine serum albumin for 3 h, the membranes were blotted with indicated primary Abs overnight at 4 °C, followed by incubation with HRP-conjugated secondary Abs. Signals were detected by SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL). Primary Abs against UCP1 (23673-1-AP), TSG101 (14497-1-AP), CD9 (60232-1-Ig), TH (25859-1-AP), GAPDH (60004-1-Ig) (Proteintech Group, Wuhan, China), STAT3 (4904), p-STAT3 (9145) (Cell Signaling Technology, Beverly, MA), HSP90 (WL0132b,Wanleibio, Shenyang, China), CD63 (BA1584, BOSTER, Wuhan, China) and Arg-1 (sc-271430, Santa Cruz Biotechnology, Dallas, TX) were used in the experiments.

CCK8 assay

ADSCs at a density of 3000 cells per well in 96-well plates were treated with CM from macrophages for 24 h, 36 h, 48 h. CCK-8 (10 μL/well, Dojindo, Tokyo, Japan) was added 1 h before the end of the incubation. Viable cells were determined by measurement of the absorbance at 450 nm.

EdU incorporation assay

ADSCs at a density of 3000 cells per well in 96-well plates were treated with CM from macrophages for 24 h. EdU (10 μM) was added for 6 h of incubation before the harvest. Cell-Light EdU Apollo567 Cell Tracking Kit (RiboBio, Guangzhou, China) was used to detect EdU, and fluorescence signals were visualized with fluorescence microscope.

DNA-binding ELISA

Human ADSCs were isolated from omentum fat of a male adult who received whipple procedure operation in Qilu Hospital of Shandong University. Exosomes from human ADSCs were isolated and characterized. The active form of STAT3 in exosomes was detected using TransAM® STAT3 Kit (Active Motif, Carlsbad, CA). Human studies were approved by Ethics Committee of Shandong University. Informed consent from the subject was obtained prior to surgery.

Assay of luciferase activity

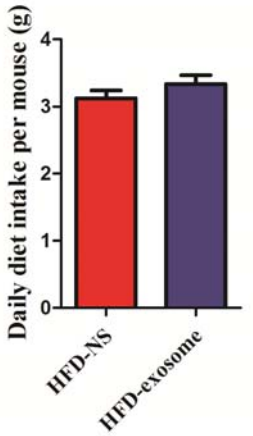
Luciferase reporter plasmid pGL3-mArg1 promoter/enhancer (-31/-3810) was provided by Peter Murray from St. Jude Children's Research Hospital via addgene (Cambridge, MA)(1). RAW264.7 or HEK-293T cells were co-transfected with pGL3-mArg1 promoter/enhancer and pRL-TK-Renilla-luciferase plasmids using Jet-PRIME. After 24 h, the cells were treated with ADSC-derived exosomes for another

24 h. Luciferase activities were measured with Dual Luciferase Reporter Assay System (Promega, Madison, WI) according to the manufacturer's instructions.

References

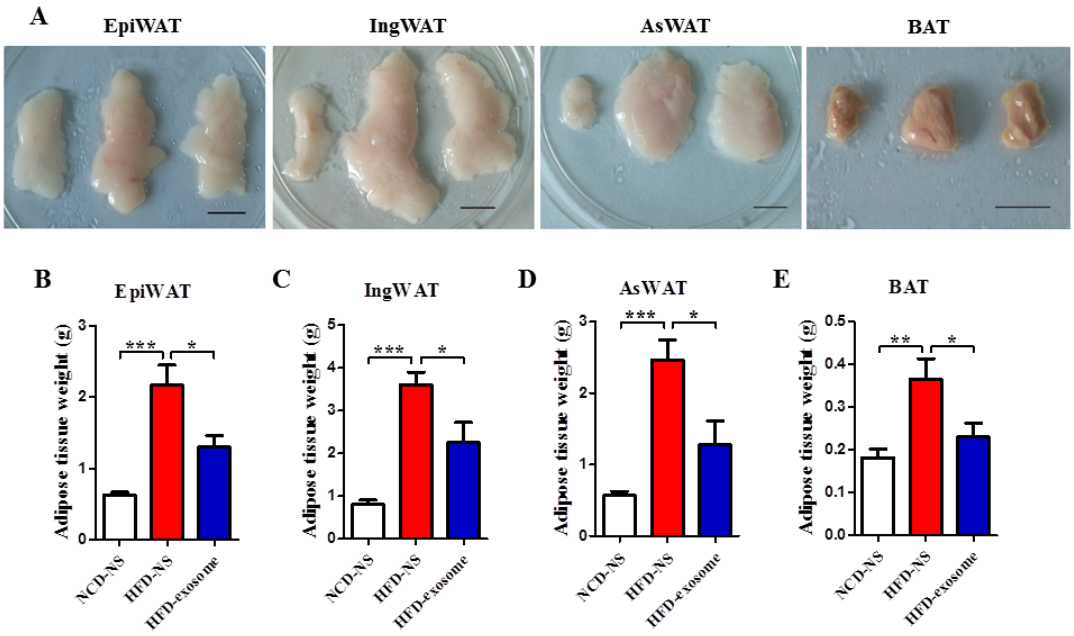
1. Pauleau AL, Rutschman R, Lang R, Pernis A, Watowich SS, Murray PJ: Enhancer-mediated control of macrophage-specific arginase I expression. Journal of immunology 2004;172:7565-7573.

Supplementary Figure 1. Influence of ADSC-derived exosomes on food intake of HFD-fed mice. C57BL/6 male mice (n=6/group) were fed on HFD for 20-26 weeks. During the last 6-8 weeks of HFD feeding, mice were intraperitoneally injected with ADSC-derived exosomes (30 μg per mice, every 3 days); mice treated with NS were used as controls. Daily food intake of the mice was calculated during the intervention.

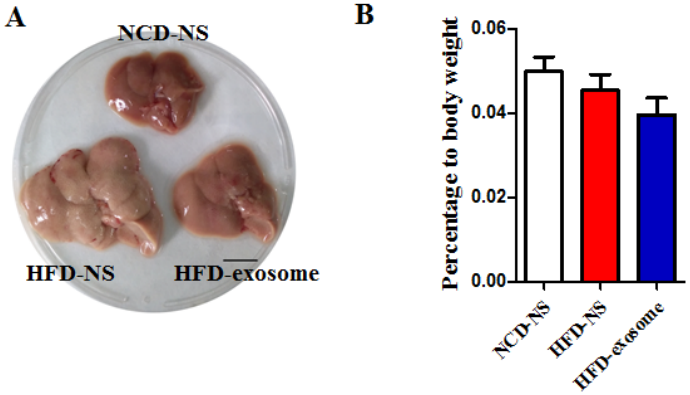


SUPPLEMENTARY DATA

Supplementary Figure 2. Delivery of ADSC-derived exosomes reduces fat weight in HFD-fed mice. C57BL/6 male mice (n=4-6/group) were fed on NCD or HFD for 20-26 weeks, HFD-fed mice were intraperitoneally injected with ADSC-derived exosomes (30 μg per mice, every 3 days) during the last 6-8 weeks; NCD or HFD-fed mice treated with NS were used as controls. *A*: Gross morphology of fat pads. NCD-NS (left), HFD-NS (middle), HFD-exosome (right) in each panel. Scale bar: 1.25cm. *B-E*: Adipose tissue weight of EpiWAT (*B*), IngWAT (*C*), AsWAT (*D*) and BAT (*E*). **P*<0.05, ***P*<0.01, ****P*<0.001.

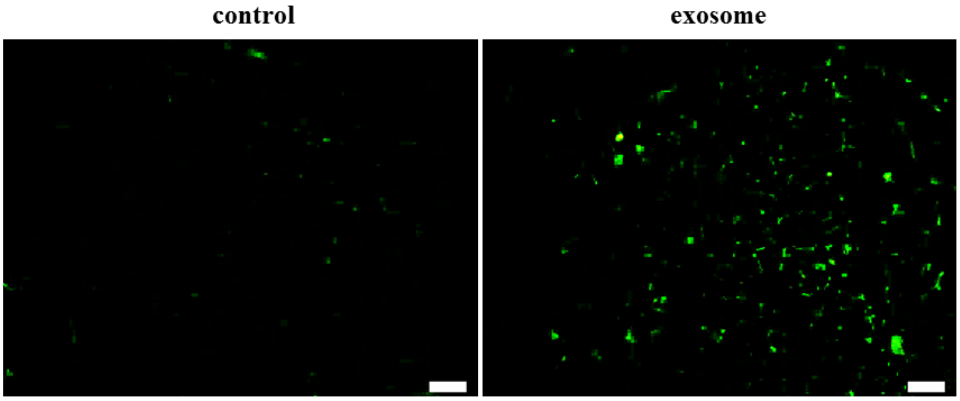


Supplementary Figure 3. Delivery of ADSC-derived exosomes attenuates hepatic steatosis in HFD-fed mice. Mice (n=4-6/group) were treated as described in Supplementary Figure 2. *A*: Gross morphology of liver. Scale bar: 1.25cm. *B*: Percentage of liver weight to body weight.

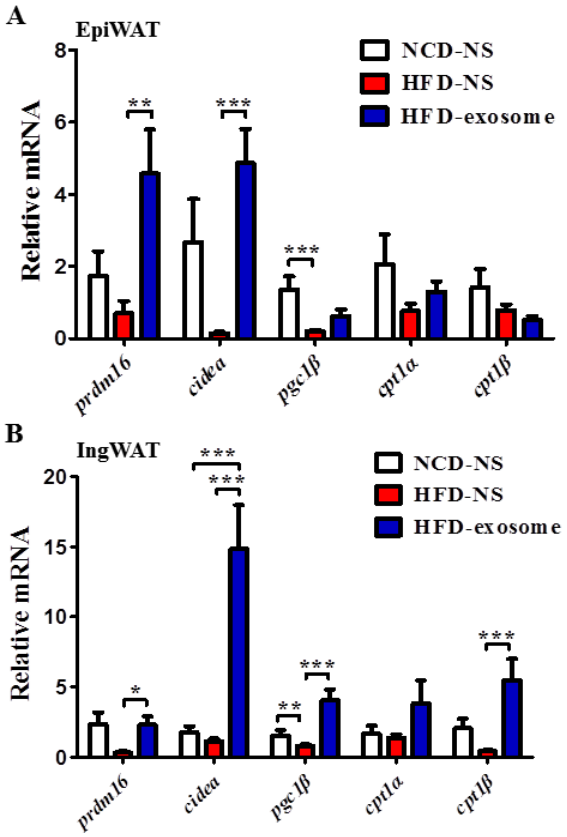


SUPPLEMENTARY DATA

Supplementary Figure 4. ADSC-derived exosomes settle in epididymal adipose tissues of HFD-fed mice. ADSC-derived exosomes labeled with PKH67 were administrated into HFD-fed mice by intraperitoneal injection. After 24 h, epididymal adipose tissues in paraffin sections were detected by fluorescence microscope. Scale bar: 100 μm.

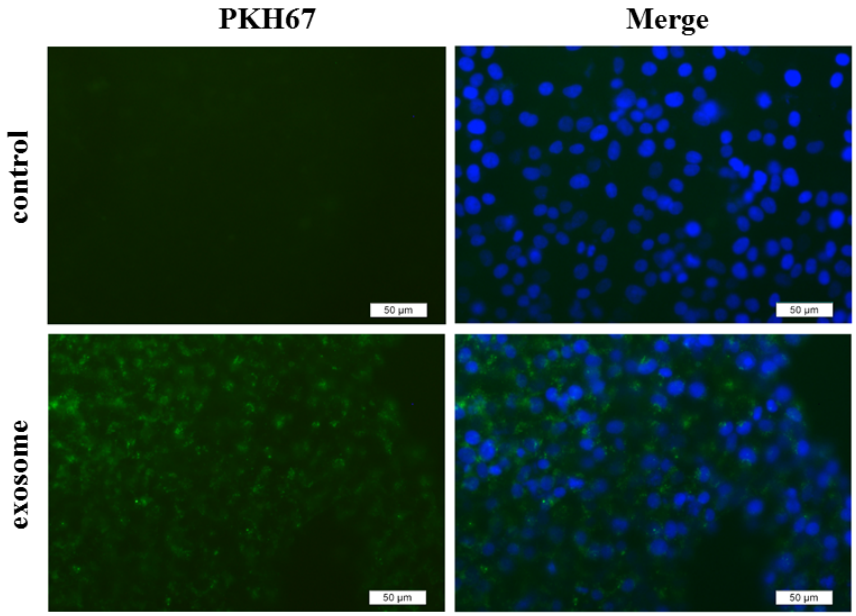


Supplementary Figure 5. Effects of ADSC-derived exosomes on gene expression in WAT from HFD-fed mice. Mice (n=3-6 mice/group) were treated as described in Supplementary Figure 2. *A*: The mRNA levels of *prdm16*, *cidea*, *pgc1β*, *cpt1α*, *cpt1β* in EpiWAT. *B*: The mRNA levels of *prdm16*, *cidea*, *pgc1β*, *cpt1α*, *cpt1β* in IngWAT. **P*<0.05, ***P*<0.01, ****P*<0.001.

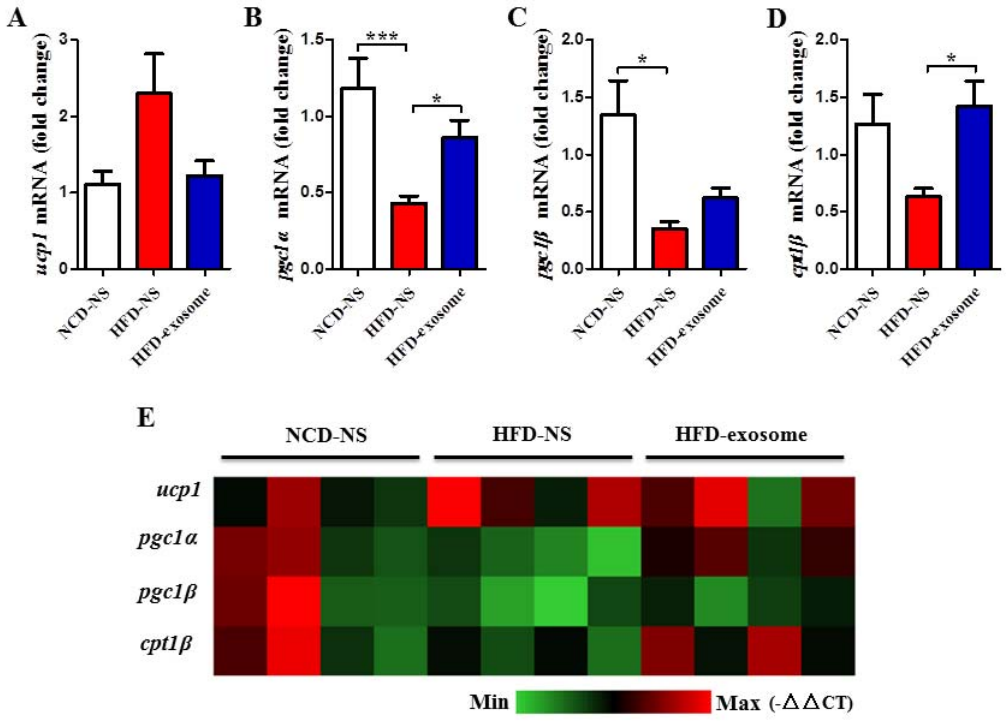


SUPPLEMENTARY DATA

Supplementary Figure 6. Uptake of ADSC-derived exosomes by HEK-293T cells. ADSC-derived exosomes were labeled with PKH67, and co-cultured with HEK-293T cells for 24 h, fluorescence signals were examined by fluorescence microscope. Scale bar: 50 μm.



Supplementary Figure 7. Effects of ADSC-derived exosomes on gene expression in BAT from HFD-fed mice. Mice were treated as described in Supplementary Figure 2. *A-D*: The mRNA levels of *ucp1* (*A*), *pgc1α* (*B*), *pgc1β* (*C*), *cpt1β* (*D*) in BAT (n=3-6 mice/group). *E*: Heat map of gene expression in BAT (n=4 mice/group). **P*<0.05, ****P*<0.001.



SUPPLEMENTARY DATA

Supplementary Table 1. Sequences of siSTAT3

siSTAT3-1	Sense: GUUGAAUUAUCAGCUUAAAdTdT
	Anti-sense:UUUAAGCUGAUAAUUCAACdTdT
siSTAT3-2	Sense: CAUCAAUCCUGUGGUAUAAdTdT
	Anti-sense:UUAUACCACAGGAUUGAUGdTdT
siControl	Sense:UUCUCCGAACGUGUCACGUTT
	Anti-sense:ACGUGACACGUUCGGAGAATT

Supplementary Table 2. Primer pairs for qRT-PCR

arginase-1	Sense: GACCACAGTCTGGCAGTTGG
	Anti-sense: TACGTCTCGCAAGCCAATGT
il-10	Sense: TAGAAGTGATGCCCCAGG
	Anti-sense: TCATTCTTCACCTGCTCCACTGC
inos	Sense: TCCTGGACATTACGACCCCT
	Anti-sense: CTCTGAGGGCTGACACAAGG
tnf-α	Sense: CCAGACCCTCACACTCAGATCATC
	Anti-sense:GCGTAGACAAGGTACAACCCATCG
il-12	Sense: TGTGGAATGGCGTCTCTGTC
	Anti-sense: GGCGGGTCTGGTTTGATGAT
il-6	Sense: AGACTTCCATCCAGTTGCCTT
	Anti-sense: TTCTCATTTCCACGATTTCCC
leptin	Sense:TCAAGCAGTGCCTATCCAGAAAGTC
	Anti-sense:GGGTGAAGCCCAGGAATGAAGTC
ucp1	Sense: ACTGCCACACCTCCAGTCATT
	Anti-sense: CTTTGCCTCACTCAGGATTGG
pgc1α	Sense: CCAGCCTCTTTGCCCAGAT
	Anti-sense: GTCGCTACACCACTTCAATCCA
pgc1β	Sense: TGACGTGGACGAGCTTTCAC
	Anti-sense: GGGTCTTCTTATCCTGGGTGC
prdm16	Sense: GCACCAACAGTTCCTCTCCA
	Anti-sense: ACATCCGTGTAGCGTGTTCC
cidea	Sense: GCCGTGTTAAGGAATCTGCTG
	Anti-sense: TGCTCTTCTGTATCGCCCAGT
tbx1	Sense: CTGTGGGACGAGTTCAATCAG
	Anti-sense: TTGTCATCTACGGGCACAAAG
tmem26	Sense: TGCAGCACCCAATCTGAGAG
	Anti-sense: TAGCAGGAGTGTTTGGTGGAG
cpt1α	Sense: AGATCAATCGGACCCTAGACAC
	Anti-sense: CAGCGAGTAGCGCATAGTCA
cpt1β	Sense: TCTTCTTCCGACAAACCCTGA
	Anti-sense: GAGACGGACACAGATAGCCC

SUPPLEMENTARY DATA

th	Sense: GTCTCAGAGCAGGATACCAAGC
	Anti-sense: CTCTCCTCGAATACCACAGCC
gapdh	Sense: GTGTTTCCTCGTCCCGTAGA
	Anti-sense: ATGAAGGGGTCGTTGATGGC
18s	Sense: GCCTGAGAAACGGCTACCACAT
	Anti-sense: CCGCTCCCAAGATCCAACTACG