Supplemental Information –Hu and Duan et al

SUPPLEMETAL METHODS

Isolation of human AD-MSCs and UC-MSCs

For AD-MSC isolation, adipose aspirates were extensively washed with phosphate-buffered saline (PBS) and mechanically minced into small pieces. The tissue was then digested with 0.075% collagenase I (Invitrogen, CA, USA) at 37°C for 30 min. Next, a high-density stromal vascular fraction (SVF) was collected by centrifugation. The cell pellet was washed with PBS and filtered through a 100-µm nylon mesh to remove cellular debris and a cell count was performed. After plating and incubation overnight at 37°C, the cells were washed with PBS in order to remove non-adherent cells. The cells were maintained at 37°C in a 5% CO_2-containing fully humidified atmosphere in DMEM containing 10% fetal bovine serum, and the medium was changed every 3 to 4 days. When the cells reached nearly 80% confluence, they were harvested and plated into new flasks at a density of 5×10^4/mL. The number of cells and cellular viability were also determined.

For UC-MSC isolation, human umbilical cords were obtained from a vicinal maternity hospital with the informed consent of the parturients. Before the umbilical cord samples were obtained, the donors underwent mandatory screening for human immunodeficiency virus (HIV), syphilis, human papilloma virus (HPV), and hepatitis B virus (HBV). Briefly, after the blood vessels were removed, the Wharton jelly was minced into 1- to 2-mm^3 fragments and then suspended in an animal serum-free MSC growth medium and incubated in a humidified atmosphere with 5% CO_2 at 37°C. The medium was changed every 4 days and tissue fragments were removed 10 days later. Once 80% confluence had been reached, the cells were harvested with 0.05% trypsin-EDTA and re-plated in the new flasks at a density of 5×10^4/mL.

All assays were performed using MSCs between passage 3 and 5, and prior to cell culture, the identity of the MSCs was confirmed by immunophenotypic analysis: 5×10^5cells were harvested to perform flow cytometry analysis for CD105, CD73, CD90, CD45, CD34, and CD19. It was found that MSCs are positive for CD105, CD73, and CD90 (>95%), and negative for CD45, CD34, and CD19 (<2%).

MSCs were cultured in a 24-well plate in complete α-MEM medium supplemented with adipogenic, ostegenic or chondrocytic differentiation-inducing agents (Sigma-Aldrich, MO, USA) at an initial cell density of 1×10^4 cells/well. After 2-3 weeks, the cells were washed twice with PBS and fixed with 4% paraformaldehyde at room temperature for 30 min. Oil-red-O, alkaline phosphatase or toluidine blue staining was applied to detect adipogenic, ostegenic or chondrocytic differentiation.

MSCs were isolated, cultured and characterized as described above in accordance with the Ethics Committee of the Beijing Institute of Radiation Medicine.

Tissue preparation, histological analysis, and immunostaining assays

Hepatic, fatty and pancreatic tissues were fixed in 10% formalin. The tissues were processed, embedded in paraffin, and stained with hematoxylin-eosin (HE).

For double-labeled immunohistofluorescence analysis, the pancreatic tissues were snap-frozen in isopentane cooled in liquid nitrogen, embedded at the optimum cutting temperature, and sectioned on a cryostat at a thickness of 5 μm. Pancreatic sections were incubated with guinea pig polyclonal anti-insulin antibody (Abcam, MA, USA) and rabbit anti-glucagon antibody (Sigma-Aldrich, MO, USA) (1:1,000) for 2 h. The sections were then washed with PBS-containing Tween-20 and incubated with Alexa Fluor 488-conjugated anti-guinea pig IgG and PE-conjugated anti-rabbit IgG (Invitrogen, CA, USA) for 1 h. Subsequently, pancreatic cryosections were stained with DAPI (Vector, Burlingame, CA) at 0.1 mg/mL, and they were observed by fluorescence microscopy.

Western blot analysis

Proteins were extracted from homogenized tissues or lysed cells in RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitors (Complete Mini, Roche). The homogenate and lysis were centrifuged at 4°C for 30 min at 12,000 rpm. The protein concentration of the resultant supernatant was determined using the bicinchoninic acid (BCA) protein assay kit (YuanPingHao Bio, China). Proteins (80 μg) were separated by 10% SDS-PAGE and subsequently transferred onto a PVDF membrane. The membrane was then blocked with 5% non-fat dry milk in TBS/Tween 20 (0.1%, v/v) for 2 h at room temperature and incubated overnight at 4°C with the following primary antibodies (Cell Signaling Technology): anti-AMPK-α, anti-SPK1, anti-ACC1, anti-HSL, anti-phosphorylated

AMPK-α(Thr-172),anti-phosphorylated ACC1(Ser-79), anti-phosphorylated HSL(Ser-600), anti-phosphorylated p70, anti-phosphorylated S6K (Thr-389) and anti-GAPDH. The incubated membranes were washed with TBST and probed with an appropriate horseradish peroxidase-conjugated anti-IgG secondary antibody. The membrane was then washed and developed with a chemiluminescent substrate (ECL Plus). An autoradiograph was then obtained. Assays were performed in triplicate for each experiment.

Real-time PCR

Total RNA was extracted from tissues and cells using RNA isolation kits (Trizol, Invitrogen, CA, USA). The isolated total RNA was reverse-transcribed into cDNA using the qPCR Reverse Transcriptase MIX Kit (Toyobo, OSAKA, Japan). The qPCR reactions were carried out using the Fast SYBR Green PCR Master Mix (Applied BiosystemsInc., CA, USA) on an ABI 7500 fast real-time PCR system (Applied BiosystemsInc., CA, USA). For normalization, threshold cycles (Ct-values) were normalized to GAPDH for each sample to obtain sample-specific ΔCt values (ΔCt= Ct gene of interest - Ct GAPDH). The $2^{-\Delta\Delta Ct}$ values were calculated to obtain the fold expression levels, where ΔΔCt = (ΔCt treatment - ΔCt control). The primer sequences used are listed in Table S2.

SUPPLEMENTAL FIGURES

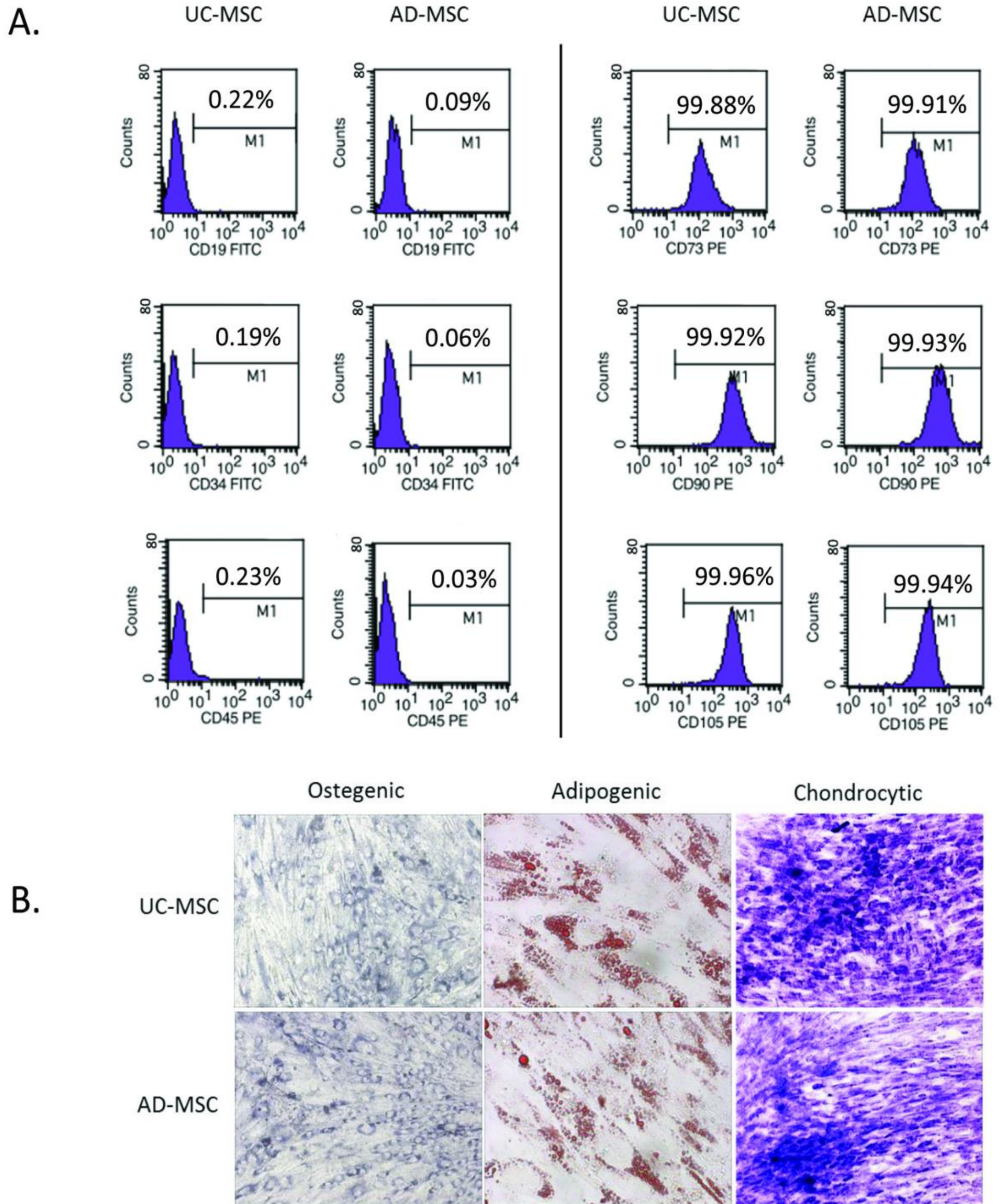

Figure S1: Identification of MSC by cell surface immuophenotype measurement and differentiation ability assay. (A) Flow cytometric analysis of the membrane expressions of CD19, CD34, CD45, CD73, CD90, and CD105 in AD-MSC and UC-MSC. (B) Differentiation capacity of AD-MSC and UC-MSC into adipocytes, osteoblasts, and chondrocytes (×400).

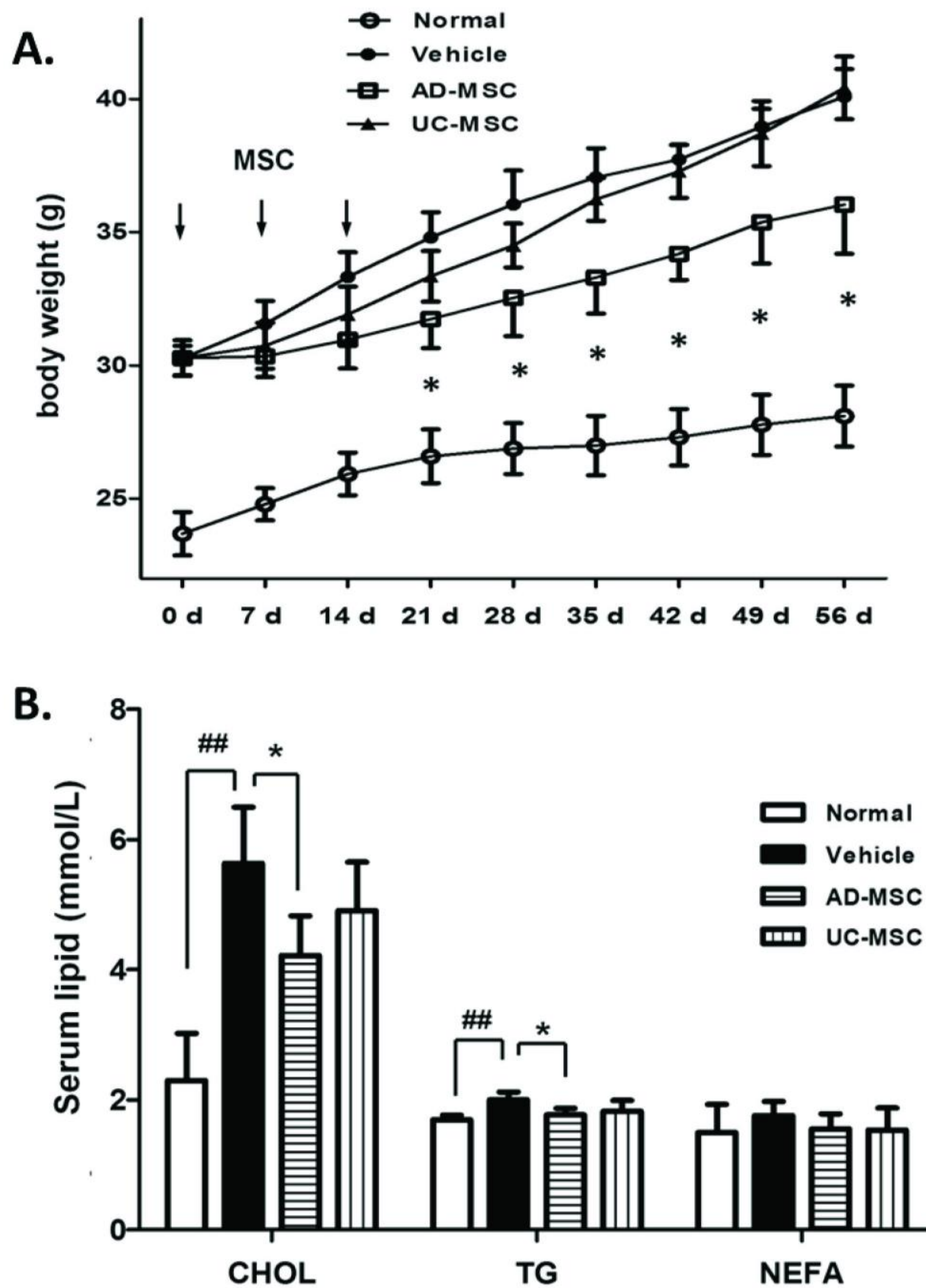

Figure S2: AD-MSC administration suppresses body weight increase and improves serum lipid profiles in DIO mice. (A) Time course of body weights over 8 weeks in normal mice and DIO mice injected i.p. with AD-MSC and UC-MSC. The cells were injected for three times as indicated. (B) Serum TG, CHOL, and NEFA levels in indicated groups. Results are means±SEM (n=8 per group). *p<0.05 vs vehicle group. ##p<0.01 vs normal group.

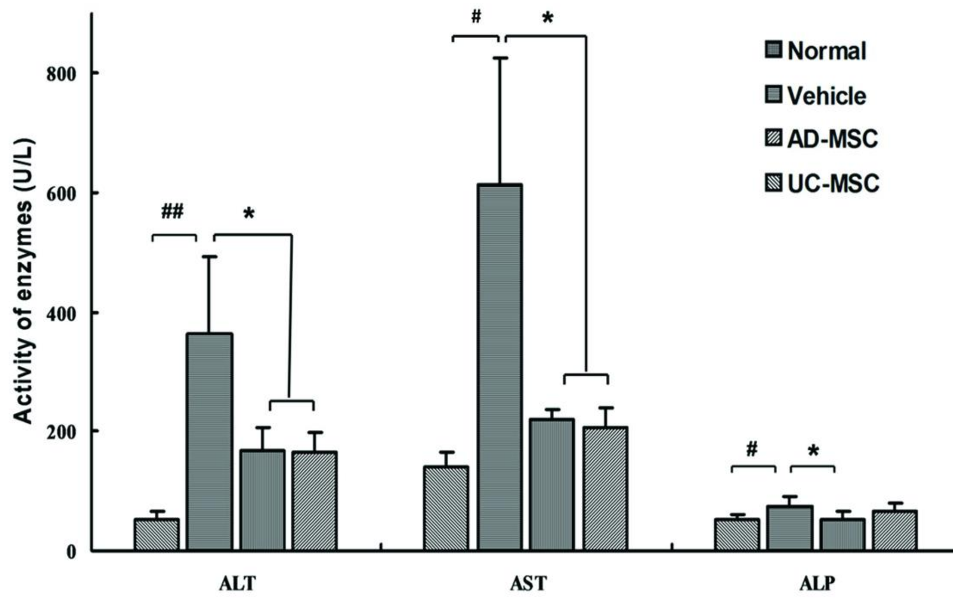

Figure S3: MSC administration repairs hepatic injuries in db/db obese mice. Serum AST, ALT, and ALP activities in normal mice and db/db mice treated with AD-MSC and UC-MSC at the endpoint of the test. *p<0.05 vs vehicle group. ##p<0.01 vs normal group.

Supplemental Tables

Table S1. Characteristics of AD-MSC donors

		Donor 1	**Donor 2**	**Donor 3**
Physical characteristics	**Sex**	Male	Female	Male
	Age (yrs)	26	22	54
	Height (cm)	176	165	171
	Weight (kg)	76	59	74
	BMI (kg/m^2)	24.5	21.7	25.3
Clinical characteristics	**CHOL(mmol/L)**	5.18	4.98	4.85
	TG(mmol/L)	1.34	1.54	1.73
	HDL-C (mmol/L)	1.22	1.46	1.55
	LDL-C(mmol/L)	2.16	2.85	2.39
	Virus(HIV/HBV/HCV)	None	None	None
	Located	Subcutaneous	Subcutaneous	Subcutaneous

Note: All the three AD-MSC donors are from the Chinese PLA General Hospital in Beijing.

Table S2. Primer list

Gene	Forward primer	Reverse primer
mUCP-1	ACT GCC ACA CCT CCA GTC ATT	CTT TGC CTC ACT CAG GAT TGG
mPredm16	CAG CAC GGT GAA GCC ATT C	GCG TGC ATC CGC TTG TG
mPGC-1α	CCC TGC CAT TGT TAA GAC C	TGC TGC TGT TCC TGT TTT C
mGAPDH	GTC TCC TGC GAC TTC AACA	GGT GGT CCA GGG TTT CTTA