

Supplementary Materials

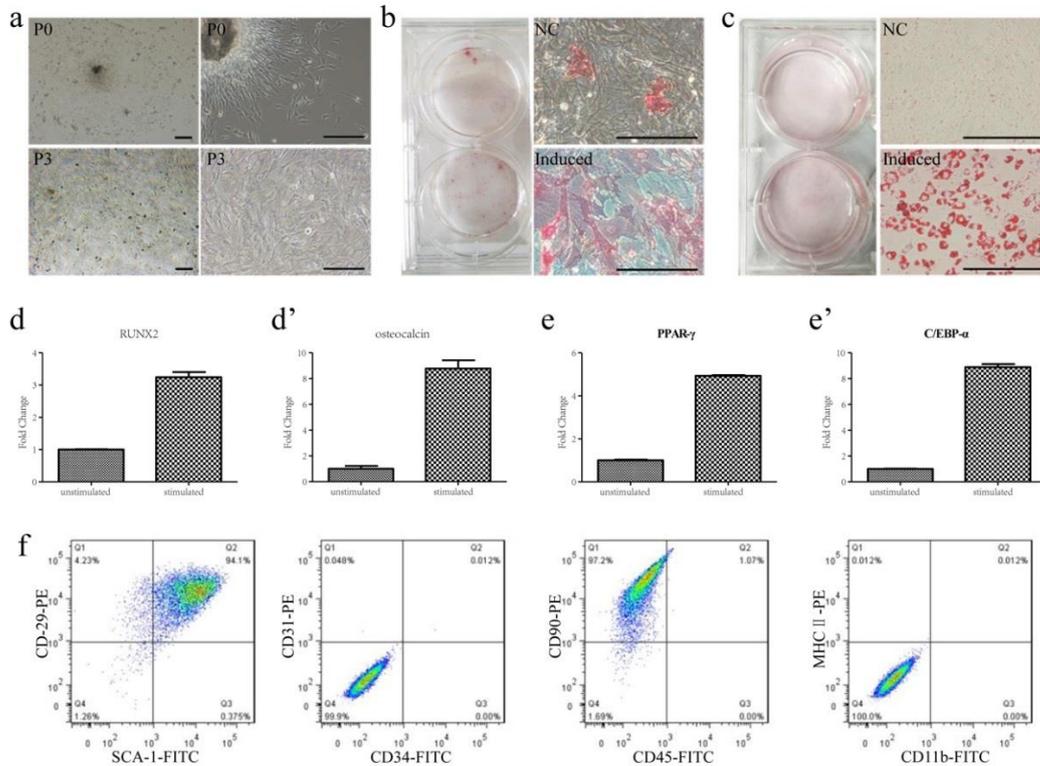
Supplementary material 1

Methods for Isolation and identification of MSCs from murine compact bones

Murine MSCs were isolated from 1-week-old C57BL/6 (H2b) compact bones following a previous protocol [22]. Long bones of 1-week-old C57BL/6 (H2b) mice were dissected and the bone marrow was flushed out. The compact bones were digested with 1% Type II collagenase (Sigma-Adrich) for 2 hrs, and the digested bone debris were cultured in alpha-minimal essential medium (α -MEM, Gibco) containing 10% fetal bovine serum (FBS, StemCell Technologies) supplemented with 1 U/ml penicillin (Sigma-Adrich) and 1 μ g/ml streptomycin (Sigma-Adrich) in a humidified atmosphere of 37 °C, 5% CO₂ incubator. The fresh medium was added to the tissue fragments till 72 hrs, and the adherent cells were detached using 0.25% trypsin-EDTA (Sigma-Adrich) digestion, and subcultured at ratio of 1:3 to passage every 2~3 days. The adherent cells isolated at passages 3~5 were used for in vivo experiment.

Supplementary material 2

Isolation and identification of MSC from murine compact bones



The adherent cells isolated from the bone compact at passages 0 (up) and passage 3 (down) were spindle shaped (a). The alkaline phosphatase staining to test the ALP activity (Sigma-Adrich) and the osteogenesis of MSCs (b), with the induced-differentiated (down) and the spontaneous-differentiated NC results (up). (c) shows the adipogenesis of MSC, with oil red O (Sigma-Adrich) staining for lipid droplets to test the induced-differentiation results (down) and the spontaneous-differentiation results (up). The real-time quantitative PCR detecting the expression of RUNX2 (d), osteocalcin (d'), PPAR- γ (e) and C/EBP- α (e'), exhibit the osteogenic differentiation capability and adipogenic differentiation capability of MSCs. The MSC cell surface markers were detected by flowcytometry analysis in (f). MSCs were positive for CD29, SCA-1 and CD90, but negative for CD31, CD34, CD45, MHC-class II and CD11b. Scale bars: a-c, 200 μ m.

Supplementary material 3

The qRT-PCR primers sequence

Primer	Sequence
Gapdh top	GGAGACAACCTGGTCCTCAG
Gapdh bottom	ACCCAGAAGACTGTGGATGG
TGF- β 1 top	CGGTGCTCGCTTTGTA
TGF- β 1 bottom	GCCACTCAGGCGTATC
Collagen I (Coll a2) top	CTTGTGGCTTCTGACTATCT
Collagen I (Coll a2) bottom	AGGAAAATGAGGCTGTTA
IFN- γ top	CTGGTGGACCACTCGGATGA
IFN- γ bottom	TTACTACCTTCTTCAGCAACAGCAA
TNF- α top	TTCTCATTCTGCTTGTGG
TNF- α bottom	TTGGGAACTTCTCATCCCT
mmu-miR-148a-3p top	ACACTCCAGCTGGGTGTCAGTTTGTC AA
snoRNA202 top	ACACTCCAGCTGGGGCTGTACTGACTTGATG
URP	TGGTGTCGTGGAGTCG

Supplementary material 4

The stem-loop RT primer sequence

stem-loop RT primer	Sequence
mmu-miR-148a-3p RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGA GTGGGGTAT
snoRNA202 RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGA GCATCAGAT

Supplementary material 5

Statistics of p value between MP group and DM group, n=20

Day	p blood glucose	p body weight
14 th	0.014	0.621
21 st	<0.0001	0.031
28 th	0.0096	0.02767
35 th	0.010473	0.05385
42 nd	0.003497	0.02893
49 th	0.036424	0.007234
56 th	<0.0001	0.000648