Supplementary materials

Materials and methods

Isolation and cultivation of BMSCs

For BMSCs isolation, the hind limbs of Wistar rats (200–250 g, n = 7) were dissected and maintained in PBS containing 5% (v/v) penicillin and streptomycin under sterile conditions. After removal of the musculature and connective tissue, the femur and tibia were rinsed with PBS, and their epiphyses were carefully cut with a bone cutter. The bone marrow was flushed using a 10 mL syringe containing PBS and then centrifuged for 5 min at 1,500 rpm at room temperature. The pellet was suspended in PBS, filtered through 40-μm filters, and then re-centrifuged at 1,500 rpm for 5 min. Next, the pellet was suspended in 10 mL proliferation medium and cultured in a 100-mm culture dish. Half of the medium was changed every 3 days. The cells were passaged when they reached ~90% confluence.

Western blot

Proteins (20 μg/ lane) were fractionated by SDS-PAGE and electrotransferred to a polyvinylidene difluoride membrane. These blots were first incubated for 1 h in a blocking buffer consisting of 0.1% Tween-20 (Sigma) and 5% nonfat powdered milk, and then incubated with primary antibody against NGF (Abcam, Cambridge, UK) at 4◦C overnight. A horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Proteintech, Chicago, USA) was used, and antigen-antibody complexes were detected by chemiluminescence using the BeyoECL Plus kit (Bi Yuntian, Shanghai, China).

Supplementary Figure 1: Isolation and cultivation of BMSCs. (a) After culture for 1 week, the spindled-shaped adherent cells were observed and formed cell colonies. (b) BMSCs at passage 2 (BMSCs-P2) showed a typical fibroblast-like morphology and were arranged in swirl. (c) BMSCs at passage 7 (BMSCs-P7) partially changed to a flattened and spread out morphology. Scale bar = 200 μm ((a), (c)), 100 μm (b).

Supplementary Figure 2: Identification of neural crest-derived DPCs. ((a), (b)) Immunofluorescent cytochemical staining revealed expression of NCSCs-specific markers in DPCs. After passage, they were negative for Sox10 (a), but were positive for P75 (a) and Nestin (b). Nuclei were stained with Hoechst 33342. Scale bar = 100 μm (a), 50 μm (b).

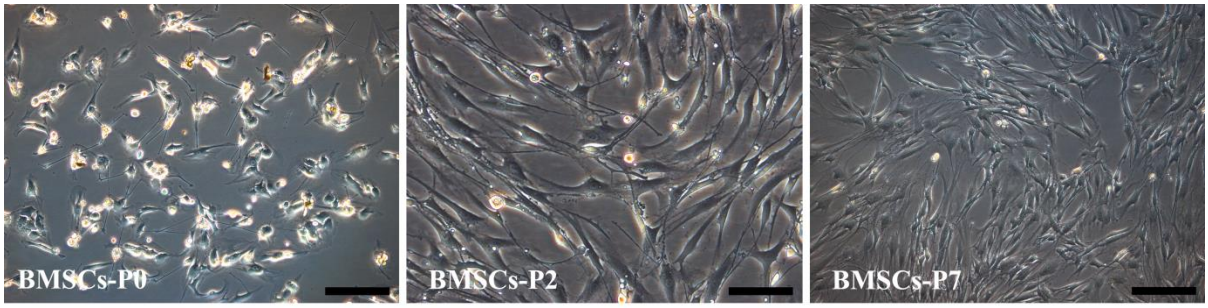
Supplementary Figure 3: Mesenchymal stem cell phenotype and multipotential differentiation assay of BMSCs. ((a)–(d)) BMSCs exhibited a mesenchymal stem cell phenotype. Immunofluorescent staining demonstrated that BMSCs expressed CD44 (a), CD90 (b), did not express CD31 (c). Flow cytometry analysis further demonstrated a high positive expression rate of CD44 and CD90, as well as a low positive expression rate of CD31 (d). ((e)–(h)) Multipotential differentiation capacity of BMSCs. (e) Adipogenic differentiation of BMSCs. The intracellular lipid droplets were formed after induction for 2 weeks and were detected by Oil-Red O staining. (f) Osteogenic differentiation of BMSCs. Calcium nodules were formed after induction for 2 weeks and detected by Alizarin Red-S staining. (g) SMC differentiation of BMSCs. A proportion of the BMSCs were positive for α-SMA after induction for 1 week. (h) Neuronal differentiation of BMSCs. A proportion of BMSCs (white arrow)

exhibited a neuron-like morphology and were positive for neuron-specific β3-tubulin after induction for 1 week. Scale bar = 50 μm ((a)–(c), (f)), 20 μm ((e), (g), (h)).

Supplementary Figure 4: Western blot revealed expression of NGF in DPCs. (a) By western blot analysis, a higher expression level of NGF was detected in DPCs compared with BMSCs. (b) The relative expression to GAPDH was 0.41 ± 0.07 (DPCs) and 0.26 ± 0.06 (BMSCs), respectively. $*P < 0.05$.

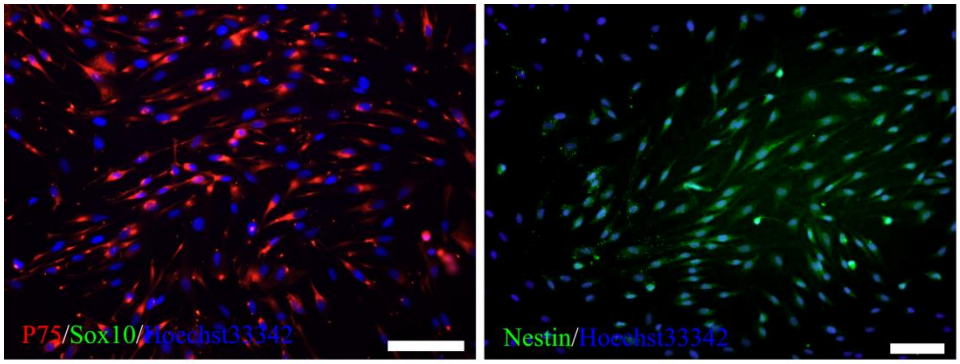
Supplementary Figure 5: BMSC-CM stimulated proliferation of PC12 cells. ((a), (b)) Phase contrast images of PC12 cells cultured for 15 days in BMSC-CM (a) and DPC-CM (b). PC12 cells proliferated actively in BMSCs-CM (a), by contrast, PC12 cells morphologically differentiated into neuron-like cells in DPCs-CM (b). Scale bar=20μm.

Supplementary Figure 1



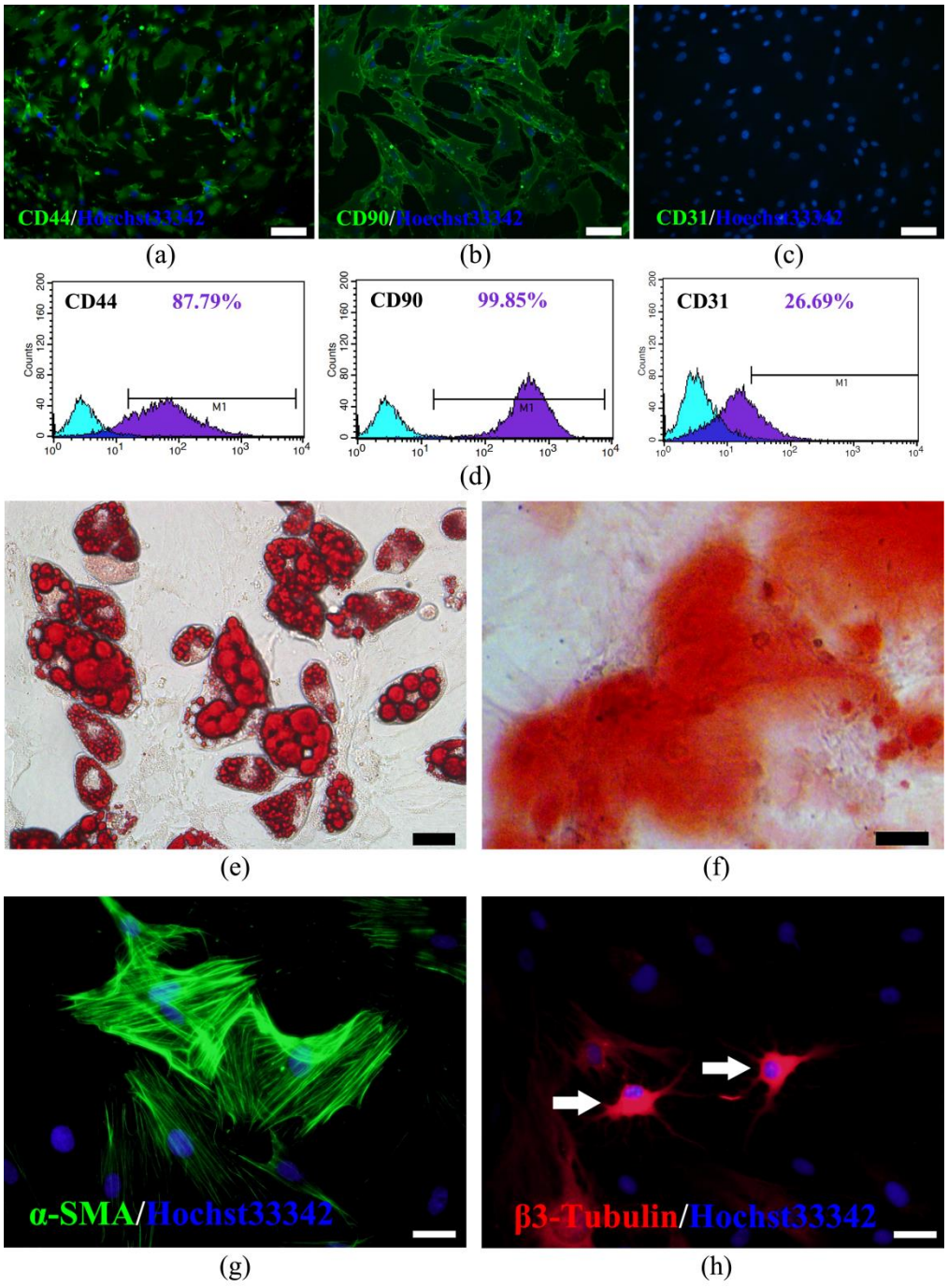
(a) (b) (c)

Supplementary Figure 2

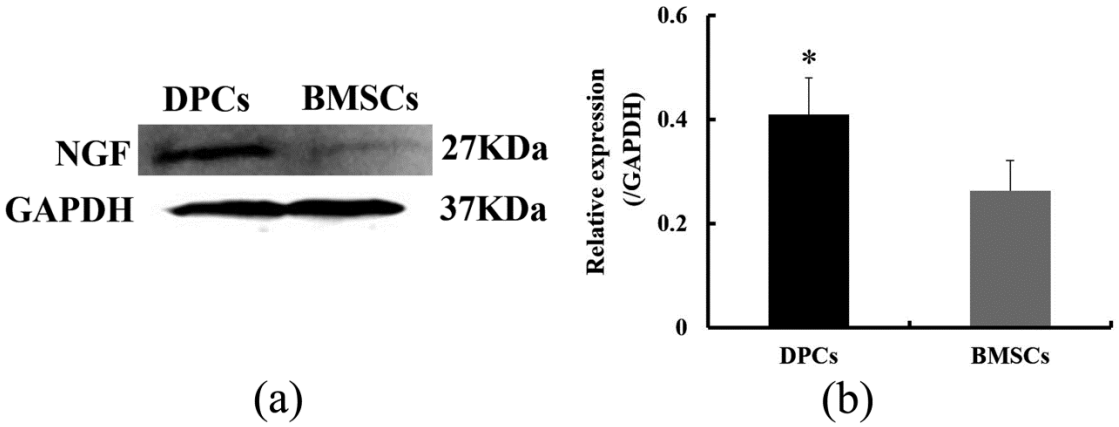


(a) (b)

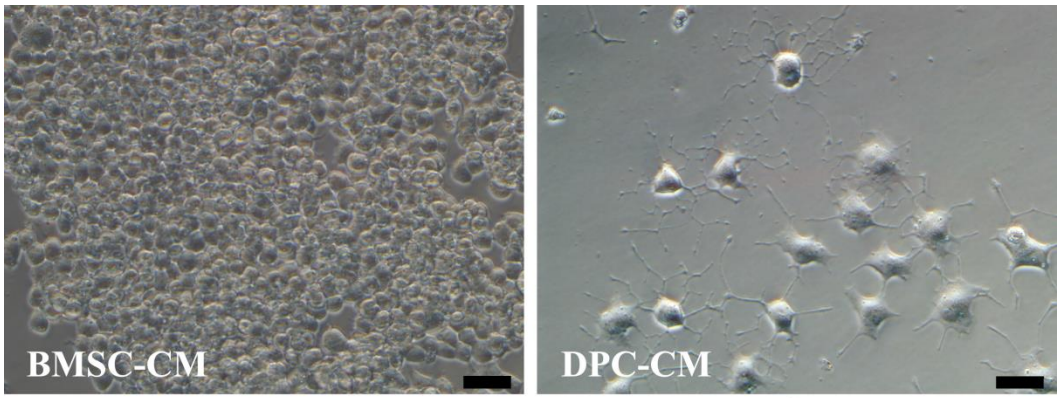
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



(a) (b)