

Supplemental Figures

The characteristics of BMMSCs

To confirm the characteristics of the BMMSCs in our system, we cultured the BMMSCs with a standard method. After 1 week of primary culture, BMMSCs adhered to culture dishes and exhibited polygonal shapes with limited spreading areas (Fig.S1A). The passage 2 BMMSCs displayed as long spindle-shaped fibroblastic cells with large nucleus and abundant cytoplasm (Fig.S1A). The passage 3 cells principally formed bipolar spindle-like cells, which were consistent with typical morphology (Fig.S1A). When the confluence reached 90%, cells exhibited as spiral shape (Fig.S1A). These cells were used in our following experiments. Both flow cytometry and immunofluorescence staining analyses showed that BMMSCs at passage 3 were strongly positive for BMMSCs markers, such as CD44, CD73 and CD90, and negative for CD34 and CD45 (Figure S1B and C). Furthermore, the isolated BMMSCs displayed the potential to differentiate into adipogenic and osteogenic lineages after treatment with the respective induction factors. Cells induced with adipogenic medium contained numerous Oil-Red-O-positive lipid globules at the end of 2 weeks (Fig. S1D). Expression of adipocytic makers, such as AP2, PPAR γ 2, and C/EBP α was evidenced (Fig. S1E). Similarly, dense cell packing and calcium deposits stained by Alizarin red were found in osteogenic BMMSCs after 3 weeks of cultivation (Fig. S1D). Expression of osteoblastic makers RUNX2 and Osteocalcin were confirmed (Fig. S1E). Together, our results demonstrated that the BMMSCs used in current study were indeed multipotent and responsive to differential stimuli.

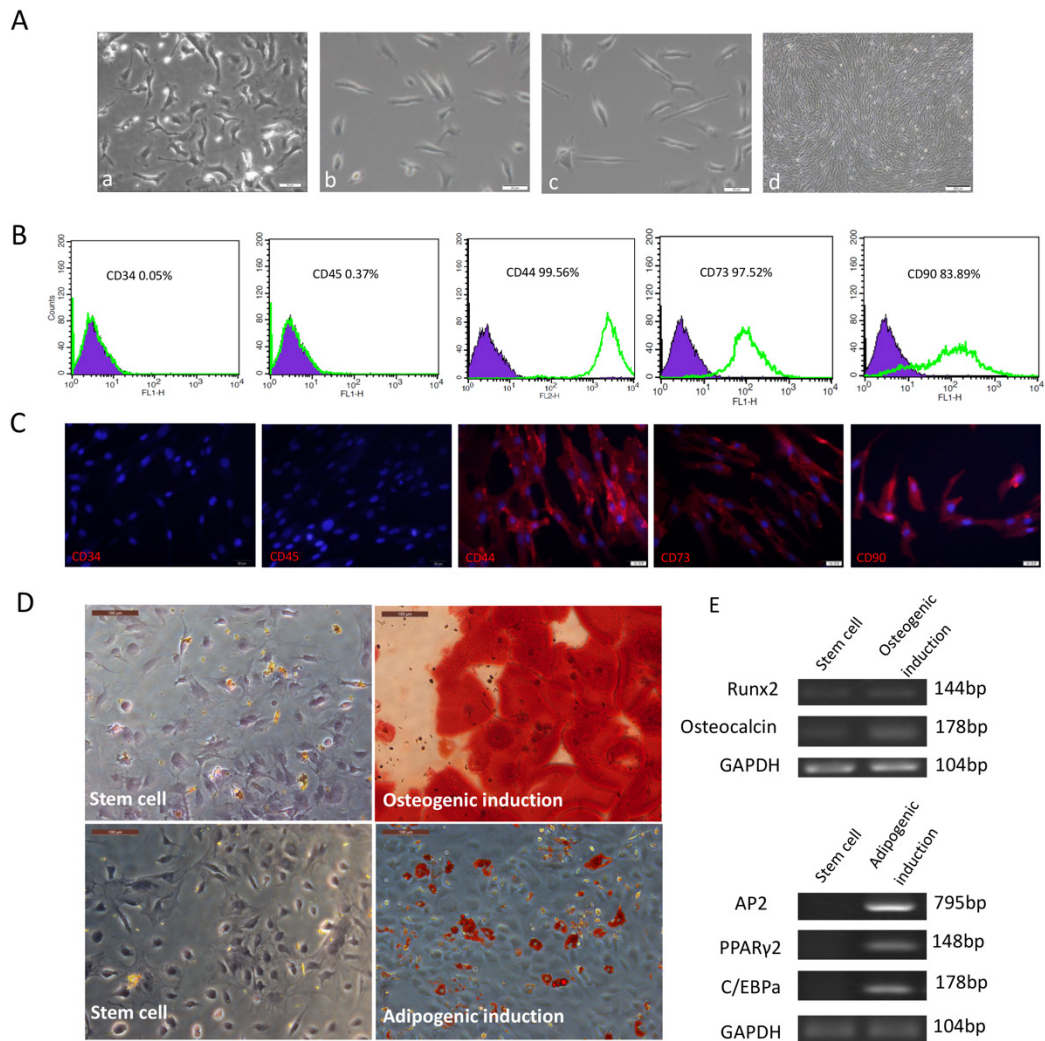


Fig. S1 Characteristics of BMMSCs. (A) Morphological appearance of BMMSCs. (a) Primary BMMSCs were isolated from mouse bone marrow and cultured in expansion medium for 1 week. (b) The 2nd passage BMMSCs were long spindle-shaped fibroblastic cells. (c) The 3rd passage BMMSCs formed bipolar spindle-like cells. (d) Cells form spiral in structure. Scale bar = 20 μ m. (B, C) Cell surface markers are identified in MSCs at passage 3. BMMSCs were fixed and stained with anti-CD34, CD44, CD45, CD73, and CD90 PE linked antibodies and analyzed with (B) flow cytometry analysis and (C) Immunofluorescence. (D) Differentiation of BMMSCs into adipocytes and osteogenic cells. Osteogenesis was detected by the formation of calcium deposits stained with Alizarin red after 3 weeks of cultivation. Adipogenesis was detected with Oil Red O after 2 weeks. (E) Osteogenic markers RUNX2, Osteocalcin and adipogenic markers AP2, PPAR γ 2 and C/EBP α were analyzed by PCR. Scale bar = 100 μ m.