Supplementary materials

cAT-MSCs isolation and culturing

Canine adipose tissue was obtained from a healthy dog <1-year-old under a protocol approved by the IACUC of Seoul National University (SNU; protocol no. 170724-6 ). The tissue was washed three times with PBS (PAN Biotech) containing penicillin and streptomycin, and then cut into small pieces and digested for 1 h at 37°C with collagenase type IA (1 mg/mL; Sigma-Aldrich). Enzymatic activity was inhibited with Dulbecco’s Modified Eagle’s Medium (DMEM; PAN Biotech) containing 10% fetal bovine serum (FBS; PAN Biotech). After centrifugation at 1200 ×g for 5 min, the pellet was filtered through a 100-µm Falcon cell strainer (Fisher Scientific, Waltham, MA, USA) to remove debris and then incubated in DMEM containing 10% FBS at 37°C in a humidified atmosphere of 5% CO₂. After 48 h, the cultures were washed with PBS to remove non-adherent cells and incubated with fresh medium, which was changed every 48 h until cells reached 70–80% confluence, after which they were repeatedly subcultured under standard conditions. Isolated cAT-MSCs were used at passage 3–4 for the following experiments.

Characterization of cAT-MSCs

Isolated cells were characterized for the expression of stem cell markers by flow cytometry using fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated antibodies against the following proteins: CD29-FITC, CD31-FITC, CD34-PE, and CD73-PE (BD Biosciences) and CD44-FITC, CD45-FITC, and CD90-APC (eBiosciences). Cells were analyzed with a FACSARia II system (BD Biosciences). Cellular differentiation was evaluated using the StemPro Adipogenesis Differentiation, StemPro Osteogenesis Differentiation, and StemPro Chondrogenesis Differentiation kits (all from Gibco/Life Technologies, Carlsbad, CA, USA).
according to the manufacturer’s instructions followed by Oil Red O staining, Alizarin Red staining, and Alcian Blue staining, respectively.