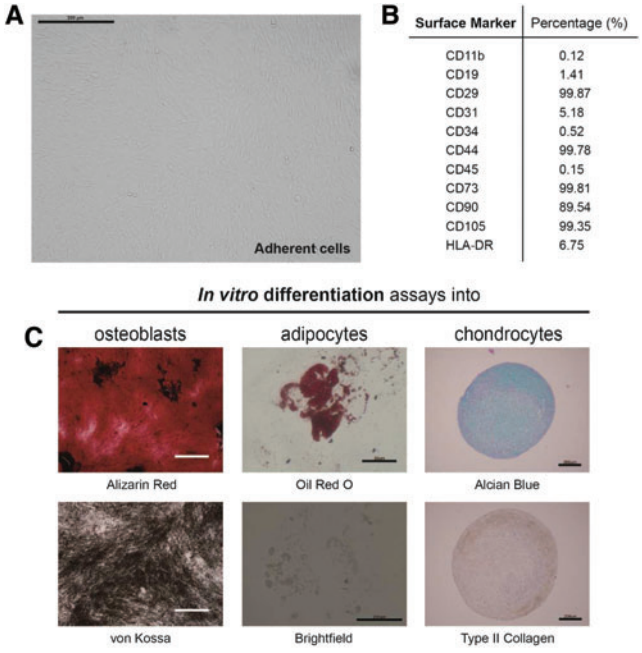
Supplementary Data

Mesenchymal Stem/Stromal Cell Characterization

Mesenchymal stem/stromal cell (MSC) characterization was performed according to Dominici *et al.*[1] Results are summarized in Supplementary Figure S1. MSCs showed a plastic-adherent behavior when maintained under standard culture conditions using conventional tissue culture flasks (Supplementary Fig. S1A). The analysis of cell phenotype was performed using flow cytometry. The cells' potential to differentiate into chondrocytes, adipocytes, or osteoblasts under appropriate stimuli was tested.



SUPPLEMENTARY FIG. S1. Mesenchymal stem/stromal cell (MSC) characterization **(A)** microscopic images taken of adherent MSCs during monolayer expansion in tissue culture flasks (bar represents 200 μm); **(B)** Flow cytometry data containing the percentage of cells positive for the indicated cell surface markers; **(C)** Images of the *in vitro* differentiation into the osteogenic (Alizarin red and von Kossa stainings–bars represent 300 μm), adipogenic (Oil red O staining and brightfield images–bars represent 50 and 200 μm, respectively), and chondrogenic lineages (alcian blue staining and immunohistochemical staining against type II collagen–bars represent 200 μm).

Phenotypic Analysis

MSC phenotype regarding the expression of surface markers was confirmed with fluorescence-activated cell sorting (FACS) analysis, as depicted in Supplementary Fig. S1B.

Briefly, the cells were fixed with a 3.7% paraformaldehyde solution. Subsequently, the cells were stained with specific antibodies for 20 min at 4°C in the dark. A total of 10,000 events were acquired in a Flow Cytometer (FACSCalibur; BD Biosciences) and analyzed with CellQuest Pro software (BD Biosciences). The following antibodies were used: CD11b-FITC (eBioscience), CD19-APC (Miltenyi Biotec), CD29-APC (BD), CD31-FITC (Chemicon), CD34-FITC (Milteny Biotec), CD44-PE (BD Biosciences), CD45-FITC (BD Biosciences), CD73-APC (Miltenyi Biotec), CD90-FITC (Chemicon), CD105-APC (Miltenyi Biotec), and HLA-DR-APC (Miltenyi Biotec). Baseline for positive cells was set via staining with the corresponding isotype control antibodies: IgG1-FITC (BD Biosciences; for CD31, CD45 and CD90), IgG2a-FITC (Milteny Biotec; for CD34), IgG1-PE (BD Biosciences; for CD44), IgG1-APC (BD Biosciences; for CD19, CD29, CD73, and CD105), and IgG2a-APC (R&D; for HLA-DR).

MSC Differentiation Assay

To demonstrate their differentiation potential, MSCs were seeded on to flat-bottomed 24-well plates (30,000 cells/cm^2) and incubated in StemPro osteogenic or adipogenic differentiation medium (Gibco) for 3 weeks. Alternatively, MSCs were grown as cell pellets (1×10^6 cells/pellet/mL) in the chondrogenic medium previously used in the present work (see Materials and Methods section). Osteogenic differentiation was demonstrated with Alizarin red and von Kossa staining, adipogenic differentiation with brightfield microscopy and Oil red O staining, and chondrogenic differentiation with alcian blue and type II collagen immunohistochemical staining, as represented in Supplementary Figure S1C.

Reference

1. Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F.C., Krause, D.S., Deans, R., Keating, A., Prockop, D.J., and Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy **8,** 315, 2006.