

Supplemental Appendix 1 Cell culture

MSCs were isolated from human fetal BM as described previously.^{4,37} Briefly, fetal BM was obtained from aborted fetus (gestational age: 14 to 22 weeks) after receiving informed consent from the patients according to the academic guidelines on the use of human subjects in research. Mononuclear cells were separated by a Ficoll-Paque gradient centrifugation (specific gravity 1.077 g/mL; Nycomed Pharma AS, Oslo, Norway) and depleted of hematopoietic cells using magnetic-activated cell separation (MACS) CD45, GlyA, and CD34 micromagnetic beads (Miltenyi Biotec, Auburn, CA). Flow cytometry analysis (Becton-Dickinson, San Jose, CA, USA) indicated that after negative selection over 95% of the remaining cells were CD45⁻GlyA⁻CD34⁻. To ensure single cell originality of each cell colony, sorted cells were plated at concentrations of 1 cell/well by limiting dilution in a total of 96 × 12 wells coated with ECM gel (Sigma, St. Louis, MO, USA). Culture medium was DF12 containing 40% MCDB medium (Sigma), 2% fetal calf serum (FCS; Gibco Life Technologies, Paisley, UK), 1 × insulin transferrin selenium (Gibco), 10⁻⁹ M dexamethasone, 10⁻⁴ M ascorbic acid 2-phosphate, 10 ng/mL epidermal growth factor, 10 ng/mL platelet-derived growth factor BB, 5 ng/mL VEGF, 50 ng/mL Flt-3 ligand (Sigma), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco) at 37°C and a 5% CO₂ humidified atmosphere. Wells with single adherent cell were identified during the first 24 hours. Culture media were changed every 4-6 days. Single colony was harvested by trypsinization and culture-expanded. The main immunophenotype of the clonal cells was persistently CD34, CD45, CD31, von Willebrand factor (vWF), GlyA, CD11a, CD11b negative, and Flk1 positive for more than 50 cell doublings, consistent with our previous report.^{4,37} So we termed them Flk1⁺ MSCs.