

## **Supplementary Methods**

### **Transduction and sorting of the expanded MSCs**

Mesenchymal cells from passage 1 were transduced with an HIV-1-derived lentiviral vector ( $1 \times 10^6$  infectious particles/mL) expressing the enhanced green fluorescent protein (EGFP) under the control of the cytomegalovirus promoter in medium containing 4 mg/mL polybrene. Cells were incubated with the vector overnight at 37°C, washed with PBS and replenished with new medium. When MSCs reached ~80% confluence, the cells were trypsinized and washed twice with medium. EGFP-expressing mesenchymal cells were sorted into 96-well plates at one cell per well using a FACSAria (Becton Dickinson, Heidelberg, Germany) and incubated overnight at 37°C. On day 1 post-sorting each well was monitored for GFP-positive cells under a fluorescence microscope, and wells containing more than one cell were excluded from the study. Wells containing single cells were monitored and counted every 3 days, and those that showed growth after 16-35 days in culture were trypsinized, counted using a hemacytometer, and further passaged at  $2 \times 10^3$  cells/cm<sup>2</sup>.

### **Clonogenic and expansion potential of CD271-MSCs derived clones**

Single-cell sorted CD271-MSCs and PA-MSC in 96-well plates were assessed for their potential to form CFU-Fs (Colony Forming Unit-Fibroblast) after 14-21 days in culture with DMEM low-glucose supplemented with 10% MSC-qualified fetal bovine serum. To evaluate clonogenic potential of both types of MSCs we compared the number of single-sorted CD271-MSCs with the number of single-sorted PA-MSCs that were able to give rise to CFU-Fs. To obtain a greater number of cells from clonally derived CFU-Fs, MSCs of both types were trypsinized at definite time-points and further expanded in tissue culture flasks.

### **Determination of the proliferation potential of MSC-clones**

To determine the proliferation potential of the clones we analyzed a total 111 MSC-derived clones from three bone marrow donors: 64 clones were derived from CD271-MSC and 47 clones were derived from PA-MSCs.

To estimate the expansion potential of CD271-MSC, the population doubling (PD) and doubling time (DT) were determined by means of the following equations: PD for each subculture =  $(\log_{10} N_H - \log_{10} N_I) / \log_{10} 2$ ; whereby  $N_H$  is the number of cells harvested and  $N_I$  is the number of inoculated cell. This number shows the number of cell doublings from first day of cell seeding until the day of cell harvest.

Doubling time (DT) is the time within which the cells duplicate themselves and is calculated using the following formula:  $DT = \log_2 \times t / (\log N_H - \log N_I)$ , where  $t$  is time in culture,  $N_H$  is the number of cells harvested and  $N_I$  is the number of inoculated cell.

### **Immunocytochemistry of MSC- derived clones**

To assess the expression of tissue-specific antigens on clonally derived GFP-positive MSCs, the CFU-Fs were washed twice with PBS and fixed with 4% paraformaldehyde for 15 minutes. The paraformaldehyde was removed by two washes with PBS, and the cells were permeabilized with 0.3% triton/PBS solution for 10 min. The CFU-Fs were washed twice with PBS and stained for 30 min with the following fluorochrome-conjugated mouse anti-human antibodies at a dilution of 1:20: a phycoerythrin (PE)-conjugated antibody against a typical MSC-antigen CD73, against the novel stromal marker CD9 and a PE-conjugated anti-CD31 antibody (BD Pharmingen, Heidelberg, Germany). The slides were washed three times with PBS and then examined under the microscope. To assess whether any CFU-Fs contain cells of endothelial origin, cells were fixed and permeabilized as described above, and endothelial-like colonies were incubated overnight at +4°C with a primary mouse anti-human von Willebrand antibody (dilution 1:20) (Acris, Herford, Germany). Next day, the colonies were washed twice with PBS and incubated with the secondary Cy3- conjugated goat anti-mouse antibody at a dilution 1:500 (Acris, Herford, Germany) for 60 minutes at room temperature. Unbound antibodies were removed by washing the wells three times with PBS, and immunostaining was evaluated using

an Olympus IX71 microscope equipped with Soft Imaging System F-View II camera and cellSens Dimension imaging software.

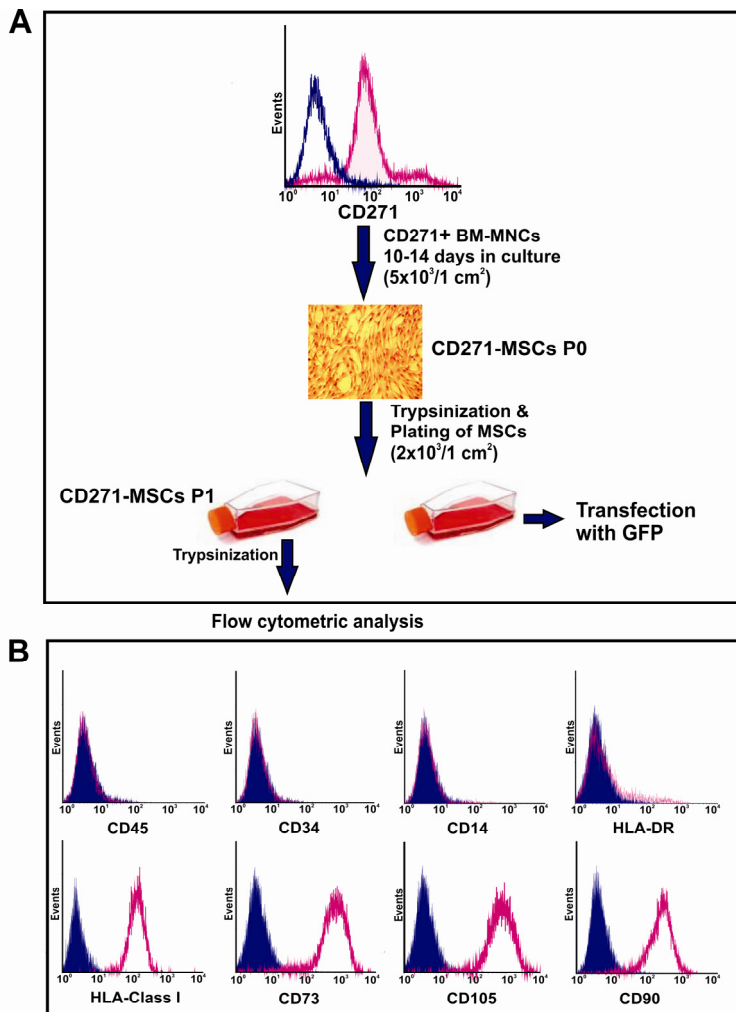
### **Immunophenotyping of clones**

Clones derived from CD271-MSC and PA-MSCs were stained with monoclonal antibodies against CD34, CD45, CD73, CD90, CD146, STRO-1, and HLA-A, HLA-B, HLA-C and incubated for 30 minutes at +4°C. After two washes with PBS the stained cells were analyzed on a FACSCalibur (Becton-Dickinson) equipped with Macintosh software for data analysis (CellQuest). All fluorochrome- conjugated antibodies and mouse immunoglobulins that were used as isotype control were purchased from BD Pharmingen (Heidelberg, Germany), except the CD105 antibody, which was purchased from Caltag-Invitrogen and the STRO-1 antibody (IgM isotype), which was purchased from Biolegend (Biozol, Eching, Germany).

### **Determination of prostaglandin E2 and IDO in the supernatants of MLR**

We used the experimental design described above for the BrdU assay to generate an identical set of MLRs in a second 96-well plate for the collection of supernatants for quantification of the soluble molecules PGE2 and IDO. On day 7 supernatants were collected, centrifuged at 14000 x g for 5 minutes to remove cell debris and then frozen at -80 °C until the time of the assay. PGE2 levels in the MLR supernatants were determined using Prostaglandin E2 FPIA Kit after diluting them 1:32, according to the manufacturer's instructions (Biomol GmbH, Hamburg, Germany). To quantify IDO levels, supernatants from the wells without MSCs were diluted 1:50, and supernatants from the wells containing PB-MNCs and MSCs were diluted 1:200. IDO levels were quantified using the ELISA-kit according to the manufacturer's instructions (Biozol, Eching, Germany).

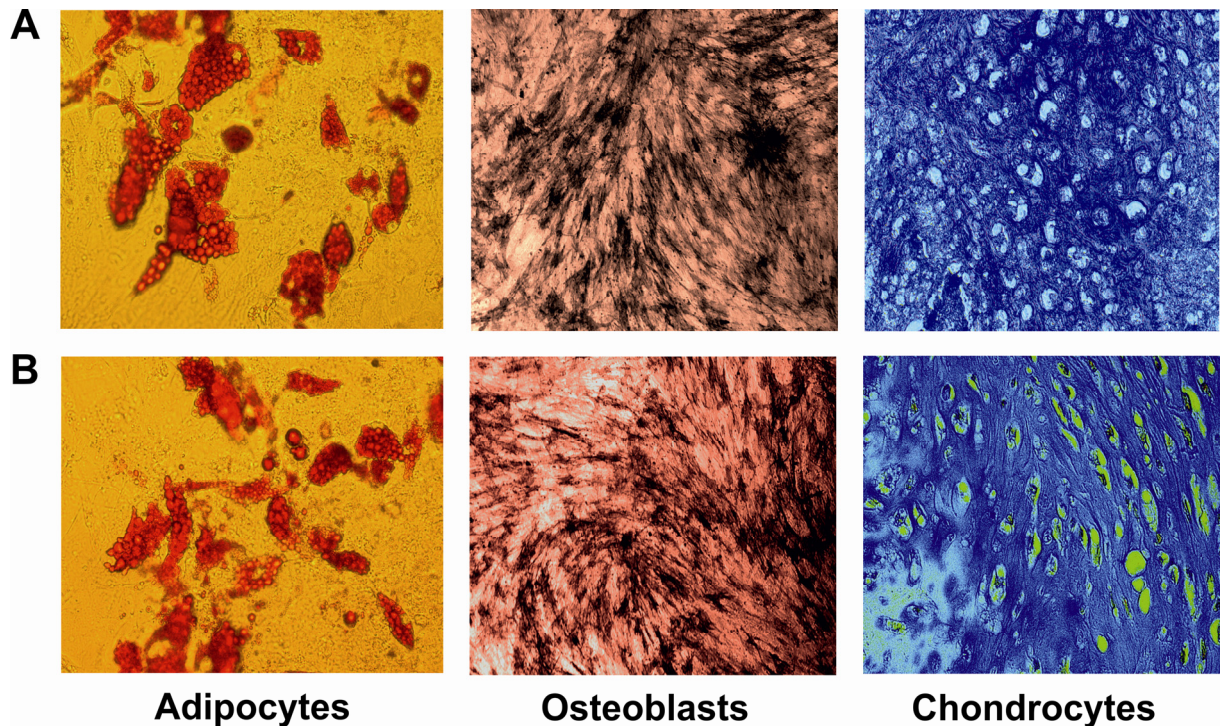
## Supplementary Figure 1



A) Highly purified bone marrow CD271<sup>+</sup> mononuclear cells by means of the immunomagnetic method (MACS) were plated in tissue culture flasks at a concentration  $5 \times 10^3/1 \text{ cm}^2$  in DMEM-low glucose supplemented with 10% FBS. The medium was changed after 7 days and later on every 3<sup>rd</sup> day until the mesenchymal stromal cells (MSCs) reached the confluence 70-80% (10-14 day). These MSCs generated from CD271<sup>+</sup> BM-MNCs represent passage 0 or P0 and were termed as CD271-MSC. After detachment of these MSCs with trypsin and their washing with PBS, they were further cultured in tissue culture flasks ( $2 \times 10^3/\text{cm}^2$ ) in the same medium and these are MSCs of passage 1 or P1. The majority of these MSCs was transfected with green fluorescent protein (GFP) for clonal studies, whereas one flask was trypsinized and used for determination of the phenotype by flow cytometry.

B) These MSCs were stained with fluorochrome-conjugated monoclonal antibodies against typical antigens expressed by MSCs. All MSCs were negative for hematopoietic cell markers (CD45, CD14, CD34) and HLA-DR, but they expressed high levels of HLA-Class I molecules and other typical MSC antigens such as CD73, CD105 and CD90.

## Supplementary Figure 2



In order to assess whether the MSCs of passage 1 that were used for clonal studies are multipotent we cultured both CD271-MSCs (A) and PA-MSCs (B) of CD271-MSCs of passage 1 in induction media for adipocytes, osteoblasts and chondrocytes. On day 10 osteoblasts can be identified by their cuboidal appearance and their association with newly synthesized new bone matrix. Furthermore, committed osteogenic cells are characterized by expression of high levels of alkaline phosphatase, an enzyme that is involved in the bone matrix mineralization. After 2-3 weeks, lipid vacuoles started to appear and on day 21 the developed adipocytes were stained with Oil Red O solution. In the chondrocytic induction medium the MSCs gave rise to a nodular micromass, which was fixed on day 24 in 4% paraformaldehyde, parafinized and then cut with the microtome in 5  $\mu\text{m}$  thick sections. These slides were stained with alcian blue, which stains acidic polysaccharides such as glycosaminoglycans in cartilage. Magnification for microphotographs of adipocytes was 20X, for osteoblasts 10x and for chondrocytes was 40x.