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## **Supplemental Materials**

### **Materials and methods**

CD34<sup>+</sup> MACS-immunisolated hHSCs from leukapheresis products were cultured on hMSCs for 3 days in serum-free medium [5,7] supplemented with early-acting cytokines (50 ng/ml SCF, 50 ng/ml Flt3-L, 15 ng/ml IL-3) prior to 24 h incubation with nocodazole (40 ng/ml) for cell synchronization. Before the labeling procedure, cells were washed 3 times with serum-free medium and incubated for additional 50 min in complete medium. Cells were fixed with 4% paraformaldehyde (PFA) for 30 min, quenched with 50 mM NH<sub>4</sub>Cl for 10 min and permeabilized with 0,2% saponin in blocking buffer (PBS containing 2% fetal calf serum) for 30 min. Cells were sequentially incubated for 30 min with mouse monoclonal (mAb) antibody CD133/1 (anti-CD133; Miltenyi Biotec) at room temperature followed by 1 h incubation with Cy<sup>TM</sup>3-conjugated AffiniPure donkey- $\alpha$ -mouse IgG (H+L) Fab fragment (Jackson ImmunoResearch Laboratories; red) at 4°C. All reactants were diluted in blocking buffer containing 0,2% saponin. Remaining mouse epitopes were saturated by incubation with unconjugated AffiniPure rabbit- $\alpha$ -mouse IgG (H+L) Fab fragment (Jackson ImmunoResearch) overnight at 4°C. After a post-fixation step with 0.2% PFA (10 min) and quenching (NH<sub>4</sub>Cl, 10 min), cells were incubated for 30 min with mouse mAb MX-49.129.5 (anti-CD63; Santa Cruz Biotechnology) followed by Cy<sup>TM</sup>2- conjugated goat- $\alpha$ -mouse antibody IgG (H+L) (Jackson ImmunoResearch; green) at room temperature. In order to identify the cell division phase, cells were

incubated for 30 min with rat mAb MCA78S directed to  $\alpha$ -tubulin (clone Num. YOL1/34; Serotec) followed by Alexa Fluor® 633-conjugated goat- $\alpha$ -rat antibody IgG (Molecular Probes; white). The nuclei were visualized with 4,6-diamidino-2-phenylindole (DAPI; blue). The cells were mounted in Mowiol 4.88. Images were captured using a Leica SP5 upright confocal microscope. Individual sections (1  $\mu$ m each) or a composite of 6-8 optical sections are shown.

As controls, no signal was observed when both anti-CD133 and anti-CD63 primary antibodies were omitted or only the CD133 immunoreactivity was detected when anti-CD63 primary antibody was omitted indicating that the first mouse primary antibody was fully saturated (data not shown). Note that the same data were obtained without addition of nocodazole although the number of observable cell divisions was low.