

Phase contrast images of CD133-selected HemSCs show growth to high density.



BM-MSCs, cbEPCs and NHDFs do not form blood vessels in Matrigel implanted into immunodeficient mice.

H&E stained sections with corresponding macroscopic views of Matrigel explanted after 7 days in vivo. Microvessels containing red blood cells were seen in Matrigel with HemSCs but not with BM-MSCs, NHDF, cord blood EPCs (not shown), or 6×10^6 BM-MSCs (4X BM-MSCs). Scale bar = 50um.



Merosin expression in microvessels formed from clonal Hem SCs.

Sections from mouse "hemangiomas" after 1 month in vivo were stained for A) merosin (red) and DAPI; B) Ulex europeus-I, a lectin that binds human endothelial cells (green), and DAPI. C) merged image and D) corresponding phase image. Scale bar = 10 um.



Cells retrieved from Matrigel implants stained with anti-human nuclear antigen antibody.

CD31-selected cells from Matrigel explants were expanded in vitro for reimplantation into secondary recipient mice. To verify that the cells were human, cells were immunostained with anti-human nuclear (Hu-NUC) antigen (left panel) and stained with DAPI (middle panel). The merged image (right panel) shows nearly all nuclei reacted with the anti-human NUC. Scale bar = 50um.



Flow cytometry of GFP-labeled HemSCs

HemSCs were infected with lentiviral construct encoding turbo GFP[™]. The solid black line shows fluorescence of non-infected HemSCs while the solid black line filled with grey shows strong fluorescence of GFP-labeled HemSCs.

Supplmental Figure 6:

Controls for immunofluorescence staining and confocal microscopy analyses.

Panel A shows GLUT-1 and GFP immunostaining (left panel) and GFP immunostaining alone (right panel) of sections from HemSCs implanted in Matrigel into nude mice for 14 days. Sections are positive for GLUT-1 (red) as expected but there is no GFP labeling, verifying that the anti-GFP does not non-specifically stain the sections. DAPI verifies presence of cells within the Matrigel sections.

Panel B shows perilipin-A and GFP immunostaining (left panel) and GFP immunostaining alone (right panel) of sections from HemSCs implanted in Matrigel into nude mice for 2 months. The



Supplemental Figure 6

sections are positive for perilipin-A (red) as expected but there is no GFPlabeling verifying that the anti-GFP does not non-specifically stain the sections. DAPI verifies the presence of cells within the Matrigel sections.

Panel C shows perilipin-A and control rabbit IgG (left panel) and rabbit IgG only (right panel). This verifies rabbit IgG as a primary antibody does not generate fluorescence (green) signal.