Supplementary Data

CD105 is a surface marker for receptor-targeted gene transfer into human long-term repopulating hematopoietic stem cells

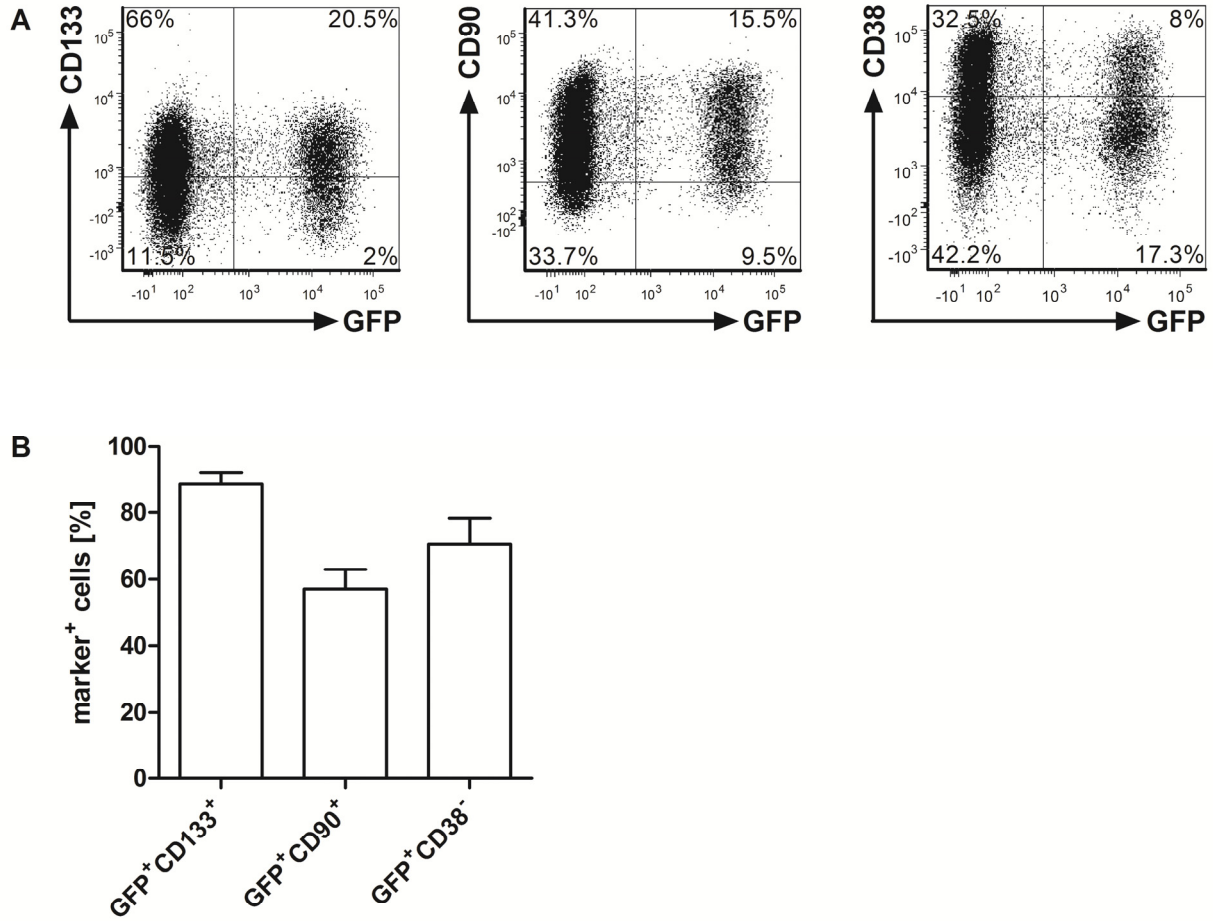
Sarah-Katharina Kays[1], Kerstin B. Kaufmann[2,*], Tobias Abel[1], Christian Brendel[2,#] Halvard Bonig[3], Manuel Grez[2], Christian J. Buchholz[1] and Sabrina Kneissl[1,4]

[1]Molecular Biotechnology and Gene Therapy, Paul-Ehrlich-Institut, Langen, Germany
[2]Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Frankfurt, Germany
[3]Institute for Transfusion Medicine and Immune Hematology, Goethe University and Red Cross Blood Donor Service Baden-Wuerttemberg-Hessen, Frankfurt, Germany
[4]Division of Haematology/Transfusion Medicine, Paul-Ehrlich-Institut, Langen, Germany

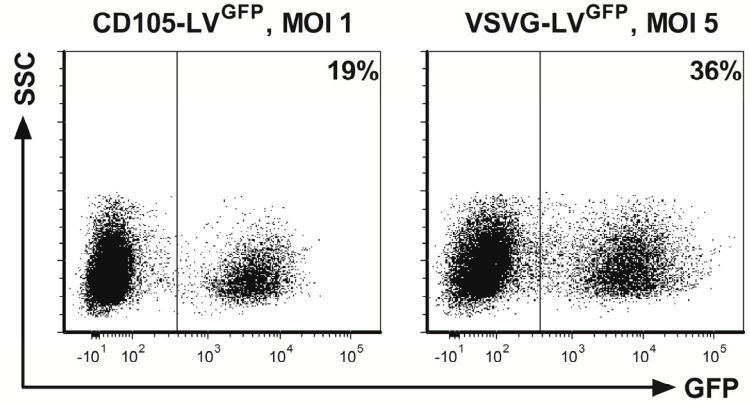
*Present address: Princess Margaret Cancer Centre, University Health Network, Toronto, Canada
[#]Present address: Division of Pediatric Hematology/Oncology, Boston Children's Hospital, Harvard Medical School, Boston, USA

Running title: CD105 as marker for long-term engrafting human HSC

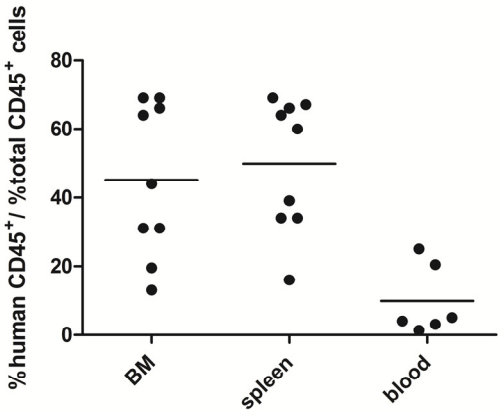
Correspondence should be addressed to Christian J. Buchholz
Head of Molecular Biotechnology and Gene Therapy
Paul-Ehrlich-Institut
Paul-Ehrlich-Str. 51-59
63225 Langen
Germany
Phone: +49-6103-774011
Fax: +49-6103-771255
Email: Christian.Buchholz@pei.de



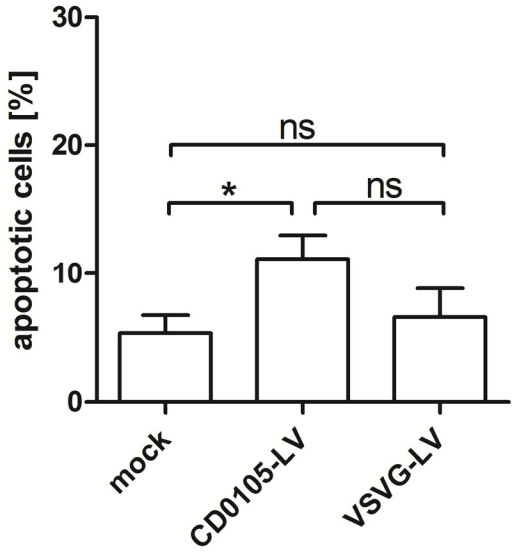
Supplementary Figure 1: Staining of transduced CD34$^+$ cells for additional surface markers. CD34$^+$ cells isolated from G-CSF mobilized peripheral blood were transduced with CD105-LV^{GFP} (MOI 1). Seventy-two hours after transduction cells were analyzed by flow cytometry for expression of GFP and the surface markers CD133, CD90 and CD38. A) FACS plots of one representative experiment. B) Mean values ± SD of four biological replicas.



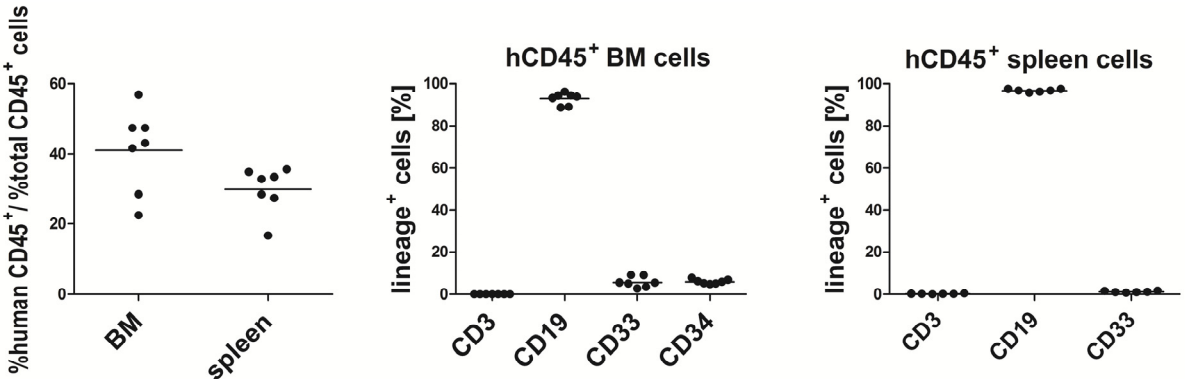
Supplementary Figure 2: Transduction efficiency of CD105-LV^{GFP} and VSVG-LV^{GFP} before start of the colony forming assay. CD34^{+} cells purified from G-CSF mobilized peripheral blood were transduced with either CD105-LV^{GFP} (MOI 1) or VSVG-LV^{GFP} (MOI 5). Seventy-two hours after transduction the percentage of GFP^{+} cells was determined by flow cytometry before the cells were applied to a colony forming assay. MOI = multiplicity of infection.



Supplementary Figure 3: Engraftment of human cells in NSG mice. Irradiated NSG mice were transplanted intravenously with 1.2 - 1.7x10^6 CD105-LV^{GFP} transduced human $CD34^+$ cells 4 h after conditioning. 7 - 18 weeks after transplantation cells were isolated from BM, spleen und blood and analyzed by flow cytometry for human and murine CD45 expression. The percentage of human $CD45^+$ cells in relation to all $CD45^+$ cells is shown. BM = bone marrow



Supplementary Figure 4: Apoptosis induction upon transduction. $CD34^{+}$ cells purified from G-CSF mobilized peripheral blood were transduced either with CD105-LV^{GFP} or VSVG-LV^{GFP} resulting in similar transduction efficiencies of 11-18%. After 48 h the fractions of early and late apoptotic/necrotic cells were determined by Annexin V/PI staining. Early and late apoptotic cells as well as necrotic cells were included into analysis of vector mediated toxicity. According to one-way ANOVA no significant differences were observed between both mock and VSVG-LV transduced cells and between CD105-LV and VSVG-LV transduced cells. CD105-LV transduced cells showed significantly more apoptotic cells ($p<0.05$) compared to the mock control.



Supplementary Figure 5: Engraftment and lineage distribution of human cells in NSG mice for competitive repopulation experiments. $CD34^+$ cells purified from G-CSF mobilized peripheral blood were transduced either with CD105-LV^{GFP}, CD105-LV^{BFP}, VSVG-LV^{GFP} or VSVG-LV^{BFP}. The next day VSVG-LV^{BFP} and CD105-LV^{GFP} and vice versa transduced cells were mixed to equal parts. 0.5 - 0.6 x 10^6 cells/mouse were injected intravenously. Eight weeks post transplantation cells were isolated from BM and spleen and analyzed by flow cytometry for human and murine CD45 expression as well as for expression of human lineage markers in the $hCD45^+$ population. BM = bone marrow; h = human.