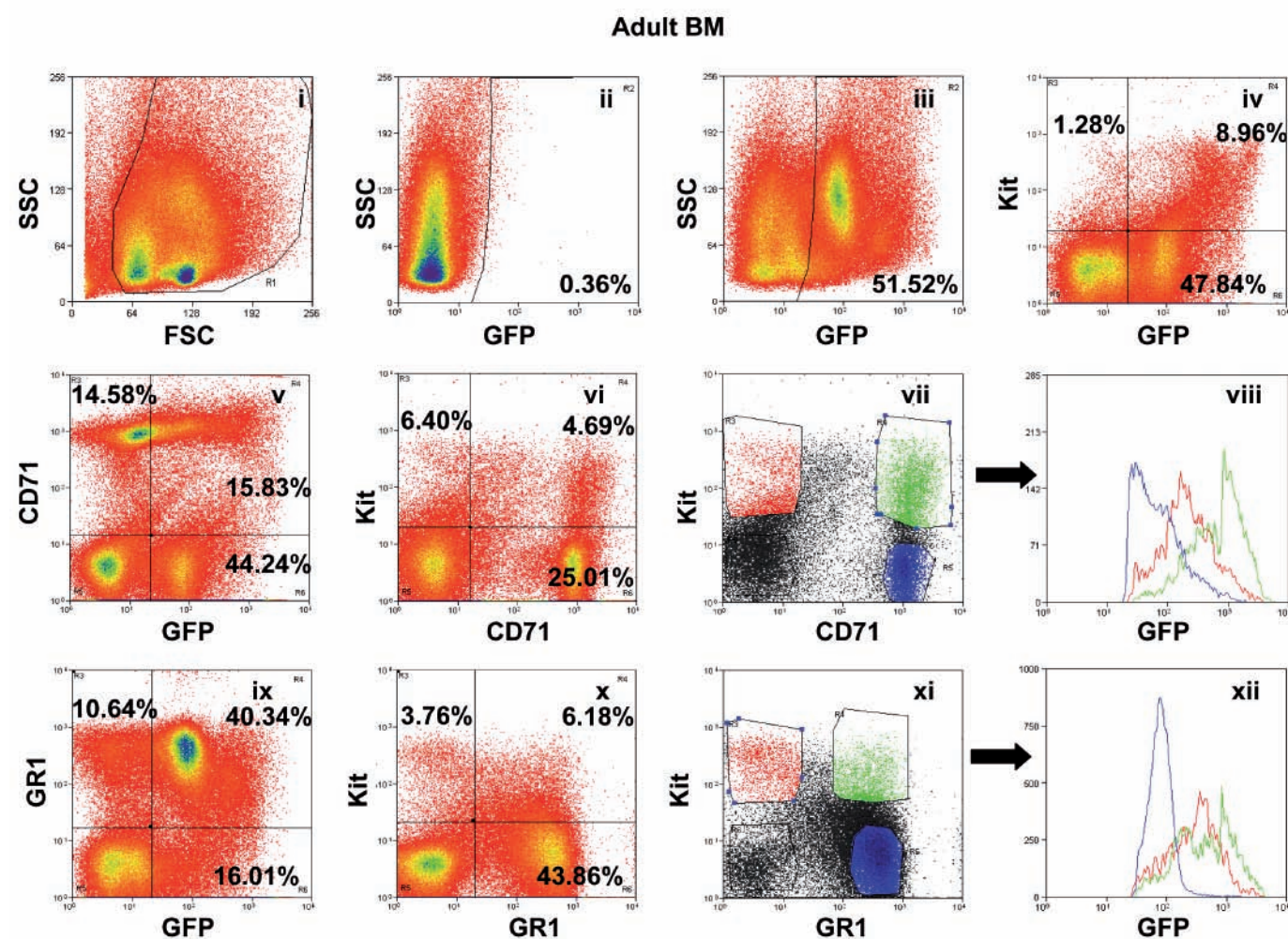


Green fluorescent protein transgene driven by Kit regulatory sequences is expressed in hematopoietic stem cells

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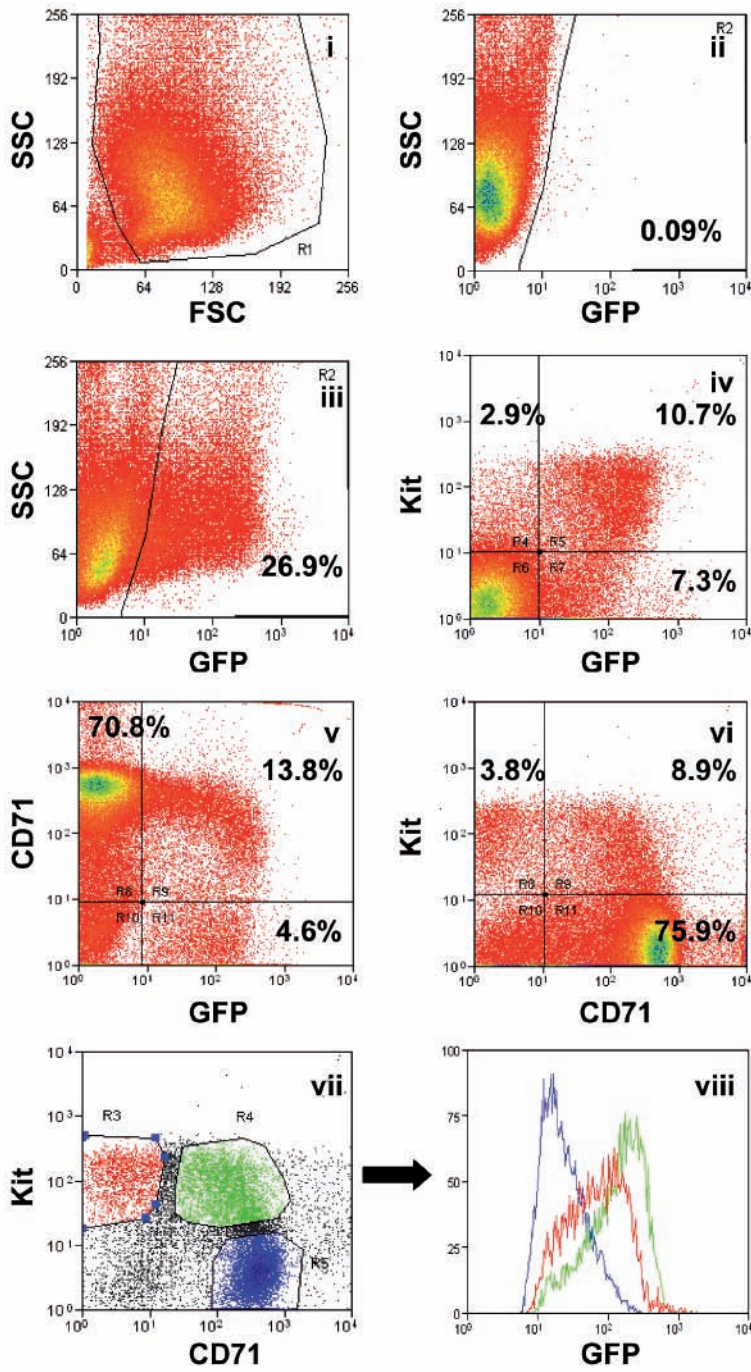
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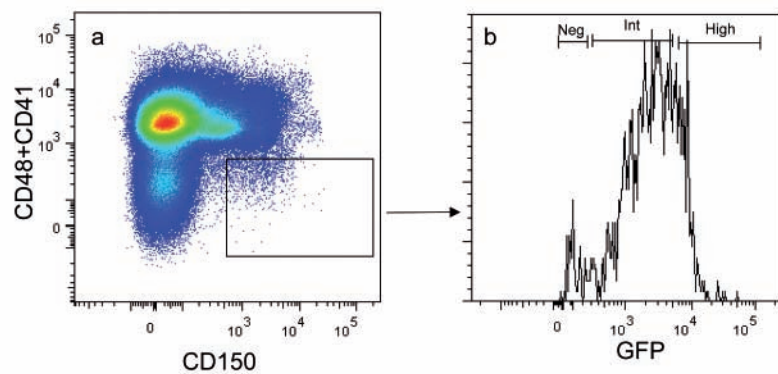


Online Supplementary Figure S1. Cytofluorimetric analysis of Kit, CD71, Gr-1 and GFP expression in bone marrow. Panel i: forward and side scattering of the population. Panels ii and iii: GFP fluorescence of control non-transgenic and transgenic fetal livers, respectively. Non-transgenic livers were from the same litter as the transgenic ones. Panels iv and v: expression of Kit versus GFP and CD71 versus GFP, respectively. Panel vi: expression of Kit versus CD71 in the total cell population (panel iii). Panel vii: expression of Kit versus CD71 in the GFP⁺ population from panel iii (R2); color identification of fractions to be further analyzed for GFP fluorescence. Panel viii: plot of number of GFP-expressing cells versus GFP level for each of the three populations (Kit⁺/CD71⁻, Kit⁻/CD71⁺ and Kit⁺/CD71⁺) identified by colors in panel vii. Panels ix and x: expression of Gr-1 versus GFP and Kit versus Gr-1 in the total population, respectively. Panel xi: expression of Kit versus Gr-1 in the GFP⁺ population (R2) of panel iii, and color identification of fractions to be further analyzed for GFP fluorescence, respectively. Panel xii: plot of number of GFP-expressing cells versus GFP level for each of the three populations (Kit⁺/Gr-1⁻, Kit⁻/Gr-1⁺ and Kit⁺/Gr-1⁺) identified by colors in panel xi. The analysis was performed with a quartz camera flowcytometer analyzer (Dako).I.

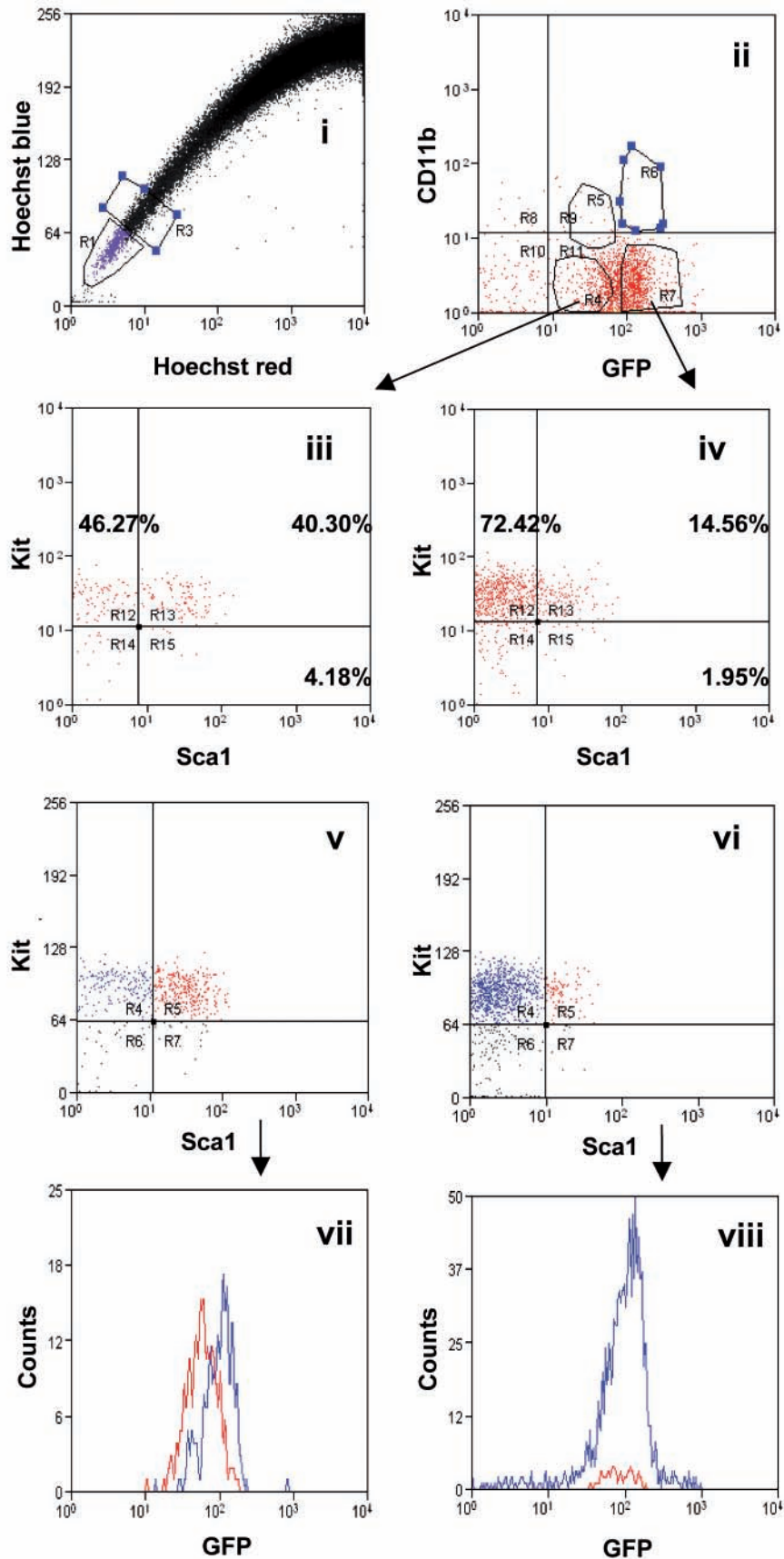
Fetal liver E12.5



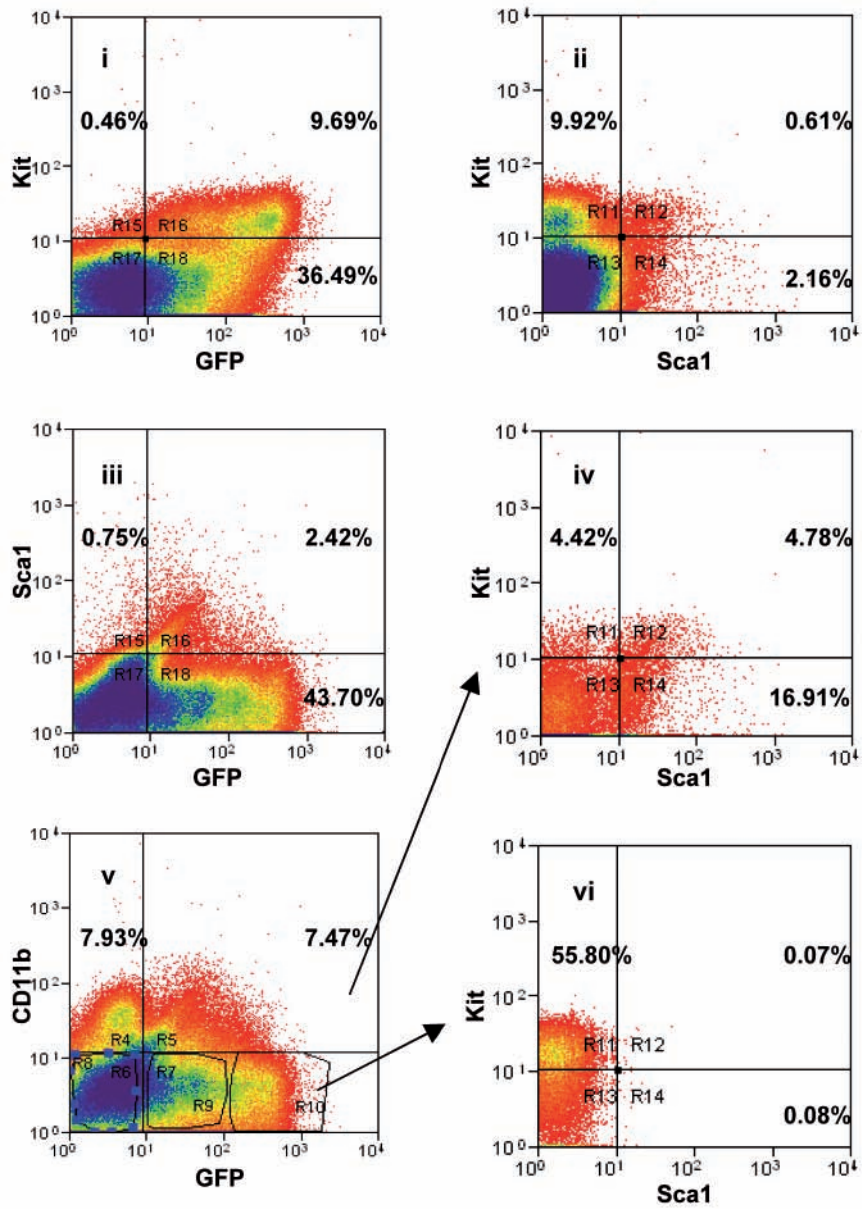
Online Supplementary Figure S2. Cytofluorimetric analysis of Kit, CD71 and GFP expression in fetal liver at E12.5. Panel i: forward and side scattering of the population. Panels ii and iii: GFP fluorescence of control non-transgenic and transgenic fetal livers, respectively. Non-transgenic livers were from the same litter as the transgenic ones. Panels iv-vi: expression of Kit versus GFP, CD71 versus GFP, and Kit versus CD71, respectively, in the total population. Panel vii: expression of Kit versus CD71 in the GFP⁺ population only (R2 in panel iii), and color identification of fractions to be further analyzed for GFP fluorescence. Panel viii: plot of number of GFP-expressing cells versus GFP for each of the three populations (Kit⁺/CD71⁻, Kit⁺/CD71⁺ and Kit⁻/CD71⁺) identified by colors in panel vii. The analysis was performed at the quartz camera cyan flowcytometer analyzer (Dako).I.



Online Supplementary Figure S3. Sorting for transplantation experiments of SLAM fractionated cells on the basis of GFP expression levels. Bone marrow cells from two adult males were depleted of differentiated cells by double density gradient ultracentrifugation. Left panel: gating of SLAM fractions. Right panel: cell fractions separated on the basis of GFP levels.



Online Supplementary Figure S4. Four color cytofluorimetric analysis of the adult bone marrow side population. Panel i: the side population can be divided in two parts for further analysis: tip (R1) and base (R3). Panel ii: CD11b versus GFP expression within the side population. Panels iii and iv: Kit versus Sca1 expression in CD11b⁻ fractions R11 (GFP^{hi}) and R7 (GFP^{low}), respectively from the side population. Panels v and vi: Kit versus Sca1 expression in cells from the tip and base of the side population, respectively. Panel vii: relative GFP expression in Kit⁺/Sca1⁺ (red) versus Kit⁺/Sca1⁻ (blue) cell fractions from the tip. Panel viii: relative GFP expression in Kit⁺/Sca1⁺ (red) versus Kit⁺/Sca1⁻ (blue) cell fractions from the base of the side population.



Online Supplementary Figure S5. Cytofluorimetric analysis of Kit, CD11b, Sca1 and GFP in fetal liver, about E12.25. Panels i,ii,iii: expression of Kit versus GFP, Kit versus Sca1 and Sca1 versus GFP, respectively. Panel v: CD11b versus GFP. Panels iv and vi: Kit versus Sca1 expression in fractions R5 (CD11b⁺/GFP⁺) and R10 (CD11b⁺/GFP^{int}), respectively, from panel v. The analysis was performed with a Moflo flow cytometer cell sorter.