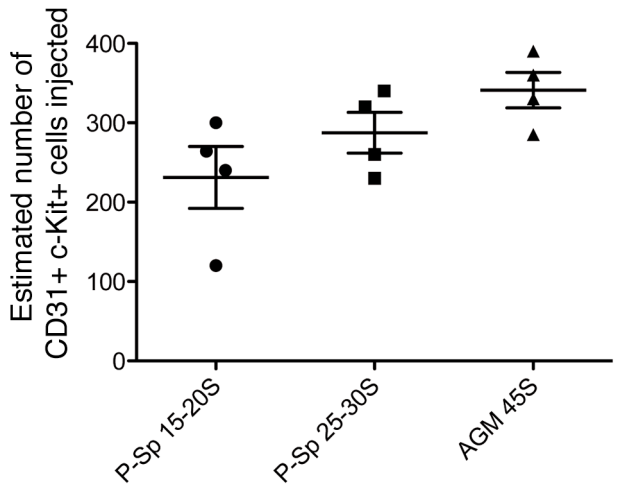

Fig. S1. Estimated number of multipotent progenitors transplanted into *Rag2γc*$^{-/-}$ recipients. CD31^{+} Kit^{+} multipotent progenitors were quantified by flow cytometry (top panel). To inject similar number of progenitors at the three stages analyzed (lower panel), the recipients were transplanted with 12 P-Sp per mouse at 9 dpc (15-20S), 10 P-Sp per mouse at 9.5 dpc (20-25S) or 3 AGM per mouse at 11 dpc (45S).

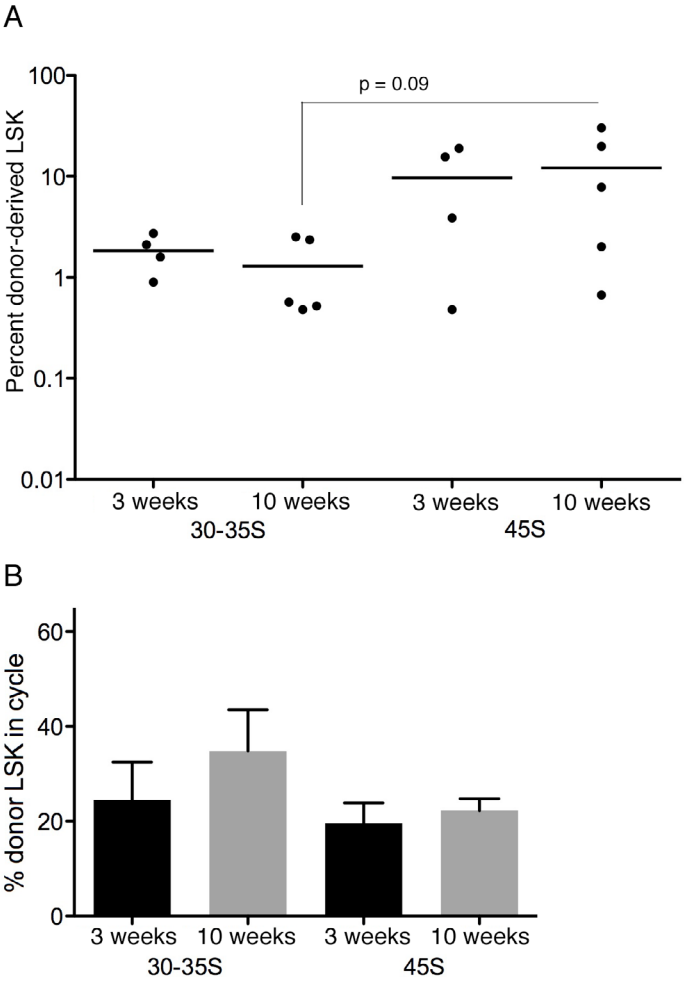

Fig. S2. Frequency of LSK cells in cycle after reconstitution. Sublethally irradiated *Rag2γc*$^{-/-}$ recipients were intravenously transplanted with cells isolated from 30-35S P-Sp/AGM (4 e.e.) or 45S AGM (2 e.e.). Donor-derived LSK cells in the BM were analyzed using the intracellular Ki67/DAPI staining 3 and 10 weeks after transplantation. The frequency of cells expressing high levels of Ki67/DAPI is shown.

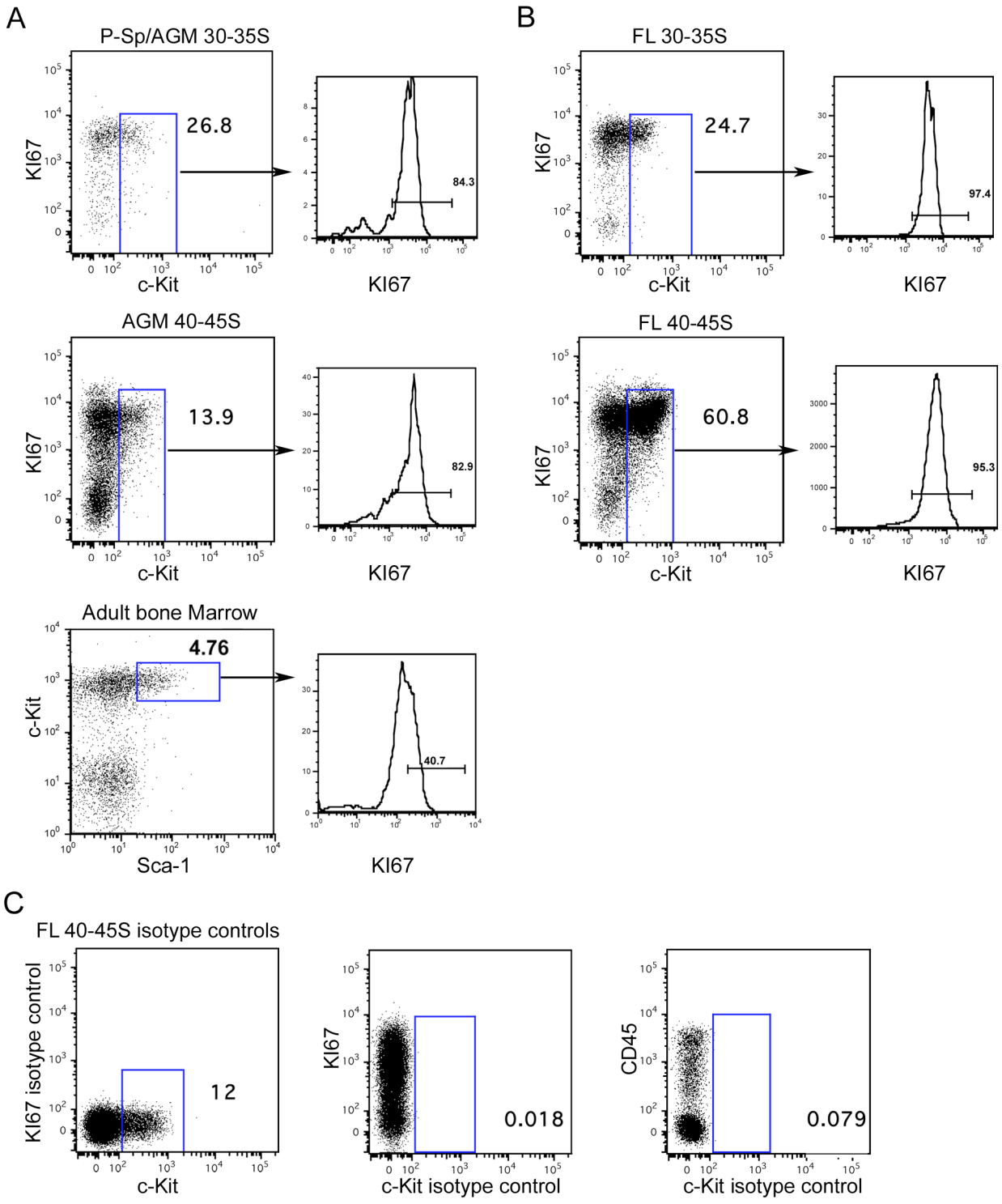

Fig. S3. Proliferative activity of AGM and FL cells. (**A**) Flow cytometry profiles of KI67 expression in $CD31^{+}Kit^{+}CD45^{-/low}$ imHSC from 30-35S P-Sp/AGM (top panel) and in $CD31^{+}Kit^{+}$ $CD45^{low/+}$ HSC from 40-45S AGM (middle panel). (Lower panel) KI67 expression in LSK cells from adult BM. (**B**) Flow cytometry profiles of KI67 expression in $CD31^{+}Kit^{+}CD45^{+}$ cells from 30-35S FL (top panel), from 40-45S FL (lower panel). (**C**) Staining of 40-45S FL cells with isotype controls.

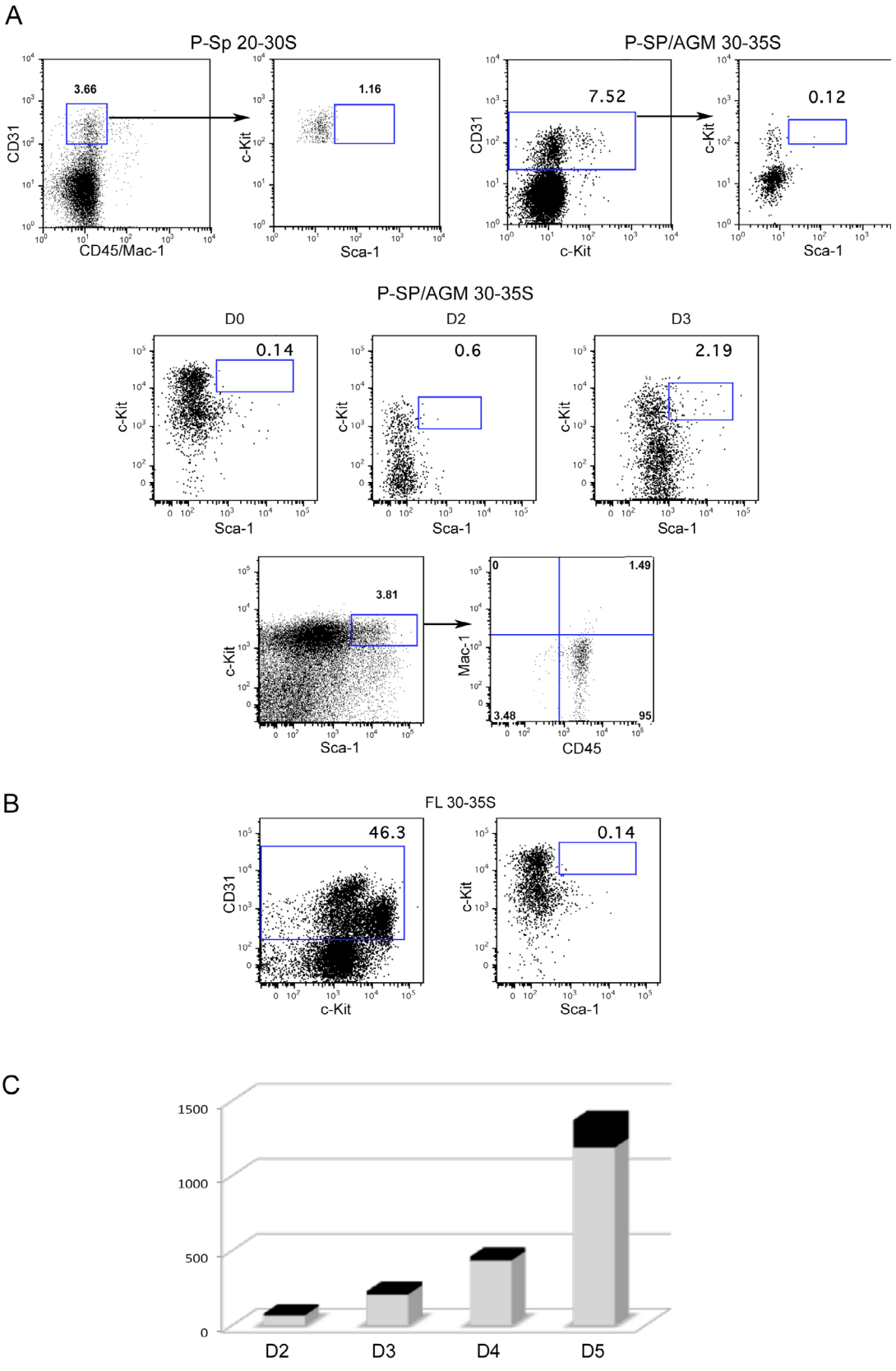

Fig. S4. Flow cytometry profile of imHSC from the P-Sp/AGM, freshly isolated or after culture. (**A**) ImHSC from 20-30S P-Sp or from 30-35S P-Sp/AGM do not overtly express Sca-1 (upper panel). After culture with TPO on OP9 stromal cells, the expression of Sca-1 is progressively acquired (middle panel). LSK cells derived from the culture of Kit^+ $CD45^{-/low}$ $Mac\text{-}1^{-/low}$ imHSC (30-35S P-Sp/AGM) express CD45 and Mac-1 (lower panel). (**B**) Similar to imHSC from the 30-35S P-Sp/AGM, imHSC from the 30-35S FL do not express Sca-1. (**C**) Number of LSK (grey bars) and LSK $CD48^-$ $CD150^+$ (black bars) per 100 $CD31^+Kit^+CD45^-$ sorted P-Sp/AGM at days 2, 3 4 and 5 of culture with OP9 stroma and TPO.

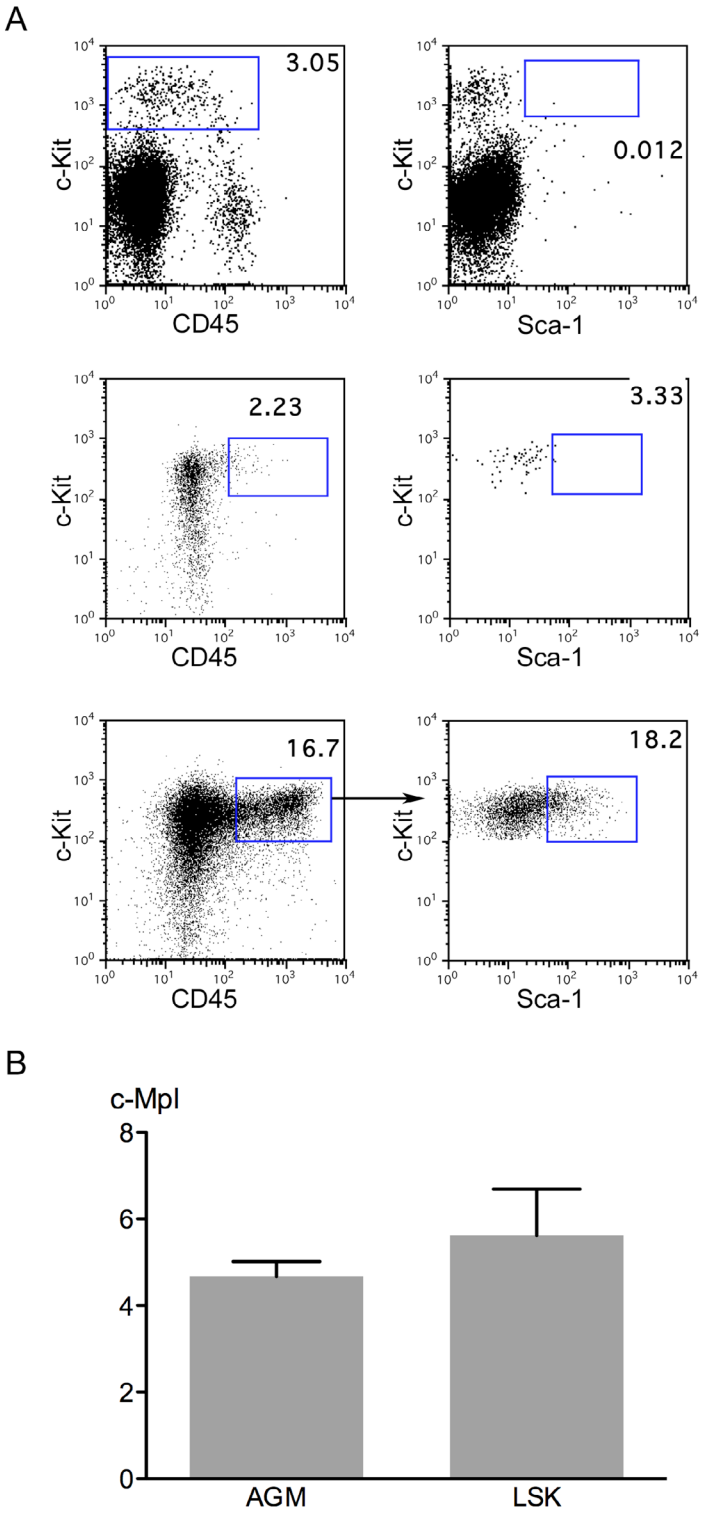

Fig. S5. TPO promotes in vitro maturation of 30-35S P-Sp/AGM multipotent progenitors. (**A**) Freshly isolated CD31^{+}Kit^{+} cells from 30-35S P-Sp/AGM initially lack the expression of CD45 and Sca-1 (top panel). After culture on OP9 stroma, in the absence of TPO, these 30-35S P-Sp/AGM cells give rise to a limited number of CD45^{+}Sca-1^{+} cells (middle panel), whereas when TPO is added to the culture medium, a well-defined population of CD45^{+}Sca-1^{+} cells appears (lower panel). (**B**) Sorted 30-35S Kit^{+}CD31^{+} P-Sp/AGM cells and adult bone marrow LSK were subjected to Q-RT-PCR for the detection of Mpl and HPRT mRNA (the receptor of TPO). Results are expressed as arbitrary units (ratio of Mpl to HPRT).

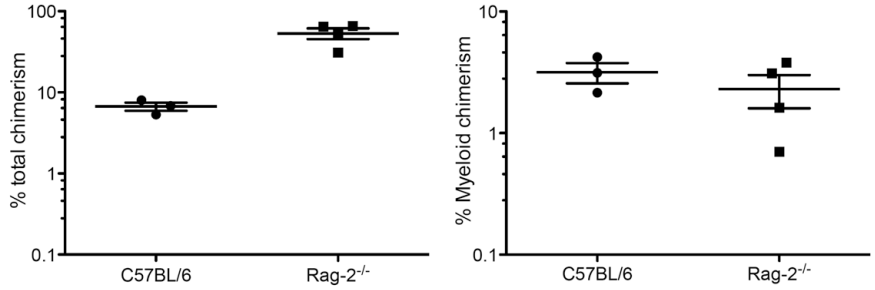

Fig. S6. Comparison of total and myeloid chimerism levels in C57BL/6 and *Rag2*$^{-/-}$ recipients. This figure relates to Fig. 3C. Analysis of the chimerism level in *Rag2*$^{-/-}$ and C57BL/6 recipients 6 months after engraftment of total cells recovered from 30-35S P-Sp/AGM cells cultured on OP9 stroma with TPO. The total chimerism level in the BM of *Rag2*$^{-/-}$ recipients often reaches levels close to 90%, owing to the 100% donor-derived contribution to the lymphoid compartment, when it reaches about 10% in similarly injected C57BL/6 recipients (left panel). The corresponding myeloid chimerism in the two types of recipients (right panel) is similar (P=0.4148) and ranges on average from 2% to 4%.

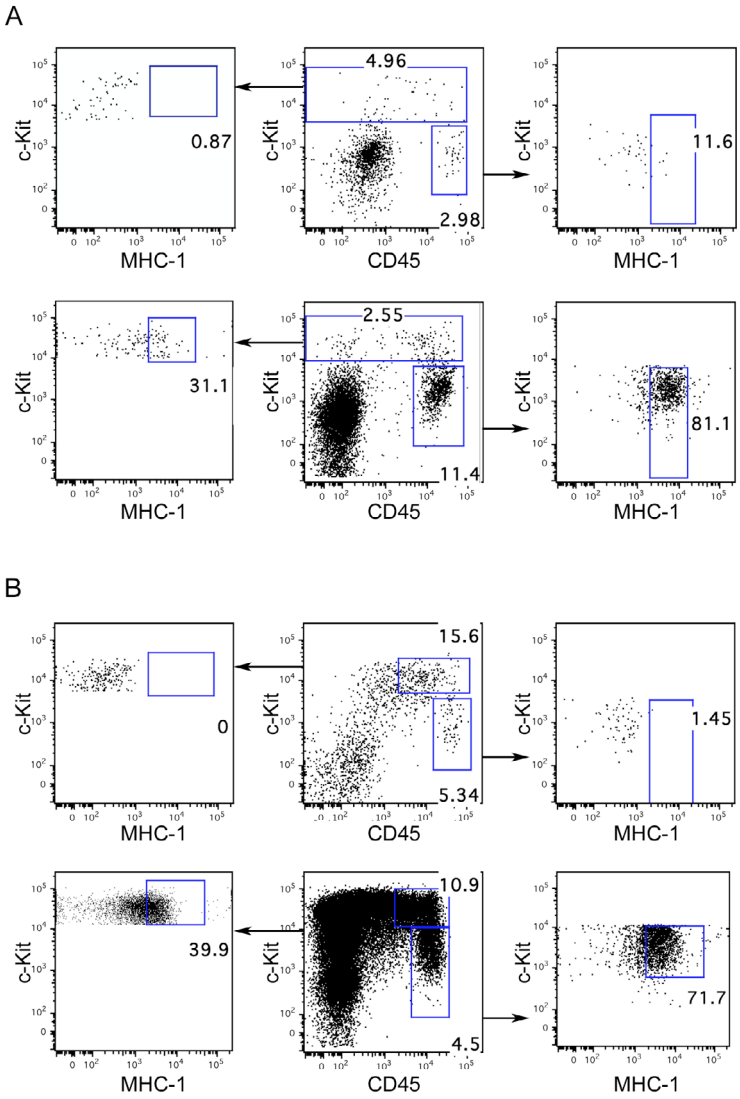

Fig. S7. Acquisition of MHC class I correlates with CD45 expression. This figure relates to Figs 4 and 6. (**A**) CD45 against MHC class I (H2D^{b}) profile (middle panels) of Kit^{+} progenitors from 30-35S P-Sp/AGM (top panel) and 45S AGM (bottom panel). Within the hematopoietic progenitor compartment, MHC class I expression is restricted to CD45^{+} cells (right panels). At earlier stages, Kit^{+} cells express low levels of CD45, and MHC class I is virtually undetectable (Fig. 4A). (**B**) CD45 against MHC class I (H2D^{b}) profile (middle panels) of Kit^{+} progenitors from 30-35S (top panel) and

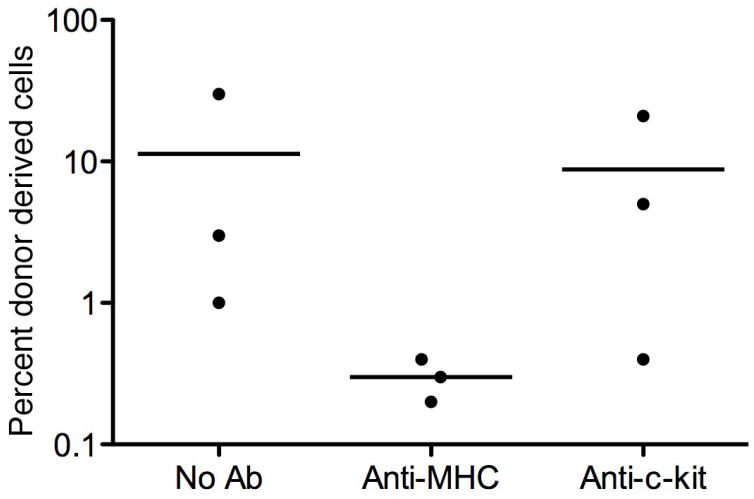

Fig. S8. Incubation of AGM with Anti-H2D^{b} antibody interferes with reconstitution capacity. CD45.2^{+} recipient mice were injected with 3 e.e. AGM cells from 45S embryos. Prior to injection, cells were incubated with anti-Kit or anti-H2D^{b} antibodies. Results show percentage donor-derived cells in the blood 24 weeks after transfer.