

SUPPLEMENTAL MATERIAL

Ishida et al., <http://www.jem.org/cgi/content/full/jem.20151493/DC1>

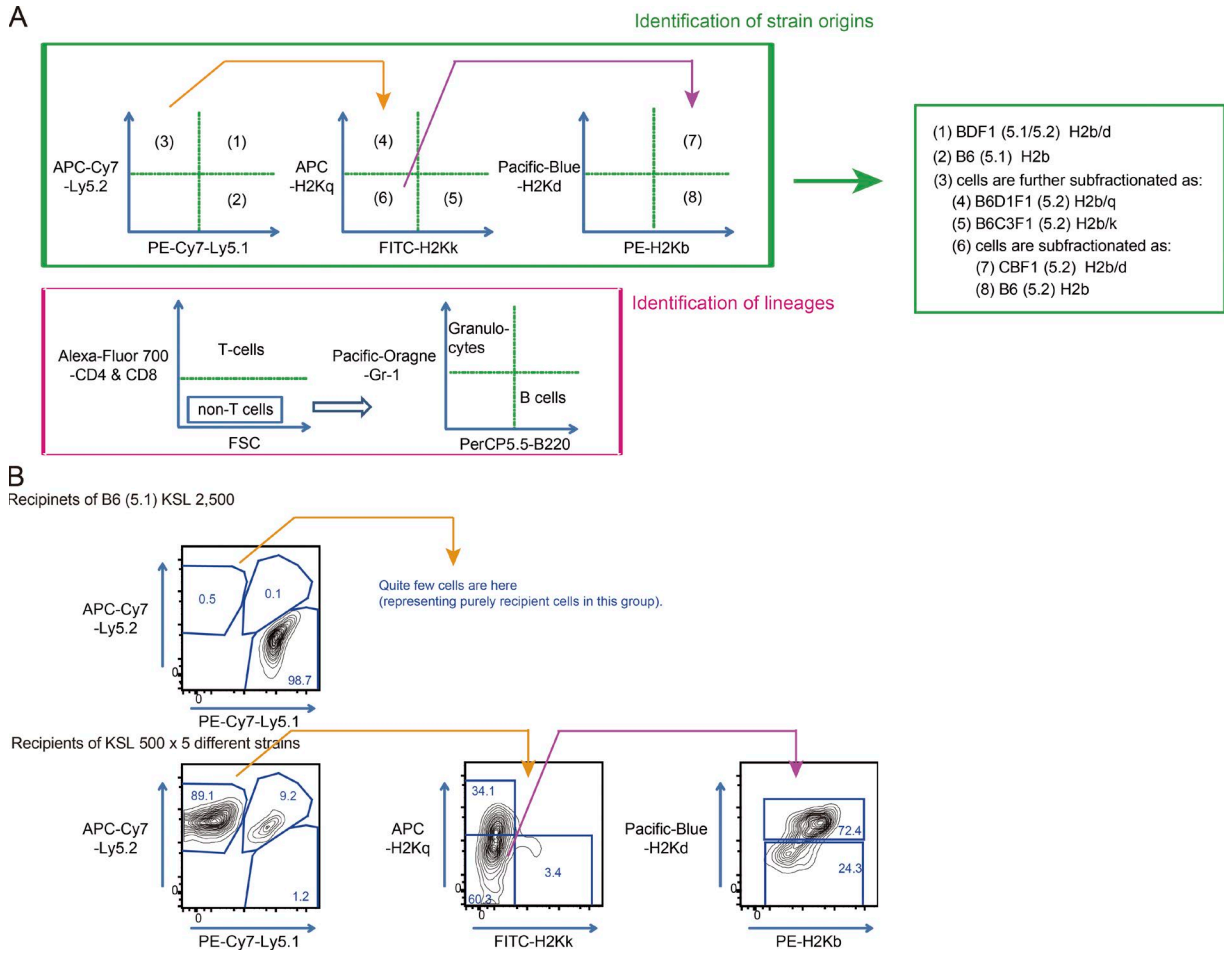


Figure S1. Gating strategies to distinguish mouse-strain cell derivations. (A, top) Using a series of antibodies separately detecting Ly5 subtypes (Ly5.1 or Ly5.2) and MHC haplotypes (H2Kq, H2Kk, H2Kd, and H2Kb), cell origins (mouse strains whence derived) were determined by flow cytometry analysis in samples obtained from recipient mice. (bottom) Each origin could be determined specifically in granulocytes, B cells, and T cells with the indicated gating strategies. (B) Representative flow cytometry plots obtained at 14 wk. (top) A recipient transplanted with 2,500 congenic KSL cells alone. (bottom) A recipient transplanted with 500 KSL cells of a congenic B6 strain and four allogeneic grafts (500 cells each).

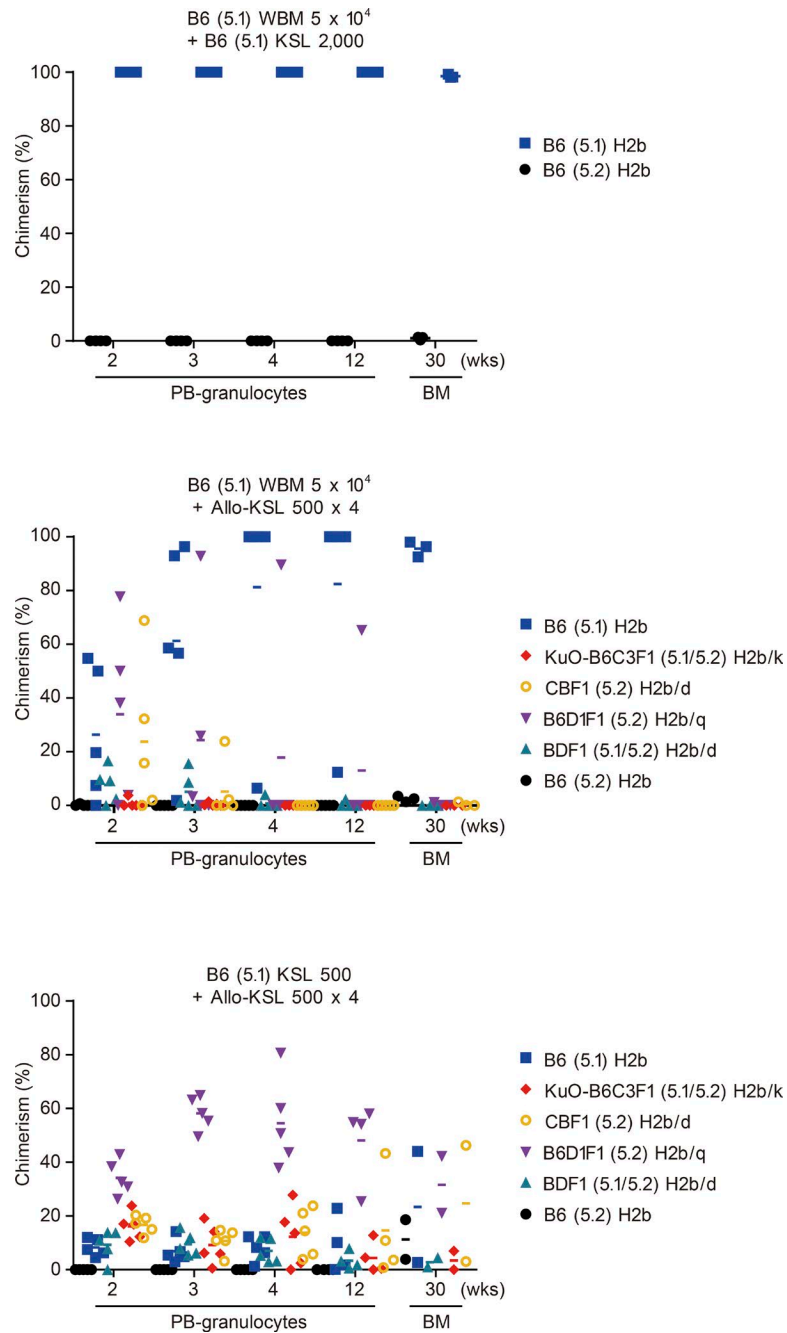


Figure S2. **Transplantation of congenic WBM and combined allogeneic KSL cells favored formation of single congenic-donor chimerism in recipient BM.** Shown are percentages of kinetics of donor chimerism in granulocytes and BM in individual recipients up to 30 wk after transplantation. We selected four mouse strains as allogeneic donor cell sources: BDF1, B6D1F1, CBF1, and B6C3F1 (C3H \times KuO-B6 F1, H2b/k). Outcomes were compared among recipient mice (B6-Ly5.2) in three cohorts; B6 (5.1) WBM 5×10^4 + B6 (5.1) KSL 2,000 (top), B6 (5.1) WBM 5×10^4 + Allo-KSL 500 $\times 4$ (middle), and B6 (5.1) KSL 500 + Allo-KSL 500 $\times 4$ (bottom). $n = 5$ mice in each group. Death supervened in two or three mice in each group during 30-wk observation. Mean values are indicated as bars.

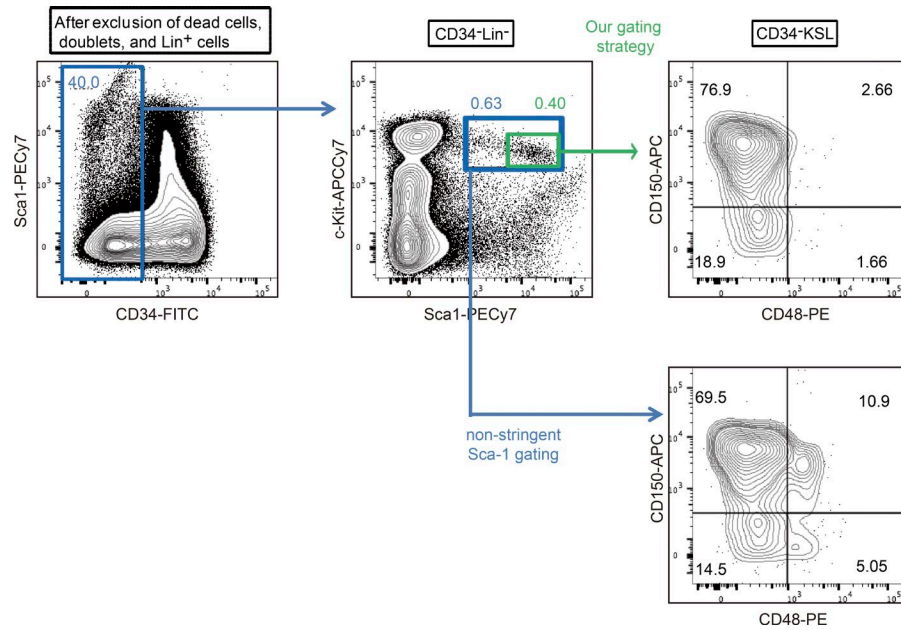


Figure S3. **SLAM marker expression in CD34⁻ KSL cells obtained with a stringent gating strategy.** Schematic representation of gating strategy for isolating CD34⁻ KSL cells from C57BL6/NCr male mice. After excluding dead cells, doublets, and Lin⁺ cells, a CD34⁻ gate is set (left). Using the population obtained after this gating, a two-color plot is prepared by setting c-Kit⁺ for y-axis and Sca-1⁺ for x-axis (middle). As shown, strict gating can be performed for a population showing Sca-1-bright and c-Kit⁺ (shown as a green gate). With this sequential gating, CD34⁻ KSL cells are ~100% CD48⁻ and ~80% CD150⁺ (top right). When gated broadly by including Sca-1-dull cells (shown in blue), the population lacked SLAM-HSC purity (bottom right).

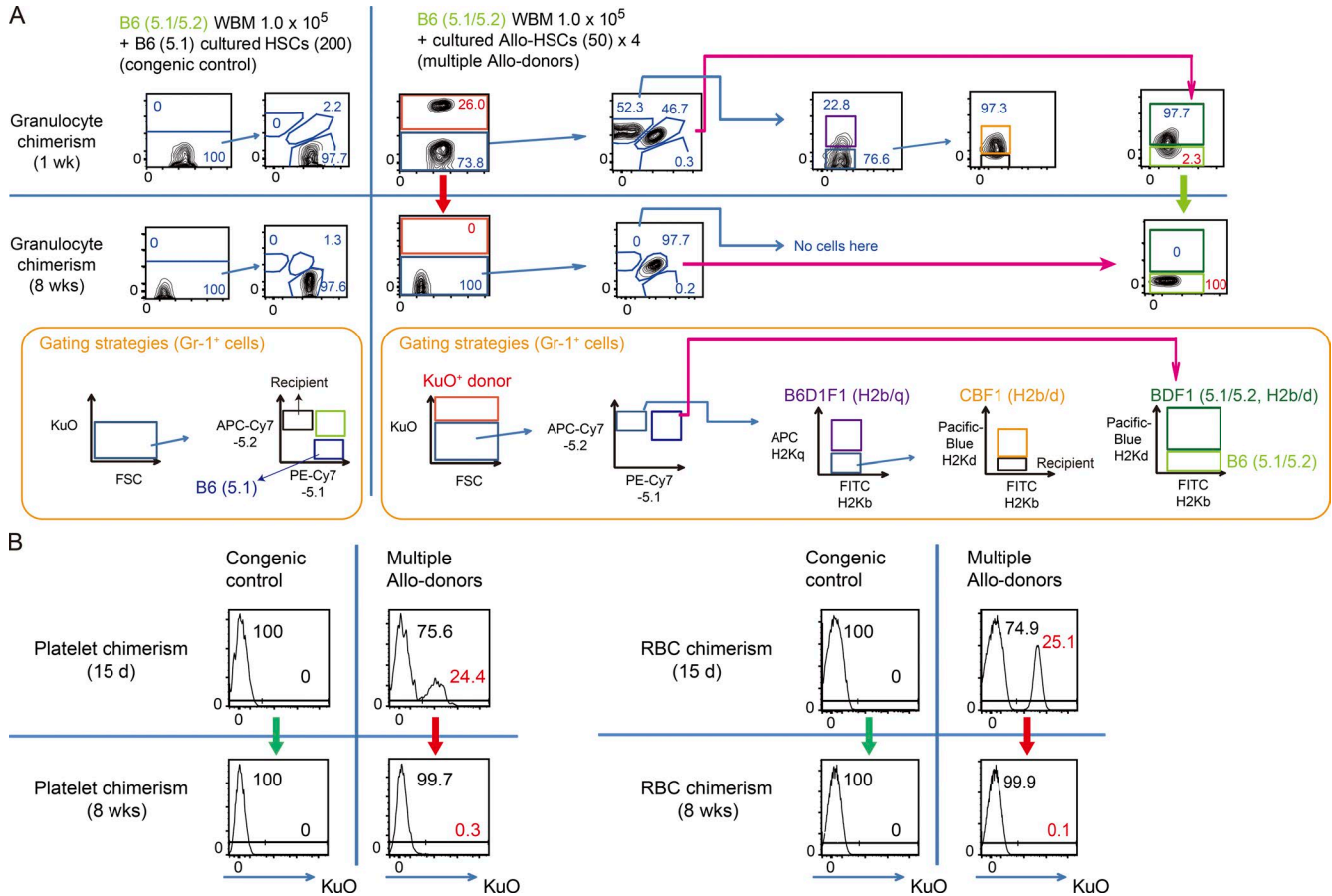


Figure S4. Cultured allogeneic HSCs contributed to early hematopoiesis after transplantation. (A) Experimental protocol to distinguish donor origin in recipient granulocytes by flow cytometry using mouse MHC, CD45 subtype, and KuO in combination. Representative flow cytometry patterns in recipients' peripheral blood 1 wk (top) and 8 wk (bottom) after transplantation. Recipients transplanted with the equivalent input dose of congenic grafts (left) and combined allogeneic grafts from multiple donors (right) are shown. Allogeneic HSCs, Allo-HSCs; Allogeneic donors, Allo-donors. (B) Representative flow cytometry patterns in recipients' platelets and red blood cells at 15 d (top) and 8 wk (bottom) after transplantation. Contributions of HSCs harvested from KuO mice (one out of five donors) were examined. Recipients transplanted with the equivalent input dose of congenic grafts (left) and combined allogeneic grafts including KuO transgenic mouse cells (right) are shown.

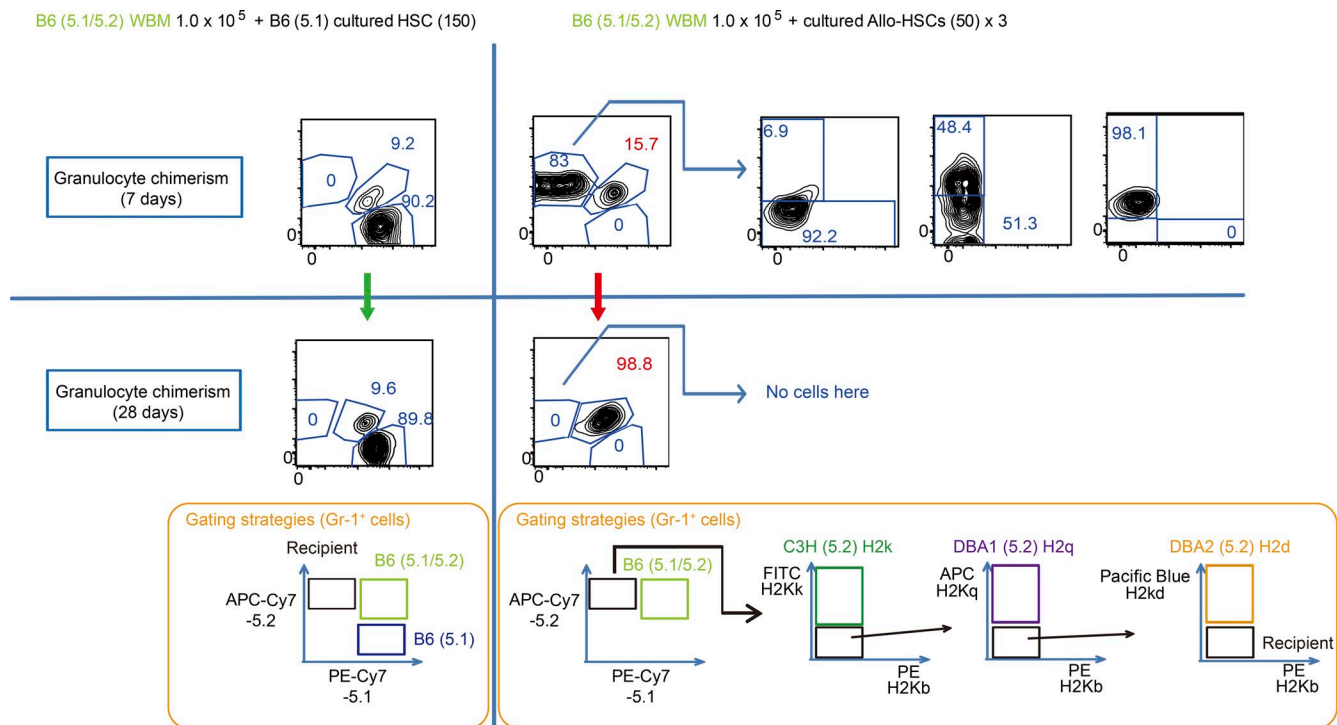


Figure S5. **Representative flow cytometry patterns of chimerism analysis in recipients transplanted with cultured allogeneic grafts obtained from multiple fully mismatched donors.** Shown are gating strategies to distinguish origins of granulocytes derived from each strain of mouse. (left) Representative flow cytometry plots obtained from recipients of Ly5.1/5.2-F1 whole bone marrow (WBM) and cultured congenic (Ly5.1-B6) HSCs. (right) Representative flow cytometry plots obtained from recipients of Ly5.1/5.2-F1 WBM and a mixture of cultured allogeneic HSCs. (top) Results obtained 7 d after transplantation. (bottom) Results obtained 28 d after transplantation.