Supplemental figure legends

Fig. 1. Gene expression analysis of NF-κB components
Real time PCR analysis of the expression of *rela, p50, relb* and *p52* in control KLS, control progenitors (c-kit$^+$Lineage$^-$Sca1$^-$), and dKO KLS cells. (n=3, *p<0.01).

Fig. 2. Characterization of hematopoiesis in the absence of RelB and p52 (dKO)
(A) Whole bone marrow cell counts of control, p52 single knockout (p52 KO), RelB single knockout (RelB KO) and RelB/p52 double knockout (dKO) mice. Values are mean ±SD. n=7-13. (B) Bone marrow cells from control or dKO mice were analyzed for frequency of myeloid cells (Mac1 and Gr1) and B (B220) cells. Values are mean ±SD. n=4. *p<0.01. (C) Bone marrow cells from control or dKO mice were analyzed for the frequency of myeloid progenitors. GMP: granulocyte-macrophage progenitor. CMP: common myeloid progenitor. MEP: macrophage-erythroid progenitor. n=3. (D) Bone marrow cells from control or dKO mice were analyzed for the frequency of common lymphoid progenitors (CLP). n=3. (E) Analysis of long-term stem cells (c-Kit$^+$, Lin$^-$, Sca1$^+$, CD34$^-$ and CD135$^-$) with different markers. n=3.

Fig. 3. Increased hematopoietic progenitors in the spleen from dKO mice
(A) Whole spleen cells from control or dKO mice were analyzed for KLS (c-Kit$^+$, Lineage$^-$, Sca1$^+$) population, which includes long-term HSCs. (B) The frequency of KLS cells in the spleen was determined from flow cytometric analysis. Values are mean ±SD. n=4. *p=0.0008.

Fig. 4. The dKO microenvironment protects bone marrow cells from the effects of 5-FU treatment.
A single dose of 5-FU (150mg/kg) was administered to dKO and control mice. Peripheral blood and bone marrow were analyzed 7 days after treatment and in untreated mice. (A) Peripheral red blood cell counts were similar in untreated control (Con) and dKO mice and did not change after treatment. (B) Peripheral white blood cell counts were significantly higher in untreated dKO than in control mice and were reduced by 5-FU treatment to similar levels in both control and dKO mice. (C) Untreated control and dKO mice had similar total numbers of bone marrow cells. Bone marrow cell numbers were reduced significantly by 5-FU treatment, but they were significantly higher in the dKO than in control mice after treatment. (D) After 5-FU treatment very few c-Kit positive cells were left in the bone marrow of control mice, while significantly more KLS cells remained in the dKO mice. (E) Phenotypic stem cells remaining in the dKO mice were unable to reconstitute irradiated mice, indicating that they were functionally impaired, similar to untreated dKO cells. (F) Experimental scheme for bone marrow transplantation (BMT) of control donor cells to control and dKO mice followed by 5-FU treatment. (G) The dKO microenvironment protects transplanted control marrow cells from the effects of 5-FU treatment. A to E, n=5, values are means ±SD., *p < 0.01 (G) Bone marrow donor-derived KLS cells. n=3, values are means ±SD., *p < 0.01.

Fig. 5. The dKO microenvironment alters donor cell behaviors.

Normal CD45.1 whole bone marrow cells (3 x 10^7) were transplanted into 5-FU (150mg/kg) pre-treated control or dKO mice. 16 weeks after transplantation, spleen cells from control or dKO mice were analyzed for (A) chimerism, (B) myeloid and (C) lymphoid components among donor cells. Plots are representative of three experiments.

Fig. 6. Whole bone marrow cell numbers are higher after transplantation of control cells into dKO mice than into WT mice. 16 weeks after transplantation of WT bone marrow into WT or dKO mice, whole bone marrow cells from control or dKO recipients were counted. Values are mean ±SD. n=4. *p=0.0018.

Fig. 7. CFU-F assay using cells isolated from bone marrow.
1 x 10^6 whole bone marrow cells from control or dKO mice were plated in 6-well plates and cultured for 10 days at 37ºC in 5% CO_2. Colonies were stained with Giemsa and counted. Values are mean ±SD., n=6. *p=0.0012. Note: the method for CFU-F assay is the same as described by STEMCELL Technologies, which shows that the average colony number per well in a six-well plate is 3.05 ± 1.86.

Fig. 8. dKO whole bone marrow cells display reduced osteoblast differentiation ability.
2 x 10^6 whole bone marrow cells from control or dKO mice were plated in 24-well plates and cultured with osteoblast-inducing medium for 7 days. The osteoblast colonies were stained for alkaline phosphatase activity.

Fig. 9. dKO mice display decreased frequency of marrow stromal progenitor cells and impaired stromal colony forming ability.
(A) The frequency of bone marrow stromal progenitor cells in control or dKO mice. The FACS profiles were pre-gated on the $CD45^-Ter119^-CD31^-$ population. (B) CFU-F assay using FACS-sorted $CD45^-Ter119^-CD31^-$ $Sca1^+PDGF\alpha^+$ stromal progenitor cells (1200 cells/10 cm dish). (n=3, $p < 0.01$).

Fig. 10. dKO mice have reduced inflammatory cell infiltration in lung and liver after control donor bone marrow cell transplantation.
Before (upper panels) and 16-weeks after (lower panels) control or dKO mice received control donor bone marrow cells. Sections of dKO liver (A) and lung (B) show marked inflammatory cell infiltrates, which are not present in sections from control mice either before or after transplantation. However, after transplantation, dKO recipients had much less inflammation in both liver (A) and lung (B). Scale bar represents 50μM.

Fig. 11. NF-κB non-canonical signaling positively regulates HSPC self-renewal intrinsically and HSPC lineage commitment extrinsically.
(A) Through the effects of various molecules, including SCF, CXCL12 and thrombopoietin, HSPCs under normal NF-κB signaling bind firmly to endosteal bone surface osteoblastic (Ob) niche cells, which differentiate from bone marrow stromal cells. HSPCs self renew and differentiate into multi-potent progenitors (MPP) and then to lymphoid and myeloid cells. (B) Deletion of RelB/NF-κB2 results in reduced Ob niche cell expression of SCF, CXCL12 and thrombopoietin leading to weaker adhesion of

HSPCs to osteoblastic niche cells and placing the HSPCs in a competent, active status. Despite increased numbers of osteoblastic niche cells, RelB/NF-κB2-deficiency is associated with severely and intrinsically impaired HSPC self-renewal ability, decreased stromal cell numbers, and enhanced osteoblast differentiation. Depletion of RelB/NF-κB2 also extrinsically increases cytokine expression (G-CSF, IL-6 and GM-CSF) by hematopoietic cells, which enhances MPP cell proliferation and differentiation and skews progenitor commitment to the myeloid lineage.