Supporting Information

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SI Materials and Methods

MicroRNA Profiling. One hundred nanograms of total RNA was reverse-transcribed into cDNA using 283-plex or 315-plex stem-loop RT primers and TaqMan miRNA reverse-transcription kit (Applied Biosystems) in 5 μL total volume. The resulting cDNA was preamplified 14 cycles using 283-plex or 315-plex PCR primers and TaqMan PreAmp Master Mix (Applied Biosystems) in a 25-μL PCR reaction. The preamplified cDNA was diluted fourfold with 0.1X TE (pH 8.0). Quantitative real-time PCR was performed using Applied Biosystems 7900HT system and TaqMan Universal PCR Master Mix on 384-well plates with 100-fold diluted cDNA input per TaqMan miRNA assay. Four PCR replicates were performed for each assay.

Microarray Analysis. All RNA samples were subjected to reverse transcription, two consecutive rounds of linear amplification, and production and fragmentation of biotinylated cRNA (Affymetrix). Complementary RNA from each sample was hybridized to Affymetrix Mouse Genome 430 2.0 Arrays. Hybridization and scanning were performed according to the manufacturer's instructions (Affymetrix). The arrays were normalized using RMA. Fold-change was computed by obtaining the ratio of expression level in control versus the average of the two miR-125b–overexpressing arrays. To identify miR-125b targets, we chose 6,000 down-regulated (top 15%) probesets and intersected them with the Targetscan v5.1 computed miR-125b targets.

Quantitative PCR Analysis. Total RNA was isolated from sorted cell populations using TRIzol according to the manufacturer's instructions and precipitated using glycogen as a carrier. RNA samples were subjected to first-strand synthesis according to the manufacturer's instructions (Superscript III; Invitrogen). The cDNA was then processed through a preamplification step with the final PCR reactions using Taqman probes run on an ABI 7900 machine. The qPCR results were normalized using GAPDH as endogenous control.

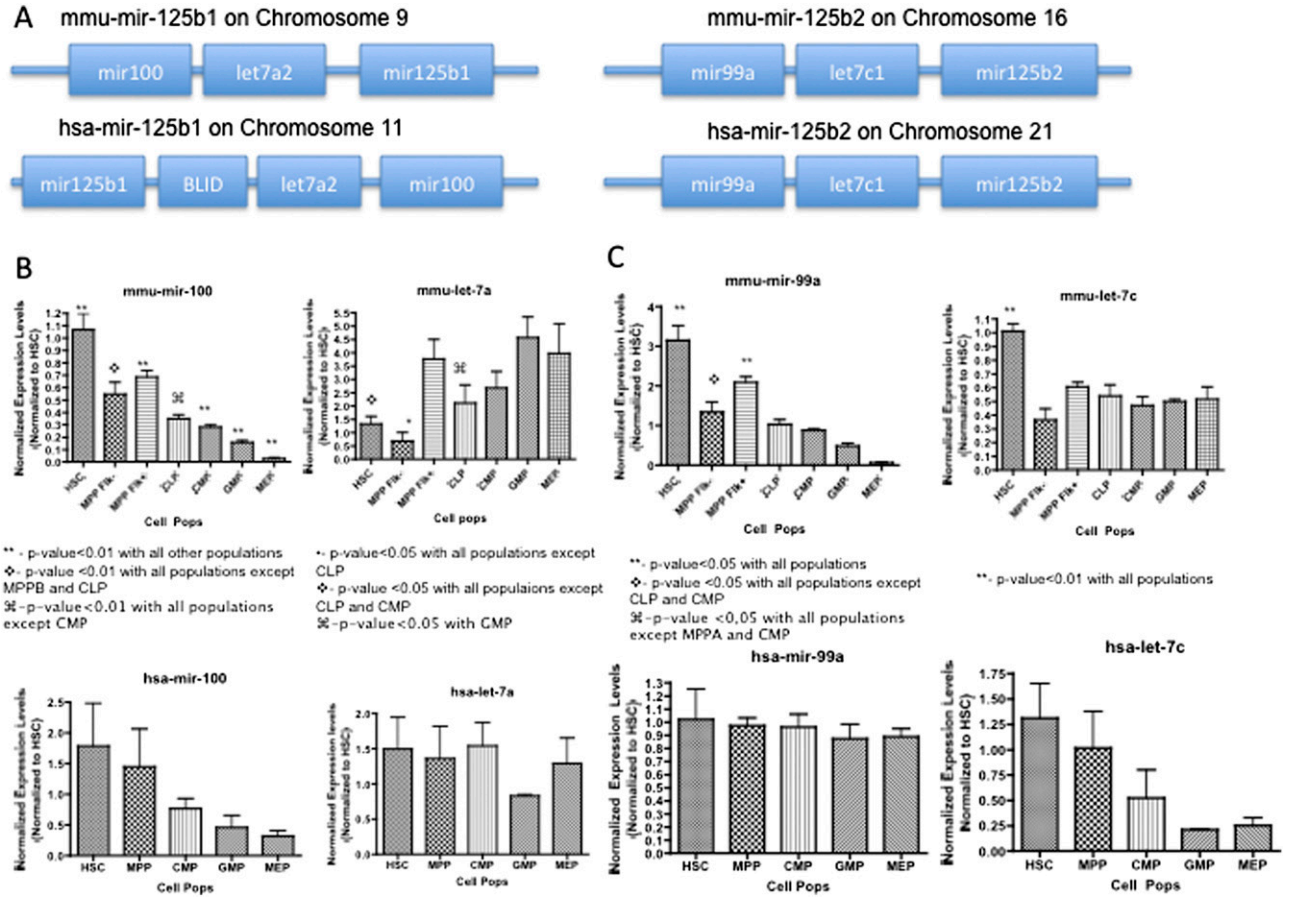


Fig. S1. miR-100 and miR-99a demonstrate expression trends in hematopoietic stem and progenitor populations similar to miR-125b. (*A*) Representation of mir-125b clusters in both mice and human. (*B*, *C*) Normalized expression levels of miR-125b1 and miR-125b2 cluster members were determined by quantitative PCR using miRNA Taqman probes in double-sorted mouse and human hematopoietic cell populations: hematopoietic stem cell (HSC), multipotent progenitor (MPP) Flk^-, MPP Flk^+, common lymphoid progenitor (CLP), common myeloid progenitor (CMP), granulocyte-monocyte progenitor (GMP), and megakaryocyte-erythrocyte progenitor (MEP) cells. Expression was normalized against mmu-mir-16 for mouse cell populations and sno-R2 for human cell populations ($n = 5$). Cell populations defined as in Fig. 1. Error bars denote SEM.

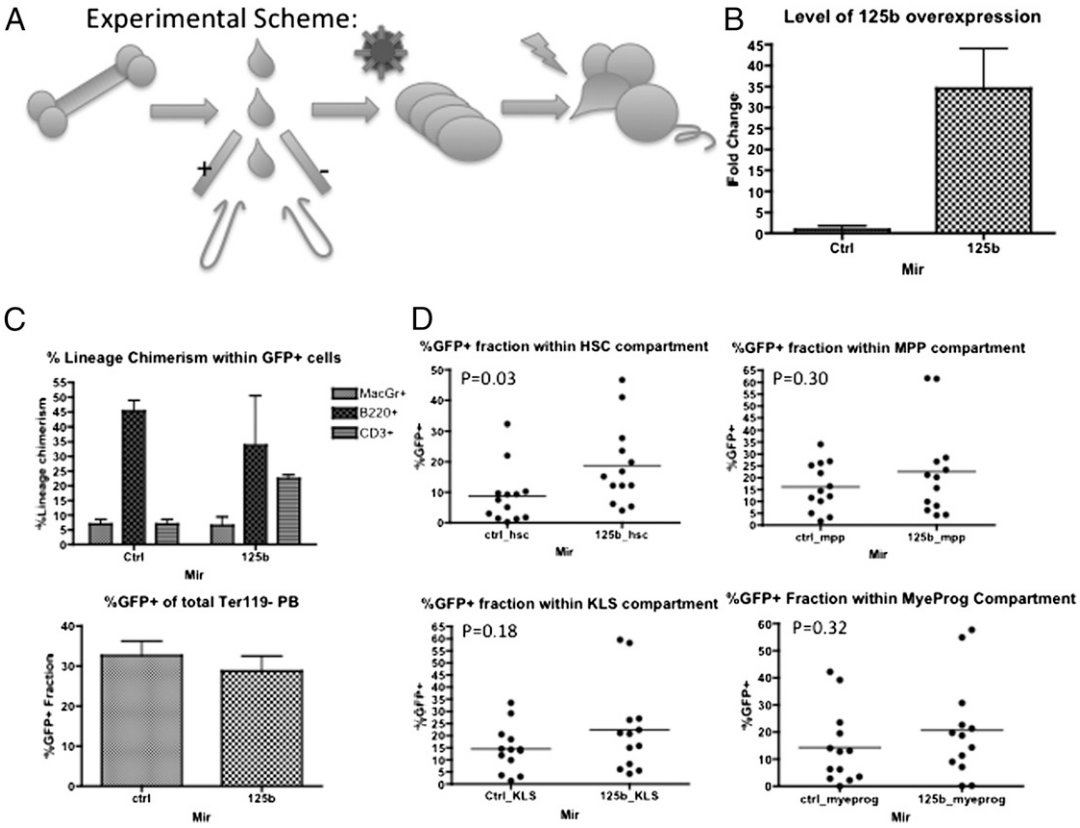


Fig. S2. Transplanted microRNA-125b overexpressing HSC result in higher HSC chimerism and give rise to lymphomyeloid cells. (*A*) Experimental scheme. Phenotypic HSC (c-kit$^+$Sca1$^+$Lin$^-$CD34$^-$Flk2$^-$ cells) were double-sorted from mouse bone marrow and transduced with control or miR-125b overexpressing lentivirus. The cells were transplanted into lethally irradiated recipients with helper marrow. (*B*) The level of miR-125b overexpression in transduced, unfractionated bone-marrow cells was measured using quantitative PCR and miRNA Taqman probes (n = 3). (*C*) Tail bleeds from transplanted mice were analyzed for GFP$^+$ cells in the peripheral blood and their distribution among granulocyte (Mac$^+$Gr$^+$), lymphoid B (B220$^+$), and lymphoid T (CD3$^+$) cells. (*D*) After more than 10 wk, the bone marrow of transplanted mice were analyzed by flow cytometry for GFP$^+$ chimerism levels within the different HSC and progenitor compartments (n = 14). Error bars denote SEM.

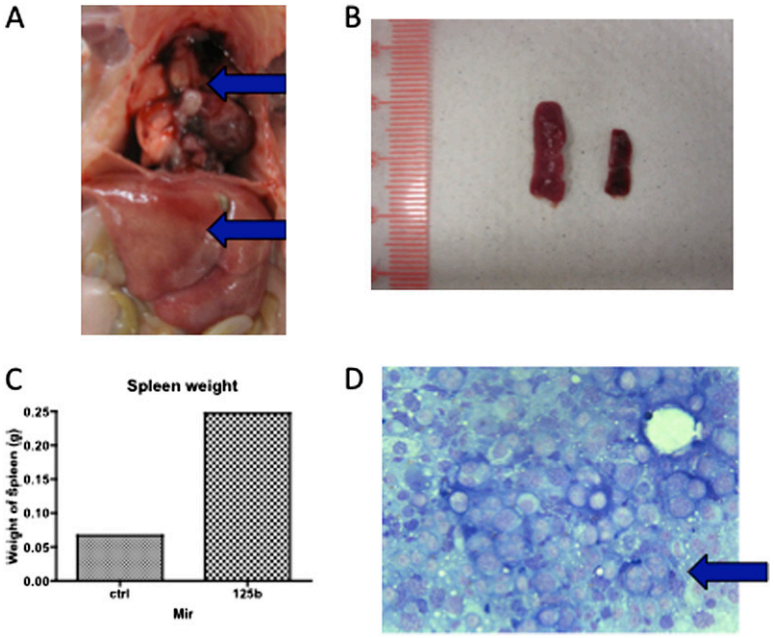
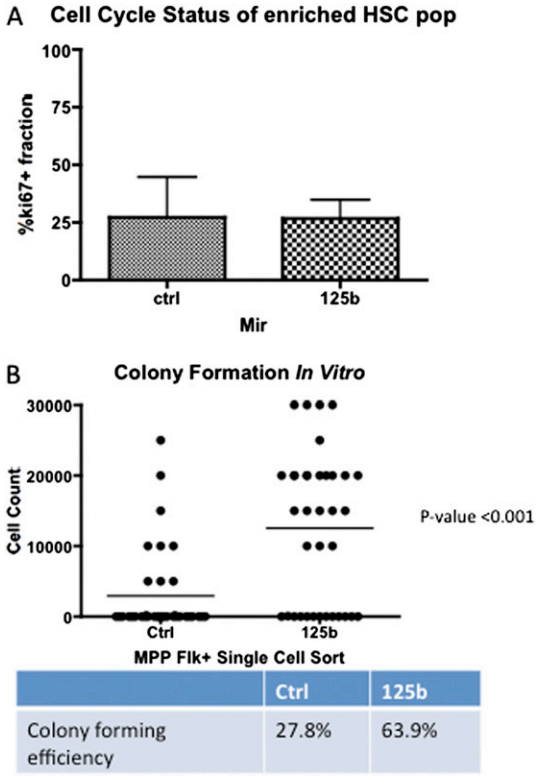


Fig. S3. MicroRNA-125b chimeric mice develop a lymphoproliferative disease with low penetrance. (*A*) Development of a lymphoproliferative disease. The mice developed enlarged lymph nodes (*Upper* arrow) and lymphocyte infiltration of the liver (*Lower* arrow). (*B*) The mice also developed splenomegaly. (*C*) Graphic representation of the spleen weight of one mouse with lymphoproliferative disease compared with control mouse. (*D*) A touch prep of the infiltrated liver exhibits numerous large, abnormal, multinucleated lymphocytes (arrow).



	Ctrl	125b
Colony forming efficiency	27.8%	63.9%

Fig. S4. Overexpression of miR-125b does not alter the cell-cycle status of bone marrow progenitors but increases their survival in culture. (*A*) Cell-cycle analysis by flow cytometry of the immature c-Kit^{+}CD34^{-}Lin^{-} compartment in the bone marrow using Ki67 as a proliferation marker (n = 5). Error bars denote SEM. (*B*) Single MPP Flk^{+} cells expressing control and miR-125b overexpressing constructs were sorted for in vitro culture and incubated at 37 °C for 1 wk (n = 36).