SUPPLEMENTAL MATERIAL

Li et al. http://www.jem.org/cgi/content/full/jem.20100504/DC1

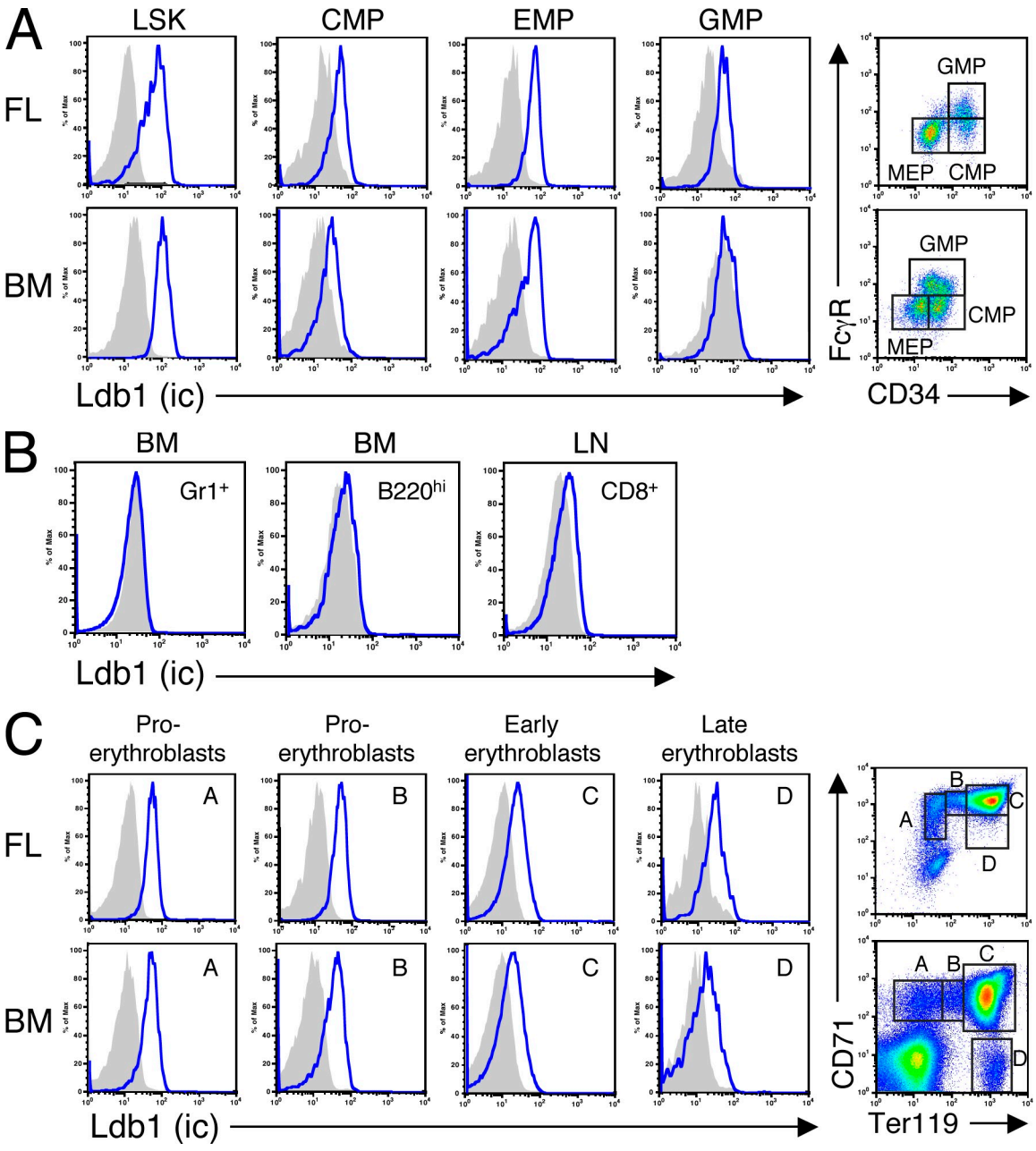

Figure S1. Ldb1 expression in hematopoietic cells. Ldb1 intracellular staining was performed on single cell suspensions prepared from adult C57BL/6 bone marrow cells or from E15 C57BL/6 fetal liver cells. Histograms show Ldb1 expression in gated populations. Rabbit anti-Ldb1, solid line; rabbit IgG, shaded area. (A) Ldb1 staining of gated LSK progenitor cells and lineage committed progenitor populations. Two color plots show gates used for designation of CMP, GMP, and MEP. (B) Ldb1 staining of granulocytes ($Gr1^+$) and B lymphocytes ($B220^{hi}$) in bone marrow and T lymphocytes ($CD8^+$) in lymph nodes. (C) Ldb1 staining of erythroblasts defined by Ter119 and CD71 staining. FL, fetal liver; BM, bone marrow. Plots shown are representative of at least three experiments for each staining. Two-color plots show gates used for erythrocyte developmental staging.

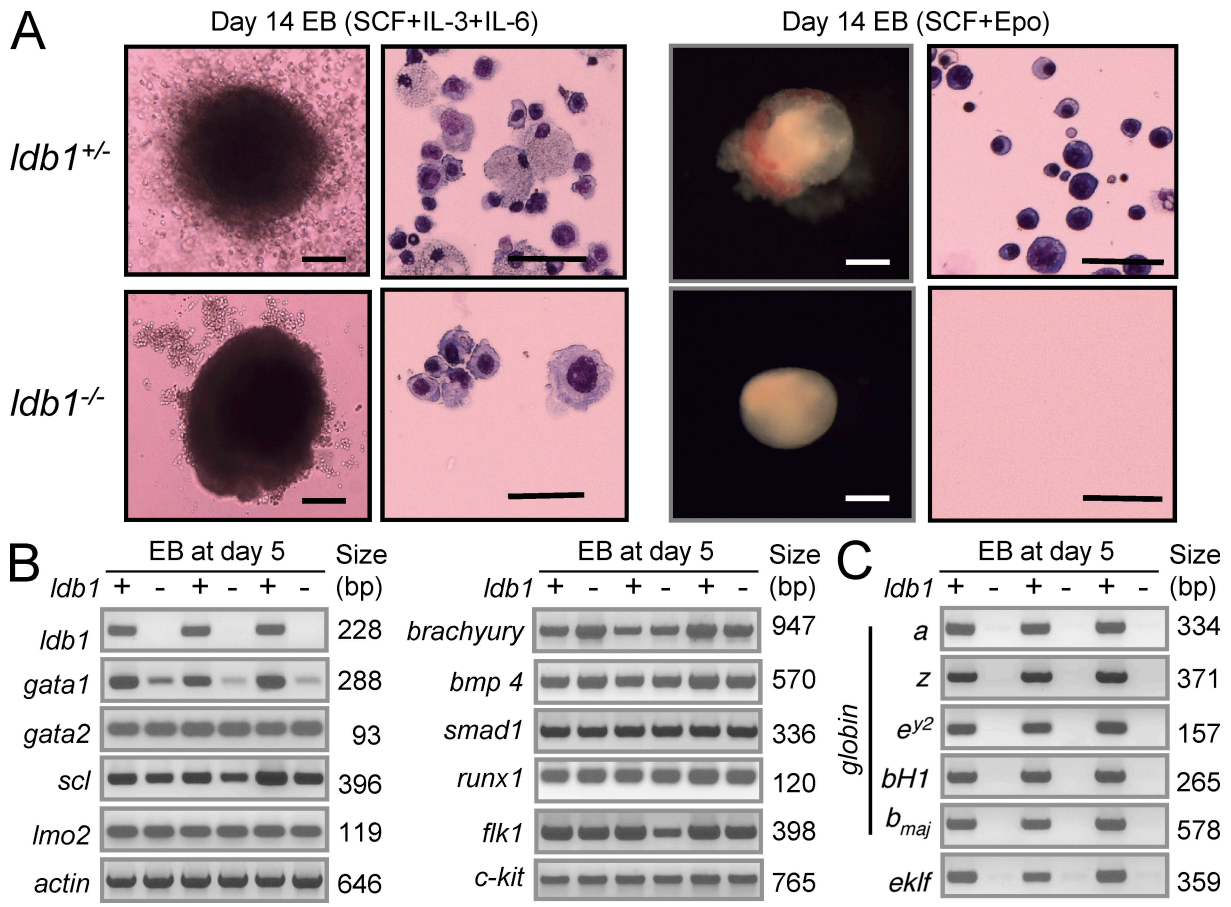

Figure S2. Impaired erythroid developmental potential of *ldb1*$^{-/-}$ ESCs. (A) *ldb1*$^{-/-}$ ESCs are capable of generating embryoid bodies and myeloid lineage cells in vitro but fail to produce erythroid lineage cells. Embryoid bodies were generated in primary liquid cultures in medium supplemented with SCF and then cultured in methylcellulose medium plus SCF, IL-3, and IL-6 (left) or methylcellulose medium plus SCF and erythropoietin (Epo; right). Shown are images of representative embryoid bodies and giemsa-stained cytospins of hematopoietic cells from methylcellulose cultures. Bars: (columns 1 and 3) 200 µm; (columns 2 and 4) 50 µm. One representative of at least three experiments is shown. (B and C) Gene expression in day 5 embryoid bodies generated from *ldb*$^{+/-}$ or *ldb*$^{-/-}$ ES cells assessed by RT-PCR. (B) Expression of Ldb1 complex subunit genes and ventral mesoderm/hemangioblast genes. (C) Expression of erythroid genes. Lanes 1, 3, and 5: *ldb1*$^{+/-}$; lanes 2, 4, and 6: *ldb1*$^{-/-}$. Embryoid bodies were cultured in suspension with SCF (lanes 1 and 2) or SCF, IL-3, IL-6, and Epo (lanes 3 and 4) or in methylcellulose medium with SCF, IL-3, IL-6, and Epo (lanes 5 and 6). In B and C, results shown are representative of two experiments.

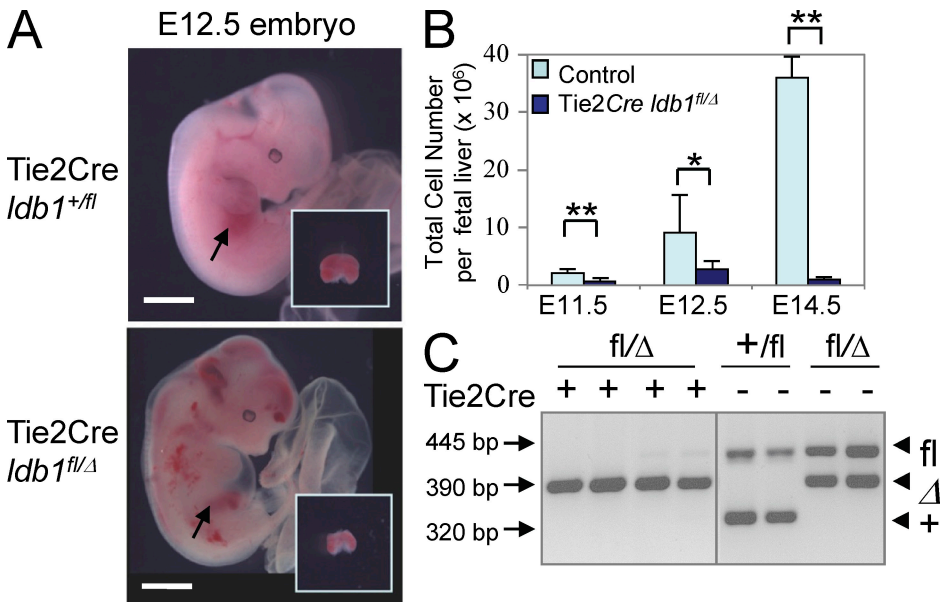

Figure S3. Defective erythropoiesis in Tie2Cre *ldb*$^{fl/\Delta}$ embryos. (A) Anemia, hemorrhage, and decreased fetal liver size in E12.5 Tie2Cre *ldb*$^{fl/\Delta}$ embryos. Embryos shown are littermates from a Tie2Cre *ldb*$^{+/\Delta}$ × *ldb*$^{fl/fl}$ mating. Fetal livers are shown in situ (arrows) and after removal (insets). Bars, 600 µm. (B) Decreased fetal liver cellularity in Tie2Cre *ldb*$^{fl/\Delta}$ embryos. Total numbers of fetal liver cells in control (*ldb*$^{+/fl}$, *ldb*$^{fl/\Delta}$, and Tie2Cre *ldb*$^{+/fl}$) embryos and Tie2Cre *ldb*$^{fl/\Delta}$ littermates at the gestation day shown. *, $P < 0.05$; **, $P < 0.01$. Values are expressed as means ± SD. Control, E11.5 ($n = 12$), E12.5 ($n = 7$), and E14.5 ($n = 4$); Tie2Cre *ldb*$^{fl/\Delta}$, E11.5 ($n = 5$), E12.5 ($n = 3$), and E14.5 ($n = 3$). (C) *ldb1* gene deletion in E12.5 Tie2Cre *ldb*$^{fl/\Delta}$ fetal liver cells. DNA from fetal liver cells was analyzed by PCR for the presence of the *ldb1* floxed allele (fl), wild type allele (+), and deleted allele (Δ).

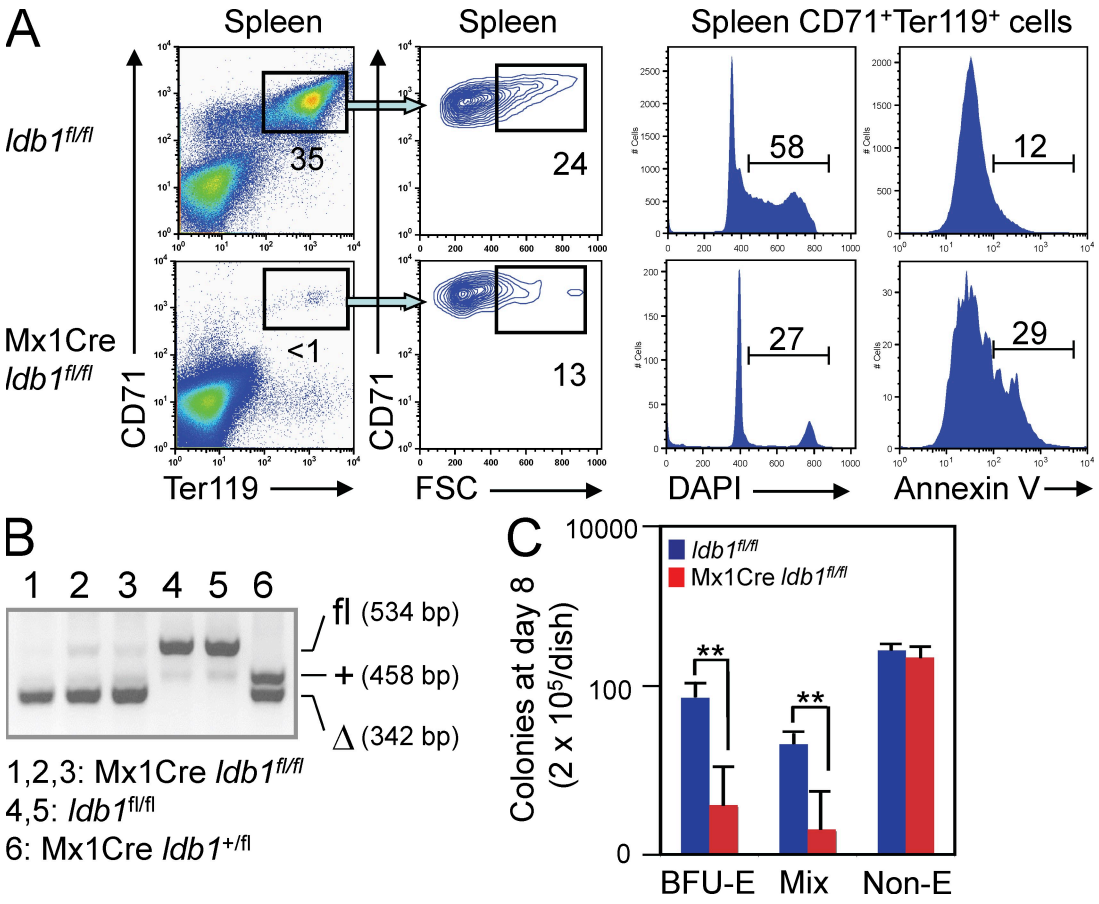

Figure S4. Ldb1 is continuously required for survival and expansion of erythroblasts in adults. (A) $CD71^{hi}Ter119^{lo}$ proerythroblasts and $Ter119^{+}CD71^{+}$ erythroblasts in spleen of indicated mice after pI:pC injection. Numbers are percentage of cells in the indicated gate. Histograms show percentage of cycling ($DAPI^{int-hi}$) and apoptotic (annexin V^{+}) $Ter119^{+}CD71^{+}$ erythroblasts. Mice were injected with pI:pC on days 1, 3, and 5 and then sacrificed on day 8. Splenocytes were stained with CD71, Ter119, DAPI, and annexin V. $CD71^{+}Ter119^{+}$ blasts are reduced in pI:pC-injected Mx1Cre *ldb1*$^{fl/fl}$ mice. In addition, there is a reduction in cycling ($DAPI^{int-hi}$) cells and an increased percentage of apoptotic (annexin V^{+}) cells in pI:pC-injected Mx1Cre *ldb1*$^{fl/fl}$ mice. Data shown are representative of four MxCre *ldb1*$^{fl/fl}$ mice and six littermate controls. Controls included *ldb1*$^{fl/fl}$ mice and *ldb1*$^{fl+}$ mice ± MxCre. (B) Deletion of *ldb1* in randomly selected myeloid colonies derived from total bone marrow cells obtained from pI:pC-treated mice. (C) Loss of erythroid colony forming potential after deletion of *ldb1*. Mice were injected with pI:pC on days 1 and 2 and sacrificed on day 3. Bone marrow cells were cultured in methylcellulose medium containing SCF, IL-3, IL-6, and Epo. Numbers of erythroid (BFU-E), mixed (Mix), and non-erythroid (Non-E) colonies on day 8 of methylcellulose culture. **, $P < 0.01$. Data are obtained from five MxCre *ldb1*$^{fl/fl}$ mice and seven littermate controls. Values are expressed as means ± SD.