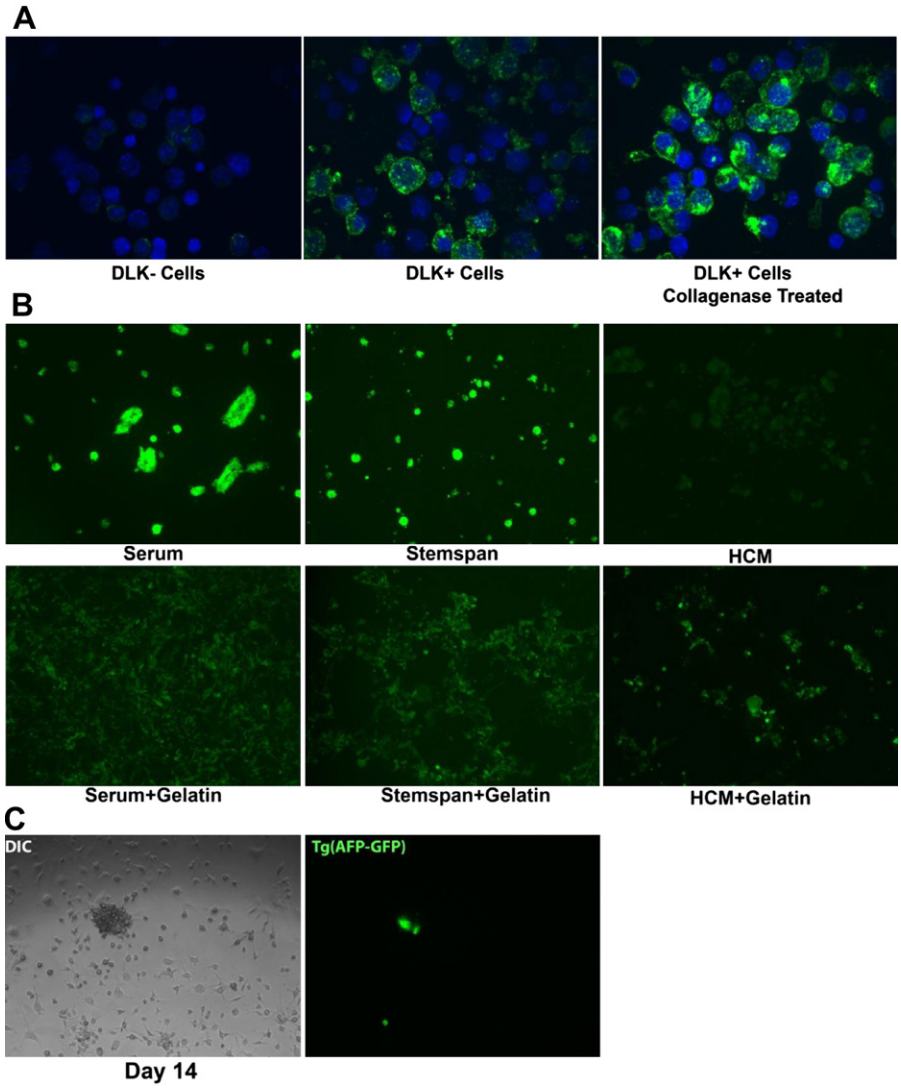
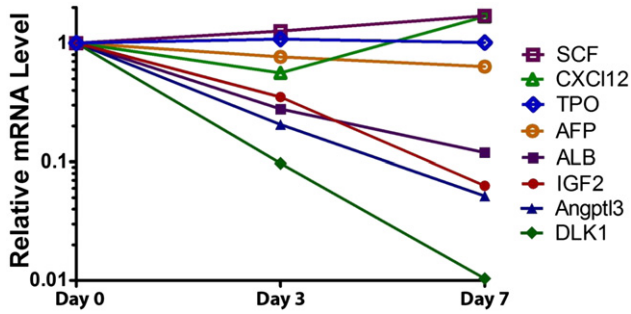
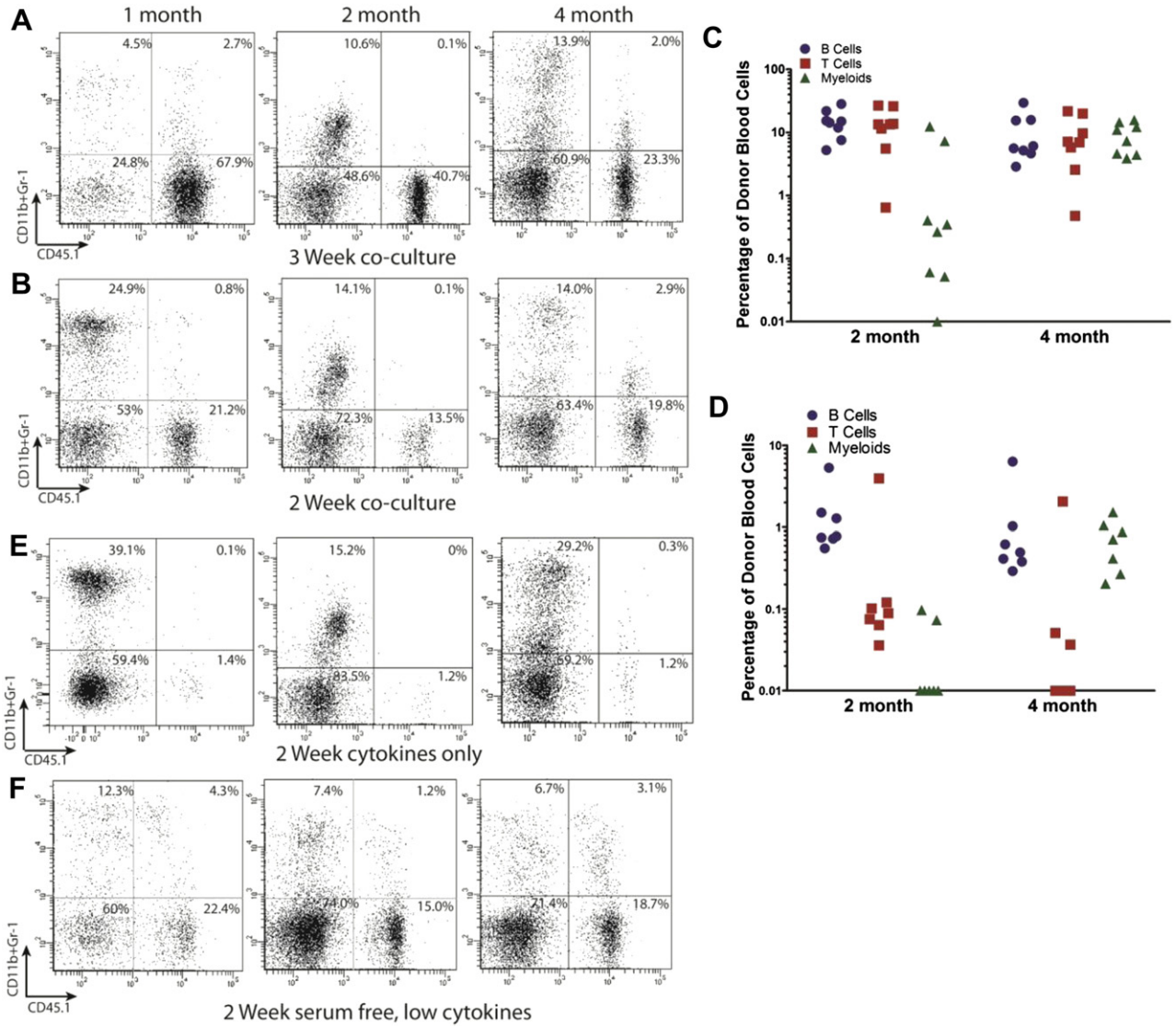


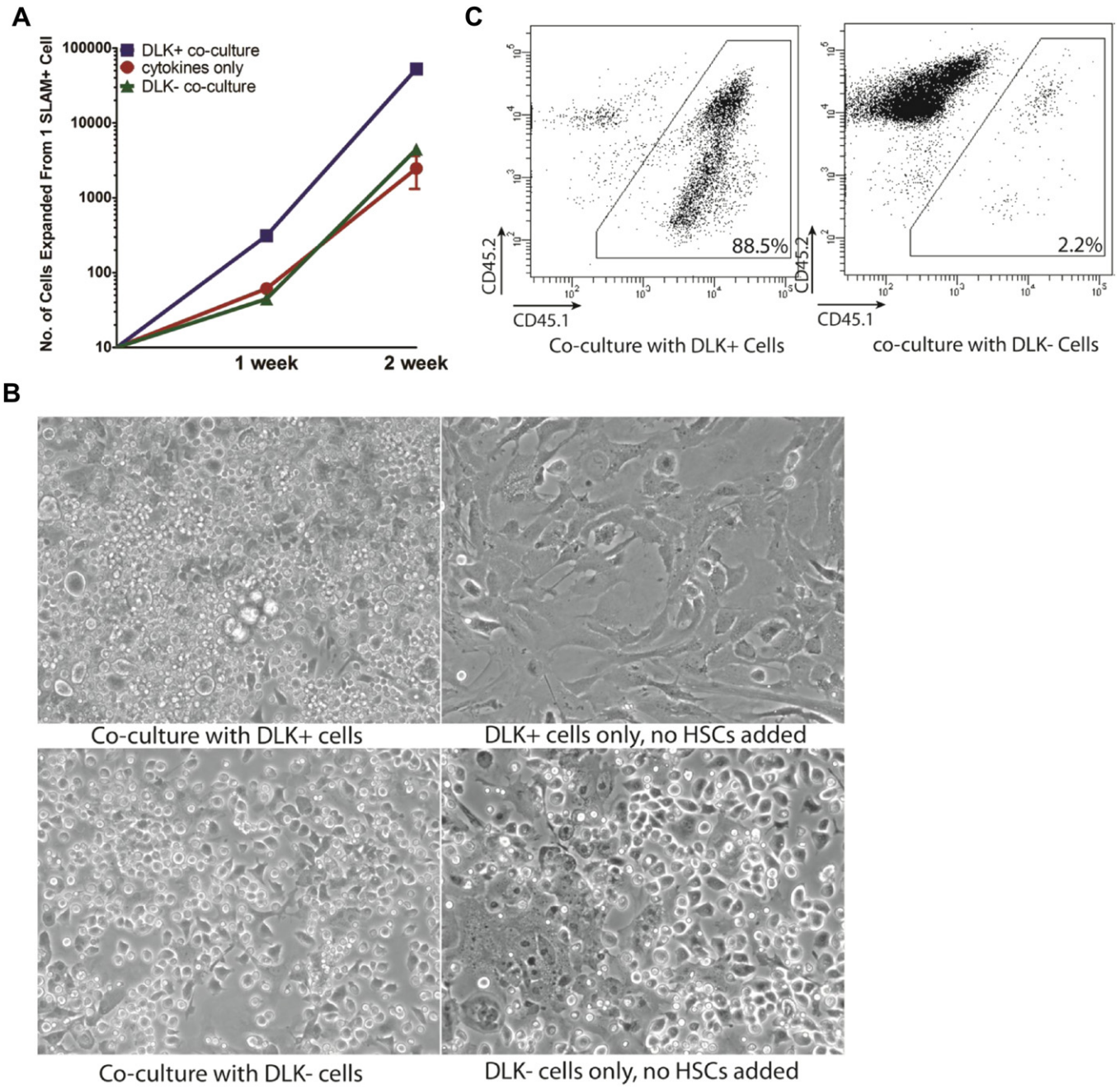
Supplementary Figure 1. Ex vivo–cultured fetal DLK^+ cells can survive for an extend period in both serum containing medium and serum-free stem span medium. **(A)** Magnetic bead purification of DLK^+ cells from E15.5 liver. Total fetal liver cells were stained with FITC-conjugated DLK1 antibody followed by anti-FITC magnetic beads. The DLK^- (left) and DLK^+ (middle) cells were separated using an automatic cell separator. Collagenase treatment before purification further improved the purity of $DLK1^+$ cells. **(B)** Comparison of the survival of DLK^+ cells in serum-containing medium, serum-free StemSpan medium, both of which are also capable of supporting hematopoietic cells and hepatocyte-defined medium (HDM; BD Biosciences), a serum-free medium specifically formulated for adult hepatocytes. GFP^+ hepatic remained alive after 7 days in both serum and StemSpan medium after 7 days, but died in HDM (top row). Culturing DLK^+ cells on gelatin-coated plates improved their survival, and hepatic cells spread and formed monolayers (bottom row). On both regular culture plates and gelatin-coated plates, hepatic cells survived the best in serum medium. **(C)** Very little hematopoietic cell growth surrounding DLK^+ hepatic cells in StemSpan medium after 14 days.



Supplementary Figure 2. The expression of many growth factors by DLK^+ cells is downregulated during ex vivo coculture. Indicated is the relative abundance of mRNA for each gene before culture and after 3 and 7 days of culture in serum-containing medium. The relative expression of each gene at each time point was calculated by setting the level of each mRNA before culture as one. Although expression of several HSC supportive factors such as SCF, TPO, and CXCl12 was maintained during culture, expression levels of DLK1, Angptl3, and IGF2 dropped quickly.



Supplementary Figure 3. Long-term culture of HSCs in serum containing STF medium causes a temporary myeloid reconstituting defect. Representative FACS analysis of donor-derived ($CD45.1^+$) myeloid ($CD11b^+$ or $Gr\text{-}1^+$) reconstitution of recipient mice at 1, 2, and 4 months after transplantation. Mice transplanted with HSCs expanded by 2 weeks **(A)** and 3 weeks **(B)** of coculture showed a myeloid reconstituting defect at month 2. This reconstituting defect is limited to myeloid lineage (and possible erythroid lineage) only; B and T lineages appear to be completely normal **(C)**. A similar defect was also observed in mice transplanted of HSCs cultured in cytokines only **(D, E)**. Coculture in serum-free, low-cytokine medium completely eliminated this problem **(F)**.



Supplementary Figure 4. DLK^- cells failed to expand hematopoietic cells in serum containing STF medium after 3 weeks of coculture. HSCs were co-cultured with DLK^+ cells, a proportional amount of DLK^- cells, or cytokines only in serum medium for 2 weeks. The numbers of hematopoietic cells expanded were counted using a hemocytometer. Fibroblastic cells from DLK^- cell population grew rapidly, and a small portion of hematopoietic progenitors remained and expanded in serum medium; therefore, significant amount of cells were present in control wells with only DLK^- cells (**B**, bottom right). We therefore subtracted the number of cells from control wells without HSCs added from the total number of cells after coculture. As **(A)** shows, coculture with DLK^- cells did not expand hematopoietic cells when compared with cytokines only. For week 3, coculture with DLK^+ cells expanded sixfold more cells than coculture with DLK^- cells. FACS analysis shows that 88.5% of total cells expanded by coculture with DLK^+ cells are donor-derived $CD45.1^+$ cells (C, left panel). In contrast, only 2.2% of cells expanded by coculture with DLK^- cells are $CD45.1^+$ (**C**, right panel). The rest of the cells were mainly $CD45.2^+$ contaminating hematopoietic cells from the fetal liver. Therefore, coculture with DLK^+ cells expanded more than 200-fold of hematopoietic cells than coculture with DLK^- cells.