Figure S1. SHP2 knockdown inhibits CD34+ cell differentiation during the extend culture.

(A) CD34+DsRED+ cells were cultured with high concentrations of GFs (IL-3+SCF+G-CSF+GM-CSF+Epo) and cell differentiation towards both myeloid (CD11b+ and CD14+) and erythroid lineages (GPA+) was analyzed during the culture for up to 11 days. SHP2 shRNA transduced CB CD34+ cells generated significantly lower amount of both myeloid and erythroid lineage cells during the culture compared with sh-Ctrla. Combined results from 3 experiments are shown. Results shown represent the Mean ± SEM. Significance: * P<0.05 compared with sh-Ctrla.

(B) CD34+DsRED+ cells were cultured under GF conditions that promoted myeloid (IL-3+SCF+G-CSF+GM-CSF) differentiation. The number of myeloid cells (CD11b+ and CD14+) generated in culture were analyzed. Results shown represent the Mean ± SEM of 4 experiments. Significance: * P<0.001 for sh-Shp2-1 and sh-Shp2-2 compared with sh-Ctrla.

(C) CD34+DsRED+ cells were cultured under GF conditions that promoted erythroid (SCF+EPO) differentiation. The number of erythroid lineage differentiation (GPA+) generated under these conditions was analyzed. Results shown represent the Mean ± SEM of 4 experiments. Significance: * P<0.01 and **P<0.001 for sh-Shp2-1 and sh-Shp2-2 compared with sh-Ctrla.
Figure S2. SHP2 knockdown results in impaired engraftment in NOD/SCID/γ chainnull (NSG) mice
5 × 10⁴ to 2 × 10⁵ FACS sorted Sh-Ctrl and Sh-Shp2-2 transduced CD34⁺DsRED⁺ cells were transplanted into sublethally irradiated NSG mice by tail vein injection. Human CD45⁺ cell engraftment in bone marrow from each mouse was evaluated at 8 to 14 weeks post-transplantation using flow cytometry. Total numbers (A) and percentages (B) of human CD45⁺ cells in bone marrow from 2 femurs are shown. Knockdown of Shp2 resulted in significantly reduced engraftment of human cells in NSG mice (*P<0.05 for the comparison of total numbers of engrafted human CD45⁺ cells; and **P<0.01 for the comparison of the percentage of human CD45⁺ cell engraftment in bone marrow between sh-Ctrl and sh-SHP2-2 using Mann Whitney test).
Figure S3. SHP2 knockdown results in impaired cell expansion and cell survival in response to individual growth factors and cytokines
FACS sorted Sh-Ctrla and Sh-Shp2-2 transduced CD34⁺DsRED⁺ cells were cultured with single growth factors (SCF or Flt-3 ligand) or cytokines (IL-3 or GM-CSF) at high concentration (100ng/ml). Cell expansion (A) expressed as fold changes relative to input cell numbers, and apoptosis (B) represented by Annexin V⁺ staining (B) were evaluated 48 hours later. SHP2 knockdown resulted in significantly reduced cell expansion in response to IL-3 and GM-CSF compared to control shRNA, and decreased survival of cells cultured with IL-3 and SCF.
Figure S4. SHP2 knockdown results in increased phosphorylation of STAT5 and STAT3 at early but not late time points
Phosphorylation levels of STAT5 (A) and STAT3 (B) in CD34+ cell population were evaluated after culture for 3 days and 5 days using flow cytometry. Phosphorylation levels of STAT5 and STAT3 were expressed as values relative to IgG control. A representative plot is shown in Fig. 6C.