Table S1. Primers for qRT-PCR

Gene	Forward (5'-3')	Reverse (5'-3')
Angptl3	ACAGAGCAAAGGGACAGCTC	TGGAGCATCATTTTGGATGA
HoxA9	TGGTTCTCCTCCAGTTGATAG	AGAAACTCCTTCTCCAGTTCC
Mef2c	AGATCTGACATCCGGTGCAG	TCTTGTTCAGGTTACCAGGTG
Meis1	CAGGACTTACCATCCTTCAAGTG	GCGCTCTGATGCCCATGTGC
Pbx3	AGAGCCAAATTGACCCAGAT	ATGGGACGCGTTCTACTCTG
PU.1	CCCTCCATCGGATGACTTGGTTAC	GCTTCTCCATCAGACACCTCCAG(
GATA1	CAGAACCGGCCTCTCATCC	TAGTGCATTGGGTGCCTGC
C/EBP ɑ	AAGAAGTCGGTGGACAAGAACAG	GTTGCGTTGTTTGGCTTTATCTC
FOG1	CACCCTGTGCAGGAACCAGT	GGGTTTCTCTTCCGTCGCCG
GATA3	AGAACCGGCCCCTTATCAA	AGTTCGCGCAGGATGTCC
Ikaros	CTTTCCAGTGCAACCAGTGT	GTGAGGCTTACCAACGGAGT
Hes1	GAGGCTGCCAAGGTTTTTGG	GCTGGTGTAGACCGGGATGA
Nestin	CTTCCCTGATGATCCAACCT	ACCTCTGTGGCTGCTTCTTT
SDF1	CAAGGTCGTCGCCGTGCTG	CGTTGGCTCTGGCGATGTGG
SCF	GTCATTGTTGGCTACGAGATA	AACACGAGGTCATCCACTATT
TPO	TCCCAGGAATTTGTCTCAGG	GATCGCTAGCTGCTCTGATG
Delta1	CACTATGGACAGTTGCTTTGA	TGGCTCATAGTAATCCAAGATA
Jagged1	TGGTTGGCTGGGAAATTGA	TGGACACCAGGGCACATTC
TGF-β	CACCGGAGAAGAGCCCTGGATA	TGCCGCACACAGCAGTTC
Wnt3a	CACCACCGTCAGCAACAGCC	AGGAGCGTGTCACTGCGAAAG
β-actin	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA

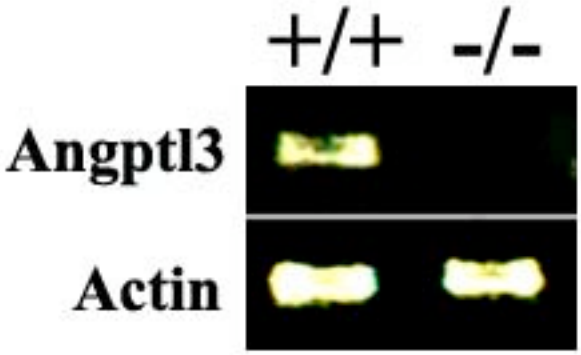

Figure S1. Angptl3-null mice do not express Angptl3 mRNA. Liver cells isolated from control (+/+) and Angptl3-null (-/-) mice were used for RT-PCR analysis to determine the level of expression of Angptl3 mRNA. Results are representative of three experiments.

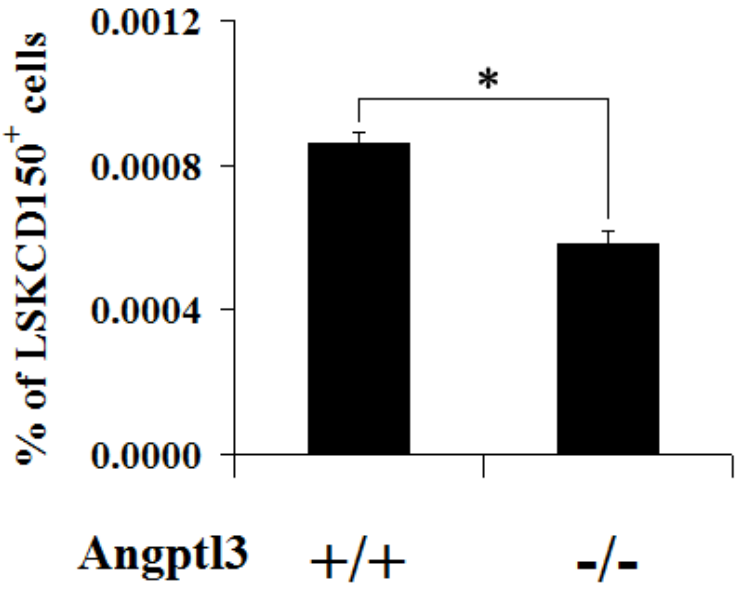

Figure S2. Decreased frequency of LT-HSCs in Angptl3-null mice. Shown are relative frequencies of LT-HSCs as $Lin^{-}Sca\text{-}1^{+}Kit^{+}$ $CD150^{+}$ cells in the BM of WT and Angptl3-null mice at 8-12 weeks (*, $p < 0.05$, $n = 4$).

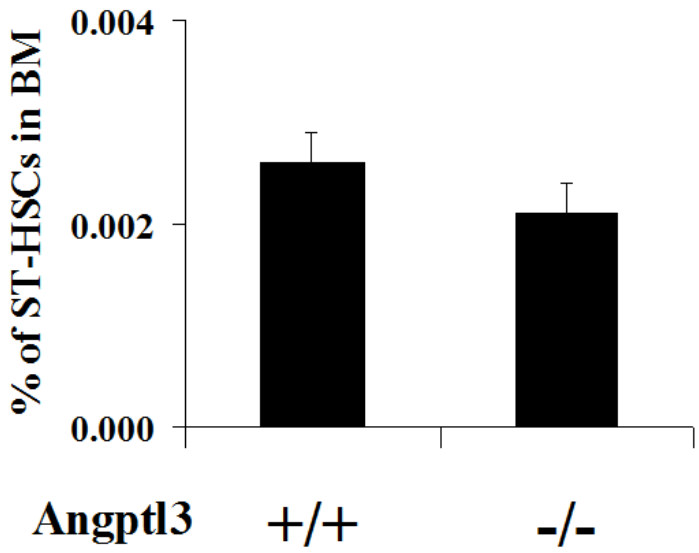

Figure S3. No significant difference of the frequencies of ST-HSCs in WT and Angptl3-null mice. Shown are relative frequencies of ST-HSCs as $Lin^{-}Sca\text{-}1^{+}Kit^{+}$ $Flk2^{+}CD34^{+}$ cells in WT and Angptl3-null BM at 8-12 weeks (*, $p < 0.05$, $n = 16$).

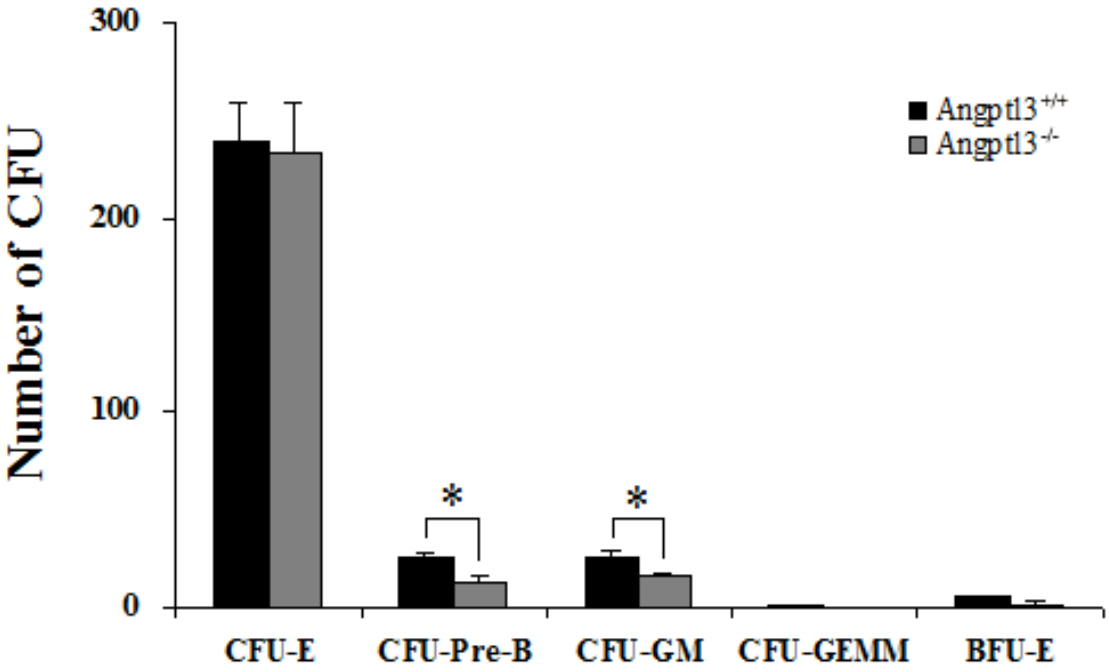

Figure S4. Myeloid progenitors and Pre-B progenitors, but not erythroid precursors, are decreased in Angptl3-null bone marrow compared to WT. Total BM cell populations were plated in methylcellulose medium M3434 (StemCell Technologies, Vancouver, Canada) for quantifying CFU-GM, CFU-GEMM, and BFU-E colonies, in M3630 for quantifying CFU-Pre-B colonies, and in M3334 for CFU-E colonies (n = 3).

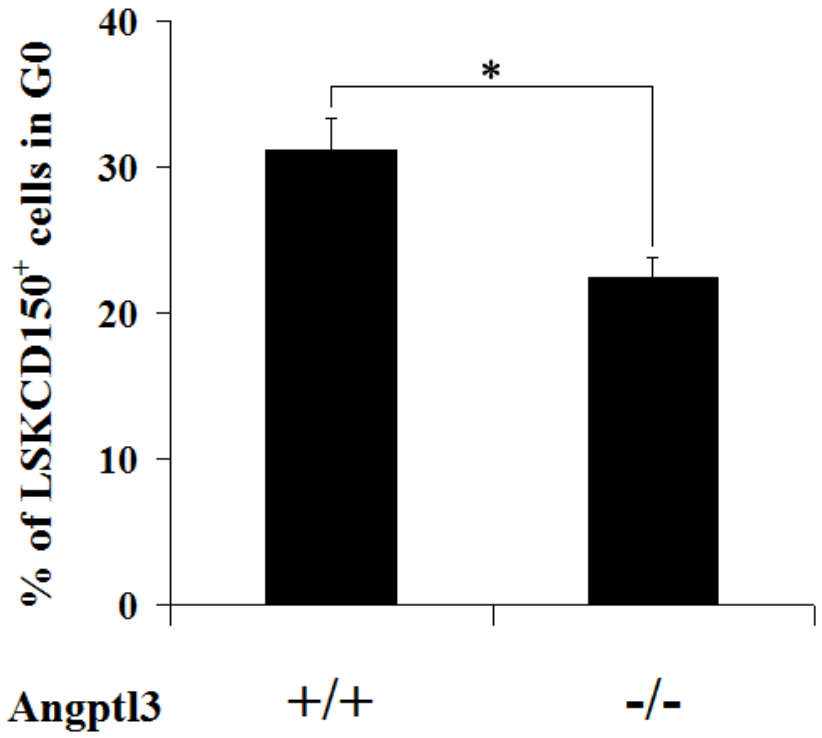

Figure S5 Angptl3-null BM HSCs are less quiescent than WT HSCs. LT-HSCs as $Lin^{-}Sca\text{-}1^{+}Kit^{+}CD150^{+}$ cells stained with Hoechst 33342 and pyronin Y, were analyzed for cell cycle stage.The percentages of G0 cells for Angptl3-null and WT cells are shown (*, $p < 0.05$, $n = 4$).

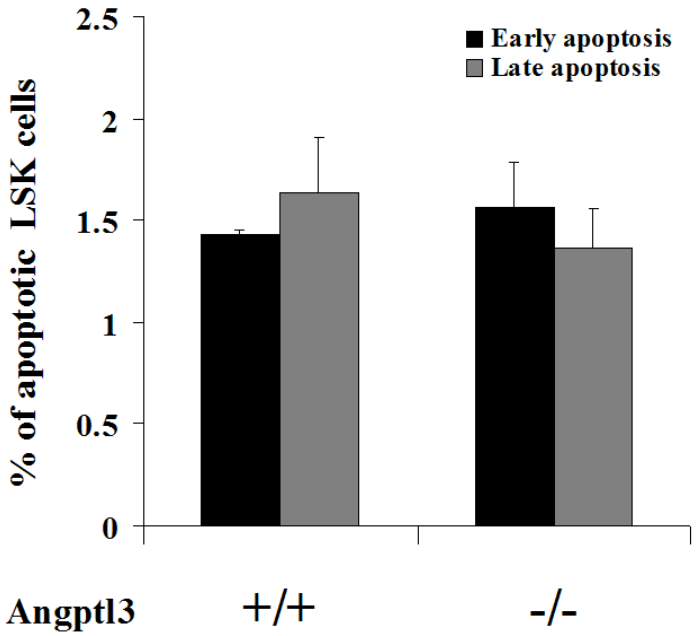

Fig. S6. WT and Angptl3-null HSCs have similar apoptotic status. WT or Angptl3-null BM $Lin^{-}Sca\text{-}1^{+}Kit^{+}$ cells were analyzed for apoptosis by using Annexin V and 7-AAD staining. Results showed there was no difference of early (Annexin $V^{+}/7\text{-}AAD^{-}$) or late apoptosis (Annexin $V^{+}/7\text{-}AAD^{+}$) between WT (+/+) and null (-/-) LSK cells (n = 4).

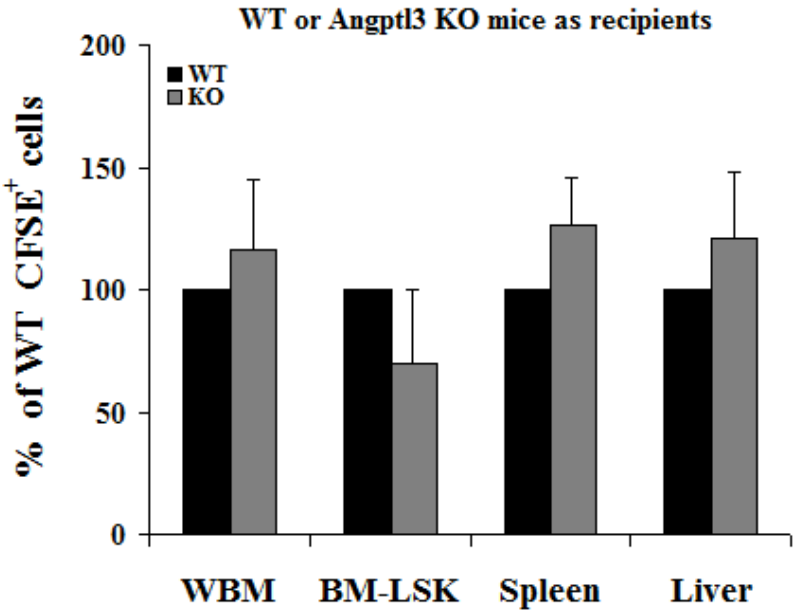

Figure S7. HSCs home equivalently to WT or to Angptl3-null recipient BM. BM from WT was labeled with 5-(and -6) carboxyfluorescein succinimidyl ester (CFSE), and 1 x 10^7 cells were transplanted into lethally irradiated WT or Angptl3-null mice (KO). After 16 hours, the total percentage of $CFSE^+$ cells in the BM, spleen or liver or $CFSE^+$ LSK ($CFSE^+Lin^-Sca\text{-}1^+Kit^+$) cells in BM was determined by flow cytometry (n = 5).

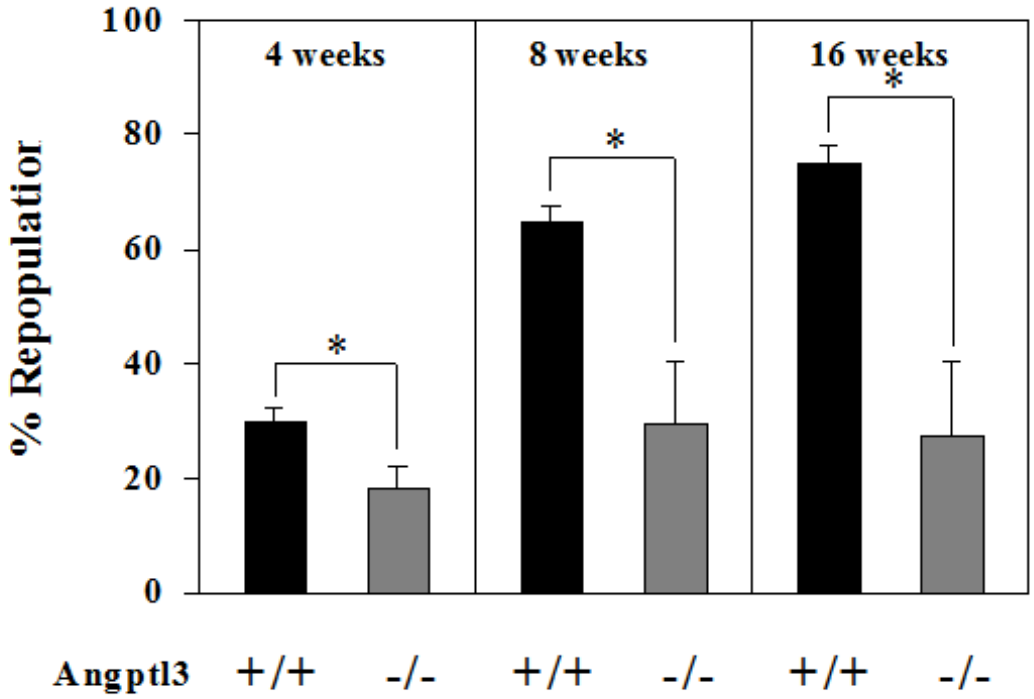

Figure S8. The deficiency of Angptl3 in HSCs results in decreased repopulation. WT or Angptl3-null donor CD45.2 $Lin^{-}Sca\text{-}1^{+}Kit^{+}Flk\text{-}2^{-}CD34^{-}$ cells (500 cells) were co-transplanted along with 1.5 x 10^5 freshly isolated CD45.1 bone marrow competitors into lethally irradiated CD45.1 wild-type recipient mice. The engraftment at 4, 8, and 16 weeks post-transplant is shown (*, $p < 0.05$. $n = 5$).

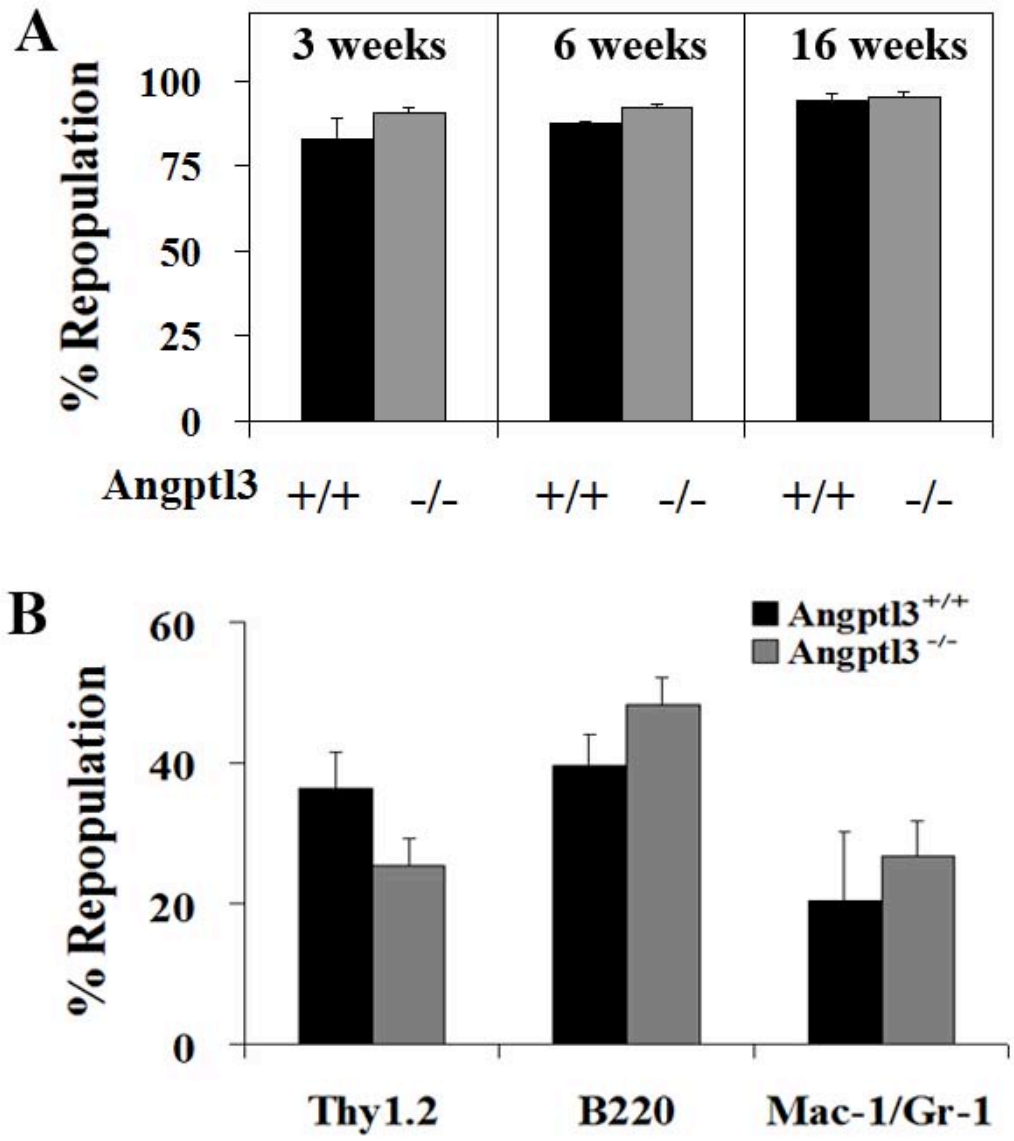

Fig. S9. Angptl3-null spleen cells have similar repopulation ability compared to WT counterparts. (A) Fifteen million of WT or Angptl3-null CD45.2 spleen cells were co-transplanted along with 1.5 x 10^5 freshly isolated CD45.1 bone marrow competitors into lethally irradiated CD45.1 wild-type recipient mice. The engraftment at 3, 6, and 16 weeks post-transplant is shown (n = 4). (B) Multilineage contribution of WT or Angptl3-null spleen cells in the transplanted recipients at 16 weeks post-transplant. The lineage contribution was shown as percentages of Thy1.2$^+$, B220$^+$, and Mac-1/Gr-1$^+$ cells in donor compartment.

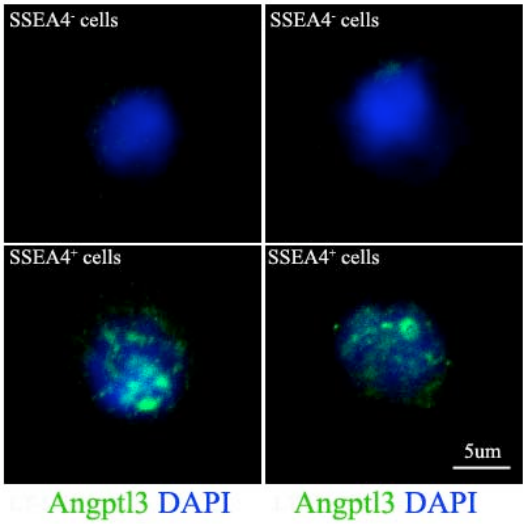

Figure S10. Angptl3 expression (green) in FACS-collected $CD45^{-}SSEA4^{-}$ cells (top panel) was much lower than that in $CD45^{-}SSEA4^{+}$ cells (bottom panel). Nuclei were counterstained with DAPI (blue). Scale bar applies to all the images.

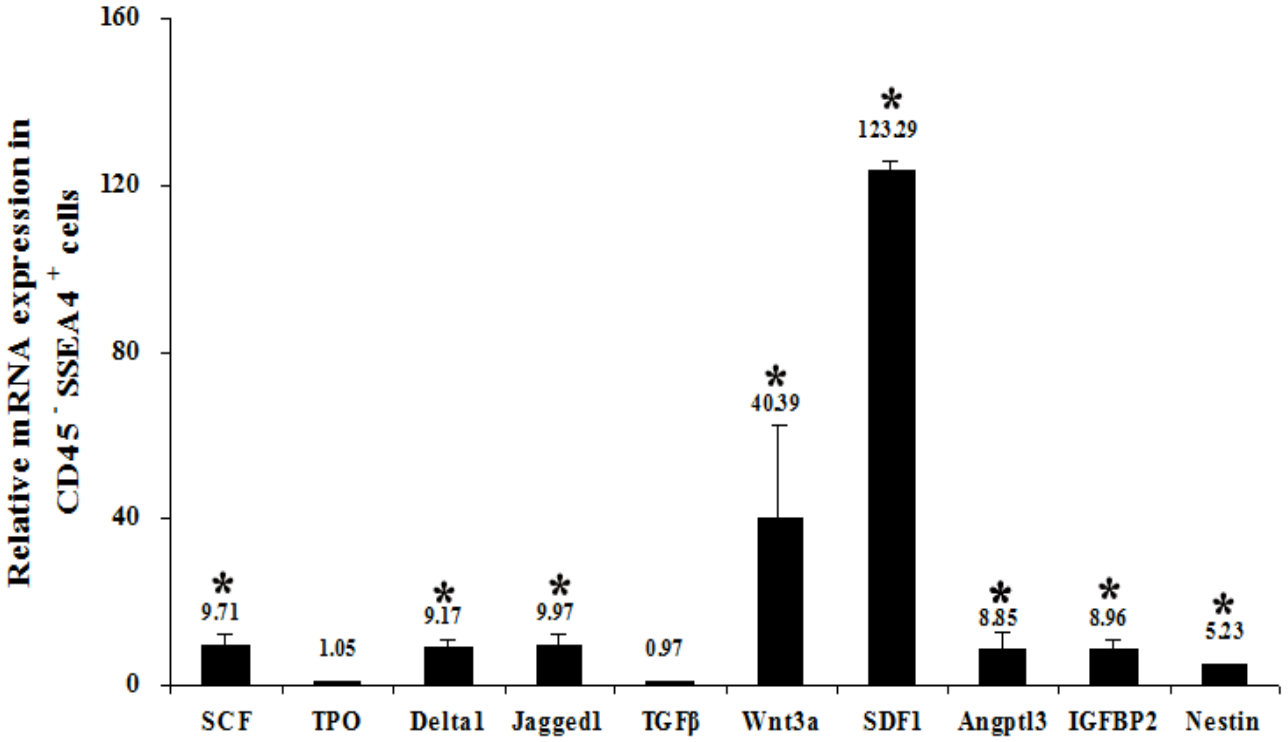

Fig. S11. Comparison of expression of cytokines in BM $CD45^{-}SSEA4^{+}$ cells and in CD45 $SSEA4^{-}$ cells by real-time RT-PCR analysis. All listed mRNAs were expressed at significantly higher levels in $SSEA4^{+}$ cells than in $SSEA4^{-}$ cells with the exception of TPO and TGF-β.

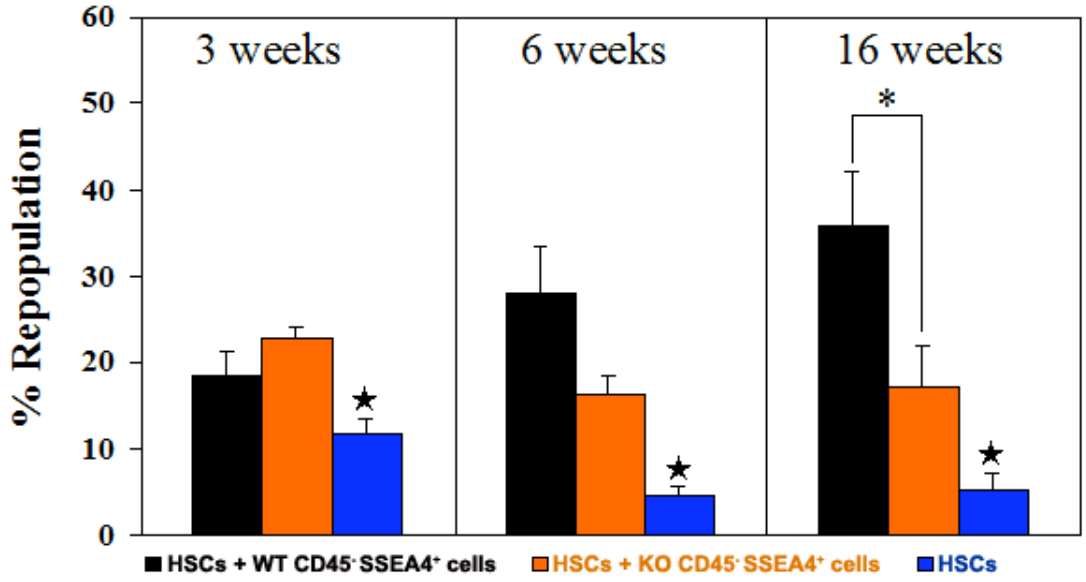

Figure S12. Angptl3-deficient BM $CD45^{-}SSEA4^{+}$ cells have decreased ability to support expansion of HSCs. One hundred and fifty BM CD45.1 $Lin^{-}Sca\text{-}1^{+}Kit^{+}Flk\text{-}2^{-}CD34^{-}$ cells were cultured alone (blue bar), co-cultured with 450 $CD45^{-}SSEA4^{+}$ BM cells isolated from CD45.2 WT mice (black bar), or co-cultured with the same number of $CD45^{-}SSEA4^{+}$ cells from Angptl3-null mice (orange bar) in serum-containing StemSpan supplemented with SCF, TPO, and FGF1. After 5 days, the co-cultured cells were co-transplanted with 1.5 x 10^{5} CD45.2 competitors into lethally irradiated CD45.2 recipient mice. Shown is the engraftment at 3, 6, and 16 weeks post-transplant (*, significantly different between two groups; ★, significantly different from co-cultured values. $p < 0.05$, $n = 5\text{-}7$).

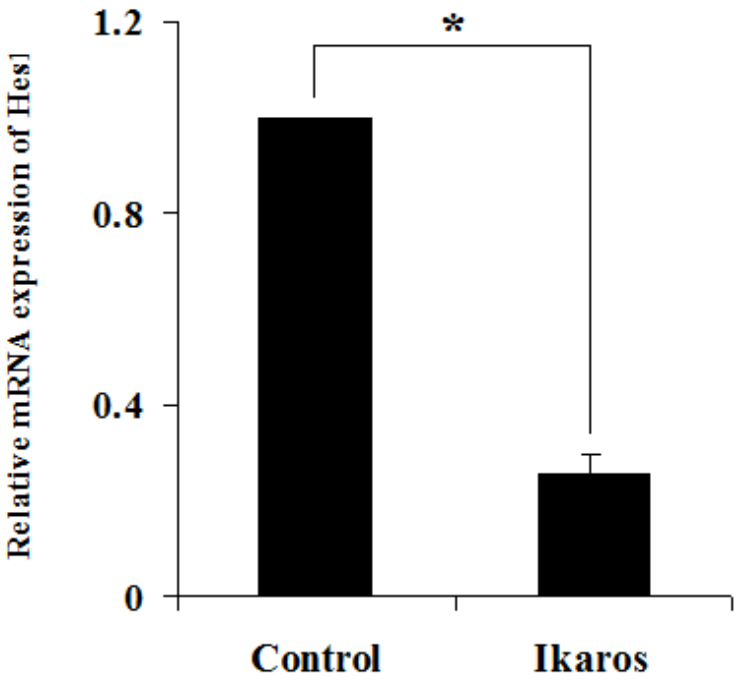

Fig. S13. Overexpression of Ikaros down-regulates Hes1 expression. Mouse E16 fetal liver Lin^- cells were infected by retroviruses encoding GFP or Ikaros. The cells at day 3 post-infection were used for real-time RT-PCR analysis. The gene expression in GFP retrovirus infected cells was normalized to 1 (*, $p < 0.05$, $n = 3$).