Animals. Female C57BL6/J (CD45.2), B6.SJL (CD45.1+), C57BL6/J TCRβ-/- and C57BL6/J CD40L-/- mice were purchased from Jackson Laboratories (Bar Harbor, ME). C57BL6/J Wnt10b-/- mice [24] were provided by Dr. Timothy Lane (University of California, Los Angeles). Mice were either sham operated or ovx at 8 weeks of age and sacrificed after 2 or 8 weeks. Uterine weight was determined at death to verify successful ovx. All mice were maintained under specific pathogen free conditions and fed sterilized food and autoclaved water ad libitum.

T cell transfer. WT spleen T cells purified by negative immunoselection using MACS Pan T cell isolation kit (Miltenyi Biotech, Auburn, CA) were injected (5×10^6 cells per mouse) IV into TCRβ-/- recipient mice. T cells were transferred 3 weeks before surgery to allow the engraftment and the peripheral expansion of the transferred T cells. Successful T cell engraftment was confirmed by flow cytometry of the spleens of the recipient mice harvested at sacrifice (Supplemental Figure 2).

SC purification. BM was collected from long bones by centrifugation and cultured for 7 days in α-MEM medium containing 10% FBS, 100mg/ml of penicillin, and 100 IU/ml of streptomycin, to allow the proliferation of SCs and their differentiation into cells of the osteoblastic lineage. After discarding non adherent cells, adherent macrophages were eliminated by positive immunoselection by MACS Microbeads (Miltenyi Biotech, Auburn, CA) coupled to anti-CD11c antibody. This marker is expressed on non-adherent dendritic cells and adherent monocytes and macrophages. The remaining adherent cells were defined as SCs as they express ALP, type-I collagen, Runx2, and have the capacity to form mineralization nodules when further cultured under mineralizing conditions.

In vitro SCs and T cells cocultures. SCs (5×10^5) and an equal number of T cells isolated from C57BL6/J WT were cocultured in α-MEM medium containing 10% FBS,

100mg/ml of penicillin, and 100 IU/ml of streptomycin; and in the presence of 2 μg/mL of irrelevant Ab, 2 μg/mL of CD40L Ab (R&D Systems, Minneapolis, MN) or 200 ng/mL of rWnt10b protein (R&D Systems) for 7 days. The T cells were then removed by washing the SCs with growth medium twice. The SCs were collected after further 48 hours incubation in growth medium, and the mRNA levels of IL-6, IL-7 and GM-CSF were measured using real-time RT-PCR.

Flow cytometry and cell sorting. For flow cytometry analyses and cell sorting, BM cells or peripheral blood cells were stained with monoclonal antibodies against the following molecules: CD45.2 (104), CD45.1 (A20), B220 (RA3-6B2), CD3 (145-2C11), CD4 (GK1.5), CD8 (53-6.7), CD11b (M1/70), CD11c (N418), Gr-1 (Rb6-8C5), TER-119 (TER-119), CD117/c-Kit (2B8), Sca1 (D7), CD150 (TC15-12F12.2), and CD48 (HM48-1) from Biolegend (San Diego, CA). Antibodies were used either direct conjugated or biotinylated. For secondary detection, streptavidin conjugated with APC-Cy7 (Biolegend) was used. 7AAD (Biolegend) was used for dead cells exclusion. Stained cells were measured by an Accuri C6 flow cytometer (BD Biosiences) or a FACS LSRII, or sorted by a FACS AriaII (Becton Dickinson, San Jose, CA), and analyzed using C6 software or FlowJo software (TreeStar, Ashland, OR).

Cytokine Assays. IL-6, IL-7, and GM-CSF were measured in culture media by ELISA (R&D Systems, Minneapolis, MN).

Real-time RT-PCR and Primers. The mRNA expression levels of aryl-hydrocarbon receptor (Ahr), axin2 (Axin2), cystein rich protein 61 (Cyr61), naked cuticle 2 homolog (Nkd2), transgelin (Tagln), transforming growth factor beta 3 (Tgfβ3), thrombospondin 1 (Thbs1), twist gene homolog 1 (Twist1) and Wnt1 inducible signaling pathway protein 1 (Wisp1) in SCs and LSK cells, were quantified by real-time RT-PCR using a ABI Prism 7000 Sequence Detection System and SYBR GREEN PCR Master Mix (Applied

Biosystems, Foster City, CA) as previously described [26]. Changes in relative gene expression between ovx and sham groups were calculated using the $2^{-\Delta\Delta CT}$ method with normalization to 18S rRNA as previously described [27]. All the primers used were designed by Primer Express Express® Software v2.0 (PE Biosystems). The primers used were: 5'-ATTCGAACGTCTGCCCTATCA-3' (forward) and 5-GTCACCCGTGGTCACCATG-3' (reverse) for 18s rRNA, 5'-TTTTCCGGCTTCTTGCAAA -3' (forward) and 5- CATTGGACTGGACCCACCTC -3' (reverse) for Ahr, 5'- CAGTGTGAAGGCCAATGGC -3' (forward) and 5'-TGGGTTCTCGGAAAATGAGG -3' (reverse) for Axin2, 5'GTGAAGTGCGTCCTTGTGGA -3' (forward) and 5'-TGCCCTTTTTTAGGCTGCTG -3' (reverse) for Cyr61, 5'-AATTTCAGTCCAAGCACGCC -3' (forward) and 5'-CGGGACTCTCTCTCCTCTTGC -3' (reverse) for Nkd2, 5'-CAGCCCAGACACCGAAGCTA -3' (forward) and 5'- AGGCTTGGTCGTTTGTGGAC -3' (reverse) for Tagln, 5'- GCAAAGGGCTCTGGTAGTCCT-3' (forward) and 5'-AGGCTGATTGTGGCCAAGTT -3' (reverse) for TGFβ3, 5'-GGACCGGGCTCAACTCTACA -3' (forward) and 5'- AGCTCCGCGCTCTCCAT -3' (reverse) for Thbs1, 5'-TCGACTTCCTGTACCAGGTCCT -3' (forward) and 5'-CCATCTTGGAGTCCAGCTCG -3' (reverse) for Twist1, 5'-ATGCCTGGCTGTGTACCAGC -3' (forward) and 5'- CCTGCGAGAGTGAAGTTCGTG-3' (reverse) for Wsp1, 5'-TTCCATCCAGTTGCCTTCTTG-3' (forward) and 5'-GAAGGCCGTGGTTGTCACC-3' (reverse) for IL-6, 5'-TTCCAGCCACGTGAGCATC-3' (forward) and 5'-TCGGTCAAATACATCCAGCG-3' (reverse) for IL-7, 5'-GGAGGATGTGGCTGCAGAAT-3' (forward) and 5'- GGCTGTAGACCACAATGCCC-3' (reverse) for GM-CSF.

Transplantation experiments. For BM transplantation, donor mice were killed and BM cells (BMCs) were collected from the femurs and tibias. BMCs ($2.5x10^5$) were suspended in PBS and transplanted by tail vein injection into lethally irradiated recipients on day 0. Before transplantation, recipients received 1300 cGy total-body irradiation (^{137}Cs source) as a split dose with 3 hours between doses (the second dose was given 2 hours before transplantation). Mice were housed in sterilized microisolator cages and received sterilized chow and autoclaved water (pH 3.0). Recipient mice were killed at 8 weeks after transplantation, and the reconstitution in spleen, blood, and BM was analyzed by flow cytometry using the lineage markers B220, CD11b, Gr-1, and CD3. For competitive transplantation assays, mice expressing the marker CD45.2 were either sham operated or ovx. BMCs ($2.5x10^5$) from C57BL6/J, TCRβ-/-, T cell reconstituted TCRβ-/-, CD40L-/-, and Wnt10b-/- mice were mixed with BMCs ($5x10^5$) from B6.SJL mice expressing the marker CD45.1 to obtain 1:2 competition and intravenously injected in CD45.1-expressing recipient mice. Recipient mice (8-10 weeks old) were lethally irradiated as previously described. Repopulation was evaluated at the times indicated after transplantation in peripheral blood by flow cytometry using the hemopoietic makers CD45.1, CD45.2, CD11b, B220, Gr-1, and CD3. The primary recipients were killed 36 weeks after transplantation and the BMCs were analyzed for the population of LSK cells and used for secondary transplantation.

For secondary transplantation, BMCs from primary recipients were mixed with BMCs from a competitor animal expressing the marker CD45.1 at a ratio of 1:2. A total of $7.5x10^5$ cells were transplanted into lethally irradiated CD45.1-expressing secondary recipients. Peripheral blood from the secondary recipients was analyzed at the times indicated after transplantation by flow cytometry using the hemopoietic makers as

previously described. The secondary recipients were killed after 12 weeks transplantation and the reconstitution in spleen and BM was analyzed.

For competitive transplantation assays using ST-HSCs, 500-750 CD45.2+CD150^{-}CD48- cells were injected into lethally irradiated CD45.1+ recipient mice (10 mice per group) with 250,000 unfractionated CD45.1+ BM cells to provide a competitor and survival population. Peripheral blood samples were obtained at 2, 4 and 8 weeks post-transplantation. Cells were stained with antibodies against CD45.2 and CD45.1 as well as lineage markers (CD11b, Gr-1, and B220), and subsequently analyzed on a FACS LSRII (BD Bioscience).

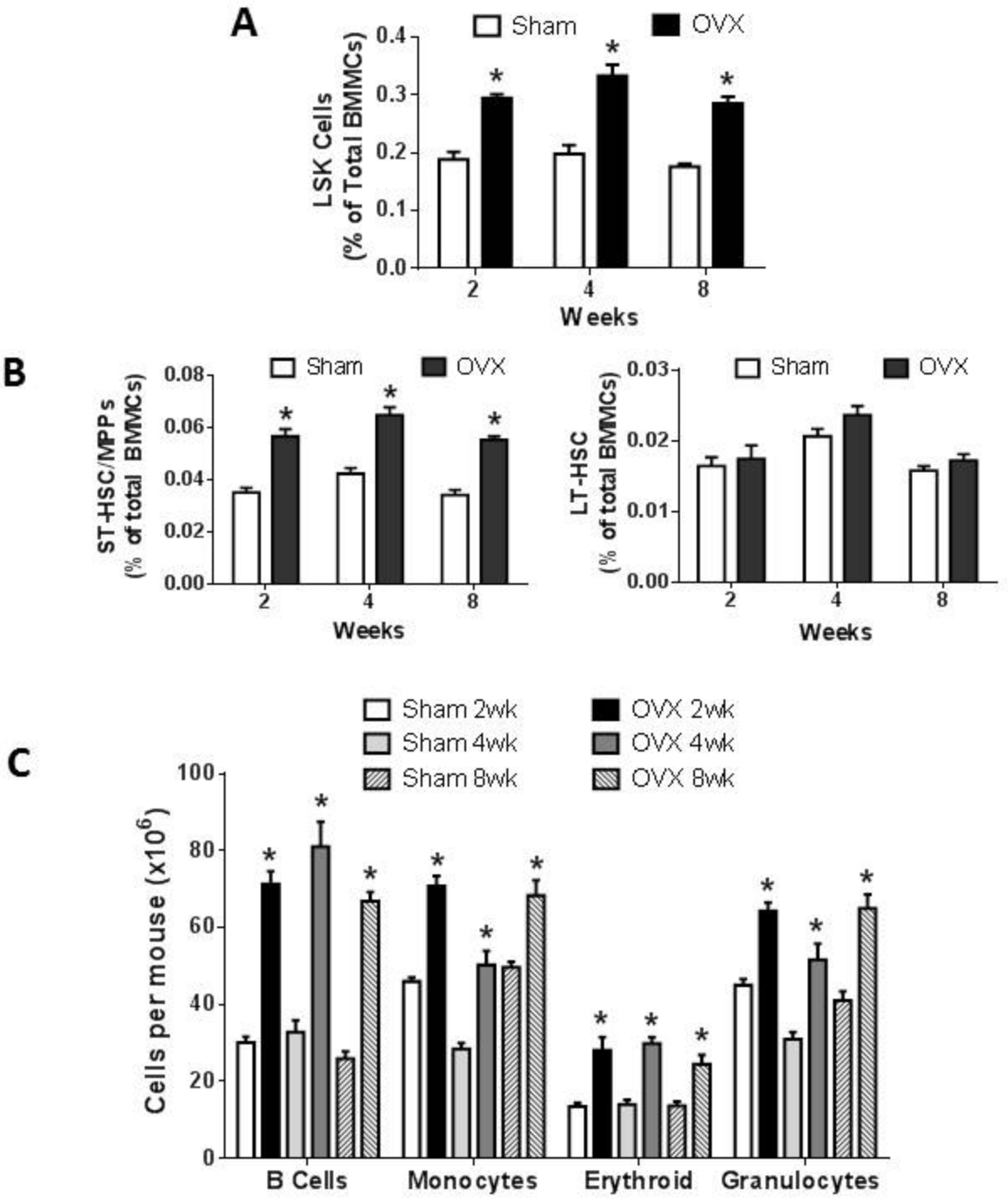
Enrichment and purification of subpopulations of Lin^{-} Sca-1^{+} c-Kit^{+} (LSK) BM cells

Lineage-depleted (Lin^{-}) BM cells were isolated from 12 to 18 week old C57BL6J (CD45.2+) using Lineage Cell Depletion Kit (Miltenyi Biotec Inc.). Lin^{-} cells were then stained with a biotin-conjugated lineage mixture (CD11b, B220, CD3, Gr-1, and TER119) plus streptavidin-FITC, PE-conjugated Sca-1, APC-Cy7-conjugated c-Kit, APC-conjugated CD150, and PE-Cy7-conjugated CD48 antibodies (Biolegend Inc.). LSK cells were sorted into ST-HSCs (CD150^{-}CD48^{-}) and LT-HSCs (CD150^{+}CD48^{-}) populations on a FACS AriaII Cell Sorter (BD Bioscience). The purity of sorted LSK subpopulations was 95-99%.

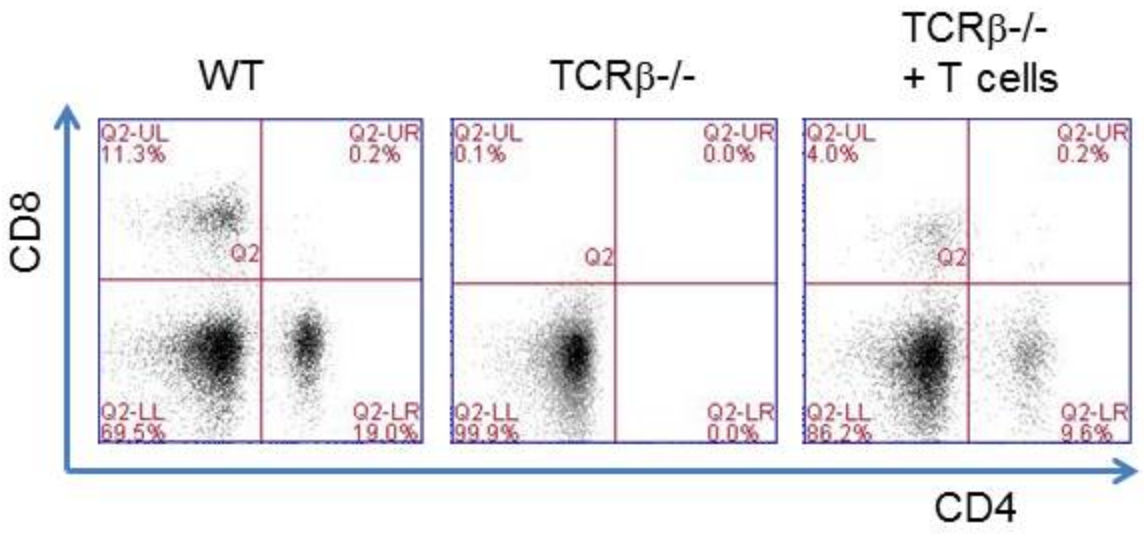
Detection of Wnt dependent genes in ST-HSCs and LT-HSCs

For real-time PCR analysis of Wnt dependent genes, ST-HSCs (CD150^{-}CD48^{-}) and LT-HSCs (CD150^{+}CD48^{-}) cells were subjected to total RNA amplification and subsequent cDNA synthesis utilizing Superscript III Cell Direct cDNA synthesis Kit (InVitrogen). Briefly, 7000-10000 cells were lysed in first strand buffer followed by reverse transcription of mRNA to generate the first strand cDNA. Real-time PCR assays were performed as described above.

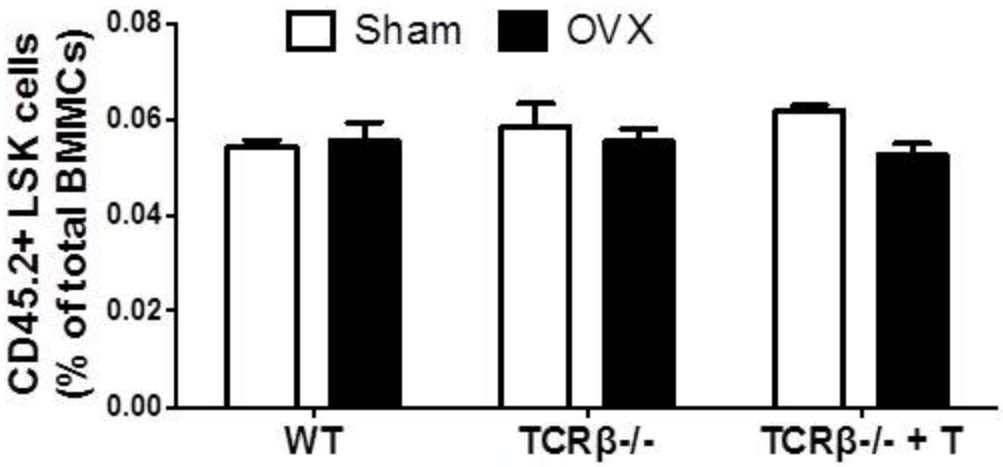
Statistical Analysis. All values are expressed as mean ± SEM. The Mantel-Cox log-rank test was used for survival data. For all other comparisons, a two-way ANOVA or an ANOVA for repeated measures were applied as appropriate. These ANOVAs included the main effects for animal strain and treatment plus the statistical interaction between animal strain and treatment. When the statistical interaction between animal strain and surgery group was not statistically significant ($P > 0.05$) nor suggestive of an important interaction ($P > 0.10$) p values for the main effects tests were reported. When the statistical interaction was statistically significant or suggestive of an important interaction then t-tests were used to compare the differences between the treatment means for each animal strain, applying the Bonferroni correction for multiple comparisons.



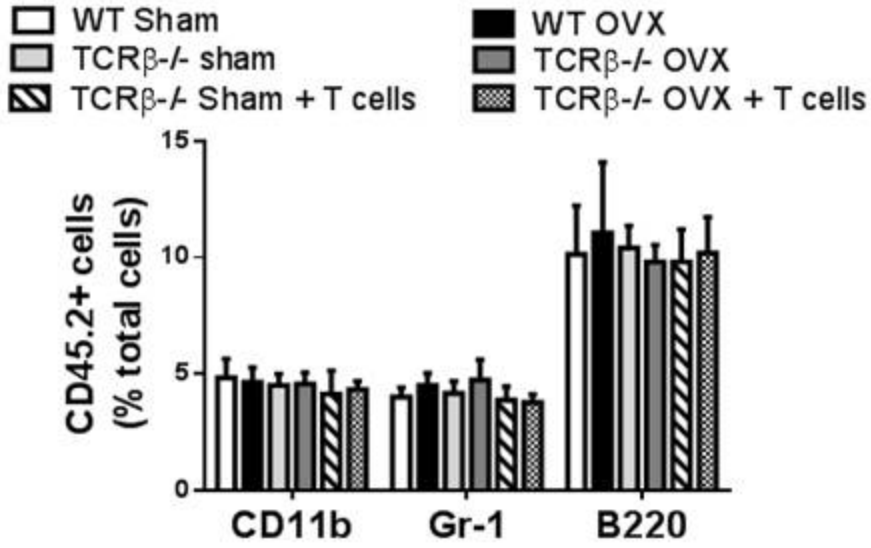
Supplemental figure 1. A Effects of ovx on the relative frequency of LSK cells in WT mice at 2, 4 and 8 weeks after surgery. Lin- cells were gated and analyzed for Sca-1 and c-Kit expression using isotype control settings. Data are expressed as % of total BM mononucleated cells (BMMCs (Mean ± SEM for each group). **B** Effects of ovx on the relative frequency of CD150-CD48- LSK cells (ST-HSPCs/MPPs) and CD150+CD48- LSK cells (LT-HSPCs) in WT mice at 2, 4 and 8 weeks after surgery. Data are expressed as % of total BMMCs (Mean ± SEM for each group). **C** Effect of ovx on the number of BM B cells, monocytes, erythroid cells and granulocytes. n= 10 mice per group. *= $p < 0.05$ compared to the corresponding sham-operated group.



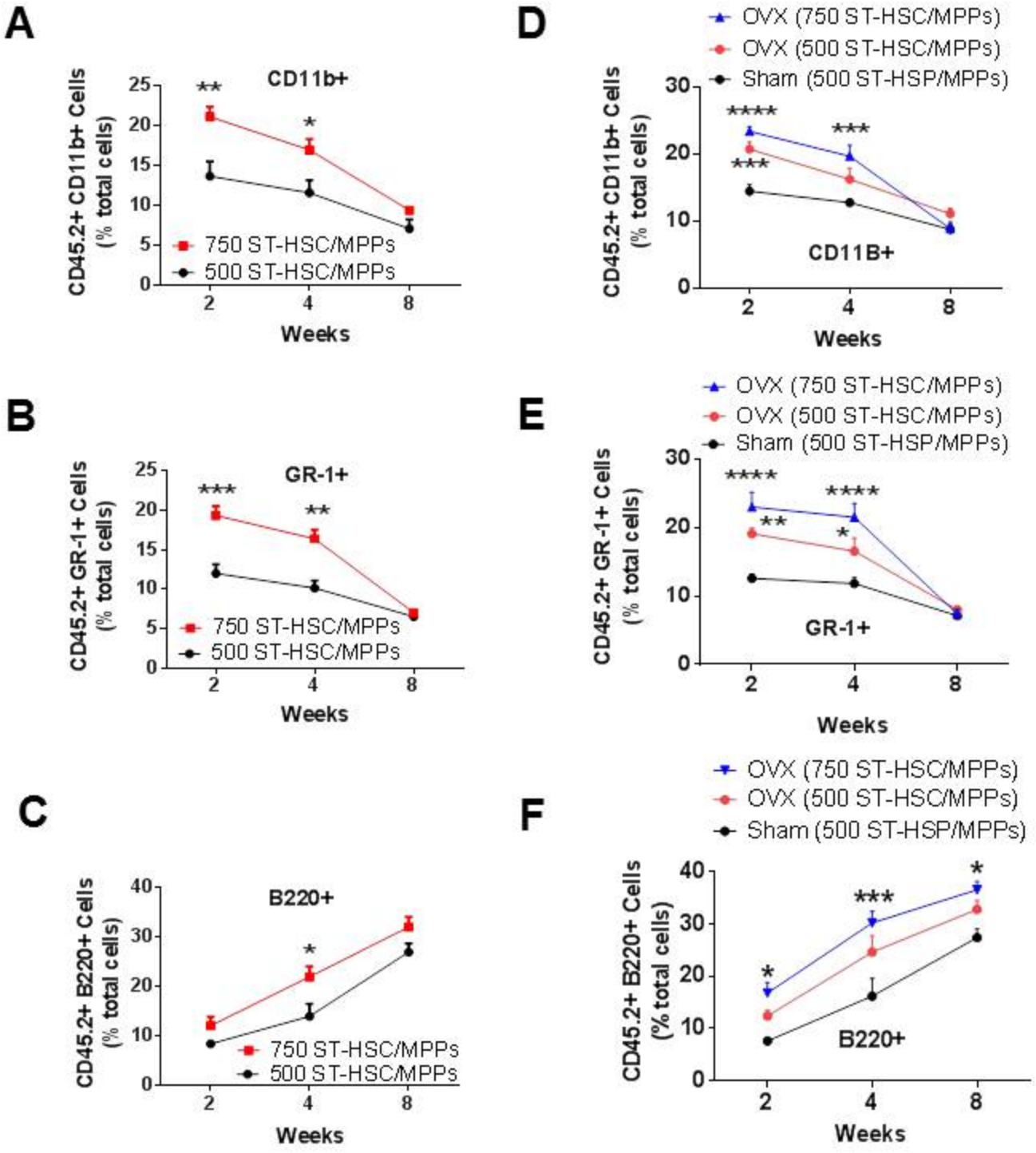
Supplemental Figure 2. Flow cytometric analysis of splenocytes harvested at sacrifice from WT mice, TCRβ -/- mice, and TCRβ -/- mice subjected to adoptive transfer of WT T cells 3 weeks before sacrifice. The adoptive transfer of T cells was carried out by injecting in the tail vein 5×10^6 T cells purified by negative immunomagnetic selection.



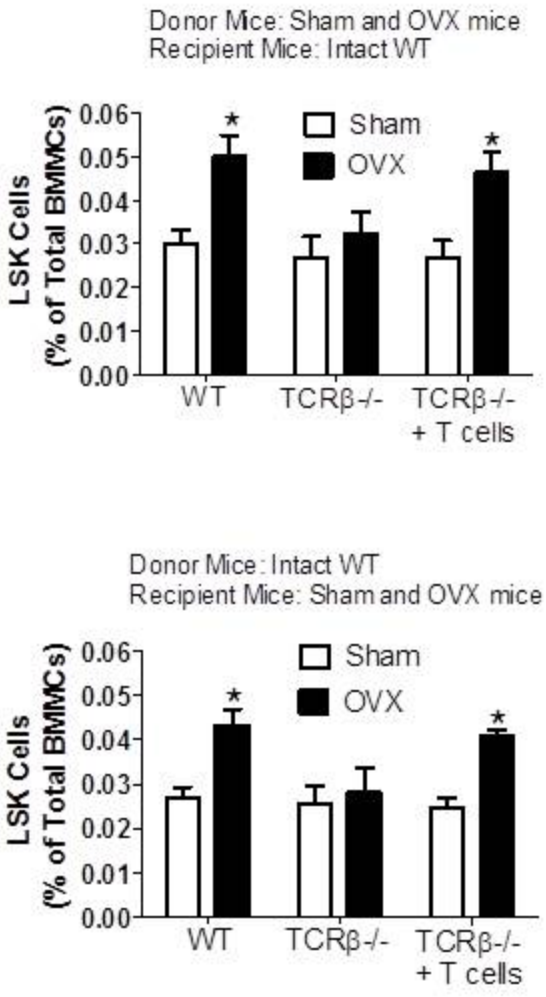
Supplemental Figure 3. Relative frequency of donor derived CD45.2+LSK cells in the BM of primary recipients at sacrifice. In these experiments lethally irradiated WT mice were transplanted with CD45.2+ BM donor cells mixed in a 1:2 ratio with CD45.1+ competitor BM cells. CD45.2+ BM cells were obtained from WT, TCRβKO, and reconstituted TCRβKO mice subjected to sham operation or ovx 2 weeks earlier. CD45.1+ BM cells were obtained from intact WT mice.



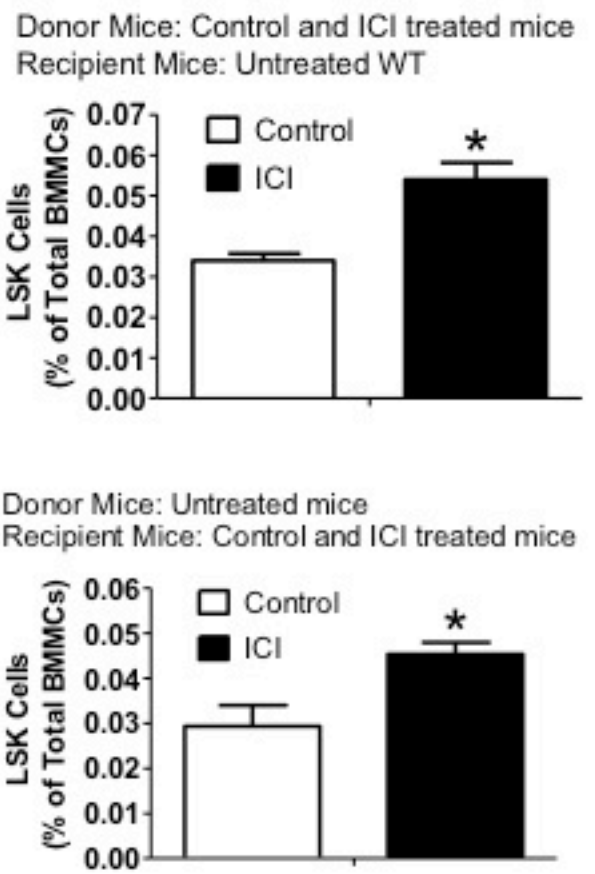
Supplemental Figure 4. Effect (Mean ± SEM) of ovx on peripheral blood cell expansion after secondary competitive repopulation. The figure shows the percentage of donor derived CD45.2+ cells in the peripheral blood of secondary recipients 10 weeks after transplantation. In these experiments CD45.1/CD45.2 BM cells were obtained from primary recipients at sacrifice, mixed in a 1:2 ratio with CD45.1+ competitor BM cells from intact WT mice, and transplanted into CD45.1+ intact WT recipients. n= 5 donor and 10 recipient mice per group.



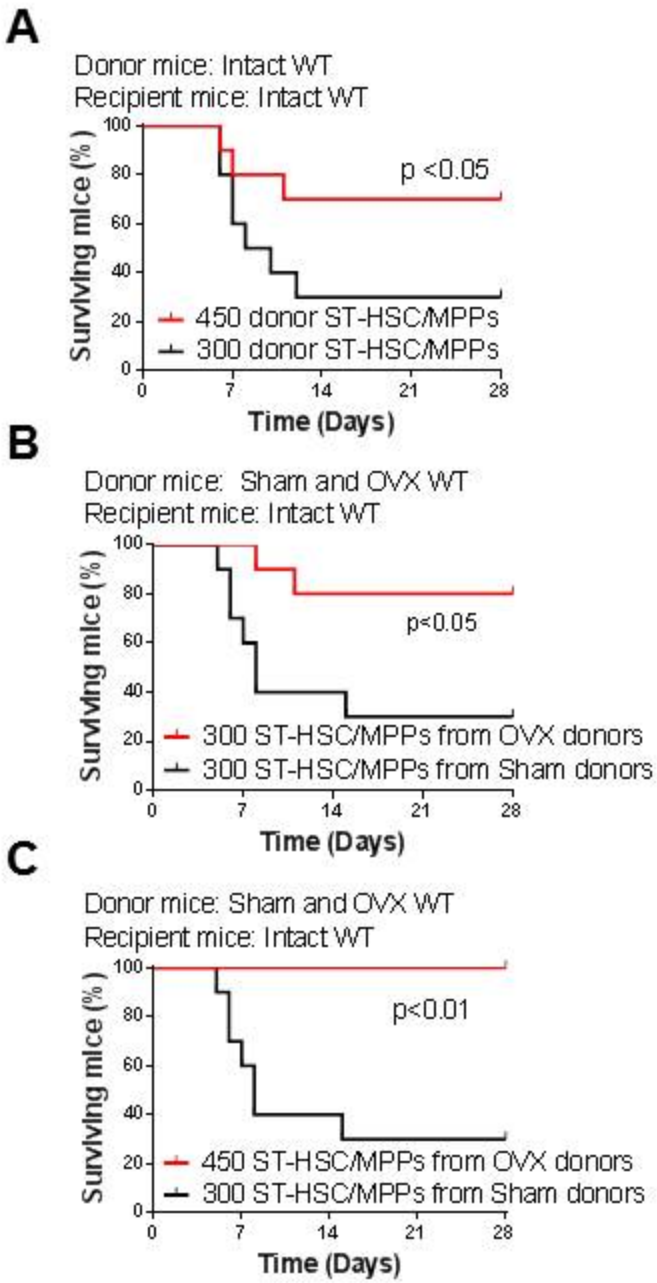
Supplemental figure 5. In vivo reconstituting ability of ST-HSC/MPPs (Lin$^-$Sca1$^+$ c-Kit$^+$ CD150$^-$ CD48$^-$) cells. Lethally irradiated mice expressing CD45.1 were transplanted with 500-750 ST-HSPCs CD45.2+ donor cells and 250,000 CD45.1+ competitor BM cells. CD45.2+ donor cells were obtained from Intact, ovx and sham operated WT mice. CD45.1+ BM cells were obtained from intact WT mice. Following 2, 4 and 8 weeks, recipient mice were investigated for donor cell contribution to total peripheral blood reconstitution. **A-D** CD11b + cell reconstitution. **B-E** GR-1+ cell reconstitution. **C-F** B220+ cells reconstitution. n= 5 donor and 10 recipient mice per group. . * = P<0.05, . ** = P<0.01, .*** = P<0.001 and .**** = P<0.0001 compared to the corresponding sham operated group



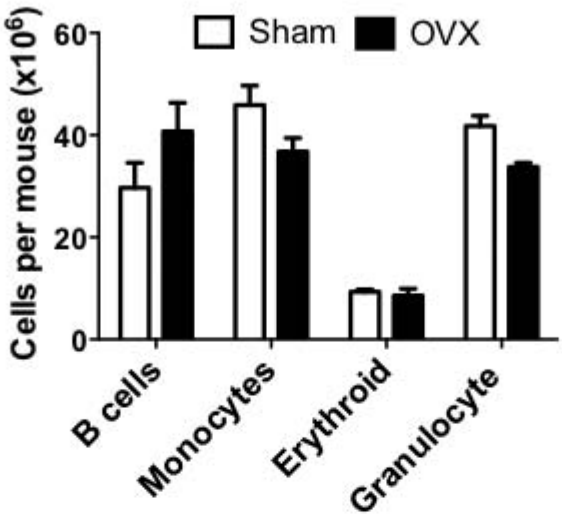
Supplemental figure 6. Relative frequency of LSK cells in the BM of surviving recipient mice 28 days after transplantation of a limiting number of donor cells derived from WT, TCRβ-/- mice and T cell reconstituted TCRβ-/- mice. Top panel: Donor mice were sham operated or ovx; recipient mice were intact. Bottom panel: Donor mice were intact; recipient mice were sham operate or ovx. Data are expressed as % as (mean $\pm$ SEM) of total BM mononucleated cells (BMMCs). Data are shown. n= 10 mice per group. * = $P<0.05$ compared to the corresponding sham operated group.



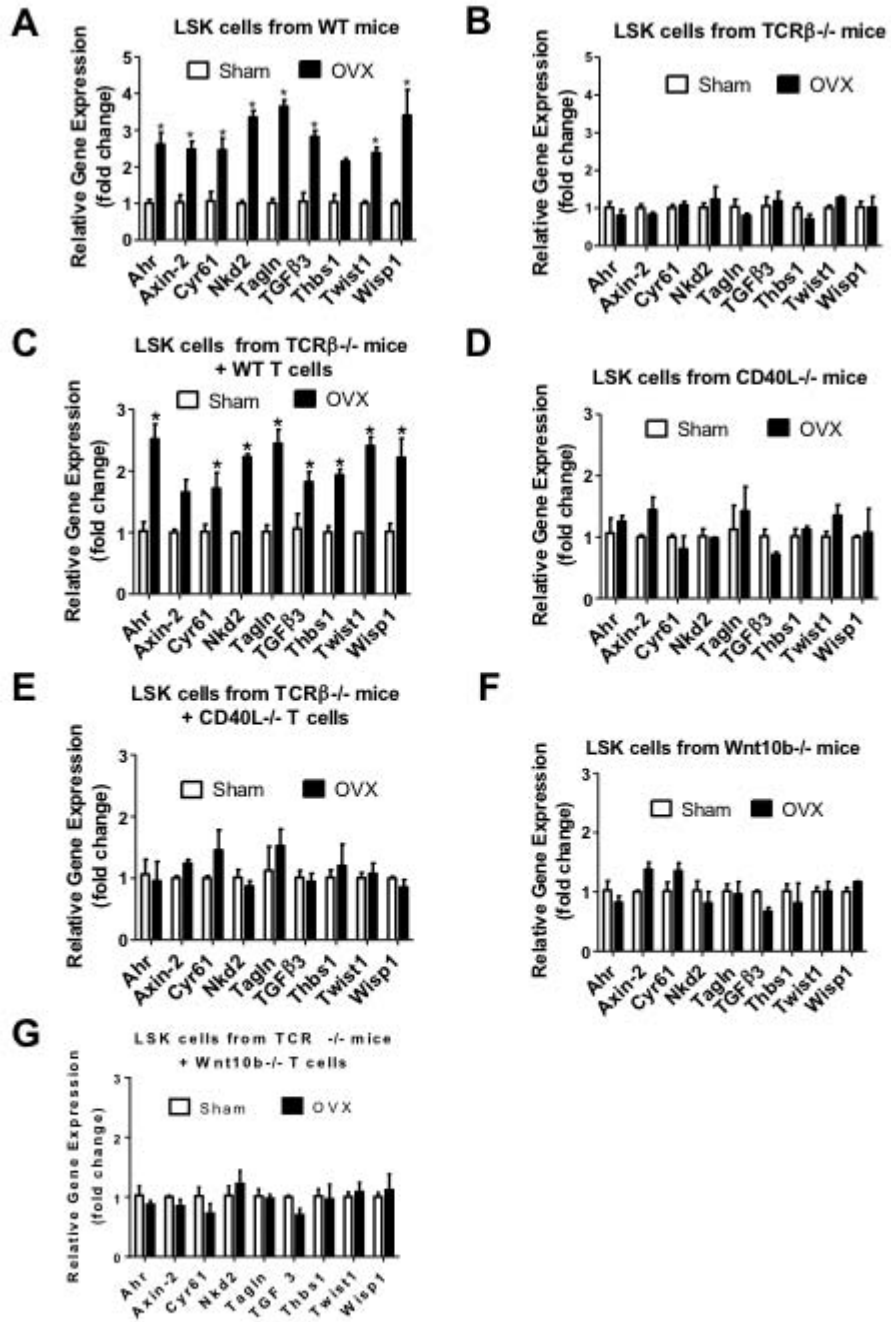
Supplemental Figure 7. Top Panel: Effects of the anti estrogen ICI182,780 and control vehicle (100 µg/mouse SC, twice a week for 4 weeks) on the relative frequency of LSK cells in the BM of surviving recipient mice 28 days after transplantation of a limiting number of donor BM cells. Top panel: Donor mice were control or ICI treated WT mice; recipient mice were untreated WT mice. Bottom panel: Donors were untreated WT mice; recipients were vehicle and ICI treated WT mice * = $p<0.05$ compared to control. n = 10 per group.



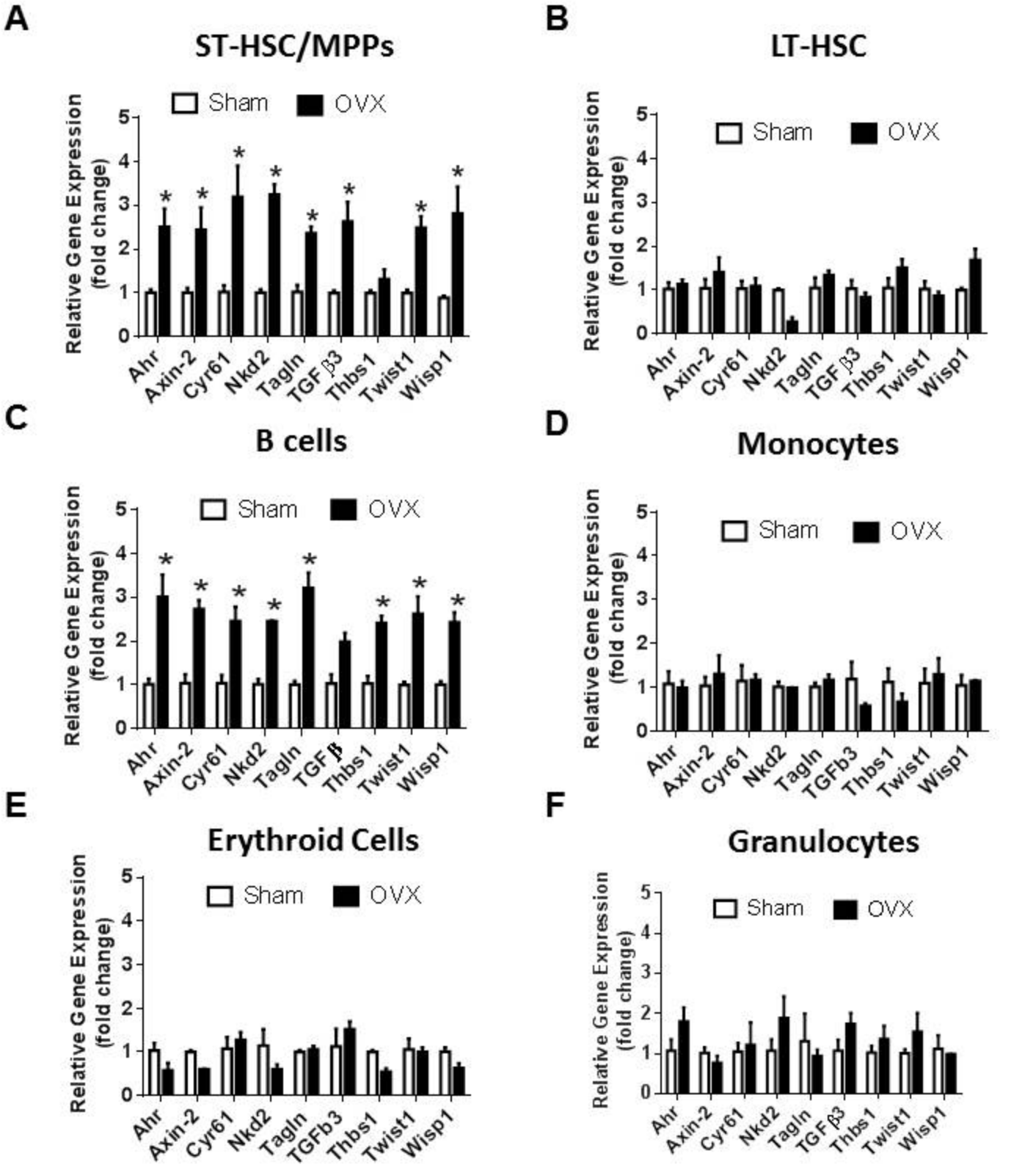
Supplemental Figure 8. Kaplan-Meyer survival analysis of WT mice transplanted with 300-450 ST-HSPC/MPPs ($Lin^-Sca1^+c\text{-}Kit^+CD150^-CD48^-$ cells) derived from intact, ovx and sham operated WT mice. **A** Donor cells were obtained from intact mice. B-C Donor cells were obtained from ovx or sham operated mice. n = 10 per group.



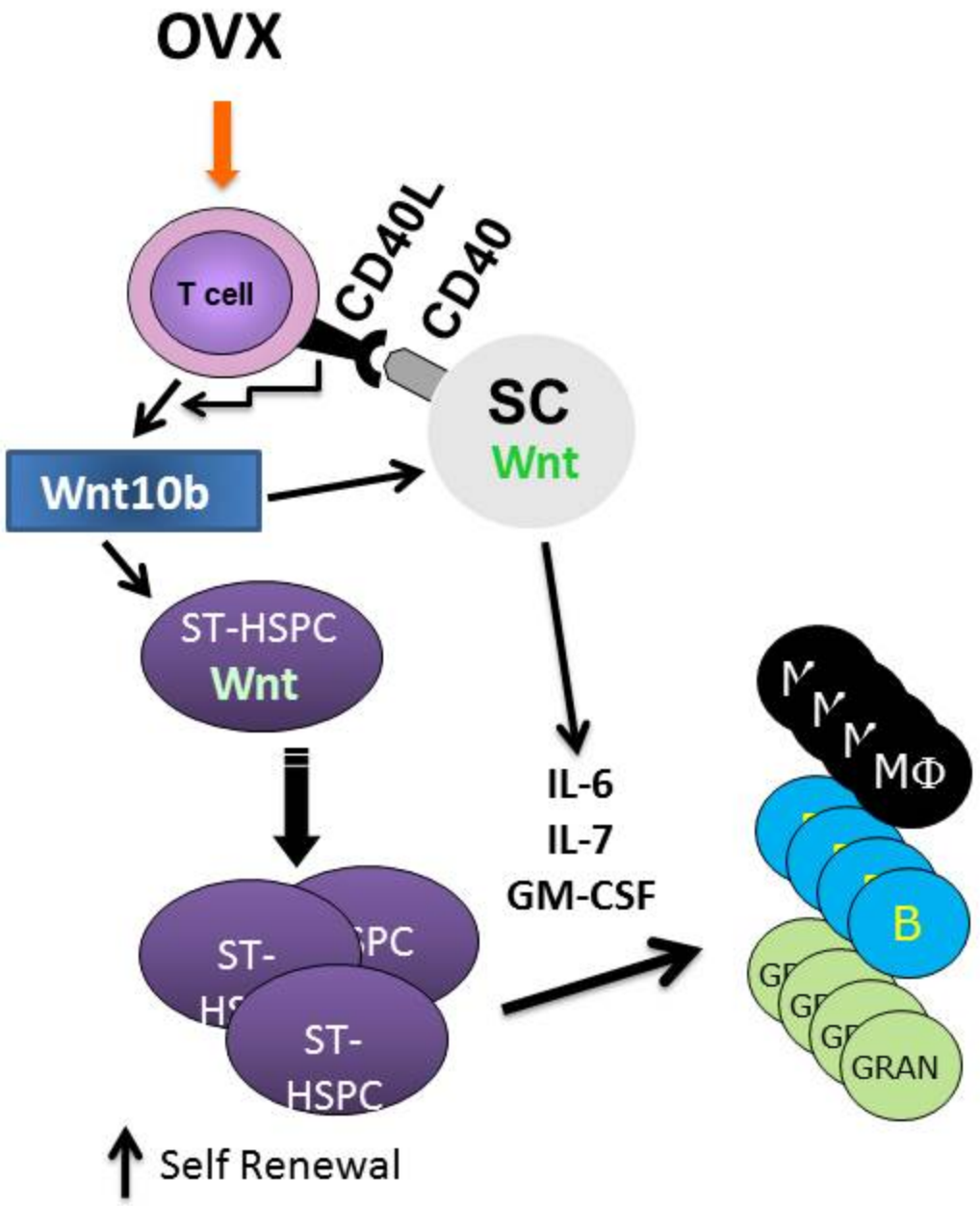
Supplemental figure 9. Effect of ovx on the number of BM B cells, monocytes, erythroid cells and granulocytes in Wnt10b-/- mice. n= 10 mice per group.



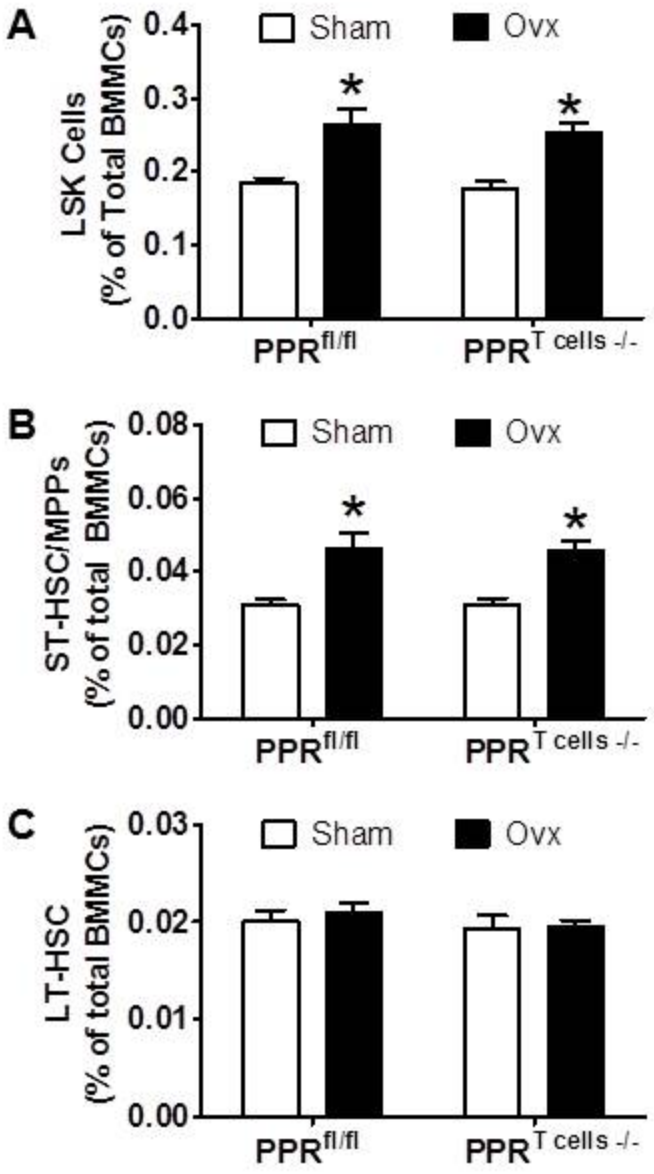
Supplemental figure 10. Effect (mean ± SEM) of ovx on the LSK cells expression of mRNA of genes known to be upregulated by Wnt signaling. LSK cells were purified by FACS sorting from the BM of sham operated and ovx WT mice (**A**), TCRβKO mice (**B**), TCRβKO mice previously subjected to adoptive transfer of WT T cells (**C**), CD40L-/- mice (**D**), TCRβ-/- mice previously reconstituted with CD40L-/- T cells (**E**), Wnt10b-/- mice (**F**), and TCRβKO mice previously subjected to adoptive transfer of Wnt10b-/- T cells (**G**). The Wnt dependent analyzed genes were the same as shown in figure 7. n=6 mice pre group. *= p <0.05 compared to the corresponding sham operated group.



Supplemental figure 11. Effect (mean ± SEM) of ovx on expression of mRNA of genes known to be upregulated by Wnt signaling in BM cells. **A** ST-HSC/MPPs **B** LT-HSCs, **C** B cells, **D** Monocytes, **E** Erythroid cells, **F** Granulocytes. The Wnt dependent analyzed genes were the same as shown in figure 7. n=6 mice pre group. *= $p < 0.05$ compared to the corresponding sham operated group.



Supplemental figure 12. Schematic representation of the role of T cells in the mechanism by which ovx stimulates HSPCs expansion. When CD40L is expressed on T cells, ovx stimulates T cells to secrete Wnt10b, a Wnt ligand required to activate Wnt signaling. In addition, CD40L stimulates SC production of the hemopoietic factors IL-6, IL-7 and GM-CSF. In the presence of T cell produced Wnt10b, ovx activates Wnt signaling in stromal cells and HSPCs. Wnt signaling activation is known to upregulate the expression of the Notch ligand Jagged 1 in SCs. These events result in HSPCs expansion.



Supplemental figure 13. Effects (mean ± of SEM) of ovx on HSPCs in PPR$^{T\ cells-/-}$ and control mice. **A** Effects of ovx on the relative frequency of BM LSK cells. **B-C** Effect of frequency of ST-HSPCs/MPP and LT-HSPCs.