Loss of the Alox5 gene impairs leukemia stem cells and prevents chronic myeloid leukemia

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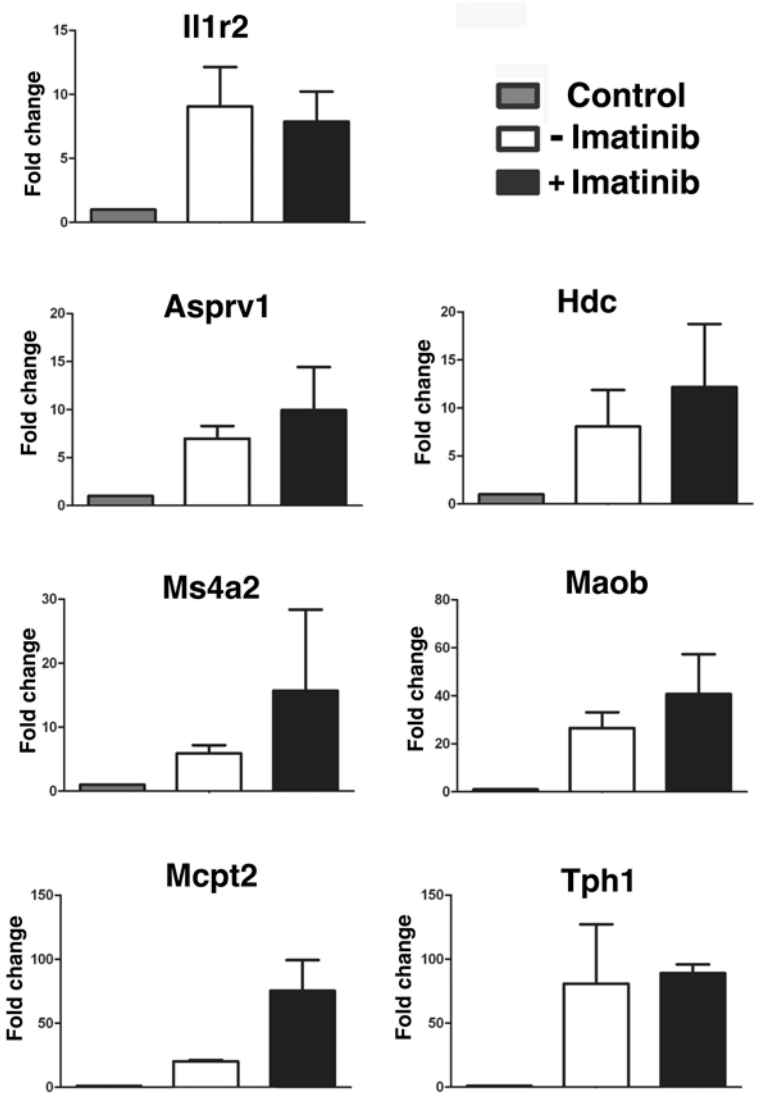
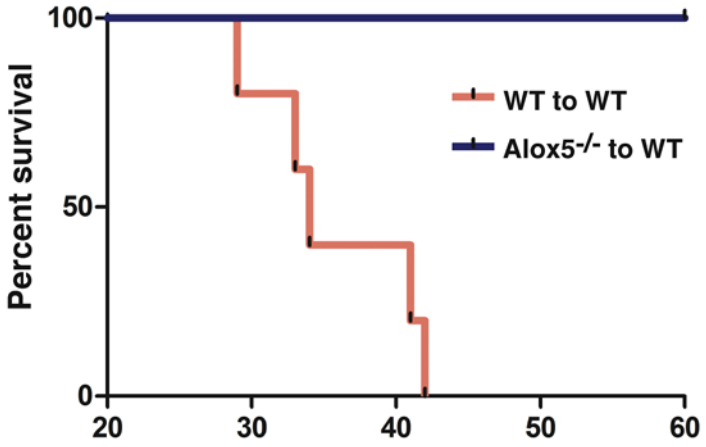
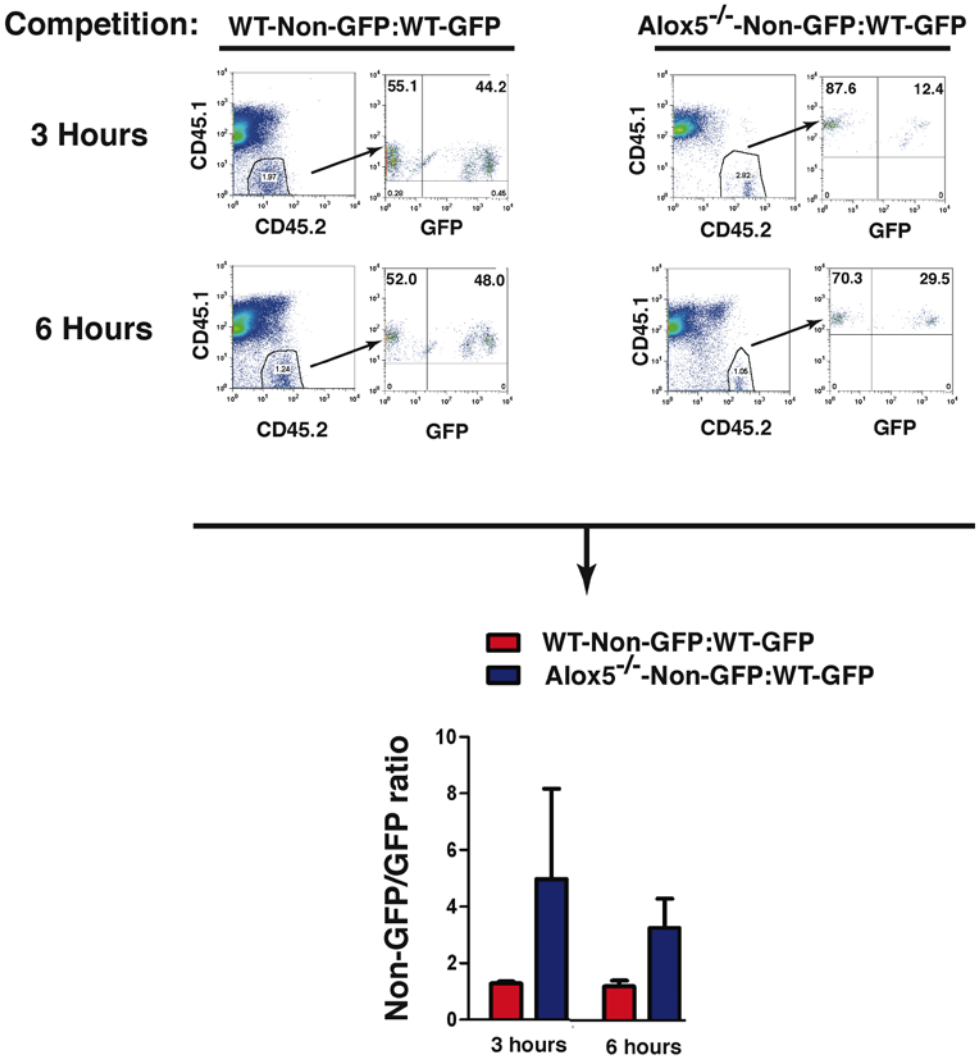


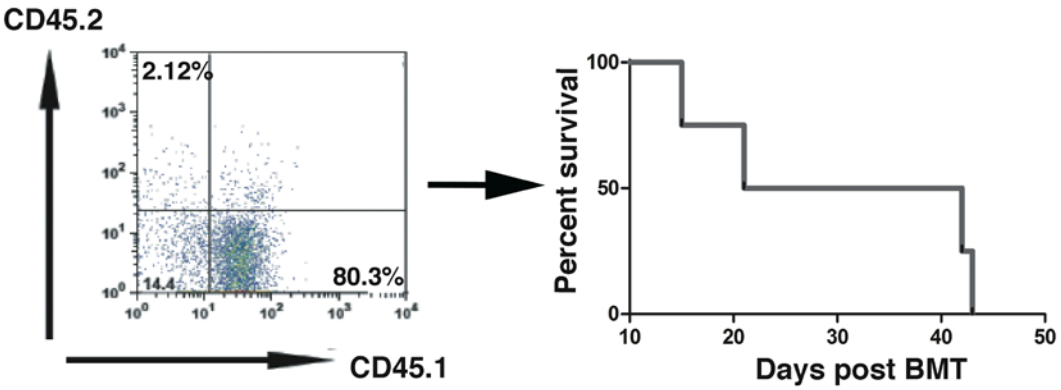
Supplementary Fig. 1. Genes that are up-regulated by BCR-ABL in LSCs and not changed in expression following imatinib treatment. Bone marrow cells from C57BL/6 mice (B6) were transduced with retrovirus containing BCR-ABL/GFP or GFP alone to induce CML as described in **Figure 1a**. A group of these mice were treated with imatinib as described in **Figure 1a**. Bone marrow cells were isolated from the mice, and were sorted by FACS for $GFP^{+}Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$ cells. Total RNA was isolated from these sorted cells for comparing *Alox5* expression between GFP vector-transduced normal stem cells and BCR-ABL-transduced LSCs by DNA microarray.



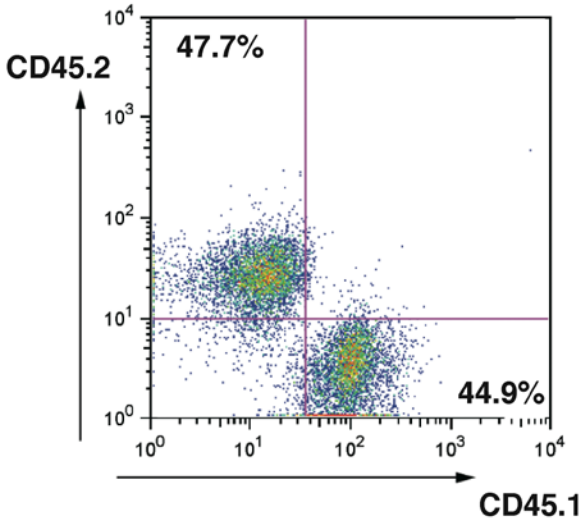
Supplementary Fig. 2. Loss of *Alox5* causes failure of BCR-ABL-expressing BM cells to induce CML in secondary recipient mice. Kaplan-Meier survival curves for secondary recipients of $2x10^6$ bone marrow cells from mice receiving *BCR-ABL*-transduced wild type (n=5) or *Alox5*$^{-/-}$ (n=6) donor BM cells.



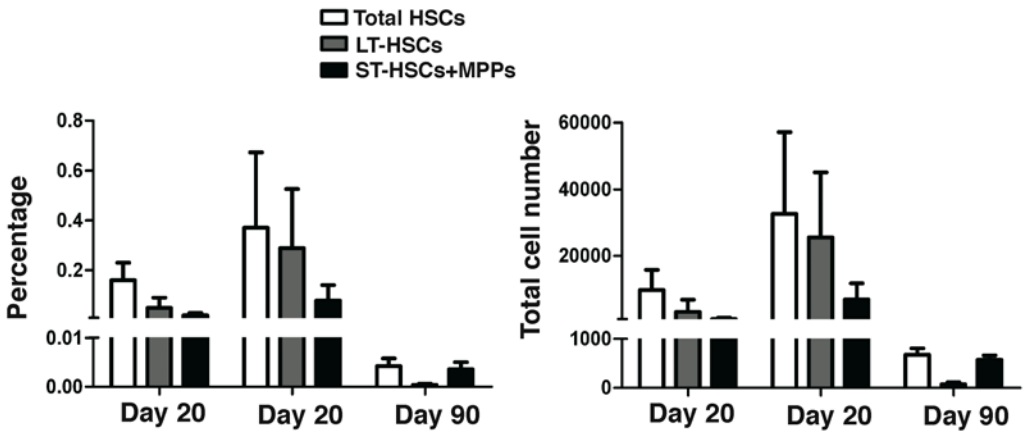
Supplementary Fig. 3. *Alox5*$^{-/-}$ bone marrow cells do not have a homing defect. Bone marrow cells ($6x10^6$) from GFP mice (CD45.2) were 1:1 mixed with either bone marrow cells from wild type B6 mice (CD45.2) or those from *Alox5*$^{-/-}$ mice (CD45.2), and then transferred by tail vein injection into each wild type recipient mouse (CD45.1). 3 or 6 hours after the transplantation, By FACS analysis, CD45.2$^+$ bone marrow cells, representing the donor cells, were first identified and then analyzed for the percentages of GFP$^+$ and GFP$^-$ populations. The ration of non-GFP and GFP populations were shown.



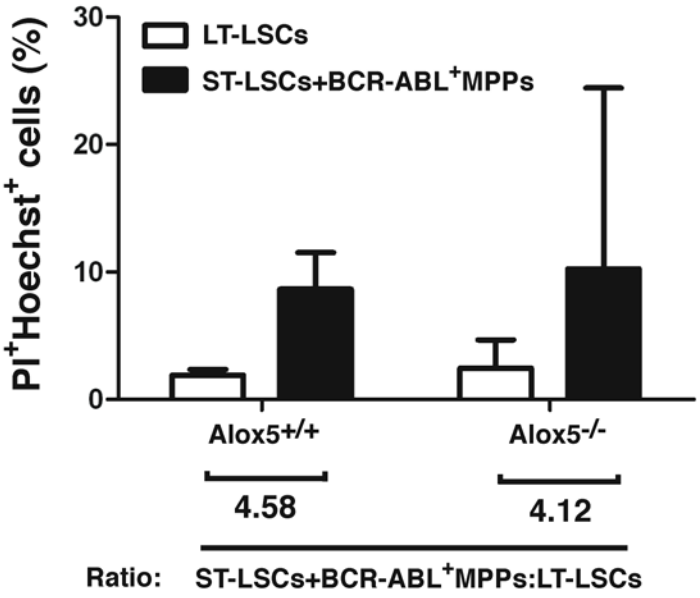
Supplementary Fig. 4. Loss of *Alox5* impairs the function of LSCs. BCR-ABL-expressing wild type ($CD45.1^{+}$) and *Alox5*$^{-/-}$ ($CD45.2^{+}$) BM cells were 1:1 mixed ($5x10^{5}$ each), followed by transplantation into lethally irradiated recipient mice. At 40 day after BMT, more than 80% of cells in PB were wild type ($CD45.1^{+}$) leukaemia cells, and all these mice died of CML.



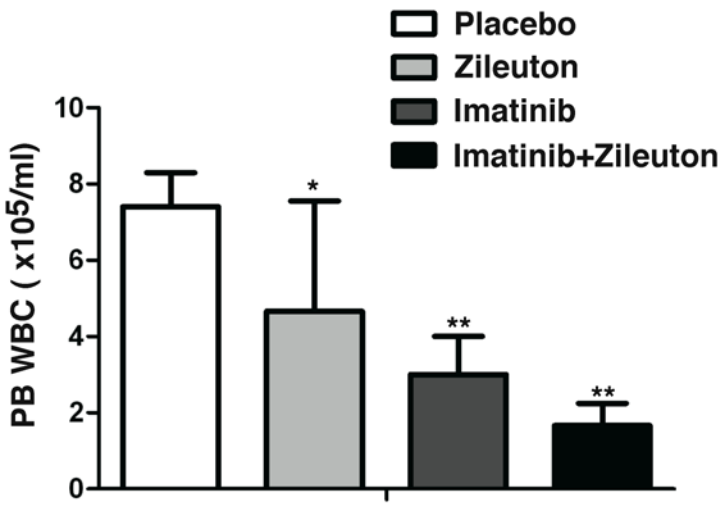
Supplementary Fig. 5. Loss of Alox5 does not impair the function of normal stem cells. Alox5$^{-/-}$ (CD45.2) and wild type (CD45.1) BM cells were 1:1 mixed and then transferred into lethal recipient mice. 4 weeks after BMT, FACS analysis was carried out to compare the percentages of wild type and Alox5$^{-/-}$ cells in BM of the recipient mice.



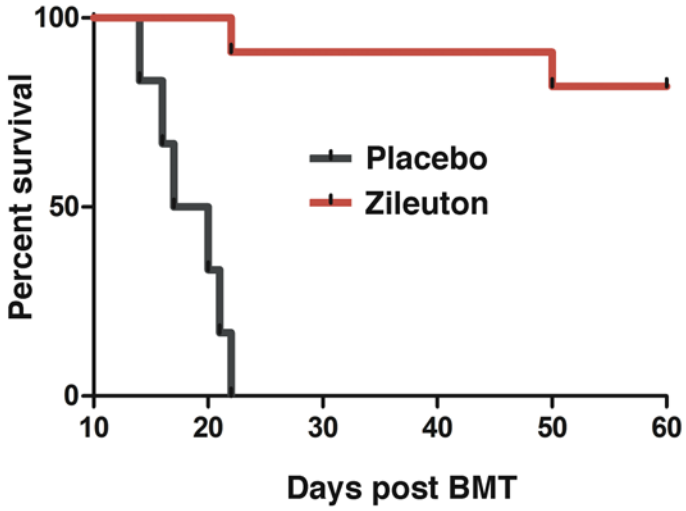
Supplementary Fig. 6. *Alox5* deficiency does not lead to blockade of differentiation of normal LT-HSCs. Bone marrow cells were isolated from recipients of *BCR-ABL*-transduced bone marrow cells from wild type or *Alox5*$^{-/-}$donor mice, and GFP$^-$ cell population (representing normal hematopoietic cells in CML mice) were analyzed by FACS analysis. At 90 days after induction of CML, the percentages and total numbers of LT-HSCs (GFP$^-$Lin$^-$c-Kit$^+$Sca-1$^+$CD34$^-$) were much lower than those of ST-HSCs /MPP cells (GFP$^-$Lin$^-$c-Kit$^+$Sca-1$^+$CD34$^+$) in a similar degree in mice receiving either BCR-ABL-transduced wild type or *Alox5*$^{-/-}$ donor bone marrow cells. These results indicate that *Alox5* deficiency does not lead to blockade of differentiation of normal LT-HSCs.



Supplementary Fig. 7. Analysis of apoptosis of LSCs in wild type and *Alox5*$^{-/-}$ mice. At day 14 after BMT, bone marrow cells were isolated from recipients of *BCR-ABL*-transduced bone marrow cells from wild type or *Alox5*$^{-/-}$donor mice. The cells were stained with PI and Hoechst Blue, and the percentages of LT-LSCs ($GFP^+Lin^-c\text{-}Kit^+Sca\text{-}1^+CD34^-$) and ST-LSCs /BCR-ABL-expressing MPP cells ($Lin^-c\text{-}Kit^+Sca\text{-}1^+CD34^+$) that were positive for PI and Hoechst Blue were determined by FACS. The ratios between ST-LSCs /BCR-ABL-expressing MPP cells and LT-LSCs in the presence and absence of *Alox5* were compared.



Supplementary Fig. 8. Comparison of white blood cell (WBC) counts. CML mice were treated with a placebo, Zileuton alone, imatinib alone, or both Zileuton and imatinib in combination as described in Figure 4b. WBCs were collected from the treated mice 14 days after induction of CML.



Supplementary Fig. 9. Inhibition of Alox5 prolongs survival of mice with CML induced with BCR-ABL-T315I. Kaplan-Meier survival curves for CML mice treated with a placebo (n=6), or Zileuton (n=11).

Gene	GeneBank accession	Forward primer	Reverse primer
β-catenin	NM_007614	5'-AACAGGGTGCTATTCCACGACTA-3'	5'-TGTGAACGTCCCGAGCAA-3'
GATA-1	NM_008089	5'-ACTGTGGAGCAACGGCTACT-3'	5'-TCCGCCAGAGTGTTGTAGTG-3'
FOG-1	NM_009569	5'-CATAGAGGAGCCCCCAAGTC-3'	5'-GGCTGCCTCTTCTTCCTTTT-3'

Supplementary Table. 1. Sequence of the primers used in real-time quantitative PCR assays.