Supplemental Information

PIAS1 SUMO ligase regulates the self-renewal and differentiation of hematopoietic stem cell

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Supplementary information includes:

Supplementary Materials and Methods

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Supplementary Materials and Methods

Mice and Reagents

The generation of *Pias1*$^{-/-}$ mice has been described (Liu et al., 2004). C57SJL (CD45.1) mice were purchased from the Jackson Labs.

The following antibodies were purchased from BioLegend: fluorescein isothiocyanate (FITC)-conjugated anti-IgM (RMM-1), anti-Mac1 (CD11b; M1/70), anti-CD3 (145-2C11), anti-CD4 (RM4-5), anti-Sca1 (D7); phycoerythrin (PE)-conjugated anti-B220 (CD45R; RA3-6B2), anti-GR1 (Ly-6G/Ly-6C; RB6-8C5), anti-Mac1 (M1/70), anti-CD11c (N418), anti-Ter119 (TER119), anti-NK-1.1 (PE136), anti-CD5 (53-7.3), anti-CD8a (53-6.7), anti-IL7Ra (CD127; SB/199); Alexa Fluor 647-conjugated anti-IL7Ra (CD127; SB/199); Pacific Blue-conjugated anti-CD48 (HM48-1); allophycocyanin (APC)-conjugated anti-FCgRII/III (CD16/32; 93), anti-CD45.2 (104); PE/Cy7-conjugated anti-FCgRII/III (CD16/32; 93); PerCP-conjugated anti-CD45.1 (A20); PE/Cy5-conjugated anti-Sca1 (D7), anti-CD34 (MEC14.7); APC/Cy7-conjugated anti-c-Kit (2B8); and isotype controls. PE-conjugated anti-CD4 (GK1.5) and Alexa Fluor 488-conjugated anti-Ki67 (B56) are from BD Pharmingen. APC-conjugated anti-CD150 (mShad150) is from eBioscience.

Flow cytometry analysis and sorting of HSC and progenitors

Various cell populations are defined as the following: common lymphoid progenitors (CLP): Lin$^-$ Sca1^{low} c-Kit^{low} IL7Ra$^+$; common myeloid progenitors (CMP): Lin$^-$ Sca1$^-$c-Kit$^+$ CD34$^+$FcgRII/III^{low}; granulocyte monocyte progenitors (GMP): Lin$^-$ Sca1$^-$c-Kit$^+$ CD34$^+$FcgRII/III$^+$; megakaryocyte erythrocyte progenitors (MEP): Lin$^-$ Sca1$^-$c-Kit$^+$ CD34$^-$FcgRII/III$^-$; Pro-B, Lin$^-$IgM$^-$B220$^+$CD43$^+$; Pre-B, Lin$^-$IgM$^-$B220$^+$CD43$^-$; LSK: Lin$^-$Sca1$^+$c-Kit$^+$; long-term hematopoietic stem cells (LT-HSC): Lin$^-$Sca1$^+$c-Kit$^+$CD34$^-$ and short-term multi-potent progenitors (ST/MPP): Lin$^-$Sca1$^+$c-Kit$^+$CD34). Dormant hematopoietic stem cells (d-HSC) are defined as Lin$^-$Sca1$^+$c-Kit$^+$CD48$^-$CD34$^-$CD150$^+$. Lineage markers include CD3, CD4, CD5, CD8a, B220, GR1, Mac1 and Ter119, except that only CD3, CD4, CD8a and GR1 were used for Pro-B and Pre-B populations.

Short term competitive reconstitution assays

Short term competitive reconstitution assays were performed as described with slight modifications (Yang et al., 2005). Briefly, FACS-sorted myeloid-restricted Lin$^-$Sca1$^-$cKit$^+$ (L$^-$S$^-$K$^+$) cells (10,000) from WT or *Pias1*$^{-/-}$ littermates (CD45.2$^+$) were mixed with 2x10^5 of WT C57SJL bone marrow (BM) cells (CD45.1$^+$) and injected into lethally irradiated WT C57SJL mice. The percentage of myeloid cells (Mac1$^+$) from donor mice in peripheral blood (PBL), BM and spleen were assayed by flow cytometry 13 days post reconstitution.

Primer sequences

The following primers for murine genes are used for Q-PCR:

Hprt1-f: 5'- CAGTACAGCCCCAAAATGGT
Hprt1-r: 3'- CAAGGGCATATCCAACAACA

Gata1-f: 5'- AGCAACGGCTACTCCACTGT
Gata1-r: 5'- TGCTGACAATCATTCGCTTC

Gata2-f: 5'- GATACCCACCTATCCCTCCTATGTG
Gata2-r: 3'- GTGGCACCACAGTTGACACACTC

Csf1r-f: 5'- CTTTGGTCTGGGCAAAGAAGAT
Csf1r-r: 5'- CAGGGCCTCCTTCTCATCAG

Mpo-f: 5'- GTTCCGCCTGAACAATCAGT
Mpo-r: 5'- ATTCAGTTTGGCTGGAGTGG

Cebpa-f: 5'- CAAGAACAGCAACGAGTACCG
Cebpa-r: 3'- GTCACTGGTCAACTCCAGCAC

Ikzf1-f: 5'- CACAACGAGATGGCAGAAGA
Ikzf1-r: 3'- CTGACAGGCACTTGTCTCCA

Gata3-f: 5'- CTACCGGGTTCGGATGTAAGTC
Gata3-r: 5'- GTTCACACACTCCCTGCCTTCT

Il7r-f: 5'- TGGCTCTGGGTAGAGCTTTC
Il7r-r: 5'- GTGGCACCAGAAGGAGTGAT

Ebf1-f: 5'- CGGAAATCCAACTTCTTCCA
Ebf1-r: 5'- GTCTTTTCGCTGTTGGCTTC

Pax5-f: 5'- AACTTGCCCATCAAGGTGTC
Pax5-r: 5'- GGCTTGATGCTTCCTGTCTC

Igll1-f: 5'- GAGCTTCAGTGGGAAGCAAC
Igll1-r: 5'- CCCACCACCAAAGACATACC

Epor-f: 5'- TGTCTCCTACTTGCTGGGGC
Epor-r: 5'- CAAGCGTTGGGTGAAGCACA

Hbb-b1-f: 5'- AACGATGGCCTGAATCACTT
Hbb-b1-r: 5'- ACGATCATATTGCCCAGGAG

Slc4a1-f: 5'- CCTCATCCTCACAGTGCCTC
Slc4a1-r: 5'- CAGGCCATTCTCCTCGTCAA

Pias1-f: 5'- CATCAACACCTCCCTCATCC
Pias1-r: 5'- CCTCCTGCACTTAGCTGGTC

The following primers are used for bisulfite sequencing:

Gata1-f: 5'- TTTATTTTAATTTTTTGGGATTTTTAGG
Gata1-r: 5'- AACTACAAACCACCTCTATAAAACAATCTA

The following primers are used for Chromatin immunoprecipitation (ChIP):

Gata1 promoter-f: 5'- ACCTGCAAAATGGGTACAGC
Gata1 promoter-r: 5'- TTCAGTGAGGAAAGCCCCTA

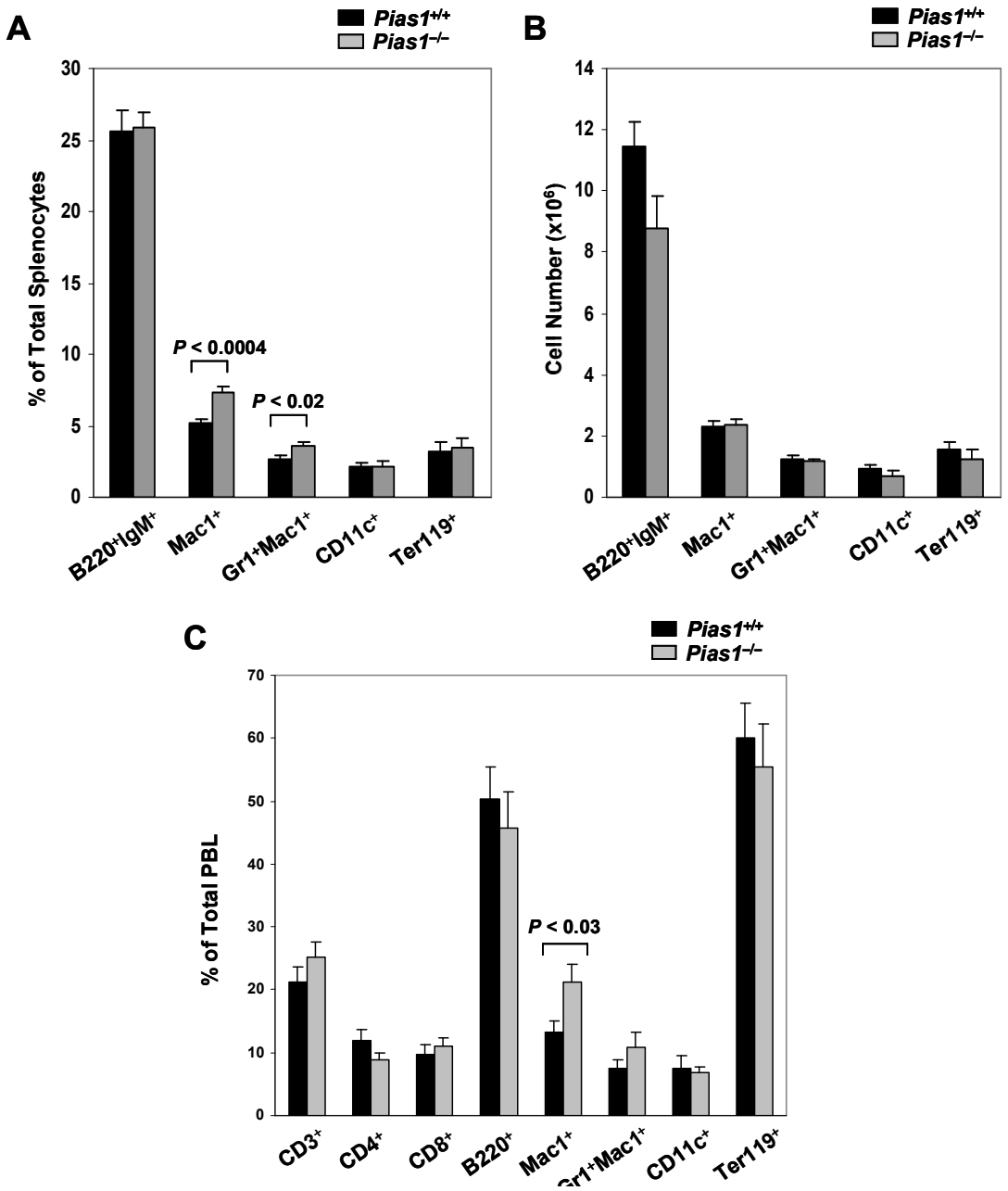

Figure S1. Normal peripheral lineage differentiation in *Pias1*$^{-/-}$ mice. (**A**) Frequencies of mature B cells (B220^{+}IgM^{+}), myeloid cells (Mac1^{+}), granulocyte/monocyte linage (Gr1^{+}Mac1^{+}), dendritic cells (CD11c^{+}) and erythroid cells (Ter119^{+}) in freshly isolated splenocytes from 8-12 weeks old WT and *Pias1*$^{-/-}$ littermates were assayed by flow cytometry. (**B**) Same as in **A** except that cell numbers of each population were presented. (**C**) Same as in **A** except that peripheral blood lymphocytes (PBL) were analyzed. Shown in each panel is a pool of 3 independent experiments (n=9-13). Error bars represent SEM. *P* values were determined by non-paired *t*-test.

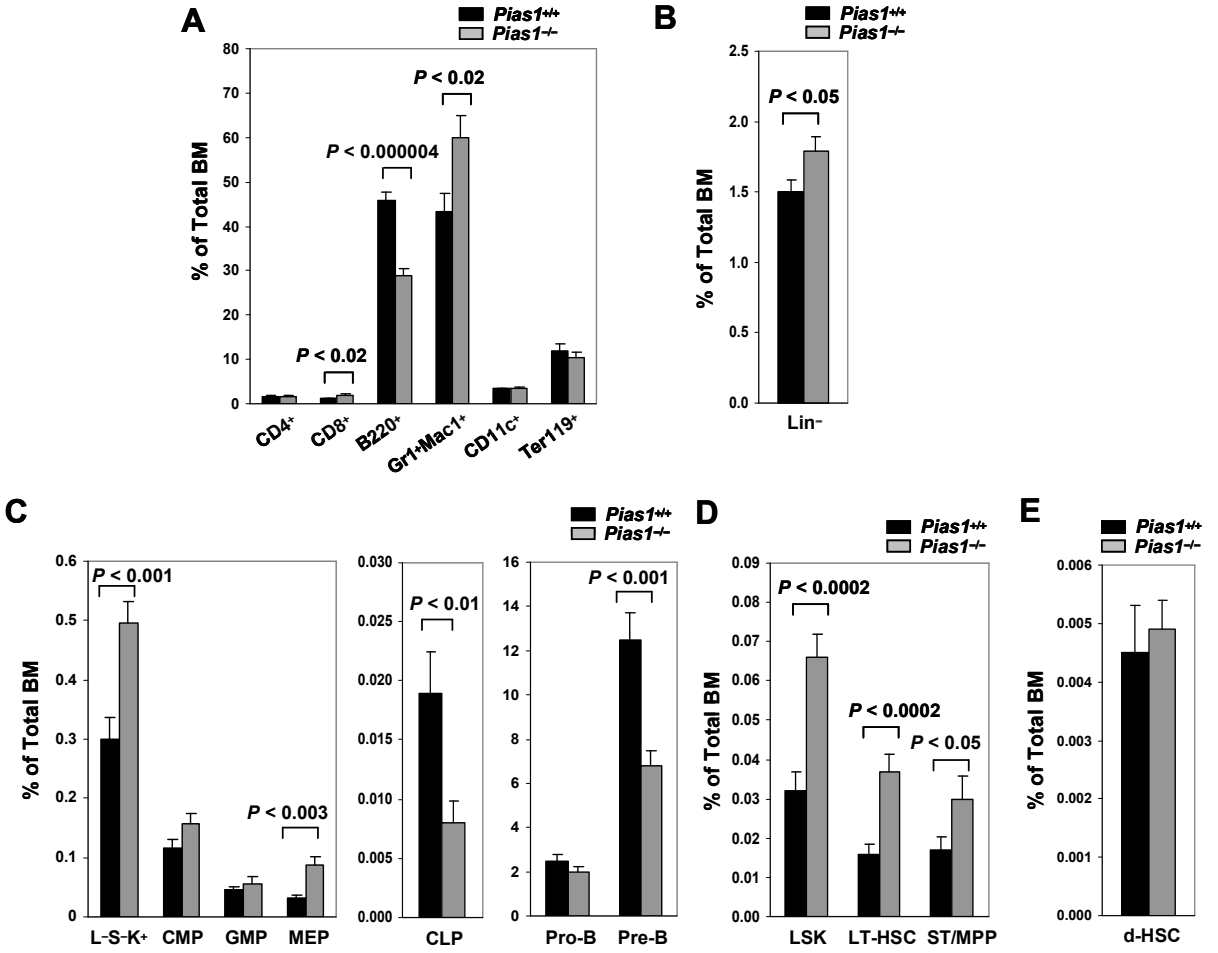

Figure S2. Altered lineage-restricted progenitors and LSK populations in *Pias1*$^{-/-}$ mice. (**A**) Percentage of T cells (CD4$^+$ or CD8$^+$), B cells (B220$^+$), granulocytes/monocytes (Gr1$^+$Mac1$^+$), dendritic cells (CD11c$^+$) and erythroids (Ter119$^+$) in freshly isolated bone marrow (BM) from 8-12 weeks old WT (*Pias1*$^{+/+}$) and *Pias1*$^{-/-}$ littermates were assayed by flow cytometry. (**B**) Same as in **A** except that frequencies of myeloid-restricted Lin$^-$Sca1$^-$c-Kit$^+$ (L$^-$S$^-$K$^+$) populations, common myeloid progenitors (CMP), granulocyte monocyte progenitors (GMP), megakaryocyte erythrocyte progenitors (MEP), common lymphoid progenitors (CLP), Pro-B and Pre-B cells as defined in Supplementary Materials and Methods were assayed. (**C**) Same as in **A** except that cell numbers of LSK, long-term hematopoietic stem cells (LT-HSC) and short-term multi-potent progenitors (ST/MPP) in total BM as defined in Supplementary Materials and Methods were assayed. (**D**) Same as in **A** except that the percentage of Lin$^-$ population was assayed. (**E**) Same as in **A** except that the percentage of dormant hematopoietic stem cells (d-HSCs; Lin$^-$Sca1$^+$c-Kit$^+$CD150$^+$CD48$^-$CD34$^-$) was assayed. Shown in each panel is a pool of 3 independent experiments (n=9-13). Error bars represent SEM. *P* values were determined by non-paired *t*-test.

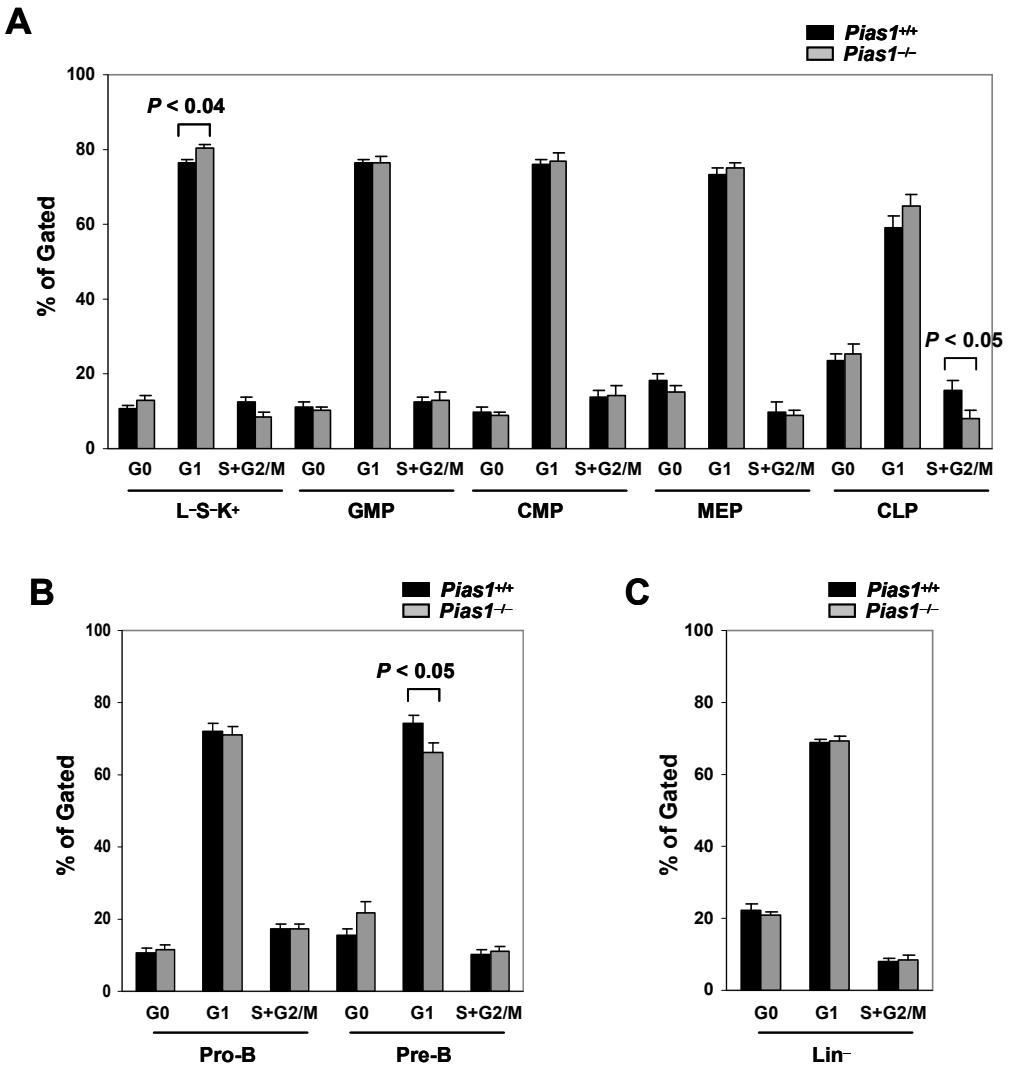

Figure S3. Normal cell proliferation of various progenitor populations. (**A**) Cell proliferation of indicated BM subsets from WT and *Pias1*$^{-/-}$ mice as defined in Supplementary Materials and Methods was revealed by intracellular Ki67 (icKi67) and Hoechst DNA staining followed by flow cytometry. G0, icKi67^{-}, 2N DNA content; G1, icKi67^{+}, 2N DNA content; S+G2/M, icKi67^{+}, > 2N DNA content. (**B**) Same as in **A** except that Pro-B and Pre-B cells were assayed. (**C**) Same as in **A** except that Lin^{-} cells were assayed. Shown in each panel is a pool of 3 independent experiments (n=10-13). Error bars represent SEM. *P* values were determined by non-paired *t*-test.

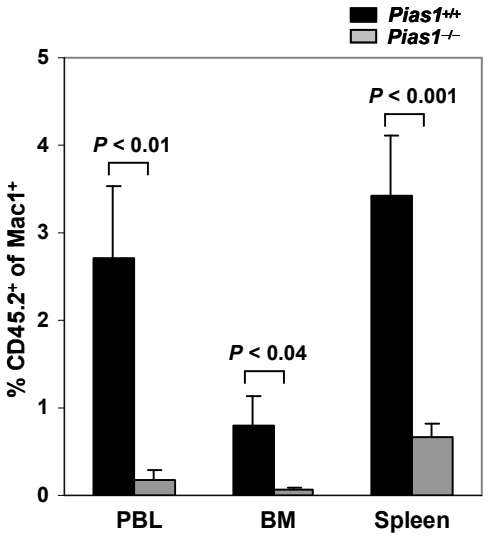

Figure S4. Defective short-term reconstitution capability of *Pias1*$^{-/-}$ myeloid-restricted Lin^{-}Sca1^{-}c-Kit^{+} (L^{-}S^{-}K^{+}) cells revealed by i*n vivo* short-term competitive reconstitution assays. FACS-sorted L^{-}S^{-}K^{+} cells (10,000) from WT or *Pias1*$^{-/-}$ littermates (CD45.2^{+}) were mixed with 2x10^{5} of WT C57SJL bone marrow (BM) cells (CD45.1^{+}) and injected into lethally irradiated WT C57SJL mice. The percentage of myeloid cells (Mac1^{+}) from donor mice in peripheral blood (PBL), BM and spleen were assayed by flow cytometry 13 days post reconstitution. Shown is a pool of 3 independent experiments in all panels (n=10). Error bars represent SEM. *P* values were determined by non-paired *t*-test.

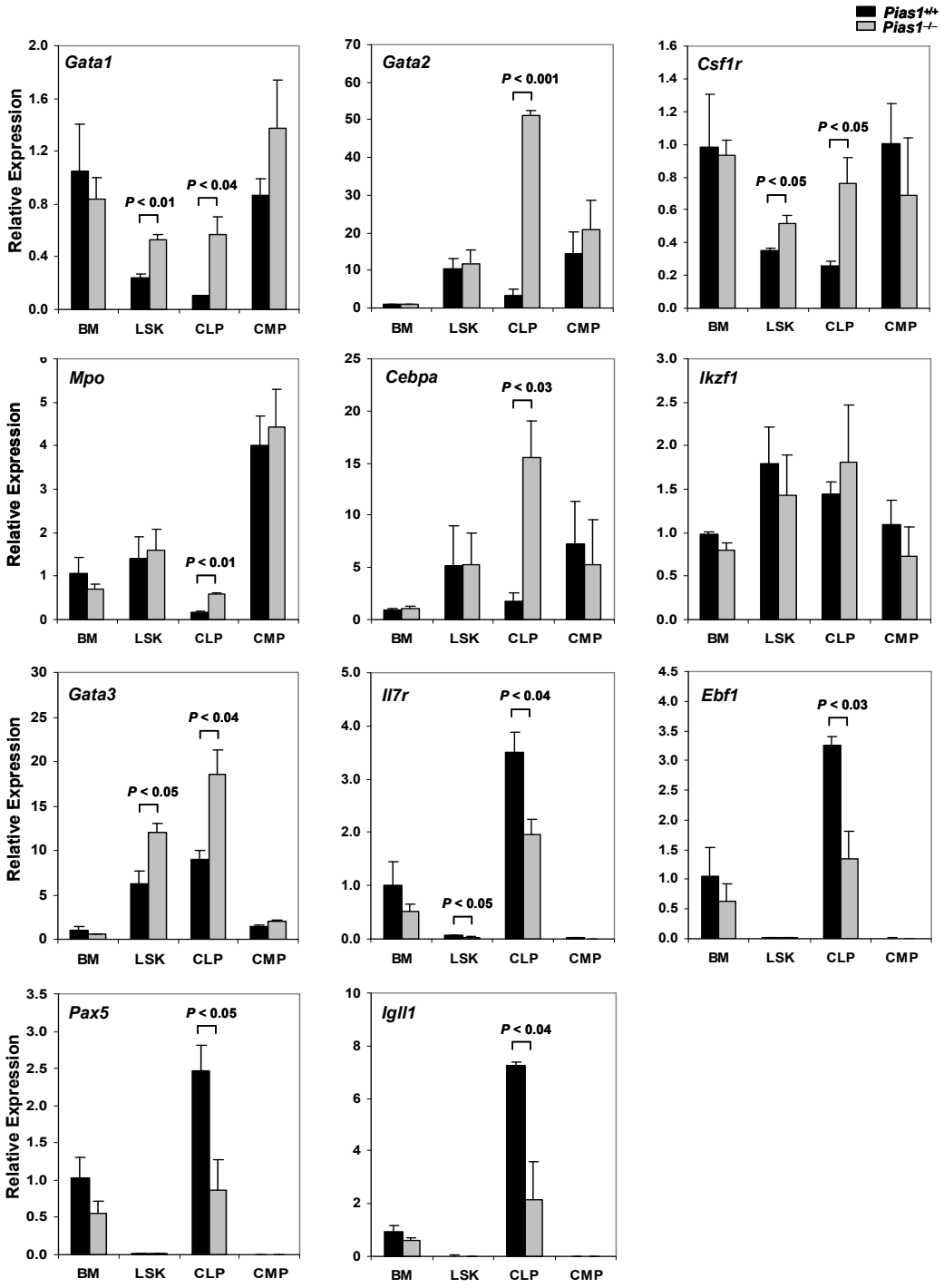

Figure S5. Transcription of the lineage-affiliated genes is regulated by PIAS1. Quantitative real time polymerase chain reaction (Q-PCR) analyses were performed with total RNA from WT or *Pias1*$^{-/-}$ bone marrow (BM), or FACS-sorted HSC-enriched Lin^{-}Sca1^{+}c-Kit^{+} (LSK), common lymphoid progenitors (CLP) and common myeloid progenitors (CMP) as defined in Supplementary Materials and Methods, using gene-specific primers. Each gene is indicated at the top left of each panel, and the results were adjusted by *Hprt1*. Shown is the average of 2 independent experiments (n=5 for each experiment). Error bars represent SD. *P* values were determined by non-paired *t*-test.

References

Liu, B., Mink, S., Wong, K.A., Stein, N., Getman, C., Dempsey, P.W., Wu, H. and Shuai, K. (2004) PIAS1 selectively inhibits interferon-inducible genes and is important in innate immunity. *Nat. Immunol.*, **5**, 891-898.

Yang, L., Bryder, D., Adolfsson, J., Nygren, J., Mansson, R., Sigvardsson, M. and Jacobsen, S.E. (2005) Identification of Lin(-)Sca1(+)kit(+)CD34(+)Flt3- short-term hematopoietic stem cells capable of rapidly reconstituting and rescuing myeloablated transplant recipients. *Blood*, **105**, 2717-2723.