

Supplementary Table Legends

Supplementary Table 1: Differentially expressed genes in HSCs >2-fold with $p < 0.05$ (Transcriptome Analysis Console)

Supplementary Table 2: Differentially expressed genes in LMPPs >2-fold with $p < 0.05$ (Transcriptome Analysis Console)

Supplementary Table 3: GSEA of HSC gene sets with $p < 0.05$ and FDR $q < 0.25$

Supplementary Table 4: GSEA of LMPP gene sets with $p < 0.05$ and FDR $q < 0.25$

Supplementary Table 5: IPA upstream analysis

IPA was performed using genes that were downregulated or upregulated in DKO LMPPs compared with WT LMPPs >2-fold with $p < 0.05$.

Supplementary Table 6: IPA upstream analysis

IPA was performed using genes that were downregulated or upregulated in $\delta^{-/-}$ (Delta) LMPPs compared with WT LMPPs >2-fold with $p < 0.05$

Supplementary Table 7: List of flow cytometry antibodies used

Supplementary Figures and Legends

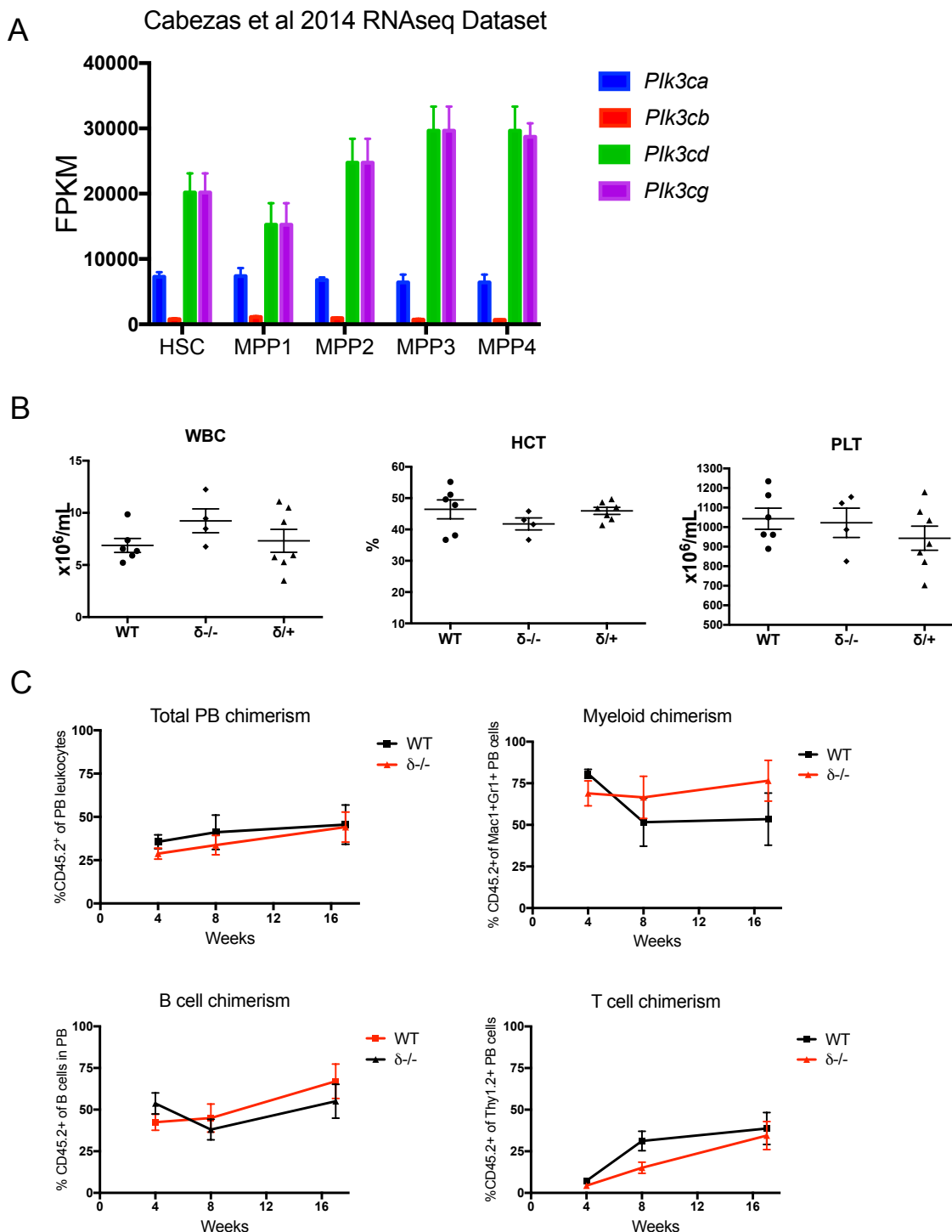
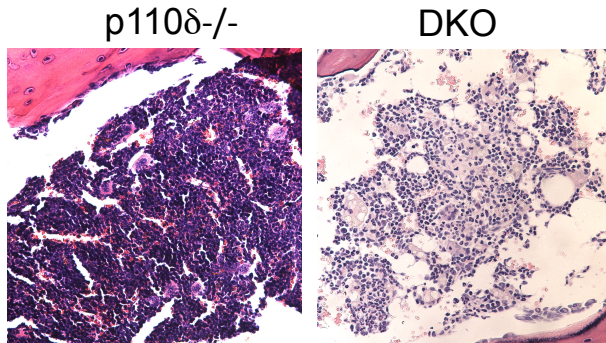


Figure S1: Germ line deletion of *Pik3cd* is dispensable for HSC repopulating activity (A) RNA expression of the Class I PI3K isoforms in sorted mouse HSCs and MPP populations extracted from the published dataset by Cabezas-Wallscheid, et al (Cabezas-Wallscheid et al., 2014). **(B)** Peripheral blood counts of 6-8 week-old *Pik3cd*^{-/-} ($\delta^{-/-}$, N=4), *Pik3cd*^{+/-} ($\delta^{-/+}$, N=7) littermates, and wild-type (WT, N=6) C57 Bl/6 mice. ANOVA analysis with the Tukey's multiple comparison test was used to compare the groups. **(C)** Competitive repopulation of $\delta^{-/-}$ bone marrow (CD45.2⁺) or C57 Bl/6 (CD45.2⁺) bone marrow mixed in a 1:1 ratio with CD45.2⁺/CD45.1⁺ WT competitor BM, and transplanted into lethally irradiated B6.SJL (CD45.1⁺) recipients. Donor chimerism from serial peripheral blood samples from recipient mice

(N=8 per group) is shown for total peripheral blood (PB), Mac1+Gr1+ (Myeloid chimerism), and B220+ (B cell chimerism), and Thy1.2+ (T cell chimerism). The % of CD45.2 single positive cells indicates the % donor chimerism in each cell population. At each time point, the Student's two-tailed unpaired t-test was used to compare the data between the two groups. Data represent mean $\pm$ SEM.

A Bone Marrow, 400X



B Spleen, 100X

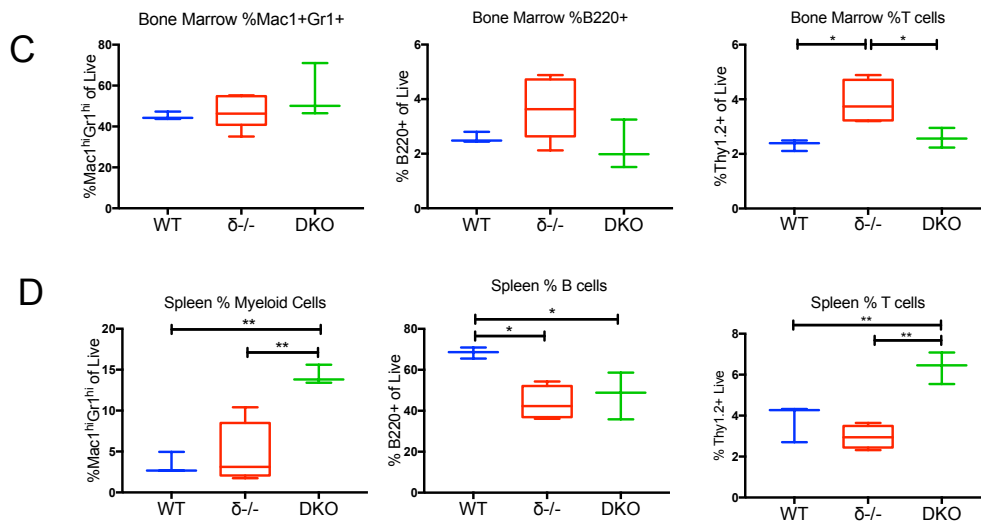
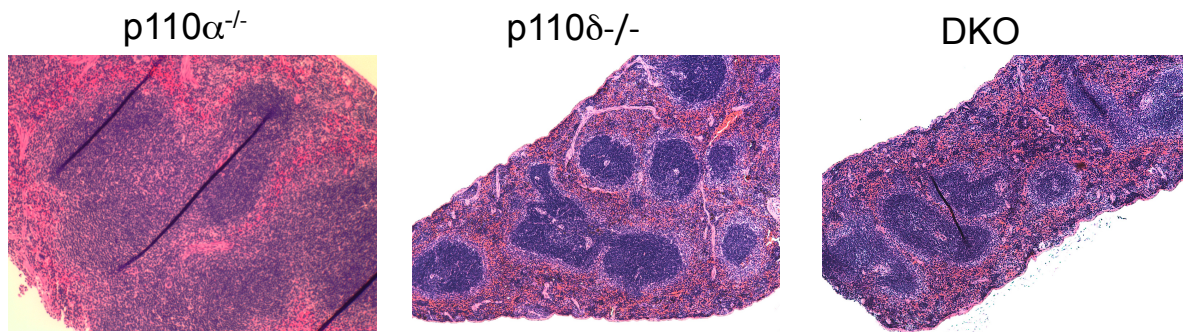


Figure S2: Images of Wright-Giemsa stained histopathology slides of **(A)** bone marrow sections from p110 $\delta^{-/-}$ and DKO mice and **(B)** spleen sections from p110 $\alpha^{-/-}$, p110 $\delta^{-/-}$, and DKO mice. **(C)** Flow cytometry analysis of bone marrow and spleen **(D)** cells from WT (N=3), p110 $\delta^{-/-}$ (N=4), and DKO (N=3) mice at 7-8 weeks of age, 3 weeks after plpC. In C and D, ANOVA analysis with Tukey's comparison test was used to compare the groups. Data represent mean $\pm$ SEM. *p<0.05; **p<0.01

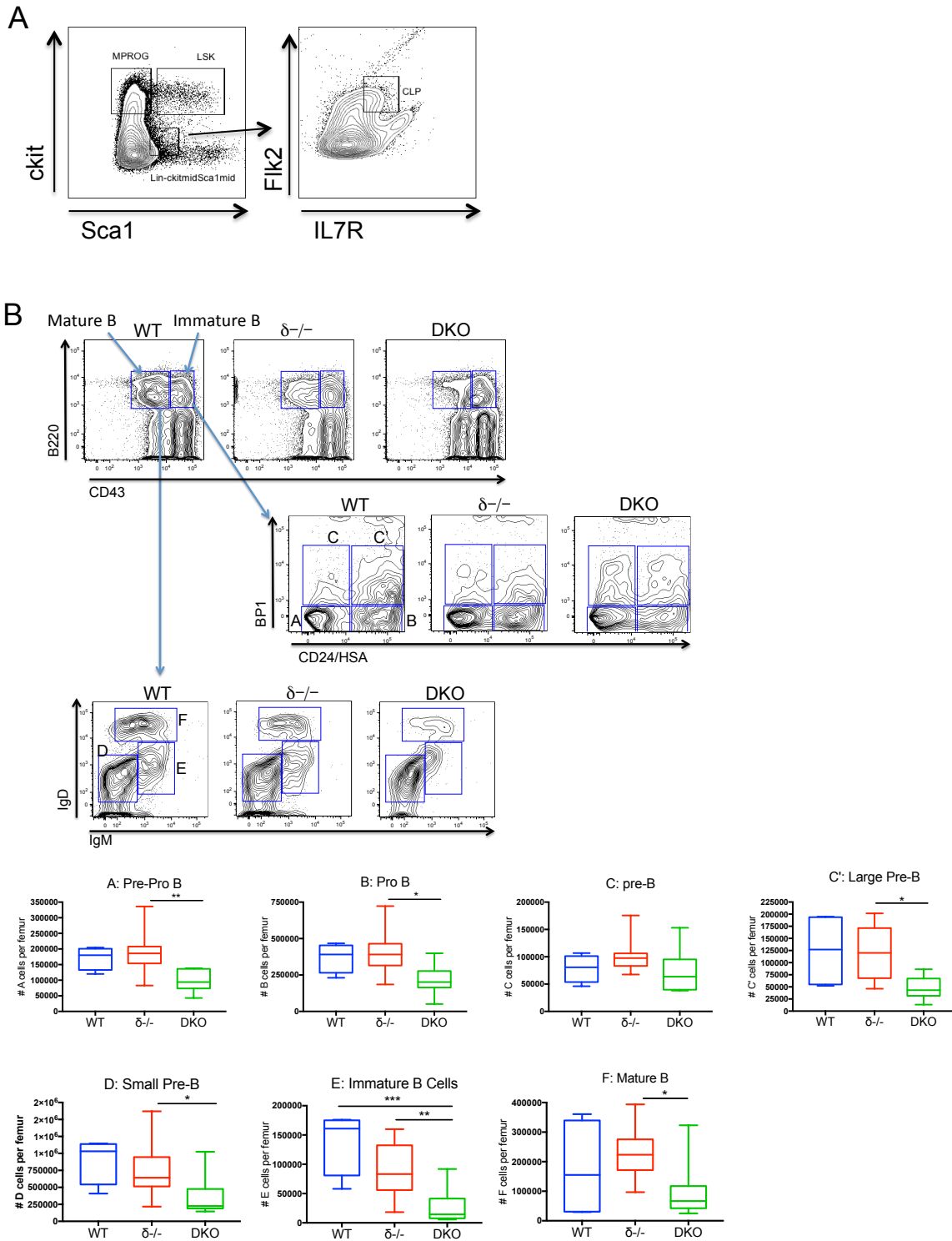


Figure S3: Analysis of bone marrow B cell development in $\delta^{-/-}$ and DKO Mice (A) CLP gating strategy on bone marrow cells (B) Hardy fraction analysis of bone marrow B cell development in WT (N=4), $\delta^{-/-}$ (N=11) and DKO (N=9) mice at 7-8 weeks of age, 3 weeks after plpC. Combined data from two independent experiments is shown. ANOVA analysis with the Tukey's multiple comparison test was used. Data represent mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$, * $p < 0.001$**

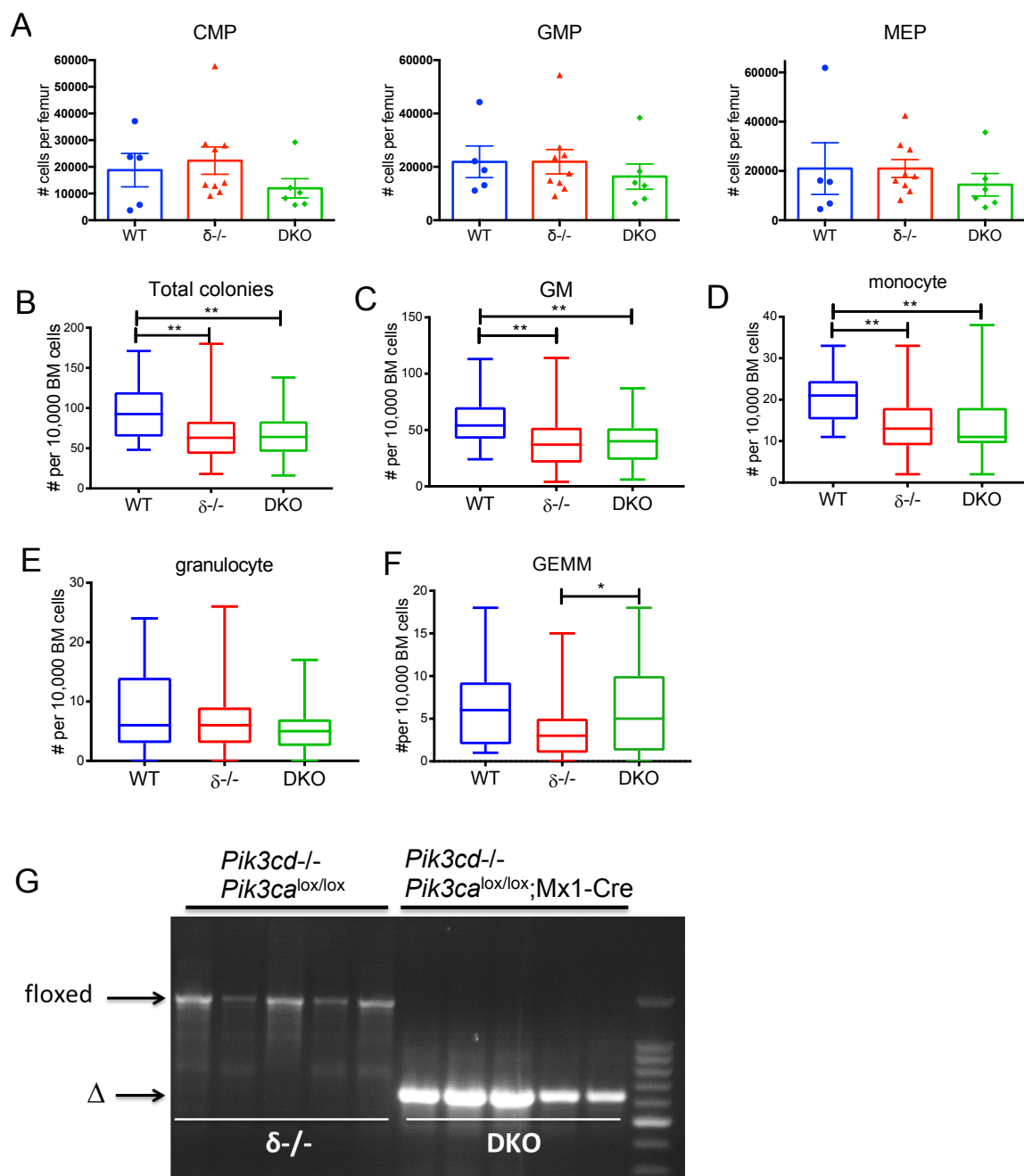


Figure S4: (A) Absolute numbers of common myeloid progenitors (CMP: $Lin^{lo}ckit+Sca1-CD34^{mid}FCRg^{mid}$), granulocyte macrophage progenitors (GMP: $Lin^{lo}ckit+Sca1-CD34^{lo}FCRg^{hi}$) and megakaryocyte erythroid progenitors (MEP: $Lin^{lo}ckit+Sca1-CD34^{lo}FCRg^{lo}$) in the bone marrow of WT (N=5), $\delta^{-/-}$ (N=9), and DKO (N=6) mice at 7-8 weeks of age, 3 weeks after plpC. Combined data from multiple experiments is shown. **(B-F)** Plating of bone marrow cells in M3434 methylcellulose media (Stem Cell Technologies). Colony numbers for each colony type at 7 days are shown. Combined data from multiple experiments is shown (N=12 for WT; N=51 for $\delta^{-/-}$; N=36 for DKO). For experiments shown in A-F, ANOVA analysis with the Tukey's multiple comparison test was used. Data represent mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$ **(G)** Excision PCR of genomic DNA extracted from peripheral blood collected at 3 weeks after a single plpC injection.

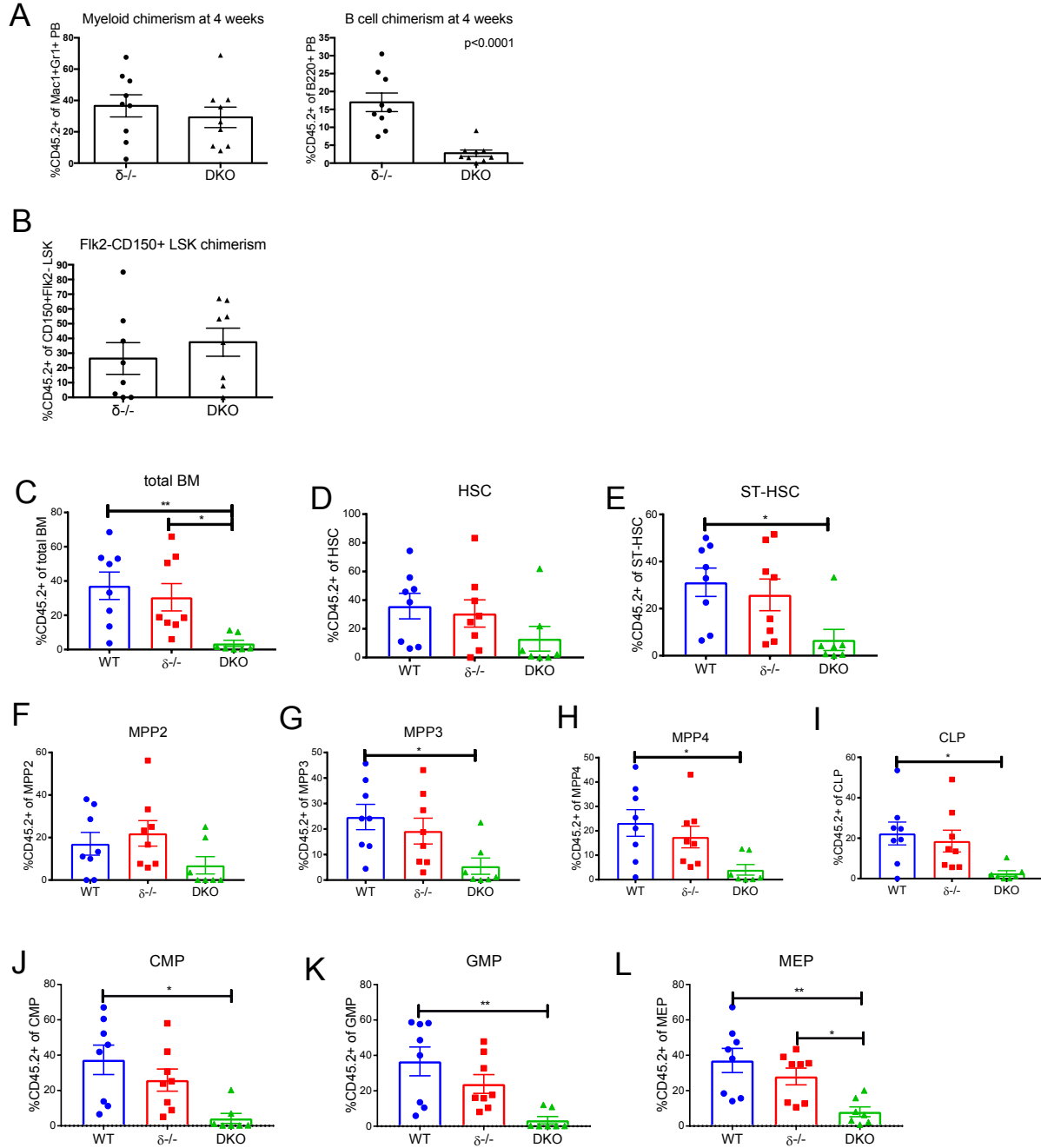


Figure S5: (A) P110 $\delta^{-/-}$ or DKO donors (CD45.2+) were injected with plpC two times starting at 4 weeks of age, and then bone marrow was harvested 3 weeks later for competitive transplantation together with bone marrow from C57 Bl/6;B6.SJL hybrids (CD45.1+45.2+). Donor chimerism in the peripheral blood of the recipient mice at 4 weeks post-transplantation is shown. **(B)** Donor chimerism of the Fik2-CD150+ LSK population in the bone marrow is shown for the same bone marrow transplant recipients in A at 16 weeks post-transplantation. For A and B, the Student's two-tailed unpaired t-test was used to compare the two groups. **(C-L)** Donor chimerism in bone marrow populations is shown at 16 weeks post-transplantation from the competitive secondary transplantation experiment shown in Figure 2D. ANOVA analysis with the Bartlett multiple comparison test was used. Data represent mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$

HSCs. **(F)** IPA analysis of differentially expressed genes between DKO and WT LMPPs (>2-fold change, $p < 0.05$)

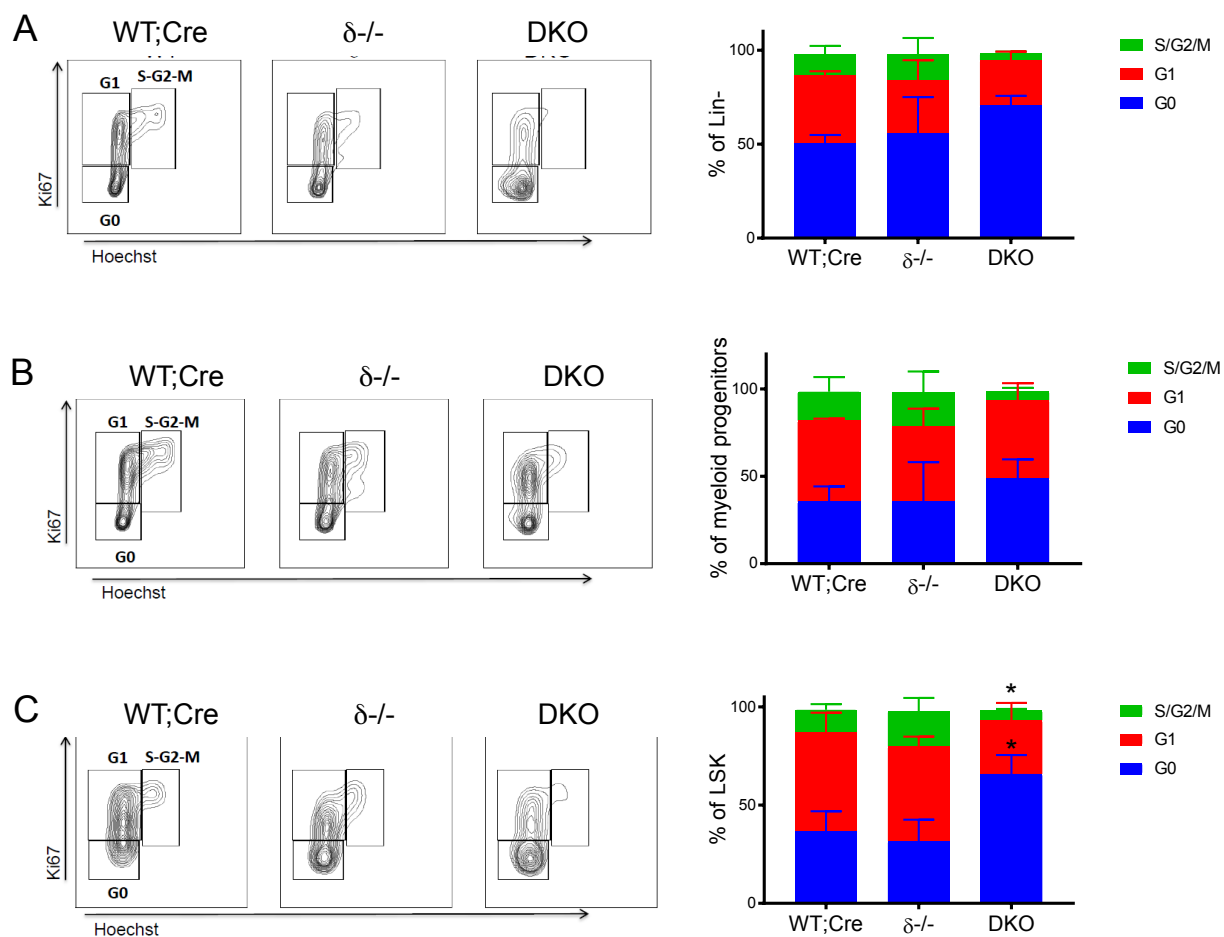
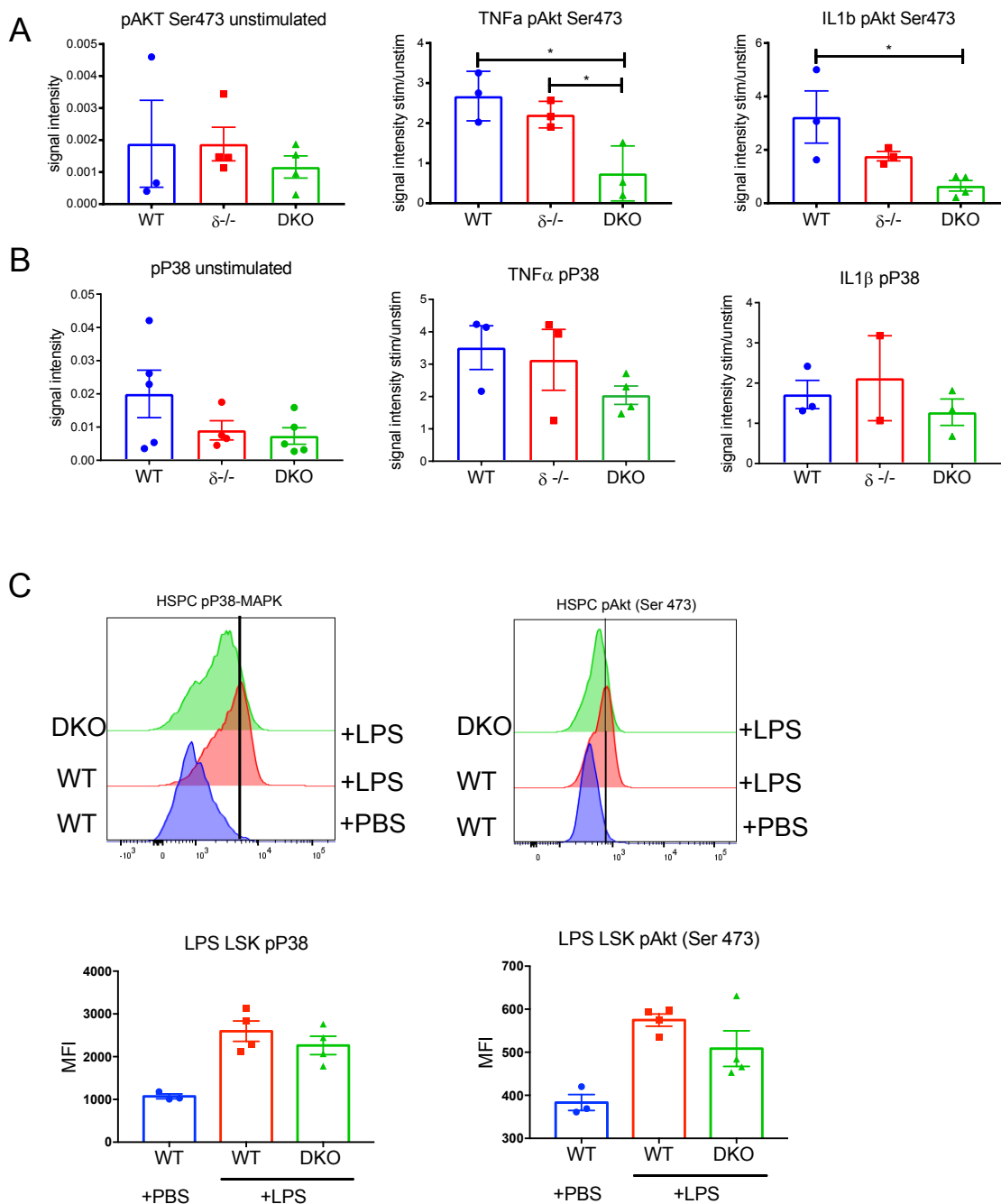
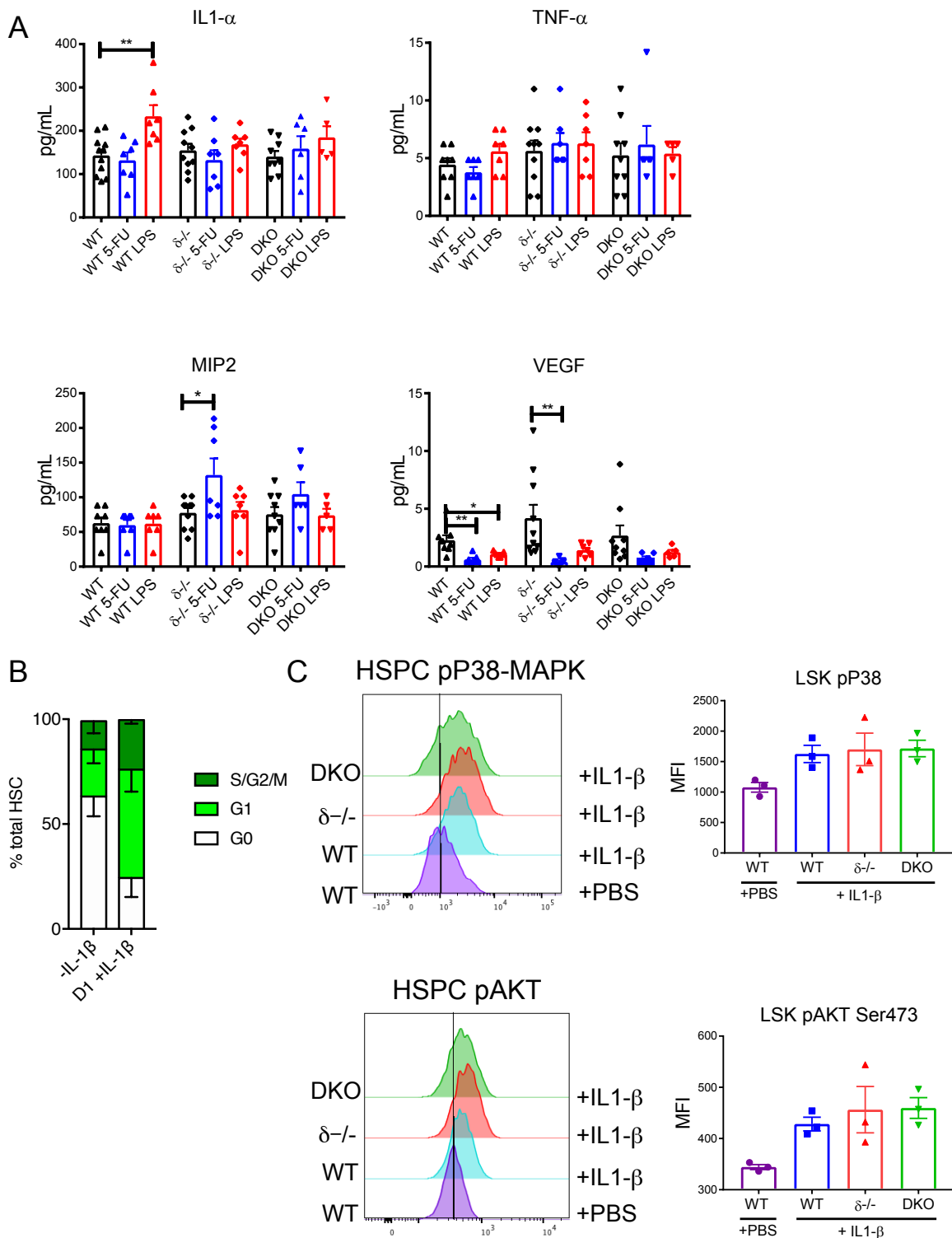


Figure S7: Cell cycle analysis of bone marrow populations using Hoechst and Ki67 staining from mice sacrificed at 7 days after 5-FU injection (N=3 per group in each experiment, with 3 experimental trials) (see Figure 5) **(A)** Lin⁻ BM cells **(B)** myeloid progenitors (Lin-ckit+Sca1-) **(C)** LSK cells (Lin-Sca1+ckit+). Left panels: representative flow cytometry plots. Right panels: quantification of one representative experiment. ANOVA analysis with the Tukey's multiple comparison test was used to compare the groups. Data represent mean $\pm$ SEM.





Supplementary References:

- Cabezas-Wallscheid, N., D. Klimmeck, J. Hansson, D.B. Lipka, A. Reyes, Q. Wang, D. Weichenhan, A. Lier, L. von Paleske, S. Renders, P. Wunsche, P. Zeisberger, D. Brocks, L. Gu, C. Herrmann, S. Haas, M.A.G. Essers, B. Brors, R. Eils, W. Huber, M.D. Milsom, C. Plass, J. Krijgsveld, and A. Trumpp. 2014. Identification of regulatory networks in HSCs and their immediate progeny via integrated proteome, transcriptome, and DNA methylome analysis. *Cell Stem Cell* 15:507-522.
- Pietras, E.M., D. Reynaud, Y.A. Kang, D. Carlin, F.J. Calero-Nieto, A.D. Leavitt, J.M. Stuart, B. Gottgens, and E. Passegue. 2015. Functionally Distinct Subsets of Lineage-Biased Multipotent Progenitors Control Blood Production in Normal and Regenerative Conditions. *Cell Stem Cell* 17:35-46.