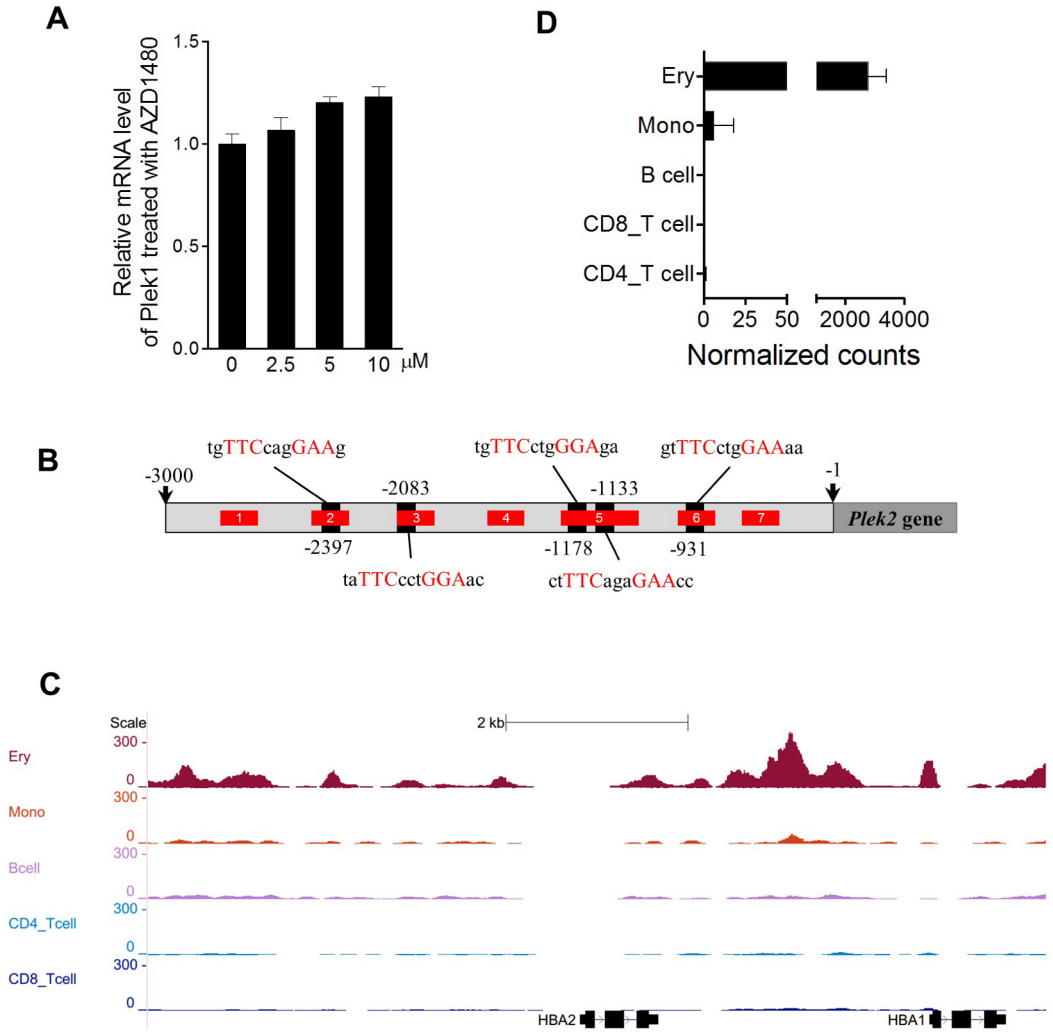
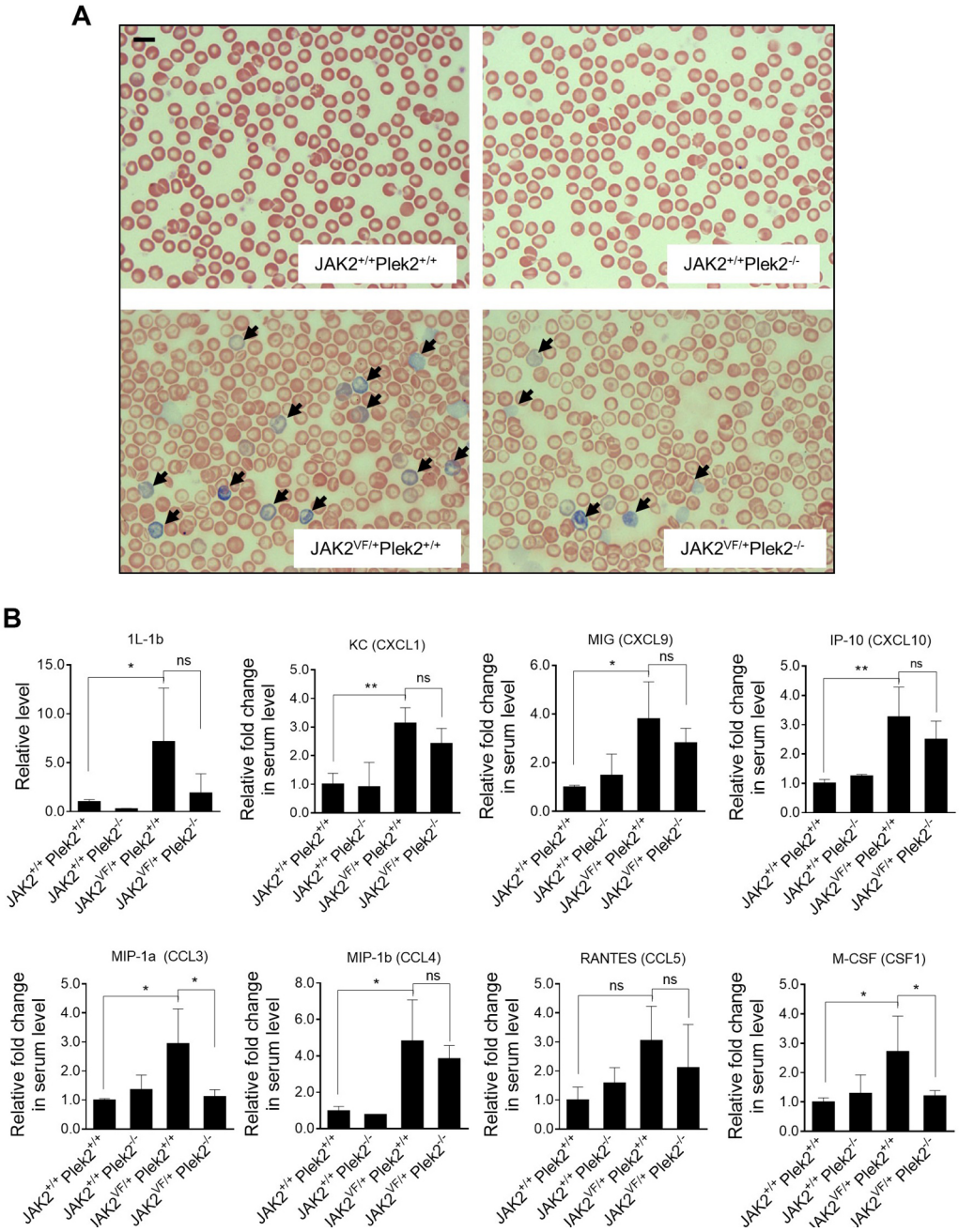
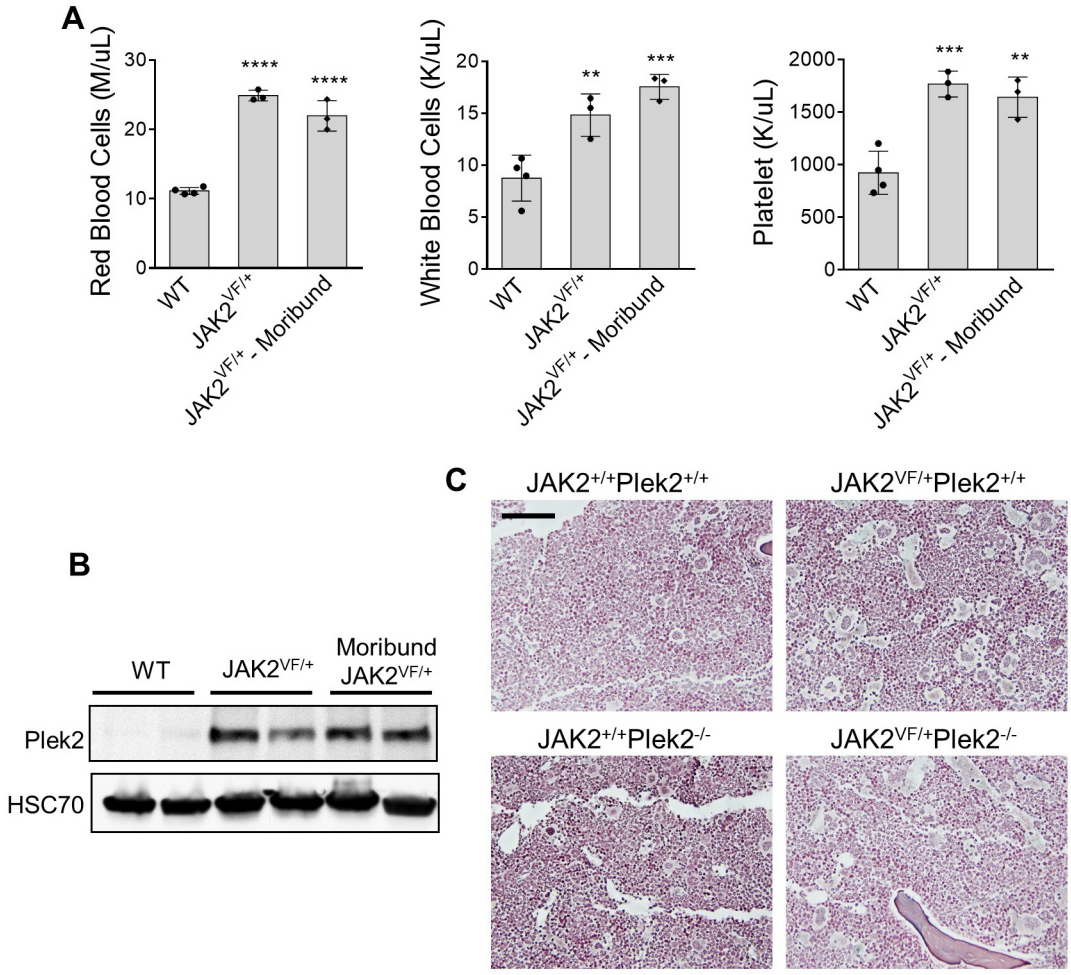
Supplemental Data



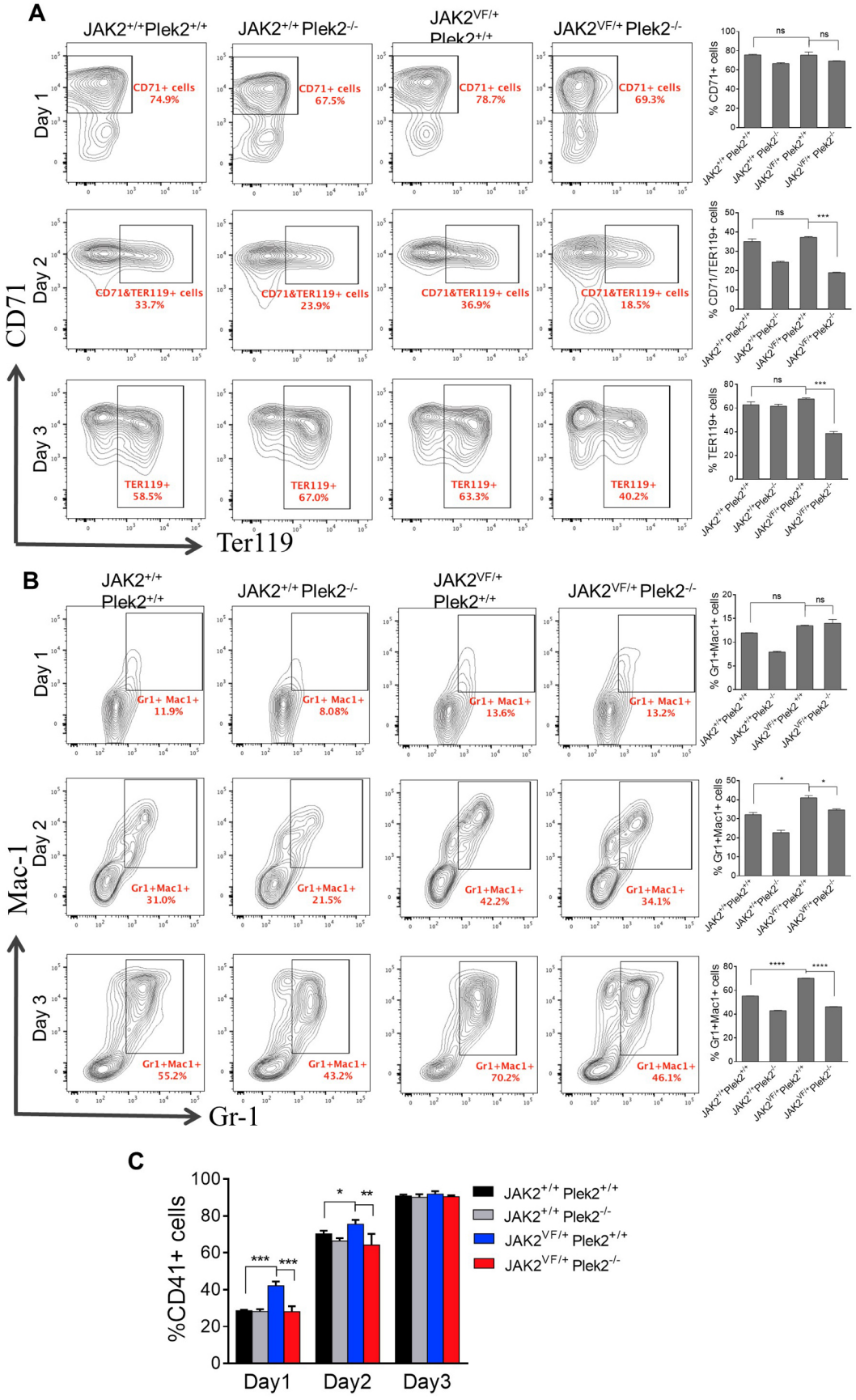
Supplemental Figure 1. Plek2 is a downstream target of STAT5. (**A**) Different concentrations of JAK2 inhibitor AZD1480 were added to the cultured erythroblasts in the presence of Epo (2 U/ml). Quantitative PCR analysis of the Plek1 mRNA expression was performed after 20 hours. (**B**) The predicted STAT5 binding sites were indicated by black blocks. Red blocks indicated fragments (1-7) amplified in the chromatin immunoprecipitation (ChIP)-qPCR assay. P1: -2555 ~ -2625; P2: -2373 ~ -2434; P3: -2012 ~ -2079; P4: -1590 ~ -1670; P5: -1050 ~ -1179; P6: -922 ~ -1009; P7: -541 ~ -609. (**C-D**) Normalized ATAC-sequencing peaks in the *HBA2 and HBA1* loci and relative expression of *HBA2* (D) in indicated cell type. Data are representative of all technical and biological replicates for each cell type: Erythroid cells (Ery), 8; Monocyte (Mono), 6; B Cell, 4; CD4_T cell, 5; CD8_T cell, 5. Y-axis represents normalized arbitrary units. Boxed regions show cell-type-specific peaks around the hemoglobin genes. Data were obtained from reference 22.



Supplemental Figure 2. Loss of Plek2 ameliorates JAK2^{V617F}-induced reticulocytosis and over-secretion of cytokines. (**A**) Wright-Giemsa stain of peripheral blood smear from indicated mice. The arrows indicate polychromatic reticulocytes. Scale bar: 10 μm. (**B**) Cytokines are reduced in JAK2^{V617F} knockin mice with the loss of Plek2. Serum was collected from the indicated mice and the listed cytokines were measured using multiplex bead-based Liminex technology (Millipore). Normalized fold changes to age-matched (3-4 months) wild type littermates were shown as mean ± SD, N = 4 in each group. * $P < 0.05$, ** $P< 0.01$, all P values were determined by 1-way ANOVA with Tukey's multiple comparisons test.



Supplemental Figure 3. Lack of myelofibrosis in JAK2^{V617F} knockin mice. (**A**) Complete blood count of indicated mice at 4-5 months old. Data were obtained from 3 moribund JAK2^{V617F} knockin mice, 3 viable age-matched JAK2^{V617F} knockin mice, and 4 age-matched wild-type littermates. * $P < 0.05$, ** $P < 0.01$, ***$P < 0.0005$ and ****$P < 0.0001$, all P values were determined by 1-way ANOVA with Tukey's multiple comparisons test. (**B**) Western blot analysis of Plek2 in the peripheral blood nucleated cells from mice in A. Hsc70 was used as a loading control. (**C**) Reticulin stain of bone marrow from indicated mice at 4-5 months old. Scale bar: 200 μm.


A
JAK2+/+Plek2+/+
JAK2+/+ Plek2-/-
JAK2VF/+ Plek2+/+
JAK2VF/+ Plek2-/-
Day 1
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Day 3
CD71
Ter119
CD71+ cells 74.9%
CD71+ cells 67.5%
CD71+ cells 78.7%
CD71+ cells 69.3%
CD71&TER119+ cells 33.7%
CD71&TER119+ cells 23.9%
CD71&TER119+ cells 36.9%
CD71&TER119+ cells 18.5%
TER119+ 58.5%
TER119+ 67.0%
TER119+ 63.3%
TER119+ 40.2%
% CD71+ cells
% CD71/TER119+ cells
% TER119+ cells
ns
***
B
JAK2+/+ Plek2+/+
JAK2+/+ Plek2-/-
JAK2VF/+ Plek2+/+
JAK2VF/+ Plek2-/-
Day 1
Day 2
Day 3
Mac-1
Gr-1
Gr1+ Mac1+ 11.9%
Gr1+ Mac1+ 8.08%
Gr1+ Mac1+ 13.6%
Gr1+ Mac1+ 13.2%
Gr1+Mac1+ 31.0%
Gr1+Mac1+ 21.5%
Gr1+Mac1+ 42.2%
Gr1+Mac1+ 34.1%
Gr1+Mac1+ 55.2%
Gr1+Mac1+ 43.2%
Gr1+Mac1+ 70.2%
Gr1+Mac1+ 46.1%
% Gr1+Mac1+ cells
ns
*
****
C
%CD41+ cells
Day1
Day2
Day3
JAK2+/+ Plek2+/+
JAK2+/+ Plek2-/-
JAK2VF/+ Plek2+/+
JAK2VF/+ Plek2-/-
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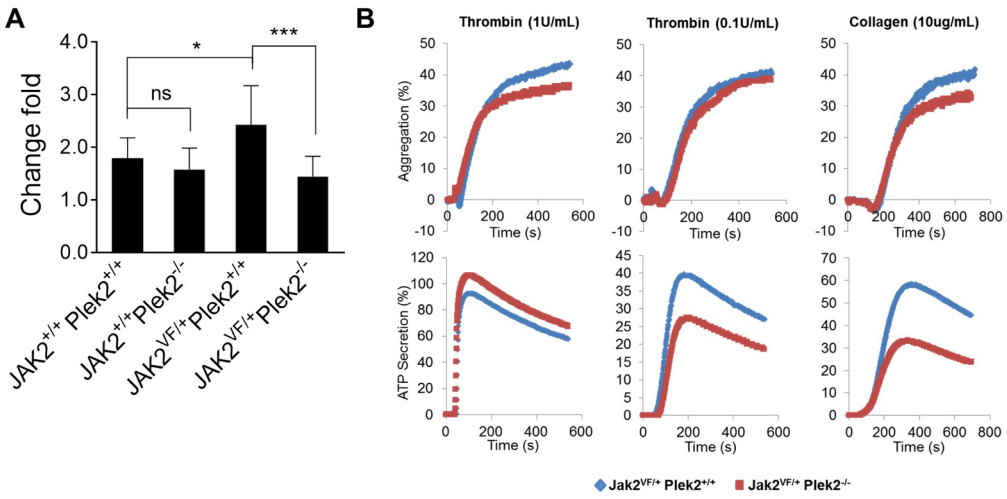
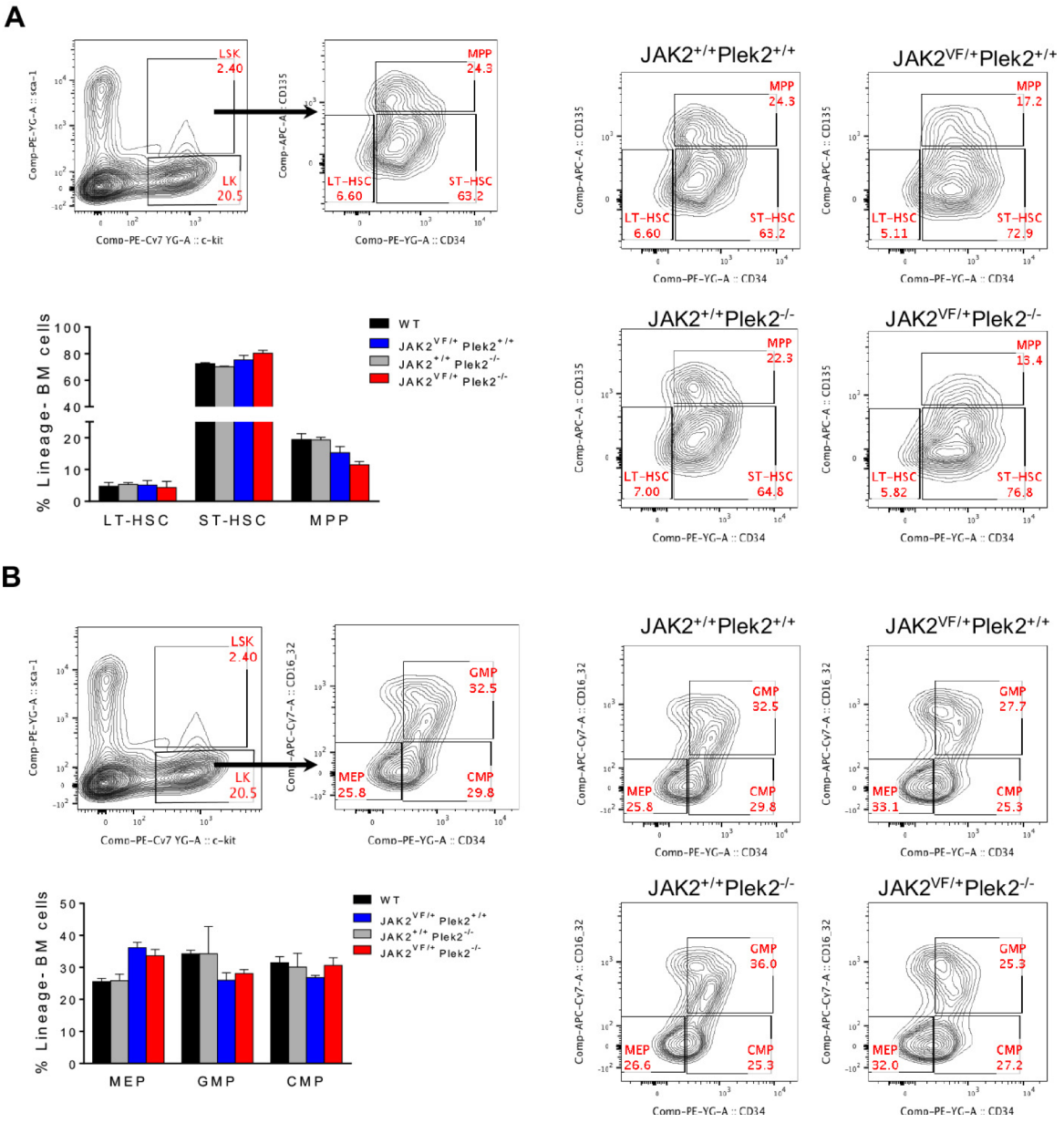
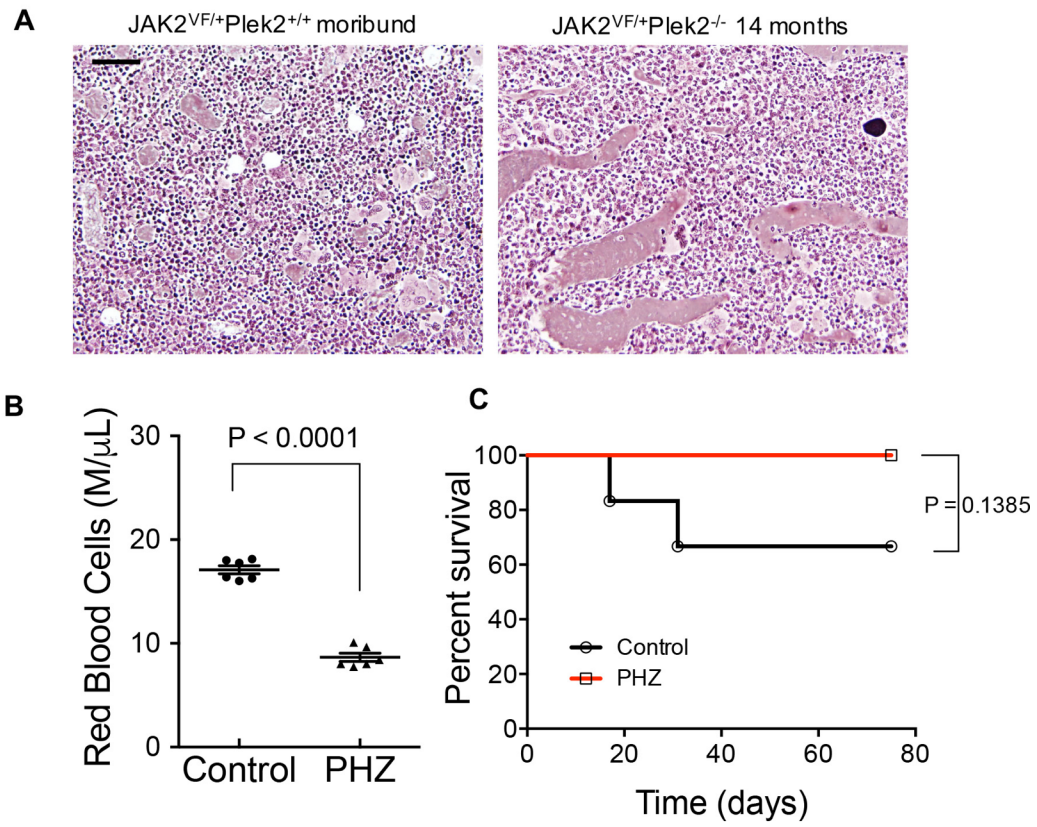
Supplemental Figure 4. Loss of Plek2 causes mild inhibition of the erythroid and granulocytic differentiation *ex vivo*. (**A**) Lineage negative bone marrow progenitor cells were purified from indicated mice and cultured in Epo-containing medium for 3 d. The differentiation was monitored by flow cytometry using CD71 and Ter119. Quantification is shown on the right. N = 3 in each group. (**B**) Lineage negative bone marrow progenitor cells were purified from indicated mice and cultured in GM-CSF-containing medium for 3 d. The differentiation was monitored by flow cytometry using Mac1 and Gr1. Quantification is shown on the right. N = 3 in each group. (**C**) Lineage negative bone marrow progenitor cells were purified from indicated mice and cultured in Tpo-containing medium for 3 d. The differentiation was monitored by flow cytometry using CD41. Quantification is presented. N = 3 in each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0005$ and **** $P < 0.0001$, all P values were determined by 1-way ANOVA with Tukey's multiple comparisons test.



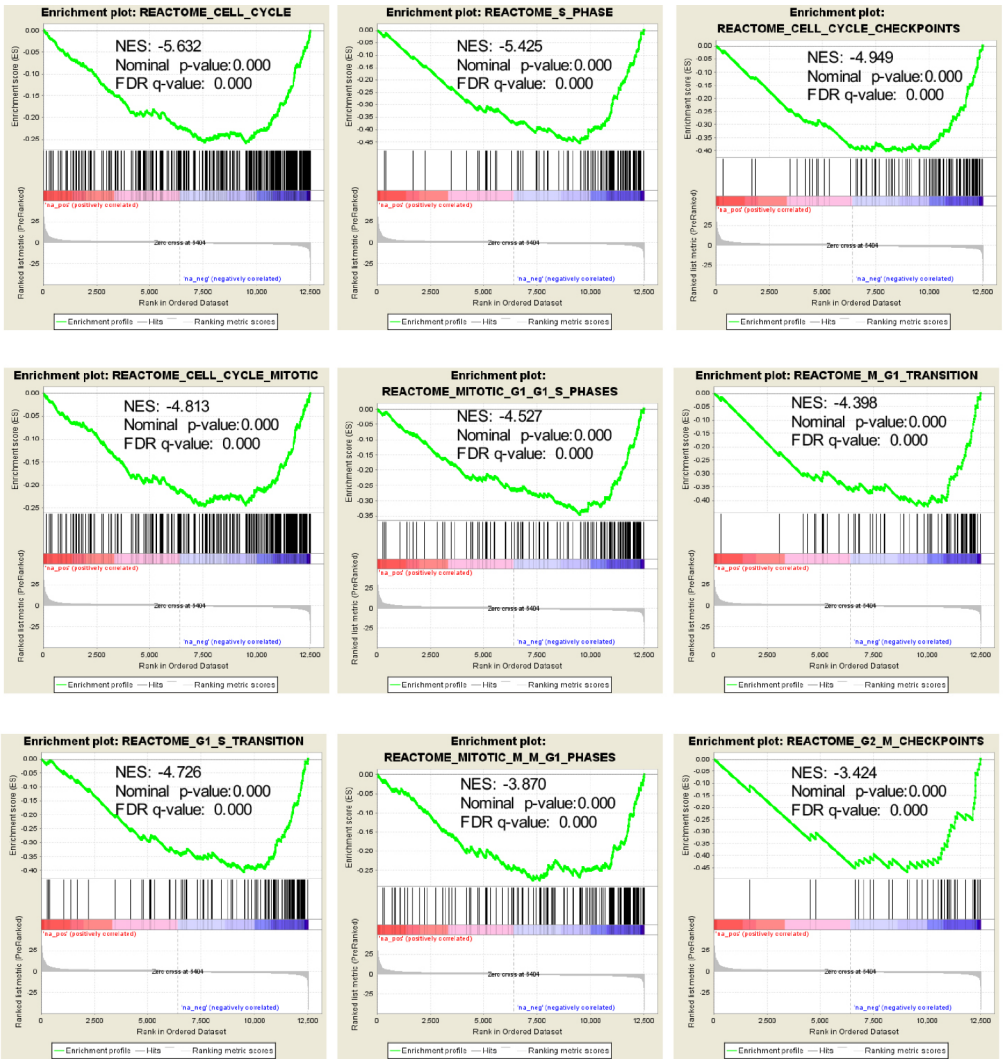
Supplemental Figure 5. Loss of Plek2 on the functions of neutrophils, platelets, and stem/progenitor cells in JAK2^{V617F} mice. (**A**) Mature neutrophils were purified from indicated mice. 50 µl cell suspension (3 x 10^5) was placed in the upper compartment of a transwell chamber. 100 nM fMLP or vehicle control was added to the bottom chamber. After incubation for 2.5 h at 37°C and 5% CO_2, the cells in the lower chamber was harvested and counted. The cell counts were compared between fMLP and vehicle chambers in each group and their change folds are illustrated. Data were obtained from 3 independent experiments and presented as Mean ± SD. * P < 0.05, ***P < 0.0005, all P values were determined by 1-way ANOVA with Tukey's multiple comparisons test. (**B**) Thrombin and collagen induced platelet aggregation (top) and dense granule secretion (bottom) in Jak2$^{VF/+}$Plek2$^{+/+}$ and Jak2$^{VF/+}$Plek2$^{-/-}$ mice. Platelets from indicated mice were tested in duplicate at each concentration. Results were represented of 3 independent experiments.



Supplemental Figure 6. The effect of Plek2 on hematopoietic stem and progenitor populations. (**A-B**) Bone marrow multi-parameter flow cytometry of indicated mice. Representative plots of LSK (Lineage-Sca1^{+}ckit^{+}) and LK (Lineage-Sca1^{-}ckit^{+}) subsets are shown on the right. LT-HSC: long-term hematopoietic stem cells (HSC); ST-HSC: short-term HSC; MPP: multipotent progenitors; MEP: megakaryocytic/erythroid progenitors; GMP: granulocyte/macrophage progenitors; CMP: common myeloid progenitors. Quantitation of the frequency of each lineage negative bone marrow cell population is shown on the left. Data were obtained from 5 mice (age matched including both male and female) in each group.



Supplemental Figure 7. PHZ treatment further reverted erythrocytosis and improved survival in JAK2$^{VF/+}$Plek2$^{-/-}$ mice. (**A**) Representative bone marrow reticulin stains of indicated mice. JAK2$^{VF/+}$Plek2$^{+/+}$ moribund mice were from 4-5 months old. (**B**) Red blood cell count of 5 months old JAK2$^{VF/+}$Plek2$^{-/-}$ mice treated with chronic injection of PHZ for 75 days. Control group mice were treated with PBS. (**C**) Kaplan-Meier survival analysis of indicated mice. Both males and females were included in each group. N=6 in each group.



Supplemental Figure 8. Gene set enrichment analysis (GSEA) of differentially expressed genes in bone marrow erythroid cells from JAK2$^{VF/+}$Plek2$^{-/-}$ and JAK2$^{VF/+}$Plek2$^{+/+}$ mice. RNA sequencing analysis was performed using Ter119 positive erythroid cells were purified from 3-month old JAK2$^{VF/+}$Plek2$^{+/+}$ and JAK2$^{VF/+}$Plek2$^{-/-}$ bone marrow. NES: normalized enrichment score; FDR: false discovery rate.

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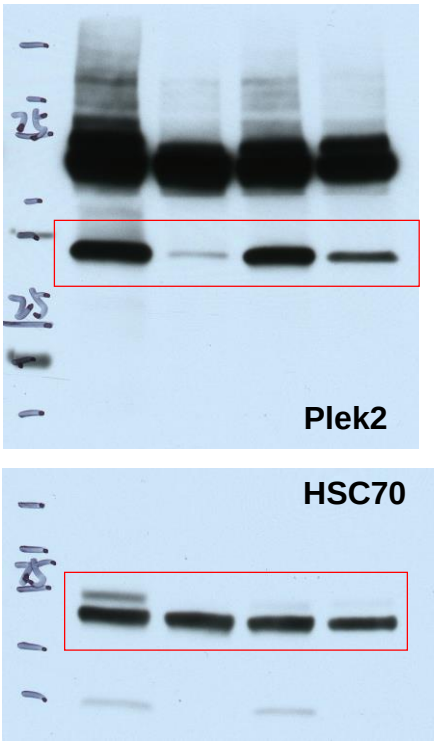

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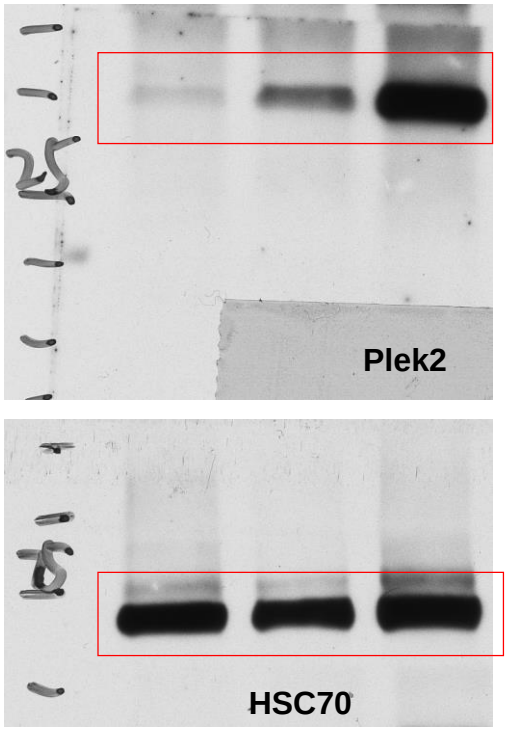

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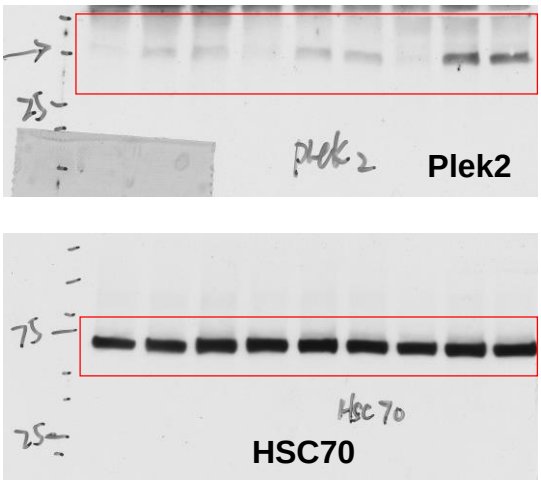

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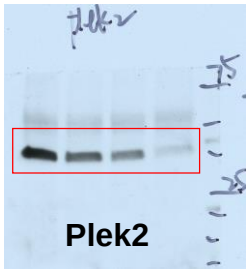

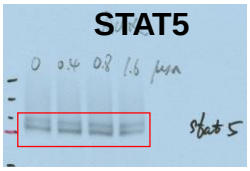

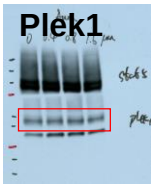

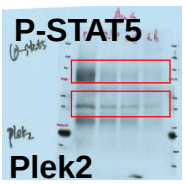

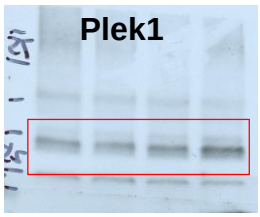

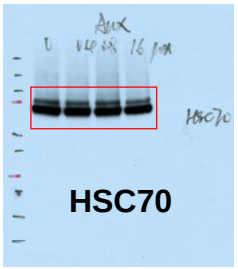

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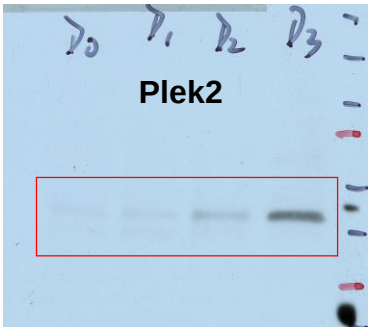

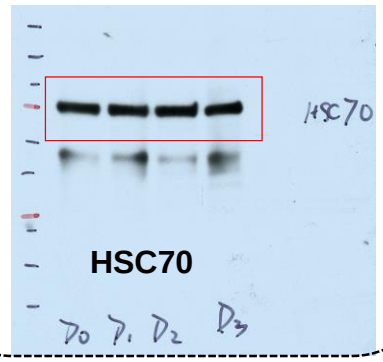

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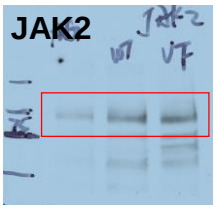

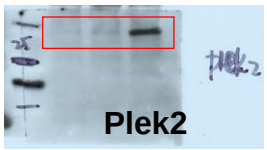

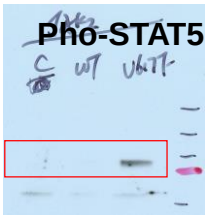

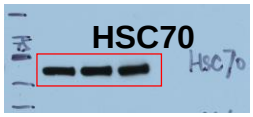

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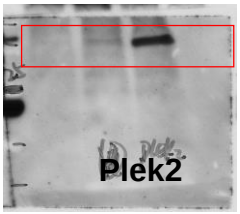

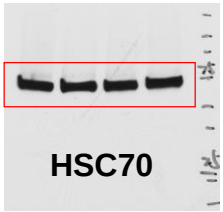

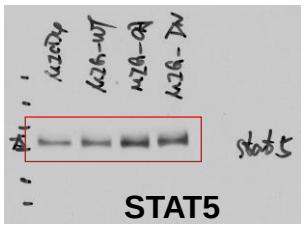

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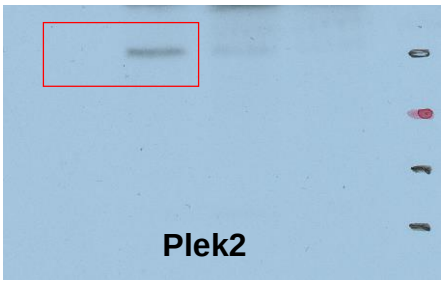

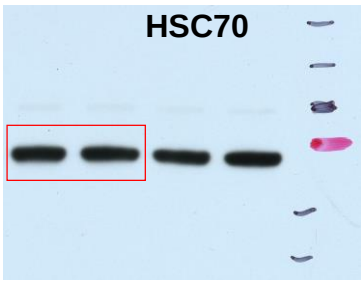

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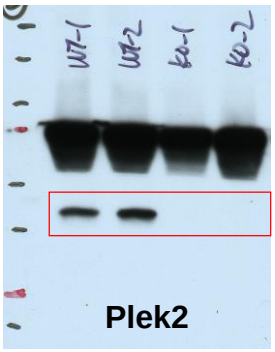

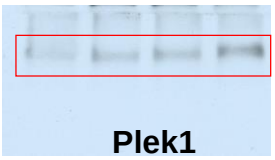

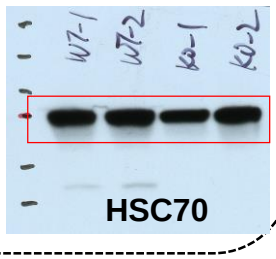

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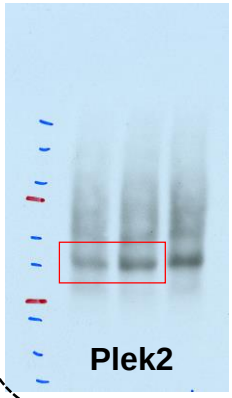

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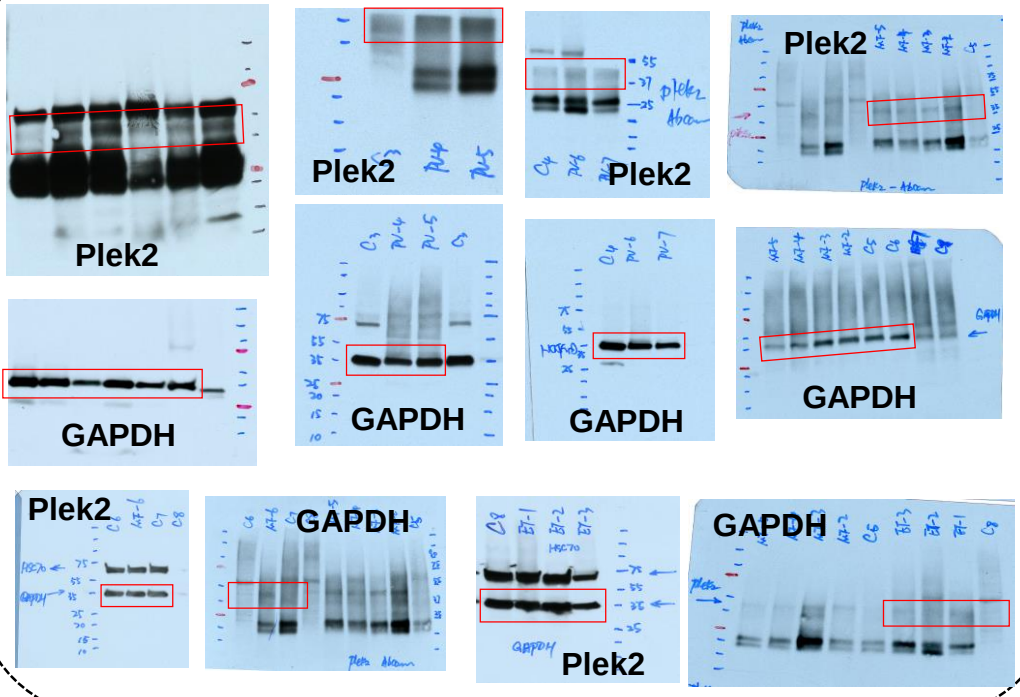

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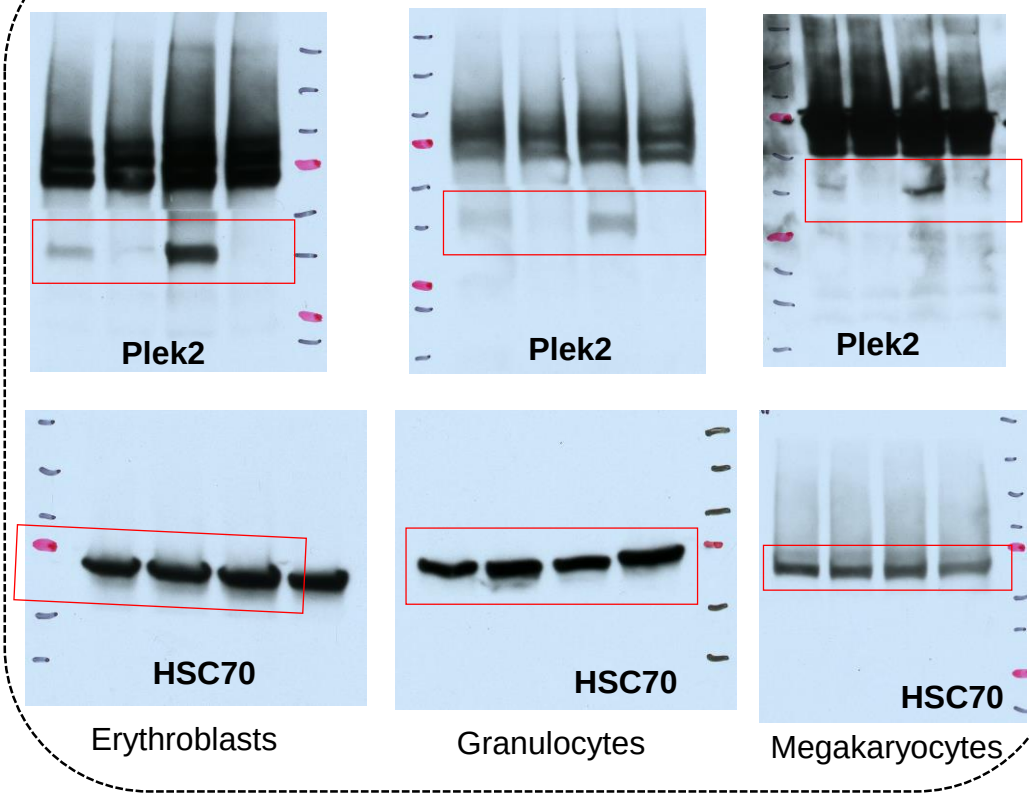

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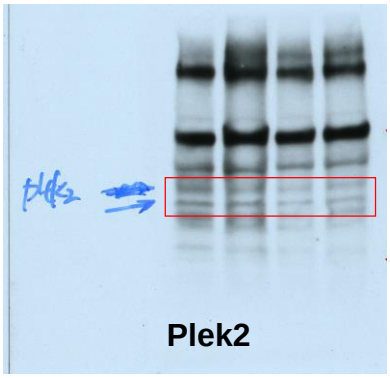

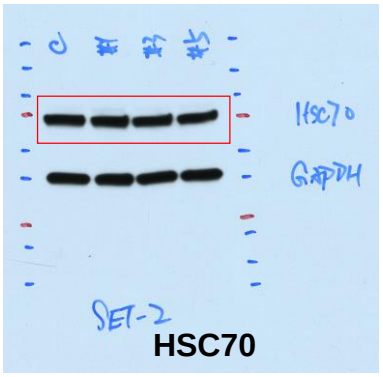

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