

Supplemental Figure Legends

Supplementary Figure 1.

(A) Jak2 sequencing analysis of cDNA synthesized from Jak2^{+/+} ST-HSC + MPP cells or Jak2^{+*Δ*VF} ST-HSC + MPP cells.

(B) Jak2 sequencing analysis of cDNA synthesized from Jak2^{+/+} LT-HSC cells or Jak2^{+*Δ*VF} LT-HSC cells.

(C) WBC counts, HCT and platelet counts from secondary recipients of Jak2^{+*Δ*VF}E2Acre⁺ and Jak2^{+/+}E2Acre⁺ LSK cells in 75:25, 50:50 and 25:75 ratios respectively, measured 5-33 weeks post transplantation. (mean +/- SEM; n = 2-6 in each group).

Supplementary Figure 2.

(A - C) Histopathologic (HE) sections of BM (A), spleen (B) and liver (C) from a mouse transplanted with Jak2^{+*Δ*VF} LT-HSC cells demonstrating accelerated phase MPN. (A) The BM is hypercellular with prominent myeloid infiltrates. Myeloid cells are immature appearing with high nuclear/cytoplasmic ratio, dense nuclear chromatin and prominent nucleoli. Megakaryocytic dysplasia with prominent emperipolesis and myelofibrosis consisting of a fine reticulin fiber network are also evident (insert and higher magnifications). (B) Splenomegaly (spleen weight 3.2G) with complete disruption of the splenic architecture as a result of myeloid infiltration. (C) The enlarged liver shows diffuse myeloid infiltrates in direct association with a dilated sinus (insert and magnification).

(D - F) CD34-staining of BM (D), spleen (E) and liver (F) from a representative mouse transplanted with Jak2^{+*Δ*VF} ST-HSC + MPP cells and from a mouse transplanted with Jak2^{+*Δ*VF} LT-HSC cells that developed accelerated phase MPN. (D) The BM from the Jak2^{+*Δ*VF} ST-HSC + MPP recipient is normocellular with 5% CD34⁺ cells and positively stained vessels. Megakaryocytes as shown in the 40X magnification stain negative. In

contrast, the CD34-staining of the BM from Jak2^{+VF} LT-HSC recipient is hypercellular with left-shifted myelopoiesis and strong neovascularization. Megakaryocytes stain partially positive for CD34 and are localized to atypical localizations in close proximity to vessels (40X magnification). (E) In the spleen from the Jak2^{+VF} ST-HSC + MPP recipient, immunohistochemistry against CD34 stains only the splenic vasculature. In contrast, in the spleen from the Jak2^{+VF} LT-HSC recipient, the splenic architecture is completely disrupted. The spleen is highly vascularized and the dilated blood vessels are filled with a myeloid infiltrate. (F) In the liver from the Jak2^{+VF} ST-HSC + MPP recipient, CD34-immunohistochemistry highlights liver sinusoids surrounded by normal hepatic parenchyma. In contrast in the liver from the Jak2^{+VF} LT-HSC recipient, even at low magnification, the immature myeloid infiltrates surrounding the sinusoids are apparent.

Supplementary Figure 3.

EPO-R expression during the stages of human hematopoietic differentiation. Data obtained from the differentiation map portal

(DMAP:<http://www.broadinstitute.org/dmap/home>).

Supplementary Figure 4.

(A) Allele specific qPCR performed on Jak2^{+/+}E2Acre⁺ MEP and Jak2^{+VF}EpoRCre⁺ MEP and Jak2^{+VF}EpoRCre⁺ CD71⁺ Ter119⁺ cells purified from primary mice (mean +/- SEM, n=3 in each group), demonstrating no Jak2V617F expression in Jak2^{+/+} cells and a Jak2 VF:WT expression ratio of approximately 0.3 in Jak2^{+VF}EpoRCre⁺ MEP cells and of approximately 1.0 in Jak2^{+VF}EpoRCre⁺ CD71⁺ Ter119⁺ cells (lower VF:WT ratio in MEP cells likely due to lower EpoR expression in MEPs as compared with CD71⁺ Ter119⁺ cells).

(B) Jak2 sequencing analysis of CD71⁺ Ter119⁺ BM cells from Jak2^{+/+} E2Acre⁺, Jak2^{+VF} E2Acre⁺ and Jak2^{+VF} EpoRcre⁺ mice.

(C) Spleen weights from age-matched Jak2^{+/+} E2Acre⁺, Jak2^{+VF} EpoRcre⁺ and Jak2^{+VF}

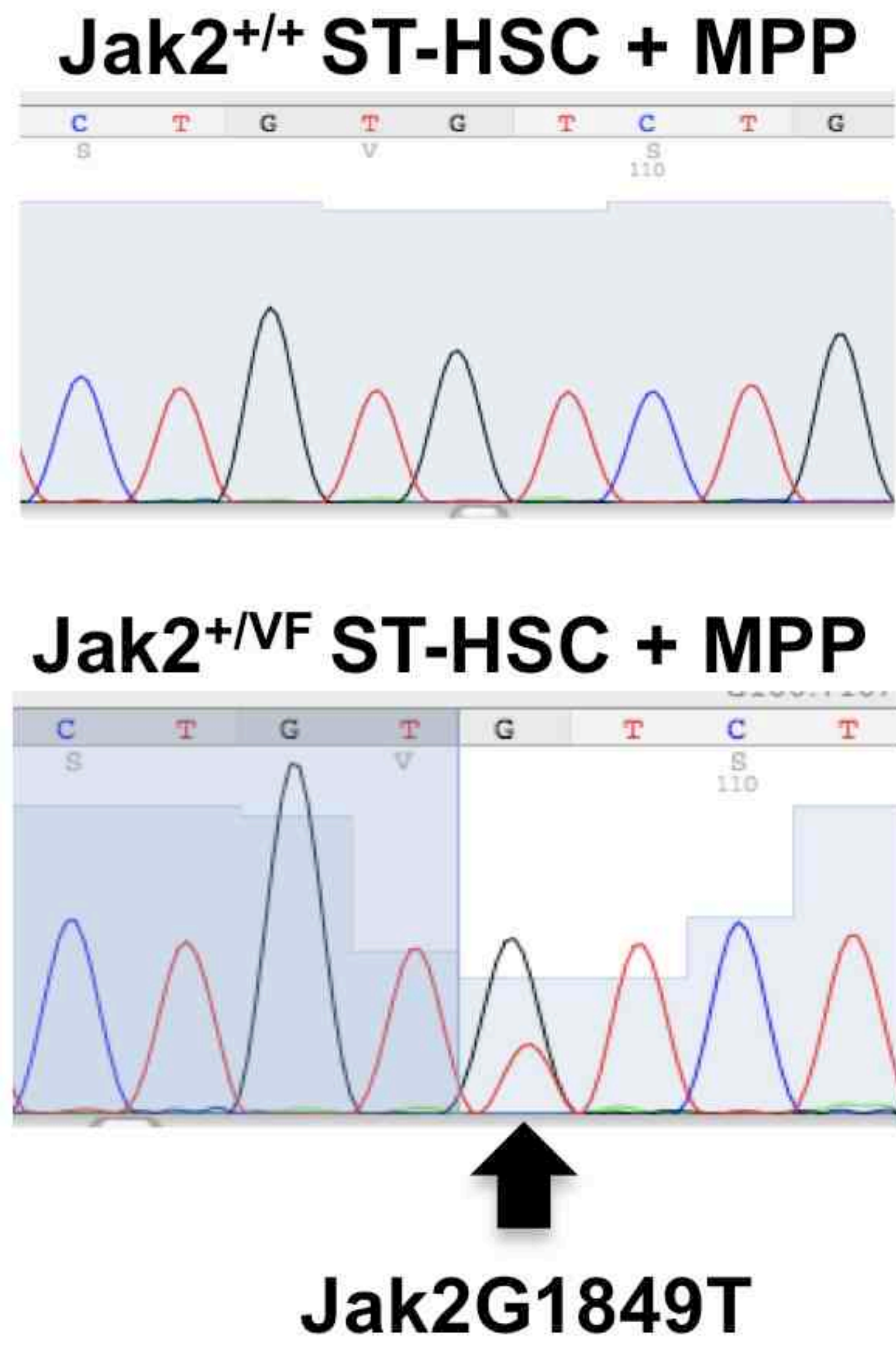
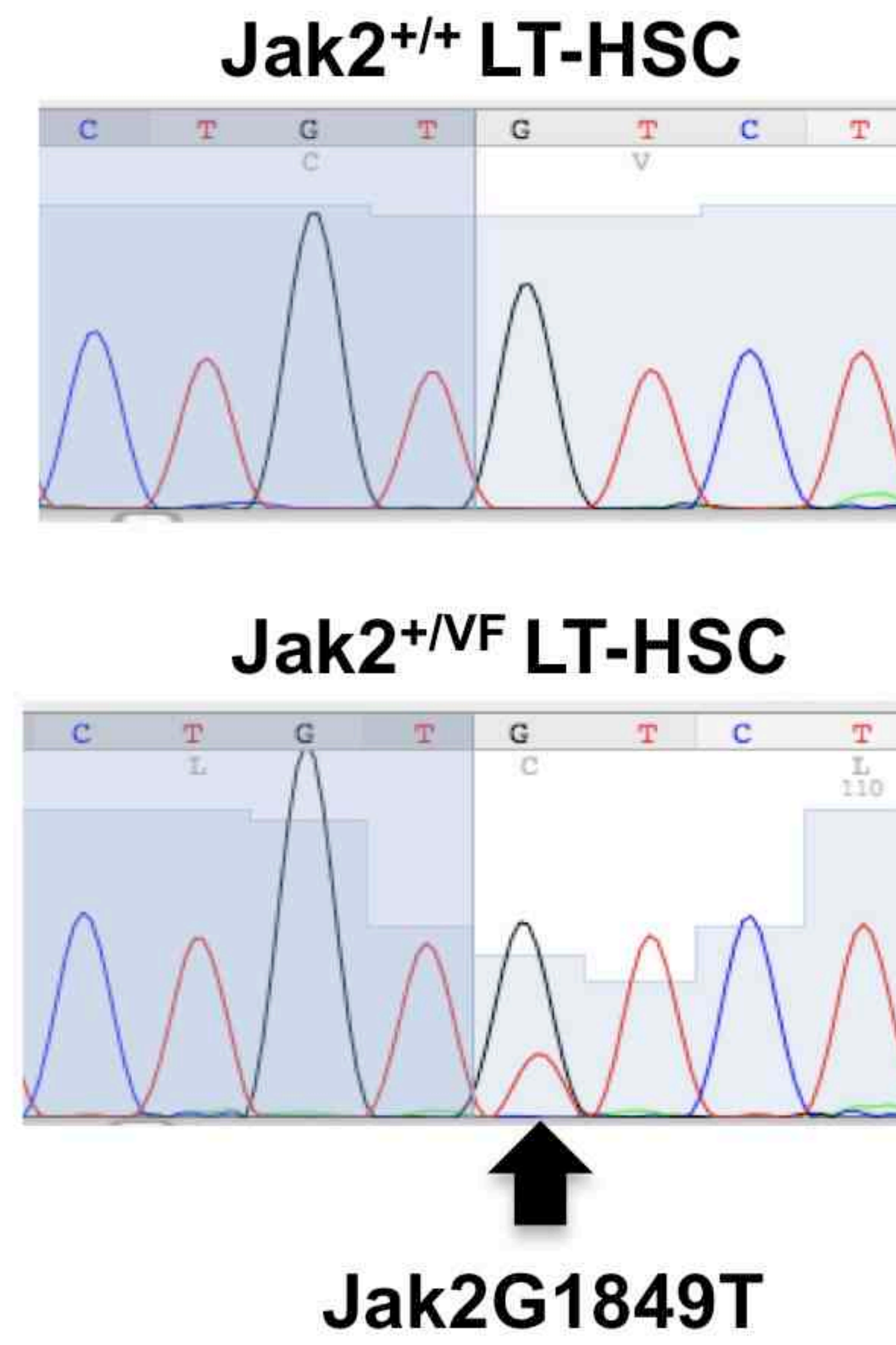
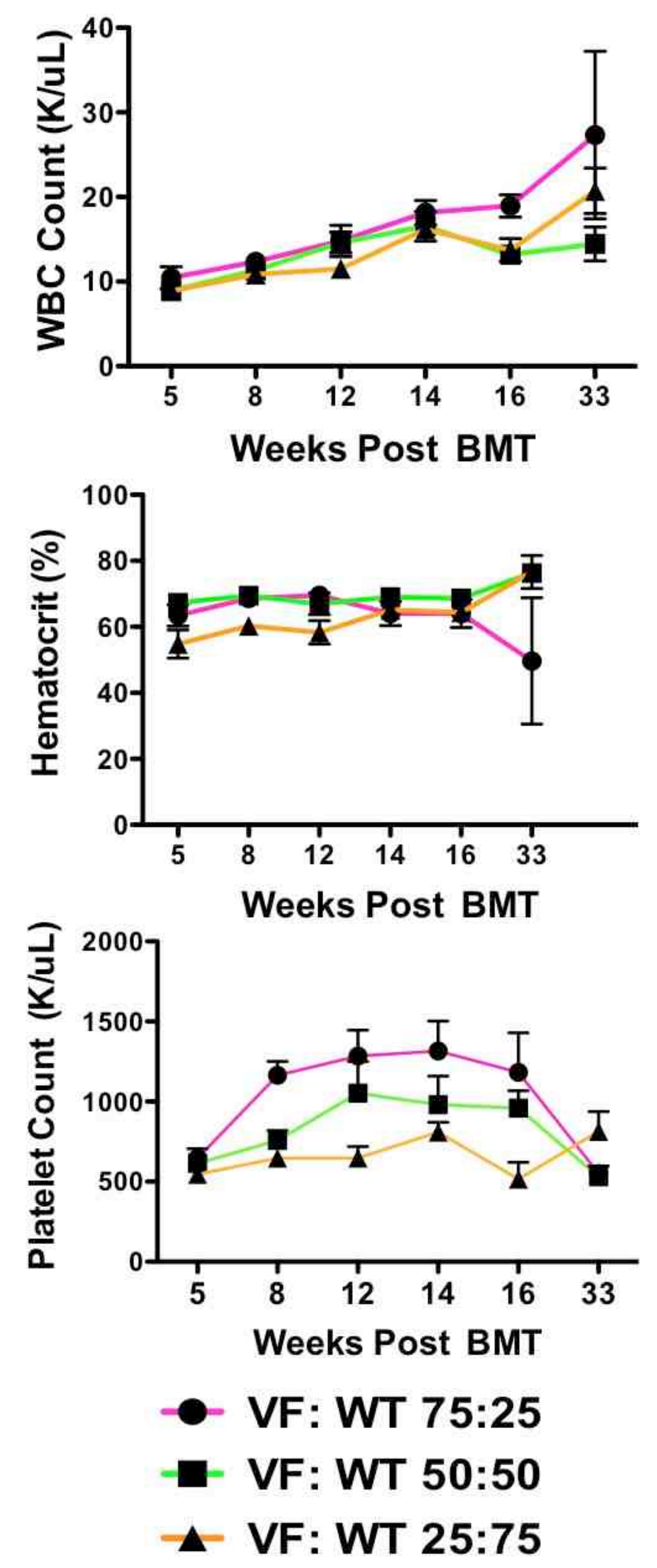
E2Acre+ mice (mean +/- SEM; n=4 in each group). * * p = 0.0001

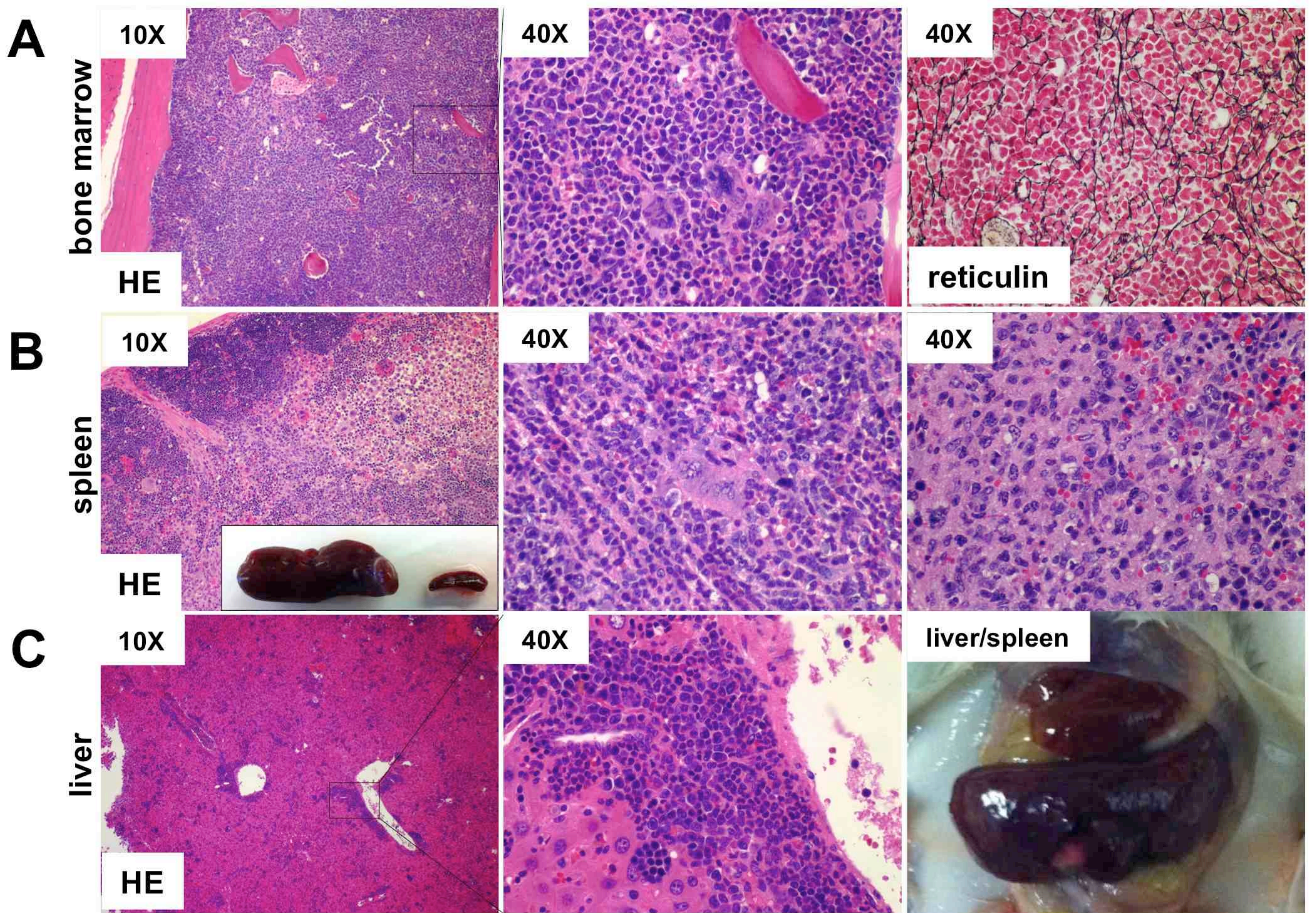
(D) Frequency of LSK cells in spleen from age-matched Jak2^{+/+} E2Acre+, Jak2^{+/+}

EpoRcre+, Jak2^{+/-} EpoRcre+ and Jak2^{+/-} E2Acre+ mice (mean +/- SEM; n=4 in each group). * p = 0.01

(E) Spleen weights from mice transplanted with Jak2^{+/-} LT-HSC cells or Jak2^{+/-} STHSC + MPP cells (mean +/- SEM; n= 3 or 4 in each group).

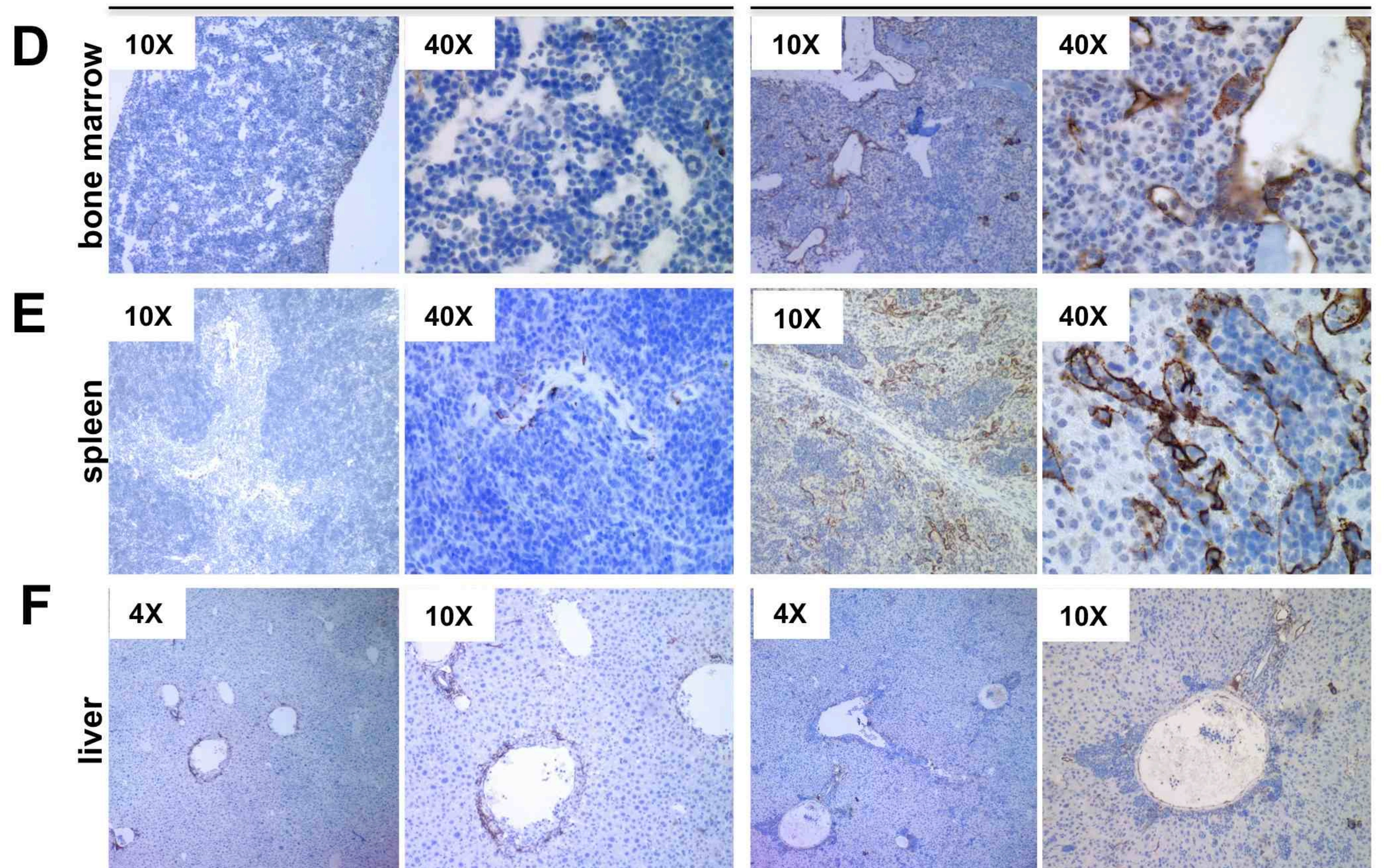
(F) HE and reticulin staining of BM from Jak2^{+/+} E2Acre+, Jak2^{+/-} ErGFPcre+ or Jak2^{+/-} E2Acre+ mice, demonstrating mild erythroid and megakaryocytic hyperplasia in Jak2^{+/-} ErGFPcre+ mice and erythroid and megakaryocytic hyperplasia with marked megakaryocytic dysplasia in Jak2^{+/-} E2Acre+ mice. Reticulin fibrosis is not seen in any group.

A**B****C**



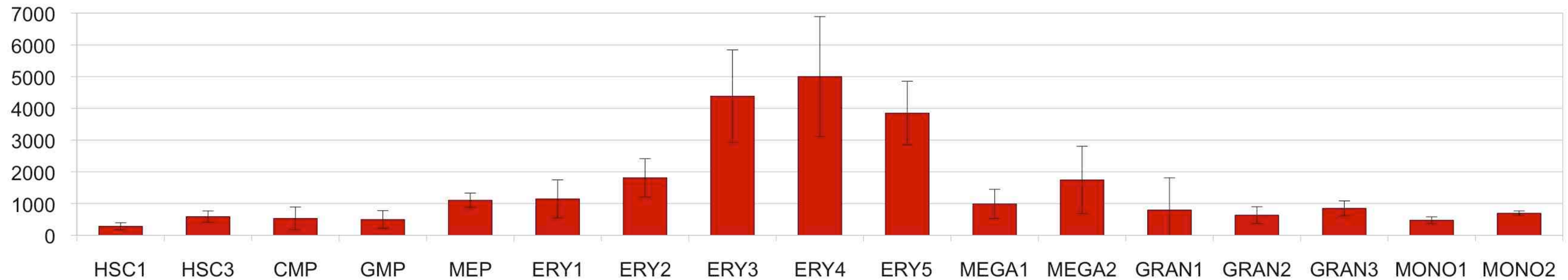
Jak2^{+VF} ST-HSC + MPP

Jak2^{+VF} LT-HSC



Supp. Figure 2

EPO-R



HSC1 = hematopoietic stem cell; lin⁻, CD133⁺, CD34 dim

HSC3 = hematopoietic stem cell; lin⁻, CD38⁻, CD34⁺

CMP = common myeloid progenitor; CD34⁺, CD38⁺, IL3R α ⁺, CD45RA⁻

GMP = granulocyte monocyte progenitor; CD34⁺, CD38⁺, IL3R α ⁺, CD45RA⁺

MEP = megakaryocyte erythroid progenitor; CD34⁺, CD38⁺, IL3R α ⁻, CD45RA⁻

ERY1 = erythroid; CD34⁺, CD71⁺, GlyA⁻

ERY2 = erythroid; CD34⁻, CD71⁺, GlyA⁻

ERY3 = erythroid; CD34⁻, CD71⁺, GlyA⁺

ERY5 = erythroid; CD34⁻, CD71 lo, GlyA⁺

ERY4 = erythroid; CD34⁻, CD71⁻, GlyA⁺

MEGA1 = colony forming unit megakaryocyte; CD34⁺, CD41⁺, CD61⁺, CD45⁻

MEGA2 = megakaryocyte; CD34⁻, CD41⁺, CD61⁺, CD45⁻

GRAN1 = colony forming unit granulocyte; CD34⁻, SSC hi, CD45⁺, CD11b⁻, CD16⁻

GRAN2 = neutrophilic metamyelocyte; CD34⁻, SSC hi, CD45⁺, CD11b⁺, CD16⁻

GRAN3 = neutrophil; CD11b⁺, CD16⁺

MONO1 = colony forming unit monocyte; CD34⁻, CD33⁺, CD13⁺

MONO2 = monocyte; CD14⁺, CD45 dim

