# Expansion of EPOR-negative macrophages besides erythroblasts by elevated EPOR signaling in erythrocytosis mouse models

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**Supplementary Methods**

**Quantitative RT–PCR**

Total RNA was extracted using RNeasy Mini kit (Qiagen, Valencia, CA, USA), and mRNA was reverse transcribed using the Invitrogen SuperScript III First-Strand Synthesis System (Invitrogen). Quantitative PCR was performed using Applied Biosystems Step One Plus thermal cycler (Applied Biosystems, Carlsbad, CA, USA), Taqman universal master mix (Applied Biosystems), and the following gene expression assays (Applied Biosystems): *Epo* (Mm01202755_m1) and *Actb* (Mm00607939_s1). Power SYBR Green PCR master mix (Applied Biosystems) and the following primers were used: for mouse *Il1b*, 5'-AGTTGACGGACCCCAAAAG-3' and 5'-AGCTGGATGCTCTCATCAGG-3'; for mouse *Il6*, 5'-CCAGAAACCGCTATGAAGTTCC-3' and 5'-GTTGTCACCAGCATCAGTCC-3'; for mouse *Il12a*, 5'-TCAGAATCACAACCATCAGCA-3' and 5'-CGCCATTATGATTCAGAGACTG-3'; for mouse *Actb*, 5'-GCTCTTTTCCAGCCTTCCTT-3' and 5'-CTTCTGCATCCTGTCAGCAA-3'.

**Multiplex immunoassay**

To obtain plasma, PB was obtained from each mouse using heparinized capillary, then samples were centrifuged for 10 minutes at 2,000 g within 1 hour. Plasma concentrations of cytokines/chemokines were measured with Bead-Based Multiplex Assays (EMD Millipore, Billerica, MA, USA) Milliplex MAP Mouse Cytokine/Chemokine 32-plex assay (MCYTMAG-70K-PX32, Millipore) was used according to manufacturer's protocol.

**ELISA**

To obtain serum, PB samples from each mouse were clotted at room temperature for 2 hours. The samples were then centrifuged for 20 minutes at 2,000 g. Serum Epo levels were measured using Quantikine ELISA mouse erythropoietin immunoassay (R&D Systems, Minneapolis, MN, USA) according to manufacturer's instruction.

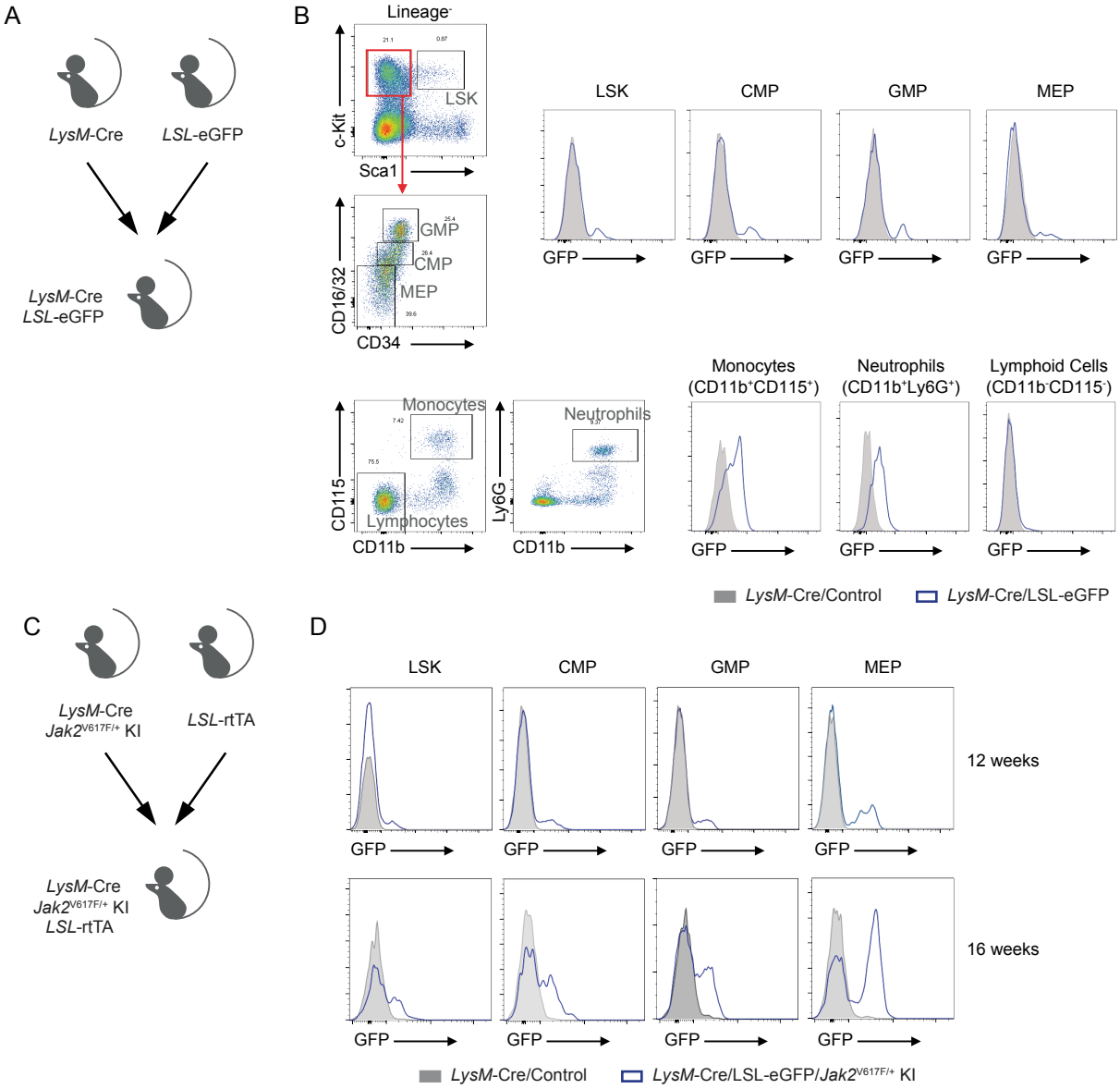
**Histology and Wright Giemsa staining**

Spleen and liver were fixed in formalin and embedded in paraffin. Organs were sectioned and stained with hematoxylin and eosin. Cytospin slides of mouse BM cells were stained using Camco Stain Pak (Cambridge Diagnostic, Fort Lauderdale, FL, USA). Morphology images were obtained using Motic BA310 microscope system (Motic).

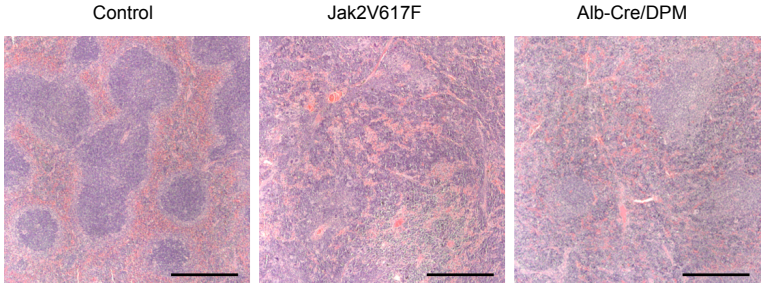
**Colony forming assay**

For BFU-E forming assay, 2 x $10^5$ BM cells were cultured in the MethoCult M3334 (StemCells technologies, Cambridge, MA, USA). Colonies were counted on day 10. For CFU-E forming assay, 2 x $10^5$ BM cells were cultured in the MethoCult M3234 (StemCells technologies). Colonies were counted on day 2.

Supplementary Figure S1 Wang et al

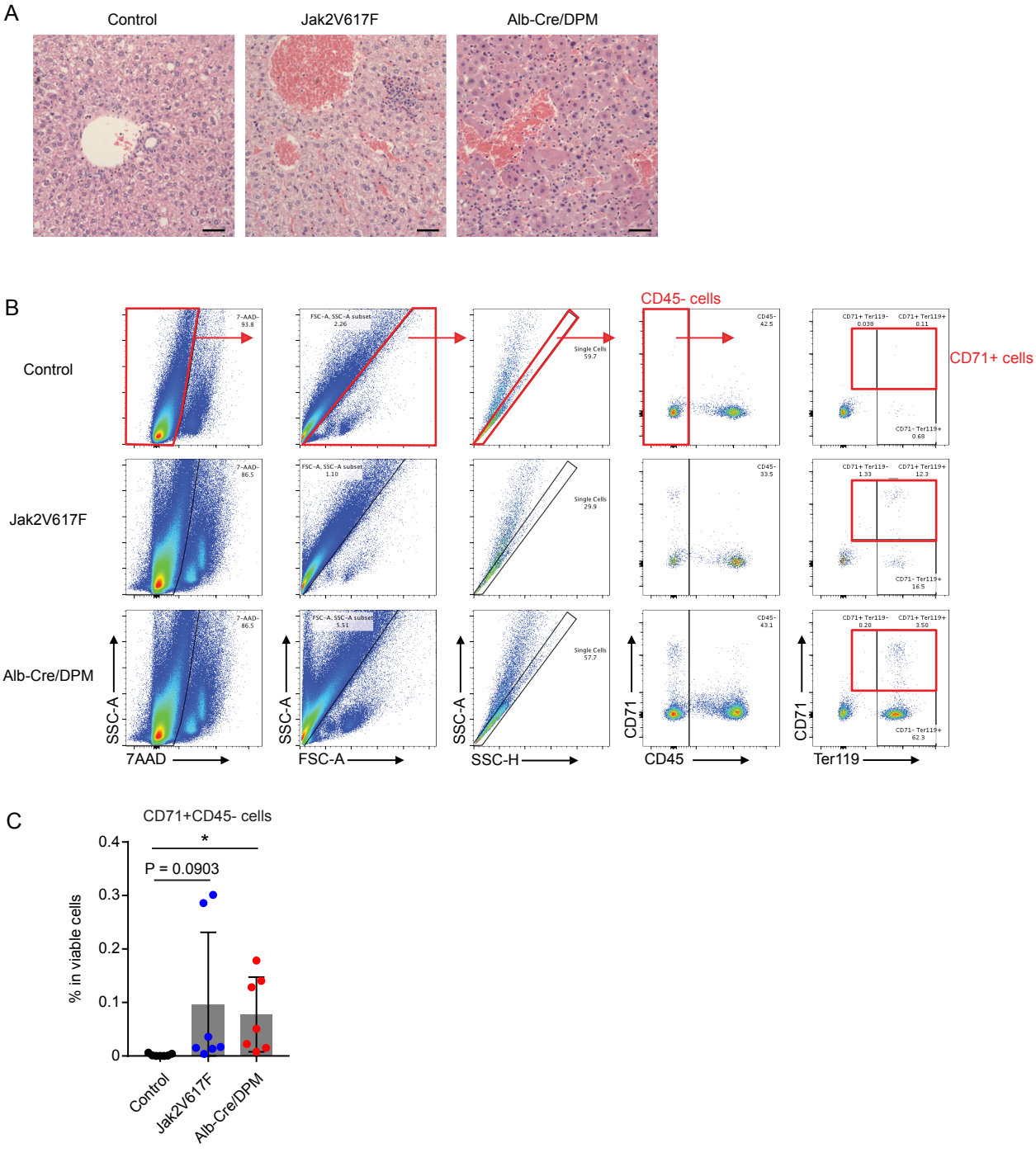


**Supplementary Figure S1. LysM-Cre expression in hematopoietic cells.**
(**A**) GFP-reporter mice using *LysM*-Cre knock-in allele and *Rosa26*/*loxP*-*Stop*-*loxP* (*LSL*)/*eGFP* allele. (**B**) In *LysM*-Cre;*Rosa26LSL-eGFP* (*LysM*-Cre;LSL-eGFP) and *LysM*-Cre;*Rosa26*-*wild-type* (*LysM*-Cre;Control) mice, GFP expressions in the following populations are shown: Lineage marker$^-$ Sca-1$^+$ c-Kit$^+$ cells (LSK), common-myeloid progenitors (CMP), granulocyte-macrophage progenitors (GMP), and megakaryocyte-erythrocyte progenitors (MEP) in BM; and monocytes, neutrophils, and lymphoid cells in PB. (**C**) GFP-reporter mice using *LysM*-Cre allele, Cre-inducible *Jak2*$^{V617F/+}$ knock-in allele, and LSL-eGFP allele. (**D**) In *LysM*-Cre;*Jak2*V617F;*Rosa26LSL-eGFP* (*LysM*-Cre; *Jak2*V617F;LSL-eGFP) and *LysM*-Cre;*Rosa26*-*wild-type* (*LysM*-Cre;Control) mice at 12 week-old age and 16 week-old age, GFP expressions in the LSK, CMP, GMP, and MEP in BM are shown. (**E**) Absolute number of BFU-E and CFU-E from indicated mice BM cells. Data are average of triplicate.



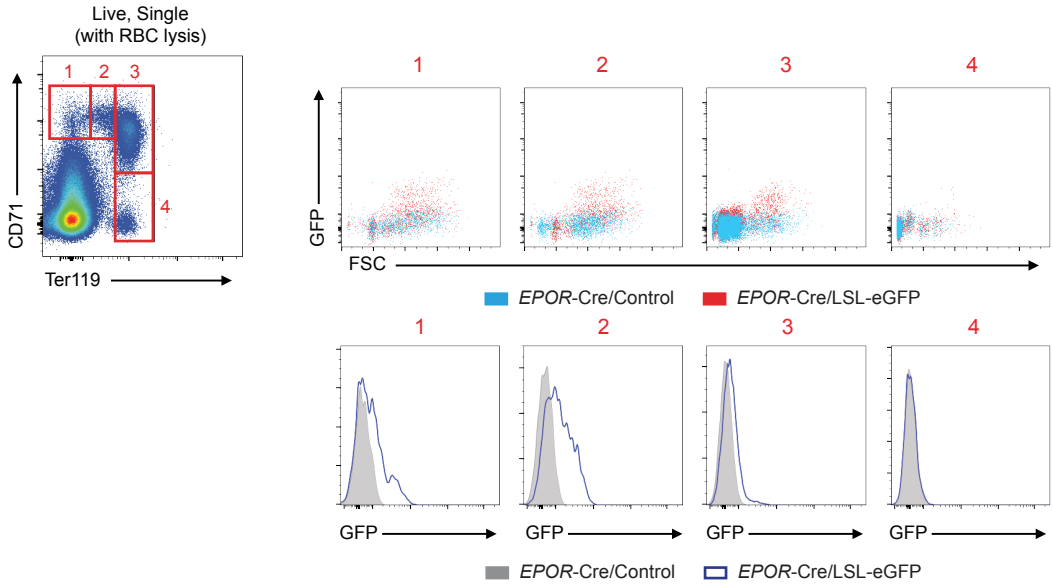

**Supplementary Figure S2. Splenic architecture in the erythrocytosis mouse models**
HE staining of spleen sections from the indicated mice. Scale bar, 500 μm; original magnification, ×40.

Supplementary Figure S3 Wang et al



**Supplementary Figure S3. Extramedullary erythropoiesis in liver**

(**A**) HE staining of liver sections from the indicated mice. Scale bar, 50 μm; original magnification, ×200. (**B-C**) Flow cytometric analysis of liver erythropoiesis. Representative plots from indicated mice ($n$ = 7 each) (B), and frequencies of $CD45^{-}CD71^{+}$ cells in the viable cells (C) are shown. Data are mean ± s.d. *$P$ < 0.05 (Student's t-test).




**Supplementary Figure S4. Distribution of EPOR expression in erythroid lineage**
Expression pattern of *EPOR* (GFP) in BM erythroid cells from *EPOR*-Cre;*Rosa26LSL-eGFP* (*EPOR*-Cre;LSL-eGFP) and *EPOR*-Cre;*Rosa26-wild-type* (*EPOR*-Cre;Control) mice. GFP expressions in the individual fractions are shown.