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Supplementary Materials for

Inflammation induces stress erythropoiesis through heme-dependent activation of SPI-C

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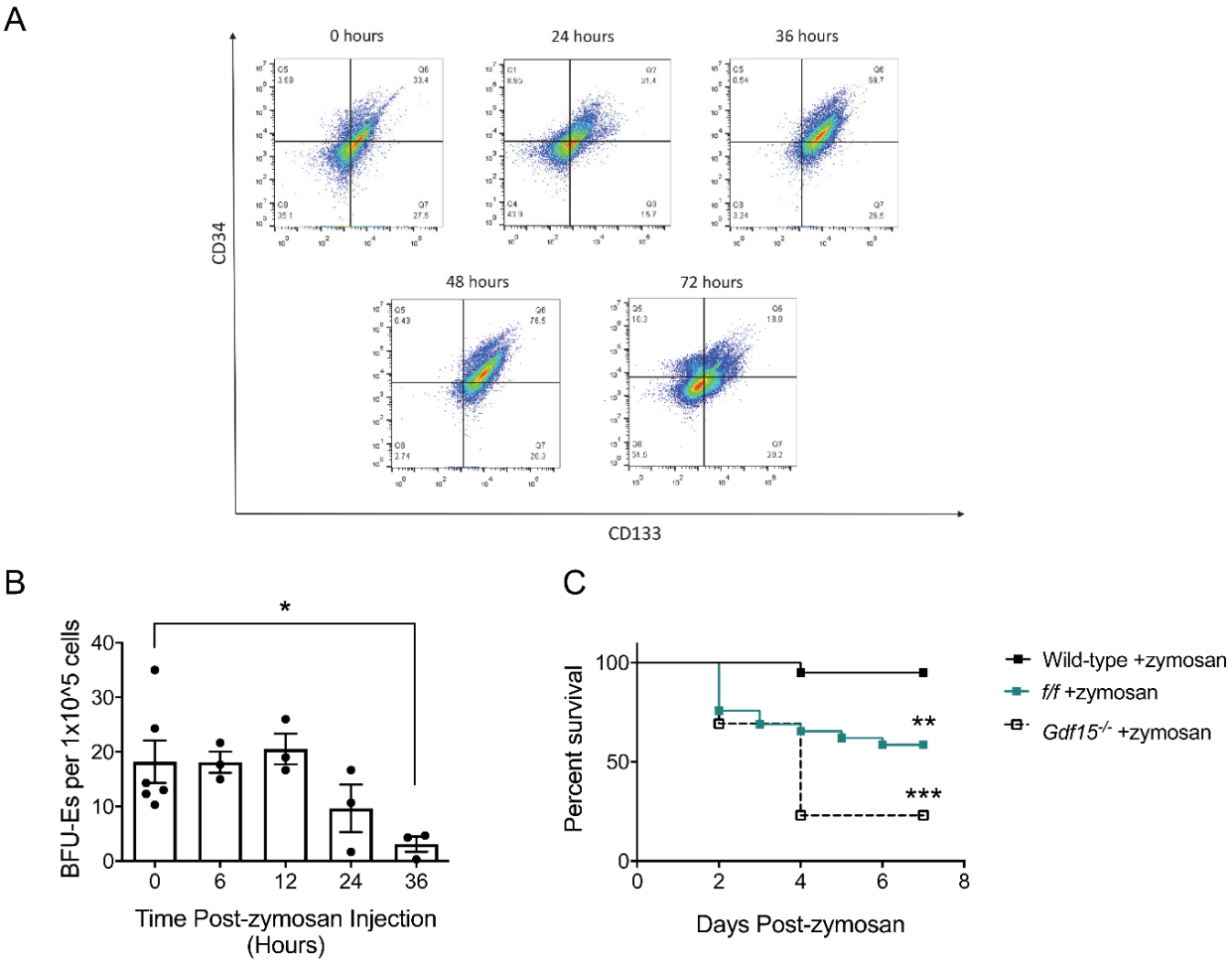

Fig. S1. Inflammation induces expansion of SEPs in the spleen and inhibits bone marrow erythropoiesis. (A) Flow cytometry analysis of erythroid progenitors in the spleen at the indicated time points following treatment with zymosan. Cells were first gated on Kit^+Sca^+ populations. Frequencies of $CD34^+$ and $CD133^+$ cells are shown. Representative images are shown. n=3 mice per time point. **(B)** Bone marrow cells from zymosan-treated mice were plated in methylcellulose media containing IL-3 and Epo and grown under normoxia for 7 days. Benzidine staining was used to assess BFU-Es. Data represent the mean±SEM, one-way ANOVA with Dunnett's multiple comparison test, n=3-6 mice per time point. **(C)** WT (C57BL/6), *f/f*, and *Gdf15*$^{-/-}$ mice were treated with zymosan, and animal survival was assessed over the first week after treatment. Mantel-Cox test, n=13-29 mice per genotype. **P* < 0.05, ***P* < 0.01, and ****P* < 0.005.

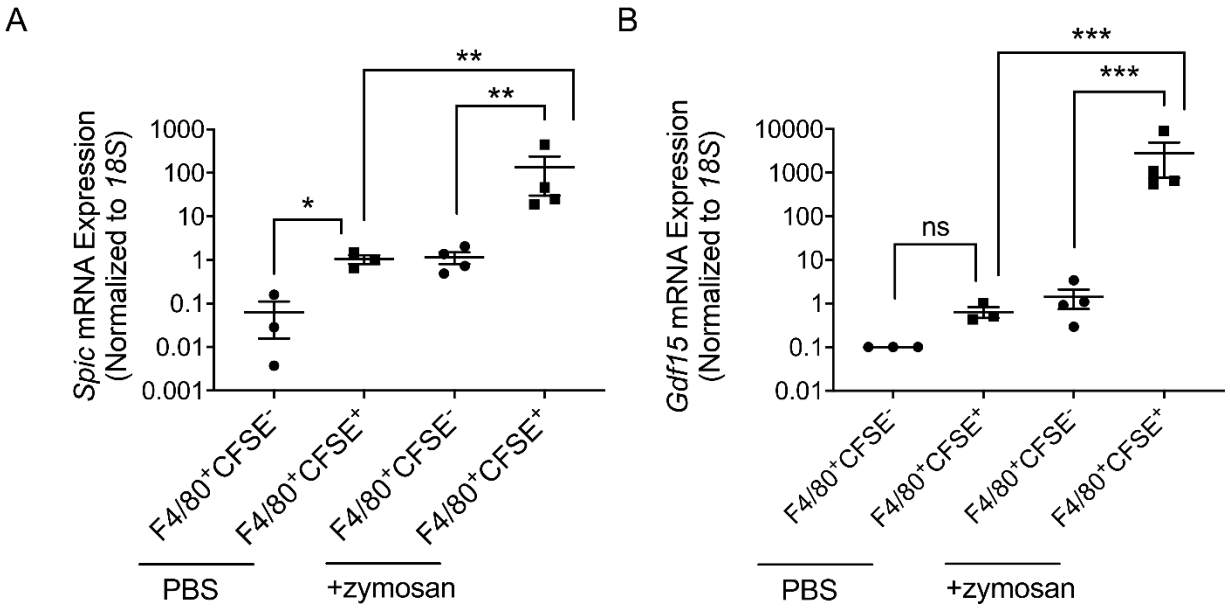

Fig. S2. Induction of *Spic* and *Gdf15* expression is restricted to phagocytosing cells in zymosan-treated mice. (A, B) Splenocytes were isolated from mice treated with either PBS or zymosan, and F4/80^{+}CFSE^{-} and F4/80^{+}CFSE^{+} cells were sorted by flow cytometry. RNA from sorted cells was used to quantify the expression of *Spic* (A) and *Gdf15* (B) relative to *18S*. Data represent the mean±SEM, one-way ANOVA with Tukey's multiple comparison test on log-transformed data, n=3 mice per treatment group. *P < 0.05, **P < 0.01, and ***P < 0.005.

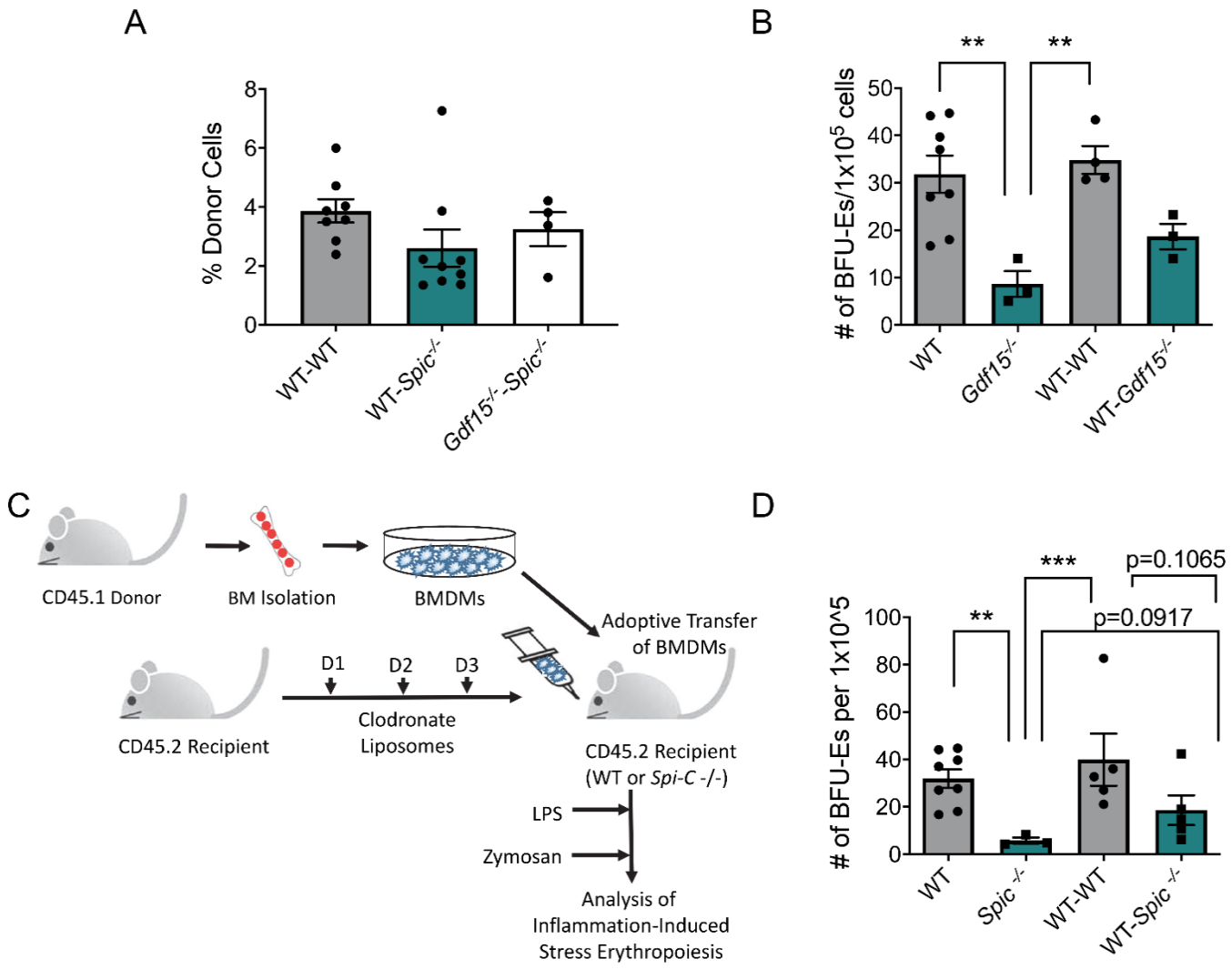

Fig. S3. Adoptive transfer of wild-type macrophages rescues inflammation-induced stress erythropoiesis in *Spic*$^{-/-}$ mice. (A) Homing of donor WT CD45.1 or CFSE^{+}*Gdf15*$^{-/-}$ monocytes to the spleen in WT, *Spic*$^{-/-}$, and *Gdf15*$^{-/-}$ recipients was measured by flow cytometry 36 hours after adoptive transfer. Data represent the mean±SEM, one-way ANOVA with Dunnett's multiple comparison test, n=3-6 mice per treatment group. **(B)** Splenocytes were isolated 36 hours after adoptive transfer and treatment with zymosan then plated under conditions to induce stress erythropoiesis. Stress BFU-Es were counted after 5 days using benzidine staining. Data represent the mean±SEM, one-way ANOVA with Tukey's multiple

comparison test, n=3-6 mice per treatment group. **(C)** Schematic of experimental design for adoptive transfer of BMDMs. **(D)** Splenocytes were isolated after treatment with zymosan and $1x10^5$ cells/well were plated under conditions to induce stress erythropoiesis. Stress BFU-Es were counted after 5 days using benzidine staining. WT-WT, WT donor BMDMs transferred into WT recipients; WT-KO, WT donor BMDMs transferred into *Spic*$^{-/-}$ mice. Data represent the mean±SEM, one-way ANOVA with Tukey's multiple comparison test, n=3-8 mice per treatment group. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.005$.

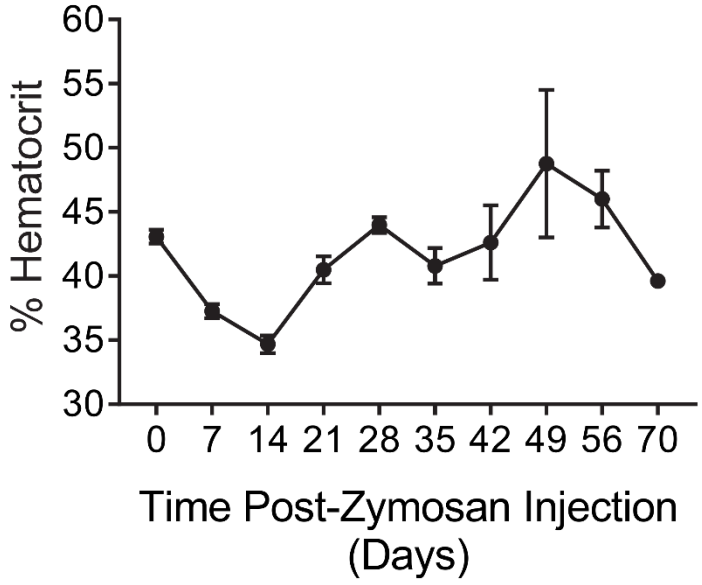

Fig. S4. Chronic inflammation results in cyclic anemia. Mice were treated with zymosan, and blood was collected weekly in heparin-coated microcapillary tubes. Tubes were spun and measured to determine the percent hematocrit. n=2-35 mice per time point.