LETTER TO THE EDITOR

Mitapivat, a pyruvate kinase activator, improves transfusion burden and reduces iron overload in β-thalassemic mice

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SUPPLEMENTARY FIGURES

Figure 1S

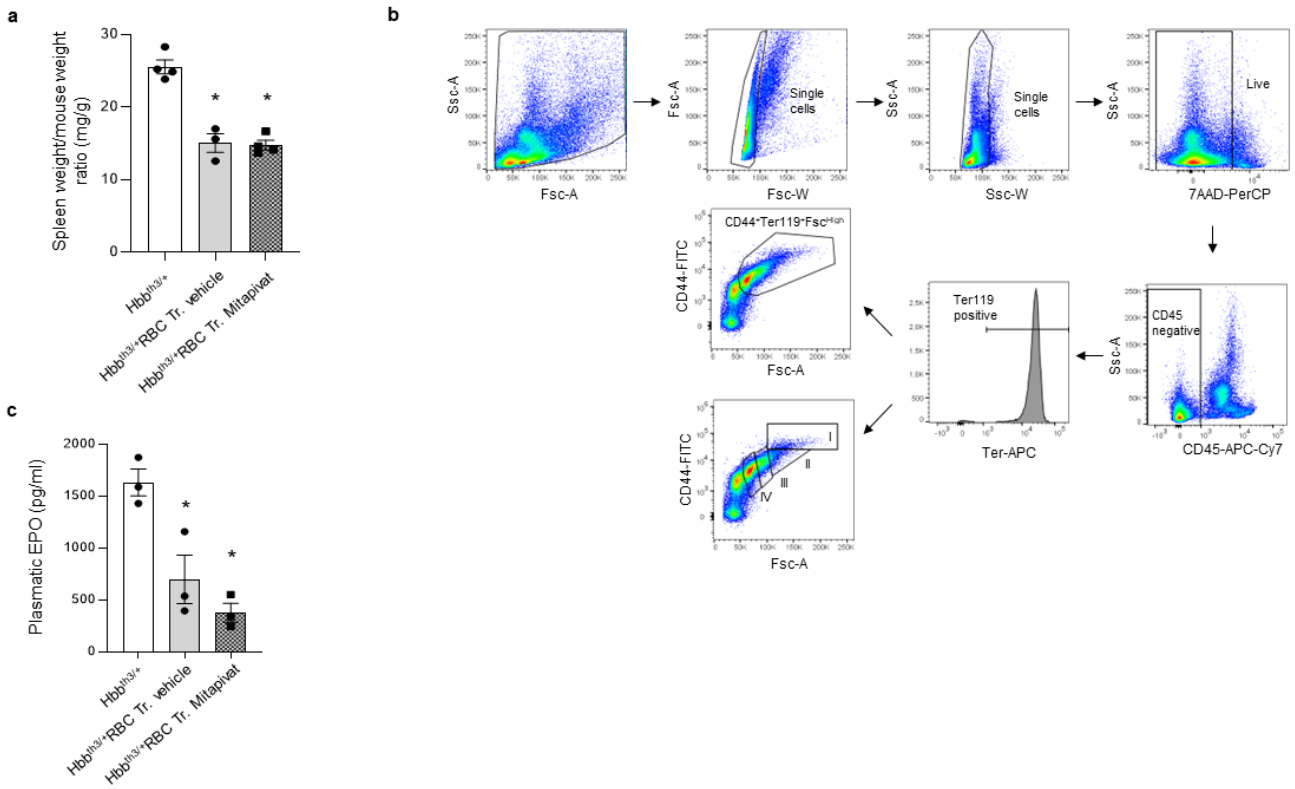

Figure 1S. a. Spleen weight to mouse weight ratio in Hbb$^{th3/+}$ mice treated with vehicle or exposed to chronic transfusion with or without mitapivat. Data are mean ± SEM (*n*=3-4). **P* < 0.05 compared with vehicle Hbb$^{th3/+}$ mice and **P* < 0.05 compared with vehicle-treated transfused Hbb$^{th3/+}$ mice. **b.** Plasma erythropoietin (EPO) levels in Hbb$^{th3/+}$ mice treated with vehicle or exposed to chronic transfusion with or without mitapivat. Data are mean ± SEM (*n*=3-4). **P* < 0.05 compared with vehicle Hbb$^{th3/+}$ mice. **c.** Typical flow cytometric gating strategy used to analyze erythropoietic activity and the maturation index in the bone marrow and in the spleen of Hbb$^{th3/+}$ mice exposed to chronic transfusion with or without mitapivat. The following antibodies were used: anti-CD16/CD32 blocking agent, anti–CD44-FITC, CD71-PE, Ter119-APC, CD45 APC-eFluor 780, GR1 APC-Cy7, and CD11b APC-Cy7 (all from eBiosciences, Thermo Fisher Scientific, USA). Analysis was performed with FlowJo software version 10 (BD Biosciences, USA).

Figure 2S

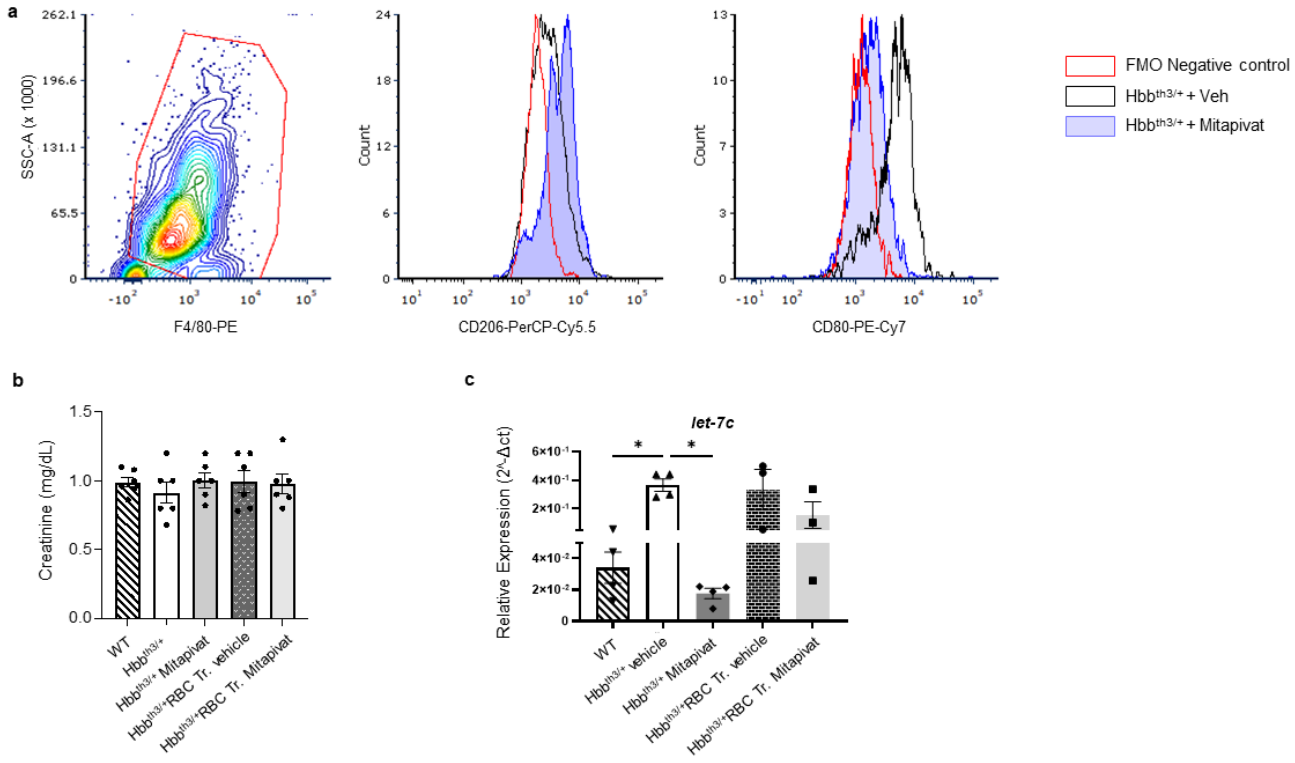

Figure 2S. a. Cytofluorimetric quantification strategy of M1 (CD80) and M2 (CD206) expression on spleen MΦ cell surface from wild-type (WT) or Hbb$^{th3/+}$ mice exposed to either vehicle or to chronic transfusion with and without mitapivat treatment. Data are mean ± SEM (n=3-4). MFI, mean fluorescence intensity. **b.** Plasma creatinine in WT, Hbb$^{th3/+}$ mice with or without mitapivat, Hbb$^{th3/+}$ exposed to transfusion with or without mitapivat. Data are mean ± SEM (n=3-4). **c**. Real time PCR analysis of let-7b and -7d relative expression (using the ΔCt method following normalization of cDNA input using RNU-6) in kidneys from WT or Hbb$^{th3/+}$ mice exposed to either vehicle or to chronic transfusion with and without mitapivat treatment determined with real time PCR. Data are mean ± SEM (n=3-4). * $P < 0.01$.

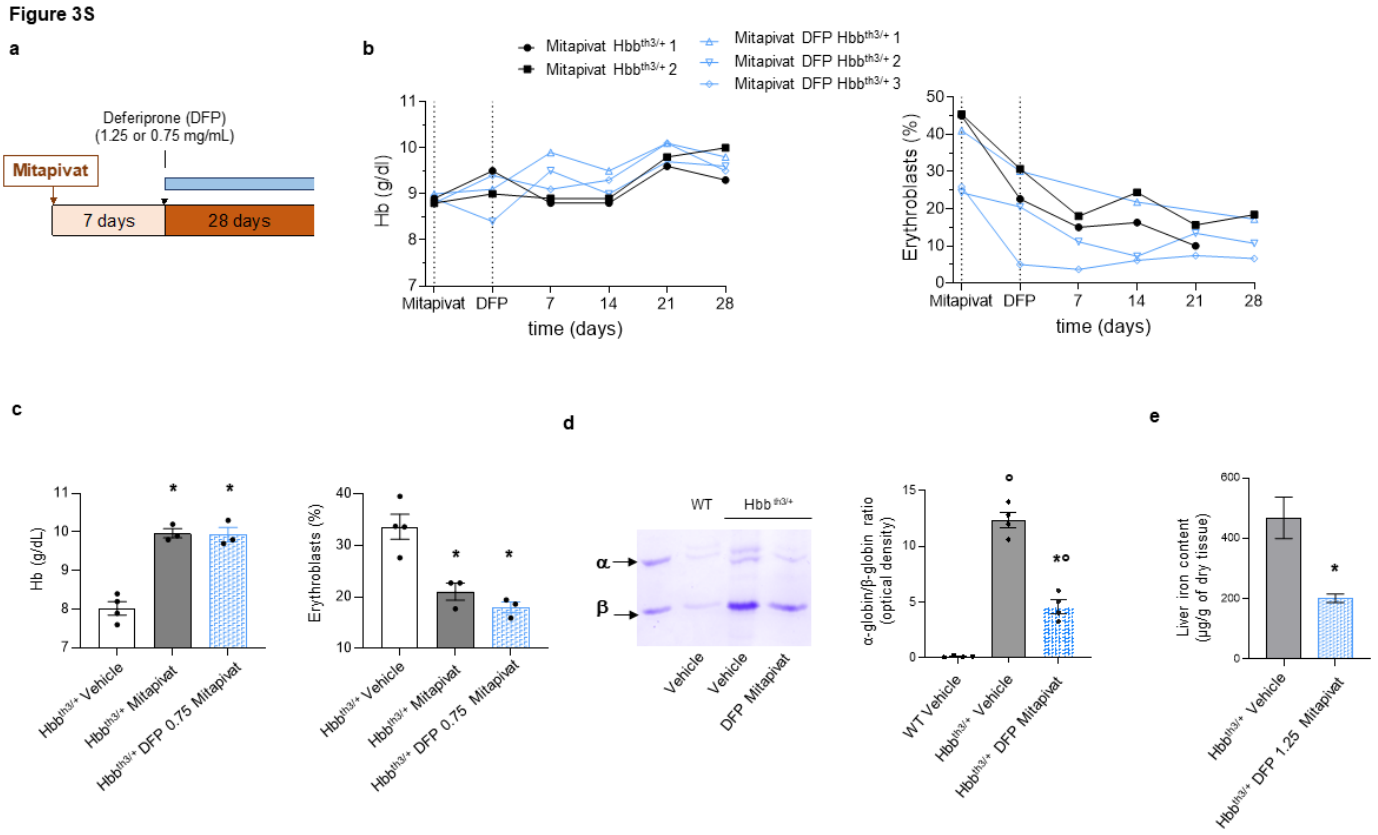

Figure 3S. a. Experimental study design to assess the effects of mitapivat on hematologic phenotype of β-thal mice treated DFP, an oral iron chelator. **b.** Hemoglobin (Hb, left panel) and circulating erythroblasts (right panel) in $Hbb^{th3/+}$ mice treated with mitapivat or mitapivat plus DFP (1.25 mg/mL). Data are shown as single animals (mitapivat $Hbb^{th3/+}$ n=2; mitapivat plus DFP n=4). **c.** Hemoglobin (Hb, left panel) and circulating erythroblasts (right panel) in $Hbb^{th3/+}$ mice treated with mitapivat or mitapivat plus DFP (0.75 mg/mL). **P* < 0.05 compared with vehicle $Hbb^{th3/+}$ mice. **d.** Triton acid-urea gel electrophoresis (left panel) of red cell membrane from WT and $Hbb^{th3/+}$ mice treated with vehicle or mitapivat plus DFP. Arrows show α-globin and β-globin associated with red cell membrane. Right panel: Gel quantification expressed as α-globin to β-globin ratio to hemoglobin. Data are mean ± SEM (n=4); **P* < 0.05 compared with vehicle $Hbb^{th3/+}$ mice; °*P* < 0.05 compared to WT. **e**. Liver iron concentration (LIC) in $Hbb^{th3/+}$ mice treated with either vehicle or DFP 1.25 mg/mL *plus* mitapivat. Data are mean ± SEM (n=4-3). **P* < 0.05 compared with vehicle $Hbb^{th3/+}$ mice.