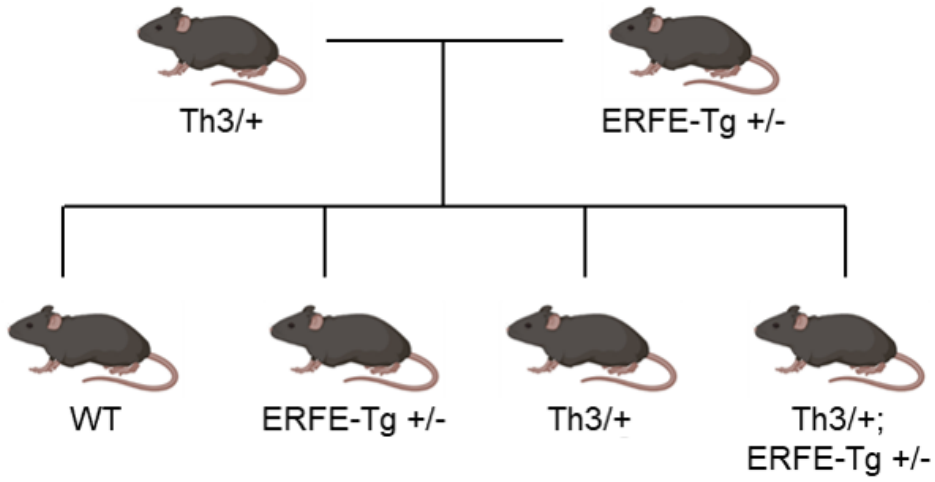
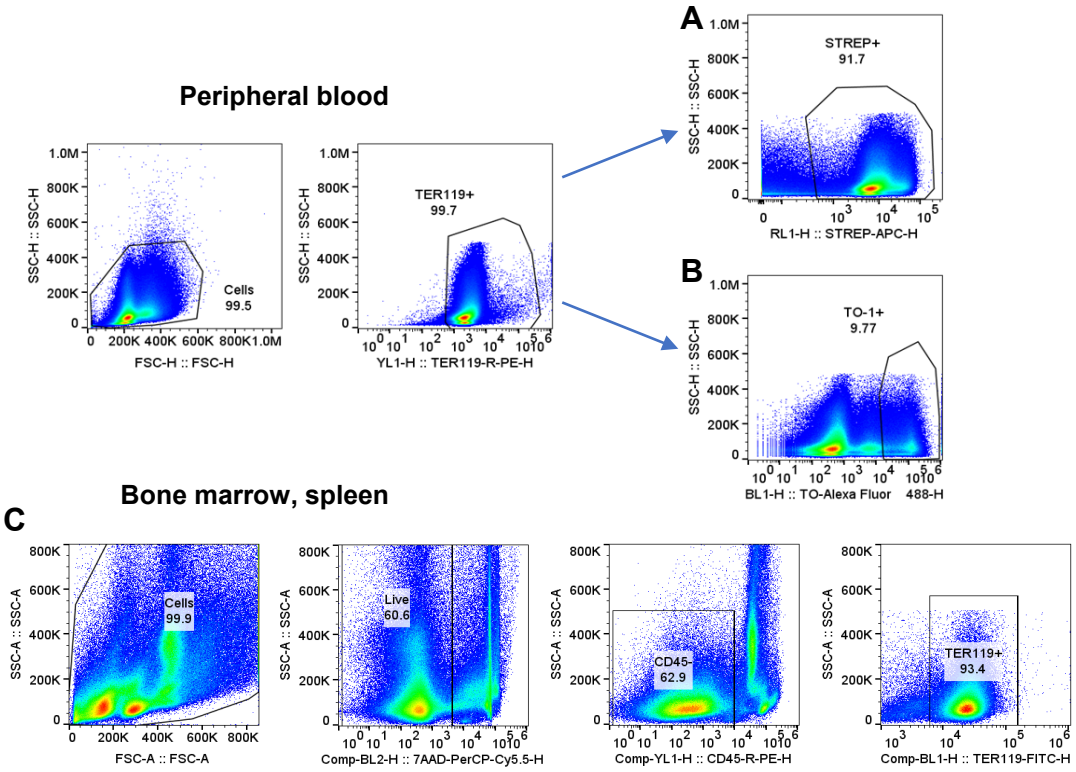


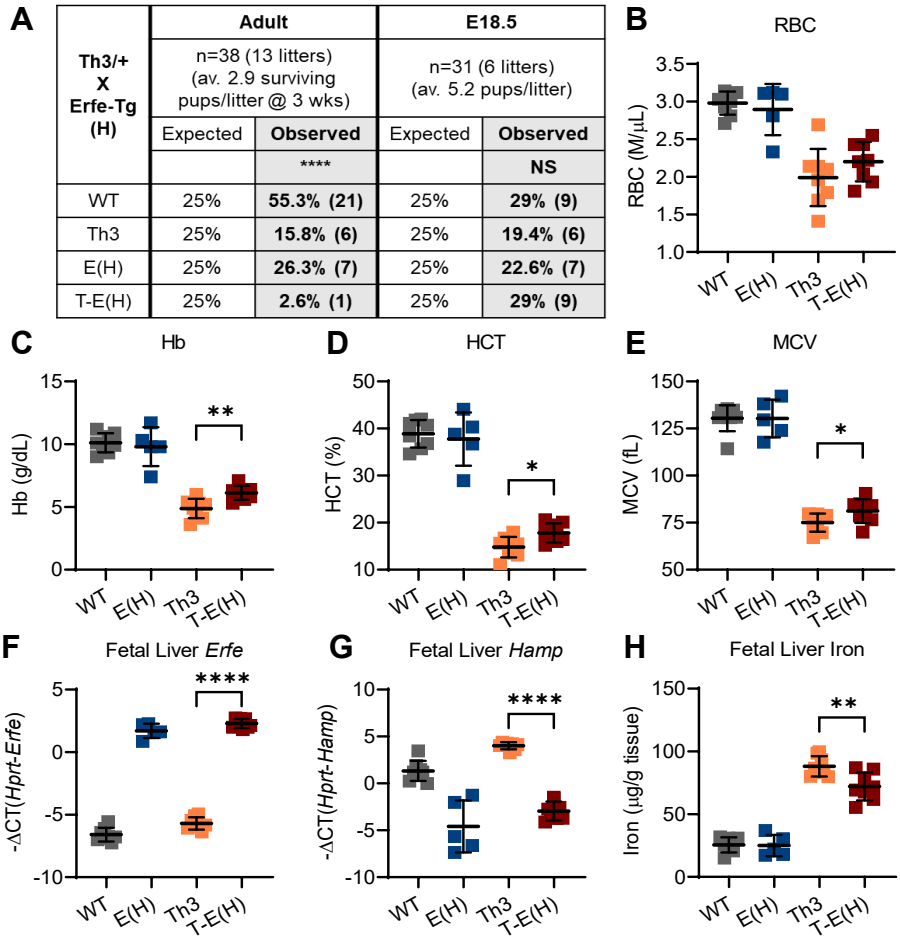
Supplemental Figure 1. Breeding scheme for Th3/+;ERFE-Tg (Th3-ERFE) mice and littermate controls.



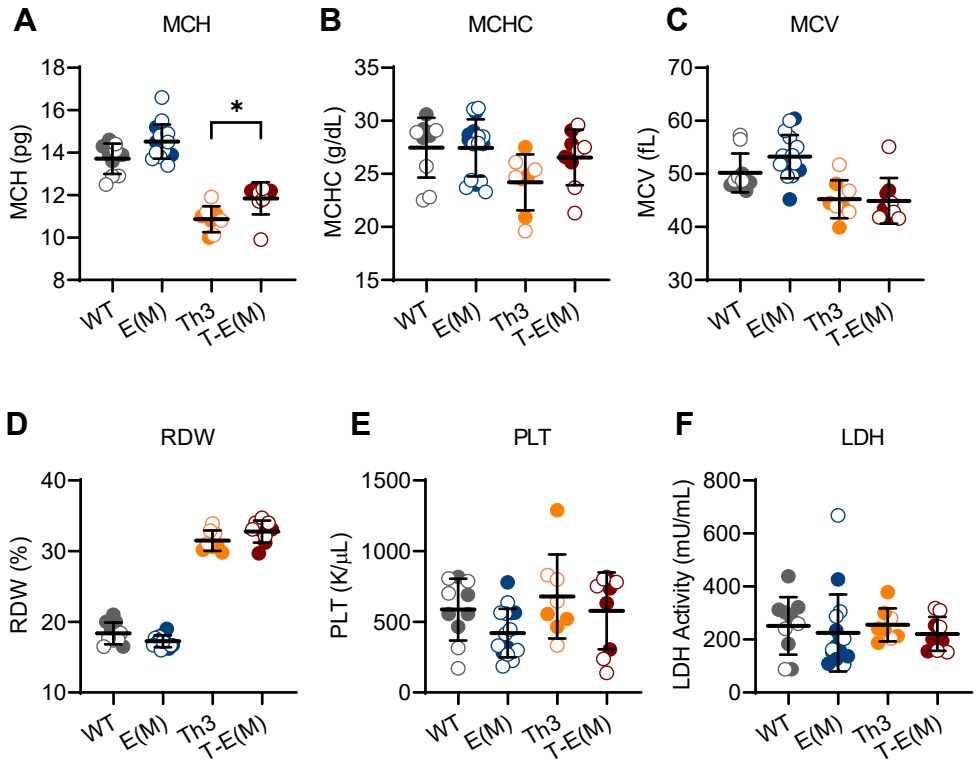
Supplemental Figure 2: Gating scheme for flow cytometry analysis of erythrocytes. (A-B) In peripheral blood: **(A)** biotinylated erythrocytes were identified as TER119+; Strep+ cells and **(B)** reticulocytes were identified as TER119+; TO-1+ cells. **(C)** In bone marrow and spleen single cell suspensions: 7-AAD was used to exclude dead cells, and erythroid cells were identified as live CD45-; TER119+ cells (and also used for further detailed analysis of maturation).

A

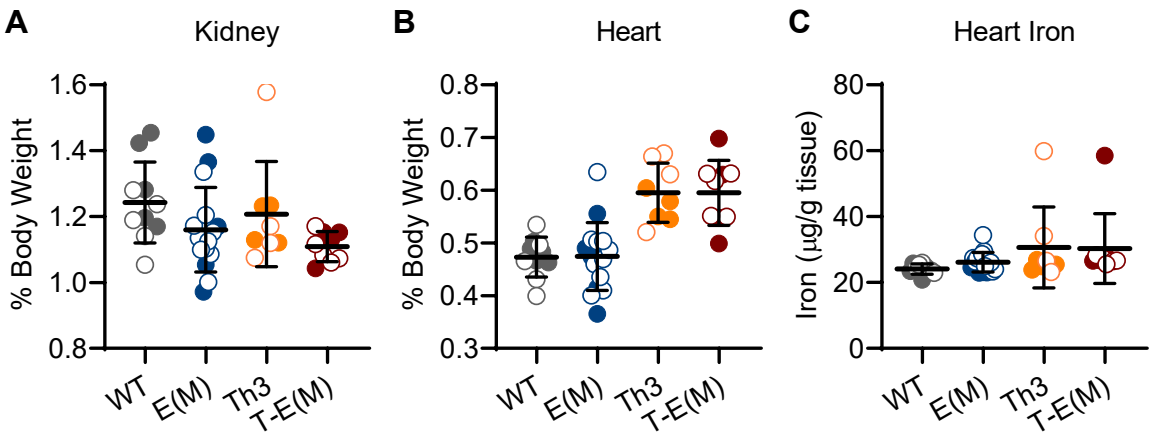
Th3/+ X Erfe-Tg (H)	Adult		E18.5	
	n=38 (13 litters) (av. 2.9 surviving pups/litter @ 3 wks)		n=31 (6 litters) (av. 5.2 pups/litter)	
	Expected	**Observed**	Expected	**Observed**
		****		**NS**
WT	25%	**55.3% (21)**	25%	**29% (9)**
Th3	25%	**15.8% (6)**	25%	**19.4% (6)**
E(H)	25%	**26.3% (7)**	25%	**22.6% (7)**
T-E(H)	25%	**2.6% (1)**	25%	**29% (9)**



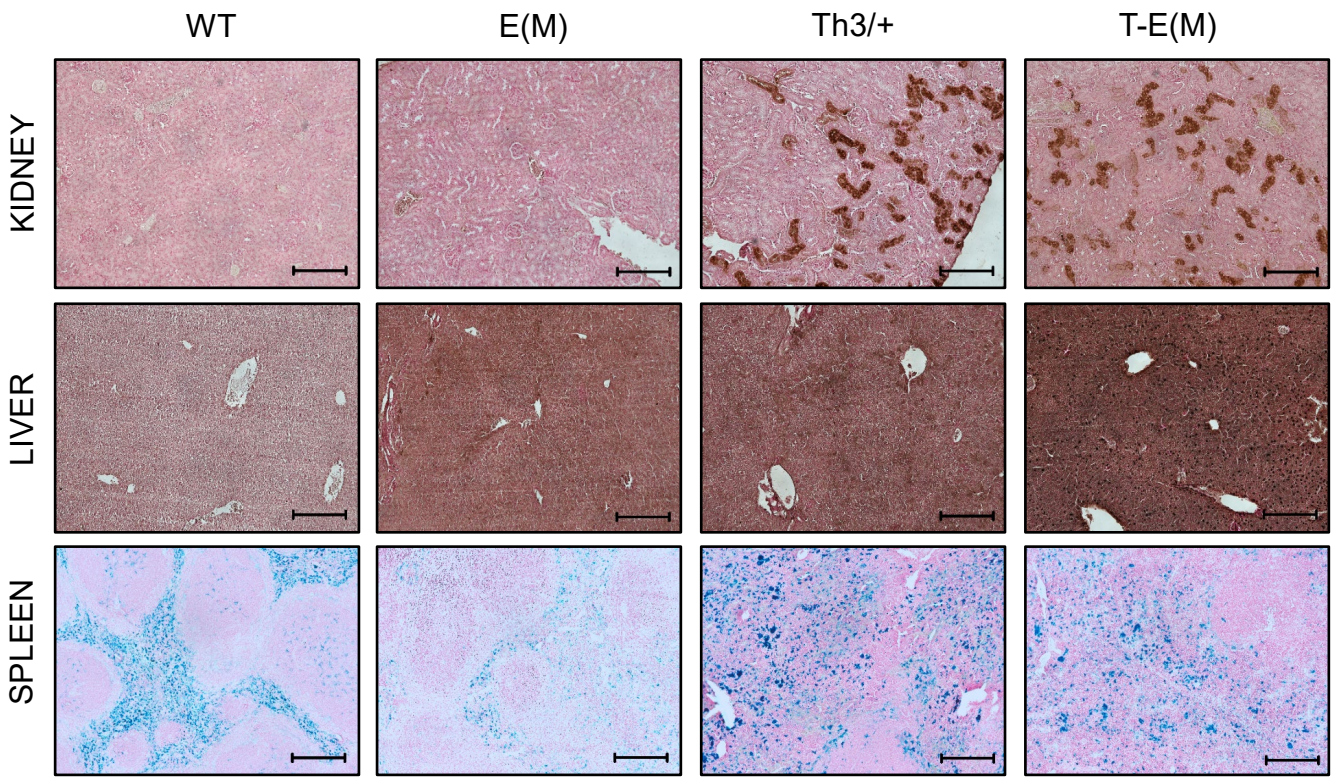
Supplemental Figure 3. Impaired postnatal survival of T-E(H) mice. Th3/+ (Th3) mice were bred with ERFE-overexpressing Line-H (E(H)) mice. **(A)** Expected and observed percentages of offspring with specified genotypes at either 3 weeks after birth or *in utero* at embryonic day E18.5. Total numbers are in parenthesis next to percentages. **(B-H)** Pups were harvested at embryonic day 18.5, blood and fetal livers were collected for analysis. Blood: **(B)** red blood cell (RBC) counts, **(C)** hemoglobin (Hb) concentration, **(D)** hematocrit (HCT), **(E)** mean corpuscular volume (MCV). Fetal liver: **(F)** *Erfe* mRNA, **(G)** *Hamp* mRNA and **(H)** non-heme iron concentrations. Statistics: **(A)** Significant differences from expected proportions were assessed by Chi-square testing (****: p<0.0001). **(B-H) p**-values were assessed by two-tailed Student's unpaired t-test (****: p<0.0001) between the Th3 and T-E(H) groups only.



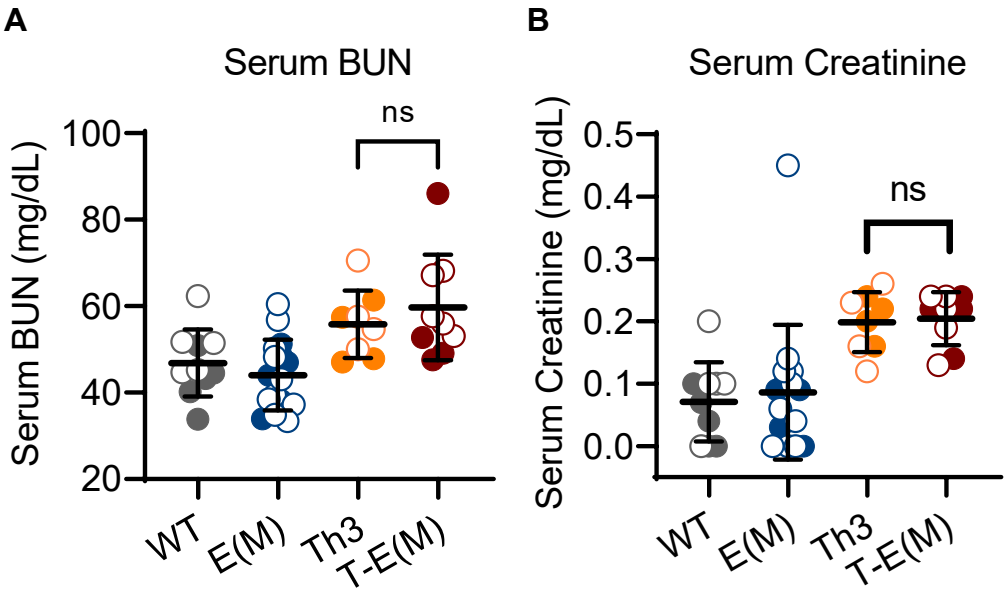
Supplemental Figure 4. Overexpression of ERFE does not substantially alter blood cell parameters in 16 week-old thalassemic mice. Blood **(A)** mean corpuscular hemoglobin (MCH), **(B)** mean corpuscular hemoglobin concentration (MCHC), **(C)** mean corpuscular volume, **(D)** red blood cell distribution width (RDW), **(E)** platelet count (PLT) and **(F)** serum lactate dehydrogenase (LDH) activity at 16 weeks of age in male (closed circles) and female (open circles) T-E(M) mice and WT, E(M), and Th3/+ littermate controls. P-values were assessed by two-tailed Student's unpaired t-test (*: $p<0.05$) between the Th3 and T-E(M) groups only.



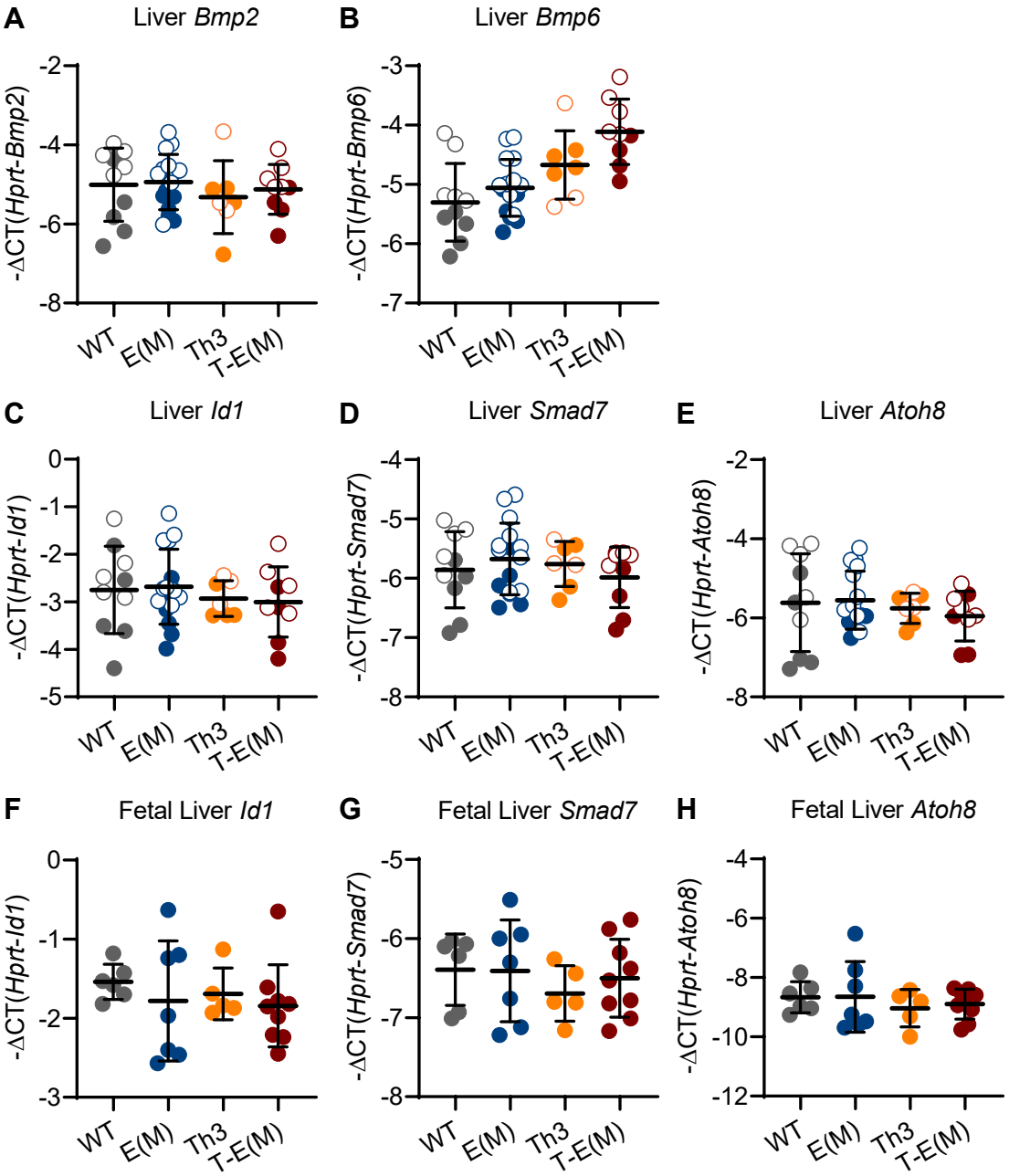
Supplemental Figure 5. ERFE overexpression does not alter kidney size, heart size or heart nonheme iron concentrations in thalassemic mice. Kidney **(A)** and heart **(B)** mass as a percentage of total body weight, and **(C)** heart nonheme iron concentration, in male (closed circles) and female (open circles) T-E(M) mice and WT, E(M), and Th3/+ littermate controls at 16 weeks of age.



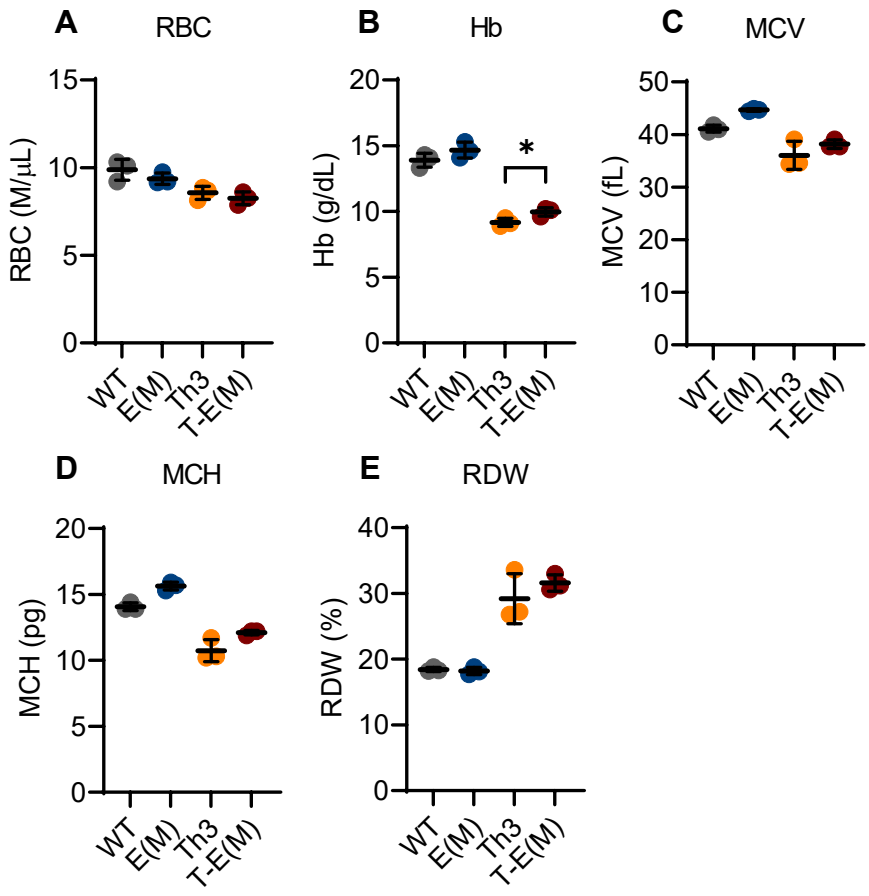
Supplemental Figure 6. ERFE overexpression exacerbates iron overload in thalassemic mice. Iron was visualized in FFPE sections of kidney *(top),* liver *(middle)* and spleen *(bottom)* from female WT, E(M), Th3/+ and T-E(M) littermates using DAB-enhanced Perls' stain (kidney and liver) and Perls' stain (spleen). Images taken at 10x magnification; scale bars represent 200 μm.



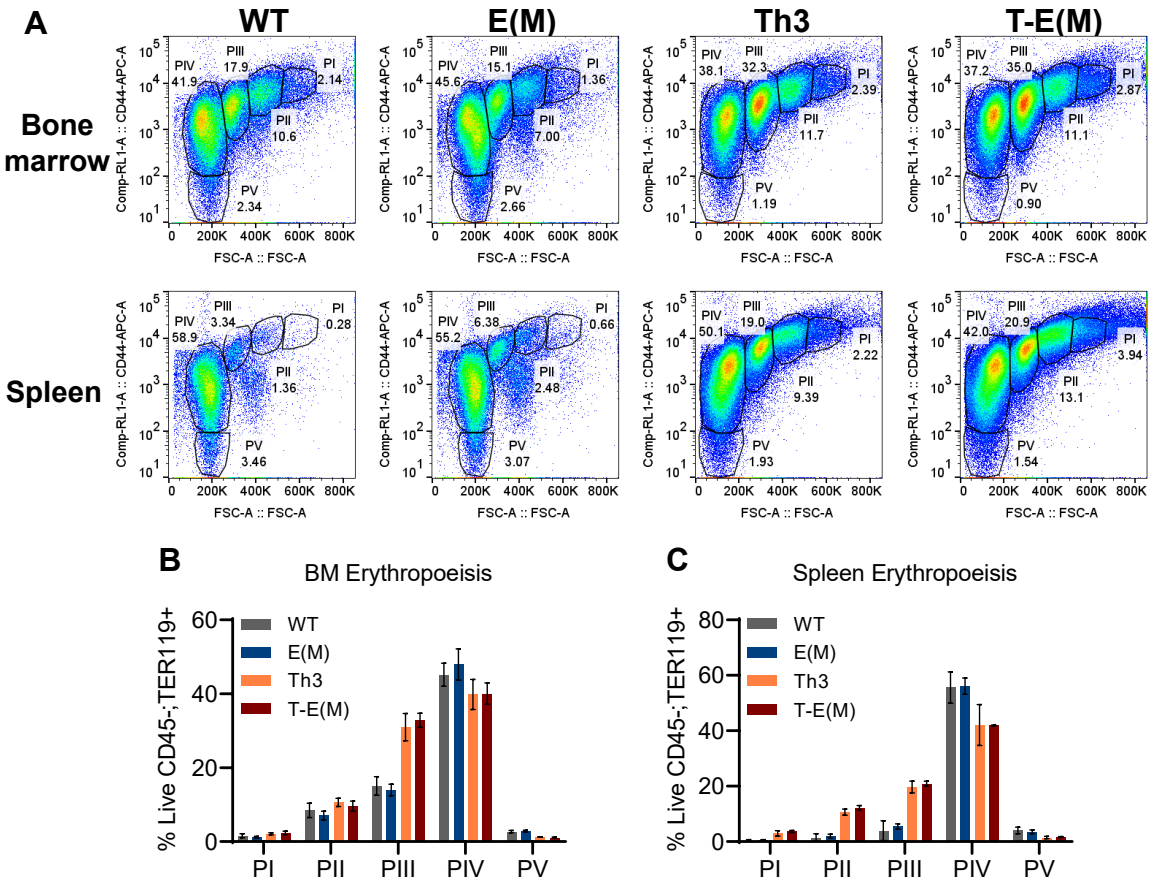
Supplemental Figure 7. Kidney function is impaired in thalassemic mice but is not further altered by overexpression of ERFE. Blood urea nitrogen (A) and serum creatinine (B) at 16 weeks of age in male (closed circles) and female (open circles) T-E(M) mice and WT, E(M), and Th3/+ littermate controls. p-values were assessed by two-tailed Student's unpaired t-test comparing Th3 and T-E(M) mice to each other and to WT mice.



Supplemental Figure 8. Overexpression of ERFE does not alter biomarkers of hepatic BMP signaling in adult and E18.5 thalassemic mice. (A-E) BMP signaling was assessed in the livers of 16-week-old WT, E(M), Th3 and T-E(M) mice. Males are depicted in closed circles and females in open circles. **(A)** *Bmp2,* **(B)** *Bmp6,* **(C)** *Id1*, **(D)** *Smad7,* and **(E)** *Atoh8* mRNA levels were measured by qRT-PCR. **(F-H)** BMP signaling was assessed in fetal livers from WT, E(M), Th3 and T-E(M) mice at E18.5. **(C)** *Id1*, **(D)** *Smad7,* and **(E)** *Atoh8* mRNA were measured by qRT-PCR. Error bars show means and standard deviations.



Supplemental Figure 9. ERFE overexpression does not substantially alter erythrocyte parameters in older adult thalassemic mice. Mice generated from Th3 x E(M) breedings were analyzed at 37-45 weeks of age. **(A-E)** Erythrocyte parameters: **(A)** RBC, **(B)** Hb, **(C)** MCV, **(D)** MCH and **(E)** RDW. p-values were assessed by two-tailed Student's unpaired t-test (*: $p<0.05$, **: $p<0.01$) between the Th3 and T-E(M) groups only.



Supplemental Figure 10. Increased proportions of immature erythroid populations in thalassemic mice are not altered by ERFE overexpression. Bone marrows and spleens from mice generated from Th3 x E(M) breedings were analyzed at 37-45 weeks of age (n=3 each group). **(A)** Representative plots of CD44 vs FSC of the live CD45-TER119+ cells in the bone marrow (top) and spleen (bottom). Erythroid populations were labelled as defined in Casu et al, *Haematologica* (2020). **(B-C)** Erythropoietic maturation profiles (means and standard deviations, n=3 mice per group) in the **(B)** bone marrow and **(C)** spleen.