SUPPLEMENTARY APPENDIX

# Erythroferrone contributes to hepcidin repression in a mouse model of malarial anemia

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**Supplementary table 1 : Primer sequences**

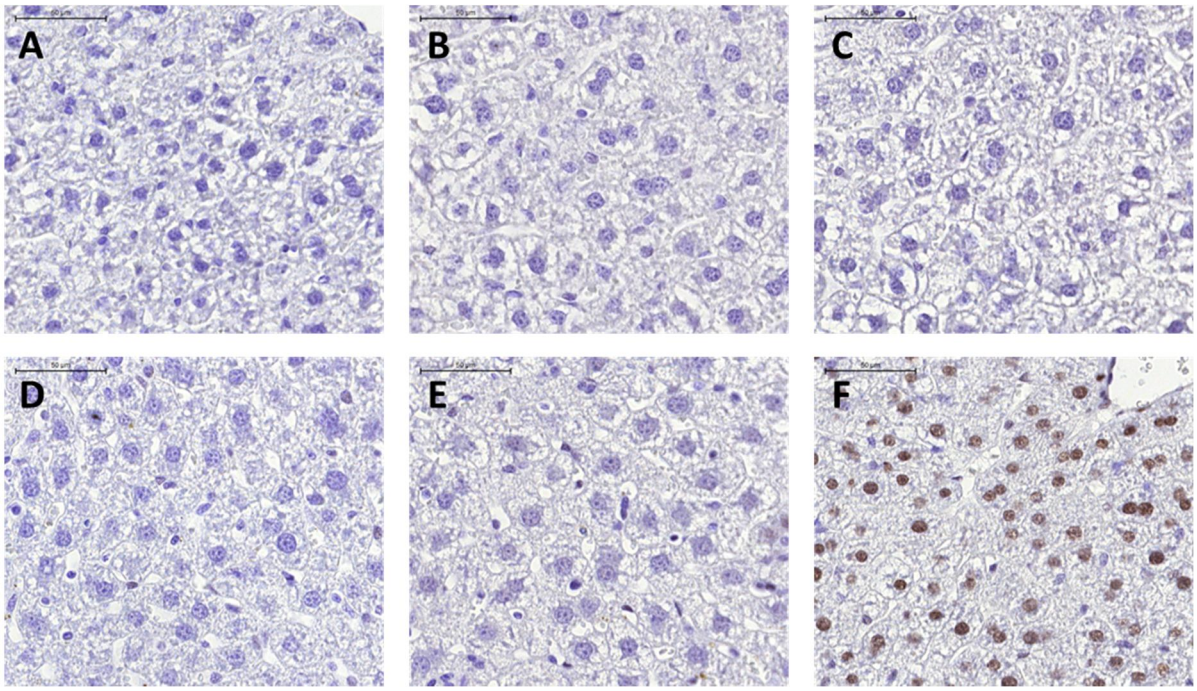
| gene | forward | reverse |
|---|---|---|
| Hprt | CTGGTTAAGCAGTACAGCCCCAA | CAGGAGGTCCTTTTCACCAGC |
| Rpl4 | TGAAAAGCCCAGAAATCCAA | AGTCTTGGCGTAAGGGTTCA |
| Hamp | AAGCAGGGCAGACATTGCGAT | CAGGATGTGGCTCTAGGCTATGT |
| Epo | GCCTCACTTCACTGCTTCGG | GGAGGCGACATCAATTCCTTC |
| Erfe | ATGGGGCTGGAGAACAGC | TGGCATTGTCCAAGAAGACA |
| Gypa | GGAGGAATGCCGTCACCAA | TAATCCCTGCCATCACGCC |
| Tnfa | AATGGCCTCCCTCTCATCAG | GCTACGACGTGGGCTACAGG |
| Infg | CAGCAACAGCAAGGCGAAA | AGCTCATTGAATGCTTGGCG |
| Gdf15 | GCTGTCCGGATACTCAGTCCA | TTGACGCGGAGTAGCAGCT |
| Twsg1 | AGCGACAAAGAGCGCATGTG | CACTGGTGGATGGACATGCAG |

**Supplementary methods:**

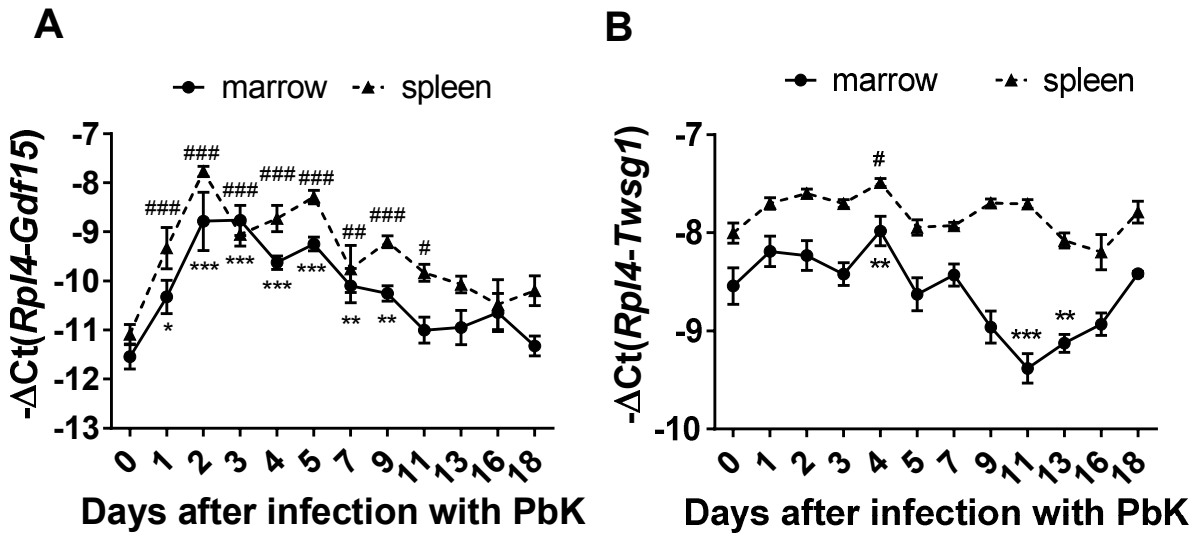
**Immunohistochemistry**

Four-micrometer sections of paraffin-embedded tissues were mounted on glass slides. Antigen retrieval was performed by incubating tissue sections with Citrate buffer pH6 for 10 min at 98°C. Cell membrane was permeabilized using Triton 0.3% for 10 min. Endogenous peroxidase activity was quenched by incubating specimens with Dako REAL Peroxidase Blocking Solution (Dako, Trappes, France). Tissue sections were then blocked with normal horse blocking serum (Vector Laboratories, Burlingame, CA, USA) and incubated overnight at 4°C with the primary anti-Phospho-Stat3 (Tyr705) (D3A7) antibody (1/150; Cell Signaling Technology) diluted in PBS-1% BSA. Immunohistochemical staining was performed using the

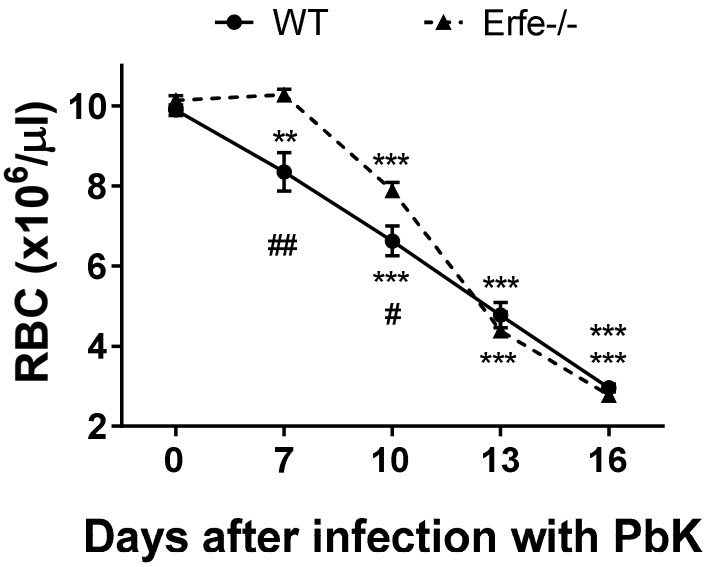
ImmPRESS Reagent (ImmPRESS Anti-Goat Ig peroxidase Kit; Vector Laboratories) according to the manufacturer's instructions. Sections were counterstained with hematoxylin.




**Supplementary Figure 1: Immunohistochemical detection of p-STAT3 in the liver of mice infected with PbK.** Phosphorylation of STAT3 was not detectable in the liver of mice at days 1 (A), 2 (B), 3(C), 4 (D), 5 (E) after infection with PbK. On the contrary, p-STAT3 was found in hepatocytes nuclei 1h after LPS administration (F). Original magnification x40. Scale bars represent 50 µm.




**Supplementary Figure 2: *Gdf15* and *Twsg1* mRNA expression in the bone marrow and spleen of WT mice infected with PbK.** *Gdf15* mRNA expression (A) increased in the bone marrow (solid line, dotted symbols) and the spleen (dashed line, triangle symbols) between day 1 and day 9 (bone marrow) or 13 (spleen). *Twsg1* expression (B) was unchanged in the spleen and slightly decreased in the bone marrow at days 11 and 13. Data shown are means ± s.e.m and were compared for each time point to values for control mice at t = 0 (n = 5 to 10 mice per group except at day 18 where only 3 mice had survived) by one-way ANOVA. ****P* < .001, ***P*< .01 for the bone marrow and ### *P* < .001, ## *P*< .01, # *P*< .05 for the spleen.




**Supplementary Figure 3: Red blood cell count in WT and Erfe-/- mice during infection with PbK.** RBC dramatically decreased during infection with PbK. Compared to WT mice, the drop in RBC between day 10 and 13 was more prominent in *Erfe*$^{-/-}$ mice despite lower parasitemia. The same mice were monitored between day 0 and 16 (n=6 mice per genotype). Data shown are means ± s.e.m and were compared for each mice at each time point to values at t = 0 (***$P$ < .001, **$P$< .01, *$P$< .05) by One-way ANOVA and between WT and *Erfe*$^{-/-}$ mice (### $P$ < .001, (## $P$ < .01, # $P$< .05) by two-tailed Student t-test.