Supplemental Figure Legends.

Supplemental Figure 1. Hematological and iron-related parameters in groups of wt and *th3*/+ mice generated by breeding and fed diets with 35 and 2.5 ppm iron for 1 and 5 months. Complete blood counts show (**A**) the Hb, RBC, and reticulocytes values, and (**B**) the MCH, MCV, and MCHC values. (**C**) The total iron content of the liver and spleen, as measured by atomic absorption. Error bars represent SD. The P values (*P<.05, **P<.01, and ***P<.001) were calculated using Student's t-test. Groups of mice on the 2.5-ppm diet were compared with the corresponding groups on the 35-ppm diet fed for the same length of time. In (C) the P values relative to comparisons between spleens are above the columns, while those between the livers are below the X-axis. The P values in the small rectangles above the columns refer to the total iron content of liver and spleen.

Supplemental Figure 2. Epo levels measured in mice fed defined iron diets and mice over-expressing *Hamp1*. (**A)** Wt and *th3*/+ mice on the 35-ppm and 2.5-ppm iron diets. (*P<.05). (**B**) Tg-*Hamp* and Tg-*Hamp*/*th3* mice, and the corresponding controls, Tg(-) and Tg(-)/*th3* mice, on a 35-ppm diet after 1 and 5 months, respectively. HHE mice showed Epo levels of 3800 (HHE-1), 13700 (HHE-2), and 13300 (HHE-3) pg/mL.

Supplemental Figure 3. The total iron content of the kidneys (top) and heart (bottom) of (**A, B**) mice on the 35-ppm and 2.5-ppm iron diets, and (**C, D**) mice over-expressing *Hamp1* as measured by atomic absorption. (P values *P<.05, **P<.01, and ***P<.001).

Supplemental Figure 4. Effects of dietary iron and over-expression of *Hamp1* on the size of the liver (normalized to body weight). (**A**) Wt and th3/+ mice fed the 35- and 2.5-ppm iron diets for 1 and 5 months. (**B**) Tg-*Hamp*, Tg-*Hamp*/*th3*, and relative control mice fed the 35-ppm iron diet for 1 and 5 months. Body weights are shown in (**C**) for wt and th3/+ mice fed the 35- and 2.5-ppm iron diets, and in (**D**) for Tg-*Hamp*, Tg-*Hamp*/*th3* and control mice. (**P<.01).

Supplemental Figure 5. (**A)** Spleen and (**B**) liver sections stained with Prussian blue to detect iron (in blue) (magnification 100X). The red arrows indicate areas of co-localization of iron and EMH in the liver.

Supplemental Figure 6. (**A**) Spleen cells from Tg(-)/*th3*, *th3*/+ on the 2.5-ppm iron diet, and Tg-*Hamp*/*th3* mice were stained with the CD44 and Ter119 markers, and analyzed by flow cytometry to identify the CD44+/Ter119+ and CD44-/Ter119+ populations. (**B**) The aforementioned populations were subsequently plotted according to forward side scatter on the X-axis and CD44 expression on the Y-axis, so as to identify 5 different stages of erythroid differentiation, corresponding to proerythroblasts (fraction I), basophilic (II), polychromatic (III), orthochromatic cells and reticulocytes (IV), and mature RBC (V). (**C**) The total number of cells in each fraction. Both *th3*/+ mice on the 2.5-ppm iron diet and Tg-*Hamp*/*th3* mice showed a reduction in the number of immature populations (I-IV), and a trend toward an increase in mature RBC (V).

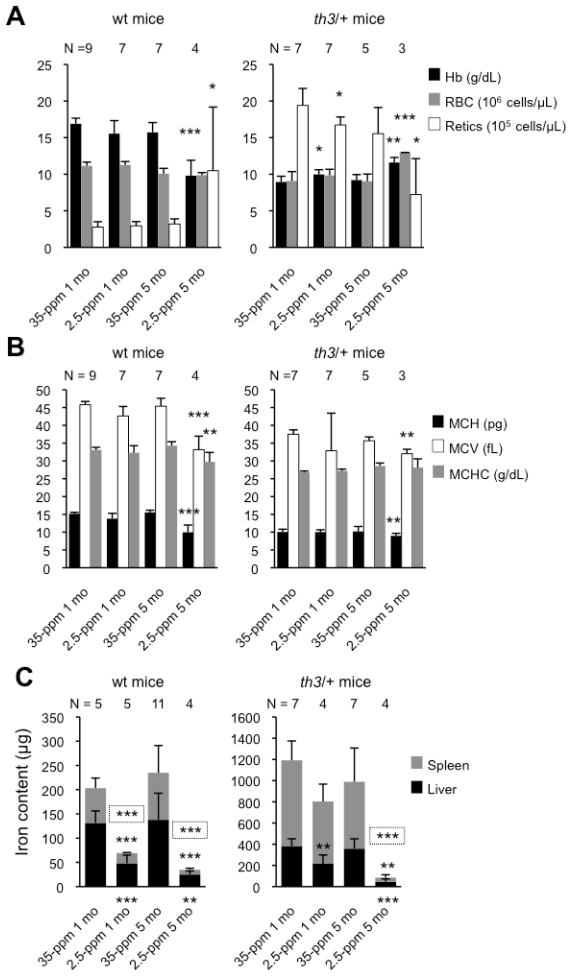
Supplemental Figure 7. The architecture of the spleen is more normal and EMH is reduced in the liver of Tg-*Hamp/th3* mice maintained on a diet containing 35-ppm iron. (**A)** Spleen and (**B**) liver staining with hematoxylin & eosin (H&E) (magnification 40X and 400X respectively). The red arrows indicate the presence of erythroid cells in the liver (EMH).

Supplemental Figure 8. High performance liquid chromatography **(**HPLC) analysis of RBC membrane-associated globins from two representative mice (**A**) Tg(-)/*th3* and (**B**) Tg-*Hamp*/*th3*. The percentages of the area under each peak are indicated. All the Tg-*Hamp*/th3 mice analyzed showed a remarkable reduction of membrane-associated alpha-chains compared to Tg(-)/*th3* control mice. The relative amounts of alpha- and beta-globin chains in the RBCs were also evaluated by HPLC. The same number of RBCs from each mouse was processed and run on a Vydac C4 column (4.6 x 250 mm, 5 mm, 300 Å, no. 214TP54) (Grace Vydac, Deerfield, IL), as described by Han et al. (1).

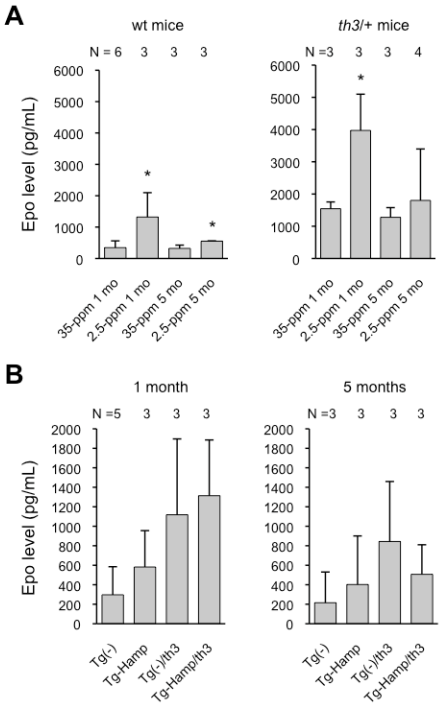
Supplemental Figure 9. Effects of over-expression of *Hamp1* on *Fpn1* levels in the duodenum of Tg-*Hamp*, Tg-*Hamp*/*th3* and relevant control mice after 1 and 5 months on the 35-ppm iron diet.

References

1. Han, A.P., Yu, C., Lu, L., Fujiwara, Y., Browne, C., Chin, G., Fleming, M., Leboulch, P., Orkin, S.H., and Chen, J.J. 2001. Heme-regulated eIF2alpha kinase (HRI) is required for translational regulation and survival of erythroid precursors in iron deficiency. *EMBO J* 20:6909-6918.



Supplemental Figure 1



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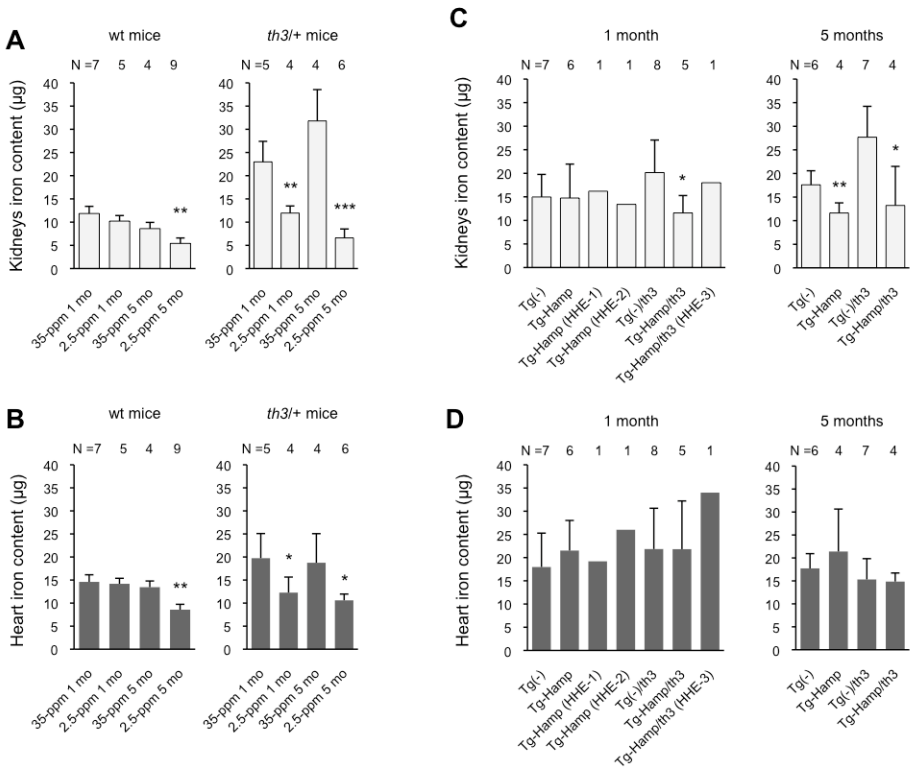
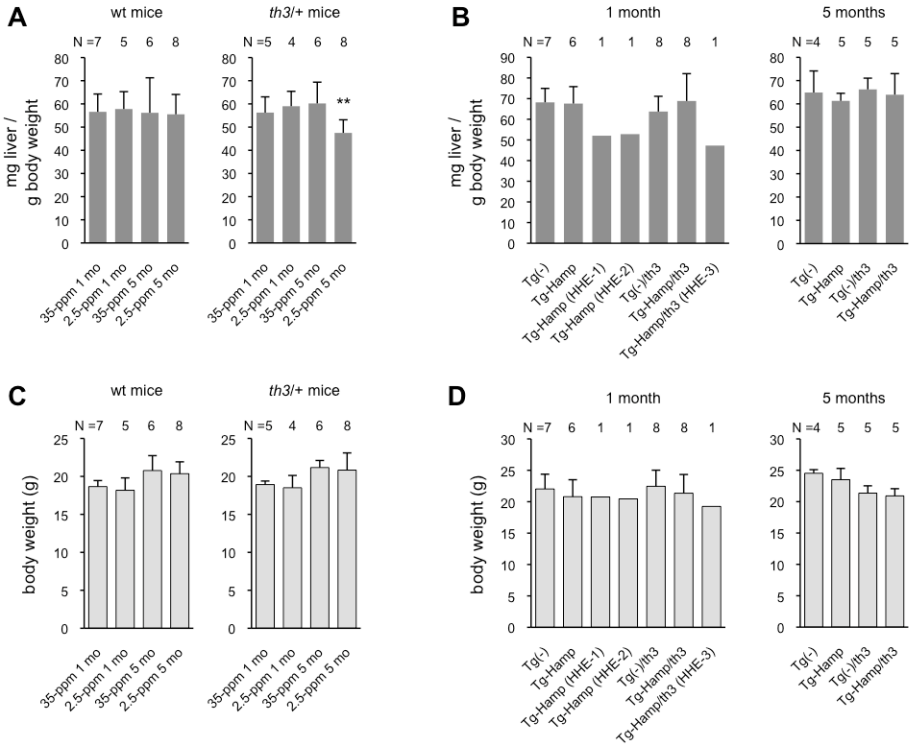


Supplemental Figure 3



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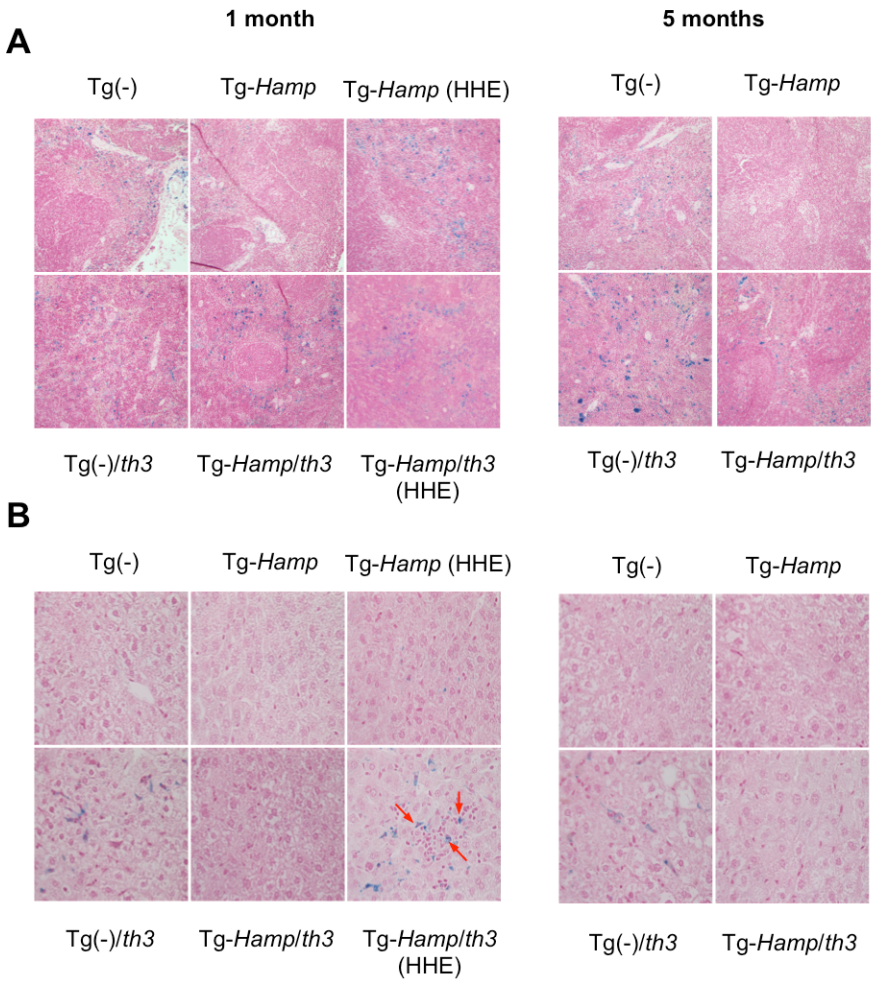

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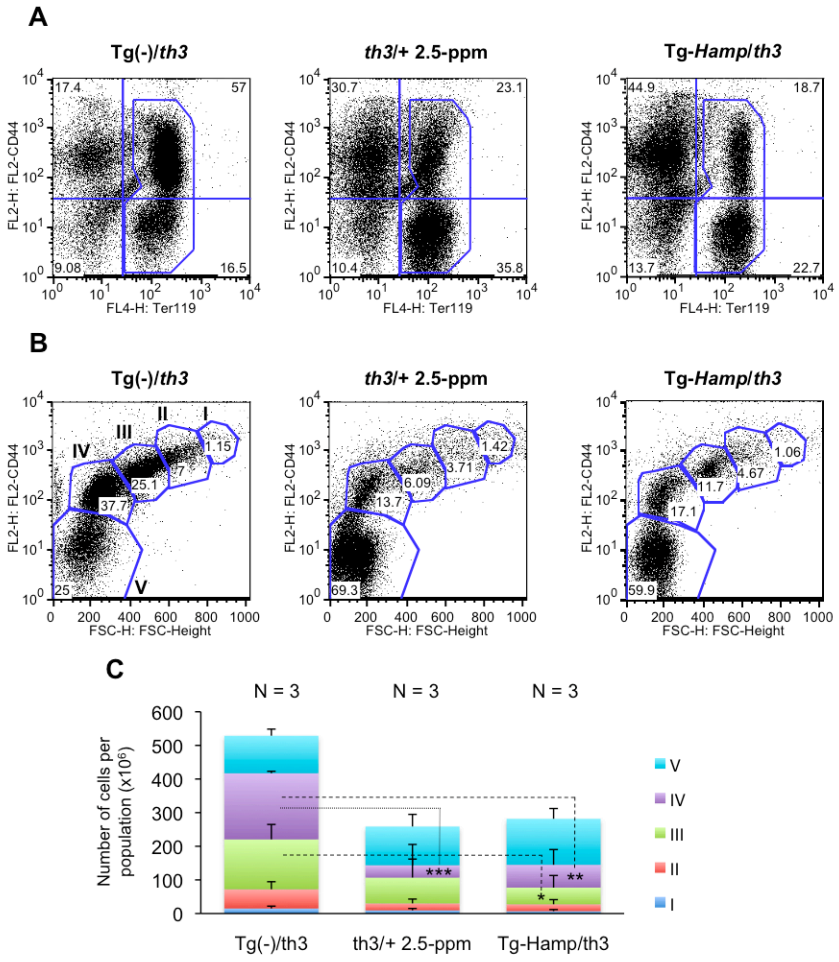

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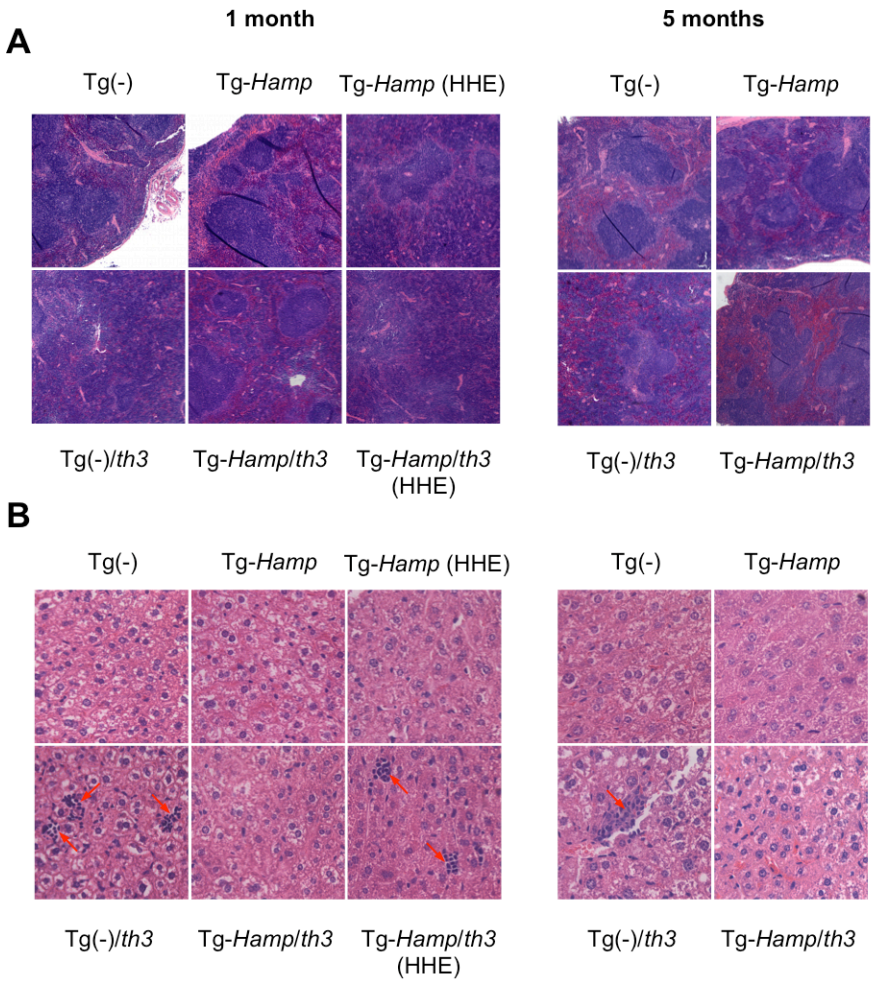

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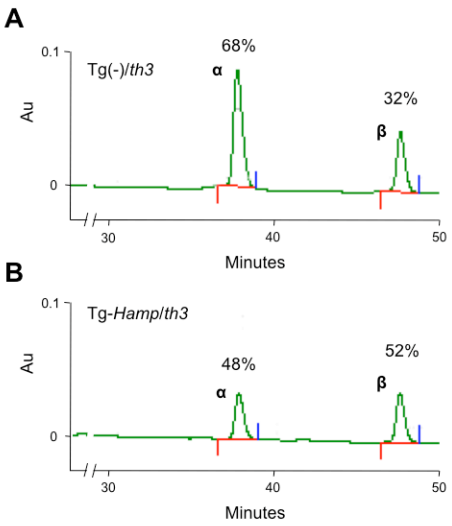
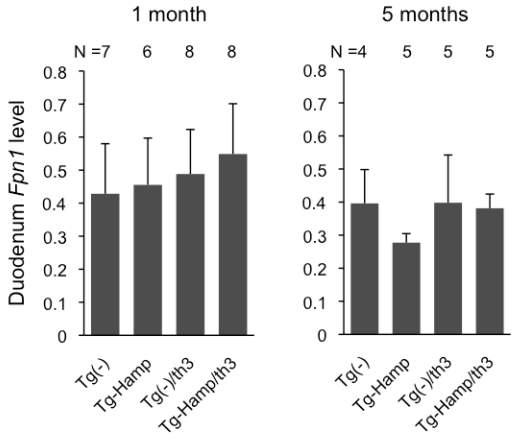


Supplemental Figure 8



Supplemental Figure 9