Molecular basis of iron homeostasis



and its translation to the clinic

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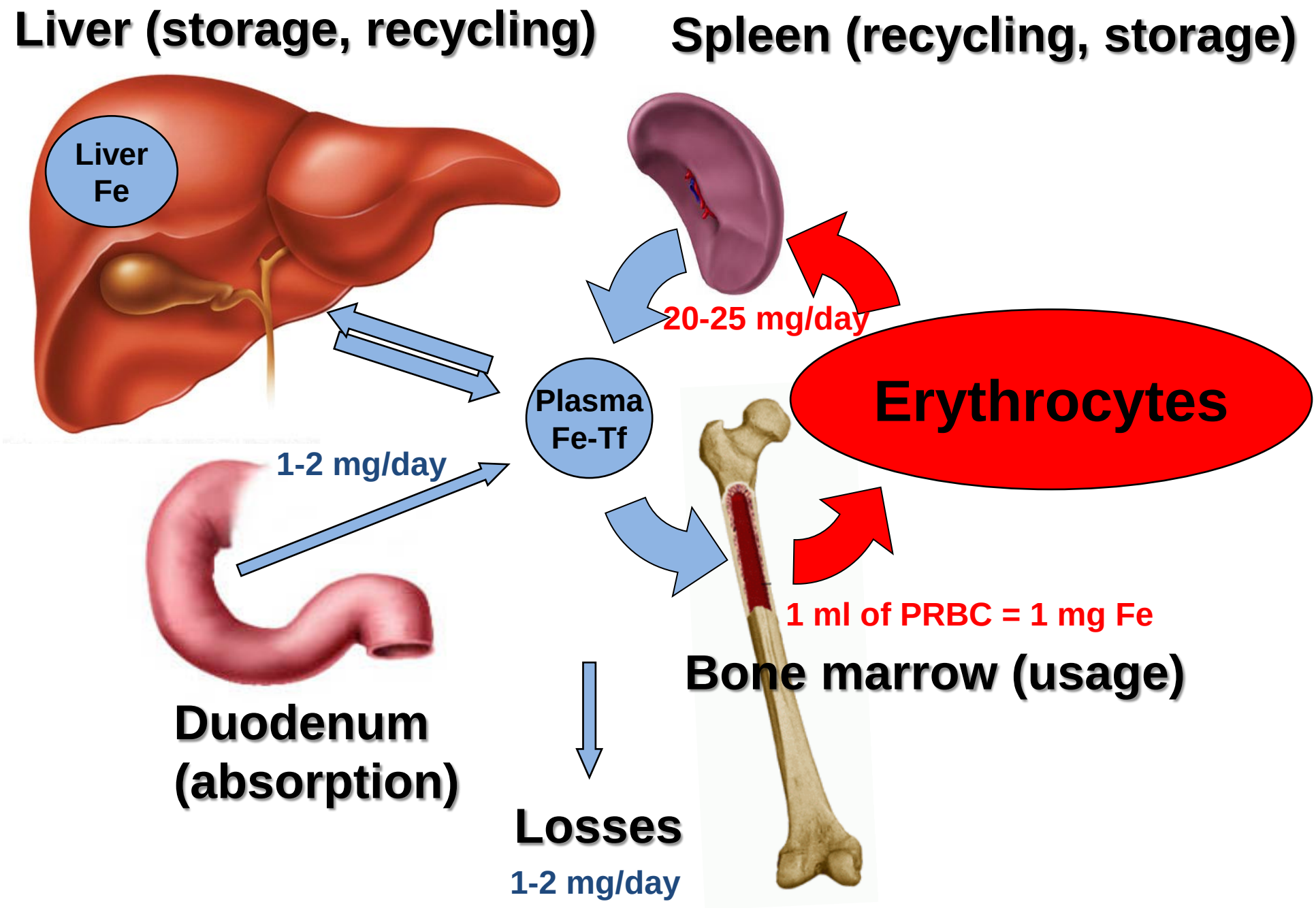
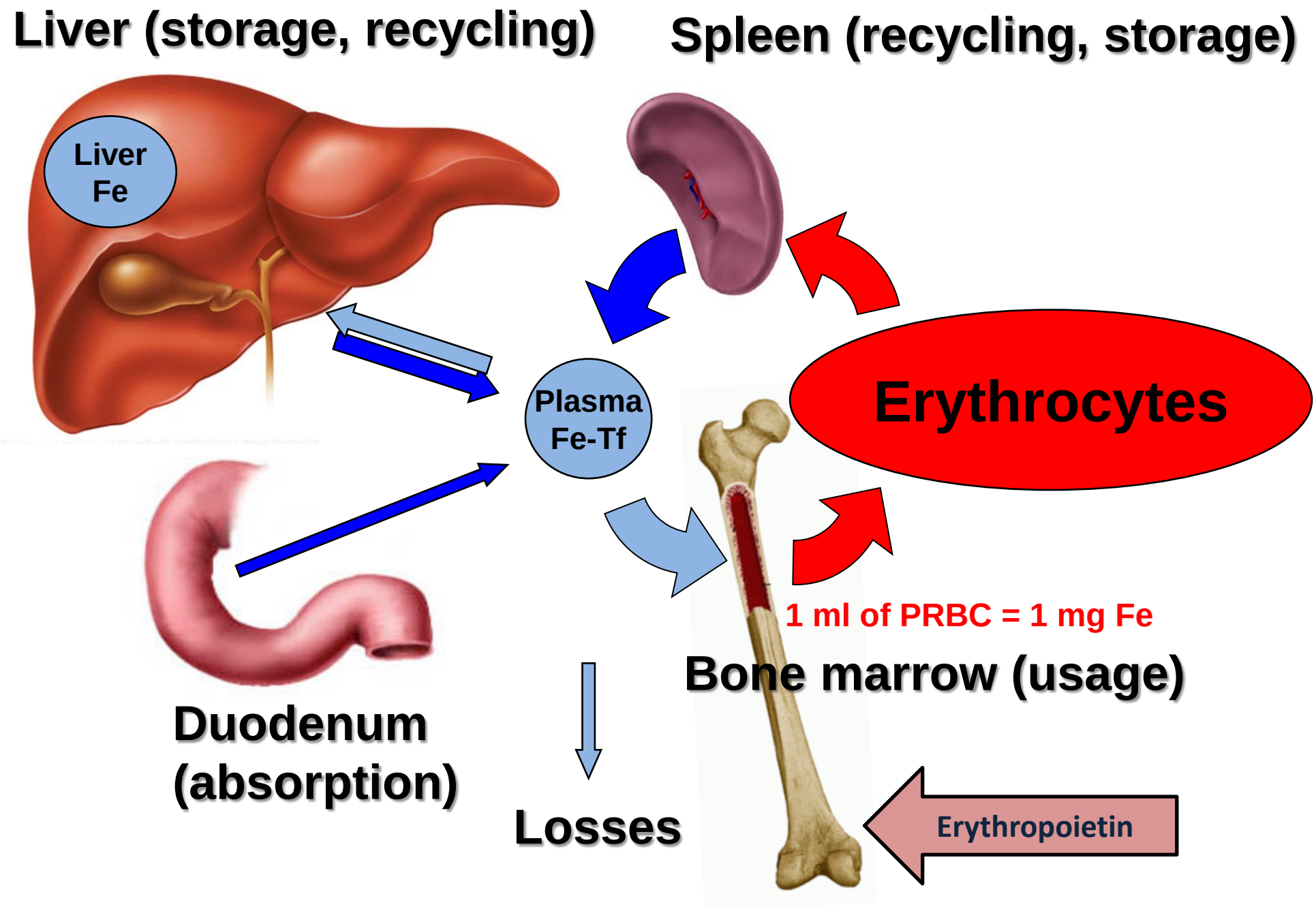
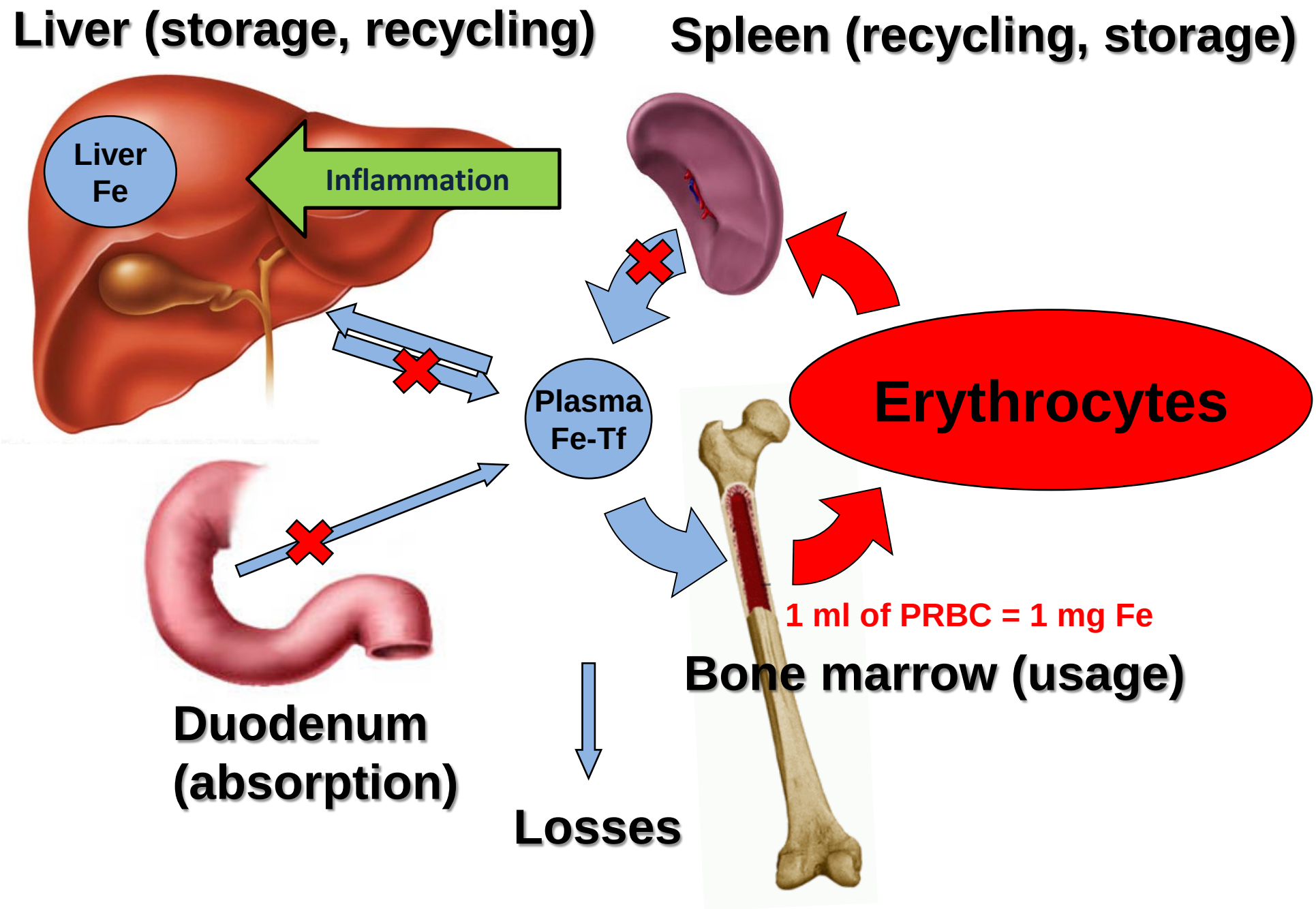
Systemic iron homeostasis



During erythropoietic stimulation

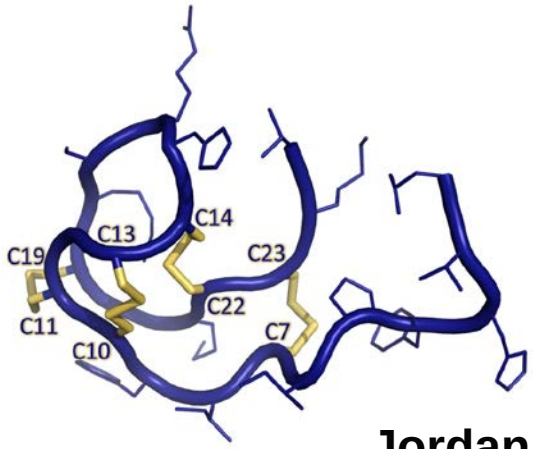


During infection/inflammation



Hepcidin—an iron-regulatory peptide hormone

- Made in the liver as 84 aa preprohepcidin
- Cleaved to 25 aa bioactive hepcidin by furin
- Secreted by hepatocytes
- Regulates intestinal iron absorption and the distribution of iron to tissues

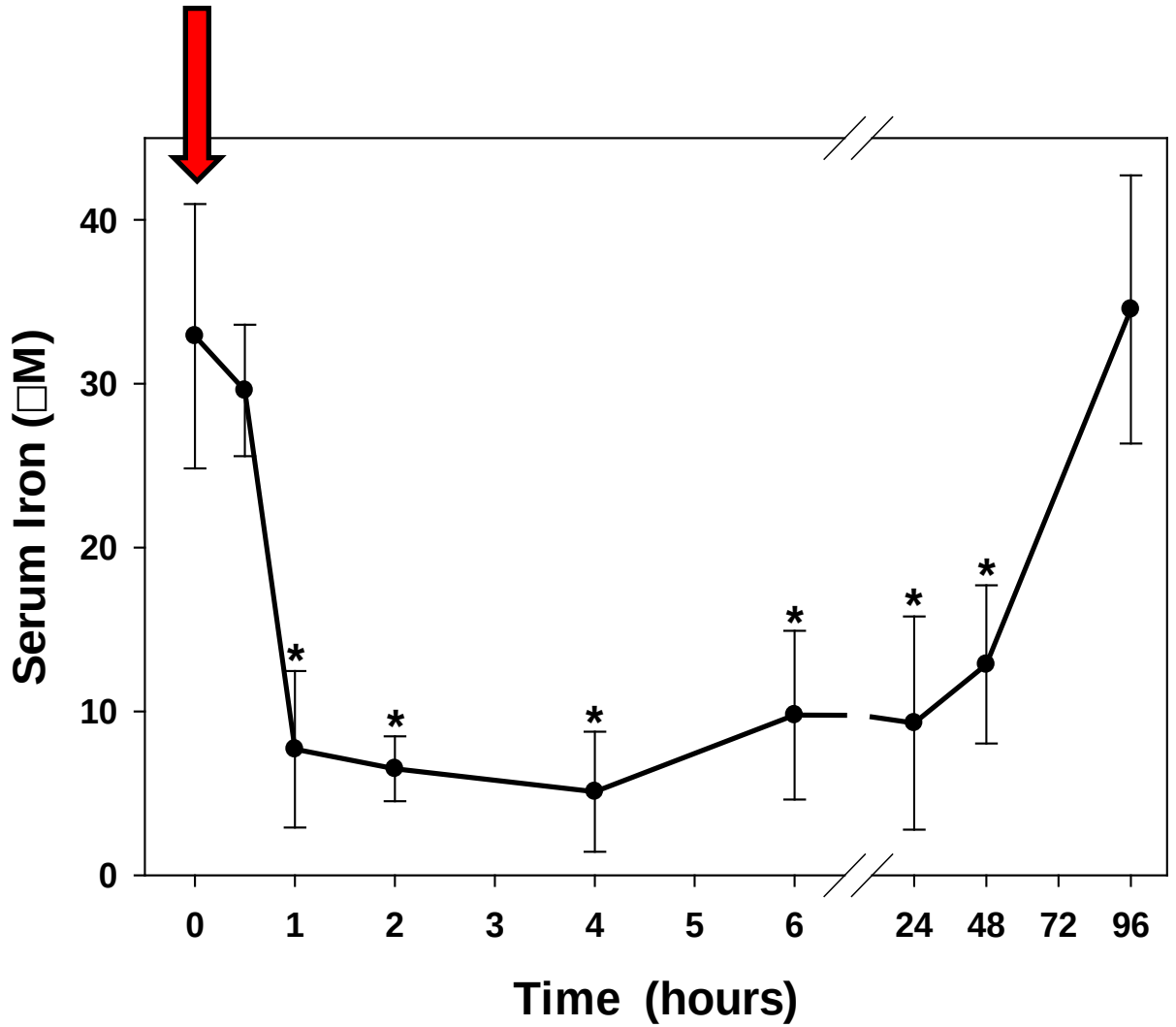


DTHFPICIFCCGCCHRSKCGMCCKT

Jordan et al. JBC 2009

Hepcidin peptide lowers plasma iron

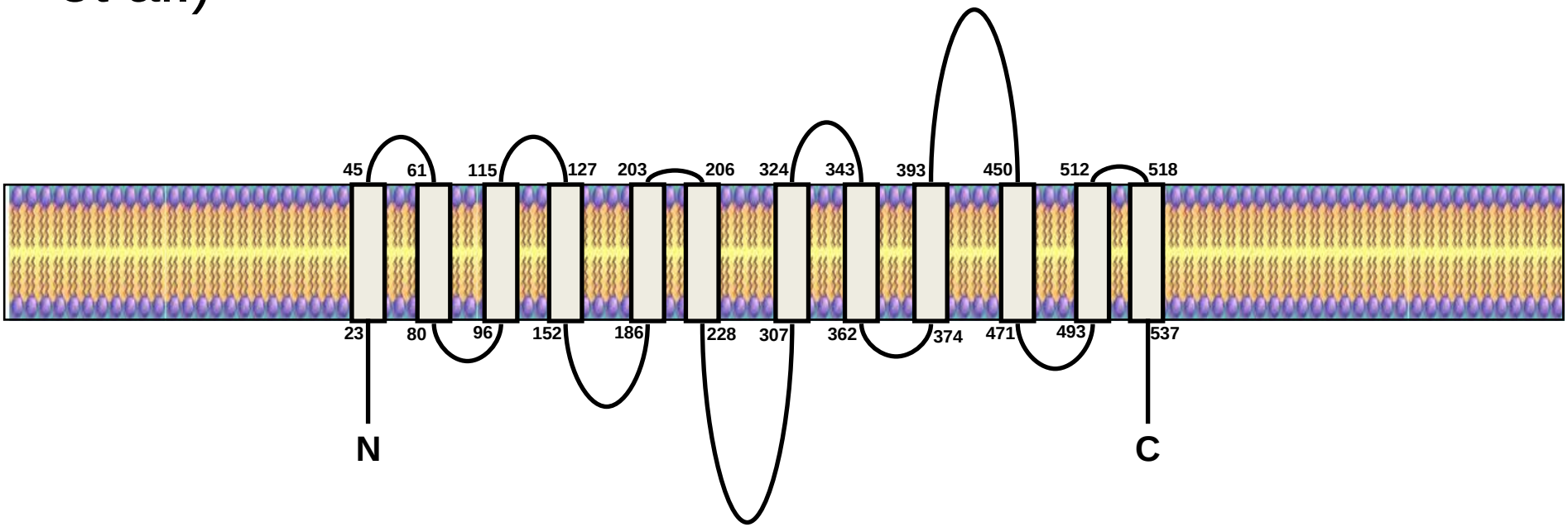
Synthetic hepcidin 50 μg IP/mouse



Rivera et al. Blood 2005

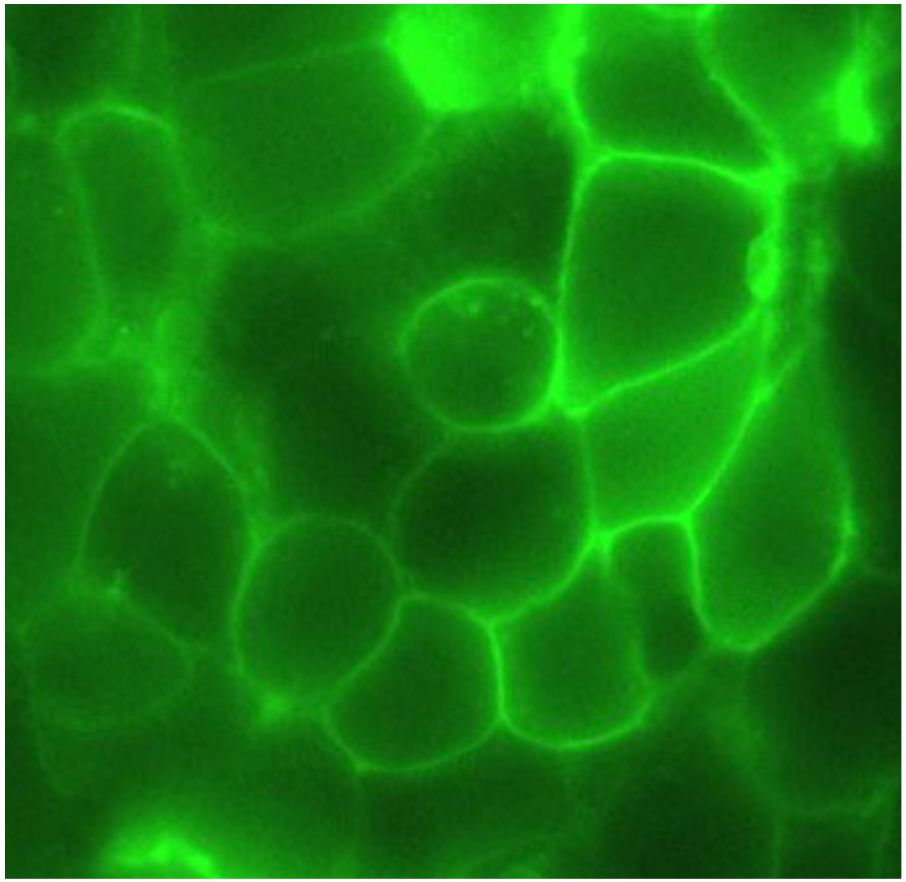
Ferroportin

- 12 transmembrane segment protein
- Found in plants, invertebrates, vertebrates
- In vertebrates, the receptor for hepcidin
- The only cellular iron exporter known (Donovan et al.)
- Present in the macrophages, duodenum, hepatocytes, and the placenta (Donovan et al., McKie et al., Abboud et al.)

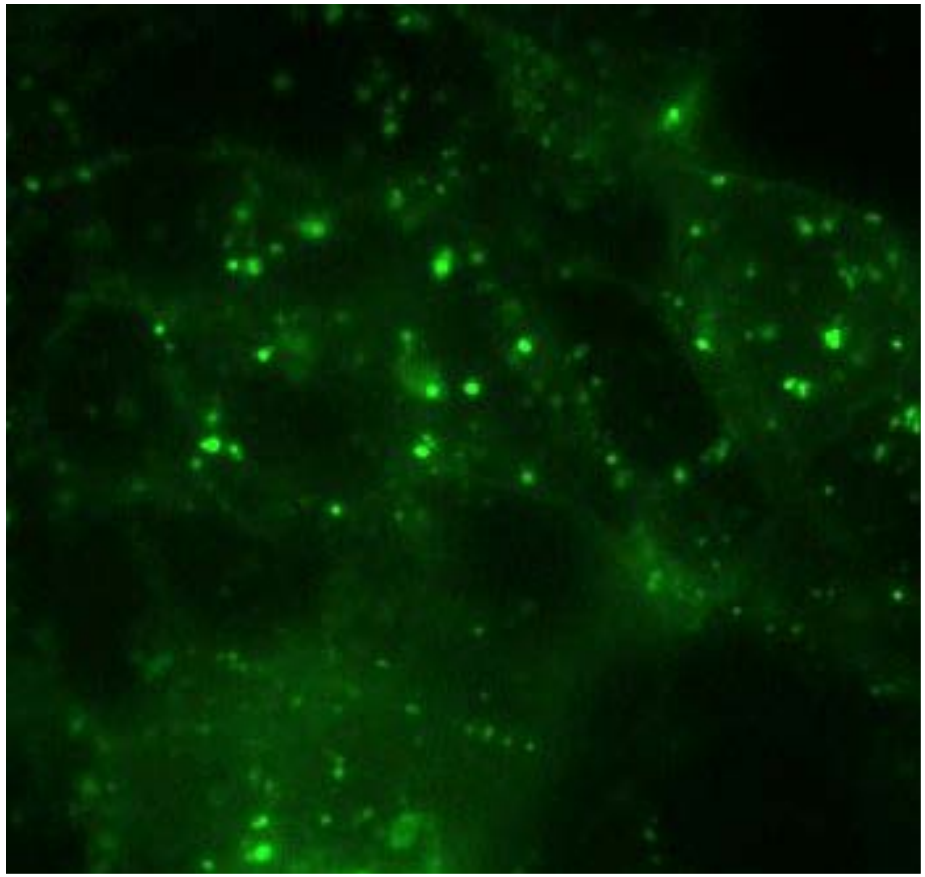


Ferroportin is internalized by hepcidin and degraded in lysosomes

control

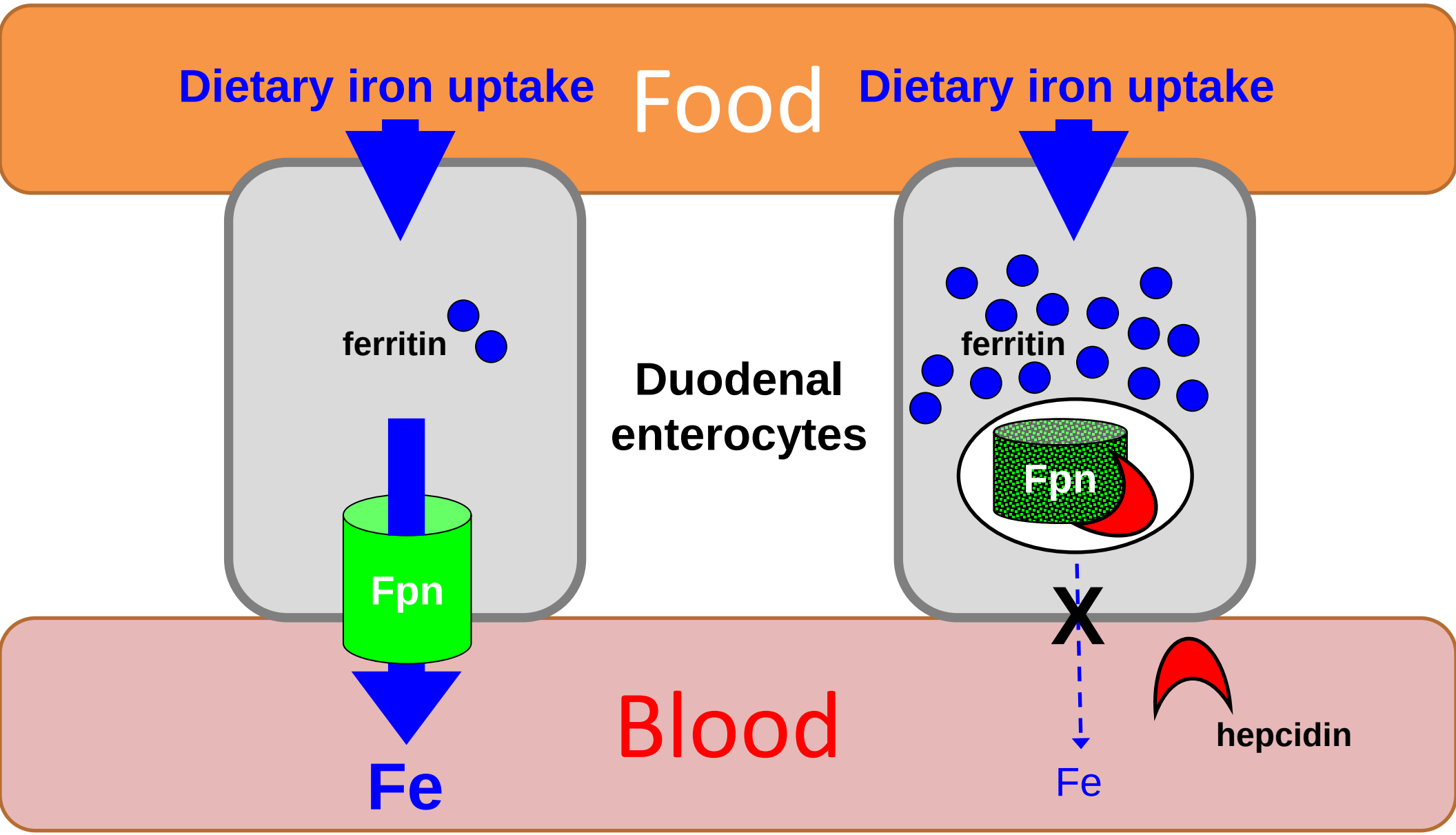


+ 1 μg/ml **hepcidin**

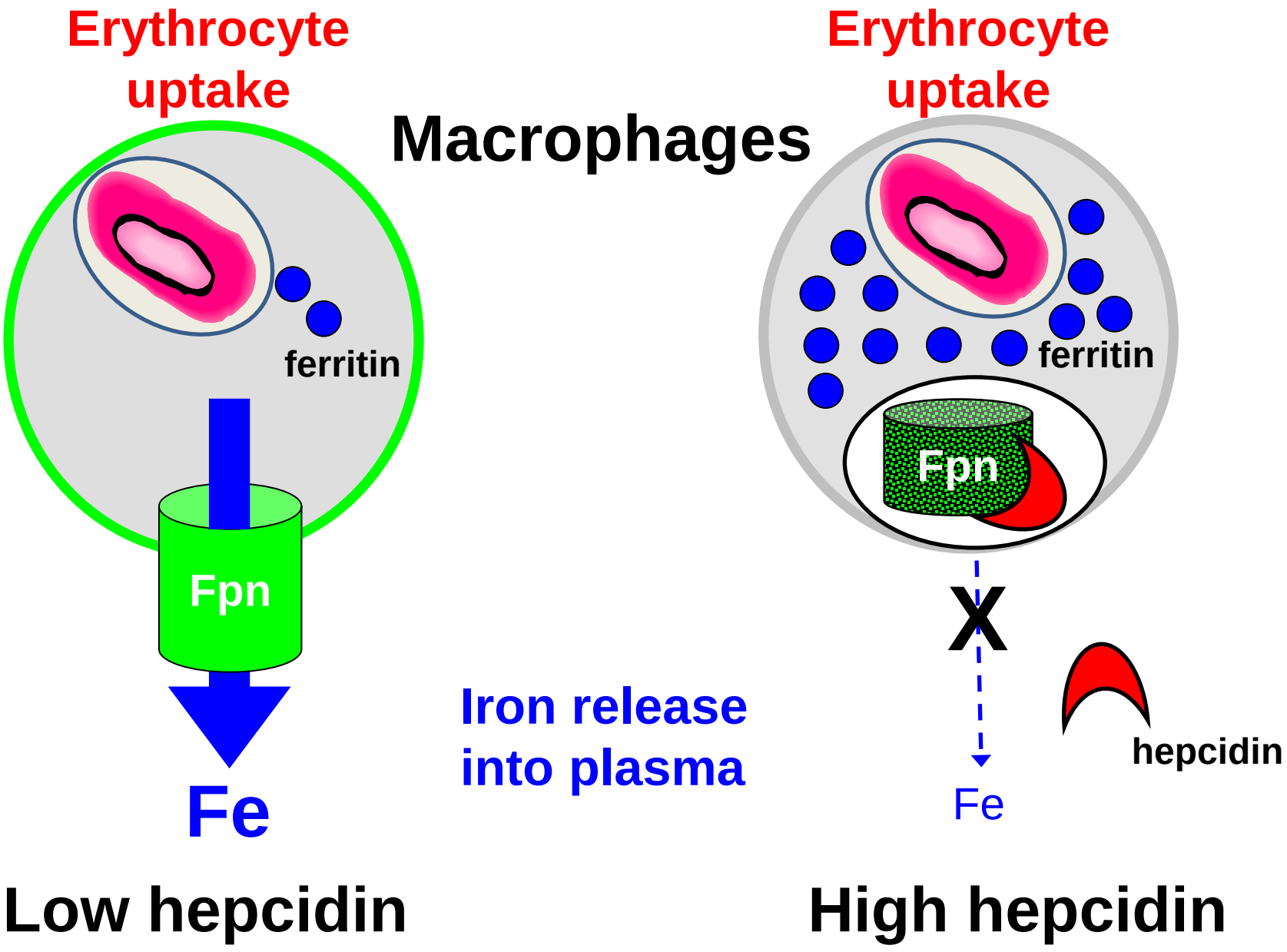


Nemeth et al. Science 2004

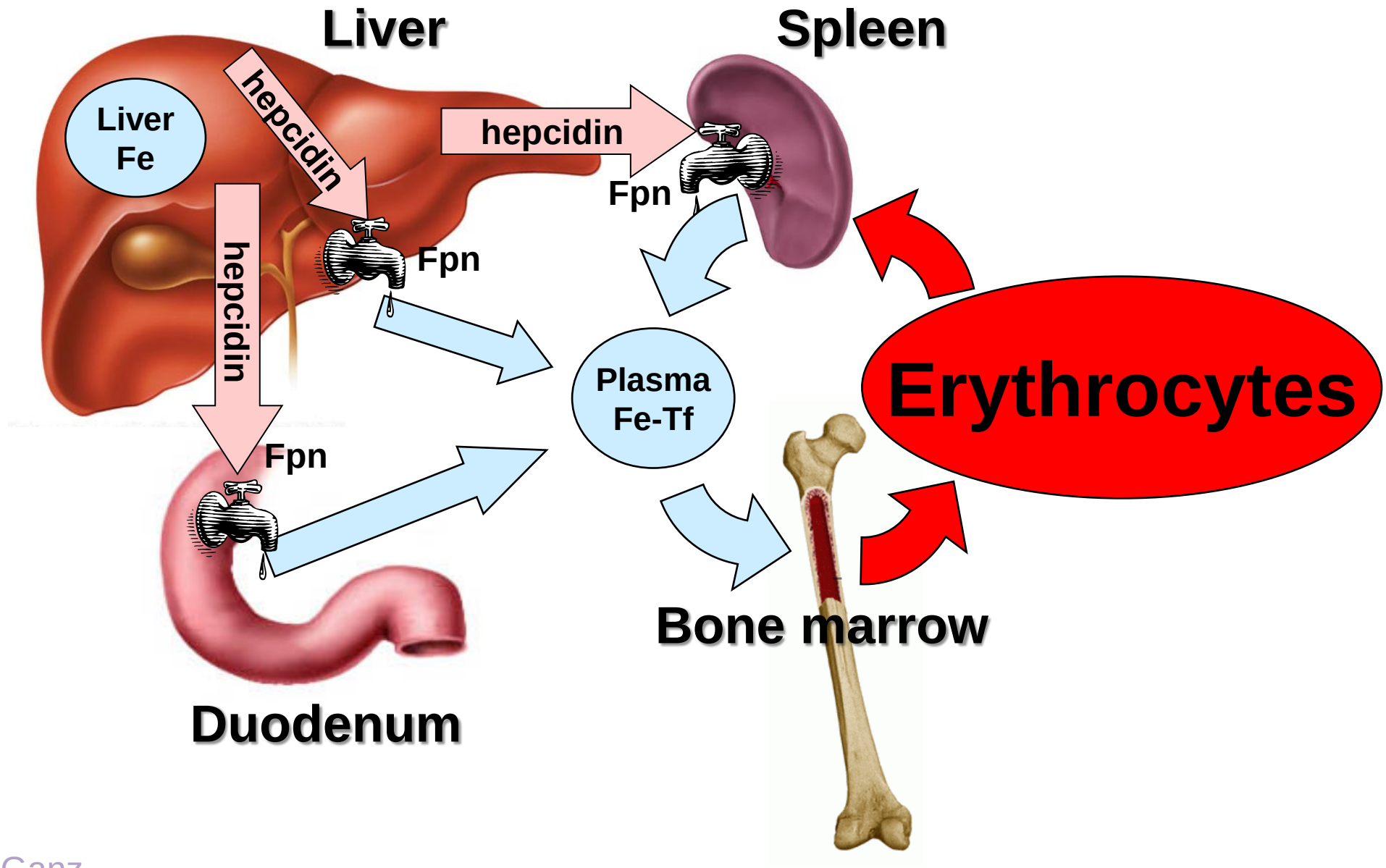
Regulation of intestinal iron absorption



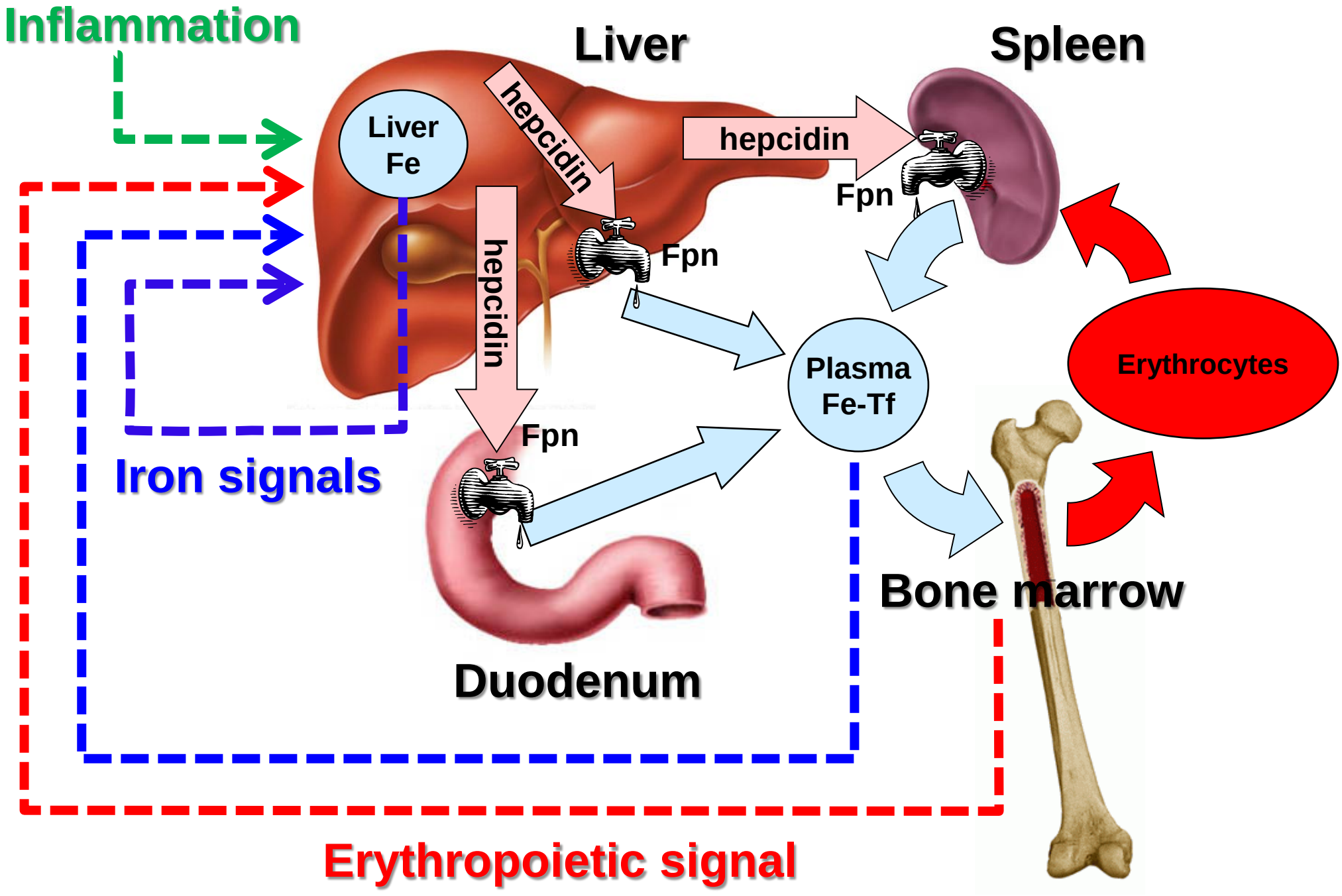
Regulation of erythrocyte iron recycling



Hepcidin regulates dietary iron absorption and influx to plasma



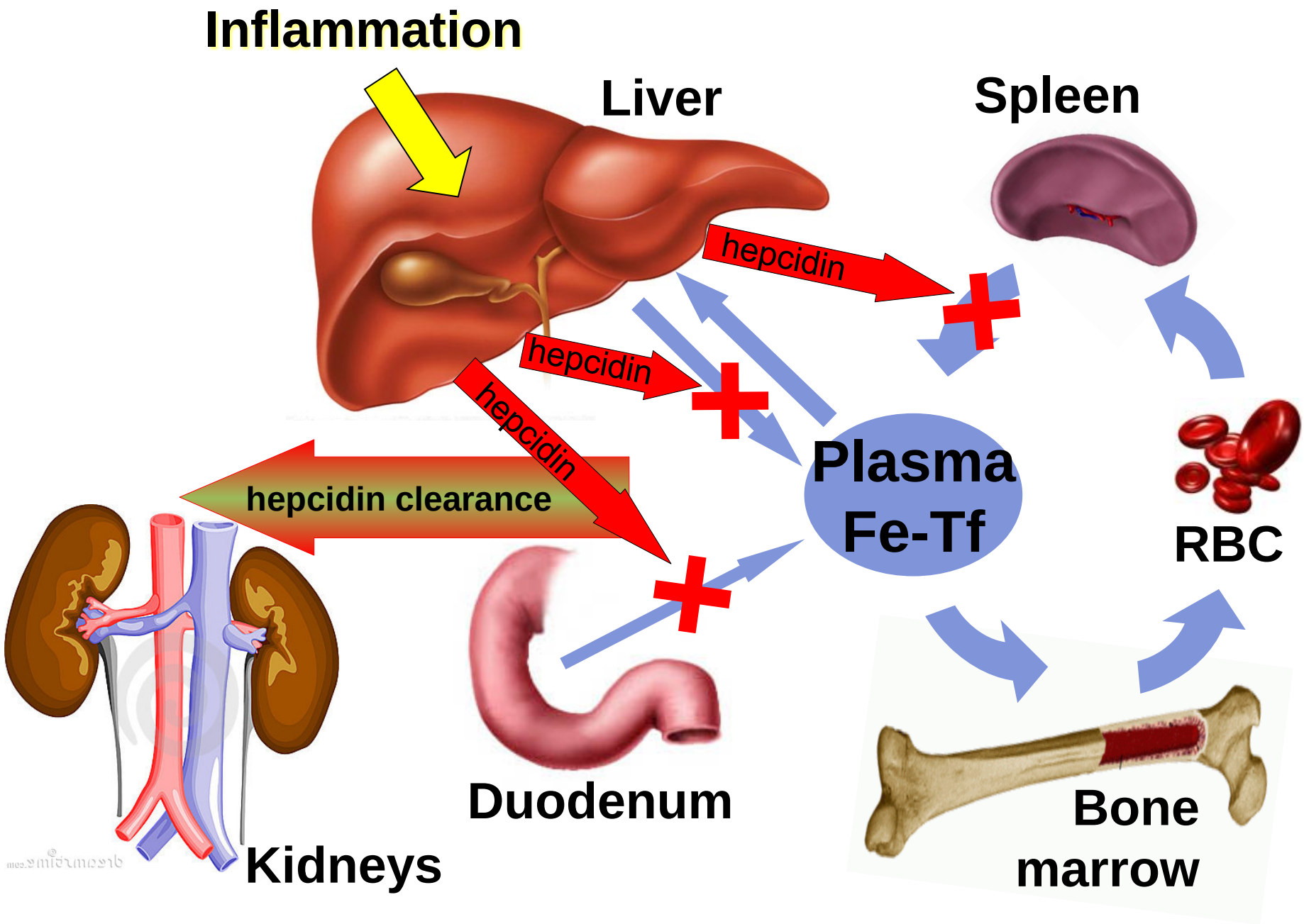
Signals regulating hepcidin



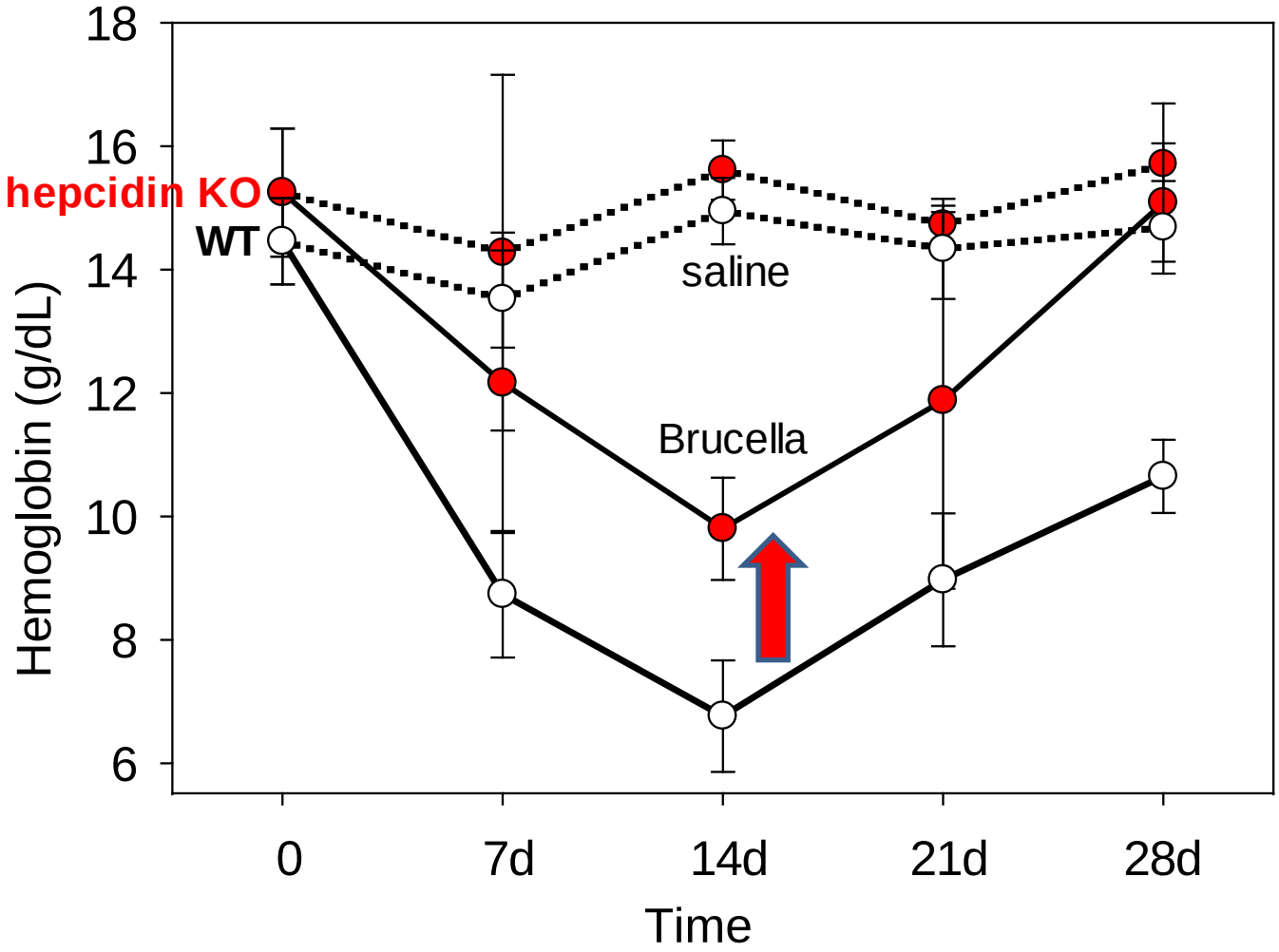
Hepcidin excess in anemia of inflammation

- **Hepcidin-dependent effects**
  - Inflammatory cytokines increase hepcidin and cause Fe trapping in macrophages
  - ↓Fe restricts hemoglobin synthesis and erythropoiesis is inhibited
- **Hepcidin-independent effects**
  - Shortened erythrocyte lifespan
  - Direct suppression of erythropoiesis by cytokines

Iron-restricted anemias

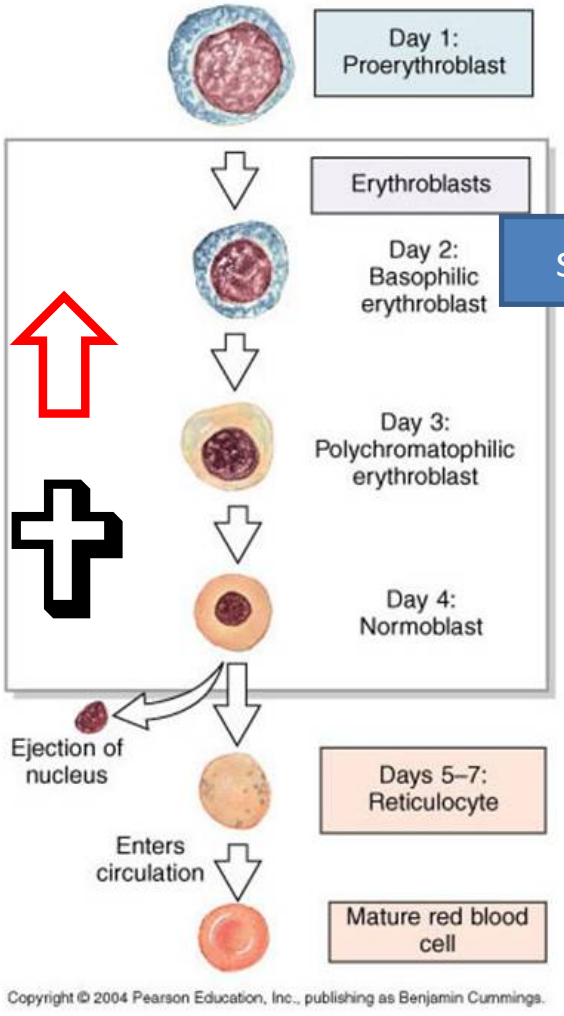


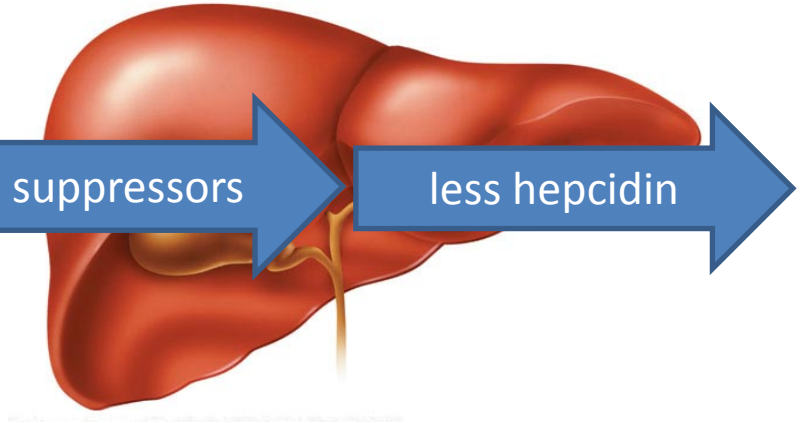
Ablation of hepcidin is beneficial in anemia of inflammation induced by heat-killed *Brucella abortus*



Kim et al. Blood 2014

Hepcidin deficiency in β-thalassemia





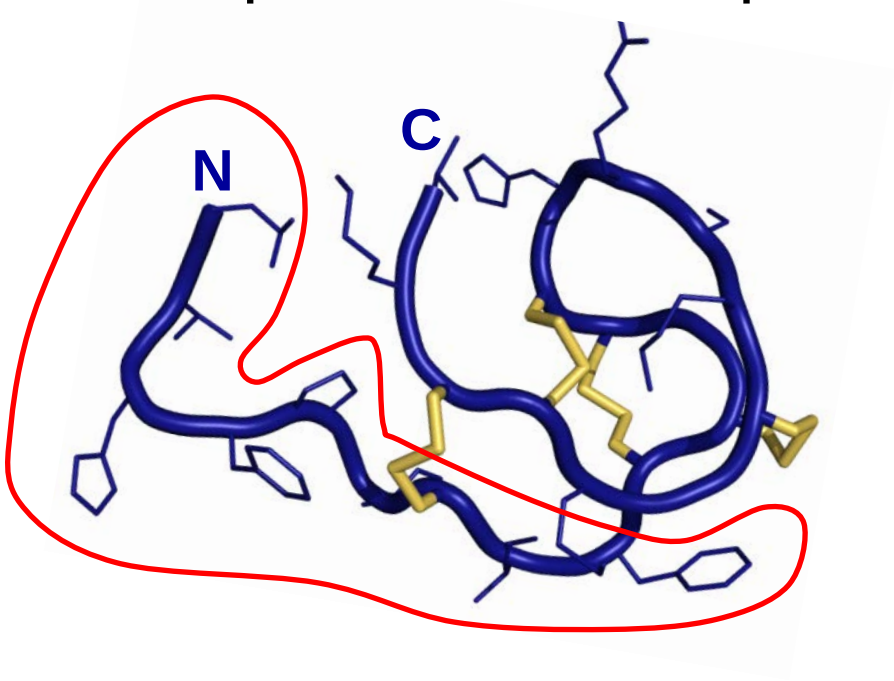
Fe overload
Worse anemia

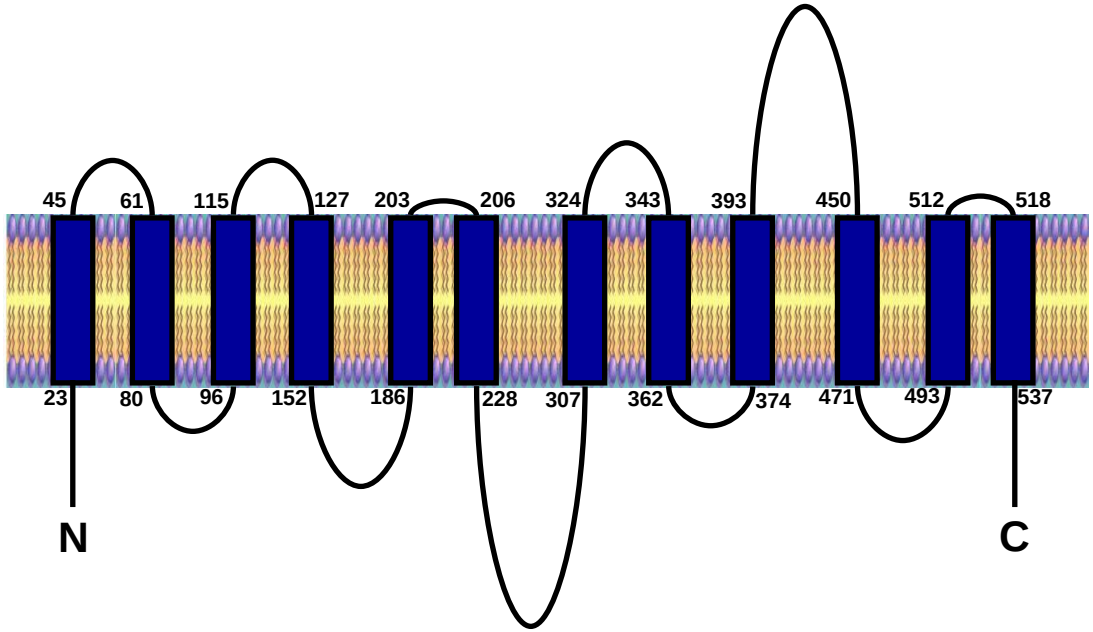
Anemia

- Failure of red cell precursors to mature into functional RBCs
- Anemia, increased erythropoietin
- Expansion of immature RBC precursors
- Hepcidin-suppressive signal causes hepcidin deficiency
- Increased Fe absorption, Fe overload

Rational design of peptide agonists

- Strategy for development: structure-function study of hepcidin and ferroportin





Hepcidin

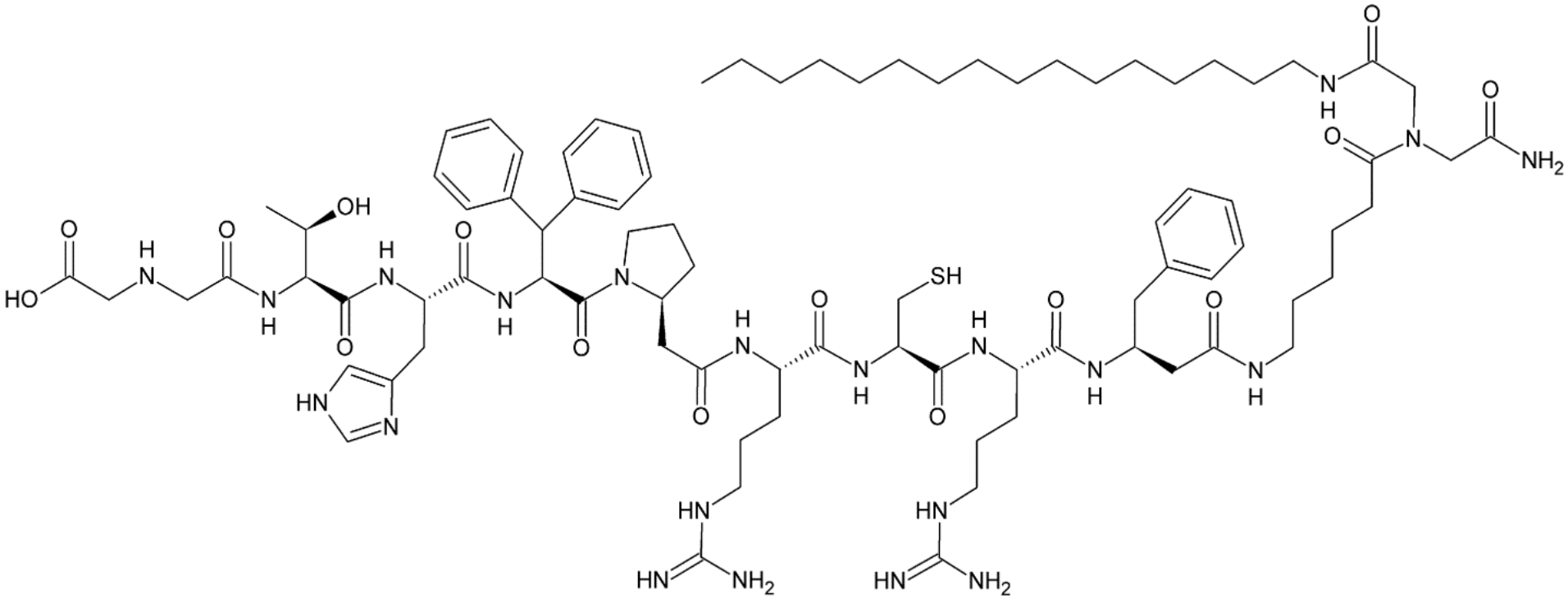
25 aa peptide hormone
4 disulfide bonds

Ferroportin

571 aa protein
12 transmembrane segments

Preza at al. JCI 2011, Ramos et al. Blood 2012

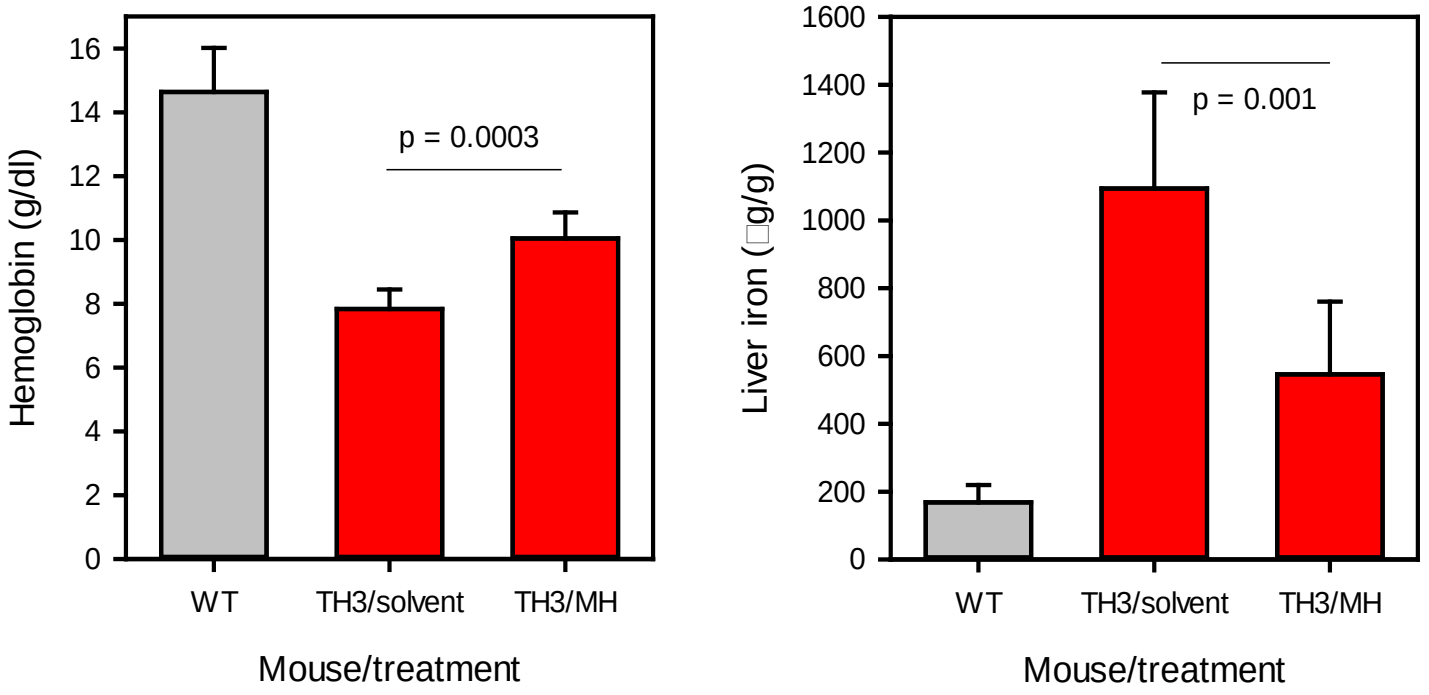
Minihepcidin PR73



Ida-Thr-His-Dpa-bhPro-Arg-Cys-Arg-bhPhe-Ahx-Ida(NHPal)-CONH2

iminodiacetic acid, L-threonine, L-histidine, L-3,3-diphenylalanine, L-β-homoproline, L-arginine, L-cysteine, L-arginine, L-β-homophenylalanine, 6-aminohexanoic acid, iminodiacetic acid palmitylamide (PEG-free)

Mouse model of β-thalassemia: minihepcidin improves anemia and decreases iron overload

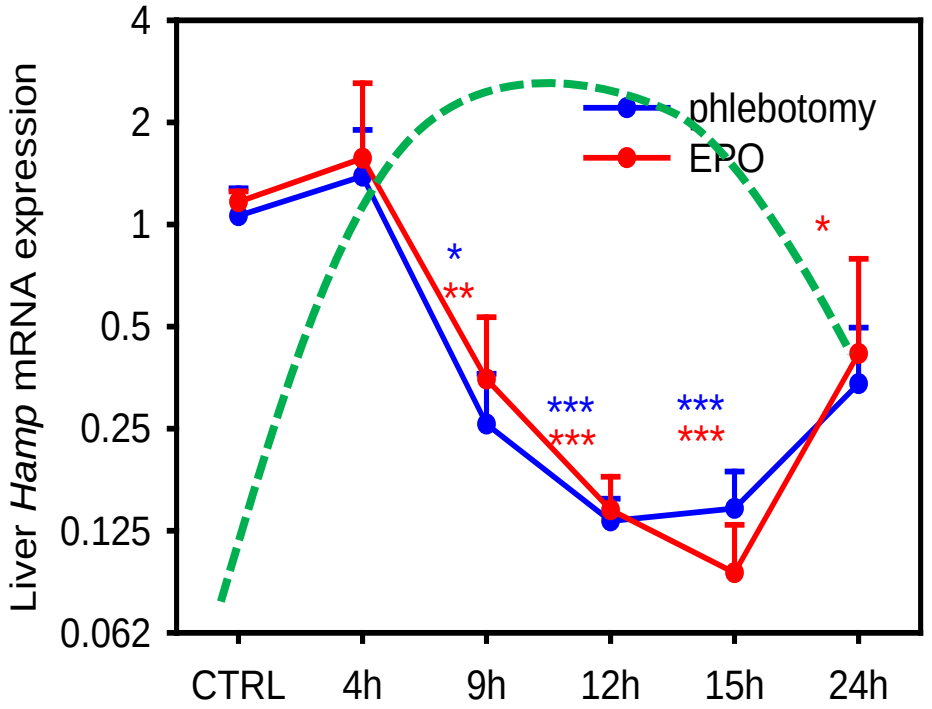


M004 (2.65 mg/kg) or solvent was administered 2x weekly SQ x 6 wks

C Casu, S. Rivella et al. and Merganser Biotech

A search for erythroid factor suppressing hepcidin

- Stimulated erythropoiesis in mice by phlebotomy (0.5 ml) or EPO injection (200 U)

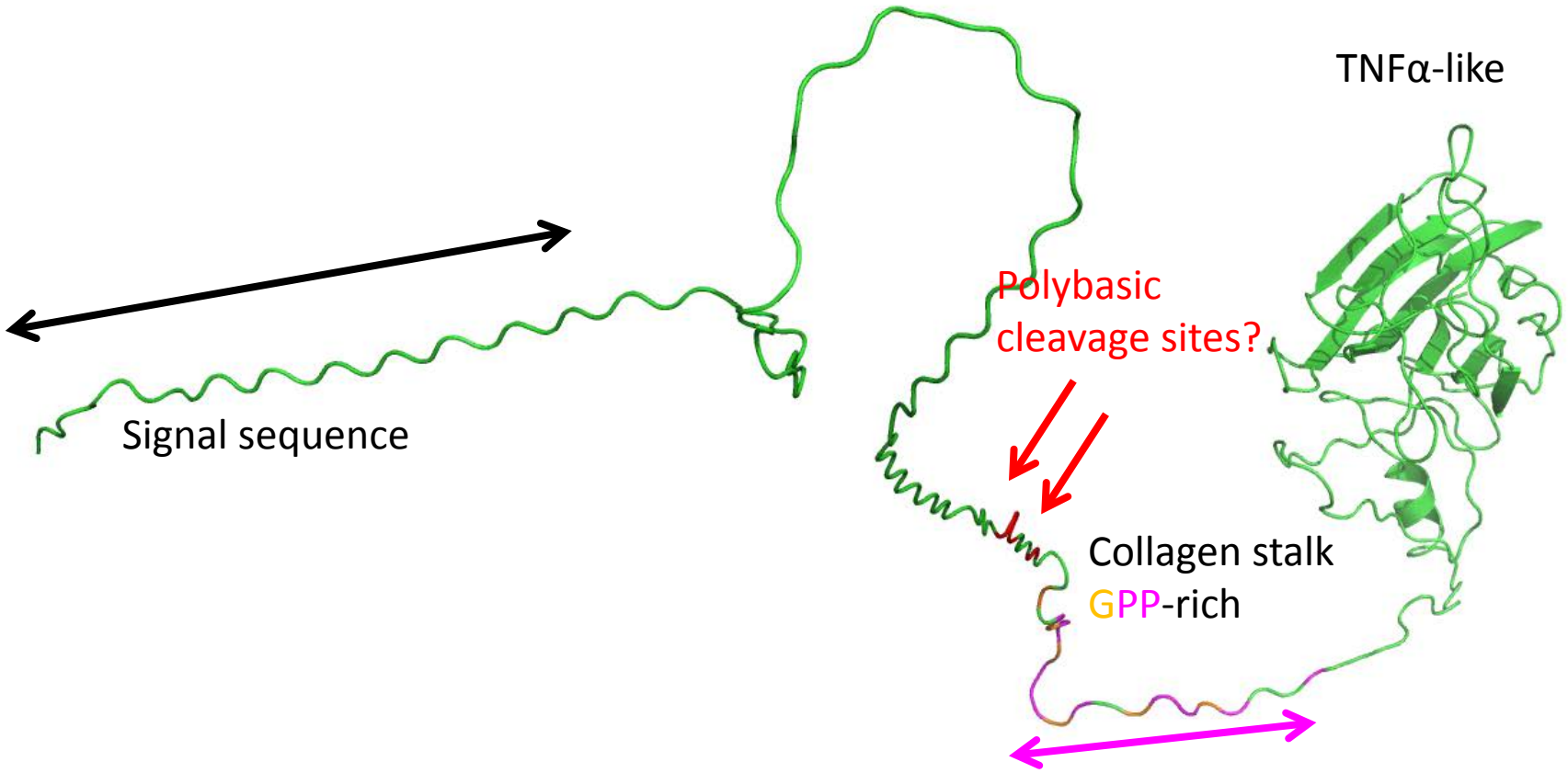


* p<0.05
** p<0.01
***p<0.001

- Gene chip expression profiling of the bone marrow to identify erythroid transcripts that 1) change before the hepcidin suppression and 2) encode secreted proteins

Erythroferrone (Erfe)

Erythroferrone (Fam132b, CTRP15)

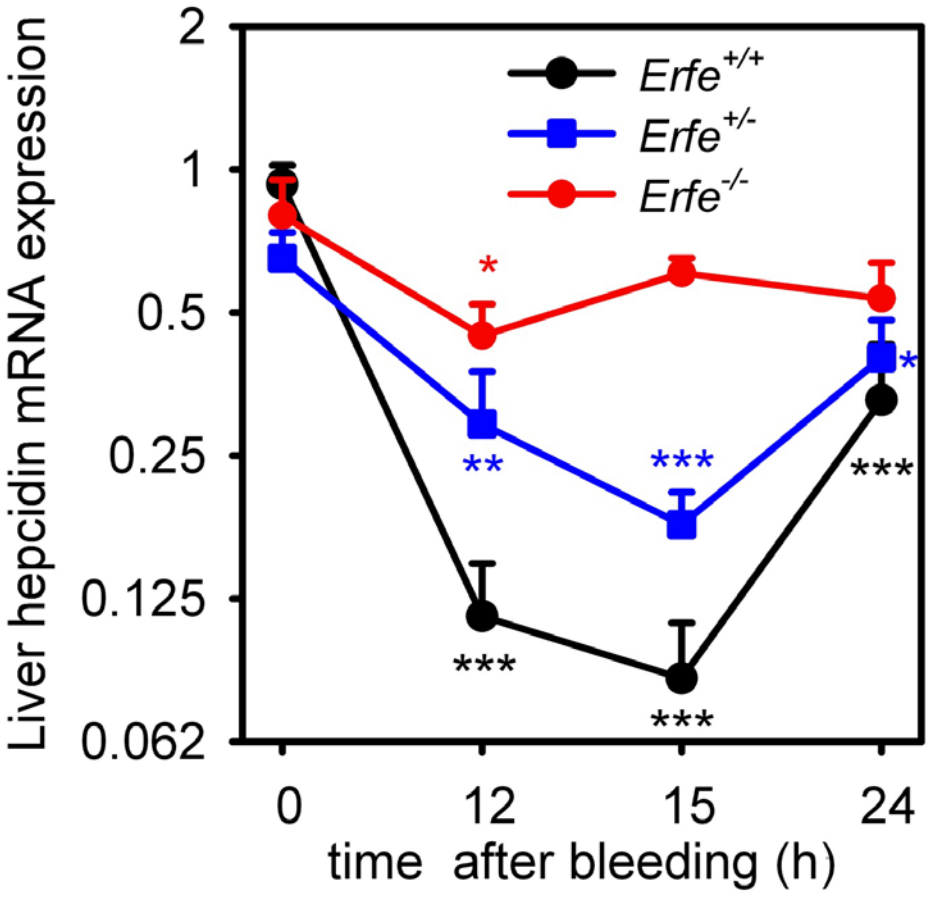


- 50 kD glycoprotein highly expressed in erythropoietin-stimulated erythroblasts

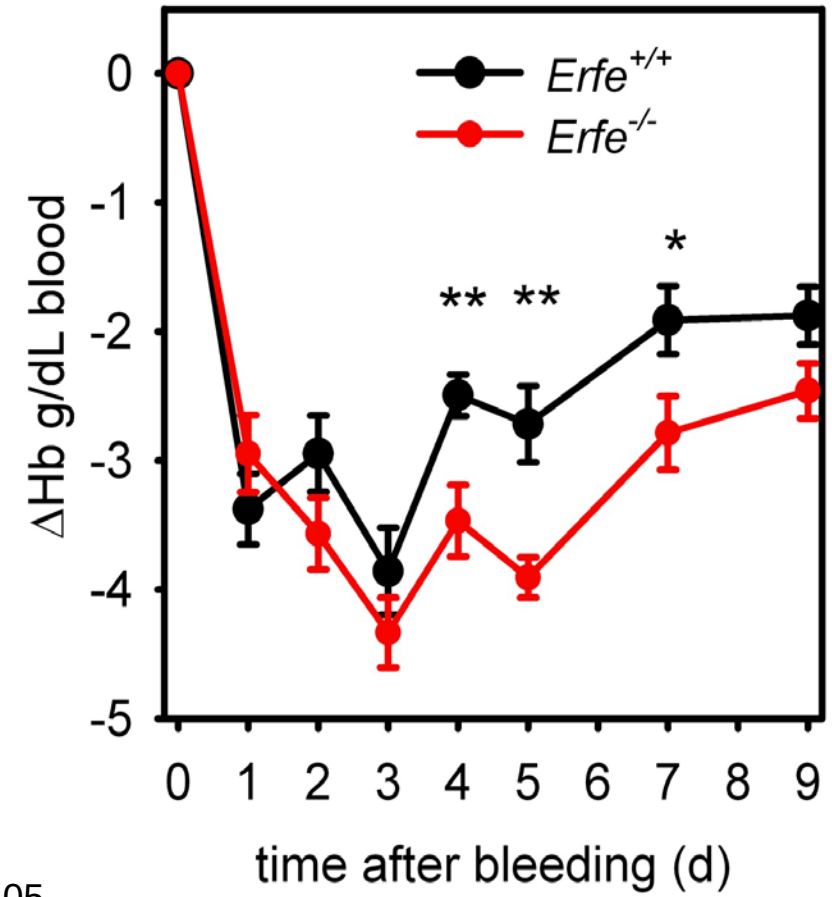
Erfe knockout mice do not suppress hepcidin in response to bleeding

- Erfe1$^{+/+}$, $^{+/-}$ and $^{-/-}$ mice were phlebotomized (0.5 ml)

Hepcidin mRNA

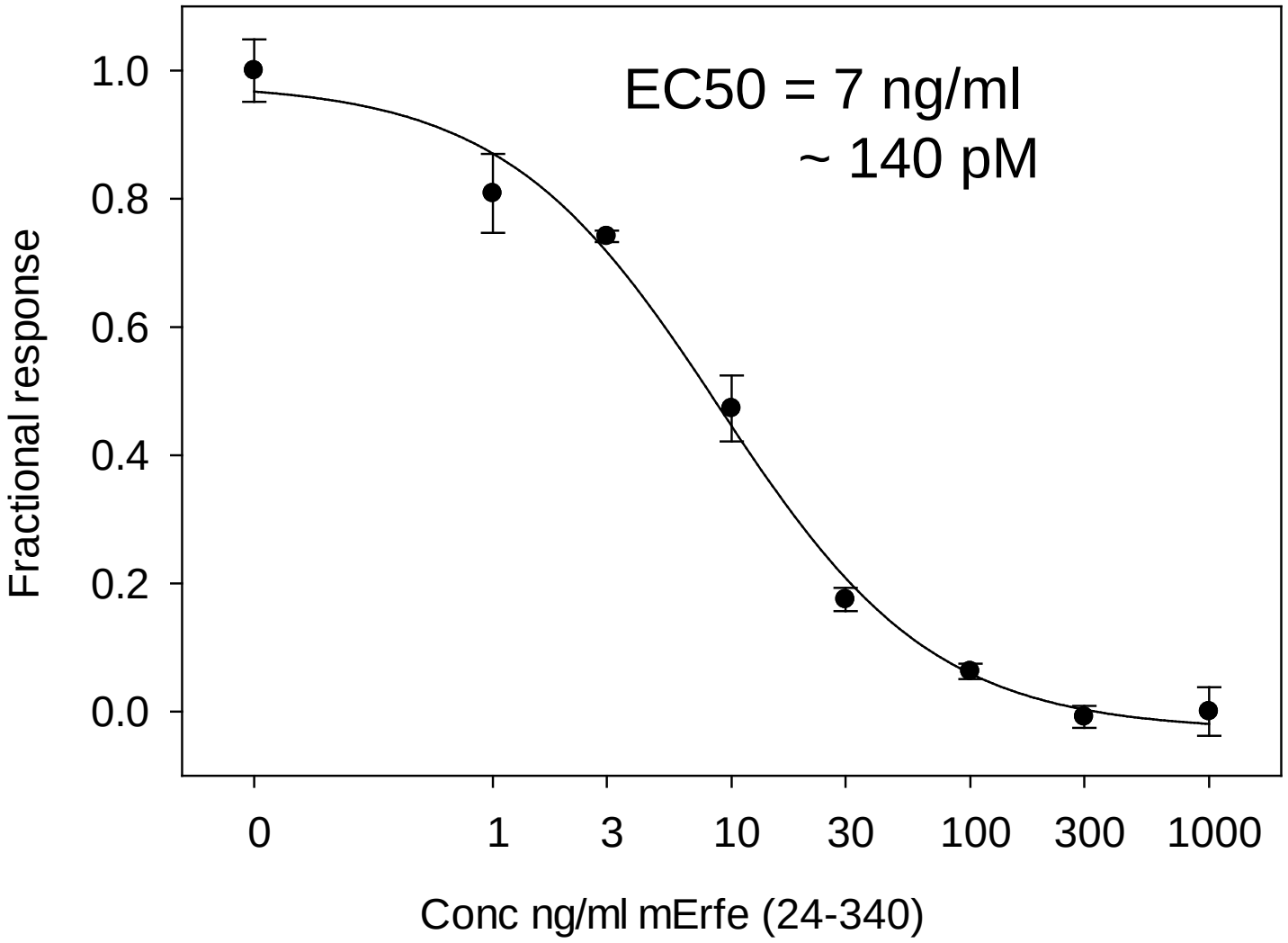


Hemoglobin recovery

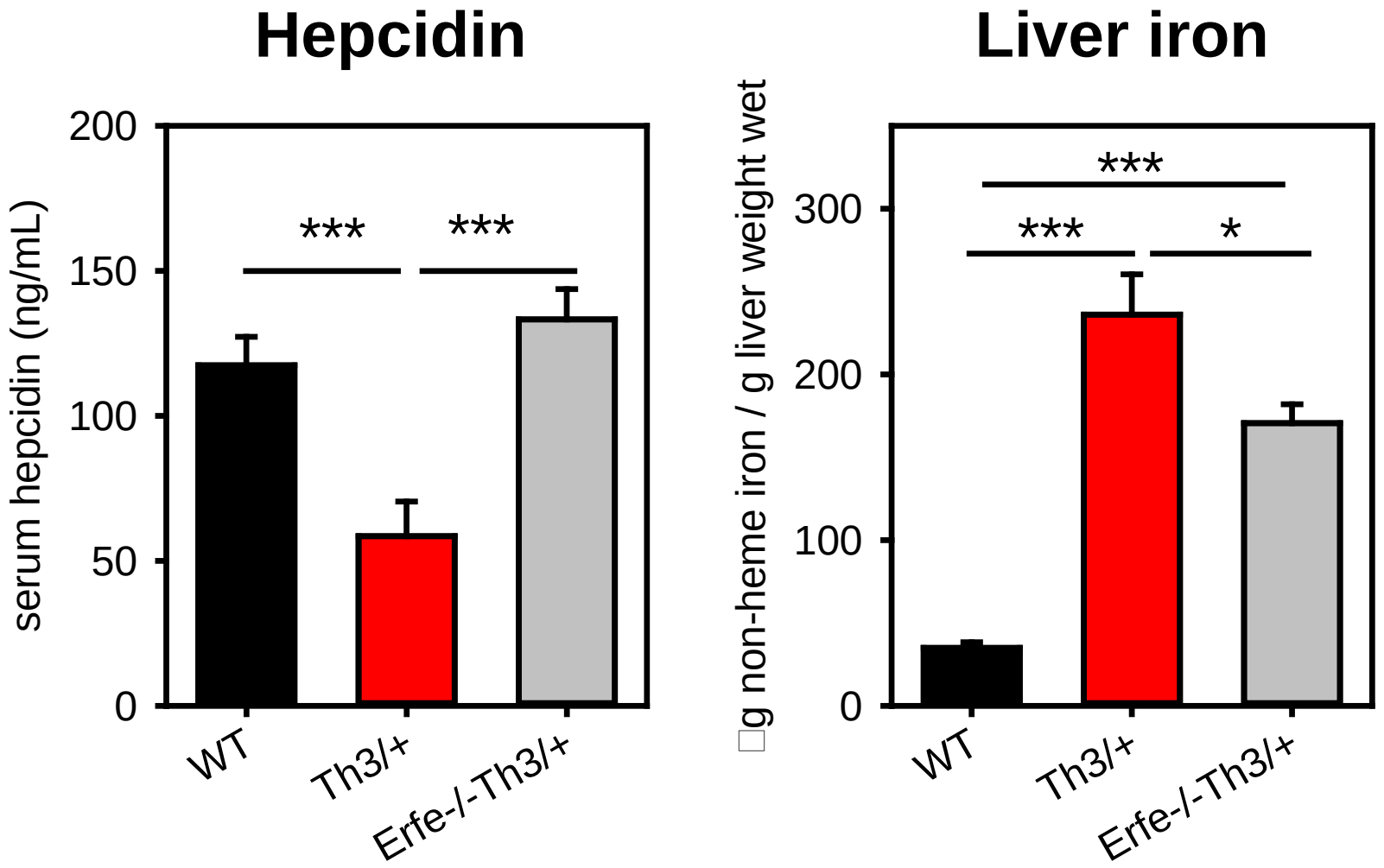


* p<0.05
** p<0.01
*** p<0.001

Dose-response for mouse erythroferrone on hepatocytes



Mouse model of β-thalassemia: Ablation of Erfe reverses hepcidin suppression and improves iron overload



Development of new treatments

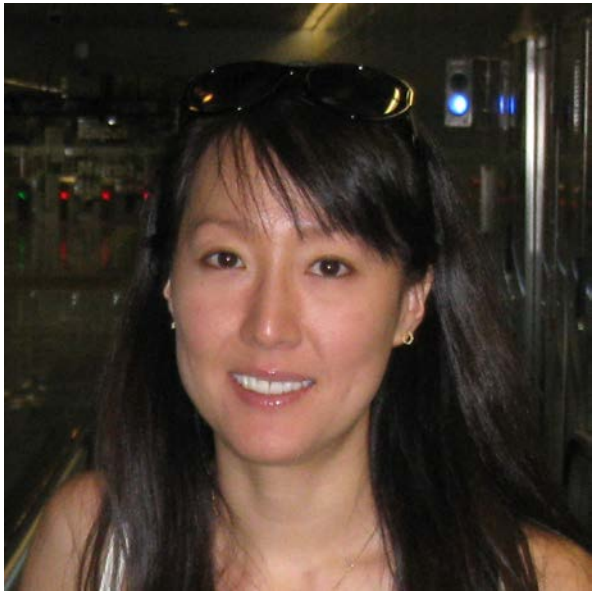
- Prevent iron overload and improve erythropoiesis in β-thalassemia
  - minihepcidins (hepcidin agonists)
  - erythroferrone antagonists
- Release sequestered iron in anemia of inflammation and anemia of chronic kidney disease
  - hepcidin antagonists
  - erythroferrone agonists
- UCLA licensed the intellectual property related to minihepcidin and erythroferrone to two startup companies for drug development

Key contributors

Ella Nemeth: Everything

Piotr Ruchala: Minihepcidin design

Airie Kim: Mouse models of anemia of inflammation



León Kautz: Erythroferrone