Supplemental Figure 1.

A

B

Heart

Pancreas

C

D

$T_{fc}$

$F_{pn}$
Supplemental Figure 1. Temporal deletion of liver hepcidin alters iron homeostasis and does not affect intestinal HIF-1α target genes or HIF-2α inflammatory targets. (A) qPCR analysis of transferrin receptor (Tfrc) and ferroportin (Fpn) in livers of HampΔLiv mice (n = 3 to 8 per group). (B) Representative Prussian blue iron stain and H&E analysis of hearts and pancreata from HampΔLiv mice. Images, 20x (n = 3 per group). (C) qPCR analysis for HIF-1α-specific transcripts in duodenal samples two-weeks after tamoxifen injection in Hampfl/fl and HampΔLiv mice (n = 5 to 8 per group). (D) qPCR analysis for HIF-2α-specific inflammatory transcripts in duodenal samples two-weeks after tamoxifen injection in Hampfl/fl and HampΔLiv mice (n = 5 to 8 per group). Male samples are designated as squares and female samples are designated as circles. Mean ± SEM are plotted. Significance determined using either one-way ANOVA with Tukey’s post hoc (A) or 2-tailed unpaired t test (C and D). *p < 0.05; **p < 0.01 compared to Hampfl/fl group.
Supplemental Figure 2. Inducible deletion of liver hepcidin does not affect HIF-2α-specific genes in the kidney and spleen. (A and B) qPCR analysis for HIF-2α-specific and iron handling genes in kidney (A) and spleen (B) samples of Hamp^{fl/fl} and Hamp^{ΔLiv} mice (n = 5 to 6 per group). Male samples are designated as squares and female samples are designated as circles. Mean ± SEM are plotted. Significance determined using 2-tailed unpaired t test. *p < 0.05; **p < 0.01 compared to Hamp^{fl/fl} group.
Supplemental Figure 3. The response of intestinal epithelial FPN to systemic iron deficiency does not activate intestinal HIF-1α or HIF-2α inflammatory targets. (A) Analysis of mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) (n = 4 to 7 per group). (B) qPCR analysis for HIF-1α-specific transcripts in duodenal samples (n = 4 to 6 per group). (C) qPCR analysis for HIF-2α-specific inflammatory transcripts in duodenal samples (n = 3 to 6 per group). Male samples are designated as squares and female samples are designated as circles. Mean ± SEM are plotted. Significance determined using two-way ANOVA with Tukey’s post hoc.
Supplemental Figure 4.

A

- **Pdk1**
  - Relative Expression
  - $Fpn^{fl/fl}$ vs $Fpn^{ΔIE}$

- **Pgk1**
  - Relative Expression
  - $Fpn^{fl/fl}$ vs $Fpn^{ΔIE}$

- **Hk1**
  - Relative Expression
  - $Fpn^{fl/fl}$ vs $Fpn^{ΔIE}$

B

- **Steap4**
  - Relative Expression
  - $Fpn^{fl/fl}$ vs $Fpn^{ΔIE}$

- **Cxcl1**
  - Relative Expression
  - $Fpn^{fl/fl}$ vs $Fpn^{ΔIE}$
Supplemental Figure 4. Intestinal epithelial FPN does not mediate activation of intestinal HIF-1α or HIF-2α inflammatory targets in response to systemic erythropoietic demand.

(A) qPCR analysis for HIF-1α-specific transcripts in duodenal samples (n = 5 to 6 per group).

(B) qPCR analysis for HIF-2α-specific inflammatory transcripts in duodenal samples (n = 5 to 6 per group). Male samples are designated as squares and female samples are designated as circles. Mean ± SEM are plotted. Significance determined using two-way ANOVA with Tukey’s post hoc.
Supplemental Figure 5.

A

B

- Ftn^ff\^f
- Ftn^de

- Dmt1^ff\^f
- Dmt1^de

Fth1
Actin
Supplemental Figure 5. Temporal deletion of FPN and DMT1 for three months both lead to iron-deficiency, microcytic and hypochromic anemia with differences in intestinal iron mobilization. (A) Analysis of mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) (n = 3 to 5 per group). (B) Western blot analysis for duodenal ferritin (FTH1) in Fpn^{fl/fl}, Fpn^{ΔIE}, Dmt1^{fl/fl}, and Dmt1^{ΔIE} mice (n = 2 per group). Male samples are designated as squares and female samples are designated as circles. Mean ± SEM are plotted. Significance determined using 2-tailed unpaired t test. ****p < 0.0001 compared between Fpn^{fl/fl} and Fpn^{ΔIE} cohorts. # # # #p < 0.0001 compared between Dmt1^{fl/fl} and Dmt1^{ΔIE} cohorts.
Supplemental Figure 6.

A

Vehicle  Dox

FPN  GAPDH

B

FPN

Rel. Densitometry

fi/fl  ΔLiv  Veh  Dox  Veh  Dox

Hamp  HEK293  IEC-6

C

Dead Cells (% Alive)

V  Dox  DFO

D

Cytosol  Nucleus

HIF-1α  GFP  GAPDH  Lamin A/C

E

Luciferase Activity (Beta Gal)

V  D  DFO  F

HIF-2α IRE

F

PHD Reporter Activity

Fold Change

V  D  V  D  V  D

Empty  NCOA4 sg1  NCOA4 sg2

G

HIF-1α

Absorbance

V  D  D+H  DFO
Supplemental Figure 6. Iron efflux through FPN is physiologically relevant and selective for HIF-2α. (A) Western blot analysis for FPN in FPN
GFP HEK293 cells treated with vehicle or 250 ng/mL doxycycline for 24-hours. (B) Relative densitometry to calculate fold induction of FPN protein in in vivo and in vitro models using data from Figure 1H, Figure 6I, and Supplemental Figure 6A. (C) Quantitation of dead cells using trypan blue following treatment of FPN
GFP HEK293 cells with doxycycline or 200 µM deferoxamine (DFO) for 48-hours. (D) Western blot analysis in cytosolic and nuclear fractions of FPN
GFP HEK293 cells treated with vehicle (V), doxycycline (D), or 100 µM FG4592 (FG) for 24-hours. (E) Relative luciferase activity in FPN
GFP HEK293 following treatment with vehicle (V), doxycycline (D), DFO, or 200 µM ferric ammonium citrate (FAC) (F). Fold change in luciferase activity in Empty, NCOA4 sg1, and NCOA4 sg2 FPN
GFP HEK293 cells infected with the PHD reporter and treated with doxycycline for 24-hours (G) ELISA in lysates from FPN
GFP IEC-6 cells treated with vehicle (V), doxycycline (D), doxycycline and 1mg/mL hepcidin (D+H), or DFO for 24-hours. Mean ± SEM are plotted. All cell culture experiments were repeated at least three times. Significance determined using one-way (C, E, G) or two-way (F) ANOVA with Tukey’s post hoc. **p < 0.01; ***p < 0.001; ****p < 0.0001 compared to vehicle. #p < 0.05 compared to doxycycline. $p <0.05 compared to Empty doxycycline. ^^^p<0.001 compared to NCOA4 sg1 doxycycline.
Supplemental Figure 7.

A

Change in Body Weight

- Vehicle \(Hamp^{fl/fl}\)
- Vehicle \(Hamp^{ALiv}\)
- PT \(Hamp^{ALiv}\)

Body Weight (% of Day 1)

Day

B

MCV

- Veh \(Hamp^{fl/fl}\)
- Veh \(Hamp^{ALiv}\)
- PT \(Hamp^{ALiv}\)

MCH

- Veh \(Hamp^{fl/fl}\)
- Veh \(Hamp^{ALiv}\)
- PT \(Hamp^{ALiv}\)
Supplemental Figure 7. Administration of PT2385 does not affect body weight or red blood cell size. (A) Body weight measurements of mice during two-week administration of either vehicle or PT2385 (n = 5 to 7 per group). (B) Analysis of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) (n = 5 to 7 per group). Male samples are designated as squares and female samples are designated as circles. Mean ± SEM are plotted. Significance determined using one-way ANOVA with Tukey’s post hoc.