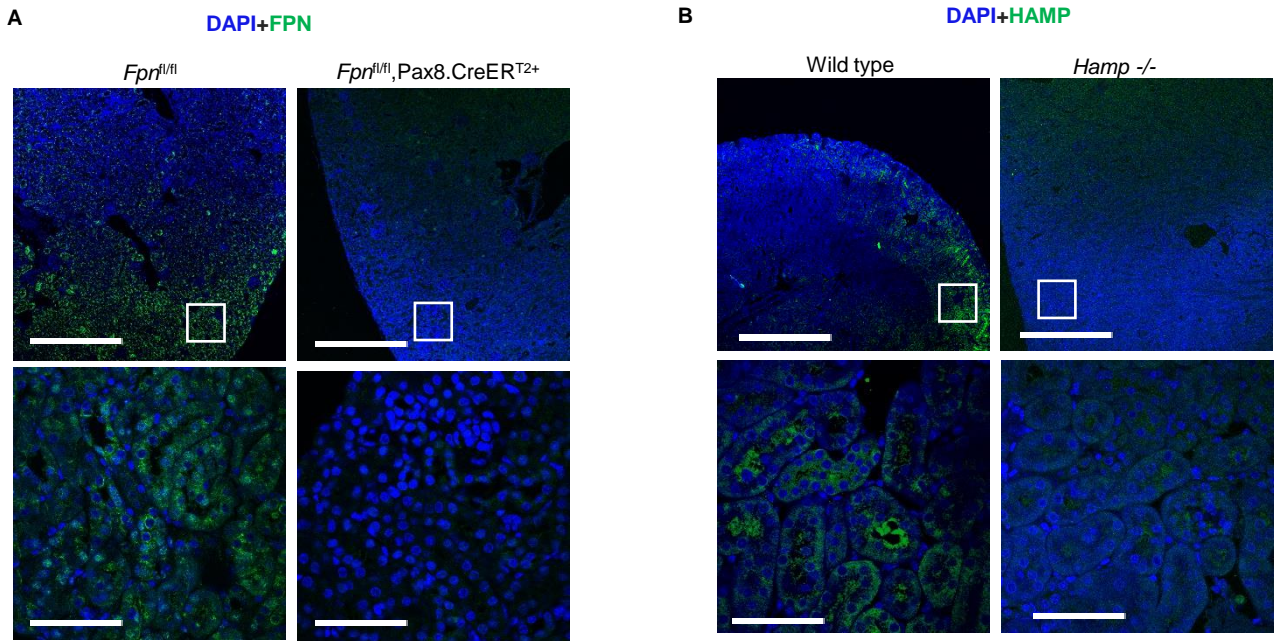


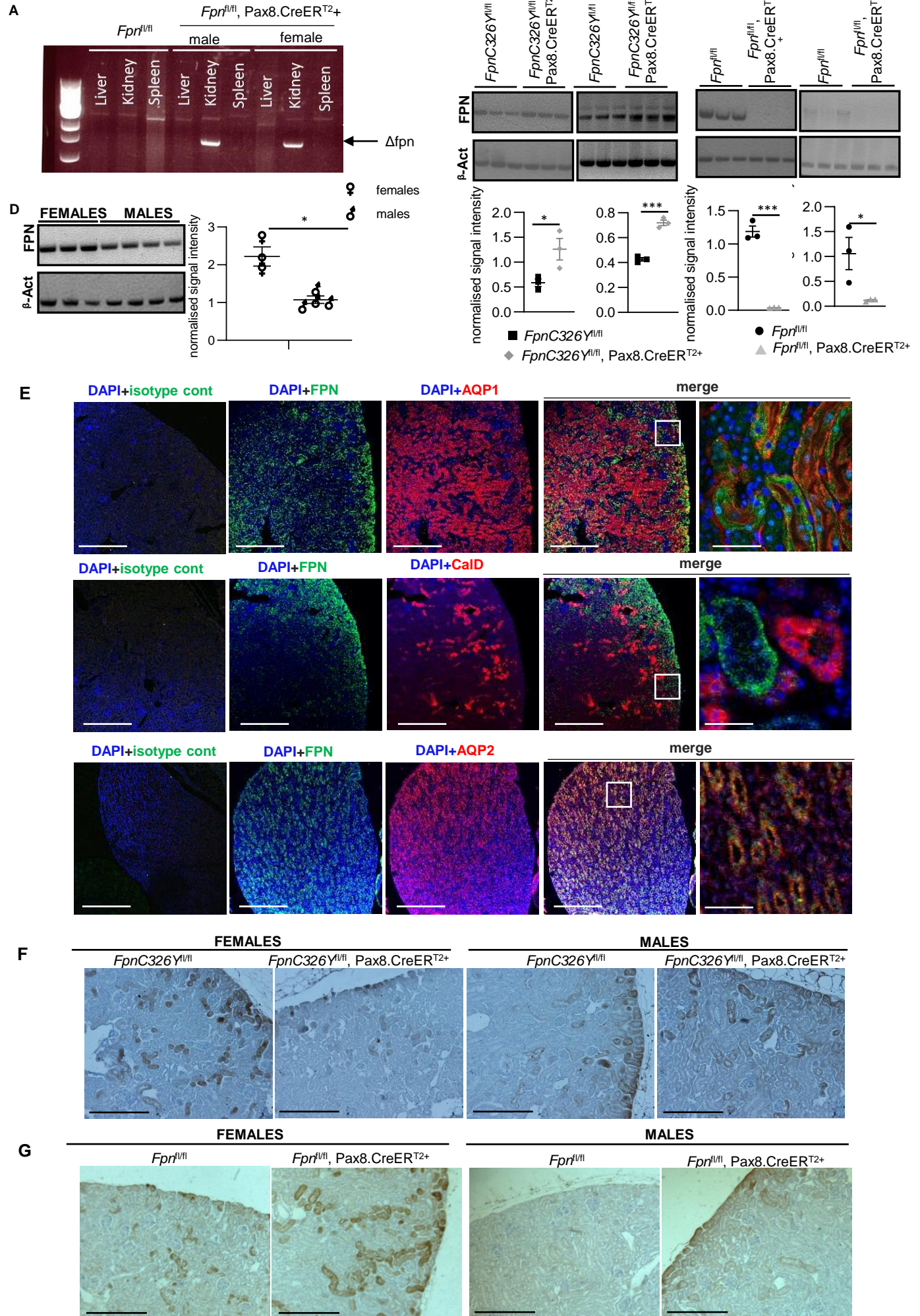
Supplemental Figure 1



Supplemental figure 1-Confirmation of FPN and HAMP antibody specificity

- A. Representative images of FPN immuno-fluorescent staining in the renal cortex of *Fpn^{fl/fl}, Pax8.CreER^{T2+}* animals and *Fpn^{fl/fl}* controls at 3 months of age. Top panels, scale bar=200 μ m, original magnification x10. Lower panels, scale bar=25 μ m, original magnification x60.
- B. Representative images of HAMP immuno-fluorescent staining in the renal cortex of *Hamp^{-/-}* animals and wild type controls at 3 months of age. Top panels, scale bar=200 μ m, original magnification x10. Lower panels, scale bar=25 μ m, original magnification x60.

Supplemental Figure 2

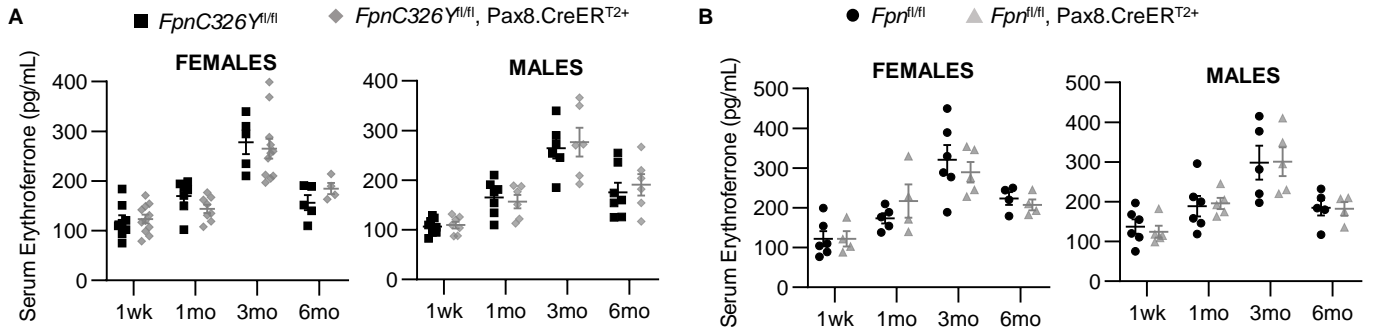


Supplemental Figure 2- Confirmation of Pax8.CreER^{T2+} activity and specificity

- A. Genotyping of *Fpn^{fl/fl}* and *Fpn^{fl/fl}, Pax8.CreER^{T2+}* mice using genomic DNA extracted from the liver, spleen and kidney at 1 week post tamoxifen induction.
 - B. Western Blot for FPN in kidneys of female and male *FpnC326Y^{fl/fl}, Pax8.CreER^{T2+}* mice and *FpnC326Y^{fl/fl}* controls at 3 months post-tamoxifen treatment. Quantitation of signal intensity shown in bottom panel.
 - C. Western Blot for FPN in kidneys of female and male *Fpn^{fl/fl}, Pax8.CreER^{T2+}* mice and *Fpn^{fl/fl}* controls at 3 months post-tamoxifen treatment. Quantitation of signal intensity shown in bottom panel.
 - D. Western Blot for FPN in kidneys of wild type females and male littermates at 3 months of age. Quantitation of signal intensity shown in bottom panel.
- E-G. Larger versions of panels Fig1A, 1G and 1E respectively.

Values are shown as mean± standard error of the mean. *p<0.05, ***p<0.001.

Supplemental Figure 3

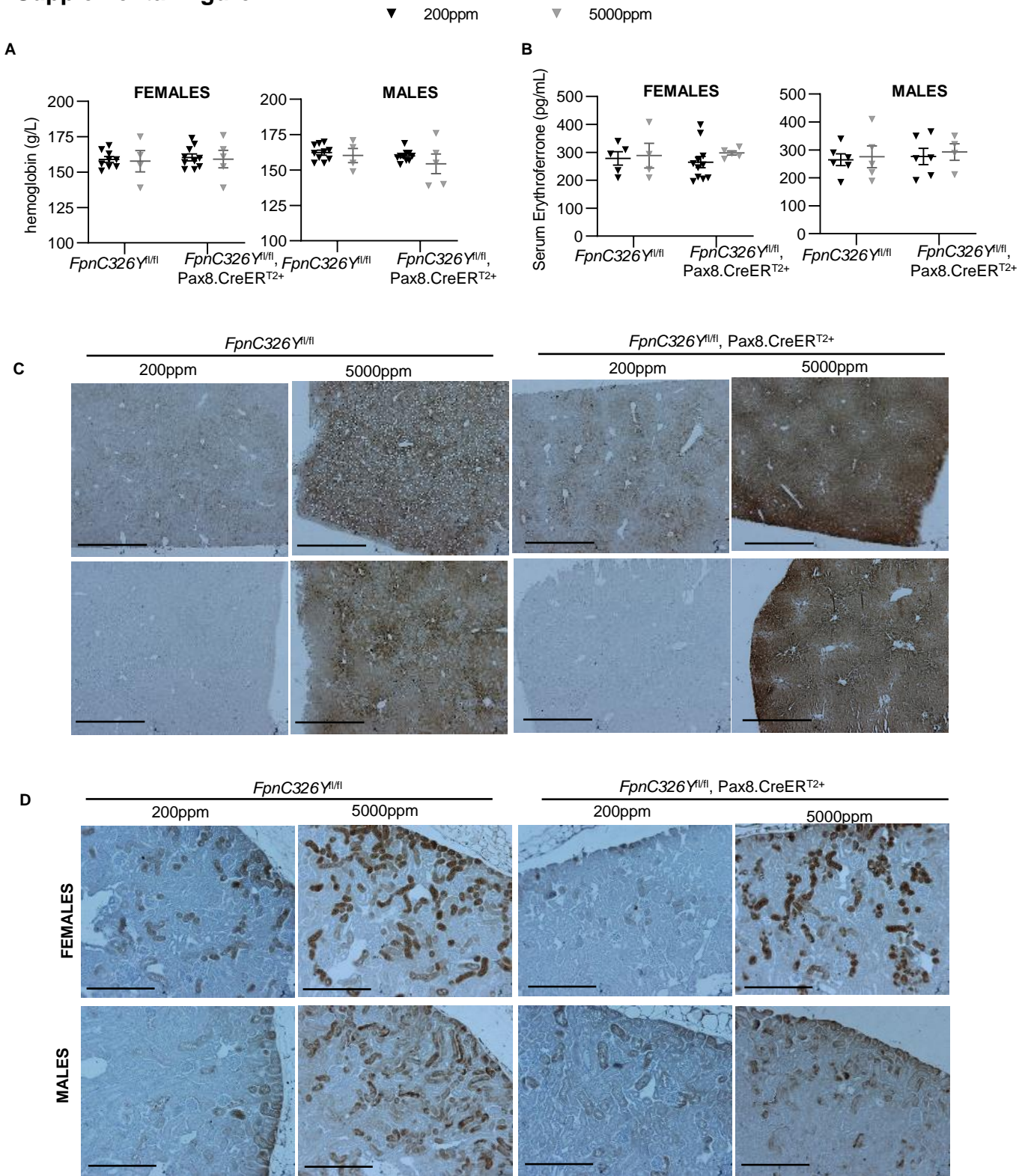


Supplemental figure 3- Serum erythroferrone levels are not altered in *FpnC326Y^{fl/fl}, Pax8.CreER^{T2+}* and *Fpn^{fl/fl}, Pax8.CreER^{T2+}* mice.

A. Serum erythroferrone levels in female and male *FpnC326Y^{fl/fl}, Pax8.CreER^{T2+}* mice and *FpnC326Y^{fl/fl}* controls at 1 week, 1 month, 3 months and 6 months post-tamoxifen treatment.

B. Serum erythroferrone levels in female and male *Fpn^{fl/fl}, Pax8.CreER^{T2+}* mice and *Fpn^{fl/fl}* controls at 1 week, 1 month, 3 months and 6 months post-tamoxifen induction. Values are shown as mean ± standard error of the mean.

Supplemental Figure 4

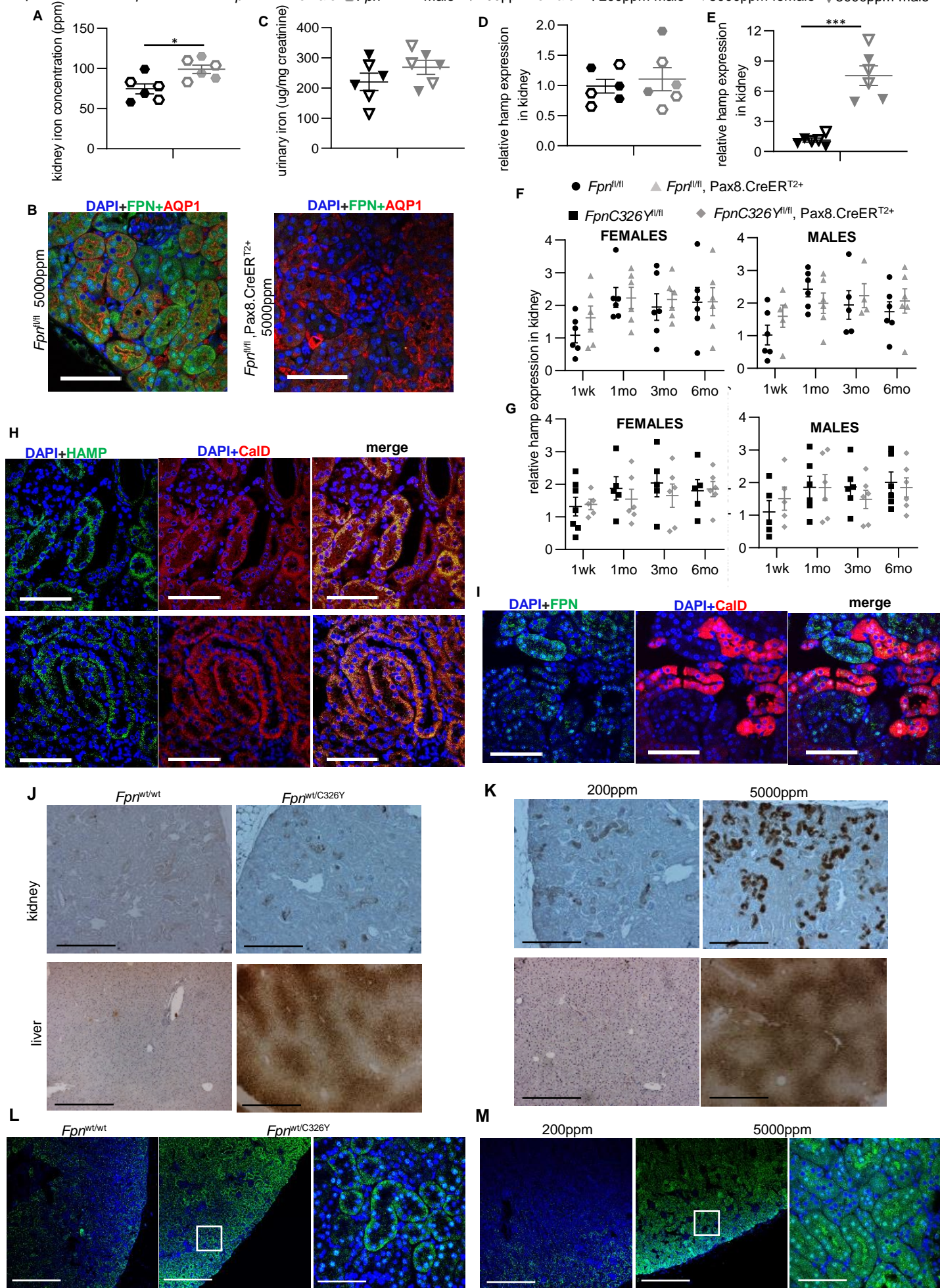


Supplemental figure 4- Haemoglobin and serum erythroferrone levels are not altered in *FpnC326Y^{fl/fl}, Pax8.CreER^{T2+}* fed an iron-loaded diet.

- A. Haemoglobin levels in female and male *FpnC326Y^{fl/fl}, Pax8.CreER^{T2+}* mice and *FpnC326Y^{fl/fl}* controls fed either a control diet (containing 200ppm iron) or an iron-loaded diet (containing 5000ppm iron) from weaning for 3 months.
- B. Serum erythroferrone levels in female and male *FpnC326Y^{fl/fl}, Pax8.CreER^{T2+}* mice and *FpnC326Y^{fl/fl}* controls fed either a control diet (containing 200ppm iron) or an iron-loaded diet (containing 5000ppm iron) from weaning for 3 months.
- Values are shown as mean ± standard error of the mean.
- C-D. Larger versions of panels Fig3H and 3I respectively.

Supplemental Figure 5

● *Fpn*^{wt/wt} female ○ *Fpn*^{wt/wt} male ● *Fpn*^{wt/C326Y} female ○ *Fpn*^{wt/C326Y} male ▼ 200ppm female ▼ 200ppm male ▼ 5000ppm female ▼ 5000ppm male

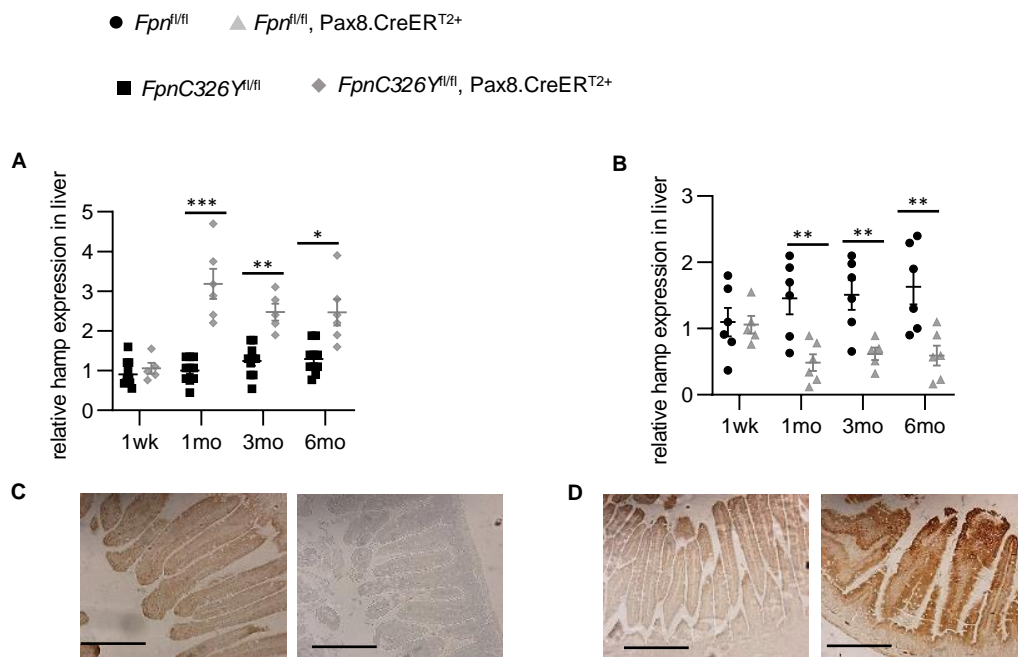


Supplemental figure 5- Renal iron handling and renal *hamp* expression in the different mouse models

- A. Renal iron levels in *Fpn*^{wt/C326Y} animals and *Fpn*^{wt/wt} controls at 6 months of age.
- B. Representative images of FPN and AQP1 immuno-fluorescent staining in the renal cortex of *Fpn*^{fl/fl}, Pax8.CreER^{T2+} animals and *Fpn*^{fl/fl} controls fed an iron-loaded diet containing 5000ppm from weaning for 3 months. Scale bar=25µm, original magnification x60.
- C. Urinary iron levels (normalised to urinary Creatinine) in wild type animals fed an iron-loaded diet containing 5000ppm iron, or a control diet containing 200ppm iron from weaning for 3 months.
- D. Renal *hamp* gene expression in *Fpn*^{wt/C326Y} animals and *Fpn*^{wt/wt} controls at 3 months of age.
- E. Renal *hamp* gene expression in wild type animals fed an iron-loaded diet containing 5000ppm iron, or a control diet containing 200ppm iron from weaning for 3 months.
- F. Renal *hamp* gene expression in female and male *Fpn*^{C326Y/fl/fl}, Pax8.CreER^{T2+} mice and *Fpn*^{C326Y/fl/fl} controls at 1 week, 1 month, 3 months and 6 months post-tamoxifen treatment.
- G. Renal *hamp* gene expression in female and male *Fpn*^{fl/fl}, Pax8.CreER^{T2+} mice and *Fpn*^{fl/fl} controls at 1 week, 1 month, 3 months and 6 months post-tamoxifen induction.
- H. Representative images of HAMP and CalD immuno-fluorescent staining in the renal cortex of wild type animals at 3 months of age. Scale bar=25µm, original magnification x60.
- I. Representative images of FPN and CalD immuno-fluorescent staining in the renal cortex of *Fpn*^{C326Y/fl/fl}, Pax8.CreER^{T2+} animals at 1 months of age. Scale bar=25µm, original magnification x60
- J-M. Larger versions of panels Fig4C, 4D, 4F and 4G respectively.

Values are shown as mean± standard error of the mean. *p<0.05.

Supplemental Figure 6



Supplemental figure 6- Liver *hamp* gene expression and gut FPN levels are altered in mice with renal tubule-specific knockout of *fpn* and in mice with renal tubule-specific knock-in of *fpnC326Y*.

- A. Relative expression of *hamp* mRNA in livers of female *FpnC326Y^{fl/fl}, Pax8.CreERT²⁺* mice and *FpnC326Y^{fl/fl}* controls at 1 week, 1 month, 3 months and 6 months post tamoxifen treatment.
- B. Relative expression of *hamp* mRNA in livers of female *Fpn^{fl/fl}, Pax8.CreERT²⁺* mice and *Fpn^{fl/fl}* controls at 1 week, 1 month, 3 months and 6 months post tamoxifen treatment.
- C. Representative images of FPN immuno-staining in the gut of female *FpnC326Y^{fl/fl}, Pax8.CreERT²⁺* mice and *FpnC326Y^{fl/fl}* controls 1 month post tamoxifen treatment. Scale bar=200µm, original magnification x10.
- D. Representative images of FPN immune-staining in the gut of female *Fpn^{fl/fl}, Pax8.CreERT²⁺* mice and *Fpn^{fl/fl}* controls 1 month post tamoxifen treatment. Scale bar=200µm, original magnification x10.

Values are shown as mean± standard error of the mean. *p<0.05, **p<0.01, ***p<0.001.