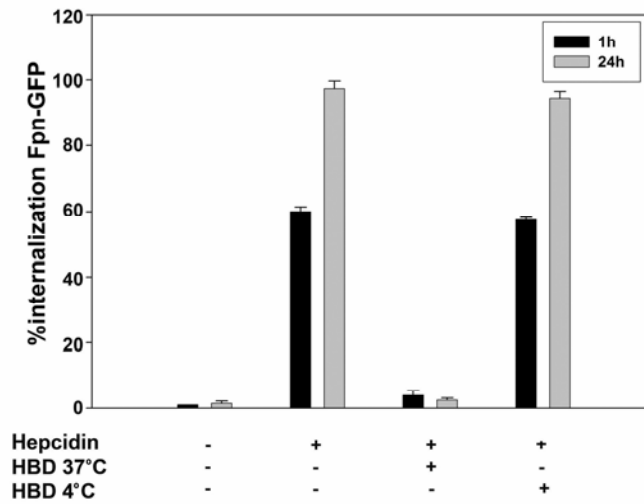


## The Hecpudin-Binding Site on Ferroportin Is Evolutionarily Conserved

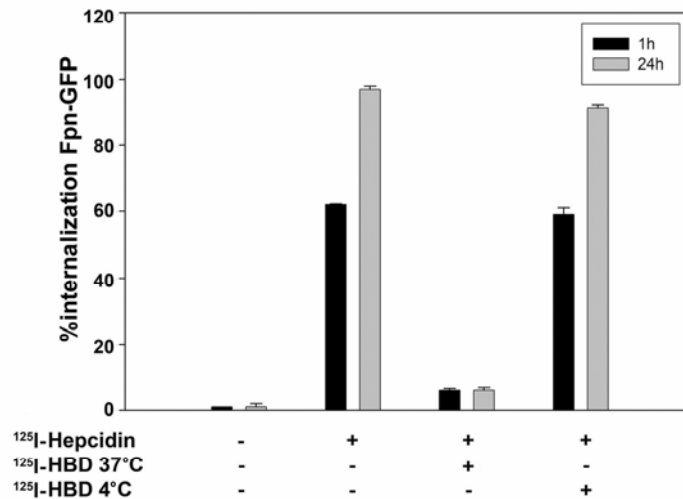
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### Supplemental Figures

**A**

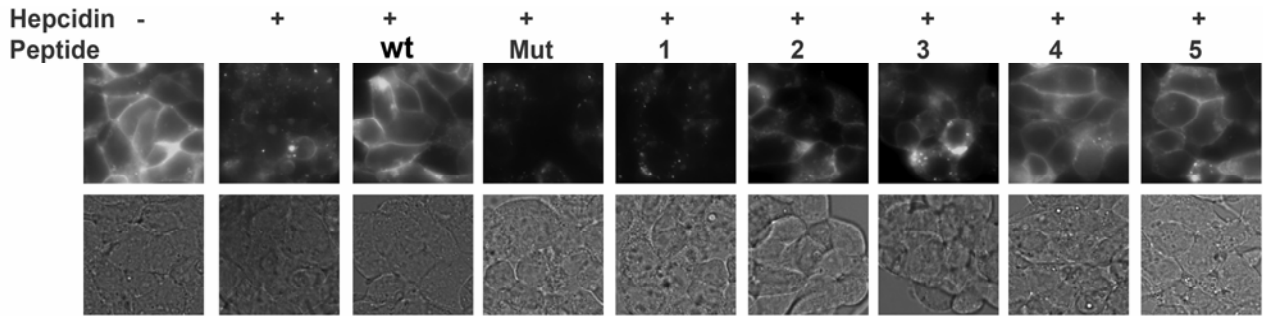
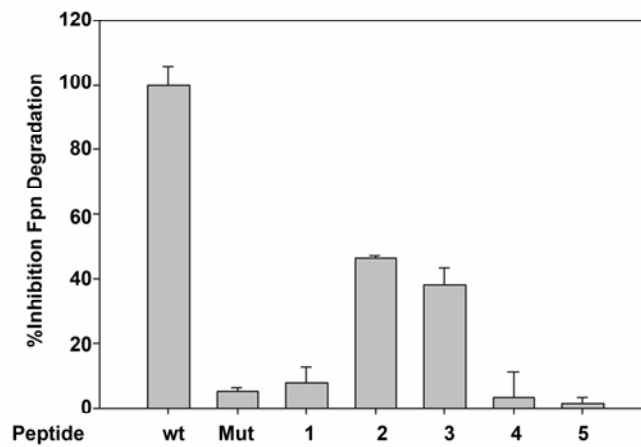


**B**



**Figure S1. Temperature dependent removal of internalization activity of hepcidin by the I-HBD.**

**A.** HEK293TFpn-GFP cells were incubated with hepcidin, hepcidin preincubated with HBD at 37°C or hepcidin preincubated with HBD at 4°C. Fpn-GFP internalization at one (**black bars**) and 24 (**grey bars**) hrs was quantified. **B.** <sup>125</sup>I-hepcidin was preincubated with HBD at 37°C or 4°C for one hr and then the mixture added to cells for one hr (**black bars**) or 24 hrs (**grey bars**). In the far right panel samples that had been incubated at 37°C were then shifted to 4°C for four hrs. The samples were then added to HEK293TFpn-GFP cells and the internalization of Fpn-GFP was determined.

**A****B**

**Figure S2. Amino acid substitutions in the HBD affect hepcidin-mediated Fpn-GFP internalization.**

**A.** HEK293TFpn-GFP cells were incubated with or without hepcidin (1  $\mu\text{g}/\text{ml}$ ) or with hepcidin that had been pre-incubated either with wild type (Wt) or mutant (Mut) HBD. The HBDs and hepcidin were mixed at an equimolar ratio for two hrs at 37°C and then added to cells for one hr. Fpn internalization at one hr was analyzed by epifluorescence. **B.** The data were quantified by counting 10 fields containing 20-30 cells/field and the number of cells with cell surface fluorescence was determined.