Hepatic hypoxia-inducible factor-2 down-regulates hepcidin expression in mice through an erythropoietin-mediated increase in erythropoiesis

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SUPPLEMENTARY APPENDIX

Online Supplementary Figure S1. Hepatocyte-specific deletion of HIF-2α (A) Recombination efficiency for Hif2a<sup>lox/lox</sup> alleles quantified by real-time polymerase chain reaction of genomic DNA isolated from the liver of Hif2a<sup>lox/lox</sup>Albumin Cre- (WT) and Hif2a<sup>lox/lox</sup>Albumin Cre+ (KO) mice. (B) HIF-2α mRNA levels in primary hepatocytes of WT and KO mice as determined by real-time polymerase chain reaction.

Online Supplementary Figure S2. Iron deficiency does not transcriptionally regulate furin or TMPRSS6

Hif2a<sup>lox/lox</sup>Albumin Cre- and Hif2a<sup>lox/lox</sup>Albumin Cre+ littermates were fed an iron deficient (-Fe diet) or control diet (Ctr) for 2 months after weaning. (A) BMP6, Furin and TMPRSS6 relative mRNA expression normalized to 18S mRNA. Results expressed as a fold-change compared with levels in Hif2a<sup>lox/lox</sup>Albumin Cre- mice on a control diet. n ≥ 4 for each group.
Online Supplementary Figure S4. Transcriptional regulation of hepcidin in primary hepatocytes. Incubation of primary hepatocytes with BMP2 (20 ng/mL) for 24 h, infected or not with a TMPRSS6 adenovirus (AV-Tmprss6) at a multiplicity of infection of 1. Hepcidin relative mRNA expression normalized to cyclophilin-A. n=3 per group.

Online Supplementary Figure S3. Transcriptional regulation of hepcidin, BMP6 and Hjv in the liver of Vhlh^lox/lox^ Albumin Cre+ and Vhlh^lox/lox^ Hif1a^lox/lox^Albumin Cre+ mice. Relative mRNA expression normalized to 18S mRNA in the liver of Vhlh^lox/lox^ Albumin Cre+ (KO grey bar) or Vhlh^lox/lox^ Hif1a^lox/lox^Albumin Cre+ (KO white bars) compared to Vhlh^lox/lox^ Albumin Cre- (dark gray bar) or Ctr (black bars). Ctr: n=9; KO: n=8.