SUPPLEMENTAL DATA

Figure S1. Characterization of *Vhl*$^{-/-}$ mice. (A) Left panel shows a schematic outline of the tamoxifen treatment schedule used to induce recombination. Arrows indicate on which days tamoxifen was injected. * indicates time point of analysis. Mutant mice were euthanatized for phenotyping on day 8. Right panel shows recombination analysis of the *Vhl* gene locus in control (Co) and *Vhl*$^{-/-}$ tissues by genomic PCR on day 8. 1-lox represents the recombined allele, 2-lox indicates the non-recombined conditional allele. (B) Complete blood counts were performed prior to tamoxifen injection on day 0 and on day 8. Shown are mean hematocrit (Hct), hemoglobin (Hb), rbc numbers, mean corpuscular volume (MCV) and reticulocyte counts (Retic) at day 0 and at day 8. (C) mRNA levels of *Dmt1* and *Trfc* in *Vhl*$^{-/-}$ livers. (D) Liver, kidney and spleen to body weight ratios in control and *Vhl*$^{-/-}$ mice at day 8 (n = 4 and 3 respectively). (E) Fraction (%) of CD71^{high}/Ter119^{high}-positive cells in bone marrow (BM) and spleen (n = 3 each). Shown are arithmetic mean values $\pm$ SEM, $* P < 0.05$; $** P< 0.01$ and $*** P< 0.001$ for comparisons of mutants to controls. ***Abb.*:** *Dmt1,* divalent metal transporter 1*; Trfc*, transferrin receptor 1.

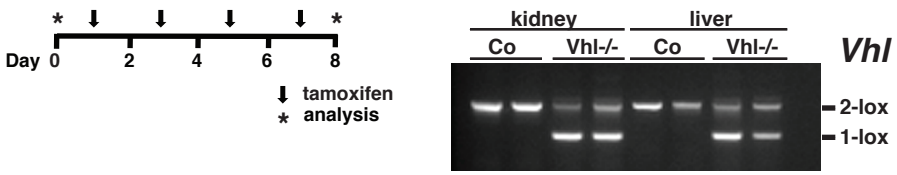
Figure S2. Characterization of *Vhl/Epo-/-* mice. (A) Left panel shows recombination analysis of the *Epo* gene locus in control (Co) and *Vhl/Epo*$^{-/-}$ kidneys and livers by genomic PCR on day 8. Right panel shows recombination analysis of the *Vhl* gene locus in the same mice. Shown are two representative control and mutant mice. 1-lox indicates

the recombined allele, 2-lox represents the non-recombined conditional allele. Table shows hematocrit (Hct), hemoglobin (Hb), rbc numbers, mean corpuscular volume (MCV) and reticulocyte counts (retic) at day 0 and day 8. (C) *Vegf* and *Dmt1* mRNA levels in control and *Vhl/Epo*$^{-/-}$ livers (n = 6 each). (D) Fraction (%) of CD71^{high}/Ter119^{high}-positive cells in bone marrow (BM) and spleen from control, *Vhl/Epo*$^{-/-}$ and *Vhl/Epo*$^{-/-}$ mice treated with recombinant human EPO (n = 10, 6 and 5 respectively). Shown are arithmetic mean values $\pm$ SEM, $* P < 0.05$; $** P < 0.01$ and $*** P < 0.001$ for comparisons of mutants to controls. ***Abb.:*** *Dmt1*, divalent metal transporter 1*;* rhEPO, human recombinant EPO; *Vhl/Epo*$^{-/-}$ (rhEPO), *Vhl/Epo* double mutant mice treated with recombinant human EPO.

Figure S3. Gdf15 suppresses hepcidin in Hep3B cells. (A) *Gdf15* mRNA levels in Ter119-positive (+) and Ter119-negative (-) spleen and bone marrow (BM)-derived cells from *Vhl*$^{-/-}$ and control mice (Co), enriched with immunomagnetic beads. While *Gdf15* mRNA levels were increased in *Vhl*-deficient Ter119-enriched splenic cell preparations compared to control, higher levels of *Gdf15* message were detected in splenic cells that did not bind to Ter119 magnetic beads. It is therefore possible that most of splenic Gdf15 is either of non-erythroid origin or is produced by Ter119^{low} erythroid progenitor cells that do not efficiently bind to Ter119 magnetic beads. (B) Shown are *Twsg1* mRNA levels in total spleen and BM cell isolates. Left panel, *Vhl*$^{-/-}$ mutants and *Cre*$^{-}$ littermate controls (n = 4 each); middle panel, *Vhl/Epo*$^{-/-}$ mice and *Cre*$^{-}$ littermate controls (n = 4 each); right panel, WT mice treated with recombinant human erythropoietin (rhEPO) or with vehicle (n = 3 and 4 respectively). (C) Real-time PCR analysis of *HAMP* levels in vehicle- or Gdf15-

treated (750 pg/ml) Hep3B cells (shown are the means of 3 independent experiments). (D) Real-time PCR analysis of *Tmprss6* and *furin* mRNA levels in $Vhl^{-/-}$ and control mice (n = 3 and 4 respectively). Shown are mean values ± SEM, ** $P< 0.01$ and *** $P< 0.001$ for comparisons of mutants to controls. ***Abb.:*** *Gdf15*, growth differentiation factor 15; *Tmprss6*, transmembrane protease serine 6 / matriptase-2; *Twsg1*, twisted gastrulation homolog 1.

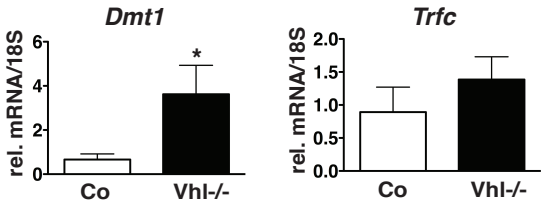
A



B

	day 0		day 8	
	Co (n=3)	Cre+ (n=3)	Co (n=3)	Vhl-/- (n=3)
Hct (%)	57.13 ± 0.5608	53.97 ± 1.146	54.93 ± 1.067	54.23 ± 0.1764
Hb (g/dL)	15.40 ± 0.3512	14.63 ± 0.2404	15.93 ± 0.6173	15.93 ± 0.1764
RBC (M/µL)	10.64 ± 0.1069	10.06 ± 0.2829	10.64 ± 0.1660	9.750 ± 0.02082
MCV (fL)	53.67 ± 0.08819	53.67 ± 0.3844	51.60 ± 0.2646	55.67 ± 0.08819 ***
Retic (%)	3.043 ± 0.03756	3.153 ± 0.1126	4.613 ± 0.1354	15.63 ± 1.786 **

C



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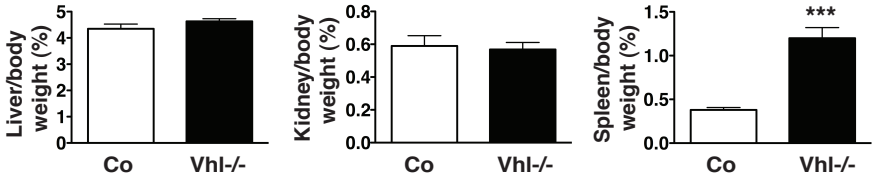

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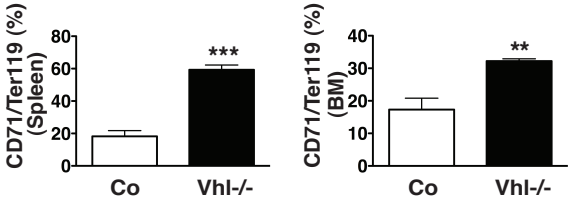

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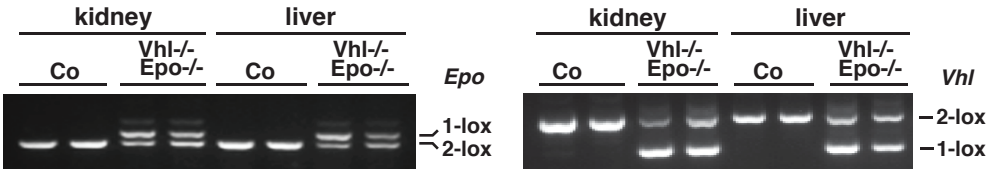
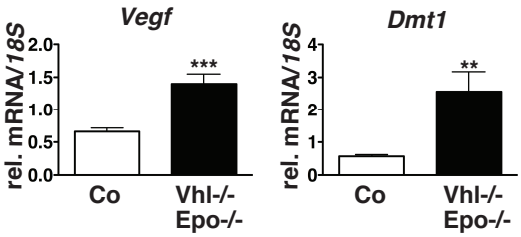


B

	day 0		day 8	
	Co (n=3)	Cre+ (n=5)	Co (n=3)	Vhl-/-, Epo-/- (n=5)
Hct (%)	45.40 ± 1.484	45.48 ± 1.476	42.93 ± 0.7688	35.38 ± 0.6320***
Hb (g/dL)	13.03 ± 0.2603	12.92 ± 0.4005	12.23 ± 0.4485	9.860 ± 0.1720***
RBC (M/µL)	9.343 ± 0.3075	9.302 ± 0.2656	9.060 ± 0.3005	7.326 ± 0.08465***
MCV (fL)	48.60 ± 0.4359	48.88 ± 0.6545	47.43 ± 0.8876	48.30 ± 0.6693
Retic (%)	2.843 ± 0.4222	2.946 ± 0.1053	4.813 ± 0.1785	5.576 ± 0.2675*

C



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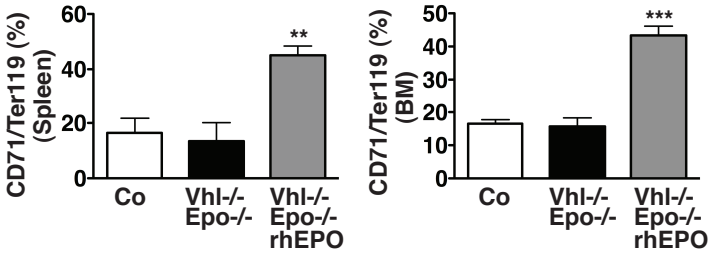

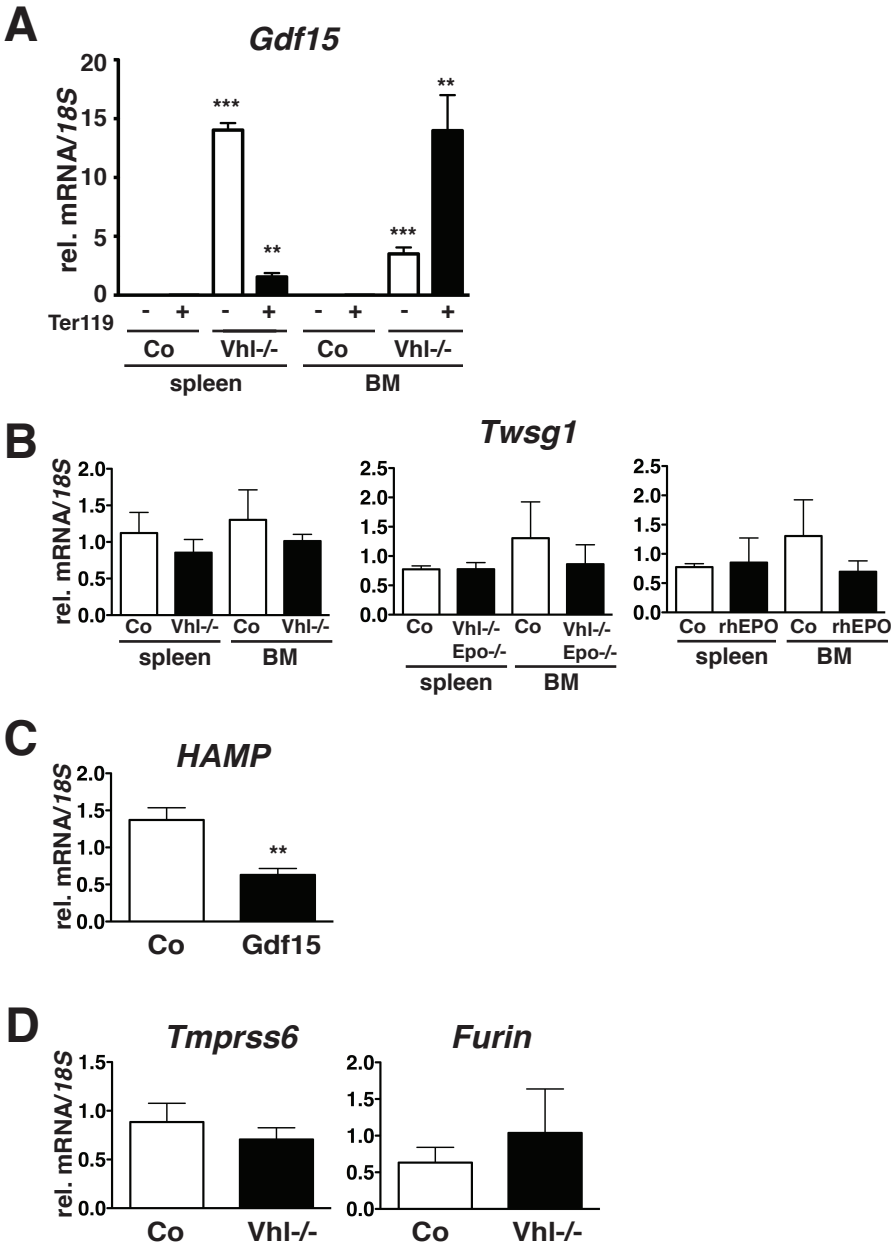
A
Gdf15
rel. mRNA/18S
20
15
10
5
0
***
**
***
**
Ter119
- + - + - + - +
Co
Vhl-/-
Co
Vhl-/-
spleen
BM
B
Twsg1
rel. mRNA/18S
2.0
1.5
1.0
0.5
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Co
Vhl-/-
Co
Vhl-/-
spleen
BM
2.5
2.0
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Co
Vhl-/- Epo-/-
Co
Vhl-/- Epo-/-
spleen
BM
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rhEPO
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HAMP
rel. mRNA/18S
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Co
Gdf15
D
Tmprss6
rel. mRNA/18S
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1.0
0.5
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Co
Vhl-/-
Furin
2.0
1.5
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Co
Vhl-/-