SUPPLEMENTAL INFORMATION

Regulation of iron homeostasis by hepatocyte TFR1 requires HFE and contributes to hepcidin suppression in β-thalassemia

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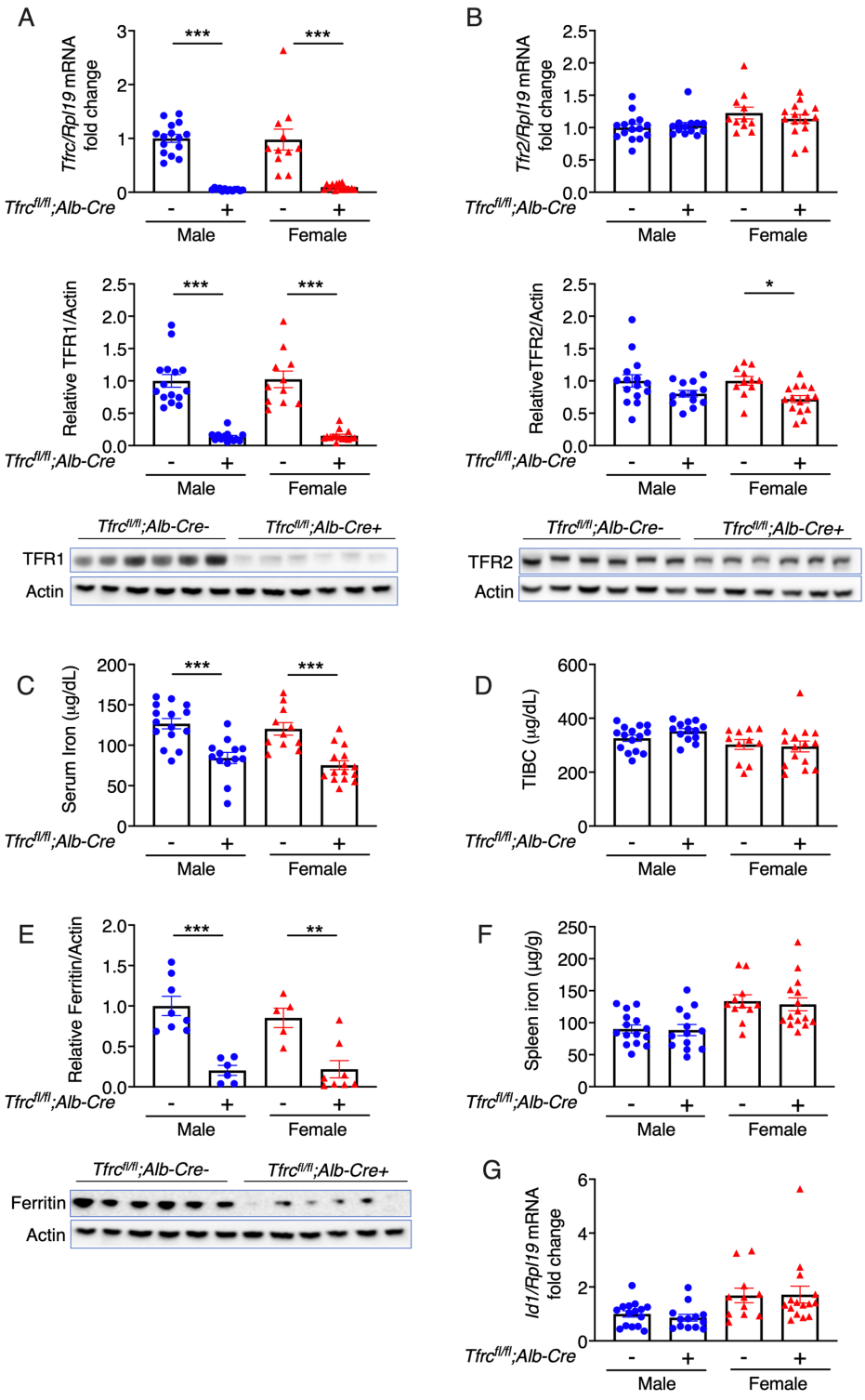
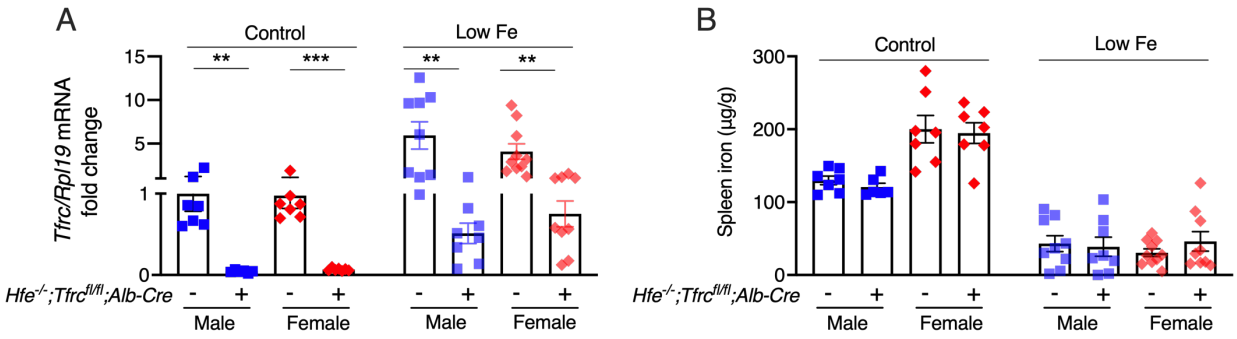
Supplemental Table S1: Primer list

Hamp F	agcccctcaaccccattatt
Hamp R	tcgtctttatttcaaggtcattgg
Rpl19 F	cccgtcagcagatcaggaa
Rpl19 R	gtcacaggcttgcggatga
Id1 F	gaacgtcctgctctacgacatg
Id1 R	tgggcaccagctccttga
Tfrc F	gctgagccagaatacatacac
Tfrc R	catctcgccagactttgctg
Tfr2 F	ccaagaaacccagagacctgtt
Tfr2 R	ccgagtcctgagtgggaaga
Erfe F	aatctgaccagcggccagta
Erfe R	ggcagcaagcgcatagaag
Bmp6 F	atggcaggactggatcattgc
Bmp6 R	ccatcacagtagttggcagcg
Gypa F	atggcagggattatcggaac
Gypa R	caccctcaggagattggatg

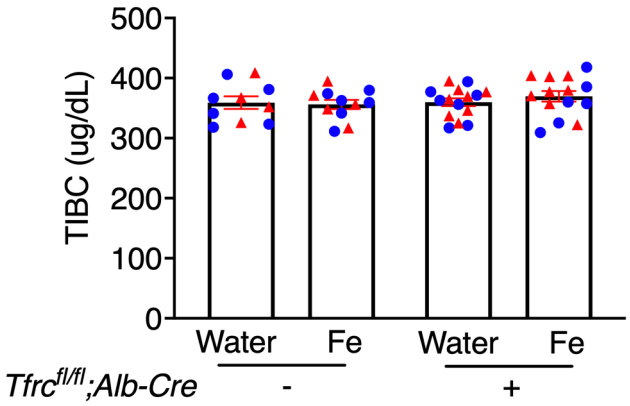


Supplemental Figure S1: *Tfrc*$^{fl/fl}$;*Alb-Cre*$^{+}$ mice exhibit hypoferremia, reduced liver ferritin, unchanged spleen iron, and unchanged *Id1* levels without compensatory upregulation of TFR2.

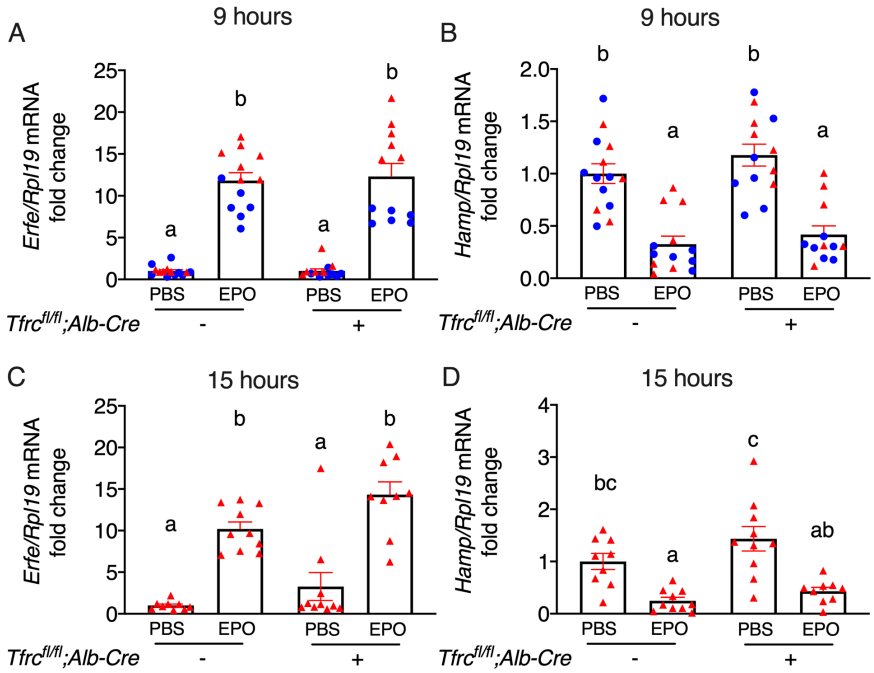
Eight-week-old male (blue) and female (red) *Tfrc*$^{fl/fl}$*;Alb-Cre*$^{+}$ mice and littermate controls (*Tfrc*$^{fl/fl}$*;Alb-Cre*$^{-}$) were analyzed for liver (A, upper panel) *Tfrc* and (B, upper panel) *Tfr2* relative to *Rpl19* mRNA by qRT-PCR. Livers were analyzed for (A, lower panels) TFR1 and (B, lower panels) TFR2 relative to total actin protein by immunoblot and chemiluminescence quantitation. Representative immunoblots are shown. (C) Serum iron and (D) total iron binding capacity (TIBC) were analyzed by colorimetric assays. (E) Livers were analyzed for ferritin relative to total actin protein by immunoblot and chemiluminescence quantitation. A representative immunoblot is shown. (F) Spleen iron levels were analyzed by colorimetric assay. (G) Liver *Id1* relative to *Rpl19* mRNA was analyzed by qRT-PCR. For panels A-B, E and G, the average of the control male mice was set to 1. For all graphs, individual data points are shown, and bars represent mean ± SEM. **P*<0.05, ***P*<0.01, *** *P*<0.001 relative to sex-matched *Tfrc*$^{fl/fl}$*;Alb-Cre*$^{-}$ mice by Student's *t*-test.



Supplemental Figure S2: Deletion of hepatocyte *Tfrc* in *Hfe* knockout mice reduces total liver *Tfrc* mRNA but does not alter spleen iron compared to *Hfe* single knockout mice. Four to five-week-old male (blue) and female (red) double global *Hfe* knockout; hepatocyte *Tfrc* knockout mice (*Hfe*$^{-/-}$;*Tfrc*$^{fl/fl}$;*Alb-Cre*$^{+}$) and littermate single *Hfe* knockout mice (*Hfe*$^{-/-}$;*Tfrc*$^{fl/fl}$;*Alb-Cre*$^{-}$) were either maintained on a standard diet (Control, ~380 ppm iron) or low iron diet (Low Fe, 2-6 ppm iron) for 3 weeks. (A) Total liver *Tfrc* relative to *Rpl19* mRNA was measured by qRT-PCR. The average of *Hfe*$^{-/-}$;*Tfrc*$^{fl/fl}$;*Alb-Cre*$^{-}$ male mice on the standard diet was set to 1. (B) Spleen iron levels were analyzed by colorimetric assay. Individual data points are shown, and bars represent mean ± SEM. ** P<0.01, *** P<0.001 for *Hfe*$^{-/-}$;*Tfrc*$^{fl/fl}$;*Alb-Cre*$^{+}$ mice relative to diet- and sex-matched *Hfe*$^{-/-}$;*Tfrc*$^{fl/fl}$;*Alb-Cre*$^{-}$ mice by Student's *t*-test.

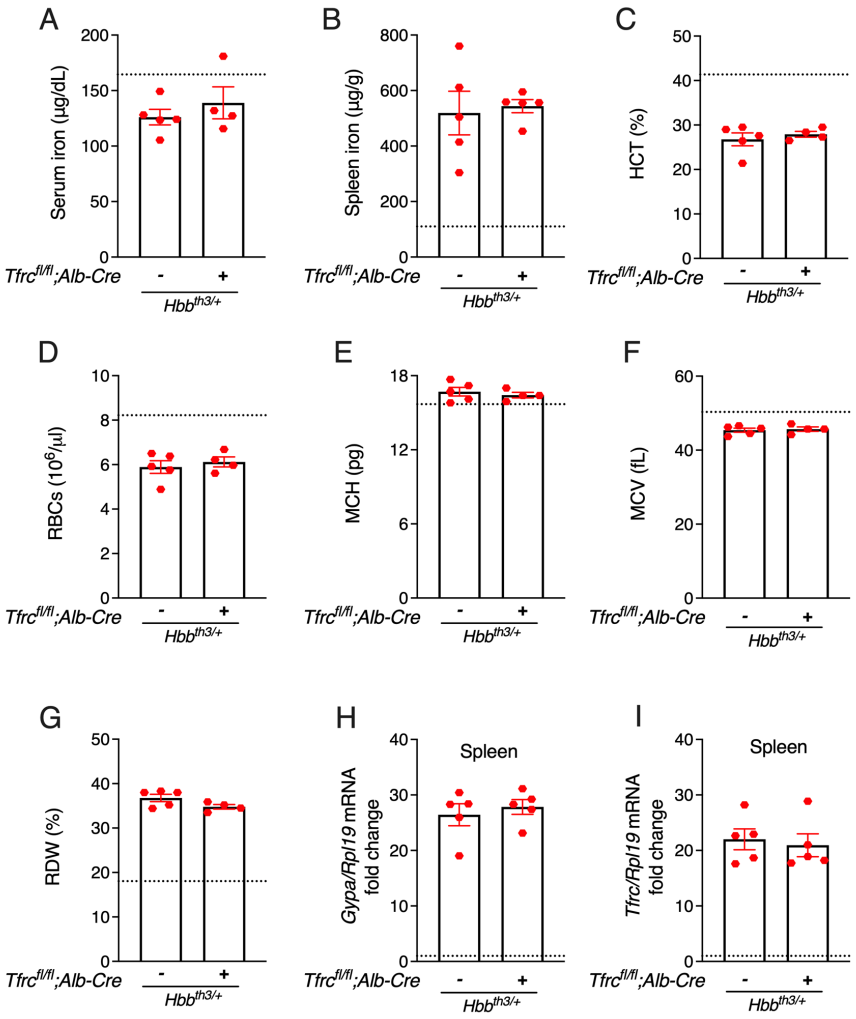


Supplemental Figure S3: Acute serum iron loading by oral iron gavage does not change total iron binding capacity in either *Tfrc*$^{fl/fl}$*;Alb-Cre*$^{+}$ or *Tfrc*$^{fl/fl}$*;Alb-Cre*$^{-}$ mice.
Seven-week-old male (blue) and female (red) *Tfrc*$^{fl/fl}$*;Alb-Cre*$^{+}$ mice and littermate controls (*Tfrc*$^{fl/fl}$*;Alb-Cre*$^{-}$) were maintained on a low Fe diet (2-6 ppm iron). After 12 days, mice were orally gavaged with 2 mg/kg elemental iron (as ferrous sulfate) or distilled water. After 5 hours, total iron binding capacity (TIBC) was analyzed by colorimetric assay. Individual data points are shown, and bars represent mean ± SEM. No significant differences were observed among groups by two-way ANOVA with Tukey's post-hoc test.



Supplemental Figure S4: Hepatocyte TFR1 is not required for acute EPO-mediated hepcidin suppression in mice.

Six-week-old male (blue) and female (red) *Tfrc*$^{fl/fl}$*;Alb-Cre*$^{+}$ mice and littermate controls (*Tfrc*$^{fl/fl}$*;Alb-Cre*$^{-}$) were injected with 200 U of EPO. After (A-B) 9 hours or (C-D)15 hours, (A,C) bone marrow samples were analyzed for *Erfe* and (B, D) livers were analyzed for *Hamp* relative to *Rpl19* mRNA by qRT-PCR. The average of the PBS-treated control mice was set to 1. For all graphs, individual data points are shown, and bars represent mean ± SEM. Data were analyzed by two-way ANOVA with Tukey's post-hoc test. Means without a common superscript differ significantly ($P < 0.05$).



Supplemental Figure S5: Deletion of hepatocyte *Tfrc* does not change serum iron, red blood cell parameters or spleen parameters in β-thalassemic mice.

Six-week-old littermate female hepatocyte *Tfrc* knockout thalassemia mice (*Hbb*$^{th3/+}$;*Tfrc*$^{fl/fl}$;*Alb-Cre*$^{+}$) mice, thalassemia mice (*Hbb*$^{th3/+}$;*Tfrc*$^{fl/fl}$;*Alb-Cre*$^{-}$) and non-thalassemic controls (*Hbb*$^{+/+}$;*Tfrc*$^{fl/fl}$;*Alb-Cre*$^{-}$:represented by dotted line) were analyzed for (A) serum iron and (B) spleen iron by colorimetric assays. (C) Hematocrit (HCT), (D) red blood cell (RBC) number, (E) mean corpuscular hemoglobin (MCH), (F) mean corpuscular volume (MCV), and (G) red cell distribution width (RDW) were analyzed by complete blood count. Spleen (H) *Gypa* and (I) *Tfrc* relative to *Rpl19* mRNA were measured by qRT-PCR. For all graphs, the average of the non-thalassemic group is shown as a dotted line. For panels H-I, the average of the non-thalassemic control mice was set to 1. For other genotypes, individual data points are shown, and bars represent mean ± SEM. No significant differences were seen for *Hbb*$^{th3/+}$;*Tfrc*$^{fl/fl}$;*Alb-Cre*$^{+}$ mice relative to *Hbb*$^{th3/+}$;*Tfrc*$^{fl/fl}$;*Alb-Cre*$^{-}$ mice by Student's *t*-test.