Table S1. Hematologic Features of *Hfe*$^{-/-}$ truncated *Hfe* (*Hfe*$^{\Delta CD}$ tg) or *Hfe*$^{-/-}$ *Hfe*^{C294Y} transgenic animals								
Genotype	n	Hgb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	RDW (%)	Retic (%)	Chr (pg)
WT	7	14.8±0.2	48.2±0.6	50.1±0.6	15.4±0.2	13.0±0.2	3.2±0.1	15.7±0.1
Hfe$^{-/-}$	6	15.7±0.3*	51.3±1.6	53.3±0.7§	16.3±0.1§	12.9±0.3	3.7±0.3	16.9±0.1◊
Hfe$^{-/-}$ *Hfe*$^{\Delta CD}$ tg	8	14.1±0.6∫	47.4±1.8	48.3±0.6†	14.3±0.3*†	15.6±0.4§‡	3.4±0.1	15.9±0.3‡
Hfe$^{-/-}$	13	15.5±0.1§	53.4±0.5◊	55.7±0.6◊	16.2±0.1§	12.4±0.1§	3.4±0.2	16.6±0.1◊
Hfe$^{-/-}$ *Hfe*^{C294Y} tg	15	15.4±0.1*	53.5±0.6◊	57.0±0.6◊	16.4±0.1◊	12.4±0.1*	3.8±0.2*	16.7±0.1◊

Table S1. Hematologic features of *Hfe*$^{-/-}$ truncated *Hfe* (*Hfe*$^{\Delta CD}$ tg) or **_Hfe_$^{-/-}$ _Hfe_^{C294Y} transgenic animals**

The red blood cell parameters hemoglobin (Hgb), hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), red cell distribution width (RDW), reticulocyte count (Retic), and reticulocyte mean cell hemoglobin (CHr) were measured in 8-week-old female wild-type (WT), *Hfe*$^{-/-}$, *Hfe*$^{-/-}$ *Hfe*$^{\Delta CD}$ and or *Hfe*$^{-/-}$ *Hfe*^{C294Y} tg mice. Data are presented as mean ± SEM. p values were calculated by Student's t test using Microsoft Excel. *P < 0.05, §P< 0.01 or ◊P < 0.001 versus WT; ∫P < 0.05, ‡P < 0.01 or †P < 0.001 versus littermate *Hfe*$^{-/-}$.

Table S2. Hematologic features of *Hjv*$^{-/-}$ *Hfe* transgenic (tg) animals								
Genotype	n	Hgb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	RDW (%)	Retic (%)	Chr (pg)
WT	5	14.7±0.2	45.4±0.5	51.2±0.2	16.6±0.2	13.5±0.2	5.9±0.9	16.9±0.1
Hjv$^{-/-}$	9	16.5±0.1§	53.1±0.4*	52.3±0.3‡	16.2±0.1	12.0±0.1*	1.9±0.2‡	16.5±0.1
Hjv$^{-/-}$ *Hfe*^{WT} tg	9	16.4±0.2§	52.1±0.8*	53.0±0.5§	16.7±0.1†	12.6±0.2§†	2.7±0.3‡†	17.2±0.2∫
Hjv$^{-/-}$	9	16.2±0.2§	49.4±0.6*	48.1±0.6*	15.8±0.2‡	12.0±0.1*	2.5±0.2‡	16.9±0.1
Hjv$^{-/-}$ *Hfe*$^{\Delta CD\text{-}MYC}$ tg	7	16.3±0.2§	51.0±0.7*	49.0±0.8‡	15.7±0.4	12.4±0.1*	3.0±0.4‡	16.7±0.2

Table S2. Hematologic features of *Hjv*$^{-/-}$ *Hfe* transgenic (tg) animals

The red blood cell parameters hemoglobin (Hgb), hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), red cell distribution width (RDW), reticulocyte count (Retic), and reticulocyte mean cell hemoglobin (CHr) were measured in 8-week-old female wild-type (WT), *Hjv*$^{-/-}$, and *Hjv*$^{-/-}$ *Hfe*^{WT} tg (tg), or *Hjv*$^{-/-}$ *Hfe*$^{\Delta CD\text{-}MYC}$ tg mice. Data are presented as mean ± SEM. p values were calculated by Student's t test using Microsoft Excel. **P* < 0.001, §*P* < 0.01 or ‡*P* < 0.05 versus WT. †*P* < 0.05 or ∫*P* < 0.01 vs littermate *Hjv*$^{-/-}$.

Table S3. Oligonucleotide primers	
Name	Sequence (5'⇒3')
HFEtgNOTAIL	CTTCCTTTTCTATAAGATTAGG
HFEtgFOR	CGGTGGACCCAGCTG
HFEmycR	ACCGGTATGCATATTCTACAGATCCTCTTCTGAGATGAGTTTTTGTTCC TTCCTTTTCCTTAAGATTAGGAACAGAATTCCAACTAAGAAGATGGCAC
C294YR	GGGTGCTCCACTTGATAGGTGAACCTTGTC
C294YF	GACAAGGTTCACCTATCAAGTGGAGCACCC
PS133	GGAATGGGACGAGCAC
PS134	GATGGCACAGATGGTG
FH03	CGGATGGTTTTTGGCAGTTAG
FH04	GCCTTTACGATATCTCAGTCC
FH07	GAATGGCTTCCTTCCATCAA
FH08	ATCTTCAAAGGCTGCAGGAA
HFENeo	CTAGCTTCGGCCGTGACG
HFEKO	AGTTGGGAGTGGTGTCCGA
Map3	ATCAAATGGCATCTCTGGCA
Map4	GTGGCGAGTCACTTTCACCA
β-actin F	ACCCACACTGTGCCCATCTA
β-actin R	CACGCTCGGTCAGGATCTTC
Hamp1 F	CTGAGCAGCACCACCTATCTC
Hamp1 R	TGGCTCTAGGCTATGTTTTGC
Id1 F	ACCCTGAACGGCGAGATCA
Id1 R	TCGTCGGCTGGAACACATG
Bmp6 F	ATGGCAGGACTGGATCATTGC
Bmp6 R	CCATCACAGTAGTTGGCAGCG
mXBP-1 F	GAACCAGGAGTTAAGAACACG
mXBP-1 R	AGGCAACAGTGTCAGACTCC
mHfeqPCR F	CACCGCGTTCACATTCTCTAA
mHfeqPCR R	CTGGCTTGAGGTTTGCTCC
TFR2 Y245XF	GTGACAAGGGGGCATATTATGCATGGGATT
TFR2 Y245XR	TGTTGTGTAGCCCAAGCAGGTCCTGTACAA

Figure S1. Phenotypic analysis of mice expressing a truncated hepatocyte-specific *Hfe* transgene (*Hfe*$^{\Delta CD}$ tg)

Schematic drawing of the *Hfe*$^{\Delta CD}$ tg (A) lacking cytoplasmic domain (CD). Box plots depicting the (B) transferrin saturation (%) and (C) non-heme liver iron (µg/g wet weight). The bar within the box represents the median, while the top and bottom of the box are the 75th and 25th percentiles, respectively. The top and bottom whiskers depict the 90th and 10th percentiles, respectively. Data points outside of the 10th and 90th percentiles are drawn as circles. WT (*n*=7), *Hfe*$^{-/-}$ (*n*=7), and *Hfe*$^{-/-}$ *Hfe*$^{\Delta CD}$ tg (*n*=8) are depicted. p-values were calculated with Microsoft Excel (Student's t-test).

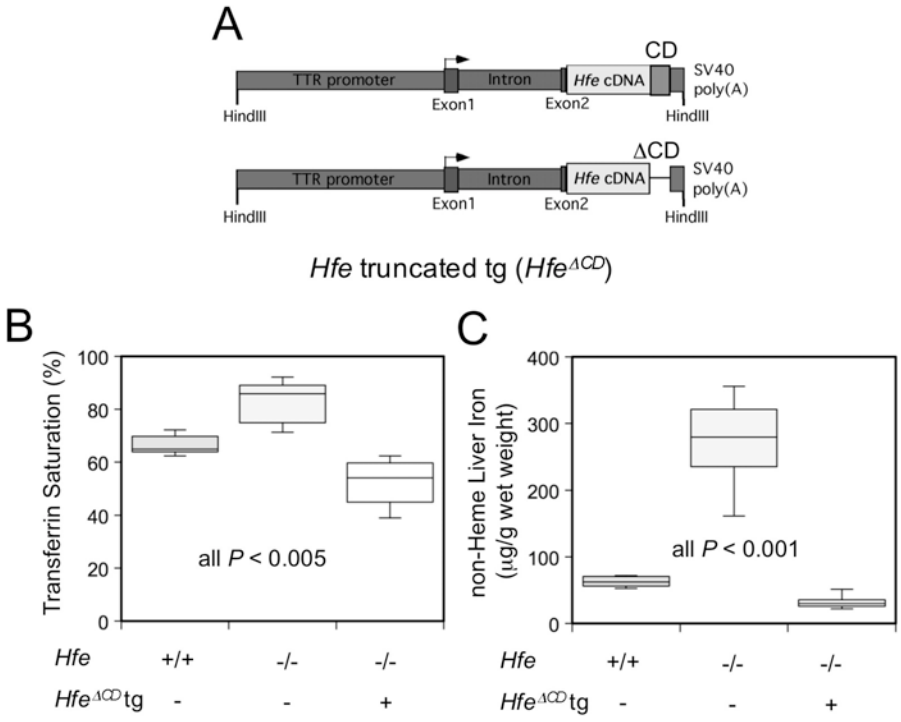
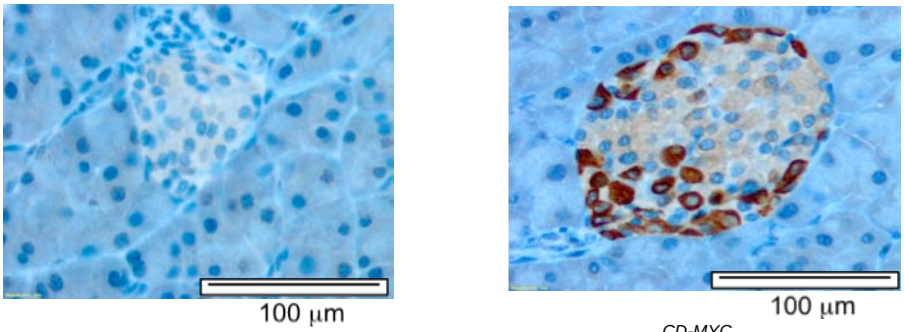


Figure S2. Additional analysis of mice expressing the *Hfe*$^{CD\text{-}MYC}$ transgene

Pancreas immunohistochemistry (A) probed for *Hfe*$^{-/-}$ animals (left panel) and cMyc epitope *Hfe*$^{-/-}$ *Hfe*$^{CD\text{-}MYC}$ (right panel) animals (Magnification 40X). Full scans of western blots (B) from Figure 1B. Additional pictures of liver immunohistochemistry (C) for cMyc epitope as in Figure 1C-D (magnification 80X).

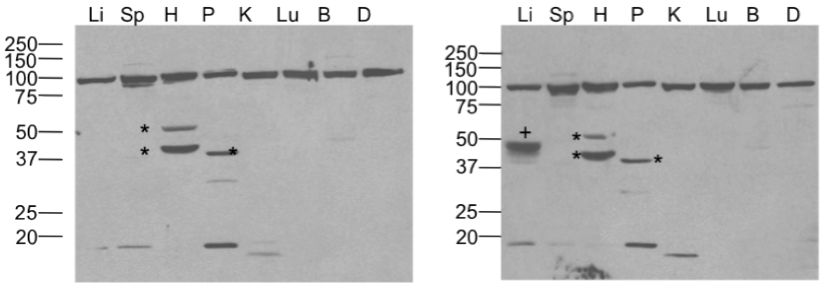
A



Hfe$^{-/-}$

Hfe$^{CD\text{-}MYC}$ *tg*
Hfe$^{-/-}$

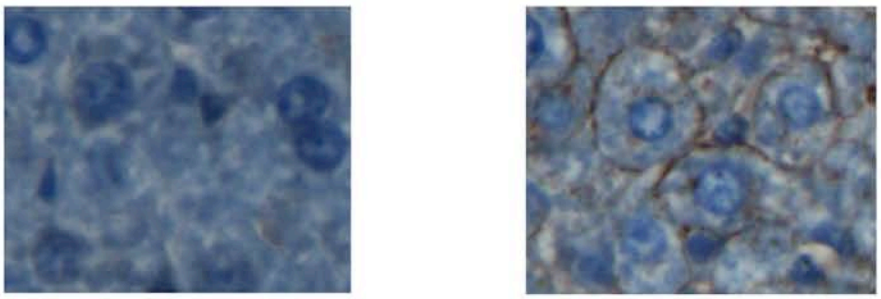
B



Hfe$^{-/-}$

Hfe$^{CD\text{-}MYC}$ *tg*
Hfe$^{-/-}$

C

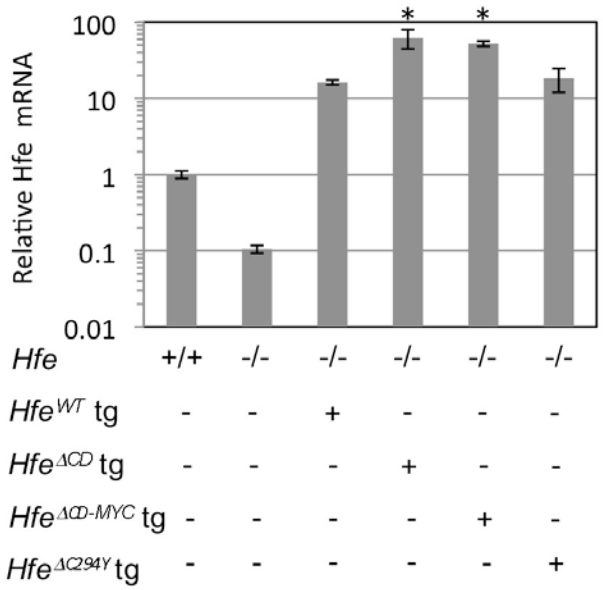


Hfe$^{-/-}$

Hfe$^{CD\text{-}MYC}$ *tg*
Hfe$^{-/-}$

Figure S3. Analysis of *Hfe* tg mRNA expression

Total mRNA was harvested from wild-type (*Hfe*$^{+/+}$), *Hfe*$^{-/-}$, *Hfe*$^{-/-}$ *Hfe*^{WT}, *Hfe*$^{-/-}$ Hfe-truncated transgenic (*Hfe*$^{\Delta CD}$ tg), *Hfe*$^{-/-}$ *Hfe-cMyc* (*Hfe*$^{\Delta CD\text{-}MYC}$ tg), or *Hfe*$^{-/-}$ *Hfe*^{C294Y} transgenic livers (*n*=5 for each genotype). *Hfe* mRNA was assessed by quantitative real-time PCR, normalized to β-actin (*Actb*), and then expressed relative to the WT value whose mean was defined as 1.0. Ratios are expressed ± SEM and are presented in both logarithmic and linear scale. P values: all $P < 0.05$ vs *Hfe*$^{+/+}$, all $P < 0.01$ vs *Hfe*$^{-/-}$, $*P < 0.05$ vs *Hfe*^{WT} or *Hfe*^{C294}. *Hfe*$^{\Delta CD}$ versus *Hfe*$^{\Delta CD\text{-}MYC}$ or *Hfe*^{WT} versus *Hfe*^{C294} not significant.



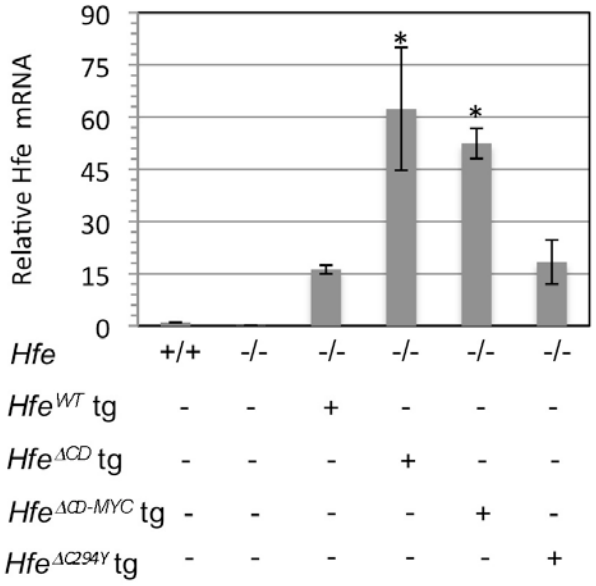


Figure S4. *Hfe* transgenes do not induce endoplasmic reticulum (ER) stress or the unfolded protein response (UPR)

ER stress in transgenic mouse livers was evaluated by measuring Xbp-1 mRNA splicing (A) using semiquantitative RT-PCR. Unspliced (205 bp, uXbp-1) or spliced (179 bp, sXbp-1) Xbp-1 mRNA forms from liver. ER stress was assessed in mouse embryonic fibroblasts (MEF) cells upon treatment with 0 or 10mM DTT as in Figure 5A. Total liver lysates (B) were analyzed for the KDEL motif-containing proteins Grp94 (Hsp90b1) and BiP (Hspa5) by western blot as quantified in Figures 5C-5D. Equivalent loading of liver lysates was confirmed by immunoblot analysis for β-actin.

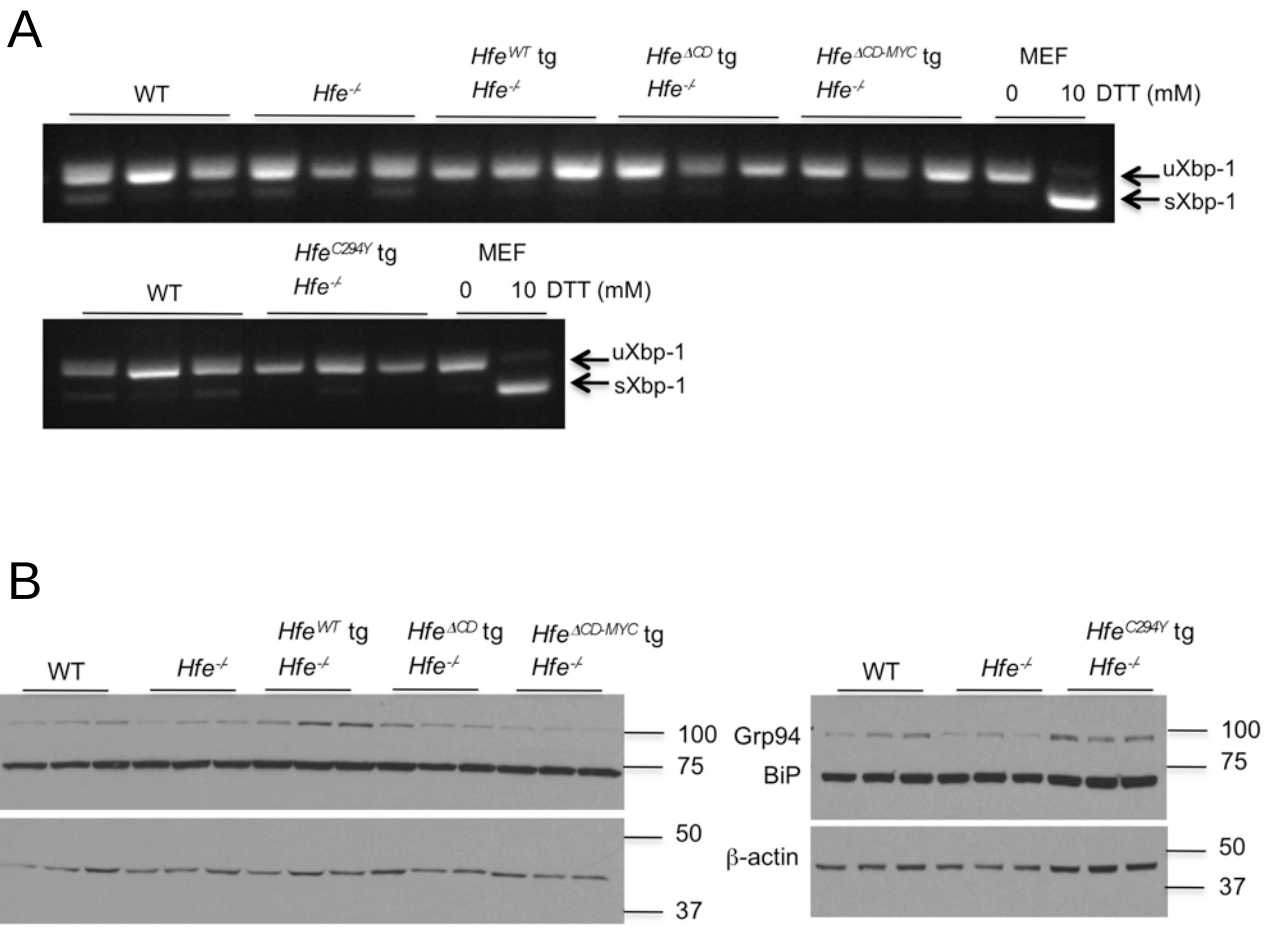


Figure S5. Tfr1 and Tfr2 protein expression in WT, *Hjv*$^{-/-}$ and *Hjv*$^{-/-}$ animals expressing full-length (*Hfe*^{WT}) or truncated *Hfe* (*Hfe*$^{\Delta CD\text{-}MYC}$) transgenes

Liver protein lysates were analyzed for Tfr2 (top panel) or Tfr1 (middle panel) protein expression in 8-week-old wildtype (WT), *Hjv*$^{-/-}$, *Hjv*$^{-/-}$ *Hfe*^{WT} tg, or *Hjv*$^{-/-}$ *Hfe*$^{\Delta CD\text{-}MYC}$ animals by western blot. Equivalent loading of liver lysate was confirmed by immunoblot analysis using an anti-β-actin antibody (bottom panel).

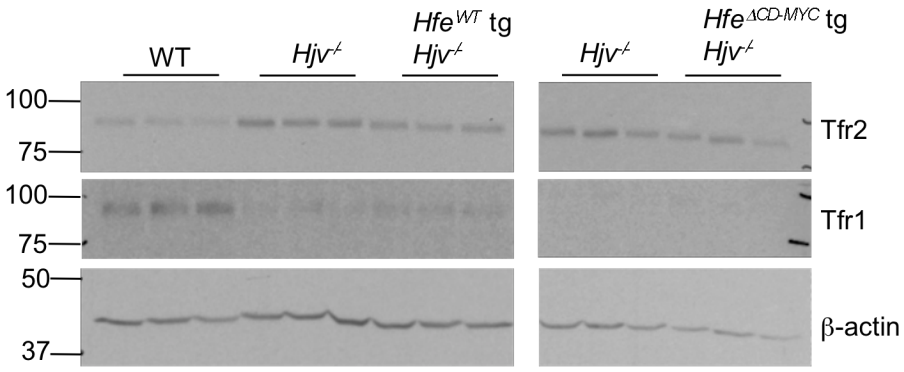


Figure S6. The anti-mouse Tfr2 antibody is specific and sensitive

Liver protein lysates were analyzed for Tfr2 (top) protein expression in 8-week-old wildtype (WT) or *Tfr2*$^{Y245X/Y245X}$ animals by Western blot. Equivalent loading of liver lysate was confirmed by immunoblot analysis using an anti-β-actin antibody (bottom).