**Supplemental digital content**

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**Supplemental References**

**Materials and Methods**

**Mice**

Animals were housed under specific pathogen-free and light-, temperature- (21°C), and humidity (50-60% relative humidity)-controlled conditions. Food (containing around 200 ppm of iron) and water were available *ad libitum*. Littermates from *Dmt1*IRE+/∆ inter-crosses were analyzed. Blood was collected by cardiac puncture after deep anesthesia of the mice with a Ketamine/Xylazine mixture. For molecular analyses, mice were sacrificed by cervical dislocation to minimize protein and RNA degradation. Animal care, husbandry, and killing were performed according to national guidelines and were approved by an institutional review board headed by the local animal welfare officers.

**Hematology and serum parameters**

Blood profiles and hemoglobin content were determined using an ABC Vet apparatus (HORIBA ABX SAS, Montpellier, France). Serum was prepared using Z-gel containing microvettes (Sarstedt, Nümbrecht, Germany). Serum concentration of iron, ferritin and transferrin was determined at the “Centre de Recherche sur l’Inflammation” (Paris, France) using an Olympus 400 analyzer (Olympus, Tokyo, Japan). Hepcidin levels were measured using the Hepcidin Murine-Compete™ ELISA kit (Intrinsic Life Sciences, LaJolla, CA, USA).

**Tissue iron levels**

Tissues were dried and non-heme iron levels were measured using the bathophenanthroline method as described previously1. Iron was stained on paraffin sections using Prussian blue, and pictures were acquired using a DM5000 microscope equipped with a DFC420C camera and a 10X objective. Images were processed using the Leica Application Suite (Leica Biosystems, Wetzlar, Germany).

**DNA analyses**

For Southern blotting, DNA was isolated by phenol/chloroform/isoamyl alcohol extraction after proteinase K digestion. The DNA was digested with NcoI, separated on an agarose gel, and transferred onto Hybond Nylon (GE Health Care Life Sciences, Freiburg, Germany) membranes. The membranes were UV crosslinked and hybridized with the P3’ probe in ULTRAHybTM solution (Fisher Scientific GmbH, Schwerte, Germany). The P3’ probe was obtained by PCR using the primers listed in Table S4 and was random labeled with [α-32P]dCTP using Ready-to-go™ DNA Labeling Beads (Sigma-Aldrich). Autoradiography was performed with a phosphor imager.

**RNA analyses**

*qRT-PCR:* Total RNA was extracted with TRIzol reagent (Life Technologies, Darmstadt, Germany), treated with DNase I to remove genomic DNA, and reverse transcribed using random primers and MultiScribe reverse transcriptase polymerase (Fisher Scientific GmbH) according to the manufacturer’s instructions. For each sample, RT reactions were performed in duplicate and the average of the duplicate was used for subsequent calculations. Real-time quantitative PCR was carried out on a CFX Real-Time PCR system (Bio-Rad, Munich, Germany) using SYBR Green and the following cycling parameters: denaturation, 95°C for 10 min, followed by 45 cycles of 15 s at 95°C, 15 s at 60°C and 15 s at 72° C. Semi-quantification was done using the ∆∆Ct-method. The primers used for qRT-PCR are listed in Table S4. Results are presented as relative mRNA expression (%) normalized to a housekeeping gene as indicated in figure legends. To selectively analyze *Dmt1*-IRE pre-mRNA levels, we used an intronic forward primer located 8 bp upstream of the splice acceptor of the exon containing the IRE; the reverse primer was located 5 bp downstream of the splice acceptor in the IRE containing exon that is used to generate the no IRE isoform (Table S4). For calibration, we used qPCR primers located in the last exon of the *Actb* pre-mRNA.

*RT-PCR*: Total RNA was reverse transcribed using the SuperScript™ III Reverse Transcriptase kit (Fisher Scientific GmbH) and an oligo-dT primer following the manufacturer’s instructions. The cDNA obtained was PCR amplified using the SequalPrep™ Long PCR Kit (Fisher Scientific GmbH) together with forward primers corresponding to exon 1A or 1B, respectively, and reverse oligonucleotides corresponding to either exon 16 (IRE isoform) or 17 (noIRE) and in both cases located near the polyA site (Table S4).

*Electromobility shift assay*:

The last 515 nucleotides of the coding region plus the first 1575 nucleotides of the 3’UTR of the *Dmt1*-IRE mRNA isoform were PCR amplified using total RNA from *Dmt1*IRE+/+ versus *Dmt1*IRE∆/∆ mice and the primers listed in Table S4. The cDNA obtained was sub-cloned into a blunt-end TOPO vector (Fisher Scientific GmbH) and served as template for in vitro synthesis of unlabeled RNA that was used in a non-radioactive competition electromobility shift assay as described previously2.

**Protein analysis.**

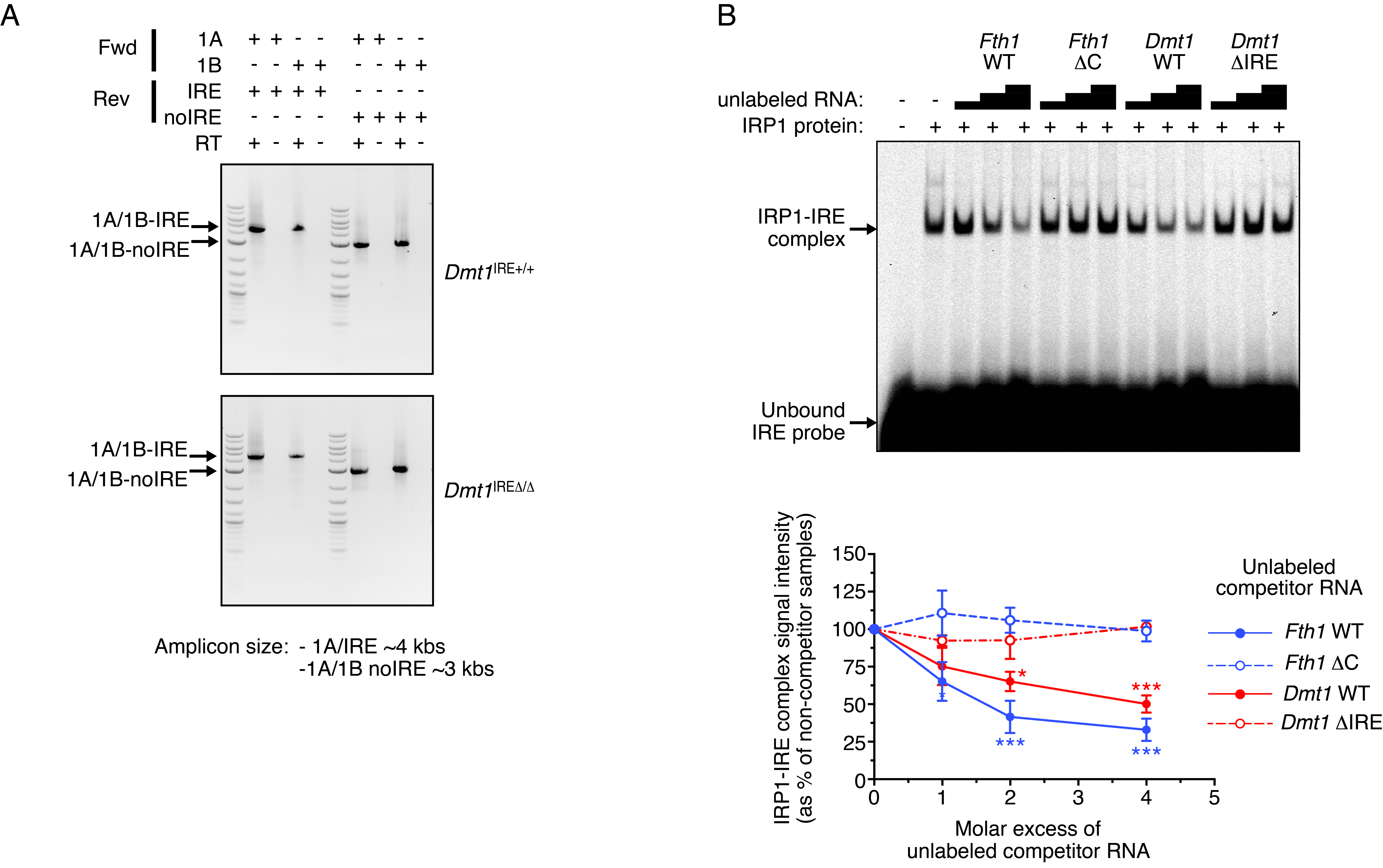
For western blotting, mucosal scrapings from the duodenum were homogenized in ice-cold lysis buffer (10 mM Tris pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.1% SDS) supplemented with protease inhibitors (100 mg/ml Pefabloc SC, 10 mg/ml Aprotinin, 1 mg/ml Leupeptin, 10 mg/ml E-64, 2 mg/ml Pepstatin), 100 mg/ml EDTA; all from Sigma-Aldrich Chemie GmbH, Munich, Germany) as described previously1. Equal amounts of total protein were separated by SDS-PAGE and transferred onto PVDF membranes. The membranes were incubated with the antibodies listed in Table S4. Tissue immuno-staining was performed on paraffin sections using the AEC kit (Vector Lab Inc., Burlingame, CA). Pictures were acquired as described above for iron staining.

**Polyribosome analysis**

Polyribosome analysis was performed as described previously1. Briefly, the duodenum was opened longitudinally and washed in ice-cold buffer A (40 mM [HEPES](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/hepes) pH 7.4, 100 mM KCl, 5 mM MgCl2, 2mM Na3-citrate) containing 300 µg/ml [cycloheximide](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/cycloheximide). The duodenal [mucosa](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/mucous-membrane) was scrapped off and homogenized in ice-cold buffer A complemented with 10 mM DTT, 500 U/ml Rnasin (Promega GmbH, Walldorf, Germany), and a complete EDTA-free protease inhibitor cocktail (Sigma-Aldrich Chemie GmbH). The homogenate was centrifuged at 5,000 × g for 20 min at 4°C. The supernatant was complemented with 1% Na deoxycholate and 1% Triton X-100 and resolved through a 12 ml 10-50% sucrose gradient at 39,000 rpm for 2 hr at 4°C in an SW40Ti rotor. 1 ml fractions were collected using a density gradient fractionation system (Teledyne Isco, Lincoln, NE). and RNA was extracted using phenol-chloroform. RNA fractions were pooled to generate samples corresponding to messenger ribonucleoprotein (mRNP) complexes, 80S, and light or heavy polysomes, respectively. The obtained RNA was used for qRT-PCR analysis as described above.

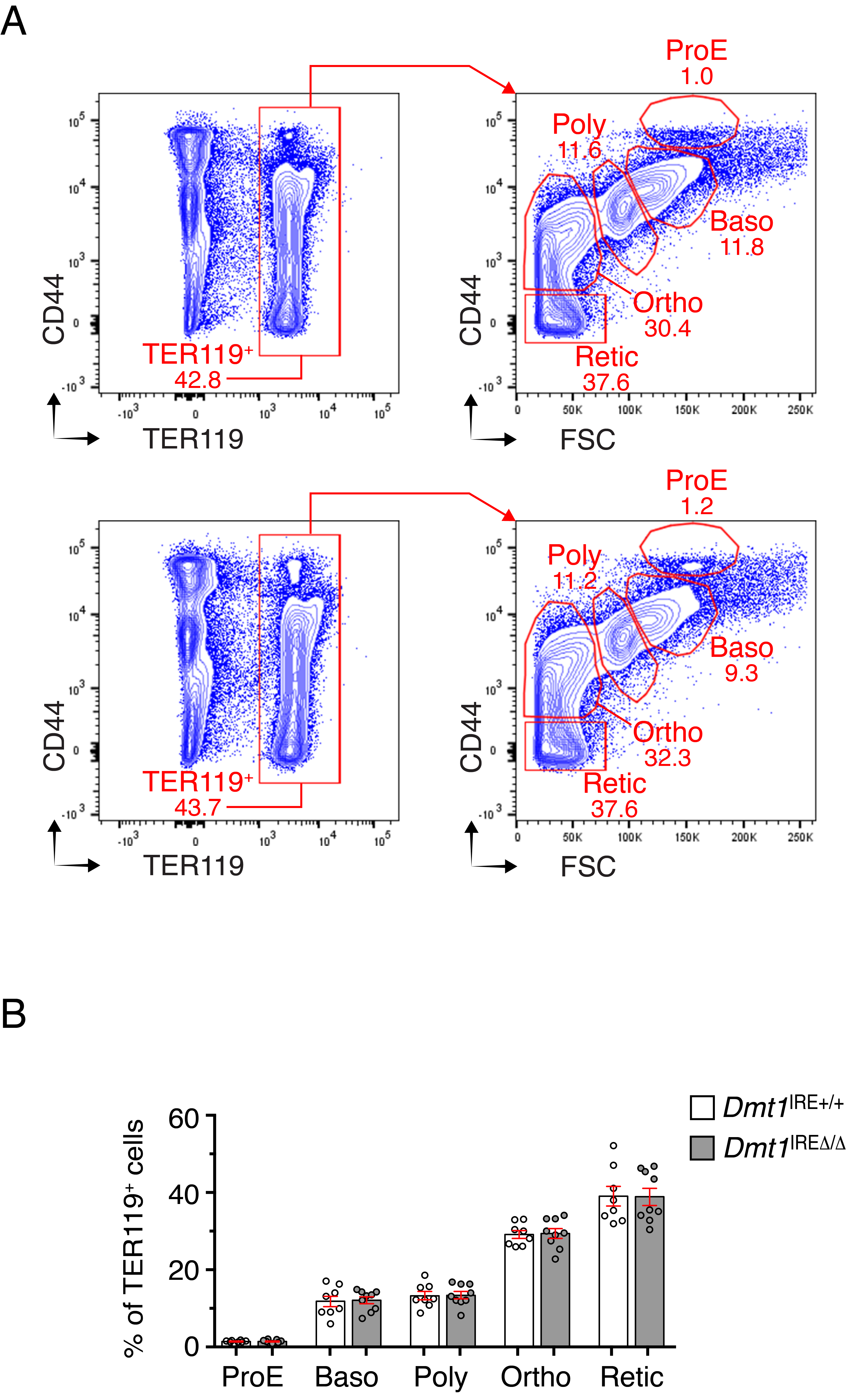
**Flow cytometry analysis**

To isolate total bone-marrow cells, the femurs were crushed in Iscove's Modified Dulbecco's Medium (Fisher Scientific GmbH) using a mortar and pestle, and cells were filtered through a 40 µM EasystrainerTM cell sieve (Greiner bio-one GmbH, Frickenhausen, Germany). 107 cells were incubated for 20 minutes on ice with phyco-erythrin (PE)-conjugated anti-mouse/human CD44 (clone IM7) and PE/Cyanine7-conjugated anti-mouse TER119 (clone TER119) antibodies (both from Fisher Scientific GmbH) in PBS containing 2% Fetal Bovine Serum (FBS). After washing in PBS/FCS, the stained cells were analyzed using a LSRFortessa™ apparatus (BD Biosciences, Heidelberg, Germany) equipped with a 561 nm laser. Single cells were gated according to the forward scatter area (FSC-A) and side scatter area (SSC-A). The differentiation stages of TER119+ erythroid cells were discriminated based on CD44 expression and FSC-A3, 4.

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**Supplemental Figure S1: Effect of mutagenesis of the *Dmt1* 3’IRE on *Dmt1* transcription and IRP binding.**

(A)To ascertain that mutagenesis of the *Dmt1* 3’IRE does not lead to overall alterations of transcript synthesis, we reverse transcribed total RNA from intestinal tissue using oligo-dT and PCR amplified all four *Dmt1* isoforms using primers corresponding to the most distal parts of the RNA. Forward primers (Fwd) are located in exons 1A or 1B, respectively; reverse primers (Rev) correspond to either exon 16 (IRE) or 17 (noIRE), respectively, and in both cases are located near the polyA site (See Figure 1A and Supplemental Table S4). We obtained the amplicons of the expected size (as indicated) from both wild-type (top) and mutant (bottom) mice. Negative control reactions were performed omitting the reverse transcriptase (RT). The boundaries of the PCR products were partially sequenced to confirm the identity of the amplicons. (B) To ascertain that mutagenesis of the *Dmt1* IRE effectively impairs IRP binding, the last 515 nucleotides of the coding region plus the first 1575 nucleotides of the 3’UTR of the *Dmt1*-IRE mRNA isoform were PCR amplified from *Dmt1*IRE+/+ versus *Dmt1*IRE∆/∆ mice (See primers in Supplemental Table S4). The cDNA obtained was sub-cloned and served as template for in vitro synthesis of unlabeled RNA used in a competition electromobility shift assay as described previously. Competitor transcripts corresponding to the full length *Fth1* mRNA (*Fth1* WT) versus a mutant version lacking the C residue in the IRE loop (*Fth1* ΔC) were used as control to validate the assay. As expected, the unlabeled wild-type *Fth1* mRNA competes for the interaction between recombinant purified IRP1 and a fluorescently labeled *Fth1* IRE probe, whereas the mutant version does not. Similarly, the wild-type *Dmt1* RNA (*Dmt1* WT) competes efficiently, but not the RNA from *Dmt1*IRE∆/∆ mice (*Dmt1* ΔIRE), confirming efficient disruption of the *Dmt1* 3’IRE. The graph displays the data as average ±SEM relative to the reaction without competitor (p: Student’s t-test; \*: p<0.05; \*\*\*: p<0.001).

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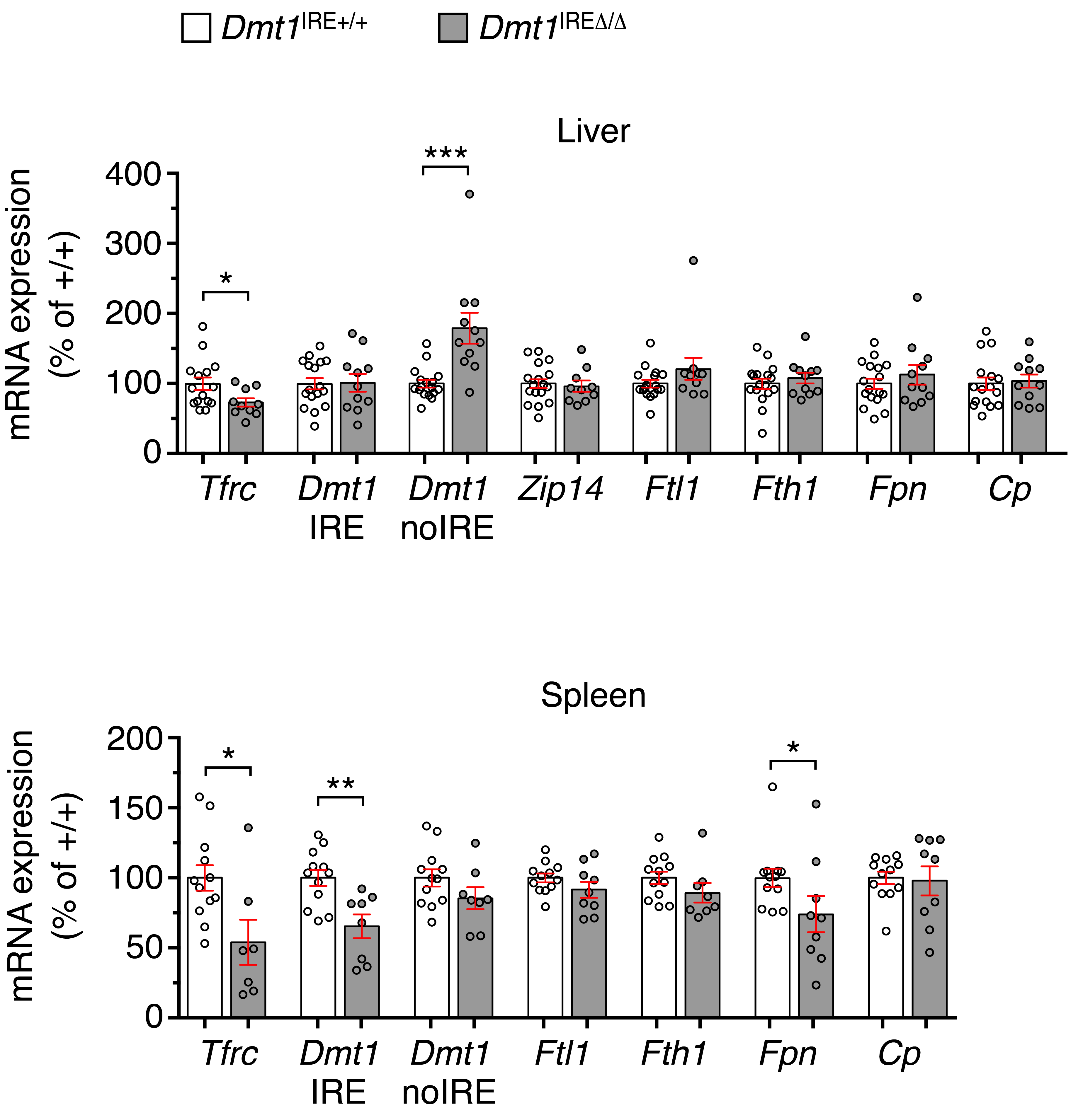
**Supplemental Figure S2: Terminal erythroid differentiation during early adulthood.**

(A) Representative flow cytometry profiles of bone marrow-derived erythroid progenitor cells from 3-month-old *Dmt1*IRE∆/∆ males versus wild-type littermates. Cells were co-stained with Ter119 and CD44. The graphs show the separation of Ter119+ cells (left panels) as a function of CD44 levels and cell size (forward scatter, FSC, right panels)3,4. Distinct erythroid populations corresponding to pro-erythroblasts (proE) progressing to basophilic (Baso), polychromatic (Poly) and orthochromatic (Ortho) stages, and finally reticulocytes (Retic), are highlighted. (B) Relative quantification of the frequencies of each erythroid population (averages ± SEM, 8 to 9 mice per group). This analysis shows that mutagenesis of DMT1 3’IRE does not alter terminal erythroid differentiation in adult animals (Student’s t-test). This is in agreement with the notion that iron assimilation in erythroid cells is dependent on DMT1 isoforms encoded by mRNA variants lacking the 3’IRE5.



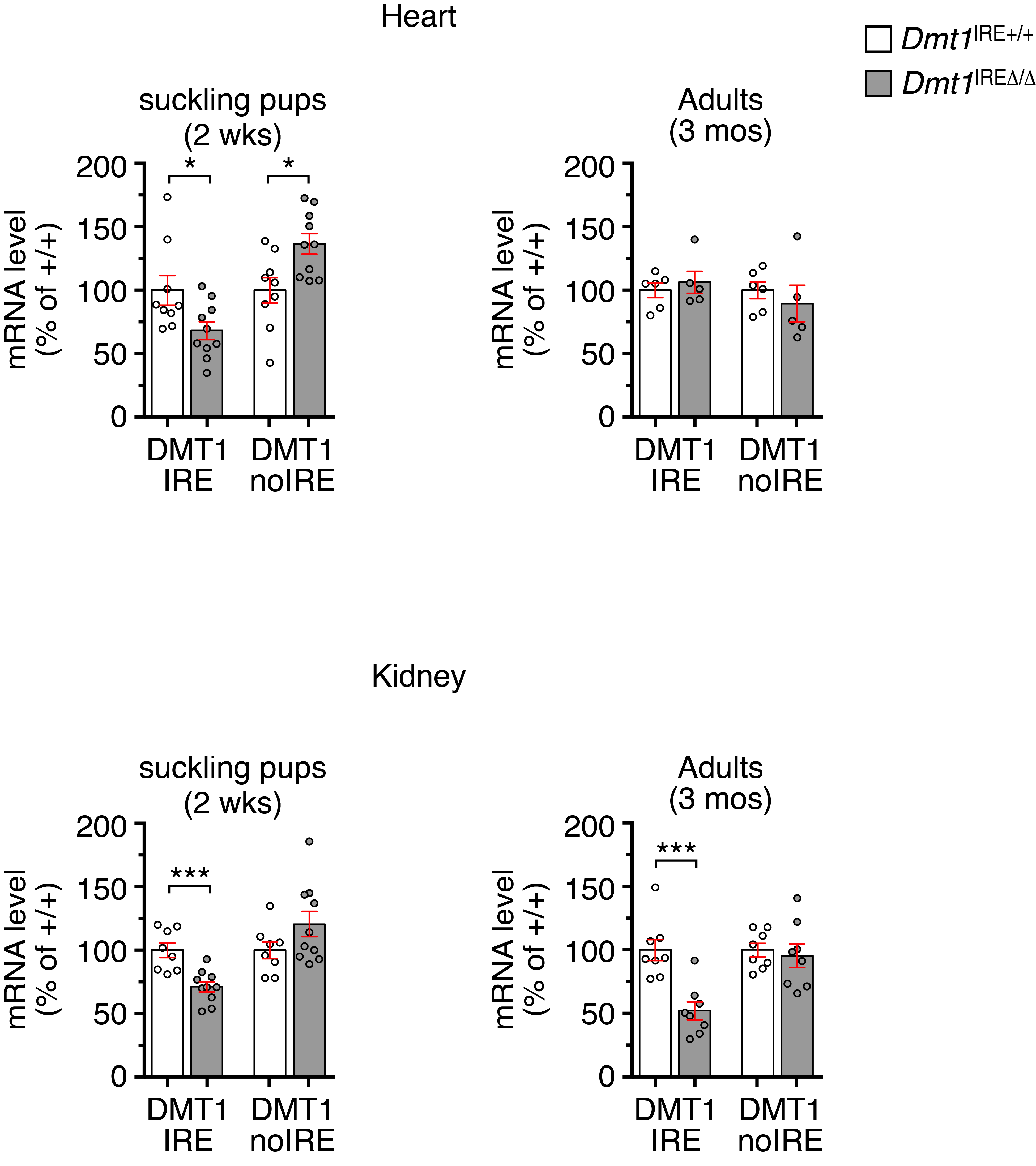
**Supplemental Figure S3: Iron metabolism parameters in female mice**

(A) Serum iron parameters were assessed in female mice during postnatal growth (2 weeks of age) or during early adulthood (3 months of age), respectively (7 to 13 mice per group). Similar to male mice (Figure 1C), *Dmt1*IRE∆/∆ female mice display a tendency towards decreased serum iron concentration and transferrin saturation during the suckling period (2 wks). On the opposite, they tend to have higher serum and transferrin saturation values during adulthood. Furthermore, the increase in serum iron levels is accompanied by a modest but significant augmentation of the liver and spleen iron stores (B) (10 to 21 mice per group), as observed in males (Figure 1D). Histograms display averages ± SEM. p: Student’s t-test (\*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001).



**Supplemental Figure S4: Expression of key iron metabolism molecules in the liver and spleen of adult mice.**

qPCR analysis of iron metabolism molecules in liver (top) and spleen (bottom) tissues of *Dmt1*IRE∆/∆ versus *Dmt1*IRE+/+ male mice at 3 months of age. *Tfrc* mRNA levels are reduced in liver, possibly as a response to hepatic iron loading. DMT1-IRE mRNA expression is unaltered, but *Dmt1*-noIRE mRNA levels are increased. Since DMT1-noIRE contributes to iron acquisition downstream of TFRC5 and *Tfrc* is downregulated in *Dmt1*IRE∆/∆ mice, hepatic iron accumulation unlikely results from stimulation of transferrin-bound iron uptake. *Zip14* (a.k.a. *Slc39a14*), which mediates the uptake of non-transferrin bound iron in liver cells6, is unchanged. The mRNA levels of the ferritin-L (*Ftl*) and -H (*Fth1*) iron sequestration molecules are comparable between mutant and wildtype. *Fpn* and the ferroxidase ceruloplasmin (Cp), both required for cellular iron export7,8, are unaltered. In spleen, iron accumulation cannot be explained by increased expression of iron import molecules, as both *Tfrc* and *Dmt1*-IRE are downregulated. We observed a mild reduction (~25%) of *Fpn* mRNA levels, which unlikely suffices to cause iron retention in splenic macrophages since 3-month-old mice lacking one *Fpn* allele display normal iron homeostasis parameters9. The *Ftl* and *Fth1* mRNAs are unaffected. Overall these data suggest that enlargement of tissue iron stores in adult *Dmt1*IRE∆/∆ animals does not result from iron mismanagement in liver and spleen cells and is rather secondary to the increase in serum iron levels. Data are presented as average ±SEM relative to wild-type control after calibration to *Actb* (n= 7 to 17 mice per group). p: Student’s t-test (\*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001).

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**Supplemental Figure S5: *Dmt1******mRNA*****expression in heart and kidney tissues during postnatal life and adulthood.**

qPCR analysis of *Dmt1* 3’ mRNA variants in the heart (top) and kidney (bottom) of *Dmt1*IRE∆/∆ versus *Dmt1*IRE+/+ male mice at 2 weeks or at 3 months of age, respectively. In heart, we observe a mild but significant decrease in *Dmt1*-IRE mRNA expression in 2-week-old *Dmt1*IRE∆/∆ mice, associated with an opposite increase in the *Dmt1* non-IRE mRNA levels. Similar to the duodenum, *Dmt1* mRNA expression is unaltered when mice reach adulthood. In kidney, *Dmt1*-IRE mRNA expression is reduced both during postnatal growth and early adulthood, suggesting that the activity of 3’IRE of *Dmt1* is not only age but also tissue dependent. Data are presented as average ±SEM relative to wild-type control after calibration to *Actb* (heart: 5 to 6 mice per group; kidney: 9 to 10 mice per group). p: Student’s t-test (\*: p<0.05; \*\*\*: p<0.001).

**Supplemental Table S1: Inheritance of the *Dmt1*IRE∆ allele.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | ***Dmt1*+/+** | ***Dmt1*+/∆** | ***Dmt1*∆/∆** | **total** | **p value** |
| Males | |  |  |  |  |  |  |
|  | Observed |  | 59 | 103 | 61 | 223 | 0.51 |
|  | Expected |  | 55.75 | 111.5 | 55.75 |  |  |
|  |  |  |  |  |  |  |  |
| Females | |  |  |  |  |  |  |
|  | Observed |  | 55 | 110 | 61 | 226 | 0.79 |
|  | Expected |  | 56.5 | 113 | 56.5 |  |  |

Genotype distribution in the progeny from *Dmt1*+/**∆** intercrosses (p value: Chi-square test)

**Supplemental Table S2: Baseline hematological parameters in *Dmt1*IRE∆/∆ mice are globally normal.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **2 wks** | |  | **3 mos** | |  | **9 mos** | |
|  |  |  | ***Dmt1*IRE+/+** | ***Dmt1*IRE∆/∆** |  | ***Dmt1*IRE+/+** | ***Dmt1*IRE∆/∆** |  | ***Dmt1*IRE+/+** | ***Dmt1*IRE∆/∆** |
| *Males* | | | (n=8) | (n=18) |  | (n=11) | (n=21) |  | (n=16) | (n=12) |
|  | WBC (109/L) |  | 3.7 ±0.5 | 3.5 ±0.2 |  | 4.5 ±0.3 | 4.4 ±0.3 |  | 3.2 ±0.2 | 3.8 ±0.3 |
|  | RBC (1012/L) |  | 5.5 ±0.3 | 6.1 ±0.1 \*\* |  | 8.7 ±0.2 | 8.4 ±0.1 |  | 8.7 ±0.1 | 8.9 ±0.1 |
|  | HGB  (g/L) |  | 10.6 ±0.2 | 11.2 ±0.2 |  | 15.2 ±0.3 | 15.2 ±0.1 |  | 14.8 ±0.2 | 15.2 ±0.2 |
|  | HCT  (L/L) |  | 32.7±0.9 | 35.2 ±0.6 \* |  | 46.6 ±0.8 | 45.8 ±0.4 |  | 46.3 ±0.5 | 47.4 ±0.7 |
|  | MCV  (fL) |  | 59.1 ±0.9 | 57.6 ±0.5 |  | 53.8 ±0.7 | 54.4 ±0.4 |  | 53.4 ±0.5 | 53.1 ±0.7 |
|  | MCHC  (pg/L) |  | 32.5 ±0.4 | 31.7 ±0.3 |  | 32.5 ±0.3 | 33.2 ±0.3 |  | 31.9 ±0.3 | 32.1 ±0.4 |
|  | PLT  (109/L) |  | 862 ±131 | 1026 ±41 |  | 1142 ±43 | 1127 ±53 |  | 1108 ±44 | 1126 ±48 |
|  |  |  |  |  |  |  |  |  |  |  |
| *Females* | |  | (n=11) | (n=11) |  | (n=13) | (n=11) |  | (n=9) | (n=10) |
|  | WBC (109/L) |  | 4.0 ±0.3 | 3.9 ±0.2 |  | 4.5 ±0.2 | 4.5 ±0.5 |  | 4.5 ±0.5 | 4.6 ±0.4 |
|  | RBC (1012/L) |  | 5.7 ±0.2 | 5.6 ±0.1 |  | 8.6 ±0.1 | 8.7 ±0.1 |  | 8.5 ±0.3 | 9.0 ±0.2 |
|  | HGB  (g/L) |  | 11.0 ±0.3 | 10.7 ±0.2 |  | 15.3 ±0.3 | 15.8 ±0.2 |  | 14.3 ±0.3 | 14.8 ±0.3 |
|  | HCT  (L/L) |  | 34.9 ±1.0 | 33.6 ±0.8 |  | 45.2 ±0.6 | 46.3 ±0.5 |  | 44.2 ±0.9 | 46.0 ±1.0 |
|  | MCV  (fL) |  | 60.9 ±0.9 | 60.5 ±1.1 |  | 52.2 ±0.5 | 52.8 ±0.5 |  | 52.2 ±1.2 | 51.2 ±0.7 |
|  | MCHC  (pg/L) |  | 31.7 ±0.4 | 31.8 ±0.3 |  | 33.9 ±0.5 | 34.2 ±0.4 |  | 32.3 ±0.3 | 32.3 ±0.2 |
|  | PLT  (109/L) |  | 1001 ±30 | 1119 ±33 \* |  | 956 ±54 | 1007 ±76 |  | 1317 ±38 | 1401 ±32 |

Hematological parameters were assessed in both male (top) and female (bottom) mice at 2 weeks of age, during early adulthood (3 months of age) and during aging (9 months of age), using EDTA blood. *Dmt1*IRE∆/∆ mice display globally unchanged hematological parameters, with only a very mild and transient increase in red blood cell counts and hematocrit values in 2-week-old male pups. This shows that the 3’IRE of DMT1 is largely dispensable for normal erythropoiesis under standard laboratory conditions and is compatible with the notion that erythroid cells rely on the non-IRE isoform of DMT1 for the uptake of transferrin-bound iron. The fact that the mice do not display signs of iron deficiency anemia in spite of the reduction in serum iron levels might be an indication that the hypoferremia is only transient and/or compensated for by the erythropoietic system.

WBC: white blood cells; RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; PLT: platelets. Results are presented as means ± standard error. Sample size (n) is indicated.

\* p<0.05; \*\* p<0.01; unpaired, two-tailed t-test.

**Supplemental Table S3: Body and organ weight parameters**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **2 wks** | |  | **3 mos** | |  | **9 mos** | |
|  |  |  | ***Dmt1*IRE+/+** | ***Dmt1*IRE∆/∆** |  | ***Dmt1*IRE+/+** | ***Dmt1*IRE∆/∆** |  | ***Dmt1*IRE+/+** | ***Dmt1*IRE∆/∆** |
| *Body weight (g)* | | |  |  |  |  |  |  |  |  |
|  | Males |  | 7.85 ±0.21  (n=18) | 8.63 ±0.20 \*  (n=30) |  | 27.41 ±0.49  (n=38) | 27.37 ±0.46  (n=35) |  | 36.75 ±0.91  (n=22) | 36.97 ±0.75  (n=24) |
|  | Females |  | 8.11 ±0.25  (n=19) | 8.23 ±0.35  (n=13) |  | 22.20 ±0.36  (n=25) | 23.02 ±71  (n=18) |  | 32.49 ±1.02  (n=19) | 33.06 ±1.38  (n=16) |
| *Spleen index* | |  |  |  |  |  |  |  |  |  |
|  | Males |  | 0.17 ±0.04  (n=23) | 0.17 ±0.01  (n=36) |  | 0.49 ±0.05  (n=17) | 0.50 ±0.01  (n=21) |  | 0.60 ±0.02  (n=21) | 0.61 ±0.03  (n=22) |
|  | Females |  | 0.10 ±0.01  (n=5) | 0.10 ±0.01  (n=7) |  | 0.44 ±0.01  (n=23) | 0.45 ±0.02  (n=16) |  | 0.55 ±0.02  (n=19) | 0.57 ±0.03  (n=16) |
| *Liver index* | |  |  |  |  |  |  |  |  |  |
|  | Males |  | 0.42 ±0.02  (n=14) | 0.46 ±0.02  (n=15) |  | 1.96 ±0.05  (n=7) | 1.93 ±0.05  (n=12) |  | 2.34 ±0.10  (n=21) | 2.43 ±0.06  (n=22) |
|  | Females |  | 0.05 ±0.01  (n=5) | 0.05 ±0.01  (n=7) |  | 1.47 ±0.03  (n=24) | 1.53 ±0.06  (n=17) |  | 2.02 ±0.06  (n=19) | 2.09 ±0.08  (n=16) |
| *Kidney index* | |  |  |  |  |  |  |  |  |  |
|  | Males |  | 0.25 ±0.01  (n=8) | 0.26 ±0.01  (n=6) |  | 1.03 ±0.03  (n=13) | 1.02 ±0.03  (n=17) |  | 1.27 ±0.02  (n=21) | 1.24 ±0.02  (n=22) |
|  | Females |  | 0.02 ±0.01  (n=5) | 0.02 ±0.01  (n=7) |  | 0.72 0.01  (n=15) | 0.76 ±0.03  (n=9) |  | 0.96 ±0.02  (n=19) | 0.97 ±0.03  (n=16) |
| *Heart index* | |  |  |  |  |  |  |  |  |  |
|  | Males |  | n.d. | n.d. |  | 0.64 ±0.02  (n=9) | 0.67 ±0.03  (n=11) |  | 0.81 ±0.01  (n=20) | 0.79 ±0.02  (n=22) |
|  | Females |  | n.d. | n.d. |  | 0.50 ±0.01  (n=8) | 0.52 ±0.02  (n=5) |  | 0.66 ±0.03  (n=8) | 0.69 ±0.02  (n=6) |

Body weight and organ weight were assessed in both male and female mice at 2 weeks of age, during early adult hood (3 months of age) and during aging (9 months of age). Organ index = .

The asterisk (\*) corresponds to a p value <0.05 (unpaired, two-tailed t-test). The sample size is indicated (n). n.d.: not determined

**Supplemental Table S4: Primers and antibodies used in the study**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Oligonucleotides** | | | | |
| *Gene name* | *primer name* | *sequence (5’ to 3’)* | *purpose* | *note* |
| *Dmt1 (Slc11a2)* | forward | tccttggcacttgcatactcac | Southern  blotting | generation of probe P3’, Figure 1C |
| reverse | aggtaacacgaaaggctaaggtg |
| exon 1A forward | AAGCCAAACCAGTCCTGCACC | RT-PCR | Analysis of *Dmt1* mRNA isoforms, Supplemental Figure S1A |
| exon 1B forward | AGGGCAGGAGGTTGACTGG |
| exon 16 reverse | GTTCACTAAAGCCTCCTTGAAGC |
| exon 17 reverse | TCTGGTTGGGATTAAAGCAAAAACC |
| exon 12 forward | Acctattctggccagtttgtcatg | Cloning | DNA template for in vitro transcription of competitor RNA, Supplemental Figure S1B |
| exon 16 reverse | GGGGACAACTATTTCAGGTC |
| qPCR IRE forward | ATGTTGCCACCGCTGGTATC | qPCR | analysis of *Dmt1* IRE mRNA isoforms, Figure 2 + Supplemental Figure S5 |
| qPCR IRE reverse | AGCTAGGCCATGTGGCACTCT |
| qPCR noIRE forward | GCGGTCAGTCCCAGGCGGTACG | qPCR | analysis of *Dmt1* noIRE mRNA isoforms, Figure 2 + Supplemental Figure S5 |
| qPCR noIRE reverse | GTGGTGGCTGCAGTGGTTAGCG |
| Forward | ggagagtgacgactgtgttttg | qPCR | Analysis of *Dmt1* IRE pre-mRNA, Figure 2C |
| reverse | tcagcaggactttcgagatg |  |
| *Fpn (Slc40a1)* | forward | GGGTGGATAAGAATGCCAGACTT | qPCR | Analysis of iron export molecules, Figure 2D |
| reverse | GTCAGGAGCTCATTCTTGTGTAGGA |
| *Heph* | forward | TCTATACATGCCCATTGGAGTTCT | qPCR | Analysis of iron export molecules in Figure 2E |
| reverse | TGGGATGTTCCACTGGTAAGT |
| *Cybrd1* | forward | GAAAAGCTGTTCTTTGTCCTGAAAC | qPCR | Analysis of HIF2 targets, Figure 2F |
| reverse | GCCCAGCGTATTTGTAAAAACAC |
| *Ccnd1* | forward | CATCCATGCGGAAAATCG | qPCR |
| reverse | GCGGGAAGACCTCCTCTT |
| *Actb* | forward | GGCCAGGATGGAGCCACCGATC | qPCR | qPCR standard |
| reverse | cagccattgctgacaggatgca |
| *Actb Pre* | forward | AAGCATCCTTAGCTTGGTGAG |  | Analysis of *Actb* pre-mRNA, Figure 2C |
| *mRNA* | reverse | TGCTGGGATTCCCCTTAAAC |  |
| *Hepcidin (Hamp)* | forward | CCTATCTCCATCAACAGAT | qPCR | Analysis of *Hepcidin*, Figure 1F |
| reverse | TGCAACAGATACCACACTG |
| *Tfrc* | forward | CCCATGACGTTGAATTGAACCT | qPCR | Analysis of iron molecules, Supplemental Figure S4 |
| reverse | GTAGTCTCCACGAGCGGAATA |
| *Zip14 (Slc39a14)* | forward | TGGAACCCTCTACTCCAACG | qPCR |
| reverse | CTGAGGGTTGAAGCCAAAAG |
| *Cp* | forward | GAAAGGCAGCTTACTTGCTGA | qPCR |
| reverse | TCAAACACTGTGGGAAACAAGT |
| *Fth1* | forward | TGGAACTGCACAAACTGGCTACT | qPCR |
| reverse | ATGGATTTCACCTGTTCACTCAGATAA |
| *Ftl* | forward | CGTGGATCTGTGTCTTGCTTCA | qPCR |
| reverse | GCGAAGAGACGGTGCAGACT |
| **Antibodies** | | | | |
| *protein*  *(host)* | *supplier* | *concentration* | *purpose* | *note* |
| DMT1 (rabbit) | Self-generated  against antigen: MVLDPEEKIPDDGASGDHGDSC | 1/500 | western blotting | reacts with all DMT1 protein isoforms |
| 1/400 | immuno-staining |
| FPN  (rabbit) | alpha-diagnostics | 1/300 | western blotting | affinity purified antibody |
| ACTB  (mouse) | Sigma-Aldrich | 1/5000 | western blotting | clone AC-15 |
| TER119  (rat) | Fisher Scientific GmbH | 1/300 | Flow cytometry | Clone TER119, coupled to Cyanine7/Phycoerythrin |
| CD44  (rat) | Fisher Scientific GmbH | 1/300 | Flow cytometry | Clone IM7, coupled to Phycoerythrin |

**Supplemental References**

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