

Supporting Information

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SI Materials and Methods

Labeling of Transferrin with ^{59}Fe and ^{59}Fe Uptake in Vivo and Its Intracellular Distribution Using Nondenaturing Fast Protein Liquid Chromatography Size-Exclusion Chromatography. Established methods were used to label apotransferrin (Sigma-Aldrich) with ^{59}Fe (Perkin-Elmer) (1, 2) and to measure organ ^{59}Fe uptake in mice (2). Briefly, 9-wk-old WT and mutant mice were injected via the tail vein with ^{59}Fe -Tf (0.6 mg) and killed after 96 h. The mice were then thoroughly perfused with ice-cold 20 mM Hepes buffer/0.14 M NaCl (pH 7.4) and the hearts washed to remove any traces of blood. Subsequently, the hearts were homogenized at 4 °C and centrifuged at $800 \times g$ for 20 min at 4 °C. The supernatant was then centrifuged at $16,500 \times g$ for 45 min at 4 °C and cytosolic and stromal mitochondrial membrane fractions were used for size-exclusion chromatography via fast protein liquid chromatography (FPLC). Fractions were incubated at 4 °C for 2 h in the presence or absence of the chelator, desferrioxamine (DFO) (2 mM), and loaded onto a Superdex 200 HR 10/300 column (GE Healthcare) implementing a BioLogic chromatography system (Bio-Rad). ^{59}Fe in fractions was measured using a Wallac Wizard 1480 γ -counter (Perkin-Elmer) and the radioactivity examined following elution of the lysate from the column.

Transmission Electron Microscopy. Muscle creatine kinase (MCK) mutant and WT mice were anesthetized with isoflurane. To remove blood from the system, an incision was made in the right atrium and warmed fixative solution [a mixture of 2% (wt/vol) formaldehyde (obtained by dissolving paraformaldehyde at 60 °C in a slightly alkaline milieu) and 2% (vol/vol) glutaraldehyde in 0.1 M phosphate buffer, pH 7.2] was injected into the left ventricle. Heart and liver tissues were harvested and fixed whole for 1 h at 4 °C before careful dissection into 1 mm³ pieces and further fixation overnight at 4 °C. Samples were then washed three times with 0.4 M Hepes (pH 7.2) buffer and postfixed with 1% osmium tetroxide for 30 min at 4 °C. Samples were again washed three times in Hepes buffer and then dehydrated in graded ethanol solutions for 10 min each at 4 °C. Infiltration was performed gradually in: (i) LR White resin (Electron Microscopy Sciences): ethanol solutions (1:3, 1:1, and 3:1) for 30 min each at 4 °C; (ii) LR White resin (100%) overnight at 4 °C; and (iii) three changes of LR White resin (100%) for 12 h each at 4 °C. Samples were embedded in LR White resin and polymerized at 60 °C for 48 h following standard protocols for transmission electron microscopy (TEM) sample preparation. Ultrathin sections (40–60 nm) were collected on Formvar-coated gold grids. The TEM micrographs were captured using a Tecnai 20 transmission electron microscope (Philips) operated at 200 kV. This microscope was equipped with an energy dispersive X-ray

spectrometer (EDAX) that allowed assessment of iron and other elements in the field of view (3).

^{57}Fe Mössbauer Spectroscopy. Tissue samples were freeze dried, ground to powder, and packed into 10-mm diameter sample holders. The thickness of the sample was adjusted so that the 14.4 keV Mössbauer γ -rays were attenuated by $\sim 1/e$. Sample temperatures were maintained at 5 K during data acquisition using a continuous helium flow cryostat. Mössbauer spectra were recorded using a ^{57}Co in rhodium foil source using standard methods (4). The source was driven at constant acceleration from -13 to $+13$ mm/s with a double ramp waveform. Spectra were subsequently folded to eliminate the parabolic background due to variation in the solid angle subtended by the detector window about the source. The resulting spectra consisted of 250 data points with a background count of $\sim 1.7 \times 10^7$ per channel. The velocity scale was calibrated with reference to the spectrum of an α -iron foil at room temperature, the center of the spectrum being taken as zero velocity (4).

The Mössbauer spectra were fitted with a doublet of Lorentzian peaks with the widths and intensities of the peaks being constrained to be equal using sum of squares minimization. To determine sextets of low intensity peaks caused by magnetic hyperfine field splitting that is characteristic of mammalian ferritin, the spectra were analyzed in terms of the total number of counts within particular regions of interest following an established protocol (4). Velocity boundaries for the different regions were implemented as follows: $v_1 = -10.8$, $v_2 = -5.8$, $v_3 = 6.5$, and $v_4 = 10.7$ mm/s.

Alternating Current Magnetic Susceptibility Measurements. Tissue samples were freeze dried, ground to powder to ensure homogeneity, and placed in gel capsules ready for measurement. Magnetic susceptibility measurements were carried out using a Magnetic Property Measurement System-XL magnetometer (Quantum Design) equipped with an alternating current (AC) susceptibility option. Measurements were performed with an exciting AC field amplitude of 0.45 mTesla and frequency of 10 Hz at 1.8–300 K. Following magnetic characterization, tissues were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) to ensure magnetism could be ascribed to the presence of iron and to calculate the magnetic effective moment (5). Diamagnetic contributions, which originate from the sample holder, were corrected for.

Statistical Analysis. Data were compared by using Student's *t* test. The results were considered statistically significant when $P < 0.05$. Results were expressed as mean \pm SD or mean \pm SEM.

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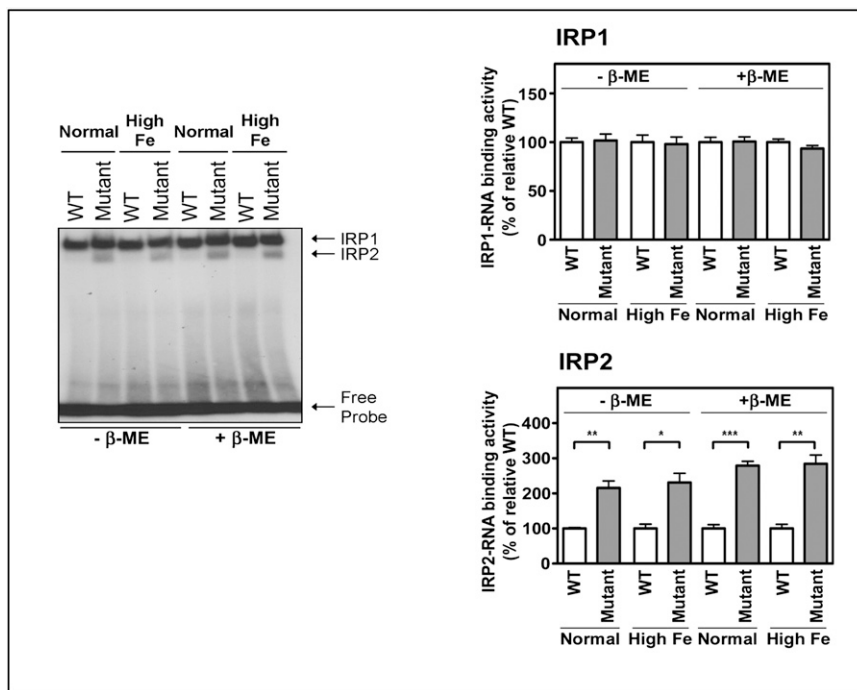


Fig. S1. Iron regulatory protein 2 (IRP2)-RNA-binding activity in the heart is increased in 8.5-wk-old mutant mice relative to their WT littermates fed a normal iron diet. In addition, there is no significant difference in IRP2-RNA-binding activity between the WT and mutants fed a normal or high iron diet. IRP-RNA-binding activity was assayed by gel-retardation analysis. WT and mutant mice were fed a normal (0.02% Fe/kg) or high (2.00% Fe/kg) Fe diet from 4.5 to 8.5 wk of age. Upon sacrifice, the heart was washed and homogenized and equal amounts of heart extract were assayed in the absence and presence of β-mercaptoethanol (β-ME) (2). Results are from typical experiments, whereas the densitometry is mean ± SD of three to five experiments. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

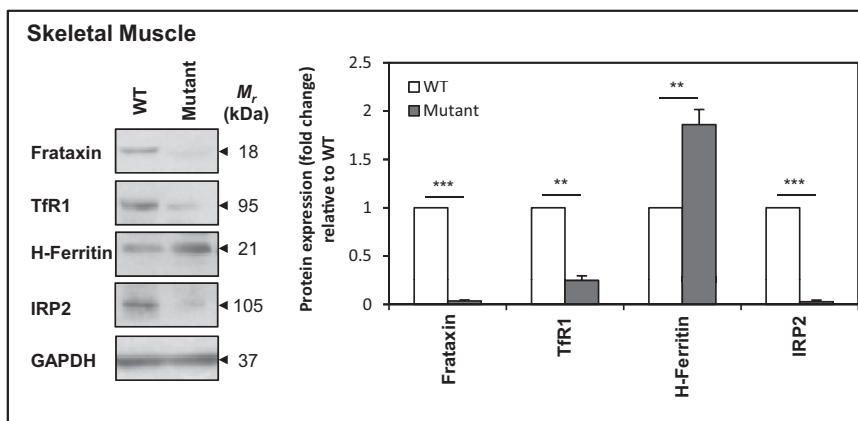


Fig. S2. Frataxin deficiency in mutant skeletal muscles from 9-wk-old mutant mice fed a normal diet demonstrates altered expression of Fe metabolism proteins relative to their WT littermates fed the same diet. The alterations observed are different from those found in the WT and mutant hearts at the same age and under the same experimental conditions (see main text and Fig. 2). Westerns are typical experiments and densitometry is mean ± SD (three to five experiments). **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

