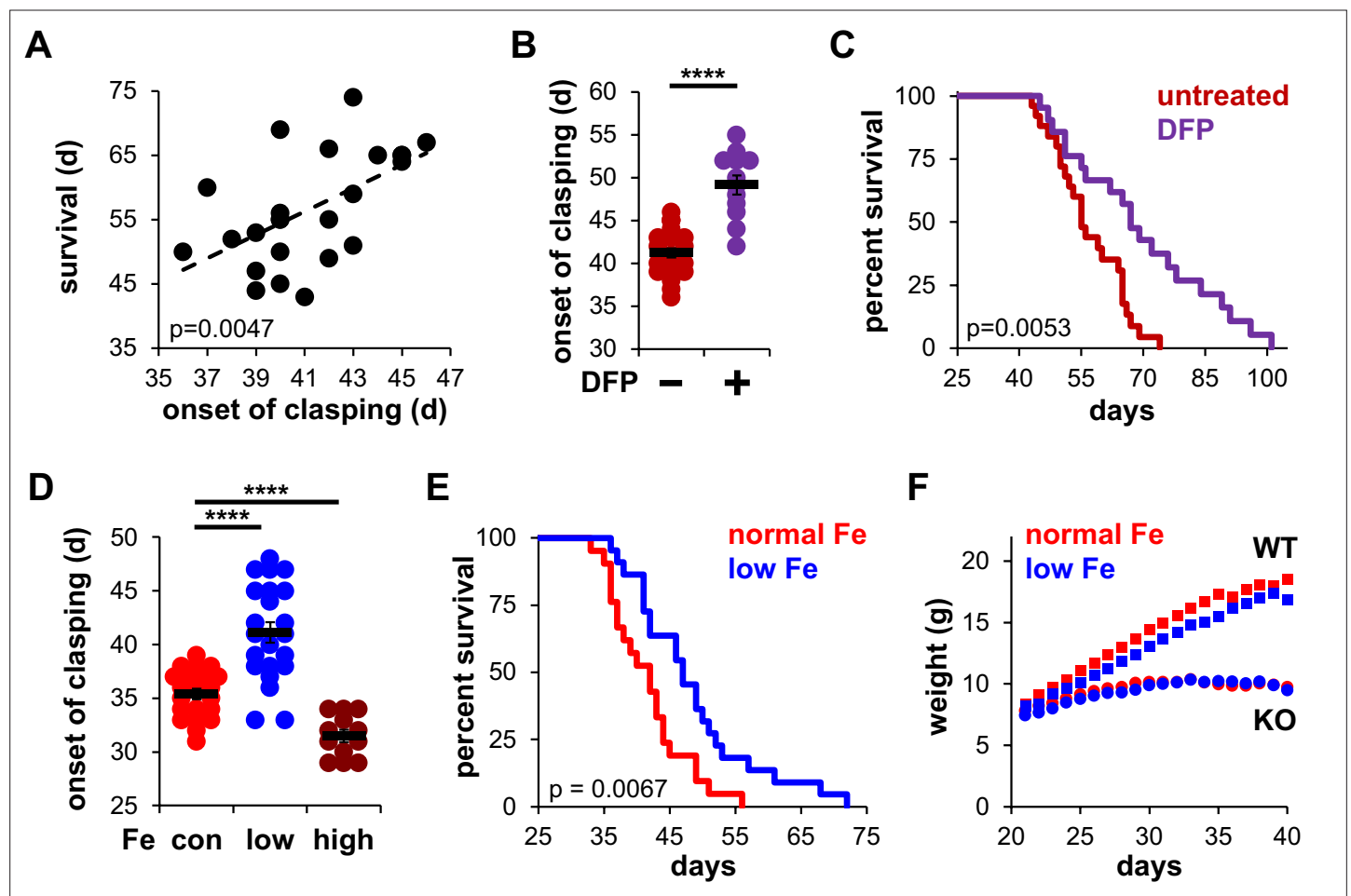


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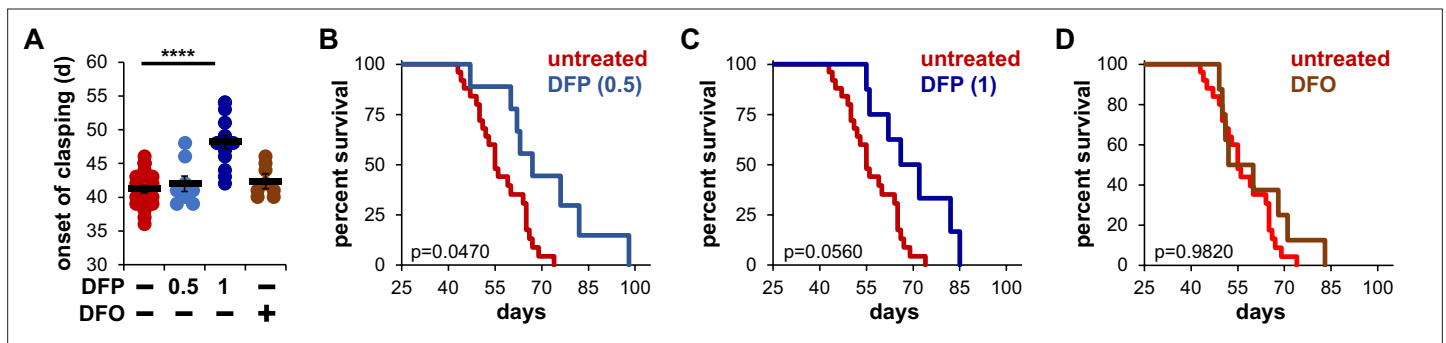
## Figures and figure supplements

Iron status influences mitochondrial disease progression in Complex I-deficient mice

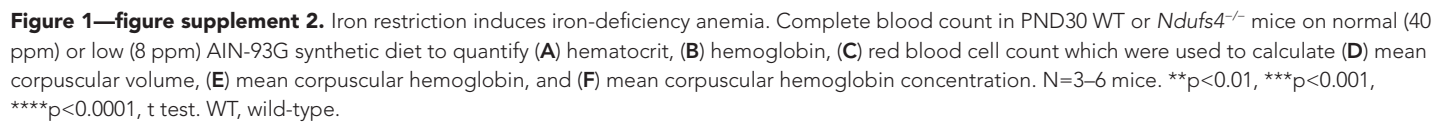
**CJ Kelly *et al.***

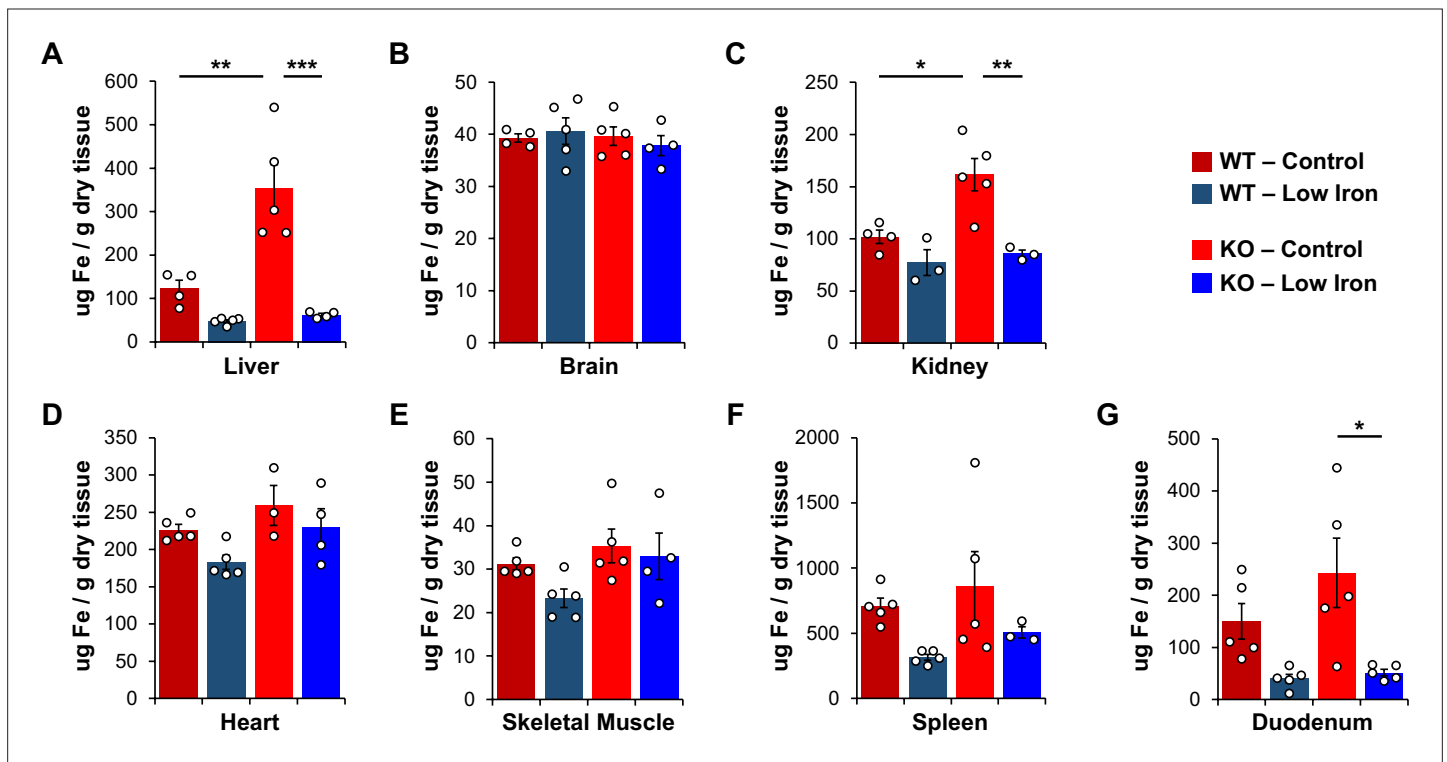


**Figure 1.** Iron restriction delays mitochondrial disease in mice. **(A)** Correlation between the onset of clasp and survival. Each point represents data from a single mouse.  $p=0.0047$ , Pearson's test. **(B)** Age at which *Ndufs4*<sup>-/-</sup> mice exhibited the clasp phenotype on chow diet. Mice were treated with either vehicle or deferiprone (DFP) in the water (2 mg/mL) from weaning. **(C)** Survival curves of *Ndufs4*<sup>-/-</sup> mice fed a chow diet and treated with deferiprone in the water (2 mg/mL) from weaning. **(D)** Onset of clasp in *Ndufs4*<sup>-/-</sup> mice on AIN-93G synthetic diet containing normal (40 ppm, con) or low (8 ppm) iron starting from weaning. Mice on control diet (40 ppm, Fe) were also treated with iron-dextran (100 mg/kg every 3 days via i.p. injection, high) from weaning. **(E)** Survival curves of mice on normal (40 ppm) or low (8 ppm) AIN-93G synthetic diet. **(F)** Weight gain in wild-type (WT, square markers) or *Ndufs4*<sup>-/-</sup> mice (KO, circle markers) on AIN-93G synthetic diet containing normal (40 ppm, red) or low (8 ppm, blue) concentrations of iron.  $p$  Value was calculated by log-rank for lifespan analyses. \*\*\*\* $p<0.0001$ ,  $t$  test with Bonferroni Correction.

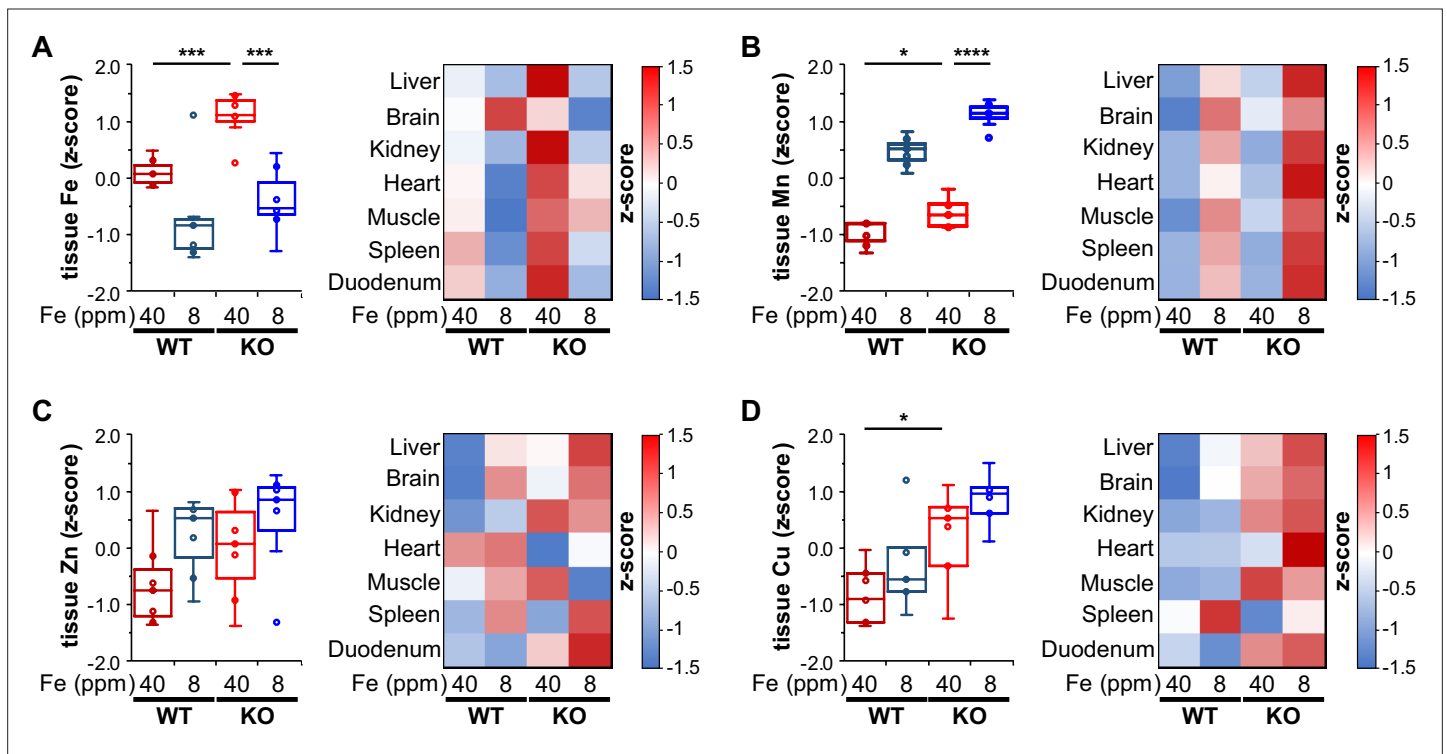


**Figure 1—figure supplement 1.** Brain-permeable iron chelators are effective in delaying mitochondrial disease. (A) Age at which *Ndufs4*<sup>-/-</sup> mice exhibited the clasping phenotype on chow diet. Mice were treated with either vehicle, deferiprone (DFP) in the water (0.5 or 1 mg/mL), or deferoxamine (DFO) via daily i.p. injection (125 mg/kg) from weaning. (B) Survival curves of *Ndufs4*<sup>-/-</sup> mice fed a chow diet and treated with deferiprone in the water (0.5 mg/mL) from weaning. (C) Survival curves of *Ndufs4*<sup>-/-</sup> mice fed a chow diet and treated with deferiprone in the water (1 mg/mL) from weaning. (D) Survival curves of *Ndufs4*<sup>-/-</sup> mice fed a chow diet and treated with deferoxamine via daily i.p. injection (125 mg/kg) from weaning. p Value was calculated by log-rank for lifespan analyses. \*\*\*\*p<0.0001, t test.

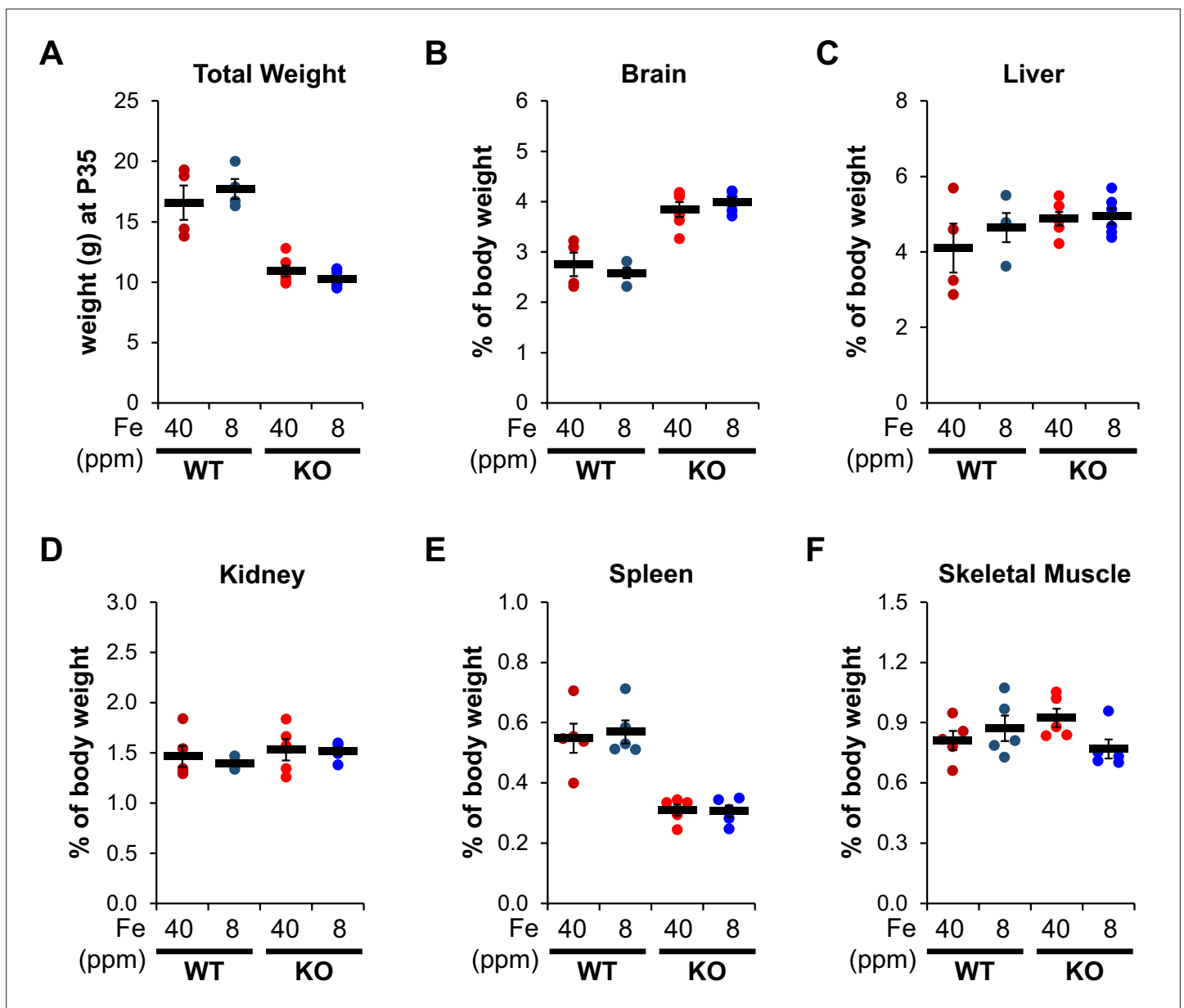




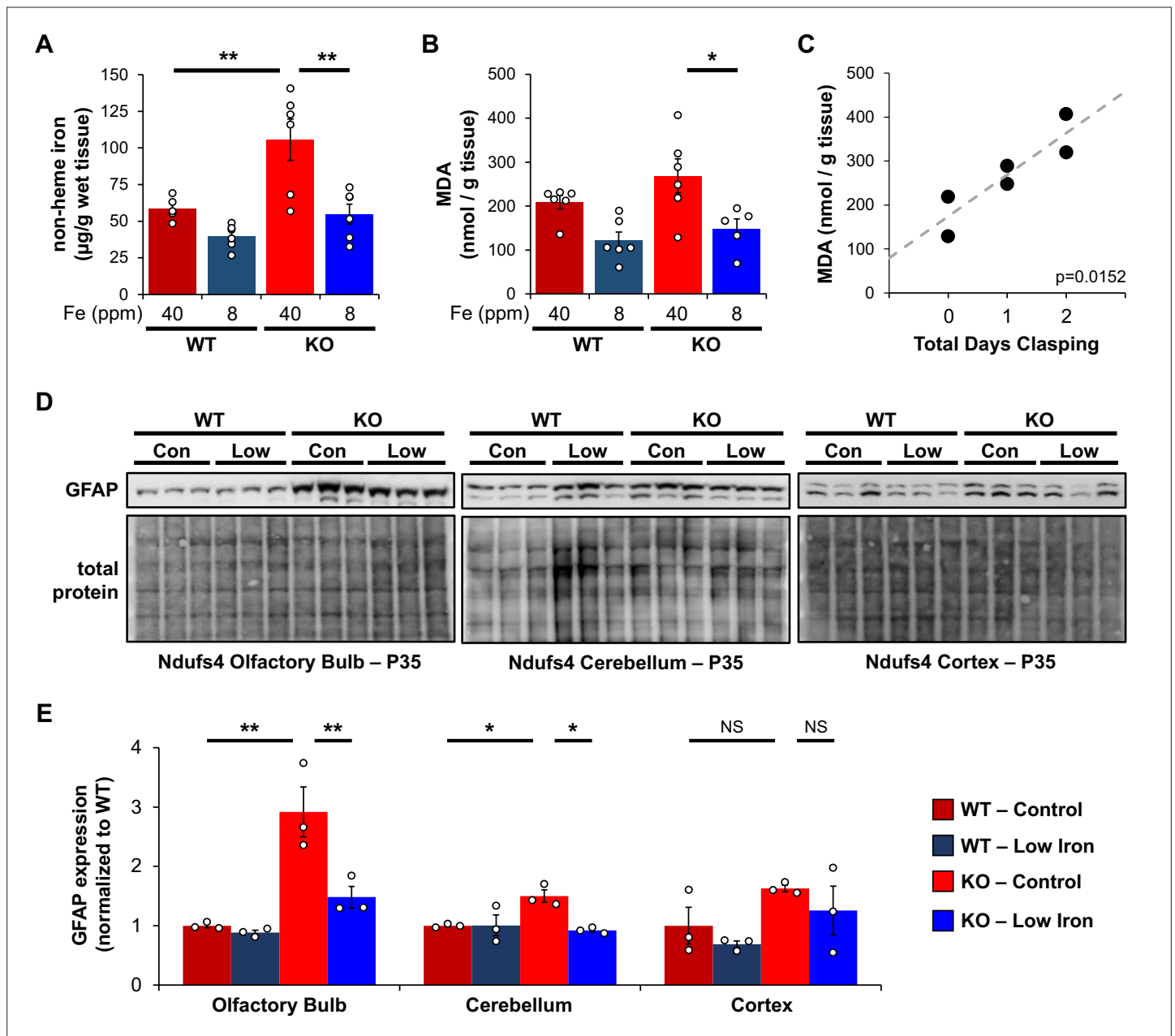
**Figure 2.** Total iron quantification in tissues. Quantification of total iron by ICP-MS from WT and *Ndufs4*<sup>-/-</sup> mice at PND35 fed control (40 ppm) or low (8 ppm) AIN-93G in (A) liver, (B) whole brain, (C) kidney, (D) heart, (E) quadriceps, (F) spleen, and (G) duodenum. N=3–5 mice. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, ANOVA with post hoc Tukey. ICP-MS, inductively coupled plasma mass spectrometry; WT, wild-type.



**Figure 2—figure supplement 1.** Analysis of biologically-relevant transition metals. (A) Left, Box, and whisker plot of combined z-score-normalized ICP-MS values of tissue iron, (B) manganese, (C) zinc, and (D) copper in WT and *Ndufs4*<sup>-/-</sup> mice on control (40 ppm) or low (8 ppm) AIN-93 diet (each point represents z-score value for an individual tissue). (A–D) Right, heat map of individualized z-scores by tissue. N=3–5 mice. \*p<0.05, \*\*\*p<0.001, \*\*\*\*p<0.0001, t test with Bonferroni correction. ICP-MS, inductively coupled plasma mass spectrometry; WT, wild-type.

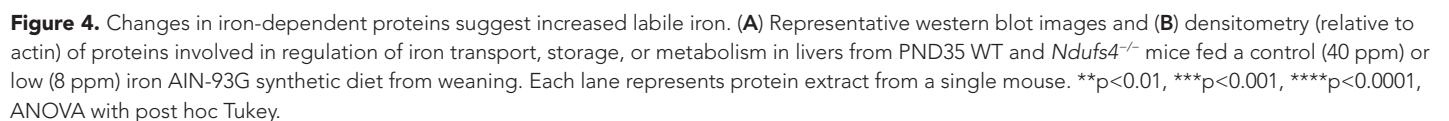


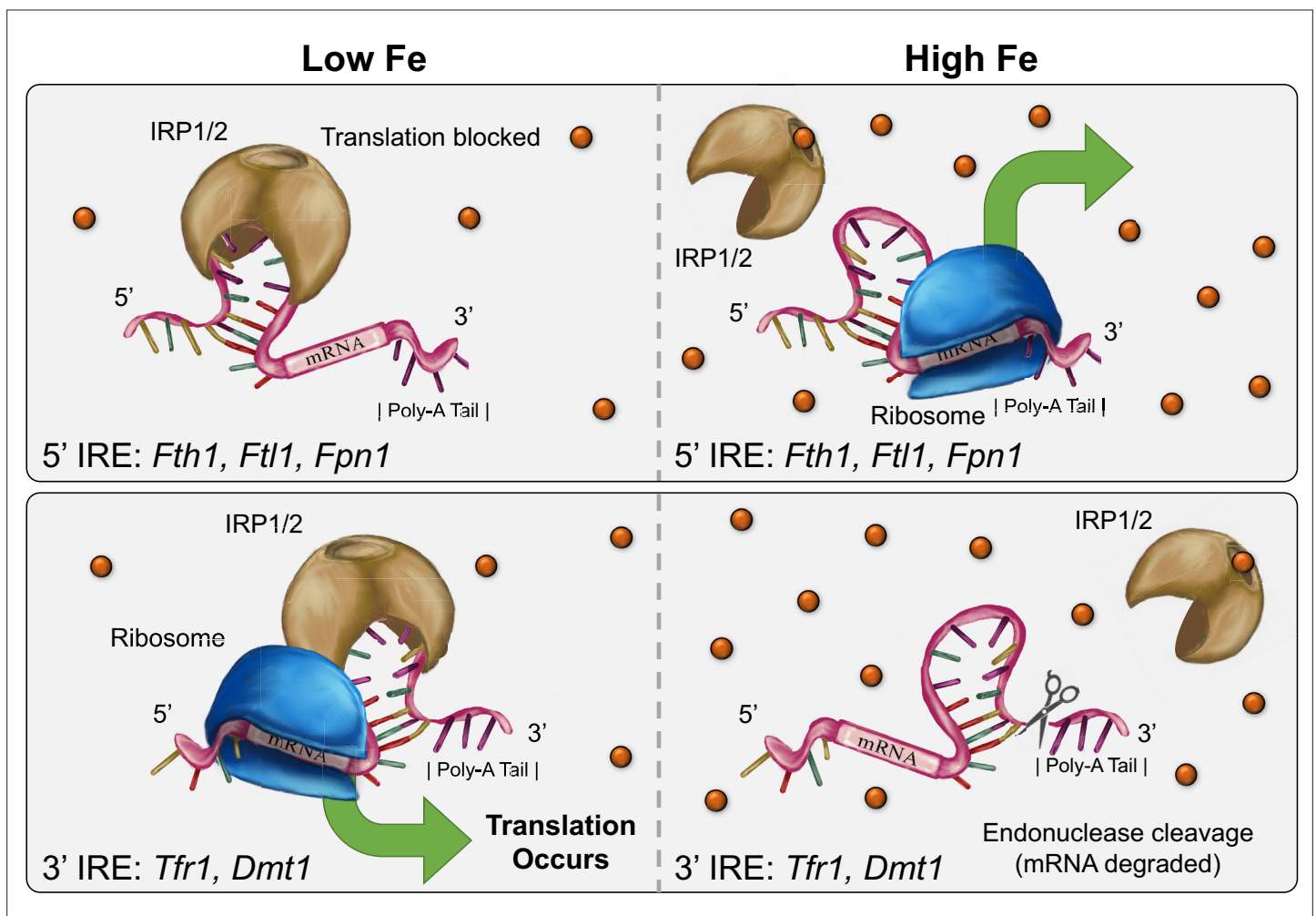
**Figure 2—figure supplement 2.** Iron restriction has no effect on organ size. (A) Weight at time of tissue collection (PND35) of WT or *Ndufs4*<sup>-/-</sup> mice on a normal (40 ppm) or low (8 ppm) AIN-93G synthetic diet. (B) Percent of tissue weight from mice in (A) relative to total body weight at time of collection (PND35) in brain, (C) liver, (D) kidney, (E) spleen, and (F) quadriceps.



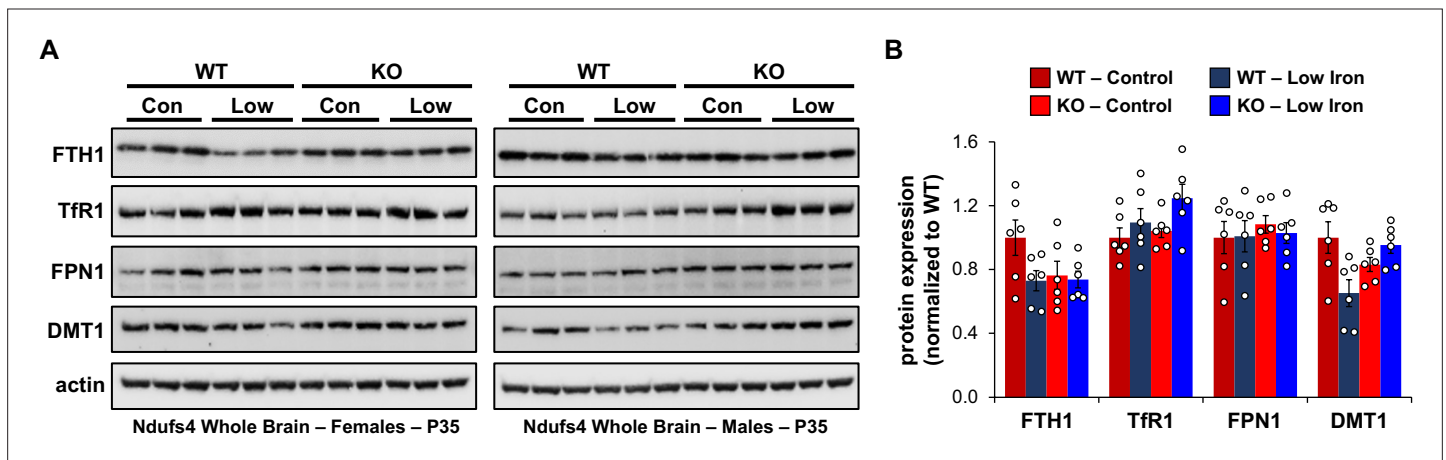
**Figure 3.** Iron restriction reduces iron-dependent oxidative damage and neuroinflammation. (A) Quantification of non-heme iron by ferrozine assay and (B) MDA-TBA adduct in livers from WT and *Ndufs4*<sup>-/-</sup> mice at PND35 that were fed control (40 ppm) or low (8 ppm) AIN-93G synthetic diet. (C) Correlation between days since *Ndufs4*<sup>-/-</sup> mice began displaying the clamping phenotype with detected liver MDA levels from (B).  $p=0.0152$ , Pearson's test. (D) Representative western blot images and (E) densitometry (relative to total protein) of the astrogliosis marker GFAP from brain sections that normally exhibit brain lesions (olfactory bulb, cerebellum) and that do not (cortex) from PND35 WT and *Ndufs4*<sup>-/-</sup> mice fed a control (40 ppm) or low (8 ppm) AIN-93G synthetic diet. Each lane represents protein extract from a single mouse.  $N=3-6$  mice. \* $p<0.05$ , \*\* $p<0.01$ , ANOVA with post hoc Tukey. MDA-TBA, malondialdehyde thiobarbituric acid; WT, wild-type.



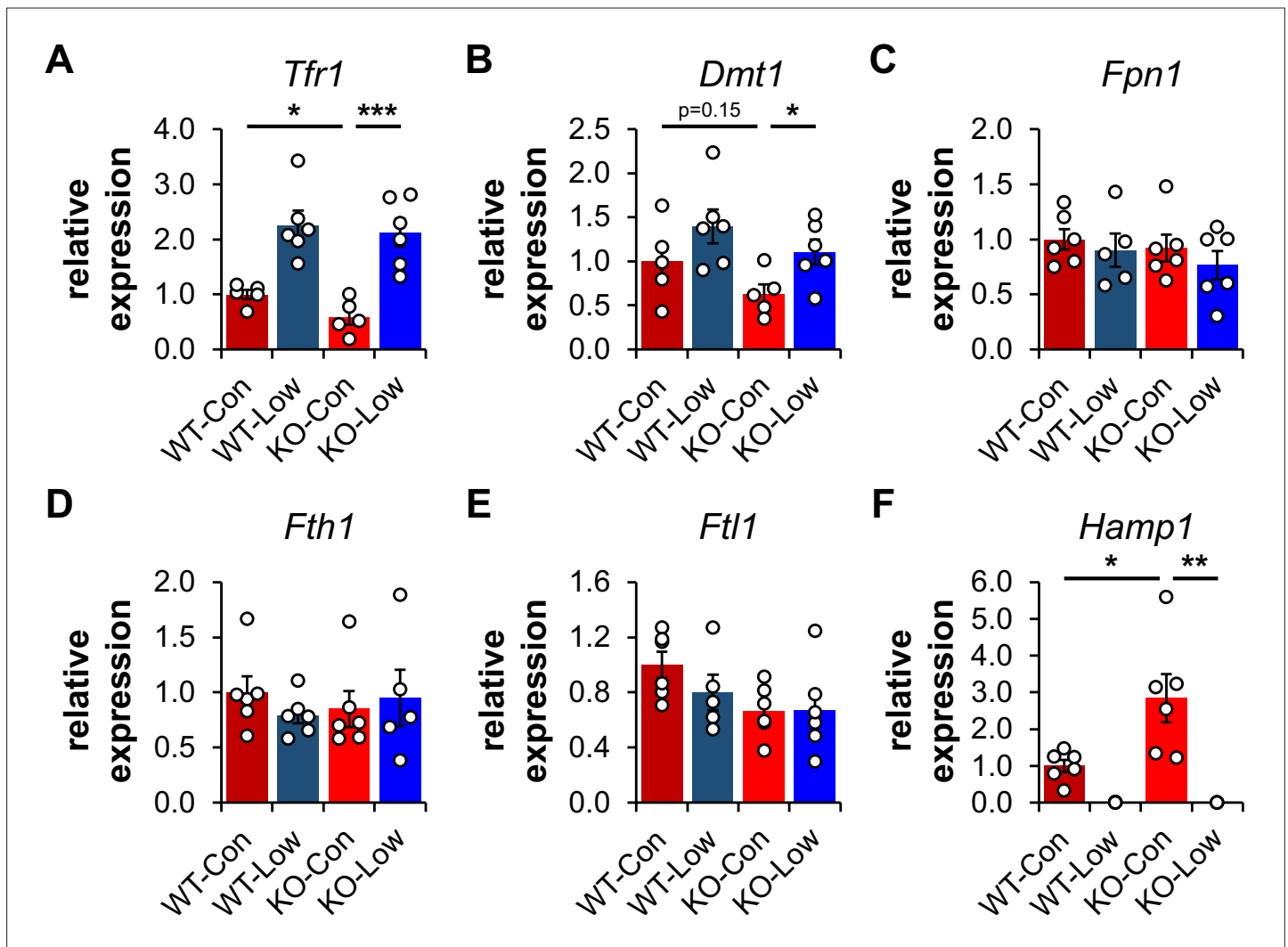




**Figure 4—figure supplement 1.** Simplified schematic of IRE-dependent translational regulation of proteins involved in iron transport, storage, or metabolism. Translation is blocked for 5'-IREs (e.g., *Fth1*, *Ftl1*, and *Fpn1*) when iron regulatory proteins (IRPs) are bound to the IRE in low iron conditions (top left). Protein expression increases due to mRNA stabilization when IRPs are bound to 3'-IREs (e.g., *Tfr1* and *Dmt1*) in low iron conditions (bottom left). Exposure to high iron allows for translation in genes containing 5'-IREs (top right) and leads to decreased protein expression for 3'-IREs due to endonuclease-mediated mRNA degradation (bottom right). IRE, iron-responsive element.



**Figure 4—figure supplement 2.** Immunoblot of iron-dependent proteins in whole brain extracts. **(A)** Representative western blot images and **(B)** densitometry (relative to actin) of proteins involved in regulation of iron transport, storage, or metabolism in whole brains from PND35 WT and *Ndufs4*<sup>-/-</sup> mice fed a control (40 ppm) or low (8 ppm) iron AIN-93G synthetic diet from weaning. Each lane represents protein extract from a single mouse. There were no statistically significant differences (aka  $p < 0.05$ ) between WT-Control versus KO-Control, or KO-Control versus KO-Low Iron mice groups. WT, wild-type.



**Figure 5.** Expression profiling of IRE-containing genes by qPCR. (A) Quantification of relative mRNA expression of *Tfr1*, (B) *Dmt1*, (C) *Fpn1*, (D) *Fth1* (ferritin heavy chain 1), (E) *Ftl1* (ferritin light chain 1), and (F) *Hamp* (hepcidin) in livers from PND35 WT and *Ndufs4*<sup>-/-</sup> (KO) mice fed a control (40 ppm) and low (8 ppm) iron AIN-93G synthetic diet from weaning. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , t test with Bonferroni correction.