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Supplementary Data

Prion Protein Regulates Iron Transport by Functioning as a Ferrireductase

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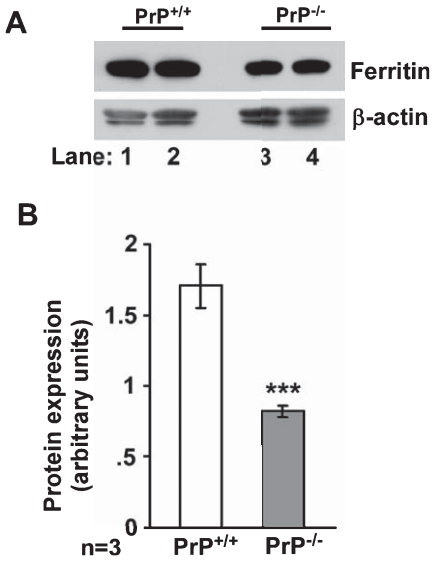
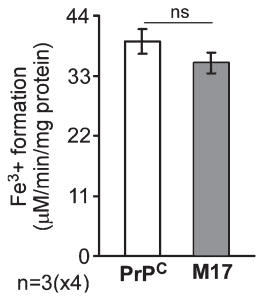
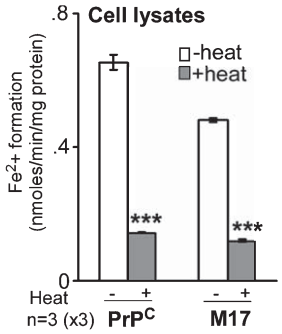


Supplementary Figure 1. Ferritin is reduced in bone marrow (BM) cells from $PrP^{-/-}$ mice. A) Flushed bone marrow cell lysates from $PrP^{-/-}$ mice show relatively less reactivity for ferritin than matched $PrP^{+/+}$ samples on western blots. B) Quantification by densitometry shows significantly less ferritin in $PrP^{-/-}$ samples relative to $PrP^{+/+}$ controls after normalization with β-actin. (***$p < 0.001$).



Supplementary Figure 2. PrP^C does not show ferroxidase activity. Lysates of PrP^C and M17 cells show similar ferroxidase activity when evaluated by published procedures [1, 2].



Supplementary Figure 3. Ferrireductase activity of PrP^C is heat labile. Heating at 100°C for 10 min reduces the ferrireductase activity of PrP^C and M17 lysates significantly relative to untreated controls. (***$p < 0.001$).

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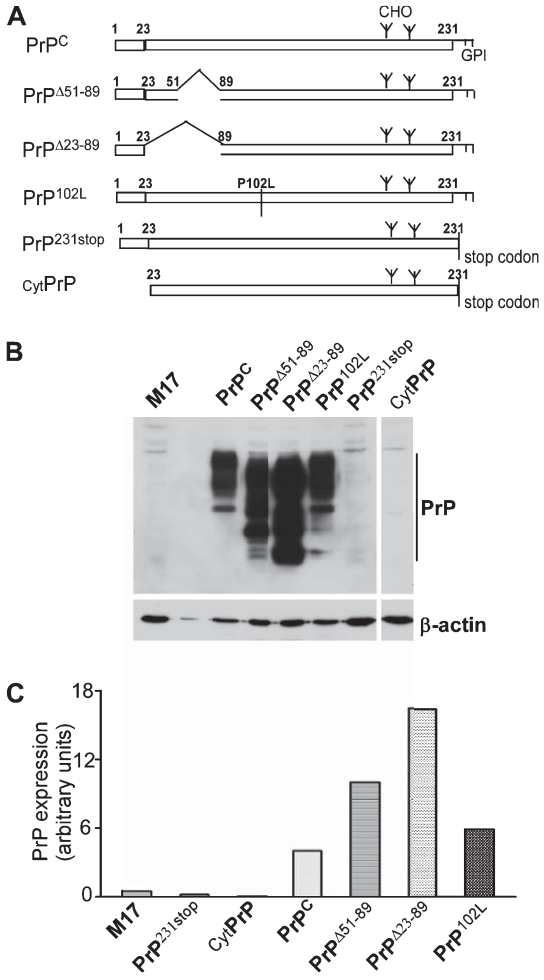
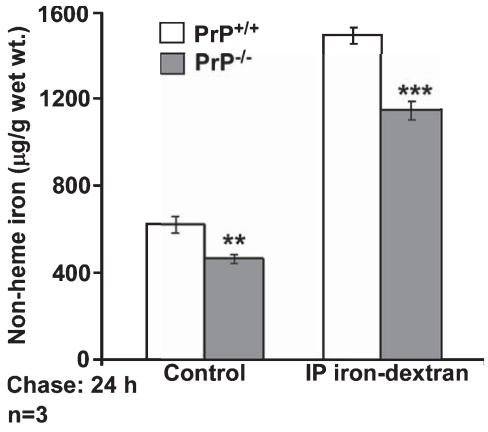


Supplementary Figure 4. PrP levels in PrP^{C}, M17, and different mutant PrP cell lines. A) Diagrammatic representation of mutant PrP forms checked for ferrireductase activity in this study. B) Lysates of M17 and transfected cells were subjected to western blotting and probed for PrP. PrP^{C} and all mutant PrP forms show the expected migration on SDS-PAGE. C) Quantitation of PrP bands after normalization with β-actin shows 5–15-fold higher expression of PrP^{C} and mutant PrP forms relative to non-transfected M17 controls.



Supplementary Figure 5. Iron stores are reduced in $PrP^{-/-}$ mice. Non-heme iron in the liver tissue of $PrP^{-/-}$ mice is significantly less than matched $PrP^{+/+}$ controls ($^{***}p < 0.001$). Intraperitoneal (IP) inoculation of iron dextran increases liver iron by ∼2.3 fold in both mouse lines, but $PrP^{-/-}$ samples fail to reach the levels similar to matched $PrP^{+/+}$ controls. ($^{***}p < 0.001$, $^{**}p < 0.01$).

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