

### Supplemental Information for Chakrabarti *et al.* 2014

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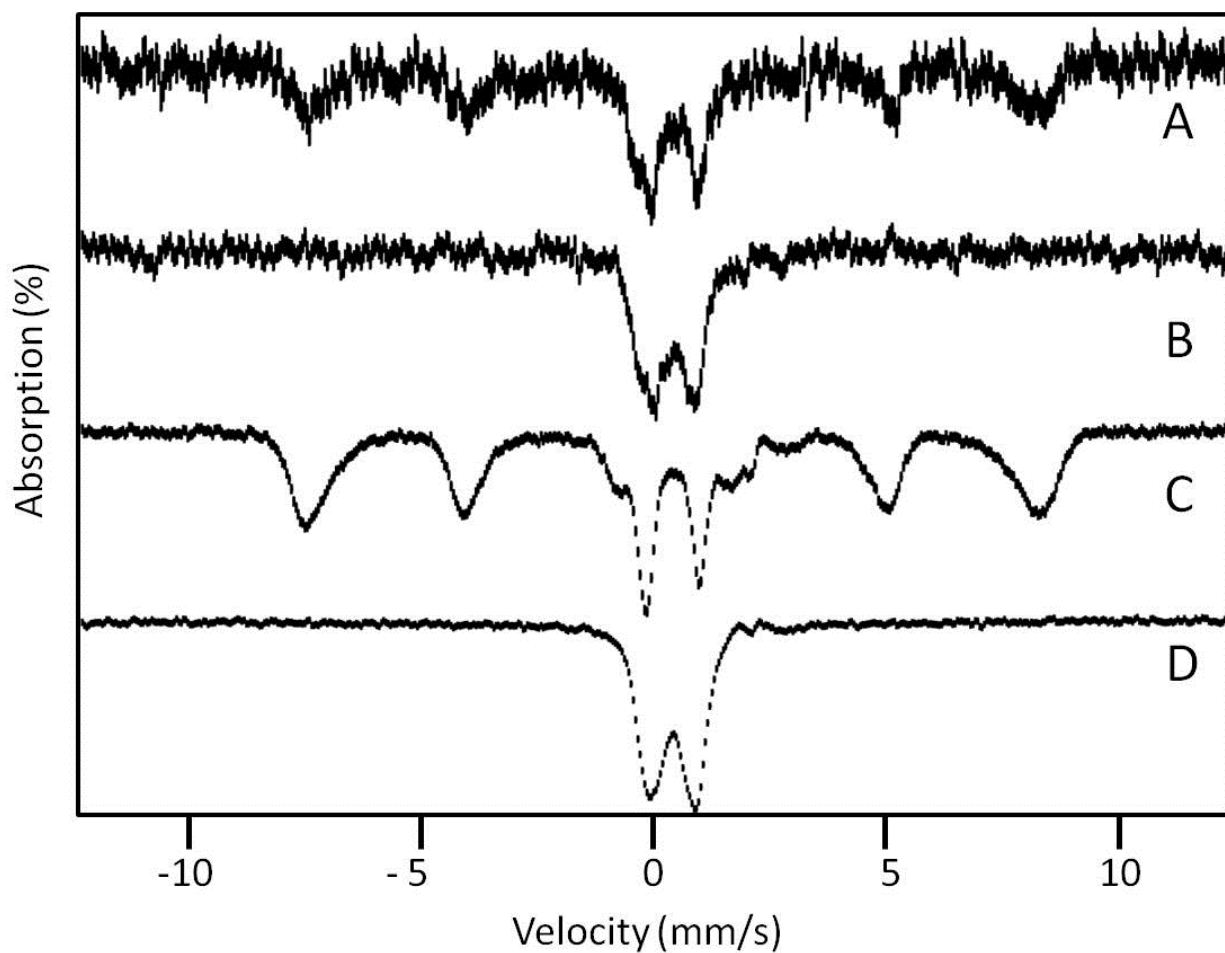
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**1. Details of Mitochondria Isolation:** Mitochondria were isolated by combining the livers of 14 WT mice (both genders, with ages ranging from 3 – 6 months). Freshly isolated livers were suspended in 2-3 volumes of degassed mitochondria-isolation-buffer (MIB), consisting of 215 mM mannitol, 75 mM sucrose, 5 mM HEPES and 1 mM EGTA at pH 7.4. Cells were disrupted anaerobically by nitrogen cavitation at 800 psi for 15-20 min. The resulting extract was centrifuged at 2000 g for 5 min. The supernatant was centrifuged again, at 18000 g for 15 min. The pellet, containing crude mitochondria, was resuspended in a minimum volume of MIB and layered over discontinuous gradients of equal volumes of 17% and 35% histodenz in MIB. The layered assembly was centrifuged at 30000 rpm for 1 hr using a Beckman Optima L-90K ultracentrifuge with a SW 32 Ti rotor. Mitochondria were collected from the 17%-35% interface and were washed with MIB. Using the same rotor, they were packed into MB cups at 12,000 rpm for 1 hr, and then frozen in LN<sub>2</sub>.

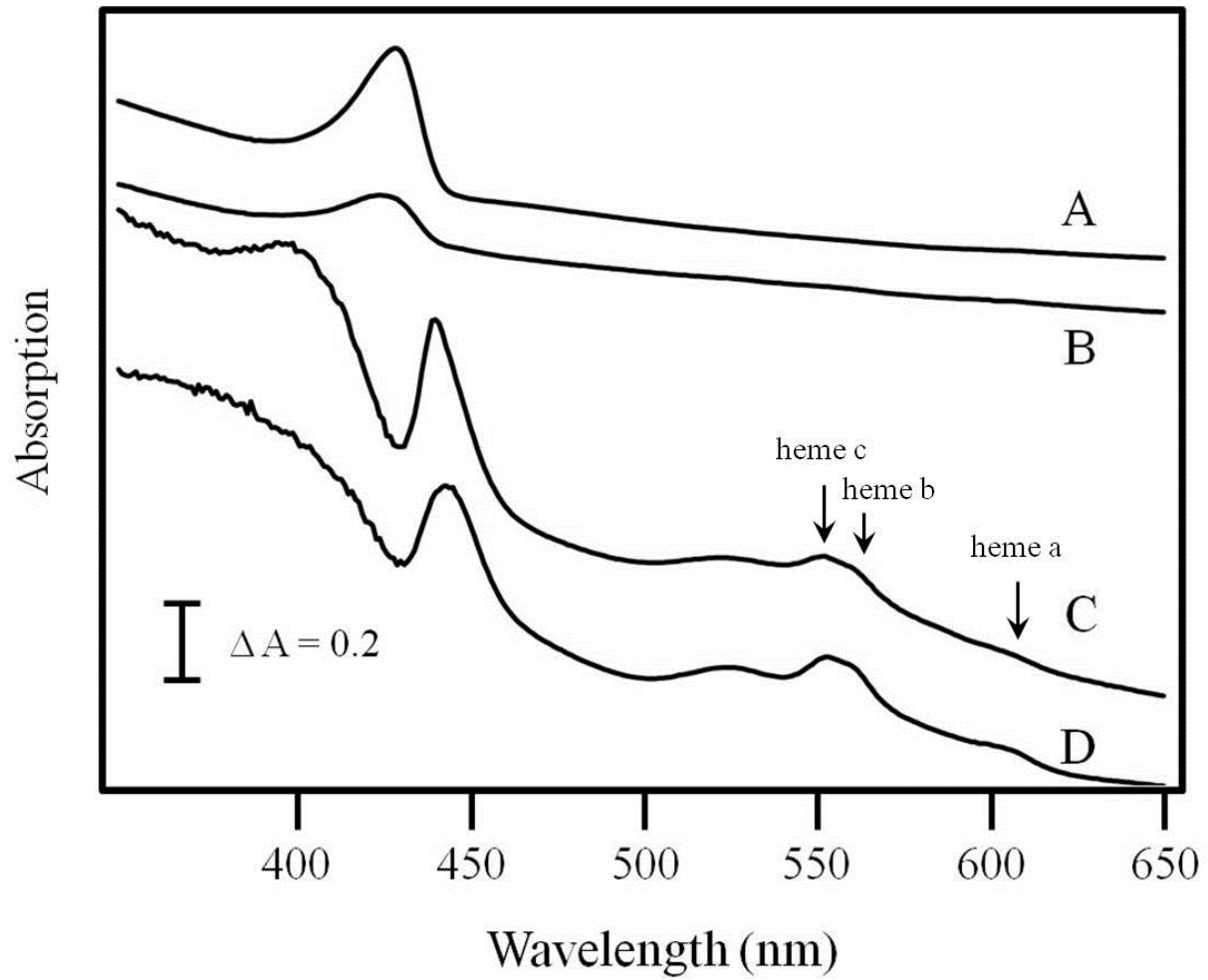
**2. Table S1.** Iron concentration of mice livers. All values are given in  $\mu\text{M}$ .

| Age (d) | Number of samples (n) | Observed Liver Iron concentration (Not flushed) | Observed Liver Iron concentration (flushed) | Blood Iron concentration | Endogenous Liver Iron concentration | Reference  |
|---------|-----------------------|---|---|--------------------------|-------------------------------------|------------|
| 0       | 12                    | $2300 \pm 640$                                  |   | $990 \pm 120$            | $1300 \pm 380$                      | This study |
| 1       | 1                     | 1820  |   | 760                      | 1060                                | This study |
| 7       | 4                     | $900 \pm 190$                                   |   | $270 \pm 90$             | $630 \pm 150$                       | 35         |
| 7       | 1                     | 540   |   | 190                      | 350                                 | This study |
| 14      | 4                     |   | $440 \pm 60$                                | $90 \pm 40$              | $360 \pm 60$                        | 35         |
| 21      | 4                     |   | $380 \pm 40$                                | $80 \pm 40$              | $300 \pm 40$                        | 35         |
| 21      | 1                     |   | 380   | 130                      | 250                                 | This study |
| 21      | 1<br>Fe def.          |   | 70  | 10                       | 60                                  | This study |
| 28      | 4                     |   | $340 \pm 60$                                | $70 \pm 30$              | $270 \pm 60$                        | 35         |
| 35      | 4                     |   | $440 \pm 120$                               | $40 \pm 40$              | $350 \pm 110$                       | 35         |
| 42      | 3                     |   | $540 \pm 190$                               | $90 \pm 50$              | $430 \pm 160$                       | 35         |
| 42      | 1                     |   | 550   | 110                      | 440                                 | This study |
| 49      | 2                     |   | $560 \pm 50$                                | $110 \pm 60$             | $440 \pm 70$                        | 35         |
| 112     | 1                     | $3800 \pm 280$                                  |   |                          |                                     | This study |
| 443     | 1<br>IRP2(-/-)        |   | 3900  | 590                      | 3300                                | This study |
| 773     | 1                     |   | 710   | 160                      | 660                                 | This study |

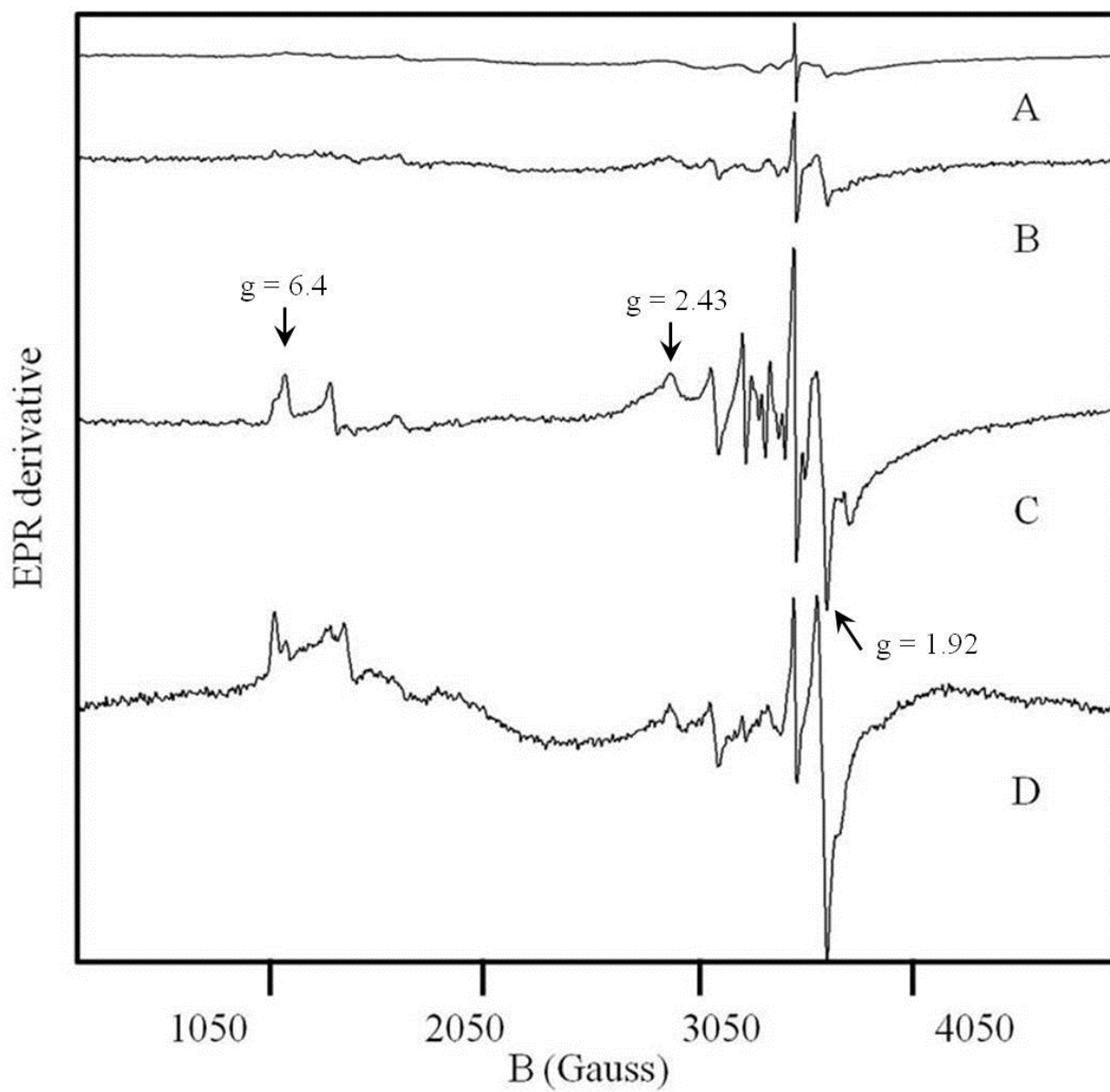
3. **Figure S1.** 0.05 T Mössbauer spectra of 1 wk (A, B) and 16 wk (C, D) mouse liver. A & C were collected at 5 K. B and D were collected at 70 K. Near-flat base line of the 70 K spectra show that mouse livers did not contain significant levels of hemosiderin.



4. **Figure S2.** UV-vis spectra of livers isolated at different ages. **A**, 1 dy; **B**, 1 wk; **C**, 3 wk; **D**, 96 wk. Contribution due to hemoglobin has been removed.



5. **Figure S3.** EPR spectra of packed liver homogenates from mice of different ages. **A**, 1 dy; **B**, 1 wk; **C**, 3 wk; **D**, 96 wk. Conditions: Temperature, 10 K; microwave frequency, 9.43 GHz; microwave power, 0.2 mW; modulation amplitude, 10 G.



**6. Table S2.** Percentage spectral contribution and corresponding concentration the different spectral features obtained by Mössbauer and ICP-MS analysis. Values derived from Mössbauer analyses have an absolute estimated uncertainty of  $\pm 4\%$ .

|                  | Ferritin<br>%, [ $\mu\text{M}$ ] | C.D<br>%, [ $\mu\text{M}$ ] | NHHS<br>%, [ $\mu\text{M}$ ] | Total Fe<br>[ $\mu\text{M}$ ] | Total Mn<br>[ $\mu\text{M}$ ] |
|------------------|----------------------------------|-----------------------------|------------------------------|-------------------------------|-------------------------------|
| 1 dy             | 96, [1020]                       | 3, [30]                     | 0                            | 1060                          | 4                             |
| 1 wk             | 60, [210]                        | 30, [110]                   | 3, [10]                      | 350                           | 8                             |
| 3 wk             | < 20,<br>[< 50]                  | 70, [180]                   | 8, [20]                      | 250                           | 140                           |
| 3 wk<br>(Fe def) | 0-5, [0-3]                       | 80, [50]                    | 15, [10]                     | 60                            |                               |
| 6 wk             | 50, [220]                        | 40, [180]                   | 0-5, [0-20]                  | 440                           | 20                            |
| 96 wk            | 66, [430]                        | 26, [170]                   | 0-5, [0-40]                  | 660                           | 20                            |
| IRP 2(-/-)       | 95, [3200]                       | < 3,<br>[< 100]             | 0-1, [0-30]                  | 3330                          | 10                            |

7. **Figure S4.** 5K, 0.05 T Mössbauer spectrum of the brain from a 63 wk IRP2(-/-) mouse. The red line is a simulation with 68% and 22% spectral contribution due to ferritin and the CD respectively. Contribution due to blood (20% of the spectral intensity was removed). The line at ca. 0.7 mm/s is probably an artifact; the spectrum of a 15 wk IRP2(-/-) brain was very similar but did not include this line.

