Supporting Information

For

The ferroxidase hephaestin but not amyloid precursor protein is required for ferroportin supported iron efflux in primary hippocampal neurons

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Fig.s1 Ceruloplasmin (Cp) is not detected at the protein level in rat primary hippocampal neuronal cultures (a) RT-PCR demonstration of GPI-Cp (440bp) and sCp (196bp) transcripts in neuronal the mRNA pool. (b) Probing for Cp by western blot in rat primary hippocampal neuronal lysates and conditioned media. No detectable GPI-linked or secreted Cp was observed. Human sCp (10ng) was used as a positive control.



Fig.s2 Validation of Hp antibody (a) 100 µg total (T) or intracellular (I) protein, or surface (S) protein from 100 µg a total protein sample were loaded in each lane. (b) PNGase F catalyzed N-deglycosylation in rat primary hippocampal neurons. Total cellular protein (20 µg) was incubated with PNGase F at 37 °C for 16 h. Arrow heads indicate Hp of the expected moecular mass. Stars indicate oligomer or post-translationally modified forms of the protein, respectively.



Fig.s3 Quantitative RT-PCR for transferrin receptor 1 (TfR1) mRNA isolated from wild type or *HEPH* knockout mouse neurons. Statistical significance was tested by unpaired t test. ***, p<0.001.