Supporting Information

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Methods:

All chemicals were obtained from Sigma Aldrich (Spain). Recombinant human H-chain apoferritin (apo-huFH) and L-chain apoferritin (apo-huFL) were purchased from MoLiRom (Italy). UV-vis absorption was measured with a NanoDrop 2000c spectrometer (Thermo Scientific).

Preparation of Pt- and Au-apoferritin. After removing the dimers and trimers by size exclusion gel filtration, monomeric apoferritin was incubated with aqueous K_2PtCl_4 solutions with molar ratios of 1/15000 at pH 7.4 with phosphate buffered saline (PBS). The solution was mixed in darkness at 30 ^oC for 2 hours, before excessive salt was removed with the Zeba Desalting Column (40kDa MWCO, Thermo Scientific). In order to avoid the formation of metal particles outside the protein shell, apoferritin, desalted with the spin columns after the incubation with metal salts, was immediately mixed with 1/20 (v/v) 1 M $NaBH_4$ and stirred for 20 min at 30 ^oC. After the reduction, the apoferritin-containing platinum nanoparticles (Pt-apo) were purified twice with spin columns (40kDa MWCO). A similar procedure was used for preparation of Au-apo. For the incubation, a molar ratio of apoferritin to $HAuCl_4$ of 1:50 was applied.

All samples were again purified with a desalting column (7kDa MWCO) in advance of all activity assays. Before use, the desalting columns were extensively washed with water to remove all NaN_3, which was added as preservative (0.05%) to the original column liquid phase. After the preparation, the protein concentration was determined with the Pierce 660nm Protein Assay Kit (Thermo Scientific Pierce).

Kinetic ferroxidase assay: The assay took place at 30 °C with a mixture of 0.5 mg/ml protein (ca. 1 µM), 50 mM Tris-HCl buffer pH7.0 and 0.1 mM $(NH_4)_2Fe(SO_4)_2$. The reaction started with the addition of freshly prepared Fe^{2+}. The absorption in the range of 220-700 nm was taken continuously in a 10 s interval for total 5 min.

***Xylenol orange assay*:** The assay took place at 30 °C with a mixture of 0.1 mg/ml protein (ca. 0.2 µM), 50 mM Tris-HCl buffer pH7.0 and 40 µM $(NH_4)_2Fe(SO_4)_2$. The reaction started with the addition of Fe^{2+}. A 10 µl aliquot was taken from the reaction at 0, 5, 10, 20, 30 and 40 min. and mixed with a 100 µl solution of 125 µM Xylenol Orange (XO) and 25 mM H_2SO_4. After incubation at RT for 20 min, the concentration of ferric ions in the aliquots was determined by measuring the absorbance at 595 nm. The autoxidation of the ferrous ions was controlled in the same assay conditions, but without addition of proteins. The aqueous solutions of $(NH_4)_2Fe(SO_4)_2$ with 25 mM H_2SO_4 were freshly prepared for each assay.

The Zn^{2+} resistance was measured by adding 10 mM $ZnCl_2$ to the reaction mixture. The influence on the XO assay was determined by adding 10 µl 550 mM $ZnCl_2$ to 100µl 125µM XO with 25 mM H_2SO_4 and the absorbance at 595 nm was measured for 15 min.

Mineralization assay: To determine the iron encapsulated in the apoferritin or Pt-ferritin, after every hour 0.1 mM $(NH_4)_2Fe(SO_4)_2$ was added to the solution of 0.1 mg/ml protein in 50 mM Tris-HCl buffer pH7.0 and incubated at room temperature. After five hours the solution was passed through a gel filtration column (7 kDa MWCO). 5 mM EDTA was added to the solution, which was collected for 2h, and followed by a further purification with the gel filtration column. The iron content of apoferritin after the ferroxidase assay was determined with the Ferene S assay.

Cell culture

The grade of the chemicals used for cell tests were all suitable for cell culturing. The solutions for in vitro experiments were either obtained sterile or filtrated with sterile filters with 0.1 μm pore size.

The human colon adenocarcinoma cell line Caco-2 was obtained from the European Collection of Cell Cultures (Sigma-Aldrich, Spain). For the experiments (passages 48-62) were cultured at 37°C (90% humidity, 5% CO_2) in minimum essential medium (MEM; PAA) supplemented with 10% fetal bovine serum (FBS) (Biochrom), 1% nonessential amino acids and 50 μg/mL gentamicin (PAA). For the assays, Caco-2 cells at 80–90% confluence were detached with trypsin/EDTA (0.05%/0.02%) and seeded onto disposable non-fluorescent multi-well plates at a density of 0.75×10^5 cells cm^{-2} and cultured for 8 d. The culture medium was replaced 1 d after seeding, then every 2 d, and 1 d before the assay.

The human liver carcinoma cell line Hep G2 was obtained from the American Type Culture Collection (ATCC, No. HB-8065). The cells between passages 12-16 were used for the experiments. The cells were maintained in MEM supplemented with 10% FBS, 1 mM pyruvate, 1% nonessential amino acids and 50 μg/mL gentamicin. For the assays, Hep G2 cells at 60-70% confluence were detached with trypsin/EDTA (0.05%/0.02%) and seeded onto disposable non-fluorescent multi-well plates at a density of 0.75×10^5 cells cm^{-2} and cultured for 3 d. The culture medium was replaced 1 d after seeding and 1 d before the assay.

For the iron challenging, 50 μM ferric citrate was added to the medium. Zinc sulfate was used for the cell treatment in a final concentration of 40 μM. The intracellular iron was determined with the Ferene S assay.

The cell viability was determined with the cell counting kit-8 (CCK-8) according to the manufacturer's protocol. The absorbance at 450 nm was measured with a plate-reader (Victor X5, PerkinElmer).

The two cell lines were authenticated by the LGC Standards (Germany) at the end of the project.

Ferene S assay: For intracellular iron estimation, the cells were washed twice with PBS and a freshly prepared assay solution of 1 mM Ferene S (3-[2-pyridyl]-5, 6-bis(2-[-furyl sulfonic acid]-2, 4-triazine), 6 M guanidin hydrochlorid and 0.5 M ascorbic acid was added to the wells (250 µL/well for 24-well plate). The absorbance at 595 nm was measured after incubation for 2 h at room temperature with a plate-reader (Victor X5, PerkinElmer).

For the assay with protein samples, the 10 µl protein sample was mixed with 150 µl assay solution, followed with the same procedure as mentioned above.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were separated with a 14% SDS-polyacrylamide gel. After electrophoresis, the gel was stained with Imperial Protein Stain (Thermo Scientific). The intensity quantification of the proteins was performed with the Kodak Molecular Imaging Software.

Protein detection with Western Blotting: The cellular membrane fractions were collected with the Mem-PER Plus Membrane Protein Extraction Kit (Thermo Scientific). 25 µg membrane proteins were separated with 4-20% gradient Tris-glycine polyacrylamide gels for SDS-PAGE (Thermo Scientific) and electroblotted onto polyvinylidene fluoride (PVDF) (BioRad). PVDF membranes were blocked for 2 h at room temperature in 5% BSA–Tris-buffered saline/Tween 20 (TBST; 25 mM

Tris HCl, pH 7.5/150 mM NaCl/0.05% Tween 20). Next, the membranes were incubated overnight at 4°C with 1 µg/ml rabbit anti-ferroportin and anti-transferrin receptor antibodies. The day after, the membranes were washed four times with TBST and incubated at room temperature for 1 h with a peroxidase-conjugated anti-rabbit antibody (Thermo Scientific) in 1:1000 as the secondary antibody. The rabbit anti-Na^+/K^+ATPase antibody (1:15000) from Abcam was used to detect ATPase in membrane fractions. For the enhanced chemiluminescence detection the Amersham ECL Prime detection reagent was used and the signal was recorded with the C-DiGit Blot Scanner (LiCor Bioscience).

Statistic analysis: All values were expressed as the mean ± SD. The significance was analyzed with one-way ANOVA. The comparison between groups was performed with the unpaired two-tailed Student's t-test.

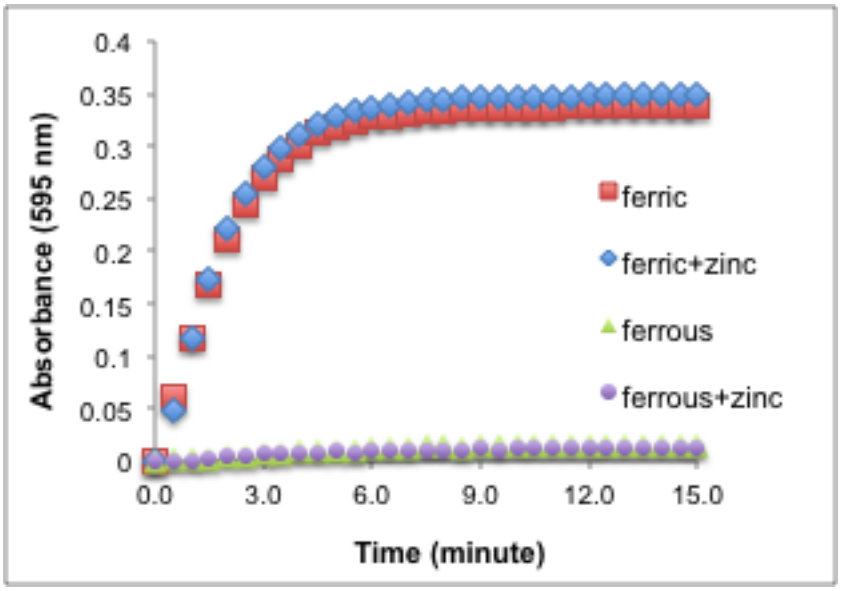

Figure S1: Influence of Zn^{2+} on the XO assay. 50 mM $ZnCl_2$ together with 25 µM $FeCl_3$ or 100 µM $(NH_4)_2Fe(SO_4)_2$ was added to the assay solution. The absorption at 595 nm was measured for 15 min in intervals of 30 s.

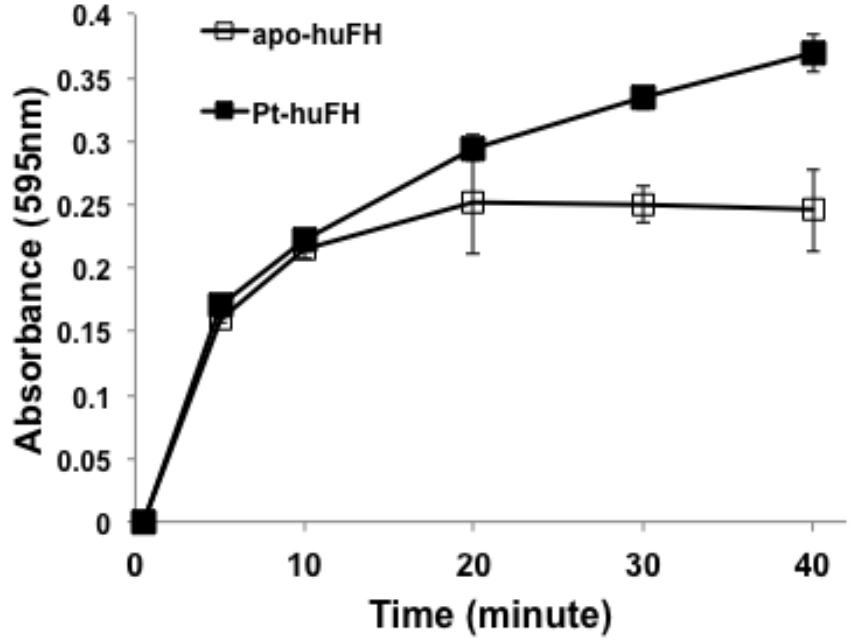

Figure S2: The ferroxidase activities of apo- and Pt-huFH measured with the XO assay. The assay was performed with 0.2 μM protein, 10 μM Fe^{2+} and 50 mM Tris at pH 7.0. The results are presented by the mean ± SD**.**

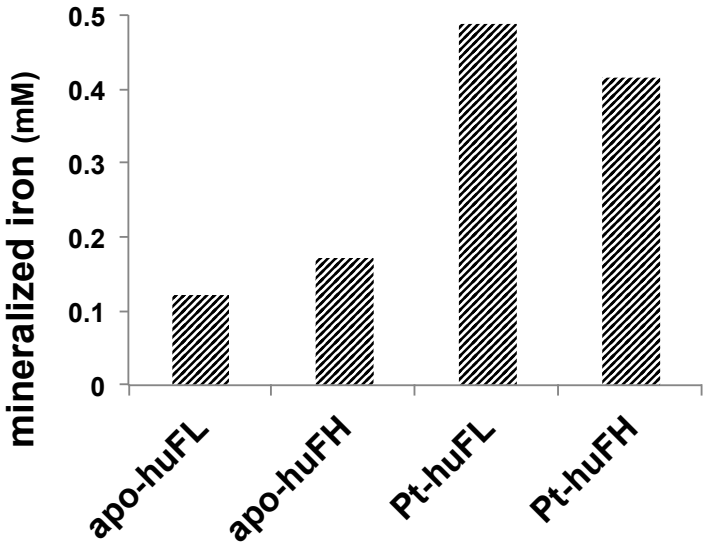

Figure S3: Quantification of mineralized iron in the protein after the mineralization reaction.

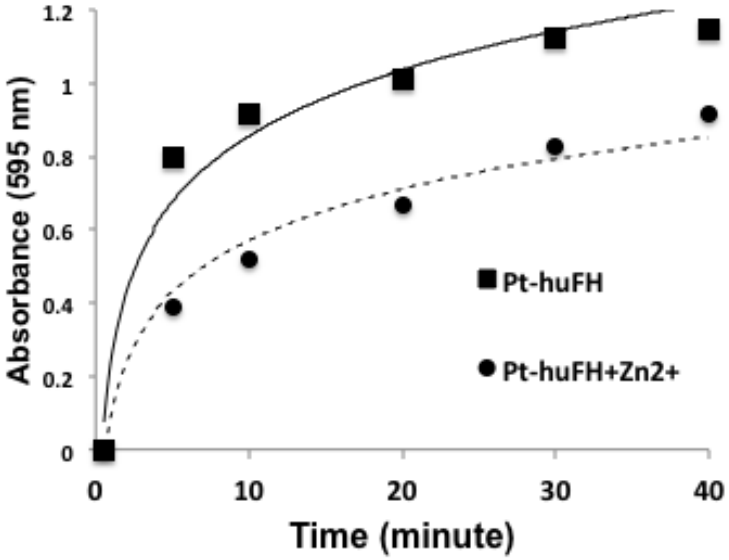

Figure S4: Inhibitory effect of 10 mM Zn^{2+} on the ferroxidase activity of Pt-huFH.

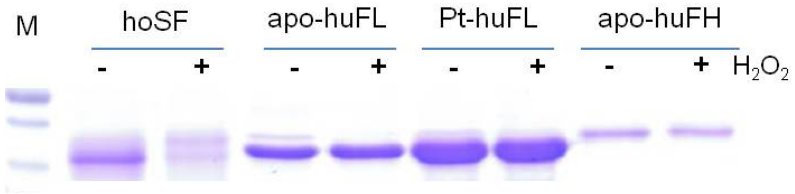

Figure S5: SDS-PAGE of native horse spleen ferritin (hoSF), recombinant human L and H apoferritin (apo-huFL and apo-huFH), Pt-huFL, and Pt-huFH after treatment with 100 mM H_2O_2. M: protein weight marker.

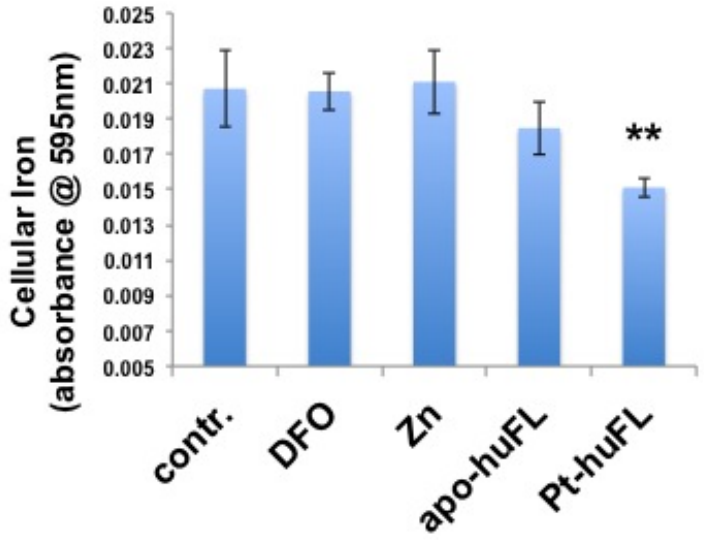

Figure S6: Cellular iron content of HepG2 cells after treatment with deferoxamine (DFO, 300 µM), apo- and Pt-huFL (50 µg/ml) for 24 h. The values are presented by mean ± SD. **: $p < 0.001$.

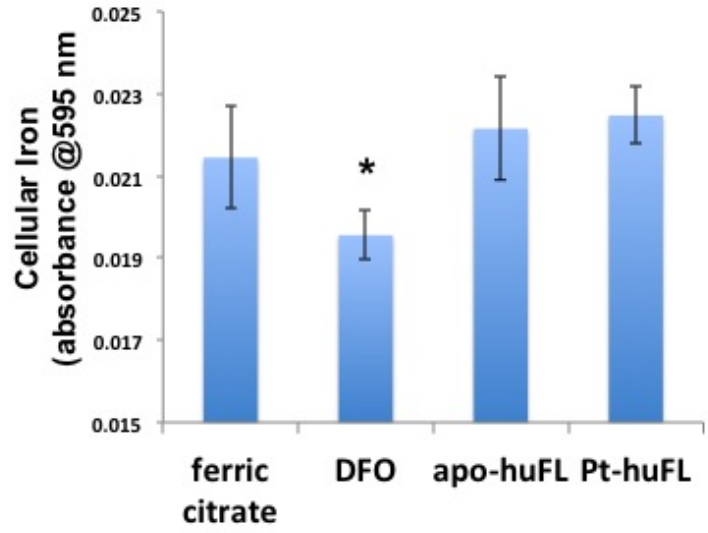

Figure S7: Cellular iron content of Caco-2 cells after the iron challenging with 40 µM Fe^{2+} together with 300 µM DFO , apo- and Pt-huFL (50 µg/ml) for 24 h. The values are presented by mean ± SD. *: $p < 0.05$.