Supplemental Figure S1.

Supplemental Figure S1. **Knockdown of ZIP14 decreases TBI uptake by wild-type HepG2 cells.** *A*, HepG2 cells were transfected with negative control or ZIP14 siRNA for 72 h prior to Western blot analysis for ZIP14 and actin as a lane loading control. *B*, Knockdown of ZIP14 decreases the uptake of TBI. HepG2 cells transfected with control or ZIP14 siRNA for 72 h were incubated with 100 nM ^{55}Fe-TF for 1 h. Cells were harvested and cell-associated radioactivity was determined by scintillation counting. The amount of TBI taken up by cells is expressed as pmol ^{55}Fe-TF/10^6 cells/hour. Data represent the mean ± SD of three independent experiments.

Supplemental Experimental Procedures

Cell culture—HepG2 cells were maintained in Minimum Essential Medium Eagle (MEM) (Life Technologies) supplemented with 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids (Life Technologies) and 10% fetal bovine serum (FBS).

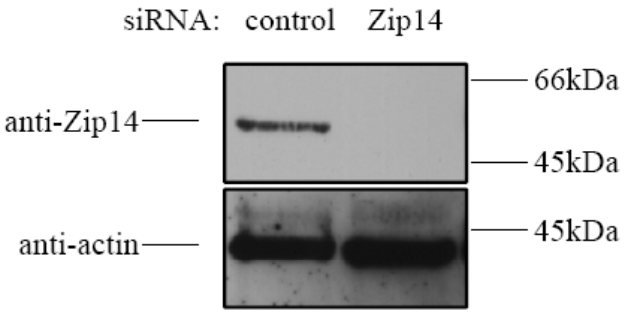
^{55}Fe-Tf uptake—Cells grown in a 12-well plate were washed twice and equilibrated in DMEM with 20 mM Hepes (pH 7.4) for 15 min at 37 °C, 5% CO_2. Wash medium was replaced with 0.5 ml of uptake medium containing 100 nM ^{55}Fe-Tf in DMEM supplemented with 20 mM Hepes (pH 7.4) and 2 mg/ml ovalbumin, pH 7.4. After 1 hour, cells were placed on ice, and externally bound ^{55}Fe-Tf was stripped with an acidic buffer (0.2 N acetic acid, 500 mM NaCl, 1 mM $FeCl_3$) for 3 min. Cells were solubilized in 0.5 ml of solubilization detergent (0.1% Triton X-100, 0.1% NaOH). Lysates were mixed with 6 ml UniverSol scintillation fluid and the radioactivity was counted for 10 min in a scintillation counter.

Knockdown of Zip14 using siRNA—Lipofectamine RNAiMAX transfection reagent (Invitrogen) was used to transfect siRNA specific for human Zip14 (Dharmacon/Thermo Scientific) or negative control siRNA into cells at final concentration of 10 nM following the manufacturer's instructions. Briefly, 2 µl Lipofectamine RNAiMAX and 12 pmol RNAi duplex were mixed in 200 µl Opti-MEM medium and added into each well of a 12-well plate. After incubation at room temperature for 15 min, ~2 x 10^5 cells in 1 ml MEM supplemented with 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids and 10% FBS were added to each well. Three days later, Zip14 protein levels were detected using immunoblots to determine the efficiency of knockdown.

Western blotting—Cells were washed with cold PBS twice and lysed on ice in NET-Triton buffer (150 mM NaCl, 5 mM EDTA, 10 mM Tris, 1% Triton X-100, pH 7.4) with 1 x Complete Mini Protease Inhibitor Cocktail (Roche Applied Science) and 1 mM phenylmethylsulfonyl fluoride (PMSF). The cell lysate was centrifuged at 16,000 x *g* for 5 min, and the supernatant was kept. Protein concentrations of the cell extracts were measured using the BCA Protein Assay (Pierce). The cell extracts were reduced and denatured with Laemmli buffer for 5 min at 95 °C and subjected to SDS-PAGE on 12% gels. Protein was transferred to nitrocellulose. Immunoblot analysis was carried out using rabbit anti-Zip14 (1:2,000) and mouse anti-actin (1:10,000, Sigma) followed by goat anti-rabbit or anti-mouse secondary antibodies conjugated to horseradish peroxidase (1:10,000, Chemicon, Temecula, CA). Bands were detected by enhanced chemiluminescence (SuperSignal West Pico, Thermo Scientific).

Supplemental Figure S1

A



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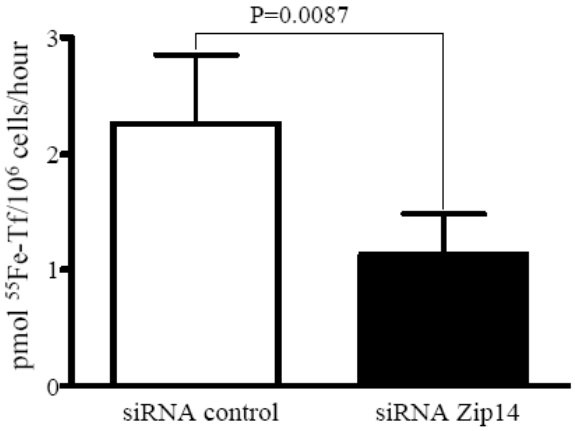

Supplemental Table 1

Table 1. List of primers used for PCR to generate and screen for endogenous Zip14-3×Flag in HepG2 cells.

Primer set	Forward primer	Reverse primer
Primers for knock-in		
Left arm	5'-GGGAAAGUGCAACCTTGAACTCCGGAGC-3’	5’-GGAGACAUTCCCCAATCTGGATCTGTCCTGAAT-3’
Right arm	5'-GGTCCCAUGGCTCTGCCAAGAGCCTG-3’	5'-GGCATAGUGCACCCCATTTCTACAAGTCAGC-3’
Cre-specific primers	5'-ATATTGCGGCCGCAAGCTTGGCCCATTGCATAC-3’	5'-AATAAGCGGCCGCCGCGTTAATGGCTAATCGCC-3’
Screening primers	P1: 5'-CCATTCAGCGGTTTTTAAGGGGGC-3’	P2: 5'-GTTGTGCCCAGTCATAGCCG-3’
	P3: 5'-GGATTCATCGACTGTGGCCG-3’	P4: 5'-CAAGGGCTCCACAGTGGCTAAG-3’
	P5: 5'-GGCCTCCTGACTGGATTCACC-3’	P6: 5'-GAGACTGGTTACCAGGGCAGC-3’