Electronic Supplementary Material (ESI) for Metallomics.

Supplementary data to:

Compound-specific adaptation of hepatoma cell lines to toxic iron

Sarah Guttmann[1], Elisabeth Therese Dewald[1], Cathrin Wohlfarth[1], Jennifer-Christin Müller[2], Uwe Karst[2], Hartmut H. Schmidt[1], Andree Zibert[1]

[1]Medizinische Klinik B für Gastroenterologie und Hepatologie, Universitätsklinikum Münster, Münster, Germany

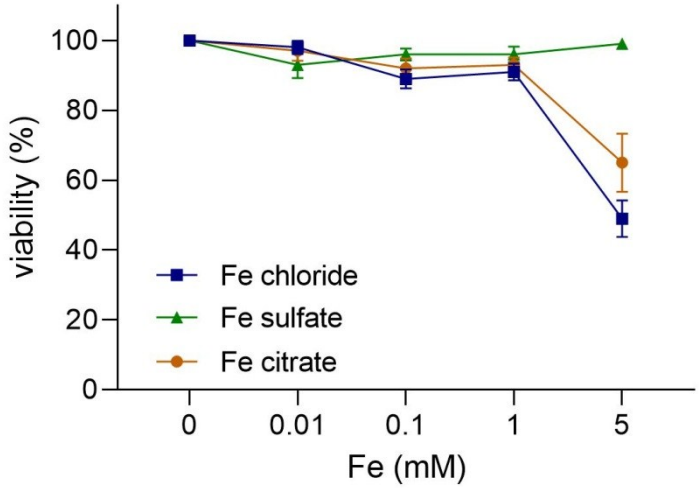
[2]Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany

Supplementary Table 1. Primers used for RT-qPCR analysis

Gene Symbol	Synonym	Accession Number	Forward/Reverse (5'-3')
BMP6	Bone morphogenetic protein 6	NM_001718	AGCCTGCAGGAAGCATGAG/AACCAAGGTCTGCACAATCG
CP	Ceruloplasmin	NM_000096	CACGGCCATAGCTTCCAATAC/CCAAATTCCAGGTGTTCTTGG
DMT1	Divalent metal transporter 1	NM_001174127	GGGTTGGCAATGTTTGATTG/GCGTCCATGGTGTTCAGAAG
FBXL5	F-box and leucine rich repeat protein 5	NM_012161	CTGCAGGATTTGGTTTCAGC/CTGCAAATTCTGGCATCCAC
FPN1	Ferroportin 1 (SLC40A1)	NM_014585	TGTCCCGGAGACAAGTCCTG/CAAAGGACCAAAGACCGATTC
FTH1	Ferritin heavy chain 1	NM_002032	TGCACAAACTGGCCACTGAC/CGTGGTCACCCAATTCTTTG
FTL	Ferritin light chain	NM_000146	GATCTTCATGCCCTGGGTTC/GGTGGTCACCCATCTTCTTG
FXN	Frataxin	NM_000144	CGCCAAACAAGCAAATCTGG/AGCAGCTCATGGAGGGACAC

GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046	CCCACTCCTCCACCTTTGAC/CCACCACCCTGTTGCTGTAG
HAMP	Hepcidin antimicrobial peptide	NM_021175	CAACAGACGGGACAACTTGC/CTTCGCCTCTGGAACATGG
HFE	Hemochromatosis	NM_000410	CGTCTGGCACCCTAGTCATTG/TCTTGAACCCTGCCTCTTCC
HIF1A	Hypoxia inducible factor 1 alpha subunit	NM_001530	GGCAATCAATGGATGAAAGTG/CAGTAGGTTTCTGCTGCCTTG
HMOX1	Heme oxygenase 1	NM_002133	GAGCTGCTGACCCATGACAC/GGGCAGAATCTTGCACTTTG
HP	Haptoglobin	NM_001126102	GACACCTGGTATGCGACTGG/CCCAGTCCTGGATGGAAGTC
IRP1	Iron responsive element binding protein (Aconitase 1)	NM_001278352	CACAGGGCAAGAACGATACAC/TGACAGCCTGGAAGGTCTTG
IRP2	Iron responsive element binding protein 2	NM_004136	CAGAGACTGGGCTGCCAAAG/GAAGTGGAGCTATGCCAATTCC
LRP1	LDL receptor related protein 1	NM_002332	CTGGTATAAGCGGCGAGTCC/GCTCTCCGCCTTCGTACATC
MDR1	Multi Drug Resistance Protein 1	NM_000927	TCGTGCCCTTGTTAGACAGC/CCAAGAAGCCCTGGACAAAG

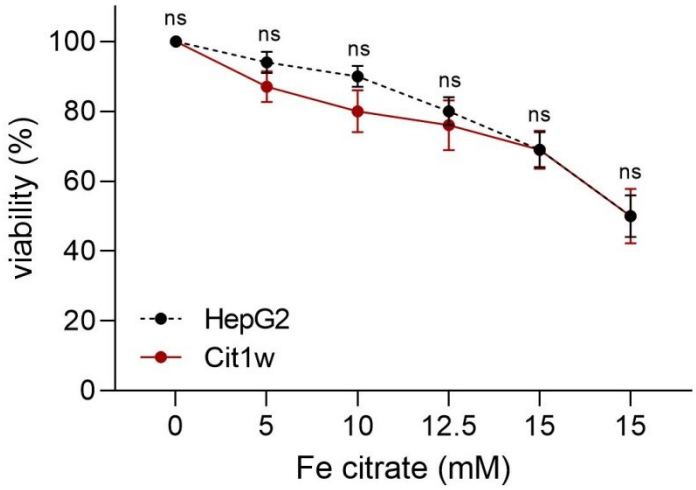
MFRN2	Mitoferrin-2 (SLC25A28)	NM_031212	TCATGATGCAGCCATGAACC/GTCTGTCACCCGGTGGTATG
MT1	Metallothionein 1	NM_005952	CTCCTTGCCTCGAAATGGAC/GCATTTGCACTCTTTGCATTTG
NCOA4	Nuclear receptor coactivator 4	NM_001145260	CCTGCCAGGAAAGAAGATGG/CTTCCTGGGCCTTCTTTCG
NEO1	Neogenin 1	NM_002499	GAAGTGCAGGAGACCACAAGG/AGGTGGGCCATCTCTTTGG
SLC13A5	Solute carrier family 13 member 5	NM_001143838	GCATCGTGCTGCTACTAGGG/ CAAGGGCTCCATCTGCTTC
SOD2	Superoxide dismutase 2	NM_000636	CAAATTGCTGCTTGTCCAAATC/TAAGCGTGCTCCCACACATC
TF	Transferrin	NM_001063	GATAAGGAAGCTTGCGTCCAC/TTGCCCGAGCAGTCAGTTAC
TFR1	Transferrin receptor	NM_001128148	GAGAGGTACAACAGCCAACTGC/TGTAAACTCAGGCCCATTTCC
TFR2	Transferrin receptor 2	NM_003227	AGAGACGAGCGACTGACACG/ATGAAGATGTGGCGGAACG



Supplementary Figure S1

Compound-specific toxicity after long-term treatment of HepG2 cells.

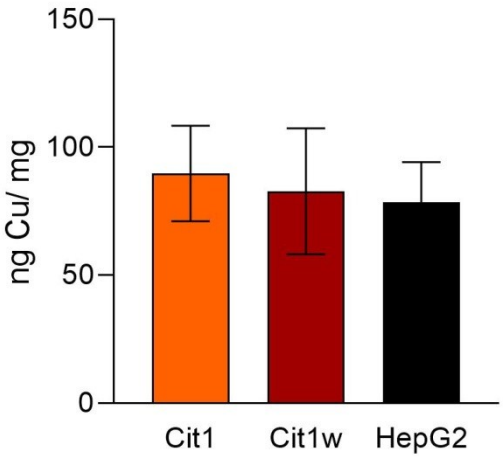
HepG2 cells were cultivated in presence of Fe chloride, Fe sulfate or Fe citrate for 5 days and subjected to MTT assay. Viability of cells was determined relative to untreated cells (100%). Mean ± SEM is given (n=3).



Supplementary Figure S2

Weaned Cit1w cells are sensitive to iron citrate.

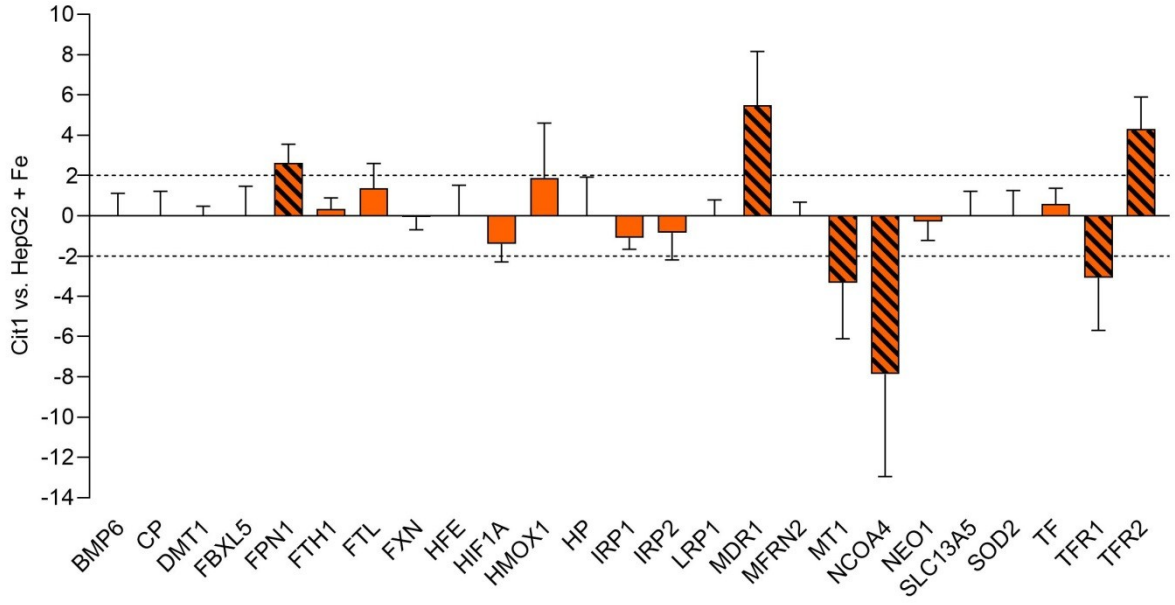
Cit1 cells that were cultivated in the absence of iron citrate (weaned) for several days were subjected to MTT assay. Cells were treated with iron for 48 h. Viability of cells was determined relative to untreated cells (100%). For comparison, results of parental HepG2 cells are denoted (dotted line). Mean ± SEM is given (n=3). *P < 0.05. ns, not significant.



Supplementary Figure S3

Cellular copper accumulation is not affected after long-term treatment of hepatoma cells.

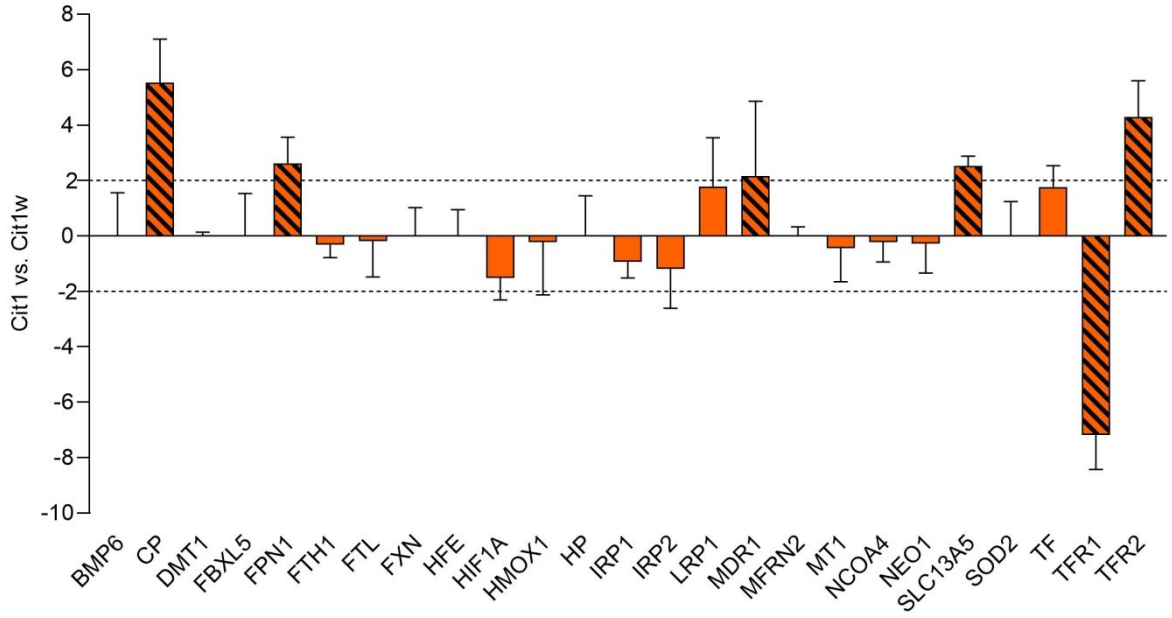
Intracellular copper was determined by TXRF in Cit1, Cit1w and HepG2 cells after 4 h incubation with 10 mM iron citrate. Mean ± SEM is given (n=3).



Supplementary Figure S4

Comparison of gene expression in Cit1 cells versus short-term iron treatment of parental HepG2 cells.

Gene expression of Cit1 cells was compared to parental HepG2 cells that were treated with iron citrate for 24 h. The difference of fold change expression in Cit1 cells relative to untreated HepG2 cells is depicted. Dotted line indicates threshold of a fold ±2. Genes above threshold are highlighted (stippled). Mean/SE are given (n=3-6).



Supplementary Figure S5

Comparison of gene expression in Cit1 cells versus weaned Cit1w cells.

Gene expression of Cit1 cells was compared to weaned Cit1w cells. Note that Cit1 cells were permanently cultivated in 10 mM iron citrate whereas Cit1w cells were weaned for several days. The difference of fold change expression in Cit1 cells relative to untreated Cit1w cells is depicted. Dotted line indicates threshold of a fold ±2. Genes above threshold are highlighted (stippled). Mean/SE are given (n=3-6).