Supplementary Materials and Methods

Preparation of cellular extracts

Cellular extracts for Enzyme Linked Immunosorbent Assay (ELISA), Western Blotting (WB), Oxyblot, Immunoprecipitation (IP) and ferritin estimation with radiolabeled iron were obtained by lysing cells in 100 mM Tris–HCl pH 8.0, 140 mM NaCl, 0.5 mM EDTA, 1% Deoxycholic Acid, 1% Triton X-100 and 0.1% SDS supplemented with protease inhibitor cocktail (Roche, Mannheim, Germany), followed by centrifugation at 10000g for 10 min. Protein content was determined by BCA assay kit (Pierce, Rockford, IL).

Cellular extracts for Electro Mobility Shift Assay (EMSA) were obtained as described above, except that a different lysis buffer was used (20 mM Tris–HCl pH 7.4, 0.5% Triton X-100).

Oxidative status

Reactive Oxygen Species were measured by incubating cells with the redox-sensitive probe CM-H_2DCFDA (Invitrogen). Briefly, cells treated in triplicate were incubated in Hanks balanced saline solution (HBSS), 10 mM glucose and 20 μM CM-H_2DCFDA for 15 min at 37°C. After washes, cells were maintained in HBSS supplemented with 10 mM glucose and plated in 96-well black plates, three wells for each replica. Fluorescence was revealed using the Victor 3 Multilabel Counter (Wallac, Perkin Elmer) at 485 nm and 535 nm for excitation and emission, respectively. Finally, results were normalized on protein content determined for each replica on a fraction of cells maintained in HBSS, but not plated.

Oxidized protein levels were evaluated using the Oxyblot Protein Oxidation Detection Kit (Merk Millipore) following the manufacturer's instructions. Briefly, the carbonyl groups in the protein side chains were derivatized to 2,4-dinitrophenylhydrazone (DNP), loaded on SDS-PAGE, blotted and incubated with an anti-DPN antibody. Signals were revealed by ECL Advance (GE Healthcare, Buckinghamshire, UK).

Western Blotting

TfR1, DMT1, Fpn, Catalase, Super Oxide Dismutase 1 (SOD1) and β actin protein content were evaluated by WB after SDS-PAGE using specific antibodies (anti-TfR1, Invitrogen) (anti-NRAMP2, Santa Cruz Biotechnology, Santa Cruz, CA) (anti-MTP1, Alpha Diagnostic, San Antonio, TX) (anti-Catalase, Calbiochem, Darmstadt, Germany) (anti-

SOD1, Santa Cruz Biotechnology) (anti-β actin, Sigma-Aldrich). After WB, signals were revealed by ECL Advance (GE Healthcare) using the ChemiDoc-It imaging system (UVP Inc, Upland, CA). Protein levels were quantified by densitometry normalized on β actin using the LabWorks software (UVP Inc).

Electro Mobility Shift Assay (EMSA)

The IRE probe was generated by *in vitro* transcription of the plasmid pSPT-fer. The plasmid was linearized with BamHI and transcribed by T7 RNA polymerase (Invitrogen) in the presence of 80 μCi [^{32}P]UTP (ICN Radiochemicals, Irvine, CA). To perform EMSA cell extracts (2 μg of total proteins) were incubated with a molar excess (100000 cpm) of ^{32}P labeled IRE probe in the presence or absence of 2% β-mercaptoethanol (2-ME). The samples were run on non denaturing 6% polyacrylamide gel electrophoresis in TBE buffer and exposed to autoradiography.

RNA extraction and Real Time PCR

Total RNA was isolated by Qiagen RNeasy Kit (Qiagen, Hilden, Germany). RNA quantity was assessed with Nanodrop (NanoDrop Technologies, Wilmington, DE) and 1 μg of each sample was incubated with 1U DNaseI (Invitrogen) for 15 min at RT. After DNase inactivation at 65°C for 10 minutes, RNA was used as a template to generate complementary DNA (cDNA) by High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Weiterstadt, Germany). Reverse-transcription samples without reverse transcriptase were also included as negative controls. Reverse transcriptase products were used to evaluate expression levels of *FTH* and *TFR1* genes as relative to *GAPDH* expression by qRT-PCR using SYBR Green PCR Master Mix (Applied Biosystems) and the comparative $\Delta\Delta C_T$ method was employed to achieve the relative quantification results.

Table S1. Percentage of viable bone marrow-derived mononuclear cells of patients affected by Multiple Myeloma, as measured by Trypan blue exclusion assay.

Patient	CD138+					CD138-				
	Alive Cells (% ± Standard Deviation)					Alive Cells (% ± Standard Deviation)				
	NT	Btz	Btz after DFO pre	Btz plus FAC after DFO pre	*Cell phenotype*	NT	Btz	Btz after DFO pre	Btz plus FAC after DFO pre	*Cell phenotype*
2a	46 ± 12	20 ± 5	21 ± 5	18 ± 9	Sensitive	75 ± 10	72 ± 12	75 ± 8	76 ± 6	Resistant
3	90 ± 5	76 ± 3	84 ± 9	42 ± 13	Resistant	91 ± 4	86 ± 2	88 ± 8	79 ± 3	Resistant
4	32 ± 4	31 ± 5	19 ± 4	15 ± 3	Resistant	34 ± 9	23 ± 5	27 ± 9	21 ± 5	Resistant
5	46 ± 8	21 ± 6	14 ± 9	7 ± 4	Sensitive					

NT: not treated. Btz: bortezomib. DFO: deferoxamine. FAC: ferric ammonium citrate.