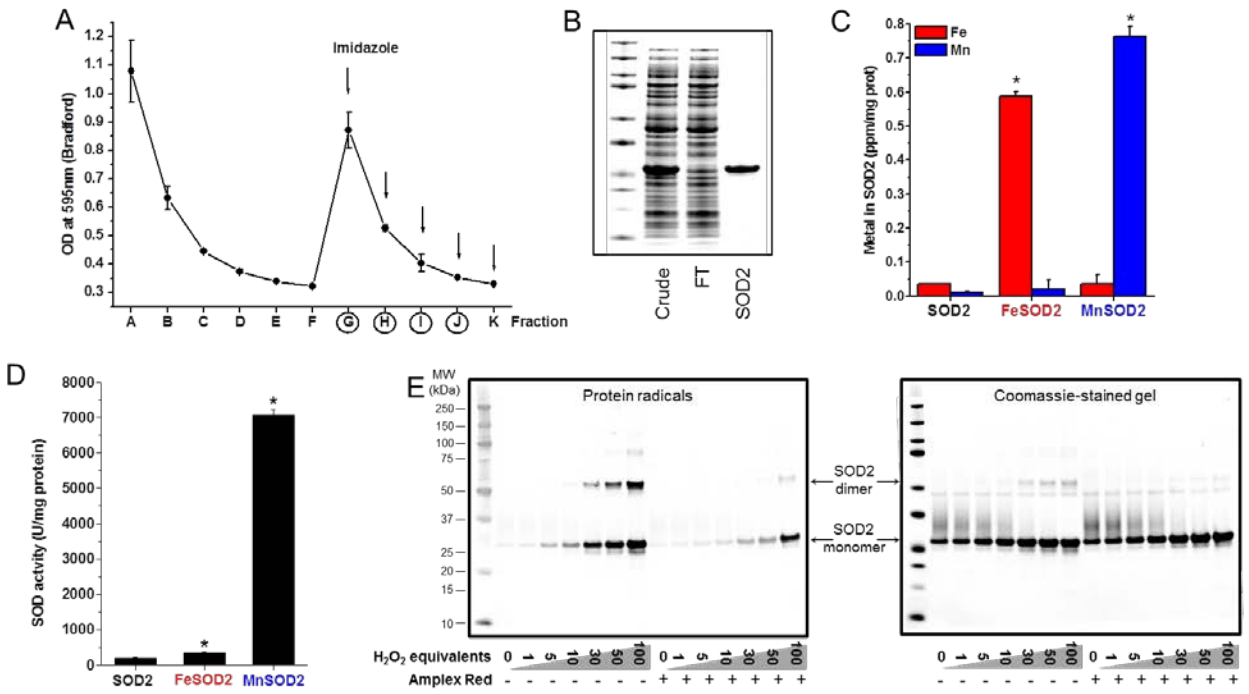


Figure S1, related to Figure 1. Protein purification, preparation and protein radical formation by human SOD2. For preparation of human SOD2, *E. coli* was transformed with overexpressing plasmids for the human SOD2 under control of a *lac* promoter and grown in Luria-Bertani broth under agitation at 37°C. Overexpression was induced by the addition of IPTG (0.1 mM) to the bacterial cultures at OD 0.6-0.8, followed by incubation of the cultures under gentle agitation at 25°C for 2 h. In (A) protein concentration was determined in the fractions eluted from the protein purification column using the Bradford assay. Where indicated by the arrow, the bound proteins were eluted from the column using imidazole, and fractions G-J were pooled for further desalting and washing as described in the Experimental Procedures section. In (B) SDS-PAGE confirmed the purity of the purified protein: Crude = whole cell homogenates (9 µg/lane), FT = flow through of the affinity column (fraction A, 9 µg/lane) and SOD2 = purified protein (pooled fractions G-J after concentration, desalting, washes and final concentration/buffer exchange, 2 µg/lane). Human SOD2 was then incorporated *in vitro* with manganese or iron as described in the Experimental Procedures section. In (C) metal content and (D) specific superoxide dismutase activities were determined. In (E), the effect of different concentrations of H_2O_2 and the peroxidase substrate Amplex red on the protein radical formation in FeSOD2 was studied. Different concentrations of H_2O_2 (expressed as M:M equivalents to FeSOD2) were added to samples containing 10 µM FeSOD2 and 100 mM DMPO. Amplex red was used at 100 µM where indicated. Samples were prepared in Chelex-treated 100 mM phosphate buffer, pH 7.4, with 25 µM DTPA. Gels were run with 1.75 µg of protein. Representative Western blot anti-DMPO (protein radicals) and Coomassie-stained gel (total protein) are depicted. Data are represented as mean ± SD. * indicates *p*-value < 0.05 *versus* SOD2 control.

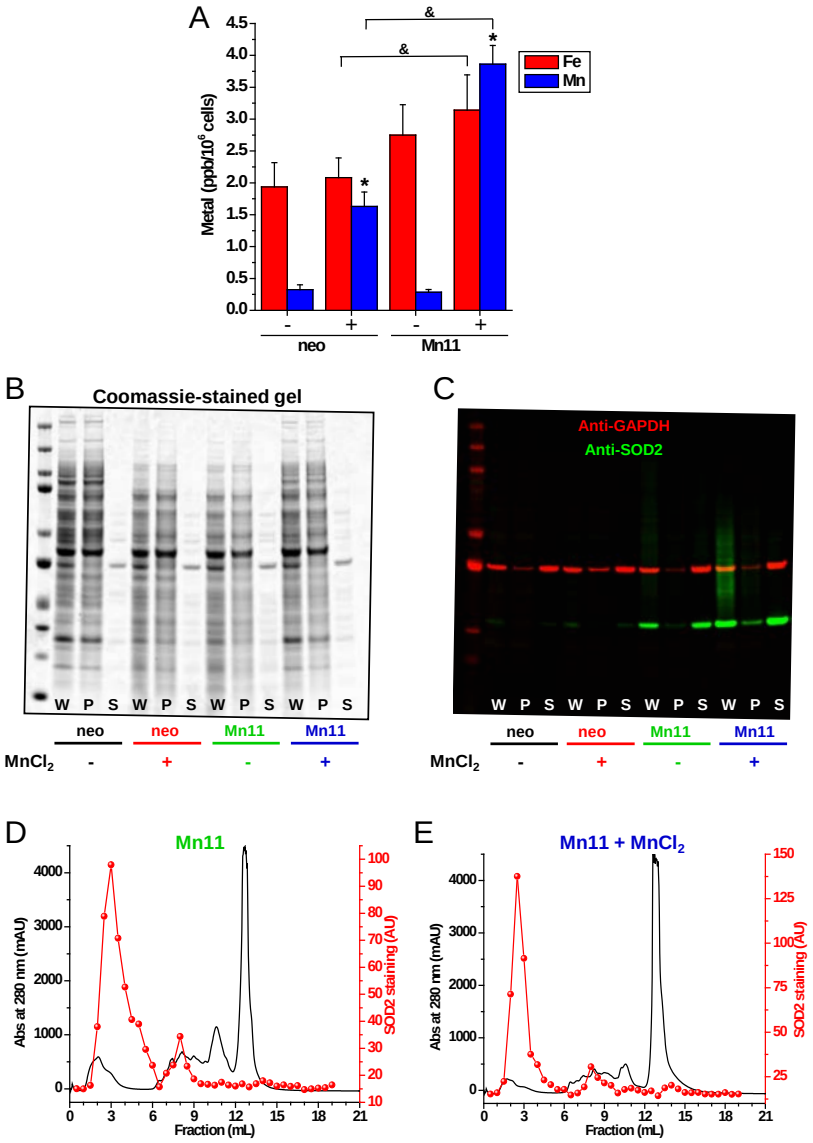


Figure S2, related to Figure 2. Intracellular total metal concentration and purification of native SOD2 from crude homogenates of cells. In (A) intracellular concentrations of iron and manganese were determined by ICP-OES in whole cells extensively digested. In (B) and (C) it is shown that ammonium sulfate precipitation (60% saturation) was used to precipitate out proteins of crude native cell homogenates and quantitatively recover SOD2 five times purer in the supernatant. W = crude homogenate, P = precipitated proteins and S = supernatant. Note that in (C) SOD2 in the S samples was still contaminated with other proteins such as GAPDH, but this fraction had less than 20% of the proteins present in the whole cell homogenate (W samples). S samples were dialyzed, injected and fractionated using anion exchange (MonoQ column) in an FPLC using a gradient of NaCl up to 1M. Samples of those fractions were immobilized in nitrocellulose membranes (3 µL per spot) and immunoprobed for SOD2. In (D) and (E) the chromatograms for two representative samples of the overexpressing cell line (Mn11) are shown for total protein (Abs at 280 nm) and SOD2. The fractions corresponding to SOD2 were pooled. The final purity was 85% as determined by SDS-PAGE and Coomassie staining. The figures shown here are representative of one of the three independent experiments performed. Data are represented as mean ± SD. * indicates *p*-value < 0.05 *versus* control and & indicates significant difference at *p*-value < 0.05.

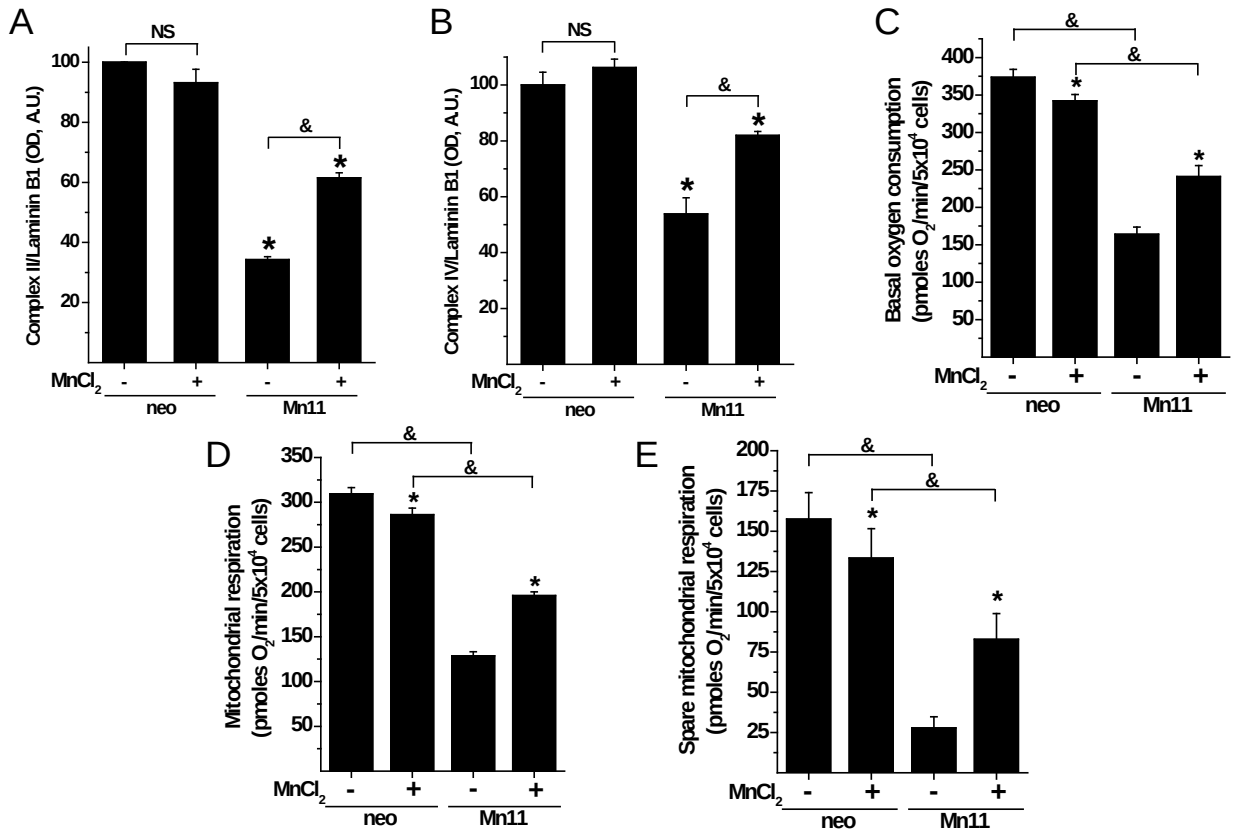


Figure S3, related to Figure 3. Mitochondrial metabolism and bioenergetics of cells overexpressing SOD2 and cultivated in the presence of manganese. The MCF-7-derived parental cell line, neo, and the SOD2-overexpressing cell line, Mn11, were cultivated with or without manganese supplementation (25 μM $MnCl_2$) for at least 21 days. Amounts of (A) Complex II (SDHB) and (B) Complex IV (MTCO1) were determined and normalized by laminin B1 content (data are represented as mean ± SD). Determination of bioenergetics of cells in culture was performed using a SeaHorse Analyzer (vide Figure 3D), which reliably measures oxygen consumption rate (OCR) of intact cells in culture. In (C) basal oxygen consumption of cells is shown. In (D) mitochondrial respiration was calculated as the difference of the OCR measured after the addition of rotenone and antimycin (10 μM each) and the basal values of OCR. In (E), spare mitochondrial capacity was calculated by subtracting the high levels of OCR seen after addition of the uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 10 μM) and the basal OCR. * indicates *p*-value < 0.05 *versus* neo; $ indicates *p*-value < 0.05 *versus* the respective basal level; & indicates *p*-value < 0.05 and NS indicates no statistically significant difference, *p*-value > 0.05.

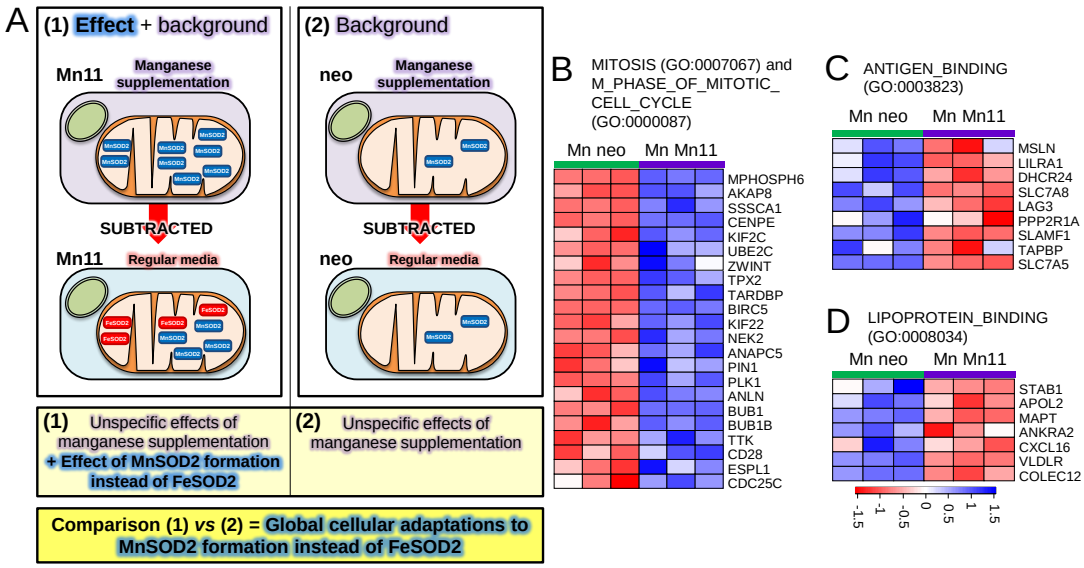


Figure S4, related to Figure 4. Alterations in global gene expression of cells containing the antioxidant MnSOD2 instead of the prooxidant FeSOD2. In (A) the rationale for the complex GSEA comparison matrix is explained as an infograph. In (B-D) supplementary heatmaps of the top gene sets significantly altered in Mn11 cells with MnSOD2 instead of the peroxidase FeSOD2 are depicted (Fig. 4).

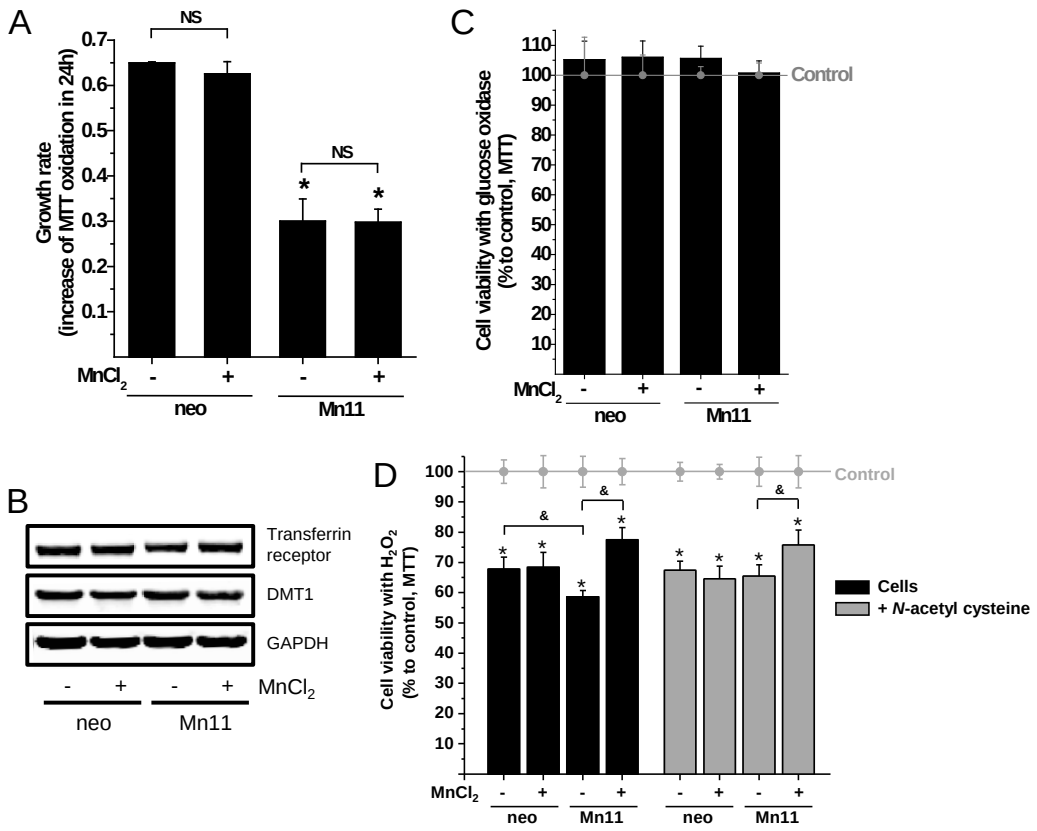
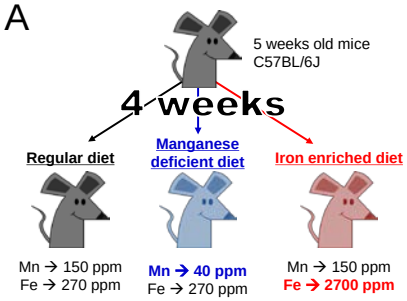


Figure S5, related to Figure 5. Growth rate and iron uptake of cells overexpressing SOD2 cultivated with or without manganese, and their viability and cytotoxicity after being challenged with low levels of H_2O_2 generated continuously or high H_2O_2 added *in bolus*. Cells (parental cell line, neo, and SOD2-overexpressing cell line, Mn11) were grown in the presence of 25 μM $MnCl_2$ for at least 21 days. In (A) cells were then assayed for their growth rate using the oxidation of the vital dye MTT (5 mg/mL) as a measurement of cellular density over 72 h, every 24 h. The rate of increase in MTT oxidation every 24 h is shown. In (B) a representative Western blot for the two major proteins involved in iron uptake by cells is depicted (n = 3). Samples have 15 μg of protein. Staining for GAPDH is used as a loading control. In (C) and (D) cells were seeded in 96-well plates (2 x 10^5 cells/well). In (C), cells were exposed to 50 mU/mL GOX for 3 h, 5 mg/mL of thiazolyl blue tetrazolium bromide (MTT) was then added, and plates were incubated in the dark for an additional 1 h in the CO_2 incubator. In (D), cells were pretreated with 10 mM *N*-acetyl cysteine for 3 h, and washed twice with PBS before fresh medium was added. Then, cells were exposed to 100 μM H_2O_2 for 1 h, before MTT (5 mg/mL) was added. Plates with MTT were incubated in the dark for an additional 1 h in the CO_2 incubator. In (C) and (D), after final incubation, medium was removed and 100 μL of DMSO was added to each well to solubilize the intracellular crystals of formazan. After plates were shaken for 30 min, absorbance at 560 nm was measured using a plate reader. In (A) and (C), data are represented as mean ± SD. * indicates *p*-value < 0.05 *versus* neo and NS indicates no statistically significant difference, *p*-value > 0.05.

A



B

		Regular diet	Manganese deficient diet	Iron enriched diet
Serum	ALT (U/L)	15.67 ± 3.06	17.0 ± 2.0	15.67 ± 7.23
	Creatinine (mg/dL)	0.23 ± 0.058	0.2 ± 0.1	0.27 ± 0.058
	Glucose (mg/dL)	224 ± 58	255 ± 38	245 ± 65
	Sodium (mEq/L)	154 ± 1.73	152.67 ± 1.53	153.33 ± 2.08
	Total cholesterol (mg/dL)	94.2 ± 4.55	94.2 ± 10.85	74.8* ± 11.8
	Iron (µg/dL)	127.5 ± 5.0	126.6 ± 8.2	123.4 ± 6.27
	TIBC (µg/dL)	293.5 ± 10.47	309.67 ± 14.85	251.17* ± 20.25
Total Hemoglobin (g/dL)		14.92 ± 1.08	14.57 ± 1.56	14.35 ± 1.47
Weight gain (g)		3.18 ± 0.68	3.24 ± 0.29	3.12 ± 0.38

C

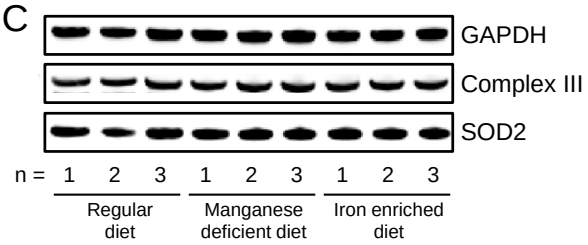


Figure S6, related to Figures 6 and 7. Animal protocol, blood biochemistry analyses, and mitochondrial markers in the liver of animals subjected to short-term manganese deprivation or iron enrichment through diet. In (A), scheme showing the animal feeding regimen with diets containing regular levels of metals (regular diet), trace levels of manganese from the corn used in the composition of the diet (manganese deficient diet) and enriched with iron sulfate up to 10 times higher than the regular diet (iron enriched diet). In (B), biochemical parameters measured in serum, the total levels of hemoglobin in total blood and final weight gain of animals in each group are shown. In (C), representative Western blot for GAPDH and the mitochondrial markers Complex III and SOD2 in whole liver homogenates (for OD quantifications vide Fig. 6D). Data are represented as mean ± SD, and * indicates *p*-value < 0.05 *versus* Regular diet.