SUPPLEMENTARY MATERIAL

Mitochondrial impairment and oxidative stress mediated apoptosis induced by α-Fe_2O_3 nanoparticles in *Saccharomyces cerevisiae*

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mRNA expression assays

Briefly, approximately 1×10^7 cells were collected using density gradient centrifugation after exposure for 24 h. Total RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA), and RNA integrity and concentration were measured using a NanoDrop spectrophotometer (ND-1000, NanoDrop Technologies Inc., Wilmington, DE). SYBR Premix Ex Taq II kit (Takara, Dalian, China) and CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA) were used for real-time PCR. Primers for apoptosis-related genes (SOD, Yca1, Nma111 and Nuc1) and 18S rRNA were designed as previous studies (Zhu et al., 2016) and listed in Table S2. The 18S rRNA was used as internal standard, and following cycling conditions were ran: initial denaturation at 95°C for 5 min, 40 cycles of 95°C denaturation for 20 s, 57°C annealing for 20 s and 72°C elongation for 20 s. Melting curves were analyzed for all the reactions. Relative expression was obtained by using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008) and normalized to the expression of the internal standard gene 18S rRNA in the same sample.

Table S1. The structural parameters for α-Fe_2O_3-NPs.

Parameter	Unit	α-Fe_2O_3-NPs
Particle length (TEM)	nm	63.3 (21.5-116)
Hydrodynamic diameter (DLS)	μm	5.2 (0.35-14)
Purity	wt%	99.9
Specific surface area	m^2/g	20-60
Particle morphology	-	nanorods

Table S2. Real time PCR primer pairs used in this study.

Genes	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)
SOD	CTCGTCCAGACTGCCAAAC	GCATTACACCAAGCACCAT	274
Yca1	CCAGGATGGAGATGAGGAA	CAGTGTTGCGGGTGAGGTA	284
Nma111	TGGTCCTGGTCCGTTCGTAG	TTTCTGAGCCGACCTTAGCC	182
Nuc1	ATGTTCGAGGACCCAATAAG	TATCCACCTACCCAGAAACC	174
18S	TTCTGCCCTATCAACTTTCG	GATGTGGTAGCCGTTTCTCA	112

35 **Table S3.** Contents of Fe^{3+} released from α-Fe_2O_3-NPs.

α-Fe_2O_3-NPs suspension (mg/L)	25	50	100	200	400	600
Fe^{3+} content (mg/L)	1.54	2.86	3.78	4.34	4.62	4.81

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Table S4. Twenty-four-hour IC_{50} (inhibition of growth by 50%) and LC_{50} (lethal concentration of 50%) calculated for α-Fe_2O_3-NPs on *S. cerevisiae*.

IC_{50} (mg/L)	LC_{50} (mg/L)
352 (266—488)	541 (489—608)

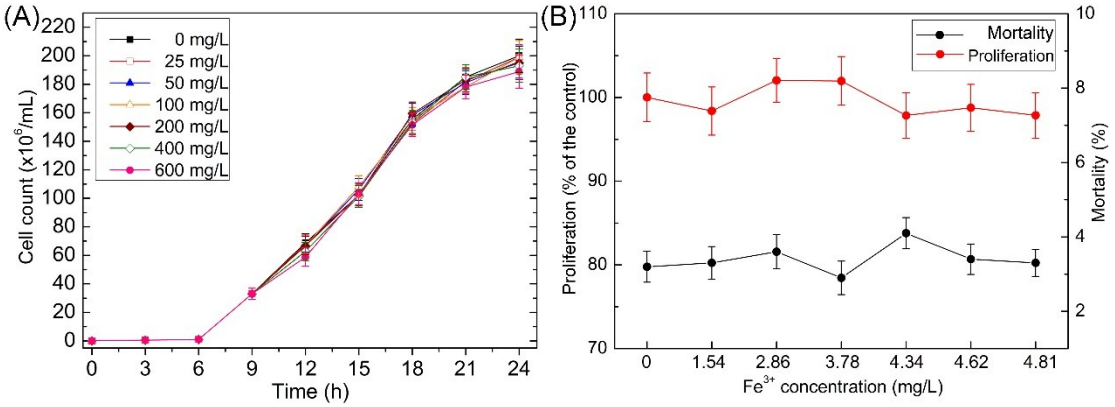

Fig. S1. (A) The growth curves of *S. cerevisiae* exposed to different concentrations of iron ion released from α-Fe_2O_3-NPs. (B) Effects of iron ion released from α-Fe_2O_3-NPs on cell proliferation and viability after exposure for 24 h. Values are presented as mean ± SD.

References

Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative CT method. Nature protocols 3, 1101-1108.

Zhu, S., Zhu, B., Huang, A., Hu, Y., Wang, G., Ling, F., 2016. Toxicological effects of multi-walled carbon nanotubes on Saccharomyces cerevisiae: The uptake kinetics and mechanisms and the toxic responses. Journal of Hazardous Materials 318, 650-662.