1 An assessment of the toxicity of CuO nanoparticles using

2 multiple-gene-deleted mutants of Saccharomyces cerevisiae

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Strain	Genotype	Source
BY4741	ΜΑΤa his3 Δ 0 leu2 Δ 0 met15 Δ 0 ura3 Δ 0	lab of aquatic animal protein
		engineering, Institute of
		Hydrobiology, Chinese Academy
		of Sciences
		(1)
yap1 mutant	ΜΑΤa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0	(2)
	yap1∆::hisG	
quadruple mutant	ΜΑΤa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0	(1)
	snq2Δ::KanMX, pdr5Δ::LEU2, cwp1Δ::hisG,	
	cwp2∆::HIS3	
quintuple mutant	ΜΑΤa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0	(3)
	snq2Δ::KanMX, pdr5Δ::LEU2, cwp1Δ::hisG,	
	cwp2∆::HIS3, yap1∆::hisG	

6 **Table S1. Yeast strains used in this study**

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8 Growth of yeast *S. cerevisiae* wild-type and mutants

9 Yeast cells were inoculated in 20 ml of SD medium by overnight (16-18 hour) culturing at 30 °C, 200 rpm under dark conditions, from a single agar plate colony. Diluted the initial 10 culture optical density (OD) at 600 nm to 0.110±0.001 (d=0.5 cm) with fresh SD medium, 11 12 then aliquots of 100 ml cell culture were continued cultivating at 30 °C, 200 rpm under dark conditions, and measured the value of optical density (OD) at 600 nm by the 13 14 spectrophotometer every two hours. Growth curves of yeast strains were presented in Figure 15 S1. Figure S1 showed that all strains' growth curves obeyed logistical growth curve (r > 16 0.9900), and except the *yap1* mutant, the biomasses of mutants were higher than that of the 17 wild-type strain, while during the exposure time (0-4 h), the biomass of different strains were 18 comparable (p>0.05).

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Fig. S1 Yeast *Saccharomyces cerevisiae* wild-type BY4741 and mutants' growth in SD medium at 30 °C with 200
 rpm continuous shaking during 28 h.



Fig. S2 Dynamic variation of pH values in the SD medium over time in the presences of yeast wild-type and
 mutants. Data are means ± standard error of 3 independent replicates.

30 **References**

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- Zhang M, Zhang C, Li J, Hanna M, Zhang X, Dai H, Xiao W. 2011. Inactivation of YAP1
 Enhances Sensitivity of the Yeast RNR3-lacZ Genotoxicity Testing System to a Broad Range of
 DNA-Damaging Agents. Toxicological Sciences 120:310-321.
- Zhang M, Hanna M, Li J, Butcher S, Dai H, Xiao W. 2010. Creation of a Hyperpermeable Yeast
 Strain to Genotoxic Agents through Combined Inactivation of PDR and CWP Genes.
 Toxicological Sciences 113:401-411.
- Wei T, Zhang C, Xu X, Hanna M, Zhang X, Wang Y, Dai H, Xiao W. 2013. Construction and
 evaluation of two biosensors based on yeast transcriptional response to genotoxic chemicals.
 Biosensors and Bioelectronics.

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