

## Supplementary Information

### **Proteomic and genetic analysis of *S. cerevisiae* response to soluble copper leads to improvement of antimicrobial function of cellulosic copper nanoparticles**

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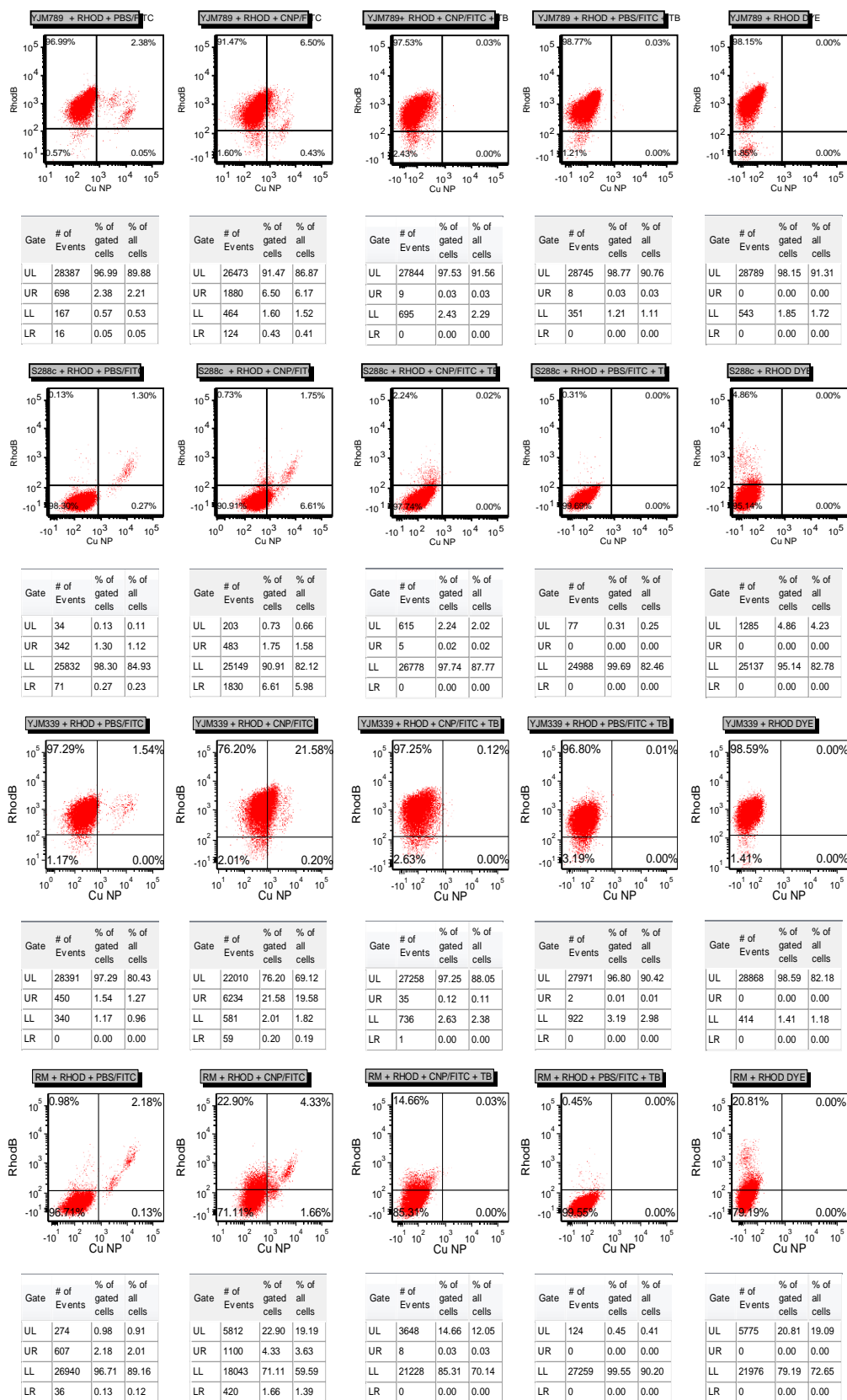
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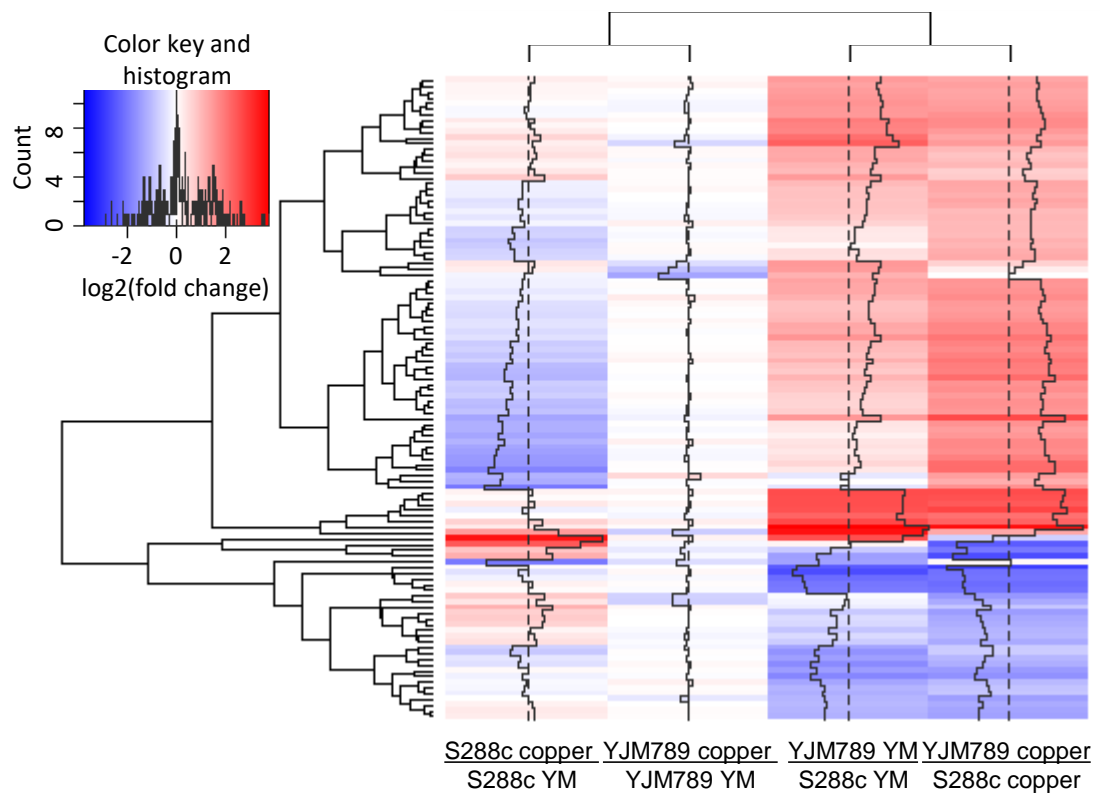
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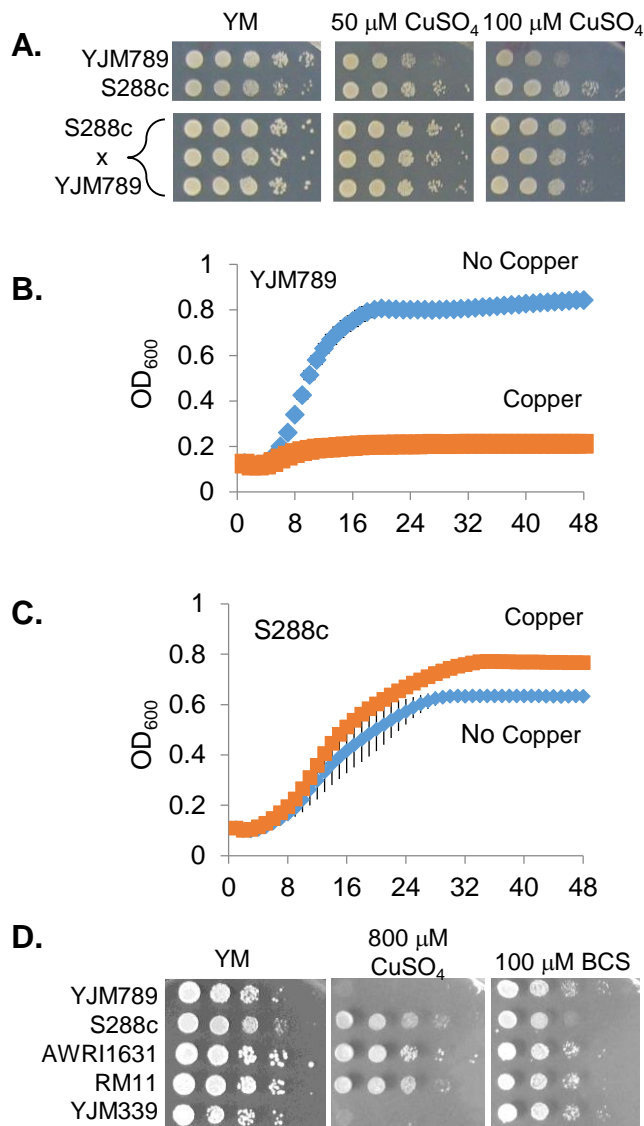
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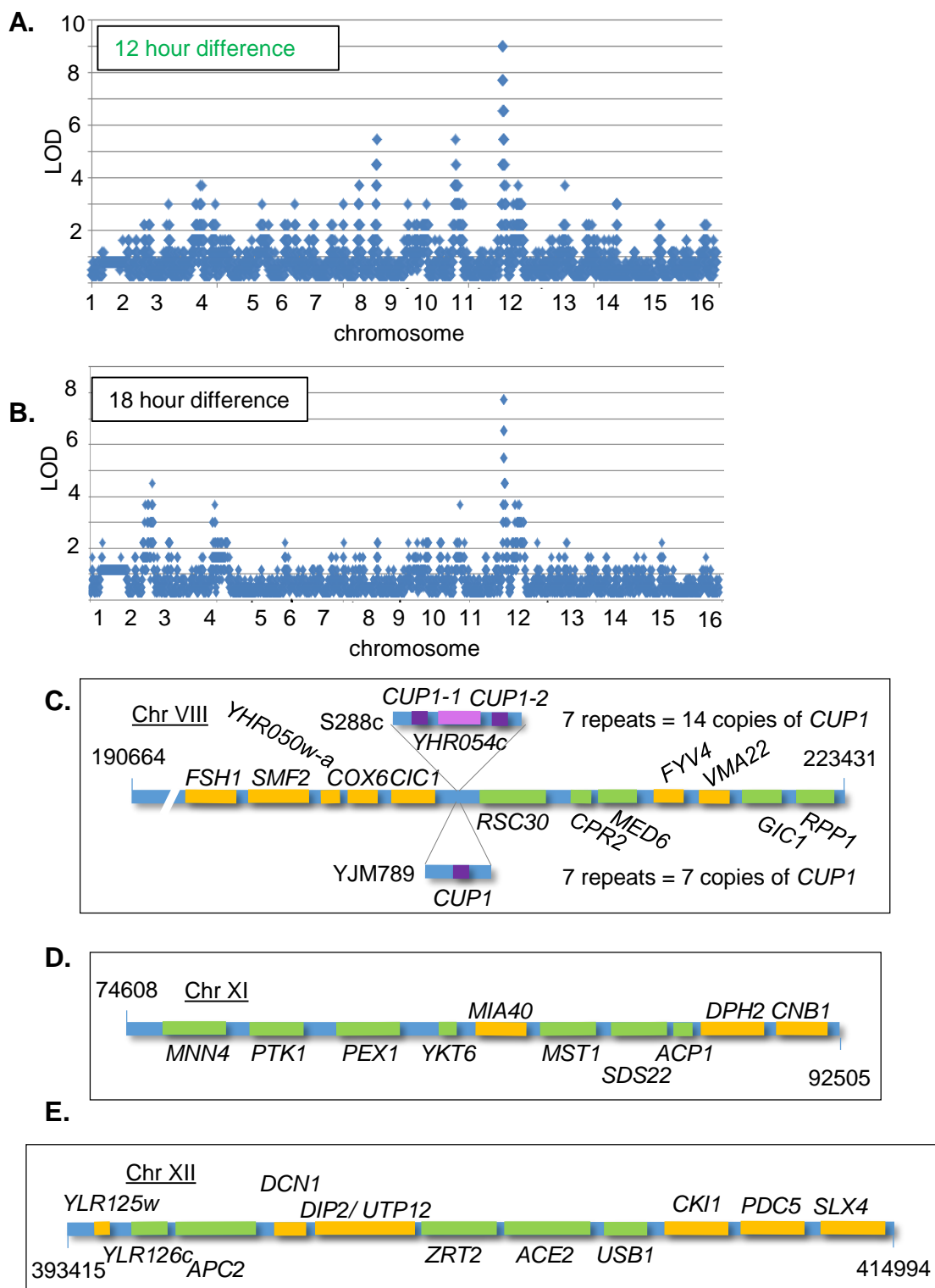
**Fig. S1** Flow cytometry of individually stained yeast.



**Fig. S2** Hierarchical clustering of protein levels from proteomic analysis of yeast treated with copper reveals inherent characteristics of yeast strains. Protein levels from S288c (S96) and YJM789 treated with 800  $\mu$ M of  $\text{CuSO}_4$  for 90 minutes were clustered hierarchically.

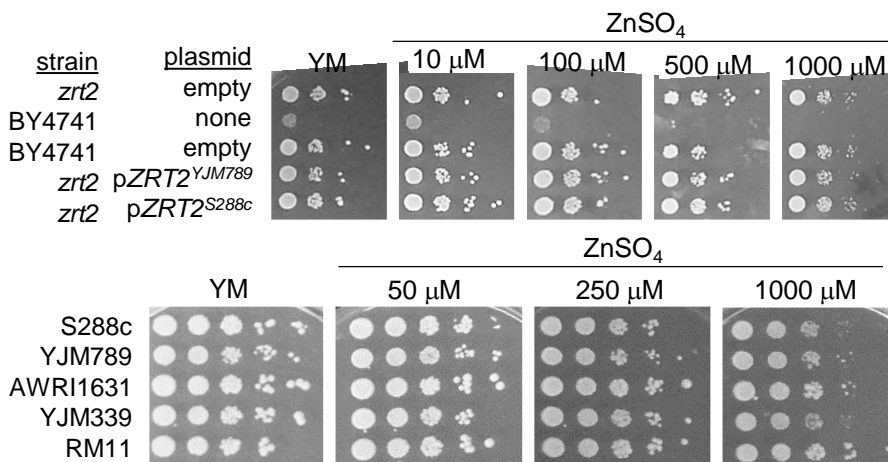


**Fig. S3** Growth assays of genetically diverse yeast in media with excess and limited copper. **A.** Copper resistance was determined to be a dominant trait in hybrids generated from mating YJM789, a copper sensitive strain, with S288c (S96), a copper resistant strain. Growth patterns of the hybrids in the presence of excess copper were similar to S288c. **B.** and **C.** Example growth curves developed for the two parent strains grown in YM and YM with 50  $\mu\text{M}$   $\text{CuSO}_4$ . Growth patterns were analyzed for 125 unique segregants developed; segregants displayed a wide variety of resistance, ranging from extremely sensitive to extremely resistant. **D.** Serial dilution of genetically diverse yeast strains (YJM789, S288c (GSY1470, AWRI1631, RM11, and YJM339) grown on YM plates containing 400  $\mu\text{M}$   $\text{CuSO}_4$  or 100  $\mu\text{M}$  bathocuproinedisulfonic acid (BCS), a copper chelator.



**Fig. S4** Genomic loci linked to copper response. **A.** Genetic linkage with 12-hours of copper treatment. **B.** Genetic linkage with 18-hours of copper treatment. **C.** Genes on chromosome XII that are linked to copper response. Chromosomal coordinates are labeled. Green genes are encoded on the top

strand and yellow genes are encoded on the bottom strand. **C.** Genes located on chromosome VIII linked to copper response from 12-hours of growth. Chromosomal coordinates are labeled. Green genes are encoded on the top strand and yellow genes are encoded on the bottom strand. Purple genes are also encoded on the bottom strand within segmentally amplified *CUP1* region. **D.** Genes on chromosome XI that are linked to copper response at 12-hours of growth. **E.** Genes on chromosome XII that were linked to copper response in both 12 and 18-hours of growth.



**Fig. S5** Serial dilutions of yeast growth in the presence of elevated zinc. **A.** Alleles of *Zrt2* were expressed from a plasmid using the endogenous promoter and terminated in BY4741 yeast in which *ZRT2* was deleted. Wild-type yeast without a plasmid served as the control for plasmid selection. YM was supplemented with necessary nutrients. **B.** Genetically diverse yeast S288c (GSY147), YJM789, RM11, AWRI1631 and YJM339 were serially diluted onto varying amounts of zinc sulfate. Plates were incubated for 2-3 days and photographed.

Zrt2_S288c	MVDLIARDDSVDTCCASNGYNHAGLRILAVFIILISSGLGVYFPILSSRYSFIRLPNWC
Zrt2_YJM789	MVDLIARDDSVDTCCASNDYNHAGLRILAVFIILISSGLGVYFPILSSRYSFIRLPNWC
	*****.***** TM1 25-47
Zrt2_S288c	FFIAKFFGSGVIVATAFVHLLQPAAEALGDECLGGTFAEYPWAFGICLMSLFLFFTEII
Zrt2_YJM789	FFIAKFFGSGVIVATAFVHLLQPAAEALGDECLGGTFAEYPWAFGICLMSLFLFFTEII
	*****.***** TM2 60-82 TM3 102-124
Zrt2_S288c	THYFVAKTLGHDHGDHGEVTSIDVDAPSSGFVIRNMDSDPVSFNNEAAYSIHNDKTPYTT
Zrt2_YJM789	THYFVAKTLGHDHGDHGEVNSIDVDAPSSGFVIRNMDSDPVSFNNEAAYSIHNDKTPYTT
	*****.*****
Zrt2_S288c	RNEEIVATPIKEKEPGSNVTNYDLEPGKTESLANELVPTSSHATNLASVPGKDHYSHEND
Zrt2_YJM789	RNEEIVATPIKEKEPDNSVTNYDLEPGKTESLANELVPTSSHATNLASVPGKDHYSHEND
	*****.***** TM4 255-288
Zrt2_S288c	HQDVSQLATRIEEEDKEQYLNQILAVFILEFGIIFHVSFVGLSLSVAGEEFETLFIVLTF
Zrt2_YJM789	HQDVSQALATRIEEEDKEQYLNQILAVFILEFGIIFHVSFVGLSLSVAGEEFETLFIVLTF
	*****.***** TM5 325-347
Zrt2_S288c	HQMFEGGLGLTRVAETNWPESKKYMPWLMGLAFTLTSPIAVAVGIGVRHSWIPGSRRALI
Zrt2_YJM789	HQMFEGGLGLTRVAETNWPESKKYTPWLMGLAFTLTSPIAVAVGIGVRHSWIPGSRRALI
	*****.***** TM6 359-381 TM7 401-418
Zrt2_S288c	ANGVFDSISSGILIYTGVELMAHEFLYSNQFKGPDGLKKMLSAYLIMCCGAALMALLGK
Zrt2_YJM789	ANGVFDSISSGILIYTGVELMAHEFLYSNQFKGPDGLKKMLSAYLIMCCGAALMALLGK
	*****.*****
Zrt2_S288c	WA
Zrt2_YJM789	WA
	**

**Fig. S6** Protein alignment of Zrt2 from S288c and YJM789. Amino acid that differ are boxed. Below sequences, identical amino acids were signified by (\*) and similar amino acids are signified by (.). Transmembrane domain sequences are underlined. Sites of phosphorylation are in red.

## Supplemental Tables

**Table S1.** Relative protein levels of S288c (S96) and YJM789 cells grown in YM with lysine or YM with excess CuSO<sub>4</sub>, shown as log2. A total of 112 proteins showed greater than a two-fold change between strains and/or copper treatments.

**Table S2.** Common contaminant list for proteomics that were screened during searches.

**Table S3.** Growth values of S288c (S96) and YJM789 haploid recombinant segregants at 12 and 18-hours with 50  $\mu$ M CuSO<sub>4</sub>.

**Table S4.** SNP LOD mean for 12-hour exposure.

**Table S5.** SNP LOD mean 18-hour end point exposure.