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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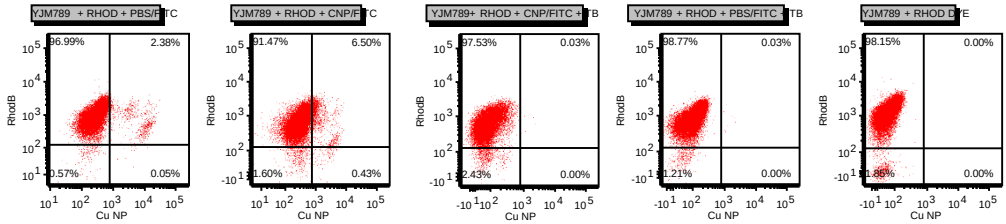

Gate	# of Events	% of gated cells	% of all cells
UL	28387	96.99	89.88
UR	698	2.38	2.21
LL	167	0.57	0.53
LR	16	0.05	0.05

Gate	# of Events	% of gated cells	% of all cells
UL	26473	91.47	86.87
UR	1880	6.50	6.17
LL	464	1.60	1.52
LR	124	0.43	0.41

Gate	# of Events	% of gated cells	% of all cells
UL	27844	97.53	91.56
UR	9	0.03	0.03
LL	695	2.43	2.29
LR	0	0.00	0.00

Gate	# of Events	% of gated cells	% of all cells
UL	28745	98.77	90.76
UR	8	0.03	0.03
LL	351	1.21	1.11
LR	0	0.00	0.00

Gate	# of Events	% of gated cells	% of all cells
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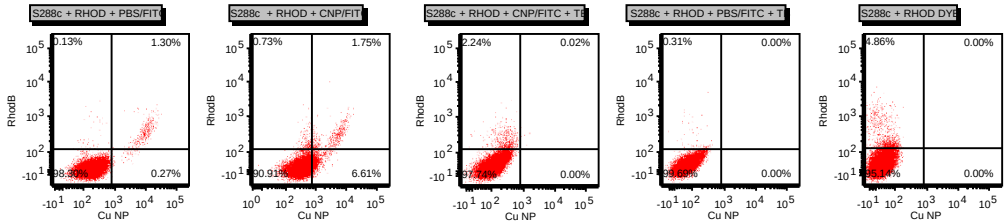
UR	0	0.00	0.00
LL	543	1.85	1.72
LR	0	0.00	0.00



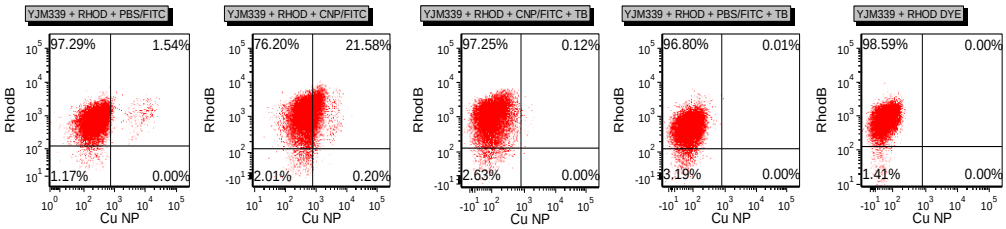
Gate	# of Events	% of gated cells	% of all cells
UL	34	0.13	0.11
UR	342	1.30	1.12
LL	25832	98.30	84.93
LR	71	0.27	0.23

Gate	# of Events	% of gated cells	% of all cells
UL	203	0.73	0.66
UR	483	1.75	1.58
LL	25149	90.91	82.12
LR	1830	6.61	5.98

Gate	# of Events	% of gated cells	% of all cells
UL	615	2.24	2.02
UR	5	0.02	0.02
LL	26778	97.74	87.77
LR	0	0.00	0.00

Gate	# of Events	% of gated cells	% of all cells
UL	77	0.31	0.25
UR	0	0.00	0.00
LL	24988	99.69	82.46
LR	0	0.00	0.00

Gate	# of Events	% of gated cells	% of all cells
UL	1285	4.86	4.23
UR	0	0.00	0.00
LL	25137	95.14	82.78
LR	0	0.00	0.00



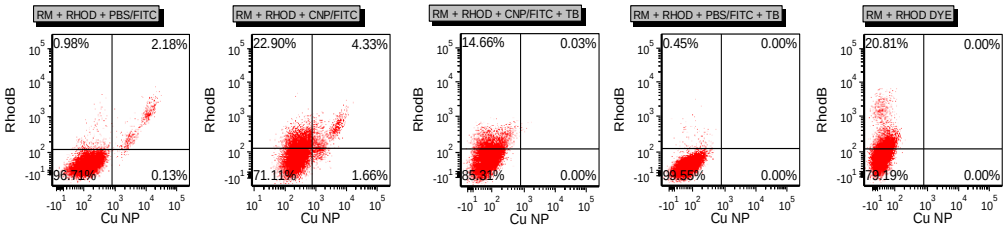
Gate	# of Events	% of gated cells	% of all cells
UL	28391	97.29	80.43
UR	450	1.54	1.27
LL	340	1.17	0.96
LR	0	0.00	0.00

Gate	# of Events	% of gated cells	% of all cells
UL	22010	76.20	69.12
UR	6234	21.58	19.58
LL	581	2.01	1.82
LR	59	0.20	0.19

Gate	# of Events	% of gated cells	% of all cells
UL	27258	97.25	88.05
UR	35	0.12	0.11
LL	736	2.63	2.38
LR	1	0.00	0.00

Gate	# of Events	% of gated cells	% of all cells
UL	27971	96.80	90.42
UR	2	0.01	0.01
LL	922	3.19	2.98
LR	0	0.00	0.00

Gate	# of Events	% of gated cells	% of all cells
UL	28868	98.59	82.18
UR	0	0.00	0.00
LL	414	1.41	1.18
LR	0	0.00	0.00



Gate	# of Events	% of gated cells	% of all cells
UL	274	0.98	0.91
UR	607	2.18	2.01
LL	26940	96.71	89.16
LR	36	0.13	0.12

Gate	# of Events	% of gated cells	% of all cells
UL	5812	22.90	19.19
UR	1100	4.33	3.63
LL	18043	71.11	59.59
LR	420	1.66	1.39

Gate	# of Events	% of gated cells	% of all cells
UL	3648	14.66	12.05
UR	8	0.03	0.03
LL	21228	85.31	70.14
LR	0	0.00	0.00

Gate	# of Events	% of gated cells	% of all cells
UL	124	0.45	0.41
UR	0	0.00	0.00
LL	27259	99.55	90.20
LR	0	0.00	0.00

Gate	# of Events	% of gated cells	% of all cells
UL	5775	20.81	19.09
UR	0	0.00	0.00
LL	21976	79.19	72.65
LR	0	0.00	0.00

Fig. S1 Flow cytometry of individually stained yeast.

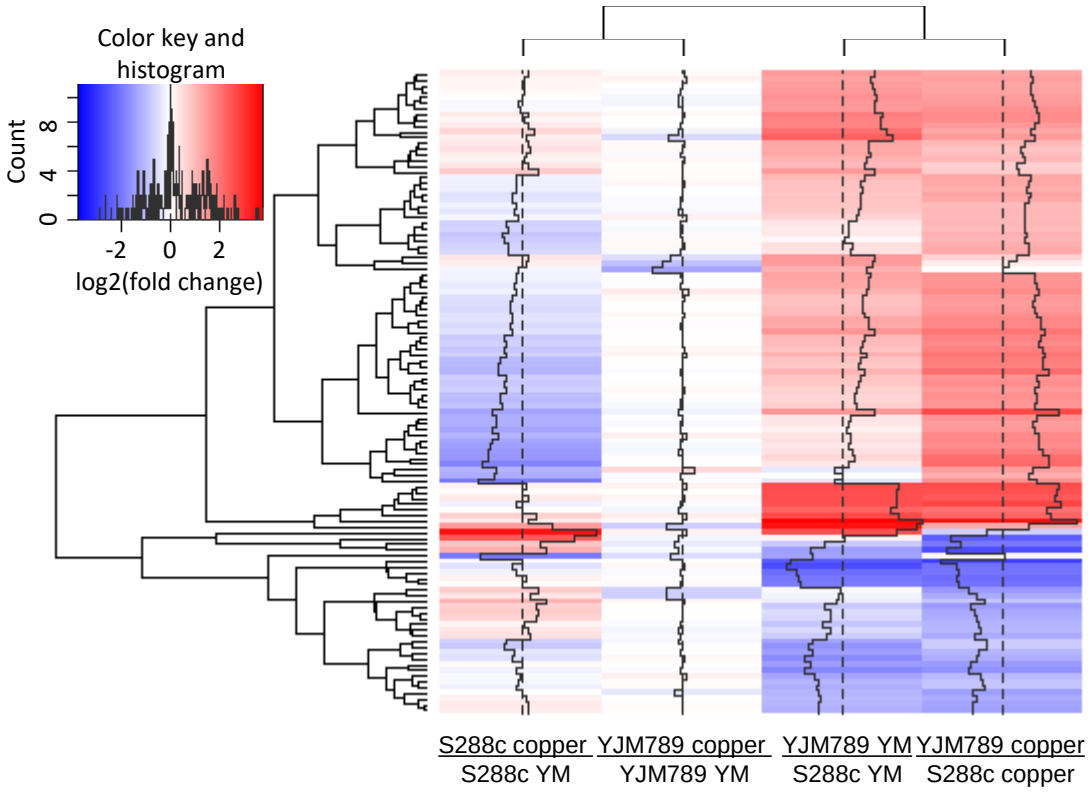


Fig. S2 Hierarchical clustering of protein levels from proteomic analysis of yeast treated with copper reveals inherent characteristics of yeast strains. Protein levels from S288c (S96) and YJM789 treated with 800 uM of $CuSO_4$ for 90 minutes were clustered hierarchically.

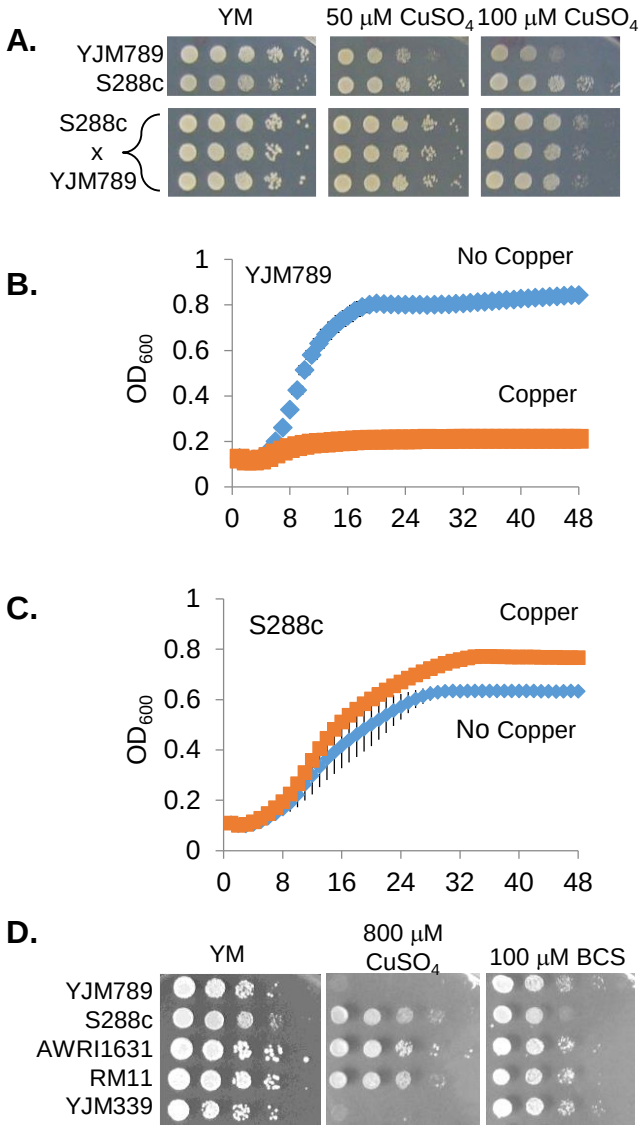


Fig. S3 Growth assays of genetically diverse yeast in media with excess and limited copper. **A.** Copper resistance was determined to be a dominant trait in hybrids generated from mating YJM789, a copper sensitive strain, with S288c (S96), a copper resistant strain. Growth patterns of the hybrids in 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 uM $CuSO_4$. 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 uM $CuSO_4$ or 100 uM bathocuproinedisulfonic acid (BCS), a copper chelator.

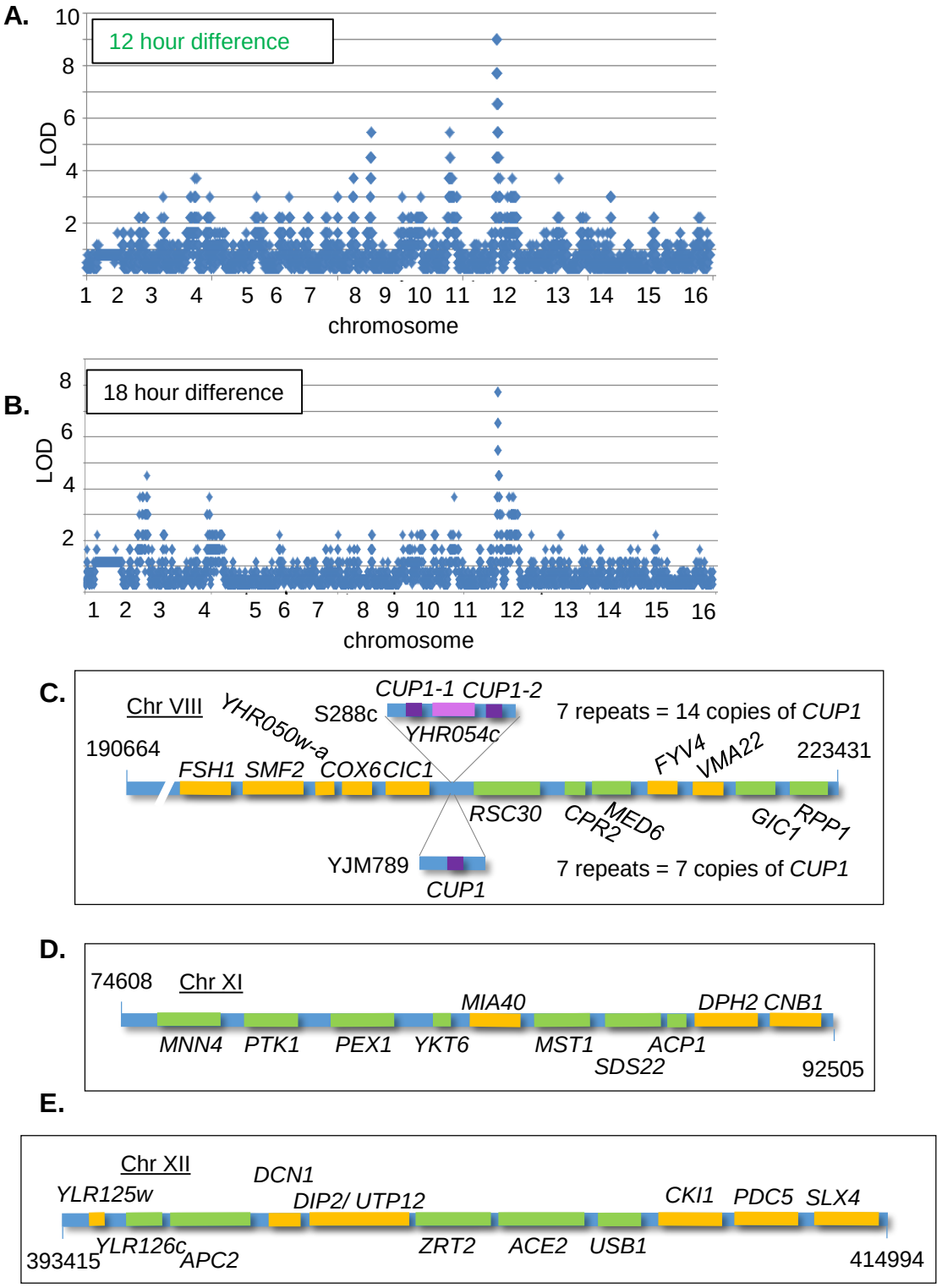


Fig. S4 Genomic loci linked to copper response. **A.** Genetic linkage with 12-hours of copper treatment. **B.** Genetic linkage with 18-hours of copper treatment. **C.** Genes on chromosome XII that are linked to copper response. Chromosomal coordinates are labeled. Green genes are encoded on the top

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.

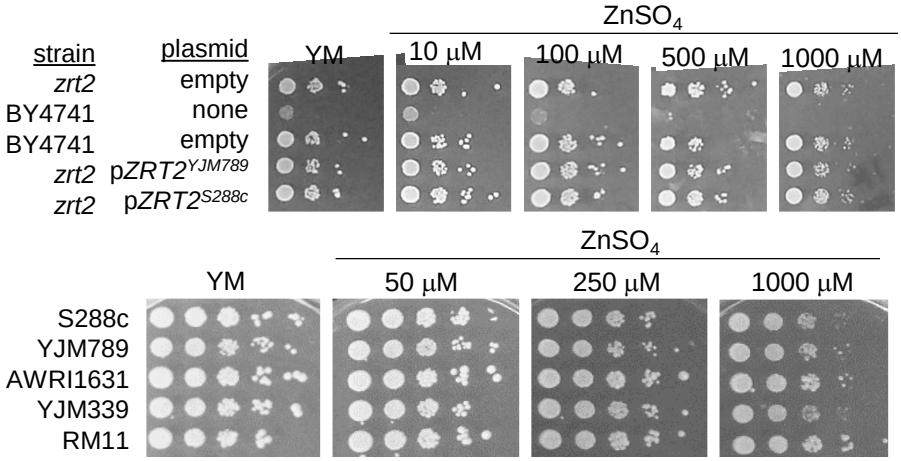


Fig. S5 Serial dilutions of yeast growth in the presence of elevated zinc. **A.** Alleles of Zrt2 were expressed from a plasmid using the endogenous promoter and terminated in BY4741 yeast in which *ZRT2* was deleted. Wild-type yeast without a plasmid served as the control for plasmid selection. YM was 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.

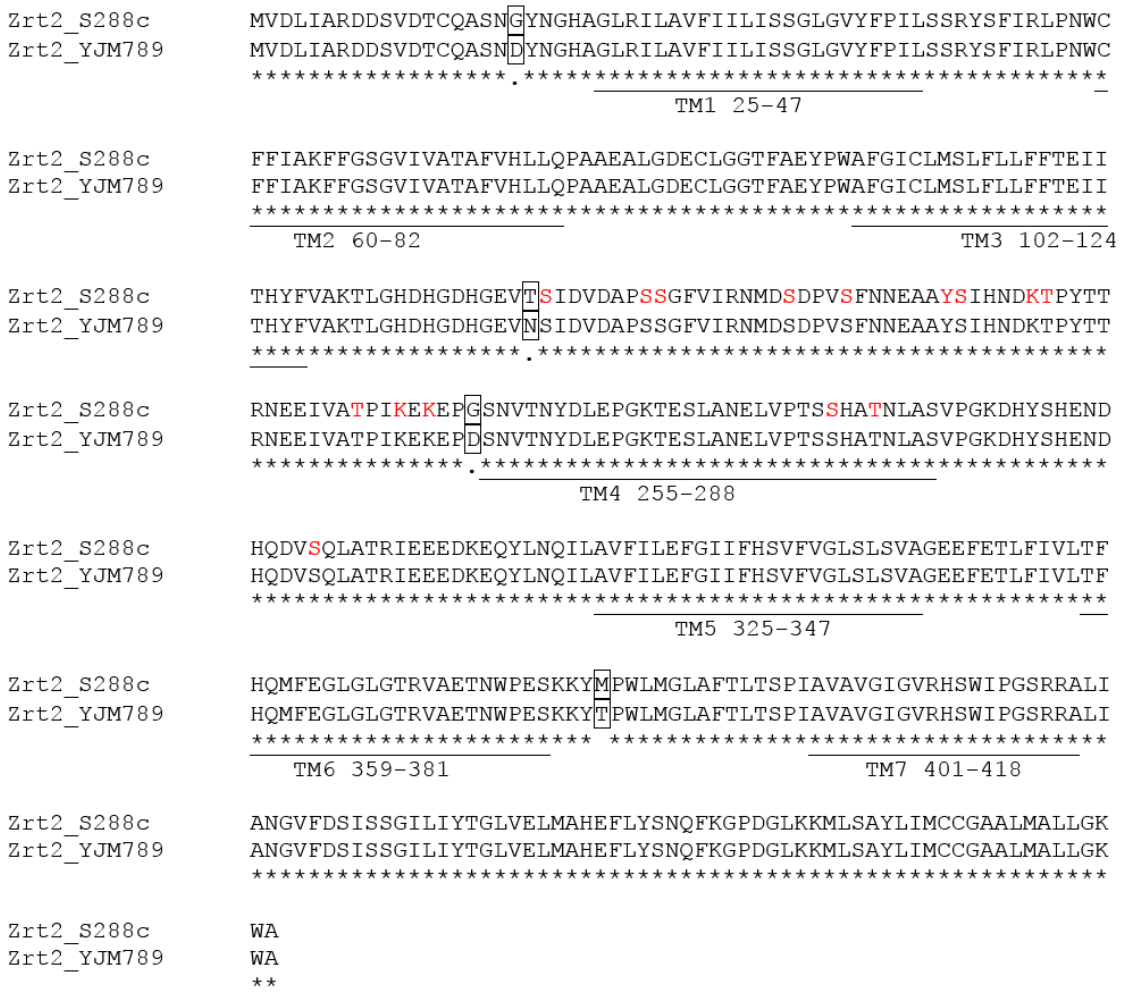


Fig. S6 Protein alignment of Zrt2 from S288c and YJM789. Amino acid that differ are boxed. Below sequences, identical amino acids were signified by (*) and similar amino acids are signified by (.). Transmembrane domain sequences are underlined. Sites of phosphorylation are in red.

Supplemental Tables

Table S1. Relative protein levels of S288c (S96) and YJM789 cells grown in YM with lysine or YM with excess $CuSO_4$, 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 ***u***M $CuSO_4$.

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

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