Supplementary Information

Supplementary Figure Legends

Figure S1A. Growth in the presence of ZPT does not affect superoxide dismutase activity until high doses of ZPT are reached. Cells were grown for five hours in CM at the specified dose of ZPT. SOD activity was measured by non-denaturing gel electrophoresis and staining with nitro blue tetrazolium.

Figure S1B. Effect of ZPT on the activity of a *CUP1-lacZ* reporter. Cells (DY14570 transformed with a plasmid containing a *CUP1-lacZ* reporter construct were grown in CM without uracil for 12 hours at the specified concentrations of ZPT. Cell growth was determined by measuring OD at 600 nm. The cells were harvested, and ß-galactosidase activity and cell protein were determined. The data are presented as specific acitivity, and the error bars represent one SD, n=3.

Figure S1C. Effect of ZPT on the activity of a *CTR1-lacZ* reporter. Cells (DY14570 transformed with a plasmid containing a *CTR1-lacZ* reporter construct were grown in CM without uracil for 12 hours at the specified concentrations of ZPT. Cell growth was determined by measuring OD at 600 nm. The cells were harvested, and ß-galactosidase activity and cell

protein were determined. The data are presented as specific activity, and the error bars represent one SD, n=3.

Figure S1D. Atomic emission spectroscopy measures of metal content of BY4741 grown anaerobically in YPD for three days with the indicated amounts of ZPT and bathocuproine disulfonate (BCS). Cell pellets reported in milligrams; metal content in ppm. There were two replicates for all samples except the ZPT, 30 μM (n=4) and ZPT, 30 μM + BCS, 1.8 mM (n=3). Error bars represent one SD.

Figure S2A. ZPT sensitivity of *ZRC COT1* double deletion mutant grown in YPD with the indicated amount of ZPT. Wild type (DY150) and *Δcot1 Δzrc1* cells were grown for eight hours in the designated concentrations of ZPT, with growth measured by OD at 600 nm.

Figure S2B. Introduction of *ACE1* restores wild-type ZPT sensitivity to a diploid *Δace1*. Wild type diploid BY4743 and a Δ*ace1* diploid were transformed with either a control plasmid or a plasmid containing *ACE1* and grown in CM without uracil. Cells were incubated for 12 hours in the specified concentrations of ZPT, and cell growth was measured by OD at 600 nm. "ACE1Δ/ACE1" refers to the *ACE1*-deleted strain complemented by an *ACE1*-containing plasmid. Error bars represent one SD, n=2.

Figure S2C. ZPT sensitivity of a *MTM1* hypomorph. Shown is the increase in OD at 600 nm, normalized to the increase for the wild type strain. Wild type (DY150) and a *MTM1* hypomorph. Error bars represent one SD, n=2.

Figure S2D. ZPT sensitivity of hypomorphic strains defective in iron metabolism. Shown is the increase in OD at 600 nm, normalized to the increase for the wild type strain. Wild type (OCY356) and hypomorphs for *GSH2*, *ISU1*, *MTM1*, and *NFS1*. Error bars represent one SD, n=2.

Supplementary Tables

Table S1. Microarray analysis of *S. cerevisiae* BY4741 gene expression in response to ZPT and comparison to published data.[1]

Systematic Name	Common Name	Published Fold Change	Fold Change: 12 µM ZPT, 0.75 Hours	Fold Change: 12 µM ZPT, 2.1 Hours	Fold Change: 12 µM ZPT, 4.9 Hours
Most up regulated genes by ZPT, 0.75 hours					
YLR205C	*HMX1*		**21**		
YJR005C-A			**12**		
YOR384W	*FRE5*		**10**		
YLR327C	*TMA10*		**9.1**		
YOR381W	*FRE3*		**7.7**		
Most up regulated genes by ZPT, 2.1 hours					
YFL053W	*DAK2*			6.8	
YLL006W-A				**4.5**	
YNL269W	*BSC4*			4.2	
YIL102C				**3.7**	
YNL067W-B				3.6	
Most up regulated					

genes by ZPT, 4.9 hours					
YJR005C-A					**88**
YMR317W					**42**
YLR205C	*HMX1*				**38**
YJR150C	*DNA1*				**26**
YOR384W	*FRE5*				**22**
Most down regulated genes by ZPT, 0.75 hours					
YMR120C	*ADE17*		**0.08**		
YPR124W	*CTR1*		**0.08**		
YDL227C	*HO*		**0.14**		
YFR055W	*IRC7*		**0.15**		
YPL189C-A	*COA2*		**0.18**		
Most down regulated genes by ZPT, 2.1 hours					
YPR124W	*ADE17*			**0.10**	
YMR120C	*CTR1*			**0.14**	
YPL189C-A	*COA2*			**0.14**	
YJR048W	*CYC1*			0.15	
YGL009C	*LEU1*			**0.15**	
Most down regulated genes by ZPT, 4.9 hours					
YOL152C	*FRE7*				**0.07**

YHR092C	*HXT4*				**0.12**
YHL028W	*WSC4*				**0.16**
YOR065W	*CYT1*				**0.22**
YPR124W	*CTR1*				**0.23**
Up regulated by ZPT in Yasokawa *et al.* (10)					
YDR534C	***FIT1***	**85**	**4.7**	**2.7**	**19**
YOR382W	*FIT2*	33	**3.3**	**2.4**	**4.3**
YHL047C	*ARN2*	32	**4.9**	**2.4**	**8.2**
YOR383C	*FIT3*	19	**1.8**	1.5	**2.1**
YHL040C	*ARN1*	15	**4.1**	**2.7**	**6.1**
YKL220C	*FRE2*	14	**2.2**	1.3	**9.7**
YCL026C-A	*FRM2*	11	**3.3**	1.7	**16**
YLR205C	*HMX1*	11	**21**	**2.3**	**38**
YLR303W	*MET17*	6.9	**1.8**	0.9	1.2
YOR153W	*PDR5*	6.7	1.1	1.0	1.1
YEL065W	*SIT1*	6.5	**2.2**	1.8	**5.0**
YLL060C	*GTT2*	6.1	**4.4**	1.1	**8.8**
YML058W-A	*HUG1*	5.8	**1.5**	0.8	**2.1**
YPL171C	*OYE3*	5.4	**4.6**	1.5	**7.6**
YGR256W	*GND2*	5.3	**1.5**	0.6	**5.7**
YKL120W	*OAC1*	5.2	**0.8**	**0.6**	0.9
YBR072W	*HSP26*	5.0	**3.0**	0.9	4.1
YBR047W	*FMP23*	5.0	**5.6**	**2.4**	**0.7**
YOL104C	*NDJ1*	4.9	1.3	1.1	1.9
YDR476C		4.9	**2.3**	1.6	**3.9**
YER145C	*FTR1*	4.6	**1.8**	**1.8**	**2.3**
YML131W		4.5	**4.7**	**2.4**	**4.3**

YHR139C	*SPS100*	4.5	**1.5**	0.6	**8.1**
YMR058W	*FET3*	4.2	**1.4**	**1.4**	**1.8**
YER175C	*TMT1*	4.3	**2.4**	1.4	**6.6**
YOR381W	*FRE3*	4.2	**7.7**	**3.0**	**7.2**
YPL277C		4.0	**1.9**	1.5	1.7
YLR127C	*APC2*	3.9	1.2	1.0	1.5
YHR199C	*FMP34*	3.9	**2.3**	1.4	**3.3**
YLR136C	*TIS11*	3.9	**6.3**	**2.3**	**15**
Down regulated by ZPT in Yasokawa *et al.* (10)					
YOL152W	*FRE7*	0.05	**0.21**	0.21	**0.07**
YPR124W	*CTR1*	0.07	**0.08**	**0.10**	**0.23**
YKL141W	*SDH3*	0.08	**0.31**	**0.37**	**0.46**
YPR123C		0.1			
YOR065W	*CYT1*	0.1	**0.29**	**0.23**	**0.22**
YER156C		0.1	**0.21**	**0.29**	**0.46**
YGR088W	*CTT1*	0.11	**0.39**	**0.44**	1.2
YLL041C	*SDH2*	0.11	**0.38**	**0.37**	**0.36**
YEL024W	*RIP1*	0.11	**0.45**	**0.46**	**0.34**
YFR055W	*IRC7*	0.12	**0.15**	**0.23**	**0.33**
YDR178W	*SDH4*	0.11	**0.33**	**0.35**	**0.41**
YKR066C	*CCP1*	0.13	**0.21**	**0.23**	**0.40**
YJL200C	*ACO2*	0.13	**0.25**	**0.32**	**0.41**
YJR048W	*CYC1*	0.14	0.19	**0.15**	**0.28**
YER174C	*GRX4*	0.14	**0.44**	**0.44**	0.65
YDR529C	*QRC7*	0.14	**0.52**	**0.50**	**0.48**
YOR176W	*HEM15*	0.14	**0.29**	**0.31**	**0.41**

YHR051W	*COX6*	0.15	**0.48**	**0.40**	**0.39**
YDR234W	*LYS4*	0.15	**0.31**	**0.37**	**0.52**
YGL187C	*COX4*	0.15	**0.53**	**0.42**	**0.36**
YOL092W		0.16	**0.72**	0.78	**0.62**
YGL256W	*ADH4*	0.16	**0.55**	**0.54**	0.62
YLR214W	*FRE1*	0.17	**0.71**	0.83	**0.67**
YJL217W	*REE1*	0.17	**0.37**	**0.38**	**0.39**
YDR044W	*HEM13*	0.17	**0.30**	**0.43**	0.71
YOR196C	*LIP5*	0.18	**0.44**	**0.46**	0.63
YKL001C	*MET14*	0.18	1.0	0.83	**2.0**
YLR220W	*CCC1*	0.19	**0.47**	**0.49**	**0.50**
YPL053C	*KTR6*	0.19	**0.48**	**0.55**	**0.54**
YER182W	*FMP10*	0.19	**0.69**	0.65	0.69
Aft1p/Aft2p-regulated genes in response to iron deprivation (5)					
YDR534C	*FIT1*		**4.7**	**2.7**	**19**
YOR382W	*FIT2*		**3.3**	**2.4**	**4.3**
YOR383C	*FIT3*		**1.8**	1.5	**2.1**
YLR214W	*FRE1*		**0.71**	0.83	**0.67**
YKL220C	*FRE2*		**2.2**	1.3	**9.7**
YOR381W	*FRE3*		**7.7**	**3.0**	**7.2**
YNR060W	*FRE4*		1.1	**1.6**	1.5
YOR384W	*FRE5*		**11**	**3.3**	**22**
YMR058W	*FET3*		**1.4**	**1.4**	**1.8**
YER145C	*FTR1*		**1.8**	**1.8**	**2.3**
YNL259C	*ATX1*		**2.0**	**1.8**	**1.9**

YDR270W	*CCC2*		**3.3**	**2.1**	**4.3**
YHL040C	*ARN1*		**4.1**	**2.7**	**6.1**
YHL047C	*ARN2*		**4.9**	**2.4**	**8.2**
YEL065W	*ARN3*		**2.2**	1.8	**5.0**
YOL158C	*ARN4*		**3.4**	**2.2**	**4.7**
YLL051C	*FRE6*		**1.9**	1.5	2.2
YLR034C	*SMF3*		1.2	1.2	1.6
YFL041W	*FET5*		**2.3**	**1.9**	2.2
YBR207W	*FTH1*		2.4	1.8	**2.7**
YGR065C	*VHT1*		**1.9**	1.5	**3.4**
YOR316C	*COT1*		**2.7**	1.8	3.3
YKR052C	*MRS4*		**2.7**	**2.2**	**3.9**
YLR205C	*HMX1*		**21**	**2.3**	**38**
YLR136C	*CTH2*		**6.3**	**2.3**	**15**
Genes up regulated by copper in van Bakel *et al.* (7)					
YMR058W	*FET3*	2.8	**1.4**	**1.4**	**1.8**
YOR382W	*FIT2*	2.2	**3.3**	**2.4**	**4.3**
YEL065W	*ARN3*	2.3	**2.2**	1.8	**5.0**
YHR053C	*CUP1*	4.4	**1.1**	1.1	**1.4**
Genes down regulated by copper in van Bakel *et al.*, (7)					
YPR124W	*CTR1*	0.5	**0.08**	**0.10**	**0.23**
YLR214W	*FRE1*	0.5	**0.71**	0.83	**0.67**
YLR213C	*CRR1*	0.4	**0.66**	**0.68**	**0.62**

YOR383C	*FIT3*	0.5	**1.8**	1.5	**2.1**
YOL158C	*ARN4*	0.4	**3.4**	**2.2**	**4.7**
YLR136C	*CTH2*	0.4	**6.3**	**2.3**	**15**
Genes up regulated by copper in Gross *et al.* (1)					
YHR053C	*CUP1*	6.3	**1.1**	1.1	**1.4**
YOR031W	*CRS5*	2.2	1.1	1.1	**1.4**
YJR104C	*SOD1*	1.2	**1.1**	1.0	1.1
YMR058W	*FET3*	4.5	**1.4**	**1.4**	**1.8**
YER145C	*FTR1*	3.8	**1.8**	**1.8**	**2.3**
Genes up regulated by copper in Yasokawa *et al.* (9)					
YHR053C	*CUP1-1*	24	**1.1**	1.1	**1.4**
YHR055C	*CUP1-2*	22			
YLR303W	*MET17*	14	**1.8**	0.88	1.2
YOR031W	*CRS5*	8	1.1	1.1	**1.4**
YGR055W	*MUP1*	6	1.1	0.86	1.5
YL289W	*PCL1*	6	0.94	1.0	**0.60**
YPL171C	*OYE3*	5	**4.6**	1.5	**7.6**
Genes down regulated by copper in Yasokawa *et al.* (9)					
YLR214W	*FRE1*	0.07	**0.71**	0.83	**0.67**

YOL152W	*FRE7*	0.08	**0.21**	0.21	**0.07**
YPR124W	*CTR1*	0.10	**0.08**	**0.10**	**0.23**
YFR055W	*IRC7*	0.14	**0.15**	**0.23**	**0.33**
YPR123C		0.17			
YJL217W		0.20	**0.37**	**0.38**	**0.39**
Zinc metabolism genes listed in Yasokawa *et al.* (10)					
YGL255W	*ZRT1*	0.29	**0.43**	**0.55**	1.1
YLR130C	*ZRT2*	0.63	**0.37**	**0.48**	**0.50**
YKL175W	*ZRT3*	2.1	1.1	0.98	1.1
YMR243C	*ZRC1*	0.73	**0.73**	0.85	0.80
YGR211W	*ZPR1*	0.47	0.95	0.98	1.3
YDR151C	*CTH1*	0.32	**0.70**	0.81	1.3
YDR492W	*IZH1*	1.6	**0.72**	0.92	0.89
YOR316C	*COT1*	2.8	**2.7**	1.8	3.3
YDR391C		1.6	**2.4**	1.7	2.4
YOR079C	*ATX2*	2.3	**1.8**	1.7	2.4
Zinc-regulated genes in Pagani *et al.* (4)					
YAL061W		4.0	**1.9**	**1.0**	2.0
YOR120W	*GCY1*	4.3	**1.8**	1.1	3.5
YMR169C	*ALD3*	3.5	**1.4**	0.83	**4.7**
YGR248W	*SOL4*	3.2	**1.5**	0.90	2.7
YML100W	*TSL1*	3.2	0.92	1.0	0.95
YER103W	*SSA4*	7.1	**1.7**	1.0	2.6

YBR072W	*HSP26*	7.1	**3.0**	0.86	4.1
YDR533C	*HSP31*	3.4	**3.9**	1.6	2.2
YBR101C	*FES1*	3.3	1.0	1.1	**1.7**
YGR055W	*MUP1*	3.2	1.1	0.86	1.5
YCR021C	*HSP30*	4.8	**1.3**	0.56	4.2
YFL014W	*HSP12*	4.7	1.1	0.79	**3.0**
YOL052C-A	*DDR2*	4.4	**1.3**	0.79	**4.6**
YJL144W		3.1	**1.9**	0.88	**5.0**
YLR108C		3.7	**2.1**	1.4	**2.7**
YOR121C		4.0			
YHR087W		3.7	**2.1**	1.3	2.8

[1] The data will be deposited at the NCBI site, Gene Expression Omnibus. The growth medium was YPD. ZPT treatments are compared to controls (treated with an equal volume of DMSO) harvested at the same time (n=4 for both treatment and control at each time point). In the top rows are the five genes showing the greatest fold change in expression at the indicated times. In the lower section is a comparison with published data. The published data sets are the 30 genes most up regulated in response to 1.3 µM ZPT (10) the 30 genes most down regulated in response to 1.3 µM ZPT (10), genes induced upon iron starvation and regulated by Aft1p/Aft2p (5), genes showing >2-fold up regulation in response to 8 µM copper sulfate (7), genes showing >2-fold down regulation in response to 8 µM copper sulfate (7), genes showing > 1.5-fold up regulation in response to 100 µM copper sulfate (1), genes showing > 2-fold up regulation in response to 10 mM copper sulfate (9), genes showing > 2-fold down regulation in response to 10 mM copper sulfate (9), zinc metabolism genes present in Table 2 of Yasokawa *et al.* (10), and genes induced > 3-fold during a one-hour treatment with 5 mM zinc (4). For the right three columns, statistically significant ($P<0.05$) fold changes are indicated with bold text.

Table S2. Atomic emission of *S. cerevisiae* BY4741 treated with metal chelators.[1]

Treatment	Cell Pellet, g	Copper, ppm	Fe, ppm	Zn, ppm
None	0.46 ± 0.00	2.3 ± 0.06	116 ± 0	210 ± 1
BPS, 4.1 mM	0.35 ± 0.00	<1	3.2 ± 0.1	23 ± 1
BPS, 11 mM	0.34 ± 0.01	<1	3.3 ± 0.2	24 ± 0
BPS, 21 mM	0.33 ± 0.01	<1	3.7 ± 0.3	24 ± 0
BCS, 1.8 mM	0.35 ± 0.00	<1	127 ± 0	261 ± 1
1,10-Phenanthroline, 27 µM	0.37 ± 0.02	2.4 ± 0.0	47 ± 6	247 ± 5
None	0.36 ± 0.02	3.0 ± 0.2	127 ± 1	275 ± 0
EDTA, 1.7 mM	0.24 ± 0.01	3.8 ± 0.2	5.7 ± 0.6	22 ± 0
EDTA, 3.4 mM	0.24 ± 0.03	3.3 ± 0.0	4.8 ± 0.2	20 ± 1
EDTA, 6.7 mM	0.23 ± 0.00	3.2 ± 0.1	5.5 ± 0.0	25 ± 3

1 Cultures were grown overnight in YPD. Mean values and SD are shown, n=2.

Table S3. The twenty most zinc chloride and ZPT-sensitive strains from the deletion library, ranked as described in Supplementary Methods.[1]

Ranking	Most Zinc Chloride-Sensitive Strains, ORF	Most Zinc Chloride-Sensitive Strains, Gene	Most ZPT-Sensitive Strains, ORF	Most ZPT-Sensitive Strains, Gene
1	YKL080W	*VMA5*	YPL031C	*PHO85*
2	YLR447C	*VMA6*	YPL045W	*VPS16*
3	YBR127C	*VMA2*	YBL025W	*RRN10*
4	YEL051W	*VMA8*	YLR403W	*SFP1*
5	YGR020C	*VMA7*	YKL155C	*RSM22*
6	YHR026W	*PPA1*	YGL165C	[2]*ACE1*
7	YGR105W	*VMA21*	YBR200W	*BEM1*
8	YKL118W	[2]*VPH2*	YMR097C	*MTG1*
9	YKL119C	*VPH2*	YGL166W	*ACE1*
10	YEL027W	*CUP5*	YHR187W	*IKI1*
11	YOR331C	[2]*VMA4*	YPL059W	*GRX5*
12	YDL185W	*TFP1*	YJR122W	*IBA57*
13	YPR099C		YLR369W	*SSQ1*
14	YHR060W	*VMA22*	YBR268W	*MRPL37*
15	YMR123W	*PKR1*	YLR025W	*SNF7*
16	YHR039C-B	[2]*VMA10*	YLL027W	*ISA1*
17	YPR036W	*VMA13*	YMR066W	*SOV1*
18	YPL234C	*TFP3*	YPL118W	*MRP51*
19	YOR270C	*VPH1*	YML028W	*TSA1*
20	YGL124C	*MON1*	YLR396C	*VPS33*

[1]Growth was in YPD.

[2]These corresponding ORF's are annotated as dubious open reading frames, unlikely to encode proteins, but overlapping with the gene indicated (6).

Table S4. Growth inhibition: IC_{50} (μM) of several materials against a wild type (BY4741) and *ACE1* deletion mutant of *S. cerevisiae*.[1]

Material	**Wild Type**	***ΔACE1***
ZPT	4.5	0.4
CuPT	2	0.08
Sodium Pyrithione	20	1
Copper Chloride	16000	800
Zinc Chloride	2000	2000
1,10-Phenanthroline	600	600
Octopirox	100	100
EDTA[2]	1000	1000
BPS[3]	>8000	>8000
BCS	>4000	>4000

[1]Growth was in YPD, n=4.

[2]Both curves show some growth inhibition but not as much as 50%. Wild type shows greater inhibition.

[3]Both curves show similar inhibition but not as much as 50%.

Table S5. Atomic emission of *S. cerevisiae* BY4741 grown overnight aerobically in YPD and treated with ZPT and copper chloride. Mean and SD are shown.

Treatment	Cell Pellet, g[1]	Copper, ppm	Fe, ppm
DMSO	0.32 ± 0.00	4 ± **0**	166 ± **3**
ZPT, 130 µM	**0.07 ± 0.00**	**20 ± 1**	**27 ± 0**
Copper Sulfate, 500 µM	**0.36 ± 0.01**	**154 ± 1**	**95 ± 4**
ZPT, 130 µM + Copper Chloride, 500 µM	**0.03 ± 0.00**	**420 ± 18**	**66 ± 5**

1 The weighed cell pellet is an indication of growth. Bold text indicates that the results were statistically significantly ($P<0.05$) different from the DMSO-treated (control) samples. n=2.

Table S6. Effect of $CuCl_2$ on inhibition of *S. cerevisiae* BY4741 growth by metal chelators in YPD medium.

Chelator	IC50, µM	$CuCl_2$ Doses (µM) That Enhance Anti-yeast Activity[1]	$CuCl_2$ Doses (µM) That Depress Anti-yeast Activity
ZPT	8	=1	-
Sodium Pyrithione	6	=2	-
Octopirox	200	-	=300
BPS	10,000	-	=5000
EDTA	1000	-	=1000
1,10-Phenanthroline	50	-	=150

[1] The indicated doses of $CuCl_2$ are the doses that increase or decrease the anti-yeast activity of the test chelator at its IC_{50} ($P<0.05$). A "-" indicates that there was no such dose of $CuCl_2$. n=2.

Table S7. Expression of selected *M. globosa* genes in response to ZPT.[1]

	P value (ZPT vs. Control)	Fold change (ZPT vs. Control)	Description
Copper			
Gene1106	4.7E-07	0.3	High affinity copper importer
Gene2566	2.9E-05	2.0	Cu(+2)-transporting P-type ATPase, required for export of copper from the cytosol into an extracytosolic compartment
Iron			
Gene704	1.8E-05	2.5	Siderophore peptide synthetase
Gene1049	7.9E-07	3.8	Ornithine 5-monooxygenase oxidoreductase involved in siderophore synthesis
Gene1727	1.9E-06	3.1	Siderophore transporter
Gene4358	1.8E-02	1.3	Fet3p/Fet5p high affinity copper uptake
Gene2939	9.8E-05	2.0	Fet3p/Fet5p high affinity copper uptake
Gene3856	3.2E-04	1.5	Multicopper oxidase, may be involved in iron uptake
Gene554	3.1E-06	2.9	Multicopper oxidase, may be nvolved in iron uptake

Gene3855	6.0E-03	1.3	Multicopper oxidase, may be involved in iron uptake
Gene2935	1.2E-07	3.7	Multicopper oxidase, may be involved in iron uptake
Gene2935	5.1E-06	3.9	Multicopper oxidase, may be involved in iron uptake
Gene2934	2.0E-06	4.8	Multicopper oxidase, may be involved in iron uptake
MGL2538	2.4E-04	3.1	Multicopper oxidase, may be involved in iron uptake
MGL661	1.0E-03	1.6	Multicopper oxidase, may be involved in iron uptake
Gene2551	6.5E-10	0.1	Ferric reductase, may be involved in copper and iron intake

[1]The growth medium was mDixon. Shown are the expression changes of selected genes annotated for their role in metal metabolism. Cells were treated with 3 µM ZPT or DMSO (control), n=4.

Table S8. Atomic emission measurements of metal content of *M. globosa* treated with ZPT, octopirox, and BPS.[1] Mean and SD are shown.

Treatment	Dose, µM	Optical Density, 600 nm	Cu, ppm	Fe, ppm	Zn, ppm
DMSO		1.12 ± 0.01	19 ± 3	58 ± 5	246 ± 6
ZPT	1	**0.96**[2] ± 0.03	31 ± 3	38 ± 6	129 ± 27
	5	**0.56** ± 0.02	**50** ± 0	**23** ± 4	**40** ± 10
Octopirox	2	1.08 ± 0.03	18 ± 1	**34** ± 2	**102** ± 1
	11	**0.68** ± 0.03	<10	**21** ± 1	**24** ± 2
BPS	27	**0.92** ± 0.04	<10	**35** ± 1	**128** ± 18
	140	**0.96** ± 0.01	<10	**12** ± 0	**61** ± 1

[1]From the same experiment shown in Figure 5.

[2]Bold text indicates that the results were statistically significantly ($P<0.05$) different from the DMSO-treated (control) samples; n=2.

Supplementary Materials and Methods

Gene Expression Methods and Results. A fresh overnight culture (YPD for *S. cerevisiae*, mDixon for *M. globosa*) was used to inoculate a 25-ml culture to an OD of 0.1. The culture was incubated at 30° C with shaking to an OD of 0.2 whereupon the cultures were treated with ZPT or an equal volume of DMSO. Cells were collected by centrifugation. The cell pellet was suspended in Trizol (Invitrogen, Carlsbad, CA), homogenized in a Retsch (Wunsiedel, Bavaria) MM300 Bead-Beater Mill using 5 mm stainless steel beads, and then frozen.

RNA Isolation. Lysates were thawed, and chloroform was added. The mixture was shaken for one to two minutes and the aqueous phase, containing crude nucleic acids, was removed and precipitated with an equal volume of isopropanol. Nucleic acids were pelleted by centrifugation, and the pellets were washed with 70% ethanol and suspended in 200 µl of diethylpyrocarbonate treated-water. RNA was purified using QIAgen (Hilden, Germany) RNEasy Cleanup minicolumns and the manufacturer's recommended protocol. The quantity of RNA was determined by UV spectroscopy, and the quality was determined using an Agilent (Palo Alto, CA) Bioanalyzer 2100.

GeneChip Target Synthesis and GeneChip Processing. One µg of purified total RNA was converted to cRNA target using the protocol provided by Affymetrix, Inc. (Santa Clara, CA). Twenty µg of cRNA target was fragmented and hybridized to either Affymetrix Yeast Genome 2.0 Arrays for *S. cerevisiae* cultures or custom *M. globosa* Genechips as described

below. Following a sixteen-hour hybridization, chips were washed, stained, and scanned according to procedures provided by Affymetrix. Complete protocols for target synthesis and GeneChip processing can be found at:

www.affymetrix.com/support/download/manuals/expression_s2_manual.pdf

Custom *M. globosa* GeneChip. A custom Affymetrix GeneChip was designed using sequence data (9). The GeneChip contains 4285 genes from the *M. globosa* genome. GeneChips were created by Affymetrix, Inc. using standard GeneChip probe selection guidelines.

Conditions of gene expression experiments. Cells were treated with either ZPT (in DMSO) or an equal volume of DMSO, and the OD was recorded at treatment and at harvest, with SD referring to the standard deviation of data in the column to the left of the SD column. Hours refers to hours of treatment. For all treatments, n=4.

Treatment	**Hours**	**OD at Treatment**	**SD**	**Final OD**	**SD**
S. cerevisiae					
No treatment	0.75	0.21	0.005	0.30	0.02
ZPT, 12 µM	0.75	0.21	0.003	0.32	0.009
No treatment	2.1	0.21	0.009	0.52	0.002
ZPT, 12 µM	2.1	0.21	0.006	0.41	0.001
No treatment	4.9	0.20	0.004	1.7	0.09
ZPT, 12 µM	4.9	0.21	0.004	0.64	0.02
M. globosa					
No treatment	12	0.21	0.008	0.99	0.08
ZPT, 3 µM	12	0.21	0.006	0.45	0.03

Supplementary Analysis of Microarrays

Microarray data were normalized using the Affymetrix MAS5 algorithm. The treated samples were compared to the controls using a t-test. Enriched biological processes were identified using hypergeometrical testing based on the Gene Ontology (GO) annotation from the yeast genome database (6). We tested the effects on *S. cerevisiae* gene expression from ZPT treatment (12 µM) with three different lengths of exposure (0.75, 2.1, and 4.9 hours), with four replicates for each length of exposure. DMSO-treated cultures were harvested at each of these times and served as controls. Yasokawa *et al.* (10) recently described the *S. cerevisiae* gene expression response to ZPT (1.3 µM). Although there were differences between the experimental conditions, including the use of different yeast strains and ZPT concentrations, the results were similar (Table S1). Yasokawa listed the 30 most up-regulated genes in response to ZPT, and 27 of these 30 were significantly ($P<0.05$) up regulated in at least one of our time points. Among the 30 most down-regulated genes identified by Yasokawa *et al.* (10), we observed 28 of these significantly down regulated in at least one of our time points.

As also noted by Yasokawa *et al.* (10), the most striking theme from our gene expression data is the induction of genes whose products are involved in iron uptake. Philpott and Protchenko (6) list a set of 25 Aft1p/Aft2p-regulated genes that are induced upon iron starvation. All but two (*FRE1, SMF3)* of these genes are up regulated in our studies. *FRE1* is known to be down regulated by increased copper ((9); see below). Three of these iron starvation-induced genes (*HMX1, FRE3, FRE5)* were among the top five most ZPT-induced genes at one or more time point (Table S1).

Microarray studies provided further evidence that ZPT caused a copper increase. Yasokawa *et al.* (10) observed that *FRE7* and *CTR1* (encoding a copper importer) were the two most down-regulated genes upon ZPT treatment, and each of these genes was strongly down regulated by copper exposure (9). We also observed that *FRE7* and *CTR1* were strongly down regulated by ZPT, with *CTR1* among the five most down-regulated genes at each of the three time points of ZPT treatment (Table S1). Furthermore there was some correlation with ZPT-induced gene expression changes and gene expression changes in other studies where *S. cerevisiae* was treated with copper salts. From three different studies, eleven genes were identified as being up regulated by copper treatment: nine of these genes were up regulated by ZPT in our studies (Table S1). From the published studies, eight genes were identified as being down regulated by copper. We observed that five of these were down regulated by ZPT (Table S1). The other three genes (*FIT3, ARN4, CTH2*) are all known to be inducible by iron deprivation (5).

As noted by Yasokawa *et al.* (10), ZPT differs from copper treatment in that the metallothionein gene *CUP1* shows little or no induction by ZPT treatment. Possibly the lack of a larger effect is due to the relatively small copper increase in ZPT-treated cells.

We looked for insights into cellular zinc levels, with several lines of evidence suggesting an increased zinc level upon treatment with 12 μM ZPT. Yasokawa *et al.* (10) listed a set of genes involved in zinc metabolism (Table S1). ZPT treatment causes a significant down regulation of two genes (*ZRT1, ZRT2*) whose products are involved in zinc uptake. Pagani *et al.* (5) described a set of genes induced by high zinc levels, with many of these genes encoding stress response proteins. Looking at the seventeen genes in which Pagani *et al.* (5) observed at least three-fold induction with zinc chloride, we saw significant up regulation of fourteen of

these genes in our ZPT-treatment experiments, with none of these showing any down regulation (Table S1). However, one conflicting data point is that *ZRC1*, whose product catalyzes transport of excess cytoplasmic zinc into the vacuole, is down regulated by ZPT (Table S1). These results are most consistent with an increase in cellular zinc upon exposure to 12 µM ZPT. However, there is no evidence that zinc has much role in growth inhibition imparted by ZPT.

From the microarray data, at 12 µM ZPT, the transcriptional profile is consistent with iron starvation, an increase in copper levels, and a likely increase in zinc levels.

ZPT effects on human cells. The observed mechanism of action in yeast may not extend, at least with the same magnitude, to human cells. ZPT causes an overload of zinc in keratinocytes, and this effect is antagonized by a zinc chelator (2). In a microarray study, a water-soluble analogue of pyrithione (PCI-5002) was synthesized and used to treat A549 human lung cancer cells (3). In the study, PCI-5002 induces the expression of several metallothionein genes and the zinc transporter gene SLC30A1, consistent with a zinc overload (3). However, the expression of the *CTR1* ortholog SLC31A1 and the copper transporter genes ATP7A and ATP7B are little changed upon PCI-5002 treatment (shown in the table below). This may indicate that pyrithione's interaction with human cells does not lead to the copper increase that we observed in *S. cerevisiae.*

Affy Accession	**Gene Symbol**	**p-value**	**Fold-change**
235013_at	SLC31A1	0.11	0.74
236217_at	SLC31A1	0.72	0.94
203971_at	SLC31A1	0.83	0.92
205197_s_at	ATP7A	0.11	0.61

205198_s_at	ATP7A	0.022	0.61
204624_at	ATP7B	0.96	1.00
234075_at	ATP7B	0.47	0.88
233796_at	ATP7B	0.983937	1.00504

Effect of PCI-5002 on several genes involved in copper homeostasis. The statistics were calculated using the deposited Affymetrix .CEL file in the GEO database with an accession number of GSE6972 (3). Microarray data were normalized using RMA.

Supplementary References

1. **Gross, C., M. Kelleher, V. R. Iyer, P. O. Brown, and D. R. Winge.** 2000. Identification of the copper regulon in Saccharomyces cerevisiae by DNA microarrays. J Biol Chem **275:**32310-6.
2. **Lamore, S. D., and G. T. Wondrak.** 2011. Zinc pyrithione impairs zinc homeostasis and upregulates stress response gene expression in reconstructed human epidermis. Biometals: an international journal on the role of metal ions in biology, biochemistry, and medicine.
3. **Magda, D., P. Lecane, Z. Wang, W. Hu, P. Thiemann, X. Ma, P. K. Dranchak, X. Wang, V. Lynch, W. Wei, V. Csokai, J. G. Hacia, and J. L. Sessler.** 2008. Synthesis and anticancer properties of water-soluble zinc ionophores. Cancer research **68:**5318-25.

4. **Pagani, M. A., A. Casamayor, R. Serrano, S. Atrian, and J. Arino.** 2007. Disruption of iron homeostasis in Saccharomyces cerevisiae by high zinc levels: a genome-wide study. Mol Microbiol **65:**521-37.

5. **Philpott, C. C., and O. Protchenko.** 2008. Response to iron deprivation in Saccharomyces cerevisiae. Eukaryotic Cell **7:**20-7.

6. **SGD.** *Saccharomyces* Genome Database. **http://www.yeastgenome.org**.

7. **van Bakel, H., E. Strengman, C. Wijmenga, and F. C. Holstege.** 2005. Gene expression profiling and phenotype analyses of S. cerevisiae in response to changing copper reveals six genes with new roles in copper and iron metabolism. Physiol Genomics **22:**356-67.

8. **Xu, J., C. W. Saunders, P. Hu, R. A. Grant, T. Boekhout, E. E. Kuramae, J. W. Kronstad, Y. M. Deangelis, N. L. Reeder, K. R. Johnstone, M. Leland, A. M. Fieno, W. M. Begley, Y. Sun, M. P. Lacey, T. Chaudhary, T. Keough, L. Chu, R. Sears, B. Yuan, and T. L. Dawson, Jr.** 2007. Dandruff-associated Malassezia genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. Proceedings of the National Academy of Sciences of the United States of America **104:**18730-5.

9. **Yasokawa, D., S. Murata, E. Kitagawa, Y. Iwahashi, R. Nakagawa, T. Hashido, and H. Iwahashi.** 2008. Mechanisms of copper toxicity in Saccharomyces cerevisiae determined by microarray analysis. Environ Toxicol **23:**599-606.

10. **Yasokawa, D., S. Murata, Y. Iwahashi, E. Kitagawa, K. Kishi, Y. Okumura, and H. Iwahashi.** 2010. DNA microarray analysis suggests that zinc pyrithione causes iron starvation to the yeast Saccharomyces cerevisiae. J Biosci Bioeng **109:**479-86.