Supplementary Information

Expanded role of the Cu-sensing transcription factor Mac1p in *Candida albicans*

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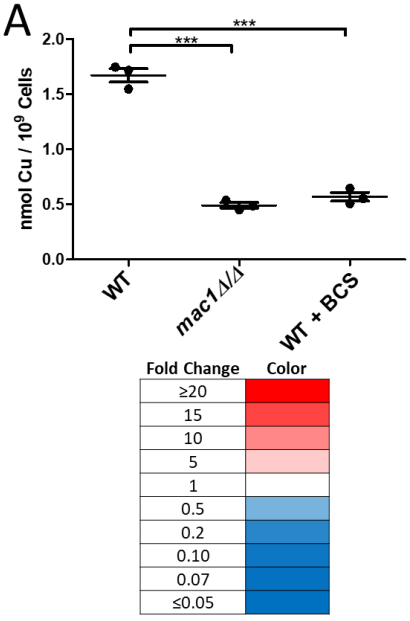
Includes:

Supplementary Figures and Figure Legends for Fig. S1 to S2

Supplementary Tables S2 to S4

References

A



B

Transcript	Wt + BCS	*mac1*Δ/Δ	Sequence	Position
SOD1	0.12	1.42	TTTGCTCA	147
AAH1	0.23	1.22		
FGR23	0.35	1.22		

C

Transcript	Wt + BCS	*mac1*Δ/Δ	Sequence	Position
CTR1	165	0.54	TTTGCTCA	-396
			R-TTTGCTCA	-275
			TTTGCTCA	-237
FRE7	78	0.17	R-CTTGCTCA	-383
			R-TTTGCTCA	-176
			TTTGCTCA	-131
FRE30	70	1.13	TTTGCTCA	-643
			R-TTTGCTCT	-238
			R-ATTGCTCA	-174
SOD3	65	0.20	ATTGCTCA	-153
AOX2	39	3.25	TTTGCTCA	-774
			R-TTTGCTCA	-176
			R-TTTGCTCA	-163
			R-TTTGCTCG	-149
MAC1	6.3	0.06	TTTGCGCA	-167

Figure S1. Genes regulated by Cu starvation in a *MAC1*-dependent manner

(A) Intracellular Cu levels were determined by AAS as described in *Experimental Procedures* and are shown for the individual cell samples utilized for RNA-seq analysis (n=3). One-way ANOVA with Tukey posttest was used to determine the statistical significance; ***p≤0.001. The absence of a bracket indicates that comparisons are not significantly different. Within the data points, the bar represents mean with error bars showing the SEM. (B, C) Summary of genes most strongly repressed (B) or induced (C) in WT cells treated with BCS, but not in *mac1*Δ/Δ strains. Comparisons are made to untreated WT control cells. The position and sequence of the Mac1p consensus sites (Woodacre *et al.*, 2008) is shown with an “R” designating if they are located on the opposite strand of DNA.

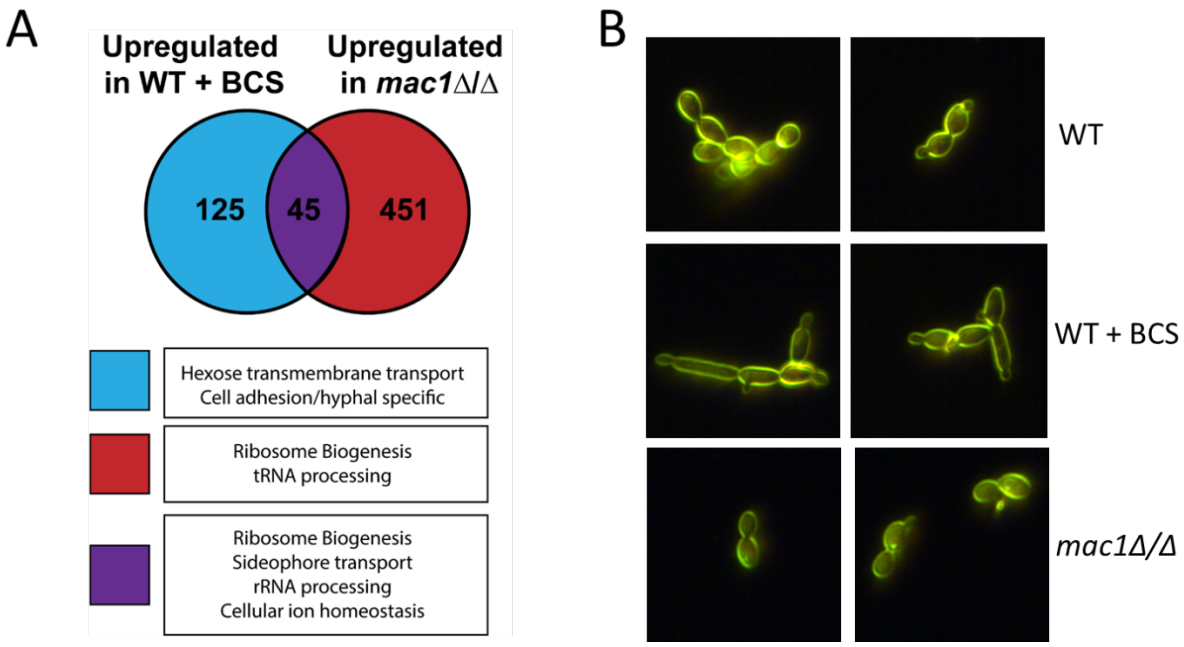


Figure S2. Genes differentially regulated by Cu starved WT versus *mac1Δ/Δ* mutants and hyphal morphology of Cu starved WT cells

(A) Shown is a summary of genes upregulated by >3-fold in WT + 400 μM BCS and *mac1Δ/Δ* strains compared to WT untreated control cells. (TOP) Venn diagram shows the total number of genes up-regulated under the two conditions. (BOTTOM) Boxes illustrate enrichment of specific gene categories as determined in part by GO analysis. B) Representative images of cellular morphology after 24 hours of growth in YPD media of WT, WT + 400 μM BCS, and *mac1Δ/Δ* cells viewed by dark field microscopy. Strains utilized: WT, SC5314; *mac1Δ/Δ,* EC004.

Table S2: Primers used for Plasmid construction

Primer Name	Description	Sequence
M1-5L-SacI	*MAC1* -441 to -205 with SacI site for pEC-M1L	GGAGCTCGTTTGACAACTTGCAGAT
M1-5L-NotI	*MAC1* -441 to -205 with NotI site for pEC-M1L	GGCGGCCGCACAAGGGAGGGATCAGGA
M1-5S-SacI	*MAC1* -205 to -1 with SacI site for pEC-M1S	GGAGCTCAGTTTCACCTAACCATTCCC
M1-5S-NotI	*MAC1* -205 to -1 with NotI site for pEC-M1S	GCGGCCGCTCCTTATTCAGTCTTGCTTT
M1-3L-XhoI	*MAC1* 1423 to 1593 with XhoI site for pEC-M1L	GGCTCGAGCCTGCATACAGCACCAAT
M1-3L-KpnI	*MAC1* 1423 to 1593 with KpnI site for pEC-M1L	GGCTCGAGCCTGCATACAGCACCAAT
M1-3S-XhoI	Amplify *MAC1* 1201 to 1419 with XhoI site for pEC-M1S	GGCTCGAGGACAAGAAGGCAACAAAG
M1-3S-KpnI	Amplify *MAC1* 1201 to 1419 with KpnI site for pEC-M1S	GGGTACCTCATAGTTCCAAATACCAC

Table S3: Primers used for CRISPR

Primer	Sequence
Mac1 gDNA	CGTAAACTATTTTTAATTTGAAAGCAAGACTGAATAAGGAGTTTTAGAGCTAGAAATAGC
SOD1 gDNA	CGTAAACTATTTTTAATTTGCAACATATATATAATTAAAAGTTTTAGAGCTAGAAATAGC
MAC1 dDNA F	TCGTACCTTAAGTTAGACTATTTACAAAAATATTTACATATACAGTTCATCCCCTTATTCAGTCTTGCTTTTTGGAGGGGATCTAGTGACATGTAAACAT
MAC1 dDNA R	AGTTTTACATGTCACTAGATCCCCTCCAAAAAGCAAGACTGAATAAGGGGATGAACTGTATATGTAAATATTTTTGTAAATAGTCTAACTTAAGGTACGA
SOD1 dDNA F	TTCTTGGGTTGGATTGTTGATGATGATGGCAATCTTGGCTCATCTATTCCTTTTAATTATATATATGTTGATAATTGAATTGAATTGAATTGATCTTT
SOD1 dDNA R	AAAGATCAATTCAATTCAATTCAATTATCAACATATATATAATTAAAAGGAATAGATGAGCCAAGATTGCCATCATCATCAACAATCCAACCCAAGAA

Table S4: Primers used for qRT-PCR reactions

Primer Name	Sequence	Reference
TUB2 F	GAGTTGGTGATCAATTCAGTGCTAT	(Li *et al.*, 2015)
TUB2 R	ATGGCGGCATCTTCTAATGGGATTT	(Li *et al.*, 2015)
CFL4 F	CGAGAGTAAAGAGCCGTTGC	(Moran, 2012)
CFL5 R	CATTGCTGGATGACCACAAG	(Moran, 2012)
SOD4 F	CTTGACGAAGGTGACGATACTGCAA	(Schatzman *et al.*, 2020)
SOD4 R	TTAAAGCAGCAACAACACCGGCAAT	(Schatzman *et al.*, 2020)
CTR1 F	CAAAAGCTCGTGGAACCGGTAAATC	(Li *et al.*, 2015)
CTR1 R	TCAGCAACAAATCTTCCAACACCGG	(Li *et al.*, 2015)

References:

Li, C.X., Gleason, J.E., Zhang, S.X., Bruno, V.M., Cormack, B.P., and Culotta, V.C. (2015) Candida albicans adapts to host copper during infection by swapping metal cofactors for superoxide dismutase. *Proc Natl Acad Sci U S A*.

Moran, G.P. (2012) Transcript profiling reveals rewiring of iron assimilation gene expression in Candida albicans and C. dubliniensis. *FEMS Yeast Res* **12**: 918-923.

Schatzman, S.S., Peterson, R.L., Teka, M., He, B., Cabelli, D.E., Cormack, B.P., and Culotta, V.C. (2020) Copper-only superoxide dismutase enzymes and iron starvation stress in Candida fungal pathogens. *J Biol Chem* **295**: 570-583.

Woodacre, A., Mason, R.P., Jeeves, R.E., and Cashmore, A.M. (2008) Copper-dependent transcriptional regulation by Candida albicans Mac1p. *Microbiology* **154**: 1502-1512.