SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Melanin production is decreased in *sre1*Δ and *stp1*Δ strains.
Melanin production was estimated by spotting serially diluted yeast cells from the indicated strains onto norepinephrine-containing medium. The plates were then incubated for 2 days at 30^{o}C in the dark.

Figure S2. Capsule size of *sre1*Δ and *stp1*Δ cells is similar to wild-type.
Capsule size of 100 cells was measured in India Ink preparations. Error bars denote standard deviation.

Figure S3. Scp1 levels in *stp1*Δ cells are similar to wild-type.
Wild-type, *sre1*Δ, *stp1*Δ and *scp1*Δ cells were grown to log phase under normoxic conditions. Microsomal fractions were harvested from cell lysates and subjected to immunoblot analysis using anti-Scp1 (amino acids 771-1444) antiserum.

Figure S4. Brefeldin A treatment accumulates Sre1 precursor in *stp1*Δ cells.
Wild-type, *sre1*Δ and *stp1*Δ strains were grown for two hours in the absence or presence of 100 μg/ml Brefeldin A (BFA), either alone or without 15 nM itraconazole. Immunoblot analysis to detect Sre1 precursor was performed on whole cell extracts using anti-Sre1 antiserum. Asterisks indicate non-specific, cross-reacting proteins detected by anti-Sre1 antiserum.

SUPPLEMENTAL METHODS

Melanin Production Assay

Two-fold serial dilutions of yeast cells (starting at 3x10^{5} cells) were spotted onto norepinephrine-containing plates and grown for 2 days at 30^{o}C in the dark (Erickson *et al.*, 2001).

Capsule Measurements

To determine capsule size in vitro, cells were grown on RPMI medium at 30^{o}C for 1 day to induce capsule production. Capsule size was measured in India Ink preparations under oil immersion (100X). Pictures were taken with a Zeiss Axiovert 200 and AxioCam camera (Carl Zeiss, NY). At least three different fields were randomly chosen and photographed. The distance from the edge of the capsule to the cell wall of 100 cells from each strain was measured and averaged (Chang *et al.*, 2007).

SUPPLEMENTAL REFERENCES

Chang,Y.C., Bien,C.M., Lee,H., Espenshade,P.J., and Kwon-Chung,K.J. (2007) Sre1p, a regulator of oxygen sensing and sterol homeostasis, is required for virulence in *Cryptococcus neoformans Mol.Microbiol.* **64**: 614-629.

Erickson,T., Liu,L., Gueyikian,A., Zhu,X.D., Gibbons,J., and Williamson,P.R. (2001) Multiple virulence factors of Cryptococcus neoformans are dependent on VPH1 *Molecular Microbiology* **42**: 1121-1131.

Figure S1

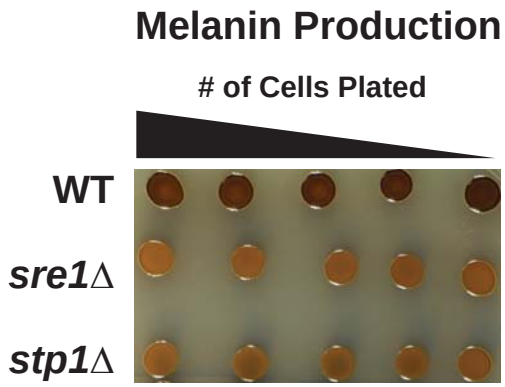

Figure S2

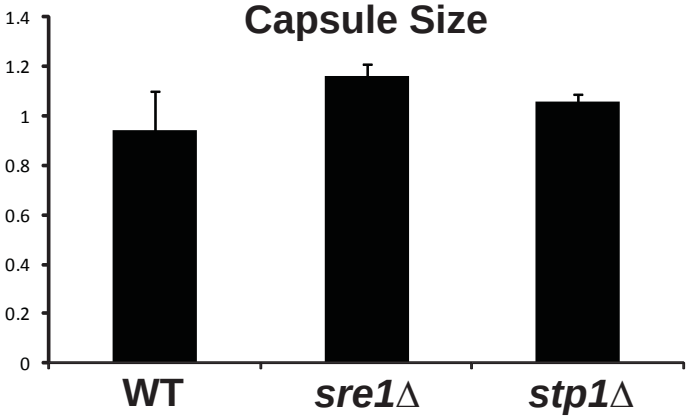

Figure S3

Western Blot

Strain	WT	*sre1*Δ	*stp1*Δ	*scp1*Δ
Lane	1	2	3	4

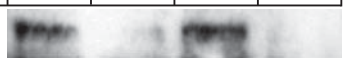

Figure S4

Western Blot

Strain	WT				*sre1*Δ				*stp1*Δ			
BFA	-		+		-		+		-		+	
Itra.	-	+	-	+	-	+	-	+	-	+	-	+
Lane	1	2	3	4	5	6	7	8	9	10	11	12

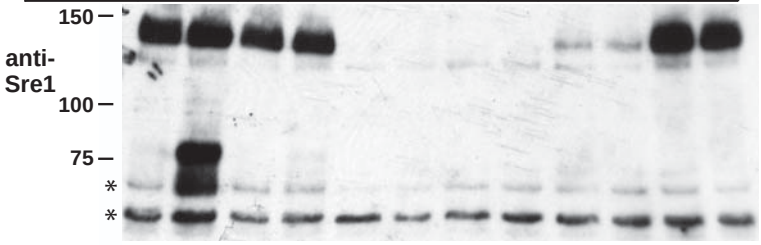

Table S1 –Strains used in this study

Strain name	Genotype	Reference
H99	Wild-type serotype A	Perfect *et al.*, 1980
CBY22-1	*sre1*Δ	This study
CBY4-1	*stp1*Δ	This study
CBY23-1	*sre1*Δ + *SRE1*	This study
CBY75-3	*sre1*Δ+ *SRE1N*	This study
CBY81-3	*stp1*Δ+*STP1*	This study
CBY78-3	*stp1*Δ+*SRE1N*	This study

Table S2 –Oligos used for quantitative PCR analysis

Gene	Oligo ID	Sequence 5’ to 3’
SRE1	SRE1-F	GAAGCGGATAAGCGTGATCT
SRE1	SRE1-R	CCAATCCTGGCAAGAATGTC
STP1	STP1-F	ACGAGAGAAGCCAAAGGTAG
STP1	STP1-R	ATCATACCCCATCCGATGAC
ERG25	ERG25-F	AGTTTCAGGTGGTGGGACTA
ERG25	ERG25-R	TCATTCTTCTCAACAGCTGCA
ERG3	ERG3-F	GCAGACCAGTACTTTGACTC
ERG3	ERG3-R	GGTTCCCAGCTTCATCTATC
CTR4	CTR4-F	CGTGGTTTCATCTATGGCAG
CTR4	CTR4-R	GACAGTCTGACCCAAGAAGA
ACT1	ACT1-F	CGTATGCAGAAGGAGATCAC
ACT1	ACT1-R	TAGAACCACCGATCCAGACA