

Fig S1. Deletion of genes encoding vesicular transport regulating proteins, increases susceptibility to fluconazole in *S. cerevisiae*. (A) Etest analysis of six vesicular transport related deletion mutants in *S. cerevisiae*. Pictures were taken after 72 h of growth on SD_{glu} medium. (B) Broth microdilution assay results of the BY4742 wild-type strain and six deletion mutants. Relative OD₆₀₀ values compared to the 0 μg/ml fluconazole condition are shown. Dotted lines indicate 50% (upper line) and 90% (lower line) growth inhibition. The MIC₅₀ and MIC₉₀ values can be deduced from the cross-section between the respective dotted lines and the data curves.

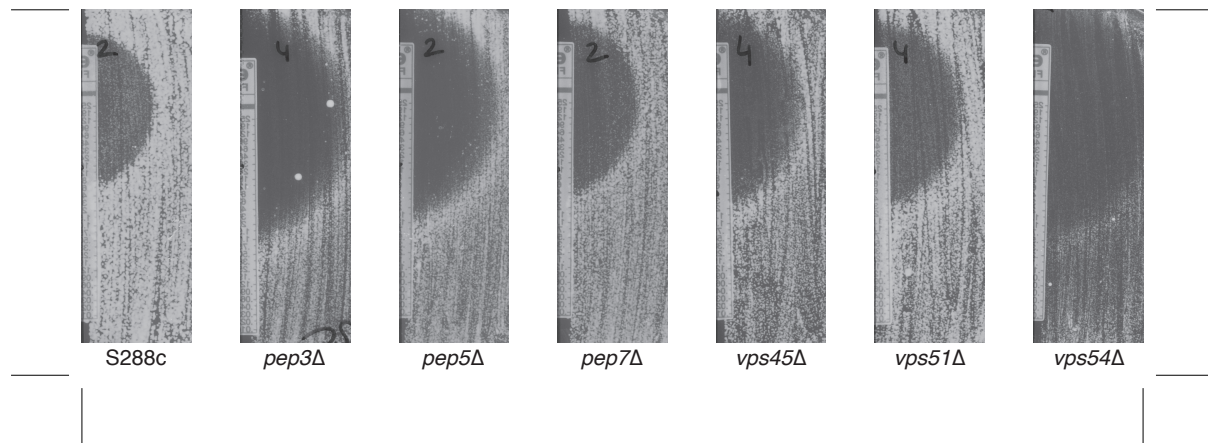


Fig S2. Increased fluconazole susceptibility of vesicular transport related *S. cerevisiae* mutants appears independent of auxotrophies. Etest analysis of six vesicular transport related deletion mutants and S288c wild-type strain. Pictures were taken after 72 h of growth on SD_{glu} medium.

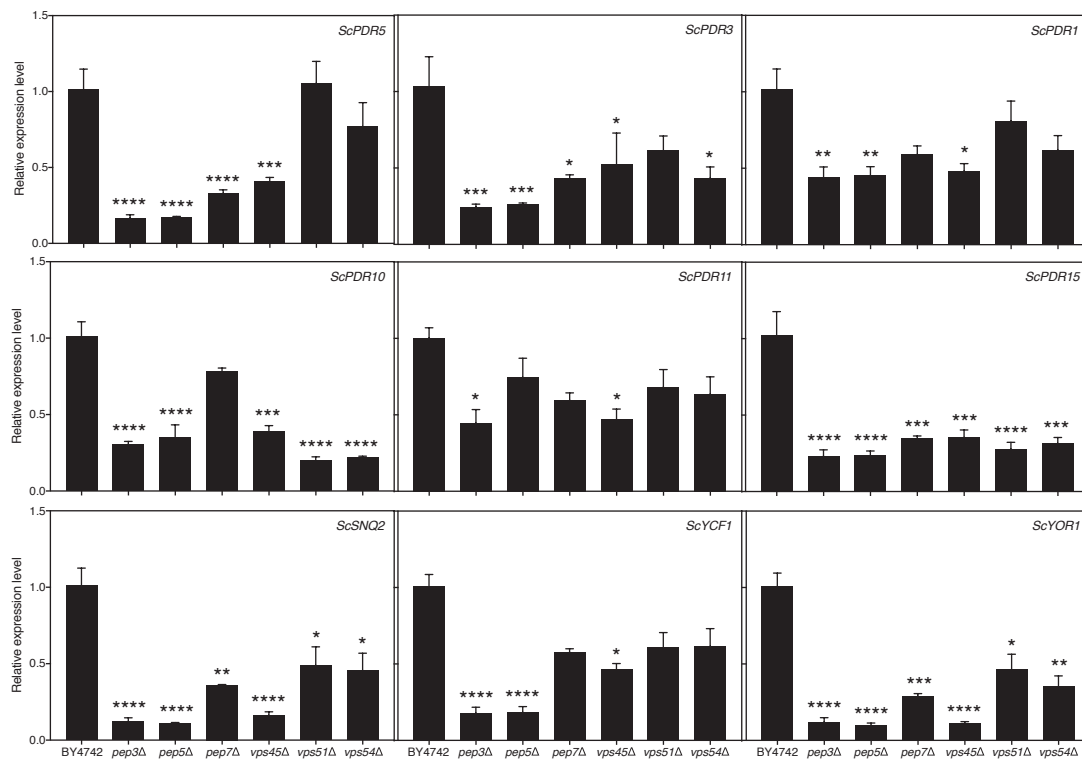


Fig S3. Expression of drug efflux related genes is downregulated in *S. cerevisiae* vesicular transport mutant strains. The deletion strains, as well as the wild-type strain, were incubated in SD_{glu} medium for 24 h, at 30°C in the presence of 20 µg/ml fluconazole. Gene expression was analyzed using qRT-PCR. Average results are displayed relative to the wild-type control and together with the SEM. Statistical analysis was conducted on log₂(Y)-transformed values using one-way ANOVA with Bonferroni correction; *, P < 0.05; **, P < 0.01; ***, P < 0.001 and ****, P < 0.0001.

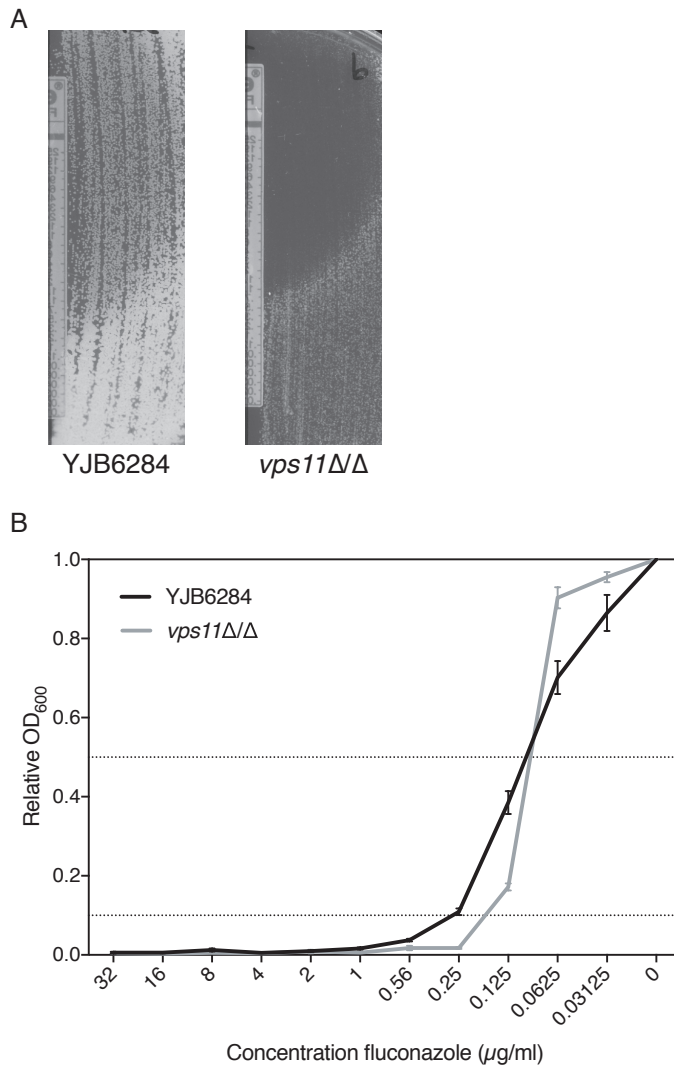


Fig S4. Deletion of *VPS11* increases susceptibility to fluconazole in *C. albicans*. (A) Etest analysis of *Cavps11Δ/Δ*. Pictures were taken after 48 h of growth on RPMI medium. (B) Broth microdilution assay results of the YJB6284 wild-type strain and the deletion mutant. Relative OD₆₀₀ values compared to the 0 $\mu\text{g/ml}$ fluconazole condition are shown. Dotted lines indicate 50% (upper line) and 90% (lower line) growth inhibition. The MIC₅₀ and MIC₉₀ values can be deduced from the cross-section between the respective dotted lines and the data curves.

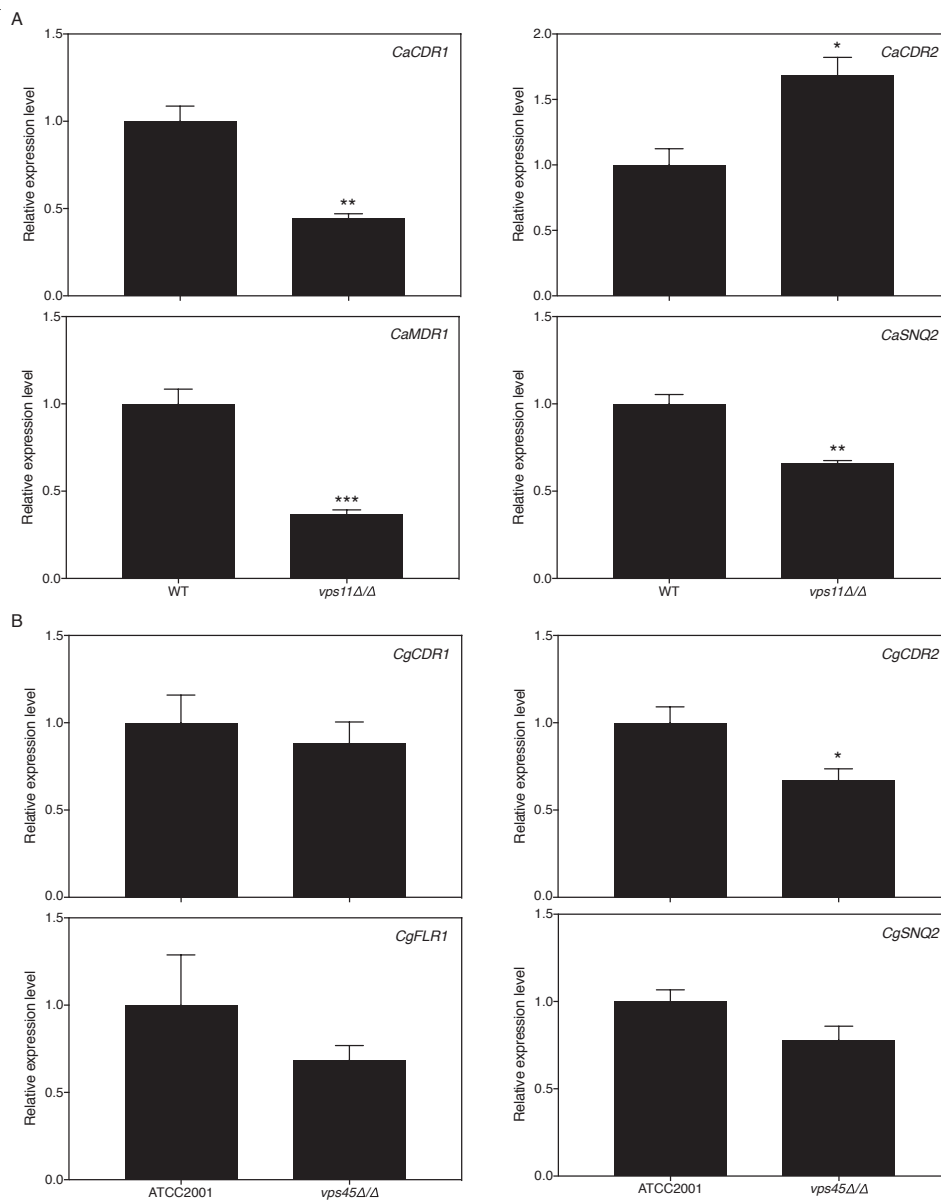


Fig S5. Expression of drug efflux related genes in *C. albicans vps11Δ/Δ* (A) and *C. glabrata vps45Δ/Δ* (B). The deletion strains, as well as the wild-type strain, were incubated in SD_{glu} medium for 24 h, at 30°C in the presence of 8 μg/ml fluconazole. Gene expression was analyzed using qRT-PCR. Average results are displayed relative to the wild-type control and together with the SEM. Statistical analysis was conducted on log₂(Y)-transformed values using one-way ANOVA with Bonferroni correction; *, P<0.05; **, P<0.01; ***, P<0.001 and ****, P<0.0001.

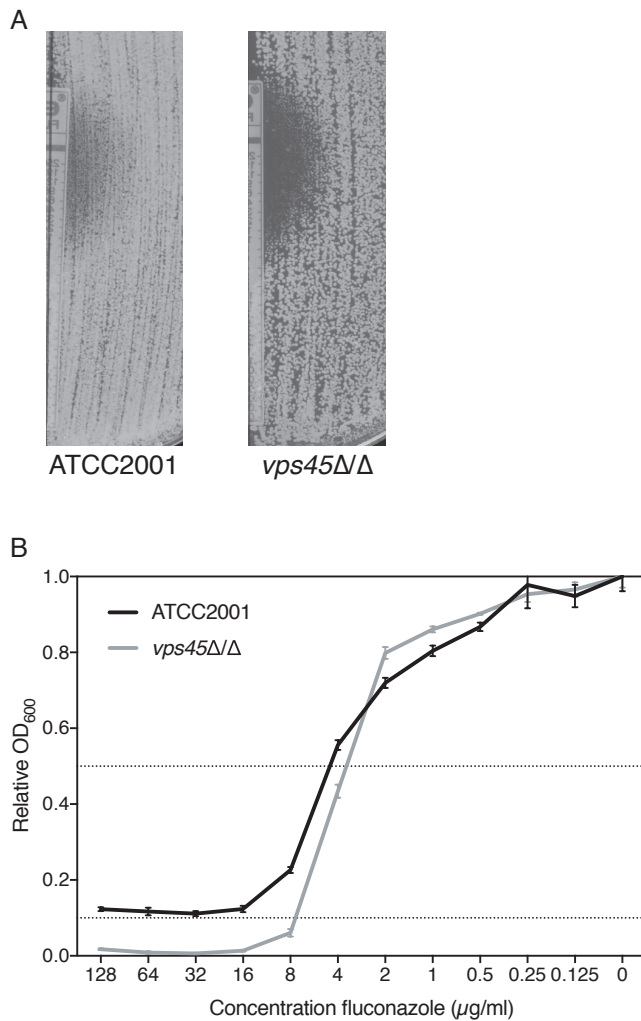


Fig S6. Deletion of *VPS45* increases susceptibility to fluconazole in *C. glabrata*. (A) Etest analysis of *Cg vps45Δ/Δ*. Pictures were taken after 48 h of growth on RPMI medium. (B) Broth microdilution assay results of the ATCC2001 wild-type strain and the deletion mutant. Relative OD₆₀₀ values compared to the 0 $\mu\text{g/ml}$ fluconazole condition are shown. Dotted lines indicate 50% (upper line) and 90% (lower line) growth inhibition. The MIC₅₀ and MIC₉₀ values can be deduced from the cross-section between the respective dotted lines and the data curves.

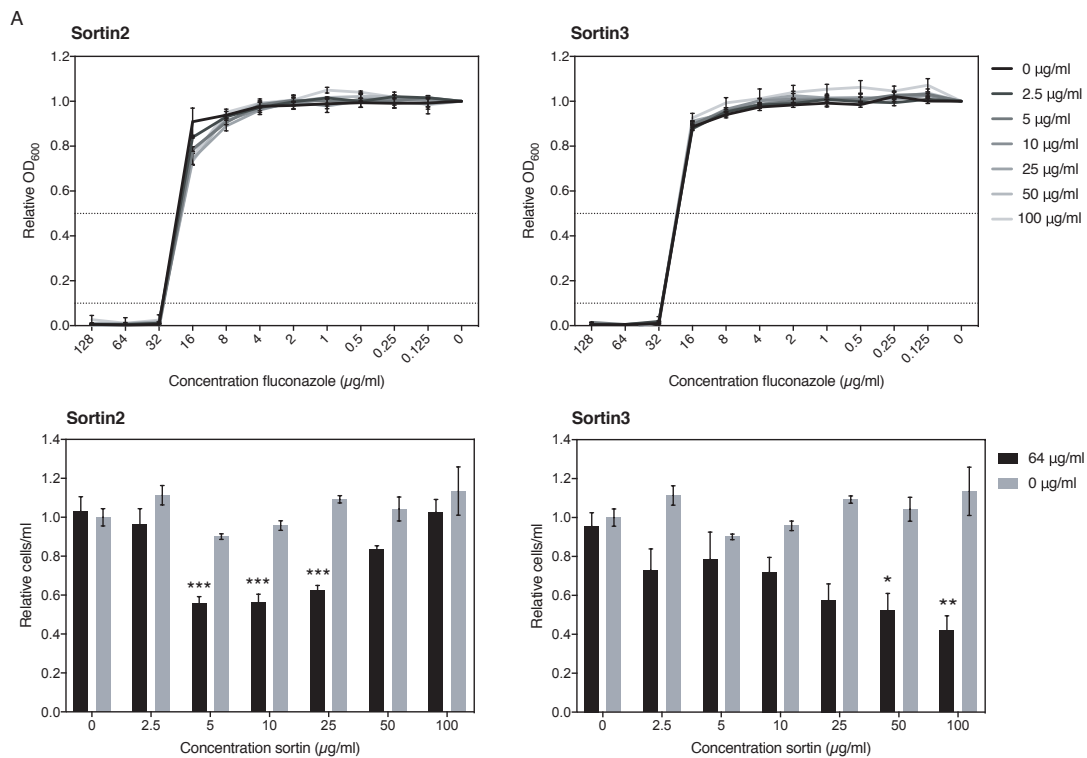


Fig S7. Sortin2 and sortin3 act mildly synergistically with fluconazole against *S. cerevisiae*.

(A) Broth microdilution assay results of the BY4742 wild-type strain in the presence of several concentrations of sortin2 or sortin3, after 72 h of growth at 30°C. Relative OD₆₀₀ values compared to the 0 μg/ml fluconazole condition are shown. Dotted lines indicate 50% (upper line) and 90% (lower line) growth inhibition. The MIC₅₀ and MIC₉₀ values can be deduced from the cross-section between the respective dotted lines and the data curves. (B) Tolerance assay results of the BY4742 wild-type strain in the presence of several concentrations of sortin2 or sortin3. CFU counts were compared in the absence and presence of fluconazole (64 μg/ml), relative to the 0 μg/ml sortin condition. Statistical analysis was conducted using one-way ANOVA with Bonferroni correction; *, P<0.05; **, P<0.01 and ***, P<0.001.

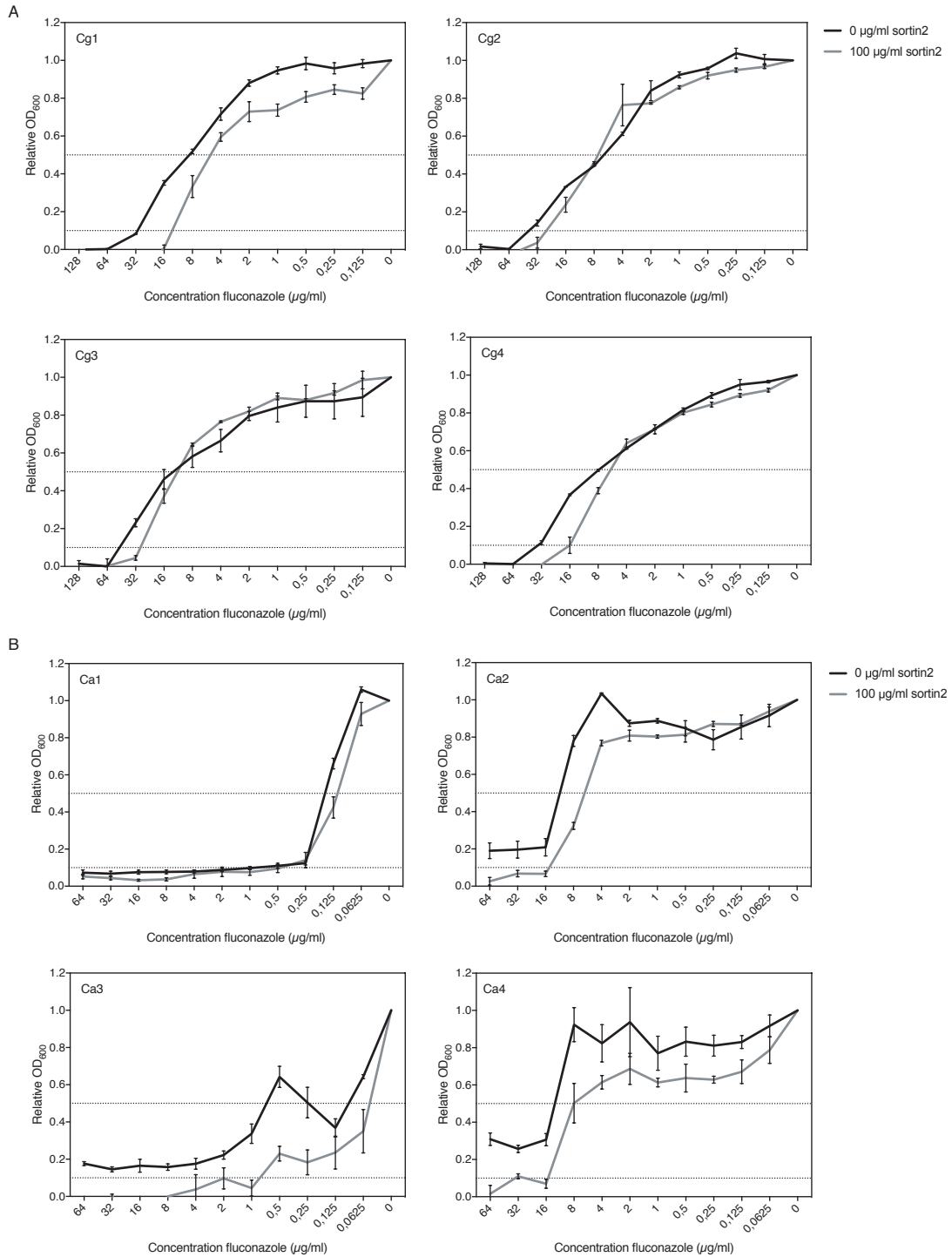


Fig S8. Sortin2 acts synergistically with fluconazole against *C. glabrata* and *C. albicans* clinical isolates. (A) Broth microdilution assay results of *C. glabrata* clinical strain in the

presence and absence of 100 µg/ml sortin2, after 48 h of growth at 37°C. (B) Broth microdilution assay results of *C. albicans* clinical strains in the presence and absence of 100 µg/ml sortin2, after 48 h of growth at 37°C. Relative OD₆₀₀ values compared to the 0 µg/ml fluconazole condition are shown. Dotted lines indicate 50% (upper line) and 90% (lower line) growth inhibition. The MIC₅₀ and MIC₉₀ values can be deduced from the cross-section between the respective dotted lines and the data curves

Standard name	Systematic name	Standard name	Systematic name
<i>ACO1</i>	YLR304C	<i>PEP7</i>	YDR323C
<i>BRE1</i>	YDL074C	<i>PLC1</i>	YPL268W
<i>CNM67</i>	YNL225C	<i>REG1</i>	YDR028C
<i>DPL1</i>	YDR294C	<i>SIN3</i>	YOL004W
<i>EMP24</i>	YGL200C	<i>SNF12</i>	YNR023W
<i>ERG24</i>	YNL280C	<i>SRV2</i>	YNL138W
<i>GAS1</i>	YMR307W	<i>SSZ1</i>	YHR064C
<i>HHY1</i>	YEL059W	<i>STE11</i>	YLR362W
<i>MED2</i>	YDL005C	<i>STE3</i>	YKL178C
<i>MKS1</i>	YNL076W	<i>VPS45</i>	YGL095C
<i>PDR16</i>	YNL231C	<i>VPS51</i>	YKR020W
<i>PDR5</i>	YOR153W	<i>VPS54</i>	YDR027C
<i>PEP3</i>	YLR148W	<i>VRP1</i>	YLR337C
<i>PEP5</i>	YMR231W	<i>YPK1</i>	YKL126W

Table S1. List of *S. cerevisiae* genes that upon deletion convert increased susceptibility to fluconazole and doxycycline.

GO term ^a	P value ^b	In category from cluster	k ^c	f ^d
GO biological process				
Golgi to endosome transport [GO:0006895]	2.516E-07	PEP7 VPS45 PEP3 PEP5	4	14
vesicle docking involved in exocytosis [GO:0006904]	3.42E-07	PEP7 VPS45 PEP3 PEP5	4	15
Golgi to vacuole transport [GO:0006896]	0.0001302	VPS54 PEP7 VPS45	3	24
regulation of SNARE complex assembly [GO:0035542]	0.0002574	PEP3 PEP5	2	6
signal transduction involved in filamentous growth [GO:0001402]	0.0007642	STE11 PLC1	2	10
vesicle organization [GO:0016050]	0.001115	EMP24 VPS51	2	12
regulation of vacuole fusion, non-autophagic [GO:0032889]	0.001314	PEP3 PEP5	2	13
negative regulation of transcription from RNA polymerase II promoter [GO:0000122]	0.001715	REG1 MKS1 SIN3	3	57
vacuole inheritance [GO:0000011]	0.002544	PEP7 VPS45	2	18
retrograde transport, endosome to Golgi [GO:0042147]	0.002544	VPS54 VPS51	2	18
late endosome to vacuole transport [GO:0045324]	0.003143	PEP3 PEP5	2	20
mitotic spindle organization [GO:0007052]	0.00424	CNM67	1	1
activation of MAPKK activity [GO:0000186]	0.00424	STE11	1	1
Golgi vesicle fusion to target membrane [GO:0048210]	0.00424	VPS45	1	1
negative regulation of phospholipid translocation [GO:0061093]	0.00424	YPK1	1	1
protein transport [GO:0015031]	0.004402	VPS54 VPS45 EMP24 VPS51 PEP3 PEP5	6	379
response to drug [GO:0042493]	0.004898	PDR16 PDR5	2	25
pheromone-dependent signal transduction involved in conjugation with cellular fusion [GO:0000750]	0.006123	STE3 STE11	2	28
sterol biosynthetic process [GO:0016126]	0.00656	PDR16 ERG24	2	29
vacuole fusion, non-autophagic [GO:0042144]	0.00656	PEP3 PEP5	2	29
positive regulation of SNARE complex assembly [GO:0035543]	0.008464	VPS45	1	2
negative regulation of transcription during meiosis [GO:0051038]	0.008464	SIN3	1	2
response to pheromone [GO:0019236]	0.008946	STE3 STE11	2	34
GO cellular component				

GARP complex [GO:0000938]	0.0001035	VPS54 VPS51	2	4
HOPS complex [GO:0030897]	0.0002574	PEP3 PEP5	2	6
CORVET complex [GO:0033263]	0.0002574	PEP3 PEP5	2	6
vacuolar membrane [GO:0005774]	0.002275	PEP7 VPS45 PEP3 PEP5	4	134
ER to Golgi transport vesicle [GO:0030134]	0.003801	EMP24 GAS1	2	22
perinuclear endoplasmic reticulum [GO:0097038]	0.00424	DPL1	1	1
external side of endosome membrane [GO:0010009]	0.00424	PEP7	1	1
primary cell septum [GO:0000936]	0.00424	GAS1	1	1
vacuole [GO:0005773]	0.004514	PEP7 VPS45 PEP3 PEP5	4	162
cellular bud scar [GO:0005621]	0.008464	GAS1	1	2

Table S2. Results of cluster analysis of genes deleted in enhancer strains of fluconazole susceptibility, using FunSpec (P < 0.01). ^aGO, gene ontology. ^bP value cutoff set at 0.01. ^cNumber of genes from the input cluster in given category. ^dTotal number of genes in given category.

Strain name	Genotype	Reference
<i>S. cerevisiae</i> strains		
BY4742	<i>MATα; his3Δ; leu2Δ; lys2Δ; ura3Δ</i>	Euroscarf
<i>pep3Δ</i>	Same as BY4742 with <i>pep3Δ::kanMX4</i>	Y.K.O./this study
<i>pep5Δ</i>	Same as BY4742 with <i>pep5Δ::kanMX4</i>	Y.K.O./this study
<i>pep7Δ</i>	Same as BY4742 with <i>pep7Δ::kanMX4</i>	Y.K.O./this study
<i>vps45Δ</i>	Same as BY4742 with <i>vps45Δ::kanMX4</i>	Y.K.O./this study
<i>vps54Δ</i>	Same as BY4742 with <i>vps54Δ::kanMX4</i>	Y.K.O./this study
<i>vps51Δ</i>	Same as BY4742 with <i>vps51Δ::kanMX4</i>	Y.K.O./this study
<i>C. albicans</i> strains		
YJB6284	<i>ura3Δ/ura3Δ arg4Δ/arg4Δ::ARG4URA3 his1Δ/his1Δ::HIS1</i> <i>VPS11/VPS11</i>	(1)
<i>vps11Δ/</i>	<i>ura3Δ/ura3Δ arg4Δ/arg4Δ his1Δ/his1Δ::HIS1</i> <i>vps11Δ::ARG4/vps11Δ/URA3</i>	(1)
SC5314	Wild-type, clinical isolate	(2)
Ca1	Clinical isolate number 731	(3)
Ca2	Clinical isolate number 732	(3)
Ca3	Clinical isolate number 2321	(3)
Ca4	Clinical isolate number 2322	(3)
<i>C. glabrata</i> strains		
ATCC2001	Wild-type, clinical isolate	(4)
<i>vps45Δ/</i>	Same as ATCC2001 with <i>vps45Δ::NAT^R</i>	This study
BG2	Wild-type, clinical isolate	(5)
Cg1	Clinical isolate number 2.08 (from blood)	Prof. dr. K. Lagrou
Cg2	Clinical isolate number 2.10 (from blood)	Prof. dr. K. Lagrou
Cg3	Clinical isolate number 2.24 (from blood)	Prof. dr. K. Lagrou
Cg4	Clinical isolate number 2.52 (from blood)	Prof. dr. K. Lagrou

Table S3. Strains used in this study.

- Palmer GE, Cashmore A, Sturtevant J. 2003. *Candida albicans* VPS11 is required for vacuole biogenesis and germ tube formation. Eukaryot Cell 2:411-21.

2. Fonzi WA, Irwin MY. 1993. Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* 134:717-28.
3. Coste A, Selmecki A, Forche A, Diogo D, Bougnoux ME, d'Enfert C, Berman J, Sanglard D. 2007. Genotypic evolution of azole resistance mechanisms in sequential *Candida albicans* isolates. *Eukaryot Cell* 6:1889-904.
4. Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, De Montigny J, Marck C, Neuveglise C, Talla E, Goffard N, Frangeul L, Aigle M, Anthouard V, Babour A, Barbe V, Barnay S, Blanchin S, Beckerich JM, Beyne E, Bleykasten C, Boisrame A, Boyer J, Cattolico L, Confanioleri F, De Daruvar A, Despons L, Fabre E, Fairhead C, Ferry-Dumazet H, Groppi A, Hantraye F, Hennequin C, Jauniaux N, Joyet P, Kachouri R, Kerrest A, Koszul R, Lemaire M, Lesur I, Ma L, Muller H, Nicaud JM, Nikolski M, Oztas S, Ozier-Kalogeropoulos O, Pellenz S, Potier S, Richard GF, Straub ML, et al. 2004. Genome evolution in yeasts. *Nature* 430:35-44.
5. Cormack BP, Falkow S. 1999. Efficient homologous and illegitimate recombination in the opportunistic yeast pathogen *Candida glabrata*. *Genetics* 151:979-87.

Primer name	Sequence (5'→3')
Primers used for recreation <i>S. cerevisiae</i> deletion strains	
ScPEP3_Fw	CTGATTCTCTTCGTATTGCT
ScPEP3_Rev	CATGGGCAAGAAGAAGTTTC
ScPEP5_Fw	GATTGTCGTCGTTTGTCT
ScPEP5_Rev	CTTCGTCTTCACCTTTGTCT
ScPEP7_Fw	GAATCACTCTGGCTATCACT
ScPEP7_Rev	CTCAGCTAGTTCCTATATAC
ScVPS45_Fw	CAGGAAAGGGTAACGTCATT
ScVPS45_Rev	AGAAGGATCACCGAACAAAG
ScVPS54_Fw	CAAAACAAACTACGACACGG
ScVPS54_Rev	CTCGCTCGATCTTCATTGTT
ScVPS51_Fw	GGGCCTTTTTGTGATGATTC
ScVPS51_Rev	TATACTACAAACAGCCGGGA
Primers used for creation <i>C. glabrata</i> deletion strain	
CgVPS45_Fw	GATAAGAAATATTCAATGAATTTCAATTCTGTCTACTTTTAAGATT GTTTTATAATTAGTGCAATAACGGATTCAACTGATATTGAAAGTA ACACTTGCAGCTCTAGAACTAGTGGATCC

CgVPS45_Rev	TCAATTATGTTATAGATATATACTCACCTATCACATATATATATAC TCACCTATCTCATATATATATACTCACCTATCTCATATATATAAGA AGCTCTCAGGAACAAAAGCTGGGTACC
Primers used for qRT-PCR of <i>S. cerevisiae</i>	
ScPDR5_qPCR_Fw	TGGGTCTGCTTGTCATTCA
ScPDR5_qPCR_Rev	TGGCACTTGGGGTAGTCATAA
ScPDR3_qPCR_Fw	TCTAAGTGACCTTTTGC GTGAG
ScPDR3_qPCR_Rev	GCCTGATTTTTCAACGGATT
ScPDR1_qPCR_Fw	AGCACCTTCCCCAGTGAGA
ScPDR1_qPCR_Rev	TTTGCCATTTGTTGGTTG
ScPDR10_qPCR_Fw	AAGGCAGACCACAGCAGATT
ScPDR10_qPCR_Rev	TTCATAGGCCGTTTGTGGGA
ScPDR11_qPCR_Fw	GGCGCTGGACTATTCATTGG
ScPDR11_qPCR_Rev	AGGGGAGGAAACACAAAGCA
ScPDR15_qPCR_Fw	CGGCGATTTAGGTGAAGGGT
ScPDR15_qPCR_Rev	ACATCCATTCGGCAGGGTTT
ScYOR1_qPCR_Fw	TGAAGTTTTGGGTGGGATGGA
ScYOR1_qPCR_Rev	TCCCGCCTCATTTCATTGT
ScSNQ2_qPCR_Fw	CGTTGCAGTTTTCTGCTCGT
ScSNQ2_qPCR_Rev	CCGCATACTCTTCCATTCCCA
ScYCF1_qPCR_Fw	AGGTGAGCGGTTATCCATC
ScYCF1_qPCR_Rev	AATGGCGAATCAGCGTCCTT
Sc18S_qPCR_Ref_Fw	CACTTCTTAGAGGGACTATCGGTTTC
Sc18S_qPCR_Ref_Rev	CAGAACGTCTAAGGGCATCACA
ScSCR1_qPCR_Ref_Fw	TGGGATGGGATACGTTGAGAA
ScSCR1_qPCR_Ref_Rev	CTAGCCGCGAGGAAGGATT
ScALG9_qPCR_Ref_Fw	CACGGATAGTGGCTTTGGTGAACAATTAC
ScALG9_qPCR_Ref_Rev	TATGATTATCTGGCAGCAGGAAAGAACTTGGG
Primers used for qRT-PCR of <i>C. albicans</i>	
CaCDR1_qPCR_Fw	AGTGAGGTATGGTGTTCGAG
CaCDR1_qPCR_Rev	ACACCGACGACAATATGAGACC
CaCDR2_qPCR_Fw	GTCGCAACAGCTAGACGAAAA
CaCDR2_qPCR_Rev	TGACTTGCAGTAGCATCAAAACC

CaMDR1_qPCR_Fw	GCTTGGGGTAGTTCCGTGTT
CaMDR1_qPCR_Rev	TTGCTCTCAACTTTGGTCCGT
CaSNQ2_qPCR_Fw	AGCTATTGGTGCTGGTGCAA
CaSNQ2_qPCR_Rev	ATCACGTTTGGCATCAGTTTGT
Ca18S_qPCR_Ref_Fw	GATGCCCTTAGACGTTCTGG
Ca18S_qPCR_Ref_Rev	CACGACGGAGTTTCACAAGA
CaLSC2_qPCR_Ref_Fw	CTGCCACCAAGAACTTCAAC
CaLSC2_qPCR_Ref_Rev	CAGCTGGGTCCAAATCTTCG
CaTUB1_qPCR_Ref_Fw	TTACCCAGCTCCACAAGTGTC
CaTUB1_qPCR_Ref_Rev	AAGTACAATCGGCGTGTTC
Primers used for qRT-PCR of <i>C. glabrata</i>	
CgCDR1_qPCR_Fw	GGGATAACGCAACGAGAGGT
CgCDR1_qPCR_Rev	GCAGCAGCGTTGGAAATACT
CgCDR2_qPCR_Fw	TTGAAGACCCACAGAACCG
CgCDR2_qPCR_Rev	GTGGGAAAGACCGTAGGTGG
CgFLR1_qPCR_Fw	TGTAGCATGGGGTTCTTCGT
CgFLR1_qPCR_Rev	ACATGGAACGCAGTGAAGGA
CgSNQ2_qPCR_Fw	AGCCGGTGAGATGGTTCTTG
CgSNQ2_qPCR_Rev	CCTTCAACACCACCAGCGAA
CgPGK1_qPCR_Ref_Fw	CAAACGGTGAAAGAAACGAGAA
CgPGK1_qPCR_Ref_Rev	CCGACACAGTCGTTCAAGAAAG
CgRPL10_qPCR_Ref_Fw	GAGATTCTTTCCACTTGAGAGTCAGA
CgRPL10_qPCR_Ref_Rev	CTCTCATACCTTGTTGCAATCTATCC
CgRPL2A_qPCR_Ref_Fw	GCCGGTAAGAAGGCCTCTTT
CgRPL2A_qPCR_Ref_Rev	CTGTCACCTGGCTTTTCTTCAA

Table S4. Primers used in this study.