SUPPLEMENTAL DATA

Table S1 : MICs determined by the EUCAST reference technique

Strain		Mean MIC_{50} (mg/L)			
		Fluconazole	**Posaconazole**	**Anidulafungin**	**Micafungin**
DAY286	Control strain	0.1875	0.007875	≤0.0078125	0.03125
DAY5	*rim101-/-*	0.125	0.007875	0.01575	0.03125
DAY23	*rim20-/-*	0.125	0.007875	NA	0.03125
DAY61	*rim8-/-*	0.1875	0.007875	0.01575	0.03125
GKO88	*rim13-/-*	0.125	0.007875	0.01575	0.03125
MC21	*rim21-/-*	0.1875	0.007875	0.01575	0.03125
MC23	*rim9-/-*	0.125	0.01575	0.01575	0.03125

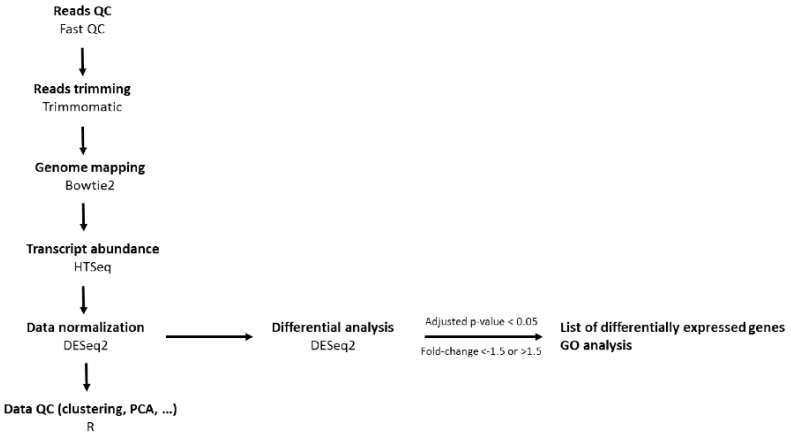
Tables S2, S3, S4 and S5: xlsx files

Table S6: Primers and probes used for RT-qPCR

Gene	Primers and probes	Reference
ACT1	F-ACT1 : TTGGTGATGAAGCCCAATCC R-ACT1 : CATATCGTCCCAGTTGGAAACA S-ACT1 : FAM-TTGACCTTGAGATACCCAATTGAACACGGTA-Tamra	(1)
HSP90	F-HSP90 : GCTGACGTTTCTATGATTG R-HSP90 : CATCCAAAGTAACAGTGAAC S-HSP90 : FAM-TCTACTCCTTGTTCTTGGTTGCTGAT-Tamra	This study
IPT1	F-IPT1 : TGGAGAGAATGCTAATGC R-IPT1 : CCATCTTGAATAATATCCAACA S-IPT1 : FAM-ATTATGATACTCCAGGTTATGCCGCT-Tamra	This study
RIM101	F-RIM101 : CCCAATCACCTCACATTA R-RIM101 : GCATCAGTGATAGGAGAA S-RIM101 : FAM-CCATTCCTCATTCACTTCTCAATCTGC-Tamra	This study

SUPPLEMENTAL FIGURES

A



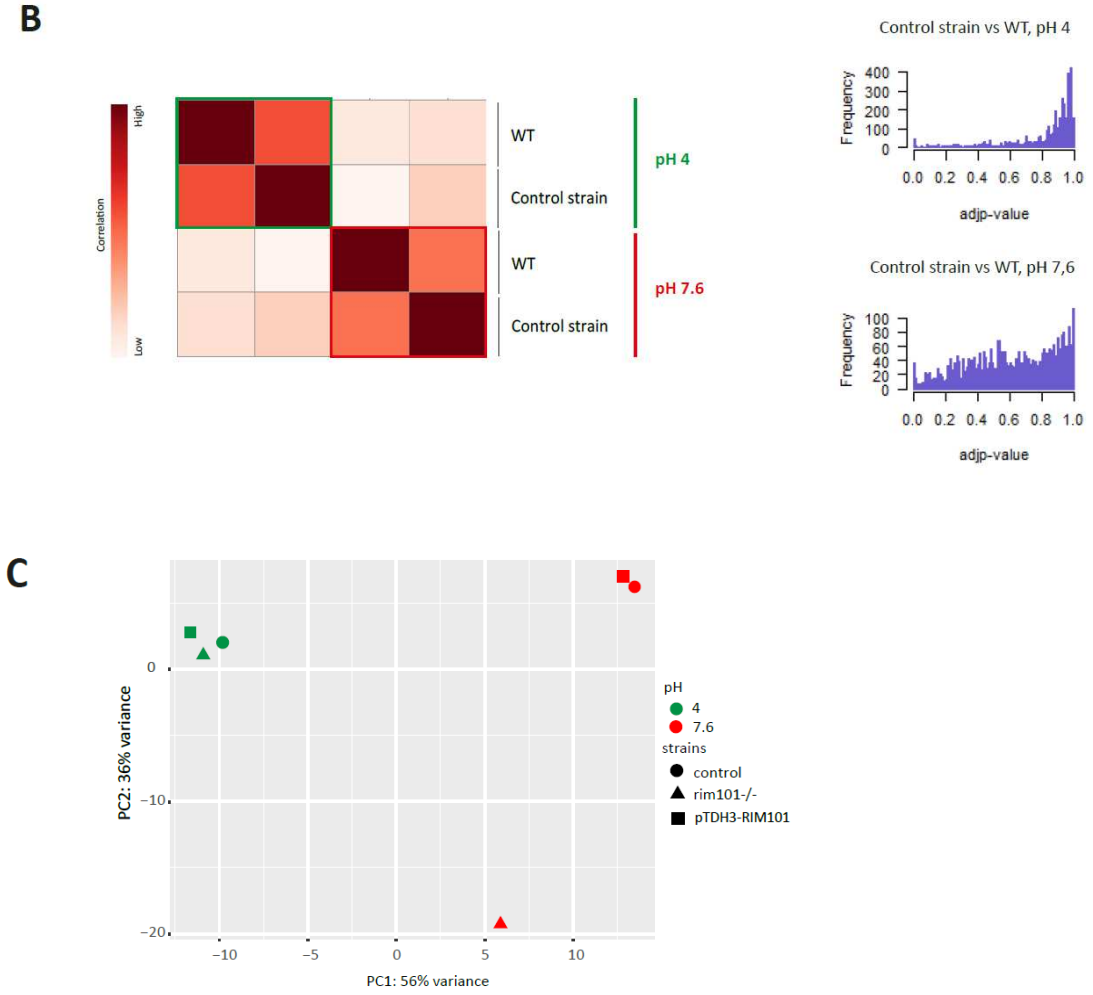


Figure S1: Transcriptomic analysis

(A) Schematic representation of the bioinformatic pipeline used for RNA-SEQ data analysis, **(B)** Left panel: unsupervised hierarchical clustering of the distance between SC5314 (WT) and DAY185 (control strain) samples at acidic and alkaline pH. All replicates are collapsed. Right panel: results of the differential analysis of DAY185 versus SC5314 transcriptomic programs at pH 4 and 7.6 expressed based on the adjusted p-value. No statistically significant difference was evidenced between these two strains. **(C)** Principal Component Analysis applied on the normalized counts of each sample. Replicates are collapsed. Principal components are represented on the x- and y-axis. Data obtained at acidic pH are shown in green; data obtained at alkaline pH are shown in red, strains are identified by the following shapes: wild-type (SC5314), round; *rim101Δ*-disrupted strain (DAY 25), triangle; *RIM101*-overexpressed strain (CGY1), square.

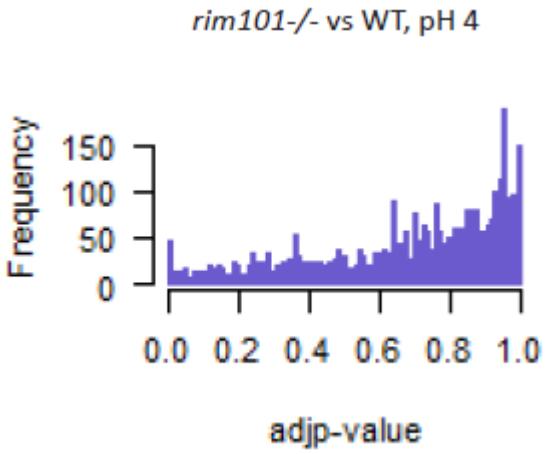


Figure S2: Results of the differential analysis of the *rim101Δ*-disrupted strain (DAY25) versus the SC5314 reference strain transcriptomic programs at pH 4 expressed based on the adjusted p-value

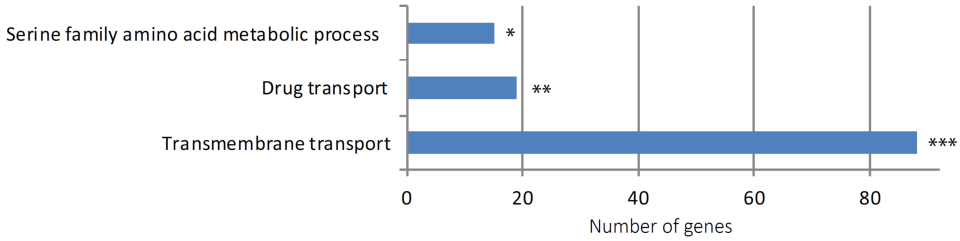


Figure S3: GO-term enrichment analysis at acidic pH
GO-term enrichment analysis of biological processes among genes differentially expressed at acidic pH between the RMI101-overexpressed strain (CGY1) and the SC5314 reference strain. The number of genes is expressed on the x-axis. The symbols *, ** and *** respectively indicate p-values < 0.05, p-values < 0.01 and p-values < 0.001.

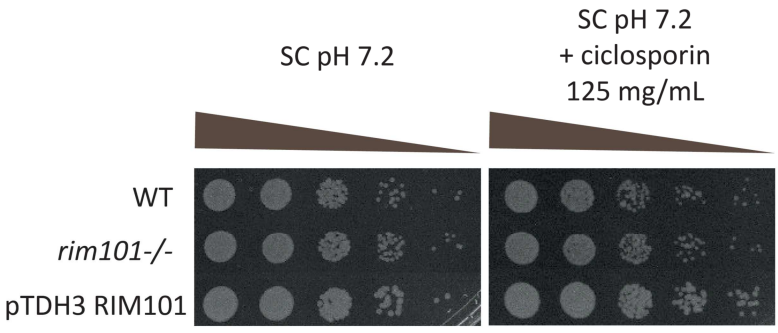
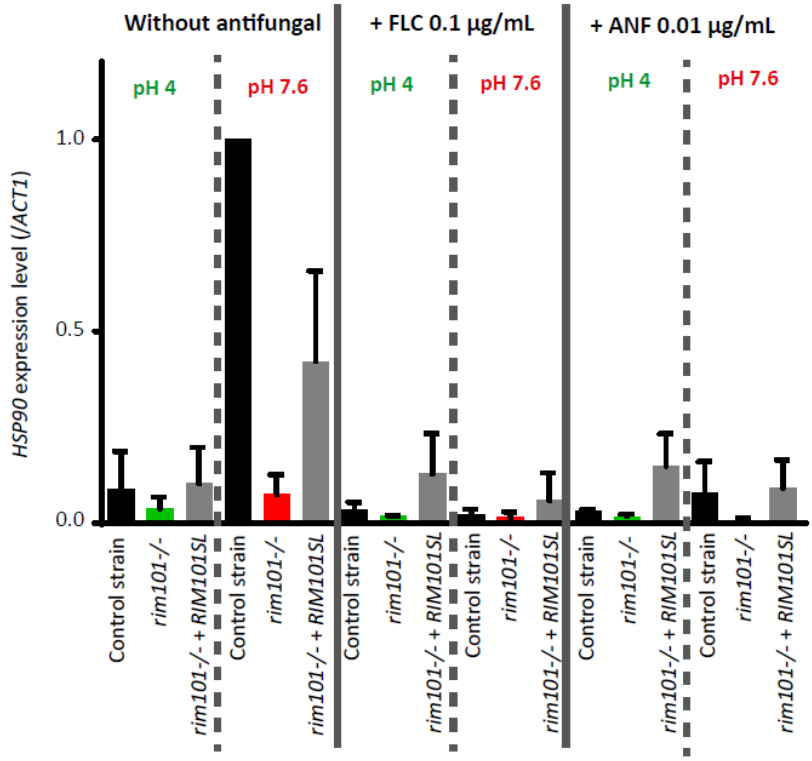
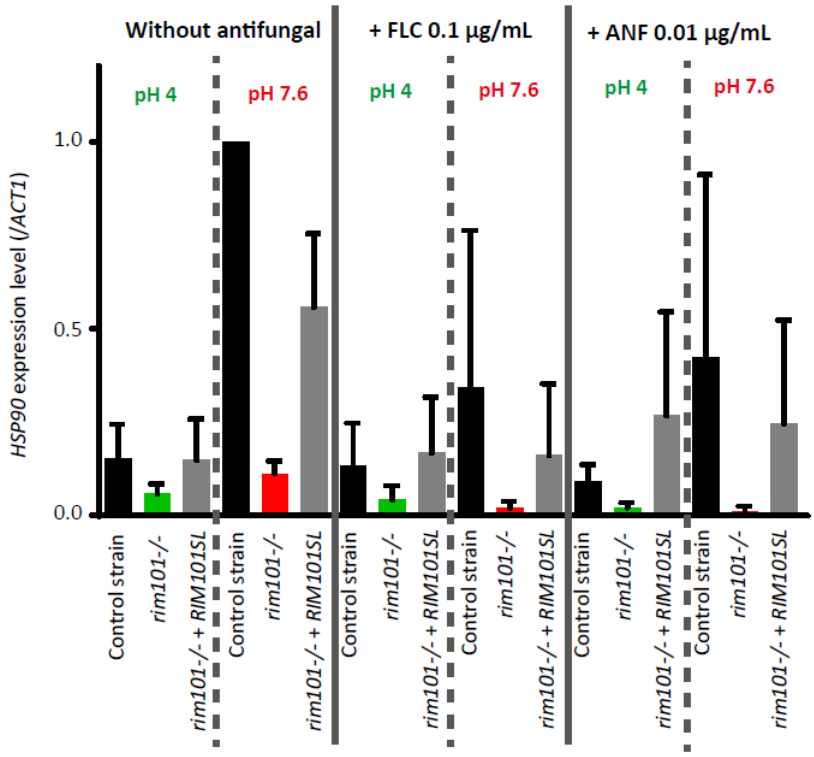


Figure S4: Pharmacological inhibition of calcineurin
Colony forming assays in presence of ciclosporin A, a pharmacological inhibitor of calcineurin. Cells were plated in ten-fold dilutions in presence of ciclosporin A (125 mg/mL) in SC-medium buffered at pH 7.2. No specific phenotype was observed.

A. *RIM101*



B. *IPT1*



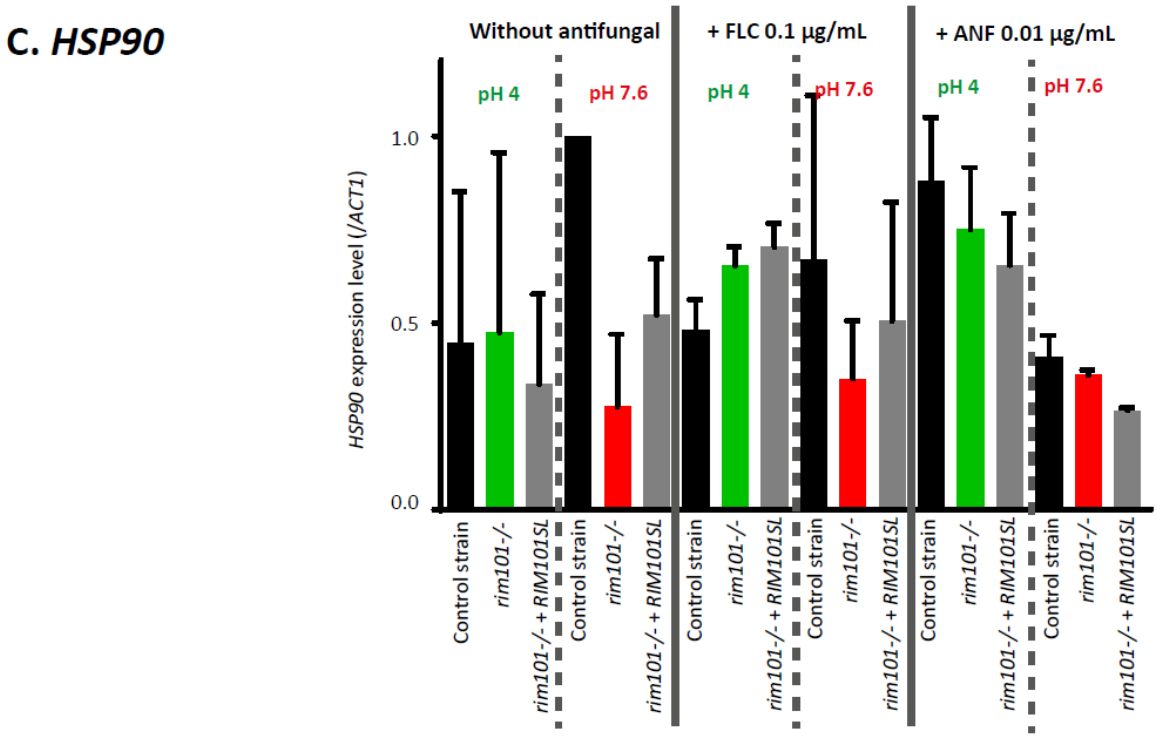


Figure S5: *RIM101*, *IPT1* and *HSP90* expression levels normalized to *ACT1* obtained in RT-qPCR at acidic and alkaline pH, under drug-stress conditions or not, in the DAY25 *rim101Δ*-disrupted strain, the DAY5SL complemented strain and the DAY185 control strain.

Each strain (DAY185, DAY25 and DAY5SL (Table 1)) was pre-cultured overnight in liquid YPD medium at 30°C under agitation, then diluted to an OD600 of 0.1 in SC medium buffered at pH 7.6 or pH 4 respectively with HEPES or citrate buffer, in presence or absence of fluconazole (0.1 µg/mL) or anidulafungin (0.001 µg/mL). Cells were then incubated at 30°C (to be consistent with the other experiments of the study and avoid intense yeast-to-hyphae transition), under agitation until an OD600 of 0.6-0.8 was reached, and collected by centrifugation. RNA extraction was performed on the pellet using the MasterPure™ Yeast RNA Purification Kit (Epicentre), according to manufacturer's instructions. Two biological replicates were performed for each strain and condition.

RT-QPCR was performed using the KAPA Probe Fast qPCR Master Mix (Clinisciences). Primers and probes are listed in Table S6. Gene expression levels were normalized to the ACT1 ones.

REFERENCES

1. Morio F, Pagniez F, Lacroix C, Miegeville M, Le Pape P. 2012. Amino acid substitutions in the *Candida albicans* sterol Δ5,6-desaturase (Erg3p) confer azole resistance: characterization of two novel mutants with impaired virulence. J Antimicrob Chemother 67(9):2131–8.