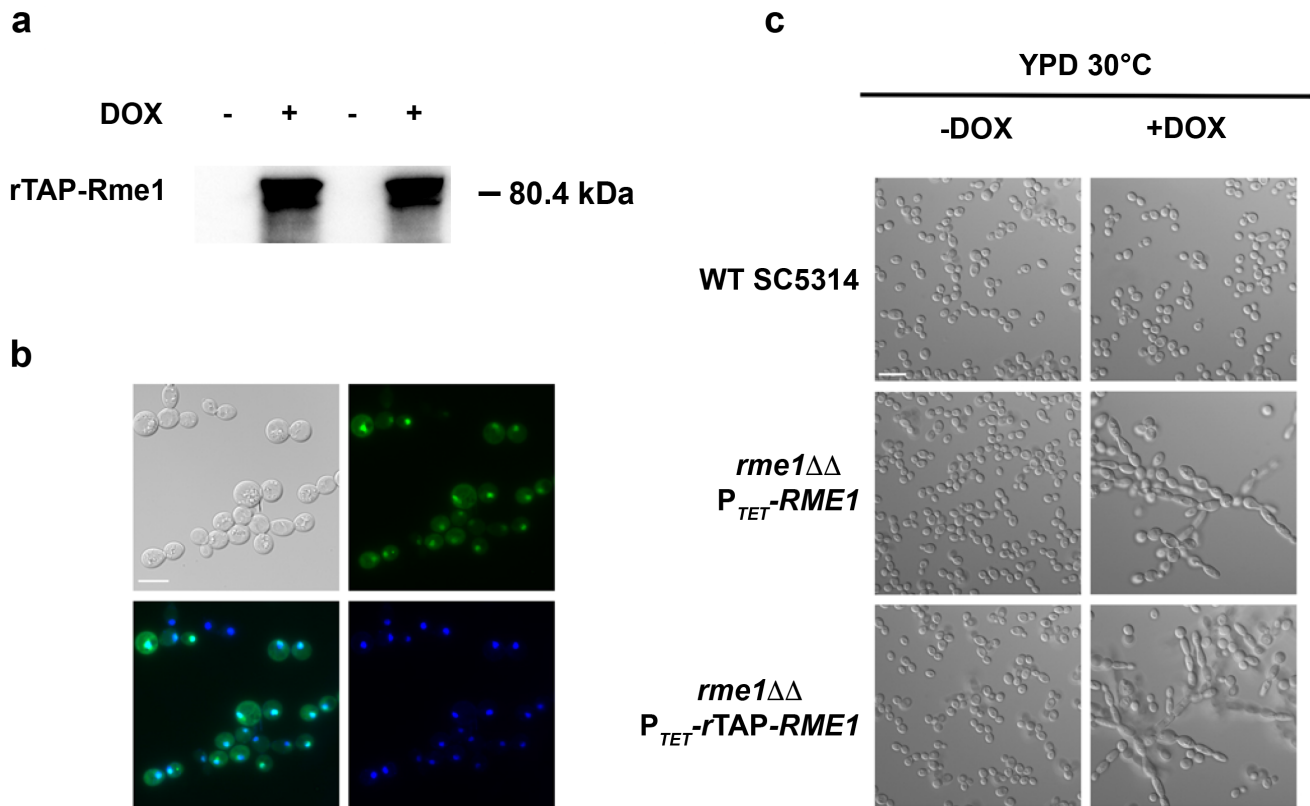
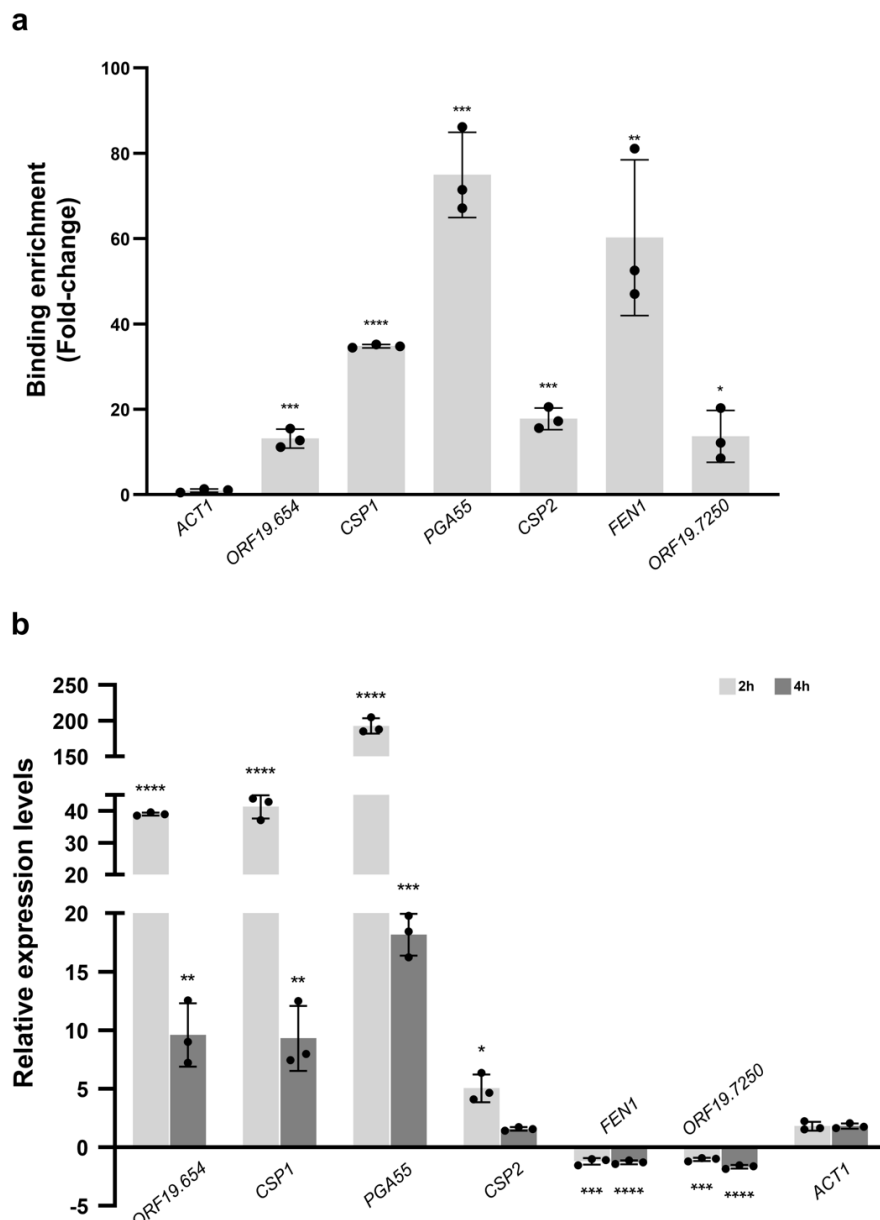


A conserved regulator controls asexual sporulation in the fungal pathogen *Candida albicans*

SUPPLEMENTARY INFORMATION



**Supplementary Fig. 1. Characterization of *C. albicans* strains expressing the rTAP-tagged and untagged versions of Rme1.** **a)** Western blot analysis of *rme1*ΔΔ mutant expressing P<sub>TET</sub>-rTAP-RME1. Two independent clones were cultured in YPD liquid medium in the presence or absence of doxycycline, and then total protein extracts were subjected to SDS-PAGE. Western blotting was conducted using an anti-TAP polyclonal antibody to detect rTAP-Rme1. **b)** Localization of GFP-Rme1 fusion protein. A strain containing P<sub>TET</sub>-GFP-RME1 (CEC5252) was grown for 6h in YPD + 40 μg/mL doxycycline. Cells were incubated with 10 μg/mL Hoechst for 15 min and rinsed with PBS prior to imaging. Top left panel: Nomarski; Top right panel: GFP-Rme1; Bottom right panel: Hoechst staining of the nuclei; Bottom left panel: overlay. Scale bar = 10 μm. **c)** Phenotypic characterization of the strains expressing the rTAP-tagged and untagged versions of Rme1. The WT strain SC5314 (top panels) and *rme1*ΔΔ strains with an inducible Rme1 either untagged (middle panels) or fused to a N-terminal rTAP-tag (bottom panels) were grown in YPD liquid medium at 30°C without induction (left panels) or in the presence of 40 μg/mL doxycycline (right panels). Scale bar = 10 μm.



**Supplementary Fig. 2. Validation of the data obtained in the ChIP-chip and transcriptomics analysis.** **a)** Quantification of DNA enrichment after the immunoprecipitation of rTAP-tagged Rme1 at the promoters of *ORF19.654* ( $P=0.000701$ ), *CSP1* ( $P<0.000001$ ), *PGA55* ( $P=0.000213$ ), *CSP2* ( $P=0.000344$ ), *FEN1* ( $P=0.00493$ ) and *ORF19.7250* ( $P=0.022279$ ) by qPCR analysis in strains expressing the tagged and untagged versions of Rme1. Bars represent relative enrichment values (n-fold) of rTAP-Rme1 coimmunoprecipitated DNA as compared to DNA from mock immunoprecipitation; the *ACT1* locus was used as a reference. Data are expressed as the mean  $\pm$  SD,  $n=3$  biological replicates, over 3 experiments. **b)** The relative expression levels of the *ORF19.654* ( $P<0.000001$ ;  $P=0.007677$ ), *CSP1* ( $P=0.000048$ ;  $0.009535$ ), *PGA55* ( $P=0.000006$ ;  $0.000097$ ), *CSP2* ( $P=0.010644$ ;  $0.173319$ ), *FEN1* ( $P=0.000368$ ;  $0.000039$ ), *ORF19.7250* ( $P=0.000257$ ;  $0.000022$ ) and *ACT1* (negative control) genes were quantified by RT-qPCR by using total RNA from independent clones expressing  $P_{TET}\text{-}RME1$  at 2h (light grey bars) and 4h (dark grey bars). The bars represent the average relative change in RNA abundance of the indicated genes in doxycycline-treated samples as compared to those that were untreated. Data are expressed as the mean  $\pm$  SD,  $n=3$  biological replicates, over 3 experiments. (\*)  $P<0.05$ , (\*\*)  $P<0.01$ , (\*\*\*)  $P<0.001$ , (\*\*\*\*)  $P<0.0001$ .

Ca 1 MFSYNLESNNAGYLNHHHSRHLNSNSNSNNNNNNNN NNSIAISNNNKAAHLEQEKQRQSQ  
 Cd 1 MFSYNLESNNAGYLNHHHSLHH-HHHNNNNNNNNNN TSSITNNKSKAAHLEQEKQRQSQ  
 Cb 1 MLAYTS-----DSKEQQDQQQQQQQQQQQQQQQQQLQQ  
 Ct 1 MVGYNL-----EESNSTIAHQQ-----NE  
 Cp 1 MSPLTAIL-----QSYHAKVEDESSYHLLKKSGPSLSLDEVVVVRVPS

Ca 61 EHEQPRNPQYQNYHFIOQQQHFQYLO-----NALANTMSQLOHH-----  
 Cd 60 EHEQPRNPQYQNYHFIOQQQHFQYLO-----NALANTMSQLOHH-----  
 Cb 35 QQQQQQQQQYQNYHFIOQQQHFQYLO-----NALANTMSQLOHH-----  
 Ct 22 QHOEPNSHSHYHHYQFVQQQHFQYLO-----NALASTMSQQQQQQLPDQQDSQ  
 Cp 46 VGEPSFTCQSAMLLNELAKASNHCSTEGIGTQIRPVSAIFGAKDRADNSTIELVNH

Ca 102 -----PPYH-GHAVFKPNYMQDVELNDSCSLGSPVNSIENSIGCTTTKTPPIIPM--  
 Cd 101 -----PPYH-GHAVFKPNYMQDVELNDSCSLGSPVNSIENSIGCTTTKTPPIIPM--  
 Cb 75 -----PPYF-MNSVFKPNYMQDVELNDSCSLDSESVNYTDSNQTTKTPPIIPT--  
 Ct 72 DQPNSQSPSPYHPSAVFKPNYMQDVELLNETCILNSPMSMTSACTTKTPPIIPMHP--  
 Cp 106 YSQLSTSYEDKSTCFMVYVKCEIDNTLLHDPEILRNESSSSSMSANTVLSLSPVSOYQ

Ca 151 -----SLN--D-NVLPPEPNHHEDETE-MGNFV-----DYTSSTYN  
 Cd 150 -----SLN--D-NVLPPEPNHHEDETE-MGNFV-----DYTSSTYN  
 Cb 124 -----SLTESIDQCHRYHNOHEEDNE-MNEHHH---HHHHHPQTI  
 Ct 130 -----SVH--EFDQLPLNNHHLHHHPPEHANIAARSDSITTLN  
 Cp 166 NPDKAIEQSRLSQIQTNSELYSLVQDLRFTEALYRMSPEASHEVD-----DHVEES-

Ca 182 PEET--LPSPSPTITVCNDG--RRHDDHNEASTFSVH-----PASKCATORYGNSN  
 Cd 181 PEHA--LPSPSPTITVGSDS--RRHEDYNEASTFSVH-----PASKCATOPNANSN  
 Cb 162 KOHS--LPSPTSPVTEFYNQNTKHHDDYKVSSEFLHTPGNFATTPTTTTNNHNNNN  
 Ct 169 EDKCKILPSPSPTITVTDGK--QPHDEYNEVITFSIH-----EOSTSSLVFRNHHHI  
 Cp 219 -----FPHSICPW-----QLQSNNTITMSQPTFVH-----LKLKRGLENIQDVF

Ca 232 CELTGYPNPNSLVYN---TTNNCI-----NNNSNNNNKYSSEYKQTRMEMA  
 Cd 231 CEFTGYPNPNSLVYN---TTNNCIINRNSNTTDANNNNNSSMKYPLIEYKQNRMEMT  
 Cb 220 NILPHKNEINPLSYN-----NLTDYNNNN-----KPKKIFEMN  
 Ct 220 NSAVFH-----ASMDLGCISENV-----LAA  
 Cp 258 NLTDDDDDKGVSEHEGSTIADC MCM--SVSPSISPROYALSRLCSPKMNNLAIFKFA

Ca 279 TPESFH--KNSLPN--TRVPIIPSPPTFPALNLSHSNANTKRHKLGSAATMLQPPLE  
 Cd 288 TPESFH--KNSLPN--TRVPIIPSPPTFPALNLSHSNASSAKRHKLGSAATMLQPPLE  
 Cb 255 TPQSPF--KNDLFAN--TKIPIIPSPPTFPALNLEPHGKVSERKYPNTNNSFIIKPSFD  
 Ct 241 TPESSENIKGLFEDNSSRPIIPSPPTFPALNLSHGKVVVQKMPKQSGP--VLQPAFE  
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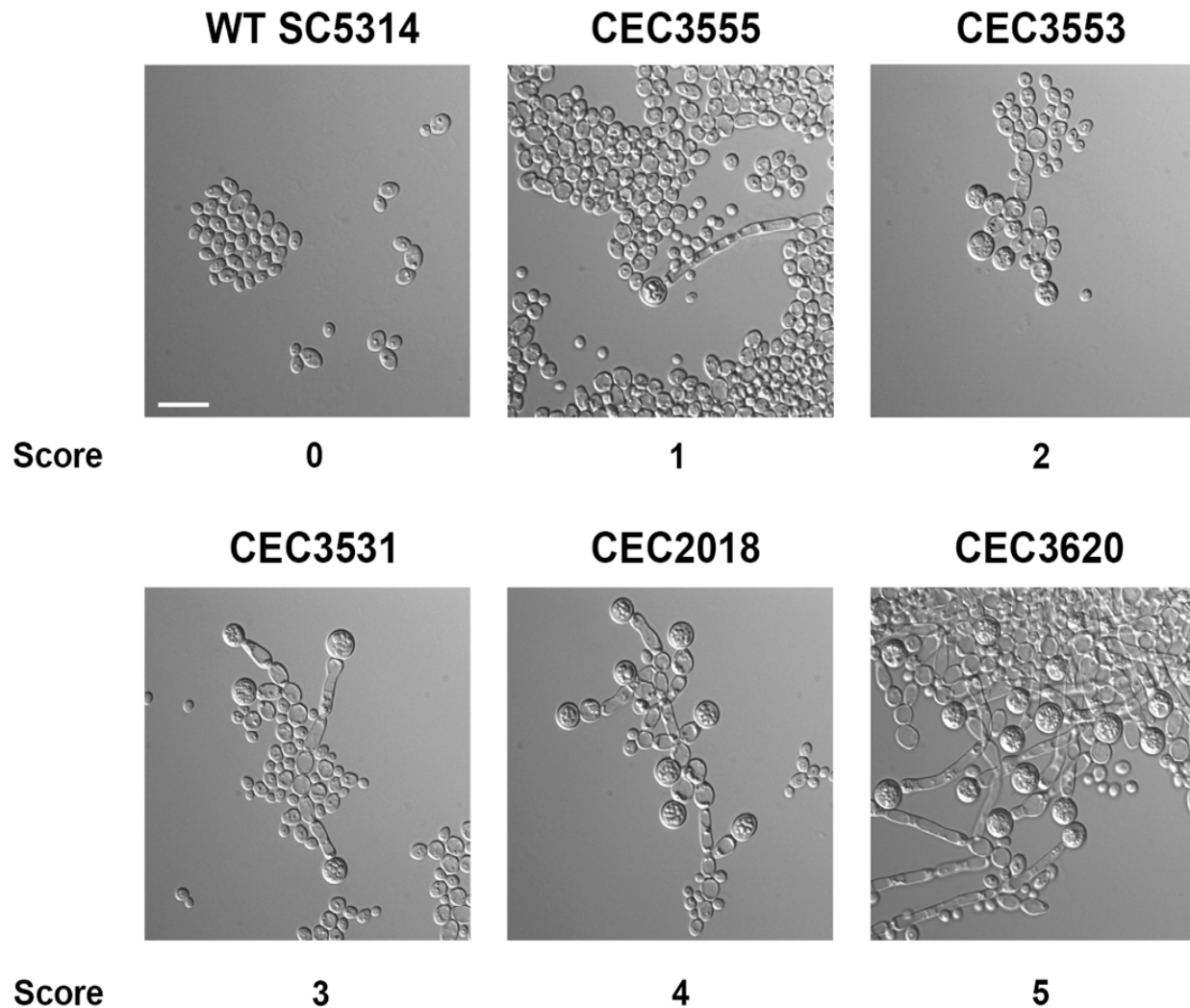
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 Ct 299 IKNMTTIKKINPEQSAATCRSKSYSRKNVNTANVLSQGYFEMKRLARVSRILLQRMRI  
 Cp 364 DDDTPHPTEATSKASWNSSDFC--NAATSGIDASKLEYYKKAFFSQYSKVSIITKIKV

Ca 366 AESDKCRKHONTREFNYLELIDHFEHGHLQRYLSRRTFICPVKECPMNMIGFDKRAELR  
 Cd 375 AESDKCRKHONTREFNYLELIDHFEHGHLQRYLSRRTFICPVKECPMNMIGFDKRAELR  
 Cb 360 AENDKCRKHCDIKFANCLELIDHFEHGYLHENLQSRFICPVKECPNITIGFDKRAELR  
 Ct 359 SESDKCRKHCEVDEFTNYLELIDHFEHNGLQMHLEIRNEKCPVKECPMNMIGFDKRAELR  
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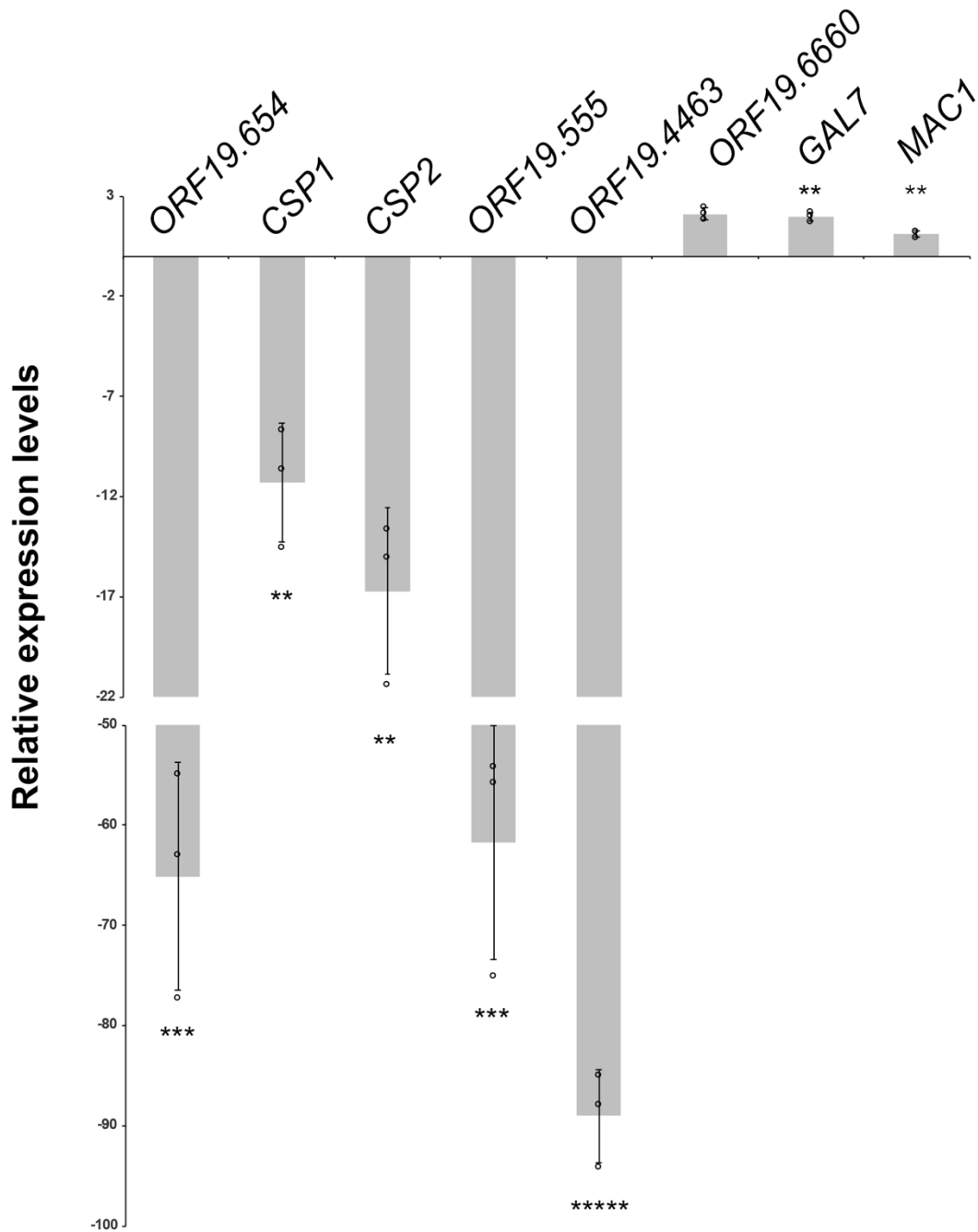
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 Cb 420 HHVHSEHHTGLVLSQYAKYANELEKELFVFCDEEENCGKGFYRSDTLTRHVKLVHKRQKQP  
 Ct 419 HHVHSDHHTGLVLSAQYSKYSDEIKRYLFCDEEPSGKGFYRSDTLTRHVKLVHKRQSNF  
 Cp 480 HHVHSEHLKNGEVKIGCREYEDIEIMLILFVCEAGCGKAFYRCDLNRHHLVHGNKRKG

Ca 486 TKRKRROVVAHOEDKA----TKKSKG-----  
 Cd 495 TKRKRROVVAHODDKA----TKKSRG-----  
 Cb 480 IRRKRKFSNNNNNNNDKSLSKKTKN-----  
 Ct 479 TRRRRRINKNHA-----  
 Cp 540 GGAKRKRFVYNDVEEILADVELVENYNDIRELNCDDGDIIEVCTTLKRRKKVI

**Supplementary Fig. 3. Multiple alignment of Rme1 sequences.** MUSCLE69 was used to generate a multiple alignment of the Rme1 protein sequences from *C. tropicalis* (CTRG\_03993) [obtained from the NCBI (<https://www.ncbi.nlm.nih.gov>)], *C. parapsilosis* (Cpar2\_212670p) [obtained from CGD (<http://www.candidagenome.org>)], *C. buenavistaensis* [*CbRME1* was PCR amplified and sequenced; the nucleotide sequence has been deposited at GenBank under the accession number MK070497], *C. dubliniensis* (Cd36\_06830p), and *C. albicans* (C1\_07330wp), with default parameters, without trimming. Boxshade ([https://embnet.vital-it.ch/software/BOX\\_form.html](https://embnet.vital-it.ch/software/BOX_form.html)) was used to highlight identical- and similar-residues (black and grey boxes, respectively).

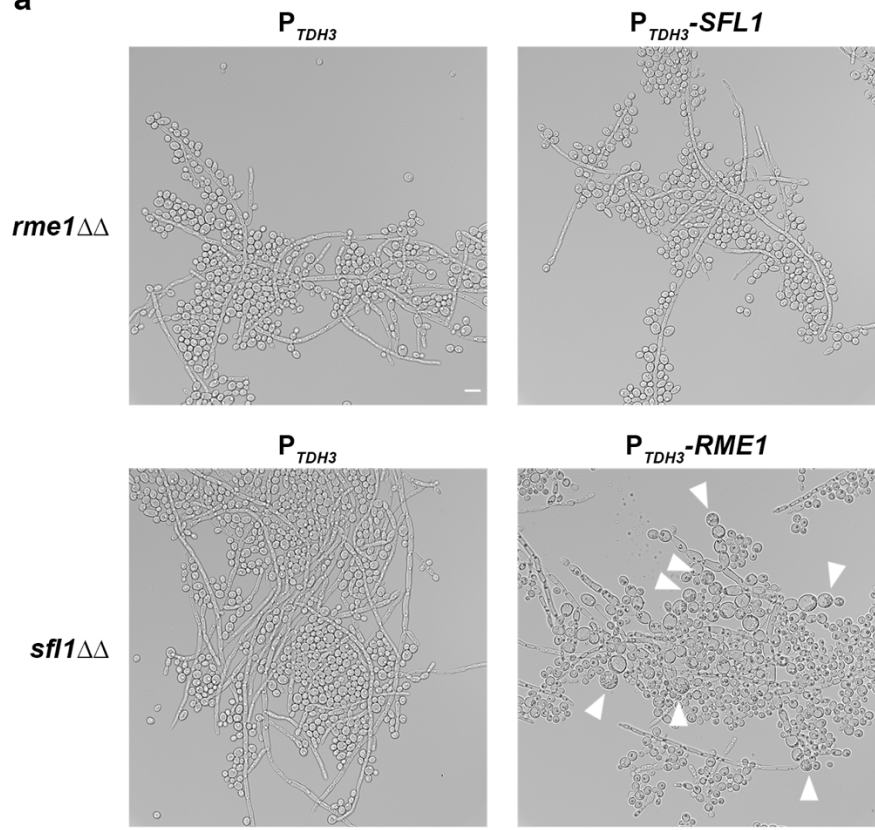


**Supplementary Fig. 4. Chlamydospore formation scoring in the collection of *C. albicans* clinical isolates.** Representative examples of *C. albicans* strains showing the chlamydospore formation status observed in the screen. The WT SC5314 strain is scored as 0, whereas the increase in chlamydospore formation is scored from 1 to 5. The screen was conducted in triplicate for three biological replicates. Scale bar = 10  $\mu$ m.

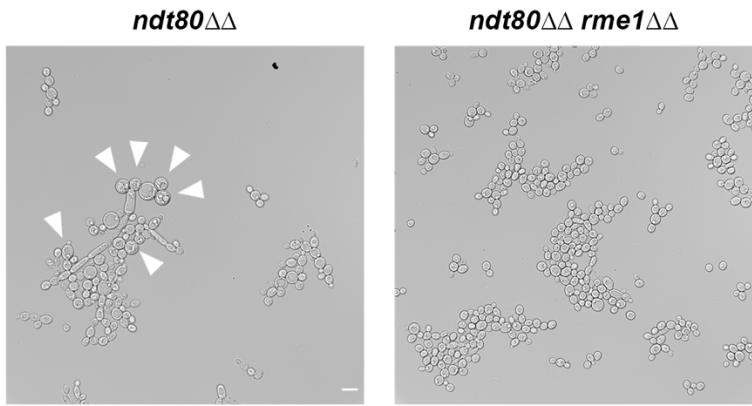


**Supplementary Fig. 5. Validation of the data obtained in the microarray experiment performed in the CEC2018-*rme1*ΔΔ strain.** The relative expression levels of the *IFL* family genes (*CSP1*, *CSP2*, *ORF19.654*, *ORF19.555* and *ORF19.4463*), and the *ORF19.6660* and *GAL7* genes used as negative controls were quantified by RT-qPCR using total RNA from independent CEC2018-*rme1*ΔΔ clones. *MAC1* was used as a reference. The bars represent the average change in RNA abundance of the indicated genes in the CEC2018-*rme1*ΔΔ strain as compared to those from the CEC2018 strain. *ORF19.654* ( $P=0.000537$ ), *CSP1* ( $P=0.001957$ ), *CSP2* ( $P=0.00173$ ), *PGA55* ( $P=0.000732$ ), *ORF19.4463* ( $P=0.0000047$ ), *ORF19.6660* ( $P=0.00624$ ) and *GAL7* ( $P=0.00677$ ). Data are expressed as the mean ± SD, n=3 biological replicates, over 3 experiments. (\*)  $P<0.05$ , (\*\*)  $P<0.01$ , (\*\*\*)  $P<0.001$ , (\*\*\*\*)  $P<0.0001$ .

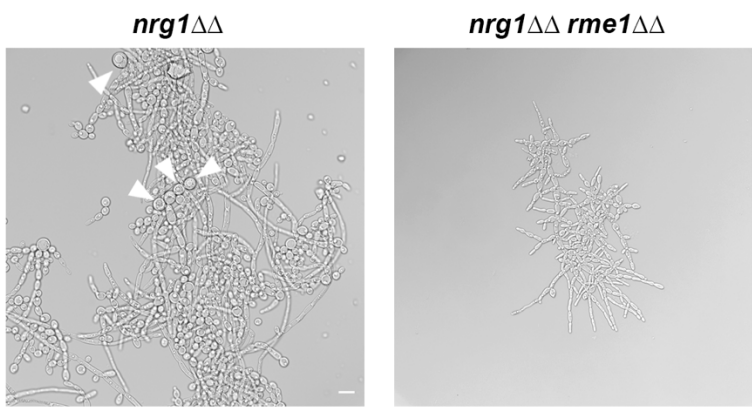
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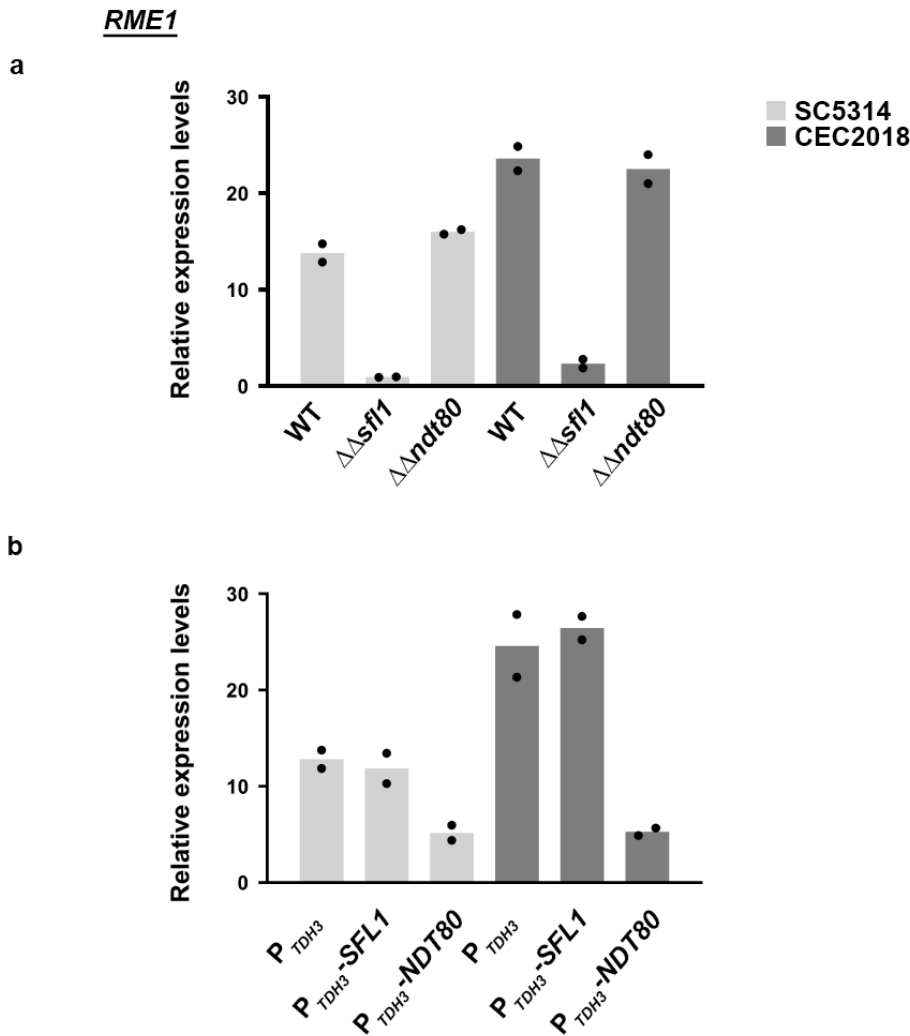
**b**



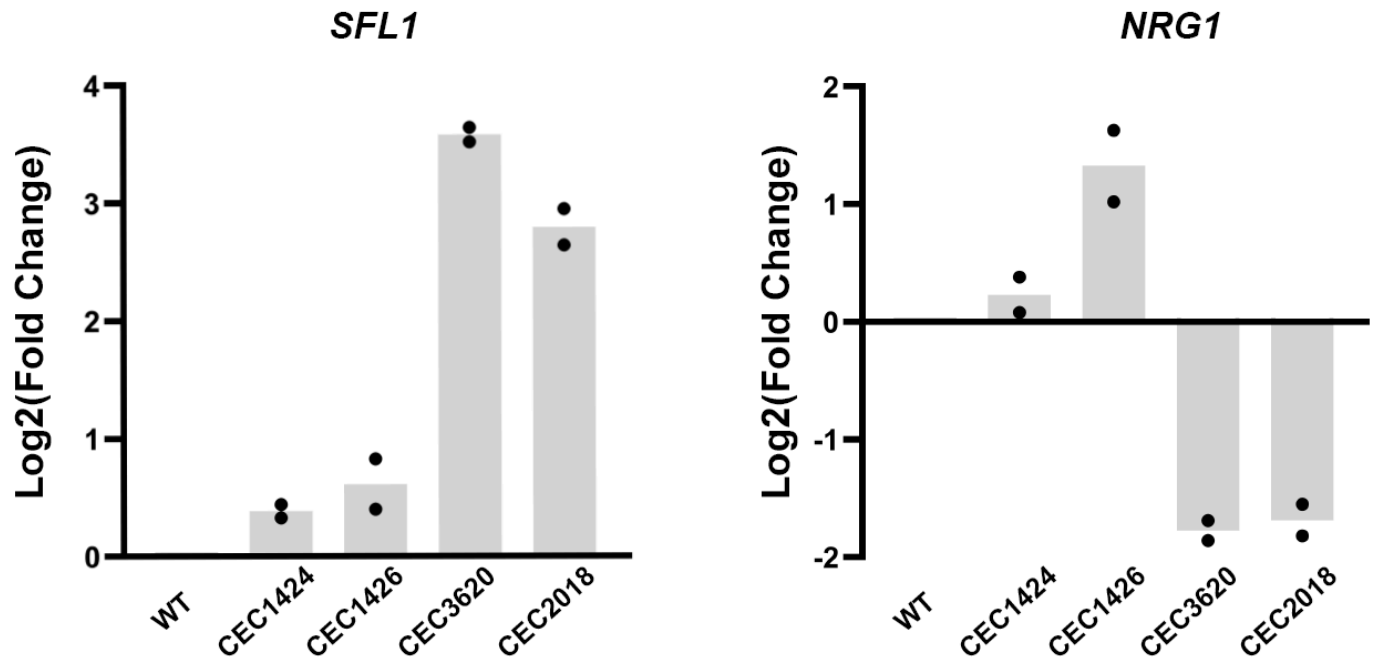
**c**



**Supplementary Fig. 6. *RME1* acts downstream of *SFL1*, *NDT80* and *NRG1*.** **a)** Strains constitutively overexpressing either *SFL1* (top right panel,  $P_{TDH3}$ -*SFL1*) or *RME1* (bottom right panel,  $P_{TDH3}$ -*RME1*) in the *rme1Δ/rme1Δ* (*rme1ΔΔ*) or the *sfl1Δ/sfl1Δ* (*sfl1ΔΔ*) strain backgrounds, respectively, were cultured overnight in liquid chlamyospore-inducing conditions together with the parental control strains *rme1Δ/rme1Δ* and *sfl1Δ/sfl1Δ* transformed with the empty overexpression plasmid (top and bottom left panels,  $P_{TDH3}$ ) before being examined by light microscopy for chlamyospore formation (white arrowheads). Scale bar=10μm. **b)** The *C. albicans* *ndt80Δ/ndt80Δ* (*ndt80ΔΔ*, left panel) together with the *ndt80Δ/ndt80Δ rme1Δ/rme1Δ* double mutant (*ndt80ΔΔ rme1ΔΔ*, right panel) strains as well as **c)** the *C. albicans* *nrg1Δ/nrg1Δ* (*nrg1ΔΔ*, left panel) and *nrg1Δ/nrg1Δ rme1Δ/rme1Δ* double mutant (*nrg1ΔΔ rme1ΔΔ*, right panel) were similarly grown in chlamyospore-inducing conditions before being microscopically examined for their efficiency to form chlamyospores (white arrowheads). Scale bar=10μm.



**Supplementary Fig. 7. *Sfl1* and *Ndt80* antagonistically affect expression of *RME1*.** **a)** Mutant strains *sfl1ΔΔ* and *ndt80ΔΔ* in SC5314 or CEC2018 backgrounds, and **b)** strains constitutively overexpressing *SFL1* or *NDT80* in SC5314 or CEC2018 backgrounds were grown overnight at 25°C in liquid chlamyospore-inducing conditions. Total RNA was extracted and the relative expression levels of *RME1* were determined by RT-qPCR using *ACT1* as a calibrator. n=2 biological replicates, over 3 experiments.



**Supplementary Fig. 8. *SFL1* and *NRG1* expression levels correlate with the efficiency of clinical isolates to form chlamyospores.** Relative expression levels of *SFL1* and *NRG1* were determined by RT-qPCR in *C. albicans* clinical isolates and the reference strain SC5314 (WT control). CEC1424 and CEC1426 are defective for chlamyospore formation, whereas CEC3620 and CEC2018 are efficient for chlamyospore formation (*x*-axes). Log<sub>2</sub>-transformed expression levels (fold change, *y*-axes) of *SFL1* (left) and *NRG1* (right) in the indicated strains relative to their expression in the reference strain SC5314 are shown on the *y*-axes. n=2 biological replicates, over 3 experiments.



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