Supplementary Information for
A common vesicle proteome drives fungal biofilm development

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This PDF file includes:
Extended Materials and Methods
Figures S1 to S3
Tables S1 to S3

Extended Materials and Methods

Culture media and conditions. Stocks of Candida strains were stored in 15% glycerol frozen at -80°C and routinely maintained on YPD agar plates (1% yeast extract, 2% Bacto™ peptone, 2% dextrose, 2% Bacto™ agar). Liquid cultures were grown in broth YPD (1% yeast extract, 2% Bacto™ peptone, 2% dextrose) rotating at 200 rpm at 30°C. For biofilm assays, strains were cultured in filter-sterilized Roswell Park Memorial Institute medium 1640 (RPMI), buffered with 4-morpholinepropanesulfonic acid (MOPS) and pH adjusted to 7.0¹.

Fungal mutant construction strategies. Strains used in this study along with their genotypes are listed in Table S2. The parental strains C. tropicalis CAY3764, C. parapsilosis CPL2H1, C. glabrata HTL, and C. auris B11804 (wild type Colombian isolate of the South American clade IV) were used to generate homozygous deletion mutants using available auxotrophic and drug resistance marker-based strategies²⁴. Gene replacement cassettes were prepared using a PCR-assisted gene splicing by overlap extension (SOE) DNA assembly procedure⁵. At least two independent mutants were created for each gene of interest. Gene deletion complementation with a single wild type gene copy and an antibiotic resistance marker was done either with nourseothricin in case of C. tropicalis and C. parapsilosis or with hygromycin B in case of C. glabrata and C. auris, respectively. Correct integration sites during gene deletion and complementation procedures were confirmed by routine PCR. The primers utilized for strain construction and genetic manipulations are listed in Table S3.
Large scale biofilm cultures and extracellular matrix and extracellular vesicle isolation. Candida biofilms were grown using a large scale rolling bottle biofilm model system. Culture media were carefully decanted from the polystyrene bottles after 24 and 48 h of incubation at 37°C. The remaining fungal biomass was dislodged from the roller bottle surface with a sterile spatula and used to isolate the extracellular matrix. The intact biofilms were then gently subjected to sonication to remove matrix from fungal cells. Sonication with done with a 6-mm microtip head at 20 kHz with an amplitude of 30% for 8 min, followed by centrifugation to separate the biomass from the matrix, filter-sterilization, and the isolated matrix was then lyophilized. Culture supernatants were filter sterilized, and concentrated down to about 25 ml using a Vivaflow 200 unit (Sartorius AG) equipped with a Hydrosart 30 kDa cut-off membrane. Samples were centrifuged in order to remove smaller cellular debris particulates first at 10,000 × g for 1 h at 4°C. The pellet was discarded, and the resulting supernatant was centrifuged again at 100,000 × g for 1.5 h at 4°C. Next, the supernatant was discarded, and the pellet was then washed in 5 ml of PBS and re-centrifuged at 100,000 × g for 1 h at 4°C. The collected extracellular vesicles were next polished by flash size-exclusion chromatography on a qEV/35 nm column (Izon Science), filter sterilized and stored until further use at 4°C.

Intermediate scale biofilm cultures. Intermediate scale biofilm cultures were grown in six-well polystyrene plates and were used to determine biofilm extracellular matrix carbohydrate composition in wild type and mutant strains of all five Candida species. Biofilms were seeded with 10⁶ yeast cells per well. The nonadherent cells were removed after a 60-min-long static adherence incubation and 1 ml of fresh RPMI medium was applied to each well. The biofilms were grown on an orbital shaker set at 50 rpm at 37°C for 24 h, then the medium was replaced with fresh RPMI and the incubation was continued for another 24 h. Biofilms were removed from wells with a sterile spatula and harvested in sterile water (1 ml/well). The aliquots were combined in a 15-ml Falcon tube and sonicated in a water bath sonicator for 20 min. To separate the dissolved ECM from fungal biomass, the sample was centrifuged at 2880 × g at 4°C for 20 min. Five-ml of the collected ECM suspension was placed in a clear 8-ml glass screw thread vial and dried overnight at 60°C. Such prepared samples were used for gas chromatography-based carbohydrate profiling as described below.

Biofilm antifungal drug susceptibility assay. Antifungal drug susceptibility of Candida biofilms was measured in 96-well flat-bottom polystyrene plates. Biofilms were treated with fluconazole, one of the most prescribed antifungal azoles, at 1000 mg/ml. Fungal cell inocula (10⁶ cells/ml) were prepared out of overnight yeast cultures in YPD at 30°C, followed by dilution in RPMI-MOPS based on count numbers with an automated Countess™ II cell counter (Invitrogen). One hundred μl of yeast cells per well were seeded. For C. albicans and C. tropicalis cultures, after a 6-h biofilm formation period in the wells, the biofilms were washed twice with phosphate-buffered saline (PBS, pH 7.2) in order to remove nonadherent cells, followed by the addition of the antifungal drug and fresh RPMI medium. The drug treatment was repeated after 24 h and the plates were incubated for an additional period of 24 h. For C. parapsilosis, C. glabrata, and C. auris cultures, the growth medium was replaced and fluconazole was dosed after 24 h followed by an additional 24 h incubation period. Biofilms exposed to the antifungal azole were then evaluated using the colorimetric tetrazolium reduction XTT assay and the percent reduction in biofilm growth was calculated using the reduction in absorbance compared to that of controls with no antifungal treatment. Briefly, XTT (2,3-bis[2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilide inner salt) was prepared fresh prior to use at the concentration of 0.75 mg/ml. To enhance XTT reduction, phenazine methosulfate (2
mM) was used as electron acceptor in *C. albicans* and *C. tropicalis* assays, whereas menadione (1 mM in acetone) was used in assays involving *C. parapsilosis, C. glabrata*, and *C. auris*. Absorbance at 492 nm was measured using an automated Cytation 5 imaging reader (BioTek).

**Biofilm dispersion assay.** Biofilm dispersion was determined in 96-well plates. Fungal cell inocula (10⁶ cells/ml) were prepared out of overnight yeast cultures in YPD at 30°C, followed by dilution in RPMI-MOPS based on count numbers with an automated Countess™ II cell counter (Invitrogen). One hundred μl of yeast cells per well were seeded and the plates were incubated for 6 h at 37°C, followed by gentle washing with PBS and fresh RPMI was applied. After continued incubation for another 24 h, the biofilms were washed with PBS and RPMI was replaced with fresh one and allowed to incubate for 24 h at 37°C. Supernatants were then carefully removed from biofilm cultures and 100-μl aliquots were transferred to a fresh 96-well plate. The amount of dispersed biofilm cells was determined by the described above modified XTT assay, in which both XTT and PMS or menadione were applied at double concentration. Dispersion capacity of biofilms was calculated using the change in absorbance compared to that of controls.

**Exogenous extracellular vesicle addback assays and functional network construction.** Biological impact of exogenous extracellular vesicles on Candida biofilm properties (susceptibility to fluconazole and dispersion) was determined in 96-well plates. Fungal cell inocula (10⁶ cells/ml) were prepared out of overnight yeast cultures in YPD at 30°C, followed by dilution in RPMI-MOPS based on count numbers with an automated Countess™ II cell counter (Invitrogen). One hundred μl of yeast cells per well were seeded. TOS1 deletion strains were used to evaluate biofilm susceptibility to fluconazole, whereas CHT3 deletion mutants were used in dispersion assays. Extracellular vesicles isolated from all five tested Candida species were used in combinations in biofilms of all five Candida species at normalized concentrations ranging between 1×10^4 and 3×10^6 particles/ml. For the biofilm antifungal susceptibility assay, exogenous extracellular vesicles were added after an initial 5-h biofilm formation period, incubated for an additional hour followed by the drug treatment as described above. Biofilm cultures were treated with fluconazole (1,000 μg/ml). For the biofilm dispersion assay, exogenous extracellular vesicles were added after 24 h of growth. Biofilms growth in cultures with and without exogenous extracellular vesicles was evaluated by the XTT assay as described above. The obtained phenotypic outcomes were organized into visual Candida biofilm phenotypic networks using the Cytoscape platform⁹.

**Time course assessment of extracellular vesicle production in biofilms.** Quantitative analysis of EVs produced in Candida biofilms was determined at various culture growth time points in 96-well plates. Fungal cell inocula (10⁶ cells/ml) were prepared out of overnight yeast cultures in YPD at 30°C, followed by dilution in RPMI-MOPS based on count numbers with an automated Countess™ II cell counter (Invitrogen). One hundred μl of yeast cells per well were seeded and incubated for 6, 12, 24, and 48 h, followed by collection of supernatants, which were then filter sterilized and subjected to extracellular vesicle analysis as described below. Data were normalized based on XTT assay readouts as described above.

**Extracellular vesicle analyses.** Exosomes were quantified using nanoparticle tracking analysis. Initial analyses were performed on a Zetasizer Nano-ZS (Malvern Instruments)¹⁰. EV samples were diluted in PBS to a final volume of 1 ml and pretested to obtain an ideal
30-100 particles per frame rate using a NanoSight NS300 system (Malvern). The following settings were applied: camera level was increased to 16 and camera gain to 2 until tested images were optimized and nanoparticles were distinctly visible without exceeding particle signal saturation. Each measurement consisted of five 1-min videos with a delay of 5 s between sample introduction and the start of the first measurement. For detection threshold analysis the counts were limited to 10-100 red crosses and no more than 5-7 blue crosses. Acquired data were analyzed using the NanoSight Software NTA 3.4 Build 3.4.003. At least 1000 events in total was tracked per sample in order to minimize data skewing based on single large particles.

**Extracellular vesicle uptake by Candida cells.** Candida cells and extracellular vesicles were imaged using the multispectral ImageStreamX Mk II flow cytometry system (Amnis Corporation) at ×60 magnification, with default low flow rate/high sensitivity using the INSPIRE software. Candida cells grown in YPD at 30°C overnight and transferred to 37°C for 30-60 min in order to induce and mimic an early biofilm formation stage. Cells were next counted with an automated Countess™ II cell counter (Invitrogen) and 90-µl aliquots containing ~2×10⁶ cells were prepared and kept on ice. Extracellular vesicles were labeled with the EZLabel™ Protein-FITC Labeling Kit (BioVision). Excessive dye particles were removed from stained vesicles using Illustra microspin G-50 columns (GE Healthcare). Ten-µl aliquots containing ~1×10⁸ FITC-labelled extracellular vesicles were used. Cells and extracellular vesicles were mixed right before the experiment and immediately introduced into the flow cytometer. The image acquisition started approximately within 90-120 s afterward. On average 1×10⁴ cells were collected for each sample and the data obtained were analyzed using the IDEAS image analysis software (Amnis Corporation). The uptake experiment was validated by using extracellular vesicles stained with lipid dyes (DiI, DiD, rhodamine R18), which could be detected in recipient Candida cells exposed to the lipid-labelled extracellular vesicles.

**In vivo Candida catheter model.** Candida biofilm growth during infection of implanted medical devices was measured using an external jugular vein rat catheter infection model. For drug treatment experiments, fluconazole at a concentration of 250 μg/ml was instilled and dwelled in the catheter over a 24-h period. The post treatment viable burden of Candida biofilm on the catheter surface was compared to untreated control growth. Three replicates were performed for treatment and control conditions. Quantitative cultures of Candida spp. after 24 h of in vivo growth were utilized to measure viable biofilm cell burden.

**Scanning electron microscopy of Candida biofilms.** The surface of Candida biofilms grown in 6-well plates was imaged using scanning electron microscopy (SEM). Briefly, 40 μl of an inoculum of 10⁸ cells/ml in RPMI was added to the coverslips and incubated at 37°C for 60 min. One ml RPMI was added to each well, and the plates were incubated at 37°C for 20 h. One ml fixative (4% formaldehyde, 1% glutaraldehyde in PBS) was then added to each well prior to incubation at 4°C overnight. Coverslips were then washed with PBS prior to incubation in 1% OsO₄ for 30 min. Samples were then serially dehydrated in ethanol (30% to 100%). Critical point drying was used to completely dehydrate the samples prior to palladium-gold coating. Samples were imaged on a SEM LEO 1530, with Adobe Photoshop 2022 (v. 23.2.2) used for image compilation.

**Cryo-electron microscopy.** Cryo-electron microscopy (cryo-EM) was done at the University of Wisconsin-Madison Cryo Research Center. CF200-CU grids (EMS) were glow-discharged for 30 seconds in at 15mA with a chamber pressure of 0.004 mBar.
Plunge freezing occurred in an FEI Vitrobot Mark IV cryo plunge freezing robot at 4°C. Three μl of sample were then spotted onto the grid, blotted for 5 seconds with a blotted pressure of 1, and plunge-frozen into liquid ethane. Grids were transferred into liquid nitrogen for storage. Cryo EM imaging was performed on a Talos Arctica (ThermoScientific) at 200 kV. Images (defocus of −2 μm) were recorded on a post-GIF Gatan K3 camera in EFTEM mode (3.7 Å/pixel at 24kx magnification and 1.1 Å/pixel at 79kx magnification) with a 20-eV slit, CDS counting mode, using SerialEM 3.8. Two magnifications were set for high-resolution imaging: 24kx magnification (spot size 7, C2 aperture 100, C2 lens power 38.133%, objective aperture 100, pixel size 3.7Å) and 79kx magnification (spot size 4, C2 aperture 70, C2 lens power 42.546%, objective aperture 100, pixel size 1.1Å). A total dose of 21.6 e-/Å2 at 24kx magnification and 48 e-/Å2 at 79kx magnification were used, respectively.

**Gel-free proteomics.** Enzymatic “in liquid” digestion and mass spectrometric analysis was done at the Mass Spectrometry Facility, Biotechnology Center, University of Wisconsin–Madison. Two hundred μg of proteins were extracted by precipitation with 15% TCA/60% acetone and then incubated at −20°C for 30 min. The matrix or vesicle preparation was centrifuged at 16,000 × g for 10 min, and the resulting pellets were washed twice with ice-cold acetone, followed by an ice-cold MeOH wash. Pelleted proteins were resolubilized and denatured in 10 μl of 8 M urea in 100 mM NH₄HCO₃ for 10 min, then diluted to 60 μl for tryptic digestion with the following reagents: 3 μl of 25 mM DTT, 4.5 μl of acetonitrile, 36.2 μl of 25 mM NH₄HCO₃, 0.3 μl of 1M Tris-HCl, and 6 μl of 100 ng/μl Trypsin Gold solution in 25 mM NH₄HCO₃ (Promega). Digestion was conducted in two stages, first overnight at 37°C, then additional 4 μl of trypsin solution were added and the mixture was incubated at 42°C for an additional 2 h. The reaction was terminated by acidification with 2.5% TFA to a final concentration of 0.3% and then centrifuged at 16,000 × g for 10 min. Trypsin-generated peptides were analyzed by nanoLC-MS/MS using the Agilent 1100 nanoflow system (Agilent) connected to a hybrid linear ion trap-orbitrap mass spectrometer (LTQ-Orbitrap, Thermo Fisher Scientific) equipped with a nanoelectrospray ion source. Capillary HPLC was performed using an in-house fabricated column with an integrated electrospray emitter, as described elsewhere. Sample loading and desalting were achieved using a trapping column in line with the autosampler (Zorbax 300SB-C18, 5 μm, 5 × 0.3 mm, Agilent). The LTQ-Orbitrap was set to acquire MS/MS spectra in a data-dependent mode as follows: MS survey scans from 300 to 2,000 m/z were collected in profile mode with a resolving power of 100,000. MS/MS spectra were collected on the five most abundant signals in each survey scan. Dynamic exclusion was employed to increase the dynamic range and maximize peptide identifications. Raw MS/MS data were searched against a concatenated C. albicans amino acid sequence database using an in-house MASCOT search engine. Identified proteins were further annotated and filtered to 1.5% peptide and 0.1% protein false-discovery-rate with Scaffold Q+ version 4.10.0 (Proteome Software Inc.) using the protein prophet algorithm. Proteomic data were mapped using Heatmapper. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the partner repository with the dataset identifier XXX and XXX.

**Proteome functional mapping.** The obtained Candida extracellular vesicle and matrix proteomes were analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG). Both KEGG pathway maps and BRITE hierarchies databases were used. Each protein predicted from the C. albicans genome assigned a KEGG Ontology ID (KOID) was obtained, and the specific pathway and superpathway membership information retained. For non-albicans Candida species, BLASTP-based mapped protein
orthologies and homologies among Candida species were obtained from the Candida Genome Database (CGD)\textsuperscript{22}. This was then correlated with the experimental proteome data, and the number of proteins expressed within a given pathway was then determined. Tabulated proteins were presented as a percentage out of the total number of proteins predicted to belong to a given pathway from the \textit{C. albicans} genome, as determined by KEGG/BRITE assignments. The visualization of relative quantities of biofilm proteins was done using KEGG/BRITE protein functional categorization\textsuperscript{23}. Based on this hierarchical classification scheme, Voronoi treemaps were constructed using Paver (v. 2.1.9, DECODON Software UG). This approach divides screen space according to hierarchy levels in which the main functional categories determine screen sections on the first level, subsidiary categories on the second level, and so forth. The polygonic cells of the deepest level represented functionally classified proteins and were colored according to relative abundance of each protein that was determined based on total counts of corresponding trypsin-digested peptides.

**Extracellular vesicle carbohydrate profiling.** Carbohydrates in biofilm extracellular vesicles were analyzed based on the modified procedures reported elsewhere\textsuperscript{24}. Monosugars were converted to alditol acetate derivatives\textsuperscript{25} and then identified and quantified by gas chromatography on a Shimadzu GC-2010 system (Shimadzu). A Crossbond\textsuperscript{TM} 50% cyanopropylmethyl/50% phenylmethyl polysiloxane column was used (15 m × 0.25 mm with 0.25 μm film thickness, RTX-225, Restek). The GLC conditions were as follows: injector at 220°C, FID detector at 240°C, and a temperature program of 215°C for 2 min, then 4°C/min up to 230°C before holding for 11.25 min, run at constant linear velocity of 33.4 cm/sec and split ratio of 50:1.

**Statistics.** Data sets of equal of different sample sizes were analyzed using the nonparametric Kruskal-Wallis one-way analysis of variance with uncorrected Dunn’s multiple comparisons without prior elimination of outliers. Data were processed with GraphPad Prism 9 for Windows 64-bit (version 9.3.1 (471)).

**Ethics statement.** All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison according to the guidelines of the Animal Welfare Act, The Institute of Laboratory Animal Resources Guide for the Care and Use of Laboratory Animals, and Public Health Service Policy. The approved animal protocol number is DA0031.

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**Fig. S1.** Mutations of select Candida EV cargo proteins impacts the carbohydrate biochemistry of Candida biofilm extracellular matrices (ECMs) determined by gas chromatography. (A) Levels of total carbohydrates in the tested Candida biofilm ECMs. Data are presented as the mean ± SD; n = 6; *P<0.05; **P<0.01; ***P<0.005; ****P ≤ 0.0001, using non-parametric Kruskal–Wallis one-way analysis of variance with post hoc uncorrected Dunn’s multiple comparison test. (B) Changes in the tested Candida biofilm ECM mannans. Data are presented as the mean ± SD; n = 6; *P<0.05; **P<0.01; ***P<0.005; ****P ≤ 0.0001, using non-parametric Kruskal–Wallis one-way analysis of variance with post hoc uncorrected Dunn’s multiple comparison test. (C) Changes in the tested Candida biofilm ECM glucans. Data are presented as the mean ± SD; n = 6; ***P<0.005; ****P ≤ 0.0001, using non-parametric Kruskal–Wallis one-way analysis of variance with post hoc uncorrected Dunn’s multiple comparison test. CA – *Candida albicans*; CT – *Candida tropicalis*; CP – *Candida parapsilosis*; CG – *Candida glabrata*; CR – *Candida auris*.
Fig. S2. Effects of exogenous Candida biofilm EVs on biofilm fluconazole susceptibility of TOS1 null mutants. Biofilm cultures of fluconazole-sensitive mutant strains (grouped in rows) were amended with WT EVs (columns) isolated from five different Candida species biofilm culture supernatants. Lines represent the mean of 8 technical replicates and the shaded blue area represents minimal and maximal value range distribution. Data are presented as the mean ± SD; n = 5; *P<0.05; **P<0.01; ***P<0.005; ****P ≤ 0.0001, using non-parametric Kruskal–Wallis one-way analysis of variance with post hoc uncorrected Dunn’s multiple comparison test. CA – Candida albicans; CT – Candida tropicalis; CP – Candida parapsilosis; CG – Candida glabrata; CR – Candida auris.
Fig. S3. Effects of exogenous Candida biofilm EVs on biofilm dispersion of CHT3 null mutants. Biofilm cultures of dispersion-altered mutant strains (grouped in rows) were amended with WT EVs (columns) isolated from five different Candida species biofilm culture supernatants. Lines represent the mean of 8 technical replicates and the shaded blue area represents minimal and maximal value range distribution. Data are presented as the mean ± SD; n = 5; *P<0.05; **P<0.01; ***P<0.005; ****P ≤ 0.0001, using non-parametric Kruskal–Wallis one-way analysis of variance with post hoc uncorrected Dunn’s multiple comparison test. CA – Candida albicans; CT – Candida tropicalis; CP – Candida parapsilosis; CG – Candida glabrata; CR – Candida auris.
**Table S1.** KEGG/BRITE-based functional category assignments to the identified proteins in Candida extracellular vesicle proteomes

| Database          | Hierarchy level 1 | Hierarchy level 2 | Hierarchy level 3 | No. of proteins per group |
|-------------------|-------------------|-------------------|-------------------|--------------------------|
|                   |                   |                   |                   | CA | CT | CP | CG | CR |
| **KEGG Pathways** | Metabolism        | Carbohydrate      |                   |    |    |    |    |    |
|                   |                   | metabolism       |                   |    |    |    |    |    |
|                   |                   |                   | Glycolysis / Gluconeogenesis | 23 | 24 | 12 | 8  | 8  |
|                   |                   |                   | Citrate cycle (TCA cycle)   | 11 | 14 | 4  | 0  | 6  |
|                   |                   |                   | Pentose phosphate pathway   | 9  | 11 | 6  | 3  | 5  |
|                   |                   |                   | Pentose and glucuronate     | 4  | 2  | 2  | 0  | 1  |
|                   |                   |                   | interconversions            |    |    |    |    |    |
|                   |                   |                   | Fructose and mannose        | 8  | 10 | 6  | 2  | 3  |
|                   |                   |                   | metabolism                 |    |    |    |    |    |
|                   |                   |                   | Galactose metabolism        | 7  | 6  | 5  | 0  | 1  |
|                   |                   |                   | Ascorbate and aldarate      | 3  | 1  | 1  | 0  | 1  |
|                   |                   |                   | metabolism                 |    |    |    |    |    |
|                   |                   |                   | Starch and sucrose          | 15 | 12 | 11 | 4  | 5  |
|                   |                   |                   | metabolism                 |    |    |    |    |    |
|                   |                   |                   | Amino sugar and nucleotide  | 17 | 14 | 10 | 2  | 7  |
|                   |                   |                   | sugar metabolism            |    |    |    |    |    |
|                   |                   |                   | Pyruvate metabolism         | 19 | 16 | 4  | 1  | 5  |
|                   |                   |                   | Glyoxylate and dicarboxylate| 10 | 12 | 3  | 1  | 6  |
|                   |                   |                   | metabolism                 |    |    |    |    |    |
|                   |                   |                   | Propanoate metabolism       | 8  | 8  | 1  | 0  | 2  |
|                   |                   |                   | Butanoate metabolism        | 3  | 1  | 0  | 0  | 0  |
|                   |                   |                   | Inositol phosphate          | 2  | 1  | 1  | 1  | 0  |
|                   |                   |                   | metabolism                 |    |    |    |    |    |
|                   |                   |                   | Energy metabolism           |    |    |    |    |    |
|                   |                   |                   | Oxidative phosphorylation   | 18 | 14 | 2  | 4  | 7  |
|                   |                   |                   | Methane metabolism          | 10 | 12 | 5  | 3  | 3  |
|                   |                   |                   | Nitrogen metabolism         | 2  | 1  | 0  | 0  | 0  |
|                   |                   |                   | Sulfur metabolism           | 1  | 1  | 1  | 1  | 0  |
|                   |                   |                   | Lipid metabolism            |    |    |    |    |    |
|                   |                   |                   | Fatty acid biosynthesis     | 3  | 3  | 1  | 0  | 2  |
|                   |                   |                   | Fatty acid elongation        | 1  | 0  | 0  | 0  | 1  |
|                   |                   |                   | Fatty acid degradation      | 6  | 3  | 0  | 0  | 2  |
|                   |                   |                   | Synthesis and degradation   | 2  | 1  | 0  | 0  | 0  |
|                   |                   |                   | of ketone bodies            |    |    |    |    |    |
|                   |                   |                   | Steroid biosynthesis        | 0  | 0  | 0  | 0  | 2  |
|                   |                   |                   | Glycerolipid metabolism     | 1  | 1  | 2  | 0  | 3  |
|                   |                   |                   | Glycerophospholipid         | 3  | 2  | 0  | 2  | 3  |
|                   |                   |                   | metabolism                 |    |    |    |    |    |
|                   |                   |                   | Arachidonic acid            | 1  | 1  | 0  | 0  | 0  |
|                   |                   |                   | metabolism                 |    |    |    |    |    |
|                   |                   |                   | alpha-Linolenic acid        | 1  | 0  | 0  | 0  | 1  |
|                   |                   |                   | metabolism                 |    |    |    |    |    |
|                   |                   |                   | Biosynthesis of unsaturated| 1  | 0  | 0  | 0  | 2  |
|                   |                   |                   | fatty acids                 |    |    |    |    |    |
|                   |                   |                   | Nucleotide metabolism       |    |    |    |    |    |
|                   |                   |                   | Purine metabolism           | 8  | 8  | 3  | 1  | 3  |
|                   |                   |                   | Pyrimidine metabolism       | 2  | 4  | 1  | 0  | 1  |
|                   |                   |                   | Amino acid                  |    |    |    |    |    |
|                   |                   |                   | metabolism                 |    |    |    |    |    |
|                   |                   |                   | Alanine, aspartate          | 9  | 3  | 2  | 0  | 1  |
|                   |                   |                   | and glutamate metabolism    |    |    |    |    |    |
|                   |                   |                   | Glycine, serine and         | 6  | 8  | 2  | 1  | 2  |
|                   |                   |                   | threonine metabolism        |    |    |    |    |    |
|                   |                   |                   | Cysteine and methionine     | 11 | 7  | 6  | 1  | 2  |
|                   |                   |                   | metabolism                 |    |    |    |    |    |
|                   |                   |                   | Valine, leucine and         | 4  | 3  | 1  | 0  | 2  |
|                   |                   |                   | isoleucine degradation      |    |    |    |    |    |
|                   |                   |                   | Valine, leucine and         | 1  | 0  | 0  | 0  | 0  |
|                   |                   |                   | isoleucine biosynthesis     |    |    |    |    |    |
|                   |                   |                   | Lysine biosynthesis         | 5  | 1  | 2  | 0  | 0  |
|                   |                   |                   | Lysine degradation          | 5  | 3  | 2  | 0  | 2  |
|                   |                   |                   | Arginine biosynthesis       | 6  | 2  | 2  | 0  | 0  |
| Metabolism of other amino acids                        | Arginine and proline metabolism | Histidine metabolism | Tyrosine metabolism | Phenylalanine metabolism | Tryptophan metabolism | Phenylalanine, tyrosine and tryptophan biosynthesis | beta-Alanine metabolism | Taurine and hypotaurine metabolism | Selenocompound metabolism | Cyanoamino acid metabolism | Glutathione metabolism | N-Glycan biosynthesis | Various types of N-glycan biosynthesis | Mannose type O-glycan biosynthesis | Other types of O-glycan biosynthesis | Other glycan degradation | Glycosaminoglycan degradation | Glycosphingolipid biosynthesis - globo and isoglobo series | Glycosphingolipid biosynthesis - ganglio series | Other glycan degradation | Thiamine metabolism | Riboflavin metabolism | Vitamin B6 metabolism | Pantothenate and CoA biosynthesis | Folate biosynthesis | One carbon pool by folate | Porphyrin and chlorophyll metabolism | Ubiquinone and other terpenoid-quinone biosynthesis | Terpenoid backbone biosynthesis | Biosynthesis of ansamycins | Carbapenem biosynthesis | Monobactam biosynthesis | Neomycin, kanamycin and gentamicin biosynthesis |
|-------------------------------------------------------|---------------------------------|----------------------|---------------------|-------------------------|------------------------|-------------------------------------------------|-------------------------|-----------------------------------|---------------------------|----------------------------------|------------------------|------------------|------------------------------------------|--------------------------|-----------------------------|------------------|-----------------------------|-----------------------------|---------------------------|------------------|------------------|----------------------|------------------------|-----------------|------------------|----------------------------------|------------------------|-----------------------|------------------|------------------------|------------------------|------------------|
| Glycan biosynthesis and metabolism                    |                                 |                      |                     |                         |                        | N-Glycan biosynthesis                             |                         | Various types of N-glycan biosynthesis | Mannose type O-glycan biosynthesis | Other types of O-glycan biosynthesis | Other glycan degradation | Glycosaminoglycan degradation | Glycosphingolipid biosynthesis - globo and isoglobo series | Glycosphingolipid biosynthesis - ganglio series | Other glycan degradation | Thiamine metabolism | Riboflavin metabolism | Vitamin B6 metabolism | Pantothenate and CoA biosynthesis | Folate biosynthesis | One carbon pool by folate | Porphyrin and chlorophyll metabolism | Ubiquinone and other terpenoid-quinone biosynthesis | Terpenoid backbone biosynthesis | Biosynthesis of ansamycins | Carbapenem biosynthesis | Monobactam biosynthesis | Neomycin, kanamycin and gentamicin biosynthesis |
| Metabolism of cofactors and vitamins                   |                                 |                      |                     |                         |                        | Other glycan degradation                           |                         | Glycosaminoglycan degradation | Glycosphingolipid biosynthesis - globo and isoglobo series | Glycosphingolipid biosynthesis - ganglio series | Other glycan degradation | Thiamine metabolism | Riboflavin metabolism | Vitamin B6 metabolism | Pantothenate and CoA biosynthesis | Folate biosynthesis | One carbon pool by folate | Porphyrin and chlorophyll metabolism | Ubiquinone and other terpenoid-quinone biosynthesis | Terpenoid backbone biosynthesis | Biosynthesis of ansamycins | Carbapenem biosynthesis | Monobactam biosynthesis | Neomycin, kanamycin and gentamicin biosynthesis |
| Genetic Information Processing                        |                                 |                      |                     |                         |                        | Thiamine metabolism                               |                         | Riboflavin metabolism | Vitamin B6 metabolism | Pantothenate and CoA biosynthesis | Folate biosynthesis | One carbon pool by folate | Porphyrin and chlorophyll metabolism | Ubiquinone and other terpenoid-quinone biosynthesis | Terpenoid backbone biosynthesis | Biosynthesis of ansamycins | Carbapenem biosynthesis | Monobactam biosynthesis | Neomycin, kanamycin and gentamicin biosynthesis |
| Transcription                                         |                                 |                      |                     |                         |                        | Spliceosome                                      |                         | Ribosome              | Aminoacyl-tRNA biosynthesis | RNA transport | mRNA surveillance pathway | Ribosome biogenesis in eukaryotes | Protein export |
| Translation                                           |                                 |                      |                     |                         |                        | Spliceosome                                      |                         | Ribosome              | Aminoacyl-tRNA biosynthesis | RNA transport | mRNA surveillance pathway | Ribosome biogenesis in eukaryotes | Protein export |
| Folding, sorting and degradation                      |                                 |                      |                     |                         |                        | Spliceosome                                      |                         | Ribosome              | Aminoacyl-tRNA biosynthesis | RNA transport | mRNA surveillance pathway | Ribosome biogenesis in eukaryotes | Protein export |
| Environmental Information Processing | Membrane transport | ABC transporters | 2 | 1 | 2 | 1 | 3 |
|-------------------------------------|-------------------|------------------|---|---|---|---|---|
| Signal transduction                | MAPK signaling pathway - yeast | 10 | 5 | 7 | 4 | 12 |
|                                    | Phosphatidylinositol signaling system | 1 | 0 | 0 | 0 | 1 |
| Cellular Processes                 | Transport and catabolism | Endocytosis | 11 | 15 | 9 | 5 | 13 |
|                                    | Phagosome | 6 | 4 | 0 | 1 | 2 |
|                                    | Peroxisome | 5 | 0 | 3 | 1 | 4 |
|                                    | Autophagy - yeast | 6 | 10 | 6 | 1 | 6 |
|                                    | Autophagy - other | 1 | 0 | 0 | 0 | 0 |
| Cell growth and death              | Cell cycle - yeast | 5 | 2 | 1 | 1 | 0 |
|                                    | Meiosis - yeast | 4 | 4 | 2 | 0 | 1 |
| Aging                              | Longevity regulating pathway - multiple species | 5 | 5 | 5 | 3 | 5 |

| BRITE | Protein families: metabolism | Enzymes | Protein kinases | 3 | 1 | 1 | 1 | 3 |
|-------|------------------------------|---------|----------------|---|---|---|---|---|
|       |                              |         | Protein phosphatases and associated proteins | 7 | 8 | 3 | 2 | 5 |
|       |                              |         | Peptidases and inhibitors | 41 | 30 | 13 | 4 | 26 |
|       |                              |         | Glycosyltransferases | 16 | 11 | 7 | 4 | 5 |
|       |                              |         | Lipid biosynthesis proteins | 4 | 4 | 1 | 0 | 4 |
|       |                              |         | Prenyltransferases | 1 | 1 | 0 | 0 | 1 |
|       |                              |         | Amino acid related enzymes | 7 | 12 | 3 | 0 | 1 |
|       | Protein families: genetic information processing | Transcription factors | Transcription factors | 1 | 0 | 1 | 0 | 0 |
|       |                              |         | Transcription machinery | 3 | 0 | 2 | 2 | 1 |
|       |                              |         | Messenger RNA biogenesis | 19 | 16 | 5 | 3 | 4 |
|       |                              |         | Spliceosome | 4 | 5 | 2 | 2 | 2 |
|       |                              |         | Ribosome | 15 | 28 | 1 | 4 | 6 |
|       |                              |         | Ribosome biogenesis | 8 | 6 | 1 | 4 | 4 |
|       |                              |         | Transfer RNA biogenesis | 9 | 14 | 3 | 1 | 2 |
|       |                              |         | Translation factors | 11 | 6 | 5 | 3 | 2 |
|       |                              |         | Chaperones and folding catalysts | 18 | 20 | 15 | 6 | 12 |
|       |                              |         | Membrane trafficking | 54 | 56 | 36 | 12 | 49 |
|       |                              |         | Ubiquitin system | 5 | 5 | 2 | 1 | 2 |
|       |                              |         | Proteasome | 13 | 13 | 2 | 3 | 16 |
| Protein families: signaling and cellular processes | Transporters |
|-----------------------------------------------|--------------|
| DNA replication proteins                     | 4 1 0 0 0    |
| Chromosome and associated proteins            | 19 14 4 4 9  |
| DNA repair and recombination proteins         | 7 3 2 0 1    |
| Mitochondrial biogenesis                      | 12 11 6 5 7  |
| Translators                                   | 19 15 12 9 28|
| Secretion system                             | 1 0 0 0 0    |
| Cytoskeleton proteins                         | 10 11 5 3 4  |
| Exosome                                       | 60 64 41 15 37|
| Ion channels                                  | 1 1 1 0 1    |
| GTP-binding proteins                          | 12 13 10 3 15|
| Glycosylphosphatidylinositol (GPI)-anchored proteins | 9 2 2 1 2 |
| Lectins                                       | 2 0 0 0 0    |
| Domain-containing proteins not elsewhere classified | 1 1 0 0 3 |

| Not Included in KEGG or BRITE |
|--------------------------------|
| Unclassified: metabolism      | Enzymes with EC numbers |
|                                | Others                   |
| Unclassified: signaling and cellular processes | Signaling proteins |
| Poorly characterized           | Function unknown |

|                        | Enzymes with EC numbers | Others | Signaling proteins | Function unknown |
|------------------------|-------------------------|--------|--------------------|------------------|
|                        | 9 9 9 3 11              | 1 0 0 0 0 | 1 0 0 0 0 | 1 2 1 1 2 |
| Strain/Gene | Phenotype | Genetic makeup* | Reference |
|------------|-----------|-----------------|-----------|
| **Candida albicans** | | | |
| SN152 | reference strain | his1Δ::his1Δ; leu2Δ::leu2Δ; arg4Δ::arg4Δ | 1 |
| SN250 | reference strain | his1Δ::his1Δ; leu2Δ::CD HIS1/leu2Δ::CM LEU2; arg4Δ::arg4Δ | 2 |
| URZ585 | cht3 homozygote Arg' | his1Δ::his1Δ; leu2Δ::CD HIS1/leu2Δ::CM LEU2; arg4Δ::arg4Δ | 3 |
| URZ688 | cht3 complement prototroph | his1Δ::his1Δ; leu2Δ::CD HIS1/leu2Δ::CM LEU2; arg4Δ::arg4Δ | 3 |
| URZ375 | mp65 homozygote Arg' | his1Δ::his1Δ; leu2Δ::CD HIS1/leu2Δ::CM LEU2; arg4Δ::arg4Δ | 3 |
| URZ889 | mp65 complement prototroph | his1Δ::his1Δ; leu2Δ::CD HIS1/leu2Δ::CM LEU2; arg4Δ::arg4Δ | 3 |
| URZ376 | sun41 complement prototroph | his1Δ::his1Δ; leu2Δ::CD HIS1/leu2Δ::CM LEU2; arg4Δ::arg4Δ | 4 |
| URZ562 | tos1 homozygote Arg' | his1Δ::his1Δ; leu2Δ::CD HIS1/leu2Δ::CM LEU2; arg4Δ::arg4Δ | 3 |
| URZ680 | tos1 complement prototroph | his1Δ::his1Δ; leu2Δ::CD HIS1/leu2Δ::CM LEU2; arg4Δ::arg4Δ | 3 |
| URZ386 | zrt2 homozygote Arg' | his1Δ::his1Δ; leu2Δ::CD HIS1/leu2Δ::CM LEU2; arg4Δ::arg4Δ | 3 |
| URZ874 | zrt2 complement prototroph | his1Δ::his1Δ; leu2Δ::CD HIS1/leu2Δ::CM LEU2; arg4Δ::arg4Δ | 3 |
| **Candida tropicalis** | | | |
| CAY2597 | reference strain C. tropicalis wild type strain | | 5 |
| CAY3764 | reference strain | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF | 5 |
| URZ922 | cht3 homozygote prototroph | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF, cht3::CM LEU2/cht3::CD HIS1 | This work |
| URZ978 | cht3 complement prototroph/Nat1* | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF, cht3::CM LEU2/cht3::CD HIS1, CM leu2::CHT3-NAT1 | This work |
| URZ928 | mp65 homozygote prototroph | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF, cht3::CM LEU2/cht3::CD HIS1 | This work |
| URZ982 | mp65 complement prototroph/Nat1* | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF, cht3::CM LEU2/cht3::CD HIS1, CM leu2::TOS1-NAT1 | This work |
| URZ993 | sun41 homozygote prototroph | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF, sun41::CM LEU2/sun41::CD HIS1 | This work |
| URZ984 | sun41 complement prototroph/Nat1* | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF, sun41::CM LEU2/sun41::CD HIS1, CM leu2::SUN41-NAT1 | This work |
| URZ925 | tos1 homozygote prototroph | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF, tos1::CM LEU2/tos1::CD HIS1 | This work |
| URZ980 | tos1 complement prototroph/Nat1* | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF, tos1::CM LEU2/tos1::CD HIS1, CM leu2::TOS1-NAT1 | This work |
| URZ937 | zrt2 homozygote prototroph | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF, zrt2::CM LEU2/zrt2::CD HIS1 | This work |
| URZ986 | zrt2 complement prototroph/Nat1* | his1Δ::FRF/his1Δ::FRF, leu2Δ::FRF/leu2Δ::FRF, zrt2::CM LEU2/zrt2::CD HIS1, CM leu2::ZRT2-NAT1 | This work |
| **Candida parapsilosis** | | | |
| CLIB214 | reference strain C. parapsilosis wild type strain | | 6 |
| CPL2H1 | reference strain His'Leu- | leu2Δ::FRT/leu2Δ::FRT, his1Δ::FRT/his1Δ::FRT | 6 |
| URZ904 | cht3 homozygote prototroph | leu2Δ::FRT/leu2Δ::FRT, his1Δ::FRT/his1Δ::FRT, cht3::CM LEU2/cht3::CD HIS1 | This work |
| URZ992 | cht3 complement prototroph/Nat1* | leu2Δ::FRT/leu2Δ::FRT, his1Δ::FRT/his1Δ::FRT, cht3::CM LEU2/cht3::CD HIS1, CM leu2::CHT3-NAT1 | This work |
| URZ913 | mp65 homozygote prototroph | leu2Δ::FRT/leu2Δ::FRT, his1Δ::FRT/his1Δ::FRT, mp65::CM LEU2/mp65::CD HIS1 | This work |
1. Noble SM & Johnson AD (2005) Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen Candida albicans. *Eukaryot Cell* 4(2):298-309.

2. Noble SM, French S, Kohn LA, Chen V, & Johnson AD (2010) Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity. *Nat Genet* 42(7):590-598.

3. Zarnowski R, et al. (2021) Coordination of fungal biofilm development by extracellular vesicle cargo. *Nat Commun* 12(1):6235.

4. Zarnowski R, et al. (2018) Candida albicans biofilm-induced vesicles confer drug resistance through matrix biogenesis. *PLoS Biol* 16(10):e2006872.

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### Candida glabrata

| Strain | Gene Deletions | Reference | Notes |
| --- | --- | --- | --- |
| URZ999 | mp65 complement prototroph/Nat1* | leu2Δ::FRT/leu2Δ::FRT, his1Δ::FRT/his1Δ::FRT, mp65::CM | This work |
| URZ911 | sun41 homozygote prototroph | leu2Δ::FRT/leu2Δ::FRT, his1Δ::FRT/his1Δ::FRT, sun41::CM | This work |
| URZ908 | tos1 homozygote prototroph | leu2Δ::FRT/leu2Δ::FRT, his1Δ::FRT/his1Δ::FRT, tos1::CM | This work |
| URZ996 | tos1 complement prototroph/Nat1* | leu2Δ::FRT/leu2Δ::FRT, his1Δ::FRT/his1Δ::FRT, tos1::CM | This work |
| URZ917 | zrt2 homozygote prototroph | leu2Δ::FRT/leu2Δ::FRT, his1Δ::FRT/his1Δ::FRT, zrt2::CM | This work |
| URZ002 | zrt2 complement prototroph/Nat1* | leu2Δ::FRT/leu2Δ::FRT, his1Δ::FRT/his1Δ::FRT, zrt2::CM | This work |

### Candida albicans

| Strain | Deletions | Reference | Notes |
| --- | --- | --- | --- |
| ATCC2001 | reference strain | C. glabrata wild type strain | (7) |
| HTL | reference strain | His3::FRT, leu2Δ::FRT, trp1Δ::FRT | (8) |
| URZ953 | cht3 homozygote Trp/Nat1* | his3Δ::FRT, leu2Δ::FRT, trp1Δ::FRT, cht3Δ::Nat1 | This work |
| URZ964 | cht3 complement Trp/HygB | his3Δ::FRT, leu2Δ::FRT, trp1Δ::FRT, cht3Δ::Nat1::CHT3-HygB | This work |
| URZ959 | mp65 homozygote Trp/Nat1* | his3Δ::FRT, leu2Δ::FRT, trp1Δ::FRT, mp65Δ::Nat1 | This work |
| URZ969 | mp65 complement Trp/HygB | his3Δ::FRT, leu2Δ::FRT, trp1Δ::FRT, mp65Δ::Nat1 | This work |
| URZ957 | tos1 homozygote Trp/Nat1* | his3Δ::FRT, leu2Δ::FRT, trp1Δ::FRT, tos1Δ::Nat1 | This work |
| URZ965 | tos1 complement Trp/HygB | his3Δ::FRT, leu2Δ::FRT, trp1Δ::FRT, tos1Δ::Nat1::TOS1-HygB | This work |
| URZ962 | zrt2 homozygote Trp/Nat1* | his3Δ::FRT, leu2Δ::FRT, trp1Δ::FRT, zrt2Δ::Nat1 | This work |
| URZ973 | zrt2 complement Trp/HygB | his3Δ::FRT, leu2Δ::FRT, trp1Δ::FRT, zrt2Δ::Nat1::ZRT2-HygB | This work |

### Candida auris

| Strain | Deletions | Reference | Notes |
| --- | --- | --- | --- |
| B11804 | Reference strain | *Candida auris* wild type Colombian isolate of the South American clade IV | (9) |
| URZ034 | cht3 homozygote Nat1* | cht3Δ::Nat1 | This work |
| URZ017 | cht3 complement HygB | cht3Δ::Nat1::Nat1::CHT3-HygB | This work |
| URZ036 | mp65 homozygote Nat1* | mp65Δ::Nat1 | This work |
| URZ013 | mp65 complement HygB | mp65Δ::Nat1::MP65-HygB | This work |
| URZ038 | sun41 homozygote Nat1* | sun41Δ::Nat1 | This work |
| URZ015 | sun41 complement HygB | sun41Δ::Nat1::SUN41-HygB | This work |
| URZ040 | tos1 homozygote Nat1* | tos1Δ::Nat1 | This work |
| URZ011 | tos1 complement HygB | tos1Δ::Nat1::TOS1-HygB | This work |
| URZ042 | zrt2 homozygote Nat1* | zrt2Δ::Nat1 | This work |
| URZ021 | zrt2 complement HygB | zrt2Δ::Nat1::ZRT2-HygB | This work |

*CD – Candida dubliniensis; CM – Candida maltosa*
5. Mancera E, Porman AM, Cuomo CA, Bennett RJ, & Johnson AD (2015) Finding a Missing Gene: EFG1 Regulates Morphogenesis in Candida tropicalis. G3 (Bethesda, Md.) 5(5):849-856.
6. Holland LM, et al. (2014) Comparative phenotypic analysis of the major fungal pathogens Candida parapsilosis and Candida albicans. PLoS Pathog 10(9):e1004365.
7. Anonymous (1996) Resource Sharing in Biomedical Research, eds Berns KI, Bond EC, & Manning FJ Washington (DC).
8. Schwarzmuller T, et al. (2014) Systematic phenotyping of a large-scale Candida glabrata deletion collection reveals novel antifungal tolerance genes. PLoS Pathog 10(6):e1004211.
9. Dominguez E, et al. (2018) Conservation and Divergence in the Candida Species Biofilm Matrix Mannan-Glucan Complex Structure, Function, and Genetic Control. mBio 9(2).
## Table S3. PCR primers used in this study

| Target Gene       | Primer Name           | Function          | Sequence                        |
|-------------------|-----------------------|-------------------|---------------------------------|
| **Candida tropicalis (CT) primers** |                       |                   |                                 |
| CT_CHT3 5' FLANK F | deletion              | GCAAATCTTCAAATCTAATCATC |
| CT_CHT3 5' FLANK R | deletion              | CACGGCGCGCCCTAGCAGCGG AGGATTATGAACTTAGCAAGCT |
| CT_CHT3 3' FLANK F | deletion              | GTCAAGCGCGCAGCATCCCTG GCCACCTGTGCACTACCCATTA |
| CT_CHT3 3' FLANK R | deletion              | TTGAATCTGATGACTGACCA |
| CT_CHT3 NESTED F  | deletion              | TAAATAAGCACGAGAACAACAA |
| CT_CHT3 NESTED R  | deletion              | TGATTTTCGTGCTAGCTGCCC |
| CT_CHT3 INTERNAL F| deletion              | TATCTGCTAGCATCTGTTGG |
| CT_CHT3 INTERNAL R| deletion              | TTTGATGGATGACGCTAGTA |
| CT_CHT3 UPSTREAM CHECK F | deletion | AGAACGTTGCACAATAATCATT |
| CT_CHT3 DOWNSTREAM CHECK R | deletion | GGTGTCAAAATAAACCGGAAT |
| CT_CHT3 compl 5' FLANK F/ORF | complementation | CGACTCAGTAAACCAGCATCA |
| CT_CHT3 compl 5' FLANK R/ORF | complementation | CACGGCGCGCCTAGCAGCGG TAAATGAATGGACAGGTGGG |
| CT_CHT3 compl 3' FLANK F | complementation | GTCAGCGCGCAGCATCCCTGC CAAGTGAATAATTGCATGTTTTG |
| CT_CHT3 compl 3' FLANK R | complementation | TTGGGAATGGTTTGATTTGGA |
| CT_CHT3 compl NESTED F | complementation | TCACAACATCCACAAAGAT |
| CT_CHT3 compl NESTED R | complementation | TTAATCGTGACTGACCA |
| CT_CHT3 compl UPSTREAM CHECK F | complementation | GGCATCCACAAATATCAG |
| CT_CHT3 compl UPSTREAM CHECK R | complementation | GCGGCCATCAAAATGTATG |
| CT_CHT3 compl DOWNSTREAM CHECK F | complementation | GTTTGGCTACTGGAAGCAGT |
| CT_CHT3 compl DOWNSTREAM CHECK R | complementation | ACCAAATGAACTGATGACCTG |
| **CT_MP65 5' FLANK F** | deletion              | TTCATCAGAGTCAACGCTAG |
| **CT_MP65 5' FLANK R** | deletion              | CACGGCGCGCCTAGCAGCGG AGGATTAGAAGGAAGACTGA |
| **CT_MP65 3' FLANK F** | deletion              | GTCAAGCGCGCAGCATCCCTGC GTTATCGTGATGACTGAA |
| **CT_MP65 3' FLANK R** | deletion              | TTGGGAATGGTTTGATTTGGA |
| **CT_MP65 NESTED F** | deletion              | TTTCAAGATTGGCGCTTGG |
| **CT_MP65 NESTED R** | deletion              | CAGGGATGATGTTCCAGTT |
| **CT_MP65 INTERNAL F** | deletion              | TCACAAAGACTACGTTGTT |
| **CT_MP65 INTERNAL R** | deletion              | GATTTGTGGTGTGGCTTGG |
| **CT_MP65 UPSTREAM CHECK F** | deletion              | AGACAGATGAGGAATACATTA |
| **CT_MP65 DOWNSTREAM CHECK R** | deletion              | ATTTCCGAGATTTCAATACATTAG |
| **CT_MP65 compl 5' FLANK F/ORF** | complementation | CGCAAAACATTACGTAACGAA |
| **CT_MP65 compl 5' FLANK R/ORF** | complementation | CACGGCGCGCCTAGCAGCGG TACAGATCCAGCGATAAC |
| **CT_MP65 compl 3' FLANK F** | complementation | GTCAGCGCGCAGCATCCCTGC TCCACTGTGTTATTTCCAA |
| **CT_MP65 compl 3' FLANK R** | complementation | ATATACTTCTCTCATCCATG |
| **CT_MP65 compl NESTED F** | complementation | GAATGTGACTGTTGAGG |
| **CT_MP65 compl NESTED R** | complementation | GGTAGTGCTGTGTTAGG |
| **CT_MP65 compl UPSTREAM CHECK F** | complementation | ACACACACATCCCTGTT |
| **CT_MP65 compl UPSTREAM CHECK R** | complementation | TGATGGGGCTAAATGTACGG |
| **CT_MP65 compl DOWNSTREAM CHECK F** | complementation | GCCATGTCATAAATCTCG |
| **CT_MP65 compl DOWNSTREAM CHECK R** | complementation | ATACACTTTTCTCATCCATG |
| **CT_SUN41 5' FLANK F** | deletion              | GGTATTTGTGTGTGGTG |
| **CT_SUN41 5' FLANK R** | deletion              | CACGGCGCGCCTAGCAGCGG ATAAAAGGAAACGACTAAGGT |
CT_SUN41 3' FLANK F deletion GTCAGCGGCCGATCCCTGCT GTTGCTGATACCTTTGCC
CT_SUN41 3' FLANK R deletion GCTATTGAAACTCCAGGCAC
CT_SUN41 NESTED F deletion TGTTGTCAGAAACACTAAGTGA
CT_SUN41 NESTED R deletion AATTTATGCTGATACCTTTGGA
CT_SUN41 INTERNAL F deletion TCAGCTGAAACTCAG
CT_SUN41 INTERNAL R deletion AACAACGAGACTAACCAC
CT_SUN41 DOWNTSTREAM CHECK F deletion TTCTCTTAAGTGTGTTTA
CT_SUN41 DOWNTSTREAM CHECK R deletion TCATTGGCTCTTGTCTTTCA
CT_SUN41 compl 5' FLANK F/ORF complementation ATTTGTTGTGTGTTGGTTG
CT_SUN41 compl 5' FLANK R/ORF complementation CACGGCGGCGCTAGACGCGG TCAGAAACATGATTCTA
CT_SUN41 compl 3' FLANK F complementation GTCAGCGGCCGCTCCCTGC TCCCCGACGTCTAGCTATGT
CT_SUN41 compl 3' FLANK R complementation TCAGCTGAAACTCAG
CT_TOS1 5' FLANK F deletion GAAAGATCAAATCACCAGCA
CT_TOS1 5' FLANK R deletion CACGGCGGCGCTAGACGCGG TCAGAAACATGATTCTA
CT_TOS1 3' FLANK F deletion GTCAGCGGCCGCTCCCTGC TCCCCGACGTCTAGCTATGT
CT_TOS1 3' FLANK R deletion TCAGCTGAAACTCAG
CT_TOS1 NESTED F deletion GTTTGAGCGGTGTATGAAAG
CT_TOS1 NESTED R deletion TCTTTACATTGTTCAATTGGTA
CT_TOS1 INTERNAL F deletion AGAAGCCACTGAAACTCAG
CT_TOS1 INTERNAL R deletion ACCAGATCCACTCAACCAGC
CT_TOS1 UPSTREAM CHECK F deletion ATTATTITGGCTGAGAAGAA
CT_TOS1 DOWNTSTREAM CHECK R deletion ATCCTTTAAGTGCATCCATTA
CT_TOS1 compl 5' FLANK F/ORF complementation CGACACTGACTCTACAT
CT_TOS1 compl 5' FLANK R/ORF complementation CACGGCGGCGCTAGACGCGG TCAGAAACATGATTCTA
CT_TOS1 compl 3' FLANK F complementation GTCAGCGGCCGCTCCCTGC TCCCCGACGTCTAGCTATGT
CT_TOS1 compl 3' FLANK R complementation TCTTTACATTGTTCAATTGGTA
CT_TOS1 compl NESTED F complementation AATCCTTTACACATCCCA
CT_TOS1 compl NESTED R complementation TCACCTTTGGATTTGCATTT
CT_TOS1 compl UPSTREAM CHECK F complementation AGATTAAGCGACGTAAAGTG
CT_TOS1 compl UPSTREAM CHECK R complementation CAATTCAACGCGTCTGTT
CT_TOS1 compl DOWNTSTREAM CHECK F complementation TATGTCTATGCAATGCTCAT
CT_TOS1 compl DOWNTSTREAM CHECK R complementation TGATGATGATGACAATG
CT_ZRT2 5' FLANK F deletion AGAAATGTCAAAGAGATGGGT
CT_ZRT2 5' FLANK R deletion CACGGCGGCGCTAGACGCGG TCAGAAACATGATTCTA
CT_ZRT2 3' FLANK F deletion GTCAGCGGCCGCTCCCTGC TCCCCGACGTCTAGCTATGT
CT_ZRT2 3' FLANK R deletion AATTTGAAACTCAG
CT_ZRT2 NESTED F deletion CCATCGAGTCGATGCAGA
CT_ZRT2 NESTED R deletion CCATCGAGTCGATGCA
CT_ZRT2 INTERNAL F deletion GTTTCTGGTGTATTGTGTTG
CT_ZRT2 INTERNAL R deletion GCACATAAGAATAAGCC
| Primer Name                          | Type                  | Sequence                        |
|------------------------------------|-----------------------|---------------------------------|
| CT_ZRT2 UPSTREAM CHECK F           | deletion              | ACAGGCACACAGAATAATACG          |
| CT_ZRT2 DOWNSTREAM CHECK R         | deletion              | ACTTGTTAAGAGATATTGACGA          |
| CT_ZRT2 compl 5’ FLANK F/ORF       | complementation       | GATCTTCCATTCACCAT              |
| CT_ZRT2 compl 5’ FLANK R/ORF       | complementation       | CACGGCGGCCTAGCAAGGGA            |
| CT_ZRT2 compl 3’ FLANK F           | complementation       | GTCAGCGGCCGATCCCTCG             |
| CT_ZRT2 compl 3’ FLANK R           | complementation       | AATCCGGGAACTCAAATT              |
| CT_ZRT2 compl NESTED F             | complementation       | TTGGTGGTATCGGGAATT              |
| CT_ZRT2 compl NESTED R             | complementation       | CCACTAGTTCAATGTCAAGG            |
| CT_ZRT2 compl UPSTREAM CHECK F     | complementation       | GACAAGGTAGTTTCCGAGAA            |
| CT_ZRT2 compl UPSTREAM CHECK R     | complementation       | GGGATGTATGGGCTAAATGT            |
| CT_ZRT2 compl DOWNSTREAM CHECK F   | complementation       | TATACGATGGTACTGCTTCC            |
| CT_ZRT2 compl DOWNSTREAM CHECK R   | complementation       | ACTTGTTAAGAGATATTGACGA          |
| CP_CHT3 5’ FLANK F                | deletion              | GTAAAGCTGAAAGACCGTG            |
| CP_CHT3 5’ FLANK R                | deletion              | CACGGCGGCCTAGCAAGGGGAAGATCAAATT|
| CP_CHT3 3’ FLANK F                | deletion              | GTCAGCGGCCGATCCCTCG             |
| CP_CHT3 3’ FLANK R                | deletion              | TTTGCAGCTTGGTTGTTTACT           |
| CP_CHT3 NESTED F                  | deletion              | TCACTCACCTCAAGTTT              |
| CP_CHT3 NESTED R                  | deletion              | TGAAACATTGCAAGCTAAA            |
| CP_CHT3 INTERNAL F                | deletion              | CACGTCAAGTGGTCAAGCTA           |
| CP_CHT3 INTERNAL R                | deletion              | CGCGAGATGGTATGATGAT             |
| CP_CHT3 UPSTREAM CHECK F           | deletion              | AAGTCATTTCGTTGTTTCGAC          |
| CP_CHT3 DOWNSTREAM CHECK R         | deletion              | TTATGAAACAAACTGAGATAAG         |
| CP_CHT3 compl 5’ FLANK F/ORF       | complementation       | GCTGAAAGACCGTGTAACACT          |
| CP_CHT3 compl 5’ FLANK R/ORF       | complementation       | CACGGCGGCCTAGCAAGGGGAGTAGTAAGCAT|
| CP_CHT3 compl 3’ FLANK F           | complementation       | GTCAGCGGCCGATCCCTCG             |
| CP_CHT3 compl 3’ FLANK R           | complementation       | TCACTAGTTCAATGTCAAGG           |
| CP_CHT3 compl NESTED F             | complementation       | TCTTCTCACCACCTCAAG             |
| CP_CHT3 compl NESTED R             | complementation       | TTGCGCTGTTTGTGTTTACT           |
| CP_CHT3 compl UPSTREAM CHECK F     | complementation       | GACACCATTTCGATGGTTCA           |
| CP_CHT3 compl UPSTREAM CHECK R     | complementation       | GTGGATCAACTGGAACCTCTT          |
| CP_CHT3 compl DOWNSTREAM CHECK F   | complementation       | TTCCTGCTGCGAGACCTG             |
| CP_CHT3 compl DOWNSTREAM CHECK R   | complementation       | ACTATGGTGTGGAATTTGCAAT         |
| CP_MP65 5’ FLANK F                | deletion              | AATCTTGAAACAAGACCC             |
| CP_MP65 5’ FLANK R                | deletion              | CACGGCGGCCTAGCAAGGGGTAAGTAGGTAGGG |
| CP_MP65 3’ FLANK F                | deletion              | GTCAGCGGCCGATCCCTCG             |
| CP_MP65 3’ FLANK R                | deletion              | AAAGATGGAAGGGAAGATT             |
| CP_MP65 NESTED F                  | deletion              | TGAACAAGATTTGATGCAGA           |
| CP_MP65 NESTED R                  | deletion              | ACTGAAACACCTCAATCAAG           |
| CP_MP65 INTERNAL F                | deletion              | CTACCACTTGGAGGCTTA            |
| CP_MP65 INTERNAL R                | deletion              | ATCAGCTTTCCCAAGATCAT          |
| CP_MP65 UPSTREAM CHECK F           | deletion              | GAAGATAAATTTCCACATCGT         |
| CP_MP65 DOWNSTREAM CHECK R         | deletion              | TTTAAAATCCAGACAGTGA           |
| CP_MP65 compl 5’ FLANK F/ORF       | complementation       | AATCTTGAAACAAGACCAAC          |
| CP_MP65 compl 5’ FLANK R/ORF       | complementation       | CACGGCGGCCGCTAGCAAGGGGTCAGAAGCTCAT |
CP_MP65 compl 3' FLANK R complementation AAAGATGGAGGGAAAGCATT
CP_MP65 compl NESTED F complementation CAAGTGGAAGATAAATTTCACCA
CP_MP65 compl NESTED R complementation ACCTAATCCAGTGAGATGTA
CP_MP65 compl UPSTREAM CHECK F complementation TATGTGCTTTCGTTAGCA
CP_MP65 compl UPSTREAM CHECK R complementation GTACCACTGAACTCTCCTCA
CP_MP65 compl DOWNSTREAM CHECK F complementation GTATGAGAATCCGATGATGTA
CP_MP65 compl DOWNSTREAM CHECK R complementation GGTCATGGGTATTGATGTA
CP_SUN41 5' FLANK F deletion AAGAGCGGACAAACCAAAAA
CP_SUN41 5' FLANK R deletion CACGGCGGCCTAGCCAGCAGAAATGTTTGACTCGGGGA
CP_SUN41 3' FLANK F deletion GTAGCGGCAGCCTCCCTGCCTTCTTTAGATGTGTGTGTTGT
CP_SUN41 3' FLANK R deletion TCATATCGGCAGCTAATTCC
CP_SUN41 NESTED F deletion TCAATACACTCACTGGACAC
CP_SUN41 NESTED R deletion CTTTTGCAGCTTGGGATAC
CP_SUN41 INTERNAL F deletion TGTCAAGAACCAGTTTTCCA
CP_SUN41 INTERNAL R deletion GTAGCCGATACAAAGATTTCA
CP_SUN41 UPSTREAM CHECK F deletion AAGAGTTAACAACCAGCAGA
CP_SUN41 UPSTREAM CHECK R deletion GTGGATCAACTGGAACTTCT
CP_SUN41 DOWNSTREAM CHECK F deletion CCACTGAGGTTCTTCTTTCA
CP_SUN41 DOWNSTREAM CHECK R deletion TCATATCGGCAGCTAATTCC
CP_TOS1 5' FLANK F deletion GGCAAATGACTCGATCTAGT
CP_TOS1 5' FLANK R deletion CACGGCGGCCTAGCCAGCAGAAATGTTTGACTCGGGGA
CP_TOS1 3' FLANK F deletion GTACCGGCAGCCTCCCTGCCTTCTTTAGATGTGTGTGTTGT
CP_TOS1 3' FLANK R deletion ACCACTTGAACACAGAAGA
CP_TOS1 NESTED F deletion GCGACTTACATCACAATGTT
CP_TOS1 NESTED R deletion CTTTTGCAGCTTGGGATAC
CP_TOS1 INTERNAL F deletion TTGTTGTGTTTGGATGAAGC
CP_TOS1 INTERNAL R deletion TTGAAGTTGCTCCAGAACAT
CP_TOS1 UPSTREAM CHECK F deletion TTAAACTTGTCGATCTCTTAC
CP_TOS1 DOWNSTREAM CHECK R deletion CTAGGTGGAATAAACAAGTAG
CP_TOS1 compl 5' FLANK F/ORF complementation GCGCAAATGACTCGATCTAGT
CP_TOS1 compl 5' FLANK R/ORF complementation CACGGCGGCCTAGCCAGCAGAAATGTTTGACTCGGGGA
CP_TOS1 compl 3' FLANK F complementation GTACCGGCAGCCTCCCTGCCTTCTTTAGATGTGTGTGTTGT
CP_TOS1 compl 3' FLANK R complementation CTGACCAAACTCGTACAAGTGGC
CP_TOS1 compl NESTED F complementation TCCCTCAACTACCTCCTTA
CP_TOS1 compl NESTED R complementation GGAATCTTTAGCAATACGG
CP_TOS1 compl UPSTREAM CHECK F complementation AATCAGACAGTGAGCTTCTG
CP_TOS1 compl UPSTREAM CHECK R complementation GGACAATTCACGCGCTT
CP_TOS1 compl DOWNSTREAM CHECK F complementation TTGAAGTTGCTCCAGAACAT
| Primer Name | Function | Mutation Type | Sequence |
|-------------|----------|---------------|----------|
| CP_TOS1 compl DOWNSTREAM CHECK R | complementation | deletion | TTTGGCAAATAATCGTTGCATG |
| CP_ZRT2 5' FLANK F | deletion | CAACGGGCAGCCATAGCAGGG AATTGAAATTGCTGATATGGTAT |
| CP_ZRT2 3' FLANK F | deletion | GTCAGGCGGCCATCCCTGC ACAATAGAATCATAATCAGG |
| CP_ZRT2 3' FLANK R | deletion | TGAAGGTGTCATGTGATAG |
| CP_ZRT2 NESTED F | deletion | TAGGGCAAAATTTTGTTGAG |
| CP_ZRT2 NESTED R | deletion | CCGTCAATGAAGCTGAATGG |
| CP_ZRT2 INTERNAL F | deletion | TGGAAAGGTGTCATGTGATAG |
| CP_ZRT2 INTERNAL R | deletion | ATCTTACCATGAAAGCCAT |
| CP_ZRT2 UPSTREAM CHECK F | deletion | CCAGAGTACTTTAATTAAAC |
| CP_ZRT2 DOWNSTREAM CHECK R | deletion | TCATGGATATGGCTCTATAG |
| CP_ZRT2 compl 5' FLANK F/ORF | complementation | deletion | CAGGTTGTGCAGTCGTTGAG |
| CP_ZRT2 compl 5' FLANK R/ORF | complementation | deletion | CACGGCAGGCATCCCTGC ACAATAGAATCATAATCAGG |
| CP_ZRT2 compl 3' FLANK F | complementation | deletion | ATCTTACCATGAAAGCCAT |
| CP_ZRT2 compl 3' FLANK R | complementation | deletion | CCAGGCTTTATGAAATGG |
| CP_ZRT2 compl NESTED F | complementation | deletion | TGIGAAGGTGTCATGTGATAG |
| CP_ZRT2 compl NESTED R | complementation | deletion | ATCTTACCATGAAAGCCAT |
| CP_ZRT2 compl UPSTREAM CHECK F | complementation | deletion | CCAGGCTTTATGAAATGG |
| CP_ZRT2 compl UPSTREAM CHECK R | complementation | deletion | TCATGGATATGGCTCTATAG |
| CP_ZRT2 compl DOWNSTREAM CHECK F | complementation | deletion | TCATGGATATGGCTCTATAG |
| CP_ZRT2 compl DOWNSTREAM CHECK R | complementation | deletion | TCATGGATATGGCTCTATAG |

**Candida glabrata (CG) primers**

| Primer Name | Function | Mutation Type | Sequence |
|-------------|----------|---------------|----------|
| CG_CHT3 5' FLANK F | deletion | AACAAAACAGGATCAAAACAAAT |
| CG_CHT3 5' FLANK R | deletion | CACGGGCGGCGCTGCAAGG GGGTGTATTGACGCGCATAA |
| CG_CHT3 3' FLANK F | deletion | GTCAGGCGGCCGCTCCCTGC GGTTGATATTGACGCGCATAA |
| CG_CHT3 3' FLANK R | deletion | AGTGAAGATTGAAACGTTAAT |
| CG_CHT3 NESTED F | deletion | CCAGGCTTTATGAAATGG |
| CG_CHT3 NESTED R | deletion | TGGTTAACAATAGTGCCTT |
| CG_CHT3 INTERNAL F | deletion | GTGCTATTATGAAATTG |
| CG_CHT3 INTERNAL R | deletion | TGAAGGTGTCATGTGATAG |
| CG_CHT3 5' FLANK F | deletion | TTAACCGACACTGGCTAAAT |
| CG_CHT3 5' FLANK R | deletion | TGAAGGTGTCATGTGATAG |
| CG_CHT3 3' FLANK F | deletion | GATGGGAATTACCTGGCTCTG |
| CG_CHT3 3' FLANK R | deletion | GACTATAAGGTGCTAAGGAG |
| CG_CHT3 compl 5' FLANK F/ORF | complementation | deletion | AACAAGATCAAAGGATCAGCAG |
| CG_CHT3 compl 5' FLANK R/ORF | complementation | deletion | CACGGGCGGCGCTGCAAGG GGGTGTATTGACGCGCATAA |
| CG_CHT3 compl 3' FLANK F | complementation | deletion | GTCAGGCGGCCGCTCCCTGC GGTTGATATTGACGCGCATAA |
| CG_CHT3 compl 3' FLANK R | complementation | deletion | ACCTGAAGACTTTAGACCCAG |
| CG_CHT3 compl NESTED F | complementation | deletion | ACCTGAAGACTTTAGACCCAG |
| CG_CHT3 compl NESTED R | complementation | deletion | TGCAAACTACGAAAGGATAG |
| CG_CHT3 compl UPSTREAM CHECK F | complementation | deletion | GACGACACTGGCTAAGGAG |
| CG_CHT3 compl UPSTREAM CHECK R | complementation | deletion | AGTTTTCCTCGTAAAGCAGC |
| CG_CHT3 compl DOWNSTREAM CHECK F | complementation | deletion | GACGACACTGGCTAAGGAG |
| CG_CHT3 compl DOWNSTREAM CHECK R | complementation | deletion | GACGACACTGGCTAAGGAG |

| Primer Name | Function | Mutation Type | Sequence |
|-------------|----------|---------------|----------|
| CG_MP65 5' FLANK F | deletion | AGATTTCCTCTGGTAGACAGC |
| CG_MP65 5' FLANK R | deletion | CACGGGCGGCGCTGCAAGG GGGTGTATTGACGCGCATAA |
CG_MP65 3' FLANK F deletion GTCAGGGCAGCATCCCTGC CTTTCTTGTAGTCAAAGTTATGA
CG_MP65 3' FLANK R deletion ATACAATTTGATTCTCTGTAGCC
CG_MP65 NESTED F deletion ATCGAAGCTTCTCTGGTATTTA
CG_MP65 NESTED R deletion AGTTACTTCTCAGGGAATTA
CG_MP65 INTERNAL F deletion GTTTTTCACTGTTGTAAGGG
CG_MP65 INTERNAL R deletion AATGTATGGAATTAGAGGT
CG_MP65 5' FLANK F deletion TAAATAGGGTTCTTCTAATGAG
CG_MP65 5' FLANK R deletion GGGTAGGTTCTTGTAAAGCT
CG_MP65 3' FLANK F deletion CATTTATGTTATATCGGTGA
CG_MP65 3' FLANK R deletion TCAATGTAGAACTAGAGG
CG_MP65 compl 5' FLANK F/ORF complementation AGATTTCTCTCTGAGACCCCTT
CG_MP65 compl 5' FLANK R/ORF complementation CACGGGGGCCCAGGCTACAATTAGCTTCTGTAGGCA
CG_MP65 compl 3' FLANK F complementation GTCAGGGGCCATCCCTGC
CG_MP65 compl 3' FLANK R complementation CCTATCACTGGAATTATGGGAC
CG_MP65 compl NESTED F complementation TGTCTGATATCTGTGCAGCCA
CG_MP65 compl NESTED R complementation AGTTGCAGTTTACAGATGAGGA
CG_MP65 compl UPSTREAM CHECK F complementation TCCGAGGTAAATCGAGATGAGGA
CG_MP65 compl UPSTREAM CHECK R complementation AAGAAGGAAGAAGAAGA
CG_MP65 compl DOWNSTREAM CHECK F complementation AGACTTAAATAATAGGACCCAAG
CG_MP65 compl DOWNSTREAM CHECK R complementation AATTAGGAGTTCACATACTGTT
CG_SUN41 5' FLANK F deletion CCTACCAATCAAATCGAGTTTT
CG_SUN41 5' FLANK R deletion CATCTATCTTCTTTGTGTTCG
CG_SUN41 3' FLANK F deletion GTCAGGGCAGCATCCCTGC
CG_SUN41 3' FLANK R deletion AGGTGTACATCTTTCGACAAAA
CG_SUN41 NESTED F deletion GTTTTGCCCTAATTCAGTCATC
CG_SUN41 NESTED R deletion TACATGGAAATGCAAAGCTAAG
CG_SUN41 INTERNAL F deletion TCATCATCATCATCTCCATCTC
CG_SUN41 INTERNAL R deletion CATTTTCGTAGGAAACATCCAC
CG_SUN41 5' FLANK F deletion TTGCCACACTATGAAAATGAAA
CG_SUN41 5' FLANK R deletion CTTTTGTGCTGGGAGGATTT
CG_SUN41 3' FLANK F deletion AGTCTTAATTGCATGTCCATCA
CG_SUN41 3' FLANK R deletion TGTTCCGACAGTTTTACGTAG
CG_TOS1 5' FLANK F deletion TTGGTGTGTGGTACTGAAA
CG_TOS1 5' FLANK R deletion TTTTCTGGGCTTTGTTCAAAG
CG_TOS1 3' FLANK F deletion CAGGGGCGGCCTAGCAGCAGGCGATTAGATGGTTGTGATG
CG_TOS1 3' FLANK R deletion GTGTTGTGATTCTAGACGATTGA
CG_TOS1 NESTED F deletion TCTCATTCTGTGAGTCTCTG
CG_TOS1 NESTED R deletion AGTGAGAGAGAGTAAATCGGTT
CG_TOS1 INTERNAL F deletion GAGGTCTCTTAAACTATCCAA
CG_TOS1 INTERNAL R deletion TTAGCACTTCAATGATT
CG_TOS1 5' FLANK F deletion AGTTTTGTGTTGTAGTCTAGCAA
CG_TOS1 5' FLANK R deletion CCACTAAACACTAAACACTAACTT
CG_TOS1 3' FLANK F deletion CCGCTAACACCTAACACTAAACACTT
CG_TOS1 3' FLANK R deletion AAGATGACCAACTACACTAACTAATG
CG_TOS1 compl 5' FLANK F/ORF complementation GCCCTTCAGTGGTTGTG
CG_TOS1 compl 5' FLANK R/ORF complementation CAGGGGCGGCCTAGCAGCAGGCGAAAGAAGGTTG
CG_TOS1 compl 3' FLANK F | complementation | GTCAGCGGCGCGATCCCTGCA ACCAACCCTTGCGCTAACAA
CG_TOS1 compl 3' FLANK R | complementation | TGCATACAAAAATGGCCAAAT
CG_TOS1 compl NESTED F | complementation | CGGTTTGTGCTGGGAGGAG
CG_TOS1 compl NESTED R | complementation | GCGGCCATTTGATGTTGAGA
CG_TOS1 compl UPSTREAM CHECK F | complementation | GATGCGGCCGCAACTTCGTT
CG_TOS1 compl UPSTREAM CHECK R | complementation | AAATGGATTGTGGCCTTCG
CG_TOS1 compl DOWNSTREAM CHECK F | complementation | GACCTAAAAATGCAACCCAGAG
CG_TOS1 compl DOWNSTREAM CHECK R | complementation | CAGTTCTGATGCTGGTGTTGC

CG_ZRT2 5' FLANK F | deletion | AGTCTTTCTTTCTATACCT
CG_ZRT2 5' FLANK R | deletion | CACGGCGGCCCTAGCAAGCC ATCACTCCTGAAACCAAAGATT
CG_ZRT2 3' FLANK F | deletion | GTCAAGGCCGATCCCTGC TGGATCGATGAAAATGCTCT
CG_ZRT2 3' FLANK R | deletion | ACCAATTCTTGATGTTGAGA
CG_ZRT2 NESTED F | deletion | CTTTATGAACTCCCTATCTT
CG_ZRT2 NESTED R | deletion | TGAATATGGTAAATAGTTGCTG
CG_ZRT2 INTERNAL F | deletion | TCTCAAAATTCAGTTTTCT
CG_ZRT2 INTERNAL R | deletion | TAGCATACCTCAGCTATC
CG_ZRT2 5' FLANK F | deletion | ACCAACCCTATACCTGCAAT
CG_ZRT2 5' FLANK R | deletion | GTCTAGTAGCTCACCTGTTAAT
CG_ZRT2 3' FLANK F | deletion | CTCAGAAACATGTTAACAGCAT
CG_ZRT2 3' FLANK R | deletion | ACCCGAGAAAAATACCTTTTTA
CG_ZRT2 compl 5' FLANK F/ORF | complementation | CATCAACTTTCTTACCGCATA
CG_ZRT2 compl 5' FLANK R/ORF | complementation | CACGGCGGCCCTAGCAAGCC ATCACTCCTGAAACCAAAGATT
CG_ZRT2 compl 3' FLANK F | complementation | GTCAAGGCCGATCCCTGCTGTCTTTCGAAA
CG_ZRT2 compl 3' FLANK R | complementation | ACCAATTCTTGATGTTGAGA
CG_ZRT2 compl NESTED F | complementation | AAGTGTTGCTGAAAGACGAG
CG_ZRT2 compl NESTED R | complementation | TCTTTAAAGGCTGTAGAGA
CG_ZRT2 compl UPSTREAM CHECK F | complementation | AGAATGCACAAGGATGAG
CG_ZRT2 compl UPSTREAM CHECK R | complementation | TGAAGTGACGCTGGAAAGTACG
CG_ZRT2 compl DOWNSTREAM CHECK F | complementation | AGGATCCGAGTGAAATCTCTG
CG_ZRT2 compl DOWNSTREAM CHECK R | complementation | AGGTTGGTTGACAGCAGACT

Candida auris (CR) primers

CR_CHT3 5' FLANK F | deletion | GGTATGTAATGCTCGCAA
CR_CHT3 5' FLANK R | deletion | CAGCGGCCGCGCTACGAGAGG AATTGTTGATCAAAGGGTGTCG
CR_CHT3 3' FLANK F | deletion | GTCAAGGCCGATCCCTGC TGGATCGATGAAAATGCTCT
CR_CHT3 3' FLANK R | deletion | GACCATGATGAGGAGCATC
CR_CHT3 NESTED F | deletion | CACTCTGTGCGATAATTTG
CR_CHT3 NESTED R | deletion | ACATCTTTGCAATGTTTGA
CR_CHT3 INTERNAL F | deletion | ACTCTTTGGCTCTCTGTT
CR_CHT3 INTERNAL R | deletion | CAGAAGTAGAGGTGATGTCG
CR_CHT3 5' FLANK F | deletion | GCCTCTCTTTTACCAATT
CR_CHT3 5' FLANK R | deletion | ACCACTAAAGGAGAAACG
CR_CHT3 3' FLANK F | deletion | GATTGCTGCCGCTAATT
CR_CHT3 3' FLANK R | deletion | ATATCGTAAGTGAG

CR_CHT3 compl 5' FLANK F/ORF | complementation | TGGGCGGCTCTTTACCAATT
CR_CHT3 compl 5' FLANK R/ORF | complementation | CAGCGGCCGCGCTACGAGAGG AATTGTTGATCAAAGGGTGTCG
CR_CHT3 compl 3' FLANK F | complementation | GTCAAGGCCGCGCTACGAGAGG AATTGTTGATCAAAGGGTGTCG
| CR_CHT3 compl 3' FLANK R | complementation | ATCGTACTTGACTTGGCCCA |
|--------------------------|----------------|-----------------------|
| CR_CHT3 compl NESTED F   | complementation | TTAGTGCCCTCAGTATTGACATCA |
| CR_CHT3 compl NESTED R   | complementation | GCTTAGATCTGGCTAGG |
| CR_CHT3 compl UPSTREAM CHECK F | complementation | TTTGCAACCTCAGCCACGC |
| CR_CHT3 compl UPSTREAM CHECK R | complementation | CATTTAAATGTATTTGGCTTTT |
| CR_CHT3 compl DOWNSTREAM CHECK F | complementation | AGACTATAAAATAGCCACCA |
| CR_CHT3 compl DOWNSTREAM CHECK R | complementation | CGCATTACCTGAGCTCAT |
| CR_MP65 5' FLANK F       | deletion        | CTTTTCGAGTGTTCAGAA |
| CR_MP65 5' FLANK R       | deletion        | CACGGGCGGCCTAGCAGCGG |
| CR_MP65 3' FLANK F       | deletion        | GTCAGGGGCGGCATCCTGG TAAATTTGCTAGG |
| CR_MP65 NESTED F         | deletion        | AAGGTTATCACCTGTTTTT |
| CR_MP65 NESTED R         | deletion        | AGGTAAGAAAGCTGAGG |
| CR_MP65 INTERNAL F       | deletion        | AACTGGAACACTGTTGTT |
| CR_MP65 INTERNAL R       | deletion        | TGGCAGAGGATAGTCAAGA |
| CR_MP65 5' FLANK F       | deletion        | ATCTGATAAAACACCCACC |
| CR_MP65 5' FLANK R       | deletion        | AAAACAGGCCAGATTGTTT |
| CR_MP65 3' FLANK F       | deletion        | TTTGTCGAGTGGTTTT |
| CR_MP65 3' FLANK R       | deletion        | CGCATTACCTGAGCTCAT |
| CR_MP65 compl 5' FLANK F | complementation | CTTTTCGAGTGTTCAGAA |
| CR_MP65 compl 5' FLANK R | complementation | CACGGGCGGCCTAGCAGCGG |
| CR_MP65 compl 3' FLANK F | complementation | GTCAAGGCGGCCATCCTGG TAAATTTGCTAGG |
| CR_MP65 compl 3' FLANK R | complementation | TAACAACCTATCAAAGCGG |
| CR_MP65 compl NESTED F   | complementation | ATCTGATAAAACACCCACC |
| CR_MP65 compl NESTED R   | complementation | CTTGCTCATTTGTTGTTT |
| CR_MP65 compl UPSTREAM CHECK F | complementation | GGGCAAATTTGGAATCTT |
| CR_MP65 compl UPSTREAM CHECK R | complementation | TGGATTTGGCTTTGCTAT |
| CR_MP65 compl DOWNSTREAM CHECK F | complementation | CACCCAAGGCAATTCTATA |
| CR_MP65 compl DOWNSTREAM CHECK R | complementation | GCCAACGCTACCTTTTATA |
| CR_SUN41 5' FLANK F      | deletion        | CAGAATCTCTGGGGCTTCTT |
| CR_SUN41 5' FLANK R      | deletion        | CAGCAGCAGCCTAGCAGCGG |
| CR_SUN41 3' FLANK F      | deletion        | GTCAAGGCGGCCATCCTG |
| CR_SUN41 3' FLANK R      | deletion        | TTTACGTTGTGGTAGTTGA |
| CR_SUN41 NESTED F        | deletion        | CCTGCGAGGTTGGAATA |
| CR_SUN41 NESTED R        | deletion        | GAGAAGCATCAGG |
| CR_SUN41 INTERNAL F      | deletion        | GCAGTAGACTGTCTACT |
| CR_SUN41 INTERNAL R      | deletion        | CAAACGTCTGACGGAAG |
| CR_SUN41 5' FLANK F      | deletion        | GGGTAGAATTTGTTGGAAC |
| CR_SUN41 5' FLANK R      | deletion        | CCACGAGATAGCAAGGT |
| CR_SUN41 3' FLANK F      | deletion        | GATACAAATACGTAGCAAG |
| CR_SUN41 3' FLANK R      | deletion        | ACTGTTTTGCAGCTGTTACT |
| CR_SUN41 compl 5' FLANK F | complementation | TCCCCCTAGTTGGTTCA |
| CR_SUN41 compl 5' FLANK R | complementation | CAGGCGCGCCCTAGCAGCGG |
| CR_SUN41 compl 3' FLANK F | complementation | GTCAAGGCGGCCATCCTG |
| CR_SUN41 compl 3' FLANK R | complementation | TTGCTTTGTTGCTCAT |
| CR_SUN41 compl NESTED F  | complementation | AACAACGACACTTTTGCAAAT |
| CR_ZRT2 compl UPSTREAM CHECK R | complementation | TGGATTTGGCTTTCGGTAT |
|---------------------------------|-----------------|----------------------|
| CR_ZRT2 compl DOWNSTREAM CHECK F | complementation  | CCACCAAGGCATTTCTATA  |
| CR_ZRT2 compl DOWNSTREAM CHECK R | complementation  | AAAGACCAAGTATATCAATCT |
**Dataset S1.** Candida albicans extracellular vesicle proteome.

**Dataset S2.** Candida tropicalis extracellular vesicle proteome.

**Dataset S3.** Candida parapsilosis extracellular vesicle proteome.

**Dataset S4.** Candida glabrata extracellular vesicle proteome.

**Dataset S5.** Candida auris extracellular vesicle proteome.