

## Supplementary Material

### ***Candida albicans* and *Candida glabrata* triosephosphate isomerase—a moonlighting protein that can be exposed on the candidal cell surface and bind to human extracellular matrix proteins**

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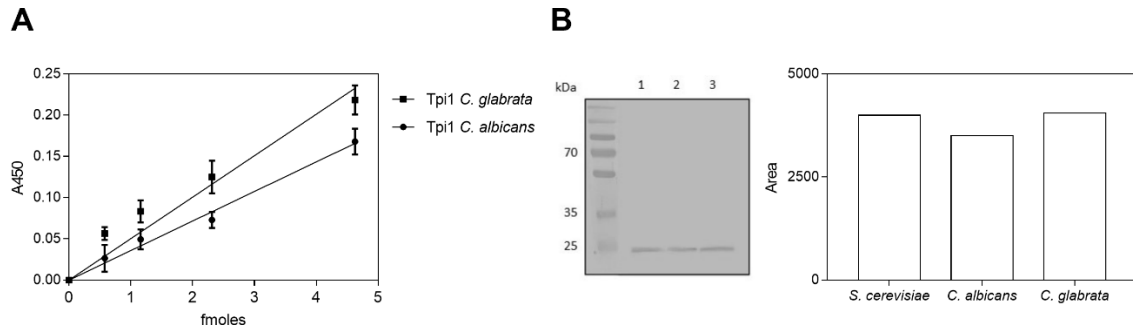
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**Table S1. List of peptide matches obtained with the Mass Matrix server.**

In the sequences identified, amino acids were underlined for which mass shifts due to the sulfo-SDAD-derived link are indicated.

| Peptide                                                                  | Charge | Score | pp value | pp2 value |
|--------------------------------------------------------------------------|--------|-------|----------|-----------|
| <sup>4</sup> QFFVGGNFKANGTK <sub>17</sub>                                | +3     | 46.4  | 10.3     | 7.6       |
| <sup>224</sup> ANVDGFLV <u>G</u> GASLK <u>P</u> EFVDIIKSR <sub>247</sub> | +3     | 70.4  | 16.3     | 14.1      |

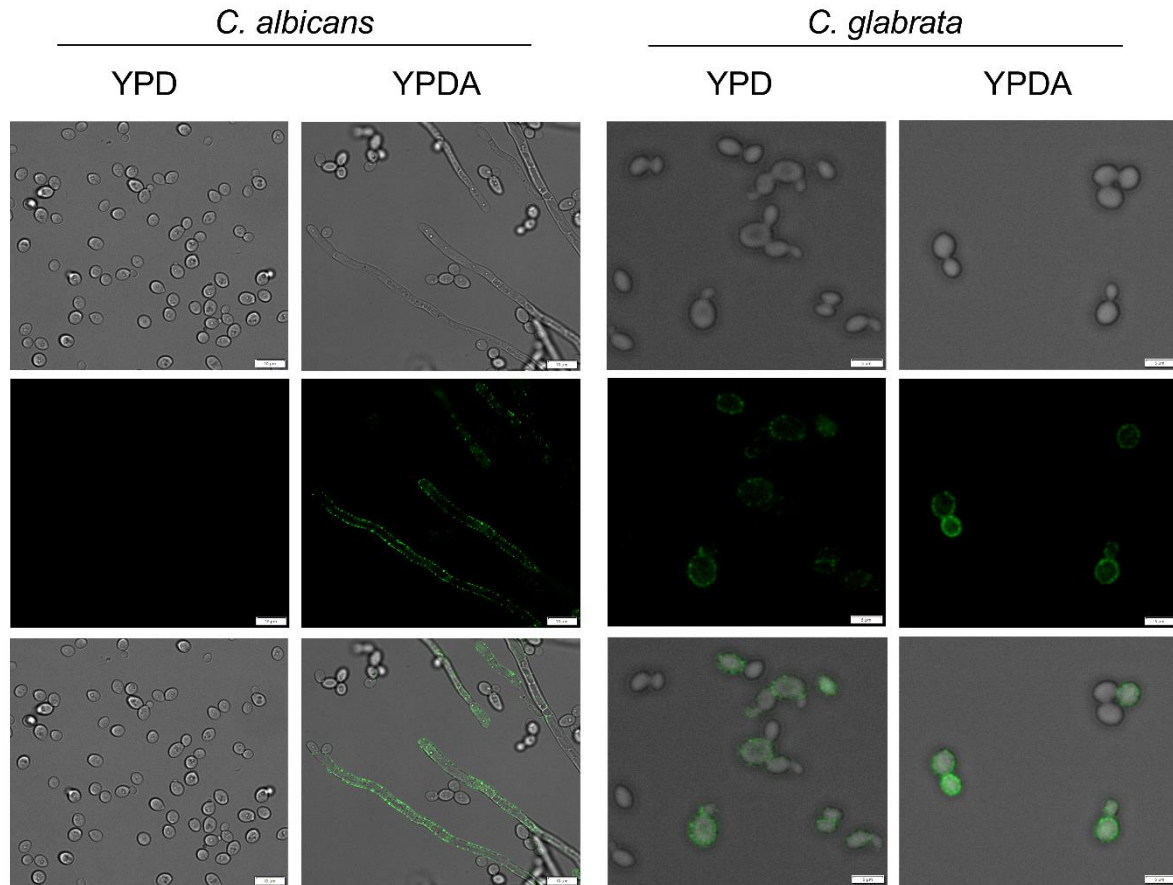


**Figure S1. The analysis of cross-reactivities of anti-*S. cerevisiae* Tpi1 antibody to *C. albicans* and *C. glabrata* Tpi1.**

(A) A calibration curve for microplate assays. Microplate wells were coated with Tpi1 at amounts in a range of 0.5–4.5 fmoles (50  $\mu$ L of protein solution in PBS) in an overnight incubation at 4°C. After each step of the following assay, the wells were washed three times with 200  $\mu$ L PBS, pH 7.4, containing 1% BSA. The unoccupied microplate well surface was blocked by incubation with 200  $\mu$ L of 3% BSA in PBS for 1 h at 37°C. After addition of 50  $\mu$ L of anti-yeast Tpi1 antibody (1  $\mu$ g/mL), the plates were incubated for 1 h at 37°C. Next, 50  $\mu$ L of HRP-conjugated secondary antibody were added, for further plate incubation at 37°C for 1 h and color development with SA-HRP/TMB system.

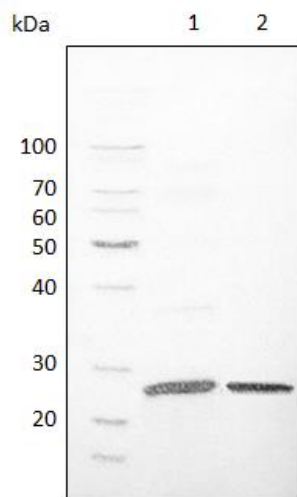
(B) A calibration of western-blotting experiments. Equal amounts (0.3  $\mu$ g) of purified Tpi1 were applied to the gel wells and separated by SDS-PAGE in the Laemmli system under reducing conditions. The protein bands were then transferred to a PVDF membrane and probed with primary anti-*S. cerevisiae* Tpi1 antibodies and an alkaline phosphatase-labelled secondary antibody. Protein bands were visualized using a BCIP/NBT system. The uncropped scan of the blot is presented (panel left). Lane 1, 0.3  $\mu$ g of *S. cerevisiae* Tpi used as a control; lane 2, 0.3  $\mu$ g of *C. albicans* Tpi1; lane 3, 0.3  $\mu$ g of *C. glabrata* Tpi1. The amount of Tpi1 recognized by anti-*S. cerevisiae* Tpi antibodies were determined by densitometric analysis

using ImageJ software (panel right). The obtained results were used to normalize the results with the use of anti-*S. cerevisiae* Tpi antibodies.



**Fig. S2. Identification of Tpi1 on the surface of *C. albicans* and *C. glabrata* cells, grown in YPD and YPDA medium.**

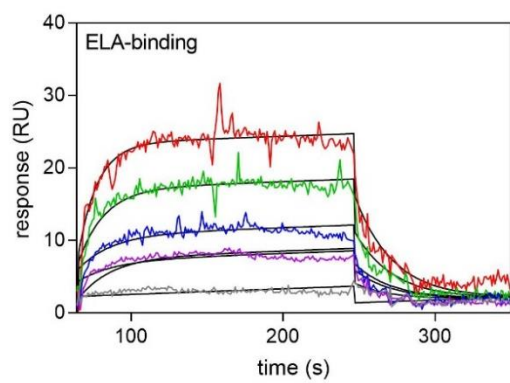
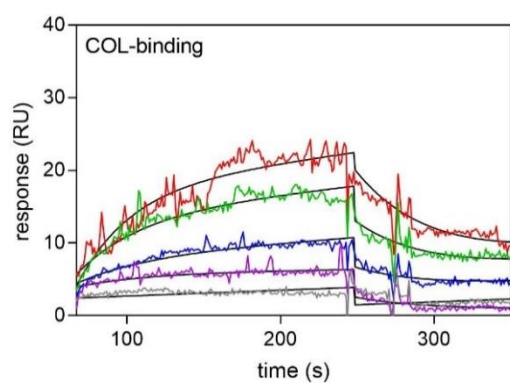
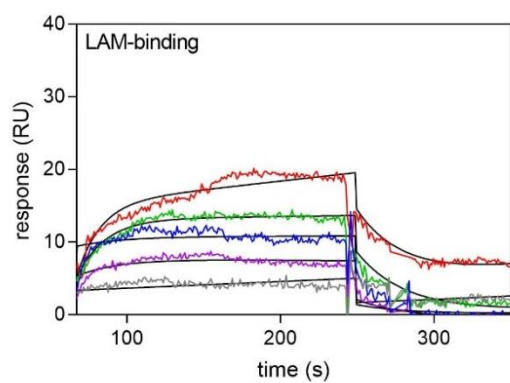
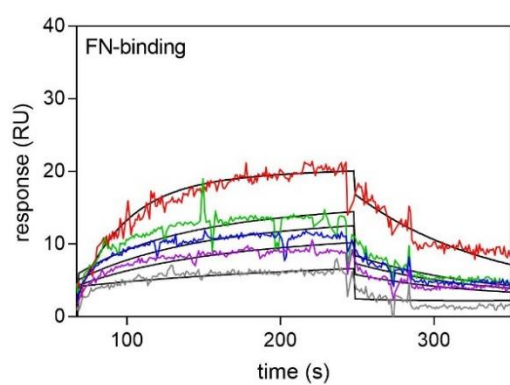
*C. albicans* yeast-like cells, grown for 16 h in YPD medium at 30°C and then transferred to Eppendorf tubes ( $3 \times 10^7$  cells per tube) or transferred to the wells of a glass-bottom 96-well plate coated with poly-L-lysine ( $10^4$  cells per well) and incubated in YPDA medium at 37°C overnight. *C. glabrata* yeast-like cells were grown overnight in YPD or YPDA medium in a glass flask and then transferred to Eppendorf tubes ( $3 \times 10^7$  cells per tube). The presence of surface-exposed Tpi1 was detected using rabbit polyclonal anti-Tpi antibody (1 µg/mL) and an Alexa Fluor 488 fluorescently labeled anti-rabbit secondary antibody (1 µg/mL). Photo of bright field (top row) and green fluorescence (middle row) were taken using an Olympus IX73 microscope. The bottom row shows the overlapped images.



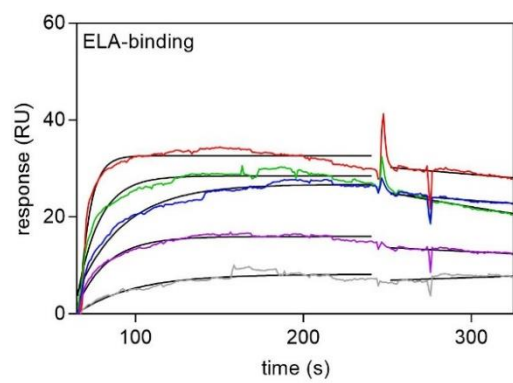
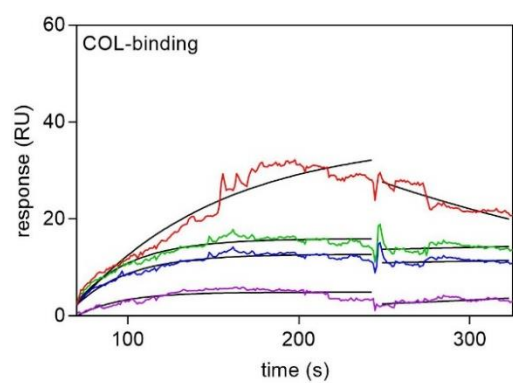
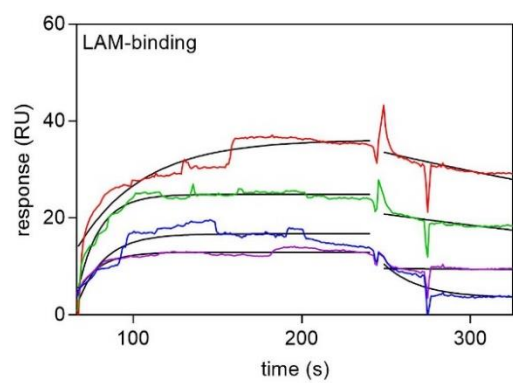
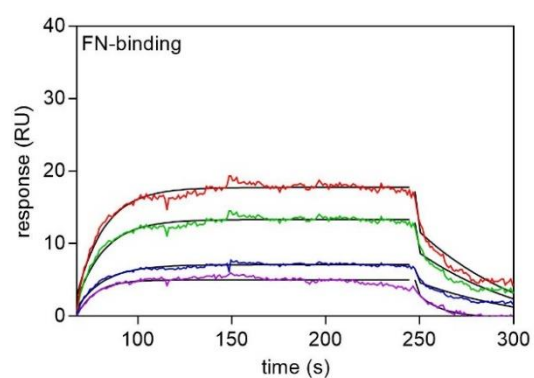
**Figure S3. SDS-PAGE analysis of purified Tpi1 from *C. albicans* and *C. glabrata*.**

Purified Tpi1 samples from *C. albicans* and *C. glabrata* were analysed with the Laemmli system using a 12% gel under reducing conditions. After electrophoresis, the protein bands were visualized by silver staining. The uncropped scan of the gel is presented. Molecular markers are shown in the leftmost lane. Lane 1, purified *C. albicans* Tpi1; lane 2, purified *C. glabrata* Tpi1.

### *C. albicans* Tpi1



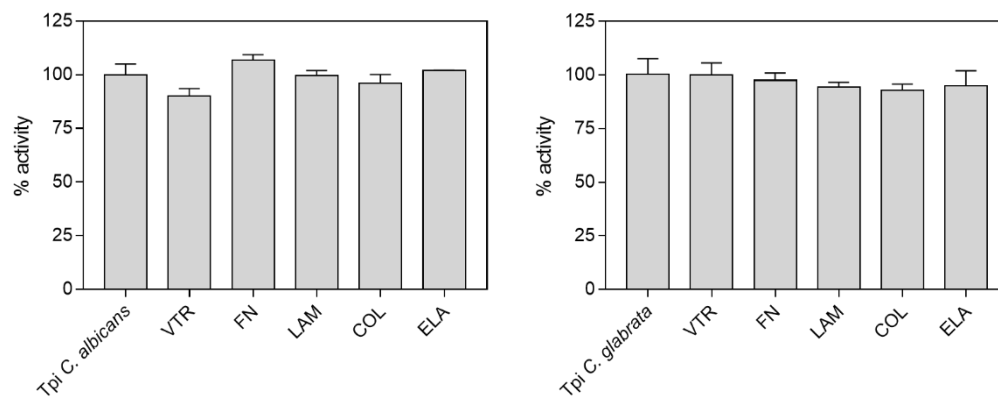
### *C. glabrata* Tpi1



Concentration of ECM protein: — 50 nM — 100 nM — 250 nM — 500 nM — 750 nM

**Figure S4. SPR sensograms for the interactions of *C. albicans* and *C. glabrata* Tpi1 with human ECM proteins: FN, LAM, COL and ELA.**

Human protein solutions in the buffer containing 10 mM HEPES, 150 mM NaCl and 0.005% (w/v) P20 surfactant, pH 7.4, at a concentration range of 50-750 nM, were injected onto a CM5 chip containing immobilized Tpi1 (150 RU) at a flow rate of 30  $\mu$ L/min for 180 seconds. A 1:1 Langmuir binding model was fitted to the experimental data (black lines).



**Figure S5. Relative enzymatic activity of *C. albicans* and *C. glabrata* Tpi1 after interaction with human ECM proteins.**

Tpi1 was incubated with ECM proteins at a 1:1 molar ratio for 1 hour in PBS at 37°C, with gentle shaking. The results show the values obtained after 40 min of activity monitoring and are presented as a percentage of the control experiment ("no human protein") taken as 100%.

The bars represent mean values  $\pm$  standard deviations (3 determinations).