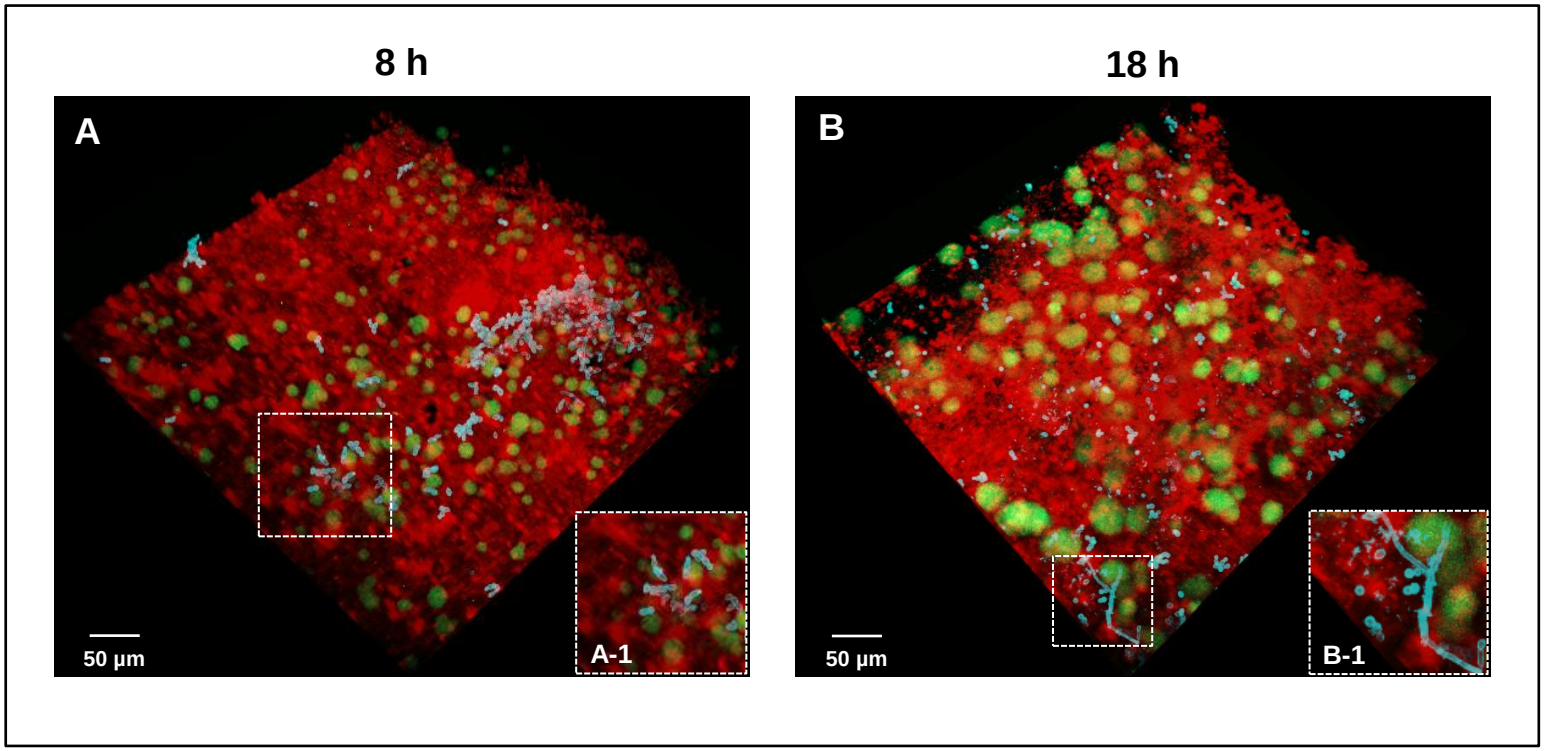


Figure S1. Representative 3D renderings of early stages of co-species biofilm development. Co-species biofilms at 8 h (A) and 18 h (B) after inoculation with *S. mutans* UA159 and *C. albicans* SC5314. *S. mutans* cells expressing GFP are green, while fungal cells labeled with ConA-tetramethylrhodamine are blue. EPS labeled with Alexa Fluor 647 dextran is red.

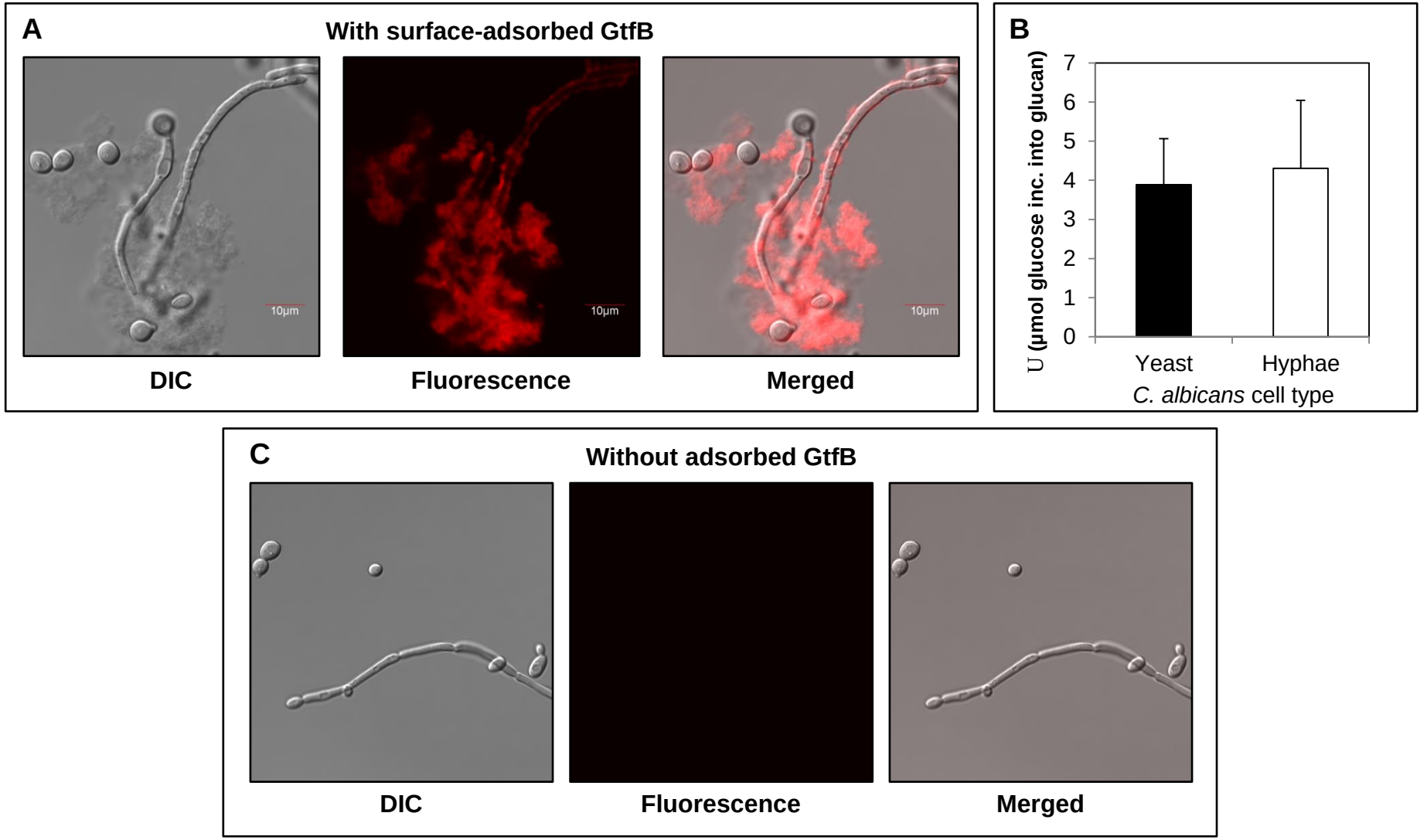


Figure S2. Exopolysaccharide (EPS) synthesis in situ by GtfB adsorbed to *C. albicans* SC5314. *C. albicans* cells (either yeast or hyphae forms) were incubated with 15 U of GtfB (or buffer) and washed to remove unbound GtfB using our previously published methods (Gregoire et al., 2011). Cells with adsorbed GtfB (or control cells without GtfB bound) were then exposed to either sucrose containing 1 µM Alexa Fluor 647-labeled dextran conjugate (for visualization) or [^{14}C] glucose-sucrose substrate (for quantification). Panel A depicts the EPS synthesized by GtfB adsorbed on both yeast and hyphal cells of *C. albicans*. DIC image of *C. albicans* cells after incubation with sucrose (100x oil objective, numerical aperture 1.4). Image obtained with laser excitation at 633 nm for detection of EPS labeled with Alexa Fluor 647 dextran. DIC and fluorescence images merged. Panel B depicts the enzymatic activity of GtfB adsorbed to *C. albicans* yeast and hyphal cells. One unit (U) of GtfB activity was defined as the amount of enzyme that incorporates 1 µmol of glucose into glucan over a 2 h reaction time at 37^{o}C. The amount of enzymatic activity of surface bound and unbound Gtf was measured by incorporation of [^{14}C] glucose from labeled sucrose into the glucan produced, using scintillation counting Gregoire et al., 2011). The data are mean values ± sd (n = 12); the values between yeast and hyphae forms are not significantly different ($p > 0.05$). Panel C shows the images of *C. albicans* cells without GtfB bound (as controls).

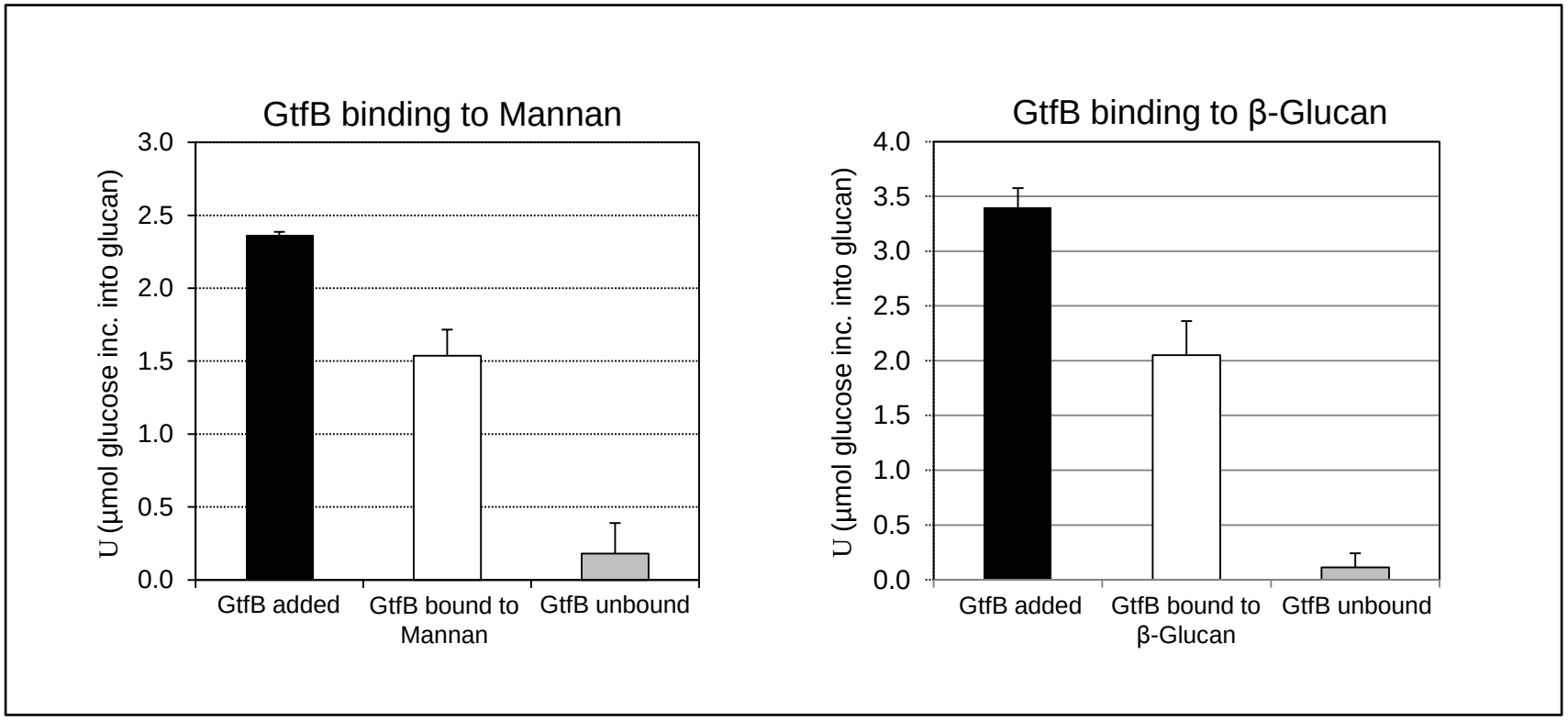


Figure S3. Amount of enzymatic activity of GtfB bound to mannan and β-glucan. See methods in Protocol S1.

Protocol S1: Assessment of GtfB binding/activity to mannan and β-glucan

Materials

BcMag™ DVS-Activated Magnetic Beads (Bioclone Inc.; San Diego, CA)

30% PEG 20,000 solution (Hampton Research; Aliso Viejo, CA)

Mannan from Saccharomyces cerevisiae (Sigma-Aldrich M7504)

Zymosan A from Saccharomyces cerevisiae (Sigma-Aldrich Z4250)

Other products were bought from Sigma-Aldrich (St. Louis, MO)

Methods

It is possible that *S. mutans* derived-GtfB may bind to the *C. albicans* cell wall carbohydrate components such as mannan and β-glucan. The identification of putative binding targets for GtfB is highly desirable to further elucidate how this exoenzyme binds and remains active, thereby modulating the observed bacterial-fungal interactions. In this experiment, we used commercially available, purified mannans and β-glucans (Zymosan A). DVS-magnetic beads were used to immobilize the purified polymers, which were then used to determine whether the GtfB binds (in enzymatically active form) to the ligand-coated surface. The magnetic beads are known for high coating ability, sensitivity, reproducibility, nonspecific binding, and the ability to rapidly pull-down from reaction mixtures with minimal sample preparation using simple magnets (Safarik and Safarikova, 2004; Aytur et al., 2006; Kulkarni et al., 2010). We have extensively tested different magnetic beads to avoid non-specific GtfB binding, and have selected DVS-magnetic beads for coupling the polysaccharides.

BcMag™ DVS-Activated Magnetic Beads: Coupling of mannan and β-glucans (Zymosan A) to DVS-magnetic beads was succesfully accomplished following the manufacturer's protocol

(Bioclone, Inc.). Briefly, 2.5 mg of ligand (mannan or zymosan A) was dissolved in 1 ml of coupling buffer containing 5% PEG. This mixture was added to 30 mg of beads (previously equilibrated in coupling buffer) and incubated overnight at room temperature with gentle rotation. Unbound ligand was removed using the magnetic separator followed by 2X washing steps using 0.1 M Na_2HPO_4, 0.5 NaCl, pH 7.2 washing buffer. Excess of active groups on the beads were blocked using a blocking buffer containing 1 M ethanolamine, pH 9.0 followed by incubation at room temperature for 30 minutes. Removal of the blocking buffer was attained using the magnetic separator, followed by 4X washing steps.

GtfB binding assay: The GtfB binding and activity assays were conducted as detailed previously by us (Gregoire et al., 2011). After ligand coupling, beads were immediately re-suspended and equilibrated for 5 minutes in fresh adsorption buffer (AB; 50 mM KCl, 1.0 mM KPO_4, 1.0 mM $CaCl_2$, 0.1 mM $MgCl_2$, pH 6.5) with gentle rotation. Beads were divided in two groups (experimental and control groups) containing 15 mg of beads each group. To the experimental group, the ligand-coated beads were mixed with saturating amounts of GtfB (75 µg/mL, 15 U) in 1 mL of AB buffer, while to the control group 1 mL of AB buffer alone was used. Beads were then incubated at 37^{o}C for 60 minutes with continuous gentle rocking. After incubation, the beads were removed using the magnetic separator, and the solutions containing unbound GtfB (experimental) or AB buffer (control) were collected for GtfB activity assays (see later). The beads were then washed (3X) with 1 mL of AB buffer. The washed beads (from each of the groups) were re-suspended in 250 µL of AB buffer and mixed with 250 µL of 2X [^{14}C]glucose labeled-sucrose substrate (0.4 µCi/ml; 200.0 mmol of sucrose/L, 40 µmol of dextran 9000/L, and 0.04% sodium azide in adsorption buffer, pH 6.5), and incubated for 2 h at 37^{o}C with continuous rocking. Additionally, each of the collected solutions (250 µL of the solution containing unbound GtfB or AB buffer) as well as the original GtfB solution used for the assay were also incubated with 250 µL of 2X [^{14}C] sucrose substrate as described above. After

incubation, 1 mL of 100% cold ethanol was added to stop the reaction and tubes were stored at -20 ˚C overnight. The enzymatic activity of surface-bound and unbound GtfB, and the original GtfB added (without ligand) was measured by the incorporation of [^{14}C] glucose from labeled sucrose into glucans using scintillation counting (Schilling and Bowen, 1992; Gregoire et al., 2011). One unit of enzyme was defined as the amount of Gtf enzyme that incorporates 1 µmol of glucose into glucans over the 2 h reaction period. Experiments were repeated by triplicate in at least three different experiments to ensure reproducibility.

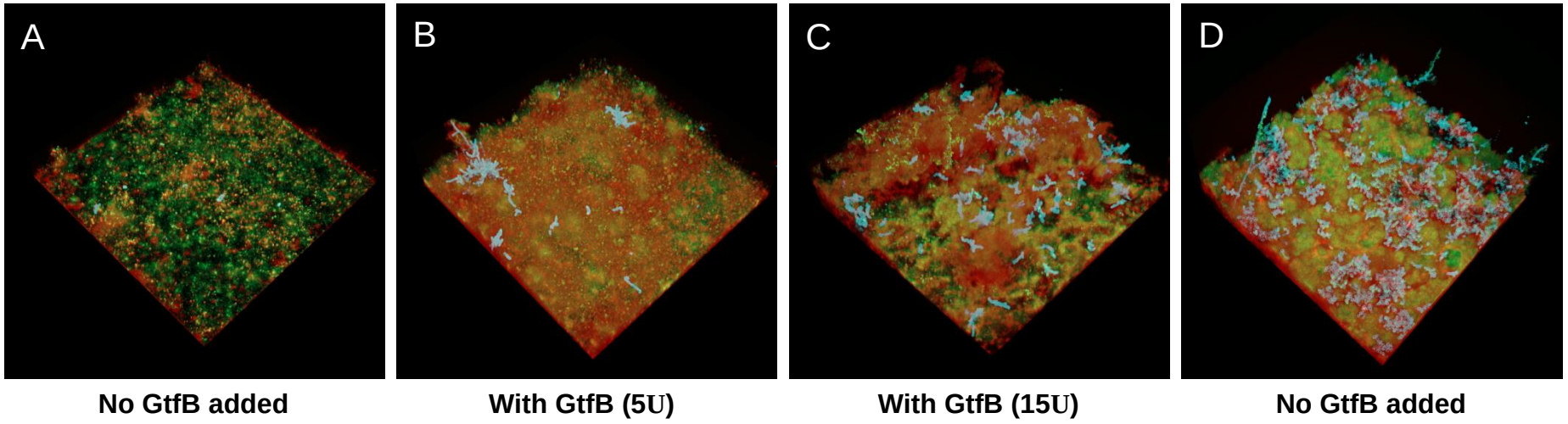
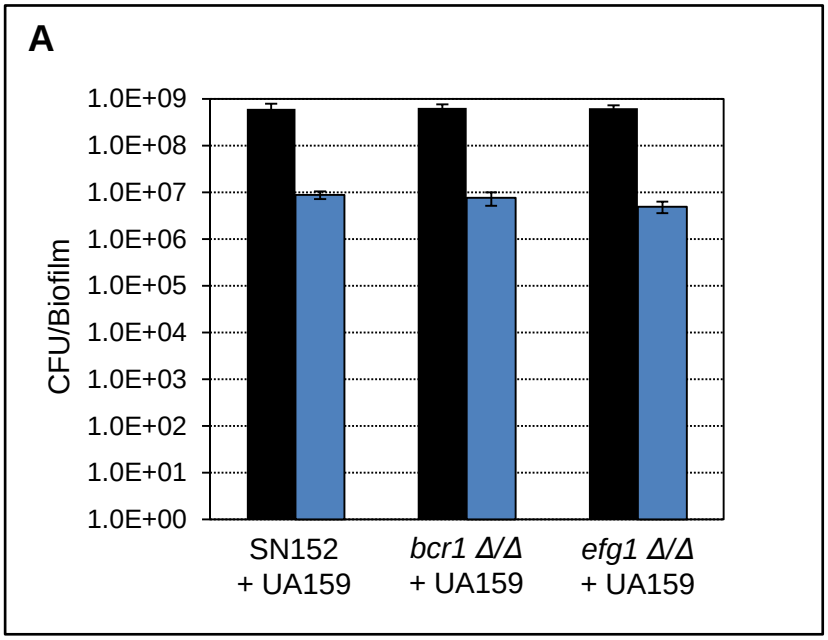


Figure S4. Supplementation with the GtfB enzyme helps to restore the phenotype/architecture of co-species biofilms formed with the Δ*gtfB*::kan mutant. This figure shows representative 3D images of the architecture of co-species biofilms (at 42 h) formed by Δ*gtfB*::kan mutant strain and *C. albicans* SC5314 without any exogenous enzyme (Panel A), with 5 U of purified GtfB added to the culture medium (Panel B), and with 15 U of purified GtfB (Panel C). Panel D depicts the co-species biofilms formed by parental UA159 strain and *C. albicans* SC5314 for comparison. The data illustrate that as increasing amounts of exogenous enzyme are added to the surrounding media, the biofilm architecture begins to better resemble the wild type. This figure also indicates that purified GtfB can help to complement the defect in GtfB activity introduced by genetic interruption of its coding sequence in *S. mutans*.



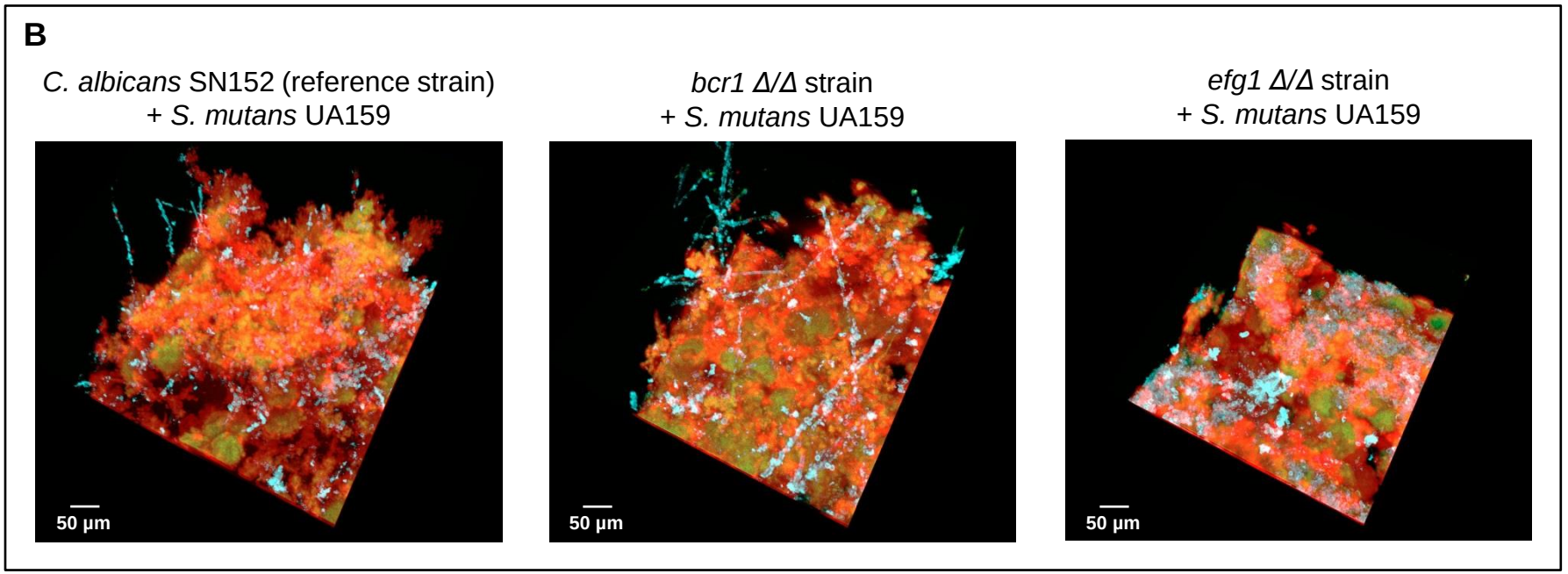


Figure S5. Co-species biofilms formed with *bcr1*Δ/Δ and *efg1*Δ/Δ mutant strains of *C. albicans* SN152 (reference strain). Panel A provides a representative image of the architecture of co-species biofilms formed by *S. mutans* UA159 and each of the *C. albicans* mutant strains (or the parental SN152 strain) at 42 h. Panel B shows the total viable counts of *S. mutans* and *C. albicans* in co-species biofilms formed with *S. mutans* UA159 and each of the *C. albicans* strains. The data are mean values ± sd (n = 16).

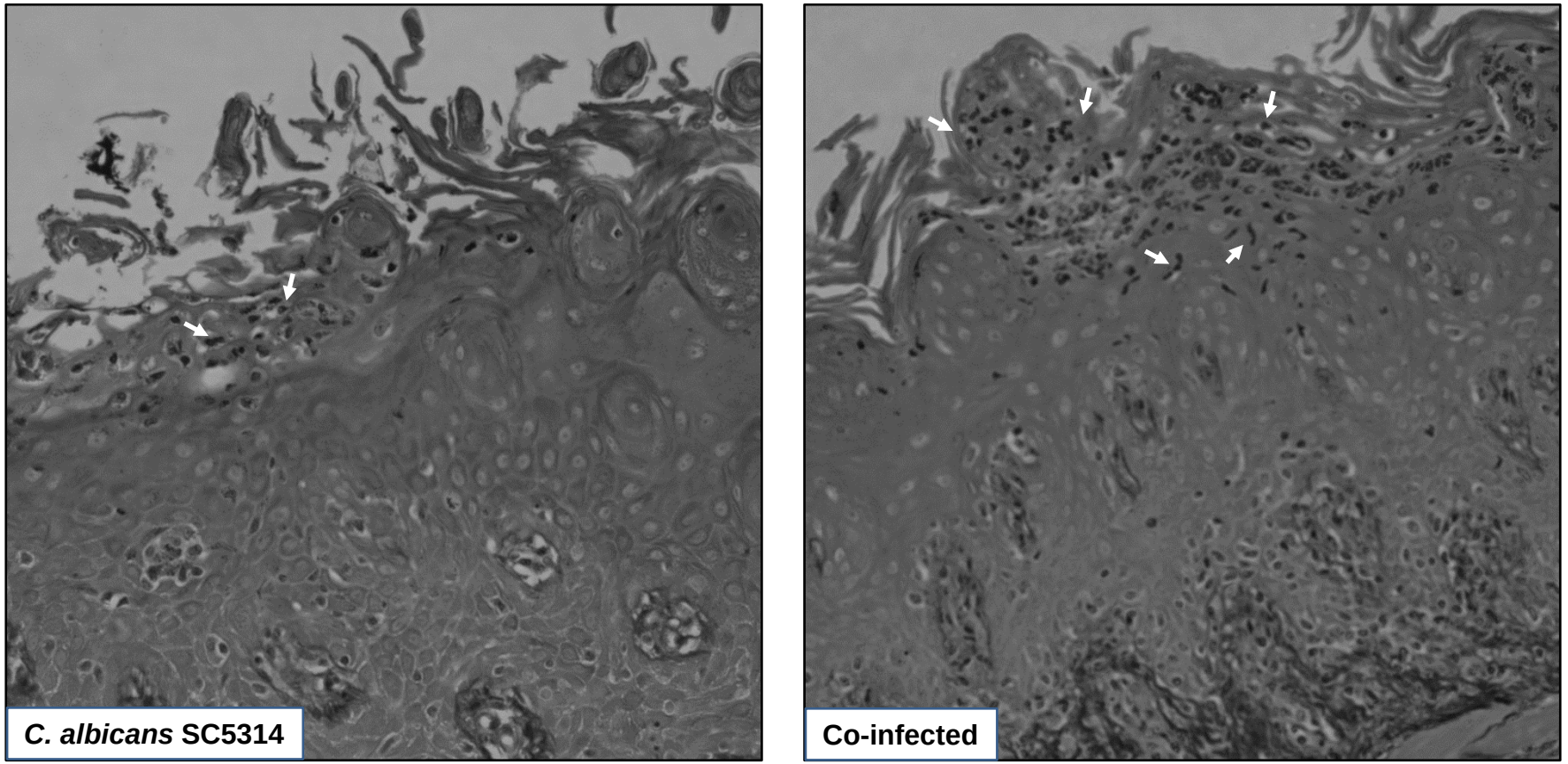


Figure S6. Cross-sections of tongue tissues from rodent infected with *S. mutans* and *C. albicans* or *C. albicans* alone. This figure provides representative images of rodent tongues collected during the in vivo study. Tongues were fixed in 3% paraformaldehyde, then washed in phosphate buffered saline (PBS), and serially dehydrated in 30, 50, and 80% ethanol. Samples were then processed for histology and embedded in paraffin wax (73). Five µm sections were stained with Modified Grocott's Methenamine Silver stain (VWR International, Radnor PA) and visualized using an Olympus (Olympus America Inc., Center Valley, PA) CKX41 microscope equipped with a 14.2 Color Mosiac Insight camera. Images were taken with the 40X objective (0.55 numerical aperture). Yeast and hyphae cells invading the tongue mucosa (ventral surface of the tongue is at the top of the image) are stained black and are denoted by the white arrows. By comparing the *C. albicans* only infected animal (left image) to the co-infected animal (right image), it is clear that there is a greater number of yeast cells invading the tongue tissue in co-infected animals. Furthermore, the arrows denote the presence of elongated cell types not present in the singly-infected animal, which are consistent with pseudohyphal or hyphal forms.

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