Supplementary Information

Candida albicans stimulates *Streptococcus mutans* microcolony development via cross-kingdom biofilm-derived metabolites

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Figure S1

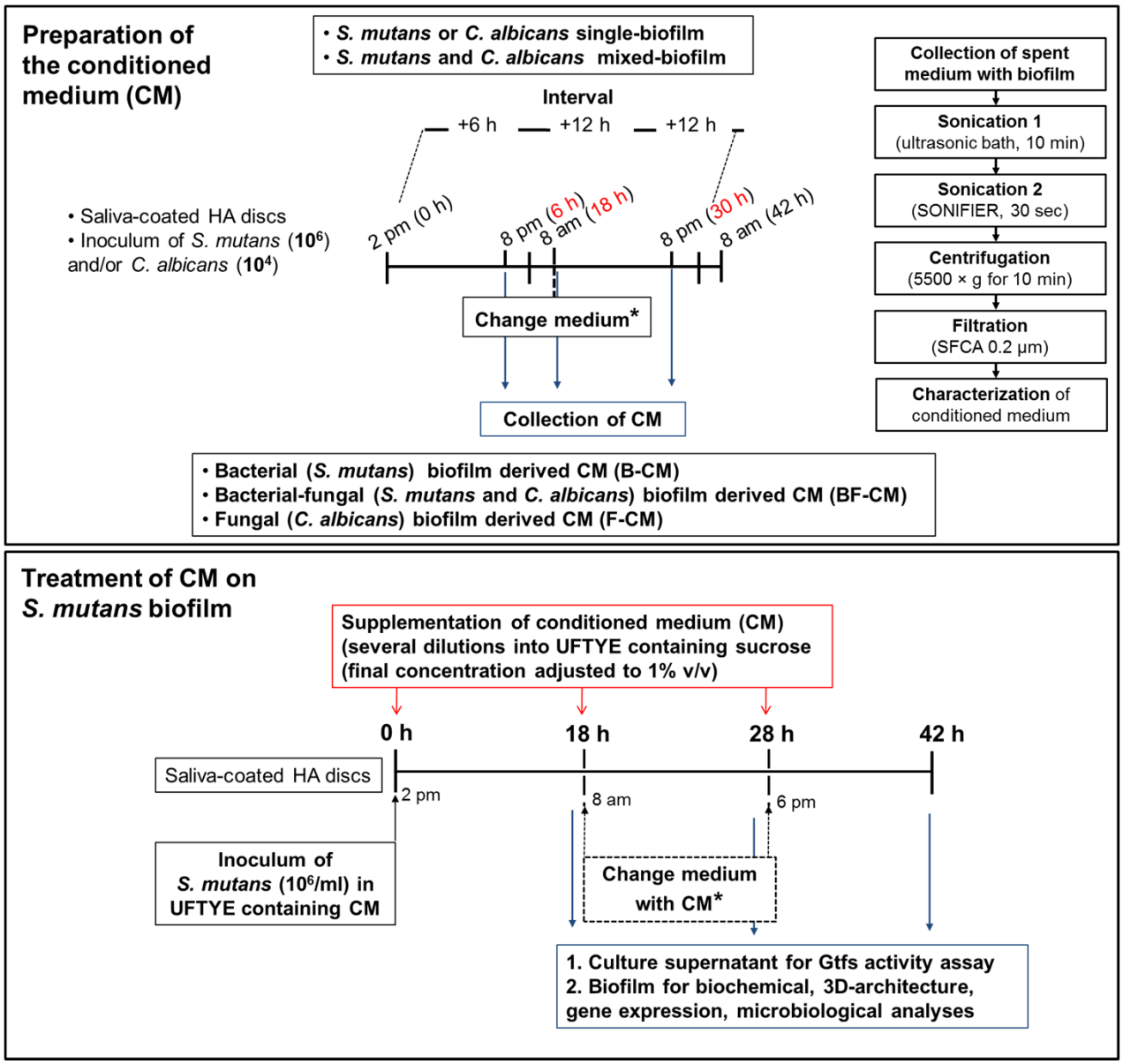


Figure S1. **Experimental design for preparation and treatment of conditioned medium.** Each cell-free conditioned medium (a) was treated to *S. mutans* biofilm (b). *The discs were dip-washed (removal of non-adhered cells) and transferred to fresh culture medium containing 1% sucrose (change medium).

Figure S2

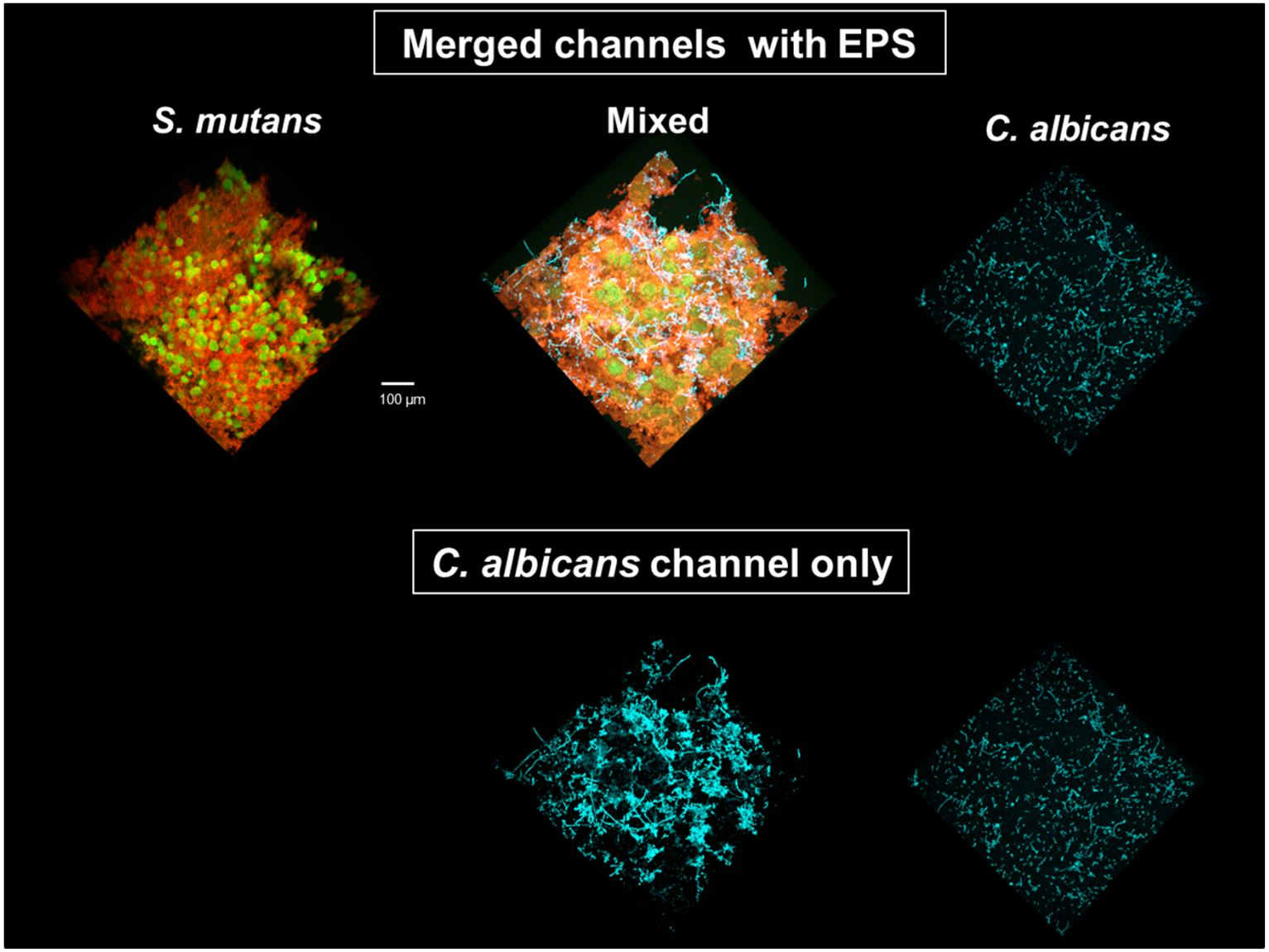


Figure S2. **Representative images of single and co-species biofilms grown for 42 h.** *S. mutans* cells stained with SYTO 9 are depicted in green, while EPS labelled with Alexa Fluor 647 is in red. *C. albicans* cells stained with Concanavalin A-tetramethylrhodamine appear blue.

Figure S3

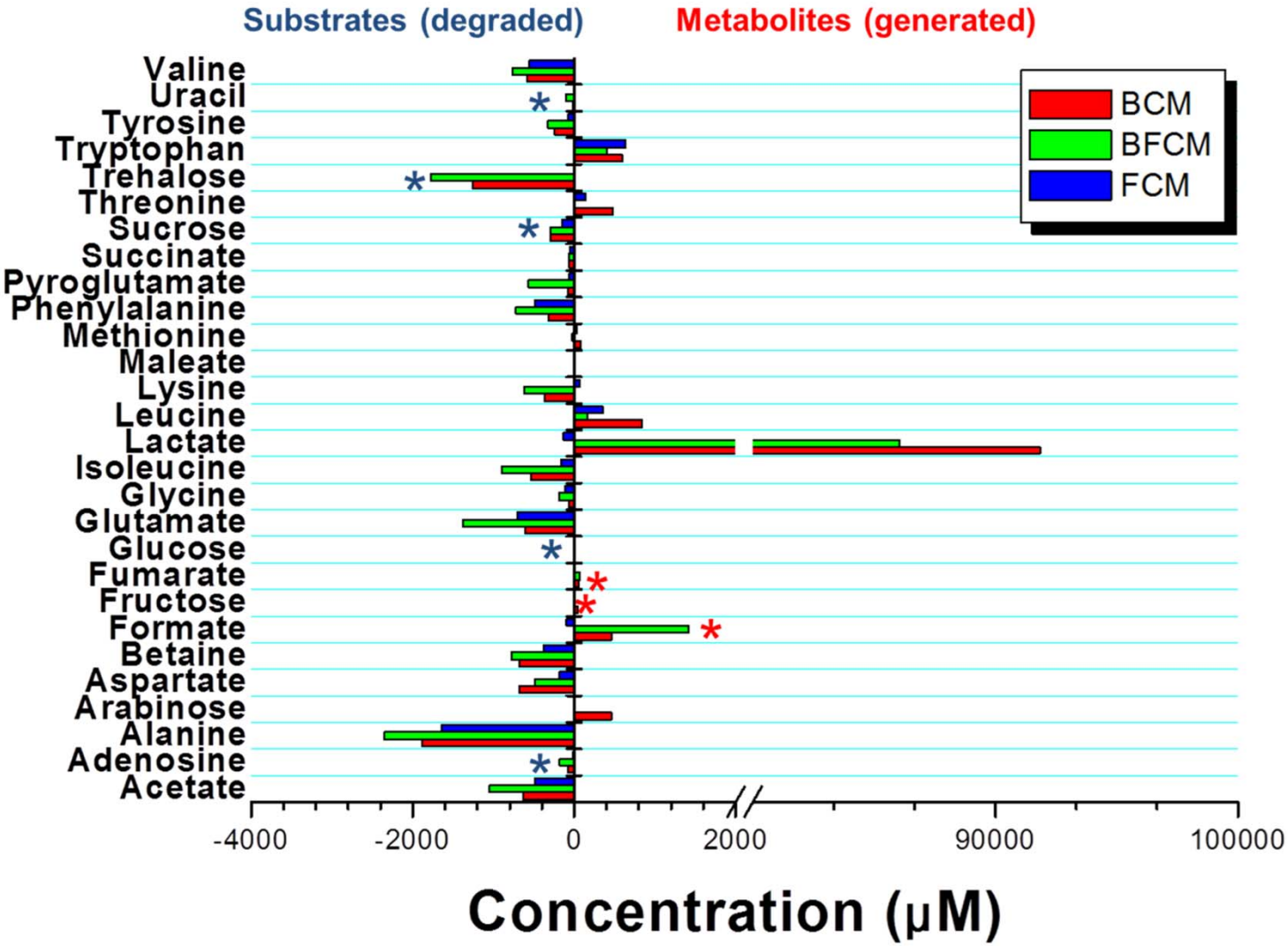


Figure S3. **Changes in the concentrations of extracellular metabolites in the conditioned medium (CM).** The metabolites were determined by a direct comparison of blank (UFTYE) and CM; higher concentration than the value of blank means metabolites while lower concentration means substrates. The data were subjected to analysis of variance (ANOVA) in the Tukey's HSD test for a multiple comparison (*P<0.01).

Figure S4

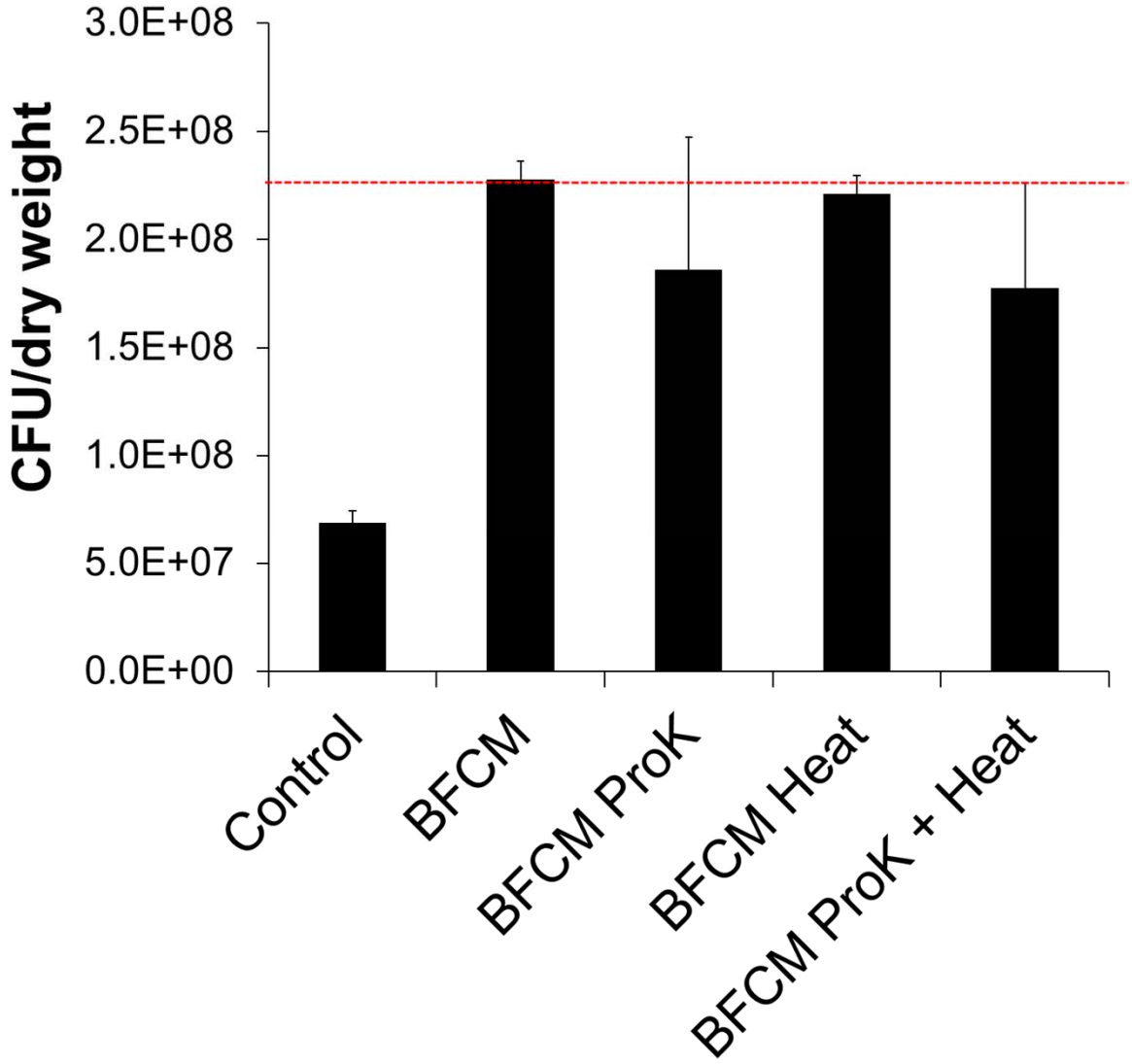


Figure S4. **Physicochemical characterization of bacterial-fungal biofilm derived conditioned medium (BF-CM).** BFCM, original CM; BFCM ProK, proteinase K (100 μg/ml, 37°C for 2 h) treated CM; BFCM Heat, heat (85°C for 0.5) treated CM; BFCM ProK+Heat, proteinase K and heat sequentially treated CM.

Figure S5

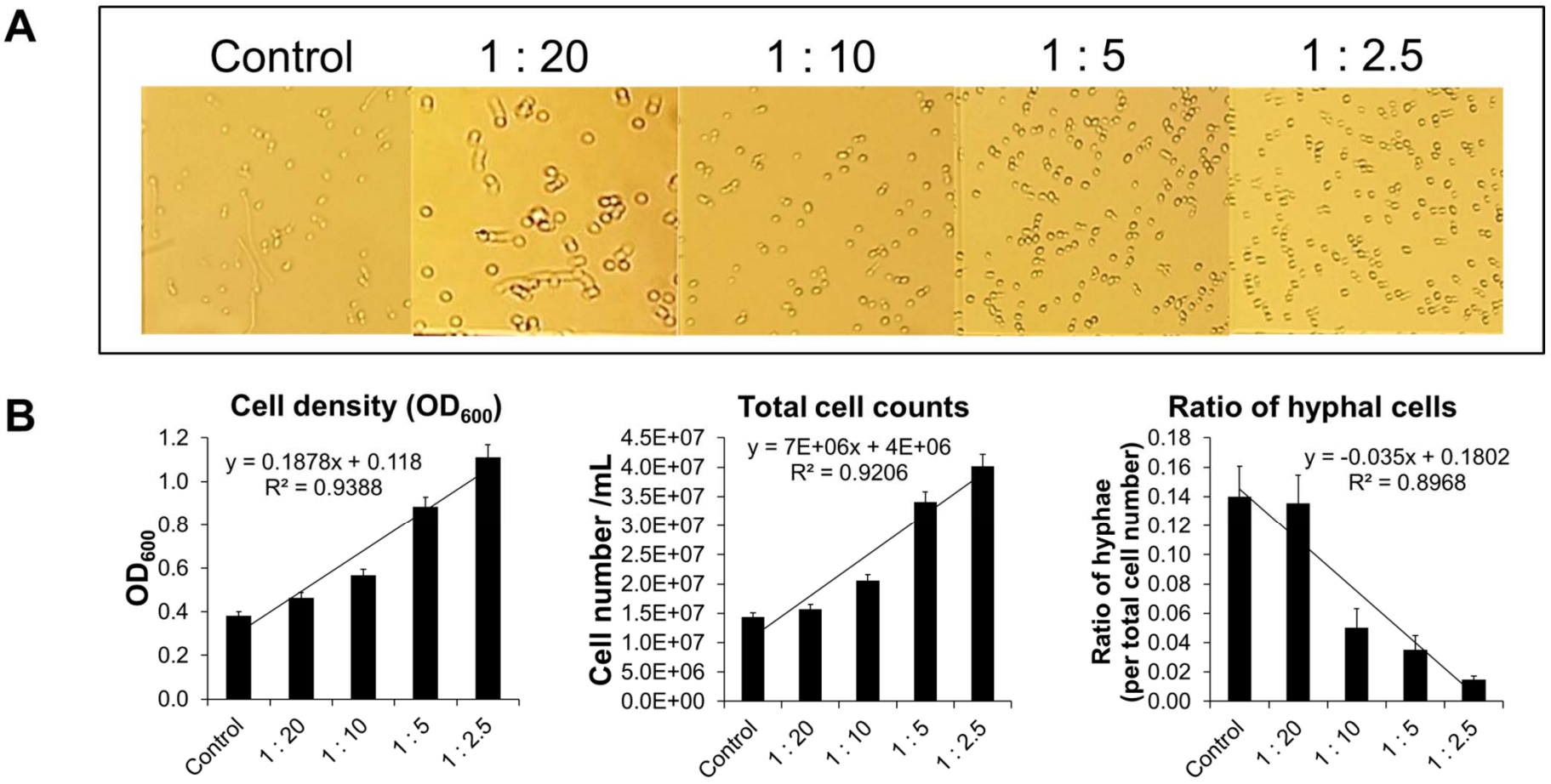
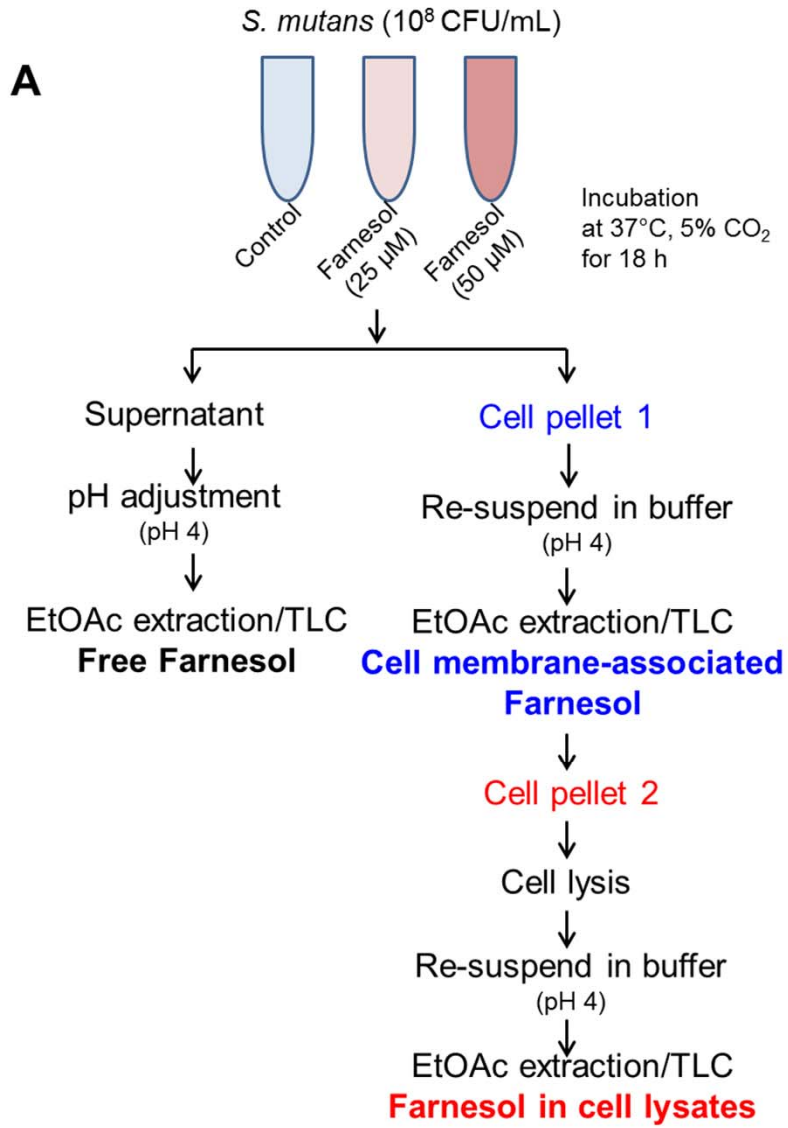


Figure S5. **Morphogenesis of *C. albicans* in the supplementation of BF-CM as a dose-dependent manner.** (A) Influences of BF-CM on morphological change of *C. albicans*. (B) Cell density, total cell number (counted in a hemocytometer) and ratio of hyphal cells were analyzed.

Figure S6



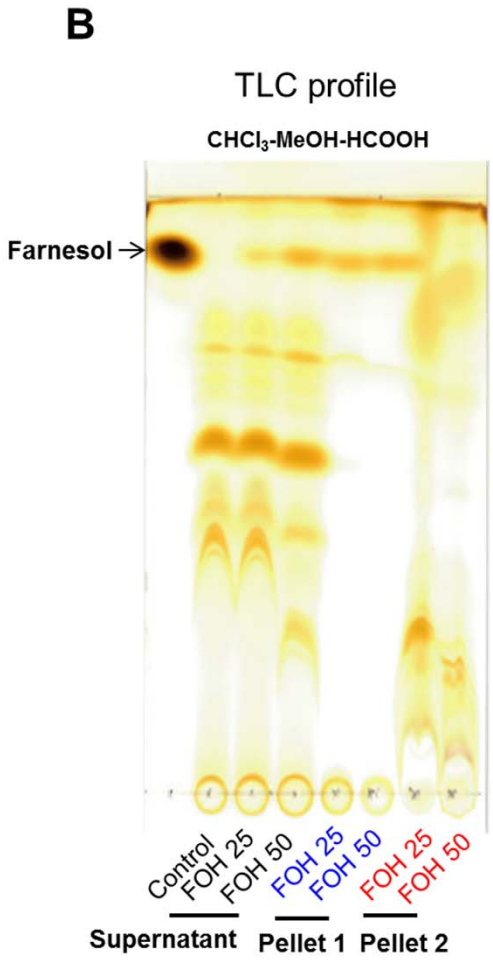


Figure S6. **Detection of farnesol incorporated by *S. mutans*.** (A) Bacterial cell pellets were collected following overnight incubation with farnesol, and then subjected to EtOAc extraction (3 times, 1/5 volume EtOAc) followed by TLC analysis. (B) The EtOAc extracts of cell pellet (Pellet 1; membrane contents were separated into EtOAc layer) contained detectable farnesol, while cell lysates (Pellet 2) showed negligible amounts of farnesol.