Supplemental materials

Materials and Methods

The effect of farnesol on the morphology and growth of *C. albicans*. This effect was evaluated as previously described with some modifications (1-2). *C. albicans* SC5314 was resuspended in RPMI1640 medium buffered with morpholinepropanesulfonic acid, yeast nitrogen base (YNB) medium containing 50 mM glucose, or synthetic medium plus dextrose (SD) medium (6.7 g/L YNB, supplemented with 5 g/L ammonium sulfate, mixture of amino acids and 20 g/L dextrose, pH adjusted to 7.0). The cell concentrations were adjusted to $10^6$ cells/ml. 200 µl of aliquot was dispended into wells of 96-well plates (NEST). The cells were treated with 40, 80, 120, 160 µM farnesol or the same volume of DMSO solvent as control. After 48h, the cell growth was detected by measuring the light absorbance at 570 nm on a plate reader (Bio-Rad Laboratories, Richmond, CA). The supernatants were removed and the adherent cell layers were observed under an Olympus microscope for morphology analysis.

Construction of green fluorescent protein-tagged *C. albicans* strain. The *C. albicans* strain *TDH3-GFP-CAI4* (MG1004) was created by homologous recombination of green fluorescent protein (GFP) sequences into the 3′ end of their open reading frame using the method as previously described (3). The DNA used for the transformation was created by PCR using primers containing ~70 base pairs of sequence homologous to the 3′ end of the *TDH3* open reading frame to amplify a cassette containing GFP and a URA3 selectable marker using pGFP-URA3 as the template. The primer sequences used were

5′-GTACGATAACGAATAACGGTTACTCCACCAGATGTGACTTGTTGGAACACGGTC-3′ and 5′-GTAAGACATTCTGCTGGGTGGTCTTCTAAAAGGTGAAGATTATT-3′ and 5′-
AAGTCTTCTAGAAGGACCACCTTTGATTG-3'. The PCR product was transformed into auxotrophic mutant strain CAI4. The transformed strain was then spotted on SD minus uracil (SD−Ura) solid medium and grown at 37 °C for 3 d. The colonies resulting from the transformation were then screened for GFP-positive cells by fluorescent microscopy and further confirmed by PCR amplification.

**Measurement of Tdh3 expression.** The expression of Tdh3 was indicated by the fluorescence intensity of GFP measured by flow cytometry as previously described (4). *TDH3-GFP-CAI4* was adjusted to $10^6$ cells/ml using SD medium. 200 µl of aliquot was dispensed into wells of 96-well plates (NEST). One group was added with 80 μM farnesol and the other one is treated with vehicle. At indicated time, the supernatants were removed and adherent cells were scraped for flow cytometry analysis. The resultant data were processed with WinMDI 2.9 software (Joseph Trotter, The Scripps Institute, La Jolla, CA).

**Persistor measurement using colony counting method.** The persistor fraction measurement was performed as previously described with a slight modification (5). Briefly, overnight cultured *C. albicans* cells were adjusted to $10^6$ cells/ml and supplemented with indicated concentration of farnesol. 200 µl of aliquot was dispensed into wells of 96-well plates (NEST). After indicated time of incubation at 37 °C with 100 rpm rotation, supernatants were discarded and wells were thoroughly washed three times with PBS to remove the non-adherent cells prior to antifungal challenge. The cells in three replica wells were detached and spread on YPD agar plates by serially dilution method for cells number counting. Subsequently the culture media containing 100 μg/ml of AmB was added another three replica wells and the plates were incubated at 37 °C with 100 rpm rotation for additional 24 h. Then supernatants were aspirated the adherent cells were
detached using tips. The collected cells were washed by PBS and re-suspended in PBS. The suspension was vortexed vigorously for 30 seconds following with serial dilution and plating on YPD agar plates for colony forming unit (CFU) determination. The frequency of the persisters was estimated through the number of surviving cells based on CFU counting divided by the cells number prior to drug challenge.

**Persisters detecting assay based on flow cytometry.** *C. albicans TDH3-GFP-CAI4* was cultured and treated as described above. The difference for this method was that scraped cells from wells were stained with PI instead of CFUs determination. After 30 min incubation in the dark, cells were washed with PBS and analyzed using flow cytometry with F1 channel for GFP fluorescence and F3 channel for PI signal. At the same time they were observed with a Zeiss LSM700 Meta confocal laser scanning microscope (CLSM). Bright field and fluorescent images were taken as our previously described method (6).

**Observation of the persisters’ revival by confocal time lapse analysis.** The time lapse observation was performed as previously described with some modification (7). Briefly, cells were prepared according to the persister detection assay described above. The PI stained cells were washed by sterile PBS and re-suspended in YPD medium containing 100 µg/ml of ampicillin, in avoid of bacterial contamination. The cells were transferred to a poly-L-Lysine coated petri dish for confocal observation. Cells were incubated at 30 °C for the first 2 h in order to have a relatively steady field due to the random movement of cells. And then a field was chosen to perform the time-lapse observation with 488 and 555 nm excitation wavelengths for GFP and PI imaging, respectively. At the beginning, the pictures were manually photographed for the first 4 h and the time-lapse program was started for subsequent imaging with 20 min intervals for another 2
h, then with 10 min intervals for the remaining time. Image processing was performed using Image J software.

**Optimization of high persister-producing culture conditions.** Overnight cultured *C. albicans* cells were suspended in SD medium and diluted to $10^6$ cells/ml and supplemented with farnesol (80 µM). Then persister determination was then performed according to flow cytometry based assay as described above. The effect of pH (ranging from 3 to 10) and osmotic stress were evaluated on the persister formation.

**GFP tagging with Tdh3 in cdr1Δ/Δ strain.** *C. albicans cdr1Δ/Δ* (DSY448) (8) was selected on YPD medium plus 5-fluoroorotic acid (5-FOA) to excise *URA3*. The resulting colonies were checked by PCR to get DSY449 (8) (Table 1). Then the GFP sequences were inserted into the 3’ end of their open reading frame of *TDH3* in DSY449 through homologous recombination following the previous described method (3). The correction of inserted GFP sequences was confirmed by PCR.

**Statistical analysis.** The experimental data were statistically analyzed using Student’s *t*-test. *p* <0.05 was considered significant.

**References**


   http://dx.doi.org/10.1007/s12275-009-0059-0.


**Legends for Videos**

Supplemental Video. 1. The revival observation of *C. albicans* persisters using time lapse mode by confocal microscopy. Adherent cells of *C. albicans* TDH3-GFP-CAI4 were scraped and transferred to fresh YPD medium in 35 mm dishes after 2 h of incubation at 30 °C. Cells were
manually photographed. The images obtained during the first 3 h were captured at a rate of one frame per hour. After the first 3 h, images were obtained at 20 min intervals for 100 min.

Supplemental Video. 2. The revival observation of cells indicated by red arrow in Fig. S6. Cells were grown for 8 h, from which the indicated area was imaged by performing time lapse observation using confocal microscope. Each frame was obtained at 10 min intervals.
FIG S1 The growth of *C. albicans* under farnesol treatment. *C. albicans* SC5314 was inoculated into 96-well plates (NEST). The cells were treated with 40, 80, 120, 160 μM farnesol or DMSO solvent as control. After 48h, the cell growth were imaged and detected by measuring the light absorbance at 570 nm on a plate reader.
**FIG S2** The morphology of *C. albicans* SC5314 cultured in RPMI1640 medium, YNB medium plus glucose, or SD medium with indicated concentration of farnesol addition.
FIG S3 The effect of farnesol on persister formation of *C. albicans*. The persister fractions of *C. albicans* SC5314 cultured in RPMI1640 medium (A) or SD medium (B) assessed by colony counting method. The data are expressed with means ± standard errors from three independent experiments.
FIG S4 The Tdh3 expression of *C. albicans, C. albicans TDH3-GFP-CAI4* was cultured in SD medium. At indicated time, adherent cells were taken out from 96 well plates for the detection of GFP fluorescence intensity using flow cytometry. The data was analyzed using WinMDI 2.9 software to obtain the geometric mean value of the fluorescence intensity.
FIG S5 The revival observation of *C. albicans* persister at the indicated time. The adherent cells of *C. albicans* TDH3-GFP-CAI4 containing persisters were suspended in YPD medium for time lapse observation by confocal microscopy. Arrows indicate the persisters chosen for the observation.