

Supplementary Materials for

Polarization-sensitive stimulated Raman scattering imaging resolves amphotericin B orientation in *Candida* membrane

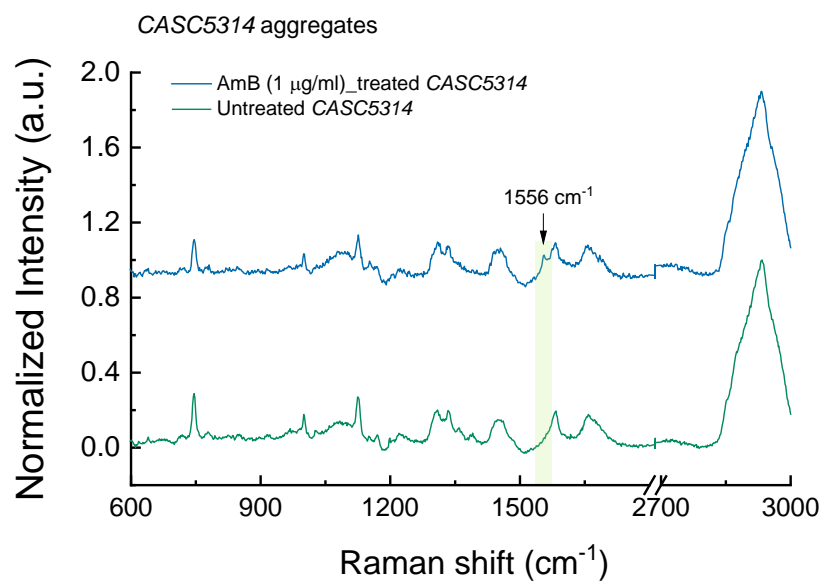
Pu-Ting Dong, Cheng Zong, Zeina Dagher, Jie Hui, Junjie Li, Yuewei Zhan, Meng Zhang, Michael K. Mansour, Ji-Xin Cheng*

*Corresponding author. Email: jxcheng@bu.edu

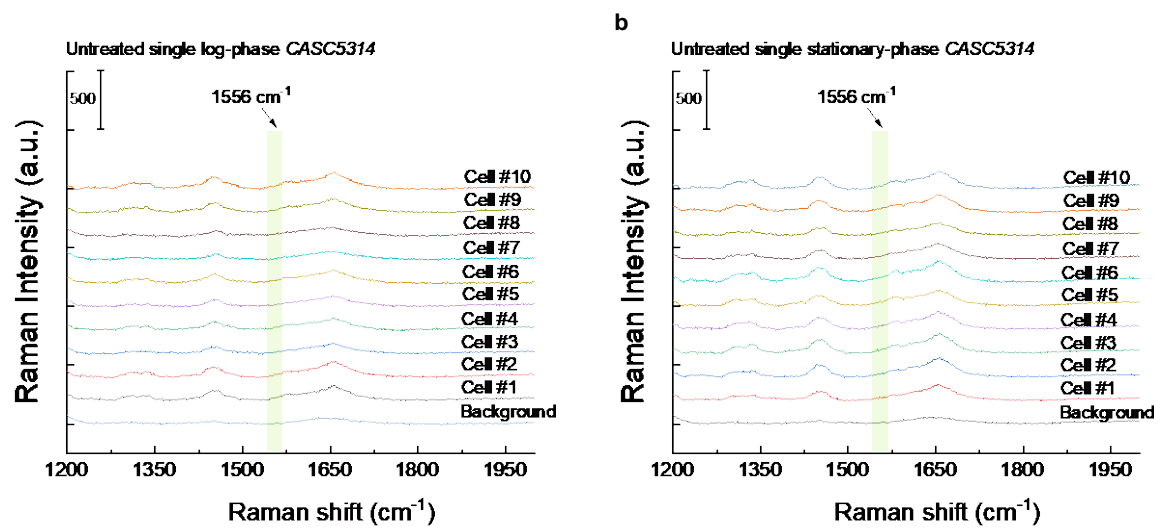
Published 6 January 2021, *Sci. Adv.* 7, eabd5230 (2021)
DOI: 10.1126/sciadv.abd5230

This PDF file includes:

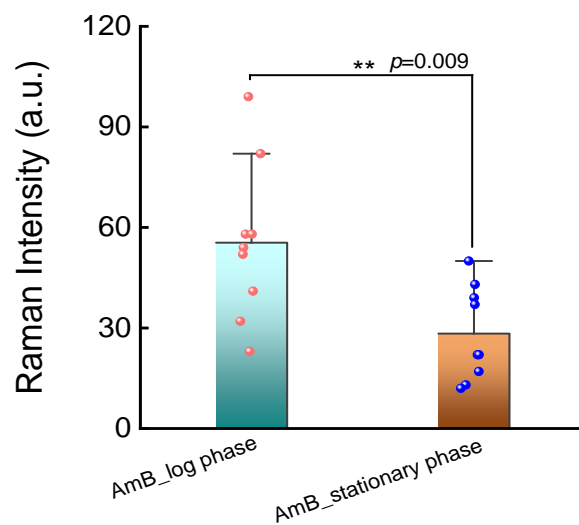
Figs. S1 to S10



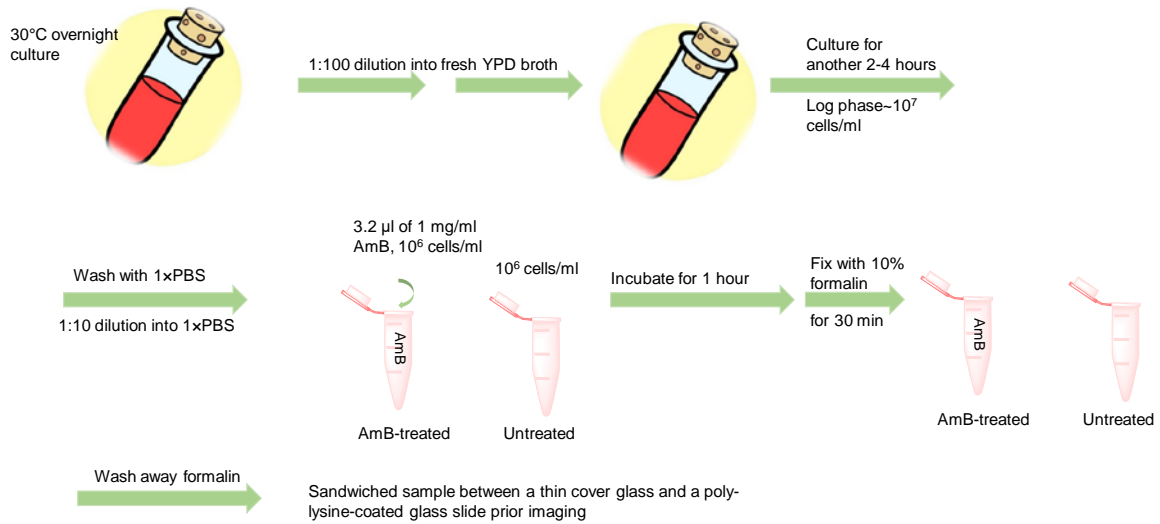
Supplementary Figure 1. Raman spectra of AmB-treated and untreated log-phase CASC5314 aggregates. Sample was dried onto the surface of aluminum substrate. AmB: 3.2 $\mu\text{g/ml}$, 1-h treatment. Excitation: 532 nm. Power: 8 mW. Spectrum acquisition time: 30 s. 1556 cm^{-1} was highlighted by green box.



Supplementary Figure 2. Raman spectra of untreated single log-phase (a) and stationary-phase (b) *CASC5314*. Samples were sandwiched between a poly-lysine-coated cover slide and a cover glass. Power: 8 mW. Spectrum acquisition time: 30 s. 1556 cm⁻¹ was indicated by a green box.

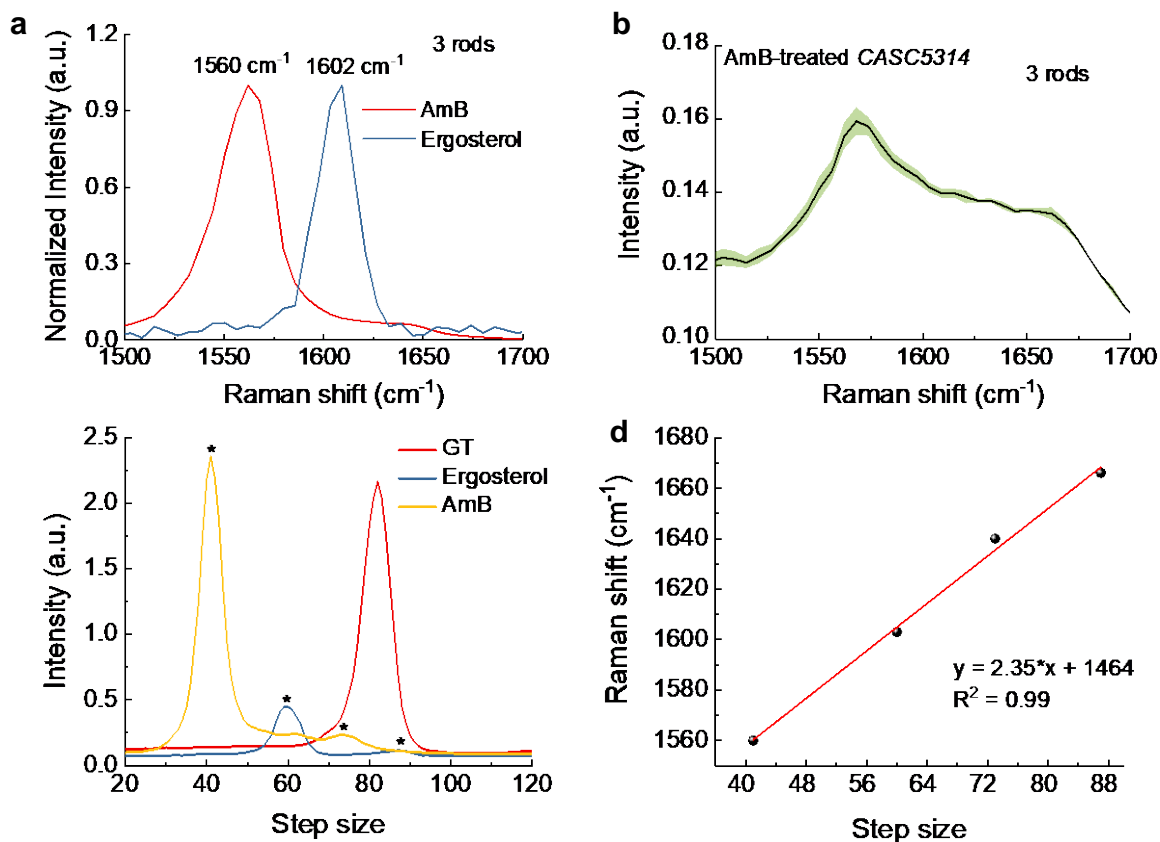


Supplementary Figure 3. Quantitative analysis of AmB amount (Raman signal at 1556 cm^{-1}) from AmB-treated single log-phase and stationary-phase *CASC5314*. Each dot came from a single yeast. Student unpaired *t*-test. **: $p<0.01$.

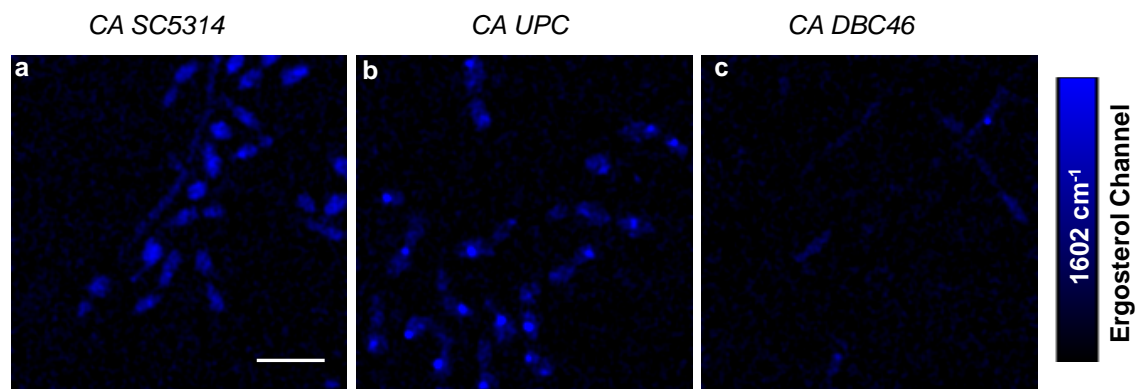


❖ Sample prepared the day before imaging

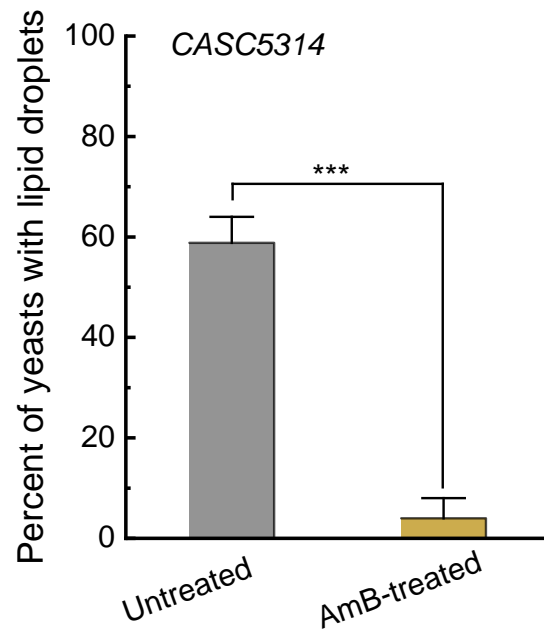
Supplementary Figure 4. Schematic illustration of sample preparation procedure of AmB-treated *Candida spp.* and *Candida auris*.



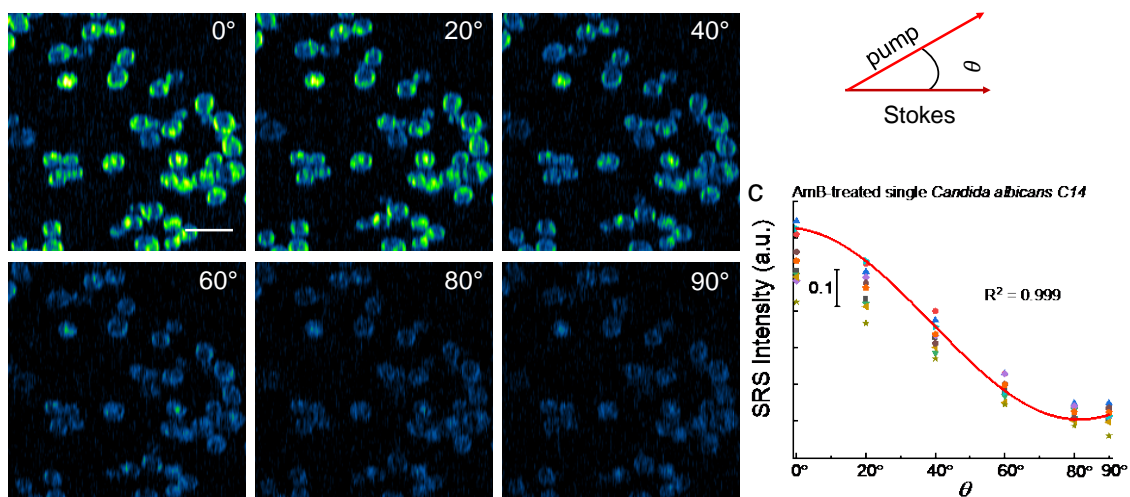
Supplementary Figure 5. Performance of three-rod chirping hyperspectral SRS microscope and calibration of six-rod chirping hyperspectral SRS system by pure glycerol trioleate (GT), ergosterol crystal and 10 mg/ml AmB. **a.** SRS spectra of 10 mg/ml AmB in DMSO and pure ergosterol crystal, with marked Raman peaks. Three-rod chirping condition. **b.** SRS spectra of AmB-treated single *CASC5314* cells. Data: Mean \pm SD from at least ten yeasts. Amp: 3.2 $\mu\text{g/ml}$ for 1 hour. Data acquired under three-rod chirping condition. **c.** The step size profiles of pure chemicals in the interested spectral window. **d.** The calibration curve of Raman shifts versus step size in the interested spectral window.



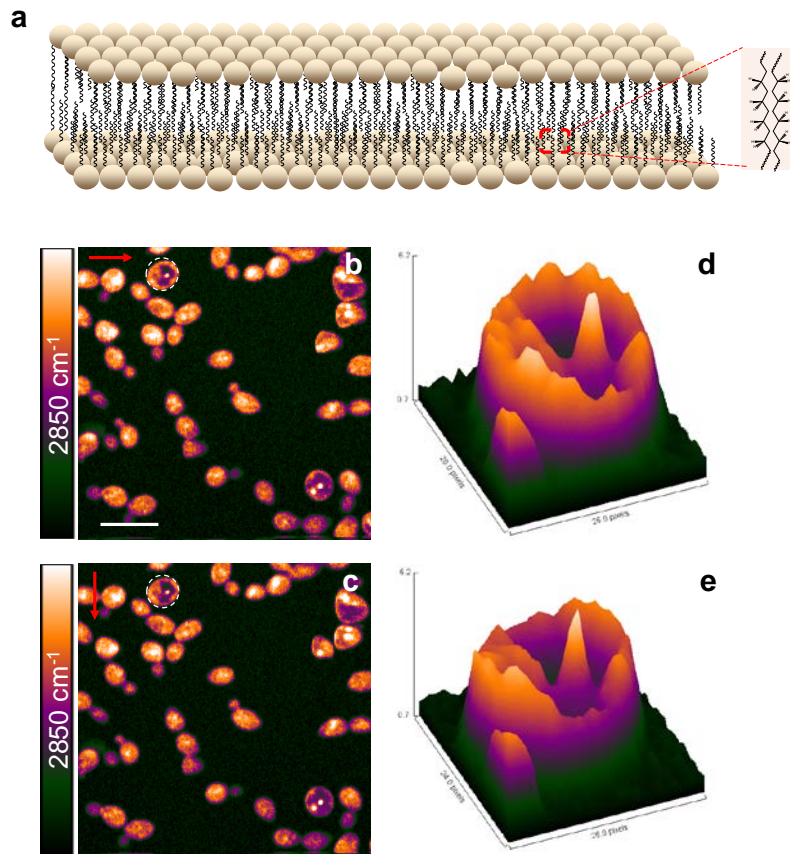
Supplementary Figure 6. SRS images of ergosterol at 1602 cm⁻¹ in *CASC5314* (a) and isogenic mutants *CA UPC* and *CA DBC46* (b-c).



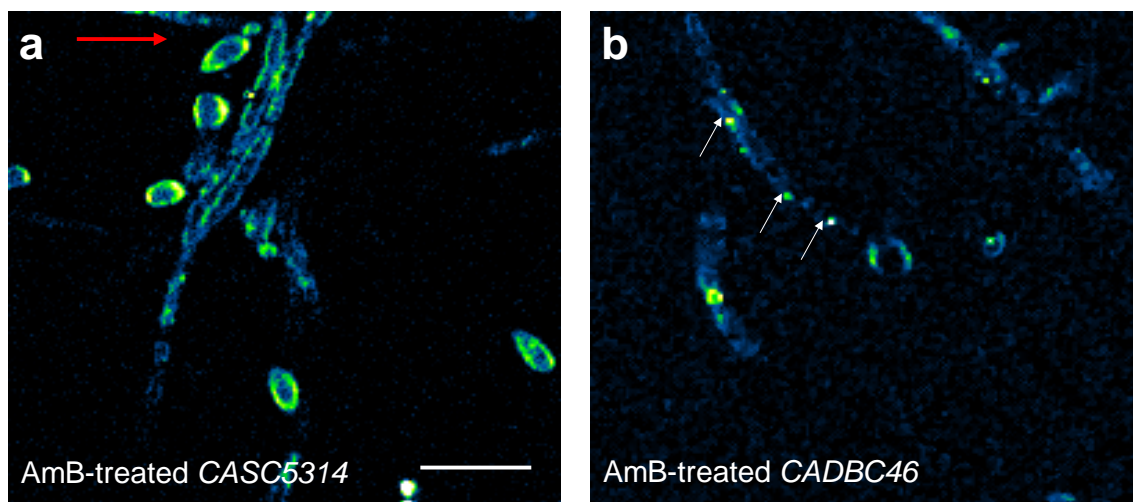
Supplementary Figure 7. Quantitative analysis of the percent of *CASC5314* yeast cells with lipid droplets by SRS imaging at 1650 cm^{-1} with and without AmB-treatment. Data: Mean \pm SD, from at least five different field of views. Student unpaired *t*-test. ***: $p < 0.001$.



Supplementary Figure 8. Polarization-sensitive SRS imaging of AmB-treated *Candida albicans* C14 at 1556 cm⁻¹ when the polarization of pump beam was rotated with respect to that of Stokes beam. a. SRS images of AmB-treated *Candida albicans* C14 at 1556 cm⁻¹ when the polarization of pump beam was rotated with respect to that of Stokes beam **(b)**. **c.** SRS intensity of single *Candida albicans* C14 at 1556 cm⁻¹ under different angles shown in **(b)**. Data was fitted by a cosine function. Pump=895 nm, Stokes=1040 nm. Pixel dwell time: 10 μ s. Scalar bar=10 μ m.



Supplementary Figure 9. Polarization-sensitive SRS imaging of CH₂ in untreated *CASC5314* at 2850 cm⁻¹. **a.** Schematic of phospholipid bilayer along with its zoom-in view of CH₂ backbone. **b-c.** SRS images of untreated *CASC5314* at 2850 cm⁻¹ at horizontal and vertical polarization directions, respectively. **d-e.** Zoom-in view of circled (by white dashed line) single yeast from (b-c). Pixel dwell time: 50 μs. Pump=802 nm. Stokes=1040 nm. Scalar bar=10 μm. Laser polarization direction is indicated by red arrow.



Supplementary Figure 10. SRS imaging of AmB at 1556 cm^{-1} in AmB-treated *CASC5314* (a) and *CADBC46* (b). Amp: $3.2\text{ }\mu\text{g/ml}$ for 1 hour at 37°C . AmB 'microdomain' was highlighted by white arrow. Pump= 895 nm , Stokes= 1040 nm . Pixel dwell time: $50\text{ }\mu\text{s}$. Scalar bar= $10\text{ }\mu\text{m}$. Laser polarization direction is indicated by red arrow.