Supplementary Information

Mechanisms involved in the triggering of neutrophil extracellular traps (NETs) by *Candida glabrata* during planktonic and biofilm growth.

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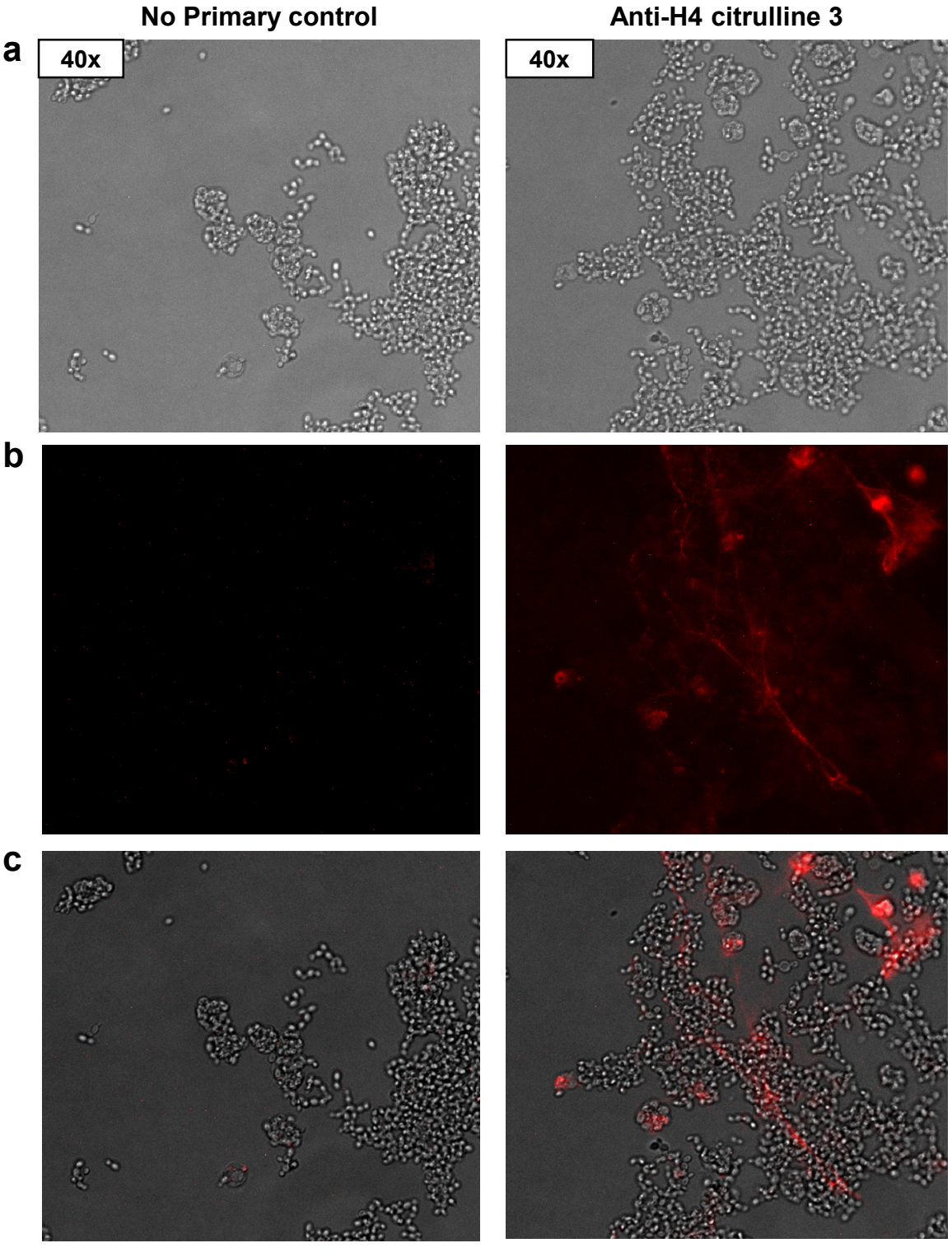
Supplementary Video 1. Time-lapse imaging of neutrophil-*C. glabrata* interactions (30 min). Calcein AM-labeled neutrophils (green) were added to *C. glabrata*. After a 30 min incubation, neutrophil interactions were imaged with time lapse at 20x. Brightfield and fluorescent images were then collected every 1 min for 1 h and compiled at 5 frames per second.

Supplementary Video 2. Time-lapse imaging of neutrophil-*C. glabrata* interactions (2.5 h). Calcein AM-labeled neutrophils (green) were added to *C. glabrata* and co-cultured for 2 h 30 min. Free DNA stain propidium iodide (red) was added prior to imaging at 2.5-3 h at 20x. Brightfield and fluorescent images were then collected every 1 min for 1 h and compiled at 5 frames per second.

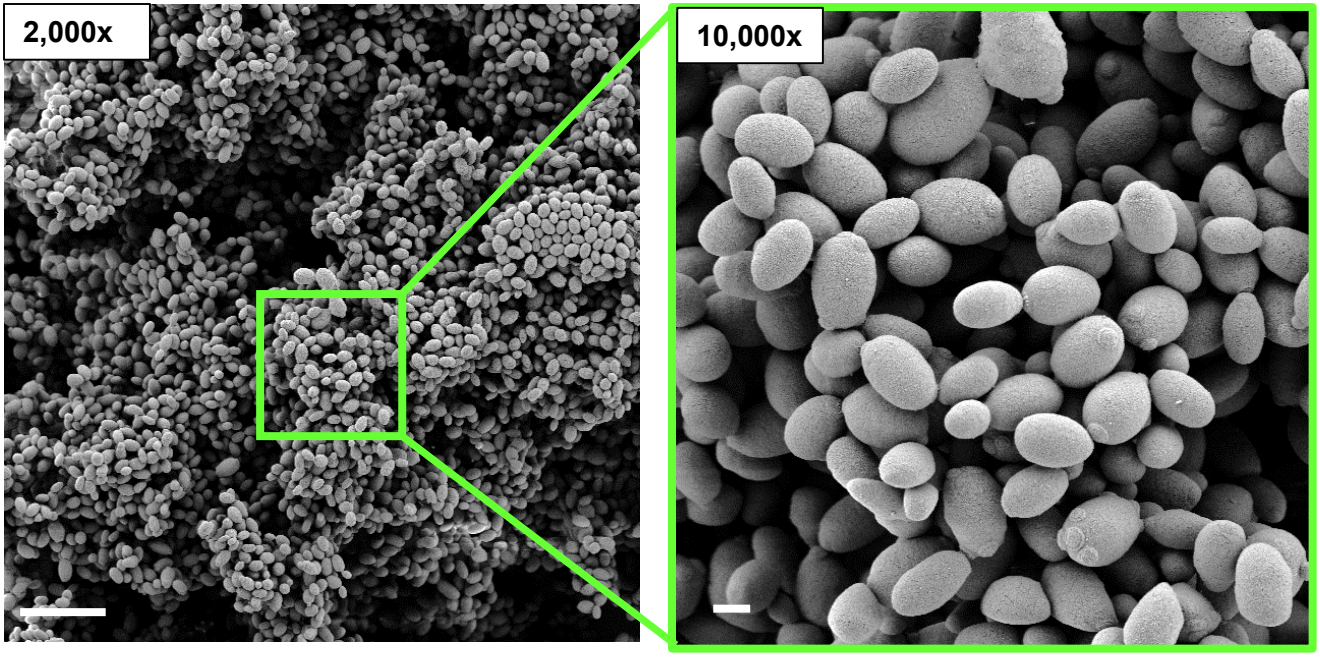
Supplementary Video 3. Neutrophils release tethered groups of *C. glabrata*. As in Video 2, Calcein AM-labeled neutrophils (green) were added planktonic *C. glabrata* and co-cultured for 2 h 30 min. Free DNA stain propidium iodide (red) was added prior to imaging at 2.5-3 h at 20x. To more closely view the movement of propidium idodide-stained *C. glabrata*, images from Video 2 are shown with fluorescent channels only. Arrows show groups of propidium iodide-labeled *C. glabrata* at the start of the image series.

Supplementary Video 4. Cytochalasin D inhibits phagocytosis of *C. glabrata*. Calcein AM-labeled neutrophils (green) were treated with cytochalasin D and added to planktonic *C. glabrata*. Neutrophil interactions were imaged with time-lapse for 1h at

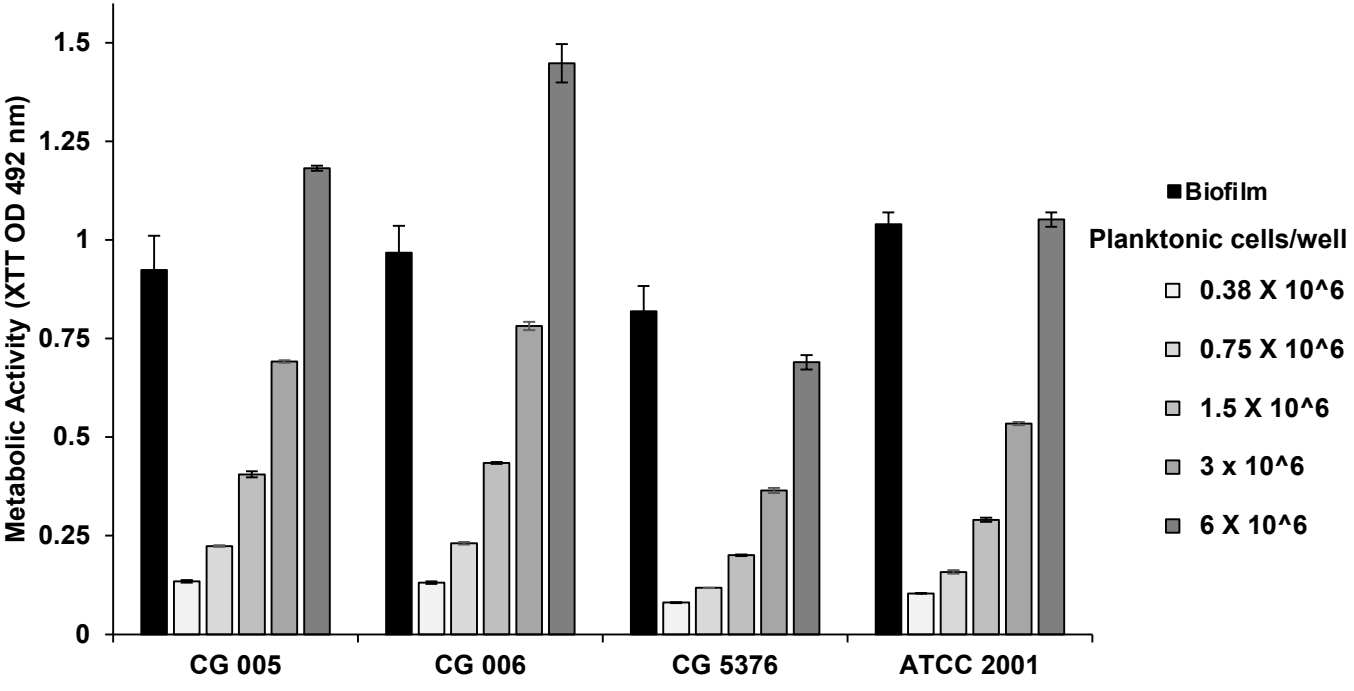
40x. Brightfield and fluorescent images were then collected every 1 min for 1 h and compiled at 5 frames per second.



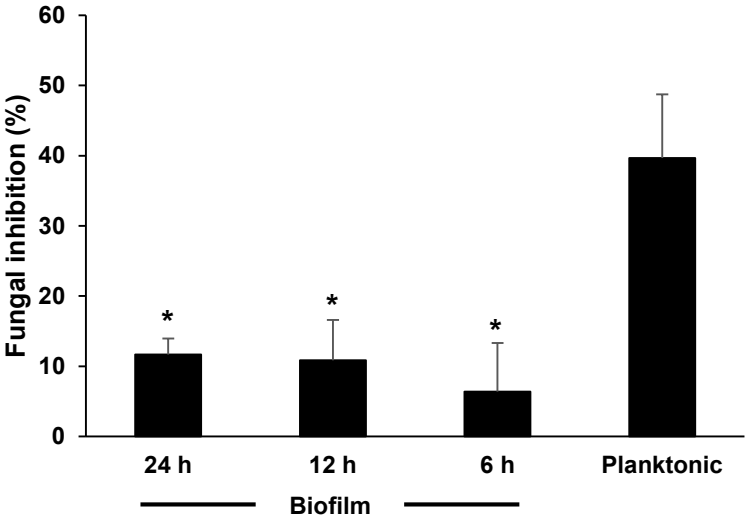
Supplementary Figure 1. Following co-culture with *C. glabrata* for 4 h, samples were immunolabeled with an anti-citrullinated H4 antibody and a fluorescently labeled (DyLight) secondary antibody. The secondary antibody alone control is shown on the left. Brightfield (**a**) and fluorescent 565/620 nm (**b**) images are merged in **c**.



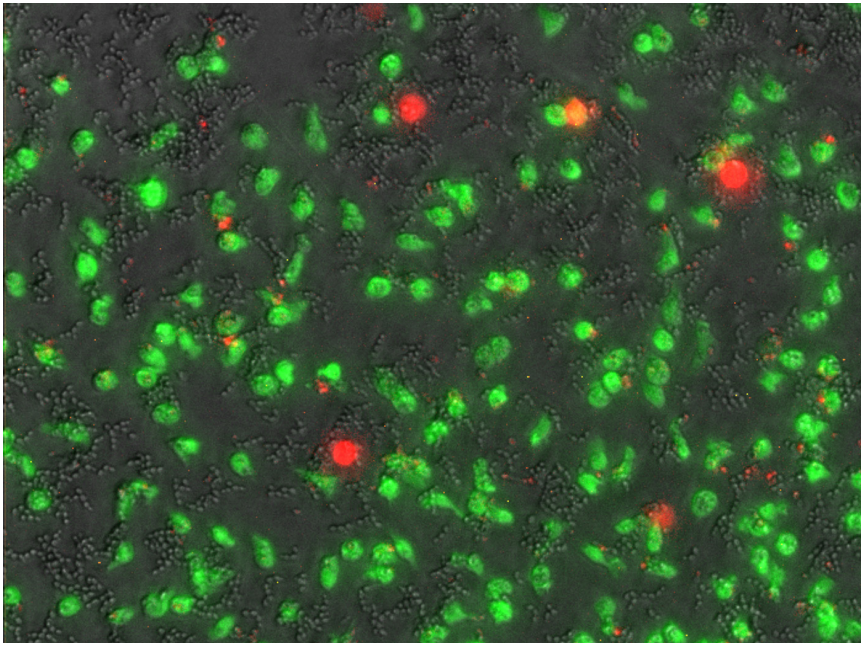
Supplementary Figure 2. ***C. glabrata*** **forms biofilms are composed of yeast.** *C. glabrata* (CG 006) biofilms were grown on coverslips for 24 h and imaged by scanning electron microscopy. Measurement bars represent 10 μm and 1 μm for 2,000x and 10,000x images, respectively.



Supplementary Figure 3. Biofilm and planktonic normalization. Biofilm formation after 24 h was measured using XTT assays. The metabolic activities of biofilms were compared to various concentrations of planktonic cells to determine a similar burden for studies. Assays were performed in triplicate on 3 occasions and mean values of a representative experiment are shown. Error bars represent SD.



Supplementary Figure 4. Neutrophil inhibition of *C. glabrata* during stages of biofilm formation and planktonic growth. Planktonic and biofilm *C. glabrata* were co-cultured with human neutrophils for 4 h and fungal inhibition was estimated by an XTT assay. Results were normalized to the no neutrophil controls, and data from 3 experiments performed in triplicate were combined. Statistical significance was determined using a two-tailed Student's t-test assuming unequal variances, *P<0.05 for biofilm condition compared to planktonic control, SEM shown.



Supplementary Figure 5. Neutrophil-*C. glabrata* interactions at 2.5 h. Calcein AM-labeled neutrophils (green) were added to *C. glabrata* and co-cultured for 2 h 30 min. Free DNA stain propidium iodide (red) was added prior to imaging at 20x.