Science Advances

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Supplementary Materials for

Single yeast cell nanomotions correlate with cellular activity

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References

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/26/eaba3139/DC1)

Movies S1 to S4
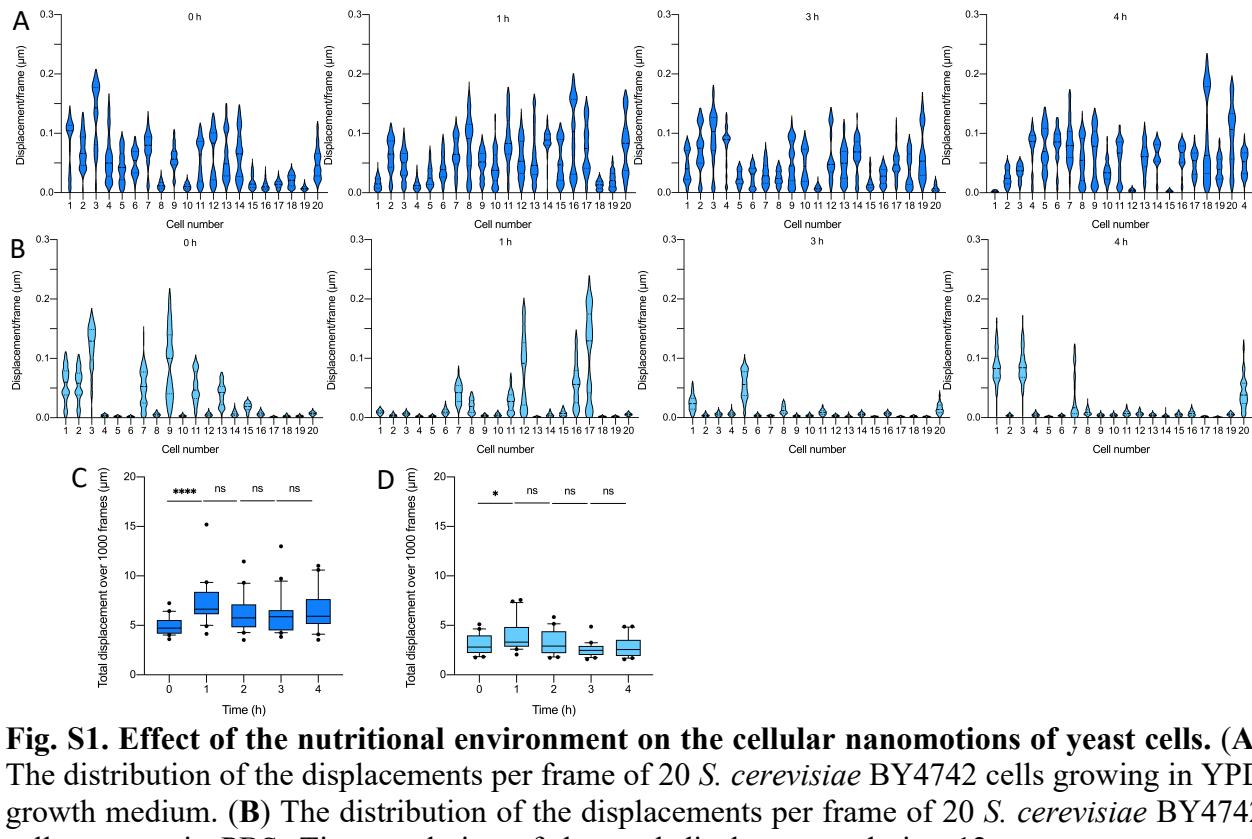
Fig. S1. Effect of the nutritional environment on the cellular nanomotions of yeast cells. (**A**) The distribution of the displacements per frame of 20 *S. cerevisiae* BY4742 cells growing in YPD growth medium. (**B**) The distribution of the displacements per frame of 20 *S. cerevisiae* BY4742 cells present in PBS. Time evolution of the total displacement during 12 s measurement as a function of time for cells (**C**) in YPD growth medium, and (**D**) in PBS. Wilcoxon test: **** P < 0.0001; * P < 0.1; ns: not significant.
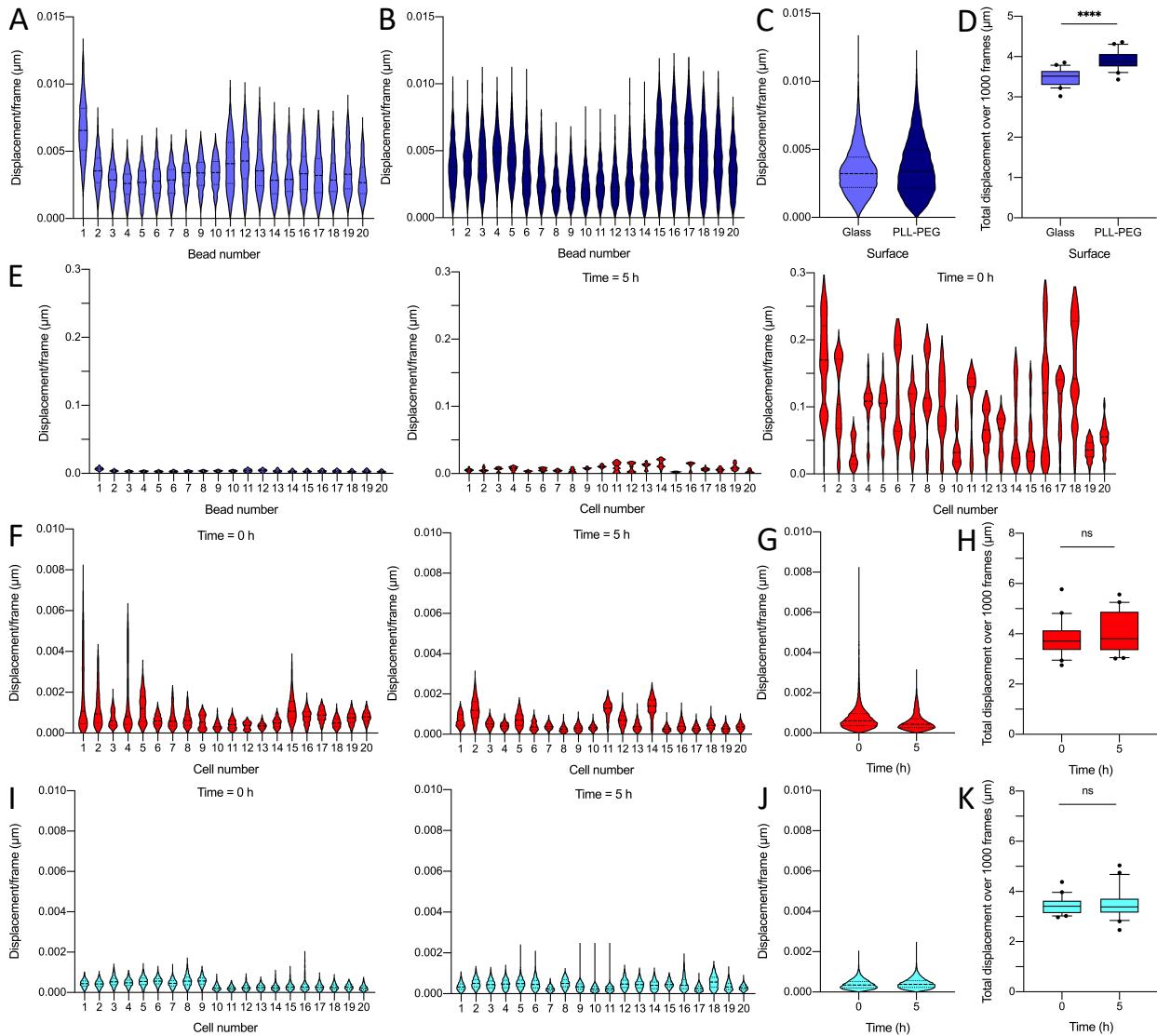
Fig. S2. Movements of silica microbeads and adhesion of cells to the glass surface by concanavalin A. A. The displacement/frame for 20 beads on (**A**) a glass surface and (**B**) PLL-PEG treated surface. (**C**) Displacements per frame of all 20 beads on a glass and PLL-PEG treated surface. (**D**) The total displacement during 12 s measurement of beads on a glass and PLL-PEG treated surface. Wilcoxon test: **** P < 0.0001. (**E**) Comparison of the displacement/frame for 20 beads on glass (left panel) to amphotericin (500 µg/ml) treated (center panel) *C. albicans* DSY294 and untreated cells (right panel). The displacement/frame of 20 cells untreated (left panel) and caspofungin (10 µg/ml) treated cells after 5 h (right panel) for (**F**) the wild-type *C. albicans* DSY294 and (**I**) the hypersusceptible *C. albicans* DSY1024 strain. Displacements per frame of all 20 (**G**) *C. albicans* DSY294 and (**J**) *C. albicans* DSY1024 cells. The total displacement during 12 s measurement of 20 (**H**) *C. albicans* DSY294 and (**K**) *C. albicans* DSY1024 cells.
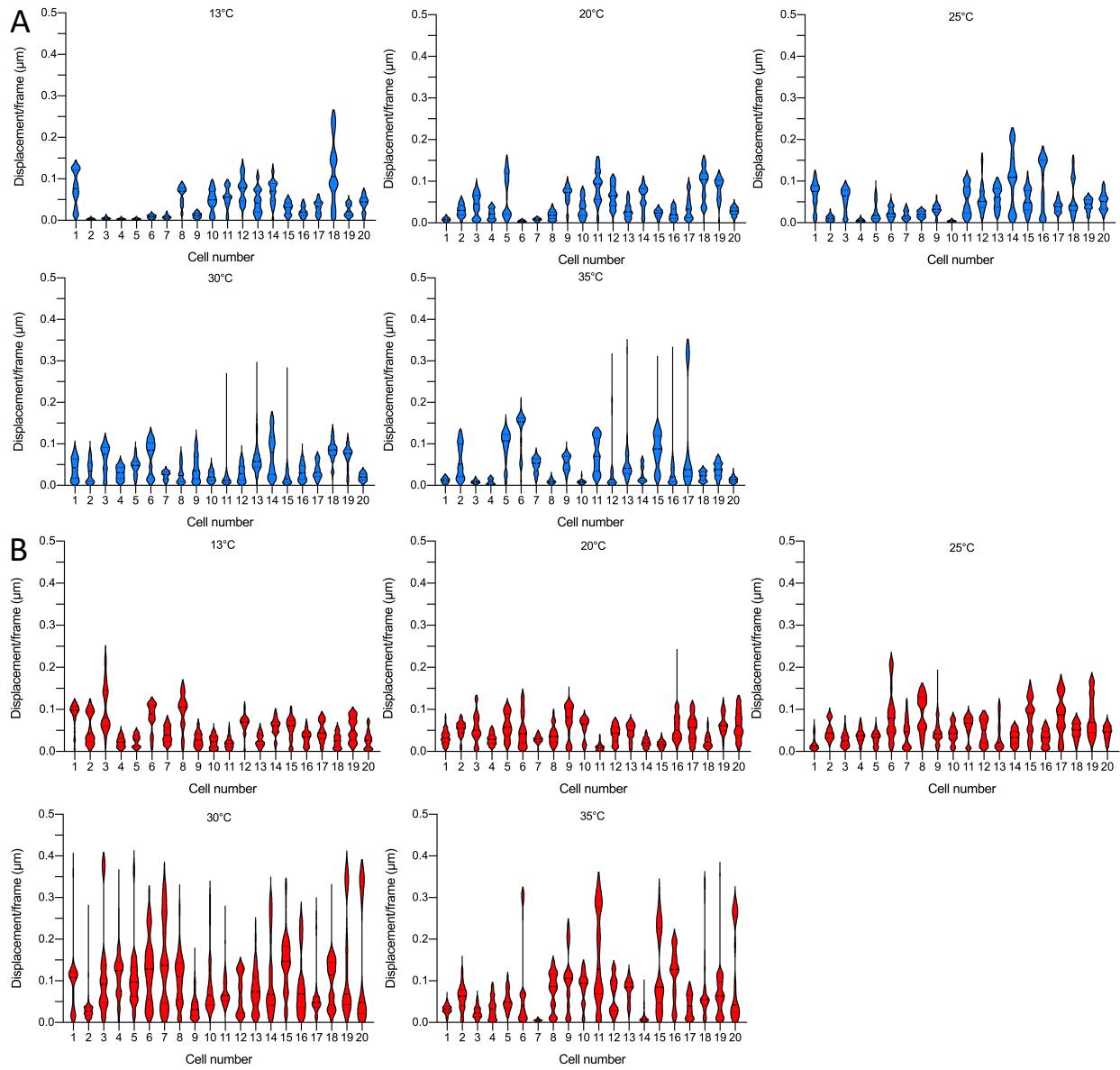
Fig. S3. Effect of the temperature on the cellular nanomotions of yeast cells. The distribution of the displacements per frame of 20 cells as function of the temperature (13°C, 20°C, 25°C, 30°C, 35°C) for (**A**) *S. cerevisiae* BY4742 and (**B**) *C. albicans* DSY294.
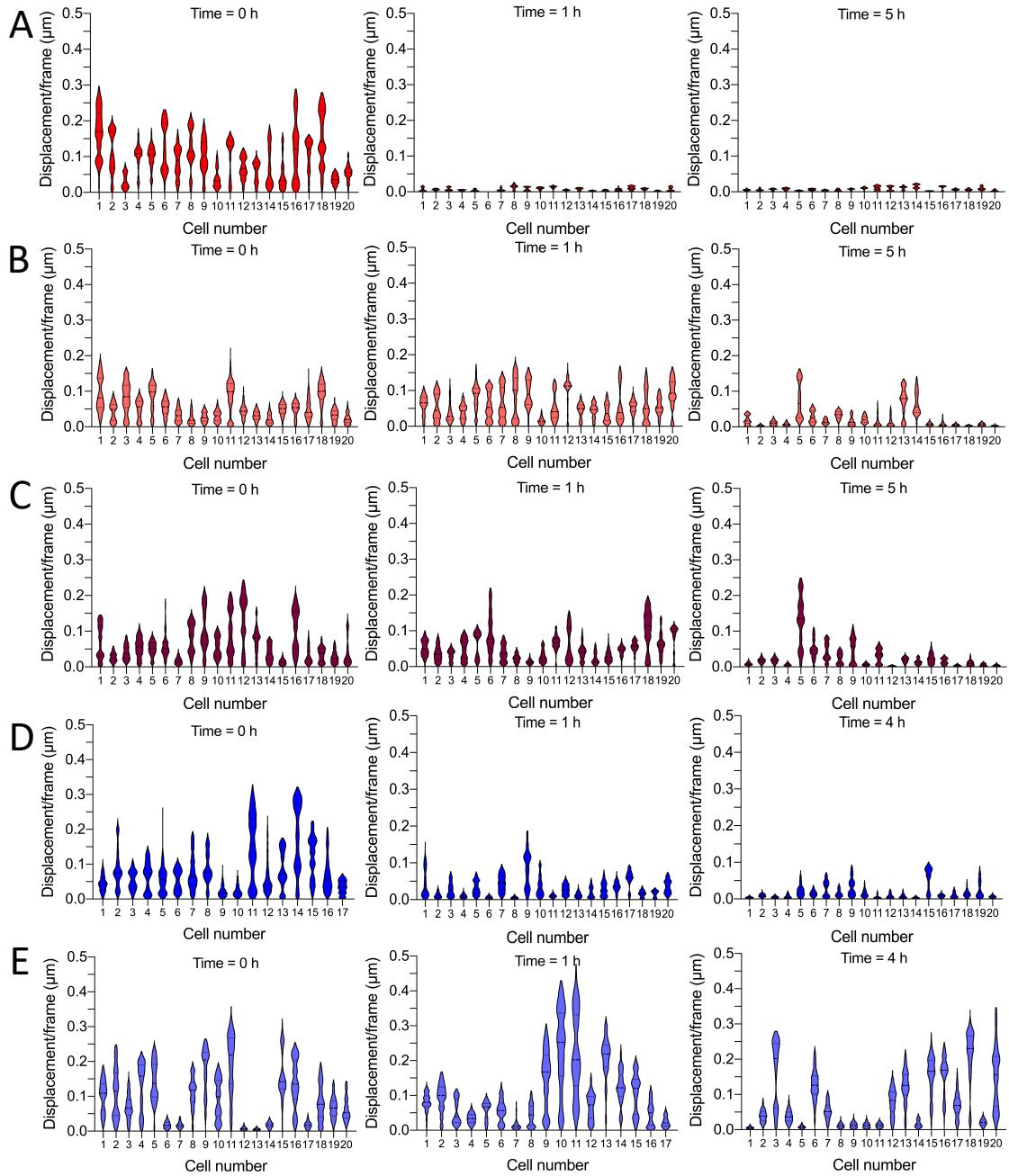
Fig. S4. Effect of antifungals on the cellular nanomotion of *C. albicans*. The displacement/frame of 20 *C. albicans* DSY294 cells treated with (**A**) amphotericin B (500 µg/ml), (**B**) caspofungin (100 µg/ml), and (**C**) fluconazole (400 µg/ml). The displacement/frame of 20 (**D**) hypersusceptible *C. albicans* DSY1024 cells and (**E**) the candin-resistant *C. albicans* DSY4614 cells treated with caspofungin (10 µg/ml).
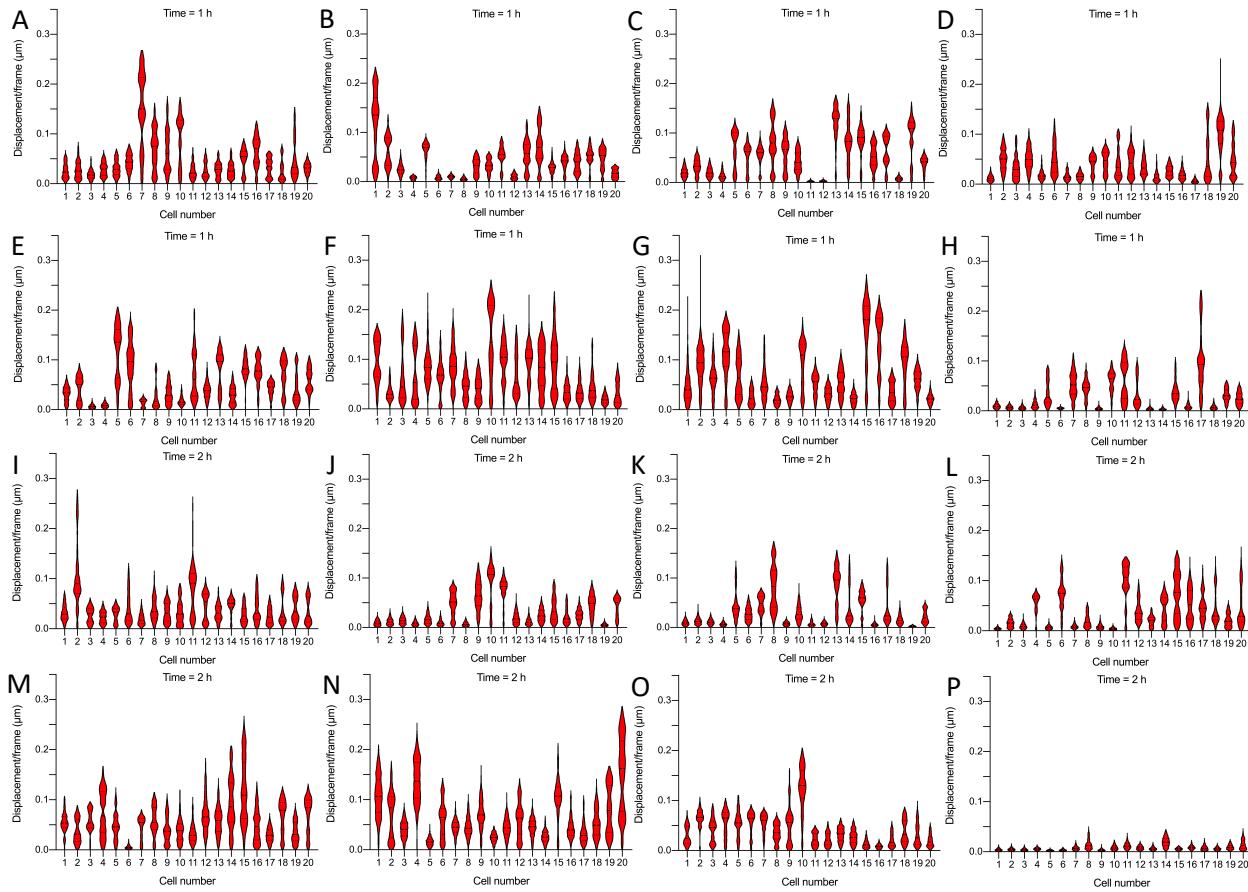
Fig. S5. Effect of increasing amphotericin B concentrations on *C. albicans* DSY294 wild-type strain. Cellular displacements per frame after 1 h for (**A**) untreated cells, (**B**) 0.1 µg/ml, (**C**) 0.5 µg/ml, (**D**) 1 µg/ml, (**E**) 4 µg/ml, (**F**) 10 µg/ml, (**G**) 50 µg/ml, (**H**) 100 µg/ml. Cellular displacements per frame after 2 h for (**I**) untreated cells, (**J**) 0.1 µg/ml, (**K**) 0.5 µg/ml, (**L**) 1 µg/ml, (**M**) 4 µg/ml, (**N**) 10 µg/ml, (**O**) 50 µg/ml, (**P**) 100 µg/ml.
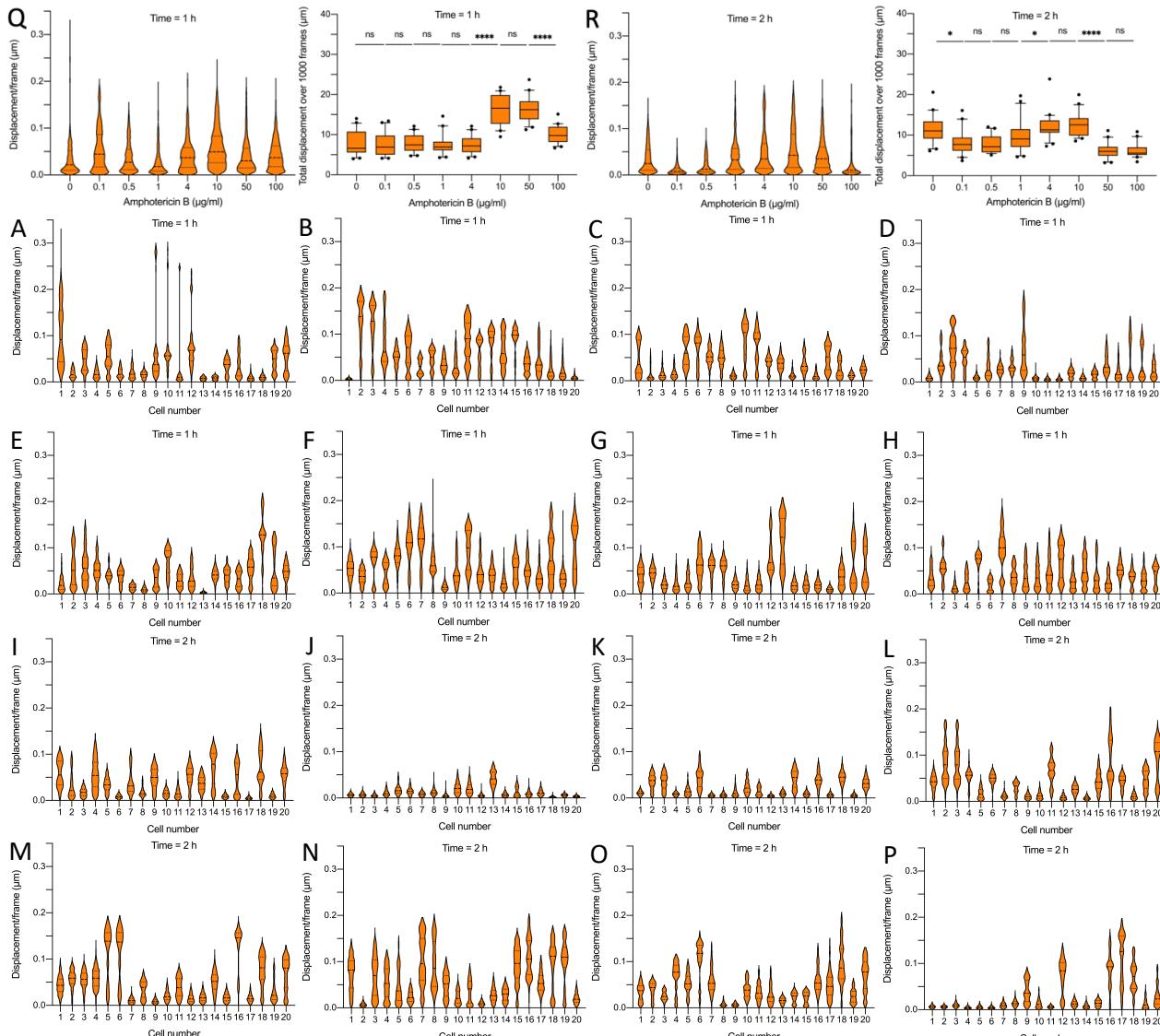
Fig. S6. Effect of increasing amphotericin B concentrations on *C. albicans* CAF2-1 wild-type strain. Cellular displacements per frame after 1 h for (**A**) untreated cells, (**B**) 0.1 µg/ml, (**C**) 0.5 µg/ml, (**D**) 1 µg/ml, (**E**) 4 µg/ml, (**F**) 10 µg/ml, (**G**) 50 µg/ml, (**H**) 100 µg/ml. Cellular displacements per frame after 2 h for (**I**) untreated cells, (**J**) 0.1 µg/ml, (**K**) 0.5 µg/ml, (**L**) 1 µg/ml, (**M**) 4 µg/ml, (**N**) 10 µg/ml, (**O**) 50 µg/ml, (**P**) 100 µg/ml. (**Q**) Cellular displacements per frame (left panel) and the total displacement per frame for 20 cells (right panel) after 1 h treatment, and (**R**) after 2 h treatment. Wilcoxon test: **** P < 0.0001; * P < 0.1; ns: not significant.
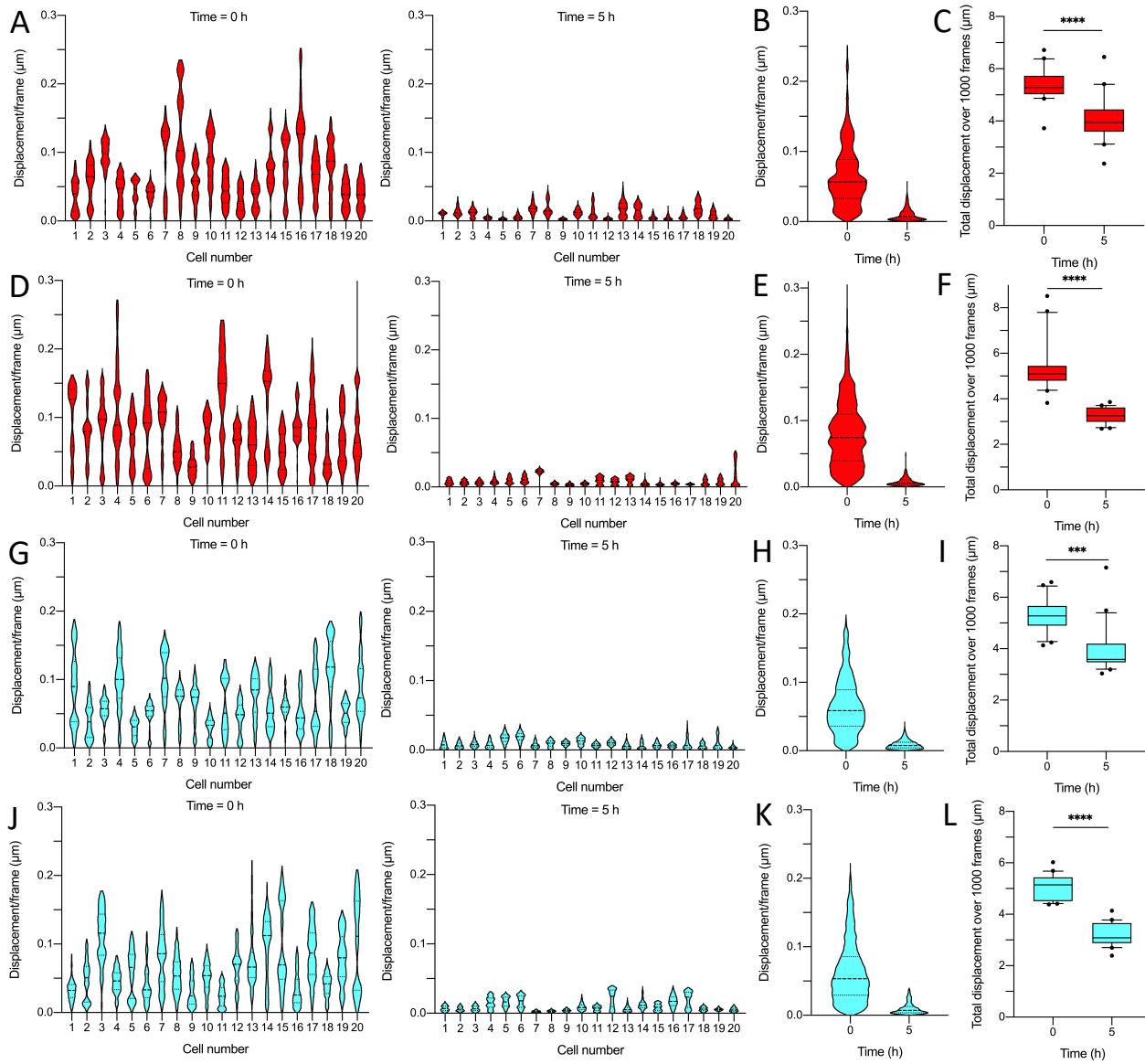
Fig. S7. Effect of the surface on cellular movement of *C. albicans*. The displacement/frame of 20 *C. albicans* DSY294 cells untreated (left panel) and caspofungin (10 µg/ml) treated cells (right panel) on (**A**) a PLL-PEG treated and (**D**) glass surface. Displacements per frame of all 20 cells on (**B**) a PLL-PEG and (**E**) a glass treated surface. The total displacement during 12 s measurement of 20 cells on (**C**) a PLL-PEG and (**F**) glass treated surface. The displacement/frame of 20 *C. albicans* DSY1024 cells untreated (left panel) and caspofungin (10 µg/ml) treated cells (right panel) on (**G**) a PLL-PEG treated and (**J**) glass surface. Displacements per frame of all 20 cells on (**H**) a PLL-PEG and (**K**) a glass treated surface. The total displacement during 12 s measurement of 20 cells on (**I**) a PLL-PEG and (**L**) glass treated surface. Wilcoxon test: **** P < 0.0001, *** P < 0.001.
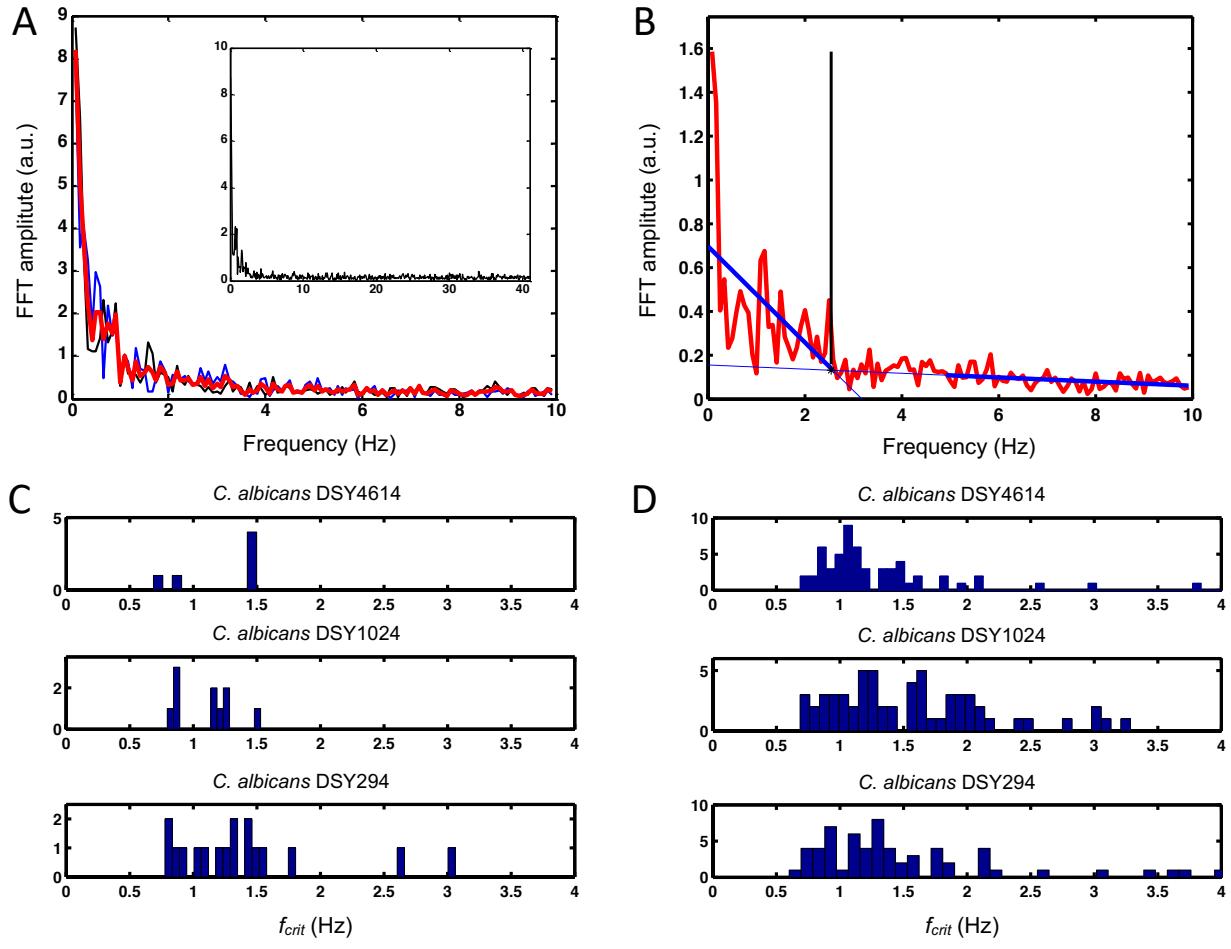
Fig. S8. Determination of the frequency range of cell movements and critical frequency. (A) Typical FFT spectra obtained from two optically recorded signals of one *C. albicans* cell movements in the horizontal (black) and vertical (blue) directions, as well as their average (red). Insert: The same FTT spectrum up to 40 Hz. **(B)** Determination of the critical frequency via the DRIM method of one typical *S. cerevisiae* BY4742 cell in PBS after 3 h (f_{crit} = 2.54 Hz). **(C)** Histograms of f_{crit} for untreated caspofungin resistant *C. albicans* DSY4614, hypersusceptible *C. albicans* DSY1024 and wild-type *C. albicans* DSY294. **(D)** Histograms of f_{crit} for caspofungin treated (10 µg/ml) resistant *C. albicans* DSY4614, hypersusceptible *C. albicans* DSY1024 and wild-type *C. albicans* DSY294. The results are pooled from 1-5-hour treatments.
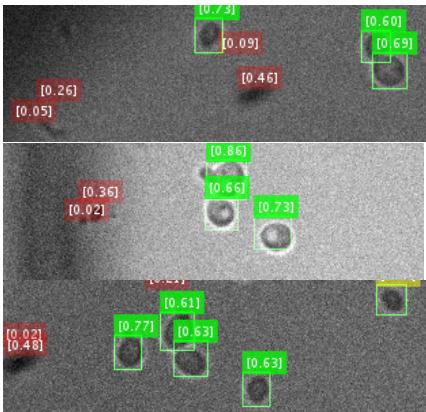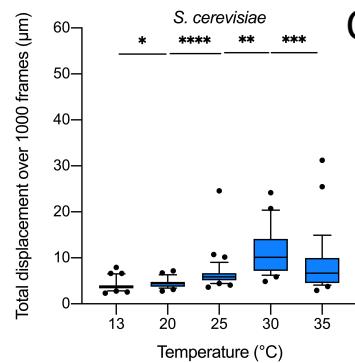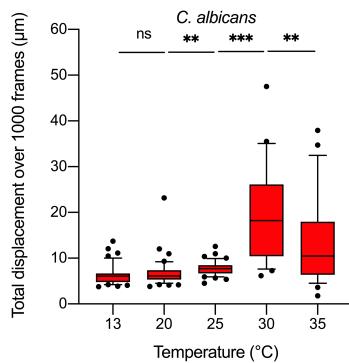
Fig. S9. Effect of the temperature and nutritional environment on the optical nanomotion of yeast cells. (**A**) Deep learning cells detection method. Cells automatically detected with high confidence (in green) when processing different videos. Although a few cells are missed, most of them are properly detected with high confidence. Green denotes high confidence cell, yellow denotes in threshold confidence, red indicates that detection is most likely to be an artefact. Effect of the temperature on the total displacement: results obtained with the automated detection method. Number of cells detected: (**B**) 147 cells (average 29 cells per condition) were analysed for *S. cerevisiae*, and (**C**) 194 cells (on average 39 cells per condition) were analysed for *C. albicans*. Wilcoxon test: **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, ns: not significant.

Table S1. Yeast strains used in this study.

Micro-organism	Type	Strains	Characteristics	Genotype/Description	Reference
Candida albicans	Lab strain	CAF2-1	Wild type strain	*Δura3::imm434/URA3*	(*35*)
Candida albicans	Lab strain	DSY1024	Mutant for efflux systems	*Δcdr1:hisG/Δcdr1::hisG;Δcdr2 ::hisG; Δflu1::hisG/Δflu1::hisG; Δmdr1::hisG-URA3-hisG/Δmdr1::hisG*	(*36*)
Candida albicans	Clinical strain	DSY294	Azole-susceptible strain	Wild type	(*37*)
Candida albicans	Clinical strain	DSY4614	Candin-resistant strain	FSK1 mutant P649H	Unpublished
Candida glabrata	Clinical strain	DSY562	Azole-susceptible strain	Wild type	(*20*)
Candida lusitaniae	Clinical strain	DSY4606	Wild type strain	Wild type	Unpublished
Saccharomyces cerevisiae	Lab strain	BY4742	Wild type strain	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	(*38*)

Movie S1. ("S. cerevisiae_before ethanol.m4v")

Examples of recorded videos of yeast nanomotions: effect of ethanol on *S. cerevisiae*: *S. cerevisiae* before ethanol (70% v/v) treatment.

Movie S2. ("S. cerevisiae-after ethanol-60 min.m4v")

Examples of recorded videos of yeast nanomotions: effect of ethanol on *S. cerevisiae*: *S. cerevisiae* after (60 min) ethanol (70% v/v) treatment.

Movie S3. ("video_ai-cells-detection_Candida albicans dsy294-20C.mp4")

Examples of detection of cells using the deep learning algorithm: *C. albicans* (at a temperature of 20°C).

Movie S4. ("video_ai-cells-detection_Saccharomyces cerevisiae-30C.mp4")

Examples of detection of cells using the deep learning algorithm: *S. cerevisiae* (at a temperature of 30°C).

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