



## SUPPLEMENTARY DATA

### Supplementary movies

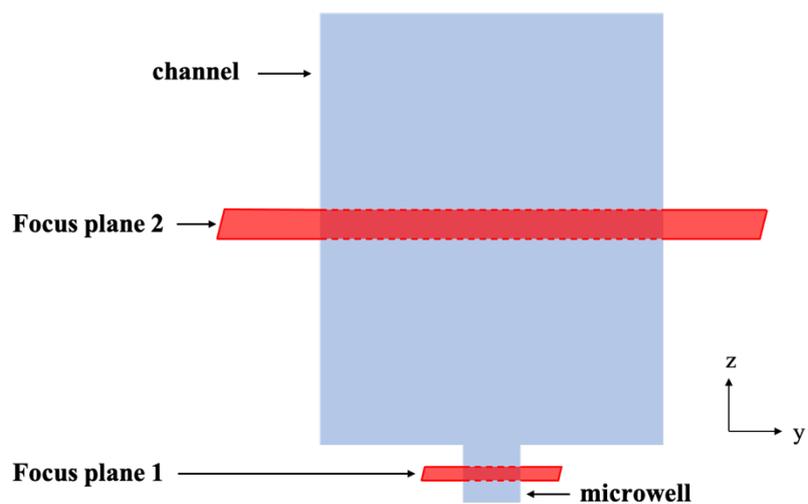
**Video S1.** Simulation of the movement of 100 yeast cells with the flowing medium in the channel and the circular movement of one yeast cell in the microwell due to the created fluid vortex. The colours of the streamlines correspond to the magnitude of the velocity as shown in the scale bar of Figure 4A.

**Video S2.** Experimental observation of the circular movement of the yeast cell in the microwell during the continuous flow of liquid in the channel at a flow rate of 120  $\mu\text{l}/\text{min}$  (movie recording at 20 fps).

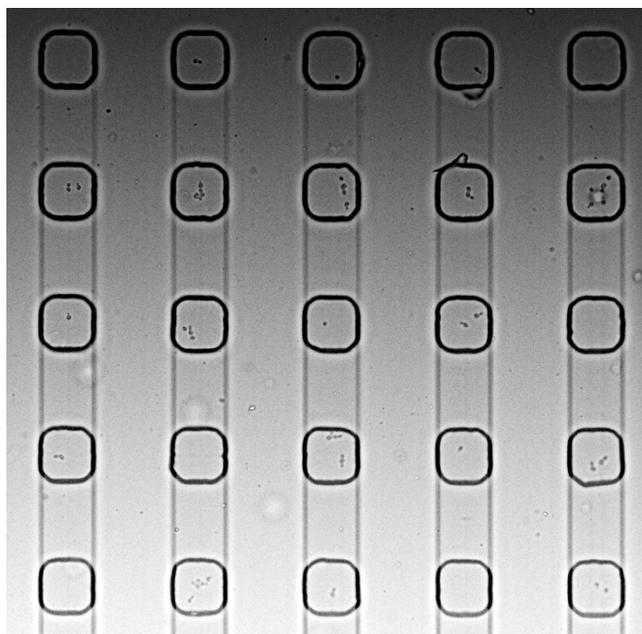
**Video S3.** Example of the nanomotion of *C. albicans* CAF2-1 cell 8 in a microwell before treatment (1  $\mu\text{g}/\text{ml}$  caspofungin).

**Video S4.** Example of the nanomotion of *C. albicans* CAF2-1 cell 8 in a microwell after 10 min of treatment (1  $\mu\text{g}/\text{ml}$  caspofungin).

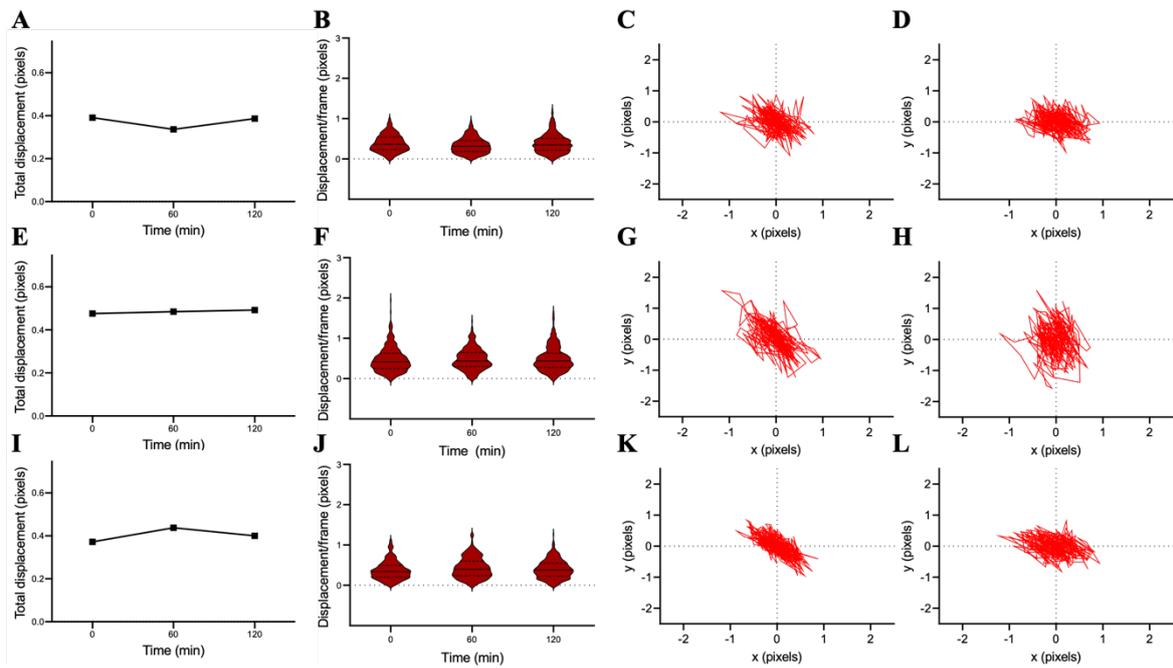
## Supplementary figures



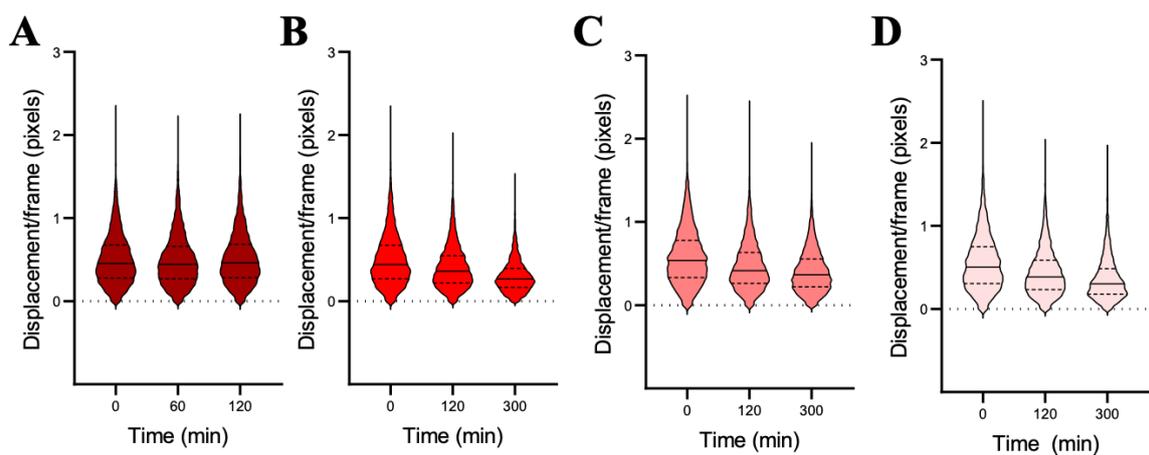
**Figure S1.** Transversal (zy) cross-section of the channel and microwell with focus plane 1 where we observed the evolution of the propidium iodide (PI) concentration in the well and focus plane 2 where we observed the evolution of the PI concentration in the channel.



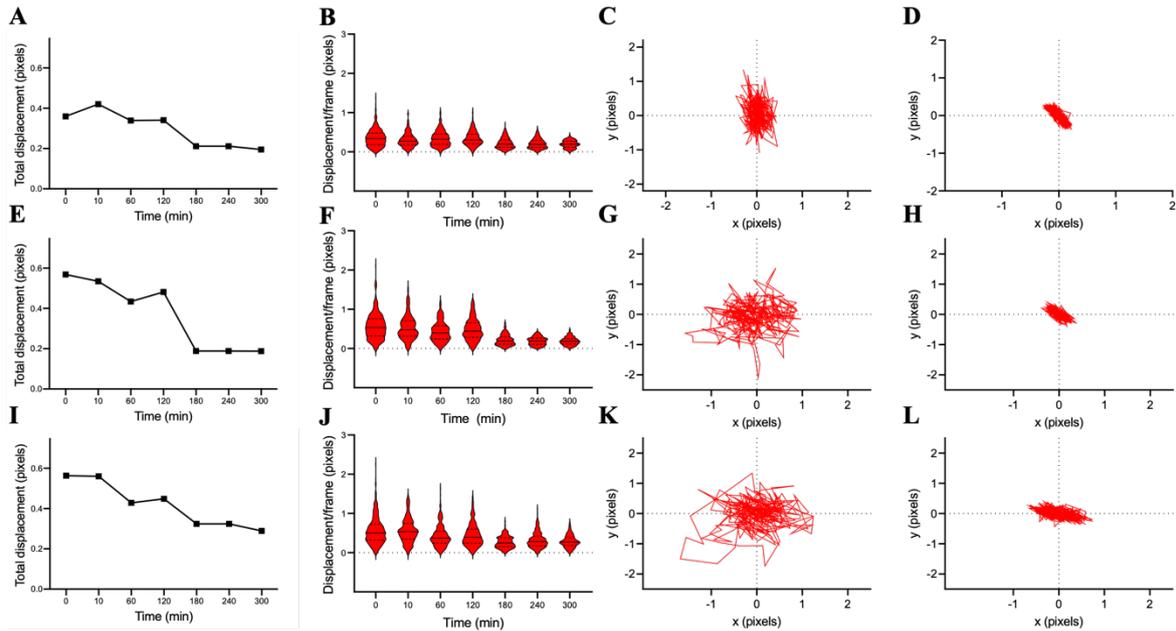
**Figure S2.** Example of a part of the microwell array showing the occupancy of the microwells by the yeast cells.



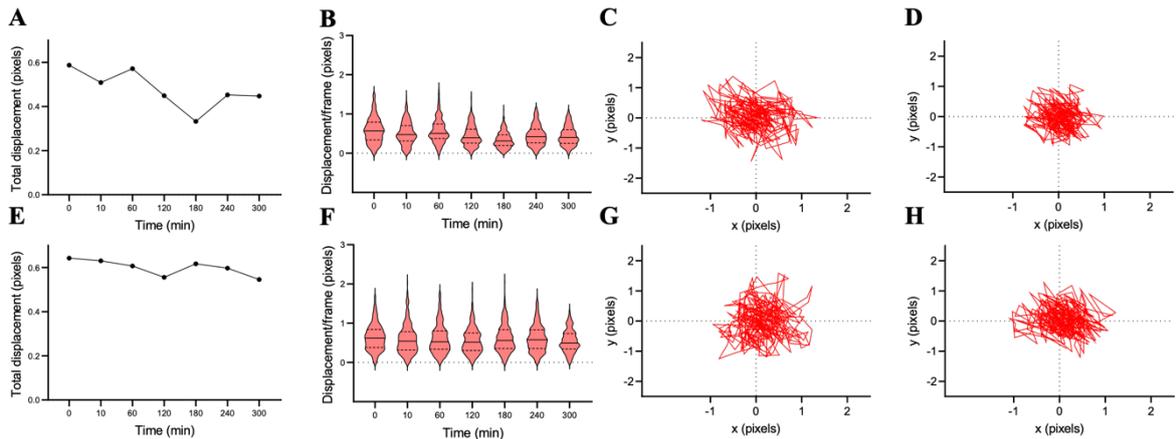
**Figure S3.** Control experiment for the *C. albicans* CAF2-1 strain grown in YPD medium for 3 selected cells: cell 1, 3 and 4 (A) Total displacements (pixels) as a function of time for cell 1. (B) Distribution of the displacement/frame as a function of time for cell 1. (C) X-y displacement at the time point 0 min for cell 1. (D) X-y displacement at the time point 120 min for cell 1. (E) Total displacements (pixels) as a function of time for cell 3. (F) Distribution of the displacement/frame as a function of time for cell 3. (G) X-y displacement at the time point 0 min for cell 3. (H) X-y displacement at the time point 120 min for cell 3. (I) Total displacements (pixels) as a function of time for cell 4. (J) Distribution of the displacement/frame as a function of time for cell 4. (K) X-y displacement at the time point 0 min for cell 4. (L) X-y displacement at the time point 120 min for cell 4.



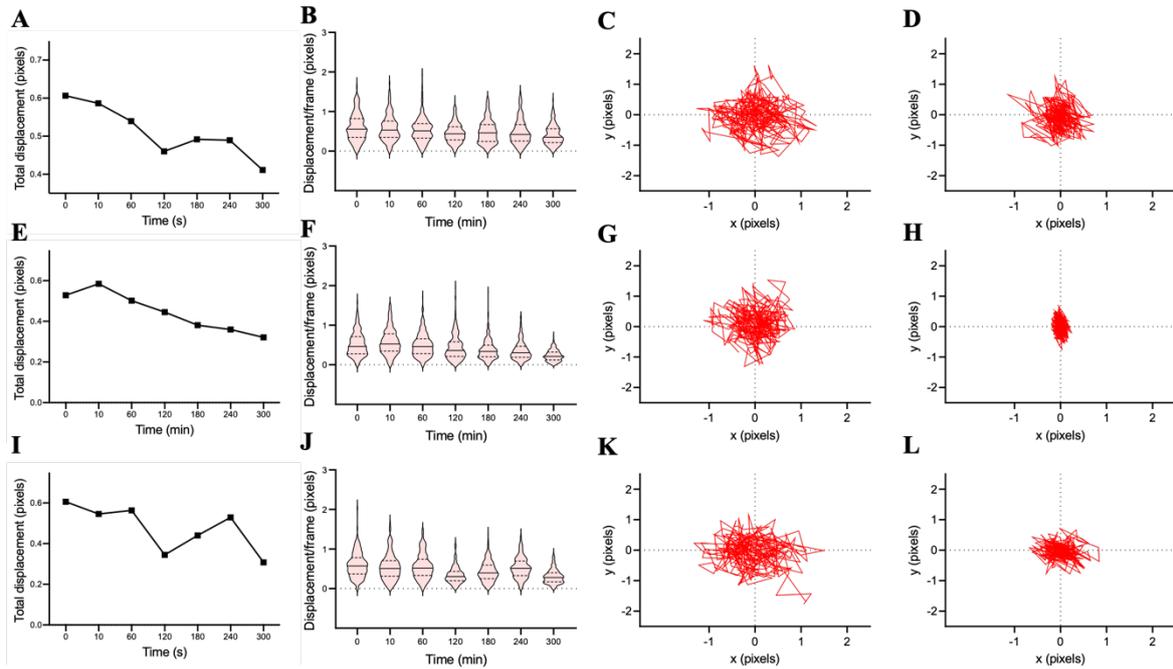
**Figure S4.** Averaged cellular nanomotion for 20 cells of *C. albicans* CAF2-1. (A) Distribution of the displacements/frames during 2 h growth (control). Distribution of the displacements/frames during (B) 0.5 µg/ml (C) 1 µg/ml, and (D) 100 µg/ml caspofungin treatment.



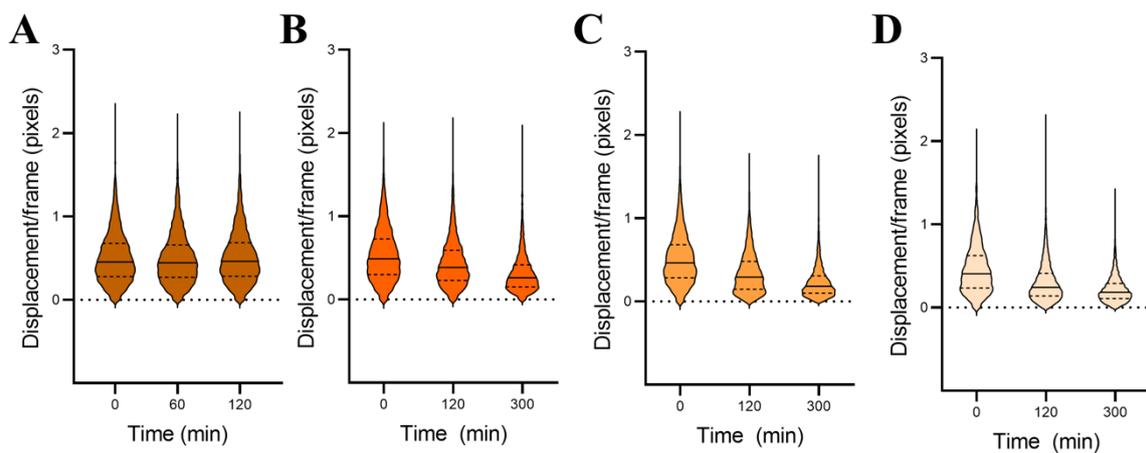
**Figure S5.** Effect of the antifungal caspofungin (0.5  $\mu\text{g/mL}$ ) on the cellular nanomotion for 3 selected cells: cell 1, 10, and 12 of *C. albicans* CAF2-1. (A) Total displacements (pixels) as a function of time for cell 1. (B) Distribution of the displacement/frame as a function of time for cell 1. (C) X-y displacement at the time point 0 min for cell 1. (D) X-y displacement at the time point 300 min for cell 1. (E) Total displacements (pixels) as a function of time for cell 10. (F) Distribution of the displacement/frame as a function of time for cell 10. (G) X-y displacement at the time point 0 min for cell 10. (H) X-y displacement at the time point 300 min for cell 10. (I) Total displacements (pixels) as a function of time for cell 12. (J) Distribution of the displacement/frame as a function of time for cell 12. (K) X-y displacement at the time point 0 min for cell 12. (L) X-y displacement at the time point 300 min for cell 12.



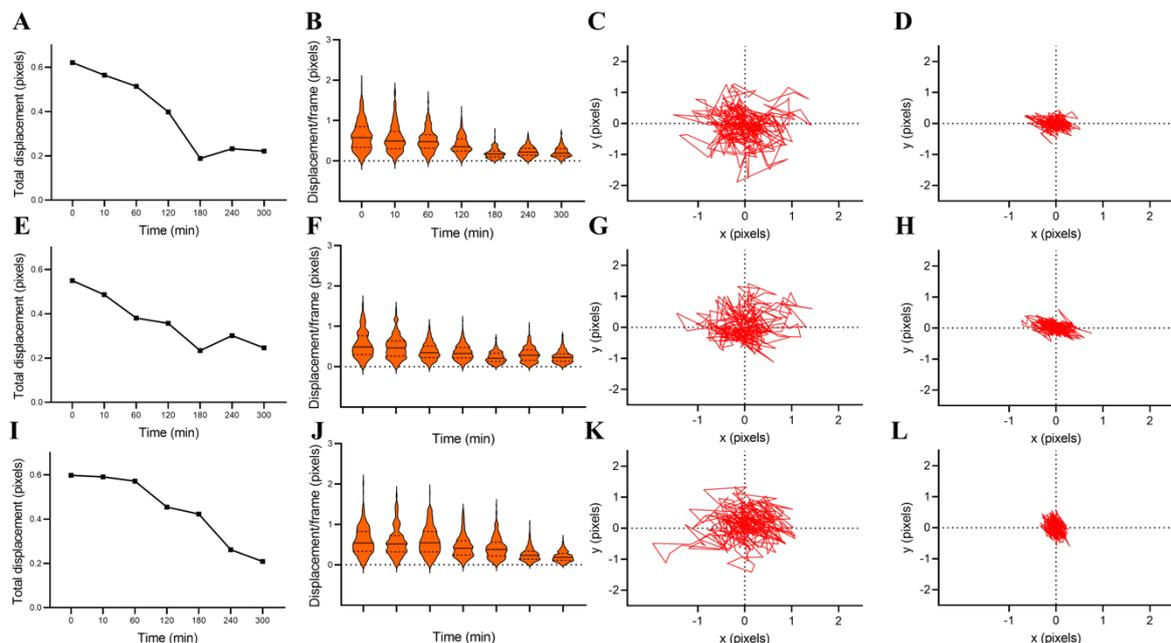
**Figure S6.** Effect of the antifungal caspofungin (1  $\mu\text{g/mL}$ ) on the cellular nanomotion for 2 selected cells: cell 1 and 2 of *C. albicans* CAF2-1. (A) Total displacements (pixels) as a function of time for cell 1. (B) Distribution of the displacement/frame as a function of time for cell 1. (C) X-y displacement at the time point 0 min for cell 1. (D) X-y displacement at the time point 300 min for cell 1. (E) Total displacements (pixels) as a function of time for cell 2. (F) Distribution of the displacement/frame as a function of time for cell 2. (G) X-y displacement at the time point 0 min for cell 2. (H) X-y displacement at the time point 300 min for cell 2.



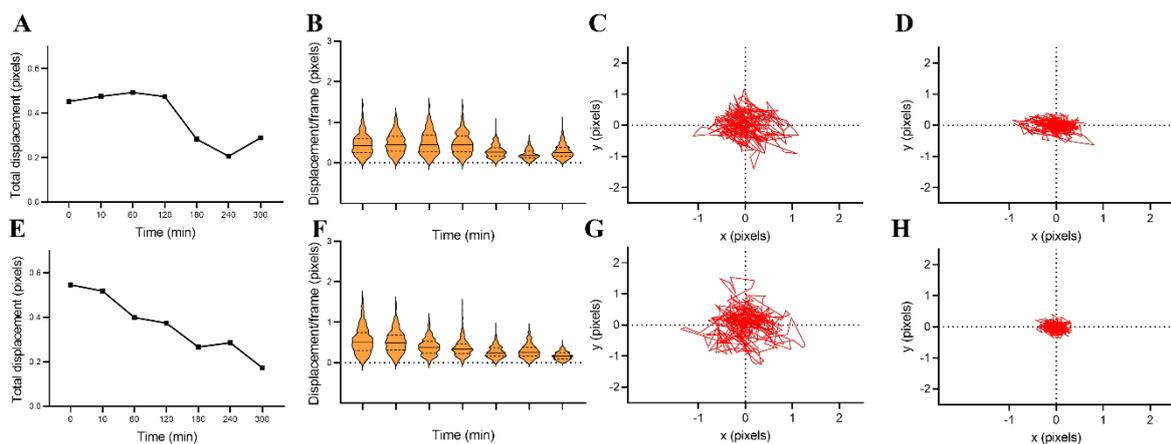
**Figure S7.** Effect of the antifungal caspofungin (100  $\mu\text{g/mL}$ ) on the cellular nanomotion for 3 selected cells: cell 5, 10 and 12 of *C. albicans* CAF2-1. (A) Total displacements (pixels) as a function of time for cell 5. (B) Distribution of the displacement/frame as a function of time for cell 5. (C) X-y displacement at the time point 0 min for cell 5. (D) X-y displacement at the time point 300 min for cell 5. (E) Total displacements (pixels) as a function of time for cell 10. (F) Distribution of the displacement/frame as a function of time for cell 10. (G) X-y displacement at the time point 0 min for cell 10. (H) X-y displacement at the time point 300 min for cell 10. (I) Total displacements (pixels) as a function of time for cell 12. (J) Distribution of the displacement/frame as a function of time for cell 12. (K) X-y displacement at the time point 0 min for cell 12. (L) X-y displacement at the time point 300 min for cell 12.



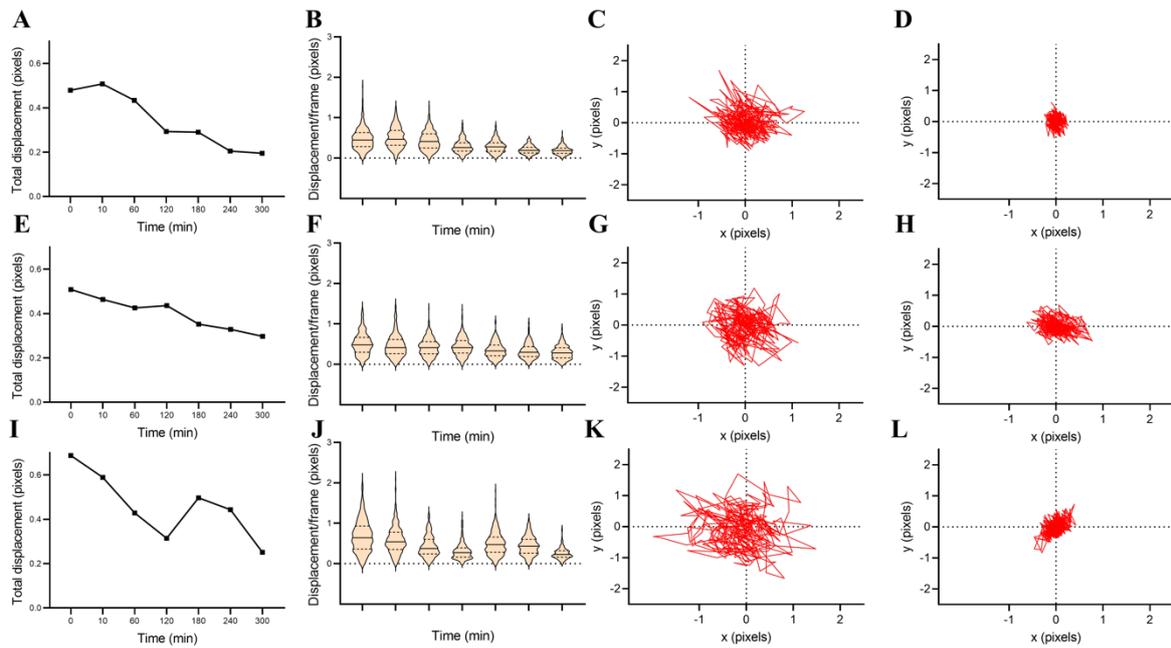
**Figure S8.** Averaged cellular nanomotion for 20 cells of *C. albicans* CAF2-1. (A) Distribution of the displacements/frames during 2 h growth (control). (B) Distribution of the displacements/frames during 0.5  $\mu\text{g/ml}$  fluconazole treatment. (C) Distribution of the displacements/frames during 1  $\mu\text{g/ml}$  fluconazole treatment. (D) Distribution of the displacements/frames during 100  $\mu\text{g/ml}$  fluconazole treatment.



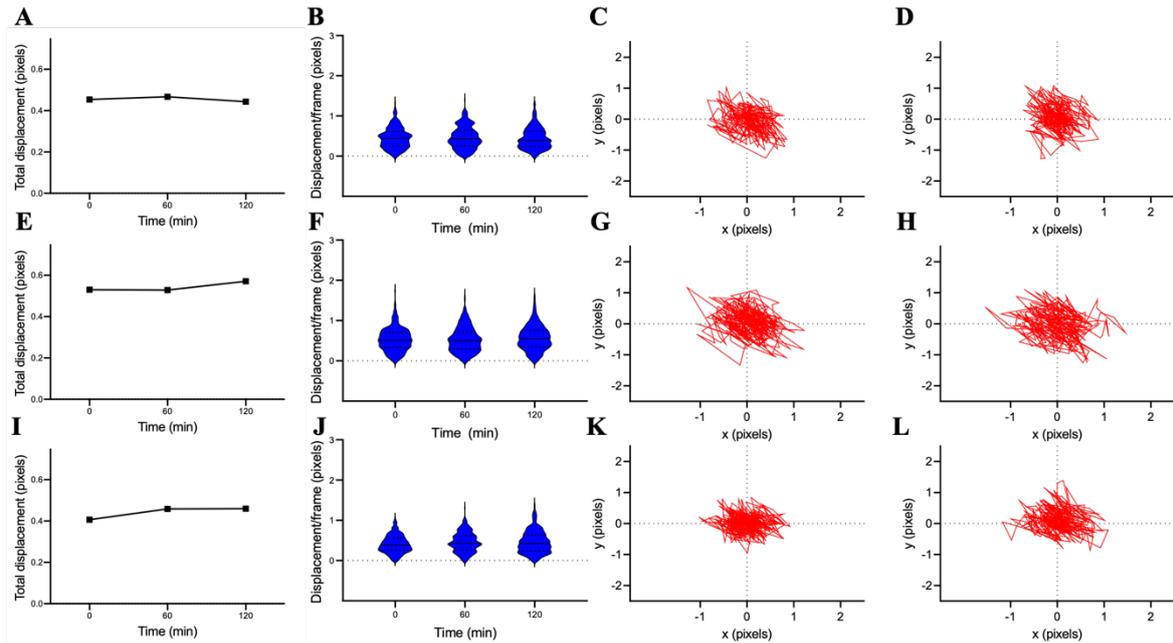
**Figure S9.** Effect of the antifungal fluconazole (0.5  $\mu\text{g/mL}$ ) on the cellular nanomotion for 3 selected cells: cell 5, 6, and 7 of *C. albicans* CAF2-1. (A) Total displacements (pixels) as a function of time for cell 5. (B) Distribution of the displacement/frame as a function of time for cell 5. (C) X-y displacement at the time point 0 min for cell 5. (D) X-y displacement at the time point 300 min for cell 5. (E) Total displacements (pixels) as a function of time for cell 6. (F) Distribution of the displacement/frame as a function of time for cell 6. (G) X-y displacement at the time point 0 min for cell 6. (H) X-y displacement at the time point 300 min for cell 6. (I) Total displacements (pixels) as a function of time for cell 7. (J) Distribution of the displacement/frame as a function of time for cell 7. (K) X-y displacement at the time point 0 min for cell 7. (L) X-y displacement at the time point 300 min for cell 7.



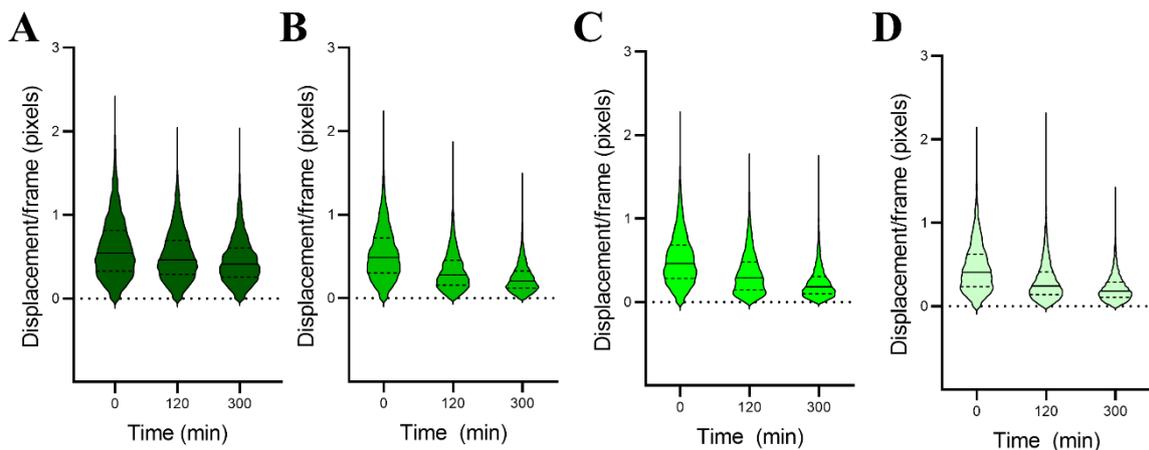
**Figure S10.** Effect of the antifungal fluconazole (1  $\mu\text{g/mL}$ ) on the cellular nanomotion for 2 selected cells: cell 18, and 19 of *C. albicans* CAF2-1. (A) Total displacements (pixels) as a function of time for cell 18. (B) Distribution of the displacement/frame as a function of time for cell 18. (C) X-y displacement at the time point 0 min for cell 18. (D) X-y displacement at the time point 300 min for cell 18. (E) Total displacements (pixels) as a function of time for cell 19. (F) Distribution of the displacement/frame as a function of time for cell 19. (G) X-y displacement at the time point 0 min for cell 19. (H) X-y displacement at the time point 300 min for cell 19.



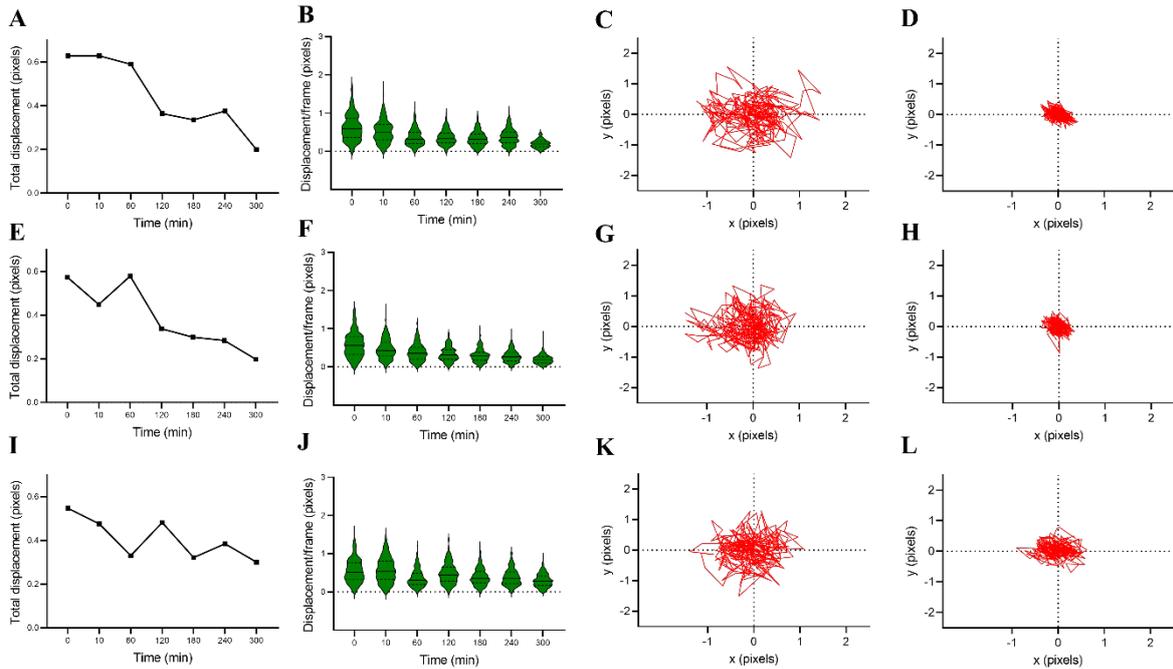
**Figure S11.** Effect of the antifungal fluconazole (100  $\mu\text{g/mL}$ ) on the cellular nanomotion for 3 selected cells: cell 2, 3, and 4 of *C. albicans* CAF2-1. (A) Total displacements (pixels) as a function of time for cell 2. (B) Distribution of the displacement/frame as a function of time for cell 2. (C) X-y displacement at the time point 0 min for cell 2. (D) X-y displacement at the time point 300 min for cell 2. (E) Total displacements (pixels) as a function of time for cell 3. (F) Distribution of the displacement/frame as a function of time for cell 3. (G) X-y displacement at the time point 0 min for cell 3. (H) X-y displacement at the time point 300 min for cell 3. (I) Total displacements (pixels) as a function of time for cell 4. (J) Distribution of the displacement/frame as a function of time for cell 4. (K) X-y displacement at the time point 0 min for cell 4. (L) X-y displacement at the time point 300 min for cell 4.



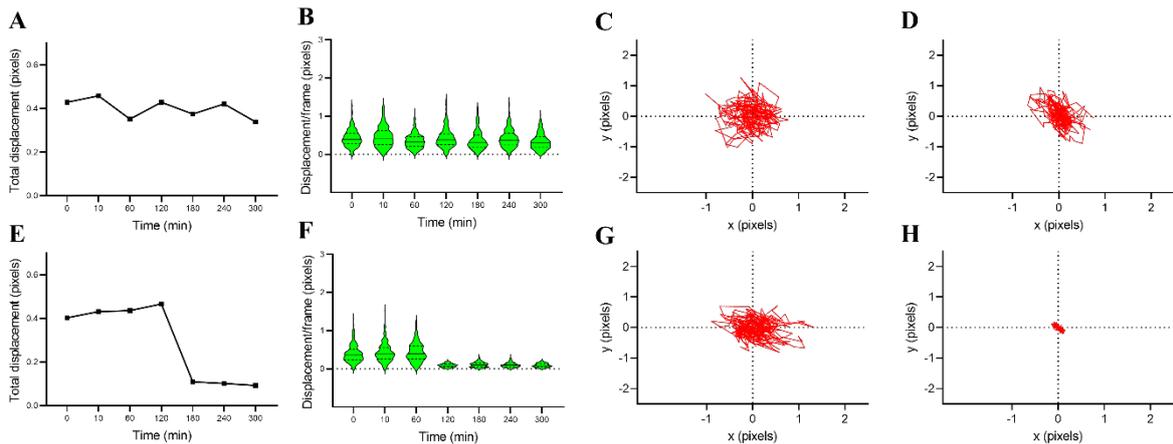
**Figure S12.** Control experiment for the *C. albicans* DSY1024 strain grown in YPD medium for 3 selected cells: cell 1, 5 and 10. (A) Total displacements (pixels) as a function of time for cell 1. (B) Distribution of the displacement/frame as a function of time for cell 1. (C) X-y displacement at the time point 0 min for cell 1. (D) X-y displacement at the time point 120 min for cell 1. (E) Total displacements (pixels) as a function of time for cell 5. (F) Distribution of the displacement/frame as a function of time for cell 5. (G) X-y displacement at the time point 0 min for cell 5. (H) X-y displacement at the time point 120 min for cell 5. (I) Total displacements (pixels) as a function of time for cell 10. (J) Distribution of the displacement/frame as a function of time for cell 10. (K) X-y displacement at the time point 0 min for cell 10. (L) X-y displacement at the time point 120 min for cell 10.



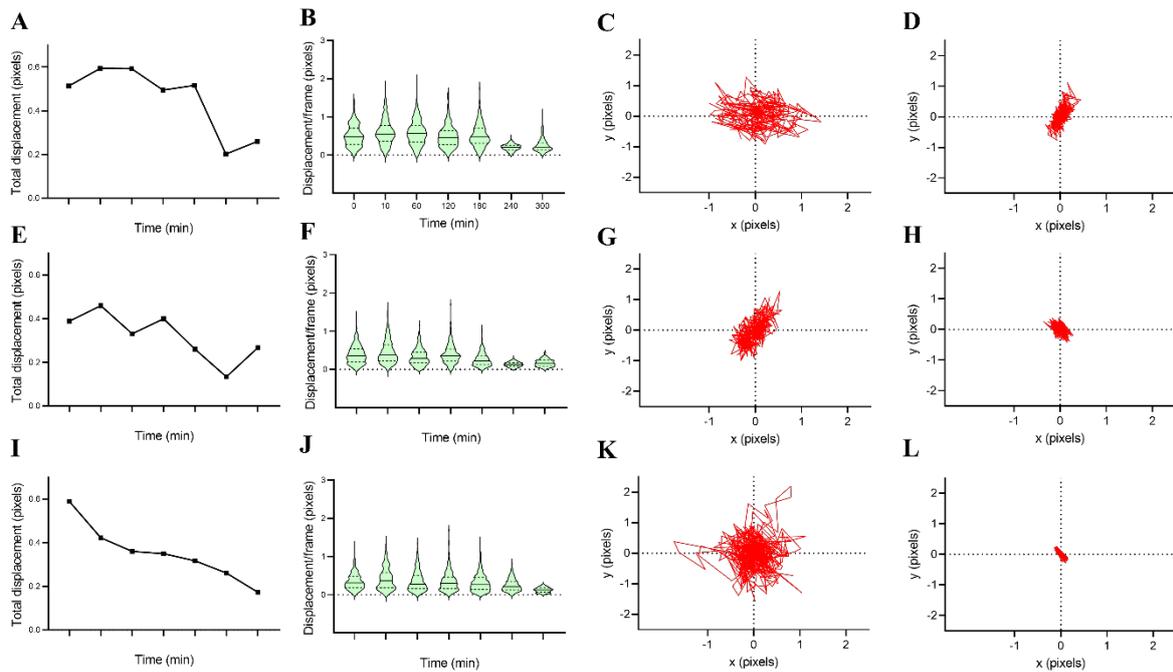
**Figure S13.** Averaged cellular nanomotion for 20 cells of *C. albicans* DSY1024. (A) Distribution of the displacements/frames during 2 h growth (control). (B) Distribution of the displacements/frames during 0.5  $\mu\text{g/ml}$  fluconazole treatment. (C) Distribution of the displacements/frames during 1  $\mu\text{g/ml}$  fluconazole treatment. (D) Distribution of the displacements/frames during 100  $\mu\text{g/ml}$  fluconazole treatment.



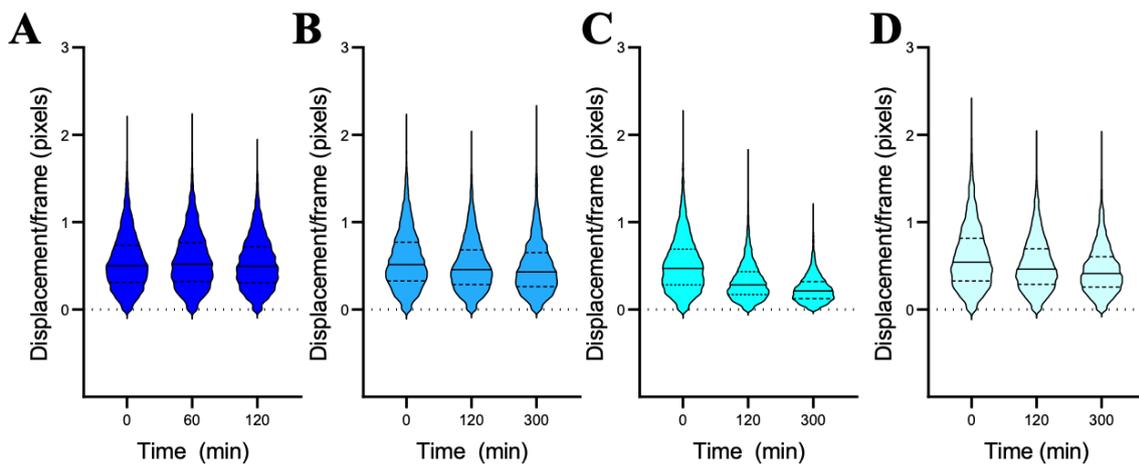
**Figure S14.** Effect of the antifungal fluconazole (0.5  $\mu\text{g}/\text{mL}$ ) on the cellular nanomotion for 3 selected cells: cell 2, 3 and 4 of *C. albicans* DSY1024. (A) Total displacements (pixels) as a function of time for cell 2. (B) Distribution of the displacement/frame as a function of time for cell 2. (C) X-y displacement at the time point 0 min for cell 2. (D) X-y displacement at the time point 300 min for cell 2. (E) Total displacements (pixels) as a function of time for cell 3. (F) Distribution of the displacement/frame as a function of time for cell 3. (G) X-y displacement at the time point 0 min for cell 3. (H) X-y displacement at the time point 300 min for cell 3. (I) Total displacements (pixels) as a function of time for cell 4. (J) Distribution of the displacement/frame as a function of time for cell 4. (K) X-y displacement at the time point 0 min for cell 4. (L) X-y displacement at the time point 300 min for cell 4.



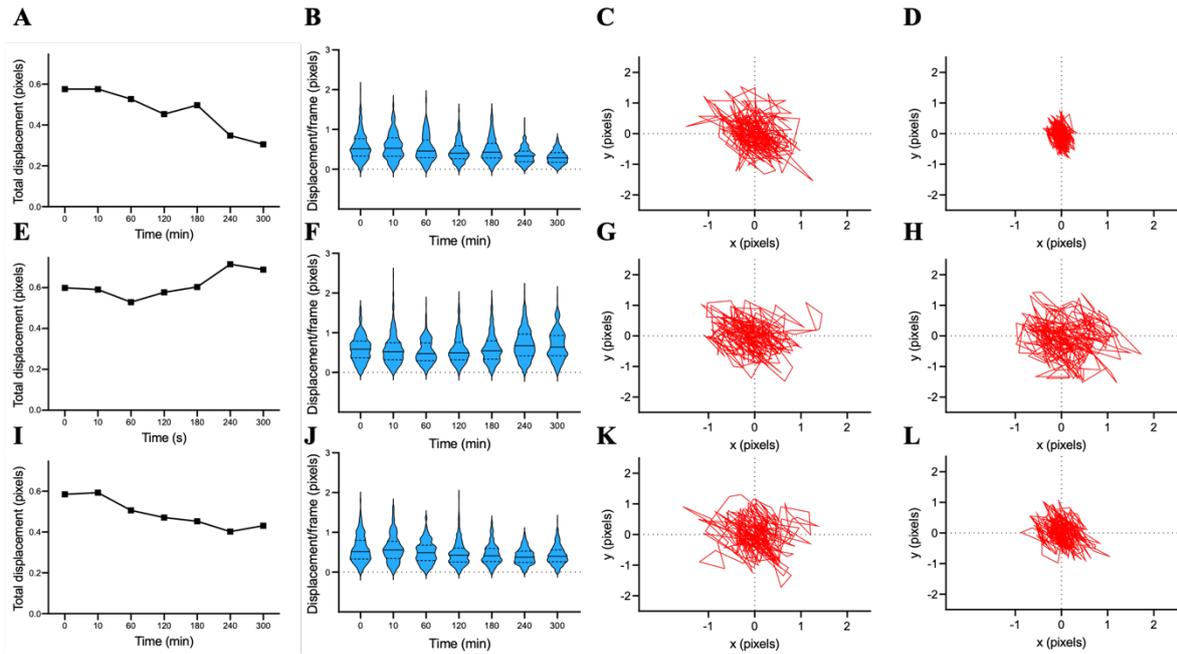
**Figure S15.** Effect of the antifungal fluconazole (1  $\mu\text{g}/\text{mL}$ ) on the cellular nanomotion for 2 selected cells: cell 2 and 5 of *C. albicans* DSY1024. (A) Total displacements (pixels) as a function of time for cell 2. (B) Distribution of the displacement/frame as a function of time for cell 2. (C) X-y displacement at the time point 0 min for cell 2. (D) X-y displacement at the time point 300 min for cell 2. (E) Total displacements (pixels) as a function of time for cell 5. (F) Distribution of the displacement/frame as a function of time for cell 5. (G) X-y displacement at the time point 0 min for cell 5. (H) X-y displacement at the time point 300 min for cell 5.



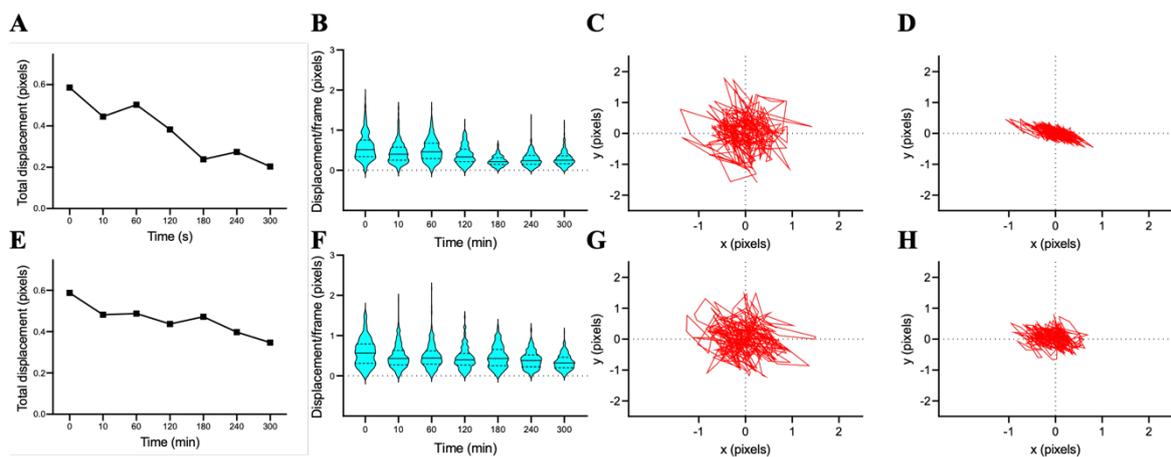
**Figure S16.** Effect of the antifungal fluconazole (100  $\mu\text{g}/\text{mL}$ ) on the cellular nanomotion for 3 selected cells: cell 1, 2, and 3 of *C. albicans* DSY1024. (A) Total displacements (pixels) as a function of time for cell 1. (B) Distribution of the displacement/frame as a function of time for cell 1. (C) X-y displacement at the time point 0 min for cell 1. (D) X-y displacement at the time point 300 min for cell 1. (E) Total displacements (pixels) as a function of time for cell 2. (F) Distribution of the displacement/frame as a function of time for cell 2. (G) X-y displacement at the time point 0 min for cell 2. (H) X-y displacement at the time point 300 min for cell 2. (I) Total displacements (pixels) as a function of time for cell 3. (J) Distribution of the displacement/frame as a function of time for cell 3. (K) X-y displacement at the time point 0 min for cell 3. (L) X-y displacement at the time point 300 min for cell 3.



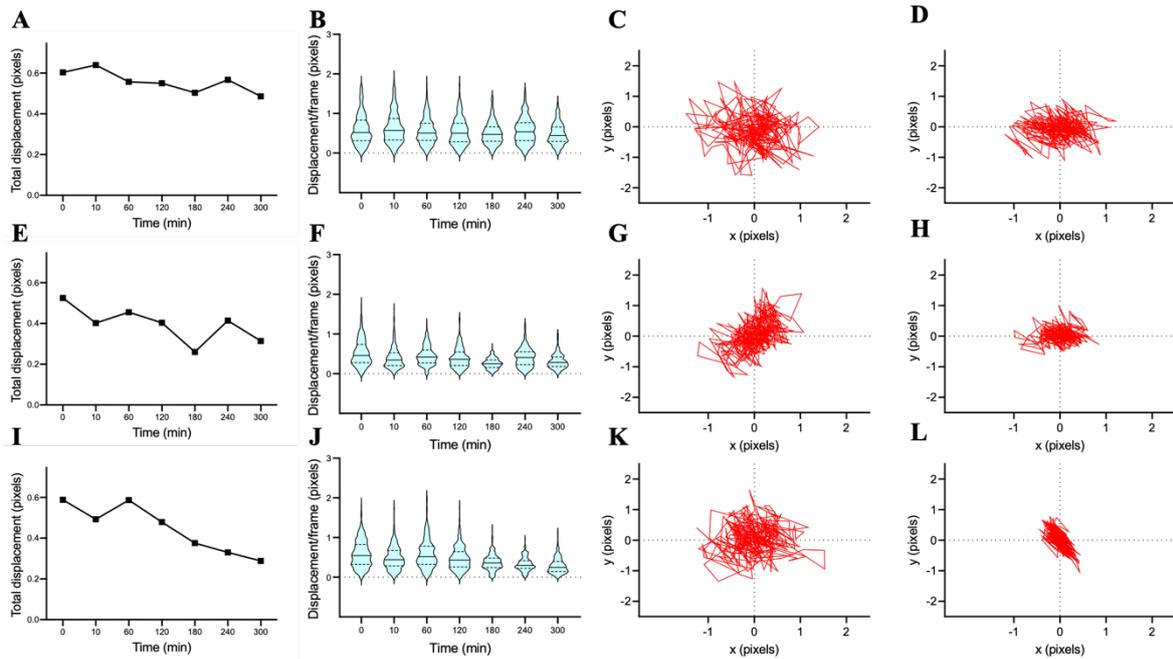
**Figure S17.** Averaged cellular nanomotion for 20 cells of *C. albicans* DSY1024. (A) Distribution of the displacements/frames during 2 h growth (control). (B) Distribution of the displacements/frames during 0.5  $\mu\text{g}/\text{ml}$  caspofungin treatment. (C) Distribution of the displacements/frames during 1  $\mu\text{g}/\text{ml}$  caspofungin treatment. (D) Distribution of the displacements/frames during 100  $\mu\text{g}/\text{ml}$  caspofungin treatment.



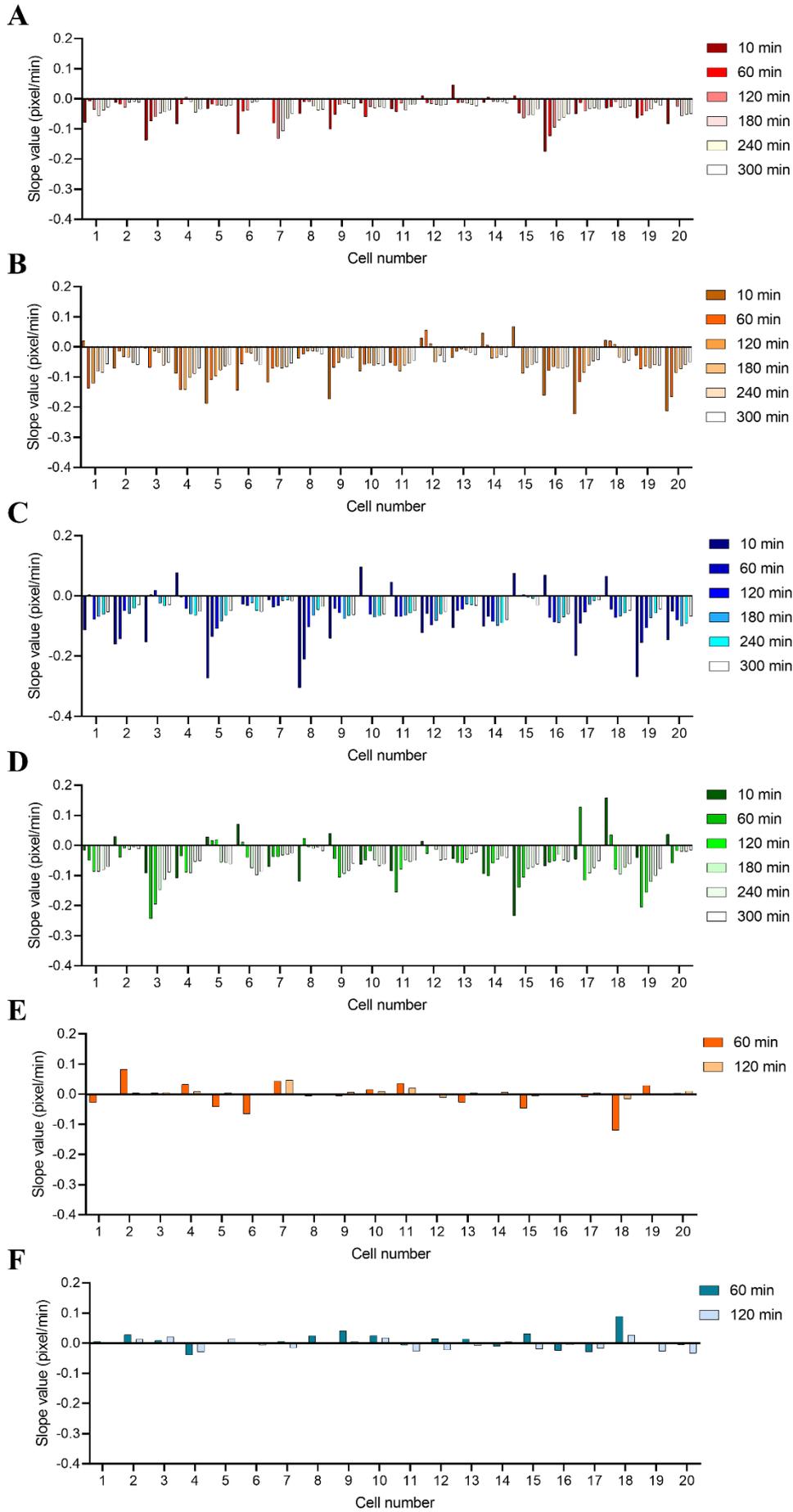
**Figure S18.** Effect of the antifungal caspofungin (0.5  $\mu\text{g}/\text{mL}$ ) on the cellular nanomotion for 3 selected cells: cell 3, 12 and 14 of *C. albicans* DSY1024. (A) Total displacements (pixels) as a function of time for cell 3. (B) Distribution of the displacement/frame as a function of time for cell 3. (C) X-y displacement at the time point 0 min for cell 3. (D) X-y displacement at the time point 300 min for cell 3. (E) Total displacements (pixels) as a function of time for cell 12. (F) Distribution of the displacement/frame as a function of time for cell 12. (G) X-y displacement at the time point 0 min for cell 12. (H) X-y displacement at the time point 300 min for cell 12. (I) Total displacements (pixels) as a function of time for cell 14. (J) Distribution of the displacement/frame as a function of time for cell 14. (K) X-y displacement at the time point 0 min for cell 14. (L) X-y displacement at the time point 300 min for cell 14.



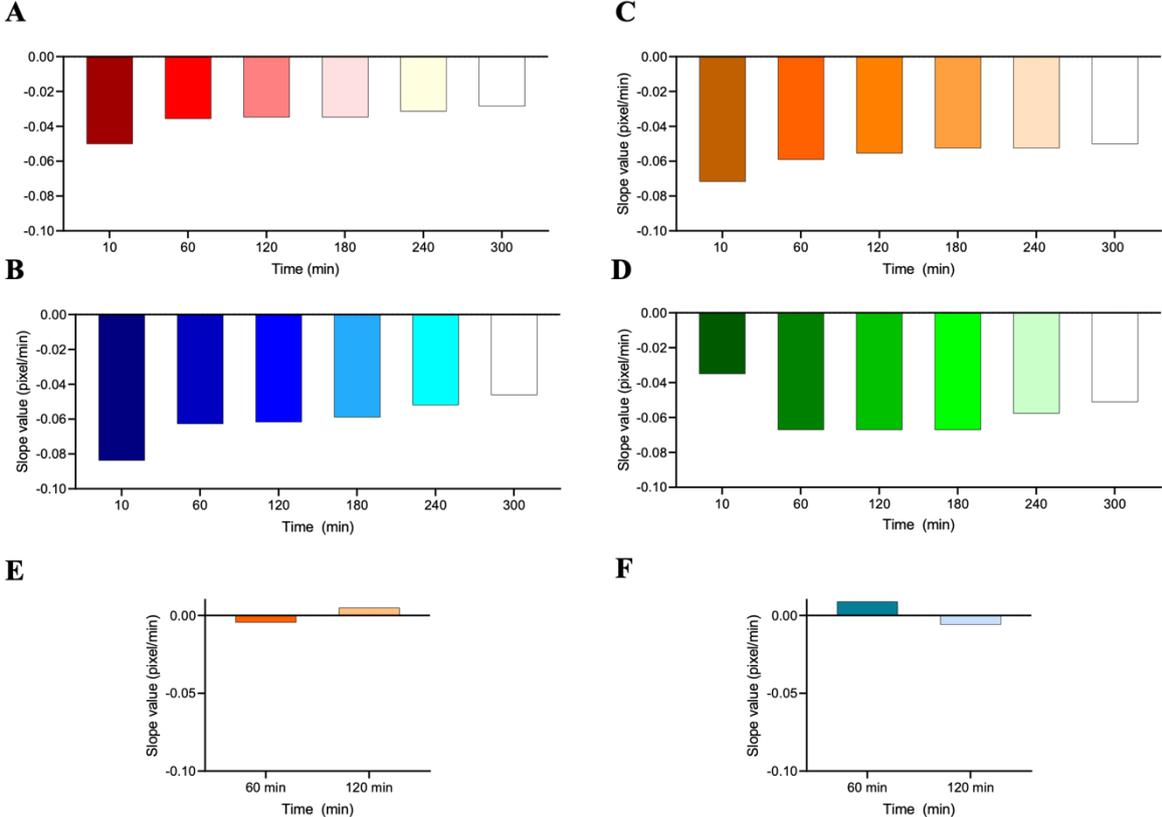
**Figure S19.** Effect of the antifungal caspofungin (1  $\mu\text{g}/\text{mL}$ ) on the cellular nanomotion for 2 selected cells: cell 9 and 13 of *C. albicans* DSY1024. (A) Total displacements (pixels) as a function of time for cell 9. (B) Distribution of the displacement/frame as a function of time for cell 9. (C) X-y displacement at the time point 0 min for cell 9. (D) X-y displacement at the time point 300 min for cell 9. (E) Total displacements (pixels) as a function of time for cell 13. (F) Distribution of the displacement/frame as a function of time for cell 13. (G) X-y displacement at the time point 0 min for cell 13. (H) X-y displacement at the time point 300 min for cell 13.



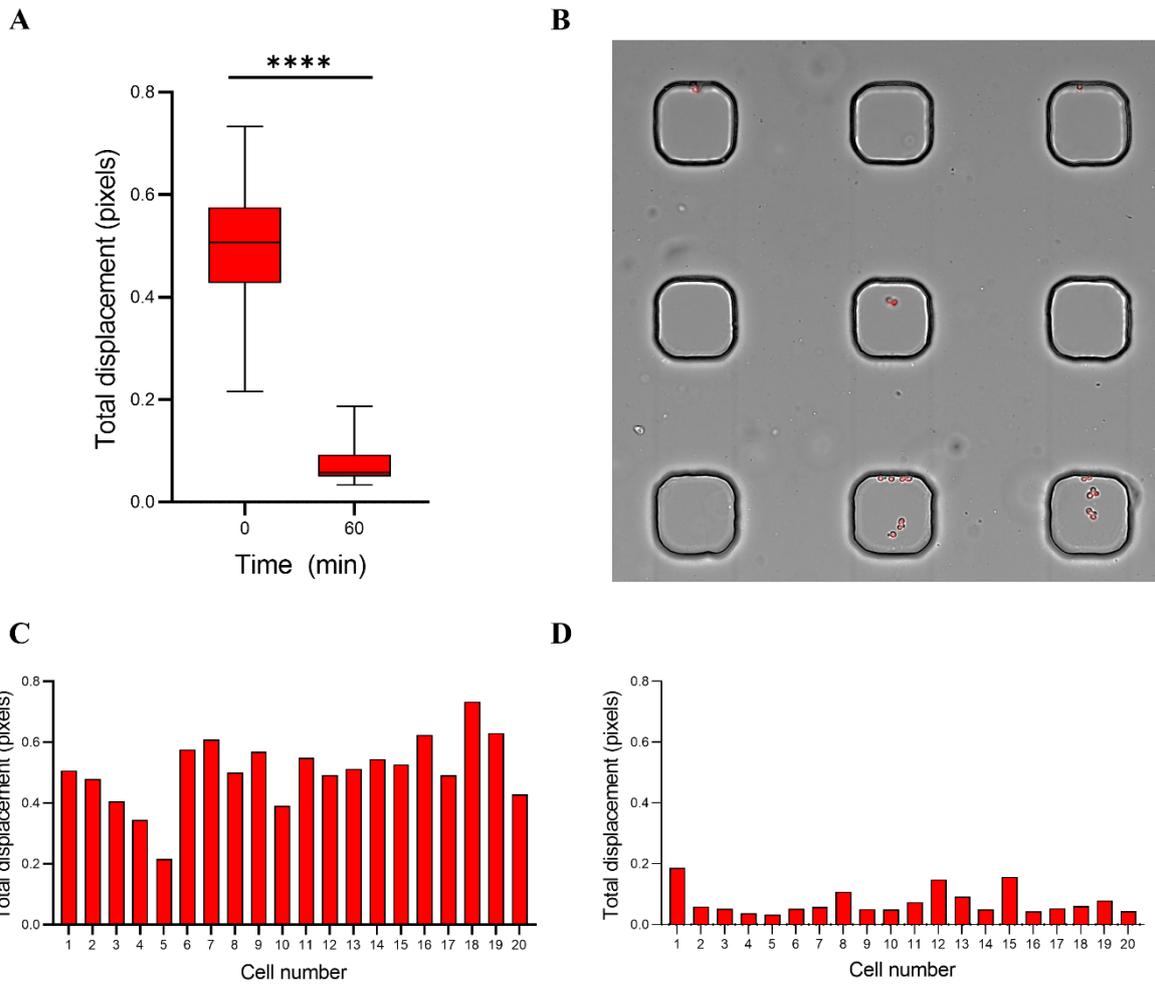
**Figure S20.** Effect of the antifungal caspofungin (100  $\mu\text{g/mL}$ ) on the cellular nanomotion for 3 selected cells: cell 2, 6, and 19 of *C. albicans* DSY1024. **(A)** Total displacements (pixels) as a function of time for cell 2. **(B)** Distribution of the displacement/frame as a function of time for cell 2. **(C)** X-y displacement at the time point 0 min for cell 2. **(D)** X-y displacement at the time point 300 min for cell 2. **(E)** Total displacements (pixels) as a function of time for cell 6. **(F)** Distribution of the displacement/frame as a function of time for cell 6. **(G)** X-y displacement at the time point 0 min for cell 6. **(H)** X-y displacement at the time point 300 min for cell 6. **(I)** Total displacements (pixels) as a function of time for cell 19. **(J)** Distribution of the displacement/frame as a function of time for cell 19. **(K)** X-y displacement at the time point 0 min for cell 19. **(L)** X-y displacement at the time point 300 min for cell 19.



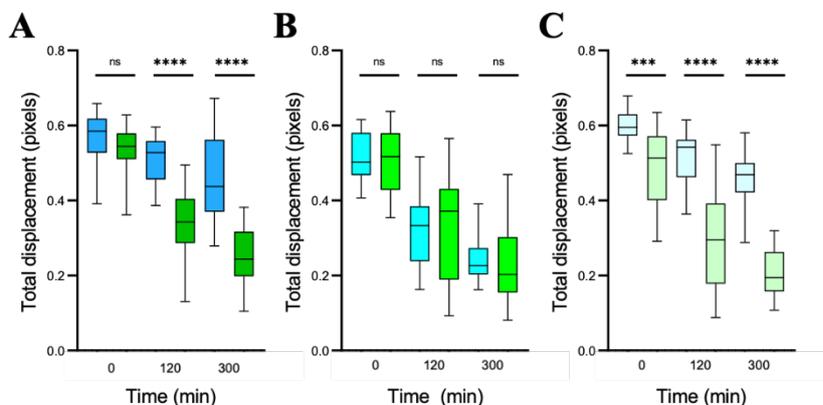
**Figure S21.** The slopes of the decrease in the total displacement of 20 cells as a function of time are calculated based on the time points 10, 60, 120, 180, 240, and 300 min during 1  $\mu\text{g/ml}$  caspofungin or fluconazole treatment of the *C. albicans* strains: (A) caspofungin treated CAF2-1, (B) fluconazole treated CAF2-1, (C) caspofungin treated DSY1024, (D) fluconazole treated DSY1024; and during the 2 h growth in YDP medium (E) CAF2-1 and (F) DSY1024.



**Figure S22.** The slopes of the decrease in the total displacement as a function of time are calculated based on the time points 10, 60, 120, 180, 240, and 300 min during 1  $\mu\text{g/ml}$  caspofungin or fluconazole treatment for the averages of total displacements of all 20 cells: (A) caspofungin treated CAF2-1, (B) caspofungin treated DSY1024, (C) fluconazole treated CAF2-1, (D) fluconazole treated DSY1024, ; and during the 2 h growth in YDP medium of (E) CAF2-1 and (F) DSY1024.



**Figure S23.** Comparison of the nanomotion of 20 living *C. albicans* CAF2-1 cells to 20 dead cell. (A) Total displacement of 20 cells of untreated living cells (time = 0 min) and 70 % (v/v) ethanol killed cells after 1 h of treatment; Wilcoxon test: \*\*\*\*  $p < 0.0001$ . (B) Image of PI stained dead cells in the microwells (after 1 h treatment with 70% (v/v) ethanol). (C) Total displacement of 20 single living cells (time = 0 min). (D) Total displacement of 20 single dead cells (time = 60 min).



**Figure S24.** Comparison of the averaged cellular nanomotion for 20 cells of the *C. albicans* azole hypersusceptible DSY1024 strain treated with fluconazole (green) or caspofungin (blue) at a concentration of (A) 0.1  $\mu\text{g/mL}$ , (B) 0.5  $\mu\text{g/mL}$ , and (C) 100  $\mu\text{g/mL}$ .