## Microscopy

Filename: Strain

WT pHsp104: Wild type strain with GFP-Hsp104

ssa1ssa2 (2/3) pHsp104: Clone 2 or 3 of SSA1/SSA2 double knockout strain with GFP-Hsp104

ssa1ssa2 SSA4 oe pHsp104/ssa1ssa2 SSA4 pHsp104: *SSA1/SSA2* double knockout strain with over expression of *SSA4* through the GPD promoter and with GFP-Hsp104

Filename: Treatment

No suffix: Control sample taken at mid-exponential growth

42C 30min: Sample taken after 30 minutes of heatshock at 42 °C

## **Imaging details**

Images were obtained using a conventional fluorescence microscope, a Zeiss Axio Observer.Z1 inverted microscope with Apotome and Axiocam 506 camera, and a Plan-Apochromat 100x/1.40 Oil DIC M27 objective. All images were acquired as Z-stacks with 10 slices over a 5  $\mu$ m range.

Channel 1: Transmitted light DIC, exp. time 10 ms

Channel 2: EGFP (38 HE Green Fluorescent Protein, λEx.: BP 470/40, Beamsplitter: FT 495, λEm.: BP

525/50), exp. time: 180 ms