

## ICC microscopy

Hsp42 primary antibody: rabbit anti Hsp42

Act1 primary antibody: mouse anti-Act1

Nsp1 primary antibody: mouse anti-Nsp1

Secondary 1: goat anti-rabbit Alexa Flour488 (Thermo Fisher Scientific Cat# A-11034, RRID: AB\_2576217))

Secondary 2: goat anti-mouse Alexa Flour 568 (Thermo Fisher Scientific Cat# A-11004, RRID: AB\_2534072)).

Replicate	Stain no. 1	Stain no. 2	Stain no. 3
170505 hsp42 IF	Anti-Hsp42, AF488	DAPI	
170518 Hsp42 IF with actin ab 45min spherop A slides	Anti-Hsp42, AF488	Anti-Act1, AF568	
170608 IF Hsp42 with anti Nsp1	Anti-Hsp42, AF488	DAPI	Anti-Nsp1, AF568

Filename: Strains

WT: Wild type strain (BY4741)

hsp42del: *HSP42* knockout strain

ssa1-2del: *SSA1/SSA2* double knockout strain

ssa1-2del Ssa4OE: *SSA1/SSA2* double knockout strain with over expression of *SSA4* through the GPD promoter

All samples were from cells in mid-exponential growth phase.

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.

170505 hsp42 IF

Channel 1: AF488 (38 HE Green Fluorescent Protein  $\lambda$ Ex.: BP 470/40, Beamsplitter: FT 495,  $\lambda$ Em.: BP 525/50), exp. time: 1800 ms

Channel 2: DAPI (49 DAPI,  $\lambda$ Ex.: G 365, Beamsplitter: FT 595,  $\lambda$ Em.: BP 445/50), exp. time: 566.6 ms

Channel 3: Transmitted light DIC, exp. time 15.9 ms

170518 Hsp42 IF with actin ab 45min spherop A slides

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

Channel 2: AF488 (38 HE Green Fluorescent Protein  $\lambda$ Ex.: BP 470/40, Beamsplitter: FT 495,  $\lambda$ Em.: BP 525/50), exp. time: 159.1 ms

Channel 3: AF568 ((45 Texas Red,  $\lambda$ Ex.: BP 560/40, Beamsplitter: FT 585,  $\lambda$ Em.: BP 630/75), exp. time: 567.4 ms

170608 IF Hsp42 with anti Nsp1

Channel 1: Transmitted light DIC, exp. time 2.9 ms

Channel 2: AF488 (38 HE Green Fluorescent Protein  $\lambda$ Ex.: BP 470/40, Beamsplitter: FT 495,  $\lambda$ Em.: BP 525/50), exp. time: 131.1 ms

Channel 3: AF568 ((45 Texas Red,  $\lambda$ Ex.: BP 560/40, Beamsplitter: FT 585,  $\lambda$ Em.: BP 630/75), exp. time: 312 ms

Channel 4: DAPI (49 DAPI,  $\lambda$ Ex.: G 365, Beamsplitter: FT 595,  $\lambda$ Em.: BP 445/50), exp. time: 89 ms