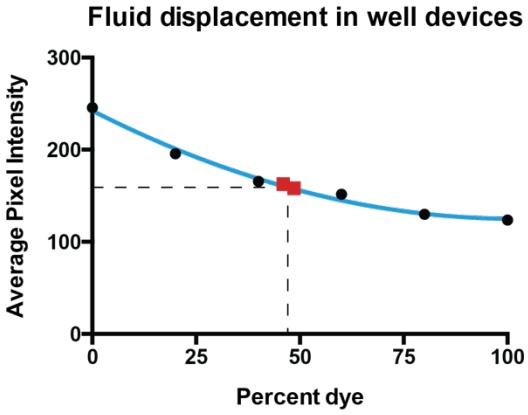
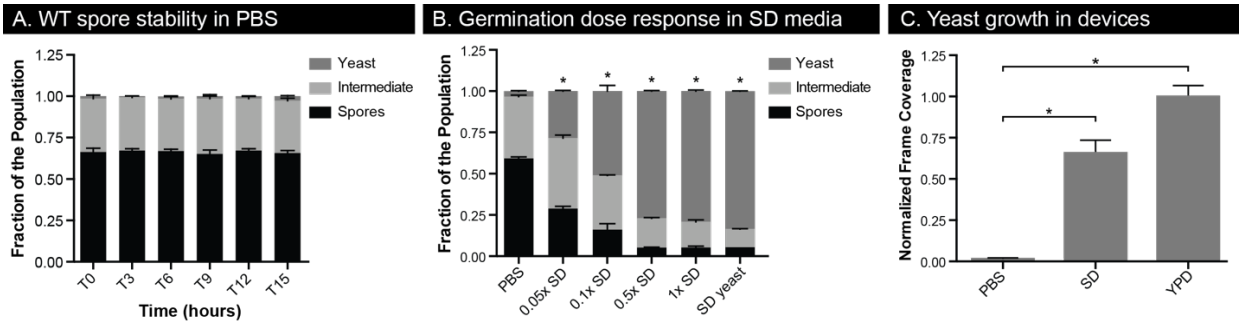
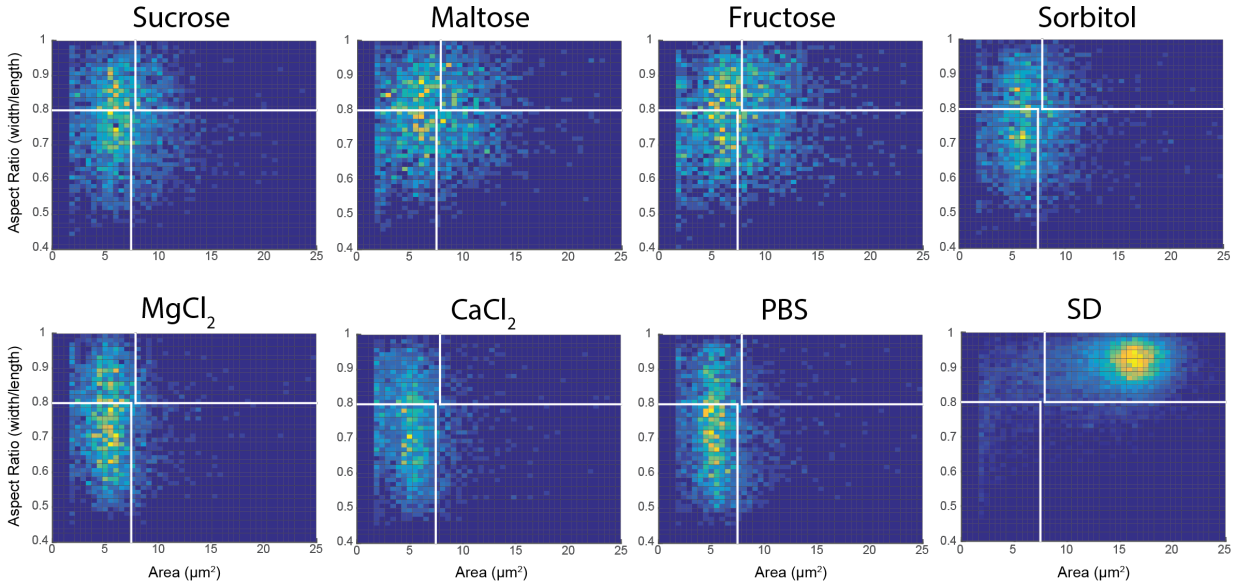
Supplemental Figures



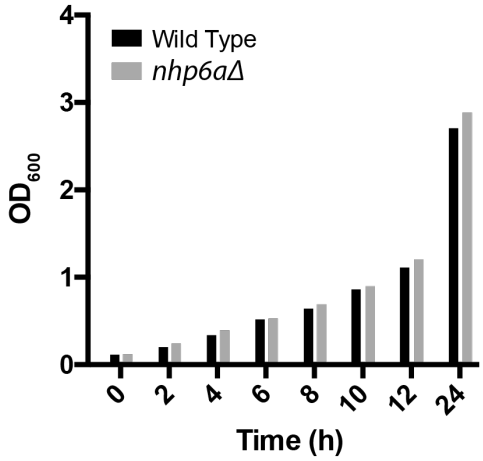
Supplemental Figure 1. Fluid addition to previously filled microwell devices displaces ~50% the liquid. Dye was loaded at various concentrations and used to establish a standard curve of image intensity (black circles are average values of two technical replicates; quadratic fit, blue line). The pixel intensity of pure dye is 124 while the pixel intensity of PBS is 246. Two wells were filled completely with 10 µL pure dye and then 5 µL PBS was used to displace the layer of fluid above the culture well. The resulting image intensity values (red squares) are 157.5 and 160.5 indicating a dilution factor of approximately 2 each time fluid is replaced in the microwell devices.



Supplemental Figure 2. Germination and yeast growth within devices is nutrient-dependent. (A) Population composition over time of spores incubated in devices at 30°C for 15 hours in PBS to assay for spore stability. (B) Population composition of spores germinated at 30°C for 16 hours in PBS or various concentrations of SD media. “SD yeast” were grown in SD liquid culture at 30°C for 12 hours. (C) WT yeast growth as measured by percent frame coverage (image area covered in cells) after 16 hours of growth in PBS, SD, and YPD at 30°C normalized to YPD. In all plots, error bars are standard deviation of experiments carried out with three independent wells. * indicates p-value <0.05 compared with PBS control.



Supplemental Figure 3. Sugars promote initiation but not completion of germination. Spores were incubated in devices for 16 hours at 30°C with compounds at 100 mM in PBS except $CaCl_2$ and $MgCl_2$ which were used at 1 mM. All plots include cell populations from three independent wells. Dark blue represents area and aspect ratio combinations not observed in the population, whereas yellow represents the combinations most frequently observed.



Supplemental Figure 4. *nhp6a*Δ strains do not show defects in vegetative growth. Wild type JEC21 and *nhp6a*Δ strains were grown in liquid YPD medium for 24 hours at 30°C with shaking (225 RPM). Cultures were then diluted in YPD broth to OD600=0.1 and assessed spectrophotometrically every two hours.