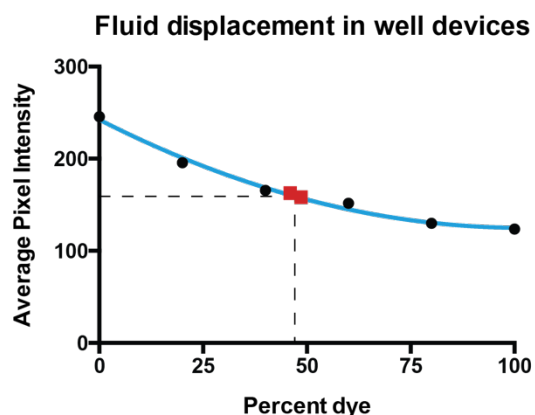
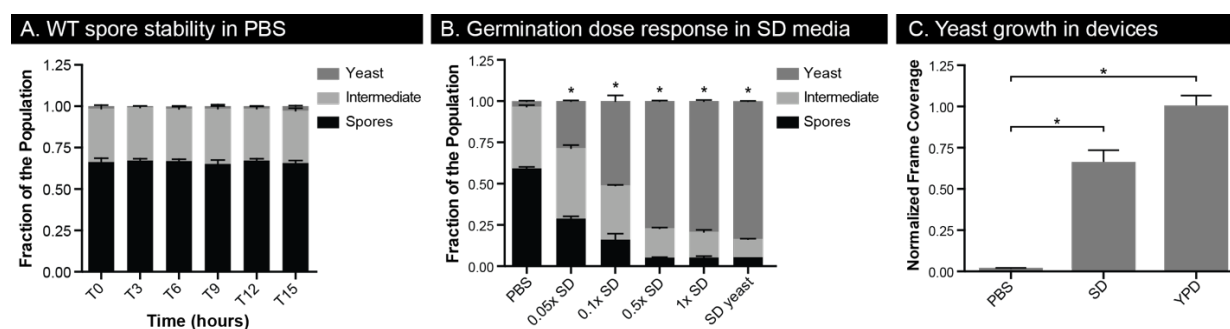


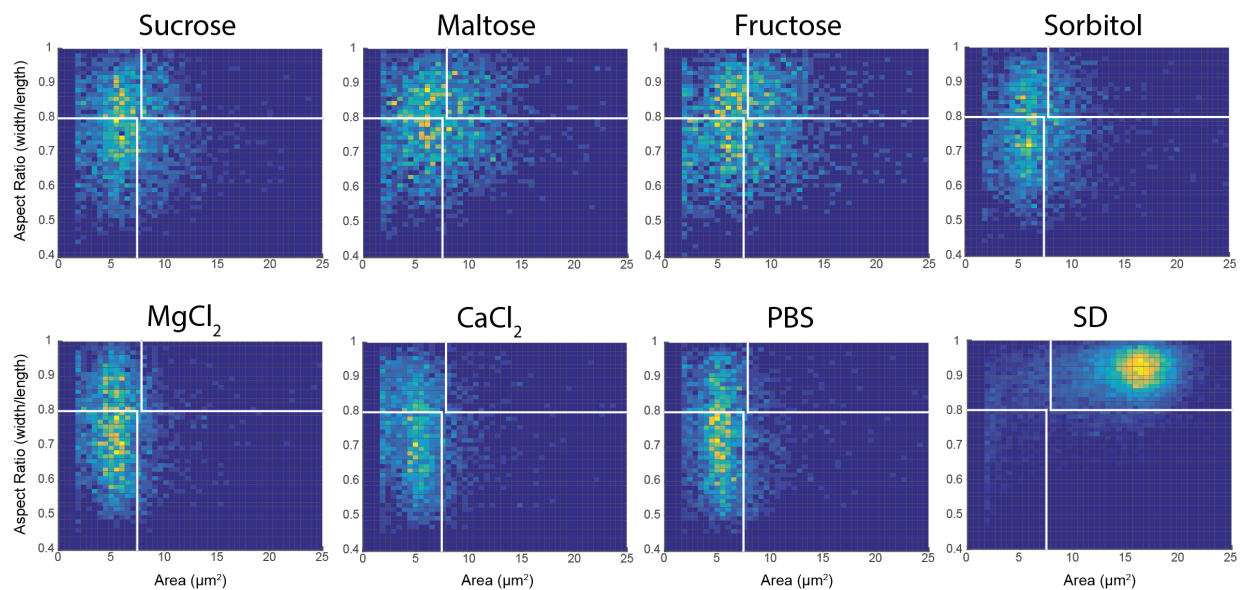
## Supplemental Figures



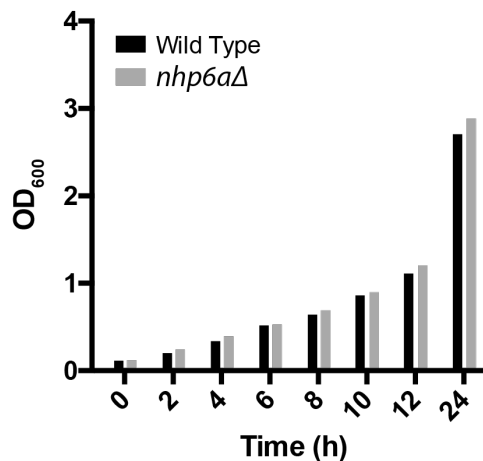
**Supplemental Figure 1.** Fluid addition to previously filled microwell devices displaces ~50% the liquid. Dye was loaded at various concentrations and used to establish a standard curve of image intensity (black circles are average values of two technical replicates; quadratic fit, blue line). The pixel intensity of pure dye is 124 while the pixel intensity of PBS is 246. Two wells were filled completely with 10  $\mu$ L pure dye and then 5  $\mu$ L 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  $\text{CaCl}_2$  and  $\text{MgCl}_2$  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* $\Delta$  strains do not show defects in vegetative growth. Wild type JEC21 and *nhp6a* $\Delta$  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  $\text{OD}_{600}=0.1$  and assessed spectrophotometrically every two hours.