File S2

Pictures of the *MAT***a**/**a** and *MAT*α/α diploids were acquired by growing cells to log phase (~5x10^6 cells/mL) at 30^{o}C in YPD. Cells were then loaded into a microfluidics chamber (CellAsic, Hayward, CA) (Lee *et al.* 2008), which was pretreated by perfusing YPD through the chamber at 34kPa for 10 minutes. Once cells were loaded, YPD was perfused through the microfluidics chamber at 14kPa. Pictures were taken using a 20x Plan Apo VC 0.75NC air lens after 10 hours growth using differential interference contrast with a 10ms exposure.

Pictures and movies of various strains responding to 10nM α-factor were produced in a similar way. Cells were grown to log phase (~5x10^6 cells/mL) at 30^{o}C in YPD, washed into SC + 0.1% BSA, and loaded into a microfluidics chamber, which had been pretreated by perfusing PBS + 2% BSA through the chamber at 34kPa for 10 minutes and then SC + 0.1% BSA through the chamber at 34kPa for 10 minutes. Once the cells were loaded, SC + 0.1% BSA + 10nM α-factor was perfused through the chamber at 14kPa. Pictures were taken every 10 minutes for 8 hours using a 60x Plan Apo VC 1.4NA oil lens with differential interference contrast with a 10ms exposure.

For pictures of the *MAT***a**-playing-α cells responding to **a**-factor, *MAT*α P_{ACT1}-*mCherry* cells were mixed with *MAT***a**-playing-α cells on a filter as described for the quantitative mating assay. Cells were washed off the filters into SC after 2.5 hours and placed directly onto glass slides (Corning, Tewksbury, MA). Pictures were taken immediately using a 20x Plan Apo VC 0.75NC air lens with a 10ms exposure for differential interference contrast and a 300ms exposure for fluorescent images.

LITERATURE CITED

Lee, P. J., N. C. Helman, W. A. Lim and P. J. Hung, 2008 A microfluidic system for dynamic yeast cell imaging. BioTechniques **44:** 91-95.