File S1

To measure unstimulated α-factor production, cells were grown to log phase (~$5x10^6$ cells/mL), washed into YPD + 0.1% BSA, and resuspended in BSA-coated culture tubes at $5x10^6$ cells/mL in YPD + 0.1% BSA. The supernatant of the media was then harvested after a 15 minute, 30 minute, or 120 minute incubation at 30°C on a roller drum using BSA-coated 1mL syringes (BD, Franklin Lakes, NJ) and filtered through 0.45µm BSA-coated syringe filters (Pall Corporation, Port Washington, NY). $5x10^5$ log phase *MAT***a** *bar1Δ* cells were then incubated in 1x, 0.5x, and 0.2x dilutions of the supernatant for 2 hours, sonicated, fixed using 60% ethanol at -20°C, and resuspended into 20% glycerol in phosphate buffered saline (PBS). The percent of the cells shmooing was counted and compared to a calibration curve produced by treating *MAT***a** *bar1Δ* cells with different concentrations of synthetic α-factor.

Stimulated α-factor production was measured in the same way except that the cells being tested were grown to log phase (~$5x10^6$ cells/mL), counted, washed into YPD + 0.1% BSA, and $5x10^6$ cells/mL of the cells being tested were mixed with $5x10^5$ cells/mL *MAT***a** *bar1Δ* cells. After 2 hours incubation at 30°C on roller drums, the cells were washed into fresh YPD + 0.1% BSA and put into BSA-coated culture tubes. The supernatant was then harvested and analyzed as described above.

To generate a standard curve, *MAT***a** *bar1Δ* cells were incubated in integer values of synthetic α-factor concentrations between 0nM and 7nM, and the best fit line was generated with an R^2 of 0.9 to determine a constant (K) for the relationship between α-factor concentration and the percentage of shmoos (K=0.07). α-factor production in molecules/cell/minute was then determined by $\frac{N \times K \times \%\, shmoos \times volume}{r \times 2^D \times t \times l}$, where *N* is Avagadro's constant, *r* is the starting number of cells, *D* is the number of doublings expected, *t* is the incubation time, and *l* is the dilution. The expected number of doublings was calculated by the incubation time divided by an expected 90 minute doubling time for yeast.

Culture tubes and syringes were BSA-coated by incubating overnight at 4°C with PBS + 2% BSA. The PBS + 2% BSA was poured out immediately prior to use of the culture tube or syringe. Filters were BSA-coated by filtering 1mL of PBS + 2% BSA through them prior to use. At least 200 cells were counted to determine the percentage of cells shmooing. Error bars are standard deviations. Statistical significance was determined using Student's *t*-test.