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.

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^oC 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^oC 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.

File S3

_MAT_a cells are capable of enduring arrest in response to pheromone stimulation.

*MAT***a** *bar1Δ* cells were grown in a microfluidics chamber for 8 hours perfused with SC plus 10nM α-factor and imaged using DIC every 10 minutes with 60x magnification.

File S3 is available for download as an avi file at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.155846/-/DC1.

File S4

_MAT_α-playing-a cells arrest only transiently in response to pheromone stimulation.

*MAT*α-playing-**a** *bar1Δ* cells were grown in a microfluidics chamber for 8 hours perfused with SC plus 10nM α-factor and imaged using DIC every 10 minutes with 60x magnification.

File S4 is available for download as an avi file at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.155846/-/DC1.

Table S1 Strains used in this study

Strain Name	**Genotype** (all cells are in the W303 background)
LBHY29	MATα P_{FUS1}-YFP @ LEU2 MFA1-TRP1::mfα1Δ MFA2-HIS3::mfα2Δ STE2-NatMX4::ste3Δ ade2-1 can1-100 his3-11,15 trp1-1 ura3-1
LBHY41	MATα ade2-1 can1-100 his3-11,15 leu2-112 trp1-1
LBHY44	MAT**a** P_{FUS1}-YFP @ LEU2 bar1Δ::HphMX4 MFα1-HIS3::mfa1Δ MFα2-TRP1::mfa2Δ STE3-NatMX4::ste2Δ asg7Δ::URA3(Kluyveromyces lactis) ade2-1 can1-100 his3-11,15 trp1-1 ura3-1
LBHY47	MATα P_{FUS1}-YFP @ LEU2 $P_{MF\alpha1}$-STE6-URA3 MFA1-TRP1::mfα1Δ MFA2-HIS3::mfα2Δ STE2-NatMX4::ste3Δ P_{FUS1*}-BAR1-KanMX6 ade2-1 can1-100 his3-11,15 trp1-1 ura3-1
LBHY49	MATα P_{FUS1}-YFP @ LEU2 $P_{MF\alpha1}$-STE6-$ura3^{-}$ MFA1-TRP1::mfα1Δ MFA2-HIS3::mfα2Δ STE2-NatMX4::ste3Δ P_{FUS1*}-BAR1-KanMX6 can1-100 his3-11,15 trp1-1 ura3-1
LBHY89	MATα mfα1Δ::NatMX4 ade2-1 can1-100 his3-11,15 leu2-112 trp1-1 ura3-1
LBHY92	MATα mfα1Δ::NatMX4 can1-100 his3-11,15 leu2-112 trp1-1 ura3-1
LBHY93	MATα mfα1Δ::NatMX4 ade2-1 can1-100 his3-11,15 leu2-112 trp1-1
LBHY98	MAT**a** P_{FUS1}-YFP @ LEU2 bar1Δ::HphMX4 MFα1-HIS3::mfa1Δ MFα2-TRP1::mfa2Δ STE3-NatMX4::ste2Δ asg7Δ::URA3(K. lactis) P_{ACT1}-yCerulean @ ADE2 can1-100 his3-11,15 trp1-1 ura3-1
LBHY108	MAT**a** P_{ACT1}-mCherry-HIS3MX6 @ P_{ACT1} ade2-1 can1-100 his3-11,15 leu2-112 trp1-1 ura3-1
LBHY156	MAT**a** mfa1Δ::KanMX6 mfa2Δ::HphMX4 ade2-1 can1-100 his3-11,15 leu2-112 trp1-1 ura3-1
LBHY177	MATα P_{FUS1}-YFP @ LEU2 MFA1-TRP1::mfα1Δ MFA2-HIS3::mfα2Δ STE2-NatMX4::ste3Δ bar1Δ::KanMX6 ade2-1 can1-100 his3-11,15 trp1-1 ura3-1
LBHY286	MAT**a** mfa1Δ::KanMX6 ade2-1 can1-100 his3-11,15 leu2-112 trp1-1
LBHY290	MAT**a** P_{FUS1*}-BAR1-KanMX6 ade2-1 can1-100 his3-11,15 leu2-112 trp1-1
LBHY316	MATα/α P_{FUS1}-YFP @ LEU2/leu2-112 $P_{MF\alpha1}$-STE6-URA3/P_{STE6}-STE6 MFA1-TRP1::mfα1Δ/MFα1 MFA2-HIS3::mfα2Δ/MFα2 STE2-NatMX4::ste3Δ/STE3 P_{FUS1*}-BAR1-KanMX6/ bar1Δ::ADE2 SPA2/ SPA2-YFP:HIS3 ade2-1/ade2-1 can1-100 /can1-100 his3-11,15/his3-11,15 trp1-1/trp1-1 ura3-1/ura3-1
LBHY318	MAT**a**/**a** P_{FUS1}-YFP @ LEU2/ leu2-112 bar1Δ::HphMX4/BAR1 MFα1-HIS3::mfa1Δ/MFA1 MFα2-TRP1::mfa2Δ/MFA2 STE3-NatMX4::ste2Δ/STE2 asg7Δ::URA3(K. lactis)/ASG7 SPA2-CFP:KanMX6/SPA2 ADE2/ade2-1 can1-100/can1-100 his3-11,15/P_{FUS1}-YFP @ HIS3 trp1-1/trp1-1 ura3-1/ura3-1
LBHY346	MATα P_{FUS1}-YFP @ LEU2 $P_{MF\alpha1}$-STE6-URA3 MFA1-TRP1::mfα1Δ MFA2-HIS3::mfα2Δ STE2-NatMX4::ste3Δ P_{FUS1*}-BAR1-KanMX6 afb1Δ::HphMX4 ade2-1 can1-100 his3-11,15 trp1-1 ura3-1
LBHY350	MATα afb1Δ::HphMX4 ade2-1 can1-100 his3-11,15 leu2-112 trp1-1
LBHY352	MATα afb1Δ::HphMX4 ade2-1 can1-100 his3-11,15 leu2-112 trp1-1
LBHY395	MAT**a** P_{FUS1}-YFP @ LEU2 bar1Δ::HphMX4 MFα1-HIS3::mfa1Δ MFα2-TRP1::mfa2Δ STE3-NatMX4::ste2Δ asg7Δ::URA3(K. lactis) P_{TDH3}-MFα1:KanMX6 @ P_{TDH3} ade2-1 can1-100 his3-11,15 trp1-1 ura3-1
LBHY397	MAT**a** P_{ACT1}-AFB1:KanMX6 @ P_{ACT1} ade2-1 can1-100 his3-11,15 leu2-112 trp1-1 ura3-1
LBHY409	MAT**a** P_{ACT1}-AFB1:KanMX6 @ P_{ACT1} ade2-1 can1-100 his3-11,15 leu2-112 trp1-1
LBHY410	MATα P_{FUS1}-YFP @ LEU2 $P_{MF\alpha1}$-STE6-$ura3^{-}$ MFA1-TRP1::mfα1Δ MFA2-HIS3::mfα2Δ STE2-NatMX4::ste3Δ P_{FUS1*}-BAR1-KanMX6 afb1Δ::HphMX4 can1-100 his3-11,15 trp1-1 ura3-1

MP 381	*MAT*α *bar1Δ::ADE2 SPA2-YFP:HIS3 ade2-1 can1-100 his3-11,15 leu2-112 trp1-1 ura3-1*
MP 384	*MAT***a** *bar1Δ::ADE2 SPA2-YFP:HIS3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*
MP 420	*MAT***a** *SPA2-CFP:KanMX6* P_{FUS1}*-YFP @ HIS3 can1-100 leu2-112 trp1-1 ura3-1*
W303	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-112 trp1-1 ura3-1 (W303 wildtype)*
W303	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-112 trp1-1 ura3-1 (W303 wildtype)*

All strains are from this study except for MP 381, MP 384, and MP 420, which are from M. Piel and the W303 wildtype strains, which are from Thomas and Rothstein 1989 (Thomas and Rothstein 1989).

LITERATURE CITED

Thomas, B. J., and R. Rothstein, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619-630.

Table S2 Genes identified as differentially expressed in *MAT*a and *MAT*α-playing-a cells using RNA sequencing

Gene	Log_2 Fold Change: Unstimulated *MAT*α-playing-**a** ÷ Unstimulated *MAT***a**	Log_2 Fold Change: Stimulated *MAT*α-playing-**a** ÷ Stimulated *MAT***a**
MATα1	**7.56***	**7.86***
YCR097W-A	**3.97***	**6.96***
YDR008C	**4.30***	**5.70***
SAG1	**3.63***	**5.49***
YCL065W	**2.91***	**5.33***
TRP1	**4.35***	**5.31***
MATα2	**1.28***	**3.08***
YLR040C	**3.41***	**2.38***
YLR041W	**3.21***	**2.03***
YML131W	0.66*	0.69*
TPO2	-0.74*	0.53*
YPR158C-D	-0.61*	-0.48*
ADE17	-0.75*	-0.48*
BAP2	-0.94*	-0.54*
FSF1	-0.64*	-0.58*
ILV3	-0.75*	-0.58*
GDH1	-0.87*	-0.60*
FIT3	-0.84*	-0.63*
ALD5	**-1.03***	-0.88*
BAT1	**-1.70***	**-1.20***
OAC1	**-2.27***	**-1.57***
LEU1	**-2.47***	**-1.82***
ADE2	**-1.38***	**-1.93***
GYP8	**-1.02***	**-2.05***
STE2	**-2.67***	**-2.18***
MFA1	**-1.23***	**-2.80***
YNL146C-A	**-2.57***	**-4.00***
MFA2	**-5.51***	**-6.85***
ASG7	**-2.68***	**-9.11***
AGA2	**-7.29***	**-9.28***
Genes whose expression changed significantly only in pheromone-stimulated cells		
YHR145C	-0.61	**3.63***

YCR041W	**2.14**	**3.53***
PCL1	-0.12	**1.85***
SNL1	0.05	**1.75***
YLR346C	**1.20**	**1.70***
TOS6	-0.03	**1.30***
INO1	0.16	**1.30***
PST1	-0.06	**1.16***
OYE3	-0.22	**1.05***
TOS4	-0.04	**1.03***
YOX1	-0.12	0.97*
IMD2	0.02	0.95*
PIR3	0.47	0.95*
LEU2	0.21	0.91*
SPO11	0.05	0.88*
MCD1	0.07	0.87*
HES1	0.29	0.85*
GRE2	0.13	0.80*
AAD16	0.00	0.80*
YJL218W	0.41	0.79*
YGL230C	0.05	0.78*
AAD6	0.03	0.77*
EGT2	0.12	0.76*
YJR154W	0.19	0.74*
MCH2	0.35	0.73*
HTA2	-0.17	0.73*
YDR134C	0.06	0.69*
PIR1	-0.11	0.69*
KDX1	0.52	0.68*
HSP31	0.22	0.68*
YGR035C	0.17	0.66*
APL1	0.07	0.66*
YNR064C	0.02	0.64*
YBR071W	-0.04	0.63*
HAL1	0.48	0.62*
YOL159C	0.04	0.62*
CRG1	0.22	0.62*
YNL134C	-0.04	0.60*
AAD10	-0.15	0.60*
SVS1	-0.09	0.59*

SPI1	0.44	0.58*
YDR034W-B	-0.51	0.57*
RNR1	0.07	0.56*
SRL3	0.08	0.56*
YNL058C	0.21	0.55*
YKE4	-0.02	0.54*
ACA1	0.28	0.52*
SSA4	-0.10	0.50*
HHF1	-0.09	0.50*
DSE4	0.29	0.48*
OAZ1	0.13	0.47*
SEO1	0.24	0.47*
NIS1	-0.11	0.47*
YEH1	0.01	0.46*
HXT1	-0.08	-0.47*
PHO12	-0.24	-0.50*
YOL103W-B	-0.31	-0.50*
SIT1	-0.46	-0.51*
PHO11	-0.24	-0.52*
ENA1	0.00	-0.52*
LEU9	-0.22	-0.52*
HIS4	-0.50	-0.52*
YPL257W-B	-0.31	-0.53*
ALT2	-0.20	-0.54*
PDH1	0.08	-0.56*
PRY2	-0.29	-0.57*
HXK1	-0.03	-0.57*
TAT1	-0.35	-0.57*
YJR003C	-0.15	-0.66*
THI22	-0.26	-0.81*
FET3	-0.32	-0.83*
AXL1	-0.52	-0.93*
Genes whose expression changed significantly only in unstimulated cells		
RDN5-2	**2.90***	**1.33**
RDN5-5	**2.75***	0.73
RDN5-4	**2.66***	**1.02**
RDN5-3	**2.51***	0.93
RDN5-1	**2.34***	0.99
RDN5-6	**2.34***	0.99

YLR042C	0.66*	0.31
HER1	0.64*	0.15
HAP4	0.55*	0.23
YJL171C	-0.59*	-0.20
DRE2	-0.59*	-0.19
HMX1	-0.64*	-0.32
GAP1	-0.64*	-0.25
ARN2	-0.65*	-0.41
FUS1	-0.65*	-0.06
MAE1	-0.70*	-0.15
AGA1	-0.85*	-0.15
FIT2	-0.89*	-0.40
ISU2	-0.92*	-0.28
YGP1	**-1.04***	-0.10
DIC1	**-1.26***	-0.28

* Indicates statistical significance determined by the default settings in Cufflinks ($p<0.001$) (Trapnell *et al.* 2010). Numbers in bold indicate that there is a greater than two-fold change between the expression of the gene in *MAT*α-playing-**a** and *MAT***a** cells.

LITERATURE CITED

Trapnell, C., B. A. Williams, G. Pertea, A. Mortazavi, G. Kwan *et al*, 2010 Transcript assembly and quantification by RNA-seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. **28:** 511-515.