Materials and Methods:

Yeast constructs and strains

All yeast constructs used in this study (Supplementary Table 1) were cloned using standard integration techniques. The strains were constructed from a W303 haploid genetic background. All plasmid constructions were confirmed by sequencing and all yeast genomic integrations and deletions were confirmed by yeast colony PCR. For the visualization of the nucleus, the endogenous copy of histone protein Htb2 was C-terminally tagged with a florescent protein. The transcriptional reporters for monitoring pathway activity were constructed by fusing a promoter of a gene that is known to be responsive to a specific stimulus with a florescent protein. The reporters were integrated in a single copy into the genome (Supplementary Table 2). The reporter for the osmotic pathway was constructed by fusing a yECitrine to the STL1 promoter (1000bp) and integration at the URA locus (15). The reporter for the invasive growth pathway was constructed by fusing a filamentation and invasion response elements element (FRE) upstream to a minimal CYC1 promoter and mCherry with integration at the LEU locus (7). The reporter for the mating pathway was constructed by fusing a yECFP to the AGA1 promoter (1000bp) and integration at the HIS locus (19).

Microfluidics and fluorescent microscopy

The microfluidics yeast cell cultures were performed in Y04C perfusion plates with an ONIX flow control system (Millipore). Overnight cultures were diluted and grown for 6 hours to mid-log phase in synthetic complete media (OD 600nm = 0.1–0.3). Cells were diluted to an OD600 nm = 0.05 and sonicated before being loaded into the flow chamber pre-coated concanavalin A. After loading, cells were left to recover with synthetic complete media flow for 1-2h with before stress exposure. Image acquisition was performed with a TE2000-E automated inverted microscope (Nikon) with Perfect Focus. A ×40 air objective was used for tracking lineage growth and a x60 oil immersion objective was used for experiments monitoring the transcriptional reporters.

In order to preform the mutational screen we conducted a comparative growth assay in the microfluidics flow cells (Fig. S4). This experimental setup insures that the co-cultured strains are exposed to the exact same environment and received identical osmotic stimuli. In order to distinguish between co-cultured strains the endogenous copy of the histone protein Htb2 was tagged with different florescent tags (mCherry or yEGFP). In each experiment we grew the two strains separately until the sonication stage. After sonication of the cultures, we measured their OD and mixed them in a 1:1 ratio before loading them into the flow cell.

Mathematical model

In order to explore which underlying cellular mechanisms can potentially underlie the band-pass dynamics of growth inhibition we used a model previously developed to study the adaptive nature of the osmotic response by Muzzey et al. (3). Using the model the time-dynamic of HOG1-PP levels after encounter with osmostress can be readily calculated. In our work we used to model predictions of HOG1-PP level to infer the time

dynamics of a downstream process (e.g., transcription) that is responds to HOG1 phosphorylation. For simplicity, we assumed that the level of downstream activation is linearly proportional to HOG1-PP levels (although other relationships can be readily modeled as well). As the dilution and degradation of the transcribed proteins are independent of HOG1-PP dynamics and operate on a much longer time scale, they will persist after pathway adaptation. Thus we use the integral under HOG1-PP as an approximation for the expected level of responsive protein that will accumulate in a cell.

Profiles of time variant inputs

Time variant inputs were achieved by precise control of media flow into the imaging chamber. We used synthetic complete media with 2% glucose in all experiments and supplemented the media according to the required stimulus: 0.4M KCl for osmotic stress experiments, 1% butanol for full activation of invasive growth response (20), and 1uM alpha factor for full activation of the mating response (19). The control of media flow was done with the ONIX FG flow control software (Cellasic). For media replacement in the flow chamber we used the maximal recommended pressure (8 psi) for 1min. Under this high pressure the media in chamber can be completely replaced within 2-3 seconds (Fig. S1). Media flow in the reminder of the experiment was kept by applying an intermediate pressure of 4 psi. Protocols for media replacements were as followed:

Control experiments – constitutive flow of complete media.

Single step input experiments – flow of complete media for 1h was switched to treated media for the reminder of the experiment (e.g. complete media supplemented with 0.4M KCl).

Oscillation experiments – flow of complete media for 1h followed by alternating inputs of treated complete media and complete media

Primed input experiments – flow of complete media for 1h followed by alternating inputs of treated complete media (0.2M KCl) and complete media for 2.5h (oscillation period was 16min). After 2.5h the media was switched to 1.2M KCl for the reminder of the experiment.

Staircase input experiments – flow of complete media for 1h followed by serial switch to media supplemented with KCl in ascending osmolarity. The following order was used: 0.4M, 0.6M, 0.8M, 1M (each for 32min). The staircase input peaked with media supplemented with 1.8M KCl for 64min. Quick ramp down was done by serial media switching to media supplemented with lower levels of KCl (1M, 0.8M. 0.6M, 0.4M, each for 2min) and finally switched to complete media for the reminder of the experiment.

Image processing

Automated image segmentation and fluorescence quantification was preformed with custom program written in the MATLAB software (Mathworks). Monitoring of cell pedigree was done by tracking the florescent-tagged nuclei in a time-lapse microscopy movie. Single cells plated sparsely in the beginning of the experiment were tracked for a

period of up to 10 hours while monitoring the number of nuclei in each micro-colony in 15min time resolution. Changes in nuclei number were used to calculate the mean doubling time of the pedigree. Monitoring of transcriptional fold expression was done by first determining the cell boundaries from the bright-field differential interference contrast microscopy images and subsequently calculating the total cell florescence. The fold induction was calculated by measuring the ratio between the total cell florescence at each time point relative to the reference time point (the last time point before application of stress).

Supplementary figures

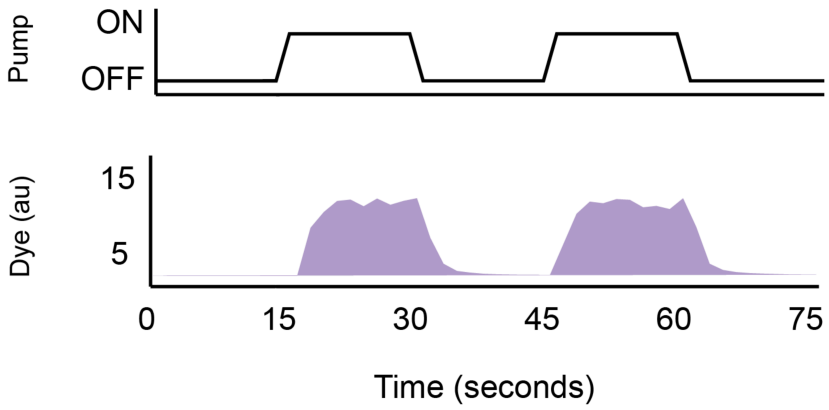

Fig. S1
Characterization of media switching dynamics inside the flow chamber. The temporal dynamics of media switching in a Y04C microfluidics plate was characterized by flowing media with or without florescent dye (fluorescein). Similarly to our experiments with live-cells, we used the maximal recommended pressure (8 psi) to switch the media and monitored the level of florescent signal at a 1 second time resolution. Analysis of changes in signal levels was done with a custom program written in the MATLAB software. The analysis revealed that media is switched almost completely within 3 seconds.

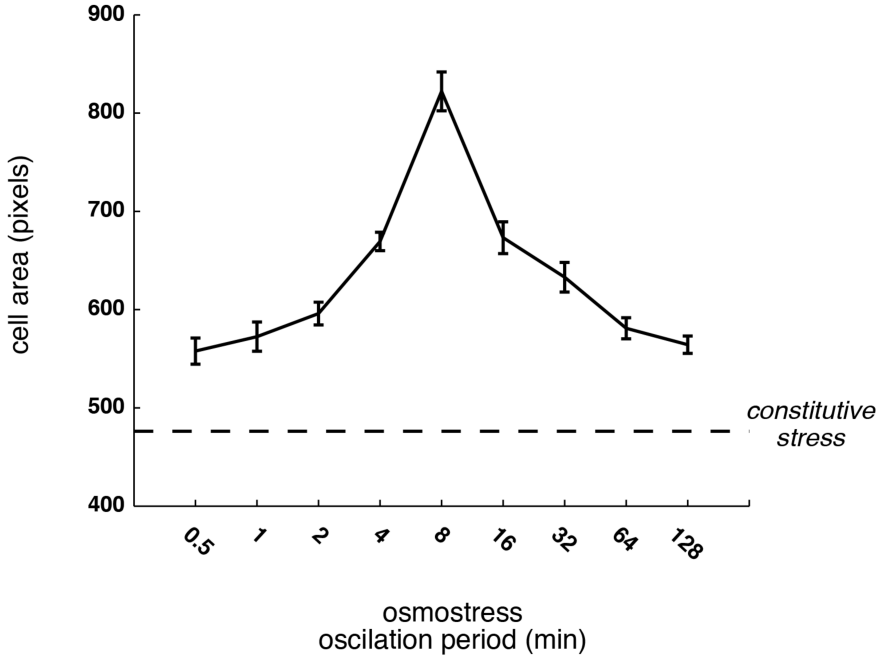

Fig. S2
Cell size measurements from a systematic frequency scan of mild osmotic oscillations (0.4M KCl). The graph shows the measured cell size area after 8 hours of growth in the indicated frequency. The cell boundaries were determined by analyzing the bright-field differential interference contrast microscopy images with a custom program written in the MATLAB software. The graph shows the median cell area and the bars mark the standard error (n=100-200 cells).

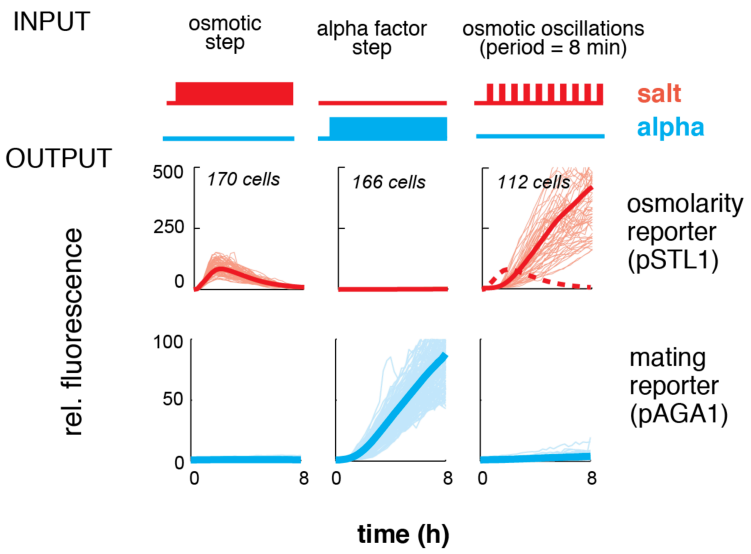

Fig. S3

The mating pathway remains inactivated under osmotic oscillations at the sensitive frequency. The transcriptional output is monitored in cells carrying reporters to both the osmotic pathway and the mating pathways. The graphs show the mean fold induction in florescence per cell and the single cell traces of cells within interquartile range. Both pathways remain isolated under all stimuli: Under the osmotic input only the osmotic reporter is transcribed while under saturating levels of mating pheromone (1 uM alpha factor) only the mating reporter is transcribed.

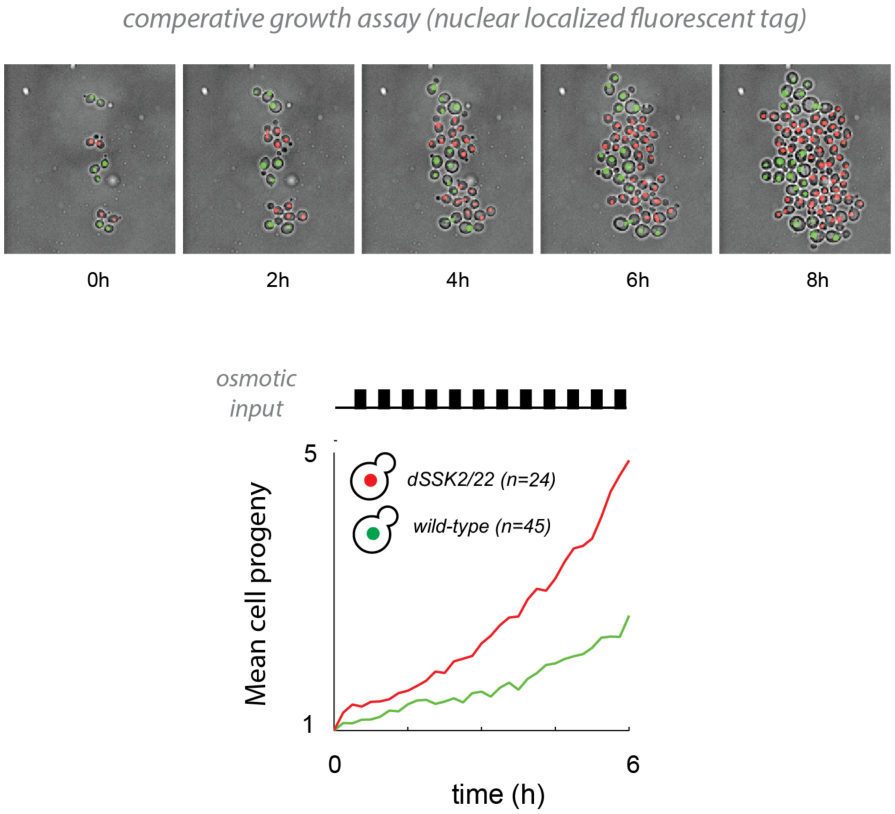

Fig. S4.
The experimental setup for the comparative growth assay. This experimental setup was used to insure that co-cultured strains are exposed to an identical environment and received identical osmotic stimuli. In order to distinguish between co-cultured strains the endogenous copy of the histone protein Htb2 was tagged with different florescent tags. Experimental setup was identical to that of other microfluidics experiments. After sonication and before loading cells into the flow chamber, cultures of the two strains were mixed in a 1:1 ratio. Analysis of the growth rate of each strain was done by a separate analysis of each florescent channel using the custom program written in the MATLAB software. The ratio between the growth rates of the two strains was taken as an indication of the relative fitness.

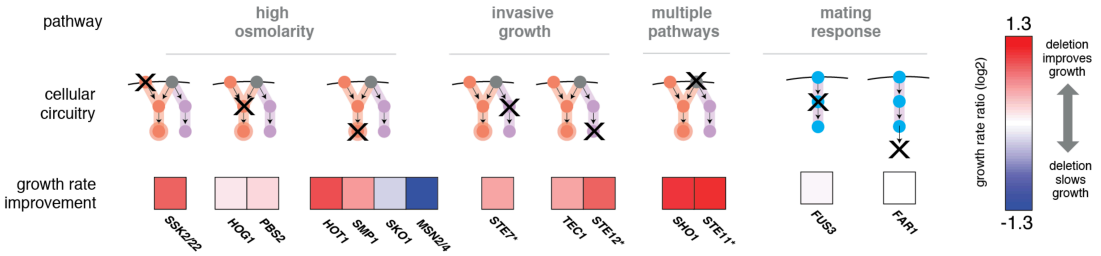

Fig. S5

Results from a mutational analysis points to contribution of both the osmotic and invasive growth pathways in growth inhibition under osmotic oscillations (0.4M KCl, 8min period). Mutations specific to the mating pathway do not affect fitness. The color code marks the fold improvement of the deleted strain versus a co-cultured wild-type strain. Deletions marked with star indicate that the deleted gene is also a component of the mating pathway.

Supplementary movies

Movie S1
Cell growth and osmoreponse under time-variant osmostress in a wild-type strain. A representative time-lapse microscopy movie depicting the cell growth and the osmotic response under different osmostress profiles (no stress, single step osmo-stress, and osmotic oscillations). The movie shows an overlay of images acquired from the DIC and yECitrine channels (images acquired every 15min). Stress starts one hour after the experiment started.

Movie S2
Cell growth and osmoreponse of wild-type and OspF strains. A representative time-lapse microscopy movie depicting the cell growth and the osmotic response under osmotic oscillations (period 16min) in the wild-type and feedback engineered strains. The movie shows an overlay of images acquired from the DIC and yECitrine channels (images acquired every 15min). Stress starts one hour after the experiment started.

Supplementary Table 1 - Plasmids used in this study

Plasmid	Parent vector / Selection	Promoter	Gene
pAM195	pNH605 (LEU)	*FRE-pCYC1*	mCherry
pAM184	pSV606 (URA)	*pSTL1*	yECitrine
pAM197	pNH603 (HIS)	*pAGA1*	yECFP
pWP207	pNH605 (LEU)	*pSTL1*	ΔN-OspF-zipper (EE)

Supplementary Table 2 - Yeast strains used in this study

Strain	Genotype
AM116	W303 MATa, ADE2
AM174	W303 MATa ADE2 HTB2-yECFP::KanMX
AM175	W303 MATa ADE2 HTB2-yEGFP::KanMX
AM176	W303 MATa ADE2 HTB2-mCherry::KanMX
AM185	W303 MATa ADE2 HTB2-mCherry::KanMX ΔN-OspF-Zipper(EE)::LEU2 PBS2-Zipper(RR)::HIS3
AM186	W303 MATa ADE2 HTB2-yEGFP::KanMX pbs2::HIS3
AM202	W303 MATa ADE2 HTB2-mCherry::KanMX PBS2-Zipper(RR)::HIS3 ste12::TRP1
AM203	W303 MATa ADE2 HTB2-mCherry::KanMX PBS2-Zipper(RR)::HIS3 ste11::NatMX6
AM210	W303 MATa ADE2 HTB2-mCherry::KanMX PBS2-Zipper(RR)::HIS3 fus3::TRP1
AM211	W303 MATa ADE2 HTB2-mCherry::KanMX PBS2-Zipper(RR)::HIS3 kss1::NatMX6
AM212	W303 MATa ADE2 HTB2-mCherry::KanMX PBS2-Zipper(RR)::HIS3 ste7::NatMX6
AM215	W303 MATa ADE2 HTB2-mCherry::KanMX PBS2-Zipper(RR)::HIS3 ssk2::HphMX6 ssk22::TRP1
AM216	W303 MATa ADE2 HTB2-mCherry::KanMX PBS2-Zipper(RR)::HIS3 sho1::TRP1
AM217	W303 MATa ADE2 HTB2-mCherry::KanMX PBS2-Zipper(RR)::HIS3 tec1::TRP1
AM220	W303 MATa ADE2 HTB2-mCherry::KanMX hog1::HphMX6
AM232	W303 MATa ADE2 HTB2-mCherry::KanMX msn2::HIS3 msn4::TRP1
AM236	W303 MATa ADE2 HTB2-mCherry::KanMX sko1::HIS3
AM238	W303 MATa ADE2 HTB2-mCherry::KanMX hot1::HIS3
AM259	W303 MATa ADE2 HTB2-mCherry::KanMX smp1::HIS3
AM261	W303 MATa ADE2 HTB2-mCherry::KanMX PBS2-Zipper(RR)::HIS3 ssk2::HphMX6 ssk22::TRP1 ste7::NatMX6
AM209	W303 MATa ADE2 pSTL1-yECitrine::URA3 FRE-pCYC1-mCherry::LEU2 pAGA1-CFP::HIS3

All strains are congenic W303 (leu2-3,112 his3-11,15 ura3-1 trp1-1 can1-1) and were constructed by standard cloning methods. The ADE2 conversion was used to reduce autofluorescence in microscopy measurements.