Title: A quorum of mechano-sensing fungal consortia

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Abstract: Bacteria use a chemical language of Quorum sensing (QS) to assemble biofilms, coordinate infections and survive in harsh environmental conditions. We discovered a new type of QS in yeast that does not involve chemical signal but instead relies on mechanical contact detection between cells that is controlled by FLO11 adhesin and SLN1 histidine kinase. This quorum mechano-sensing (QMS) controls downstream gene expression in cell density-dependent manner to resolve growth conflicts in mixed fungal consortia based on asymmetries in FLO11 activity. FLO11 mediates SLN1 inhibition, providing an adaptive limiter for the cell-density dependent regulation of gene expression in the population of cells. Genetic deletion of FLO11 removes inhibition of SLN1, yielding spontaneous activation of gene expression. Our study reveals an alternative QS mechanism that links FLO11 and SLN1 to intracellular regulation of gene expression, challenging classical view of chemically-driven QS systems and providing new strategies for controlling cellular collectives.

One-Sentence Summary:

Quorum macho sensing (QMS) mediates growth conflict resolution in mixed fungal consortia

Main Text:

Quorum sensing (QS) allows bacteria to communicate chemical messages across entire populations to recognize familiar kin (*1*). Actively growing microbial cells produce diffusing QS signals at a critical concentration threshold that directly scales with population density (*1*). This threshold triggers synchronized gene expression across the microbial consortia, providing a mechanism for cellular noise buffering and robust and coordinated colony-level responses (*2,3*). Much less is known in fungi such as yeasts that can produce QS-like molecules *(4*) since molecular mechanisms underlying QS in fungi are largely unknown (*5*). Different from bacteria which use extensively QS, the kin recognition in fungal consortia involves intercellular(trans) and across cell(cis) mechanical interactions mediated by the FLO11 cell wall-resident adhesin (*6-10*). Intriguingly, FLO11 expression scales with cell density, typically peaking at the entrance to the stationary growth phase (*9*). Nevertheless, molecular queues and mechanisms downstream of FLO11-mediated kin detection remain an enigma. Furthermore, it is unknown if this kin recognition mechanism would share somehow similar characteristic to that of QS mechanisms in bacteria.

Interestingly, bacteria, fungi and plants utilize similar multi-step histidine kinase (HK) phosphorylation signaling to report changes in cell wall integrity and osmotic conditions (*11-17*). Arguably the most studied example of this type of HK signaling is the SLN1-dependent osmosensory pathway in yeast (*18-20*). Although, the molecular basis of SLN1-mediated mechanical stress perception in the cell wall remains still unknown, it has been suggested that SLN1 activity can be modulated by cell-wall resident mannoproteins (*21*). Despite this putative connection between SLN1, cell wall and mechanoperception, the SLN1 activity has never been studied in the context of kin recognition and QS-like phenomena.

Identifying molecular bases underlying social communication and kin detection is a critical step for the rational control of the biofilm dynamics. Here, we reveal molecular mechanisms acting downstream of kin detection and social communication in yeast that control macroscopic growth dependencies in fungal consortia. Unexpectedly, we found that these growth patterns emerge from mechanical conflicts that are resolved through previously unknown type of QS that links together adhesin and osmosensor activities in the fungal cell wall.

Kin detection in mixed consortia relies on a noncanonical SLN1-dependent intracellular signaling route

To explore the molecular mechanism of kin detection and social communication through adhesins in fungi we used two different strains of yeast *S. Cerevisiae* to establish a mixed fungal consortium model (Fig. 1A). Cen.pk2 strain('Kin') has lost major flocculation genes during the evolution (*22*) but retained FLO11 which is the central regulator of biofilm formation and invasive growth (*8, 23-26*). In contrast, the BY4741 strain('Intruder') is a descendent of wild-type yeast S288C that retained all major flocculation genes but their expression remains dormant due to deleterious mutations in FLO8 transcriptional activator (*6, 9*). However, the weak activity of FLO11 was previously reported in BY4741 strain (*27*). Both strains show similar growth kinetics when cultivated alone (Fig. S1), yet they could exhibit interesting asymmetries in the amount of FLO11 (Fig. 1A), thus providing an intriguing model for the exploration of dynamics

underlying kin detection and growth conflict resolution. One hypothesis is that emerging asymmetries in adhesin levels between two strains would trigger the alarm at the detection threshold that further transmits information to the cell interior for subsequent growth regulation. To test this idea, we initially checked whether the SLN1-dependent pathway (Fig. 1B) in the 'Kin' is active in this model consortia using the short-lived NeonGreen reporter (dNeon) placed under the control of the OCH1 promoter (*28*) or its engineered synthetic variant (*29*). The activity of both promoters presumably depends on transcription factor SKN7 which is phosphorylated(activated) by SLN1 through trans-phosphorylation (*20, 28*) (Fig. 1B). In either scenario, the 'Kin' reporter was, however, inactive when cocultured with the 'Intruder' strain (Fig. S2A). However, previous studies suggest that SKN7 may require co-activators and does not act as sole transcriptional regulator (*30-32*). We sought that SKN7 may require coactivator to strongly induce gene expression following phosphorylation. To investigate this possibility, we engineered a synthetic promoter (pSynSKN7) to facilitate the galactose-promoted association of SKN7 with the DNA by recruitment of the GAL4 transcriptional co-activator (*33*) (Fig. 1C, Fig. S2B). Remarkably, we found that dNeon reporter in the 'Kin' driven by pSynSKN7 was activated with the high-potency in presence of both the 'Intruder' and galactose yet it remained silent in either of control conditions (Fig. 1D). Furthermore, this dNeon activation does not trigger the HOG1-mediated osmotic response pathway (*12*) nor the cell wall integrity (*13*) because neither pSLT1 hyperosmotic (*12*) nor pRML1 cell wall stress responsive (*13*) promoters were activated in the 'Kin' - 'Intruder' cocultures (Fig. 1D).

Then, we inspected the growth competition in cocultures of 'Kin' and 'Intruder' on agar over several days starting from 1:1 initial inoculation. We recorded a generation of flower-like patterns that were dominated by protruding 'Kin' populations (Fig. 1E). These dNeon patterns are created by 'Kin' (cyan) which established the sharp boundary interface with the 'Intruder' (shown in red) on the colony periphery (Fig. 1F and Fig. S3). By creating these tight boundaries/barriers 'Kin' eventually outcompeted and blocked 'Intruder', making space for peripheral cells to grow freely (Fig. 1E). Thus, these findings hint at the presence of previously unknown SLN1-dependent signaling route of mechanical, cell contact-based nature that could underly the complex pattern generation at the interface between strains.

Dynamic, cell density-dependent mechanism connects Flo and SLN1 at cell-to-cell contacts

To understand how and when the 'Kin' becomes activated, we performed time-course experiments to track dNeon reporter dynamics in uniformly mixed liquid co-cultures. Unexpectedly, we found that the reporter was rapidly activated when cultures reach a specific cell density threshold (THR) represented as OD_{600} measured at 10% of peak reporter fluorescence. Notably, this THR corresponds to the late-exponential phase/early stationary phase ($OD_{600} \sim 1.17$, Fig. 2A). The dNeon reporter response was dramatically increased following higher cell densities (Fig. 2A) that is reminiscent of the QS phenomena and switch-like dynamics (Fig. 2B).
Chemical compounds have been suggested to possibly act as QS molecules in yeast, including 2-phenylethanol, tryptophol and tyrosol (*34*). These putative QS signals seem to require both intact FLO8 protein (acting upstream of FLO11) and functional cAMP signaling (*35*). However, 'Intruder' carries non-functional FLO8 (*6, 9*), and 'Kin' has several mutations in regulators of cAMP signaling, including CYR1 gene (*22*). Therefore, it is very unlikely the classical QS

would be responsible for the activation of dNeon reporter. Nevertheless, we explored this possibility by culturing 'Kin' with extracts of media in which 'Intruder' was grown, possibly enriched in putative QS-like molecules. However, dNeon reporter was inactive under this condition (Fig. S4), indicating that the signal is unlikely to be of chemical nature.

We then hypothesized that asymmetric amount of adhesin FLO11 in 'Kin' versus 'Intruder' could trigger transient instabilities in contacts between strains, possibly leading to SLN1-pathway activation. To test this concept, we cocultured both strains in a microfluidic device (see M&M for more information, Fig. S5) under continuous media perfusion. Indeed, experiments on a chip revealed transient damped oscillatory dynamics of 'Kin' activation through dNeon reporter at contact sides of both strains, indicative of transient instability that underlies the dNeon activation (Fig. 2C, D, Fig. S6A and Movie S1). Furthermore, the activation of dNeon reporter preceded aggregation and 'Kin' microbiofilm formation, eventually creating a growth barrier and outcompeting the "Intruder" (Fig. 2E, Fig. S6B) similar to that observed on cocultures grown on agar (Fig. 1E). To further probe these transient switching dynamics associated with unstable contact formation observed in microfluidic experiments, we sought to manipulate the initial ratios of each strain in the consortia. Notably, we could detect a transient peak in the dNeon activation dynamics (Fig. 2F) and a remarkably steep response when 'Kin' was in majority (Fig. 2F, Fig. S6F). These data indicate that the 'Kin' strain could behave as a density-dependent trigger in the presence of 'Intruder' by detecting unstable contacts (incompatibility) between these two strains, whereas 'Kin' cultured alone would rely on stable cell-to-cell contacts which go undetected. Therefore, this putative mechanical contact-based mechanism shares striking similarities to QS, we refer to it onwards as Quorum mechano-sensing (QMS).

Given that FLO11 is activated during the late-exponential growth phase in yeast *(6, 9*), we tested whether the observed hypersensitive response of the 'Kin' strain requires FLO11. FLO11 mechanical contacts can be disrupted by exogenous treatments with mannose (*6, 36, 37*), or alternatively by increasing the pH of the solution that disrupts Ca+-dependent attachment to the cell wall mannans (*6, 23, 38*), or adding the anti-adherence agent, such as lectin concanavalin A (*39*). Thus, the chemical modulation of adhesin interactions in a dose-dependent manner resulted in the altered 'Kin' response in the presence of Intruder (Figs. 2G and S6C, D). Furthermore, disruption of adhesin interactions due to mild mannose treatment also increased THR by 8%-15% and eventually dampen the dNeon response at higher mannose concentrations (Figs. 2G and S6E). Therefore, FLO11 asymmetries between 'Kin' and 'Intruder' could be detected by SLN1-dependent intracellular signaling through QMS mechanism.

We then tested whether 'Kin' response can be attenuated by imposing the hyperosmotic stress which deactivates SLN1(*12*). Indeed, treatments with KCL led to a gradual decrease of dNeon reporter response and increment of THR (Figs. 2H, and S6E), further suggesting that the activation of 'Kin' relies on phosphorylated(active) form of SLN1. Thus, either mannose or KCL treatments had a strikingly similar effect on the SLN1 pathway activity, implicating involvement of mechanically contracting and extending FLO11(*7, 40*) in the regulation of SLN1 pathway. The hyperosmotic stress can lead to the tight clustering of SLN1 and its deactivation (*18*). To further test whether forcing the dimerization of SLN1 could impair QMS mechanism by deactivating SLN1 we constructed a chimeric SLN1 in which we swapped the extracellular

domain for complementary synthetic leucine zippers (*41*)(Fig. S7A). Indeed, this synthetic pair of chimeric SLN1s decreased activity of SLN1, and disarmed the QMS detection mechanism (Fig. S7B).

FLO11 controls the timing and steepness of QMS response

FLO11 is a plausible candidate for regulation of SLN1 inhibition in 'Kin' (Fig. 3A, B), we tested how the deletion of FLO11 from 'Kin' would impact reporter expression dynamics. Remarkably, flo11-deficient 'Kin Δf1o11' strain was strongly activated even in the absence of 'Intruder' (Fig. 3C) which indicates that FLO11 is required to inhibit the SLN1-mediated activation of dNeon reporter. Also, we noticed earlier activation and significantly flattened response characteristics compared to cocultures of 'Kin' with 'Intruder' in which FLO11 was intact (Fig. 3C), which confirmed the inhibitory role of FLO11 in SLN1 pathway activation. Therefore, FLO11 in 'Kin' may directly controls THR and steepness of QMS response (Fig. 3C, F). Because FLO11 can form homotypic contacts in the same kin as reported in earlier studies (*8, 24*) (Fig. 3A, B), a putative inhibition mechanism could depend on interplay between FLO11 and SLN1 extracellular domains (Fig. 3A, B). To test whether FLO11 restoration would be sufficient to inhibit QMS response, we cocultured 'Kin Δf1o11' with 'Kin w/o rep' lacking reporter. We found that FLO11 of 'Kin w/o rep' restored partial inhibition of responsiveness in 'Kin Δf1o11' by substantially delaying THR by nearly 18% (Fig. 3F, Fig. S8A, B), suggesting that the FLO11-dependent regulation of activation threshold requires, at least in part the FLO11 presence in the contacting strain. Notably, the deletion of Flo11 in 'Intruder' delayed activation dynamics when cocultures with 'Kin' but did not affected response amplitude (Fig. 3F, Fig. S8A).

Next, we checked whether increased production of FLO11 in the 'Kin' strain ('Kin FLO11OX') strain would increase the inhibition of the SLN1-mediated reporter response but reducing chance for unstable contacts with 'Intruder'. Notably, we found that coculturing 'Kin FLO11OX' with 'Intruder' resulted in a ~2-fold weaker response compared to controls (Fig. 3D, F). Furthermore, this response was delayed and less aggressive than in controls (Fig. 3D, F). Alternatively, producing more FLO11 in 'Intruder' showed delayed response (Fig. 3D, F). We then asked which part of FLO11 may be involved in the inhibition of SLN1 in 'Kin'. For that purpose, we constructed a synthetic membrane receptor spanning cell wall by fusing nanospring-like ectodomains of WSC1 and MID2 cell-wall proteins (*42, 43*) with the adhesin-like N-terminal part of FLO11 (*36*)(Fig. S8C) to allow for mechanical unfolding in the cell wall during FLO11-mediated homotypic contacts. We expressed this chimeric protein in either 'Kin sFLO11' or 'Intruder sFLO11' strains and monitor dNeon reporter activity in 'Kin'. Intriguingly, the induced expression of the chimeric membrane-bound FLO11 receptor showed consistent but much more noticeable changes in dNeon reporter activity as compared to FLO11 full-length overexpression (Fig. 3E, F). Firstly, we noticed a complete inhibition of reporter signal in 'Kin sFLO11' in coculture with 'Intruder' (Fig. 3E), whereas cocultures of 'Kin' with 'Intruder sFLO11', resulted in even more delayed but very sharp responsiveness, likely due to accumulation of less stable contacts between non-isogenic strains (Fig. 3E, F).

These data indicate that increasing unstable contact formation through FLO11 trans interactions between 'Kin' and 'Intruder' further release inhibition and boost sensitivity and amplitude of

QMS response. Furthermore, our data indicate that relative FLO11 asymmetries between strains may be at the core of QMS mechanism controlling both THR and steepness of the QMS response.

Studying QMS by incorporating a synthetic orthogonal histidine kinase system from bacteria

Our findings indicate that QMS mechanism mediates SLN1 activation through unstable FLO11 contacts between non-isogenic strains. QMS leads to a subsequent two-step phosphorylation cascade involving phosphor relay onto the SKN7 transcription factor (*12, 20*) which activity is quantified by dNeon reporter. Since SLN1 mutants are lethal and mutations in downstream components such as SKN7 could produce unwanted pleiotropic effects, we sought to test pathway downstream of SLN1 by constructing a synthetic orthogonal pathway to plug QMS to downstream regulation of gene expression. Bacterial two-step HK kinase systems (*44-46*) offer an excellent opportunity to introduce heterologous HK-based regulation into fungi because of evolutionary similarities between fungi and bacterial HK systems. We chose a specific FixL-FixJ system from the nitrogen-fixing bacteria *Rhizobium* meliloti(*47,48*) as this system has a lower likelihood of being activated by the yeast YPD1 kinase (Fig. 4A, Fig. S9). To assemble a synthetic rerouting, we created a chimeric HK by fusing the signal peptide, transmembrane, and HAMP domains of SLN1 with the HK domain of FixL HK (Fig. S9). FixJ transcriptional regulator was fused to transactivation domain from Herpes virus (VP64)(*49*). We then placed our dNeon reporter under the control of synthetic FixJ-regulated promoter (*50,51*).

We tested if QMS activated SLN1 would directly transfer active phosphoryl-group to chimeric FixL which would then activate FixJ transcription factor (Fig. 4A). Firstly, we checked whether the presence of FixJ and reporter alone would result in leaky reporter expression. Our quantifications suggest that FixJ alone is not phosphorylated by native YPD1 phosphoryl group transfer mechanism (Fig. 4B, Fig. S10B, C), confirming suitability of this bacterial HK system for orthologous pathway connecting QMS to any gene of interest. Indeed, using the full system ('synFix') that includes FixL chimera showed clear activation that was dependent on QMS and FixL (Fig. 4B, Fig. S10C). To mimic orthologous SKN7(D427N) unphosphorylated mutant (*21*) we mutated phosphor-accepting aspartate residue in FixJ ($Fixj_{nd}$) completely blocked the reporter response (Fig. 4B, Fig. S10A, C). Because QMS does not seems associated with cell wall integrity and hypoosmotic responses (Fig. 1D), we tested whether imposing cell wall stress through applications of chitin-binding dye calcofluor white would trigger reporter response in 'Kin' without presence of 'Intruder' in a synthetic signaling route. In this scenario, reporter remained silent, indicating that cell wall stress may not trigger QMS response (Fig. S10D). Finally, we investigated if this synFix system responds to mannose and SLN1-deactivation by hyperosmotic conditions. Indeed, we found that Mannose and KCL inhibited FixJ reporter response (Fig. 4C and D) in similar manner to that observed in a native system (see Fig. 2G, H), that is by delaying response, increasing THR and reducing response amplitude (Fig. S10E). These findings indicate that QMS might be studied orthogonal intracellular signaling pathway but also could be used to control gene expression dynamics in the response to mechanical contact-based and cell density-dependent cues.

Conclusions

Understanding molecular machineries of social interaction through QS in microbes is a key to control pathogenic infections and control biofilm formation for various synthetic biology applications. In fungal kingdom, putative QS mechanisms remained largely unknown (*4, 5*). We revealed a previously unidentified QS-like behavior that involves kin detection and mechanical contacts in the cell wall of baker's yeast as opposed to classical chemical perception (*1*). We showed that this QMS mechanism requires strain to strain asymmetries in adhesin FLO11 activity which then inhibits SLN1, thus adjusting gene expression in the presence of intruder. Unstable contacts resulting from these asymmetries between non-isogenic strains may trigger QMS and resolve growth competition producing astonishing patterns reminiscent of flower petals. Furthermore, the QMS mechanism does not seem to involve hypoosmotic nor cell wall stresses through canonical pathways. Also, our results are in a strong agreement with previously study that suggests involvement of other cell wall mannoproteins in the regulation of SLN1 activity (*21*). We demonstrated that the extracellular domain (ECD) of FLO11 tunes the response sensitivity and QMS density threshold. It is an intriguing possibility that FLO11 ECD interacts with SLN1 ECD either directly or indirectly allowing SLN1 to detect asymmetries in FLO11 activity underlying the nature of cell-to-cell contacts either unstable (ON) or stable (OFF). Further studies should address the structural nature of FLO11-SLN1 interaction. Finally, we demonstrate that QMS mechanism can be studied through synthetic modular regulatory pathway to control gene expression orthogonally.

These results open opportunities for development of new systems that can be used to tailor heterologous biofilm dynamics for various applications including biomaterial and preventing biofilm-mediated pathogen infections. It remains a fascinating question whether similar QMS-like mechanism could operate in multicellular fungi, and plants that use analogous HK osmosensing signaling circuits (*14, 17, 52*) as such mechanisms would act in specific kin or cell-type detection and to resolve mechanical growth conflicts during morphogenesis.

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Supplementary Materials

Materials and Methods

Figs. S1 to S10

Tables S1-S4

References (*53–57*)

Movie S1

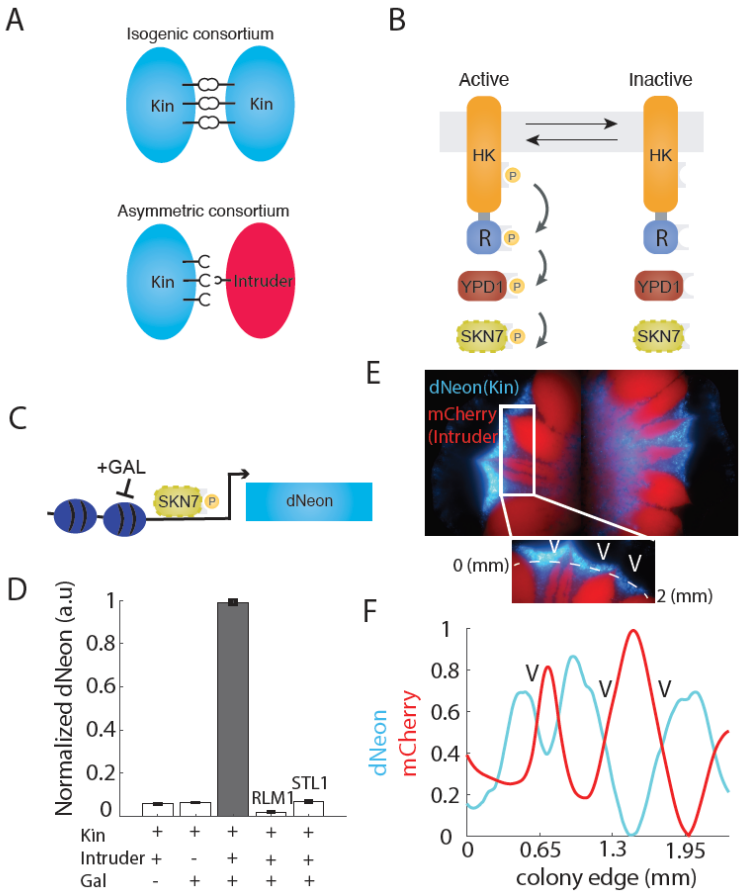

Fig. 1. Contact-based detection mechanism resolves growth conflicts in non-isogenic consortia through SLN1-dependent intracellular signaling

A, Schematic of asymmetric strain consortia with 'Kin' (CEN.PK2) and 'Intruder' (BY4741) haploid laboratory strains. 'Kin' can express FLO11 normally whereas 'Intruder' has none or very little expression of FLO11 due to FLO8 defect. **B**, Schematics of canonical SLN1-YPD1-SKN7 signaling cascade. SLN1 in active phosphorylated state transfer phosphoryl group from receiver domain (R) to YPD1 and subsequently onto SKN7 transcription factor. **C**, Basic schematic of engineered SKN7-responsive promoter. Galactose promotes coactivator recruitment guided by active SKN7 attachment to DNA. This promoter drives dNeon reporter to monitor dynamic changes of fluorescence signal. **D**, Normalized dNeon activation in different co-culturing conditions. Note that observed response does not associate with wall stress (pRML1 reporter (*13*)) or changes in osmotic conditions (pSTL1 reporter (*12*)) responses. Note that only presence of both galactose and 'Intruder' triggers dNeon activation. **E**, Growth patterns of mixed consortia (1:1 initial inoculation at OD_{600}=0.1 each) show astonishing growth arrangements with 'Kin' protruding from the colony peripheral after 5-7 days of growth in standard conditions. 'Kin' dNeon reporter is shown in cyan and 'Intruder' expressing constitutively mCherry is

shown in red. **F**, Representative trend of growth pattern on the colony peripheral (n=10), arc on colony periphery(edge) was depicted by dashed white curve. Note generation of flower-like pattern of ‘’Kin’ and ‘Intruder’ growth extrusions.

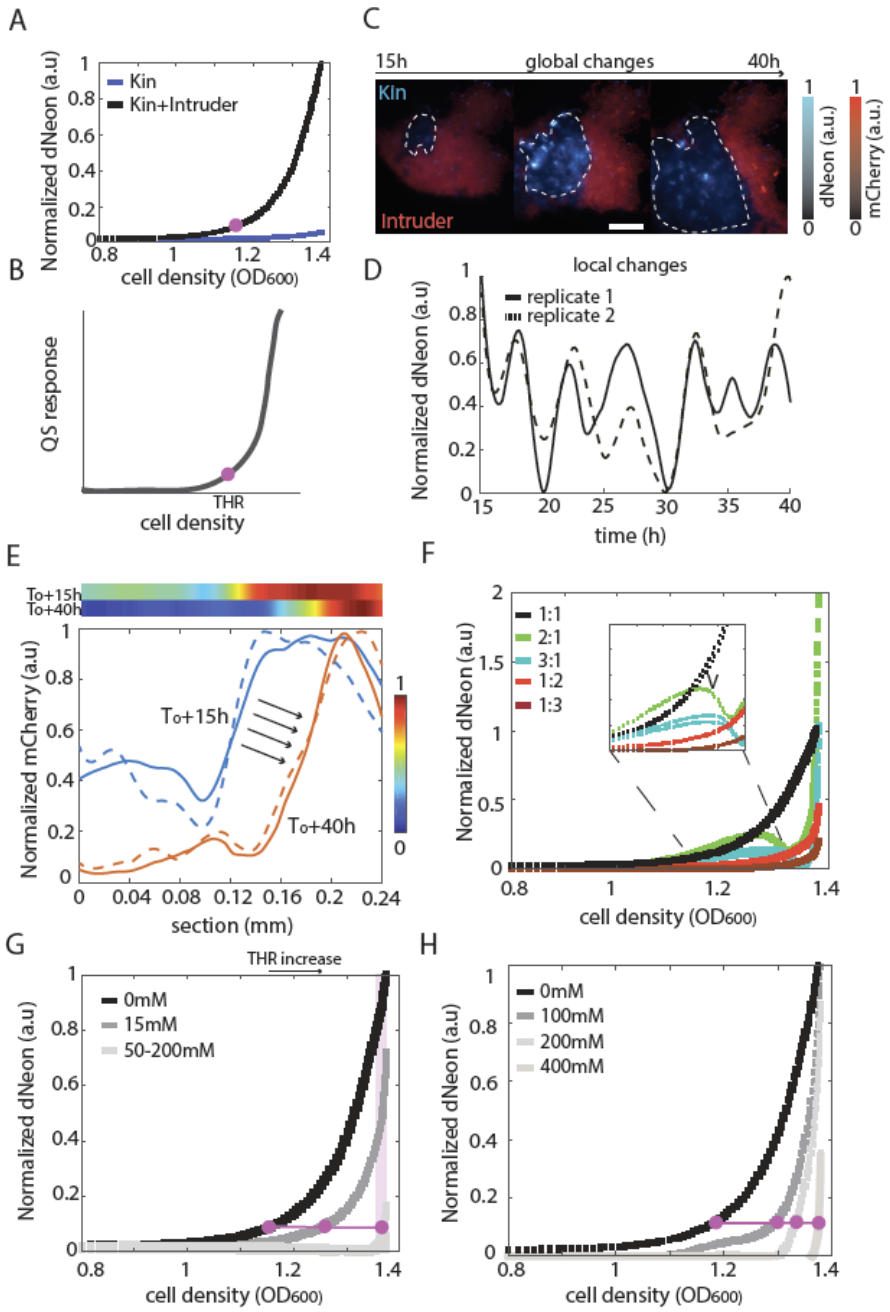

Fig. 2. Quorum mechano-sensing(QMS) controls dynamic population-level response of 'Kin' to 'Intruder' presence

A, B Time-lapse kinetics of 'Kin' activation (QMS curve) in the presence or absence of 'Intruder' (A) and the model of classical QS response (B). Note that cell density threshold (THR) for dNeon reporter activation corresponds to early stationary phase of cell growth ($OD_{600} > 1$) and 10% of maximal observed response. **C**, Snapshots from time lapse experiments of mixed

‘Kin’ and ‘Intruder’ (1:1) consortia performed in the microfluidic device. dNeon fluorescence (’Kin’) is shown in cyan and ‘Intruder’ is marker in red with constitutive mCherry reporter. Note unstable contacts between strains trigger dNeon response that underlie switch-like dynamics. **D**, two representative time-course plots from microfluidic experiments presented in (C). Note the transient oscillatory dynamics of ‘Kin’ in response to presence of ‘Intruder’. **E**, Time evolution of growth conflict resolution between ‘Kin’ and ‘Intruder’ in microfluidic environment (two examples of trap sections are shown as solid and dash curves, respectively). The ‘Intruder’ presence shown with mCherry reporter was quantified over time. In ~24h (red curves) ‘Intruder’ occupancy drastically decreases to nearly 50% of its original size (blue curves). This is a consequence of aggressive growth of the ‘Kin’. **F**, QMS curves for different initial inoculation ratios of ‘Kin’ and ‘Intruder’. Note that early prominence of ‘Kin’ (green curve) reveals transient unstable dynamics, triggering reporter activation as seen in microfluidic experiments. **G, H**, QMS kinetics after mannose(G) and KCL(H) treatments suggest the involvement of adhesin FLO11 and SLN1 pathway in the activation of ‘Kin’. Progressive increase in Mannose and KCL gradually elevated THRs (pink dots) and reduced amplitude of dNeon activation. QMS curves show all data points (filled squares) collected from three independent replicates. Standard deviation correlates with thickness of the curve.

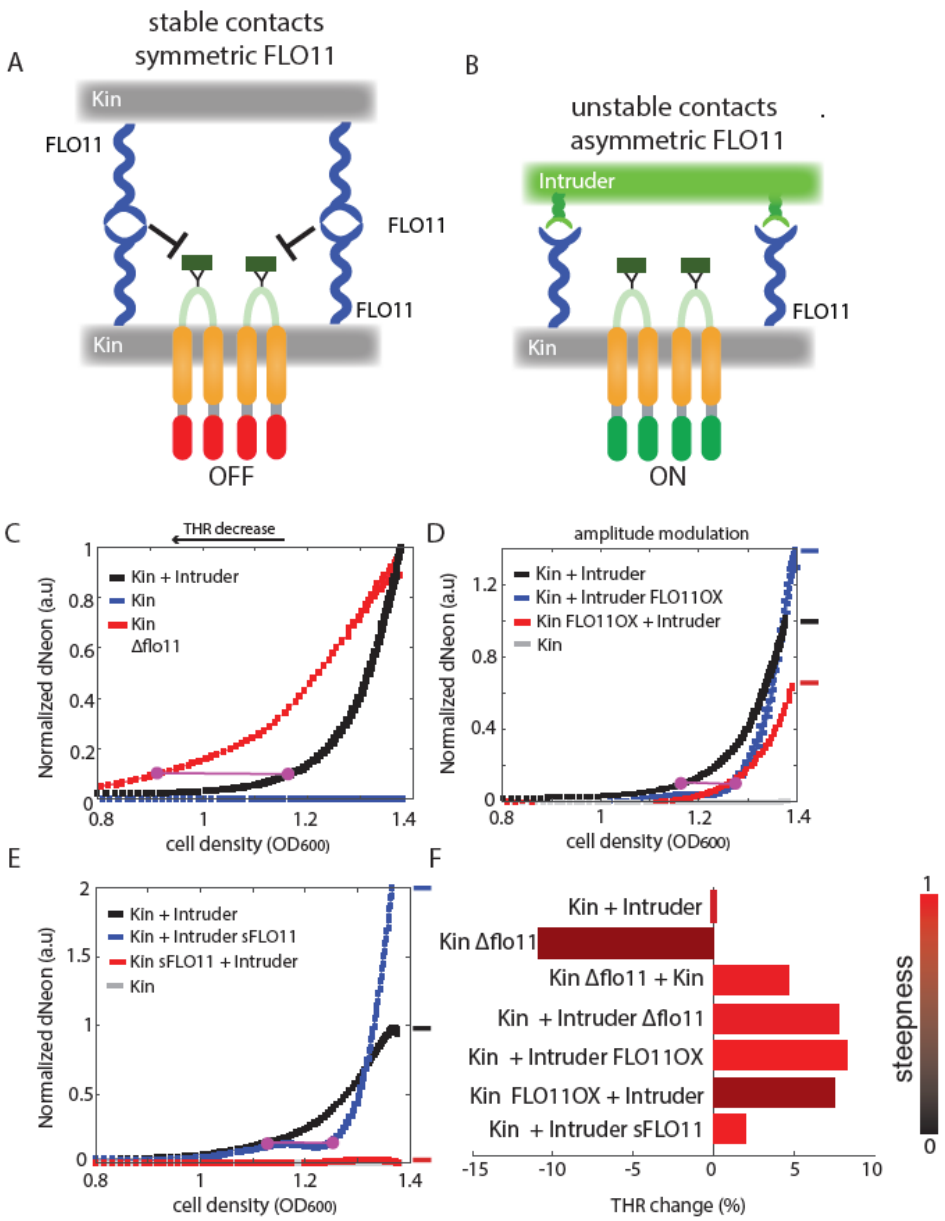

Fig. 3. FLO11 promotes SLN1 inhibition and modulates QMS responsiveness characteristics

A, B, Schematic of putative QMS mechanism. In control condition FLO11 in isogenic 'Kin' cocultures creates stable contacts and inhibits SLN1. In contrast presence of 'Intruders' with low or no FLO11 inhibition establishes unstable contacts between non-isogenic strains releasing SLN1 from repression by triggering QMS response at high cell density (B)**.**
C, QMS curves in controls and flo11 mutant of 'Kin' (Kin Δflo11). The deletion of FLO11 in 'Kin' resulted in early dNeon response and attenuated repression. **D, E**, Coculturing experiments with strains overexpressing FLO11 full length(D) or sFLO11 genes(E). Note that boosting FLO11 in 'Kin' has opposite effect to its deletion (C). Increase in FLO11 in 'Kin' compared to

‘Intruder’ resulted in stronger inhibition of QMS probably due to higher frequency of isogenic contacts between ‘Kin’. Increasing contacts with homotypic FLO11 interactions through explicitly FLO11 extracellular domain in ‘Intruder’ boost inhibition but allowed for the sharp response, due to existence of unstable contacts between strains. This effect was prominent in chimeric protein sFLO11 that contains N-terminus domain of FLO11. **F**, THR changes in different cocultures (pink dots connected by lines). dNeon response steepness is color coded in red. QMS curves show all data points (filled squares) collected from three independent replicates. Standard deviation correlates with thickness of the curve.

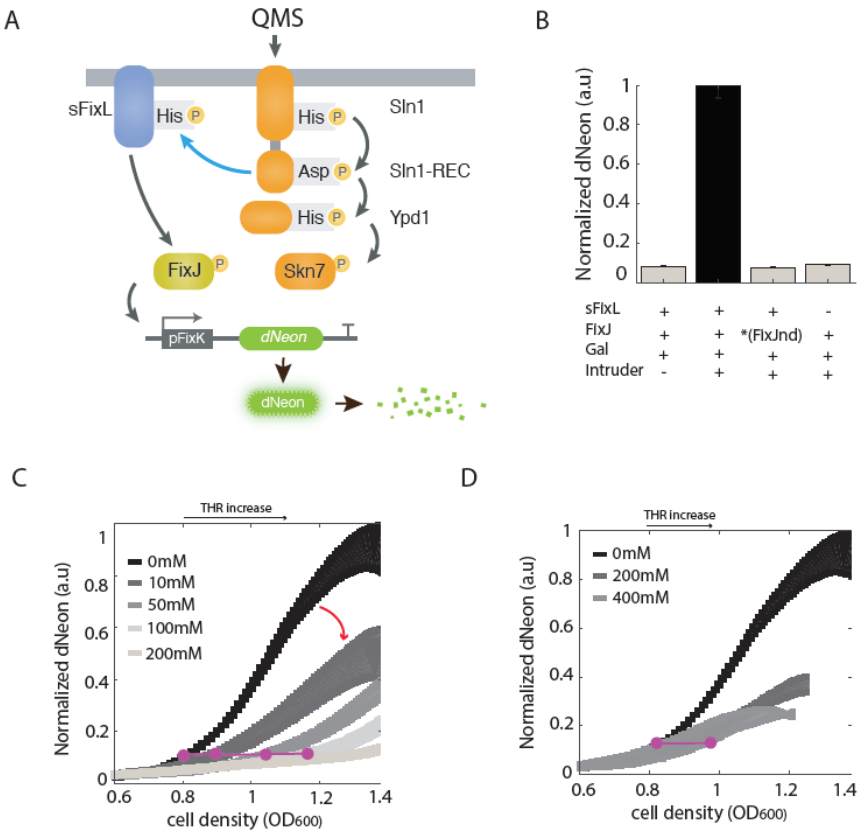

Fig. 4. Wiring QMS to synthetic intracellular circuit to control orthologous gene expression

A, Schematics of synthetic system design integrating upstream QMS mechanism (orange). Bacterial HK sFixL(in blue) fused with the HAMP and transmembrane domains of yeast SLN1. FixJ receiver (in green) was fused to herpes-virus VP16 activation domain. The synthetic promoter containing FixJ operators (pFixK) controls dNeon reporter. **B**, dNeon Reporter activation level in different configurations of synthetic circuit. $FixJ_{nd}$ stands for non-phosphorylatable FixJ mutant (D54Q) orthologous to SKN7(D427N). **C**, **D** dNeon time-lapse activities in the complete system subjected to different concentrations of mannose (C) or KCL (D). Note gradual shift in cell density threshold (THR) and decay of response amplitude as observed in QMS system; see Fig. 2G, H for comparison. QMS curves show all data points (filled squares) collected from three independent replicates. Standard deviation correlates with thickness of the curve. THR is represented by pink dots.

Supplementary Materials for

Title: A quorum of mechano-sensing fungal consortia

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This PDF file includes:

Materials and Methods
Figs. S1 to S10
Tables S1-S4
Caption for Movie S1

Other Supplementary Materials for this manuscript include the following:

Movie S1

Materials and Methods

Plasmids and gene constructions

Plasmids were constructed using isothermal Gibson assembly cloning (*53*). A middle-copy (~10-30 copies) episomal vector pGADT7 (Takara Bio Inc.) was used as the basic expression backbone for the introduction of chimeric proteins, synthetic genes and reporter constructs. The DNA fragments used for the construction of sFLO11, ZIPsln, and sFixL/FixJ/ pFixK and dNeon reporter plasmids (including backbone and inserts) were either amplified from yeast genomic DNA using a polymerase chain reaction (PCR) or synthesized through Integrated DNA technologies (IDT) services. Specific adapters at the 5' and 3' ends of DNA fragments were designed to facilitate efficient Gibson assembly and minimize secondary structure formation. High-fidelity Phusion PCR Master Mix with HF Buffer (Thermo Scientific, USA) was used for all PCR reactions, following the manufacturer's protocol. The size of the amplified PCR products was confirmed in 1% agarose gel electrophoresis, purified using the EasyPure PCR Purification Kit (TransGen Biotech, China), and concentrated to approximately 100 ng/µL before Gibson assembly. Plasmids and DNA parts are summarized in Tables S1 and S2.

Yeast genomic integrations and Flo11 knockouts

To study 'Intruder' presence in microfluidic and agar plate experiments, the constitutively expressed mCherry reporter was integrated in genome of BY4741 and selected with G418 antibiotic (250mg/L). The correct insertion was confirmed by sequencing and fluorescence microscopy. To generate Δflo11 CEN.PK2-1C strain ('Kin' Δflo11), gRNA was designed to target the 5' of FLO11 exon (sequence:" CGGATTTCCCAGGCTTCTAT") by minimizing off-side effects and cloned into gRNA shuttle plasmid provided by Easy-Clone Marker free kit (*54*) https://www.addgene.org/kits/borodina-easyclone-markerfree/). Then, 'Kin Δflo11' mutant was obtained with CRISPR-based genomic knockout (*54*) and confirmed by sequencing. BY4741 Δflo11 strain was purchased through Horizon Yeast Knockout library (https://horizondiscovery.com/en/non-mammalian-research-tools/products/yeast-knockout). The Galactose-inducible full length FLO11 ORF clone with URA selectable marker was a kind gift from Dr. Jean Marie Francois (Institut National des Sciences Appliquées de Toulouse: Toulouse, FR). All episomal and integration plasmids were transformed in ultra-competent cells from E. coli DH5a strain using standard protocols.

Yeast growth, transformation and culturing conditions

Yeast strains were cultivated in Yeast Nitrogen Base (Formedium, UK) plates supplemented with 2% of glucose at 30 °C. They were then grown in liquid low fluorescence Yeast Nitrogen Base media (Formedium, UK) supplemented with 2% sucrose at 30 °C in a shaking incubator. Minimal drop-out media compositions were used at all stages to prevent plasmid loss. Appropriate antibiotics were added to key dominant selection markers (e.g., G418 (250mg/L) or Hygromacin B (300mg/L)). The activation of the system was monitored using mNeonGreen fluorescent protein was fused to non-cleavable ubiquitin UbG76V for fast degradation (*55*) referred to as the dNeon reporter. Plasmids were transformed into competent S. cerevisiae BY4741 and CEN.PK2-1C cells (a kind gift from Dr. Luis Rubio). Competent yeast cells were prepared and transformed with the Frozen-EZ Yeast Transformation II Kit (Zymo Research,

USA), following the manufacturer's instructions. All strains were transformed with three plasmids, each containing a selection marker for the complementation of either uracil, histidine or leucine auxotrophy. Transformant cells were selected on Yeast Nitrogen Base (Formedium, UK) 2% agar plates deficient in the corresponding selection nutrients. Engineered yeast strains are presented in Table S3.

Microfluidics device fabrication and cell loading and imaging protocols

Fluorescence microscopy images of the engineered strains were acquired in 40 h time-lapse experiments using microfluidics chips to grow colonies of cells under a constant perfusion of medium according to protocols previously described (*56*). Cocultures were grown for first 15h before imaging and then imaged every 10 min up to 40h. Each cell trap (500x500x8 microns) contains > 10000 cells. A general scheme of the microfluidics setup is provided in Fig. S5. The internal features of the chips (media channels and cell traps) were laser-printed on polystyrene sheets. Next, the sheets were heated to approx. 160 °C. The printed pattern shrinked and increased in height by approx. 10-fold, rendering a high-relief mold that was used as a template for soft lithography. After thorough cleaning, the device mold was covered in PDMS silicone (Sylgard), degassed in a vacuum pump for approx. 20 minutes, and cured overnight at 80 °C. The resulting PDMS wafers were cut to the final shape of individual chips and input ports were opened using a 0.7 mm biopsy puncher (World Precision Instruments). Cover glasses were cleaned in a sonic bath, thoroughly rinsed with water and ethanol, and blow dried using a nitrogen gun. In order to bond the PDMS chips to the cover glasses, the surface of both the chip and the glass were exposed to oxygen plasma with a Corona SB plasma treater (ElectroTechnics) for about 15 seconds. Then, both surfaces were brought together and kept at 80 °C overnight. The microfluidics chip was vacuumed for at least 20 minutes to facilitate the loading process. Flexible transfer tubing lines (ColePalmer) with an internal diameter of 0.5 mm were cleaned with 70% ethanol and connected in sterile conditions to syringes containing fresh media and to output containers. The input syringes were placed in a height control device to regulate the flow by adjusting their relative height to the output containers. The lines were then plugged into the chip one by one, ensuring that the liquid reached all features of the chip and no air bubbles were trapped. A separate input line had been connected to a falcon tube containing a liquid culture of the desired strain at an OD_{600} of 0.2. The tube containing the cells was lifted until the cells began to flow into the chip at a significant rate and started seeding the trapping region. Once 10-20 cells were retained in each trap, the flow from the loading port was reversed by decreasing the height of the syringe to prevent the entry of bubbles during the experiment. During experiment we kept a low flow conditions to supply nutrients but do not impose stress on cells.

Fluorescent reporter data acquisition, processing and analysis in agar-based and liquid microwell plate experiments

In coculturing experiments either agar or liquid cultures, overnight cultures were diluted to a total starting OD_{600} of 0.2 (for co-cultures, OD_{600} = 0.1 of each strain, unless different ratios were used see Fig. 2F). In microwell plate experiments, additional compounds (mannose, KCl, Concanavalin A) at indicated concentrations were added. 180 µL aliquots were pipetted from these diluted cultures to a multi-well plates, and 20 µL of galactose stock dilutions were added to each well to reach a concentration of 0.25%. In cocultures of 'Kin' with media extracts from 'Intruder'. 'Intruder' was grown over night at standard conditions (200ul) to the stationary phase and next day cell were separated by filtering (0.45 micron filtration) and 20ul were used in

cocultures with 'Kin' as described above. The plates were incubated at 30 °C in a FLUOstar® Omega Plate Reader (BMG LABTECH) performing time-lapse measurements of OD_{600} and dNeon fluorescence intensity (λ_{Ex} = 488 nm; λ_{Em} = 515 nm) every 10 minutes for up to 24 hours. All data points from fluorescence plate reader were processed to remove media background signal and normalized to the maximum observed fluorescence level based on positive control conditions, yielding values in the range of 0-1 for more comprehensive comparison between experiments. All plots contain complete data points from three biological replicates per condition. All experiments were repeated on two separated days (technical replicates) in triplicates yielding similar results. Cell density thresholds (THR) (Table S4) was set at OD_{600} corresponding to the 10% of maximal dNeon reporter signal measured. Steepness of QMS curve was estimated as difference of OD_{600} at 10% and 50% of maximal dNeon signal per condition, respectively. Accordingly, the smaller the difference in OD_{600} between points the steeper the QMS curve. Typically, we observed an inverse correlation between THR and steepness of the QMS curve (e.g., Fig. 3F). This suggests that steeper (hypersensitive) response required effective delay in the activation of SLN1 that is modulated by FLO11. In agar-based experiments cocultures (1:1 ratio) were grown on 1% agar with standard minimal media as described in above for 5-7 days to allow for colony outgrowth competition and the formation of 'Kin' protrusions at the edges. Images were taken using GFP (λEx = 488 nm; λEm = 515 nm) and mCherry (λEx = 583 nm; λEm = 610 nm) channels. Pictures were taken with an automated inverted Leica DMi8 fluorescence microscope equipped with a Hamamatsu Orca Flash V3 camera. Images were analyzed in Fiji by quantifying fluorescence signal along the arcs fitted to colony edge as shown in Fig. 1E, taking as reference tips of 'Intruder' fluorescence signal (mCherry).

Microscopy image acquisition during microfluidic experiments

Experiments were run over 40 h under a constant perfusion of fresh media inside the microfluidic chip. Each trap was imaged every 10 minutes using three different channels: differential interference contrast (DIC), GFP (λEx = 488 nm; λEm = 515 nm) and mCherry (λEx = 583 nm; λEm = 610 nm). Pictures were taken with an automated inverted Leica DMi8 fluorescence microscope equipped with a Hamamatsu Orca Flash V3 camera. The image acquisition was controlled by the software µManager. Images were captured using a 20x dry objective with NA=0.8 (Leica, Germany). Fluorescence microscopy images were generated using a CoolLed pE600 LED source and a standard epifluorescent filter set (Chroma, USA). The multichannel image sequences were afterwards processed and analyzed using the Fiji software (*57*), and custom R-studio and phyton scripts as described previously (*56*). Briefly, each image was divided into 25 regions of interest (ROIs) and analyzed separately to isolate regions where cells were actively growing and could be tracked over time. The posterior analysis was done with custom R-studio scripts. Firstly, raw data were detrended using the detrend function from "pracma" R-studio v4.0.3 package and then smoothed with Savitzky-Golay Smoothing function (savgol), from the same package, with a filter length of 15 was applied and the signal was normalized between 0 and 1. To plot growth competition curves we quantified mCherry signal ('Intruder)' across each co-culture region (traps) in two different time points 15h after the start of experiment and a day later when experiment was completed. Generally, 24h was sufficient time for 'Kin' to outcompete 'Intruder' and decrease 'Kin' presence by nearly 50%.

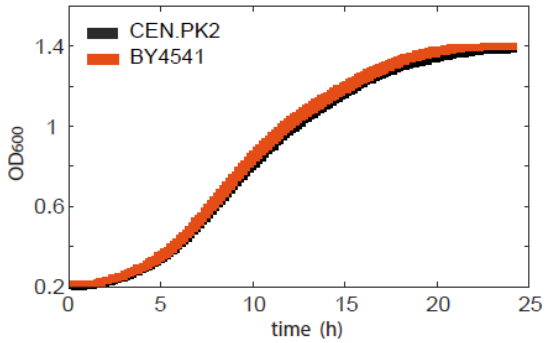

Fig. S1. Comparison of growth kinetics in BY4741 and CEN.PK2 strains

Both CEN.PK2(black) and BY4741 (red) show indistinguishable OD_{600} growth kinetics under growth conditions used in the study. Curves show all data points (filled squares) collected from three independent replicates. Standard deviation correlates with thickness of the curve.

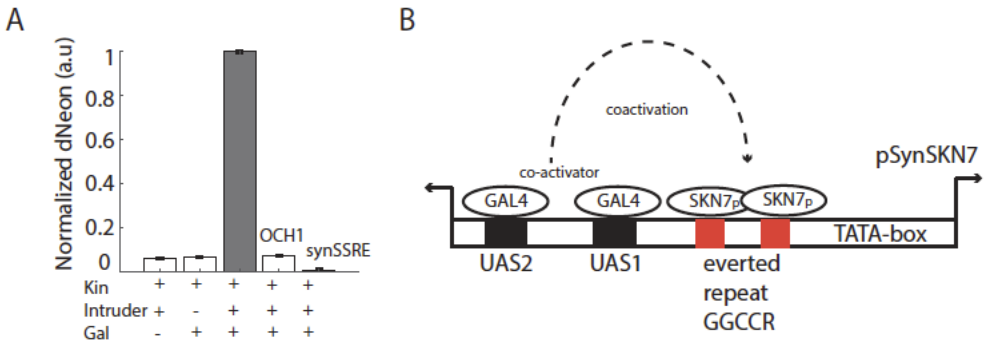

Fig. S2. Design and testing of synthetic SKN7 responsive promoter

A, Normalized dNeon activation with pSynSKN7 promoter and OCH1-based promoters. Note that observed dNeon response is not triggered in configuration with pOCH1 or synthetic OCH1 variant promoters (psynSSRE) (*28, 29*). **B**, Schematic of synthetic promoter (pSynSKN7) design used to track 'Kin' activation dynamics. Two everted repeats of SKN7-binding motif were place upstream of minimal promoter (TATA-box). Two GAL4-UAS sites were placed upstream with reverse orientation relative to the minimal promoter. Proximity of UAS sites (black boxes) could facilitate SKN7-dependent activation of pSynSKN7 promoter possibly by co-activation or DNA bending. However, galactose addition alone does not activate promoter, indicating requirement for proximity of active SKN7 bound to GCCR sites (red boxes).

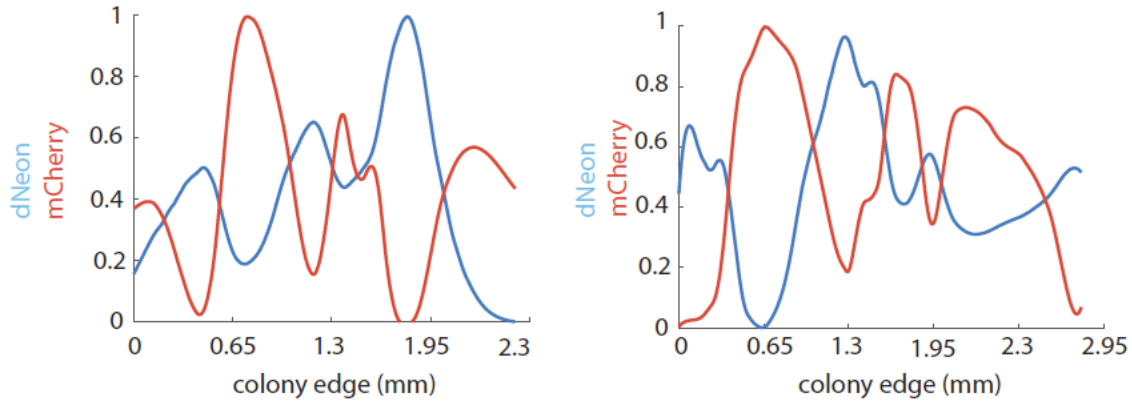

Fig. S3. Examples of growth patterns measured at the colony periphery

Two examples of mutually excluding binary patterns of 'Kin' and 'Intruder' growth regions on the edge of mixed colony. Plots represent dNeon (blue) and mCherry(red) average signals across the edge of the colony (arc) as indicated in Fig. 1E.

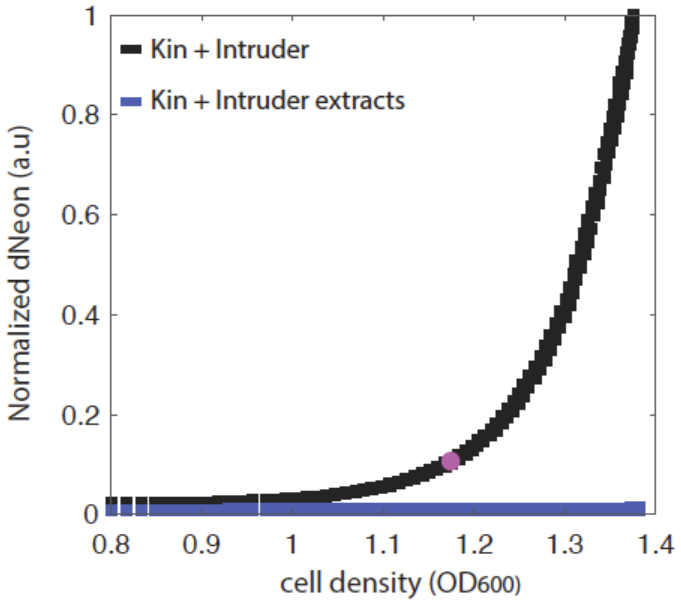

Fig. S4. dNeon reporter activation is not mediated by chemical messenger

'Kin' was cocultured with media extracts in which 'Intruder' was previously grown to stationary phase. These extracts could potentially contain QS-like molecules released by 'Intruder' cells. However, dNeon reporter showed no activity under this condition (blue) compared to control. This data indicated that signal is likely not of chemical nature. Curves show all data points (filled squares) collected from three independent replicates. Standard deviation correlates with thickness of the curve. THR is depicted with pink dot.

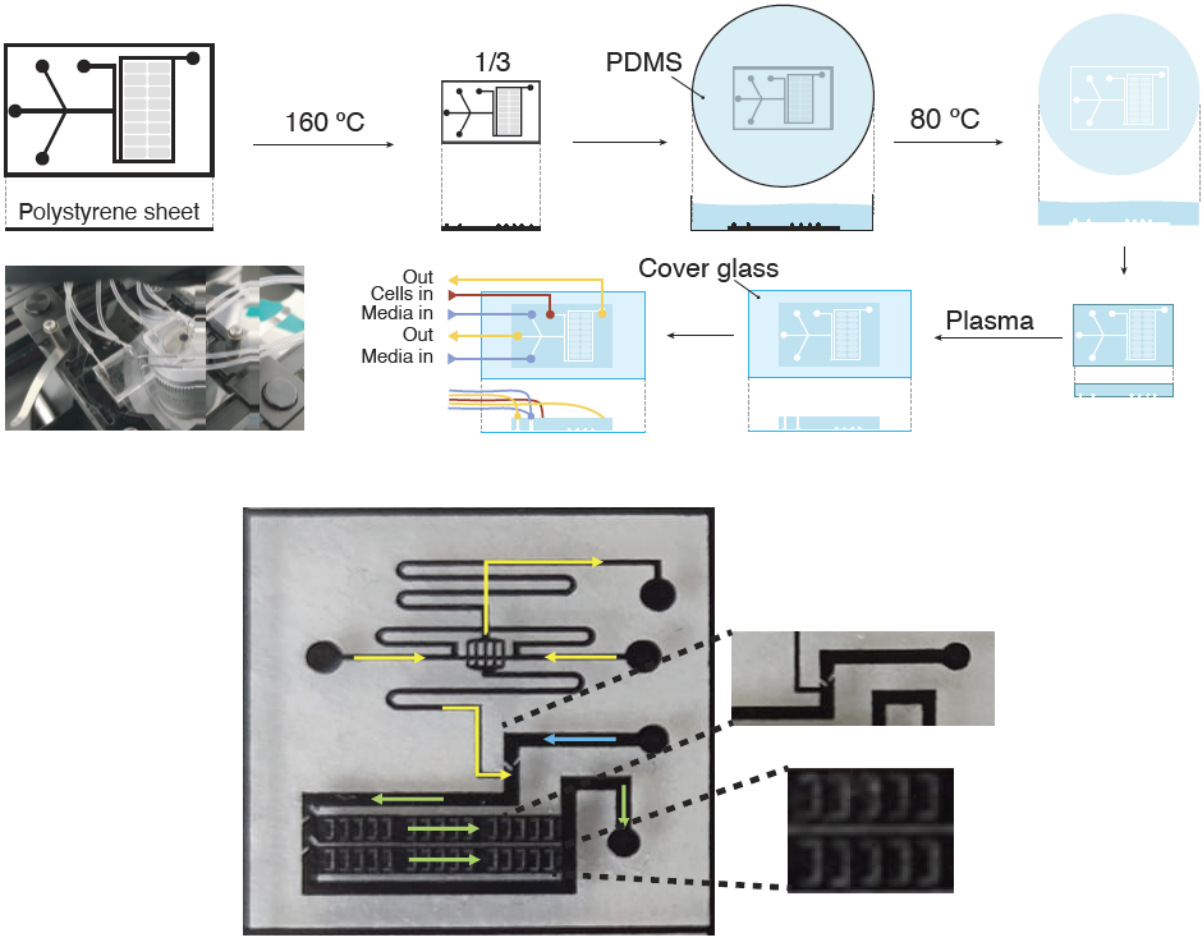

Fig. S5. Microfluidics setup

Upper panel show the process of microfluidic device fabrication as described previously (*56*). The design of the chips is laser-printed on polystyrene sheets, thermally treated, and used as a mold for soft lithography with PDMS. The chips are cut and plasma-bonded to a cover glass. Ports are opened on the surface of the chip with a biopsy puncher, and input and output lines are plugged. Middle panel depicts inlets and outlets on the chip used during microfluidic experiments. Bottom panel, show typical experimental setup. Initially, cells are loaded through cell port (blue line) after they seeded the traps the flow is reversed (yellow arrows) and kept at low rate to avoid cell stress. Channel widths were 120µm for in the mixer module and 500µm in main channels. The approximate height of channels was 25 microns. Cell traps had 500µm x 500µm size and height of approximately 8 microns.

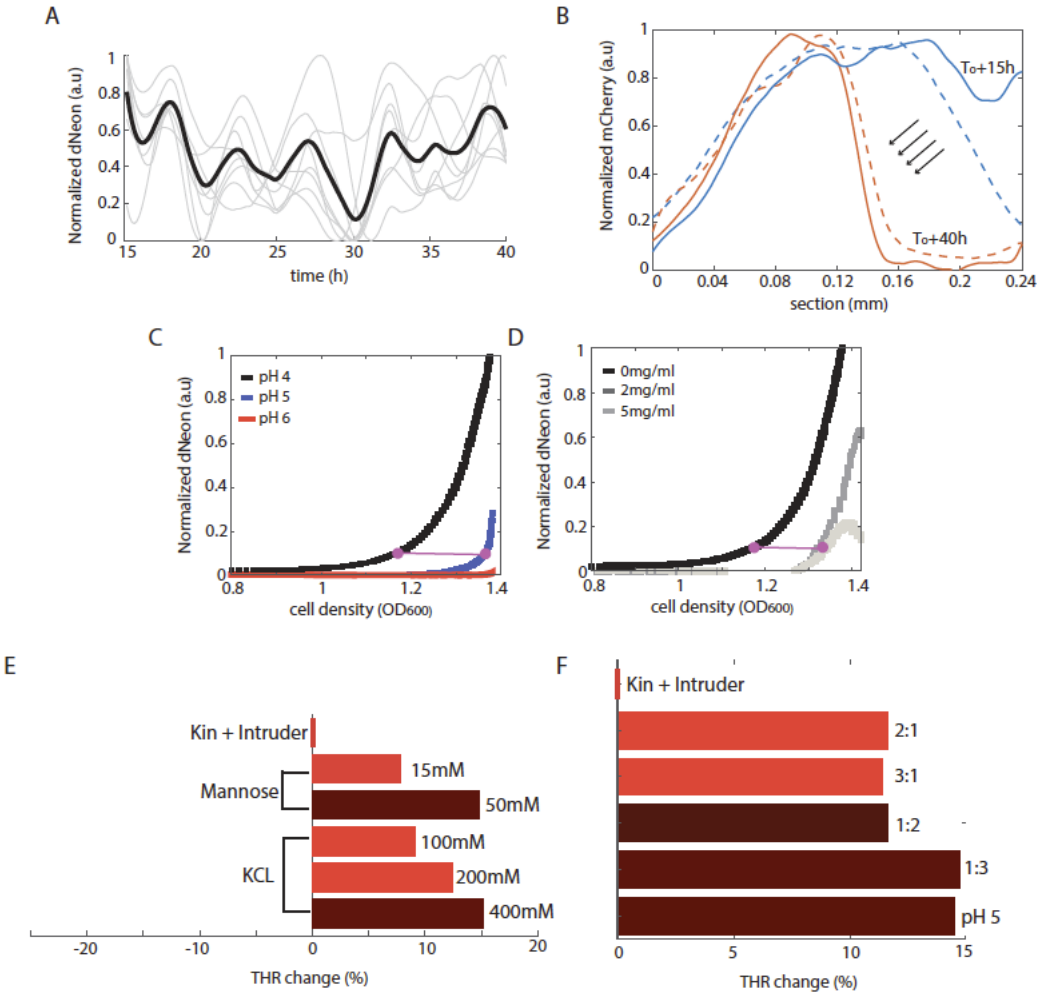

Fig. S6. Analysis of QMS mechanism dynamics

A, Time-course plots from microfluidic experiments of 'Kin' and 'Intruder' cocultures in 10 independent trapping regions (n=10, each trapping region contains > 10000 cells) and average trace is shown in black. Note the transient oscillatory dynamics of 'Kin' in response to presence of 'Intruder'. **B**, Other examples of time evolution of growth competition between 'Kin' and 'Intruder' in microfluidics traps. Only intruder presence is shown with mCherry reporter. Note that over the course of ~24h (red curves) 'Intruder' occupancy drastically decreases to nearly 50% less of original size caused by outgrowing 'Kin' population. Color coding is as in Fig. 2E. **C-D**, QMS curves of various conditions and treatments. pH increase (C), treatments with adhesion inhibitor lectin/Concanavalin A(D). Note that negative interference with Flo protein interactions by pH increase or lectin caused increase in THR accompanied by decrease in dNeon fluorescence amplitudes. THR position and changes in THR are depicted by pink dots connected by lines**.** QMS curves show all data points (filled squares) collected from three independent replicates. Standard deviation correlates with thickness of the curve. **E**, THR changes in an antithetic pattern after applications of Mannose or KCL. **F**, increasing an inoculation ratio of 'Kin' to 'Intruder' shifts THR by > 10% inducing aggressive and steep response of 'Kin'. dNeon response steepness is color-coded in red as in Fig. 3F.

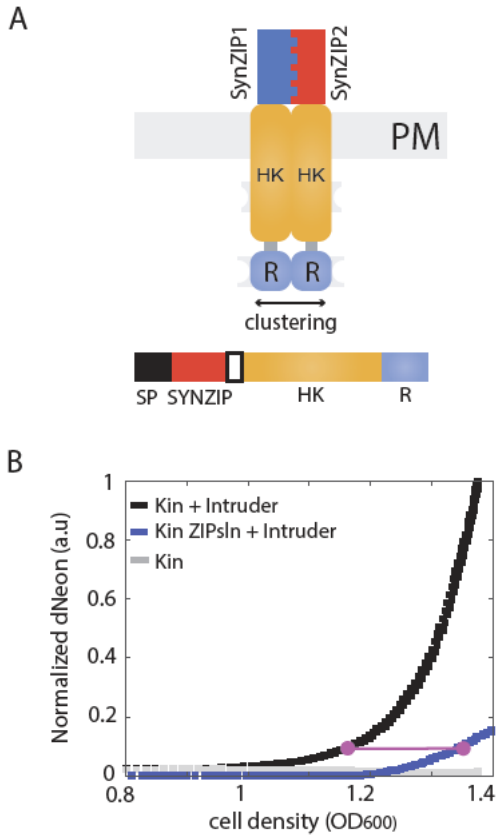

Fig. S7. Forcing extracellular SLN1 dimerization with synthetic zippers

A, Schematic of engineered SLN1 designs. Signal peptide (SP) has been fused to SynZIP1 or SynZIP2 zippers (*41*) following Transmembrane and HK, and Receiver (R) domain of SLN1. This configuration allows for a direct dimerization of SLN1 through extracellular domains. **B**, QMS curves in controls and engineered ZIPsln coculture with 'Intruder'. Note that imposing extracellular dimerization of SLN1 attenuated dNeon response by ~5-fold. THR position and changes in THR are depicted by pink dots connected by lines. QMS curves show all data points (filled squares) collected from three independent replicates. Standard deviation correlates with thickness of the curve.

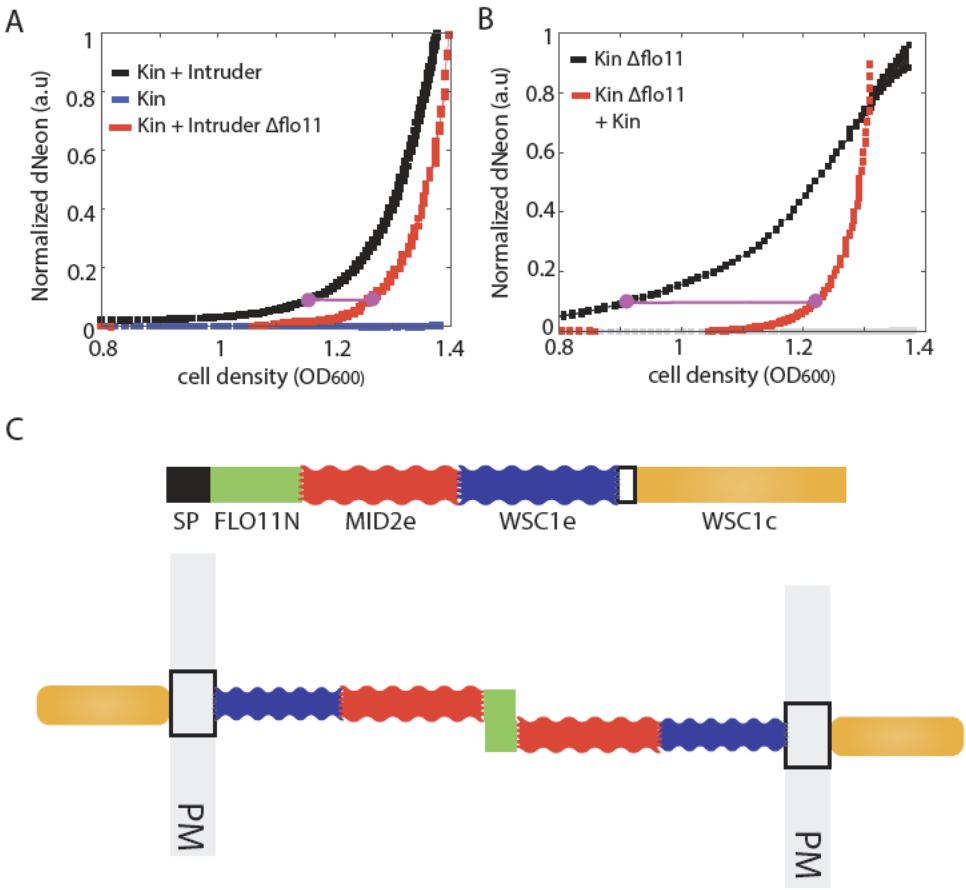

Fig. S8. FLO11 is core regulator of QMS mechanism in 'Kin'

A, B, QMS curves in controls and FLO11 deletion in either 'Kin' or 'Intruder' (A). Inhibition is rescued by coculturing with FLO11-containing 'Kin', indicating that FLO11 asymmetries are key for QMS mechanism. THR position and changes in THR are depicted by pink dots connected by lines. QMS curves show all data points (filled squares) collected from three independent replicates. Standard deviation correlates with thickness of the curve.
C, sFLO11 construction schematic. Signal peptide and FLO11 extracellular region (1-211) were fused to extracellular domains of MID2 (43-219) and WSC1 (22-245) following transmembrane and intracellular domains of WSC1 (245-368). This chimeric construct extends to the cell wall of neighboring cell > 100nm, providing possibility for either cis and trans interactions between sFLO11 in adjacent cells.

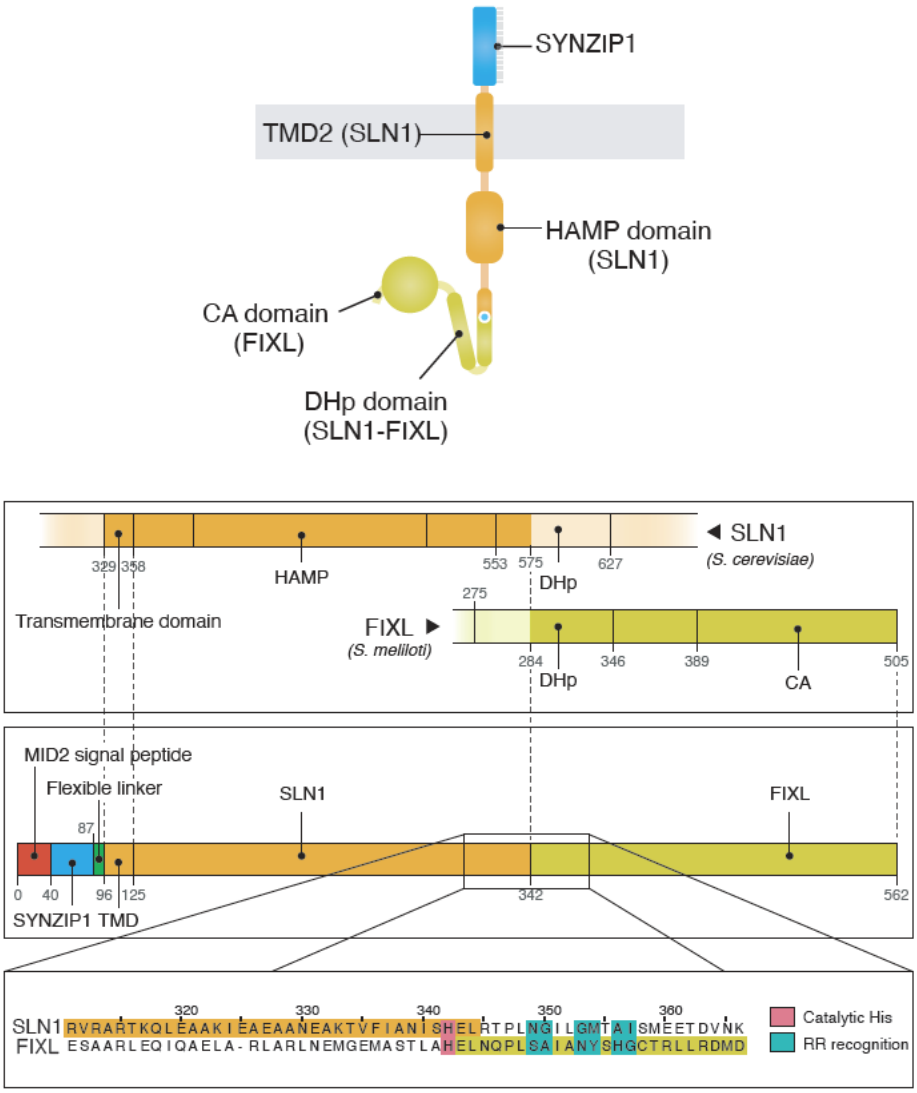

Fig. S9. Construction of synthetic QMS-driven system in yeast based on FixL/FixJ bacterial HK system to study downstream SLN1 pathway

MID2sp(signal peptide)-SynZIP1 was fused to transmembrane domain (TM) of SLN1 using flexible GS linker followed by SLN1 HAMP domain connected through the complementary helical region to HK part of FixL composed of DHp and CA domains (*47*) based on secondary structure alignment. In this configuration sFixL receives phosphoryl group from receiver domain of native SLN1 (see Fig. 4A, B). FixJ transcription factor was fused to tandem repeats of VP16 (VP64) transactivation domain of herpes simplex virus (*49*).

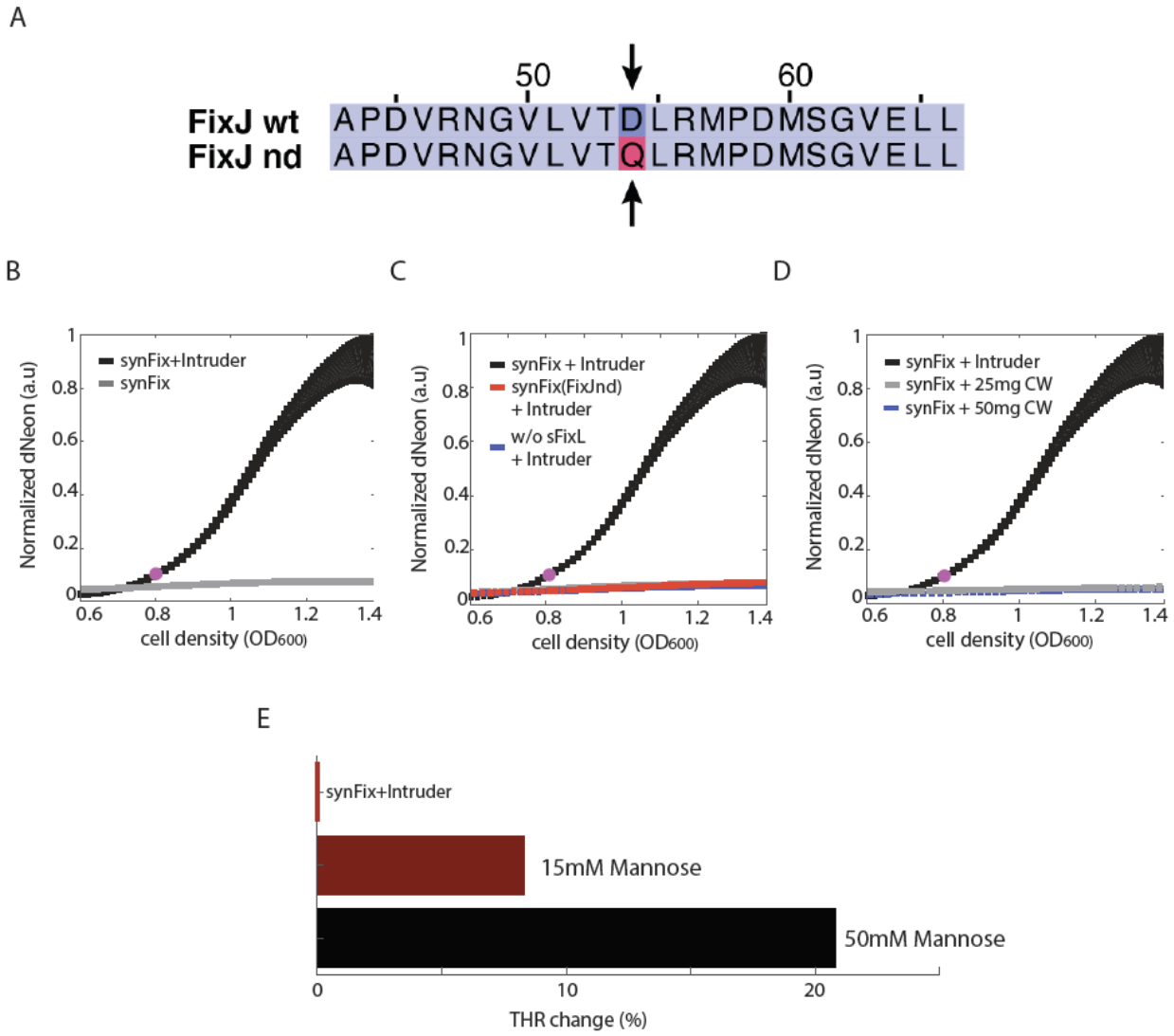

Fig. S10. Sensitivity of synthetic FixL/FixJ system to phosphorylation targeting mutation in FixJ and to mannose treatments

A, Sequence annotation of FixJ dephosphorylating/deactivating mutation D54Q orthologous to SKN7(D427N). **B, C,** dNeon reporter time-lapse kinetics in controls (B), with or without sFixL or with $FixJ_{nd}$(D54Q) mutants (C). **D,** Activation of 'synFix' strain does not depend on cell wall stress caused by calcofluor white treatments. QMS curves show all data points (filled squares) collected from three independent replicates. Standard deviation correlates with thickness of the curve. THR position and changes in THR are depicted by pink dots connected by lines. **E**, Mannose addition to cocultures shifts cell density THR by 20% and inhibits the response in the synthetic FixL/FixJ system in a similar manner to the native QMS system.

Table S1. Summary of plasmids generated in this study

Construct name	Components	Auxotrophy (yeast)	Notes
dNeon reporter	pSynSKN7 :: UbG76V-mNeonGreen	HIS	Main reporter plasmid used in this study
OCH1 reporter	pOCH1 :: UbG76V-mNeonGreen	HIS	OCH1 gene promoter
synSSRE reporter	psynSSRE :: UbG76V-mNeonGreen	HIS	psynSSRE synthetic promoter
STL1 reporter	pSTL(1-1000) :: UbG76V-mNeonGreen	HIS	STL1 gene promoter fragment responsive to osmotic stress
RLM1 reporter	pRLM1(1-864) :: UbG76V-mNeonGreen	HIS	Promoter Fragment of RLM1 downstream of wall stress response pathway
ZIPsln (SynZIP1/2)	pGAL7 :: SP(SLN1)-SynZIP1/2-SLN1(TMD)-SLN1cyt	URA(SynZIP1), LEU(SynZIP2)	This study Chimeric SLN1 protein where ectodomain has been replaced with complementary synthetic leucine zippers
sFLO11	pGAL1 :: FLO11N(1-211)-MID2ecto(43-219)-WSC1ecto(22-245)-WSC1TMD-WSC1cyt(245-368)	URA	This study Signal peptide and FLO11 extracellular region (1-211) were fused to extracellular domains of MID2 (43-219) and WSC1 (22-245) following transmembrane and intracellular domains of WSC1 (245-368).
pFixK	Minimal TATA + FixJ binding sites[48]	HIS	This study

			Synthetic yeast promoter with FixJ operator sites
sFixL	pGAL7 :: SP(MID2)-SynZIP1-SLN1TMD-SLN1HAMP-linker- FixLHK	URA	This study MID2sp(signal peptide)-SynZIP1 was fused to transmembrane domain (TM) of SLN1 using flexible GS linker followed by SLN1 HAMP domain connected through the complementary helical region to HK part of FixL composed of DHp and CA domains based on secondary structure alignment.
FixJ $FixJ_{nd}$ mutant	pGAL7 :: VP64-FixJ pGAL7 :: VP64-$FixJ_{nd}$	LEU	This study FixJ fused to VP16 repeats (VP64) transactivation domain
eLeu	Empty insert pGADT7	LEU	This study
eUra	Empty insert pGADT7	URA	This study
eHis	Empty insert pGADT7	HIS	This study

Table S2. Summary of DNA sequences

DNA fragment	Sequence
pSynSKN7	tcatgttgatgctctgcataataatgcccataaatatttccgacctgcttttatatctttgctagccaaact aactgaacatagctacacattattttcagcttggctattttgtgaacactgtatagccagtccttcggatc acggtcaacagttgtccgagcgctttttggacccttttcccttatttttgggttaaggaaaatgacagaaa atatatctaatgagccttcgctcaacagtgctccgaagtatagctttccaaaaggaactggccgtcgtt ttacagccttcatgctccttgatttccctagctcagtcctaggtatagtgcggaaatcaaggagcatga aggctgtaaaacgacggccagtatcgcagcccctgtccctatcagtgatagagaacgtataaggag tttactccctatcagtgatagagatattacttcttattcaaatgtaaggcatatatatatgtgcgcgtatata catgattatatggcatgtatgtgctctgtatgtatataaaactctttttttctttttttctctaaatttttttcctta tacattaggacctttgcagcataaattactatacttctatagacacgcaaatacaaatacacacactaa
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	gcgaaaatgaaagccaagtccttgccccatttggtcaggatggccctagcaggtggctttggaccg agttaa

Table S3. **Yeast strains summary**

Strain name	Composition	Source
CEN.PK2-1C	MATa; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2	Kind gift form Dr. Luis Rubio
BY4741	S288C isogenic yeast strain: MATa; his3D1; leu2D0; met15D0; ura3D0	Kind gift form Dr. Luis Rubio
'Kin'	CEN.PK2-1C eLeu; eUra; dNeon reporter	This study
'Intruder'	BY4741 eLeu; eUra; eHis	This study
'RLM1'	CEN.PK2-1C eLeu; eUra; RLM1 reporter	This study
'STL1'	CEN.PK2-1C eLeu; eUra; STL1 reporter	This study
'OCH1'	CEN.PK2-1C eLeu; eUra; OCH1 reporter	This study
'synSSRE'	CEN.PK2-1C eLeu; eUra; synSSRE reporter	This study
'Kin ZIPsln'	CEN.PK2-1C ZIPsln(SynZIP2); ZIPsln(SynZIP1); dNeon reporter	This study
'Kin Δflo11'	CEN.PK2-1C Δflo11 eLeu; eUra; dNeon reporter	This study
'Intruder Δflo11'	BY4741 Δflo11 eLeu; eUra; eHis	This study
'Kin FLO11OX'	CEN.PK2-1C eLeu; pGAL1::FLO11; dNeon reporter	This study
'Intruder FLO11OX'	BY4741 eLeu; pGAL1::FLO11; eHis	This study
'Kin sFLO11'	CEN.PK2-1C eLeu; sFLO11; dNeon reporter	This study
'Intruder sFLO11'	BY4741 eLeu; sFLO11; eHis	This study
'synFix'	CEN.PK2-1C FixJ; sFixL; pFixK	This study
'synFix($FixJ_{nd}$)'	CEN.PK2-1C	This study

	$FixJ_{nd}$; sFixL; pFixK	
‘w/o sFixL’	CEN.PK2-1C FixJ; eUra; pFixK	This study

Table S4. THR values for all tested conditions and strain combinations

THR value (OD_{600})	Coculture condition
1.168	'Kin' + 'Intruder'
1.267	'Kin' + 'Intruder' + 15mM Mannose
1.37	'Kin' + 'Intruder' + 50mM Mannose
1.366	'Kin' + 'Intruder' + pH 5
1.284	'Kin' + 'Intruder' + KCL 100mM
1.333	'Kin' + 'Intruder' + KCL 200mM
1.376	'Kin' + 'Intruder' + KCL 400mM
1.322	'Kin' + 'Intruder' Ratio 1:2
1.37	'Kin' + 'Intruder' Ratio 1:3
1.322 (latter peak)	'Kin' + 'Intruder' Ratio 2:1
1.318	'Kin' + 'Intruder' Ratio 3:1
1.053	'Kin Δflo11'
1.226	'Kin Δflo11' + 'Kin'
1.266	'Kin' + 'Intruder Δflo11'
1.388	'Kin ZIPsln'+'Intruder'
1.263	'Kin FLO11OX'+'Intruder'
1.273	'Kin'+'Intruder FLO11OX'
1.224	'Kin' + 'Intruder sFLO11'
0.809	'synFix'+'Intruder'
0.882	'synFix'+'Intruder' + 10mM Mannose
1.0215	'synFix'+'Intruder' + 50mM Mannose
0.992	'synFix'+'Intruder' + 200mM KCL

Movie S1. Time-lapse imaging of ‘Kin’ and ‘Intruder’ growth competition in the microfluidic device**.** ‘Kin’ is expression dNeon reporter (in cyan), ‘Intruder’ is marked by constitutively expressed mCherry (in red). Pictures were taken every 10 min from 15h up to 40h. Color coding as in Fig. 2C.