**Supplementary Discussion, Data or Methods**

A model was constructed to simulate the dynamic and steady state behavior of the cytokinin receiver and quorum sensing (QS) systems. In the first configuration, the model was used to simulate the receiver’s main regulatory mechanisms including ligand binding and dissociation from the AtCRE1 receptor, auto-phosphorylation of ligand-bound AtCRE1, AtCRE1-YPD1-SKN7 two-component phospho-transfer reactions, SKN7 transcriptional activation, degradation of IP signaling molecule, and cell growth. The second configuration was used to simulate the full QS system by adding the synthesis of IP and continuous culture dilution (only for steady state behavior).

Both systems are described by twelve biochemical species and cell population density $N$ (Table 1). Tables 2 and 3 show the systems’ biochemical reactions and the corresponding ordinary differential equations. We assumed the following: (1) increases in cell density follow logistic kinetics\(^1\) with a rate constant $k_n$ and saturate with a maximum volume density $N_{max}$ (Table 3, DE1); (2) IP binds AtCRE1 with a cooperativity of one (Table 3, R6 & R8) based on structure predictions showing a one-to-one interaction between the receptor ligand-binding domain and cytokinin\(^2\). Overall system behavior did not change significantly when IP/AtCRE1 binding cooperativity was increased two-fold due to existing cooperativity in SKN7 transcriptional activation as described below. (3) SKN7 forms a protein dimer (SKN7D) that binds two 13bp repeat SSRE sequences (R19 & R20). Activation of SSRE promoter requires SKN7D phosphorylation at two sites (SKN7D\(_{pp}\)) which follows a two-step, distributive mechanism using phosphorelay by YPD1 (R25 & R27). Mono-phosphorylated SKN7D\(_p\) does not activate transcription; (4) AtIPT4 catalyzes the first and rate-limiting step of IP biosynthesis\(^3\); in the QS configuration, production of IP is therefore directly dependent on the concentration of SKN7D\(_{pp}\) (R32). Overall IP production in the culture is also proportional to $N$ (DE2).

Table 4 lists the kinetic constants used in the simulations. Most of the values are based on previously published kinetic rates\(^4,9\) with the assumption that 1 nM corresponds to 40 molecules per cell\(^6\). A few parameters for which no published data was available were determined by manually
fitting to our experimental results and by normalizing the observed fluorescence intensities to GFP concentrations (1 fluorescence a.u. equals to 1 nM). The dynamic behavior of the systems was simulated by using MATLAB’s stiff differential equation solver ode15s.

The simulated dynamic and steady state cytokinin receiver behavior is shown in Figure 1. The IP synthesis rate was set to zero and cells were grown without dilution for 6 hours from a density of 2x10^6 cells/ml to a density of 8x10^6 cells/ml. Figure 1a shows the time-dependent response of receiver cells grown in media supplemented with 10 M IP. Figure 1b shows the steady-state GFP dosage response to 300 different IP concentrations.

Figure 2 shows the simulated dynamic and steady state QS behavior (with IP production). For the dynamic experiment (Fig. 2a), the SSRE and TR-SSRE QS strains were initially set to low cell densities and to contain high GFP levels and phosphorylated signaling proteins. Figure 2b depicts steady state GFP concentrations as a function of cell density. The steady state response curves were obtained by simulating 300 different constant cell densities where cells were growing and the media was constantly diluted, with each simulation lasting 36 hours. In order to model constitutive IP expression without positive feedback (GAL1 QS strain), basal IP synthesis rate \( k_{ip} \) was increased to 40 and SKN7Dpp dependent IP synthesis rate \( k_{sip} \) was set to zero. Both the steady state and dynamic simulation results correlate well with our experiments (Figs. 4b and 4c of the main text), demonstrating the important role of positive feedback regulation of AtIPT4 for the switch-like QS response.
Reference