Supplementary Table 1:

Equations governing the dynamics of the phosphorelay module

We assume mass conservation for the molecules of the three-component-system during the relevant time span. ATP is considered to be in excess and its concentration is included in the rate constant for phosphorylation of Sln1.

$$\frac{d}{dt}Sln1 = -v_1^{TCS} + v_2^{TCS} - Sln1 \cdot V_{ratio} \tag{1.1}$$

$$\frac{d}{dt}Sln1P = v_1^{TCS} - v_2^{TCS} - Sln1P \cdot V_{ratio} \tag{1.2}$$

$$\frac{d}{dt}Ypd1 = v_3^{TCS} - v_2^{TCS} - Ypd1 \cdot V_{ratio} \tag{1.3}$$

$$\frac{d}{dt}Ypd1P = v_2^{TCS} - v_3^{TCS} - Ypd1P \cdot V_{ratio} \tag{1.4}$$

$$\frac{d}{dt}Ssk1 = v_4^{TCS} - v_3^{TCS} - Ssk1 \cdot V_{ratio} \tag{1.5}$$

$$\frac{d}{dt}Ssk1P = v_3^{TCS} - v_4^{TCS} - Ssk1P \cdot V_{ratio} \tag{1.6}$$

$$v_1^{TCS} = k_1^{TCS}(t) \cdot Sln1 \tag{1.7}$$

$$\text{with } k_1^{TCS}(t) = k_1^{TCS,0} \cdot \left(\frac{\Pi_t(t)}{\Pi_t^0} \right)^{n_1} \quad \text{(compare with main text)}$$

$$v_2^{TCS} = k_2^{TCS} \cdot Sln1P \cdot Ypd1 - k_{-2}^{TCS} \cdot Sln1 \cdot Ypd1P \tag{1.8}$$

$$v_3^{TCS} = k_3^{TCS} \cdot Ssk1 \cdot Ypd1P \tag{1.9}$$

$$v_4^{TCS} = k_4^{TCS} \cdot Ssk1P \tag{1.10}$$

Conservation relations

$$Sln1_{tot} = Sln1^0 + Sln1P^0 \tag{1.11}$$

$$Ypd1_{tot} = Ypd1^0 + Ypd1P^0 \tag{1.12}$$

$$Ssk1_{tot} = Ssk1^0 + Ssk1P^0 \qquad (1.13)$$

$k_1^{TCS,0} = 5\,\mathrm{s}^{-1}$ $\qquad n_1 = 2$

$k_2^{TCS} = 50\,(\mu\mathrm{M}\cdot\mathrm{s})^{-1}$

$k_{-2}^{TCS} = 50\,(\mu\mathrm{M}\cdot\mathrm{s})^{-1}$

$k_3^{TCS} = 50\,(\mu\mathrm{M}\cdot\mathrm{s})^{-1}$

$k_4^{TCS} = 0.415\,\mathrm{s}^{-1}$

$Sln1_{tot} = 0.016\,\mu\mathrm{M}$ (656 molecules per cell)[1]

$Ypd1_{tot} = 0.156\,\mu\mathrm{M}$ (6330 molecules per cell)[1]

$Ssk1_{tot} = 0.029\,\mu\mathrm{M}$ (1200 molecules per cell)[1]

Numbers of molecules per cell are taken from the Yeast Localization Database[1] at http://yeastgfp.ucsf.edu/

Initial concentration values

$Sln1P^0 = 2.25\cdot 10^{-3}\,\mu M$

$Ypd1P^0 = 36\cdot 10^{-3}\,\mu M$

$Ssk1P^0 = 1.88\cdot 10^{-3}\,\mu M$