Supplementary Figure Legends

Figure S1. Nuclear Localization of Msn2 in Response to Different Stresses.
Experiments presented in Figure 1 as kymographs are shown here as averaged traces over the population. Media in the flow-cells was changed at time 0 from control (SD media) to the indicated condition. A, B, C, D, E, F, J: control media (SD), -nitrogen, +1M sorbitol, -nitrogen and +1M sorbitol, 0.05% glucose, -glucose, 0.05% glucose and +1M sorbitol, respectively, all examined on a wide-field fluorescence microscope. G, H, I: -nitrogen, +1M sorbitol, -nitrogen and +1M sorbitol, respectively, examined on a 2-photon microscope. K-M: Kymographs of Msn2 nuclear localization in WT cells immobilized in a flow-chamber in SD media, and subjected to one of three different sorbitol concentrations 0.5M, 0.7M, or 1M. Data were acquired at one min intervals. N: traces averaged over the population of cell for each experiment shown in K-M.

Figure S2. Transcriptional response to Msn2 nuclear localization in stress conditions.
Four STRE elements were inserted upstream of a chromosomally-integrated LacZ gene under control of a LEU2 promoter and LacZ mRNA was assayed by Real-Time PCR after batch cultures were shifted from SD to the indicated condition at time 0. LacZ mRNA was normalized to that for ACT1, although normalizing to TFB3 or ARP4 mRNA gave similar results. mRNA levels are presented as a function of time as a log2 ratio relative to time 0. A: LacZ mRNA levels in an MSN2 msn4Δ strain (Y3645). B: LacZ mRNA levels in an msn2Δ msn4Δ strain (Y3647). C: MSN2 msn4Δ strain (Y3645) responding to glucose limitation of 0%, 0.05%, or 0.2%. D: MSN2 msn4Δ strain (Y3645) responding to 1M sorbitol stress, nitrogen deprivation, or 1M
sorbitol and nitrogen deprivation combined. E: MSN2 msn4Δ strain (Y3645) treated with 1M sorbitol, 0.05% glucose limitation, or 1M sorbitol and 0.05% glucose limitation combined. F: 200nM rapamycin, which inactivates the TORC1 complex, added either alone or in combination with 1M sorbitol stress to MSN2 msn4Δ strain (Y3645). G: Comparison of the transcriptional response of the Msn2 and Msn2-GFP-tagged strains (Y3645 and Y3722) to 0.05% glucose limitation. H: YFP(Venus) mRNA measured by RT-PCR as above from strains carrying a STRE(4)-PLEU2-YFP (Venus) construct inserted into an MSN2-mCherry (Y3992) or msn2 (Y4010) background, following transfer of batch cultures for SD + 2% glucose to SD + 0.05% glucose at time 0. I: Comparison of Venus and LACZ mRNA levels of chromosomally-integrated STRE(4)-PLEU2-YFP (Venus) (Y3992) and STRE(4)-PLEU2-LacZ (Y3645) in response to glucose limitation and control conditions. Cells were grown in batch culture and shifted from SD media to 0.05% glucose or SD (the control experiment).

Figure S3. Msn2 nuclear localization responds to changing glucose levels even in the absence of PKA modulation.

This figure is an addendum to Figure 3 and presents different time frames of glucose or 1NM-PP1 switching and different pka-wimp strains. Panels A, B: wt strain (Y3645) with SD and glucose-free media switched every 1 min and every 3 min, respectively. C, D: pka-as strain (Y3817) with the inhibitor 1NM-PP1 added and removed every 1 min and 3 min. E, F: The pka-wD139H strain (Y3845) with SD and glucose-free media alternated every 1 min and 3 min. G, H: The pka-wD139H snf1Δ strain (Y3964) with SD and glucose-free media exchanged every 1 min and 3 min. I-K: The pka-wE235Q strain (Y3842) subject to transition from SD to SD-glucose or from SD-glucose back to SD or to 2 min cycles of SD and SD-glucose. L-N: The pka-wV218G
strain (Y3841) subject to transition from SD to SD-glucose or from SD-glucose back to SD or to 2 min cycles of SD and SD-glucose. Data were acquired at 20 sec intervals except for L and M, which were at one min intervals.

**Figure S4.** Snf1 accelerates Msn2 exit from the nucleus and suppresses noise.

Kymograph of Msn2 nuclear localization in WT (Y3630) (A,D) or *snf1Δ* (Y3847) (B,E) under steady-state SD (A,B) or following transfer from SD + 2% glucose to SD + 0.05% glucose (D,E). Data were acquired at one min intervals. The coefficient of variation (the standard deviation divided by the mean) of the Msn2 nuclear localization as a function of time in WT (Y3630) (C) or *snf1Δ* (Y3847) (F). The *snf1Δ* mutant cells (red lines in panels C and F) have higher variability.

**Figure S5.** Stochastic behavior of Msn2 nuclear localization.

A-C: *pka-wimp*<sup>E235Q</sup> cells (Y3842) were immobilized in a flow-chamber in SD media and imaged after transition to nitrogen-free media at time 0. Three individual cells are represented here as montages over the same 40-minute time period, with frames 60 seconds apart, left to right. D-F: Three different *pka-wimp*<sup>V218G</sup> cells over the same 76-minute time period, following SD addition to cells previously deprived of glucose. Frames are 60 seconds apart, left to right. G,H: Strains Y4024 (*MSN2-GFP/MSN2-GFP msn4Δ/msn4Δ*) and Y4025 (*MSN2-GFP/msn2Δ msn4Δ/msn4Δ*) were immobilized in a flow-chamber in SD media and imaged after transition to SD + 0.05% glucose at time 0. The shaded area shows the total number of cells with nuclear GFP fluorescence above a threshold of background fluorescence as a function of time. I: For all time points between 60 and 120 minutes, the population-wide coefficient of variation of Msn2
nuclear localization was calculated for experiments shown in panels G and H (gray circles). Also shown are the mean (red lines), its 95% confidence interval (pink shaded areas), and the mean +/- one standard deviation interval (blue shaded areas). J-L: Cells from the diploid strain (Y4012) expressing both MSN2-GFP and MSN2-mCherry from identical MSN2 promoters were transferred from 2% glucose (SD media) to 0.05% glucose at time 0. The cells in the kymographs J and K are sorted in the same order, so that row 1 in J shows the same cell as row 1 in K, etc. For each cell in panels J and K, a correlation coefficient was computed between Msn2-GFP and Msn2-mCherry nuclear localization profiles. Histogram of these correlation coefficients is presented in L. All data were acquired at one min intervals.
FIG. S1: Nuclear Localization of Msn2 in Response to Different Stresses. Experiments presented in Figure 1 as kymographs are shown here as averaged traces over the population. Media in the flow-cells was changed at time 0 from control (SD media) to the indicated condition. A, B, C, D, E, F, J: control media (SD), -nitrogen, +1M sorbitol, -nitrogen and +1M sorbitol, 0.05% glucose, -glucose, 0.05% glucose and +1M sorbitol, respectively, all examined on a wide-field fluorescence microscope. G, H, I: -nitrogen, +1M sorbitol, -nitrogen and +1M sorbitol, respectively, examined on a 2-photon microscope. K-M: Kymographs of Msn2 nuclear localization in WT cells immobilized in a flow-chamber in SD media, and subjected to one of three different sorbitol concentrations 0.5M, 0.7M, or 1M. Data were acquired at one min intervals. N: traces averaged over the population of cell for each experiment shown in K-M.
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FIG. S4: Snf1 accelerates Msn2 exit from the nucleus and suppresses noise. Kymograph of Msn2 nuclear localization in WT (Y3630) (A,D) or snf1Δ (Y3847) (B,E) under steady-state SD (A,B) or following transfer from SD + 2% glucose to SD + 0.05% glucose (D,E). Data were acquired at one min intervals. The coefficient of variation (the standard deviation divided by the mean) of the Msn2 nuclear localization as a function of time in WT (Y3630) (C) or snf1Δ (Y3847) (F). The snf1Δ mutant cells (red lines in panels C and F) have higher variability.
FIG. S5: Stochastic behavior of Msn2 nuclear localization. A–C: *pka- we^{E235Q}* cells (Y3842) were immobilized in a flow-chamber in SD media and imaged after transition to nitrogen-free media at time 0. Three individual cells are represented here as montages over the same 40-minute time period, with frames 60 seconds apart, left to right. D–F: Three different *pka- we^{V218C}* cells over the same 76-minute time period, following SD addition to cells previously deprived of glucose. Frames are 60 seconds apart, left to right. G,H: Strains Y4024 (*MSN2-GFP/MSN2-GFP msn4Δ/msn4Δ*) and Y4025 (*MSN2-GFP/msn2Δ msn4Δ/msn4Δ*) were immobilized in a flow-chamber in SD media and imaged after transition to SD + 0.05% glucose at time 0. The shaded area shows the total number of cells with nuclear GFP fluorescence above a threshold of background fluorescence as a function of time. I: For all time points between 60 and 120 minutes, the population-wide coefficient of variation of Msn2 nuclear localization was calculated for experiments shown in panels G and H (gray circles). Also shown are the mean (red lines), its 95% confidence interval (pink shaded areas), and the mean +/- one standard deviation interval (blue shaded areas). J–L: Cells from the diploid strain (Y4012) expressing both *MSN2-GFP* and *MSN2-mCherry* from identical *MSN2* promoters were transferred from 2% glucose (SD media) to 0.05% glucose at time 0. The cells in the kymographs J and K are sorted in the same order, so that row 1 in J shows the same cell as row 1 in K, etc. For each cell in panels J and K, a correlation coefficient was computed between Msn2-GFP and Msn2-mCherry nuclear localization profiles. Histogram of these correlation coefficients is presented in L. All data were acquired at one min intervals.
TABLE S1: Strains used in this study.

| Number | Designation          | Genotype                                           |
|--------|----------------------|----------------------------------------------------|
| Y3630  | MSN2-GFP             | MATα MSN2-GFP msn4::KANMX                         |
| Y3611  | MSN2                 | MATα msn4::KANMX can1-100                         |
| Y3539  | msn2Δ msn4Δ          | MATα msn2::KANMX msn4::KANMX can1-100             |
| Y3645  | STRE-LacZ            | msn4::KANMX ura3::URA3-UAS4×STRE-PLEU2-LacZ       |
| Y3647  | msn2Δ msn4Δ STRE-LacZ| MATα msn2::KANMX msn4::KANMX ura3::URA3-UAS4×STRE-PLEU2-LacZ |
| Y3722  | MSN2-GFP STRE-LacZ   | MSN2-GFP ura3::URA3-UAS4×STRE-PLEU2-LacZ msn4::KANMX |
| Y3817  | pka-as MSN2-GFP      | tpk1(M164G)tpk2(M147G)tpk3(M165G) MSN2-GFP msn4::KANMX |
| Y3841  | pka-wimpV218G MSN2-GFP| MATα MSN2-GFP HHF2-CPP msn4::KANMX tpk1::HIS3 tpk2(V218G) tpk3::TRP1 bcy1::LEU2 |
| Y3842  | pka-wimpE235Q MSN2-GFP| MSN2-GFP HHF2-CPP msn4::KANMX tpk1::HIS3 tpk2(E235Q) tpk3::TRP1 bcy1::LEU2 |
| Y3845  | pka-wimpD139H MSN2-GFP| MSN2-GFP msn4::KANMX tpk1::HIS3 tpk2(D139H) tpk3::TRP1 bcy1::LEU2 |
| Y3964  | pka-wimpD139H MSN2-GFP snf1Δ| MSN2-GFP tpk1::HIS3 tpk2(D139H) tpk3::TRP1 bcy1::LEU2 snf1::TRP1 msn4::KANMX |
| Y3847  | snf1Δ MSN2-GFP       | snf1::TRP1 MSN2-GFP msn4::KANMX                   |
| Y3815  | SNF1-as MSN2-GFP     | MATα snf1(I132G) MSN2-GFP msn4::KANMX            |
| Y3895  | MSN2-GFP hog1Δ       | MATα MSN2-GFP msn4::KANMX hog1::HPHMX            |
| Y3992  | MSN2-mCherry STRE-VENUS| MSN2-mCHERRY ura3::URA3-UAS4×STRE-PLEU2-VENUS-KANMX msn4::KANMX |
| Y4010  | msn2Δ STRE-VENUS     | msn2::KANMX msn4::KANMX ura3::URA3-UAS4×STRE-PLEU2-VENUS-KANMX |
| Y3995  | MSN2-mCherry MSN4-GFP| MSN2-mCHERRY::HPHMX MSN4-GFP::KANMX              |
| Y4012  | MSN2-mCherry MSN2-GFP| MATα/α MSN2-mCHERRY::HPHMX/MSN2-GFP HHF2-CFP/HHF2 msn4::KANMX/msn4::KANMX |
| Y4024  | MSN2-GFP/MSN2-GFP    | MATα/α MSN2-GFP/MSN2-GFP msn4::KANMX/msn4::KANMX HHF2-CFP/HHF2 |
| Y4025  | MSN2-GFP/msn2Δ       | MATα/α MSN2-GFP/msn2::KANMX msn4::KANMX/msn4::KANMX HHF2-CFP/HHF2 |
TABLE S2: Numerical values of the parameters given in Garmendia-Torres et al. [1] that we used in our model.

| Parameters | Values | Units         |
|------------|--------|---------------|
| $K_1$      | 0.2    | $\mu M$       |
| $K_2$      | 0.2    | $\mu M$       |
| $K_3$      | 0.015  | $\mu M$       |
| $K_4$      | 0.015  | $\mu M$       |
| $K_5$      | 0.25   | $\mu M$       |
| $K_6$      | 0.25   | $\mu M$       |
| $K_7$      | 0.005  | $\mu M$       |
| $K_8$      | 0.005  | $\mu M$       |
| $K_9$      | 0.05   | $\mu M$       |
| $K_{10}$   | 0.05   | $\mu M$       |
| $K_{11}$   | 0.05   | $\mu M$       |
| $K_{12}$   | 0.05   | $\mu M$       |
| $k_o$      | 0.01   | $\mu M^{-1} \cdot min^{-1}$ |
| $k_i$      | 1.0    | $min^{-1}$    |
| $K_{md}$   | 20.0   | $\mu M$       |
| $k_{i1}$   | 10.0   | $min^{-1}$    |
| $k_{i2}$   | 0.001  | $min^{-1}$    |
| $k_{i3}$   | 0.001  | $min^{-1}$    |
| $k_{i4}$   | 10.0   | $min^{-1}$    |
| $V_3$      | 3.5    | $min^{-1}$    |
| $V_4$      | 1.3    | $\mu M \cdot min^{-1}$ |
| $V_5$      | 240.0  | $min^{-1}$    |
| $V_6$      | 600.0  | $min^{-1}$    |
| $V_7$      | 3.333  | $min^{-1}$    |
| $V_8$      | 3.333  | $min^{-1}$    |
| $V_9$      | 1.5    | $\mu M \cdot min^{-1}$ |
| $V_{10}$   | 3.333  | $min^{-1}$    |
| $V_{11}$   | 0.6    | $\mu M \cdot min^{-1}$ |
| $V_{12}$   | 3.333  | $min^{-1}$    |
| $V_{13}$   | 2.0    | $\mu M \cdot min^{-1}$ |
| $Str$      | 0.5    |               |
| Number | Reaction | Propensity |
|--------|----------|------------|
| 1      | Gef₁ → Gefₐ | $w_1 = V_1 \text{Glu} \Omega_1 \frac{(Gef₁ - Gefₐ)}{K_{M,M1} + (Gef₁ - Gefₐ)}$ |
| 2      | Gefₐ → Gef₁ | $w_2 = V_2 \text{Str} \Omega_1 \frac{Gefₐ}{K_{M,M1} + Gefₐ}$ |
| 3      | Gap₁ → Gapₐ | $w_3 = V_3 \text{C} \frac{(Gap₁ - Gapₐ)}{K_{M,M1} + (Gap₁ - Gapₐ)}$ |
| 4      | Gapₐ → Gap₁ | $w_4 = V_4 \Omega_1 \frac{Gapₐ}{K_{M,M1} + Gapₐ}$ |
| 5      | RGDP → RGTP | $w_5 = V_5 \text{Gefₐ} \frac{(RGDP - RGTP)}{K_{M,R} + (RGDP - RGTP)}$ |
| 6      | RGTP → RGDP | $w_6 = V_6 \text{GAPₐ} \frac{RGTP}{K_{M,R} + RGTP}$ |
| 7      | CYCL₁ → CYCLₐ | $w_7 = k_{i1} \text{CYCLₐ}$ |
| 8      | CYCLₐ → CYCL₁ | $w_8 = k_i \text{CYCLₐ}$ |
| 9      | PDEₐ → PDE₁ | $w_9 = V_7 \text{C} \frac{(PDEₐ - PDE₁)}{K_{M,M1} + (PDEₐ - PDE₁)}$ |
| 10     | PDE₁ → PDEₐ | $w_{10} = V_8 \Omega_1 \frac{PDE₁}{K_{M,R} + PDE₁}$ |
| 11     | ∅ → cAMP | $w_{11} = k_i \text{CYCLₐ}$ |
| 12     | cAMP → ∅ | $w_{12} = k_d \text{PDEₐ} \frac{cAMP}{K_{d,cAMP} + cAMP}$ |
| 13     | 4cAMP + R₂C₂ → R₂cAMPₐ + 2C | $w_{13} = \frac{k_i}{R_k} (R₂C₂) (cAMP)^4$ |
| 14     | R₂cAMPₐ + 2C → 4cAMP + R₂C₂ | $w_{14} = \frac{k_i}{R_k} (R₂cAMPₐ) C^2$ |
| 15     | MC → MN | $w_{15} = k_{i1} \text{MC}$ |
| 16     | MN → MC | $w_{16} = k_{i2} \text{MN}$ |
| 17     | MC → MCP | $w_{17} = (V_{i1} \text{C + Snf₁ₐ}) \frac{MC}{K_{M,M1} + MC}$ |
| 18     | MCP → MC | $w_{18} = (V_{i2} \text{Str} \frac{MC}{K_{M,M1} + MC} + PPI₁ \frac{MC}{K_{M,M1} + MC}$ |
| 19     | MN → MNP | $w_{19} = (V_{i3} \text{C + Snf₁ₐ}) \frac{MN}{K_{M,M1} + MN}$ |
| 20     | MNP → MN | $w_{20} = (V_{i3} \text{Str} \frac{MNP}{K_{M,M1} + MNP}$ |
| 21     | MCP → MNP | $w_{21} = k_{i3} \text{MCP}$ |
| 22     | MNP → MCP | $w_{22} = k_{i4} \text{MNP}$ |
| 23     | Snf₁ₐ → Snf₁ₐ | $w_{23} = k_{aS} (Snf₁ₐ - Snf₁ₐ)$ |
| 24     | Snf₁ₐ → Snf₁ₐ | $w_{24} = k_{aG} \text{Glu Snf₁ₐ}$ |
| 25     | PPI₁ → PPIₐ | $w_{25} = k_{aP} (PPI₁ - PPIₐ)$ |
| 26     | PPIₐ → PPI₁ | $w_{26} = k_{f} \text{Glu PPIₐ}$ |
SUPPLEMENTARY METHODS

Image analysis

The output of the wide-field fluorescence microscope is a .DV (DeltaVision) file. This file contains a stack of optical sections (also called a z-series) for each time point. We use MATLAB to preprocess each image and to increase the overall quality of all images. The first preprocessing step is the correction of non-uniform illumination due to photobleaching and other factors. It appears that this problem affects only a few images in our experiments. We use a morphological opening operation (an erosion followed by a dilation) to estimate the background illumination. After the background illumination correction step, we build a mask that helps us keep track of the photobleaching effect. We mark the region which contains all the cells, and, using the total intensity in this region, change the overall intensity in the movie such that the total intensity (given by the total number of cells) remains constant during the entire experiment, as if photobleaching were absent.

In order to build this mask, we do simple thresholding and obtain a black and white (or binary) image. Then we dilate and morphologically open this image. Dilation adds pixels to the boundaries of objects in an image (and removes the black isolated pixels), and area opening removes the white isolated pixels from the binary image. Finally, the image is filtered with a weak rotationally symmetric Gaussian low-pass filter of size 7x7 pixels and with the standard deviation of 1.5 pixels. After we filter each image as described above, the final reconstructed images are obtained by selecting the brightest pixels from each filtered z-stack corresponding to each point in the horizontal plane. With these reconstructed images, the detection of the cells is accomplished using a MATLAB function imfindcircles (http://www.mathworks.com/help/images/ref/imfindcircles.html).

Transcription network

We used as a starting point the transcription network from Garmendia-Torres et al. [1] and Gonze et al. [2]. In addition to the original network, we added signaling pathways which include Protein Phosphatase 1 (PP1) and Snf1 protein kinase. These are necessary in order to explain why the mutant cells in which the PKA-cAMP pathway was eliminated and the PKA level is kept constant still respond to glucose starvation. The following species are modeled by the transcription network: active/inactive GEF protein (GEF_a/GEF_i); active/inactive GAP protein (GAP_a/GAP_i); Ras protein bound to GTP or GDP (RGTP/RGDP); active/inactive adenylate cyclase (CYCL_a/CYCL_i); cyclic AMP (cAMP); active/inactive phosphodiesterase (PDE_a/PDE_i); PKA in the form of a holoenzyme complex between the regulatory (R) and catalytic (C) subunits, free of cAMP; holoenzyme with 4 cAMP molecules bound to the regulatory subunits (R_4cAMP_4). MN and MC represent the nuclear and cytoplasmic Msn2 molecules that are not phosphorylated, and MNP and MCP represent the nuclear and cytoplasmic Msn2 molecules that are phosphorylated. PP1_a/PP1_i are the active/inactive PP1 and Snf1_a/Snf1_i are the active/inactive Snf1 proteins.
The equations that govern this network are as follows:

\[
\begin{align*}
\frac{d[GEF_a]}{dt} &= V_1[Glu] \frac{[GEF_i]}{K_1 + [GEF_i]} - V_2[Str] \frac{[GEF_a]}{K_2 + [GEF_a]} \\
\frac{d[GAP_a]}{dt} &= V_3[C] \frac{[GAP_i]}{K_3 + [GAP_i]} - V_4[GAP_a] \\
\frac{d[RGTP]}{dt} &= V_5[GEF_a] \frac{[RGDP]}{K_5 + [RGDP]} - V_6[GAP_a] \frac{[RGTP]}{K_6 + [RGTP]} \\
\frac{d[CYCL_a]}{dt} &= k_a[RGTP][CYCL_a] - k_i[CYCL_a] \\
\frac{d[PDE_a]}{dt} &= V_7[C] \frac{[PDE_i]}{K_7 + [PDE_i]} - V_8[K_a + [PDE_a]} \\
\frac{d[cAMP]}{dt} &= k_s[CYCL_a] - k_d[PDE_a] \frac{[cAMP]}{K_{md} + [cAMP]} - 4v_{PKA} \\
\frac{d[R_2C_2]}{dt} &= -v_{PKA} \\
\frac{d[PP1a]}{dt} &= k_{PP1a}[PP1a] - k_i[P][Glu][PP1a] \\
\frac{d[Snf1a]}{dt} &= k_{Snf1a}[Snf1a] - k_i[S][Glu][Snf1a] \\
\frac{d[MC]}{dt} &= -k_{MC}[MC] + k_{MC}[MN] - V_{11total} \frac{[MC]}{K_{11} + [MC]} + V_{12total} \frac{[MC]}{K_{12} + [MC]} \\
\frac{d[MN]}{dt} &= k_{MN}[MC] - k_{MN}[MN] - V_{9total} \frac{[MN]}{K_{9} + [MN]} + V_{10total} \frac{[MN]}{K_{10} + [MN]} \\
\frac{d[MNP]}{dt} &= k_{MNP}[MNP] - k_{MNP}[MNP] + V_{9total} \frac{[MN]}{K_{9} + [MN]} - V_{10total} \frac{[MN]}{K_{10} + [MN]} \\
\frac{d[MC]}{dt} &= -k_{MC}[MC] + k_{MC}[MNP] + V_{11total} \frac{[MC]}{K_{11} + [MC]} - V_{12total} \frac{[MC]}{K_{12} + [MC]} \\
\end{align*}
\]

with

\[ v_{PKA} = a[R_2C_2][cAMP] - r[C]^2[R_2cAMP] \]

\[ V_{9total} = V_9[C] + V_9[S][Snf1a], \]

\[ V_{10total} = V_{10}[Str] + V_{10}[PP1a], \]

\[ V_{11total} = V_{11}[C] + V_{11}[Snf1a], \]

\[ V_{12total} = V_{12}[Str] + V_{12}[PP1a]. \]

The parameter \([Glu]\) is proportional to the glucose concentration and normalized such that it has a value of 1 in the medium containing 2% glucose. We set the parameters \(V_9, V_10, V_11, V_12\) to 1 min\(^{-1}\) and fit the activation/deactivation rates of PP1 and Snf1, which determine activity and dynamics of these species. The conservation relations are:

\[
\begin{align*}
[GEF_a] + [GEF_i] &= [GEF_i] \\
[GAP_a] + [GAP_i] &= [GAP_i] \\
[RGTP] + [RGDP] &= [Ras_i] \\
[CYCL_a] + [CYCL_i] &= [CYCL_i] \\
[PDE_a] + [PDE_i] &= [PDE_i] \\
[R_2C_2] + [R_2cAMP_i] &= [R_2C_2] + \frac{[C]}{2} = [PKA_i] \\
[MN] + [MNP] + [MC] + [MCP] &= [MSN_i] \\
[PP1a] + [PP1i] &= [PP1i] \\
[Snf1a] + [Snf1i] &= [Snf1i]. \\
\end{align*}
\]

All the parameters of the model are presented below and in Table S2.
Parameter estimation procedure

First, we model the experiments with the \textit{pka-wimp} mutants, in which glucose is removed and added again, alternating every 1, 2, or 3 minutes. By fitting the data, we estimate the following parameters: $PP1_t$, $k_aP$, $k_iP$, $Snf1_t$, $k_aS$, $k_iS$, representing the total amount of PP1 and Snf1 and their corresponding activation and inactivation rates. The three mutants that we used have different PKA activities, which correspond in our model to different levels of active PKA concentration. We estimate these three concentration of active PKA: $C_{D139H}$, $C_{V218G}$, $C_{E235Q}$. Because PKA has an inhibitory role on Snf1 and the mutants have different levels of active PKA, we allow $k_iS$ to have different values for each mutant.

We obtain the following numeric values:

\begin{align*}
PP1_t &= 10 \mu M \\
Snf1_t &= 7.4 \mu M \\
k_aP &= 0.18 \mu M^{-1} \cdot min^{-1} \\
k_iP &= 3.78 \mu M^{-1} \cdot min^{-1} \\
k_aS &= 0.30 \mu M^{-1} \cdot min^{-1} \\
k_iS_{D139H} &= 4.06 \mu M^{-1} \cdot min^{-1} \\
k_iS_{V218G} &= 5.59 \mu M^{-1} \cdot min^{-1} \\
k_iS_{E235Q} &= 5.84 \mu M^{-1} \cdot min^{-1} \\
C_{D139H} &= 0.24 \mu M \\
C_{V218G} &= 0.28 \mu M \\
C_{E235Q} &= 0.28 \mu M
\end{align*}

After we estimate these parameters, we fit a Hill function which gives the dependence of $k_iS$ on $C$. We obtain the relationship

\[ k_iS = V \frac{C^n}{C^n + K^n}, \]

where $V = 6.27 \mu M^{-1} \cdot min^{-1}$, $K = 0.22 \mu M$, and $n = 9.7$.

We use the function $k_{iS}(C)$ together with the other fitted parameters ($PP1_t$, $Snf1_t$, $k_aP$, $k_iP$, $k_aS$) to extend the signaling network for the wild-type cells that was previously reported [1]. The new signaling pathways are shown in Figure 5. Using the full network, we fit Msn2 nuclear localization dynamics in wild-type cells subjected to switching between glucose-containing and glucose-free medium every 1, 2, and 3 minutes. At this stage, we optimize the following parameters: $V_1$, $V_2$, $a$, $r$, $k_s$, $k_d$ to obtain the numeric estimates:

\begin{align*}
V_1 &= 2.5 \mu M \cdot min^{-1} \\
V_2 &= 10 \mu M \cdot min^{-1} \\
a &= 285 \mu M^{-4} \cdot min^{-1} \\
r &= 0.1 \mu M^{-2} \cdot min^{-1} \\
k_s &= 4.1 \ min^{-1} \\
k_d &= 310 \ min^{-1}
\end{align*}

These parameters have the same order of magnitude as the ones used in Garmendia-Torres et al. [1], except for $a$ and $r$ that are different because in that paper the authors used a different set of reactions for the activation/deactivation of PKA. There are 4 molecules of cAMP which bind to one $R_2C_2$ molecule, not 2 as used in [1], and this modifies the corresponding reaction rates.

To model the addition of sorbitol, we vary activation rates $k_aP$ and $k_aS$ as shown in the insets of Figure 6D-F. Specifically, we approximate the variation of the two activation rates by sigmoidal Hill-like functions and fit their
parameters. The dependence of $k_{ap}$ and $k_{as}$ on the time measured from the moment of sorbitol addition is given by:

$$\tilde{k}_{ap}(t) = k_{ap} + (k_{ap}^{sorb} - k_{ap}) \frac{t^{n_p}}{(T^{PP1}_{1/2})^{n_p} + t^{n_p}},$$

$$\tilde{k}_{as}(t) = k_{as} + (k_{as}^{sorb} - k_{as}) \frac{t^{n_s}}{(T^{Snf1}_{1/2})^{n_s} + t^{n_s}}.$$

We study the addition of sorbitol in wild-type cells and in two pka-wimp strains (D139H, E235Q). We obtain the following fitting parameters which dictate the variation of the activation rates of PP1 and Snf1. For the wild-type cells, we obtain:

$k_{ap}^{sorb} = 0.39 \mu M^{-1} \cdot min^{-1}$

$T^{PP1}_{1/2} = 3.18 \ min$

$n_p = 3.68$

$k_{as}^{sorb} = 0.96 \mu M^{-1} \cdot min^{-1}$

$T^{Snf1}_{1/2} = 7.86 \ min$

$n_p = 3.11.$

The corresponding fitting parameters for the pka-wimp mutant D139H are:

$k_{ap}^{sorb} = 0.24 \mu M^{-1} \cdot min^{-1}$

$T^{PP1}_{1/2} = 7.65 \ min$

$n_p = 1.62$

$k_{as}^{sorb} = 0.52 \mu M^{-1} \cdot min^{-1}$

$T^{Snf1}_{1/2} = 30.35 \ min$

$n_p = 3.16.$

Finally, for the E235Q mutant we have:

$k_{ap}^{sorb} = 0.23 \mu M^{-1} \cdot min^{-1}$

$T^{PP1}_{1/2} = 0.56 \ min$

$n_p = 4.93$

$k_{as}^{sorb} = 0.48 \mu M^{-1} \cdot min^{-1}$

$T^{Snf1}_{1/2} = 50.83 \ min$

$n_p = 5.60.$

Stochastic simulations

Stochastic simulation of a group of chemical reactions is the process in which one evolves the species in a discrete manner, i.e. one reaction at a time. One starts with a given number of molecules of each type. Randomly selecting one of the reactions, by the rules given below, and changing the numbers of molecules according to that specific reaction, one evolves the system for as long as necessary. We computed the amounts of chemical species using Gillespie stochastic simulations, as described in [3]. Sometimes this method of stochastic simulation is referred as the BKL method [4]. The same method was used in Gonze et al. [2] to study the oscillations of Msn2 transcription factor. The basic steps of the Gillespie stochastic simulation algorithm (SSA) are as follows:

(i) Compute the propensity functions for all the possible reactions $w_i(t)$ (Table S3) [2], and the sum of these propensities $w_0 = \sum_i w_i$;

...
(ii) Generate $r_1$ and $r_2$ uniformly distributed in $(0, 1)$;

(iii) Compute the time interval $\tau$ that passes until the next reaction occurs,

$$\tau = \frac{1}{w_0} \ln \left( \frac{1}{r_1} \right);$$

(iv) Select the reaction which occurs next, i.e. find $i$ such that

$$\frac{1}{w_0} \sum_{j=1}^{i-1} w_j \leq r_2 < \frac{1}{w_0} \sum_{j=1}^{i} w_j;$$

(v) Finally, update the number of molecules of each type, according to the modifications induced by the $i$-th reaction, and update the current time of the simulation ($t \rightarrow t + \tau$). Repeat the steps (i)-(v) until the current time increases to the desired total time of the simulation.

The stochastic version of the model is represented by the reactions and propensities given in Table S3. Most of the table is identical with the table of parameters in [2]. As in [2], we introduce two effective volume parameters, $\Omega_1$ and $\Omega_2$, that control the number of molecules in the simulations. These parameters allow us to vary the number of Msn2 molecules relative to the number of molecules of all other species. We use $\Omega_1 = 1000$ and $\Omega_2 = 100$, which are of the same order of magnitude as the parameters employed in [2].

MATLAB implementations of our stochastic and deterministic models are available as supplementary files.

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