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Supplementary Materials for

Regulation of Yeast G Protein Signaling by the Kinases That Activate the AMPK Homolog Snf1

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Published 3 September 2013, *Sci. Signal.* **6**, ra78 (2013)
DOI: 10.1126/scisignal.2004143

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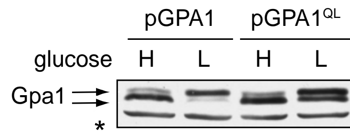

Fig. S1. Phosphorylation of Gpa1 is not affected by nucleotide binding. Western blotting analysis of Gpa1-GDP and Gpa1-GTP. WT cells transformed with the plasmids pAD4M-Gpa1 or pAD4M-Gpa1^{Q323L} (which encodes a GTPase-deficient mutant Gpa1) were grown in SC–Leu medium containing 2% (high, H) or 0.05% (low, L) glucose and then were analyzed by Western blotting with anti-Gpa1 antibody. Gpa1 was detected in two bands indicated by the arrows; the upper band corresponds to the phosphorylated protein. The asterisk indicates a nonspecific band. Data are representative of two independent experiments.

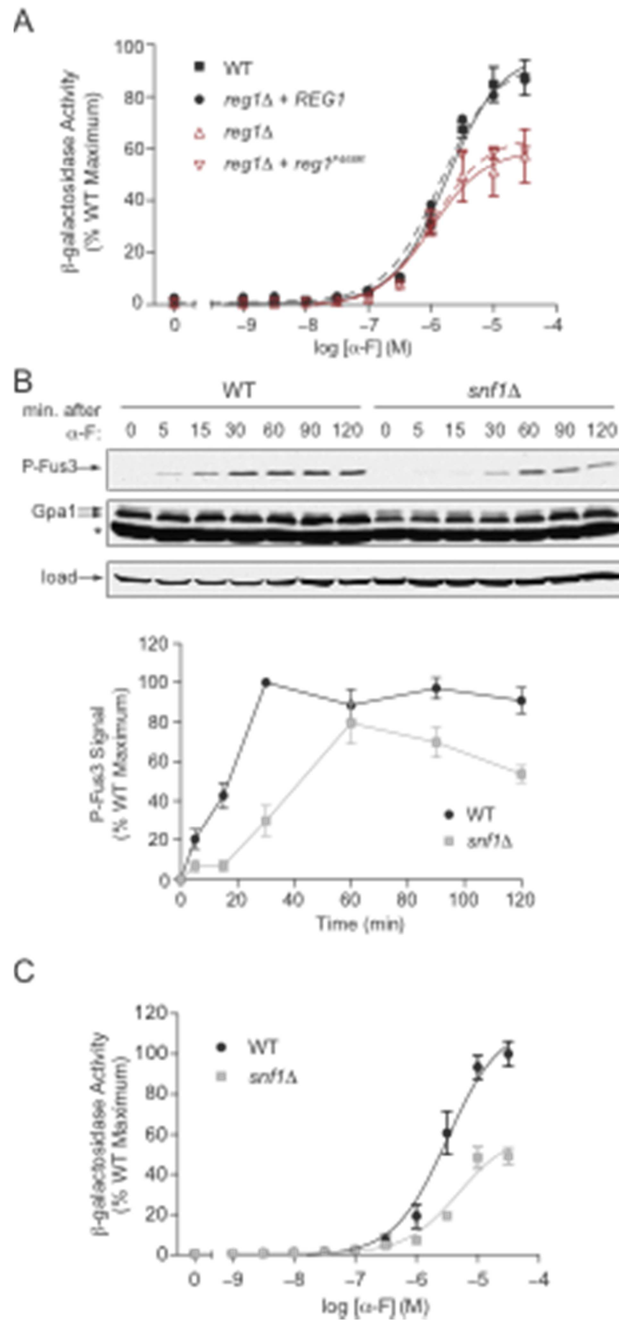

Figure S2. Reg1 and Snf1 promote maximal mating responses. (**A**) Analysis of pheromone-dependent gene transcription. WT and *reg1*Δ cells were cotransformed with a *FUS1-lacZ* reporter and empty vector or with plasmids encoding *REG1* or the *reg1*^{F468R} mutant. Cells were treated with the indicated concentrations of α-factor (α-F) for 90 min. Data are means ± SEM from three independent experiments, each performed in quadruplicate. Data are expressed as a percentage of the β-galactosidase activity of WT

cells at the maximum concentration of pheromone. (**B**) Top: Time course of the phosphorylation of Fus3 in WT and *snf1*Δ cells. Early-log phase cultures were treated with 3 μM α-F for the indicated times before samples were collected. MAPK activation was determined by Western blotting analysis of cell lysates with anti–phospho-p44/42 antibodies to detect pFus3. The same samples were analyzed with anti-Gpa1 and anti-G6PDH antibodies. Bottom: Densitometric analysis of pFus3 abundance. The abundance of pFus in each sample is expressed as a percentage of that in WT cells at the maximal response. Results are means ± SEM from three independent experiments. (**C**) Analysis of pheromone-dependent gene transcription in WT and *snf1*Δ cells. Cells expressing a *FUS1-lacZ* reporter were treated with the indicated concentrations of α-F for 90 min. Data are expressed as a percentage of the β-galactosidase activity of WT cells at the maximum concentration of pheromone. Data are means ± SEM from three independent experiments, each performed in quadruplicate.

Table S1. Yeast strains used in this study.

Strains	Parent	Description
BY4741		MAT**a** *leu2*Δ *met15*Δ *his3*Δ *ura3*Δ
BY4742		MATa *leu2*Δ *his3*Δ *ura3*Δ *lys2*Δ *MET* $^{+}$
*elm1*Δ	BY4741	*elm1*Δ::KanMX4
*tos3*Δ	BY4741	*tos3*Δ::KanMX4
*sak1*Δ	BY4741	*sak1*Δ::KanMX4
*ste2*Δ	BY4741	*ste2*Δ::KanMX4
*ste4*Δ	BY4741	*ste4*Δ::KanMX4
*sst2*Δ	BY4741	*sst2*Δ::KanMX4
*vps15*Δ	BY4741	*vps15*Δ::KanMX4
*elm1*Δ*sak1*Δ	BY4741	*elm1*Δ::*URA3 sak1*Δ::KanMX4
*elm1*Δ*tos3*Δ	BY4741	*elm1*Δ::*URA3 tos3*Δ::KanMX4
*sak1*Δ*tos3*Δ	BY4741	*sak1*Δ::*URA3 tos3*Δ::KanMX4
*elm1*Δ*sak1*Δ*tos3*Δ	BY4741	*elm1*Δ::*URA3 sak1*Δ::*LEU2 tos3*Δ::KanMX4
*snf1*Δ*sak1*Δ	BY4741	*snf1*Δ::KanMX4 *sak1*Δ::*LEU2*
*snf1*Δ	BY4741	*snf1*Δ::KanMX4
*reg1*Δ	BY4741	*reg1*Δ::KanMX4

Table S2. Plasmids used in this study.

Name	Description	Source
pAD4M	2 μm Amp^R LEU2 ADH1 promoter/terminator	*(50)*
pAD4M-GPA1	2 μm Amp^R LEU2 ADH1 GPA1	*(22)*
pLIC-6XHIS-GPA1	Amp^R 6HIS-TEV-GPA1	*(46)*
pLIC-REG1-MBP	Amp^R REG1-TEV-MBP	This Study
pRS316-ADH1-GPA1-FLAG	CEN6 Amp^R URA3 ADH1 GPA1-$FLAG^{Internal}$	*(7)*
pRS313	CEN6 Amp^R HIS3	*(51)*
pRS315	CEN6 Amp^R LEU2	*(51)*
pRS316	CEN6 Amp^R URA3	*(51)*
pRS313-SAK1	CEN6 Amp^R HIS3 SAK1 +/- 500bp	This Study
pRS423-FUS1-lacZ	2 μm Amp^R HIS3 FUS1-lacZ	*(52)*
pRS426-SAK1-TAP	2 μm Amp^R URA3 SAK1-TAP	*(53)*
pRS426-$SAK1^{D277A}$-TAP	2 μm Amp^R URA3 $SAK1^{D277A}$-TAP	*(53)*
pRS316-REG1	CEN6 Amp^R URA3 REG1 +/- 500bp	This Study
pRS316-$REG1^{F468R}$	CEN6 Amp^R URA3 $REG1^{F468R}$ +/- 500bp	This Study
pAD4M-GPA1-FLAG	2 μm Amp^R LEU2 ADH1 GPA1-$FLAG^{Internal}$	This Study
pRS316-ADH1-REG1-HA	CEN6 Amp^R URA3 ADH1 REG1-HA	This Study
yCP50	CEN6 Amp^R URA3	*(28)*
yCP50-STE11-4	CEN6 Amp^R URA3 STE11-4	*(28)*

Table S3. Sequences of oligonucleotide used in this study.

Name	Sequence (5'→3')
SacII-SAK1-F	GCCCGCGGGACAAGCTGTTGAGAGCAGGC
SmaI-SAK1-R	GCCCCGGGGCCCTCTTTAATTCCGGACG
XhoI-REG1-F	TTCACCTCGAGTCCTCCACTTCATGC
KpnI-REG1-R	TCTCGGTACCGGTGTGACACTGCCAG
REG1-F468R-F*	CCTACTAAAAATAGACATATACAT**CG**TAATGACAGGGTGGAAC
SmaI-ADH1-F	TATCCCGGGTAGTCGACGGATCCCATG
SacI-GPA1-R	ACGAGCTCTATAATACCAATTTTTTTAAGGTTTTGCTGGATG
REG1-HA-F*	GAAAATGGAAATGACAGCAGT**TACCCATACGATGTTCCAGATTACG** **CT**TAGGACGTCGACGGATCCGAGC
REG1-MBP-F	TACTTCCAATCCAATCGCATGTCAACAAATCTAGCAAATTACTTC
REG1-MBP-R	TTATCCACTTCCAATGCGCTACTAACTGCTGTCATTTCC

*Only forward sequences are shown for QuikChange primers. Mutations are indicated in bold.