Supporting Information

Simpson-Lavy and Johnston 10.1073/pnas.1304839110

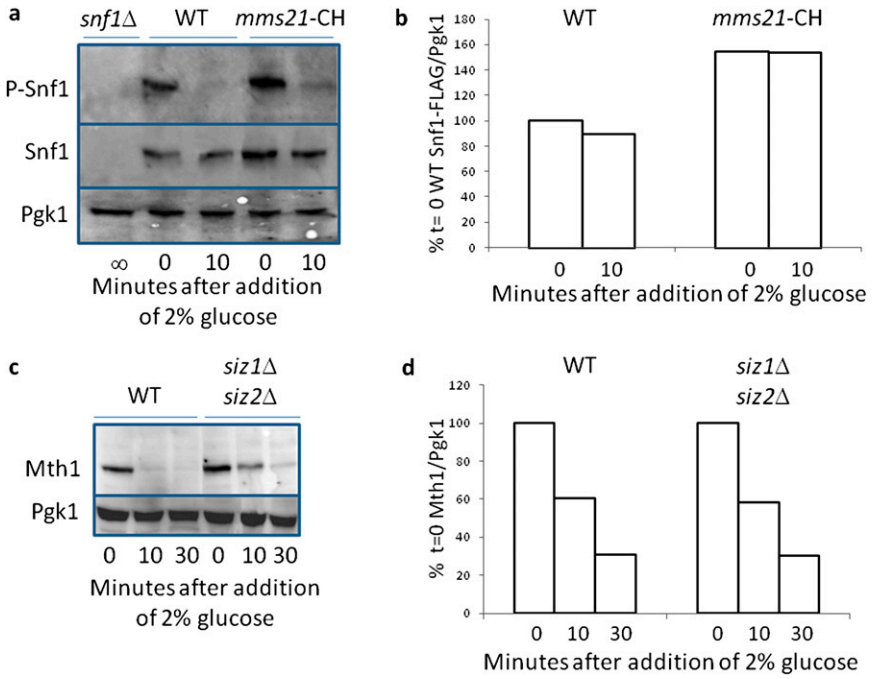

Fig. S1. Other small ubiquitin-like modifier protein (SUMO) ligases do not regulate Mth1 stability in glucose. (*A*) Cells were grown in 2% galactose at 30 °C overnight. The temperature was elevated to 34 °C for 1 h before addition of preheated glucose to 2%. Samples were processed for immunoblots at the indicated times. (*B*) Quantification of Snf1 levels in *A*. Amount of Snf1 is shown relative to wild-type in galactose-grown cells ($t = 0$). (*C*) Cells were grown in 2% galactose at 30 °C overnight. Glucose was added to 2% and samples were processed for immunoblots at the indicated times. (*D*) Quantification of *C*. Amount of Mth1 is shown relative to each strain in the absence of glucose ($t = 0$).

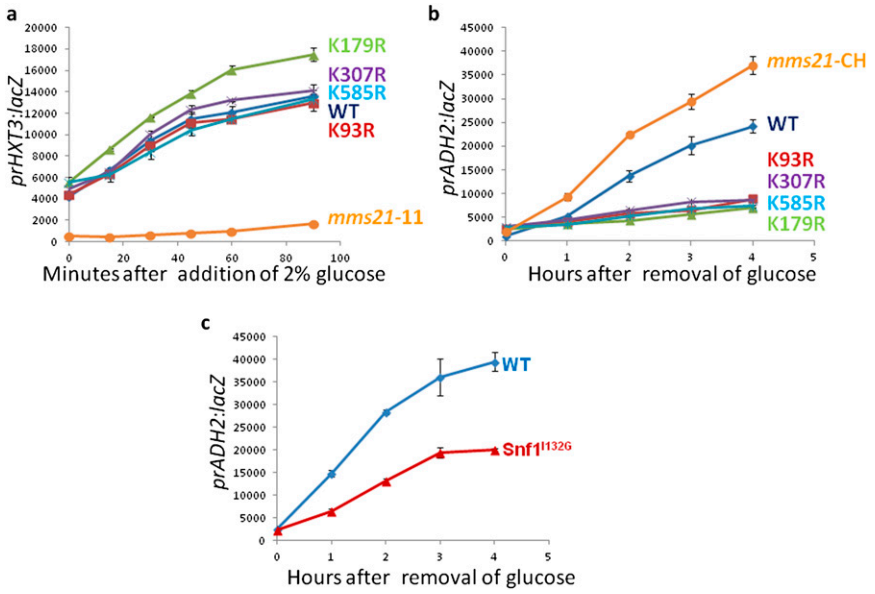

Fig. S2. Other SUMO motifs in Snf1 are nonfunctional. (*A*) *snf1Δ* cells with plasmids bearing wild-type *SNF1* or *SNF1* with the indicated mutations, or *snf1Δmms21-11* cells with a plasmid bearing wild-type *SNF1* were grown overnight at 30 °C in 2% galactose. Glucose was added to 2%, and samples were taken for β-galactosidase assays at the indicated times. $n = 3$. Plasmids are listed in Table S3. (*B*) *snf1Δ* cells with plasmids bearing wild-type *SNF1* or *SNF1* with the indicated mutations, or *snf1Δmms21*-CH cells with a plasmid bearing wild-type *SNF1* were grown overnight at 30 °C in 4% glucose, then for 1 h at 34 °C. Cells were washed three times with water at 34 °C, and resuspended in medium containing 3% glycerol at 34 °C. Samples were taken for β-galactosidase assays at the indicated times. $n = 3$. Plasmids are listed in Table S3. (*C*) *snf1Δ* cells with plasmids bearing wild-type *SNF1* or $SNF1^{I132G}$ were grown overnight at 30 °C in 4% glucose. Cells were washed three times with water and resuspended in medium containing 3% glycerol at 30 °C. Samples were taken for β-galactosidase assays at the indicated times. $n = 3$.

Fig. S3. Functional domains and amino acid residues of Snf1. Schematic diagram of Snf1 illustrating locations of domains and residues discussed herein.

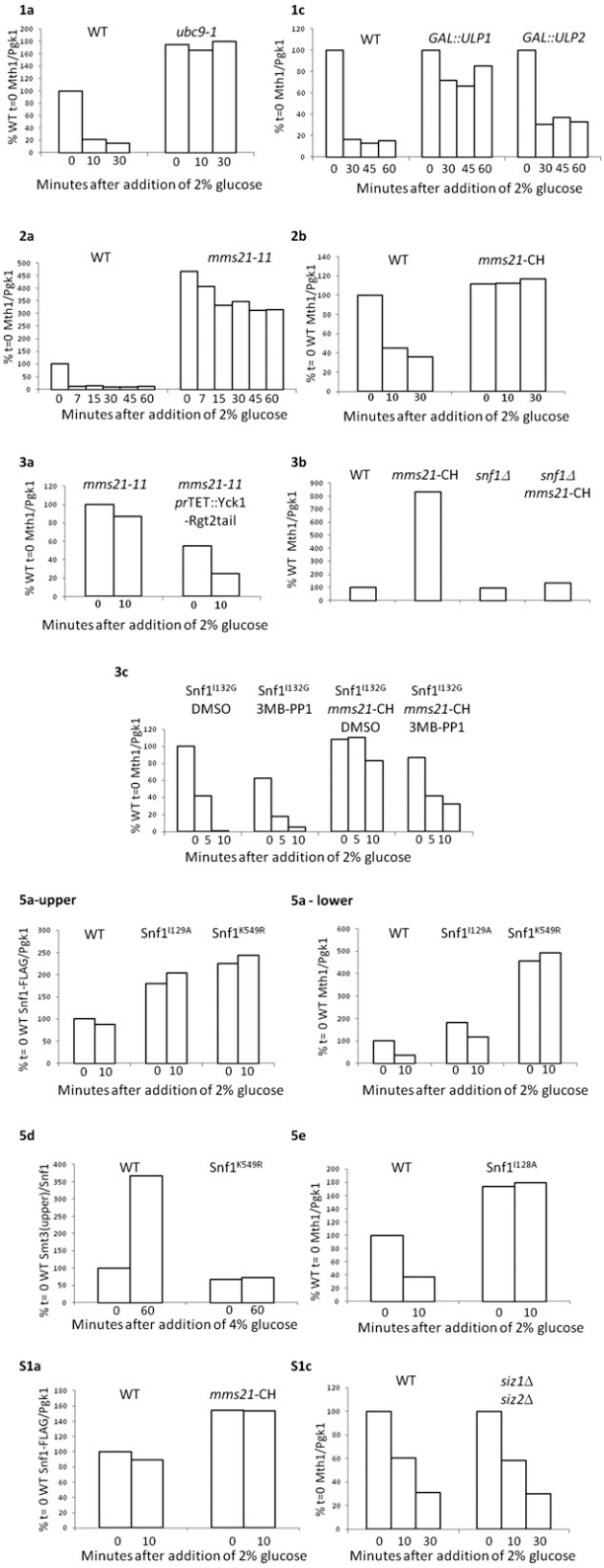

Fig. S4. Quantifications of blots. Quantifications of blots shown in this article were performed using ImageJ. The boldface numbers in the upper left corner of each panel signifies the figure and panel referenced in this article (i.e., "1a" represents Fig. 1*A* in this article).

Table S1. Yeast strains

Name	Genotype	Source
W303	*leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15* [*phi*$^{+}$] *rad5-535, bud4, ssd1-d2*	Lab collection
G16	W303 *MAT*a RAD5	(1)
T79	W303 *MAT*a RAD5 *mms21-11*::LEU2	(1)
T80-14	W303 *MAT*a RAD5 *mms21-11*::URA3	(1)
M21-CH	W303 *MAT*a RAD5 *mms21*-CH::URA3	
M21-CHu3	W303 *MAT*a RAD5 *mms21*-CH::URA3::*ura3*	
Snf1KAN (CKY18)	W303 *MATα snf1*Δ::KAN	(2)
Snf1U3	W303 *MAT*a *snf1Δ::URA3*	J. Rutter
Snf1KANm21-CH	W303 *MATα snf1*Δ::KAN *mms21*-CH::URA3	
Snf1KANm21-CHura3	W303 *MATα snf1*Δ::KAN *mms21*-CH::URA3::*ura3*	
BY4741	*MAT*a *his3Δ1 leu2Δ0/LYS2 met15Δ0 ura3Δ0*	
BY4742	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 MET15*	
ubc9-1 (ts)	BY4741 *ubc9-1*::NAT	T. Ravid
YAC391	MATα *siz1*Δ::HygB *siz2*Δ::KAN, *lyp1*Δ::STE3pr:LEU2, his3Δ1 leu2Δ0 ura3Δ0	(3)
EY1274	BY MATα *ulp1-ts*::MET15 LYS2 STE3pr::LEU2 *his3Δ1 ura3Δ0*	(4)
Ubp8	BY4741 *ubp8*::KAN	
Ubp8M21CH	BY4741 *ubp8*::KAN *mms21*-CH::URA3::ura3	
Ubp8slx8	BY4741 *ubp8*::KAN *slx8*::URA3	

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1. Zhao X, Blobel G (2005) A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. *Proc Natl Acad Sci USA* 102(13):4777–4782.
2. Ratnakumar S, Kacherovsky N, Arms E, Young ET (2009) Snf1 controls the activity of adr1 through dephosphorylation of Ser230. *Genetics* 182(3):735–745.
3. Chavez A, George V, Agrawal V, Johnson FB (2010) Sumoylation and the structural maintenance of chromosomes (Smc) 5/6 complex slow senescence through recombination intermediate resolution. *J Biol Chem* 285(16):11922–11930.
4. Wykoff DD, O'Shea EK (2005) Identification of sumoylated proteins by systematic immunoprecipitation of the budding yeast proteome. *Mol Cell Proteomics* 4(1):73–83.

Table S2. Plasmids

Name	Genotype	Backbone*	Mutations	Source
pBM4560	Mth1-9myc	pRS **313**		Lab collection
pBM5020	Mth1-Stag	pRS **313**		Lab collection
pBGM18	pr*ADH2*::*lacZ*	pRS 313, 315, **316**		(1)
pBM2819	pr*HXT3*::*lacZ*	2 μ URA3		Lab collection
pBM3118	pr*HXT3*::*lacZ*	2 μ LEU2		Lab collection
pBM4924	*prTET*^{on}::*YCK1*-Rgt2tail	2 μ TRP1		Lab collection
Mms21-47	mms21-CH	pUC19 URA3	C200A H202A	(2)
pBM3926	pr*GAL1*:His6-FLAG-Smt3(G98)	pRS 313, **315**, 316		(3)
S12F	Snf1-2FLAG	pRS 314, 315, **316**	I129A, K549R	(4)
pYL199 (S18M)	Snf1-8myc	pRS **313**, 314, 316	I129A, K549R	(5)
Snf1-as1	Snf1^{I132G}	pRS **316**		(6)
Snf1	Snf1	pRS **316**, 313		(6)
GALulp1	pr*GAL1*:GST-Ulp1	2 μ URA3		M. Hochstrasser
GALulp2	pr*GAL1*:Ulp2-FLAG	2 μ TRP1		M. Hochstrasser

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*Selectable markers in pRS plasmids were changed by gap repair; the original plasmid that is the backbone of the named plasmid is in bold.

1. Dombek KM, Voronkova V, Raney A, Young ET (1999) Functional analysis of the yeast Glc7-binding protein Reg1 identifies a protein phosphatase type 1-binding motif as essential for repression of ADH2 expression. *Mol Cell Biol* 19(9):6029–6040.
2. Santa Maria SR, Gangavarapu V, Johnson RE, Prakash L, Prakash S (2007) Requirement of Nse1, a subunit of the Smc5-Smc6 complex, for Rad52-dependent postreplication repair of UV-damaged DNA in *Saccharomyces cerevisiae*. *Mol Cell Biol* 27(23):8409–8418.
3. Johnson ES, Blobel G (1999) Cell cycle-regulated attachment of the ubiquitin-related protein SUMO to the yeast septins. *J Cell Biol* 147(5):981–994.
4. Nayak V, et al. (2006) Structure and dimerization of the kinase domain from yeast Snf1, a member of the Snf1/AMPK protein family. *Structure* 14(3):477–485.
5. Raines RT, McCormick M, Van Oosbree TR, Mierendorf RC (2000) The S.Tag fusion system for protein purification. *Methods Enzymol* 326:362–376.
6. Shirra MK, et al. (2008) A chemical genomics study identifies Snf1 as a repressor of GCN4 translation. *J Biol Chem* 283(51):35889–35898.

Table S3. Supplemental plasmids

Name	Genotype	Backbone	Mutations	Source
S12F	Snf1-2FLAG	pRS 316	K93R, K179R, K307R, K585R	(1)

1. Nayak V, et al. (2006) Structure and dimerization of the kinase domain from yeast Snf1, a member of the Snf1/AMPK protein family. *Structure* 14(3):477–485.