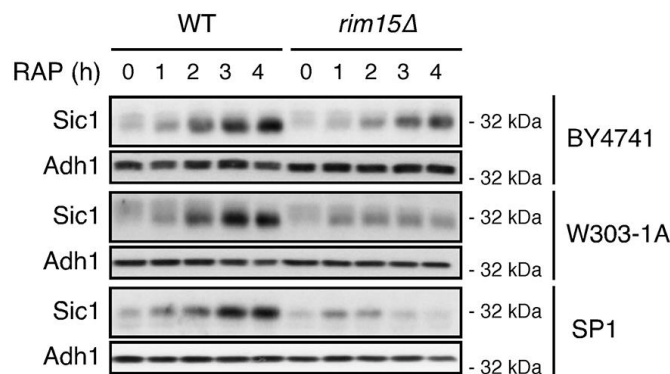
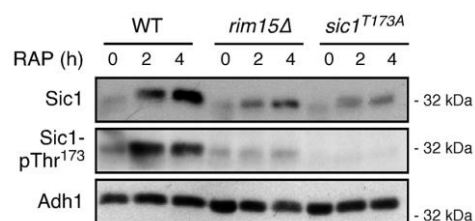


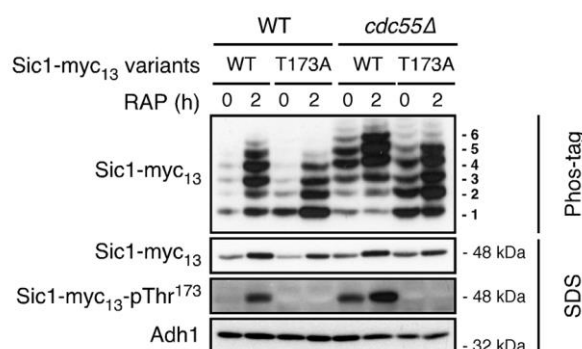
Supplementary Figures



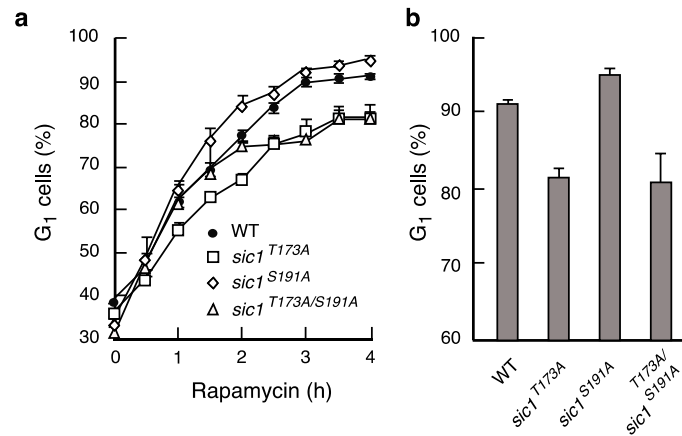
Supplementary Figure 1 | Rim15 ensures Sic1 accumulation following TORC1 inactivation independently of the yeast strain background. The levels of endogenous Sic1, in exponentially growing (0 h) and rapamycin-treated (RAP; 1-4 h) BY4741, W303-1A, and SP1 wild-type (WT) and respective isogenic *rim15Δ* mutant cells, were determined by immunoblot analyses using polyclonal anti-Sic1 antibodies. Adh1 levels served as loading controls.



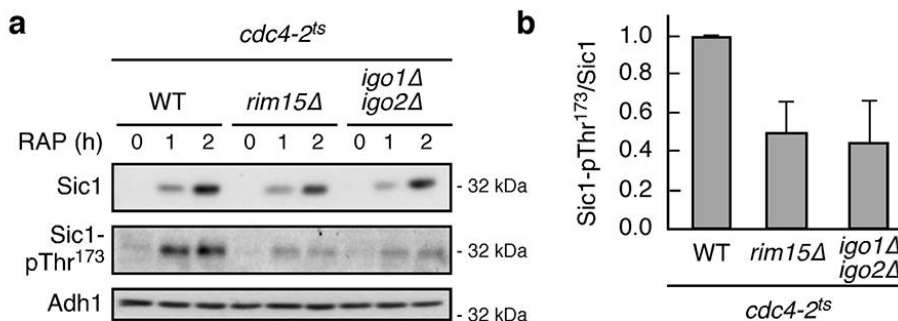
Supplementary Figure 2 | Mutation of Thr¹⁷³ to Ala in Sic1, like loss of Rim15, compromises normal Sic1 accumulation in rapamycin-treated cells. Sic1 levels and phosphorylation of Thr¹⁷³ in Sic1 (Sic1-pThr¹⁷³) were determined in exponentially growing (0 h) and rapamycin-treated (RAP; 2 and 4 h) cells with the indicated genotypes by immunoblot analyses using polyclonal anti-Sic1 and phosphospecific anti-Sic1-pThr¹⁷³ antibodies, respectively. Adh1 levels served as loading controls.



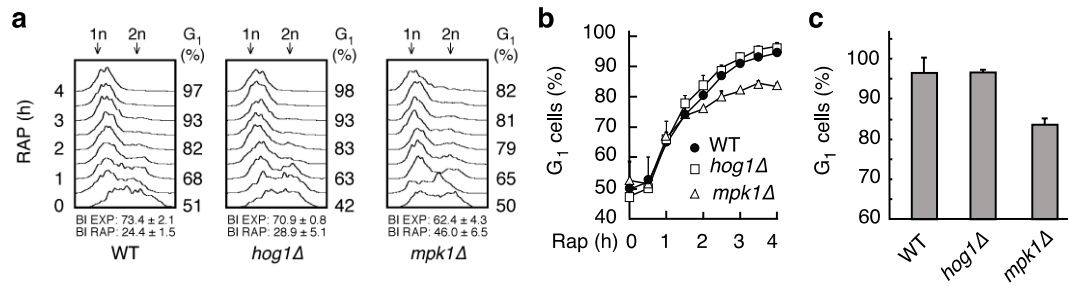
Supplementary Figure 3 | Phos-tag phosphate affinity gel electrophoresis analysis of genomically myc₁₃-tagged Sic1 or Sic1^{T173A}. Sic1-myc₁₃ or Sic1^{T173A}-myc₁₃ were analyzed by phos-tag phosphate affinity and SDS gel electrophoresis (followed by immunoblot analysis using anti-myc or anti-Sic1-pThr¹⁷³ antibodies) in extracts from exponentially growing (0 h) and rapamycin-treated (RAP; 2 h) WT and *cdc55Δ* strains. The 6 differentially phosphorylated Sic1-myc₁₃ isoforms are numbered sequentially from 1 to 6 (right side of the panels). Adh1 levels served as loading controls.



Supplementary Figure 4 | The *Sic1*^{T173A} allele compromises G₁ arrest in rapamycin-treated cells. (a) FACS analyses were performed in exponentially growing (0 h) and rapamycin-treated (times indicated) WT, *sic1*^{T173A}, *sic1*^{S191A}, and *sic1*^{T173A/S191A} cells. The experiments were performed independently 3 times for each strain (one representative FACS profile is shown in Fig. 2g) and the quantifications (means ± SD) of the percentage of G₁ cells in the respective populations are presented. (b) Bar graphs show the percentage of G₁ cells in the populations of rapamycin-treated (4 h) strains with the indicated genotypes with error bars indicating the 95% confidence interval. The data points from the 4-h rapamycin treatment were further used to perform an ANOVA analysis, which was followed by a Tukey's post-hoc test to examine the differences for each pair of strains. We found a highly significant difference among the four strains (ANOVA, p-value <0.001). Tukey's post-hoc test indicated that the values for WT and *sic1*^{S191A} cells were not significantly different from each other (p-value=0.133); similarly the values for *sic1*^{T173A} and *sic1*^{T173A/S191A} cells were also not significantly different from each other (p-value=0.97). All other pairwise comparisons were statistically significant (all p-values <0.001), showing that the values for WT and *sic1*^{S191A} cells significantly diverged from the ones of the *sic1*^{T173A} and *sic1*^{T173A/S191A} cells.

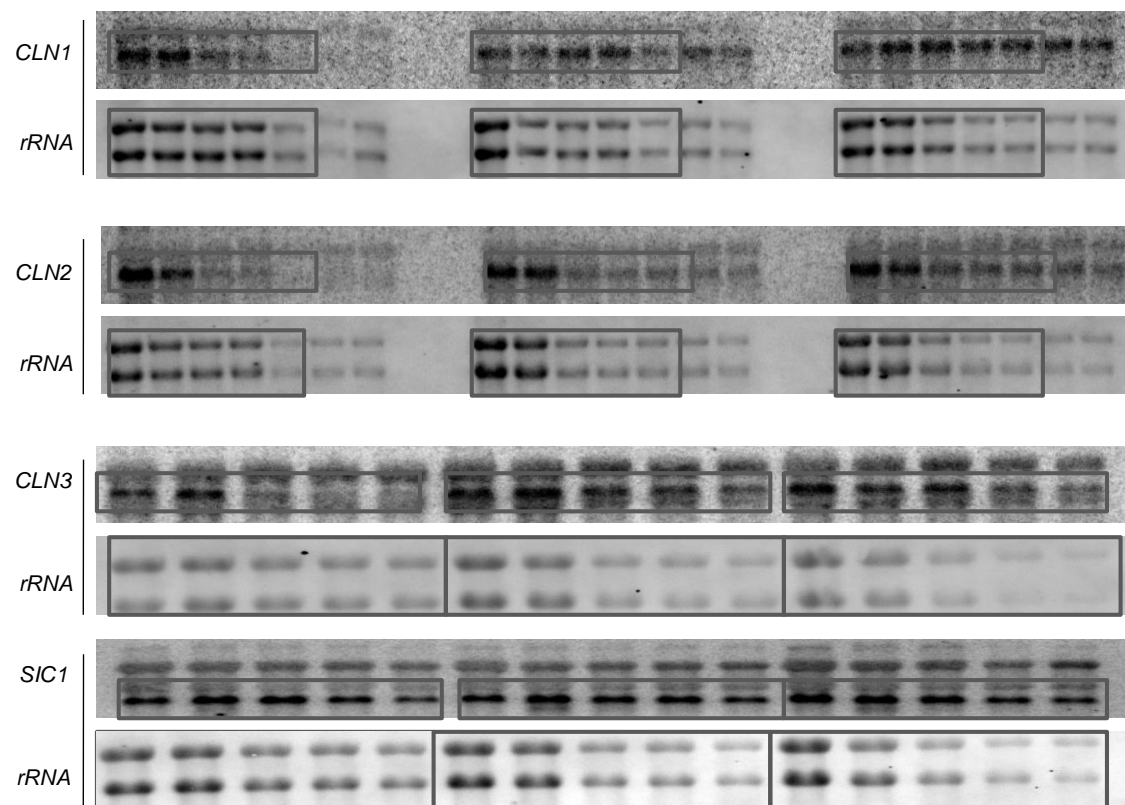


Supplementary Figure 5 | Inactivation of the SCF^{Cdc4} ubiquitin ligase suppresses the defect in Sic1 accumulation, but not in Sic1-Thr¹⁷³ phosphorylation, in rapamycin-treated *rim15Δ cdc4-2^{ts}* and *igo1Δ igo2Δ cdc4-2^{ts}* mutant cells. (a) Sic1 levels and phosphorylation of Thr¹⁷³ in Sic1 (Sic1-pThr¹⁷³) were determined by immunoblot analyses using polyclonal anti-Sic1 and phosphospecific anti-Sic1-pThr¹⁷³ antibodies, respectively. Cells (genotypes indicated) were pre-grown exponentially at 24°C (0 h) and then shifted to 37°C for 1 or 2 h (to inactivate Cdc4-2^{ts}) in the presence of rapamycin (RAP). Adh1 levels served as loading controls. The experiment was performed independently 3 times and one representative set of blots is shown. (b) Bars represent the ratio between the mean Sic1-pThr¹⁷³ levels and Sic1 protein levels (± SD; 3 independent experiments), determined in rapamycin-treated (2h at 37°C) *cdc4-2^{ts}*, *rim15Δ cdc4-2^{ts}*, and *igo1Δ igo2Δ cdc4-2^{ts}* cells and expressed relative to the value in *cdc4-2^{ts}* cells (set to 1.0).

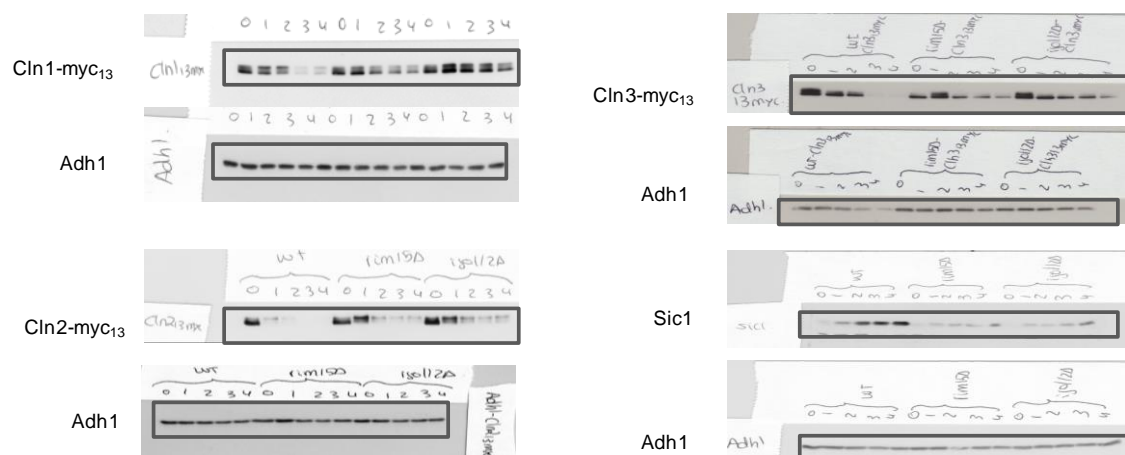


Supplementary Figure 6 | Loss of Mpk1, but not of Hog1, compromises timely G₁ arrest in rapamycin-treated cells. (a) FACS analyses were performed in exponentially growing (time 0 h) and rapamycin-treated (times indicated) WT, *hog1Δ*, and *mpk1Δ* cells. FACS and BI analyses were performed as in Fig. 1a. The experiments were performed independently 3 times for each strain (one representative FACS profile is shown). (b) Quantifications (means ± SD) of the percentage of G₁ cells in the respective populations in (a) are presented. (c) Bar graphs showing the percentage of G₁ cells in the populations of rapamycin-treated (4 h) strains with the indicated genotypes with error bars indicating the 95% confidence interval. The data points from the 4-h rapamycin treatment were further used to perform an ANOVA analysis, which was followed by a Tukey's post-hoc test to examine the differences for each pair of strains. We found a highly significant difference among the three strains (ANOVA, p-value <0.001). Tukey's post-hoc test indicated that the values for WT and *hog1Δ* cells were not significantly different from each other (p-value=0.53), but that the values for the *mpk1Δ* cells were significantly different from the other two strains (both p-values <0.002).

Supplementary Figures 7-25: Original Blots

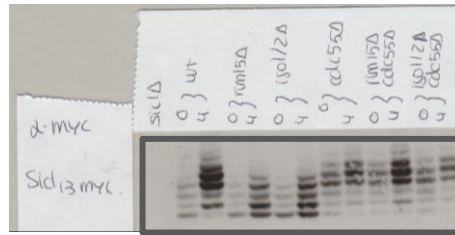


Supplementary Figure 7 | Full-sized scans of Northern blots in Figure 1e.

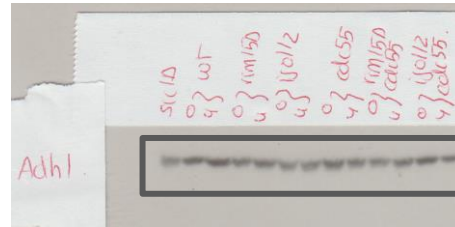


Supplementary Figure 8 | Full-sized scans of Western blots in Figure 1f.

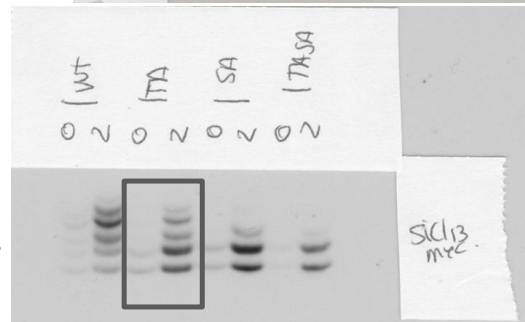
Sic1-myc₁₃



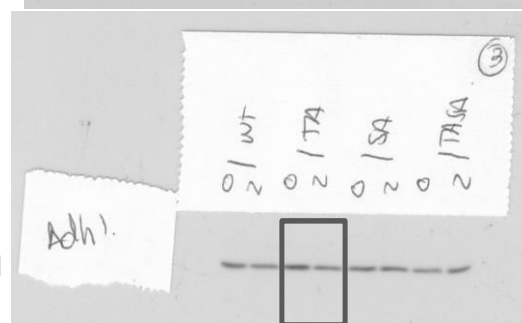
Adh1



Sic1-myc₁₃

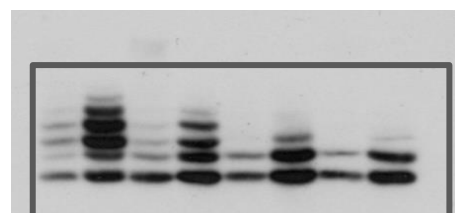


Adh1

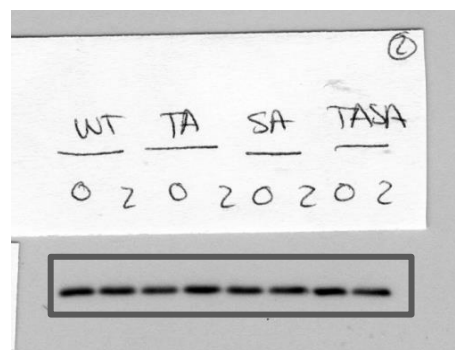


Supplementary Figure 11 | Full-sized scans of Western blots in Figure 2d.

Sic1-myc₁₃



Adh1



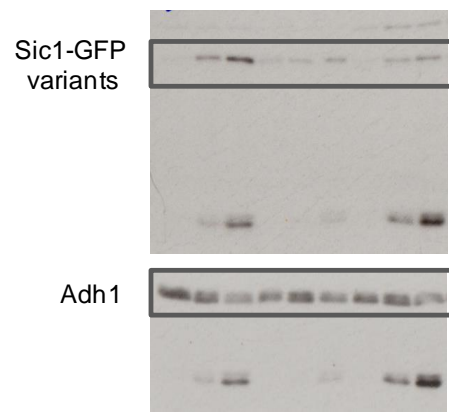
Supplementary Figure 12 | Full-sized scans of Western blots in Figure 2e.



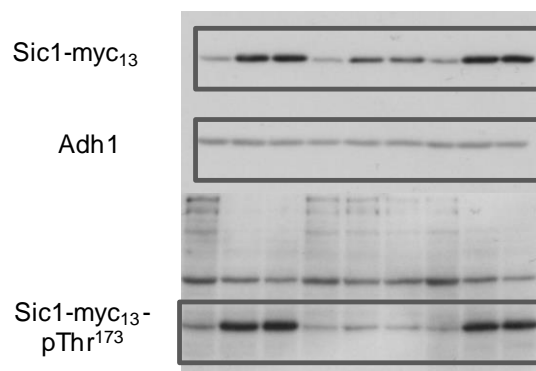
Supplementary Figure 13 | Full-sized scans of Western blots in Figure 2f.



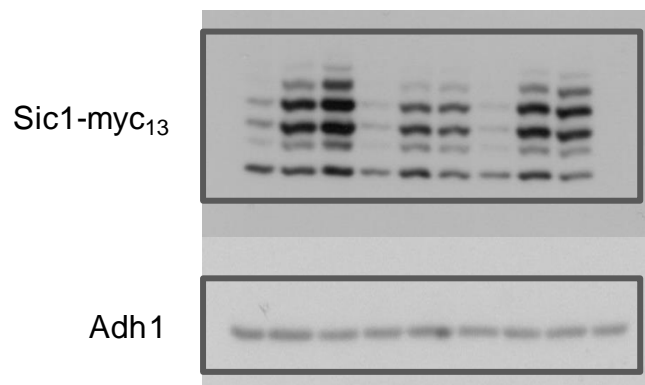
Supplementary Figure 14 | Full-sized scans of Western blots in Figure 3a.



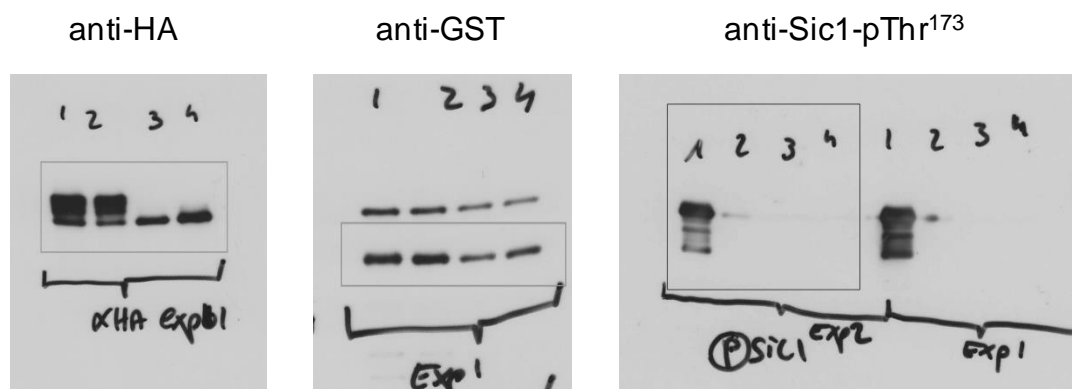
Supplementary Figure 15 | Full-sized scans of Western blots in Figure 3c.



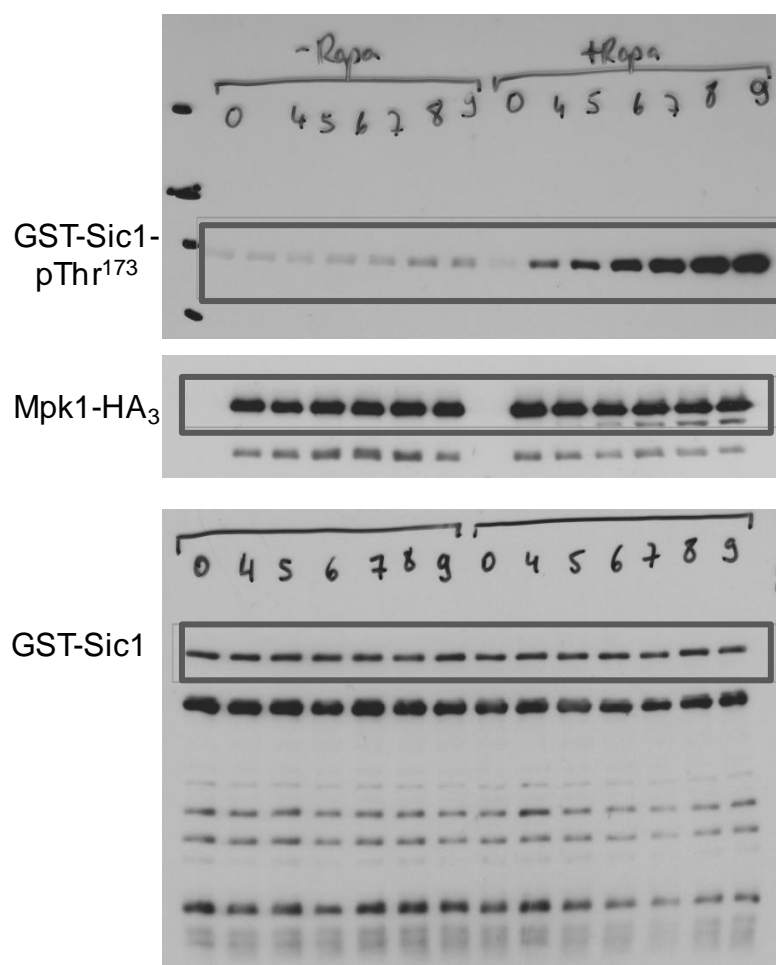
Supplementary Figure 16 | Full-sized scans of Western blots in Figure 4a.



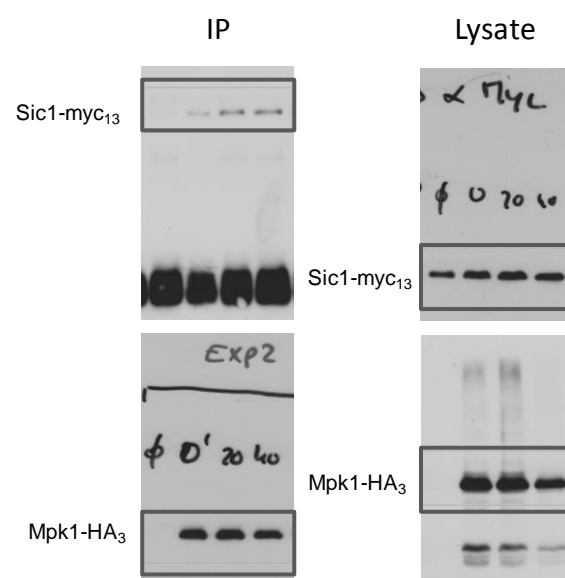
Supplementary Figure 17 | Full-sized scans of Western blots in Figure 4b.



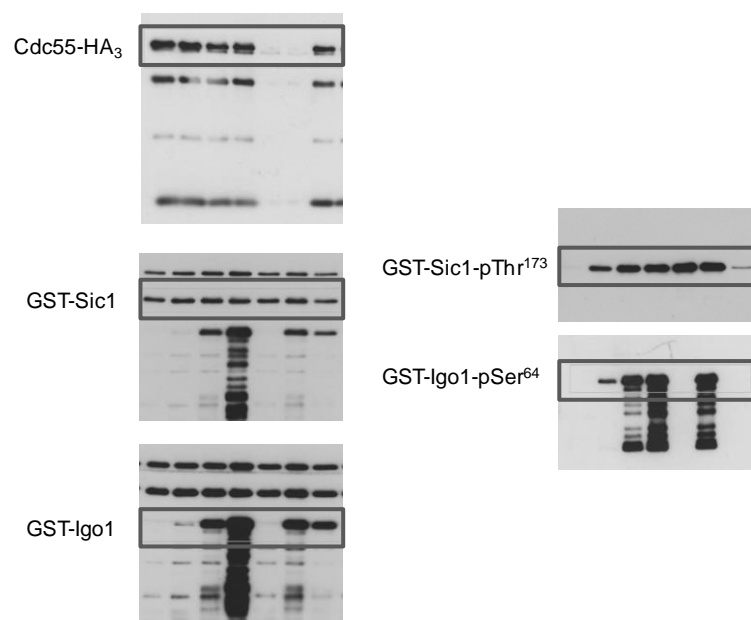
Supplementary Figure 18 | Full-sized scans of Western blots in Figure 4c.



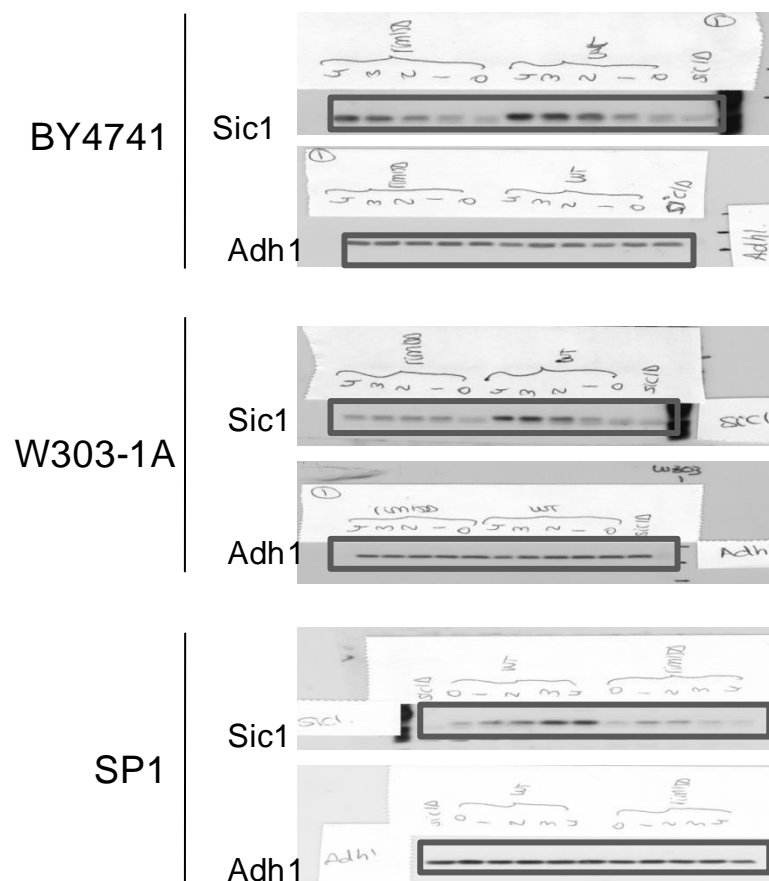
Supplementary Figure 19 | Full-sized scans of Western blots in Figure 4d.



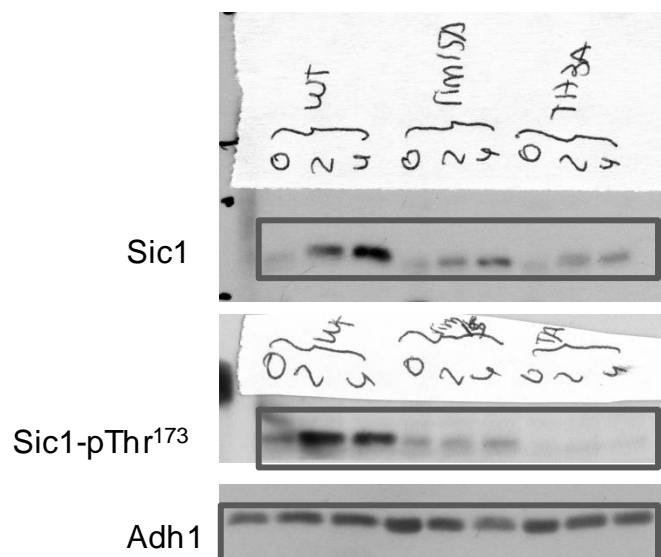
Supplementary Figure 20 | Full-sized scans of Western blots in Figure 4e.



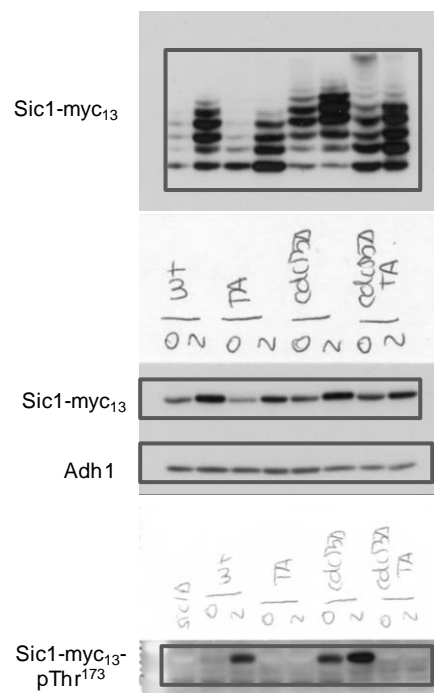
Supplementary Figure 21 | Full-sized scans of Western blots in Figure 5a.



Supplementary Figure 22 | Full-sized scans of Western blots in Supplementary Figure 1.



Supplementary Figure 23 | Full-sized scans of Western blots in Supplementary Figure 2.



Supplementary Figure 24 | Full-sized scans of Western blots in Supplementary Figure 3.



Supplementary Figure 25 | Full-sized scans of Western blots in Supplementary Figure 5.

Supplementary Tables

Supplementary Table 1 | Strains Used in This Study.

Strain	Genotype	Source	Figure
BY4741	<i>MATa; his3Δ1, leu2Δ0, met15Δ0, ura3Δ0</i>	reference ¹	1a, S1
W303-1A	<i>MATa; ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3-1</i>		1a, S1
SP1	<i>MATa; leu2, his3, trp1, ade8, ura3, can1</i>	reference ²	1a, S1
JK9-3D	<i>MATa; leu2, his4, trp1, ura3, rme1, GAL,HMLa</i>	reference ³	1b,1d-f,1i, 2a-b, 2f-g, S2, S4, S6
YMM203	[SP1] <i>rim15Δ::kanMX</i>	this study	S1
YSB147	[BY4741] <i>rim15Δ::natMX, MET15</i>	reference ⁵	S1
CDV95-4A	[W303-1A] <i>rim15Δ::kanMX</i>	this study	S1
IP11	[JK9-3D] <i>rim15Δ::kanMX</i>	reference ⁴	1c-f, 1i, 2a, S2
YMM57-2A	[JK9-3D] <i>igo1Δ::natMX, igo2Δ::Hph-NT1</i>	this study	1c-f, 1i, 2a
YMM59	[JK9-3D] <i>CLN1-myc₁₃::kanMX</i>	this study	1f-g
YMM60	[JK9-3D] <i>CLN2-myc₁₃::kanMX</i>	this study	1f-g
YMM61	[JK9-3D] <i>CLN3-myc₁₃::kanMX</i>	this study	1f, 1h
YMM87-2D	[JK9-3D] <i>rim15Δ::kanMX, CLN1-myc₁₃::kanMX</i>	this study	1f-g
YMM88-12B	[JK9-3D] <i>rim15Δ::kanMX, CLN2-myc₁₃::kanMX</i>	this study	1f-g
YMM78-2A	[JK9-3D] <i>rim15Δ::kanMX, CLN3-myc₁₃::kanMX</i>	this study	1f, 1h
YMM85-5D	[JK9-3D] <i>igo1Δ::natMX, igo2Δ::Hph-NT1, CLN1-myc₁₃::kanMX</i>	this study	1f-g
YMM79-9A	[JK9-3D] <i>igo1Δ::natMX, igo2Δ::Hph-NT1, CLN2-myc₁₃::kanMX</i>	this study	1f-g
YMM80-5C	[JK9-3D] <i>igo1Δ::natMX, igo2Δ::Hph-NT1, CLN3-myc₁₃::kanMX</i>	this study	1f, 1h
YMM55-1C	[JK9-3D] <i>rim15Δ::kanMX, cdc55Δ::natMX</i>	this study	2a
YMM90-3D	[JK9-3D] <i>igo1Δ::natMX, igo2Δ::Hph-NT1, cdc55Δ::natMX</i>	this study	2a
YMM46	[JK9-3D] <i>cdc55Δ::natMX</i>	this study	2a, 5a
YMM98	[JK9-3D] <i>sic1^{T173A}-myc₁₃::kanMX, EMP46::natMX</i>	this study	2c-e, S3
YMM143-2A	[JK9-3D] <i>sic1^{T173A}-myc₁₃::kanMX, cdc55Δ::natMX, EMP46::natMX</i>	this study	S3
YMM63	[JK9-3D] <i>SIC1-myc₁₃::kanMX</i>	this study	2c-e, S3
YMM68-9D	[JK9-3D] <i>rim15Δ::kanMX, SIC1-myc₁₃::kanMX</i>	this study	2c-d
YMM70-6B	[JK9-3D] <i>igo1Δ::natMX, igo2Δ::Hph-NT1, SIC1-myc₁₃::kanMX</i>	this study	2c-d
YMM69-1C	[JK9-3D] <i>cdc55Δ::natMX, SIC1-myc₁₃::kanMX</i>	this study	2c-d, S3
YMM100-9D	[JK9-3D] <i>rim15Δ::kanMX, cdc55Δ::natMX, SIC1-myc₁₃::kanMX</i>	this study	2c-d
YMM96	[JK9-3D] <i>igo1Δ::natMX, igo2Δ::Hph-NT1, cdc55Δ::natMX, SIC1-myc₁₃::kanMX</i>	this study	2c-d
YMM91	[JK9-3D] <i>sic1^{T173A}, EMP46::natMX</i>	this study	2f-g, S2, S4
YMM101	[JK9-3D] <i>sic1^{S191A}, EMP46::natMX</i>	this study	2f-g, S4
YMM103	[JK9-3D] <i>sic1^{T173A/S191A}, EMP46::natMX</i>	this study	2f-g, S4
YMM105	[JK9-3D] <i>sic1^{S191A}-myc₁₃::kanMX, EMP46::natMX</i>	this study	2e
YMM133	[JK9-3D] <i>sic1^{T173A/S191A}-myc₁₃::kanMX, EMP46::natMX</i>	this study	2e
YMM114	[JK9-3D] <i>cdc4-2::kanMX</i>	this study	3a-b, S5
YMM117-3A	[JK9-3D] <i>rim15Δ::kanMX, cdc4-2::kanMX</i>	this study	3a-b, S5
YMM116-4A	[JK9-3D] <i>igo1Δ::natMX, igo2Δ::Hph-NT1, cdc4-2::kanMX</i>	this study	3a-b, S5
YMM118-2D	[JK9-3D] <i>sic1^{T173A}, EMP46::natMX, cdc4-2::kanMX</i>	this study	3a-b
YMM77	[JK9-3D] <i>SIC1-GFP(S65T)::kanMX</i>	this study	3c-d
YMM97-7B	[JK9-3D] <i>rim15Δ::kanMX, SIC1-GFP(S65T)::kanMX</i>	this study	3c
YMM99	[JK9-3D] <i>sic1^{T173A}-GFP(S65T)::kanMX</i>	this study	3c-d
YMM67-1C	[JK9-3D] <i>sic1Δ::kanMX</i>	this study	2a
YMM53	[JK9-3D] <i>mpk1Δ::kanMX</i>	this study	4c-f, S6
YMM65-2D	[JK9-3D] <i>mpk1Δ::kanMX, SIC1-myc₁₃::kanMX</i>	this study	4a-b
YMM204-14C	[JK9-3D] <i>hog1Δ::kanMX, SIC1-myc₁₃::kanMX</i>	this study	4a-b
YMM64-3C	[JK9-3D] <i>rim15Δ::kanMX, mpk1Δ::kanMX</i>	this study	4f
YMM111-2A	[JK9-3D] <i>igo1Δ::natMX, igo2Δ::Hph-NT1, mpk1Δ::kanMX</i>	this study	4f
YMM130	[JK9-3D] <i>hog1Δ::kanMX</i>	this study	S6

Supplementary Table 2 | Plasmids Used in This Study.

Plasmid	Genotype	Source	Figure
pRS416	<i>CEN/ARS, URA3</i>	reference ¹	1b
pMM5	[pRS416] <i>RME1</i>	this study	1b
p1308	[pRS414]	reference ¹	1d, 2d
p1309	[pRS415]	reference ¹	1d, 2d
p1310	[pRS416]	reference ¹	1d, 2d
pMM10	[pRS415] <i>HIS4</i>	this study	1d, 2d
pSB004	[pRS416] <i>ADH1p-CDC55</i>	reference ⁵	2a-b
p834	[pRS416] <i>ADH1p</i>	reference ⁶	2a-b
pMM6	[pRS416] <i>MPK1-HA₃</i>	this study	4c-e
pMM7	[pRS416] <i>mpk1^{K54R}-HA₃</i>	this study	4c
pMM8	pGEX- <i>SIC1</i>	this study	4c-d, 5a
pMM9	pGEX- <i>sic1^{T173A}</i>	this study	4c
pMJA2610	[pRS416] <i>CDC55-HA₃</i>	this study	5a
pCDV487	pHAC195- <i>GALI-GST-RIM15</i> , 2 μ , <i>URA3</i>	reference ⁴	5a
pLC1092	pGEX- <i>IGO1</i>	reference ⁷	5a
pLC1134	pGEX- <i>igo1^{S64A}</i>	reference ⁷	5a

Supplementary References

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