Supplementary Information

TORC1 Coordinates the Conversion of Sic1 from a Target to an Inhibitor of Cyclin-CDK-Cks1

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INVENTORY OF SUPPLEMENTARY INFORMATION

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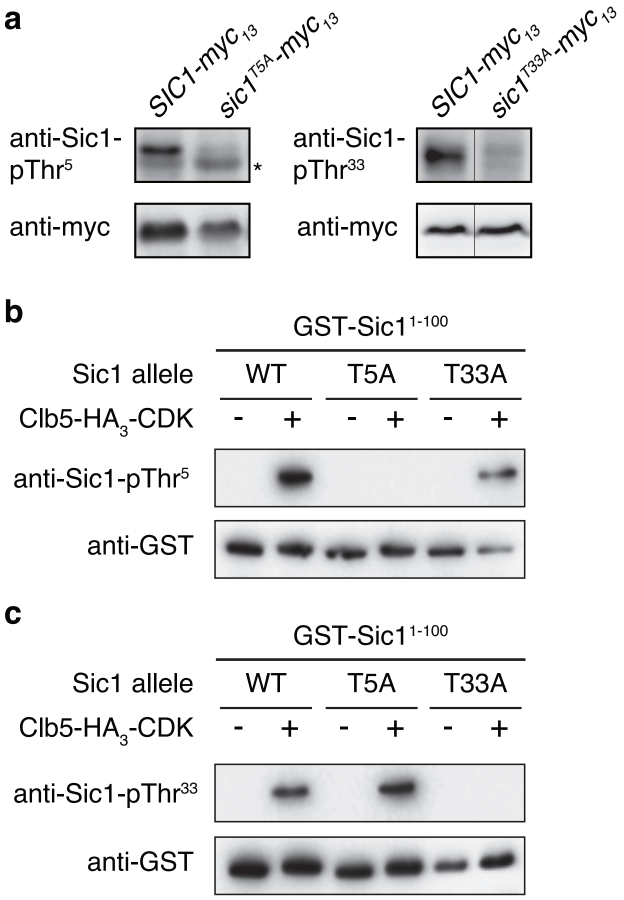

Figure S1 *In vivo* and *in vitro* specificity of the anti-Sic1-pThr^{5}/-pThr^{33} antibodies. (**a**) Sic1-pThr^{5} and Sic1-pThr^{33} levels were determined by immunoblot analyses using respective phospho-specific antibodies and extract of exponentially growing cells that expressed plasmid-encoded versions of myc$_{13}$-tagged Sic1 and Sic1^{T5A} (left panels), or myc$_{13}$-tagged Sic1 and Sic1^{T33A} (right panels). The levels of the Sic1-myc$_{13}$ variants were determined by using polyclonal anti-myc antibodies. The asterisk denotes an unspecific band. (**b**, **c**) Clb5-HA$_{3}$-CDK immunocomplexes from exponentially growing yeast cells were used for *in vitro* kinase assays in which the bacterially-purified, N-terminal parts (encompassing the first 100 amino acids) of Sic1 (WT), Sic1^{T5A} (T5A), and Sic1^{T33A} (T33A) served as substrates. Sic1-pThr^{5} (b) and Sic1-pThr^{33} (c) levels were determined by immunoblot analyses using respective phospho-specific antibodies and the indicated GST-Sic1$^{1-100}$ variants that have been phosphorylated (+), or not (-), by Clb5-HA$_{3}$-CDK. The input levels of the GST-Sic1$^{1-100}$ variants were determined by using polyclonal anti-GST antibodies.

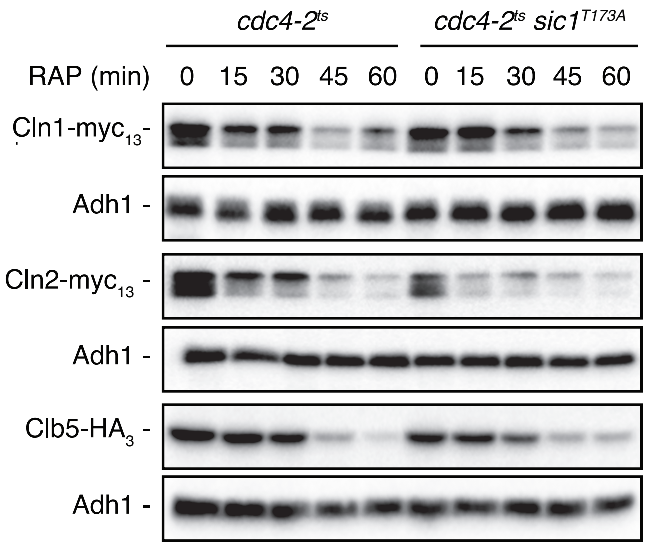

Figure S2 Sic1^{T173A}-expressing *cdc4-2^{ts}* cells are not defective in the clearance of Cln1, Cln2 or Clb5 when treated with rapamycin. *cdc4-2^{ts}* and *cdc4-2^{ts} sic1^{T173A}* strains expressing Cln1-myc$_{13}$, Cln2-myc$_{13}$, or Clb5-HA$_3$ were grown as in Fig. 1A. the levels of the tagged proteins were determined by immunoblot analyses using monoclonal anti-myc or anti-HA antibodies. Adh1 levels served as loading control.

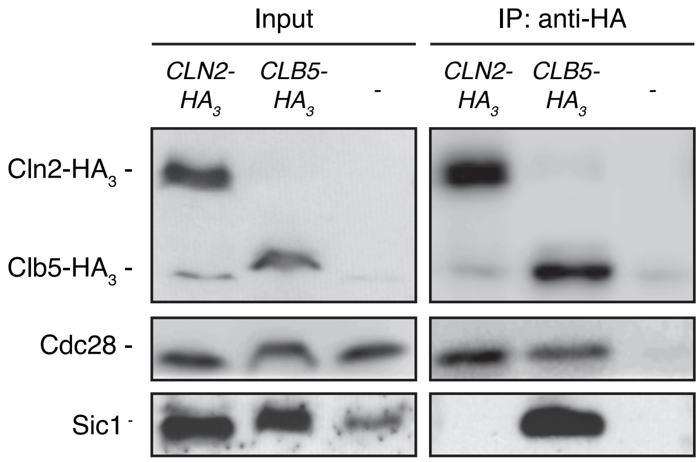

Figure S3 Cln2-HA$_3$ and Clb5-HA$_3$ interact with Cdc28 *in vivo*. Plasmid-expressed, HA$_3$-tagged Cln2 or Clb5 were immunoprecipitated (IPed) from extracts of exponentially growing wild-type cells. Cell lysates (Input) and anti-HA immunoprecipitates (IP: anti-HA) were analyzed by immunoblotting with anti-HA (top panels), anti-Cdc28 (panels in the middle), and anti-Sic1 (bottom panels) antibodies. Please note that both Cln2-HA$_3$ and Clb5-HA$_3$ interact with Cdc28, while only Clb5-HA$_3$ is able to bind Sic1 as expected. Neither Cdc28 nor Sic1 were recovered in anti-HA immunoprecipitates from extracts of cells that carried an empty plasmid (-; 3rd lanes in all panels).

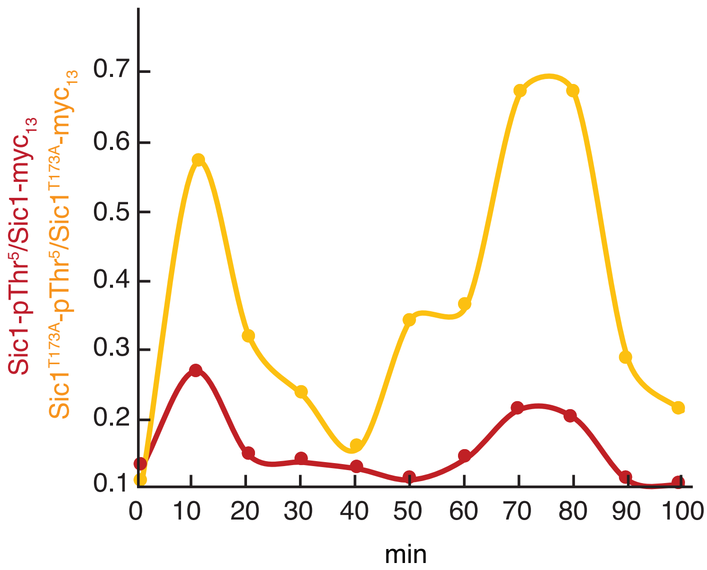

Figure S4 Proliferating, Sic1^{T173A}-expressing cells exhibit enhanced levels of Cln-/Clb5/6-CDK activity. For synchronization, exponentially growing WT cells expressing genomically-tagged Sic1-myc$_{13}$ or Sic1^{T173A}-myc$_{13}$ were treated for 2 h with α-factor (5 μg ml^{-1}). Following α-factor release, samples were collected at the indicated time points. The phosphorylation levels of Thr^5 in Sic1 and Sic1^{T173A} were determined by using phosphospecific anti-Sic1-pThr^5 antibodies and normalized with respect to the total levels (quantified by using anti-myc antibodies) of Sic1-myc$_{13}$ and Sic1^{T173A}-myc$_{13}$, respectively.

Supplementary Tables

Table S1. Strains used in this study

Strain	Genotype	Source	Figure
JK9-3D	*MAT***a**, *leu2, his4, trp1, ura3, rme1, GAL, HMLa*	Ref. (1)	2A/B, 3B, 2C, 4G, S1B/C, S3
RL343-E1	[JK9-3D] *his3*, *HIS4*, *cdc28*Δ, pRS416-*cdc28*^{as} (F88G)	Ref. (2)	4C
YMM114	[JK9-3D] *cdc4-2*^{ts}*::kanMX*	Ref. (3)	1A/C
YMM118-2D	[JK9-3D] *cdc4-2*^{ts}*::kanMX, sic1*^{T173A}*, EMP46::natMX*	Ref. (3)	1A/C
YMM67-1C	[JK9-3D] *sic1*Δ*::kanMX*	Ref. (3)	1A/B, S1A
YMM237-1A	[JK9-3D] *cdc4-2*^{ts}*::kanMX, cdc28*Δ*::kanMX,* pRS416-*cdc28*^{as}	This study	1B/D
YMM250-10C	[JK9-3D] *cdc4-2*^{ts}*::kanMX, cdc28*Δ*::kanMX,* pRS416-*cdc28*^{as}, *sic1*^{T173A}*, EMP46::natMX*	This study	1B/D
YMM246-1C	[JK9-3D] *cdc4-2*^{ts}*::kanMX, CLB5-HA*$_{3}$*::TRP1, CLN1-myc*$_{13}$*::kanMX*	This study	S2
YMM247-4B	[JK9-3D] *cdc4-2*^{ts}*::kanMX, CLB5-HA*$_{3}$*::TRP1, CLN1-myc*$_{13}$*::kanMX, sic1*^{T173A}*, EMP46::natMX*	This study	S2
YMM249-2B	[JK9-3D] *cdc4-2*^{ts}*::kanMX, CLB5-HA*$_{3}$*::TRP1, CLN2-myc*$_{13}$*::kanMX*	This study	S2
YMM252-2A	[JK9-3D] *cdc4-2*^{ts}*::kanMX, CLB5-HA*$_{3}$*::TRP1, CLN2-myc*$_{13}$*::kanMX, sic1*^{T173A}*, EMP46::natMX*	This study	S2
MJA4090	[JK9-3D] *cdc4-2*^{ts}*::kanMX, CLB5-HA*$_{3}$*::TRP1*	This study	S2
MJA4091	[JK9-3D] *cdc4-2*^{ts}*::kanMX, CLB5-HA*$_{3}$*::TRP1, sic1*^{T173A}*, EMP46::natMX*	This study	S2
YMM91	[JK9-3D] *sic1*^{T173A}*, EMP46::natMX*	Ref. (3)	2A/B, 3B,
MJA490	[JK9-3D] *CKS1-HA*$_{3}$*::kanMX*	This study	2D-F, 4A
MJA491	[JK9-3D] *CKS1-HA*$_{3}$*::kanMX, sic1*^{T173A}*, EMP46::natMX*	This study	2D-E, 4A
MJA524-2B	[JK9-3D] *sic1*$^{R262A/L264A}$*-myc*$_{13}$*::kanMX*	This study	3A
YMM63	[JK9-3D] *SIC1-myc*$_{13}$*::kanMX*	Ref. (3)	3A, 4B, S4
YMM98	[JK9-3D] *sic1*^{T173A}*-myc*$_{13}$*::kanMX, EMP46::natMX*	Ref. (3)	3A, S4
MJA523	[JK9-3D] *sic1*$^{R262A/L264A}$*, EMP46::natMX*	This study	3B
MJA536	[JK9-3D] *cln1Δ::kanMX, cln2Δ::kanMX*	This study	3B
MJA528	[JK9-3D] *sic1*$^{T173A/R262A/L264A}$*, EMP46::natMX*	This study	3B
YMM232-6A	[JK9-3D] *clb5Δ::kanMX, clb6Δ::kanMX, sic1*^{T173A}*, EMP46::natMX*	This study	3B
MJA544-2B	[JK9-3D] c*ln1Δ::kanMX, cln2Δ::kanMX, sic1*^{T173A}*, EMP46::natMX*	This study	3B
YMM253-11A	[JK9-3D] *clb5Δ::kanMX, clb6Δ::kanMX*	This study	3B
MJA547	[JK9-3D] *clb5Δ::kanMX, cln1Δ::kanMX, cln2Δ::kanMX, sic1*^{T173A}*, EMP46::natMX*	This study	3B
MJA545	[JK9-3D] *CKS1-HA*$_{3}$*::kanMX, CLB5-myc*$_{13}$*::kanMX, sic1*^{T173A}*, EMP46::natMX*	This study	3D
MJA546	[JK9-3D] *CKS1-HA*$_{3}$*::kanMX, CLB5-myc*$_{13}$*::kanMX*	This study	3D
MJA531	[JK9-3D] *CKS1-HA*$_{3}$*::kanMX, CLN2-myc*$_{13}$*::kanMX, sic1*^{T173A}*, EMP46::natMX*	This study	3E
MJA530	[JK9-3D] *CKS1-HA*$_{3}$*::kanMX, CLN2-myc*$_{13}$*::kanMX,*	This study	3E
YMM231-1A	[JK9-3D] *CKS1-HA*$_{3}$*::kanMX, mpk1Δ::kanMX*	This study	4A
YMM230-8A	[JK9-3D] *CKS1-HA*$_{3}$*::kanMX, cdc55Δ::natMX*	This study	4A
YMM233-3A	[JK9-3D] *CKS1-HA*$_{3}$*::kanMX, cdc55Δ::natMX, sic1*^{T173A}*, EMP46::natMX*	This study	4A
MJA518	[JK9-3D] *cdc28*Δ, *pRS416-cdc28*^{as}	This study	4C
MJA519	[JK9-3D] *cdc28*Δ, *pRS416-cdc28*^{as}*, rim15Δ::kanMX*	This study	4C/H
YMM58-1B	[JK9-3D] *rim15Δ::kanMX*	This study	4E/F
MM3D	[JK9-3D] *cdc28*Δ, pRS416-*cdc28*^{as}*, rim15Δ::kanMX, LEU2::CYC1p-HHF2-tDimer*	This study	4D

Table S2. Plasmids used in this study

Plasmid	Genotype	Source	Figure
pRS415	CEN, *LEU2*	Ref. (5)	
pMJA2881	[pRS415] *ADH1p-SIC1-myc*$_{13}$	This study	S1A
pMJA3173	[pRS415] *ADH1p-sic1*^{T5A}*-myc*$_{13}$	This study	S1A
pMJA3174	[pRS415] *ADH1p-sic1*^{T33A}*-myc*$_{13}$	This study	S1A
pMJA2995	[pRS415] *ADH1p-SIC1*$^{150-285}$*-myc*$_{13}$	This study	2F
pMJA2996	[pRS415] *ADH1p-sic1*$^{150-285 - T173A}$*-myc*$_{13}$	This study	2F
pRS416	CEN, *URA3*	Ref. (5)	S3
pMJA3038	[pRS416] *ADH1p-CLB5-HA*$_3$	This study	2B/C, 3A, 4F/G, S3, S1B/C, S3
YCplac33	*CEN, URA3*	Ref. (6)	
JCE456	[YCplac33] *ADH1p-CLN2-HA*$_3$	Ref. (7)	2A/C, 4D, 4F/G, S3
pAS2654	[YCplac33] *ADH1p-LST7-HA*$_3$	Ref. (8)	4F
pGEX	*GST*	Ref. (9)	4G, S1B/C
pMMT2629	[pGEX] *GST-SIC1*	This study	2C
pMMT2630	[pGEX] *GST-sic1*^{T173A}	This study	2C
pMJA3029	[pGEX] *GST-sic1*$^{R262A/L264A}$	This study	2C
pMJA3037	[pGEX] *GST-SIC1*$^{1-100}$	This study	2C, 3A, 4G, S1B/C
pMJA3219	[pGEX] *GST-sic1*$^{T5A(1-100)}$	This study	S1B/C
pMJA3220	[pGEX] *GST-sic1*$^{T33A(1-100)}$	This study	S1B/C
pVW995	[pGEX] *GST-RIM15*$^{944-1149}$	Ref. (10)	4G
pVW827	CEN, *LEU2, ADH1p-GST-RIM15*	Ref. (10)	4F
pVW904	2μ, *LEU2, TDH3p-RIM15-myc*$_{13}$	Ref. (10)	4H
pVW910	2μ, *LEU2, TDH3p-rim15* ^{T1075A}*-myc*$_{13}$	Ref. (10)	4H
pFD1008	CEN, *TRP1, ADH1p-rim15*^{K823Y}*-GFP*	Ref. (10)	4D/E

Table S3. Antibodies used in this study

Name	Dilution	Source
Anti-Sic1	1:1'000	sc-50441 Santa Cruz
Anti-Adh1	1:200'000	Calbiochem
Anti-Sic1-pThr^{173}	1:1'000	GenScript
Anti-Sic1-pThr^{5}	1:1'000	GenScript
Anti-Sic1-pThr^{33}	1:1'000	GenScript
Anti-myc	1:3'000	9E10; sc-40; Santa Cruz
Anti-HA	1:1'000	Enzo Life Sciences
Anti-GST	1:3'000	Bethyl Laboratories
Anti-Igo1-pSer^{64}	1:1'000	GenScript
Anti-Igo1	1:1'000	Eurogentec
Anti-Cln2	1:1'000	Santa Cruz
Anti-Cdc28	1:300	Santa Cruz
Anti-Clb5	1:1'000	Santa Cruz
Anti-Sch9-pThr^{737}	1:1'0000	GenScript
Anti-Sch9	1:1'000	GenScript
Anti-Rim15-pThr^{1075}	1:10'000	Eurogentec
Goat anti-rabbit IgG HRP	1:3'000	Biorad
Goat anti-mouse HRP	1:3'000	Biorad
Donkey anti-goat HRP	1:5'000	Abcam
Goat anti-mouse IgG-Fcy HRP	1:5'000	Jackson Immunoresearch
Goat anti-mouse IgG, light chain HRP	1:5'000	Jackson Immunoresearch

Supplementary References

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