Supplemental Materials

Molecular Biology of the Cell

Schilke and Craig

Supplementary Material

2 figures; 2 tables

Essentiality of Sis1, a J-domain protein Hsp70 cochaperone, can be overcome by Tti1, a specialized PIKK chaperone

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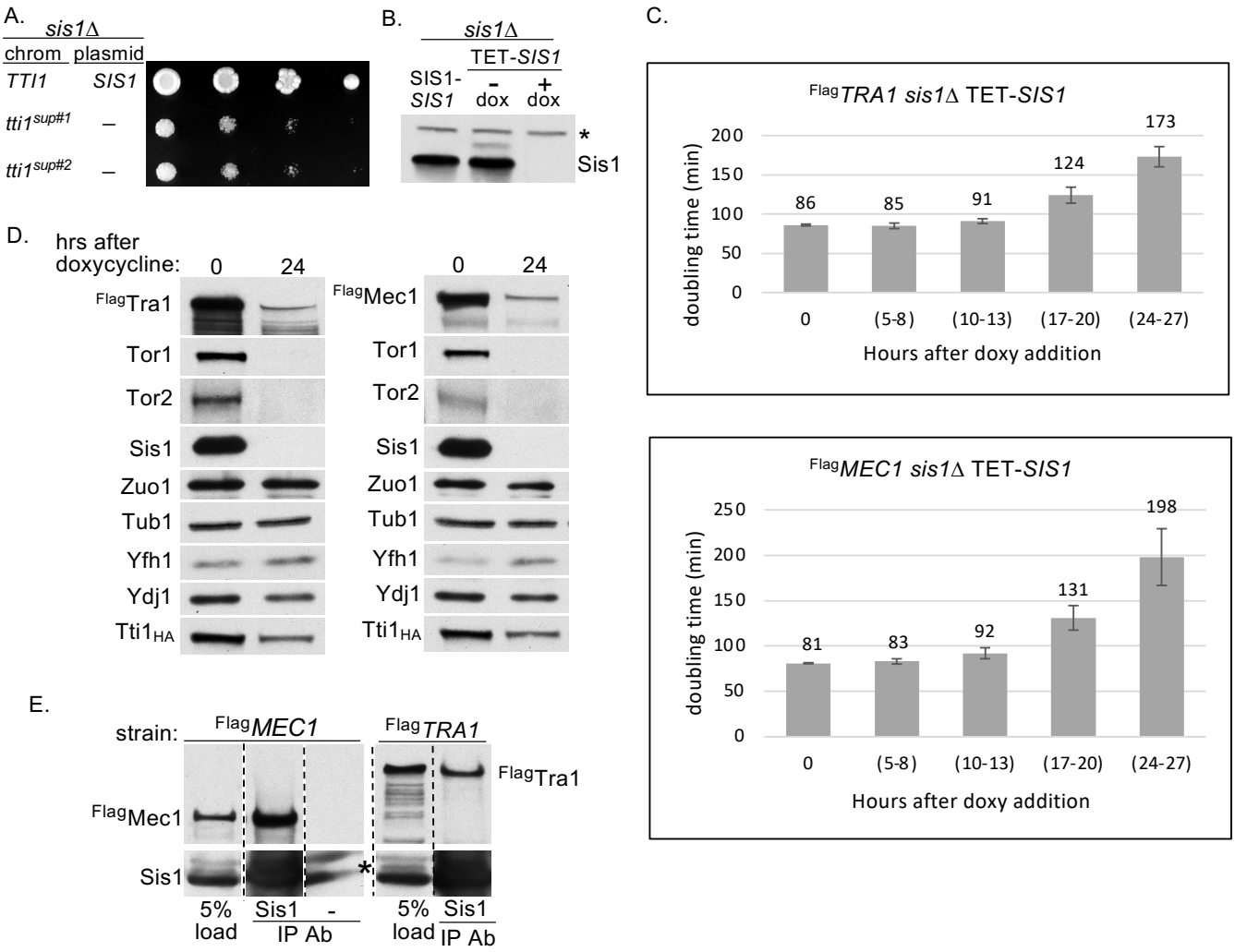
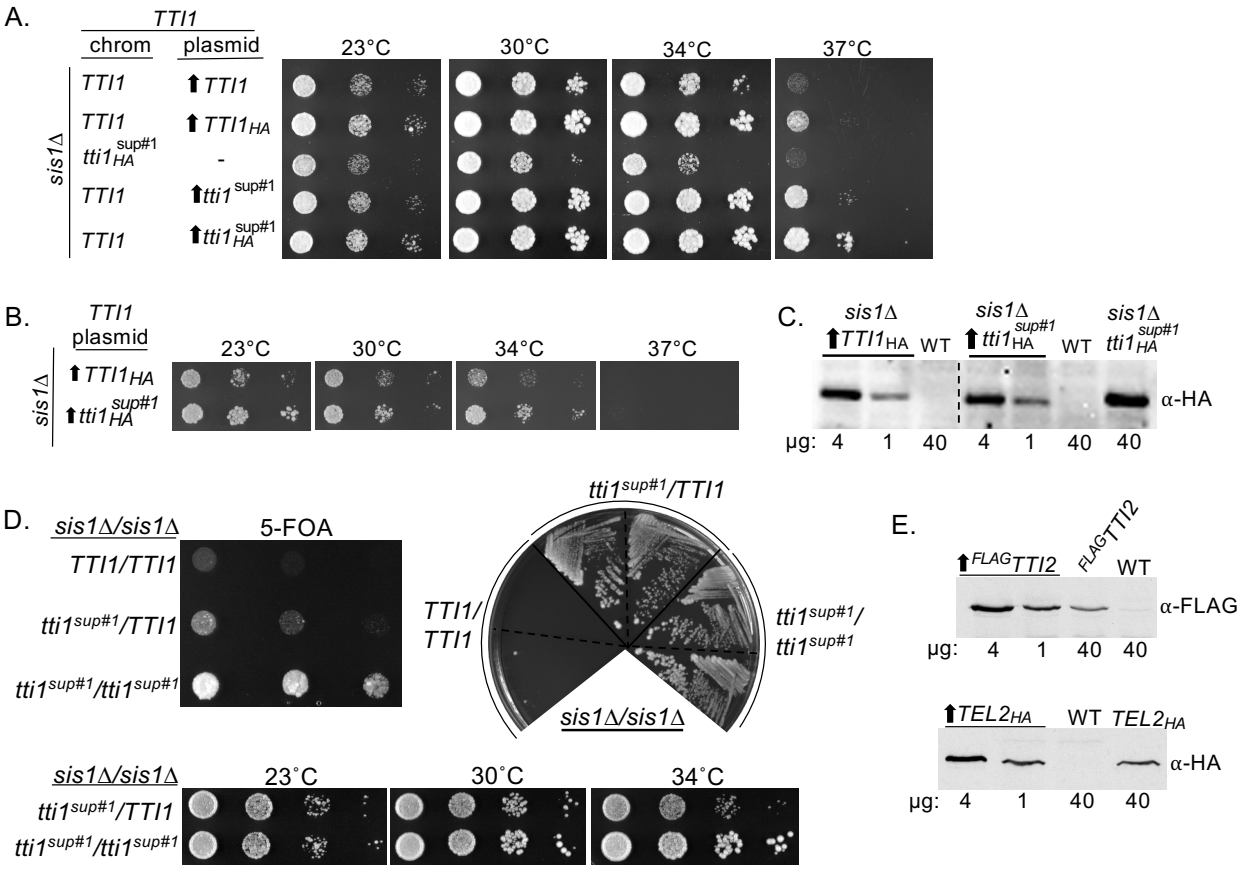


Supplementary Figure 1. Changes in cell growth and protein levels upon reduction of Sis1 expression. (A) 10X serial dilutions of *sis1Δ* cells, carrying either *SIS1* on a centromeric plasmid or containing the indicated variants on the chromosome, were spotted on Leu omission media and incubated for 4 days at 30°C. (B) Extracts of *sis1Δ* cells expressing *SIS1* from either the native promoter (SIS1-*SIS1*) or the doxycycline repressible promoter (TET-*SIS1*) incubated in the presence (+) or, as a control, absence (-) of doxycycline for 21 hours were subjected to electrophoresis and immunoblot analysis using anti Sis1 antibodies. The band indicated by the asterisk (*) is Ydj1, which reacts weakly with the antibody. (C) Doubling times of six *sis1Δ* strains having either FLAG-tagged Mec1 or Tra1 in the genome used for analysis of protein levels in Figure 2B. Numbers in parentheses indicated the time frame in hours after doxycycline addition that OD_{600} measurements were taken. (D) Doxycycline was added at time zero to log phase cultures of BY4741 *sis1Δ* having Sis1 expressed from the TET promoter. Cell lysates from the indicated strains were subjected to electrophoresis and immunoblot analysis using antibodies specific for the proteins indicated on the left. Cells had either Flag-tagged *TRA1* (^{Flag}Tra1) or *MEC1* (^{Flag}Mec1) in the chromosome, left and right, respectively, as well as HA-tagged Tti1 ($Tti1_{HA}$). (E) Cell lysates from strains having either chromosomal Flag tagged Tra1 or Mec1 were subjected to coimmunoprecipitation with purified polyclonal Sis1 antibody. Inputs (5%) and anti-Sis1 IPs were subjected to electrophoresis and immunoblot analysis with Flag or Sis1 antibodies. All samples were run on same gel with lanes irrelevant to the figure removed as indicated by dotted lines. Presence of Sis1 signal indicated by asterisk in no antibody control lane (-) is due to spill over from adjacent lane.



Supplementary Figure 2. **Effects of overexpression of Tti1, Tti2 and Tel2.** (A) 10X serial dilutions of *sis1Δ* cells expressing the indicated genes either expressed from the chromosome (chrom) from their native promoter or overexpressed from a centromeric plasmid under the control of the strong *ADH1* promoter (indicated by upward arrow), with presence of an HA tag indicated, were plated on Trp omission media and incubated for 4 days at the indicated temperatures. (B) 10X serial dilutions of *sis1Δ* cells of the BY4741 genetic background and harboring centromeric plasmids expressing either HA-tagged WT Tti1 or Tti1$^{sup\#1}$ under the control of the *ADH1* promoter (indicated by upward arrow) were plated on Trp omission media. Plates were incubated for 5 days at 23°C, or 3 days at 30°C, 34°C or 37°C. (C) The indicated amounts of extracts (μg) from *sis1Δ* cells expressing either HA-tagged *TTI1* or *tti1*$^{sup\#1}$ from a plasmid driven by the *ADH1* promoter (indicated by upward arrow), or having *tti1*$^{sup\#1}$ in the chromosome under its native promoter or, as a control, WT cells, were subjected to electrophoresis and immunoblot analysis using HA-specific antibodies. All samples were run on the same gel; dotted line indicates removal of irrelevant lanes. (D) Testing of recessive/ dominance of *tti1*$^{sup\#1}$. Top, left: Starting with 1 OD_{600} cultures, 1:4 serial dilutions of diploid strains homozygous for *sis1Δ* carrying *SIS1* on a URA3-based plasmid and homozygous or heterozygous for *TTI1* or *tti1*$^{sup\#1}$ were spotted onto plates containing 5-FOA and incubated at 30˚C for 4 days. Top, right: Patches were then streaked onto rich media and incubated at 30°C for 5 days. Bottom: 10 fold serial dilutions of suspensions of individual colonies from the plate shown at top, right were plated on rich media and incubated at indicated temperatures for 4 days. (E) The indicated amounts of extracts (μg) from cells having indicated FLAG-tagged *TTI2* (^{FLAG}TTI2) or HA-tagged *TEL2* (TEL2$_{HA}$) gene in the chromosome or expression driven by the *ADH1* promoter from a plasmid (indicated by upward arrow), or as a control WT cells, were subjected to electrophoresis and immunoblot analysis using antibodies specific for FLAG (left) or HA (right) antibodies.

Supplementary Table 1. Yeast Strains used in this study

Strain	Description	Reference
PJ51-3A	MATa *trp1-1 ura3-1 leu2-3,112 his3-11,15 ade2- 1 can1-100 GAL2+ met2-Δ1 lys2-Δ2.*	(James *et al.*, 1997)
WY26 *(sis1-Δ)*	α *trp1-1 ura3-1 leu2-3,112 his3-11,15 ade2- 1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 sis1Δ*::*LEU2* with *URA3* marked pYCp50-*SIS1.*	(Yan and Craig, 1999)
sis1-Δ tti1^{T598R}	Isogenic to WY26 except *tti1*^{T598R} and without plasmid	This study
sis1-Δ tti1^{T598R}-HA	Isogenic to *sis1-Δ tti1*^{T598R} except with 3x-HA tag:*HIS3* on C-term of *tti1*^{T598R}.	This study
sis1-Δ tti1^{G858V}	Isogenic to WY26 except *tti1*^{G858V} and without plasmid.	This study
sis1-Δ TTI1-HA	Isogenic to WY26 except with 3x-HA tag:*HIS3* on C-term of *TTI1.*	This study
TEL2-HA	Isogenic to PJ51-3A except with 3x-HA tag:*HIS3* on C-term of *TEL2.*	This study
Flag^{3}-*TTI2*	Isogenic to PJ51-3A except with 3x-Flag tag on N-term of *TTI2.*	This study
sis1-Δ TEL2-HA	Isogenic to WY26 except with 3x-HA tag:*HIS3* on C-term of *TEL2.*	This study
sis1-Δ Flag^{3}-*TTI2*	Isogenic to WY26 except with 3x-Flag tag on N-term *TTI2.*	This study
TEL2-HA Flag^{3}-*TTI2*	Isogenic to PJ51-3A except for 3x-HA tag:*HIS3* on C-term of *TEL2* and 3x-Flag tag on N-term of *TTI2.*	This study
BY4743*sis1-Δ*	MATa/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0 trp1::Kn/trp1::Kn sis1-Δ::LEU2/SIS1.*	This study
BY*sis1-Δ*	*his3Δ1 leu2Δ0 lys2? met15? ura3Δ0 trp1::Kn sis1-Δ::LEU2* carrying *TRP1* marked pTETrSIS1.	This study
BY4743*trp1-Δ*	MATa/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0 trp1::Kn/trp1::Kn.*	(Winzeler *et al.*, 1999)
CY6808	*his3Δ1leu2Δ0 lys2Δmet15Δura3Δ0 URA3*-Flag^{5}-*TRA1*	(Berg *et al.*, 2018)
BY*sis1-Δ* Flag^{5}-*TRA1*	*his3Δ1leu2Δ0 lys2Δmet15Δura3Δ0 trp1::Kn sis1-Δ::LEU URA3*-Flag^{5}-*TRA1* carrying pTETr*SIS1.*	This study
BY*sis1-Δ* Flag^{5}-*MEC1*	*his3Δ1 leu2Δ0 lys2? met15? ura3Δ0 trp1::Kn sis1-Δ::LEU2* URA3-Flag^{5}-*MEC1* carrying pTETr*SIS1.*	This study
BY*sis1-Δ* Fl-T *TTI1*-HA	Isogenic to BY*sis1-Δ* Flag-*TRA1* except for 3x-HA tag:*HIS3* on C-term of *TTI1* and plasmid TETr*SIS1.*	This study
BY*sis1-Δ*Fl-T *tti1*^{T598R}-HA	Isogenic to BY*sis1-Δ* Flag-*TRA1* except for 3x-HA tag:*HIS3* on C-term of *tti1*^{T598R} and plasmid TETr*SIS1.*	This study
BY*sis1-Δ* Fl-M *TTI1*-HA	Isogenic to BY*sis1-Δ* Flag-*MEC1* except for 3x-HA tag:*HIS3* on C-term of *TTI1* and plasmid TETr*SIS1.*	This study
BY*sis1-Δ*Fl-M *tti1*^{T598R}-HA	Isogenic to BY*sis1-Δ* Flag-*MEC1* except for 3x-HA tag:*HIS3* on C-term of *tti1*^{T598R} and plasmid TETr*SIS1.*	This study

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Supplementary Table 2. Plasmids used in this study

Plasmid	Description	Reference
Fa6 3xHA:*HIS3*MX6	Plasmid to amplify 3XHA:*HIS3* for directed integration of 3' HA tag into yeast genome.	(Longtine *et al.*, 1998)
pXIPHOS+TTI2	CRISPR plasmid (Cas9/sgRNA) used for tagging N-term of *TTI*2	This study
pYCp50-*SIS1*	pYCP50 (*URA3*) carrying *SIS1* under control of native promoter.	(Yan and Craig, 1999)
pYW65	pRS314 (*TRP1*) carrying *SIS1* under control of native promoter.	(Yan and Craig, 1999)
TETr*SIS1*	pCM184 (*TRP1*) carrying *SIS1* under control of TET regulated promoter.	(Aron *et al.*, 2007)
pRS314-*ydj1*^{G70N}	Ydj1 variant that rescues *sis1-Δ* cells	(Schilke *et al.*, 2017)
p414ADH	Yeast CEN vector (*TRP1*) for cloning open reading frames under ADH promoter followed by a CYC1 transcription terminator.	(Mumberg *et al.*, 1995)
p414ADH-*TTI1*	p414ADH carrying *TTI1* under the ADH promoter.	This study
p414ADH-*TTI1*-HA	p414ADH-*TTI1* with C-term 3x-HA tag.	This study
p414ADH-*tti1*^{T598R}	p414ADH-*TTI1* with point mutation T598R.	This study
p414ADH-*tti1*^{T598R}-HA	p414ADH-*tti1*^{T598R} with C-term 3x-HA tag.	This study
p414ADH-*TEL2*	p414ADH carrying *TEL2* under the ADH promoter.	This study
p414ADH-*TEL2*-HA	p414ADH-*TEL2* with C-term 3x-HA tag.	This study
p414ADH-*TTI2*	p414ADH carrying *TTI2* under the ADH promoter.	This study
p414ADH-Flag-*TTI2*	p414ADH-*TTI2* with N-term 3x-Flag tag.	This study
CB2363	Plasmid containing template for targeting integration of *URA3*-Flag^{5}*MEC1* at N-term.	(Genereaux *et al.*, 2012)
pURA3-4XHSE-Cyc1-GFP	4X consensus HSE cloned upstream of crippled Cyc1 promoter which drives GFP expression.	(Brandman *et al.*, 2012)
p4XHSE-Cyc1-GFP	pRS313 (*HIS3*) carrying 4xHSE-CYC1-GFP	This study
pA4HSE-Cyc1-GFP	pRS313 (*HIS3*) carrying A4HSE-CYC1-GFP	This study
pA3HSE-Cyc1-GFP	pRS313 (*HIS3*) carrying A3HSE-CYC1-GFP	This study
pSis1HSE-Cyc1-GFP	pRS313 (*HIS3*) carrying SIS1HSE-CYC1-GFP	This study

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