Supplemental material

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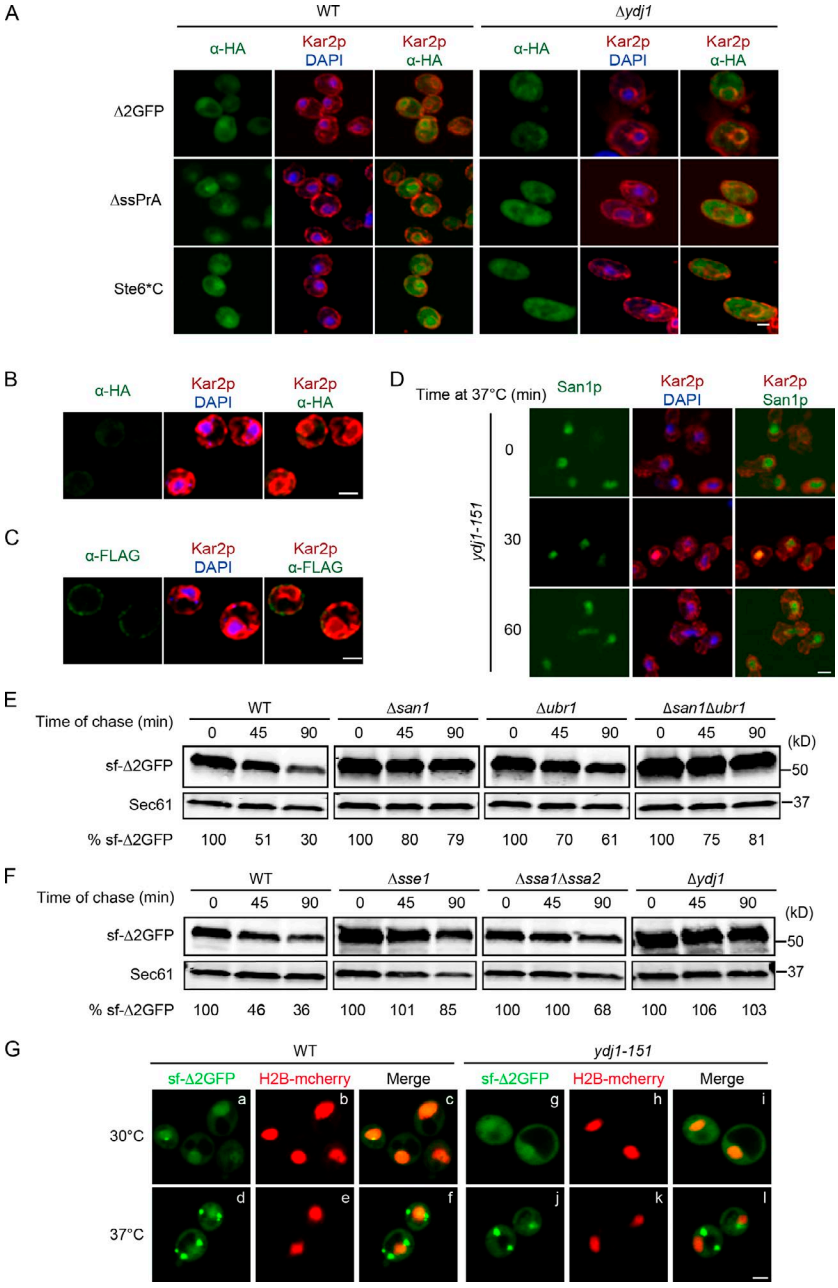

Figure S1. **Substrate imaging and dynamics. (A)** Intracellular localization of Δ2GFP, ΔssPrA, and Ste6*C in WT and *Δydj1* cells. WT and Δ*ydj1*cells expressing Δ2GFP, ΔssPrA, or Ste6*C were grown to log phase at 30°C and prepared for immunostaining as described in Fig. 2. Δ2GFP, ΔssPrA, or Ste6*C were detected using anti-HA antibody (green channel), ER/nuclear envelope was visualized by anti-Kar2 antibody (red channel), and nuclei were marked by DAPI staining (purple channel). **(B and C)** WT cells expressing empty vector were probed with anti-HA (B) and anti-FLAG (C) antibodies (green channel). ER membranes were visualized using Kar2 antibody (red channel), and nuclei were labeled by DAPI staining (purple channel). **(D)** Intracellular localization of San1 in *ydj1-151* cells. *ydj1-151* cells expressing San1-V5 were grown at room temperature and shifted to 37°C for as time indicated. After fixation, cells were subjected to immunostaining as described in Fig. 2. Cellular localization of San1 was visualized by anti-V5 antibody staining (green channel). ER membranes were visualized using Kar2 antibody (red channel), and nuclei were labeled by DAPI staining (purple channel). **(E and F)** sf-Δ2GFP is a CytoQC substrate. Cycloheximide decay experiments were performed in WT, Δ*san1*, Δ*ubr1*, Δ*san1*Δ*ubr1*, Δ*sse1*, Δ*ssa1*Δ*ssa2*, and Δ*ydj1* cells expressing sf-Δ2GFP. Cycloheximide was added to 200 µg/ml to initiate the chase, and cells were collected at the times indicated. Total protein extracts were prepared by TCA precipitation, and a portion of each lysate was resolved by SDS-PAGE. Quantitative immunoblotting was performed to determine the protein level over the chase. **(G)** Localization of sf-2GFP in WT and *ydj1-151* cells was determined by confocal microscopy at the indicated temperature. HTB2-mCherry marks the position of nuclei. Bars, 2 µm.

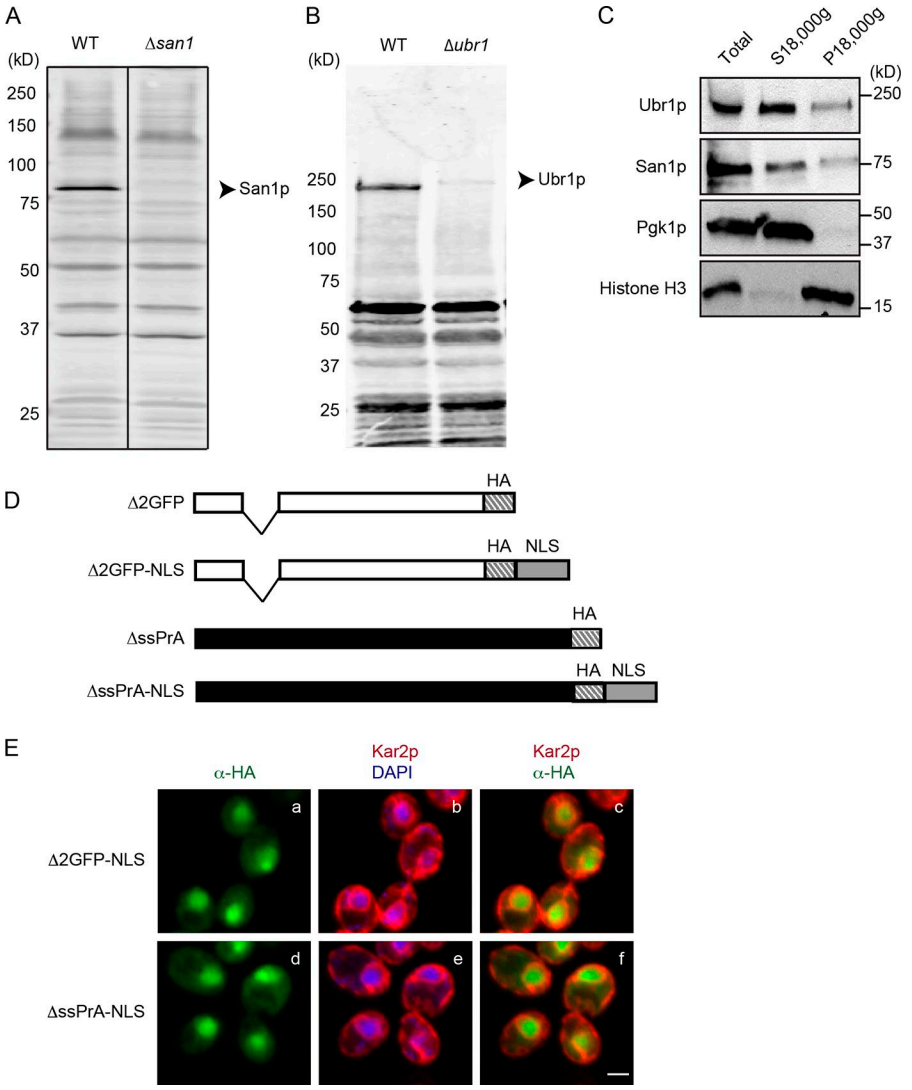

Figure S2. **Characterization of Ubr1 and San1 localization by conventional subcellular fractionation and schematic representation of NLS-tagged misfolded proteins along with their localization in WT cells. (A and B)** Total protein extract prepared from WT, Δsan1, and Δubr1 cells and analyzed by immunoblotting with anti-San1 and anti-Ubr1 antibodies. The black line indicates that intervening lanes have been spliced out. **(C)** WT cells were disrupted by vortexing using zirconium beads, and the lysates were fractionated by differential centrifugation. The unbroken cells were removed by low-speed centrifugation at 300 *g* for 5 min. The supernatant was saved as total fraction and subjected to a subsequent high-speed centrifugation at 18,000 *g* to separate membrane fraction (designated as P18,000 *g*) and cytosol (designated as S18,000 *g*). Equal portions of each fraction were analyzed by immunoblotting with various antibodies. Antibodies against Ubr1, San1 (nuclear protein), Pgk1 (cytosolic protein), and histone H3 (nuclear protein) were used. **(D)** Schematic representation of NLS-tagged misfolded proteins. **(E)** Cellular localization of Δ2GFP-NLS or ΔssPrA-NLS in WT cells was examined by immunostaining. Δ2GFP-NLS and ΔssPrA-NLS were stained using anti-HA antibody (green channel). ER membranes were labeled using anti-Kar2 antibody (red channel), and nuclei were marked by DAPI staining (purple channel). Bar, 2 µm.

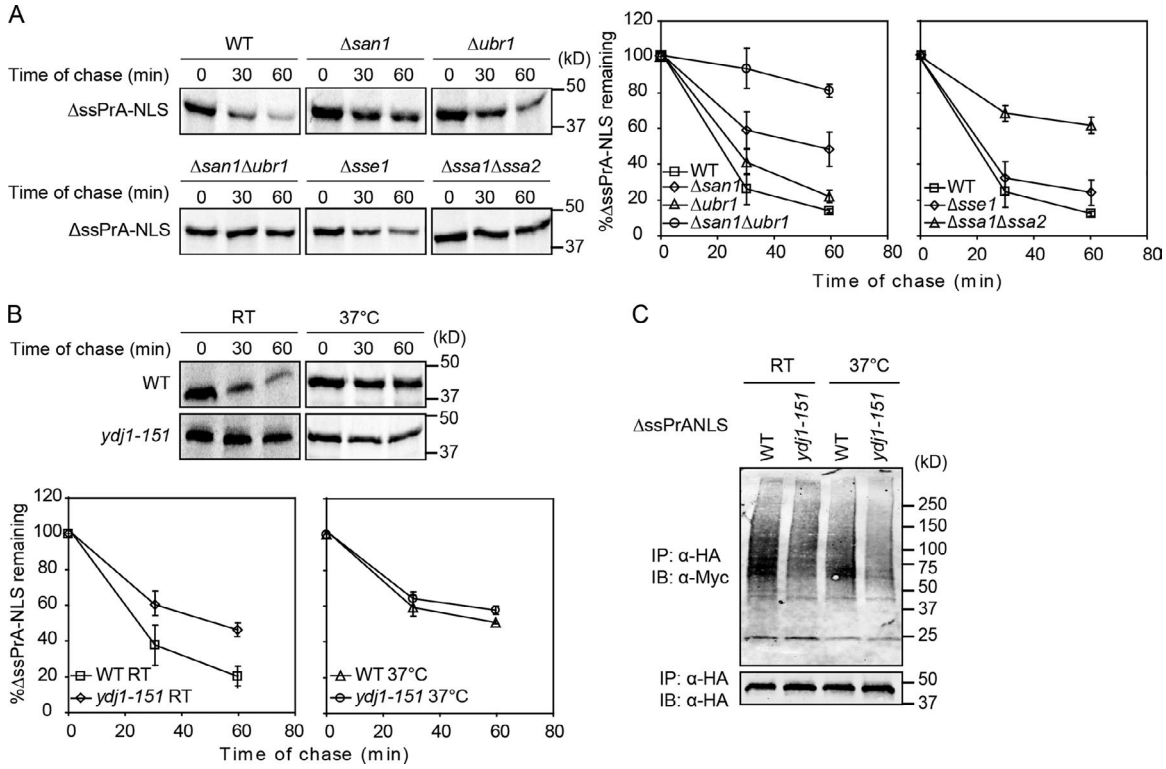

Figure S3. **Analysis of ΔssPrA-NLS degradation dependency. (A and B)** Turnover of ΔssPrA-NLS in WT, Δ*san1*, Δ*ubr1*, Δ*san1Δubr1*, Δ*ssa1Δssa2*, Δ*sse1*, and *ydj1-151* was determined by pulse–chase analysis as described in in Fig. 5 (A and B). All data plotted were processed using Excel, reflecting three independent experiments with means and SD indicated. **(C)** Ubiquitination of ΔssPrA-NLS in WT or *ydj1-151* cells was examined as described in Fig. 1 B. IB, immunoblot; IP, immunoprecipitation.

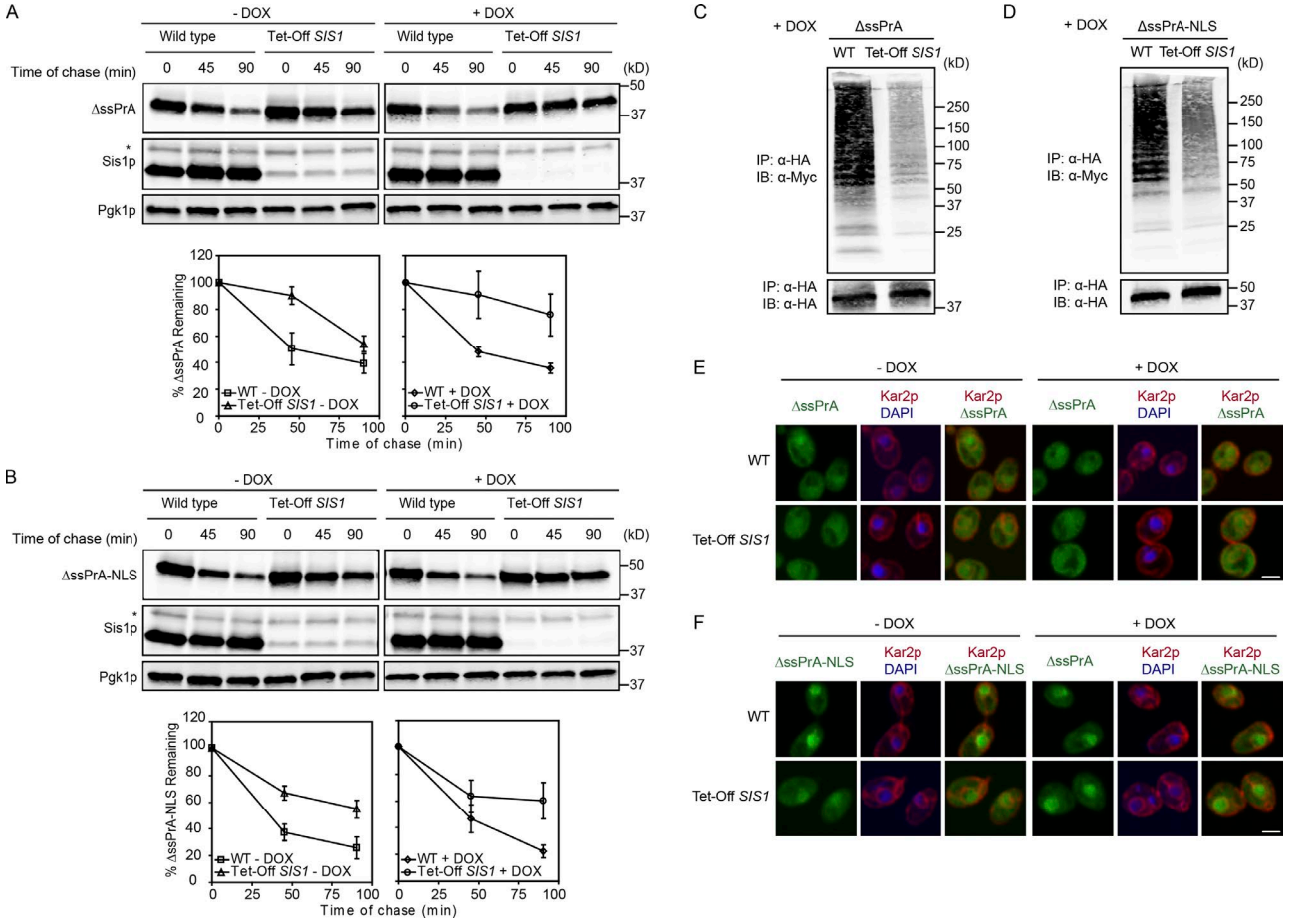

Figure S4. **Sis1 is required for CytoQC and nuclear QC but dispensable for ΔssPrA nuclear import. (A and B)** Cycloheximide decay experiments were performed in WT cells (R1158) and Tet-Off *SIS1* cells expressing ΔssPrA or ΔssPrA-NLS in the absence and presence of doxycycline (DOX; 10 µg/ml) as described in Fig. 6 A. All data plotted were processed using Excel, reflecting three independent experiments with means and SD indicated. **(C and D)** The ubiquitination of ΔssPrA or ΔssPrA-NLS in WT and Tet-Off *SIS1* cells was examined as described in Fig. 6 C. IB, immunoblot; IP, immunoprecipitation. **(E and F)** Intracellular localization of ΔssPrA or ΔssPrA-NLS in WT and Tet-Off *SIS1* cells was determined by indirect immunostaining as described in Fig. 6 E. Asterisks indicate the position of a nonspecific band. Bars, 2 µm.

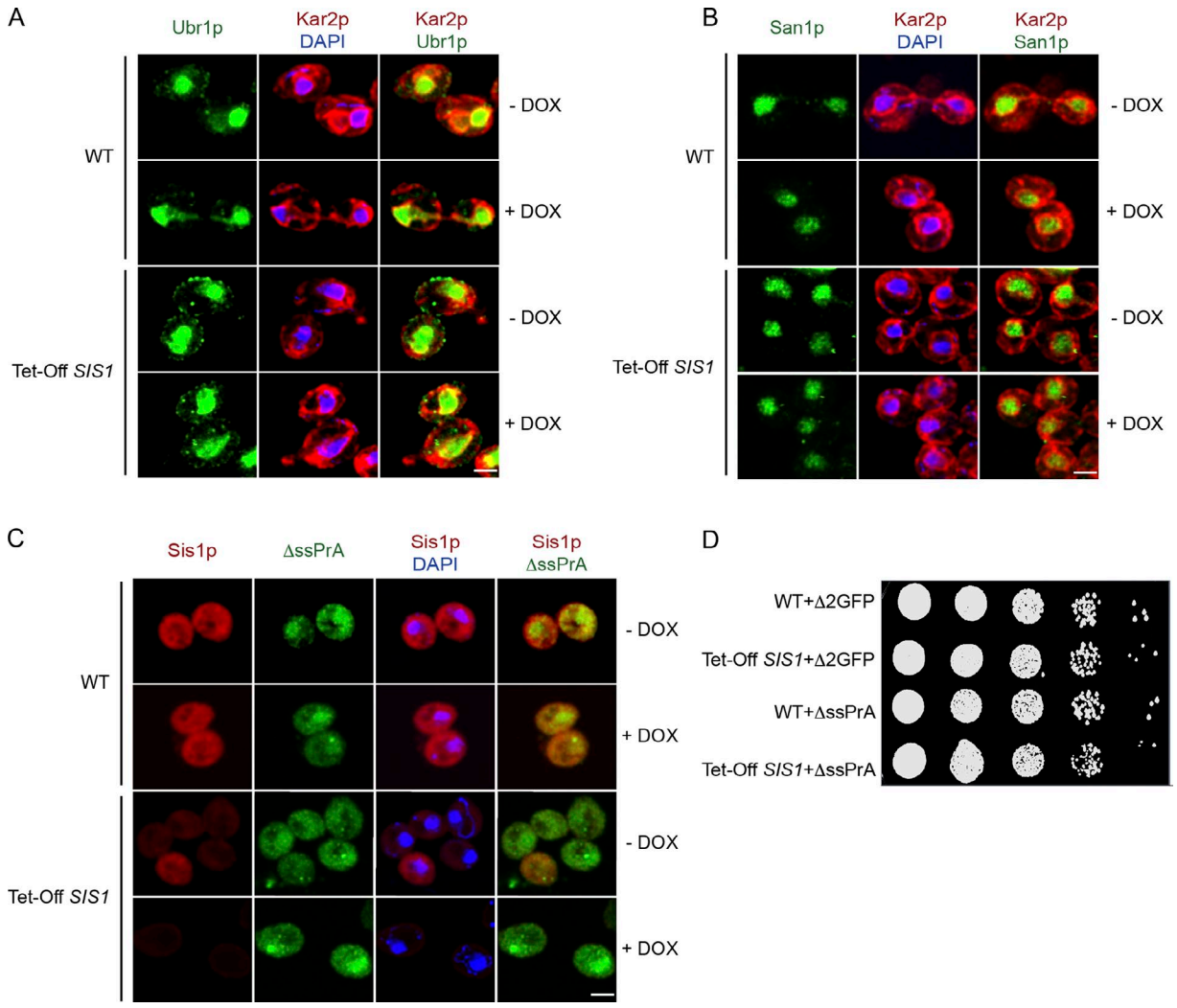

Figure S5. **Localization of Ubr1 and San1 is intact in Tet-OFF *SIS1* strains. (A–C)** Intracellular localization of Ubr1, San1, and Sis1 in WT and Tet-Off *SIS1* cells was determined by indirect immunostaining as described in Fig. 6 E. Bars, 2 µm. **(D)** Equal concentrations of WT and Tet-Off *SIS1* cells were serially diluted 10-fold and spotted onto Synthetic Complete plates and incubated at 30°C for ∼2 d until colonies were formed.

Table S1. **Strains used in this study**

Strain	Genotype	Source
W303	*MATa, leu2-3,112, his3-11, trp1-1, ura3-1, can1-100, ade2-1*	P. Walter
ACY17b	*MATα, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151,* W303 background	Caplan et al. (1992)
R1158	*MATa,URA3::CMV-tTA, his3-1 leu2-0 met15-0,* BY4741 background	Open Biosystem
TH_7868 (Tet- OFF–SIS1)	*MATa,URA3::CMV-tTA, his3-1 leu2-0 met15-0, R1158 pSIS1::KanRTetO7TATA,* BY4741 background	Open Biosystem
RPY145	*MATa, pRP22,* W303 background	Prasad et al. (2012)
RPY205	*MATa, pRP42,* W303 background	Prasad et al. (2010)
RPY206	*MATa, pRP44,* W303 background	Prasad et al. (2012)
RPY301	*MATa, ydj1::KANMX, pRP42,* W303 background	Prasad et al. (2010)
RPY302	*MATa, ydj1::KANMX, pRP44,* W303 background	Prasad et al. (2010)
RPY368	*MATa, ydj1::KANMX, pRP22,* W303 background	Prasad et al. (2012)
RPY450	*MATa, pRP88,* W303 background	This study
RPY451	*MATa, pRP86,* W303 background	This study
RPY585	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151,* W303 background	This study
RPY589	*MATa, pSK146*, W303 background	This study
RPY596	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pSK146,* W303 background	This study
RPY655	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pRP86,* W303 background	This study
RPY656	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pRP88,* W303 background	This study
RPY657	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pRP92,* W303 background	This study
RPY658	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pRP93,* W303 background	This study
RPY667	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, pRP44,* BY4741 background	This study
RPY668	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, R1158 pSIS1::KanRTetO7TATA, pRP44,* BY4741 background	This study
RPY669	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, pRP42,* BY4741 background	This study
RPY670	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, R1158 pSIS1::KanRTetO7TATA, pRP42,* BY4741 background	This study
RPY671	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, pRP90,* BY4741 background	This study
RPY672	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, R1158 pSIS1::KanRTetO7TATA, pRP90,* BY4741 background	This study
RPY673	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, pRP91,* BY4741 background	This study
RPY674	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, R1158 pSIS1::KanRTetO7TATA, pRP91,* BY4741 background	This study
RPY675	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, pRP44* and *pRP84,* BY4741 background	This study
RPY676	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, R1158 pSIS1::KanRTetO7TATA, pRP44* and *pRP84,* BY4741 background	This study
RPY677	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, pRP42* and *pRP84,* BY4741 background	This study
RPY678	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, R1158 pSIS1::KanRTetO7TATA, pRP42* and *pRP84,* BY4741 background	This study
RPY679	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, pRP90* and *pRP84,* BY4741 background	This study
RPY680	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, R1158 pSIS1::KanRTetO7TATA, pRP90* and *pRP84,* BY4741 background	This study
RPY681	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, pRP91* and *pRP84,* BY4741 background	This study

Strain	Genotype	Source
RPY682	*MATa, URA3::CMV-tTA, his3-1 leu2-0 met15-0, R1158 pSIS1::KanRTetO7TATA*, pRP91 and *pRP84*, BY4741 background	This study
RPY683	*MATa, pRP120*, W303 background	This study
RPY684	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pRP120*, W303 background	This study
RPY685	*MATa, pRP120* and *YEp105*, W303 background	This study
RPY686	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pRP120* and *YEp105*, W303 background	This study
RPY688	*MATa, pRP92*, W303 background	This study
RPY689	*MATa, ssa1::KANMX, ssa2::KANMX, pRP92*, W303 background	This study
RPY690	*MATa, pRP93*, W303 background	This study
RPY691	*MATa, ssa1::KANMX, ssa2::KANMX, pRP93*, W303 background	
RPY692	*MATa, pRP92* and *YEp105*, W303 background	This study
RPY693	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pRP92* and *YEp105*, W303 background	This study
RPY694	*MATa, pRP93* and *YEp105*, W303 background	This study
RPY695	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pRP93* and *YEp105*, W303 background	This study
RPY696	*MATa, pRP94*, W303 background	This study
RPY699	*MATa*, pRP86 and YEp105, W303 background	This study
RPY700	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151*, pRP86 and YEp105, W303 background	This study
RPY701	*MATa, pRP88* and *YEp105*, W303 background	This study
RPY702	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pRP88* and *YEp105*, W303 background	This study
RPY703	*MATa, pRP97* and *pRP103*, W303 background	This study
RPY704	*MATa, ade2-1, his3-1,1 leu2-3,112 ura3-1, trp1-,1 can 1-100, ydj1-2::HIS3, LEU2::ydj1-151, pRP97* and *pRP103*, W303 background	This study
RPY706	*MATa, san1::KANMX, pRP92*, W303 background	This study
RPY707	*MATa, ubr1::KANMX, pRP92*, W303 background	This study
RPY708	*MATa, san1::KANMX, ubr1::KANMX, pRP92*, W303 background	This study
RPY709	*MATa, sse1::KANMX, pRP92, W303 background*	This study
RPY710	*MATa, san1::KANMX, pRP93*, W303 background	This study
RPY711	*MATa, ubr1::KANMX, pRP93*, W303 background	This study
RPY712	*MATa, san1::KANMX, ubr1::KANMX, pRP93*, W303 background	This study
RPY713	*MATa, sse1::KANMX, pRP93, W303 background*	This study
RPY714	*MATa, pRP61* and *pKW431*, W303 background	This study
RPY718	*MATa, pRP96*, W303 background	This study
RPY719	*MATa, san1::KANMX, pRP96*, W303 background	This study
RPY720	*MATa, ubr1::KANMX, pRP96*, W303 background	This study
RPY721	*MATa, san1::KANMX, ubr1::KANMX, pRP96*, W303 background	This study
RPY722	*MATa, sse1::KANMX, pRP96, W303 background*	This study
RPY723	*MATa, ssa1::KANMX, ssa2::KANMX, pRP96*, W303 background	This study
RPY724	*MATa, ydj1::KANMX, pRP96, W303 background*	This study
CXY351	*MATa, sse1::KANMX, pRP97, pRP103 W303 background*	This study

Table S2. **Plasmids used in this study**

Plasmid	Encoded protein	Promoter	Vector	Source
pRP22	Ste6*C-HA	*TDH3*	pRS313*	Ng Laboratory plasmid collection
pRP42	ΔssPrA-HA	*TDH3*	pRS313*	Ng Laboratory plasmid collection
pRP42	Δ2GFP-HA	*TDH3*	pRS313*	Ng Laboratory plasmid collection
pRP61	GFP	*TDH3*	pRS313*	Ng Laboratory
pRP86	Δ2GFP-HA	*TDH3*	pRS316*	This study
pRP88	ΔssPrA-HA	*TDH3*	pRS316*	This study
pRP90	Δ2GFP-HA-NLS	*TDH3*	pRS313*	This study
pRP91	ΔssPrA-HA-NLS	*TDH3*	pRS313*	This study
pRP92	Δ2GFP-HA-NLS	*TDH3*	pRS316*	This study
pRP93	ΔssPrA-HA-NLS	*TDH3*	pRS316*	This study
pRP94	FLAG-Ubr1	*ADH1*	Yeplac181	A. Varshavsky (Du et al., 2002)
pRP96	Sf-Δ2GFP-HA	*TDH3*	pRS313*	This study
pRP97	Sf-Δ2GFP-HA	*TDH3*	pRS314*	This study
pRP103	*HTB2-mcherry*	*TDH3*	pRS316*	Ng Laboratory plasmid collection
YEp105	Myc-Ub	*CUP1*	YEp105 (2μ Trp)	M. Hochstrasser (Hochstrasser et al., 1991)
pRP120	Ste6*C-HA	*TDH3*	pRS316*	This study
pSK146	San1-V5H6	*GAL*	pTS210	Ng Laboratory plasmid collection
pAC376	Myc-Ub	*CUP1*	YEp351 (2μ Leu)	Chellappa et al. (2001)
pKW431	NLS-GFP-P12	*ADH1*	pRS426	K. Weis (University of California, Berkeley, Berkeley, CA)

Table S3. **Oligonucleotide primers used in this study**

Primer	Construct	Sequence (5′–3′)
RP143	pRP103	CGGGATCCCGATGTCCTCTGCCGCCGAAAA
RP145	pRP103	GCTCTAGAGCCTACTTGTACAGCTCGTCCATGCCG
RP163	pRP90	CCCATATGATGTTCCAGATTACGCTTCACCAAAGAAGAAGCGTAAGGTAGAAGCATCAGGATCATAAGCTCTAGATAATCTCTGCTTTT
RP164	pRP91	CCCATATGATGTTCCAGATTACGCTTCACCAAAGAAGAAGCGTAAGGTAGAAGCATCAGGATCATGATCCCCCCTAGATAATCTCTGCT

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