Table S1. Strains Used in This Study, Related to Experimental Procedures

Strain number	Strain name	Genotype	Source
	W303-1a	*MATa ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100*	R. Rothstein
	W303 diploid	*MATa/MATalpha ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100*	R. Rothstein
RJD1144	*Pre1-Flag-His*	*MATa his3Δ200 leu2-3,112 lys2-801 trp1Δ63 ura3-52 PRE1^{FH}::Ylplac211 (URA3)*	(Verma et al., 2000)
JSY1175	*Ubp2-His-TEV-9xMyc*	*W303 MATa ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100, Ubp2-6xHis-2xTEV-9xMyc::TRP, FLAG-Rsp5 ::URA*	This study
JSY568	*Δdef1 ::URA3*	*W303 MATa leu2-3,112 his3-11,15 trp1-1 ade2-1 can1-100 Δdef1::URA3*	(Woudstra et al., 2002)
JSY645	*Δdef1::TRP1*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100 Δdef1::TRP1*	(Woudstra et al., 2002)
JSY1176	*def1 1-100 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-100}$-3HA::HIS3*	This study
JSY1177	*def1 1-200 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-200}$-3HA::HIS3*	This study
JSY1178	*def1 1-300 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-300}$-3HA::HIS3*	This study
JSY1179	*def1 1-600 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-600}$-3HA::HIS3*	This study
JSY1180	*def1 1-700 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-700}$-3HA::HIS3*	This study
JSY1181	*def1 1-738 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, DEF1-3HA::HIS*	This study
JSY1182	*DEF1/def1$_{1-500}$-HA*	*MATa/MATalpha ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100, DEF1/def1$_1$-500-3HA::HIS3*	This study
JSY1183	*def1 1-530 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-530}$-3HA::HIS3*	This study
JSY1184	*def1 1-540 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-540}$-3HA::HIS3*	This study
JSY1185	*def1 1-550 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-550}$3HA::HIS3*	This study
JSY1186	*def1 1-560 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-560}$-3HA::HIS3*	This study
JSY1187	*def1 1-570 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-570}$-3HA::HIS3*	This study
JSY1188	*def1 1-580 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-580}$-3HA::HIS3*	This study
JSY1189	*def1 1-590 HA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100, def1$_{1-590}$-3HA::HIS3*	This study
JSY1190	*Def1 WT*	As JSY 568, but with *DEF1* knock-in (KI) at genomic *DEF1* locus	This study
JSY1191	*Def1-TEV*	As JSY568, but with *def1-TEV522* KI at genomic *DEF1* locus	This study
JSY1192	*rsp5-1*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100 rsp5 I733S::pRS303*	(Harreman et al., 2009)
GAC202	*GAC202*	*Matα his3-11 leu2-3,112 ura3-52 lys2-801 trp1-1 pdr5Δ::KanMX6 pre3-Δ2::HIS3 pup1Δ::leu2-HIS3 [pRS317-pup1-T30A] [YCplac22-pre3-T20A] gal¯*	(Collins et al., 2010)
GAC201	*GAC201*	*Matα his3-11 leu2-3,112 ura3-52 lys2-801 trp1-1 pdr5Δ::KanMX6 pre3-Δ2::HIS3 pup1Δ::leu2-HIS3 [pRS317-PUP1] [YCplac22-PRE3] gal¯*	(Collins et al., 2010)
JSY1193	*4xUbm*	As JSY568, but with *def1 K281R, K288R, K328R, K329R* at genomic *DEF1* locus	This study
JSY1194	*eGFP-DEF1*	As JSY568, but with *eGFP-DEF1* at genomic *DEF1* locus	This study
JSY1195	*eGFP-DEF1 GAC202*	*Matα his3-11 leu2-3,112 ura3-52 lys2-801 trp1-1, but with eGFP-DEF1* at genomic *DEF1* locus	This study
	DEF1-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, DEF1-GFP::HIS3*	(Huh et al., 2003)

JSY1196	*def1$_{CUEm}$*	As JSY568, but with *def1 I54A, I55A, F33A, P34A* at genomic *DEF1* locus	This study
JSY1197	*eGFP-def1$_{CUEm}$*	As JSY568, but with *eGFP-def1 I54A, I55A, F33A, P34A* at genomic *DEF1* locus	This study
JSY1198	*9xMyc-TEV-His-DEF1*	As JSY568, but with *9xMyc-2xTEV-6xHis-DEF1* at genomic *DEF1* locus	This study
JSY1199	*9xMyc-TEV-His-def1$_{CUEm}$*	As JSY568, but with *9xMyc-2xTEV-6xHis -def1 I54A, I55A, F33A, P34A* at genomic *DEF1* locus	This study
JSY642	*DEF1-6xHA*	*W303 MATa ura3 leu2-3,112 his3-11,15 ade2-1 can1-100 def1::pHAHIS304*	(Reid and Svejstrup, 2004)
JSY919	*RPB1-3xHA*	*MATa ura3-52, trp1-1, prb1-1122, prc1-407, pep4-3, leu2-3, 112 nuc1::LEU2 RPB1-3HA::URA*	(Reid and Svejstrup, 2004)
RAT7-1	*rat7-1*	*MATα ura3-52, leu2∆1, his∆200, rat7-1.*	(Gorsch et al., 1995)
MNY8	*Mny8*	*MATa leu2, his3, trp1, ura3. pDC-CRM1T539C::LEU2*	(Neville and Rosbash, 1999)
JSY1201	*Mny8 GFP-Def1*	*Mata leu2, his3, trp1, ura3. pDC-CRM1T539C::LEU2. GFP-Def1::URA*	This study
JSY1202	*Def1 CUEm, 9xMyc-TEV-His-Elc1*	As JSY1196 with 9xMyc-2xTEV-6xHis at N-terminus of Elc1::URA (see JSY 1116)	This study
JSY 1116	*9xMyc-TEV-His -Elc1*	*MATa ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100. 9xMyc-2xTEV-6xHis at N-terminus of Elc1::URA*	Harreman et al, 2009
JSY 1205	*∆ela1*	*MATa ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100. ∆ela1::URA3*	(Hobson et al., 2012)
JSY 1203	*Ela1$_{1-250}$*	*MATa ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100, Ela1 1-250::HIS*	This study
JSY 1204	*Ela1 WT*	*MATa ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100, Ela1::HIS*	This study
JSY 1208	*Def1∆400-445*	As JSY568, but with def1 lacking residues 400-445	This study
JSY 1209	*Def1∆430-490*	As JSY568, but with def1 lacking residues 430-490	This study
JSY 1210	*Def1∆480-520*	As JSY568, but with def1 lacking residues 480-520	This study
JSY 1211	*Def1∆506-575*	As JSY568, but with def1 lacking residues 506-575	This study
JSY1215	*9xMyc-TEV-His-Elc1, Ela1 WT*	*MATa ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100, 9xMyc-2xTEV-6xHis at N-terminus of Elc1::URA, Ela1::HIS*	This study
JSY1216	*9xMyc-TEV-His-Elc1, Ela1$_{1-250}$*	*MATa ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100, 9xMyc-2xTEV-6xHis at N-terminus of Elc1::URA, Ela1 1-250::HIS*	This study

Table S2. Plasmids Used in This Study, Related to Experimental Procedures

Name	Description	Source
pRS423	AmpR, 2μ, URA3	(Sikorski and Hieter, 1989)
pRS414-DEF1	AmpR, CEN, TRP; DEF1 expressed from own promoter and terminator	This study
pRS414-MTH-DEF1	AmpR, CEN, TRP; as above, but 9xMyc-2xTEV-6xHis-DEF1	This study
pRS414-MTH-DEF1-TEV	AmpR, CEN, TRP; as above, but TEV site inserted at DEF1 amino acids 522-523 (note: N-terminal TEV sites were removed during JSY1191 strain construction)	This study
pRS414-def1$_{CUEm}$	AmpR, CEN, TRP; as pRS414-DEF1, but CUE domain mutated (I54A, I55A, F33A, P34A)	This study
pRS414-def1$_{Ubm}$	AmpR, CEN, TRP; as pRS414-DEF1, but Def1 mutated at K281R, K288R, K328R, K329R	This study
YIPlac204-TEV118	AmpR, TRP; pGAL10, NLS-9xMyc-TEV-2xNLS	(Uhlmann et al., 2000)
pRS425-Myc-TEV	AmpR, 2μ, LEU; pGAL10, NLS-9xMyc-TEV-2xNLS	This study
pGEX-6P1	AmpR, GST	GE healthcare
pGEX-Def1$_{1-500}$	AmpR, GST-Def11-500	This study
pGEX-Def1$_{1-500/CUEm}$	AmpR, GST-Def11-500. CUE mutated at I54A, I55A, F33A, P34A	This study
pGEX-Dsk2	AmpR, GST-Dsk2	(Anindya et al., 2010)
pGST-MD	AmpR, GST-6xHis-MultiDsk	(Wilson et al., 2012)
pGEX-Rsp5	AmpR, GST-Rsp5	(Somesh et al., 2005)
pET21-Ubc5	AmpR, 6xHis-Ubc5	(Parker and Ulrich, 2009)
pST44-Elc1-Ela1	AmpR, His-Elc1, Ela1. Poly-cistronic	This study
pST44-Elc1-Ela1$_{1-250}$	AmpR, His-Elc1, Ela1 residues 1-250	This study
pST44-Elc1-Ela1$_{1-300}$	AmpR, His-Elc1, Ela1 residues 1-300	This study
pST44-Elc1-Ela1$_{1-350}$	AmpR, His-Elc1, Ela1 residues 1-350	This study
pGEX-Ubi	AmpR, GST-Ubiquitin	(Anindya et al., 2010)
pGEX-Ubi$_{I44A}$	AmpR, GST-Ubiquitin (I44A mutated)	(Anindya et al., 2010)
pGEX-Ela1$_{350-379}$	AmpR, GST-Ela1 C-terminus residues 250-379	This study
pPS815	AmpR, 2μ, URA, pADH1, SV40 NLS-GFP-LacZ	(Lee et al., 1996)
pPS1372	AmpR, 2μ, URA, pADH1, SV40 NLS-NES-GFP2	(Taura et al., 1998)
pBOW3 NLS-GFP-def1$_{500-738}$	AmpR, 2μ, HIS, pADH1, SV40 NLS-GFP-DEF1500-738	This study
pYES2	AmpR, 2μ, URA, pGAL1	Invitrogen
pYES2-DEF1	AmpR, 2μ, URA, pGAL1, DEF1	This study
pYES2-def1$_{1-400}$	AmpR, 2μ, URA, pGAL1, DEF11-400	This study
pYES2-def1$_{1-500}$	AmpR, 2μ, URA, pGAL1, DEF11-500	This study
pYES2-def1$_{1-600}$	AmpR, 2μ, URA, pGAL1, DEF11-600	This study
pYC2/NT	AmpR, CEN, URA, pGAL1	Invitrogen
pYC2-DEF1	AmpR, CEN, URA, pGAL1, DEF1	This study
pYC2-def1$_{1-500}$	AmpR, CEN, URA, pGAL1, DEF11-500	This study
pYC2-def1$_{CUEm}$	AmpR, CEN, URA, pGAL1, DEF1 CUE mutated (I54A, I55A, F33A, P34A)	This study
pYC2-def1$_{1-500CUEm}$	AmpR, CEN, URA, pGAL1, DEF11-500 CUE mutated (I54A, I55A, F33A, P34A)	This study
pYC2-NES-DEF1	AmpR, CEN, URA, pGAL1, NES-DEF1 (NES=LALKLAGLDI)	This study
pYC2-NES-def1$_{1-500}$	AmpR, CEN, URA, pGAL1, NES-DEF11-500	This study
pYM28	AmpR, CEN, HIS, eGFP	(Janke et al., 2004)

Table S3. Primary Antibodies Used in This Study, Related to Experimental Procedures

Antibody	Epitope	Source	Supplier	Dilution
4H8	Rpb1 CTD	Mouse, m	Abcam	1: 10,000
8WG16	Rpb1 CTD	Mouse, m	Abcam	1: 1,000
9E10	Myc tag	Mouse, m	Abcam	1: 5,000
9E11	Myc tag	Mouse, m	Abcam	1: 5,000
α-Pgk1	Pgk1	Mouse, m	Invitrogen	1: 10,000
α-His	6xHis	Mouse, m	Clonetech	1: 1,000
12CA5	HA tag	Mouse, m	Abcam	1: 1,000
α-Ela1	$Ela1_{115-214}$	Mouse, p	Abcam	1: 1,000
P4D1	Ubiquitin	Mouse, m	Millipore	1: 1,000
α-Def1	$Def1_{388-738}$	Rabbit, p	This study	1: 1,000

m, monoclonal; p, polyclonal. CTD, C-terminal domain