Supplementary Figure S1 (Kitabatake)

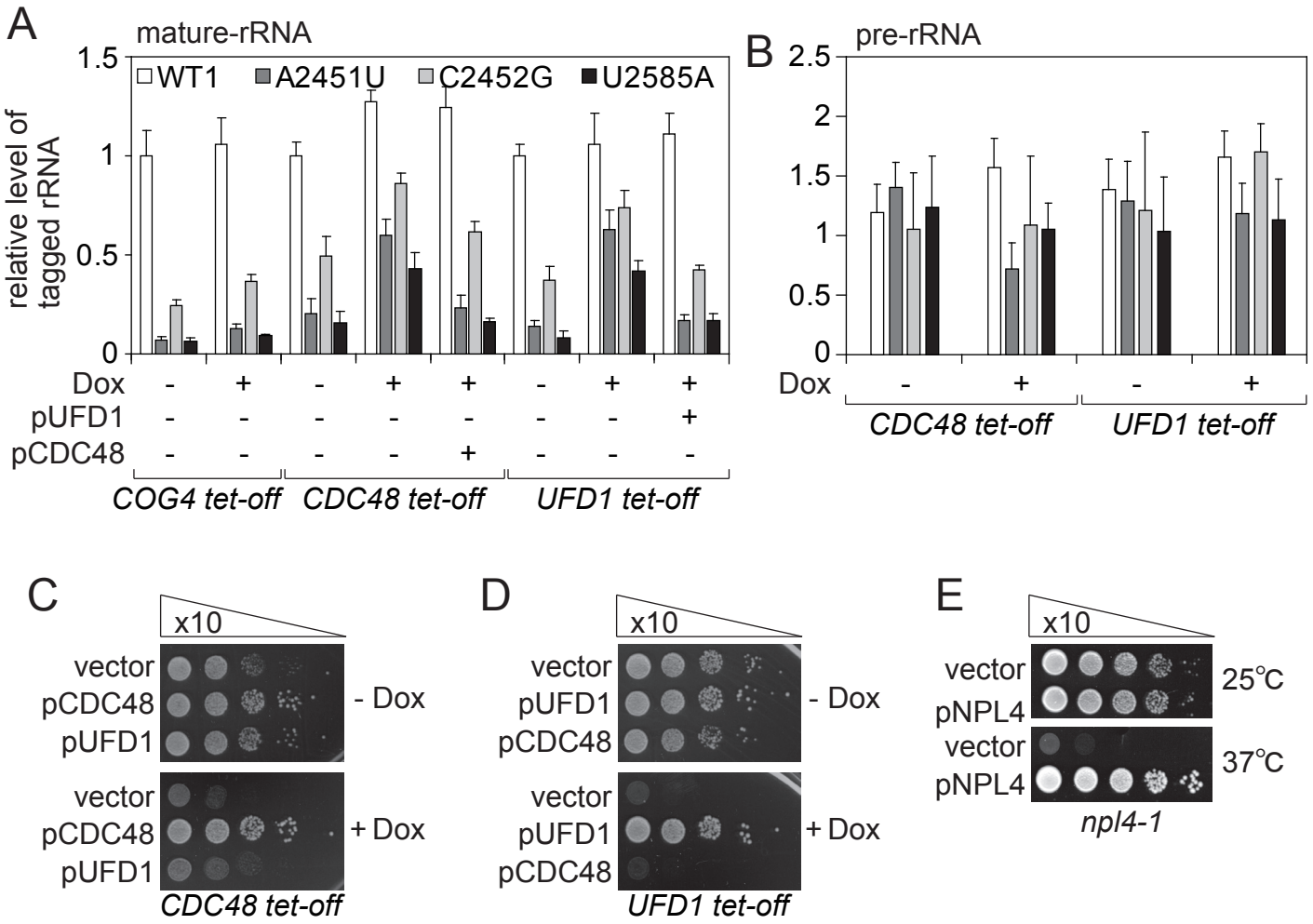


Figure S1. Cdc48 complex is involved in the 25S NRD pathway

(A) Quantitative reverse transcription (qRT)–PCR showing the involvement of Cdc48 and Ufd1 in 25S NRD. Nonfunctional 25S rRNAs, A2451U, C2452G, and U2585A, were expressed in tet-off strains. Each mutant strain was collected at A_{600} = 0.5. Total RNAs were purified and examined by qRT–PCR for 25S rRNA containing the 18-nt tag sequence (Supplementary Figure S5B). The *COG4 tet-off* strain was used as a control strain to show that growth repression by the tet-off system did not necessarily induce the accumulation of nonfunctional 25S rRNA. Values are means ±SD for triplicates. (B) qRT–PCR showing that Cdc48 and Ufd1 are not involved in the accumulation of pre-25S rRNA. The total RNAs purified in (A) were used for qRT–PCR to examine the amounts of pre-25S rRNAs accumulated (Supplementary Figure S5B). The repression of Cdc48 or Ufd1 did not induce the accumulation of pre-25S rRNAs, suggesting that the observed accumulation of nonfunctional 25S rRNAs in (A) is attributable to the inefficient degradation of mature 25S rRNAs. Values are means ±SD for triplicates. (C) A complementation assay for the *CDC48 tet-off* strain. pCDC48 or pUFD1, a *CEN* plasmid expressing the corresponding gene, was cotransformed into the strain, as indicated. (D) A complementation assay for the *UFD1 tet-off* strain. This was performed as in (C). (E) A complementation assay for the *npl4-1* temperature-sensitive strain. The *npl4-1* strain containing the pNPL4 plasmid or an empty vector was grown at 25 °C and spotted onto YPD plates after serial dilution. The plates were then incubated at the indicated temperatures for 3 days.

Supplementary Figure S2 (Kitabatake)

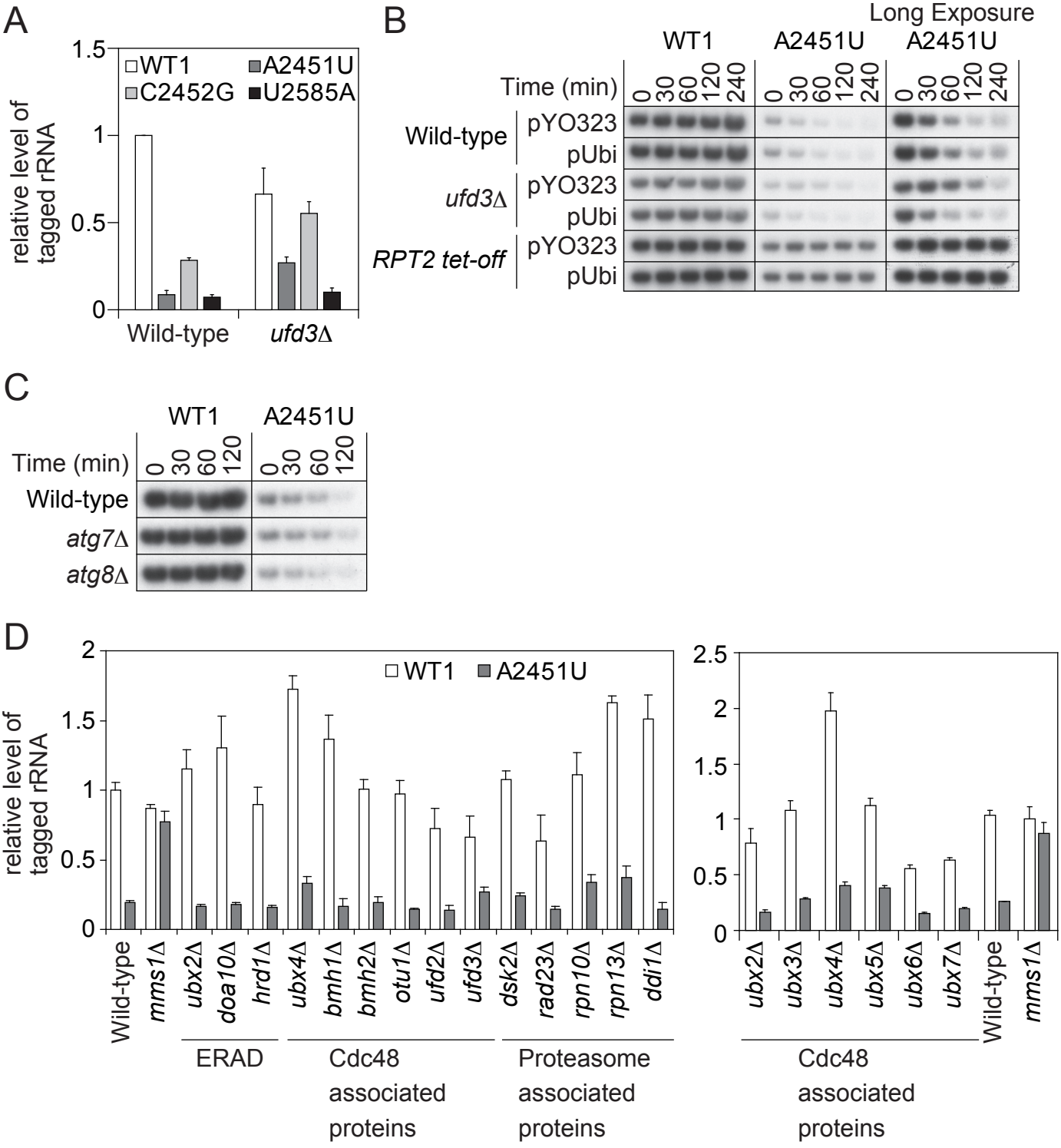


Figure S2. Cdc48 related factors were examined whether they are involved in 25S NRD.

(A) qRT–PCR showing the effects of *UFD3* disruption on 25S NRD. 25S NRD was inefficient to some extent in the *ufd3*Δ strain. The wild-type or *ufd3*Δ strain expressing the indicated 25S rRNA with the 18-nt insertion was harvested and the accumulation of tagged 25S rRNA was measured by qRT–PCR assay. Values are means ±SD for triplicates. (B) The overexpression of ubiquitin rescued 25S NRD in *ufd3*Δ strain. The indicated strains expressing the 18-nt-tagged wild-type (WT1) or A2451U mutant 25S rRNA (A2451U) with pUbi or empty vector (pYO323), were grown in SD–galactose and the transcription of the tagged rRNAs was shut-off. At the indicated time points, the RNAs were isolated and the tagged RNAs were examined by northern hybridization. $CuSO_4$ was added to the medium 2 h before the medium was changed, to induce the expression of untagged ubiquitin from a *CUP1* promoter in pUbi. (C) Northern blot analysis showing that autophagy is not required for 25S NRD. In *atg7*Δ and *atg8*Δ strains, wild-type (WT1) or nonfunctional mutant (A2451U) 25S rRNA was expressed and the RNAs were prepared and examined as in (B). (D) qRT–PCR assay showing these genes not involved in 25S NRD. Wild-type (WT1) or nonfunctional mutant (A2451U) 25S rRNA was expressed in the indicated strains and analyzed as in (A). *mms1*Δ is a control strain that lacks a component of E3 ubiquitin ligase essential for 25S NRD. Values are means ±SD for triplicates.

Supplementary Figure S3 (Kitabatake)

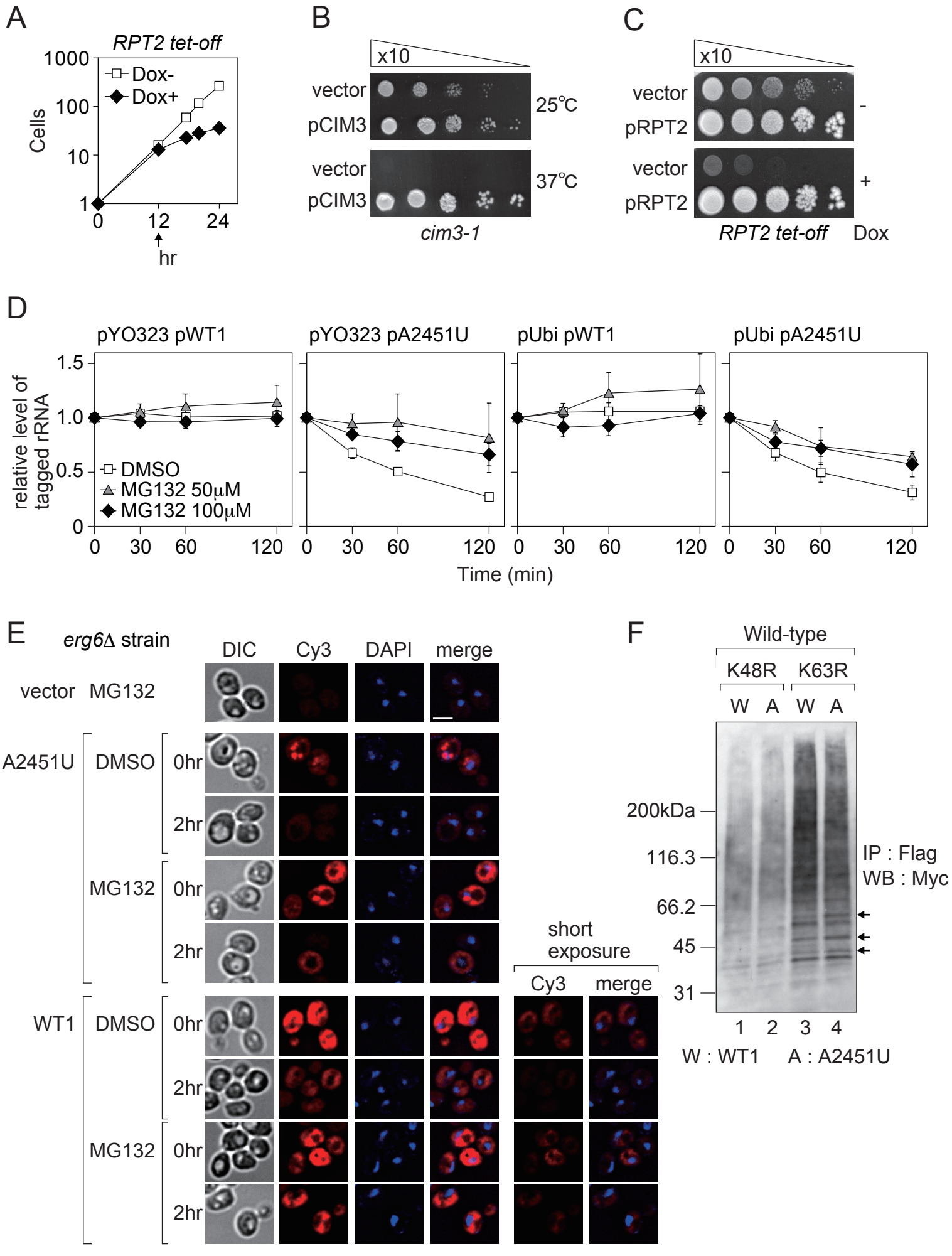


Figure S3. Proteasome activity is required for 25S NRD pathway.

(A) Growth curve for the *RPT2 tet-off* strain. The *RPT2 tet-off* strain was grown in SD–glucose medium with or without Dox. The culture was diluted every 12 h to maintain the cells in log phase. A_{600} was monitored at the indicated time points. The arrow indicates the time point at which the cells were harvested for the following analyses. (B) Complementation of *cim3-1* by the pCIM3 plasmid. pCIM3 or an empty vector was introduced into the *cim3-1* strain and grown at 25 °C in SD–glucose. The culture was spotted onto an SD–glucose plate after serial dilution. The plates were incubated for 3 days at 25 °C or 37 °C. (C) Complementation of the *RPT2 tet-off* strain with the pRPT2 plasmid. pRPT2 or an empty vector was introduced into the *RPT2 tet-off* strain, which was grown in SD–glucose. The cultures were spotted onto YPD plates with or without Dox and incubated for 2 days. (D) Stability of nonfunctional rRNA in MG132 treated cells with or without ubiquitin over expression in Figure 4C were quantified using BAS2500 (FUJIFILM). Values are means ±SD for triplicates. (E) Subcellular localization of an 18-nt-tagged wild-type (WT1) or nonfunctional (A2451U) 25S rRNA in the presence of proteasome inhibitor. 18-nt-tagged 25S rRNAs were visualized with in situ hybridization using Cy3-labeled oligonucleotide probes. An *erg6*Δ strain was grown in SD–raffinose to A_{600} = 0.5. The medium was then replaced with SD–galactose. The expression of tagged 25S rRNAs was induced for 6 h and the cells were treated with MG132 for 2 h in the same medium. 6 h after the induction the medium was replaced again with SD-glucose containing MG132 or DMSO and incubated 2 h to shut-off the transcription of plasmid derived rRNA. Scale bar, 4 μm. (F) Immunoblotting of ribosomal fractions purified from the wild-type strain expressing various tagged rRNAs, amino acid substituted Myc-ubiquitins, and Rpl28-Flag.

Ubiquitinated proteins were probed by anti-Myc antibody. Myc-ubiquitin had amino acid substitutions from lysine to arginine at the codon 48 (lanes 1 and 2) or 63 (lanes 3 and 4). The arrow indicates the bands enhanced by nonfunctional rRNA expression.

Supplementary Figure S4 (Kitabatake)

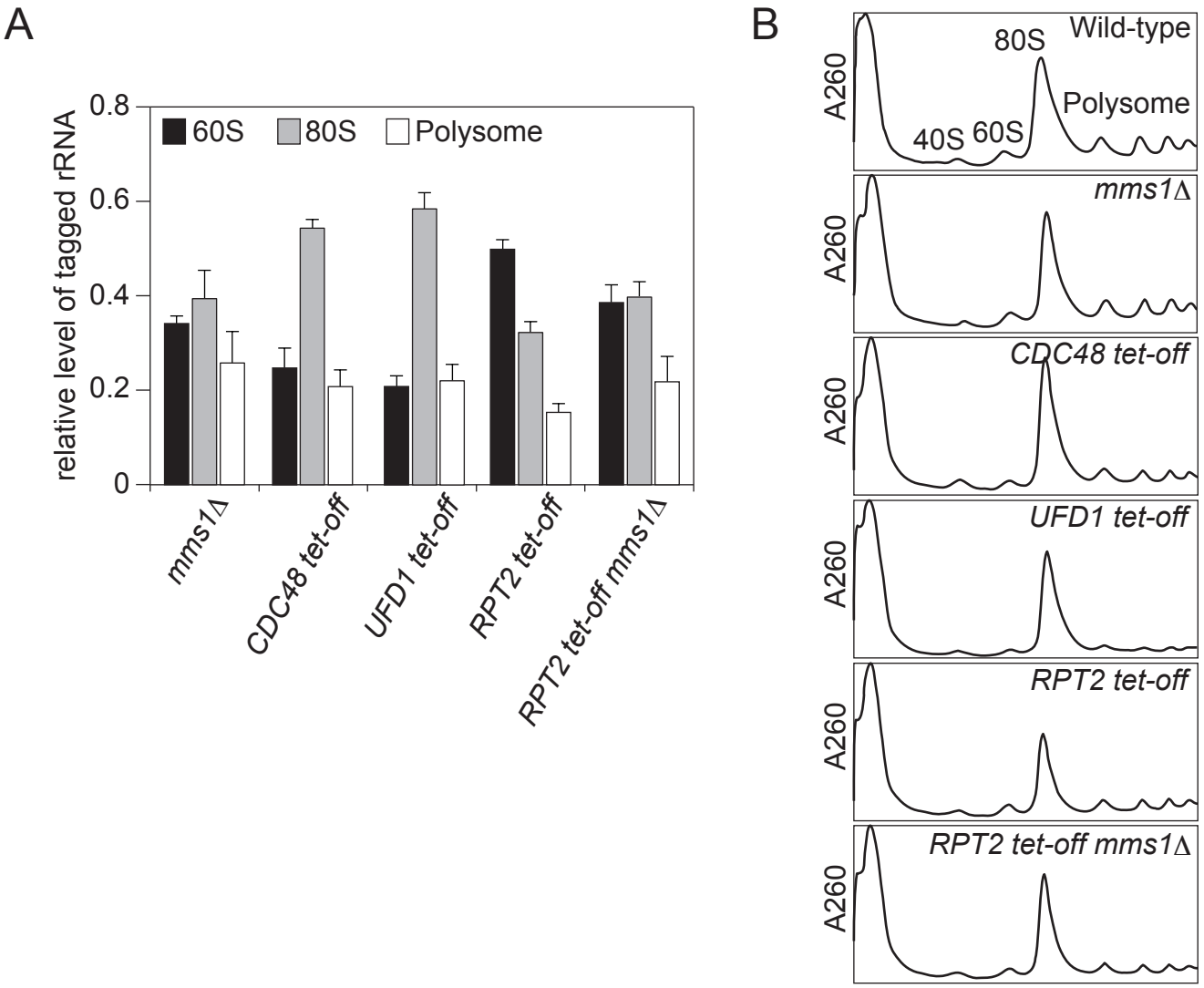
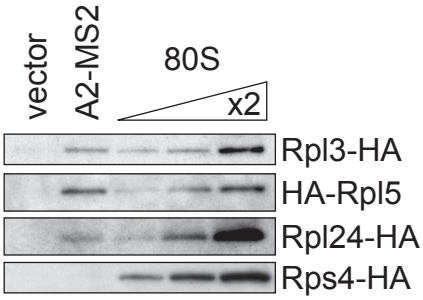


Figure S4. Nonfunctional ribosomes are accumulated in 60S fraction in proteasome deficient cells

(A) The signals of 60S, 80S, and polysome fractions in Figure 4F were quantified using BAS2500 (FUJIFILM). Values are means ±SD for triplicate. (B) Polysome profiles of wild-type, *mms1*Δ, *UFD1 tet-off*, *CDC48 tet-off*, *RPT2 tet-off*, and *RPT2 tet-off mms1*Δ strains in Figure 4F were monitored by A_{260}.

Supplementary Figure S5 (Kitabatake)

A



B

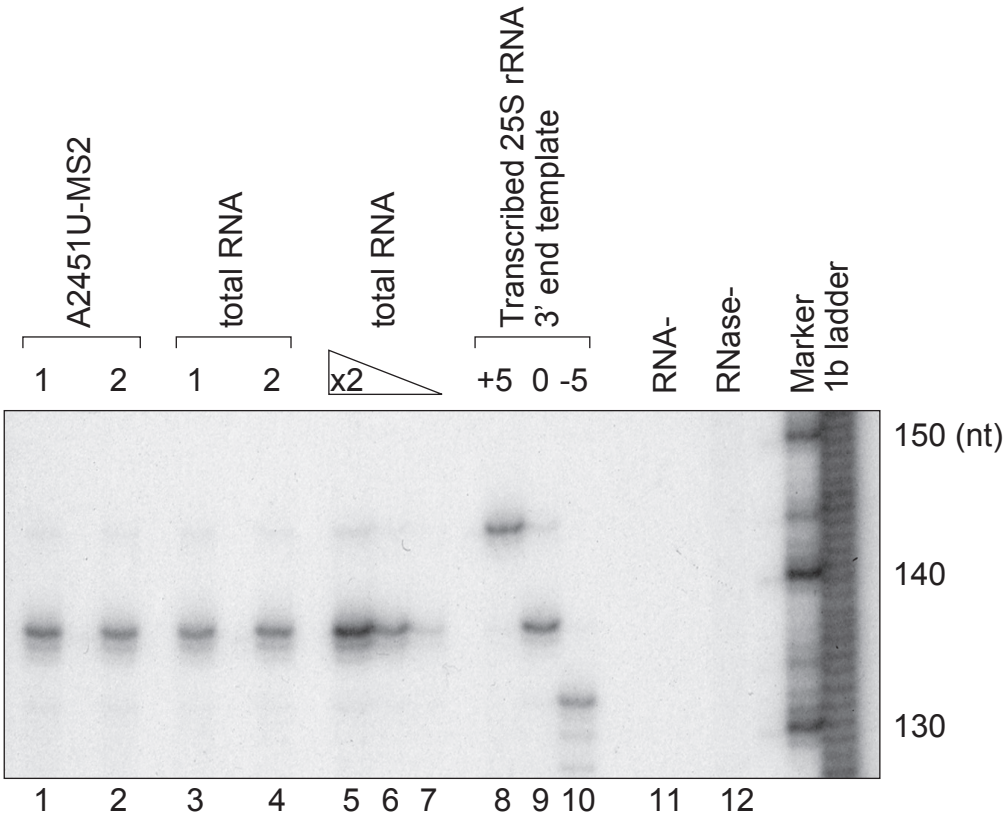


Figure S5. Analysis of components in the nonfunctional ribosome in proteasome deficient cell

(A) Ribosomal proteins L3, L5, and L24 were retained in nonfunctional ribosome were accumulated in *Rpt2 tet-off* strain. *Rpt2 tet-off* strain expressing HA-tagged ribosomal proteins, GST-MS2, and MS2-tagged nonfunctional rRNAs were grown in SD-Galactose to A_{600} =0.5. The medium was replaced with SD-Glucose and incubated 4 h to shut off the nonfunctional rRNA expression. Nonfunctional ribosomes were purified from 60S fraction by GST-MS2 pull down assay and contained ribosomal proteins were analyzed by anti-HA antibody. As shown in the 80S fraction, plasmid derived ribosomal proteins were stably constructed in the ribosome. (B) Nonfunctional 25S rRNA accumulated in proteasome deficient cell had perfect 3' end in single nucleotide resolution in the same assay with Figure 5E. In lane 1 and 2, purified nonfunctional 25S rRNAs from *Rpt2 tet-off* strain were used for the RNase protection assay. Total RNA was used as template in lane 3 to 7. Transcribed 3' region of 25S rRNA including +5nt and -5nt were used as template in lane 8 to 10. At lane 11, no target RNA was added to the reaction. Lane 12 is the no-RNase control. Internal labeled marker was made by in vitro transcription. A single base ladder was made by alkaline hydrolysis of 5'-end labeled RNA, which had same sequence with the probe.

Supplementary Figure S6 (Kitabatake)

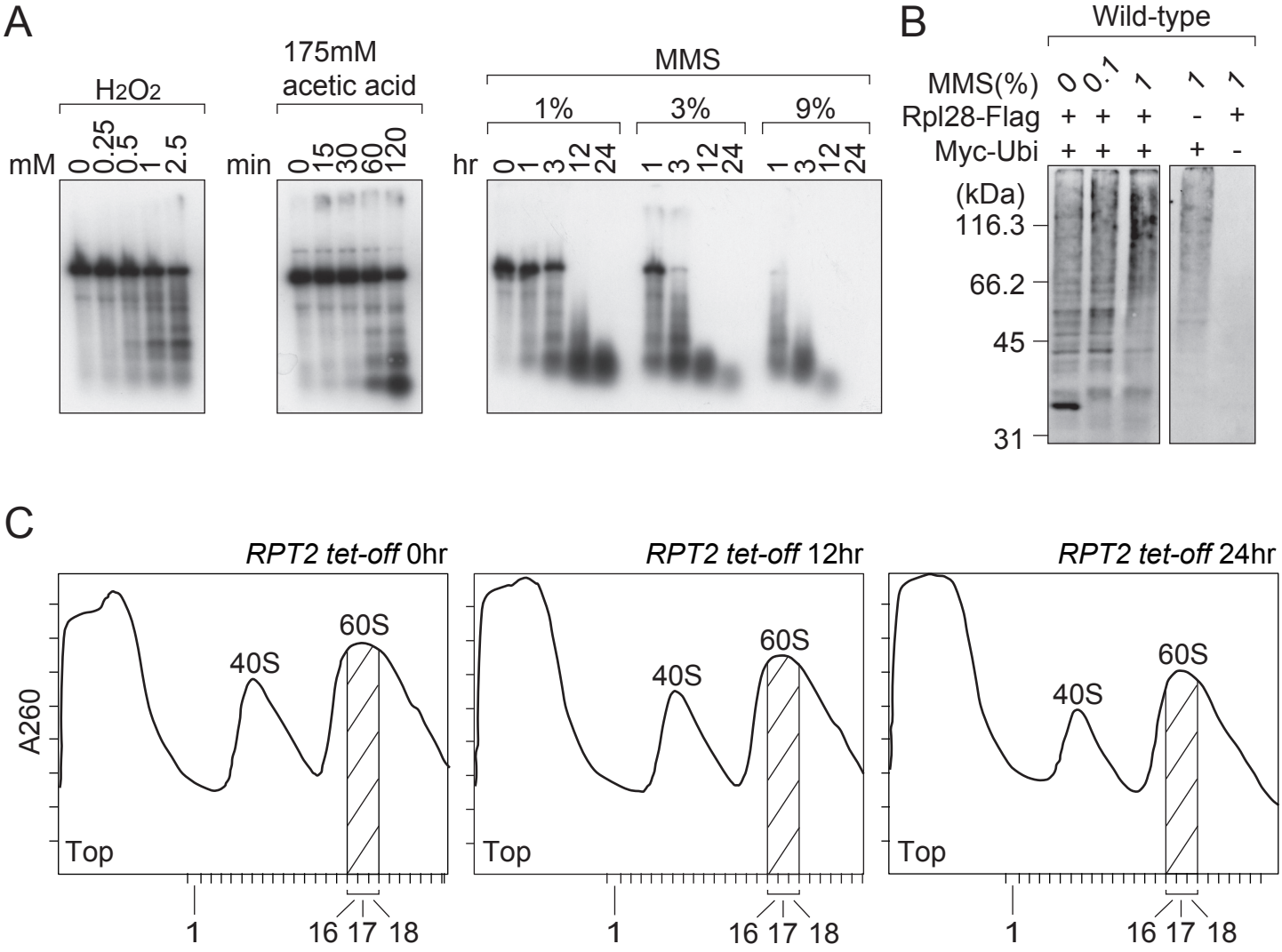


Figure S6. Ribosomes are degraded by the ubiquitin–proteasome system

(A) Northern blotting of endogenous 25S rRNA after stress treatments. The wild-type strain was grown in SD–glucose and harvested after the indicated treatments. Total RNAs were isolated from the cells and separated on 1% denaturing agarose. Northern hybridization was performed using the Kota388 probe, which detects endogenous 25S rRNAs. (B) Western blotting of ubiquitinated ribosomes after MMS treatment. pMyc–Ubi and pRpl28–Flag were transformed to the wild-type strain. The transformants were grown in SD–glucose and harvested 2 h after treatment with MMS. The ribosomes were isolated from the strain by immunoprecipitation using anti-Flag agarose. The ubiquitinated proteins were detected with an anti-Myc polyclonal antibody after 12.5% SDS–PAGE. (C) 60S fractions used for the purification in Figure 6C. The *RPT2 tet-off* strain expressing Myc–ubiquitin and Rpl28–Flag were grown in SD–glucose medium containing Dox. The lysates were resolved on a 10%–40% sucrose gradient containing 40 mM EDTA. The fractions indicated were pooled and used as the 60S subunits for immunoprecipitation. Exactly the same result was obtained for the 40S subunit and the wild-type strain.

Supplementary Figure S7 (Kitabatake)

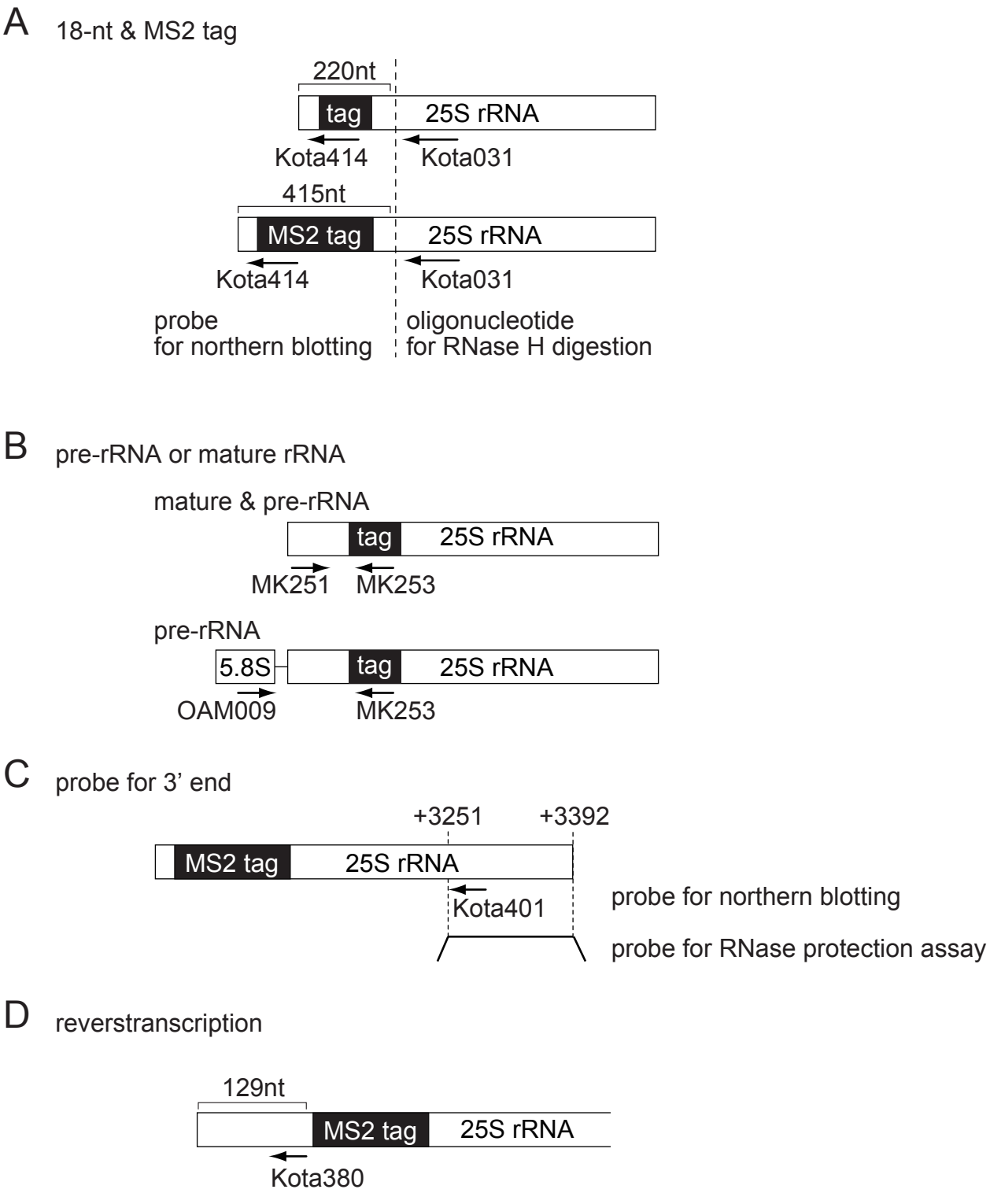


Figure S7. Primers used for RNA analyses are shown in schematic representation

(A) Oligonucleotides for RNase H digestion and northern blotting (Figure 2A). (B) Primers for qRT–PCR used to measure tagged 25S rRNAs and pre-25S rRNAs (Supplementary Figure S1A and B). (C) Probes for the northern blotting in Figure 5D and the RNase protection assay (Figure 5E and Supplementary Figure S5B). (D) The primer used for the reverse transcription in Figure 5F and G.

Supplementary Methods

Plasmids

pWT1,pA2451U, pC2452G, pU2585A, pWT4, and pA1492C have been described previously (Fujii et al, 2009). To construct pCDC48, pUFD1, pNPL4, pCIM3, and pRPT2, each open reading frame (ORF), including 1 kb each of the upstream and downstream sequences, was amplified by PCR and cloned into YCplac111 (Gietz & Sugino, 1988) or pRS313 (Sikorski & Hieter, 1989).

To construct pRpl28–Flag, pRps2–Flag, pRpl3-HA, Rpl24-HA, and Rps4-HA the Flag or HA tags was added to the C-terminus of each gene by overlap extension. PCR fragments of each gene, containing the promoter, ORF, tag sequence, and terminator, were cloned into YCplac111 (Gietz & Sugino, 1988) or pRS315 (Sikorski & Hieter, 1989).

To construct pHA-Rpl5, the HA tag was inserted into the N-terminus region at same position with previous paper (Deshmukh et al, 1993) and endogenous promoter was replaced with *GAL7* promoter by overlap extension. PCR fragment were cloned into pYO325 (Qadota et al, 1992).

pMyc-UbiK48R and pMyc-UbiK63R are identical to pMyc-Ubi except for the Lys-to-Arg mutation at codon 48 or 63 respectively.

pWT1–MS2 and pA2451U–MS2 were created by inserting a 195-bp fragment containing six repeats of the MS2 coat protein-binding site into the unique *Xho*I site in the 18-nt tag of pWT1 or pA2451U. The inserted fragment

(5′-ctcgagCGTACACCATCAGGGTACGAGCTAGCCCATGGCGTACACCATCAGGGTA
CGACTAGTAGATCTCGTACACCATCAGGGTACGGAAGCTGGTACCTGTACCTTCG
GTGCGTACACCATCAGGGTACGAGCTAGCCCATGGCGTACACCATCAGGGTACGA

CTAGTAGATCTCGTACACCATCAGGGTACGctcgag-3′) was amplified with primers MK327 and MK330 using the pδ-crystallin MS2 plasmid as the template (Yoshimoto et al, 2009).

pGST–MS2 was constructed by the assembly of three PCR fragments with overlap extension PCR. These three fragments included 1) the *GPD* promoter amplified by MK331–MK340, 2) the sequence encoding *GST–MS2*, amplified with MK336–MK339, and 3) the *CYC1* terminator amplified with MK337–MK338. MK331 and MK338 were used for the final assembly PCR. The fragment was cloned into pYO325 (Qadota et al, 1992).

Yeast strains and growth conditions

Yeast strain collections, YKO and yTHC, were purchased from Open Biosystems. NOY401 was kindly provided by Dr M. Nomura (Nogi et al, 1991), the *npl4-1* strain was from Dr P. Silver (DeHoratius & Silver, 1996), and the *cim3-1* strain (Ghislain et al, 1993) was obtained from NBRP, Japan. The *TRP1* gene was disrupted by targeting it with a hygromycin-resistance cassette from pFA6 (Goldstein & McCusker, 1999). The same cassette was used to delete the *MMS1* gene in the *RPT2 tet-off* strain from the yTHC collection.

To induce expression from the *GAL7* promoter, the cells were pregrown in SD medium containing 2% raffinose. This culture was inoculated into SD medium containing 2% galactose. The cells were grown until they reached mid-log phase (A_{600} = 0.5). When necessary, the medium was replaced with SD containing 2% glucose to repress the *GAL7* promoter. MG132 was added to the culture medium 2 h before the medium was changed from 2% galactose to 2% glucose. When ubiquitin overexpression was required,

it was achieved by adding 0.1 mM $CuSO_4$ at the same time as MG132. For the Tet-off assay, the yTHC strains were grown in SD medium containing 10 μg/mL Dox (Hughes et al, 2000). The detailed procedures used to screen yTHC are described in the Supplemental Materials. For treatment with H_2O_2, acetic acid, or MMS, the cells were grown in SD medium containing 2% glucose until they reached mid-log phase (A_{600} = 0.5) and were then stressed with H_2O_2 (0.25–1 mM) for 2 h, with 90 mM acetic acid for the indicated time, or with MMS (1%–9%) for the indicated time.

In vivo complementation assay

For the complementation assay, the (pol I ts) NOY401 strain was transformed with a variety of rRNA expressing plasmids. The transformants were pregrown in SD–galactose culture medium at 25 °C, spotted onto a plate in a series of dilutions, and incubated at 25 °C or 37 °C for 3 or 5 days.

Tet-off culture conditions and screening for yTHC

Each tet-off strain showed different sensitivity to Dox treatment, depending upon the stability of the repressed gene product in the strain. To establish the growth conditions for the preparation of factor-depleted cells, we inspected the growth curves of the strains empirically in the presence of Dox. When the cells reached A_{600} = 0.5, they were diluted 20-fold in the same medium containing fresh Dox, to maintain log phase growth. Typically, the culture was diluted every 12 h until the cells ceased to grow. For the analyses, the *UFD1 tet-off*, *RPT2 tet-off*, and *CDC48 tet-off* strains were harvested 12 h, 12 h, and 24 h after Dox treatment, respectively.

To screen the yTHC collection, all 800 yTHC strains were transformed with pA2451U–Leu. The precultured cells grown in 2% galactose medium were diluted 100-fold in the same medium containing 10 μg/mL Dox. After incubation for 24 h, the cells were harvested and spotted onto a membrane, although not all of these strains showed a reduction in growth under these conditions. This membrane was processed with a previously described colony northern technique (Fujii et al, 2009).

Supplementary Tables

Supplementary Table S I , Plasmid list

Plasmid	Notes	Reference	
pNOY102	*URA3*, *2μ*, *GAL7-rDNA*	(Nogi et al, 1991)	pMK001
pWT1	*LEU2*, *2μ*, *GAL7-rDNA 25S-Tag*	(Fujii et al, 2009)	pMK010
pA2451U	*LEU2*, *2μ*, *GAL7-rDNA 25S-Tag A2451U*	(Fujii et al, 2009)	pMK011
pC2452G	*LEU2*, *2μ*, *GAL7-rDNA 25S-Tag C2452G*	(Fujii et al, 2009)	pMK012
pU2585A	*LEU2*, *2μ*, *GAL7-rDNA 25S-Tag U2585A*	(Fujii et al, 2009)	pMK013
pWT4	*URA3*, *2μ*, *GAL7-rDNA 25S-Tag*, *18S-Tag*	(Fujii et al, 2009)	pMK008
pA1492C	*URA3*, *2μ*, *GAL7-rDNA 25S-Tag*, *18S-Tag A1492C*	(Fujii et al, 2009)	pMK009
pWT-MS2	*URA3*, *2μ*, *GAL7-rDNA 25S-MS2 Tag*	This Study	pMK014
pA2451U-MS2	*URA3*, *2μ*, *GAL7-rDNA 25S-MS2 Tag A2451U*	This Study	pMK015
pYO323	*HIS3*, *2μ*	(Qadota et al, 1992)	pMK176
pWT-MS2-His	*HIS3*, *2μ*, *GAL7-rDNA 25S-MS2 Tag*	This Study	pMK016
pA2451U-MS2-His	*HIS3*, *2μ*, *GAL7-rDNA 25S-MS2 Tag A2451U*	This Study	pMK017
pUbi	*HIS3*, *2μ*, *CUP1-Ubi-CYC1*	(Fujii et al, 2009)	pMK089
pMyc-Ubi	*HIS3*, *2μ*, *CUP1-Myc-Ubi-CYC1*	(Fujii et al, 2009)	pMK088
pMyc-UbiK63R	*HIS3*, *2μ*, *CUP1-Myc-UbiK63R-CYC1*	This Study	pMK092
pMyc-UbiK48R	*HIS3*, *2μ*, *CUP1-Myc-UbiK48R-CYC1*	This Study	pMK093
pYO325	*LEU2*, *2μ*	(Qadota et al, 1992)	pMK178
pGST-MS2-Leu	*LEU2*, *2μ*, *GST-MS2*	This Study	pMK151

pRpl28-Flag	*LEU2, 2μ, RPL28-Flag*	This Study	pMK077
pRS315	*LEU2, CEN*	(Sikorski & Hieter, 1989)	pMK196
pRps2-Flag	*LEU2, CEN, RPS2-Flag*	This Study	pMK231
pRps4-HA	*LEU2, CEN, RPS4-HA*	This Study	pMK038
pRpl3-HA	*LEU2, CEN, RPL3-HA*	This Study	pMK042
pHA-Rpl5	*LEU2, 2μ, GAL7-HA-RPL5*	This Study	pMK200
pRpl24-HA	*LEU2, CEN, RPL24-HA*	This Study	pMK047
pYO324	*TRP1*, *2μ*	(Qadota et al, 1992)	pMK177
pGST-MS2-Trp	*TRP1*, *2μ*, *GST-MS2*	This Study	pMK153
pRS313	*HIS3*, *CEN*	(Sikorski & Hieter, 1989)	pMK195
pCDC48	*HIS3*, *CEN*, *CDC48*	This Study	pMK133
pUFD1	*HIS3*, *CEN*, *UFD1*	This Study	pMK139
pRPT2	*HIS3*, *CEN*, *RPT2*	This Study	pMK143
YCplac111	*LEU2*, *CEN*, *lacZ*	(Gietz & Sugino, 1988)	pMK169
pNPL4	*LEU2*, *CEN*, *NPL4*	This Study	pMK144
pCIM3	*LEU2*, *CEN*, *CIM3*	This Study	pMK142
pAG26	*hphMX6*, *CEN*, *URA3*	(Goldstein & McCusker, 1999)	pMK179

Supplementary Table S Ⅱ, Yeast Strains list

Strain	Genotype and Notes	Reference	
BY20693	*MATa, his3-1, leu2-Δ0, ura3-Δ0*	(Brachmann et al, 1998)	MKY13
mms1Δ	*MATa, his3-1, leu2-Δ0, ura3-Δ0, met15Δ0, mms1Δ::KanMX4*	(Winzeler et al, 1999)	MKY47
NOY401	*MATa, rpa190-3, ura3, leu2, trp1, can1*	(Nogi et al, 1991)	MKY7
CYH2-Flag	*MATa, his3-1, leu2-Δ0, ura3-Δ0, RPL28-CYH2-Flag*	(Fujii et al, 2009)	MKY17
CYH2-Flag, mms1Δ	*MATa, his3-1, leu2-Δ0, ura3-Δ0, RPL28-CYH2-Flag, mms1Δ::KanMX4*	(Fujii et al, 2009)	MKY18
CYH2-Flag, trp1Δ	*MATa, his3-1, leu2-Δ0, ura3-Δ0, RPL28-CYH2-Flag, trp1Δ::hphMX6*	This Study	MKY137
CDC48 tet-off	*MATa, his3-1, leu2-Δ0, ura3-Δ0, URA3::CMV-tTA, KanMX::tetO$_7$CYC$_{TATA}$CDC48*	(Hughes et al, 2000)	MKY69
CDC48 tet-off, mms1Δ	*MATa, his3-1, leu2-Δ0, ura3-Δ0, URA3::CMV-tTA, KanMX:: tetO$_7$CYC$_{TATA}$CDC48, mms1Δ::hphMX6*	This Study	MKY70
UFD1 tet-off	*MATa, his3-1, leu2-Δ0, ura3-Δ0, URA3::CMV-tTA, KanMX:: tetO$_7$CYC$_{TATA}$UFD1*	(Hughes et al, 2000)	MKY37
PSY825	*MATa, ura3-52, leu2Δ1, npl4-1*	(DeHoratius & Silver, 1996)	MKY64
RPT2 tet-off	*MATa, his3-1, leu2-Δ0, ura3-Δ0, URA3::CMV-tTA, KanMX:: tetO$_7$CYC$_{TATA}$RPT2*	(Hughes et al, 2000)	MKY92

RPT2 tet-off, mms1Δ	*MATa, his3-1, leu2-Δ0, ura3-Δ0, URA3::CMV-tTA, KanMX:: $tetO_7CYC_{TATA}$RPT2, mms1Δ::hphMX6*	This Study	MKY108
cim3-1	*MATα cim3-1 ura3-52 leu2-Δ1*	(Ghislain et al, 1993)	MKY70
erg6Δ	*MATa, his3-1, leu2-0, ura3-Δ0, met15Δ0, erg6Δ::KanMX4*	(Winzeler et al, 1999)	MKY34

Supplementary Table SⅢ, Oligo list

Name	Sequence	Notes
OAM009	5'-AGGGGGCATGCCTGTTGAG	5.8S rRNA sequence
MK251	5'-GACCTCAAATCAGGTAGGAGTACCC	25S rRNA sequence
MK253	5'-CACCGAAGGTACACTCGAGAGCTTC	complementary to pWT1, pWT4 25S rRNA tag sequence
Kota030	5'-GAAATCTGGTACCTTCGGTG	untagged 25S rRNA sequence
Kota031	5'-GATTCTCACCCTCTATGACG	complementary to untagged 25S rRNA sequence
Kota153	5'-CGAGGATTCAGGCTTTGG	complementary to pWT4 18S tagged sequence
Kota414	5’-GTACACTCGAGAGCTTCAGTACCAC	prove for MS2 tag & 18nt tag sequence
Kota401	5’-GACATTGCAATTCGCCAGCAAGCA	complementary to 25S rRNA sequence
Kota379	5'-GATGACTAGCTTGCTGGCGAATTGCAATGTC	fw prime for the template of RPA probe *in vitro* transcription
Kota383	5’-TAATACGACTCACTATAGGGAGA tcctgccagtacccacttagaaag	rev prime for the template of RPA probe *in vitro* transcription
Kota381	5’-TAATACGACTCACTATAGGGAGA tgcttgctggcgaattgcaatgtc	rev prime for the template of 25S rRNA 3' region *in vitro* transcription
Kota382	5’-ACAAATCAGACAACAAAGGC	complementary to 25S rRNA sequence
Kota380	5’-GTACCAGATTTCAAATTTGAGC	complementary to 25S rRNA sequence
Kota388	5'-TTCCTCCGCTTATTGATATGC	complementary to 25S rRNA 5' reagion
Kota179	5’-ATCTTCCTCATCGTCTTCAG	upstream sequence of the *UFD1* gene
Kota182	5’-TCCAGCATCCTAATGTGCAC	complementary sequence to downstream of the *UFD1* gene
Kota219	5’-CACCAATGATGGCGATAGTC	upstream sequence of the *CDC48* gene
Kota220	5’-ACATGTCTCTCGCCATTCTTG	complementary sequence to downstream of the *CDC48* gene

Kota221	5’-TCTGCTGCTGGTGGTTATCC	upstream sequence of the *CIM3* gene
Kota222	5’-ACGTTCACCAGTTTTCGTAG	complementary sequence to downstream of the *CIM3* gene
Kota357	5’-GATTGCACCTATTGCAGAAG	upstream sequence of the *RPT2* gene
Kota358	5’-GTCATATGGTGTTTCTGGCC	complementary sequence to downstream of the *RPT2* gene
Kota213	5’-CACACAGTAGGCACTAATTG	upstream sequence of the *NPL4* gene
Kota214	5’-TCGTTGACAATCCTTACAGG	complementary sequence to downstream of the *NPL4* gene
Kota488	5’-TC GGTATCTCAG CATCTAGG	upstream sequence of the *RPS2* gene
Kota535	5’- GAT TAC AAG GAC GAC GAT GAC AAG taagcttgttgtctacaaattataaaatag	construction of pRps2-Flag
Kota532	5’-CTT GTC ATC GTC GTC CTT GTA ATC gaatctcttcttttgagcagaagc	construction of pRps2-Flag
Kota472	5’-AACAATGTAA GTTCGGTACG	complementary sequence to downstream of the *RPS2* gene
MK704	5-CACTTACGTTATCATTCTAAAG	upstream sequence of the *RPL3* gene
MK705	5-ctacgcatagtcaggaacatcgtatgggtac aagtccttcttcaaagtacc	construction of pRpl3-HA
MK714	5-GCTGTTGCTATTGCCAAGTTAA G	upstream sequence of the *RPL24* gene
MK715	5-ctacgcatagtcaggaacatcgtatgggtaa cgagaagtagcagcaacctttg	construction of pRpl24-HA
MK696	5-CTAATGGAGAGCTGGGTACTGC	upstream sequence of the *RPS4* gene
MK697	5-ctacgcatagtcaggaacatcgtatgggtat aaaccttgttgagctcttcttctg	construction of pRps4-HA
MK722	5-CATACGATGTTCCTGACTATG CGtagATCTAAAAACTTCATCAAA G	HA tag and *RPL13* gene 3' UTR sequence
MK395	5-ttcatacattcttaaattgc	upstream sequence of the *GAL7* gene
MK685	5-tatccctatgacgtcccggactatgcaTCC TCTGCTTACTCCTCTCGTTTC	construction of pHA-Rpl5
MK686	5-tgcatagtccgggacgtcatagggatactta	construction of pHA-Rpl5

	gcgtctttttggaaagccat	
MK687	5-gttagaatgccattctagag	complementary sequence to downstream of *RPL5* gene
MK723	5-ccaaaacttgaaggacttattgg	complementary sequence to downstream of the *RPL13* gene
MK327	5-acatcagctttaagCTCGAG	Used for amplifying MS2 tag sequence
MK330	5-aacatttccgtgtcctcgag	Used for amplifying MS2 tag sequence
MK331	5-GCGGCCGCacacgctttttcagttcgag	Used for amplifying *GPD* promoter fragment
MK336	5-caatgtgggatccacctgttttagtagatgccggagtttg	Used for amplifying GST-MS2 fragment
MK337	5-AACAGGTGGATCCCACATTGtcatgtaattagttatgtcac	Used for amplifying *CYC1* terminator fragment
MK338	5-ggtatcctcgagggccgcaaattaaagcctttc	Used for amplifying *CYC1* terminator fragment
MK339	5-gtttcgaataaacacacataaacaaacaaaATGtcccctatactaggttattg	Used for amplifying GST-MS2 fragment
MK340	5-caataacctagtataggggacattttgtttgtttatgtgtgtttattcgaaac	used for amplifying *GPD* promoter fragment
MK590	5-atctttgccggtAGAcagctcgaggac	construction of pMyc-UbiK48R
MK591	5-gtcctcgagctgtctaccggcaaagat	construction of pMyc-UbiK48R
MK592	5-attacaacattcagAGAgagtcgaccttac	construction of pMyc-UbiK63R
MK593	5-gtaaggtcgactctctctgaatgttgtaat	construction of pMyc-UbiK63R
MK560	5-aacaagcgctcatgagcccg	upstream sequence of the Myc-Ubi *CUP1* promoter
MK561	5-gacagcttatcatcgataag	complementary sequence to downstream of the Myc-Ubi *CYC1* terminator

Supplementary References

Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P, Boeke JD (1998) Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **14:** 115-132

DeHoratius C, Silver PA (1996) Nuclear transport defects and nuclear envelope alterations are associated with mutation of the Saccharomyces cerevisiae NPL4 gene. *Mol Biol Cell* **7:** 1835-1855

Deshmukh M, Tsay YF, Paulovich AG, Woolford JL, Jr. (1993) Yeast ribosomal protein L1 is required for the stability of newly synthesized 5S rRNA and the assembly of 60S ribosomal subunits. *Molecular and cellular biology* **13:** 2835-2845

Fujii K, Kitabatake M, Sakata T, Miyata A, Ohno M (2009) A role for ubiquitin in the clearance of nonfunctional rRNAs. *Genes Dev* **23:** 963-974

Ghislain M, Udvardy A, Mann C (1993) S. cerevisiae 26S protease mutants arrest cell division in G2/metaphase. *Nature* **366:** 358-362

Gietz RD, Sugino A (1988) New yeast-Escherichia coli shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. *Gene* **74:** 527-534

Goldstein AL, McCusker JH (1999) Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. *Yeast* **15:** 1541-1553

Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, Armour CD, Bennett HA, Coffey E, Dai H, He YD, Kidd MJ, King AM, Meyer MR, Slade D, Lum PY, Stepaniants SB, Shoemaker DD, Gachotte D, Chakraburtty K, Simon J, Bard M, Friend SH (2000) Functional discovery via a compendium of expression profiles. *Cell* **102:** 109-126

Nogi Y, Yano R, Nomura M (1991) Synthesis of large rRNAs by RNA polymerase II in mutants of Saccharomyces cerevisiae defective in RNA polymerase I. *Proc Natl Acad Sci U S A* **88:** 3962-3966

Qadota H, Ishii I, Fujiyama A, Ohya Y, Anraku Y (1992) RHO gene products, putative small GTP-binding proteins, are important for activation of the CAL1/CDC43 gene product, a protein geranylgeranyltransferase in Saccharomyces cerevisiae. *Yeast* **8:** 735-741

Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* **122:** 19-27

Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H, Chu AM, Connelly C, Davis K, Dietrich F, Dow SW, El Bakkoury M, Foury F, Friend SH, Gentalen E, Giaever G, Hegemann JH, Jones T, Laub M, Liao H, Liebundguth N, Lockhart DJ, Lucau-Danila A, Lussier M, M'Rabet N, Menard P, Mittmann M, Pai C, Rebischung C, Revuelta JL, Riles L, Roberts CJ, Ross-MacDonald P, Scherens B, Snyder M, Sookhai-Mahadeo S, Storms RK, Veronneau S, Voet M, Volckaert G, Ward TR, Wysocki R, Yen GS, Yu K, Zimmermann K, Philippsen P, Johnston M, Davis RW (1999) Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. *Science* **285:** 901-906

Yoshimoto R, Kataoka N, Okawa K, Ohno M (2009) Isolation and characterization of post-splicing lariat-intron complexes. *Nucleic Acids Res* **37**: 891-902