Supporting Information

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SI Materials and Methods

Plasmid Constructions. Plasmid-borne TAP-Stop and TAP-Non-Stop reporter constructs (TAP; tandem affinity purification) were obtained by PCR-based yeast recombination, respectively, from Stop ProtA (pAV183) and Non-Stop ProtA (pAV184) plasmids (Tables S1 and S2). For this purpose, pAV183 or pAV184 was linearized with XbaI and BamHI and transformed in yeast together with the PCR-amplified TAP-tag. The latter, an N-terminal fusion, was PCR amplified from pBS1761 plasmid (1) with JM028 (5′-GGAGAAAAAACCCCGGATCATAATCGGCCG-CTCTAGAATGGCAGGCCTTGCGCAACACG-3′) and JM029 (5′-GATAAGAAAGCAACACCTGGCAATTCCTTACCGGA-TCCTAGGGCGAATTGGGTACCG-GG-3′) oligos.

Genetic Interaction Mapping Screens. Genetic interaction mapping (GIM) was done as previously described (2) with two exceptions: The barcoded 979 DAmP strains were added to the pool of mutants and a new system for the cultures of the final pools of haploid double mutants was used. Constant turbidity cultures were done at 30 °C by using a custom-made turbidostat system. This system uses sterile air injection in 10-mL reaction flasks to ensure mixing of the cells and allows yeast growth at rates that are similar to the ones measured in batch cultures. The reference population either came from a parallel screen done with the deletion of *YEL068C* open reading frame or consisted of a mix of at least 10 double-mutant populations obtained with different query gene deletions. Data analysis of the microarray results was done using R to perform Lowess normalization with marray (3) independently for the UP and DOWN measured sets of ratios (corresponding to the two barcodes situated in the 5′ and 3′ regions of KANMX, Kanamycin resistance cassette). We corrected the results for systematic bias and batch effects by applying a weight on each ratio value. The weights were determined using the whole-screens collection containing at this moment 838 experiments. We supposed that the collection was big enough and the screens sufficiently independent for globally showing no effect on any gene (or spot). Assuming that, we calculated the mean ratio for each spot, expecting a zero value for it. A nonzero value indicates a systematic bias on the spot. We used each nonzero mean spot value to calculate a correction factor, or weight, that would decrease the effect of unwanted biases. We defined empirically the weight applied to a given spot value through the following formula:

$$x_j = \frac{1}{n}\sum_{i=0}^{n} \text{ratio}_{ij}$$

$$\mu = \frac{1}{n * m}\sum_{j=0}^{m}\sum_{i=0}^{n} \text{ratio}_{ij}$$

$$\sigma = \frac{1}{n * m}\sum_{j=0}^{m}\sum_{i=0}^{n} \left(\text{ratio}_{ij} - \mu\right)^2.$$

Weight determination is for the jth spot, log-ratio$_{ij}$ is the log-ratio value of the jth spot of the ith experiment, n is the total number of experiments, and m is the total number of spots on a microarray.

We obtained weights for the spots, which are near 1 for a mean spot log-ratio value near 0 and a weight near 0 for a mean spot log-ratio value showing an important bias. Advantages of this method are a progressive way for calculating weights and no need for the use of a threshold for choosing which spot needs to be corrected. We used the same method to determine a weight for batch correction but using x_j as the mean of the j spot ratios of the series and μ and σ as the mean and SD of the ratios for the same j spot in all of the experiments of the screen collection:

$$x_j = \frac{1}{p}\sum_{k=0}^{p} \text{log-ratio}_{kj}$$

$$\mu_j = \frac{1}{m}\sum_{i=0}^{m} \text{log-ratio}_{ij}$$

$$\sigma_j = \frac{1}{m}\sum_{i=0}^{p} \left(\text{log-ratio}_{ij} - \mu_j\right)^2.$$

Weight determination is for the jth spot, log-ratio$_{kj}$ is the log-ratio value of the jth spot of the kth experiment, p is the total number of experiments in the batch, and m is the total number of spots on a microarray.

For each mutant strain, the median of the batch and globally corrected up and down values were calculated (Dataset S1).

Tandem Affinity Purifications. Cells expressing C-terminal TAP fusions (4) of each bait protein were cultivated in 2 L of rich medium (YPD) until OD_{600} = 1, and cultures were centrifuged at 4 °C, rinsed in cold water, and frozen at −80 °C. Cell pellets were thawed on ice, resuspended in 20 mL of lysis buffer (20 mM HepesK, pH 7.4, 100 mM KOAc, 10 mM $MgCl_2$; Sigma-Aldrich protease inhibitors), and broken with a French press (twice at 1,200 psi), and lysates were clarified by centrifugation at 15,000 × g for 20 min at 4 °C. Supernatants were collected with addition of 0.5% Triton X100, 1/2,500 antifoam, and 50 μL of magnetic beads coupled with IgG, and binding was performed on a wheel for 40 min at 4 °C (5). Beads were collected on a magnet to discard lysis buffer and were washed three times in washing buffer containing 0.5% Triton X100 and three times in washing buffer without Triton X100 (20 mM HepesK, pH 7.4, 100 mM KOAc, 10 mM $MgCl_2$, 1 mM DTT). Elution was performed in 400 μL of washing buffer (without Triton X100) with 10 μL of AcTEV Protease (Life Technologies) for 1 h 45 min at 17 °C. Eluted beads were discarded on a magnet and eluate proteins were precipitated by the methanol/chloroform technique (6). One-fifth of the precipitated proteins were resuspended in 10 μL sample buffer [100 mM Tris·HCl, pH 6.8, 20% (vol/vol) glycerol, 0.02% bromophenol blue, 8% (wt/vol) SDS, 100 mM DTT], migrated on acrylamide NuPAGE Novex 4–12% Bis-Tris gels (Life Technologies), and analyzed by silver staining using ethanol (7). The rest of the eluates were analyzed by mass spectrometry.

Mass Spectrometry Experiment and Data Analysis. Briefly, protein samples were treated with Endoprotease Lys-C and Trypsin (Trypsin Gold Mass Spec Grade; Promega). Peptide samples were desalted by OMIX C18 pipette tips (Agilent Technologies) and then analyzed on an LTQ-Orbitrap velos instrument (Thermo Fisher Scientific) connected online to an Ultimate 3000 nanoHPLC system (Dionex; Thermo Fisher Scientific). Raw mass spectrometry (MS) data from the LTQ-Orbitrap were analyzed using MaxQuant software (8, 9) version 1.3.0.5, which uses the

Andromeda search engine (10). Only protein identifications based on a minimum of two peptides were selected for further quantitative studies. Bioinformatic analysis of the MaxQuant/Andromeda work flow output and the analysis of the abundances of the identified proteins were performed with the Perseus module (version 1.30.4, available as part of the MaxQuant suite). After data processing, label-free quantification (LFQ) values from the "proteinGroups.txt" output file of MaxQuant were further analyzed. To distinguish specifically interacting proteins from the background, protein abundances were compared between sample and control groups, using the Student's *t* test statistic, and the results were represented as volcano plots (11).

Northern Blots. Total RNAs were extracted using the hot phenol procedure from six OD_{600} exponential cultures. Two micrograms of total RNA was separated on a 1% agarose gel, transferred on a Hybond N^+ membrane, and probed with ^{32}P-radiolabeled oligonucleotides that are specific to ProtA mRNA (YY233 5′-TCTACTTTCGGCGCCTGAGCATCATTT-3′) or Scr1 RNA (YY234 5′-GTCTAGCCGCGAGGAAGG-3′).

mRNA Degradation Assays. Yeast strains transformed with the ProtA-Non-Stop reporter gene (pAV184) were grown in the presence of 2% galactose at 30 °C. To switch off the expression of the reporter gene that is under the control of a *GAL1* promoter, cells were switched to glucose media at $T = 0$ min. Aliquots were withdrawn from the cell culture, for each indicated time point. Total RNAs were extracted and analyzed by Northern blot as described above.

1. Puig O, et al. (2001) The tandem affinity purification (TAP) method: A general procedure of protein complex purification. *Methods* 24(3):218–229.
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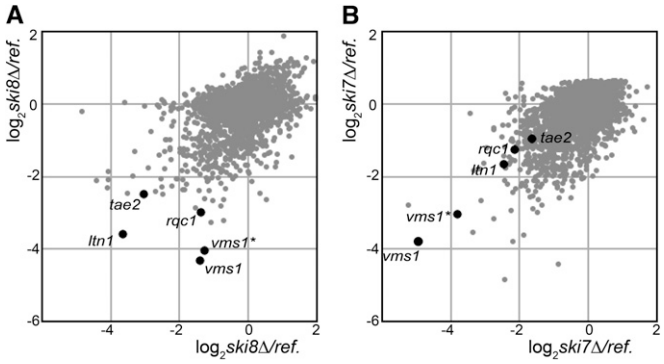

Fig. S1. *SKI7* and *SKI8* are functionally linked to translation-associated element 2 (*TAE2*), ribosomal quality control 1 (*RQC1*), and *LTN1*. (*A*) Comparison of two independent GIM screens, using *ski8*Δ as a query gene. The experiment and analysis were done as described in Fig. 1 in the main text. (*B*) Comparison of two independent GIM screens, using *ski7*Δ as query gene. The experiment and analysis were done as described in Fig. 1 in the main text.

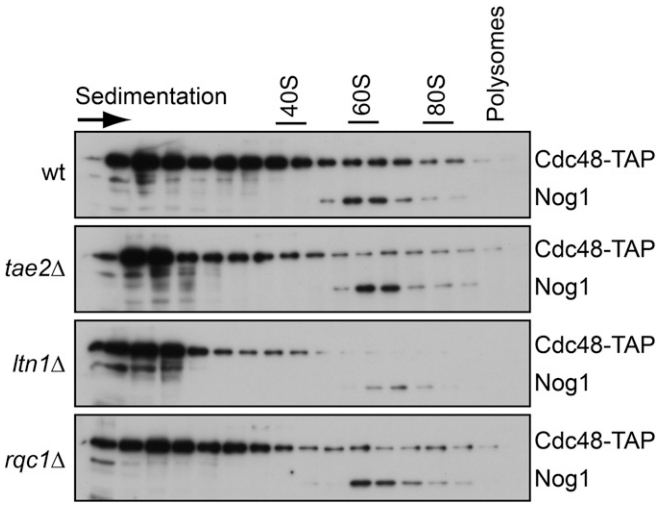

Fig. S2. Sedimentation of the AAA-ATPase Cdc48-TAP fusion protein in the absence of Ltn1, Tae2, or Rqc1 protein. Total cellular extracts from cells expressing Cdc48-TAP in WT or in the absence of Ltn1, Tae2, and Rqc1 were separated on a 10–30% sucrose gradient as described in Fig. 2 in the main text.

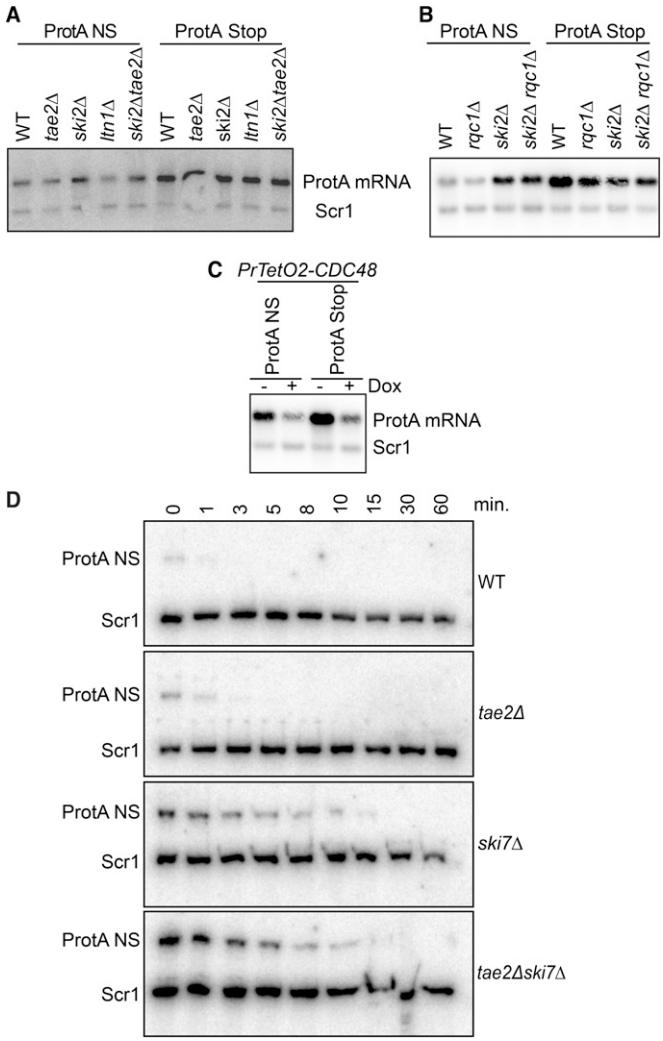

Fig. S3. The level of ProtA-Non-Stop mRNA is not affected in the absence of Tae2, Ltn1, or Rqc1 as well as upon depletion of Cdc48. (*A–D*) Northern blot analysis of total mRNAs using probes against ProtA mRNA or Scr1 RNA as indicated in *SI Materials and Methods*. Plasmid-borne reporter constructs expressing ProtA-NS (pAV184) or ProtA-Stop (pAV183) are described in Tables S1 and S2. (*A*) ProtA-Non-Stop mRNA does not accumulate in the *tae2*Δ mutant. ProtA-NS (Non-Stop) and ProtA-Stop mRNA levels in WT, *tae2*Δ, *ski2*Δ, *ltn1*Δ, and *ski2*Δ*tae2*Δ cells were analyzed by Northern blot. (*B*) *rqc1*Δ does not accumulate Non-Stop ProtA mRNA. ProtA-NS and ProtA-Stop mRNAs were analyzed as described in *A*. (*C*) Depletion of Cdc48 does not accumulate ProtA-NS mRNA. The *PrTetO2-CDC48* strain, where *CDC48* is under the control of a tetracyclin-repressible promoter, was incubated in the absence or the presence of Doxycyclin (5 μg/mL) for 13 h. ProtA-NS or ProtA-Stop mRNAs were detected as indicated in *A*. (*D*) The half-life of Non-Stop ProtA mRNA is not affected by the absence of Tae2. Shown is Northern blot analysis of ProtA-NS mRNA amounts during an expression shutoff time course, in WT, *tae2*Δ, *ski7*Δ, or *ski7*Δ*tae2*Δ cells.

Table S1. Yeast strains used in this study

Strains	Genotypes	Source
Wild type		
BY4741	*MATa, ura3Δ0, his3Δ1, leu2Δ0, met15Δ0*	(1)
BY4742	*MATα, ura3Δ0, his3Δ1, leu2Δ0, lys2Δ0*	(1)
Fusion proteins		
LMA1949	As *BY4741*, ***Tae2-TAP****:HIS3MX6*	(2)
LMA1951	As *BY4741*, ***Rqc1-TAP****:HIS3MX6*	(2)
LMA2195	As *BY4741*, ***Rpl16a-TAP****:HIS3MX6*	(2)
LMA2196	As *BY4741*, ***Cdc48-TAP****:HIS3MX6,*	(2)
LMA2544	As *BY4741*, ***Ltn1-TAP****:HIS3MX6*	(2)
LMA2685	As *BY4741*, ***Tae2-TAP****:HIS3MX6,* ***ltn1****Δ::KANMX4*	This study
LMA2686	As *BY4741*, ***Tae2-TAP****:HIS3MX6,* ***rqc1****Δ::KANMX4*	This study
LMA2688	As *BY4741*, ***Rqc1-TAP****:HIS3MX6,* ***tae2****Δ::KANMX4*	This study
LMA2689	As *BY4741*, ***Rqc1-TAP****:HIS3MX6,* ***ltn1****Δ::KANMX4*	This study
LMA2711	As *BY4741*, ***Cdc48-TAP****:HIS3MX6,* ***ltn1****Δ::URA3*	This study
LMA2712	As *BY4741*, ***Cdc48-TAP****:HIS3MX6,* ***tae2****Δ::KANMX4*	This study
LMA2713	As *BY4741*, ***Cdc48-TAP****:HIS3MX6,* ***rqc1****Δ::KANMX4*	This study
Mutants		
LMA1713	As *BY4741*, ***tae2****Δ::HIS3*	This study
LMA1740	As *BY4741*, ***ski2****Δ::URA3*	This study
LMA1986	As *BY4741*, ***ltn1****Δ::URA3*	This study
LMA1741	As *BY4741*, ***tae2****Δ::HIS3,* ***ski2****Δ::URA3*	This study
LMA2026	As *BY4741*, ***tae2****Δ::HIS3,* ***ltn1****Δ::URA3*	This study
LMA1744	As *BY4741*, ***tae2****Δ::URA3*	This study
LMA2203	As *BY4741*, ***ltn1****Δ::HIS3*	This study
LMA2204	As *BY4741*, ***ltn1****Δ::HIS3,* ***ski2****Δ::URA3*	This study
LMA2714	As *BY4741*, ***rqc1****Δ::HIS3*	This study
LMA2715	As *BY4741*, ***rqc1****Δ::HIS3,* ***ski2****Δ::URA3*	This study
LMA2649	As *BY4741, PrTetO2* ***CDC48****:: KANMX4*	This study
LMA843	As *BY4742, yel068cΔ::PrαNATMX4,ydl242wΔ::KANMX4*	This study
LMA836	As *BY4742,* ***tae2****Δ::PrαNATMX4, yel068cΔ::KANMX4*	This study
LMA837	As *BY4742,* ***ski2****Δ::KANMX4, yel068cΔ::PrαNATMX4*	This study
LMA832	As *BY4742,* ***ski7****Δ::KANMX4,* ***tae2****Δ::PrαNATMX4*	This study
LMA839	As *BY4742,* ***ski7****Δ::KANMX4, yel068cΔ::PrαNATMX4*	This study
LMA1921	As *BY4742,* ***ltn1****Δ::KANMX4, yel068cΔ::PrαNATMX4*	This study
LMA830	As *BY4742,* ***tae2****Δ::PrαNATMX4,* ***ski2****Δ::KANMX4*	This study
LMA1920	As *BY4742,* ***tae2****Δ::PrαNATMX4,* ***ltn1****Δ::KANMX4*	This study
LMA1997	As *BY4742,* ***ski2****Δ::PrαNATMX4,* ***ltn1****Δ::KANMX4*	This study
LMA2001	As *BY4742,* ***ski7****Δ::PrαNATMX4,* ***ltn1****Δ::KANMX4*	This study
LMA2306	As *BY4742,* ***rqc1****Δ::KANMX4, yel068cΔ::PrαNATMX4*	This study
LMA1996	As *BY4742,* ***rqc1****Δ::KANMX4,,* ***ski2****Δ::PrαNATMX4*	This study
LMA2709	As *BY4742, KANMX4:PrTetO2:****CDC48***	This study
LMA2719	As *BY4742, PrαNATMX4:PrTetO2:****CDC48***	This study
LMA2746	As *BY4742, PrαNATMX4:PrTetO2:****CDC48****,* ***ski2****Δ::KANMX4*	This study
GIM query strains		
GIM123	As *BY4741,* ***ski2****Δ::PrαNATMX4*	This study
GIM155	As *BY4741,* ***tae2****Δ::PrαNATMX4*	This study
GIM225	As *BY4741,* ***ski8****Δ::PrαNATMX4*	This study
GIM477	As *BY4741,* ***ski7****Δ::PrαNATMX4*	This study
GIM479	As *BY4741,* ***rqc1****Δ::PrαNATMX4*	This study
GIM503	As *BY4741,* ***ski3****Δ::PrαNATMX4*	This study
GIM448	As *BY4741,* ***ltn1****Δ::PrαNATMX4*	This study

The modified genes of interest are indicated in bold.

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Table S2. Yeast plasmids used in this study

Plasmid	Markers	Source
pGID1	URA3, HygR	(1)
pAV183	URA3	(2)
pAV184	URA3	(2)
pAV188	URA3	(2)
pTAP Stop	URA3	This study
pTAP Non-Stop	URA3	This study

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Table S3. Antibodies used for immunodetection

Target	Antibody	Dilution
TAP-tagged proteins	Peroxydase anti-peroxidase complex (PAP) (Sigma-Aldrich)	1/10,000
HA-tagged proteins	Anti-HA peroxidase (Roche)	1/500
Cdc48	Rabbit polyclonal, gift of Alexander Buchberger (University of Würzburg, Germany)	1/4,000
Nog1	Rabbit polyclonal	1/5,000
Rps8	Rabbit polyclonal, gift of Georgio Dieci (Università degli Studi di Parma, Italy)	1/5,000
G6PDH	Rabbit polyclonal	1/100,000
Ubiquitin	P4D1, monoclonal (Covance)	1/1,000

Nog1 is a pre60S factor which marks the 60S fractions. Rps8 is a ribosomal protein of the small subunit which marks 40S, 80S and polysomal fractions. G6PDH (Glucose-6-Phosphate-Deshydrogenase) is used as loading control.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)
Dataset S3 (XLSX)
Dataset S4 (XLSX)