Supporting information

Supplemental Materials and Methods

Plasmid constructions

Plasmid-borne TAP-Stop and TAP-Non-Stop reporter constructs were obtained by PCR-based yeast recombination, respectively from Stop ProtA (pAV183) and Non-Stop ProtA (pAV184) plasmids (Table S2). For this purpose, pAV183 or pAV184 were linearized with *Xba*I and *Bam*HI, and transformed in yeast together with the PCR-amplified TAP-tag. The latest, an N-terminus fusion, was PCR-amplified from pBS1761 (1) with JM028 (5'-GGAGAAAAAACCCCGGATCATAATCGGCCGCTCTAGAATGGCAGGCCTTGCGCAACACG-3') and JM029 (5'- GATAAGAAAGCAACACCTGGCAATTCCTTACCGGATCCTAGGGCGAATTGGGTACCG-GG-3') oligos.

GIM screens

The genetic interactions mapping 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 came either from a parallel screen done with the deletion of *YEL068C* 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 (Exploratory analysis for two-color spotted microarray data. R package version 1.34.0., by Yee Hwa Yang with contributions from Agnes Paquet and Sandrine Dudoit, 2009) independently for the UP and DOWN measured sets of ratios (corresponding to the two barcodes situated in the 5' and 3' region of the KANMX 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 non-zero value indicates a systematic bias on

the spot. For each non-zero value we calculated a weight to apply on each ratio of a given spot. The weight was determined as the ratio of 2 probability densities. The first is the probability density for the mean of all ratios for a spot j (x_j) on a gauss distribution having for parameters μ and σ respectively determined as the mean and the standard deviation of all the spots values. The second is the probability density on the same distribution of the value μ. As shown on Equation 1 the ratio can be simplified as a more simple expression.

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

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

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

$$\text{weight}_j = \frac{\frac{1}{\sqrt{2\pi\sigma^2}}e^{-\frac{(x_j-\mu)^2}{2\sigma^2}}}{\frac{1}{\sqrt{2\pi\sigma^2}}e^{-\frac{(\mu-\mu)^2}{2\sigma^2}}} = e^{-\frac{(x_j-\mu)^2}{2\sigma^2}}$$

Equation 1: weight determination for the j^{th} spot. log-ratio$_{ij}$ is the log-ratio value of the j^{th} spot of the i^{th} experiment, n the total number of experiments and m the total number of spot on a microarray.

Therefore we obtained weights for the spots which are near to 1 for a mean spot log-ratio value near to 0 and a weight near 0 for a mean spot log-ratio value showing a an important bias. Advantages of this method are a progressive way for calculating weights and avoid 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 as x_j the mean of the j spot ratios of the series, and as μ and σ the mean and standard deviation of the ratios for the same j spot in all the experiments of the screen collection (Equation 2).

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

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

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

$$\text{weight}_j = \frac{\frac{1}{\sqrt{2\pi{\sigma_j}^2}} e^{-\frac{(x_j-\mu_j)^2}{2\sigma^2}}}{\frac{1}{\sqrt{2\pi{\sigma_j}^2}} e^{-\frac{(\mu_j-\mu_j)^2}{2{\sigma_j}^2}}} = e^{-\frac{(x_j-\mu_j)^2}{2{\sigma_j}^2}}$$

Equation 2: weight determination for the j^{th} spot. log-ratio$_{kj}$ is the log-ratio value of the j^{th} spot of the k^{th} experiment, p the total number of experiments in the batch and m the total number of spot on a microarray

Tandem Affinity Purifications

Cells expressing C-terminus TAP fusions (3) of each bait protein were cultivated in 2L of rich medium (YPD) until OD_{600}=1, cultures were centrifuged at 4°C, rinsed in cold water and frozen at -80°C. Cell pellets were thawed on ice, resuspended in 20mL of Lysis Buffer (20mM HepesK pH7.4, 100mM KOAc, 10mM $MgCl_2$, Sigma-Aldrich protease inhibitors) and broken with a French Press (twice at 1200 psi), lysates were clarified by centrifugation at 15000g for 20min at 4°C. Supernatants were collected with addition of Triton X100 0,5%, antifoam 1/2500, and 50µL of magnetic beads coupled with IgG, and binding was performed on a wheel during 40 minutes at 4°C (4). Beads were collected on a magnet to discard lysis buffer, and were washed 3 times in Washing Buffer containing Triton X100 0,5% and 3 times in Washing Buffer without Triton X100 (20mM HepesK pH7.4, 100mM KOAc, 10mM $MgCl_2$, 1mM DTT). Elution was performed in 400µL of Washing Buffer (without Triton X100) with 10µL of AcTEV Protease (Life Technologies) during 1h45 at 17°C. Eluted beads were discarded on a magnet and eluate proteins were precipitated by the methanol/chloroform technique (5). A fifth of the precipitated proteins was resuspended in 10µL Sample Buffer (100mM Tris-HCl pH6.8, 20% glycerol, 0,02% bromophenol blue, 8% SDS, 100mM DTT), migrated on acrylamide NuPAGE Novex 4-12% Bis-Tris gels (Life Technologies) and analyzed by silver staining using ethanol (6). The rest of the eluates was 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 a LTQ-Orbitrap velos instrument (Thermo

Fisher Scientific, Bremen) connected online to an Ultimate 3000 nanoHPLC system (Dionex, Thermo Fisher Scientific). Raw MS data from the LTQ-Orbitrap were analyzed using the MaxQuant software (7) (8) version 1.3.0.5, which uses the Andromeda search engine (9). 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 analysed. 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 (10).

Northern Blots

Total RNAs were extracted using hot phenol procedure from 6 OD_{600} exponential cultures. 2 µg of total RNA were separated on 1% agarose gel, transferred on Hybond N^+ membrane and probed with ^{32}P radiolabelled oligonucleotides that are specific of ProtA mRNA (YY233 5'-TCTACTTTCGGCGCCTGAGCATCATTT-3') or Scr1 RNA (YY234 5'- GTCTAGCCGCGAGGAAGG-3').

mRNA degradation assays

Yeast strains transformed with ProtA-Non-Stop reporter gene (pAV184) were grown in the presence of Galactose 2% 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.

Supplementary Figure Legends

Figure S1. *SKI7* and *SKI8* are functionally linked to *TAE2*, *RQC1* and *LTN1*

(A) Comparison of two independent GIM screens using *ski8*Δ as query gene. The experiment and analysis were done as described in **Fig. 1.**

(B) Comparison of two independent GIM screens using *ski7*Δ as query gene. The experiment and analysis were done as described in **Fig. 1.**

Figure S2. 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) ProtA-Non-Stop mRNA does not accumulate in *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. *PrTetO2-CDC48* strain, where *CDC48* is under the control of a tetracyclin-repressible promoter, was incubated in absence or in 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. Northern blot analysis of ProtA-NS mRNA amounts during an expression shut-off time-course, in WT, *tae2*Δ, *ski7*Δ or *ski7*Δ*tae2*Δ cells.

(A-D). Northern blot analysis of total mRNAs using probes against ProtA mRNA or Scr1 RNA as indicated in supplementary materials and methods. Plasmid-borne reporter constructs expressing ProtA-NS (pAV184) or ProtA-Stop (pAV183) are described on Table S2.

Fig. S3 Sedimentation of Cdc48-TAP in absence of Ltn1, Tae2 or Rqc1

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.

Table S1. Yeast strains used in this study

Strains	Genotypes	References
Wild type		
BY4741	*MATa, ura3Δ0, his3Δ1, leu2Δ0, met15Δ0*	(11)
BY4742	*MATa, ura3Δ0, his3Δ1, leu2Δ0, lys2Δ0*	(11)
Fusion proteins		
LMA1949	as *BY4741*, ***Tae2-TAP****:HIS3MX6*	(12)
LMA1951	as *BY4741*, ***Rqc1-TAP****:HIS3MX6,*	(12)
LMA2195	as *BY4741*, ***Rpl16a-TAP****:HIS3MX6*	(12)
LMA2196	as *BY4741*, ***Cdc48-TAP****:HIS3MX6,*	(12)
LMA2544	as *BY4741*, ***Ltn1-TAP****:HIS3MX6*	(12)
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*, *yel068c*Δ*::PraNATMX4,ydl242w*Δ*::KANMX4*	This study
LMA836	as *BY4742*, ***tae2***Δ*::PraNATMX4, yel068c*Δ*::KANMX4*	This study
LMA837	as *BY4742*, ***ski2***Δ*::KANMX4, yel068c*Δ*::PraNATMX4*	This study
LMA832	as *BY4742*, ***ski7***Δ*::KANMX4,* ***tae2***Δ*::PraNATMX4*	This study
LMA839	as *BY4742*, ***ski7***Δ*::KANMX4, yel068c*Δ*::PraNATMX4*	This study
LMA1921	as *BY4742*, ***ltn1***Δ*::KANMX4, yel068c*Δ*::PraNATMX4*	This study
LMA830	as *BY4742*, ***tae2***Δ*::PraNATMX4,* ***ski2***Δ*::KANMX4*	This study
LMA1920	as *BY4742*, ***tae2***Δ*::PraNATMX4,* ***ltn1***Δ*::KANMX4*	This study
LMA1997	as *BY4742*, ***ski2***Δ*::PraNATMX4,* ***ltn1***Δ*::KANMX4*	This study
LMA2001	as *BY4742,* ***ski7***Δ*::PraNATMX4,* ***ltn1***Δ*::KANMX4*	This study
LMA2306	as *BY4742*, ***rqc1***Δ*::KANMX4, yel068c*Δ*::PraNATMX4*	This study
LMA1996	as *BY4742*, ***rqc1***Δ*::KANMX4, ,* ***ski2***Δ*::PraNATMX4*	This study
LMA2709	as *BY4742*, *KANMX4:PrTetO2:CDC48*	This study
LMA2719	as *BY4742*, *PraNATMX4:PrTetO2:CDC48*	This study
LMA2746	as *BY4742*, *PraNATMX4:PrTetO2:CDC48,* ***ski2***Δ*::KANMX4*	This study

GIM query strains

GIM123	as *BY4741,* ***ski2***Δ*::PraNATMX4*	This study
GIM155	as *BY4741,* ***tae2***Δ*::PraNATMX4*	This study
GIM225	as *BY4741,* ***ski8***Δ*::PraNATMX4*	This study
GIM477	as *BY4741,* ***ski7***Δ*::PraNATMX4*	This study
GIM479	as *BY4741,* ***rqc1***Δ*::PraNATMX4*	This study
GIM503	as *BY4741,* ***ski3***Δ*::PraNATMX4*	This study
GIM448	as *BY4741,* ***ltn1***Δ*::PraNATMX4*	This study

Table S2. Plasmids used in this study

Plasmid	Markers	Reference
pGID1	URA3, HygR	(2)
pAV183	URA3	(13)
pAV184	URA3	(13)
pAV188	URA3	(13)
pTAP Stop	URA3	This study
pTAP Non-Stop	URA3	This study

Table. S3. Antibodies used for immunodetection

Target	Antibody	Dilution
TAP-tagged proteins	PAP (Peroxydase anti-Peroxidase complex), Sigma-Aldrich	1/10,000
HA-tagged proteins	anti-HA Peroxidase, Roche	1/500
Cdc48	Rabbit Polyclonal, Gift of Alexander Buchberger	1/4,000
Nog1	Rabbit Polyclonal	1/5,000
Rps8	Rabbit Polyclonal, Gift of Georgio Dieci	1/5,000
G6PDH	Rabbit Polyclonal	1/100,000
Ubiquitin	P4D1, monoclonal, Covance	1/1,000

References

1. Puig O et al. (2001) The Tandem Affinity Purification (TAP) Method: A General Procedure of Protein Complex Purification. *Methods* 24:218–229.

2. Decourty L et al. (2008) Linking functionally related genes by sensitive and quantitative characterization of genetic interaction profiles. *Proc Natl Acad Sci U S A* 105:5821–6.

3. Rigaut G et al. (1999) A generic protein purification method for protein complex characterization and proteome exploration. *Nat Biotechnol* 17:1030–2.

4. Oeffinger M et al. (2007) Comprehensive analysis of diverse ribonucleoprotein complexes. *Nat Methods* 4:951–6.

5. Wessel D, Flügge UI (1984) A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. *Anal Biochem* 138:141–3.

6. Yan JX et al. (2000) A modified silver staining protocol for visualization of proteins compatible with matrix-assisted laser desorption/ionization and electrospray ionization-mass spectrometry. *Electrophoresis* 21:3666–72.

7. Cox J, Mann M (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol* 26:1367–72.

8. Cox J et al. (2009) A practical guide to the MaxQuant computational platform for SILAC-based quantitative proteomics. *Nat Protoc* 4:698–705.

9. Cox J et al. (2011) Andromeda: a peptide search engine integrated into the MaxQuant environment. *J Proteome Res* 10:1794–805.

10. Hubner NC, Mann M (2011) Extracting gene function from protein-protein interactions using Quantitative BAC InteraCtomics (QUBIC). *Methods* 53:453–9.

11. Brachmann CB et al. (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* 14:115–32.

12. Ghaemmaghami S et al. (2003) Global analysis of protein expression in yeast. *Nat New Biol* 425:737–41.

13. Van Hoof A, Frischmeyer P a, Dietz HC, Parker R (2002) Exosome-mediated recognition and degradation of mRNAs lacking a termination codon. *Science* 295:2262–4.