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’-GGAGAAAAAACCCGGATCATAATCGGCCGCTCTAGAATGGCAGGCCTTGCGCAACACG-3’) and JM029 (5’-GATAAGAAAAGCAACACCTGGCAATTCCTTACCGGATCCTAGGGCGAATTGGGTACCG-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 $\mu$ and $\sigma$ 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 $\mu$. As shown on Equation 1 the ratio can be simplified as a more simple expression.

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

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

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

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

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 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 $\mu$ and $\sigma$ the mean and standard deviation of the ratios for the same $j$ spot in all the experiments of the screen collection (Equation 2).

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

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

$$\sigma_j = \frac{1}{m} \sum_{i=0}^{m} (\text{log-ratio}_{ij} - \mu_j)^2$$
weight_j = \frac{1}{\sqrt{2\pi \sigma_j^2}} e^{-\frac{(x_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\text{th} spot. log-ratio_{kj} is the log-ratio value of the j\text{th} spot of the k\text{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\textsubscript{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\textsubscript{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$\text{600}$ 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’-TCTACTTTCCGCGCCTGAGCATTT-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 *ski8Δ* as query gene. The experiment and analysis were done as described in Fig. 1.
(B) Comparison of two independent GIM screens using *ski7Δ* 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 *tae2Δ* mutant. ProtA-NS (Non-Stop) and ProtA-Stop mRNA levels in WT, *tae2Δ*, *ski2Δ*, *ltn1Δ* and *ski2Δtae2Δ* cells were analyzed by Northern blot.
(B) *rqc1Δ* 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, *tae2Δ*, *ski7Δ* or *ski7Δtae2Δ* 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

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This study

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This study

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This study

LMA2715 as *BY4741, rqc1Δ::HIS3, ski2Δ::URA3*

This study

LMA2649 as *BY4741, PrTetO2 CDC48:: KANMX4*

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LMA843 as *BY4742, yel068cΔ::PraNATMX4, ydl242wΔ::KANMX4*

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This study

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This study

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This study

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This study

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This study

LMA2719 as *BY4742, PraNATMX4:PrTetO2: CDC48*

This study

LMA2746 as *BY4742, PraNATMX4:PrTetO2: CDC48, ski2Δ::KANMX4*

This study

**GIM query strains**

GIM123 as *BY4741, ski2Δ::PraNATMX4*

This study

GIM155 as *BY4741, tae2Δ::PraNATMX4*

This study

GIM225 as *BY4741, ski8Δ::PraNATMX4*

This study

GIM477 as *BY4741, ski7Δ::PraNATMX4*

This study

GIM479 as *BY4741, rqc1Δ::PraNATMX4*

This study

GIM503 as *BY4741, ski3Δ::PraNATMX4*

This study

GIM448 as *BY4741, ltn1Δ::PraNATMX4*

This study
Table S2. Plasmids used in this study

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Table. S3. Antibodies used for immunodetection

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References


