Supplementary Information

Protein quality control at the inner nuclear membrane

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1. Supplementary Methods

Here we describe the construction of a genome-wide library of *S. cerevisiae* strains each expressing a different protein tagged with a tFT, hereafter referred to as tFT library.

tFT library construction: overview and workflow

First, we selected a tFT suitable for studies of protein dynamics in this organism. The mean and median half-life of the *S. cerevisiae* proteome is ~43 min, as determined with cycloheximide chase experiments using strains expressing proteins fused to the TAP tag[1]. A tFT composed of the slower maturing red fluorescent protein mCherry[2] and the faster maturing green fluorescent protein sfGFP[3] can be used to study the degradation of proteins with half-lives between ~10 min and ~8 h[4] (see also Supplementary Note 1). The dynamics of most yeast proteins could thus be analyzed with this tFT. Therefore, we constructed a module for seamless protein tagging with the mCherry-sfGFP timer (described in section 1.1).

Second, we selected a strain background for library construction. Various genome-wide libraries of yeast strains carrying genome manipulations such as gene deletions or tagged loci have been constructed over the last fifteen years[5,6]. With synthetic genetic array (SGA) technology, different genome manipulations present in such libraries can be combined using automated procedures[7], greatly expanding the potential applications of each individual library. Therefore, we decided to construct the tFT library in a strain background compatible with SGA. We introduced the genetic elements required for seamless protein tagging into the SGA entry strain Y8205[8], generating the library background strain yMaM330 (described in section 1.2).

Third, we selected the open reading frames (ORFs) to be tagged with the tFT. We sought to reduce potential artifacts (e.g. protein mislocalization) caused by tagging and avoid tagging proteins localized to subcellular compartments that affect the properties of the timer. Moreover, we decided to exclude proteins unlikely to be expressed under standard yeast growth conditions. Using information from previous systematic protein tagging enterprises[9-11] and annotations collected in the *Saccharomyces* Genome Database (http://www.yeastgenome.org), we selected a total of 4081 ORFs (described in section 1.3).

Next, we proceeded to tag each ORF at the respective endogenous chromosomal locus with the mCherry-sfGFP timer. Strain manipulations were automated and performed in 96-well format whenever possible. Each ORF was assigned unique plate and well coordinates to facilitate the construction process. Following this coordinate system, ORF-specific primers required for tagging by PCR targeting (described in section 1.4) were obtained from IDT (Integrated DNA Technologies) in 96-well format. The module for seamless protein tagging with the mCherry-sfGFP timer was integrated into each selected genomic locus using conventional PCR targeting[12] and lithium acetate transformation of yeast[13]. The protocols were optimized for 96-well format such that up to 480 different strains could be constructed in parallel. Briefly, the tagging module was PCR amplified with ORF-specific primers containing short overhangs homologous to each genomic locus (described in section 1.5). Competent yMaM330 cells, prepared from a single colony, were transformed with each PCR product (described in section 1.6). Each transformation mixture (specific for a different ORF) was then manually plated onto a separate 9 cm plate with selective agar medium and incubated at 30°C until distinct colonies were visible. From each plate, six clones were manually purified for single colonies and four purified clones were subsequently inoculated into four separate 96-well plates according to the coordinates assigned to each ORF. Therefore, starting with 45 96-well plates of ORF-specific primers for PCR targeting, we obtained 45x4 plates of purified clones grown in liquid medium that were stored at -80°C at the end of the first round of transformations. Verification primers were obtained from IDT (Integrated DNA Technologies) in 96-well format using the same coordinate system (described in section 1.7) and used to test for correct integration of the tagging module into each locus by diagnostic PCR (described in section 1.8). For a subset of ORFs, the PCR gave unclear or ambiguous results and was therefore repeated using new validation primers (described in section 1.7). ORFs for which correct integration of the tagging module could not be confirmed for at least 2 clones were taken through a

second round of transformations using ORF-specific primers with longer overhangs homologous to each genomic locus (described in section 1.4). ORFs for which correct integration of the tagging module could not be confirmed for at least 2 clones after two rounds of transformation were taken through a third and final round of manual transformations. Finally, fluorescence intensities of all strains were measured with a fluorescence plate reader to identify clones validated by diagnostic PCR that nevertheless failed to express a tFT fusion due to mutations in the ORF-specific primers used for PCR targeting (described in section 1.9). In total, we obtained at least 2 validated clones for 3952 ORFs, 1 validated clone for 92 ORFs and no validated clones for 37 ORFs (Supplementary Table 2).

1.1. Tagging strategy

Studies in *S. cerevisiae* suggest that N-terminal residues of most proteins are likely to encode signals regulating protein turnover[14,15]. Although a variety of signals can also occur at the C-terminus of many proteins, in the absence of a systematic comparison between the effects of N- and C-terminal tagging, the tFT library was constructed using C-terminal tagging. We applied a seamless tagging approach that reduces the impact of introducing foreign sequences into the yeast genome and allows expression of protein fusions for their endogenous chromosomal loci under the control of both upstream and downstream gene regulatory elements[16]. We designed a module for seamless protein tagging with the mCherry-sfGFP timer that contains the following elements: S3 primer annealing site, *mCherry* sequence, I-SceI cut site, terminator sequence of the *CYC1* gene from *Saccharomyces paradoxus*, *URA3* gene with endogenous promoter and terminator from *S. cerevisiae*, second I-SceI cut site, *mCherry*$^{\Delta N}$*-sfGFP* sequence coding for a C-terminal fragment of mCherry followed by sfGFP, S2 primer annealing site (plasmid pMaM168 in Supplementary Table 5, Extended Data Fig. 3a). After integration of this module into a locus of interest using conventional PCR targeting, an mCherry-tagged protein is expressed. All auxiliary sequences required for clonal selection can then be excised from the genome through conditional expression of the I-SceI endonuclease, leading to the expression of an mCherry-sfGFP-tagged protein (Extended Data Fig. 3a). The seamless excision process is practically error free for the majority of yeast genes and thus does not require validation[16].

1.2. Library background strain

The library background strain is based on the SGA entry strain Y8205[8]. The Y8205 strain (MATα *can1*Δ::*STE2pr-SpHIS5 lyp1*Δ::*STE3pr-LEU2 his3*Δ*1 leu2*Δ*0 ura3*Δ*0*) can be crossed with strains of the opposite mating type carrying genome manipulations such as deletions of non-essential genes[17], temperature-sensitive (*ts*) alleles[18,19] or decreased abundance by mRNA perturbation (DAmP) alleles[20] of essential genes. Importantly, this strain contains the genetic elements (*can1*Δ::*STE2pr-SpHIS5 lyp1*Δ::*STE3pr-LEU2*) necessary for selection of haploid double mutant progeny during the SGA procedure.

The seamless tagging strategy used to construct the tFT library relies on conditional expression of the I-SceI endonuclease[16] (Extended Data Fig. 3a). We constructed the plasmid pND32-8 carrying the *I-SCEI* sequence, placed under the control of the galactose-inducible promoter from the *GAL1* gene, and the nourseothricin resistance gene *natNT2*[12] (Supplementary Table 5). The *GAL1pr-I-SCEI-natNT2* sequence was then integrated into the *leu2*Δ*0* locus in the Y8205 strain by PCR targeting with primers ISce1-Nat-A (tcaaaaagatccatgtataatcttcattattacagccctcttgacttatttcaggaaagttt cggaggag) and ISce1-Nat-B (gtttcgtctaccctatgaacatattccattttgtaatttcgtgtcgcaagaattttcgttttaaaccta ag) and pND32-8 as template. Correct integration was verified by PCR. Tagging of all selected ORFs with the mCherry-sfGFP timer was performed in the resulting strain yMaM330 (Supplementary Table 4).

1.3. Selection of ORFs

We sought to reduce potential artifacts in the tFT library and to rationalize the labor required for library construction. Previously, most *S. cerevisiae* ORFs were successfully fused to a common tag at respective endogenous loci in a haploid reference strain[9-11]. The C-terminally tagged proteins

could be detected by fluorescence microscopy, flow cytometry or immunoblotting at levels close to endogenous[9,10,21,22]. However, some protein fusions could not be detected or analyzed because the C-terminus is important for protein function[9,10]. We sought to avoid tagging ORFs encoding such proteins. Moreover, the properties of the mCherry-sfGFP timer depend on the intracellular environment[23]. We sought to avoid tagging ORFs encoding proteins that localize to compartments with extreme environments such as the lumen of the vacuole or the cell wall/extracellular space.

Therefore, we selected all verified or uncharacterized ORFs from the *Saccharomyces* Genome Database assigned to the following gene ontology (GO) terms: GO:0005829 [cytosol], GO:0005634 [nucleus], GO:0005886 [plasma membrane], GO:0005737 [cytoplasm], GO:0016021 [integral to membrane] (as of 09/08/2010) and GO:0005739 [mitochondrion] (as of 07/09/2010). ORFs from the mitochondrial genome and the 2μ plasmid were not included. For each protein localization pattern defined in a systematic localization study of GFP protein fusions in yeast[9], ORFs absent from our list were manually inspected for possible inclusion. ORFs encoding the following proteins were subsequently removed from the selection:

- cell wall proteins (manually curated list of ORFs assigned to the GO terms GO:0005618 [cell wall] or GO:0009277 [fungal-type cell wall] as of December 2009);
- glycosylphosphatidylinositol (GPI)-anchored proteins[24];
- tail-anchored proteins[20,25];
- proteins with one of the following motifs at the C-terminus: HDEL, KKXX, CaaX ('X' stands for any amino acid, 'a' stands for an aliphatic amino acid) or the PTS1 peroxisomal targeting signal (selected using the Yeast Genome Pattern Matching tool at http://www.yeastgenome.org/cgi-bin/PATMATCH/nph-patmatch as of 30/08/2010);
- proteins fatty acylated at the C-terminus (manually curated list of proteins obtained from the UniProt Knowledgebase, http://www.uniprot.org as of 14/09/2010).

The resulting list was further curated using information from previous genome-wide tagging efforts[9-11] and a proteome-wide mass spectrometry study[22]. We decided to exclude ORFs encoding proteins that were tagged but could not be detected in any previous genome-wide tagging study[9-11]. ORFs encoding proteins that were not detected by mass spectrometry[22] and either were not detected by fluorescence microscopy after tagging with GFP[9] or no information on the expression of tagged proteins was available in any genome-wide library of protein fusions[9-11] were also excluded. This resulted in a list of 4081 ORFs (Supplementary Table 2).

1.4. Primers for PCR targeting of the tagging module

S2/S3 primers for PCR amplification of the tagging module were designed as previously described[13]. Sequences of all ORFs with untranslated regions 1000 bases upstream of the initial ATG and 1000 bases downstream of the stop codon were downloaded from the *Saccharomyces* Genome Database (orf_genomic_1000.fasta.gz as of December 2009) and used for primer design. For each ORF, an S3 primer was composed of 55 nucleotides before the stop codon (excluding stop) followed by cgtacgctgcaggtcgac and an S2 primer was composed of the reverse complement of 55 nucleotides downstream of the stop codon (including stop) followed by atcgatgaattcgagctcg. All primers were obtained from IDT (Integrated DNA Technologies) in 96-well format. Unique plate and well coordinates were assigned to each ORF such that each well contained a mixture of S2/S3 primers for a different ORF at 5 μM concentration. A distinct well was left empty on each plate for identification purposes (plate 1 – well A1 empty, plate 2 – well A2 empty, etc.). Four wells on each plate were served as controls in PCR amplification of the tagging module: well H9 was left empty; well H10 contained a mixture of S2/S3 primers for *HSP104*, which performed robustly in PCR amplification of tagging modules (data not shown); well H11 contained a mixture of S2/S3 primers for *SPC110*, which yielded a PCR product only under optimal conditions (data not shown); well H12 was left empty.

New tagging primers were obtained for the ORFs for which no positive clones were identified by diagnostic PCR in the first round of transformations. For each ORF, the new S3 primer was composed of 62 nucleotides before the stop codon (excluding stop) followed by

cgtacgctgcaggtcgac and the new S2 primer was composed of the reverse complement of 61 nucleotides downstream of the stop codon (including stop) followed by atcgatgaattcgagctcg.

1.5. Amplification of the tagging module

The module for seamless protein tagging with mCherry-sfGFP was PCR amplified in 96-well format using the plasmid pMaM168 (Supplementary Table 5) as template and ORF-specific S2/S3 primers in each well, as follows. Cooled 96-well PCR plates (4titude, 4ti-0960) were filled with 45 µl per well of a PCR mix (each well received 10 µl of 5x Herculase II buffer (Agilent Technologies), 0.5 µl of 100mM stock of dNTPs (Fermentas, R0141/0151/0161/0171), 0.075 µl of 1M stock $MgCl_2$, 5 µl of 5M stock of betaine (Sigma-Aldrich, 61962), 0.5 µl of template DNA (200 ng/µl stock), 28.675 µl of H_2O and 0.25 µl of Herculase II Fusion DNA polymerase (Agilent Technologies, 600679)) using a Multiprobe II liquid handling 8-channel robot (Perkin Elmer). A mixture of ORF-specific S2/S3 primers (5 µl of 5µM stock) was added to each well from 96-well primer source plates (see section 1.4) using the liquid handling 8-channel robot. The plates were sealed with peelable aluminum seals (Agilent, 24210-001) using a Velocity11 PlateLoc sealer. PCR was then carried out in PTC-225 PCR cyclers (MJ Research) using the following program: 2 min at 95°C, 30 cycles of 20 s at 95°C/20 s at 64°C/2 min 20 s at 72°C, 3 min at 72°C and incubation at 4°C. Control reactions in wells H8-H11 of each 96-well plate were examined by agarose gel electrophoresis to ensure successful amplification of the tagging module.

1.6. Transformation

Preparation of yeast competent cells and transformations were carried out essentially as previously described[13]. Briefly, a pre-culture of the strain yMaM330 was grown to saturation in rich YPD medium (1% yeast extract, 2% peptone, 2% glucose) and used to inoculate a 5 L YPD culture to optical density of 0.2 (OD_{600nm}). After growth at 30°C to OD_{600nm} of 2.0, the cells were collected by centrifugation (500 g for 5 min at room temperature), washed first with 5 L of sterile water and finally with 1 L of LiSorb (100mM lithium acetate (L4158, Sigma), 1M sorbitol (1.07758.1000, Merck), 10mM Tris/HCl pH 8, 1mM EDTA/NaOH pH 8, adjusted to pH 8 with acetic acid). The cell pellet was resuspended in 45 ml of LiSorb, aliquoted and stored at -80°C. For transformations, a thawed aliquot of cells was mixed 18:2 with pre-boiled carrier DNA (salmon sperm DNA, #15632-011, Invitrogen).

Transformations were performed in 96-deepwell plates (Eppendorf, 0030 502.132). 50 µl of competent yMaM330 cells were pipetted into each well. Using a Platetrak 96-channel liquid handling robot (Perkin Elmer), 5 µl of amplified tagging module were transferred from each PCR plate (see section 1.5) into the corresponding transformation plate. 300 µl of LiPEG (100mM lithium acetate (L4158, Sigma), 10mM Tris/HCl pH 8, 1mM EDTA/NaOH pH 8, 40% (w/v) polyethylene glycol (P4338, Sigma)) were subsequently added to each well and thoroughly mixed. The plates were sealed with gas permeable adhesive seals (AB-0718, Thermo Scientific) and incubated for 40 min in a 42°C water bath. After a centrifugation step (5 min at 500 g), the supernatant was removed and the cell pellet was resuspended in 100 µl per well of synthetic medium devoid of uracil (SC-Ura) using the 96-channel liquid handling robot. The cell suspension from each well was manually plated onto a separate 9 cm plate with SC-Ura agar medium. The plates were incubated at 30°C for 3 days until clear colonies were visible in the control transformations (wells H10 and H11 in each 96-deepwell transformation plate).

Six clones from each 9 cm plate were manually streaked for single colonies on SC-Ura agar medium and incubated at 30°C for 2 days. For each ORF, four purified clones were inoculated in 96-well format (each well contained 150 µl of SC-Ura medium with 15% (v/v) of glycerol), with each clone at the same well position in a separate 96-well plate. The plates were sealed with gas permeable adhesive seals, incubated at 30°C for 2 days and stored at -80°C.

1.7. Verification primers

ORF-specific verification primers that anneal within each ORF were designed using BatchPrimer3 v1.0[26] (http://batchprimer3.bioinformatics.ucdavis.edu/index.html as of 22/10/2010),

a high-throughput web implementation of Primer3[27]. For each ORF, a sequence composed of 1000 nucleotides before the STOP codon followed by the tag was entered into BatchPrimer3. Using the 1st set of constraints indicated below (min/optimal/max values are specified for each parameter), a unique primer with two consecutive G and/or C nucleotides at the 3' end that would yield a PCR product around 450 nucleotides long, when used together with a generic reverse primer (atggccatgttatcctcctcg) that anneals 71 nucleotides downstream of the start of the tag, was selected for each ORF. When no satisfactory primer could be found, the 2nd set of relaxed constraints or finally the 3rd set were used.

Selection of verification primers (first round)

	1st set	2nd set	3rd set
PCR product length (nucleotides)	300/450/600	300/450/600	300/450/670
primer length (nucleotides)	19/20/21	18/20/22	18/20/24
melting temperature (°C)	60/63/65	59/63/66	59/63/66
GC content (%)	25/50/75	25/50/75	25/50/75
satisfactory primers	3911	158	12

These primers were used in the first round of diagnostic PCR. For 360 ORFs with ambiguous results, the diagnostic PCR was repeated with new ORF-specific primers, designed using the constraints indicated below (no optimal PCR product length in the 1st set, optimal PCR product length of 250 or 600 nucleotides in the 2nd and 3rd sets of constraints).

Selection of verification primers (second round)

	1st set	2nd set	3rd set
PCR product length (nucleotides)	300/0/600	250/<>/600	250/<>/600
primer length (nucleotides)	19/20/21	18/20/22	18/20/24
melting temperature (°C)	60/63/65	59/63/66	59/63/66
GC content (%)	25/50/75	25/50/75	25/50/75
satisfactory primers	253	104	3

1.8. Diagnostic PCR

Integration of the tagging module into each genomic locus was tested by PCR. The junction between each ORF and the tag was verified using a forward ORF-specific primer annealing within the ORF and a generic reverse primer annealing within the tag (see section 1.7). Cooled 96-well PCR plates (4titude, 4ti-0960) were filled with 35 µl per well of a PCR mix (each well received 4 µl of 10x long incubation buffer (200mM Tris pH8.8, 100mM $(NH_4)_2SO_4$, 100mM KCl), 0.16 µl of 100mM stock of dNTPs (Fermentas, R0141/0151/0161/0171), 0.1 µl of 1M stock $MgCl_2$, 4 µl of 5M stock of betaine (Sigma-Aldrich, 61962), 0.2 µl of 100µM stock of the generic reverse primer (see section 1.7), 25.94 µl of H_20 and 0.6 µl of Taq DNA polymerase (self-made, ~5 U/µl) using a Perkin Elmer Multiprobe II liquid handling 8-channel robot. A distinct forward ORF-specific validation primer (4 µl of 5µM stock) was added to each well from 96-well validation primer source plates (see section 1.7). A dense culture of each strain (stored at -80°C as glycerol stock, see section 1.6) added to each well (1 µl) provided the genomic DNA template. For each ORF-specific

primer, a control PCR was set up with the library background strain yMaM330 as a source of template DNA. The plates were sealed with peelable aluminum seal using a Velocity11 PlateLoc sealer. PCR was then carried out in MJ Research PTC-225 PCR cyclers using the following program: 7 min at 97°C, 38 cycles of 30 s at 95°C/30 s at 58°C/30 s at 72°C, 5 min at 72°C and incubation at 4°C.

All PCR products were examined by agarose gel electrophoresis using 96-well precast gels (2% E-Gel® 96 gels (G7008-02), Invitrogen) and a reference DNA ladder (FastRuler low range DNA ladder (SM1103), Fermentas). Clones with correct chromosomal integration of the tagging module were identified by the presence of a PCR product of expected size in the sample but not in the control PCR.

1.9. Whole colony imaging

Using a RoToR pinning robot (Singer Instruments), all clones from a single 96-well transformation plate were combined in 1536-colony format with 4 technical replicates of each clone. All strains underwent seamless marker excision by sequential growth on galactose (synthetic complete medium containing 2% galactose and 2% raffinose instead of glucose) and 5-FOA plates (synthetic medium containing 5-fluoroorotic acid)[16]. Fluorescence intensities of all colonies were measured after ~21 h of growth on glucose medium (synthetic complete medium containing 2% glucose) using an Infinite M1000 Pro microplate reader (Tecan).

2. Supplementary Notes

2.1. Supplementary Note 1

We have previously shown that measurements of protein turnover with the mCherry-sfGFP timer exceed cycloheximide or pulse-chase experiments in both dynamic range and sensitivity. For instance, the turnover of 20 N-degrons differing in the N-terminal residue, which were previously classified into five stability groups based on pulse-chase experiments[28,29], could be reliably resolved with the tFT, showing that each of the 20 N-degrons possesses a specific turnover (Supplementary Figure 7 in Ref.[4]).

Cycloheximide chase experiments are limited by the availability of free ubiquitin in the cell. Upon inhibition of translation, ubiquitin is depleted from yeast cell with a half-life of ~2 h[30]. This prevents reliable turnover measurements for relatively stable proteins. In contrast, the tFT approach is not limited by ubiquitin availability but by the maturation kinetics of the used fluorescent proteins. Degradation of proteins with half-lives between ~10 min and ~8 h can be analyzed with the mCherry-sfGFP timer[4]. Thus, although 3HA-tagged Are2, Yip4, Alg2 and Ybr287w appear rather stable in cycloheximide chase experiments (data not shown), tFT-tagged Are2, Yip4, Alg2 and Ybr287w were identified as Asi substrates in our screens (Fig. 3b) and, accordingly, accumulated at the nuclear rim in the *asi1*Δ mutant (Extended Data Fig. 4c).

Besides the aforementioned differences, we note that protein stability measurements with the tFT based on whole colony fluorescence measurements are not directly comparable with cycloheximide experiments also due to differences in growth conditions. Cycloheximide chase experiments are typically performed with exponentially growing cultures. However, measurements of colony fluorescence detect the signal mostly from the colony surface, where nutrient supply is limited and cells grow slower[31], with a potential influence on protein expression and turnover. For instance, Aqy2 was identified as an Asi substrate in our screens (Fig. 3b) but was not expressed during exponential growth in synthetic complete medium (data not shown).

2.2. Supplementary Note 2

SILAC mass spectrometry[32] or quantitative microscopy can be used to identify potential substrates of degradation pathways based on changes in protein abundance[33-35]. However, these methods cannot distinguish between changes in abundance that result from altered protein stability and those caused by changes in protein expression. The tFT strategy directly identifies proteins with altered stability. However, in contrast to approached based on mass spectrometry, it has the disadvantage that fusion to the tFT might compromise protein function[36]. Nevertheless, our screens identified Erg11 among the substrates of the Asi ubiquitin ligase, in agreement with the recent work of Foresti et al.[37] Nsg1, another Asi substrate identified by Foresti et al., is a false-negative in our screens, as retesting showed stabilization of Nsg1-tFT upon deletion of *ASI1* (data not shown).

3. Supplementary Tables

Supplementary Table 1: Correlation coefficients between genetic interaction profiles

Pearson correlation coefficients between genetic interaction profiles of *ASI1, ASI2, ASI3, UBC6, UBC7, CUE1, HRD1, DOA10* genes and of all the other genes in the genome-wide genetic interaction map[38]. The data (Excel file) is available online.

Supplementary Table 2: Strains in the tFT library

Number of validated clones for each ORF in the tFT library, including ORFs with zero validated clones. The data (Excel file) is available online.

Supplementary Table 3: Protein stability changes in screens with the tFT library

Stability changes of each protein in the tFT library in *asi1*Δ, *asi3*Δ, *hrd1*Δ, *doa10*Δ, *ubc6*Δ and *ubc7*Δ mutants. z-scores and p-values adjusted for multiple testing are provided. The data (Excel file) is available online.

Supplementary Table 4: Yeast strains used in this study

Strain	Background	Genotype	Source
PLY127	S288c	*MATa ura3-52 lys2Δ201*	Ljungdahl lab
PLY966	S288c	*MATa ura3-52 lys2Δ201 leu2-3,112 ubc6Δ::LEU2*	Ref.[39]
PLY967	S288c	*MATa ura3-52 lys2Δ201 leu2-3,112 ubc7Δ::LEU2*	Ref.[39]
PLY1558	S288c	*MATa ura3-52 lys2Δ201 leu2-3,112 ubc6Δ::LEU2 ubc7Δ::natMX4*	Ref.[39]
PLY1327	S288c	*MATa ura3-52 lys2Δ201 asi1Δ80::hphMX*	Ljungdahl lab
PLY1329	S288c	*MATa ura3-52 lys2Δ201 asi3Δ::kanMX*	Ljungdahl lab
BY4741	S288c	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Ref.[40]
MHY2241	BY4741	*ubc4Δ::kanMX*	Hochstrasser lab
MHY3032	BY4741	*hrd1Δ::kanMX*	Ref.[41]
MHY3033	BY4741	*doa10Δ::kanMX*	Ref.[41]
Y8205	S288c	*MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 can1Δ:: STE2pr-spHIS5 lyp1Δ:: STE3pr-LEU2*	Ref.[40]
scEB115	Y8205	*MATalpha can1Δ::STE2pr-spHIS5 lyp1Δ::STE3pr-HPH rpn7Δ::RPN7-tDimer2(12)-LEU2*	Rabut lab
scEB133	scEB115	*ubc1Δ::VC-UBC1-natMX*	Rabut lab
scEB129	scEB115	*rad6Δ::RAD6-VC-natMX*	Rabut lab
scEB130	scEB115	*cdc34Δ::CDC34-VC-natMX*	Rabut lab
scEB153	scEB115	*ubc4Δ::UBC4-VC-natMX*	Rabut lab
scEB121	scEB115	*ubc5Δ::UBC5-VC-natMX*	Rabut lab
scEB152	scEB115	*ubc6Δ::VC-UBC6-natMX*	Rabut lab
scGR1267	scEB115	*ubc7Δ::VC-UBC7-natMX*	Rabut lab
scEB125	scEB115	*ubc8Δ::UBC8-VC-natMX*	Rabut lab
scEB126	scEB115	*pex4Δ::PEX4-VC-natMX*	Rabut lab
scEB127	scEB115	*ubc11Δ::UBC11-VC-natMX*	Rabut lab
scEB123	scEB115	*ubc13Δ::UBC13-VC-natMX*	Rabut lab
VN_0484	BY4741	*hul4Δ::HUL4-VN-KlURA3*	Bioneer
VN_0394	BY4741	*hul5Δ::HUL5-VN-KlURA3*	Bioneer
scGR1173	BY4741	*pGR703 (expressing RSP5-VN)*	Rabut lab
VN_0034	BY4741	*tom1Δ::TOM1-VN-KlURA3*	Bioneer
VN_0045	BY4741	*ufd4Δ::UFD4-VN-KlURA3*	Bioneer
VN_1027	BY4741	*asi1Δ::ASI1-VN-KlURA3*	Bioneer
VN_0927	BY4741	*asi3Δ::ASI3-VN-KlURA3*	Bioneer
VN_2780	BY4741	*asr1Δ::ASR1-VN-KlURA3*	Bioneer
VN_0860	BY4741	*bre1Δ::BRE1-VN-KlURA3*	Bioneer
VN_3152	BY4741	*cwc24Δ::CWC24-VN-KlURA3*	Bioneer
VN_2108	BY4741	*dma1Δ::DMA1-VN-KlURA3*	Bioneer
VN_1460	BY4741	*dma2Δ::DMA2-VN-KlURA3*	Bioneer
VN_4936	BY4741	*doa10Δ::DOA10-VN-KlURA3*	Bioneer
VN_1175	BY4741	*etp1Δ::ETP1-VN-KlURA3*	Bioneer
VN_0376	BY4741	*fap1Δ::FAP1-VN-KlURA3*	Bioneer
scGR1165	BY4741	*far1Δ::VN-FAR1-URA3*	Rabut lab
VN_1990	BY4741	*gid2Δ::GID2-VN-KlURA3*	Bioneer
VN_1419	BY4741	*gid9Δ::GID9-VN-KlURA3*	Bioneer
VN_1031	BY4741	*hel2Δ::HEL2-VN-KlURA3*	Bioneer
VN_5317	BY4741	*hrd1Δ::HRD1-VN-KlURA3*	Bioneer
VN_0043	BY4741	*irc20Δ::IRC20-VN-KlURA3*	Bioneer
VN_0884	BY4741	*mag2Δ::MAG2-VN-KlURA3*	Bioneer
scGR1166	BY4741	*mot2Δ::VN-MOT2-URA3*	Rabut lab
VN_0246	BY4741	*mtc5Δ::MTC5-VN-KlURA3*	Bioneer
scGR1172	BY4741	*nam7Δ::VN-NAM7-URA3*	Rabut lab
VN_5066	BY4741	*pep3Δ::PEP3-VN-KlURA3*	Bioneer
VN_5052	BY4741	*pep5Δ::PEP5-VN-KlURA3*	Bioneer
VN_5657	BY4741	*pex2Δ::PEX2-VN-KlURA3*	Bioneer
VN_5559	BY4741	*pex10Δ::PEX10-VN-KlURA3*	Bioneer
VN_5469	BY4741	*pex12Δ::PEX12-VN-KlURA3*	Bioneer
VN_2059	BY4741	*psh1Δ::VN-PSH1-KlURA3*	Bioneer
VN_0234	BY4741	*rad5Δ::RAD5-VN-KlURA3*	Bioneer
VN_0644	BY4741	*rad16Δ::RAD16-VN-KlURA3*	Bioneer
VN_1639	BY4741	*rad18Δ::RAD18-VN-KlURA3*	Bioneer
VN_0021	BY4741	*rkr1Δ::RKR1-VN-KlURA3*	Bioneer
VN_0153	BY4741	*rtc1Δ::RTC1-VN-KlURA3*	Bioneer
VN_1107	BY4741	*san1Δ::SAN1-VN-KlURA3*	Bioneer
VN_1072	BY4741	*slx5Δ::SLX5-VN-KlURA3*	Bioneer
VN_3137	BY4741	*slx8Δ::SLX8-VN-KlURA3*	Bioneer
scGR1171	BY4741	*ste5Δ::VN-STE5-URA3*	Rabut lab
VN_1785	BY4741	*ssl1Δ::SSL1-VN-KlURA3*	Bioneer
scGR1167	BY4741	*tfb3Δ::VN-TFB3-URA3*	Rabut lab
VN_0629	BY4741	*tul1Δ::TUL1-VN-KlURA3*	Bioneer
VN_0060	BY4741	*ubr2Δ::UBR2-VN-KlURA3*	Bioneer
VN_0020	BY4741	*uls1Δ::ULS1-VN-KlURA3*	Bioneer
VN_4927	BY4741	*vps8Δ::VPS8-VN-KlURA3*	Bioneer
VN_4447	BY4741	*ybr062cΔ::YBR062C-VN-KlURA3*	Bioneer
scGR1168	BY4741	*prp19Δ::VN-PRP19-URA3*	Rabut lab

Strain	**Background**	**Genotype**	**Source**
VN_0419	BY4741	*ufd2Δ::UFD2-VN-KlURA3*	Bioneer
VN_1367	BY4741	*hel1Δ::HEL1-VN-KlURA3*	Bioneer
VN_1749	BY4741	*itt1Δ::ITT1-VN-KlURA3*	Bioneer
VN_3891	BY4741	*apc11Δ::APC11-VN-KlURA3*	Bioneer
VN_0690	BY4741	*cul3Δ::CUL3-VN-KlURA3*	Bioneer
VN_0556	BY4741	*rtt101Δ::RTT101-VN-KlURA3*	Bioneer
scEB300	scEB115	*asi1Δ::ASI1-VN-KlURA3 ubc6Δ::VC-UBC6-natMX*	Rabut lab
scEB289	scEB115	*asi1Δ::ASI1-VN-KlURA3 ubc4Δ::UBC4-VC-natMX*	Rabut lab
scEB323	scEB115	*asi1Δ::ASI1-VN-KlURA3 ubc4Δ::UBC4-VC-natMX ubc6Δ::kanMX*	Rabut lab
scEB258	scEB115	*asi3Δ::ASI3-VN-KlURA3 ubc6Δ::VC-UBC6-natMX*	Rabut lab
scEB265	scEB115	*asi3Δ::ASI1-VN-KlURA3 ubc6Δ::VC-UBC6-natMX asi1Δ::kanMX*	Rabut lab
scEB266	scEB115	*asi3Δ::ASI1-VN-KlURA3 ubc6Δ::VC-UBC6-natMX asi2Δ::kanMX*	Rabut lab
scGR1245	BY4741	*asi3Δ::ASI3-TAP-HIS3MX*	Open Biosystems
scGR1258	BY4741	*asi3Δ::ASI3-TAP-HIS3MX asi1Δ::kanMX*	Rabut lab
scGR1260	BY4741	*asi3Δ::ASI3-TAP-HIS3MX asi2Δ::kanMX*	Rabut lab
scAB17	W303	*tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 PRE8-FRBGFP::kanMX6 stp2(1-45)-TAP::URA3*	Rabut lab
scAB22	scAB17	*asi3::HPH*	Rabut lab
scAB23	scAB17	*ubc6::HPH*	Rabut lab
scAB24	scAB17	*ubc7::HPH*	Rabut lab
AK1234	BY4741	*asi1Δ::kanMX6*	Knop lab
YMaM767	BY4741	*ire1Δ::natNT2 hrd1Δ::hphNT1*	Knop lab
YMaM768	BY4741	*hac1Δ::natNT2 hrd1Δ::hphNT1*	Knop lab
YMaM814	BY4741	*ire1Δ::natNT2 hrd1Δ::hphNT1 asi1Δ::kanMX6*	Knop lab
YMaM815	BY4741	*hac1Δ::natNT2 hrd1Δ::hphNT1 asi1Δ::kanMX6*	Knop lab
AK1222	BY4741	*hrd1Δ::hphNT1*	Knop lab
AK1225	BY4741	*doa10Δ::hphNT1*	Knop lab
YMaM891	BY4741	*hrd1Δ::hphNT1 asi1Δ::kanMX6*	Knop lab
YMaM892	BY4741	*doa10Δ::hphNT1 asi1Δ::kanMX6*	Knop lab
YMaM330	Y8205	*leu2Δ::GAL1pr-I-SCEI-natNT2*	Knop lab
YMaM344	YMaM330	*ura3Δ0::mCherryΔN-I-SceIsite-CYC1term-ScURA3-I-SceIsite-mCherryΔN*	Knop lab
AK1235	BY4741	*asi2Δ::kanMX6*	Knop lab
AK1236	BY4741	*asi3Δ::kanMX6*	Knop lab
AK1237	BY4741	*ubc6Δ::kanMX6*	Knop lab
AK1238	BY4741	*ubc7Δ::kanMX6*	Knop lab
AK1239	BY4741	*cue1Δ::kanMX6*	Knop lab
YMaM758	YMaM330	*VTC1-mCherry-sfGFP*	Knop lab
YMaM757	YMaM330	*ERG11-mCherry-sfGFP*	Knop lab
YMaM759	YMaM330	*VCX1-mCherry-sfGFP*	Knop lab
YMaM762	YMaM330	*ARE2-mCherry-sfGFP*	Knop lab
YMaM765	YMaM330	*YIP4-mCherry-sfGFP*	Knop lab
YMaM756	YMaM330	*ALG2-mCherry-sfGFP*	Knop lab
YMaM764	YMaM330	*VTC4-mCherry-sfGFP*	Knop lab
YMaM761	YMaM330	YBR287W-mCherry-sfGFP	Knop lab
YMaM763	YMaM330	AQY2-mCherry-sfGFP	Knop lab
YMaM760	YMaM330	ERG1-mCherry-sfGFP	Knop lab
YMaM828	YMaM330	*VTC1-mCherry-sfGFP asi1Δ::hphNT1*	Knop lab
YMaM827	YMaM330	*ERG11-mCherry-sfGFP asi1Δ::hphNT1*	Knop lab
YMaM830	YMaM330	*VCX1-mCherry-sfGFP asi1Δ::hphNT1*	Knop lab
YMaM831	YMaM330	*ARE2-mCherry-sfGFP asi1Δ::hphNT1*	Knop lab
YMaM833	YMaM330	*YIP4-mCherry-sfGFP asi1Δ::hphNT1*	Knop lab
YMaM829	YMaM330	*ALG2-mCherry-sfGFP asi1Δ::hphNT1*	Knop lab
YMaM832	YMaM330	*VTC4-mCherry-sfGFP asi1Δ::hphNT1*	Knop lab
YMaM835	YMaM330	YBR287W-mCherry-sfGFP *asi1Δ::hphNT1*	Knop lab
YMaM834	YMaM330	AQY2-mCherry-sfGFP *asi1Δ::hphNT1*	Knop lab
YMaM836	YMaM330	ERG1-mCherry-sfGFP *asi1Δ::hphNT1*	Knop lab
YMaM818	YMaM330	*VTC1-mCherry-sfGFP hrd1Δ::hphNT1*	Knop lab
YMaM817	YMaM330	*ERG11-mCherry-sfGFP hrd1Δ::hphNT1*	Knop lab
YMaM820	YMaM330	*VCX1-mCherry-sfGFP hrd1Δ::hphNT1*	Knop lab
YMaM821	YMaM330	*ARE2-mCherry-sfGFP hrd1Δ::hphNT1*	Knop lab
YMaM823	YMaM330	*YIP4-mCherry-sfGFP hrd1Δ::hphNT1*	Knop lab
YMaM819	YMaM330	*ALG2-mCherry-sfGFP hrd1Δ::hphNT1*	Knop lab
YMaM822	YMaM330	*VTC4-mCherry-sfGFP hrd1Δ::hphNT1*	Knop lab
YMaM825	YMaM330	YBR287W-mCherry-sfGFP *hrd1Δ::hphNT1*	Knop lab
YMaM824	YMaM330	AQY2-mCherry-sfGFP *hrd1Δ::hphNT1*	Knop lab
YMaM826	YMaM330	ERG1-mCherry-sfGFP *hrd1Δ::hphNT1*	Knop lab
YMaM791	BY4741	*VTC1-3HA-hphNT1*	Knop lab
YMaM790	BY4741	*ERG11-3HA-hphNT1*	Knop lab
YMaM793	BY4741	*VCX1-3HA-hphNT1*	Knop lab
YMaM795	BY4741	*VTC4-3HA-hphNT1*	Knop lab
YMaM801	BY4741	*VTC1-3HA-hphNT1 asi1Δ::kanMX6*	Knop lab
YMaM800	BY4741	*ERG11-3HA-hphNT1 asi1Δ::kanMX6*	Knop lab
YMaM803	BY4741	*VCX1-3HA-hphNT1 asi1Δ::kanMX6*	Knop lab
YMaM805	BY4741	*VTC4-3HA-hphNT1 asi1Δ::kanMX6*	Knop lab
YMaM864	BY4741	*VTC1-3HA-hphNT1 hrd1Δ::natNT2*	Knop lab

Strain	**Background**	**Genotype**	**Source**
YMaM863	BY4741	*ERG11-3HA-hphNT1 hrd1Δ::natNT2*	Knop lab
YMaM866	BY4741	*VCX1-3HA-hphNT1 hrd1Δ::natNT2*	Knop lab
YMaM868	BY4741	*VTC4-3HA-hphNT1 hrd1Δ::natNT2*	Knop lab
YDK179	BY4741	*VTC1-3HA-hphNT1 asi1Δ::kanMX6 hrd1Δ::natNT2 doa10Δ::KlURA3*	Knop lab
YDK178	BY4741	*ERG11-3HA-hphNT1 asi1Δ::kanMX6 hrd1Δ::natNT2 doa10Δ::KlURA3*	Knop lab
YDK180	BY4741	*VCX1-3HA-hphNT1 asi1Δ::kanMX6 hrd1Δ::natNT2 doa10Δ::KlURA3*	Knop lab
YDK181	BY4741	*VTC4-3HA-hphNT1 asi1Δ::kanMX6 hrd1Δ::natNT2 doa10Δ::KlURA3*	Knop lab
YDK182	BY4741	*VTC1-myeGFP-kanMX*	Knop lab
YDK188	BY4741	*VTC4-myeGFP-kanMX*	Knop lab
YDK223	YMaM330	*sfGFPΔC-I-SceIsite-SpCYC1term-ScURA3-SpTEF1pr-I-SceIsite-sfGFP-VTC1*	Knop lab
YDK224	YMaM330	*sfGFPΔC-I-SceIsite-SpCYC1term-ScURA3-SpTEF1pr-I-SceIsite-sfGFP-VTC4*	Knop lab
YDK266	YMaM330	*sfGFP-VTC1*	Knop lab
YDK267	YMaM330	*sfGFP-VTC4*	Knop lab

Supplementary Table 5: Plasmids used in this study

Plasmid	**Description**	**Source**
pRS316	CEN ARS URA4 low copy yeast/*E. coli* shuttle plasmid	Ref.[42]
pRS317	CEN ARS LYS2 low copy yeast/*E. coli* shuttle plasmid	Ref.[42]
pCA047	pRS316 (*URA3*) containing *STP1-3HA*	Ref.[43]
pCA111	pRS316 (*URA3*) containing *STP2-3HA*	Ref.[43]
pDO74	pRS316 (*URA3*) containing *STP1-RI_{17-33}-3HA*	Ref.[44]
pAG04	pRS316 (*URA3*) containing *STP2Δ_{2-13}-3HA*	Ljungdahl lab
YCpAGP1-LacZ	AGP1pr-lacZ in CEN URA3	Ref.[45]
pFA6a	E. coli plasmid containing the ampicillin resistance gene ampR	Ref.[46]
pFA6a-KanMX6	pFA6a-KanMX6	Ref.[46]
pKS133	pFA6a-HphNT1	Ref.[12]
pKS134	pFA6a-NatNT2	Ref.[12]
pYM24	pFA6a-3HA-HphNT1	Ref.[12]
pMaM168	pFA6a-mCherry-I-SceIsite-SpCYC1term-ScURA3-I-SceIsite-mCherryΔN-sfGFP	Knop lab
pND32-8	pRS305N-GAL1pr-I-SCEI	Ref.[16]
pMaM173	pFA6a-sfGFPΔC-I-SceIsite-SpCYC1term-ScURA3-SpTEF1pr-I-SceIsite-sfGFP	Knop lab
pYM12monomeric	pFA6a-yeGFP-kanMX6 with A206R mutation	Knop lab
pGR731	pGEX4TG containing GST-Ubc6$^{\Delta TM}$	Rabut lab
pGR732	pGEX4TG containing GST-Ubc7	Rabut lab
pGR738	pETDuet-1 containing GST-Ubc7 + Cue1^{U7BR}	Rabut lab
pGR773	pMALXTG containing MBP-Hrd1^{CT}	Rabut lab
pGR759	pMALXTG containing MBP-Asi1^{RING}	Rabut lab
pGR766	pMALXTG containing MBP-Asi3^{RING}	Rabut lab
pGR703	pRS316 (*URA3*) containing Rsp5-VN	Rabut lab
pGR295	p415TEF1 *(LEU2)* containing 10His-Ubiquitin	Rabut lab

4. References

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