CSN Facilitates Cullin/RING Ubiquitin Ligase Function by Counteracting Autocatalytic Adapter Instability

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The COP9 signalosome (CSN) is known to bind cullin/RING ubiquitin ligases (CRLs) and promote their activity *in vivo* [1-3]. The mechanism of this stimulation has remained enigmatic, because CSN's intrinsic and associated enzymatic activities paradoxically inhibit CRL activity *in vitro* [4,5]. Reconciling this paradox, we show here that Csn5-catalyzed cullin (Cul) deneddylation and Ubp12-mediated deubiquitylation cooperate in maintaining the stability of labile substrate adapters thus facilitating CRL function. Various fission yeast *csn* and *ubp12* deletion mutants have reduced levels of the Cul3p adapter Btb3p. This reduction is due to increased autocatalytic, Cul3p-dependent, ubiquitylation and subsequent degradation of Btb3p. The CSN/Ubp12p pathway also maintains the stability of the Cul1p adapter Pop1p, a mechanism required for efficient destruction of its cognate substrate Rum1p. Emphasizing the physiological importance of this mechanism, the dispensable *csn5* and *ubp12* genes become essential for viability when adapter recruitment to Cul1p is compromised. Our data suggest maintenance of adapter stability as a general mechanism of CRL control by the CSN.

CRLs represent an extensive class of multisubunit E3 ubiquitin ligases each consisting of a core module containing a member of the Cul family and the RING domain protein Rbx1 (= Hrt1, Roc1), which recruits E2 ubiquitin conjugating enzymes (UBCs) to the ligase (reviewed in Ref. [6]). This core is joined by one of several hundred adapter proteins each of which targets a distinct array of substrates for ubiquitylation and proteasomal degradation. Whereas F-box adapters are tethered to the Cul1 core through the linker protein Skp1 to form SCF complexes [6], Cul3 adapters are recruited via their inherent BTB domains [7-9]. Unlike CRL core subunits, several members of both adapter families are unstable proteins, apparently due to autoubiquitylation by the intrinsic

ubiquitin ligase activity of their associated core modules [9-13].

CRL-dependent substrate degradation is subject to several levels of post-translational control. At the level of the substrate, phosphorylation is ordinarily required for recognition by CRLs [6]. At the level of the ligases, modification of cullins with the ubiquitin-related peptide Nedd8 is essential for CRL activity in all organisms, except budding yeast (reviewed in Ref. [14]). Cullin neddylation is reversed by the CSN, a highly conserved protein complex that binds cullins [15-17] and thereby exposes them to a deneddylating activity intrinsic to subunit 5 of the CSN (Csn5p) [18]. Consistent with neddylation being a stimulatory modification, hyperneddylated Cul1p and Cul3p complexes isolated from fission yeast *csn* deletion mutants have increased substrate-independent ubiquitin ligase activity *in vitro* [5,15]. Readdition of purified CSN leads to partial cullin deneddylation and complete inhibition of their ubiquitin ligase activity [5,15]. Inhibition is complete, because CSN also neutralizes CRL activity through its associated deubiquitylating enzyme Ubp12p. Cul1p and Cul3p complexes isolated from *ubp12* mutants show the same extent of stimulation as cullin complexes retrieved from *csn* mutants [5]. Thus, both CSN enzymatic activities directed at regulating cullins, deneddylation and deubiquitylation, inhibit CRL activity *in vitro* [4,5].

However, the CSN was shown to promote CRL-dependent substrate degradation *in vivo* (reviewed in [1,2]). To determine how the established *in vitro* activities of CSN/Ubp12p impact on CRLs *in vivo*, we assessed the status of the fission yeast Cul3p ubiquitin ligase associated with the adapter protein Btb3p [9] in *csn* and *ubp12* mutants. As shown in Fig. 1a, Btb3p steady-state protein levels were considerably reduced in *csn1*, *csn4*, and *csn5* deletion mutants relative to wild-type cells (Fig. 1a). Whereas a similar downregulation was found in *ubp12* mutants, an even greater decrease was observed in

csn5 ubp12 double mutants (Fig. 1b), suggesting that both genes cooperate in regulating Btb3p levels. Whereas Cul3p deneddylation was inhibited in *csn*, but not *ubp12* mutants, as described [5,17], Cul3p and Rbx1p protein levels were unchanged in the absence of either *csn5* or *ubp12* (Fig. 1a, b).

Btb3p formed Cul3p-dependent interactions with both Csn5p and Ubp12p, suggesting that CSN recruits Ubp12p to Cul3p/Btb3p complexes (Fig. 1c). Consistent with this notion, binding of Ubp12p to Btb3p was largely dependent on *csn5* (Fig. 1d). However, we noticed that a small fraction of Ubp12p still associated with Btb3p in the absence of CSN (Fig. 1d, lane 3). These data suggested partially overlapping, but not completely redundant, functions of Csn5p-mediated deneddylation and Ubp12p-mediated deubiquitylation in maintaining Btb3p levels. Consistent with this notion, expression of Csn5p from a plasmid rescued Btb3p levels in *csn5 ubp12* mutants close to the level observed in *ubp12* single mutants, but not to the level in a wild-type strain (Fig. 1e). The mutant Csn5p-H118A protein, which is deficient in deneddylation activity (Fig. 1e, Cul3 panel), was completely unable to rescue Btb3p levels in *csn5 ubp12* mutants (Fig. 1e, lane 5). Thus, Csn5p-mediated deneddylation and Ubp12p-dependent deubiquitylation have partially overlapping, but biochemically separable, functions in the control of Btb3p levels.

Several lines of evidence indicated that the observed changes reflect direct control of Btb3p by the CSN/Ubp12p pathway. For one, neither *csn5* or *ubp12* single mutants, nor *csn5 ubp12* double mutants, display any obvious cellular phenotypes[17,19], thus excluding secondary consequences of grossly disturbed physiology. Secondly, unlike Btb3p protein levels, *btb3* mRNA levels were not decreased in *csn5* and *ubp12* single or double mutants (Supplementary Fig. 1).

Decreased Btb3p levels in *csn5* and *ubp12* mutants may therefore reflect increased proteolytic turn-over. Indeed, Btb3p stability was reduced by ~30% in *csn5* mutants and ~60% in *ubp12* mutants (Fig. 2a). Btb3p destabilization corresponded with an increase in Btb3p ubiquitylation, which became apparent by the accumulation of high molecular weight forms of Btb3p in the *mts3-1* proteasome mutant (Fig. 2b). These intermediates represent polyubiquitylated Btb3p, because they were disassembled *in vitro* by recombinant Ubp12p (Fig. 2c). In addition, polyubiquitylated Btb3p present in *mts3-1* cell lysate was deubiquitylated upon incubation of the lysate in the absence of exogenous ATP (Fig. 2c). Deubiquitylation was not observed in cell lysate from *mts3-1 ubp12* double mutants (Fig. 2c), indicating that Ubp12p is the exclusive Btb3p deubiquitylating enzyme *in vivo* and *in vitro*.

Btb3p is known to be subject to autocatalytic degradation by its associated Cul3p/Rbx1p core module [5]. Remarkably, deletion of *cul3* completely abolished Btb3p downregulation in *csn5* and *ubp12* mutants (Fig. 2d). In fact, Btb3p did not appreciably decay in the absence of *cul3*, either in wild-type or in *csn5* and *ubp12* mutants (Fig. 2e). This indicates that the CSN/Ubp12p pathway prevents proteolytic downregulation of Btb3p by directly and specifically acting upon the Cul3p-dependent, autocatalytic destruction mechanism. This conclusion is strengthened by the observation that the level of Btb2p, another Cul3p adapter that is not subject to autocatalytic destruction [9], was unchanged in *csn5* and *ubp12* mutants (Fig. 1b).

The finding that the same CSN/Ubp12p-associated activities that inhibit Cul3p activity *in vitro* are required to maintain Btb3p adapter stability *in vivo* suggested that CSN/Ubp12p paradoxically promotes rather than inhibits CRL-dependent substrate degradation. In fact, CSN was already shown to be required for the efficient destruction

of the *C. elegans* CUL3 substrate MEI1 via its autocatalytically unstable BTB adapter MEL26 [20], although the mechanism remained unclear. To determine the effect of CSN-mediated deneddylation and deubiquitylation on substrate degradation, we turned to the Cul1p-associated SCF^{Pop1p} ubiquitin ligase, for which, unlike with Cul3p and other putative Cul1p ligases, substrates are already known in fission yeast. Similar to Btb3p, and consistent with our previous report [5], the half-life of the F-box adapter Pop1p was reduced by ~30% in *csn5* and *ubp12* mutants (Fig. 3). Instead, the degradation of its substrate, the CDK inhibitor Rum1p, was delayed in the same cells (Fig. 3). While the extent of Rum1p stabilization was as moderate as could be expected from the modest destabilization of Pop1p, a similar stabilization was reported for the budding yeast CDK inhibitor Sic1p in *csn5* mutants [18], although the effect of *ubp12* deletion was not tested. Regardless, this finding confirmed the prediction that reduced adapter stability due to loss of CSN/Upb12p compromises substrate degradation.

As observed with yeast CDK inhibitors, lack of CSN also delays the degradation of CRL substrates in higher eukaryotes, in which cullins encode essential genes (reviewed in [1,2]). Presumably as a consequence, CSN genes are indispensable for viability in *A. thaliana*, *D. melanogaster*, *C. elegans*, and mice (reviewed in Ref. [3]). In contrast, yeast *csn5* and *ubp12* mutants are viable and show no obvious growth or morphological defects [5,17,19,21]. This is likely due to the relatively relaxed requirement for CRL activity in yeasts. For example, unlike with higher eukaryotes, *cul3* is completely dispensable in *S. pombe* [17], and accumulation of its substrates, which are currently unknown, is not expected to be consequential for viability. In contrast, *cul1* mutants are inviable, presumably resulting from the accumulation of Cul1p targeted substrates [22]. However, only two of the sixteen *S. pombe* F-box adapters are essential for vegetative growth [23].

Thus, despite adapter instability, the CRL activity retained in *csn5* and *ubp12* mutants appears to be sufficient to maintain viability under normal growth conditions.

We therefore asked whether the critical role of CSN established for CRL function in higher eukaryotes could be uncovered in fission yeast, if SCF activity was compromised. To accomplish this, we turned to a panel of recently described temperature-sensitive *skp1* mutants [23]. Although these mutants showed various degrees of cell elongation, slow growth, and Rum1p accumulation, they maintained viability at the restrictive temperature (Fig. 4a). In addition, the mutant Skp1p proteins efficiently assembled into SCF core complexes with Cul1p and Rbx1p at both permissive and restrictive temperatures (Fig. 4b). Since the *ts* point mutations cluster around the F-box adapter binding region [23], Skp1p-ts proteins appear to be defective in recruitment of adapter proteins to Cul1p core complexes. In fact, the Skp1p-A7 mutant protein was shown to exhibit reduced binding of some F-box adapters [23].

Deletion of *csn5* or *ubp12* in the three *skp1-ts* mutants dramatically exacerbated their phenotype, causing lethality at the restrictive temperature (Fig. 4c). A similar synthetic effect was observed with *csn4* mutants, suggesting that it is due to loss of function of the entire CSN complex (Supplementary Fig. 2). The *csn5 skp1-A7* strain was rescued from lethality when *csn5* was ectopically provided from a plasmid (Fig. 4d). This rescue depended on the integrity of the JAMM metalloprotease motif [18] of the deneddylating Csn5p enzyme, as Csn5p carrying single point mutations destroying the JAMM domain were unable to complement the lethality of *skp1-ts* mutants (Fig. 4d). Similarly, the *ubp12 skp1-A7* mutant was rescued by plasmid-borne *ubp12,* but not by *ubp3* or *uch2*, two genes encoding additional ubiquitin processing cysteine proteases (Fig. 4e). These data suggest that the CSN/Ubp12p deneddylation and deubiquitylation

activities become essential in fission yeast when adapter recruitment is compromised.

Our results provide an explanation for the paradoxical finding emerging from numerous recent reports that CSN and its associated enzymatic activities inhibit CRL activity *in vitro*, while stimulating substrate degradation *in vivo* (reviewed in [1,2]). As shown here, the CRL inhibitory deneddylation and deubiquitylation activities identified *in vitro* serve overlapping, but not completely redundant, functions in restraining the degradation of labile substrate adapters *in vivo*. Partial redundancy is suggested by the findings that both activities have additive effects on Btb3p stability (Fig. 1b, e), while loss of either is sufficient to cause lethality of *skp1-ts* mutants (Fig. 4c). Adapter availability appears to be rate-limiting for the activity of at least a subset of CRLs, as overexpression of some adapters forces the ectopic degradation of their cognate substrates [24,25]. Promoting adapter availability therefore provides an attractive mechanism for CSN-mediated stimulation of CRL function *in vivo*.

Importantly, we pinpointed the mechanism of adapter stabilization by CSN/Ubp12p to the suppression of autocatalytic destruction by Cul/Rbx1 core complexes. In the absence of Cul3p, the CSN/Ubp12p pathway is not required to maintain Btb3p stability (Fig. 4e). This finding demonstrates that inhibition of Cul3p ligase activity by removal of the stimulatory Nedd8 modification as well as neutralization of Cul3p activity by Ubp12p, both of which occur when the cullin is bound to CSN, limit the extent to which the Cul3p/Rbx1p core complex can autoubiquitylate its associated adapter Btb3p.

The same mechanism may ensure the stability of Pop1p and other autocatalytically unstable Cul1p adapters, although this is difficult to address *in vivo*, because *cul1* is an essential gene. Nevertheless, the physiological significance of the

CSN/Ubp12p pathway for Cul1p function is strikingly exposed by the synthetic phenotypes of *csn5* and *ubp12* mutants with *skp1-ts* alleles. Since the Skp1p-ts proteins are proficient in assembling with Cul1p/Rbx1p at the restrictive temperature, their cellular phenotypes are most likely due to inefficient adapter recruitment, as previously documented for the Skp1p-A7 protein [23]. In the absence of the adapter stabilizing function of CSN/Ubp12p, this defect appears to be aggravated to an extent inconsistent with survival. The increased reliance of higher eukaryotes on CRL activity and their vast repertoire of adapters may explain why the CSN is essential in these organisms even when adapter recruitment is not compromised.

CRL core complexes associate with hundreds of different adapters presumably targeting an even greater number of substrates. A switch in substrate specificity will therefore require adapter exchange. When substrate concentration for a particular adapter is low, autocatalytic adapter removal provides a ready means for rapidly changing substrate specificity. The cell is then faced with the challenge of recruiting new adapters to an inherently unfavorable environment, i.e. autocatalytically active CRL core complexes. Alternatively, autocatalytic adapter degradation may be merely an unavoidable by-product of the "hit-and-run" mechanism proposed to underlie the normal SCF^{Cdc4p}-dependent destruction of budding yeast Sic1p [26]. According to this model, ubiquitin-charged UBCs are transiently recruited to the CRL core, but then released into the vicinity of the adapter/substrate complex. Whereas the spatial flexibility afforded by this mechanism may facilitate the assembly of multiubiquitin chains on substrates, adapters containing exposed lysine residues may be inadvertently ubiquitylated due to their close proximity.

In either case, *de novo* assembly of CRLs with labile adapters or maintenance of

CRLs containing adapters prone to autocatalytic destruction would profit greatly from the CSN/Ubp12p-dependent mechanisms of adapter stabilization demonstrated in this study. Our results strengthen the model that, by virtue of its enzymatic activities, CSN serves as an assembly and maintenance platform for cullins and their labile adapters, thus promoting CRL activity [1,2].

Materials and Methods

Yeast methods

The deletion strains and epitope-tagged strains summarized in Table 1 were constructed by one-step gene replacement using PCR-generated fragments containing *ura4* or kanamycin cassettes [27]. The *skp1-ts* mutants were created as described by Lehmann *et al.* [23]. The *skp1-ts csn4, skp1-ts csn5,* and *skp1-ts ubp12* double mutants were created by mating and tetrad dissection, followed by verification of the recombinants by colony PCR. For growth analysis of temperature sensitive strains, cells were grown at the permissive temperature to an OD_{595} of 0.4. Cells were adjusted to a concentration of 800 cells/ul medium, and 10 ul were spotted onto plates. Five iterative two-fold dilutions were subsequently spotted onto the same plates. Plates were incubated either at 36.5 ^{0}C or at room temperature for 4-5 days.

Plasmids for complementation studies were prepared by amplifying the respective genes from *S. pombe* cDNA. PCR products were sequenced, and cloned into pRep3 plasmids, which drive the expression of N-terminally Myc epitope-tagged proteins from the thiamine repressible *nmt1* promoter. Cells were grown in EMM containing thiamine (promoter off) and spotted onto plates lacking thiamine to induce expression.

Immunological techniques

Epitope-tagged proteins were detected by the monoclonal anti-Myc antibody 9E10 or with monoclonal anti-Protein A antibodies (Sigma). Cell lysates for immunoprecipitation were prepared as described [17]. Lysates were cleared by centrifugation, and proteins were precipitated with the respective antisera. Immunocomplexes were collected by binding to

protein A beads, washed, and analyzed by immunoblotting as described [17]. Protein A-tagged proteins were precipitated using whole rabbit immunoglobulin absorbed to Dynabeads. Affinity-purified rabbit antisera against Cul3p, Skp1p, and Rbx1p were described before [9,17]. Conditions for observing ubiquitylated Btb3p were previously described [9]. For loading controls either anti-tubulin (Sigma) or PSTAIR (Santa Cruz) antibodies were used at dilutions of 1:10.000 and 1:1.000, respectively.

In Vitro Deubiquitylation Assay

Cells were grown to an OD_{595} of 0.6 and shifted to the non-permissive temperature of 37^0C for 2.5 hours. Cells were washed in STOP buffer, and protein extraction was performed as described above for immunoprecipitation. Fifty microgram of pre-cleared protein lysate was incubated in the presence or absence of 200 ng bacterially expressed His6-Ubp12p in a total volume of 10 ul at 30^0C for the indicated times. The reaction were stopped by the addition of SDS sample buffer. Btb3p-ProA ubiquitin bands were visualized by immunoblotting with ProA antibodies.

Acknowledgments

We wish to thank S. Moreno for Rum1p antisera, and G. Cope and R. Deshaies for *csn5* JAMM mutants. The assistance of C. Zhou during much of these studies is gratefully acknowledged. This work was supported by NIH grant GM59780 to D.A.W., by grants from Cancer Research UK and the Human Frontier Science Program to T.T., and by the NIEHS Center Grant ES00002 and the NIEHS Training Grant ES07155.

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Figure Legends

Fig. 1 Effect of CSN/Ubp12p on Cul3p/Btb3p

a, b) Total protein lysate prepared from the indicated strains was analyzed by immunoblotting for the expression of Btb3p-ProA, Cul3p, Rbx1p, and Btb2p-ProA. Tubulin and Cdc2p signals were used as loading controls. The asterisks on the Cul3p blots indicate cross-detection of Btb3p-ProA by the HRP anti-rabbit secondary antibodies.

c) Cul3p-dependent interactions of Btb3p with Csn5p and Ubp12p. Myc epitope-tagged Csn5p or Ubp12p were expressed from pREP81 plasmids in strains containing ProA-tagged Btb3p integrated at the endogenous genomic locus. Some strains carry deletions of *cul3* as indicated (lanes 3 and 6). Wild-type strains expressing Myc-Csn5p (lane1) or Myc-Ubp12p (lane 4) are included to demonstrate binding specificity. Btb3p-ProA was affinity-purified on IgG resin and co-purification of Myc-Csn5p (left panel) and Myc-Ubp12p (right panel) was examined by immunoblotting with Myc antibodies. Total cell lysates and loading controls are shown in the top panels.

d) CSN-dependent interactions of Btb3p with Ubp12p. Myc epitope-tagged Ubp12p was expressed from pREP81 in strains containing tagged Btb3p-ProA. A csn5 deletion strain was also included in the analysis (lane 3). Btb3p-ProA was affinity-purified on IgG resin and co-purification of Myc-Ubp12p was examined by immunoblotting with Myc antibodies. Note that there is residual binding of Btb3p to Ubp12p in CSN deficient strains (lane 3). Total cell lysates and loading controls are shown in the top panels.

e) Partial, JAMM-dependent rescue of Btb3p levels in *csn5 ubp12* double mutants by Csn5p. Wild-type, *ubp12*, and *csn5 ubp12* deletion strains carrying tagged Btb3p were

transformed with plasmids expressing Myc-tagged Csn5p. The H118A mutation destroys the JAMM metalloprotease motif, resulting in lack of deneddylation activity [18]. Total protein lysates were examined for the expression of Btb3p, Cul3p, Myc-Csn5p, and Cdc2p by immunoblotting.

Fig. 2 Btb3p ubiquitylation and stability in *csn5* and *ubp12* mutants

a) Wild-type, *csn5*, and *ubp12* deletion strains expressing Btb3p-ProA from the endogenous genomic locus were incubated with 100 ug/ml cycloheximide (CHX) for the indicated periods prior to preparation of total protein lysates. Btb3p stability was assessed by immunoblotting with ProA antibodies. Tubulin is shown as loading control. The experiment was performed in triplicates, quantitated by densitometry, and Btb3p half-lives were calculated and displayed in a bar graph (right panel).

b) Effect on Btb3p ubiquitylation. The strains described above were crossed into *mts3-1* proteasome mutants and shifted to the restrictive temperature for the indicated times. Protein lysates were prepared in urea containing buffer as described in the Methods section. Btb3p-ProA and its ubiquitylated forms were detected by immunoblotting. Polyubiquitylated species are indicated (Btb3p-ProA-Ub_n).

c) Deubiquitylation of Btb3p by Ubp12p *in vitro*. Lysate prepared from *btb3-proA ubp12 mts3-1* cells was incubated with bacterially expressed His6-Ubp12p in the absence of exogenous ATP for the indicated times (left panel). The status of Btb3p-ProA ubiquitylation was assessed by immunoblotting. The short exposure on the bottom shows the effect on Btb3p monoubiquitylation. In the right panel, lysates from *btb3-proA mts3-1* and *btb3-proA ubp12 mts3-1* were incubated in the absence of exogenous ATP, and Btb3p deubiquitylation was determined by immunoblotting. Twice the amount of lysate

from *btb3-proA mts3-1* was loaded to account for the lower ubiquitylation levels of Btb3p in the presence of *ubp12*. Cdc2p levels are shown as loading control.

d) Strains of the indicated backgrounds were analyzed for Btb3p-ProA levels by immunoblotting.

e) Btb3p-ProA stability in the indicated strains was determined as in a).

Fig. 3 Effect of CSN/Ubp12p on the stability of Pop1p and Rum1p

Wild-types, *csn5*, and *ubp12* deletion strains simultaneously harboring Myc epitope-tagged Pop1p and Rum1p at the endogenous genomic loci were incubated with cycloheximide for the indicated periods. Pop1p and Rum1p levels were assessed by immunoblotting with Myc antibodies. Tubulin is shown as a loading control.

Fig. 4 Analysis of the effects of *csn5* and *ubp12* on *skp1-ts* mutants

a) Phenotypes of the *skp1-ts* mutants A3, A4, and A7. Despite varying degrees of cell elongation, all mutants are viable at the restrictive temperature. Rum1p accumulation was examined by immunoblotting upon shift to the restrictive temperature for the indicated periods.

b) SCF core complex interactions in *skp1-ts* mutants. Cell lysates of the indicated strains were prepared for immunoprecipitation with affinity-purified Rbx1p antisera, followed by immunoblotting with Rbx1p, Skp1p, and Cul1p antibodies. The experiment was performed with cells grown at the permissive and restrictive temperatures, as indicated. The mobility shift of the Skp1p-A4 protein is due to a read-through mutation of the Stop codon [23].

c) Synthetic lethality of *csn5* and *ubp12* with *skp1-ts* mutants. Dilution series of cells of

the indicated genotypes were spotted onto plates and incubated at the permissive (24^{o}C) or restrictive (36.5^{o}C) temperature. Cell growth was monitored after 4 – 5 days.

d) Rescue by *csn5*. Cells of the genotype *csn5 skp1-A7* were transformed with pREP3 plasmids driving the expression of wild-type or JAMM mutant *myc-csn5* as indicated. Cells were spotted onto plates lacking thiamine, and incubated at the permissive and restrictive temperatures. Expression of the Myc-Csn5p proteins was confirmed by immunoblotting (blot to the right).

e) Rescue by *ubp12*. Cells of the genotype *ubp12 skp1-A7* were transformed with pREP3 plasmids driving the expression of *ubp12* (upper panel), *ubp3*, or *uch2* (lower panel). Transformants were kept in the absence or presence of thiamine, leading to high or low expression levels as indicated. Low level expression of Ubp12p in the presence of thiamine is due to leakiness of the *nmt1* promoter under repression by thiamine. Rescue by *ubp12* only occurs at low expression levels, since high level expression was found to be toxic to wild-type cells. Expression of the exogenous proteins was confirmed by immunoblotting (blots on the right).

Supplementary Figure 1

Total mRNA was isolated from the indicated strains and used in RT/PCR reactions with primers specific for *btb3* and actin as a loading control. Samples were removed after the number of PCR cycles indicated. Reactions lacking reverse transcriptase are shown as negative controls for DNA contamination of the RNA samples (top panel).

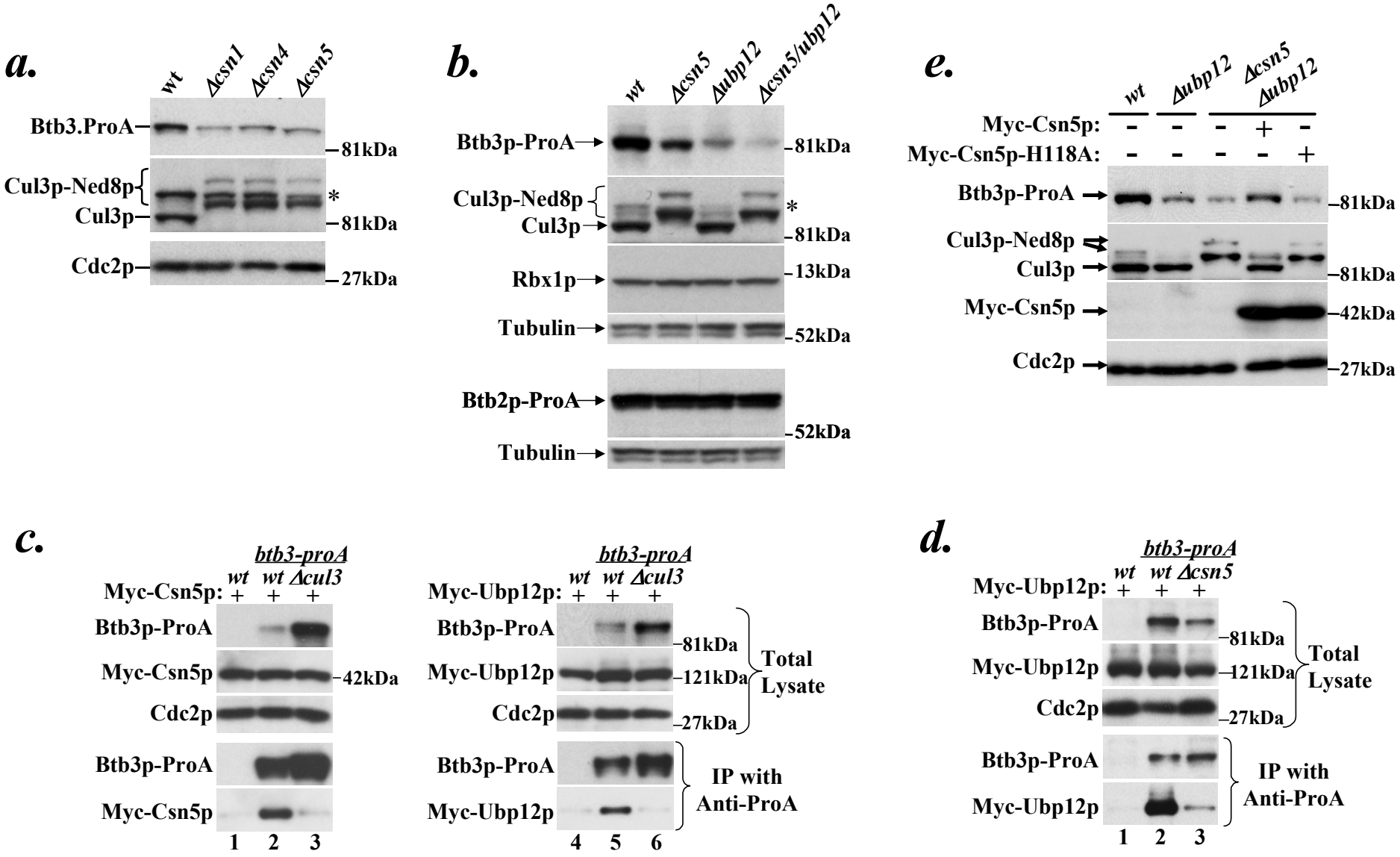
Supplementary Figure 2

Synthetic lethality of *csn4* with *skp1-a7* mutants. Dilution series of cells of the indicated

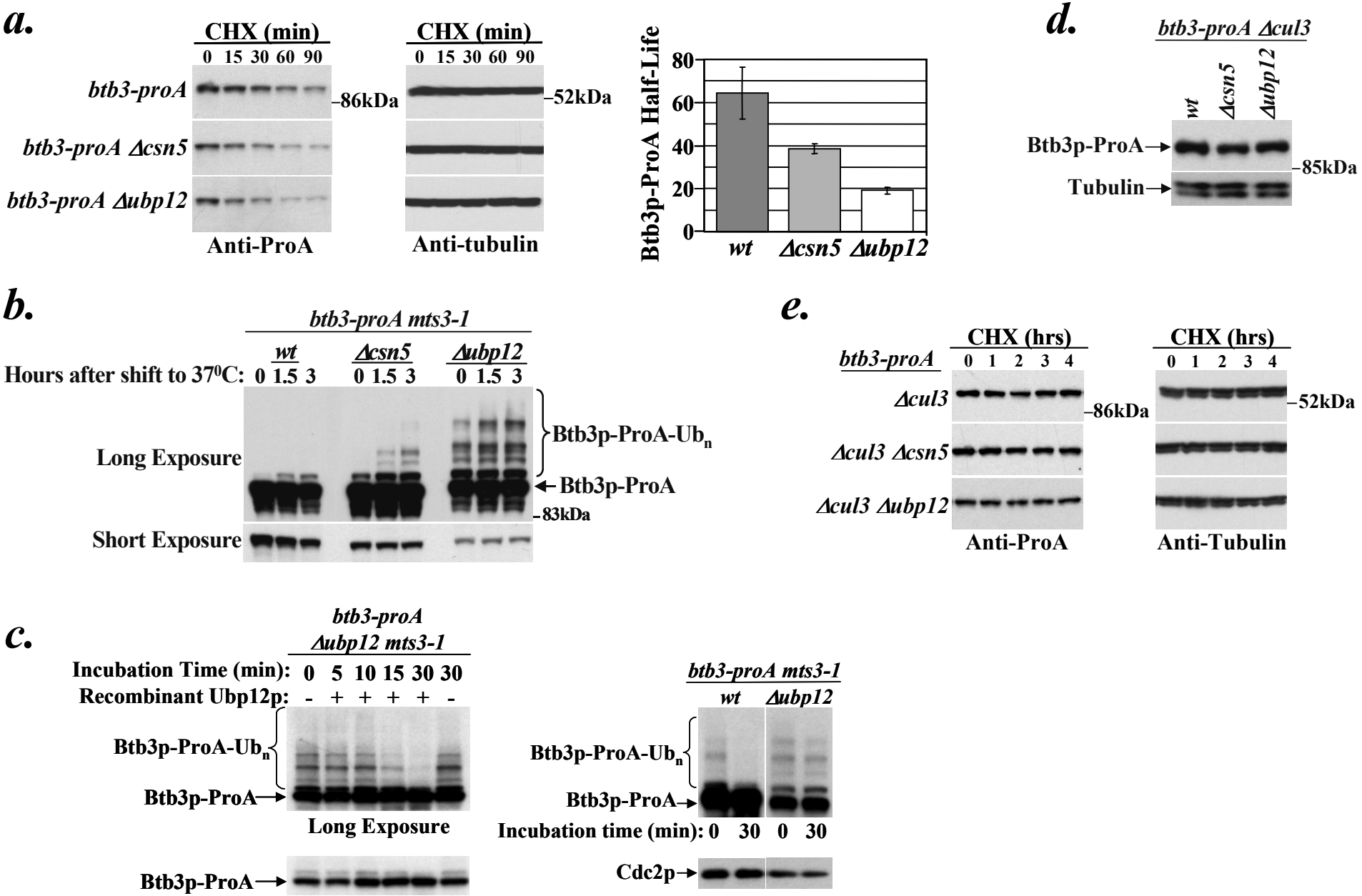
genotypes were spotted onto plates and incubated at the permissive (24^{o}C) or restrictive (36.5^{o}C) temperature. Cell growth was monitored after 4 – 5 days.

Table 1. Strains Used

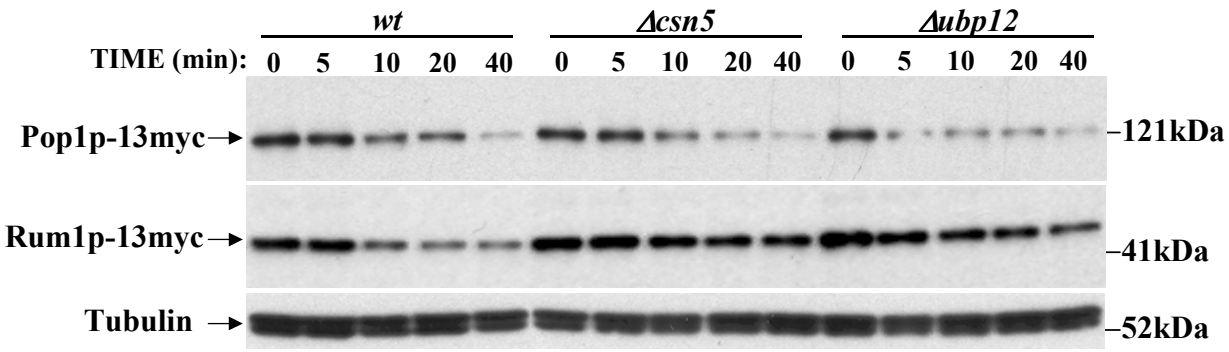
Name	Genotype		Source
DS448/1	*leu1-32 ura4-d18 ade6-704*	*h+*	Lab stock
DS448/2	*leu1-32 ura4-d18 ade6-704*	*h-*	Lab stock
DS362/2	*leu1-32 ura4-d18 ade6-704*	*rum1::ura4 h-*	Lab stock
SWF16	*leu1-32 ura4-d18 ade6-704*	*pop1::ura4 h-*	Lab stock
SWF64	*leu1-32 ura4-d18 ade6-704*	*pop1.13myc kan h+*	Lab stock
SWF85	*leu1-32 ura4-d18 ade6-704*	*csn5::ura4 rum1.13myc pop1.13myc kan h+*	This study
SWF87	*leu1-32 ura4-d18 ade6-704*	*ubp12:ura4 rum1.13myc pop1.13myc kan h+*	This study
SWF91	*skp1-A4 leu1-32 ura4-d18 ade6-704*	*ubp12::ura4 h-*	This study
SWF 98	*skp1-A4 leu1-32 ura4-d18*	*csn4::ura4 h-*	This study
SWF114	*skp1-A3 leu1-32 ura4-d18*	*h-*	Lab stock
SWF115	*skp1-A4 leu1-32 ura4-d18*	*h-*	Lab stock
SWF118	*skp1-A4 leu1-32 ura4-d18 ade6-704*	*csn5::ura4 h-*	This study
SWF121	*skp1-A3 leu1-32 ura4-d18 ade6-704*	*csn5::ura4 h-*	This study
SWF126	*skp1-A3 leu1-32 ura4-d18 ade6-704*	*ubp12::ura4 h-*	This study
SWF130	*skp1-A7 leu1-32 ura4-d18*	*h-*	Lab stock
SWF138	*skp1-A7 leu1-32 ura4-d18 ade6-704*	*csn5::ura4 h-*	This study
SWF140	*skp1-A7 leu1-32 ura4-d18 ade6-704*	*ubp12::ura4 h-*	This study
SWF191	*skp1-A7 leu1-32 ura4-d18 ade6-704*	*csn4::ura4 h-*	This study
SWF196	*leu1-32 ura4-d18 ade6-704*	*csn5::ura4 btb3.tev5proA h+*	This study
SWF199	*leu1-32 ura4-d18 ade6-704*	*btb3.tev5xproA kan h+*	Lab stock
SWF200	*leu1-32 ura4-d18 ade6-704*	*ubp12::ura4 btb3.tev5proA h+*	This study
SWF217	*leu1-32 ura4-d18 ade6-704*	*csn5::ura4 cul3::ura4 btb3.tev5proA h+*	This study
SWF219	*leu1-32 ura4-d18 ade6-704*	*ubp12::ura4 cul3::ura4 btb3.tev5proA h+*	This study
SWF235	*leu1-32 ura4-d18 ade6-704*	*csn5::ura4 ubp12::ura4 btb3.tev5proA h+*	This study
SWF241	*mts3-1 leu1-32 ura4-d18 ade6-704*	*btb3.tev5xproA kan h-*	Lab stock
SWF242	*mts3-1 leu1-32 ura4-d18 ade6-704*	*csn5::ura4 btb3.tev5proA h-*	This study
SWF245	*mts3-1 leu1-32 ura4-d18 ade6-704*	*ubp12::ura4 btb3.tev5proA h-*	This study
SWF248	*leu1-32 ura4-d18 ade6-704*	*btb2.tev5xproA kan h+*	Lab stock
SWF250	*leu1-32 ura4-d18 ade6-704*	*csn5:ura4 btb2.tev5xproA kan h-*	This study
SWF253	*leu1-32 ura4-d18 ade6-704*	*ubp12::ura4 btb2.tev5xproA kan h-*	This study
SWF257	*leu1-32 ura4-d18 ade6-704*	*csn5:ura4 ubp12::ura4 btb2.tev5xproA kan h-*	This study



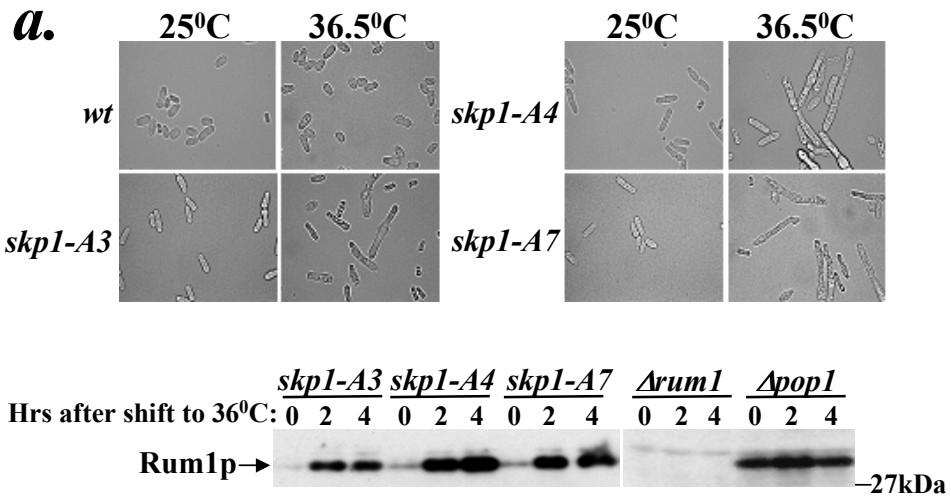
Wee et al., Fig. 1

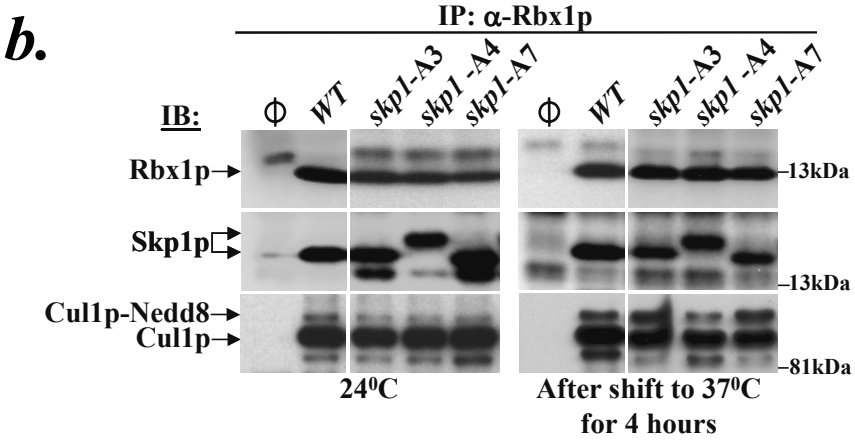


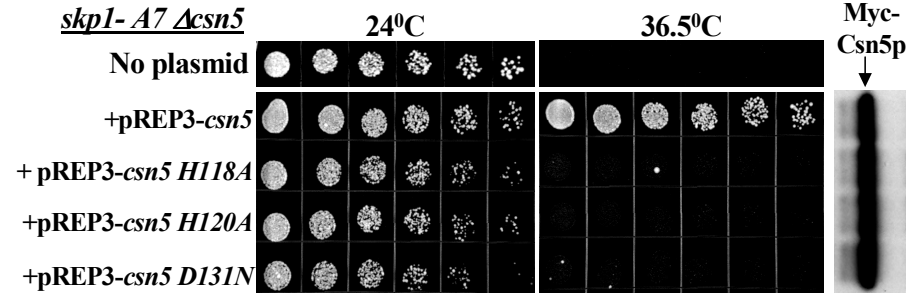
Wee et al., Fig. 2

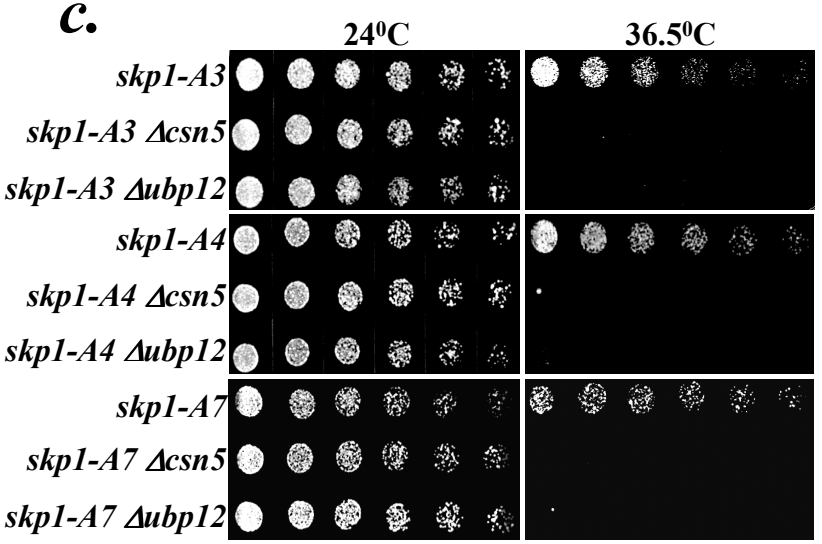


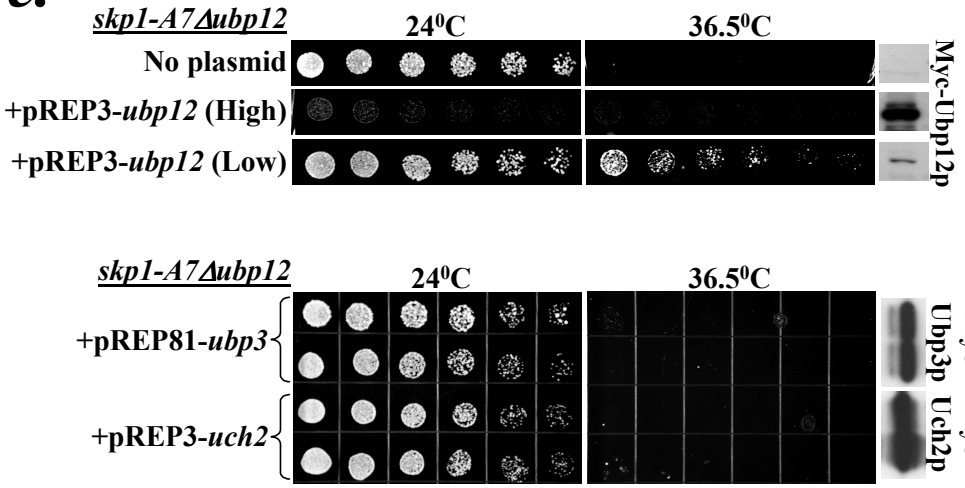
Wee et al., Fig. 3











Wee et al., Fig. 4