Supplement to:

F-Box-directed CRL complex assembly and regulation by the CSN and CAND1

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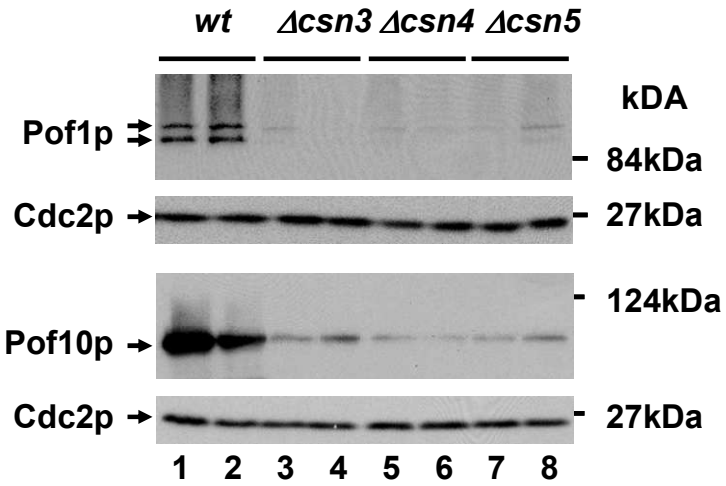
Supplementary Figure 1

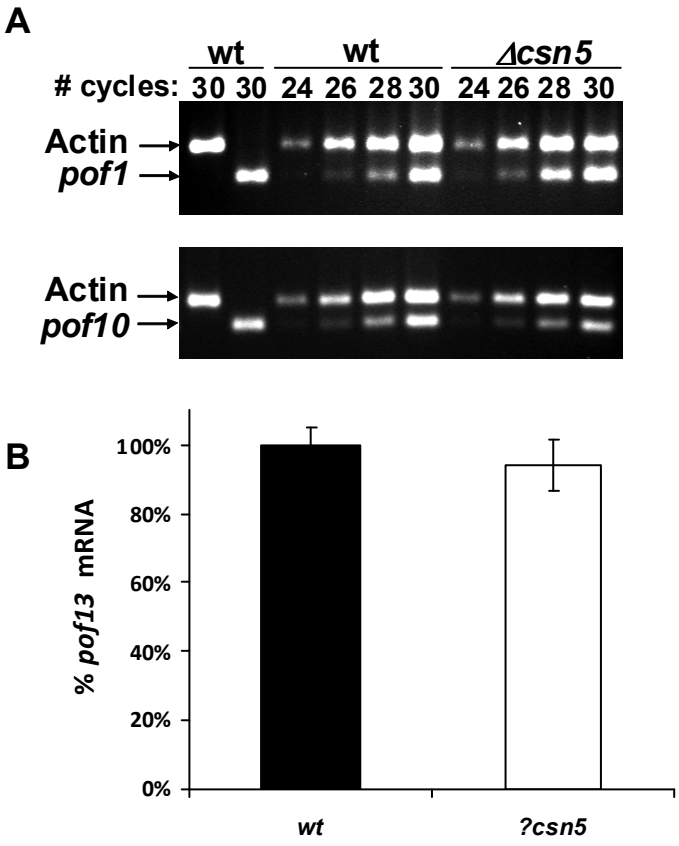
Effect of *csn* deletions on Pof1p and Pof10p levels

The indicated wild-type and *csn* deletion strains harboring Myc-tagged Pof1p or Pof10p were examined for FBP expression by immunoblotting with Myc antibodies. Two independently derived strains are shown for each mutant. Note that Pof1p is known to occasionally resolve into duplet bands on SDS gels (Harrison et al., 2005). The exact nature of these forms remains to be determined.



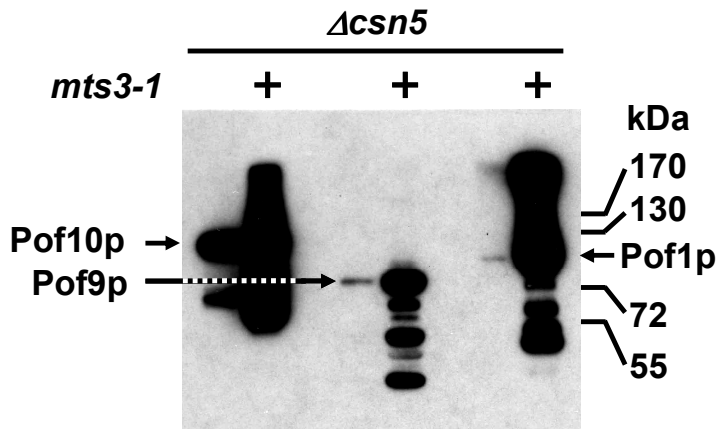
Supplementary Figure 2

Steady-state mRNA levels of (A) *pof1* and *pof10*, and (B) *pof13* in wildtype cells and in csn5 mutants. mRNA levels were determined by semiquantitaive RT-PCR (A) or by quantitative real time RT-PCR (B).



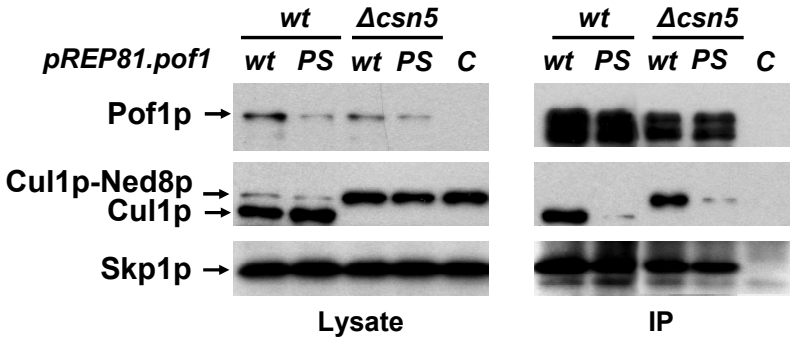
Supplementary Figure 3

Accumulation of Myc-tagged FBPs in the *mts3-1* proteasome mutant strain. *Csn5* and *csn5 mts3-1* strains harboring Myc-tagged alleles of Pof1p, Pof9p, or Pof10p were maintained at 37^{o}C for 90 min. The expression of FBPs was determined by immunoblotting with Myc antibodies. Only 1/10 of cell lysate was loaded for *mts3-1* strains. The high molecular weight smears likely represent polyubiquitylated FBP species.



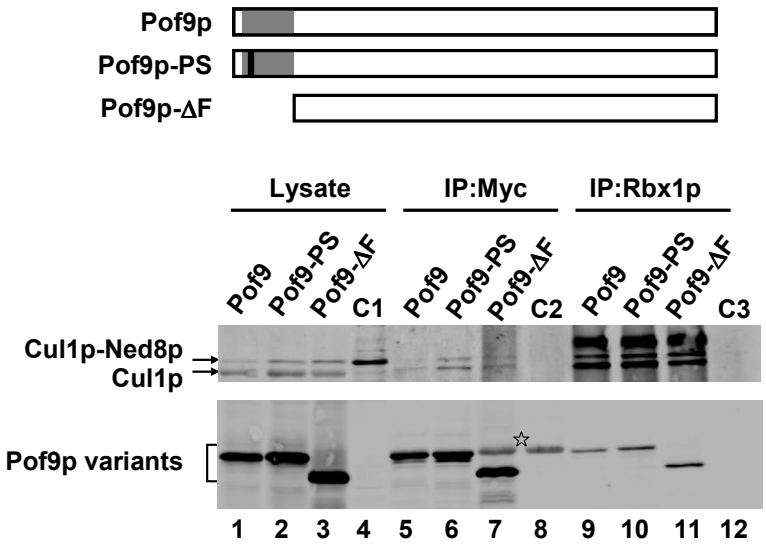
Supplementary Figure 4

Interaction of plasmid derived wild-type and proline mutant Pof1p with Cul1p and Skp1p in wildtype cells and in *csn5* mutants. Myc-tagged FBPs were immunoprecipitated and copurification of Cul1p and Skp1p was assayed by immunoblotting. As noted in the legend to Supplementary Fig. 1, Pof1p occasionally resolves into a duplet, probably due to unknown posttranslational modification. "C" denotes a specificity control (cell lysate from untransformed cells).



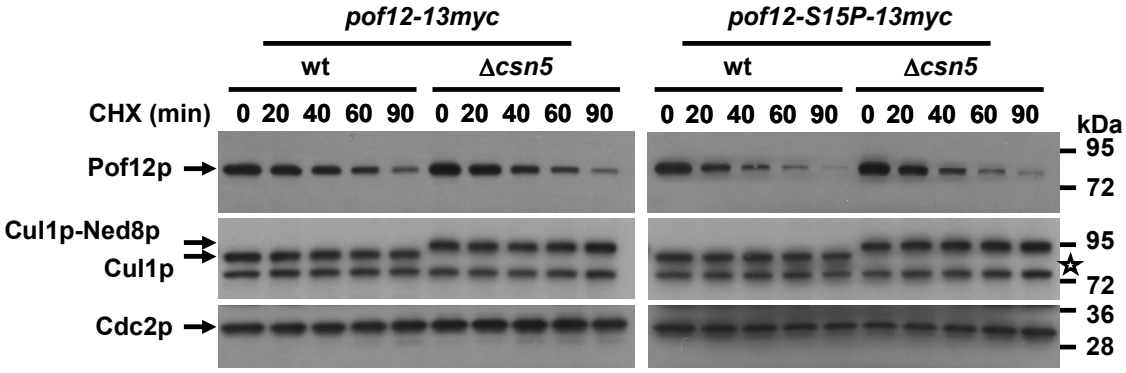
Supplementary Figure 5

The indicated variants of Pof9p were expressed as Myc-tagged proteins from pRep81 plasmids and binding to Cul1p was assessed by immunoprecipitation with Rbx1p and Myc antibodies. “C1, C2, C3” denote specificity controls (cell lysate from untransformed cells). The asterisk denotes an unspecific band.



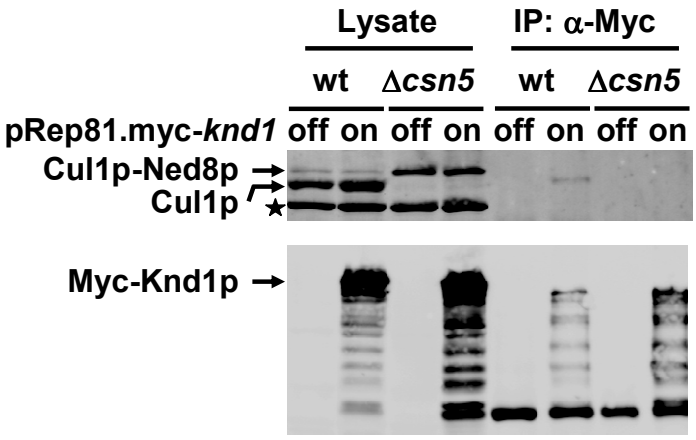
Supplementary Figure 6

Myc epitope-tagged alleles of Pof12p and Pof12p-S15P were created by homologous recombination and crossed into a *csn5* mutant background. The stability of Pof12p and Pof12p-S15P in wildtype (wt) *csn5* mutants were determined by CHX chase. Cul1p and Cdc2p blots are shown for reference. The asterisk denotes an unspecific band.



Supplementary Figure 7

Interaction of Knd1p with Cul1p. Myc-tagged Knd1p was expressed in wildtype cells (wt) and in *csn5* mutants from a thiamine repressible pRep81 plasmid. Cells were kept in the presence (promoter off) or absence (promoter on) of thiamine for 20 h followed by preparation of cell lysate. Cell lysates were subjected to immunoprecipitation with Myc antibodies, and co-purifying Cul1p was revealed by immunoblotting with Cul1p antisera. Lysates prepared from cells not expressing Myc-Knd1p (promoter off) are shown as negative controls. Note than only the unneddylated form of Cul1p present in wildtype cells bound to Knd1p. The asterisk denotes an unspecific band.



References

Harrison, C., Katayama, S., Dhut, S., Chen, D., Jones, N., Bahler, J., and Toda, T. (2005). SCF(Pof1)-ubiquitin and its target Zip1 transcription factor mediate cadmium response in fission yeast. Embo J *24*, 599-610.