

## The proteasome lid triggers COP9 signalosome activity during the transition of *S. cerevisiae* cells into quiescence

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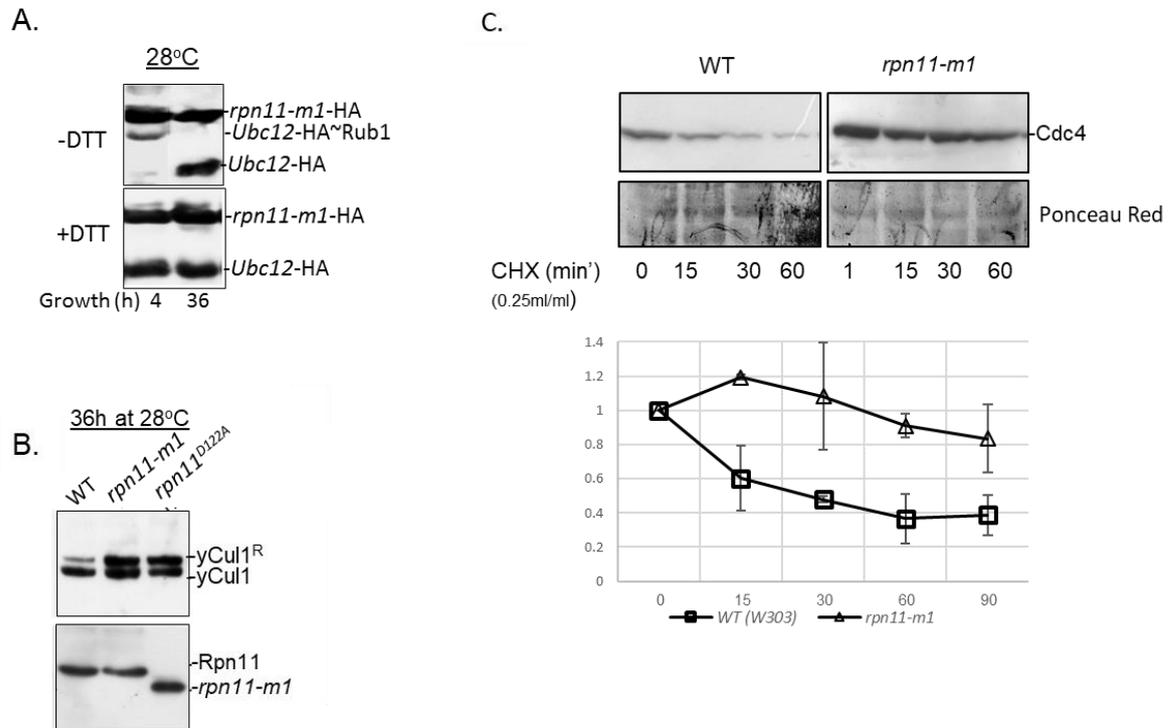
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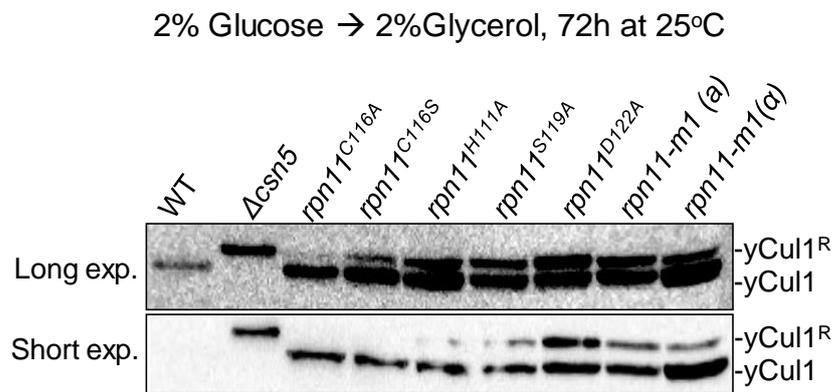
### Includes –

Five supplementary figures

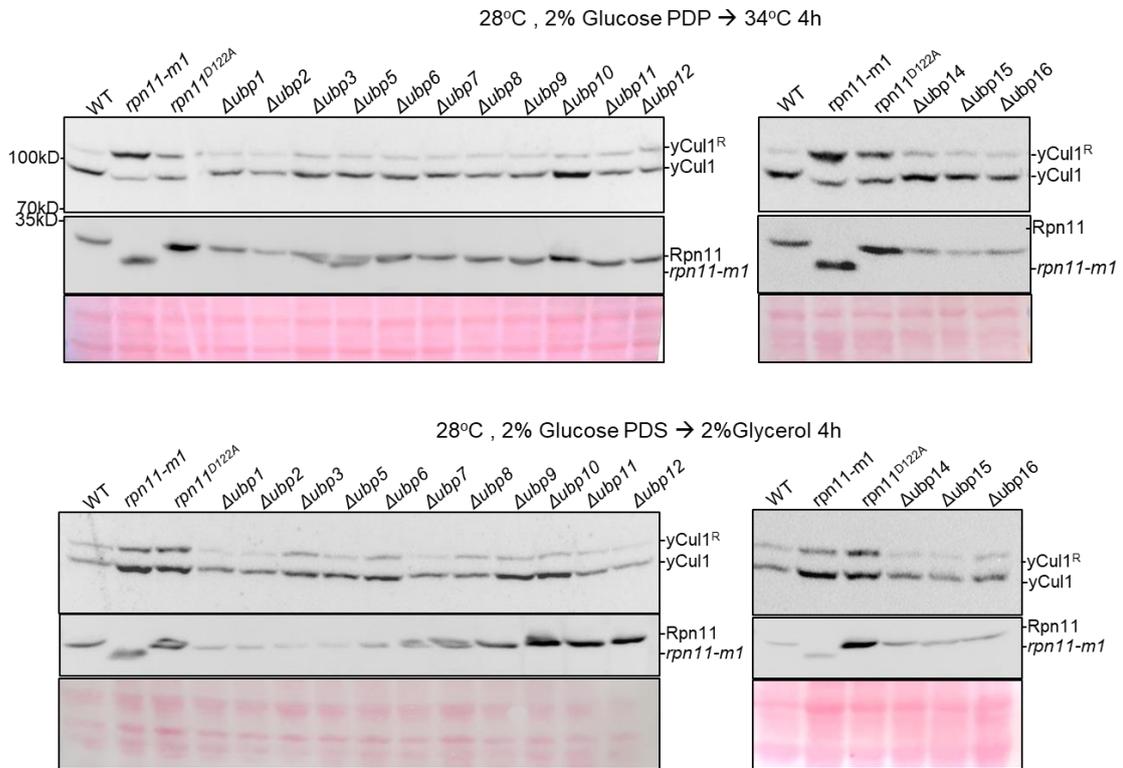
Supplementary references



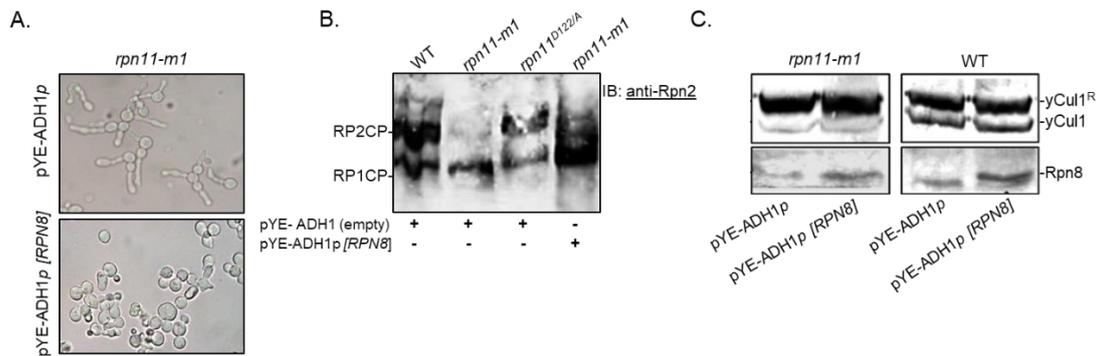
**Figure S1 (for figure 1):** (A) Overnight grown culture of genomic *rpn11-m1*-HA mutant cells expressing endogenous levels of Ubc12-HA was diluted to  $OD_{600} = 0.5$  in YPD and cultured at a permissive temperature of 28°C for 4 and 36 hours. Ubc12~Rub1 thioester forms were examined in non-reduced (-DTT) and reduced (+DTT) conditions. (B) WT and *rpn11* mutant cells were diluted to  $0.5OD_{600}$  in YPD and grown at 28°C and yCul1 modification status was monitored after 36 hours. Notably, yCul1 status at the post diauxic phase (36 hours) is high in *rpn11* mutants comparing with WT; however, the E2 enzyme (Ubc12-HA) is not forming thioesters with Rub1. (C) WT and *rpn11* mutant cells were diluted to  $OD_{600} = 0.5$  in YPD and grown at 28°C for 6 hours. After 6 hours cyclohexamide (CHX) was added in a final concentration of 0.25 mg/ml. Equal amount of cells were extracted at indicated times and total protein extraction were used for immunoblotting with anti-Cdc4. Ponceau red staining of nitrocellulose membranes was added to confirm the equal loading of total proteins. Three independent blots were quantified using Image J software and the intensity of Cdc4 is shown (bottom).



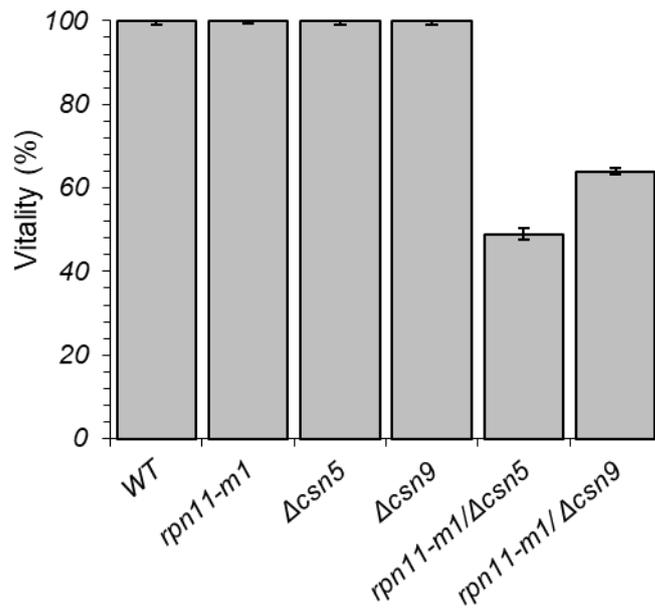
**Figure S2 (for figure 1):** (A) WT and various *rpn11* mutant strains were grown in YP-Glycerol for 72 hours at 25°C and yCul1 modification status was assessed by immunoblotting. Note that *rpn11-m1* and mutants of the MPN+/JAMM active site (*rpn11*<sup>H111/A</sup>, *rpn11*<sup>S119/A</sup>, and *rpn11*<sup>D122/A</sup>) exhibit a higher status of yCul1<sup>R</sup> than silent mutations (*rpn11*<sup>C116A</sup>, *rpn11*<sup>C116S</sup>) or WT.



**Figure S3 (for figure 1):** Various deletion mutants of DUBs were grown in glucose YPD medium until the post-diauxic phase (PDP) followed by four hours at 34°C to induce cell cycle defects (top); or replacement of the growth medium from to YPG medium, containing glycerol as a carbon source. Total cell extracts were used for immunoblotting for yCul1 and Rpn11. Ponceau red staining of nitrocellulose membranes was added to confirm the equal loading of total proteins.



**Figure S4 (for figure 2):** Suppression of cell cycle and proteasome defects by Rpn8 did not alter yCul1 modification status in *rpn11-m1*. The mutant of *rpn11-m1* has transformed with Rpn8 [RPN8] to suppress proteasome and cell cycle defects [1]. Cells were grown overnight at permissive temperature of 28°C followed by additional 8 hours at a restrictive temperature of 37°C to induce cell cycle defects. (A) Suppression of cell cycle defects confirmed by light microscopy (magnification x400, bottom) (B) proteasome structure confirmed by native PAGE, immunoblotted by anti-Rpn2 antibody. The protocol for native PAGE is according to Yu et al. 2011 [2]. (C) Overexpression of Rpn8 did not alter yCul1 modification status in *rpn11-m1*.



**Figure S5 (for figure 5):** Vitality test for double mutants of *rpn11-m1* and *csn* components. Two hundred cells of each mutant in the BY4741 genotypic background at logarithmic phase were plated on YPD in triplicates. Agar plates were incubated at 28°C for 2–3 days and the colonies were then counted and percentage of viable cells was calculated. Experiments were repeated three times.

## **References -**

1. Rinaldi, T.; Pick, E.; Gambadoro, A.; Zilli, S.; Maytal-Kivity, V.; Frontali, L.; Glickman, M.H. Participation of the proteasomal lid subunit Rpn11 in mitochondrial morphology and function is mapped to a distinct C-terminal domain *Biochem J.* **2004**, *381*, 275-285.
2. Yu, Z.; Kleifeld, O.; Lande-Atir, A.; Bsoul, M.; Kleiman, M.; Krutauz, D.; Book, A.; Vierstra, R.D.; Hofmann, K.; Reis, N., et al. Dual function of Rpn5 in two PCI complexes, the 26S proteasome and COP9 signalosome. *Mol Biol Cell* **2011**, *22*, 911-920, doi:10.1091/mbc.E10-08-0655.