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online data

## SUPPLEMENTARY ONLINE DATA

# The Cdc48–Vms1 complex maintains 26S proteasome architecture

Joseph R. TRAN*†[1] and Jeffrey L. BRODSKY*[2]

*Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, U.S.A.
†Graduate Program in Biochemistry and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, U.S.A.

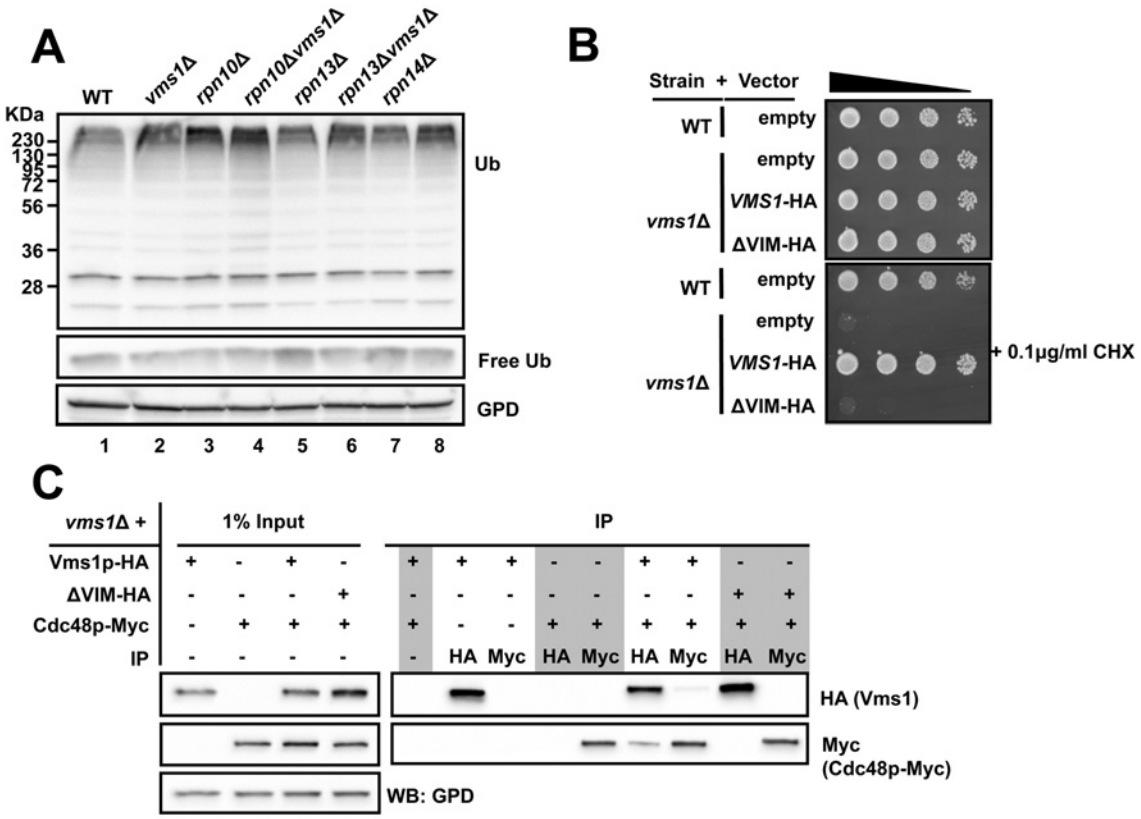


**Figure S1 Ubiquitinated protein accumulation in vms1Δ yeast is magnified when other components of the UPS are absent**

(**A**) The indicated yeast strains were grown to log-phase and the designated proteins were examined as in Figure 1(A) of the main text. (**B**) Cdc48 interaction is required for Vms1 function. Wild-type (WT) or vms1Δ yeast strains were transformed with either a vector control (empty), or a vector engineered for the expression of full-length Vms1 or lacking the VIM. The cells were serially plated on selective medium in the presence or absence of 0.1 $\mu$g/ml cycloheximide (CHX). (**C**) Vms1 interaction with Cdc48 requires the VIM. Lysates were prepared from strains expressing either an untagged or the tagged version of Cdc48 (Myc) and Vms1 (HA), and proteins were immunoprecipitated (IP) with either anti-Myc–agarose or anti-HA–agarose. The bound proteins were resolved by SDS/PAGE and detected with the indicated antibodies. Total lysate, corresponding to 1 % input, is shown in the left-hand panel and immunoprecipitations are shown in the right-hand panel. GDP, glycerol-3-phosphate dehydrogenase; WB, Western blot.

[1] Present address: Department of Embryology, Carnegie Institution for Science, 3520 San Martin Drive, Baltimore, MD 21228, U.S.A.
[2] To whom correspondence should be addressed (email jbrodsky@pitt.edu).

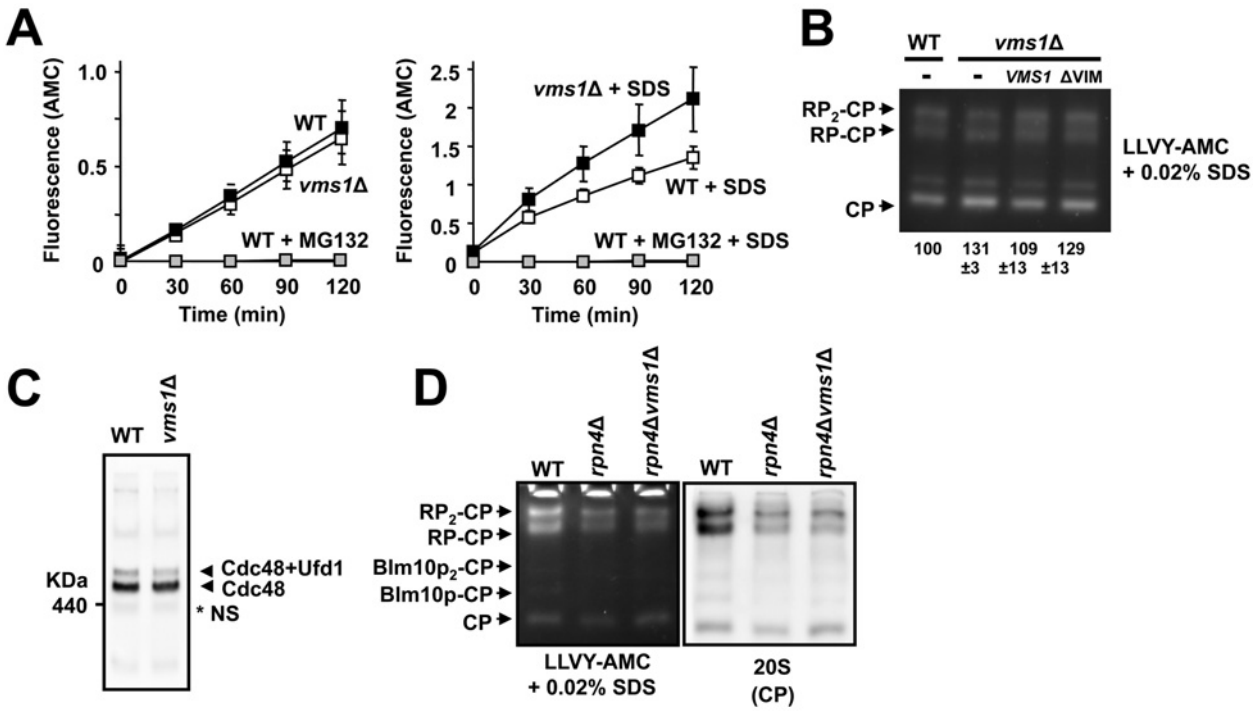


**Figure S2 The Cdc48–VMS1 complex affects proteasome activity**

(**A**) The loss of VMS1 increases 20S proteasome activity in a real-time solution-based assay. Total cellular lysates from the indicated strains were incubated with a fluorogenic substrate Suc-LLVY-AMC in the absence (left-hand panel) or presence (right-hand panel) of 0.02 % SDS, and the fluorescence generated by the liberated AMC was measured at the indicated times. In addition, proteasome activity was assessed in the presence of 100 $\mu$M MG132 ( + MG132). Results are means$\pm$S.D. for three independent experiments. (**B**) The accumulation of 20S core particles in vms1$\Delta$ yeast is reversed upon the re-introduction of VMS1. Cell lysates were prepared by glass-bead lysis and resolved by native PAGE. Proteasome activity was assessed in the presence of 0.02 % SDS as described in Figure 2 of the main text. Values below the gel indicate the mean$\pm$S.D. for three independent experiments in which 20S-mediated activity was measured. (**C**) Loss of VMS1 does not affect the integrity of the Cdc48 hexamer. Cell lysates from the indicated strains were resolved by native PAGE and a Western blot was performed to detect Cdc48. The slower migrating band is most likely a Cdc48 hexameric complex bound to a cofactor, Ufd1, based on Western blotting with antibodies against Ufd1 (results not shown). The *NS represents non-specific immunoreactive protein. (**D**) The increase in free 20S core particles in vms1$\Delta$ lysates is not the result of an Rpn4-mediated response. Lysates were prepared from the indicated strains and resolved by native PAGE. Fluorogenic proteasome activity was assessed in the presence of SDS as in (**B**) and a Western blot analysis to detect 20S resident proteins was performed with an anti-20S antibody. WT, wild-type.

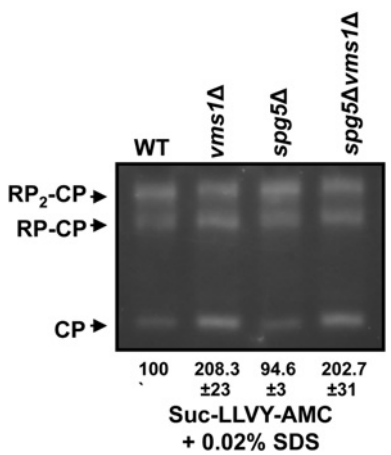


**Figure S3 Loss of VMS1 in the spg5$\Delta$ background still leads to an increase in 20S core particle (CP) activity**

The indicated strains were grown to logarithmic phase and lysates were prepared. The fresh extracts were resolved on a 4 % native gel and incubated with Suc-LLVY-AMC in the presence of 0.02 % SDS and imaged as in Figure 2 of the main text. WT, wild-type.

**Table S1 Yeast strains used in the present study**

WT, wild-type.

| Strain | Genotype | Source/reference |
|---|---|---|
| BY4742 (WT) | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0* | Open Biosystems |
| *vms1Δ* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *vms1ΔKanMX* | Open Biosystems |
| *vms1ΔHis3MX* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *vms1ΔHis3MX* | [1] |
| *ubx2Δ* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *ubx2ΔKanMX* | Open Biosystems |
| *ubx2Δ*,*vms1ΔHis3MX* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *ubx2ΔKanMX*, *vms1ΔHis3MX* | [1] |
| *cdc48-3* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *cdc48-3* | [1] |
| *cdc48-3*,*vms1Δ* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *cdc48-3*, *vms1ΔKanMX* | [1] |
| *rad23Δ*,*dsk2Δ* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *rad23ΔKanMX*, *dsk2ΔKanMX* | S. Michaelis |
| *rad23Δ*,*dsk2Δ*,*vms1ΔHis3MX* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *rad23ΔKanMX*, *dsk2ΔKanMX*, *vms1ΔHis3MX* | The present study |
| *rpn4Δ* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *rpn4ΔKanMX* | The present study |
| *rpn4Δ*,*vms1ΔHis3MX* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *rpn4ΔKanMX*, *vms1ΔHis3MX* | The present study |
| *spg5Δ* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *spg5ΔKanMX* | The present study |
| *spg5Δ*,*vms1ΔHis3MX* | *Matα*, *his3Δ1*, *leu2Δ0*, *ura3Δ0*, *lys2Δ0*, *spg5ΔKanMX*, *vms1ΔHis3MX* | The present study |

**Table S2 Plasmids used in the present study**

| Plasmids | Description | Source/reference |
|---|---|---|
| pRS316-*VMS1*-HA | Endogenous promoter, Vms1-C-terminal 1×-HA | [1] |
| pRS416-*VMS1*ΔVIM-HA | Endogenous promoter, Vms1ΔVIM-C-terminal 3×-HA | [2] |
| pRS316-*VMS1*ΔVIM-HA | Endogenous promoter, Vms1ΔVIM-C-terminal 3×-HA | The present study |

**Table S3 Antibodies used in the present study**

| Antibody against | Source (catalogue number) |
|---|---|
| 20S | Enzo Life Sciences (BML-PW8195) |
| Blm10 | Enzo Life Sciences (BML-PW0570) |
| Cdc48 | A. Buchberger and R. Hartmann-Petersen |
| GPD | Sigma–Aldrich (A9521) |
| HA | Roche (12013819001) |
| Hsm3 | D. Finley |
| Myc (A-14) | Santa Cruz Biotechnology (SC-789) |
| Sec61 | R. Schekman |
| Rpt3 | Abcam (ab22677) |
| Rpt5 | Enzo Life Sciences (BML-PW8245) |
| Rpn3 | Abcam (ab79769) |
| Rpn10–GST | D. Skowyra |
| Rpn12 | D. Finley |
| Ubiquitin (P4D1) | Santa Cruz Biotechnology (SC-8017) |

## REFERENCES

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