Supplemental information

Assembly checkpoint of the proteasome regulatory particle is activated by coordinated actions of proteasomal ATPase chaperones

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Figure S1 related to Main Text Figure 3. Additional controls validating that the NOT module regulates the cellular abundance of RP chaperone proteins.

A, Cellular abundance of the RP chaperones remains unaffected when Not4 activity is partially disrupted. Whole cell lysates from wild-type, not4Δ, not4-ring (I64A substitution in the RING domain) and not4-rrm mutants (G167A, F202A, C244A substitutions in the RNA recognition motif) were subjected to SDS-PAGE and immunoblotting with antibodies specific to each chaperone. Pgk1, loading control in all panels.

B, Chaperone protein abundance is generally unaffected by Rpn4. Experiments were conducted as in A. Quantification of the results are shown in graph (mean ± SD; n=3 biological replicates; ***, p < 0.0005; **, p < 0.005; *, p < 0.05; ns, not significant).

C, Regulation of the chaperone levels via the NOT module does not require Not5’s N-terminal domain (NTD). Experiments were conducted as in A. Not5-NTD recruits the Ccr4-Not complex to the ribosome translating mRNAs with non-optimal codons, so that Ccr4 RNase can promote their degradation (Buschauer et al., 2020). If chaperone mRNAs tend to translate with non-optimal codons, their levels should be increased in not5-ΔNTD cells. However, this possibility is not supported by our data, since chaperone protein abundance is generally unaffected in not5-ΔNTD cells (C, lane 2), as in ccr4Δ cells.
Our data using not5-ΔNTD further validate the ccr4Δ data, supporting that the NOT module transcriptionally regulates chaperone expression, and is unlikely to involve Ccr4 RNase activity for downstream translational control.

**D.** Rpn4 induces CP subunit expression in the NOT module mutants, as a compensatory mechanism for deficient proteasome function (Ju et al., 2004; London et al., 2004); for example, in the present study, functionally impaired proteasome holoenzymes form due to RP assembly defects (Figures 4, 5, S5). β2 is a CP peptidase subunit. All CP α subunits except α4 are recognized by the MCP231 antibody (see Key Resources Table). Experiments were conducted as in A. A pair of CP assembly chaperones, Pba1/2 (Wani et al., 2015), was noticeably increased in not2Δ and not5Δ cells, and to some extent in not4Δ cells (lanes 3, 5 vs. 7). Whether the NOT module directly or indirectly regulates Pba1/2, increased free CP level in NOT mutants (particularly in not2Δ and not5Δ cells; Figure 3E, lanes 2, 5) can be explained by both the increased Pba1/2 level and Rpn4-induced CP subunit expression.

**E.** Proteasome holoenzyme level and activity are increased upon transient inactivation of the NOT module via the anchor-away strategy. Rapamycin (7.5 µM) was added to yeast cultures as in Figure 3F (see the Main Text for details). Whole cell lysates were subjected to Native-PAGE, and in-gel peptidase activity assays using the fluorogenic peptide substrate, LLVY-AMC [i], followed by immunoblotting [ii]. Cellular abundance of proteasome subunits remains unchanged [iii], based on SDS-PAGE and immunoblotting of whole cell lysates. Rpt5, a base subunit. Rpn8, a lid subunit.
Figure S2 related to Main Text Figure 4. Validation of the specificity and comparable pulldown efficiency of FLAG-tagged chaperone-bound complexes.

A-C, Validation of the specificity of 3xFLAG-tagged chaperone pulldowns using an untagged control (unmodified control strain, SUB62, see Table S2). Nas6 pulldowns (A) were conducted in the presence of ATPγS or ATP (1 mM), as in Figure 4B and 4E. Rpn14 and Hsm3 pulldowns (B, C) were conducted in the presence of ATP (1 mM) as in Figure 4F and 4G. Pulldown materials from the indicated strains were analyzed by native gels, followed by immunoblotting (a, b). In parallel, native gels were stained with Sypro-Ruby (c). Some non-specific proteins (n.s.) are found in both untagged control and FLAG-tagged chaperone pulldowns, due to the gentle purification conditions used (no detergent, low salt washes using 150mM NaCl) (A, B, C, n.s. in [b, c]); they migrate more slowly than chaperone-bound complexes and do not affect detection of these complexes by immunoblotting. Non-specific proteins (n.s.) are present comparably (d, ~70kDa region of SDS-PAGE gels).

D, E, Validation that comparable amounts of Nas6-bound complexes were analyzed in the indicated Main Text Figures. Two input controls are shown: 1 ATPase (Rpt5) and 1 non-ATPase subunit (Rpn1) of the base complex using SDS-PAGE and immunoblotting. For example, these input controls (D, lane 1, 2) show that the total sum of base subunits (=base + base-lid) is comparable. Within the same total sum, the ratio of base subunits in base vs. base-lid can be different in the ATPγS condition; they are mainly in the base in wild-type, but are distributed more into the base-lid in not4Δ cells (Figure 4B, [a, b], lane 1, 2). In Main Text Figure 4E [d], relative to the vector control (lane 1), both base and base-lid levels were increased upon Rpn14 and Hsm3 overexpression (lane 2); input controls are largely comparable (E, lane 1, 2). In the ATPγS condition, which mimics early-stage base assembly (Figure 4A, top), the base complex may be more metastable since inter-subunit contacts are being established; during migration on native gels, it may be more easily disturbed and may not be well resolved. Excess Rpn14 and Hsm3 may drive base assembly through such a metastable state, stabilizing Nas6 binding to both base and base-lid (Figure 4B, lane 2 in [c, d]; Figure 4E, lane 2 in [a, b]).

F, G, Validation that comparable amounts of Rpn14-bound complexes, and Hsm3-bound complexes were analyzed in the indicated Main Text Figures. Two input controls are shown: 1 ATPase (Rpt5) and 1 non-ATPase subunit (Rpn1) of the base complex using SDS-PAGE and immunoblotting. These controls further support our conclusion that chaperones exhibit differential affinity to their cognate Rpt protein, depending on the Rpt’s nucleotide state.

H, Nas6 at its endogenous expression level can distinguish the nucleotide state of its cognate Rpt3 protein. Nas6 pulldown was conducted via its 3xFLAG affinity tag, using wild-type cells, rpt3-EQ and rpt6-EQ cells. Nas6 pulldown material was resolved by native gels, followed by immunoblotting for the indicated proteins (a, b). Input controls (c) for Nas6 pulldown materials confirm that comparable amounts of Nas6-bound complexes are analyzed.
Figure S3 related to Main Text Figure 4H. Validation of the successful isolation of RP and individual chaperones for ATPase assays.

_A_, RP was obtained from the yeast strain (sDL135) harboring an affinity-tag, TEV-ProA on a RP subunit, Rpn11, through high-salt washes of the proteasome holoenzyme (Kleijnen et al., 2007; Leggett et al., 2002). RP chaperones were individually expressed and purified from _E. coli_. To ensure the successful isolation of the RP and individual chaperones, these samples were resolved by SDS-PAGE and were stained with Coomassie. Note the absence of the CP subunits between 20 and 30 kDa, confirming that CP dissociated from RP, leaving only the RP in the purified materials.

_B_, Confirmation that high-salt washes during RP preparation removed the RP chaperones, and Ubp6, a RP-associated protein that stimulates RP’s ATP hydrolysis (Bashore et al., 2015). RP was subjected to SDS-PAGE and immunoblotting for the indicated proteins. Rpt5 and Rpn8 are two representative subunits of RP.
Figure S4 related to Main Text Figures 4E, 5F and 6A. Validation that chaperone levels are increased in wild-type cells harboring low-copy plasmids, and are decreased in the diploid *not4ΔΔ* cells harboring a single copy of each chaperone.

A, Validation that Rpn14 and Hsm3 levels are increased via plasmid-driven overexpression in wild-type, *rpt6-EQ* and *rpt3-EQ* cells (lanes 6, 8, 10), at least to the levels seen in *not4Δ* cells (lanes 2, 4). Lanes 5-10 correspond to whole cell lysates of samples in lanes 1-6 in Figure 4E, as analyzed by SDS-PAGE and immunoblotting. Wild-type and *not4Δ* cells were grown in YPD (lanes 1, 2), or in synthetic complete media (lanes 3, 4). Cells bearing plasmids were grown in synthetic media lacking specific amino acids for their auxotrophic markers (lanes 5-10). Asterisk, non-specific signal in panels, *A* and *B*. Pgk1, loading control in panels, *A* and *B*.

B, Validation that Rpn14, Hsm3 and Nas6 levels are decreased in the diploid *not4ΔΔ* strains harboring a single copy of each of these chaperone genes, as in the experiments in Figures 5F and 6A (m, n, o). Experiments were conducted as in *A*. The graph shows quantification of the chaperone levels from immunoblotting data (mean ± SD; n=3 biological replicates; ***, p < 0.0005; **, p < 0.005; *, p < 0.05, ns, not significant).
Figure S5 related to Main Text Figure 5. Additional evidence supporting that the RP assembly checkpoint restricts RP association with CP until RP remodeling occurs properly.

A, Cartoon depicts the current model for the final RP-CP association step of proteasome holoenzyme assembly. At the RP assembly checkpoint during RP subunit rearrangement (left), Nas6 obstructs CP binding (Lu et al., 2017; Nemec et al., 2019; Park et al., 2013; Roelofs et al., 2009). The s1-mut (rpn5-E127F,N128W,K129A) is known to enhance Nas6’s obstruction of RP-CP association by disrupting s1-specific base-lid contacts, which normally help evict Nas6 from the proteasome holoenzyme (Nemec et al., 2019). The rpt3EQ mutant overrides Nas6’s obstruction of RP-CP association, since Rpt3 conformation in the ATP-bound state relieves Nas6’s hindrance against the CP (Li et al., 2017; Nemec et al., 2019). Upon completion of proteasome holoenzyme assembly (right), chaperones are evicted. In the free CP (left), the substrate-entry gate is closed and it opens upon RP association in the proteasome holoenzyme.

B, Defective s1-mut RP proceeds to proteasome holoenzyme in not4Δ cells, similarly as in rpt3EQ cells. Whole cell lysates were subjected to native gels, followed by LLVY-AMC activity assay [a] and immunoblotting [b; short and long exposures]. Rpn12, a RP subunit. The graph shows the quantification of RP level, using the data as in panel b (n=3 biological replicates; mean ± SD; *, p < 0.05; ns, not significant). Since the RP is a transient complex en route to proteasome holoenzyme, we quantified RP level, relative to their corresponding RP2-CP level. Pgk1, loading control for panels B, C, and D.

C, D, Unrestricted progression of RP into proteasome holoenzyme in the combined rpt3EQnot4Δ mutants, as shown by native gel experiments (C, [a, b], lane 4), which were conducted as in B. These proteasomes efficiently degrade the model substrate LLVY-AMC (C, [b], lane 4), but not cellular ubiquitinated proteins in rpt3EQnot4Δ mutants (C, [e], lane 4), supporting that RP cannot undergo proper conformational switching. The observed increase in proteasome holoenzyme level is not due to their increased subunit expression, which remains largely comparable in our samples (C, [e]; see Rpt5), as shown by SDS-PAGE and immunoblotting of whole cell lysates. Also, this nucleotide-dependent effect during RP-CP association is specific to rpt3EQ, and not to rpt6EQ (D, [a, b]); levels and activities of RP2-CP and RP1-CP are comparable in lane 1 vs. 2, and also lane 3 vs. 4. This result suggests that the final RP-CP association step is not noticeably influenced by Rpt6’s nucleotide state, which mainly ensures the RP assembly checkpoint via Not4, to allow its cognate Rpn14 chaperone to recognize a RP in need of further remodeling (Figure 4F).
Figure S6 related to Main Text Figure 6. Additional controls relevant to the fate of proteasomal complexes during glucose starvation.

A, Not4 ubiquitinating activity is dispensable for incorporation of the proteasomal complexes into PSGs. Live-cell confocal microscopy shows that proteasomal complexes form PSGs (arrowheads) in not4-L35A cells during glucose starvation; Not4’s ubiquitin ligase activity is specifically disabled in these cells. Experiments were conducted as in Main Text Figure 6A (panels g-i and j-l). Scale bar = 5 µm.

B, Chaperone localization is indistinguishable between wild-type and not4Δ cells during glucose starvation. Experiments were conducted as in Main Text Figure 6A (panels d-f and j-l). GFP is appended to Nas6 and Rpn14 in their endogenous chromosomal locus. Scale bar = 5 µm.

C, D, Rpt3 and Rpt6 tails are important for protecting proteasomal complexes from autophagic degradation. In C, GFP-fused proteasomal subunits remain intact in cells grown in normal growth condition. In D, free GFP (27 kDa, dotted red box) is released from GFP-fused proteasomal subunits in rpt3Δrpt6Δ cells, indicating autophagic degradation of proteasomal complexes, upon 4-day glucose starvation. Additional bands between 35kDa and ~60kDa may result from some post-lysis cleavage of full-length GFP-fused proteasome subunits, as these bands are also detected in samples from wild-type cells under glucose starvation samples (Figure 6B, lane 1, 5). Whole cell extracts were resolved by SDS-PAGE and immunoblotting. Pgk1, loading control. Molecular weight markers (kDa) are shown at right.
Table S1 related to Main Figure 4B. Identified peptides of the RP band from *not4Δ* cells, as in Figure 4B, lane 2.

| Subunit Name | No. of unique peptides | No. of total peptides |
|--------------|------------------------|----------------------|
| **Base**     |                        |                      |
| *RPN1*      | 57                     | 175                  |
| *RPN2*      | 76                     | 376                  |
| *RPN13*     | 7                      | 17                   |
| *RPT1*      | 33                     | 113                  |
| *RPT2*      | 32                     | 108                  |
| *RPT3*      | 31                     | 271                  |
| *RPT4*      | 25                     | 42                   |
| *RPT5*      | 21                     | 25                   |
| *RPT6*      | 35                     | 338                  |
| **Chaperones** |                    |                      |
| *NAS6*      | 18                     | 52                   |
| *RPN14*     | 25                     | 151                  |
| *HSM3*      | 28                     | 114                  |
| *NAS2*      | 3                      | 3                    |
| **Base-associated protein** | |                      |
| *UBP6*      | 27                     | 34                   |
| **Lid**     |                        |                      |
| *RPN3*      | 17                     | 18                   |
| *RPN5*      | 22                     | 40                   |
| *RPN6*      | 17                     | 26                   |
| *RPN7*      | 15                     | 19                   |
| *RPN8*      | 11                     | 14                   |
| *RPN9*      | 23                     | 31                   |
| *RPN11*     | 15                     | 22                   |
| *RPN12*     | 10                     | 10                   |
| **Base-lid** |                        |                      |
| *RPN10*     | 3                      | 3                    |
| Strain     | Genotype                                                                 | Source                                      |
|------------|---------------------------------------------------------------------------|---------------------------------------------|
| SUB6²      | MATα lys2-801 leu2-3, 2-112 ura3-52 his3-3/200 trp1-1                     | (Finley et al., 1987)                       |
| DF⁵        | MATα/a lys2-801/lys2-801 leu2-3, 2-112/leu2-3, 2-112, ura3-52/ura3-52, his3-3/200/ his3-3/200 trp1-1/trp1-1 | (Finley et al., 1987)                       |
| SP1658C    | MATα not4::kanMX6                                                         | (Fu et al., 2018)                           |
| SP2407A    | MATα not4::not4-6/44A (TRP1)                                              | (Fu et al., 2018)                           |
| SP3534A²   | MATα not4::not4-6/44A,G167A,F202A,C244A (TRP1)                             | Derived from (Chen et al., 2018)            |
| SP3587A²   | MATα not4::not4-6/44A,G167A,F202A,C244A (TRP1)                             | Derived from (Chen et al., 2018)            |
| SP3640A    | MATα rpn4::RPN4-HA6 (TRP1)                                               | This study                                  |
| SP3649A    | MATα rpn4::RPN4-HA6 (TRP1) not4::kanMX6                                  | This study                                  |
| SP3828A    | MATα rpn4::RPN4-HA6 (TRP1) not4::not4-6/44A,G167A,F202A,C244A (TRP1)    | This study                                  |
| SP3814A    | MATα rpn4::RPN4-HA6 (TRP1) pdr5::kanMX6                                  | This study                                  |
| SP3855A    | MATα rpn4::RPN4-HA6 (TRP1) pdr5::kanMX6 not4::kanMX6                     | This study                                  |
| SP3827A    | MATα rpn4::RPN4-HA6 (TRP1) pdr5::kanMX6 not4::not4-6/44A,G167A,F202A,C244A (TRP1) | This study                                  |
| sJ2R²      | MATα rpn4::kanMX6                                                         | (Fu et al., 2018)                           |
| SP2186A    | MATα not4::kanMX6 rp4::kanMX6                                             | (Fu et al., 2018)                           |
| SP3594A    | MATα not2::kanMX6                                                         | This study                                  |
| SP2248A    | MATα not3::kanMX6                                                         | This study                                  |
| SP2272A    | MATα not5::kanMX6                                                         | This study                                  |
| SP2246A    | MATα ccr4::kanMX6                                                         | This study                                  |
| SP2245A    | MATα caf130::kanMX6                                                       | This study                                  |
| SP2243A    | MATα caf1::kanMX6                                                         | This study                                  |
| SP2244A    | MATα caf40::kanMX6                                                        | This study                                  |
| SP3891A    | MATα not5::not5-ANDT-HA6 (TRP1)                                           | This study                                  |
| SP3625A    | MATα not5::not5-HA6 (TRP1)                                               | This study                                  |
| SP3621A    | MATα not2::kanMX6 rp4::kanMX6                                             | This study                                  |
| SP3589A    | MATα not5::kanMX6 rp4::kanMX6                                             | This study                                  |
| sJ2R²      | MATα rpn4::kanMX6                                                         | (Fu et al., 2018)                           |
| JR1721     | MATα tor1-1 fpr1::NAT RPL13-2×FKBP12::TRP1 ade2-11; his3-11,15; leu2-3,112; ura3-1; trp1-1; can1-100 | (Jiang et al., 2019)                        |
| SP3880     | MATα tor1-1 fpr1::NAT RPL13-2×FKBP12::TRP1 ade2-11; his3-11,15; leu2-3,112; ura3-1; trp1-1; can1-100; not5::NOT5-FRB (hphMX) | This study                                  |
| SP1677A    | MATα nas6::NAS6-6×Gly-3×FLAG (hphMX)                                      | (Li et al., 2017)                           |
| SP2130A    | MATα nas6::NAS6-6×Gly-3×FLAG (hphMX) not4::kanMX6                        | This study                                  |
| SP3834A    | MATα rpn4::RPN4-6×Gly-3×FLAG (hphMX) rp6::HIS3 [pRS316-RPT6]             | This study                                  |
| SP4003     | MATα rpn4::RPN4-6×Gly-3×FLAG (hphMX) rp4::HIS3 [pRS316-RPT6]             | This study                                  |
| SP3990A    | MATα nas6::NAS6-6×Gly-3×FLAG (hphMX) rp3::HIS3 [pRS316-RPT3]             | This study                                  |
| SP3972A    | MATα nas6::NAS6-6×Gly-3×FLAG (hphMX) rp6::HIS3 [pRS316-RPT6]             | This study                                  |
| SP4201A    | MATα hsm3::HSM3-6×Gly-3×FLAG (HIS3) rp2::HIS3 [YCplac111-RPT2]           | This study                                  |
| SP4202A    | MATα hsm3::HSM3-6×Gly-3×FLAG (HIS3) rp2::HIS3 [YCplac111-rpt2(E283Q)]    | This study                                  |
| SP4190B    | MATα hsm3::HSM3-6×Gly-3×FLAG (HIS3) rp6::HIS3 [pRS316-RPT6]              | This study                                  |
| sDL133     | MATα rap1::RPN11-TEV-ProA (HIS3)                                         | (Leggett et al., 2002)                      |
| SP4098A    | MATα nas6::NAS6-6×Gly-3×FLAG (hphMX), rp2(R407C)::natMX4, rp7(D123C)-6xGly-5 linkkanMX6 | This study                                  |
| SP4096A    | MATα nas6::NAS6-6×Gly-3×FLAG (hphMX), rp2(R407C)::natMX4, rp7(D123C)-6xGly-5 linkkanMX6 | This study                                  |
| RTY2112    | MATα rap2::rap2(R407C)::natMX4, rp7::rap2(D123C)-6xGly-5::kanMX6          | (Eisele et al., 2018)                      |
| SP4095A    | MATα rap2::rap2(R407C)::natMX4, rp7::rap2(D123C)-6xGly-5::kanMX6 not4::kanMX6 | This study                                  |
| SP4389A²   | MATα/a not4::kanMX6 not4::kanMX6, rp2(R407C)::natMX4/rap2(R407C)::natMX4, rp7(D123C)-6xGly-5 linkkanMX6 | This study                                  |
| SP4390A²   | MATα/a not4::kanMX6 not4::HIS3, rp2(R407C)::natMX4/rap2(R407C)::natMX4, rp7(D123C)-6xGly-5 linkkanMX6 | This study                                  |
| RTY1155    | MATα rap5::hphMX4 [pRS316-RPN5-GFP(S65T)-FLAG]                            | (Nemec et al., 2019)                       |
| SP4117     | MATα rap5::hphMX4 [pRS316-RPN5-GFP(S65T)-FLAG]                            | This study                                  |
| SP4099A | MATa rpm5::hphMX4 [pRS316-RPN5-GFP(S65T)-FLAG] nas6::HIS3 | This study |
| SP3967A | MATa rpm5::hphMX4 [pRS316-RPN5-GFP(S65T)-FLAG] not4::KanMX6 | This study |
| SP4093A | MATa rpm5::hphMX4 [pRS316-RPN5-GFP(S65T)-FLAG] not4::NOT4-L35A(KanMX6) | This study |
| SP3999A | MATa rpt3::HIS3 [pRS316-RPT3] | Derived from RTY1504 (Eisele et al., 2018) |
| SP3996A | MATa rpt3::HIS3 [pRS316-RPT3] not4::kanMX6 | This study |
| SP3998A | MATa rpt6::HIS3 [pRS316-RPT6] | Derived from RTY1173 (Eisele et al., 2018) |
| SP3994 | MATa rpt6::HIS3 [pRS316-RPT6] not4::kanMX6 | This study |
| SP2017 | MATa rpt6::Prpt6-yEGFP1F-RPT6-LEU2 | (Nahar et al., 2019) |
| SP2782B | MATa rpt6::Prpt6-yEGFP1F-RPT6-LEU2 not4::kanMX6 | This study |
| SP3909A | MATa rpm5::RPN5-GFP (kanMX6) | This study |
| SP3935C | MATa rpm5::RPN5-GFP (kanMX6) not4::kanMX6 | This study |
| SP3908A | MATa pre10::PRE10-GFP (kanMX6) | This study |
| SP3934A | MATa pre10::PRE10-GFP (kanMX6) not4::kanMX6 | This study |
| SP3477A | MATa/a not4::kanMX6 not4::kanMX6, RPN14/rpn14::hphMX4, HSM3/hsm3::TRP, NAS6/nas6::HIS3, rpt6::Prpt6-yEGFP1F-RPT6-LEU2/rpt6::Prpt6-yEGFP1F-RPT6-LEU2 | This study |
| SP3481A | MATa/a not4::kanMX6 not4::kanMX6, RPN14/rpn14::hphMX4, HSM3/hsm3::kanMX6, NAS6/nas6::TRP, rpm5::RPN5-GFP (HIS3)/rpm5::RPN5-GFP (HIS3) | This study |
| SP3485A | MATa/a not4::kanMX6 not4::kanMX6, RPN14/rpn14::hphMX4, HSM3/hsm3::kanMX6, NAS6/nas6::TRP, pre10::PRE10-GFP (HIS3)/pre10::PRE10-GFP (HIS3) | This study |
| SP3077A | MATa rpm6::Prpt6-yEGFP1F-RPT6-LEU2 pep4::kanMX6 | This study |
| SP3431A | MATa rpm6::Prpt6-yEGFP1F-RPT6-LEU2 pep4::kanMX6 not4::kanMX6 | This study |
| SP3472B | MATa rpm5::RPN5-GFP (HIS3) pep4::kanMX6 | This study |
| SP3473A | MATa rpm5::RPN5-GFP (HIS3) pep4::kanMX6 not4::kanMX6 | This study |
| SP3437A | MATa pre10::PRE10-GFP (kanMX6) pep4::kanMX6 | This study |
| SP3438A | MATa pre10::PRE10-GFP (kanMX6) pep4::kanMX6 not4::kanMX6 | This study |
| SP3091A | MATa rpm6::Prpt6-yEGFP1F-RPT6-LEU2 pep4::kanMX6 not4::kanMX6 | This study |
| SP4225B | MATa rpm5::RPN5-GFP (HIS3) pep4::kanMX6 not4::kanMX6 | This study |
| SP4226B | MATa pre10::PRE10-GFP (kanMX6) pep4::kanMX6 not4::kanMX6 | This study |
| SP4216A | MATa rpm6::Prpt6-yEGFP1F-RPT6-LEU2 pep4::kanMX6 not4::kanMX6 | This study |
| SP4232A | MATa rpm5::RPN5-GFP (HIS3) pep4::kanMX6 not4::kanMX6 | This study |
| SP4143A | MATa pre10::PRE10-GFP (kanMX6) pep4::kanMX6 not4::kanMX6 | This study |
| SP4127A | MATa rpm6::Prpt6-yEGFP1F-RPT6-LEU2 pep4::kanMX6 not4::kanMX6 | This study |
| SP4244A | MATa rpm5::RPN5-GFP (HIS3) pep4::kanMX6 not4::kanMX6 | This study |
| SP4141A | MATa pre10::PRE10-GFP (kanMX6) pep4::kanMX6 not4::kanMX6 | This study |

All strains are isogenic to SUB62 genetic background except SP3880, which is isogenic to JR1721.

bThese diploid strains are isogenic to DF5.

cThese chromosomal strains were generated by integrating the PCR-amplified ORFs into the endogenous chromosomal locus.
### Table S3 related to Main Figures 1 through 6. Plasmids used in this study

| Plasmid | Genotype | Source |
|---------|-----------|--------|
| YE96    | Copper-inducible ubiquitin plasmid (pCUP1-Ub, 2µm; TRP1) | (Ecker et al., 1987) |
| pRT702  | YCplac111-RPT2 | (Tomko et al., 2010) |
| pRT364  | YCplac111-RPT3 | (Tomko et al., 2010) |
| pRT1496 | YCplac111-RPT6 | (Eisele et al., 2018) |
| pRT1410 | YCplac111-rpt2(E283Q) | (Eisele et al., 2018) |
| pRT1411 | YCplac111-rpt3(E273Q) | (Eisele et al., 2018) |
| pRT1497 | YCplac111-rpt6(E249Q) | (Eisele et al., 2018) |
| pRT1592 | pRS314-RPN5-6xGly-3xFLAG | (Nemec et al., 2019) |
| pRT1790 | pRS314-rpn5(E127F,N128W,K129A)-6xGly-3xFLAG | (Nemec et al., 2019) |
| pSP265  | RPN14 ORF with its own promoter and terminator in pRS414 | This study |
| pSP262  | HSM3 ORF with its own promoter and terminator in pRS416 | This study |
| pJR40   | GST-Nas6 in pGEX6P-1 | (Roelofs et al., 2009) |
| pJR56   | GST-Rpn14 in pGEX6P-1 | (Roelofs et al., 2009) |
| pJR89   | GST-Hsm3 in pGEX6P-1 | (Roelofs et al., 2009) |
| pSP128  | GST-Nas2 in pGEX6P-1 | (Fu et al., 2018) |