Supplemental Data for accompanying manuscript titled:

**Dual function of Rpn5 in two PCI complexes, the 26S proteasome and COP9 signalosome**

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On the following pages, this file contains the following sections:

A) Four supplementary Figures
B) Six Supplementary Tables
C) Supplementary materials and methods
D) Supplementary references
A) SUPPLEMENTARY FIGURES (S1-S4):
Figure S1:

- (a) A schematic diagram in scale, of wild-type and mutant Rpn5 used in this study. Rpn5 bears a carboxyl terminal PCI domain (black), which is truncated in \textit{rpn5-1} and \textit{rpn5ΔC} mutants (Isono et al., 2007; Ben-Aroya et al. 2010). \textit{rpn5-1}: Bears an early stop codon, and lacks 30 carboxyl terminal amino acids. In addition this mutant bears two point mutations: Ile/Leu in position 180 and Arg/Gly in position 344. \textit{rpn5ΔC}: Bears an early stop codon, and lacks 28 amino acids which are substituted by an octapeptide. This mutant also bears a point mutation of Phe/Ser in position 275.

- (b) Phylogenetic relationship of Rpn5 and Csn4 paralog subunits. Most eukaryotes contain both Csn4 and Rpn5 but in \textit{S. cerevisiae} only Rpn5 exists.
Figure S2: Rpn5-CBP-A2, CBP-A2-Rpt6 and Csn10-CBP-A2 complexes were subjected to western blot using anti-Rpn5 antibody recognizes both Rpn5 and protein A peptides, anti-CBP antibody recognizes proteins tagged by CBP, or anti-HA that recognizes proteins tagged by protein A. The results show that the carboxyl terminal protein-A tags were cleaved in approximately half of the Rpn5-CBP-A2 population.
Figure S3: (a) Calmodulin purified CBP-A2-Rpt6, Rpn5-CBP-A2 and Csn10-CBP-A2 complexes, were separated on a 12% SDS PAGE and stained with Coomassie Brilliant Dye. A conventionally purified proteasome was separated aside, as for comparison. As seen from the gel, both Rpn5-CBP-A2 and CBP-A2-Rpt6 samples are enriched with RP- components. (b) Rpn5-CBP-A2 complexes were separated on 4.5% native PAGE that was obtained for peptidase activity (left) or western-blot (right) using anti-HA antibody. Most of the peptidase activity is found in doubly regulatory particle proteasomes. Interestingly, immunoblotting with anti-HA that is being recruited by the Protein A tags, exposes low molecular Rpn5- containing complexes which are proteolytically inactive, this in addition to full structures of proteasome. These complexes include 19S, Lid and CSN.
Figure S4: Crude protein extract from green *Arabidopsis* seedlings was fractionated in a gradient of 10% - 40% Glycerol. Proteasome distribution was determined by western blot with antibodies recognizing proteasome lid (Rpn5, Rpn6 and Rpn12), base (Rpn1) and CP (Pba1) subunits, as well as Arabidopsis CSN (Csn5, Csn4) subunits. Complexes are indicated by the brackets.
B) SUPPLEMENTARY TABLES (Tables S1-6)
Table S1:

| DOMAIN | A.thaliana | A nidulans | N. crassa | S. pombe | C. merolae | C. albicans | S. cerevisiae |
|---------|------------|------------|-----------|----------|------------|-------------|---------------|
|         | 19S Lid    | CSN        |           |          |            |             |               |
| MPN     | Rpn11      | Csn5a/b    | Csn5      | Csn5     | Csn5       | Csn5        | Csn5          |
| PCI     | Rpn6       | Csn2       | Csn2      | Csn2     | Csn2       | Csn2        | Csn2          |
| PCI     | Rpn7       | Csn1       | Csn1      | Csn1     | Csn1       | Csn1        | Csn11         |
| PCI     | Rpn9a/b    | Csn7       | Csn7      | Csn7     | Csn7       | Csn7        | Csn9          |
| PCI     | Rpn3a/b    | Csn3       | Csn3      | Csn3     | Csn3       | Csn3        |                |
| PCI     | Rpn5a/b    | Csn4       | Csn4      | Csn4     | Csn4       | Rpn5        |                |
| MPN     | Rpn8a/b    | Csn6a/b    | Csn6      |          |            |             |               |
| PCI     | Rpn12a/b   | Csn8       | Csn8      |          |            |             |               |
|         | Sem1       |            |           |          |            |             | Csi1          |

Table S1: **Loss of CSN subunits in unicellular organisms**: direct orthologs of *Arabidopsis thaliana* CSN subunits in various unicellular organisms (*Aspergillus nidulans; Neurospora crassa; Schizosaccharomyces pombe; Cyanidioschyzon merolae; Candida albicans, Saccharomyces cerevisiae*). Rpn paralogs in plant proteasome are shown to guide the reader. Note that only one direct ortholog of CSN subunits is found in budding yeast (Csn5). The other three PCI subunits (Csn9, 10 and 11) are highly diverged, and one subunit (Csi1) does not bear any of the conserved recognition domains. In this manuscript we suggest that Rpn5 is the substitution for Csn4 which is missing in the budding yeast genome. The table is not intended to show phylogenic relationship.
Table S2:

| PLASMID  | CHARACTERISTICS                          | SOURCE        |
|----------|------------------------------------------|---------------|
| M1070    | AtCSN4-LEU2 ADH Amp                      | This study    |
| M1077    | ScRPN5-URA3 CEN Amp                      | (Yang et al., 2004) |
| M1079    | AtRPN5a-LEU2 2mic-ScRpn10 promoter Amp   | (Yang et al., 2004) |
| M1081    | AtRPN5b-LEU2 2mic ScRpn10 promoter Amp   | (Yang et al., 2004) |
| M1083    | ScRPN5-LEU2 2mic ScRpn10 promoter Amp    | (Yang et al., 2004) |
| M1043    | CBP-A2-RPT6-LEU2 2mic Rpt4 promoter Amp  | This study    |

Table S2: List of plasmids used in this study
Table S3: list of yeast strains used in this study

| STRAIN  | MAT   | RELEVANT GENOTYPE                                      | SOURCE/COMMENTS |
|---------|-------|-------------------------------------------------------|-----------------|
| MY58    | a     | his3ko1; leu2ko0; met15ko0; ura3ko0                  | Euroscarf       |
| MY60    | a     | Csn5::KAN'                                             | Euroscarf       |
| MY299   | a     | rub1::KAN'                                              | Euroscarf       |
| MY1045  | a     | csn9::CSN9-MYC13-HIS3                                   | This study      |
| MY1061  | a     | csn9::CSN9-MYC13- KAN' csn10::CSN10-CBP-A2-HIS3        | This study      |
| MY1068  | a     | rpn7::rpn7-3-URA3                                       | (Isono et al., 2004) |
| MY1070  | a     | rpn5::rpn5-1-TRP1                                       | (Isono et al., 2007) |
| MY1082  | a     | Rpt6::CBP-A2-RPT6-LEU2                                  | This study      |
| MY1094  | a     | csn9::CSN9-MYC13-HIS3 csn5:: KAN'                        | This study      |
| MY1107  | a     | rpn3::rpn3-4-TRP1                                       | (Bailly and Reed, 1999) |
| MY1108  | a     | rpn3::rpn3-7-TRP1                                       | (Bailly and Reed, 1999) |
| MY1109  | a     | rpn12::rpn12-1-URA3                                     | (Bailly and Reed, 1999) |
| MY1122  | a     | rpn6::rpn6-1 URA3                                       | (Isono et al., 2005) |
| MY1123  | a     | rpn9::rpn9ΔC-LEU2                                       | (Takeuchi et al., 1999) |
| MY1130  | a     | rpn5::RPN5-CBP-A2-URA3                                  | (Gavin et al., 2002) |
| MY1131  | a     | RPN5:: KAN' [M1077]                                     | (Yang et al., 2004) |
| MY1135  | a     | RPN5:: KAN' [M1079]                                     | (Yang et al., 2004) |
| MY1137  | a     | RPN5:: KAN' [M1081]                                     | (Yang et al., 2004) |
| MY1139  | a     | RPN5:: KAN' [M1083]                                     | (Yang et al., 2004) |
| MY1194  | a     | RPN6::RPN6-CBP-A2-URA3                                  | (Gavin et al., 2002) |
| MY1195  | a     | RPN8::RPN8-CBP-A2-URA3                                  | (Gavin et al., 2002) |
| MY1231  | a     | rpn5::rpn5-1-TRP1 csn9::CSN9-MYC13-HIS3                 | This study      |
| Ts944   | α     | rpn5::rpn5ΔC-URA3                                      | (Ben-Aroya et al., 2010) |
Table S4:

| ORF        | Name | Coverage | Unique peptides | Group              |
|------------|------|----------|-----------------|--------------------|
| YDR179C    | CSN9 | 10.5%    | 2               | CSN                |
| YDL216C    | RRI1 | 7.5%     | 2               | CSN                |
| YDL147W    | RP5S | 8.3%     | 5               | Proteasome         |
| YDL229W,YNL209W | SSB1 | 29.2%   | 16              | ATPase HSP family  |
| YLR259C    | HSP60| 20.6%    | 9               | Mitochondrial chaperone |
| YER090W    | TRP2 | 5.9%     | 2               | Metabolism         |
| YJR121W    | ATP2 | 4.9%     | 2               | Mitochondrial F1F0 ATP synthase |
| YER013W    | PRP22| 0.8%     | 2               | RNA helicase       |
| YCR073C    | SSK22| 1.5%     | 2               | MAP kinase         |

**Table S4:** Proteins from MY58 wild-type strain (control), and MY1045 wild type strain expressing a genomic tagged Csn9-Myc13 were affinity purified under native conditions using anti-Myc antibodies coupled to agarose beads. Myc tagged Csn9 and other co-purified proteins were separated by SDS-PAGE and 30-70 kDa gel slices were excised and analyzed by mass spectrometry. Unique proteins identified by tandem MS analysis only in MY1045 are presented, showing the number of unique peptides and the relative coverage (in percent of full sequence).
## Table S5:

| ORF     | PROTEIN | START | FRAC4 | FRAC8 | GROUP  |
|---------|---------|-------|-------|-------|--------|
| YGL011C | a1      | 30/65%| 27/58%| CP    |        |
| YML092C | a2      | 3/40% | 13/39%| CP    |        |
| YGR135W | a3      | 21/47%| 17/35%| CP    |        |
| YOL038W | a4      | 21/61%| 12/31%| CP    |        |
| YGR253C | a5      | 28/72%| 25/52%| CP    |        |
| YMR314W | a6      | 30/92%| 24/60%| CP    |        |
| YOR362C | a7      | 18/51%| 16/42%| CP    |        |
| YJL001W | b1      | 11/40%| 8/30% | CP    |        |
| YOR157C | b2      | 8/18% | 4/14% | CP    |        |
| YER094C | b3      | 11/48%| 7/23% | CP    |        |
| YER112W | b4      | 15/40%| 16/50%| CP    |        |
| YPR103W | b5      | 13/36%| 13/33%| CP    |        |
| WBL041W | b6      | 19/59%| 14/38%| CP    |        |
| YFR050C | B7      | 11/41%| 11/31%| CP    |        |
| YHR127C | RPN1    | 45/37%| 33/29%| base  |        |
| YIL075C | RPN2    | 76/57%| 41/36%| base  |        |
| YLR421C | RPN13   | 7/33% | 1/7%  | 4/25% | base   |
| YKL145W | RPT1    | 48/59%| 34/45%| base  |        |
| YDL007W | RPT2    | 32/52%| 21/34%| base  |        |
| YDR394W | RPT3    | 38/63%| 18/34%| base  |        |
| YOR259C | RPT4    | 42/60%| 29/40%| base  |        |
| YOR117W | RPT5    | 35/59%| 5/13% | 29/40%| base   |
| YGL048C | RPT6    | 37/61%| 25/44%| base  |        |
| YHR200W | RPN10   | 14/34%| 8/24% | 8/22% | base   |
| YER021W | RPN3    | 51/56%| 18/29%| Lid   |        |
| YDL147W | RPN5    | 42/62%| 15/23%| Lid   |        |
| YDL097C | RPN6    | 51/71%| 18/32%| Lid   |        |
| YPR108W | RPN7    | 43/48%| 27/38%| Lid   |        |
| YOR261C | RPN8    | 48/73%| 21/52%| Lid   |        |
| YDR427W | RPN9    | 57/67%| 28/51%| Lid   |        |
| YFR004W | RPN11   | 25/56%| 18/44%| Lid   |        |
| YFR052W | RPN12   | 31/64%| 10/43%| Lid   |        |
| YDR363W | SEM1    |       |       | Lid   |        |
| YOL117W | CSN10   | 6/16% | 1/2%  | CSN   |        |
| YDL216C | CSN5    | 7/12% | 1/1%  | CSN   |        |
| YDR179C | CSN9    | 3/12% |       | CSN   |        |
| YIL071C | *CSN11  | 5/13% | 2/4.3%| CSN   |        |
| YMR025W | *CSN1   | 4/13.9%| 4/13.9%| CSN |        |
| YJR084W | CSN12   |       |       | CSN   |        |
| YGL004C | RPN14   | 13/31%| 3/8%  | 19S assembly |    |
| YGR232W | NAS6    | 13/45%| 2/8%  | 19S assembly |    |
| YIL007C | NAS2    | 4/20% |       | 19S assembly |    |
| YBR272C | HSM3    | 15/28%|       | 19S assembly |    |
| YOR269W | PAC1    |       |       | 20S assembly |    |
| YER007W | PAC2    |       |       | 20S assembly |    |
| YMR294W | PAC3    |       |       | 20S assembly |    |
| YBR173C | UMP1    |       |       | 20S assembly |    |
| YHL030W | ECM29   | 1/2%  |       |       |        |
| YFL007W | BLM10   |       |       |       |        |
| YFR010W | UBP6    |       |       |       |        |
| YGL141W | Hu5     |       |       |       |        |
| YDL126C | CDC48   | 2/2%  |       |       |        |

**Table S5:** Tandem MS identification of proteasome, CSN subunits and known proteasome assembly or activity-related factors in affinity purified RPN5-CBP-A2 eluant and two fractions bearing
deRubylation or peptidase activity. The number of unique peptides and the relative coverage (in percent of full sequence) is shown. An asterisk refers to hits that were identified from an identical parallel independent experiment.
Table S6:

| ORF            | PROTEIN | START      | FRAC4     | GROUP    |
|----------------|---------|------------|-----------|----------|
| YOL117W        | CSN10   | 22/19.2%   | 6/41.6%   | CSN      |
| YDL216C        | CSN5    | 34/63.6%   | 32/47.5%  | CSN      |
| YIL071C        | PCE8    | 11/19.6%   | 33/40.8%  | CSN      |
| YMR025W        | CS1     | 5/12.2%    | 28/53.9%  | CSN      |
| YDR179C        | CSN9    | 6/24.7%    | 10/34%    | CSN      |
| YDL147W        | RPN5    | 13/27.4%   | 40/60.2%  | Proteasome |
| YDL097C        | RPN6    | 6/7.1%     |           | Proteasome |
| YKL145W        | RPT1    | 5/10.5%    |           | Proteasome |
| YIL075C        | RPN2    | 1/1.6%     |           | Proteasome |
| YDR394W        | RPT3    | 1/4.4%     |           | Proteasome |
| YGR232W        | NAS6    | 1/4.4%     |           | 19S assembly |
| YIL148W, YKR094C, YLL039C, YLR167W | ubiquitin | 9/50.8%     | 8/69.6% | Ubiquitin |
| YER151C        | UBP3    | 9/10.5%    | 21/35%    | Ubiquitin |
| YNR051C        | BRE5    | 2/2.5%     | 11/31.1%  | Ubiquitin |
| YDL074C        | BRE1    | 3/5.1%     | 2/5.1%    | Ubiquitin |
| YHL002W        | HSE1    | 9/14.8%    | 3/7.1%    | Ubiquitin |
| YDR143C        | SAN1    | 3/5.2%     | 1/2%      | Ubiquitin |
| YKL090W        | CUE2    | 1/3.8%     | 2/5.9%    | Ubiquitin |
| YGR054W        | YGR054W | 1/3.3%     | 5/10.4%   | eIF2a    |
| YMR012W        | TIF31   | 7/7.5%     | 2/2.4%    | eIF3     |
| YBR079C        | RPG1    | 19/13.9%   | 6/8.2%    | eIF3a    |
| YOR361C        | PRT1    | 7/6.6%     | 1/1.7%    | eIF3b    |
| YOL139C        | CDC33   | 4/12.7%    | 4/13.6%   | eIF4e    |
| YEL034W        | HYP2    | 2/19.1%    |           | eIF5a    |
| YPL096W        | PNG1    | 4/12.7%    | 7/24.2%   |           |
| YBR025C        | OLA1    | 22/49.2%   | 8/28.2%   |           |
| YLR215C        | CDC123  | 1/3.1%     | 1/3.1%    |           |
| YER165W        | PAB1    | 7/15.9%    | 1/1.7%    |           |

Table S6: Tandem MS identification of proteasome, CSN subunits and known proteasome assembly or activity-related factors in affinity purified RPN5-CBP-A2 (before fractionation in a gradient of glycerol density) and fraction 4 that is bearing deRubylation activity. The number of unique peptides and the relative coverage (in percents of full sequence) is shown.

Note that total peptide counts of all core CSN subunits are enriched in fraction 4 relative to the Csn10-pullout used as start in this experiment. This is not the case for most other trace hits.
C) SUPPLEMENTARY INFORMATION FOR MASS SPECTROMETRY ANALYSIS

Protein preparation and digestion

Rpn5 interacting proteins from Rpn5-CBP-A2 strain were purified as described in experimental procedure section (Glickman et al., 1998). The affinity purified proteins were denatured by addition of 8M Urea, reduced with 10 mM DTT (at 60°C for 30min), modified with 100 mM iodoacetamide in 10 mM ammonium bicarbonate (room temperature for 30min) and trypsinized in 10 mM ammonium bicarbonate containing trypsin [modified trypsin (Promega)] at a 1:50 enzyme-to-substrate ratio, overnight at 37°C.

Mass spectrometry analysis

The resulting tryptic peptides were resolved by reverse-phase chromatography on 0.075 X 200-mm fused silica capillaries (J&W) packed with Reprosil reversed phase material (Dr Maisch GmbH, Germany). The peptides were eluted with linear 65 minutes gradients of 5 to 45% and 15 minutes at 95% acetonitrile with 0.1% formic acid in water at flow rates of 0.25 μl/min. Mass spectrometry was performed by an ion-trap mass spectrometer (Orbitrap, Thermo) in a positive mode using repetitively full MS scan followed by collision induces dissociation (CID) of the 7 most dominant ion selected from the first MS scan.

Database search

The mass spectrometry data was analyzed using the Trans Proteomic Pipeline (TPP) Version 4.3(Keller et al., 2005). TPP-processed centroid fragment peak lists in mzXML format were searched against Saccharomyces cerevisiae translations of all systematically named ORFs (release date Jan 5th, 2010; Downloaded form SGD). The 5904 proteins were supplemented with their 5904 corresponding decoy sequences (as described in http://www.matrixscience.com/help/decoy_help.html). The database searches were performed using X! Tandem with k-score plugin through the TPP. Search parameters include: trypsin cleavage specificity with two missed cleavage, cysteine carbamidomethyl as fixed modification, methaionine oxidation and protein N-terminal acetylation as variable modifications, peptide tolerance and MS/MS. Absolute Protein Expression (APEX) abundances of the CSN10-CPB-A2 pullout proteins were calculated using the protXML file
generated from the PeptideProphet™ and ProteinProphet™ validation of the X!Tandem search results. A <1% false positive rate (FPR) was chosen Employing the APEX tool (Braisted et al., 2008) Stoichiometry of the CSN complex was calculated by dividing the protein abundances found by the calculated abundance of CSN10.

D) SUPPLEMENTARY REFERENCES

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