Proteasome activity is influenced by the HECT_2 protein Ipa1 in budding yeast

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Table legends

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Pfam protein name and organism identifier are given for each node of the network shown in Figure 1b.

Supplemental Table S2

Microarray data for Ipa1-psd strain compared to control strain ESM356-1, both exposed to blue-light for 5 hours.

Supplemental Table S3

Microarray data for Ipa1-psd strain compared to control strain ESM356-1, both grown in darkness before the experiment.

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Microarray data for ESM356-1 strain exposed to blue-light compared to strain ESM356-1 grown in darkness.

Supplemental Table S5

List of genes upregulated in Ipa1-psd cells compared to ESM356-1.

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List of genes downregulated in Ipa1-psd cells compared to ESM356-1.

Supplemental Table S7

List of GO terms enriched in the upregulated gene list.

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List of GO terms enriched in the downregulated gene list.

Supplemental Table S9

List of yeast strains.

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List of plasmids.

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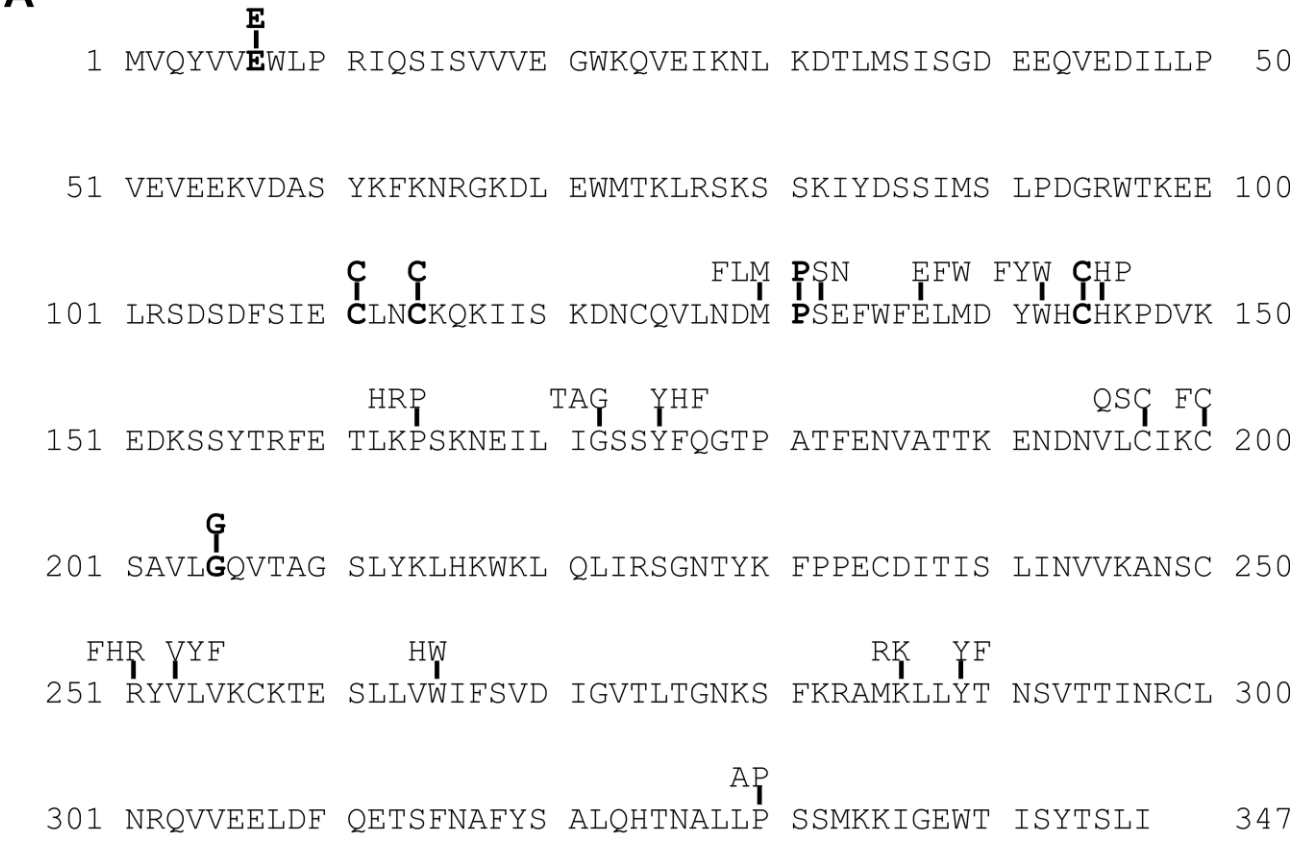
List of oligos used for the quantitative real-time PCR analysis.

A



B

domain architecture	number of members
HECT_2	371
HECT_2 x 2	11
HECT_2 + other	11

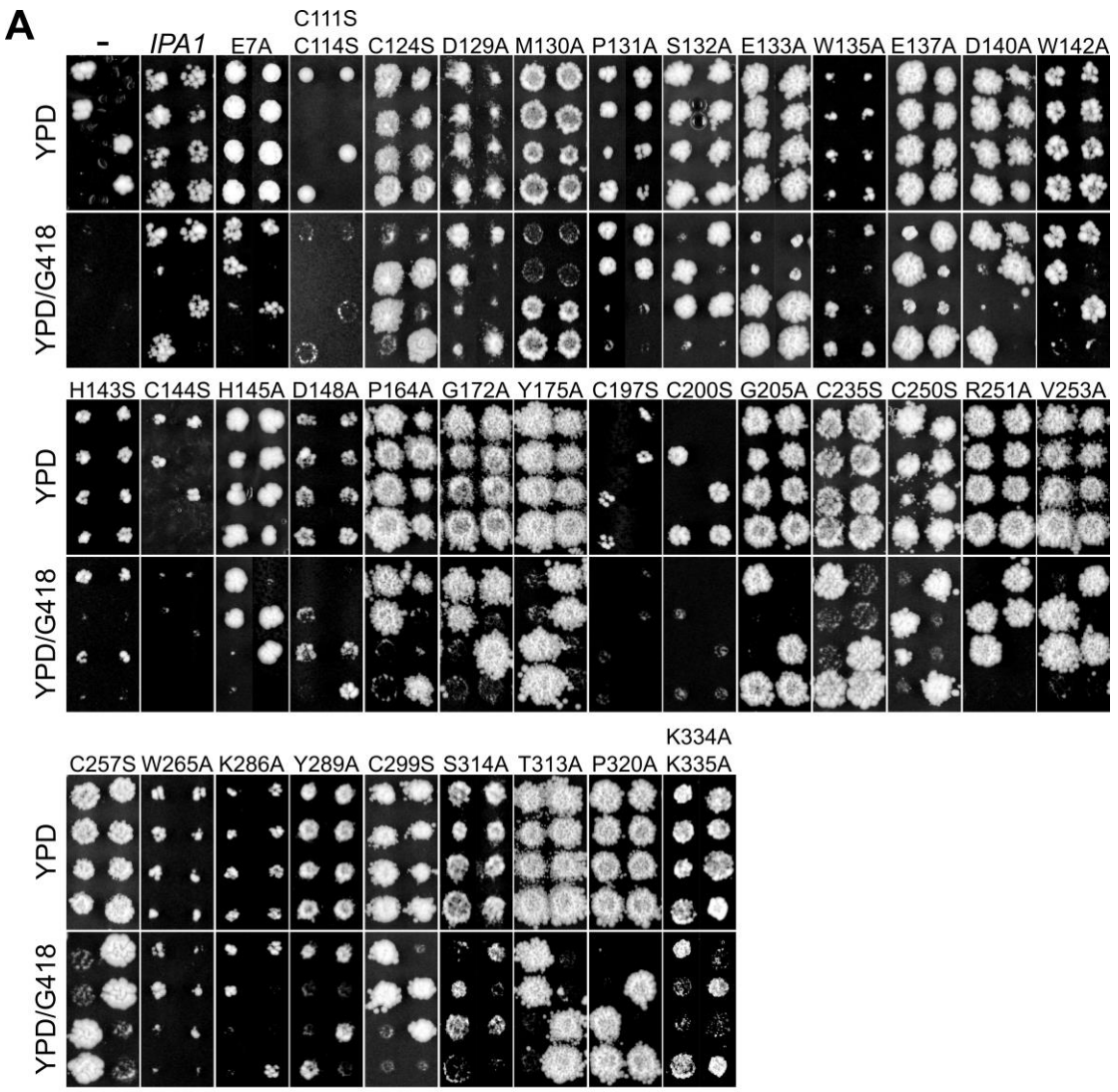
source:
http://pfam.xfam.org/family/HECT_2#tabview=tab1

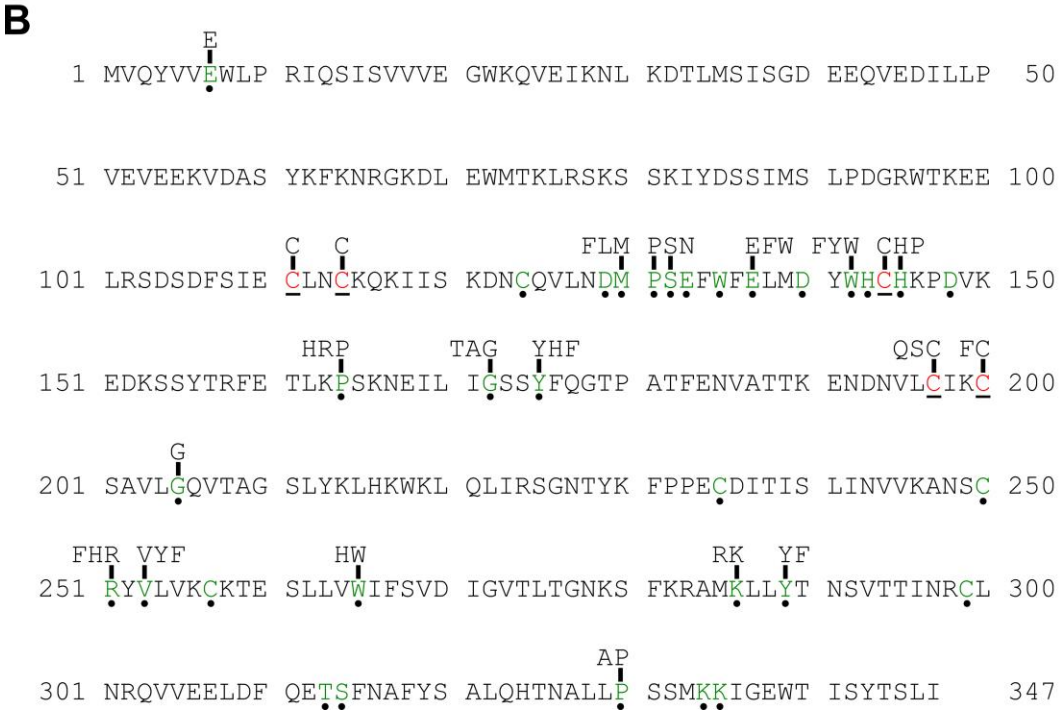
Supplemental Figure S1

The HECT_2-family signature

(**A**) The HECT_2-family signature compared to Yjr141w/Ipa1 sequence. The family signature (source http://pfam.xfam.org/family/pf09814#tabview=tab4) is placed above the corresponding residue (indicated by a line) in Yjr141w/Ipa1. Invariant residues are shown in bold letters.

(**B**) Most family members currently listed in the Pfam database contain only the HECT_2 domain.



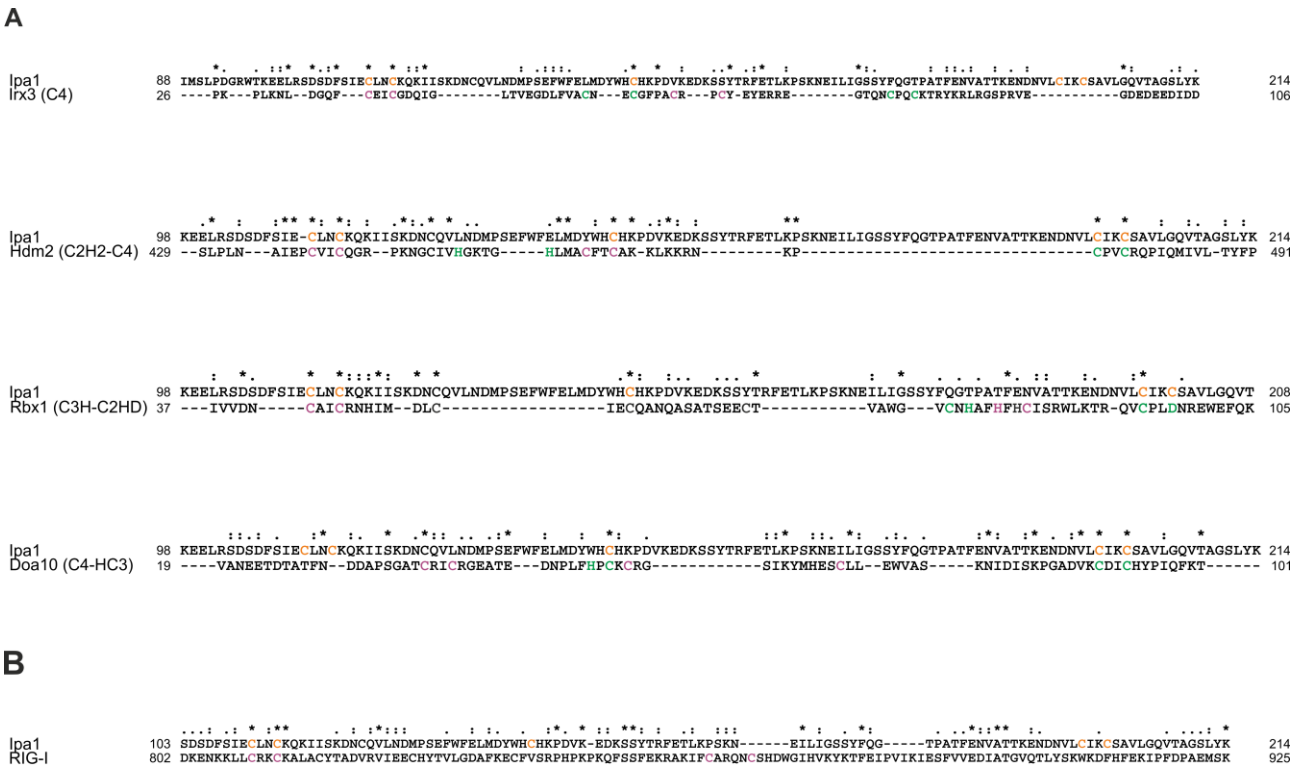


Supplemental Figure S2

Complementation assay with *IPA1* alleles

(**A**) Plasmid-encoded *IPA1* alleles (as indicated) were transformed into diploid yeast cells with a heterozygous *IPA1* deletion, sporulated and subjected to tetrad analysis. Upper row: growth on YPD medium, lower row: growth on YPD + 200 mg/l Geneticin, which selects for cells carrying the *IPA1* deletion.

(**B**) Mutational approach mapped to the sequence of Ipa1. The family signature of the HECT_2 domain is indicated, invariant residues are shown in bold. Residues shown underlined and in red were essential for viability, residues dotted and in green were not (black = not tested).

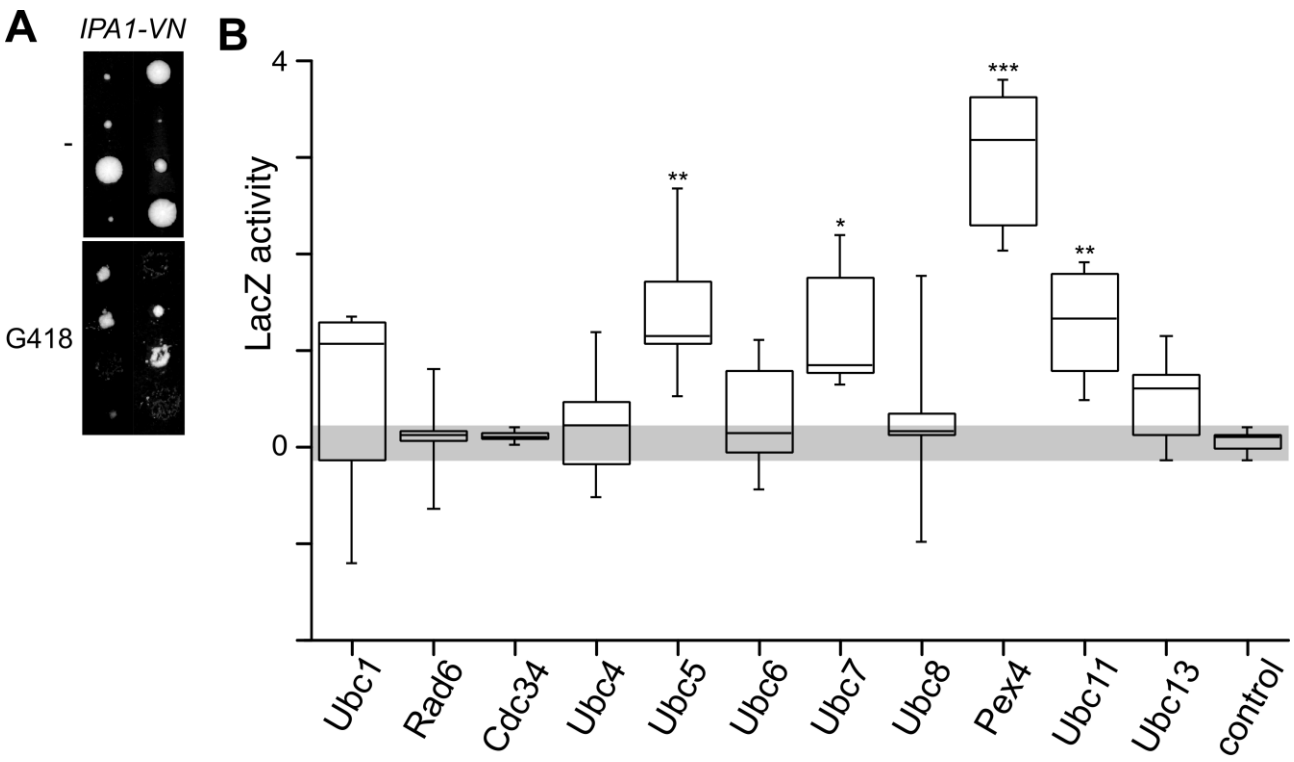


Supplemental Figure S3

Sequence alignment of Ipa1 (residues 88-214) with zinc-binding domains

(**A**) Pair-wise sequence alignment of Ipa1 with several RING-domain variants (*Arabidopsis thaliana* Irx3, *Homo sapiens* Hdm2 and Rbx1, and *Saccharomyces cerevisiae* Doa10). The five essential cysteines of Ipa1 are highlighted in orange. In the RING domains, the residues coordinating the first and second Zn^{2+} are highlighted in purple and green, respectively.

(**B**) Sequence alignment of the regulatory domain of *Homo sapiens* RIG-I with Ipa1. The cysteine residues of RIG-I coordinating the Zn^{2+} are highlighted in purple; the essential cysteines of Ipa1 are highlighted in orange.

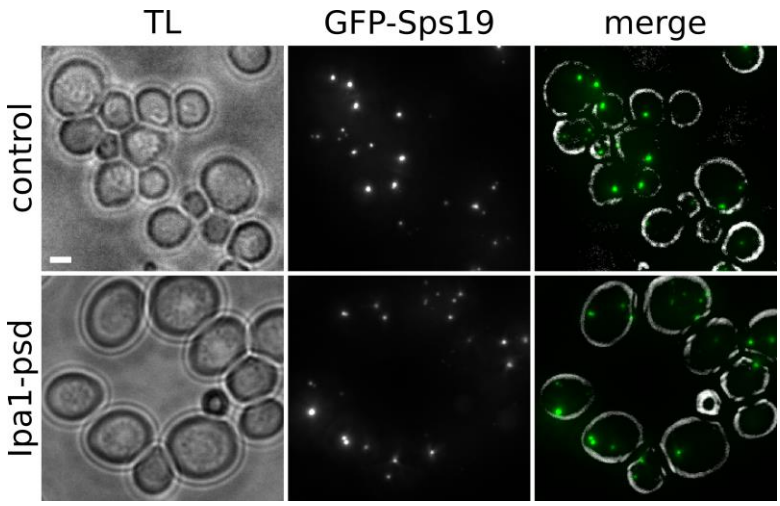


Supplemental Figure S4

Ipa1 interacts with ubiquitin-conjugating enzymes

(**A**) Complementation assay with plasmid-encoded P_{GAL1}-*IPA1-VN* using a heterozygous *IPA1*/*ipaΔ* deletion mutant. Experimental conditions as described for Supplemental Figure S2a using galactose containing medium to induce *IPA1-VN* expression.

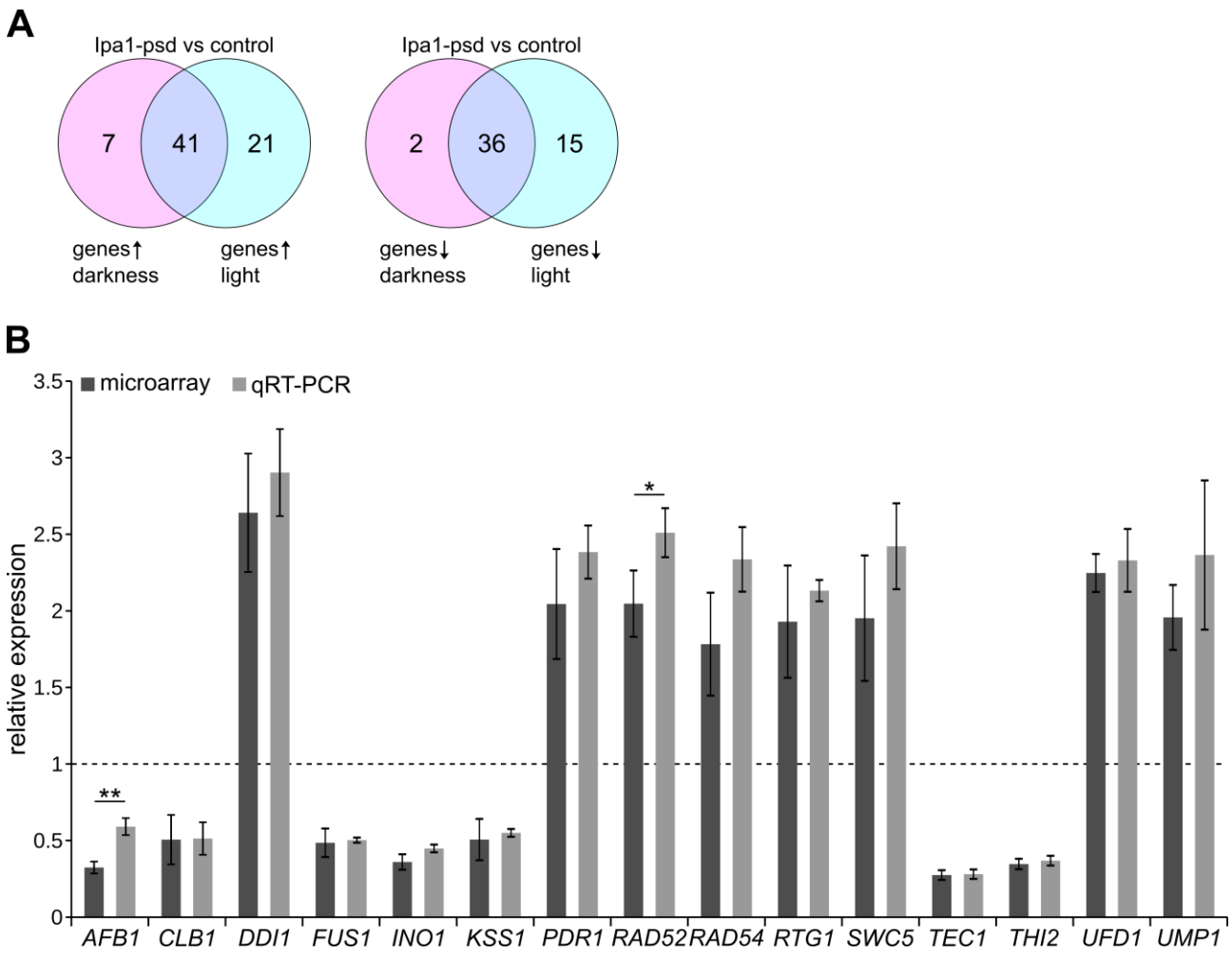
(**B**) Quantitative yeast two-hybrid assay measuring LacZ activity. Combinations between $Gal4^{AD}$-HA-Ipa1 and $LexA^{BD}$-myc fused to ubiquitin-conjugating enzymes were tested. For each pair an appropriate control ($Gal4^{AD}$-HA + $LexA^{BD}$-myc-E2) was used to obtain background activity, which was subtracted from the activity of $Gal4^{AD}$-HA-Ipa1 + $LexA^{BD}$-myc-E2. Two empty vectors ($Gal4^{AD}$-HA + $LexA^{BD}$-myc) were used to obtain background activity in case of the control strain ($Gal4^{AD}$-HA-Ipa1 + $LexA^{BD}$-myc). The grey bar indicates the range of LacZ units measured for the control strain. Whiskers indicate the full range of values, the median of the measurements is indicated by a line within the box ($n \geq 4$); statistical significance of differences was tested by a two-sided student's t-test, ***: P<0.001; **: P<0.01; *: P<0.05).



Supplemental Figure S5

Peroxisome number and protein import into peroxisomes is not affected in Ipa1-depleted cells

The peroxisomal 2,4-dienoyl-CoA reductase Sps19 was tagged with GFP. Cells in logarithmic growth-phase exposed to blue-light for five hours were subjected to fluorescence microscopy. TL images, maximum projections of z-stacks for fluorescence channel, and TL/YFP overlays are shown (bar size 2 µm).

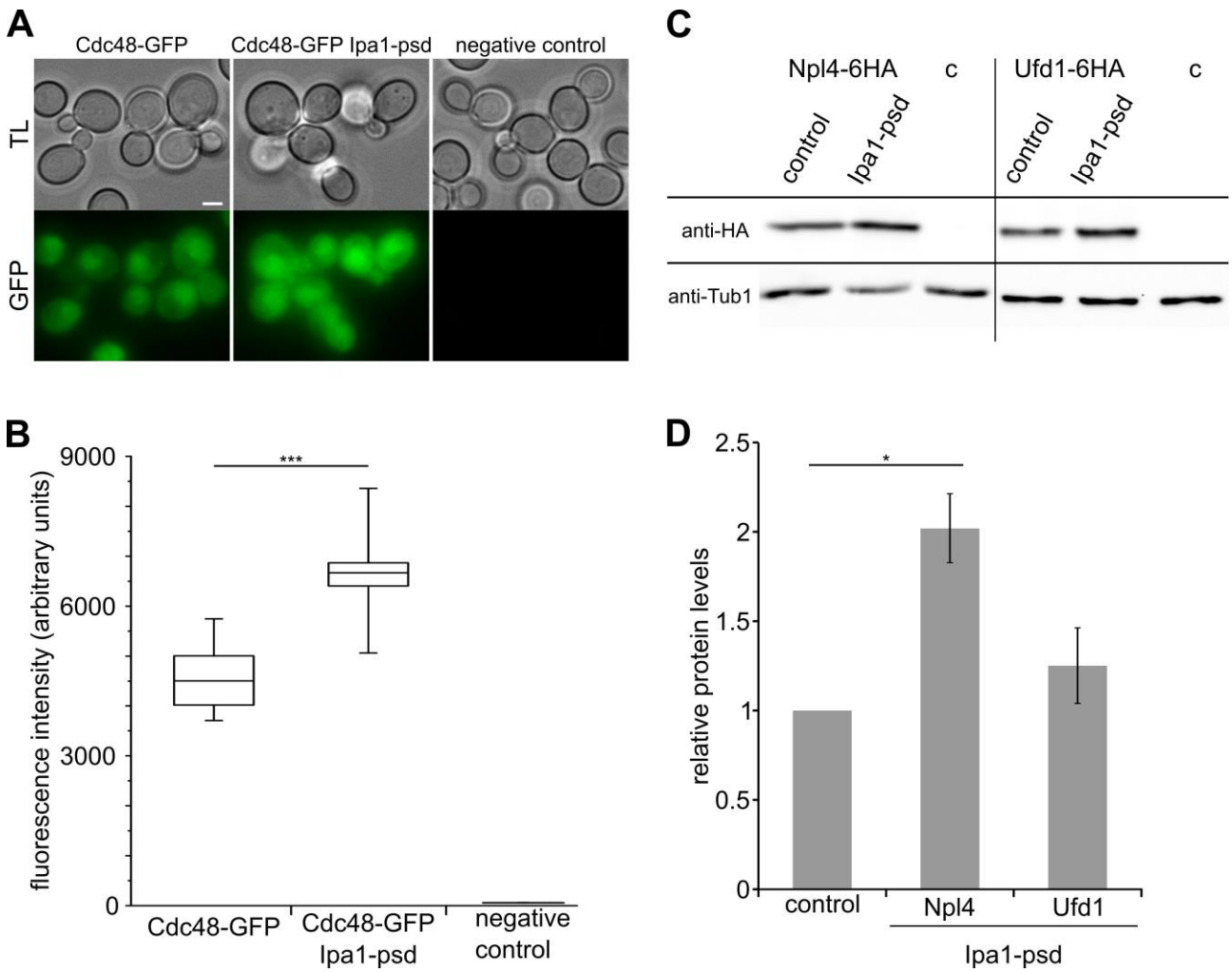


Supplemental Figure S6

Transcriptome analysis of Ipa1-psd cells

(**A**) Venn diagrams showing the overlap between differentially regulated genes in Ipa1-psd cells exposed to blue-light or kept in darkness compared with control cells exposed to the same conditions. Left side: genes with higher expression (>2), right side: genes with lower expression (<0.5).

(**B**) Comparison of relative expression changes found in the microarray experiments with data obtained by real-time qRT-PCR for selected genes. Messenger RNA was obtained from logarithmically growing Ipa1-psd cells and control cells exposed to blue-light (n=3; error bar = standard deviation; statistical significance of differences was tested by a two-sided student's t-test, **: P<0.01; *: P<0.05).



Supplemental Figure S7

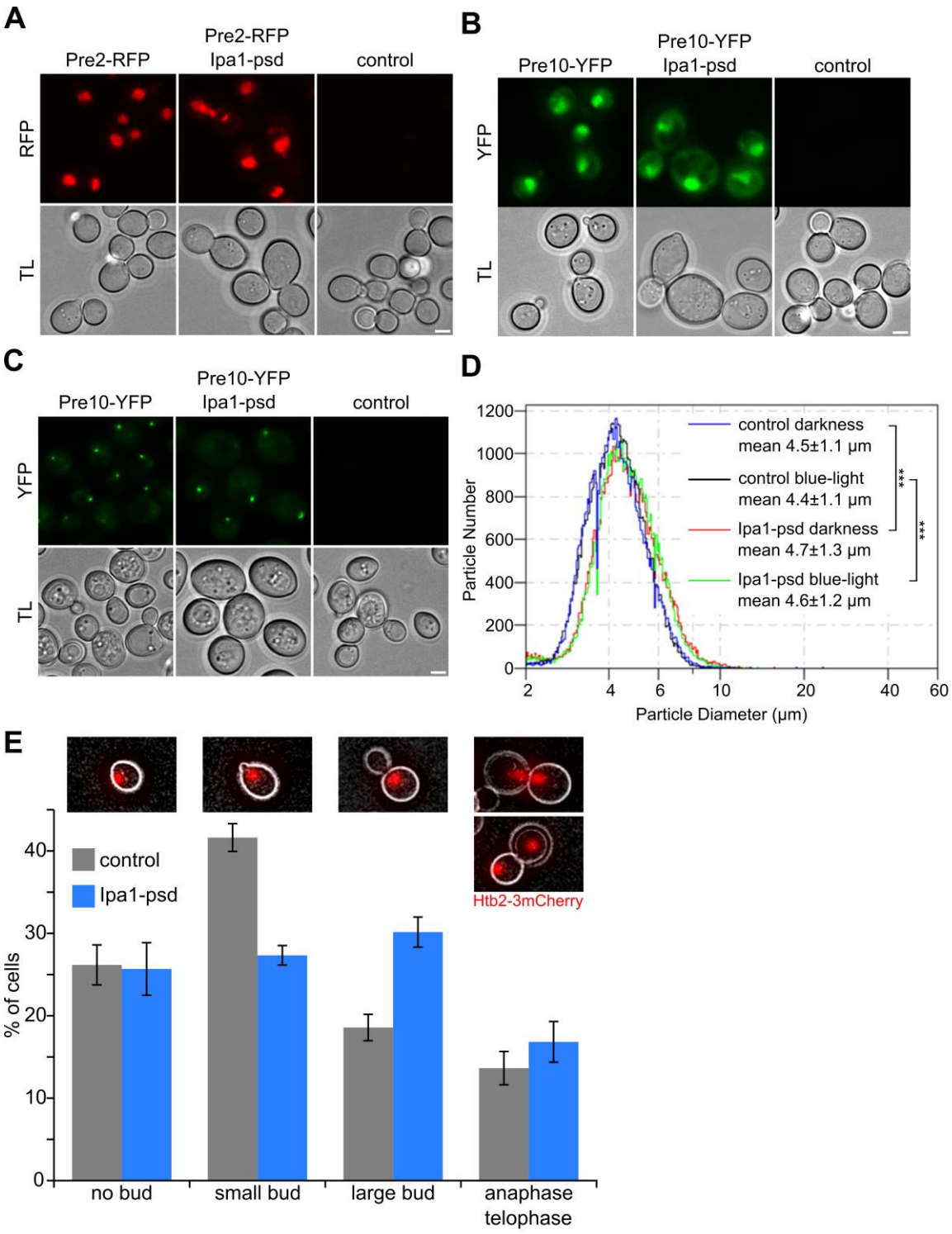
The abundance of the Cdc48-Npl4-Ufd1 complex is increased in Ipa1-depleted cells

(**A**) Abundance of Cdc48-GFP in control cells compared to Ipa1-psd cells. Cells in logarithmic growth-phase were subjected to fluorescence microscopy. TL images and maximum projections of z-stacks for fluorescence channel are shown (bar size 2 µm).

(**B**) Quantification of Cdc48-GFP levels. Example images are shown in A. An ImageJ macro was used for segmentation and fluorescence intensity measurement in projected z-stacks. For each strain 1000 cells were analyzed. Whiskers comprise full range of measurements (statistical significance of differences was tested by a two-sided student's t-test, ***: $p \leq 0.001$).

(**C**) Abundance of Npl4-6HA and Ufd1-6HA in control cells compared to Ipa1-depleted cells. Whole cell extracts were prepared from cells growing logarithmically in liquid cultures exposed to blue-light. Blots were probed with anti-HA and anti-Tub1 (loading control) antibodies.

(**D**) Quantification of Npl4-6HA and Ufd1-6HA abundance in Ipa1-psd cells exposed to blue-light. Exemplary blot shown in C. Graph shows the mean of at least four independent measurements, Npl4-6HA and Ufd1-6HA levels were normalized to Tub1 signal (error bar = standard error of the mean; statistical significance of differences was tested by a two-sided student's t-test, *: $p \leq 0.05$).



Supplemental Figure S8

Localization of proteasomal subunits Pre2 and Pre10, cell-size analysis, and cell-cycle stage distribution of Ipa1-psd cells

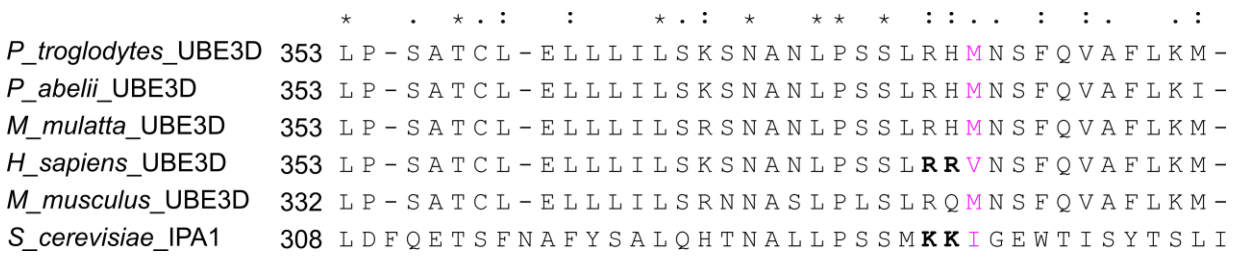
(**A**) Pre2-RFP localization was analyzed in control cells and cells depleted for Ipa1-psd in the logarithmic growth phase. Cells were exposed for 5 hours to blue-light before the start of the experiment. Maximum projections of deconvolved z-stacks are shown for fluorescence channels together with TL images (bar size 2 µm).

(**B**) As in A. Analyzing localization of Pre10-YFP.

(**C**) Pre10-YFP localization was analyzed in control and Ipa1-psd cells after prolonged starvation to induce proteasomal foci formation. Cells were kept in darkness during growth and starvation phase. Maximum projections of deconvolved z-stacks are shown for fluorescence channels (bar size 2 µm).

(**D**) Analysis of cell size distribution in a population of yeast cells. Cell diameter was determined with a Coulter Counter, 50 000 cells were analyzed for each curve (error: standard deviation; statistical significance of differences was tested by a two-sided student's t-test, ***: $p \leq 0.001$).

(**E**) Cell cycle analysis of control (Htb2-3mCherry) and Ipa1-depleted cells (Ipa1-psd Htb2-3mCherry). Cells were exposed to blue-light (465 nm 30 µmol m^{-2} s^{-1}) for 5 hours. Cell cycle stage was determined considering cell morphology and Htb2-3mCherry signals. Example images for each stage are shown at the top of the bar graph (bar size 2 µm). The images are an overlay of transmitted light images with maximum projections of the fluorescence image z-stacks.



Supplemental Figure S9

Alignment of the C-termini of selected HECT_2 domain proteins

The sequences of the C-termini of the HECT_2 domain proteins *Homo sapiens* (*H_sapiens*) UBE3D, *Macaca mulatta* (*M_mulatta*) UBE3D, *Mus musculus* (*M_musculus*), *Pongo abelii* (*P_abelii*) UBE3D, *Pan troglodytes* (*P_troglodytes*) UBE3D, and *Saccharomyces cerevisiae* (*S_cerevisiae*) Ipa1 were aligned with ClustalX. The conservation grade of a residue is shown by asterisk, double point and point to indicate strict conservation, higher, and lower similarity, respectively. The residues corresponding to *H. sapiens* V379 are highlighted in magenta, the adjacent arginines in UBE3D and the lysines in Ipa1 are shown in bold.