Supplementary figures and table

Loss of Rpt5 interactions with the core particle and Nas2 causes the formation of faulty proteasomes that are inhibited by Ecm29

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Supplementary table S1 Strain list

Strain	Genotype[a] (*lys2-801 leu2-3, 2-112 ura3-52 his3-Δ200 trp1-1)*	Figure	source
SUB61		2,3,4a,6	(1)
sDL133	*rpn11::RPN11-TEVProA (HIS3)*	4b,c,5	(2)
sMK60	*ecm29::TRP rpn11::RPN11-TEVProA (HIS3)*	5	(3)
sJR233	*hsm3::KAN nas6::TRP*	6	(4)
sJR236	*hsm3::KAN rpn14::HYG*	6	(4)
sJR239	*nas6::TRP rpn14::HYG*	6	(4)
sJR245	*hsm3::KAN*	6	(4)
sJR299	*hsm3::KAN nas6::TRP ecm29::TRP*	6	(5)
sJR301	*nas6::TRP rpn14::HYG ecm29::TRP*	6	(5)
sJR303	*hsm3::KAN rpn14::HYGRO ecm29::TRP*	6	(5)
sJR515	*hsm3::KAN*	3	(5)
sJR518	*nas2::NAT, hsm3::KAN rpt5::rpt5Δ1-HYG*	3	(5)
sJR519	*nas2::NAT, hsm3::KAN rpt5::rpt5Δ3-HYG*	3	(5)
sJR528	*hsm3::KAN rpt5::rpt5Δ1-HYG*	3	(5)
sJR530	*hsm3::KAN rpt5::rpt5Δ3-HYG*	3	(5)
sJR534	*nas2::NAT*	2,3,4a	(5)
sJR535	*rpt5:: rpt5Δ1-HYG*	2,3,4a	(5)
sJR536	*rpt5:: rpt5Δ3-HYG*	2,3,4a	(5)
sJR539	*nas2::NAT, rpt5::rpt5Δ1-HYG*	3	(5)
sJR540	*nas2::NAT, rpt5::rpt5Δ3-HYG*	3	(5)
sJR544	*ecm29::TRP rpt5::rpt5Δ3-HYG*	6	(5)
sJR545	*hsm3::KAN ecm29::TRP rpt5::rpt5Δ0-HYG*	6	(5)
sJR546	*hsm3::KAN ecm29::TRP rpt5::rpt5Δ3-HYG*	6	(5)
sJR548	*ecm29::TRP rpt5::rpt5Δ3-HYG rpn11::RPN11-TEVProA (HIS3)*	4b,c	(5)
sJR550	*hsm3::KAN ecm29::TRP rpt5::rpt5Δ3-HYG rpn11::RPN11-TEVProA (HIS3)*	4b,c	(5)
sJR552	*rpt5::rpt5Δ3-HYG rpn11::RPN11-TEVProA (HIS3)*	4b,c	(5)
sJR554	*hsm3::KAN rpt5::rpt5Δ3-HYG rpn11::RPN11-TEVProA (HIS3)*	4b,c	(5)
sJR555	*rpt5::rpt5Δ0-HYG*	6	(5)
sJR556	*rpt5::rpt5Δ3-HYG*	6	(5)
sJR557	*hsm3::KAN rpt5::rpt5Δ0-HYG*	6	(5)
sJR558	*hsm3::KAN rpt5::rpt5Δ3-HYG*	6	(5)
sJR559	*ecm29::TRP*	6	(5)
sJR578	*nas2::NAT hsm3::KAN ecm29::TRP*	6	(5)
sJR579	*nas2::NAT hsm3::KAN*	6	(5)

a. All strains have the DF5 background genotype (*lys2-801 leu2-3, 2-112 ura3-52 his3-Δ200 trp1-1*)

1. Finley, D., Ozkaynak, E., and Varshavsky, A. (1987) *Cell* **48**, 1035-1046
2. Leggett, D. S., Hanna, J., Borodovsky, A., Crosas, B., Schmidt, M., Baker, R. T., Walz, T., Ploegh, H., and Finley, D. (2002) *Mol Cell* **10**, 495-507
3. Kleijnen, M. F., Roelofs, J., Park, S., Hathaway, N. A., Glickman, M., King, R. W., and Finley, D. (2007) *Nat Struct Mol Biol* **14**, 1180-1188
4. Roelofs, J., Park, S., Haas, W., Tian, G., McAllister, F. E., Huo, Y., Lee, B. H., Zhang, F., Shi, Y., Gygi, S. P., and Finley, D. (2009) *Nature* **459**, 861-865
5. This study

Supplementary table S2 LC-MS/MS Ananlysis of Ecm29-Buond RP_2-CP

			WT				*rpt5*Δ3			
	mass		score	matches	sequences	emPAI	score	matches	sequences	emPAI
Ecm29	ECM29_YEAST	211610	938	15	15	0.24	2319	52	40	0.77
alpha1	PSA6_YEAST	25759	361	7	6	1.11	332	6	6	0.7
alpha2	PSA2_YEAST	27145	305	10	5	1.42	263	6	4	0.74
alpha3	PSA4_YEAST	31688	366	7	6	1.08	379	9	8	1.31
alpha4	PSA7_YEAST	28697	289	6	5	0.52	266	6	4	0.52
alpha5	PSA5_YEAST	28770	36	1	1	0.11	38	1	1	0.11
alpha6	PSA1_YEAST	28154	357	5	5	0.79	452	9	7	1.26
alpha7	PSA3_YEAST	28650	394	7	7	0.95	258	7	5	0.46
beta1	PSB6_YEAST	26968	74	2	1	0.13	142	3	2	0.29
beta2	PSB7_YEAST	22560	101	2	2	0.23	60	1	1	0.11
beta3	PSB3_YEAST	22819	327	8	4	1.19	317	8	5	1.19
beta4	PSB2_YEAST	29425	87	2	2	0.3	174	3	3	0.49
beta5	PSB5_YEAST	31902	230	4	4	0.46	246	4	4	0.46
beta6	PSB1_YEAST	23761	161	3	3	0.25	174	4	4	0.56
beta7	PSB4_YEAST	28478	300	6	5	0.85	167	6	3	0.67
Rpt1	PRS7_YEAST	52293	472	15	12	1.13	288	6	6	0.34
Rpt2	PRS4_YEAST	49026	509	9	8	0.75	86	2	2	0.13
Rpt3	PRS6B_YEAST	47864	444	11	8	0.56	306	5	5	0.37
Rpt4	PRS10_YEAST	49492	319	8	5	0.36	95	2	2	0.13
Rpt5	PRS6A_YEAST	48283	687	13	10	1.13	289	6	6	0.37
Rpt6	PRS8_YEAST	45471	512	11	8	0.71	288	6	6	0.4
Rpn1	RPN1_YEAST	109880	512	12	12	0.4	537	10	10	0.32
Rpn2	RPN2_YEAST	104623	948	18	17	0.55	884	20	17	0.65
Rpn3	RPN3_YEAST	60754	235	4	4	0.16	399	7	6	0.42
Rpn5	RPN5_YEAST	51850	438	10	8	0.7	460	13	11	1.03
Rpn6	RPN6_YEAST	50085	219	7	7	0.36	146	4	4	0.28
Rpn7	RPN7_YEAST	49213	97	3	3	0.2	220	4	4	0.2
Rpn8	RPN8_YEAST	38460	525	8	8	0.88	559	10	9	1.2
Rpn9	RPN9_YEAST	45811	611	12	11	1.07	421	10	10	0.49
Rpn10	RPN10_YEAST	29786	205	3	2	0.36	249	4	3	0.5
Rpn11	RPN11_YEAST	34433	244	5	5	0.42	153	5	3	0.3
Rpn12	RPN12_YEAST	31956	407	8	8	0.76	304	7	6	0.76
Rpn13	RPN13_YEAST	18005	143	4	3	0.93	152	3	3	0.64

Supplementary table 2. To determine the presence of the CP subunit β3 in Ecm29-RP_2-CP proteasomes complexes derived from the *rpt5*-Δ3 strain, purified proteasomes samples were treated with apyrase and separated on a native gel. As a positive control wildtype proteasomes without apyrase treatment were loaded on gel. The band containing doubly capped proteasomes was excised from each lane and submitted for analysis by mass spectrometry. Mass spectormetry of samples from two independent purifications for both wildtype and Rpt3-Δ3 proteasomes proteasome showed very similar results. All proteasome subunits, with the exception of sem1, were identified in all fours sample. Sem1 was only detected in the second purification of wildtype and mutant proteasomes (data not shown). The initial absence can probably be explained by the small size of Sem1 which can hinder its detection, even if present. For Mass spectrometry analysis CBB stained gel pieces were destained using 50% acetonitrile (ACN) at 30 °C. After destaining, the gel pieces were shrunk by addition of 50 µl of 100% ACN for 10 min and solvent was discarded. The gel pieces were dried by speed vacuum concentrator. To rehydrate the gel in 20 µL 20 mM ammonium bicarbonate supplemented with 200 ng sequencing grade trypsin (Trypsin Gold, Promega, Madison, WI), was added. Next, 20 µL of 20 mM ammonium bicarbonate and 10% ACN was added, and gel pieces were incubated at 30°C for 17 h. Tryptic peptides were recovered from gel plugs by extraction with 100 µL of 50% ACN in 2% trifluoroacetic acid (TFA) at 30 °C for 30 min. Extracted peptides were concentrated by speed vacuum concentrator and added to 100 µL of 2% ACN in 0.1% formic acid. 30 µL Peptide solution was loaded on a C18 reversed-phase capillary column (75 µm ID × 15 cm, PepMap: Dionex) in conjunction with an Acclaim C18 PepMap trapping column (300 µm id×10 mm, Dionex) using Nano-HPLC was performed automatically using a microcolumn switching device (Switchos; LC Packings) coupled to an autosampler (Famos: LC Packings) and a nanogradient generator

(UltiMate Nano HPLC; LC Packings). . Peptides were separated by a nanoflow linear ACN gradient using buffer A (0.1% formic acid, 2% ACN) and buffer B (0.1% formic acid, 80% ACN) starting from 5% buffer B to 70% over 55 min at a flow rate of 200 nL/min. Then column was washed by 95% of buffer B for 5 min. The system control software, Hystar 3.2, was used to control the entire process. The eluted peptides were injected into an HCT Ultra Ion Trap Mass Spectrometer (Bruker Daltronics). The mass spectrometer was set up in the data dependent MS/MS mode to alternatively acquire full scans (*m*/*z* acquisition range from 300 to 1500 Da). The four most intense peaks in any full scan were selected as precursor ions and fragmented by collision energy. MS/MS spectra were interpreted and peak lists were generated by DataAnalysis 3.4 and Biotools 3.0 software (Bruker Daltronics).

Peptide masses were compared to Swiss Prot Database using MASCOT 2.2 (http://www.matrixscience.com). *Saccharomyces Cerevisiae* was selected for the taxonomy. The following parameters were used in all searches: the maximum number of missed cleavages allowed was 2; the mass tolerance was 1.2 Da for MS and 0.6 Da for MS/MS. Fixed modification was set on cystein with carbamidomethylation. Variable modification was done on methionine with oxidation. Positive protein identifications using a threshold of 0.05 were used. Peptides scoring <20 were automatically rejected, ensuring all protein identifications were based on the reliable peptide identifications.