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Supplementary Materials for

Regulation of the 26*S* Proteasome Complex During Oxidative Stress

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The PDF file includes:

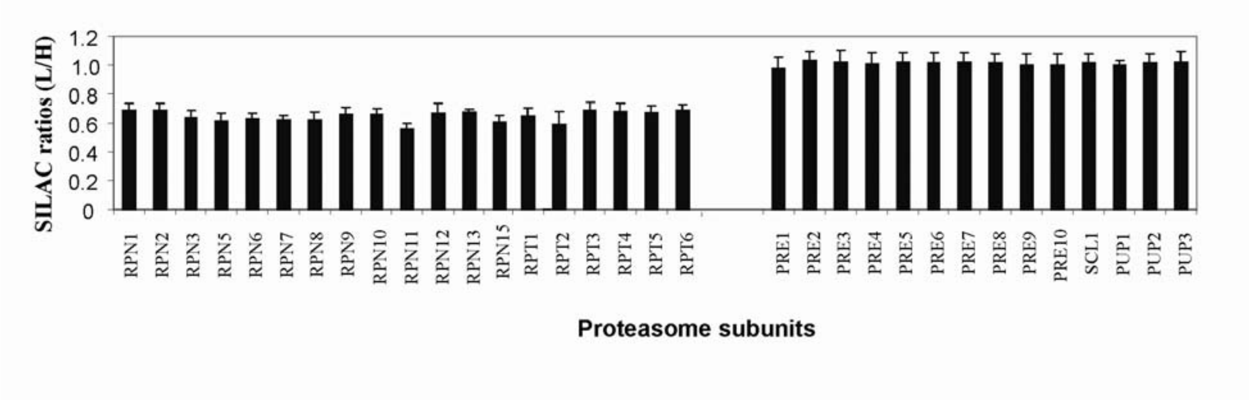

Fig. S1. Average SILAC ratios (L/H) of the yeast 19*S* and 20*S* subunits purified with the 20*S* subunit Pre10-TAP after oxidative stress. Proteasomes were purified from cells whose abundance of Pre10-TAP was similar to that of endogenous Pre10. The light (L) cultures were treated with 3 mM H_2O_2 and the heavy (H) culture was untreated. All ratios were normalized to that of Pre10. Detailed results are summarized in table S3.

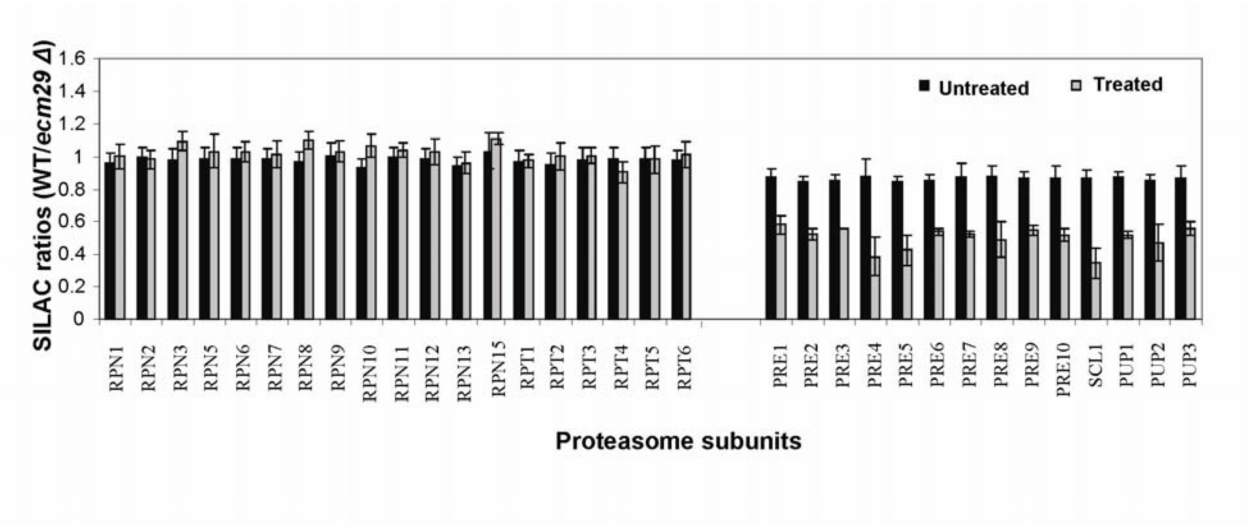

Fig. S2. Quantitative comparison of the composition of the 26*S* proteasome purified by Rpn11-TAP from wild-type and *ecm29Δ* cells with the SILAC-MS method. The plots of the average SILAC ratios (L/H) represent changes in the abundances of proteasome subunits when comparing wild-type and *ecm29Δ* cells without treatment (black) or after exposure to 3 mM H_2O_2 for 30 min (gray). The wild-type cells were grown in light (L) media and *ecm29Δ* cells were grown in heavy (H) media. Cells contained amounts of Rpn11-TAP that were similar to that of endogenous Rpn11. All ratios were normalized to that of Rpn11. Detailed results are summarized in tables S4 and S5.

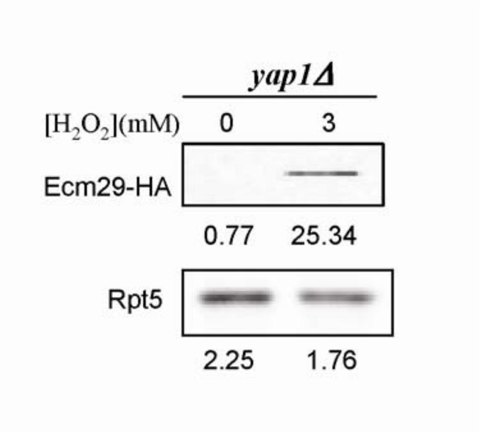

Fig. S3. Yap1-independent enrichment of Ecm29 in purified 19*S* complexes. The proteasome complexes were purified from *yap1*Δ cells expressing Rpn11-TAP before and after treatment with 3mM H_2O_2 for 30 min. The abundances of Ecm29-HA protein bound to the purified proteasomes from untreated and treated cells were measured by Western blotting with an antibody against the HA tag. The 19*S* subunit Rpt5 was detected with a specific antibody.

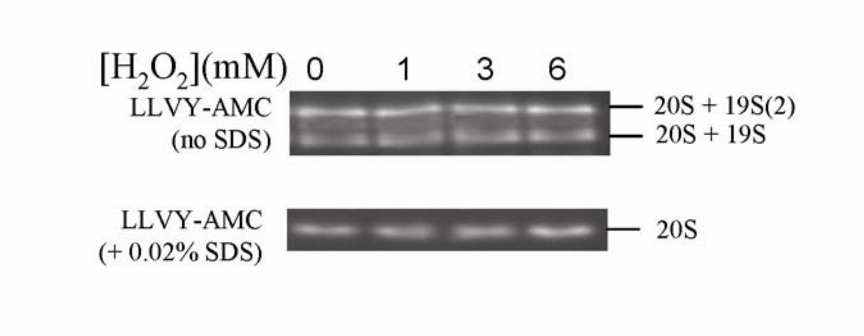

Fig. S4. In-gel proteasome activities in *ecm29Δ* cells after H_2O_2-induced stress. Total lysates from cells treated with the indicated concentrations of H_2O_2 were resolved by native gel electrophoresis. The chymotrypsin-like activity was measured by a native gel overlay assay with the fluorogenic peptide substrate SUC-LLVY-AMC in the absence or presence of 0.02% SDS for 26*S* and 20*S* proteasomes, respectively.

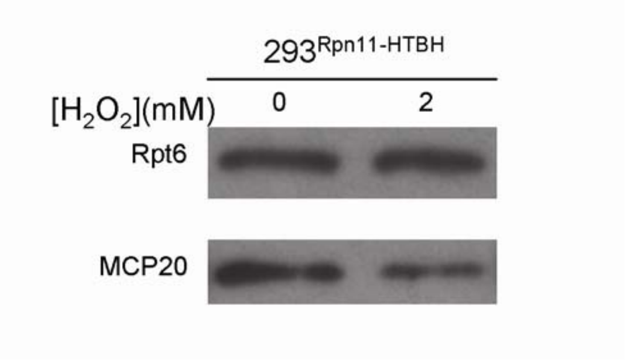

Fig. S5. Validation of H_2O_2-triggered separation of the 20*S* core from the 19*S* particle in human cells by Western blotting analysis. The 26*S* proteasome was affinity purified from HEK $293^{Rpn11\text{-}HTBH}$ cells with Rpn11-HTBH and the composition of the complex was analyzed by Western blotting with antibodies against Rpt6 (a 19*S* subunit) and MCP20 (a 20*S* subunit).

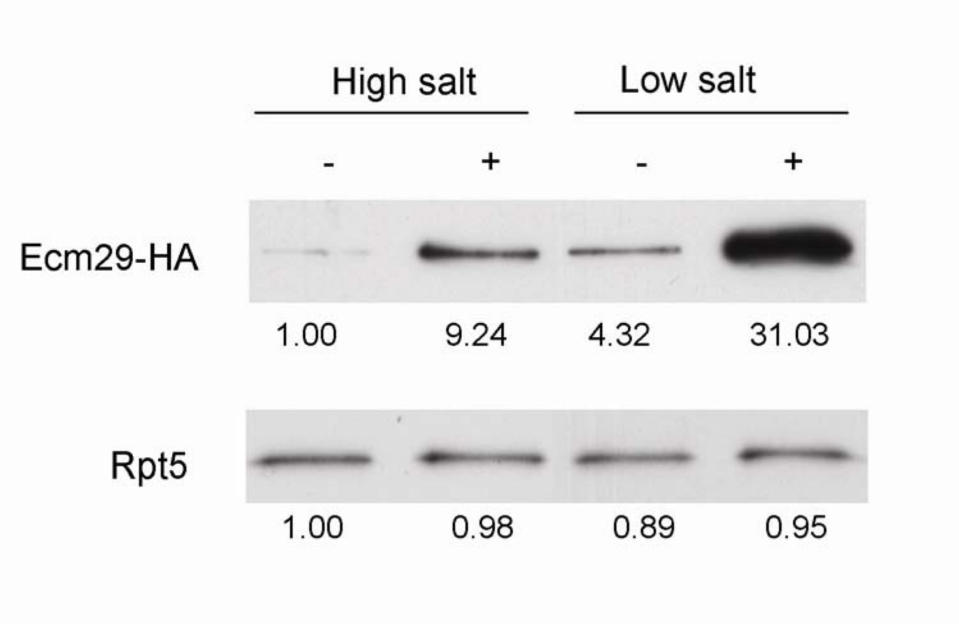

Fig. S6. Presence of Ecm29 in purified 19*S* subunits is independent of the salt concentration used during affinity purification. Affinity purification of 26*S* proteasomes was performed with cells containing Rpn11-TAP that were either untreated or exposed to 3 mM H_2O_2 for 30 min. Proteasomes were purified in low-salt buffer (25 mM tris, 5 mM NaF, 2.5 mM $Na_4P_2O_7$) or high-salt buffer (100 mM NaCl, 50 mM tris, 50 mM NaF, 10 mM $Na_4P_2O_7$). The purified samples were analyzed by quantitative Western blotting with antibodies against Rpt5 (a 19*S* subunit) and Pre10 (a 20*S* subunit). The number below each band represents the band intensity obtained with an Odyssey Infrared Imaging System for quantitation.

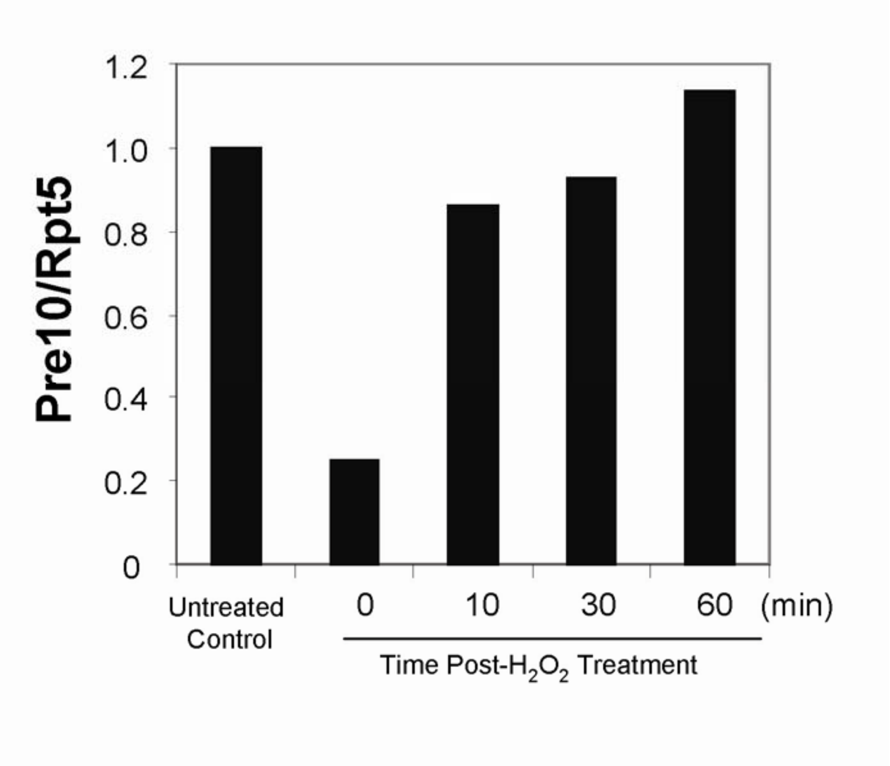

Fig. S7. Reassembly of the 26*S* proteasome after 3 mM H_2O_2-induced stress. Proteasomes were affinity purified from yeast cells that contained Rpn11-TAP at an abundance similar to that of endogenous Rpn11. Cells were first treated with 3mM H_2O_2 for 30 min at 30°C, after which the medium containing H_2O_2 was removed, cells were washed twice with fresh medium, and were incubated in fresh medium for the indicated times. Proteasomes were purified and the ratio between the abundances of Pre10 (a 20*S* subunit) and Rpt5 (a 19*S* subunit) was determined by quantitative Western blotting.

Table S1. Identification and quantitation of proteasome subunits by SILAC-MS in Rpn11-TAP–expressing cells before and after treatment with H_2O_2.

				1 mM H2O2						3 mM H2O2						6 mM H2O2				
	Name	Acc #	Num Unique	% Cov	Best Expect Val	# Pep used for SILAC Ratios	Average SILAC Ratios	STDEV	Num Unique	% Cov	Best Expect Val	# Pep used for SILAC Ratios	Average SILAC Ratios	STDEV	Num Unique	% Cov	Best Expect Val	# Pep used for SILAC Ratios	Average SILAC Ratios	STDEV
19S	RPN1	YHR027C	128	65.3	9.5E-11	81	1.026	0.086	196	80.9	4E-14	99	0.992	0.084	168	70.1	5.9E-12	85	0.997	0.093
	RPN2	YIL075C	146	60.3	2E-09	95	1.008	0.083	187	66	2E-12	93	0.981	0.087	151	61.3	1.9E-10	76	1.000	0.092
	RPN3	YER021W	71	57.6	8.9E-10	48	0.997	0.105	102	75.3	1E-13	57	0.965	0.093	77	63.9	2.8E-11	54	1.025	0.096
	RPN5	YDL147W	63	65.6	2.6E-09	44	0.992	0.092	95	75.7	8E-13	63	0.967	0.100	66	61.1	8.1E-11	38	1.037	0.099
	RPN6	YDL097C	67	59.2	5.9E-10	45	0.971	0.079	91	73.3	9E-14	41	0.970	0.076	65	70.3	2.7E-10	36	0.984	0.083
	RPN7	YPR108W	62	55.5	6.6E-09	44	0.981	0.098	82	66.7	2E-12	51	0.966	0.102	74	67.8	5E-10	38	1.012	0.074
	RPN8	YOR261C	57	79.3	8.4E-10	36	1.006	0.089	65	80.8	1E-13	39	0.963	0.087	59	79.3	5.4E-11	35	1.002	0.095
	RPN9	YDR427W	70	59	5.5E-09	44	0.993	0.092	104	69.5	1E-12	62	0.993	0.107	78	66.2	6.1E-10	42	1.010	0.087
	RPN10	YHR200W	36	54.9	2.7E-10	20	1.021	0.099	39	56.7	3E-13	24	0.965	0.054	38	55.6	2.5E-11	19	1.033	0.128
	RPN11	YFR004W	48	61.1	8.8E-11	24	1.000	0.068	66	74.8	5E-13	34	1.000	0.098	54	69	1E-09	29	1.000	0.076
	RPN12	YFR052W	52	74.8	3.9E-09	37	0.995	0.096	69	82.1	1E-13	37	0.980	0.087	60	79.9	1.8E-12	29	1.047	0.117
	RPN13	YLR421C	22	59.6	1.3E-08	6	1.061	0.084	23	68.6	2E-12	14	0.920	0.081	21	65.4	1.2E-11	12	0.959	0.077
	RPN15	YDR363W-A	9	73	3.6E-09	5	0.966	0.085	12	78.7	6E-13	6	1.008	0.118	11	77.5	1.4E-10	4	0.943	0.039
	RPT1	YKL145W	62	67.9	2.4E-09	46	1.024	0.083	82	81.2	3E-12	44	0.941	0.077	69	72.8	8.9E-11	34	0.960	0.073
	RPT2	YDL007W	73	76.4	2E-10	46	1.040	0.087	82	73.7	1E-13	40	0.997	0.084	71	71.4	1.3E-11	33	1.006	0.096
	RPT3	YDR394W	57	81.8	7.6E-11	31	1.023	0.079	85	88.1	1E-13	33	0.989	0.110	72	80.8	4.8E-11	26	1.021	0.110
	RPT4	YOR259C	62	63.8	1.4E-09	35	1.063	0.097	88	74.1	4E-13	47	0.931	0.079	79	74.6	3.9E-11	19	1.089	0.124
	RPT5	YOR117W	81	74.4	1.5E-10	46	1.060	0.082	104	74.7	9E-14	58	0.973	0.106	91	72.6	2.2E-11	37	1.009	0.113
	RPT6	YGL048C	60	66.7	5.8E-10	41	1.039	0.067	77	73.3	7E-13	46	0.972	0.082	63	67.7	3.9E-10	31	1.002	0.090
20S	PRE1	YER012W	16	40.4	1.1E-07	4	0.882	0.035	19	59.1	9E-12	10	0.340	0.060	14	42.9	5.2E-09	10	0.107	0.037
	PRE2	YPR103W	24	56.8	1.7E-09	16	1.040	0.103	24	56.1	2E-11	12	0.368	0.067	18	53.3	9.6E-09	11	0.098	0.033
	PRE3	YJL001W	21	71.2	1.5E-09	12	1.028	0.155	25	74.9	2E-11	12	0.380	0.100	20	74.9	2.4E-09	10	0.167	0.076
	PRE4	YFR050C	25	47.4	9E-10	15	0.932	0.059	34	56.4	9E-12	15	0.324	0.064	21	56.4	4.9E-09	18	0.124	0.055
	PRE5	YMR314W	26	70.9	9.9E-10	17	0.959	0.122	37	82.9	9E-13	18	0.344	0.062	28	76.1	7.2E-10	20	0.142	0.079
	PRE6	YOL038W	15	51.6	2.4E-11	7	0.938	0.062	20	66.9	3E-13	14	0.357	0.086	16	49.6	2.7E-11	13	0.147	0.050
	PRE7	YBL041W	26	51.5	1.3E-09	17	0.972	0.095	34	65.1	7E-12	19	0.365	0.058	25	55.2	4.1E-09	19	0.130	0.042
	PRE8	YML092C	29	51.6	2.5E-09	19	0.966	0.075	34	52	3E-12	20	0.356	0.073	27	52.4	2.2E-09	22	0.129	0.059
	PRE9	YGR135W	30	67.1	2.1E-09	19	0.957	0.095	40	73.3	8E-12	19	0.306	0.047	30	72.9	1.5E-09	22	0.167	0.085
	PRE10	YOR362C	24	51	1.2E-08	11	0.978	0.088	37	60.4	1E-11	19	0.331	0.078	28	60.1	1.7E-10	21	0.163	0.090
	SCL1	YGL011C	50	73.8	3.4E-10	22	0.946	0.099	52	77.8	7E-12	27	0.344	0.068	44	76.6	1.2E-09	32	0.150	0.074
	PUP1	YOR157C	12	28	4E-07	7	0.984	0.066	15	31	1E-10	9	0.377	0.070	11	31	4.3E-07	6	0.088	0.054
	PUP2	YGR253C	24	78.5	3.5E-10	15	0.948	0.075	30	86.5	1E-10	15	0.367	0.067	21	66.5	8.6E-10	16	0.142	0.056
	PUP3	YER094C	20	61.5	1.8E-10	8	0.991	0.063	20	61.5	5E-13	13	0.336	0.067	12	39.5	1.8E-10	9	0.101	0.035

SILAC ratios: treated /untreated, normalized to Rpn11

Table S2. Identification and quantitation of proteasome subunits and Ecm29 by SILAC-MS in Pre10-TAP–expressing cells before and after treatment with H_2O_2.

	Protein Name	Acc #	Num Unique	% Cov	Best Expect Val	# Pep used for SILAC Ratios	Average SILAC Ratios	STDEV
19S	RPN1	YHR027C	89	42.4	2.7E-10	54	0.686	0.048
	RPN2	YIL075C	94	43.1	1.3E-10	70	0.687	0.049
	RPN3	YER021W	46	49.9	6.7E-09	34	0.641	0.048
	RPN5	YDL147W	43	43.4	3E-09	36	0.619	0.050
	RPN6	YDL097C	36	38.2	3.2E-09	29	0.629	0.046
	RPN7	YPR108W	36	35.9	1.6E-08	30	0.624	0.036
	RPN8	YOR261C	34	57.1	5E-08	25	0.627	0.054
	RPN9	YDR427W	48	45.5	4.5E-09	41	0.661	0.052
	RPN10	YHR200W	22	41.8	2.7E-09	20	0.662	0.041
	RPN11	YFR004W	27	42.8	4.7E-08	20	0.564	0.035
	RPN12	YFR052W	32	52.6	9.8E-10	22	0.674	0.062
	RPN13	YLR421C	11	46.8	2.6E-07	6	0.678	0.017
	RPN15	YDR363W-A	4	40.4	4.9E-08	3	0.609	0.050
	RPT1	YKL145W	45	47.5	3.1E-09	37	0.647	0.060
	RPT2	YDL007W	46	51.9	4E-12	28	0.595	0.086
	RPT3	YDR394W	45	64	1.3E-08	32	0.688	0.053
	RPT4	YOR259C	58	56.3	4.4E-09	46	0.679	0.057
	RPT5	YOR117W	60	63.1	7.6E-10	42	0.669	0.053
	RPT6	YGL048C	43	60.7	1.5E-08	26	0.684	0.044
20S	PRE1	YER012W	23	50.5	1.7E-09	20	0.978	0.074
	PRE2	YPR103W	23	36.6	2.1E-08	17	1.033	0.063
	PRE3	YJL001W	30	70.7	7.2E-09	23	1.027	0.075
	PRE4	YFR050C	31	59	1.2E-09	27	1.005	0.080
	PRE5	YMR314W	36	80.8	7.6E-11	31	1.023	0.063
	PRE6	YOL038W	19	62.2	1.8E-10	17	1.020	0.070
	PRE7	YBL041W	34	58.9	9.7E-09	28	1.022	0.064
	PRE8	YML092C	36	47.2	9.8E-10	32	1.013	0.069
	PRE9	YGR135W	48	75.6	6.6E-10	36	0.997	0.081
	PRE10	YOR362C	25	53.8	7.5E-09	16	1.000	0.076
	SCL1	YGL011C	52	72.6	2.6E-10	40	1.017	0.066
	PUP1	YOR157C	17	30.7	2.3E-07	14	1.003	0.033
	PUP2	YGR253C	32	71.5	2.5E-09	26	1.015	0.065
	PUP3	YER094C	15	39.5	9.4E-10	14	1.023	0.074

	Protein Name	Acc #	Num Unique	% Cov	Best Expect Val	# Pep used for SILAC Ratios	Average SILAC Ratios	St Dev
PIPs	BLM10	YFL007W	149	34.3	4.40E-11	115	0.986	0.128
	CDC48	YDL126C	9	10.3	4.00E-07	6	0.830	0.070
	ECM29	YHL030W	12	6	5.40E-07	9	1.127	0.070
	EFT2	YDR385W	4	5.1	2.20E-08	3	2.497	0.281
	HSP26	YBR072W	2	13.1	1.20E-07	2	1.890	0.212
	SSA1	YAL005C	33	31.9	2.80E-08	23	1.158	0.125
	SSA2	YLL024C	33	29.7	1.10E-08	23	1.160	0.195
	SSB1	YDL229W	27	33.3	4.00E-09	17	1.238	0.236
	SSB2	YNL209W	26	33.3	4.00E-09	17	1.205	0.231
	SSC1	YJR045C	6	6.6	6.40E-06	4	1.455	0.178
	TEF2	YBR118W	17	32.1	6.60E-07	11	1.485	0.095

SILAC ratios: treated/untreated, normalized to Pre10

Table S3. Identification and quantitation of the known PIPs by SILAC-MS in Rpn11-TAP–expressing cells before and after treatment with H_2O_2.

	1 mM H2O2						3 mM H2O2						6 mM H2O2						
ACC#	Num Unique	% Cov	Best Expect Val	# Pep used for SILAC Ratios	SILAC Ratios (Avg)	STDEV	Num Unique	% Cov	Best Expect Val	# Pep used for SILAC Ratios	SILAC Ratios (Avg)	St Dev	Num Unique	% Cov	Best Expect Val	# Pep used for SILAC Ratios	SILAC Ratios (Avg)	STDEV	name
YHL030W	9	4.6	2.10E-05	8	1.84	0.073	63	35.9	1.30E-08	42	16.39	4.880	75	39.8	6.90E-09	48	52.76	17.595	ECM29
YBR272C	18	30.8	7.40E-08	14	2.14	0.040	24	43.3	8.80E-08	17	2.62	0.351	21	33.8	2.00E-09	14	4.30	0.598	HSM3
YPL106C	10	16.6	6.90E-07	6	1.38	0.061	26	31.9	6.50E-08	17	2.68	0.306	24	31	2.80E-07	19	3.56	0.466	SSE1
YJL008C	9	10.4	4.00E-07	5	1.23	0.088	11	23.2	8.60E-08	9	1.96	0.129	11	15.5	2.40E-07	7	2.70	0.271	CCT8
YIL142W	5	9.5	1.60E-06	3	1.33	0.031	14	26.8	9.60E-08	9	2.25	0.331	9	19	1.10E-07	5	2.64	0.447	CCT2
YDL143W	3	11.4	8.30E-05	3	1.01	0.064	7	16.7	6.50E-10	6	2.29	0.231	11	25.9	2.60E-07	8	2.63	0.267	CCT4
YDL229W	48	48.9	3.00E-10	40	0.87	0.086	51	54.2	2.80E-10	35	1.97	0.165	49	52.9	5.00E-10	41	2.57	0.259	SSB1
YNL209W	49	52.4	3.00E-10	41	0.87	0.086	52	54.2	2.80E-10	36	1.98	0.169	51	56.3	5.00E-10	42	2.57	0.257	SSB2
YDR188W	4	6	3.20E-05	3	1.28	0.057	6	12.5	3.70E-05	6	2.42	0.393	7	11.9	4.80E-06	2	2.31	0.028	CCT6
YJR045C	8	13.9	4.50E-07	6	1.35	0.092	18	26.1	2.30E-07	14	2.04	0.258	21	26.5	1.10E-08	16	2.29	0.168	SSC1
YDL126C	20	24.3	3.60E-07	18	1.66	0.087	24	29.9	4.20E-09	20	1.84	0.201	28	31.7	6.80E-09	17	2.10	0.287	CDC48
YBR072W	13	40.7	3.70E-08	10	1.59	0.080	20	52.8	3.20E-10	12	1.59	0.212	19	57	5.60E-10	11	1.92	0.278	HSP26
YJL111W	9	15.3	3.00E-08	5	1.27	0.059	11	17.6	2.40E-09	8	1.70	0.193	5	7.8	4.00E-09	3	1.78	0.121	CCT7
YAL005C	56	52.3	1.40E-09	53	1.20	0.091	60	45.5	5.50E-11	43	1.44	0.105	68	48.8	4.00E-10	47	1.56	0.206	SSA1
YLL024C	57	55.9	1.40E-09	50	1.13	0.097	67	51.6	5.50E-11	48	1.42	0.115	74	54.1	3.00E-11	49	1.54	0.178	SSA2
YER151C	11	12.6	8.60E-07	7	0.45	0.262	22	17.4	5.60E-10	14	1.17	0.120	17	17.7	2.20E-10	12	1.11	0.131	UBP3
YGR232W	33	88.2	1.80E-09	26	1.22	0.080	45	95.6	2.10E-10	32	1.16	0.098	36	94.7	1.20E-10	29	0.90	0.080	NAS6
YFL007W	10	4.3	8.20E-07	8	0.89	0.121	24	10.5	3.20E-08	17	0.47	0.060	12	6.8	1.30E-07	8	0.20	0.036	BLM10

SILAC ratios: treated /untreated, normalized to Rpn11

Table S4. SILAC-based quantitative comparison of the composition of the 26*S* proteasome in wild-type and *ecm29Δ* cells in the absence of H_2O_2-induced stress.

	Name	Acc #	Num Unique	% Cov	Best Expect Val	# Pep used for SILAC Ratios	Average SILAC Ratios	STDEV
19S	RPN1	YHR027C	128	69.6	4.8E-10	87	0.971	0.056
	RPN2	YIL075C	127	62.4	1.6E-10	96	1.001	0.067
	RPN3	YER021W	59	62.3	3.2E-10	42	0.988	0.061
	RPN5	YDL147W	61	61.1	1.3E-08	46	0.992	0.063
	RPN6	YDL097C	52	73.0	4E-09	35	0.992	0.062
	RPN7	YPR108W	56	59.7	4.9E-09	44	1.011	0.078
	RPN8	YOR261C	44	76.0	8.9E-09	32	0.978	0.055
	RPN9	YDR427W	60	60.8	4.5E-09	41	1.010	0.071
	RPN10	YHR200W	31	54.9	1.1E-10	18	0.943	0.049
	RPN11	YFR004W	47	62.7	6.3E-09	41	1.000	0.058
	RPN12	YFR052W	35	74.1	6.5E-10	24	0.994	0.053
	RPN13	YLR421C	15	59.6	2.9E-09	9	0.950	0.045
	RPN15	YDR363W-	9	80.9	1.50E-07	7	1.040	0.101
	RPT1	YKL145W	65	67.0	6.9E-10	48	0.985	0.059
	RPT2	YDL007W	63	77.8	3E-09	42	0.969	0.061
	RPT3	YDR394W	61	86.4	1.9E-10	46	0.990	0.089
	RPT4	YOR259C	66	70.0	5.8E-09	50	0.996	0.061
	RPT5	YOR117W	71	79.7	1.6E-09	49	0.993	0.066
	RPT6	YGL048C	60	72.6	2.1E-08	44	0.983	0.058
20S	PRE1	YER012W	16	43.9	2.00E-08	6	0.896	0.044
	PRE2	YPR103W	25	56.4	6.2E-10	15	0.877	0.022
	PRE3	YJL001W	27	75.8	1.3E-09	12	0.883	0.019
	PRE4	YFR050C	28	69.5	1.2E-08	14	0.969	0.119
	PRE5	YMR314W	33	80.8	9.6E-10	17	0.880	0.029
	PRE6	YOL038W	28	74.0	5.8E-10	15	0.885	0.034
	PRE7	YBL041W	32	65.1	1.2E-10	19	0.926	0.072
	PRE8	YML092C	39	87.6	4E-09	14	0.910	0.059
	PRE9	YGR135W	43	87.2	1.1E-09	11	0.899	0.019
	PRE10	YOR362C	25	56.6	5.9E-08	12	0.926	0.075
	SCL1	YGL011C	43	89.7	3E-09	17	0.895	0.047
	PUP1	YOR157C	14	45.2	5.7E-08	6	0.888	0.032
	PUP2	YGR253C	36	87.3	5.7E-08	19	0.890	0.024
	PUP3	YER094C	19	55.1	2.9E-10	12	0.968	0.030

SILAC ratios: wt/ecm29Δ, normalized to Rpn11.

Table S5. SILAC-based quantitative comparison of the composition of the 26*S* proteasome in wild-type and *ecm29Δ* cells after H_2O_2-induced stress.

		Acc #	Num Unique	% Cov	Best Expect Val	# Pep used for SILAC Ratios	Average SILAC Ratios	STDEV
19S	RPN1	YHR027C	56	31.4	4.8E-10	24	0.963	0.075
	RPN2	YIL075C	53	24.6	4.1E-09	27	0.945	0.056
	RPN3	YER021W	30	37.1	4.9E-10	12	1.051	0.058
	RPN5	YDL147W	22	24.5	4.7E-09	10	0.992	0.099
	RPN6	YDL097C	29	33.6	2.2E-09	24	0.988	0.061
	RPN7	YPR108W	28	31.5	8.2E-09	15	0.975	0.084
	RPN8	YOR261C	23	49.7	1.1E-08	6	1.055	0.050
	RPN9	YDR427W	29	32.1	5.9E-10	14	0.992	0.063
	RPN10	YHR200W	13	29.5	2E-09	9	1.020	0.070
	RPN11	YFR004W	12	18.6	9.1E-09	8	1.000	0.044
	RPN12	YFR052W	23	40.9	2.4E-09	11	0.989	0.077
	RPN13	YLR421C	7	32.7	2.2E-09	4	0.925	0.067
	RPN15	YDR363W	4	41.6	1.5E-08	3	1.065	0.038
	RPT1	YKL145W	44	51.8	1.2E-08	19	0.937	0.038
	RPT2	YDL007W	42	52.6	7.7E-10	18	0.960	0.079
	RPT3	YDR394W	32	34.3	3.6E-09	14	0.967	0.046
	RPT4	YOR259C	34	39.4	2.1E-09	19	0.868	0.068
	RPT5	YOR117W	47	60.1	2.5E-09	21	0.945	0.081
	RPT6	YGL048C	35	46.2	4.9E-09	20	0.974	0.079
20S	PRE1	YER012W	5	16.7	0.00035	3	0.558	0.058
	PRE2	YPR103W	10	17.8	9.1E-07	5	0.501	0.035
	PRE3	YJL001W	2	12.6	2.80E-08	1	0.534	--
	PRE4	YFR050C	8	36.1	7.7E-08	4	0.371	0.119
	PRE5	YMR314W	12	43.2	8.1E-10	6	0.409	0.091
	PRE6	YOL038W	10	41.7	3.1E-08	5	0.517	0.021
	PRE7	YBL041W	7	17.8	0.000002	5	0.503	0.015
	PRE8	YML092C	9	28	2.6E-08	7	0.471	0.109
	PRE9	YGR135W	10	26.4	7.6E-10	4	0.525	0.028
	PRE10	YOR362C	9	22.9	2.1E-07	5	0.497	0.037
	SCL1	YGL011C	22	48	5.6E-08	5	0.333	0.094
	PUP1	YOR157C	2	4.6	3.1E-06	2	0.498	0.020
	PUP2	YGR253C	9	25.8	3.9E-08	5	0.449	0.114
	PUP3	YER094C	7	32.2	2.1E-10	4	0.534	0.044

SILAC ratios: wt/ecm29Δ, normalized to Rpn11.

Table S6. Yeast strains generated for this study.

Name	**Relevant genotype**
LH#001	*a bar1 pep4::URA3 RPN11-TAP::TRP1*
LH#002	*a bar1 pep4::URA3 RPN11-TAP::KAN arg4::hyg lys2::Zeo*
LH#003	*a bar1 pep4::URA3 RPN11-TAP::TRP1 ecm29::KAN*
LH#004	*a bar1 pep4::URA3 RPN11-TAP::TRP1 ECM29-HA:KAN*
LH#005	*a bar1 pep4::URA3 RPN11-TAP::TRP1 yap1::KAN*
LH#006	*a bar1 pep4::URA3 Ecm29-TAP::KAN arg4::hyg lys2::Zeo*
LH#007	*a bar1 pep4::URA3 RPN1-HBH::TRP1*
LH#008	*a bar1 pep4::URA3 RPN10-HBH::TRP1*
LH#009	*a bar1 pep4::URA3 RPT5-TAP::TRP1 arg4::hyg lys2::Zeo*
LH#010	*a bar1 pep4::URA3 Pre10-TAP::TRP1 arg4::hyg lys2::Zeo*