

**Supplementary File for Accompanying Manuscript titled:**

Disassembly of Lys11- and mixed-linkage polyubiquitin conjugates provide insights into function of proteasomal deubiquitinases Rpn11 and Ubp6

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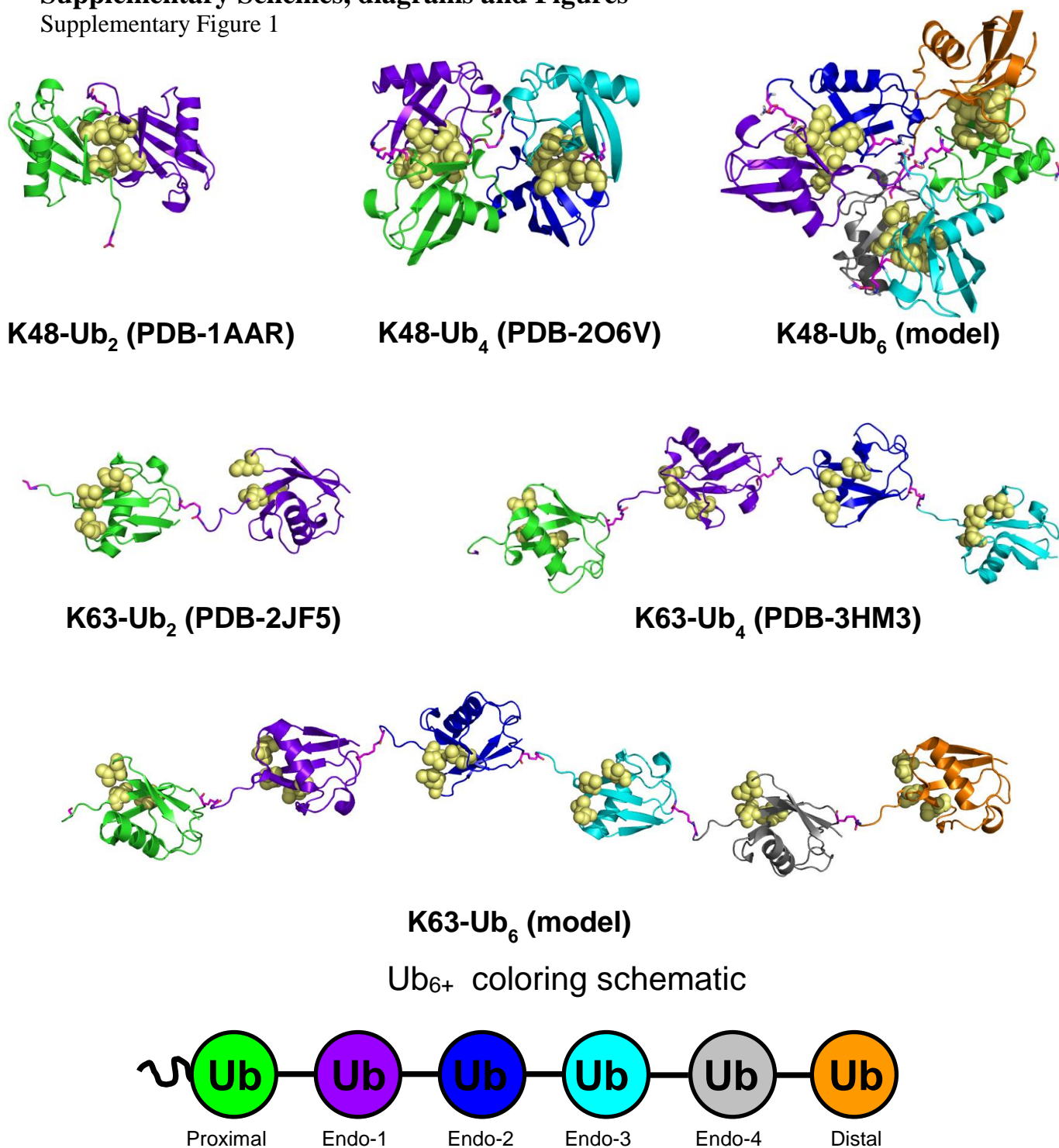
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This supplementary file contains five Figures S1-S5 (pages 2-7) and a supplementary Table S1 (page 8).

## Supplementary Schemes, diagrams and Figures

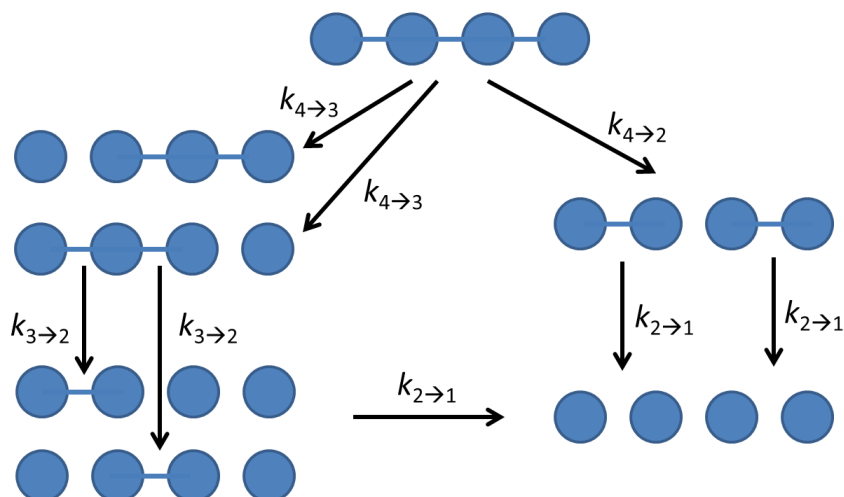
### Supplementary Figure 1



**Figure S1 – Structures of high molecular weight polyUb chains.**

Lys48 polyUb chains adopt an extremely compact conformation in dimeric, tetrameric, and hexameric forms. The L8, I44, V70 hydrophobic patch (in yellow) facilitates their compact nature. All forms of Lys63 polyUb retain an extended conformation with no interdomain contacts of the hydrophobic patch.

## Supplementary Figure 2



**Figure S2:** A kinetic scheme of disassembly of Ub<sub>4</sub> and subsequent products (above) can be described by equation 2 in the main text. In order to solve equation 2 it can be written in the matrix form as:

$$\frac{d}{dt} \begin{bmatrix} [Ub_4] \\ [Ub_3] \\ [Ub_2] \\ [Ub] \end{bmatrix} = \begin{bmatrix} -2k_{4 \rightarrow 3} - k_{4 \rightarrow 2} & 0 & 0 & 0 \\ 2k_{4 \rightarrow 3} & -2k_{3 \rightarrow 2} & 0 & 0 \\ 2k_{4 \rightarrow 2} & 2k_{3 \rightarrow 2} & -k_{2 \rightarrow 1} & 0 \\ 2k_{4 \rightarrow 3} & 2k_{3 \rightarrow 2} & 2k_{2 \rightarrow 1} & 0 \end{bmatrix} \begin{bmatrix} [Ub_4] \\ [Ub_3] \\ [Ub_2] \\ [Ub] \end{bmatrix}, \quad (S1)$$

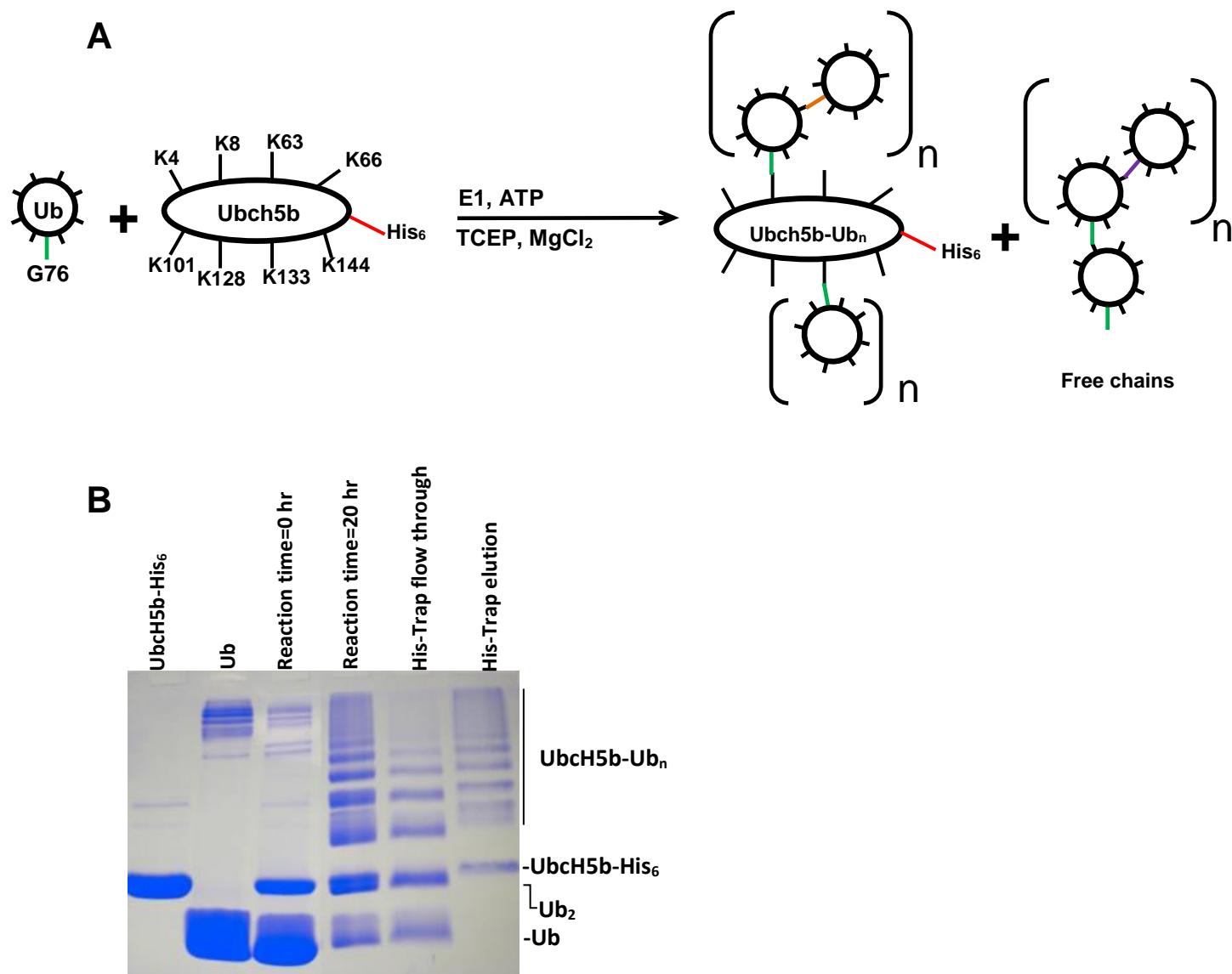
and the general solution is written as:

$$\begin{bmatrix} [Ub_4]_t \\ [Ub_3]_t \\ [Ub_2]_t \\ [Ub]_t \end{bmatrix} = \exp \left( \begin{bmatrix} -2k_{4 \rightarrow 3} - k_{4 \rightarrow 2} & 0 & 0 & 0 \\ 2k_{4 \rightarrow 3} & -2k_{3 \rightarrow 2} & 0 & 0 \\ 2k_{4 \rightarrow 2} & 2k_{3 \rightarrow 2} & -k_{2 \rightarrow 1} & 0 \\ 2k_{4 \rightarrow 3} & 2k_{3 \rightarrow 2} & 2k_{2 \rightarrow 1} & 0 \end{bmatrix} t \right) \begin{bmatrix} [Ub_4]_{t=0} \\ [Ub_3]_{t=0} \\ [Ub_2]_{t=0} \\ [Ub]_{t=0} \end{bmatrix} \quad (S2)$$

where  $t$  is time, and  $t=0$  corresponds to the initial time point. These equations account for the fact that Ub<sub>4</sub> can be cleaved into Ub<sub>3</sub>+Ub in two possible ways, the same applies to disassembly of Ub<sub>3</sub> into Ub<sub>2</sub>+Ub, as schematically illustrated in this figure. The rate constants for cleavage of the individual isopeptide bonds in Ub<sub>4</sub>, Ub<sub>3</sub>, and Ub<sub>2</sub> chains in 1/min units are summarized in the following table:

DUB	Ub substrate	Panel in Figure 4	$k_{4 \rightarrow 3}$ (exo)	$k_{4 \rightarrow 2}$ (endo)	$k_{3 \rightarrow 2}$	$k_{2 \rightarrow 1}$
Ubp6	K48-Ub <sub>4</sub>	C	2.19	5.33	5.98	4.70
Ubp6	K63-Ub <sub>4</sub>	D	1.02	1.05	1.35	1.46
Lid-core	K48-Ub <sub>4</sub>	E	0.12	0.10	0.13	0.11
Lid-core	K63-Ub <sub>4</sub>	F	0.24	0.24	0.23	0.15
Rpn8-11	K48-Ub <sub>4</sub>	G	0.06	0.05	0.06	0.06
Rpn8-11	K63-Ub <sub>4</sub>	H	0.14	0.10	0.15	0.07

Supplementary Figure 3

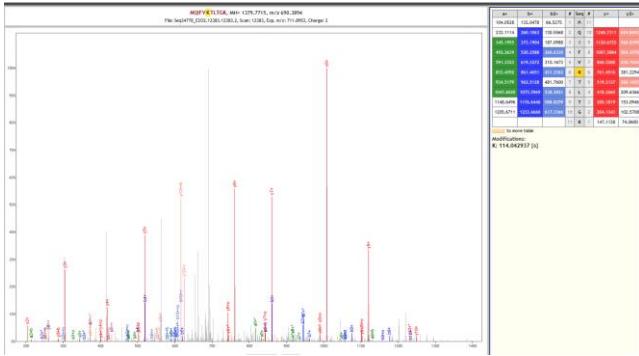


**Figure S3 – purification scheme of His<sub>6</sub> tagged Ubch5b-Ub<sub>n</sub>.**

(A) Schematic describing the synthesis of Ubch5b-Ub<sub>n</sub> with free polyUb chains as a secondary. (B) SDS-PAGE analysis shows Ubch5b efficiently converts a majority of monomeric Ub to either free polyUb chains or the desired autoubiquitinated products. The His<sub>6</sub> tag on Ubch5b allows for easy separation from the free polyUb chains as shown between stages of preparation.

Figure S4 – MS/MS reveals all Ub linkage types on UbcH5b-Ub<sub>n</sub>

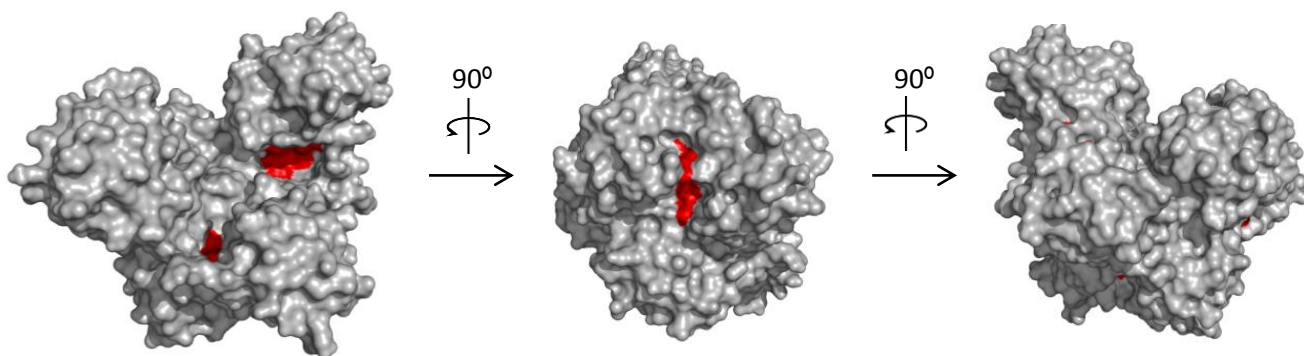
Lys6



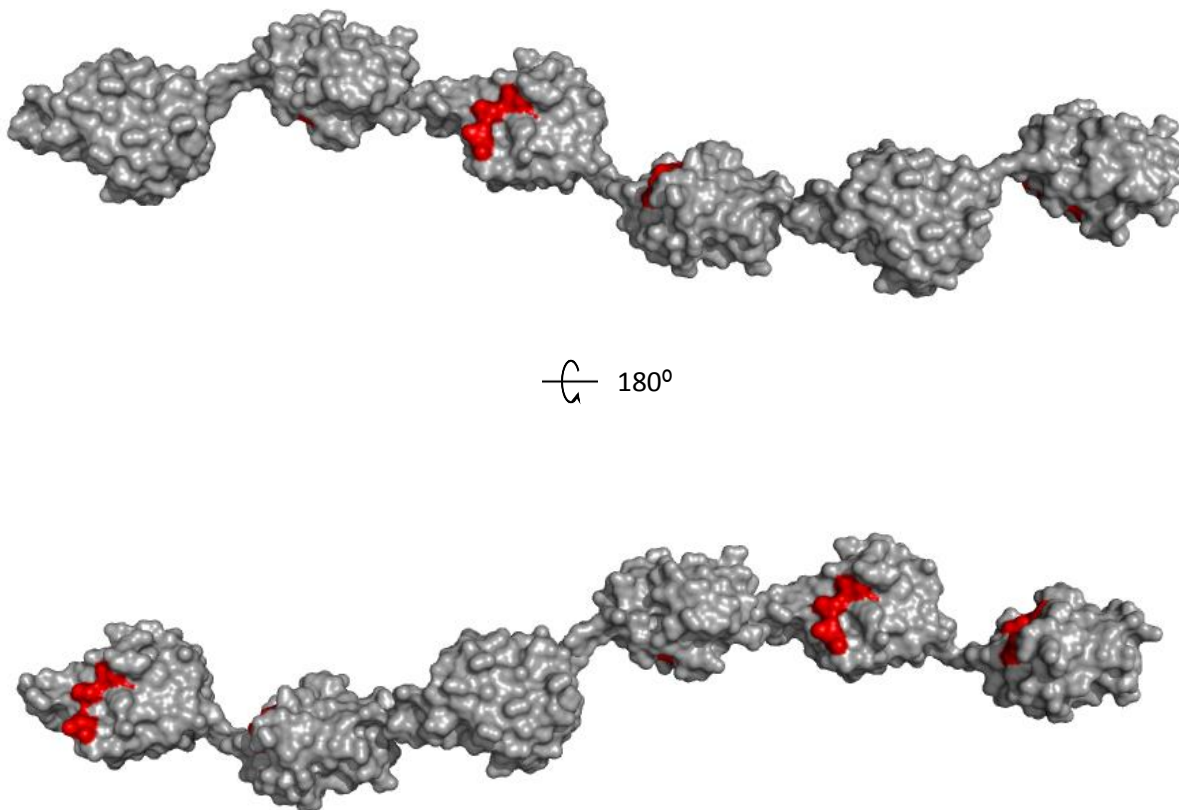


Supplementary Figure 5

A



B



**Figure S5 – Modeling of high molecular weight Ub<sub>6</sub> chains present alternate structural conformations. (A) L8, I44, V70 hydrophobic patch (red) is buried Lys48-Ub<sub>6</sub> (B) Opposite to Lys48, the hydrophobic patch residues are entirely solvent exposed in Lys63-Ub<sub>6</sub>.**

**Supplementary Table S1 – MS of Lid-core to show coverage of all subunits expected**

**Band #1**

group	sibling	seqid	uniprot_id_url	description gene	length	probability	percent_cc	num_uniq	num_pepti	percent_sf	organism	other_seqids
3 a	Q12377	RPN6_YE/	http://unipr	26S protease RPN6	434	1	61.5	51	110	22.4	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
2 a	Q12250	RPN5_YE/	http://unipr	26S protease RPN5	445	1	33	20	20	3.93	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
1 a	Q08723	RPN8_YE/	http://unipr	26S protease RPN8	338	1	18.3	5	6	1.19	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
13 a	P43588	RPN11_YE/	http://unipr	26S protease RPN11	306	0.9746	4.2	1	1	0.21	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
14 a	P60010	ACT_YEA/	http://unipr	Actin ACT1	375	0.9746	2.7	1	1	0.21	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	

**Band #2**

group	sibling	seqid	uniprot_id_url	description gene	length	probability	percent_cc	num_uniq	num_pepti	percent_sf	organism	other_seqids
2 a	Q12250	RPN5_YE/	http://unipr	26S protease RPN5	445	1	60.9	52	180	40.03	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
3 a	Q12377	RPN6_YE/	http://unipr	26S protease RPN6	434	1	32.5	16	18	4.08	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
1 a	Q08723	RPN8_YE/	http://unipr	26S protease RPN8	338	1	12.7	3	3	0.69	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
12 a	P43588	RPN11_YE/	http://unipr	26S protease RPN11	306	0.9664	4.2	1	1	0.23	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	

**Band #3**

group	sibling	seqid	uniprot_id_url	description gene	length	probability	percent_cc	num_uniq	num_pepti	percent_sf	organism	other_seqids
1 a	Q04062	RPN9_YE/	http://unipr	26S protease RPN9	393	1	50.6	53	102	14.69	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
2 a	Q08723	RPN8_YE/	http://unipr	26S protease RPN8	338	1	71.6	65	151	21.87	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
3 a	Q12250	RPN5_YE/	http://unipr	26S protease RPN5	445	1	25.2	14	14	1.92	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
4 a	Q12377	RPN6_YE/	http://unipr	26S protease RPN6	434	1	36.2	22	22	3.85	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	

**Band #4**

group	sibling	seqid	uniprot_id_url	description gene	length	probability	percent_cc	num_uniq	num_pepti	percent_sf	organism	other_seqids
1 a	P43588	RPN11_YE/	http://unipr	26S protease RPN11	306	1	67.3	55	103	14.16	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
2 a	Q04062	RPN9_YE/	http://unipr	26S protease RPN9	393	1	16	7	7	0.85	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
3 a	Q08723	RPN8_YE/	http://unipr	26S protease RPN8	338	1	68.9	38	40	5.42	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
4 a	Q12250	RPN5_YE/	http://unipr	26S protease RPN5	445	1	27.6	17	17	2.2	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	
5 a	Q12377	RPN6_YE/	http://unipr	26S protease RPN6	434	1	21	9	9	1.13	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	

