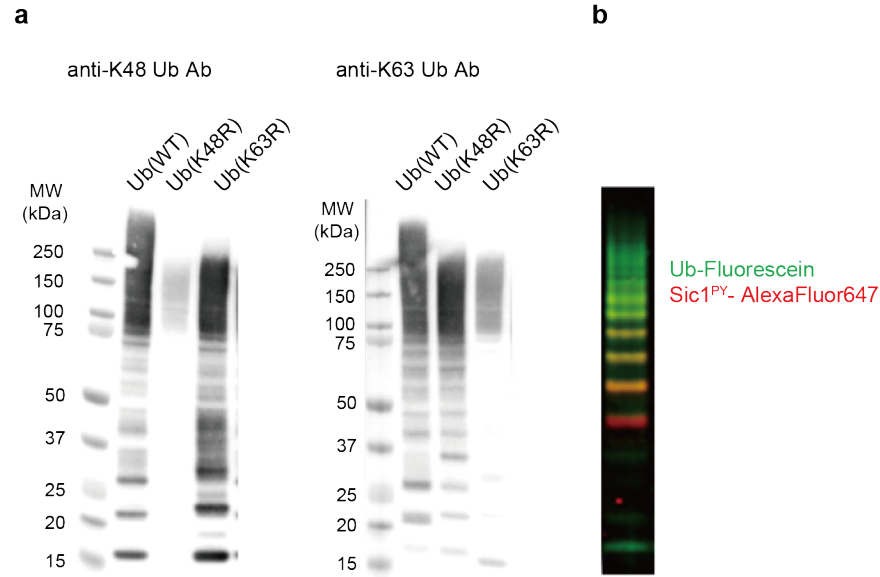
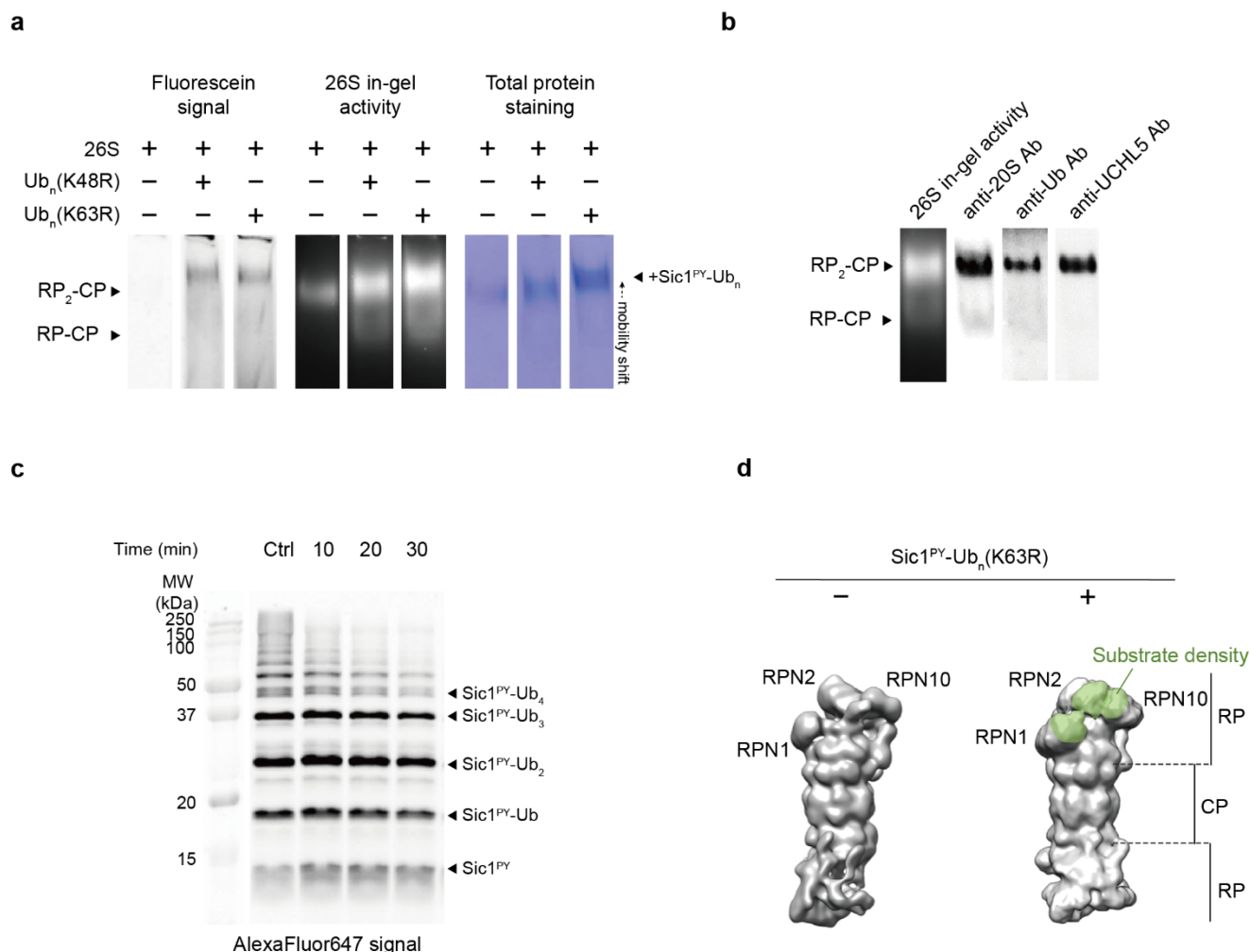
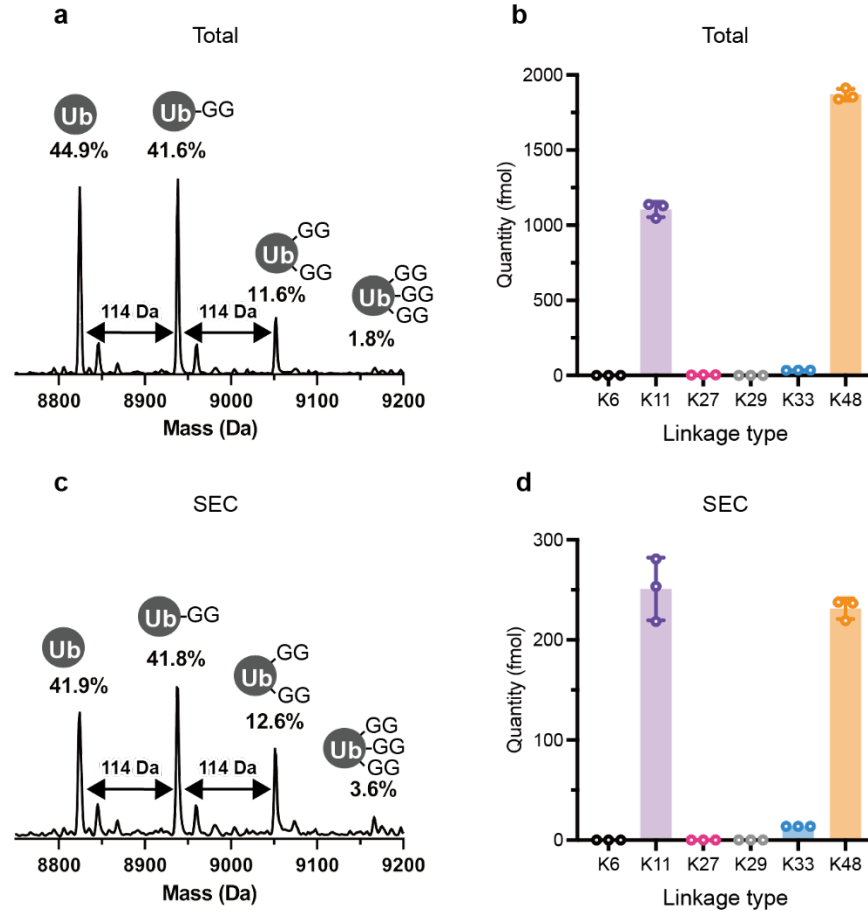




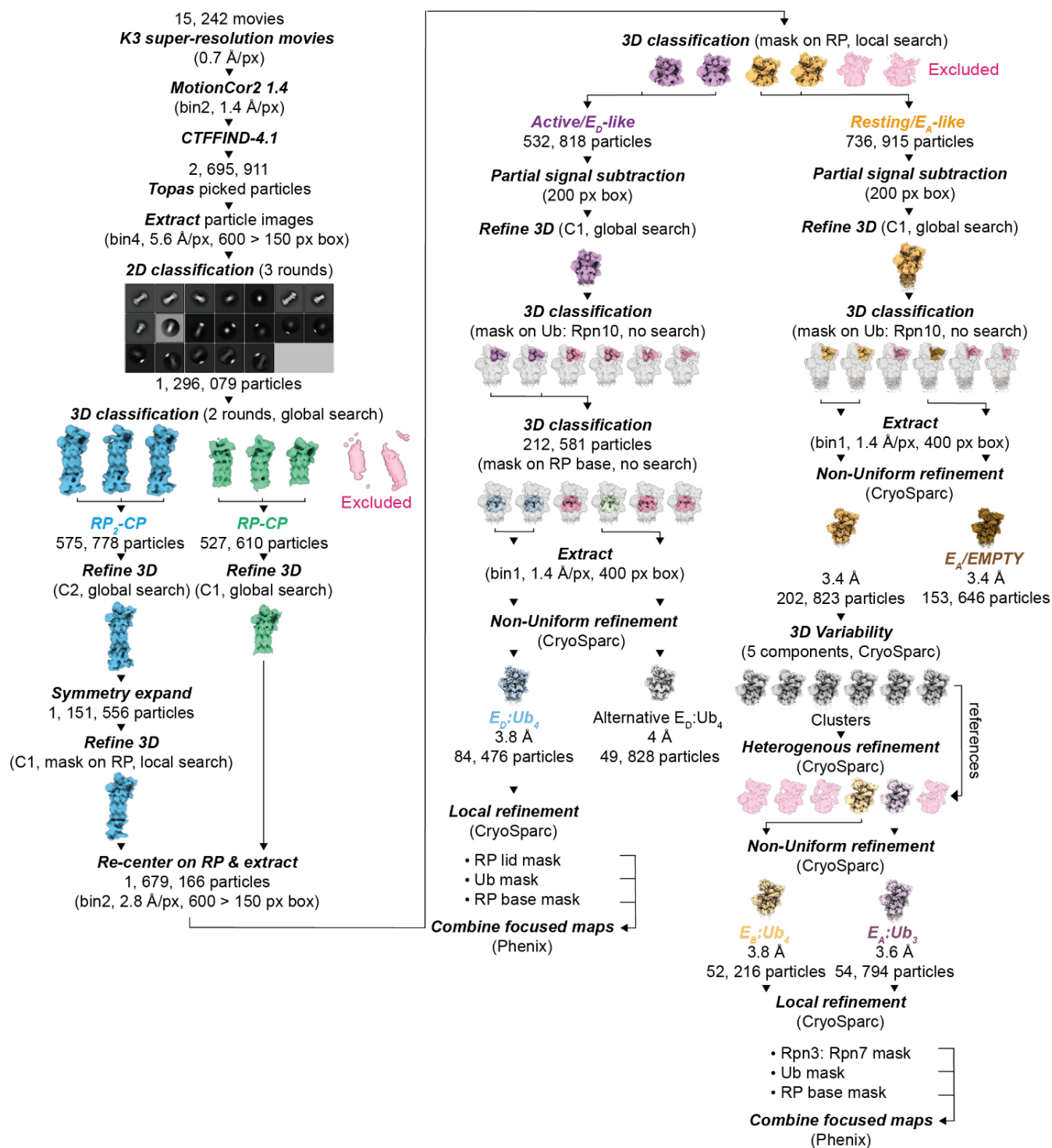
Supplementary Fig. 1. Characteristics of the ubiquitinated Sic1^{PY} substrate. **a**, Use of Ub(K63R) prevented the formation of unwanted K63-linked Ub conjugates. The opposite was true when Ub(K48R) was used in the ubiquitination assay. Anti-K48-linked and anti-K63-linked Ub antibodies were used to probe the linkage types, which are indicated above. **b**, Two-color fluorescence probing of the ubiquitination product using fluorescein-labeled Ub (green) and AlexaFluor647-labeled Sic^{PY} (red). The SDS-PAGE was imaged in two separate channels at the same time to image Ub-Fluorescein and Sic^{PY}-AlexaFluor647-labeled.



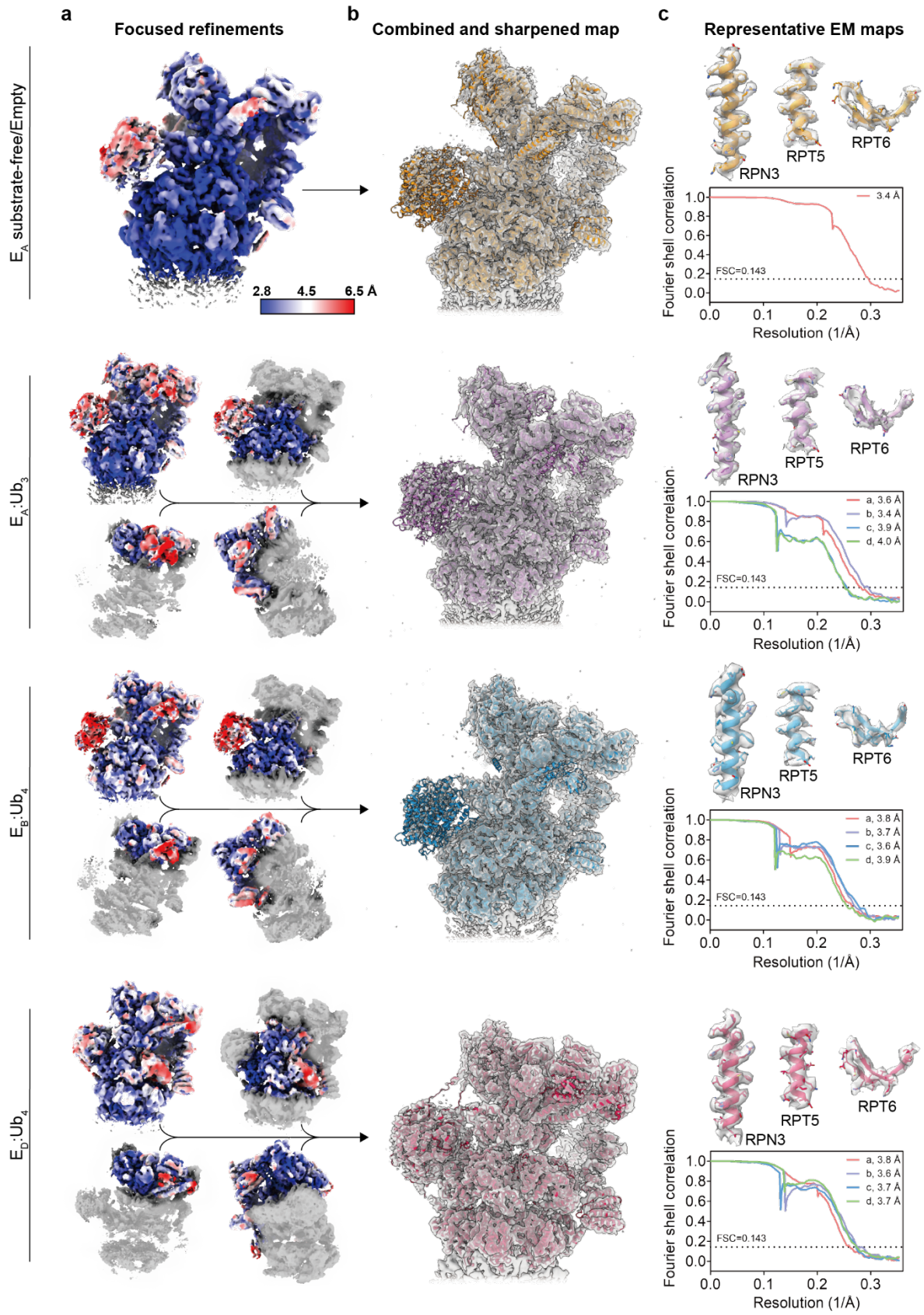
Supplementary Fig. 2. Validation of the functional 26S complex formation. **a**, Native gel electrophoresis detected by fluorescein signals of Ub, in-gel proteolytic activity, and Coomassie Blue staining showed distinct changes in the mobility of the 26S proteasome upon the addition of Sic1^{PY}-Ub_n(K63R) or Sic1^{PY}-Ub_n(K48R). **b**, Immunodetection of the native gel-separated samples confirmed the formation of the tertiary complex of 26S-Sic1^{PY}-Ub_n(K63R)-UCHL5(C88A). **c**, Kinetics of Sic1^{PY}-Ub_n(K63R) degradation by 26S proteasome monitored by the AlexaFluor647 signal of Sic1^{PY}. **d**, EM maps of apo and substrate-bound 26S proteasome derived from NSEM. The additional EM map density in the substrate-bound 26S was highlighted in green.



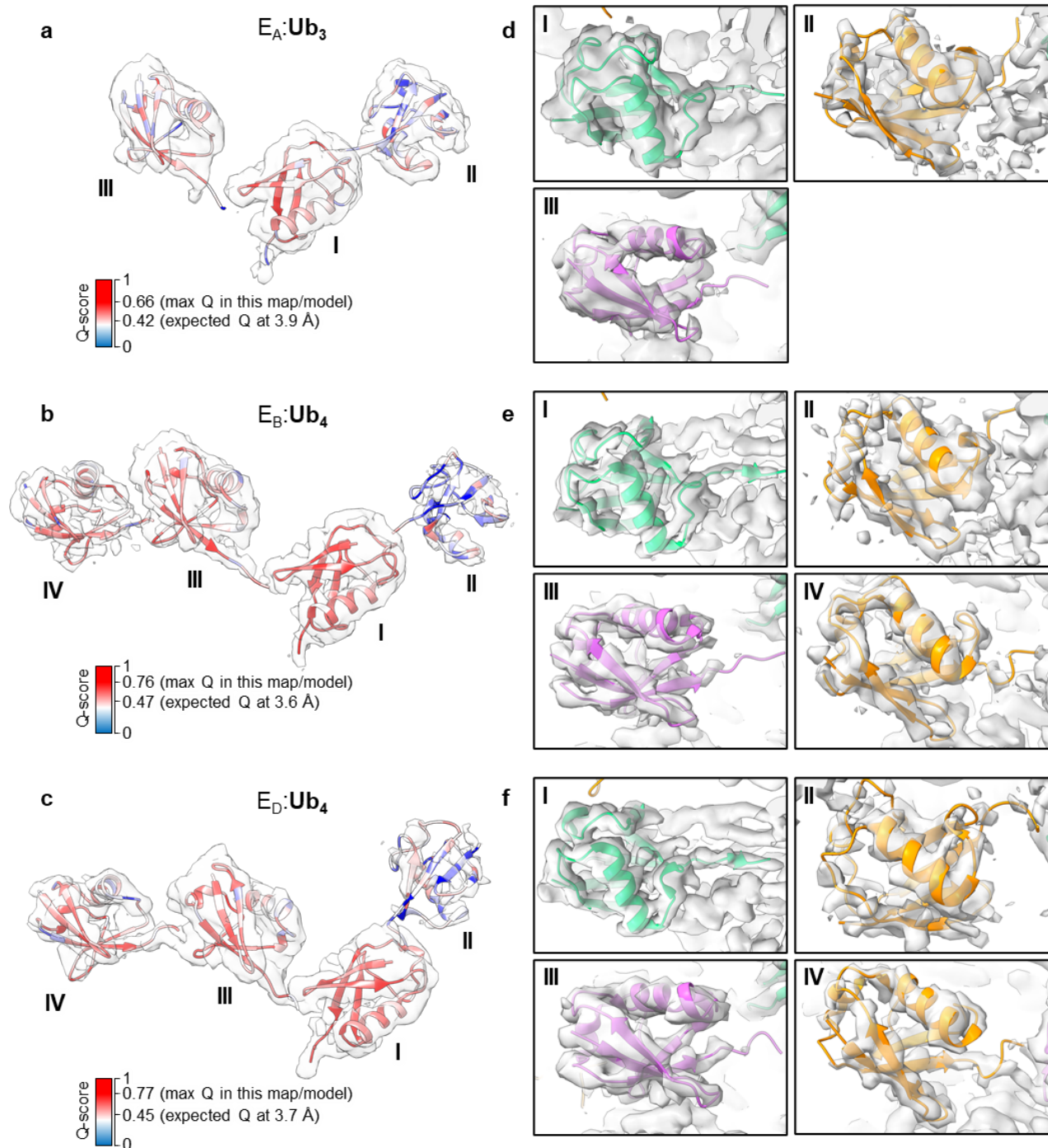
Supplementary Fig. 3. MS-based quantitative analyses of Ub branching and linkage type distributions. The amount of branching was quantified by intact MS analysis using Lb^{pro*}-digested Sic1^{PY}-Ub_n. Comparison of the intact mass spectra of total (**a**) and SEC-enriched (**c**) Sic1^{PY}-Ub_n showed an increased amount doubly and triply branched Ub after the SEC enrichment. The quantities of the individual peaks were estimated by integrating the areas under the curve. Ub-AQUA analyses of total (**b**) and SEC-enriched (**d**) Sic1^{PY}-Ub_n. A significant increase of K11-linked Ub was found after SEC enrichment. The experiments were carried in triplicate (n = 3) with the original data points shown in open circles and the error bars indicating the standard deviations. Source data are provided as a Source Data file.



Supplementary Fig. 4. Cryo-EM data processing pipeline.

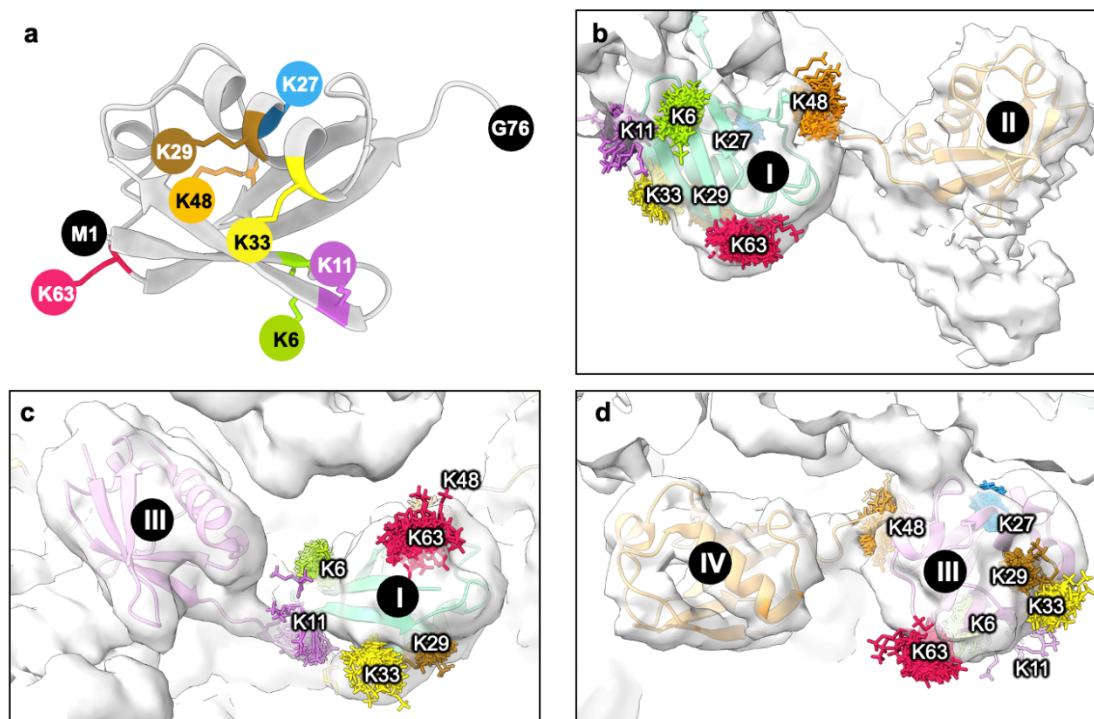


Supplementary Fig. 5. Quality assessment of cryo-EM maps. **a**, Local resolution estimation of the individual cryo-EM maps of different functional states as indicated on the left. **b**, The composite cryo-EM maps were fused and sharpened (except the E_A substrate-free state) using Phenix Combine Focused Maps and Map Sharpening tools. **c**, Representative cryo-EM maps superimposed with the structural models with the Fourier Shell Correlation curves shown below.

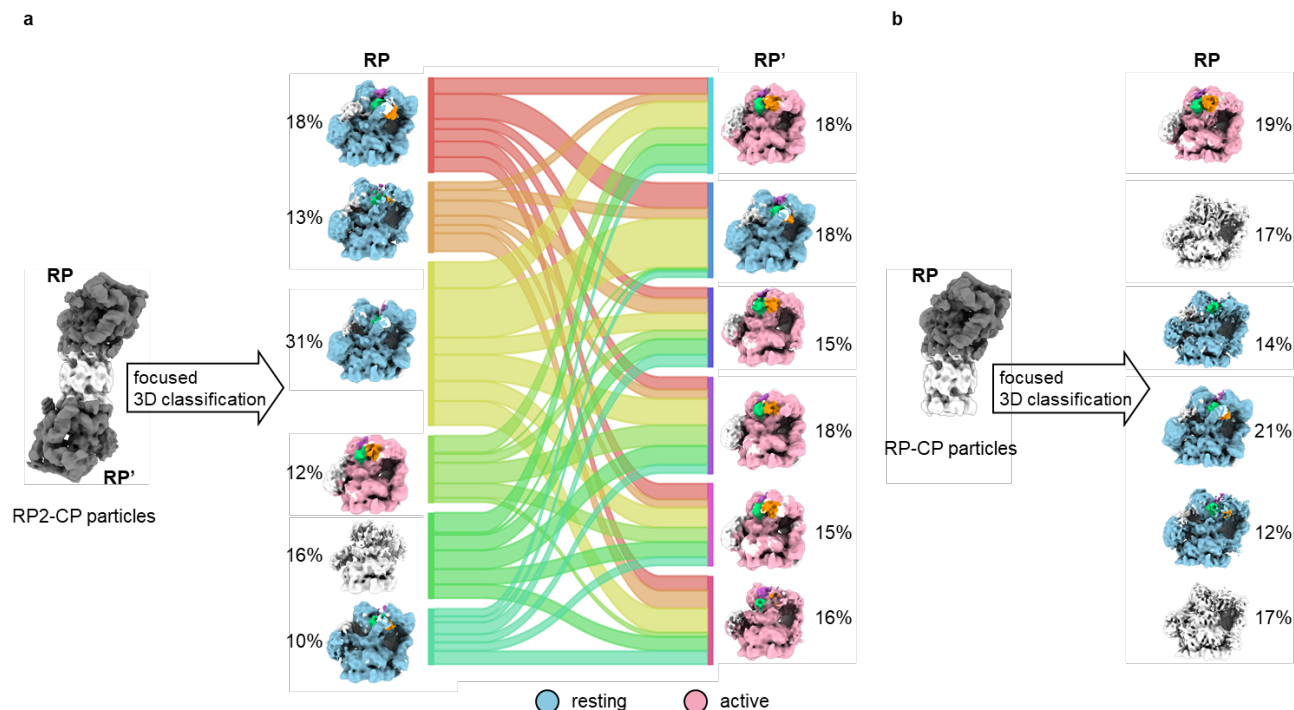


Supplementary Fig. 6. Structural quality assessments of the K11/K48-branched Ub chain bound to the proteasome. **a**, The atomic model of the tri-Ub in the $E_A:Ub_3$ state is shown in a cartoon representation, colored from blue to white to red according to the Q-score, which reports on the quality of the structural model in relation to the corresponding cryo-EM map, shown in a transparent surface. The expected Q-score at the resolution of the cryo-EM map and the maximum Q-score of the tri-Ub model are indicated in the scale bar on the lower left corner. Red and blue colors indicate regions that are better and

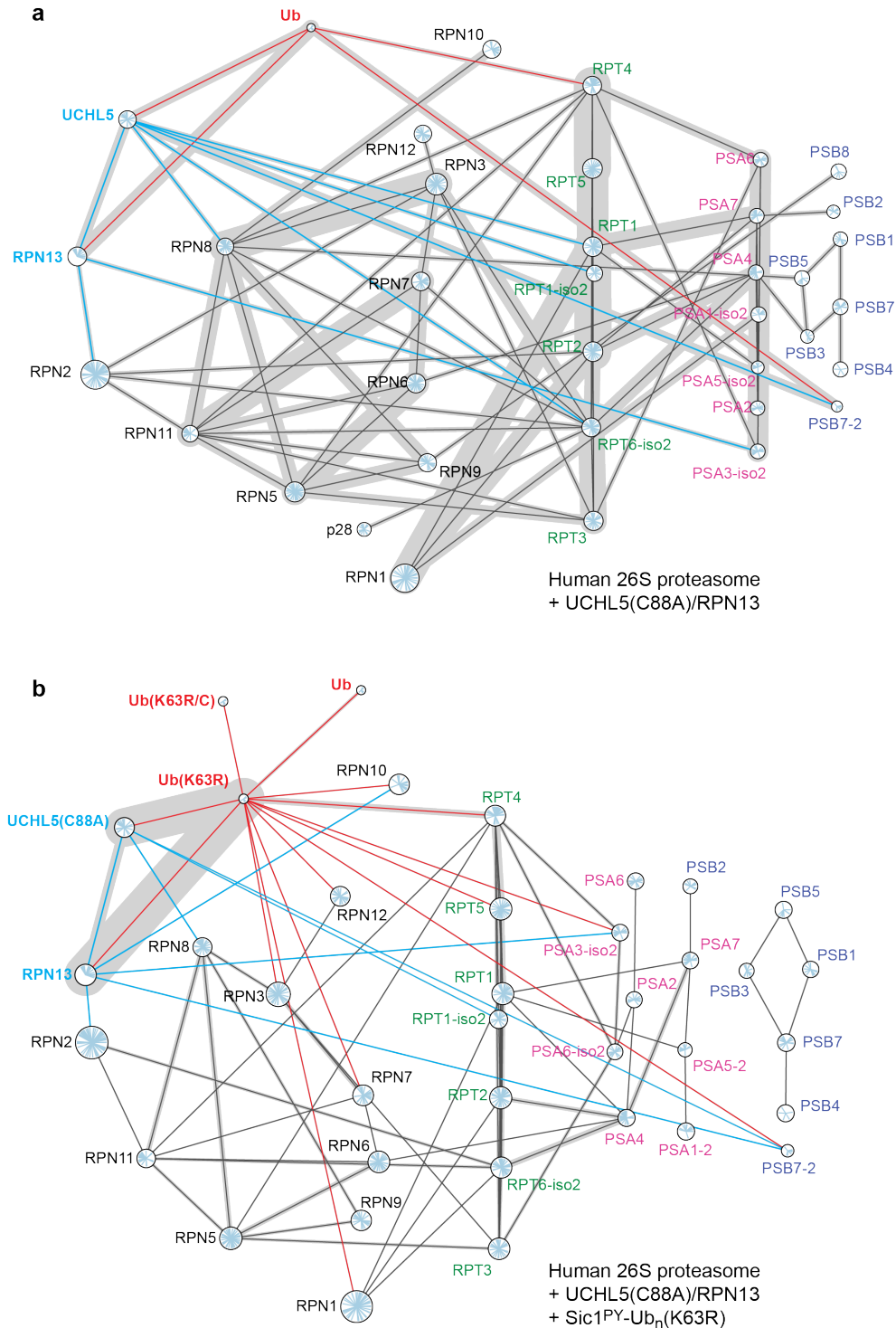
worse than the expected structural quality at this resolution, respectively. The same Q-score analyses were applied to the tetra-Ub in the E_B:Ub₄ (**b**) and E_D:Ub₄ (**c**) states. In panels **a-c**, the contour levels were set to 5 for all the cryo-EM maps for better comparisons. **d-f**, Superposition of the sharpened cryo-EM maps and the corresponding atomic structures of the individual Ub. The signature thumb-like α -helix of individual Ubs was clearly visible in all the maps.



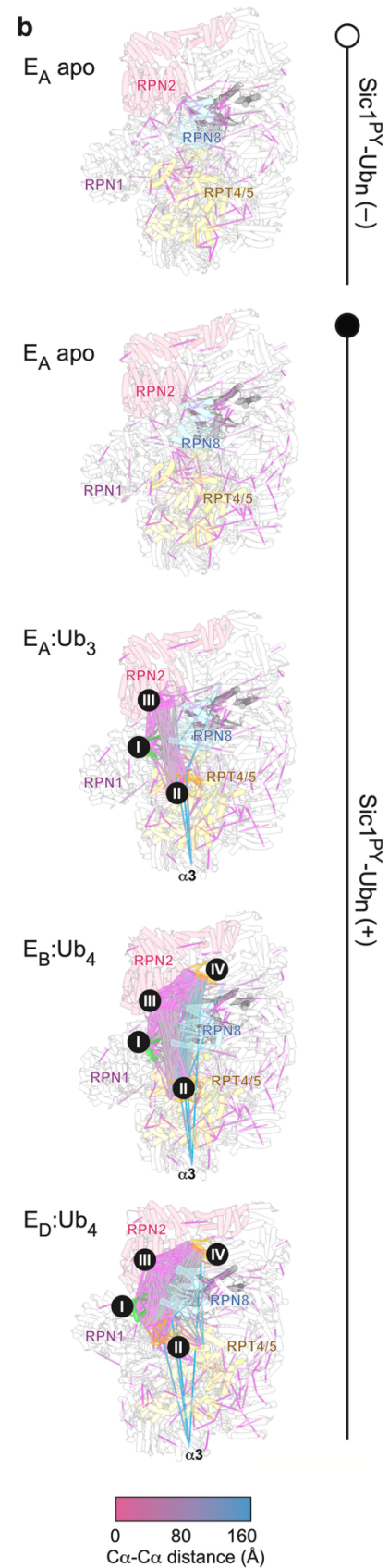
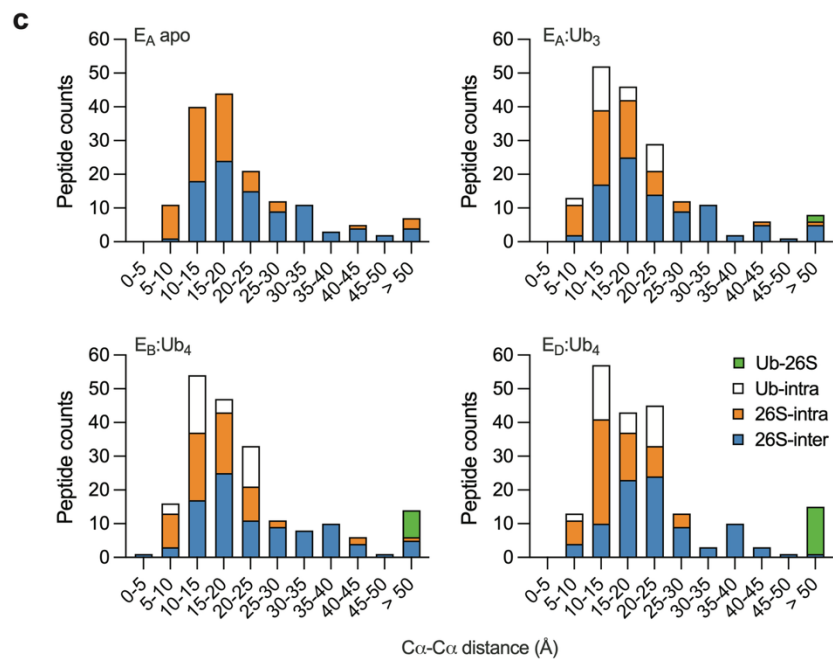
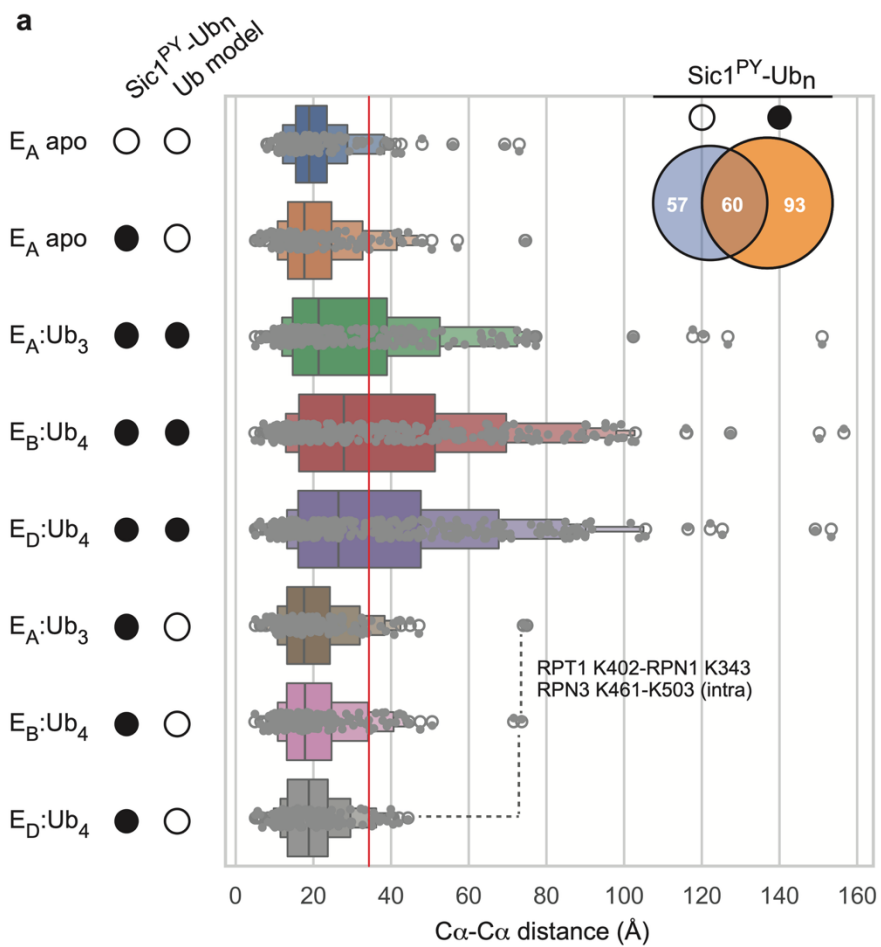
Supplementary Fig. 7. Ensemble analysis of potential isopeptide bond linkages of observed Ub EM map. **a**, Cartoon representation of Ub with the individual lysine positions indicated by different colors. **b**, Ensemble conformations of the individual lysine side-chains of Ub_I by superpositioning the NMR ensemble structure (PDB entry: 2K39) on the cryo-EM structure of Ub_I with the individual lysine side-chains colored and labeled according to (a). The same structural alignment was applied to Ub_I (c) and Ub_{III} (d). In all cases, the only possible isopeptide linkage corresponds to the one that was reported in this study, which has clear EM density connecting the C-terminus of the other Ub to the isopeptide-linked lysine.



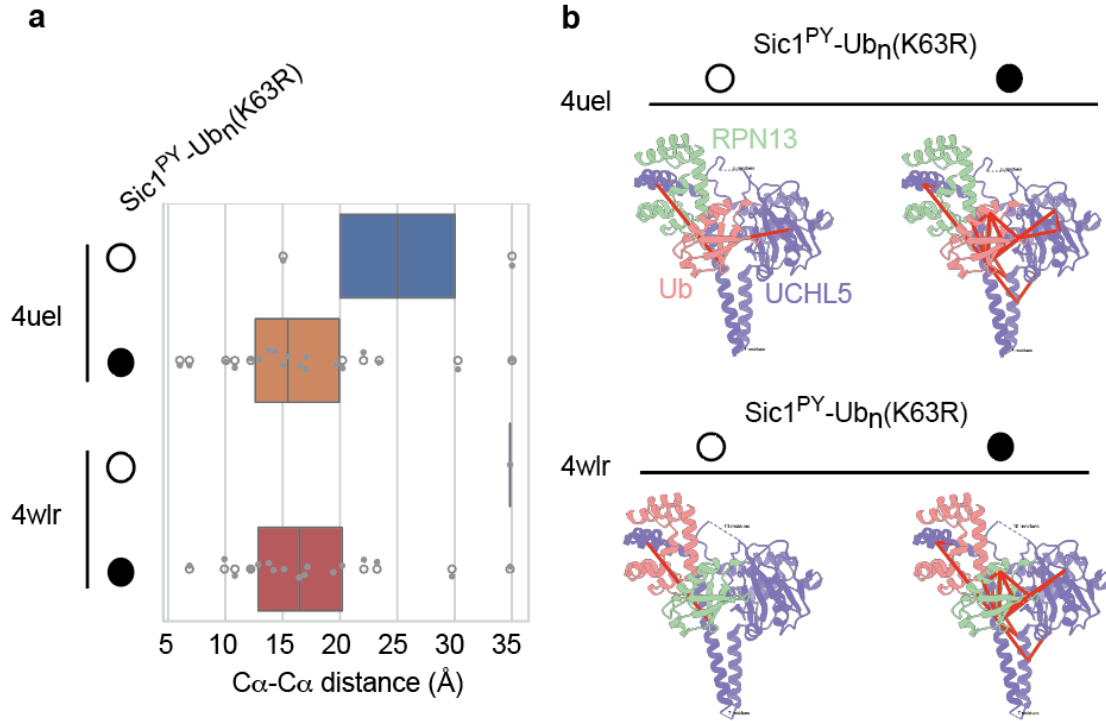
Supplementary Fig 8. Classifications of the functional states of the 19S RP in the doubly capped (RP₂-CP) and singly capped (RP-CP) 26 proteasome. a, Sankey diagram of the functional states of the 19S RPs on two sides of the RP₂-CP (RP and RP'). The 19S RPs in the resting, active and unclassified states are colored cyan, pink and white, respectively. The percentages of the individual 3D classes are indicated next to the 3D maps of the 19S RPs. A small fraction of the RP₂-CP has both RPs in active state. **b,** Analogous 3D classification analysis for the RP-CP complex.



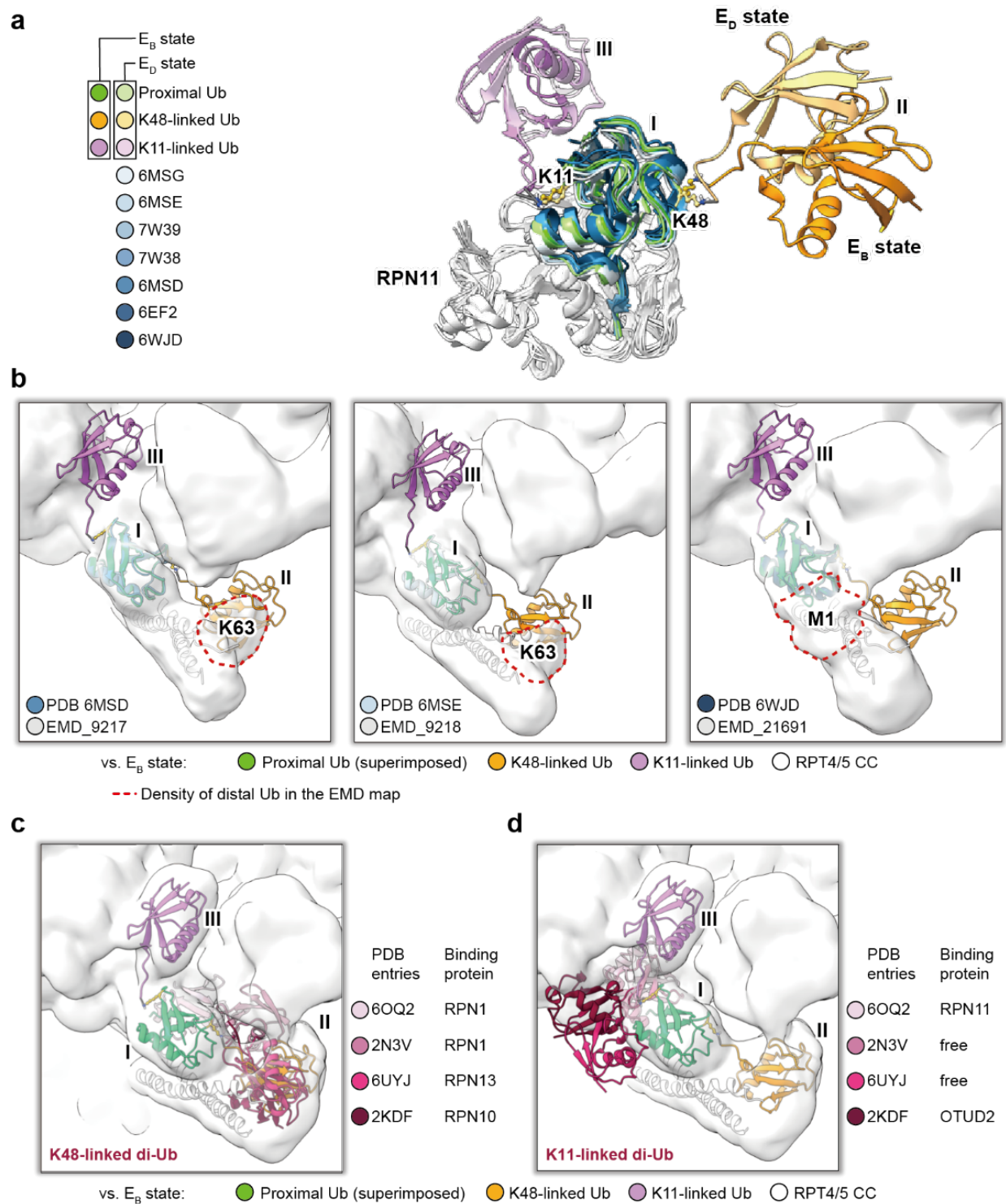
Supplementary Fig. 9. Graphical representation of XL-MS network of 26S proteasome. The XL-MS data depict the XL network in the human 26S proteasome without (a) and with (b) the addition of Sic1^{PY}-Ub_n(K63R) spiked with 10% cysteine mutant Ub(K63R) for fluorescent labeling. XLs associated with Ub variants are red, those with UCHL5 and RPN13 variants are blue. Thickness of XLs corresponds to the number observed in datasets. Generated by xiNET (<https://crosslinkviewer.org/>) with color modifications for subunit labels and XL types.



Supplementary Fig. 10. Structural mapping of XL-MS data onto the cryo-EM models of 26S proteasome. **a**, Distance distributions of observed XL lysine pairs (derived from a single sample, $n = 1$) in different functional states – apo E_A (PDB entry: 8JRI), $E_A:Ub_3$ (PDB entry: 8JRT), $E_B:Ub_4$ (PDB entry: 8JTI) and $E_D:Ub_4$ (PDB entry: 8K0G) – without and with $Sic1^{PY}-Ub_n$ indicated by open and filled circles, respectively, next to their functional states. The XL distances were mapped by XMAS as a plug-in module within ChimeraX. The distances are defined as the $C\alpha-C\alpha$ distances between the lysines that are linked by DSSO. To highlight the long distances attributed to XL with Ubs, additional distance distributions were calculated for the $E_A:Ub_3$, $E_B:Ub_4$, and $E_D:Ub_4$ states, excluding the coordinates of the Ubs (bottom three rows), as indicated by open circles in the second column next to their functional state annotations. The generally accepted XL range of 35 Å is indicated by a red vertical line. Two long XL distances beyond 75 Å were observed in the $E_A:Ub_3$ and $E_B:Ub_4$ states, corresponding to an inter-chain XL of RPT1 K402-RPN1 K343 and an intra-chain XL of RPN3 K461-K503. These long XL distances were reduced to < 45 Å in the $E_D:Ub_4$ state, indicating that these XLs captured the conformation corresponding to the $E_D:Ub_4$ state distinct to the $E_A:Ub_3$ and $E_B:Ub_4$ states. Inset: Venn diagram of the overlap of the observed XL between the E_A states without (blue) and with (orange) $Sic1^{PY}-Ub_n$. Only 60 XLs were commonly observed in these two conditions, dominated by the shift of XLs corresponding to the supplemented UCHL5(C88A), RPN13, and Ub(K63R) in addition to $Sic1^{PY}-Ub_n$ in the reconstituted 26S proteasome sample. **b**, Structural mapping of the observed XLs in the apo E_A ($\pm Sic1^{PY}-Ub_n$), $E_A:Ub_3$, $E_B:Ub_4$ and $E_D:Ub_4$ states rendered by XMAS using ChimeraX. The XL distances are color-ramped from fuchsia to blue, corresponding to 0 to 160 Å, as indicated by the scale bar on the bottom. The subunits of interests and Ub_I-Ub_{IV} are colored following the same scheme in Figure 1e (except RPN1 in white) and in highly transparent cartoon representations. **c**, Histogram of observed XL as a function of $C\alpha-C\alpha$ distance in Å. The inter- and intra-subunit XLs within the 26S proteasome are colored blue and orange, respectively. The intra-subunit XLs within individual Ubs are colored white. The inter Ub-26S XLs are colored green. The majority of the long XLs (> 50 Å) correspond to the inter Ub-26S XLs, which may be attributed to the assignments of the same 26S subunit linked to the multiple Ubs, evidenced by the only few XLs > 35 Å in $E_A:Ub_3$ where Ub_{IV} is absent in the structure. Source data are provided as a Source Data file.

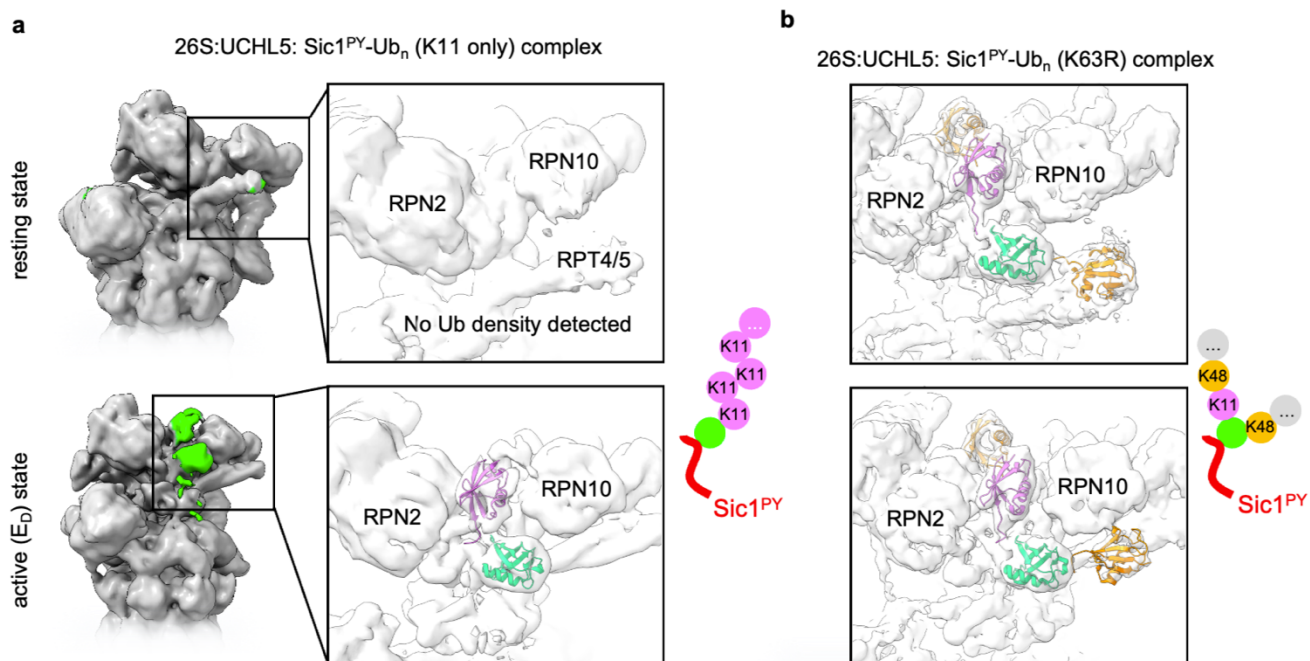


Supplementary Fig. 11. Structural mapping of XL-MS data onto the crystal structures of UCHL5-RPN13-Ub. **a**, Distance distributions of observed XL lysine pairs in the two reported crystal structures of UCHL5-RPN13-Ub (PDB entries: 4UEL and 4WLR) without and with Sic1^{PY}-Ub_n indicated by open and filled circles, respectively. The XL distances were mapped by XMAS within ChimeraX. **b**, Structural mapping of the observed XLs on the quaternary structures of UCHL5-RPN13-Ub. UCHL5, RPN13 (DEUBAD) and Ub are colored purple, green and salmon, respectively. The observed XLs are shown in solid red lines.

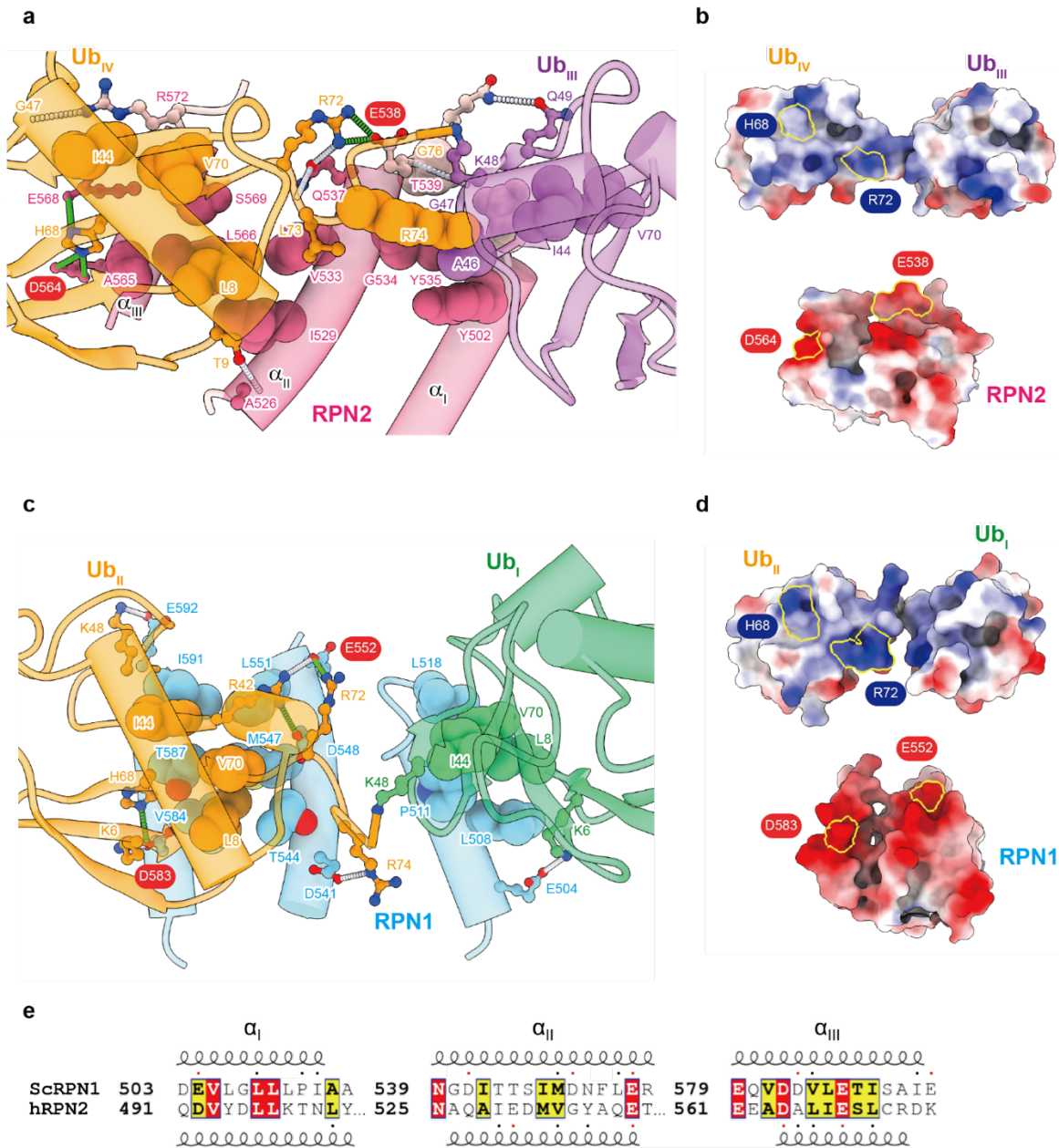


Supplementary Fig. 12. A comparison of the proteasome-bound K11/K48-branched Ub with the available structural data. **a**, Superposition of the structures of reported RPN11-bound Ub showed a highly conserved pose of the RPN11-bound proximal Ub. The individual structures are color coded as indicated on the left with their corresponding PDB ID. **b**, Superposition of reported EM maps of the Ub-bound RPT4/5 CC with partially resolved EM density of the proximal Ub and K48-linked Ub (the

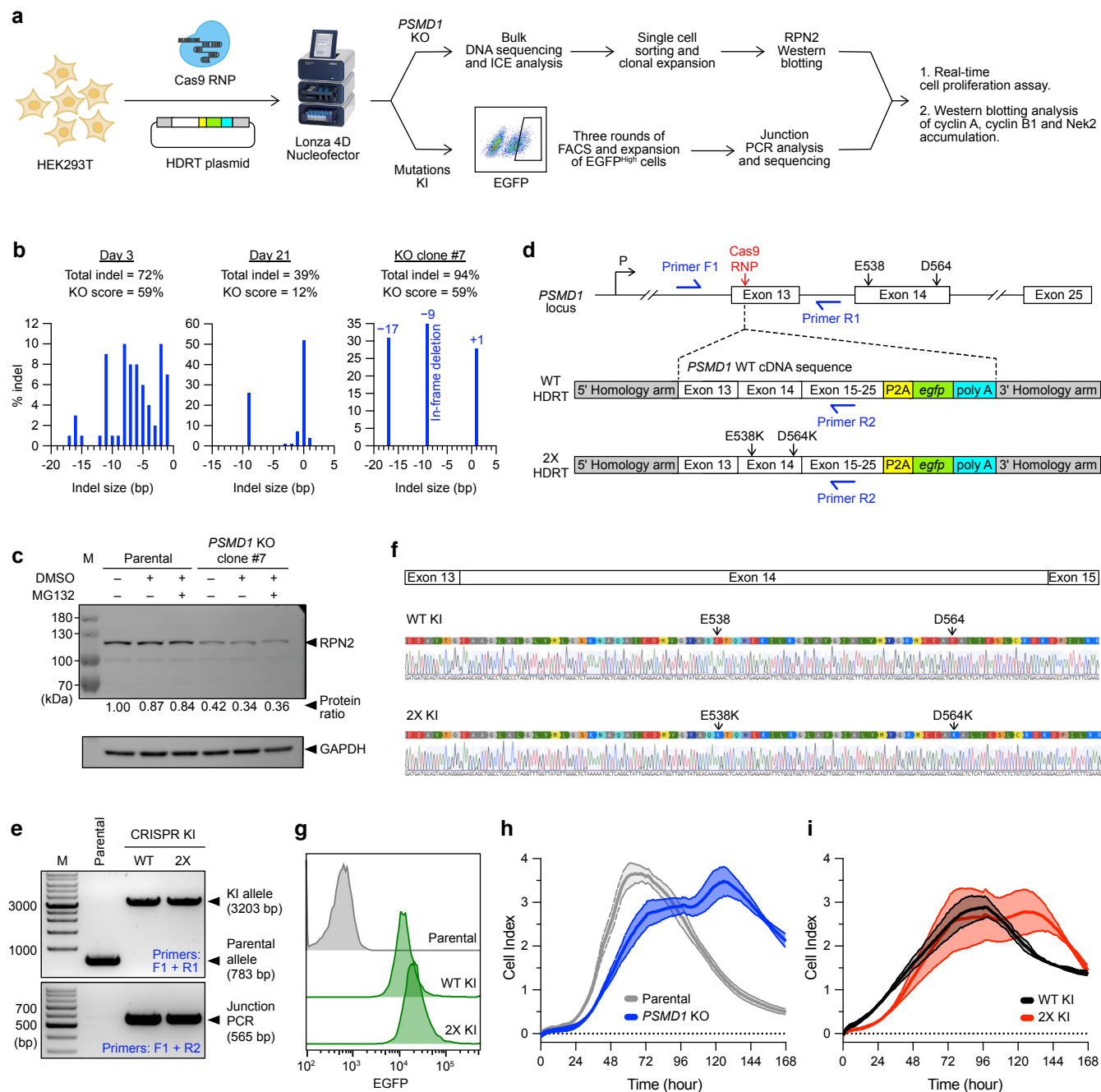
equivalent of Ub_{II} in this study). The PDB IDs and the corresponding EMDB IDs are indicated on the lower left corner of individual panels. The positions of the observed Ub in the earlier studies are outlined in dashed red lines, and they deviate significantly from the Ub_{II} in this study. **c**, Superposition of K48-linked di-Ub in complex with different Ub receptors with respect to Ub_I resolved in this study shows a broad conformation space sampled by the distal Ubs in the previously reported structures. Nevertheless, the distal Ubs broadly sample the same conformational space as that of Ub_{II} resolved in this study. **d**, Superposition of reported K11-linked di-Ub structures shows that the distal Ubs sample different conformations distinct from the K11-linked Ub_{III} resolved in this study.



Supplementary Fig. 13. Newly identified K11-linked ubiquitin binding groove is specifically tailored for K11-linked ubiquitin chains. The comparison of cryo-EM maps of the 26S proteasome in complex with the K11-only ubiquitin chain (**a**) and the K11/K48-branched ubiquitin chain (**b**) reveals significant differences. The K11-only ubiquitin chain was observed in the E_D state within the K11-linked ubiquitin binding groove (highlighted in green), but it was absent in the resting (E_A) state. Furthermore, no EM density was detected near the RPT4/5 CC, which binds to the K48-linked ubiquitin extending from the proximal ubiquitin.

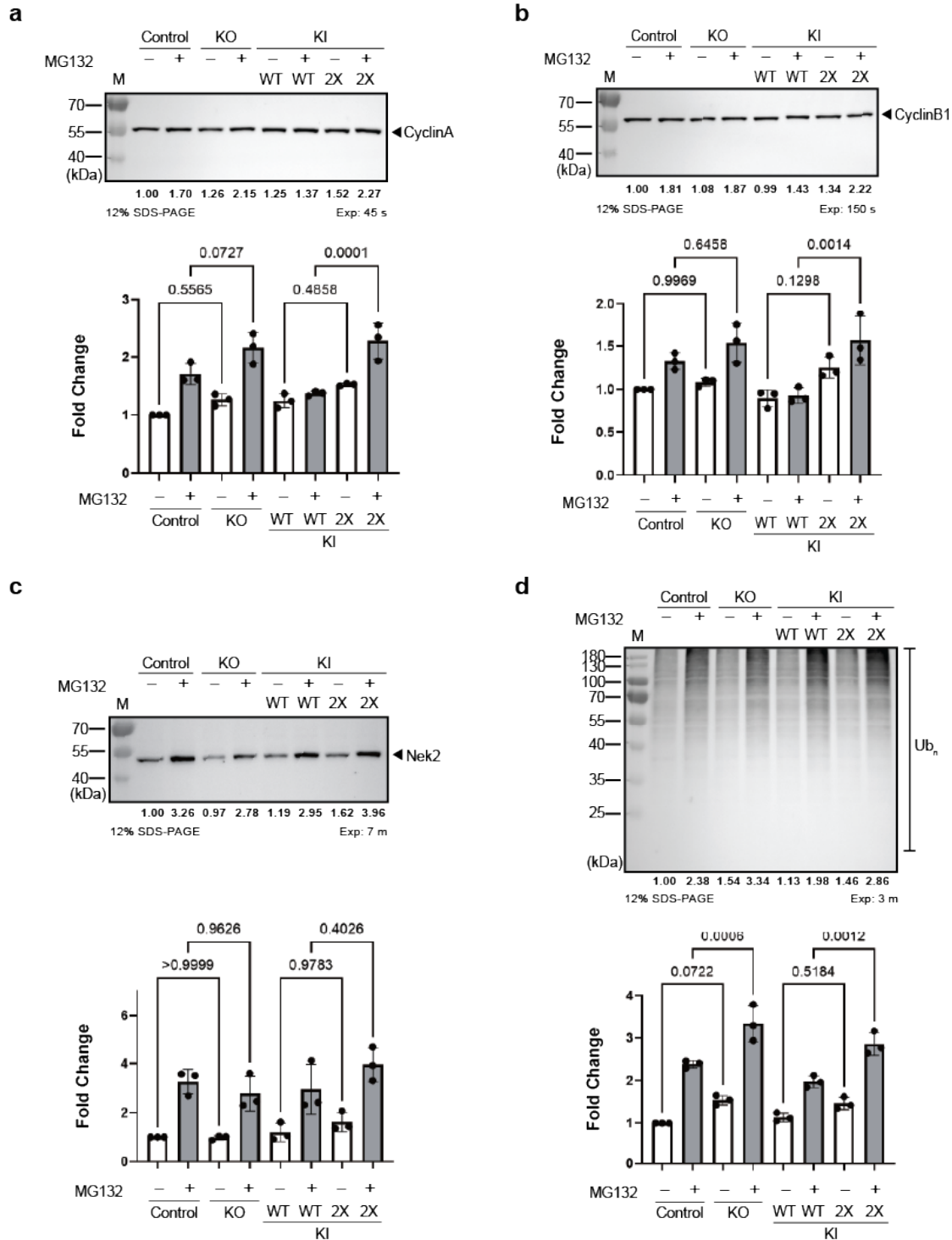


Supplementary Fig. 15. Structural insights into the conservation of the K48-link di-Ub recognition by RPN2 and RPN1 **a**, Expanded view of K48-linked Ub_{III}-Ub_{IV} in complex with RPN2 in the E_D:Ub₄ state. **b**, Open book view of the electrostatic surface representations of K48-linked Ub and RPN2, illustrating highly complementary surface electrostatic potentials of the two molecules. The locations of the residues involved in the conserved salt bridge pairs are outlined in solid yellow lines. **c**, Expanded view of K48-linked di-Ub in complex with the T1 binding site of yeast RPN1 (ScRPN1; PDB ID: 2N3V). **d**, Open book view of the electrostatic surface representation of K48-linked di-Ub and ScRPN1 following the same scheme as **(b)**. **e**, Sequence alignment of the K48-linked di-Ub binding motif of human RPN2 (hRPN2) and ScRPN1. The structure-based alignment was made by ESPrpt3.0 (<https://esprpt.ibcp.fr/ESPrpt/ESPrpt/>). Identical acidic residues are boxed with a red background, which is also outlined in red in **(a)** and **(c)**. Similar hydrophobic residues are boxed with a yellow background.



Supplementary Fig. 16. CRISPR gene editing of *PSMD1* (RPN2). **a.** Experimental workflow of *PSMD1* knockout (KO) and mutation knock-in (KI). Cas9 ribonucleoproteins (RNP) were electroporated into HEK293T cells to disrupt the *PSMD1* gene. Edited cells were analyzed by DNA sequencing, single-cell sorted for clonal expansion and confirmed by Western blotting. For KI experiments, plasmid DNA encoding the homology-directed repair templates (HDRT) was co-electroporated with Cas9 RNPs to introduce targeted mutations into the endogenous *PSMD1* locus. Mutant cells were enriched using an EGFP reporter strategy and confirmed by genomic PCR and DNA sequencing. The schematic workflow is drawn by using Affinity Designer. **b.** Sanger sequencing and ICE analysis of the *PSMD1* KO cell population on day 3 and 21 post editing and the isolated KO clone #7. Bar graphs show the percentages of insertion or deletion (% indel) and indel size at the Cas9 target site. Total indels and KO scores were

determined by ICE analysis. **c**, Western blotting analysis of RPN2 abundance in the parental HEK293T and PSMD1 KO clone #7 under MG132 or DMSO treatment. GAPDH serves as a loading control. Relative protein ratios were determined by protein band intensity using ImageJ. The experiment was carried out once (n = 1). **d**, Design of the HDRT, encoding the cDNA sequence of *PSMD1* exons 13–25 and an *egfp* reporter for selection. WT HDRT contains the wild-type cDNA sequence and 2X HDRT the point mutations E538K and D564K. CRISPR-mediated KI enables the insertion of *PSMD1* cDNA and a polyA sequence at exon 13, bypassing the endogenous *PSMD1* transcription. Primer F1, R1 and R2 (positions indicated) were used for PCR validation. **e**, Genomic PCR analysis of WT and 2X KI clones. Successful KI yielded a 3,203-bp product, while the endogenous allele generated a 783-bp fragment. Junction PCR using primers F1 and R2 produced a distinct 565-bp amplicon. The experiment was carried out once (n = 1). **f**, Sanger sequencing of junction PCR products confirmed the targeted insertion at the *PSMD1* locus. **g**, Flow cytometry analysis showing stable EGFP expression in mutant KI clones. **h**, Cell proliferation analysis of parental HEK293T cells versus *PSMD1* KO clone using xCELLigence Real-Time Cell Analysis (RTCA). Cell density was measured by electrical impedance and presented as cell index. **i**, Cell proliferation analysis of WT versus 2X KI clones using RTCA.



Supplementary Fig. 17. Western blot analyses of the effects of RPN2 mutations. Representative Western blot results are shown for cyclin A (a), cyclin B1 (b), Nek2 (c) and ubiquitin (d) with and without MG132 treatments. All cells are treated with nocodazole for cell cycle arrest. Untreated control, CRISPR/Cas9-edited heterozygous KO, KI with WT and 2X are indicated. Quantitation of band intensities are shown below individual lanes. Further quantitative analyses of technical triplicates (n = 3) are shown below as bar charts with the p-values indicated above the individual pairs. Source data are provided as a Source Data file.

a

RMSD (Å) values between Cα atoms of aligned 19S/RP

	apo E _A	E _A :Ub ₃	E _B :Ub ₄	E _D :Ub ₄	E _A /E _{A1} 6msb	E _{A2} 6msd	E _B 6mse	E _{C1} 6msg	E _{C2} 6msh	E _{D1} 6msj	E _{D2} 6msk
apo E _A		1.3	5.0	18.0	3.2	3.3	10.6	19.0	18.4	18.3	17.9
E _A :Ub ₃			5.0	17.5	3.1	3.2	10.7	19.0	18.4	18.2	17.4
E _B :Ub ₄				19.7	7.0	5.1	8.7	19.7	18.6	20.0	22.3
E _D :Ub ₄					17.0	18.3	20.1	9.3	8.0	3.7	3.6

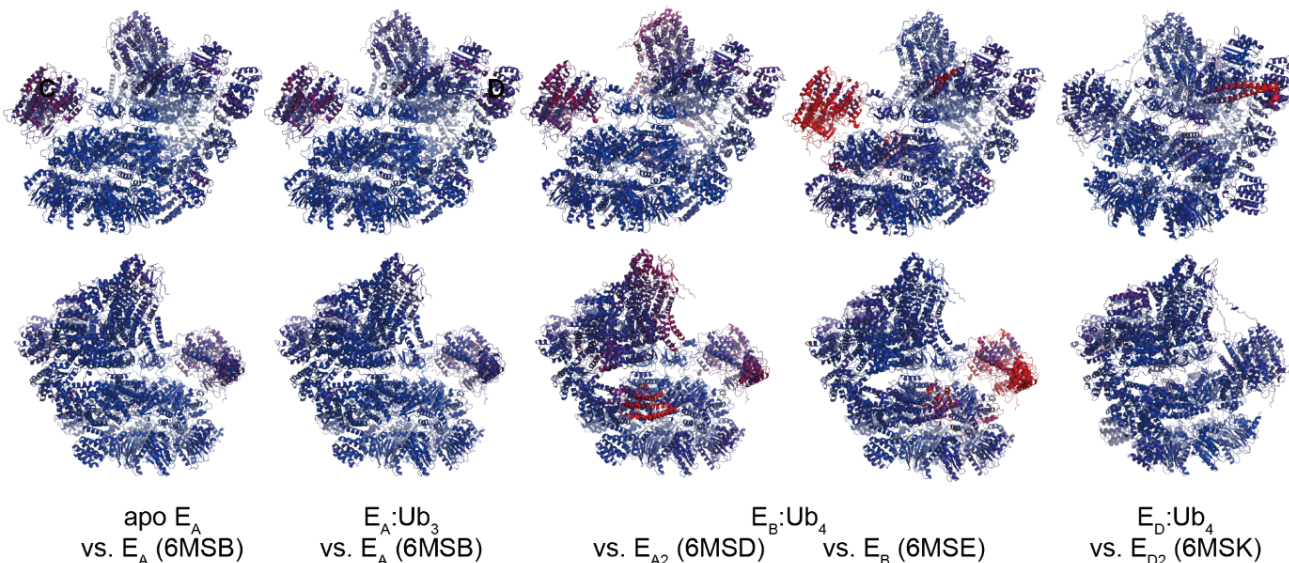
b

c

d

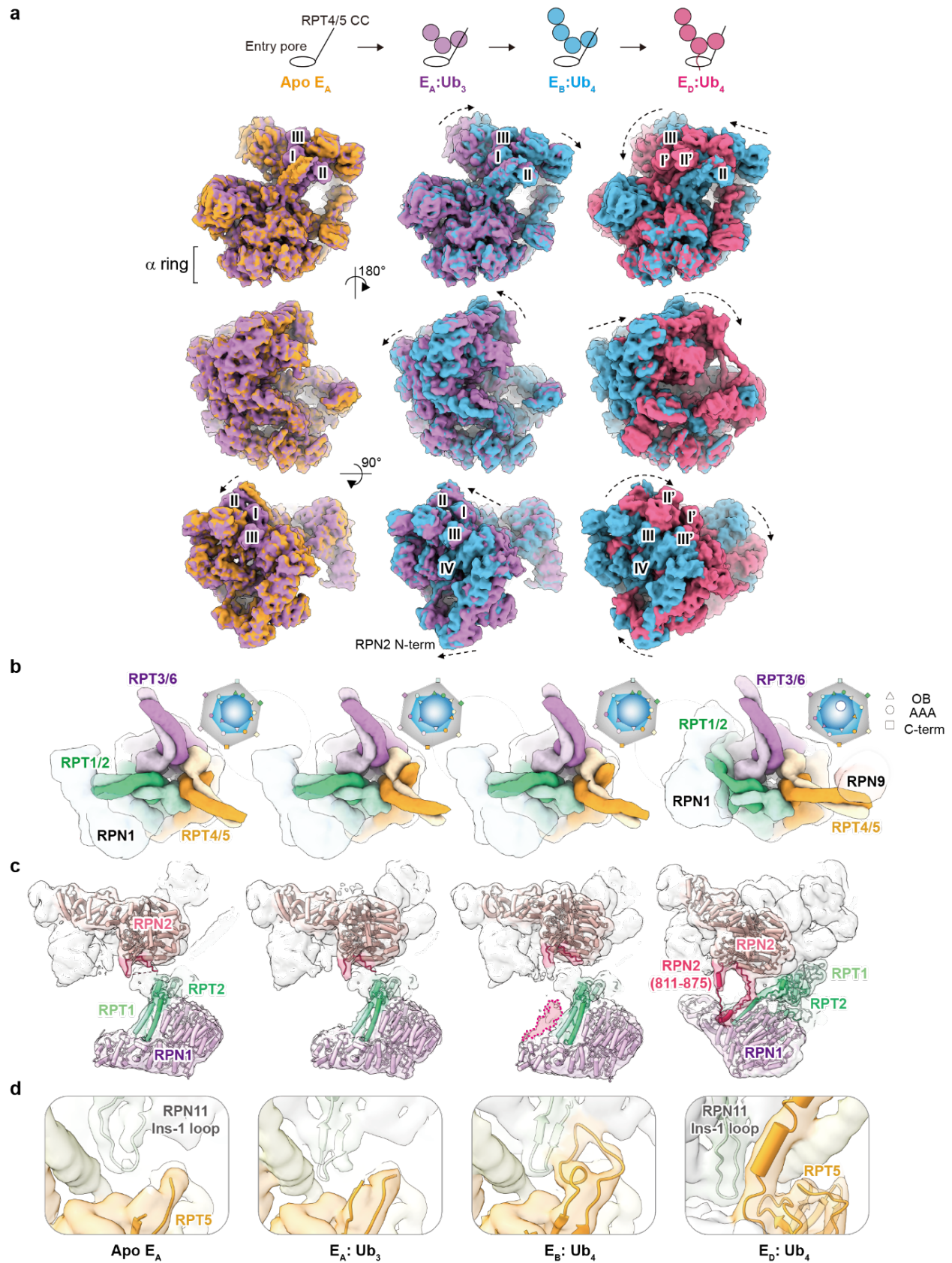
e

f

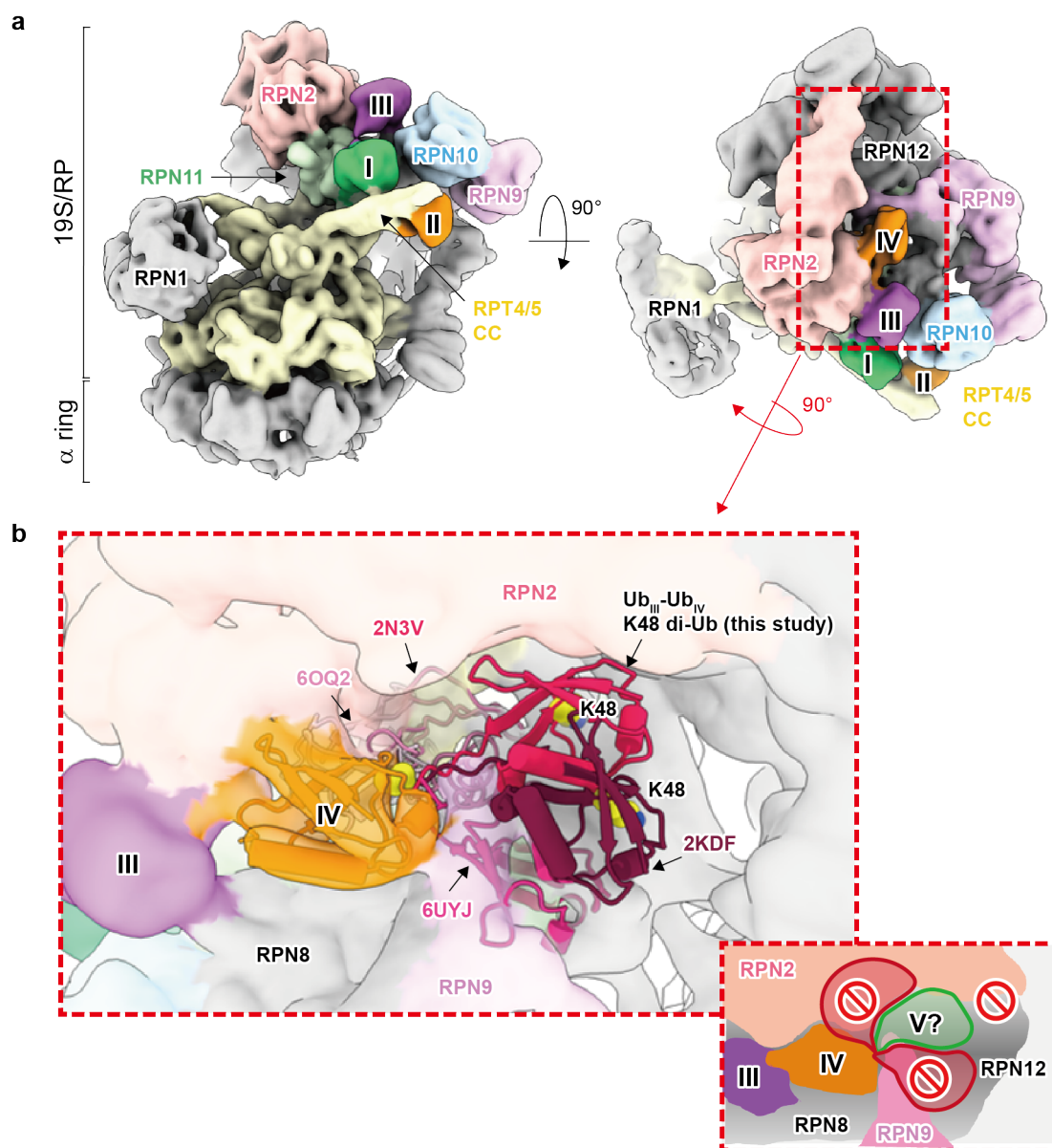


Supplementary Fig. 18. Comparison between the proteasome states observed in the presence of K11/K48-branched ubiquitin chain and previously published states of the human 26S proteasome.

a, The Root Mean Square Deviation (RMSD) between aligned Cα within proteasomal 19S/RP of the analyzed atomic models is shown in the table. Colors varying from green to red indicate the lowest (most similar structures) and highest RMSD values, respectively. **b-f**, The 26S states reported here are colored according to the RMSD values calculated for Cα atoms of the model and the model that showed to be the most similar, as indicated by black frames in the table A. The E_A:Ub₄ state was compared to E_{A1} (**d**), and E_B (**e**) state, even though it showed the lowest RMSD when compared to the RP in the E_{A1} state. Panels (**d**) and (**e**) show that E_A:Ub₄ shares features of both states. The RP base of E_A:Ub₄ is similar to the E_{A1} state (PDB ID: 6MSD), especially in nucleotide distribution, but its lid conformation resembles the E_B state (PDB ID: 6MSE). The main differences between the E_A:Ub₄ and the earlier reported E_B state are the conformations of the RPT2, RPT6, and RPN1. Compared to the E_A state, the E_A:Ub₄ shows a shift and downward movement of the whole lid comparable to the conformation of the E_B state.



Supplementary Fig. 19. Conformational transitions of the 19S RP induced by substrate binding. **a**, Superpositions of the substrate-free E_A (orange), $E_A:Ub_3$ (purple), $E_B:Ub_4$ (light blue), and $E_D:Ub_4$ complex (hot pink) with the positions of the four Ubs indicated in Roman numbers. **b**, Conformational transitions of the three CCs around the base of the 19S RP. Schematic representations of the OB, AAA, and C-terminal regions of the six subunits of the AAA⁺ ATPase are shown in triangles, circles, and squares, as indicated on the upper right corners. Note that the three structural motifs are coaxially aligned in the $E_D:Ub_4$ state, resulting in opening of the proteolytic chamber entrance as indicated by an open circle in the schematic drawing. **c**, RPT1, RPT2, and RPT1 conformational changes accompany the structural ordering of the RPN2 811-875 loop. The intrinsically disordered RPN2 loop is resolved in the $E_D:Ub_4$ complex, allowing for atomic modeling as shown in the hot pink cartoon representation. **d**, Structural ordering of the RPN11 Ins-1 loop exhibits different conformations in the $E_B:Ub_4$ and $E_D:Ub_4$ complex, while it is disordered in the apo and $E_B:Ub_4$ complex.



Supplementary Fig. 20. Geometrical constrain of the K11-linked Ub binding groove for K11-linked Ub chains. **a**, Orthogonal views of the cryo-EM map of the 19S RP in the E_B:Ub₄ state with the individual components colored in the same scheme as Figure 1d. **b**, Expanded view of the K11-linked Ub binding groove by RPN2, RPN8, RPN9, RPN10 and RPN11 (the last two subunits are not visible in this view). The segments of the EM map corresponding to Ub_{III} and Ub_{IV} are colored magenta and orange, respectively. The atomic model of Ub_{IV} is shown in cartoon representation and superimposed with the structures of the previously reported K48-linked di-Ub (the PDB IDs are indicated alongside). Some of the K48-linked Ub extending from Ub_{IV} will clash with the K11-Ub binding groove (such as 6UYJ, 6OQ2, and 2N3V), as schematically illustrated in the inset. However, the structure of 2KDF could still fit into the K11-linked Ub binding groove without steric clash, but further extension will clash with RPN12. The structural modeling suggests that the K11-linked Ub binding groove can accommodate up to three Ub moieties through the ubiquitin alternating K11-K48-linkage with a further extension from Ub_{IV}.

Supplementary Table 1. List of Ub AQUA (heavy) peptides used in this study.

Abbreviation	Peptide sequence	Precursor m/z	Charge state	Product ions used for PRM	RT (min)
K6 linkage	M[Oxid]QIFVK[GG]TLTGK	465.9270	3+	y2+, y3+, y5+, y6+, y7+, y8+	39.1
K6 linkage (heavy)	M[Oxid]QIFVK[GG]TLTGK	468.2661			
K11 linkage	TLTGK[GG]TITLEVEPSDTIENVK	801.4269	3+	y4+, y6+, y7+, y8+, y9+, y10+	42.5
K11 linkage (heavy)	TLTGK[GG]TITLEVEPSDTIENVK	803.4315			
K27 linkage	TITLEVEPSDTIENVK[GG]AK	701.0390	3+	y6+, y7+, y8+, y9+, y10+	41.4
K27 linkage (heavy)	TITLEVEPSDTIENVK[GG]AK	703.0436			
K29 linkage	AK[GG]IQDK	408.7323	2+	y2+, y3+, y4+, y5+	28.1
K29 linkage (heavy)	AK[GG]IQDK	412.2409			
K33 linkage	IQDK[GG]EGIPPDQQR	546.6129	3+	y1+, y2+, y3+, y4+, y5+, y6+	31.8
K33 linkage (heavy)	IQDK[GG]EGIPPDQQR	548.6175			
K48 linkage	LIFAGK[GG]QLEDGR	487.6001	3+	y2+, y3+, y4+, y5+, y6+, y8+	39.2
K48 linkage (heavy)	LIFAGK[GG]QLEDGR	489.9391			
K63 linkage	TLSDYNIQK[GG]ESTLHLVLR	748.7376	3+	y5+, y6+, y7+, y8+, y9+	42.6
K63 linkage (heavy)	TLSDYNIQK[GG]ESTLHLVLR	751.0767			
M1 linkage	GGM[Oxid]QIFVK	448.2389	2+	y2+, y3+, y4+, y5+, y6+	36.2
M1 linkage (heavy)	GGM[Oxid]QIFVK	451.2458			

Supplementary Table 2. Summary of the cryo-EM data collection parameters and model statistics.

		E_A:Ub₃	E_B:Ub₄	E_D:Ub₄
	Apo E_A	PDB 8JRT	PDB 8JTI	PDB 8K0G
	PDB 8JRI	EMD- 36605	EMD-36645	EMD-36764
	EMD- 36598	EMD-37269,	EMD-37317	EMD-37334
		EMD-37273,	EMD-37319	EMD-37335
		EMD-37276,	EMD-37327	EMD-37341
		EMD-37277	EMD-37328	EMD-37344
Data collection and processing				
Magnification		64,000x		
Voltage (kV)		300		
Electron exposure (e ⁻ /Å ²)		48–50		
Defocus range (μm)		-1.2 to -1.8		
Pixel size (Å)		0.7 super-resolution pixel		
Symmetry imposed	C1	C1	C1	C1
Initial particle images (no.)		2 695 911		
Final particle images (no.)	153 646	54 794	52 216	84 476
Map resolution (Å)	3.4	3.6	3.8	3.8
FSC threshold	0.143	0.143	0.143	0.143
Refinement				
Initial model used (PDB ID)	6MSB	6MSB	6MSE	6MSK
Model resolution (Å)	3.6	3.6	3.8	3.8
FSC threshold	0.143	0.143	0.143	0.143
Model composition				
Non-hydrogen atoms	62 679	66 765	67 839	68 848
Protein atoms	8 787	9 029	9 115	9 220
Ligands	MG:5	MG:5	MG:5	MG:4
	ATP:4	ATP:4	ATP:4	ATP:3
	ADP:2	ADP:2	ADP:2	ADP:2
<i>B</i> factors (Å ²)				
Protein	80.24	113.61	99.06	107.54
Ligand	31.10	58.82	47.71	64.33
R.m.s. deviations				
Bond lengths (Å)	0.003	0.006	0.010	0.008
Bond angles (°)	0.607	0.737	0.926	0.884
Validation				
MolProbity score	1.81	2.02	2.35	2.39
Clashscore	6.3	7.30	10.65	11.91
Poor rotamers (%)	0.81	1.50	2.12	2.22
Ramachandran plot				
Favored (%)	92.53	92.08	89.61	90.52
Allowed (%)	7.16	7.66	10.02	9.18
Disallowed (%)	0.31	0.27	0.38	0.30

Supplementary Table 3. Characteristics of interaction interfaces between K11/K48-branched tetra-Ub and human 26S proteasome. Solvent Accessible Surface Area (SASA) occupied by each of the Ubs.

E _B :Ub ₄ state												
Ub		26S subunits				Interface area (Å ²)	Δ ⁱ G (kcal/mol)	Δ ⁱ G P-value	N _{HB}	N _{SB}	% of total interface area	
ⁱ N _{at}	ⁱ N _{res}	ⁱ N _{at}	ⁱ N _{res}									
Ub _I	102	26	Rpn11	114	34	1157.4	-16.6	0.138	7	2	38%	
Ub _I	12	5	Rpt5	11	4	131.5	-0.3	0.510	1	2	4%	
Ub _{II}	22	8	Rpt5	23	6	226.8	-0.0	0.626	2	2	7%	
Ub _{II}	17	5	Rpt4	12	6	124	2.2	0.700	3	4	4%	
Ub _{III}	30	9	Rpn2	37	8	289.4	-2.4	0.351	2	0	9%	
Ub _{III}	22	6	Rpn8	27	9	198.7	0.7	0.780	1	1	6%	
Ub _{III}	8	2	Rpn11	13	4	79.3	1.6	0.769	2	2	3%	
Ub _{III}	1	1	Rpn10	4	1	15.4	-0.0	0.621	0	0	1%	
Ub _{IV}	65	18	Rpn2	66	19	640.7	-4.8	0.353	7	5	21%	
Ub _{IV}	20	6	Rpn9	15	6	154.7	1.5	0.775	3	6	5%	
Ub _{IV}	9	3	Rpn8	7	2	59.2	0.4	0.715	1	1	2%	
Total interface area (Å ²)=						3077.1					Ub _I	42%
							% of total interface area occupied by each Ub (Å ²)				Ub _{II}	11%
											Ub _{III}	19%
											Ub _{IV}	28%
E _D :Ub ₄ state												
Ub		26S subunits				Interface area (Å ²)	Δ ⁱ G (kcal/mol)	Δ ⁱ G P-value	N _{HB}	N _{SB}	% of total interface area	
ⁱ N _{at}	ⁱ N _{res}	ⁱ N _{at}	ⁱ N _{res}									
Ub _I	89	23	Rpn11	115	34	1117.2	-17.4	0.099	11	2	38%	
Ub _{II}	25	11	Rpn10	28	11	254.3	1.6	0.759	3	2	9%	
Ub _{II}	23	7	Rpt5	16	6	210	-1.6	0.393	1	0	7%	
Ub _{II}	8	4	Rpt4	6	2	57.8	1.2	0.742	2	0	2%	
Ub _{III}	36	10	Rpn2	40	10	304.2	-1.4	0.570	0	0	10%	
Ub _{III}	18	5	Rpn8	23	9	168.8	-0.5	0.701	1	0	6%	
Ub _{III}	12	4	Rpn11	12	5	90.3	0.1	0.743	1	1	3%	
Ub _{IV}	63	17	Rpn2	61	15	612	-4.7	0.409	5	6	21%	
Ub _{IV}	14	4	Rpn9	7	3	103.3	0.8	0.710	3	4	4%	
Ub _{IV}	6	2	Rpn8	2	1	20.3	0.9	0.834	0	0	1%	
Total interface area (Å ²)=						2938.2					Ub _I	38%
							% of total interface area occupied by each Ub (Å ²)				Ub _{II}	18%
											Ub _{III}	19%
											Ub _{IV}	25%

ⁱN_{at} indicates the number of interfacing atoms in the corresponding structure. ⁱN_{res} indicates the number of interfacing residues in the corresponding structure. Interface area in Å², calculated as difference in total accessible surface areas of isolated and interfacing structures divided by two. ΔⁱG indicates the solvation free energy gain upon formation of the interface. ΔⁱG P-value indicates the P-value of the observed solvation free energy gain (P=0.5 means that the interface is not "surprising" at all with ΔiG equal to an average value for given structures; P>0.5 means that the interface is less hydrophobic then it could be, therefore the interface is likely to be an artefact of crystal packing; P<0.5 indicates interfaces with surprising (higher than would-be-average for given structures) hydrophobicity). N_{HB} indicates the number of potential hydrogen bonds across the interface. N_{SB} indicates the number of potential salt bridges across the interface.

Supplementary Table 4. List of residues forming hydrogen bonds (HB, in black) and salt bridges (SB, in blue) between K11/K48-branched Ub chain and the human 26S proteasome in the observed conformational states of the complex.

	E _A :Ub ₃ state				E _B :Ub ₄ state				E _P :Ub ₄ state			
	Ub residue (atom)	Dist. (Å)	Interacting subunit	Sub. residue (atom)	Ub residue (atom)	Dist. (Å)	Interacting subunit	Sub. residue (atom)	Ub residue (atom)	Dist. (Å)	Interacting subunit	Sub. residue (atom)
Ub _I	GLN 40 (NE2)	3.59	Rpn11	ASN 128 (OD1)	LEU 8 (O)	3.72	Rpn11	VAL 108 (N)	LEU 8 (O)	3.49	Rpn11	VAL 108 (N)
	GLN 49 (NE2)	3.36	Rpn11	ASP 88 (OD2)	LEU 73 (O)	3.66	Rpn11	ALA 86 (N)	GLN 40 (OE1)	2.80	Rpn11	SER 132 (OG)
	ARG 72 (NE)	3.00	Rpn11	GLU 85 (OE2)	ARG 74 (O)	3.22	Rpn11	THR 129 (OG1)	LEU 73 (O)	3.82	Rpn11	ALA 86 (N)
	ARG 72 (NH2)	3.13	Rpn11	GLU 85 (OE1)	GLY 75 (O)	3.22	Rpn11	VAL 84 (N)	ARG 74 (O)	2.73	Rpn11	THR 129 (OG1)
	ARG 74 (N)	3.78	Rpn11	THR 129 (OG1)	ARG 74 (NH2)	2.43	Rpn11	GLU 85 (OE2)	GLY 75 (O)	2.93	Rpn11	VAL 84 (N)
	ARG 74 (NE)	2.57	Rpn11	GLU 85 (OE1)	GLY 75 (N)	3.40	Rpn11	VAL 84 (O)	GLN 40 (NE2)	3.69	Rpn11	ASN 128 (O)
	GLY 75 (N)	2.75	Rpn11	VAL 84 (O)	GLY 76 (N)	3.09	Rpn11	ASP 126 (OD1)	GLN 40 (NE2)	3.52	Rpn11	ASN 128 (OD1)
	GLY 76 (N)	3.59	Rpn11	GLU 52 (OE2)	ARG 74 (NE)	2.89	Rpn11	GLU 85 (OE2)	ARG 74 (N)	3.37	Rpn11	THR 129 (OG1)
	GLY 76 (N)	2.64	Rpn11	SER 83 (OG)	ARG 74 (NH2)	2.43	Rpn11	GLU 85 (OE2)	ARG 74 (NH1)	2.77	Rpn11	GLU 85 (OE2)
	GLY 76 (N)	3.19	Rpn11	ASP 126 (OD1)	ASP 58 (OD2)	2.43	Rpt5	ARG 61 (NH2)	GLY 75 (N)	3.12	Rpn11	VAL 84 (O)
	LEU 8 (O)	3.66	Rpn11	VAL 108 (N)	ASP 58 (OD2)	3.89	Rpt5	ARG 61 (NE)	GLY 76 (N)	3.52	Rpn11	ASP 126 (OD1)
	LEU 73 (O)	2.76	Rpn11	ALA 86 (N)	ASP 58 (OD2)	2.43	Rpt5	ARG 61 (NH2)	ARG 74 (NE)	4.00	Rpn11	GLU 85 (OE2)
	ARG 74 (O)	2.39	Rpn11	THR 129 (OG1)					ARG 74 (NH1)	2.77	Rpn11	GLU 85 (OE2)
	GLY 76 (OXT)	2.72	Rpn11	SER 123 (OG)								
	ARG 72 (NE)	3.00	Rpn11	GLU 85 (OE2)								
	ARG 72 (NE)	3.83	Rpn11	GLU 85 (OE1)								
	ARG 72 (NH2)	3.58	Rpn11	GLU 85 (OE2)								
	ARG 72 (NH2)	3.13	Rpn11	GLU 85 (OE1)								
	ARG 74 (NE)	2.57	Rpn11	GLU 85 (OE1)								
	ARG 74 (NH2)	2.72	Rpn11	GLU 85 (OE1)								
	ASP 58 (OD2)	3.08	Rpt5	ARG 61 (NH2)								
	ASP 58 (OD2)	3.08	Rpt5	ARG 61 (NH2)								
Ub _{II}	GLU 34 (O)	2.91	Rpt4	ARG 30 (NH1)	THR 9 (OG1)	3.38	Rpt4	LYS 20 (NZ)	THR 22 (OG1)	3.50	Rpt4	ARG 25 (NH1)
	GLU 34 (OE1)	3.98	Rpt4	LYS 27 (NZ)	GLU 34 (OE2)	2.66	Rpt4	ARG 30 (NH2)	THR 22 (OG1)	3.54	Rpt4	ARG 25 (NH2)
	GLN 40 (NE2)	3.32	Rpt5	GLU 58 (OE2)	GLU 34 (OE2)	2.23	Rpt4	LYS 27 (NZ)	ASP 52 (OD2)	2.42	Rpt5	THR 63 (OG1)
					GLU 34 (OE1)	3.77	Rpt4	LYS 27 (NZ)				
					GLU 34 (OE2)	3.33	Rpt4	ARG 30 (NH1)				
					GLU 34 (OE2)	2.66	Rpt4	ARG 30 (NH2)				
					GLU 34 (OE2)	2.23	Rpt4	LYS 27 (NZ)				
					THR 9 (OG1)	2.55	Rpt5	GLU 51 (OE2)				
					ARG 74 (NH1)	2.91	Rpt5	GLU 58 (OE2)				
					ARG 74 (NH2)	3.28	Rpt5	GLU 58 (OE2)				
Ub _{III}	ARG 74 (NH1)	3.02	Rpn11	PHE 61 (O)	ARG 72 (NH1)	3.84	Rpn11	GLU 60 (OE1)	ARG 42 (NH2)	3.08	Rpn11	GLU 60 (OE2)
	ARG 74 (NH2)	3.12	Rpn11	PHE 61 (O)	ARG 72 (NH2)	3.53	Rpn11	GLU 60 (OE1)	ARG 42 (NH2)	3.08	Rpn11	GLU 60 (OE2)
	ARG 54 (NH2)	2.75	Rpn8	ASN 31 (OD1)	ARG 72 (NH1)	3.84	Rpn11	GLU 60 (OE1)	GLY 53 (O)	3.57	Rpn8	ASN 31 (N)
	GLU 51 (OE1)	2.96	Rpn8	LYS 28 (NZ)	ARG 72 (NH2)	3.53	Rpn11	GLU 60 (OE1)				
	GLU 51 (OE2)	3.72	Rpn8	LYS 28 (NZ)	GLU 51 (OE2)	3.16	Rpn8	LYS 28 (NZ)				
	GLU 51 (OE1)	2.96	Rpn8	LYS 28 (NZ)	GLU 51 (OE2)	3.16	Rpn8	LYS 28 (NZ)				
Ub _{IV}					GLN 49 (OE1)	3.69	Rpn2	GLN 540 (NE2)				
					LYS 48 (N)	3.79	Rpn2	GLU 538 (O)				
					GLY 47 (O)	3.39	Rpn2	ARG 572 (NH2)	GLY 47 (O)	3.43	Rpn2	ARG 572 (NH2)
					THR 9 (OG1)	2.30	Rpn2	ALA 526 (O)	THR 9 (OG1)	3.38	Rpn2	ALA 526 (O)
					LEU 73 (N)	3.46	Rpn2	GLN 537 (OE1)	LEU 73 (N)	3.65	Rpn2	GLN 537 (OE1)
					ARG 72 (NH1)	2.55	Rpn2	GLN 537 (OE1)	ARG 74 (NE)	2.37	Rpn2	GLU 538 (OE2)
					ARG 72 (NH1)	2.57	Rpn2	GLU 538 (OE2)	LYS 6 (NZ)	3.61	Rpn2	ASP 564 (OD1)
					ARG 72 (NH2)	2.67	Rpn2	GLU 538 (OE2)	ARG 74 (NE)	3.45	Rpn2	GLU 538 (OE1)
					HIS 68 (NE2)	3.22	Rpn2	ASP 564 (OD1)	ARG 74 (NE)	2.37	Rpn2	GLU 538 (OE2)
					ARG 72 (NH1)	2.57	Rpn2	GLU 538 (OE2)	ARG 74 (NH2)	2.65	Rpn2	GLU 538 (OE2)
					ARG 72 (NH2)	2.67	Rpn2	GLU 538 (OE2)	LYS 6 (NZ)	3.61	Rpn2	ASP 564 (OD1)
					HIS 68 (NE2)	3.22	Rpn2	ASP 564 (OD1)	HIS 68 (ND1)	3.31	Rpn2	GLU 568 (OE2)
					HIS 68 (NE2)	3.95	Rpn2	ASP 564 (OD2)	HIS 68 (NE2)	2.84	Rpn2	GLU 568 (OE2)
					HIS 68 (ND1)	3.83	Rpn2	GLU 568 (OE2)	ASP 58 (OD2)	3.41	Rpn9	LYS 361 (NZ)
					ASP 58 (OD2)	2.95	Rpn9	LYS 361 (NZ)	ARG 54 (NH1)	3.09	Rpn9	GLU 368 (OE1)
					ARG 54 (NH1)	3.47	Rpn9	GLU 368 (OE2)	ARG 54 (NH2)	3.80	Rpn9	GLU 368 (OE2)
					ARG 54 (NH2)	2.44	Rpn9	GLU 368 (OE1)	ASP 58 (OD2)	3.41	Rpn9	LYS 361 (NZ)
					ASP 58 (OD2)	2.95	Rpn9	LYS 361 (NZ)	ARG 54 (NH1)	3.09	Rpn9	GLU 368 (OE1)
					ARG 54 (NH1)	3.85	Rpn9	GLU 368 (OE1)	ARG 54 (NH2)	3.53	Rpn9	GLU 368 (OE1)
					ARG 54 (NH1)	3.47	Rpn9	GLU 368 (OE2)	ARG 54 (NH2)	3.80	Rpn9	GLU 368 (OE2)
					ARG 54 (NH2)	2.44	Rpn9	GLU 368 (OE1)				
					ARG 54 (NH2)	3.28	Rpn9	GLU 368 (OE2)				

Supplementary Table 5. List of residues at the interface between K11/K48-branched Ub chain and the human 26S proteasome with their solvation energy change (Δ^iG in kcal/mol) upon complex formation. Energetically favorable changes are highlighted in green.

	$E_A:Ub_3$ state					$E_B:Ub_4$ state					$E_D:Ub_4$ state				
	Ub residue	Δ^iG	Interacting subunit	Sub. residue	Δ^iG	Ub residue	Δ^iG	Interacting subunit	Sub. residue	Δ^iG	Ub residue	Δ^iG	Interacting subunit	Sub. residue	Δ^iG
Ub₁	LEU 73	1.8	Rpn11	MET 107	1.5	LEU 73	1.8	Rpn11	LEU 136	1.62	LEU 73	1.91	Rpn11	LEU 136	1.56
	LEU 8	1.08	Rpn11	LEU 136	1.5	LEU 8	1.19	Rpn11	MET 107	1.51	LEU 8	1.06	Rpn11	MET 107	1.48
	LEU 71	1.05	Rpn11	PRO 89	1.23	ILE 44	0.84	Rpn11	PRO 89	1.23	THR 9	1.03	Rpn11	PRO 89	1.27
	ILE 44	0.85	Rpn11	VAL 125	0.81	ILE 36	0.82	Rpn11	VAL 90	1.12	LEU 71	0.95	Rpn11	VAL 125	0.98
	THR 9	0.84	Rpn11	VAL 90	0.75	LEU 71	0.82	Rpn11	THR 129	0.96	ILE 44	0.93	Rpn11	VAL 90	0.87
	ILE 36	0.83	Rpn11	MET 54	0.7	VAL 70	0.71	Rpn11	VAL 125	0.87	VAL 70	0.72	Rpn11	ALA 93	0.82
	VAL 70	0.65	Rpn11	ALA 93	0.66	GLY 76	0.71	Rpn11	ALA 93	0.86	LYS 11	0.51	Rpn11	HIS 113	0.72
	GLY 75	0.36	Rpn11	PHE 133	0.6	GLY 10	0.32	Rpn11	MET 54	0.83	ILE 36	0.42	Rpn11	MET 54	0.7
	GLY 10	0.32	Rpn11	VAL 108	0.5	GLY 75	0.18	Rpn11	PHE 133	0.61	ARG 42	0.28	Rpn11	PHE 133	0.65
	ARG 42	0.2	Rpn11	SER 132	0.49	THR 9	0.18	Rpn11	SER 132	0.56	GLY 75	0.28	Rpn11	VAL 108	0.59
	LEU 69	0.17	Rpn11	LEU 96	0.38	LYS 11	0.15	Rpn11	ALA 135	0.35	GLY 10	0.23	Rpn11	HIS 115	0.47
	THR 7	0.15	Rpn11	ALA 86	0.38	ARG 42	0.14	Rpn11	VAL 108	0.34	THR 7	0.23	Rpn11	LEU 96	0.34
	HIS 68	0.12	Rpn11	SER 137	0.31	LEU 69	0.13	Rpn11	MET 75	0.31	HIS 68	0.18	Rpn11	SER 137	0.34
	PRO 37	0.04	Rpn11	SER 83	0.22	THR 7	0.1	Rpn11	LEU 96	0.29	LEU 69	0.06	Rpn11	ALA 135	0.31
	ARG 74	0.02	Rpn11	VAL 82	0.15	LYS 48	0.06	Rpn11	SER 83	0.29	GLY 47	0.03	Rpn11	MET 75	0.28
	GLU 34	-0.05	Rpn11	SER 123	0.14	PRO 37	0.06	Rpn11	SER 137	0.26	PRO 37	0	Rpn11	SER 83	0.28
	GLY 47	-0.07	Rpn11	MET 75	0.14	GLY 35	0.05	Rpn11	LEU 56	0.24	GLY 35	-0.15	Rpn11	SER 132	0.24
	ARG 72	-0.19	Rpn11	LEU 56	0.13	GLY 47	0.04	Rpn11	TRP 111	0.24	ARG 72	-0.19	Rpn11	ALA 86	0.22
	GLN 49	-0.24	Rpn11	THR 129	0.12	ILE 13	0	Rpn11	ALA 86	0.11	GLY 76	-0.2	Rpn11	TRP 111	0.19
	GLY 76	-0.27	Rpn11	TRP 111	0.12	LEU 43	0	Rpn11	HIS 113	0.09	GLN 49	-0.3	Rpn11	LEU 56	0.19
	LYS 11	-0.29	Rpn11	ALA 135	0.06	GLU 34	-0.09	Rpn11	ASP 97	0.04	GLU 34	-0.3	Rpn11	SER 123	0.14
	GLN 40	-0.69	Rpn11	GLY 55	0.03	GLN 49	-0.12	Rpn11	LYS 94	0.02	GLN 40	-0.67	Rpn11	THR 129	0.04
			Rpn11	HIS 115	0.01	ARG 72	-0.18	Rpn11	GLY 55	0.02	ARG 74	-0.74	Rpn11	GLU 85	0.01
			Rpn11	HIS 113	0.01	GLN 40	-0.66	Rpn11	ASP 88	0.01			Rpn11	GLY 55	0.01
			Rpn11	GLN 131	-0.01	HIS 68	-0.93	Rpn11	HIS 115	-0.01			Rpn11	PHE 73	0.01
			Rpn11	GLU 138	-0.05	ARG 74	-1.09	Rpn11	VAL 87	-0.02			Rpn11	VAL 109	0
			Rpn11	THR 80	-0.05			Rpn11	ASP 126	-0.08			Rpn11	ASP 126	-0.05
			Rpn11	ASP 126	-0.06			Rpn11	VAL 84	-0.08			Rpn11	VAL 82	-0.14
			Rpn11	GLU 52	-0.12			Rpn11	SER 123	-0.08			Rpn11	ASP 88	-0.14
			Rpn11	VAL 84	-0.14			Rpn11	VAL 82	-0.1			Rpn11	ASP 97	-0.15
			Rpn11	ASP 88	-0.26			Rpn11	GLU 52	-0.13			Rpn11	GLU 52	-0.2
			Rpn11	GLU 85	-0.31			Rpn11	GLN 92	-0.23			Rpn11	VAL 84	-0.2
			Rpn11	GLN 92	-0.33			Rpn11	ASN 128	-0.33			Rpn11	GLN 92	-0.25
			Rpn11	ASN 128	-0.34			Rpn11	GLU 85	-0.4			Rpn11	ASN 128	-0.42
	ARG 74	-0.19	Rpt4	GLN 51	0.10										
	ASP 58	-0.2	Rpt5	ARG 61	-1.35	THR 9	0.39	Rpt4	LEU 16	0.01					
	ARG 54	0.19	Rpt5	LEU 60	0.01	GLY 35	0.35	Rpt4	ASP 23	-0.02					
	GLY 53	0.09	Rpt5	HIS 64	-0.01	ILE 36	0.06	Rpt4	LYS 34	-0.24					
	THR 22	0.02				LYS 33	0	Rpt4	LYS 20	-0.45					
	GLU 24	0.03				GLU 34	-0.51	Rpt4	LYS 27	-0.65					
	THR 55	0.11						Rpt4	ARG 30	-1.1					
						THR 55	0.33	Rpt5	LEU 60	0.6					
						ARG 54	0.33	Rpt5	GLU 65	-0.02					
						THR 22	0.16	Rpt5	HIS 64	-0.14					
						GLY 53	0.14	Rpt5	ARG 61	-0.89					
						ASP 58	-0.2								
Ub₁₁	GLY 35	0.18	Rpt4	LYS 27	0.05	THR 9	0.39	Rpt4	LEU 16	0.01	ILE 44	0.45	Rpn10	ILE 35	0.57
	GLU 34	-0.42	Rpt4	ARG 30	-0.98	GLY 35	0.35	Rpt4	ASP 23	-0.02	GLY 47	0.26	Rpn10	HIS 76	0.37
	PRO 37	0.06	Rpt4	LEU 17	0.00	ILE 36	0.06	Rpt4	LYS 34	-0.24	VAL 70	0.01	Rpn10	LEU 72	0.32
	LYS 11	0.06	Rpt4	ASP 23	-0.02	LYS 33	0	Rpt4	LYS 20	-0.45	ARG 72	-0.02	Rpn10	SER 73	0.21
	ILE 36	0.07	Rpt4	LYS 20	-0.13	GLU 34	-0.51	Rpt4	LYS 27	-0.65	TYR 59	-0.06	Rpn10	THR 68	0.18
	THR 9	-0.02	Rpt4	HIS 19	0.08			Rpt4	ARG 30	-1.1	ALA 46	-0.08	Rpn10	CYS 37	0.02
											ARG 42	-0.3	Rpn10	ASP 67	-0.01
	VAL 70	0.22	Rpt5	SER 57	0.01	LEU 71	1.05	Rpt5	ILE 54	1.4	GLU 51	-0.35	Rpn10	ASP 31	-0.09
	THR 9	0.21	Rpt5	SER 50	0.04	LEU 73	0.35	Rpt5	MET 55	0.15	GLN 49	-0.54	Rpn10	ARG 42	-0.16
	GLN 40	-0.48	Rpt5	LYS 53	0.36	PRO 37	0.08	Rpt5	SER 57	-0.09	ARG 54	-0.6	Rpn10	HIS 38	-0.21
	LEU 71	0.34	Rpt5	GLU 58	-0.30	LEU 8	0.04	Rpt5	GLU 51	-0.16	LYS 48	-0.9	Rpn10	ASN 34	-0.64
	LEU 8	1.12	Rpt5	LEU 48	0.82	ARG 72	-0.02	Rpt5	GLU 58	-0.34					
	LEU 73	1.58	Rpt5	GLU 51	-0.19	THR 9	-0.18	Rpt5	ARG 61	-0.44	THR 22	0.14	Rpt4	GLU 21	-0.11
	ILE 36	0.01	Rpt5	ILE 54	1.50	GLN 40	-0.42				THR 55	0.1	Rpt4	ARG 25	-1.17
	PRO 37	0.10	Rpt5	LEU 47	0.02	ARG 74	-1.44				ASP 52	-0.04			
	ARG 72	0.05									SER 20	-0.12			
											GLY 53	0.26	Rpt5	LEU 60	0.89
											THR 55	0.23	Rpt5	MET 55	0.48
											ARG 54	0.21	Rpt5	VAL 59	0.45
											ASP 58	-0.08	Rpt5	THR 63	0.11
											GLU 51	-0.08	Rpt5	LYS 56	0
											ASP 39	-0.31	Rpt5	GLN 67	-0.19
											ASP 52	-0.36			

	E _A :Ub ₃ state					E _B :Ub ₄ state					E _D :Ub ₄ state				
	Ub residue	ΔG	Interacting subunit	Sub. residue	ΔG	Ub residue	ΔG	Interacting subunit	Sub. residue	ΔG	Ub residue	ΔG	Interacting subunit	Sub. residue	ΔG
Ub _{III}	GLY 75	0.1	Rpn11	GLU 60	0.34	ARG 74	-0.77	Rpn11	MET 107	0.71	ARG 72	0.05	Rpn11	MET 107	0.35
	GLY 76	-0.01	Rpn11	VAL 62	0.13	ARG 72	-1.31	Rpn11	VAL 62	0.12	ARG 74	0.03	Rpn11	PHE 61	0.34
	ARG 72	-0.15	Rpn11	MET 107	0.06			Rpn11	PHE 61	-0.12	GLY 76	-0.13	Rpn11	SER 137	0.04
	ARG 42	-0.65	Rpn11	PRO 105	0.04			Rpn11	GLU 60	-0.21	ARG 42	-0.26	Rpn11	LEU 136	-0.1
	ARG 74	-0.96	Rpn11	GLU 106	0.02								Rpn11	GLU 60	-0.44
			Rpn11	SER 137	-0.06	ALA 46	0.58	Rpn2	TYR 535	0.44	GLN 49	0.4	Rpn2	TYR 535	0.51
			Rpn11	PHE 61	-0.07	GLY 47	0.53	Rpn2	TYR 502	0.43	ILE 44	0.39	Rpn2	TYR 502	0.34
			Rpn11	GLU 138	-0.15	ILE 44	0.48	Rpn2	THR 539	0.11	GLY 47	0.34	Rpn2	THR 539	0.08
						LYS 48	0.4	Rpn2	ASP 504	0.05	ALA 46	0.26	Rpn2	GLY 534	-0.01
	LEU 8	0.44	Rpn2	GLU 538	0.36	VAL 70	0.34	Rpn2	GLU 538	0.04	HIS 68	0.24	Rpn2	THR 499	-0.02
	ILE 44	0.35	Rpn2	TYR 535	0.34	LEU 8	0.27	Rpn2	THR 499	-0.09	LEU 8	0.19	Rpn2	ASP 504	-0.03
	ALA 46	0.33	Rpn2	ASP 504	0	LEU 69	0	Rpn2	GLN 540	-0.32	VAL 70	0.16	Rpn2	ASN 470	-0.13
	LYS 48	0.29	Rpn2	GLY 534	0	GLN 49	-0.11	Rpn2	GLN 503	-0.64	LYS 48	0.11	Rpn2	GLN 540	-0.27
	GLY 47	0.24	Rpn2	THR 499	-0.03	HIS 68	-0.11				THR 7	0.02	Rpn2	GLU 538	-0.29
	HIS 68	0.21	Rpn2	GLN 540	-0.13	THR 55	0.22	Rpn8	ASN 31	0.34	LYS 6	-0.24	Rpn2	GLN 503	-0.67
	VAL 70	0.2	Rpn2	TYR 502	-0.13	GLY 53	0.18	Rpn8	GLY 27	0.15					
	GLN 49	0.1	Rpn2	GLN 503	-0.74	ASP 52	0.08	Rpn8	ILE 26	0.12	GLY 53	0.2	Rpn8	ASN 31	0.32
	LYS 6	-0.83				ASP 58	-0.02	Rpn8	VAL 29	0.08	THR 55	0.13	Rpn8	TYR 27	0.27
						GLU 51	-0.21	Rpn8	GLY 30	0	ASP 52	0.11	Rpn8	GLY 30	0.18
	GLY 53	0.31	Rpn8	GLY 30	0.2	ARG 54	-1.29	Rpn8	ARG 25	0	GLU 51	-0.09	Rpn8	LYS 28	0.16
	THR 55	0.06	Rpn8	VAL 29	-0.08			Rpn8	GLN 32	-0.02	ARG 54	-0.71	Rpn8	ILE 26	0.04
	GLU 51	-0.01	Rpn8	GLY 27	-0.18			Rpn8	ASN 24	-0.04			Rpn8	VAL 29	0.02
	ASP 52	-0.28	Rpn8	ASN 31	-0.32			Rpn8	LYS 28	-0.28			Rpn8	ARG 25	-0.01
	ARG 54	-0.55	Rpn8	LYS 28	-0.42								Rpn8	GLN 32	-0.04
						GLU 24	-0.21	Rpn10	LYS 103	0.23			Rpn8	ASN 24	-0.05
Ub _{IV}						LEU 8	1.32	Rpn2	ALA 565	0.98	LEU 8	1.26	Rpn2	ALA 565	0.92
						LEU 73	1.19	Rpn2	ILE 529	0.51	LEU 73	1.11	Rpn2	VAL 533	0.64
						ILE 44	0.82	Rpn2	VAL 533	0.43	VAL 70	0.88	Rpn2	LEU 566	0.44
						VAL 70	0.58	Rpn2	GLY 534	0.36	HIS 68	0.88	Rpn2	ILE 529	0.4
						HIS 68	0.58	Rpn2	ALA 526	0.35	ILE 44	0.54	Rpn2	GLY 534	0.3
						ARG 74	0.3	Rpn2	LEU 566	0.28	ARG 72	0.36	Rpn2	ALA 526	0.2
						GLY 76	0.21	Rpn2	SER 569	0.27	GLN 49	0.13	Rpn2	GLU 530	0.15
						THR 9	0.11	Rpn2	GLU 538	0.14	GLY 76	0.1	Rpn2	TYR 535	0.12
						GLY 10	0.11	Rpn2	GLU 530	0.1	GLY 10	0.07	Rpn2	SER 569	0.09
						LYS 48	0.03	Rpn2	ASP 573	0.04	THR 7	-0.01	Rpn2	LEU 570	0.05
						LYS 11	0.03	Rpn2	MET 556	0.02	LEU 69	-0.03	Rpn2	ASP 564	-0.12
						GLN 49	0.02	Rpn2	LEU 570	0.01	THR 9	-0.05	Rpn2	GLU 568	-0.3
						THR 7	0.01	Rpn2	GLU 562	-0.03	LEU 71	-0.05	Rpn2	GLN 537	-0.59
						GLY 47	-0.03	Rpn2	LYS 534	-0.08	ALA 46	-0.06	Rpn2	GLU 538	-0.65
						LEU 71	-0.04	Rpn2	ASP 564	-0.17	GLY 47	-0.12	Rpn2	ARG 572	-0.84
						LYS 6	-0.14	Rpn2	ARG 572	-0.25	ARG 74	-0.56			
						ARG 42	-0.62	Rpn2	GLU 568	-0.27	LYS 6	-0.57			
						ARG 72	-1.3	Rpn2	LYS 574	-0.41	GLU 51	-0.02	Rpn8	LYS 199	-0.8
								Rpn2	GLN 537	-0.67	ARG 54	-0.1			
						ARG 54	-0.03	Rpn8	LYS 129	0.4					
						ASP 39	-0.09	Rpn8	LYS 199	-0.29	THR 55	0.07	Rpn9	MET 365	1.13
						GLU 51	-0.37				ASP 58	-0.02	Rpn9	GLU 368	-0.32
											GLY 53	-0.07	Rpn9	LYS 361	-0.56
											ARG 54	-0.98			
						THR 55	0.12	Rpn9	MET 365	1.22					
						LYS 48	0.06	Rpn9	THR 376	0.29					
						ASP 58	0.04	Rpn9	GLU 364	-0.1					
						GLY 53	-0.08	Rpn9	GLU 368	-0.18					
						GLU 51	-0.1	Rpn9	HIS 372	-0.18					
						ARG 54	-1.7	Rpn9	LYS 361	-0.85					