

Supplementary Information

Structural dynamics of the midnolin–proteasome during ubiquitin-independent substrate turnover

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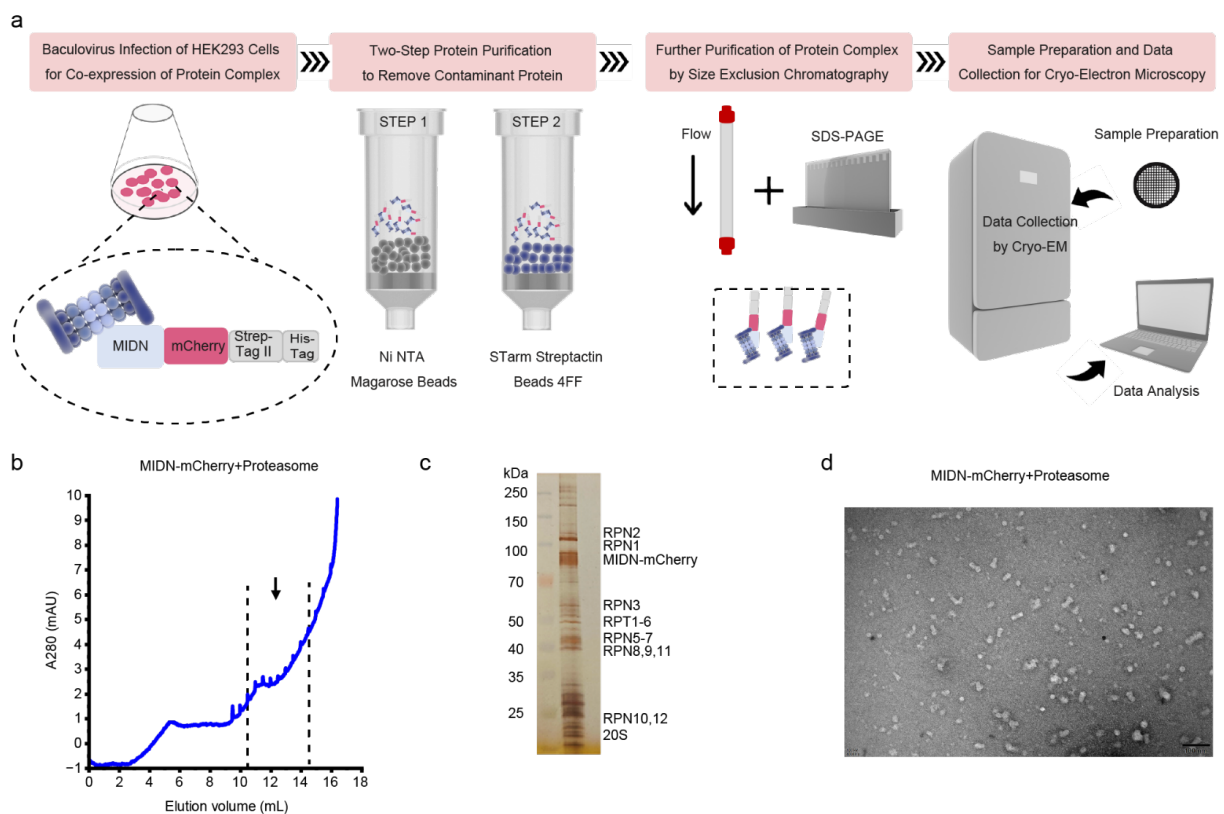
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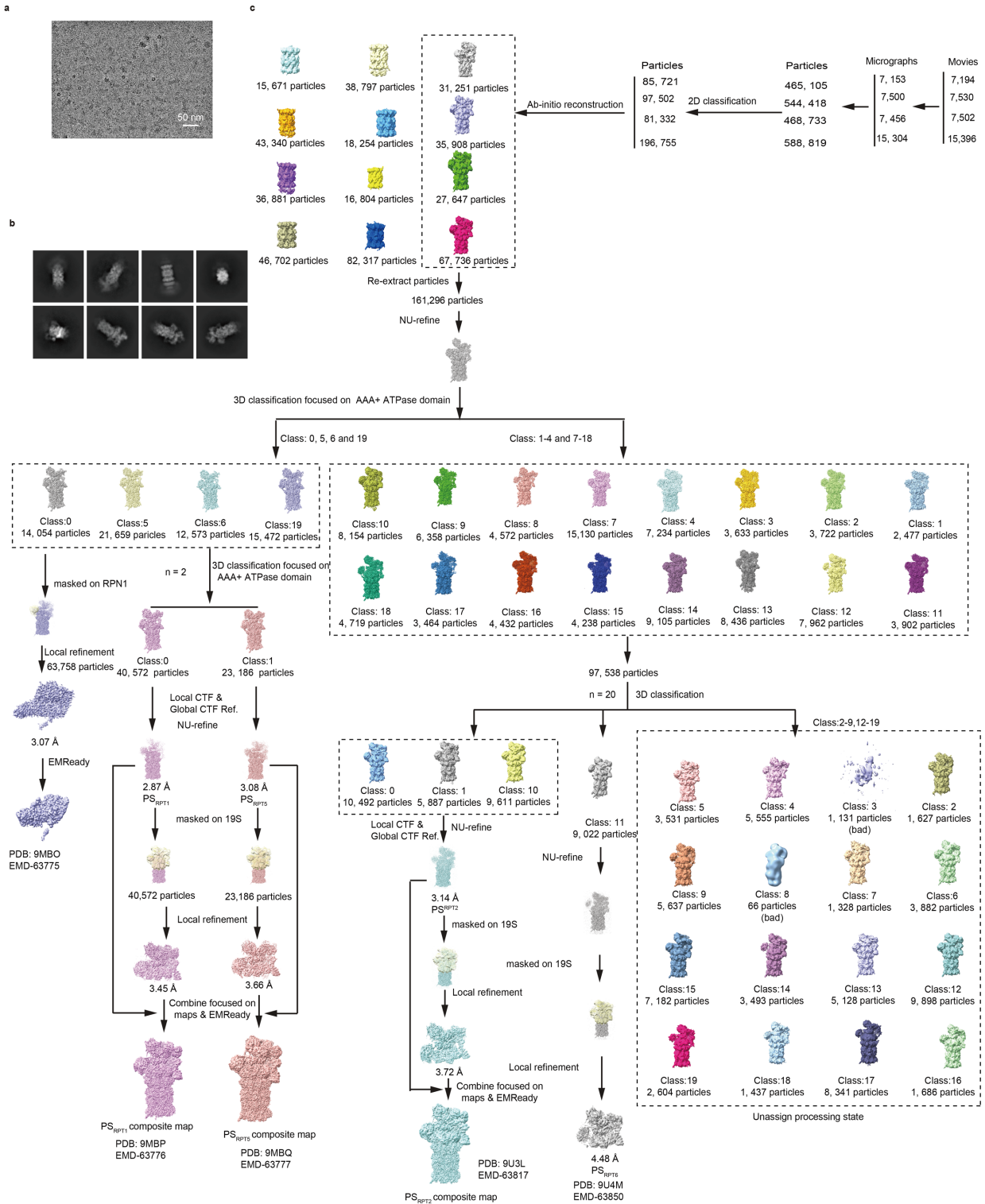
[#]These authors contributed equally to this work

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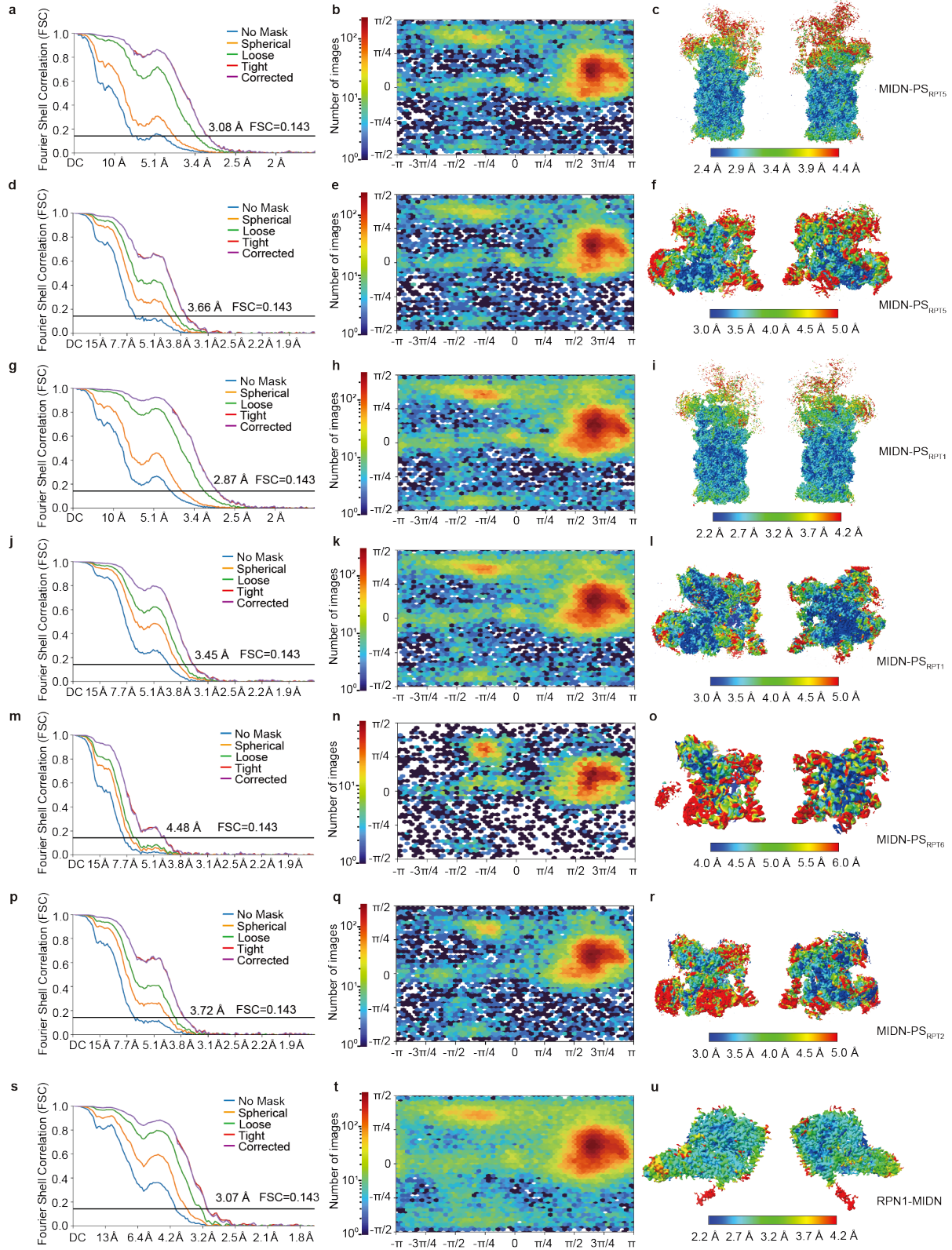
Supplementary Fig. 1 | Purification of MIDN-FL-proteasome complexes.

(a) Workflow diagram of protein complex purification and data acquisition for structural determination. (b) Size-exclusion chromatography (SEC, Superose 6) profile of MIDN-FL-Proteasome complex purification. (c) Silver-staining analysis of MIDN-FL-Proteasome complexes following SEC purification. (d) Representative transmission electron microscopy (TEM) micrograph of MIDN-mCherry-proteasome complexes.



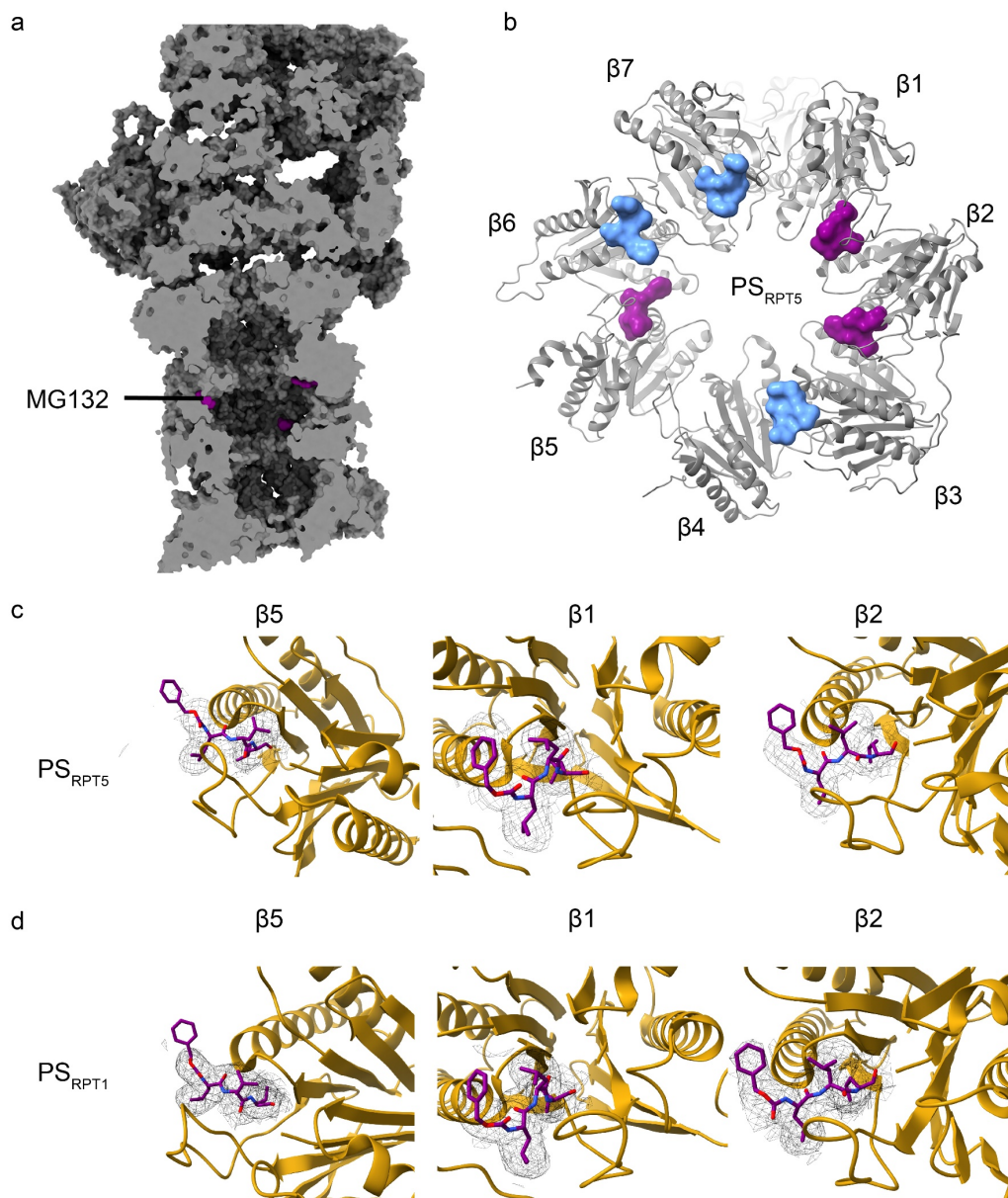
Supplementary Fig. 2 | Cryo-EM data processing workflow of MIDN-FL-human 26S proteasome complex.

(a) Representative micrographs for datasets. (b) Representative 2D class averages from the final subset of particles used in the reconstruction. (c) Cryo-EM processing pipeline. Focused masks are shown on 19S of the 3D volumes.



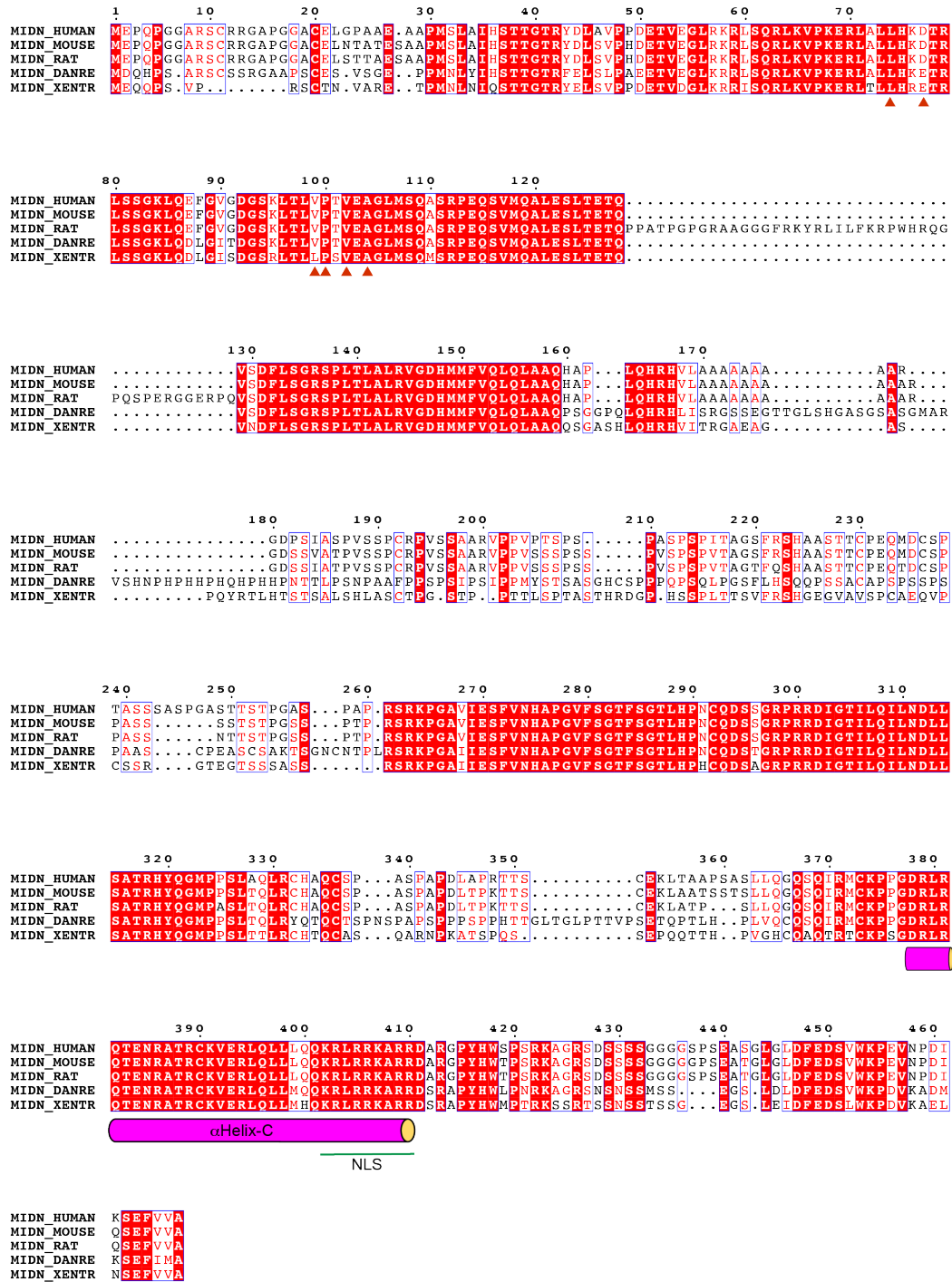
Supplementary Fig. 3 | Cryo-EM reconstructions and local resolution estimation.

(a, d, g, j, m, p, s) Gold-standard Fourier Shell Correlation (FSC) of the refined map. (b, e, h, k, n, q, t) Orientation distribution plot. (c, f, i, l, o, r, u) Local resolution estimates for the cryo-EM map.



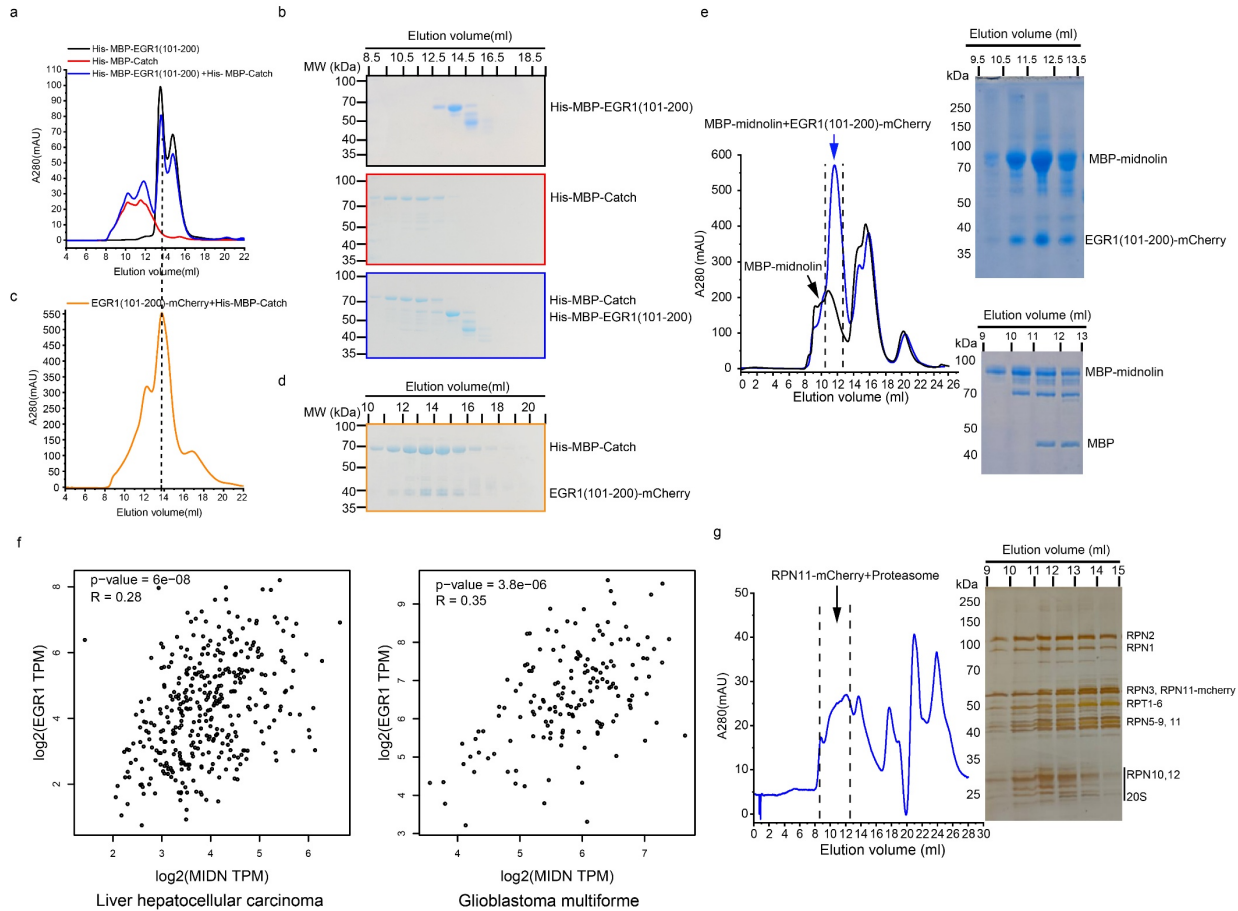
Supplementary Fig. 4 | Cryo-EM densities of MG132 bound to the proteasome.

(a) Overall surface representation of the proteasome (grey), shown in cross-section, with MG132 (purple) bound to three β -subunits of the core particle (CP) in the MIDN-PS_{RPT5} state. (b) Top view of the CP with seven subunits depicted in cartoon representation (grey). In this view from the MIDN-PS_{RPT5} state, MG132 molecules bound to the three β -subunits ($\beta 1$, $\beta 2$, $\beta 5$) of the distal ring are colored purple, while those bound to the equivalent subunits of the proximal (symmetry-related) ring are colored blue. The $\beta 1$ (PSMB6), $\beta 2$ (PSMB7), and $\beta 5$ (PSMB5) subunits are annotated with their respective catalytic activities: aspartyl protease-like, neutral protease, and carboxypeptidase activities. (c) Cryo-EM density map (grey mesh) of MG132 (purple) bound to three β -subunits of the CP (dark goldenrod) in the MIDN-PS_{RPT5} state. (d) Cryo-EM density map (grey mesh) of MG132 (purple) bound to three β -subunits of the CP (dark goldenrod) in the MIDN-PS_{RPT1} state.



Supplementary Fig. 5 | Cross-species conservation analysis of MIDN protein sequences.

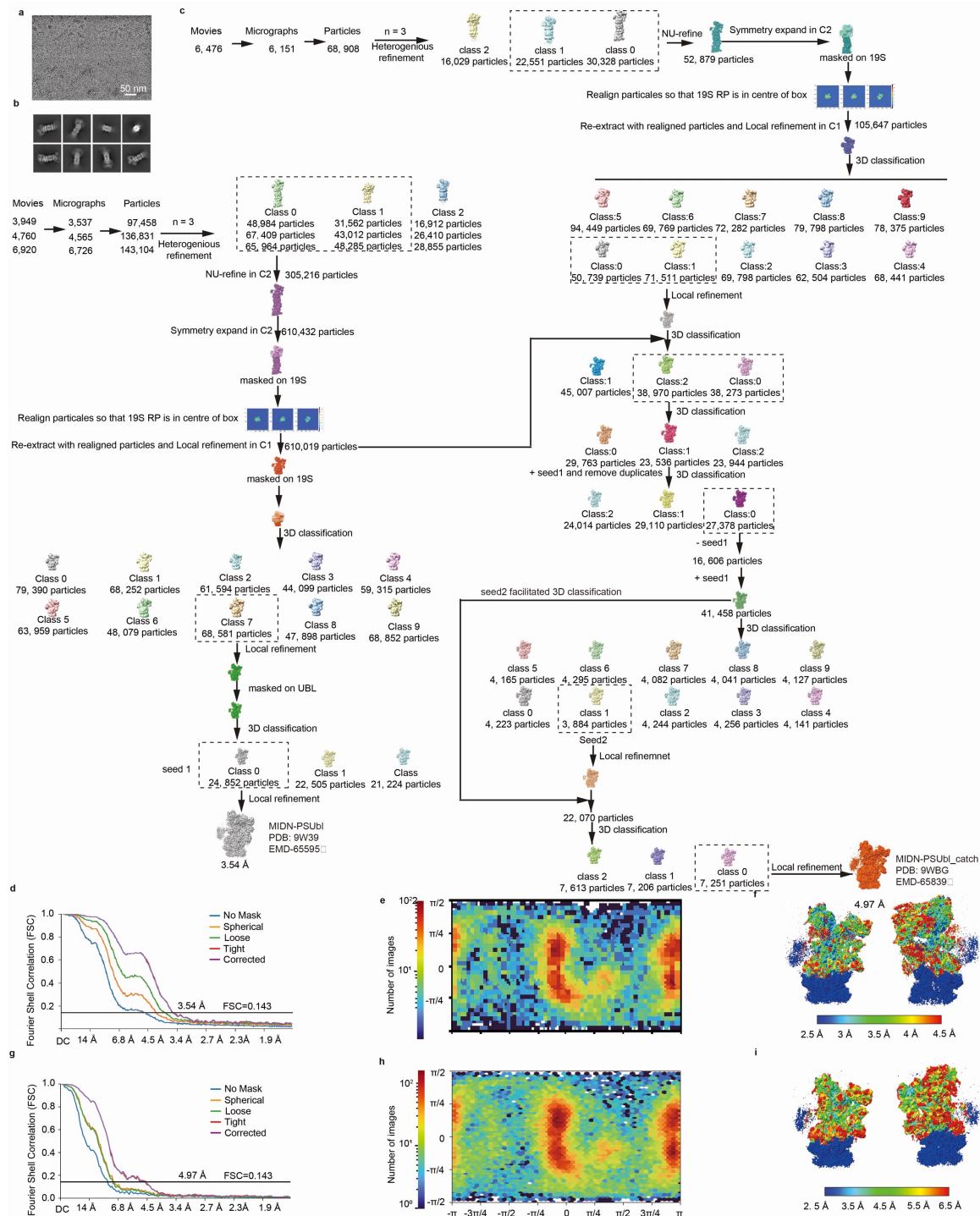
The MIDN sequences are derived from the following species: Human (*Homo sapiens*); Mouse (*Mus musculus*); Rat (*Rattus norvegicus*); DANRE (*Danio rerio*); XENTR (*Xenopus tropicalis*). αHelix-C for binding with RPN1 and NLS are labelled and overlapped. Residues for binding with RPN11 are denoted by red triangle.



Supplementary Fig. 6 | Midnolin correlates with substrates and purification of proteasome.

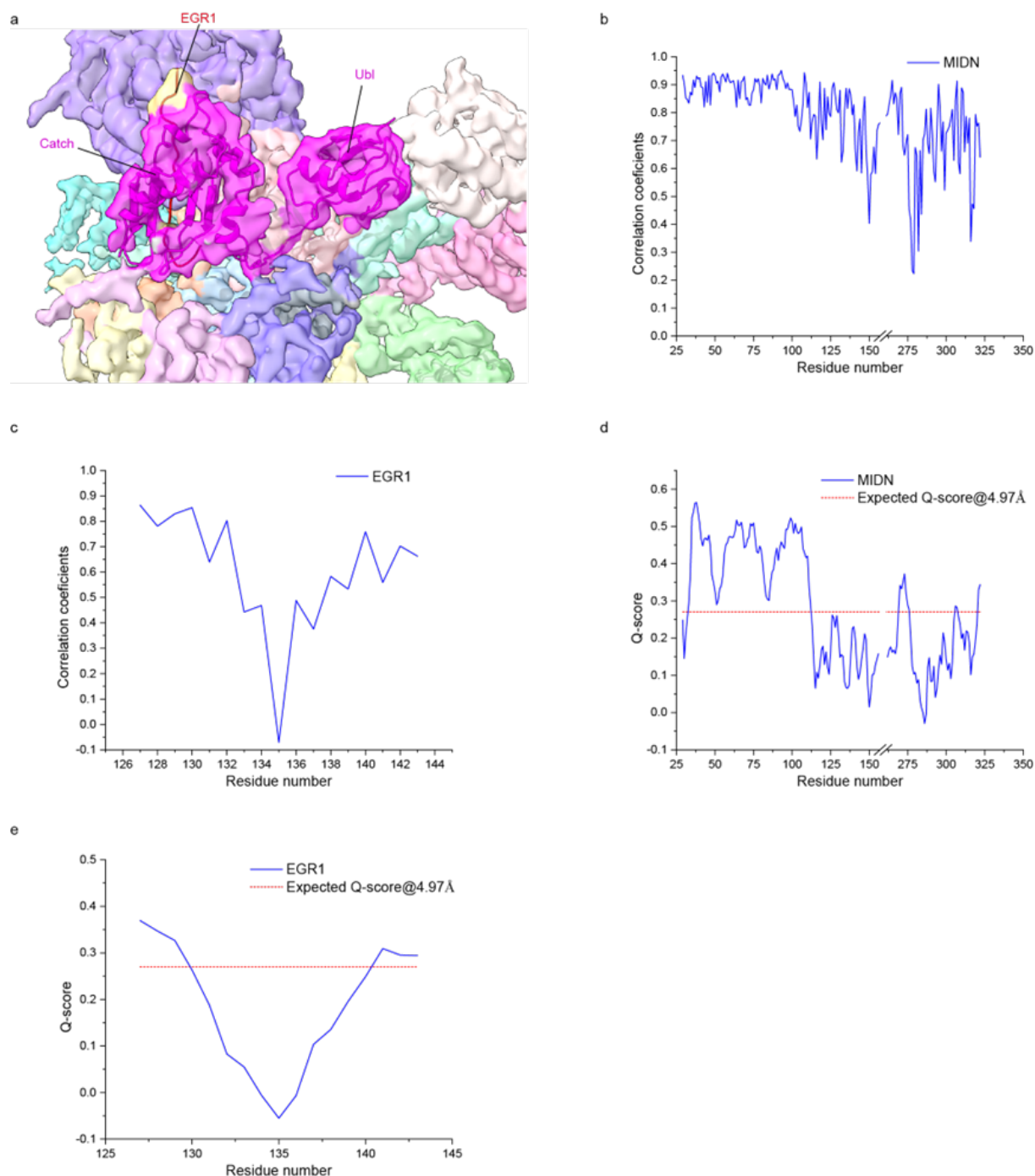
(a) and (b) *In vitro* interaction between Catch and its substrate EGR1(101-200). Gel filtration profiles show the interaction between His-MBP-Catch and His-MBP-EGR1(101-200) in a Superdex200 Increase 10/300 size exclusion chromatography column. His-MBP-EGR1(101-200) is excessive. Fractions (0.5 ml each) corresponding to the elution were analyzed by SDS-PAGE and stained by Coomassie brilliant blue. His-MBP-Catch and His-MBP-EGR1(101-200) do not exhibit co-elution, indicating that they cannot form a complex efficiently *in vitro*. Size-exclusion chromatography elution profiles were generated for both individual proteins. Black: His-MBP-EGR1 (101-200); Red: His-MBP-midnolin-Catch; *In vitro* incubated complex (blue) ($n = 2$). (c) and (d) *In vivo* interaction between Catch and its substrate EGR1(101-200). The complex is purified by affinity purification through His tag. Gel filtration profiles show the interaction between His-MBP-Catch and EGR1(101-200)-mCherry. Fractions (0.5 ml each) corresponding to the elution were analyzed by SDS-PAGE and stained by Coomassie brilliant blue MBP-Catch and EGR1(101-200)-mCherry co-elutes, indicating that they form a stable complex efficiently *in vivo* ($n = 2$). (e) Overlay of size-exclusion chromatography profiles for MBP-midnolin purified alone (left, black line) and the co-expressed MBP-midnolin and EGR1(101-200)-mCherry complex (left, blue line). Coomassie brilliant blue staining analysis of the purified samples revealed that MBP-midnolin co-expressed with the substrate EGR1(101-200)-mCherry exhibited a homogeneous and monodisperse state (right top), whereas MBP-midnolin expressed alone contained high-order oligomeric states (right bottom). (f) Scatter plot illustrating the positive correlation between MIDN and EGR1 gene expression levels in liver hepatocellular carcinoma and glioblastoma, analyzed by

GEPIA. The x-axis represents the log₂-transformed Transcripts Per Million (TPM) values of MIDN, while the y-axis corresponds to the log₂-transformed TPM values of EGR1. The correlation coefficient (R) is 0.28 and 0.35, indicating a moderate positive correlation, and the p-value is 6e-08 and 3.8e-06, denoting statistical significance. Each point representing an individual sample. This analysis supports the hypothesis that active MIDN expression is contingent upon its substrates, such as EGR1. (g) Purification of proteasome with RPN11-mCherry.



Supplementary Fig. 7 | Cryo-EM data processing workflow of MIDN-EGR1-human 26S proteasome complex.

(a) Representative micrographs for datasets. (b) Representative 2D class averages from the final subset of particles used in the reconstruction. (c) Cryo-EM processing pipeline. (d,g) Gold-standard Fourier Shell Correlation (FSC) of the refined map. (e,h) Orientation distribution plot. (f,i) Local resolution estimates for the cryo-EM map.

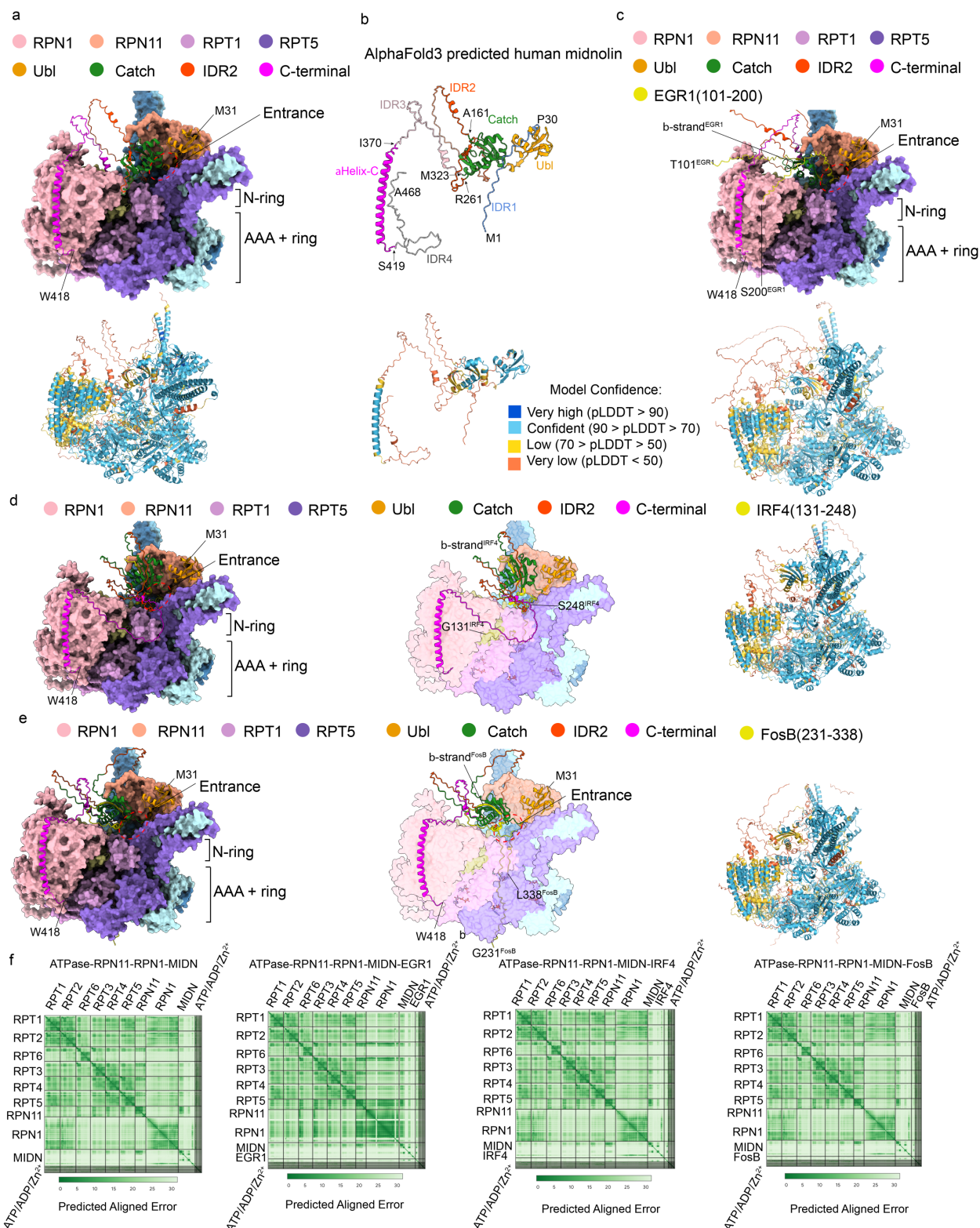


Supplementary Fig. 8 | Local quality of model fitting to cryo-EM density maps.

(a) close-up view of the model in relation to the map for MIDN and EGR1.

(b-c) Fitted models were analyzed with PHENIX and their per-residue correlation coefficients (CC) are plotted, b for MIDN and c for EGR1.

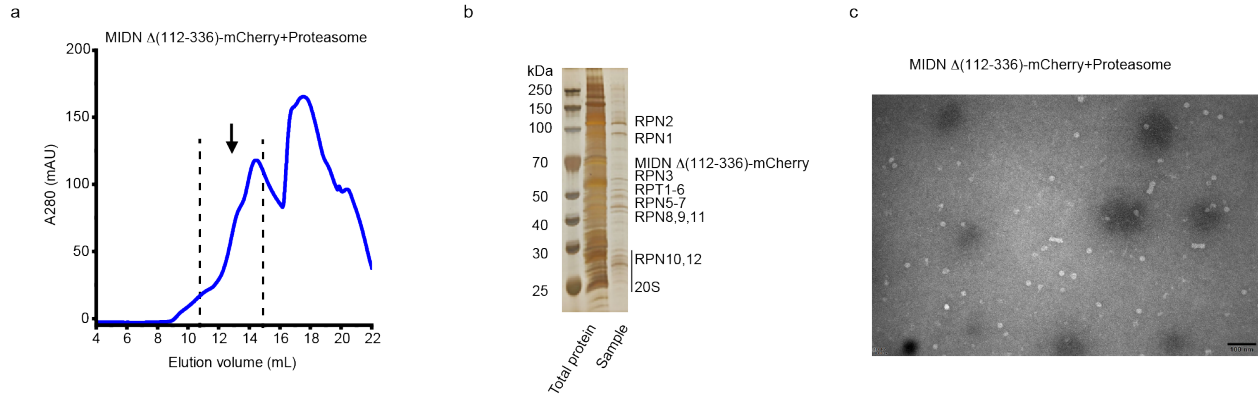
(d-e) Q-score plot of MIDN (d) and EGR1 (e) chains. The expected Q-score at 4.97 Å resolution is shown as red dashed lines. The 5-residue average of Q-scores is plotted here.



Supplementary Fig. 9 | The molecular mechanism of Ubl and Catch domains coordinate the transfer of substrates to proteasomes.

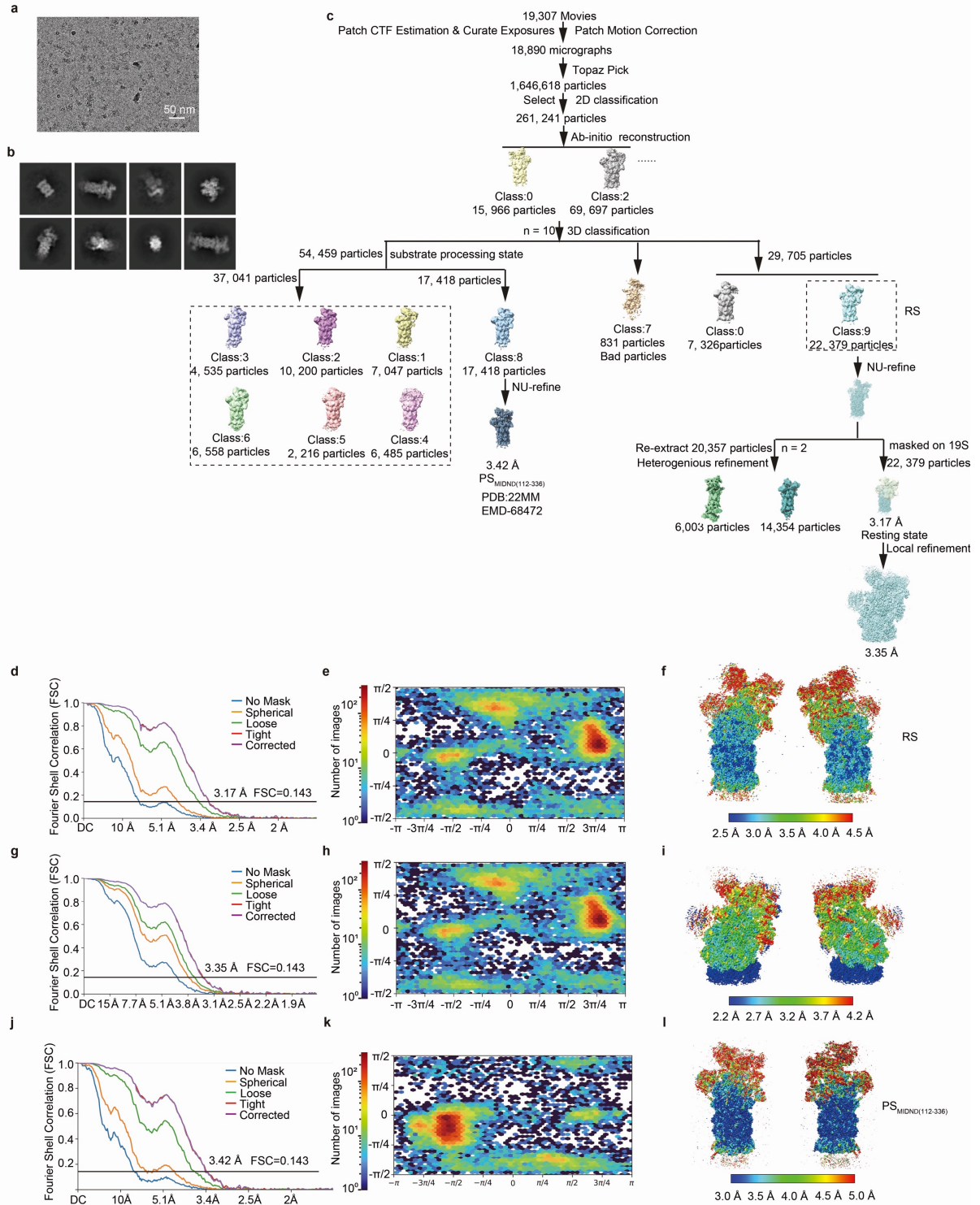
(a) AlphaFold3 prediction of midnolin-RPN1-RPN11-ATPase. The Catch domain's IDR2 (161-261, dark orange) dock into the the N-ring pore entrance. α Helix-C(magenta); Ubl (dark orange);

Catch (green); RPN1 (light pink); RPN11 (dark pink); RPT1 (light pruple); RPT5(dark purple). (b) AlphaFold3 predicted midnolin shows four IDRs. The numbers denote the boundaries of these IDRs. IDR2 is inserted into the Catch domain (green). (c-e) AlphaFold3 prediction of midnolin-RPN1-RPN11-ATPase with EGR1 (101-200) (c), IRF4(131-248) (d) and FosB (231-338) (e), respectively. These substrates dock into the the N-ring pore entrance. α Helix-C: magenta, Ubl: dark orange; Catch: green, EGR1 (101-200, yellow), IRF4(131-248, yellow), FosB (231-338, yellow), RPN1 (light pink), RPN11 (dark pink), RPT1 (light pruple), and RPT5(dark purple). (a-e), the predicted structures are also colored by pLDDT (AlphaFold3 pattern as indicated). (f), PAE plot of the AlphaFold3 model. The color scale indicates the expected aligned error in Å.



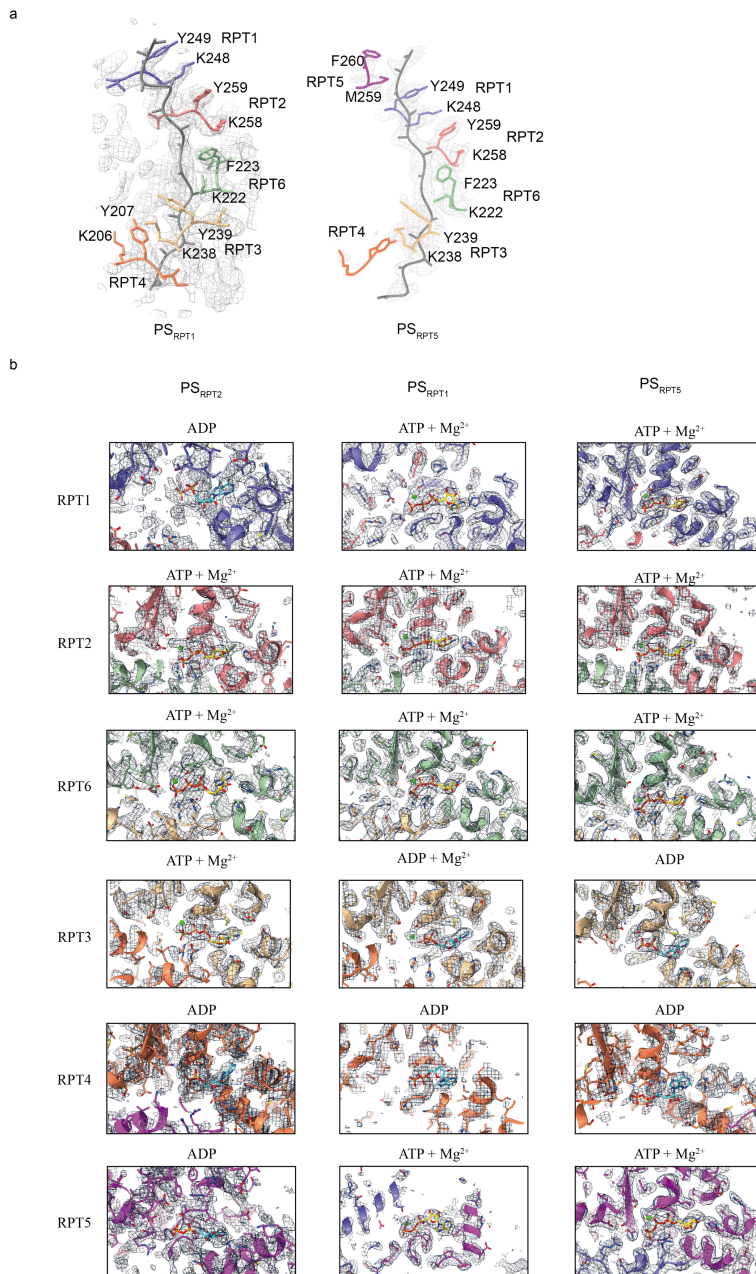
Supplementary Fig. 10 | Purification of MIDN Δ (112-336)-proteasome.

(a) Size-exclusion chromatography (Superose6) profile of MIDN Δ (112-336)-proteasome complex purification. (b) Silver-staining analysis of MIDN Δ (112-336)-proteasome complexes following SEC purification. (c) Representative transmission electron microscopy (TEM) micrograph of MIDN Δ (112-336)-proteasome complexes.



Supplementary Fig. 11 | Cryo-EM data processing workflow of MIDN $\Delta(112-336)$ -26S proteasome complex.

(a) Representative micrographs for datasets. (b) Representative 2D class averages from the final subset of particles used in the reconstruction. (c) Cryo-EM data processing pipeline. (d, g, j) Gold-standard Fourier Shell Correlation (FSC) of the refined map. (e, h, k) Orientation distribution plot. (f, i, l) Local resolution estimates for the cryo-EM map.



Supplementary Fig. 12 | Mg^{2+} coordination during proteasomal substrate degradation.

(a) Cryo-EM density maps for pore-1 loop residues of RPTs interacting with substrate in PS_{RPT1} and PS_{RPT5} . (b) The ADP/ATP- Mg^{2+} binding architecture with coordinating residues in PS_{RPT2} / PS_{RPT1} / PS_{RPT5} and corresponding cryo-EM densities.

Supplementary Table 1 | Cryo-EM data collection, refinement and validation statistics

	RPN1-MIDN 9MBO EMD-63775	MIDN- PS _{RPT1} 9MBP EMD- 63776	MIDN- PS _{RPT5} 9MBQ EMD- 63777	MIDN- PS _{RPT2} 9U3L EMD- 63817	MIDN- PS _{RPT6} 9U4M EMD- 63850	RS 9U7R EMD- 63943
Data collection and processing						
Magnification	105,000 x	105,000 x	105,000 x	105,000 x	105,000 x	105,000 x
Voltage (kV)	300	300	300	300	300	300
Electron exposure (e-/Å ²)	40	40	40	40	40	40
Defocus range (mm)			-0.8 to -1.8			
Pixel size (Å)			0.85			
Symmetry imposed	C1	C1	C1	C1	C1	C1
Initial particle images (no.)	2,467,120	2,467,120	2,467,120	2,467,120	2,467,120	1,394,981
Final particle images (no.)	63,758	40,572	23,186	25,990	9,022	22,379
Map resolution (Å)	3.07	2.87	3.08	3.14	4.48	3.17
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143
Refinement						
Initial model used (PDB code)	AlphaFold2	6MSK	9MBP	6MSJ	9E8K	6MSG
Model resolution (Å)	2.83	2.72	2.41	2.93	4.26	2.96
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143
Map sharpening B factor (Å ²)	-67.8	-37	-68.3	-27.9	-15	-29.5
Model composition						
Non-hydrogen atoms	6770	105160	105726	104944	46918	66563
Protein residues	871	13456	13523	13434	5894	8618
Ligands	0	LDZ: 6 Zn ²⁺ : 1 Mg ²⁺ : 5 ATP: 4 ADP: 2	LDZ: 6 Zn ²⁺ : 1 Mg ²⁺ : 4 ATP: 4 ADP: 2	LDZ: 6 Zn ²⁺ : 1 Mg ²⁺ : 3 ATP: 3 ADP: 3	Zn ²⁺ : 1 Mg ²⁺ : 1 ADP: 3 ATP: 3	LDZ: 6 Zn ²⁺ : 1 Mg ²⁺ : 4 ATP: 4 ADP: 2
B-factors (Å ²)						
Protein	106.83	85.93	77.19	102.25	269.72	70.58
Ligand	-	55.44	79.12	90.58	261.47	71.53
R.m.s. deviations						
Bond lengths (Å)	0.003	0.004	0.010	0.009	0.004	0.004
Bond angles (°)	0.560	0.801	0.983	0.972	0.803	0.716
Validation						
MolProbity score	1.56	2.46	2.67	2.52	2.38	2.14
Clashscore	4.34	12.00	18.90	19.24	26.22	8.79
Poor rotamers (%)	2.04	3.35	3.23	2.14	0.12	2.47
Ramachandran plot						
Favored, %	97.45	92.38	91.45	92.06	92.43	94.6
Allowed, %	2.55	6.99	7.97	7.53	6.9	5.29
Disallowed, %	0	0.64	0.58	0.41	0.67	0.11

Supplementary Table 1 (continued) | Cryo-EM data collection, refinement and validation statistics

	MIDN- PS _{Ubl} 9W39 EMD- 65595	MIDN- PS _{Ubl_Catch} 9WBG EMD- 65839	MIDN- PS _{Δ(112-336)} 22MM EMD- 68472
Data collection and processing			
Magnification	105,000 x	105,000 x	105,000 x
Voltage (kV)	300	300	300
Electron exposure (e-/Å ²)	40	40	40
Defocus range (mm)	-0.8 to -1.8		
Pixel size (Å)	0.85		
Symmetry imposed	C1	C1	C1
Initial particle images (no.)	1,012,249	1,296,193	1,394,981
Final particle images (no.)	24,852	7,251	17,418
Map resolution (Å)	3.54	4.97	3.42
FSC threshold	0.143	0.143	0.143
Refinement			
Initial model used (PDB code)	6MSG	9W39	9MBP
Model resolution (Å)	3.26	4.19	3.21
FSC threshold	0.143	0.143	0.143
Map sharpening B factor (Å ²)	-38.6	-11.1	-25.4
Model composition			
Non-hydrogen atoms	49255	50210	103249
Protein residues	6178	6303	13207
Ligands	UNK: 7	UNK: 7	LDZ: 6
	Zn ²⁺ : 1	Zn ²⁺ : 1	Zn ²⁺ : 1
	Mg ²⁺ : 3	Mg ²⁺ : 3	Mg ²⁺ : 5
	ATP: 4	ATP: 4	ATP: 4
	ADP: 2	ADP: 2	ADP: 2
B-factors (Å ²)			
Protein	99.43	202.16	111.19
Ligand	102.94	186.92	72.32
R.m.s. deviations			
Bond lengths (Å)	0.005	0.004	0.004
Bond angles (°)	0.898	0.837	0.835
Validation			
MolProbity score	2.15	2.26	2.11
Clashscore	15.95	21.08	14.14
Poor rotamers (%)	0.52	0.07	0.42
Ramachandran plot			
Favored, %	93.16	93.11	92.97
Allowed, %	6.47	6.63	6.61
Disallowed, %	0.37	0.26	0.41

Supplementary Table 2 | Detailed information of Thermal Stability Analysis of Proteins

Samples	T _m	Excitation Power	Start Temperature	End Temperature	Temperature Slope
RPN1	49.5°C	10%	20°C	95°C	1.0°C/min
	49.5°C				
	49.6°C				
MIDN (337-432)	53.6°C				
	53.5°C				
	53.5°C				
MIDN (337-468)	54.3°C				
	54.3°C				
	54.3°C				
MIDN (337-432) + RPN1	55.0°C				
	55.3°C				
	55.2°C				
MIDN (337-468) + RPN1	55.8°C				
	55.8°C				
	55.7°C				

Supplementary Table 3 | Detailed information of ITC experimental results

Samples	Thermodynamic parameters	Value (KJ/mol)
MIDN (337-468) to RPN1	ΔH	-96.8 $\pm$ 5.9
	Offset	-8.6 $\pm$ 2.1
	ΔG	-38.9
	-T ΔS	57.9
	ΔH	-96.0 $\pm$ 6.3
MIDN (337-432) to RPN1	Offset	0
	ΔG	-36.5
	-T ΔS	59.4

Supplementary Table 4 | Detailed information of SPR experimental results

Samples	K _D (M)	K _a (M ⁻¹ s ⁻¹)	K _d (s ⁻¹)	R _{max}	Chi ²
MIDN (337-468) to RPN1	3.904e-07	1.151e+04	4.492e-03	53.5424	1.6
MIDN (337-432) to RPN1	6.630e-07	8.405e+03	5.573 e-03	37.0597	0.57

Supplementary Table 5 | Summary of key structural features of MIDN-bound proteasome states

	MIDN-PS _{RPT6}	MIDN-PS _{RPT2}	MIDN-PS _{RPT1}	MIDN-PS _{RPT5}
Pore-loop staircase contacting substrate (from top to bottom)	RPT6 RPT3 RPT4 RPT5	RPT2 RPT6 RPT3 RPT4	RPT1 RPT2 RPT6 RPT3 RPT4	RPT5 RPT1 RPT2 RPT6 RPT3
RPT subunits disengaged from pore loop staircase	RPT2 RPT1	RPT1 RPT5	RPT5	RPT4
RPT subunits with ADP	RPT2 RPT1 RPT5	RPT1 RPT5 RPT4	RPT4 RPT3	RPT4 RPT3
Exchange compared to previous state	N/A	RPT2	RPT1 RPT5	no
Hydrolysis compared to previous state	N/A	RPT4	RPT3	no
Mg ²⁺	N/A	N/A	RPT4 no RPT3 yes	RPT4 no RPT3 no
Move down (RPT3, 238Ca)	N/A	N/A	yes, 6.1 Å	yes, 2.3 Å
Particles	9022	25990	40572	23186
Resolution	4.48 Å	3.14 Å	2.87 Å	3.08 Å

Supplementary Table 6 | Summary of key structural features of TXNL1-bound proteasome states

	PS _{RPT6} (9E8K)	PS _{RPT2} (9E8O)	PS _{RPT1} (9E8J)	PS _{RPT5} (9E8G)
Pore-loop staircase contacting substrate (from top to bottom)	RPT6 RPT3 RPT4 RPT5 RPT1	RPT2 RPT6 RPT3 RPT4 RPT5	RPT1 RPT2 RPT6 RPT3 RPT4	RPT5 RPT1 RPT2 RPT6 RPT3
RPT subunits disengaged from pore loop staircase	RPT2	RPT1	RPT5	RPT4
RPT subunits with ADP	RPT2 RPT1 RPT5 RPT4	RPT1 RPT5 RPT4 RPT3	RPT4 RPT3	RPT4 RPT3 RPT6
Exchange compared to previous state	N/A	RPT2	RPT1 RPT5	no
Hydrolysis compared to previous state	N/A	RPT3	no	RPT6

Supplementary Table 7 | List of primers used in this study.

Name	Sequence (5'→3')
MIDN-F	CAGCTCTTAAGGGAATTC _{Gccacc} ATGGAGCCGCAGCCCCGCCG
MIDN-R	GAAAATACAGGTTTTCTCTAGA _{agc} CACCACGaACTCTGACTTG
His-RPN1-F	AAACCTGTATTTTCAGGGCATGGAGGAGGGAGGCGGGACAA GGC GCCG
His-RPN1-R	TGGTGGTGGTGGTGGTCTCGAGTCAGAGATCATAATTGGGGTTCT TCCGAAGGATAACAAA
His-MBP-MIDN(337-432)-F	AAACCTGTATTTTCAGGGCGAATTCATGTGCAAGCCCCCGGG GGA
His-MBP-MIDN(337-432)-R	TGAAAATACAGGTTTTCTCTAGAGCTGCTACTGCTGTCGCTGCG G
His-MBP-MIDN(337-468)-F	AAACCTGTATTTTCAGGGCGAATTCATGTGCAAGCCCCCGGG GGA
His-MBP-MIDN(337-468)-R	TGAAAATACAGGTTTTCTCTAGA _{agc} CACCACGaACTCTGACTT
His-MBP-MIDN(108-335)-His-F	ACCGAAAACCTGTATTTTCAGGGCTCTCAGGCTTCTCGTCCGG AA
His-MBP-MIDN(108-335)-His-R	ATCTCAGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT CT
His-MBP-EGR1(101-200)-F	ACCGAAAACCTGTATTTTCAGGGCACCGCTGAATCTTTCCC GG ACA
His-MBP-EGR1(101-200)-R	AGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT AGACGGA
MBP-MIDNΔ(112-336)-F	AAACCTGTATTTTCAGGGCGAATTCATGGAGCCGCAGCCCC GG CGG
MBP-MIDNΔ(112-336)-R	TGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT GAgcCACCACGaACTCTGACTT
MBP-MIDN(1-111)-EGR1(101- 200)-MIDN(337-468)-F	AAACCTGTATTTTCAGGGCGAATTCATGGAGCCGCAGCCCC GG CGG
MBP-MIDN(1-111)-EGR1(101- 200)-MIDN(337-468)-R	TGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT GAgcCACCACGaACTCTGACTT
EGR1(101-200)-mCherry-F	ATAAGAAGGAGATATACATATGACCGCTGAATCTTTCCC GG GGA
EGR1(101-200)-mCherry-R	TGAAAATACAGGTTTTTCAGAAGAGTCGTTAGACGGAACA

Protein Sequences

The following proteins were expressed in bacteria using a pET-28a(+) plasmid:

His-RPN1 (PSMD2)

MGSSHHHHHHSSGENLYFQGMEEGGRDKAPVQPQQSPAAAPGGTDEKPSGKERRDAG
DKDKEQELSEEDKQLQDELEMLVERLGEKDTSLYRPALEELRRQIRSSSTTSMTSVPKPLK
FLRPHYGKLKEIYENMAPGENKRFAADIISVLAMTMSGERECLKYRLVGSQEELASWG
HEYVRHLAGEVAKIEWQELDDAEKVQREPLLTLVKEIVPYNMAHNAEHEACDLLMEIE
QVDMLEKIDENAYAKVCLYLTSCVNYVPEPENSALLRCALGVFRKFSRFPEALRLAL
MLNDMELVEDIFTSCKD VVVQKQMAFMLGRHGVFLELSEDEVEEYEDLTEIMSNVQLNS
NFLALARELDIMEPKVPDDIYKTHLENNRFGSGSQVDSARMNLASSFVNGFVNAAF
QDKLLTDDGNKWLYKNKDHGMLSAAASLGMILLWDVDGGLTQIDKYLYSSEDIKSG
ALLACGIVNSGVRNECDPALALLSDYVLHNSNTMRLGSIFGLGLAYAGSNREDVLTLL
PVMGDSKSSMEVAGVTALACGMIAVGSCNGDVTSTILQTIMEKSETELKDTYARWLPL
GLGLNHLGKGAEIAEILAALEVSEPFRRSFANTLVDCAYAGSGNVLKVQQLLHICSEHF
DSKEKEEDKDKKEKKDKKKEAPADMGAHQGVAVLGIALIAMGEEIGAEMALRTFGH
LLRYGEPTLRRVPLALALISVSNPRLNILDTLKFSHDADPEVSYSNIFAMGMVGSNTN
NARLAAMLRLQAQYHAKDPNNLFMVRLAQGLTHLGKGTLTLCYPYHSDRQLMSQVAV
AGLLTVLVSF LDVRNII LGKSHYVLYGLVAAMQPRMLVTFDEELRPLPVSVRVGQAVD
VVGQAGKPKTITGFQTHHTPVLLAHGERAE LATEEFLPVTPILEGFVILRKNPNYDL

His-MBP-MIDN (337-432)-mCherry-His-Strep

MGHHHHHHHKIEEGKLVIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLEEKFPQV
AATGDGPDIIFFWAHDFRGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIA
VEALSIIYNKD LLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYA
FKYENGKYDIKDVGVNDNAGAKAGLTFLVDLIK NKHMNADTDYSIAEAAFNKGETAMTI
NGPWAWSNIDTSKVN YGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLL
TDEGLEAVNKDKPLGAVALKS YEEELAKDPRIAATMENAQKGEIMPNI PQMSAFWYAV
RTAVINAASGRQTVDEAL KDAQTDYDIPTTENLYFQGEFMSPASPAPDLAPRTTSCEKLT
AAPSASLLQGQSQIRMCKPPGDRLRQTENRATRCKVERLQLLLQKRLRRKARRDARG
PYHWSPSRKAGRS DSSSENLYFQGAMVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEF
EIEGEGEGRPYEGTQTAKLK VTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDY LKLS
FPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFP SDGPVMQKKTMGW
EASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDI
TSHNEDYTIVEQYERA EGRHSTGGMDELYKHHHHHHS AW SHPQFEKGGGSGGGSGGS
AW SHPQFEK

His-MBP-MIDN (337-468)-mCherry-His-Strep

MGHHHHHHHKIEEGKLVIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLEEKFPQV
AATGDGPDIIFFWAHDFRGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIA
VEALSIIYNKD LLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYA
FKYENGKYDIKDVGVNDNAGAKAGLTFLVDLIK NKHMNADTDYSIAEAAFNKGETAMTI
NGPWAWSNIDTSKVN YGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLL
TDEGLEAVNKDKPLGAVALKS YEEELAKDPRIAATMENAQKGEIMPNI PQMSAFWYAV
RTAVINAASGRQTVDEAL KDAQTDYDIPTTENLYFQGEFMSPASPAPDLAPRTTSCEKLT
AAPSASLLQGQSQIRMCKPPGDRLRQTENRATRCKVERLQLLLQKRLRRKARRDARG
PYHWSPSRKAGRS DSSSSGGGSPSEASGLGLDFEDSVWKPEANPDIKSEFVVAENLYF

QGAMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTK
GGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVT
QDSSLQDGEFIYKVKLRGTNFPDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLK
LKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTG
GMDELYKHHHHHSAWSHPQFEKGGGSGGGSGGSAWSHPQFEK

His-MBP-Catch-His

MGHHHHHHKIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQV
AATGDGPDIIFFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIA
VEALSLIYNKDILLNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYA
FKYENGKYDIKDVGVNDNAGAKAGLTFLVDLIKNNKHMNADTDYSIAEAAFNKGETAMTI
NGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLL
TDEGLEAVNKDKPLGAVALKSYYYEELAKDPRIAATMENAAQKGEIMPNIQMSAFWYAV
RTAVINAASGRQTVDEALKDAQTDYDIPTTENLYFQGSQASRPEQSVMQALESLTETQV
SDFLSGRSPLTLALRVGDHMMFVQLQLAAQHAPLQHRHVLAAAAAAAAAARGDPSIASP
VSSPCRPVSSAARVPPVPTSPSPASPSPTAGSFRSHAASTTCPEQMDCSPTASSSASPGAS
TTSTPGASAPRSRKPGAVIESFVNHAPGVFSGTFSGTLHPNCQDSSGRPRRDIGTILQILN
DLLSATRHYQGMPPSLAQLRCHAQHSHHHHH

His-MBP-EGR1(101-200)

MGHHHHHHKIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQV
AATGDGPDIIFFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIA
VEALSLIYNKDILLNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYA
FKYENGKYDIKDVGVNDNAGAKAGLTFLVDLIKNNKHMNADTDYSIAEAAFNKGETAMTI
NGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLL
TDEGLEAVNKDKPLGAVALKSYYYEELAKDPRIAATMENAAQKGEIMPNIQMSAFWYAV
RTAVINAASGRQTVDEALKDAQTDYDIPTTENLYFQGTAESEFDPISLNNEKVLVETSYP
QTTRLPPITYTGRFSLEPAPNSGNTLWPEPLFSLVSGLVSMNTNPPASSSSAPSPAASSASAS
QSPPLSCAVPSNDSS

His-MBP-MIDN^{P100E}

MGHHHHHHKIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQV
AATGDGPDIIFFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIA
VEALSLIYNKDILLNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYA
FKYENGKYDIKDVGVNDNAGAKAGLTFLVDLIKNNKHMNADTDYSIAEAAFNKGETAMTI
NGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLL
TDEGLEAVNKDKPLGAVALKSYYYEELAKDPRIAATMENAAQKGEIMPNIQMSAFWYAV
RTAVINAASGRQTVDEALKDAQTDYDIPTTENLYFQGEFMPEPQPGGARSCRREGAPGGA
CELGPAAEAAPMSLAHSTTGTRYDLAVPPDETVEGLRKRLSQRLKVPKERLALLHKDT
RLSSGKLQEFVGVGDGSKLTLVETVEAGLMSQASRPEQSVMQALESLTETQVSDFLSGRS
PLTLALRVGDHMMFVQLQLAAQHAPLQHRHVLAAAAAAAAAARGDPSIASPVSSPCRPV
SSAARVPPVPTSPSPASPSPTAGSFRSHAASTTCPEQMDCSPTASSSASPGASTTSTPGAS
PAPRSRKPGAVIESFVNHAPGVFSGTFSGTLHPNCQDSSGRPRRDIGTILQILNDLLSATR
HYQGMPPSLAQLRCHAQCSPAPDLAPRTTSCEKLTAAPSASLLQGQSQIRMCKPPG
DRLRQTENRATRCKVERLQLLLQKRLRRKARRDARGPYHWSPSRKAGRSDDSSSSGGG
GSPSEASGLGLDFEDSVWKPEANPDIKSEFVVA

His-MBP-MIDN^{P100E/V102E/A104E}

MGHHHHHHKIEEGKLVINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQV
AATGDGPDIIFFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIA
VEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYA
FKYENGKYDIKDVGVNDNAGAKAGLTFLVDLIKNNKHMNADTDYSIAEAAFNKGETAMTI
NGPWAWSNIDTSKVNNGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLL
TDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAV
RTAVINAASGRQTVDEALKDAQTDYDIPTTENLYFQGEFEPQPGGARSCRRGAPGGACE
LGPAAEAAPMSLAIHSTTGTRYDLAVPPDETVEGLRKRLSQRLKVPKERLALLHKDTRL
SSGKLQEFVGVGDGSKLTLVPTVEAGLMSQASRPEQSVMQALESLETQVSDFLSGRSPL
TLALRVGDHMMFVQLQLAAQHAPLQHRHVLAAAAAARGDPSIASPVSSPCRVPSS
AARVPPVPTSPSPASPSITAGSFRSHAASTTCPEQMDCSPTASSSASPGASTTSTPGASPA
PRSRKPGAVIESFVNHAPGVFSGTFSGLTLPNCQDSSGRPRRDIGITILQILNDLLSATRHY
QGMPPSLAQLRCHAQCSPASPAPDLAPRTTSCEKLTAAAPSASLLQGQSQIRMCKPPGDRL
RQTENRATRCEVEREQLLLQKRLRRKAERDARGPYHWSRKAAGRSDDSSSSGGGGSPS
EASGLGLDFEDSVWKPEANPDIKSEFVVA

His-MBP-MIDN^{K391E/L395E/R409E}

MGHHHHHHKIEEGKLVINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQV
AATGDGPDIIFFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIA
VEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYA
FKYENGKYDIKDVGVNDNAGAKAGLTFLVDLIKNNKHMNADTDYSIAEAAFNKGETAMTI
NGPWAWSNIDTSKVNNGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLL
TDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAV
RTAVINAASGRQTVDEALKDAQTDYDIPTTENLYFQGEFEPQPGGARSCRRGAPGGACE
LGPAAEAAPMSLAIHSTTGTRYDLAVPPDETVEGLRKRLSQRLKVPKERLALLHKDTRL
SSGKLQEFVGVGDGSKLTLVPTVEAGLMSQASRPEQSVMQALESLETQVSDFLSGRSPL
TLALRVGDHMMFVQLQLAAQHAPLQHRHVLAAAAAARGDPSIASPVSSPCRVPSS
AARVPPVPTSPSPASPSITAGSFRSHAASTTCPEQMDCSPTASSSASPGASTTSTPGASPA
PRSRKPGAVIESFVNHAPGVFSGTFSGLTLPNCQDSSGRPRRDIGITILQILNDLLSATRHY
QGMPPSLAQLRCHAQCSPASPAPDLAPRTTSCEKLTAAAPSASLLQGQSQIRMCKPPGDRL
RQTENRATRCEVEREQLLLQKRLRRKAERDARGPYHWSRKAAGRSDDSSSSGGGGSPS
EASGLGLDFEDSVWKPEANPDIKSEFVVA

His-MBP-MIDN(1-111)-EGR1(101-200)-MIDN(337-468)

MGHHHHHHKIEEGKLVINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQV
AATGDGPDIIFFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIA
VEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYA
FKYENGKYDIKDVGVNDNAGAKAGLTFLVDLIKNNKHMNADTDYSIAEAAFNKGETAMTI
NGPWAWSNIDTSKVNNGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLL
TDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAV
RTAVINAASGRQTVDEALKDAQTDYDIPTTENLYFQGEFMPPQPGGARSCRRGAPGGA
CELGPAAEAAPMSLAIHSTTGTRYDLAVPPDETVEGLRKRLSQRLKVPKERLALLHKDT
RLSSGKLQEFVGVGDGSKLTLVPTVEAGLMSQASTAESFPDISLNNEKVLVETSYPSTTR
LPPITYTGRFSLEPAPNSGNTLWPEPLFSLVSGLVSMNTNPPASSSSAPSPAASSASASQSP
LSCAVPSNDSSSPASPAPDLAPRTTSCEKLTAAAPSASLLQGQSQIRMCKPPGDRLRQTEN
RATRCKVERLQLLLQKRLRRKARRDARGPYHWSRKAAGRSDDSSSSGGGGSPSEASG
LGLDFEDSVWKPEANPDIKSEFVVA

The following proteins were expressed in bacteria using a pETDuet-1 plasmid:

EGR1 (101-200)-mCherry

MTAESFPDISLNNEKVLVETSYPSQTTRLPPITYTGRFSLEPAPNSGNTLWPEPLFSLVSGL
VSMTNPPASSSSAPSPAASSASASQSPPLSCAVPSNDSSMVSKGEEDNMAIIKEFMRFKV
HMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVK
HPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPDSDG
PVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLDGGHYDAEVKTTYKAKKPVQL
PGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK

The following proteins were expressed in HEK293F using a modified pFastBac Dual-CMV-mCherry-Strep-EF1a plasmid:

MIDN

MEPQPGGARSCRRGAPGGACELGPAAEAAPMSLAIHSTTGTRYDLAVPPDETVEGLRK
RLSQRKVPKERLALLHKDTRLSSGKLQEFVGDGSKLTLVPTVEAGLMSQASRPEQSV
MQALESLETQVSDFLSGRSPLTLALRVGDHMMFVQLQLAAQHAPLQHRHVLAAAAA
AAAARGDPSIASPVSSPCRVPSSAARVPPVPTSPSPASPSPTAGSFRSHAASTTCPEQMDC
SPTASSSASPGASTTSTPGASPAPRSRKPGAVIESFVNHAPGVFSGTFSGTLHPNCQDSSG
RPRRDIGTILQILNDLLSATRHYQGMPPSLAQLRCHAQCSPASPAPDLAPRTTSCEKLTA
APSASLLQGQSQIRMCKPPGDRLRQTENRATRCKVERLQLLLQKRLRRKARRDARGP
YHWSPSRKAGRSDSSSSGGGGSPSEASGLGLDFEDSVWKPEANPDIKSEFVVA

MIDN Δ (112-336)

MEPQPGGARSCRRGAPGGACELGPAAEAAPMSLAIHSTTGTRYDLAVPPDETVEGLRK
RLSQRKVPKERLALLHKDTRLSSGKLQEFVGDGSKLTLVPTVEAGLMSQASSPASPA
PDAPRTTSCEKLTAAPSASLLQGQSQIRMCKPPGDRLRQTENRATRCKVERLQLLLQK
KRLRRKARRDARGPYHWSPSRKAGRSDSSSSGGGGSPSEASGLGLDFEDSVWKPEVNP
DIKSEFVVA

RPN11-mCherry

MDRLLRLGGGMPGLGQGPPTDAPAVDTAEQVYISSLALLKMLKHGRAGVPMEVMGL
MLGEFVDDYTVRVIDVFAMPQSGTGVSVEAVDPVFQAKMLDMLKQTGRPEMVVGWY
HSHPGFGCWLSGVDINTQQSFEALSERAVAVVVDPIQSVKGKVVIDAFRLINANMMVL
GHEPRQTTSNLGHLNKPSIQALIHGLNRHYYSITINYRKNELEQKMLNLHKKSWMEGL
TLQDYSEHCKHNESVVKEMLELAKNYNKAVEEEDKMTPEQLAIKNVGKQDPKRHLEE
HVDVLMTSNIVQCLAAMLDTVVFKSRENLYFQGAMVSKGEEDNMAIIKEFMRFKVHM
EGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPA
DIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPDSDGPVM
QKKTMGWEASSERMYPEDGALKGEIKQRLKLDGGHYDAEVKTTYKAKKPVQLPGA
YNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKHHHHHHSAWSHPQFEKGGG
SGGGSGGSAWSHPQFEK