

Structural insights into human PA28-20S proteasome enabled by efficient tagging and purification of endogenous proteins

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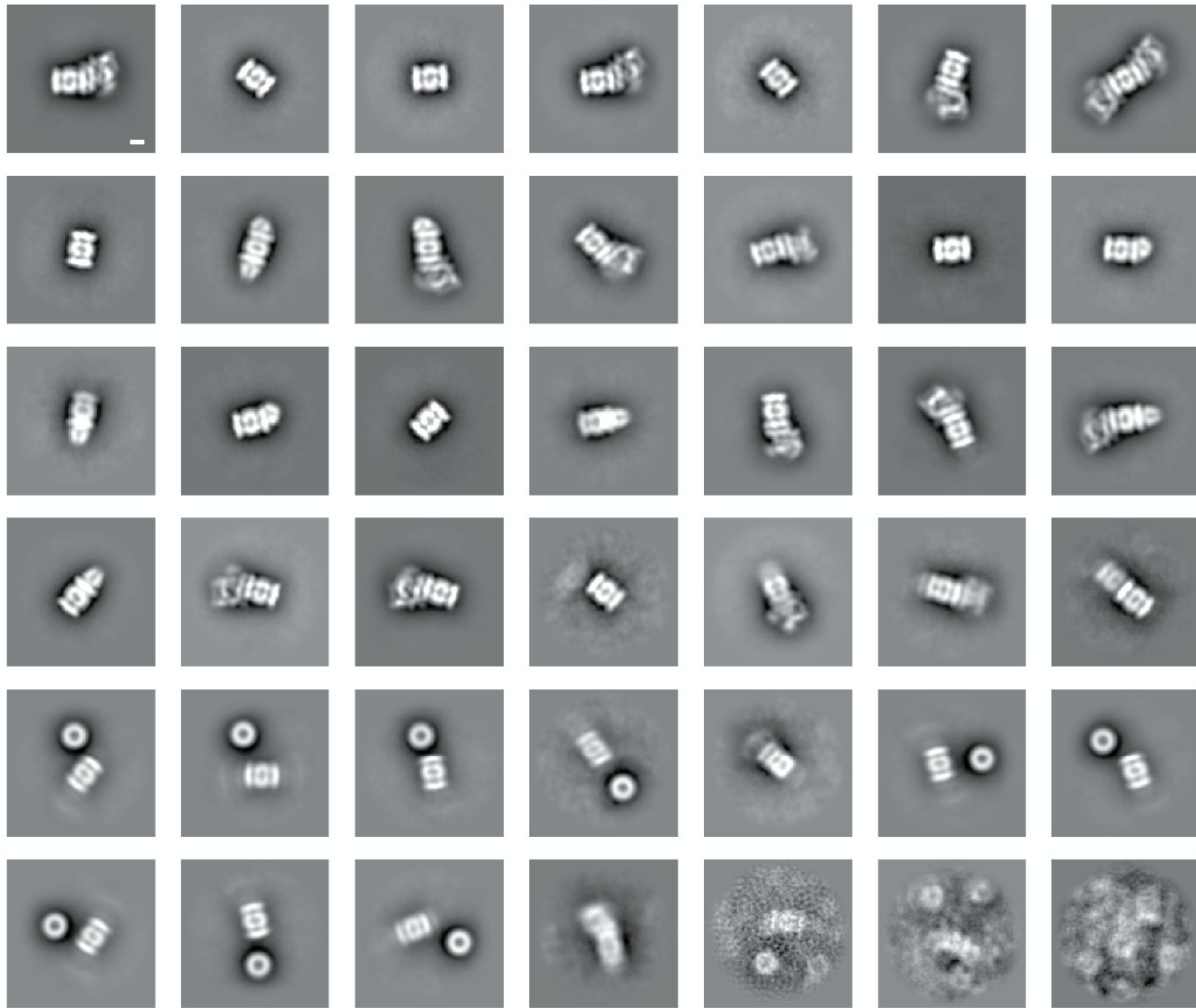
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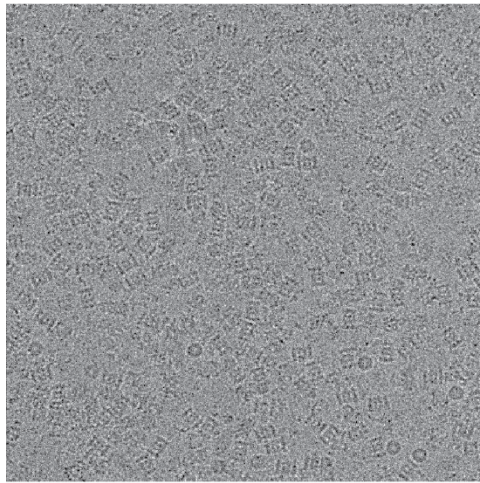
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Supplementary Figures



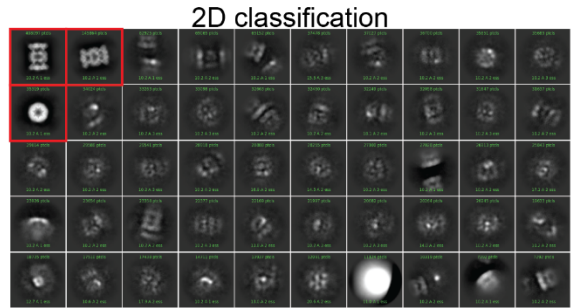
Supplementary Figure 2. Negative stain EM of β 4-tagged proteasomal complexes. 2D classification of the images show a diverse population of different proteasomal complexes formed between 20S, 19S, PA28, and PA200. Scale bar, 50 Å.

PSMB2-StrepII



3,761 micrographs

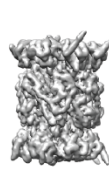
template picking
→
1,912,794
particle
images



2D classification

↓ heterogeneous
refinement

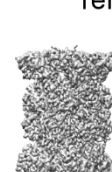
homogeneous
refinement



499,629
particles



50,767
particles

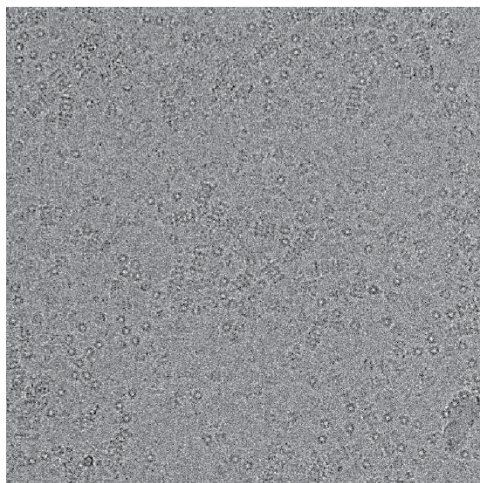


20S
2.7 Å

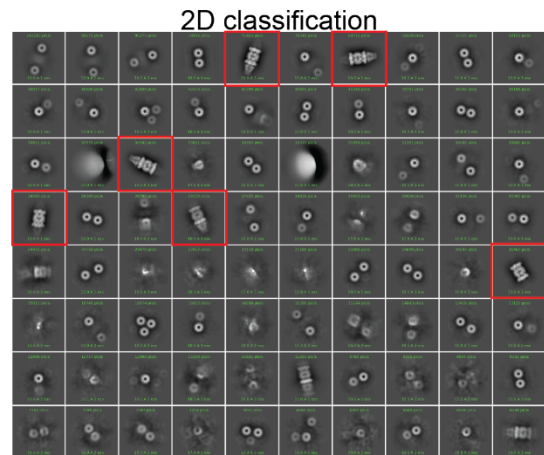


PA200-20S
3.1 Å

ALFA-PSME1



template picking
→
2,540,092
particle
images



2D classification

↓ 248,552
particle
images

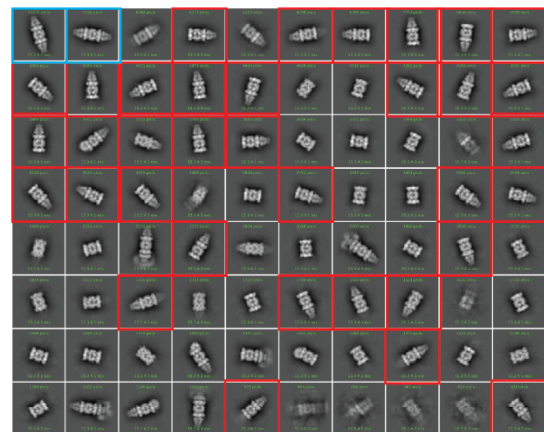


PA28-20S (2.9 Å)
135,937 particle images

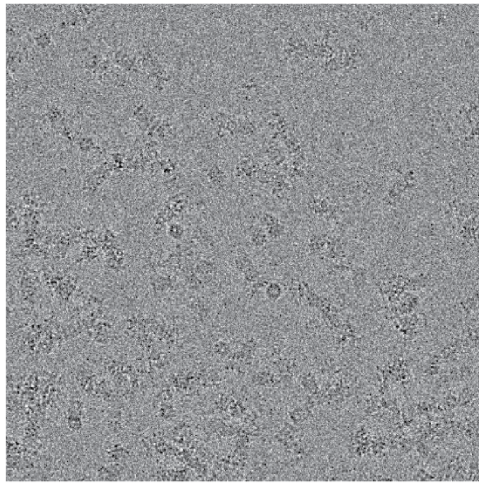


PA28-20S-PA28 (3.2 Å)
22,946 particle images

homogeneous
refinement



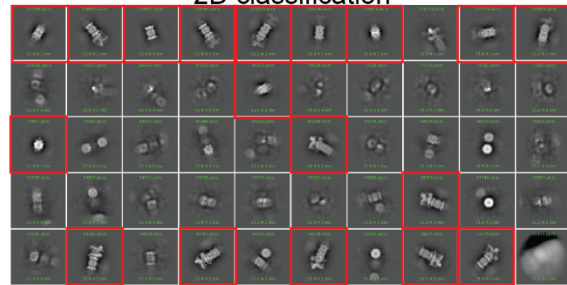
PSMB2-StrepII + MG132



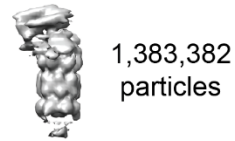
8,128 micrographs

template picking
 →
 3,537,033
 particle
 images

2D classification

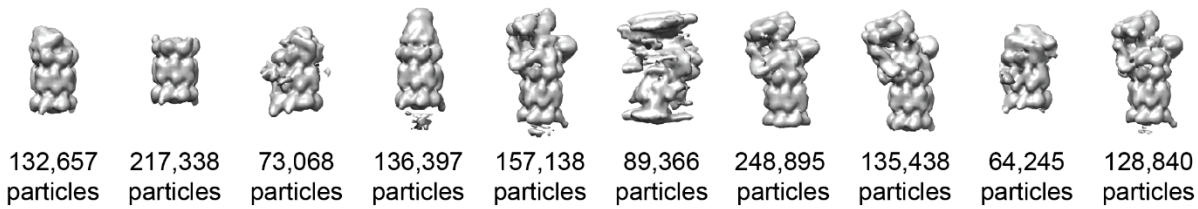


homogeneous
 refinement



1,383,382
 particles

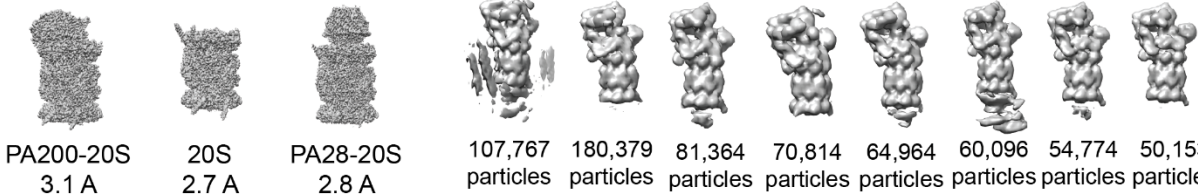
heterogeneous
 refinement



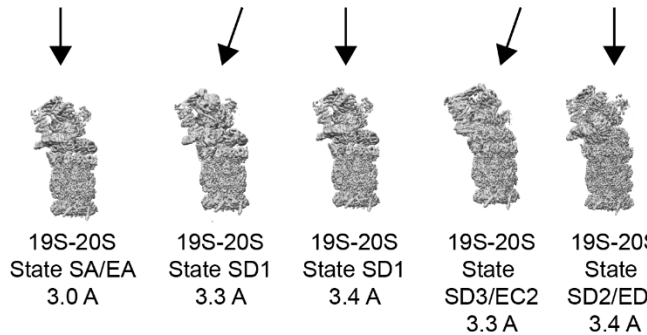
132,657 particles 217,338 particles 73,068 particles 136,397 particles 157,138 particles 89,366 particles 248,895 particles 135,438 particles 64,245 particles 128,840 particles

homogeneous
 refinement

heterogeneous refinement

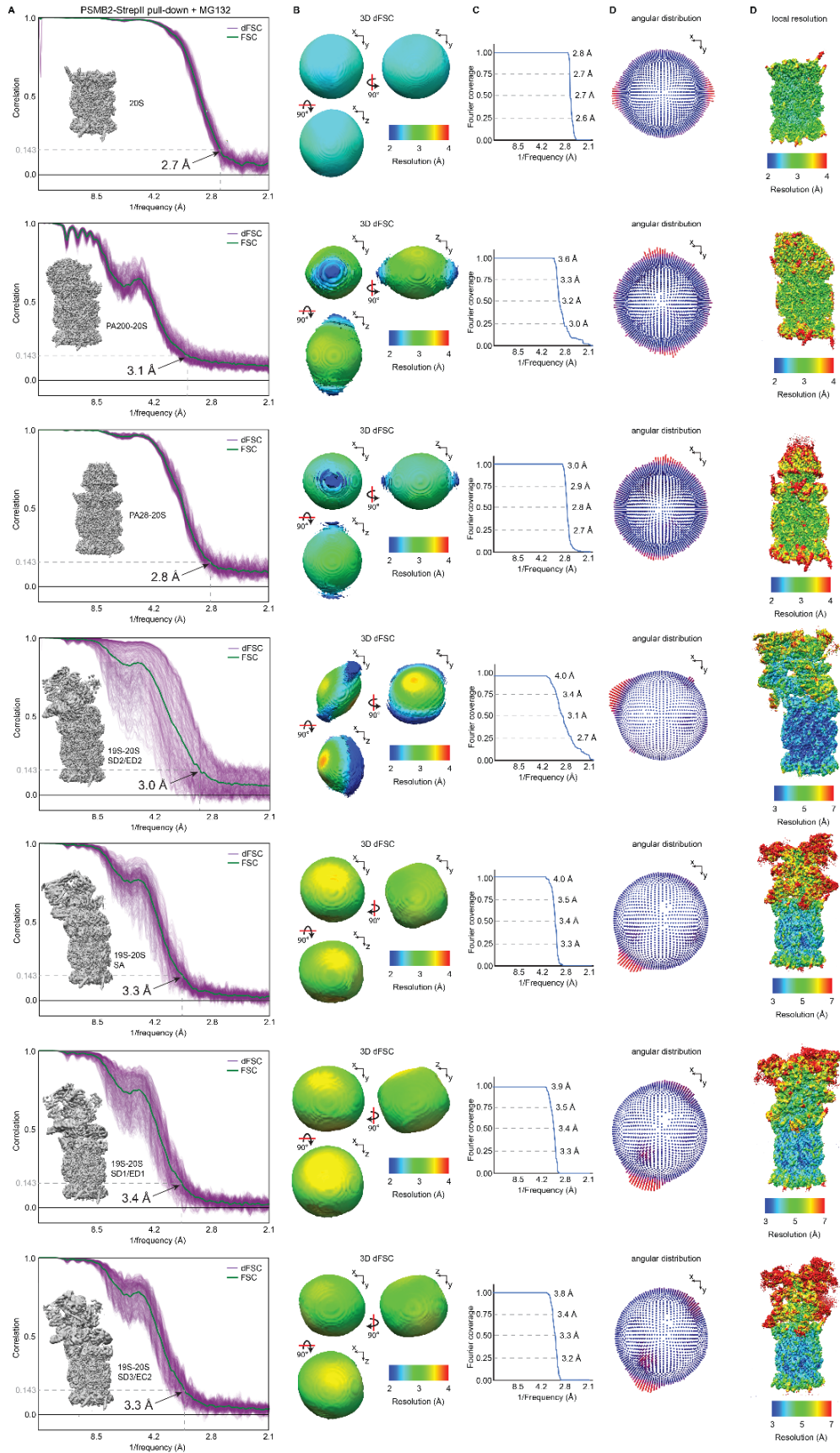


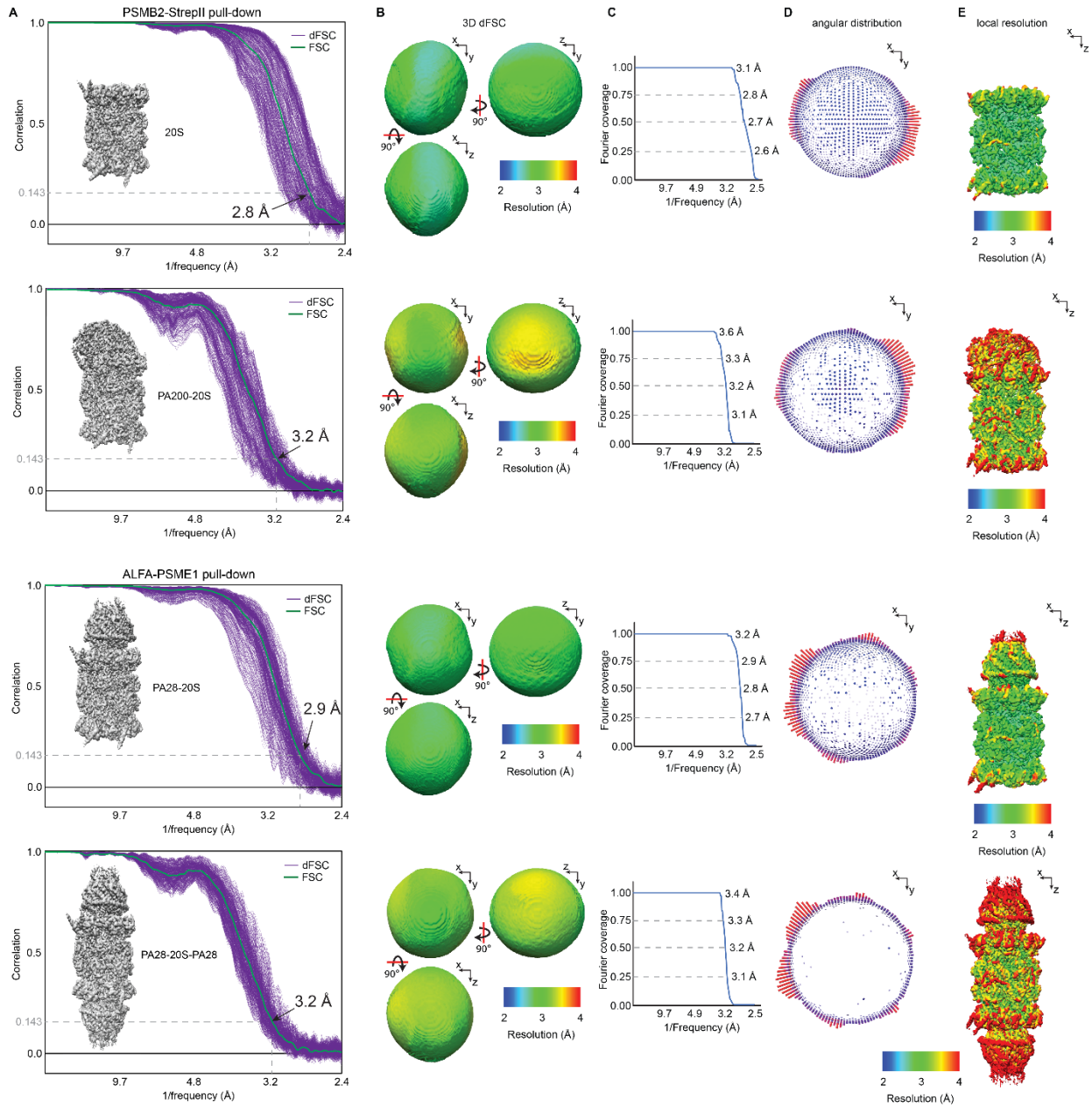
PA200-20S 3.1 Å 20S 2.7 Å PA28-20S 2.8 Å 107,767 particles 180,379 particles 81,364 particles 70,814 particles 64,964 particles 60,096 particles 54,774 particles 50,153 particles



19S-20S State SA/EA 3.0 Å 19S-20S State SD1 3.3 Å 19S-20S State SD1 3.4 Å 19S-20S State SD3/EC2 3.3 Å 19S-20S State SD2/ED2 3.4 Å

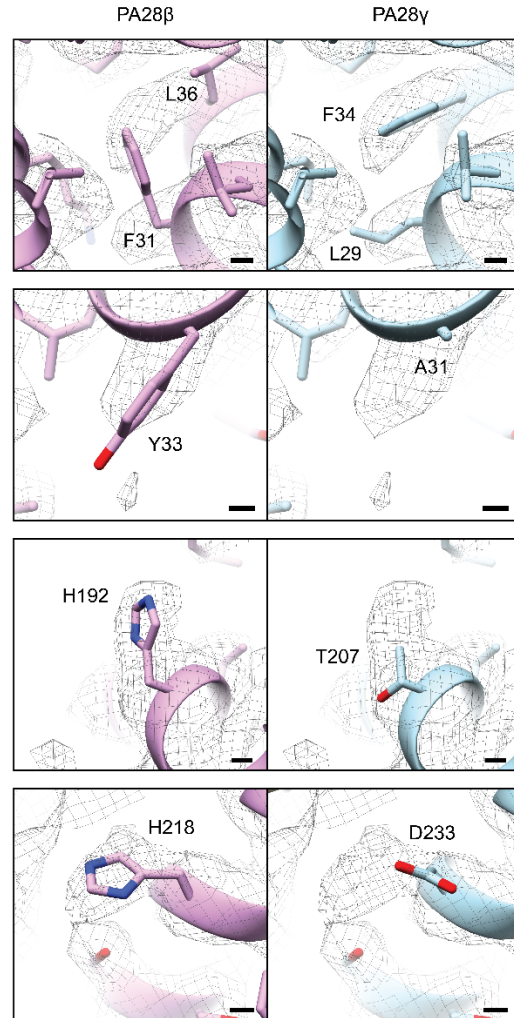
Supplementary Figure 3. Cryo-EM image processing workflow. 2D and 3D classification for PSMB2-StrepII, ALFA-PSME1, and PSMB2-StrepII + MG132 samples.



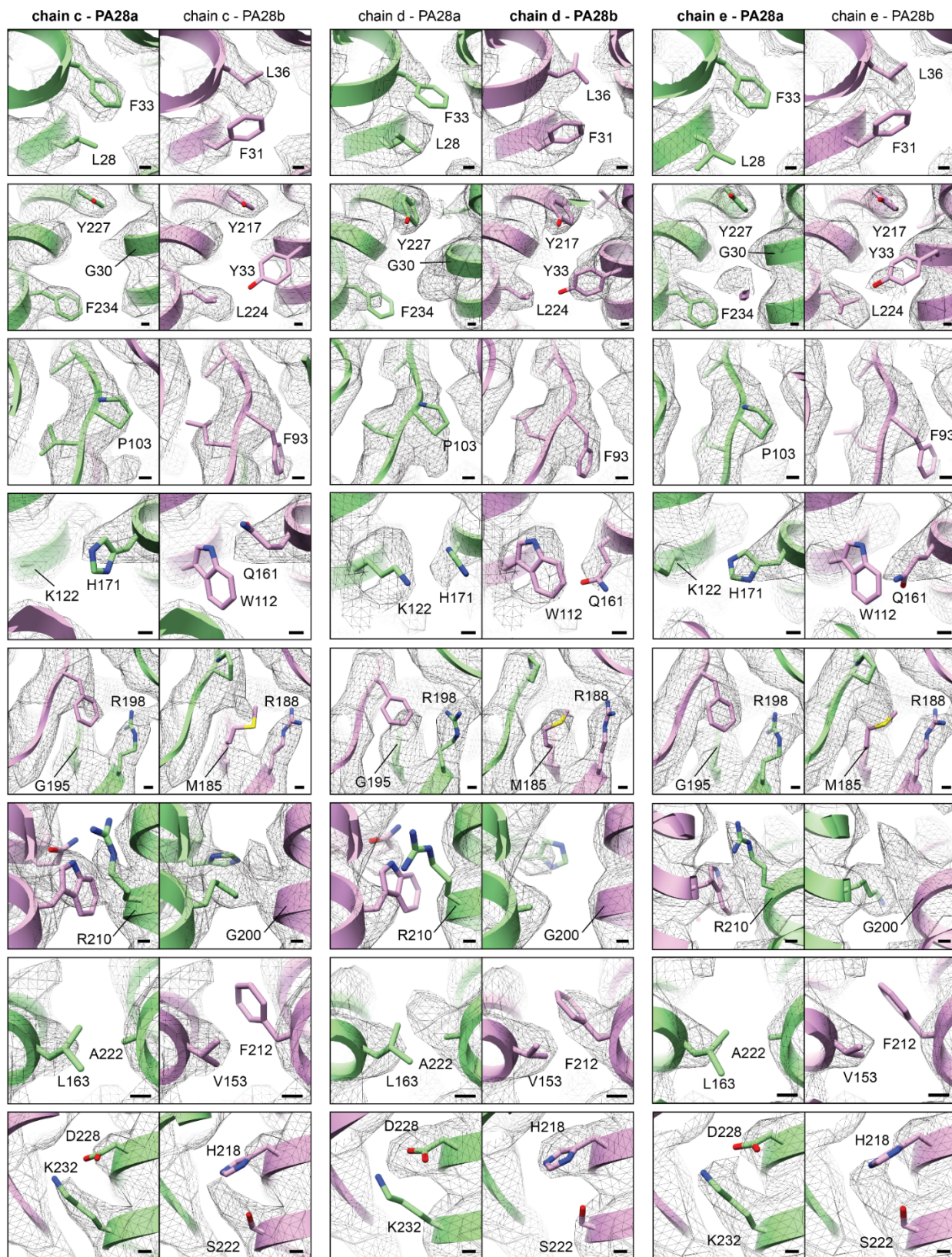


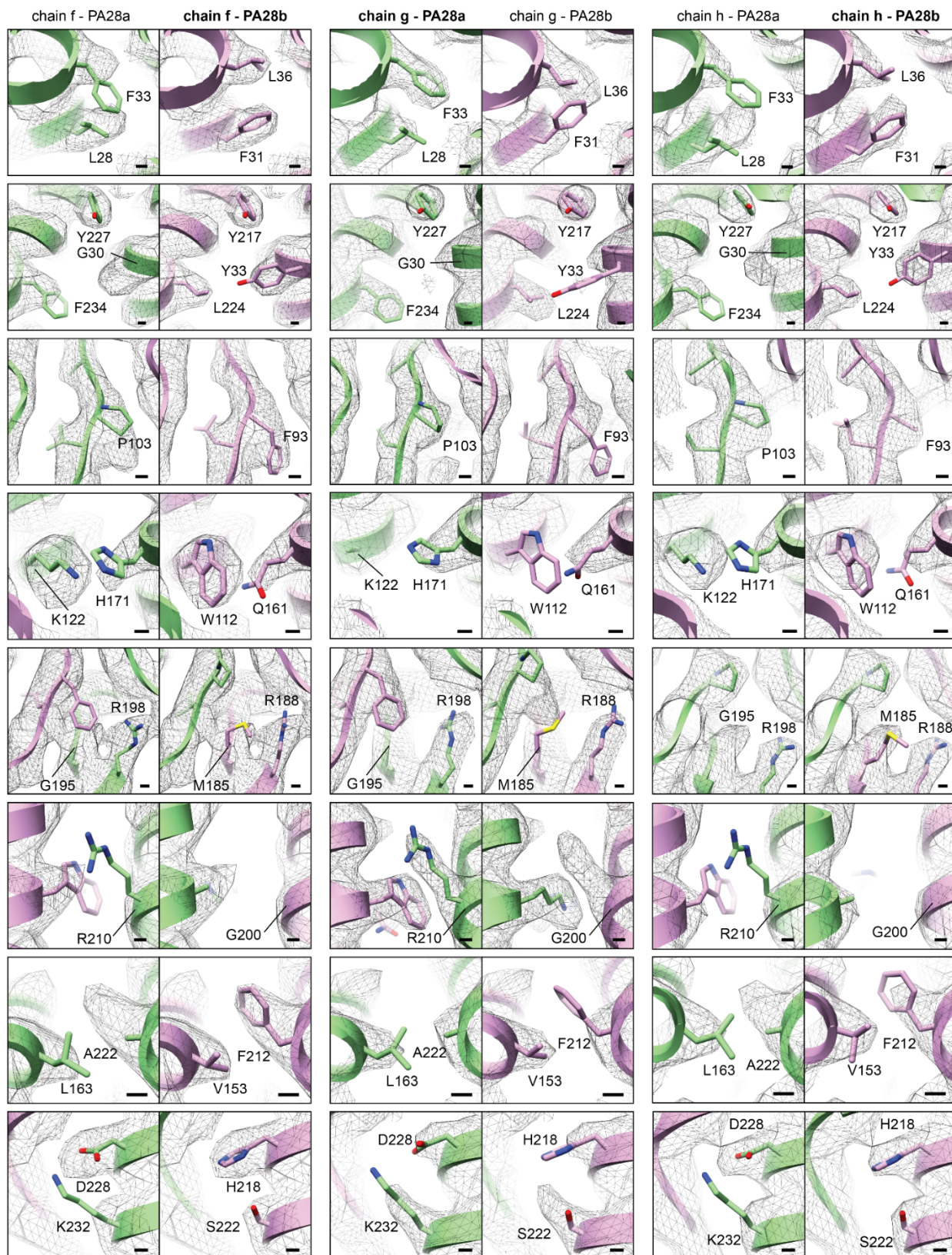
Supplementary Figure 4. Cryo-EM map statistics. (A) Fourier shell correlation (FSC) and 1D directional FSC (dFSC) plots. **(B)** Visualization of 3D dFSC. **(C)** Plots showing coverage of Fourier space. **(D)** Plots showing distribution of viewing angles from final refined datasets. **(E)** Corresponding local resolution map with color scale.

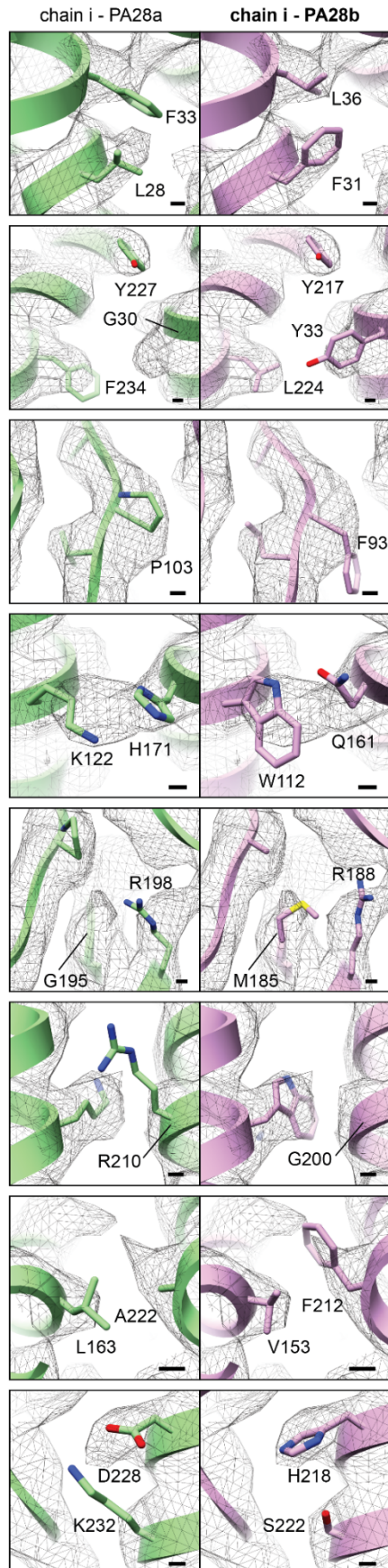
| Gene | Seq. coverage (%) | Gene | Seq. coverage (%) |
|-------|-------------------|---------------|-------------------|
| PSMB5 | 75.3 | PSMD11 | 70.1 |
| PSMA7 | 74.6 | PSMD13 | 76.9 |
| PSMA5 | 87.1 | PSMC3 | 67.2 |
| PSMB1 | 66.4 | PSMC5 | 70 |
| PSMA6 | 91.9 | PSMD12 | 55.3 |
| PSMA1 | 77.9 | PSME3 | 64.6 |
| PSMA4 | 72.4 | PSMD4 | 52.8 |
| PSMB6 | 77.4 | PSMC1 | 66.6 |
| PSMB7 | 71.8 | PSMC6 | 65 |
| PSMB4 | 75 | PSMD7 | 58 |
| PSMD1 | 59.7 | PSMC4 | 73.9 |
| PSMD2 | 76 | PSME1 | 67.5 |
| PSMA2 | 80.8 | PSME2 | 69 |
| PSMA3 | 80 | HSPA1B;HSPA1A | 61.3 |
| PSMB2 | 88.6 | PSMD14 | 69.4 |
| PSME4 | 52.6 | ADRM1 | 53.1 |
| PSMB3 | 70.2 | RPS27A;UBA52 | 55.8 |
| PSMC2 | 71.4 | TXNL1 | 66.1 |
| PSMD6 | 68.9 | PSMD8 | 46.9 |



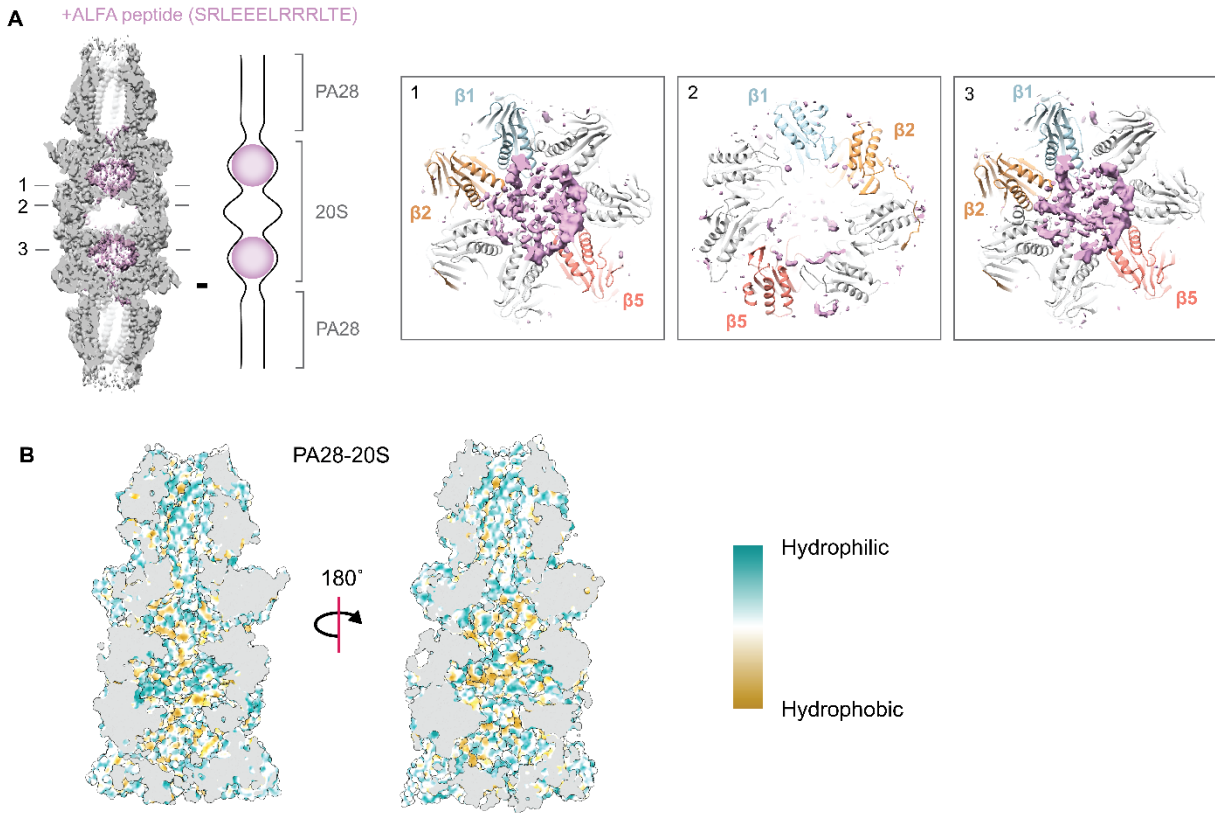
Supplementary Figure 5. Mass spectrometry (left) of 20S(β 4-StreptII) pull-down shows co-purification with PA28 α (PSME1), PA28 β (PSME2), and PA28 γ (PSME3). Cryo-EM density map of PA28-20S(β 4-StreptII) was modeled with both PA28 $\alpha\beta$ and PA28 γ (right). The density fits PA28 γ sidechains in some regions (F34, L29) while PA28 β sidechains are better fit in other regions (Y33, H192, H218), indicating that the map is an average of PA28 $\alpha\beta$ and PA28 γ .



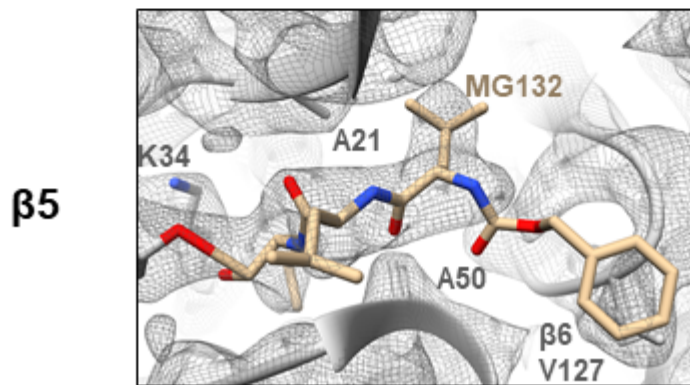
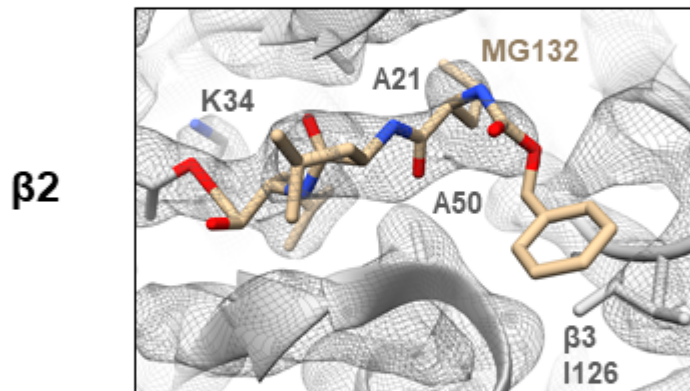
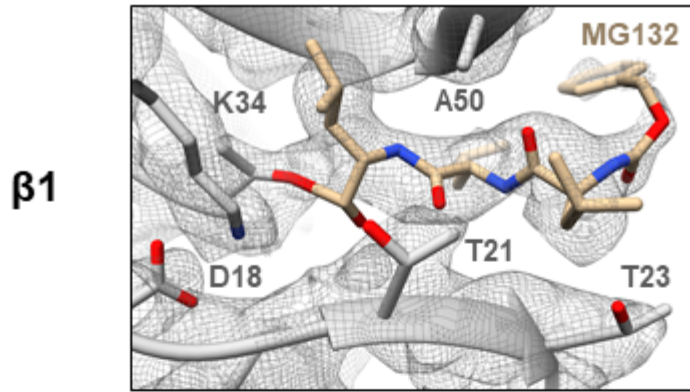




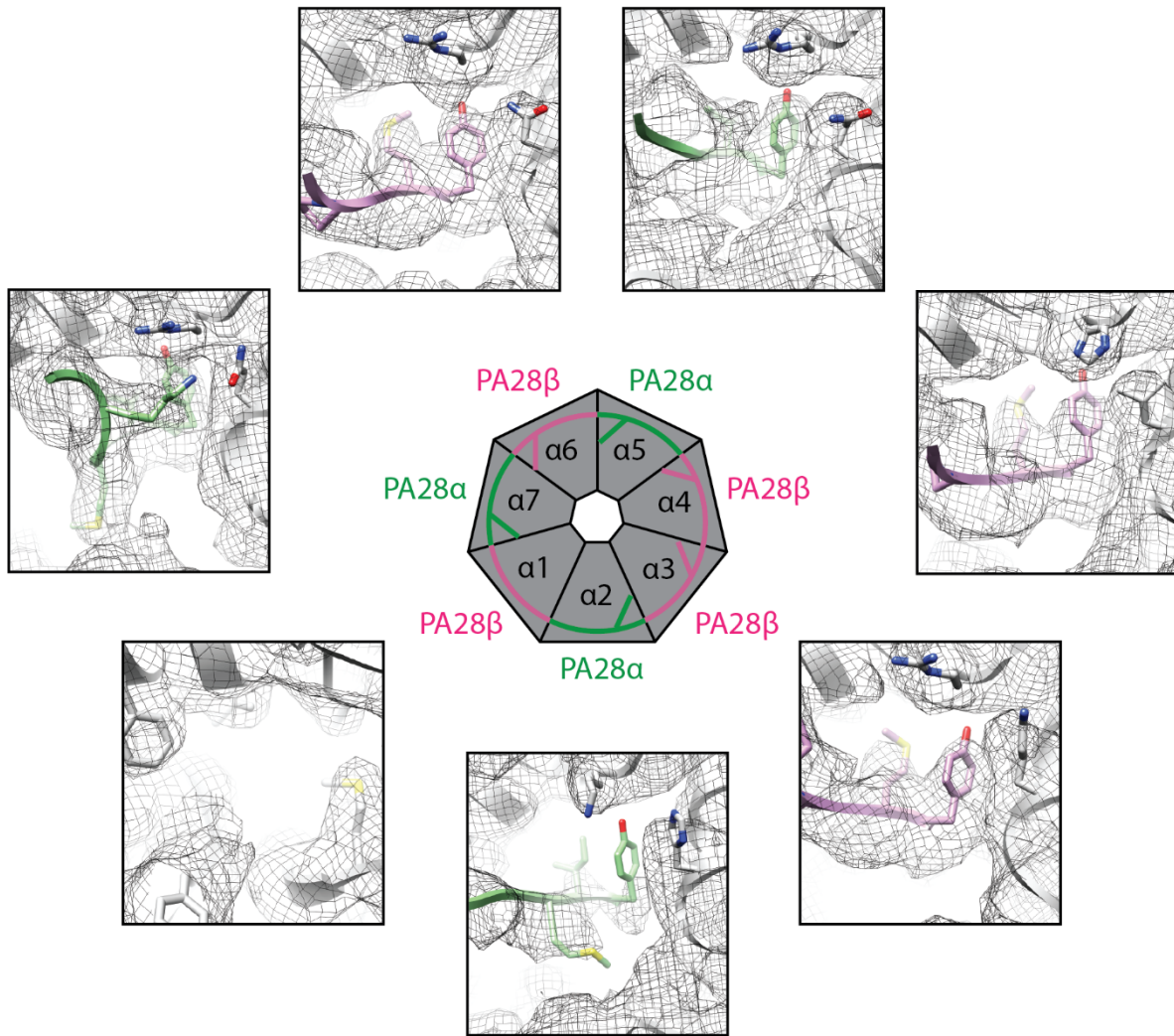
Supplementary Figure 6. PA28 model fit to map density. For each chain in PA28, PA28a (PSME1) and PA28b (PSME2) were modeled into the cryo-EM density map of single capped PA28-20S proteasome. Key differences in the model-to-map fit are highlighted and support a stoichiometry of {a,b,a,b,a,b,b} for chains {c,d,e,f,g,h,i}, respectively. Scale bar, 1 Angstrom.



Supplementary Figure 7. (A) Cryo-EM map of double capped PA28-20S-PA28 shows ALFA peptide density in both antechambers. **(B)** The walls of the 20S proteasome inner chambers are made up of both hydrophilic and hydrophobic regions.



Supplementary Figure 8. Fit of MG132 into the PA28-20S cryo-EM density at the proteolytic sites of $\beta 1$, $\beta 2$, and $\beta 5$.



Supplementary Figure 9. PA28 C-terminal tail model and map density. Density is observed for the C-terminal tails of PA28 subunits for 6 out of 7 binding sites.

Supplementary Table 1. Cryo-EM imaging parameters

| PSMB2-StrepII pulldown | |
|---|--|
| Microscope | Polara (300 kV), UCSF |
| Camera (software) | Gatan K2 Summit (SerialEM, 1 shot/hole) |
| Magnification (at detector) | 40,984X |
| Exposure rate (total) | 8 e/pixel/s (43 e/Å ²) |
| Frames | 30 |
| Pixel size | 1.22 Å/pixel |
| Defocus range (um) | 1.2 – 3.1 |
| Micrographs (# initial particles) | 3761 (1,912,794) |
| # of 3D classes (# particles) | 3 (550,396) |
| Class 1 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | 20S (499,629), EMD-24275, PDB 7NAN 2.8/3.8 Å (mask), 3.5/4.1 Å (no mask) 2.7 – 29 Å 2.5 – 2.8 Å 0 |
| Class 2 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | PA200-20S (50,767), EMD-24278, PDB 7NAQ 3.2/4.4 Å (mask), 4.2/7.6 Å (no mask) 2.8 – 50 Å 3.0 – 3.6 Å 0 |
| Class 3 (# particles) | 19S-20S (did not refine) |
| ALFA-PSME1 pulldown | |
| Microscope | Polara (300 kV), UCSF |
| Camera (software) | Gatan K2 Summit (SerialEM, 1 shot/hole) |
| Magnification (at detector) | 40,984X |
| Exposure rate (total) | 8 e/pixel/s (43 e/Å ²) |
| Frames | 30 |
| Pixel size | 1.22 Å/pixel |
| Defocus range (um) | 1.3 – 2.0 |
| Micrographs (# initial particles) | 5994 (2,540,092) |
| # of 3D classes (# particles) | 2 (158,883) |
| Class 1 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | PA28-20S (135,937), EMD-24276, PDB 7NAO 2.9/3.9 Å (mask), 3.8/5.3 Å (no mask) 2.7 – 43 Å 2.6 – 3.2 Å 0 |
| Class 2 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | PA28-20S-PA28 (22,946), EMD-24277, PDB 7NAP 3.2/4.5 Å (mask), 6.1/8.5 Å (no mask) 2.7 – 52 Å 3.0 – 3.4 Å 0 |
| PSMB2-StrepII pulldown + MG132 | |
| Microscope | Titan Krios (300 kV), SBP |
| Camera (software) | Gatan K3 (SerialEM, 4-shots/hole) |

| | |
|---|--|
| Magnification (at detector) | 47,170X |
| Exposure rate (total) | 25 e/pixel/s (30 e/Å ²) |
| Frames | 20 |
| Pixel size | 1.06 Å/pixel |
| Defocus range (um) | 1.3 – 2.3 |
| Micrographs (# initial particles) | 8128 (3,537,033) |
| # of 3D classes | 7 (729,026) |
| Class 1 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | 20S (214,790), EMD-27013, PDB 8CVR 2.7/3.1 Å (masked), 3.3/4.1 Å (unmasked) 2.5 – 45 Å 2.5 – 2.8 Å 0 |
| Class 2 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | PA200-20S (70,412), EMD-27015, PDB 8CVS 3.1/4.3 Å (masked), 4.1/8.0 (unmasked) 2.4 – 40 Å 2.2 – 3.6 Å 0 |
| Class 3 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | PA28-20S (136,392), EMD-27014 2.8/3.4 Å (masked), 4.1/7.8 Å (unmasked) 2.6 – 45 Å 2.3 – 3.0 Å 0 |
| Class 4 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | 19S-20S, S _A /E _A (65,390), EMD-27016 3.3/4.1 Å (masked), 7.1/9.4 Å (unmasked) 3.1 – 57 Å 3.0 – 4.0 Å 0 |
| Class 5 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | 19S-20S, S _{D1} (45,734), EMD-27017 3.4/4.2 Å (masked), 7.3/11 Å (unmasked) 3.1 – 57 Å 3.2 – 3.9 Å 0 |
| Class 6 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | 19S-20S, S _{D2} /E _{D2} (147,696), EMD-27018, PDB 8CVT 3.1/3.8 Å (masked), 4.7/8.9 Å (unmasked) 2.8 – 51 Å 2.2 – 4.0 Å 0 |
| Class 7 (# particles), codes Resolution (0.143/0.5) Local resolution (min-max) 3DFSC spread (0.143) Sharpening B-factor | 19S-20S, S _{D3} /E _{C2} (48,612), EMD-27019 3.3/4.1 Å (masked), 7.2/11 Å (unmasked) 3.1 – 57 Å 3.1 – 3.8 Å 0 |

Supplementary Table 2. Comparing PA28(4a3b) and PA28(3a4b) models

| Model composition | 20S-PA28(4a3b) | 20S-PA28(3a4b) |
|-------------------------------------|----------------|----------------|
| PDB code | 8CXB | 7NAO |
| Atomic modeling package | Coot | Coot |
| CCvolume/CCmask | 0.85/0.85 | 0.85/0.85 |
| B-factor (masked/unmasked) | 70/75 | 70/75 |
| RMSD bond lengths, angles | 0.007/1.281 | 0.006/1.090 |
| Molprobit score | 1.41 | 1.04 |
| Clashscore | 4.59 | 2.51 |
| Rotamers outliers (%) | 1.71 | 0 |
| C-beta outliers (%) | 0.25 | 0 |
| Ramachandrans (Favored, outliers) % | 98.57/0.09 | 98.63/0.07 |
| CaBLAM outliers (%) | 1.39 | 1.41 |
| EMRinger score | 3.05 | 3.09 |