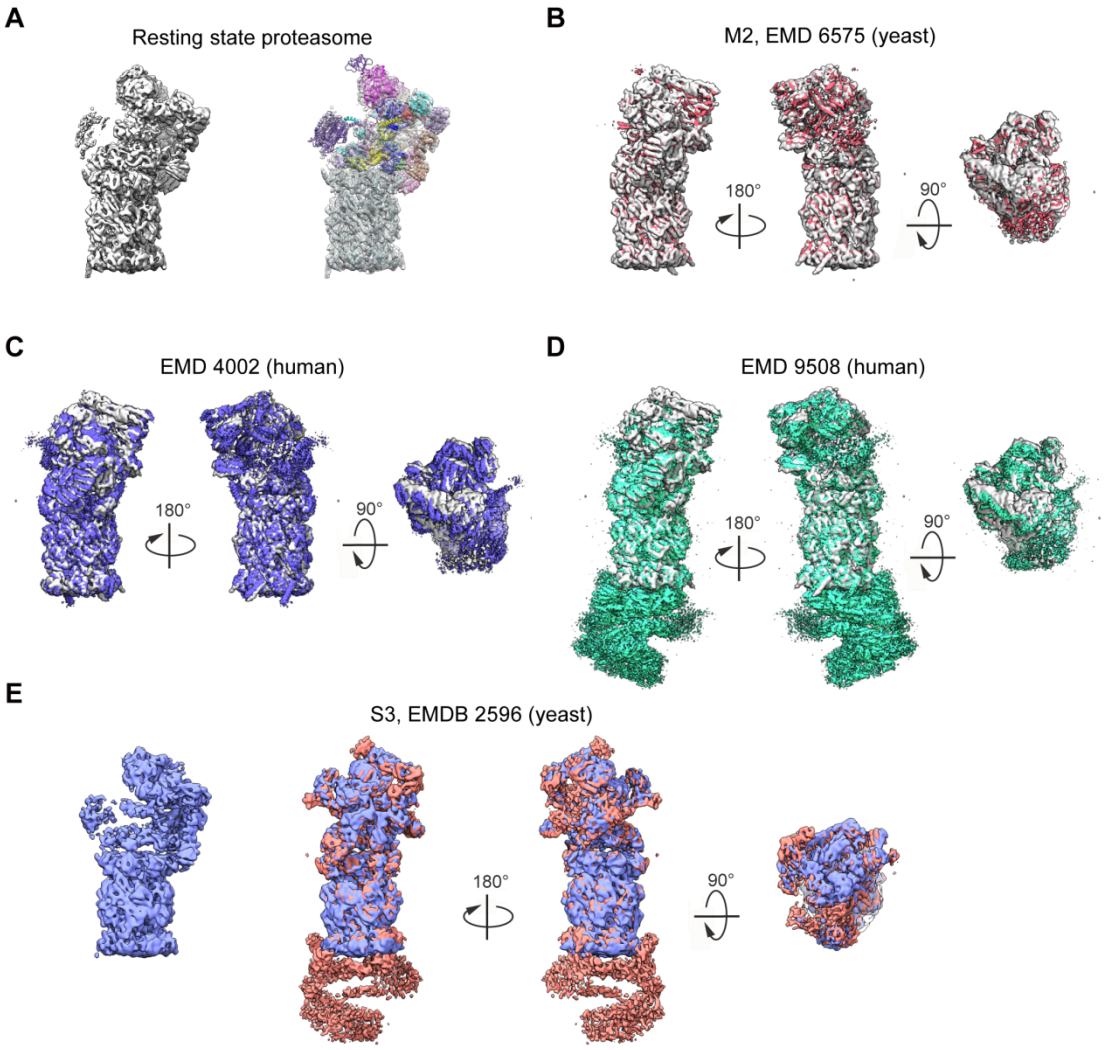

Supplementary information, Figure S5 Cryo-EM maps of yeast proteasome in the presence of ATP and conformation comparison with related previous cryo-EM maps. **(A)** Our resting state cryo-EM map (left, grey) obtained from the majority (69.2%) of the ATP presented single-capped proteasome dataset, and the corresponding pseudo atomic model fitted into the map (right). **(B)** Comparison of our yeast resting state map (grey) with the previous yeast resting state cryo-EM map (M2, firebrick, EMDB ID: 6575) [23], which reveals a similar conformation between them. **(C-D)** Comparison of our yeast resting state map with the recently reported two cryo-EM maps of human proteasome in the same nucleotide state (medium blue, EMDB ID: 4002 [30], **C**; green, EMDB ID: 9508 [31], **D**), which shows noticeable structure variations. **(E)** The other cryo-EM map (purple, left) obtained from 30.8% of the same ATP presented single-capped proteasome dataset (see **Materials and Methods**). This

map showed a conformation comparable to that of the previous yeast proteasome-ATPγS map obtained from the ATPγS presented dataset (salmon, EMDB ID: 2596) [18], indicating this map is likely in the ATPγS state. All the previous maps are rendered in their recommend threshold. We should mention that there is a higher resolution cryo-EM map of yeast proteasome in the ATPγS conformation published recently (M1, EMDB ID: 6574) [23]. Still, as the authors pointed out, the six AAA-ATPase subunits in the base exhibited discontinuous density in their map. Since many of our analyses in this study were focused on the AAA-ATPase ring region of the base, we then chose to perform all our related comparisons with the previous proteasome-ATPγS structure (S3, EMDB ID: 2596) [18] with the AAA-ATPase ring being better resolved.