



**Supplementary information, Figure S7** The AAA-ATPase ring conformational change between proteasome-ADP-AIFx and the resting state structures. **(A)** The AAA-ATPase ring of the resting state (left column) and proteasome-ADP-AIFx (right column) structures. The AAA-ATPase subunits follow the same color schema as in Fig. 2. The dashed lines indicate the orientation of the AAA domains in the resting state (black lines) and proteasome-ADP-AIFx (red lines) structures, and the arrows denote the direction that these domains would rotate from the resting state to the ADP-AIFx state according to these structures. The interaction interface between 19S and 20S calculated by PISA [81] is also listed (in  $\text{\AA}^2$ ). **(B)** Comparison of the AAA-ATPase ring conformations in the resting state (grey) and proteasome-ADP-AIFx (subunits with color) structures with respect to their 20S with the differences in the positions of their AAA-ATPase rings indicated in two directions

in red and black, respectively. **(C)** The top view of the AAA-ATPase ring of the resting state (left column) and proteasome-ADP-AIFx (right column) structures. The circles indicate the diameters of the AAA-ATPase pore. **(D)** Top view of the comparison as in **(B)**, with the N-ring removed for better visualization. The outer boundaries of the AAA-rings of the resting state and proteasome-ADP-AIFx structures are shown in black and red hexagons, respectively. The dashed lines depict the diameters of the AAA-rings: 126.1 Å for the resting state (black) and 130.9 Å for ADP-AIFx state (red). The AAA-ring rotation is also labeled. **(E)** Location of the Rpt1 C-terminal tail (red) resolved in our resting state map. Its location relative to the entire map is shown on the upper left, a magnified view of Rpt1 (dodger blue) with its relationship to the 20S  $\alpha$ 6 subunit (sandy brown) in the middle, and the top view of the tail relative to 20S on the right. The location of the gate is indicated by the black circle.