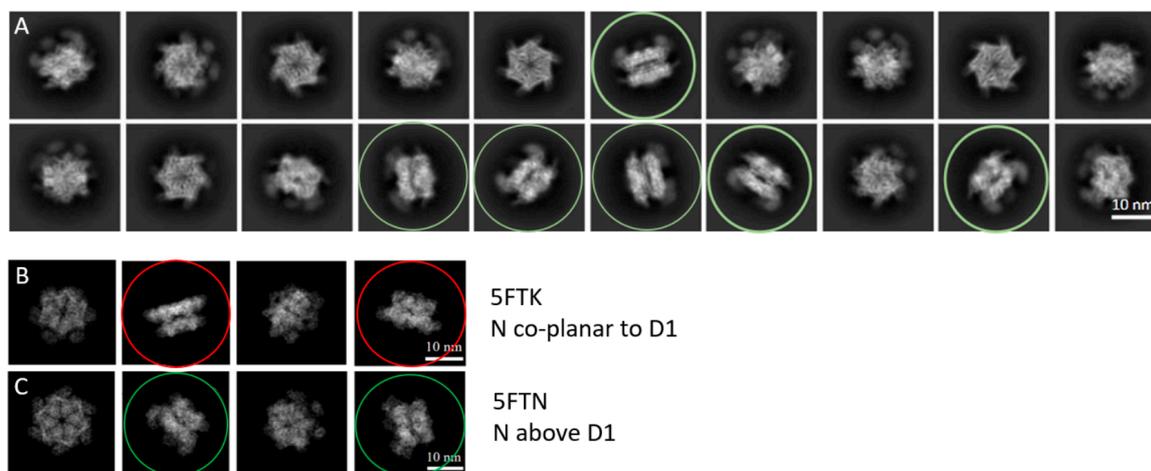
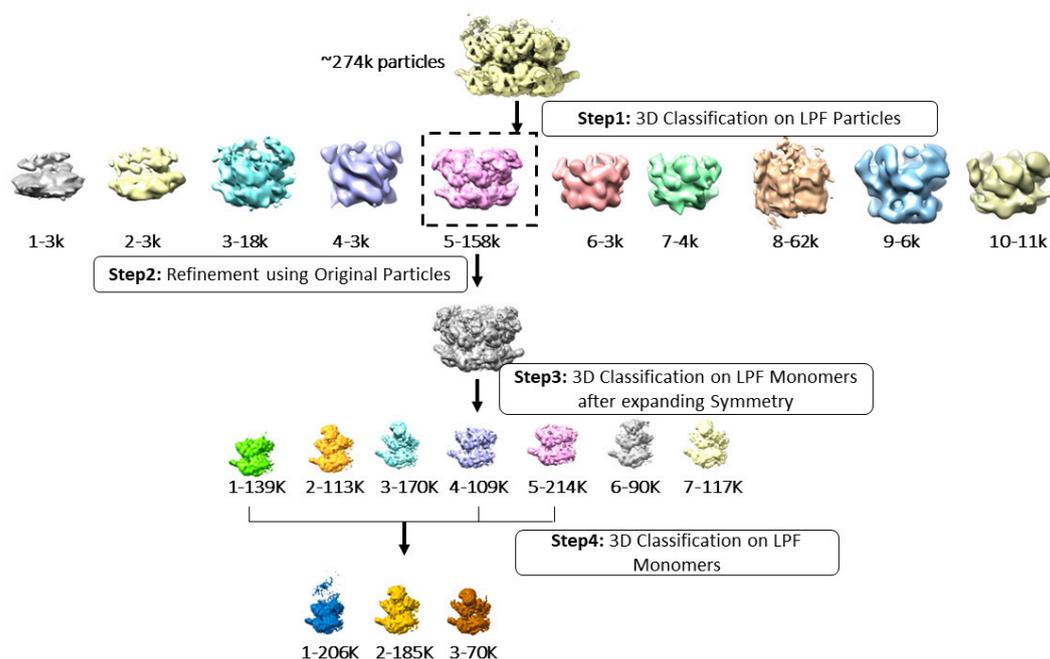


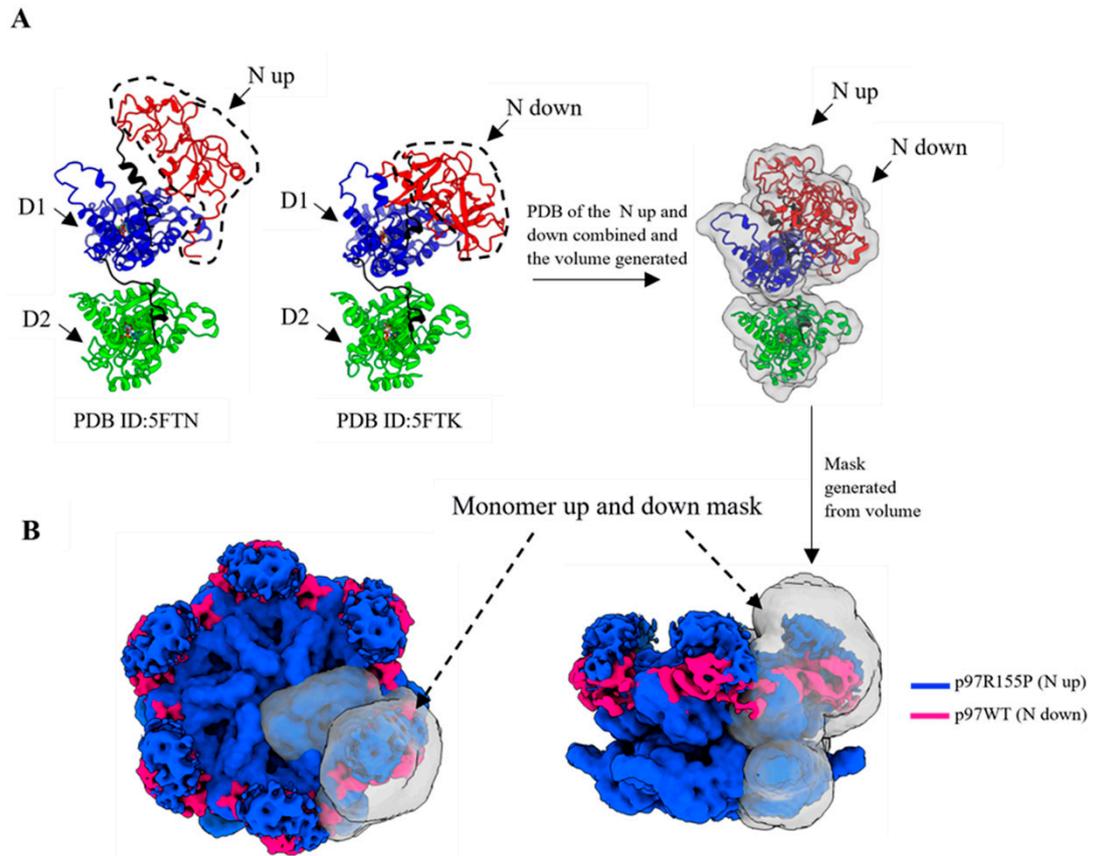
## Supplementary Materials



**Figure S1.** 2D classification of p97R155P-ATP $\gamma$ S. **(A)** Representative main classes obtained after removing false positive from the automatic particle picking. These classes comprise the 274,640 particles analyzed in this study. The N-domains position above the D1 ring as seen in the side views (circled light green). **(B,C)** Simulated projection images of the models of p97 with the N-domains in the co-planar conformation (pdb id: 5FTK, side views encircled red) and the N-domains in the up conformation (pdb id: 5FTN, side views encircled dark green).



**Figure S2.** Overall processing steps of the focused classification using low-pass filtered images. Particle images were low-pass filtered (LPF) using a 6Å cut-off, a resolution at which alpha helices should still be visible. A first classification using the complete signal for the hexamer was performed. A second classification was performed focusing on the signal of a monomer, after pre-processing the images using the C6 symmetry expansion and signal subtraction of 5 monomers as described in Figure 2.



**Figure S3.** Generating a mask for monomers with the N domains in the up and down positions. **(A)** The volume generated from the combined PDB file of N up (PDB ID: 5FTN) and N down (PDB ID: 5FTK) in Chimera. The N, D1 and D2 domains are colored red, blue, and green. The generated volume is shown as transparent. **(B)** The generated volume from PDB file was used to create a mask in Relion. The monomer up and down mask is shown as transparent on the superimposed map of p97WT-ATP $\gamma$ s (pink) and R155P-ATP $\gamma$ s (blue) map.