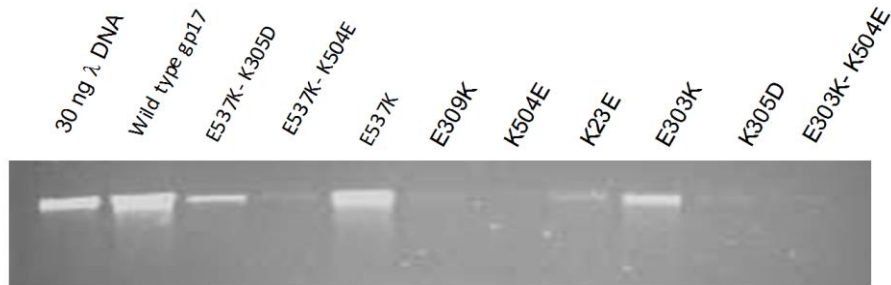
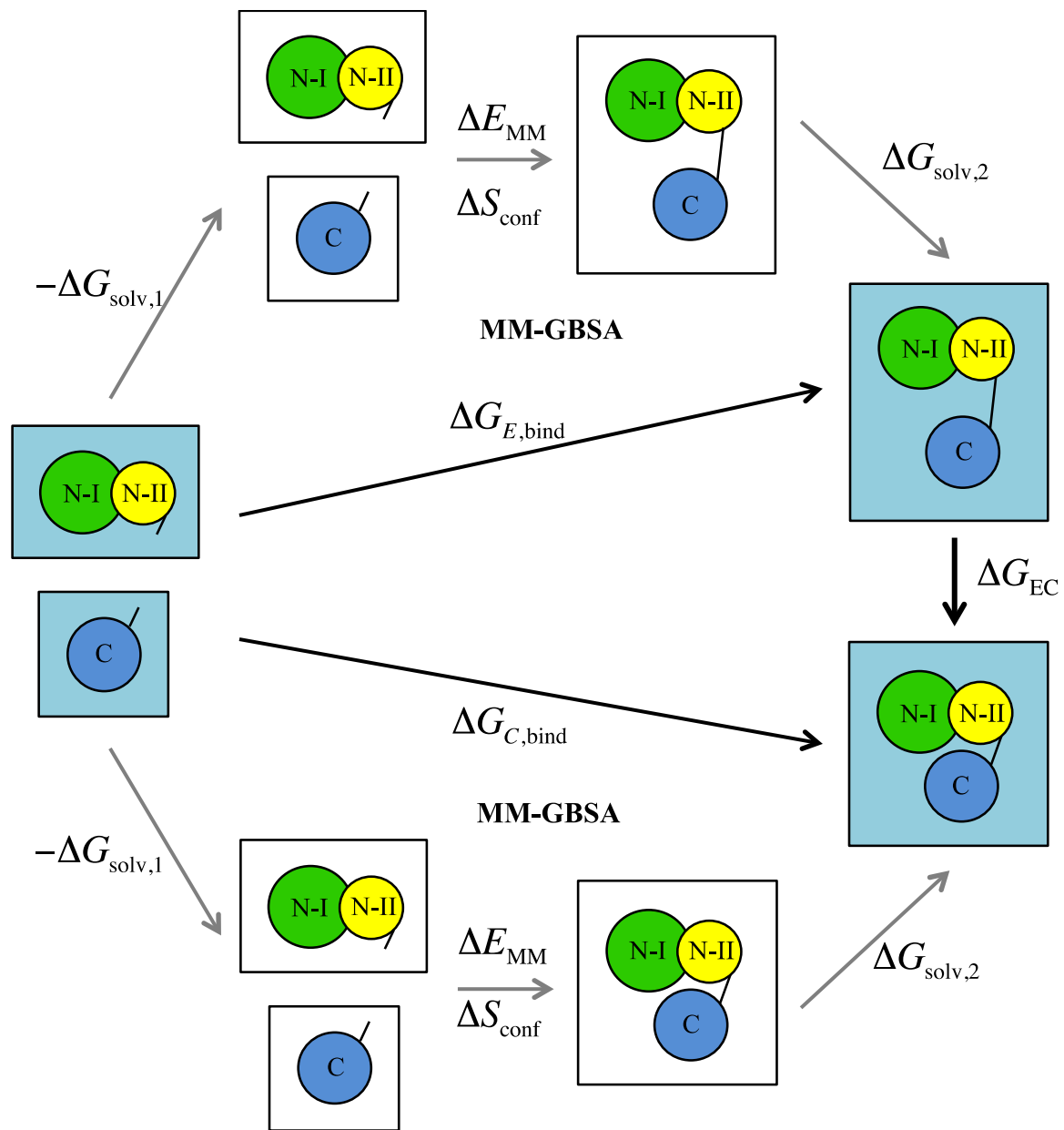


Supplementary Figure 1. Additional views of the gp17 structures (PDB code: 3CPE), orthogonal to the views in Fig. 2A, in the compact (top row, two views rotated 180°) and extended states (bottom row, two views rotated 180°) showing the interfacial residues mutated in this study. Colors indicate the sub-domains N-I (green), N-II (yellow), and C-terminal (blue).



Supplementary Fig. 2. Bulk measurements of in vitro DNA packaging using the DNase protection assay. Each packaging reaction used 1×10^{10} proheads, 300 ng of phage λ DNA, 2 μ M gp17, and 1 mM ATP. The far left lane is a control sample containing 30 ng λ DNA for comparison.



Supplementary Figure 3. Free energy cycle depicting out MM-GBSA-based approach for computing the free energy difference ΔG_{EC} between the extended (E) and compact (C) conformations of gp17 (for wild type and mutant motors). Note that the net solvation free energy ΔG_{solv} (see text) is given by $\Delta G_{solv,2} - \Delta G_{solv,1}$. The blue shading indicates where implicit solvent was included in the calculations.

Supplementary Table 1. Numbers of independent packaging measurements on different individual complexes for WT and each mutant studied at each of the six applied forces.

	5 pN	15 pN	30 pN	40 pN	50 pN	60 pN
WT	752	93	200	120	150	105
E537K	405	73	133	84	46	35
E537K-K305D	487	108	147	91	80	33
K303E	230	63	33	51	47	30