Supporting Information

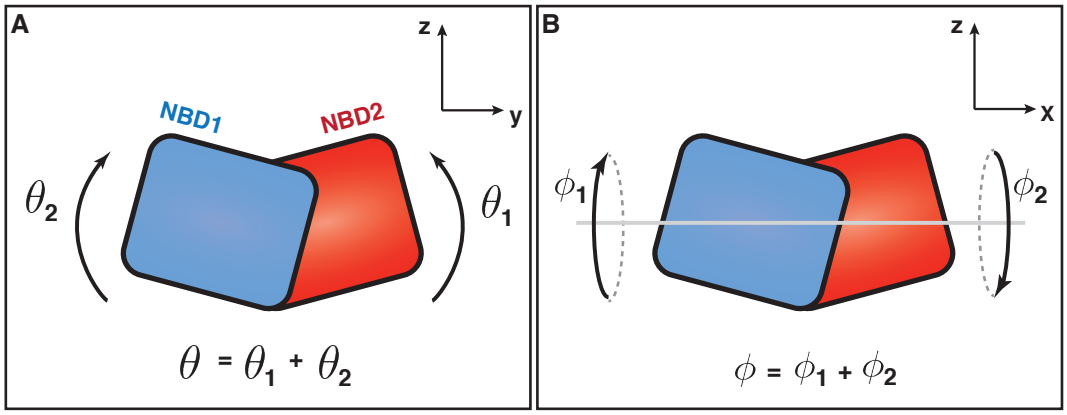

FIGURE S1 Schematic representation of two Euler angles, θ and ϕ, employed to study NBDs global conformational changes. (A) NBDs orientational changes in the θ direction. The θ angle can be calculated by adding the orientational changes in NBD1 and NBD2, θ_1 and θ_2, respectively, with respect to the y axis. (B) NBDs orientational changes in the ϕ direction. The ϕ angle can be calculated by adding the orientational changes in NBD1 and NBD2, ϕ_1 and ϕ_2, respectively, with respect to the x axis. NBD1 and NBD2 are colored in blue and red, respectively, in both (A) and (B).

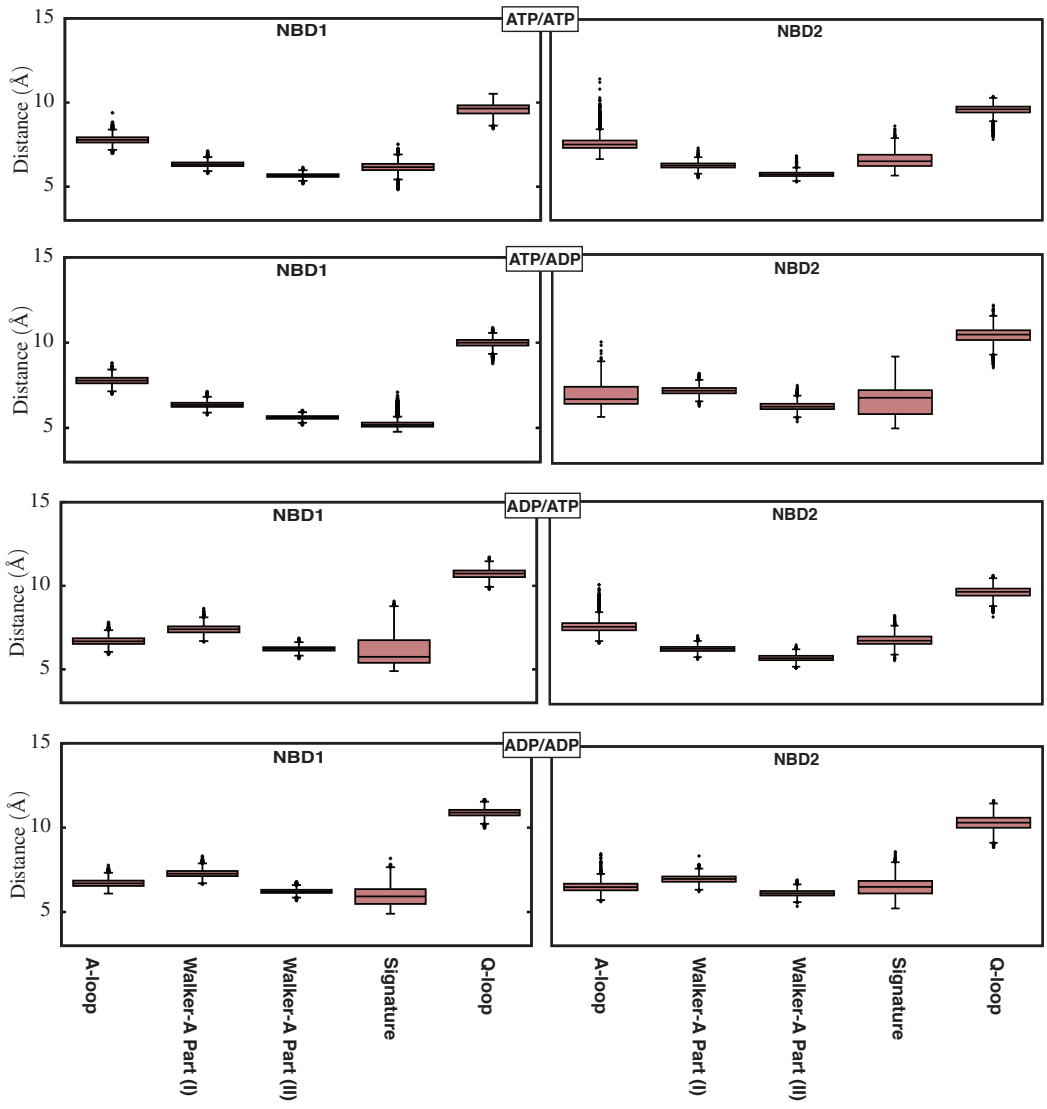

FIGURE S2 COM pairwise distances between nucleotide and different regions at its binding site in both NBDs. Each box plot is obtained from the last 100 ns of all three simulation replicas for each bound state. The distances are calculated between the nucleotides and the A-loop, Walker-A motif part (I), Walker-A motif part (II), the signature motif, and the Q-loop. All of the atoms within each region are used to calculated the COM. The signature motif interacting with each NBD fluctuates the most after ATP hydrolysis in the corresponding NBD.

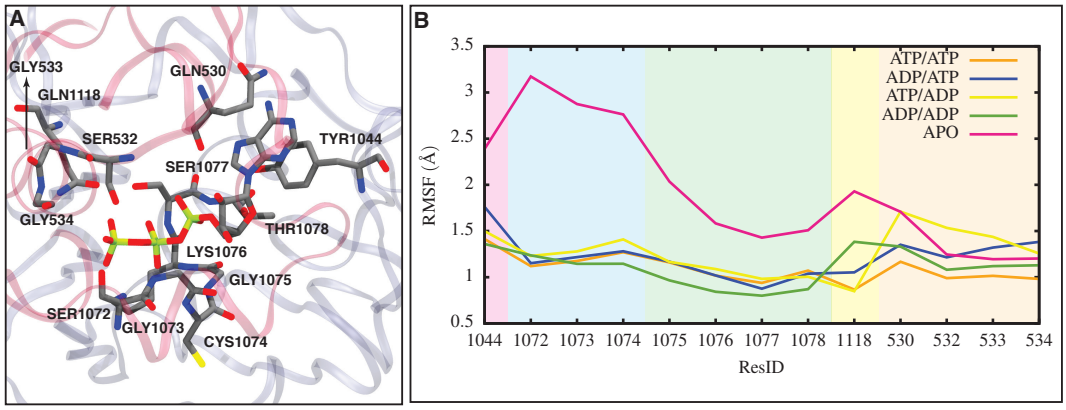

FIGURE S3 Nucleotide binding site in NBD2 for all simulated systems. (A) Nucleotide binding site in NBD2 for one of ATP/ATP systems. (B) RMSF of nucleotide binding site in NBD2 for all simulated systems averaged over the last 100 ns of all three replicas. Background colors correspond to different regions at the nucleotide binding site with residues belonging to A-loop, Walker-A motif part (I), Walker-A motif part (II), signature motif, and Q-loop shown in magenta, blue, green, orange, and yellow, respectively. RMSF values show the highest fluctuation (1-3 Å) in the APO system in comparison to other simulated systems.

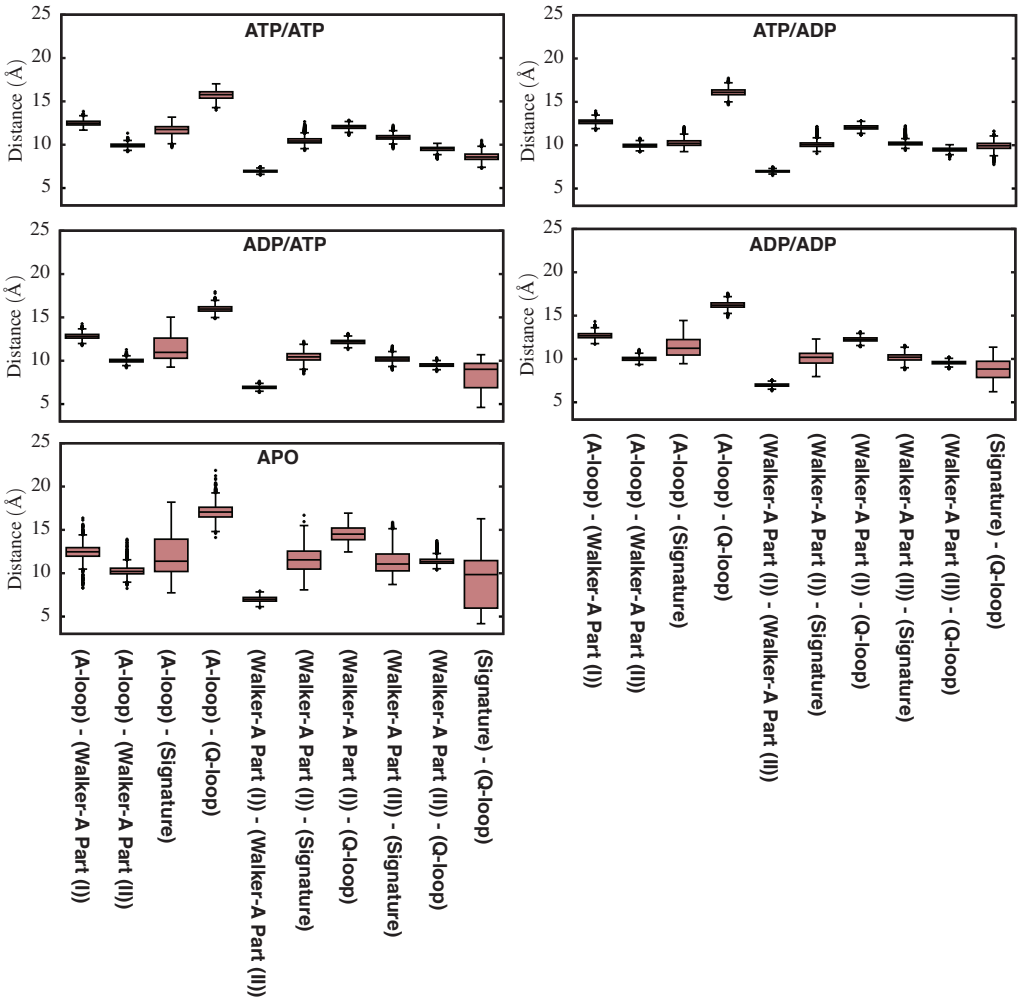

FIGURE S4 Distances between different regions of the nucleotide binding site in NBD1. Each box plot is calculated for a bound state (ATP/ATP, ATP/ADP, ADP/ATP, ADP/ADP, or APO) and from the last 100 ns of all three simulation replicas. Distance with respect to the signature motif shows the most deviation in the ADP/ATP, ADP/ADP, and APO systems due to instability of this motif after ATP hydrolysis and dissociation of the nucleotides.

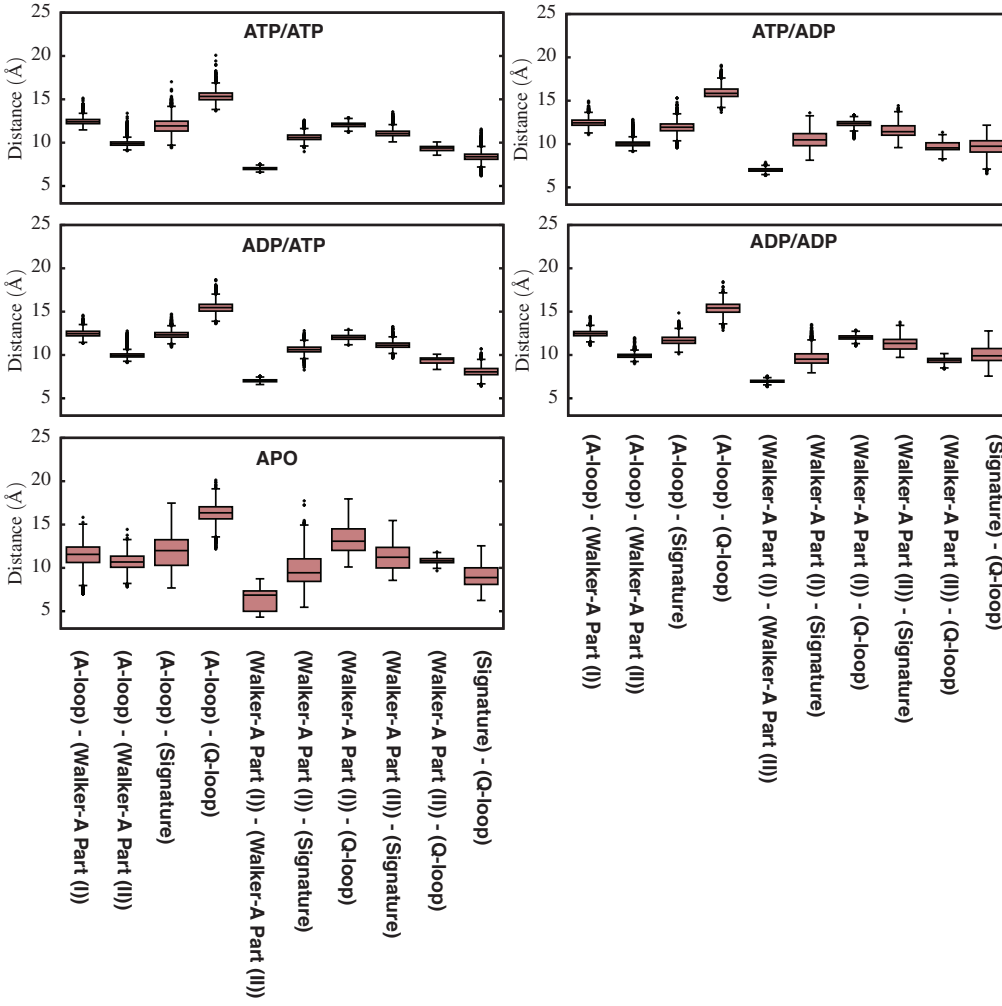

FIGURE S5 Distances between different regions of the nucleotide binding site in NBD2. Each box plot is calculated from the last 100 ns of all three replicas.APO system shows the highest fluctuation in the nucleotide binding site motifs, indicating the importance of the nucleotides in stabilizing the nucleotide binding site in NBD2.

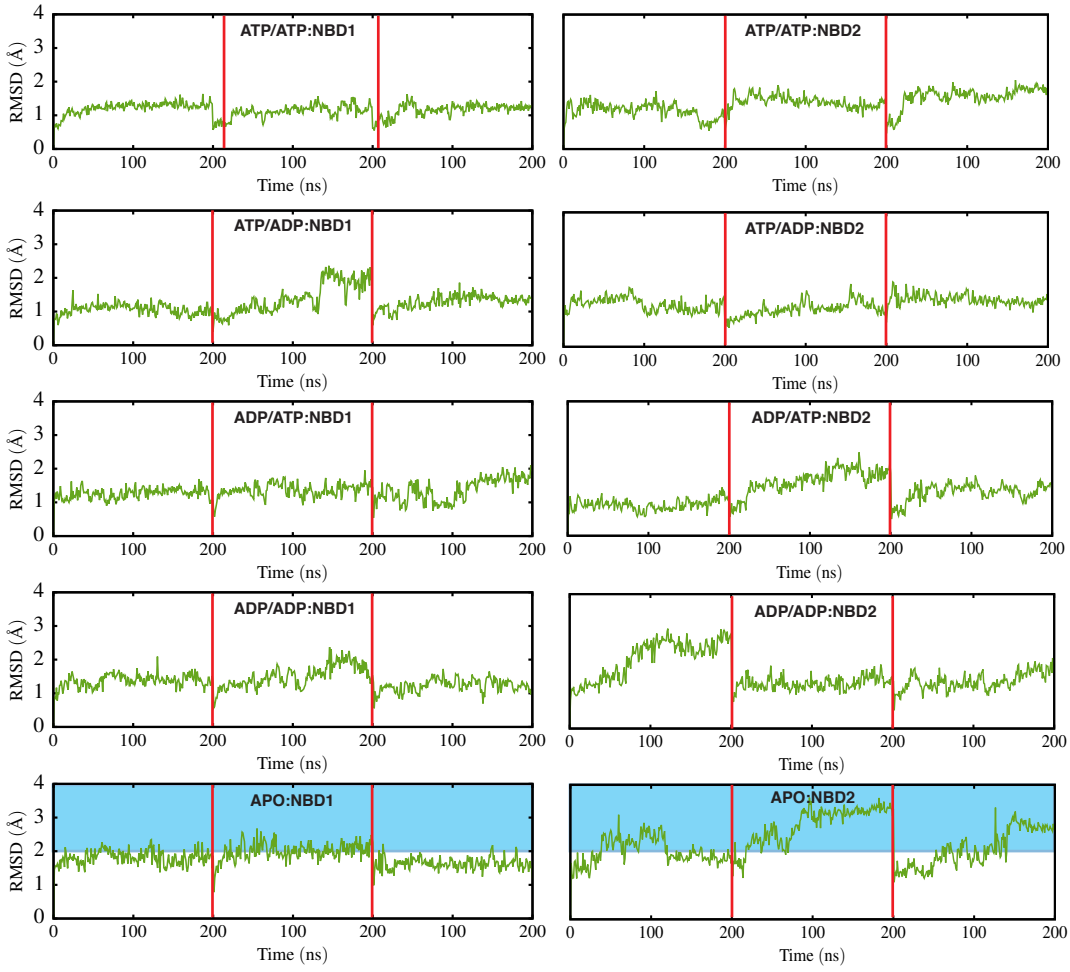

FIGURE S6 Signature and Walker-A motifs RMSD calculations. RMSD values are calculated with respect to the starting structure for the C_α atoms of the signature and Walker-A motifs in both NBD1 (left panels) and NBD2 (right panels), and for all 3 replicates of the simulated systems through the course of simulations. Replicates in each plot are separated by red lines. The highest RMSD values are observed in the case of the APO system, where all the replicates reach more than 2 Å RMSD (highlighted in blue boxes), indicating that nucleotide interaction is necessary for stabilizing the signature and Walker-A motifs. Furthermore, the signature and Walker-A motifs in NBD2 show more fluctuations in comparison to the same motifs in NBD1.

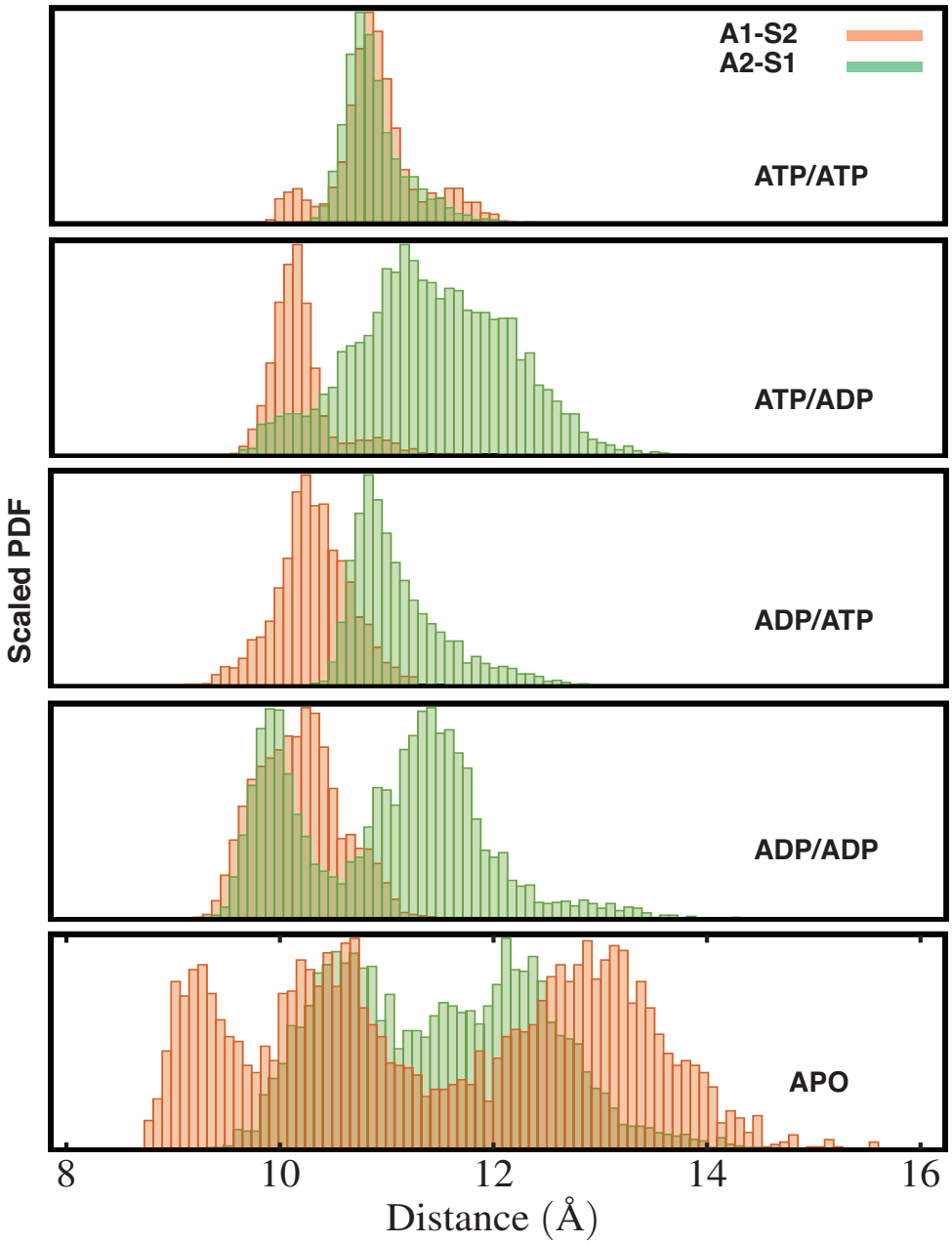

FIGURE S7 COM distances between the Walker-A and signature motifs for all simulated systems. All histograms were obtained from the last 100 ns of all three replicas for each bound state. The y axis in each histogram represents scaled PDF, with the highest probability within each distribution set to one. A1, A2, S1, and S2 represent the following motifs: Walker-A in NBD1, Walker-A in NBD2, signature in NBD1, and signature in NBD2, respectively. Orange histograms correspond to COM distances between all the atoms of the Walker-A motif in NBD1 and the signature motif in NBD2, whereas, green histograms show COM distances between the Walker-A motif in NBD2 and the signature motif in NBD1. NBDs dissociation starts mostly in the APO system after the release of the nucleotides.

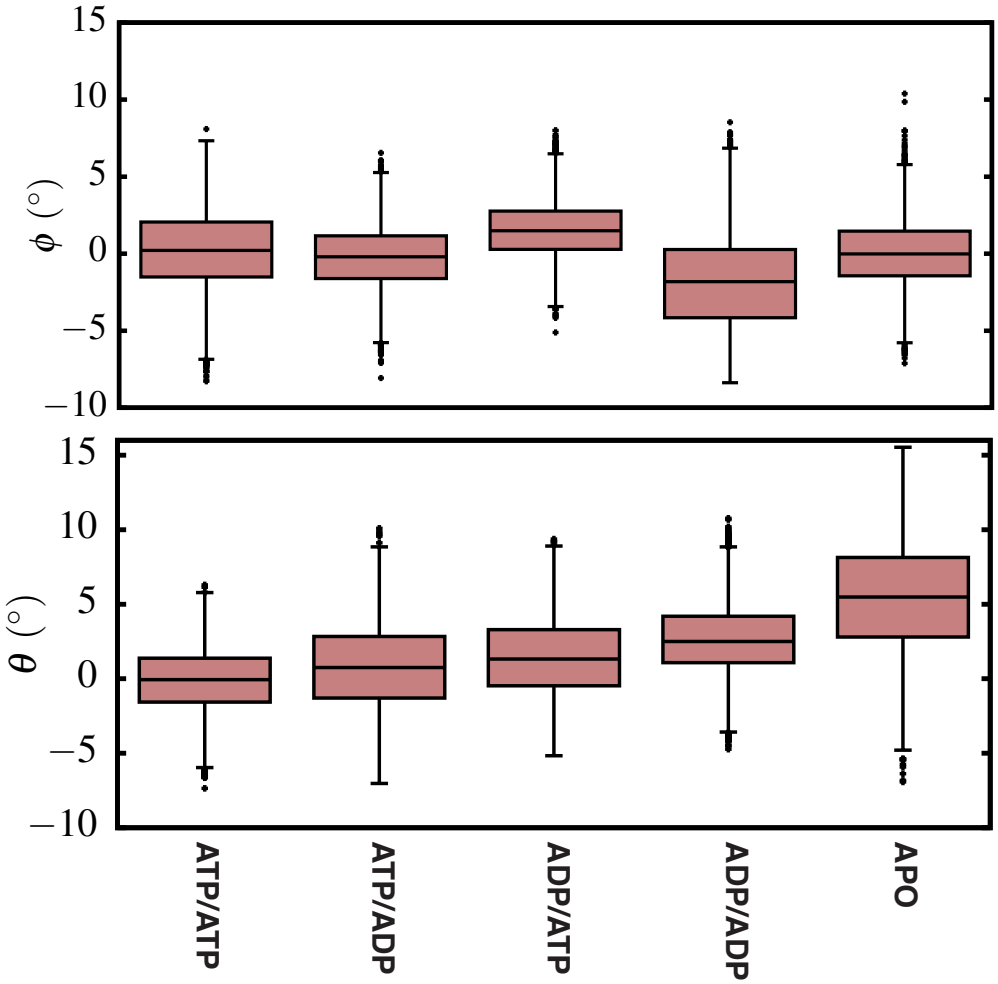

FIGURE S8 Box plots for all bound states showing ϕ and θ, two orientational collective variables representing NBDs global conformational changes. The values correspond to the last 100 ns of each simulation and include all three simulation replicas. θ values increase monotonically after each ATP hydrolysis, as well as after the nucleotides dissociation, whereas ϕ changes in a non-monotonic fashion.

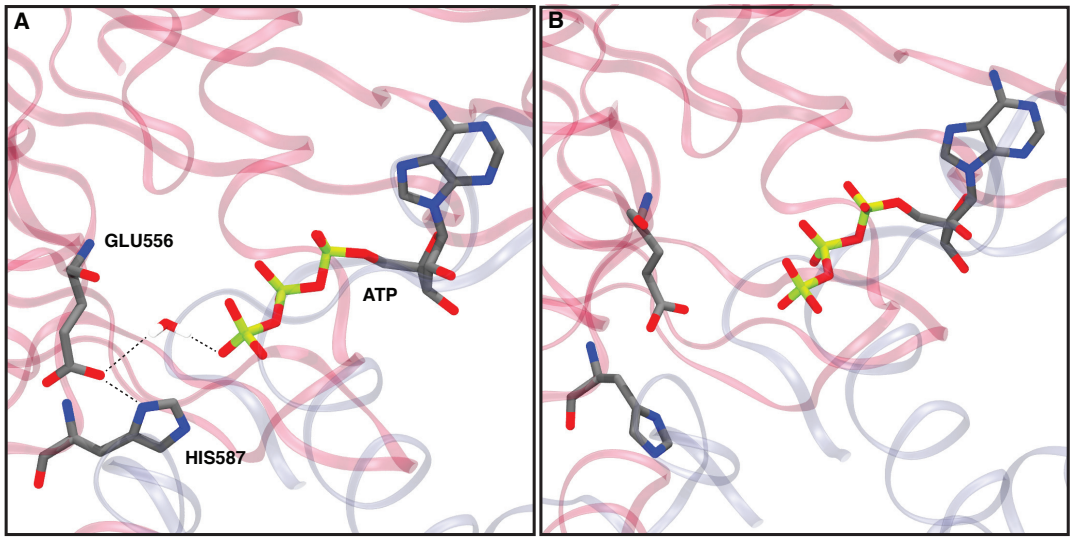

FIGURE S9 Catalytic glutamate GLU556 coordination with HIS587 (H-loop) in NBD1 for one of the ATP/ATP systems. (A) A hydrolysis-competent state in which the catalytic glutamate coordinates with the H-loop and the putative catalytic water. (B) A hydrolysis-non-competent state in which the catalytic glutamate does not coordinate with the H-loop and therefore, is not present in a proper position to capture the catalytic water. The coordination between the H-loop and the catalytic glutamate is thus necessary to capture the putative catalytic water.