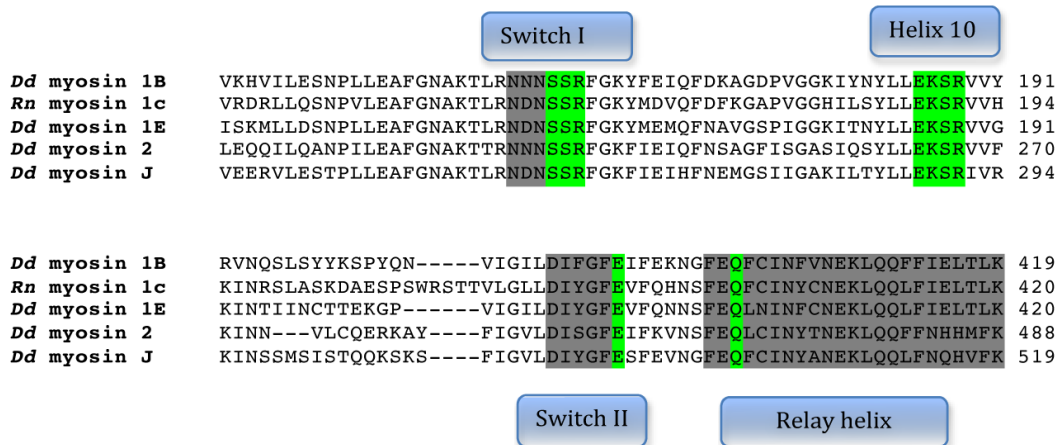
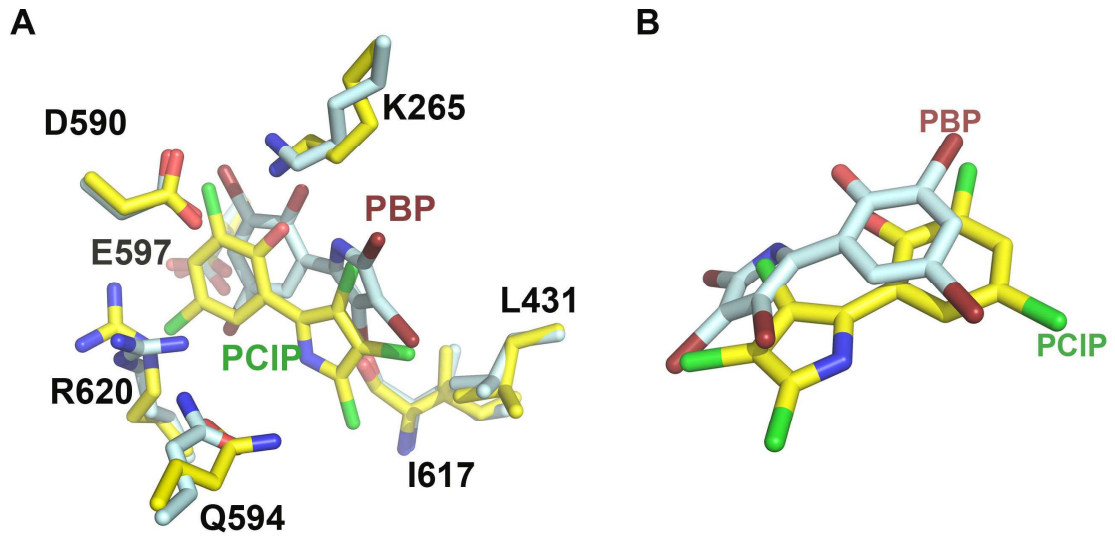


## Figure S1



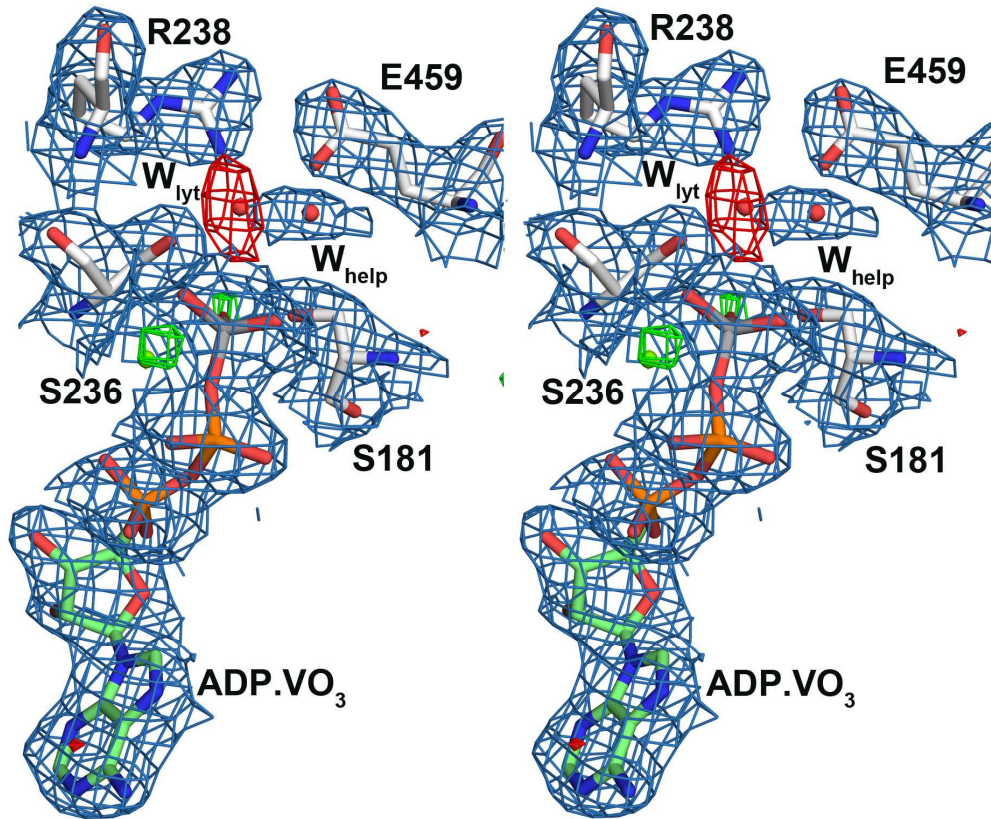
Alignment of residues in the allosteric relay path in different myosin isoforms. Residues in the allosteric path from PCIP binding residues in helix 10 to the nucleotide-binding site are shown. Residues colored in green are involved in the allosteric relay. Further, adjacent residues belonging to the same motif or secondary structure element are shown in grey color.

**Figure S2**



Differences in the allosteric binding site of PBP and PCIP bound myosin motor domain structures. (A) Close-up view of the superimposed binding site residues in the PCIP structure (2XEL) and PBP structure (2JHR) in the pseudilin-binding pocket. (B) Close-up view of the PCIP and PBP molecules in the allosteric binding pocket.

**Figure S3**



Stereo view of the nucleotide binding region with the lytic water ( $W_{Lyt}$ ) included for refinement of the PC1P-bound myosin structure. A clear negative density peak is observed around  $W_{Lyt}$  after refinement. Negative difference density is shown in red and positive difference density in green.  $2F_o-F_c$  density (blue) is shown for ADP•VO<sub>3</sub>, helper water ( $W_{help}$ ) and the residues surrounding the lytic water ( $W_{lyt}$ ).