Supporting Information

Mechanistic Insights into the Differential Catalysis by RheB and its Mutants: Y35A and Y35A-D65A

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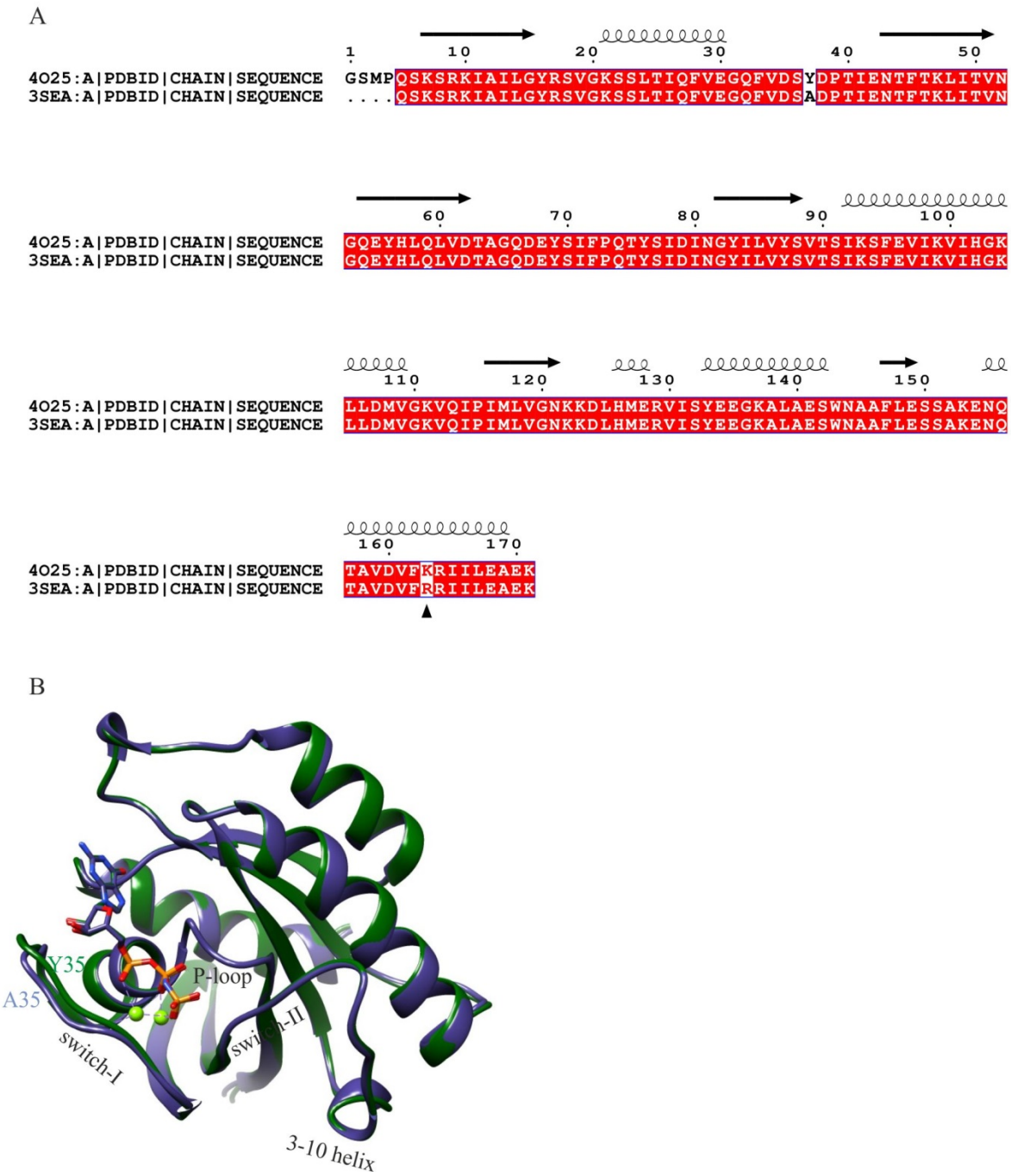

Figure S1. Comparision between the wild-type and Y35A mutant of RheB GTPase. (A) Alignment of protein sequences from the crystal structures of wild-type (PDB ID 4O25) and the Y35A mutant (PDB ID 3SEA). (black triangle indicates the conservative substitution between the two structures). (B) Overlay of the crystal structures of wild-type and the Y35A mutant of RheB GTPase of RheB GTPase. (dark blue –Y35A, dark green – wild-type)

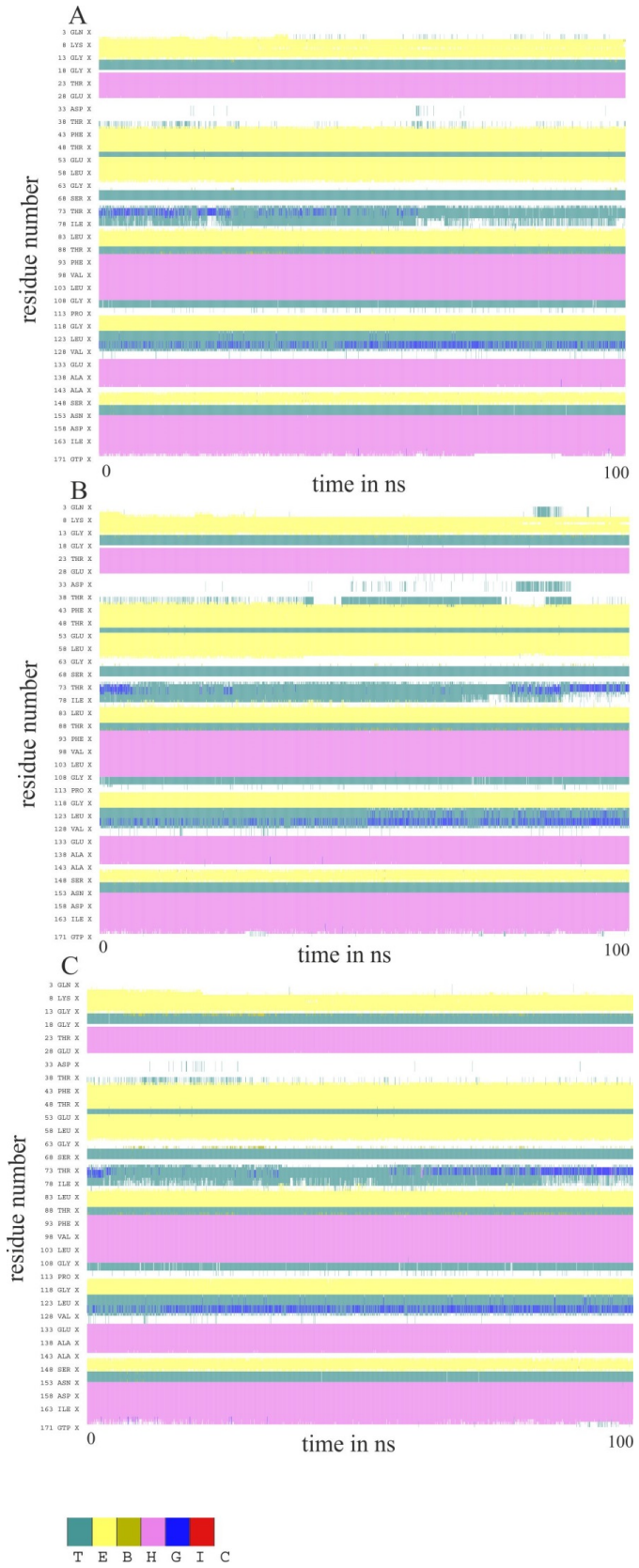

Figure S2. Secondary structure timeline analysis of three trajectories (A, B, C) of the WT. Graphic representation symbol 'T' is hydrogen bonded turn, 'E' is extended parallel or anti-parallel β-sheet, 'B' is single pair β-bridge, 'H, G, I' represents 4, 3, 5-turn helix and 'C' is coil.

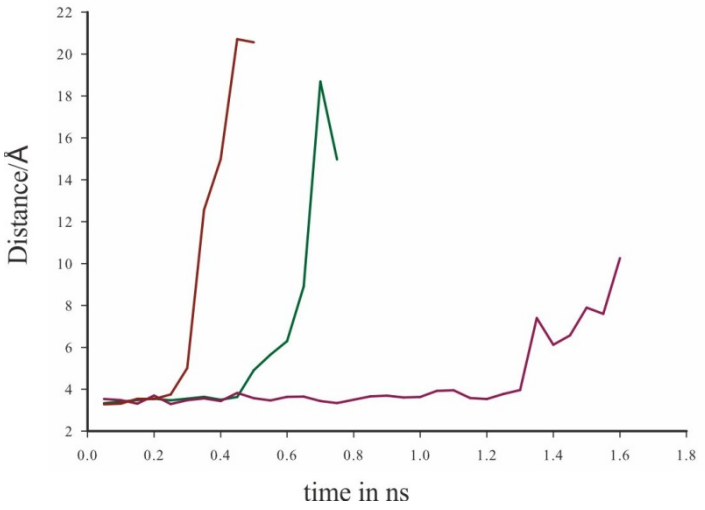

Figure S3. Residence times of equitorial water. Distance between the γ-phosphate atom of GTP and oxygen atom of equitorial water as a function of time. (dark green – WT, dark purple –Y35A-mdl, dark brown –Y35A-D65A-mdl)

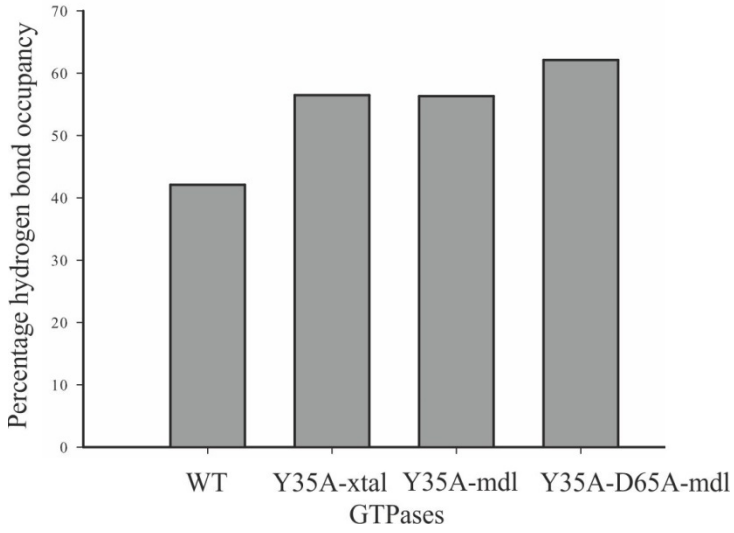

Figure S4. Hydrogen bond interaction between E40 of Switch-I and Y74 of 3-10 helix. Percentage hydrogen bond occupancy of WT, Y35A-xtal, Y35A-mdl, Y35A-D65A-mdl systems.

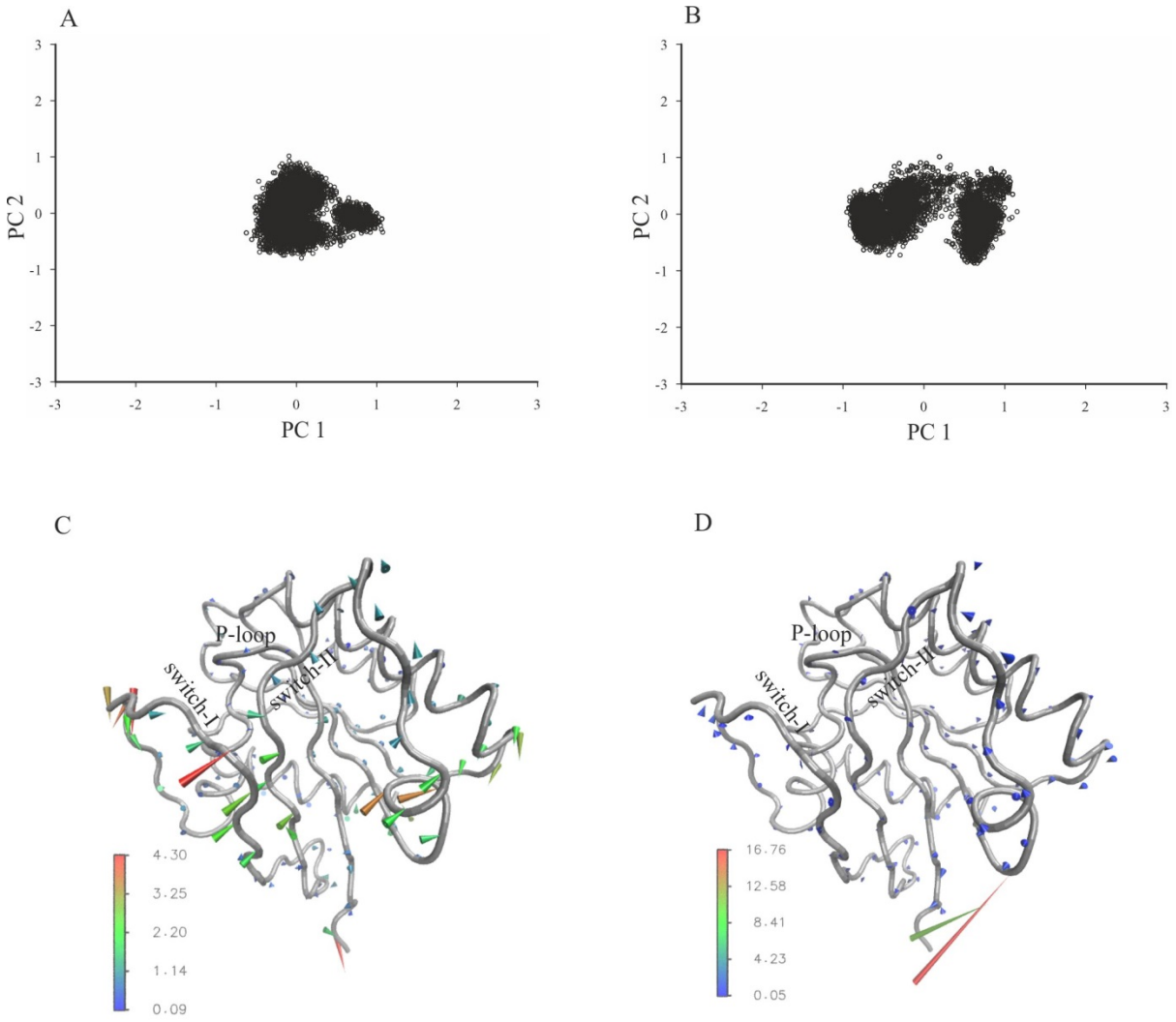

Figure S5. Principal Component Analysis. (A) Projection of MD trajectories on the first two PC's of WT. (B) Projection of MD trajectories on the first two PC's of Y35A-mdl. (C) Porcupine plot showing the significant motions in the WT. (D) Porcupine plot showing the significant motions in the Y35A-D65A-mdl. Arrows on the protein backbone show the direction and the magnitude of the correlated motion. The red color of the arrows depicts the highest movement followed by green while blue depicts the least movement.

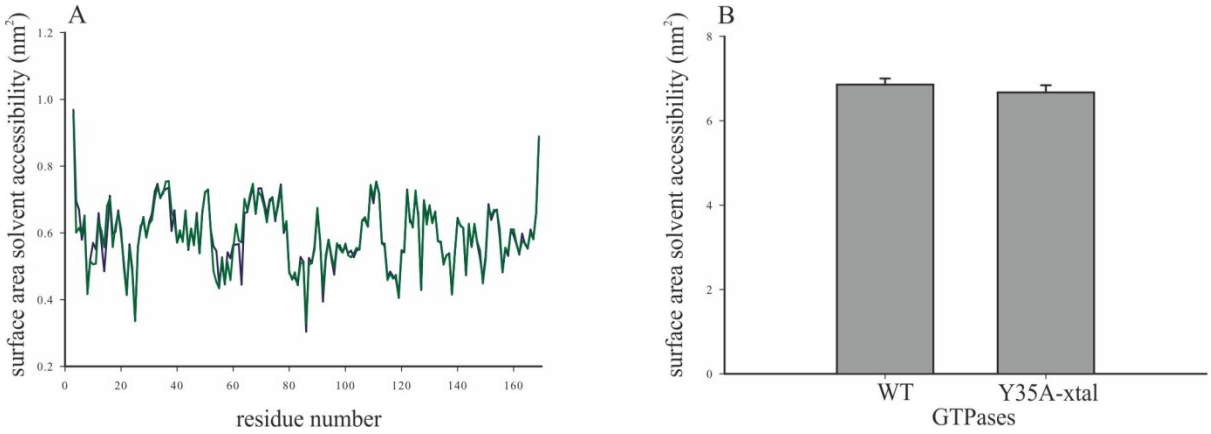

Figure S6. Surface area solvent accessibility analysis (SASA).(A) Overlay of Cα surface area accessibility between WT (dark green) and Y35A-xtal (dark blue) of RheBGTPase. (B) Bar graph representation of surface area accessibility of GTP ligand in the WT and Y35A-xtal.

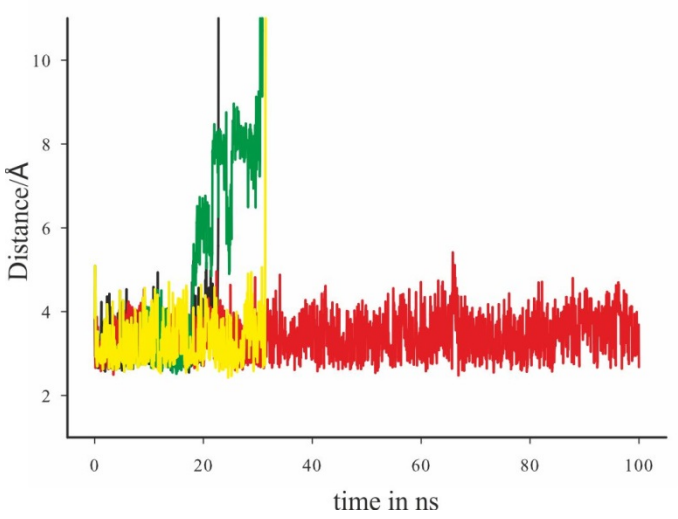

Figure S7. Interaction of equatorial water with T38 in Y35A-xtal. Distance between the backbone oxygen atom of T38 and oxygen atom of the equatorial water molecule as a function of time. (black, red, green, yellow represents four independent trajectories).

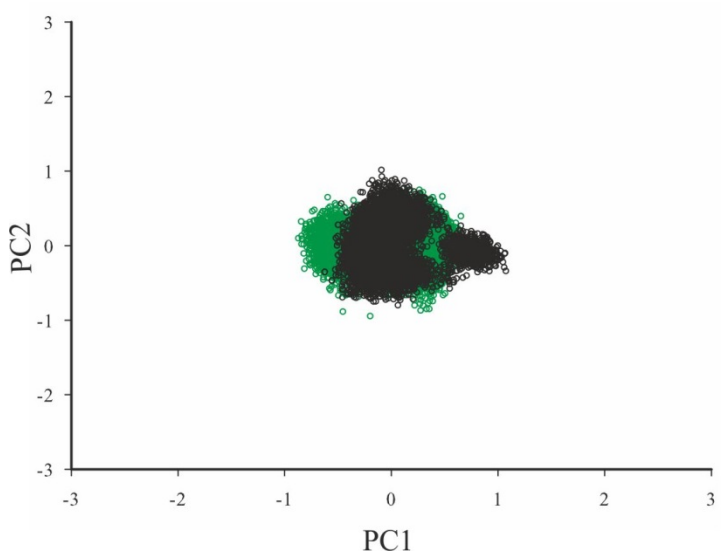

Figure S8. Dihedral Principal Component Analysis of RheB Y35A. Overlay of PCA between the Y35A-xtal (green) and Y35A-mdl (black).

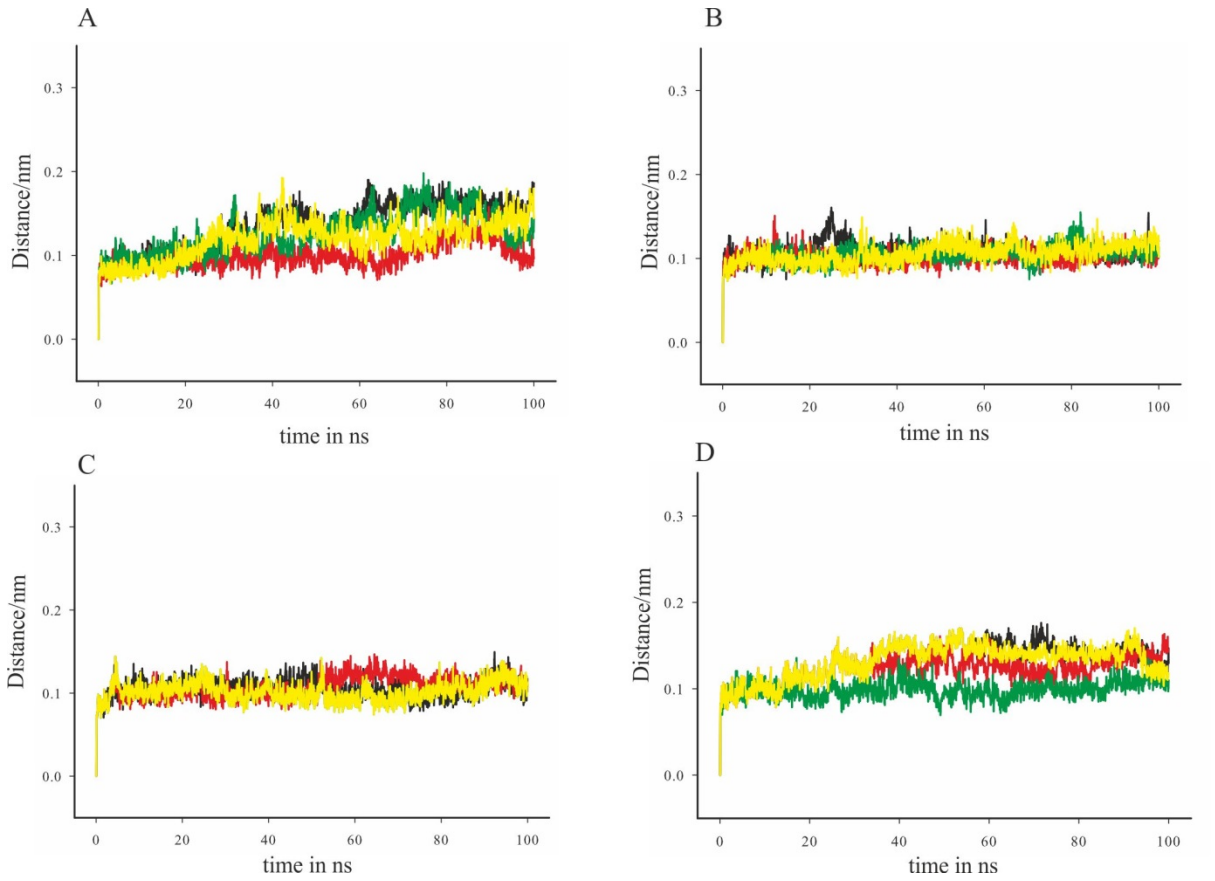

Figure S9. RMSD plot of Cα atoms. (A) Overlay of RMSD plots of the WT. (B) Overlay of RMSD plots of the Y35A-xtal. (C) Overlay of RMSD plots of the Y35A-mdl. (D) Overlay of RMSD plots of the Y35A-D65A-mdl. (black, red, green, yellow represents four independent trajectories)

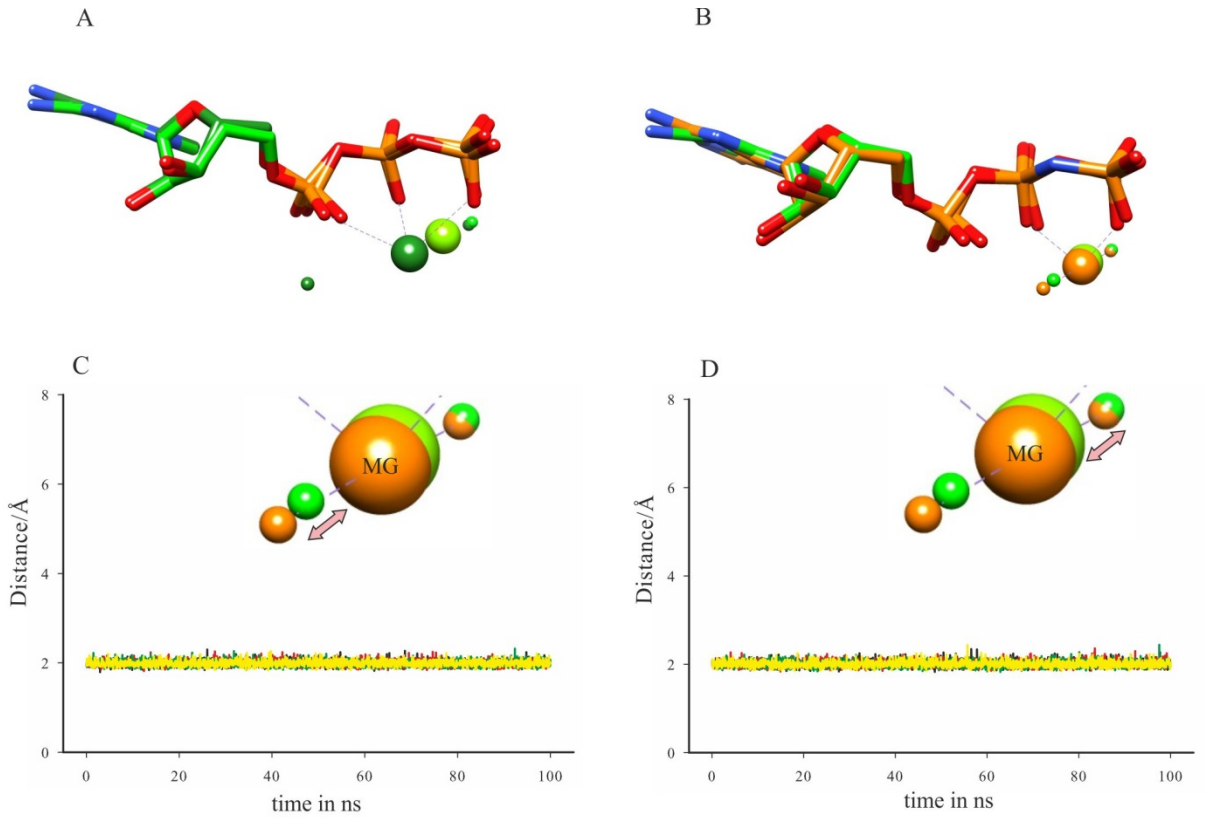

Figure S10. Dynamics of Mg^{2+} coordinated water molecules in WT simulations. (A) Overlay representation of GTP, Mg^{2+} and its coordinated water molecules in the crystal structure of RheB (PDB ID 4O25 B, dark green) and WT (light green) during start of production run. (B) Overlay representation of GTP, Mg^{2+} and its coordinated water molecules in the crystal structure of H-Ras (PDB ID 5P21, orange) and WT (light green) during start of production run. (C) Distance between the Mg^{2+} atom (large sphere) and oxygen of coordinated water molecule (light green, small sphere, arrow representation) as a function of time in WT (black, red, green, yellow represents four independent trajectories). (D) Distance between the Mg^{2+} atom (large sphere) and oxygen of coordinated water molecule (light green, small sphere, arrow representation) as a function of time in WT (black, red, green, yellow represents four independent trajectories).