SUPPLEMENTARY INFORMATION

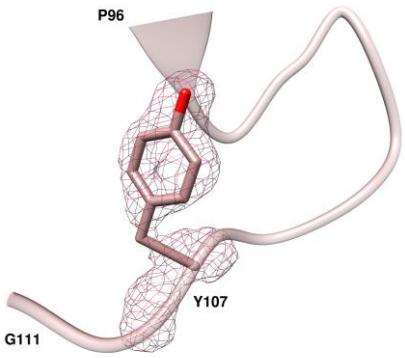

Figure S1. *Fo-Fc* map of residue Y107 at 1 σ is shown as pink mesh. The ribbon shown in pink represents the loop created by residues 96 to 111 in the complex structure of ADP-Glu PPase P96A with pyruvate.

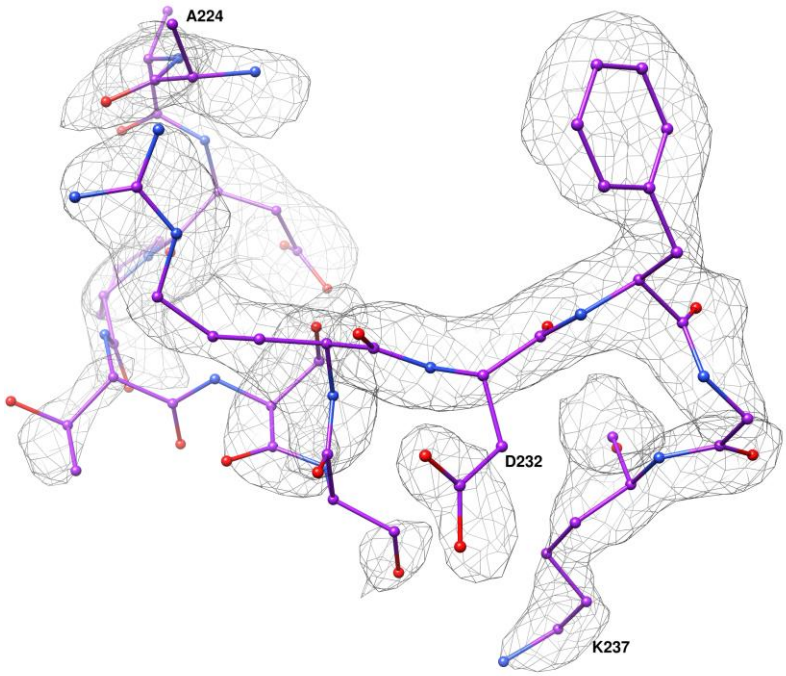

Figure S2. *Fo-Fc* map of residues 224 to 237 at 1 σ is shown as a grey mesh. The atoms that form this loop are shown as purple ball and sticks. The heteroatoms oxygen and nitrogen are shown in red and blue, respectively.

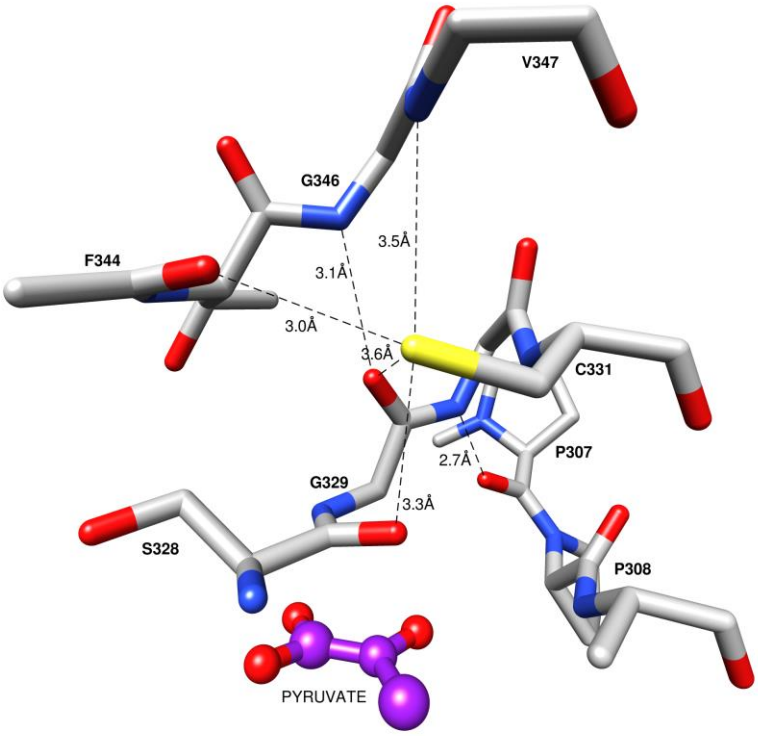

Figure S3. Hydrogen bonding network near the binding site of pyruvate. The location of the Ser328/Gly329 peptide bond relative to pyruvate is enforced by the hydrogen bonding interactions with nearby residues. The enzyme atoms are shown as grey sticks and the heteroatoms oxygen and nitrogen are shown in red and blue, respectively. Pyruvate is shown as a purple ball and stick model with oxygen atoms in red. The hydrogen bonding distances (Å) are shown as black dashed lines.

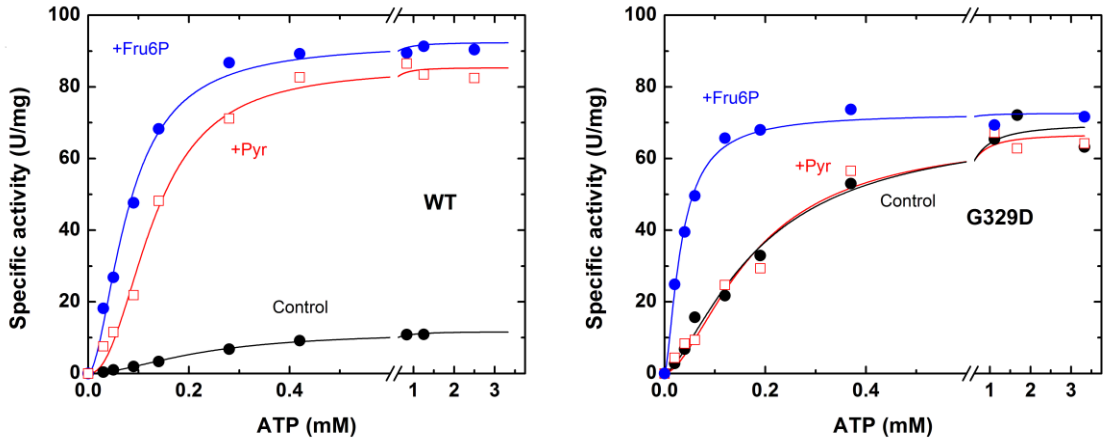

Figure S4. Effect of activators on substrate ATP curves for the G329D mutant and wild type *A. tumefaciens* ADP-Glc PPase. Saturation curves for ATP were obtained as described in Experimental Procedures. The assays contained 1.5 mM of Fru6P (blue) or 1.5 mM of pyruvate (red) when indicated. The control curve (black) contained no activator present.

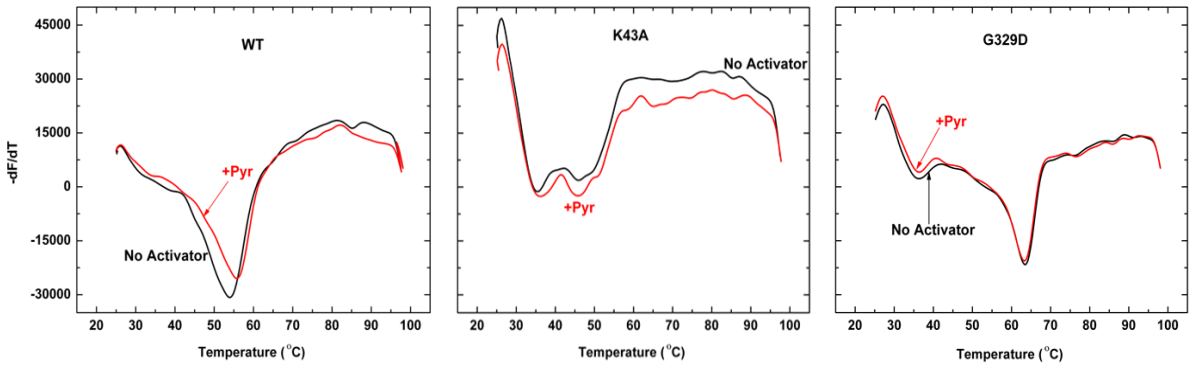

Figure S5. Thermal shift analysis of mutants and wild-type ADP-Glc PPase from *Agrobacterium tumefaciens*. The effect of temperature on the stability of the enzyme was assayed in the presence and absence of the activator (Pyr) for the wild type (WT), and mutants K43A, and G329D. The thermal shift assays were performed as described in the materials and methods. The black line represents the control in absence of the activator, and the red line represents the presence of pyruvate.

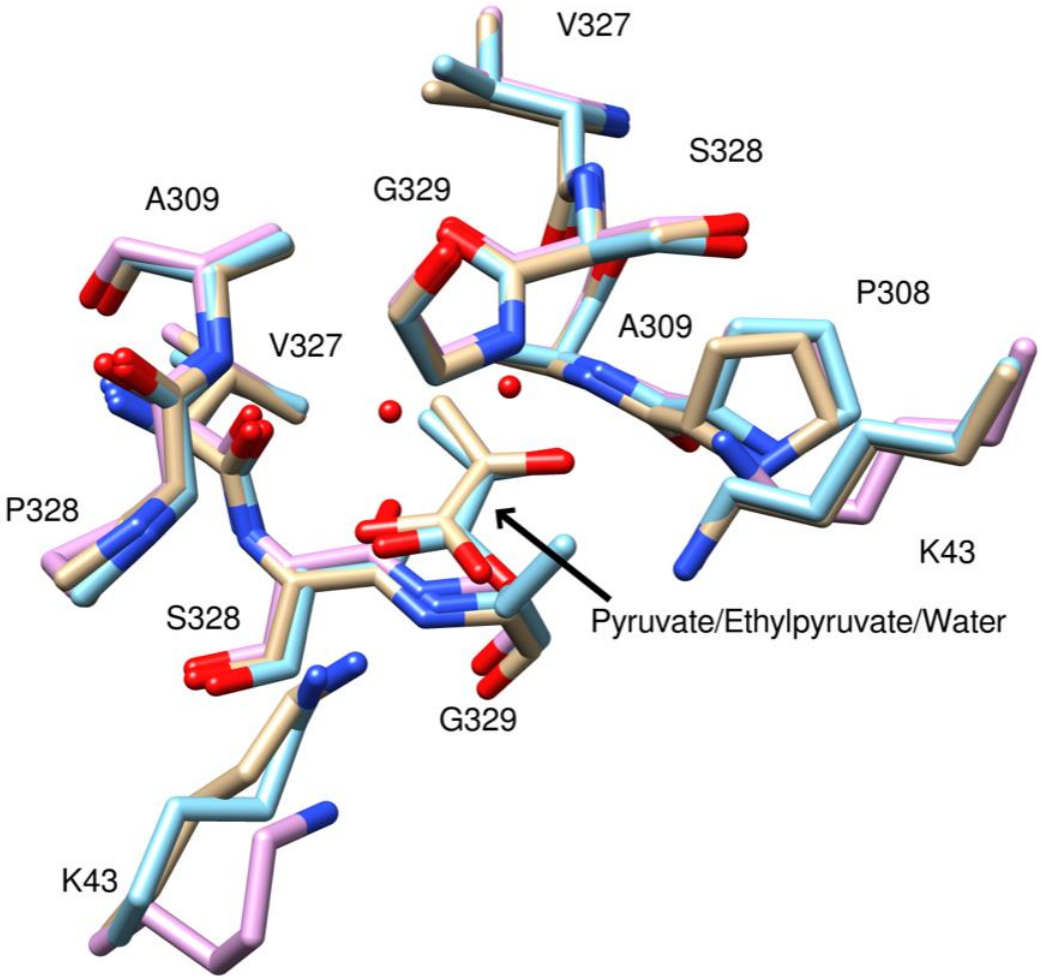

Figure S6. Overlap of the structures with pyruvate (PDB 5W5R, blue), ethyl pyruvate (PDB 5W5T, tan), and no ligand (PDB 5W6J, magenta). The structure with no ligand show water molecules that overlap with the structure of the ligands.

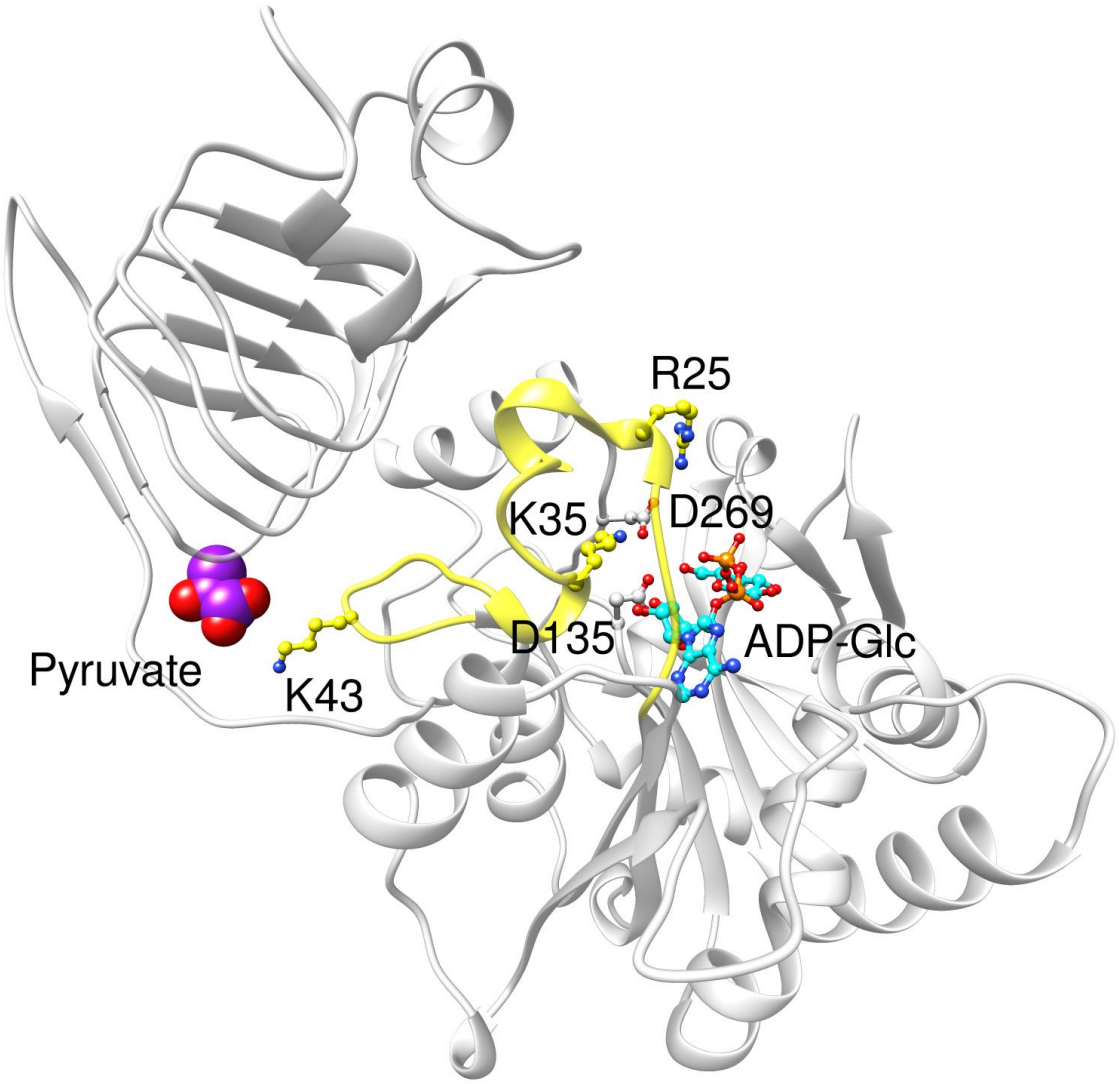

Figure S7. Connection between the pyruvate site and the catalytic site. Loop from residues 20-46 is depicted in yellow. Residues R25, K35, D135, and D269 are in the active site and critical for catalysis. The product ADP-Glc is shown here for illustration purposes, to highlight the position of the active site. Its coordinates were inherited from the small subunit potato tuber ADP-glucose pyrophosphorylase (PDB 1YP4, subunit B) after overlapping the structures 1YP4 and 5W5R.

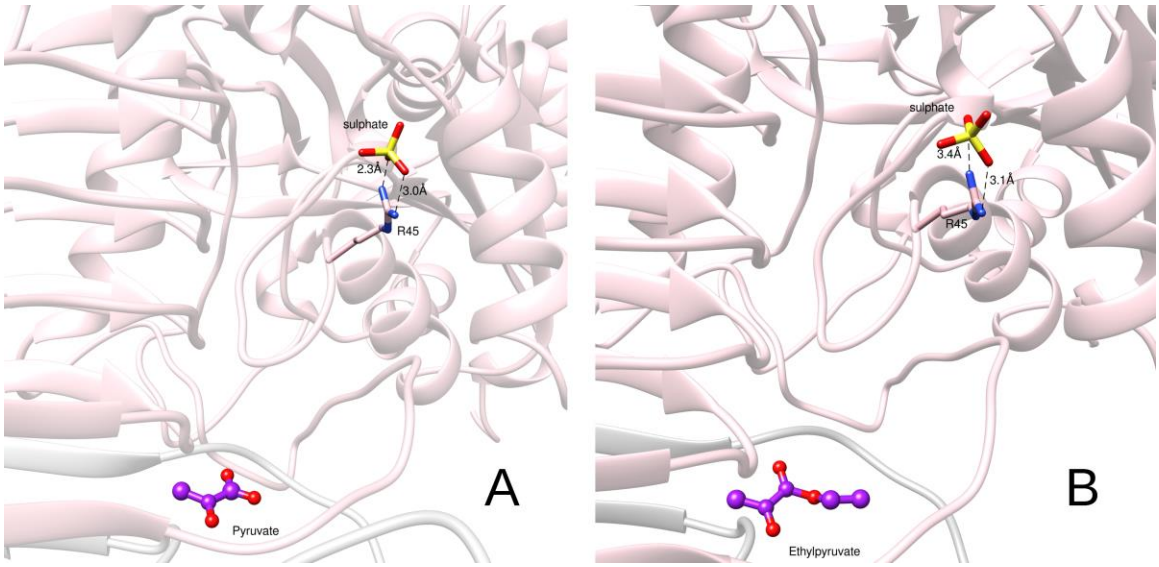

Figure S8. Structure of *A. tumefaciens* P96A ADP-Glc PPase showing simultaneous binding of pyruvate and sulfate. A. The sulfate displayed in this picture (PDB accession code 5W5R and 5W5J) interacts with Arg45, which is critical for Fru6P activation. There are other sulfates bound in the structure are not shown. B. This is the structure of the wild type enzyme with ethyl pyruvate.

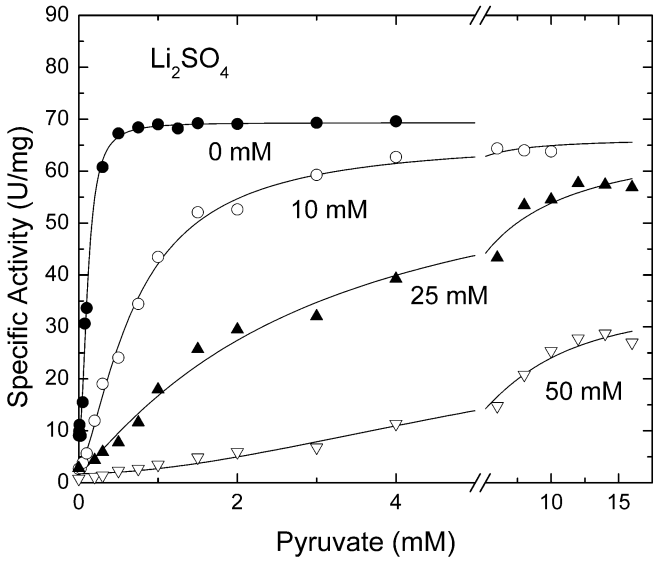

Figure S9. Effect of Li_2SO_4 on the activation by pyruvate of the *A. tumefaciens* ADP-Glc PPase. Saturation curves for pyruvate were obtained as described in Experimental Procedures. In each curve, a constant amount of Li_2SO_4 was added as indicated.

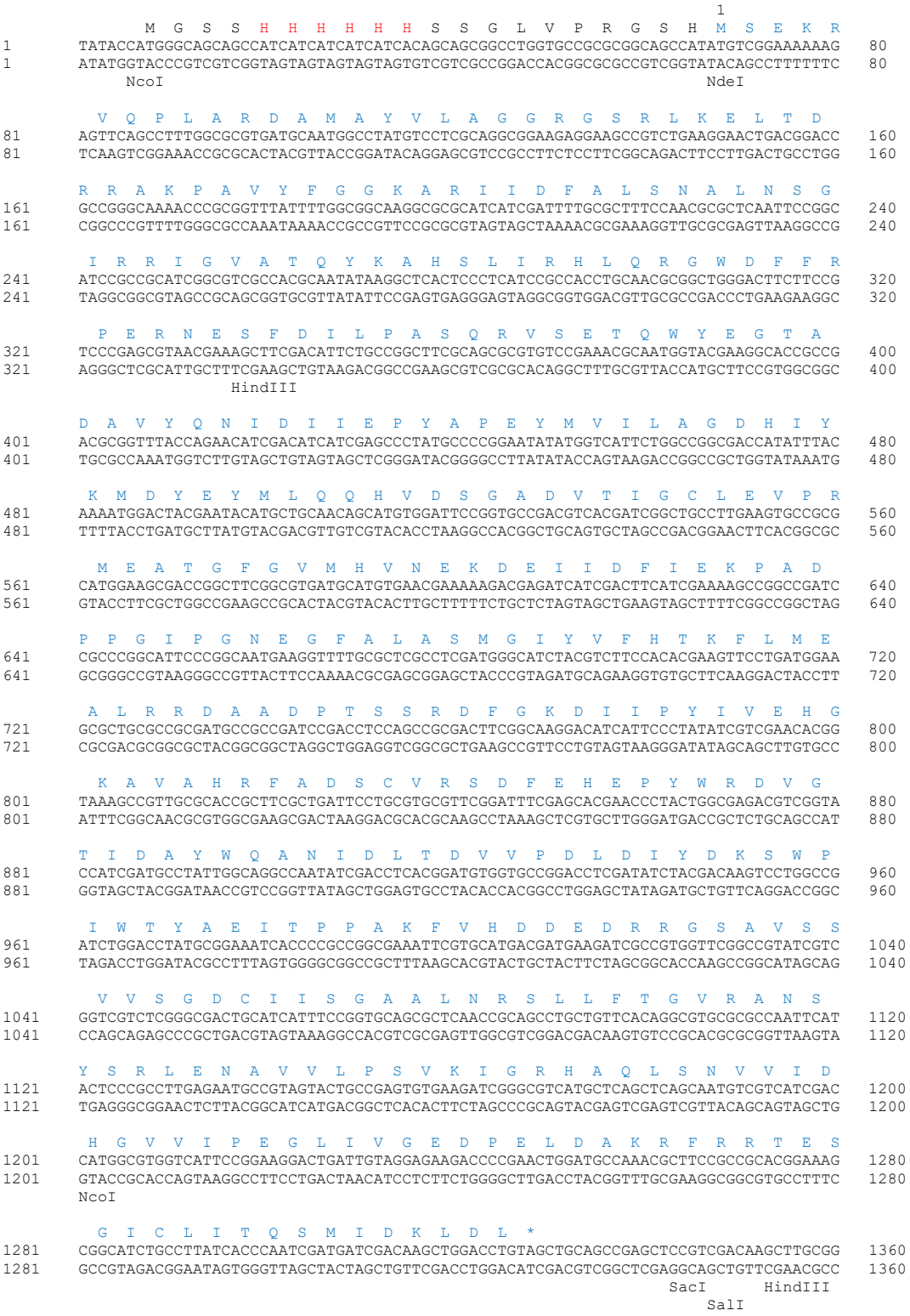

Figure S10. Sequence of the coding region of the plasmid pBLH1. The gene coding for the *A. tumefaciens* ADP-Glc PPase was subcloned into pET-28c as described in Materials and Methods. The coding region starts at the *Nco*I restriction site, includes the His-Tag (red), and the sequence from the enzyme (blue). Numeration used in the paper starts at the methionine near the *Nde*I site. DNA sequences before and after *Nde*I and *Sac*I, respectively, belong to the vector pET-28c.

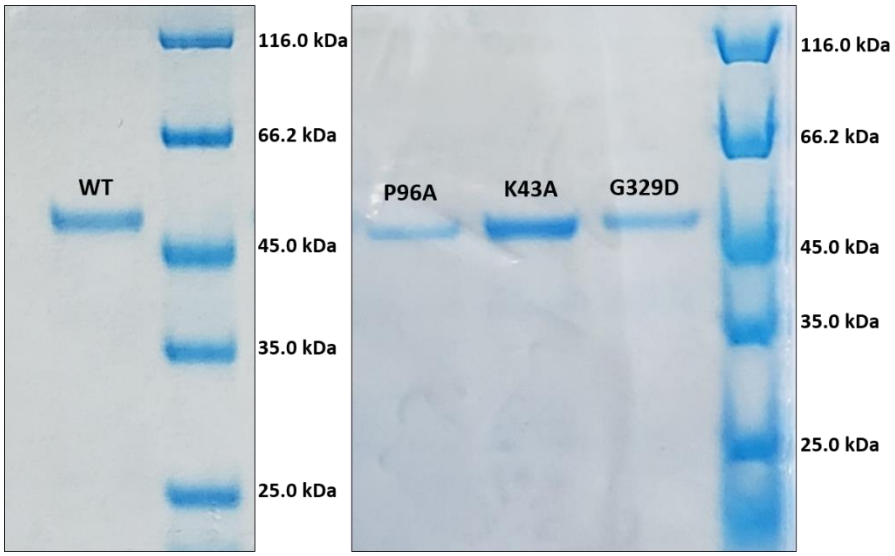

Figure S11. Electrophoretic homogeneity of the wild type and mutant (P96A, K43A, and G329D) ADP-Glc PPase from *Agrobacterium tumefaciens*. Proteins and markers were separated using 10% SDS gels, and stained with Coomassie Brilliant Blue dye. The amount of protein loaded for the WT, P96A, K43A, and G329D were 0.4, 0.7, 1.2, and 0.8 µg, respectively. The molecular mass of the protein standards are shown on the right side.