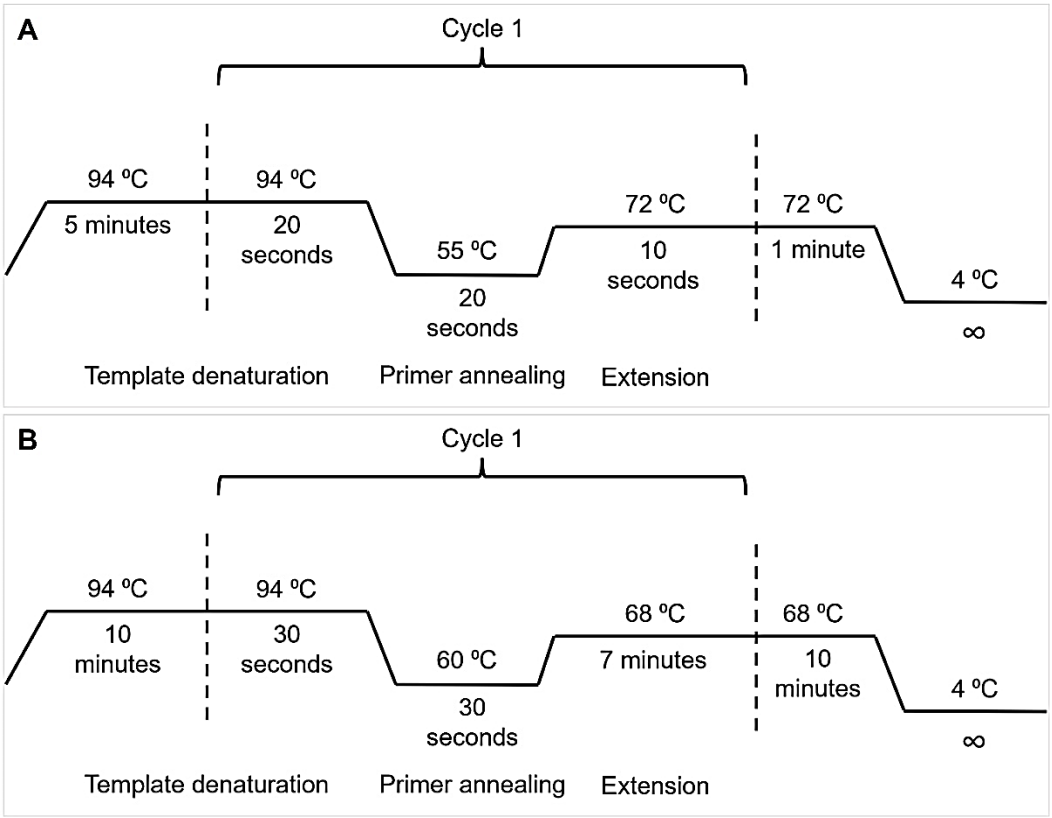
Supplementary data for the manuscript entitled: "Biophysical characterization of a novel phosphopentomutase from the hyperthermophilic archaeon *Thermococcus kodakarensis*"

1. *Construction of the expression vector, pET-His$_6$-TEV-Tk1777, using Quick-change PCR*



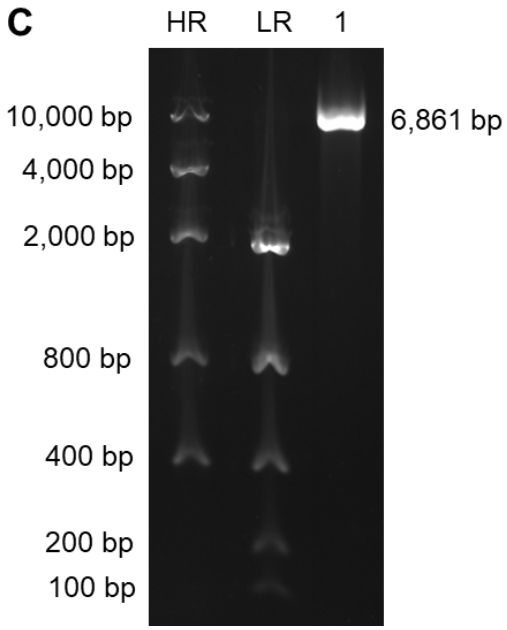


Figure S1. Pictorial representation of Quick-change PCR steps for constructing the expression vector, pET-His$_6$-TEV-Tk1777. (A) step 1: primer annealing and extension, (B) step 2: construct extension, (C) agarose gel showing: HR: Invitrogen E-Gel™ 96 High Range DNA Ladder (Product No. MAN0001082); LR: Invitrogen E-Gel™ Low Range Quantitative DNA Ladder (Product No. MAN0001085); Lane 1: purified PCR product (6,861 bp).

2. Restriction digestion analysis of the expression vector, pET-His$_6$-TEV-Tk1777

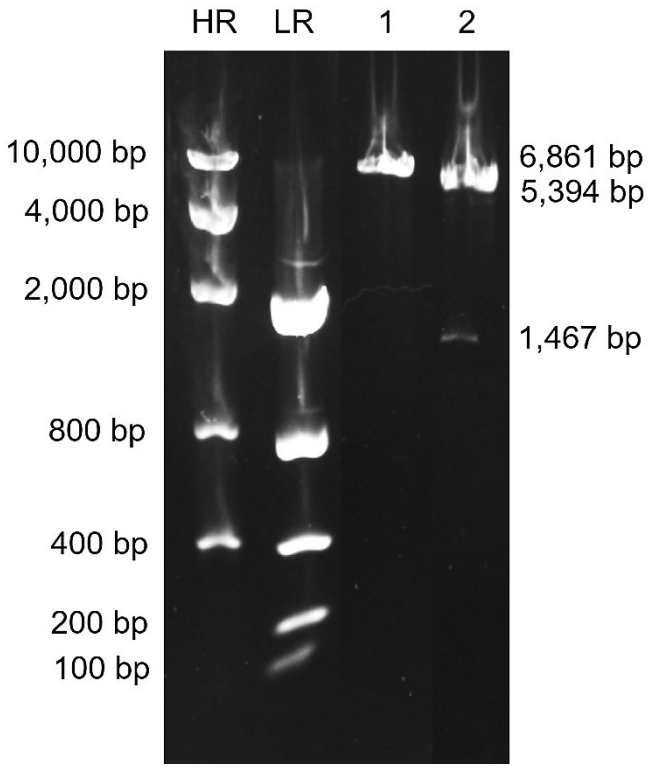
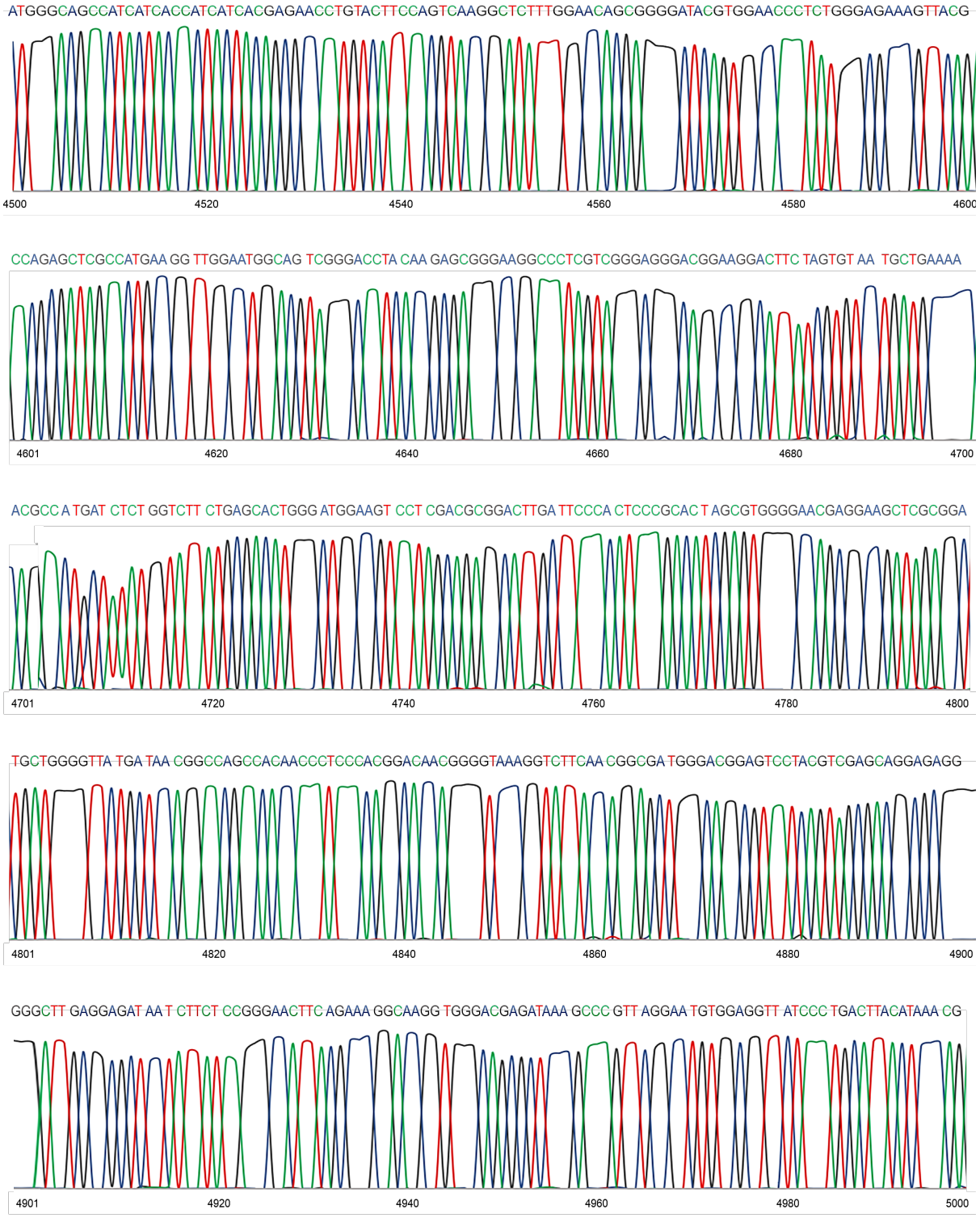
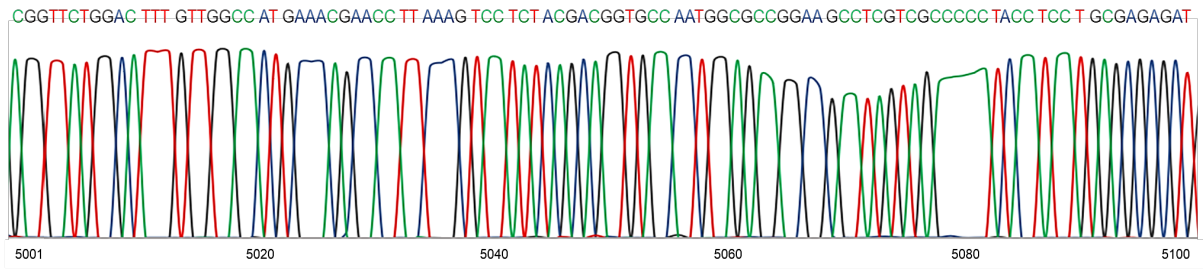
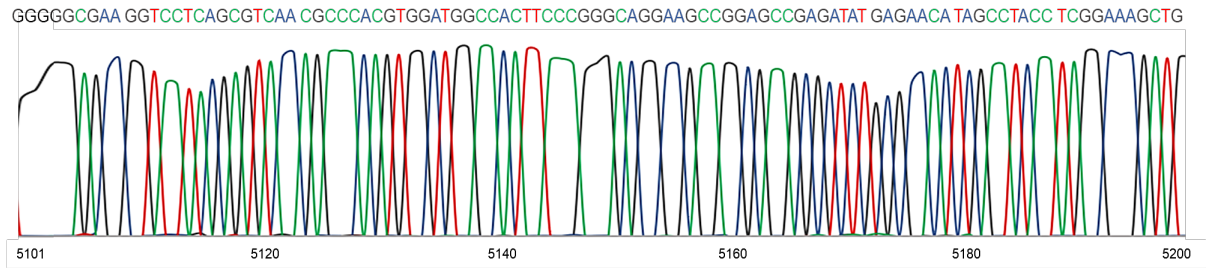


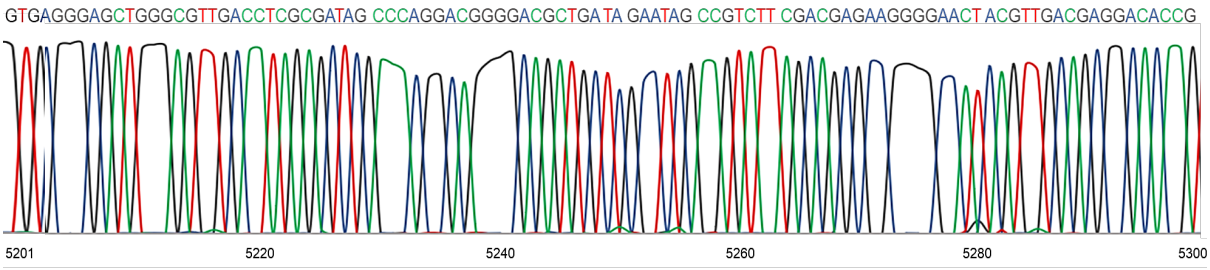
Figure S2. Agarose gel (Invitrogen E-Gel™ EX Agarose Gels, Cat. No. G401001) for restriction digestion analysis of the construct pET-His$_6$-TEV-Tk1777) with *Eco*R1 and *Nde*I. HR: Invitrogen E-Gel™ 96 High Range DNA Ladder (Product No. MAN0001082); LR: Invitrogen E-Gel™ Low Range Quantitative DNA Ladder (Product No. MAN0001085); Lane 1 shows the undigested construct (6,861 bp); and Lane 2 shows the bands of size 1,467 bp, and 5,394 bp, obtained after restriction digestion.

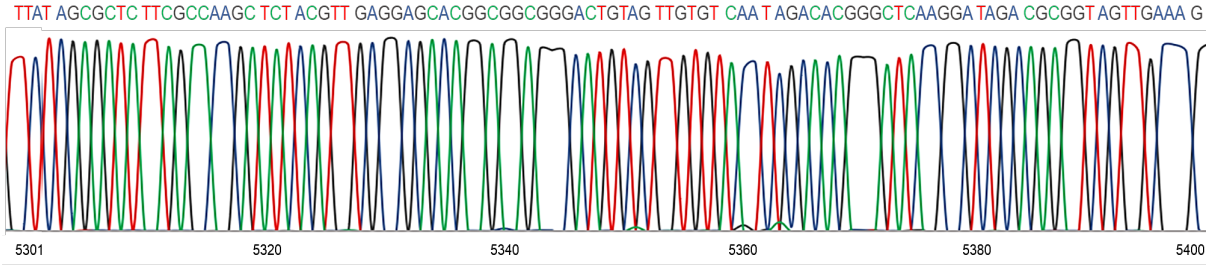
3. *Sequencing electropherogram for His_6-TEV-Tk1777*

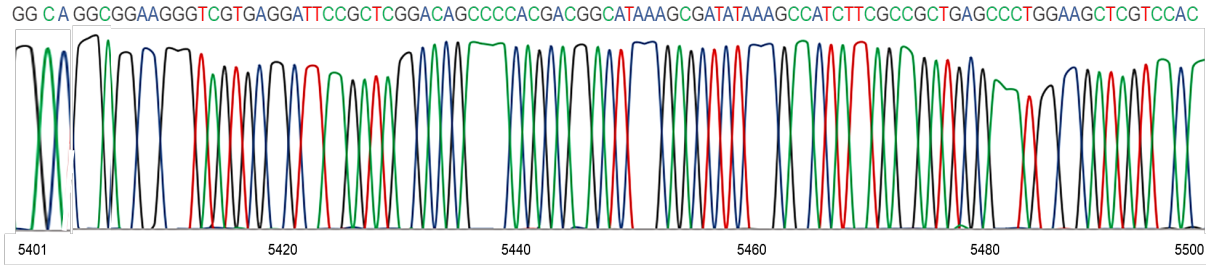


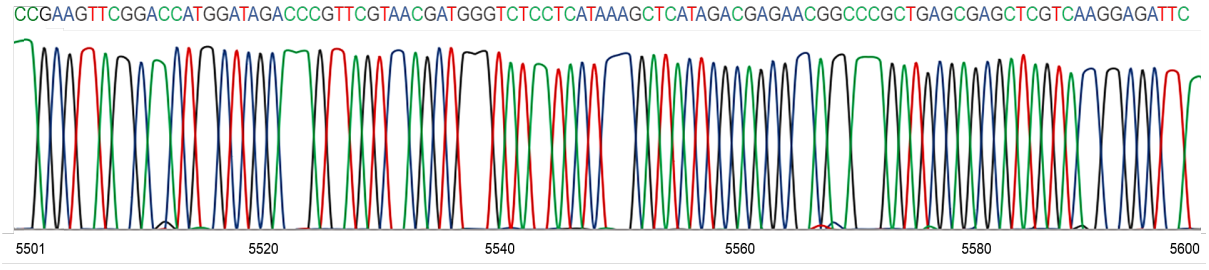

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5040
5060
5080
5100


GGGGGCGAA GGTCCTCAGCGTCAA CGCCCACGTGGATGGCCACTTCCCGGGCAGGAAGCCGGAGCCGAGATAT GAGAACA TAGCCTACC TCGGAAAGCTG
5101
5120
5140
5160
5180
5200


GTGAGGGAGCTGGGCGTTGACCTCGCGATAG CCCAGGACGGGGACGCTGA TA GAATAG CCGTCTT CGACGAGAAGGGGAACT ACGTTGACGAGGACACCG
5201
5220
5240
5260
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5301
5320
5340
5360
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5400


GG C A GGCGGAAGGGTCGTGAGGATTCCGCTCGGACAGCCCCACGACGGCATAAAGCGATATAAAGCCATCTTCGCCGCTGAGCCCTGGAAGCTCGTCCAC
5401
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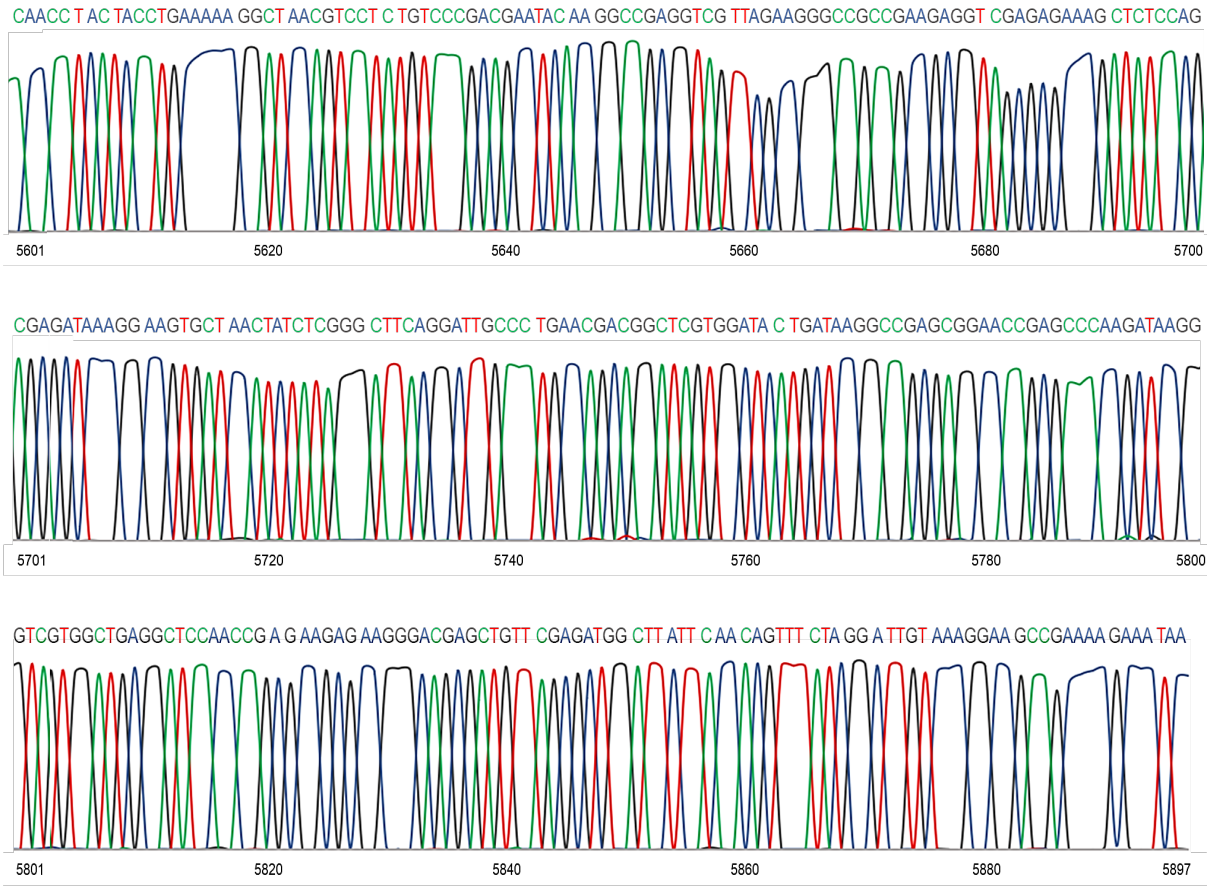


Figure S3. The computer-generated electropherogram shows the nucleotide sequencing for His_6-TEV-Tk1777. The expression construct was subjected to Sanger sequencing to confirm the product of Quick-change PCR.

4. SDS-PAGE analysis of the expression of His_6-TEV-Tk1777 in E. coli strains

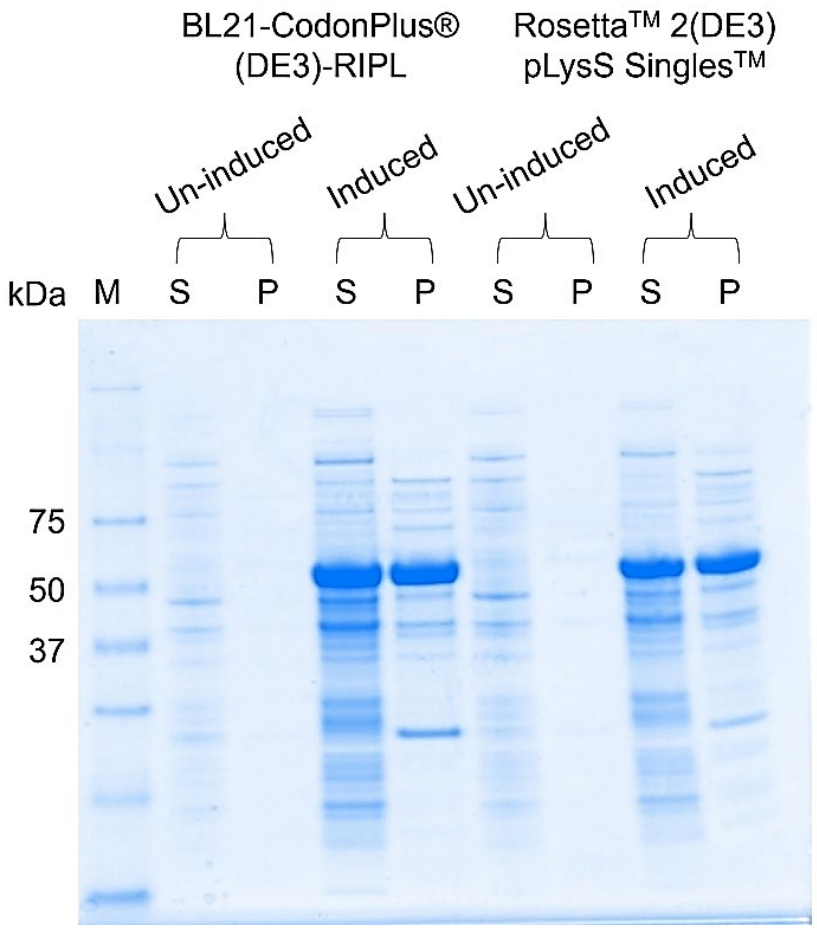


Figure S4. SDS-PAGE analysis of the expression of His_6-TEV-Tk1777 in *E. coli* strains; BL21-CodonPlus®(DE3)-RIPL, and Rosetta™ 2(DE3) pLysS Singles™. Protein expression was carried out at 18 °C, using 1 mM IPTG as an inducer. Lane S shows the soluble fractions while Lane P shows inclusion bodies. Lane M is the protein marker (BioRad_Precision Plus Protein™ Unstained Protein Standards, Cat. #1610363).

5. *Sequence alignment of PPM_{Tk} and PGM_{Ph}*



Figure S5. Pair-wise sequence alignment (PSA) of PPM_{Tk} and PGM_{Ph} done using Clustal W shows presence of four conserved motifs in PGM_{Ph}. For PPM_{Tk}, motif I and II are present while the motifs III and IV have different amino acid residues. Symbol 'I' (bar) shows the identical residues; a ':' (colon) highlights residues with strongly similar properties; a '.' (period) indicates residues with weekly similar properties; and a '-' (hyphen) shows the gap.

6. *Cofactor binding site of PPM_{Tk} and PGM_{Ph}*

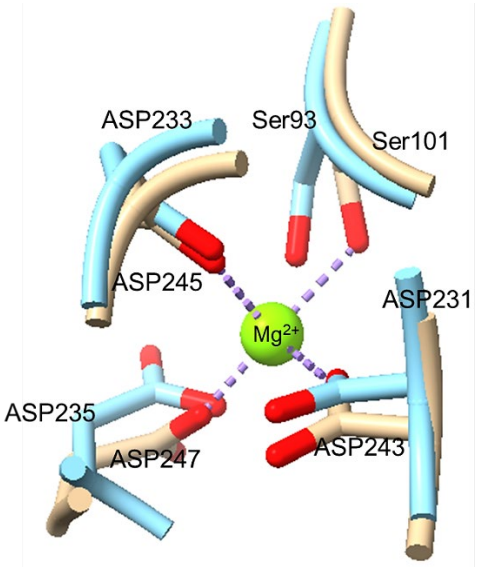


Figure S6. Structural alignment of PPM_{Tk} and PGM_{Ph} done using ChimeraX (ver. 1.9) showed the well-aligned cofactor binding site. Magnesium (MG^{2+}) helps stabilize the overall protein structure and supports binding of the substrate to the active site cleft. Cofactor binding residues of PGM_{Ph} (in tint color): Ser^{101}, Asp^{243}, Asp^{245}, and Asp^{247}; of PPM_{Tk} (in light-blue color): Ser^{93}, Asp^{231}, Asp^{233}, and Asp^{235}.