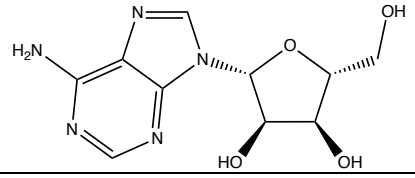
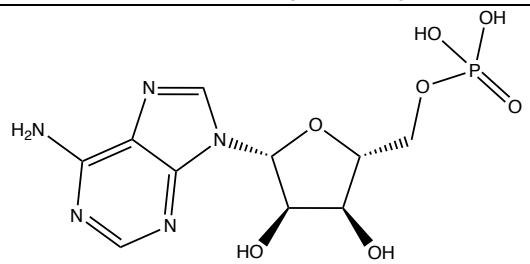
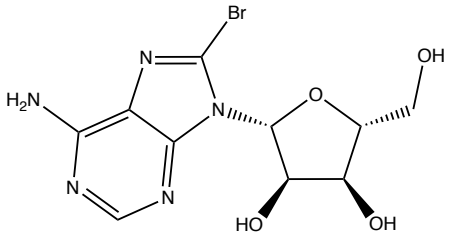
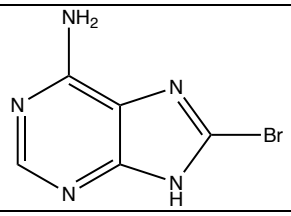
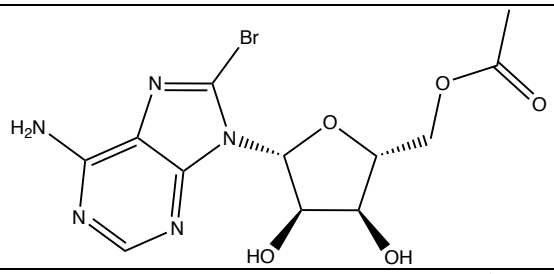
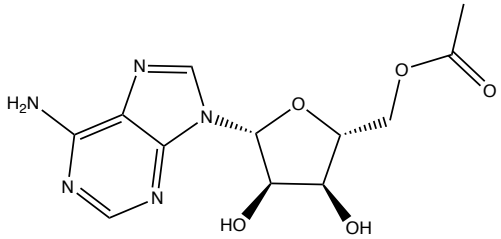
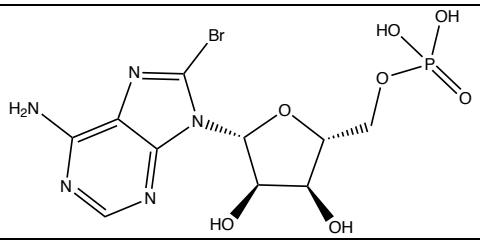
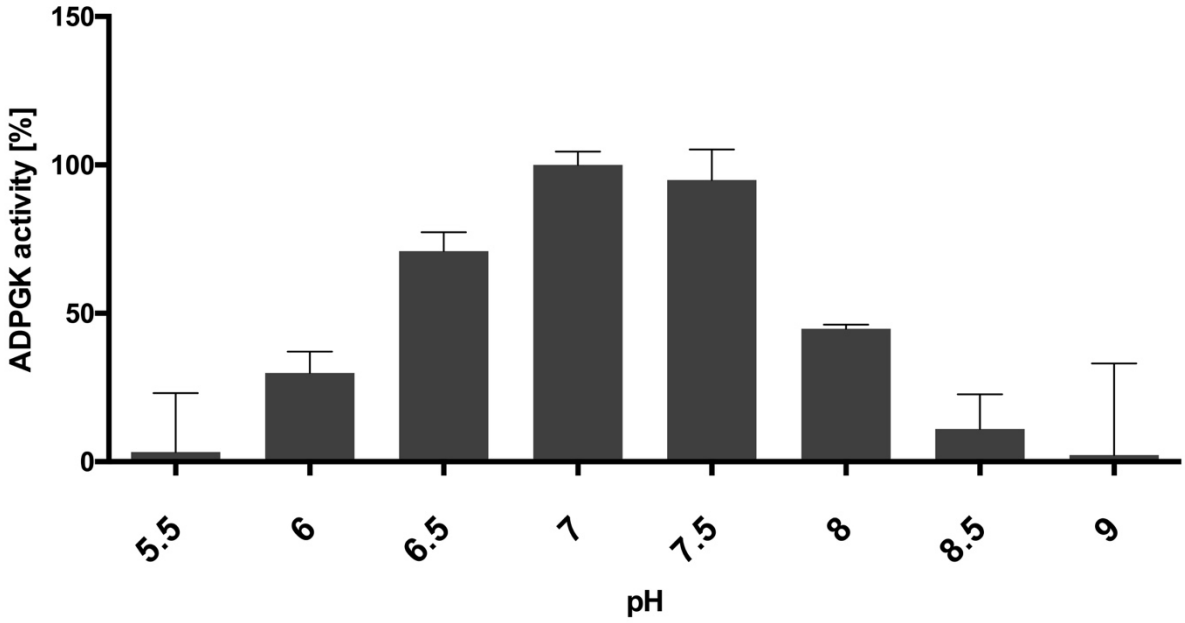
Suplementary information.

Table S1. Structures of adenosine analogues utilized in this study.

Compound name	Structure
Adenosine	
Adenosine monophosphate	
8-bromoadenosine	
8-bromo-9H-purin-6-amine	
8-bromoadenosine acetate	
5-O-acetyladenosine	
8-bromoadenosine monophosphate	

A



B

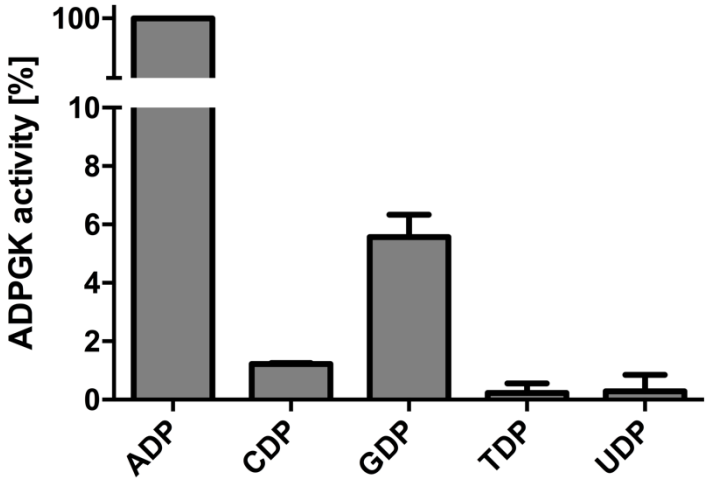


Figure S1. hADPGK properties (A) activity pH dependence (B) Substrate specificity towards different dinucleotides.

8-Br-AMP [mM]	Apparent Km [mM]
0	0,13
0,0625	0,2026
0,125	0,2568
0,25	0,4114
0,5	0,7462
1	1,132
Vmax	0.12 +/- 0.015
Ki [mM]	0.27 +/- 0.03

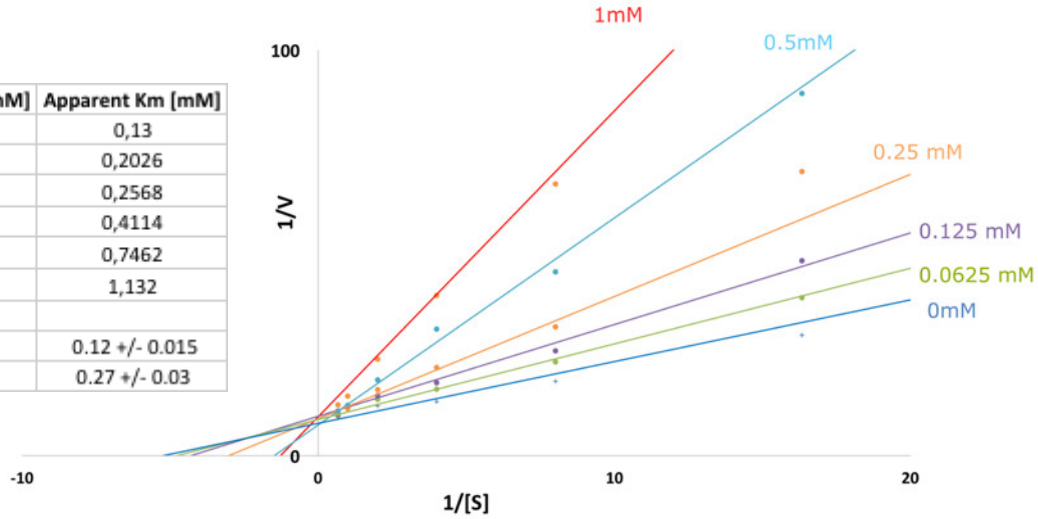


Figure S2. Lineweaver-Burk plot of hADPGK kinetics in the presence of 8-Br-AMP.

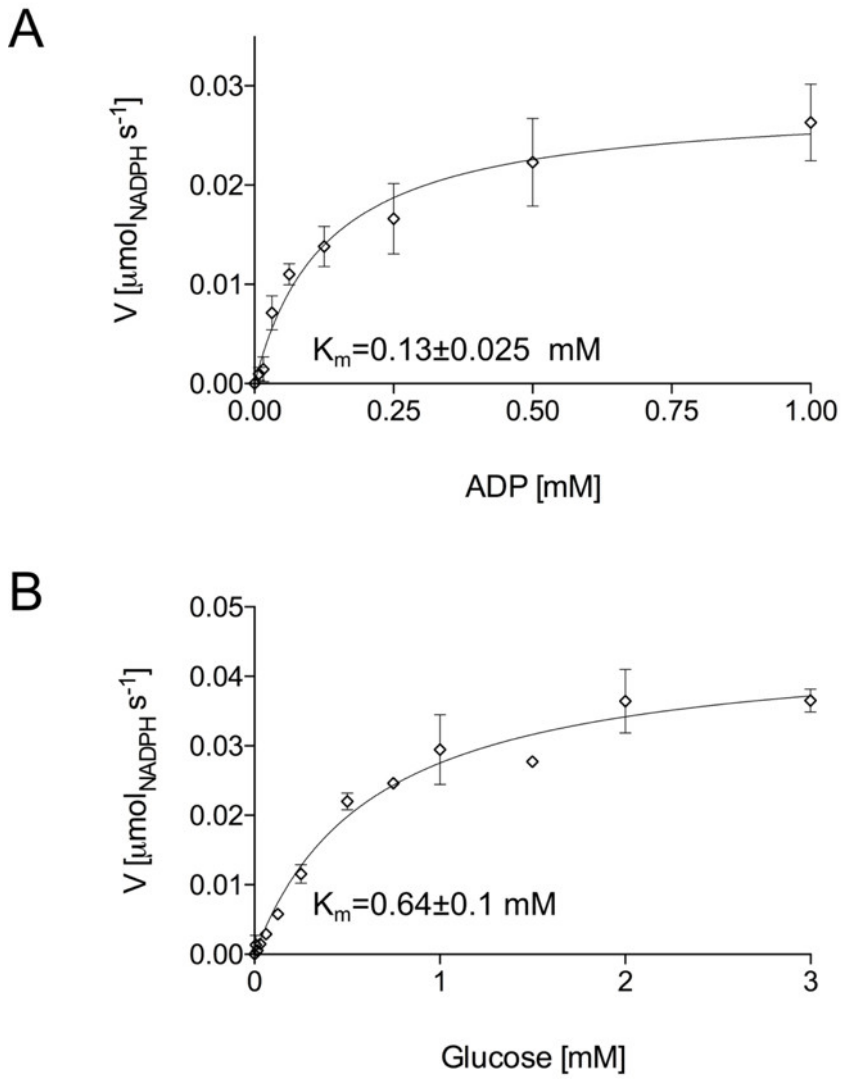
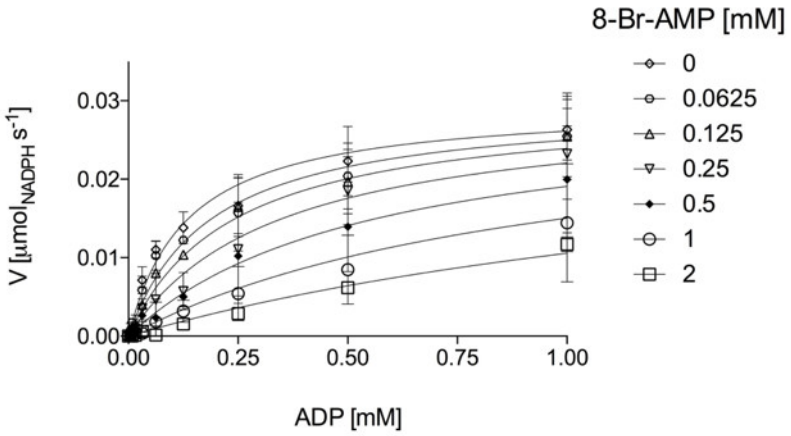


Figure S3. Enzymatic characterization of phADPGK. K_m and V_{max} determination for ADP (A) and glucose (B).

A



8-Br-AMP [mM]	Apparent Km [mM]
0	0,1292
0,0625	0,1502
0,125	0,2213
0,25	0,5204
0,5	0,5986
1	1,389
2	~ 28,95
Vmax	0.031 +/- 0.003
Ki [mM]	0.16 +/- 0.017

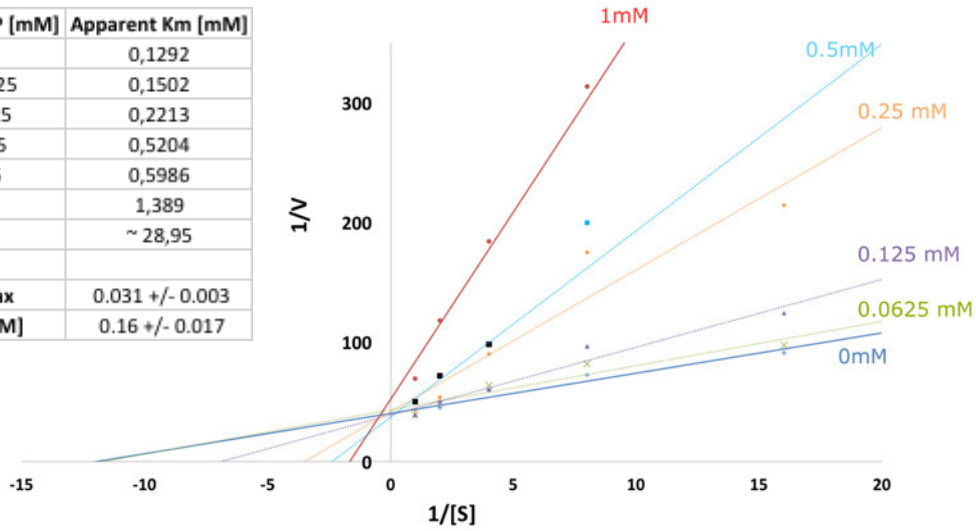


Figure S4. A. Inhibition of phADPGK by 8-Br-AMP. B. Lineweaver-Burk plot of phADPGK kinetics in the presence of 8-Br-AMP.

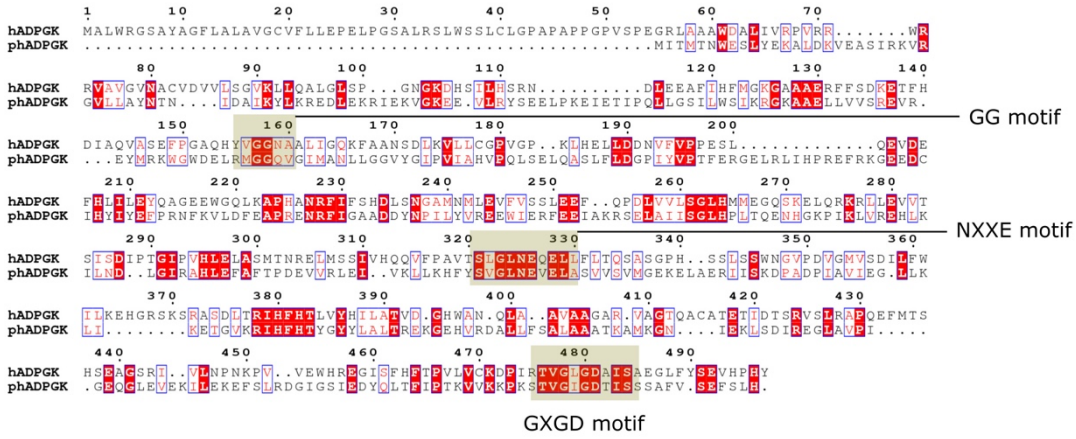


Figure S5. Sequence alignment of human and archaeal ADPGK. Highly conserved substrate binding motifs are highlighted. Overall sequence identity is 25%.

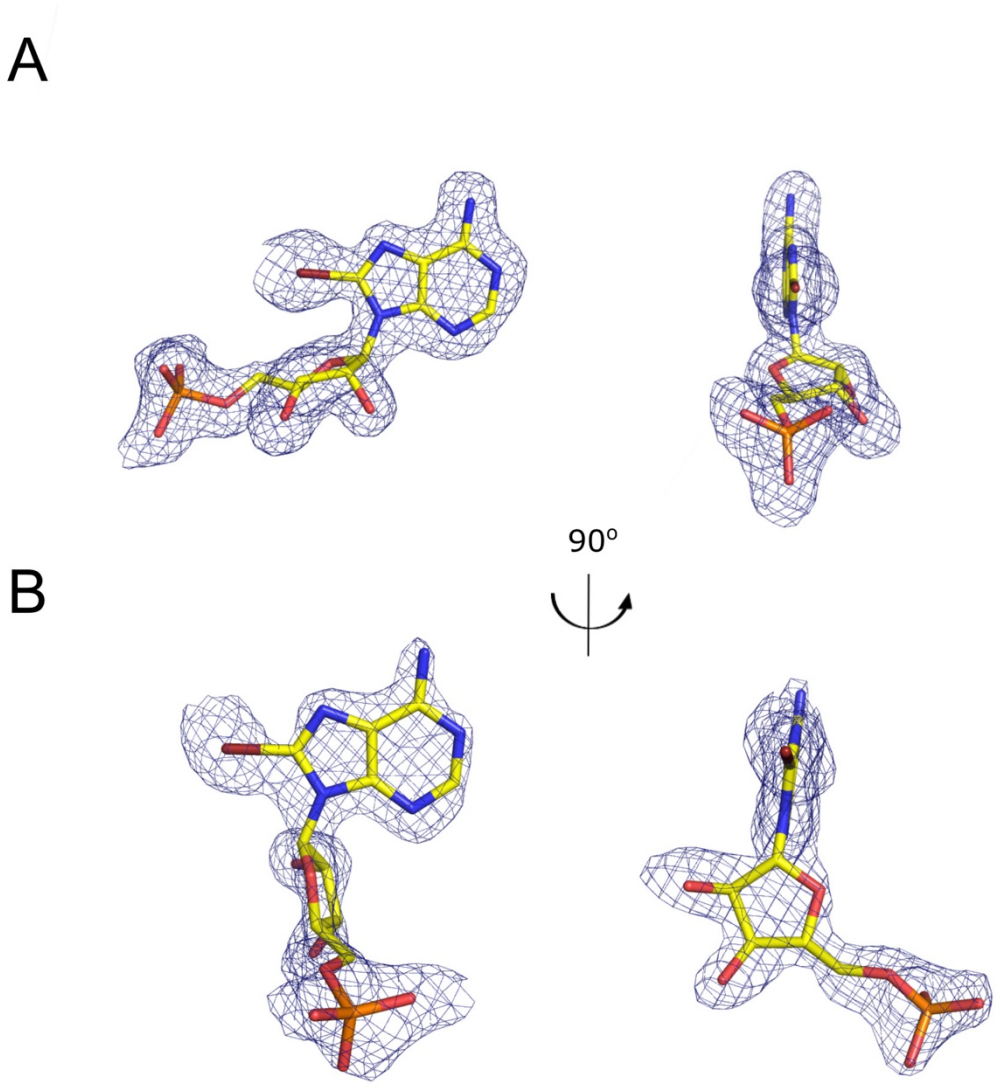


Figure S6. Simulated annealing F_o-F_c composite omit maps (3 σ) calculated for 8-Br-AMP within the active site (A) and additional 8-Br-AMP molecule (B).

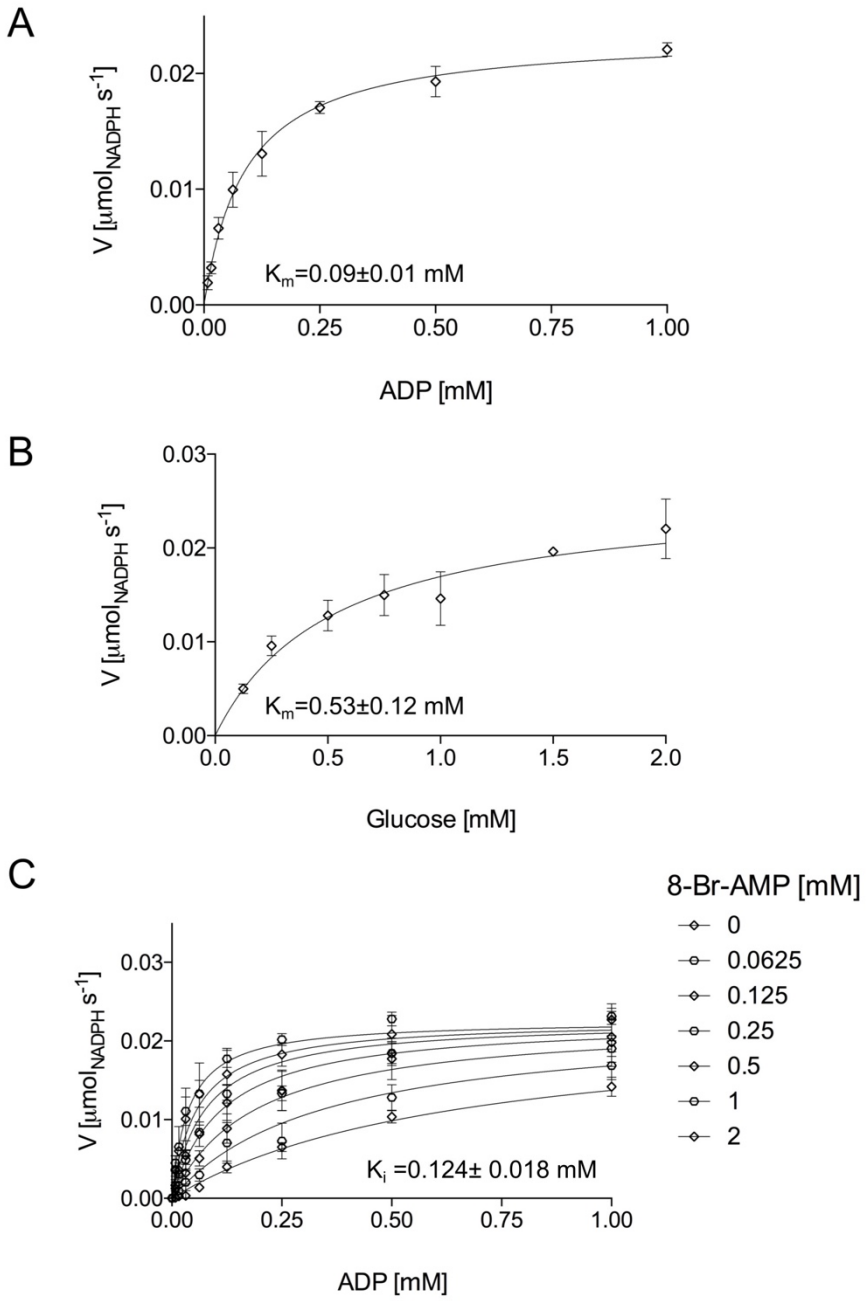


Figure S7. Enzymatic characterization of phADPGK F272A. K_m and V_{max} determination for ADP (A) and glucose (B). Inhibition of phADPGK F272A by 8-Br-AMP.

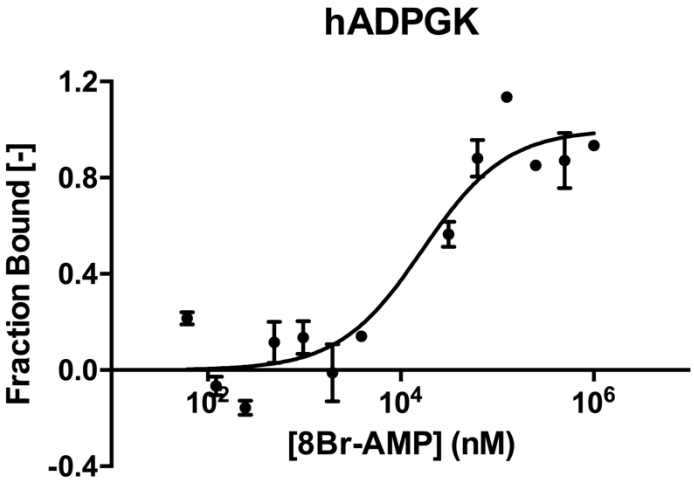


Figure S8. Binding of 8-Br-AMP to hADPGK analyzed by Microscale Thermophoresis. K_d=16.55 ± 4.78 μM

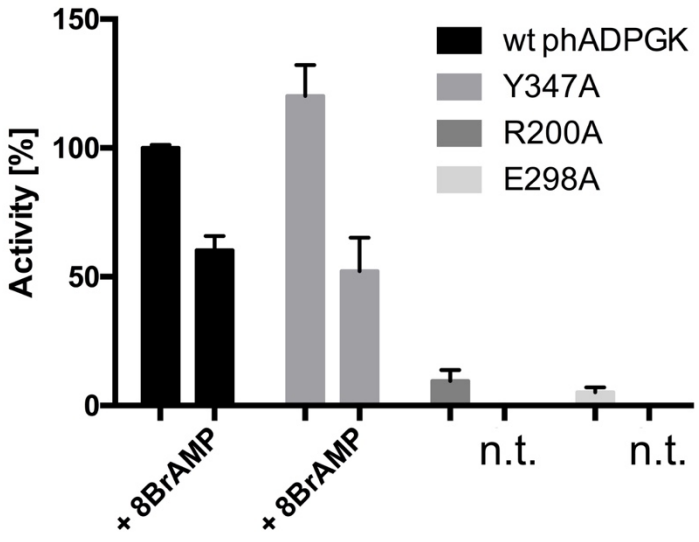


Figure S9. Effect of amino acid substitutions on the phADPGK activity. The substitution of Tyr347 for alanine did not cause any significant changes either in the protein activity or inhibition by 8-Br-AMP (1mM), however the substitutions of Arg200 and Glu298 led to inactivation of phADPGK (n.t. – not tested).

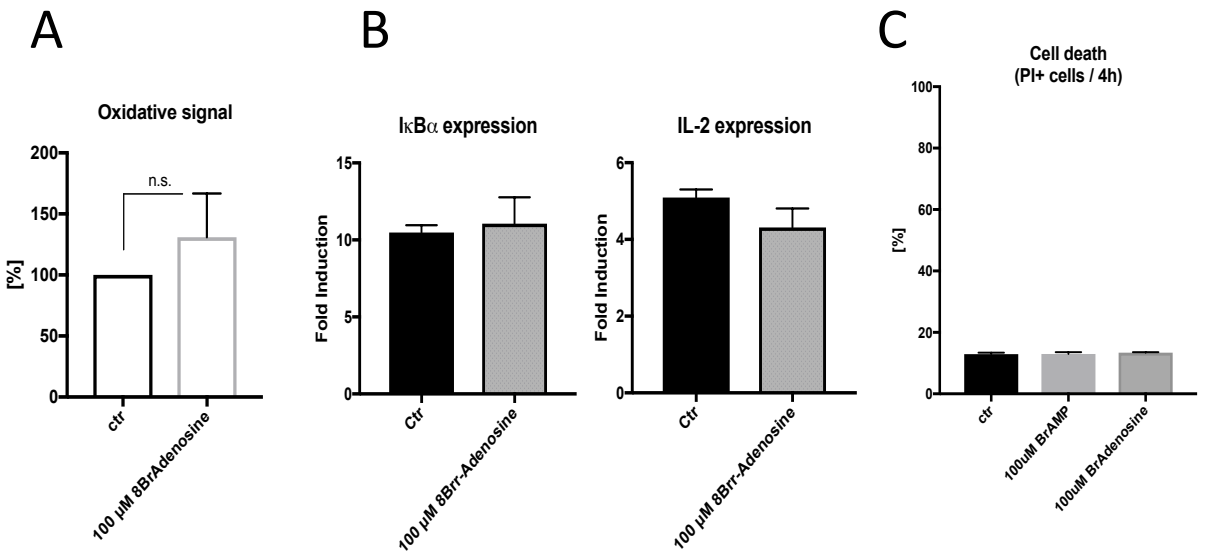


Figure S10. 8-Br-adenosine does not inhibit T cell activation-induced ROS generation and subsequent NF-κB-dependent gene expression. (A), Jurkat T cells stained with H_2DCF-DA and 30 min pre-treated with 100 µM of 8-Br-adenosine were activated by PMA treatment (1 h) and the 'oxidative signal' was measured by FACS (mean values +/- SD). Cumulative results of three independent technically triplicated experiments are show (mean values +/- SD). Statistical significance was calculated by single sample t test (ctr set up to 100%). (B), 8-Br-adenosine-pre-treated Jurkat T cells (30 min) were activated by PMA/ionomycin treatment for 1 h. Next, IL-2 and IκBα gene expression was assayed by RT-PCR (mean values +/- SD). (C), Jurkat T cells were treated with 100 µM of 8-Br-AMP or 8-Br-adenosine for 4 hours and cell death was assayed by FACS measeurement of PI+ cells (mean values +/- SD).