Supplementary data to

Beyond chelation: EDTA tightly binds Taq DNA polymerase, MutT and dUTPase and directly inhibits dNTPase activity

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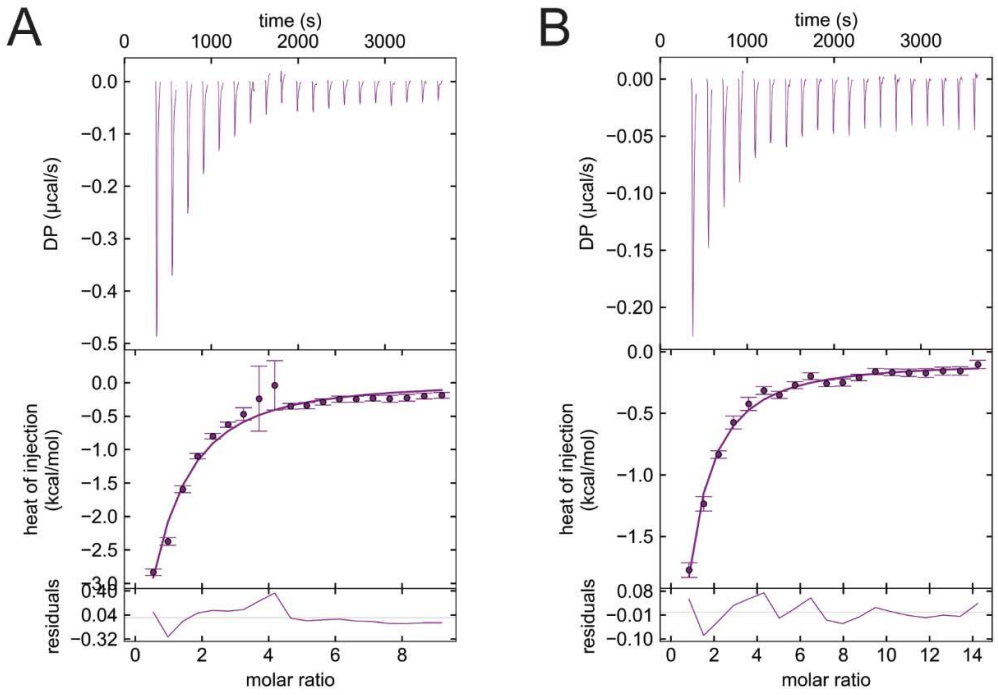

Figure S1 ITC titrations with dUTPases and dUPNPP confirm the functionality of the enzyme. A) hDUT and dUPNPP titration B) mtDUT and dUPNPP titration represent the binding between the components. Parameters of the fitting are the following: A) ΔH = –19.9 kcal/mol, $-T\Delta S$ = 14.1 kcal/mol, ΔG = –5.7 kcal/mol, K_d = 54 µM, n = 0.41; B) ΔH = –4.4 kcal/mol,
$-T\Delta S$ = –1.8 kcal/mol, ΔG = –6.2 kcal/mol, K_d = 22 µM, n = 1.

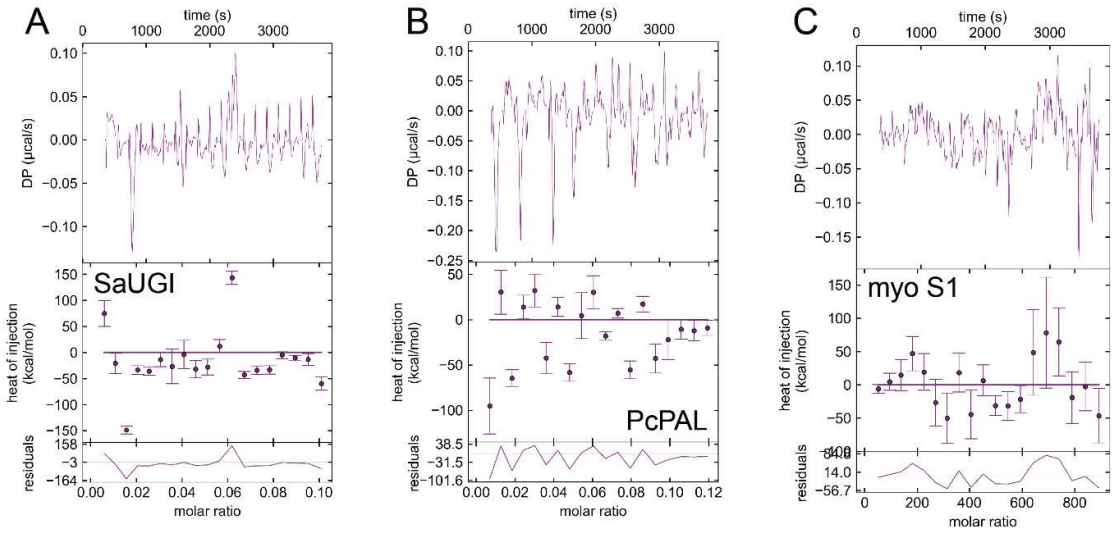

Figure S2 ITC titrations show the lack of binding between EDTA and A) *Staphylococcus aureus* uracil glycosylase inhibitor (SaUGI), B) *Petroselinum crispum* phenylalanine ammonia-lyase (PcPAL) and C) rabbit myosin subfragment 1 (myo S1).

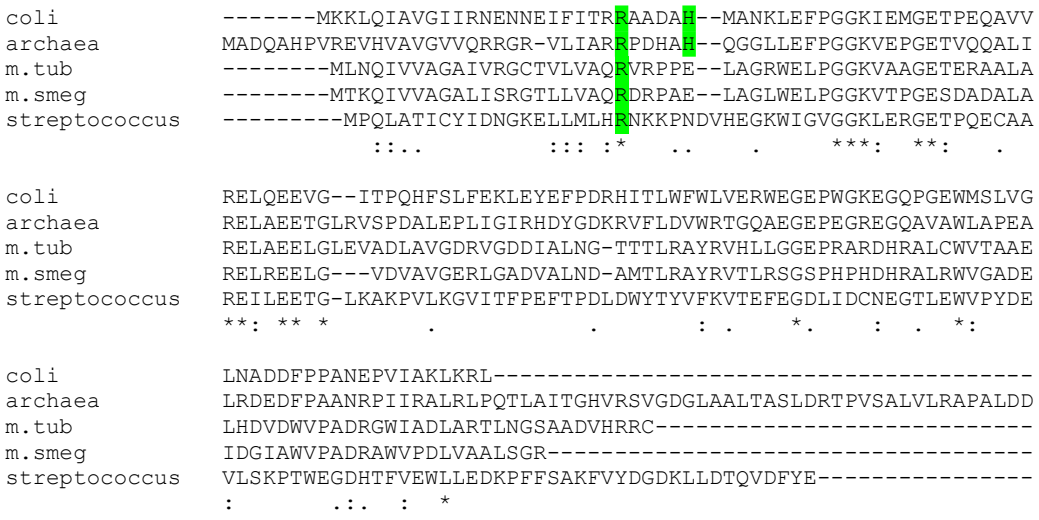

Figure S3 The alignment of MutT protein sequences indicate that the residues involved in 2' deoxyribose exclusion from the active site (green) are conserved amongst the above species. Arg23 can be found in *Escherichia coli*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Mycobacterium smegmatis* and in *Euryarchaeota archaeon*. His28 is additionally conserved in *Escherichia coli* and in *Euryarchaeota archaeon*. However no homologue residues were found in MutT proteins from *Bacillus subtilis*, *Haemophilus influenzae*, *Arabidopsis thaliana* and *Homo sapiens*. This region is not part of the conserved motif identified by Koonin 1993 NAR.

Aligned proteins:

>sp|P08337|MUTT_ECOLI 8-oxo-dGTP diphosphatase OS=Escherichia coli (strain K12) OX=83333 GN=mutT PE=1 SV=1

>sp|P41354|MUTX_STRPN 8-oxo-dGTP diphosphatase OS=Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334 / TIGR4) OX=170187 GN=mutX PE=1 SV=2

>tr|A0A2E4GCL2|A0A2E4GCL2_9EURY DNA mismatch repair protein MutT OS=Euryarchaeota archaeon OX=2026739 GN=CL958_03010 PE=4 SV=1

>sp|P9WIY1|MUTT2_MYCTU Putative 8-oxo-dGTP diphosphatase 2 OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) OX=83332 GN=mutT2 PE=1 SV=1

>tr|A0A0D6IWC3|A0A0D6IWC3_MYCSM Mutator protein MutT2/NUDIX hydrolase OS=Mycobacterium smegmatis OX=1772 GN=mutT2 PE=3 SV=1