

## Supplementary data to

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

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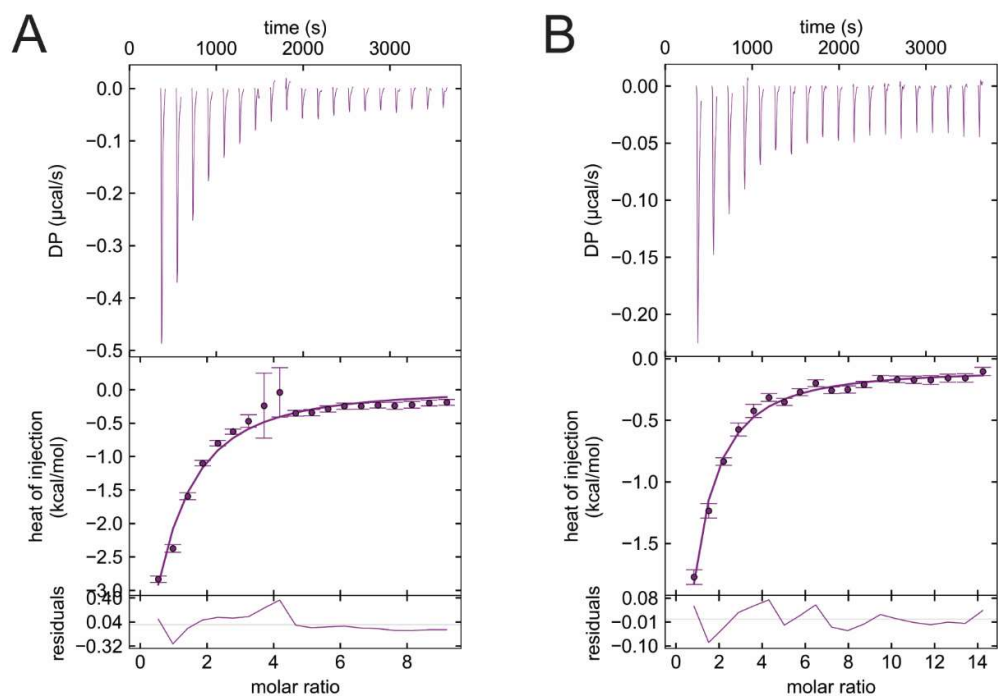
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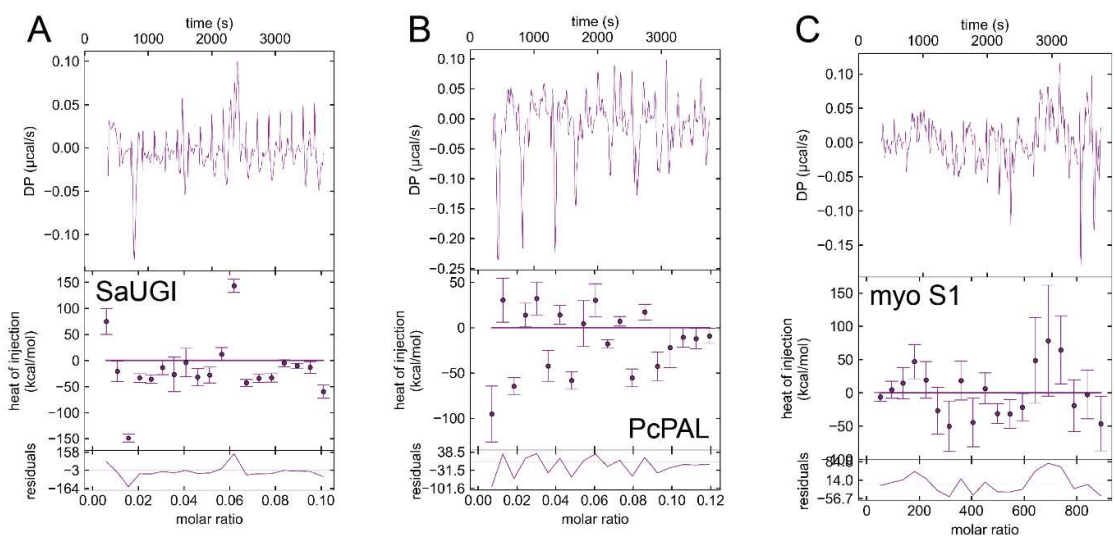
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**Figure S1** ITC titrations with dUTPases and dUPNPP confirm the functionality of the enzyme. A) hDUT and dUPNPP titration B) mtDUT and dUPNPP titration represent the binding between the components. Parameters of the fitting are the following: A)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



**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).

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coli          -----MKKLQIIVVAVGVVQRRGR-VLIARPDHAH-----MANKLEFPGGKIEMGETPEQAVV
archaea      MADQAHVPREVHVAVGVVQRRGR-VLIARPDHAH-----QGGLLEFPGGKVEPGETVQQALI
m.tub       -----MLNQIVVAGAIIVRGCTVLVAQVVRPPE--LAGRWELPGGKVAAGETERAALA
m.smeg      -----MTKQIVVAGALISRGTLLVAQVDRPAE--LAGLWELPGGKVTPEGSDADALA
streptococcus -----MPQLATICYIDNGKELLMLHNNKKPNDVHEGKWIIVGGKLERGETPQECQA
              :...      : : *   .   .   .   .   .   .   .   .   .   .   .
coli          RELQEEVG--ITPQHFSLFKLEYEFPDRHITLWFVWLVVERWEGEPWGKEGQPGEWMSLVG
archaea      RELAETGLRVSPDALEPLIGIRHIDYGDKRVLVDVWRTGQAEGEPEGREGQAVAWLAPEA
m.tub       RELAEELGLEVADLAVGDRVGDIALNG-TTTLRAYRVHLLGGEPRARDRALCWVTAAE
m.smeg      RELREELG---VDVAVGERLGADVALND-AMTLRAYRVTLRSGSPHPHHRALRWVGADE
streptococcus REILEETG-LKAKPVLKGVITFPEFTPDLWYTYVFKVTEFEGDLIDCNEGTLWVVPYDE
              **: * *   .   .   .   .   .   .   .   .   .   .   .   .
coli          LNADDFPPANEFVIAKLKRL-----
archaea      LRDEDFPAANRPIIRALRLPQTLAITGHVRSVGDGLAALTASLDRTPVSAVLVLRAPALDD
m.tub       LHDVDWVPADRGWIADLARTLNGSAADVHRRC-----
m.smeg      IDGIAWVPADRAWVPDLVAALSGR-----
streptococcus VLSKPTWEGDHTFVEWLELKDPPFAKFFVYDGDKLLDTQVDFYE-----
              :   . . : *

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**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