

SUPPLEMENTARY METHODS:

Protein purification and DNA preparation

Dpo4 was expressed and purified as previously described (Ling et al., 2001). DNA oligonucleotides were purchased from Keck oligonucleotide synthesis facility at Yale University and Integrated DNA Technologies, Inc. and were subsequently gel purified. Template-strand DNA (5'-TCTGGCTTTCCTTCCCCC-3') was modified with cisplatin to generate a site-specific 1,2-intrastrand cross-link with the adjacent guanine residues as previously described (Gelasco and Lippard, 1998) and was gel purified. The primer-strand sequences are listed in Figure 2. For the crystallization studies, DNA substrates were annealed by mixing the complementary primer and template DNA strands at an equal molar ratio at 90°C for 5 min, followed by gradual cooling to 4°C. The Dpo4-DNA complex was formed by mixing Dpo4 with the pre-annealed primer-template DNA at a 1:1.2 molar ratio and incubated for 20 min at room temperature after adding 1 mM of appropriate incoming nucleotide. For the kinetic studies, primers were 5'-radiolabeled with [γ -³²P]ATP (MP Biomedicals) and Optikinase (USB). Unreacted [γ -³²P] ATP was removed using a BioSpin-6 column (Bio-Rad Laboratories). The 5'-[³²P]-radiolabeled DNA primer and unlabeled template were annealed at a 1:1.15 molar ratio, heated to 85 °C for 6 minutes, and cooled slowly to room temperature.

Crystallization

Hanging drops were made by mixing 0.1 mM of Dpo4-DNA-dNTP complexes with an equal volume of well buffer composed of 0.2 M calcium acetate, 2.5-5% glycerol, and 10–15% PEG3350. Mineral oil composed of 80% paraffin and 20% silicon was used to overlay the mother-liquor that controlled the evaporation rate within the hanging drop.

Controlled streaking was necessary to achieve diffraction-quality crystals (Bauer et al., 2007). The cryo-buffer was the mother-liquor with 20% PEG3350 and 15% glycerol.

***In Vitro* Assays:**

Running start assay

The optimized reaction buffer contained 50 mM HEPES (pH 7.5 at 23°C), 50 mM NaCl, 5 mM MgCl₂, 5 mM DTT, 10% glycerol, 0.1 mM EDTA, and 0.1 mg/ml BSA (Fiala and Suo, 2004). All reported concentrations were the final concentrations upon mixing. All reactions were performed at 23°C unless otherwise noted. Mixing was achieved through using a rapid chemical-quench flow apparatus (Kin-Tek). The DNA polymerase (100 nM) and 5'-[³²P] DNA (100 nM) were pre-incubated prior to mixing with an equal volume (15 µl) of all four dNTPs (200 µM each). The polymerization reaction was quenched with 0.37 M EDTA at times ranging from ms to min. The incorporation pattern was resolved via denaturing polyacrylamide gel electrophoresis (PAGE) (22% acrylamide, 8 M urea, TBE running buffer). The dried gel was scanned with a Typhoon TRIO (GE Healthcare) and quantitated with ImageQuant software (Molecular Dynamics).

Nucleotide incorporation assays

A pre-equilibrated solution of Dpo4 (120 nM) and 5'-[³²P] DNA (30 nM) in buffer D (50 mM HEPES (pH 7.5 at 23 °C), 5 mM MgCl₂, 5 mM DTT, 10% glycerol, 0.1 mM EDTA, and 0.1 mg/ml BSA, and 50 mM NaCl) was combined with a single natural dNTP (1 mM) in buffer D. Aliquots of the reaction mixtures were quenched using 0.37 M EDTA at 10 min for the undamaged DNA and and 60 min for the Pt-GG DNA. Reaction

products were resolved using sequencing gel electrophoresis as described in the previous section.

***In Vivo* Experiments:**

Construction of the *S. solfataricus dpo-4* mutant

The *dpo-4* knockout mutant of *S. solfataricus* was constructed as described previously (Schelert et al., 2004; Worthington et al., 2003). Briefly, the *dpo-4* open reading frame at nt570 was disrupted by the insertion of the *lacS* gene, which encodes beta-glycosidase. The *dpo-4* knockout mutant replaced the endogenous gene in the *S. solfataricus* genome by homologous recombination. Genotype analysis by PCR of *dpo-4* revealed a 1.3 kb product for the wildtype and a 3.0 kb product for the *dpo-4* mutant that has the 1.7 kb *lacS* insert. Western blot analysis using anti-Dpo4 polyclonal sera shows the absence of Dpo4 in the mutant strain relative to wild-type.

SUPPLEMENTARY DATA

Table S1 Torsional parameters of Pt-GG in the complex structures

Structure	G base	α ($^{\circ}$) ^a	β ($^{\circ}$) ^b	ρ ($^{\circ}$)	Bending of helix ($^{\circ}$)	
GG1	3'G	172.3	112.0	134.4	11.2	
	5'G	142.9	150.3			
GG2a (AC1)	3'G	149.7	116.4	22.0	18.0	
	5'G	119.0	108.0			
GG2b (AC2)	3'G	133.2	145.7	26.4		18.0
	5'G	149.0	103.1			
GG3	3'G	159.6	111.7	25.6	17.7	
	5'G	123.9	105.6			

^a defined as the dihedral angle C8 - N7 - C5 - Pt , ideal value = 180° (Yao et al., 1994)

^b defined as the dihedral angle C8 - N7 - Pt - *cis*N, ideal value = 90° (Yao et al., 1994)

Table S2. Proteomic alterations arising from Dpo4 deficiency^a

Increased Abundance	Spot #	Protein Identity
SSO0758	1	Peptidyl-prolyl cis-trans isomerase, FKBP-type rotamase (EC: 5.2.1.8)
SSO0940	2	Fibrillarin-like pre-rRNA processing protein
SSO0067	3	(rps2AB) SSU ribosomal protein S2AB
SSO2613	4	(bcp-4) Peroxiredoxin, bacterioferritin comigratory protein homology
SSO2364	5	(SSB) Single-stranded DNA binding protein
SSO1865	6	Conserved hypothetical protein
SSO0970	7	(eiF5A) Initiation factor 5A, hypothetical
SSO2642	8	(rr) Rubrerythrin
Reduced Abundance		
SSO3107	9	(ilvD) Dihydroxy-acid dehydratase (EC: 4.2.1.9)
SSO2629	10	Oxidoreductase (flavoprotein)
SSO0535	11	Acyl carrier protein synthase
SSO1817	12	(cysA-2) Thiosulfate sulfurtransferase (EC: 2.8.1.1)

^a Proteins were sequenced using tandem mass spectrometry (MS/MS) and peptides identified by local BLAST against the *S. solfataricus* proteome as described (Worthington 2003).

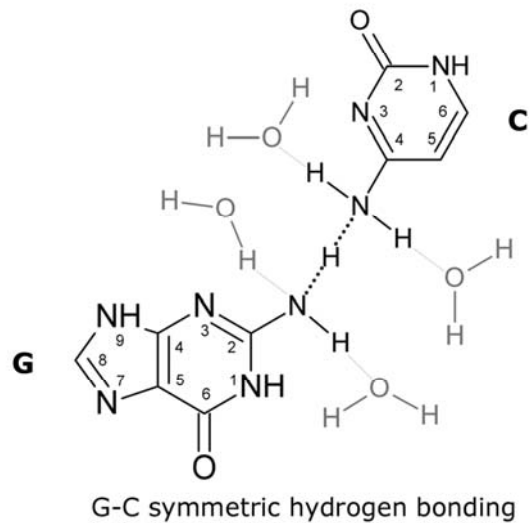
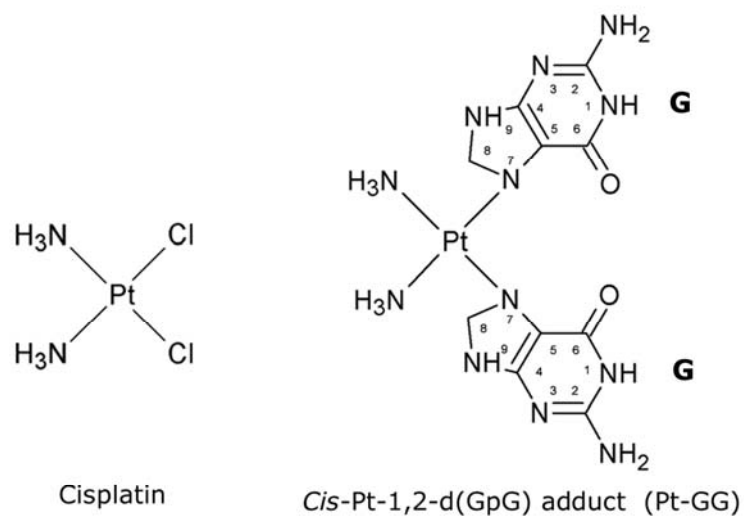


Figure S1. Chemical structures of cisplatin, Pt-GG, G-C symmetric H-bonding, and the dPTP nucleotide analog. The grey molecules in the bottom panel are water molecules associated with the bases.

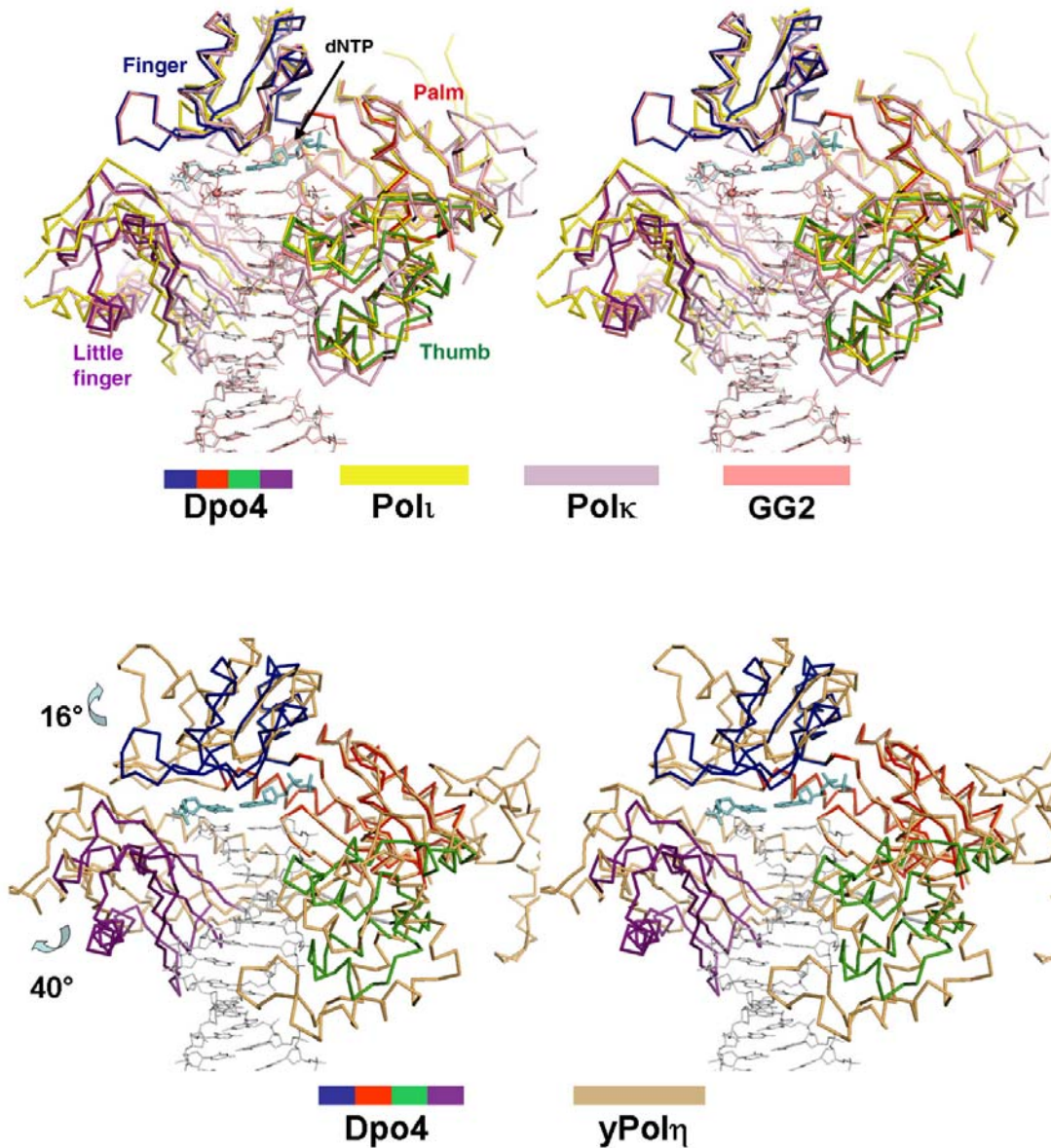


Figure S2 Structural comparison of Y-family polymerases (stereo views). Upper panel: Dpo4 structures (type 1,(1JX4), blue for finger, red for palm, green for thumb, and purple for little finger domains and GG2, salmon) are superimposed with human pol ι (3GV5, yellow), human pol κ (2OH2, light purple). The DNA models are from GG2 (salmon) and Dpo4 type 1 (1JX4, grey), with the replicating base pair in cyan. The finger domains in the upper panel are superimposed well. Lower panel: Dpo4 type 1 (1JX4) is superimposed with Yeast pol η (2R8J, tan) on their palm domains. The finger domain of ypolh off 16° from that of Dpo4 in the lower panel. The DNA models are from Dpo4 type 1(1JX4).

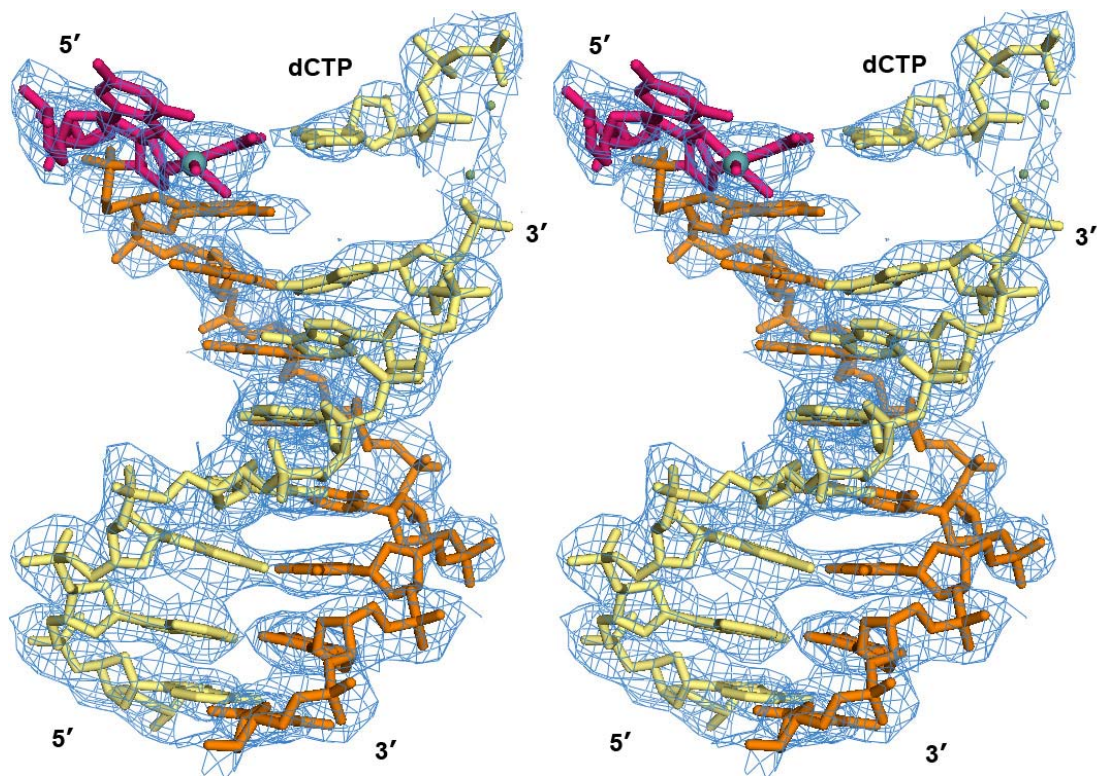


Figure S3. Stereo-view of the DNA helix in the GG1 reaction stage with 2Fo-Fc electron density contoured to 1σ at 2.9 Å resolution. The template strand is in orange, primer strand in yellow, the Pt-GG adduct in dark pink.

Template: 3' CCC CCT TCC TTT **CGG** TCT 5'
 Primer: 5' GGG GGA AGG A 3'

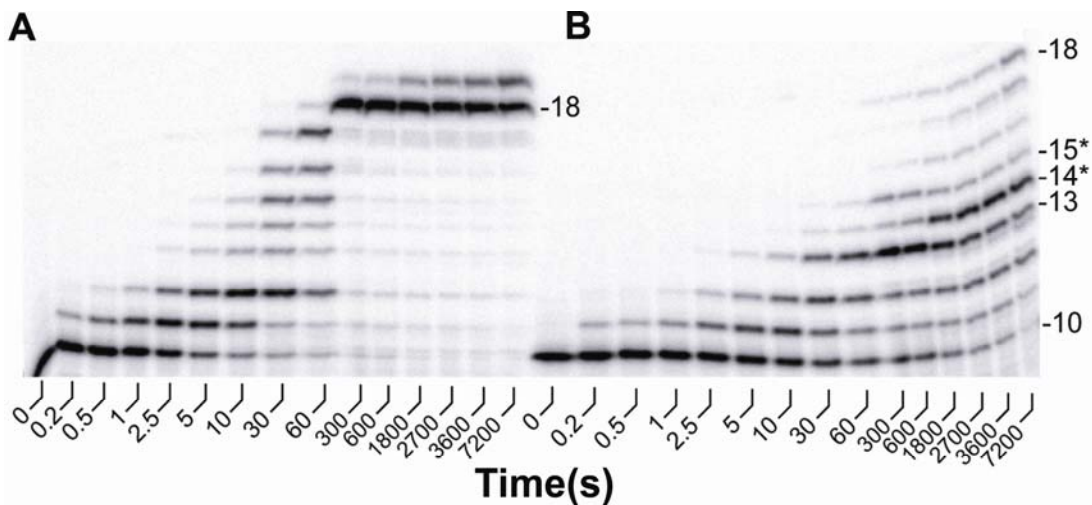


Figure S4. A running start nucleotide incorporation assay with (A) undamaged DNA and (B) Pt-GG cross-linked DNA. The 18-mer template sequence with Pt-GG position bold and underlined and a 10-mer primer are shown on the top of the figure. Lengths of the oligonucleotides (primer extension products) are denoted in the right margin.

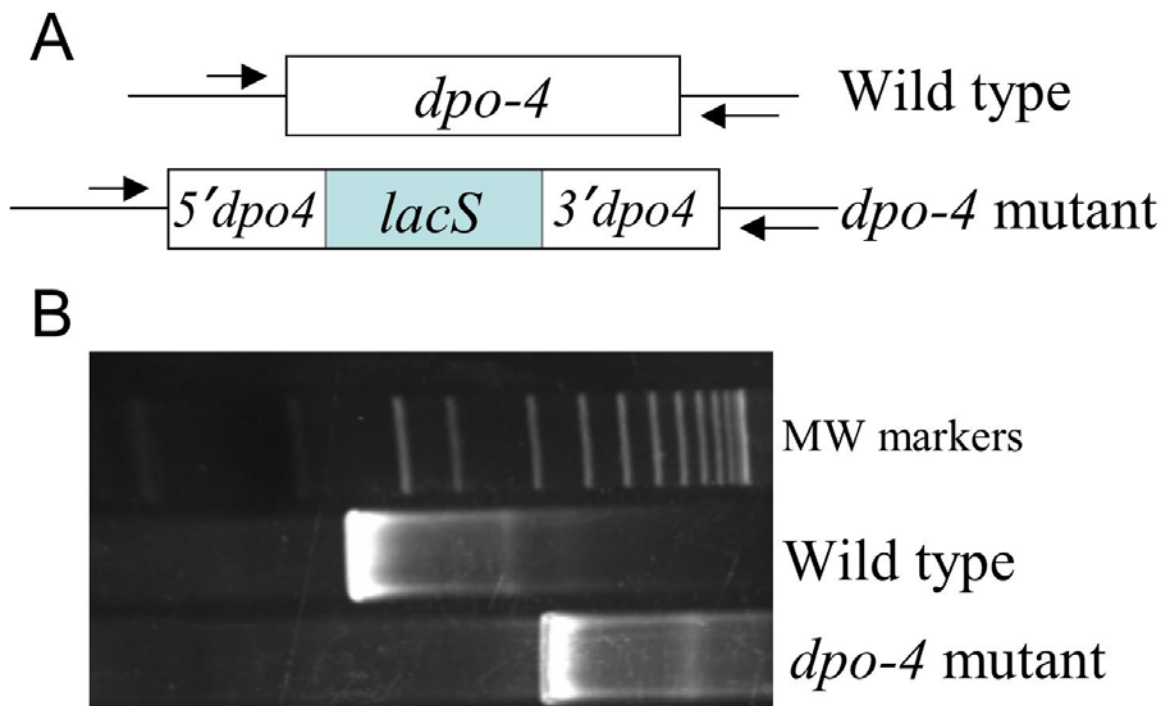


Figure S5. Genotype analysis of a *S. solfataricus dpo-4* knockout mutant. (a) Schematic of wild type *dpo-4* gene and the *lacS* disrupted copy of the *dpo-4* gene present in the wild type and *dpo-4* mutant strains. Location of PCR primers are indicated by horizontal arrows. (b) Ethidium bromide-stained agarose gel showing PCR amplicons using genomic DNA template from wild type and *dpo-4* mutant cell lines.

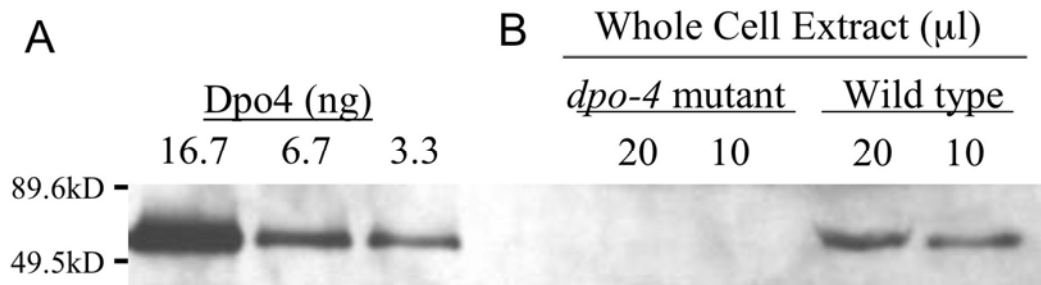


Figure S6. Chemiluminescent western analysis of wild type and *dpo-4* disruption mutant strains. (a) Purified Dpo4 protein. (b) Cell extracts (10 μl = 25 μg total protein). Protein samples were fractionated by SDS-PAGE on 12% gels, transferred to PVDF and then probed with 1:2000 polyclonal anti-Dpo4 rabbit primary antibody and HRP-conjugated secondary antibody. Chemiluminescence detection was used to visualize Dpo4.

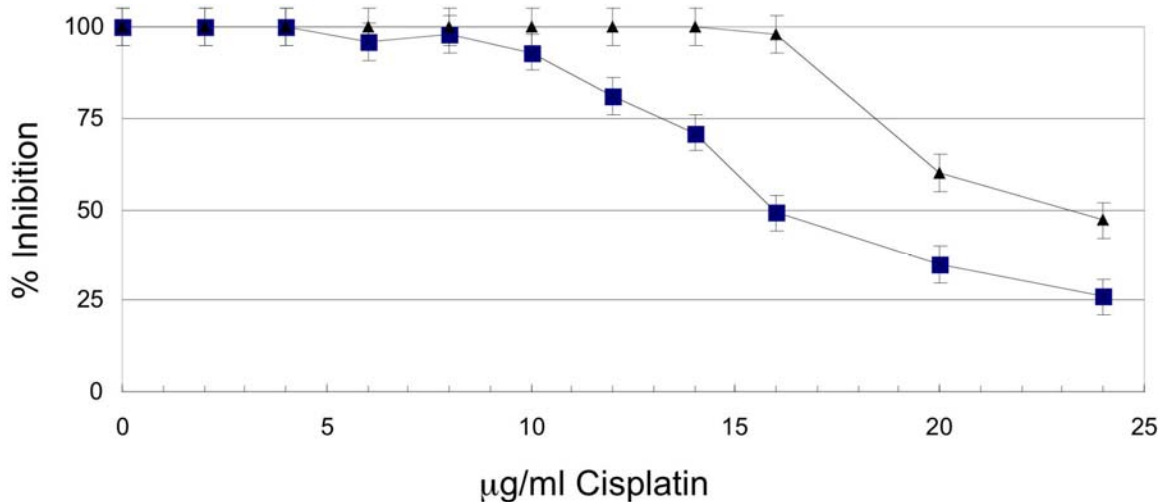


Figure S7. *In vivo* cisplatin toxicity. LC₅₀ values for the *S. solfataricus* wild type and *dpo4* mutant cell lines were derived from the cell yields resulting from treatment of replicate cultures at the indicated cisplatin doses. Values for cell yields were normalized to untreated cultures. Error between replicate cultures are indicated.

References

- Bauer, J., Xing, G., Yagi, H., Sayer, J. M., Jerina, D. M., and Ling, H. (2007). A structural gap in Dpo4 supports mutagenic bypass of a major benzo[a]pyrene dG adduct in DNA through template misalignment. *Proc Natl Acad Sci U S A* *104*, 14905-14910.
- Gelasco, A., and Lippard, S. J. (1998). NMR solution structure of a DNA dodecamer duplex containing a cis-diammineplatinum(II) d(GpG) intrastrand cross-link, the major adduct of the anticancer drug cisplatin. *Biochemistry* *37*, 9230-9239.
- Ling, H., Boudsocq, F., Woodgate, R., and Yang, W. (2001). Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication. *Cell* *107*, 91-102.
- Schelert, J., Dixit, V., Hoang, V., Simbahan, J., Drozda, M., and Blum, P. (2004). Occurrence and characterization of mercury resistance in the hyperthermophilic archaeon *Sulfolobus solfataricus* by use of gene disruption. *J Bacteriol* *186*, 427-437.
- Worthington, P., Hoang, V., Perez-Pomares, F., and Blum, P. (2003). Targeted disruption of the alpha-amylase gene in the hyperthermophilic archaeon *Sulfolobus solfataricus*. *J Bacteriol* *185*, 482-488.
- Yao, S., Plastaras, J., and Marzilli, L. G. (1994). A Molecular Mechanics AMBER-Type Force Field for Modeling Platinum Complexes of Guanine Derivatives. *Inorganic Chemistry* *33*, 6061-6077.