

Supporting Information for:

Effects of N^2 -Alkylguanine, O^6 -Alkylguanine, and Abasic Lesions on DNA Binding and Bypass Synthesis by the Euryarchaeal B-family DNA Polymerase Vent (exo⁻)

Seonhee Lim,^{†,‡} Insil Song,[†] F. Peter Guengerich,[§] and Jeong-Yun Choi^{†,*}

Division of Pharmacology, Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, Gyeonggi-do 440-746, Republic of Korea; Department of Pharmacology, School of Medicine, Ewha Womans University, Seoul 158-710, Republic of Korea; Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146, USA

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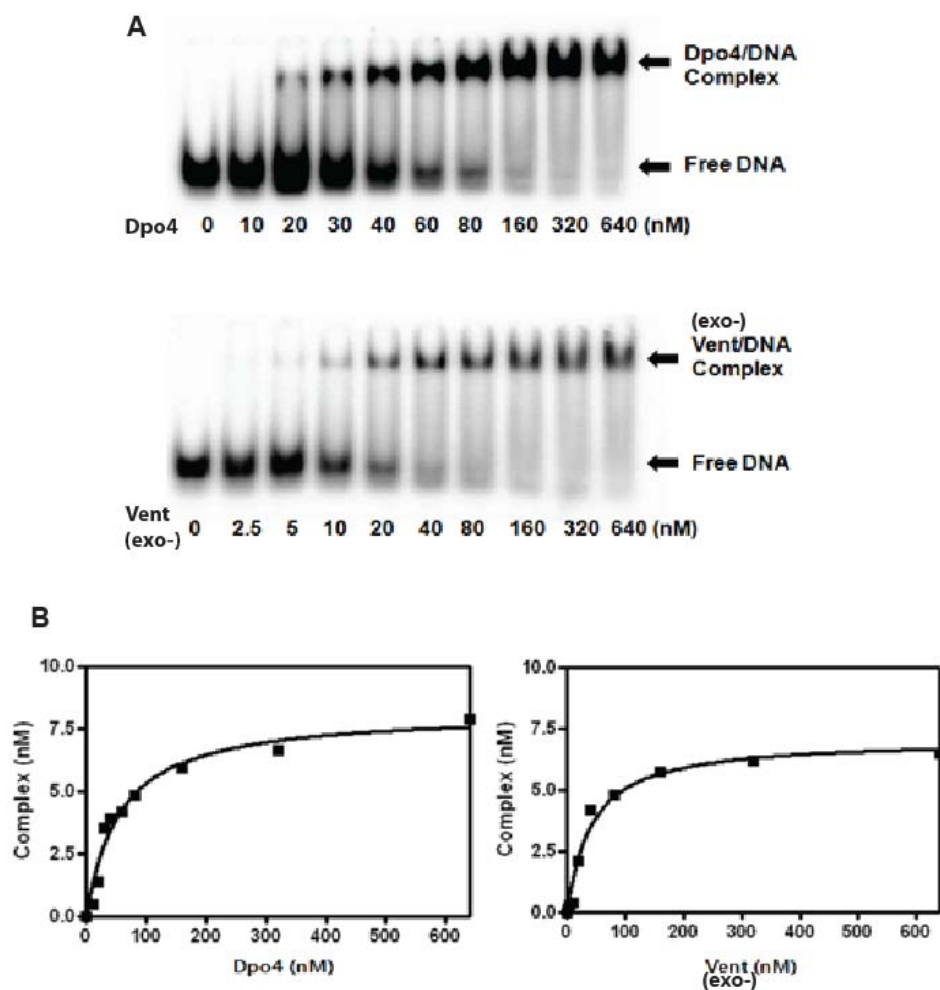


Figure S1. Estimation of the apparent K_d^{DNA} for Dpo4 and Vent (exo⁻) to 24-mer/36-G-mer DNA by electrophoretic mobility shift assays. (A) Representative gels of electrophoretic mobility shift assays with Vent (exo⁻) and Dpo4. Reactions containing 5'-[³²P]-labeled 24-mer/36-G-mer primer-template duplex DNA (10 nM) were incubated with increasing concentrations of each polymerase (0 - 640 nM). The binary complex was separated from the unbound DNA substrate by 4% non-denaturing polyacrylamide gel electrophoresis. (B) Plot of binary complex formation (Pol•24-mer/36-G-mer primer-template) versus polymerase concentration was fit to a quadratic equation, as described in the Experimental Procedures section. The following values of K_d were estimated: Vent (exo⁻), 39 ± 8 nM; Dpo4, 52 ± 9 nM.

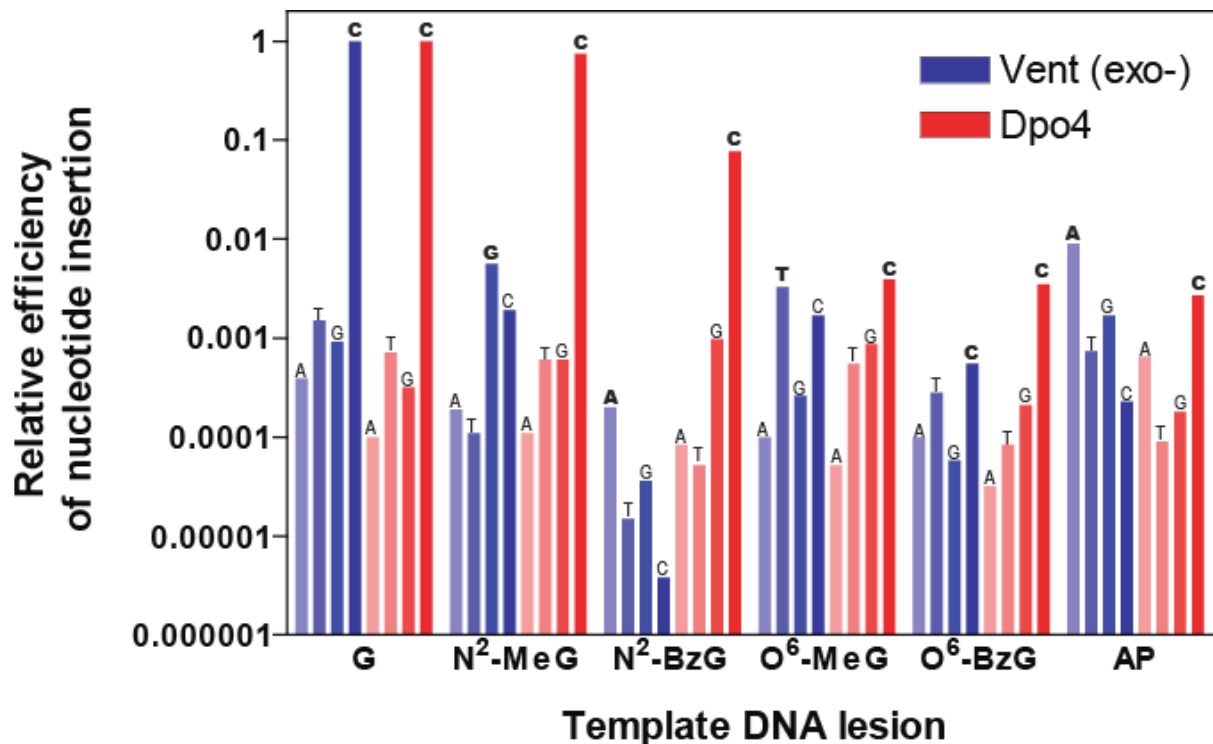


Figure S2. Comparison of the relative efficiencies of single nucleotide incorporation opposite various template DNA lesions by Vent (exo⁻) and Dpo4. Relative efficiencies (Tables 2 and 3) of single dNTP incorporation opposite each of DNA lesions by Vent (exo⁻) (blue bars) and Dpo4 (red bars) are represented in the bar graph. Each single nucleotide (dATP, dTTP, dGTP, or dCTP) insertion is represented by A, T, G, and C on top of each bar, and the most efficiently inserted nucleotide is shown in bold.