

Supplementary Materials

for

**“Transcriptional Bypass of Regioisomeric Ethylated Thymidine Lesions by T7
RNA Polymerase and Human RNA Polymerase II”**

by

Changjun You¹, Pengcheng Wang², Xiaoxia Dai¹, and Yinsheng Wang^{1,2,*}

¹Department of Chemistry and ²Environmental Toxicology Graduate Program, University of
California, Riverside, California 92521-0403

Figure Legends

Supplementary Figure 1. Representative high-resolution “ultra zoom-scan” ESI-MS results showing the detection of the $[M-3H]^{3-}$ ions of restriction fragments of interest arising from the T7 RNAP-mediated transcription of *N3-* (a), *O*²- (b) or *O*⁴-EtdT-bearing substrates (c). ‘13mer A’ represents the non-mutagenic product, i.e., d(AATTATAGCACGC), whereas ‘13mer C’, ‘13mer G’ and ‘13mer T’ designate the corresponding products carrying an A→C, A→G or A→T mutation opposite the lesion site, i.e., d(AATTATAGCCGC), d(AATTATAGCGCGC) and d(AATTATAGCTCGC), respectively.

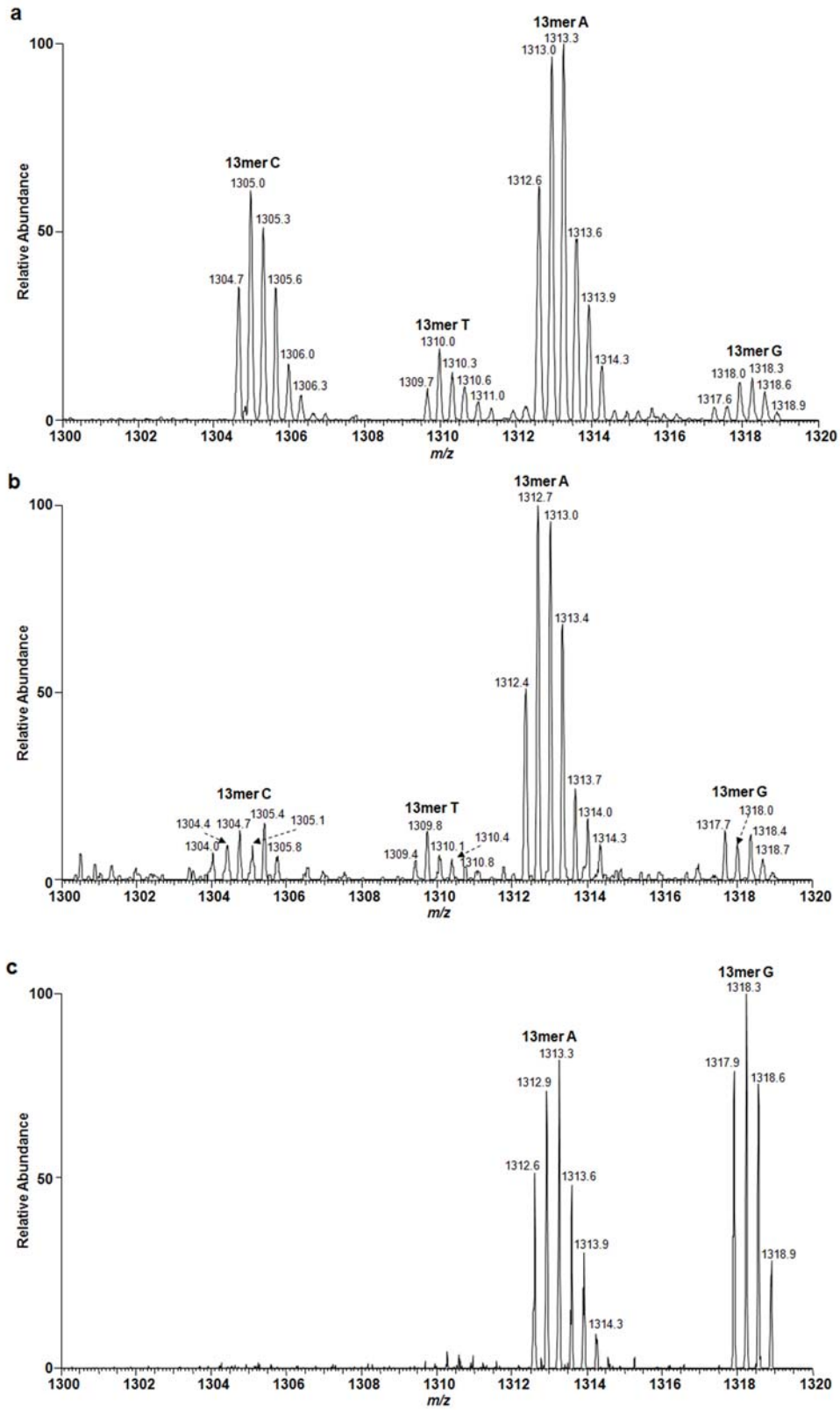
Supplementary Figure 2. Representative LC-MS/MS for monitoring the 13-mer restriction fragments of interest with A→C (a), A→T (b) or A→G (c) mutation opposite the original *N3*-EtdT site. Shown in (a), (b) and (c) are the product-ion spectra of the ESI-produced $[M-3H]^{3-}$ ions (m/z 1304.6, 1309.6 and 1317.9, respectively) of the 13-mer DNA fragments, i.e., d(AATTATAGCMCGC), where M is C, T and G, respectively. Shown in the insets are schemes summarizing the observed $[a_n - \text{Base}]$ and w_n fragment ions [nomenclature follows that described previously. *J. Am. Soc. Mass Spectrom.* 3, 60-70 (1992)].

Supplementary Figure 3. PAGE analysis for determining the effects of *N3*-EtdT, *O*²-EtdT and *O*⁴-EtdT on DNA transcription in XPA-deficient (XP12RO) and XPA-complemented (GM15876A) cells. (a) Representative gel images showing the NcoI-SfaNI-treated restriction fragments of interest. The restriction fragment arising from the competitor vector, i.e., d(CATGGCGATATGCTAT), is designated as ‘16mer-Comp’; ‘13mer-C’, ‘13mer-A’, ‘13mer-G’, and ‘13mer-T’ represent the standard ODN d(CATGGCGNGCTAT), where N is C, A, G, T, respectively. (b) Representative gel images showing the MluCI-Cac8I-treated restriction fragments of interest. ‘10mer-C’, ‘10mer-A’, ‘10mer-G’, and ‘10mer-T’ represent the standard ODN d(AATTATAGCM), where M is C, A, G, T, respectively.

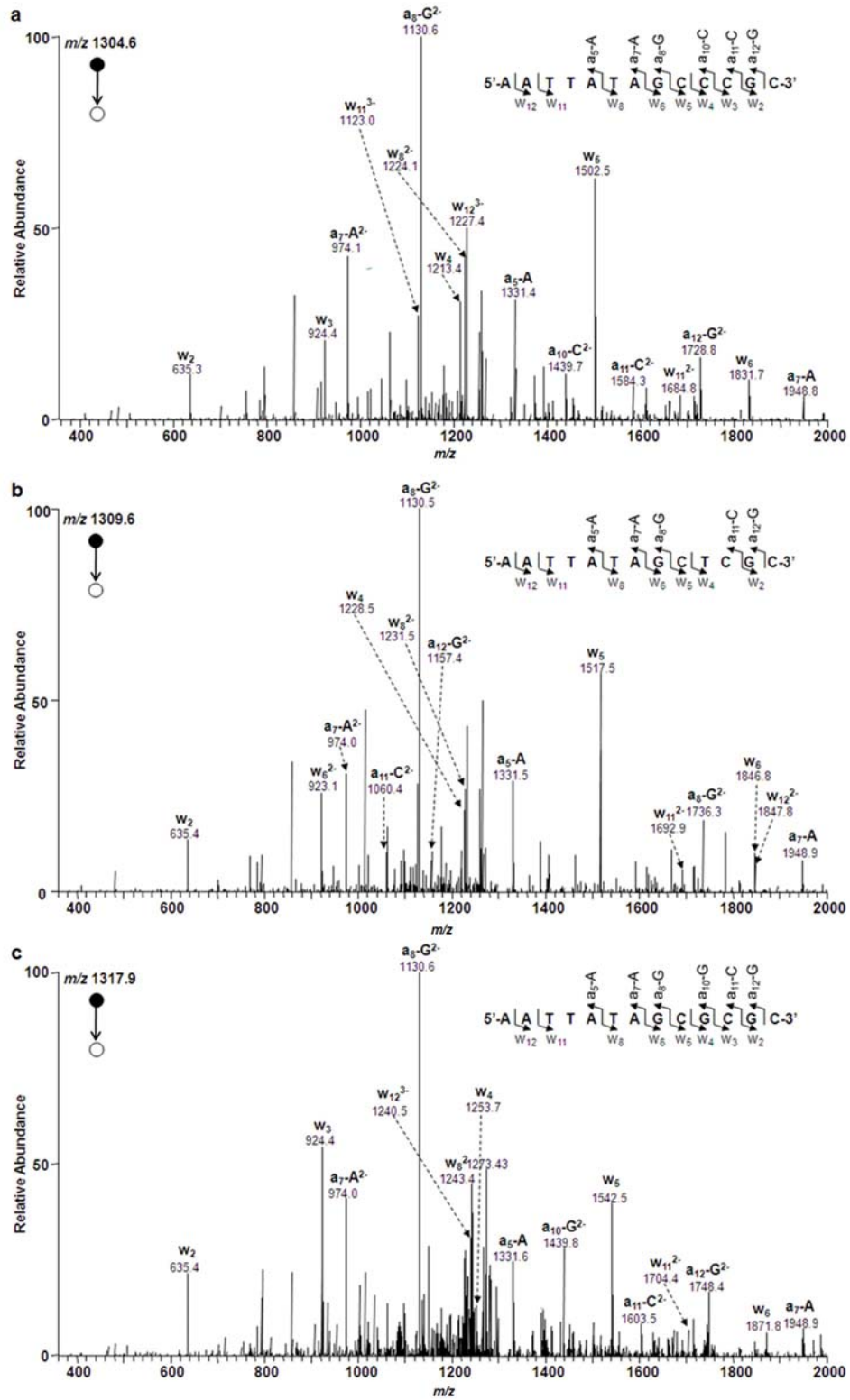
Supplementary Figure 4. Real-time RT-PCR analysis for monitoring the siRNA-mediated knockdown of *CSB* (a) or *XPC* (b) in human 293T cells. *GAPDH* gene was used as a control for real-time RT-PCR analysis. The PCR data represent the mean and standard error of results from three separate experiments.

Supplementary Figure 5. PAGE analysis for determining the effects of *N3*-EtdT, *O*²-EtdT and *O*⁴-EtdT on DNA transcription in 293T cells treated with *CSB* or *XPC* siRNAs. (a) Representative gel images showing the restriction fragments of interest after NcoI and SfaNI cleavage. The restriction fragment arising from the competitor vector, i.e., d(CATGGCGATATGCTAT), is designated as ‘16mer-Comp’; ‘13mer-C’, ‘13mer-A’, ‘13mer-G’, and ‘13mer-T’ represent the standard ODN d(CATGGCGNGCTAT), where N is C, A, G, T, respectively. (b) Representative gel images showing the MluCI-Cac8I-treated restriction fragments of interest. ‘10mer-C’, ‘10mer-A’, ‘10mer-G’, and ‘10mer-T’ represent the standard ODN d(AATTATAGCM), where M is C, A, G, T, respectively.

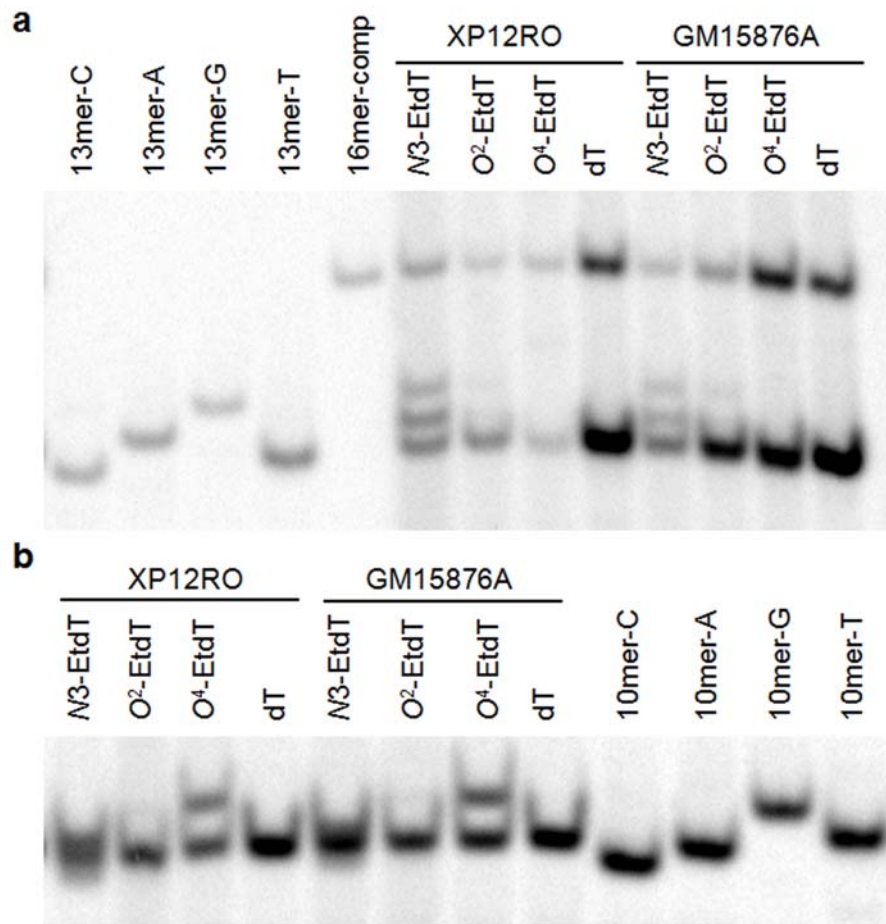
Supplementary Figure 1



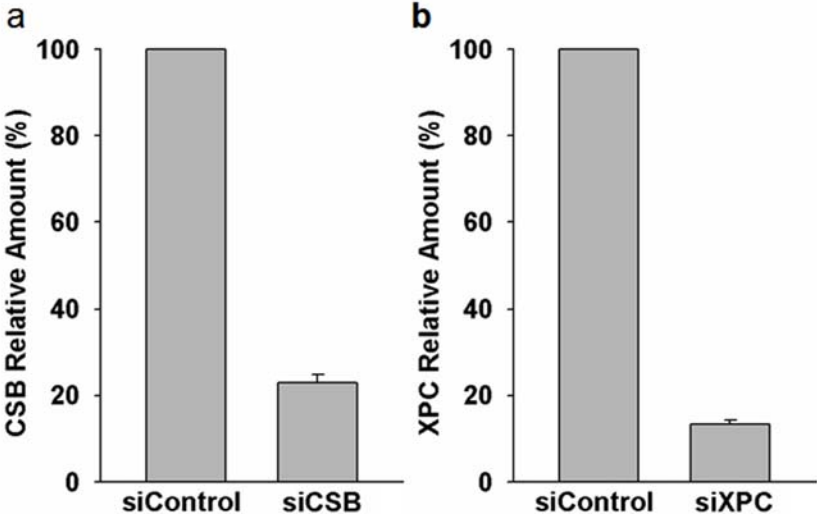
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

