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Supporting Material

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Supplemental Data for

UVA GENERATES PYRIMIDINE DIMERS IN DNA DIRECTLY

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This Supplemental Data includes:
Figure S1 to S5

S1. T4 Endonuclease V does not relax or cut any unirradiated DNA.

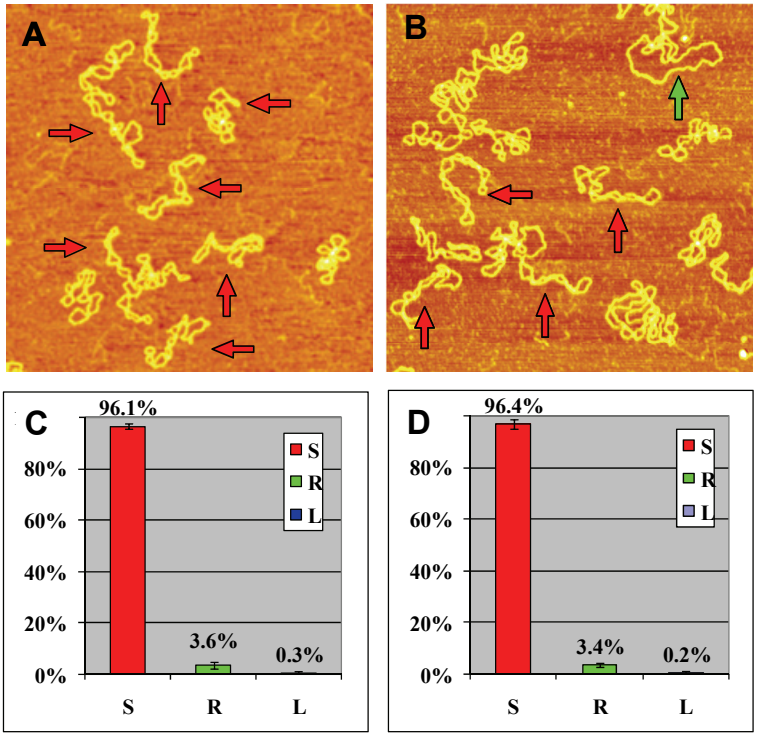

Figure S1. (A) AFM image of intact pUC18 molecules (B) AFM image of pUC18 DNA that was dialyzed in 10 mM Tris-HCl, 1 mM EDTA and 100 mM NaCl buffer and incubated with T4 Endonuclease V, but was never subjected to UV light. (C-D) are histograms of the occurrence of various configurations of pUC18 plasmids determined from the AFM images such as these shown in (A and B, respectively). Color code: red, supercoiled DNA (S); green, relaxed circular plasmids (R); blue, linear DNA (L). The error bars in the figures represent the standard deviation. Each histogram is based on 600-1000 DNA molecules from 30–36 AFM images. For intact pUC18 DNA, ~4% of DNA is relaxed or linear; this background damage likely occurred during the purification process. It is clear that a vast majority of these plasmids (>95 %, Fig. S1B) is resistant to T4 Endo V, and the percentage of relaxed circular plasmids is consistent with the fraction of damaged molecules in the original stock sample that has not been exposed to UV radiation or to a T4 Endo V treatment. Thus, we conclude that T4 Endo V does not incise undamaged plasmids and that all damage to DNA is the result of UV treatment.

S2. UVA irradiated pUC18 treated by different Endonuclease enzymes.

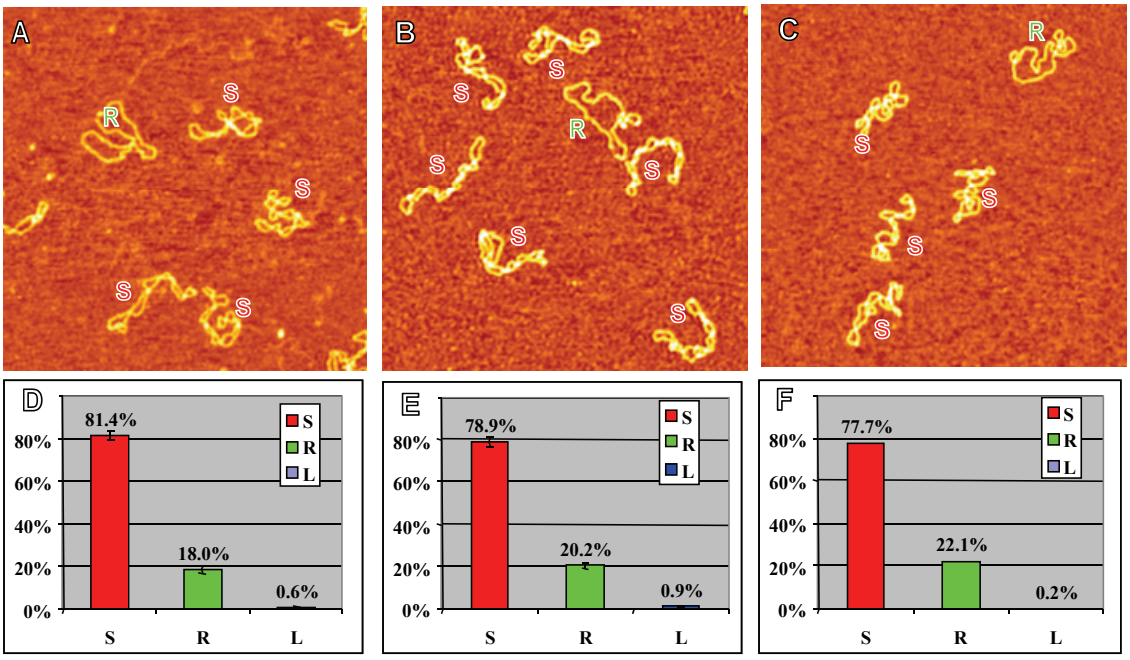

Figure S2. AFM images of different pUC18 DNAs that were subjected to 1.3 MJ/m^2 UVA radiation and different enzyme treatments prior to imaging. DNA was dialyzed in 10 mM Tris-HCl, 1 mM EDTA and 100 mM NaCl buffer and irradiated in same solution by UVA. After that, the sample was diluted back to the suitable buffer for different enzyme incubation: (A) *E. coli* endonuclease IV, (B) *E. coli* endonuclease III, (C) *E. coli* Fpg,. Scan size in all the images is 1 x 1 μm^2. Color code and error bars are the same as in Fig. S1. Each histogram is based on 600-1000 DNA molecules from 30–36 AFM images. (D, E and F) are histograms of the occurrence of various configurations of pUC18 plasmids determined from the AFM images such as these shown in (A, B, and C, respectively).

S3. Comparison between AFM and Gel electrophoresis results

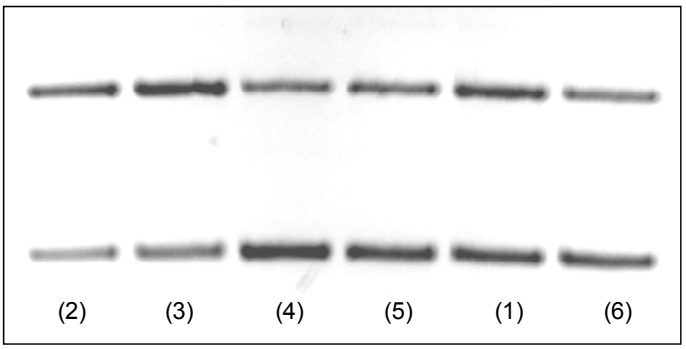

Figure S3. A photograph of the agarose gel shows separated supercoiled and relaxed pUC18 bands after: (1) 8h UVA, (2) 8h UVA and *E. coli* Endo-IV, (3) 8h UVA and T4 Endo-V, (4) 5h UVA, (5) 5h UVA and *E. coli* Endo-IV and (6) 5h UVA and T4 Endo-V treatment. DNA was dialyzed in 10 mM Tris-HCl, 1 mM EDTA and 100 mM NaCl buffer and irradiated in the same solution by UVA. After that, the sample was diluted back to the suitable buffer for different enzyme incubation. For gel electrophoresis, DNA was separated on 1% agarose gel in the presence of 10 μg mL^{-1} ethidium bromide in TBE Buffer (89 mM Tris borate, 2 mM ethylenediaminetetraacetate (EDTA), pH 8.3). Gel images were taken using 8-bit CCD camera (Gel Doc EQ system, Bio-Rad Laboratories, Inc.) and analyzed using the Quantity One software (Bio-Rad Laboratories, Inc.).

In order to confirm the accuracy of the damage-detection assay by AFM imaging, we compared the distribution of various DNA topological forms obtained by AFM imaging with the amounts of DNA measured in various bands after separating the irradiated plasmids by agarose gel electrophoresis. The results are shown in Fig. S3 and Table S1. After careful calibration and quantification, both gel and AFM results fit quite well. Therefore, our AFM imaging methods are convincible and comparable with traditional methods. We briefly mention here that when analyzing gel electrophoresis data it proved necessary to carefully examine the effect of underfluorescence of supercoiled plasmids as compared to relaxed plasmids. This was done by executing additional control gel electrophoresis measurements, in which the relative fractions of both topological forms of the plasmids were known. It proved that the slightly different ionic conditions required for various enzymes (such as a small amount of Mg^{++}) had a substantial effect on the band intensity, and a failure to correct for these effects would lead one to an erroneous conclusion that practically all UVA-generated T4 Endonuclese V sites were abasic sites, i.e. that UVA did not produce CPDs.

Table S1. Comparison between Gel and AFM results

UVA Radiation		8h	8h	8h	5h	5h	5h
Enzyme		Control	Endo-IV	Endo-V	Control	Endo-IV	Endo-V
GEL	Supercoiled %	66.8%	56.4%	47.3%	76.8%	73.8%	67.3%
	Relaxed %	33.2%	43.6%	52.7%	23.2%	26.2%	32.7%

	Linear%	x	x	x	x	x	x
AFM	Supercoiled %	72.7	58.3	47.0	77.7	69.9	60.5
	Relaxed %	27.1	40.8	52.6	22.1	29.9	37.8
	Linear%	0.2	0.9	0.4	0.2	0.2	2.7

“x“ means undetectable

S4. pUC18 dialyzed in pure water.

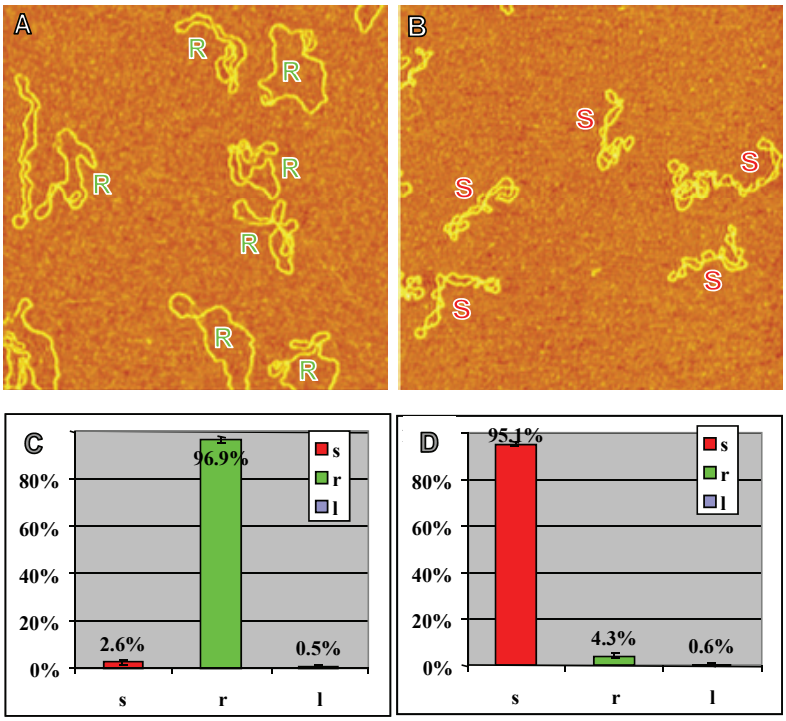

Figure S4. Tapping-mode AFM images of intact supercoiled pUC18 plasmid DNA on an APS-mica surface. (A) DNA looks similar to the relaxed circular configuration when it was deposited on APS-mica from an ultra pure Millipore water solution. (B) DNA returned back to the supercoiled form when it was diluted back to its saline buffer (10 mM Tris-HCl, 1 mM EDTA and 100 mM NaCl) before it was deposited on APS-mica. Scan size is 1 x 1 μm^2. Each histogram is based on 600-1000 DNA molecules from 30–36 AFM images. Color code and error bars are the same as in Fig. S1. (C and D) histograms count the various configurations of pUC18 molecules determined from the AFM images such as these shown in A and B, respectively.

Figure S4 is the AFM image showing the dialyzed supercoiled pUC18 DNA deposited on APS-mica directly from a solution of pure water. The intact pUC18 molecules look like they are in the relaxed circular, not supercoiled form. We counted the number of DNA in each conformation for this image and similar images. The histogram result in Fig S4c shows that ~96.9±1.0% of DNA molecules are circular. The reason for DNA taking a circular form is related to large electrostatic forces within the DNA caused by the lack of cations in pure water that normally neutralize the negative charges of the phosphate groups on the DNA backbone. Therefore, the repulsion between these negative charges makes DNA adopt a circular form to minimize the repulsion interaction. However, after diluting the plasmids back to their regular buffer (10 mM Tris-HCl, 1 mM EDTA and 100 mM NaCl), they were able to return back into their supercoiled form, as shown in Figure S4b. Figure S4d shows that ~95.1±0.9% of these molecules are supercoiled. Only less than 5% of the molecules are in a relaxed circular or linear form, in which damages may have been introduced during the extraction and purification processes. Therefore, the dialysis itself did not introduce any significant damage to supercoiled DNA.

S5. pUC18 plasmids irradiated by UVA in 10x diluted buffer and treated by different DNA enzymes.

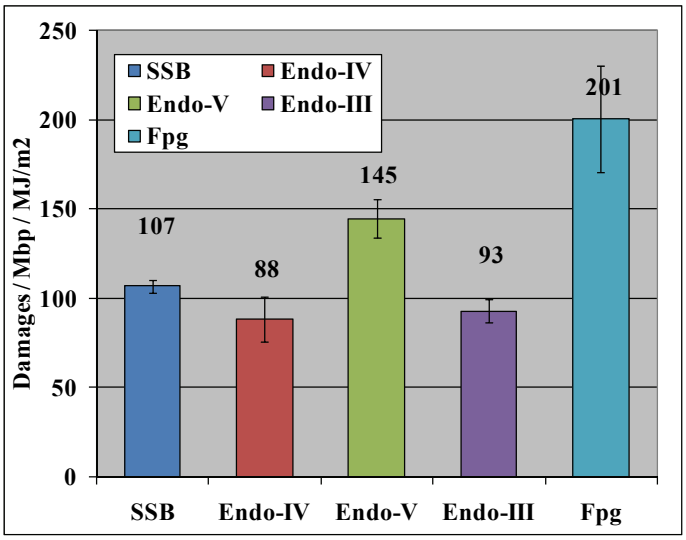

Figure S5. Histograms show the different damages after UVA irradiation in diluted Tris buffer, whose composition was: 1 mM Tris-HCl, 0.1 mM EDTA and 10 mM NaCl. DNA was treated by different enzymes at their preferred buffer conditions and then diluted by regular imaging buffer, 10 mM Tris-HCl, 1 mM EDTA and 100 mM NaCl before deposition onto APS-mica for AFM imaging.