Electronic Supplementary Material (ESI) for Photochemical & Photobiological Science




**SUPPLEMENTARY INFORMATION**

# The relative roles of DNA damage induced by UVA irradiation in human cells

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**Discriminating living or dead cells by fluorescence microscopy.**

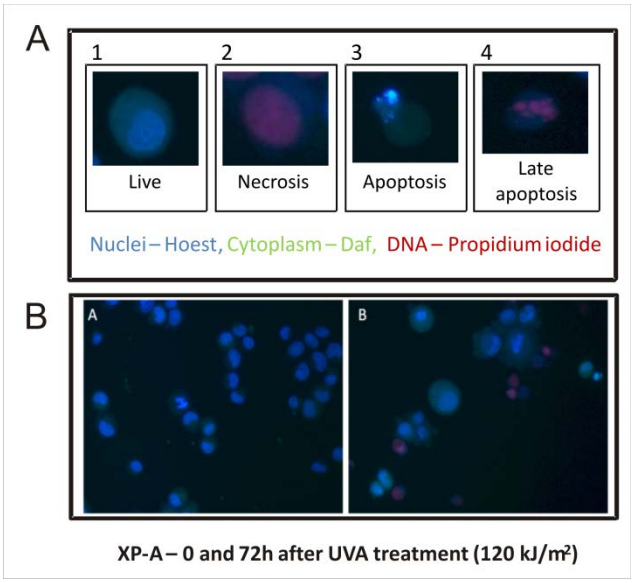


**Figure 1S:** Examples of cell characterization as live or death (necrosis, apoptosis and late apoptosis). (A) Representative cell stained with Propidium iodide (PI) – 1 mg/ml in red), cytoplasm (Diacetate Fluorescein (DAF) – 1.5 mg/ml in green) and nuclei (Hoechst 33342 (HO) – 1 mg/ml) in blue): live cells stain green while (1), while necrotic cells stain intense red; early apoptotic cells stain mostly green, however their nuclei appear condensed and fragmented (3) and late apoptotic cells stain red with fragmented nuclei (4). (B) A representative image for UVA-irradiated (120 kJ/m$^2$) XP-A cells: 0 or 72 h after exposure.

**DNA photoproducts detection – 6-4(PP) formation**

The Figure 2S shows the detection of CPDs and 6-4PPs lesions using specific antibodies on extracted DNA from CHO9 cell line (Proficient in NER) after the treatment with UV-B and UV-A radiation.

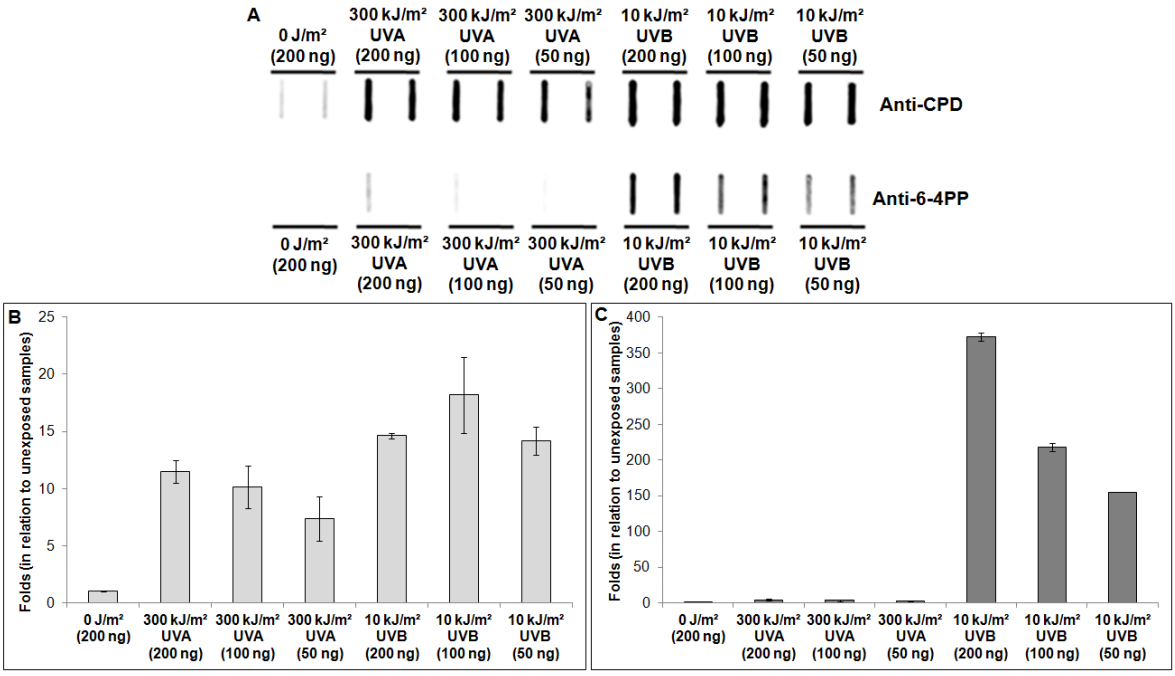


**Figure 2S:** Immunological detection of DNA lesions after exposure of CHO9 cells to UVB and UVA radiation. (A) Photoproduct detection by the antibodies anti-CPD and anti-6-4PP (as indicated). (B) Quantification of CPD induction (fold relative to unexposed control samples – 0 J/m$^2$ 200 ng). (C) Quantification of 6-4PP induction (fold relative to unexposed control samples – 0 J/m$^2$ 200 ng).

It is clear that the UVB radiation is very effective in inducing both types of DNA lesions in these cells, as the bands are very prominent in samples exposed to such waveband. For UVA radiation, the induction of CPDs is very clear, however for 6-4PPs, these lesions are almost undetectable in all analyzed samples.

To confirm the induction of these lesions in human cells following exposure to UVA and UVB light, we used human fibroblasts MRC5 (proficient in DNA repair) and XP12RO (mutant in the DNA repair gene *XPA*). And the results are shown in Figure 3S.

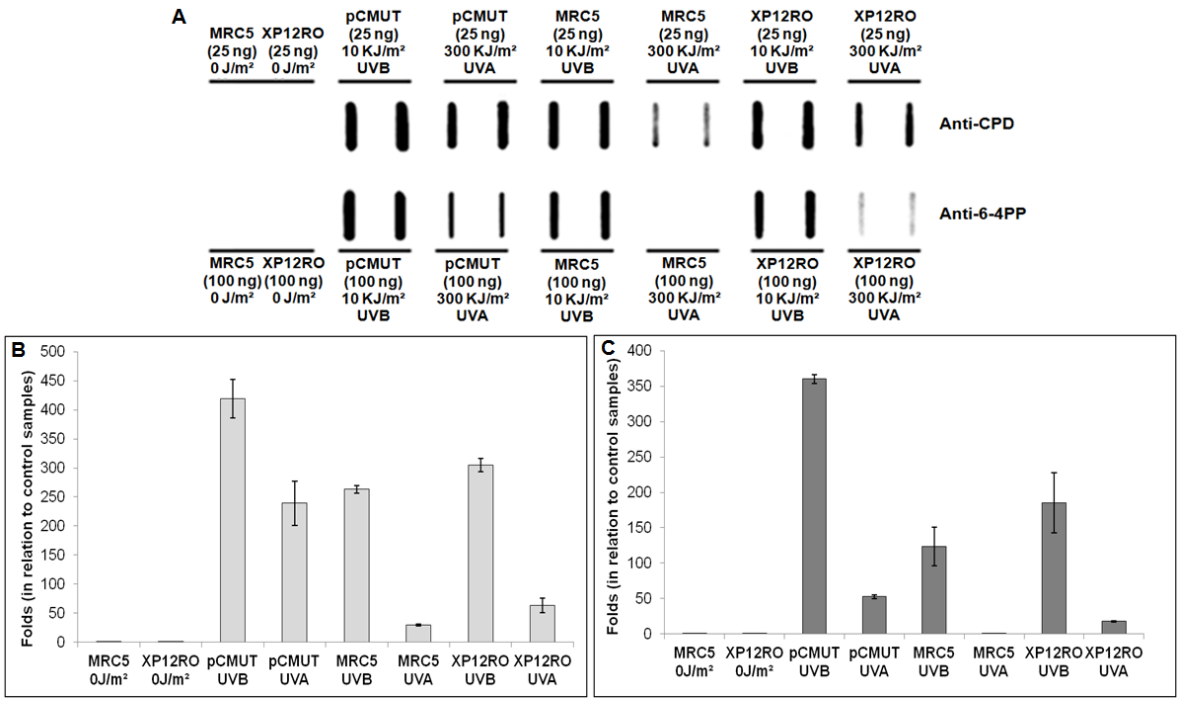


**Figure 3S:** Immunological detection of DNA lesions induced in MRC5 and XP12RO cells after exposure to UVB and UVA radiation. (A) Photoproduct detection by antibodies anti-CPD and anti-6-4PP, as indicated. (B) Quantification of CPD induction (fold relative to unexposed control samples – MRC5 and XP12RO 0 J/m$^2$). (C) Quantification of 6-4PP induction (fold relative to unexposed control samples – MRC5 and XP12RO 0 J/m$^2$).

These results clearly show that the detection of the lesions were higher in plasmid DNA (pCMUT) than in both cell lines employed (Figure 3S), and the induction of CPDs occurs efficiently after UVA or UVB exposure. Regarding induction of 6-4PPs, these lesions are efficiently induced in all UVB exposures, but after UVA irradiation the lesions are undetectable in the DNA from repair proficient cells (MRC5). However, in

the DNA from repair deficient cells (XP12RO), 6-4PPs are clearly detected in the genomic DNA after the exposure to UVA, with the bands significantly larger than the signal detected in the DNA from unexposed control samples.

**UVA irradiation on ice increase DNA photoproducts formation**

Cells were UVA irradiated on ice, in order to check the potential effect of DNA repair in the amounts of the photoproducts induced. The results revealed an important increase in the levels of CPD and 6-4PP in both MRC5 and XP-A cell lines, as can be seen in the figure 4S.

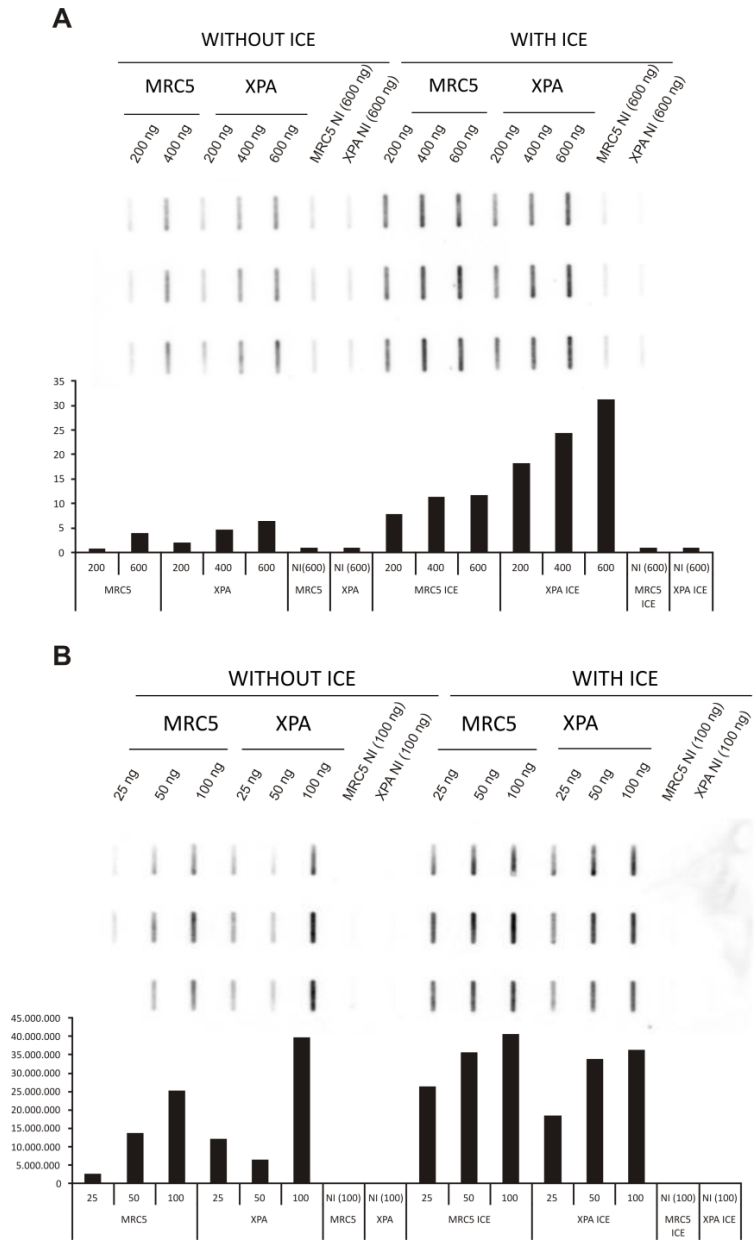


**Figure 4S:** Immunological detection of DNA lesions induced in MRC5 and XP12BE (XP-A) cells after exposure to UVA radiation (300 kJ/m²). (A) 6-4PP detection by antibodies anti-6-4PP and quantification. (B) CPD detection by antibodies anti-CPD and quantification (fold relative to unexposed control samples – MRC5 and XP-A 0 kJ/m$^2$). These results are representative of three independent experiments.

**UVA light induces photolyases activation**

To determinate if UVA radiation activates the photolyases, photolyases-transduced XP-A cells were irradiated with UVC light which generates important amounts of both CPDs and 6-4PPs.

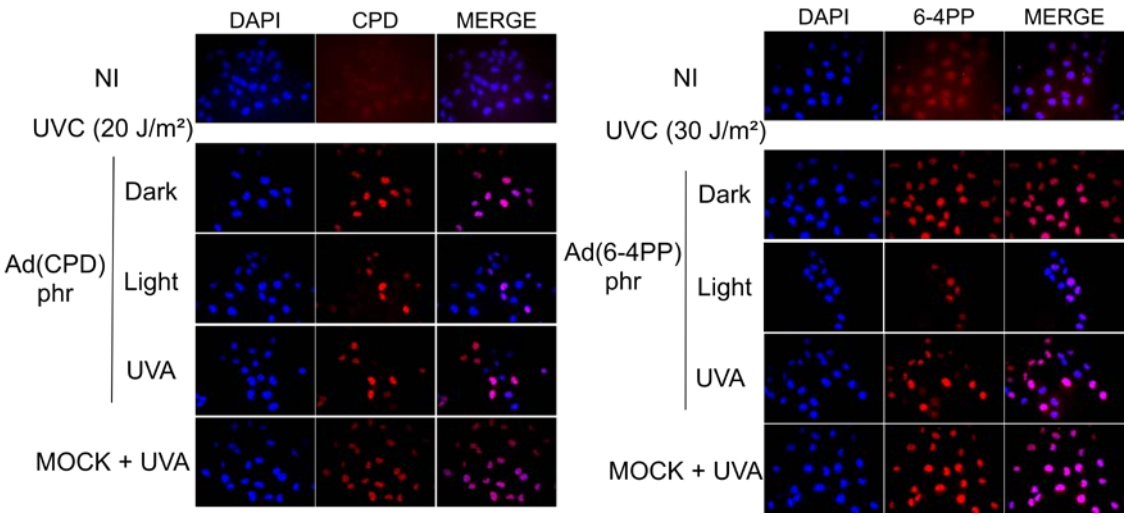


**Figure 5S:** Immunofluorescence of CPDs (left) and 6-4PPs (right) in XP-A cells mock-treated or transduced with either Ad(CPD) or Ad(6-4PP) photolyase (phr) irradiated with 20 J/m² (for CPDs detection) or 30 J/m² (for 6-4PPs detection) UVC. Cells were then exposed to visible light, UVA light or kept in the dark.

Figure 5S shows that when either photolyases are present in UVC irradiated-cells in the dark, both photoproducts can still be detected in all cells. However, when photolyases-transduced cells are exposed to visible or UVA light after UVC irradiation, there is a decrease in the number of labeled nuclei, indicating that UVA light photoactivates both CPD- and 6-4PP-photolyases in these cells, promoting the photorepair of these lesions.