Supplementary Information

Mass Spectrometry Based Proteomics Study of Cisplatin-Induced DNA-Protein Cross-Linking in Human Fibrosarcoma (HT1080) Cells

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Figure S1. Cytotoxicity of cisplatin in HT1080 cells immediately after treatment. Cells were treated with increasing concentrations of the drug (0, 250, or 500 μM) for 3 h. Cells were counted immediately after treatment, and the cytotoxicity was measured as the percentage of cells surviving cisplatin treatment compared to untreated controls.

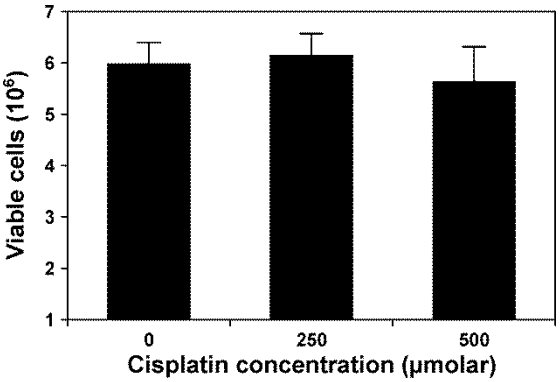

Figure S2. Cytotoxicity of cisplatin in HT1080 cells. To establish the effects of cisplatin treatment on cell viability, HT1080 cells were treated with increasing concentrations of the drug (0, 10, 250, and 500 µM) for 3 h. Following overnight incubation in a drug free media, cells were counted, and the cytotoxicity was measured as the percentage of cells surviving cisplatin treatment compared to untreated controls.

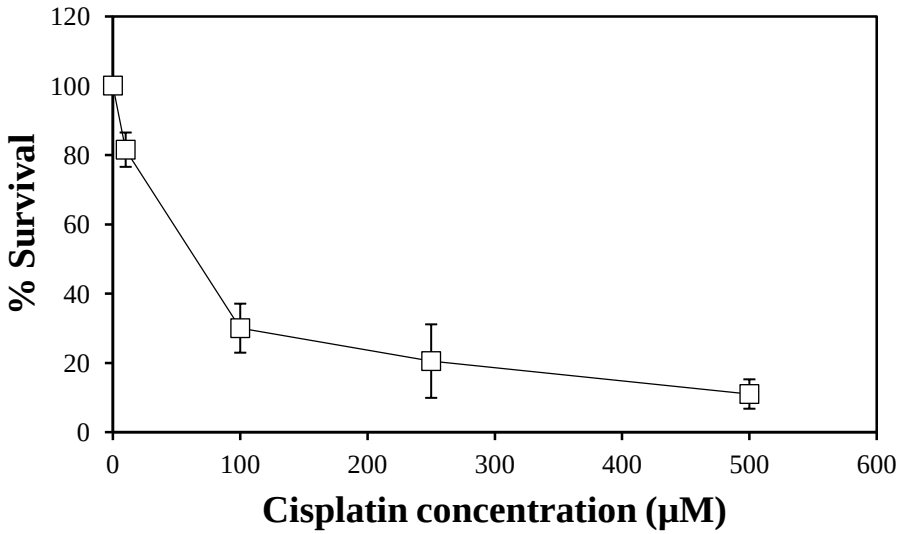

Figure S3. Representative HPLC trace for the separation of nucleoside mixtures resulting from the enzymatic digestion of nucleic acids that were isolated from cisplatin-treated HT1080 cells. After harvesting cell nuclei, DNA containing covalently trapped proteins were isolated by a phenol/chloroform extraction protocol optimized for the extraction of DPCs. The identities of nucleosides were confirmed by HPLC analysis of authentic standards. Coformycin was added to inhibit the contaminating deaminase activity present in commercial alkaline phosphatase. Accurate DNA quantification was accomplished by comparing dG peak areas in all samples to a standard calibration curve obtained by injecting known amounts of dG.