**Repair of *O*6-carboxymethylguanine adducts by**

***O6*-methylguanine-DNA methyltransferase in human colon epithelial cells**

**- Supplementary Material -**

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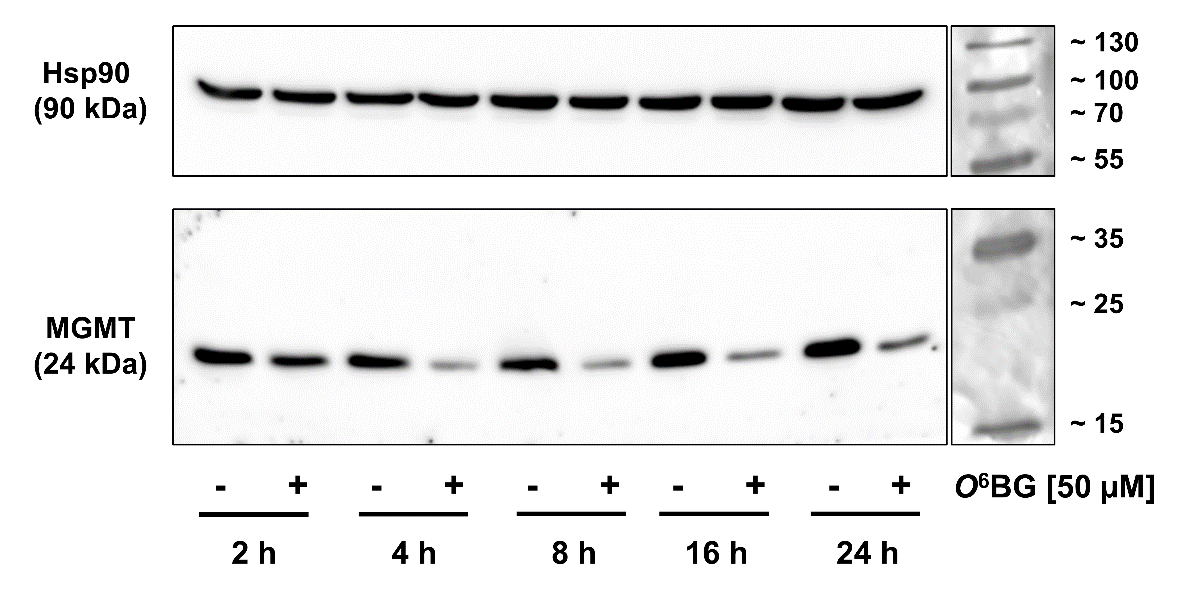
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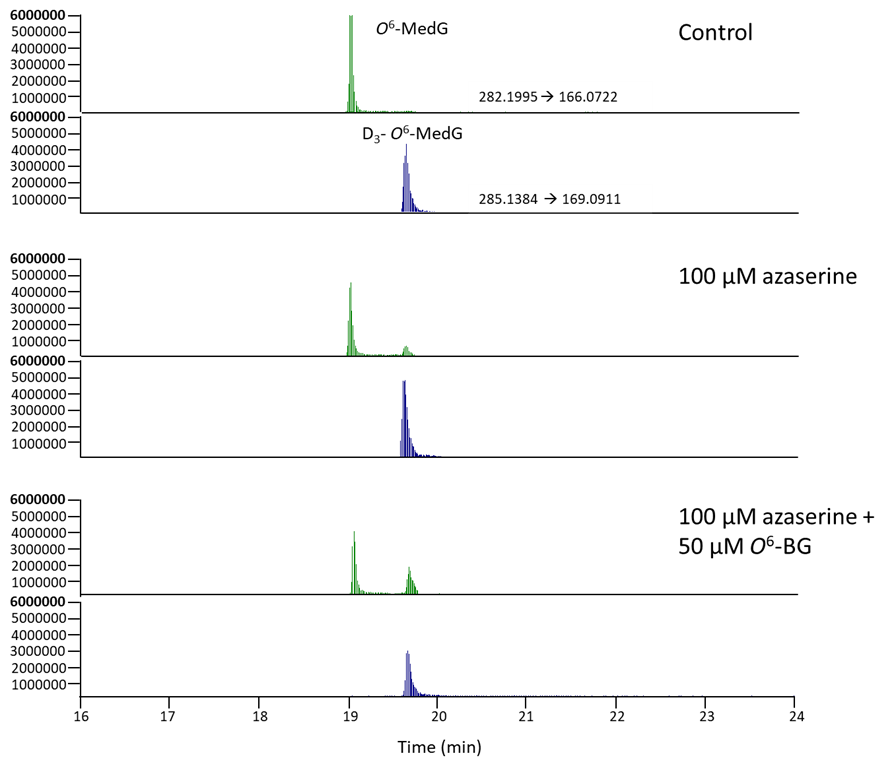
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**Supplementary Fig. 1: Western blot images showing time-dependent depletion of MGMT protein expression levels upon treatment with 0.1 % DMSO (-) or *O*6BG (+) in HCEC 1CT cells.** The intensity signal of Hsp90 served as loading control, while molecular markers verified the protein sizes.



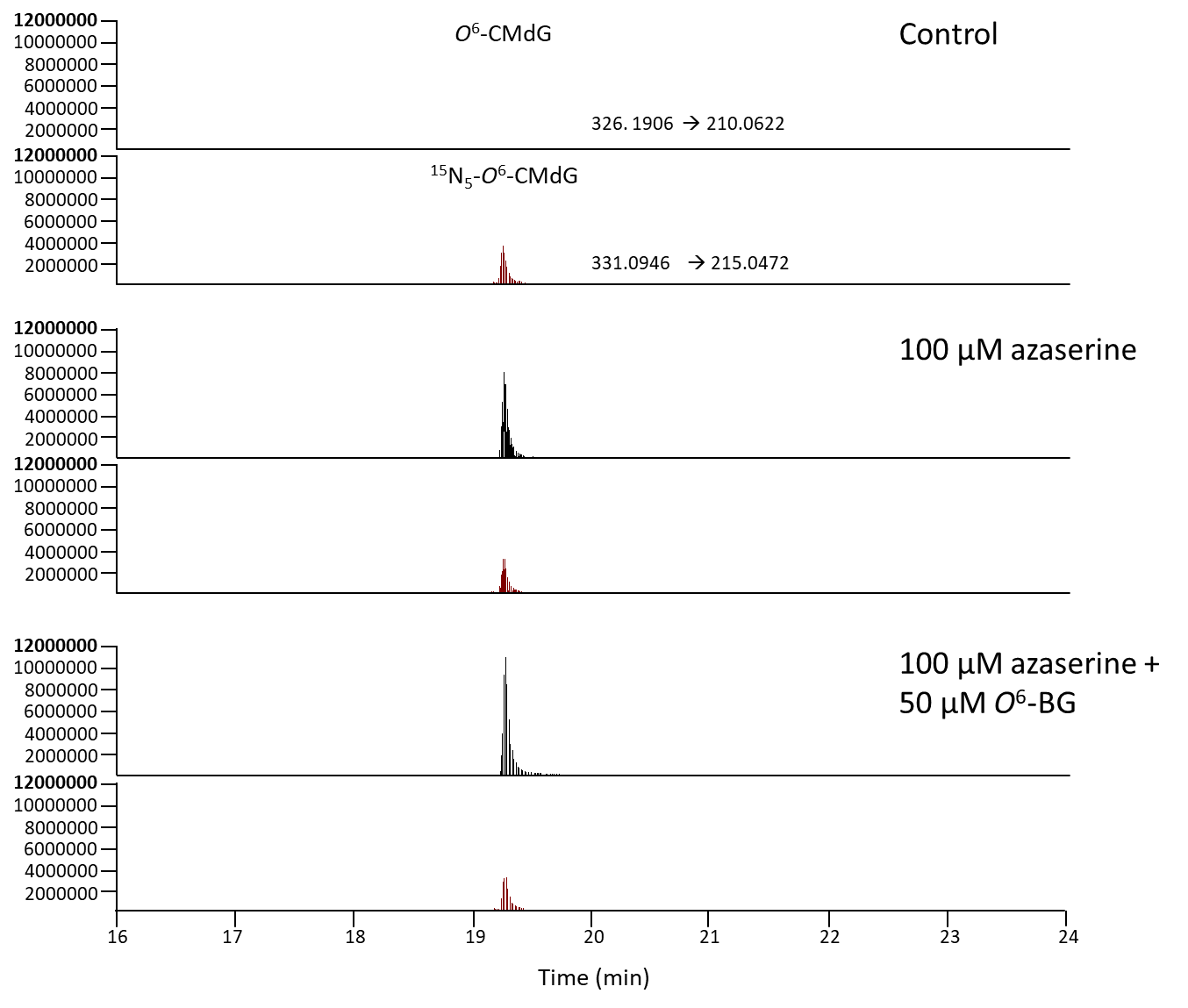
**100 µM AZA +   
50 µM *O*6BG**

**100 µM AZA**

**Control**

**Abundance**

**Supplementary Fig. 2: Representative ion chromatograms of *O*6-MedG adducts extracted from HCEC 1CT cells after cellular treatment.** D3-*O*6-MedG was used as internal standard. The peak with m/z 282.1995→166.0722 is *N*2-MedG.



**100 µM AZA +   
50 µM *O*6BG**

**100 µM AZA**

**Control**

**Abundance**

**Supplementary Fig. 3: Representative ion chromatograms of *O*6-CMdG adducts extracted from HCEC 1CT cells after cell treatment.** 15N5-*O*6-CMdG was used as internal standard.

**Supplementary Table I: Reduced MGMT activity in HCEC 1CT cells induced by various concentrations of *O*6BG.** The activity of MGMT-deficient HeLa MR cells served as blank and was used for the calculation of corrected radioactivity. Significance levels are given in **Figure 2C** of the main manuscript.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample** | **Radioactivity**  **[cpm] in triplicate** | **Corrected radioactivity [cpm]** | **MGMT activity**  **[fmol/mg protein]** | **Mean (± SEM)** |
| **DMSO** | 1627.19 | 1427.19 | 211.30 | 203.93 (± 7.43) |
| 1502.19 | 1277.02 | 189.06 |
| 1652.93 | 1428.10 | 211.43 |
| **0.1 µM O6BG** | 901.12 | 701.12 | 103.80 | 90.57 (± 7.44) |
| 752.31 | 527.15 | 78.04 |
| 831.78 | 606.95 | 89.86 |
| **1 µM *O*6BG** | 342.38 | 142.38 | 21.08 | 15.94 (± 2.60) |
| 310.63 | 85.47 | 12.65 |
| 319.91 | 95.08 | 14.08 |
| **10 µM *O*6BG** | 275.97 | 75.97 | 11.25 | 5.03 (± 3.12) |
| 240.62 | 15.46 | 2.29 |
| 235.23 | 10.40 | 1.54 |
| **HeLa MR blank** | 200.00 |  |  |  |
| 225.17 |  |  |  |
| 224.83 |  |  |  |

**Supplementary Table II: Targeted *m/z* of compounds and fractions after neutral loss of deoxyribose (dR) detected using tandem mass spectrometry in different modes.** D3-*O*6-MedG and 15N5-*O*6-CMdG were used as internal standards.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compound** | **MSn** | **Molecular ions** | ***m/z*** | **Chemical structure of**  **compounds and fractions** |
| ***O*6-MedG** | MS1 | [M+H]+ | 282 | C:\Users\TiKa\Desktop\282.tif.jpg |
| MS2 | [M+H-dR]+ | 166 |
| **D3-*O*6-MedG** | MS1 | [M+H]+ | 285 | C:\Users\TiKa\Desktop\285.jpg |
| MS2 | [M+H-dR]+ | 169 |
| ***O*6-CMdG** | MS1 | [M+H]+ | 326 | C:\Users\TiKa\Desktop\326.tif.jpg |
| MS2 | [M+H-dR]+ | 210 |
| **15N5-*O*6-CMdG** | MS1 | [M+H]+ | 331 | C:\Users\TiKa\Desktop\331.jpg |
| MS2 | [M+H-dR]+ | 215 |