

Automated Screening for Oxidative or Methylation-Induced DNA Damage in Human Cells

Supplementary Data

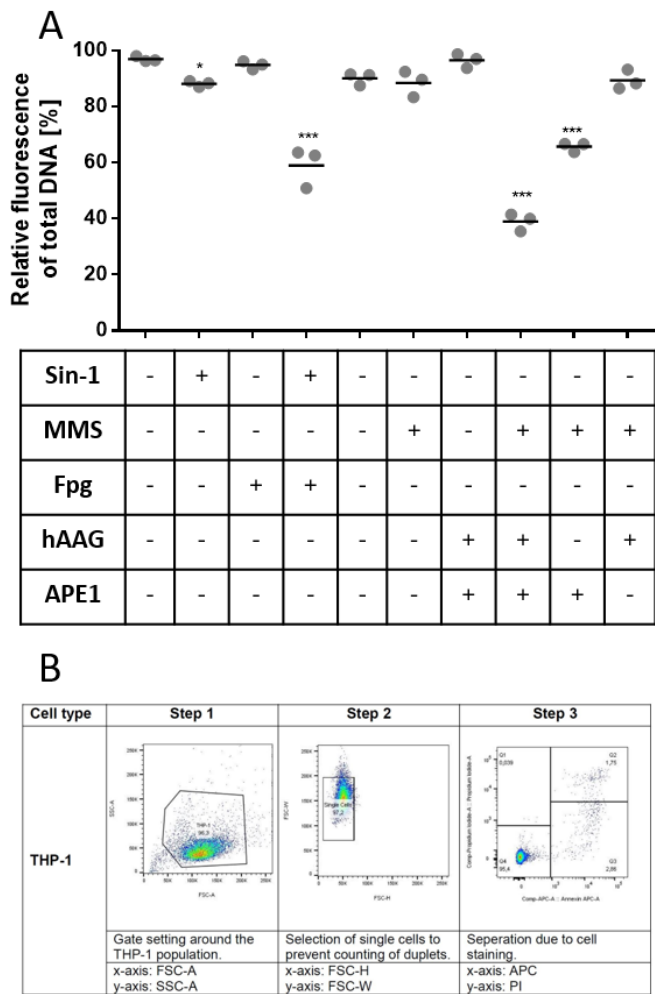


Fig. S1: Enzyme-modified FADU on purified plasmid DNA, FACS gating strategy and genotoxin screening
(A) 4 μ g plasmid DNA (14 kbp) was treated for 40 min at 30°C with 250 μ M Sin-1 or 10 mM MMS in 17 μ L H₂O. For enzyme-mediated lesion detection, 1 μ L enzyme solution (Fpg: 8 U; hAAG: 8 U; APE1: 0.5 U) and 2 μ L NEB1 buffer (10x), were added. After 30 min at 37°C, the FADU assay was performed as described by Müller et al. (2013). Three independent experiments with 3 technical replicates per data point were performed. Statistical analysis: One-way ANOVA with Dunnett's multiple comparisons test compared to untreated control (*p < 0.05, ***p < 0.001). (B) Gating strategy for the Annexin V APC/PI flow cytometric detection of apoptosis induction in THP-1 cells.

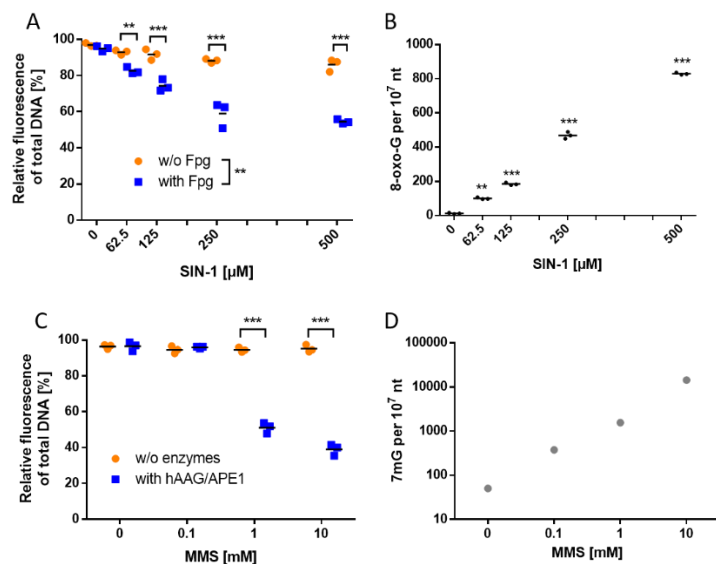


Fig. S2: Detection of DNA strand breaks, oxidative lesions, and methylation lesions upon Sin-1 or MMS treatment of plasmid DNA

104 μg plasmid DNA (4 μg for FADU, 100 μg for LC-MS/MS) was treated for 40 min at 30°C with Sin-1 or MMS. (A) Fpg-sensitive sites were measured via plasmid FADU assay. Three independent experiments with 3 technical replicates per data point were performed. Statistical analysis: Two-way ANOVA with Sidak's multiple comparisons test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (B) 8-oxoG was determined via LC-MS/MS. Statistical analysis: One-way ANOVA with Dunnett's multiple comparisons test compared to untreated control (* $p < 0.05$, *** $p < 0.001$). (C) hAAG/APE1-sensitive sites were measured via plasmid FADU assay. Three independent experiments with 3 technical replicates per data point were performed. Statistical analysis: Two-way ANOVA with Sidak's multiple comparisons test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (D) 7mG was determined via LC-MS/MS. One experiment was performed.