Supplementary Information

Function of high-mobility group A proteins in the DNA damage signaling for the induction of apoptosis

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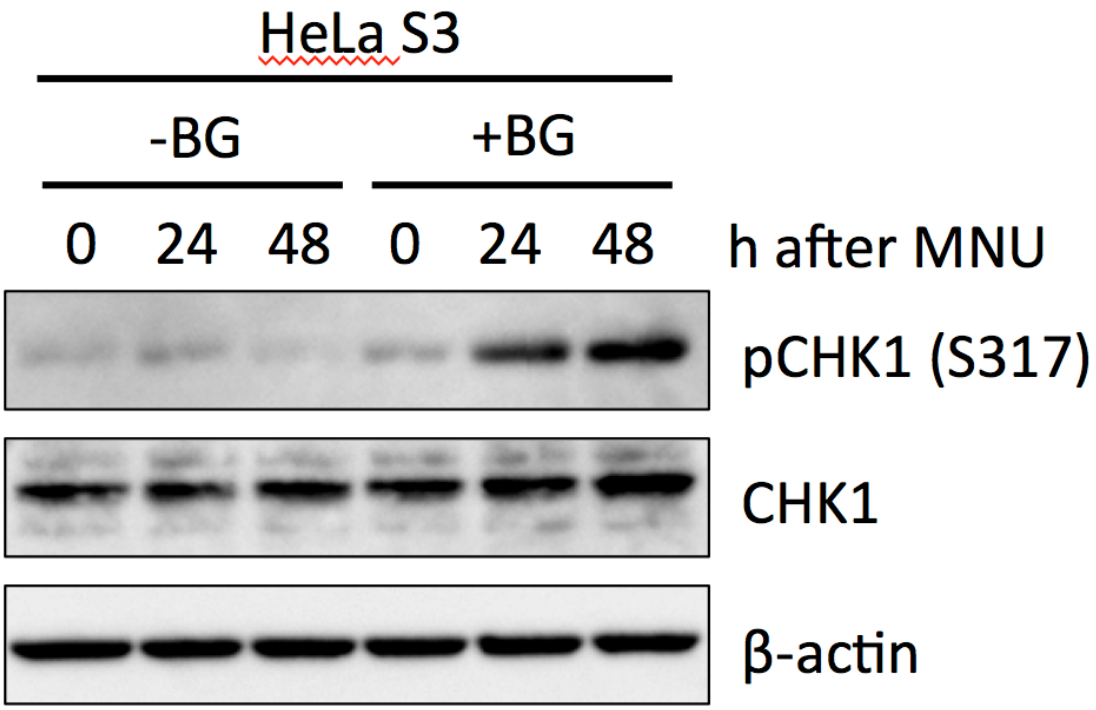
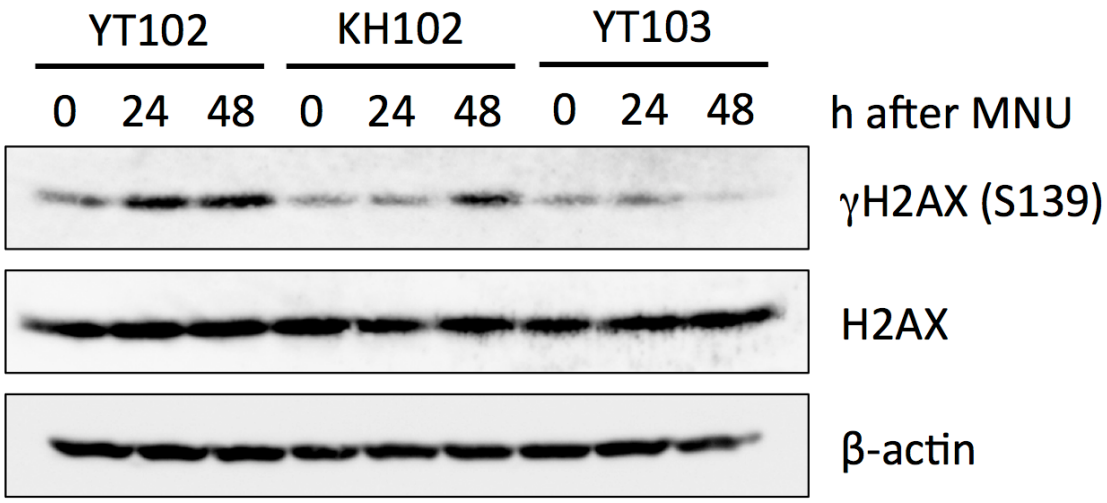
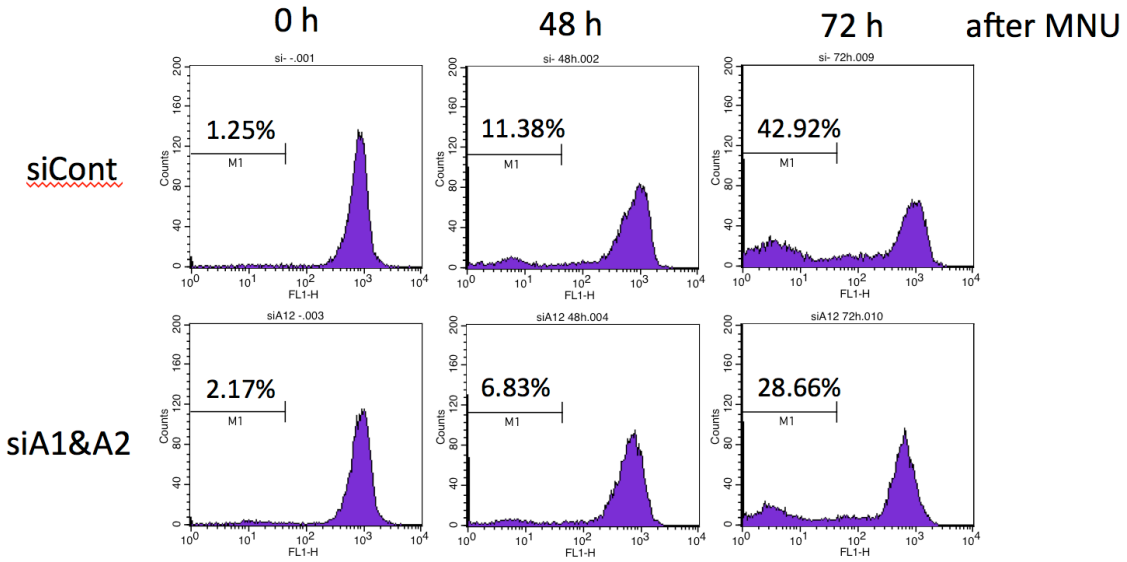


Supplementary Figure S1. O^6-methylguanine-dependent activation of CHK1. MGMT-proficient HeLa S3 cells were treated with or without 25 μM of O^6-benzylguanine (Sigma) for 2 h, and then treated with 0.2 mM MNU in serum-free medium containing 0.02 M HEPES-NaOH (pH 6.0) for 1 h at 37 °C. After cultivation in a complete media in the presence or absence of 25 μM of O^6-benzylguanine for 24 or 48 h, whole-cell extracts were prepared by lysing the cells by adding 2 × SDS sample buffer containing 100 mM Tris-HCl (pH 6.8), 2% SDS, 20% glycerol, 2% β-mercaptoethanol, and 0.4 mg/ml bromophenol blue, followed by boiling for 10 min. The whole-cell extracts were subjected to SDS-PAGE, and immunoblotting was performed using antibodies specific for phospho-serine-317-CHK1 (Cell Signaling) and total CHK1 (Santa Cruz Biotech.) proteins. β-actin (Sigma) was the loading control.



Supplementary Figure S2. The DNA-damage response in *Hmga2*-deficient cell line KH102. Mouse fibroblast-derived cell lines YT102 (*Mgmt*$^{-/-}$), KH102 (*Mgmt*$^{-/-}$ *HmgA2*$^{+/-}$), and YT103 (*Mgmt*$^{-/-}$ *Mlh1*$^{-/-}$) were treated with 0.2 mM MNU for 1 h and further cultivated in a complete media for 24 and 48 h. The whole-cell extracts were prepared as described in Supplementary Figure S1. Immunoblotting was performed using antibodies specific for γH2AX (Cell Signaling) and H2AX (Cell Signaling) proteins. β-actin (Sigma) was the loading control.



Supplementary Figure S3. The effects of *HMGA*-knockdown on the mitochondrial outer membrane permeabilization. siHMGA1/A2-transfected HeLa MR cells were treated with 0.2 mM MNU in serum-free medium containing 0.02 M HEPES-NaOH (pH 6.0) for 1 h at 37 °C, and further cultivated in a complete media for 24 and 48 h. The cells were trypsinized and suspended in 1 ml of PBS. The cells were stained with the membrane-potential-sensitive cyanine dye, $DiOC_2(3)$ in accordance with the instructions (Mitoprobe $DiOC_2(3)$ Assay kit, Thermo Fischer Scientific) and then subjected to flow cytometric analyses. The percentage of mitochondria outer membrane permeabilized cells (M1) were indicated.