Supplementary Information

Supplementary materials and methods

Plasmids and siRNAs.

Myc-MPG and Myc-UNG2 vectors were as described (16), and site-specific MPG<sup>D132N</sup>, MPG<sup>N169D</sup>, MPG<sup>E125Q/N169D</sup> and UNG2<sup>D154N</sup> mutations were generated using the QuickChange site-directed mutagenesis method (Agilent Technologies). To generate green fluorescent protein (GFP) fusions of MPG<sup>WT</sup> and MPG<sup>D132N</sup>, the relevant MPG segments were cut from Myc-MPG vectors and cloned into the EcoRI and BamHI sites of the pAcGFP-N1 vector (Clontech) for immunofluorescence detection. The GFP-APOBEC3A (A3A) mammalian expression vector was amplified from HeLa cDNA and cloned into the EcoRI and SalI sites of the pAcGFP-N1 vector (Clontech). To produce 4-OHT–inducible A3A expression, the nucleotide sequence corresponding to the estrogen receptor ligand-binding domain (ER-LBD; amino acids 282–595) amplified from MCF7 cDNA was inserted into the SalI site of pAcGFP-A3A to generate the pAcGFP-A3A-ER vector. The nucleotide sequence of the A3A-ER segment is shown in Supplementary Figure 1.

The siRNA target sequences used in this study were synthesized by Sigma-Proligo as follows: ATMIN, 5'-CCCTGATCCTCGGCCTAGA-3'; Mre11, 5'-CATTACATAACCTGCCTCGAGT-3'; Rad50, 5'-AAGGTCAGTCTCGCTCAAG-3'; NBS1, 5'-AATGATCAGTCGATCAGCCGA-3'; OGG1, 5'-GTATGGACACTGACTCAGACT-3'; Tip60, 5'-ACGGAAGGTGGAGGTGGTT-3' (51) and UNG2 #1, 5'-ATGATATGGATCCTGTCCCAGGATG-3'. The siRNA sequences that targeted ATM and MPG, as well as the control (random) sequence, were as described (16). UNG2 #2 (a pool of two duplexes HSS187640 and HSS187641) were obtained from Invitrogen.

Cell lysis and fractionation

Lysates prepared for analysis of ATM nuclear retention were fractionated using different NP-40 concentrations as described (52). Briefly, cells treated with IR or MMS for 30 min were resuspended in 100 μl of fractionation buffer (50 mM HEPES-KOH [pH 7.5], 150 mM NaCl, 1 mM EDTA and 0.2% NP-40) containing 1 mM DTT and a mixture of protease inhibitors (Roche) for 5 min on ice and centrifuged at 1000 × g for 5 min. The supernatant was collected and designated as the "low NP-40" fraction. The nuclear pellets were washed twice with fractionation buffer, extracted for 40 min on ice with 100 μl of the same buffer containing 0.5% NP-40 and centrifuged at 15 000 × g for 20 min. This supernatant was collected and designated as the "high NP-40" fraction. Equal volumes of the low and high NP-40 fractions were subjected to protein electrophoresis and western blotting.
**ATM kinase assay**

To prepare recombinant proteins for the kinase assay, an *E. coli*–expressed GST fusion p53 fragment (GST-p53) was purified as described (16).

**DNA Substrates.** The pcDNA3 plasmid was used as the DNA substrate and was freshly prepared to avoid unexpected DNA damage during repeated freeze-thaw cycles. To generate an AP site–containing plasmid, pcDNA3 was depurinated in acidic buffer (0.01 M sodium citrate and 0.1 M KCl [pH 5.0]) and heated at 70°C for 2 h. The DNA DSB and SSB substrates were made by cutting the pcDNA3 with NcoI (R0193S; New England BioLabs) and the depurinated pcDNA3 with APE1 (M0282S; New England BioLabs), respectively. All DNA substrates were purified using the QIAquick Gel Extraction kit (Qiagen).

**Kinase.** Dimeric ATM kinase was purified from Flag-ATM–transfected 293T cells that were lysed with three freeze-thaw cycles in detergent-free lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl and 10% glycerol) as described (26). Briefly, the cell lysates were centrifuged and the supernatant was transferred to a new tube, into which was added anti-Flag M2 affinity gel (A2220; Sigma-Aldrich). The precipitated proteins were washed three times with lysis buffer followed by two washes with kinase buffer (50 mM HEPES-KOH [pH 7.5], 50 mM KCl, 5 mM MgCl₂ and 10% glycerol). ATM kinase assays were carried out for 90 min at 30°C in kinase buffer with protease inhibitors and 1 mM ATP and contained purified Flag-ATM, 5 μl GST-p53 and 20 ng DNA. The reactions were stopped by adding Laemmli sample buffer, and the samples were then subjected to protein electrophoresis and western blotting.

**Indirect immunofluorescence**

Immunofluorescence staining was performed as described (16). Briefly, U2OS cells grown on coverslips were treated with MMS for 30 min at 37°C, the medium was replaced for cell recovery and the cells were incubated for an additional 30 min. The cells were fixed for 15 min at room temperature in 2.0% paraformaldehyde in PBS, washed three times with PBS and permeabilized for 10 min at room temperature in PBS containing 0.1% Triton X-100. The cells were then stained for 1 h at room temperature with anti-ATM Ser1981 (clone 10H11.E12; 1:50 dilution; Abcam) and then with TRITC-conjugated anti–mouse IgG (1:100 dilution; Jackson ImmunoResearch). For nuclear staining, cells were treated with DAPI. Coverslips were mounted over the stained cells on microscope slides using antifade reagent, and images were obtained using a Zeiss LSM510 Meta confocal microscope.

**Comet assay and colony formation analysis**

To detect the presence of DNA damage and to measure the quantity of DNA strand breaks formed in the initial steps of BER, the alkaline comet assay was performed as described (16).
For cell viability analysis, cells were transfected with the relevant siRNA or plasmid, and 2 days later cells were seeded into six-well plates (400 cells for untreated control, 1200 cells for MMS treatment). The cells were incubated overnight and then treated for 1 h with MMS at the indicated concentration. The cells were washed with PBS and then cultured in fresh medium for 10–14 days to allow colony formation. Experiments were performed three times, and the mean and standard deviation for each cell line for each treatment was calculated from three independent wells in one experiment.

**Supplementary references**


**Supplementary Figure Legends**

Supplementary Figure 1. Full-length A3A-ER nucleotide sequence. The sequence was inserted into the EcoRI and SalI sites of the pAcGFP vector to generate pAcGFP-A3A-ER, a 4-OHT–inducible A3A activation vector.

Supplementary Figure 2. MMS induces ATM-pS1981, Chk2-pT68, and Chk1-pS345 in MCF7 (left) or MDA-MB-453 (right) cells. Cells transfected with control siRNA or siRNA targeting MPG (siMPG) for 2 days were treated with 0.3 mg/ml MMS for 30 min; the cells were then harvested, and protein expression was analyzed by immunoblotting.

Supplementary Figure 3. Decreased expression of MPG, but not Mre11, abrogates MMS-induced phosphorylation of ATM S1981 in 293T cells. Cells treated with the relevant siRNAs were exposed to 0.3 mg/ml MMS for 30 min; the cells were then harvested, and protein expression was analyzed by immunoblotting.

Supplementary Figure 4. Decreased expression of Rad50 or NBS1 does not suppress MMS-induced phosphorylation of ATM S1981 and Chk2 T68 in 293T cells. Cells treated with the relevant siRNA were exposed to 0.3 mg/ml MMS for 30 min; the cells were then harvested, and protein expression was analyzed by immunoblotting.

Supplementary Figure 5. Decreased expression of ATMIN does not suppress MMS-induced phosphorylation of ATM S1981 and Chk2 T68 in 293T cells. Cells treated with the relevant
siRNA were exposed to 0.3 mg/ml MMS for 30 min; the cells were then harvested, and protein expression was analyzed by immunoblotting.

Supplementary Figure 6. Decreased expression of Tip60 does not suppress MMS-induced phosphorylation of ATM S1981 and Chk2 T68 in 293T cells. Cells treated with the relevant siRNA were exposed to 0.3 mg/ml MMS for 30 min; the cells were then harvested, and protein expression was analyzed by immunoblotting.

Supplementary Figure 7. Immunoblot showing relative MPG expression in HeLa, MCF7, and U2OS cells. MPG expression was also assessed in 293T cells transfected with control siRNA or siRNA targeting MPG. Actin was used as an internal control.

Supplementary Figure 8. Immunoblot analysis showing MMS-induced phosphorylation of ATM S1981 in U2OS cells that ectopically expressed MPG or its derivative mutants. These cells were also used to determine the levels of MMS-induced DNA damage by alkaline comet assay (Figure 2C). Cells expressing empty vector, Myc-MPGWT, or Myc-MPD132N were incubated with or without MMS for 20 min, and the cell lysates were immunoblotted to detect the indicated proteins.

Supplementary Figure 9. Agarose electrophoresis analysis showing methoxyamine protects depurinated pcDNA3 plasmid from APE1 digestion. The supercoil relaxation assay was used to analyze APE1 activity. Plasmid DNA was depurinated in sodium citrate buffer (pH 5.0) by heating at 70°C for 1 h and was then used as the APE1 substrate and was digested to produce the nicked circular plasmid (SSB). Depurinated pcDNA3 was preincubated with methoxyamine at the indicated concentrations for 30 min and then was treated with or without APE1 for 20 min. Supercoiled plasmid is shown in lane 1 (uncut depurinated pcDNA3). Lane 2, depurinated pcDNA3 cut by recombinant human APE1. Lanes 3–6, depurinated DNA preincubated with methoxyamine in the absence of (lanes 3 and 5) and in the presence of (lanes 4 and 6) APE1 digestion.

Supplementary Figure 10. Ponceau S staining showing recombinant GST and GST-p53 (9–22 aa) expression and purity. Marker at left was used to determine the approximate protein size.

Supplementary Figure 11. ATM S1981 phosphorylation induced by MMS treatment occurs independently of UNG2 expression. 293T cells were exposed to 0.3 mg/ml MMS for 30 min after transfection with control siRNA or siRNA targeting UNG2 for 2 days. After treatment, the cells were harvested, and protein expression was analyzed by immunoblotting.

Supplementary Figure 12. Decreased OGG1 expression, but not MPG expression, abolishes H2O2-induced phosphorylation of Chk2-T68 in 293T cells. Cells treated with the relevant siRNA were exposed to 0.3 mg/ml H2O2 for 30 min. The cells were then harvested, and protein expression was analyzed by immunoblotting.

Supplementary Figure 13. Inhibition of PARP1 activity promoted, whereas inhibition of APE1
activity decreased, H$_2$O$_2$-induced phosphorylation of Chk2-T68 in 293T cells. Cells were incubated with DMSO as control, ABT-888, or APE1 inhibitor III, respectively, for 1 h before exposed to 0.3 mg/ml H$_2$O$_2$ for 30 min. The cells were then harvested, and protein expression was analyzed by immunoblotting.

Supplementary Figure 14. HeLa cells transfected with siRNA targeting MPG are resistant to MMS treatment. HeLa cells transfected with control or MPG siRNA were incubated with MMS at the indicated doses for 1 h. Colony survival is expressed as a percentage of that detected in the untreated control. Bar, standard error. Asterisks indicate that the survival fractions in the indicated dosage of treatment for control group is significantly different from those seen in the siMPG group ($P < 0.05$).

Supplementary Figure 15. Association between MPG expression and prognostic outcome of ovarian cancers who received chemotherapies. Kaplan–Meier plots of RFS showing patient survival among patients who received (A) Gemcitabine, (B) platins, (C) Topotecan, (D) Avastin, (E) Docetaxel, (F) Paclitaxel, and (G) Taxol treatment. Data were obtained from the Kaplan–Meier Plotter database and $P$ values were calculated by log-rank tests. HR, hazard ratio; n, number of patients.
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1201  CTGGACAAGATCACAGACACTTTGATCCACCTGATGGCCAAGGCAGGCCTGACCCTGCAG  1260
     LDKI TDTL IHLMAKAGLTLQ
1261  CA6C6ACACCAGCGGCTGGCCCAGCTCCTCCTCATCTCTTCACATCAGGCACATGA7T  1320
     QQRLAQLLLILSHIRHMS
1321  AACAAAGGCAF76GACATCTGTACAGCAGTGAAGTGCAAGAACGTGGTGCCCCTCTATGAC  1380
     N KGMEDHLYSMKCKKNVPLYD
1381  CTGCTGCTGGAGGGCGGCGGACGCCCACCGCCTACATCGGCCACTAGCGGTGGAGGGCA  1440
     LLLEAADAHRHAPTSRGA
1441  TCCGTGAGAGACGGACCAAAAGCCACTTGCGCCCTCGACCTCTCATCGCATCC  1500
     SVETDQSHLATAGSSTSSHS
1501  TTGCAAAAGTATTACATCAGGGGAGGCAGAGGTTTCCCTTGCCACGCTGTCGACG  1560
     LQKYYITGEAEFPATVWST

- APOBEC3A coding sequence
- ER-ligand binding domain sequence
- Critical mutation for regulation by 4-OHT
Supplementary Figure 8

Supplementary Figure 9

Depurinated pcDNA3

Methoxyamine (MX)  —  —  25  25  200  200 (mM)
APE1 (0.1 U)        —  +  —  +  —  +  —  +

nicked circle
supercoiled

1  2  3  4  5  6
Supplementary Figure 10

Supplementary Figure 11
Supplementary Figure 14

Colony formation (% of control)

MMS (mg/ml)

Control
siMPG

MPG
Tubulin
HeLa

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