Supplemental Materials and Methods

Viability assays

Cells (1-5x10^5 cells) were plated into 6 well dishes in 2 ml of DMEM/FBS. Following incubation with siRNA, shRNA or controls in presence or absence of TMZ and other inhibitors, cells were collected and analyzed for cell count and cell viability using trypan blue. Cells were directly counted using the Beckman Coulter Vi-CELL (12-Sample Carousel) Cell Viability Analyzer (Beckman Coulter, Brea, CA, USA). Additional viability assays were performed in 96 well assays plating 2x10^3 cells per well and using the alamarBlue cell viability assay (Resazurin viability dye, Invitrogen, Cat# DAL1025) according to the manufacturer's instructions.

Cleaved Caspase Assay

Caspase 3/7 activity levels were measured using the Apo-One® Homogeneous Caspase 3/7 assay (Promega Corp., WI, USA) that provides a profluorescent substrate and a cell lysis/activity buffer for Caspase 3/7 (DEVDase) activity. After induction of apoptosis, 100 uL of Apo-One was added to each well, incubated for 4-12 hours and then fluorescence levels measured (485Ex/527Em) according to the manufacturer's instructions.

Immunoblotting/Immunoprecipitation

Cells were lysed with standard PLC lysis buffer containing protease and phosphatase inhibitors (Sigma-Aldrich Inc.). Protein concentration was determined using the bicinchoninic acid (BCA) assay (Pierce Chemical Co., Rockford, IL). Lysates containing 30 ug total protein were loaded onto 10% or 12% SDS-PAGE gels and electrophoresed. Proteins were then transferred onto PVDF membranes (NEN Research Products) using a semi-dry transfer apparatus (Bio-Rad). Membranes were blocked for one hour and probed for varying proteins overnight in 5% non-fat milk or 5% BSA in Tris Buffered Saline Solution, 0.5% Tween (TBST). Following incubation, membranes were washed three times for 10 mins with PBST and incubated with horseradish peroxidase-conjugated antibodies specific for the primary antibody (BioRad Laboratories, Inc., CA, USA). Binding was detected using

Chemiluminescence Reagent Plus (PerkinElmer Inc., Massachusetts, USA). Antibodies were used at the following dilutions: beta-actin (Sigma-Aldrich Inc., Cat#A2228, 1:10000), MGMT (Cell Signaling, cat#2739, 1:1000), MPG (Santa Cruz, SC-101237, 1:500), Cleaved PARP (ASN214) (Cell Signaling cat#9546, 1:1000), LIG4 (Santa Cruz, SC-28232 1:1000), APEX1 (Cell Signaling, Cat#4128, 1:1000), pSQ-ATM substrate (Cell Signaling, Cat#2851, 1:1000), ATM (Cell signaling, Cat#2873, 1:1000) pATM (Cell Signaling, Cat#4526, 1:500). Immuno-precipitations and co-immuno-precipitations (co-IP) were performed using 500ug total lysate using manufacturer specific dilutions. Denaturing IPs were performed as previously described(1-3).

Immunohistochemistry (IHC) and Immunofluorescence (IF)

Paraffin embedded blocks were cut into 5 μm sections and de-waxed in xylene followed by rehydration in a standard alcohol series. Antigen retrieval was by pressure cooking for 20 minutes in citrate buffer (pH 6.0), followed by blocking of endogenous peroxidase in 0.3% H_2O_2.

The antibodies (MPG 1:100, ATM 1:100, ki67 (Dako 1:100)) were added and incubated overnight at 4^oC. Detection used biotinylated secondary antibodies for 30 minutes and the ABC reagent kit (Vector Labs, CA, USA) and 3,3'-Diaminobenzidine (DAB) chromagen (Vector Labs). Sections were counter-stained with hematoxylin (Fisher Scientific Inc., Canada) for 30 secs, dehydrated in 70, 80 and 100% ethanol, briefly washed in xylene and mounted in Permount (Fisher Scientific Inc.). Hemotoxylin and Eosin (H&E) sections were stained using standard protocols (Eosin Yellowish Solution 1% w/v, Fisher Scientific Inc).

For immunofluorescence, 5 x 10^4 cells were grown on coverslips in 6-well media plates in D-MEM (Wisent) containing 10% FBS (Wisent) and treated with TMZ (0-100uM) for 4-12h. Cells were fixed for 10 minutes in 4% paraformaldehyde (PFA, Pierce Chemical Co., Rockford, IL) and permeabilized with 0.5% Triton X for 10 minutes. After removal of Triton X, cells were incubated with MPG monoclonal antibody (Santa Cruz Inc., 1:50), or phospho- H2AX (Millipore, Cat# JBW301, 1:500) and detected using Alexa Fluor 488 labeled secondary antibodies (Invitrogen Inc). Cells were also stained

with DAPI in the mounting medium. Images were captured on a Nikon E-600 microscope and analysed using Nikon ACT-1 software.

Stable cell line generation and siRNA/Plasmid transient transfections

The MPG-myc-flag dual tag construct was obtained from Origene (Cat#RC204933). MPG-myc tagged and ATM-Flag-His tagged constructs were obtained from The Signaling Identification Network (SIDNET, The Hospital for Sick Children, Toronto, Canada) and Addgene (Dr. Michael Kastan, addgene #31985(4)) respectively, under a material transfer agreement). MPG or empty vector controls (3 ug) were transfected into cells in 10 cm dishes using Fugene HD (3:1 ratio, Roche Canada). After 48h, puromycin was added to 1ug/ml. Resistant clones were selected 7-14 days later, pooled and assayed for MPG by immunoblotting. MPG shRNA and ATM shRNA constructs were purchased from Origene (Cat#TR311419) and Open biosystems. Briefly, 3 ug of MPG, ATM or control shRNA constructs were transfected into cells in 10 cm dishes using Fugene HD. After 48h, puromycin was added to 1ug/ml. Resistant clones were selected 5 days later, pooled and assayed for knockdown using immunoblotting. Double knockdown was performed by sequential selection of MPG knockdown expressing clones followed by transfection of ATM shRNA constructs and linear hygromycin (3 ug ATM shRNA constructs: 300ng linear hygromycin) using Fugene. Hygromycin selection was performed at 40 ug/ml. Resistant clones expressing ATM and MPG shRNA were selected 7-14 days later and pooled after confirming dual knockdown.

Real-time quantitative PCR (qRT-PCR)

Total RNA isolation was performed using an RNA extraction kit according to the manufacturer's instructions (RNeasy kit, Qiagen). cDNA was synthesized from 100 ng of total RNA using the Quantitect RT kit which includes DNAse treatment (Qiagen). Quantitative real-time PCR was performed on 10 ng of cDNA template in a final volume of 20 µl using the Chromo4 Real Time PCR detector (MJ Research a Division of Bio-Rad Laboratories Ltd) using SYBR green fluorescence. Real-time PCR data was analyzed using Opticon Monitor 3.1.3 analysis software. Data analysis was done

using the delta delta CT method with HPRT1 as a reference/control gene. Specific Primers are available upon request.

Previously published assays

Molecular beacon MPG activity assay(5), site-directed mutagenesis assays, DNA damage assays (comet assay) and *in vitro* kinase/MPG purification were performed as previously described(2, 6, 7).

Supplemental References:

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