Supplemental Figure Legends:

Supplemental Figure 1. Related to Figure 1

a. IC 50 values for TMZ for a series of adult and pediatric GBM cell lines at days 3 and day 7. IC50 values were measured by direct cell counts.

b-c. Z-score plots demonstrating genes whose knockdown results in decreased cell viability in the absence of TMZ (dotted line represents z-score <-1.65, p=0.05) in SJG2 (b) and KNS42 (c) cell lines. Genes with z-scores <-1.65 in all three biological replicates (black dots: Rep 1, blue dots: Rep 2, red dots: Rep 3) were removed from further analysis. siRNA targeting 240 DNA repair genes and control siRNAs are plotted along the x-axis.

d. Quantitative reverse transcriptase (qRT) PCR confirming significant knockdown (*P<0.05) of genes at the transcript level after 48h siRNA treatment compared to control.

e-h. Immunoblotting of top ranking base excision repair pathway members to confirm protein knockdown by siRNA in NHA, KNS42 and SJG2. Legend "C"=control scrambled siRNA, "+"= gene specific siRNA added.

Supplemental Figure 2. Related to Figure 1.

a. Heat map of cell viability to validate target genes in TMZ and non-TMZ conditions using SJG2, KNS42, NHAs and normal neural stem cells (NSC). TMZ dose used was 25uM and viability was assessed using almarBlue assay on day 7. *p<0.05 using ANOVA followed by a post-hoc Dunnett's test. Each heatmap box represents the average of three independent experiments.

b. Immunofluorescence of nuclear MPG in KNS42 cells and negative staining in SF188 cells. Scale bar =20um.

c. Molecular beacon assay to measure MPG activity. The MPG specific probe contains an ethnaoadenine DNA lesion in the middle of a hairpin RNA molecule. Excision of the lesion which is recognized by MPG (from total cell lysate), breaks the hairpin releasing a fluorescence quencher dye which is quantified at various time points. AU = arbitrary units.***p<0.001**.** All experiments were performed in triplicate, error bars represent standard error of the mean.

d. Pearson correlation plots of MPG (black) and MGMT (red) protein expression quantified by densitometry versus IC50 values following 3 days of TMZ in pediatric GBM cell lines from supplemental figure 1a. Correlation is seen with MPG levels (r=0.83 at 3 days) but not with MGMT levels (r=0.02). All experiments were performed in triplicate, error bars represent standard error of the mean.

e-f. Pearson correlation plots of MPG protein expression quantified by densitometry versus IC50 values for BCNU (e) or MMS (f) treatment of pediatric GBM cell lines from supplemental figure 1a showing correlation between increasing amounts of MPG expression and increasing resistance to alkylating agents (BCNU r=0.79, MMS r=0.75) by day 7. All experiments were performed in triplicate, error bars represent standard error of the mean.

g. Kaplan Meir survival curve analysis of patients staining positive for MPG or negative in the Sickkids Toronto dataset. Log rank p=0.14.

Supplemental Figure 3. Related to Figure 3.

a-b. Quantification of phospho-H2AX (pH2AX) in the presence (+) or absence (-) of TMZ (100uM) in SJG2 (a) and KNS42 (b) cells expressing control shRNA or MPG shRNA. RFU = relative fluorescence units of foci per 100 cells.

c. Immunofluorescence of phospho-H2AX (pH2AX) in KNS42 cells cultured in TMZ (100uM) expressing control or MPG shRNA. Scale bar =10uM.

d. Box and whisker plot showing quantification of comet tail assay measuring DNA damage in KNS42 (blue) and SJG2 (red) cells expressing control shRNA (shRNA con) or MPG shRNA constructs in the presence (+) or absence (-) of TMZ (100uM).
e. Immunoblots of MPG, cleaved PARP and beta-actin (B-actin) in SJG2 and KNS42 cells 48h post 100uM TMZ treatment. Densitometry analysis was performed in triplicate with one representative western blot and densitometric analysis shown.
f. Colony forming unit (CFU) assay of SF188 cells expressing empty vector (EV) or MPG cultured in vehicle, BCNU (200uM) or MMS (10uM). CFUs were scored after 14 days
g. Colony forming unit (CFU) assay of SJG2 cells expressing shRNA control or MPG shRNA cultured in vehicle, BCNU (200uM) or MMS (10uM).
All experiments were performed in triplicate, error bars represent standard error of the mean, ***$p<0.001$ compared to control cells.

Supplemental Figure 4. Related to Figure 3.
a. Immunoblot demonstrating stable MPG expression in RES259 and SF188 cells compared to empty vector (EV) controls.
b. Plot of cell counts of SF188 (red) or RES259 (blue) cells expressing empty vector (EV, solid lines) or MPG (dashed lines) in the presence of varying doses of TMZ. All experiments were performed in triplicate, error bars represent standard error of the mean, ***$p<0.001$ versus control cells at 72.
c-d. Quantification of phospho-H2AX (pH2AX) in the presence (+) or absence (-) of TMZ (100uM) in RES259 (c) or SF188 (d) cells expressing empty vector (EV) or stable MPG. All experiments were performed in triplicate, error bars represent standard error of the mean, ***$p<0.001$ versus control cells at 72h. RFU: relative fluorescence units.
e. Quantification of cleaved caspase 3/7 in SF188 (red) or RES259 (blue) cells expressing empty vector (EV) or stable MPG in the presence (+) or absence (-) of TMZ (100uM). **$p<0.001$ versus empty vector control cells at 72h.
f. Immunoblot of cleaved PARP as a measure of apoptosis in SF188 and RES259 cells expressing empty vector (EV) or stable MPG in the presence of TMZ (100uM) at 72h.
g. Box and whisker plot showing quantification of comet tail assay measuring DNA damage in RES259 (blue) and SF188 (red) cells expressing empty vector (EV) control or stable MPG in the presence (+) or absence (-) of TMZ (100uM). ***$p<0.01$ versus control cells.
h. Quantification of colony forming unit (CFU) assay of SF188 (red) and RES259 (blue) cells cultured in TMZ (100uM). CFUs were scored after 14 days. ***$p<0.01$ versus empty vector control cells.

Supplemental Figure 5. Related to Figure 3
a. qRT-PCR confirming effective knockdown of MPG, APEX1, ATM and POLB in SJG2 cells. **$p<0.01$ versus control siRNA cells.
b-d. Cell viability of SJG2 cells transfected with scrambled siRNA (control) or MPG siRNA and/or ATM siRNA (b), APEX1 siRNA (c), POLB siRNA (d) following exposure to varying amounts of TMZ for 7 days versus control cells. *$p<0.05$, **$p<0.01$, ***$p<0.001$. All experiments were performed in triplicate, error bars represent standard error of the mean. Dual siRNA knockdowns were also compared to single knockdowns to evaluate additive effects of double knockdown, *$p<0.05$.
e. Immunoblotting of MGMT and MPG in UW479 and RES186 pGBM cells following gene specific pooled siRNA treatments.
f-g. Cell viability of RES186 (f) and UW479 (g) cells transfected with scrambled siRNA (control) or MPG siRNA and/or MGMT siRNA following exposure to varying amounts of TMZ for 7 days versus control cells. *$p<0.05$, **$p<0.01$, ***$p<0.001$. All experiments were performed in triplicate.

Supplemental Figure 6. Related to Figure 4.
a. Chemiluminescence densitometric analysis of phospho-ATM (pATM) and phospho-ATR (pATR) normalized to total ATM and ATR in the presence (+) or absence (-) of TMZ in various cell lines. All experiments were performed in triplicate, error bars represent standard error of the mean,**$p<0.01$ versus treated compared to TMZ treated cells (100uM) at 72h.
b. Immunoblots of co-immunoprecipitations of FLAG-ATM and MYC-MPG constructs in T98G and SJG2 cells.
c. Immunoblots following denaturing immunoprecipitation of FLAG-MPG immunoblotted for FLAG-MPG, pSQ in presence (+) or absence (-) of ATM inhibitor (ATM inh, ku55933 used at 5uM). W.C.L=whole cell lysate, EV: empty vector
d. Denaturing Immunoprecipitation of MPG in normal human astrocytes under normal, TMZ (100uM for 72h) and radiation treated (RT) conditions. W.C.L=whole cell lysate.
e. Immunoblots of pGBM cell lines 462 and 477 were treated with 5uM ku55933. pATM is significantly reduced Upper blots). Lower blots are immunoprecipitation of MPG followed by pSQ blot to show reduction of phospho MPG.
f. Lysates were collected from S6e and use to perform a MPG glycosylase activity assay. ATM inhibitor treated lysates had a significant reduction in MPG activity compared to control vehicle treated cells.

Supplemental Figure 7. Related to Figure 6.
a. Histologic and immunohistochemical characterisation of xenografts generated by injection of SJG2 cells expressing scrambled shRNA (shRNA con), ATM shRNA, MPG shRNA or dual ATM/MPG shRNA. The tumors were morphologically similar but showed reduced Ki67 staining with ATM, MPG or dual shRNA expression. ATM and MPG immunohistochemistry demonstrates loss of the corresponding protein in the presence of the respective shRNA (MPG, ATM or both). H&E: hematoxylin and eosin.

b. Plot of cell counts pediatric (SJG2) GBM cells cultured in vehicle (DMSO), TMZ(100uM), methoxyamine (MA,1mM) or both from 1-7 days.
c. Histogram showing quantification of percent cell death for pediatric (SJG2) GBM cell lines transfected with scrambles siRNA (control), ATM siRNA, MPG siRNA or both and treated with vehicle (black), TMZ (green), methoxyamine (MA, blue) or both (red).
d. Immunoblot of MPG, ATM or phospho-ATM (pATM) on protein extracted from normal neural stem cells (NSC), adult GBM stem cells (G179) and pediatric GBM stem cells (G462 and G477).
e. Cell count proliferation assay of adult GBM glioma stem cell line G179 with vehicle, methoxyamine (MA), TMZ or dual treatments.

Supplemental Figure 9. Related to Figure 6.
a-b. Cell count proliferation assay of a pediatric pGBM (pGBM 462) primary culture treated with control, MPG, ATM or dual siRNA over 5 days in the absence (a) or presence of TMZ (b).
c. Cell count proliferation assay of a primary pGBM culture (pGBM 462) treated with vehicle, methoxyamine (MA), TMZ or both.
d-e. Cell count proliferation assay of normal neural fetal stem cells treated with control, MPG, ATM or dual siRNA over 5 days in the absence (d) or presence of TMZ (e).
f. Cell count proliferation assay normal neural stem cells treated with vehicle, methoxyamine (MA), TMZ or both.
All experiments were performed in triplicate, error bars represent standard error of the mean,*$p<0.05$ versus vehicle treated control cells.

Supplemental Figure 9. Related to Figure 7.

a. Quantitation of cleaved caspase 3/7 in pediatric GBM stem cell lines (G179 and G462) and normal neural stem cells (NSC) treated with vehicle (black), TMZ (green), methoxyamine (MA, blue) or both (red) for 5 days. All experiments were performed in triplicate, error bars represent standard error of the mean,*$p<0.05$ versus vehicle treated control cells.

b. Normal human astrocytes treated with vehicle, methoxyamine (MA), temozolomide (TMZ) or dual treatment.

c. Normal human astrocytes treated with vehicle or dual treatment (MA + TMZ) and transfected with siRNA control, or DNA repair genes redundant to ATM and MPG. All experiments were performed in triplicate, error bars represent standard error of the mean,*$p<0.05$ versus vehicle treated control cell.