Supplementary data

KDM6B promotes PARthanatos via suppression of O^6-methylguanine DNA methyltransferase repair and sustained checkpoint response

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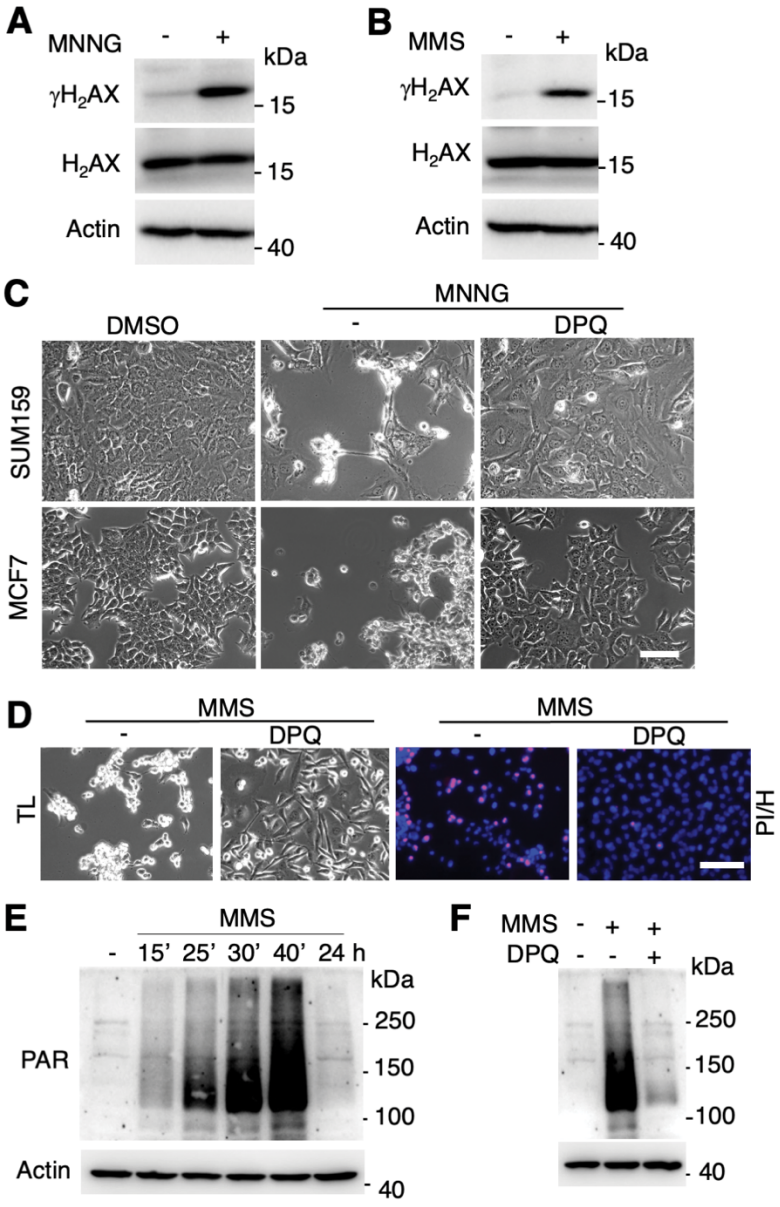
Supplementary Table 1.
Sequences of sgRNAs, siRNAs, and primers for plasmid construction.

KDM6Bg1	Forward: 5'-CACCGGGCGCCCCTGGAGCATAATA-3'
	Reverse: 5'-AAACTATTATGCTCCAGGGGCGCCC-3'
KDM6Bg2	Forward: 5'-CACCGCATTTCAGCTAACCAAGCCA-3'
	Reverse: 5'-AAACTGGCTTGGTTAGCTGAAATGC-3'
KDM6Bg3	Forward: 5'-CACCGTACCACAGCGCCCTTCGATA-3'
	Reverse: 5'-AAACTATCGAAGGGCGCTGTGGTAC-3'
MGMT-g2	Forward: 5'-CACCGTATCGAAGAGTTCCCCGTGC-3'
	Reverse: 5'-AAACGCACGGGGAACTCTTCGATAC-3'
PARP-1 siRNA pool (Dharmacon)	GAUUUCAUCUGGUGUGAUA
	GAUUUCAUCUGGUGUGAUA
	GAUUUCAUCUGGUGUGAUA
	GAUUUCAUCUGGUGUGAUA
PARP1-g1	Forward: 5'-CACCGCGAGTCGAGTACGCCAAGAG-3'
	Reverse: 5'-AAACCTCTTGGCGTACTCGACTCGC-3'
PARP1-g2	Forward: 5'- CACCGAAGTACGTGCAAGGGGTGTA-3'
	Reverse: 5'- AAACTACACCCCTTGCACGTACTTC-3'
XRCC1-g2	Forward: 5'-CACCGCTATGCAGCTGCTACCCTCC-3'
	Reverse: 5'-AAAC GGAGGGTAGCAGCTGCATAGC-3'
XRCC1-g3	Forward: 5'-CACCGCCCACTCCTTACGCACGATG-3'
	Reverse: 5'-AAAC CATCGTGCGTAAGGAGTGGGC-3'
MGMT (full length)	Forward: 5'-CCGGATGGATCCGCCACCATGCTGGGACAGCCCGCG-3'
	Reverse: 5'-ACATACGAATTCTCAGTTTCGGCCAGCAGGCGGGG-3'
MGMT (mutation)	Forward: 5'-CATCCCGGCCCACAGAGTGGTCTGCAGCAGCG-3'
	Reverse: 5'-ACTCTGTGGGCCGGGATGAGGATGGGGACAGGATTG-3'
KDM6B (full length)	Forward: 5'-GCTCTAGAACTAGTGGCCACCATGCATCGGGCAGTGGATCCT-3'
	Reverse: 5'-GATAAGCTTGATATCTCGCGACGTGCTGGCTGGGGCCTGG-3'
KDM6B-C	Forward: 5'-GTGAATTCCCCAAAAAGAAGCGGAAAGTGCGCGCCA GCAGGAATGCCAAG-3'
	Reverse: 5'-AACATGTTCAGCGGCCGCTCATCGCGACGTGCTGGCTG-3’
XRCC1	Forward: 5'-GGGTTTCATATGATGCCGGAGATCCGCC-3’
	Reverse: 5'-GGCCGGAATTCTCAGGCTTGCGGCACC-3’

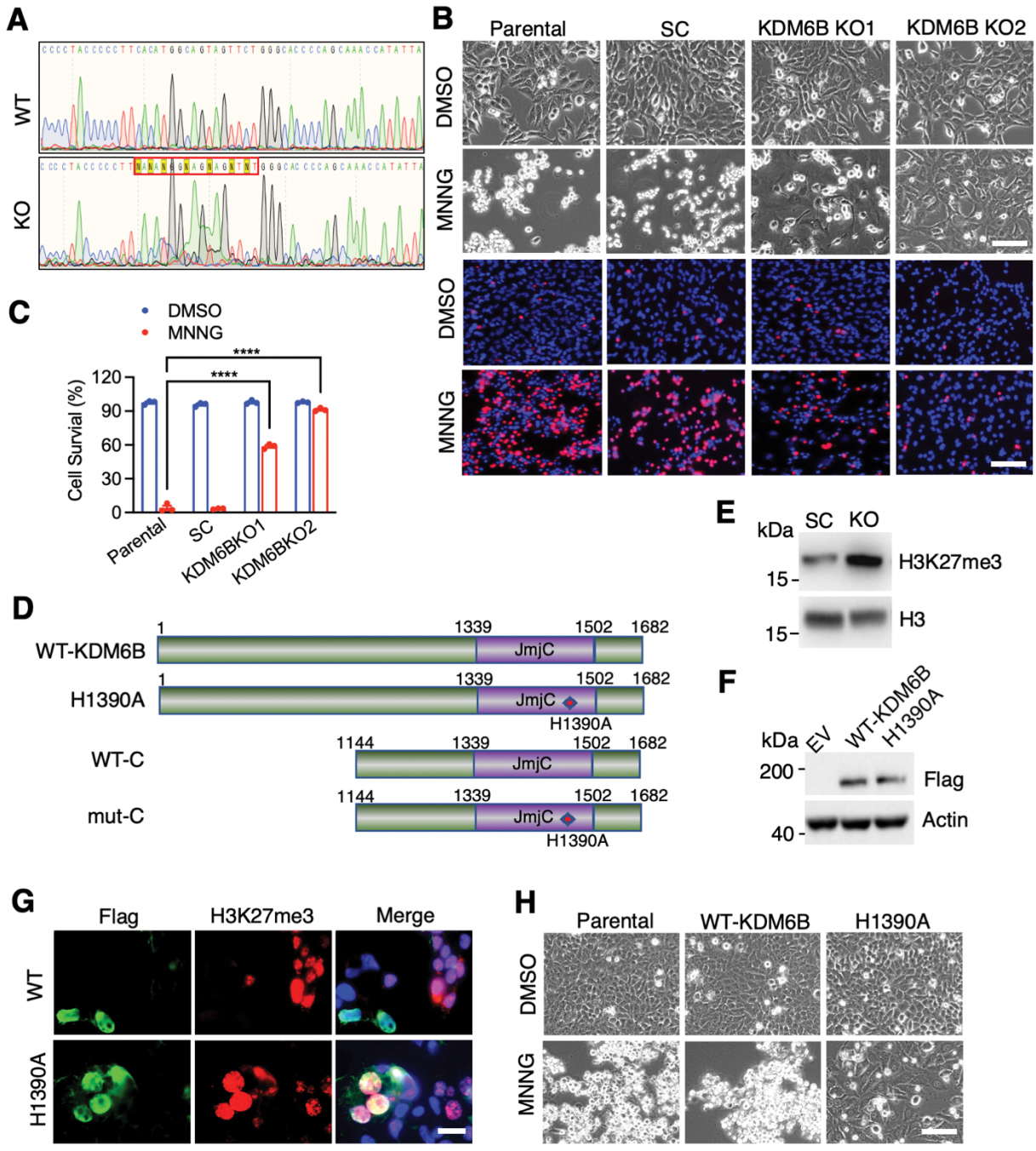
Supplementary Table 2. Sequences of qPCR primers

Name	Sequence
MGMT	Forward: 5'-AATGAGAGGCAATCCTGTCCC-3'
	Reverse: 5'-AGTCCTCCGGAGTAGTTGCC-3'
M-MGMT	Forward: 5'-TTTCGACGTTCGTAGGTTTTCGC-3'
	Reverse: 5'-GCACTCTTCCGAAAACGAAACG-3'
U-MGMT	Forward: 5'-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3'
	Reverse: 5'-AACTCCACACTCTTCCAAAAACAAAACA-3'
ACTIN	Forward: 5'-GAGCACAGAGCCTCGCCTTT-3'
	Reverse: 5'-TCATCATCCATGGTGAGCTGG-3'
ACTG (for genomic DNA)	Forward: 5'-TGGTGATGGAGGAGGCTCAGCAAGT-3'
	Reverse: 5'-AGCCAATGGGACCTGCTCCTCCCTTGA-3'
ACTB (for DNA methylation PCR)	Forward: 5'-TGGTGATGGAGGAGGTTTAGTAAGT-3'
	Reverse: 5'-AACCAATAAAACCTACTCCTCCCTTAA-3'
JMJD3-C	Forward: 5'-CACGGTCAAAATCAGCGACC-3'
	Reverse: 5'-AAACACCTCCACATCGCACT-3'
JMJD3	Forward: 5'-CACCCACTGTGGTCTGTTGT-3'
	Reverse: 5'-CGCCTCAGTAACAGCCAGAT-3'
ALKBH1	Forward: 5'-ACTCCACACTGGGAATCCAC-3'
	Reverse: 5'-TCCAAAGCTGAATGACAGCAAG-3'
ALKBH2	Forward: 5'-AAGGGGGCCTTTTGAGGAAG-3'
	Reverse: 5'-TGGCCAGTGCTCCTGTAAAAT-3'
ALKBH3	Forward: 5'-GCCACGAGTGATTGACAGAG-3'
	Reverse: 5'-TTCACGTCAACAAAGCCAGG-3'
ALKBH4	Forward: 5'-CCCAGCGAAAACATACCGTT-3'
	Reverse: 5'-CGGGTCACAAAGTCCTCGAT-3'
ALKBH5	Forward: 5'-GTGGACCCCATCCACATCTT-3'
	Reverse: 5'-ATCAGCAGCATATCCACTGAGC-3'
ALKBH6	Forward: 5'-TGGACGGATTGGGTGCAAG-3'
	Reverse: 5'-GATTACAGGTGGTGCCTGCT-3'
ALKBH7	Forward: 5'-GGCTGGAACTCTTGCTGGAG-3'
	Reverse: 5'-TCCCGAAGGATCTCATGGGAG-3'

ALKBH8	Forward: 5'-TGCAACAGCAGAGCGTAGAG-3'
	Reverse: 5'-AAATGAGTGCCTTCCCACCT-3'
ALKBH9	Forward: 5'-CTGTGAAGGCCCTGAAGAGG-3'
	Reverse: 5'-AAGGGGTATCGCCAAACCAG-3'
MPG	Forward: 5'-CGTCCATCGTCAGACGTGAT-3'
	Reverse: 5'-GTCGGCAAAACTGCTTTGGT-3'
APE1	Forward:5’-ACGGCATAGGCGATGAGGAG-3’
	Reverse:5’-TGCGAAAGGCTTCATCCCAG-3’

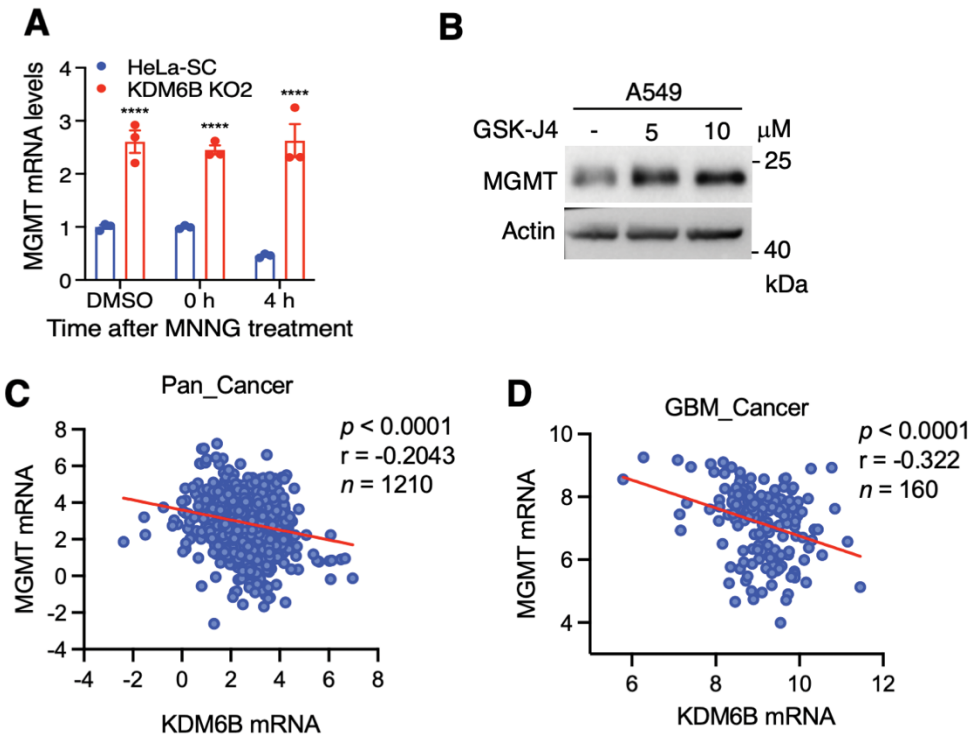


Supplementary Figure 1. PARP-1 is required for alkylating agent-induced cell death in different types of cancer cells. (**A** and **B**) Immunoblot analysis of DNA damage in HeLa cells 6 h after the treatment with vehicle (-), MNNG (50 μM, 15 min), or MMS (2 mM, 6 h). (**C**) Representative cell death images from SUM159 and MCF-7 cells treated with DMSO, MNNG (200 μM for SUM159 and 500 μM for MCF7), MNNG plus DPQ (30 μM) for 24 h. Scale bar, 20 μm. (**D**) Representative cell death images in MDA-MB-231 cells treated with MMS and/or DPQ for 24 h. Scale bar, 20 μm. TL, transmission light. PI/H, propidium iodide/Hoechst staining. (**E**) Immunoblot analysis of PARP-1 activation in MDA-MB-231 cells treated with vehicle (-) or MMS (2 mM) for indicated time. (**F**) Immunoblot analysis of PARP-1 activation in MDA-MB-231 cells treated with vehicle (-) or MMS for 1 h in the presence or absence of DPQ.

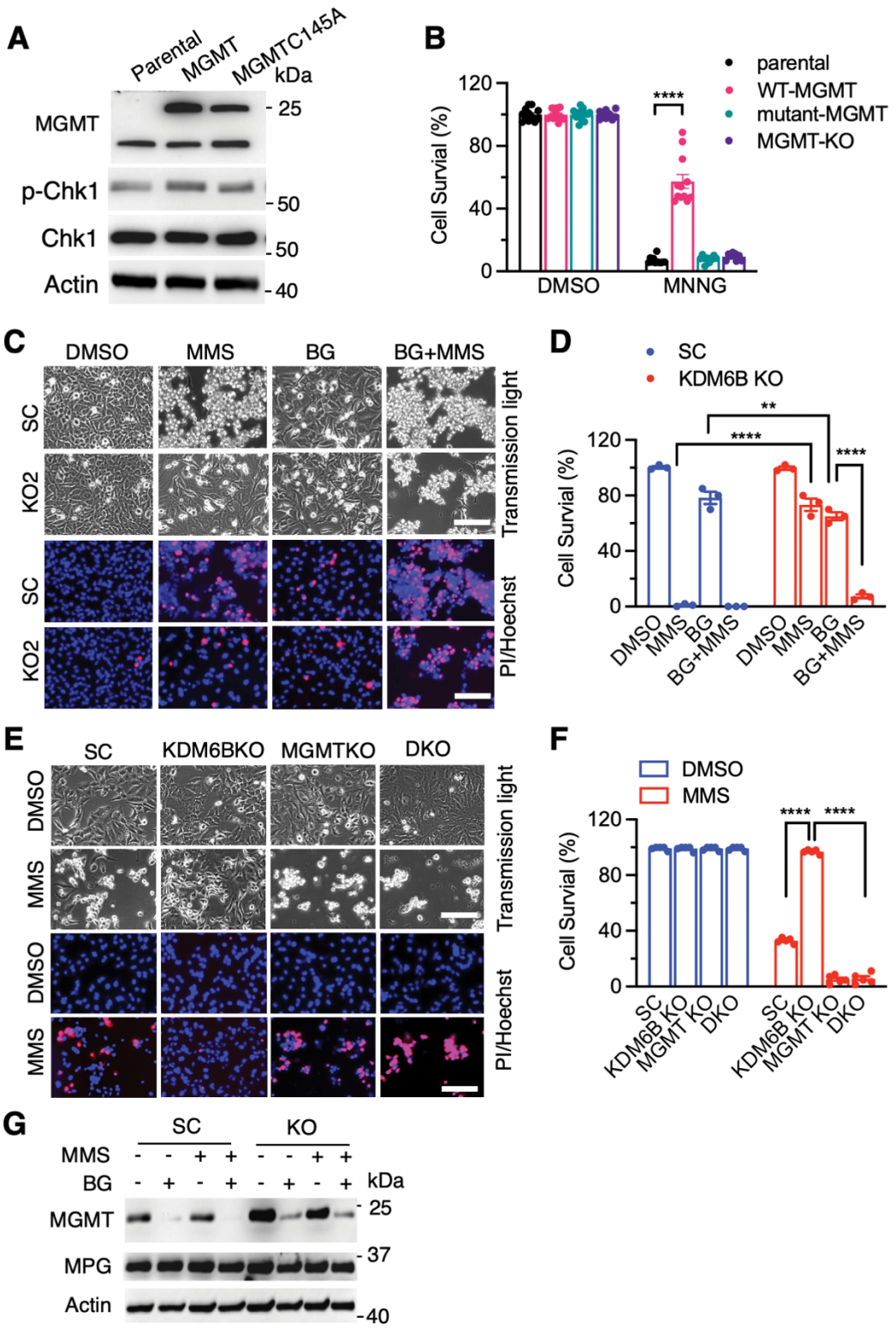


Supplementary Figure 2. Loss of KDM6B or its H3K27 demethylase activity blocks PARthanatos induced by alkylating agent MNNG. (**A**) Genotyping of KDM6B KO1 HeLa cells. A 17-nt deletion/shift was found in one allele of *KDM6B* gene. (**B** and **C**) Representative images and quantification of MNNG (50 μM, 15 min)-induced cell death in WT and KDM6B KO cells, which was established by sgRNAs targeting to different regions of *KDM6B* (mean ± SEM, $n = 3$). ****$p < 0.0001$ by two-way ANOVA Sidak's multiple comparisons test. Scale bar, 20 μm. (**D**) Schematic diagrams of human full-length and C-terminal KDM6B and their demethylase inactive H1390A mutant variants. Numbers in the diagrams correspond to the positions of amino acid

residues. JmjC, jumonji C domain; WT, wild type; mut, mutant; C, C-terminal. (**E**) Expression of H3K27me3 in SC and KDM6B KO cells. (**F**) Expression of full-length Flag-tagged WT-KDM6B and demethylase inactive KDM6B H1390A mutant in HeLa cells. (**G**) Expression of H3K27me3 (red) and Flag-tagged WT and H1390A KDM6B (green) in HeLa cells overexpressed WT or H1390A mutant KDM6B. Blue indicates DAPI staining. Scale bar, 20 μm. (**H**) PARthanatos induced by MNNG in HeLa cells overexpressing WT or H1390A mutant KDM6B. Scale bar, 20 μm.

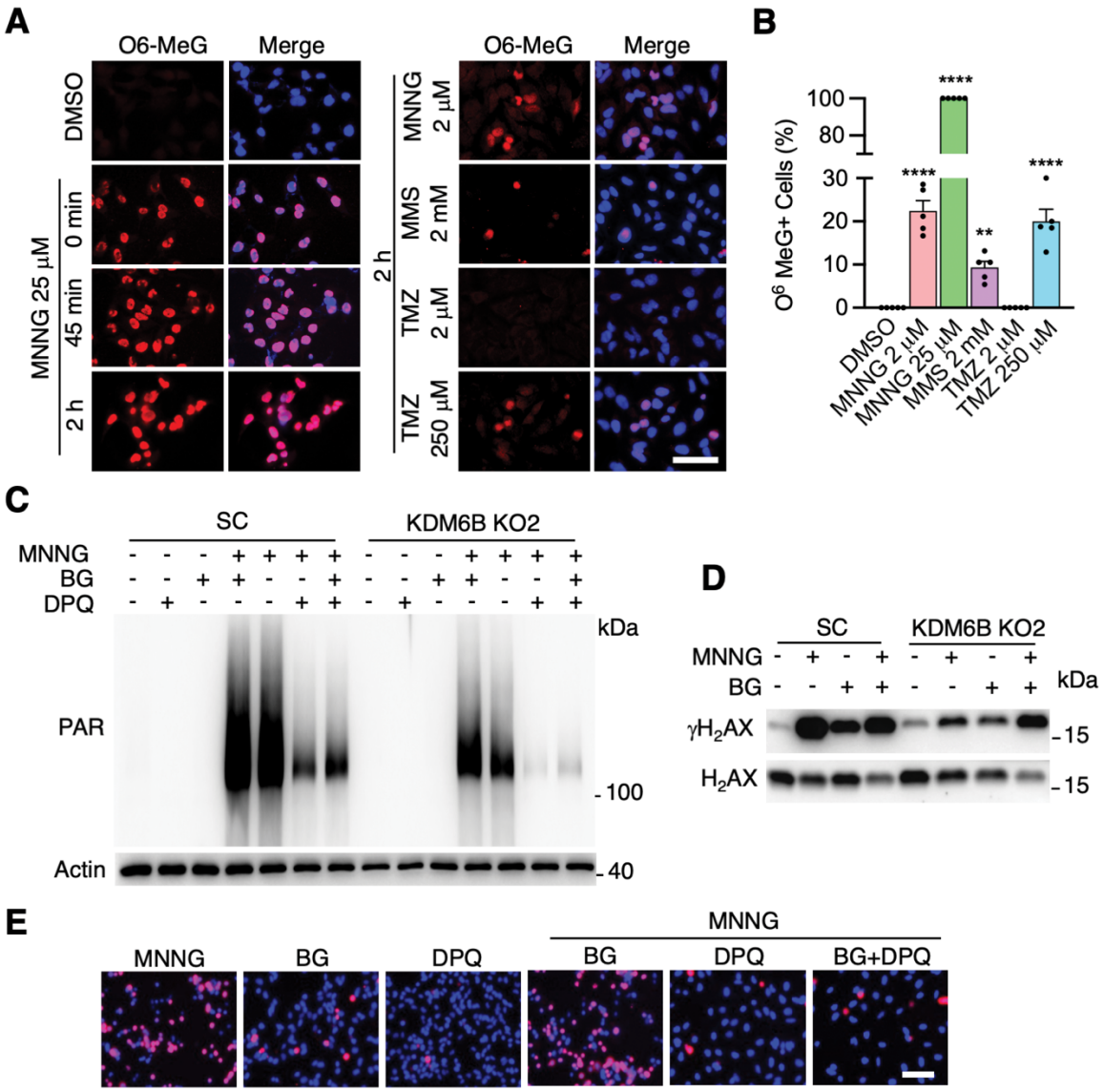


Supplementary Figure 3. KDM6B negatively regulates MGMT expression. (**A**) RT-qPCR analysis of MGMT mRNA in SC and KDM6B KO2 HeLa cells treated with DMSO or MNNG for 15 min, followed by recovery for 0 and 4 h (mean ± SEM, $n = 3$). ****$p < 0.0001$ by two-way ANOVA Sidak's multiple comparisons test. (**B**) Immunoblot analysis of MGMT in A549 cells treated with or without GSK-J4 for 24 h. (**C** and **D**) A negative correlation of KDM6B with MGMT mRNA expression in 1210 human pan tumors (**C**) and 160 GBM tumors (**D**). Data were retrieved from the TCGA cohort at cBioPortal. "r" represents Pearson correlation coefficient of MGMT vs KDM6B expression. -0.4 < r <-0.2 is considered as a moderate negative correlation.

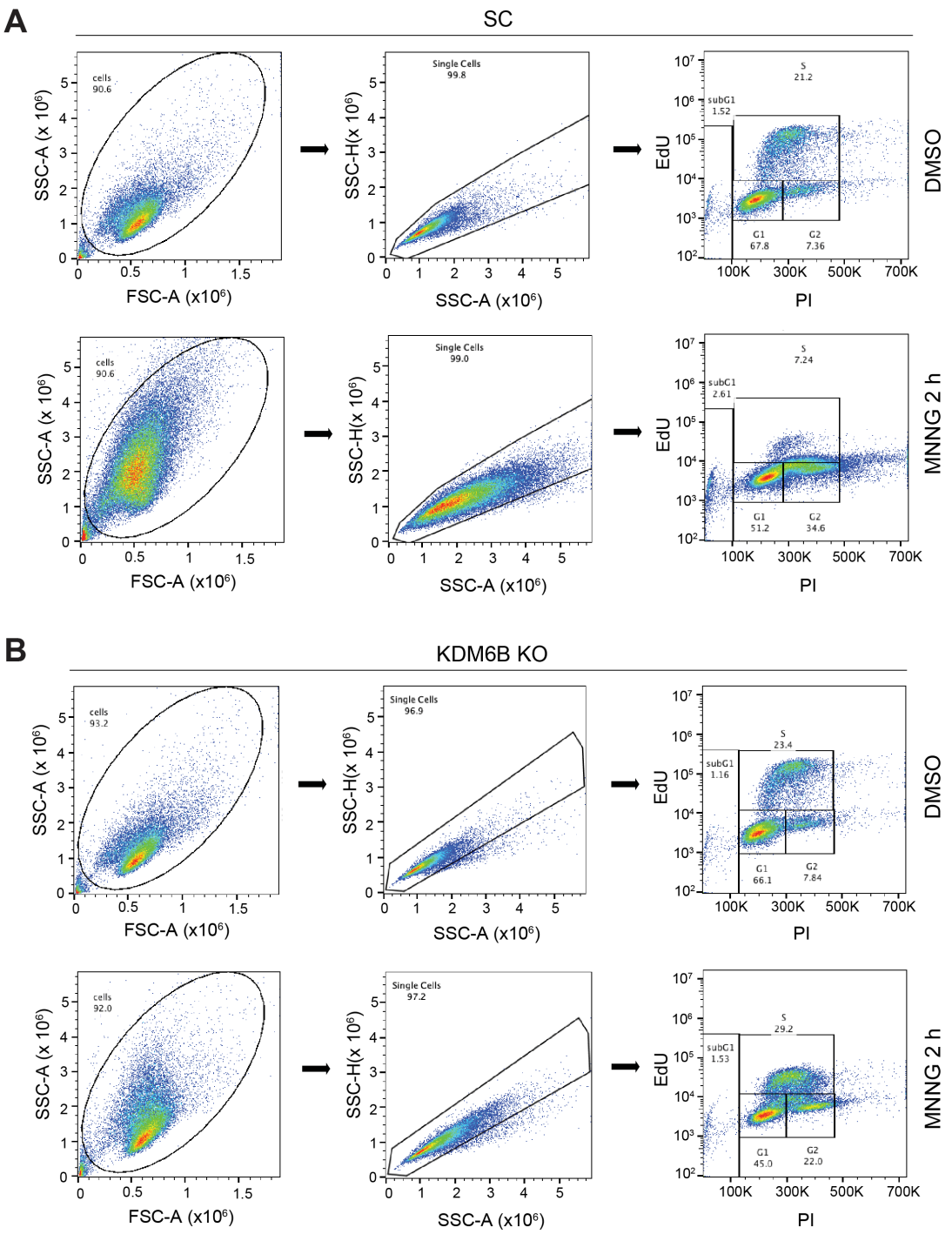


Supplementary Figure 4. MGMT inhibition overcomes alkylating agent resistance in KDM6B KO cells. (**A**) Expression of MGMT and its methyltransferase inactive mutant C145A MGMT in HeLa cells. (**B**) Effects of MGMT, mutant C145A MGMT and MGMT KO on MNNG

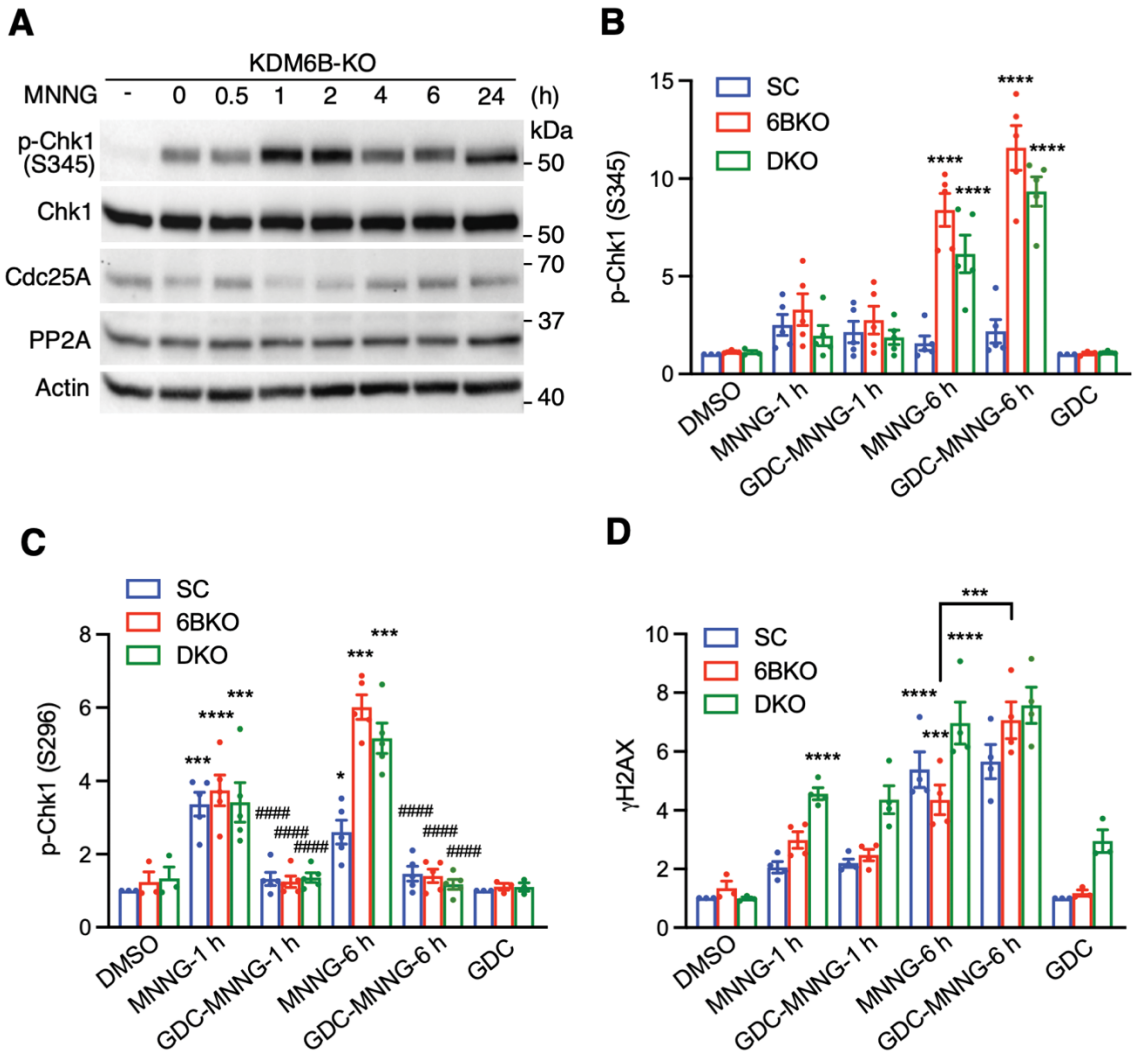
(50 μM, 15 min)-induced cell death (mean ± SEM, n = 9-12). ****p < 0.0001 by two-way ANOVA Tukey's multiple comparisons test. (**C** and **D**) Representative cell death images and quantification of cell death in SC and KDM6B KO2 HeLa cells 24 h after the treatment with MMS (2 mM, 1 h) and/or BG (200 μM) (mean ± SEM, n = 3). **p <0.01, ****p < 0.0001 by two-way ANOVA Sidak's multiple comparisons test. (**E** and **F**) Representative cell death images (E) and quantification of cell death (F) in SC, KDM6B KO2, MGMT KO and KDM6B/MGMT DKO HeLa cells 24 h after the treatment with MMS (2 mM, 1 h) (mean ± SEM, n = 5). ****p < 0.0001 by two-way ANOVA Sidak's multiple comparisons test. Scale bar, 20 μm. (**G**) Protein expression of MGMT and MPG 6 h after the treatment of MMS (2 mM, 1 h) and BG (200 μM).



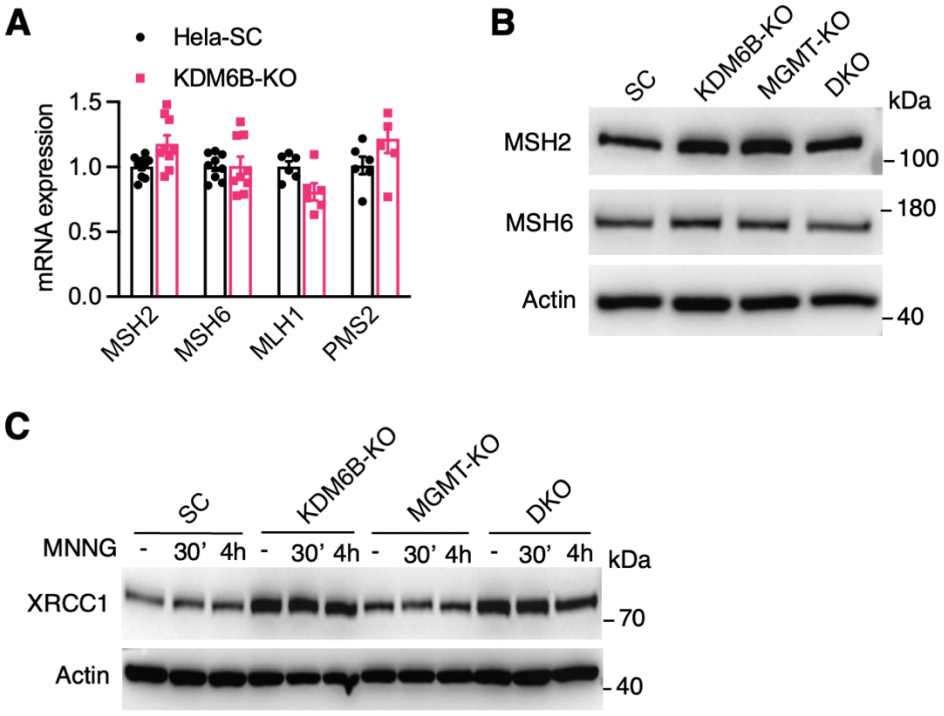
Supplementary Figure 5. Effects of alkylating agents on O^6 MeG formation and effects of PARP inhibition on alkylating agent-induced PAR formation and cell death in the presence or absence of MGMT inhibitor BG. (**A**) Representative images of O^6 MeG staining in SC cells at 0 min, 45 min and 2 h post treatment of different alkylating agents at the indicated concentrations. (**B**) O^6 MeG+ cell quantification in SC cells at 2 h post treatment of different alkylating agents at the indicated concentrations. **p < 0.01, ****p < 0.0001 by one-way ANOVA Dunnett's multiple comparisons test. (**C**) Immunoblot analysis of PARP-1 activation in SC and KDM6B KO2 HeLa cells treated with MNNG (50 μM), BG (200 μM, 24 h pre-treatment), and/or DPQ (30 μM, 30 min pre-treatment) for 15 min. (**D**) Immunoblot analysis of DNA damage in SC and KDM6B KO2 HeLa cells 6 h after MNNG in the presence or absence of 24 h BG pretreatment. (**E**) Representative cell death images in HeLa cells 24 h after treatment of MNNG, BG, and/or DPQ. Scale bar, 20 μm.



Supplementary Figure 6. Flow cytometry gating strategy for HeLa cell cycle analysis. (**A** and **B**) All cells were labeled with 10 μM EdU at 37°C for 2 hours right before fixation and further conjugated to Alexa Fluor 488 azide. First, cell debris was excluded from living cells in the forward scatter (FCS-A) vs side scatter (SSC-A) plot. Then single cells were selected within the population in the SSC-A vs SSC-H plot and applied to Propidium Iodide (PI) vs EdU histogram plot to identify G1, S and G2/M phases. Both SC (A) and KDM6B KO (B) cells at 2 h post treatment of DMSO or MNNG (50 μM, 15 min) were shown as examples.



Supplementary Figure 7. KDM6B KO promotes sustained Chk1 activation following alkylating agent treatment. (**A**) Immunoblot analysis of checkpoint response in KDM6B KO2 HeLa cells 0-24 h after MNNG (50 μM, 15 min) treatment. (**B-D**) Quantification of checkpoint response and DNA damage in SC, KDM6B KO2 (6BKO), and KDM6B/MGMT DKO HeLa cells 1 h and 6 h after MNNG treatment with or without checkpoint inhibitor GDC0575 (50 nM). ***p < 0.001, ****p < 0.0001 vs its own DMSO control or two groups as indicated, ####p < 0.0001 vs its own MNNG treatment without checkpoint inhibitor GDC0575 at the same timepoint, by one-way ANOVA Sidak's multiple comparisons test.



Supplementary Figure 8. Effects of KDM6B KO on expression of DNA repair proteins involved in the MMR and BER. (**A**) mRNA expression of MSH2, MSH6, MLH1 and PMS2 in SC and KDM6B KO HeLa cells. (**B**) Protein expression of MSH2 and MSH6 in SC and KDM6B KO HeLa cells. (**C**) Protein expression of XRCC1 in SC, KDM6B KO, MGMT KO and KDM6B/MGMT DKO HeLa cells.