# Supplementary Information for

## A DNA repair-independent role for alkyladenine DNA glycosylase in alkylation-induced unfolded protein response

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**This PDF file includes:**

Supplementary text
Tables S1 to S2
Figs. S1 to S11

**Other supplementary materials for this manuscript include the following:**

Dataset 1 – Detailed gene expression data
Dataset 2 – Gene enrichment analysis

**Supplementary Information Text**

## Material and Methods

**Animal Experiments.** Wild type and *Aag* null 6-8 week old male mice were used (n=3 for microarray experiments). Mice were fed standard diet *ad libitum* and housed in accredited facilities. Euthanasia was by $CO_2$ asphyxiation.

**MMS treatment of mice.** MMS was dissolved in 10% ethanol in phosphate-buffered saline (PBS). Animals were injected intraperitoneally (i.p.) with solvent or a single dose of 75 mg MMS per kg body weight, a dose known to be sub-lethal to both *Aag*-null and wild-type animals (1). Whole liver (mixtures of all 5 different liver lobes) was collected 6 h after treatment, cut into pieces, flash frozen in liquid nitrogen and stored at -80ºC until further processing.

**Microarray processing and data analysis.** Raw data background correction (RMA method), normalization (qspline method), probe specific correction, and summary value computation were performed using the affy::expresso function in R (2). Differential gene expression was studied using limma (3). P values were corrected for multiple hypothesis testing using the false-discovery rate (FDR) method. Lists of differentially expressed genes were analyzed for enrichment (hypergeometric test) with prior knowledge categories using gene-set libraries from the enrichr website (4, 5). Microarray data have been deposited on GEO (GSE115254). R scripts and additional data files used in this study are available at https://github.com/nohturfft/Milano_et_al_2022.

**Cell culture and cell line construction.** Medium A refers to DMEM low glucose containing 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. Cells were grown in medium A at 37°C in 5% CO2. Lentivirus expressing AAG shRNAs were produced as described previously (6), followed by infection of T98G cells and selection in medium A plus 1 µg/mL puromycin. A172/hAAG cells, stably expressing EGFP-hAAG, were generated by Lipofectamine LTX (Invitrogen) mediated transfection of A172 cells with pEGFP-hAAG, followed by selection in medium A plus 600 µg/ml G418. Puromycin resistant *AAG* knockdown shAAG3 cells were complemented by transfection with the pCAGGS empty vector or the indicated construct, as well as the empty pEGFP-C3/N1 for G418 resistance, using Lipofectamine 2000 (Invitrogen), at a ratio of 10:1 (10 parts gene expression plasmid: 1 part antibiotic selection marker plasmid). Post-transfection, cells were selected with 1000 µg/ml G418. Selected cells were cultured in the presence of 0.5 µg/mL puromycin and 400 µg/ml G418 for maintenance.

For complementation with human *AAG* cDNA, puromycin resistant *AAG* knockdown shAAG3 cells were transfected with knockdown resistant wildtype and glycosylase-defective *AAG* cDNA (in pEGFP-N1).

**Cell treatment and survival determination.** Clonogenic survival assays were performed as described (7). Briefly, cells were plated and treated with MMS (0.5-2mM) for 1 h or temozolomide (10 to 75µM) for 5 days. After 14 days, colonies were fixed with

methanol and stained with 0.1% crystal violet. Colonies containing >50 cells were scored and the percent survival calculated relative to untreated control. For viability, cells were pre-treated with salinomycin (0.1 μM) for 24 hours and then temozolomide treated in the presence of salinomycin for 5 days. Cell viability was determined after treatment using the MTS-based CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, #G3580).

**MMS sensitivity assay for complemented cells**
Sensitivity assay for complemented *AAG* knockdown cells and controls was performed as described(8). Briefly, cells were seeded in triplicate in a clear bottom black 96-well microplate at a density of 5000 cells per well. MMS treatment was for 1h in serum-free media at concentrations ranging from 0 (DMSO) to 0.25 mM. Post-treatment, media was replaced by complete media and cells were incubated for 24h. Images of entire wells were acquired at 4 x magnification with a Cytation 5 Cell Imaging Multi-Mode Reader followed by quantification of Hoechst-stained nuclei with the Gen5 Data Analysis Software v3.03 (BioTek Instruments). Cell viability was expressed as percentage of survival in MMS-treated cells relative to vehicle (DMSO)-treated cells. Results represent the mean ± SEM of at least 3 independent experiments.

**AAG Activity assay**. Briefly, nuclear extracts were prepared from cells for AAG activity analysis using a NE-PER™ Nuclear and Cytoplasmic Extraction Reagents kit and the concentration of protein in the extracts determined using the MicroBCA kit according to the manufacturer's instructions (ThermoFisher Scientific, Loughborough, UK). Synthetic oligonucleotides HX02 (sequence 5' Phos-CACGAITCAACTCAGCAACTCC*T*T-$NH_2$ 3', where Phos indicates a phosphate modification, I indicates an internal inosine, * indicates a phosphorothioate linkage between nucleotides and $NH_2$ indicates an amino group modification) and Loop01Aird (5' IRDye-T*T*GGAGTTGCTGAGTTGATTCGTGAGCACCAACCGGTGCT 3', where IRDye indicates a IRDye® 800CW modification) were synthesized and HPLC purified by Integrated DNA Technologies (Leuven, Belgium). The plate based glycosylase assays were done as previously published (9). For assessing glycosylase activity of knockdown cells complemented with wildtype or mutant mouse *Aag*, a gel-based assay was used. Briefly, 3 μM HX02 was annealed to 3 μM Loop01AIRD in a buffer containing 30 mM Tris HCl and 10 mM $MgCl_2$ with total reaction volume 40 μl. Annealing was done by using a thermal cycler programmed as 95°C for 10 min; cool to 80°C at 1°C/min; 80°C for 10 min; cool to 70°C at 1°C/min; 70°C for 10 min; cool to 21°C at 1°C /min. After annealing, 10 mM DTT, 1 mM ATP, and 6 U T4 DNA ligase (Promega, Southampton, UK) were added to the reaction mixture and incubated for 1 h at 30°C, and then overnight at 4°C. The ligated oligo (substrate) was ethanol precipitated using 0.3 M sodium acetate and 1 μg/μl glycogen (Ambion), washed in 70% ethanol and resuspended in ultrapure water (40 μl). For the glycosylase assay reactions, the oligo substrate (3 pmol) was incubated at 37°C for times from 30 minutes to 2 h (in 30 min increments) with either recombinant hAAG (New England Biolabs, Hitchin, UK) or with nuclear extracts diluted in PIPES buffer (50mM PIPES (pH 6.7), 30mM KCl, 2mM EDTA, 3.4% v/v glycerol) in a 10 μl reaction. Following the repair incubation, the substrate was denatured by incubation with 0.1M NaOH for 20 min at 70°C, an equal volume of 2 X formamide loading buffer was added (95% formamide, 5 mM EDTA, 0.1% OrangeG) and samples

incubated at 65°C for 5 min before gel electrophoresis. Reaction products were resolved in a 15% polyacrylamide/7M urea gel electrophoresed in TBE buffer. Gels were imaged using the Odyssey CLx IR imaging system (LI-COR Biosciences, Lincoln, USA), and image acquisition and signal quantification were done using Image Studio software (LI-COR Biosciences, Lincoln, USA).

**XBP1 Splicing** The detection of XBP1u (unspliced) and XBP1s (spliced) transcripts was performed as described in (10), except that Go Taq Green (Promega) was used, and PCR products were resolved on a 2.5% agarose gel.

**mRNA Expression Analysis** RNA was extracted using the PureLink RNA Mini kit (Invitrogen, Carlsbad, USA) and first strand cDNA was synthesized using the Maxima First Strand cDNA Synthesis (Thermo Fisher Scientific, Waltham, USA), according to the manufacturer's instructions. RNA amplification was done using SYBR Green Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Waltham, USA) and quantitative real-time PCR was performed using QuantStudio 7 Flex Real-Time PCR System (Life Technologies, Carlsbad, USA). All experiments were performed with biological and technical triplicates. Results were generated using the comparative Ct method and are expressed as fold change relative to the untreated control for each cell line (11). A complete list of primers used in this study is shown in Table S1.

**Immunoblotting** Cells were lysed with M-PER Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, USA), supplemented with 1X Phosphatase inhibitor (Sigma-Aldrich, St. Louis, USA) and 1x protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, USA). Protein concentration was determined using the BCA assay (Pierce). Total protein lysates (20 μg) were separated under denaturing conditions in 4-20% True-PAGE (Sigma-Aldrich) gels. Proteins were then transferred onto PVDF membranes (Life Technologies). Membranes were blocked in 1% nonfat milk and incubated overnight with specific antibodies at 4 °C. Antibodies were as follows: β-actin (1:10,000 dilution; ab52614, Abcam), AAG (1:1,000 dilution; Abcam, ab196553), BiP (1:1,000 dilution; 3183S, Cell Signaling). After primary antibody incubation, membranes were washed and then incubated with the secondary antibodies IRDye 680RD green goat anti-rabbit IgG and IRDye 800CW red goat anti mouse IgM (LI-COR Biosciences, Lincoln, USA) at 1:10,000, for 1 hour at room temperature. Proteins were detected using the Odyssey CLx IR imaging system (LI-COR Biosciences, Lincoln, USA). In some experiments, horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch) were used at 1:10,000 in PBS-T for 1 h at room temperature, followed by detection using the Western Lighting Chemiluminescence Reagent Plus (Perkin Elmer). Image files of immunoblots were opened in Adobe Photoshop CS6 and cropped to display the relevant lanes. Image mode was set to grayscale. Gray levels were then adjusted by using the Auto Levels command with black and white clip both set to 0%.

**PAR detection** Cells (1 x 10^6 cells/well) were seeded in 6-well plates and treated for 1h with MMS (1 mM) in serum-free media. After treatment, media was replaced with complete media containing 1 μM PDDX004 (N02214) (PARG inhibitor) for 1h (in Fig. S8). Experiment was also done in the absence of PARG inhibitor (Fig. 3F and G). Cell

lysates were obtained using Laemmli buffer (3 X: 150 mM Tris-HCl pH 6.8, 6% SDS, 0.3 % Bromophenol Blue, 30% Glycerol). Proteins were separated by SDS–polyacrylamide gel electrophoresis using pre-cast gels 4-12% (Invitrogen) and transferred to Nitrocellulose membrane. PAR detection in Fig. 3F was performed using a rabbit anti-poly(ADP-ribose) LP-9610 in a 1:5,000 dilution (12). PAR detection in Fig. S8 used Poly/Mono-ADP Ribose (E6F6A) Rabbit mAb (1:40,000, Cell Signalling, 83732). Mouse monoclonal anti-vinculin (1:200,000; Sigma, #V9131) was used as loading control. Horseradish peroxidase-conjugated anti-rabbit or anti mouse IgG (1:10,000; Jackson Immuno Research) were used as secondary antibodies. Signal was detected using enhanced chemiluminescence (Perkin-Elmer).

**γH2AX Foci Quantification** Cells were seeded into Corning 96-Well Half Area High Content Imaging Film Bottom Microplate at 10 000 cells/well. After 24h, cells were treated with MMS in serum-free media for 1h. Cells were then incubated in drug- free complete media for 1 h. Unless otherwise stated, all immunofluorescence dilutions were prepared in PBS and incubations performed at room temperature with intervening washes in PBS. Cell fixation was carried out by incubation with 4% paraformaldehyde for 10 min followed by 100% ice-cold methanol for 5 min at −20◦C. Cells were permeabilised in 0.2% Triton X-100 for 5 min followed by a quenching step using 0.1% sodium borohydride for 5 min. After blocking for 1 h in a solution containing 10% goat serum and 1% BSA, cells were incubated for 1 h with primary antibody anti-phospho-Histone H2A.X (Ser139) (1:5000, Millipore, #05-636) diluted in 1% BSA. Secondary antibody labelling used Alexa Fluor 568 goat anti-mouse (Invitrogen, #A-11004) diluted at 1:1000 in 1% BSA for 1 h. Nuclei were stained for 10 min with 1 mg/ml 4,6-diamidino- 2-phenylindole (DAPI). Z-stack images were acquired on a ZEISS Celldiscoverer 7 automated microscope using a 50× water immersion objective, and analysed for γH2AX foci formation with ZEN Blue software 3.2 (ZEISS).

**Table S1. Primers used in this study.**

| | |
|---|---|
| **Mutagenesis - KD resistant *AAG* (c.69C>A):** | Fwd 5' cgccAGAGCAGGGCAGCCACAC<br>Rev 5' ggtctCTGCTTCTTTTGCCCCATCC |
| **Mutagenesis - Y127I:** | Fwd 5' GACCGAGGCAatCCTGGGGCCAG<br>Rev 5' TCCACGATGCGGCCTCGG |
| **Mutagenesis - H136L:** | Fwd 5' GAAGCCGCCCtgTCAAGGGGTGG<br>Rev 5' ATCCTCTGGCCCCAGGTA |
| ***qPCR - ACTB* (NM_001101)** | Fwd 5' ATT GCC GAC AGG ATG CAG AA 3'<br>Rev 5' GCT GAT CCA CAT CTG CTG GAA 3' |
| ***qPCR - AAG* (NM_001015054*)*** | Fwd 5' CCC CGC AAC CGA GGC ATG TT 3'<br>Rev 5' AGC AAG ACG CAA GCC CCG TC 3' |
| ***qPCR - ATF4* (NM_001675)** | Fwd 5' TTCTCCAGCGACAAGGCTAAGG 3'<br>Rev 5' CTCCAACATCCAATCTGTCCCG 3' |
| ***qPCR - HSPA5* (NM_005347)** | Fwd 5' CTGTCCAGGCTGGTGTGCTCT 3'<br>Rev 5' CTTGGTAGGCACCACTGTGTTC 3' |
| ***qPCR - XBP1*s (NM_005080)** | 412–431 5' CCTTGTAGTTGAGAACCAGG 3'<br>834–853 5' GGGGCTTGGTATATATGTGG 3' |
| ***qPCR - HERPUD1* (NM_001010989)** | Fwd 5' CCAATGTCTCAGGGACTTGCTTC 3'<br>Rev 5' CGATTAGAACCAGCAGGCTCCT 3' |
| ***qPCR - DDIT3* (NM_004083)** | Fwd 5' GGTATGAGGACCTGCAAGAGGT 3'<br>Rev 5' CTTGTGACCTCTGCTGGTTCTG 3' |

**Table S2. shRNA sequences used for AAG silencing.**

| |
|---|
| V3LHS_343111: ACAGCTTCATCCTGTGCCA |
| V3LHS_343113: TCATGCAGAAGTACATGCC |
| V3LHS_343114: CTAGCTGGTCGCTGCTTCT |
| V3LHS_343116: CATGCAGAAGTACATGCCG |

**References**


1. Roth RB, Samson LD (2002) 3-Methyladenine DNA glycosylase-deficient Aag null mice display unexpected bone marrow alkylation resistance. *Cancer Res* 62(3):656–60.
2. Gautier L, Cope L, Bolstad BM, Irizarry RA (2004) Affy - Analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* 20(3).
3. Ritchie ME, et al. (2015) Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 43(7).
4. Chen EY, et al. (2013) Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* 14(1):128.
5. Kuleshov M V., et al. (2016) Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res* 44(W1):W90–W97.
6. Tamajusuku ASK, et al. (2010) Characterization of ATP-induced cell death in the GL261 mouse glioma. *J Cell Biochem* 109(5):983–991.
7. Franken N a P, Rodermond HM, Stap J, Haveman J, van Bree C (2006) Clonogenic assay of cells in vitro. *Nat Protoc* 1(5):2315–9.
8. Rodrigue A, et al. (2019) A global functional analysis of missense mutations reveals two major hotspots in the PALB2 tumor suppressor. *Nucleic Acids Res*. 47(20):10662-10677.
9. Healing E, Charlier CF, Meira LB, Elliott RM (2019) A panel of colorimetric assays to measure enzymatic activity in the base excision DNA repair pathway. *Nucleic Acids Res* 47(11).
10. Harding HP, et al. (2005) Bioactive small molecules reveal antagonism between the integrated stress response and sterol-regulated gene expression. *Cell Metab* 2(6):361–371.
11. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. *Nat Protoc* 3(6):1101–1108.
12. Affar EB, et al. (1999) Immunological determination and size characterization of poly(ADP-ribose) synthesized in vitro and in vivo. *Biochim Biophys Acta - Gen Subj*. 1428(2-3):137-46.

# Figure S1

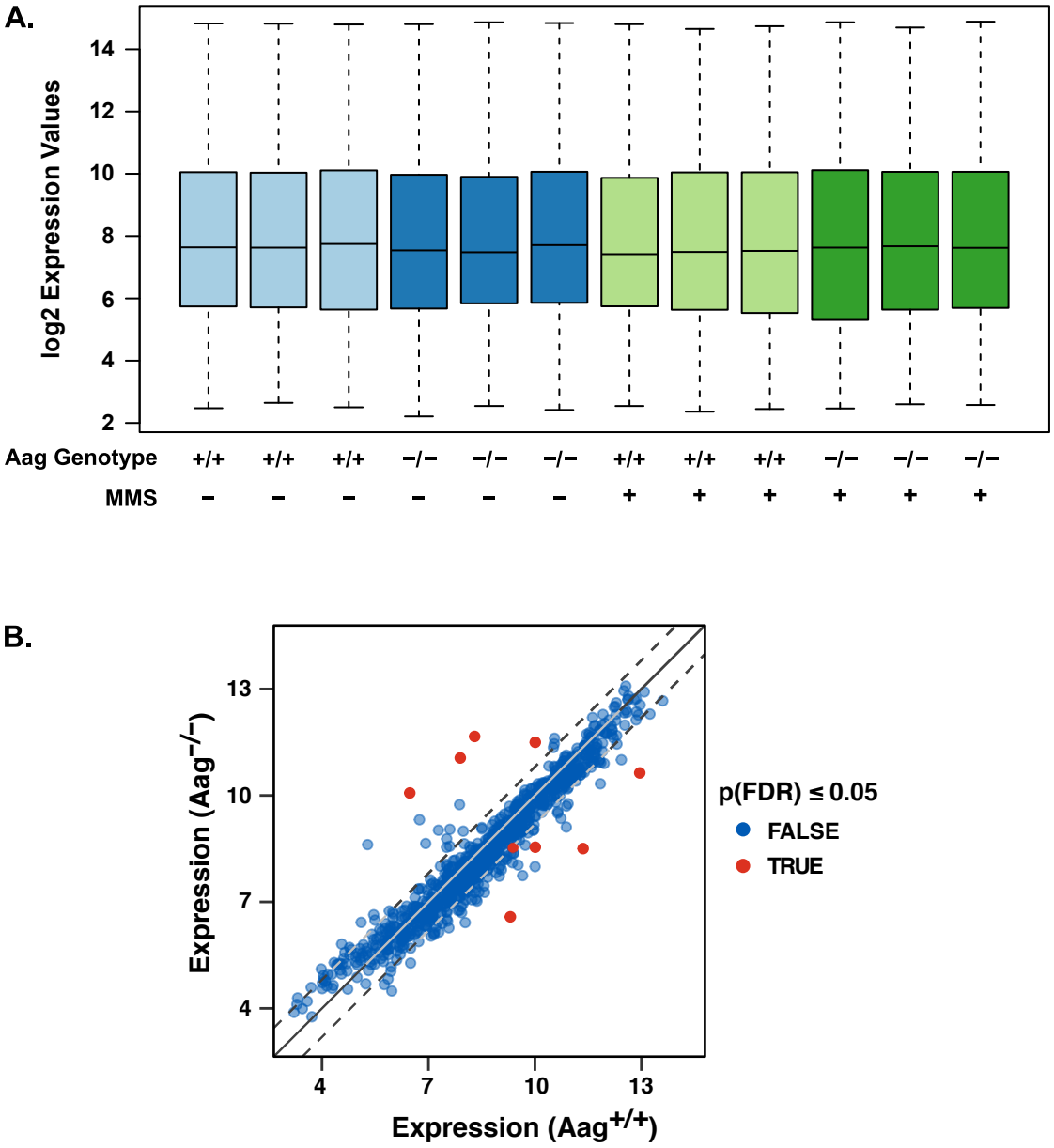


**Figure S1. Similar levels of gene expression in wild-type and Aag-deficient liver under control conditions.** Liver transcriptomes were studied by microarray analysis as described for Fig. 1. (A) Log2-transformed expression values for each replicate were generated using the R/Bioconductor affy package (expresso function). (B) Average expression values from control-treated knockout animals are plotted against expression values from control-treated wild-type animals. Each marker represents one of 980 microarray probes whose expression changed significantly according to Figure 1 A and B, under any of the conditions. Markers are colour-coded to indicate whether the difference in expression between knockout and wild-type samples was associated with an FDR-adjusted p-value of 0.05 or less. Solid line, bisection; dashed lines, bisection plus or minus $\log_2(1.75)$. The R scripts used to generate this figure are available at at https://github.com/nohturfft/Milano_et_al_2022.

# Figure S2

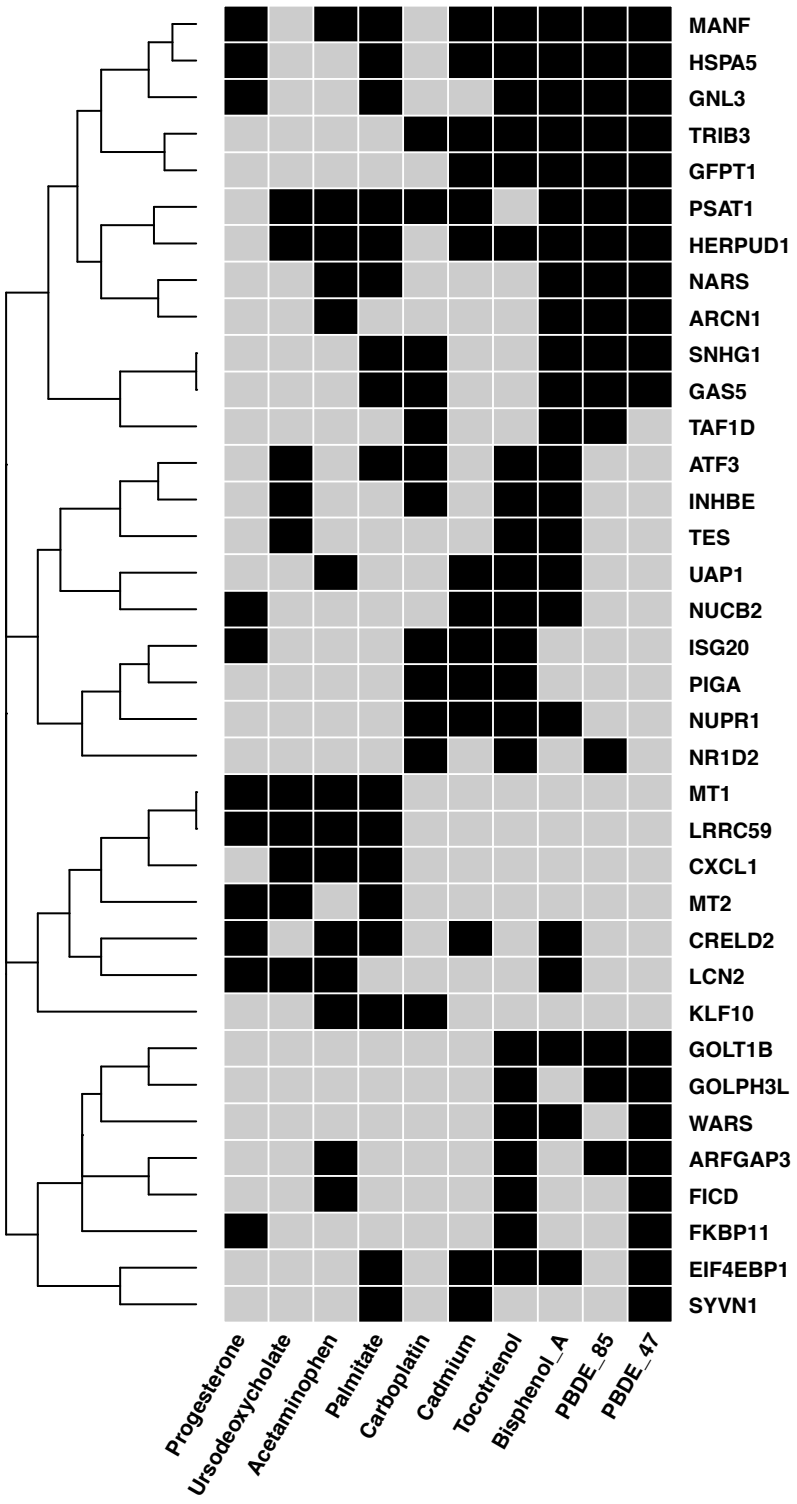


**Fig. S2. Genes induced by MMS and ER stress**. Genes induced by MMS in wild-type liver showed significant overlap with multiple genesets induced by ER stress-inducing compounds (Suppl. Data 2). In this binary heatmap the black squares indicate genes (y-axis) that are both induced by MMS in wild-type liver as well as by the respective ER stress-causing compound (x-axis). Only a subset of 36 genes are shown that overlapped with at least three ER stress-induced gene sets. For the complete results and methods (R scripts) see supporting documentation at https://github.com/nohturfft/Milano_et_al_2022.

# Figure S3

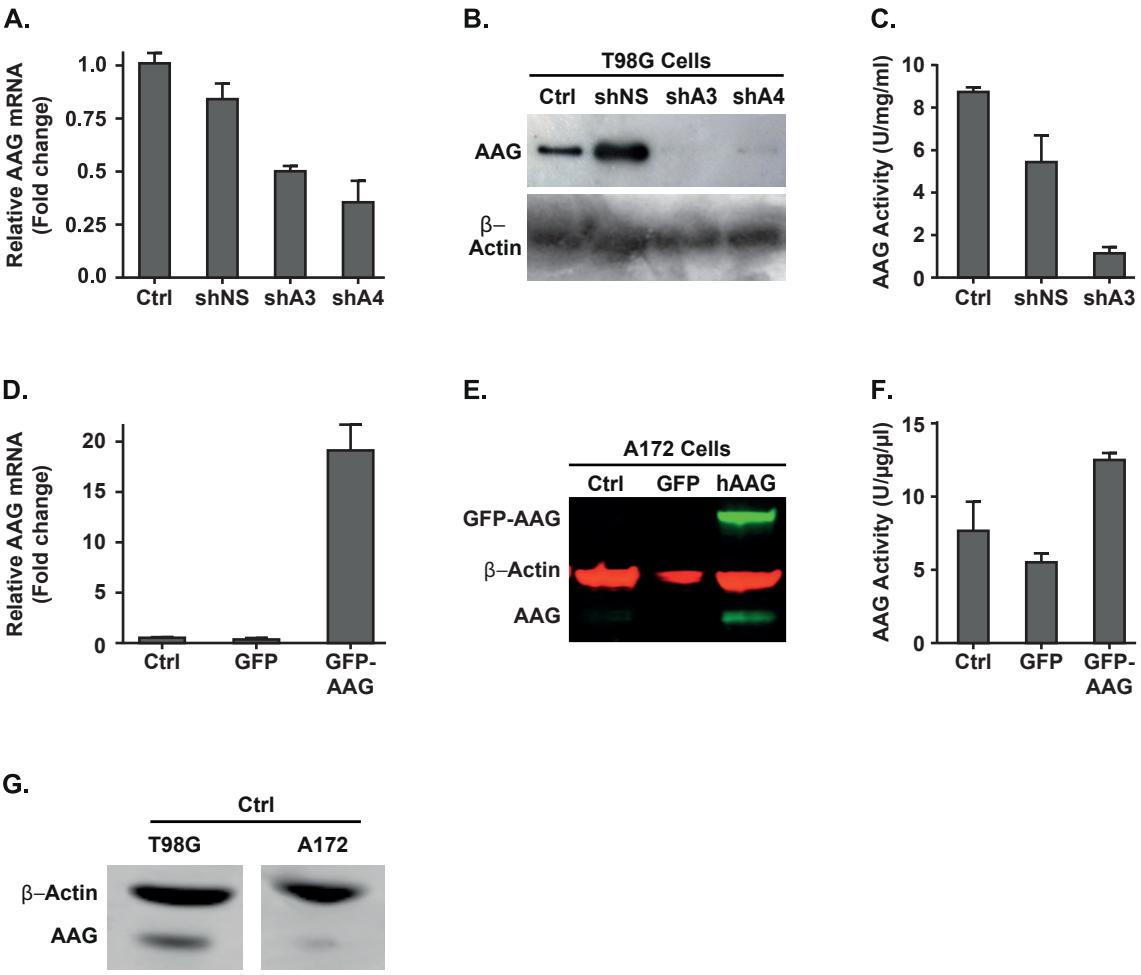


**Fig. S3. Characterization of AAG levels in glioblastoma cells.** (A-C) Glioblastoma T98G cells (Ctrl) were stably transduced with lentiviral particles expressing a non-silencing control shRNA (shNS) or an shRNA directed against AAG (shA3 and shA4). (D-F) Glioblastoma A172 cells (Ctrl) were stably transfected with plasmids expressing GFP or a GFP-AAG fusion protein. (A,D) Actin-corrected AAG mRNA levels were quantified by RT-qPCR. (B,E,G) AAG and actin protein levels were analyzed by immunoblotting. (C,F) AAG activity was measured with a plate-based assay as described in Materials and Methods.

# Figure S4

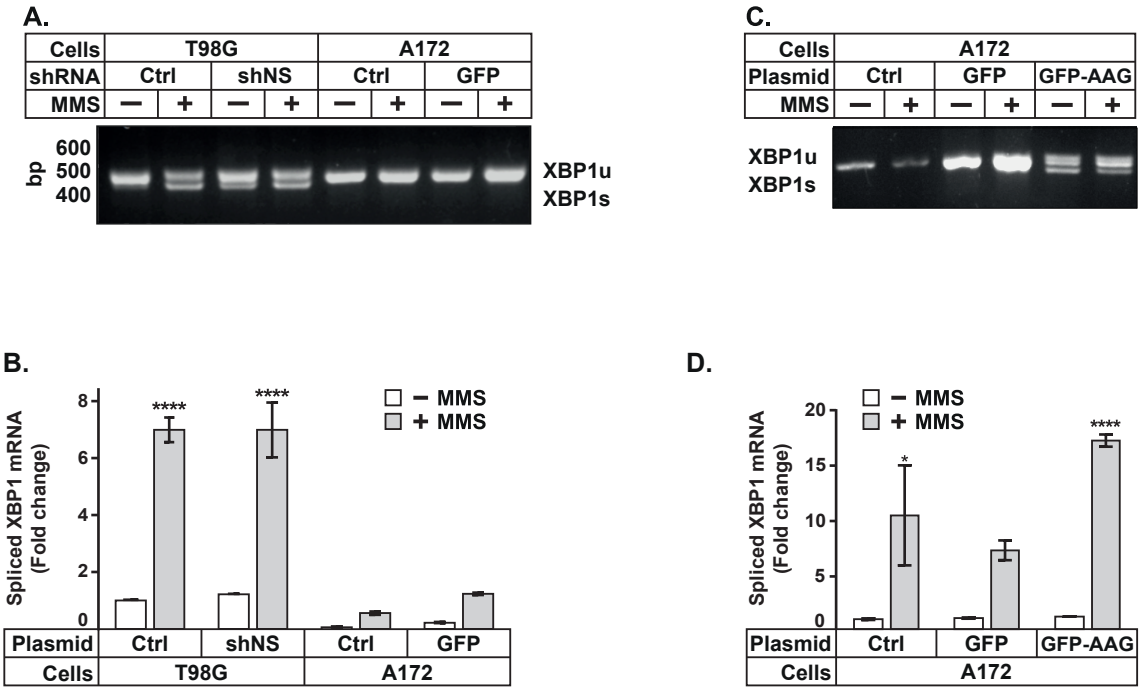


**Fig. S4. MMS treatment induces XBP1 splicing in cells expressing AAG**. Parental and stably transfected T98G and A172 cells were grown with or without 2.5 mM MMS for 1 h, followed by incubation without drug for 5 h. XBP1 splicing was analyzed by RT-PCR and agarose gel electrophoresis (A,C) and by RT-qPCR (B,D). Ctrl, untransfected cells; shNS, stably transfected with non-silencing control shRNA; GFP, stably transfected with GFP; GFP-AAG, stably transfected with GFP-AAG fusion protein; XBP1u, unspliced XBP1; XBP1s, spliced XBP1. (B,D) Data represent the average of three experiments ± standard error of the mean. **** $p < 0.0001$.

# Figure S5

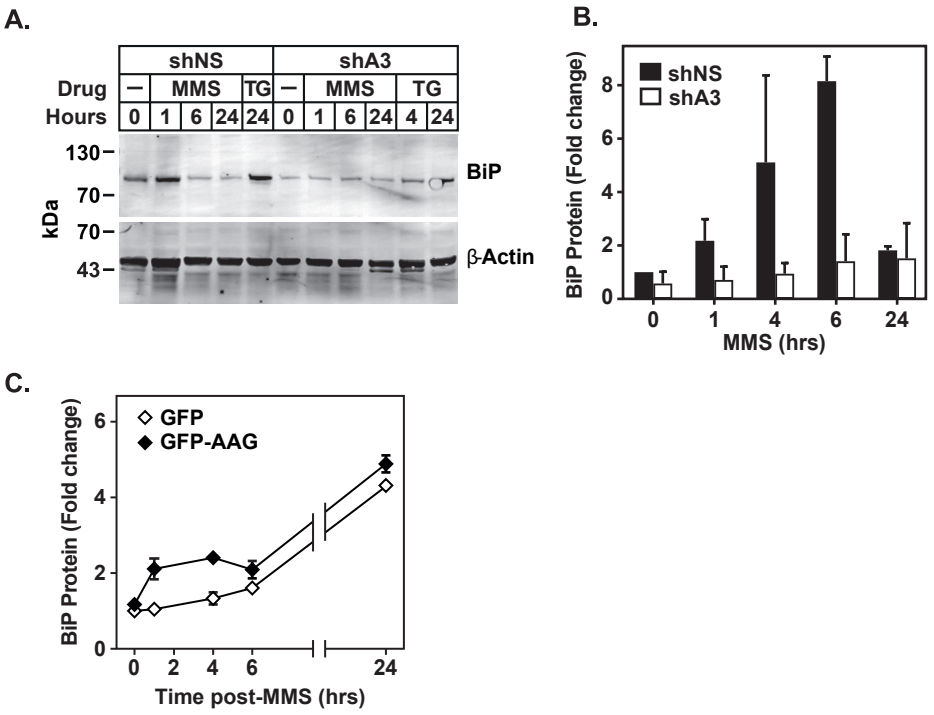


**Fig. S5. MMS treatment induces BiP in an AAG-dependent manner**. (A) Stably shRNA transfected T98G cells were treated with or without 2.5 mM MMS or 300 nM thapsigargin (TG) for the indicated time. Protein extracts were analyzed by immunoblotting with antibodies again BiP and actin. (B) Quantification of BiP; protein levels were normalised to b-actin and expressed relative to untreated control; (C) Quantification of BiP mRNA levels in AAG overexpressing GFP-AAG and control GFP cells.

# Figure S6

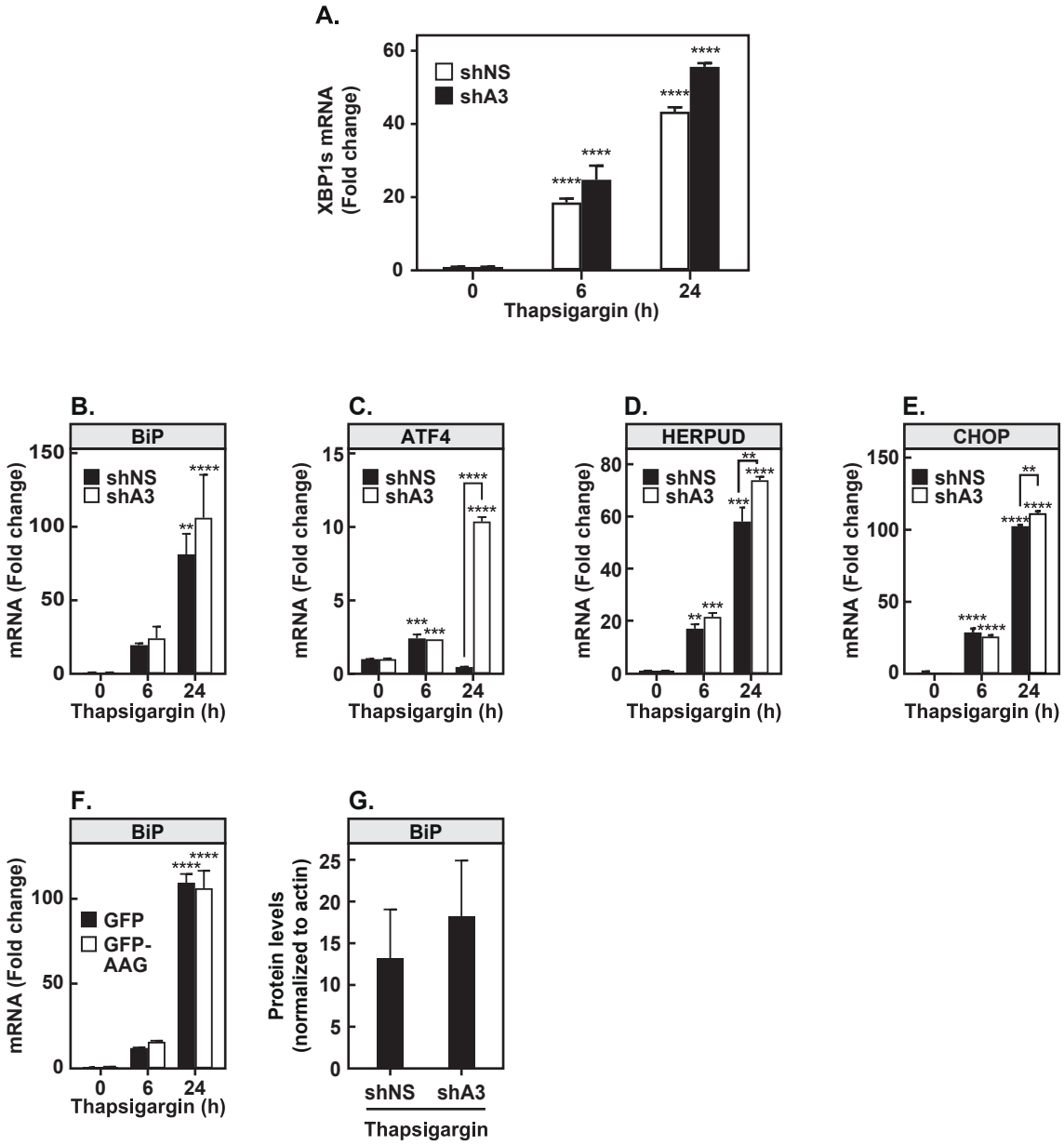


**Fig. S6. UPR transcriptional targets.** (A-E) Stably shRNA-transfected T98G cells were treated with 300 nM thapsigargin for the indicated time and spliced XBP1 (XBP1s, panel A) and the indicated genes (B-E) were quantified by qPCR. (F) A172 cells stably transfected with plasmids expressing either GFP or a GFP-AAG fusion protein were treated with 300 nM thapsigargin for the indicated time, and BiP mRNA was measured by qPCR. (G) Stably shRNA-transfected T98G cells were treated with 300 nM thapsigargin for 24 hours; then BiP and actin protein levels were quantified by immunoblotting. shNS, stably transfected non-silencing control shRNA; shA3, shRNA directed against AAG (clone 3). Results indicate the mean ± S.E. of three independent experiments. ** $p < 0.01$; **** $p < 0.0001$.

# Figure S7

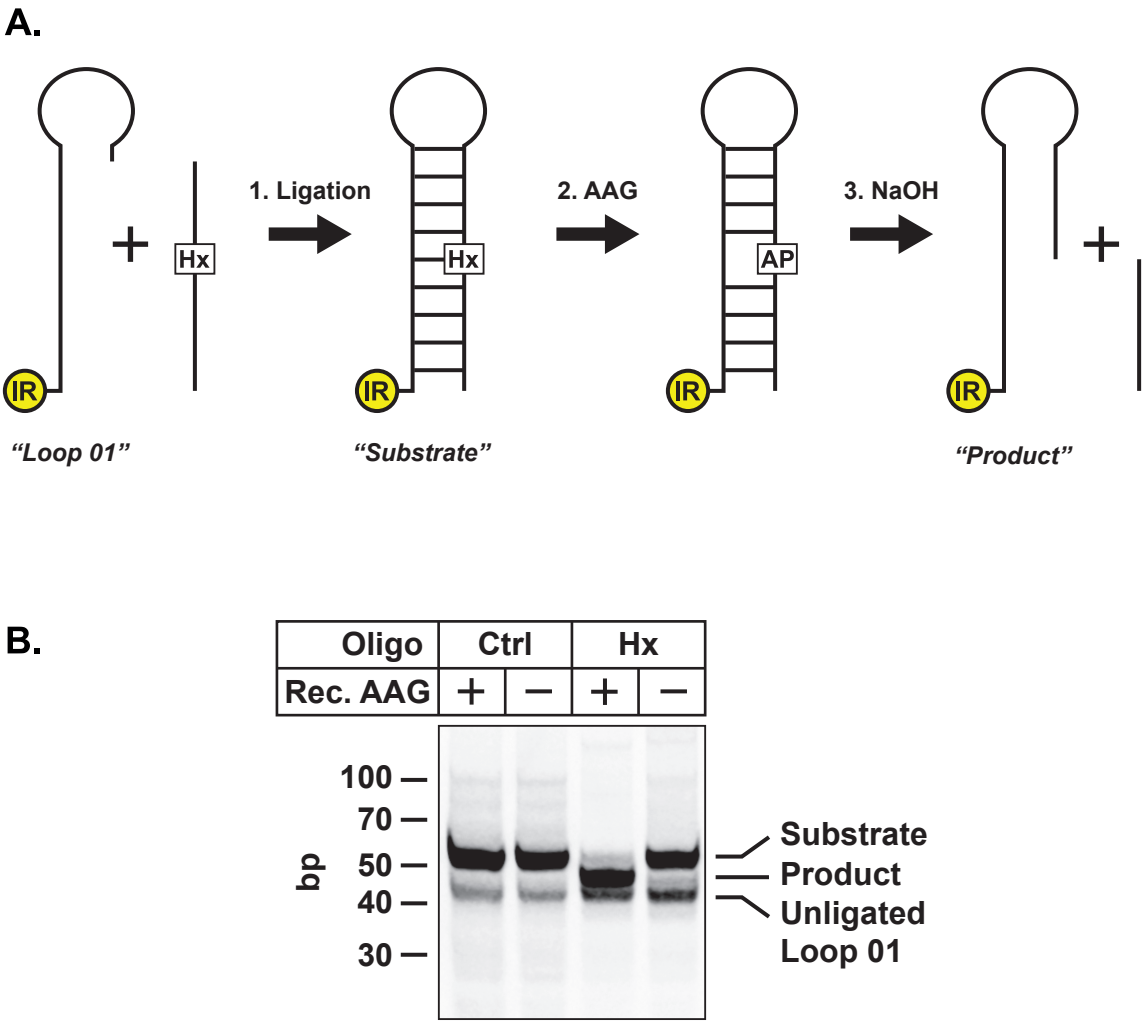


**Fig. S7. Gel-based AAG activity assay.** (A) Schematic illustrating reaction steps. A double-stranded, fluorescently labelled hairpin molecule was generated by ligating two complementary oligonucleotides (Step 1). The resulting substrate includes a thymine:hypoxanthine base pair where hypoxanthine (Hx) is a substrate for AAG-catalyzed base removal (Step 2). The resulting abasic site (AP) is labile to hydrolysis by NaOH, generating a shorter fluorescently tagged fragment (Step 3). (B) Ligated substrate (Hx) was incubated with recombinant AAG protein for 1 h at 37˚C, followed by addition of NaOH solution for 20 min. An "undamaged" control substrate (Ctrl) was included containing an adenine base instead of hypoxanthine. Reactions were separated by 1x TBE 15% polyacrylamide / 7M urea gel electrophoresis, followed by infrared fluorescence imaging

# Figure S8

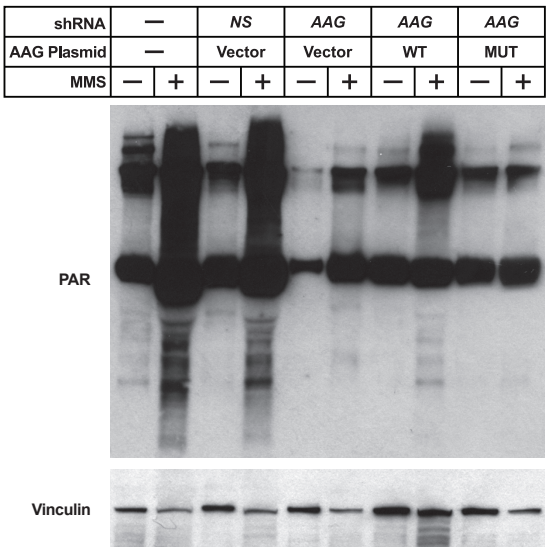


**Fig. S8. MMS treatment induces PARP activation in cells expressing wildtype AAG**. Cells (1 x 10^6 cells/well) were seeded in 6-well plates and treated for 1h with MMS (1 mM) in serum-free media. After treatment, media was replaced with complete media containing 1 μM PDDX004 (N02214) (PARG inhibitor) for 1h. Total PAR levels were examined by Western blotting against PAR. NS, stably transfected with non-silencing control shRNA. Three independent experiments were performed, and a representative blot is shown.

# Figure S9

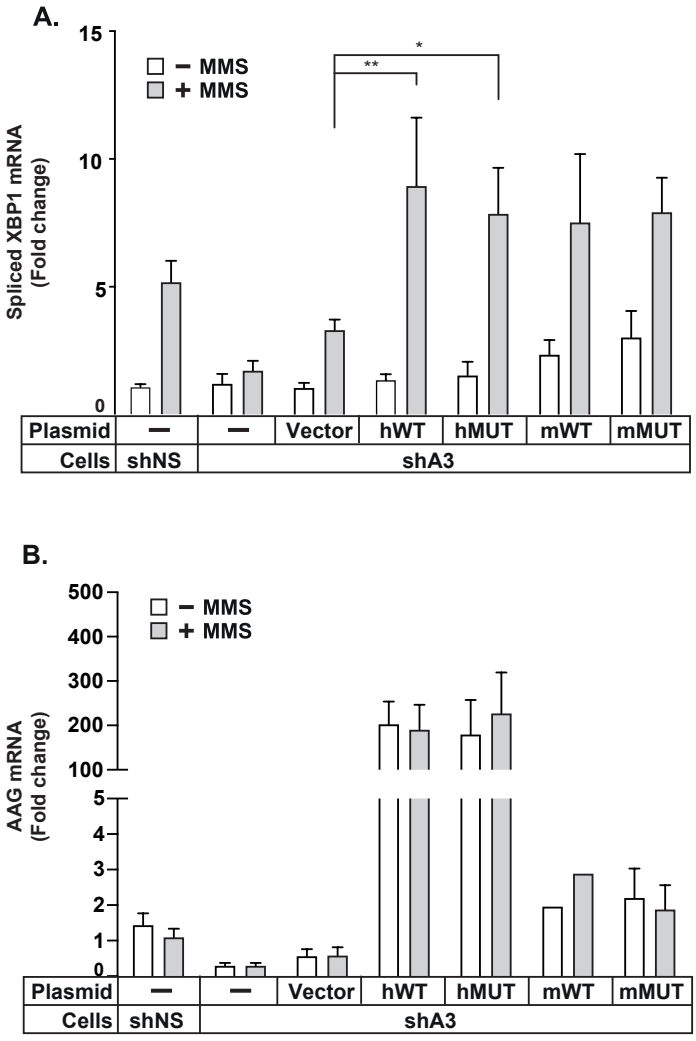


**Fig. S9. Human and mouse wildtype and glycosylase defective AAG complement the XBP1 splicing defect of *AAG* knockdown cells**. *AAG* knockdown cells (5x10^5 cells/well on a 6-well plate) were transfected using Lipofectamine (6.25:1) with plasmids (800 ng/well) expressing wildtype and glycosylase-defective versions of human or mouse AAG cDNA. Twenty-four hours later, cells were replated at 1.5 x 10^5 cells/well (6-well) and treated with or without 0.5 mM MMS for 1 h, followed by incubation without drug for 1 h before harvest for RNA extraction. XBP1 splicing (A) and AAG mRNA expression (B) were analysed by RT-qPCR. hWT, human wildtype AAG, mMUT, human Y127I/H136L double mutant AAG, mWT, mouse wildtype Aag, mMUT, mouse Y147I/H156L double mutant Aag. Data represent the average of three experiments, each of which was carried out in triplicate ± SEM. * p=0.0359, ** p=0.0081

# Figure S10

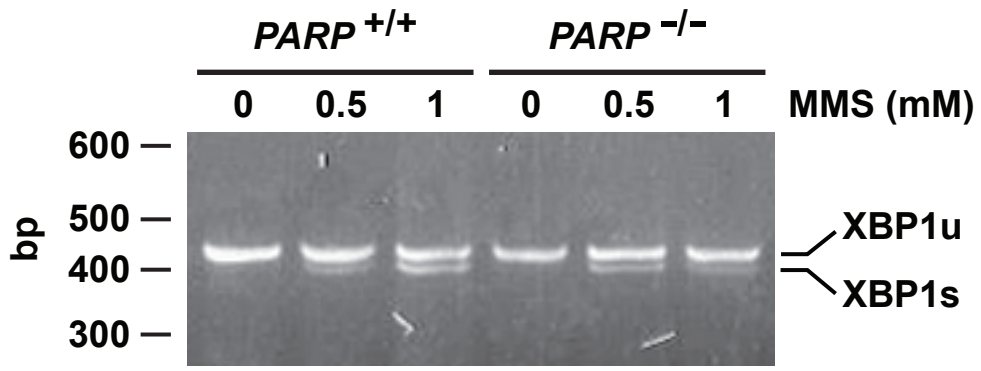


**Fig. S10. *PARP* knock-out cells are proficient at alkylation-induced XBP1 splicing**. Wild-type and PARP-deficient human osteosarcoma U2OS cells were grown with the indicated concentration of MMS for 1 h, followed by growth in drug-free medium for 1 h before RNA extraction. XBP1 splicing was then analysed by RT-PCR and agarose gel electrophoresis.

# Figure S11

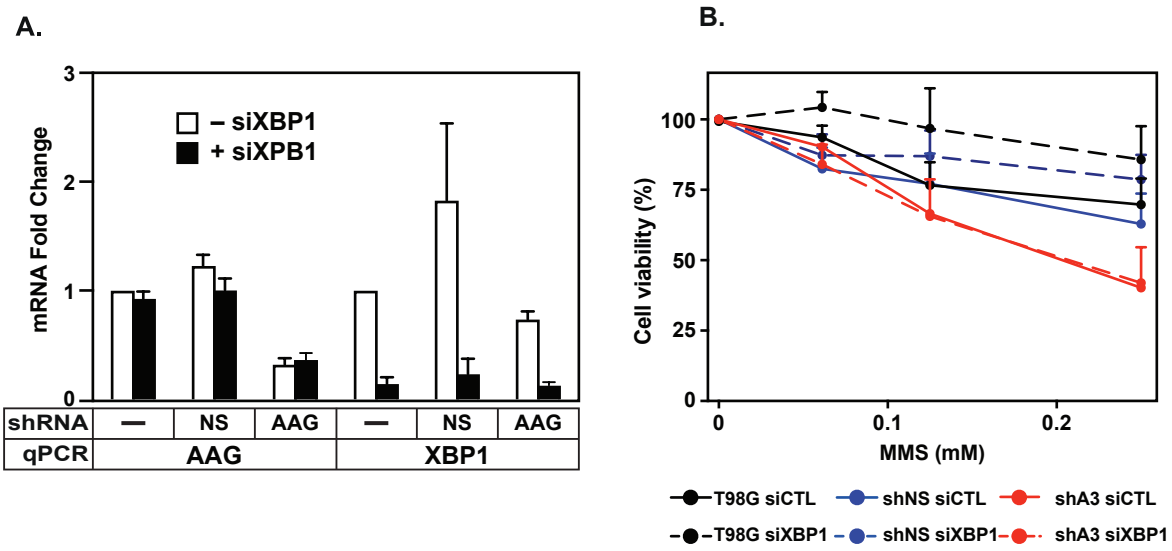


**Fig. S11. Effect of XBP1 siRNA on control and *AAG* knockdown cells.** Control and *AAG* knockdown cells were transiently transfected with control siRNA (siCTL, white bars) or siXBP1 (black bars). (A) AAG and XBP1 mRNA levels were measured by RT-qPCR; (B) Cells were treated with MMS (from 0 to 0.25 mM) for 1h and incubated in drug-free media for 24h. Viability was assessed by Hoechst staining. Results are expressed as mean values ± SEM from three independent experiments.