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Supplemental Experimental Procedures

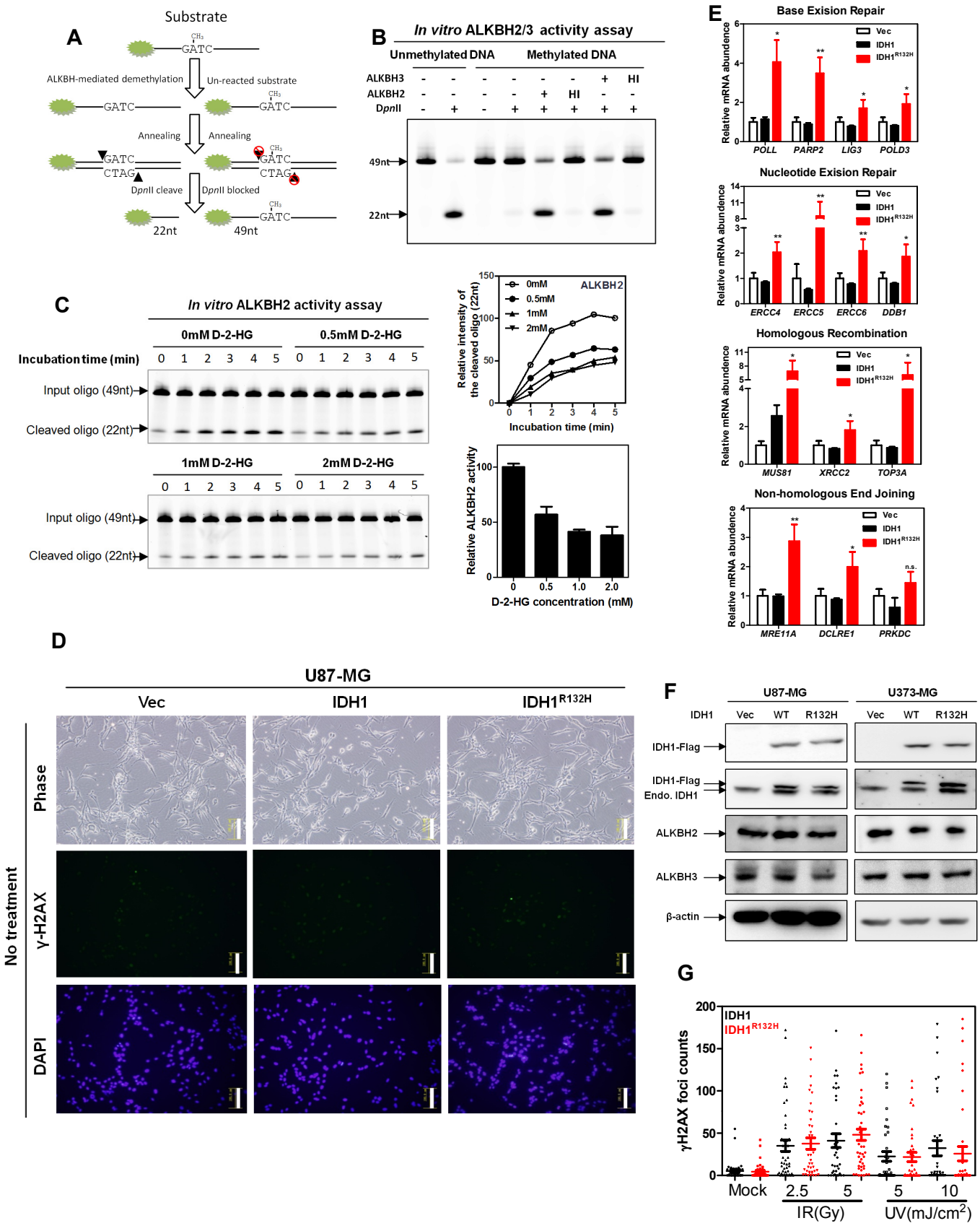
A
Substrate
ALKBH-mediated demethylation
Un-reacted substrate
Annealing
Annealing
DpnII cleave
DpnII blocked
22nt
49nt
B
In vitro ALKBH2/3 activity assay
Unmethylated DNA
Methylated DNA
ALKBH3
ALKBH2
DpnII
49nt
22nt
C
In vitro ALKBH2 activity assay
0mM D-2-HG
0.5mM D-2-HG
1mM D-2-HG
2mM D-2-HG
Incubation time (min)
Input oligo (49nt)
Cleaved oligo (22nt)
Relative intensity of the cleaved oligo (22nt)
ALKBH2
0mM
0.5mM
1mM
2mM
Incubation time (min)
Relative ALKBH2 activity
D-2-HG concentration (mM)
D
U87-MG
Vec
IDH1
IDH1R132H
No treatment
Phase
γ-H2AX
DAPI
E
Base Exision Repair
Nucleotide Exision Repair
Homologous Recombination
Non-homologous End Joining
Relative mRNA abundance
Vec
IDH1
IDH1R132H
POLL
PARP2
LIG3
POLD3
ERCC4
ERCC5
ERCC6
DDB1
MUS81
XRCC2
TOP3A
MRE11A
DCLRE1
PRKDC
n.s.
F
U87-MG
U373-MG
IDH1
Vec
WT
R132H
IDH1-Flag
IDH1-Flag
Endo. IDH1
ALKBH2
ALKBH3
β-actin
G
IDH1
IDH1R132H
γH2AX foci counts
Mock
2.5
5
5
10
IR(Gy)
UV(mJ/cm2)

Figure S1. 2-HG inhibits DNA damage repair enzyme ALKBH2 and ALKBH3, related to Figure 1.

(A) Schematic overview of reaction setup used for the ALKBH-mediated DNA demethylation assay *in vitro*. 1-MedA indicates the 1-methylated adenine base, which is part of the restriction endonuclease D*pn*II recognition site and only cleaves the unmethylated GATC sequence. After incubation with purified ALKBH2 or ALKBH3, the DNA substrate was annealed to a reverse complementary oligo, subjected to extensive D*pn*II digestion, and separated on a PAGE gel. Demethylated and undemethylated DNA was 22nt and 49nt, respectively.

(B) Verification of the ALKBH2/3 enzyme activity assay *in vitro*. HI: heat inactivated enzyme.

(C) D-2-HG inhibits the activity of ALKBH2 *in vitro*, with IC50 values being 0.81 mM. Shown are average values of triplicated results with standard deviation (S.D.).

(D) 2-HG accumulation causes increased double strand breaks after MMS treatment. U87-MG cells stably expressing the indicated proteins were exposed to increasing concentrations of MMS for 1 hr. After the treatment, the cells were cultured in fresh medium containing no MMS for another 10 hours, and the level of phosphorylated histone variant H2A.X (γ -H2AX) was determined by immunofluorescence. Scale bar:100 μm. This is the control staining related to main Fig 1D.

(E) Up-regulation of DNA repair genes in U87-MG cells transiently expressing tumor-derived *IDH1* mutant. The mRNA expression of candidate genes involved in four DNA repair pathways was determined by qRT-PCR analysis. Error bars represent standard deviation (S.D.) for triplicate experiments. *denotes the $p < 0.05$ and **denotes the $p < 0.01$ for cells expressing indicated *IDH1* mutant versus wild-type *IDH1*; n.s.= not significant.

(F) Ectopic expression of wild-type or mutant IDH1 did not affect the protein expression of endogenous ALKBH2 or ALKBH3 in both U87-MG and U373-MG cells.

(G) Overexpression of mutant IDH1 does not sensitize cells to UV or IR. U87 cells stably expressed IDH1 wild-type or R132H mutant were exposed to UV or IR at indicated does. 2 hours after treatment, cells were fixed and stained with pSer139 H2AX antibody. γH2AX foci numbers per cell were counted from 40 cells randomly selected per group. Student's *t*-test was carried out to determine the significance between each group.

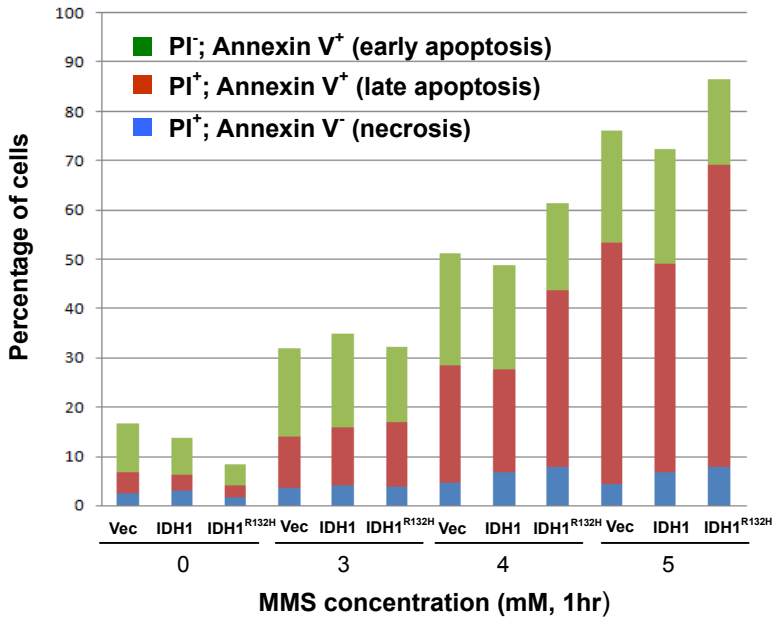

Figure S2. Cell death analysis of U87-MG stable cells expressing wild-type or mutant IDH1 in response to MMS, related to Figure 2

U87-MG cells stably expressing the indicated proteins were established by retroviral infection, and the cells were treated with MMS at the indicated concentrations for 1 hour, followed by a switch to fresh media (containing no MMS) for 47 hours prior to flow cytometry analysis. Cell death was assessed by determining the percentages of PI-positive, Annexin-positive, as well as PI- and Annexin-double positive cells.

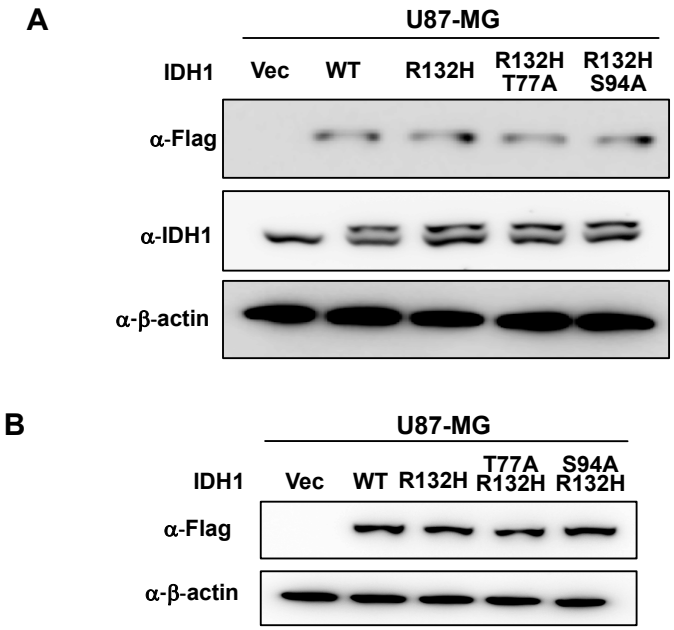

Figure S3. Establishment of U87-MG stable cell lines expressing wild-type or mutant IDH1, related to Figure 3

(A) U87-MG cells stably expressing the indicated proteins were established by retroviral infection, and the presence of ectopically expressed proteins was verified by western blot analysis.

(B) Expression of wild-type, single and double mutant IDH1 in U87-MG cells were determined by direct immunoblotting.

Supplemental Experimental Table

Table S1. Enzymes inhibited by 2-HG, related to Figure 1.

Enzyme	IC50 by L-2HG	IC50 by D-2HG	Reference
FIH	189±34	1,500±400	Chowdhury et al., (2011)
PHD2	419±150	7,300±3,300	Chowdhury et al., (2011)
ALKBH2	150±20	424±77	Chowdhury et al., (2011)
JMJD2A	26±3	24±2	Chowdhury et al., (2011)
JMJD2C	97±24	79±7	Chowdhury et al., (2011)
FBXL11	48±15	106±22	Chowdhury et al., (2011)
BBOX1	142±30	13200±1100	Chowdhury et al., (2011)
CeKDM7A	?	?	Xu et al., (2011)
JHDM1A	?	?	Xu et al., (2011)
TET1	<10mM	est. 25-50mM	Xu et al., (2011)
TET2	<10mM	est. 10-25mM	Xu et al., (2011)
C-P4H	?	?	Sasaki et al., (2012)
ATP synthase	?	?	Fu et al., (2015)

Table S2. Sequence of quantitative RT-PCR primers and shRNAs, related to "RNA isolation and qRT-PCR analysis" and "Antibodies, plasmids and chemicals" in main experimental procedure.

Gene	Primer sequence (Forward)	Primer sequence (Reverse)
ACTB	5'-GTTGTCGACGACGAGCG	5'-GCACAGAGCCTCGCCTT
DCLRE1C	5'-TTGGCAGAGGATCATCAAAG	5'-CCAAAGTACGGAGCCAAAGT
DDB1	5'-ATTAGGGTCAGACCGCAGTG	5'-GATCATCCGGAATGGAATTG
ERCC4	5'-TGCCTTCTCTTTCTTCAGGG	5'-GCTATCTCACTGCTTTGCGG
ERCC5	5'-CCGGACTCCTTTAAGTGCTTG	5'-GGAAGCTGCTGGAGTGCT
ERCC6	5'-CCTCCAGCTTCAGACGTTTC	5'-CCGAGATGATGGAGATGAAGA
LIG3	5'-GACTTCTCTTAGGGGTGGGG	5'-TTTGTCACCAGTACCAATCCC
PARP2	5'-TGTTGTTGTTGAACTGGAGATTG	5'-GGACCCAGAGTGTACAGCCA
MUS81	5'-TCTCAGGAAGGCTGAGGTTG	5'-AAGCGACTGGATGACCTTTG
PARP2	5'-TGTTGTTGTTGAACTGGAGATTG	5'-GGACCCAGAGTGTACAGCCA
POLD3	5'-TGAGACTGCCAGACACCAAG	5'-GGTTCATGTTAACCAGGCCA
POLL	5'-CATCTGGGTGAGTGATGAGC	5'-CAGGGAGGAGGCTACAGAGA
PRKDC	5'-TGGCGTGTGAAACTTAGGC	5'-GTTTCAGGAGGATTGCCAGA
TOP3A	5'-CATTCTGGCCATACAGATGA	5'-GCCGACCTGCTGTCAAAC
XRCC2	5'-CTACCTTCAAGTCGGGCAAG	5'-GGCGGGAAAGTTGAGTCTCT

shRNA	Sequence (5' to 3')
ALKBH2#1	CCGGGTGCTCATCAACAGGTATAAACTCGAGTTTATACCTGTTGATGAGCACTTTTTG
ALKBH2#2	CCGGAGTTGGAGAAAGAAGTAGAATCTCGAGATTCTACTTCTTTCTCCAACTTTTTTG

Supplemental Experimental Procedures

Protein expression and purification

ALKBH2 and ALKBH3 were expressed in E. coli strain BL21 (DE3). 0.5 mM IPTG was added into the bacterial culture to induce protein expression till OD600 reached 0.6. Cells were lysed by sonication and the protein was purified and desalted by using HisTrap FF (GE healthcare) and HiTrap desalting columns (GE healthcare), respectively, following the manufacturer's instructions.

In vitro ALKBH2 and ALKBH3 activity assay

In vitro ALKBH2 and ALKBH3 activity assay was modified from (Ringvoll et al., 2006). In brief, the oligo substrate (5'-FAM-TAGACATTGCCATTCTCGATAGGXTCCGGTCAAATCTAGACGAAT TCCG, X=1-MedA) was synthesized by Genescript. ALKBH2 and ALKBH3 was assayed in 50 mM Hepes K (pH 8.0), 50 μM $(NH4)_2Fe(SO4)_2$, 50 μM α-KG, 2 mM ascorbic acid, 10 μM oligo substrate, 3 μM enzyme, 50 μg/ml BSA, and 10 mM $MgCl_2$. The reaction was carried out at 37℃ and stopped by heating at 95℃ for at least 5min. The oligo substrate was annealed to a reverse complementary oligo, and subjected to extensive DpnII digestion. The digested DNA was separated on 15% TBE-Urea-PAGE gel, and imaged using a Typhoon Scanner (GE healthcare).

Cell culture, treatment and viability assay

Human U87-MG, U373-MG and HT1080 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen) supplemented with 10% fetal bovine serum (Biochrom) in the presence of penicillin, streptomycin, and 8 mM L-glutamine (Invitrogen).

For viability assays, cells treated with MNNG or MMS were collected and stained with propidium iodide (PI) (Sigma-Aldrich) and analyzed by flow cytometry (Beckman Coulter EPICS Altra), or stained with trypan blue (final concentration: 0.05-0.1%) for viable cell counting. MTT assays were performed following the manufacturer's instruction (Sigma-Aldrich).

Transfection and western blotting

Cell transfection was performed using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. For western blot, cell lysate was separated on 10% or 15% SDS-PAGE and transferred onto nitrocellulose membrane. Membrane was blocked in 5%, followed by incubation with primary antibody overnight at 4℃, and a horseradish peroxidase (HRP)-conjugated secondary antibody for 1 hour at room temperature. The membrane was imaged by Typhoon Scanner (GE healthcare) or LAS 4000 (GE healthcare).

Metabolite extraction and analysis

The metabolites were extracted by adding 1 ml 80% (v/v) chilled methanol into

the plates immediately after removing the culture medium. The cell debris was removed by centrifuge, and the supernatant was lyophilized and derived at 70 °C for 30 min in 80 μl pyridine (Sigma-Aldrich) supplemented with 20 μl N-methyl-N-[tert-butyldimethylsilyl] trifluoroacetamide (MTBSTFA , Sigma-Aldrich). Derivatized sample was injected into an Agilent 7890A-5975C GC-MS system with a HP-5MS column (30m×0.25mm×0.25μm). GC oven temperature was programmed from 140℃ to 260℃ at 10℃/min, from 260℃ to 310℃ at 8℃/min and hold at 310℃ for 5min. The flow rate of carrier gas was 1 ml/min. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV.

LC-MS/MS analysis of nucleosides

The LC-based MS/MS method was modified from (Ringvoll et al., 2006). In brief, 50 μg genomic DNA was hydrolyzed to nucleosides by nuclease P1 (Sigma) and alkaline phosphatase (TaKaRa Bio Company). The digested samples were subjected to LC-MS/MS analysis using a ShimazuLC (LC-20AB pump) system coupled with TSQ-Vantage triple quadrupole mass spectrometer (Thermo). C18 column (250mm x 2.1 mm I.D., 3 μm particle size, ULTIMATE) was used for separation. The mass spectrometer was optimized and set up in selected reaction monitoring (SRM) scan mode for monitoring the [M+H]+ of deoxyadenosine (m/z 252.2 → 136.1), 1-methyldeoxyadenosine (266.2 → 150.1) and dideoxycytidine (212.1 → 112.1). Dideoxycytidine (TCI) was used as an internal control. The Xcalibur Software was used for analysis.

Statistical analysis

Statistical analyses were performed with a two-tailed unpaired Student's t-test. The values of $p<0.05$ were considered statistically significant. The IC50 and half-life are calculated by GraphPad software using non-linear fit curve model and one phase decay model, respectively.