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# Supplemental Information

# Oncometabolite 2-Hydroxyglutarate Is a Competitive Inhibitor of α-Ketoglutarate-Dependent Dioxygenases

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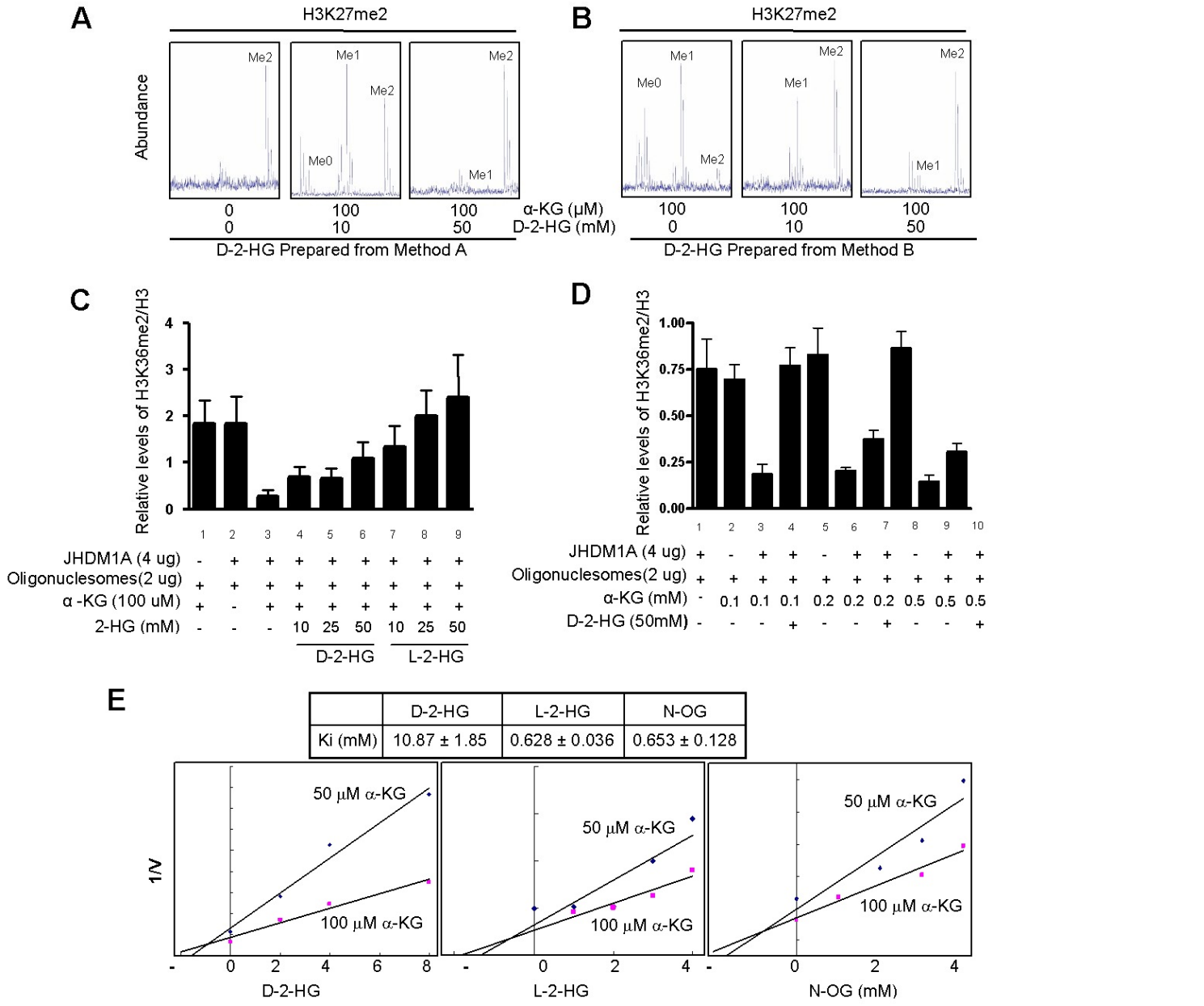



**Figure S1, related to Figure 1. 2-HG is a competitive inhibitor of a-KG for histone demethylases**

**A, B.** D-2HG synthesized start from either D-2-HG dibenzyl ester or D-Glutamic acid 5-benzyl ester and purified via distinct methods are tested for their KDM7A histone demethylase inhibitory effects toward H3K27me2 substrate. The assay was carried out as described in Figure 1.

**C**. 2-HG inhibits human JHDM1A demethylase activity. JHDM1A demethylase activity was assayed in presence of various concentrations of D-2-HG and L-2-HG as indicated in Figure 1C. The intensity of individual H3K36me2 band was quantified and normalized against total H3 protein. Error bars represent ±SD for triplicate experiments.

**D**. a-KG partially reverses the inhibitory effect of D-2-HG toward JHDM1A. JHDM1A demethylase activity was assayed in the presence of 50 mM D-2-HG and various concentrations of a-KG as indicated in Figure 1D. The intensity of individual H3K36me2 band was quantified and normalized against total H3 protein. Error bars represent ±SD for triplicate experiments.

**E.** Inhibition Constant (Ki) of D-2-HG, L-2-HG and N-OG were determined using purified JARID1B/KDM5B as an enzyme and synthetic H3K4me2 peptide as a substrate. Demethylation was analyzed by mass spectrometry. Shown are average value of triplicate assays with SD and a representative example for each inhibitor.

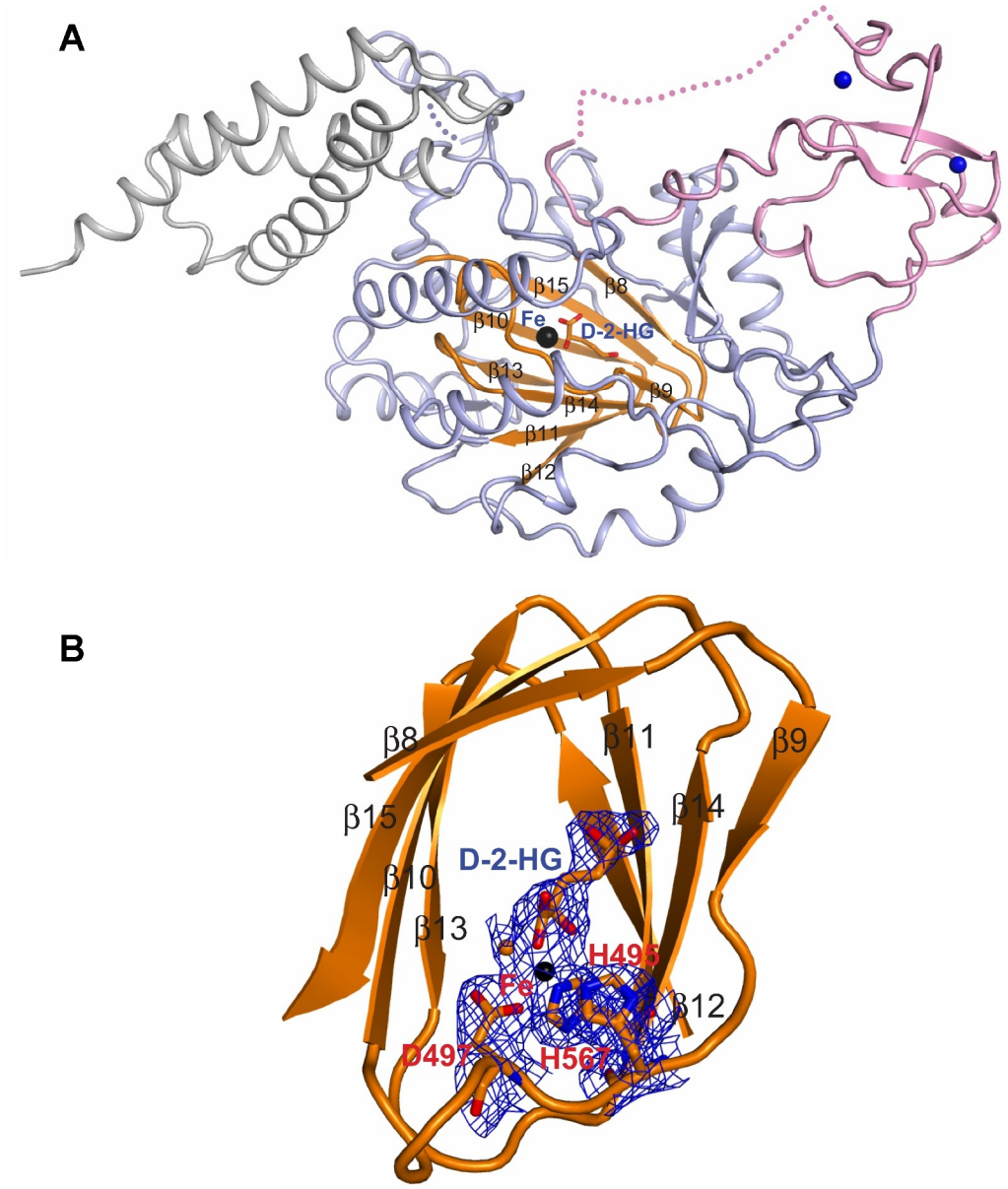



**Figure S2, related to Figure 2. 2-HG binds to the same site in a histone demethylase as a-KG binds**
**A**. Ribbon representation of the structure of CeKDM7A with D-2-HG. The JmjC domain and D-2-HG are shown in orange, zinc atoms in blue, Fe (II) in black. Secondary structural elements of CeKDM7A JmjC domain are indicated. For more detailed description, see Figure 2 in the text.
**B**. Electron density (2Fo-Fc) map covering the D-2-HG at resolution of 2.1 Å was shown. Map is contoured at 1.0 s and D-2-HG is shown as stick representation.

**Table S1, related to Figure 2.** Crystallographic data and structure refinement statistics for the structure presented in Figure 2.

**Data collection and reduction statistics**

| Protein | CeKDM7A with D-2-HG | CeKDM7A with a-KG |
|---|---|---|
| Beamline | SSRF-17U | SSRF-17U |
| Wavelength (Å) | 0.9798 | 0.9789 |
| Resolution (Å) | 50.0-2.10 (2.18-2.10) | 50.0-2.25 (2.33-2.25) |
| Space group | $P2_1$ | $P2_1$ |
| Cell dimensions (Å) | a=66.0,b=144.1, c=78.1, ß=106.218° | a=93.6, b=78.1, c=102.9, ß=92.01° |
| Completeness (%)[a] | 96.4 (77.3) | 99.1(99.6) |
| $R_{merge}$ (%) | 6.9 (31.3) | 9.3(31.4) |
| I/s (I) | 40.1 (3.0) | 17.8(6.7) |
| Unique reflections | 70867 | 69782 |
| Redundancy | 6.8 (3.6) | 7.0 (6.2) |

**Structure refinement statistics**

| Resolution range (Å) | 50.0-2.10 (2.18-2.10) | 50.0-2.25 (2.33-2.25) |
|---|---|---|
| $R_{work}/R_{free}$ (%)[b] | 21.40/25.05 | 18.75/22.36 |
| Deviation from identity | | |
| Bonds, (Å) | 0.008 | 0.007 |
| Angles, (º) | 1.117 | 1.040 |
| Average B factor, (Å$^2$) | 55.6 | 36.7 |

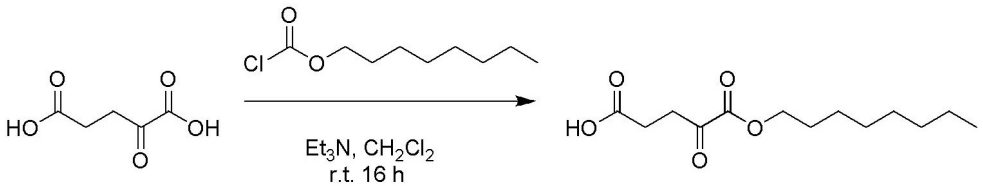
**Ramachandran plot statistics**

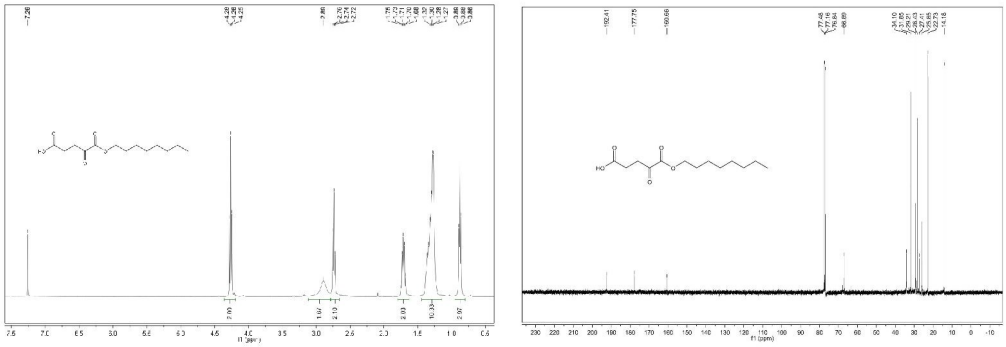
| | | |
|---|---|---|
| Most favored regions (%) | 90.0 | 91.1 |
| Allowed regions (%) | 9.3 | 8.7 |
| Generously allowed regions (%) | 0.7 | 0.2 |

[a] The values for the data in the highest resolution shell are shown in parentheses.

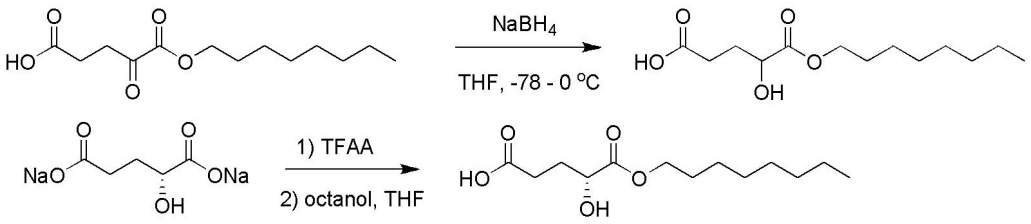
[b] $R_{free} = \sum_{Test}||F_{obs}| - |F_{calc}||/\sum_{Test}|F_{obs}|$, where "Test" is a test set of about 5% of the total reflections randomly chosen and set aside prior to refinement for the complex.

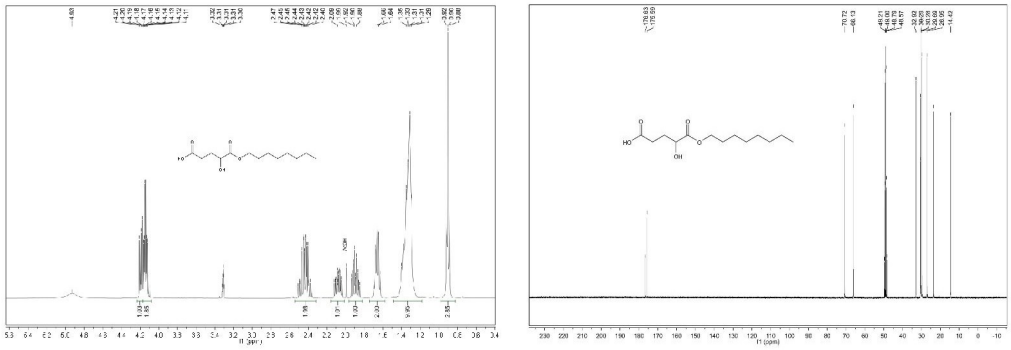
**A**



**B**



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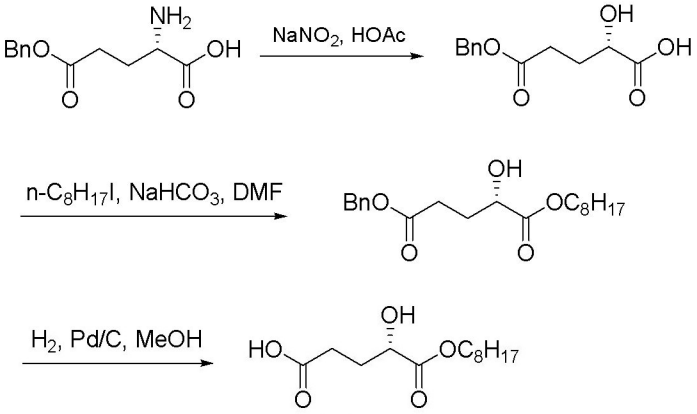
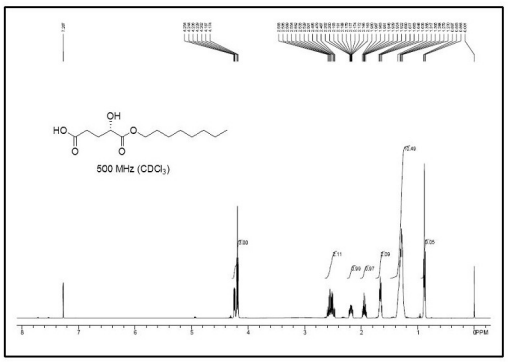
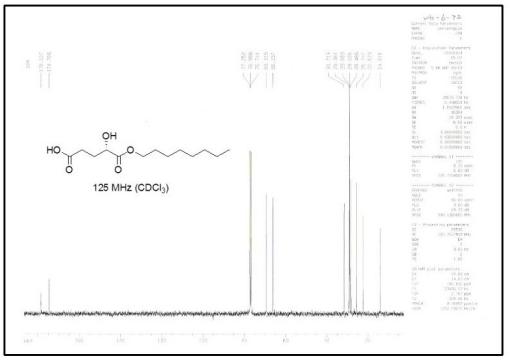
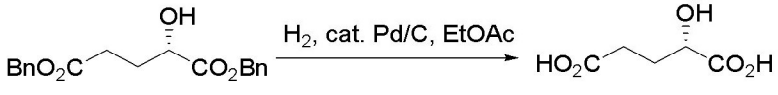


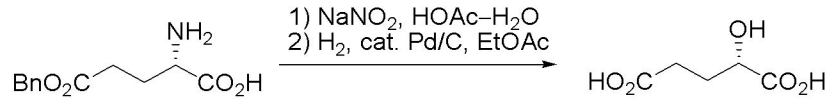




**D**



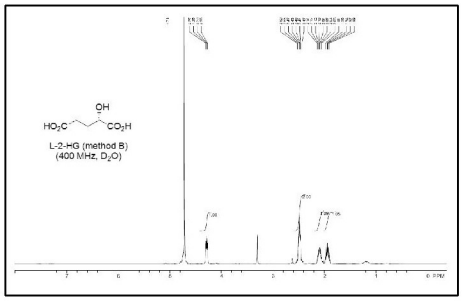

L-2-HG dibenzyl ester

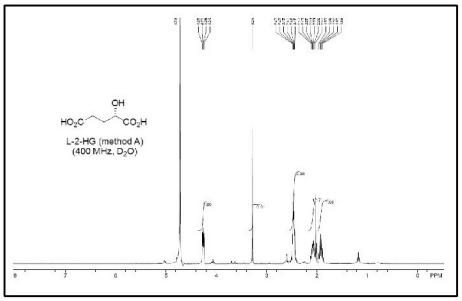
L-2-HG

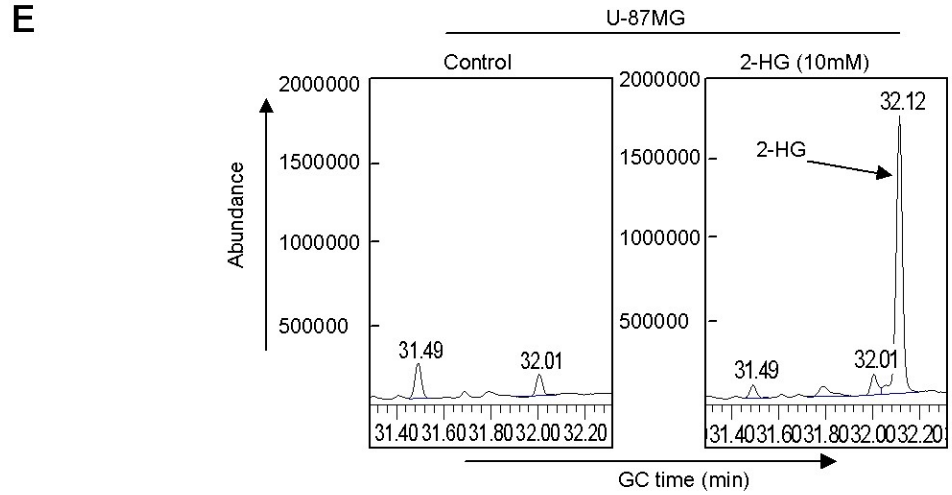


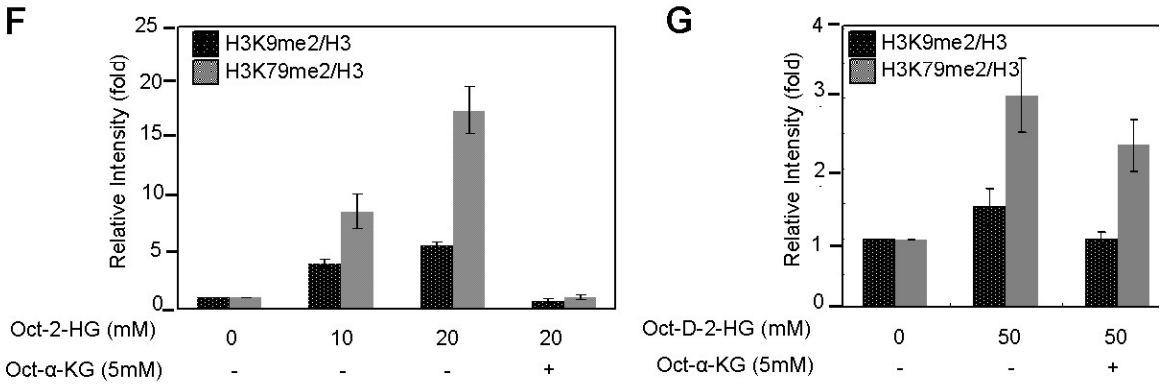

L-Glutamic acid 5-benzyl ester

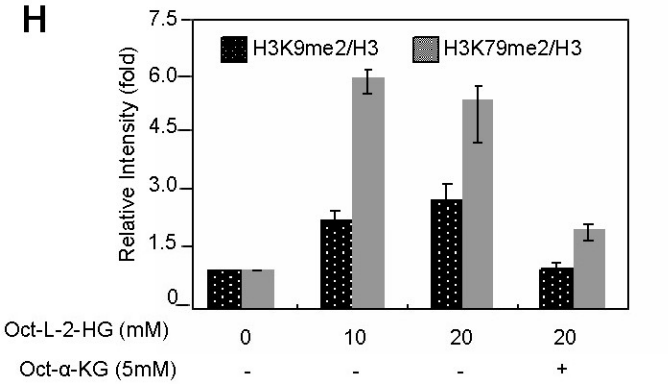
L-2-HG





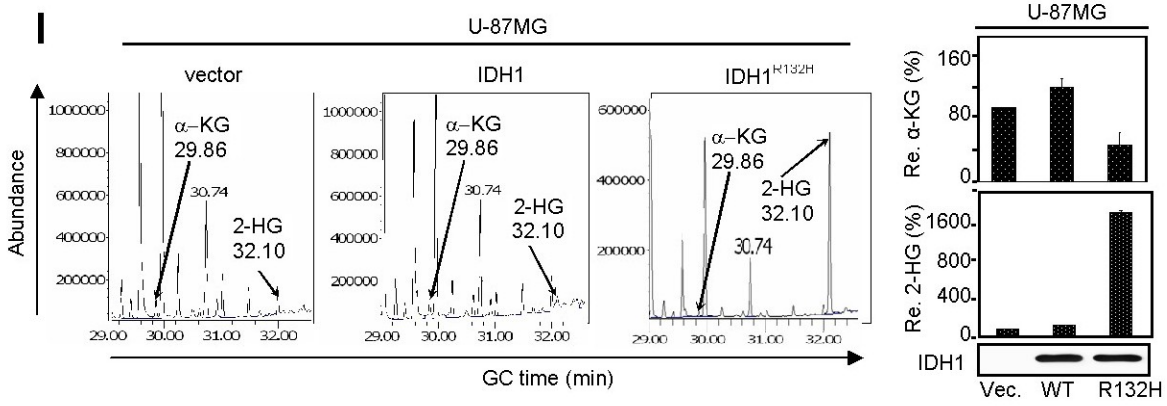




J

U-87MG

| Transfection | Vec. | IDH1$^{R132H}$ | |
|---|---|---|---|
| Oct-α-KG | - | - | + |
| H3K4me/H3 | 100% | 209%±36% | 105%±16% |
| H3K4me3/H3 | 100% | 466%±64% | 274%±25% |
| H3K9me2/H3 | 100% | 283%±56% | 126%±21% |
| H3K27me2/H3 | 100% | 232%±24% | 99%±9% |
| H3K79me2/H3 | 100% | 267%±47% | 130%±20% |



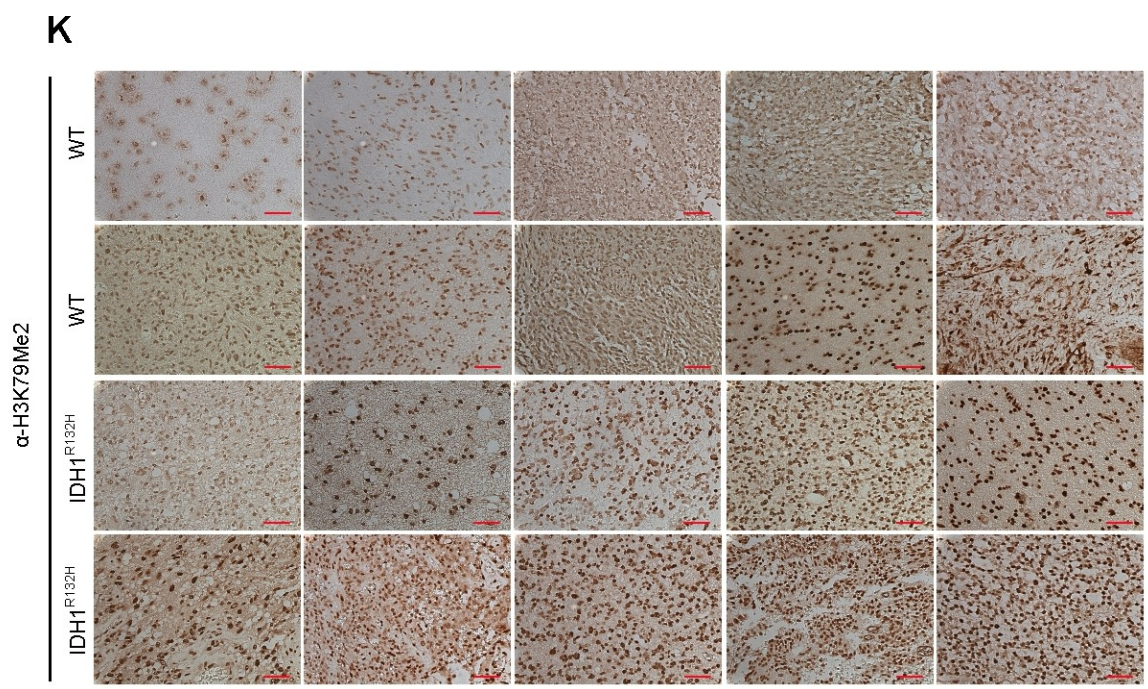



**Figure S3, related to Figure 3. reduced IDH1 activity and elevated 2-HG increase histone methylation**
**A** – **D**. Synthesis reactions and NMR verification of octyl-a-KG (**A**), racemic octyl-2-HG and octyl-D-2-HG (**B**), octyl-L-2-HG (**C**) and L-2-HG (**D**) are shown.
**E**. Cultured U-87MG cells were treated with 10 mM octyl-D-2-HG for 4 h. Cells were quenched rapidly by the addition of 10 ml 80% (v/v) pre-chilled (-80 ºC) methanol into culture plates, harvested by scrambling and lyophilized. Lyophilized samples were subjected to derivation and GC-MS analysis.
**F – H, J.** Quantification of inhibition of histone demethylases in vivo by cell-permeable octyl-2-HG. The western blot results shown in Figure 3 are quantified by ImageQuant 5.2 (Molecular Dynamics). Individual histone H3 methylation intensities are normalized to total H3 protein level. Results are shown in either bar figures or in tables. The quantification in S3F, S3G, S3H and S3I correspond to the western blots of Figures 3A, 3B, 3C and 3D, respectively. Error bars represent ± SD for triplicate experiments (**F**, **G**, and **H**). Shown are average quantifications of triplicate experiments with SD (**J**).
**I.** Ectopic expression of $IDH1^{R132H}$ decreases a-KG and accumulates 2-HG. a-KG and 2-HG levels were determined by GC-MS (left). Error bars represent ±SD for duplicate experiments.
**K.** Increased H3K79 dimetylation in gliomas harboring *IDH1* mutation. Randomly collected 20 glioma samples, 10 containing $IDH1^{R132H}$ mutation and 10 with wild type *IDH1*, were subject to immunohistochemistry (IHC) analysis for their H3K79 dimethylation status. IHC results are presented according to the order of staining intensity (from light to intense). Scale bars represent 50 μm. The quantification is presented in Figure 3E.

**Table S2, related to Figure 3.** Detailed information of clinical samples. These tumors were subjected to the analysis by immunohistochemistry for the expression of H3K79 dimethylation (Figure 3E), endostatin (Figure 5F), 5-hydroxymethylcytosine (Figure 8C) and 5-methylcytosine (Figure 8D).

| Sample ID | Diagnosis | WHO Grade | Age | Sex | *IDH1* Genotype | Nucleotide | Codon |
|---|---|---|---|---|---|---|---|
| a | Astrocytoma anaplastic | III | 32 | M | mutant | G395A | R132H |
| b | Astrocytoma anaplastic | III | 50 | M | mutant | G395A | R132H |
| c | Astrocytoma anaplastic | III | 41 | F | mutant | G395A | R132H |
| d | glioblastoma | IV | 41 | M | mutant | G395A | R132H |
| e | glioblastoma | IV | 52 | M | mutant | G395A | R132H |
| f | glioblastoma | IV | 30 | F | mutant | G395A | R132H |
| g | glioblastoma | IV | 51 | M | mutant | G395A | R132H |
| h | Astrocytoma anaplastic | III | 42 | M | mutant | G395A | R132H |
| i | glioblastoma | IV | 68 | M | mutant | G395A | R132H |
| j | Astrocytoma anaplastic | III | 43 | F | mutant | G395A | R132H |
| k | Astrocytoma anaplastic | III | 45 | M | wild type | wild type | R132 |
| l | glioblastoma | IV | 47 | M | wild type | wild type | R132 |
| m | glioblastoma | IV | 60 | F | wild type | wild type | R132 |
| n | Astrocytoma anaplastic | III | 67 | M | wild type | wild type | R132 |
| o | Astrocytoma anaplastic | III | 66 | F | wild type | wild type | R132 |
| p | glioblastoma | IV | 50 | M | wild type | wild type | R132 |
| q | Astrocytoma anaplastic | III | 21 | M | wild type | wild type | R132 |
| r | glioblastoma | IV | 51 | M | wild type | wild type | R132 |
| s | glioblastoma | IV | 71 | M | wild type | wild type | R132 |
| t | glioblastoma | IV | 59 | F | wild type | wild type | R132 |

| | U-87MG | | |
|---|---|---|---|
| Oxalomalate(mM) | 0 | 2.5 | 5.0 |
| H3K4me/H3 | 100% | 150% ± 10% | 235% ± 13% |
| H3K4me3/H3 | 100% | 271% ± 53% | 523% ± 37% |
| H3K9me2/H3 | 100% | 150% ± 30% | 303% ± 25% |
| H3K27me2/H3 | 100% | 128% ± 20% | 370% ± 45% |
| H3K79me2/H3 | 100% | 135% ± 25% | 368% ± 79% |

**Figure S4, related to Figure 4. Quantification of inhibition of histone demethylases in vivo by oxalomalate**

The western blot results shown in Figure 4A are quantified by ImageQuant 5.2 (Molecular Dynamics). Individual histone H3 methylation intensities are normalized to total H3 protein level. Average intensities of bands correspond to no oxalomalate treatment were set as 100% arbitrarily. Shown are average quantifications of triplicate experiments with SD.

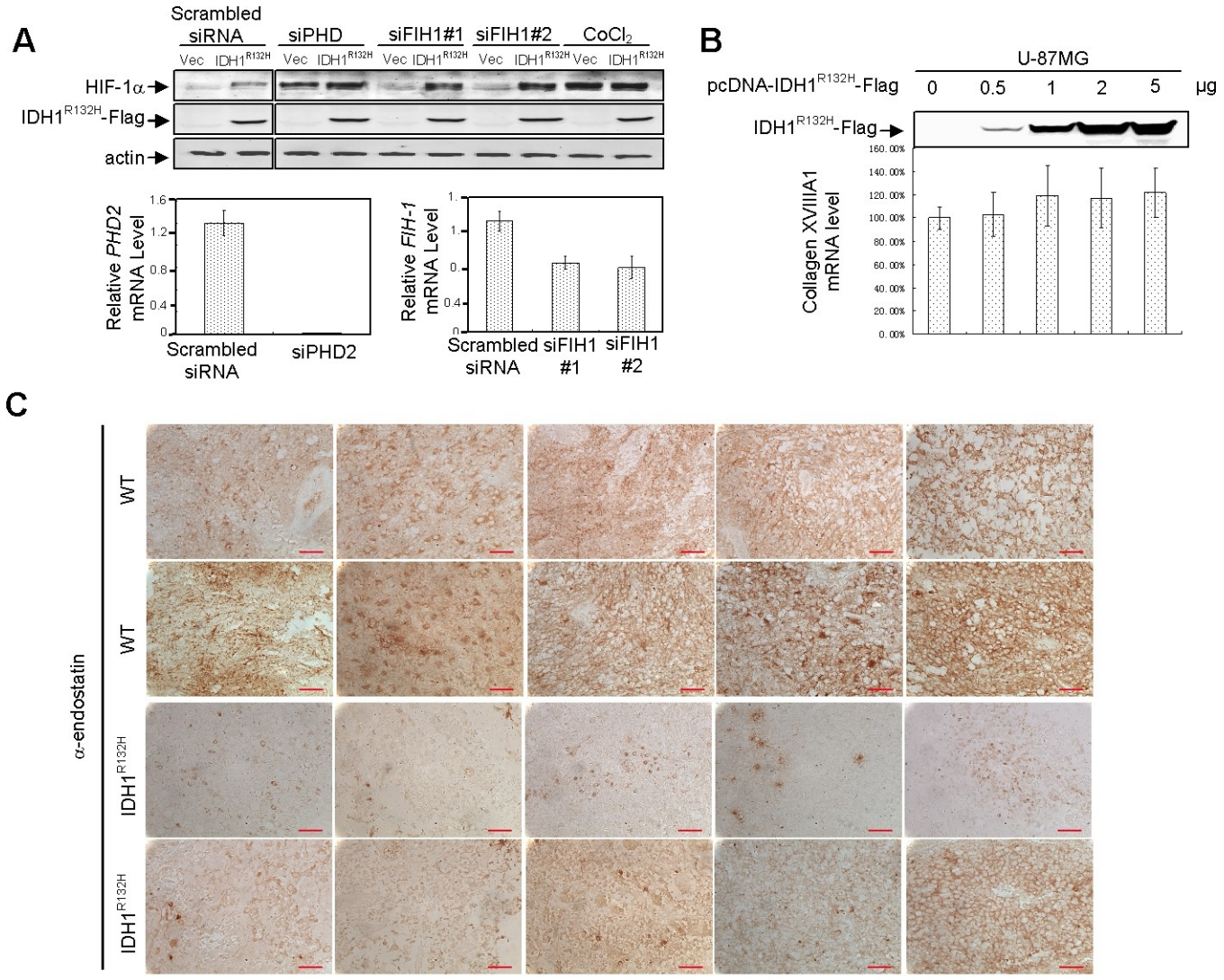



**Figure S5, related to Figure 5. IDH1 function supports the activity of a-KG-dependent dioxygenases in vivo**

**A**. FIH-1 knock down does not affect HIF-1α level. HEK293T cells were transfected with two different siRNA oligos targeting FIH-1 either alone or with $IDH1^{R132H}$. HIF-1α levels were determined by direct immunoblotting. siRNA targeting PHD2 and $CoCl_2$ treatment were included as controls for HIF-1α stabilization. This result supports the conclusion that the increase of HIF-1a in cells with reduced function of IDH1 is largely caused by the impairment of PHDs (Figure 5). Error bars represent ±SD for triplicate experiments.

**B**. Collagen XVIII transcription is not affected by $IDH1^{R132H}$ expression. $IDH1^{R132H}$ was gradient expressed in U-87MG cells, Collagen XVIII mRNA levels were quantified by qRT-PCR. Protein levels were verified by western blot. Ectopic expression of $IDH1^{R132H}$ did not significantly affect the levels of *collagen XVIII* mRNA, supporting the notion that decreased endostatin protein upon $IDH1^{R132H}$ expression is due to inhibition of C-P4H (Figure 5E). Error bars represent ±SD for triplicate experiments.

**C.** Decreased endostatin level in gliomas harboring mutation in *IDH1*. The same two groups of twenty glioma samples as Table S2 were subject to IHC analysis for their endostatin level. IHC results are presented according to the order of staining intensity (from light to intense). Scale bars represent 50 μm. The quantification is presented in Figure 5F.

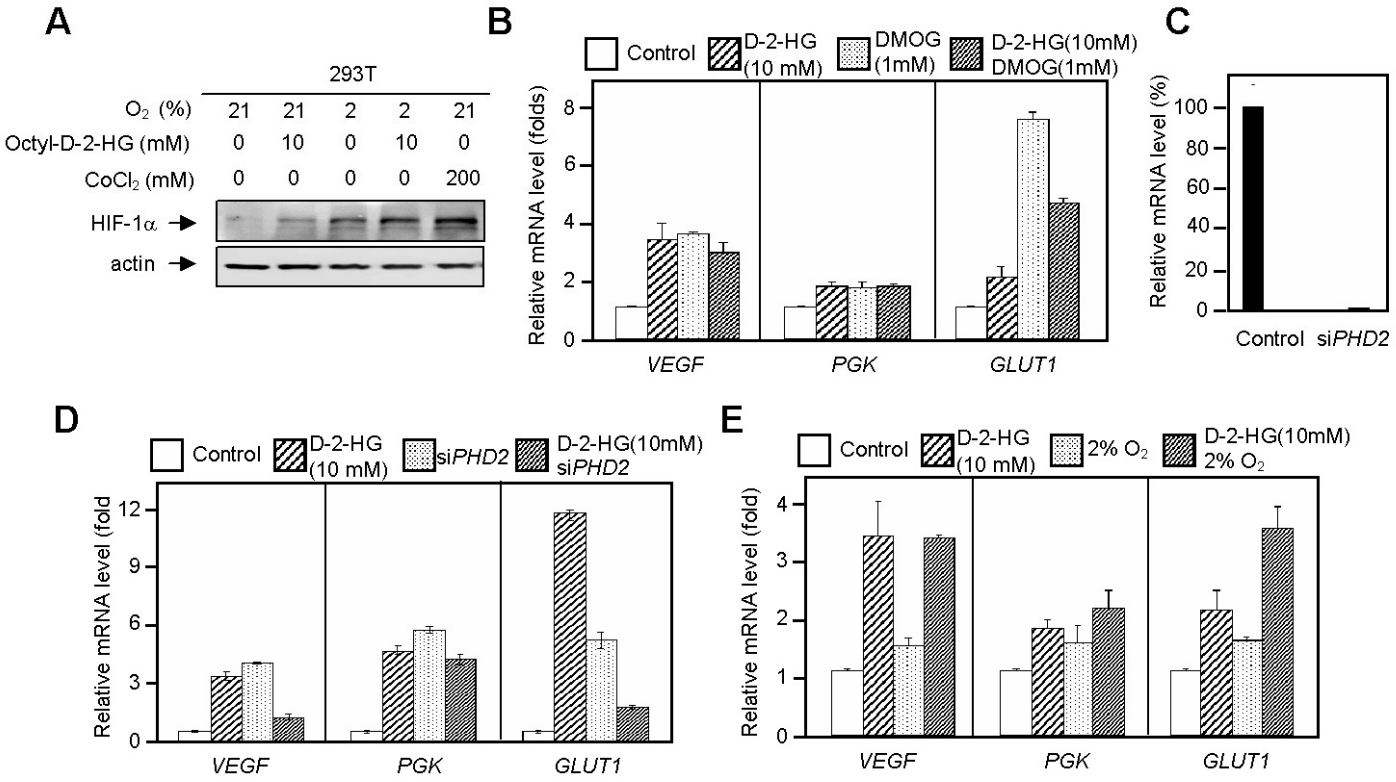



**Figure S6, related to Figure 6. 2-HG treatment inhibits PHD2 and activates HIF-1a targets genes**

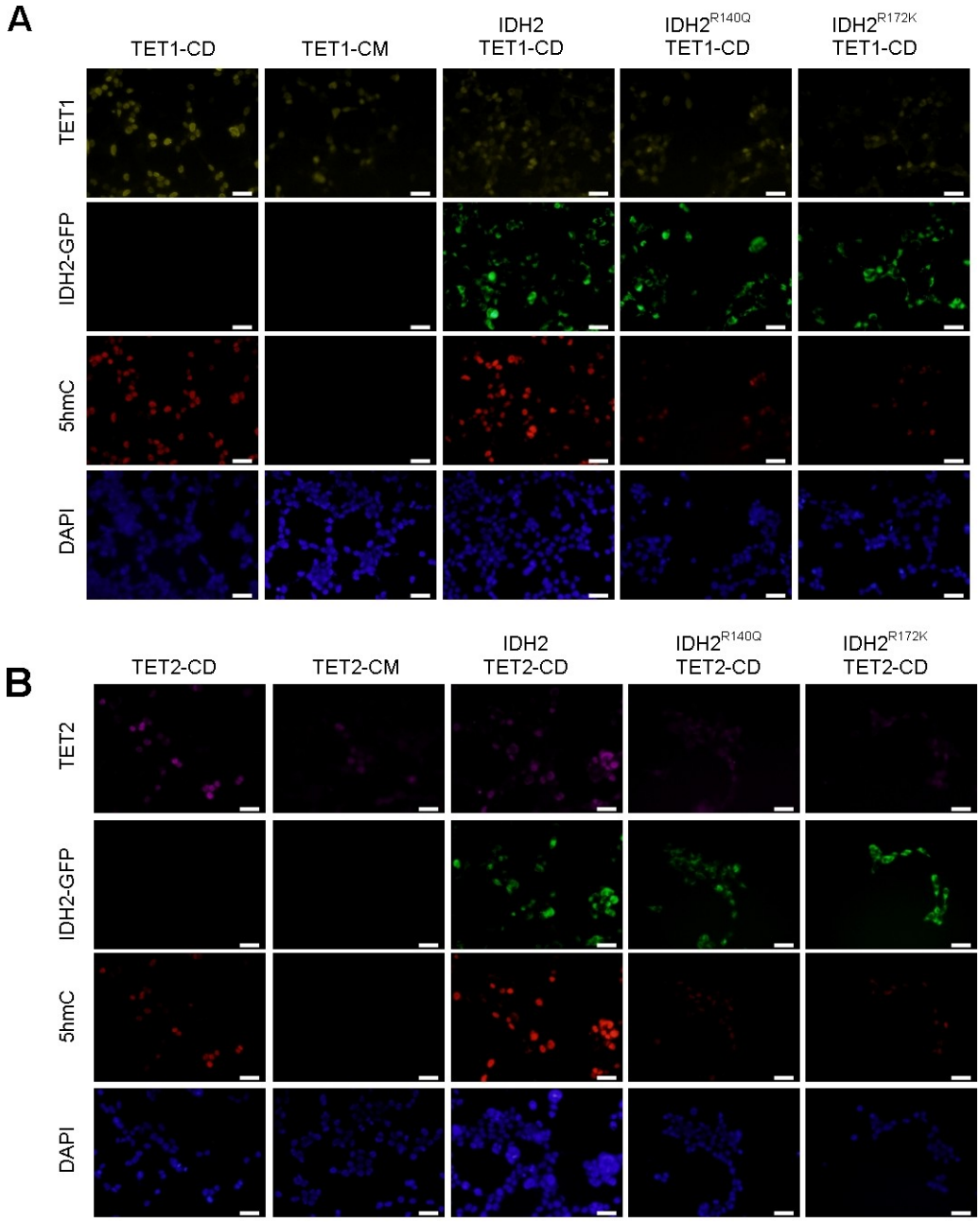
**A**. HEK293T cells were treated with octyl-D-2-HG and the steady state levels of HIF-1a were determined by direct western blotting.

**B**. DMOG inhibition abolishes D-2-HG activation on HIF-1α target genes. Error bars represent ±SD for triplicate experiments.

**C.** qRT-PCR confirmation of *PHD2* knockdown by siRNA. Error bars represent ±SD for triplicate experiments.

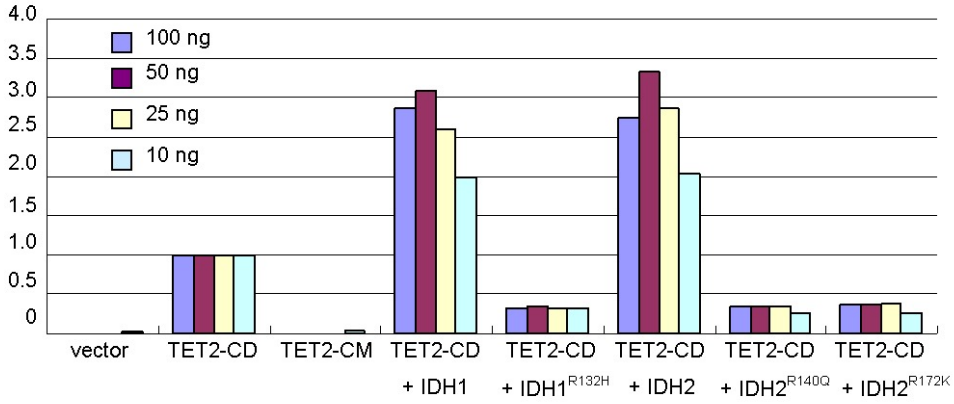
**D.** PHD2 knockdown abolishes D-2-HG activation on HIF-1α target genes. Error bars represent ±SD for triplicate experiments.

**E.** Hypoxia enhances D-2-HG activation on HIF-1α target genes. Error bars represent ±SD for triplicate experiments.


A
TET1-CD
TET1-CM
IDH2 TET1-CD
IDH2R140Q TET1-CD
IDH2R172K TET1-CD
TET1
IDH2-GFP
5hmC
DAPI
B
TET2-CD
TET2-CM
IDH2 TET2-CD
IDH2R140Q TET2-CD
IDH2R172K TET2-CD
TET2
IDH2-GFP
5hmC
DAPI

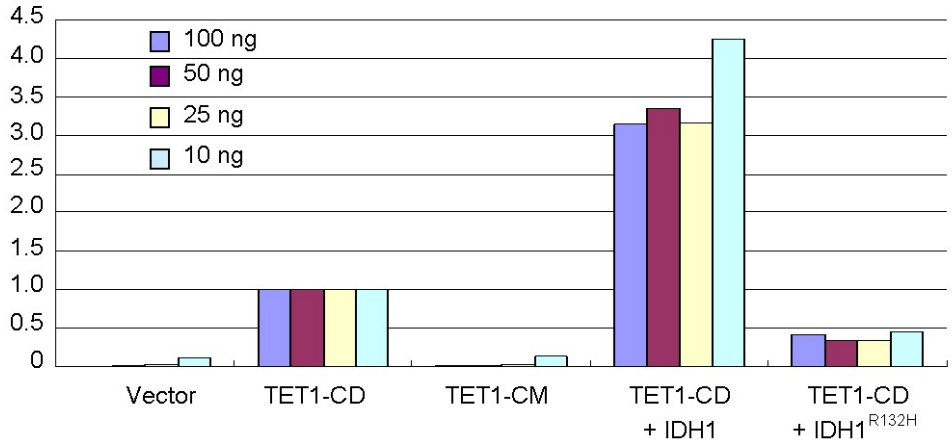
**C**

| Genomic DNA | 100 ng | 50 ng | 25 ng | 10 ng |
|---|---|---|---|---|
| Vector | 0.00517 | 0.00880 | 0.00287 | 0.01864 |
| TET2-CD | 1 | 1 | 1 | 1 |
| TET2-CM | 0.00453 | 0.00798 | 0.01007 | 0.04495 |
| TET2-CD + IDH1 | 2.85693 | 3.09081 | 2.59622 | 1.99999 |
| TET2-CD + IDH1$^{R132H}$ | 0.32237 | 0.34715 | 0.32648 | 0.33157 |
| TET2-CD + IDH2 | 2.73703 | 3.33565 | 2.87038 | 2.02278 |
| TET2-CD + IDH2$^{R40Q}$ | 0.34850 | 0.34852 | 0.34974 | 0.25734 |
| TET2-CD + IDH2$^{R172K}$ | 0.36640 | 0.35616 | 0.38449 | 0.27159 |




**D**

| Genomic DNA | 100 ng | 50 ng | 25 ng | 10 ng |
|---|---|---|---|---|
| Vector | 0.00656 | 0.01606 | 0.02927 | 0.12012 |
| TET1-CD | 1 | 1 | 1 | 1 |
| TET1-CM | 0.00882 | 0.01511 | 0.02530 | 0.13721 |
| TET1-CD + IDH1 | 3.13960 | 3.35235 | 3.16484 | 4.25557 |
| TET1-CD + IDH1$^{R132H}$ | 0.41311 | 0.33416 | 0.32921 | 0.45046 |



**E**

| Genomic DNA | 100 ng | 50 ng | 25 ng | 10 ng |
|---|---|---|---|---|
| Vector | 0.00138 | 0.00551 | 0.01937 | 0.01941 |
| TET1-CD | 1 | 1 | 1 | 1 |
| TET1-CM | 0.00187 | 0.00792 | 0.01873 | 0.00310 |
| TET1-CD + IDH2 | 2.99315 | 3.13546 | 3.30797 | 2.83720 |
| TET1-CD + IDH2$^{R40Q}$ | 0.27418 | 0.31505 | 0.26688 | 0.24594 |
| TET1-CD + IDH2$^{R172K}$ | 0.31388 | 0.35507 | 0.36766 | 0.22710 |

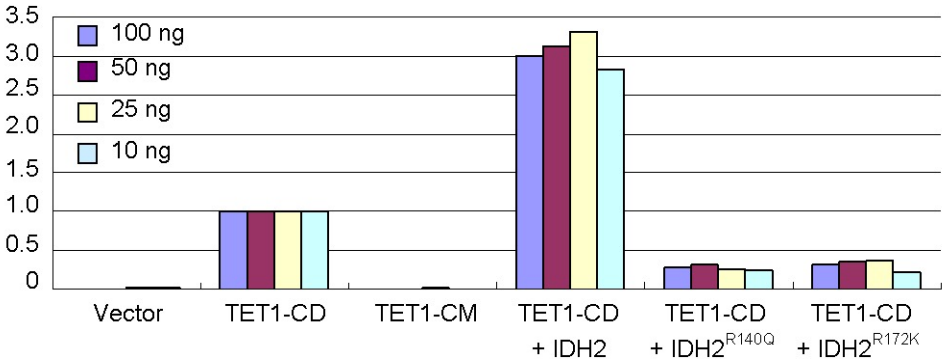



**Figure S7, related to Figure 7. Tumor-derived IDH2 mutants inhibit the 5hmC production by TET1 and TET2.**

(**A**, **B**) HEK293 cells were transiently transfected with plasmids expressing Flag-tagged TET1 (**A**) or TET2 (**B**) wild-type catalytic domain (CD) or catalytic mutant (CM), either alone or with wild-type or tumor-derived mutants IDH2 fused with GFP. 36 - 40 h after transfection, cells were fixed and stained with antibody specific to Flag to determine the expression of TET protein, to 5hmC to determine the levels of 5hmC, with DAPI (4',6-diamidino-2-phenylindole) to view the cell nuclei or visualized for green fluorescence to determine the expression of IDH proteins. Scale bars represent 50 μm. Similar results determining the effects of IDH1 on TET1 and TET2 are presented in Figure 7A.

(**C**) HEK293 cells were transiently transfected with plasmids expressing Flag-tagged TET2 wild-type catalytic domain or catalytic mutant, either alone or with wild-type or tumor-derived mutant IDH1 or IDH2 fused with GFP. 36 – 40 h after transfection, genomic DNAs were isolated from transfected cells, spotted on nitrocellulose membranes and immunolobtted with an antibody specific to 5hmC as shown in Figure 7B. Quantification of 5hmC was calculated from three independent assays and summarized in a diagram below the table.

(**D**, **E**) HEK293 cells were transiently transfected with plasmids expressing Flag-tagged TET1 wild-type catalytic domain or catalytic mutant, either alone or with wild-type or tumor-derived mutant IDH1 (**B**) or IDH2 (**C**) fused with GFP. 36 – 40 h after transfection, genomic DNAs were isolated from transfected cells, spotted on nitrocellulose membranes and immunolobtted with an antibody specific to 5hmC. Quantification of 5hmC was calculated from three independent assays and summarized in a diagram below the table.

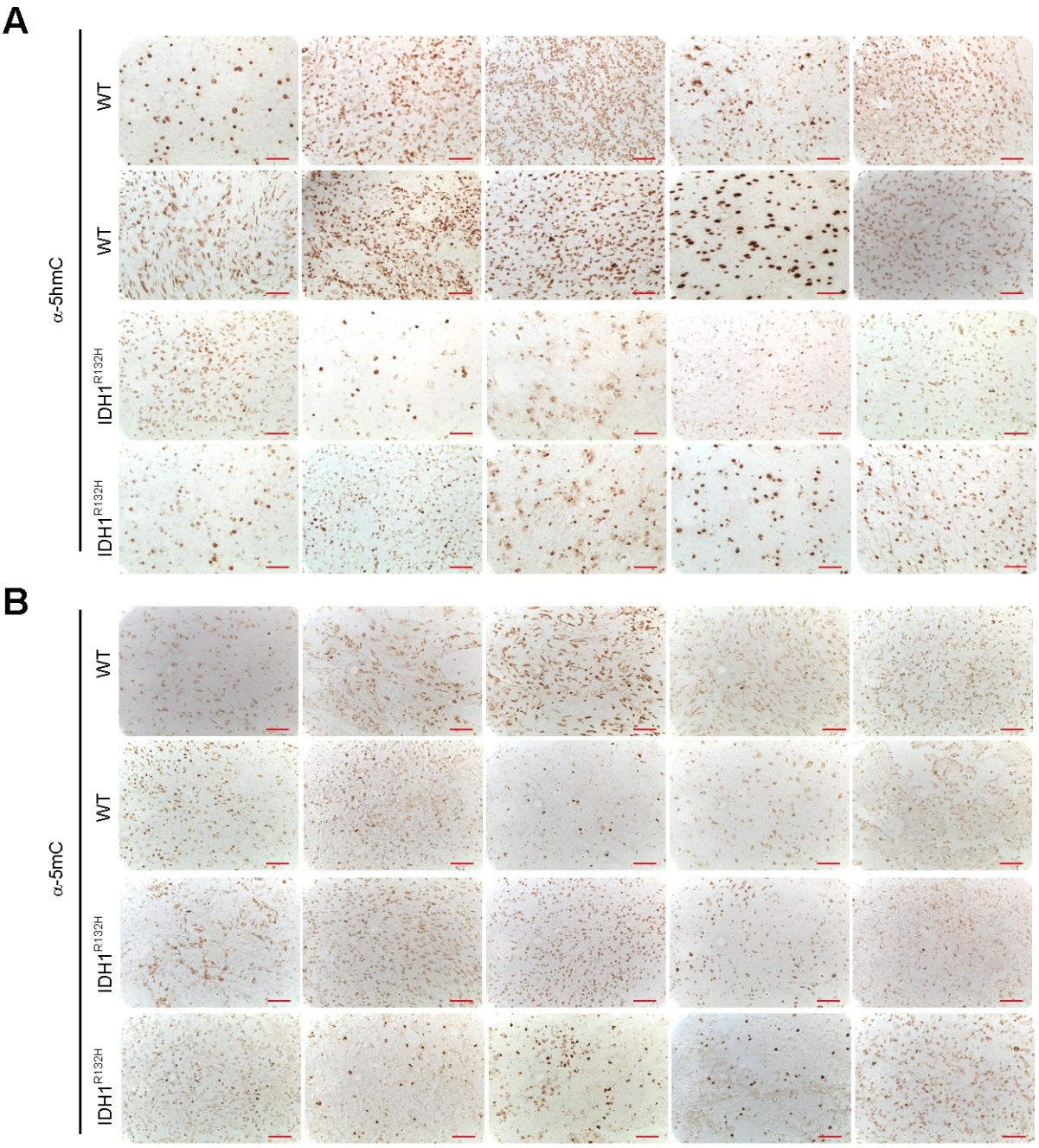



**Figure S8, related to Figure 8. Decreased 5hmC and increased 5mC levels in gliomas with mutant *IDH1*** (**A**, **B**) Randomly collected 20 glioma samples, 10 bearing *IDH1*$^{R132H}$ mutation and 10 containing wild type *IDH1*, were subject to immunohistochemistry (IHC) analysis for their 5hmC (**A**) and 5mC (**B**) level. IHC results are presented according to the order of staining intensity (from light to intense). Scale bars represent 50 μm. The quantification is presented in Figure 8C and 8D, respectively.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Cell culture, transfection, and western blotting

Human glioblastoma U-87MG cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum. U-87MG cells were transfected using Lipofectamine (Invitrogen) unless otherwise indicated specifically.

To examine the expression of endostatin, cells cultured on 60 mm plates were transfected with desired plasmids and were replaced with 2 ml conditioned media (CM) containing reduced serum (OptiMEM, Invitrogen) 12 h post-infection. Proteins were precipitated from CM by adding 100% trichloroacetic acid (TCA) to a final concentration of 20%. Samples were incubated for 1 h on ice and then centrifuged for 20 min. at 14,000 rpm in an Eppendorf microcentrifuge. Protein pellets were washed once with 80% ice-cold acetone, air dried and dissolved in 1X SDS sample buffer.

For western blotting of HIF-1a, methylated histone H3 and endostatin proteins, exponentially growing cells on 60 mm plates were washed with cold PBS once and lysed directly in 0.5 ml SDS loading buffer and heated at 99 $^{o}$C for 10 min. For western blotting of other proteins, cells were lysed by 0.5% NP40 lysis buffer and proteins were blotted following standard protocol. Antibodies to FLAG (Sigma-Aldrich), HIF-1a (Novus Biologicals), histone H3 (Genescript), H3K4me, H3K4me3, H3K9me2, H3K27me2, H3K7me2 (Abcam), H3K36me2 (Active motif), endostatin (Novus Biologicals), IDH1 (Abcam), H3 (Abcam), His (Santa Cruz), and actin (GenScript Corp) were purchased commercially.

### Crystallography study

His-tagged CeKDM7A was expressed in *E. coli* strain BL21 (DE3) grown at 15 °C. Cells were harvested and suspended in buffer A containing 25 mM Tris-Cl (pH 8.0), 150 mM NaCl, 10 mM imidazole. The soluble fraction of cell lysate was loaded onto Ni-NTA (GE Healthcare) affinity column. The protein was eluted and further purified by ion-exchange chromatography (Source 15Q, GE Healthcare) followed by gel filtration (Superdex 200, GE Healthcare) and concentrated to 15 mg ml$^{-1}$. The CeKDM7A crystals were grown by the hanging-drop vapor-diffusion method at 4 °C by mixing 1 µl of protein solution and 1 µl of reservoir solution containing 18% PEG1000, 0.1 M Bis-Tris, pH 6.6, 0.2 M Sodium Formate. Crystals were slowly equilibrated with a cryoprotectant buffer containing reservoir buffer plus 10% glycerol (v/v) and 300 mM D-2-HG and were flash frozen in a cold nitrogen stream at –173 °C.

### Primers for knocking down IDH1 and PHD2 and sequencing IDH1

*IDH1* knocking down was carried out using shRNA as described previously (Zhao et al., 2009). PHD2 knocking down was carried out using the following two synthetic siRNA oligonucleotides as previously described (Appelhoff et al., 2004; Berra et al., 2003); AAGGACATCCGAGGCGATAAG and CTTCAGATTCGGTCGGTAAAG. The knock down efficiency was verified by quantitative real-time polymerase chain reaction (qRT-PCR).

*IDH1* mutations were analyzed by PCR amplification over the entire exon 4 using a pair of primers derived from intron 3 and intron 4 (forward primer: 5'-TGAGCTCTATATGCCATCACTGC-3' and reverse primer: 5'-CAATTTCATACCTTGCTTAATGGG-3'), followed by sequencing using the same pair of primers.

### Chemical modification and synthesis procedures

For the synthesis of octyl-a-KG ester, a solution of a-ketoglutaric acid (2.92 g, 20 mmol) in methylene chloride (100 ml) was added to triethyl amine (2.8 ml, 20 mmol), followed by slow addition of octyl chloroformate (2.94 ml, 20 mmol) at ambient temperature. The resulting solution was stirred overnight. Dilute aqueous HCl was added after which the aqueous layer was extracted with ethyl acetate and the combined ethyl acetate layers were dried over $Na_2SO_4$, concentrated and purified via silica gel chromatography to provide octyl a-ketoglutarate (0.8 g, 15%). $^{1}$H NMR (400MHz, $CDCl_3$) d: 4.27 (t, 2H, *J* = 6.8 Hz), 2.90 (bs, 2H), 2.74 (t, 2H, *J* = 7.2 Hz), 1.72 (m, 2H), 1.36-1.27 (m, 10H), 0.88 (t, 3H, *J* = 6.8 Hz); $^{13}$C NMR (100MHz, $CDCl_3$) d: 192.4, 177.8, 160.7, 66.9, 34.1, 31.9, 29.2, 28.4, 27.4, 25.9, 22.7, 14.2; LC-MC (Agilent Eclipse Plus 4.6 x 50 mm, 1.8 µM, C18 column ,10%-100% MeOH in $H_2O$, 5 min, then 100% MeOH for 2 min): $t_R$ = 5.62 min, MS (ESI) 257.0 [M-H]$^{-}$.

For synthesis of racemic octyl-2-HG, sodium borohydride (0.032 g, 0.84 mmol) was added to a solution of octyl a-ketoglutarate (0.18 g, 0.7 mmol) in 10 ml THF at -78 $^{o}$C. The resulting mixture was warmed to 0 $^{o}$C

over 4 h before being quenched with water, followed by dilute aqueous HCl. The aqueous layer was extracted with ethyl acetate, dried over $Na_2SO_4$, concentrated and purified via silica gel chromatography to provide octyl 2-hydroxyglutarate (0.1 g, 55%). $^1$H NMR (400MHz, $CD_3OD$) d: 4.19 (dd, 1H, *J* = 4.4 Hz), 4.15 (m, 2H), 2.51-2.36 (m, 2H), 2.12-2.04 (m, 1H), 1.94-1.85 (m, 1H), 1.70-1.63 (m, 2H), 1.40-1.31 (m, 10H), 0.90 (t, 3H, *J* = 6.8 Hz); $^{13}$C NMR (100MHz, $CDCl_3$) d: 176.6, 175.6, 70.7, 66.1, 32.9, 30.5, 30.4, 30.29, 30.28, 29.7, 27.0, 23.7, 14.4; LC-MC (Agilent Eclipse Plus 4.6 x 50 mm, 1.8 µM, C18 column ,10%-100% MeOH in $H_2O$, 5 min, then 100% MeOH for 2 min): $t_R$ = 5.66 min, MS (ESI) 259.2 $[M-H]^-$ .

For the synthesis of octyl-D-2-HG, disodium D-2-hydroxyglutarate (50 mg, 0.26 mmol) was dissolved in 2 ml trifluoroacetic anhydride at 0 $^o$C. After stirring at 0 $^o$C for 1 h, it was warmed to ambient temperature, stirred for another 2 h and the solvent was evaporated under reduced pressure. The residue was dissolved in 3 ml dry THF and octanol (0.2 ml, 1.3 mmol) was added at 0 $^o$C. After stirring for 2 h at 0 $^o$C, it was warmed to ambient temperature and stirred overnight. Water was added to quench the reaction, the mixture was extracted with ethyl acetate, the combined ethyl acetate layers were dried over $Na_2SO_4$, concentrated and purified via silica gel chromatography to provide octyl D-2-hydroxyglutarate (7 mg, 10%). NMR and LC-MS data are identical to that of racemic octyl 2-hydroxyglutarate.

For the synthesis of octyl-L-2-HG, 711 mg d-benzyl L-glutamate was dissolved in 24 ml water with 6 ml acetic acid and cooled to 0 ºC. 1 M sodium nitrite was slowly added with stirring to allow reaction temperature to rise to room temperature and it was stirred for additional 3 h. The solvents were evaporated and the resulting products were dissolved in 6 ml N, N-dimethylformamide (DMF). 252 mg sodium bicarbonate and 1.44 g iodooctane was added and stirred at room temperature for 24 h. The organic phase was washed with water and the product was obtained by evaporating the solvent, followed by column chromatography. 380 mg out of 392 mg (*L*)-5-benzyl 1-octyl 2-hydroxypentanedioate obtained from previous steps was dissolved in 10 ml methanol and 40 mg palladium on carbon hydrogenation was added. The reaction was stirred at room temperature and 1 atmospheric H2 pressure for 24 h. After filtration, evaporation and chromatographic purification, 282 mg (*L*)-4-hydroxy-5-(octyloxy)-5-oxopentanoic acid (octyl-L-2HG) was obtained.

To synthesize D-2-HG from D-2-HG dibenzyl ester, a suspension of D-2-HG dibenzyl ester (340 mg, 1.04 mmol, synthesized from L-2-HG previously) and 5% Pd/C (50 mg) in EtOAc (10 ml) was stirred under hydrogen atmosphere (1 atm) for 4 h, filtered through celite, and the filter cake was washed thoroughly with EtOAc. The combined filtrates was concentrated under reduced pressure, the residue was dissolved in EtOAc–MeOH (2:1), and filtered through a syringe fitted with a 0.45 µm microporous membrane filter. The filtrate was concentrated under reduced pressure and dried under high vacuum to afford D-2-HG (152 mg, 99% yield) as white crystals. $^1$H NMR was used to verify the purity of the product.

To synthesize D-2-HG from D-Glutamic acid 5-benzyl ester, a cooled (0 °C) solution of D-Glutamic acid 5-benzyl ester (948 mg, 4.0 mmol) in aqueous HOAc (50% (v/v), 20 ml) was added dropwise to a solution of $NaNO_2$ (828 mg, 12.0 mmol) in $H_2O$ (3 ml). After completion of the addition, the solution was kept at 0 °C for 2 h, and warmed to ambient temperature overnight. The solution was diluted with EtOAc (25 ml), and the aqueous layer was extracted with EtOAc (25 ml) twice. The combined organic layer was washed successively with water and brine, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography and eluted with $CHCl_3$-MeOH-AcOH solution (90:9:1) to afford L-2-HG 5-benzyl ester (768 mg, 81% yield) as colorless oil. A suspension of L-2-HG 5-benzyl ester (245 mg, 1.03 mmol) and 5% Pd/C (30 mg) in EtOAc (8 ml) was stirred under hydrogen atmosphere (101,325 Pa) for 4 hr, filtered through celite, and the filter cake was washed thoroughly with EtOAc. The combined filtrates was concentrated under reduced pressure, the residue was dissolved in EtOAc–MeOH (2:1), and filtered through a syringe fitted with a 0.45 µm microporous membrane filter. The filtrate was concentrated under reduced pressure and dried under high vacuum to afford D-2-HG (148 mg, 98% yield) as white crystals. The purity of the product was verified by $^1$H NMR.

**GC-MS measurement of cellular** a**-KG and 2-HG concentrations**

Procedures for measuring both a-KG and 2-HG were modified from a previously published protocol (Dang et al., 2009). Plasmids bearing GFP, IDH1-IRES-GFP and IDH1$^{R132H}$-IRES-GFP were transfected into cells with SunBio Trans-EZ (SunBio) reagent. 48 h the after transfection, the culture medium was replaced with DMEM supplemented with10% dialyzed fetal bovine serum and cells were maintained in new medium for another 24 h. Cells were quenched by adding 10 ml 80%(v/v) pre-cold (-80 ºC) methanol into culture plates immediately after removing the culture medium and were harvested by scrapping and lyophilization. Lyophilized samples were oximated with 20 mg ml$^{-1}$ methoxyamine hydrochloride in pyridine at 30 °C for 60 min. The samples were then derivatized at 70 °C for 30 min in 80 µl pyridine (Sigma-Aldrich) and 20 µl *N*-methyl-*N*-[tert-butyldimethylsilyl]

trifluoroacetamide (Sigma-Aldrich). After filtration, 3 µl of derivatized sample was injected for the gas chromatography-mass spectrometry (GC-MS) assay. The GC-MS analysis was performed with an Agilent 6890-5973 GC-MS system. The HP-5MS column (30 m×0.25 mm×0.25 µm) was used for GC-MS analysis. GC oven temperature was programmed from 60 °C to 180 °C at 5 °C/min and from 180 °C to 260 °C at 10 °C/min. The flow rate of carrier gas was set at 1 ml $min^{-1}$. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV. Relative a-KG and 2-HG concentrations were calculated by normalizing a-KG (29.86 min) and 2-HG (30.10 min) peak areas to the average of L-threonine (29.58 min), L-serine (29.96 min) and L-phenylalanine (30.74 min) peak areas.

**In vitro TET enzyme assay**

Briefly, 5 µg of purified proteins were incubated with 0.5 µg of double stranded oligonucleotide substrates in 50 mM HEPES, pH 8, 75 µM $Fe(NH_4)_2(SO_4)_2$, 2 mM Ascorbate, and 0.1mM a-KG with or without a various amount of 2-HG for 3 h at 37 °C. Oligonucleotide substrates were purified and then digested with *Msp*I. 5'-end of the digested DNA was treated with calf alkaline phosphatase and labeled with [γ-$^{32}$P]ATP and T4 polynucleotide kinase. Labeled fragments were ethanol-precipitated and digested with 10 µg of DNase I and 10 µg Phosphodiesterase I in the presence of 15 mM $MgCl_2$, 2 mM $CaCl_2$ at 37 °C. One µl of digestion product was spotted on a PEI-cellulose TLC plate (Merck) and separated in an isobutyric acid-water-ammonium hydroxide (66:20:2) running buffer.