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**Title**: SET7-mediated TIP60 methylation is essential for DNA double-strand break repair

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**Title:** SET7-mediated TIP60 methylation is essential for DNA double-strand break repair

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**Running Title:** TIP60 methylation by SET7 induces DNA repair.

**Keywords:** SET7, TIP60, methylation, DNA repair, epigenetics

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**ABSTRACT**

The repair of DNA double-strand breaks (DSBs) by homologous recombination (HR) is crucial for maintaining genomic integrity and is involved in numerous fundamental biological processes. Post-translational modifications by proteins play an important role in regulating DNA repair. Here, we report that the methyltransferase SET7 regulates HR-mediated DSB repair by methylating TIP60, a histone acetyltransferase and tumor suppressor involved in gene expression and protein stability. We show that SET7 targets TIP60 for methylation at K137, which facilitates DSB repair by promoting HR and determines cell viability against DNA damage. Interestingly, TIP60 demethylation is catalyzed by LSD1, which affects HR efficiency. Taken together, our findings reveal the importance of TIP60 methylation status by SET7 and LSD1 in the DSB repair pathway.

## INTRODUCTION

DNA damage repair plays a key role in maintaining genomic integrity. Failure of DNA damage repair leads to the accumulation of mutagenic effects, thus increasing the risk of numerous pathological processes, including tumorigenesis. Among DNA lesions, double-strand breaks (DSBs) are generated by endogenous and exogenous DNA damaging factors, such as ionizing radiation, ultraviolet (UV) light and certain highly toxic chemicals. Non-homologous end joining (NHEJ) and homologous recombination (HR) are the two main DSB repair pathways. Error-prone NHEJ is a template-independent DSB repair mechanism, which is a specialized DSB-end ligation reaction that is active throughout all phases of the cell cycle. In contrast, error-free HR requires an intact template DNA. Therefore, HR only occurs during the late S/G2 phase. HR, which relies on DSB end resection by Mre11-Rad50-NBS1/ExoI, CtIP, and helicase activity, achieves DNA repair with high fidelity. In particular, post-translational modifications (PTMs) of non-histone proteins are necessary for promoting DNA damage repair. Since unrepaired DNA is sufficient to induce genome instability, such as chromosome rearrangement or cancer development, many proteins involved in DNA repair systems are regulated by the modulation of PTMs for a rapid DNA damage response (DDR). For instance, the P300/CBP associated factor (PCAF)-mediated acetylation of RPA1 is necessary for nucleotide excision repair and protein arginine N-methyltransferase 5 (PRMT5)-dependent methylation of RuvB-like AAA ATPase 1 (RUVBL1) for HR (1, 2).

Ubiquitously expressed acetyltransferase TIP60 plays a role in multiple signaling pathways, including transcriptional regulation, nuclear receptor function, chromatin remodeling, and DNA repair (3). TIP60 acetylates both core histones H2A, H3, and H4, and different non-histone proteins associated with tumorigenesis and metastasis, such as p53, Twist (4-6). The acetyltransferase activity of TIP60 is regulated by various PTMs; for example, S86-

phosphorylation plays a central role in regulating TIP60-induced autophagy and apoptosis under various stress conditions (7, 8). Furthermore, TIP60 plays a key role in DSB repair by maintaining genomic integrity and regulating the repair of DNA damage through its histone acetyltransferase (HAT) activity (9).

Histone H3K4-specific monomethyltransferase, SET7, is a prime methyltransferase for non-histone proteins. To date, more than 30 non-histone SET7 targets that are involved in various cellular processes, including transcriptional regulation, differentiation, and response to DNA damage, have been identified. In particular, SET7 plays a critical role in proper DDR by promoting the enzymatic activity of DDR proteins or regulating the binding affinity of DDR-associated transcription factors. For example, SET7-mediated methylation of poly [ADP-ribose] polymerase 1 (PARP1) shows enhanced enzymatic activity, and catalytically activated PARP1 is required for activating other DDR proteins (10).

In this study, we found that TIP60 is methylated by SET7 at K137 in response to DNA damage. We showed that DNA damage caused by a potent DNA-damaging agent, hydroxyurea (HU), induces SET7-mediated TIP60 methylation and facilitates HR repair. Additionally, we identified that LSD1 causes the demethylation of TIP60 and regulates the DNA damage repair process. Finally, we revealed that methylation of TIP60, which is required for HR-mediated DSB repair, promotes cellular proliferation in HCT116 colon cancer cells.

## RESULTS

### SET7 methylates TIP60 at Lys 137 *in vitro* and *in vivo*

TIP60 is an acetyltransferase that plays a role in DNA repair and apoptosis by acetylating histones. Moreover, it plays an important role in DDR signal induction (9). To further investigate the post-translational modification of TIP60 in the DNA repair process, we determined TIP60 methylation by SET7. We selected SET7 among the methyltransferases because it is known as a major methyltransferase for various non-histone proteins, and we also reported that UHRF1 methylation by SET7 is required for DSB repair (11). First, we performed an *in vitro* methylation assay by incubating recombinant GST-TIP60 and GST-SET7. Interestingly, TIP60 was methylated by SET7 in a dose-dependent manner; however, the catalytic null mutant SET7 H297A did not methylate TIP60 (Fig. 1A). To determine whether SET7 methylated TIP60 *in vivo*, we tested the methylation level of TIP60 via an IP assay with anti-methyl lysine antibodies. An increase in TIP60 methylation levels was observed in HCT116 cells overexpressing SET7 (Fig. 1B). When SET7 was ectopically overexpressed in SET7 knockdown cells, the methylation level of TIP60 was increased; in contrast, SET7 H297A did not rescue the methylation level of TIP60 (Supplementary Fig. 1A). The methyltransferase activity of SET7 is known to be inhibited by (R)-8-fluoro-N-(1-oxo-1-(pyrrolidin-1-yl)-3-(3-(trifluoromethyl)phenyl)propan-2-yl)-1,2,3,4-tetra-hydroisoquinoline-6-sulfonamide hydrochloride (R-PFI) (12). When cells were treated with R-PFI, TIP60 methylation was significantly inhibited in HCT116 cells (Fig. 1C). To accurately investigate the methylation site of TIP60, we performed an *in vitro* methylation assay using TIP60 deletion constructs TIP60 Δ1, Δ2, and Δ3. Among the deletion constructs tested, only TIP60 Δ2 was methylated by SET7 (Fig. 1D). To further determine the exact major methylation sites of TIP60, liquid chromatography coupled with tandem mass

spectrometry (LC-MS/MS) was performed on a high-resolution orbitrap instrument, after an *in vitro* methylation assay, using recombinant SET7 and TIP60. Results of mass spectrometry showed that the methylated lysine residue of TIP60 was K137 (Fig. 1E upper panel). An *in vitro* methylation assay was performed with SET7 and TIP60 Δ2 mutants in which K137 was replaced with arginine (TIP60 Δ2 K137R) to confirm the results of the LC-MS/MS analysis. Compared with TIP60 Δ2, the methylation of TIP60 in TIP60 Δ2 K137R was not detected (Fig. 1E lower panel). Interestingly, TIP60 K137 is an evolutionarily conserved residue across various species, indicating the functional importance of this residue (Supplementary Fig. 1B). This indicates that the major methylation site of TIP60 is K137. When we compared the methylation levels between TIP60 WT and TIP60 K137R *in vivo*, the methylation level of TIP60 K137R was lower than that of TIP60 WT (Fig. 1F). Taken together, our data suggest that SET7 methylates K137 of TIP60 both *in vitro* and *in vivo*. Next, we examined the interaction between TIP60 and SET7. Formerly, we confirmed that TIP60 methylation by SET7 did not affect TIP60 expression levels or TIP60-mediated acetylation (Supplementary Fig. 2A, B). TIP60 is mainly localized in the nucleus (4). Therefore, we analyzed the cellular fraction to confirm that SET7 did not affect TIP60 localization (Supplementary Fig. 2C). Co-IP assays were performed by overexpressing Flag-TIP60 and pcDNA3.1-SET7, and the results showed that TIP60 interacts with SET7 *in vivo* (Fig. 1G). To identify the interaction between TIP60 and SET7 *in vitro*, we performed a GST pull-down assay using TIP60 constructs (Supplementary Fig. 3A). Next, we performed a co-IP assay by overexpressing Flag-empty vector (EV) or Flag-TIP60 and pcDNA3.1-SET7 to prove the interaction between SET7 and TIP60 in cells, and the results showed that TIP60 interacts with SET7 *in vivo* (Supplementary Fig. 3B). Together, these data suggest that SET7 interacts with TIP60 both *in vitro* and *in vivo*.

**HU-mediated DNA damage induces TIP60 methylation and promotes HR for DSB repair**

HU is a replication inhibitor that can induce DSB by depleting the nucleotide pool and causing replication fork arrest (13). We set the experimental condition for HU treatment at 5 mM HU concentration 4 hours because we identified that the apoptosis was induced enough in this condition (Supplementary Fig. 4A). To investigate whether TIP60 methylation is important for DNA damage signals, we evaluated the methylation level of TIP60 after treating HCT116 cells with HU. The methylation level of TIP60 was significantly increased by HU-induced DNA damage (Fig. 2A). However, HU did not induce TIP60 methylation when SET7 was depleted (Supplementary Fig. 4B). To investigate whether the interaction between TIP60 and SET7 is affected by HU-induced DNA damage, we performed an IP assay in the presence or absence of HU. Remarkably, the interaction between TIP60 and SET7 was significantly increased in response to DNA damage by HU (Supplementary Fig. 4C).

HU, which is primarily dynamic in the S phase of the cell cycle, induces HR in mammalian cells by inhibiting replication (14, 15). Additionally, HR and SET7 are associated with DDR by catalyzing the methylation of DDR protein ARTD1, and HR occurs during the S and G2 phases of the cell cycle (10, 16). Therefore, we focused on the effect of TIP60 methylation by SET7 on the HR repair mechanism.

To investigate the relationship between TIP60 methylation and its role in HR, we measured the TIP60 methylation level in each phase of the cell cycle. As shown in Fig. 2B, TIP60 methylation was increased in the S phase, implying that methylation of TIP60 may play a role in HR. Our data demonstrated that methylation of TIP60 is induced by SET7 in response to

DNA damage. Accordingly, we performed an integrated reporter assay to determine whether TIP60 methylation affects HR efficiency (Fig. 2C upper panel). Since TIP60 methylation by SET7 induces the HR process, we tested whether methylation-deficient TIP60 K137R could promote HR. The HR reporter assay showed that TIP60 WT promoted HR in SET7 overexpressing cells, but not in TIP60 K137R expressing cells (Fig. 2C lower panel). In addition, when we overexpressed SET7 WT and SET7 H297A (catalytic mutant) in HCT116 cells and measured HR efficiency, the result showed that TIP60 methylation by SET7 promoted HR (Supplementary Fig. 4D). To determine the effect of TIP60 on RPA and Rad51 foci formation on DNA damage sites, HU-treated shTIP60 cells were subjected to an immunofluorescence staining assay. In contrast to the effects of TIP60 WT, RPA foci was blocked in cells transfected with TIP60 K137R (Fig. 2D). We also identified Rad51 foci in TIP60 WT (Supplementary Fig. 4E).

Next, we performed a neutral comet assay using shTIP60 cells to determine whether TIP60 methylation by HU promotes DNA repair. The length of the comet tail moment indicates the degree of DNA breakage. In TIP60 K137R cells, the comet tail moment increased significantly after HU treatment, whereas in TIP60 WT cells, the tail moment was shorter. Also, when compared to the control, the comet tail moment of TIP60 K137R cells was increased more than that of TIP60 WT after HU treatment (Fig. 2E). These data indicate that the degree of DNA breaks in TIP60 WT cells was significantly lower than that in TIP60 K137R cells. Taken together, our data suggest that HU increases SET7-mediated TIP60 methylation and this promotes HR in DNA-damaged cells.

**LSD1 mediates demethylation of TIP60**

SET7 can methylate histone H3K4, and LSD1 is the major H3K4 demethylase (17, 18).

Studies have shown that LSD1 demethylates SET7-mediated methylated proteins, including UHRF1 and DNMT (11, 19). Before investigating whether LSD1 can catalyze the demethylation of TIP60, we performed an IP assay to check the interaction between LSD1 and TIP60. The results showed that endogenous TIP60 interacted with LSD1 in HCT116 cells (Fig. 3A). Next, we found that the methylation level of TIP60 was increased in cells treated with the LSD1 inhibitor, GSK-LSD1 (Fig. 3B), suggesting that LSD1 is responsible for the demethylation of TIP60. To further test this, we overexpressed empty vector or Flag-tagged LSD1 in LSD1 knockdown HCT116 cells and performed IP assays with an anti-methyl lysine antibodies. The methylation level of TIP60 was significantly increased in LSD1 knockdown cells, whereas the methylation level was decreased in cells rescued by LSD1 overexpression (Supplementary Fig. 5). Since we identified that TIP60 methylation was damage-dependent in this study, we performed an IP assay to determine whether demethylation of TIP60 by LSD1 is also damage-dependent. We found that the increased level of TIP60 methylation in HU-treated cells was decreased when LSD1 was overexpressed (Fig. 3C). To investigate the effect of TIP60 demethylation by LSD1 on HR, we verified HR efficiency using an HR reporter assay. LSD1 reduced HR efficiency with TIP60 WT, but LSD1 did not affect HR efficiency in TIP60 K137R-transfected cells (Fig. 3D). Together, these data showed that methylation of TIP60 is regulated by SET7 and LSD1 in the DNA repair process.

**SET7-mediated TIP60 methylation promotes colon cancer cell proliferation**

Substances such as HU, thymidine, and camptothecin induce replication fork collapse and strongly induce HR in mammalian cells. These substances require HR for cell survival (15). UHRF1 methylation by SET7 is necessary for cell survival in response to UV exposure (11). Therefore, we investigated whether SET7-mediated TIP60 methylation regulates cell

proliferation and apoptosis upon HU treatment by flow cytometry. It was confirmed that TIP60 K137R increased apoptosis in a HU-dependent manner compared to TIP60 WT (Supplementary Fig. 6A, Fig. 4A upper panel). We also investigated apoptosis through the expression level of cleaved caspase-3, an apoptosis marker (Fig. 4A lower panel). Additionally, we measured apoptosis in shLSD1 cells by treating HU. In cells overexpressing TIP60 in shLSD1, there was no change in apoptosis before and after HU was treated, and in control cells, the number of apoptotic cells increased compared to shLSD1 when HU was treated (Supplementary Fig. 6B). Next, we observed the viability of cells with TIP60 WT and methylation-deficient mutant overexpression treated with 5 mM HU for 4 h. We performed a colony formation assay. The number of colonies increased in TIP60 WT cells. However, we observed a decrease in the number of colonies in the methylation-deficient mutant (TIP60 K137R)-treated cells (Fig. 4B). To further investigate these observations, we performed a MTT assay. TIP60 knockdown cells transfected with TIP60 WT showed higher cell proliferation compared to TIP60 knockdown cells. In contrast, cells transfected with the methylation-deficient mutant (TIP60 K137R) did not show a significant increase in cell proliferation compared to the TIP60 knockdown cells (Fig. 4C). TIP60 knockdown cells showed increased proliferation compared to that of control cells, which was in agreement with the finding that overexpression of TIP60 reduced HCT116 proliferation (20). In addition, we performed MTT assay with LSD1 inhibitor, GSK-LSD1. Control cells showed increased cell proliferation when treated with GSK-LSD1 and similar results were obtained in TIP60 overexpressing cells (Supplementary Fig. 6C). Altogether, our data suggest that SET7-dependent methylation of TIP60 promotes cancer cell proliferation.

## DISCUSSION

Our study presents a new perspective on TIP60 function in the DNA repair process by PTMs. We showed that SET7 methylates TIP60 and promotes the HR-mediated DSB repair process. Specifically, our current study suggests that TIP60 methylation, which is significantly increased in the S phase, effectively promotes HR. Aberrant expression of TIP60 has been reported in prostate and colorectal cancer (20, 21). However, reduced levels of nuclear TIP60 have been detected in breast cancer, which demonstrates that TIP60 functions to protect cells from genomic instability (22). Studies suggest that TIP60 acetylates ataxia-telangiectasia mutated (ATM) and induces autophosphorylation to activate DDR in cancer cells. Furthermore, TIP60-mediated DNA damage repair is essential for the maintenance of kidney cells (23). These results, along with those of our current study, suggest that TIP60 is not only involved in the maintenance of differentiation but also the proliferation of cells via the regulation of the DDR signaling process.

SET7 plays multiple roles in DDR by catalyzing the methylation of a series of non-histone proteins such as p53, E2F1, and SIRT1(24, 25). Additionally, we have identified that UHRF1 is methylated by SET7 in response to DNA damage, polyubiquitinates PCNA, and promotes HR progression (11). Several studies have indicated that SET7 is also involved in the regulation of PARP1 activity. To further elucidate the mechanism by which TIP60 methylation by SET7 induces the HR repair pathway, it is necessary to investigate PARP1 as a mediator that recruits and interacts with TIP60 in damaged lesions in more detail. In addition, SET7 is predominantly in the cytosol and TIP60 is located in nucleus, as indicated by supplementary Fig. 2C. However, further research is needed on where and how TIP60 goes when it is methylated by SET7.

A recent study suggested that the histone demethylase LSD1 is recruited to DNA damage sites, where it is involved in DSB repair via interaction with the E3 ubiquitin ligase RNF168 (26). We also reported that UHRF1 is methylated by SET7 in response to DNA damage and that LSD1 reduces UHRF1 methylation, further blocking HR progression, suggesting an interesting role of the SET7-LSD1 axis in DDR signaling (11). Since LSD1 demethylates SET7-mediated methylated proteins and plays both roles in DDR, additional research is needed on the relationship between SET7 and LSD1 on TIP60 during DNA damage.

Overall, we found that TIP60 is methylated by SET7 and that this SET7-dependent methylation of TIP60 induced by DNA damage is essential for the HR repair pathway. Furthermore, we showed that TIP60 methylation by SET7 promoted cell proliferation and the potential relationship between TIP60 methylation and the HR repair pathway (Fig. 4D). In this study, we present a novel mechanism of DNA damage repair based on the methylation status of TIP60 by SET7 and LSD1.

## MATERIALS AND METHODS

Materials and methods are available in the supplemental material.

**ACKNOWLEDGMENTS**

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## FIGURE LEGENDS

**Figure 1. SET7/9 methylates TIP60 *in vitro* and *in vivo.*** (A) Purified GST-TIP60 was incubated overnight at 30 °C with increasing amounts of GST-SET7. Reaction mixtures were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained by Coomassie or exposed to an autoradiographic film. (B) SET7 was overexpressed in HCT116 cells. IPs using anti-methyl lysine antibody were performed. Methyl lysine levels were normalized by input of TIP60. (C) HCT116 cells were treated with 1uM SET7 inhibitor R-PFI for 24 h. IPs using an anti-methyl lysine antibody were performed. Methyl lysine levels were normalized by input of TIP60. (D) Purified GST-TIP60 deletion mutants were incubated overnight at 30 °C with GST-SET7. Reaction mixtures were separated by SDS-PAGE and analyzed via a phosphorimager. (E) Mass spectrometry analysis (LC-MS/MS) was performed, and methylation status of the K137 residue was determined. Purified GST-TIP60Δ2 or GST-TIP60Δ2 point mutants were incubated overnight at 30 °C with GST-SET7. Reaction mixtures were separated by SDS-PAGE and analyzed via a phosphorimager. (F) HEK293T cells were transfected with the indicated plasmids and IPs using an anti-methyl lysine antibody were performed. Methyl lysine levels were normalized by input of TIP60. (G) pcDNA3.1-SET7 and Flag-TIP60 were overexpressed in HCT116 cells. The cell lysates were immunoprecipitated with an anti-SET7 antibody. Associated proteins were eluted, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotted using the indicated antibodies.

**Figure 2. Hydroxyurea (HU)-mediated DNA damage induces SET7-dependent methylation of TIP60 and induces homologous recombination (HR).**

(A) HCT116 cells were treated with 5 mM HU for 4 h. IPs using an anti-methyl lysine antibody were performed. Methyl lysine levels were normalized by input of TIP60. (B)

HCT116 cells were arrested at the G1/S checkpoint by double thymidine block/release, and the cells were then treated with 5 mM HU for 4 h. IPs using an anti-methyl lysine antibody were performed. Methyl lysine levels were normalized by input of TIP60. Results were shown as the mean ± SEM; n = 3, ***P<0.001, **P < 0.01. (C) Schematic diagram of the HR reporter. PcDNA3.1-SET7, Flag-TIP60 WT, or Flag-TIP60 K137R was subjected to HR assay. Results were shown as the mean ± SEM; n = 5, *P < 0.05, N.S: no significant difference. (D) HCT116 cells with TIP60 knockdown were transfected with TIP60 WT or TIP60 K137R. RPA foci was examined following 5 mM HU treatment for 4 h. (E) HCT116 shTIP60 cells overexpressing SET7 were transfected with the indicated plasmid. Then, cells were treated with HU and subjected to a neutral comet assay. The tail moment was determined using CaspLab software, and 90 individual comets were counted for each sample (lower panel). Results were shown as the mean ± SEM; n = 90, ***P < 0.001.

**Figure 3. LSD1 demethylates TIP60 methylation.**

(A) Cell extracts from HCT116 cells were immunoprecipitated with anti-TIP60 antibodies. Immunoprecipitates were eluted, resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotted with the indicated antibodies. (B) HCT116 cells were treated with 500 nM GSK-LSD1 for 24 h. IPs using an anti-methyl lysine antibody were performed. Methyl lysine levels were normalized by input of TIP60. (C) HCT116 cells were transfected with Flag-EV of Flag-LSD1 and treated with 5 mM HU for 4 h. IPs using an anti-methyl lysine antibody were performed. Methyl lysine levels were normalized by input of TIP60. (D) Flag-LSD1, Flag-TIP60 WT, or Flag-TIP60 K137R were subjected to the homologous recombination (HR) assay. Results were shown as the mean ± SEM; n = 5, ***P < 0.001, N.S: no significant difference.

**Figure 4. TIP60 methylation by SET7 promotes cell proliferation.**

(A) HCT116 cells were transfected with the indicated plasmids and treated with 5 mM HU for 4 h. Apoptotic cells were measured by fluorescence-activated cell sorting (FACS) analysis, and protein expression was confirmed through a western blot assay. Results were shown as the mean ± SEM; n = 3, **P < 0.01, N.S: no significant difference. (B) Representative colony formation assay was performed using HCT116 cells with TIP60 knockdown transfected with TIP60 WT or TIP60 K137R. Cells were treated with 5 mM HU for 4 h and incubated in fresh media for 7 days. Results were shown as the mean ± SEM; n = 3, *P < 0.05, N.S: no significant difference. (C) Cell viability was determined using the MTT assay. HCT116 cells with TIP60 knockdown were transfected with TIP60 WT or TIP60 K137R. Cells were treated with 5 mM HU for 4 h and incubated in fresh media for 0–72 h. Results were shown as the mean ± SEM; n = 3, **P < 0.01, N.S: no significant difference. (D) Schematic showing how SET7-mediated TIP60 methylation promotes the homologous recombination (HR)-mediated DNA repair pathway.

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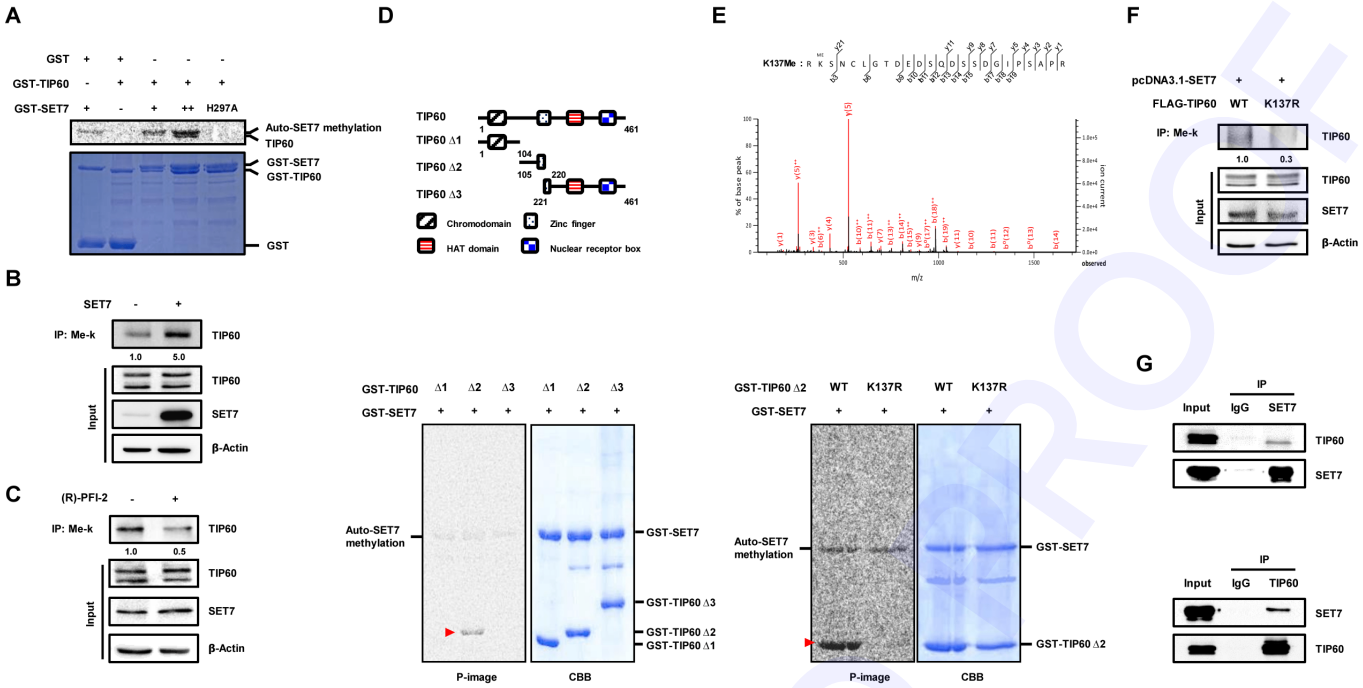


Fig. 1. Figure 1

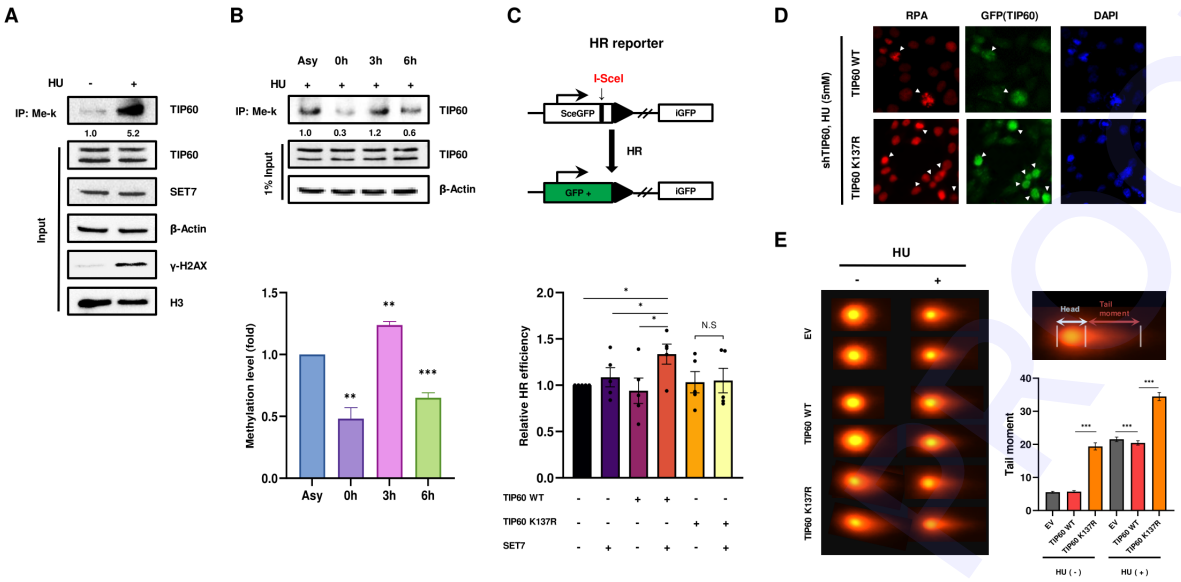


Fig. 2. Figure 2-revised

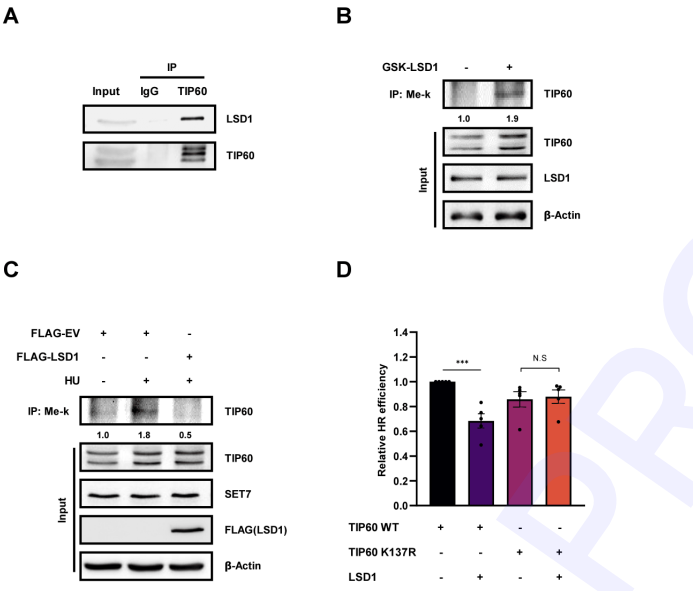


Fig. 3. Figure 3

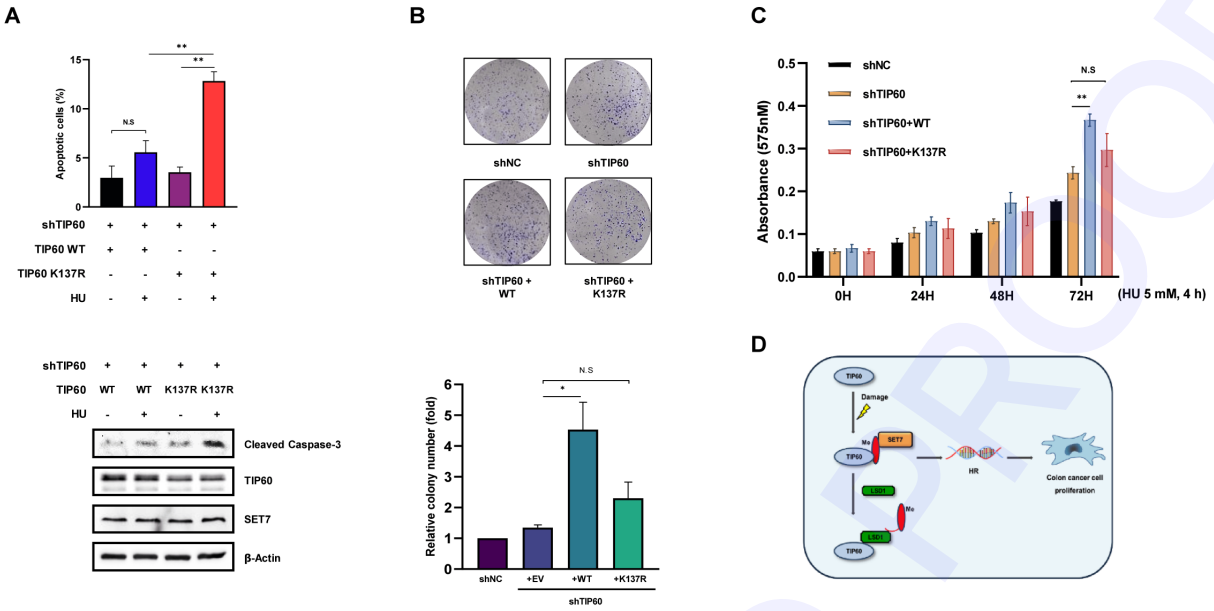


Fig. 4. Figure 4-revised

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**Running Title:** TIP60 methylation by SET7 induces DNA repair.

**Keywords:** SET7, TIP60, methylation, DNA repair, epigenetics

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## MATERIALS AND METHODS

### Plasmid constructs

TIP60 (residues 1–461), TIP60 K137R, and partial constructs of human TIP60 were sub-cloned into the pGFP-C1 vector (Clontech), bacterial expression vector pGEX-4T2 (Invitrogen). SET7 (residues 1–366) of human SET7 (#24084; Addgene) was amplified by PCR and then sub-cloned into the bacterial expression vector pGEX-4T2. A double-stranded oligonucleotide for shRNA plasmid construction was produced using primers from the 5′ to the 3′ end. The shTIP60 and shSET7 RNA oligonucleotide sequences were as follows: shTIP60: 5′–CCGGTCGAATTGTTTGGGCACTGATCTCGAG ATCAGTGCCCAAACAATTCGATTTTTG–3′ (top strand) and 5′–AATTCAAAAATCGAA TTGTTTGGGCACTGATCTCGAGATCAGTGCCCAAACAATTCGA–3′ (bottom strand); shSET7: 5′–CCGGGCCAGGGTATTATTATAGAATCTCGAGATTCTATAATAATACCC TGGCTTTTT–3′ (top strand) and 5′– AATTCAAAAAGCCAGGGTATTATTATAGAATCT CGAGATTCTATAATAATACCCTGG –3′ (bottom strand). 5′– CCGGAGGAAGGCTCTTC TAGCAATACTCGAGTATTGCTAGAAGAGCCTTCCTTTTTTG –3′ (top strand) and 5′–AATTCAAAAAAGGAAGGCTCTTCTAGCAATACTCGAGTATTGCTAGAAGAGCCT TCCT–3′ (bottom strand). These oligonucleotides were in serted into the *Age*I/*Eco*RI site of the pLKO.1 TRC vector. The siRNA sequence of the negative control (siNC) was as follows: negative control, 5′– CCUCGUGCCGUUCCAUCAGGUAGUU –3′.

### Antibodies

Antibodies against SET7 (sc-390823), p-histone H2A.X (sc-517348), RPA (sc-56770), GFP (sc-9996), β-actin (sc-47778), TIP60 (sc-166323), and LSD1 (sc-271720) were purchased from

Santa Cruz Biotechnology (Santa Cruz Biotechnology). Methyl-lysine (Me-K; ab174719, ab23366) (Abcam), Flag (F3165) (Sigma-Aldrich), anti-caspase 3 (AB1899) (millipore), Rad51 (GTX70230) (Genetex) were employed.

**Cell culture and transfection**

HEK293T and U2OS cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco), and HCT116 cells were grown in RPMI 1640 medium (Gibco) containing 10% heat-inactivated fetal bovine serum (Gibco) and 0.05% penicillin-streptomycin (Welgene) at 37 °C in a 5% $CO_2$ atmosphere. HCT116 WT and U2OS cells were transfected with the indicated DNA constructs using polyethyleneimine (PEI) (Polyscience) or Lipofectamine 2000 (Invitrogen).

***In vitro* methylation assay**

Methylation was performed at 30 °C for 3 h in 30 μL volumes containing 50 mM Tris-HCl [pH 8.5], 20 mM KCl, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 1.25 M sucrose, 100 nCi of [$^{14}$C]-SAM (Perkin Elmer), GST-TIP60, GST-TIP60 Δ1 (residues 1–104), GST-TIP60 Δ2 (residues 105–220), GST-TIP60 Δ3 (residues 221–461), and 2 μg of GST-SET7. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by autoradiography. TIP60 K137R was synthesized based on the N-terminal amino acid sequences of H3 histone (Cosmo Genetech), filtered using p81 filter paper (Upstate), and washed three times with cold 10% trichloroacetic acid (TCA) and 95% ethanol for 5 min at room temperature. The filters were allowed to air dry, after which 2 mL of Ultima Gold (Perkin Elmer) was added, and the [$^{14}$C]-SAM was quantified using a scintillation counter.

**Immunoprecipitation (IP) assay**

Cells were lysed in a lysis buffer (50 mM Tris-HCl [pH 7.5], 200 mM NaCl, 0.5% NP-40, 1× protease inhibitor cocktail) and incubated with the indicated antibodies overnight at 4 °C. Protein A/G agarose beads (GenDEPOT) were then added, and the mixture was gently rotated for 3 h at 4 °C. Bound proteins were analyzed by immunoblotting with the indicated antibodies.

**LTQ-orbitrap mass spectrometry**

Samples were separated by SDS-PAGE and were isolated via gel extraction. After overnight trypsin digestion at 37 °C, the eluted peptides were separated using a C18 column with a linear gradient (A: 100% H2O, 0.1% formic acid and B: 100% ACN) at a flow rate of 300 nl/min. Typically, 2 μL of the sample was injected. Mass spectrometry was performed with a dual-mass spectrometer (LTQ Orbitrap Velos; Thermo Scientific) coupled to a nano-LC system (EASY nLC; Thermo Scientific). This method consisted of a cycle combining one full MS scan (mass range: 150–2000 m/z). Proteins were identified from the MS/MS spectra using SEQUEST.

**DNA repair assay (HR reporter assay)**

An integrated DNA repair reporter system was used to determine the HR efficiency. U2OS cells integrated with an HR reporter were transfected with the indicated constructs and the I-SceI plasmid, which induces DSB. Cells were harvested 48 h after transfection, and the percentage of GFP-positive cells was determined by fluorescence-activated cell sorting (FACS) analysis using a BD Accuri C6 cytometer (BD Biosciences). Data were analyzed using BD Accuri C6 software (BD Biosciences). Repair frequencies represent the mean of at least three independent experiments.

**Immunofluorescence staining**

HCT116 TIP60 knockdown cells were cultured on coverslips and transfected with Flag-TIP60 WT or methylation-deficient TIP60 (Flag-TIP60 K137R) using Lipofectamine 2000 Reagent (Thermo Fisher Scientific). After treatment with 5 mM HU for 4 h, the cells were fixed in 4% paraformaldehyde for 1 h and permeabilized in 0.2% Triton X-100 solution for 10 min at room temperature. Cells were blocked with 1% bovine serum albumin in PBS and incubated with a primary antibody for 2 h. Cells were washed with PBS and incubated with a secondary antibody for 1 h. The coverslips were mounted onto glass slides and visualized using an ECLIPSE 80i fluorescence microscope (Nikon, Tokyo, Japan).

**Comet assay**

After treatment of cells with HU for 4 h, the media were replaced with fresh media, and the cells were incubated at 37 °C. After harvesting the next day, neutral comet assay was performed. The cells were mixed with 1% UltraKem LE Agarose (Young Science; 1:10 [v/v]), and upon agarose solidification, the cells were lysed with a lysis buffer (2% sarkosyl, 0.5 M EDTA-$Na_2$, and 0.5 mg/mL proteinase K, pH 8.0) at 37 °C overnight, followed by three 20 min washes with an electrophoresis buffer (90 mM Tris buffer, 90 mM boric acid, and 2 mM EDTA- $Na_2$, pH 8.5). The proteins in the washed gels were subjected to electrophoresis at 20 V for 25 min. The slides were stained with propidium iodide (Sigma-Aldrich), and fluorescence images were captured using an Olympus BX53 epifluorescence microscope (Olympus). The tail moment was quantified using CASP version 1.2.3 beta2 (CaspLab).

**MTT assay**

HCT116 cells (shTIP60) and control cells were transfected with FLAG-TIP60 WT or FLAG-TIP60 K137R and seeded in 48-well plates at a density of $5 \times 10^3$ cells per well. After 24 h, the cells were treated with 5 mM HU for 4 h, and the medium was replaced with a fresh medium.

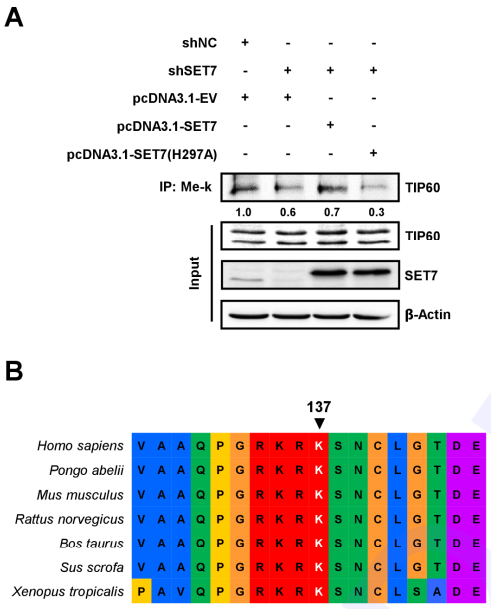
At 24, 48, and 72 h after HU treatment, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to the cells (final concentration 0.5 mg/mL), after which the cells were incubated further for 2h at 37 °C. The medium was then removed, and dimethyl sulfoxide (DMSO) was added (200 µL/well). Finally, the OD was determined using a microplate spectrophotometer (BioTek) at a wavelength of 575 nm.

**Colony formation assay**

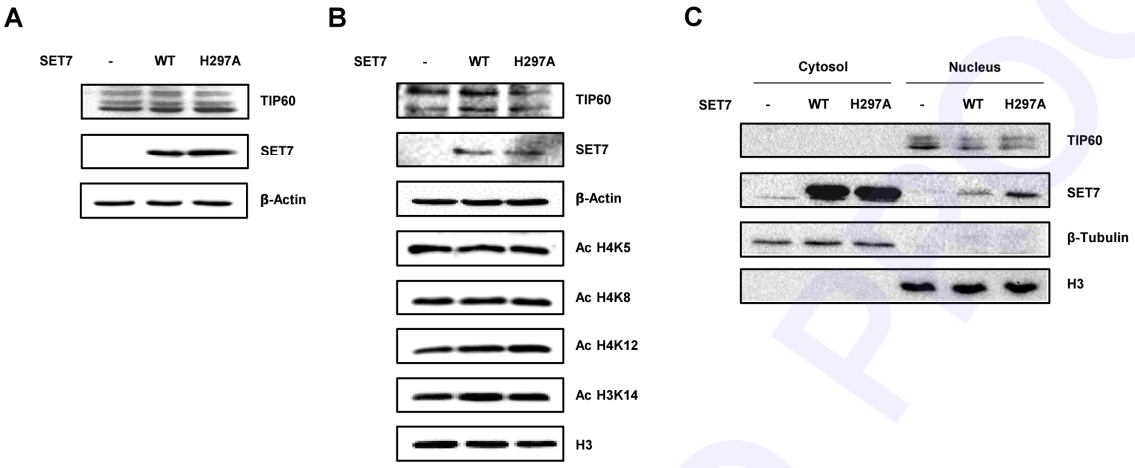
Cells transfected with FLAG-TIP60 WT or FLAG-TIP60 K137R were seeded in 35 mm culture dishes at a density of $5 \times 10^3$ cells per well. After 24 h, cells were treated with 5 mM HU for 4 h, and the medium was replaced with a fresh medium. After incubation for 6 days, surviving colonies were stained with 0.005 % crystal violet.

**Statistical analysis**

Data are expressed as the mean ± standard error of the mean (SEM) of three independent experiments. Data were analyzed using GraphPad Prism (version 9; GraphPad Software, USA). Differences between the groups were evaluated by Student's *t*-test. $P < 0.05$ was considered statistically significant.

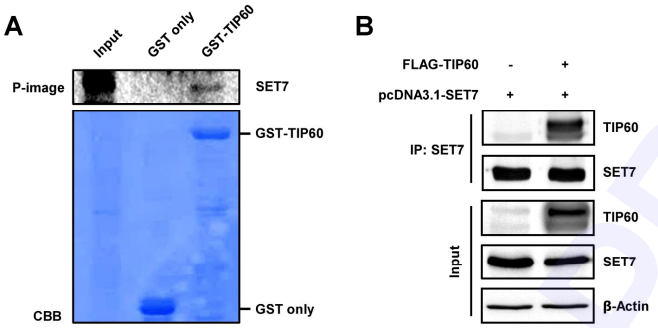



**Supplementary Fig. 1. SET7 methylates TIP60 at Lys 137, and the region is conserved in various species.** (A) HCT116 cells were transfected with the indicated plasmids and immunoprecipitated with anti-methyl lysine antibodies. Associated proteins were eluted, resolved by SDS-PAGE, and immunoblotted with indicated antibodies. Methyl lysine levels were normalized by input of TIP60. (B) Conserved region in various species. The arrow indicates TIP60 at K137.

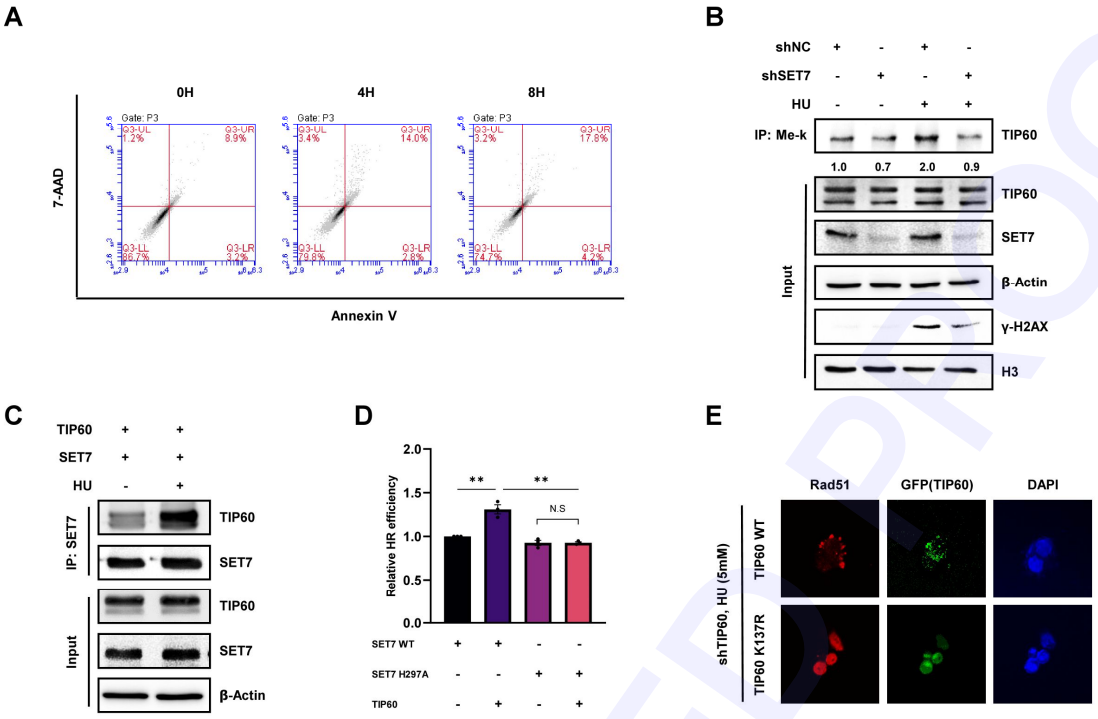



**Supplementary Fig. 2. SET7 doesn't affect TIP60-mediated acetylation and localization.**

(A, B) pcDNA3.1-SET7, pcDNA3.1-SET7 H297A transfected 293T cells and HCT116 cells were lysed and immunoblotted using the indicated antibodies. (C) HCT116 cells, transfected with pcDNA3.1-SET7, pcDNA3.1-SET7 H297A or empty vector, were separated into nuclear and cytoplasmic fractions. H3 and β-tubulin were used as loading controls.

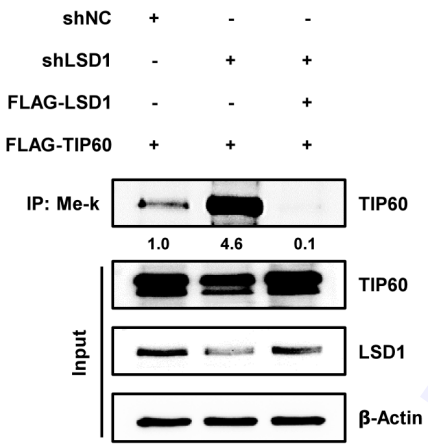



**Supplementary Fig. 3. SET7 interacts with TIP60 *in vitro* and *in vivo*.** (A) Extracts of HCT116 cells transfected with SET7 were incubated with purified GST or GST-TIP60. Associated proteins were eluted, resolved by SDS-PAGE, and immunoblotted (top). The amount of TIP60 in the cell extract was determined by C oomassie staining (bottom). (B) Flag-EV or Flag-TIP60 and pcDNA3.1-SET7 were overexpressed in HCT116 cells. The cell lysates were immunoprecipitated with an anti-SET7 antibody. Associated proteins were eluted, resolved by SDS-PAGE, and immunoblotted using the indicated antibodies.

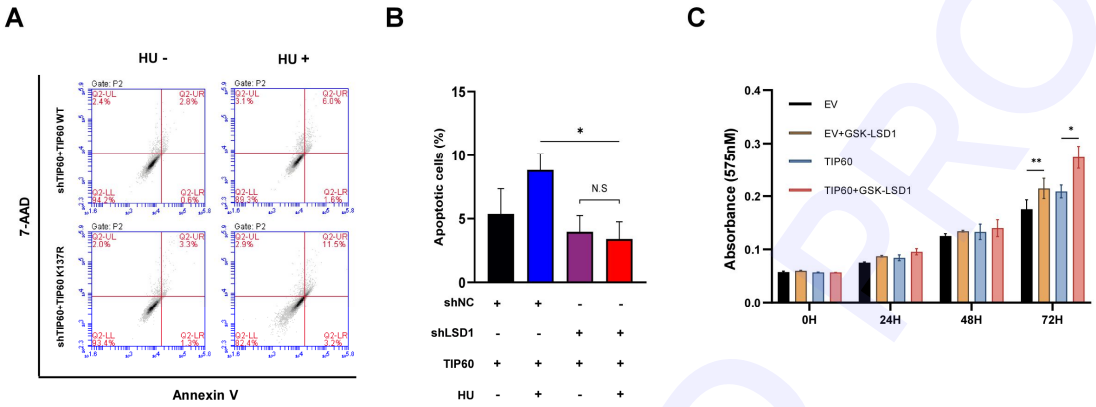



**Supplementary Fig. 4. Hydroxyurea (HU)-mediated DNA damage induces SET7-dependent methylation of TIP60 and induces homologous recombination (HR).** (A) HCT116 cells were treated with 5 mM hydroxyurea (HU) for 4 h, 8 h. Apoptotic cells were measured by fluorescence-activated cell sorting (FACS) analysis. (B) HCT116 shNC and shSET7 cells treated with 5 mM HU for 4 h were immunoprecipitated using anti-methyl lysine antibodies. Immunoprecipitates were eluted, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotted using indicated antibodies. Methyl lysine levels were normalized by input of TIP60. (C) HCT116 cells were transfected with the indicated plasmids and treated with 5 mM HU for 4 h. Cell extracts of control and damaged cells were immunoprecipitated using anti-SET7 antibodies, and associated proteins were pulled down with A/G agarose beads. Beads were washed extensively, and bound proteins were resolved by SDS-PAGE and immunoblotted using the indicated

antibodies. (D) PcDNA3.1-SET7, PcDNA3.1-SET7 H297A, or Flag-TIP60 was subjected to HR assay. Results were shown as the mean ± SEM; n = 3, **P < 0.01, N.S: no significant difference. (E) HCT116 cells with TIP60 knockdown were transfected with TIP60 WT or TIP60 K137R. Rad51 foci was examined following 5 mM HU treatment for 4 h.




**Supplementary Fig. 5. LSD1 demethylates TIP60 methylation.** HCT116 cells were transfected with Flag-EV or Flag-LSD1 in control or LSD1 stably knocked down cells. The lysates were immunoprecipitated using anti-methyl lysine antibodies. Methyl lysine levels were normalized by input of TIP60.




**Supplementary Fig. 6. HU-dependently increased apoptosis in TIP60 K137R compared to TIP60 WT.** (A) HCT116 cells were transfected with the indicated plasmids and treated with 5 mM hydroxyurea (HU) for 4 h. Apoptotic cells were measured by fluorescence-activated cell sorting (FACS) analysis. (B) HCT116 cells were transfected with the indicated plasmids and treated with 5 mM HU for 4 h. Apoptotic cells were measured by fluorescence-activated cell sorting (FACS) analysis. Results were shown as the mean ± SEM; n = 3, *P < 0.05, N.S: no significant difference. (C) Cell viability was determined using the MTT assay. HCT116 cells were transfected with Flag-empty vector (EV) or Flag-TIP60. Cells were treated with 500 nM GSK-LSD1 for 24 h and incubated in fresh media for 0–72 h. Results were shown as the mean ± SEM; n = 3, **P < 0.01, *P < 0.05, N.S: no significant difference.