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# Inhibition of FoxO1 acetylation by INHAT subunit SET/TAF-Iβ induces p21 transcription

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ABSTRACT

**Post-translational modification of forkhead family transcription factor, FoxO1, is an important regulatory mode for its diverse activities. FoxO1 is acetylated by HAT coactivators and its transcriptional activity is decreased via reduced DNA binding affinity. Here, we report that SET/TAF-Iβ inhibited p300-mediated FoxO1 acetylation in an INHAT domain-dependent manner. SET/TAF-Iβ interacted with FoxO1 and activated transcription of FoxO1 target gene, p21. Moreover, SET/TAF-Iβ inhibited acetylation of FoxO1 and increased p21 transcription induced by oxidative stress. Our results suggest that SET/TAF-Iβ inhibits FoxO1 acetylation and activates its transcriptional activity toward p21.**

*Structured summary of protein interactions:*
**FoxO1** physically interacts with **SET/TAF-Iβ** by pull down (1, 2)
**FoxO1** physically interacts with **SET/TAF-Iβ** by anti tag coimmunoprecipitation (View interaction)
**SET/TAF-Iβ** physically interacts with **FoxO1** by anti bait coip (1, 2)

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## 1. Introduction

The FoxO family of forkhead transcription factors is evolutionally conserved and consists of the functionally related proteins FoxO1 (FKHR), FoxO3a (FKHRL1), FoxO4 (AFX), and FoxO6 in mammals [1,2]. Recent studies demonstrated that FoxO factors play key roles in activation of various target genes, including metabolism, cell cycle, cell death, DNA repair, and oxidative stress response [2–5].

The transcriptional activity of FoxO1 is regulated by post-translational modifications and those modifications affect its subcellular localization. FoxO1 is acetylated by p300, CBP (cAMP-response element-binding protein), and PCAF (p300/CBP-associated factors) in response to oxidative stress [6–8]. Acetylation of FoxO1 decreased its transcriptional activity through attenuation of its DNA binding ability [9]. FoxO1 is deacetylated by the sirtuin family member, NAD-dependent deacetylase Sir2, which has a homolog in mammals known as Sirt1, and resulted in increased transcriptional activity [8].

We previously identified SET/TAF-Iβ and pp32 as subunits of the INHAT complex; binding of these proteins to histones prevents histone acetylation by p300/CBP and PCAF [10,11]. SET/TAF-Iβ inhibits not only acetylation of histones but also acetylation of non-histone proteins. We previously reported that SET/TAF-Iβ regulates p53-mediated cell cycle arrest and apoptosis through inhibition of p53 acetylation [12]. Recently, we reported that inhibition of Ku70 acetylation by SET/TAF-Iβ regulates the Ku70-mediated DNA damage response [13].

In the current study, we found that SET/TAF-Iβ inhibits FoxO1 acetylation by p300 and increases its transcriptional activity. Furthermore, SET/TAF-Iβ inhibits the FoxO1 acetylation induced by $H_2O_2$. We showed that SET/TAF-Iβ regulates apoptosis through inhibition of FoxO1 acetylation under oxidative stress conditions.

## 2. Materials and methods

### 2.1. Plasmid constructs

The pCMX-SET/TAF-Iβ plasmid was previously described [11]. shRNA against human SET/TAF-Iβ (RHS4533) was purchased from OpenBiosystems. p3XFLAG-CMV10-SET/TAF-Iβ, pCMX-FoxO1, p3X FLAG-CMV10-FoxO1, p3XFLAG-CMV10-FoxO1Δ1 (1–240 aa), p3X FLAG-CMV10-FoxO1Δ2 (236–440 aa), p3XFLAG-CMV10-FoxO1Δ3 (436–655 aa), pGEX4T1-FoxO1, pGEX4T1-FoxO1Δ1, pGEX4T1-FoxO1Δ2, pGEX4T1-FoxO1Δ3, and pCMX-p300 were used.

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### 2.2. Cell culture and transient transfection

HEK293T cells were grown in Dulbecco's modified Eagle's medium (DMEM), and HCT116 (p53−/−) cells and H1299 cells were grown in RPMI 1640 containing 10% heat inactivated fetal bovine serum and 0.05% penicillin–streptomycin at 37 °C in a 5% $CO_2$ atmosphere. HCT116 (p53−/−) cells and HEK293T cells were transfected with the indicated DNA constructs using polyethylenimine (PEI).

### 2.3. In vitro INHAT assay

INHAT assays were performed by incubating purified GST-SET/TAF-Iβ with GST-FoxO1 in HAT buffer [11] for 15 min on ice. Following pre-incubation, p300, along with $^{14}$[C]-acetyl CoA (10 μCi/μl, Perkin Elmer), were added for 2 h at 30 °C. Reaction products were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and analyzed by a phosphorimager. For scintillation counting, FoxO1 peptides [EGGK SGKSPR] were synthesized (Peptron). Peptides were filtered using p81 filter paper (Upstate Biotechnology) and washed two times with $H_3PO_4$ and one time with 95% ethanol. The filters were then allowed to air dry, followed by the addition of 2 ml of Ultima Gold (Perkin Elmer). $^{14}$[C]-acetyl CoA was then quantified using a scintillation counter.

### 2.4. Immunoprecipitation

Immunoprecipitation was performed in order to investigate the potential relationship between FoxO1 and SET/TAF-Iβ interaction and FoxO1 acetylation level. Cells were lysed in lysis buffer (20 mM Tris–HCl [pH 7.5], 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1X protease inhibitor cocktail, and 1 mM PMSF) at 4 °C. Proteins were immunoprecipitated with anti-FoxO1, anti-SET/TAF-Iβ or anti-Flag antibodies overnight at 4 °C, and then protein A/G agarose beads (GenDEPOT) were added for 2 h with rotation at 4 °C. Bound proteins were analyzed via western blotting with anti-SET/TAF-Iβ, anti-FoxO1, anti-Flag and anti-acetyl lysine antibodies.

### 2.5. Western blotting

Total protein extracts were prepared by lysing cells in radioimmunoprecipitation assay (RIPA) lysis buffer (50 mM Tris–HCl [pH 8.0], 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate (SDC), 1% NP-40, 1X protease inhibitor cocktail, and 1 mM EDTA). Proteins were resolved by SDS–PAGE and subsequently transferred to nitrocellulose membranes. Membranes were probed overnight with the indicated antibodies at 4 °C. Blots were subsequently incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse antibodies (Enzo Life Science), and bands were detected using an enhanced chemiluminescence (ECL) system (Animal Genetics Inc.).

### 2.6. Antibodies

Antibodies against FKHR (sc-374427), acetyl lysine (Ac-K) (sc-32268), SET/TAF-Iβ (sc-25564), H3 (sc-8654), β-actin (sc-47778), His (sc-53073), p21 (sc-397; Santa Cruz Biotechnology), Flag (F3165; SIGMA), Sirt1 (2496s; cell signaling), and caspase3 (04-439; Upstate) were employed for western blotting and immunoprecipitation (IP).

### 2.7. GST-pull down assay

Cell lysates from HEK293T cells ectopically expressing Flag-FoxO1 were incubated with GST-SET/TAF-Iβ or GST-SET/TAF-Iβ deletion constructs. Likewise, GST-FoxO1, GST-FoxO1 deletion constructs were incubated with SET/TAF-Iβ-his overexpressed cell lysates. Associated proteins were eluted, resolved by SDS–PAGE, and immunoblotted with the indicated antibodies.

### 2.8. Reverse transcription and real-time PCR

Total RNA was isolated from transfected HCT116 (p53−/−) cells using RNAiso Plus (TaKaRa). After cDNA synthesis, cDNA was quantified and then subjected to *p21* and *FasL* mRNA expression analysis. The following PCR primers were used: *p21* (forward, 5′-GGCCCAGTGGACAGCGAGCA-3′; reverse, 5′-CCCAGGCGAAGTC ACCCTCC-3′), and *FasL* (forward, 5′-ACACCTATGGAATTGTCCTG C-3′; reverse, 5′-GACCAGAGAGAGCTCAGATACG-3′). Disassociation curves were performed after each PCR run to ensure that a single product of the appropriate length was amplified. Mean threshold cycle ($C_T$) and standard error values were calculated from individual $C_T$ values obtained from triplicate reactions per stage. The normalized mean $C_T$ value was estimated as $\Delta C_T$ by subtracting the mean $C_T$ of β-actin. The value $\Delta\Delta C_T$ was calculated as the difference between control $\Delta C_T$ and values obtained for each sample. The *n*-fold change in gene expression, relative to an untreated control, was calculated as $2^{-\Delta\Delta C_T}$.

### 2.9. Luciferase assay

Luciferase assays were conducted using a *p21* promoter reporter system. H1229 cells and HEK293T cells were co-transfected with the *p21* promoter reporter construct and the indicated DNA constructs using Lipofectamine 2000 or PEI. After transfection, cells were harvested and assayed for luciferase activity using a luciferase assay system (Promega). Each value was expressed as the mean of five replicates from a single assay, and experiments were performed at least three times.

### 2.10. Chromatin immunoprecipitation assay

HCT116 (p53−/−) cells were treated with $H_2O_2$ (500 μM) for 6 h. Cells were cross linked with 1% formaldehyde, which was added to the medium for 10 min at RT, followed by the addition of 125 mM glycine for 5 min at RT. Cells were then lysed in SDS lysis buffer, after which samples were sonicated and immunoprecipitated using the indicated antibodies. Immunoprecipitates were eluted and cross links were reversed; subsequently, DNA fragments were purified using a PCR purification kit (Axygen). The following PCR primers were used: *p21* (forward, 5′-GTGGCTCTG ATTGGCTTTCTG-3′; reverse, 5′-CTGAAAACAGGCAGCCCAAG-3′). Disassociation curves were generated after each PCR run to ensure that a single product of the appropriate length was amplified. The mean threshold cycle ($C_T$) and standard error values were calculated from individual $C_T$ values, obtained from duplicate reactions per stage. The normalized mean $C_T$ value was estimated as $\Delta C_T$ by subtracting the mean $C_T$ of the input from that of *p21*.

### 2.11. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

HCT116 (p53−/−) cells were seeded in 48-well plates ($2.5 \times 10^4$ cells/well) and transiently transfected with SET/TAF-Iβ and shSET/TAF-Iβ. At 48 h post-transfection, cells were treated with 50 μM $H_2O_2$ for 24 h. MTT was added to cells (20 μL, final concentration 0.5 mg/mL), and cells were incubated a further 4 h at 37 °C. At this time, medium was removed by aspiration and DMSO was added (200 μL). OD values were determined with an ELISA reader (Biochrom) at a wavelength of 570 nm. The value of a blank sample,

containing DMSO alone, was measured and subtracted from all values to correct for background in measurements.

### 2.12. BrdU incorporation assay

BrdU incorporation assays were performed according to the manufacturer's protocols (Roche Diagnostic). Briefly, HCT116 (p53−/−) cells were seeded in 48-well plates ($2.5 \times 10^4$ cells/well) and subsequently transfected with either SET/TAF-Iβ or shSET/TAF-Iβ. At 48 h post-transfection, cells were treated with $H_2O_2$ (50 μM) for 24 h. Cells were then incubated in BrdU-containing medium for an additional 2 h, after which time absorbance was measured at 370 nm (reference wavelength: 492 nm) with an ELISA plate reader.

### 2.13. TUNEL assay

HCT116 (p53−/−) cells were seeded in 4-well chamber slides and then transiently co-transfected with pCMX-SET/TAF-Iβ and shSET/TAF-Iβ. After 2 days, cells were treated with 50 μM $H_2O_2$ for 24 h and subsequently fixed with 4% paraformaldehyde for 1 h at RT. TUNEL (TdT-mediated dUTP nick end labeling) reactions were then carried out using a DeadEndTM Fluorometric TUNEL System, according to the manufacturer's instructions (Promega).

## 3. Results

### 3.1. Inhibition of FoxO1 acetylation by SET/TAF-Iβ

Previous studies found that FoxO1 can be acetylated at Lys-242, Lys-246, and Lys-262 by PCAF, CBP, and p300 [6–8]. Such acetylation of FoxO1 attenuates its DNA binding ability and increases its sensitivity to phosphorylation [9,14]. Our previous report demonstrated that SET/TAF-Iβ represses p53 activity through inhibition of p53 acetylation [12]. Therefore, we hypothesized that SET/TAF-Iβ might inhibit acetylation of FoxO1. To investigate the regulation of FoxO1 acetylation through INHAT activity of SET/TAF-Iβ, we first tested whether SET/TAF-Iβ can inhibit p300-mediated FoxO1 acetylation using in vitro HAT assays. As expected, recombinant p300 acetylated FoxO1, and FoxO1 acetylation was inhibited by SET/TAF-Iβ (Fig. 1A). To further confirm the inhibition of FoxO1 acetylation by SET/TAF-Iβ, we performed a HAT assay using 10-mer FoxO1 peptides containing two acetylation target lysine residues and measured acetylation levels by scintillation counting. Incubation of FoxO1 peptides including Lys-242 and Lys-246 with p300, as well as SET/TAF-Iβ significantly decreased FoxO1 acetylation (Fig. 1B). Moreover, SET/TAF-IβΔ3 and SET/TAF-IβΔ5 which have deleted INHAT domain, did not change the FoxO1 acetylation level (Fig. 1B). Inhibition of FoxO1 acetylation by SET/TAF-Iβ was

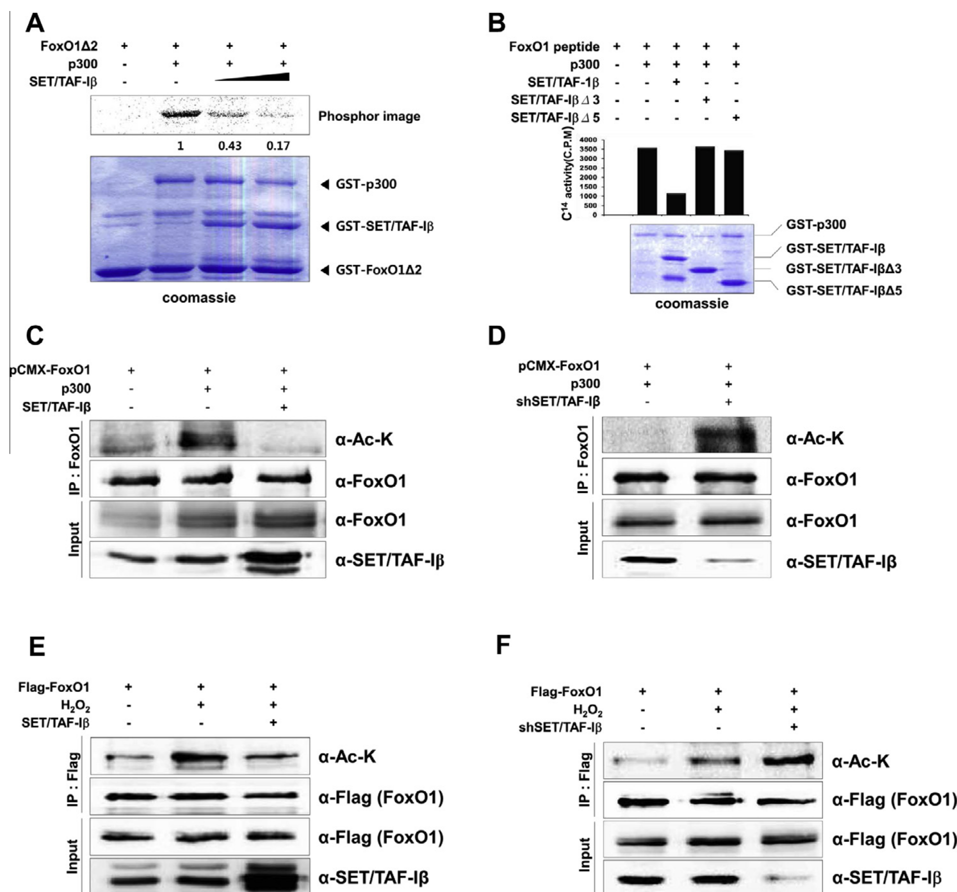


**Fig. 1.** SET/TAF-Iβ inhibits acetylation of FoxO1. (A) Acetylation assays of FoxO1 with p300 were performed with increasing concentrations of SET/TAF-Iβ. An autoradiogram of an INHAT assay using recombinant p300 with SET/TAF-Iβ on GST-FoxO1Δ2 (top) followed by Coomassie staining (bottom). The number below the phosphorimage represents the quantification of FoxO1 acetylation. (B) FoxO1 peptides were used as substrates in the INHAT assay with p300 with GST-SET/TAF-Iβ, GST-SET/TAF-IβΔ3 or GST-SET/TAF-IβΔ5. Acetylation levels were quantified via filter binding assay and were represented as raw counts per minute (cpm) incorporated. (C and D) HEK293T cells (C) and HEK293T shSET/TAF-Iβ stable cells (D) were transfected with indicated plasmids. After anti-FoxO1 immunoprecipitation, eluted proteins were subjected to western blotting with anti-acetyl lysine, anti-FoxO1, and anti-SET/TAF-Iβ antibodies. (E and F) HCT116 (p53−/−) cells (E) and HCT116 (p53−/−) shSET/TAF-Iβ stable cells (F) were transfected with indicated plasmids. After anti-Flag immunoprecipitation, eluted proteins were subjected to Western blotting with anti-acetyl lysine, anti-Flag, and anti-SET/TAF-Iβ antibodies. Cells were treated with $H_2O_2$ (500 μM) for 6 h.

confirmed in vivo by IP. When p300 was transfected transiently in HEK293T cells, the acetylation level of FoxO1 was increased. As expected, overexpression of SET/TAF-Iβ inhibited the FoxO1 acetylation level (Fig. 1C). However, the FoxO1 acetylation level was clearly elevated in the shSET/TAF-Iβ stable HEK293T cell line (Fig. 1D). Two independent shSET/TAF-Iβ RNAs have been transiently expressed and showed increased FoxO1 acetylation levels (Supplementary Fig. S1A). These results demonstrated that SET/TAF-Iβ inhibits FoxO1 acetylation in vitro and in vivo. Previous studies reported that FoxO1 acetylation was increased under oxidative stress [1,6,15,16]. Next, we tested whether SET/TAF-Iβ can inhibit FoxO1 acetylation in oxidative stress conditions. With exogenously overexpressed FoxO1, treatment with $H_2O_2$ resulted in a high level of FoxO1 acetylation, while the FoxO1 acetylation level was significantly decreased when SET/TAF-Iβ was overexpressed (Fig. 1E). However, as expected, the FoxO1 acetylation level was increased in the SET/TAF-Iβ-knock-down cells (Fig. 1F). Again, knock-down of SET/TAF-Iβ by different shSET/TAF-Iβ RNAs showed the same results (Supplementary Fig. S1B). Therefore, these results demonstrated that the oxidative stress-mediated FoxO1 acetylation level is regulated by SET/TAF-Iβ.

### 3.2. Interaction between SET/TAF-Iβ and FoxO1

Next, we tested whether SET/TAF-Iβ interacts with FoxO1 by in vitro binding assay with purified full length GST-SET/TAF-Iβ, or GST-SET/TAF-Iβ deletion constructs incubated with lysate of cells ectopically expressing FoxO1. Compared to GST-SET/TAF-Iβ, GST-SET/TAF-IβΔ3 and GST-SET/TAF-IβΔ5 which are C-terminal INHAT domain deletion mutants did not interact with FoxO1 (Fig. 2A). We also confirmed this interaction in vivo by IP with anti-SET/TAF-Iβ antibodies. As predicted, FoxO1 interacted with the INHAT domain of SET/TAF-Iβ (Fig. 2B). However, a weak interaction between SET/TAF-IβΔ3 and FoxO1 was also observed, probably due to the presence of endogenous SET/TAF-Iβ in the lysates. The results of this experiment indicated that FoxO1 interaction is

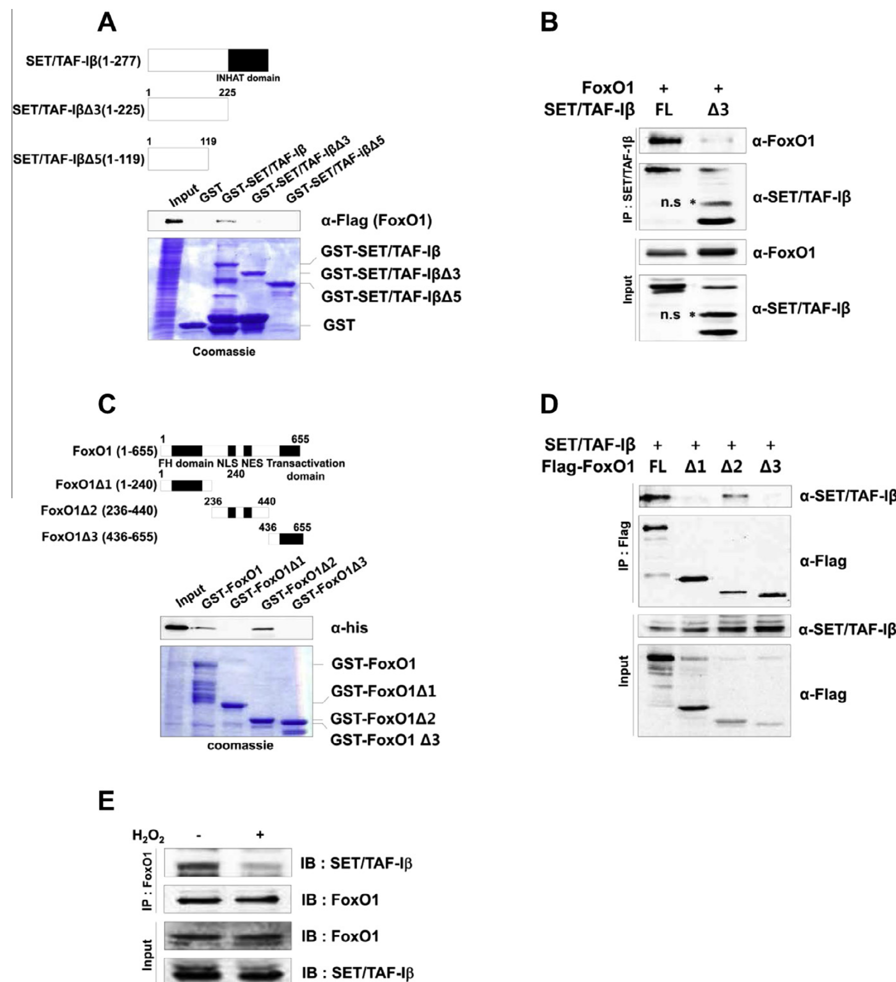


**Fig. 2.** SET/TAF-Iβ interaction with FoxO1 is mediated by its INHAT domain. (A) Cell lysates from HEK293T cells ectopically expressing Flag-FoxO1 were incubated with GST-SET/TAF-Iβ, GST-SET/TAF-Iβ deletion constructs. Associated proteins were eluted, resolved by SDS–PAGE, and immunoblotted with anti-Flag antibodies. The amounts of full-length SET/TAF-Iβ and SET/TAF-Iβ deletion mutants were determined by Coomassie staining. (B) HEK293T cells were transfected with constructs driving the expression of pCMX-FoxO1, pCMX-SET/TAF-Iβ, and pCMX-SET/TAF-IβΔ3 prior to immunoprecipitation with anti-SET/TAF-Iβ antibodies. Associated proteins were eluted, resolved by SDS–PAGE, and immunoblotted with the indicated antibodies. (C) SET/TAF-Iβ was overexpressed in HEK293T cells prior to cell lysis and pull-down assays with immobilized GST-FoxO1 deletion mutants as indicated. Associated proteins were eluted, resolved by SDS–PAGE, and immunoblotted with anti-his antibodies. The amounts of full-length FoxO1 and FoxO1 deletion constructs were determined by Coomassie staining. (D) HEK293T cells were transfected with plasmids driving the expression of full-length Flag-FoxO1, deletion mutants thereof, and SET/TAF-Iβ-his prior to immunoprecipitation with anti-Flag antibodies. Immunoprecipitated and associated proteins were eluted, resolved by SDS–PAGE, and immunoblotted with anti-Flag and anti-SET/TAF-Iβ antibodies. (E) Endogenous interaction between SET/TAF-Iβ and FoxO1 were examined in HCT116 (p53−/−) cells treated with $H_2O_2$ (500 μM) for 6 h. Cell lysates were immunoprecipitated with anti-FoxO1 antibodies and associated proteins were analyzed by Western blotting with anti-FoxO1 and anti-SET/TAF-Iβ antibodies.

dependent on the INHAT domain of SET/TAF-Iβ. We confirmed this interaction using FoxO1 deletion constructs, too. To reveal the FoxO1 interaction site with SET/TAF-Iβ, we performed a GST-pull down assay with purified GST-FoxO1 deletion mutants. SET/TAF-Iβ can inhibit acetylation through interaction with the acetylation site of the target protein. Thus, we hypothesized that SET/TAF-Iβ may bind to FoxO1 acetylation sites. We observed that FoxO1Δ2, including Lys-242 and Lys-246, bound to SET/TAF-Iβ (Fig. 2C). This interaction was confirmed by showing an interaction between FoxO1 constructs and SET/TAF-Iβ by IP in vivo (Fig. 2D). Likewise, SET/TAF-Iβ was found to bind to FoxO1Δ2 in vivo. To further confirm the interactions, we showed that endogenous SET/TAF-Iβ interacts with FoxO1 in HCT116 (p53−/−) cells in the absence of $H_2O_2$ treatment (Fig. 2E). Next, we tested interaction between SET/TAF-Iβ and FoxO1-3KA (K242A, K245A, K262A) mutant which acetylation site lysines are substituted with alanines. The mutations on these acetylation sites did not influence interaction between FoxO1 and SET/TAF-Iβ (*Supplementary* Fig. S3). These data

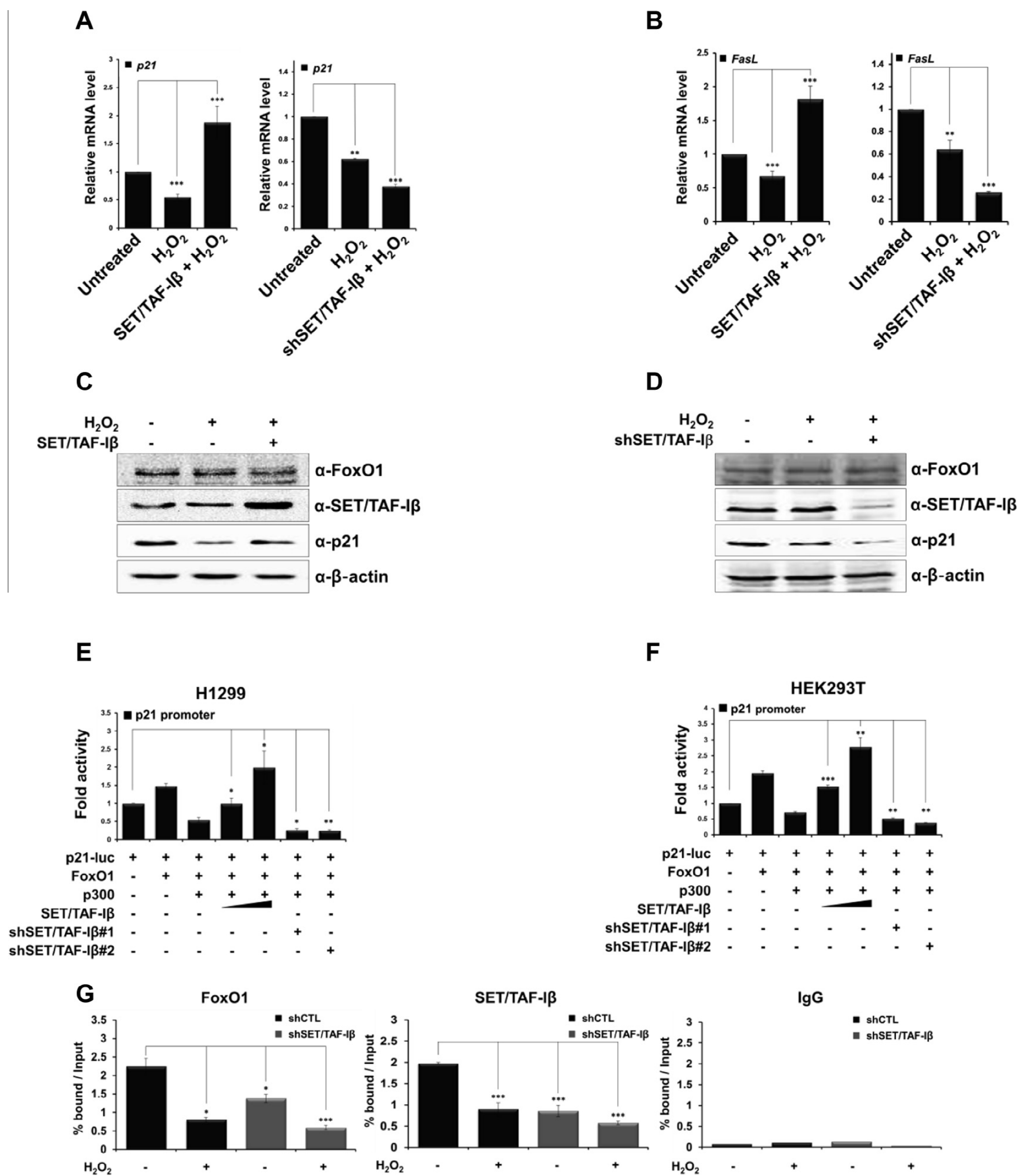


**Fig. 3.** SET/TAF-Iβ regulates FoxO1 target genes. (A and B) SET/TAF-Iβ-his transfected HCT116 (p53−/−) cells and shSET/TAF-Iβ stable HCT116 (p53−/−) cells were analyzed by real time PCR to examine the mRNA expression levels of *p21* (A) and *FasL* (B). Cells were treated with $H_2O_2$ (500 μM) for 6 h or not. Results are shown as means ± SDs; $n = 3$. $^{***}P < 0.001$; $^{**}P < 0.01$. (C and D) Protein expression levels of p21 were examined by Western blotting in HCT116 (p53−/−) cells via pCMX-SET/TAF-Iβ overexpression (C) and shSET/TAF-Iβ stable HCT116 (p53−/−) cells (D). Cells were treated with $H_2O_2$ (500 μM) for 6 h or not. (E and F) H1299 cells (E) and HEK293T cells (F) were co-transfected with the *p21* promoter-luc construct and either pCMX, pCMX-FoxO1, pCMX-p300, pCMX-SET/TAF-Iβ, shSET/TAF-Iβ#1, or shSET/TAF-Iβ#2. Cell extracts were then assayed for luciferase activity. Luciferase activities were normalized to those of β-galactosidase. Results are shown as means ± SDs; $n = 3$. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$. (G) HCT116 (p53−/−) cells and HCT116 (p53−/−) shSET/TAF-Iβ stable cells were treated with $H_2O_2$ (500 μM) for 6 h. ChIP analysis was then conducted using anti-FoxO1 and anti-SET/TAF-Iβ antibodies. Recruitment of FoxO1 and SET/TAF-Iβ to the *p21* promoter region was normalized to the assay inputs. Anti-IgG antibodies were used as a negative control. Results are shown as means ± SDs; $n = 3$. $^{***}P < 0.001$; $^{*}P < 0.05$.

suggest that SET/TAF-Iβ inhibits FoxO1 acetylation through an interaction with FoxO1 acetylation sites. We performed Sirt1 and FoxO1 interaction assay and confirmed that SET/TAF-Iβ did not affect the interaction (Supplementary Fig. S2).

### 3.3. FoxO1 target genes were regulated by SET/TAF-Iβ

A previous study found that acetylation of FoxO1 decreased its transcriptional activity through attenuation of its DNA binding ability [9]. Therefore, we hypothesized that SET/TAF-Iβ increases the transcriptional activity of FoxO1 via inhibition of FoxO1 acetylation. To confirm this hypothesis, we conducted real-time PCR. We treated HCT116 (p53−/−) cells with different concentrations of $H_2O_2$ to induce FoxO1 acetylation in cells that were either overexpressing SET/TAF-Iβ or had been subjected to knock-down of SET/TAF-Iβ. The FoxO1 target gene, *p21,* was down-regulated via induction of FoxO1 acetylation by $H_2O_2$ treatment. However, the transcriptional level of *p21* was increased when SET/TAF-Iβ was overexpressed (Fig. 3A and Supplementary Fig. S4A). In contrast, FoxO1 transcriptional activity was decreased when shSET/TAF-Iβ stable cell line was used (Fig. 3A and Supplementary Fig. S4B). We tested additional FoxO1 target genes including *FasL*, *PUMA*, and *Bim* and found *FasL* showed prominent change in expression similar to that of *p21* (Fig. 3B). To further confirm the regulation of FoxO1 target genes, we tested whether SET/TAF-Iβ regulates the FoxO1 target gene on the protein level. We observed the increase in protein level of p21 via SET/TAF-Iβ mediated the inhibition of FoxO1 acetylation despite $H_2O_2$ treatment (Fig. 3C). In contrast, when SET/TAF-Iβ was knocked down, p21 was decreased (Fig. 3D). Subcellular localization of FoxO1 remained unaltered both in SET/TAF-Iβ overexpression and knock-down cells (Supplementary Fig. S5A and B). To further confirm these results, we performed a reporter assay using a *p21* promoter-driven luciferase (luc) reporter system in different cell lines. In reporter assays performed in exogenous FoxO1-expressing H1299 and HEK293T cell lines, SET/TAF-Iβ increased *p21* promoter activity. On the other hand, *p21* promoter activity was decreased when SET/TAF-Iβ was knocked down (Fig. 3E and F). To determine if FoxO1 transcriptional activity was increased by SET/TAF-Iβ, we conducted a chromatin immnoprecipitation (ChIP) assay. When SET/TAF-Iβ was knocked down, recruitment of FoxO1 on the *p21* promoter was reduced. In comparison with the untreated condition, recruitment of FoxO1 on the *p21* promoter was further decreased under oxidative stress (Fig. 3G). Recruitment of SET/TAF-Iβ to *p21* promoter was downregulated by $H_2O_2$ treatment similar to those of FoxO1 (Fig. 3G). All together, these data demonstrated that SET/TAF-Iβ regulates transcriptional activity of FoxO1 through inhibition of FoxO1 acetylation.

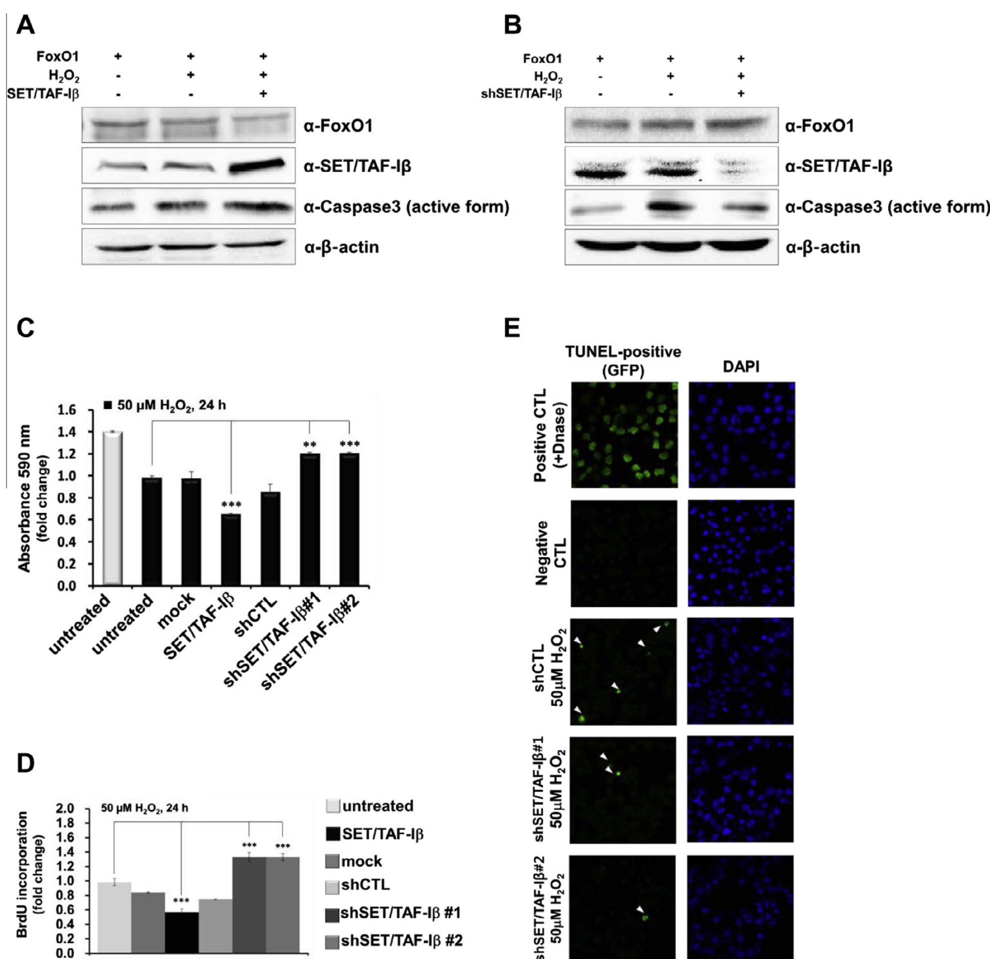


**Fig. 4.** SET/TAF-Iβ promotes apoptosis in oxidative stress conditions. (A and B) HCT116 (p53−/−) cells (A) and shSET/TAF-Iβ stable cells (B) were transfected with indicated plasmids. Each cell lysate was resolved by SDS–PAGE and immunoblotted with anti-FoxO1, anti-SET/TAF-Iβ, anti-caspase3, and anti-β-actin antibodies. (C) Cell viability was determined by MTT assay. HCT116 (p53−/−) cells were treated with $H_2O_2$ (50 μM) for 24 h and transfected with the indicated constructs. Results are shown as means ± SDs; $n = 3$. $^{***}P < 0.001$; $^{**}P < 0.01$. (D) HCT116 (p53−/−) cells were transfected with the indicated constructs upon $H_2O_2$ (50 μM) incubation for 24 h. At 72 h post-transfection, cells were fixed and BrdU assays performed. Results are shown as means ± SDs; $n = 3$. $^{***}P < 0.001$. (E) HCT116 (p53−/−) cells, transiently transfected with the indicated constructs upon $H_2O_2$ (50 μM) for 24 h were subjected to TUNEL assays. DNase I-treated cells are shown as a positive control.

### 3.4. SET/TAF-Iβ promotes apoptosis in oxidative stress condition

Previous studies revealed that $H_2O_2$ induced apoptosis through activation of caspase 3 and caspase 9 [17,18]. To further investigate the biological role of SET/TAF-Iβ in the cellular functions of FoxO1, we measured the apoptosis-inducing protein, caspase 3. When FoxO1 acetylation was reduced by SET/TAF-Iβ, apoptosis was further up-regulated (Fig. 4A). In contrast, when SET/TAF-Iβ was knocked down, apoptosis was reduced via increasing FoxO1 acetylation (Fig. 4B). To confirm the regulation of apoptosis by FoxO1 acetylation level, we performed an MTT assay to measure cell survival via treatment of $H_2O_2$. As shown in Fig. 4C, cell survival was reduced when acetylation of FoxO1 was decreased, and cell survival was increased when SET/TAF-Iβ was knocked down (Fig. 4C). Consistent results were obtained in BrdU analysis, in which decreasing of FoxO1 acetylation by SET/TAF-Iβ decreased cell viability and knock-down of SET/TAF-Iβ increased cell viability (Fig. 4D). Positive regulation of apoptosis by SET/TAF-Iβ was also investigated by TUNEL assay. The amount of apoptotic nuclei in DNase I-treated control and $H_2O_2$ treated cells decreased upon knock-down of SET/TAF-Iβ by different two shRNA sequences, a finding which supports the hypothesis that reducing of FoxO1 acetylation by SET/TAF-Iβ positively regulates apoptosis (Fig. 4E). Altogether, our study demonstrated that decreasing FoxO1 acetylation by SET/TAF-Iβ regulates the transcriptional activity of FoxO1 and these mechanisms are able to regulate apoptosis under oxidative stress.

## 4. Discussion

FoxO1 regulates cell survival and apoptosis via regulation of its target genes such as *Bim*, *PUMA*, and *p21* [19]. Regulation of FoxO1-mediated apoptosis and cell survival are important to FoxO1 acetylation status. Here, we tried to elucidate the correlation of FoxO1 and the INHAT complex subunit SET/TAF-Iβ in human cells. We found that SET/TAF-Iβ inhibits FoxO1 acetylation by p300. We demonstrated that SET/TAF-Iβ regulates the transcriptional activity of FoxO1 through regulation of FoxO1 acetylation. Furthermore, our results suggest that reducing FoxO1 acetylation by SET/TAF-Iβ regulates FoxO1-mediated apoptosis in oxidative stress conditions. Acetylated FoxO1 not only showed attenuated DNA binding ability but was also more sensitive to phosphorylation [9]. Phosphorylation of FoxO1 led to its translocation from the nucleus to the cytoplasm [20,21]. Domain mapping analysis indicated that SET/TAF-Iβ inhibits FoxO1 acetylation through masking of its acetylation site and increases recruitment of FoxO1 on the *p21* promoter. In oxidative stress conditions, acetylation of FoxO1 was increased by CBP/p300. It is intriguing that SET/TAF-Iβ inhibits acetylation of FoxO1 under oxidative stress. Among FoxO1 target genes, *p21* transcription was increased via inhibition of FoxO1 acetylation. In oxidative stress conditions, recruitment of FoxO1 on the *p21* promoter is reduced through the induction of FoxO1 acetylation. However, when SET/TAF-Iβ was overexpressed, recruitment of FoxO1 to the *p21* promoter was increased. In addition, we showed that FoxO1-mediated apoptosis is increased via inhibition of FoxO1 acetylation by SET/TAF-Iβ. As a result, SET/TAF-Iβ is able to regulate FoxO1 transcriptional activity under oxidative stress.

In conclusion, our data provide evidence that SET/TAF-Iβ negatively regulates FoxO1 acetylation. Inhibition of FoxO1 acetylation by SET/TAF-Iβ induces transcription of the FoxO1 target gene, p21. Moreover, these studies indicate that inhibition of FoxO1 acetylation by SET/TAF-Iβ regulates aspects of cellular physiology such as apoptosis and cell survival via p21 regulation.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.06.053.

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