Cancer Letters 448 (2019) 40–51

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original Articles

Reversible regulation of SATB1 ubiquitination by USP47 and SMURF2 mediates colon cancer cell proliferation and tumor progression

Le Yu[a,1], Ling Dong[a,1], Yang Wang[a], Liu Liu[a], Hua Long[a], Hui Li[a], Jinping Li[a], Xiaolong Yang[b], Zhaojian Liu[c], Guangjie Duan[d], Xiaotian Dai[d], Zhenghong Lin[a,*]

[a] Laboratory of Pathology, Chongqing University School of Life Sciences, Chongqing University, Chongqing, 401331, China
[b] Chongqing Key Laboratory of Natural Product Synthesis and Drug Research, School of Pharmaceutical Sciences, Chongqing University, Chonqqing, 401331, China
[c] Department of Cell Biology, Shandong University School of Medicine, Jinan, 250012, China
[d] Department of Pulmonology, Southwest Hospital, Third Military Medical University, Chongqing, 400038, China

ARTICLE INFO

Keywords:
USP47
SATB1
Migration
CRC
Tumorigenesis

ABSTRACT

Upregulation of special AT-rich sequence-binding protein-1 (SATB1) has been suggested to promote tumor growth and metastasis. However, the factors governing its cellular levels remain unclear. Here, we report that ubiquitin-specific peptidase 47 (USP47), a member of the deubiquitinating enzymes family, interacts with SATB1 and mediates its deubiquitination and stability. USP47 deficiency impairs transcriptional activity of SATB1 target genes and inhibits colon cancer cell proliferation, migration, and tumorigenesis in a mouse model of colon cancer. Furthermore, we identified SMURF2 as an E3 ubiquitin ligase that promotes SATB1 degradation by upregulating its ubiquitination, and its deficiency promotes colon cancer cell proliferation and SATB1 target gene transcription. SMURF2 is negatively regulated by USP47, and USP47 depletion sensitizes colon cancer cells to 5-FU treatment-induced apoptosis. Taken together, our findings provide a ubiquitination-related mechanistic link to USP47, SMURF2, and SATB1 and suggest that USP47 might be targeted for colon cancer treatment when SATB1 is overexpressed.

1. Introduction

The special AT-rich sequence-binding protein-1 (SATB1) is a genome organizer that facilitates the organization of chromatin and regulates gene expression [2,6,11,41]. SATB1 constitutes a nuclear architecture that functions as a landing platform for the organization of chromatin-remodeling factors surrounding heterochromatin [15,36]. Recent studies have implicated SATB1 dysregulation in multiple cancers, including colon cancer [5,27], breast carcinoma [16,18,23,26], and ovarian cancer [38]. SATB1 plays crucial roles in tumor growth, migration, and metastasis by reprogramming of the expression of various genes [16].

SATB1 is regulated by various post-translational modifications, including methylation, acetylation, sumoylation, and phosphorylation, all of which have been implicated in the regulation of its nuclear localization, DNA-binding ability, and tumor-promoting activity [17,31,33,37,40]. Moreover, SATB1 upregulation is dramatically correlated with clinicopathologic characteristics in various cancers. Despite the functional importance and implications of SATB1 expression in cancer progression, the mechanisms governing its cellular levels remain largely unknown.

Ubiquitin-specific peptidase 47 (USP47) is a member of the deubiquitinating enzyme family [22,29,39] and is suggested to regulate cell growth by interacting with β-Trcp, and knocking down USP47 represses cell survival and promotes the sensitivity of cells to DNA-damaging agents [32]. USP47 maintains genome integrity by modulating the efficiency of DNA-damage repair through the BER pathway [30]. USP47 also participants in Wnt signaling and regulates β-catenin stability [35]. As a regulator of Snail, USP47 enhances its expression under hypoxic conditions to induce epithelial-mesenchymal transition (EMT) in colorectal cancer (CRC) cells [8].

To gain further insights into SATB1 function and its regulators, we used a deubiquitinase library to identify SATB1-interacting partners. Here, we demonstrate that USP47 interacts with SATB1 to mediate its ubiquitination and positively regulate its stability. We further show that USP47 deficiency represses cell proliferation and migration through SATB1. Furthermore, we show that SMURF2 functions as the E3 ubiquitin ligase for SATB1 and is negatively regulated by USP47.

* Corresponding author. School of Life Sciences, Chongqing University Huxi Campus, Chongqing, 401331, China.
E-mail address: zhenghonglin@cqu.edu.cn (Z. Lin).
[1] Authors contributed equally to this work.

https://doi.org/10.1016/j.canlet.2019.01.039
Received 12 December 2018; Received in revised form 26 January 2019; Accepted 29 January 2019

Moreover, we identify an important correlation among USP47, SATB1, and SMURF2 expression in human colon cancer. Collectively, our data reveal a novel and critical mechanism for USP47 and SMURF2 function in cell proliferation and migration through mediation of SATB1 ubiquitination and abundance.

2. Materials and methods

2.1. Animal models

All animals were maintained, and experiments were conducted under the guidance of the Animal Management Regulations of Chongqing University.

2.2. Cell culture

Cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and were cultured in Dulbecco's modified Eagle's medium (purchased from Procell Life Science & Technology Co. Ltd., Wuhan, China) containing 10% fetal bovine serum (FBS). All the cell lines were maintained under the supplier's directions.

2.3. RNA extraction and RT-qPCR

Total RNA was extracted using TRIzol reagent (Ambion, CA, USA). Quantitative real-time RT-PCR was conducted using SYBR-Green qPCR master mix (Clontech Laboratories). β-actin was used as reference. A standard amplification protocol was used according to the Clontech kit's instructions. Primers were listed in Supplementary Tables 1 and 2

2.4. Western blotting analysis

Total proteins were extracted and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were then transferred onto PVDF membranes and immunoblotted with the following antibodies: α-mouse USP47 (Cell Signaling-Danvers, MA, USA), α-rabbit Actin (Sigma-Aldrich), α-rabbit SATB1 (Abcam-Cambridge, Cat. No. ab96027, MA), α-rabbit SATB2 (Abcam-Cambridge, MA), α-mouse SMURF2 (Sigma-Aldrich), α-rabbit E-cadherin (Cell Signaling-Danvers, MA, USA), α-mouse Flag (Sigma-Aldrich), α-rabbit HA (Santa Cruz Biotechnology, USA), α-mouse Myc (9E10) (Santa Cruz Biotechnology), β-actin (Santa Cruz Biotechnology). Blots were stripped and reprobed with GAPDH or β-actin antibody as a loading control.

2.5. Generation of CRISPR-Cas9-based knockout cells

We designed sgRNA of USP47, SATB1, and SMURF2 and cloned the sequences into the lenti CRISPR vector. HCT116 and DLD1 cells were transfected with the above constructs together with packaging lentivirus plasmids. After two weeks of selection with puromycin, mRNA and protein levels were analyzed. Then, individual clones were selected for further analysis.

2.6. Ubiquitination assay

For the ubiquitination assay, cells were lysed with ubiquitination buffer containing 1% SDS and were boiled at 95 °C for 10 min. The denatured cell lysates were diluted with SDS-negative RIPA buffer to reduce SDS to 0.2% and were then subjected to co-IP followed by western blotting with anti-HA or anti-ubiquitin Abs.

2.7. Cell proliferation and clonogenic assays

For cell proliferation, 1×10^4 cells were seeded into a 96-well plate supplied in triplicate, and the Celltiter 96® aq_{ueous} One Solution Cell Proliferation Assay kit (Promega, WI, USA) was used to monitor the cell proliferation rate continuously for 96 h. For the clonogenic assay, 1×10^3 cells were seeded into six-well plates to form colonies within two to three weeks. Colonies were fixed with glutaraldehyde (6.0% v/v), stained with crystal violet (0.5% w/v), and counted using a stereomicroscope. Colonies with no less than 50 cells per colony were counted.

2.8. Wound-healing assay

For the wound-healing migration assays, a single scratch wound was created using a sterile 100-μL plastic pipette tip across the cell surface. The area of a defined region within the scratch was measured using ImageJ software (NIH). The extent to which the wound had closed over 36 h was calculated and expressed as a percentage of the difference between time points 0 h and 36 h.

2.9. In vivo tumorigenesis assay

Six-week-old nude mice were inoculated subcutaneously in the right hind flank with 5×10^6 cells per 100 μL suspended in diluted Matrigel 1:1 in ice-cold PBS. Tumor development was monitored over a period of six weeks, then the mice were euthanized for further analysis. Tumor volume (mm^3) was measured with calipers and calculated as $(W^2 \times L)/2$, where W is the width and L is the length. All procedures involving animals were performed in accordance with the Institutional Animal Welfare Guidelines of Chongqing University.

2.10. TMT labeling and quantitative proteomic analysis by LC-MS/MS

The TMT labeling of tryptic peptides was performed using an TMT reagent kit (Thermo Scientific, San Jose, CA, USA). Mass spectroscopy (MS) analysis was performed using a tandem mass spectrometry (MS/MS) in Q Exactive™ plus (Thermo Scientific, San Jose, CA, USA) coupled online to the UPLC. Differentially expressed proteins were defined as the TMT ratio > 1.50 or < 0.67 compared to the control. The differentially expressed proteins were categorized using Gene Ontology (GO) information [3] and evaluated using the KEGG pathways database [21].

2.11. Statistical analysis

Statistical analyses were performed using one-way ANOVA by SPSS18.0 for Windows (IBM, New York, USA). Data were presented as the mean ± SEM of at least three independent experiments. A *P*-value of ≤0.05 was considered significant.

3. Results

DUB library screening identifies USP47 as a novel SATB1-interacting protein. To determine the molecular mechanisms underlying the aberrant protein expression of SATB1 in tumorigenesis, we used a deubiquitinase (DUB) library (Flag-tagged: USP1, 2, 3, 4, 7, 8, 10, 12, 14, 15, 17, 18, 19, 21, 22, 25, 26, 27, 28, 29, 30, 33, 36, 37, 38, 39, 42, 44, 46, 47, 51, CYLD, and A20. Myc-tagged: USP5, 11, 16, COPS5, COPS6, BRCC3, UCHL1, UCHL3, UCHL5, JOSD1, JOSD2, ATXN3, ATXN3L, OTUB1, OTUB2, OTUD6A, ZOTUD6B, STAMBP, STAMBPL1, DUB3, EIF3S3,MPND, PSMD7, BAP1, EIF3S5, MYSM1, and ZRANB1) to screen the SATB1-interacting protein that might be responsible for its dysregulation. As shown in Fig. 1A and Fig. S1, USP47 was identified as an SATB1-interacting partner among 60 DUBs. To further confirm that the interaction between SATB1 and USP47 is specific, we transfected these alone or together into 293T cells and evaluated their interaction by immunoprecipitation and western blotting. Fig. 1B shows that USP47 could be pulled down by SATB1 and reversely, SATB1 could be pulled down by USP47, suggesting that USP47 was indeed an SATB1

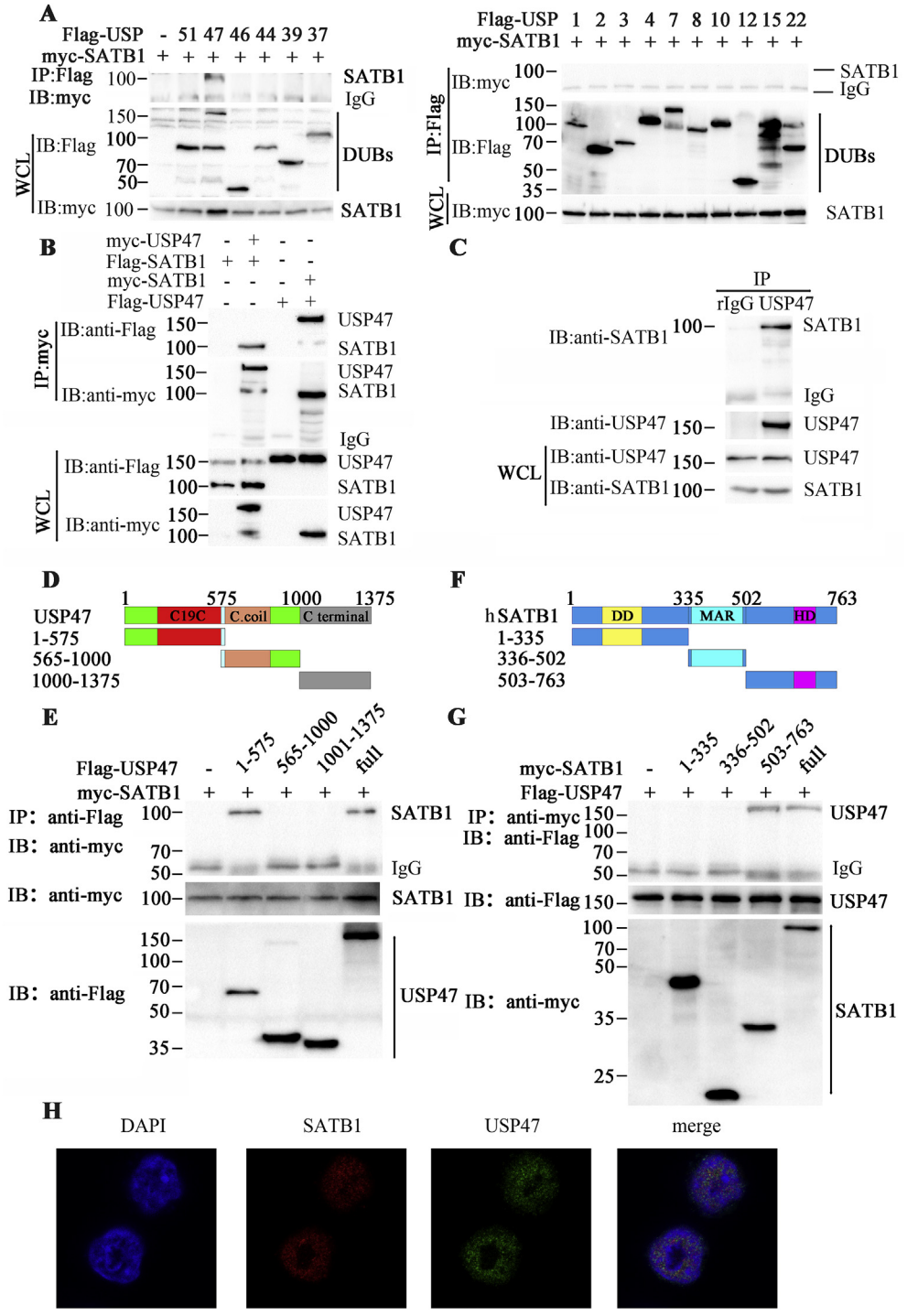

Fig. 1. USP47 is a SATB1-interacting protein. (**A**) Identification of SATB1 interaction partner. The interactions between SATB1 and DUBs (a deubiquitinases library containing about 60 members) were tested in 293T cells. Cells were transfected with SATB1 and all DUBs and immunoprecipitation and immunoblot experiments were performed with indicated antibodies. NS: nonspecific. WCL: whole-cell lysates. (**B**) Flag- or myc-tagged SATB1 expression plasmids were co-transfected with empty vectors or with myc-USP47/Flag-USP47 into 293T cells and their interaction was determined by immunoprecipitation and immunoblot as indicated. (**C**) The endogenous interaction of USP47 and SATB1 was tested in HCT116 cells. Normal rabbit IgG (rIgG) was used as control. (**D**) Schematic representation of USP47 and its mutants, showing that the USP47 protein contains an N-terminal C19 peptidase catalytic domain (C19C), a coiled-coil domain and a C-terminus. (**E**) SATB1 interactions with USP47 and its mutants were tested. SATB1 plasmids were co-transfected with USP47 or each of its mutants into 293T cells, and their interactions were analyzed as in (A). (**F**) Schematic representation of SATB1 and its mutants, indicating that SATB1 contains an N-terminal dimerization domain (DD), an MAR binding domain (MAR) and a C-terminal homeodomain (HD) [10,14]. (**G**) The interactions of SATB1 and its mutants with USP47 were tested. USP47 plasmids were co-transfected with SATB1 or each of its mutants and their interactions were analyzed as in (A). (**H**) Co-localization of SATB1 and USP47 in HCT116 cells. The cellular localization of USP47 and SATB1 was examined by immunofluorescence staining with corresponding antibodies. DAPI was used to stain the DNA. DAPI: 4, 6-diamidino-2-phenylindole.

interaction partner. In addition, we examined whether endogenous SATB1 interacted with USP47 in HCT116 cells. As expected, SATB1 was detected in the anti-USP47 samples, but not in the IgG control immunoprecipitates (Fig. 1C). Therefore, we identified USP47 as a specific interacting protein for SATB1.

Next, we mapped the regions of USP47 that mediate its interaction with SATB1 by generating truncated mutants. The USP47 protein carries an N-terminal C19 peptidase domain, a middle coil-coiled domain, and a C-terminus (Fig. 1D). Co-IP and western blotting analyses revealed that the N-terminal C19 peptidase domain of USP47 is required for its interaction with SATB1, as deletion of this region completely abolished its interaction with SATB1 (Fig. 1E). Similarly, truncated mutation analysis showed that the C-terminal homeodomain (HD) of SATB1 is required for its interaction with USP47 (Fig. 1F and G). Furthermore, USP47 and SATB1 were co-localized in HCT116 cells (Fig. 1H). Taken together, these data suggest that USP47 interacts and co-localizes with SATB1.

USP47 stabilizes SATB1 by negatively regulating its ubiquitination. Because USP47 is a deubiquitinase that protects its substrate from proteasome-mediated degradation, we determined whether this DUB removed ubiquitin from SATB1. Specifically, SATB1 and ubiquitin were transfected into 293T cells with or without USP47 addition. Fig. 2A shows that with USP47 expression, the ubiquitination of SATB1 protein was largely restrained. Conversely, knocking out endogenous

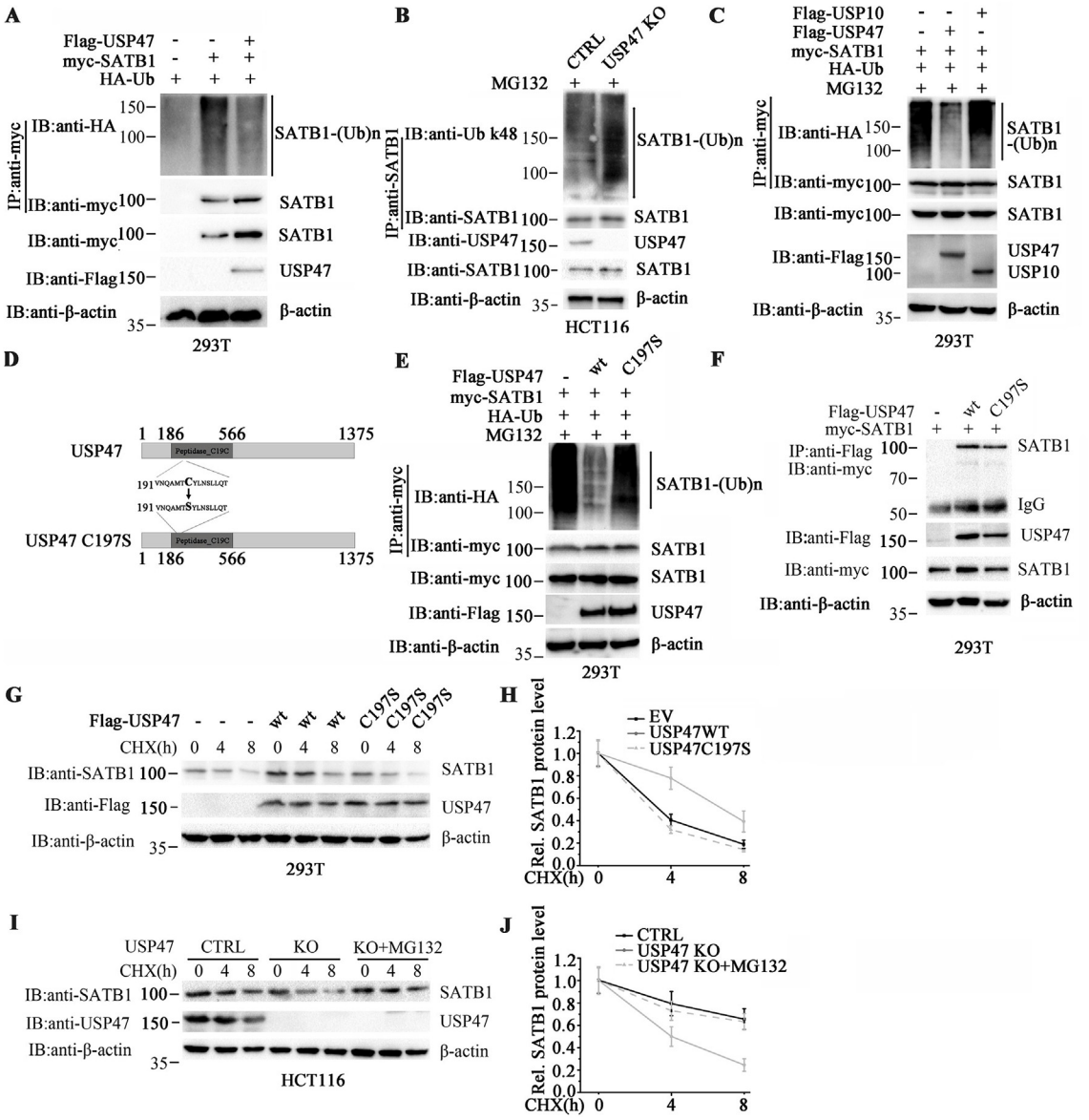

Fig. 2. USP47 mediates SATB1 ubiquitination and stability. (A) HA-Ubiquitin, FLAG-USP47, and myc-SATB1 plasmids were transfected alone or were co-transfected into 293T cells. SATB1 ubiquitination was detected by immunoprecipitation of SATB1 with anti-myc antibody and western blotting with anti-HA antibody. The expression of SATB1 and USP47 in the whole-cell lysates was confirmed. **(B)** The ubiquitination of endogenous SATB1 was analyzed by immunoprecipitation with anti-SATB1 antibody and western blotting with anti-ubiquitin antibody in USP47 wild-type and knockout HCT116 cells. The expression of USP47 and SATB1 was confirmed by western blotting. β-actin was used as a loading control. **(C)** HA-Ubiquitin and myc-SATB1 expression plasmids were co-transfected with USP47 or USP10 into 293T cells. Forty hours later, cells were treated with MG132 for 4 h before harvest. SATB1 ubiquitination was analyzed as in (A). **(D)** Schematic showing of USP47 and its point mutants. The conserved cysteine (C) residue in position 197 in the C19 peptidase domains was replaced by serine (S). **(E)** HA-Ubiquitin and myc-SATB1 expression plasmids were co-transfected into 293T cells without or with USP47 or with the USP47/CS mutant. The effects of USP47 and its mutants on SATB1 ubiquitination were analyzed as in (A). **(F)** The interactions of SATB1 with USP47 or its mutants were analyzed as in Fig. 1A. **(G&H)** 293T cells were transfected with empty vectors or with wildtype USP47 or its CA mutant plasmids, and two days later were treated with 10 mg/ml cycloheximide (CHX) for the indicated time. The effects of SATB1 protein stability were analyzed by western blotting (G) and quantified (H). **(I&J)** SATB1 protein stability was analyzed in control or USP47 knockout HCT116 cells as in (G). Knockout cells were treated with or without MG132. The expression of SATB1 and USP47 was determined by western blotting.

USP47 by CRISPR-Cas9 (Figs. S2A and S2B) dramatically enhanced Lys48 polyubiquitination of endogenous SATB1 in HCT116 cells and led to a decrease in SATB1 protein expression levels (Fig. 2B). Furthermore, as shown in Fig. S2C, overexpression of USP47 effectively removed Lys48-ubiquitination of SATB1. USP47-mediated SATB1 deubiquitination was highly specific, as co-expression of USP10 did not have any effect on SATB1 ubiquitination (Fig. 2C, lane 3). Next, we generated a deubiquitinase inactive mutant of USP47 (USP47/C197S) and assessed the effect of the mutation on SATB1 ubiquitination (Fig. 2D). As seen in Fig. 2E, this mutant failed to inhibit the ubiquitination of SATB1 protein without affecting USP47 interaction with SATB1 (Fig. 2Eand F). Together with our findings that SATB1 interacted with USP47, these results indicate that deubiquitinase activity is required for USP47 to remove ubiquitin from SATB1.

Polyubiquitination promotes protein degradation, which can be reversed by ubiquitin-specific peptidases. Our discovery that USP47 is a deubiquitinase of SATB1 implied that USP47 might regulate SATB1 protein stability. Indeed, expression of USP47 stabilized SATB1 (Fig. S3A). Next, we determined whether deubiquitinase activity is required for USP47 to regulate SATB1 stability. As shown in Fig. 2G and H, and

S3B, the catalytically inactive USP47/CS mutant failed to protect SATB1 from degradation, implying that deubiquitinase activity is required for USP47-mediated SATB1 stabilization. To further confirm our hypothesis that USP47 regulates SATB1 stability, we knocked out USP47 expression in HCT116 cells and found that the endogenous SATB1 protein levels decreased more rapidly than in the control (Fig. 2Iand J). Likewise, knockdown of USP47 in HCT116 cells promoted SATB1 degradation (Fig. S3C). In addition, we determined whether USP47-mediated SATB1 stability is achieved via proteasomes. As seen from Fig. 2I, MG132 (proteasome inhibitor) treatment could rescue the USP47 knockout-mediated degradation of the SATB1 protein. Finally, no changes in SATB1 mRNA expression levels were observed in USP47 knockout HCT116 cells (Fig. S3D), suggesting that the regulation of USP47 on SATB1 stability might occur at the post-transcriptional level. In conclusion, our data suggest that USP47 regulates SATB1 stability.

USP47 promotes colon cancer cell proliferation, tumorigenesis, and invasion partially through SATB1. We next assessed the pathological role of USP47 in CRC by investigating the effects of USP47 overexpression (Fig. S2D) or knockout on the viability, colony formation capacity, tumorigenesis, and invasion of colon cancer cells. The results of the MTS assay confirmed that the growth of HCT116 cells is apparently inhibited by knocking out USP47 or SATB1 (Fig. S2E), whereas introducing SATB1 expression in USP47 knockout cells rescues arrested cell growth (Fig. 3A). Conversely, USP47 or/and SATB1 overexpression (Fig. S2F) induces HCT116 cell proliferation (Fig. 3B). Knocking down SATB1 (Fig. S2G) in USP47-overexpressing cells partially weakened the malignant phenotype. Similar results were obtained in DLD-1 colon cancer cells, wherein USP47 or SATB1 was knocked out or overexpressed alone or in combination (Figs. S4A and B). Accordingly, knocking out USP47 or SATB1 led to cell growth arrest, whereas introducing SATB1 expression into USP47 knockout cells partially restored the faster-growing phenotype (Fig. 3C). We then used an *in vitro* anchorage-independent colony formation assay and analyzed the roles of USP47 and SATB1 in tumor formation. USP47 or SATB1 knockout resulted in a dramatic decrease in colony numbers, and SATB1 transfection rescued the colony formation capacity of USP47-knockout cells (Fig. 3D and E, S4C and S4D). Conversely, the overexpression of USP47 or/and SATB1 significantly enhanced colony formation in HCT116 cells (Fig. 3F and G) and DLD-1 cells (Figs. S4E and S4F). Notably, knocking down SATB1 in USP47-overexpressing cells partially attenuated the malignant phenotype (Fig. 3F–G & S4E and S4F).

In addition, we examined whether knocking out USP47 affects tumorigenesis *in vivo*. As observed in Fig. 3H and I, USP47-knockout dramatically repressed tumor growth in nude mice. Both tumor weight (Fig. 3J) and tumor volume (Fig. 3K) were significantly inhibited in mice that were inoculated with USP47-knockout HCT116 cells.

SATB1 has been reported to promote colorectal cancer metastasis [27]. To explore the roles of USP47-SATB1 interactions in CRC metastasis, we examined cell migration and invasion. The wound-healing assay demonstrated that knocking out USP47 or SATB1 significantly decreased cell migration compared to the control cells, whereas introducing SATB1 into USP47-knockout cells partially restored cell migration capacity (Fig. 3L and S5A). Conversely, the overexpression of USP47 or SATB1 alone or together evidently enhanced cell migration (Fig. 3M and S5B). Consistently, knocking out USP47 or SATB1 markedly decreased the invasion of HCT116 and DLD1 cells in Transwell Matrigel invasion assays, whereas the invasion of USP47 KO cells was rescued by SATB1 addition (Fig. 3N, S5C, and S6A and B). Similarly, we then used an *in vitro* Transwell assay and analyzed the role of USP47 overexpression in HCT116 and DLD1 cells. The overexpression of USP47 or SATB1 alone or together promoted cell invasion in HCT116 (Fig. 3O and S5D) and DLD1 (Figs. S6C and D) cells. Notably, when USP47 was knocked out, these cells repressed the expression of metastasis-related genes (Fig. 3P and S5E).

USP47 deficiency reprograms the expression profiles of cell growth- and tumor metastasis-related genes. To investigate changes in expression profiles of cancer-associated genes, we compared the gene expression pattern of control and USP47 knockout HCT116 cells. A total of 161 genes from quantity proteomics with TMT label identified two groups of genes with significant differential expression levels (> 1.5-fold) after USP47 knockout. Fig. 4 and S7A include 114 upregulated genes, and Fig. 4B and S7B depict 47 downregulated genes upon USP47 knockout. For functional pathway analysis, we identified genes involved in cell adhesion, cell cycle, ECM-receptor interactions, and the PPAR signaling pathway (Fig. 4C) such as ADAM19, ITGB5, SERPINA1, CCNA2, and CCNB1. We used quantitative real-time PCR and western blotting to confirm changes in the expression of cell growth- and migration-related genes (Fig. 4D and E).

SMURF2 binds to, ubiquitinates, and degrades SATB1 in colon cancer cells. We hypothesized that SMURF2, an E3 ubiquitin ligase upregulated upon USP47 depletion in mass spectrometry analysis (Fig. S7A), mediates SATB1 protein stability. To test this hypothesis, we investigated whether SMURF2 interacts with SATB1. SMURF2 was transfected alone or together with SATB1 into 293T cells for protein-protein interaction analysis by co-immunoprecipitation assay. As indicated in Fig. S8A, SATB1 could be pulled down by SMURF2. Moreover, the specific interaction between the endogenous SATB1 and SMURF2 was confirmed in HCT116 cells because the SATB1 protein was detected in the immunoprecipitates with anti-SMURF2, but not with the control rabbit immunoglobulin (Ig) G (Fig. 5A). These results suggest that SATB1 and SMURF2 interact with each other. Furthermore, we confirmed that SMURF2 and SATB1 were co-localized in the nuclei of HCT116 cells using immunofluorescence staining (Fig. 5B).

Because SMURF2 promotes the ubiquitination of its interacting proteins, which targets them for proteasome-mediated degradation [4,34], we analyzed the effect of SMURF2 expression on SATB1 ubiquitination. We detected SATB1 protein ubiquitination in transiently transfected 293T cells (Fig. 5C). Co-expression of SMURF2 with SATB1 significantly enhanced its ubiquitination (Fig. 5C, lane 3). Together with the result that SMURF2 interacts with SATB1, our data indicate that SMURF2 functions as an E3 ubiquitin ligase for SATB1.

Ubiquitination can promote protein degradation. Our discovery that SMURF2 ubiquitinates SATB1 implies that SMURF2 regulates SATB1 protein stability. In fact, ectopic expression of SMURF2 remarkably shortened the half-life of transfected (Figs. S8B and C) and endogenous SATB1 proteins (Fig. 5D and E). To further confirm our notion that SMURF2 regulated SATB1 stability, we knocked down SMURF2 in HCT116 cells and found that the half-life of endogenous SATB1 protein levels was significantly extended (Fig. 5F and G). In contrast, the mRNA expression level of SATB1 was not affected by SMURF2 depletion (Fig. 5I). Taken together, our results indicate that SMURF2 is an E3 ubiquitin ligase of SATB1 that promotes ubiquitin-mediated degradation.

Knocking out SMURF2 promotes colon cancer cell proliferation, invasion, and tumorigenesis partially through SATB1. SATB1 is a tumor promoter, and the subtle upregulation of SATB1 alters the expression profiles of genes involved in cancer cell proliferation and migration [1,18,27]. Therefore, we posited that SMURF2 might antagonize SATB1 activity by downregulating its protein level to inhibit cell proliferation and tumor progression. To test this hypothesis, we first assessed the expression of SATB1 target genes in SMURF2-knockout or -overexpression HCT116 cells by RT-qPCR and western blotting (Fig. 6A and B). As expected, the expression of genes known to have important functions in promoting metastasis and cell cycle progression was found to be upregulated by SMURF2 depletion. As shown in Fig. 6A and B, Snail was upregulated in SMURF2-knockout cells, whereas E-cadherin was downregulated.

Next, we assessed the roles of SMURF2 in CRC progression by investigating the effects of its overexpression or knockout on the viability and colony formation in HCT116 cells. The results of the MTS assay confirmed that the growth of HCT116 cells was apparently inhibited by

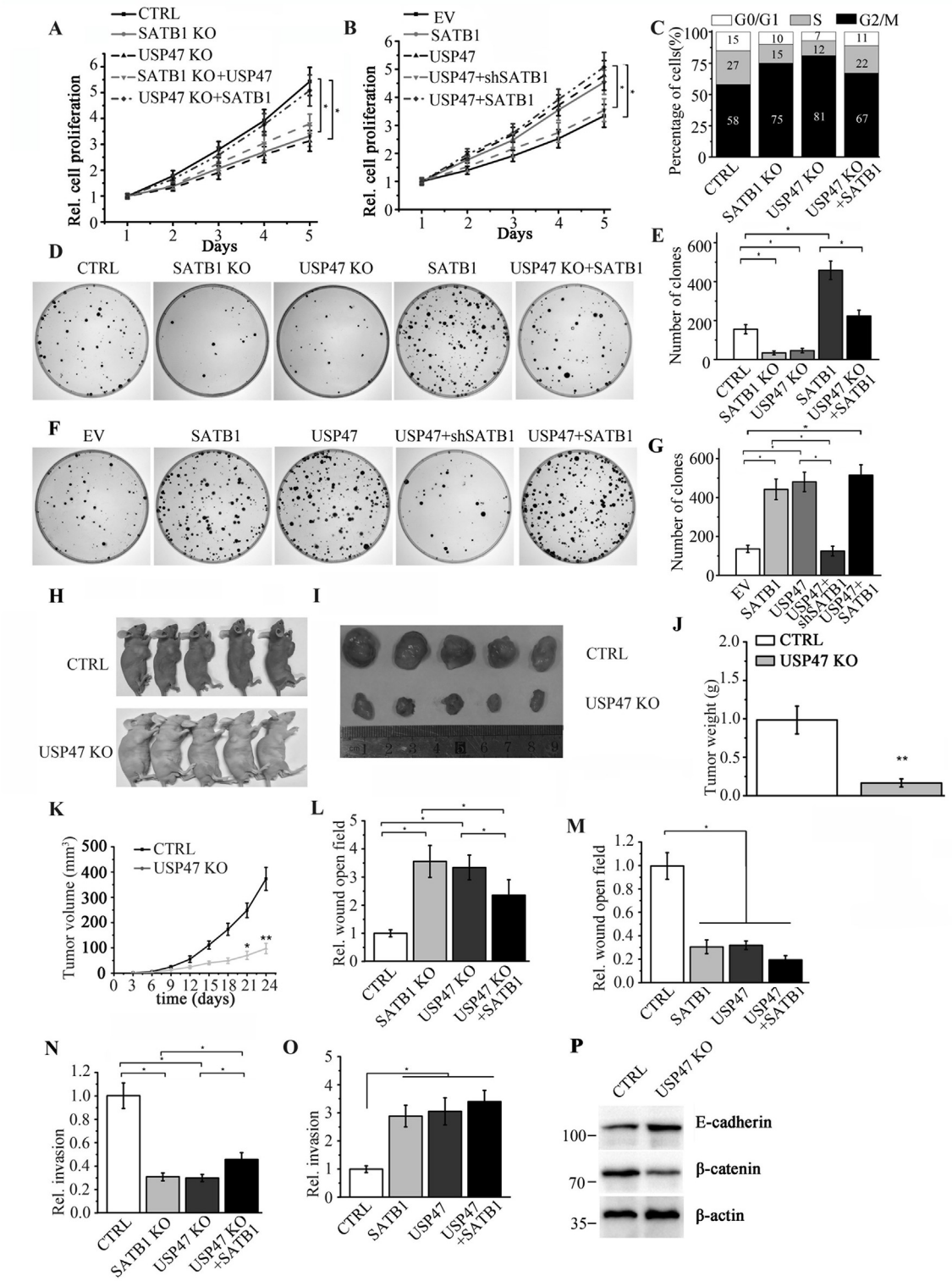

Fig. 3. USP47 promotes cell proliferation, tumorigenesis and migration. (**A&B**) MTS assay was performed to determine the cell proliferation in HCT116 cells stably expressing indicated plasmids or combination. (**C**) The effects of SATB1-knockout, USP47-knockout or USP47-knockout plus SATB1-overexpression (USP47KO + SATB1) on HCT116 cell cycle progression were measured by flow cytometry analysis. (**D-G**) Clonogenic assay was performed to measure the capacity of colony formation in HCT116 cells stably expressing indicated plasmids or a combination of plasmids. (**H-K**) Representative images showing mice euthanized (H) and xenograft tumors (I) at day 24 post subcutaneous injection (n = 5). Tumor sizes were measured and depicted as tumor weight (J) or tumor volume (K). Results presented represent the means of triplicate experiments ± SEM. *P < 0.05; **P < 0.01. (**L-M**) Wound healing assays were performed to measure the migration of HCT116 cells stably expressing indicated single or a combination of plasmids. Cells were monitored within 24 h or 18 h to evaluate the rate of migration into the scratched area. The percentage of wound open field is shown. Results presented represent the mean of triplicate experiments ± SEM. *P < 0.05. (**N-O**) The effects of stable overexpression or knockout of USP47 or SATB1 alone or indicated combinations on cell invasion were measured in HCT116 cells by Matrigel transwell assays. The quantitative results are shown. (**P**) Western blotting analysis showed knockout of USP47 in HCT116 cells leading to a significant increase in E-cadherin expression and inhibited β-catenin expression.

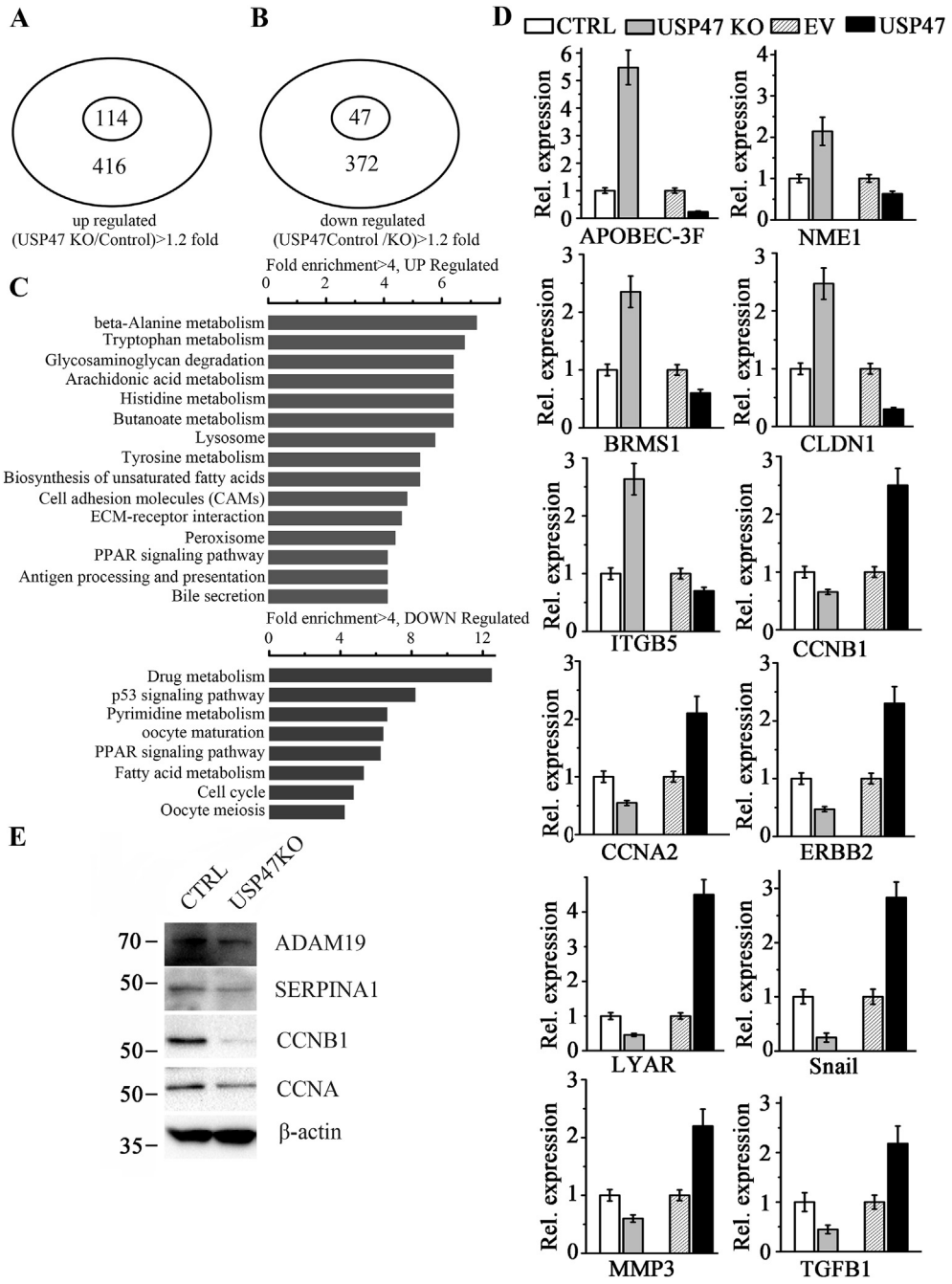

Fig. 4. Global identification of cancer-associated genes upon USP47 depletion. (**A&B**) Proteomic analysis of genes differentially expressed between control and USP47-knockout HCT116 cells, and 114 USP47-activated genes and 47 USP47-repressed genes are shown with Venn diagrams. (**C**) Representative image showing of USP47-activated and USP47-repressed genes by KEGG analysis. (**D**) Expression levels of multiple genes in the proteomic analysis, including SATB1-dependent genes, were confirmed by RT-qPCR: control versus USP47 knockout cells; empty vector versus USP47 overexpression cells. (**E**) Protein levels of multiple genes in the above assay, including cancer-related genes, were confirmed with western blotting.

SMURF2 overexpression, whereas SATB1 transfection resulted in an increase in cell proliferation in SMURF2-overexpressing HCT116 cells (Fig. S9A). By contrast, SMURF2-knockout induced HCT116 cells proliferation, but SATB1 knockdown in SMURF2-knockout cells retarded the proliferation rate, suggesting an inhibitory function of SMURF2 in cell proliferation (Fig. 6C). Similarly, SMURF2-knockout resulted in an increase in cell numbers in DLD-1 (Fig. S9B). We then used *in vitro* anchorage-independent colony formation assay and analyzed the role of SMURF2 in tumor formation. SMURF2-knockout in HCT116 cells resulted in a dramatic increase in colony numbers, and SATB1-knockdown partially attenuated the malignant phenotype in SMURF2-knockout HCT116 cells (Fig. 6D and E). Similar results were obtained using DLD-1 cell lines (Fig. S9C). Furthermore, SMURF2 knockout-mediated cell cycle progression was antagonized by SATB1-knockdown (Fig. 6F). To explore the roles of SMURF2-SATB1 interaction in CRC metastasis, we examined CRC cell migration and invasion. Knockout of SMURF2 significantly increased cell migration compared to the control HCT116 cells using the wound healing assay, whereas knockdown of SATB1 decreased cell migration compared to the control SMURF2-knockout HCT116 cells (Figs. S9D and S9E). Consistently, knocking out SMURF2 markedly induced the invasion of HCT116 cells in Transwell Matrigel invasion assays, whereas the invasion of SMURF2-knockout cells was suppressed by additional SATB1-knockdown (Fig. 6G and S9F).

Finally, we examined whether knocking out SMURF2 affected tumorigenesis *in vivo*. As observed in Fig. 6H, SMURF2-knockout dramatically induced tumor growth in nude mice. Both the tumor volume (Fig. 6I) and tumor weight (Fig. 6J) were significantly enhanced in mice that had been injected with SMURF2-knockout HCT116 cells, whereas injection with SATB1-knockdown plus SMURF2-knockout cells partially weakened the malignant phenotype. These data suggested that knockout of SMURF2 might promote cell proliferation and tumor

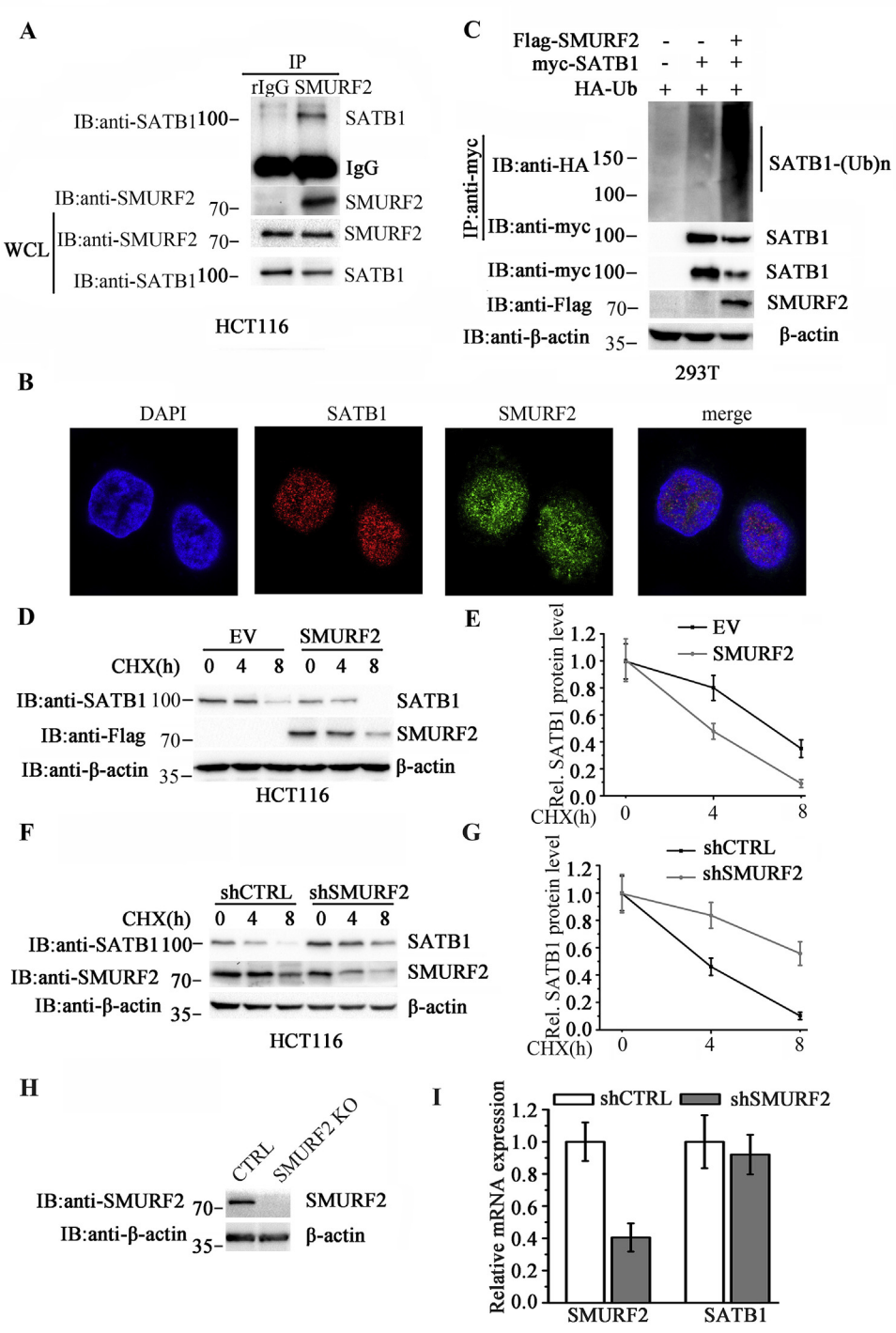

Fig. 5. SMURF2 binds to, ubiquitinates, and degrades SATB1. (**A**) The endogenous interaction of SMURF2 and SATB1 was tested in HCT116 cells. Normal rabbit IgG (rIgG) was used as a control. (**B**) Co-localization of SMURF2 and SATB1 in HCT116 cells. The cellular localization of SMURF2 and SATB1 was examined as in Fig. 1(I). (**C**) HA-Ubiquitin, Flag-SMURF2, and myc-SATB1 plasmids were transfected alone or co-transfected into 293T cells. SATB1 ubiquitination was detected by immunoprecipitation with anti-myc antibody and western blotting with anti-HA antibody. The expression of SATB1 and SMURF2 in the whole-cell lysates was confirmed. (**D&E**) SATB1 protein stabilities were analyzed in HCT116 cells stably expressing SMURF2 plasmids or the empty vector by treatment with CHX for different times and were quantified. (**F&G**) SATB1 protein stabilities were analyzed in the control or SMURF2 knockdown HCT116 cells as described in (D) and were quantified. (**H**) Western blotting analysis showed knockdown of SMURF2 in HCT116 cells. (**I**) mRNA levels of both SATB1 and SMURF2 were determined by real-time qPCR in HCT116 cells stably expressing SMURF2 knockdown or control plasmids. Their relative levels are shown. The error bar represents the SEM of triplicate experiments.

progression through SATB1.

SMURF2 is negatively regulated by USP47, and USP47 depletion sensitizes colon cancer cells to 5-FU-treatment-induced apoptosis. Thus far, we have demonstrated that USP47 was a deubiquitinase of SATB1 and SMURF2 functioned as its E3 ubiquitin ligase. Based on the relationship among USP47, SATB1 and SMURF2, we next examined whether SMURF2 was regulated by USP47 or whether USP47 was regulated by SMURF2. As indicated in Fig. 7A and B, we determined that SMURF2 was upregulated in USP47-knockout cells at the mRNA and protein levels. Conversely, forced expression of USP47 led to a repression of SMURF2 expression. Furthermore, ectopic expression of USP47 remarkably shortened the half-life of endogenous SMURF2 proteins (Fig. S8E). However, we did not observe a change in USP47 expression in SMURF2-knockout cells (Fig. 5H, S8D, and S8F). Therefore, these findings suggest that SMURF2 might be downregulated by USP47.

SATB1 is remarkably upregulated in multiple cancers, including colon cancer [27,38]. To further investigate the relationship among USP47, SMURF2, and SATB1, we examined their expression level in colon cancer tissues (n = 9) and matched adjacent normal colon tissues (n = 9). As shown in Fig. 7C, USP47 and SATB1 showed higher expression in colon cancer tissues compared with adjacent normal tissues (in seven of nine patients), and the relative USP47 and SATB1 levels were clearly positively correlated. In addition, the expression of

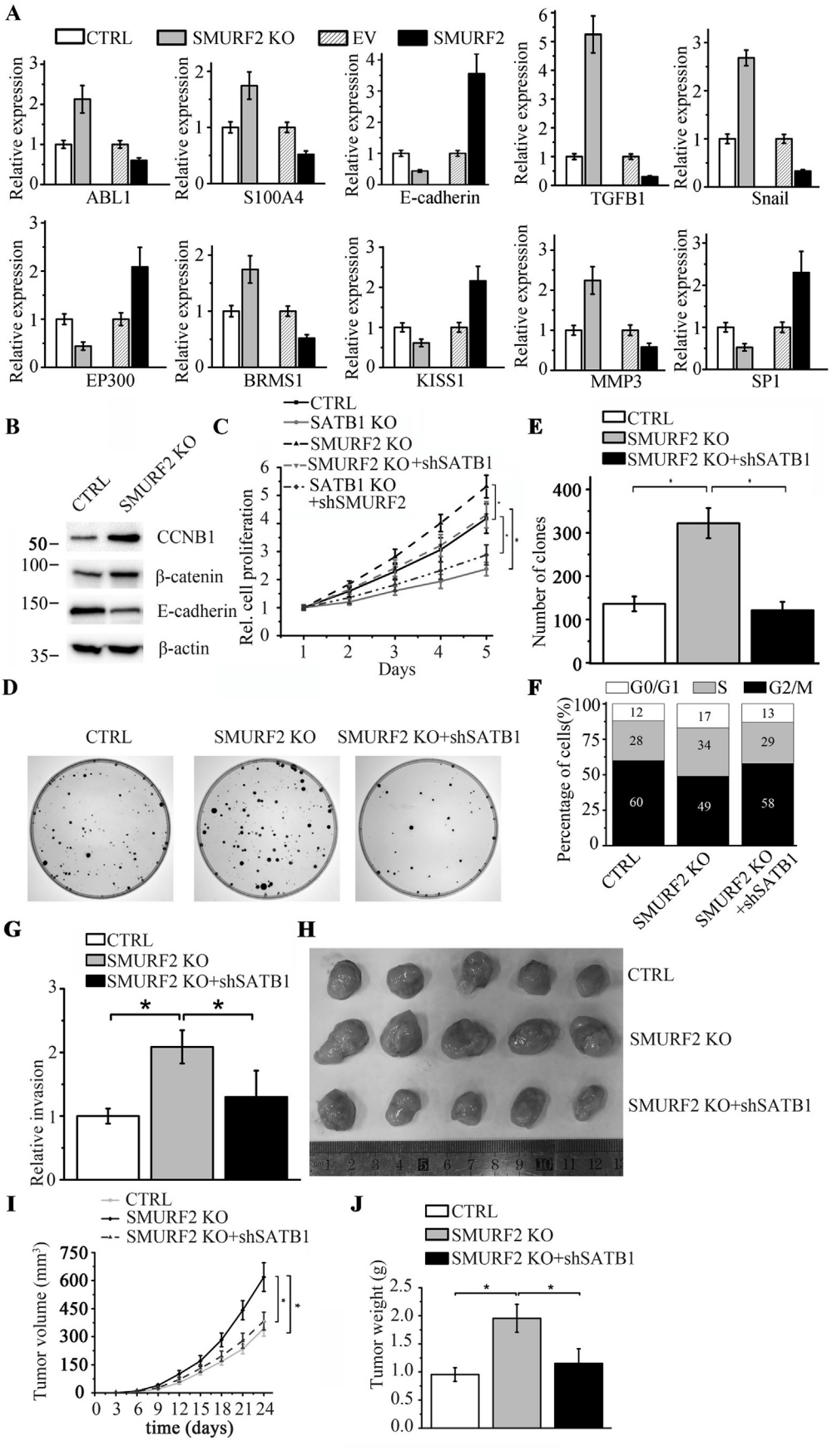

Fig. 6. SMURF2-knockout promotes cell proliferation, invasion, and tumorigenesis. (**A**) Expression of SATB1 target genes in SMURF2-knockout or -overexpression HCT116 cells was confirmed by RT-qPCR. (**B**) Western blots showed expression changes in multiple cancer-related genes. **(C)** MTS assay was performed to determine the cell proliferation in indicated HCT116 cell lines. **(D&E)** Clonogenic assay was performed to measure the capacity of colony formation in HCT116 cells stably expressing indicated plasmids or combination of plasmids (D). Quantitation of colony number is shown in panel (E). **(F)** Flow cytometry analysis was performed to determine the cell cycle progression in HCT116 cells stably expressing SMURF2-knockout alone or plus SATB1-knockdown plasmids. **(G)** The effects of stable knockout of SMURF2- or SMURF2-knockout plus SATB1-knockdown on cell invasion were measured in HCT116 cells by Matrigel Transwell assays**. (H–J)** HCT116 cells stably expressing control, SMURF2 knockout or/and SATB1 knockdown plasmids were injected subcutaneously into nude mice. Representative images showing xenograft mice tumors (H) at day 24 post subcutaneous injection (n = 5). Tumor sizes were measured and depicted as tumor volume (I) or tumor weight (J). Results presented represent the means of triplicate experiments ± SEM. *P < 0.05; **P < 0.01.

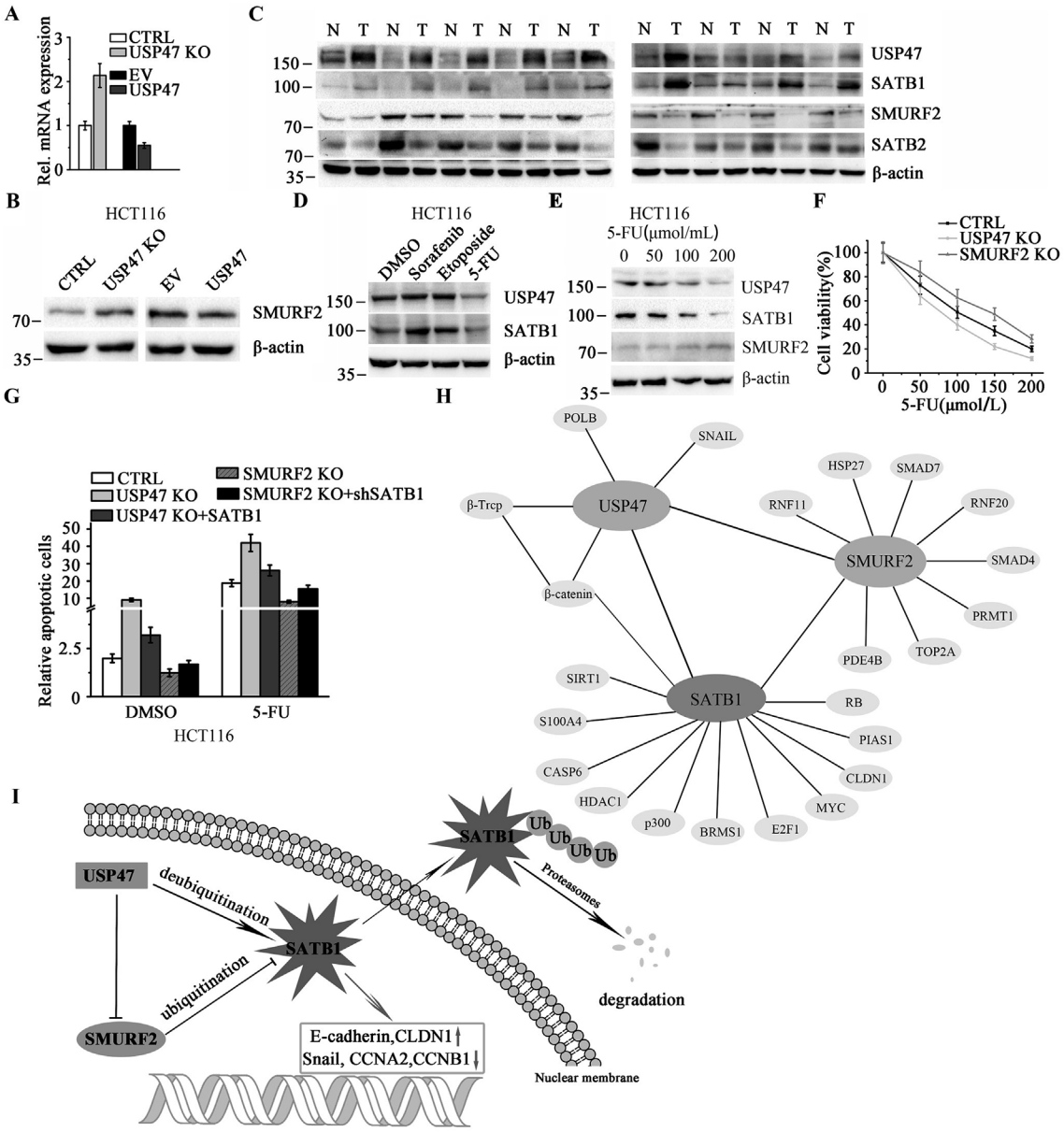

Fig. 7. Interplays among SATB1, SMURF2, and USP47. (**A&B**) The expression of SMURF2 in USP47-knockout or -overexpression HCT116 cells was measured by RT-qPCR (A) and western blotting (B). (**C**) Western blot analysis of SATB1, USP47, SATB2 and SMURF2 expression in colon cancer tissue specimens (n = 9) and matched adjacent normal tissues (n = 9). β-actin was used as a loading control. (**D**) 5×10^6 HCT116 cells were treated with Etoposide (20 nmol/L), Sorafenib (20 μmol/L), or 5-FU (100 μmol/L) for 48 h. USP47 and SATB1 expression was examined by western blotting. (**E**) 2×10^6 HCT116 cells were treated with 5-FU at different concentrations as indicated for 24 h. USP47, SATB1, and SMURF2 expression was examined by western blotting. (**F**) The viability of the control, USP47-knockout and SMURF2-knockout HCT116 cells exposed to 5-FU at different concentrations for 48 h was detected with an MTS kit. (**G**) HCT116 cells stably expressing USP47-knockout constructs, USP47-knockout plus SATB1-overexpression plasmids, SMURF2-knockout constructs, SMURF2-knockout plus SATB1-knockdown constructs, or control vectors were treated with DMSO or 5-FU for 24 h, and apoptosis was evaluated by Annexin V-FITC and PI staining followed by flow cytometry analysis. (**H**) USP47-SATB1-SMURF2 interaction network. (**I**) Schematic model showed SATB1 regulation by USP47 and SMURF2.

SMURF2 and SATB2 was downregulated in colon cancer tissues (in seven out of nine patients) whereas USP47 and SATB1 were upregulated (Fig. 7C). However, we noticed that the expression pattern of USP47, SATB1 and SMURF2 in two of nine samples is inconsistent with others, and we do not know the reasons.

To evaluate the therapeutic potential of the USP47/SATB1/SMURF2 interaction targeting in cancer treatment, we treated colon cancer cells with different anti-cancer drugs. As shown in Fig. 7D, USP47 and SATB1 expression was downregulated by 5-FU treatment. The protein expression of USP47 and SATB1 decreased and SMURF2 increased in a dose-dependent manner following 5-FU treatment (Fig. 7E). Then, we detected the effect of 5-FU treatment on cell viability in colon cancer HCT116 cells. As shown in Fig. 7F, treatment with 5-FU at the doses from 50 μmol/L to 200 μmol/L significantly inhibited cell viability in a dose-dependent manner in HCT116 cells. Next, we examined the effects of USP47 and SATB1 on apoptosis with or without 5-FU treatment. USP47-knockout sensitized HCT116 cells to apoptosis, and SATB1 overexpression plus USP47 knockout (USP47−KO + SATB1) partially rescued cells from apoptosis, suggesting a therapeutic potential of USP47 inhibition for cancer treatment. In addition, SMURF2-knockout decreased the sensitivity of HCT116 cells to apoptosis, and further SATB1-knockdown in SMURF2-knockout cells induced apoptosis (Fig. 7G). This result suggested that repression of SATB1 expression combined with 5-FU treatment may be more effective for cancer

therapy. Collectively, our data implied that USP47/SATB1 might be targeted in cancer therapy for colon cancer patients.

In conclusion, USP47 functioned as a deubiquitinase, whereas SMURF2 functioned as an E3 ubiquitin ligase of SATB1 to regulate colon cancer cell proliferation, migration, invasion, and tumor growth by interacting with SATB1. In this study, we discovered that USP47 and SMURF2 regulated SATB1 function via opposing effects on its ubiquitination (Fig. 7H&I).

4. Discussion

SATB1 is a nuclear protein that functions as a 'genome organizer' and is essential for certain T-cell development [2]. SATB1 forms a "cage-like" nuclear architecture structure by combining with the AT-rich sequence in the genome, which is also termed "the SATB1 regulatory network". SATB1 regulates the expression of thousands of genes by recruiting chromatin remodeling enzymes and transcription factors to genomic DNA, thus determining the classification of cell types and their functions [6,41]. Given the functional importance of SATB1 and its potential targeting application by drug inhibition in cancer therapy, it is important to identify the deubiquitinase that may stabilize SATB1. Furthermore, there is also a need to identify E3 ubiquitin ligase that regulates SATB1 ubiquitination and degradation.

In this study, we demonstrated that USP47 is a deubiquitinase of SATB1. Co-immunoprecipitation and ubiquitination studies confirmed that SATB1 was a direct substrate for USP47. We also provided evidence that USP47 positively regulated SATB1 stability. Furthermore, we demonstrated that USP47 deficiency decelerated CRC cell proliferation and tumor formation, and overexpression of SATB1 restored the cell proliferative phenotype in USP47-knockout cells. In addition, we demonstrated that USP47 promotes colon cancer cell metastasis and invasion by upregulating SATB1 expression.

Over the past decade, increasing studies have reported that SATB1 is highly expressed in a variety of tumors and is positively correlated with tumor growth, proliferation, migration and EMT. SATB1 deficiency leads to lower proliferation, decreased colony formation, invasion, and migration capacity in breast cancer cells [16]. Silencing of SATB1 significantly influenced cell morphology and reduced EMT [23]. In addition, SATB1 downregulation leads to decreased tumor growth and tumor formation *in vivo* [16]. SATB1 is also highly expressed in colon cancer and is a novel target of Wnt/β-catenin signal participant in colorectal cancer tumorigenesis and progression [27]. SATB1 regulates the expression of β-catenin-associated EMT in CRC [23,27]. Furthermore, USP47 is a novel deubiquitinase of β-catenin and positively regulates its stability [32], which is consistent with the regulatory function of USP47 in cell proliferation and migration. To test the hypothesis that SATB1 is responsible for the USP47-induced EMT in colon cancer cells, we generated USP47-knockout HCT116 cells and overexpressed SATB1 in this cell line. Our findings indicated that USP47 deletion decreased cell proliferation and migration, whereas introducing SATB1 expression rescued cell growth and invasion.

Ubiquitinases play major roles in several cellular processes and signaling pathways, and their defects have been associated with a number of diseases including cancer. Deubiquitinases and E3 ubiquitin ligases exert opposing effects on the ubiquitination levels of their substrates and, as such, are critical for the regulation of ubiquitination-dependent processes. Despite the importance of SATB1 function and the role that deubiquitination plays in the regulation of its activity and turnover, mechanisms that ubiquitinate SATB1 and the ubiquitinases involved in these processes remain elusive. SMURF2 is an E3 ubiquitinase that is important for the ubiquitination and regulation of turnover and function of a number of substrates, including RNF11, SMAD2, TOP2A, PDE4B, RNF20, MDM2, USP15, YY1, and SMAD7 [4,7,9,13,19,20,24,25,28]. Notably, in this study, we also identified SMURF2 as an interacting partner for SATB1. Furthermore, we discovered that SMURF2 was involved in controlling cell growth and invasion through SATB1 inhibition.

A previous study showed that ΔE2Smurf2 reduced production of pro-inflammatory cytokines to control intestinal tumor growth by enhancing TGF-β receptor type II expression [12]. Tumor-derived transforming growth factor β (TGF-β) decreased SATB1 expression by binding SMAD proteins to the SATB1 promoter. Furthermore, the SATB1-binding status of SATB1-dependent gene TGF-β1 indicates that SATB1 directly regulates its expression [36], which is consistent with our findings that SMURF2 downregulated both SATB1 and TGF-β1expression.

Proteomics analysis has indicated that SMURF2 is highly expressed in USP47-knockout HCT116 cells compared with the control cells. Likewise, we reported that SMURF2 was negatively regulated by USP47 at the mRNA and protein levels. It is possible that USP47 indirectly controls the expression of SMURF2 through transcriptional repression; however, further studies are needed to investigate how USP47 regulates SMURF2 expression.

In this study, we also reported that in addition to USP47, SMURF2 also interacts with SATB1. We showed that SMURF2 acted as an E3 ubiquitin ligase to regulate polyubiquitination of SATB1. Ubiquitination assay demonstrated that SATB1 was a direct substrate for SMURF2. Our data also supported that SMURF2 negatively regulated SATB1 stability. Furthermore, we provided evidence that SMURF2-knockout accelerated CRC cell proliferation and tumor formation, and knocking down SATB1 suppressed the growth of SMURF2-knockout cells. In addition, we demonstrated that SMURF2 deficiency promoted colon cancer cell metastasis and invasion. Interestingly, we also observed that SMURF2 is downregulated by USP47.

In summary, our results revealed that USP47 is a deubiquitinase for SATB1 and mediates SATB1 ubiquitination and degradation. USP47 stimulates cell growth, tumorigenesis and migration through SATB1. We also identified SMURF2 as an E3 ligase interacting with SATB1 and provided evidence that SMURF2 ubiquitinates SATB1 and negatively regulates its protein level. In addition, we found that USP47 or SATB1 is downregulated by 5-FU treatment, and USP47/SATB1 depletion sensitized colon cancer cells to apoptosis. In conclusion, our data suggested that repression of USP47/SATB1 expression combined with 5-FU treatment may be more effective than drug treatment alone for colon cancer therapy.

Conflicts of interest

None declared.

Acknowledgements

The Fundamental Research Funds for the Central Universities (Project No.106112017CDJQJ298833), the National Natural Science Foundation of China (Grant No.31571454 and No.31801181), the 61st Batch of China Postdoctoral Science Fund (Grant No.2017M610588), and the Special Fund for Postdoctoral Research Projects of Chongqing City (Grant No. Xm2017080) supported this study. We highly appreciate Dr. Long Zhang (Zhejiang University) for constructive suggestions and for reviewing the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2019.01.039.

References

[1] R. Agrelo, H. Kishimoto, M. Novatchkova, V. Peraza, M. Paolino, A. Souabni, A. Wutz, SATB1 collaborates with loss of p16 in cellular transformation, Oncogene 32 (2013) 5492–5500.
[2] J.D. Alvarez, D.H. Yasui, H. Niida, T. Joh, D.Y. Loh, T. Kohwi-Shigematsu, The

MAR-binding protein SATB1 orchestrates temporal and spatial expression of multiple genes during T-cell development, Genes Dev. 14 (2000) 521–535.
[3] M. Ashburner, C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, M.A. Harris, D.P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J.C. Matese, J.E. Richardson, M. Ringwald, G.M. Rubin, G. Sherlock, Gene ontology: tool for the unification of biology. The Gene Ontology Consortium, Nat. Genet. 25 (2000) 25–29.
[4] M. Blank, Y. Tang, M. Yamashita, S.S. Burkett, S.Y. Cheng, Y.E. Zhang, A tumor suppressor function of Smurf2 associated with controlling chromatin landscape and genome stability through RNF20, Nat. Med. 18 (2012) 227–234.
[5] J. Brocato, M. Costa, SATB1 and 2 in colorectal cancer, Carcinogenesis 36 (2015) 186–191.
[6] S. Cai, H.J. Han, T. Kohwi-Shigematsu, Tissue-specific nuclear architecture and gene expression regulated by SATB1, Nat. Genet. 34 (2003) 42–51.
[7] Y. Cai, G. Huang, L. Ma, L. Dong, S. Chen, X. Shen, S. Zhang, R. Xue, D. Sun, S. Zhang, Smurf2, an E3 ubiquitin ligase, interacts with PDE4B and attenuates liver fibrosis through miR-132 mediated CTGF inhibition, Biochim. Biophys. Acta 1865 (2018) 297–308.
[8] B.J. Choi, S.A. Park, S.Y. Lee, Y.N. Cha, Y.J. Surh, Hypoxia induces epithelial-mesenchymal transition in colorectal cancer cells through ubiquitin-specific protease 47-mediated stabilization of Snail: a potential role of Sox9, Sci. Rep. 7 (2017) 15918.
[9] P.A. Chong, H. Lin, J.L. Wrana, J.D. Forman-Kay, An expanded WW domain recognition motif revealed by the interaction between Smad7 and the E3 ubiquitin ligase Smurf2, J. Biol. Chem. 281 (2006) 17069–17075.
[10] L.A. Dickinson, C.D. Dickinson, T. Kohwi-Shigematsu, An atypical homeodomain in SATB1 promotes specific recognition of the key structural element in a matrix attachment region, J. Biol. Chem. 272 (1997) 11463–11470.
[11] L.A. Dickinson, T. Joh, Y. Kohwi, T. Kohwi-Shigematsu, A tissue-specific MAR/SAR DNA-binding protein with unusual binding site recognition, Cell 70 (1992) 631–645.
[12] H. Dornhoff, C. Becker, S. Wirtz, D. Strand, S. Tenzer, S. Rosfa, C. Neufert, J. Mudter, J. Markl, J. Siebler, M.F. Neurath, A variant of Smurf2 protects mice against colitis-associated colon cancer by inducing transforming growth factor beta signaling, Gastroenterology 142 (2012) 1183–1194 e1184.
[13] A. Emanuelli, A.P. Borroni, L. Apel-Sarid, P.A. Shah, D.M. Ayyathan, P. Koganti, G. Levy-Cohen, M. Blank, Smurf2-Mediated stabilization of DNA topoisomerase IIalpha controls genomic integrity, Cancer Res. 77 (2017) 4217–4227.
[14] S. Galande, L.A. Dickinson, I.S. Mian, M. Sikorska, T. Kohwi-Shigematsu, SATB1 cleavage by caspase 6 disrupts PDZ domain-mediated dimerization, causing detachment from chromatin early in T-cell apoptosis, Mol. Cell Biol. 21 (2001) 5591–5604.
[15] S. Galande, P.K. Purbey, D. Notani, P.P. Kumar, The third dimension of gene regulation: organization of dynamic chromatin loopscape by SATB1, Curr. Opin. Genet. Dev. 17 (2007) 408–414.
[16] H.J. Han, J. Russo, Y. Kohwi, T. Kohwi-Shigematsu, SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis, Nature 452 (2008) 187–193.
[17] S. Han, J. Xia, X. Qin, S. Han, A. Wu, Phosphorylated SATB1 is associated with the progression and prognosis of glioma, Cell Death Dis. 4 (2013) e901.
[18] E. Iorns, H.J. Hnatyszyn, P. Seo, J. Clarke, T. Ward, M. Lippman, The role of SATB1 in breast cancer pathogenesis, J. Natl. Cancer Inst. 102 (2010) 1284–1296.
[19] P.V. Iyengar, P. Jaynes, L. Rodon, D. Lama, K.P. Law, Y.P. Lim, C. Verma, J. Seoane, P.J. Eichhorn, USP15 regulates SMURF2 kinetics through C-lobe mediated deubiquitination, Sci. Rep. 5 (2015) 14733.
[20] H.M. Jeong, S.H. Lee, J. Yum, C.Y. Yeo, K.Y. Lee, Smurf2 regulates the degradation of YY1, Biochim. Biophys. Acta 1843 (2014) 2005–2011.
[21] M. Kanehisa, S. Goto, S. Kawashima, Y. Okuno, M. Hattori, The KEGG resource for deciphering the genome, Nucleic Acids Res. 32 (2004) D277–D280.
[22] D. Komander, M.J. Clague, S. Urbe, Breaking the chains: structure and function of the deubiquitinases, Nat. Rev. Mol. Cell Biol. 10 (2009) 550–563.
[23] Q.Q. Li, Z.Q. Chen, X.X. Cao, J.D. Xu, J.W. Xu, Y.Y. Chen, W.J. Wang, Q. Chen, F. Tang, X.P. Liu, Z.D. Xu, Involvement of NF-kappaB/miR-448 regulatory feedback loop in chemotherapy-induced epithelial-mesenchymal transition of breast cancer cells, Cell Death Differ. 18 (2011) 16–25.
[24] X. Lin, M. Liang, X.H. Feng, Smurf2 is a ubiquitin E3 ligase mediating proteasome-dependent degradation of Smad2 in transforming growth factor-beta signaling, J. Biol. Chem. 275 (2000) 36818–36822.
[25] R.J. Malonis, W. Fu, M.J. Jelcic, M. Thompson, B.S. Canter, M. Tsikitis, F.J. Esteva, I. Sanchez, RNF11 sequestration of the E3 ligase SMURF2 on membranes antagonizes SMAD7 down-regulation of transforming growth factor beta signaling, J. Biol. Chem. 292 (2017) 7435–7451.
[26] N. McInnes, T.J. Sadlon, C.Y. Brown, S. Pederson, M. Beyer, J.L. Schultze, S. McColl, G.J. Goodall, S.C. Barry, FOXP3 and FOXP3-regulated microRNAs suppress SATB1 in breast cancer cells, Oncogene 31 (2012) 1045–1054.
[27] R. Mir, S.J. Pradhan, P. Patil, R. Mulherkar, S. Galande, Wnt/beta-catenin signaling regulated SATB1 promotes colorectal cancer tumorigenesis and progression, Oncogene 35 (2016) 1679–1691.
[28] J. Nie, P. Xie, L. Liu, G. Xing, Z. Chang, Y. Yin, C. Tian, F. He, L. Zhang, Smad ubiquitylation regulatory factor 1/2 (Smurf1/2) promotes p53 degradation by stabilizing the E3 ligase MDM2, J. Biol. Chem. 285 (2010) 22818–22830.
[29] S.M. Nijman, M.P. Luna-Vargas, A. Velds, T.R. Brummelkamp, A.M. Dirac, T.K. Sixma, R. Bernards, A genomic and functional inventory of deubiquitinating enzymes, Cell 123 (2005) 773–786.
[30] J.L. Parsons, Dianova II, S.V. Khoronenkova, M.J. Edelmann, B.M. Kessler, G.L. Dianov, USP47 is a deubiquitylating enzyme that regulates base excision repair by controlling steady-state levels of DNA polymerase beta, Mol. Cell 41 (2011) 609–615.
[31] P. Pavan Kumar, P.K. Purbey, C.K. Sinha, D. Notani, A. Limaye, R.S. Jayani, S. Galande, Phosphorylation of SATB1, a global gene regulator, acts as a molecular switch regulating its transcriptional activity in vivo, Mol. Cell 22 (2006) 231–243.
[32] A. Peschiaroli, J.R. Skaar, M. Pagano, G. Melino, The ubiquitin-specific protease USP47 is a novel beta-TRCP interactor regulating cell survival, Oncogene 29 (2010) 1384–1393.
[33] P.K. Purbey, S. Singh, D. Notani, P.P. Kumar, A.S. Limaye, S. Galande, Acetylation-dependent interaction of SATB1 and CtBP1 mediates transcriptional repression by SATB1, Mol. Cell Biol. 29 (2009) 1321–1337.
[34] C. Ramkumar, H. Cui, Y. Kong, S.N. Jones, R.M. Gerstein, H. Zhang, Smurf2 suppresses B-cell proliferation and lymphomagenesis by mediating ubiquitination and degradation of YY1, Nat. Commun. 4 (2013) 2598.
[35] J. Shi, Y. Liu, X. Xu, W. Zhang, T. Yu, J. Jia, C. Liu, Deubiquitinase USP47/UBP64E regulates beta-catenin ubiquitination and degradation and plays a positive role in Wnt signaling, Mol. Cell Biol. 35 (2015) 3301–3311.
[36] T.L. Stephen, K.K. Payne, R.A. Chaurio, M.J. Allegrezza, H. Zhu, J. Perez-Sanz, A. Perales-Puchalt, J.M. Nguyen, A.E. Vara-Ailor, E.B. Eruslanov, M.E. Borowsky, R. Zhang, T.M. Laufer, J.R. Conejo-Garcia, SATB1 expression governs epigenetic repression of PD-1 in tumor-reactive T cells, Immunity 46 (2017) 51–64.
[37] J.A. Tan, Y. Sun, J. Song, Y. Chen, T.G. Krontiris, L.K. Durrin, SUMO conjugation to the matrix attachment region-binding protein, special AT-rich sequence-binding protein-1 (SATB1), targets SATB1 to promyelocytic nuclear bodies where it undergoes caspase cleavage, J. Biol. Chem. 283 (2008) 18124–18134.
[38] A.J. Tesone, M.R. Rutkowski, E. Brencicova, N. Svoronos, A. Perales-Puchalt, T.L. Stephen, M.J. Allegrezza, K.K. Payne, J.M. Nguyen, J. Wickramasinghe, J. Tchou, M.E. Borowsky, G.A. Rabinovich, A.V. Kossenkov, J.R. Conejo-Garcia, Satb1 overexpression drives tumor-promoting activities in cancer-associated dendritic cells, Cell Rep. 14 (2016) 1774–1786.
[39] K.H. Ventii, K.D. Wilkinson, Protein partners of deubiquitinating enzymes, Biochem. J. 414 (2008) 161–175.
[40] Y. Wang, X. Gu, G. Zhang, L. Wang, T. Wang, Y. Zhao, X. Zhang, Y. Zhou, M. Kadin, P. Tu, SATB1 overexpression promotes malignant T-cell proliferation in cutaneous CD30+ lymphoproliferative disease by repressing p21, Blood 123 (2014) 3452–3461.
[41] D. Yasui, M. Miyano, S. Cai, P. Varga-Weisz, T. Kohwi-Shigematsu, SATB1 targets chromatin remodelling to regulate genes over long distances, Nature 419 (2002) 641–645.