nature portfolio

Peer Review File

ATAD5-BAZ1B interaction modulates PCNA ubiquitination during DNA repair

Corresponding Author: Dr Sukhyun Kang

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
How DNA lesion bypass replication and a central component in orchestrating replication, PCNA, are regulated are important questions.
In this paper by Yeongjae Kim et al., the authors provide some novel mechanistic insights towards this. They did this by focusing on ATAD5 which is involved in regulating PCNA activity by mediating its de-ubiquitination.
By proteomic analysis they identify the BAZ1B-SMARCA5 chromatin remodelling complex as interacting proteins of ATAD5 and found that it is BAZ1B that primarily interacts with ATAD5 in this complex (Figure 1). They mapped the interaction domains of BAZ1B and ATAD5 (Figures 1, 2, 3) and they generated a specific mutant ATAD5 that does not interact with BAZ1B (ATAD5 B1m, Figure 2). They used micro-irradiation to show that BAZ1B, ATAD5 and PCNA are recruited to DNA damage sites but a PCNA-binding defective BAZ1B is not (Figure 4). They demonstrated that that ATAD5 and BAZ1B are independently recruited to sites of DNA synthesis through the interaction with PCNA. PCNA ubiquitination is critical to regulate its turnover at DNA damage and repair sites, e.g., after hydrogen peroxide mediated damage. BAZ1B depletion reduced the Ub-PCNA level after hydrogen peroxide treatment, indicating that BAZ1B exerts an opposing effect on the Ub-PCNA level compared to ATAD5 (Figure 5). The authors suggest that BAZ1B regulates the Ub-PCNA de-ubiquitination function of ATAD5 rather than the PCNA unloading function. The come to the conclusion that BAZ1B negatively regulates Ub-PCNA de-ubiquitination by binding to ATAD5. Indeed, in Ub-PCNA de-ubiquitination assays in vitro the addition of BAZ1B-SMARCA5 inhibited Ub-PCNA de-ubiquitination by ATAD5-UAF1-USP1 (Figure 6). Finally, the authors present experiments that indicate that premature de-ubiquitination of Ub-PCNA results in accumulation of DNA breaks and activation of DNA damage checkpoints (Figure 7). They conclude "..BAZ1B modulates PCNA ubiquitination by inhibiting ATAD5 Ub-PCNA de-ubiquitination activity to coordinate Ub-PCNA de-ubiquitination with DNA repair". All together, the authors state that the results demonstrate "an additional layer of regulation of Ub-PCNA dynamics at DNA repair sites".

This study is interesting, well developed and provides mechanistic, novel and original insights about regulation of the DNA damage response. BAZ1B has been implicated in DNA repair before, but its precise roles in this context are less known. The role in regulating PCNA de-ubiquitination is novel. Most of the data are of high quality, the writing is clear and the study is structured in a logical way.

There are a few items that I wish to flag up:

Figure 1B, especially the BAZ1 signal, does not look convincing. Maybe repeat or delete?

Figure 5A seems a key figure, but the drop in ubPCNA on BAZ1B knockdown does not look overwhelming (not very convincing). The conclusions are supported by many additional experiments, but it would be good to know how many times this experiment was performed with similar results. Maybe repeat and show some quantification (and statistics etc), as for Figure 6D. An indication how many times the experiments were done for 5 B-C would also be good.

Abstract:
"..a regulatory subunit of the chromatin-remodeling complex.." should be "…a regulatory subunit of the BAZ1B-SMARCA5 (also called WICH)-chromatin-remodeling complex…", or similar.

Reviewer #2

(Remarks to the Author)
Nature Communications
Ms: Kim et al.

This paper comes from the group that identified ATAD5 as the PCNA unloader, which was an important contribution to the field. They elaborated this discovery with a functional analysis and demonstrated that the N-terminal portion of ATAD5 (via USP1) regulates the deubiquitination of PCNA and that the C-terminus of ATAD5 contains the unloading activity. They also have identified an ATAD5-interacting protein, BRD4, which seems to regulate the unloading activity. Here, they wish to extend these observations by identifying another ATAD5-interacting protein, BAZ1B, that seems to regulate the deubiquitination regulatory activity of ATAD5.

In general, I'm supportive of the manuscript. As can be seen from my comments below, I don't really have a problem with the data per se. Indeed, I believe that the authors have provided reasonable data to support their claims. With that said, my reservation is that the effect is not particularly profound and thus this paper seems more suited for a solid biochemistry journal than for something "higher profile" like Nature Communications. That, however, is an editorial decision, so I will leave that up the editors.

General minor comments:
1) Without meaning to offend, it is clear that English is not the authors' first language. For example, they keep referring to a replication stall. Alas, in English, stall can be a noun or a verb and when used as a noun (as in replication stall) it means a small pen, stand or booth and not to stop or slow down. Thus, I would suggest a gentle editorial hand in cleaning up the text to make it read better/smoother.
2) It would really help the reviewer if the MS had line numbers (heck, even page numbers!) so that specific points could be accurately referenced.

Specific comments:
1) The fact that BZB1:SMARCA5 is an ISWI chromatin remodeler that in known to interact w/ PCNA and works during replication, lends veracity to the author's observations.
2) I liked the experimental approach of KOing ATAD5 in 293T cells and then complementing those cells with a CLIP-ATAD5-Strepli-FLAG cDNA. With that said, I couldn't determine if the rescuing cDNA was overexpressed (probably?) when compared with endogenous ATAD5 expression. If it is, this would detract somewhat from the results.
3) Hum, the interaction with ATAD5 does not seem robust (Fig. 1B).
4) The reciprocal coIP is also not robust (Fig. 1C), but Fig. 1D is more convincing.
5) In the FLAG IP lane (Fig. 1G) it actually looks like there might be a tad more BAZ1B coming down; can this be quantitated? The same could be said for HU in Fig. 1C sup, and H2O2 in Fig. 1D sup.
6) The bipartite binding domain on ATAD5 for BAZ1B is interesting, but it is makes the analysis more complex.
7) ATAD5 B1m looks like a good mutant; the other mutants not so much.
8) The fact that BAZ1B also also appears to have a bipartite ATAD5-interacting domain in its N-terminus begs the question of whether there is some specific stoichiometry/alignment of the interaction domains on the 2 proteins.
9) I liked the fact that the authors could cleanly separate the ATAD5 (N-terminus) and SMARCA (C-terminus) binding sites on BAZ1B.
10) The PIP box on BAZ1B is decidedly "funky". This PIP box is at the N-terminus of BAZ1B and the authors convincingly show that it does not functionally overlap with the ATAD5 bipartite sites.
11) The authors show that the interaction of BAZ1B to ATAD5 is NOT needed for interaction with damaged DNA, but that PCNA is. I found this confusing.
12) The authors then go on to show that ATAD5 is also localized to damaged DNA. However, they also claim that ATAD5 has quasi PIP boxes (but this is not obvious from Fig. 4D sup) and can interact with PCNA. This implies that ATAD5 and BAZ1B are independently recruited to damaged DNA via PCNA, which seems quite convoluted.
13) The effects of a BAZ1B knockdown on PCNA Ub are modest. Moreover, the authors do not observe an effect on chromatin PCNA so conclude that it does not affect (ATAD5-mediated) PCNA unloading – just deubiquitination.
14) The authors try to make conclusions "without replication stress" but 293T cells are a cancer cell line and therefore could be considered to always be under stress. They should probably more precisely say with even more DNA damage-induced stress. Again, while the H2O2 sensitivity is significant, it is modest.
15) The authors conclude that BAZ1B is a negative regulatory of PCNA deubiquitination and that it needs to bind to ATAD5 to do that. I do think they have shown this, but again, the effects are somewhat modest.
16) Finally, the authors note that the ATAD5 bipartite binding site for BAZ1B overlaps with the UAF1 site, which is required for ATAD5 to interact with USP1. However, they also see that UAF1 binding is reduced, but not ablated, in the presence of increasing BAZ1b, which makes the observation less certain.

Reviewer #3

(Remarks to the Author)
This manuscript by Kim et al. describes a novel interaction between the ATAD5 PCNA unloading factor and BAZ1B, a subunit of a chromatin-remodeling complex. The authors present data suggesting that BAZ1B binding to ATAD5 prohibits its association with the UAF1-USP1 PCNA deubiquitinase. They propose that this interaction fine-tunes PCNA ubiquitination and show that an ATAD5 mutant that cannot interact with BAZ1B decreases PCNA ubiquitination in response to H2O2

treatment leading to DNA replication defects, replication stress and genome instability.
The article is well-written and the many of the claims are well supported by the data. In terms of novelty, the mapping of the interactions between BAZ1B and ATAD5 is well done and comprehensive (although additional structure prediction work would strengthen their model see below). The authors also identify a PIP in BAZ1B and provide data suggesting its role in recruiting BAZ1B to DNA lesions. BAZ1B was previously shown to interact with PCNA but the motif responsible for this association remains unknown (Poot et al 2004). The regulation of PCNA ubiquitination by a BAZ1B-ATAD5 interaction that outcompetes the ATAD5-UAF1/USP1 association is also an interesting new layer of regulation for DNA damage tolerance. However, some of the key conclusions, especially those regarding the in vivo influence of BAZ1B and its interaction with ATAD5 on ubPCNA levels and DNA damage tolerance require additional experimental support to be validated.

Major points :
1. In figure 1, the authors show that ATAD5 interacts with BAZ1B and that this interaction also mediates its association with SMARCA5. They also claim that the interactions are not affected by DNA damage but all the experiments were done using overexpressed proteins potentially masking some of the regulatory pathways.
Pulldown of endogenous ATAD5 or BAZ1B and examination of their interaction in the presence of genotoxins should be provided.
2. In figure 4, the authors show that BAZ1B requires its PCNA-interacting motif but not its association with ATAD5 for recruitment to micro-irradiation stripes. They also show that ATAD5-binding to BAZ1B is not required (B1m mutant) for its recruitment at lesions. Instead, they show that two putative PIP-like motifs at the N-ter of ATAD5 mediate its recruitment.

A weaker point of these experiments is that everything is done using overexpressed proteins which may artefactually tether these factors to sites of damage, precluding some recruitment dependencies from being observed.

To strengthen the manuscript, the authors should perform microirradiation experiments and look at endogenous ATAD5 and BAZ1B to see if their recruitment is affected by ATAD5/BAZ1B/PCNA KD.

3. In figure 5, the authors try to show that BAZ1B impedes PCNA deubiquitination by monitoring the levels of this modification in H2O2-treated cells following siRNA-mediated depletion of BAZ1B and reconstitution of ATAD5KO cells with the ATAD5 B1m mutant that cannot interact with BAZ1B.

This part of the model is improperly supported by the current data. At least 2 independent BAZ1B-targeting siRNAs should be tested and reconstitution with WT or an ATAD5-interacting mutant could be provided to show that the phenotype is indeed caused by a decreased association between BAZ1B and ATAD5.

Additionally, ubPCNA quantification from at least 3 independent experiments should be added to a revised manuscript. Quantification should also be provided in 5B and C.

In 5B, the magnitude of the ubPCNA decrease in the 2 B1m ATAD5KO complemented cell lines is quite variable. Since there is a large difference in ubPCNA levels for the #1 and #14 cell lines, the experiment in Fig5C should also be redone for the #14 clone.

4. Most important point: the model posits that BAZ1B binding to ATAD5 blocks the association of ATAD5 with USP1-UAF1. This is well demonstrated in vitro (Fig. 6), but there is no in vivo data showing that the interaction between UAF1-USP1 and ATAD5 is increased in BAZ1B-depleted cells as expected. A pulldown of ATAD5 or UAF1 should be done in control and BAZ1B-depleted cells to test whether the absence of BAZ1B stabilizes the ATAD5-UAF1 interaction. Alternatively, does BAZ1B overexpression impede the ATAD5-UAF1 interaction or increase UbPCNA levels ?

5. In figure 7, the authors investigate the impact of mutating the BAZ1B-binding motif of ATAD5 on DNA synthesis and genomic instability in response to H2O2-treatment. Again, data from the #14 B1m-complemented ATAD5 KO clone should be provided in panels B and C. To fully implicate BAZ1B in these phenotypes, experiments should be done in BAZ1B KD cells. If BAZ1B KD does not induce strong defects in DNA synthesis, DNA damage or resistance to H2O2, then it would suggest that something other that the association of ATAD5 with BAZ1B participates in the observed phenotypes. In addition, it is expected that depletion of BAZ1B in ATAD5 KO cells complemented with B1m should not further enhance the decrease in UbPCNA levels or sensitivity towards H2O2.
Minor points:

1. In Fig S1 and in many other instances throughout the paper, the pulldown blots for factors that are not expected to interact with the baits should be included even though no signal is expected. (e.g. S1C, -H2AX S2A, tubulin S4E, lamin B1…).

2. loading control for Fig.1D should be fixed, noting is visible in the input.

3. In figure 3, the authors map the BAZ1B motifs that interact with ATAD5 and show that their interaction is independent of BAZ1B-binding to PCNA and SMARCA5. They map 2 motifs (A5BM1 and 2) which fold into a -sheet-based motif according to Alphafold predictions. Since the authors have narrowed down the putative contacting domains between BAZ1B and ATAD5, further predictions of the structure of the complex using Collafold (Mirdita et al. Nature Methods 2022) would reinforce this section.

A model in which the ATAD5-BAZ1B interaction is compared with that of ATAD5 bound to USP1-UAF1 would provide

structural insights into the BAZ1B/UAF1-USP1 competition for ATAD5 binding. Similarly, the proposed PIP motifs at the N-ter of ATAD5 could be co-modeled with PCNA to strengthen the idea that this association is what recruits ATAD5 to sites of damage.

4. Why are there doublets in the input of endogenous ATAD5 in Fig. 3A whereas no such doublets can be seen in most of the other blots ?

5. Details regarding the creation of the Neon-tagged constructions used in figures 4 and S4 are not provided, these should be added in a revised manuscript. Similarly the HeLa GFP-PCNA cells used in Fig S4 are not described.

6. It does not appear to be mentioned in the manuscript but full MS data for the ATAD5 and BAZ1B interactomes should be provided.
7. In fig 6B, adding more than 3.3 nM BAZ1B to the reaction does not further decrease the ATAD5-UAF1-USP1 association, why is that ? Similarly to fig 5, data quantification should be provided from 3 independent experiments.

8. There is a misannotation of the 5th and 9th lanes in Fig.6C; there is no UAF1-USP1 in these samples.

9. In Fig 6D, the blots for all the other proteins apart from PCNA should be provided. Additionally, why is the B1m mutant added at 1.3 nM compared with the WT which is at 1.5 nM ? Is that a typo ?
Typos:
Line 4. Introduction After resolution of stalled forks, damage repair…
Line 5. 2nd page results section …we searched for the BAZ1B-interacting…
Line 18 2nd page results section …UAF1-binding domain being more important…
Throughout the text, in vivo and in vitro should be in italics
Section on ATD5 B1m cells are more sensitive to hydrogen peroxide …treatment and found increased tail moments…
Methods 1st page third line from bottom …HEK293T cells were lysed….

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
"ATAD5-BAZ1B interaction modulates PCNA ubiquitination during DNA repair".

This study uncovers a novel layer of regulation at DNA repair sites and employs biochemical and cell biological means to characterise this. It, thus, provides new mechanistic insights which are valuable to the field and may ultimately lead to new drug development, as DNA repair processes are an important drug target to fight cancer.
The authors have engaged diligently with all of the reviewers' comments and returned with an improved and strengthened manuscript. The combination of results makes a convincing case for the BAZ1B-ATAD5 interaction in DNA repair.

My only comment is that they write (lines 287 etc): "In contrast, BAZ1B depletion reduced Ub-PCNA levels following hydrogen peroxide treatment (Figure 5a and Figure S6c), whereas SMARCA5 depletion did not reduce the Ub-PCNA levels." and (lines 417 etc) "Notably, SMARCA5 depletion did not reduce Ub-PCNA levels, indicating that chromatin remodeling activity is not essential for regulating PCNA ubiquitination".

Actually, SMARCA5 depletion led to an increase of Ub-PCNA in Figure 5a. This might be explained by the fact the BAZ1B-SMARCA5 interaction affects the dynamics of BAZ1B-ATA5-Ub-PCNA interaction. While I understand that it is beyond the scope of this study to figure out exactly what is happening here, the authors should describe these findings accurately and might flag them up in the DISCUSSION as something to be examined in the future etc.

Reviewer #2

(Remarks to the Author)
Nature Communications
Ms: Kim et al.

This paper comes from the group that identified ATAD5 as the PCNA unloader, which was an important contribution to the field. They elaborated this discovery with a functional analysis and demonstrated that the N-terminal portion of ATAD5 (via

USP1) regulates the deubiquitination of PCNA and that the C-terminus of ATAD5 contains the unloading activity. They also have identified an ATAD5-interacting protein, BRD4, which seems to regulate the unloading activity. Here, they wish to extend these observations by identifying another ATAD5-interacting protein, BAZ1B, that seems to regulate the deubiquitination regulatory activity of ATAD5.

This is a resubmission of a manuscript that I reviewed earlier this year. At that time, I and the other two reviewers found much to like about the paper, but we also found some shortcomings and so the paper was sent back to the authors for revision. At the very least, the authors should be commended for their appreciation of the review process. I'm pretty sure that in ~40 years of reviewing manuscripts I've never seen a 75-page long response letter. While quantity does not necessarily equal quality, the good news here is that the authors have taken the reviewers' criticisms to heart and have bolstered much of their data with repeats to statistically shore the data up or repeats to achieve better quality images. Moreover, the AlphaFold analyses are a nice new addition to the manuscript that certainly supports (however indirect and circumstantial) the authors' contentions that the interaction domains that they have identified are indeed interaction domains. My biggest original hesitation is still somewhat there: this is a solid biochemistry paper that identifies a new player in PCNA regulation and probably a new mechanistic step. It does not, however, radically alter our understanding of what PCNA is doing during replication/repair. Whether this precludes it from being published in Nature Communication as opposed to some other journal is a pure editorial decision that is above my pay grade. In summary, I believe that the authors should be commended for their efforts to improve the manuscript, which is now, in my opinion, acceptable for publication.

Reviewer #3

(Remarks to the Author)
The authors have provided many additional experiments and modifications to the manuscript that appropriately respond to my comments and suggestions. In cases where the experiments were unable to fully address my questions, the authors have provided rational explanations for why that is. The manuscript now more robustly supports their model and I believe that their work is now suitable for publication.

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response to Reviewers for

ATAD5-BAZ1B interaction modulates PCNA ubiquitination during DNA repair

Yeongjae Kim, Na Young Ha, Mi-Sun Kang, Eunjin Ryu, Geunil Yi, Juyeong Yoo, Nalae Kang, Byung-Gyu Kim, Kyungjae Myung, and Sukhyun Kang*

*Corresponding author. Email: kangsh@ibs.re.kr

We appreciate the reviewers' thoughtful comments and suggestions regarding our manuscript. To strengthen our arguments, we conducted a substantial number of additional experiments. We believe this revision process has significantly improved our manuscript.

Below are our responses to each of the reviewers' comments.

Changes in the revised manuscript and supplementary information are highlighted in red in the revised documents.

Reviewer #1 (Remarks to the Author):

How DNA lesion bypass replication and a central component in orchestrating replication, PCNA, are regulated are important questions.

In this paper by Yeongjae Kim et al., the authors provide some novel mechanistic insights towards this. They did this by focusing on ATAD5 which is involved in regulating PCNA activity by mediating its de-ubiquitination.

By proteomic analysis they identify the BAZ1B-SMARCA5 chromatin remodelling complex as interacting proteins of ATAD5 and found that it is BAZ1B that primarily interacts with ATAD5 in this complex (Figure 1). They mapped the interaction domains of BAZ1B and ATAD5 (Figures 1, 2, 3) and they generated a specific mutant ATAD5 that does not interact with BAZ1B (ATAD5 B1m, Figure 2). They used micro-irradiation to show that BAZ1B, ATAD5 and PCNA are recruited to DNA damage sites but a PCNA-binding defective BAZ1B is not (Figure 4). They demonstrated that that ATAD5 and BAZ1B are independently recruited to sites of DNA synthesis through the interaction with PCNA. PCNA ubiquitination is critical to regulate its turnover at DNA damage and repair sites, e.g., after hydrogen peroxide mediated damage. BAZ1B depletion reduced the Ub-PCNA level after hydrogen peroxide treatment, indicating that BAZ1B exerts an opposing effect on the Ub-PCNA level compared to ATAD5 (Figure 5). The authors suggest that BAZ1B regulates the Ub-PCNA de-ubiquitination function of ATAD5 rather than the PCNA unloading function. The come to the conclusion that BAZ1B negatively regulates Ub-PCNA de-ubiquitination by binding to ATAD5. Indeed, in Ub-PCNA de-ubiquitination assays in vitro the addition of BAZ1B-SMARCA5 inhibited Ub-PCNA de-ubiquitination by ATAD5-UAF1-USP1 (Figure 6). Finally, the authors present experiments that indicate that premature de-ubiquitination of Ub-PCNA results in accumulation of DNA breaks and activation of DNA damage checkpoints (Figure 7). They conclude "..BAZ1B modulates PCNA ubiquitination by inhibiting ATAD5 Ub-PCNA de-ubiquitination activity to coordinate Ub-PCNA de-ubiquitination with DNA repair". All together, the authors state that the results demonstrate "an additional layer of regulation of Ub-PCNA dynamics at DNA repair sites".

This study is interesting, well developed and provides mechanistic, novel and original insights about regulation of the DNA damage response. BAZ1B has been implicated in DNA repair before, but its

precise roles in this context are less known. The role in regulating PCNA de-ubiquitination is novel. Most of the data are of high quality, the writing is clear and the study is structured in a logical way.

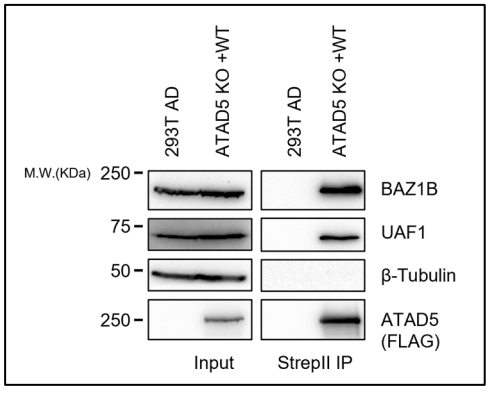
We appreciate the reviewer for his/her encouraging comments.

There are a few items that I wish to flag up:

1. Figure 1b, especially the BAZ1B signal, does not look convincing. Maybe repeat or delete?

We thank the reviewer for this suggestion. We have carefully repeated the experiment presented in Figure 1b and revised the figure with updated results. We believe that the new results more convincingly demonstrate the interaction between ATAD5 and BAZ1B.

<Figure 1b>



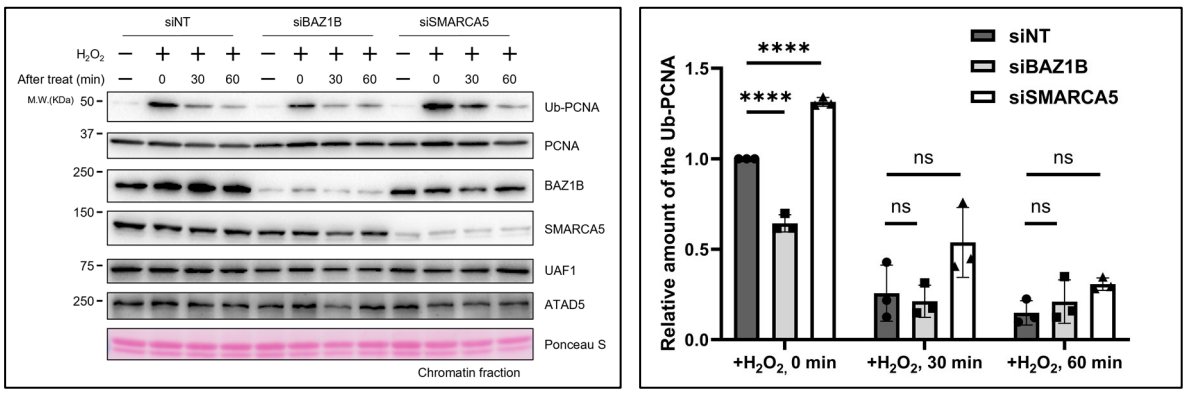
<Figure 1b legend>

b. BAZ1B is co-precipitated with ATAD5. ATAD5 was affinity purified from the indicated cells, and co-purified proteins were analyzed using immunoblotting.

2. Figure 5a seems a key figure, but the drop in ub-PCNA on BAZ1B knockdown does not look overwhelming (not very convincing). The conclusions are supported by many additional experiments, but it would be good to know how many times this experiment was performed with similar results. Maybe repeat and show some quantification (and statistics etc), as for Figure 6d. An indication how many times the experiments were done for 5 B-C would also be good.

We appreciate the reviewer's suggestion. For the experiments shown in Figure 5a, b and c, we have now included quantification from experimental replicates. The number of replicates and the corresponding statistics are presented in the quantification graphs. We consistently observed that the depletion of BAZ1B reduced the Ub-PCNA levels following hydrogen peroxide treatment (Figure 5a). In Figure 5b, we included an additional B1m cell line (previously clone number 14-9 in the initial manuscript, now renamed B1m #2) to strengthen our arguments. The abrogation of ATAD5-BAZ1B interaction consistently resulted in a reduction of Ub-PCNA levels upon hydrogen peroxide treatment across all B1m cell lines. After USP1 depletion, the Ub-PCNA levels were similar between wild-type and B1m cells in all replicates, further supporting our original argument that BAZ1B negatively regulates Ub-PCNA de-ubiquitination by inhibiting ATAD5-UAF1-USP1 activity.

<Figure 5a>

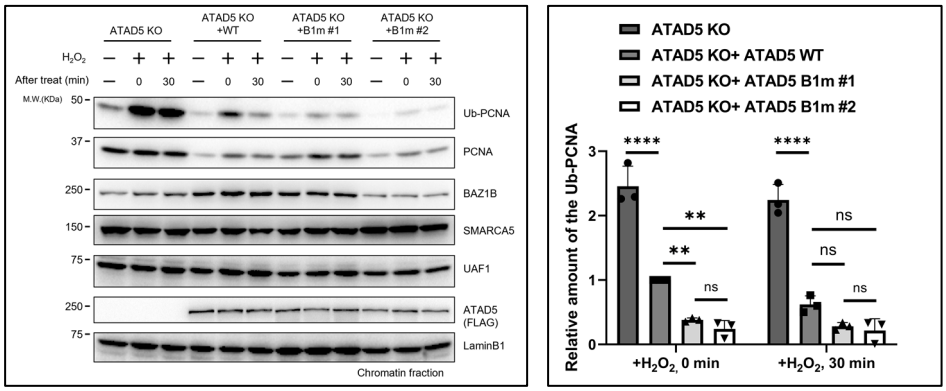


<Figure 5a legend>

a. BAZ1B depletion decreases Ub-PCNA levels after H_2O_2 treatment. BAZ1B or SMARCA5 was depleted, and cells were treated with 1 mM H_2O_2 for 20 minutes. Following incubation in fresh

media for the indicated times, chromatin fractions were prepared and analyzed by immunoblotting. *The right panel shows the quantified relative amount of Ub-PCNA. Error bars represent standard deviation (n=3). Statistical analysis: Ordinary one-way ANOVA; ****P ≤0.0001 and ns: not significant.*

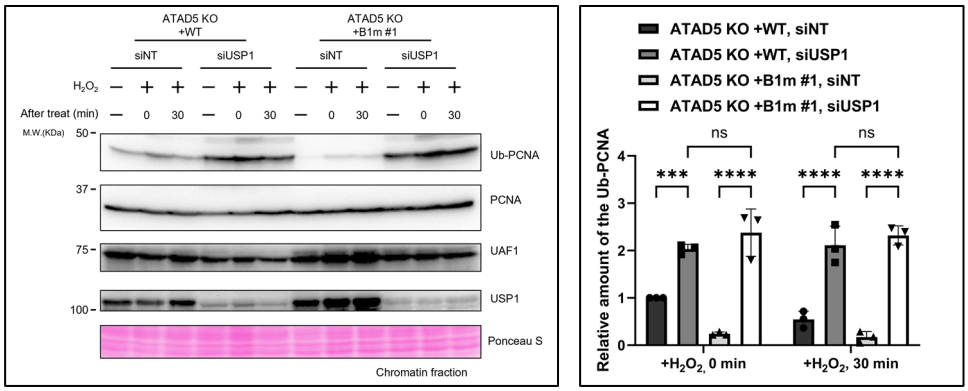
<Figure 5b>



<Figure 5b legend>

b. Abrogation of the ATAD5-BAZ1B interaction reduces Ub-PCNA levels after oxidative stress. ATAD5 knockout cells expressing either wild-type ATAD5 or ATAD5 B1m were treated with 0.3 mM H2O2 for 20 minutes. After incubating in fresh media for the indicated times, cells were harvested, and chromatin fractions were prepared. *The right panel shows the quantified relative amount of Ub-PCNA. Error bars represent standard deviation (n=3). Statistical analysis: Ordinary one-way ANOVA; ****P ≤0.0001, **P ≤0.01, and ns: not significant.*

<Figure 5c>



<Figure 5c legend>

c. BAZ1B regulates Ub-PCNA de-ubiquitination by USP1. Cells with USP1 depletion, expressing either wild-type ATAD5 or ATAD5 B1m, were treated with 0.3 mM H_2O_2 for 20 minutes. Following incubation in fresh media for the indicated times, chromatin fractions were prepared and analyzed by immunoblotting. *The right panel shows the quantified relative amount of Ub-PCNA. Error bars represent standard deviation (n=3). Statistical analysis: Two-way ANOVA; ****P ≤0.0001, ***P ≤0.001, and ns: not significant.*

3. Abstract: "..a regulatory subunit of the chromatin-remodeling complex.." should be "…a regulatory subunit of the BAZ1B-SMARCA5 (also called WICH)-chromatin-remodeling complex…", or similar.

We appreciate the reviewer for this suggestion. We have made the following change:

- Abstract (page 1, Lines 23-24): The original sentence, "… a regulatory subunit of the chromatin-remodeling complex…" has been revised to "… a regulatory subunit of the BAZ1B-SMARCA5 chromatin-remodeling complex (also known as the WICH complex) …".

Reviewer #2 (Remarks to the Author):

This paper comes from the group that identified ATAD5 as the PCNA unloader, which was an important contribution to the field. They elaborated this discovery with a functional analysis and demonstrated that the N-terminal portion of ATAD5 (via USP1) regulates the deubiquitination of PCNA and that the C-terminus of ATAD5 contains the unloading activity. They also have identified an ATAD5-interacting protein, BRD4, which seems to regulate the unloading activity. Here, they wish to extend these observations by identifying another ATAD5-interacting protein, BAZ1B, that seems to regulate the deubiquitination regulatory activity of ATAD5.

In general, I'm supportive of the manuscript. As can be seen from my comments below, I don't really have a problem with the data per se. Indeed, I believe that the authors have provided reasonable data to support their claims. With that said, my reservation is that the effect is not particularly profound and thus this paper seems more suited for a solid biochemistry journal than for something "higher profile" like Nature Communications. That, however, is an editorial decision, so I will leave that up the editors.

We thank the reviewer for her/his thoughtful comments. We consistently observed the effect of ATAD5-BAZ1B interaction in regulating Ub-PCNA de-ubiquitination. We conducted additional experiments and analyses to strengthen our arguments. We believe our findings are significant because we identify the novel regulatory mechanism of the DNA lesion bypass signal.

General minor comments:

1. Without meaning to offend, it is clear that English is not the authors' first language. For example, they keep referring to a replication stall. Alas, in English, stall can be a noun or a verb and when used as a noun (as in replication stall) it means a small pen, stand or booth and not to stop or slow down.

Thus, I would suggest a gentle editorial hand in cleaning up the text to make it read better/smoother.

We apologize for any confusion caused by unclear descriptions in the manuscript. We have conducted a thorough editorial revision throughout the manuscript to improve its clarity and readability. For example, we made the following changes:

- Abstract (page 1, Line 19): The original sentence, “After resolving a replication stall …” has been revised to “After resolving stalled forks …”.
- Introduction (page 2, Line 38): The original sentence, “After resolution of replication fork stalls, …” has been revised to “Once the stalled forks are resolved, …”.

2. It would really help the reviewer if the MS had line numbers (heck, even page numbers!) so that specific points could be accurately referenced.

We apologize for any inconvenience during the review process. We have now added line numbers and page numbers throughout the manuscript.

Specific comments:

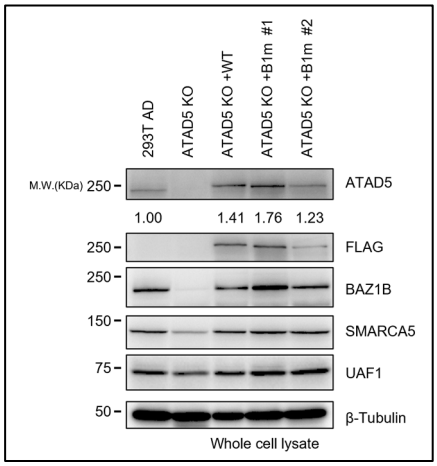
1. The fact that BAZ1B:SMARCA5 is an ISWI chromatin remodeler that in known to interact w/ PCNA and works during replication, lends veracity to the author’s observations.

We appreciate the reviewer’s positive remarks. As noted, BAZ1B-SMARCA5 complex interacts with PCNA during DNA replication, and ATAD5 modulates PCNA functions. Therefore, the functional interplay between ATAD5 and BAZ1B aligns well with their roles in DNA replication.

2. I liked the experimental approach of KOing ATAD5 in 293T cells and then complementing those cells with a CLIP-ATAD5-Strepli-FLAG cDNA. With that said, I couldn't determine if the rescuing cDNA was overexpressed (probably?) when compared with endogenous ATAD5 expression. If it is, this would detract somewhat from the results.

We understand the reviewer's concern. When generating ATAD5 add-back cells, we selected ATAD5 wild-type and ATAD5 B1m cell clones that exhibited ATAD5 expression levels comparable to endogenous ATAD5. We have now included a supplementary figure demonstrating the levels of ATAD5 in the add-back clones (Figure S6f). Figure S5f shows that the expression levels of CLIP-ATAD5-StrepII-FLAG in the ATAD5 add-back cells were comparable to those of endogenous ATAD5.

<Figure S6f>



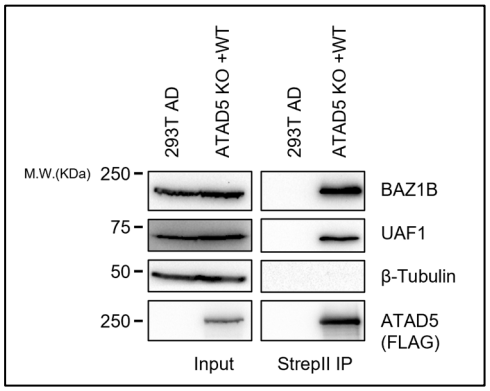
<Figure S6f legend>

(f) The expression levels of ATAD5 in ATAD5 add-back clones are comparable to endogenous ATAD5. Numbers below the ATAD5 blot indicate the relative amount of ATAD5.

3. Hum, the interaction with ATAD5 does not seem robust (Fig. 1B).

We appreciate the reviewer's comment. We carefully repeated the ATAD5 immunoprecipitation experiments and have presented updated results in Figure 1b. The revised figure more clearly demonstrates the interaction between BAZ1B and ATAD5.

<Figure 1b>



<Figure 1b legend>

b. BAZ1B is co-precipitated with ATAD5. ATAD5 was affinity purified from the indicated cells, and co-purified proteins were analyzed using immunoblotting.

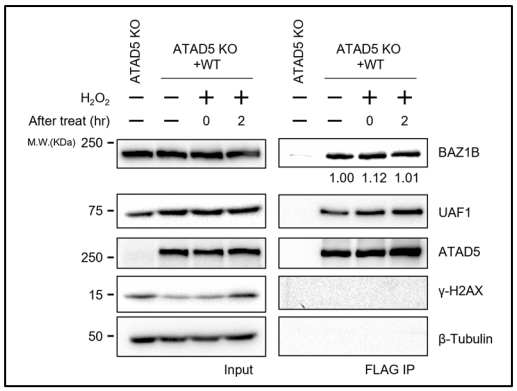
4. The reciprocal coIP is also not robust (Fig. 1C), but Fig. 1D is more convincing.

We understand the concern regarding the robustness of ATAD5 co-isolation with BAZ1B in Figure 1c. However, we clearly detected the ATAD5 peptides in BAZ1B interactome, even though the spectral count is low. Additionally, we verified the reciprocal BAZ1B-ATAD5 interaction using immunoblot as noted by the reviewer (Figure 1d). Given that the interaction between ATAD5 and BAZ1B has been confirmed through various approaches throughout the manuscript, we are confident in the specificity of BAZ1B binding to ATAD5.

5. In the FLAG IP lane (Fig. 1G) it actually looks like there might be a tad more BAZ1B coming down; can this be quantitated? The same could be said for HU in Fig. 1C sup, and H2O2 in Fig. 1D sup.

We appreciate the reviewer's comment. In response, we conducted the experiments more carefully to examine the interaction between ATAD5 and BAZ1B in the presence of damage agents using ATAD5 Knock-out cells expressing wild type Clip-ATAD5-StrepII-FLAG. The results are now presented in Figure 1g and Figure S1c-d of the revised manuscript. As suggested, we quantified the amount of BAZ1B co-isolated with ATAD5 in the presence of several genotoxic agents (Figure 1g and Figure S1c-d). Our quantification results confirmed that genotoxic stress - including hydrogen peroxide (H_2O_2), UV, hydroxyurea (HU), and camptothecin (CPT) - did not significantly affect the BAZ1B-ATAD5 interaction. These results support our original conclusion.

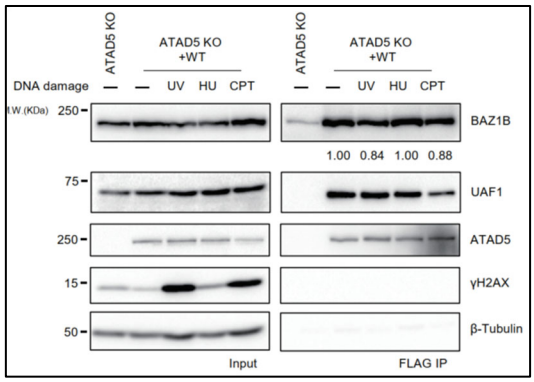
<Figure 1g>



<Figure 1g legend>

g. Oxidative stress does not affect ATAD5-BAZ1B interaction. Wild-type ATAD5 cells were treated with 1 mM hydrogen peroxide for 20 minutes, followed by incubation in fresh media for the indicated time period. ATAD5 was immunoprecipitated using anti-FLAG beads. The numbers below the BAZ1B blot indicate the relative amount of BAZ1B co-precipitating.

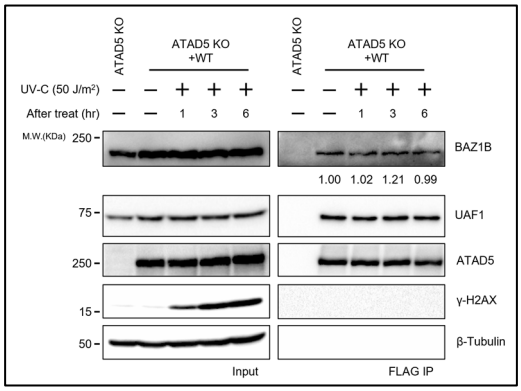
<Figure S1c>



<Figure S1c legend>

(c) UV, Hydroxyurea (HU) or Camptothecin (CPT) does not alter the interaction between ATAD5 and BAZ1B. ATAD5 knock-out (KO) cells expressing wild-type ATAD5 (ATAD5 KO+WT) were treated with UV-C (50 J/m^2), HU (2 mM for 6 hours), or CPT (2 μM for 17 hours), followed by anti-FLAG immunoprecipitation. UV treated cells were incubated in fresh media for 6 hours. Numbers below the BAZ1B blot indicate the relative amount of BAZ1B.

<Figure S1d>



<Figure S1d legend>

(d) ATAD5 KO+WT cells were treated with UV-C (50 J/m^2) and then incubated in fresh media for the indicated times. At each time point, cells were harvested, and anti-FLAG immunoprecipitation was performed. Numbers below the BAZ1B blot indicate the relative amount of BAZ1B.

6. The bipartite binding domain on ATAD5 for BAZ1B is interesting, but it is makes the analysis more complex.

8. The fact that BAZ1B also also appears to have a bipartite ATAD5-interacting domain in its N-terminus begs the question of whether there is some specific stoichiometry/alignment of the interaction domains on the 2 proteins.

We thank the reviewer for these remarks and would like to provide clarification regarding our findings on this matter to address his/her concern. Our result in Figure 2b clearly demonstrated that the ATAD5 (240-320) plays a crucial role in the interaction with BAZ1B. The deletion of ATAD5 (240-320) eliminated BAZ1B binding, underscoring its essential role in mediating the interaction between BAZ1B and ATAD5. On the other hand, our data indicate that the ATAD5 (400-692) contributed to BAZ1B binding, albeit to a lesser extent than ATAD5 (240-320). While the deletion of ATAD5 (400-692) exhibited mild defects in BAZ1B interaction, the overall impact was less pronounced compared to the deletion of ATAD5 (240-320).

To further investigate the ATAD5-BAZ1B interaction, we conducted structural prediction analysis using AlphaFold2 (Figure S3g-h, S3k and Figure R1). The predictions suggest that ATAD5 (240-320) is important for BAZ1B binding. Notably, A5BM1 and A5BM2 of BAZ1B are predicted to form β strands that consist of ATAD5-binding substructure. We identified another β strand, composed of BAZ1B residues ^{231}KIIS^{235} and referred to as A5BM3, which aligns adjacent to ATAD5 B1 motif (Figure S3g). Mutations in A5BM3 abolished ATAD5 binding, similar to mutations in A5BM1 and A5BM2 (Figure S3b, S3m). Importantly, ATAD5 Y267 and F270 in the B1 motif form parallel-displaced (staggered stacking) configuration, facilitating aromatic π-π interaction with BAZ1B F122 residue in the A5BM1 motif (Figure S3h). This parallel-displaced π-π stacking is common under physiological conditions and is known to be crucial for protein structure and protein-protein interactions [1]. Based on this prediction, we created an ATAD5 (Y267A, F270A) mutant and found that the YFAA mutation disrupted the BAZ1B binding similar to the B1m mutant. (Figure S3i-j).

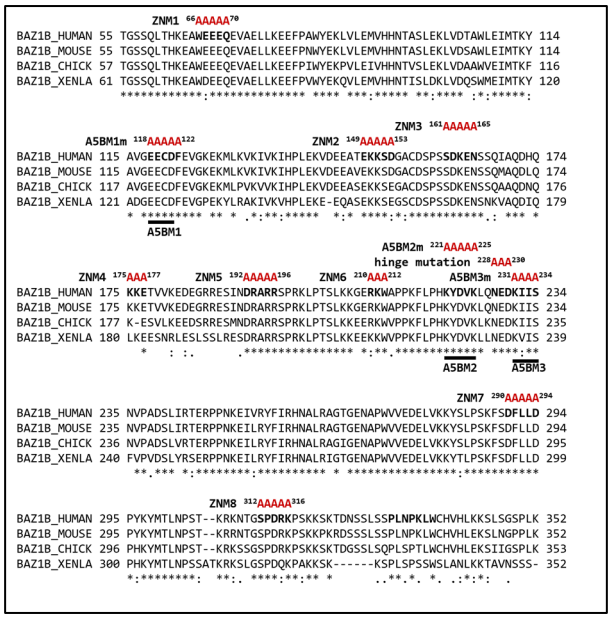
Additionally, E118 in the BAZ1B A5BM1 motif stabilizes the orientation of the β-sheet-based structure toward ATAD5, reinforcing the BAZ1B-ATAD5 interaction (Figure S3k). We also observed that Y222 in the BAZ1B A5BM2 motif forms a T-shaped π-π stacking interaction with the BAZ1B Y114, which stabilizes the positioning of the A5BM1 to support the ATAD5-BAZ1B interaction. Both

the E118A and Y222A mutations in BAZ1B abolished its binding to ATAD5 (Figure 3c and Figure S3l), supporting the structural prediction.

Overall, ATAD5 B1 motif and BAZ1B A5BM1-3 engage in the formation of a 4-stranded antiparallel structure (Figure S3g-h, S3k and Figure R1). The structural predictions align well with mutational analyses, suggesting that BAZ1B A5BM1-3 cooperatively assemble into a single structural unit that interacts with one ATAD5 molecule. Although the role of ATAD5 (400-692) in the BAZ1B interaction remains unclear, our additional analyses and experimental results further support our argument that ATAD5 (240-320) forms a singular binding interface for BAZ1B, and that BAZ1B A5BM1-3 acts as an ATAD5 binding module.

In the revised manuscript, we have included additional analyses and experimental results. The main text in the results section has been updated accordingly.

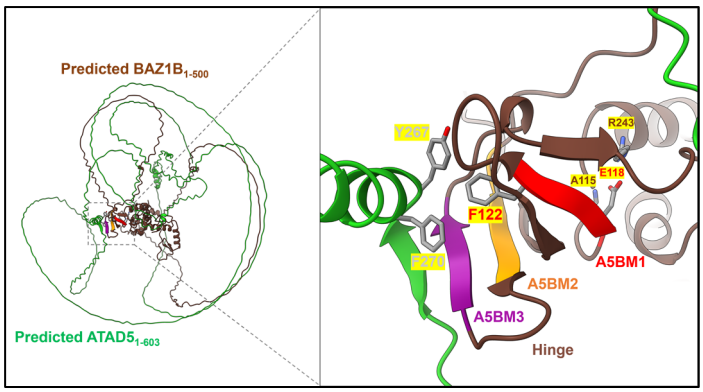
<Figure S3b>



<Figure S3b legend>

(b) The locations of ZNM1-8 mutations, A5BM1m, A5BM2m, A5BM3m, and the hinge mutation are indicated.

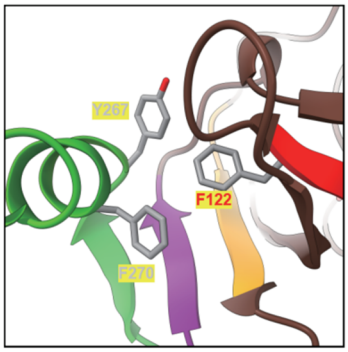
<Figure S3g>



<Figure S3g legend>

(g) AlpfaFold2 prediction of ATAD5 (1-603) bound to BAZ1B (1-500).

<Figure S3h>



<Figure S3h legend>

(h) The residues Y267 and F270 of ATAD5 form a parallel-displaced (staggered stacking) configuration of aromatic π-π interactions with the BAZ1B F122 residue.

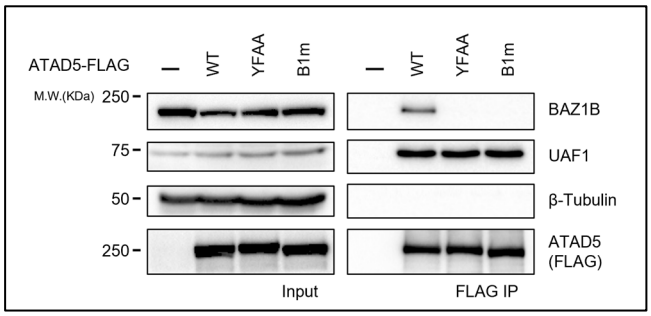
<Figure S3i>

```
                    YFAA 264TVSAEEA270
ATAD5_HUMAN  254 EAAQLNDSIITVSYEEFLKSHKENKVE 280
ATAD5_MOUSE  251 KRADLKESTITVSYEEFVKSHKAAKVE 277
ATAD5_CHICK  253 KTPQANDSIITVSFEDFLKSQGENYVE 279
ATAD5_XENLA  248 DGLHGKDSILVVSFEEFIKSQGEKDME 274
                 .  . ::* :.**:*:*:**:    :*
```

<Figure S3i legend>

(i) The residues Y264 and F270 of ATAD5 are conserved among species. The location of the YFAA mutations (Y264A, F270A) were indicated.

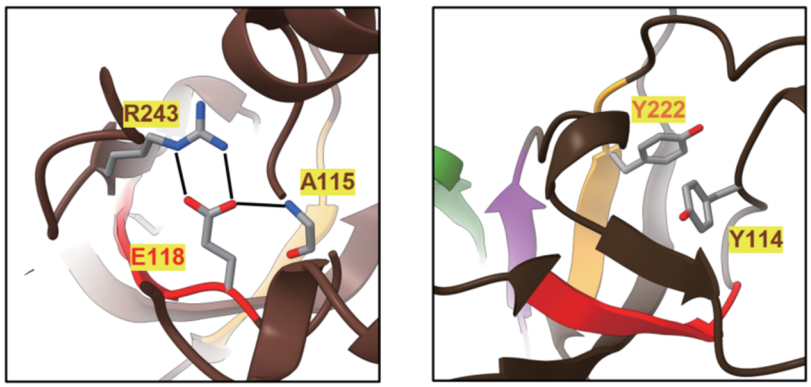
<Figure S3j>



<Figure S3j legend>

(j) ATAD5 YFAA fails to interact with BAZ1B. Anti-FLAG-immunoprecipitation was performed with cells expressing either wild-type or mutant FLAG-ATAD5.

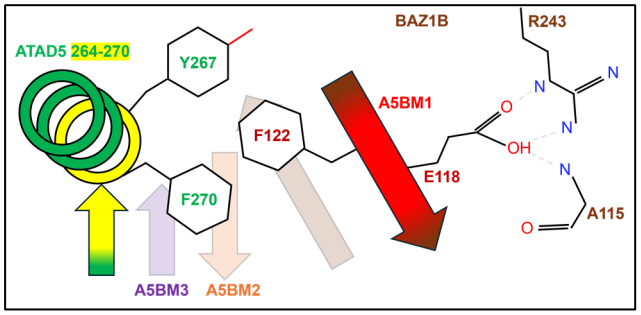
<Figure S3k>



<Figure S3k legend>

(k) The BAZ1B motifs A5BM1, A5BM2, and A5BM3 constitute the ATAD5 binding module and support the ATAD5-BAZ1B interaction. The Y222 residue in the BAZ1B A5BM2 motif forms a T-shaped π-π stacking interaction with the Y114 residue in BAZ1B, stabilizing the positioning of the A5BM1 motif. The BAZ1B E118 residue stabilizes the orientation of the β-sheet-based motif toward ATAD5.

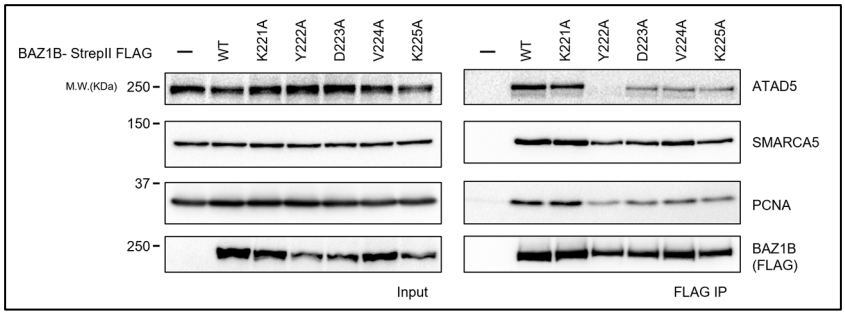
<Figure R1>: This figure is not included in the revised manuscript.



<Figure R1 legend>

BAZ1B A5BM1 is crucial for the interaction with ATAD5. The BAZ1B F122 residue forms a π-π staggered stacking interaction with the ATAD5 Y267 and F270 residues. The BAZ1B E118 residue stabilizes the orientation of the β-sheet-based motif toward ATAD5.

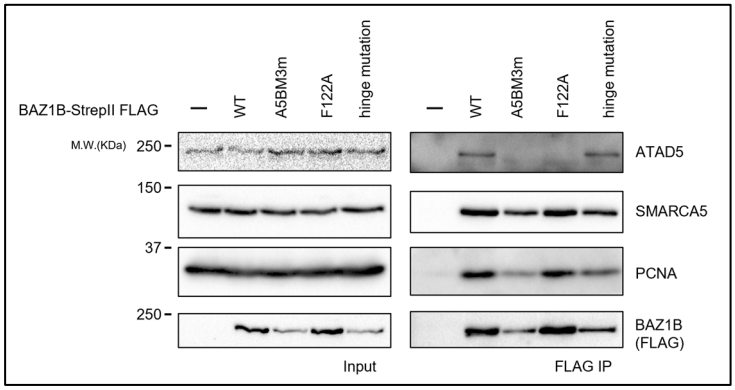
<Figure S3l>



<Figure S3l legend>

(l) The BAZ1B Y222A mutant failed to interact with ATAD5. Anti-FLAG-immunoprecipitation was performed with cells expressing either wild-type or mutant StrepII-FLAG-BAZ1B.

<Figure S3m>



<Figure S3m legend>

(m) The BAZ1B F122A mutation and A5BM3 mutant (^{231}KIIS^{234} to AAAA) fail to interact with ATAD5. The BAZ1B hinge mutant (^{228}NED^{230} to AAA) does not affect the ATAD5-BAZ1B interaction. Anti-FLAG-immunoprecipitation was performed with cells expressing either wild-type or mutant StrepII-FLAG-BAZ1B.

<Main Text>: Page 5, lines 138-144

Deletion of residues 240-320 completely abolished the BAZ1B interaction without affecting the binding of UAF1 or BRD4. Removing residues 400-693 resulted in partial reduction in BAZ1B binding. Although ATAD5 (400–693) contains a motif for BET protein binding [2], a BET-binding defective mutation did not impair BAZ1B binding (Figure S2a), suggesting that this region enhances BAZ1B binding independently of BET proteins. *Therefore, the BAZ1B binding region overlaps with the UAF1-binding domain of ATAD5.*

<Main Text>: Pages 6-7, lines 179-198

To further investigate the ATAD5-BAZ1B interaction, we conducted structural prediction analysis using AlphaFold2 (Figure S3g). The predictions suggest that the ATAD5 region encompassing residues 260-270 is critical for binding to BAZ1B. The BAZ1B binding region and the ATAD5 binding domain form a 4-stranded antiparallel structure. Notably, residues Y267 and F270 in the ATAD5 B1 motif engage in aromatic π-π interactions with the BAZ1B F122 residue (Figure S3h). These π-π stacking interactions are crucial for protein-protein interaction [1]. The ATAD5 (Y267A, F270A) mutation disrupted its interaction with BAZ1B, similar to the B1m mutant (Figure S3i-j). Furthermore, the F122A mutation in BAZ1B A5BM1 abolished the interaction with ATAD5, validating our predictions (Figure 3c).

In AlphaFold2 prediction, A5BM1 and A5BM2 of BAZ1B are predicted to form β strands that constitute the ATAD5-binding domain. Another β strand, consisting of BAZ1B residues ^{231}KIIS^{234} and referred to as A5BM3, was found to align next to ATAD5 B1, forming parallel interactions (Figure S3k). Additionally, E118 in the BAZ1B A5BM1 motif stabilizes the orientation of the β-sheet-based motif towards ATAD5, strengthening the ATAD5-BAZ1B interaction. Y222 in the BAZ1B A5BM2 motif forms a T-shaped π-π stacking interaction with BAZ1B Y114, which stabilizes the positioning of A5BM1 to support the ATAD5-BAZ1B interaction (Figure S3k). Consistent with our predictions, the E118A, Y222A, and A5BM3 AAAA mutants showed significant defects in ATAD5 interaction (Figure 3c and Figure S3b, S3l-m).

7. ATAD5 B1m looks like a good mutant; the other mutants not so much.

We appreciate the reviewer for acknowledging our effort to identify the BAZ1B-binding defective ATAD5 mutant. We conducted additional analyses regarding the interaction between ATAD5 A5BM1 and BAZ1B F122A, as discussed in response to points 6 and 8 from the reviewer. In addition, we included further analyses regarding the PCNA binding site of ATAD5 in the revised manuscript.

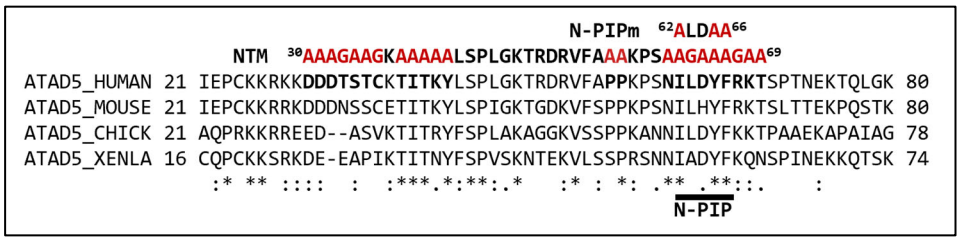
We ran AlphaFold2 prediction using ATAD5 (1-603) and PCNA and observed N-terminal region of ATAD5 largely lacked a defined secondary structure, exhibiting low pLDDT (predicted local distance difference test) scores (Figure S5e). Only the segment ATAD5 (60-69) displayed relatively high confidence (pLDDT > 70), indicating a reliable backbone prediction [3]. This segment resembles

the PCNA-interacting protein motif (N-PIP motif; 62-ILDYF-66) and is located within the binding pocket of the IDCL of PCNA (Figure S5d).

Based on this, we generated an N-PIP mutant (Figure S5d) and found that the mutant disrupted the PCNA binding similarly to the NTM mutant (Figure S5f). Furthermore, the N-PIP mutant was defective in localization to the micro-irradiated site, similar to NTM (Figure S5g). From these analyses, we conclude that the N-terminal region of ATAD5 is crucial for its interaction with PCNA and for localization to damage sites (Figure S5e-g).

In the revised manuscript, we have included additional analyses and experimental results. The main text in the results section has been updated accordingly.

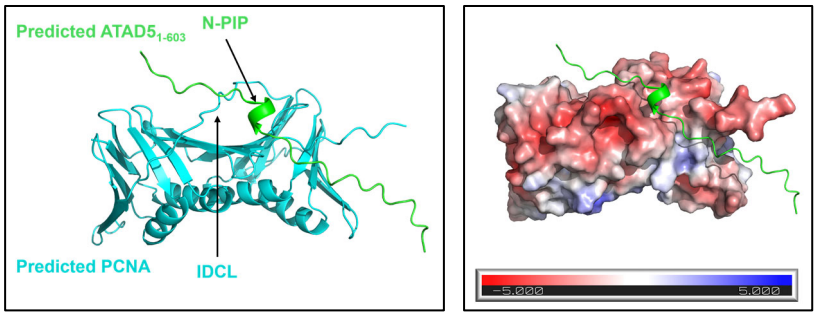
<Figure S5d>



<Figure S5d legend>

(d) The ATAD5 ^{62}IxxYF^{66} motif is conserved among species. The locations of NTM and N-PIPm mutations are indicated.

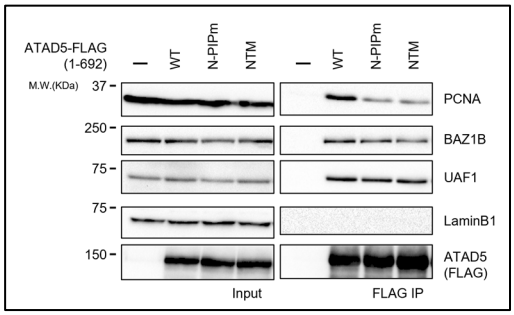
<Figure S5e>



<Figure S5e legend>

(e) Structural prediction of PCNA-bound ATAD5 N-terminal region using AlphaFold2 (Left). The electrostatic surface of PCNA was analyzed using the APBS plugin (Right). The ATAD5 N-terminal domain is predicted to contact with the PCNA IDCL.

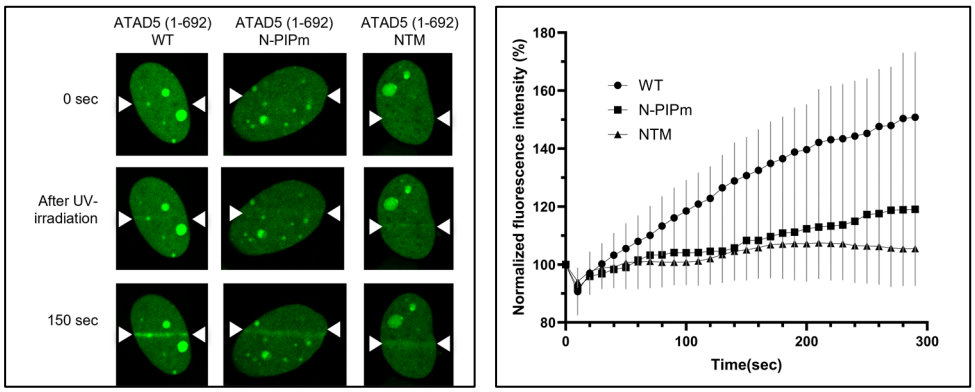
<Figure S5f>



<Figure S5f legend>

(f) ATAD5 interacts with PCNA through the N-PIP motif (residues 62-66). ATAD5-FLAG (1-692) wild-type or mutants were transiently expressed in 293T cells and immunoprecipitated using anti-FLAG beads.

<Figure S5g>



<Figure S5g legend>

(g) PCNA interaction is crucial for the localization of ATAD5 to micro-irradiated sites. ATAD5-FLAG-mNeonGreen (1-692) wild-type or the indicated mutants were transiently expressed in U2OS cells and subjected to 355 nm UV laser micro-irradiation.

<Main Text>: Pages 8-9, lines 257-272

The far N-terminus of ATAD5 contains conserved amino acids, and mutations in these residues (referred to as far N-terminal mutations, NTM) significantly reduced localization to damage sites (Figure 4d and Figure S5d). Notably, the residues ^{61}NILDYF^{66} in the ATAD5 N-terminus resemble a predicted PCNA-interacting motif. Using AlphaFold2, we predicted the structure of the ATAD5 (1-603) bound to PCNA, revealing that ATAD5 residues ^{62}ILDYF^{66} were positioned in the binding pocket of IDCL of PCNA (Figure S5e). Based on this prediction, we generated an N-PIP mutant by substituting ^{62}ILDYF^{66} with ALDAA, finding that both the NTM and N-PIP mutant significantly reduced PCNA binding (Figure S5d, S5f). Interestingly, localization to damage sites was also significantly impaired by the N-PIP mutation (Figure S5g). RFC1 depletion prevents the loading of PCNA onto DNA and reduces the PCNA accumulation at micro-irradiation sites [4,5]. Our results showed that RFC1 depletion resulted in significant reduction in the recruitment of both ATAD5 and BAZ1B to damage sites (Figure S5h-j). These findings suggest that ATAD5 and BAZ1B are independently recruited to DNA repair sites through their interaction with PCNA.

9. I liked the fact that the authors could cleanly separate the ATAD5 (N-terminus) and SMARCA (C-terminus) binding sites on BAZ1B.

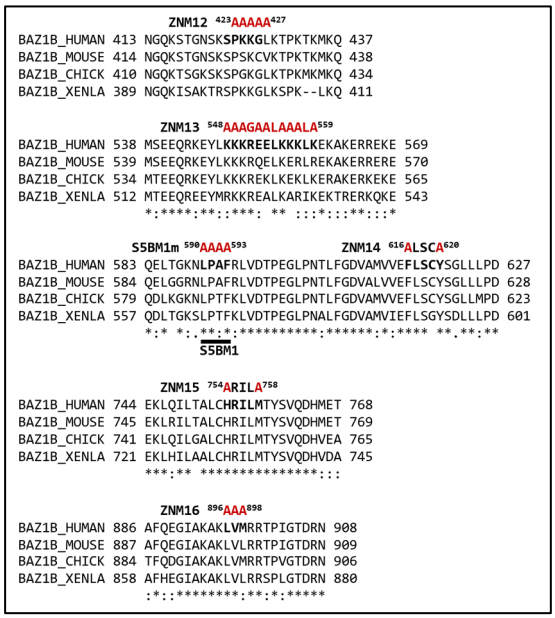
We appreciate the reviewer for recognizing this point. To further clarify the SMARCA5 binding regions in BAZ1B, we generated additional BAZ1B mutants and examined their interaction with SMARCA5. Our results from the initial submission indicated that the middle (539-592) and C-terminal (1001-1343) regions are required for SMARCA5 binding.

In the case of the middle region, we found that mutations of residues ^{590}LPAF^{593} to AAAA abolished SMARCA5 binding (Figure S4f-i). For the C-terminal region, we prepared additional

deletion mutants and determined that BAZ1B (1027-1214) contains the SMARCA5 binding motif (Figure S4k). We mutated several conserved residues within BAZ1B (1027-1214) and examined SMARCA5 binding through immunoprecipitation (Figure S4l-m). The results indicated that the mutations ^{1158}EAQTFSR^{1164} to AAAAAAA eliminated SMARCA5 interaction. On the contrary, mutations in DDT domain or WHIM1-2 domains did not affect SMARCA5 binding. Importantly, ATAD5 binding was not affected by either of the two SMARCA5-binding defective mutants. These results reinforce our argument that ATAD5 and SMARCA5 bind independently to BAZ1B.

In the revised manuscript, we have included additional analyses and experimental results. The main text in the results section has been updated accordingly.

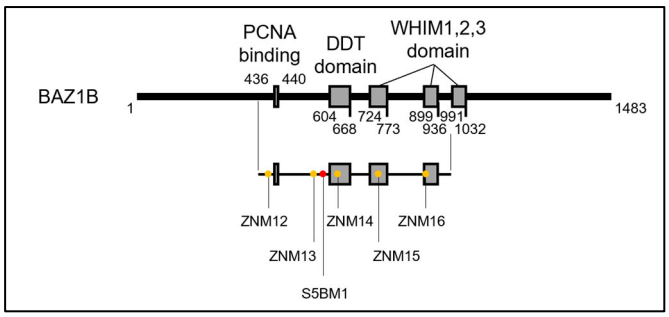
<Figure S4f>



<Figure S4f legend>

(f) Positions of ZNM12-16 mutations and S5BM1m are indicated on the aligned sequences of the middle region of BAZ1B homologues.

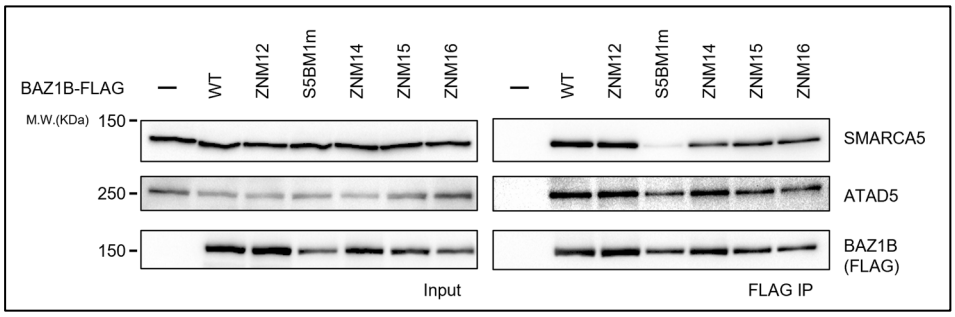
<Figure S4g>



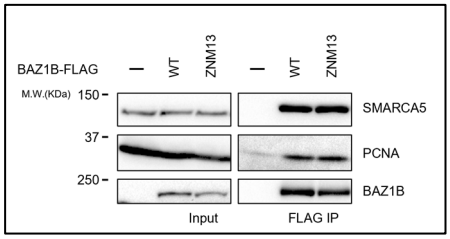
<Figure S4g legend>

(g) Diagram showing the positions of ZNM12-16 mutations and S5BM1. Grey boxes indicate the domains of BAZ1B.

<Figure S4h>



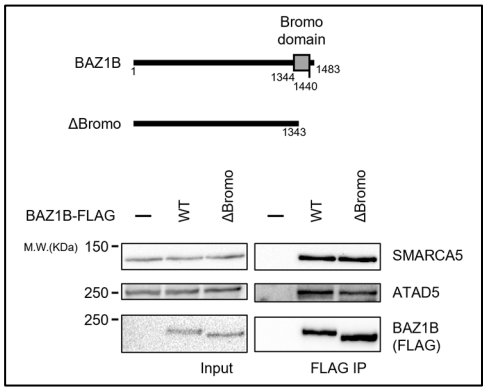
<Figure S4i>



<Figure S4h-i legends>

(h-i) The mutations in BAZ1B S5BM1 (^{590}LPAF^{593} to AAAA) eliminates the BAZ1B-SMARCA5 interaction. The indicated Clip-BAZ1B-StrepII-FLAG variants were transiently expressed, and FLAG-immunoprecipitation was performed.

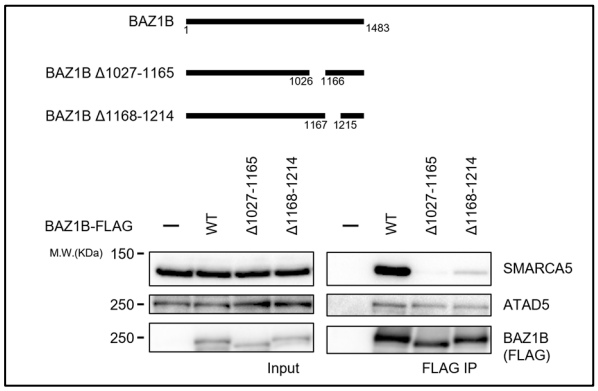
<Figure S4j>



<Figure S4j legend>

(j) The bromodomain of BAZ1B is not necessary for SMARCA5 binding. FLAG-tagged BAZ1B wild-type or bromodomain deletion mutant was transiently expressed in 293T cells, followed by FLAG-immunoprecipitation.

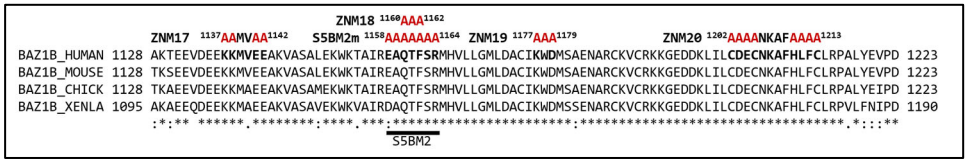
<Figure S4k>



<Figure S4k legend>

(k) The residues 1027-1214 of BAZ1B are essential for SMARCA5 binding. The indicated Clip-BAZ1B-StrepII-FLAG variants were transiently expressed, and FLAG-immunoprecipitation was performed.

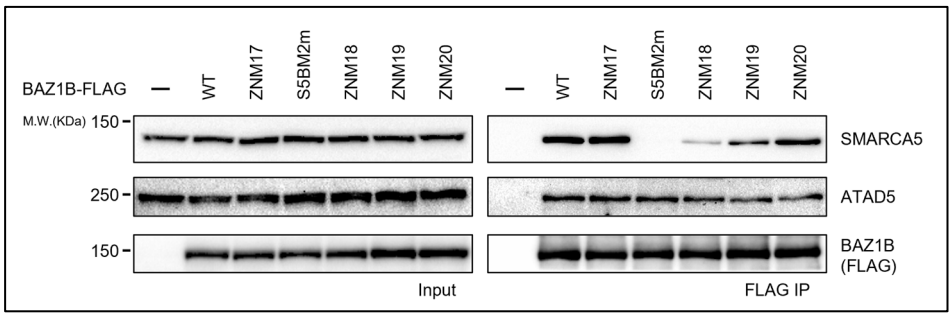
<Figure S4l>



<Figure S4l legend>

(l) Positions of ZNM17-20 mutations and S5BM2m are indicated on the aligned sequences of the C-terminal domain of BAZ1B homologues. The indicated Clip-BAZ1B-StrepII-FLAG variants were transiently expressed, and FLAG-immunoprecipitation was performed.

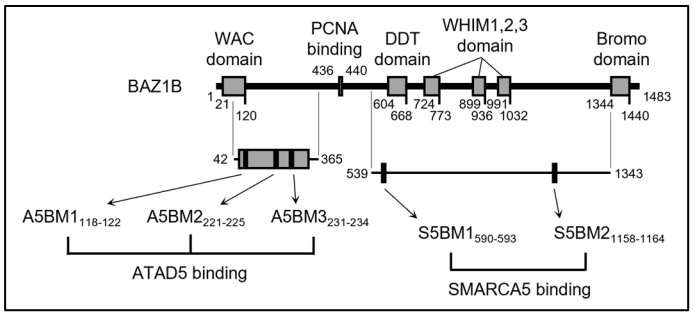
<Figure S4m>



<Figure S4m legend>

(m) The mutations in S5BM2 (^{1158}EAQTFSR^{1164} to AAAAAAA) abolishes SMARCA5 binding. The indicated Clip-BAZ1B-StrepII-FLAG variants were transiently expressed, and FLAG-immunoprecipitation was performed.

<Figure S4n>



<Figure S4n legend>

(n) Diagram illustrating the positions of ATAD5, PCNA, and SMARCA5 binding motifs in BAZ1B.

<Main Text>: Pages 7-8, lines 217-234

Regarding the SMARCA5 interaction, both the middle region of BAZ1B (residues 539–592, Figure S4e) and the C-terminal region (residues 1001–1483, Figure 3a) were necessary. To further explore this interaction, we tested several mutations in the middle region of BAZ1B. We found that mutating residues ^{590}LPAF^{593} (referred to as S5BM1) to AAAA abolished SMARCA5 binding (Figure S4f-i). Mutations in the DDT or WHIM1-2 domains did not affect SMARCA5 binding, although a previous report suggested that the WHIM domains might be involved in SMARCA5 interaction [6]. For the C-terminal region, deleting the bromodomain did not impede its interaction with SMARCA5 (Figure S4j). Additional deletion mutants revealed that residues 1027-1214 contain the SMARCA5-binding motif (Figure S4k). Further mutational analysis of conserved patches within this region was conducted through immunoprecipitation (Figure S4l-m). This analysis indicated that mutating residues ^{1158}EAQTFSR^{1164} (referred to as S5BM2) to AAAAAAA eliminated the SMARCA5 interaction. Importantly, mutations in both S5BM1 and S5BM2 did not affect ATAD5 binding.

Overall, BAZ1B interacts with ATAD5 and PCNA through its N-terminal domain and with SMARCA5 through its middle and C-terminal domains. Notably, each protein interacts with BAZ1B independently (Figure S4n).

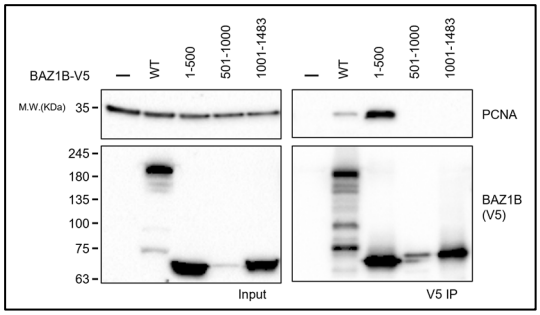
10. The PIP box on BAZ1B is decidedly “funky”. This PIP box is at the N-terminus of BAZ1B and the authors convincingly show that it does not functionally overlap with the ATAD5 bipartite sites.

We appreciate the reviewer's feedback. During the immunoprecipitation experiments aimed at investigating the interaction between BAZ1B and PCNA, we observed that PCNA co-precipitates with BAZ1B (Figure 3b). While the interaction between BAZ1B and PCNA has been previously reported, the specific PCNA binding motifs in BAZ1B have yet to be identified. To explore the influence of PCNA on ATAD5 binding, we sought to determine the PCNA-binding site on BAZ1B.

First, we transiently expressed the N-terminal, middle and C-terminal fragments of BAZ1B in 293T cells and assessed their interaction with PCNA through immunoprecipitation. The results are included in Figure S4a of the revised manuscript. Our findings indicate that PCNA binds to the BAZ1B (1-500), but not to the BAZ1B (501-1000) or BAZ1B (1001-1483).

Subsequently, we identified the PCNA-interacting motif through a series of deletions in BAZ1B, revealing that BAZ1B (323-466) contains the PCNA-binding region (Figure 3d). We then introduced mutations in the several conserved patches in BAZ1B (323-466) and determined that BAZ1B (436-440) constitutes the PCNA interacting motif (Figure 3e). In the initial manuscript, we demonstrated that mutating BAZ1B from ^{436}KQMTL^{440} to AAGAG (designated PIPm) eliminated BAZ1B-PCNA interaction. We further examined additional mutations within BAZ1B (323-466) to pinpoint the PCNA-binding motif, with the findings presented in the revised manuscript (Figure S4b-c). Other mutations within BAZ1B (323-466) did not significantly impact PCNA binding. These results reaffirm our argument that the BAZ1B ^{436}KQMTL^{440} motif represents the PCNA interacting "PIP" motif.

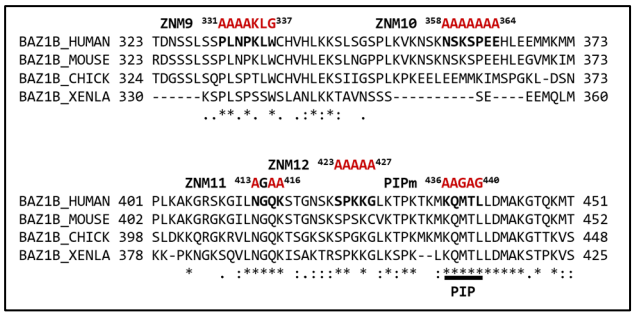
<Figure S4a>



<Figure S4a legend>

(a) The BAZ1B (1-500) is sufficient for PCNA interaction. V5-tagged wild-type and indicated BAZ1B fragments were expressed in 293T cells, followed by anti-V5 immunoprecipitation.

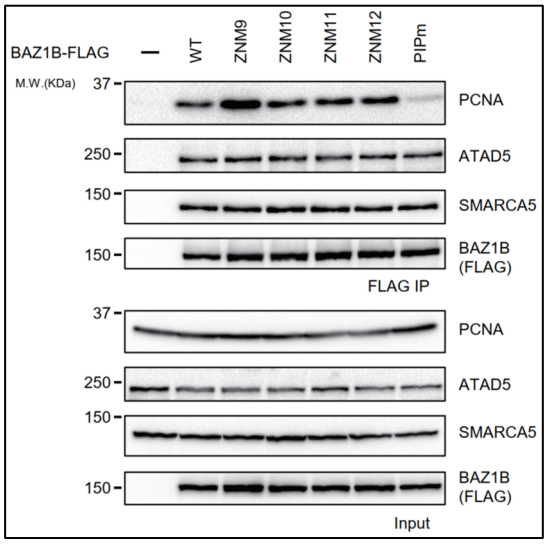
<Figure S4b>



<Figure S4b legend>

(b) Locations of ZNM9-12 mutations and PIPm are indicated.

<Figure S4c>



<Figure S4c legend>

(c) Other mutants in BAZ1B (323-466) do not significantly affect PCNA binding. Anti-FLAG-immunoprecipitation was performed with the indicated cells expressing wild-type or mutant FLAG-BAZ1B.

<Main Text>: Page 7, lines 202-211

To explore whether PCNA influences the ATAD5-BAZ1B interaction, we searched for a PCNA-binding motif in BAZ1B. We identified that PCNA binds to BAZ1B (1-500), but not to BAZ1B (501-1000) or BAZ1B (1001-1483) (Figure S4a). Further analysis revealed that BAZ1B residues 323–466 are essential for PCNA binding (Figure 3d). We tested several mutations within BAZ1B (323-466) and found that the mutation of ^{436}KQMTL^{440} to AAGAG (PIPm) eliminated the BAZ1B-PCNA interaction (Figure 3e and Figure S4b-c). This PCNA-interacting motif (BAZ1B PIP) features a distinct peptide sequence compared to the canonical PIP box (Q-x-x-ψ-x-x-θ-θ). Notably, the PCNA-interaction mutant did not affect ATAD5 binding, indicating that ATAD5 binds to BAZ1B independently of PCNA.

11. The authors show that the interaction of BAZ1B to ATAD5 is NOT needed for interaction with damaged DNA, but that PCNA is. I found this confusing.

12. The authors then go on to show that ATAD5 is also localized to damaged DNA. However, they also claim that ATAD5 has quasi PIP boxes (but this is not obvious from Fig. 4D sup) and can interact with PCNA. This implies that ATAD5 and BAZ1B are independently recruited to damaged DNA via PCNA, which seems quite convoluted.

We appreciate the reviewer's comment. We believe that our responses to point 7 and 10 from reviewer #2 adequately demonstrated that both ATAD5 and BAZ1B bind independently to PCNA through specific motifs on each protein. The role of BAZ1B in DNA replication and repair is mediated by PCNA, as BAZ1B-SMARCA5 is recruited to the site of DNA synthesis via PCNA to promote proper chromatin positioning and facilitate DNA replication and repair. Meanwhile, PCNA is a target of the ATAD5-containing complexes for the de-ubiquitination and unloading, necessitating the enrichment of ATAD5 at sites where PCNA accumulates on chromatin. Our micro-irradiation results confirmed that BAZ1B and ATAD5 accumulate at regions enriched with PCNA, highlighting that PCNA binding to each protein is essential for their recruitment to damaged sites.

The ATAD5-UAF1-USP1 complex de-ubiquitinates Ub-PCNA, thereby counteracting the DNA lesion bypass process. Upon encountering DNA lesions, PCNA becomes ubiquitinated, promoting legion bypass and allowing DNA synthesis to continue. ATAD5-UAF1-USP1 counteracts this bypass process by de-ubiquitinating Ub-PCNA. Timely de-ubiquitination of Ub-PCNA is crucial for maintaining genome integrity as abnormal Ub-PCNA accumulation may impede DNA synthesis. Conversely, premature de-ubiquitination of Ub-PCNA can stall DNA synthesis at lesions, resulting in single-stranded DNA (ssDNA) gaps. Our findings indicate that BAZ1B prevents premature Ub-PCNA de-ubiquitination of Ub-PCNA by ATAD5-UAF1-USP1.

As the reviewer pointed out, both ATAD5 and BAZ1B localize to the micro-irradiated DNA damage sites; however, disruption of the ATAD5-BAZ1B interaction does not affect the localization of either protein at these sites. This suggests that ATAD5 and BAZ1B do not always form a stable complex, although BAZ1B can regulate Ub-PCNA de-ubiquitination through its interaction with ATAD5. Because ATAD5 operates at the final stages of DNA synthesis, it targets polymerase-unassociated, free PCNA on DNA. Based on our experimental data, our current model suggests that BAZ1B protects Ub-PCNA from de-ubiquitination before bypassing DNA lesions by inhibiting the activity of ATAD5-UAF1-USP1. The interactions among ATAD5, BAZ1B, and PCNA appear to be dynamic throughout the DNA lesion bypass process. We have revised the discussion section to present our model more clearly.

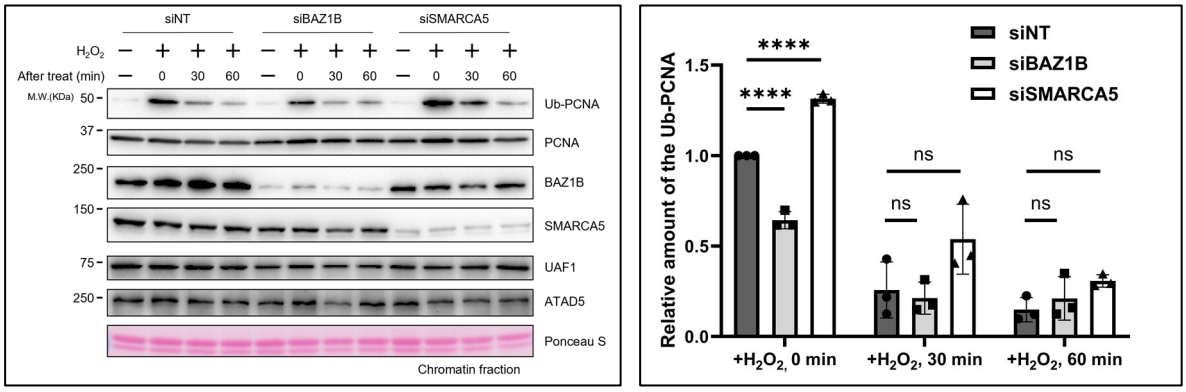
<Main Text>: Page 13, lines 404-416

Our findings demonstrate that BAZ1B inhibits premature Ub-PCNA de-ubiquitination by the ATAD5-UAF1-USP1 complex. Because the BAZ1B binding site on ATAD5 is located near the UAF1 binding site, ATAD5-BAZ1B interaction may sterically hinder or alter the ATAD5-UAF1 interaction. Supporting this hypothesis, our *in vitro* studies show that BAZ1B interferes with the ATAD5-UAF1 association. *Notably, ATAD5 is recruited to DNA damage sites independently of BAZ1B, suggesting that their interaction is not constitutive. Additionally, we did not observe an increase in the interaction between ATAD5 B1m and UAF1 in the immunoprecipitation assay, implying that BAZ1B regulates the ATAD5-UAF1 interaction locally at sites of replication stress*. The proper association of UAF1-USP1 complex to ATAD5 is crucial for the de-ubiquitination of Ub-PCNA; any alterations in this interaction by BAZ1B may inhibit the de-ubiquitination. *The interactions among ATAD5, BAZ1B, and PCNA appear to be dynamic throughout the DNA lesion bypass process*.

13. The effects of a BAZ1B knockdown on PCNA Ub are modest. Moreover, the authors do not observe an effect on chromatin PCNA so conclude that it does not affect (ATAD5-mediated) PCNA unloading – just deubiquitination.

We appreciate the reviewer's comments. As the reviewer noted, our findings indicate that BAZ1B negatively regulates Ub-PCNA de-ubiquitination but does not affect PCNA unloading. We consistently observed a reduction in Ub-PCNA levels following BAZ1B depletion. To further support our argument, we included a statistical analysis of Ub-PCNA levels derived from experimental replicates. The results demonstrate that Ub-PCNA level was significantly reduced by BAZ1B depletion.

<Figure 5a>



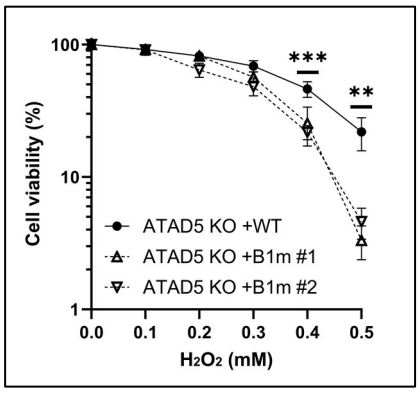
<Figure 5a legend>

a. BAZ1B depletion decreases Ub-PCNA levels after H_2O_2 treatment. BAZ1B or SMARCA5 was depleted, and cells were treated with 1 mM H_2O_2 for 20 minutes. Following incubation in fresh media for the indicated times, chromatin fractions were prepared and analyzed by immunoblotting. *The right panel shows the quantified relative amount of Ub-PCNA. Error bars represent standard deviation (n=3). Statistical analysis: Ordinary one-way ANOVA; ****P≤0.0001 and ns: not significant.*

14. The authors try to make conclusions “without replication stress” but 293T cells are a cancer cell line and therefore could be considered to always be under stress. They should probably more precisely say with even more DNA damage-induced stress. Again, while the H2O2 sensitivity is significant, it is modest.

We appreciate the reviewer’s comment. We agree that cultured 293T cells may experience replication stress even in the absence of DNA-damage-inducing agents; therefore, we have removed "without replication stress" from the revised manuscript. To strengthen our argument regarding the significance of ATAD5-BAZ1B interaction, we included the additional B1m clone in the H_2O_2 sensitivity experiment (Figure 7d). Furthermore, we observed that the viability of the BAZ1B depleted cells was reduced following H_2O_2 treatment (Figure S8e). These results clearly demonstrate that BAZ1B plays a crucial role in regulating Ub-PCNA levels and is important for maintaining genomic integrity under H_2O_2 induced replication stress.

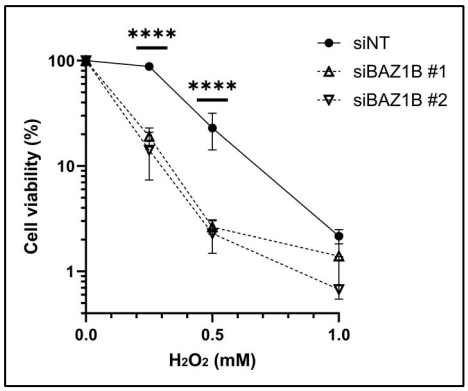
<Figure 7d>



<Figure 7d legend>

*d. Abrogation of the ATAD5-BAZ1B interaction increases sensitivity to H_2O_2. A clonogenic survival assay was conducted with wild-type or B1m ATAD5 cells following treatment with H_2O_2 (0-0.5 mM for 16 hours). Statistical analysis: Ordinary one-way ANOVA; ***P≤0.001 and **P≤0.01.*

<Figure S8e>



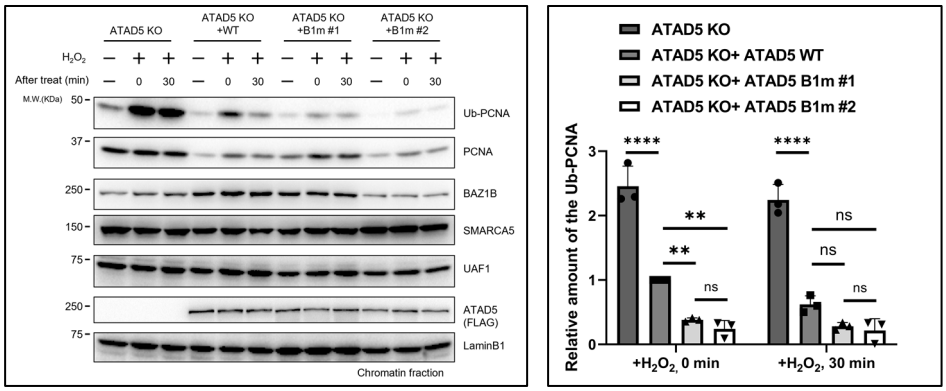
<Figure S8e legend>

*(e) BAZ1B depletion increases sensitivity to H_2O_2. A clonogenic survival assay was conducted with BAZ1B-depleted cells following treatment with H_2O_2 (0-1 mM for 16 hours). Statistical analysis: Ordinary one-way ANOVA; ****P≤0.0001.*

15. The authors conclude that BAZ1B is a negative regulatory of PCNA deubiquitination and that it needs to bind to ATAD5 to do that. I do think they have shown this, but again, the effects are somewhat modest.

We appreciate the reviewer's concern. However, we constantly observed the effect of the ATAD5-BAZ1B interaction on Ub-PCNA de-ubiquitination, both *in vitro* and in cells. To strengthen our argument, we included additional quantifications and statistical analyses in the revised manuscript (Figure 5b-c and Figure S6h). Furthermore, we incorporated additional B1m clones in experiments examining Ub-PCNA levels. We believe that these additional results and analyses further support our original argument that BAZ1B serves as a negative regulator of Ub-PCNA de-ubiquitination.

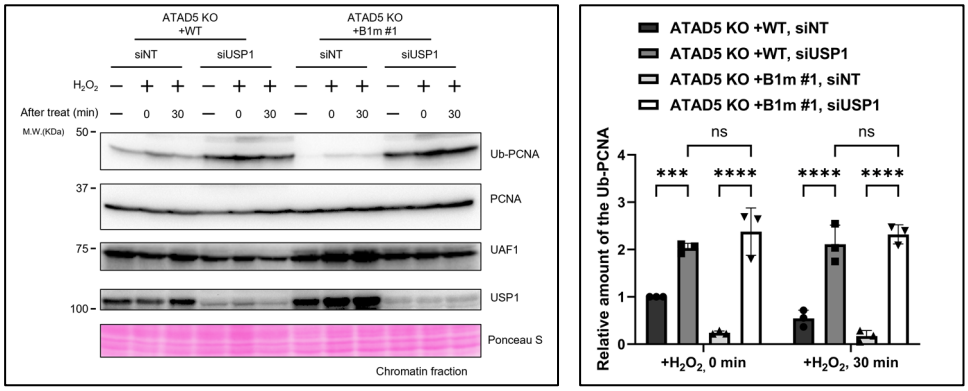
<Figure 5b>



<Figure 5b legend>

b. Abrogation of the ATAD5-BAZ1B interaction reduces Ub-PCNA levels after oxidative stress. ATAD5 knock-out cells expressing either wild-type ATAD5 or ATAD5 B1m were treated with 0.3 mM H_2O_2 for 20 minutes. After incubating in fresh media for the indicated times, cells were harvested, and chromatin fractions were prepared. *The right panel shows the quantified relative amount of Ub-PCNA. Error bars represent standard deviation (n=3). Statistical analysis: Ordinary one-way ANOVA; ****P≤0.0001, **P≤0.01, and ns: not significant.*

<Figure 5c>

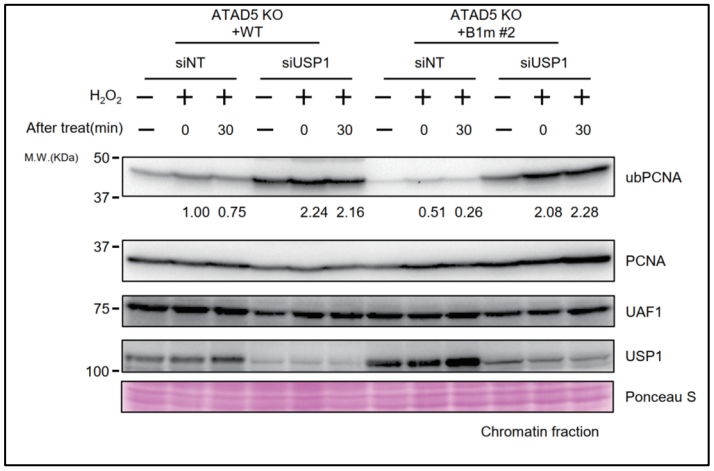


<Figure 5c legend>

c. BAZ1B regulates Ub-PCNA de-ubiquitination by USP1. Cells with USP1 depletion, expressing either wild-type ATAD5 or ATAD5 B1m, were treated with 0.3 mM H_2O_2 for 20 minutes. Following incubation in fresh media for the indicated times, chromatin fractions were prepared and analyzed by immunoblotting. *The right panel shows the quantified relative amount of Ub-PCNA.*

*Error bars represent standard deviation (n=3). Statistical analysis: Two-way ANOVA; ****P≤0.0001, ***P≤0.001, and ns: not significant.*

<Figure S6h>



<Figure S6h legend>

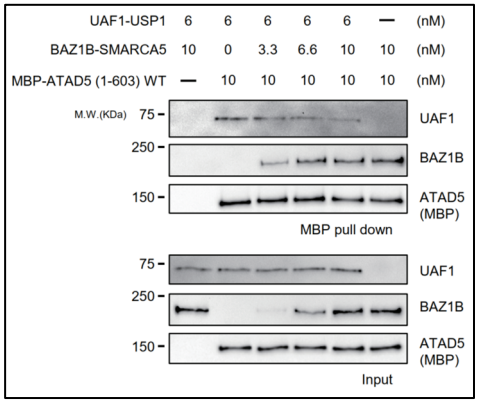
(h) BAZ1B regulates Ub-PCNA de-ubiquitination by USP1. The experiments shown in Figure 5c were conducted using the ATAD5 B1m #2 clone. Numbers below the Ub-PCNA blot indicate the relative amount of Ub-PCNA.

16. Finally, the authors note that the ATAD5 bipartite binding site for BAZ1B overlaps with the UAF1 site, which is required for ATAD5 to interact with USP1. However, they also see that UAF1 binding is reduced, but not ablated, in the presence of increasing BAZ1b, which makes the observation less certain.

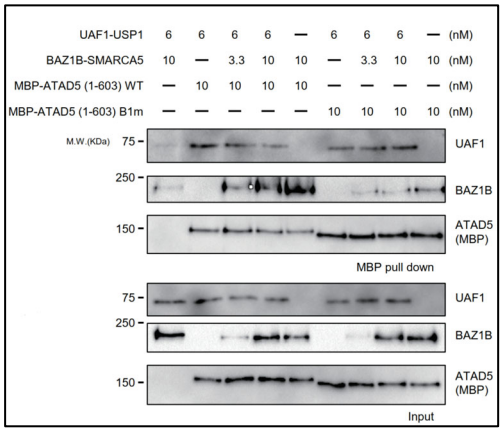
We understand the reviewer's concern. As noted, BAZ1B did not completely outcompete UAF1 for ATAD5 binding. However, we believe that BAZ1B binding can influence the activity of the ATAD5-UAF1-USP1 complex, as the presence of BAZ1B interfered with Ub-PCNA de-ubiquitination *in vitro*, and the disruption of the ATAD5-BAZ1B interaction led to a reduction in Ub-PCNA levels in cells. To

more convincingly demonstrate the effect of BAZ1B on the ATAD5-UAF1 interaction, we included a statistical analysis of the *in vitro* pulldown experiments in the revised manuscript.

<Figure 6b>



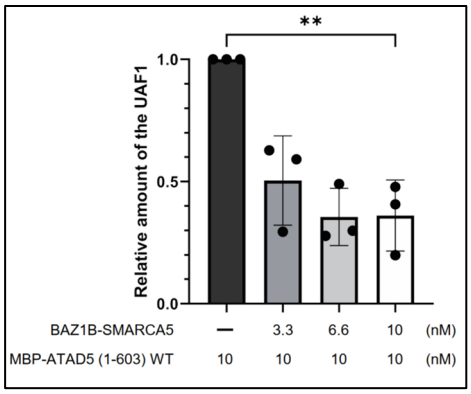
<Figure 6c>



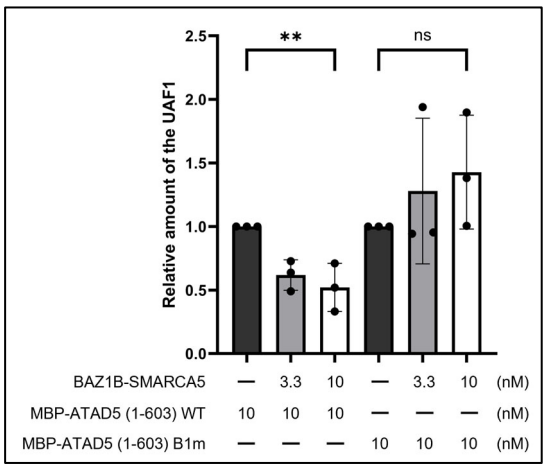
<Figure 6b-c legends>

b-c. BAZ1B-SMARCA5 interferes with the interaction between UAF1 and ATAD5. Wild-type, (b), or B1m MBP-ATAD5 (1-603), (c), were mixed with UAF1-USP1 and BAZ1B-SMARCA5, followed by amylose resin pull down.

<Figure S7c>



<Figure S7d>



<Figure S7c-d legends>

*(c-d) Quantified relative amounts of the UAF1 in Figure 6b or Figure 6c, respectively. Error bars represent standard deviation (n=3). Statistical analysis: Ordinary one-way ANOVA; **P≤0.001 and ns: not significant.*

Reviewer #3 (Remarks to the Author):

This manuscript by Kim et al. describes a novel interaction between the ATAD5 PCNA unloading factor and BAZ1B, a subunit of a chromatin-remodeling complex. The authors present data suggesting that BAZ1B binding to ATAD5 prohibits its association with the UAF1-USP1 PCNA deubiquitinase. They propose that this interaction fine-tunes PCNA ubiquitination and show that an ATAD5 mutant that cannot interact with BAZ1B decreases PCNA ubiquitination in response to H2O2 treatment leading to DNA replication defects, replication stress and genome instability.

The article is well-written and the many of the claims are well supported by the data. In terms of novelty, the mapping of the interactions between BAZ1B and ATAD5 is well done and comprehensive (although additional structure prediction work would strengthen their model see below). The authors also identify a PIP in BAZ1B and provide data suggesting its role in recruiting BAZ1B to DNA lesions. BAZ1B was previously shown to interact with PCNA but the motif responsible for this association remains unknown (Poot et al 2004). The regulation of PCNA ubiquitination by a BAZ1B-ATAD5 interaction that outcompetes the ATAD5-UAF1/USP1 association is also an interesting new layer of regulation for DNA damage tolerance. However, some of the key conclusions, especially those regarding the in vivo influence of BAZ1B and its interaction with ATAD5 on ubPCNA levels and DNA damage tolerance require additional experimental support to be validated.

We appreciate the reviewer's thoughtful comments. As suggested, we conducted additional experiments to strengthen our arguments.

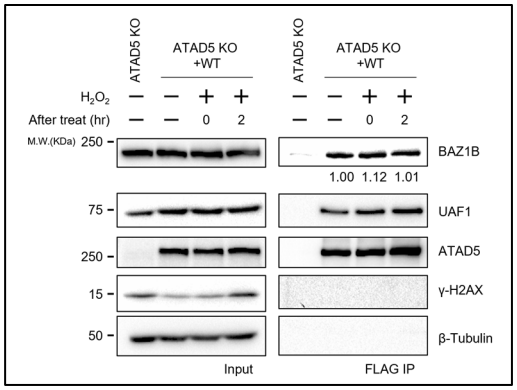
Major points :

1. In figure 1, the authors show that ATAD5 interacts with BAZ1B and that this interaction also mediates its association with SMARCA5. They also claim that the interactions are not affected by DNA damage but all the experiments were done using overexpressed proteins potentially masking

some of the regulatory pathways. Pulldown of endogenous ATAD5 or BAZ1B and examination of their interaction in the presence of genotoxins should be provided.

We appreciate the reviewer's suggestion. Our findings indicate that BAZ1B binds to ATAD5 independently of SMARCA5. As noted by the reviewer, we previously argued that ATAD5-BAZ1B interaction was unaffected by DNA-damaging agents. We agree that these experiments should be conducted using endogenous ATAD5. Since the ATAD5 antibody used in this study was raised against the N-terminal domain of ATAD5, which overlaps with the BAZ1B binding site, we utilized an ATAD5 knock-out cell line with an add-back of CLIP-ATAD5-StrepII-FLAG and performed FLAG-immunoprecipitation following DNA damage agent application. The expression level of CLIP-ATAD5-StrepII-FLAG in the add-back clone was comparable to that of endogenous ATAD5 (Figure S5f). We quantified the interaction between BAZ1B and ATAD5 after pull down (Updated Figure 1g and Figure S1c-d). Our results confirmed that genotoxic stress from agents such as hydrogen peroxide (H_2O_2), UV, hydroxyurea (HU), and camptothecin (CPT), does not significantly affect the ATAD5-BAZ1B interaction, supporting our original argument.

<Figure 1g>

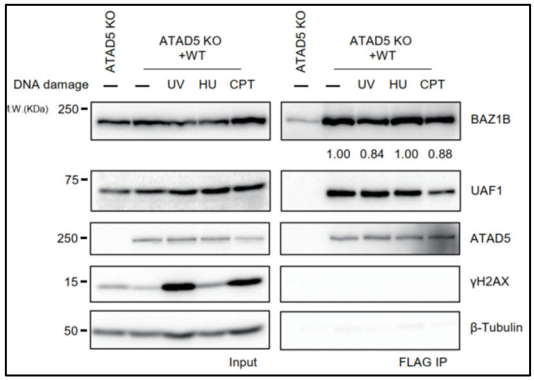


<Figure 1g legend>

g. Oxidative stress does not affect ATAD5-BAZ1B interaction. Wild-type ATAD5 cells were treated with 1 mM hydrogen peroxide for 20 minutes, followed by incubation in fresh media for the

indicated time period. ATAD5 was immunoprecipitated using anti-FLAG beads. The numbers below the BAZ1B blot indicate the relative amount of BAZ1B co-precipitating.

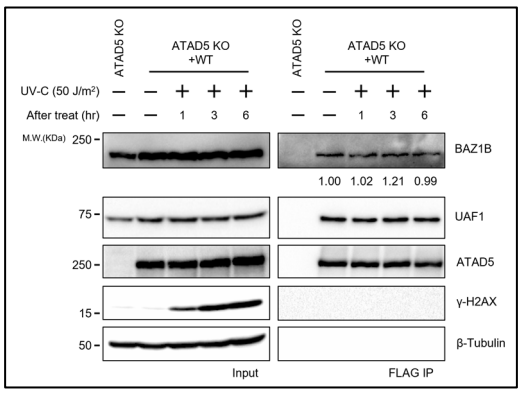
<Figure S1c>



<Figure S1c legend>

(c) UV, Hydroxyurea (HU) or Camptothecin (CPT) does not alter the interaction between ATAD5 and BAZ1B. ATAD5 knock-out (KO) cells expressing wild-type ATAD5 (ATAD5 KO+WT) were treated with UV-C (50 J/m^2), HU (2 mM for 6 hours), or CPT (2 μM for 17 hours), followed by anti-FLAG immunoprecipitation. UV-treated cells were incubated in fresh media for 6 hours. Numbers below the BAZ1B blot indicate the relative amount of BAZ1B.

<Figure S1d>



<Figure S1d legend>

(d) ATAD5 KO+WT cells were treated with UV-C (50 J/m^2) and then incubated in fresh media for the indicated times. At each time point, cells were harvested, and anti-FLAG immunoprecipitation was performed. Numbers below the BAZ1B blot indicate the relative amount of BAZ1B.

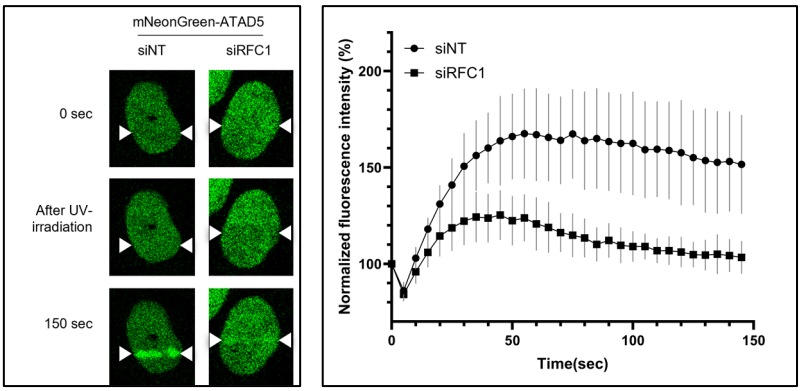
2. In figure 4, the authors show that BAZ1B requires its PCNA-interacting motif but not its association with ATAD5 for recruitment to micro-irradiation stripes. They also show that ATAD5-binding to BAZ1B is not required (B1m mutant) for its recruitment at lesions. Instead, they show that two putative PIP-like motifs at the N-ter of ATAD5 mediate its recruitment.

A weaker point of these experiments is that everything is done using overexpressed proteins which may artefactually tether these factors to sites of damage, precluding some recruitment dependencies from being observed.

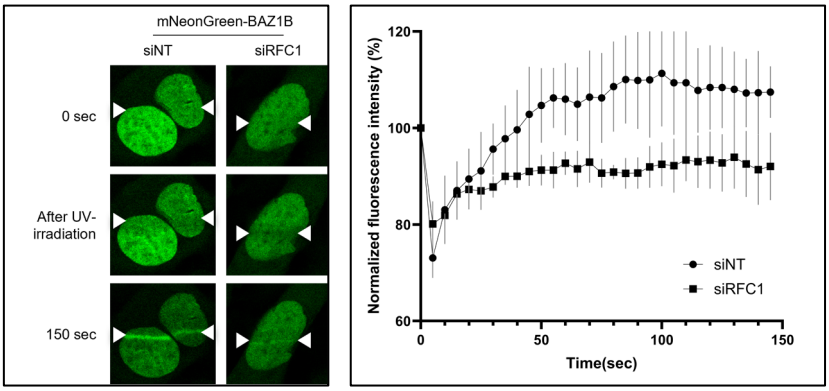
To strengthen the manuscript, the authors should perform microirradiation experiments and look at endogenous ATAD5 and BAZ1B to see if their recruitment is affected by ATAD5/BAZ1B/PCNA KD.

We appreciate the reviewer's suggestion. Unfortunately, our ATAD5 and BAZ1B antibodies were unsuitable for micro-irradiation experiments. To address the reviewer's concern, we examined the localization of ATAD5 at micro-irradiated sites using an mNeon-ATAD5 Hela cell line, where endogenous ATAD5 was fused with mNeonGreen (Park et al., 2021 NAR). For BAZ1B, we created a stable cell line expressing mNeonGreen-BAZ1B, with expression levels comparable to endogenous BAZ1B (Figure S4j). PCNA is loaded onto DNA by RFC during DNA replication and repair. A previous report (Park et al., 2023 NAR) demonstrated that depletion of RFC1, a key RFC subunit, abolished PCNA accumulation at micro-irradiation stripes. Consequently, rather than knocking down PCNA, we conducted micro-irradiation experiments following RFC1 knockdown. The results showed that recruitment of ATAD5 and BAZ1B was reduced by RFC1 depletion (Figure S5h-j), supporting our original argument that ATAD5 and BAZ1B are both recruited to the site of repair synthesis through PCNA interaction.

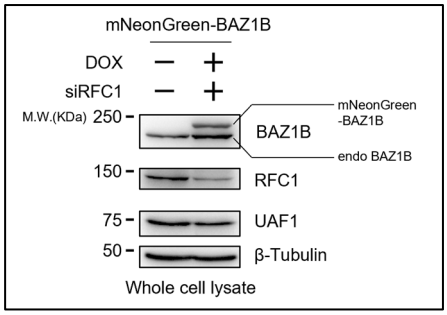
<Figure S5h>



<Figure S5i>



<Figure S5j>



<Figure S5h-j legends>

(h-i) The localization of ATAD5 and BAZ1B to damage sites depends on PCNA. HeLa cells with endogenous ATAD5 fused to mNeonGreen, (h), or stably expressing mNeonGreen-BAZ1B, (i), were subjected to 355 nm UV laser micro-irradiation following RFC1 depletion. (j) The expression level of mNeonGreen-BAZ1B is comparable to that of endogenous BAZ1B. Whole cell lysates were prepared for immunoblotting.

<Main Text>: Page 9, lines 267-272

RFC1 depletion prevents the loading of PCNA onto DNA and reduces the PCNA accumulation at micro-irradiation sites [4,5]. Our results showed that RFC1 depletion resulted in significant reduction in the recruitment of both ATAD5 and BAZ1B to damage sites (Figure S5h-j). These findings suggest that ATAD5 and BAZ1B are independently recruited to DNA repair sites through their interaction with PCNA.

3. In figure 5, the authors try to show that BAZ1B impedes PCNA deubiquitination by monitoring the levels of this modification in H2O2-treated cells following siRNA-mediated depletion of BAZ1B and reconstitution of ATAD5KO cells with the ATAD5 B1m mutant that cannot interact with BAZ1B.

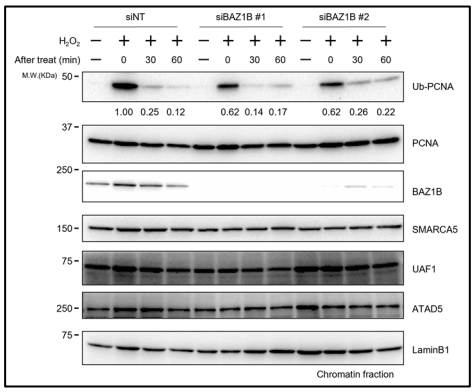
This part of the model is improperly supported by the current data. At least 2 independent BAZ1B-targeting siRNAs should be tested and reconstitution with WT or an ATAD5-interacting mutant could be provided to show that the phenotype is indeed caused by a decreased association between BAZ1B and ATAD5.

Additionally, ubPCNA quantification from at least 3 independent experiments should be added to a revised manuscript. Quantification should also be provided in 5B and C.

In 5B, the magnitude of the ubPCNA decrease in the 2 B1m ATAD5KO complemented cell lines is quite variable. Since there is a large difference in ubPCNA levels for the #1 and #14 cell lines, the experiment in Fig5C should also be redone for the #14 clone.

We appreciate the reviewer's thoughtful comments. As suggested, we included an additional BAZ1B siRNA in our experiments examining Ub-PCNA levels after BAZ1B depletion (Figure S6c). The results more convincingly demonstrated that BAZ1B depletion reduces the Ub-PCNA level.

<Figure S6c>

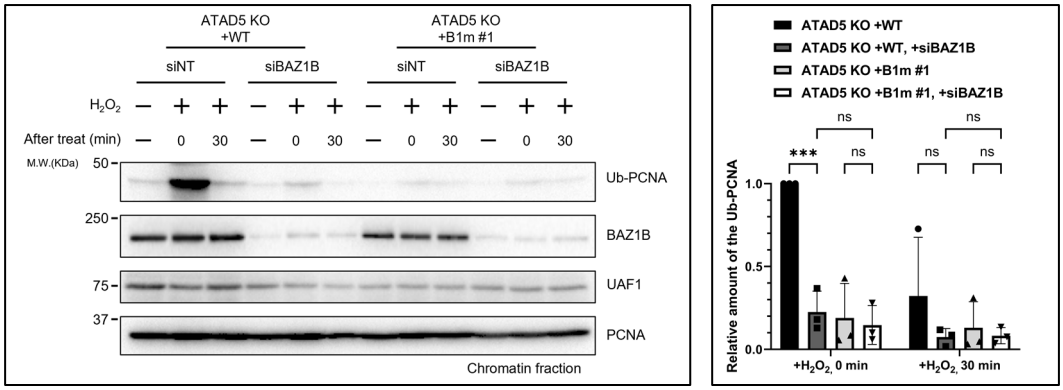


<Figure S6c legend>

(c) BAZ1B depletion reduces the Ub-PCNA levels after H_2O_2 treatment. Two independent BAZ1B-targeting siRNAs were used for BAZ1B depletion. Cells were treated with 1 mM H_2O_2 for 20 minutes and then incubated in fresh media for the indicated time. Chromatin fractions were prepared and analyzed by immunoblotting. Numbers below the Ub-PCNA blot indicate the relative amount of Ub-PCNA.

As the reviewer suggested, we depleted BAZ1B in ATAD5 B1m cells and examined the Ub-PCNA level following H_2O_2 treatment. BAZ1B depletion in both wild type and B1m cells resulted in similar Ub-PCNA levels. In addition, in ATAD5 B1m cells, BAZ1B depletion did not further reduce the Ub-PCNA level. These results support our argument that the ATAD5-BAZ1B interaction regulates Ub-PCNA de-ubiquitination in cells.

<Figure S6i>

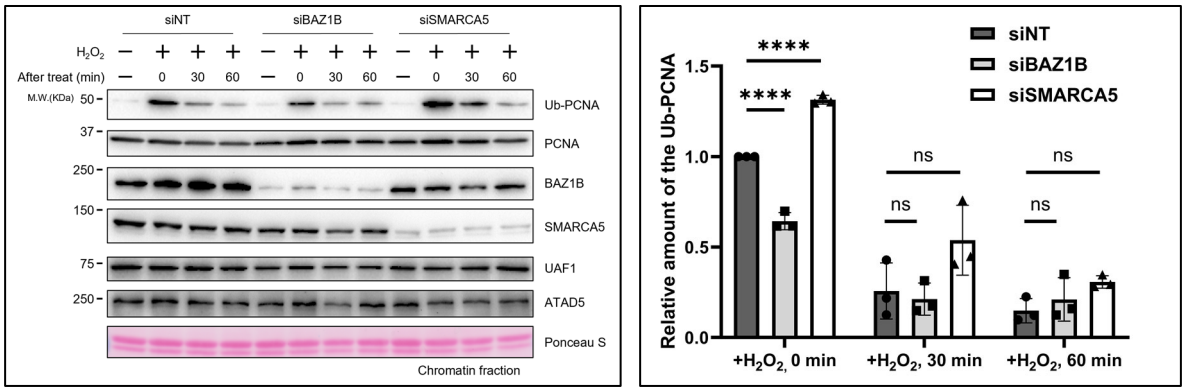


<Figure S6i legend>

*(i) Ub-PCNA levels after oxidative stress are regulated by the ATAD5-BAZ1B interaction. BAZ1B was depleted in ATAD5 KO+WT and ATAD5 KO+B1m #1 cells, which were treated with 0.3 mM H_2O_2 for 20 minutes. Following treatment, cells were incubated in fresh media for the indicated times and chromatin fractionation was performed. The right panel shows the quantified relative amount of Ub-PCNA. Error bars represent standard deviation (n=3). Statistical analysis: Two-way ANOVA; ***P≤0.001 and ns: not significant.*

As the reviewer suggested, we included the quantification and statistical analysis from experimental replicates for Figure 5a-c. In addition, we conducted a more thorough analysis using an additional ATAD5 B1m cell line (previously clone number 14-9 in the initial manuscript, now renamed B1m #2). We observed similar results with two independent ATAD5 B1m clones. We believe that the revised figures more convincingly support our original arguments.

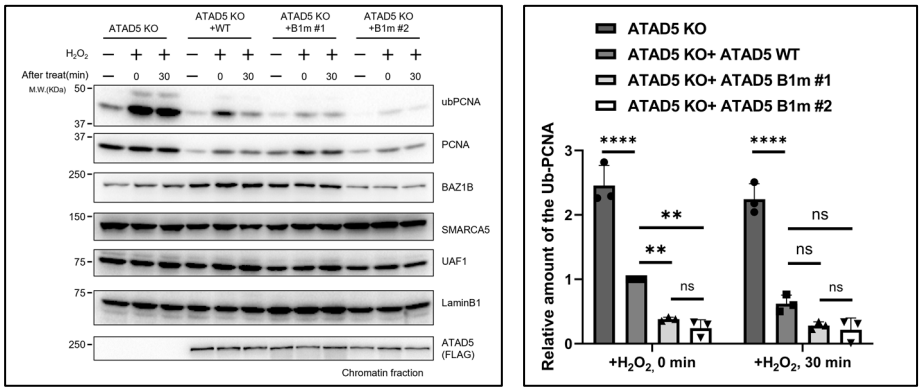
<Figure 5a>



<Figure 5a legend>

a. BAZ1B depletion decreases Ub-PCNA levels after H_2O_2 treatment. BAZ1B or SMARCA5 was depleted, and cells were treated with 1 mM H_2O_2 for 20 minutes. Following incubation in fresh media for the indicated times, chromatin fractions were prepared and analyzed by immunoblotting. *The right panel shows the quantified relative amount of Ub-PCNA. Error bars represent standard deviation (n=3). Statistical analysis: Ordinary one-way ANOVA; ****P ≤0.0001 and ns: not significant.*

<Figure 5b>

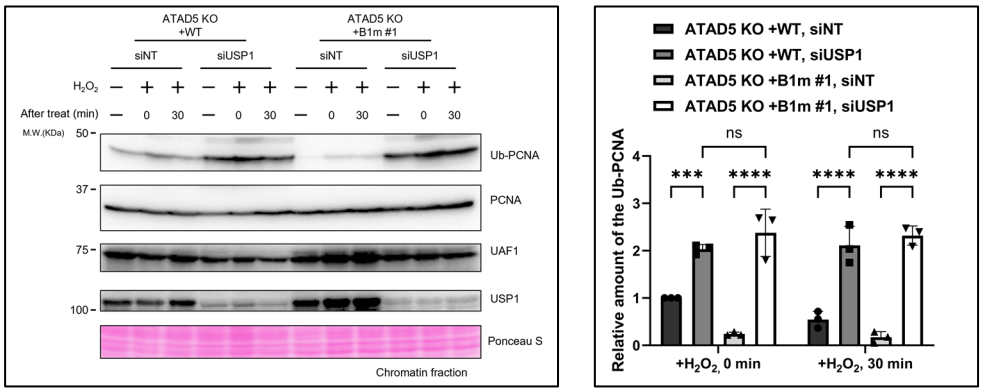


<Figure 5b legend>

b. Abrogation of the ATAD5-BAZ1B interaction reduces Ub-PCNA levels after oxidative stress. ATAD5 knock-out cells expressing either wild-type ATAD5 or ATAD5 B1m were treated with 0.3 mM H_2O_2 for 20 minutes. After incubating in fresh media for the indicated times, cells were harvested, and chromatin fractions were prepared. *The right panel shows the quantified relative*

*amount of Ub-PCNA. Error bars represent standard deviation (n=3). Statistical analysis: Ordinary one-way ANOVA; ****P ≤0.0001, **P ≤0.01, and ns: not significant.*

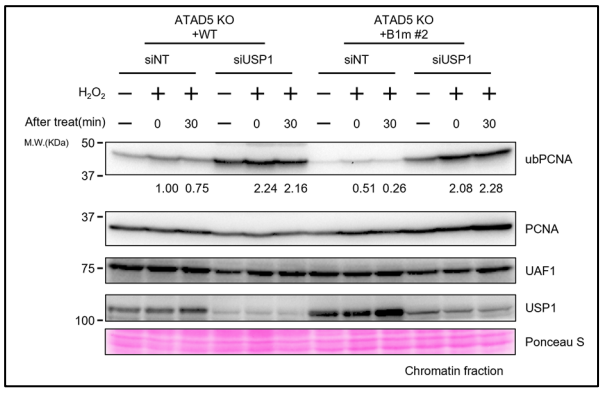
<Figure 5c>



<Figure 5c legend>

c. BAZ1B regulates Ub-PCNA de-ubiquitination by USP1. Cells with USP1 depletion, expressing either wild-type ATAD5 or ATAD5 B1m, were treated with 0.3 mM H_2O_2 for 20 minutes. Following incubation in fresh media for the indicated times, chromatin fractions were prepared and analyzed by immunoblotting. *The right panel shows the quantified relative amount of Ub-PCNA. Error bars represent standard deviation (n=3). Statistical analysis: Two-way ANOVA; ****P ≤0.0001, ***P ≤0.001, and ns: not significant.*

<Figure S6h>



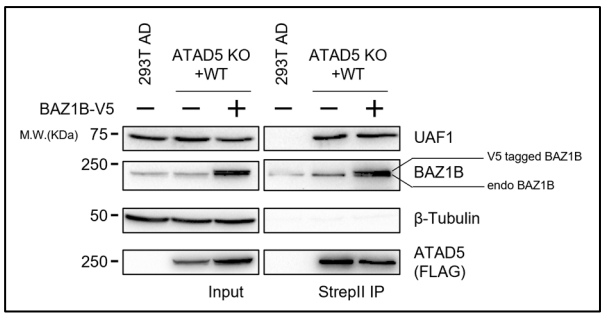
<Figure S6h legend>

(h) BAZ1B regulates Ub-PCNA de-ubiquitination by USP1. The experiments shown in Figure 5c were conducted using the ATAD5 B1m #2 clone. Numbers below the Ub-PCNA blot indicate the relative amount of Ub-PCNA.

4. Most important point: the model posits that BAZ1B binding to ATAD5 blocks the association of ATAD5 with USP1-UAF1. This is well demonstrated in vitro (Fig. 6), but there is no in vivo data showing that the interaction between UAF1-USP1 and ATAD5 is increased in BAZ1B-depleted cells as expected. A pulldown of ATAD5 or UAF1 should be done in control and BAZ1B-depleted cells to test whether the absence of BAZ1B stabilizes the ATAD5-UAF1 interaction. Alternatively, does BAZ1B overexpression impede the ATAD5-UAF1 interaction or increase UbPCNA levels?

We appreciate the reviewer's suggestion. Following this advice, we examined the interaction between UAF1 and ATAD5 after either exogenous expression of BAZ1B (Figure R2) or depletion of BAZ1B (Figure R3) through immunoprecipitation, using wild-type ATAD5 add-back in ATAD5 Knock-out cells. In these experiments, we did not observe significant changes in the amount of UAF1 co-precipitated with ATAD5. These results suggest that the effect of BAZ1B on the ATAD5-UAF1 interaction might be specific to DNA lesions where regulation of Ub-PCNA de-ubiquitination is necessary. We believe that the additional *in vitro* and cellular results presented in our study sufficiently support our argument that BAZ1B negatively regulates ATAD5-mediated Ub-PCNA de-ubiquitination.

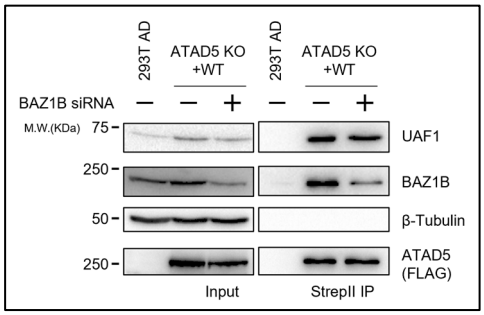
<Figure R2>: This figure is not included in the revised manuscript.



<Figure R2 legend>

The interaction between ATAD5-UAF1 is not notably altered by BAZ1B overexpression. ATAD5 knockout cells expressing wild-type ATAD5 (ATAD5 KO+WT) were transiently transfected with V5-tagged BAZ1B and followed by Strep-Tactin pull-down.

<Figure R3>: This figure is not included in the revised manuscript.



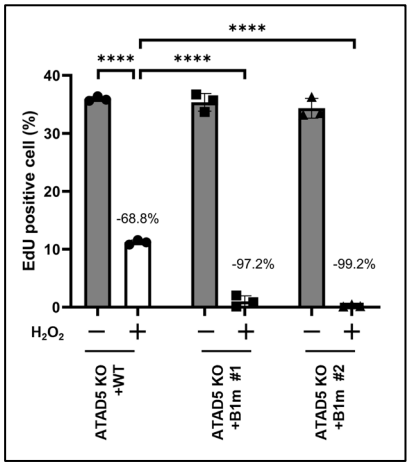
<Figure R3 legend>

Depletion of BAZ1B does not significantly affect the ATAD5-UAF1 interaction. ATAD5 knockout cells expressing wild-type ATAD5 (ATAD5 KO+WT) were transfected with BAZ1B-targeting siRNA for 48 hours and followed by Strep-Tactin pull-down.

5. In figure 7, the authors investigate the impact of mutating the BAZ1B-binding motif of ATAD5 on DNA synthesis and genomic instability in response to H2O2-treatment. Again, data from the #14 B1m-complemented ATAD5 KO clone should be provided in panels B and C. To fully implicate BAZ1B in these phenotypes, experiments should be done in BAZ1B KD cells. If BAZ1B KD does not induce strong defects in DNA synthesis, DNA damage or resistance to H2O2, then it would suggest that something other that the association of ATAD5 with BAZ1B participates in the observed phenotypes. In addition, it is expected that depletion of BAZ1B in ATAD5 KO cells complemented with B1m should not further enhance the decrease in UbPCNA levels or sensitivity towards H2O2.

We appreciate the reviewer's thoughtful comments. As suggested, we included data from an additional ATAD5 B1m clone in the revised Figure 7a, 7d, S8c, and S8d. We observed the same phenotype with the additional ATAD5 B1m clone.

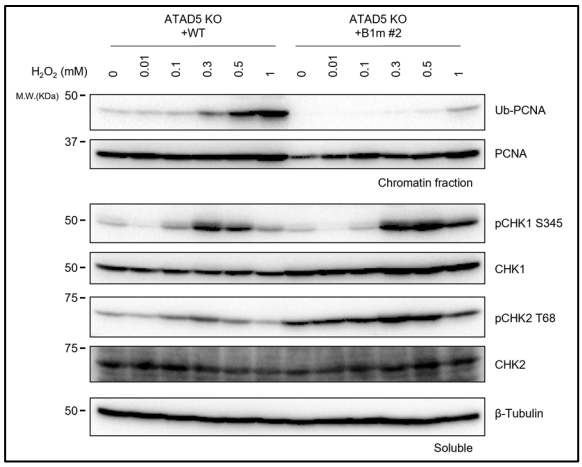
<Figure 7a>

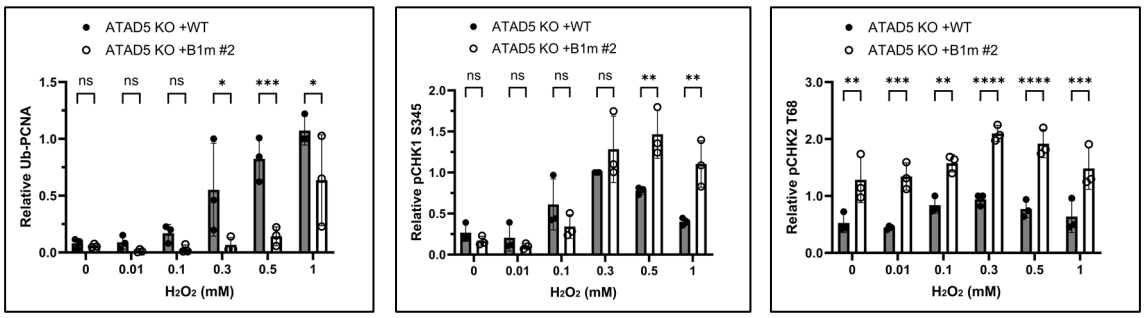


<Figure 7a legend>

a. DNA replication is more severely inhibited in ATAD5 B1m cells compared to wild-type under oxidative stress. Cells were labeled with EdU for 30 minutes and treated with H_2O_2 for 20 minutes as indicated. Following the Click reaction, EdU intensity was measured by flow cytometry. The relative decrease in the percentage of EdU-positive cells was calculated from three independent experiments. Error bars represent standard deviation (n=3). Statistical analysis: Two-way ANOVA; ****P≤0.0001.

<Figure S8c>

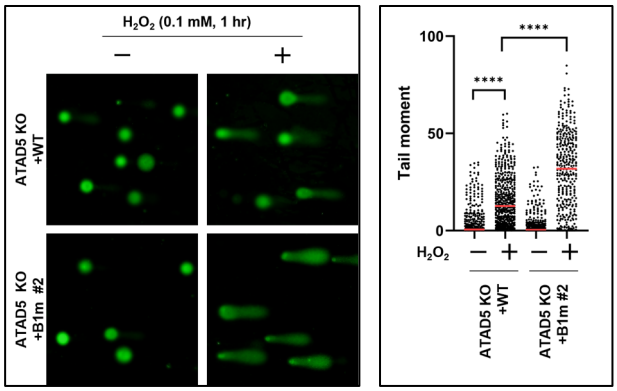




<Figure S8c legend>

*(c) ATAD5 KO+WT and ATAD5 KO+B1m #2 cells were fractionated after treatment with the indicated concentrations of hydrogen peroxide for 20 minutes. The bottom panel shows the quantified relative amounts of Ub-PCNA, phosphorylated CHK1 (S345), and phosphorylated CHK2 (T68). Error bars represent standard deviation (n=3). Statistical analysis: Ordinary one-way ANOVA; ****P≤0.0001, ***P≤0.001, **P≤0.01, *P≤0.05, and ns: not significant.*

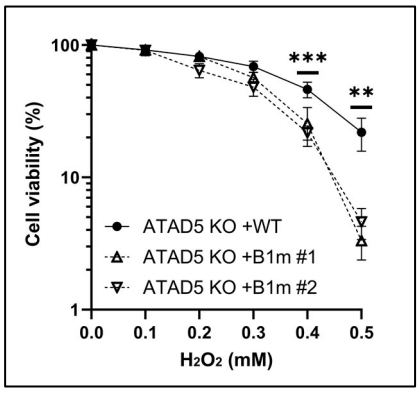
<Figure S8d>



<Figure S8d legend>

*(d) Abrogation of the ATAD5-BAZ1B interaction causes increased DNA breaks after oxidative stress. Another ATAD5 KO+B1m clone (B1m #2) also shows more DNA breaks after oxidative stress, consistent with the B1m #1 clone. The alkaline comet assay was performed with ATAD5 KO+WT and ATAD5 KO+B1m #2 cells after treatment with H_2O_2 (0.1 mM for 1 hour). The right panel shows the quantification of the tail moment. Statistical analysis: Two-way ANOVA; ****P≤0.0001.*

<Figure 7d>

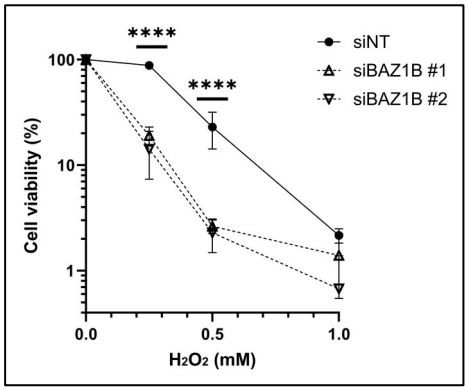


<Figure 7d legend>

*d. Abrogation of the ATAD5-BAZ1B interaction increases sensitivity to H_2O_2. A clonogenic survival assay was conducted with wild-type or B1m ATAD5 cells following treatment with H_2O_2 (0-0.5 mM for 16 hours). Statistical analysis: Ordinary one-way ANOVA; ***P≤0.001 and **P≤0.01.*

We examined the sensitivity to H_2O_2 following BAZ1B depletion and found that it increased sensitivity to hydrogen peroxide (Figure S8e). This finding supports our argument that BAZ1B mediated Ub-PCNA regulation is important for maintaining genome integrity.

<Figure S8e>

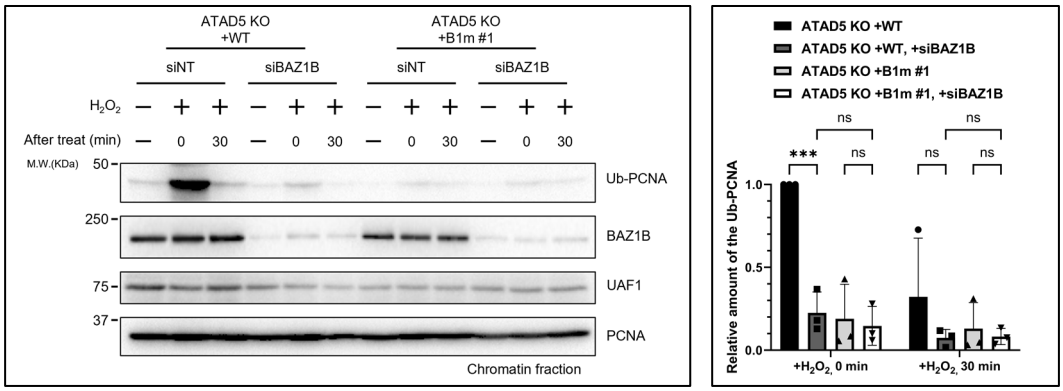


<Figure S8e legend>

*(e) BAZ1B depletion increases sensitivity to H_2O_2. A clonogenic survival assay was conducted with BAZ1B-depleted cells following treatment with H_2O_2 (0-1 mM for 16 hours). Statistical analysis: Ordinary one-way ANOVA; ****P≤0.0001.*

We examine the H_2O_2 induced Ub-PCNA levels following BAZ1B depletion in ATAD5 B1m cells (Figure S6i). The results showed that BAZ1B depletion in B1m cells did not further reduce the Ub-PCNA levels, supporting our argument that the ATAD5-BAZ1B interaction is crucial for regulating Ub-PCNA levels.

<Figure S6i>



<Figure S6i legend>

*(i) Ub-PCNA levels after oxidative stress are regulated by the ATAD5-BAZ1B interaction. BAZ1B was depleted in ATAD5 KO+WT and ATAD5 KO+B1m #1 cells, which were treated with 0.3 mM H_2O_2 for 20 minutes. Following treatment, cells were incubated in fresh media for the indicated times and chromatin fractionation was performed. The right panel shows the quantified relative amount of Ub-PCNA. Error bars represent standard deviation (n=3). Statistical analysis: Two-way ANOVA; ***P≤0.001 and ns: not significant.*

Minor points :

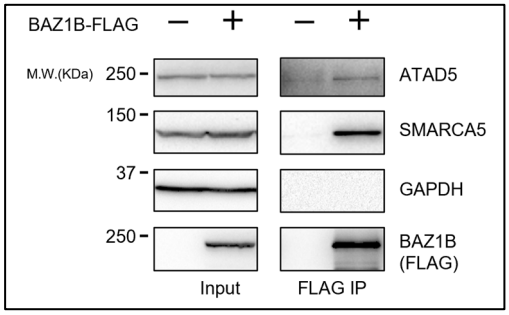
1. In Fig S1 and in many other instances throughout the paper, the pulldown blots for factors that are not expected to interact with the baits should be included even though no signal is expected. (e.g. S1C, γ-H2AX S2A, tubulin S4E, lamin B1…)

We thank the reviewer for this suggestion. In response, we have now included all pulldown blots for factors not expected to interact with the baits in the revised manuscript.

2. loading control for Fig. should be fixed, noting is visible in the input.

We conducted the experiment more carefully and revised Figure 1d to include the appropriate loading control.

<Figure 1d>



<Figure 1d legend>

d-e. BAZ1B binds to ATAD5. Transiently expressed BAZ1B-FLAG, *(d)*, or endogenous BAZ1B, *(e)*, was immunoprecipitated using anti-FLAG bead or anti-BAZ1B antibody, respectively. The isolated proteins were examined by immunoblotting.

3. In figure 3, the authors map the BAZ1B motifs that interact with ATAD5 and show that their interaction is independent of BAZ1B-binding to PCNA and SMARCA5. They map 2 motifs (A5BM1 and 2) which fold into a β-sheet-based motif according to Alphafold predictions. Since the authors have narrowed down the putative contacting domains between BAZ1B and ATAD5, further predictions of the structure of the complex using Collafold (Mirdita et al. Nature Methods 2022) would reinforce this section.

A model in which the ATAD5-BAZ1B interaction is compared with that of ATAD5 bound to USP1-UAF1 would provide structural insights into the BAZ1B/UAF1-USP1 competition for ATAD5 binding. Similarly, the proposed PIP motifs at the N-ter of ATAD5 could be co-modeled with PCNA to strengthen the idea that this association is what recruits ATAD5 to sites of damage.

We appreciate the reviewer's insightful suggestions for enhancing our structural analysis. We agree that additional structural insights, particularly by modeling the ATAD5-BAZ1B and ATAD5-PCNA interactions, would strengthen this section of our manuscript.

In response to the reviewer's recommendation, we used AlphaFold2 to predict the structure of the ATAD5(1-603) bound to BAZ1B (1-500) and the ATAD5(1-603) bound to PCNA.

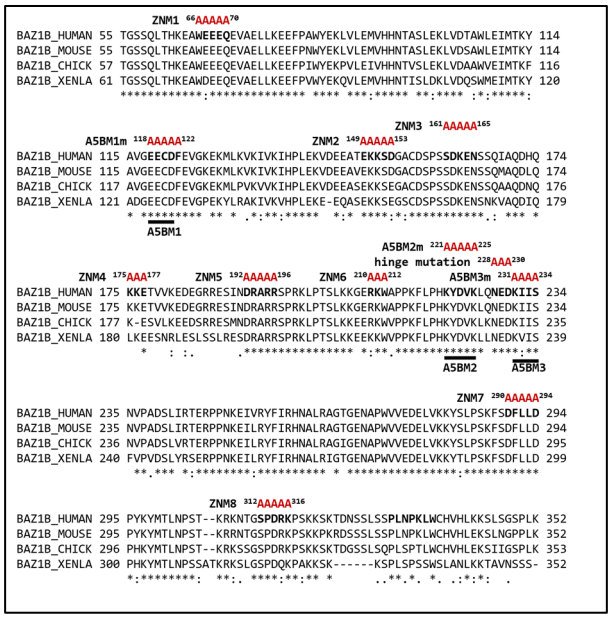
1) Prediction of BAZ1B-ATAD5 complex:

We predicted the structure of the ATAD5-BAZ1B complex using AlphaFold2 (Figure S3g). We observed that the BAZ1B F122 residue (in the A5BM1 motif) forms parallel-displaced (staggered stacking) configuration of aromatic π-π interaction with ATAD5 Y267 and F270 (in the B1 motif) (Figure S3g-h, and Figure R1). The parallel-displaced conformation is a representative physiological π-π stack structure and π-π stacking is crucial for protein structure and protein-protein interactions [1]. Based on this analysis, we created an ATAD5 (Y267A, F270A) mutant, referred to as the YFAA mutant, and found that it disrupted the BAZ1B interaction, similar to the B1m mutant. (Figure S3i-j).

Additionally, we observed that the Y222 residue in the BAZ1B A5BM2 motif forms a T-shaped π-π stacking interaction with the Y114 residue in BAZ1B, stabilizing the positioning of the A5BM1 motif and further supporting the ATAD5-BAZ1B interaction (Figure S3k). Moreover, the BAZ1B (231-234, referred to as A5BM3) motif interacts in a parallel fashion with the ATAD5 B1 motif contributing to forming a 4-stranded antiparallel structure with the BAZ1B A5BM1/2 motifs and the ATAD5 B1 motif (Figure S3g-h, S3k and Figure R1). To validate these predictions, we generated alanine substitution mutants in the BAZ1B A5BM1, A5BM2, and A5BM3 motifs. Each mutant (E118A, Y222A, and A5BM3 alanine substitution mutant) exhibited significant defects in ATAD5 interaction, consistent with our predictions (Figure 3c and Figure S3b, S3l-m).

In the revised manuscript, we have included additional analyses and experimental results. The main text in the results section has been updated accordingly.

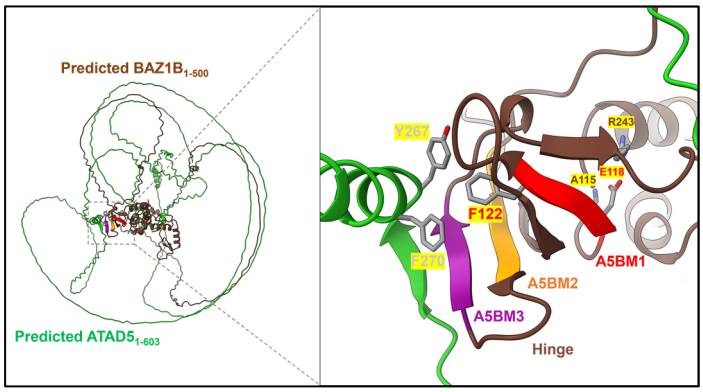
<Figure S3b>



<Figure S3b legend>

(b) The locations of ZNM1-8 mutations, A5BM1m, A5BM2m, A5BM3m, and the hinge mutation are indicated.

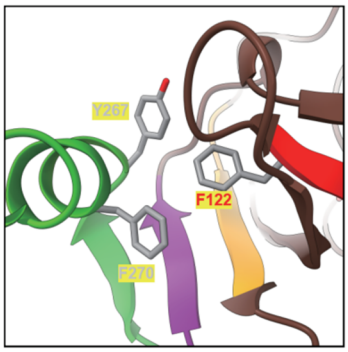
<Figure S3g>



<Figure S3g legend>

(g) AlpfaFold2 prediction of ATAD5 (1-603) bound to BAZ1B (1-500).

<Figure S3h>



<Figure S3h legend>

(h) The residues Y267 and F270 of ATAD5 form a parallel-displaced (staggered stacking) configuration of aromatic π-π interactions with the BAZ1B F122 residue.

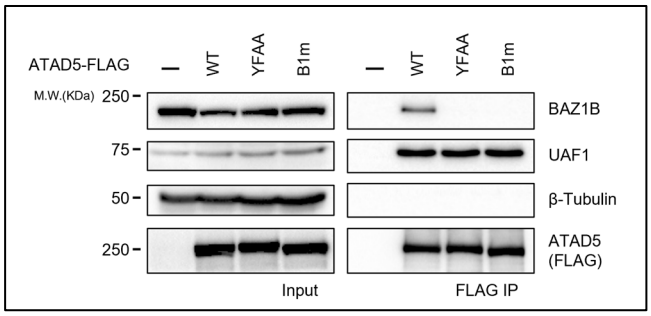
<Figure S3i>

```
                    YFAA 264TVSAEEA270
ATAD5_HUMAN   254 EAAQLNDSIITVSYEEFLKSHKENKVE 280
ATAD5_MOUSE   251 KRADLKESTITVSYEEFVKSHKAAKVE 277
ATAD5_CHICK   253 KTPQANDSIITVSFEDFLKSQGENYVE 279
ATAD5_XENLA   248 DGLHGKDSILVVSFEEFIKSQGEKDME 274
                  .  . ::* :.**:*:*:**:    :*
```

<Figure S3i legend>

(i) The residues Y264 and F270 of ATAD5 are conserved among species. The location of the YFAA mutations (Y264A, F270A) were indicated.

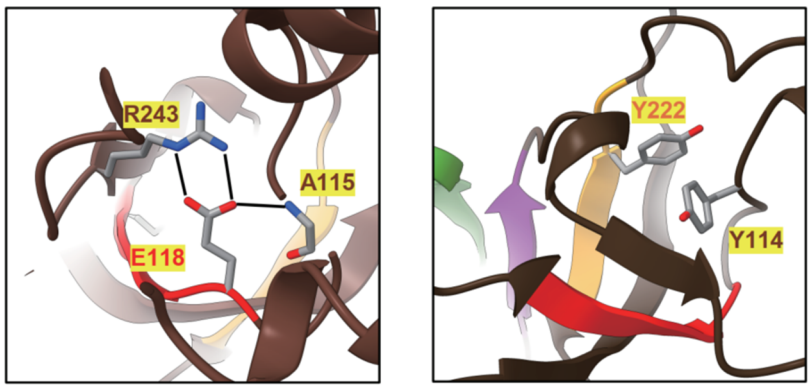
<Figure S3j>



<Figure S3j legend>

(j) ATAD5 YFAA fails to interact with BAZ1B. Anti-FLAG-immunoprecipitation was performed with cells expressing either wild-type or mutant FLAG-ATAD5.

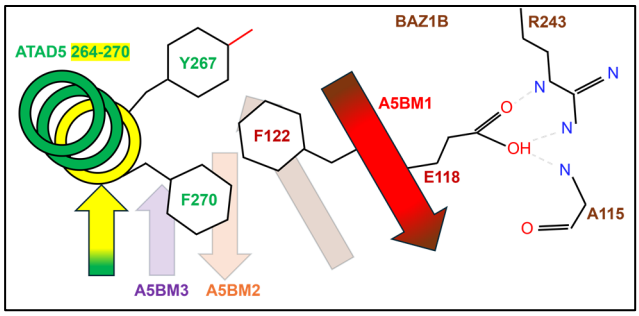
<Figure S3k>



<Figure S3k legend>

(k) The BAZ1B motifs A5BM1, A5BM2, and A5BM3 constitute the ATAD5 binding module and support the ATAD5-BAZ1B interaction. The Y222 residue in the BAZ1B A5BM2 motif forms a T-shaped π-π stacking interaction with the Y114 residue in BAZ1B, stabilizing the positioning of the A5BM1 motif. The BAZ1B E118 residue stabilizes the orientation of the β-sheet-based motif toward ATAD5.

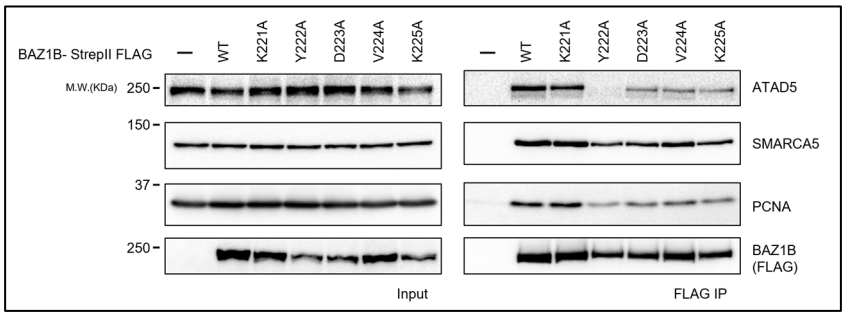
<Figure R1>: This figure is not included in the revised manuscript.



<Figure R1 legend>

BAZ1B A5BM1 is crucial for the interaction with ATAD5. The BAZ1B F122 residue forms a π-π staggered stacking interaction with the ATAD5 Y267 and F270 residues. The BAZ1B E118 residue stabilizes the orientation of the β-sheet-based motif toward ATAD5.

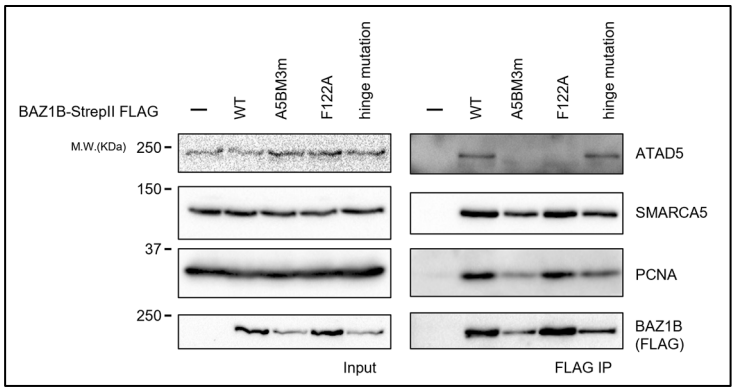
<Figure S3l>



<Figure S3l legend>

(l) The BAZ1B Y222A mutant failed to interact with ATAD5. Anti-FLAG-immunoprecipitation was performed with cells expressing either wild-type or mutant StrepII-FLAG-BAZ1B.

<Figure S3m>



<Figure S3m legend>

(m) The BAZ1B F122A mutation and A5BM3 mutant (^{231}KIIS^{234} to AAAA) fail to interact with ATAD5. The BAZ1B hinge mutant (^{228}NED^{230} to AAA) does not affect the ATAD5-BAZ1B interaction. Anti-FLAG-immunoprecipitation was performed with cells expressing either wild-type or mutant StrepII-FLAG-BAZ1B.

<Main Text>: Pages 6-7, lines 179-198

To further investigate the ATAD5-BAZ1B interaction, we conducted structural prediction analysis using AlphaFold2 (Figure S3g). The predictions suggest that the ATAD5 region encompassing residues 260-270 is critical for binding to BAZ1B. The BAZ1B binding region and the ATAD5 binding domain form a 4-stranded antiparallel structure. Notably, residues Y267 and F270 in the ATAD5 B1 motif engage in aromatic π-π interactions with the BAZ1B F122 residue (Figure S3h). These π-π stacking interactions are crucial for protein-protein interaction [1]. The ATAD5 (Y267A, F270A) mutation disrupted its interaction with BAZ1B, similar to the B1m mutant (Figure S3i-j). Furthermore, the F122A mutation in BAZ1B A5BM1 abolished the interaction with ATAD5, validating our predictions (Figure 3c).

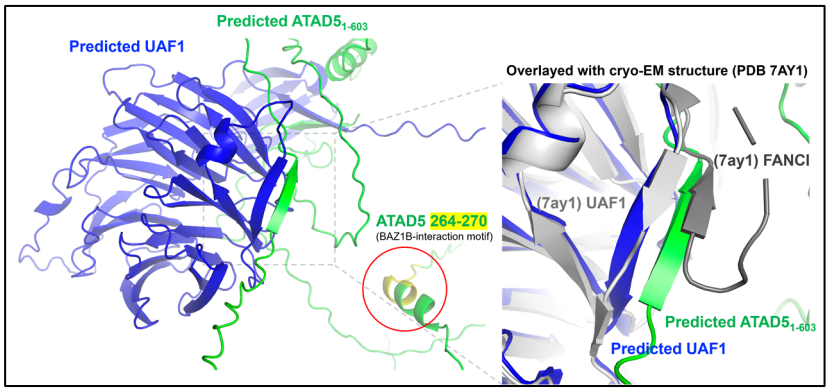
In AlphaFold2 prediction, A5BM1 and A5BM2 of BAZ1B are predicted to form β strands that constitute the ATAD5-binding domain. Another β strand, consisting of BAZ1B residues ^{231}KIIS^{234} and referred to as A5BM3, was found to align next to ATAD5 B1, forming parallel interactions (Figure S3k). Additionally, E118 in the BAZ1B A5BM1 motif stabilizes the orientation of the β-sheet-based motif towards ATAD5, strengthening the ATAD5-BAZ1B interaction. Y222 in the BAZ1B A5BM2 motif forms a T-shaped π-π stacking interaction with BAZ1B Y114, which stabilizes

the positioning of A5BM1 to support the ATAD5-BAZ1B interaction (Figure S3k). Consistent with our predictions, the E118A, Y222A, and A5BM3 AAAA mutants showed significant defects in ATAD5 interaction (Figure 3c and Figure S3b, S3l-m).

2) Prediction of USP1-UAF1-ATAD5 complex and comparison with the BAZ1B-ATAD5 complex.

Our prediction revealed that the ATAD5 binding motif of UAF1 overlaps with its FANCI interaction motif (PDB: 7ay1). The space occupied by the predicted UAF1 overlaps with that of BAZ1B. However, the predicted structure of the ATAD5 N-terminal region was too flexible to allow for a direct comparison with the BAZ1B-ATAD5 complex model (Figure R4 and R5).

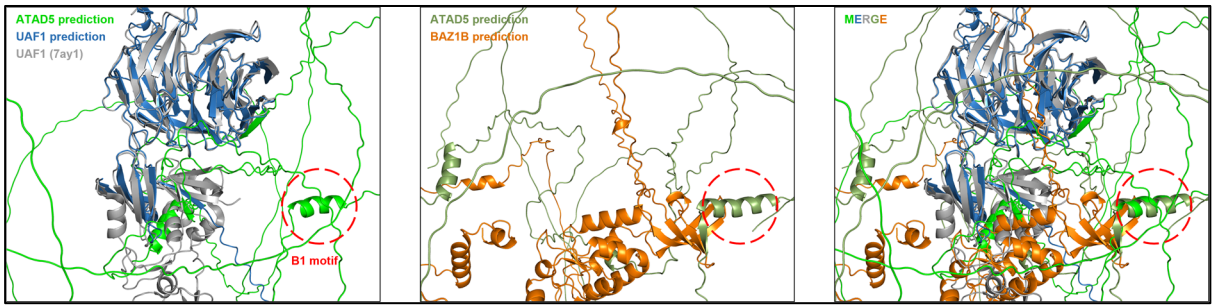
<Figure R4>: This figure is not included in the revised manuscript.



<Figure R4 legend>

The left panel shows the predicted structure of the UAF1(1-400) -ATAD5(1-603) complex generated using AlphaFold2. The right panel displays the structural overlap between the predicted UAF1-ATAD5 complex and the UAF1-FANCI complex (PDB: 7ay1).

<Figure R5>: This figure is not included in the revised manuscript.



<Figure R5 legend>

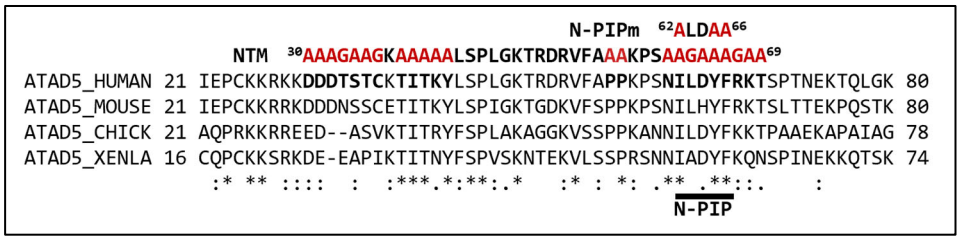
The left panel shows the predicted structure of the UAF1(1-400)- ATAD5(1-603) complex and overlayed UAF1(7ay1) as seen in Figure R4. The middle panel presents the predicted structure of the BAZ1B (1-500)- ATAD5(1-603) complex as seen in Figure S3g. The right panel displays the structural overlap at the B1 motif between the predicted UAF1-ATAD5 complex, the BAZ1B-ATAD5 complex, and UAF1(7ay1).

3) Prediction of ATAD5 PIP motifs with PCNA.

We observed that the N-terminal domain of ATAD5 largely did not form a secondary structure and had low pLDDT (predicted local distance difference test) scores, indicating low confidence (Figure S5e). Only residues 60-69 of ATAD5 showed relatively high confidence (pLDDT > 70), indicating a correct backbone prediction [3]. This region resembled the PCNA-interacting protein motif (PIP motif; 62-ILDYF-67). Furthermore, this motif is positioned in the pocket of the interdomain connecting loop (IDCL) of PCNA (Figure S5e). Based on this, we made a PIP mutant and found that it disrupted the PCNA interaction similarly to the NTM mutant (Figure S5f). Furthermore, the N-PIP mutant was defective in localization to the micro-irradiated site, similar to NTM (Figure S5g). From these analyses, we suggest that the PIP motif of ATAD5 is crucial for PCNA interaction.

In the revised manuscript, we have included additional analyses and experimental results. The main text in the results section has been updated accordingly.

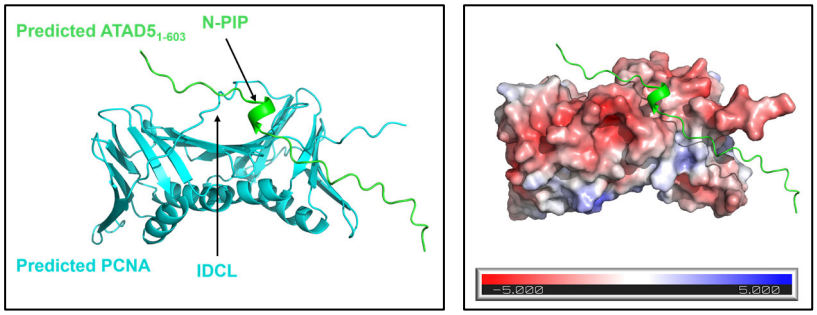
<Figure S5d>



<Figure S5d legend>

(d) The ATAD5 ^{62}IxxYF^{66} motif is conserved among species. The locations of NTM and N-PIPm mutations are indicated.

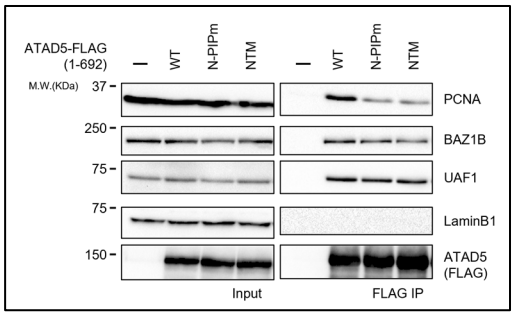
<Figure S5e>



<Figure S5e legend>

(e) Structural prediction of PCNA-bound ATAD5 N-terminal region using AlphaFold2 (Left). The electrostatic surface of PCNA was analyzed using the APBS plugin (Right). The ATAD5 N-terminal domain is predicted to contact with the PCNA IDCL.

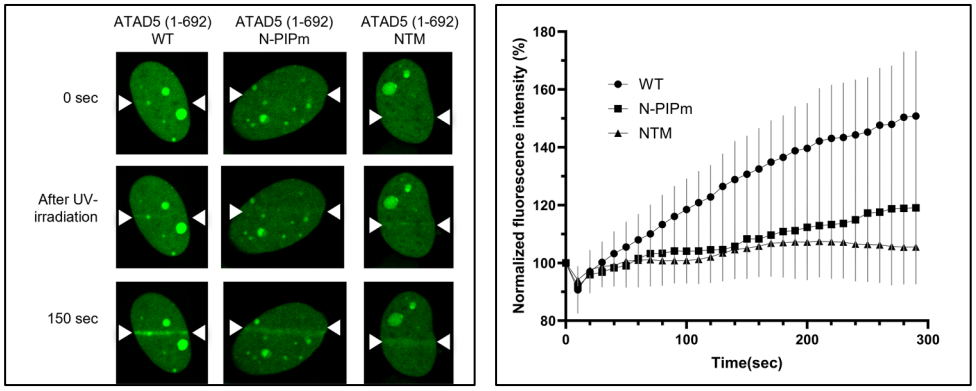
<Figure S5f>



<Figure S5f legend>

(f) ATAD5 interacts with PCNA through the N-PIP motif (residues 62-66). ATAD5-FLAG (1-692) wild-type or mutants were transiently expressed in 293T cells and immunoprecipitated using anti-FLAG beads.

<Figure S5g>



<Figure S5g legend>

(g) PCNA interaction is crucial for the localization of ATAD5 to micro-irradiated sites. ATAD5-FLAG-mNeonGreen (1-692) wild-type or the indicated mutants were transiently expressed in U2OS cells and subjected to 355 nm UV laser micro-irradiation.

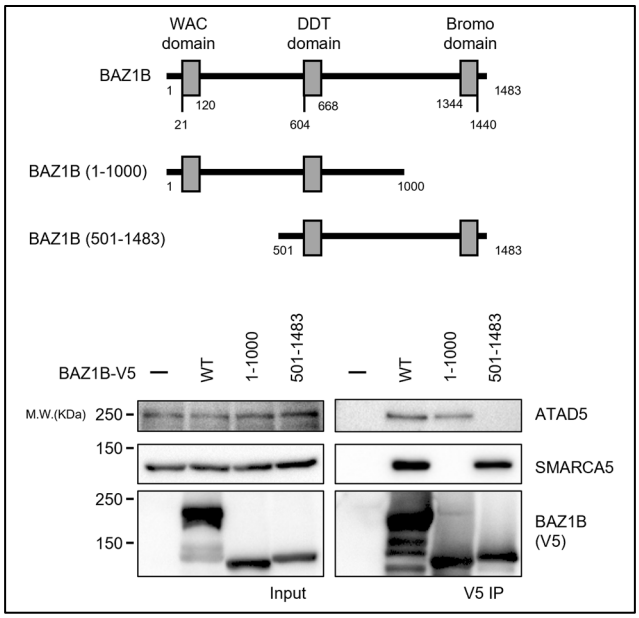
<Main Text>: Pages 8-9, lines 257-272

The far N-terminus of ATAD5 contains conserved amino acids, and mutations in these residues (referred to as far N-terminal mutations, NTM) significantly reduced localization to damage sites (Figure 4d and Figure S5d). Notably, the residues ^{61}NILDYF^{66} in the ATAD5 N-terminus resemble a predicted PCNA-interacting motif. Using AlphaFold2, we predicted the structure of the ATAD5 (1-603) bound to PCNA, revealing that ATAD5 residues ^{62}ILDYF^{66} were positioned in the binding pocket of IDCL of PCNA (Figure S5e). Based on this prediction, we generated an N-PIP mutant by substituting ^{62}ILDYF^{66} with ALDAA, finding that both the NTM and N-PIP mutant significantly reduced PCNA binding (Figure S5d, S5f). Interestingly, localization to damage sites was also significantly impaired by the N-PIP mutation (Figure S5g). RFC1 depletion prevents the loading of PCNA onto DNA and reduces the PCNA accumulation at micro-irradiation sites [4,5]. Our results showed that RFC1 depletion resulted in significant reduction in the recruitment of both ATAD5 and BAZ1B to damage sites (Figure S5h-j). These findings suggest that ATAD5 and BAZ1B are independently recruited to DNA repair sites through their interaction with PCNA.

4. Why are there doublets in the input of endogenous ATAD5 in Fig. 3A whereas no such doublets can be seen in most of the other blots ?

We believe that the doublets were caused by an issue during PAGE running. We repeated the experiment with more care and replaced the figure with new results that do not show ATAD5 doublets.

<Figure 3a>



<Figure 3a legend>

. ATAD5 binds to the N-terminal domain of BAZ1B. *Top*, Diagram of BAZ1B variants analyzed for their interaction with ATAD5. *Bottom*, the indicated BAZ1B-V5 fragments were transiently expressed, followed by V5-immunoprecipitation. SMARCA5 bound to BAZ1B (501-1483), but ATAD5 did not.

5. Details regarding the creation of the Neon-tagged constructions used in figures 4 and S4 are not provided, these should be added in a revised manuscript. Similarly the HeLa GFP-PCNA cells used in Fig S4 are not described.

We thank the reviewer for this suggestion. We have included information about the creation of Neon-tagged constructions (pages 25-26, Lines 630-638) and the HeLa GFP-PCNA, HeLa ATAD5-mNeonGreen, HeLa BAZ1B-mNeonGreen cell line in the Method section (page 24, Lines 570-579).

6. It does not appear to be mentioned in the manuscript but full MS data for the ATAD5 and BAZ1B interactomes should be provided.

We uploaded the complete mass data to a public database. The information is as follows:

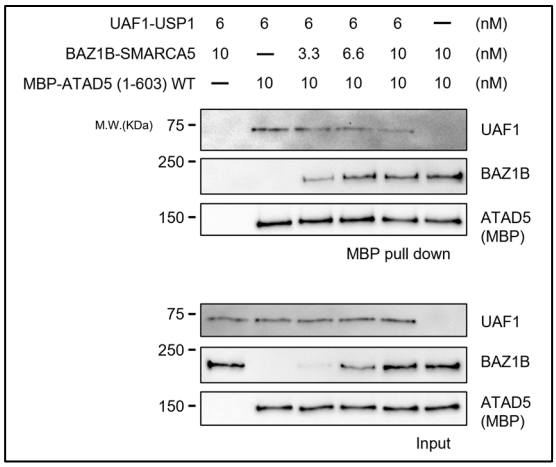
Project Name: ATAD5-BAZ1B interaction modulates PCNA ubiquitination during DNA repair
Project accession: PXD050108
Project DOI: 10.6019/PXD050108

Reviewer account details:
Username: reviewer_pxd050108@ebi.ac.uk
Password: uVre5JcX

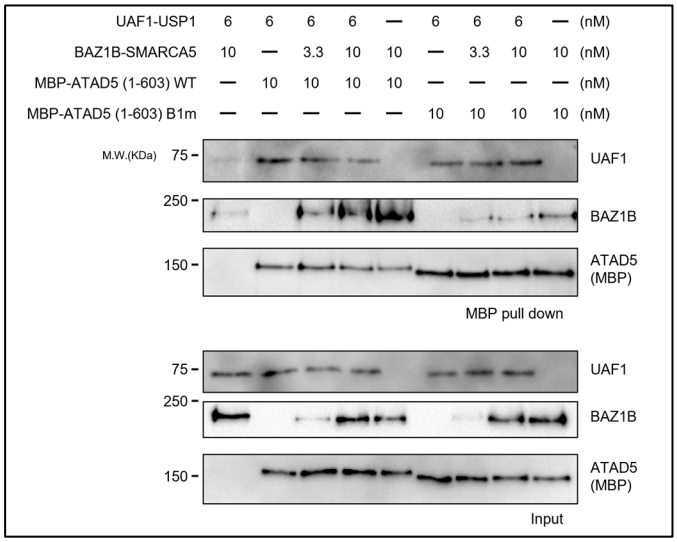
7. In fig 6B, adding more than 3.3 nM BAZ1B to the reaction does not further decrease the ATAD5-UAF1-USP1 association, why is that ? Similarly to fig 5, data quantification should be provided from 3 independent experiments.

We appreciate the reviewer's suggestion. We have now included the quantification results from experimental replicates for Figure 6b and c. We observed that increasing amounts of BAZ1B led to a decrease in the UAF1 association with ATAD5. As the reviewer pointed out, BAZ1B did not fully compete with the ATAD5-UAF1 interaction within this range. These results suggest that the ATAD5-BAZ1B interaction is not as strong as the ATAD5-UAF1 interaction. However, BAZ1B interfered with Ub-PCNA de-ubiquitination by ATAD5-UAF1-USP1 *in vitro* and in cells. Therefore, the ATAD5-BAZ1B interaction is sufficiently effective to regulate Ub-PCNA de-ubiquitination by disrupting the association between ATAD5 and UAF1-USP1.

<Figure 6b>



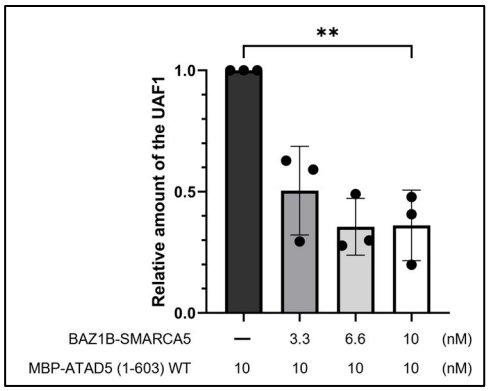
<Figure 6c>



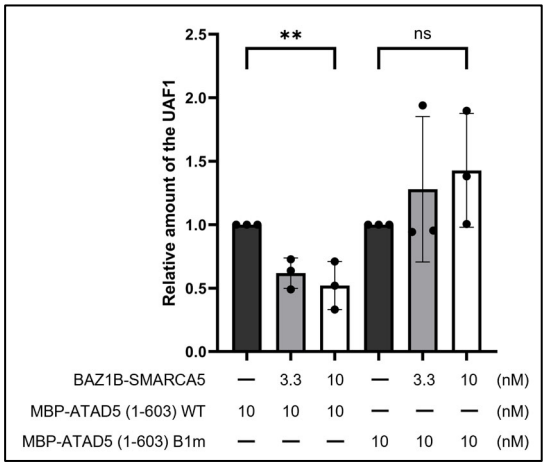
<Figure 6b-c legends>

b-c. BAZ1B-SMARCA5 interferes with the interaction between UAF1 and ATAD5. Wild-type, *(b)*, or B1m MBP-ATAD5 (1-603), *(c)*, were mixed with UAF1-USP1 and BAZ1B-SMARCA5, followed by amylose resin pull down.

<Figure S7c>



<Figure S7d>



<Figure S7c-d legend>

*(c-d) Quantified relative amounts of the UAF1 in Figure 6b or Figure 6c, respectively. Error bars represent standard deviation (n=3). Statistical analysis: Ordinary one-way ANOVA; **P≤0.001 and ns: not significant.*

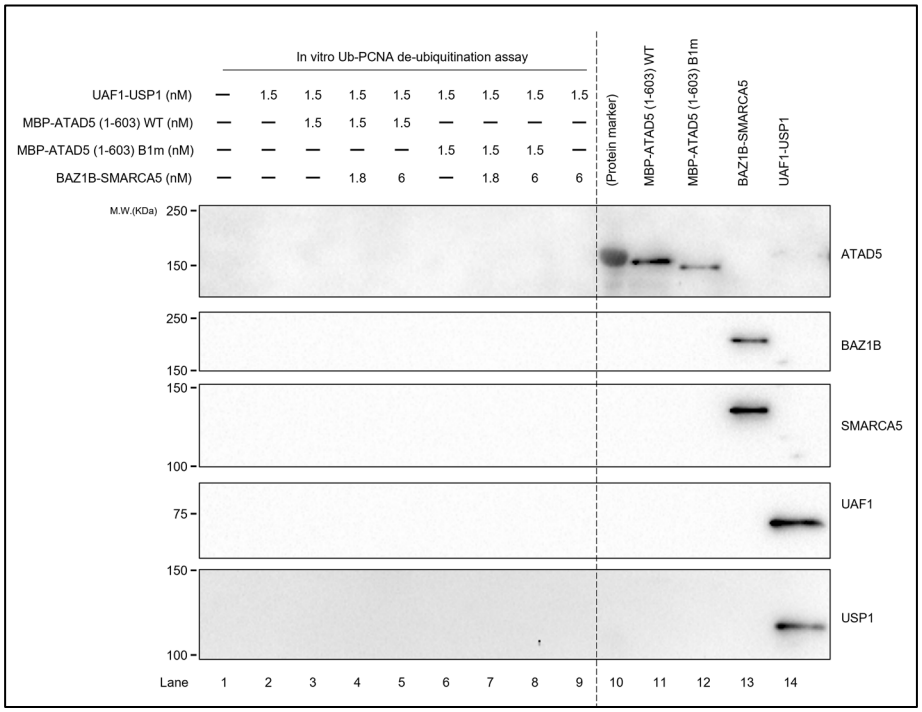
8. There is a misannotation of the 5th and 9th lanes in Fig.6C; there is no UAF1-USP1 in these samples.

We are grateful to the reviewer for his/her careful reading. We corrected the mis-annotation in Figure 6c.

9. In Fig 6D, the blots for all the other proteins apart from PCNA should be provided. Additionally, why is the B1m mutant added at 1.3 nM compared with the WT which is at 1.5 nM ? Is that a typo ?

In response to the reviewer's suggestion, we included the blots for other proteins that might remain attached to DNA after the reaction (Figure S7e). Our observations indicate that no detectable proteins remained on the DNA following the *in vitro* Ub-PCNA de-ubiquitination assay. While we cannot completely rule out the possibility that trace amounts of other proteins might persist, ATAD5, BAZ1B, and UAF1-USP1 appeared to be transiently associated with DNA-loaded PCNA in our *in vitro* reaction and were washed out afterward. Additionally, 'ATAD5 B1m 1.3 nM' was a typographical error, for which we apologize. We have corrected the figure in the revised manuscript.

<Figure S7e>



<Figure S7e legend>

(e) The UAF1-USP1 complex, ATAD5 (1-603), and the BAZ1B-SMARCA5 complex were not detected in DNA-associated fraction after the in vitro Ub-PCNA deubiquitination assay. Lanes 1-9 correspond to the same samples shown in Figure 6d. Lane 10 contains the protein marker, and Lanes 11-14 serve as positive controls.

10. Typos:

Line 4. Introduction After resolution of stalled forks, damage repair…

Line 5. 2nd page results section …we searched for the BAZ1B-interacting…

Line 18 2nd page results section …UAF1-binding domain being more important…

Throughout the text, in vivo and in vitro should be in italics

Section on ATD5 B1m cells are more sensitive to hydrogen peroxide …treatment and found increased tail moments…

Methods 1st page third line from bottom …HEK293T cells were lysed….

We apologize for these typographical errors and have corrected them.

Reviewer #4 (Remarks to the Author):

1. I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We appreciate the reviewer's careful reading.

Rebuttal reference

1. McGaughey, G.B., Gagne, M. & Rappe, A.K. pi-Stacking interactions. Alive and well in proteins. *J Biol Chem* **273**, 15458-63 (1998).
2. Kang, M.S. et al. PCNA Unloading Is Negatively Regulated by BET Proteins. *Cell Rep* **29**, 4632-4645 e5 (2019).
3. Mariani, V., Biasini, M., Barbato, A. & Schwede, T. lDDT: a local superposition-free score for comparing protein structures and models using distance difference tests. *Bioinformatics* **29**, 2722-8 (2013).
4. Shiomi, Y. & Nishitani, H. Control of Genome Integrity by RFC Complexes; Conductors of PCNA Loading onto and Unloading from Chromatin during DNA Replication. *Genes (Basel)* **8**(2017).
5. Park, S.H. et al. Short-range end resection requires ATAD5-mediated PCNA unloading for faithful homologous recombination. *Nucleic Acids Res* **51**, 10519-10535 (2023).
6. Sharif, S.B., Zamani, N. & Chadwick, B.P. BAZ1B the Protean Protein. *Genes (Basel)* **12**(2021).

Response to Reviewers for

ATAD5-BAZ1B interaction modulates PCNA ubiquitination during DNA repair

Yeongjae Kim, Na Young Ha, Mi-Sun Kang, Eunjin Ryu, Geunil Yi, Juyeong Yoo, Nalae Kang, Byung-Gyu Kim, Kyungjae Myung, and Sukhyun Kang*

*Corresponding author. Email: kangsh@ibs.re.kr

We appreciate the reviewers' positive feedback regarding our manuscript and their support for publication in Nature Communications.

Below are our responses to each of the reviewers' comments.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

"ATAD5-BAZ1B interaction modulates PCNA ubiquitination during DNA repair".

This study uncovers a novel layer of regulation at DNA repair sites and employs biochemical and cell biological means to characterise this. It, thus, provides new mechanistic insights which are valuable to the field and may ultimately lead to new drug development, as DNA repair processes are an important drug target to fight cancer.
The authors have engaged diligently with all of the reviewers' comments and returned

with an improved and strengthened manuscript. The combination of results makes a convincing case for the BAZ1B-ATAD5 interaction in DNA repair.

We appreciate the reviewer's positive remarks on our manuscript.

My only comment is that they write (lines 287 etc): "In contrast, BAZ1B depletion reduced Ub-PCNA levels following hydrogen peroxide treatment (Figure 5a and Figure S6c), whereas SMARCA5 depletion did not reduce the Ub-PCNA levels." and (lines 417 etc) "Notably, SMARCA5 depletion did not reduce Ub-PCNA levels, indicating that chromatin remodeling activity is not essential for regulating PCNA ubiquitination".

Actually, SMARCA5 depletion led to an increase of Ub-PCNA in Figure 5a. This might be explained by the fact the BAZ1B-SMARCA5 interaction affects the dynamics of BAZ1B-ATA5-Ub-PCNA interaction. While I understand that it is beyond the scope of this study to figure out exactly what is happening here, the authors should describe these findings accurately and might flag them up in the DISCUSSION as something to be examined in the future etc.

We appreciate the reviewer's suggestion. We have taken the reviewer's point into consideration and revised our manuscript as follows.

In contrast, BAZ1B depletion reduced Ub-PCNA levels following hydrogen peroxide treatment (Figure 5a and Figure S6c), whereas SMARCA5 depletion did not reduce the Ub-PCNA levels.

➔ In contrast, depletion of BAZ1B decreased Ub-PCNA levels after hydrogen peroxide treatment (Figure 5a and Figure S6c), *while SMARCA5 depletion did not decrease, but rather slightly increased Ub-PCNA levels.*

Notably, SMARCA5 depletion did not reduce Ub-PCNA levels, indicating that chromatin remodeling activity is not essential for regulating PCNA ubiquitination.

➔ *Notably, SMARCA5 depletion resulted in a slight increase in Ub-PCNA levels, in contrast to BAZ1B depletion, which decreased Ub-PCNA levels. This suggests that the interaction between BAZ1B and SMARCA5, or the chromatin remodeling activity of SMARCA5, may influence the dynamics of ATAD5 and BAZ1B during the de-ubiquitination of Ub-PCNA in the lesion bypass process. Further investigation will help elucidate the role of SMARCA5 in DNA lesion bypass.*

Reviewer #2 (Remarks to the Author):

Nature Communications
Ms: Kim et al.

This paper comes from the group that identified ATAD5 as the PCNA unloader, which was an important contribution to the field. They elaborated this discovery with a functional analysis and demonstrated that the N-terminal portion of ATAD5 (via USP1) regulates the deubiquitination of PCNA and that the C-terminus of ATAD5 contains the unloading activity. They also have identified an ATAD5-interacting protein, BRD4, which seems to regulate the unloading activity. Here, they wish to extend these observations by identifying another ATAD5-interacting protein, BAZ1B, that seems to regulate the deubiquitination regulatory activity of ATAD5.

This is a resubmission of a manuscript that I reviewed earlier this year. At that time, I and the other two reviewers found much to like about the paper, but we also found some shortcomings and so the paper was sent back to the authors for revision. At the very least, the authors should be commended for their appreciation of the review process. I'm pretty sure that in ~40 years of reviewing manuscripts I've never seen a 75-page long response letter. While quantity does not necessarily equal quality, the good news here is that the authors have taken the reviewers' criticisms to heart and have bolstered much of their data with repeats to statistically shore the data up or repeats to achieve better quality images. Moreover, the AlphaFold analyses are a nice new addition to the manuscript that certainly supports (however indirect and circumstantial) the authors' contentions that the interaction domains that they have identified are indeed interaction domains. My biggest original hesitation is still somewhat there: this is a solid biochemistry paper that identifies a new player in PCNA regulation and probably a new mechanistic step. It does not, however, radically alter our understanding of what PCNA is

doing during replication/repair. Whether this precludes it from being published in Nature Communication as opposed to some other journal is a pure editorial decision that is above my pay grade. In summary, I believe that the authors should be commended for their efforts to improve the manuscript, which is now, in my opinion, acceptable for publication.

We appreciate the reviewer for acknowledging our efforts to enhance the manuscript through the revision process. Thank you.

Reviewer #3 (Remarks to the Author):

The authors have provided many additional experiments and modifications to the manuscript that appropriately respond to my comments and suggestions. In cases where the experiments were unable to fully address my questions, the authors have provided rational explanations for why that is. The manuscript now more robustly supports their model and I believe that their work is now suitable for publication.

We appreciate the reviewer's positive feedback on our manuscript.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We appreciate the reviewer's careful reading.