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The SAGA complex regulates early steps in transcription via its deubiquitylase module subunit USP22

Timothy Stanek, Victoria Gennaro, Mason Tracewell, Daniela DiMarcantonio, Kristen Pauley, Sabrina Butt, Chrisopher McNair, Feng Wang, Andrew Kossenkov, Karen Knudsen, Tauseef Butt, Stephen Sykes, and Steven B McMahon
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1st Editorial Decision 22nd Jul 2019

22nd Jul 2019

Re: EMBOJ-2019-102509
USP22 regulates Mediator recruitment and PIC stability during activator-driven transcription

Dear Dr. McMahon,

Thank you for submitting your study on USP22's role in regulating Mediator recruitment for consideration by The EMBO Journal. We have now received three referee reports on your study, which are included below for your information.

As you will see, the referees overall appreciate that you propose a novel role for USP22 in activator-driven transcription by affecting recruitment of Mediator subunits. However, they also raise several conceptual and experimental concerns that would need to be resolved prior to considering the study further for publication. In addition to technical issues regarding the ChIPseq analyses and shRNA validation, referee #2 also finds that further in vitro experiments would be needed to support the proposed model. In particular experiments to demonstrate a direct role of USP22 in deubiquitination of the Mediator tail subunits and to define the role of the ubiqutination for complex recruitment would be important, as well as showing that this is the crucial role of USP22 in activator-driven transcription (points 1- 3).

Should you be able to address these key concerns as well as the additional more specific issues raised by the three referees, then we would like to invite you to prepare and submit a revised manuscript. Please note that EMBO Journal policy allows only a single round of major revision. We are aware that addressing all issues will possibly require a substantial amount of experimental work and include experiments with potentially unknown outcome. We can extend the revision time up to a total of six months in certain cases, but it is nonetheless important to clarify all key concerns at this stage. Please feel free to contact me should you have any further questions regarding the revision.

When preparing your letter of response to the referees' comments, bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, visit our website:
http://emboj.embopress.org/about#Transparent_Process

Kind regards,

Stefanie Boehm

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Editor
The EMBO Journal

Referee #1:

Review for The EMBO Journal (EMBOJ-2019-102509)
USP22 regulates Mediator recruitment and PIC stability during activator-driven transcription [EMBOJ-2019-102509]
Timothy J Stanek, Victoria J Gennaro, Kristen L Pauley, Daniela DiMarcantonio, Sabrina Butt, Chrisopher McNair, Feng Wang, Andrew Kossenkov, Tauseef Butt, Karen E Knudsen, Stephen M Sykes, and Dr. Steven B McMahon

The present work addresses the function of the SAGA DUB module in human. For several reasons, as concisely outlined in the introduction, the specific role of the human Ubp8p ortholog USP22 during regulation of gene expression has remained difficult to understand. Yet, increasing evidence shows that USP22 is frequently targeted in cancers, stressing a biological rationale for this study. To untangle the effects of USP22 as part of the SAGA chromatin-remodeling complex, the authors reside to the ER stress program for their genomic studies. This is an elegant system to functionally interrogate the effects of USP22 as part of SAGA during the transcription cycle. The manuscript is well-written, making the study rationale and implications easy to digest and appreciate. However, how the certain gene sets are defined needs further clarification, as it hampers the understanding of a crucial part of the paper.

1. Some considerations regarding inclusion of the relevant literature:

a) The SPT module is generally viewed as the structural module and this description is convenient explain to the audience the general role of the four modules, it strictly speaking not correct. Structural studies suggest that the TAF and SPT module combined form the structural core (1, 2).
b) The statement that the 90% of yeast genes that lack SAGA are instead regulated by TFIID seems to reach back to the older literature , conveying that only the yeast stress genes, which make up about 10% of the genome, are regulated by SAGA. This concept seems to be outdated/overinterpreted (3, 4). In fact, the authors do show awareness of this issue, as they carefully write later in the paragraph that " most- but not all- studies suggest that SAGA is present at activator-driven genes and absent from basally transcribed genes". Because the authors already cite the relevant literature, this sentence could be altered to no further perpetuates the classic overinterpretation of the early studies.
c) The point that the authors make regarding the layers of complexity regarding the function of the DUB module is well-taken. Their point is even further substantiated by studies in other model organisms. In yeast, mutations in Sgf73 alters the enzymatic activity without integration of USP homologs (5, 6) , and furthermore studies in Drosophila show that Nonstop, the homolog of USP22, forms independent complexes in the ATXN7 mutant. Thus, there is a wide range of defects associated with manipulation of ATXN7 homologs in multiple model organisms that affect ubiquitination in different fashions. Depending on the citation limits and writing line that the authors

chose to follow (the choice to focus on human literature might be very deliberate), these may or may not be worth mentioning.

2. Figure 1:
a) Reviewer's evaluation of the data:
The authors seem concerned that the number of USP22 peaks is lower than the number observed for GCN5 and ATXN7L3. True, in Drosophila, binding of the DUB module (peak counts, measured by Sgf11 and/or Nonstop, the homolog of USP22) exceeds by far the occupancy of the HAT and SPT module (7). The authors explain the abundance of the GCN5 peaks and ATXN peaks by their inclusion in other complexes, a valid explanation. In Drosophila, studies eliminated this confounder by using the ChIP data for the SPT module as good proxy for localization of strictly SAGA (as e.g. Spt3 is restricted to SAGA and not part of other HATs). The comparison between the prevalence of the DUB and SPT module showed that the DUB module binds more wide-spread. This data and previous studies suggested that the DUB module can bind chromatin independently of SAGA (7, 8). Though this would still argue that the number of USP22 peaks might be conflicting with the findings in drosophila, it also means that since the DUB module might bind without the HAT module, a lesser degree of overlap of GCN5 and USP22/ATAXN7L3 is expected regardless of the fact that GCN5 is incorporated in many HAT complexes. The discrepancy in ATAXN73L and USP22 signal could also be an antibody-related issue/epitope exposure issue. The absence of peaks in ChIP data remains hard to evaluate. USP22 mighty ChIP simply with lower affinity than ATAXN7, and if so, a part of the ATXN7 peaks could reflect true binding sites of the DUB module.
Hence, I believe that the 293 presented SAGA sites are the higher confidence sites, but might underrepresent the number of DUB-occupied genes in the human genome. These 293 sites contain two components of the DUB module and GCN5. That the intact SAGA complex is present at these sites is also reflected by the significant increase in promoter signal at these sites for each subunit upon ER stress induction, which is a clean observation. Thus, although this number of SAGA sites is low, it seems reliable gene set to further study the mechanism of DUB function as part of SAGA, and the reviewer sees no issue with the observation that the number of USp22 peaks is lower

b) Question:
The authors describe that around 10% of these 293 SAGA-bound promoters is part of the ER response pathway. It would be useful to have a reference of how many genes are part of this pathway, and how many are commonly induced (perhaps t Nagy et al., 2009 provides insight). Is this 10% a reasonable number, or was a near 100% of the promoters expected to belong to genes part of the ER response?

3. Figure 2:
a) no comments

4. Figure 3:
a) Panel a: What is the explanation for the double band for GRP78? Phosphorylation? Both bands increase in intensity simultaneously and I wonder what this means.
b) The authors state that the response is defective rather than delayed (for panel b), yet GFP78 bands increase in intensity when shUSP22 is added, especially at 4 and 8 hours.
c) Panel a: The authors show H2b and ub-H2B levels, along wit the drop in USP22 levels upon shRNA treatment for every timepoint. The loss of ub-H2B after shRNA treatment is variable over time. The rationale for showing this data and the implications are not discussed and raises questions. Why is the level of Ub-H2B shown when evaluating addressing the ER response? How do the authors explain the variable levels in ubiquitinated H2B over time? Are other hydrolases becoming active? Do their putative activities bias the ub-H2B measurements at ER-responsive

genes presented in the next result section?
d) Panel b: PERK is blotted. Please include where PERK is in the ER stress response pathway.

5. Figure 5:
a) Please clarify with a visual how the highlighted gene sets are defined.

'Interestingly, ER stress-induced recruitment of Pol II was sensitive to loss of USP22 at 283 genes that were either directly bound by SAGA ("Bound" group, 28 genes, Fig. 4b, upper panel) or not bound by SAGA ("Unbound" group, 255 genes, Fig. 4c, upper panel); an additional 1,149 ER stress-induced genes were insensitive to USP22 depletion (Supplementary Figure 5a).'

The way this data is subsetted from Fig 1 (if it is?) is not clear. Consequently, I have a hard time evaluating the data, which is rather crucial.

b) The unbound subset (255), are they in each replicate and each condition showing no binding for ATXN73L and USP22 (in the light of the evaluation of the data discussed at fig.1.) i.e., are these sites truly devoid of any DUB signal? (especially since there was no decrease in any USP22 sensitive, stress induced genes, whether bound or unbound)
b) ER stress increased Pol II occupancy at the promoters and coding regions of ER stress response genes (Fig. 4a). Are these genes the 29 genes that were SAGA bound and shwed an increased occupancy by SAGA upon induction of ER stress in Fig 1?
c) Are the 293 genes that are bound by SAGA (Fig.1) also the ones that lose PolII in the absence of USP22 when inducing the ER response? The numbers are very similar, but these gene sets could be independent

6. Figure 5:
a) What is the promoter type (what are the promoter elements of the stress-induced promoters? Do these follow a pattern? Do these all contain TATA boxes and are these enriched for Ohler elements? The DUB-dependent changes in transcription (expression changes in DUB mutants) of DUB target genes (promoters with DUB signal by ChIP) depends on promoter types in fly (7).

7) Figure 6:
a) PTM analysis is performed upon loss of USP22 to determine which proteins change in Ub levels upon loss of USP22. The authors describe this as USP22-dependent changes in the ubiquitin-modified proteome. Though the authors carefully word this, an uncareful reader could interpret this as a direct effect of USP22. It might be worth considering highlighting that the increased ubiquitin levels of Med 24 and RPB1 could be indirectly caused by a loss of USP22.

1. Han Y, Luo J, Ranish J, Hahn S. Architecture of the Saccharomyces cerevisiae SAGA transcription coactivator complex. EMBO J. 2014 Nov 03;33(21):2534-46.
2. Setiaputra D, Ross JD, Lu S, Cheng DT, Dong MQ, Yip CK. Conformational flexibility and subunit arrangement of the modular yeast Spt-Ada-Gcn5 acetyltransferase complex. J Biol Chem. 2015 Apr 17;290(16):10057-70.
3. Baptista T, Grunberg S, Minoungou N, Koster MJE, Timmers HTM, Hahn S, et al. SAGA Is a General Cofactor for RNA Polymerase II Transcription. Mol Cell. 2017 Sep 11.
4. Warfield L, Ramachandran S, Baptista T, Devys D, Tora L, Hahn S. Transcription of Nearly All Yeast RNA Polymerase II-Transcribed Genes Is Dependent on Transcription Factor TFIID. Mol Cell. 2017 Oct 5;68(1):118-29 e5.

5. Hsu CH, Chen YJ, Yang CN. Loss of function in SAGA deubiquitinating module caused by Sgf73 H93A mutation: A molecular dynamics study. J Mol Graph Model. 2019 Jun 6;91:112-8.
6. Yan M, Wolberger C. Uncovering the role of Sgf73 in maintaining SAGA deubiquitinating module structure and activity. J Mol Biol. 2015 Apr 24;427(8):1765-78.
7. Li X, Seidel CW, Szerszen LT, Lange JJ, Workman JL, Abmayr SM. Enzymatic modules of the SAGA chromatin-modifying complex play distinct roles in Drosophila gene expression and development. Genes Dev. 2017 Aug 01;31(15):1588-600.
8. Mohan RD, Dialynas G, Weake VM, Liu J, Martin-Brown S, Florens L, et al. Loss of Drosophila Ataxin-7, a SAGA subunit, reduces H2B ubiquitination and leads to neural and retinal degeneration. Genes Dev. 2014 Feb 01;28(3):259-72.

Referee #2:

The manuscript by Stanek TJ et al. addresses the role of the SAGA subunit USP22 during activator-driven transcription in human cells. Indeed, despite many studies, the exact roles of SAGA activities in the different steps of the transcription cycle remain poorly understood.

In summary, this study establishes that the SAGA subunits GCN5, ATXN7L3, and USP22 bind to the promoters of a subset or ER stress-induced genes. RT-PCR and RNA Pol II ChIP-seq analyses show that USP22 contributes to the transcriptional induction of several ER stress-induced genes. Although this work represents a comprehensive study of the role of SAGA activities in a model of activator-driven transcriptional induction, the involvement of SAGA in ER stress gene induction was expected from previous work (eg. Nagy et al., 2009). The authors further show that global and chromatin-associated H2B ubiquitination are not controlled by USP22 in HCT116 cells, both in basal conditions, as expected from previous studies (Atanassov et al., 2016), and upon ER stress induction.

Unexpectedly, the authors describe defects in PIC component recruitment at a few selected ER stress-induced promoters upon loss of USP22. These defects correlate with increased ubiquitination and defective recruitment of at least one subunit of the tail module of Mediator. Overall, a model is proposed in which, contrary to the role of SAGA DUB module in yeast, human USP22 of SAGA functions prior to PIC assembly and contributes to Mediator-controlled enhancer-promoter loop formation to promote transcriptional induction of specific genes upon activator binding. In conclusion, whereas the second part of the work brings interesting new results about the putative role of USP22 in the transcription cycle of a specific group of genes, many conclusions from this part of the study are based on correlative evidence. Several key experiments are essential to support the model proposed here.

Major concerns:

1. The in vivo experiments presented in Figure 6 convincingly show that several Mediator subunits, as well as the RBP1 subunit of RNA Pol II, are de-ubiquitinated in an USP22-dependent manner. However, in vitro experiments are necessary to demonstrate a direct role for USP22 in this process.

2. The manuscript would be strengthened if it included a characterization of the functional roles of MED16 and/or MED24 ubiquitination: how does ubiquitin affect Mediator tail module recruitment at enhancers specifically at these genes? Is ubiquitination controlling interaction of MED16/24 with specific activators or, alternatively, with the rest of Mediator? Is the effect of USP22 on MED16/24

recruitment (Figure 7) indeed dependent on their ubiquitination?

3. Along the same line, there are no experimental evidence supporting the statement that regulation and recruitment of Mediator tail subunits are the primary transcriptional events controlled by USP22 (Discussion). Showing that preventing de-ubiquitination of Mediator tail subunits rescues the defects in PIC assembly observed upon loss of USP22 would allow the authors to conclude this.

4. A comprehensive set of 3C experiments would elucidate whether USP22 does indeed control enhancer-promoter interactions, at which genes, whether these loops are ER stress-induced and promoted by interactions between Mediator core and tail modules.

5. Finally, it is unclear whether USP22 binds to core promoters together with core Mediator subunits, including MED1. This appears true at some promoters, for example those shown in Figure 1a, Figure 7a, as well as Suppl Figure 1, but is contradicted by the absence of correlation between USP22 and Med1 (Figure 7d). Alternatively, the authors seem to suggest that USP22 binds to enhancers together with Mediator tail module (Figure 7e and page 32: "empirical analysis of a selected subset of enhancers … reveals a correlation between USP22 and Mediator tail binding."). The confusion might stem from the fact that there are no genomic scales on the genome browser snapshots presented and no information in the text about distance between USP22- or Mediator-bound sites and TSSs. It is therefore difficult to understand which genes "retain conserved regulatory elements form yeast UAS" and what distance and orientation is defined as "adjacent" (page 28). Why was CHOP shown in Figure 1a but not in Figure 7a? Overall, it is important to clarify how enhancers from each gene were identified, at least those from Figure 1a, 7a and Suppl. Figure 1, and where they are located relative to the TSS and to USP22/MED16 binding sites.

Minor concerns:

6. What is the overlap between GCN5, USP22, and ATXN7L3 occupancy profiles? In particular, are USP22-bound promoters also occupied by GCN5 and ATXN7L3?

7. Why are the genes for which genome browser snapshots are shown in Figure 1a not in the list of 29 genes shown in Figure 1b?

8. From the RNA Pol II ChIP-seq presented here, 1149+283=1432 promoters show an increase in RNA Pol II occupancy upon ER stress and are thus presumably induced transcriptionally. How do these compare with the thapsigargin-induced transcriptome (from Bergman et al. 2018 for example)? Of these, 283 promoters are USP22-dependent, of which only 28 are bound by USP22. Do these 28 promoters correspond to the 29 genes shown in Figure 1b? What is the overlap between USP22-bound promoters upon ER stress and USP22-regulated genes, at least those analyzed here by RT-PCR? Finally, such a small overlap suggests that USP22 might be recruited by a specific activator (eg. ATF4, ATF6, or XBP1) and that some of the observed effects of USP22 are indirect. Please discuss this. For example, within these 28 genes, is there one encoding a transcription factor(s) that would activate the expression of downstream targets in successive waves?

9. Related to this point, it is surprising that USP22 contributes to the expression of only a subset of ER stress-responsive genes (~20%) but would be required for the subsequent induction of apoptosis. Rather, Figure 2 shows that shUSP22-treated cells can induce an apoptotic response in response to ER stress, although starting from a reduced initial apoptotic response. Of note, a 2-way ANOVA, rather than a Student test, should be used to calculate statistical significance when

comparing more than 2 means, which is the case in Figure 2b.

10. Details on how linear regression statistics were computed are missing from Figure 1 and 7.

11. I did not understand the following sentence, from the ChIP-seq section of Materials & Methods: "Regions with 500bp around TSS, 500kb around 3kb downstream from TSS and gene body (TSS to end) were used in the study."

12. A linear x axis should be used to represent time-course data, at least when these were plotted as XY plots with connecting lines, eg. in Figure 3b, Suppl. Figure 3b, and Suppl. Figure 4a.

13. The number of independent biological replicates used to construct many figures (N) is missing from several figure legends (eg. from Figure 2, 3, Suppl. Fig. 3, Suppl. Fig. 4).

14. Along the same line, there is no information about how many times each immunoblotting experiment was repeated.

15. Graph annotations are missing from Suppl. Figure 3c, so I cannot judge whether USP22 contributes to activator binding to target promoters, as mentioned in the text.

16. The word 'significant' is sometimes used without referring to any quantification. For example:
- Page 16, "Depletion of USP22 impaired stress-induced accumulation of Pol II at many ER stress response genes, both at the promoters and throughout the coding regions ". The effect is not visible at promoters (Figure 4b).
- Page 28, "depletion of USP22 resulted in a significant reduction of MED16 binding". Is MED16 binding level significantly different from background at unbound genes (Figure 7c)?

Additional non-essential suggestions:

17. The results obtained in AML cell lines are too preliminary to support a role of USP22 in the physiological response to ER stress. These should be complemented with ChIP analysis of USP22 to determine whether it is recruited constitutively to ER stress promoters in this context. In addition, USP22 promotes cell growth even in cells that do not require constitutive ER stress signaling to survive, so the authors should provide functional evidence that USP22 contributes to AML survival by sustaining ER stress signaling.

18. The rationale for using RT-PCR to determine the effect of USP22 on the initial activation of ATF6 is not clear. As mentioned by the authors, this event occurs post-transcriptionally and consists in ATF6 cleavage from the ER membrane and translocation into the nucleus via the Golgi. This process should be specifically examined in shUSP22-treated HCT116 cells to support this conclusion.

Referee #3:

The authors address the biology of the USP22 component of the SAGA coactivator complex. They provide evidence that USP22 is required for activator-driven trancription, using a colon cancer model, via modulating the levels of ubiquitylation of specific mediator complex proteins. Overall, I believe the work is solid. However, I have some issues with the ChIP-seq analysis and use of

shRNAs, which I address below.

Major concerns:
1. Regarding the ChIP-seq analysis. Firstly, it would be important to perform a USP22 ChIP-seq on their control versus USP22 depleted cells to ensure the specificity of their antibody. This is particularly relevant in Figure 1 where they compare the binding of USP22 to other members of the SAGA complex and don't find major overlap. Furthermore, in Figure 1, they should also provide a heatmap or tornado plot analysis of all their ChIP-seqs, showing the common target genes and specific target genes. Furthermore, they should validate these CHIP-SEQ analyses with independent ChIP-qPCRs to ensure the lack of overlap between their CHIP-seqs is not due to weak antibody enrichments or any other technical issues.
2. Regarding the use of shRNAs, I have an issue with this due to potential non-specific consequences of this approach. All experiments should have a knockdown of an unrelated control genes, and show by western its knockdown. Note that this is not the same as a control non-targetting shRNAs.

Some related comments. Although not required for this paper, in an ideal world, the authors should really be employing CRISPR knockout of USP22 to do their experiments, and have several independent clones of same. If USP22 is essential for growth, they should develop a conditional knockout strategy. The lack of such approaches weakens this study.

We appreciate the Reviewers' comments on our manuscript and their enthusiasm for our findings. Each Reviewer made specific requests for additional data and for clarification of aspects of the text. We have addressed each of the issues raised, and we hope the Editors and Reviewers agree that the additions and corrections have substantially improved the study.

We look forward to hearing your evaluation of our revised manuscript. We are, of course, happy to make additional changes if you find that they are needed before our manuscript is suitable for publication in EMBO J.

Individual comments from each Reviewer are listed below (in bold), with our response directly following each comment (in italics).

Reviewer #1:

Comments: The present work addresses the function of the SAGA DUB module in human. For several reasons, as concisely outlined in the introduction, the specific role of the human Ubp8p ortholog USP22 during regulation of gene expression has remained difficult to understand. Yet, increasing evidence shows that USP22 is frequently targeted in cancers, stressing a biological rationale for this study. To untangle the effects of USP22 as part of the SAGA chromatin-remodeling complex, the authors reside to the ER stress program for their genomic studies. This is an elegant system to functionally interrogate the effects of USP22 as part of SAGA during the transcription cycle. The manuscript is well-written, making the study rationale and implications easy to digest and appreciate.

However, how the certain gene sets are defined needs further clarification, as it hampers the understanding of a crucial part of the paper.

We appreciate the need to clarify how we defined our gene groups and have therefore created a flowchart of ChIP-seq subsetting and included it as Fig. EV3A. We now point out in the revised manuscript that ER stress-responsive genes are defined by significant increases in Pol II ChIP-seq density as a surrogate for transcriptional activity. We find that this increase in ChIP-seq signal at 2 hrs post-Thaps treatment accurately reflects increased transcription as measured by RT-PCR at later time points. If the Reviewers feel this would be valuable to include in the main body of the manuscript, we are happy to do so.

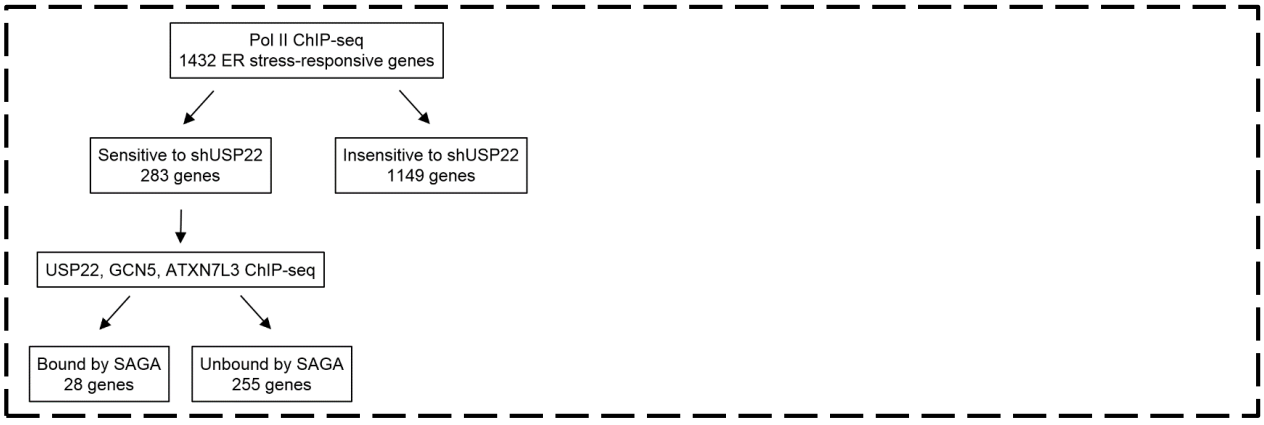


Figure EV3A, Flowchart depicting ChIP-seq subsetting.

Specific Comments:

Some considerations regarding inclusion of the relevant literature:
[C1.1] The SPT module is generally viewed as the structural module and this description is convenient explain to the audience the general role of the four modules, it strictly speaking not correct. Structural studies suggest that the TAF and SPT module combined form the structural core (1, 2).

We thank the Reviewer for this clarification and have revised our introduction to reflect the findings in the referenced studies.

[C1.2) The statement that the 90% of yeast genes that lack SAGA are instead regulated by TFIID seems to reach back to the older literature, conveying that only the yeast stress genes, which make up about 10% of the genome, are regulated by SAGA. This concept seems to be outdated/overinterpreted (3, 4). In fact, the authors do show awareness of this issue, as they carefully write later in the paragraph that " most- but not all- studies suggest that SAGA is present at activator-driven genes and absent from basally transcribed genes". Because the authors already cite the relevant literature, this sentence could be altered to no further perpetuates the classic overinterpretation of the early studies.

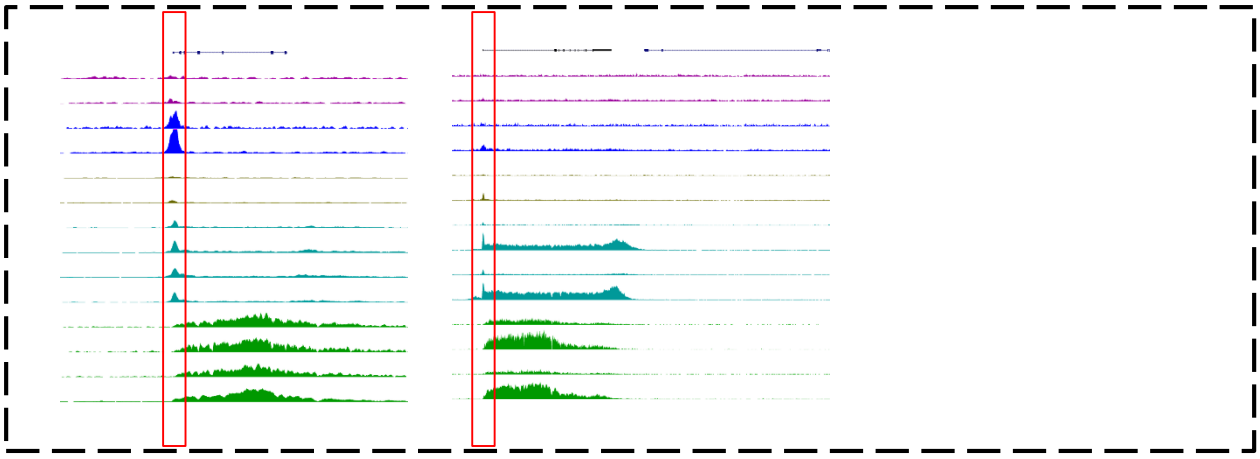
At the Reviewer's suggestion, we have revised the relevant text to account for the more recent literature surrounding SAGA-mediated gene regulation. Moreover, de-emphasis of the classical view of SAGA in gene regulation provides more context for our response to Reviewer 1's question below regarding promoter elements at SAGA-bound ER stress response genes [C1.14].

C1.3] The point that the authors make regarding the layers of complexity regarding the function of the DUB module is well-taken. Their point is even further substantiated by studies in other model organisms. In yeast, mutations in Sgf73 alters the enzymatic activity without integration of USP homologs (5, 6) , and furthermore studies in Drosophila show that Nonstop, the homolog of USP22, forms independent complexes in the ATXN7 mutant. Thus, there is a wide range of defects associated with manipulation of ATXN7 homologs in multiple model organisms that affect ubiquitination in different fashions. Depending on the citation limits and writing line that the authors chose to follow (the choice to focus on human literature might be very deliberate), these may or may not be worth mentioning.

We thank the Reviewer for this suggestion. They are correct in assuming that we chose to focus on human literature. However, we agree that inclusion of findings from other model organisms will better edify the complexity of studying SAGA-specific regulatory events. We have revised our introduction to include references to these studies.

[C1.4] Figure 1: The authors seem concerned that the number of USP22 peaks is lower than the number observed for GCN5 and ATXN7L3. True, in Drosophila, binding of the DUB module (peak counts, measured by Sgf11 and/or Nonstop, the homolog of USP22) exceeds by far the occupancy of the HAT and SPT module (7). The authors explain the abundance of the GCN5 peaks and ATXN peaks by their inclusion in other complexes, a valid explanation. In Drosophila, studies eliminated this confounder by using the ChIP data for the SPT module as good proxy for localization of strictly SAGA (as e.g. Spt3 is restricted to SAGA and not part of other HATs). The comparison between the prevalence of the DUB and SPT module showed that the DUB module binds more wide-spread. This data and previous studies suggested that the DUB module can bind chromatin independently of SAGA (7, 8).
Though this would still argue that the number of USP22 peaks might be conflicting with the findings in drosophila, it also means that since the DUB module might bind without the HAT module, a lesser degree of overlap of GCN5 and USP22/ATXN7L3 is expected regardless of the fact that GCN5 is incorporated in many HAT complexes. The discrepancy in ATXN7L3 and USP22 signal could also be an antibody-related issue/epitope exposure issue. The absence of peaks in ChIP data remains hard to evaluate. USP22 might ChIP simply with lower affinity than ATXN7, and if so, a part of the ATXN7 peaks could reflect true binding sites of the DUB module.
Hence, I believe that the 293 presented SAGA sites are the higher confidence sites, but might underrepresent the number of DUB-occupied genes in the human genome. These 293 sites contain two components of the DUB module and GCN5. That the intact SAGA complex is present at these sites is also reflected by the significant increase in promoter signal at these sites for each subunit upon ER stress induction, which is a clean observation. Thus, although this number of SAGA sites is low, it seems reliable gene set to further study the mechanism of DUB function as part of SAGA, and the reviewer sees no issue with the observation that the number of USP22 peaks is lower

To highlight the Reviewer's point here, we have included below two UCSC Genome Browser snapshots of loci that lack USP22 signal, yet contain appreciable ATXN7L3 or GCN5 binding (not included in manuscript).



As the Reviewer stated, while sites such as these may indeed represent SAGA-bound loci that warrant further exploration, our choice to include only loci bound by all three SAGA proteins allows us to utilize a set of high-confidence sites for our studies. To illustrate this, we have included as Fig. EV1D a Venn diagram depicting peak overlaps between all three SAGA factors.

***Fig. EV1D**, Venn diagram overlap of SAGA peaks prior to filtering for ER stress responsiveness as measured by increases in signal following Thaps treatment.*

Consistent with the Reviewer's observations and previous findings in Drosophila, there are many loci containing either one or both DUB module subunits but not GCN5 of the KAT module, suggesting a modular composition of SAGA, there is a higher degree of overlapping peaks between USP22 and ATXN7L3 than between USP22 and GCN5. Note that these peaks have not yet been filtered for promoter-associated ER stress peaks as detailed above.

[C1.5] Question:
The authors describe that around 10% of these 293 SAGA-bound promoters is part of the ER response pathway. It would be useful to have a reference of how many genes are part of this pathway, and how many are commonly induced (perhaps t Nagy et al., 2009 provides insight). Is this 10% a reasonable number, or was a near 100% of the promoters expected to belong to genes part of the ER response?

We apologize for the typographical errors. To clarify, in total, 1447 genes undergo an increase in Pol II occupancy following Thapsigargin treatment. Of those genes, 283 are sensitive to depletion of USP22. Within this USP22-sensitive group, only 29 promoters are directly bound by SAGA (and, as discussed in [C1.5] and [C1.11], could be termed high confidence or "DUB-strong" SAGA-bound gene promoters). As indicated above, we have included in Fig. EV3A a flowchart of the gene subsets explored in this study.

Regarding the second question, several studies have compiled lists of genes upregulated by the ER stress response. For example, 67 of 242 genes (28%) whose transcription is increased following induction of ER stress by Thapsigargin (Bergmann et al.) can be found on our list of 1447 genes. Alternatively, 229 of 876 genes (26%) upregulated by ER stress as measured by

microarray overlap our 1447 genes (Dombroski et al.). Although these overlaps appear small, it is important to note that the depth of sampling by these previous studies is limited by their technologies. In the first case, an expression array plate measured expression of only a limited set of RefSeq- and UniGene-annotated transcripts. In the second case, a microarray (Dombroski) measured PolyA-enriched transcripts only. By using changes in Pol II occupancy in the gene body as a surrogate for transcriptional activity, our data capture a wider swath of target genes across multiple annotation databases (including RefSeq and GENCODE). More importantly, however, our analysis is more sensitive to early transcription of target genes, where changes in Pol II occupancy may precede detection of stable mRNA. To this point, many of our strongest genes are defined by Dombroski et al. as members of the second wave of ER stress response transcription, yet our ChIP-seq data identify them as target genes at just 2 hours after stress induction. Our approach gives us confidence that the majority of our 1447 Thapsigargin-responsive genes are true targets of the ER stress response transcriptional program. We have revised the text to clarify these details and highlight the advantages of our approach for identifying ER stress response genes.

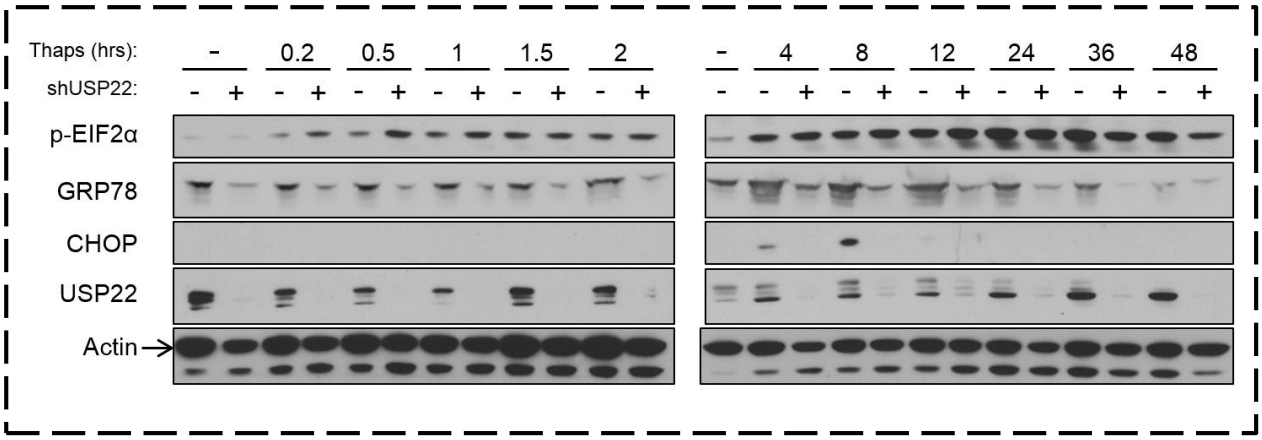
[C1.6] Figure 3, Panel a: What is the explanation for the double band for GRP78? Phosphorylation? Both bands increase in intensity simultaneously and I wonder what this means.

GRP78 (also known as BiP) is a chaperone protein that resides in the endoplasmic reticulum as well as on the cell surface. In the presence of an ER stress stimulus, unfolded proteins accumulate in the ER, leading to sequestration of GRP78 from transmembrane proteins PERK and IRE1, which then self-dimerize, autophosphorylate, and activate translation of ER stress transcription factors ATF4 and Xbp1s, respectively. GRP78 is phosphorylated and ADP-ribosylated, both of which result in its oligomerization and inactivation (Freiden et al.). The upper band we see in Figure 3A likely represents modified GRP78, as observed in Lizardo et al. Following an ER stress stimulus, the increase in total GRP78 protein can result in its proportional modification and inactivation, allowing ER stress signaling to persist. We would be happy to include a discussion of these alternate bands if the Reviewers think this would bolster the observations described in the text.

[C1.7] Figure 3, Panel b: The authors state that the response is defective rather than delayed, yet GRP78 bands increase in intensity when shUSP22 is added, especially at 4 and 8 hours.

We agree with this observation that GRP78 continues to increase following induction of ER stress, even after knockdown of USP22. However, as indicated in Figure 3C, the transcript for GRP78 increases in the shUSP22 condition, but never reaches the levels observed in the shLuc condition. To illustrate this on the protein level, we have included here immunoblots that span the entire time course detailed in Figure 3C. Probing for the GRP78 and CHOP proteins reflect the patterns observed at the transcript level, where the shUSP22 condition remains perpetually

impaired in its upregulation of these ER stress targets. Like PERK, phosphorylated (p-)EIF2α is an initial activation mark of the ER stress response and is shown here as a control for successful stimulation; it also illustrates that the pre-transcriptional response to stress is not impaired by loss of USP22. If the Reviewer finds this a helpful representation of our ER stress response data, we are happy to include it in the manuscript.



[C1.8] Figure 3, Panel a: The authors show H2b and ub-H2B levels, along with the drop in USP22 levels upon shRNA treatment for every timepoint. The loss of ub-H2B after shRNA treatment is variable over time. The rationale for showing this data and the implications are not discussed and raises questions. Why is the level of Ub-H2B shown when evaluating addressing the ER response? How do the authors explain the variable levels in ubiquitinated H2B over time? Are other hydrolases becoming active? Do their putative activities bias the ub-H2B measurements at ER-responsive genes presented in the next result section?

We apologize for the confusion here and we have revised our text related to this figure to address the presence of H2B and Ub-H2B immunoblots. We show total H2B and Ub-H2B as a demonstration that, in the untreated conditions, depletion of USP22 does not result in a global increase in H2B ubiquitylation, in line with the previous observations of Atanassov et al. Similarly, the decrease in global Ub-H2B may represent overcompensation by the alternate DUB complexes described in the same study. We attempted to address this potential compensatory action by alternate DUB complexes experimentally, but all commercially available antibodies for USP27X and USP51 were unsuccessful in ChIP-qPCR experiments. It is entirely possible that the activity of these alternative complexes affects Ub-H2B levels at other genes, but we do not believe it negatively impacts our findings. We speculate in the text that our Bound gene groups represent genes at which alternative DUB complexes cannot act, hence no change in Ub-H2B levels following USP22 knockdown (Figure 4B, lower panel). Conversely, our Unbound gene group may allow these complexes to access chromatin and compensate for the loss of USP22, hence the reduction in Ub-H2B levels (Figure 4C, lower panel). We included this speculation in our original draft, but we have revised our text to further clarify this point.

[C1.9] Figure 3, Panel b: PERK is blotted. Please include where PERK is in the ER stress response pathway.

We apologize for the lack of clarity regarding this figure. As stated above, PERK is one of the transmembrane proteins in the ER whose self-dimerization and autophosphorylation is among the initial steps in activating the ER stress response. We include PERK in several our Western blots as a marker of successful induction of ER stress, as noted by the shift in PERK migration due to autophosphorylation. We have revised the text to include this description.

[C1.10] Figure 4, Panel a: Please clarify with a visual how the highlighted gene sets are defined. 'Interestingly, ER stress-induced recruitment of Pol II was sensitive to loss of USP22 at 283 genes that were either directly bound by SAGA ("Bound" group, 28 genes, Fig. 4b, upper panel) or not bound by SAGA ("Unbound" group, 255 genes, Fig. 4c, upper panel); an additional 1,149 ER stress-induced genes were insensitive to USP22 depletion (Supplementary Figure 5a).' The way this data is subsetted from Fig 1 (if it is?) is not clear. Consequently, I have a hard time evaluating the data, which is rather crucial.

As discussed in our first response above, we have included as Fig. EV3A a flowchart of our ChIP-seq subsetting to more clearly illustrate how we defined our groups for further analysis.

[C1.11] Figure 4, Panel b: The unbound subset (255), are they in each replicate and each condition showing no binding for ATXN7L3 and USP22 (in the light of the evaluation of the data discussed at fig.1.) i.e., are these sites truly devoid of any DUB signal? (especially since there was no decrease in any USP22 sensitive, stress induced genes, whether bound or unbound)

The Reviewer raises an important point. As shown above in [C1.4], several gene promoters from the unbound subset do indeed display ChIP signal for ATXN7L3 and GCN5. In this regard, a more appropriate classification rather than "Bound" and "Unbound" might be "DUB-strong" and "DUB-weak," respectively. However, the constraints used in our DESEQ analysis to identify ER stress responsive SAGA peaks, the variation in ChIP signal across replicates for USP22, and the possibility that bound ATXN7L3 might belong to alternative DUB complexes (Atanassov et al.) prevented us assigning peaks from the "Unbound" subset to the "Bound" subset.

[C1.12] Figure 4, panel b: ER stress increased Pol II occupancy at the promoters and coding regions of ER stress response genes (Fig. 4a). Are these genes the 29 genes that were SAGA bound and shared an increased occupancy by SAGA upon induction of ER stress in Fig 1?

With the exception of XBP1s whose induction is not USP22-dependent, yes, these 28 genes are the same. We have revised the text regarding this figure to clarify the connection to Figure 1.

[C1.13] Figure 4, panel c: Are the 293 genes that are bound by SAGA (Fig.1) also the ones that lose Pol II in the absence of USP22 when inducing the ER response? The numbers are very similar, but these gene sets could be independent.

We apologize for our typographical error in the manuscript: there are 283, not 293, genes sensitive to shUSP22, 28 of which are directly bound by SAGA (Figure 1 and Bound group in Figure 4B) and 255 are not bound by SAGA (Unbound group in Figure 4C). As stated above, we have revised the text and provided a flowchart to clarify the relationship of gene groups between Figure 1 and Figure 4.

[C1.14] Figure 5: What is the promoter type (what are the promoter elements of the stress-induced promoters? Do these follow a pattern? Do these all contain TATA boxes and are these enriched for Ohler elements? The DUB-dependent changes in transcription (expression changes in DUB mutants) of DUB target genes (promoters with DUB signal by ChIP) depends on promoter types in fly (7).

We appreciate this series of questions. Across all three groups Bound, Unbound, and Insensitive, we performed motif enrichment analysis using CentriMo (Bailey and Machanick, 2012), with Ohler element sequences (Ohler et al, 2002) as motif input and regions surrounding the TSS of each target gene (-200 to + 100 bp) as input sequences. This analysis failed to identify any significantly enriched Ohler elements in any specific group. As a second tactic, we used the online promoter element detection tool ElemeNT (Sloutskin et al, 2015) to identify promoter elements across all three groups. Although multiple instances of each element were often found in a single sequence, only hits that matched known constraints relative to the TSS (Hendrix et al, 2008; Sloutskin et al, 2015; Vo Ngoc et al, 2017; Wang et al, 2017) were counted. Below is a result of this analysis. Although there are instances of a diverse set of promoter elements across all gene groups, no set of elements is significantly enriched in one specific group compared to the others. Included in this is the fact that within the 29 genes of the Bound group, only 7 genes contain TATA boxes. This particular finding may be better characterized in the context of more recent studies showing that SAGA is not limited to regulating only TATA-containing genes. If the Reviewer finds this characterization of promoter elements at SAGA-bound target genes helpful, we will include it in the manuscript.

	Promoter ElemeNT Analysis (% of group)												
Group (n)	**BRE 5'**	**TATA Box**	**BRE 3'**	**Inr**	**BBCABW Inr**	**MTE**	**DPE**	**Bridge**	**TCT**	**XCPE 1**	**XCPE 2**	**Pause**	**TFIIA RE**
Bound (29)	10.34	10.34	3.45	48.28	34.48	0.00	6.90	6.90	0.00	0.00	0.00	6.90	3.45
Unbound (255)	12.55	2.75	1.57	47.06	13.73	0.78	5.88	4.71	8.24	0.78	1.18	5.88	1.57
Insensitive (1164)	26.81	4.74	2.23	44.68	17.61	0.36	6.43	9.83	5.99	0.54	0.54	7.06	5.54

[C1.15] Figure 6: PTM analysis is performed upon loss of USP22 to determine which proteins change in Ub levels upon loss of USP22. The authors describe this as USP22-dependent changes in the ubiquitin-modified proteome. Though the authors carefully word this, an uncareful reader could interpret this as a direct effect of USP22. It might be worth considering highlighting that the increased ubiquitin levels of Med 24 and RPB1 could be indirectly caused by a loss of USP22.

We agree with the Reviewer about the importance of distinguishing direct from indirect activities of USP22 that influences ubiquitylation of Mediator and Pol II. In our initial submission, we were careful to emphasize that our data do not assign MED24 and RPB1 as direct targets of USP22.

However, in our new Ubi-Test (Fig. EV4D), we once again used Tandem Ubiquitin Binding Entities (TUBEs) to isolate ubiquitylated proteins from HCT116 cells depleted of USP22. Non-denatured eluates were then digested with either buffer, recombinant USP2 (a non-specific DUB), or hDUB module recombinantly expressed from baculoviral constructs and purified from Sf9 cells. These data show that hDUBm can directly catalyze removal of ubiquitin from MED16 and MED24, as shown by the appearance of unit length protein for each substrate. That we observe only a partial decrease in Ub from RPB1 is perhaps expected: given the greater number of altered sites of ubiquitylation on RPB1 following USP22 depletion (Figure EV4A), it is possible ubiquitylation of many of these sites are not direct targets of USP22.

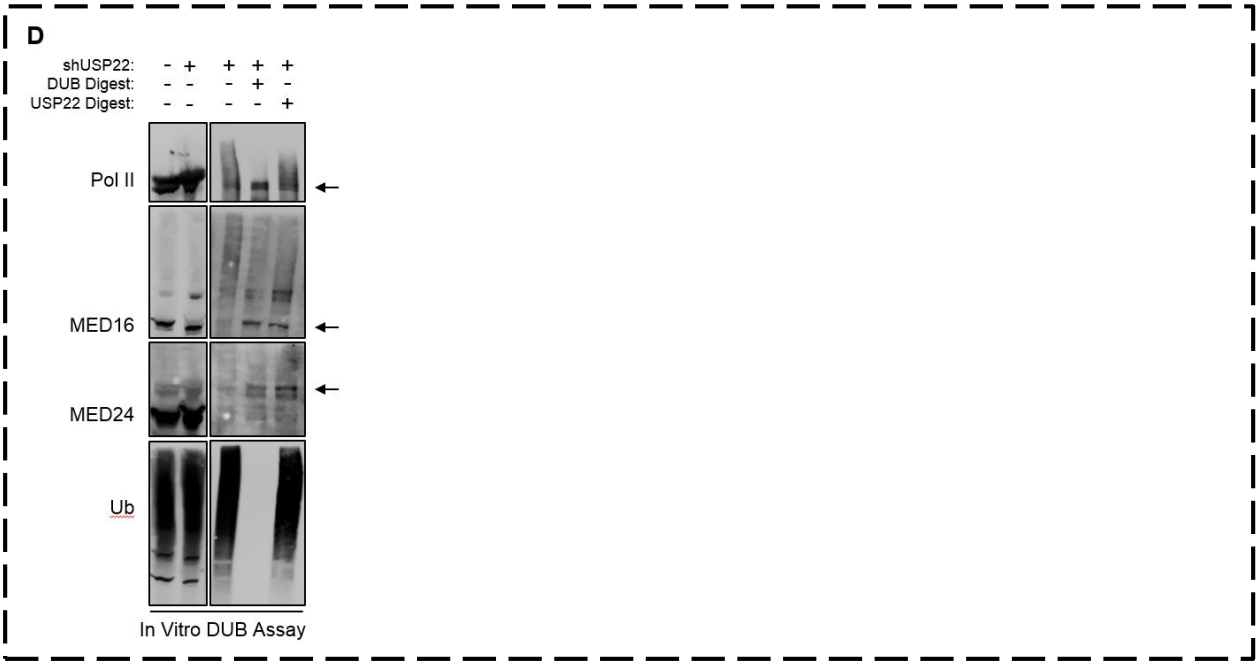


Fig. EV4D, *In vitro* deubiquitylation of endogenously ubiquitylated proteins. HCT116 cells were treated as in *C*. Ubiquitylated proteins were purified on ubiquitin binding resin and eluates were either undigested (lane 1), digested with USP2 to strip all polyubiquitin (lane 2), or digested with USP22 (lane 3) to reduce target proteins to unit length (black arrows). Digestion reactions were subjected to immunoblotting with the indicated antibodies.

In light of these new results, we can more confidently attribute direct action of USP22 to regulating ubiquitylation of Mediator and Pol II. However, we are careful to cite the limitations of our in vitro findings and make clear that this may not reflect a direct parallel in vivo.

Reviewer #2:

Comments: The manuscript by Stanek TJ et al. addresses the role of the SAGA subunit USP22 during activator-driven transcription in human cells. Indeed, despite many studies, the exact roles of SAGA activities in the different steps of the transcription cycle remain poorly understood.

In summary, this study establishes that the SAGA subunits GCN5, ATXN7L3, and USP22 bind to the promoters of a subset or ER stress-induced genes. RT-PCR and RNA Pol II ChIP-seq analyses show that USP22 contributes to the transcriptional induction of several ER stress-induced genes. Although this work represents a comprehensive study of the role of SAGA activities in a model of activator-driven transcriptional induction, the involvement of SAGA in ER stress gene induction was expected from previous work (eg. Nagy et al., 2009). The authors further show that global and chromatin-associated H2B ubiquitination are not controlled by USP22 in HCT116 cells, both in basal conditions, as expected from previous studies (Atanassov et al., 2016), and upon ER stress induction.

Unexpectedly, the authors describe defects in PIC component recruitment at a few selected ER stress-induced promoters upon loss of USP22. These defects correlate with increased ubiquitination and defective recruitment of at least one subunit of the tail module of Mediator. Overall, a model is proposed in which, contrary to the role of SAGA DUB module in yeast, human USP22 of SAGA functions prior to PIC assembly and contributes to Mediator-controlled enhancer-promoter loop formation to promote transcriptional induction of specific genes upon activator binding. In conclusion, whereas the second part of the work brings interesting new results about the putative role of USP22 in the transcription cycle of a specific group of genes, many conclusions from this part of the study are based on correlative evidence. Several key experiments are essential to support the model proposed here.

Specific comments:

Major concerns:

[C2.1] The in vivo experiments presented in Figure 6 convincingly show that several Mediator subunits, as well as the RBP1 subunit of RNA Pol II, are de-ubiquitinated in an USP22-dependent manner. However, in vitro experiments are necessary to demonstrate a direct role for USP22 in this process.

As stated above in [C1.15], our in vitro digestion of ubiquitylated substrates demonstrate the ability of USP22 to directly deubiquitylate MED16, MED24, and Pol II. We have revised our text and figures accordingly.

[C2.2] The manuscript would be strengthened if it included a characterization of the functional roles of MED16 and/or MED24 ubiquitination: how does ubiquitin affect

Mediator tail module recruitment at enhancers specifically at these genes? Is ubiquitination controlling interaction of MED16/24 with specific activators or, alternatively, with the rest of Mediator? Is the effect of USP22 on MED16/24 recruitment (Figure 7) indeed dependent on their ubiquitination?

We apologize for the confusion and agree that addressing these questions would enhance our understanding of the dependence of tail Mediator recruitment to activator-driven genes upon USP22-mediated deubiquitylation of MED16 and MED24. We made several attempts to characterize the molecular consequences of Mediator module composition and tail subunit hyperubiquitylation following depletion of USP22, including in vitro ER stress response element DNA binding assays, in vitro PIC pulldowns followed by mass spectrometry, and in vivo co-IP of various Mediator subunits spanning multiple modules. The results from these experiments were inconclusive in either supporting or refuting our hypothesis regarding USP22-mediated deubiquitylation of Mediator subunits to promote PIC stability.

Two additional limitations have impeded our progress toward directly assessing the effects of MED16/24 deubiquitylation on Mediator recruitment. First, due to the relative promiscuity of E3 ligases on target proteins, site-specific mutation of candidate lysine residues can have little effect on with regard to ubiquitylation, often requiring elimination of all known lysines in a specific protein (Jaenicke et al, 2016). Second, because the specific E3 ligase(s) responsible for initial ubiquitylation of MED16 and MED24 at our sites of interest remain unknown, we are limited in our ability to assess the biochemical consequences of subsequent deubiquitylation by USP22.

Although our results from these experiments were inconclusive, we were able to demonstrate that USP22 can directly deubiquitylate MED16 and MED24 (Fig. EV4D and [C1.15] above). Additionally, in response to [C2.4] below, we show by HiChIP analysis that USP22 participates in the stabilization of long-range promoter-enhancer loops, which are known to depend on interactions between activator and tail Mediator subunits (Jeronimo et al, 2016; Kagey et al, 2010; Phillips-Cremins et al, 2013; Robinson et al, 2016).

[C2.3] Along the same line, there are no experimental evidence supporting the statement that regulation and recruitment of Mediator tail subunits are the primary transcriptional events controlled by USP22 (Discussion). Showing that preventing de-ubiquitination of Mediator tail subunits rescues the defects in PIC assembly observed upon loss of USP22 would allow the authors to conclude this.

Although we were unable to directly connect USP22-mediated deubiquitylation of Mediator subunits to proper PIC stability, we used HiChIP sequencing to assess changes in long-range interactions between ER stress gene promoters and distal enhancers. These new data allowed us to draw further correlations between USP22-dependent changes in Mediator tail recruitment and target gene transcription. Please see our response to comment {C2.4] below for details.

[C2.4] A comprehensive set of 3C experiments would elucidate whether USP22 does indeed control enhancer-promoter interactions, at which genes, whether these loops are ER stress-induced and promoted by interactions between Mediator core and tail modules.

We agree with the Reviewer that, given the established role of the Mediator tail in bridging activator-bound enhancers with target gene promoters, assessment of long-range interactions at ER stress response gene promoters would provide insight into the potential mechanism of USP22-mediated control of tail Mediator that we hypothesize here.

As indicated above [C2.3], we performed Hi-C followed by ChIP-seq (HiChIP) for H3K4me3, a histone modification known to be found at active gene promoters. HiChIP confers practical and experimental advantages over Hi-C because it allows for targeted assessment of long-range interactions by isolating only Hi-C products containing the epitope to the ChIP antibody of choice, thus reducing the overall complexity of the dataset and reducing the number of sequencing reads required to achieve sufficient coverage across the interactome. As shown in a revised Figure 7A, several long-range interactions can be observed at the BHLHE40 promoter, a USP22-sensitive, SAGA-bound ER stress response gene. Additionally, two long range interactions change in response to ER stress, the more distal of which overlaps MED16 ChIP signal, contains an XBP1s binding site, and is deficient in forming a promoter-enhancer loop in the absence of shUSP22 (Thaps/Luc shLuc/shUSP22). To support this observation in a broader context, Figure 7B displays a heatmap of ER stress promoter-enhancer loops at the SAGA-bound group of gene promoters whose interaction frequencies increase significantly following ER stress. The right four columns of Figure 7B display the log2 enrichment score for loops across all four conditions. All transient promoter-enhancer loops that change significantly after ER stress induction are available in the new Table EV2.

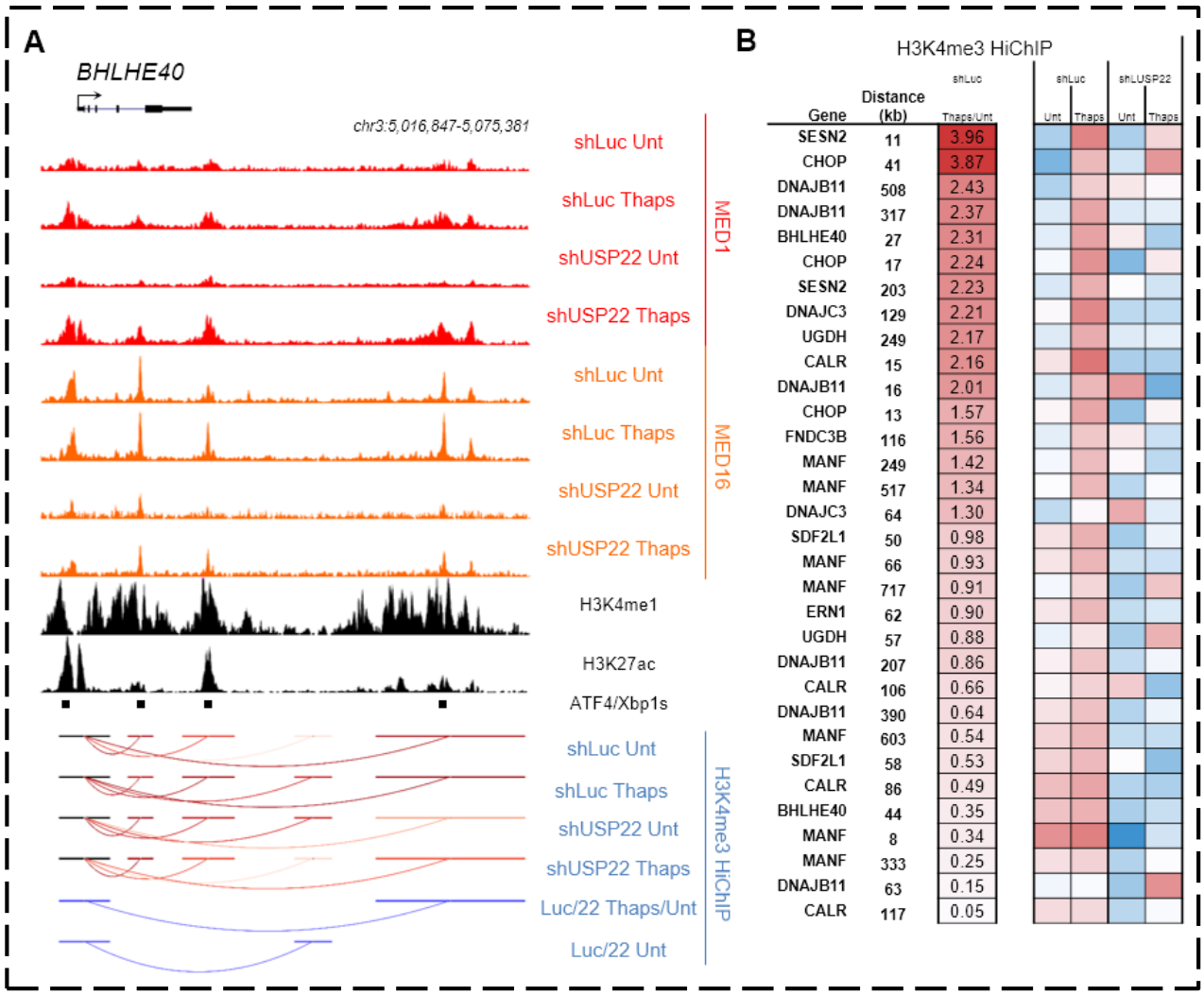


Fig. 7A, Genome browser image of ChIP-seq for middle and tail Mediator subunits and H3K4me3 HiChIP tracks before and after induction of ER stress at ER stress response gene *BHLHE40*. H3K4me1 and H3K27ac tracks are from the ENCODE Consortium (GEO accessions GSM945858 and GSM945853). ATF4/Xbp1s binding sites are from publicly available datasets (GEO accessions GSE69304 and GSE49952). **B**, H3K4me3 HiChIP signal (log2) at promoter-enhancer loops before and after induction of ER stress or USP22 depletion at indicated Bound group genes.

Overall, these new data suggest that USP22 participates in the stabilization of long-range promoter-enhancer loops, and that disruption of these interactions via depletion of USP22 correlates with deficient upregulation of target gene transcription.

[C2.5] Finally, it is unclear whether USP22 binds to core promoters together with core Mediator subunits, including MED1. This appears true at some promoters, for example

those shown in Figure 1a, Figure 7a, as well as Suppl Figure 1, but is contradicted by the absence of correlation between USP22 and Med1 (Figure 7d).

The Reviewer is correct in concluding that core Mediator binds ER stress response gene promoters together with USP22 and the rest of SAGA. In fact, MED1 ChIP signal is found at all SAGA-Bound gene promoters defined in Figure 4. Regarding Fig. 7D: these plots display the log2-fold-change in MED1 and USP22 signals following ER stress induction, not absolute ChIP signal for MED1 and USP22. The lack of correlation between changes in USP22 binding and changes in MED1 binding (Fig. 7D) is often due to the fact that MED1 is already bound to these promoters prior to ER stress. By contrast, both USP22 and MED16 are recruited to these promoters in a stress-dependent manner and to a similar extent across SAGA-bound loci (Fig. 7E). If the Reviewer feels it would clarify our findings, we are happy to revise our text to further elucidate this point.

[C2.6] Alternatively, the authors seem to suggest that USP22 binds to enhancers together with Mediator tail module (Figure 7e and page 32: "empirical analysis of a selected subset of enhancers ... reveals a correlation between USP22 and Mediator tail binding."). The confusion might stem from the fact that there are no genomic scales on the genome browser snapshots presented and no information in the text about distance between USP22- or Mediator-bound sites and TSSs. It is therefore difficult to understand which genes "retain conserved regulatory elements form yeast UAS" and what distance and orientation is defined as "adjacent" (page 28).

We apologize for the confusion regarding this figure. In light of our new HiChIP analysis demonstrating USP22-dependent changes in promoter-enhancer looping (revised Fig. 7A, 7B, and [C2.4]) and the promoter analysis requested by Reviewer 1 [C1.14], we have removed from the text our hypothesis of "conserved regulatory elements from yeast UAS". To clarify, we observe USP22 binding at target gene promoters only, where we also observe dynamic MED16 binding in response to ER stress (revised Fig. 7C); these sites of MED16 binding have been previously shown to bind ER stress response activators ATF4 and XBP1s following ER stress (Fig. 1A, 4A, 7A, and Appendix Fig. S2). As described above in [C2.5], revised Fig. 7F illustrates the correlation between changes in MED16 and USP22 binding following ER stress; this correlation was performed using the ChIP signal for both proteins at the TSS. Genomic scales have been added to all genome browser snapshots (Fig. 1A, 4A, and 7A) to aid in assessment of these data.

[C2.7] Why was CHOP shown in Figure 1a but not in Figure 7a?

In our revised Figure 7, BHLHE40 was substituted for CHOP in Figure 7 to illustrate the Mediator ChIP signal at more distal enhancer elements that overlap sites of altered promoter-enhancer looping as detected by HiChIP. The Mediator peaks at CHOP, ERP70, and GRP78

contain MED16 binding sites immediately upstream of the transcriptional start site; these latter three loci have been moved to Appendix Fig. S2.

[C2.8] Overall, it is important to clarify how enhancers from each gene were identified, at least those from Figure 1a, 7a and Suppl. Figure 1, and where they are located relative to the TSS and to USP22/MED16 binding sites.

In our original draft of Figure 7, putative enhancers for target genes were identified by overlaps of MED16, H3K27ac, and H3K4me1 located more than 5 kb distal from the TSS. However, our new H3K4me3 HiChIP analysis offers us precise identification of enhancers likely to regulate transcription of our target genes, as documented in the new Figures 7A, 7B, and Table EV2.

Minor concerns:

[C2.9] What is the overlap between GCN5, USP22, and ATXN7L3 occupancy profiles? In particular, are USP22-bound promoters also occupied by GCN5 and ATXN7L3?

As mentioned above, we have now included in a Venn diagram depicting peaks overlap between the three SAGA members (Fig. EV1D).

[C2.10] Why are the genes for which genome browser snapshots are shown in Figure 1a not in the list of 29 genes shown in Figure 1b?

We apologize for the confusion here, as several ER stress target genes are frequently identified in the literature by alternative names. Common names for DDIT3 (CHOP), PDIA4 (ERP70), and HSPA5 (GRP78, also known as BiP) are used in Fig. 1A and throughout the manuscript. For consistency, Figure 1B has been revised to display these same names.

[C2.11a] From the RNA Pol II ChIP-seq presented here, 1149+283=1432 promoters show an increase in RNA Pol II occupancy upon ER stress and are thus presumably induced transcriptionally. How do these compare with the thapsigargin-induced transcriptome (from Bergman et al. 2018 for example)?

Please see comment [C1.6] above regarding overlap between our ER stress response genes and those defined in previous studies.

[C2.11b] Of these, 283 promoters are USP22-dependent, of which only 28 are bound by USP22. Do these 28 promoters correspond to the 29 genes shown in Figure 1b?

As stated above in [C1.1], yes, this is how the SAGA-bound gene set was defined. The only SAGA-bound gene that did not display sensitivity to USP22 depletion was XBP1 (Fig EV2B), so it was excluded from the Bound group in Figures 4 and 7.

[C2.11c] What is the overlap between USP22-bound promoters upon ER stress and USP22-regulated genes, at least those analyzed here by RT-PCR?

All genes listed as USP22-bound promoters were validated as USP22-sensitive in the 48-hours Thapsigargin time course described in Fig. 3B and Appendix Fig. S4A. Additionally, FNDC3B and PAI1 (SERPINE1) are USP22-sensitive target genes within the Unbound gene group and exhibit similar defects in upregulation over time (Appendix Fig S4A).

[C2.11d] Finally, such a small overlap suggests that USP22 might be recruited by a specific activator (eg. ATF4, ATF6, or XBP1) and that some of the observed effects of USP22 are indirect. Please discuss this. For example, within these 28 genes, is there one encoding a transcription factor(s) that would activate the expression of downstream targets in successive waves?

We appreciate this observation by the Reviewer. Both ER stress response target genes CHOP and BHLHE40 code for transcription factors. CHOP itself is known to mediate transcription later in the ER stress response as cells commit to resolution of the stress versus apoptosis, which might help explain the delay in induction of apoptosis following Thapsigargin treatment (Fig. 2A-B and Appendix Fig. S3A-B). To avoid confounding effects of such secondary transcriptional events, we restricted our analyses to only 2 hrs post-induction of stress.

Additionally, in conjunction with our ChIP-seq analysis, we show at many of our "Bound" gene promoters published ChIP-seq data that demonstrate binding of ER stress response transcription factors ATF4 and Xbp1s, both of which mediate primary transcriptional events of the ER stress response. From our new HiChIP analysis, 20 of the 28 "Bound" gene promoters also form loops with distal enhancers containing ATF4/XBP1s binding sites. We have broadened our discussion of these considerations in the text.

[C2.12] Related to this point, it is surprising that USP22 contributes to the expression of only a subset of ER stress-responsive genes (~20%) but would be required for the subsequent induction of apoptosis. Rather, Figure 2 shows that shUSP22-treated cells can induce an apoptotic response in response to ER stress, although starting from a reduced initial apoptotic response. Of note, a 2-way ANOVA, rather than a Student test, should be used to calculate statistical significance when comparing more than 2 means, which is the case in Figure 2b.

As discussed in the manuscript, Thapsigargin is an irreversible inhibitor of Ca^{2+} ATPase channels that maintain homeostasis in the ER lumen. Given this, we are not surprised that USP22-depleted cells, although initially defective in their ability to undergo apoptosis, USP22-depleted cells do eventually die. To better illustrate this delay across time, we have moved our flow cytometry analyses of Annexin V/PI staining to Appendix Fig. S3A and S3B and replaced them with a finer series of time points measuring Cas3/7 cleavage, another marker of active apoptosis, over the course of prolonged ER stress (Fig. 2A and 2B). Again we observe a significant delay in the ability of USP22-depleted cells to undergo ER stress-induced apoptosis, which at later time points exhibit cleaved Cas3/7 at levels comparable to the control condition.

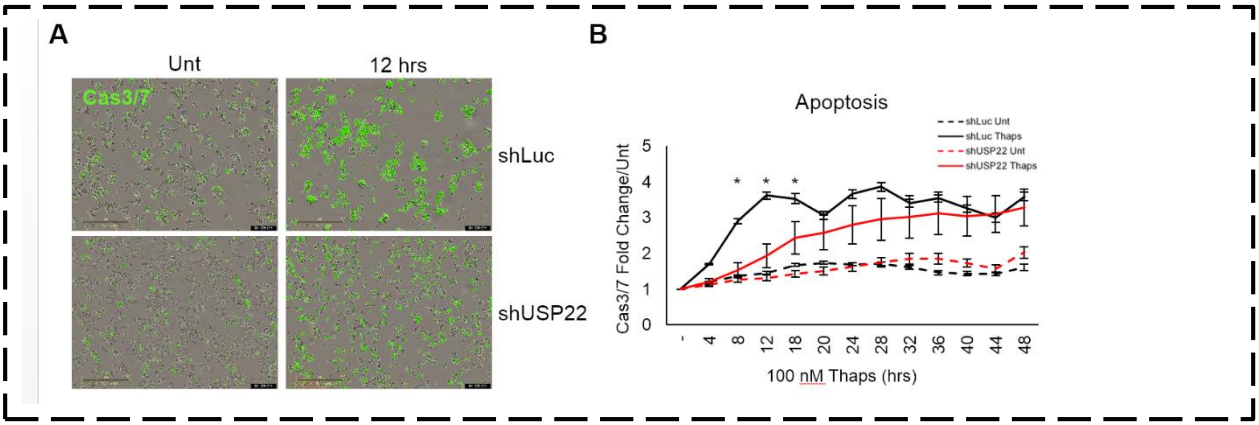


Fig. 2A, HCT116 cells were treated with 100 nM Thapsigargin for 24 hrs. Cells were stained with dye recognizing cleaved Caspases 3 and 7 and imaged every 4 hours. **B**, Quantification of apoptotic populations as indicated by Cas3/7 fluorescence from A. Data are from three biological replicates, represented as mean ± SEM. Two-way ANOVA between conditions over time ($f(3)=50.530$, $p<0.001$); significant pairwise comparisons by Tukey's HSD post-hoc assessment are indicated by *.

At the Reviewer's request, we applied two-way ANOVA analyses to these experiments followed by Tukey's HSD as post hoc assessment of pairwise comparisons. Comparisons marked statistically significant remain the same for the Annexin V/PI flow cytometry analysis (Appendix Fig. S3B).

[C2.13] Details on how linear regression statistics were computed are missing from Figure 1 and 7.

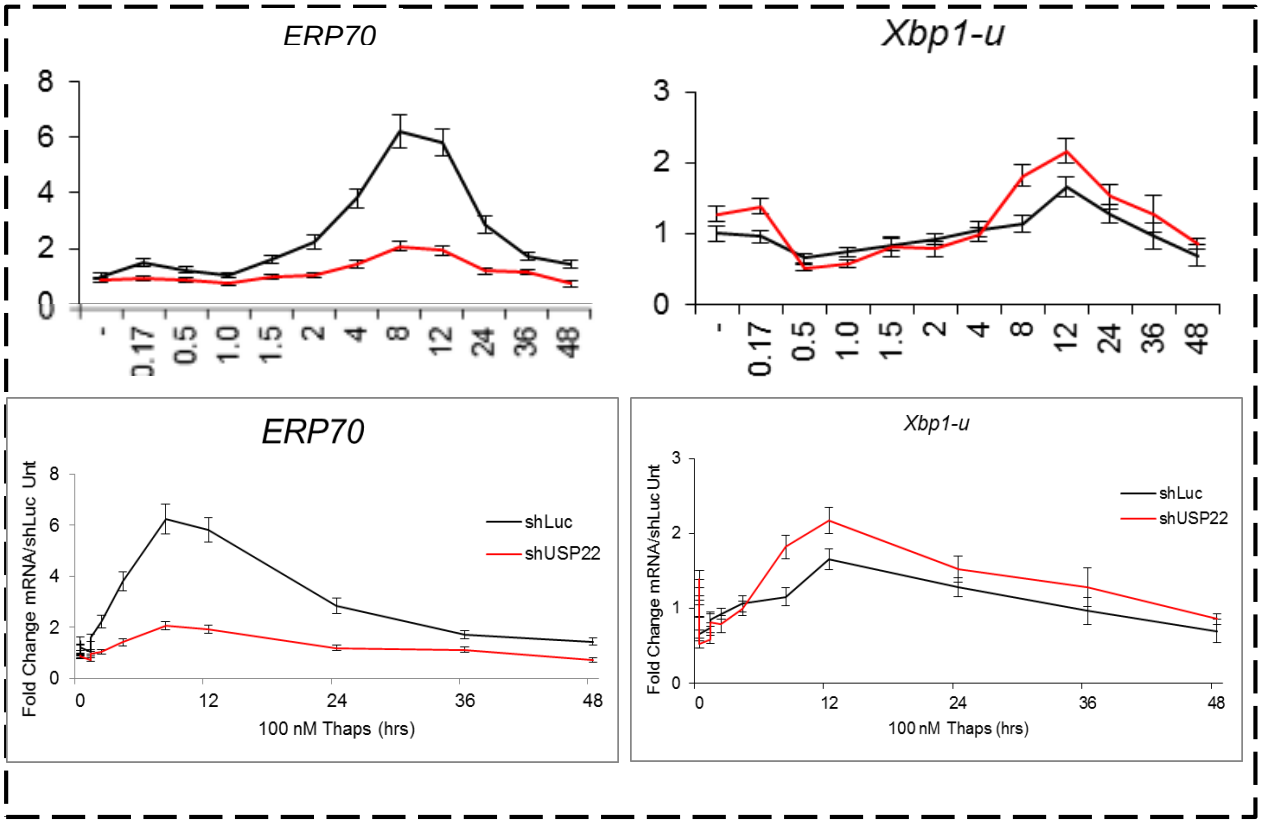
Linear regression analysis was performed in Excel using the fold-change enrichment (Thaps treated vs. Untreated) of ChIP-seq signal for designated proteins at SAGA-bound promoters. We have revised our Figure legends to include descriptions of linear regression analysis. We have also included p-values to accompany our R^2 values in the relevant Figures 1 and 7.

[C2.14] I did not understand the following sentence, from the ChIP-seq section of Materials & Methods: "Regions with 500bp around TSS, 500kb around 3kb downstream from TSS and gene body (TSS to end) were used in the study."

We apologize for the typographical error: the text has been corrected to "Regions with 500 bp around TSS, 500bp around 3kb downstream from TSS, and gene body (TSS to TES) were used in the study." These regions refer to bins used to analyze ER stress-induced changes in occupancy across all ChIP-seq experiments to determine responsiveness to Thapsigargin treatment and sensitivity to USP22 depletion.

[C2.15] A linear x axis should be used to represent time-course data, at least when these were plotted as XY plots with connecting lines, eg. in Figure 3b, Suppl. Figure 3b, and Suppl. Figure 4a.

At the request of the Reviewer, we show below two target genes across our extended time course of ER stress: on the top are the original plots, on the bottom the X axis has been modified to display a linear time scale (modified plots have not been included in the revised manuscript).



Although we agree that a linear X axis presents a more accurate representation of each gene's expression over time, the modified plots obscure critical observations in the earlier time points recorded. For example, shown here is are the original and modified plots for Xbp1-u, the

unspliced transcript of XBP1 which, prior to ER stress, contains a premature stop codon. Following induction of ER stress, Xbp1-u is quickly spliced to produce Xbp1s mRNA, which is subsequently translated in function Xbp1s protein. This loss of Xbp1-u can be observed in the original plot but is lost in the modified plot. Although we believe our original plots allow for a thorough assessment of these earlier time points with no observations lost at the later time points, if the Reviewer finds the modified plots a helpful representation of our gene expression data, we will include them in the manuscript.

[C2.16] The number of independent biological replicates used to construct many figures (N) is missing from several figure legends (eg. from Figure 2, 3, Suppl. Fig. 3, Suppl. Fig. 4).

Figure legends have been revised to indicate replicate number.

[C2.17] Along the same line, there is no information about how many times each immunoblotting experiment was repeated.

Each experiment requiring immunoblotting was performed a minimum of 2 times, but we are happy to restate this explicitly for each figure if doing so adheres to editorial guidelines.

[C2.18] Graph annotations are missing from Suppl. Figure 3c, so I cannot judge whether USP22 contributes to activator binding to target promoters, as mentioned in the text.

We apologize for this typographical error. Annotations have been added to this figure.

[C2.19] The word 'significant' is sometimes used without referring to any quantification. For example:
- Page 16, "Depletion of USP22 impaired stress-induced accumulation of Pol II at many ER stress response genes, both at the promoters and throughout the coding regions ". The effect is not visible at promoters (Figure 4b).

We apologize for the confusion regarding Fig. 4B. At this scale, impairment of Pol II recruitment at gene promoters following USP22 depletion is evident only in the Unbound group, yet it is deficient in both Bound and Unbound groups. We have included bar graphs to accompany each plot to demonstrate the fold-enrichment of ChIP-seq signal at both the TSS and gene body. We included Mann-Whitney tests of statistical significance for each region, as indicated by the p-values in the bar graphs.

[C.20] Page 28, "depletion of USP22 resulted in a significant reduction of MED16 binding". Is MED16 binding level significantly different from background at unbound genes (Figure 7c)?

MED16 binding does indeed decrease at unbound gene promoters following depletion of USP22. Our new HiChIP data documenting USP22-mediated changes in promoter-enhancer interactions at both bound and unbound ER stress response genes (Table EV2) complement our observations regarding these changes observed MED16 binding by ChIP-seq.

Additional non-essential suggestions:

[C.21] The results obtained in AML cell lines are too preliminary to support a role of USP22 in the physiological response to ER stress. These should be complemented with ChIP analysis of USP22 to determine whether it is recruited constitutively to ER stress promoters in this context. In addition, USP22 promotes cell growth even in cells that do not require constitutive ER stress signaling to survive, so the authors should provide functional evidence that USP22 contributes to AML survival by sustaining ER stress signaling.

We agree with the Reviewer that these data are too preliminary and simply wanted to show them as a second system where a well-established ER stress response necessary for survival also requires USP22. Unfortunately, recapitulating these findings in a new system is not practical at this time. Therefore, we have removed these data. If the other Reviewers feel this removal is unwarranted, we are happy to reinstate them into Appendix Fig. S3.

[C.22] The rationale for using RT-PCR to determine the effect of USP22 on the initial activation of ATF6 is not clear. As mentioned by the authors, this event occurs post-transcriptionally and consists in ATF6 cleavage from the ER membrane and translocation into the nucleus via the Golgi. This process should be specifically examined in shUSP22-treated HCT116 cells to support this conclusion.

We apologize for the confusion regarding this figure. As the Reviewer pointed out, ATF6 protein, not ATF6 transcript, is the functional unit of the ER stress response transcriptional program. We tested several antibodies against ATF6 to detect both uncleaved and cleaved species by Western blot with no success. However, that we observe no decrease in uncleaved ATF6 following ER stress indicates that our specific treatment with Thapsigargin might not stimulate ATF6 cleavage, and rather upregulation of ATF4 and XBP1s protein levels comprise the primary response in our experimental system. ATF6 transcript levels were included for the sake of completion along with transcript levels of ATF4 and both unspliced and spliced variants of XBP1. These ATF6 transcript levels indicate that the ATF6 gene is itself an ER stress response transcriptional target and behaves as such. However, as noted in our text, ATF6 protein levels remain unaffected. We have expanded our discussion of these observations in the text to clarify our findings.

Reviewer #3:

Comments: The authors address the biology of the USP22 component of the SAGA coactivator complex. They provide evidence that USP22 is required for activator-driven transcription, using a colon cancer model, via modulating the levels of ubiquitylation of specific mediator complex proteins. Overall, I believe the work is solid. However, I have some issues with the ChIP-seq analysis and use of shRNAs, which I address below.

Specific comments:

[C3.1a] Regarding the ChIP-seq analysis. Firstly, it would be important to perform a USP22 ChIP-seq on their control versus USP22 depleted cells to ensure the specificity of their antibody. This is particularly relevant in Figure 1 where they compare the binding of USP22 to other members of the SAGA complex and don't find major overlap.

Prior to performing ChIP-seq for USP22, we validated the specificity of our USP22 antibody by ChIP-qPCR. In control conditions, increased binding of USP22 can be observed at both CHOP and ERP70 promoters following induction of ER stress; this binding is ablated in cells depleted of USP22. These results have been added as Fig. EV1A and EV1B.

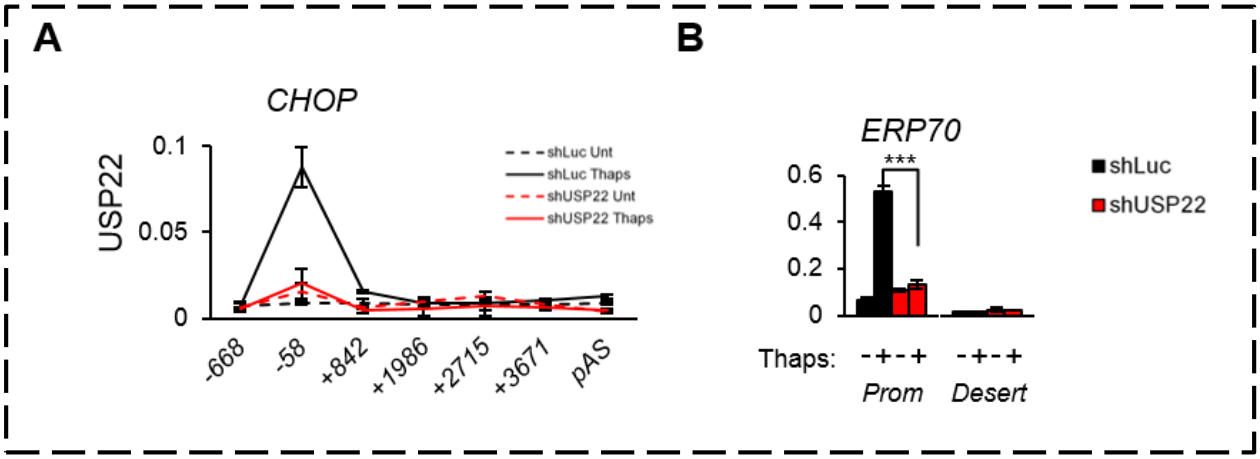
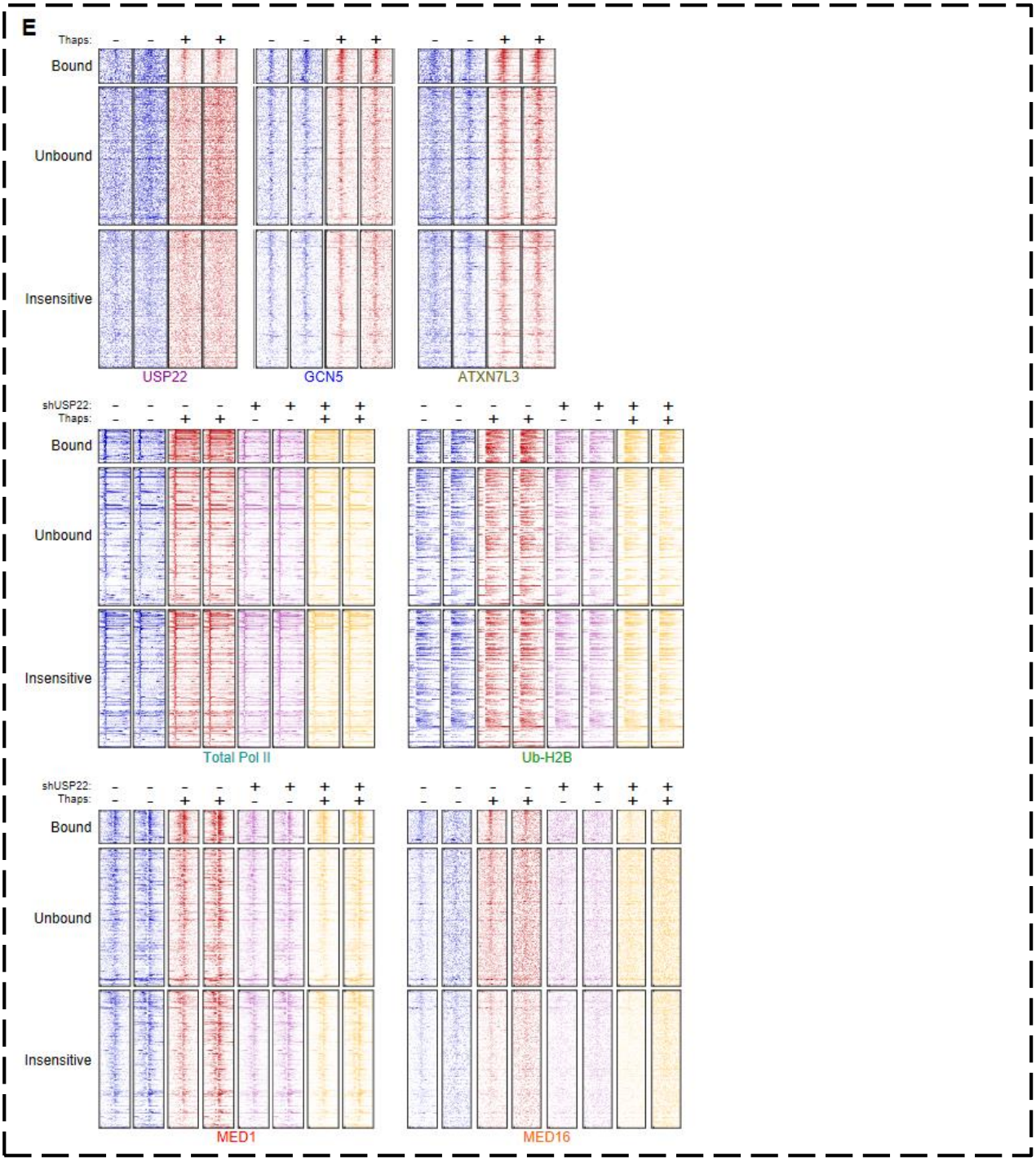


Fig. EV1A,B, ChIP-qPCR for USP22 at the CHOP and ERP70 loci before and after Thaps treatment, with and without shRNA-mediated depletion of USP22. Three independent experiments are represented as mean ± SEM; with significance measured by Student's t-test. X-axis labels on *CHOP* indicate coordinates relative to the TSS.

[C3.1b] Furthermore, in Figure 1, they should also provide a heatmap or tornado plot analysis of all their ChIP-seqs, showing the common target genes and specific target genes.

We have included as Appendix Fig. S1 tornado plots for all ChIP-seq experiments across all ER stress-responsive genes across our three defined groups: shUSP22-sensitive/SAGA-bound, shUSP22-sensitive/SAGA-unbound, and shUSP22-insensitive.



Appendix Figure S1. Related to Fig 1,4,7; SAGA and USP22 inducibly bind to the promoters region of ER stress response genes, Tornado plots of all ChIP-seq conditions from two independent experiments across Bound, Unbound, and Insensitive groups (see Fig. EV3 for description of ChIP-seq subsetting). USP22, GCN5, ATXN7L3, MED1, and MED16

tracks are centered 3 kb surrounding the TSS. Total Pol II and Ub-H2B plots span 3kb upstream to TSS to 3kb downstream of TES.

[C3.1c] Furthermore, they should validate these CHIP-SEQ analyses with independent ChIP-qPCRs to ensure the lack of overlap between their CHIP-seqs is not due to weak antibody enrichments or any other technical issues.

We have added as Fig. EV1C independent validation of USP22 and ATXN7L3 occupancy by ChIP-qPCR at genes from Bound (GRP78 and SEL1L) and Unbound (FNDC3B) groups; the GCN5 antibody we used for ChIP-seq is no longer available. After induction of ER stress with Thapsigargin, both USP22 and ATXN7L3 are recruited to GRP78 and SEL1L promoters, but not FNDC3B or a gene desert. These results are consistent with our ChIP-seq experiments in Figure 1.

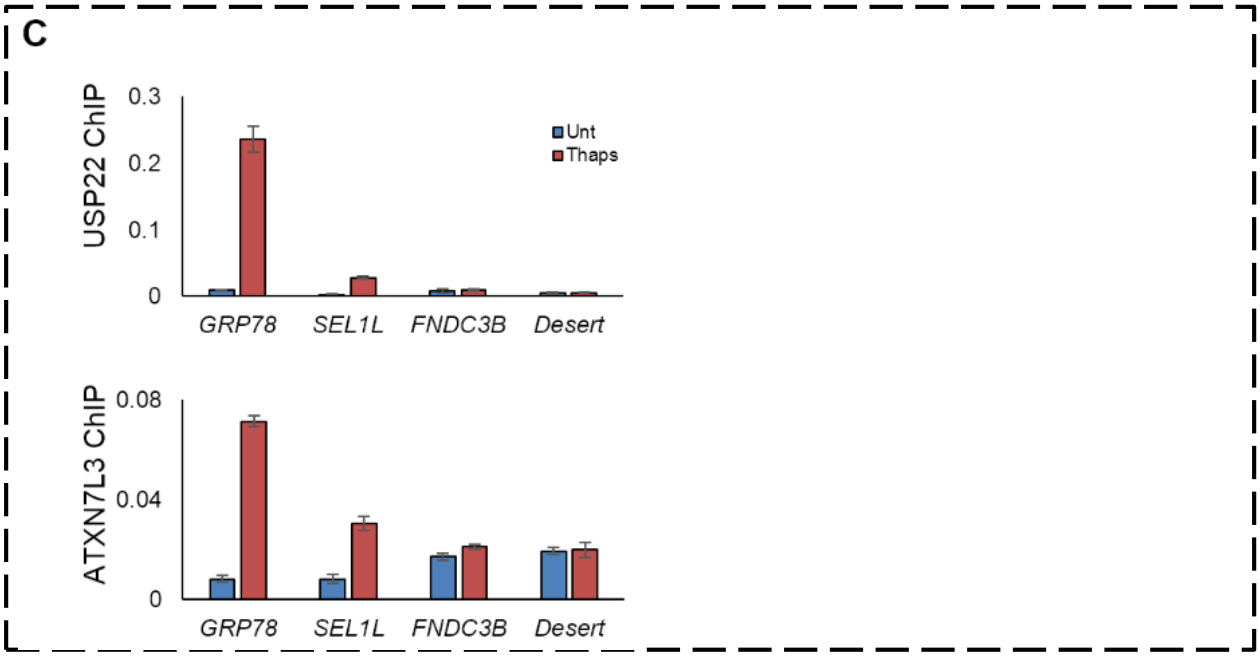


Fig. EV1C, ChIP-qPCR for USP22 and ATXN7L3 at Bound (*GRP78*, *SEL1L*) and Unbound (*FNDC3B*) genes. Three independent experiments are represented as mean ± SEM. *Desert* serves as a negative control.

[C3.2] Regarding the use of shRNAs, I have an issue with this due to potential non-specific consequences of this approach. All experiments should have a knockdown of an unrelated control genes, and show by western its knockdown. Note that this is not the same as a control non-targeting shRNAs.

To demonstrate the lack of off-target effects for our shRNAs used against USP22, we have revised Appendix Fig. S4B and S4C to display an additional control shRNA against 14-3-3-γ, which was originally included this experiment for unrelated research. Depletion of 14-3-3-γ had no effect on USP22 protein levels or induction of ER stress target genes. Additionally, although no changes in total 14-3-3 levels are observed by Western (there are six closely related

members of the 14-3-3 family, all of which are recognized by this antibody), depletion of 14-3-3-γ was validated with RT-PCR primers specific for this member (Fig. S4B, lower panel).

[C3.3] Some related comments. Although not required for this paper, in an ideal world, the authors should really be employing CRISPR knockout of USP22 to do their experiments, and have several independent clones of same. If USP22 is essential for growth, they should develop a conditional knockout strategy. The lack of such approaches weakens this study.

We agree with the Reviewer that use of CRISPR/Cas9 to completely knock out USP22 would be more specific compared to our use of shRNAs. However, all major labs in the USP22 field have encountered the issue of toxicity with constitutive knockout.

Thank you again for submitting your revised manuscript. We have now received reports from two of the original referees (see comments below). As you will see, the referees acknowledge that you have performed additional experiments and added clarifications. However, while they in principle support publication of the study, they do raise some points that should be addressed in an exceptional second round of revision. Referee #1 has a number remaining questions regarding the initial comments, as well as additional points ([extra]). These can likely all be addressed by textual edits and/or further discussion. Referee #2 is still not fully convinced by the experimental proof for the model of Mediator and Pol II subunit deubiquitination by USP22 and is concerned that a functional link to the effects of USP22 on ER stress genes activation and PIC stability is not sufficiently established. Please carefully consider the raised issues, and where additional data is available to address the points, please add this to the manuscript. For all raised issues, please revise the text and add further information and discussion as applicable. Please also provide a point-by-point response to all referee comments when submitting the revised manuscript.

Referee #1:

Review for The EMBO Journal (EMBOJ-2019-102509)
USP22 regulates Mediator recruitment and PIC stability during activator-driven transcription [EMBOJ-2019-102509]
Timothy J Stanek, Victoria J Gennaro, Kristen L Pauley, Daniela DiMarcantonio, Sabrina Butt, Chrisopher McNair, Feng Wang, Andrew Kossenkov, Tauseef Butt, Karen E Knudsen, Stephen M Sykes, and Dr. Steven B McMahon

Revised version comments Feb 2021:
It was great to read that the McMahon lab was able to address our comments. We know that is has been a hard year to work in the lab. Looking at the extra HiC experiments + how all comments have been addressed, one can see that this has been time and effort well-spent. As we mentioned already at our first revision, the authors use an elegant system to look at the roles of UPS22 during the transcription cycle, and we add to this that we are satisfied with how our initial comments have been addressed. For those initial comments that needed a decision from us, please find them listed here. In addition we recommend some textual changes and citation updates below.
[no number] Please include the workflow data subsetting graph (EV3A). It is OK if it is a supplement, whoever requires it will find it. Please see C1.4 for further comments.
[C1.1] New structural papers came out in 2020, please update the citations to include (1, 2). In response to this, there was also a wave of SAGA-related reviews that the authors could consider to include if they find them useful. Ones that come to mind are: DUB module: (3); SAGA structure (4-8). We noted that the revised version uses module names that differ from the most recent Drosophila and yeast literature (as used in these 2020 papers). However, they might still be common in Mammalia and that could be the reason why the authors stick to it. Please double check that this is the most recent nomenclature for the human SAGA modules, and if not, update it.
[C1.4] Thank you for addressing my concern regarding the categorizing & filtering. However, I still have some questions:
• The authors have included a Venn diagram EV1D. The new legend is" Venn diagram overlap of SAGA peaks prior to filtering for ER stress responsiveness as measured by increases in signal following Thaps treatment. To what signal increase do the authors refer? ChIP-seq signal for GCN5, ATXIN7L3, USP22, or the PolII seq density after ER stress induction?
• EV1D: I am confused though because the text description seems to contradict these numbers. I read:" GCN5 and ATXN7L3 peaks were identified at over 3,000 loci each, while USP22 peaks numbered only in the hundreds GCN5 and ATXN7L3 peaks were identified at over 3,000 loci each, while USP22 peaks numbered only in the hundreds." I count: GCN5 940+623+9+46. ATAXN7L3 3926+623+46+27. USP22 9+46+27+217. That is not 3000 loci for each. This needs to be explained/corrected.
• Then the text goes on to say that there are 311 shared peaks, but the diagram has only 46?

[C1.6] Thank you for explaining. The information about how GRP70 responds to ER stress and activates ATF4 and Xvp1s is very helpful. The discussion of the bands does not need to be includes specifically, but this explanation helps to understand why GFP78 and CHOP are detected in figure 3 and if would further improve the manuscript if this elaboration is included, for me the sentence " CHOP and GRP78, both central integrators of the ER stress response is a bit to concise. Also, please include the relevant citations (Freiden et al and Lizardo et al?)
[C1.7]. The explanation the authors provide in the point by point answer, and the longer time course are both helpful. Please add these sentences to the results: "Probing for the GRP78 and CHOP proteins reflect the patterns observed at the transcript level, where the shUSP22 condition remains perpetually impaired in its upregulation of these ER stress targets. Like PERK, phosphorylated (p-)EIF2α is an initial activation mark of the ER stress response and is shown here as a control for successful stimulation; it also illustrates that the pre-transcriptional response to stress is not impaired by loss of USP22." And please include this figure as supplemental figure.

[C1.14]. Thank you for taking the time to perform these additional promoter type analysis. Since no clear promoter type emerged we think it will not add much to the current data description.
[extra] We re-read the manuscript and do have some comments that we did not make before. We suggest that the authors further improve the manuscript textually by addressing these points and including citations for:

• Overall, check for a consistent use of italics Sometimes Drosophila, sometimes Drosophila
• Abstract: "proximal stages of activator-driven transcription". Especially since now HiC data is included, the term proximal for me makes me think of short-range interactions, but I believe that the aspect that is described here is one that relates to the temporal sequence, i.e. early transcription initiation events? It reads a bit as a fancy word that actually makes it harder to understand what exactly is meant.
• Introduction paragraph 1. Some citations are missing. Does the PIC contain 100 proteins? There are 8 listed. Where did this number originate from? And the sentence: " coordinates chromatin looping that brings the promoter-bound PIC into physical contact with enhancer bound activators. I know that there is a vast body of literature and it has become general knowledge, but some key citations should be provided.
• Introduction new sentence: "Moreover, USP22 contributes to the stability of long-range enhancer-promoter contacts at activated target genes." It is not clear what activation stimulus/condition is referred to here.
• Results: where the effect of the Thapsigargin is introduced on Ca2+; the sentence requires a citation. Only later these papers are cited: Furuya et al, 1994; Lytton et al, 1991.
• Results page 15: A citation is required at: " Transcript levels of the ATF6 gene itself decreased without USP22, but its initial activation as a transcription factor occurs posttranscriptionally when the ATF6 protein is cleaved from ER membrane and translocates to thenucleus via the Golgi".
• The authors inserted results regarding cleavage of ATF6. The work where such event has been observed needs to be cited. Otherwise one wonders why it should be tested/can be a potential problem for data interpretation.
• Directly after this insertion, enhancer occupancy is addressed, but it is not clear how enhancer regions were determined and why it is relevant to look at these. A short sentence addressing this would help.
• Insertion PolII. What is a strong gene? One with a strong change in gene expression, one with a lot of changes in histone mark abundance? Please rephase/ clarify the term.
• Page 15 last paragraph: Please add a citation for this event " autophosphorylation of PERK, an initial activation event of the ER stress response that precedes transcription of ER stress target genes" .
• Last result section, co-recruitment SAGA and TFIID. This lines up with other studies that came out in 2020: (9). Please include the reference.
• Discussion. At sentence" hGCN5 can be replaced within SAGA by its paralog acetyltransferase PCAF/KAT2B, and both of these enzymes exist outside SAGA as subunits of the related ATAC (Wang et al, 2008), ADA (Eberharter et al, 1999), and other complexes (Brand et al, 1999; Martinez et al, 2001)." Please include these two Drosophila citations for Ada and Chiffon complexes complexes that contain Gcn5 (10, 11).
Discussion: Add a citation to the statement: "The central underpinning for this analysis comes from comprehensive yeast studies of the USP22 ortholog Ubp8p, which deubiquitylates K123 of H2B at the 5' end of ORFs to facilitate Pol II CTD phosphorylation and transcriptional elongation.

1. Wang H, Dienemann C, Stutzer A, Urlaub H, Cheung ACM, Cramer P. Structure of the transcription coactivator SAGA. Nature. 2020.
2. Papai G, Frechard A, Kolesnikova O, Crucifix C, Schultz P, Ben-Shem A. Structure of SAGA and mechanism of TBP deposition on gene promoters. Nature. 2020.
3. Cornelio-Parra DV, Goswami R, Costanzo K, Morales-Sosa P, Mohan RD. Function and regulation of the Spt-Ada-Gcn5-Acetyltransferase (SAGA) deubiquitinase module. Biochimica et biophysica

acta Gene regulatory mechanisms. 2021;1864(2):194630.
4. Soffers JHM, Workman JL. The SAGA chromatin-modifying complex: the sum of its parts is greater than the whole. Genes Dev. 2020;34(19-20):1287-303.
5. Grant PA, Winston F, Berger SL. The biochemical and genetic discovery of the SAGA complex. Biochimica et biophysica acta Gene regulatory mechanisms. 2020:194669.
6. Cheon Y, Kim H, Park K, Kim M, Lee D. Dynamic modules of the coactivator SAGA in eukaryotic transcription. Experimental & molecular medicine. 2020.
7. Helmlinger D, Papai G, Devys D, Tora L. What do the structures of GCN5-containing complexes teach us about their function? Biochimica et biophysica acta Gene regulatory mechanisms. 2021;1864(2):194614.
8. Ben-Shem A, Papai G, Schultz P. Architecture of the multi-functional SAGA complex and the molecular mechanism of holding TBP. Febs j. 2020.
9. Donczew R, Warfield L, Pacheco D, Erijman A, Hahn S. Two roles for the yeast transcription coactivator SAGA and a set of genes redundantly regulated by TFIID and SAGA. Elife. 2020;9.
10. Soffers JHM, Li X, Saraf A, Seidel CW, Florens L, Washburn MP, et al. Characterization of a metazoan ADA acetyltransferase complex. Nucleic Acids Res. 2019.
11. Torres-Zelada EF, Stephenson RE, Alpsoy A, Anderson BD, Swanson SK, Florens L, et al. The Drosophila Dbf4 ortholog Chiffon forms a complex with Gcn5 that is necessary for histone acetylation and viability. J Cell Sci. 2018.

Referee #2:

I have now gone through the revised manuscript and appreciate the efforts made to address the reviewers' concerns. The authors clarified several important aspects of their analyses, making the manuscript easier to read. In addition, despite the circumstances, the authors were able to generate additional data that strengthen their conclusions.

Although the role of SAGA in ER-stress gene induction was already known, this work reports new, unexpected effects of USP22 on transcription initiation events. This conclusion is well supported by experimental data, including a very interesting, new HiChIP analysis. However, mechanistically, whether USP22 controls transcription initiation through Mediator and Pol II de-ubiquitination remains unclear. Less emphasis should be put on this putative mechanism in the corresponding sections, such as the abstract.

First, although I understand the argument that manipulating ubiquitination sites is inherently difficult, the lack of any genetic analysis nevertheless weakens the proposed model and the observations remain correlative.

Second, the data on Mediator and PolII de-ubiquitination by USP22 are actually weak. Figure 6 and EV4 show P-values that are above 0.05 for most identified peptides. As these were calculated using a T-test, presumably without correction for multiple comparisons, there is likely high variability between replicates. The independent assay shown in Figure 6C and EV4C is therefore critical but shows convincing results only for MED24, less so for Pol II (fuzzy bands in the last 2 lanes), and no data for MED16.
In addition, results from the in vitro DUB assays are difficult to interpret: According to the labeling, lanes 2 and 3 are identical, but they appear different (different exposures?). The pattern and intensity of Pol II look similar between lane 3 and 5. The MED16 data are more convincing but it seems that several higher-MW bands also increase upon USP2 and USP22 digestion: what are

these bands? Which band corresponds to the unmodified form of MED24 - the strong ones in lanes 1-2 or the higher-MW bands labelled with the arrow? Finally, my understanding from sequence analyses and structural studies is that Ubp8/USP22 lack the residues that are needed for ubiquitin binding, which is why it is active only in complex with the other DUB subunits. It is thus surprising that USP22 alone can catalyze MED16/24 / Pol II deubiquitination. Please explain.

Minor points:

1. P4: TRRAP is believed to represent the main activator-docking module, not TAF. Also, citing the latest yeast SAGA structures (Papai et al, Nature, 2020, and Wang et al, Nature 2020) is more relevant than Sharov et al 2017 to describe the architecture of SAGA .

2. P4: I think that "activator-driven genes" is a misleading term. All genes likely require an activator for transcription. Furthermore, there is a confusion between "present" and "regulates". The point of the Huisinga study (and many others) was that SAGA predominantly *regulates* stress-inducible genes. In fact, most ChIP-seq studies found that SAGA binds many genes, even in yeast.

3. P5: The mammalian ortholog of yeast Sgf73 is ATXN7, not ATXN7L3.

4. Results from the AML cell line have been removed according to the authors' response but this result is still mentioned at the bottom of page 5.

5. Figure 3B and EV2B: A quantitative variable, such as time, cannot be represented as a qualitative variable. The authors used a line graph to show the relation between two quantitative variables that depend on each other (expression vs time), so artificially changing the distance between two data points on the x-axis is visually misleading. If it is important to show the fate of Xbp1 splicing at early time points, though I didn't see it mentioned in the manuscript, then one can split the graph in two graphs, or highlight this part of the time-course onto a separate graph.

6. In response to [C2.5], thanks for the clarification and yes, I think that it is worth to include this explanation in the text.

7. Color scale is missing from Figure 7B.

We appreciate the Reviewers' additional comments on our revised manuscript and their enthusiasm for our findings. Each Reviewer made specific requests for inclusion of citations, additional data from our previous comments, and clarifications to the text. We have addressed each of the issues raised, and we agree that the additions and corrections suggested by the Reviewers have substantially improved the study. We hope that you will find it acceptable for publication.

Individual comments from each Reviewer are listed below (in bold), with our response directly following each comment (in italics).

Referee #1:

Revised version comments Feb 2021:
It was great to read that the McMahon lab was able to address our comments. We know that is has been a hard year to work in the lab. Looking at the extra HiC experiments + how all comments have been addressed, one can see that this has been time and effort well-spent. As we mentioned already at our first revision, the authors use an elegant system to look at the roles of UPS22 during the transcription cycle, and we add to this that we are satisfied with how our initial comments have been addressed. For those initial comments that needed a decision from us, please find them listed here. In addition we recommend some textual changes and citation updates below.

[no number] Please include the workflow data subsetting graph (EV3A). It is OK if it is a supplement, whoever requires it will find it. Please see C1.4 for further comments.

This subsetting graph is included as Fig EV3A as requested.

[C1.1] New structural papers came out in 2020, please update the citations to include (1, 2). In response to this, there was also a wave of SAGA-related reviews that the authors could consider to include if they find them useful. Ones that come to mind are: DUB module: (3); SAGA structure (4-8). We noted that the revised version uses module names that differ from the most recent Drosophila and yeast literature (as used in these 2020 papers). However, they might still be common in Mammalia and that could be the reason why the authors stick to it. Please double check that this is the most recent nomenclature for the human SAGA modules, and if not, update it.

We thank the Reviewer for bringing this to our attention and have updated our manuscript to include the most recent literature and nomenclature.

[C1.4] Thank you for addressing my concern regarding the categorizing & filtering. However, I still have some questions:
• The authors have included a Venn diagram EV1D. The new legend is" Venn diagram overlap of SAGA peaks prior to filtering for ER stress responsiveness as measured by increases in signal following Thaps treatment. To what signal increase do the authors

refer? ChIP-seq signal for GCN5, ATXN7L3, USP22, or the Pol II seq density after ER stress induction?

This "signal" refers to increases in ChIP-seq signal for GCN5, ATXN7L3, and USP22. We have revised the figure legend to clarify this point:

"Venn diagram overlap of SAGA peaks prior to filtering for ER stress responsiveness as measured by increases in SAGA subunit ChIP-seq signal following Thaps treatment."

• EV1D: I am confused though because the text description seems to contradict these numbers. I read:" GCN5 and ATXN7L3 peaks were identified at over 3,000 loci each, while USP22 peaks numbered only in the hundreds GCN5 and ATXN7L3 peaks were identified at over 3,000 loci each, while USP22 peaks numbered only in the hundreds." I count: GCN5 940+623+9+46. ATAXN7L3 3926+623+46+27. USP22 9+46+27+217. That is not 3000 loci for each. This needs to be explained/corrected.

We apologize for this textual error. We have revised the text to more accurately reflect called peaks for each SAGA subunit individually:

"GCN5 and ATXN7L3 peaks were identified at over 1,500 and 4,000 loci each, respectively, while USP22 peaks numbered only in the low hundreds."

• Then the text goes on to say that there are 311 shared peaks, but the diagram has only 46?

These 311 shared peaks were the result of lowering our threshold for identifying shared SAGA subunit binding. We have revised the relevant text to more explicitly illustrate our adjusted threshold:

"We therefore prioritized peaks likely to represent SAGA complex binding by relaxing our criteria for a SAGA binding event, identifying those shared by all three proteins in at least one replicate and at least one condition."

[C1.6] Thank you for explaining. The information about how GRP70 responds to ER stress and activates ATF4 and Xvp1s is very helpful. The discussion of the bands does not need to be includes specifically, but this explanation helps to understand why GFP78 and CHOP are detected in figure 3 and if would further improve the manuscript if this elaboration is included, for me the sentence "CHOP and GRP78, both central integrators of the ER stress response is a bit to concise. Also, please include the relevant citations (Freiden et al and Lizardo et al?)

Thank you for this recommendation. We have included in the text a brief introduction to activation of ER stress response (relevant citations included):

"In the presence of an ER stress stimulus, unfolded proteins accumulate in the ER, leading to sequestration of GRP78, a chaperone protein, from transmembrane proteins ATF6, PERK, and IRE1, the latter two of which self-dimerize, autophosphorylate (Harding et al, 1999; Sidrauski &

Walter, 1997), and activate translation of ER stress transcription factors ATF4 and Xbp1s, respectively. ATF6, itself a transcription factor, is cleaved from the ER membrane and transits through the Golgi to the nucleus (Haze et al, 1999), where in concert with ATF4 and Xbp1s, it activates ER stress response genes such as GRP78 and additional ER stress response transcription factors such as CHOP (Bergmann & Molinari, 2018; Yamamoto et al, 2007)."

[C1.7]. The explanation the authors provide in the point by point answer, and the longer time course are both helpful. Please add these sentences to the results: "Probing for the GRP78 and CHOP proteins reflect the patterns observed at the transcript level, where the shUSP22 condition remains perpetually impaired in its upregulation of these ER stress targets. Like PERK, phosphorylated (p-)EIF2α is an initial activation mark of the ER stress response and is shown here as a control for successful stimulation; it also illustrates that the pre-transcriptional response to stress is not impaired by loss of USP22." And please include this figure as supplemental figure.

We have included the extended time course Western blot as Appendix Fig S4A. We have also included the requested sentences to clarify our immunoblotting for GRP78 and CHOP as well as the initial ER stress response activation events PERK and eIF2α phosphorylation:

"Immunoblotting for the GRP78 and CHOP proteins reflect the patterns observed at the transcript level, where the shUSP22 condition remains perpetually impaired in its upregulation of these ER stress targets."

"Of note, autophosphorylation of PERK and PERK-mediated phosphorylation of translation factor EIF2a (p-)EIF2α represent initial activation events of the ER stress response that precede transcription of ER stress target genes and were probed here as controls for successful stimulation (Fig 3C and Appendix Fig S4A). Neither phosphorylation event, as observed by the shift in PERK migration or the increase in p-EIF2α band intensity, was affected by USP22 depletion, illustrating that the pre-transcriptional response to stress is not impaired by loss of USP22."

[C1.14]. Thank you for taking the time to perform these additional promoter type analysis. Since no clear promoter type emerged we think it will not add much to the current data description.

[no response required]

[extra] We re-read the manuscript and do have some comments that we did not make before. We suggest that the authors further improve the manuscript textually by addressing these points and including citations for:
• Overall, check for a consistent use of italics Sometimes Drosophila, sometimes Drosophila.

Thank you for pointing this out. We have revised all instances of "Drosophila" to be normal font.

• Abstract: "proximal stages of activator-driven transcription". Especially since now HiC data is included, the term proximal for me makes me think of short-range interactions, but I believe that the aspect that is described here is one that relates to the temporal sequence, i.e. early transcription initiation events? It reads a bit as a fancy word that actually makes it harder to understand what exactly is meant.

We agree with the Reviewer's comments about the term "proximal" and have changed the relevant instances of "proximal" to "early."

• Introduction paragraph 1. Some citations are missing. Does the PIC contain 100 proteins? There are 8 listed. Where did this number originate from?

We apologize for this error: we have revised our text and provided citations for the number of PIC subunits reported:

"The PIC consists of ~45 proteins, including the RNA polymerase II (Pol II) enzyme complex and the general transcription factors (GTFs) TFIID, TFIIA, TFIIB, TFIIF, TFIIE, and TFIIH (Duttke, 2015; Schilbach et al, 2017)."

And the sentence: "coordinates chromatin looping that brings the promoter-bound PIC into physical contact with enhancer bound activators. I know that there is a vast body of literature and it has become general knowledge, but some key citations should be provided.

We have added citations to the referenced text.

• Introduction new sentence: "Moreover, USP22 contributes to the stability of long-range enhancer-promoter contacts at activated target genes." It is not clear what activation stimulus/condition is referred to here.

We have revised this sentence to clarify our findings:

"Consistent with this, USP22 contributes to the stability of long-range enhancer-promoter contacts at ER stress-activated target genes."

• Results: where the effect of the Thapsigargin is introduced on Ca2+; the sentence requires a citation. Only later these papers are cited: Furuya et al, 1994; Lytton et al, 1991.

We have included citations for these papers at the referenced text.

• Results page 15: A citation is required at: "Transcript levels of the ATF6 gene itself decreased without USP22, but its initial activation as a transcription factor occurs post-

transcriptionally when the ATF6 protein is cleaved from ER membrane and translocates to the nucleus via the Golgi"

We have included a citation at the referenced text.

• The authors inserted results regarding cleavage of ATF6. The work where such event has been observed needs to be cited. Otherwise one wonders why it should be tested/can be a potential problem for data interpretation.

We have included a citation at the referenced text.

• Directly after this insertion, enhancer occupancy is addressed, but it is not clear how enhancer regions were determined and why it is relevant to look at these. A short sentence addressing this would help.

We have revised the text to clarify our results regarding our determination of enhancer loci shown in Fig EV2C-D:

"Moreover, binding of activators to target gene promoters and putative enhancers, determined by publicly available genome-wide binding profiles of ATF4 and Xbp1s (Chen et al, 2014; Gowen et al, 2015), was also unaffected by loss of USP22 (Fig EV2C-D)."

• Insertion Pol II. What is a strong gene? One with a strong change in gene expression, one with a lot of changes in histone mark abundance? Please rephrase/ clarify the term.

We appreciate the Reviewer's comment here and have revised our text to remove the term "strong," instead describing ER stress responsive genes identified via increased Pol II binding after Thaps treatment:

"To this point, many genes that exhibit significant increases in Pol II binding after stress induction were defined by previous studies as members of the second wave of ER stress response transcription (Bergmann et al, 2018; Dombroski et al, 2010), yet our ChIP-seq analyses identified them as target genes at just 2 hours after stress induction."

• Page 15 last paragraph: Please add a citation for this event "autophosphorylation of PERK, an initial activation event of the ER stress response that precedes transcription of ER stress target genes".

We have included a citation at the referenced text.

• Last result section, co-recruitment SAGA and TFIID. This lines up with other studies that came out in 2020: (9). Please include the reference.

We have included this citation at the referenced text.

• Discussion. At sentence" hGCN5 can be replaced within SAGA by its paralog acetyltransferase PCAF/KAT2B, and both of these enzymes exist outside SAGA as subunits of the related ATAC (Wang et al, 2008), ADA (Eberharter et al, 1999), and other complexes (Brand et al, 1999; Martinez et al, 2001)." Please include these two Drosophila citations for Ada and Chiffon complexes that contain Gcn5 (10, 11).

We have included these citations at the referenced text.

Discussion: Add a citation to the statement: "The central underpinning for this analysis comes from comprehensive yeast studies of the USP22 ortholog Ubp8p, which deubiquitylates K123 of H2B at the 5' end of ORFs to facilitate Pol II CTD phosphorylation and transcriptional elongation.

We have included a citation at the referenced text.

1. Wang H, Dienemann C, Stutzer A, Urlaub H, Cheung ACM, Cramer P. Structure of the transcription coactivator SAGA. Nature. 2020.
2. Papai G, Frechard A, Kolesnikova O, Crucifix C, Schultz P, Ben-Shem A. Structure of SAGA and mechanism of TBP deposition on gene promoters. Nature. 2020.
3. Cornelio-Parra DV, Goswami R, Costanzo K, Morales-Sosa P, Mohan RD. Function and regulation of the Spt-Ada-Gcn5-Acetyltransferase (SAGA) deubiquitinase module. Biochimica et biophysica acta Gene regulatory mechanisms. 2021;1864(2):194630.
4. Soffers JHM, Workman JL. The SAGA chromatin-modifying complex: the sum of its parts is greater than the whole. Genes Dev. 2020;34(19-20):1287-303.
5. Grant PA, Winston F, Berger SL. The biochemical and genetic discovery of the SAGA complex. Biochimica et biophysica acta Gene regulatory mechanisms. 2020:194669.
6. Cheon Y, Kim H, Park K, Kim M, Lee D. Dynamic modules of the coactivator SAGA in eukaryotic transcription. Experimental & molecular medicine. 2020.
7. Helmlinger D, Papai G, Devys D, Tora L. What do the structures of GCN5-containing complexes teach us about their function? Biochimica et biophysica acta Gene regulatory mechanisms. 2021;1864(2):194614.
8. Ben-Shem A, Papai G, Schultz P. Architecture of the multi-functional SAGA complex and the molecular mechanism of holding TBP. Febs j. 2020.
9. Donczew R, Warfield L, Pacheco D, Erijman A, Hahn S. Two roles for the yeast transcription coactivator SAGA and a set of genes redundantly regulated by TFIID and SAGA. Elife. 2020;9.
10. Soffers JHM, Li X, Saraf A, Seidel CW, Florens L, Washburn MP, et al. Characterization of a metazoan ADA acetyltransferase complex. Nucleic Acids Res. 2019.
11. Torres-Zelada EF, Stephenson RE, Alpsoy A, Anderson BD, Swanson SK, Florens L, et al. The Drosophila Dbf4 ortholog Chiffon forms a complex with Gcn5 that is necessary for histone acetylation and viability. J Cell Sci. 2018.

Referee #2:

I have now gone through the revised manuscript and appreciate the efforts made to address the reviewers' concerns. The authors clarified several important aspects of their analyses, making the manuscript easier to read. In addition, despite the circumstances, the authors were able to generate additional data that strengthen their conclusions.

Although the role of SAGA in ER-stress gene induction was already known, this work reports new, unexpected effects of USP22 on transcription initiation events. This conclusion is well supported by experimental data, including a very interesting, new HiChIP analysis. However, mechanistically, whether USP22 controls transcription initiation through Mediator and Pol II de-ubiquitination remains unclear. Less emphasis should be put on this putative mechanism in the corresponding sections, such as the abstract.

First, although I understand the argument that manipulating ubiquitination sites is inherently difficult, the lack of any genetic analysis nevertheless weakens the proposed model and the observations remain correlative.

In response to feedback from both Reviewers and the Editor, we have revised our text to de-emphasize our findings regarding our identification of non-histone substrates of USP22 as a putative mechanism for USP22-mediated regulation of PIC stability.

Second, the data on Mediator and Pol II de-ubiquitination by USP22 are actually weak. Figure 6 and EV4 show P-values that are above 0.05 for most identified peptides. As these were calculated using a T-test, presumably without correction for multiple comparisons, there is likely high variability between replicates. The independent assay shown in Figure 6C and EV4C is therefore critical but shows convincing results only for MED24, less so for Pol II (fuzzy bands in the last 2 lanes), and no data for MED16.

We thank the Reviewer for his comments and agree that several Ub-peptide hits in our proteomic analysis were subject to high variability between replicates. As stated above, we have reduced our emphasis of these results within the broader context of our findings of USP22 as a regulator of early transcriptional events. In this revised context, we believe our proteomic and enzymatic assays provide sufficient evidence for MED16, MED24, and Pol II as direct substrates of USP22.

In addition, results from the in vitro DUB assays are difficult to interpret: According to the labeling, lanes 2 and 3 are identical, but they appear different (different exposures?).

We apologize for any confusion our figure labels may have caused in Fig EV4D. We have re-labeled this figure to clarify that lanes 1 and 2 are Input samples and lanes 3 through 5 are the in vitro DUB assay.

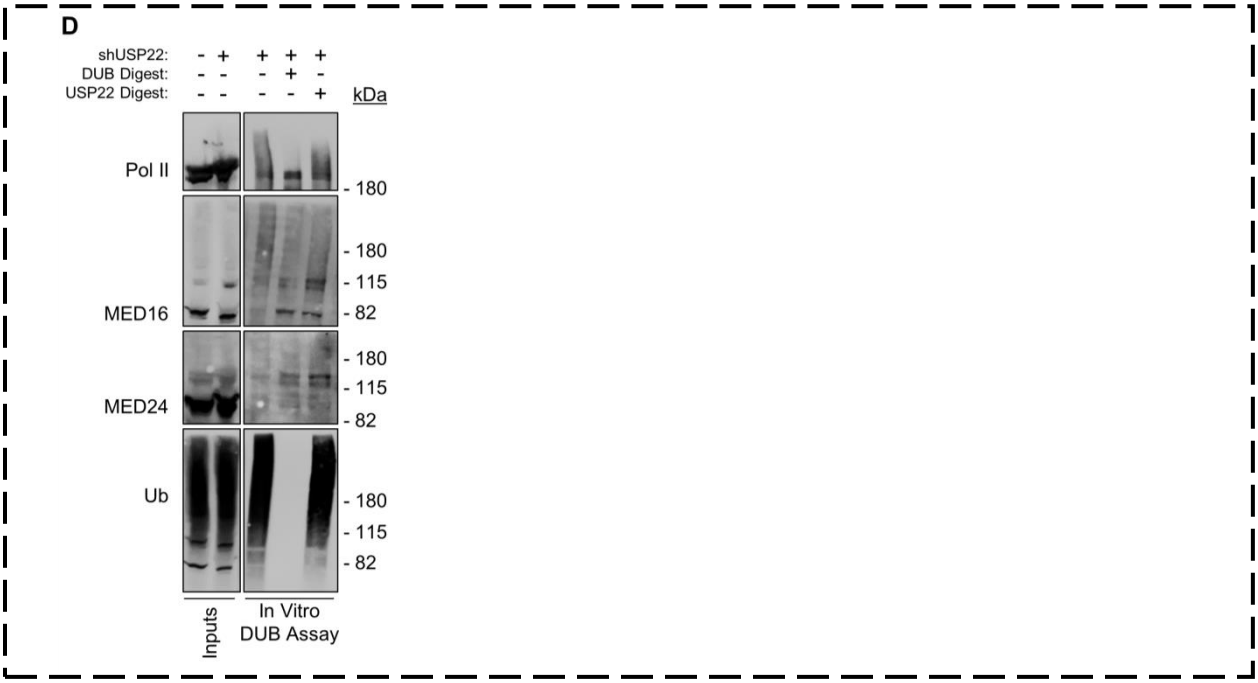
The pattern and intensity of Pol II look similar between lanes 3 and 5. The MED16 data are more convincing but it seems that several higher-MW bands also increase upon USP2 and USP22 digestion: what are these bands?

In both cases of Pol II and MED16, any higher MW smears or bands in lanes 4-5 likely represent sites of ubiquitylation or other post-translational modification insensitive to USP2 or USP22 enzymatic activity. In the case of Pol II, that we see only partial reduction of the Ub-like streak following in vitro digestion with USP22 is not unexpected: our proteomics data (Appendix Table S2, 'Summary' tab) show that Pol II, MED16, and MED24 contain several lysines whose ubiquitylation status does not change in response to USP22 depletion, suggesting they are not target sites for USP22 enzymatic activity and would therefore persist in lane 5 of our assay. Additional sites for all three proteins are also reported in a publicly available database of the ubiquitylated proteome (Kim et al 2011, https://ggbase.hms.harvard.edu/). We have revised our text to include these additional ubiquitylation sites as potential contributors to the higher MW bands observed in our digest:

"The appearance of higher molecular weight bands in the DUB-digested conditions suggest the persistence of ubiquitylated residues or other post-translational modifications insensitive to DUB or USP22 activity."

Which band corresponds to the unmodified form of MED24 - the strong ones in lanes 1-2 or the higher-MW bands labeled with the arrow?

We apologize for this confusion: the lower MW band in lanes 1-2 correspond to unmodified MED24 protein. Like Pol II and MED16 as explained above, the higher MW bands in lanes 4-5 likely represent ubiquitylation sites on MED24 that are insensitive removal by either USP2 or USP22. To aid in the interpretation of these data, MW size labels have been added to the figure (see above Fig EV4D).



Finally, my understanding from sequence analyses and structural studies is that Ubp8/USP22 lacks the residues that are needed for ubiquitin binding, which is why it is active only in complex with the other DUB subunits. It is thus surprising that USP22 alone can catalyze MED16/24 / Pol II deubiquitination. Please explain.

You are correct that USP22 is only active in complex with other DUB module subunits. Although labeled 'USP22' in the figure to distinguish it from the non-specific DUB (USP2) digest in lane 4, our reaction in lane 5 comprises all four members of the DUB module that we recombinantly co-expressed in Sf9 cells and purified as a full complex. We have revised the text and the figure legend to clarify this point explicitly.

"Furthermore, recombinantly expressed and purified human DUB module was capable of directly deubiquitylating MED16, MED24, and Pol II in an in vitro digestion reaction, as indicated by reduction of visible streaking with MED16 and Pol II as well as increases in unit-length protein (Fig EV4D).

Minor points:

1. P4: TRRAP is believed to represent the main activator-docking module, not TAF. Also, citing the latest yeast SAGA structures (Papai et al, Nature, 2020, and Wang et al, Nature 2020) is more relevant than Sharov et al 2017 to describe the architecture of SAGA.

As stated above for Reviewer 1, we have updated our nomenclature and references to include the most recent literature regarding SAGA.

2. P4: I think that "activator-driven genes" is a misleading term. All genes likely require an activator for transcription. Furthermore, there is a confusion between "present" and "regulates". The point of the Huisinga study (and many others) was that SAGA predominantly *regulates* stress-inducible genes. In fact, most ChIP-seq studies found that SAGA binds many genes, even in yeast.

We thank the Reviewer for this input. In all cases where "activator-driven" is used in the text, we have either removed it or replaced it with "stimulus-responsive"

3. P5: The mammalian ortholog of yeast Sgf73 is ATXN7, not ATXN7L3.

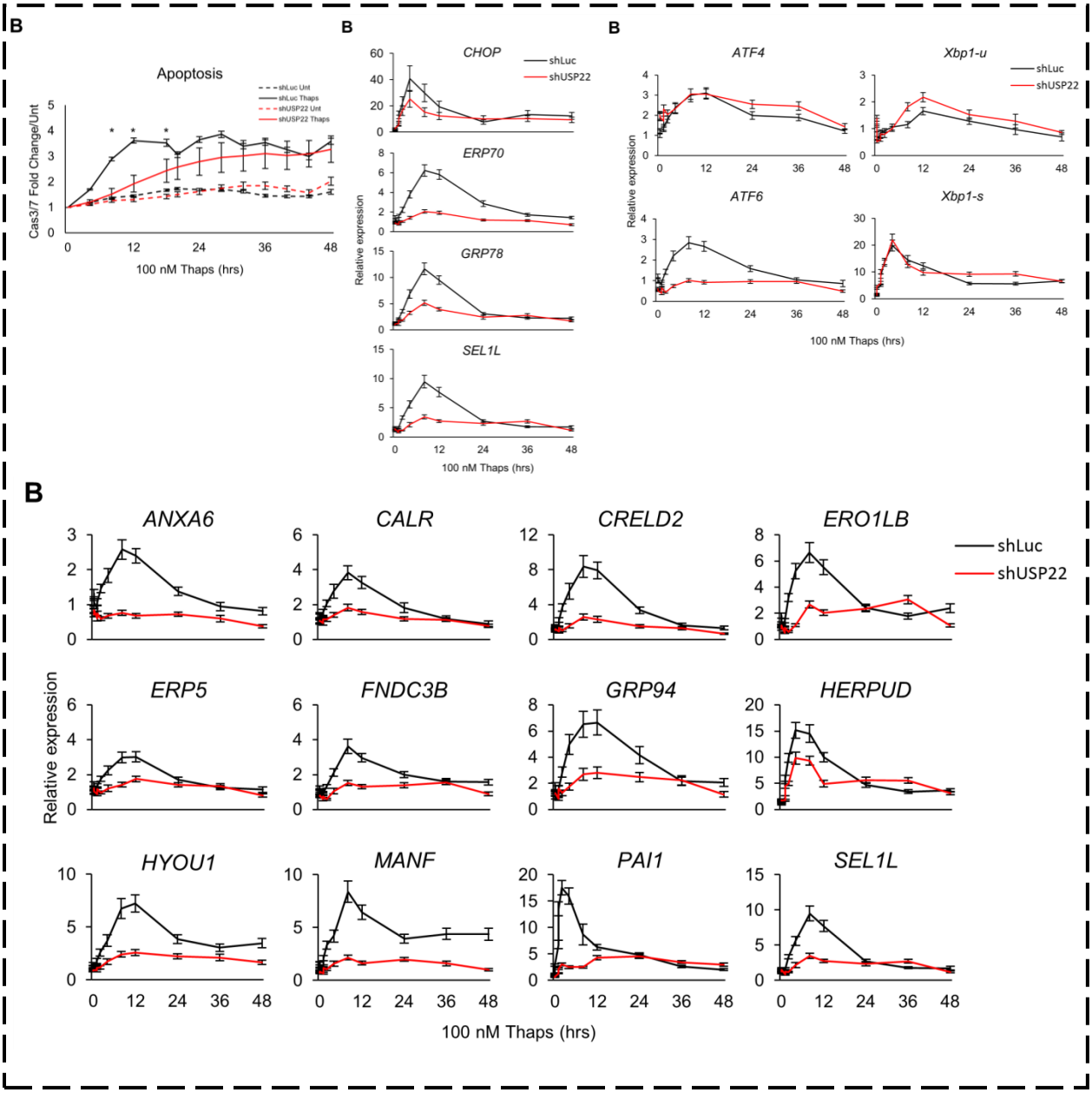
Thank you for pointing out this typographical error. It has been fixed.

4. Results from the AML cell line have been removed according to the authors' response but this result is still mentioned at the bottom of page 5.

Thank you for bringing this to our attention. This referenced text has been removed.

5. Figure 3B and EV2B: A quantitative variable, such as time, cannot be represented as a qualitative variable. The authors used a line graph to show the relation between two quantitative variables that depend on each other (expression vs time), so artificially changing the distance between two data points on the x-axis is visually misleading. If it is important to show the fate of Xbp1 splicing at early time points, though I didn't see it mentioned in the manuscript, then one can split the graph in two graphs, or highlight this part of the time-course onto a separate graph.

We appreciate the Reviewer's comments here and have changed all graphs depicting time courses to contain a qualitative x axis (Fig 2B, 3B, EV2B, Appendix Fig S4B). We have also concluded that for Xbp1, the new format sufficiently demonstrates its insensitivity to USP22 depletion and so have opted not to include a second graph highlighting early points in the time course.



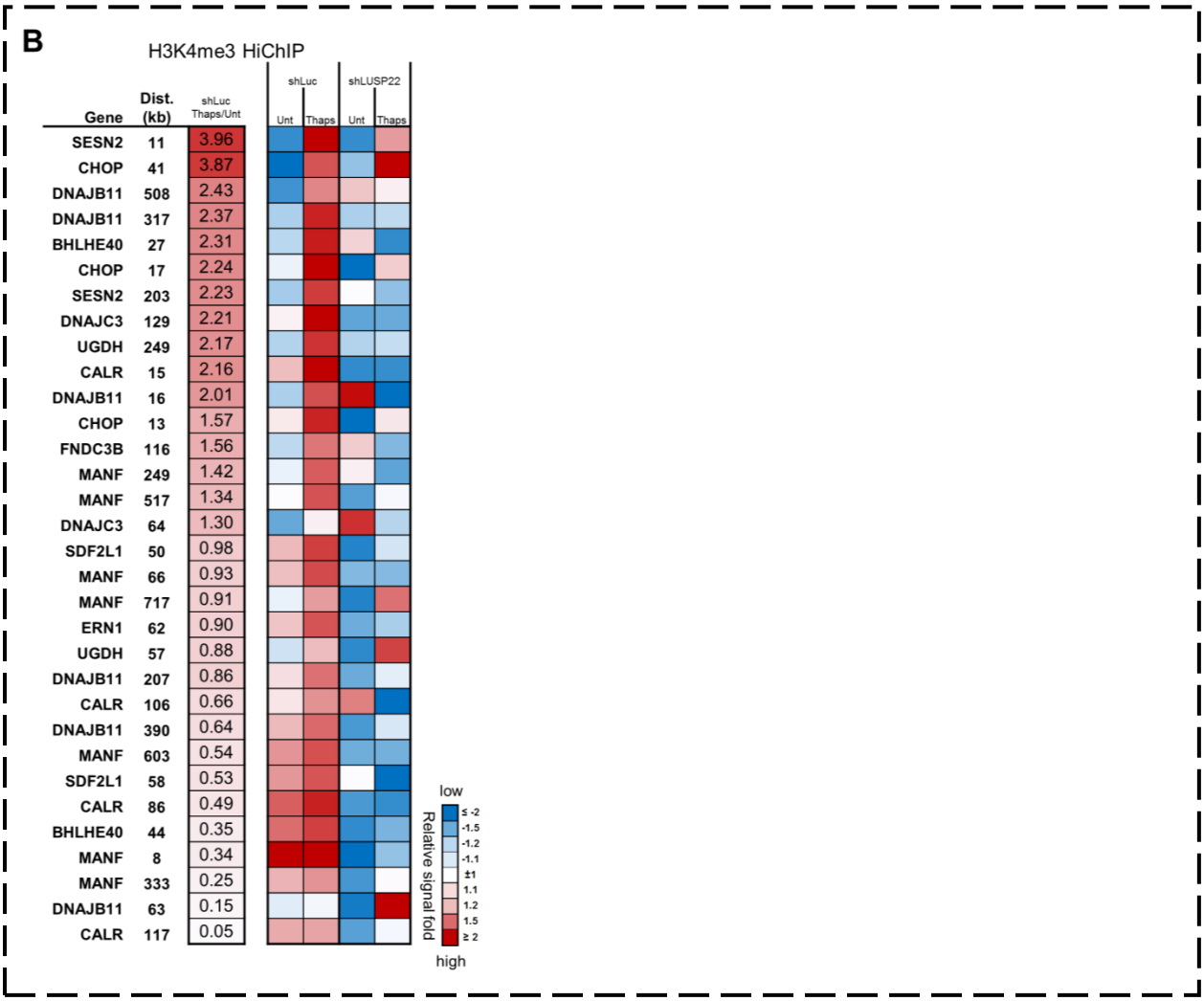
6. In response to [C2.5], thanks for the clarification and yes, I think that it is worth to include this explanation in the text.

We have included the following text in the manuscript to include the referenced clarification for Fig. 7E,F:

"Our ChIP-seq analyses show that core Mediator binds ER stress response gene promoters together with USP22 and the rest of SAGA. In fact, MED1 ChIP signal is found at all SAGA-Bound gene promoters defined in Figure 4. However, assessment of ER stress-induced changes in MED1 and USP22 binding show poor correlation (Fig 7E). This is often due to the fact that MED1 is already bound to these promoters prior to ER stress. By contrast, both USP22 and MED16 are recruited to SAGA-bound promoters in a stress-dependent manner and to a similar extent across these loci (Fig. 7F)."

7. Color scale is missing from Figure 7B.

We have included the color scale for Fig 7B.



2nd Revision - Editorial Decision 9th Apr 2021

Thank you for submitting the revised manuscript and responding to the remaining referee comments. Please also excuse the delay in communicating this decision to you, which was due to the currently high number of new submissions and delays during the pre-acceptance checks. Nonetheless, I am happy to say that once the remaining editorial issues are resolved, we will proceed to formally accept the manuscript for publication. Therefore, I would now like to ask you to address the issues that are listed in detail below in a final revised version. Please make any changes to the manuscript text in the attached document only using the "track changes" option.

The Editor made specific requests for minor changes to the main manuscript and supplemental figures and source data. We have addressed each request and we hope you find it acceptable for publication.

Individual comments from the Editor are listed below (in bold), with our response directly following each comment (in italics).

Thank you for submitting the revised version of your manuscript. You have added the file "Appendix Table S2", however this is not included in the Appendix itself nor referred to in the manuscript. If the Table should be part of the Appendix, the Appendix needs to be updated. However, as this is a dataset and the file contains multiple tabs there are 2 more options which may be more appropriate:

1) Submit it as a dataset. For this the nomenclature would need to be changed to Dataset EV1 and the revised (change legend title) excel file uploaded. A reference to this dataset must then be added to the manuscript text where appropriate.

2) Submit it as source data for the respective figure. Here the nomenclature would need to be changed to source_data-figX and the file uploaded accordingly.

At the Editor's suggestion, we have renamed "Appendix Table S2" as "Dataset EV1." We have updated the dataset figure legend and uploaded a revised version. We have added a reference to it in the manuscript text and uploaded a revised version of the manuscript.

This dataset is also referenced in the figure legend for Table EV1, so we have uploaded a revised Table EV1 file containing the updated reference.

In addition, the source data files for the EV figures and Appendix need to be combined into one zip file for all EV figures and one for all Appendix figures. The source data for the main figures is good as is.

We have uploaded one zip file containing source data for EV figures and one zip file containing source data for Appendix figures.

Thank you again for submitting the final revised version of your manuscript. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Steven B. McMahon
Journal Submitted to: EMBO J
Manuscript Number: 2019-102509

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be iustified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

USEFUL LINKS FOR COMPLETING THIS FORM

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B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was chosen in adherence to previous studies in this field of research. For high-throughput sequencing and proteomics experiments, biological duplicates were prepared. Followup validation was achieved with a minimum of 2 independent experiments. For all other experiments, a minimum of 3 independent experiments were performed.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples were excluded.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes; please see figure legends for statistical tests used where applicable..
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Linear regression analysis, Student's T-Test, Two-Way ANOVA, and nonparametric Mann-Whitney tests of significance were used.
Is there an estimate of variation within each group of data?	Please see figure legends for estimates of variation where applicable.

Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	All antibodies used are commercially available and are described in the Materials and Methods section. Included for each antibody is a catalog/clone number.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HCT116 (CCL-247) and 293T (CRL-11268) cells were acquired from ATCC. All cell lines in the lab are tested regularly for mycoplasma contamination using the LookOut® Mycoplasma PCR Detection Kit (Sigma # MP0035).

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	ChIP-seq data are available at GSE Accession # GSE121798. HiChIP data are available at GSE Accession # GSE158108. This information can be found in the Data Availability section of the manuscript.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	UbiScan® data are provided in Appendix Table S2.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No