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# The Ulp2 SUMO Protease Promotes Transcription Elongation through Regulation of Histone Sumoylation

Hong-Yeoul Ryu, Dan Su, Nicole R. Wilson-Eisele, Dejian Zhao, Francesc López-Giráldez, and Mark Hochstrasser.

**Review timeline:**

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| Submission date: | 14th March 2019 |
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## Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 3nd April 2019

Thank you again for submitting your manuscript on Ulp2 links to histone ubiquitylation and RNAPII transcription for our editorial consideration. We have now received the reports of three expert referees, copied below for your information. As you will see, while the reviewers acknowledge the applied methodologies and large amounts of data included, they are (to varying degrees) concerned that not all main conclusions of the study are equally well supported by the presented data. In particular, all referees remain unconvinced that your results provide sufficiently definitive support on H2B being the critical target of Ulp2, an issue that would thus require further investigation and (if necessary) more cautiously stated conclusions. Another general issue mentioned by all reviewers is the need for additional controls and source data, especially for ChIP and RT-qPCR experiments, and, as detailed by referee 2, improved statistical testing and increased number of replicates.

Should you be able to satisfactorily address these key concerns, as well as the various more specific experimental and presentational points noted in the three reports, then we would be happy to consider a revised manuscript further for publication in The EMBO Journal.

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REFEREE REPORTS

Referee #1:

Ryu and colleagues examined the role of Ulp2 SUMO protease in transcription elongation. The authors were able to demonstrate that ULP2 is required for optimal transcription, especially for highly transcribed genes. Furthermore, Ulp2 is recruited to these genes, while the ablation of Ulp2 leads to 'hyper-sumoylation' on the same genes. In order to understand the role of Ulp2 in transcription elongation, the authors used genetics/epistasis and other approaches to somehow link Ulp2 with histone H2Bub/SUMO. Finally, they found that the H2Bub/SUMO-Ulp2 relay is crucial for Ctk1 recruitment and S2 phosphorylation of RNA Pol II. While the authors support some of their statements with an abundance of experimental data, some findings and conclusions are not

 

sufficiently supported. The following points need to be addressed.
Major comments:
1) Figure 1. While the RNA-seq results provide a genome-wide view indicating that ulp2 may specifically impact the transcription of highly active genes, ChIP experiments were only carried out for three housekeeping genes. To test the correlation between Ulp2 and transcriptional activity, other assays (i.e., ChIP-seq) would be better suited to provide robust support that Ulp2 is preferentially recruited to actively transcribed regions.
2) Figure 2. Although a large number of known SUMO conjugates in yeast are transcription factors, SUMOylation modifies proteins involved in a wide range of cellular processes; thus, it is not surprising to see the fragility of ulp2 cells. Since the growth defect of ulp2 cells was greater than that in ulp2-C624A cells, a possible interpretation could be that in addition to a SUMO protease activity, Ulp2 plays other roles in transcription elongation as well. The authors should consider other possible mechanisms that could explain the sensitivity results.
3) Figure 3. How can the authors be sure that the Co-IP of Ulp2 with histone H2B shown in Fig 3D is not due to Ulp2 interactions with the free histone pool? A more appropriate experiment to demonstrate chromatin binding is to first isolate the chromatin fraction.
4) Figure 4. Following up on the interaction of Ulp2 with H2B, the authors tried to link Ulp2 with H2B-SUMO; however, they are also aware that H2B is not the only histone that can be SOMOylated. In addition, it has been well documented that Bre1-H2Bub also play roles in DNA replication/repair and RNA processing. The synthetic lethality of ulp2 rad6, ulp2 bre1 and ulp2 htb-K123R cannot simply be interpreted as these two pathways having overlapping functions only in the regulation of transcription.
5) Figure 5. There were abundant high molecular weight SUMO conjugates accumulated in ulp2 cells (Fig 5B), indicating that Ulp2 targets a wide range of SUMO-conjugated proteins, and histones are among these targeted factors. Thus, the ChDIP procedure (Fig 5D) may not reveal the true occupancy of H2B-SUMO. In the first ChIP, anti-Flag immunoprecipitates H2B, which is cross-linked to other histones and chromatin binding factors. Many of these cross-linked factors are likely SUMOylated, so the second ChIP that targets HA-SUMO is not specific for H2B-SUMO.
6) Figure 6. The ChDIP in Fig 6G suffers the same shortcoming mentioned in the previous point. In Figure 6H-J, the authors were able to demonstrate that SUMO-fused H2B shares similar effects with the ulp2Δ mutation in terms of preventing Rpb3 binding and CTD ser2-P. However, they did not demonstrate that SUMO-fused H2B also blocks Ctk1 recruitment to chromatin. In addition, the reduction of CTD ser2-P is proportional with that of Rpb3. Therefore, in order to accurately probe the effect of preventing Ctk1 recruitment, the CTD ser2-P signals should be normalized to those of Rpb3. To support their current conclusions, the authors should show that SUMO-fused H2B sensitizes cells to 6-AU, and also demonstrate the epistatic effect of SUMO-fused H2B with ulp2Δ. This proof would eliminate the possibility that the strong growth defect in ulp2Δ cells may be induced by hyper-sumoylation of other functional proteins (such as those bulky sumoylated proteins shown in Figure 5B).

Referee #2:

In their manuscript " The Ulp2 SUMO Protease Links Sequential Histone Ubiquitylation and Sumoylation with RNAPII CTD S2 Phosphorylation" by Ryu et al, the authors explored the function of Ulp1 and Ulp2 in regulation of transcription. Sumo was previously shown to regulate transcription of most -if not all- genes in budding yeast, but exactly how cells dynamically control sumoylation to regulate gene expression remains poorly understood.
In a series of experiments the authors show that Ulp2 localizes to a subset of genes to control their transcription. Loss of ULP2 results in increased sumoylation at several highly expressed genes, which correlates with reduced expression levels. This function of Ulp2 appears to be dependent upon prior H2B ubiquitination, and promotes CTD-S2 phosphorylation of RNA pol II to promote transcriptional elongation. The authors also show that H2B is a Sumo target, and reveal a correlation (but not evidence of causality) between H2B sumoylation and transcriptional regulation.

The manuscript is well written and easy to follow and in general the choice of methods is appropriate.
However, I do have a few major concerns that need to be addressed, as well as several other issues to help the authors improve their manuscript:

 

Major concerns
1. Statistics:
The manuscript suffers from statistical weakness at several levels, which should be improved. More specifically:
-The authors should include a section on statistics in the Methods describing how statistical significance was calculated. According to the information provided in the legends, the authors used Student's t-test for all statistical analyses, but that can only be done when data are normally distributed. Have the authors confirmed normal distribution of their data prior to analysis by the t-test? It is very well possible that other statistical tests are more appropriate.
-Without statistical analysis it is difficult to interpret the differences observed in several of the graphs (Fig. 1E, EV1, EV3).
-Several experiments were only performed one or twice and should be repeated to a total of at least three.
-According to the legends, the experiments in Fig. 2D, 2F, 5C, 5D, 6G were performed only twice, yet the authors calculated p values, which is inappropriate.

2. Data availability
In addition to the polished and normalized graphs shown in the figures, which look quite beautiful indeed, the authors must include a supplemental data file including all the raw data of the ChIP and RT-qPCR experiments so that the data can be evaluated independently by the reviewers/audience.

3. ChIP-qPCR experiments are widely used throughout the manuscript. It is therefore essential to show that the experimental set-up is thoroughly controlled in each experiment. ChIP experiments (such as those shown in Fig. 1E,F, but also many other figures) can be confounded by non-specific pull-down during the IP step, which is especially a concern for highly expressed genes such as PMA1, ADH1 and PYK1. The TAP tag in particular is often associated with high levels of background signal at highly expressed genes in such experiments. It is therefore important to include mock controls, e.g. an untagged strain or -even better- a strain expressing a TAP/FLAG tag with an NLS, or alternatively a TAP- and/or FLAG-tagged nuclear protein that does not bind chromatin. A similar control should be included for the ChIP experiments with the Rpb3 and Sumo antibodies (e.g. an isotype control is the minimum), since increased ChIP signals upon activation of the CUP1 gene could be the result of increased non-specific chromatin accessibility.

4. It remains unclear exactly what the critical target of Ulp2 is in regulation of transcription. While I agree with the authors that their experiments revealed a strong correlation between H2B ubiquitination, H2B sumoylation and gene expression, the authors have not formally shown a causal relationship between H2B sumoylation and transcription. Without identification of the critical Sumo target site in H2B and documentation of its physiological relevance, this will remain uncertain (it may be a different target altogether). It is important the authors make this very clear to the audience in the Discussion section. In addition, some of the conclusions should be toned down (such as " Deconjugation of SUMO from histones by Ulp2 is associated with Ctk1- mediated S2 phosphorylation of RNAPII, which helps drive transcription elongation."; and "...Sumo... is part of a dependent histone conjugation and deconjugation sequence during transcription in which both ubiquitin and SUMO play positive roles.")

Other issues:
5. Fig. 2A: I suppose the bottom panel was SD-URA-TRP?

6. Fig. EV1 and EV3: How many replicates were performed? It appears to be just one, if that is true then these experiments cannot be properly interpreted.

7. Fig. 3A: "The ulp2ΔN strain and cells expressing just the NTD showed severe growth defects on SD-Ura and they were almost inviable on the plate with 100 ug/ml 6-AU, suggesting that both nuclear localization and the catalytic domain of Ulp2 are likely required for efficient transcription elongation."
With such a strong growth defect even in absence of 6AU it is impossible to conclude that the deltaN mutant and NTD mutants affect transcriptional elongation; a perfectly fine alternative

 

interpretation of these data is that cells expressing this mutant simply fail to properly respond to cell stress caused by exposure to this drug. Moreover, when comparing the deltaN and NTD mutants grown at 30C on SD-URA with SD-URA+6AU there may not even be a growth defect at all.

8. Fig. 3C: As already mentioned above, it is important to include appropriate negative controls for ChIP experiments. In this particular experiment it is unclear what the contribution of the antibody's background is at these particular genes, making it difficult to understand the relative contribution of the different domains of Ulp2.

9. Fig. 3D: how many times was this experiment repeated?

10. Co-IP experiments: Can the authors rule out the possibility that the presence of Ulp proteins (and certain ubiquitin proteases) in their lysates affects sumoylation/ubiquitination levels of the proteins they studied, thereby qualitatively affecting the protein-protein interactions they intended to study? According to the Methods, no compounds were added to the lysis buffer to block such potential post-lysis effects of Ulps and/or ubiquitin proteases (the authors do state they used NEM in their in vivo ubiquitylation/sumoylation assays, but not co-IPs).

Referee #3:

In this manuscript, Ryu and colleagues examine mechanisms by which Ulp2, one of two budding yeast SUMO proteases, regulates gene expression. This is a follow-up to a previous study from this lab that found changes to the transcriptome in yeast lacking Ulp2. In the current study, chromatin-immunoprecipitation is used to show that Ulp2 is detected at active genes where it is thought to remove polySUMO modifications from histone H2B. Evidence is provided that H2B mono-ubiquitination promotes its sumoylation and recruitment of Ulp2. In cells lacking Ulp2, more chromatin sumoylation is detected, less RNAPII associates with active genes, and the CTD of RNAPII shows less Ser2 phosphorylation, which is associated with transcriptional elongation.

The study convincingly shows that Ulp2 is required for maintaining low levels of sumoylation on chromatin and that elevated chromatin-associated sumoylation levels correlate with reduced RNAPII occupancy. It also demonstrates an interesting connection between H2B mono-ubiquitination and sumoylation. Additionally, some of the data supports a role for Ulp2 in elongation and RNAPII-CTD Ser2 phosphorylation, but I am not fully convinced, however, that the role of Ulp2 is transcription-dependent. This major issue should be addressed before the article is suitable for publication in EMBO J.

Major points

Deletion of ULP2 has clear effects, particularly on H2B sumoylation and RNAPII occupancy, but whether the effects are due to transcription-recruited Ulp2 or a result of less direct roles for the SUMO protease is not clear.

1. Deletion of ULP2 results in an apparent general elevation in SUMO levels across chromatin, not just where Ulp2 was detected by ChIP. Ulp2 is shown to primarily occupy ORF regions for most tested genes, but ULP2 deletion elevates SUMO levels at promoters as well as at ORFs (Figs. 2C,F). Notably, although SUMO ChIP was not performed at an untranscribed region, H2B-SUMO ChDIP was performed at a no-ORF region, and deletion of ULP2 resulted in elevated sumoylation of H2B at this untranscribed region (5E, compare WT with ulp2delta). This argues that Ulp2 has a general role in reducing chromatin sumoylation levels that is not dependent on transcription. The significance of ORF-associated Ulp2 is therefore not clear, and this should be addressed.

2. The abstract indicates that Ulp2 is "specifically recruited to transcriptionally active genes to control local sumoylation" and in Fig. 1E, it is claimed that Ulp2-Flag occupies the length of the three genes. However, there is no untranscribed ("non_ORF") region analysis for determining what background signal levels are, or if Ulp2 is also detected at untranscribed regions. Is an IP/INPUT ratio of 2 equal to background? If so, then ADH1 doesn't show much Ulp2 occupancy, yet ULP2 deletion has a strong effect on occupancy of RNAPII and SUMO on the ADH1 gene, and ADH1

 

shows the highest dependence on Ulp2 in Fig 1C. This would support an indirect role for Ulp2. At least one non-ORF region should be compared, minimally for the Ulp2-Flag ChIP.

3. In WT cells, H2B-SUMO levels is about the same on constitutive genes (promoters and ORFs) and in the untranscribed/no-ORF region tested (compare Figs. 5D with "no ORF" in 5E and EV2), which argues that H2B sumoylation is actually not transcription-dependent. The claim of a link between transcription activation and H2B sumoylation is dependent on analysis of a single inducible gene. To support this claim, additional inducible genes (at least one) should be tested.

Minor Points

4. Can sumoylated Flag-H2B species be detected on a Flag immunoblot? To support that the sumoylated proteins, in particular the polysumoylated species, shown in the SUMO blot correspond to H2B, more of the Flag blot should be shown, including the stacking gel.

5. Related to this, on the H2B IP (Fig. 4A and p.13) the manuscript refers to sumoylated and polysumoylated species detected as "histones" and not specifically H2B. This should be clarified. The samples were prepared through denaturing TCA precipitation. Are nucleosomes retained in this procedure (i.e. are other histones coIPed?), or is the IP be specific for H2B?

6. In Fig. 5A, the mono-sumoylated species appears to be roughly the same size as the mono-ubiquitinated species seen in the lower blot of the same figure, suggesting that H2B sumoylation and ubiquitination are not necessarily occurring on the same H2B polypeptide. Furthermore, mono-sumoylation levels are affected by altering H2B mono-ubiquitination levels. This suggests probable crosstalk between the two H2B polypeptides in the nucleosome (i.e. ubiquitination of one subunit promotes sumoylation of the other). This should be discussed somewhere.

7. In WT cells, why is the Ulp2 occupancy level at ORFs about twice as high as for promoters of PMA1 and PYK1 in Figs. 1E, 5H, and 5I, but not in 5G, where they are about even?

8. The subheading at the bottom on p. 17 appears to be a mistake. According to the data Ulp2 promotes (not blocks) recruitment of Ctk1.

9. In Fig. 6K, Rpb3 occupancy on SDH1 is not significantly affected by ULP2 deletion, whereas the other tested genes are affected. How does this correlate with the RNA-seq analysis summarized in Fig. 1B?

10. On p. 13 line 2, the figure callout refers to lanes 4 and 6. It would be helpful if the lanes were labeled on the Figure.

11. The data referred to on p. 13 line 3 as "not shown" should be shown or the statement should be excluded.

1st Revision - authors' response 22nd May 2019

Referee #1:

Ryu and colleagues examined the role of Ulp2 SUMO protease in transcription elongation. The authors were able to demonstrate that ULP2 is required for optimal transcription, especially for highly transcribed genes. Furthermore, Ulp2 is recruited to these genes, while the ablation of Ulp2 leads to 'hyper-sumoylation' on the same genes. In order to understand the role of Ulp2 in transcription elongation, the authors used genetics/epistasis and other approaches to somehow link Ulp2 with histone H2Bub/SUMO. Finally, they found that the H2Bub/SUMO-Ulp2 relay is crucial for Ctk1 recruitment and S2 phosphorylation of RNA Pol II. While the authors support some of their statements with an abundance of experimental data, some findings and conclusions are not sufficiently supported. The following points need to be addressed.

 

We thank the reviewer for the positive review and constructive comments.

Major comments:
1) Figure 1. While the RNA-seq results provide a genome-wide view indicating that ulp2 may specifically impact the transcription of highly active genes, ChIP experiments were only carried out for three housekeeping genes. To test the correlation between Ulp2 and transcriptional activity, other assays (i.e., ChIP-seq) would be better suited to provide robust support that Ulp2 is preferentially recruited to actively transcribed regions.

These are good points. Therefore, we have now performed ChIP-seq of Ulp2-Flag to map the binding sites of the Ulp2 enzyme on a genome-wide scale. The results were then compared to previous RNA-seq data (Ryu et al, 2018); these data are in new Fig 1G, H and EV1. Our results confirmed that Ulp2 SUMO protease is preferentially recruited to ribosomal protein genes and highly transcribed genes. Furthermore, this pattern is likely correlated with previous RNA-seq data in which transcription of most highly expressed genes was down-regulated in *ulp2Δ* strains (Fig 1B). Therefore, we suggest that Ulp2 is directly involved in transcription of actively transcribed genes.

2) Figure 2. Although a large number of known SUMO conjugates in yeast are transcription factors, SUMOylation modifies proteins involved in a wide range of cellular processes; thus, it is not surprising to see the fragility of ulp2 cells. Since the growth defect of ulp2 cells was greater than that in ulp2-C624A cells, a possible interpretation could be that in addition to a SUMO protease activity, Ulp2 plays other roles in transcription elongation as well. The authors should consider other possible mechanisms that could explain the sensitivity results.

We agree that Ulp2 may have diverse roles in transcription. However, we do not have data that show a more severe defect for *ulp2Δ* vs. ulp2 catalytic point mutants, so there are no grounds for us to propose a noncatalytic role for Ulp2 in transcription elongation. We also agree that the sensitivity of *ulp2* cells to various stresses is very likely due to multiple different sumoylated substrates. We now have additional evidence for the significance of histone sumoylation being a key target. In Fig 6H, 6-AU sensitivity assays revealed that cells expressing 2SUMO-fused H2B showed strong sensitivity to 6-AU. Importantly, no additional growth defect was observed in *ulp2Δ* cells expressing 2SUMO-fused H2B, compared with WT cells expressing 2SUMO-fused H2B. These results imply that accumulation of polySUMO-conjugated histones may be a primary source of the growth defect and 6-AU sensitivity of *ulp2* mutants. We describe our inferences cautiously (p. 19): "These results are consistent with the accumulation of polySUMO-conjugated histones being a major cause of the growth defects and 6-AU sensitivity in *ulp2* mutants." Also, in the Discussion, we have added the proviso (p. 22) that "we cannot exclude the possibility that SUMO conjugation to other chromatin-bound factors is directly required for Ulp2 binding and contributes to *ulp2* mutant defects."

3) Figure 3. How can the authors be sure that the Co-IP of Ulp2 with histone H2B shown in Fig 3D is not due to Ulp2 interactions with the free histone pool? A more appropriate experiment to demonstrate chromatin binding is to first isolate the chromatin fraction.

This was a good suggestion, and we have now performed the recommended chromatin association assays (Fig 3E, F). After fractionation into chromatin-associated proteins and soluble proteins, we performed immunoblotting to determine the ratio of Ulp2 proteins in soluble and chromatin fractions. We observed that Ulp2 was preferentially observed in the chromatin fraction, consistent with a physical interaction between Ulp2 and histone proteins within chromatin. Furthermore, loss of either the Ulp2 CTD or NTD substantially reduced Ulp2 localization to the chromatin fraction, closely paralleling the quantified histone H2B co-IP data in Fig 3D.

4) Figure 4. Following up on the interaction of Ulp2 with H2B, the authors tried to link Ulp2 with H2B-SUMO; however, they are also aware that H2B is not the only histone that can be SOMOylated. In addition, it has been well documented that Bre1-H2Bub also play roles in DNA replication/repair and RNA processing. The synthetic lethality of ulp2 rad6, ulp2 bre1 and ulp2 htb-K123R cannot simply be interpreted as these two pathways having overlapping functions only in the regulation of transcription.

 

These are good points. We analyzed sumoylation of H4 as well as H2B in Fig EV2A, and the analysis of histone H4 sumoylation yielded results similar to those seen with H2B. The data showed not only H2B but also H4 can be sumoylated and both are substrates of Ulp2. Given the data in the SUMO research field for "group sumoylation" of subunits in protein complexes, with no single subunit that must be modified, we suspect that multiple subunits of the nucleosome (and possibly even associated factors) can be sumoylated and serve the function of SUMO in transcription elongation.

Because histone ubiquitylation also has roles in DNA replication/repair and RNA processing, we could not conclude that the synthetic lethal interaction between mutations in *ULP2* and the *RAD6*- and *BRE1*-encoded H2B ubiquitylation enzymes and *htb1-K123R* mutant is only due to defect of transcription elongation. However, disruption of *RTF1*, encoding a component of RNAPII-associated chromatin remodeling PAF complex that promotes H2B ubiquitylation (Van Oss et al, 2016), was also lethal with *ulp2Δ*, data which we have now added (Fig 4E) We now write (p. 14), "These data are consistent with histone H2B ubiquitylation and Ulp2 have overlapping functions in the regulation of transcription, but additional shared functions for Ulp2 and H2B ubiquitylation are not ruled out."

5) Figure 5. There were abundant high molecular weight SUMO conjugates accumulated in ulp2 cells (Fig 5B), indicating that Ulp2 targets a wide range of SUMO-conjugated proteins, and histones are among these targeted factors. Thus, the ChDIP procedure (Fig 5D) may not reveal the true occupancy of H2B-SUMO. In the first ChIP, anti-Flag immunoprecipitates H2B, which is cross-linked to other histones and chromatin binding factors. Many of these cross-linked factors are likely SUMOylated, so the second ChIP that targets HA-SUMO is not specific for H2B-SUMO.

This is a reasonable point regarding the technical limitations of ChDIP. Without a specific antibody for a particular histone modification, ChDIP is nevertheless useful to estimate the modifications on chromatin-bound histones, and the method has been frequently used for the study of histone ubiquitylation and sumoylation (Kao et al, 2004; Nathan et al, 2006; Shieh et al, 2011; Wyce et al, 2007). Given the likelihood of "group sumoylation" at the nucleosome level noted in the previous comment, the estimation of sumoylation at the locus through IP of the tagged histone H2B is likely as relevant as knowing the degree of modification of H2B per se. We now explicitly comment on the limits of the ChDIP technique in the Discussion (top of p. 23).

6) Figure 6. The ChDIP in Fig 6G suffers the same shortcoming mentioned in the previous point. In Figure 6H-J, the authors were able to demonstrate that SUMO-fused H2B shares similar effects with the ulp2Δ mutation in terms of preventing Rpb3 binding and CTD ser2-P. However, they did not demonstrate that SUMO-fused H2B also blocks Ctk1 recruitment to chromatin. In addition, the reduction of CTD ser2-P is proportional with that of Rpb3. Therefore, in order to accurately probe the effect of preventing Ctk1 recruitment, the CTD ser2-P signals should be normalized to those of Rpb3. To support their current conclusions, the authors should show that SUMO-fused H2B sensitizes cells to 6-AU, and also demonstrate the epistatic effect of SUMO-fused H2B with ulp2Δ. This proof would eliminate the possibility that the strong growth defect in ulp2Δ cells may be induced by hyper-sumoylation of other functional proteins (such as those bulky sumoylated proteins shown in Figure 5B).

These are good points. In response, we checked the recruitment of Ctk1 to constitutive genes in cells expressing 2SUMO-fused H2B (Fig 6K). The data show that 2SUMO-fused H2B also impairs Ctk1 recruitment to the tested genes. Also, we reanalyzed the ratio of CTD Ser2-P to RNAPII by normalization of CTD Ser2-P signals to those of Rpb3 (Fig 6J, N) and redid the ChIP assays in *ubp8Δ*, *ubc9ts* and *ulp2Δ* cells (Fig 6A and 6N), according to the reviewer's advice. The results support our previous data: low relative CTD S2-P levels were observed in the *ubp8* and *ulp2* mutants and in cells expressing 2SUMO-fused H2B (6J). As noted in our response to comment #2 above, we show that *2SUMO-H2B* cells are sensitive to 6-AU and that the *ulp2Δ 2SUMO-H2B* mutations show epistasis in growth on regular medium (6H). We believe our new data strongly suggest that failure to remove (poly)SUMO from histones leads to defects in transcription elongation.

Referee #2:

 

In their manuscript " The Ulp2 SUMO Protease Links Sequential Histone Ubiquitylation and Sumoylation with RNAPII CTD S2 Phosphorylation" by Ryu et al, the authors explored the function of Ulp1 and Ulp2 in regulation of transcription. Sumo was previously shown to regulate transcription of most -if not all- genes in budding yeast, but exactly how cells dynamically control sumoylation to regulate gene expression remains poorly understood.
In a series of experiments the authors show that Ulp2 localizes to a subset of genes to control their transcription. Loss of ULP2 results in increased sumoylation at several highly expressed genes, which correlates with reduced expression levels. This function of Ulp2 appears to be dependent upon prior H2B ubiquitination, and promotes CTD-S2 phosphorylation of RNA pol II to promote transcriptional elongation. The authors also show that H2B is a Sumo target, and reveal a correlation (but not evidence of causality) between H2B sumoylation and transcriptional regulation.

The manuscript is well written and easy to follow and in general the choice of methods is appropriate.
However, I do have a few major concerns that need to be addressed, as well as several other issues to help the authors improve their manuscript:

We thank the referee for the positive overall evaluation and the constructive comments to extend several points in the paper.

Major concerns
1. Statistics:
The manuscript suffers from statistical weakness at several levels, which should be improved. More specifically:
-The authors should include a section on statistics in the Methods describing how statistical significance was calculated. According to the information provided in the legends, the authors used Student's t-test for all statistical analyses, but that can only be done when data are normally distributed. Have the authors confirmed normal distribution of their data prior to analysis by the t-test? It is very well possible that other statistical tests are more appropriate.

These are good points. The t-test assumes that the means of the different samples are normally distributed; it does not assume that the population is normally distributed. Also, t-test is generally used for qPCR data for ChIP or RT-PCR analyses, although there are not many numbers of samples. It is difficult to get sufficiently large numbers of samples to confirm normal distributions using these techniques. Nevertheless, t-test or ANOVA is usually used for statistical analysis in those experiments. Because we always compare the data between pairs of samples in all of the experiments, we only have used t-tests. To raise confidence in statistical analyses, we increased the number of samples in all experiments to 3-4 and provided all data in Dataset EV2.

-Without statistical analysis it is difficult to interpret the differences observed in several of the graphs (Fig. 1E, EV1, EV3).

To quantify and validate ChIP signals, the PCR signals were normalized to the internal control (untranscribed intergenic region) and the input DNA, unless otherwise indicated. This strategy of quantitation is broadly used (Ahn et al, 2004; Rosonina et al, 2010; Suh et al, 2016), showing whether the tested factor is associated with specific sites compared with the intergenic region; a value of 1 indicates a lack of enrichment relative to the untranscribed region. In these Figures, we only show whether a specific factor can be associated with active genes relative to a region lacking an ORF. We added a horizontal line indicating the baseline value of 1, as previously described (Rosonina et al, 2010). Also in Fig 1E, we added a statistical analysis of binding of Paf1-TAP and Ubc9-TAP versus Ulp1-TAP, which did not show any chromatin binding above background and binding of Ulp2-Flag versus untagged strain (negative control; new Fig EV1A). Since the data for original Figs EV1 and EV3 were not essential for the current study, we removed the results in the original Fig EV1 and now performed ChIP comparing Ulp2-Flag (positive control) and Ubp8-TAP (Fig EV3; three repetitions).

-Several experiments were only performed one or twice and should be repeated to a total of at least three.

 

We have increased the number of samples analyzed to 3-4 in all experiments.

-According to the legends, the experiments in Fig. 2D, 2F, 5C, 5D, 6G were performed only twice, yet the authors calculated p values, which is inappropriate.

We have increased the number of experiments to at least three and recalculated p values.

2. Data availability
In addition to the polished and normalized graphs shown in the figures, which look quite beautiful indeed, the authors must include a supplemental data file including all the raw data of the ChIP and RT-qPCR experiments so that the data can be evaluated independently by the reviewers/audience.

We now provide all data in Dataset EV2.

3. ChIP-qPCR experiments are widely used throughout the manuscript. It is therefore essential to show that the experimental set-up is thoroughly controlled in each experiment. ChIP experiments (such as those shown in Fig. 1E,F, but also many other figures) can be confounded by non-specific pull-down during the IP step, which is especially a concern for highly expressed genes such as PMA1, ADH1 and PYK1. The TAP tag in particular is often associated with high levels of background signal at highly expressed genes in such experiments. It is therefore important to include mock controls, e.g. an untagged strain or -even better- a strain expressing a TAP/FLAG tag with an NLS, or alternatively a TAP- and/or FLAG-tagged nuclear protein that does not bind chromatin. A similar control should be included for the ChIP experiments with the Rpb3 and Sumo antibodies (e.g. an isotype control is the minimum), since increased ChIP signals upon activation of the CUP1 gene could be the result of increased non-specific chromatin accessibility.

These are important points. To show more reliable results in the ChIP experiments, we usually use the strategy of quantitation as described it above. Also, we carried out new ChIP assays in Ulp2-Flag and untagged strain (negative control) by IP using anti-Flag beads (Figs 1E and EV1A). Furthermore, when we performed new ChIP assays during *CUP1* induction, we added mock immunoprecipitation without specific antibody for a negative control of specific antibodies such as anti-Rpb3 or anti-SUMO (Fig 1F); Among four independent experiments, we used the stored frozen chromatin samples for Mock-IP three times. These control experiments strongly support that our ChIP signals were not non-specific. Because TAP-tagged Ulp1 didn't show any chromatin binding (Fig 1E), we didn't add an IgG beads-only control in these ChIP analyses.

4. It remains unclear exactly what the critical target of Ulp2 is in regulation of transcription. While I agree with the authors that their experiments revealed a strong correlation between H2B ubiquitination, H2B sumoylation and gene expression, the authors have not formally shown a causal relationship between H2B sumoylation and transcription. Without identification of the critical Sumo target site in H2B and documentation of its physiological relevance, this will remain uncertain (it may be a different target altogether). It is important the authors make this very clear to the audience in the Discussion section. In addition, some of the conclusions should be toned down (such as " Deconjugation of SUMO from histones by Ulp2 is associated with Ctk1- mediated S2 phosphorylation of RNAPII, which helps drive transcription elongation."; and "...Sumo... is part of a dependent histone conjugation and deconjugation sequence during transcription in which both ubiquitin and SUMO play positive roles.")

We thank the reviewer for the thoughtful comments. Although we cannot exclude the possibility that Ulp2 may have other critical chromatin substrates (and surely does), our data clearly show that sumoylated histone is a substrate for Ulp2 (Figs 4A, 5A and EV2A), and that recruitment of Ctk1 and Rpb3 and CTD serine-2 phosphorylation levels at active genes are significantly impaired in cells expressing 2SUMO-fused histone H2B as well as in *ulp2Δ* cells (Fig 6). The new 6-AU sensitivity assays with 2SUMO-H2B further support the idea that accumulation of polySUMO-conjugated histones can negatively affect transcription elongation (Fig 6H). We nevertheless agree that it that there may be other key target(s) of Ulp2 in transcription regulation; in the revised manuscript, we now write, "…we cannot exclude the possibility that SUMO conjugation to other chromatin-bound factors is directly required for Ulp2 binding and contributes to *ulp2* mutant defects" (p. 22).

 

Also, as requested, we have made the cited statements more cautious, now writing, "Deconjugation of SUMO from histones by Ulp2 *appears to be* associated…" and "Sumo…*appears to be* part of a dependent histone…"

Other issues:
5. Fig. 2A: I suppose the bottom panel was SD-URA-TRP?

We used SD-URA plates to ensure presence of pRS316 plasmids in all strains, after checking for *TRP1* marker.

6. Fig. EV1 and EV3: How many replicates were performed? It appears to be just one, if that is true then these experiments cannot be properly interpreted.

As noted above, the previous Fig EV1 was removed, and we performed ChIP in the Ulp2-Flag and Ubp8-TAP strains three times (Fig EV3).

7. Fig. 3A: "The ulp2ΔN strain and cells expressing just the NTD showed severe growth defects on SD-Ura and they were almost inviable on the plate with 100 ug/ml 6-AU, suggesting that both nuclear localization and the catalytic domain of Ulp2 are likely required for efficient transcription elongation."
With such a strong growth defect even in absence of 6AU it is impossible to conclude that the deltaN mutant and NTD mutants affect transcriptional elongation; a perfectly fine alternative interpretation of these data is that cells expressing this mutant simply fail to properly respond to cell stress caused by exposure to this drug. Moreover, when comparing the deltaN and NTD mutants grown at 30C on SD-URA with SD-URA+6AU there may not even be a growth defect at all.

It is true that both the Ulp2N and ulp2deltaN protein support only very poor growth, so we have refrained from any interpretation of growth effects in the revised paper. Instead, we have focused on the *ulp2ΔC* strain which grows quite well on SD-Ura but is nearly dead on 6-AU.

8. Fig. 3C: As already mentioned above, it is important to include appropriate negative controls for ChIP experiments. In this particular experiment it is unclear what the contribution of the antibody's background is at these particular genes, making it difficult to understand the relative contribution of the different domains of Ulp2.

As noted above, when we performed ChIP assays in Fig1F, we used a mock-immunoprecipitation as a negative control. The results showed that our Flag-based ChIP have negligible non-specific signal. In Fig 3C, the important comparison is between chromatin binding by WT Ulp2 versus each mutant.

9. Fig. 3D: how many times was this experiment repeated?

While we performed Co-IP assay once, chromatin association assay was newly added in Fig 3E. This assay showed results that comport extremely well with those seen in the Co-IP experiment in Fig 3D.

10. Co-IP experiments: Can the authors rule out the possibility that the presence of Ulp proteins (and certain ubiquitin proteases) in their lysates affects sumoylation/ubiquitination levels of the proteins they studied, thereby qualitatively affecting the protein-protein interactions they intended to study? According to the Methods, no compounds were added to the lysis buffer to block such potential post-lysis effects of Ulps and/or ubiquitin proteases (the authors do state they used NEM in their in vivo ubiquitylation/sumoylation assays, but not co-IPs).

Unlike the *in vivo* histone sumoylation assays or ChDIP experiments, the Co-IP experiments were not focused in the physical interaction between SUMO and other proteins. Therefore, we did not add NEM in the buffer. We did add NEM to the buffers used in the chromatin association assays (Fig 3E), and we found results highly consistent with the Co-IP experiment in Fig 3D.

Referee #3:

In this manuscript, Ryu and colleagues examine mechanisms by which Ulp2, one of two budding

 

yeast SUMO proteases, regulates gene expression. This is a follow-up to a previous study from this lab that found changes to the transcriptome in yeast lacking Ulp2. In the current study, chromatin-immunoprecipitation is used to show that Ulp2 is detected at active genes where it is thought to remove polySUMO modifications from histone H2B. Evidence is provided that H2B mono-ubiquitination promotes its sumoylation and recruitment of Ulp2. In cells lacking Ulp2, more chromatin sumoylation is detected, less RNAPII associates with active genes, and the CTD of RNAPII shows less Ser2 phosphorylation, which is associated with transcriptional elongation.

The study convincingly shows that Ulp2 is required for maintaining low levels of sumoylation on chromatin and that elevated chromatin-associated sumoylation levels correlate with reduced RNAPII occupancy. It also demonstrates an interesting connection between H2B mono-ubiquitination and sumoylation. Additionally, some of the data supports a role for Ulp2 in elongation and RNAPII-CTD Ser2 phosphorylation, but I am not fully convinced, however, that the role of Ulp2 is transcription-dependent. This major issue should be addressed before the article is suitable for publication in EMBO J.

We thank the reviewer for the positive review and constructive comments.

Major points

Deletion of ULP2 has clear effects, particularly on H2B sumoylation and RNAPII occupancy, but whether the effects are due to transcription-recruited Ulp2 or a result of less direct roles for the SUMO protease is not clear.

1. Deletion of ULP2 results in an apparent general elevation in SUMO levels across chromatin, not just where Ulp2 was detected by ChIP. Ulp2 is shown to primarily occupy ORF regions for most tested genes, but ULP2 deletion elevates SUMO levels at promoters as well as at ORFs (Figs. 2C,F). Notably, although SUMO ChIP was not performed at an untranscribed region, H2B-SUMO ChDIP was performed at a no-ORF region, and deletion of ULP2 resulted in elevated sumoylation of H2B at this untranscribed region (5E, compare WT with ulp2delta). This argues that Ulp2 has a general role in reducing chromatin sumoylation levels that is not dependent on transcription. The significance of ORF-associated Ulp2 is therefore not clear, and this should be addressed.

We definitely think Ulp2 has roles that are not directly linked to transcription. Nevertheless, we believe the argument for a role of Ulp2 associated with RNAPII transcription is strong. (1) In our revision, we now include new unbiased ChIP-Seq data for Ulp2. Ulp2 is preferentially recruited to ribosomal protein genes and highly transcribed genes throughout the genome (new Fig 1G). It is particularly enriched along gene ORFs (Fig 1H and EV1B), consistent with Ulp2 protein being directly involved in transcription. (2) Parallel ChIP analysis of Ulp2 and Rpb3 (RNAPII) at the transiently transcribed *CUP1* gene shows a very tight temporal correlation between the signals of the two proteins. (4) When we performed SUMO ChIP assays (Fig 2C, F), the PCR signals were normalized to the internal control (untranscribed intergenic region), and the data showed that the modifier levels are enhanced in *ulp2Δ* cells at expressed sites compared with the untranscribed region. (5) Using ChDIP, we observed increased H2B-SUMO conjugation at *CUP1* when copper was added, but such an increase did not occur at an untranscribed locus in *ulp2Δ* cells (Fig 5E). We have now added data for *GAL1* induction by galactose (Fig EV2C). Here too, transcription induction in *ulp2Δ* cells led to increased H2B-SUMO at *GAL1* but not the 'no ORF' site. Therefore, our data show H2B-SUMO occupancy is dependent on the transcription state at these genes. Taken together, we suggest that Ulp2 is preferentially recruited to actively transcribed genes and it regulates SUMO, including H2B-SUMO, levels at those sites during transcription.

2. The abstract indicates that Ulp2 is "specifically recruited to transcriptionally active genes to control local sumoylation" and in Fig. 1E, it is claimed that Ulp2-Flag occupies the length of the three genes. However, there is no untranscribed ("non_ORF") region analysis for determining what background signal levels are, or if Ulp2 is also detected at untranscribed regions. Is an IP/INPUT ratio of 2 equal to background? If so, then ADH1 doesn't show much Ulp2 occupancy, yet ULP2 deletion has a strong effect on occupancy of RNAPII and SUMO on the ADH1 gene, and ADH1 shows the highest dependence on Ulp2 in Fig 1C. This would support an indirect role for Ulp2. At least one non-ORF region should be compared, minimally for the Ulp2-Flag ChIP.

 

The PCR signals were normalized to the internal control (untranscribed 'no ORF' region) and the input DNA for quantitation. Therefore, our results show Ulp2 enzyme is preferentially recruited to transcriptionally active genes compared with the untranscribed region (Fig 1E; to make this clearer, we had added a horizontal line to indicate the no enrichment level). Furthermore, the new genome-wide data supports the inference that Ulp2 is preferentially associated with active genes (Figs 1G, H and EV1B).

3. In WT cells, H2B-SUMO levels is about the same on constitutive genes (promoters and ORFs) and in the untranscribed/no-ORF region tested (compare Figs. 5D with "no ORF" in 5E and EV2), which argues that H2B sumoylation is actually not transcription-dependent. The claim of a link between transcription activation and H2B sumoylation is dependent on analysis of a single inducible gene. To support this claim, additional inducible genes (at least one) should be tested.

In response to the reviewer's comment, we have now also performed ChDIP for H2B-SUMO during galactose induction (Fig EV2C). These data show H2B-SUMO levels were increased at *GAL1* gene during its induction but not at the 'no ORF' control region. These data support the idea that H2B sumoylation is associated with transcription.

Minor Points

4. Can sumoylated Flag-H2B species be detected on a Flag immunoblot? To support that the sumoylated proteins, in particular the polysumoylated species, shown in the SUMO blot correspond to H2B, more of the Flag blot should be shown, including the stacking gel.

We have not been able to detect Flag-H2B-SUMO in a straight anti-Flag blot. We provide uncropped results of immunoblotting using anti-Flag antibody in SourceData Figures. Detecting protein sumoylation of specific proteins by Western is usually very difficult because of the relatively low fraction of modification; the signal in this case would also be expected to be dispersed among differentially sumoylated histone species.

5. Related to this, on the H2B IP (Fig. 4A and p.13) the manuscript refers to sumoylated and polysumoylated species detected as "histones" and not specifically H2B. This should be clarified. The samples were prepared through denaturing TCA precipitation. Are nucleosomes retained in this procedure (i.e. are other histones coIPed?), or is the IP be specific for H2B?

We also analyzed sumoylation of H4 as well as H2B in Fig EV2A, and histone H4 sumoylation yielded results similar to those seen with H2B. The data showed not only H2B but also H4 can be sumoylated and both are substrates of Ulp2. But, in this case, we agree H2B is better than histones to describe the results exactly and have changed the text accordingly.

6. In Fig. 5A, the mono-sumoylated species appears to be roughly the same size as the mono-ubiquitinated species seen in the lower blot of the same figure, suggesting that H2B sumoylation and ubiquitination are not necessarily occurring on the same H2B polypeptide. Furthermore, mono-sumoylation levels are affected by altering H2B mono-ubiquitination levels. This suggests probable crosstalk between the two H2B polypeptides in the nucleosome (i.e. ubiquitination of one subunit promotes sumoylation of the other). This should be discussed somewhere.

This is a good suggestion. We added your idea in the discussion part: "We also do not know if SUMO and ubiquitin moieties are simultaneously conjugated to the same H2B polypeptide within the nucleosome or even to the same nucleosome." (p. 23)

7. In WT cells, why is the Ulp2 occupancy level at ORFs about twice as high as for promoters of PMA1 and PYK1 in Figs. 1E, 5H, and 5I, but not in 5G, where they are about even?

The reviewer is right that we normally see roughly double the Ulp2 occupancy in the ORF regions compared to the promoters, and we are not sure of the reason for the relatively reduced ORF occupancy in 5G. The key point of the panel is that loss of *RAD6* or *BRE1* reduced the relative recruitment of Ulp2 to both promoter and ORF.

8. The subheading at the bottom on p. 17 appears to be a mistake. According to the data Ulp2

 

promotes (not blocks) recruitment of Ctk1.

Thanks for catching this! We have edited it in the revised manuscript.

9. In Fig. 6K, Rpb3 occupancy on SDH1 is not significantly affected by ULP2 deletion, whereas the other tested genes are affected. How does this correlate with the RNA-seq analysis summarized in Fig. 1B?

This is a good question. Although we tried to study the transcription of *SDH1* gene in cells lacking *ULP2* or expressing 2SUMO-fused H2B, the mechanism of its transcription may be different with other genes, *UTH1* and *ATP2*, and the ChIP signals were generally low. As we are slightly less confident of these data because of the weaker signals, we decided to remove the *SDH1* results (and we have added ChIP-Seq data).

10. On p. 13 line 2, the figure callout refers to lanes 4 and 6. It would be helpful if the lanes were labeled on the Figure.

This is a good suggestion. We added lane numbers for better visualization.

11. The data referred to on p. 13 line 3 as "not shown" should be shown or the statement should be excluded.

We agree with your suggestion, and the results of *in vivo* histone H4 sumoylation assay have now been added as Fig EV2A.

 

## References

Ahn SH, Kim M, Buratowski S (2004) Phosphorylation of serine 2 within the RNA polymerase II C-terminal domain couples transcription and 3′ end processing. *Molecular cell* **13:** 67-76

Kao C-F, Hillyer C, Tsukuda T, Henry K, Berger S, Osley MA (2004) Rad6 plays a role in transcriptional activation through ubiquitylation of histone H2B. *Genes & development* **18:** 184-195

Nathan D, Ingvarsdottir K, Sterner DE, Bylebyl GR, Dokmanovic M, Dorsey JA, Whelan KA, Krsmanovic M, Lane WS, Meluh PB, Johnson ES, Berger SL (2006) Histone sumoylation is a negative regulator in Saccharomyces cerevisiae and shows dynamic interplay with positive-acting histone modifications. *Genes Dev* **20:** 966-976

Rosonina E, Duncan SM, Manley JL (2010) SUMO functions in constitutive transcription and during activation of inducible genes in yeast. *Genes Dev* **24:** 1242-1252

Ryu HY, Lopez-Giraldez F, Knight J, Hwang SS, Renner C, Kreft SG, Hochstrasser M (2018) Distinct adaptive mechanisms drive recovery from aneuploidy caused by loss of the Ulp2 SUMO protease. *Nat Commun* **9:** 5417

Shieh GS, Pan CH, Wu JH, Sun YJ, Wang CC, Hsiao WC, Lin CY, Tung L, Chang TH, Fleming AB (2011) H2B ubiquitylation is part of chromatin architecture that marks exon-intron structure in budding yeast. *BMC genomics* **12:** 627

Suh H, Ficarro SB, Kang UB, Chun Y, Marto JA, Buratowski S (2016) Direct Analysis of Phosphorylation Sites on the Rpb1 C-Terminal Domain of RNA Polymerase II. *Mol Cell* **61:** 297-304

Van Oss SB, Shirra MK, Bataille AR, Wier AD, Yen K, Vinayachandran V, Byeon IL, Cucinotta CE, Heroux A, Jeon J, Kim J, VanDemark AP, Pugh BF, Arndt KM (2016) The Histone Modification Domain of Paf1 Complex Subunit Rtf1 Directly Stimulates H2B Ubiquitylation through an Interaction with Rad6. *Mol Cell* **64:** 815-825

Wyce A, Xiao T, Whelan KA, Kosman C, Walter W, Eick D, Hughes TR, Krogan NJ, Strahl BD, Berger SL (2007) H2B ubiquitylation acts as a barrier to Ctk1 nucleosomal recruitment prior to removal by Ubp8 within a SAGA-related complex. *Molecular cell* **27:** 275-288

 

Accepted 26th June 2019

Thank you again for submitting your final revised manuscript for our consideration. I am pleased to inform you that in light of the positive re-reviews from the three original referees (copied below), we have now accepted it for publication in The EMBO Journal.

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REFEREE REPORTS.

Referee #1:

I find the authors have adequately responded to all the points I raised. I am satisfied with the changes and agree that the quality of the paper has been greatly improved and recommend its publication in EMBO Journal.

Referee #2:

The authors have addressed all my concerns and added a substantial amount of data to bolster their statistical analyses. I have no further comments.

Referee #3:

The authors have now expanded their study, providing additional support for their conclusions along the lines of what was requested by the reviewers. In particular, they have provided convincing arguments that, in addition to a general effect of ulp2-deletion on elevated sumoylation on chromatin, it has transcription-specific effects at gene ORFs. In my opinion, the manuscript is now suitable for publication in EMBO J.

 

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## 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 justified
- 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.**

## 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? | The sample size was at least 3 to 4 for qPCR-mediated all experiments. |
| 1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used. | We didn't carry out any anamal studies. |
| 2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? | We used all of samples for analysis. |
| 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. | We selected randomlly colonies among cells streaked from frozen stock. |
| For animal studies, include a statement about randomization even if no randomization was used. | We didn't carry out any anamal studies. |
| 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. | In Fig6B, we selected randomlly three colonies for plate streaking. |
| 4.b. For animal studies, include a statement about blinding even if no blinding was done | We didn't carry out any anamal studies. |
| 5. For every figure, are statistical tests justified as appropriate? | Yes, we used two-tailed Student's t test between two samples. |
| Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. | A t-test with two samples is commonly used with small sample sizes, testing the difference between the samples when the variances of two normal distributions are not known. Therefore, we always do random sampling for assumptions. |
| Is there an estimate of variation within each group of data? | Error bars showed variation within each group. |
| Is the variance similar between the groups that are being statistically compared? | t test resutls showed statistical significance between each samples. |

## C- Reagents

## USEFUL LINKS FOR COMPLETING THIS FORM

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http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

| | |
|---|---|
| 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). | Western blot: 9E10 anti-Myc (Covance, MMS-150R), anti-Flag (Sigma, F3165), peroxidase-anti-peroxidase complex (Sigma, P1291), anti-SUMO (Li & Hochstrasser, 1999), anti-PGK (Molecular Probes, 459250) or anti-H3 (Abcam, ab1791) ChIP: IgG-Sepharose beads (GE Healthcare, 17-0969-01), anti-Flag agarose beads (Sigma, A2220), Protein G-Sepharose (GE Healthcare, 17-0618-01), anti-HA conjugated agarose (Thermo Scientific, 26182), anti-Myc conjugated agarose (Covance, AFC-150P), anti-mouse IgM antibodies coupled to agarose beads (Sigma, A4540), anti-ubiquitin antibody (BioMol, PW8810), anti-Rpb3 (BioLegend, 665004) or anti-SUMO (Rockland, 200-401-428), anti-CTD S2-P (BioLegend, 920204) |
| 7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination. | We used following yeast cell backgrounds; MHY500 (Chen, 1993, Cell), W303a/alpha (Thomas, 1989, Cell) and BY4741 (Open Biosystems) |

* 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. | We didn't carry out any anamal studies. |
| 9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments. | We didn't carry out any anamal studies. |
| 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. | We didn't carry out any anamal studies. |

## E- Human Subjects

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| 11. Identify the committee(s) approving the study protocol. | We didn't carry out any Human studies. |
| 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. | We didn't carry out any Human studies. |
| 13. For publication of patient photos, include a statement confirming that consent to publish was obtained. | We didn't carry out any Human studies. |
| 14. Report any restrictions on the availability (and/or on the use) of human data or samples. | We didn't carry out any Human studies. |
| 15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable. | We didn't carry out any Human studies. |
| 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. | We didn't carry out any Human studies. |
| 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. | We didn't carry out any Human studies. |

## 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'.<br><br>Data deposition in a public repository is mandatory for:<br>a. Protein, DNA and RNA sequences<br>b. Macromolecular structures<br>c. Crystallographic data for small molecules<br>d. Functional genomics data<br>e. Proteomics and molecular interactions | The ChIP-seq data used in this publication have been deposited in Gene Expression Omnibus (GEO) with accession GSE130623. |
| 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). | We provide analysis of ChIP-seq and data of qPCR as Dataset EV1 and EV2, respectively. |
| 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). | not applicable |
| 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. | not applicable |

## G- Dual use research of concern

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| 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. | We checked it. |