

DSS1 is required for proper Integrator–PP2A function

Corresponding Author: Dr Fei Xavier Chen

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

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Report review to the manuscript entitled « Stabilization of Integrator/INTAC by the small but versatile DSS1 protein » by Xu et al.

In this article the authors report a novel partner for DSS1 protein besides its reported function within the 26S proteasome, BRCA2 complex and the TREX-2 complex. They explore the genomic distribution of DSS1 and compare it to INTAC. They also report the structure of INTAC in complex RNA polymerase paused elongation complex (PEC) adding a missing snapshot to the RNA polymerase II transcription steps.

This article represents the third publication this year reporting a structure of DSS1 in complex with INTAC (Fianu et al. Nature 20024; Sabath et al. Mol Cell 2024), but here the focus is put on DSS1 and further analysis of DSS1 function in INTAC complex is provided in contrast to previous reports.

Overall the manuscript has some weaknesses especially regarding the role DSS1 in stabilizing INTAC function. This represents my major criticism that makes me consider the manuscript not ready to be published as it is, but requiring a revision.

Major points:

Figure 1f: The control background with an IgG pull-down is not very convincing to provide evidence that DSS1 is enriched with INTS3 and INTS5 pull-down. This experiment should be repeated.

Figure 2a, b : It is stated that the immunofluorescence signal was reduced (P7, L140) but to the term “reduced” would be more appropriate.

P7, L150: It is not true that “promoter exhibited the highest occupancy of DSS1” as Figure 2f shows that promoters represent 26.72% and Distal intergenic regions represent 28.75%. This should be taken into account and most probably modifies/attenuates conclusions.

Figure 5a: The authors mention a reduction of the protein level upon introduction of DSS1-W39R protein in comparison to WT. This is not obvious to me in the Figure 5a. A quantification would help clarifying this point.

As DSS1 is interacting with many different complexes, this intriguing ability should/could be more explored either through experiments or at least during the discussion. It could be mentioned whether the expression level of DSS1 varies upon cell-cycle, stress ... explaining the availability of the protein to associate with different complexes in different cell compartments (?).

Minor points:

Figure 1b, 1c: Enlarge the illustration as it either difficult (1b) or impossible to read the text (1c). Please also add, by a circle for example, to which complex the represented subunits belong to. This would help clarify the figure.

Figure 1e, f: It is initially intriguing that the 70-residues flag-tagged protein DSS1 migrates around 35kDa but looking at its very acidic sequence clarifies this point. It could of interest for a general audience to mention this characteristic.

Ref. 26 is now published in Mol Cell.

Reviewer #2

(Remarks to the Author)

In this manuscript, Xu et al. demonstrate that the integrator/INTAC complex, involved in the regulation of gene transcription and RNA processing, is stabilized and regulated by DSS1, a small intrinsically disordered protein that is also a component of several protein assemblies, such as the 26S proteasome and DNA repair protein BRCA2. The authors used single-molecule cryo-EM data analysis to determine the 3D structure of the human INTAC complex in association with DSS1. From this structure, they showed that the DSS1 residues responsible for binding to INTAC are different from those that bind to the 26S proteasome. Based on this observation, they designed a separation-of-function mutant that disrupts DSS1 binding to INTAC while preserving its interaction with the 26S proteasome. Using this DSS1 mutant, they demonstrated that DSS1 recruitment to chromatin is dependent on its interaction with INTAC. Next, they investigated the structure and function of DSS1 when bound to INTAC in association with the paused elongation complex (PEC), which includes RNA polymerase II (Pol II) and other proteins. While DSS1 adopts the same conformation whether bound to INTAC or INTAC-PEC, they found that DSS1-bound INTAC-PEC undergoes a stabilizing conformational change that increases the stability of INTAC-PEC, correlating with its optimal function in transcriptional regulation. This work will be of great interest to scientists in the field of transcriptional regulation. The manuscript is clear, well-written, and presents technically sound, well-executed experiments.

Points of clarification:

Is it the case that the additional density for the N terminus of INTS1 observed in DSS1-INTAC-PEC, but not in INTAC, is absent in INTAC-PEC? Could the authors confirm if this interpretation is correct?

Given that the INTAC-bound conformation of DSS1 remains unchanged in the context of INTAC-PEC, the authors could provide further discussion on the mechanism by which DSS1 contributes to the stabilization of INTAC-PEC and supports the function of INTAC in transcriptional regulation.

Reviewer #3

(Remarks to the Author)

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

Reviewer #4

(Remarks to the Author)

In this manuscript, Xu et al. describe two cryoEM structures: i) the Integrator complex (INTAC) and ii) INTAC bound to the paused RNA polymerase II elongation complex (PEC), where both structures are associated with the DSS1 protein, primarily known for its interaction with the 26S proteasome. Notably, the Integrator complex was first identified when co-purified with DSS1, and the authors now detail the nature of this interaction. DSS1 is embedded within the Integrator's core, and despite its small size (only 70 amino acids in the context of the >1.0 MDa INTAC complex), it is postulated to play a role in the INTAC stability. Based on their structural data, the authors introduced a point mutation in DSS1 (W39R) at the interface with the INTAC backbone (specifically INTS7), which impairs complex formation. This impairment manifests as reduced INTAC occupancy on chromatin and increased RNA polymerase II abundance across gene bodies, which can be attributed to decreased transcription attenuation near transcription start sites.

Overall, the manuscript is well written, and the data is presented very clearly; however, there are several major issues that require consideration:

1. The structural part of the manuscript, suggests that the authors report two new structures in this study, however they are virtually identical to the previously reported integrator structures, and differ only in DSS1 being present in the current model. A closer look at the deposited INTAC and/or INTAC-PEC cryoEM maps (EMDB: EMD-30473, EMD-13479, EMD-33741) reveals unassigned densities that align well with the DSS1 position described in this manuscript. Indeed, a recent paper by Sabath et al. (Molecular Cell 84, 2525–2541, July 11, 2024) demonstrates a good fit of the AlphaFold2 model of DSS1 to this density. In the current study, neither the overall resolution of the entire complex nor the resolution of the DSS1 itself has improved. Therefore, the same conclusions could have been drawn without the new cryoEM reconstruction presented here. For reporting integrity, these cryoEM structures should not be presented as the new DSS1-bound forms of the Integrator structures, as this could be misleading for readers unfamiliar with the previous work.

2. In Figure 5a, the authors report changes in the INTAC protein levels observed during the complementation of DSS1-depleted cells with DSS1-WT and DSS1-W39R. While this might be the case for INTS4, where a minimal effect is visible, we struggle to see any effect for other INTS. Hence, this data does not support the INTAC-destabilisation hypothesis, which should be toned down throughout the manuscript.

3. The authors examined DSS1's role in INTAC function based on its chromatin occupancy and changes of the RNAPII chromatin occupancy in DSS1-WT and DSS1-W39R cells. While this is consistent with the DSS1 being a component of the Integrator complex, it does not provide a direct link to the integrator function. It would significantly strengthen the manuscript if the authors addressed the consequences of DSS1 depletion for gene expression through transcriptomic approaches and compare them to the effects of the depletion of other Integrator subunits.

4. The exact position of DSS1 at some of the presented loci differs substantially from that of INTS3 (used as a proxy for INTAC occupancy on chromatin) (i.e. compare Fig. 2e and 5c, FAM136A and TOB2). This is somewhat confusing and should be clarified.

The data presented in this manuscript reinforces the notion that DSS1 is a core component of the Integrator complex, and its depletion has consequences similar to the depletion of other Integrator subunits. While this work will be valuable for the community, the scope and the novelty of the findings remain limited.

Reviewer #5

(Remarks to the Author)

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Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Dear authors,

I appreciate the care in which the authors addressed all my concerns and I think and hope that altogether this improved the manuscript. I have no more concern and I recommend to accept this article for publication.

Reviewer #2

(Remarks to the Author)

The authors have thoroughly addressed the points we raised. The manuscript is improved and is well-suited for publication in *Nature Communications*.

Reviewer #3

(Remarks to the Author)

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Reviewer #4

(Remarks to the Author)

We thank the authors for addressing our comments on the manuscript. The revised title more accurately reflects the overall findings, and we appreciate that the DSS1 stabilization hypothesis has been appropriately toned down in accordance with the presented data. We appreciate the effort the authors have made to address all reviewers' comments experimentally and by adding new explanatory passages, which have improved the manuscript overall.

That said, we feel that a few more improvements are necessary before the manuscript can be accepted:

1) While the authors have addressed concerns about redundancy with previously published INTAC-PEC structures in the discussion, it should also be mentioned in the results section to provide clearer context during the first presentation of the data.

2) We still believe that some of the language used in the manuscript overstates the conclusions. For example, terms like "essential" (e.g., line 34 in the abstract) remain too strong and should be moderated.

3) The relationship between Figures 2F and 2G is unclear. A more explicit explanation would help a broader audience distinguish between DSS1 association at specific genomic regions versus its average occupancy across the genome.

4) The improvements to Figure 5A, including additional quantification of depletion effects, are appreciated. Although the observed changes are not dramatic, they help make the experiment more convincing, particularly with the toned-down interpretation of the results. However, in Figure S5A, these differences are less apparent. Quantification in this figure would be beneficial for consistency and clarity.

5) References 46 and 47 refer to the same manuscript (46 is a preprint, and 47 is the peer-reviewed version). This redundancy should be corrected.

6) The last issue that escaped our attention in the previous revision concerns the low number of differentially expressed

genes caused by knock-down of individual INTAC subunits in the Perturb-seq analysis (Supplementary Fig. 5). This, together with the small overlap (e.g., only 7 DEGs intersecting DSS1 and the rest of the Integrator complex in Figure S5E), is puzzling. Depleting the INTAC components is expected to have a broader impact on gene expression. The authors should discuss and clarify why this analysis involves only a limited number of genes?

To conclude, we believe these additional clarifications should be provided to make this manuscript publishable.

Reviewer #5

(Remarks to the Author)

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We thank the reviewers for their constructive comments and criticisms that we believe has substantially improved our study. Most notably, we have performed additional proteomics studies of the highly versatile DSS1 protein across cellular compartments which demonstrates that INTAC is the major chromatin form of DSS1. Additional gene expression analysis further demonstrates that DSS1 is an important functional component of INTAC. Together with the other findings in this manuscript demonstrating the importance of DSS1 contacts within INTAC contributing to INTAC's role in chromatin occupancy and transcription by Pol II, we believe our study is the first to demonstrate that DSS1 is an essential part of Integrator/INTAC function.

Point-by-point response to reviewers:

Reviewer #1 (Remarks to the Author):

In this article the authors report a novel partner for DSS1 protein besides its reported function within the 26S proteasome, BRCA2 complex and the TREX-2 complex. They explore the genomic distribution of DSS1 and compare it to INTAC. They also report the structure of INTAC in complex RNA polymerase paused elongation complex (PEC) adding a missing snapshot to the RNA polymerase II transcription steps.

This article represents the third publication this year reporting a structure of DSS1 in complex with INTAC (Fianu et al. Nature 20024; Sabath et al. Mol Cell 2024), but here the focus is put on DSS1 and further analysis of DSS1 function in INTAC complex is provided in contrast to previous reports. Overall the manuscript has some weaknesses especially regarding the role DSS1 in stabilizing INTAC function. This represents my major criticism that makes me consider the manuscript not ready to be published as it is, but requiring a revision.

We appreciate the reviewer's acknowledgment of our additional insights into DSS1 function within the INTAC complex beyond what others have recently published. We also thank the reviewer for pointing out the potential weaknesses in our manuscript. We have now addressed these criticisms with additional experiments and analyses as well as toning down some statements for improved accuracy, which together we believe has led to a substantially improved manuscript. Below, please see our point-by-point response to the reviewer's comments.

Major points:

1. *Figure 1f: The control background with an IgG pull-down is not very convincing to provide evidence that DSS1 is enriched with INTS3 and INTS5 pull-down. This experiment should be repeated.*

As the reviewer suggested, we have optimized the Co-IP and Western blot experiments, and these optimized assays confirmed an interaction between DSS1 and INTAC subunits.

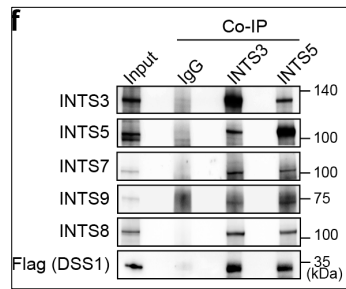


Fig.1. (f) Co-IP analysis of endogenous INTS3 and INTS5 in DSS1-Flag-dTAG cells.

2. Figure 2a, b: It is stated that the immunofluorescence signal was reduced (P7, L140) but to the term “reduced” would be more appropriate.

We agree with the reviewer and have replaced the word “eliminated” with “reduced” in the revised manuscript as suggested.

3. P7, L150: It is not true that “promoter exhibited the highest occupancy of DSS1” as Figure 2f shows that promoters represent 26.72% and Distal intergenic regions represent 28.75%. This should be taken into account and most probably modifies/attenuates conclusions.

We thank the reviewer for pointing out the inaccuracy in our description. We intended to convey that on average, DSS1 occupancy is higher at promoters compared with other genomic regions. We have revised our description of the results of Fig. 2g to clarify this distinction.

4. Figure 5a: The authors mention a reduction of the protein level upon introduction of DSS1-W39R protein in comparison to WT. This is not obvious to me in the Figure 5a. A quantification would help clarifying this point.

We agree with the reviewer that quantification would be helpful to demonstrate this reduction. We performed a simplified experiment by examining and quantifying protein levels at 0 and 36 hours following endogenous DSS1 depletion in DSS1-dTAG cells introduced with either DSS1-WT or DSS1-W39R. Our results show an approximately 30% reduction in the levels of several subunits—such as INTS7 (backbone), INTS5 (shoulder), INTS4 (endonuclease), and INTS3 (SOSS)—upon introducing DSS1-W39R. However, we acknowledge that these effects are not very large and have therefore toned down claims of INTAC subunit stability being a major function of DSS1.

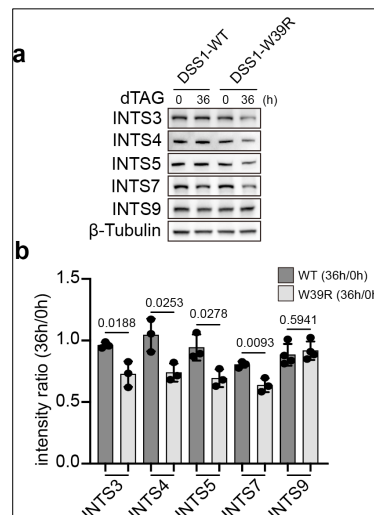
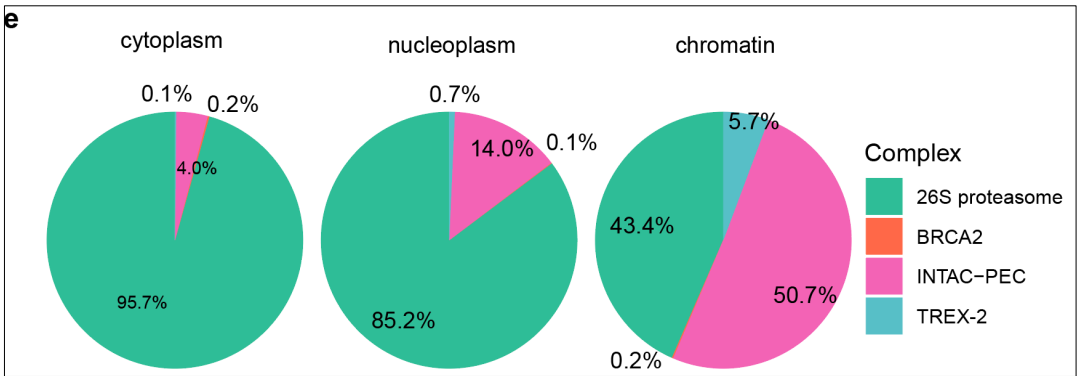


Fig. 5. (a, b) Western blotting of INTAC subunits in DSS1-WT or DSS1-W39R expressing DSS1–Flag–dTAG cells with dTAG treatment. Tubulin serves as a loading control.

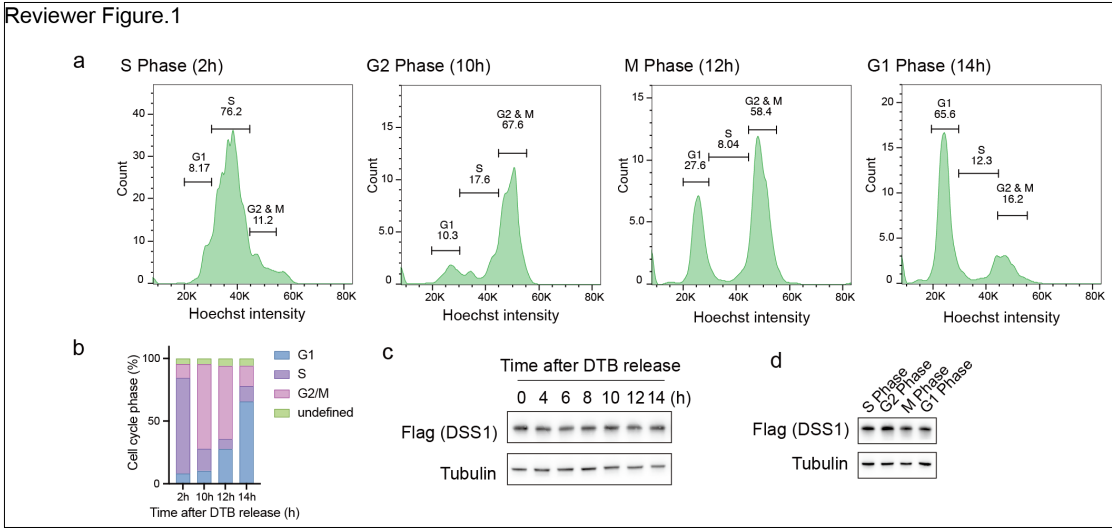
5. As DSS1 is interacting with many different complexes, this intriguing ability should/could be more explored either through experiments or at least during the discussion. It could be mentioned whether the expression level of DSS1 varies upon cell-cycle, stress ... explaining the availability of the protein to associate with different complexes in different cell compartments (?).

We thank the reviewer for raising this critical point. To better understand DSS1’s interactions with various complexes, we performed cellular fractionation followed by DSS1 immunoprecipitation and mass spectrometry analysis. Our results show that DSS1 is mainly associated with the 26S proteasome in the cytoplasm, while being more enriched in INTAC-PEC on chromatin (Supplementary Fig. 2e).

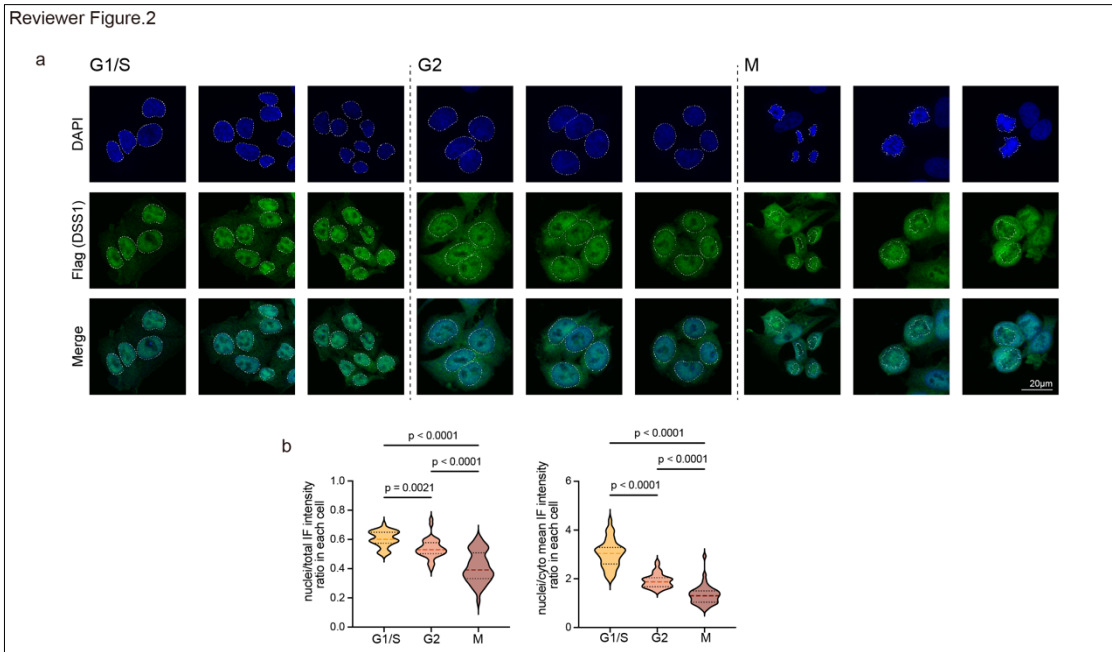


Supplementary Fig.2. (e) Pie charts showing the proportion of DSS1 interacting complexes in the cytoplasm, nucleoplasm and chromatin determined by cellular fractionation followed by immunoprecipitation and mass spectrometry analysis.

To investigate DSS1 expression across the cell cycle, we used the double-thymidine block method to synchronize cells at different stages. Western blot analyses indicate that total DSS1 expression varies only minimally across the cell cycle (Reviewer Fig. 1). Immunofluorescence results confirm that DSS1 is present in both cytoplasmic and nuclear compartments, with a higher nuclear concentration during interphase (Reviewer Fig. 2).



Reviewer Fig.1. (a) Histogram representing DNA cycle analysis after 2h, 10h, 12h, and 14h DTB washout. (b) Stacked plot displaying the proportions of cell cycle phases over time course of DTB washout. (c) Western blotting of DSS1 levels over time course of DTB washout. (d) Western blotting of DSS1 levels at different cell cycle stages.



Reviewer Fig.2. (a) Representative images showing the localization of endogenous DSS1 (green) along with the DAPI signal (blue) at different cycle stages. (b) Quantification of mean immunofluorescence (IF) intensity of DSS1 in nuclei compared with total of cells or cytoplasm of cells.

Minor points:

6. Figure 1b, 1c: Enlarge the illustration as it either difficult (1b) or impossible to read the text (1c). Please also add, by a circle for example, to which complex the represented subunits belong to. This would help clarify the figure.

We appreciate the reviewer's suggestions. In the revised manuscript, we have enlarged figures 1b and 1c to improve readability and added circles to indicate which complexes each subunit belongs to.

7. Figure 1e, f: *It is initially intriguing that the 70-residues flag-tagged protein DSS1 migrates around 35kDa but looking at its very acidic sequence clarifies this point. It could of interest for a general audience to mention this characteristic.*

We thank the reviewer for highlighting this point. The DSS1 construct used in these experiments comprises the native 70-residue DSS1, a FLAG tag, a FKBP12^{F36V} degradation tag (dTAG), and linker sequences, which together result in migration just below the 35 kDa band on SDS-PAGE. However, as the discrepancy between the predicted and observed migration is modest, we opt not to elaborate on this detail further in the revised manuscript.

8. Ref. 26 is now published in Mol Cell.

Thank you for pointing this out. We have updated the citation accordingly in the revised manuscript.

Reviewer #2 (Remarks to the Author):

In this manuscript, Xu et al. demonstrate that the integrator/INTAC complex, involved in the regulation of gene transcription and RNA processing, is stabilized and regulated by DSS1, a small intrinsically disordered protein that is also a component of several protein assemblies, such as the 26S proteasome and DNA repair protein BRCA2. The authors used single-molecule cryo-EM data analysis to determine the 3D structure of the human INTAC complex in association with DSS1. From this structure, they showed that the DSS1 residues responsible for binding to INTAC are different from those that bind to the 26S proteasome. Based on this observation, they designed a separation-of-function mutant that disrupts DSS1 binding to INTAC while preserving its interaction with the 26S proteasome. Using this DSS1 mutant, they demonstrated that DSS1 recruitment to chromatin is dependent on its interaction with INTAC. Next, they investigated the structure and function of DSS1 when bound to INTAC in association with the paused elongation complex (PEC), which includes RNA polymerase II (Pol II) and other proteins. While DSS1 adopts the same conformation whether bound to INTAC or INTAC-PEC, they found that DSS1-bound INTAC-PEC undergoes a stabilizing conformational change that increases the stability of INTAC-PEC, correlating with its optimal function in transcriptional regulation. This work will be of great interest to scientists in the field of transcriptional regulation. The manuscript is clear, well-written, and presents technically sound, well-executed experiments.

We greatly appreciate the reviewer's positive feedback in recognizing our work as being of great interest to scientists in the field of transcriptional regulation while suggesting areas for improving the impact of our study. Please find our point-by-point response below:

Points of clarification:

1. *Is it the case that the additional density for the N terminus of INTS1 observed in DSS1-INTAC-*

PEC, but not in INTAC, is absent in INTAC-PEC? Could the authors confirm if this interpretation is correct?

We thank the reviewer for raising this point. The additional density for the N terminus of INTS1 can be observed in both DSS1-INTAC-PEC and INTAC-PEC. We have clarified this point in the revised manuscript as follows:

“In our DSS1-INTAC-PEC structure, we observed an extra density corresponding to the N-terminus of INTS1, which is not visible in the standalone INTAC structure, in addition to the integration of the PEC components, including Pol II, NELF, and DSIF (Fig. 4a, b). This “tail” of INTS1 extends from the bottom of the backbone module and loops back to interact with PEC, consistent with its conformation observed in the previously reported INTAC-PEC structures^{47,53}. Overall, INTAC forms close contacts with the PEC, and features extensive interacting interfaces between them (Fig. 4a, b)”

2. Given that the INTAC-bound conformation of DSS1 remains unchanged in the context of INTAC-PEC, the authors could provide further discussion on the mechanism by which DSS1 contributes to the stabilization of INTAC-PEC and supports the function of INTAC in transcriptional regulation.

We thank the reviewer for this constructive suggestion. Within the INTAC complex, INTS1, INTS2, and INTS7 form a backbone scaffold that holds the complex together. DSS1 primarily interacts with INTS7 through broad hydrophobic contacts. Notably, this interface is positioned adjacent to the INTS2-INTS7 dimer interface. We therefore hypothesized that DSS1 stabilizes the INTAC backbone module by interacting with this region. Consistently, when this interaction was disrupted, we observed decreased enrichment of DSS1 on chromatin and impaired function of INTAC in transcriptional regulation. We have extended the Discussion section to elaborate on this point, as indicated below:

“In this study, we establish that human DSS1 is a bona fide component of the INTAC backbone module. Through our structural analysis of DSS1-INTAC, both alone and as part of a complex with the paused Pol II-DSIF-NELF elongation complex (PEC), we identify a close association between DSS1 and the INTS7 subunit of the INTAC backbone module. Notably, this interface is positioned adjacent to the INTS2-INTS7 dimer interface. We therefore propose that DSS1 stabilizes the INTAC backbone module by binding in this region. Disruption of DSS1’s interactions with INTS7 leads to reduced DSS1 enrichment on chromatin and impairs the function of INTAC in transcriptional regulation.”

Reviewer #3 (Remarks to the Author):

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Reviewer #4 (Remarks to the Author):

In this manuscript, Xu et al. describe two cryoEM structures: i) the Integrator complex (INTAC) and ii) INTAC bound to the paused RNA polymerase II elongation complex (PEC), where both structures are associated with the DSS1 protein, primarily known for its interaction with the 26S

proteasome. Notably, the Integrator complex was first identified when co-purified with DSS1, and the authors now detail the nature of this interaction. DSS1 is embedded within the Integrator's core, and despite its small size (only 70 amino acids in the context of the >1.0 MDa INTAC complex), it is postulated to play a role in the INTAC stability. Based on their structural data, the authors introduced a point mutation in DSS1 (W39R) at the interface with the INTAC backbone (specifically INTS7), which impairs complex formation. This impairment manifests as reduced INTAC occupancy on chromatin and increased RNA polymerase II abundance across gene bodies, which can be attributed to decreased transcription attenuation near transcription start sites.

Overall, the manuscript is well written, and the data is presented very clearly; however, there are several major issues that require consideration:

We thank the reviewer for the thorough evaluation of our manuscript and for recognizing its clarity and significance. We also appreciate the reviewer's constructive feedback, which we have addressed as described below:

1. The structural part of the manuscript, suggests that the authors report two new structures in this study, however they are virtually identical to the previously reported integrator structures, and differ only in DSS1 being present in the current model. A closer look at the deposited INTAC and/or INTAC-PEC cryoEM maps (EMDB: EMD-30473, EMD-13479, EMD-33741) reveals unassigned densities that align well with the DSS1 position described in this manuscript. Indeed, a recent paper by Sabath et al. (Molecular Cell 84, 2525–2541, July 11, 2024) demonstrates a good fit of the AlphaFold2 model of DSS1 to this density. In the current study, neither the overall resolution of the entire complex nor the resolution of the DSS1 itself has improved. Therefore, the same conclusions could have been drawn without the new cryoEM reconstruction presented here. For reporting integrity, these cryoEM structures should not be presented as the new DSS1-bound forms of the Integrator structures, as this could be misleading for readers unfamiliar with the previous work.

We thank the reviewer for bringing this concern to our attention. As the reviewer mentions, the unassigned density in previous maps aligns well with the DSS1 density in our structure, which was subsequently confirmed in the Sabath et al. study (although this had not been published when we submitted our manuscript to BioRxiv). We, for the first time, coexpressed DSS1 with other INTAC subunits and therefore our structures should be considered to be new, even if aligning well with previous structures resulting from fortuitously copurified endogenous DSS1. We have clarified these issues in the revised manuscript as follows:

“The detection of DSS1 in association with paused Pol II suggests its continuous engagement with INTAC throughout the premature termination process. A comparison of the DSS1–INTAC structures, with and without PEC, reveals that despite a reconfiguration in the overall structure of INTAC including activation of the RNA endonuclease module upon PEC interaction. DSS1's positioning in relation to the backbone module remains unchanged in the presence of PEC, underscoring a scaffolding role for DSS1. Supporting this notion, a density corresponding to DSS1 can also be observed in previously reported structural maps of INTAC and INTAC–PEC^{25,47,53}, which are all consistent with endogenous DSS1 stably associating with INTAC.”

2. In Figure 5a, the authors report changes in the INTAC protein levels observed during the complementation of DSS1-depleted cells with DSS1-WT and DSS1-W39R. While this might be the case for INTS4, where a minimal effect is visible, we struggle to see any effect for other INTS. Hence, this data does not support the INTAC-destabilization hypothesis, which should be toned down

throughout the manuscript.

We appreciate the reviewer highlighting this point and we have toned down the destabilization hypothesis and focus on the functional consequence of disrupting DSS1's interaction with INTAC as opposed to stability of individual subunits. We did quantify the protein levels of INTAC subunits in DSS1-depleted (DSS1-dTAG) cells introduced with DSS1-WT or DSS1-W39R and saw an approximately 30% reduction of several subunits by 36 hours post-depletion in the presence of DSS1-W39R as opposed to wild-type DSS1. However, since these effects are not particularly dramatic, we have softened our discussion of subunit stabilization in the revised manuscript.

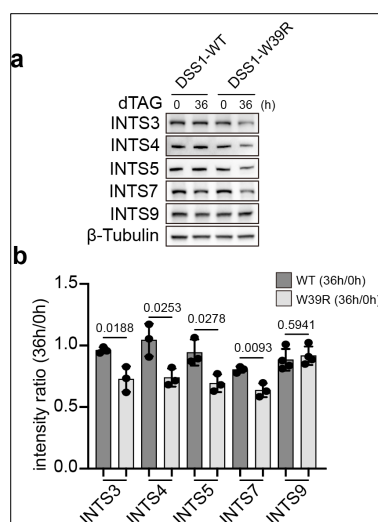
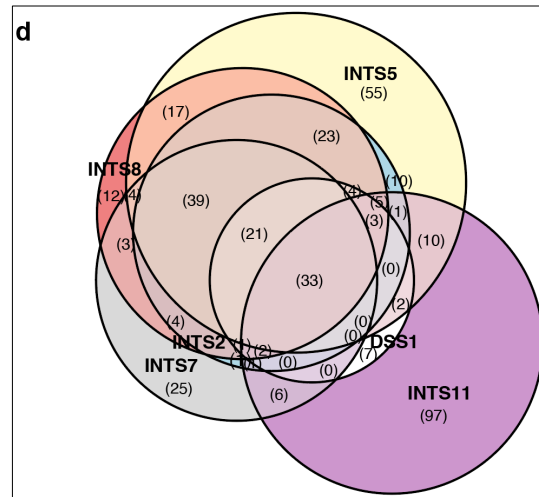


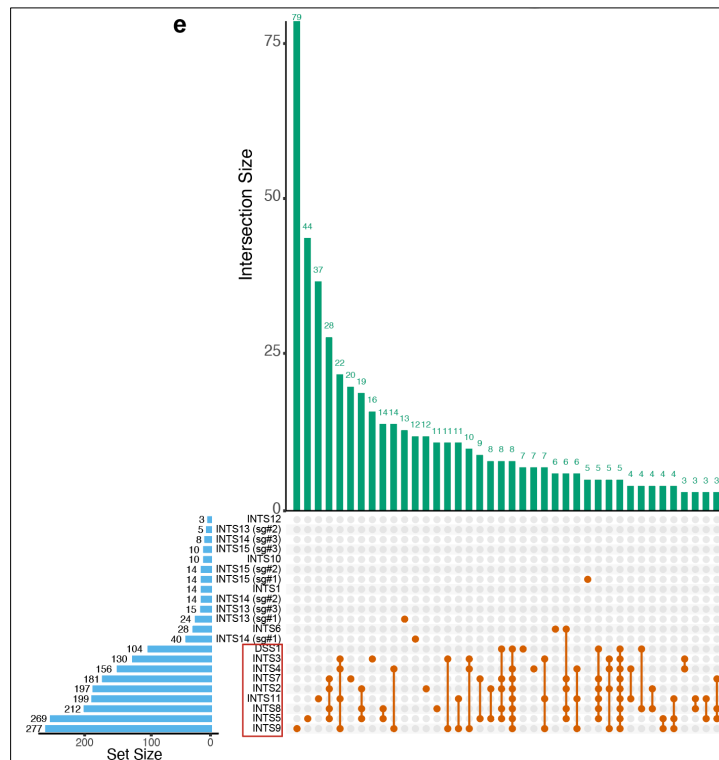
Fig. 5. (a, b) Western blotting of INTAC subunits in DSS1-WT or DSS1-W39R expressing DSS1-Flag-dTAG cells with dTAG treatment. Tubulin as loading control.

3. The authors examined DSS1's role in INTAC function based on its chromatin occupancy and changes of the RNAPII chromatin occupancy in DSS1-WT and DSS1-W39R cells. While this is consistent with the DSS1 being a component of the Integrator complex, it does not provide a direct link to the integrator function. It would significantly strengthen the manuscript if the authors addressed the consequences of DSS1 depletion for gene expression through transcriptomic approaches and compare them to the effects of the depletion of other Integrator subunits.

We thank the reviewer for this valuable suggestion. In our original manuscript, we analyzed the results of genome-scale Perturb-seq, showing that DSS1 depletion induces transcriptional changes closely resembling those seen upon depletion of INTAC "backbone" and "shoulder" subunits (Fig. 5e). To further clarify the role of DSS1 within INTAC, we compared gene expression changes triggered by DSS1 depletion to those caused by depletion of key subunits from all known DSS1-containing complexes. We found that the transcriptional profile of DSS1-depleted cells most closely resembled that of cells lacking INTAC backbone (INTS1/2/7) or shoulder (INTS5/8) subunits (Supplementary Fig.5c), and that the differentially expressed genes in DSS1-depleted cells overlapped significantly with those affected by depletion of these INTAC modules (Supplementary Fig. 5d, 5e). These results strongly suggest that the gene expression alterations observed upon DSS1 depletion primarily stem from its role in INTAC rather than other DSS1-containing complexes.



Supplementary Fig.5. (d) Venn diagram analysis of gene expression changes caused by knockdown of INTAC subunits including INTS2, INTS7, INTS8, INTS5, INTS11 and DSS1.



Supplementary Fig. 5. (e) UpSet plot displays differentially expressed genes (DEGs) set of INTAC subunits. Left bars: Number of DEGs by individual INTAC subunits perturbation, Top bars: Size of intersections across multiple INTAC subunit combinations.

4. The exact position of DSS1 at some of the presented loci differs substantially from that of INTS3 (used as a proxy for INTAC occupancy on chromatin) (i.e. compare Fig. 2e and 5c, FAM136A and TOB2). This is somewhat confusing and should be clarified.

We apologize for the confusion. We discovered an error in figure assembly, where the gene annotations for *FAM136A* and *TOB2* were misplaced in Figure 2e of the original manuscript. We have corrected these panels in the revised manuscript to accurately represent the positions of DSS1 and INTS3.

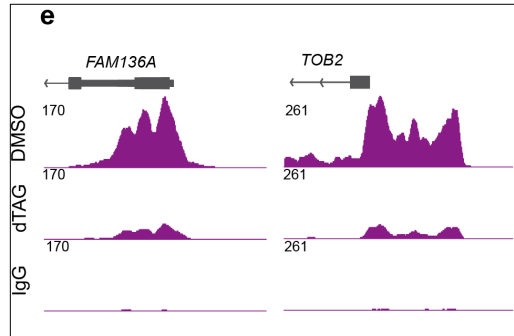


Fig.2. (e) Representative track examples showing DSS1 occupancy in DSS1–Flag–dTAG cells treated with DMSO or dTAG for 24 h.

The data presented in this manuscript reinforces the notion that DSS1 is a core component of the Integrator complex, and its depletion has consequences similar to the depletion of other Integrator subunits. While this work will be valuable for the community, the scope and the novelty of the findings remain limited.

We appreciate the reviewer’s perspective. While our study may appear overly focused, we believe it offers important new insights into the structural organization and functional role of DSS1 within INTAC and in transcriptional regulation. Our structure-guided mutagenesis approach differentiates DSS1’s role in INTAC from its functions in other DSS1-containing complexes. We believe this work lays a foundation for future investigations into how DSS1 operates across multiple essential cellular pathways and whether its functions within each complex are distinct or converge on shared biological outcomes.

We thank the reviewer again for the thorough review and constructive feedback, which have helped us to improve our manuscript. We hope that our revisions address all concerns and enhance the clarity and significance of our study.

Reviewer #5 (Remarks to the Author):

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

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Dear authors,

I appreciate the care in which the authors addressed all my concerns and I think and hope that altogether this improved the manuscript. I have no more concern and I recommend to accept this article for publication.

Reviewer #2 (Remarks to the Author):

The authors have thoroughly addressed the points we raised. The manuscript is improved and is well-suited for publication in Nature Communications.

Reviewer #3 (Remarks to the Author):

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

Reviewer #4 (Remarks to the Author):

We thank the authors for addressing our comments on the manuscript. The revised title more accurately reflects the overall findings, and we appreciate that the DSS1 stabilization hypothesis has been appropriately toned down in accordance with the presented data. We appreciate the effort the authors have made to address all reviewers' comments experimentally and by adding new explanatory passages, which have improved the manuscript overall.

That said, we feel that a few more improvements are necessary before the manuscript can be accepted:

1) While the authors have addressed concerns about redundancy with previously published INTAC-PEC structures in the discussion, it should also be mentioned in the results section to provide clearer context during the first presentation of the data.

We agree with the reviewer and now describe the presence of DSS1 in previous INTAC-PEC structures in the Results section of the revised manuscript as follows:

Lines 116-117:

“Notably, a density corresponding to DSS1 can also be found in previously reported structural maps of INTAC and INTAC–PEC^{25,47,50}, supporting the notion that endogenous DSS1 stably associates with INTAC”

2) We still believe that some of the language used in the manuscript overstates the conclusions. For example, terms like “essential” (e.g., line 34 in the abstract) remain too strong and should be moderated.

We appreciate the reviewer’s concern and have further moderated our language throughout the manuscript to avoid any potential overstatements. Our revised sentences are listed below, with the key changes highlighted in parentheses:

Lines 34-35 (we removed the word “essential”):

“Together, our findings reveal a role for the versatile DSS1 protein in supporting the structure and regulatory functions of INTAC.”

Lines 80-81 (we replaced the word “essential” with “important”):

“In this study, we highlight the role of DSS1 as an integral component of the INTAC backbone module that is important for maintaining the stability of INTAC to support its diverse functions in transcription regulation”

Lines 245-246 (we removed the word “critical”):

“Collectively, these findings underscore DSS1’s role in supporting INTAC’s multifaceted regulation of transcription.”

Lines 269-270 (we removed the word “critical”):

“Moreover, disrupting the DSS1–INTAC interaction triggers transcriptional alterations akin to those seen in INTAC deficient cells, affirming DSS1’s function in facilitating INTAC’s role in transcriptional regulation”

3) The relationship between Figures 2F and 2G is unclear. A more explicit explanation would help a broader audience distinguish between DSS1 association at specific genomic regions versus its average occupancy across the genome.

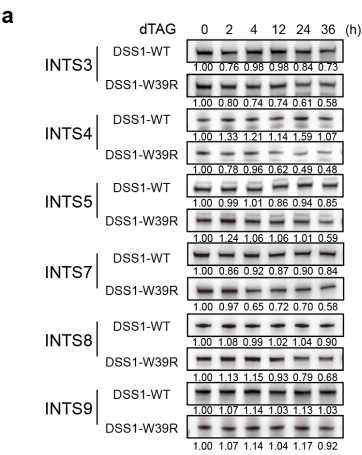
We appreciate the reviewer’s comments. In the revised manuscript, we have now specified the number of peaks for each category of genomic region in Figure 2F and clarified the purpose for calculating the average occupancy of DSS1 in Figure 2G as follows:

Lines 144-148:

“To evaluate the differential binding of DSS1 across various genomic regions, we calculated the average occupancy of DSS1 at each genomic element. Notably, although the number of DSS1-bound promoters and distal intergenic regions were comparable (14,586 vs. 15,694), we found that DSS1 occupancy is higher, on average, at promoters compared with other genomic regions (Fig. 2g)”

4) The improvements to Figure 5A, including additional quantification of depletion effects, are appreciated. Although the observed changes are not dramatic, they help make the experiment more convincing, particularly with the toned-down interpretation of the results. However, in Figure S5A, these differences are less apparent. Quantification in this figure would be beneficial for consistency and clarity.

We appreciate the reviewer’s comment and have now added quantifications for the western blotting results in Figure S5A, as suggested.



5) References 46 and 47 refer to the same manuscript (46 is a preprint, and 47 is the peer-reviewed version). This redundancy should be corrected.

We apologize for this error and have corrected it.

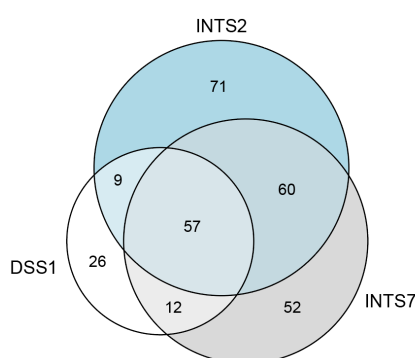
6) The last issue that escaped our attention in the previous revision concerns the low number of differentially expressed genes caused by knock-down of individual INTAC subunits in the Perturb-seq analysis (Supplementary Fig. 5). This, together with the small overlap (e.g., only 7 DEGs intersecting DSS1 and the rest of the Integrator complex in Figure S5E), is puzzling. Depleting the INTAC components is expected to have a broader impact on gene expression.

The authors should discuss and clarify why this analysis involves only a limited number of genes?

We thank the reviewer for raising this important point. As Perturb-seq relies on single-cell RNA sequencing (scRNA-seq) to quantify gene expression, it is inherently limited by the inefficient capture of transcripts that results in a low coverage of expressed genes (and consequently a very low number of differentially expressed genes).

Regarding the limited overlap of DEGs across all INTAC subunits, we suspect that this reflects the differential impact of distinct INTAC modules on gene expression. For example, we previously reported that the INTAC endonuclease and phosphatase modules play distinct roles: the endonuclease module primarily promotes promoter-proximal Pol II termination, with its loss exerting differential effects on highly versus lowly expressed genes, whereas the phosphatase module mainly suppresses Pol II release and broadly attenuates transcription (Hu et al., PMID: 37080207). Other studies have also highlighted the highly modular nature of INTAC in both its assembly and transcriptional regulation (PMID: 36180603, 37995689, 38823386, 38906142, 39032489, 39592860).

Given that DSS1 directly interacts with INTS7 and is located adjacent to the INTS2–INTS7 dimer interface, we compared the DEGs upon DSS1 loss with those observed following depletion of INTS7 or INTS2. Notably, 75% (78 out of 104) of DEGs induced by DSS1 loss overlapped with those caused by loss of INTS7 or INTS2. This finding supports the notion that the gene expression changes observed upon DSS1 depletion primarily stem from its role within INTAC (Reviewer Figure 1).



To conclude, we believe these additional clarifications should be provided to make this manuscript publishable.

We sincerely thank the reviewer for the thoughtful and constructive suggestions that have helped to improve the manuscript.

Reviewer #5 (Remarks to the Author):

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