

H3K4me1 directs H3K36me2 and H3K36me3 deposition in land plants

Corresponding Author: Professor Aiwu Dong

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)

I reviewed this paper in another journal before transferred to Nature Communications. Because the comments I raised before have not been incorporated to the manuscript, I mostly copy the previous comments below.

This paper by Wu et al describes a PHD domain protein Ehd3 in rice, which specifically binds to histone H3K4me1. They analyzed structure of Ehd3-H3K4me1 peptide and showed a unique binding mode of Ehd3 to H3K4me1. They further showed that Ehd3 bind to a H3K36 methyltransferase SDG724, and the loss of function of these genes showed similar loss of H3K36me3 where H3K4me1 localizes in gene body. Overall this paper is interesting and shows novel mechanism possibly connecting H3K4me1 and H3K36me3, co-localizing histone marks in land plants. The data are solid and well written. However, I have concern about the main conclusion of this paper “H3K4me1 directs H3K36me2/3 in land plants” is still overstatement. Especially the role of Ehd3 and SDG724 in this potential pathway. I summarize my specific comments below.

1. In the beginning of the discussion section the authors stated that “In this study, we identified a specific H3K4me1 reader protein, Ehd3, and revealed a crucial role of H3K4me1 in facilitating H3K36me2/me3 deposition in rice.” However, authors did not show direct evidence that H3K4me1 is facilitating H3K36me2/3 in rice. Experimental evidence should be presented or tone down the statement. Same statement is in several places such as title and abstract.
2. In the next sentence “Ehd3 recognizes H3K4me1 through its tandem PHD finger domain and recruits the H3K36 methyltransferase SDG724 to H3K4me1-enriched chromatin regions.” To state this, the authors should perform SDG724 ChIP-seq in ehd3 mutant background, and hopefully, in the mutant that lose H3K4me1.
3. L308-310, “Collectively, these results indicated that Ehd3 and SDG724 together bind to genes enriched with H3K4me1 and promote deposition of H3K36me2 and H3K36me3 (Fig. 5d and Extended Data Fig. 6e).” To claim that the binding of Ehd3 to H3K4me1 is important for H3K36me2/3 deposition, the authors should perform H3K36me2/3 ChIP-seq in HA-Ehd3mu/ehd3-1 plants and compare it with wild type and ehd3-1 plants.
4. This paper presents a lot of data in the discussion section, such as Extended data 8-10. I suggest to move the description of data in the results section.
5. The patterns of Ehd3 and SDG724 localizations are bit different (Fig. 5a and Extended Data Fig. 6d). Again, the effect of ehd3 mutation on SDG724 localization may support the authors’ claim.
6. Similar to point 5, L293 says “SDG724 exhibited a notable co-occupancy with Ehd3 at H3K4me1-enriched genes”, but heatmaps are not enough to show co-occupancy. Formal analysis of co-occupancy is needed.
7. Does IP-MS experiment in Fig. 4a contain negative control such as non-transgenic plants to show that the detected proteins are specifically bound to the bait proteins?
8. How is the experiment in Fig. 5g reproduced? Results of replicate experiments should be presented in Supplementary data.
9. The author describes the potential role of H3K36me3 in repressing cryptic transcription start. To test this possibility, studying transcription start by such as TSS-seq in sdg724 and ehd3 mutants will be needed.

Other comments

1. Around L184-189, a lot of abbreviation such as NZ or OD1 without explanation. Explanation is needed.

2. Line 201-203, "the Ala substitution for each H3K4me1 binding-pocket residue markedly reduced the affinity of the Ehd3 tandem PHD finger for the H3K4me1 peptide (Fig. 3g and Supplementary Table 2)." Are D496 and Y446 H3K4me1 binding-pocket? Looks like they are interacting with H3R2. Explanation for this results will be helpful.
3. Line 229, "a 4-bp deletion at target site 1 and a 1-bp insertion at target site 2 (ehd3-4)" This will make 3-bp deletion in total and frame will be normal. Explanation of why this mutant show loss-of-function phenotype will be helpful.
4. Line 277, "Significant overlap". Please indicate statistical significance for overlap if state so.
5. Line 290-291, "As a control, the H3 distribution in these mutants was comparable to that in NiP (Extended Data Fig. 6c)." Why H3 data is shown only by heatmaps and all gene metaplots, which do not tell if pattern is unaltered or not in mutants? Showing scatter plots like Fig. 4i will be preferable.
6. Extended Data Fig. 7a. Is this based on gene-by-gene? Or based on fixed length bin such as 100bp?

Reviewer #2

(Remarks to the Author)

In this manuscript, Wu et al. investigate the connection between H3K4me1 and the deposition of H3K36me2 and H3K36me3 in plants. The authors show that rice Ehd3 interacts with H3K4me1 and the H3K36 methyltransferase SDG724, and that Ehd3 and SDG724 act in the same pathway and co-localize with H3K4me1. They further demonstrate that the interaction between H3K4me1 and Ehd3 homologs is conserved in land plants. Overall, the results are convincing, and the findings are both interesting and significant. My suggestions for improving the manuscript are as follows:

1. Results from Extended Data Figures 8 and 9 should be described in the Results section rather than the Discussion.
2. The authors are suggested to test the interaction between Ehd3 homolog proteins in Arabidopsis and the H3K36 methyltransferases (e.g., SDG8) to examine whether the interaction between the H3K4me1 reader and H3K36 methyltransferase is conserved in other plant species.
3. Lines 277–279: Please provide P-values to support the claim of "significant overlap".
4. Lines 326–330: In addition to the genome browser view, a correlation analysis should be performed to strengthen the conclusion.

Reviewer #3

(Remarks to the Author)

Histone modifications play critical roles in eukaryotic gene regulation. Among the multiple common histone modifications, H3K4me1 is relatively less studied. It is associated with enhancers in animals but not plants. To investigate the functional roles of H3K4me1 in plants, Wu et al. performed comprehensive studies in rice and later extended to land plants in general. The authors first analyzed the H3K4me1 patterns in multiple species and found a unique distribution of H3K4me1 in land plants. A PHD finger protein Ehd3 was later identified as the H3K4me1 reader. The structural studies successfully revealed the molecular basis for the specific recognition of H3K4me1 by Ehd3, and the structure-based mutagenesis studies confirmed the H3K4me1 reading is essential for the in vivo function of Ehd3. Further, the H3K36 MTase SDG724 was identified as an Ehd3 binding factor that links H3K4me1 reading to H3K36me deposition. Overall, this study revealed an important and unique interplay between two histone modifications in land plants. I support the publication of this paper with the following minor concerns.

L31 and 53, H3 means histone 3. So, please change histone H3 directly to histone 3.

L171-172, it is not rigorous to say that the hydrogen bond exists between the N and O atoms, as there is no hydrogen. I would like to suggest that the author change the N and O atoms here to the amino and carbonyl groups, respectively. The following descriptions should also be revised to prevent using the atom but use the group to describe the hydrogen bonds. Fig. 4f, it looks like there is no significant difference between II and IV, and III and IV. Please analyze the differences to confirm.

L284-285, why analyze H3K36me here? Because SDG724 is an H3K36 MTase? Please add the short sentence here to describe the logic for analyzing H3K36me. Or else, the introduction of H3K36 becomes strange here.

L334, why SDG725? Please have a very brief introduction for SDG725, especially about the CW domain. A potential way is to move the sentence of L341-345, the introduction of the CW domain of SDG8 and SDG725, to L334.

Does SDG724 have a CW domain like SDG725? If yes, please give the details. If not, why could the H3K4me1 NCP stimulate the activity of SDG724? Please have a short discussion about this issue.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors provided satisfactory new data addressing my previous comments. The paper is now very much suited for the publication in Nature Communications. Congratulations for the great piece of work. I only have a suggestion on the title of the paper. I suggest to make title more informative and accurate; such as "The H3K4me1 reader protein EHD3 mediates H3K36me2 and H3K36me3 by recruiting the H3K36me writer SDG724 in rice" or "The H3K4me1 reader protein EHD3 mediates H3K4me1–H3K36me3 co-occupancy in land plants" or similar.

Reviewer #2

(Remarks to the Author)

The authors have addressed my concerns. I have no further comments.

Reviewer #3

(Remarks to the Author)

Most of my concerns have been precisely addressed. A small error is listed below.

Line 175, it is correct that here is “amino group”. But in Line 178, it should be “amide group” of Arg2, but not “amino group”.

Open Access This Peer Review File is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

AUTHOR RESPONSES to REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

I reviewed this paper in another journal before transferred to Nature Communications. Because the comments I raised before have not been incorporated to the manuscript, I mostly copy the previous comments below.

This paper by Wu et al describes a PHD domain protein Ehd3 in rice, which specifically binds to histone H3K4me1. They analyzed structure of Ehd3-H3K4me1 peptide and showed a unique binding mode of Ehd3 to H3K4me1. They further showed that Ehd3 bind to a H3K36 methyltransferase SDG724, and the loss of function of these genes showed similar loss of H3K36me3 where H3K4me1 localizes in gene body. Overall this paper is interesting and shows novel mechanism possibly connecting H3K4me1 and H3K36me3, co-localizing histone marks in land plants. The data are solid and well written. However, I have concern about the main conclusion of this paper “H3K4me1 directs H3K36me2/3 in land plants” is still overstatement. Especially the role of Ehd3 and SDG724 in this potential pathway. I summarize my specific comments below.

Response: We thank you for your interest in this work. And we thank you for your helpful comments that have improved our manuscript and led to the inclusion of new data. We have addressed the specific comments as presented below.

1. In the beginning of the discussion section the authors stated that “In this study, we identified a specific H3K4me1 reader protein, Ehd3, and revealed a crucial role of H3K4me1 in facilitating H3K36me2/me3 deposition in rice.” However, authors did not show direct evidence that H3K4me1 is facilitating H3K36me2/3 in rice. Experimental evidence should be presented or tone down the statement. Same statement is in several places such as title and abstract.

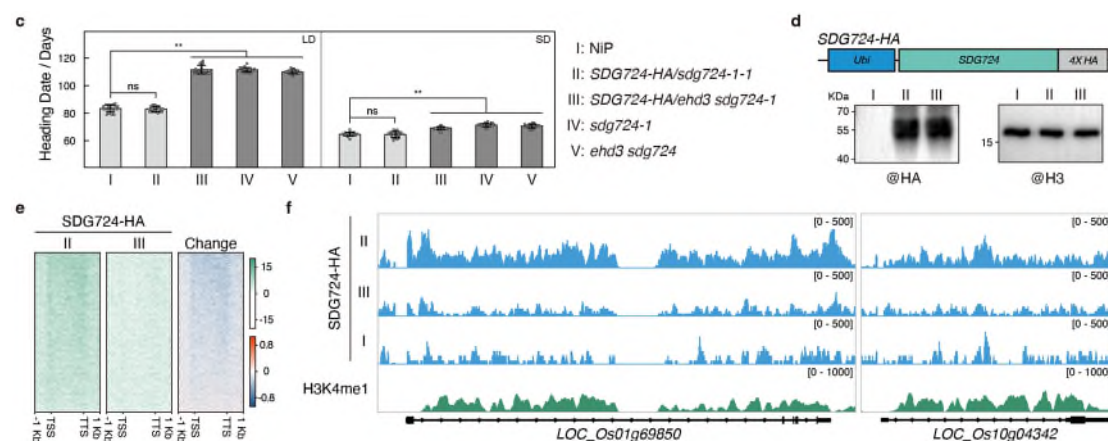
Response: Following your suggestion, we have toned down our statements in the revised abstract and discussion. In the revised manuscript, we clarified that our findings suggest a crucial role for H3K4me1 in directing H3K36me2/me3 deposition in the examined land plants (Lines 43 and 83).

As you suggested, we have provided new experimental evidence in the revised manuscript, and we think our data strongly support the function of H3K4me1 in directing H3K36me2/me3 establishment in rice. 1) Loss of the H3K4me1 reader Ehd3 or the H3K36 methyltransferase SDG724 leads to similar changes in plant phenotype, transcriptome, and epigenome, especially genome-wide changes of H3K36me2/me3 distributions (Figure 4). 2) As you suggested, we performed CUT&Tag assay to test the genome-wide distribution of SDG724 in the *ehd3-1* mutant background and found that SDG724 failed to enrich at its target regions due to the absence of Ehd3, indicating that Ehd3 is required for SDG724 to bind to chromatin (Extended Data Fig. 8 c-f). 3) Also following your comments, we performed ChIP-seq of H3K36me2/me3 in *HA-Ehd3mu/ehd3-1-1*, *HA-Ehd3/ehd3-1-1*, and WT plants, showing that the site-mutated Ehd3 (Ehd3mu), which lost its binding capacity to H3K4me1, failed to recover the H3K36me2/me3 profiles in the *HA-Ehd3mu/ehd3-1-1* plants, while the wild-type Ehd3 did in the *HA-Ehd3/ehd3-1-1* plants (Extended Data Fig. 9a). Gene-by-gene analysis further confirmed that the site-mutated Ehd3 failed to rescue the aberrant H3K36me2/me3 distributions in the *ehd3-1* mutant background (Extended Data Fig. 9b, c). 4) We have also examined the published ChIP-seq data of plants and confirmed the colocalization of H3K4me1 and H3K36me3 in other land plants including *Eutrema salsugineum*, *Populus trichocarpa*, *Phaseolus vulgaris*, *Asparagus officinalis*, *Brachypodium distachyon*, *Setaria viridis*, *Sorghum bicolor*, and *Zea mays*, further supporting our conclusion (Extended Data Fig. 10d).

Together, all these results proved that Ehd3-binding to H3K4me1 is necessary for SDG724 to establish H3K36me2/me3 *in planta*, supporting that Ehd3 recognizes H3K4me1 and recruits SDG724 to H3K4me1-marked chromatin to promote the deposition of H3K36me2 and H3K36me3 in rice.

2. In the next sentence “Ehd3 recognizes H3K4me1 through its tandem PHD finger domain and recruits the H3K36 methyltransferase SDG724 to H3K4me1-enriched chromatin regions.” To state this, the authors should perform SDG724 ChIP-seq in *ehd3* mutant background, and hopefully, in the mutant that lose H3K4me1.

Response: We thank you for your helpful comments. We have generated the *SDG724-HA/sdg724-1-1* and *SDG724-HA/ehd3 sdg724-1* transgenic plants (Extended Data Fig. 8 c, d) and performed CUT&Tag assay to examine the genome-wide distributions of SDG724 in the *sdg724-1* and *ehd3 sdg724* mutant plants, respectively. In *SDG724-HA/sdg724-1-1* plants, SDG724 was predominantly enriched at H3K4me1-marked genes, similarly as in the *YFP-SDG724/NiP* plants. However, in *SDG724-HA/ehd3 sdg724-1* plants, which lack Ehd3, SDG724-binding to chromatin was almost abolished (Extended Data Fig. 8 e, f). These results support the conclusion that Ehd3 is required for recruiting SDG724 to H3K4me1-occupied chromatin regions. We have now included the new data in our revised manuscript (Lines 350–353, Extended Data Fig. 8 c-f).

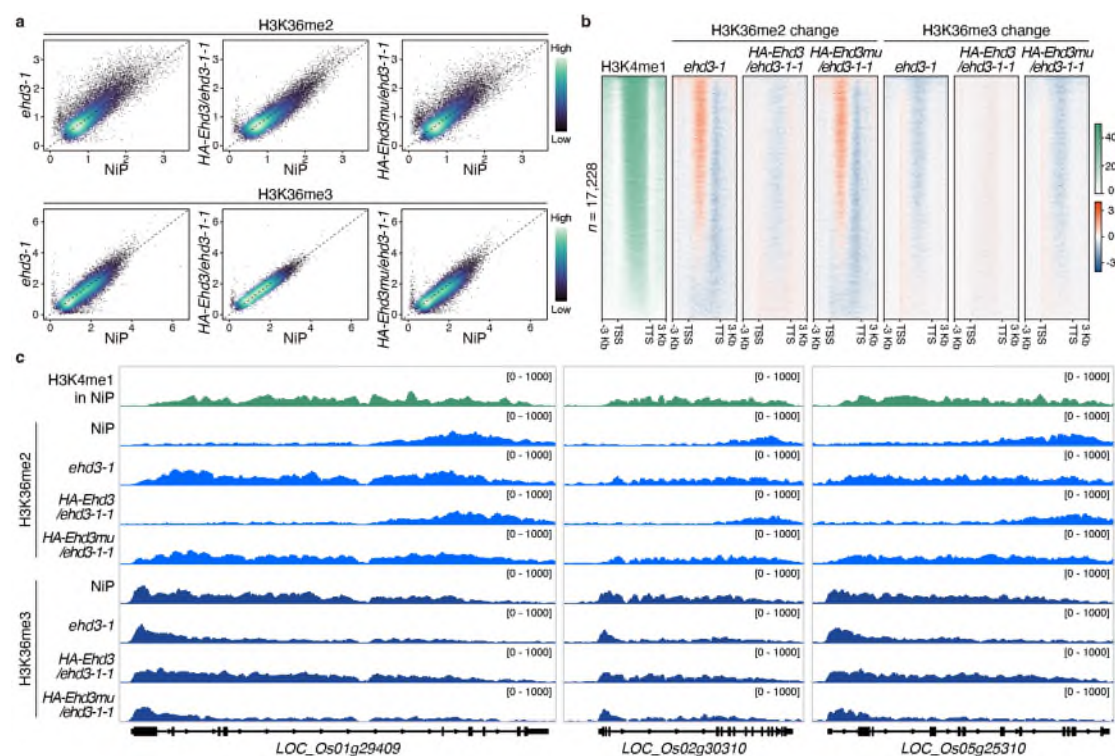


Extended Data Fig. 8 c-f. Change of SDG724 enrichment in the absence of *Ehd3*. **c**, Histograms showing days to heading in NiP (I), *SDG724-HA/sdg724-1-1* (II), *SDG724-HA/ehd3 sdg724-1* (III), *sdg724-1* (IV) and *ehd3 sdg724* (V) plants under long-day (LD) and short-day (SD) conditions. Values are shown as means $\pm$ standard deviation of 20 individual plants. Statistical significances were determined by Tukey's multiple comparisons test: ** p -value < 0.01 ; ns (not significant). **d**, Schematic representation showing the *Ubi::SDG724-HA* construct. The western blot shows comparable levels of SDG724-HA protein in the *SDG724-HA/sdg724-1-1* (II) and *SDG724-HA/ehd3 sdg724-1* (III) plants. **e**, Heatmaps showing SDG724 levels within H3K4me1-occupied genes from 1 kb upstream of TSS to 1 kb downstream of TTS in the *SDG724-HA/sdg724-1-1* (II) and *SDG724-HA/ehd3 sdg724-1* (III) plants. All genes are sorted based on enrichment of H3K4me1 identified in NiP. **f**, Integrative Genomics Viewer images showing CUT&Tag signals in NiP (I), *SDG724-HA/sdg724-1-1* (II), and *SDG724-HA/ehd3 sdg724-1* (III) plants, as well as H3K4me1 in NiP.

3. L308-310, “Collectively, these results indicated that Ehd3 and SDG724 together bind

to genes enriched with H3K4me1 and promote deposition of H3K36me2 and H3K36me3 (Fig. 5d and Extended Data Fig. 6e).” To claim that the binding of Ehd3 to H3K4me1 is important for H3K36me2/3 deposition, the authors should perform H3K36me2/3 ChIP-seq in *HA-Ehd3mu/ehd3-1* plants and compare it with wild type and *ehd3-1* plants.

Response: We thank you for your helpful comments. Following your suggestion, we performed H3K36me2/me3 ChIP-seq in the *HA-Ehd3/ehd3-1-1* and *HA-Ehd3mu/ehd3-1-1* plants, showing that the introduction of *HA-Ehd3mu*, encoding site-mutated Ehd3 protein lacking the binding capacity to H3K4me1, failed to rescue the H3K36me2/me3 profiles in the *ehd3-1* background, while *HA-Ehd3* did, and *HA-Ehd3/ehd3-1-1* plants displayed similar H3K36me2/me3 profiles as WT. All these results proved that Ehd3-binding to H3K4me1 is necessary for H3K36me2/me3 establishment *in planta*, supporting that Ehd3 recognizes H3K4me1 and recruits SDG724 to H3K4me1-marked chromatin to promote the deposition of H3K36me2 and H3K36me3 in rice. We have included the new data in our revised manuscript (Lines 357–365, Extended Data Fig. 9a-c).



Extended Data Fig. 9 a-c. Changes of H3K36me2 and H3K36me3 levels in the *ehd3-1*, *HA-Ehd3*, and *HA-Ehd3mu* backgrounds.

***Ehd3/ehd3-1-1* and *HA-Ehd3mu/ehd3-1-1* plants.** **a**, Scatter plots comparing H3K36me2 and H3K36me3 levels between the wild-type NiP and the *ehd3-1*, *HA-Ehd3/ehd3-1-1*, and *HA-Ehd3mu/ehd3-1-1* plants. **b**, Heatmaps showing changes in H3K36me2 and H3K36me3 levels (BPKM) within H3K4me1-enriched genes, compared with the wild-type NiP, from 3 kb upstream of TSS to 3 kb downstream of TTS in the *ehd3-1*, *HA-Ehd3/ehd3-1-1*, and *HA-Ehd3mu/ehd3-1-1* plants. All genes are sorted based on enrichment of H3K4me1 (BPKM) identified in the wild-type NiP. Numbers of genes are shown on the left. **c**, Integrative Genomics Viewer images of ChIP-seq data showing H3K4me1 level in the wild-type NiP, as well as H3K36me2 and H3K36me3 levels in NiP, *ehd3-1*, *HA-Ehd3/ehd3-1-1*, and *HA-Ehd3mu/ehd3-1-1* plants.

4. This paper presents a lot of data in the discussion section, such as Extended data 8-10. I suggest to move the description of data in the results section.

Response: Following your suggestion, we have moved the description of Extended data 8-9 to the Results section, and we prefer to keep the former Extended data 10 in the discussion section in our revised manuscript (Lines 311–339, Figure 5).

5. The patterns of Ehd3 and SDG724 localizations are bit different (Fig. 5a and Extended Data Fig. 6d). Again, the effect of *ehd3* mutation on SDG724 localization may support the authors' claim.

Response: Following your suggestion, we performed CUT&Tag assay in the *SDG724-HA/sdg724-1-1* and *SDG724-HA/ehd3 sdg724-1* transgenic plants. Introduction of *SDG724-HA* rescued the phenotypes of the *sdg724-1* single mutant but not of the *ehd3 sdg724* double mutant. CUT&Tag data showed that due to the absence of Ehd3, SDG724-binding to chromatin was nearly abolished in the *SDG724-HA/ehd3 sdg724-1* plants compared with the *SDG724-HA/sdg724-1* plants. These results strongly support the notion that Ehd3 is necessary for recruiting SDG724 to chromatin. We have included the new data in our revised manuscript (Lines 350–353, Extended Data Fig. 8 c-f).

6. Similar to point 5, L293 says “SDG724 exhibited a notable co-occupancy with Ehd3 at H3K4me1-enriched genes”, but heatmaps are not enough to show co-occupancy. Formal analysis of co-occupancy is needed.

Response: Following your suggestion, we have now performed a formal analysis of the

co-occupancy of Ehd3, SDG724, and H3K4me1 modification. We identified 15,967 genes occupied by Ehd3 and 17,070 genes occupied by SDG724, of which 10,325 genes were co-occupied by both Ehd3 and SDG724. Notably, these overlapping genes were significantly enriched of H3K4me1 modification. We have included the analysis in our revised manuscript (Lines 342–346, Fig. 6a).

7. Does IP-MS experiment in Fig. 4a contain negative control such as non-transgenic plants to show that the detected proteins are specifically bound to the bait proteins?

Response: Indeed, we have used the transgenic plants expressing *YFP* alone driven by the *Ubiquitin* promoter as the negative control in IP-MS experiments. This control confirmed that the detected proteins are specifically bound to the bait proteins. We have now included the negative control information in the revised manuscript (Lines 270–272 and Supplementary Table 6).

8. How is the experiment in Fig. 5g reproduced? Results of replicate experiments should be presented in Supplementary data.

Response: Following your suggestion, we have repeated the histone methyltransferase assay and included the data in our revised manuscript (Fig. 6h and Extended Data Fig. 10f). The results confirmed that both Ehd3 and H3K4me1 modification are required to stimulate the catalytic activity of SDG724.

9. The author describes the potential role of H3K36me3 in repressing cryptic transcription start. To test this possibility, studying transcription start by such as TSS-seq in *sdg724* and *ehd3* mutants will be needed.

Response: We agree with you that analyzing transcription start sites using approaches such as TSS-seq in the *ehd3-1* and *sdg724-1* mutants will be insightful to understand the potential role of H3K36me3 in regulation of cryptic transcription initiation in plants. However, this is not the main scope of the current study, and we hope to report the related results in our future work.

Other comments

1. Around L184-189, a lot of abbreviation such as NZ or OD1 without explanation. Explanation is needed.

Response: Following your suggestion, we have revised the relevant descriptions by referring to the functional groups instead of individual atoms in our revised manuscript (Lines 175–203), which will be more approachable for all readers. We would like to explain that ‘NZ’ and ‘OD1’ are not context-specific abbreviations, but standard atomic nomenclature in structural biology, as defined by IUPAC-IUBMB (<https://iupac.qmul.ac.uk/AminoAcid/>).

2. Line 201-203, “the Ala substitution for each H3K4me1 binding-pocket residue markedly reduced the affinity of the Ehd3 tandem PHD finger for the H3K4me1 peptide (Fig. 3g and Supplementary Table 2).” Are D496 and Y446 H3K4me1 binding-pocket? Looks like they are interacting with H3R2. Explanation for this results will be helpful.

Response: We thank you for your helpful comments. Indeed, both Y446 and D496 are interacting with H3R2. To avoid possible confusion, we revised the description as “Consistent with the structural observation, mutation of H3R2-interacting residues (Tyr446 and Asp496) or H3K4me1 binding-pocket residues (His441, Tyr471, Asp486, and Ile489) either abolished or markedly reduced the binding affinity of the Ehd3 tandem PHD finger to the H3K4me1 peptide (Fig. 3g and Supplementary Table 2), which further confirmed that both H3R2-binding and H3K4me1-binding residues are critical for H3K4me1 recognition by Ehd3” in our revised manuscript (Lines 206–211).

3. Line 229, “a 4-bp deletion at target site 1 and a 1-bp insertion at target site 2 (*ehd3-4*)” This will make 3-bp deletion in total and frame will be normal. Explanation of why this mutant show loss-of-function phenotype will be helpful.

Response: Following your suggestion, we have added an explanation in our revised manuscript (Lines 238–239). Indeed, in the *ehd3-4* allele, a 4-bp deletion combined with a 1-bp insertion resulted in a net 3-bp deletion. However, this frameshift led to amino acid changes within the Ehd3 protein (residues 52–92; see Fig. R1 below), which

could abolish the function of Ehd3.

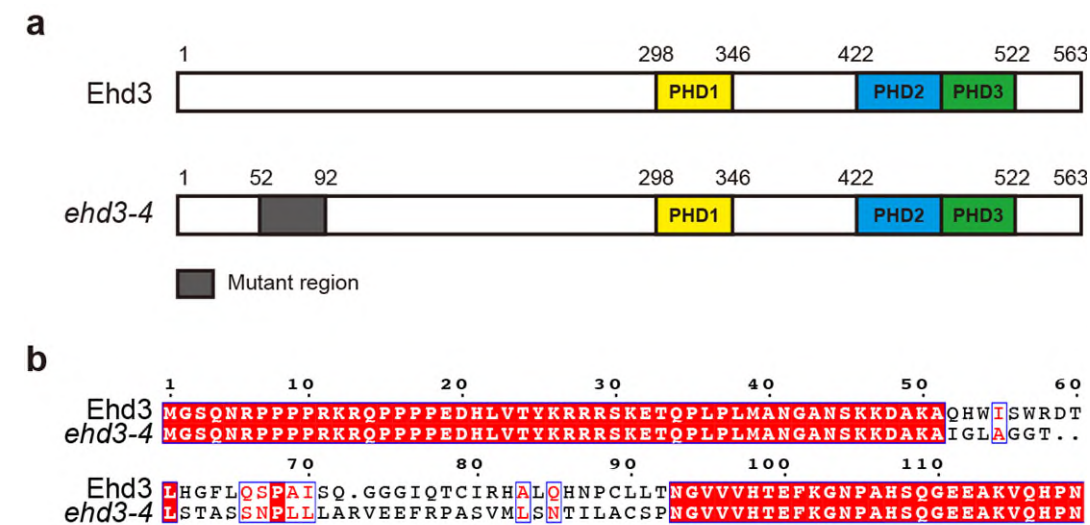


Figure R1: Mutation of Ehd3 protein in *ehd3-4* allele. **a**, Schematic representation of Ehd3 protein expressed in the wild-type and *ehd3-4* mutant plants. **b**, Sequence alignment of Ehd3 protein expressed in the wild-type and *ehd3-4* mutant plants within the mutated regions. Strictly conserved residues are indicated by white text with red background. Residues that exhibit similarity are indicated by red text with blue frame.

4. Line 277, “Significant overlap”. Please indicate statistical significance for overlap if state so.

Response: Following your suggestion, we have performed Fisher’s exact test and provided the *p*-values in the revised figures to support significance for overlap (Extended Data Fig. 5b, c).

5. Line 290-291, “As a control, the H3 distribution in these mutants was comparable to that in NiP (Extended Data Fig. 6c).” Why H3 data is shown only by heatmaps and all gene metaplots, which do not tell if pattern is unaltered or not in mutants? Showing scatter plots like Fig. 4i will be preferable.

Response: Following your suggestion, we have added the scatter plots of H3 data, supporting that H3 distribution in the mutants was comparable to that in NiP (Extended Data Fig. 6d).

6. Extended Data Fig. 7a. Is this based on gene-by-gene? Or based on fixed length bin

such as 100bp?

Response: Following your suggestion, we have revised the figure legend to provide a detailed description for the analysis (Extended Data Fig. 10a). Specifically, all genes were scaled to fixed length and then processed for histone modification quantification, Z-normalized, and Pearson correlation coefficients calculation.

Reviewer #2 (Remarks to the Author):

In this manuscript, Wu et al. investigate the connection between H3K4me1 and the deposition of H3K36me2 and H3K36me3 in plants. The authors show that rice Ehd3 interacts with H3K4me1 and the H3K36 methyltransferase SDG724, and that Ehd3 and SDG724 act in the same pathway and co-localize with H3K4me1. They further demonstrate that the interaction between H3K4me1 and Ehd3 homologs is conserved in land plants. Overall, the results are convincing, and the findings are both interesting and significant. My suggestions for improving the manuscript are as follows:

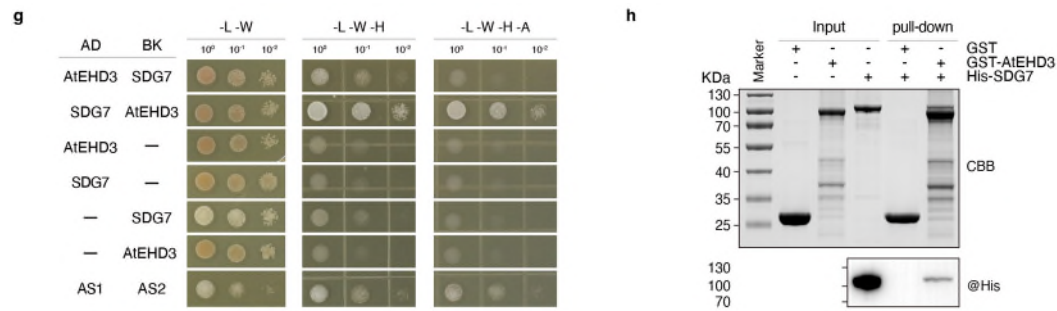
Response: We thank you for your helpful comments that have improved our manuscript and led to the inclusion of new data. We have addressed the specific comments as presented below.

1. Results from Extended Data Figures 8 and 9 should be described in the Results section rather than the Discussion.

Response: Following your suggestion, we have described Extended Data Figures 8 and 9 in the Results section and have combined part of the data into a main figure (Lines 311–339, Figure 5).

2. The authors are suggested to test the interaction between Ehd3 homolog proteins in Arabidopsis and the H3K36 methyltransferases (e.g., SDG8) to examine whether the interaction between the H3K4me1 reader and H3K36 methyltransferase is conserved in other plant species.

Response: Following your suggestion, we examined the interaction between AtEHD3 (the Arabidopsis homolog of rice Ehd3) and SDG7 (the Arabidopsis homolog of rice SDG724) using yeast two-hybrid and GST pulldown assays. Our results proved that AtEHD3 interacted with SDG7, supporting that the interaction between the H3K4me1 reader and the H3K36 methyltransferase is conserved in Arabidopsis. We have now included the data in our revised manuscript (Lines 276–279, Extended Data Fig. 4g, h).



Extended Data Fig. 4 g-h. Interaction of AtEHD3 and SDG7 in Arabidopsis. **g**, Yeast two-hybrid assay showing the interaction between Arabidopsis AtEHD3 and SDG7 in yeast cells. AS1 and AS2 were used as the positive control. **h**, *In vitro* GST pulldown assay showing the interaction between Arabidopsis AtEHD3 and SDG7. His-SDG7 was pulled down by GST-AtEHD3 but not GST. The upper panel showing the SDS-PAGE stained with Coomassie Brilliant Blue (CBB) and the lower panel showing the western blot using the anti-His antibody.

3. Lines 277–279: Please provide P-values to support the claim of “significant overlap”.

Response: We thank you for your comments. We have performed Fisher’s exact test and provided the *p*-values in the revised figures to support our claim of “significant overlap” (Extended Data Fig. 5b, c).

4. Lines 326–330: In addition to the genome browser view, a correlation analysis should be performed to strengthen the conclusion.

Response: Following your suggestion, we have performed correlation analyses across different species, supporting that land plants exhibit stronger positive correlation between H3K4me1 and H3K36me2/me3 compared to animals and *C. reinhardtii*. We have now included the data in our revised manuscript (Extended Data Fig. 10c).

Reviewer #3 (Remarks to the Author):

Histone modifications play critical roles in eukaryotic gene regulation. Among the multiple common histone modifications, H3K4me1 is relatively less studied. It is associated with enhancers in animals but not plants. To investigate the functional roles of H3K4me1 in plants, Wu et al. performed comprehensive studies in rice and later extended to land plants in general. The authors first analyzed the H3K4me1 patterns in multiple species and found a unique distribution of H3K4me1 in land plants. A PHD finger protein Ehd3 was later identified as the H3K4me1 reader. The structural studies successfully revealed the molecular basis for the specific recognition of H3K4me1 by Ehd3, and the structure-based mutagenesis studies confirmed the H3K4me1 reading is essential for the in vivo function of Ehd3. Further, the H3K36 MTase SDG724 was identified as an Ehd3 binding factor that links H3K4me1 reading to H3K36me deposition. Overall, this study revealed an important and unique interplay between two histone modifications in land plants. I support the publication of this paper with the following minor concerns.

[Response: We thank you for your helpful comments that have improved our manuscript. We have addressed the specific comments as presented below.](#)

L31 and 53, H3 means histone 3. So, please change histone H3 directly to histone 3.

[Response: We have changed it in our revised manuscript \(Lines 31, 53, and 64\).](#)

L171-172, it is not rigorous to say that the hydrogen bond exists between the N and O atoms, as there is no hydrogen. I would like to suggest that the author change the N and O atoms here to the amino and carbonyl groups, respectively. The following descriptions should also be revised to prevent using the atom but use the group to describe the hydrogen bonds.

[Response: We thank you for your helpful suggestion. We have revised the descriptions of individual atoms to functional groups in our revised manuscript \(Lines 175–203\).](#)

Fig. 4f, it looks like there is no significant difference between II and IV, and III and IV.

Please analyze the differences to confirm.

Response: Following your suggestion, we have performed the statistical analysis to compare groups II and IV, as well as III and IV. As expected, there is no significant difference between these groups. We have now revised Fig. 4f in our revised manuscript.

L284-285, why analyze H3K36me here? Because SDG724 is an H3K36 MTase? Please add the short sentence here to describe the logic for analyzing H3K36me. Or else, the introduction of H3K36 becomes strange here.

Response: Following your suggestion, we have added a brief introduction to clarify that SDG724 is a known H3K36 methyltransferase in our revised manuscript (Line 300).

L334, why SDG725? Please have a very brief introduction for SDG725, especially about the CW domain. A potential way is to move the sentence of L341-345, the introduction of the CW domain of SDG8 and SDG725, to L334.

Response: Thank you for your helpful comments. Now we have added a brief introduction for SDG725 and moved the introduction of the CW domain of SDG8 and SDG725 as you suggested in our revised manuscript (Lines 396–402).

Does SDG724 have a CW domain like SDG725? If yes, please give the details. If not, why could the H3K4me1 NCP stimulate the activity of SDG724? Please have a short discussion about this issue.

Response: SDG724 does not contain a CW domain (Extended Data Fig. 11a). We have repeated the histone methyltransferase experiment and proved that H3K4me1-NCP did not stimulate SDG724 activity. We have now included the data in our revised manuscript (Line 406, Fig. 6h).

AUTHOR RESPONSES to REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors provided satisfactory new data addressing my previous comments. The paper is now very much suited for the publication in Nature Communications. Congratulations for the great piece of work. I only have a suggestion on the title of the paper. I suggest to make title more informative and accurate; such as "The H3K4me1 reader protein EHD3 mediates H3K36me2 and H3K36me3 by recruiting the H3K36me writer SDG724 in rice" or "The H3K4me1 reader protein EHD3 mediates H3K4me1–H3K36me3 co-occupancy in land plants" or similar.

Response: We sincerely appreciate your positive comments on our revised manuscript. At the same time, we thank you for your thoughtful suggestion regarding the title of the manuscript. After careful consideration, we hope to keep the title “H3K4me1 directs H3K36me2 and H3K36me3 deposition in land plants”, as we think it directly and clearly conveys the core conclusion of our work: H3K4me1 has a completely different function in plants compared to animals. Once again, we thank you for your constructive suggestions throughout the review process.

Reviewer #2 (Remarks to the Author):

The authors have addressed my concerns. I have no further comments.

Response: We thank you for your constructive comments throughout the review process.

Reviewer #3 (Remarks to the Author):

Most of my concerns have been precisely addressed. A small error is listed below.

Line 175, it is correct that here is “amino group”. But in Line 178, it should be “amide group” of Arg2, but not “amino group”.

Response: We thank you for your constructive comments throughout the review process. We have now corrected the mistake in our revised manuscript (Line 178).