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WRKY transcription factors and OBERON histone-binding proteins form complexes to balance plant growth and stress tolerance

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Dear Dr. He,

Thank you again for the submission of your manuscript entitled "WRKY transcription factors and histone-binding proteins form complexes to balance plant growth and stress tolerance" (EMBOJ-2023-113639). We have now received three reports from the referees, which I copy below.

As you can see from their comments, all referees appreciated the significance of the interaction between OBE and WRKY proteins. However, all of them explicitly point out that the conclusions are somewhat preliminary and will require further experimental support before your manuscript can be published in The EMBO Journal. From my side, I would encourage to explore the influence the described interactions have under drought stress conditions more thoroughly.

Based on the overall interest expressed in the reports, I would like to invite you to address the comments of all referees in a revised version of the manuscript. I should add that it is The EMBO Journal policy to allow only a single major round of revision and that it is therefore important to resolve the main concerns at this stage. I believe the concerns of the referees are reasonable and addressable, but please contact me if you have any questions, need further input on the referee comments or if you anticipate any problems in addressing any of their points. Please, follow the instructions below when preparing your manuscript for resubmission.

I would also like to point out that as a matter of policy, competing manuscripts published during this period will not be taken into consideration in our assessment of the novelty presented by your study ("scooping" protection). We have extended this 'scooping protection policy' beyond the usual 3 month revision timeline to cover the period required for a full revision to address the essential experimental issues. Please contact me if you see a paper with related content published elsewhere to discuss the appropriate course of action.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Again, please contact me at any time during revision if you need any help or have further questions.

Thank you very much again for the opportunity to consider your work for publication. I look forward to your revision.

Best regards,

William

William Teale, Ph.D.
Editor
The EMBO Journal

When submitting your revised manuscript, please carefully review the instructions below and include the following items:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point response to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

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5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript.

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under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see https://www.embopress.org/page/journal/14602075/authorguide#datadeposition). If no data deposition in external databases is needed for this paper, please then state in this section: This study includes no data deposited in external repositories. Note that the Data Availability Section is restricted to new primary data that are part of this study.

Note - All links should resolve to a page where the data can be accessed.

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10) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online (see examples in https://www.embopress.org/doi/10.15252/embj.201695874). A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc. in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: .

- Additional Tables/Datasets should be labelled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

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Please make sure you upload a letter of response to the referees' comments together with the revised manuscript.

Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

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IMPORTANT: When you send the revision we will require
- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines
(https://www.embopress.org/page/journal/14602075/authorguide).
- Expanded View files (replacing Supplementary Information)
Please see out instructions to authors
https://www.embopress.org/page/journal/14602075/authorguide#expandedview

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We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (1st Jun 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

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Referee #1:

The manuscript from Du and collaborators investigates the interaction between WRKY transcription factors and histone binding proteins OBEs and its relevance in plant growth.
The work is interesting with lots of different datasets however it doesn't fully clarify the role of this interaction in plants.
More in details:
Figure 1B: this experiment gives a hint on the stabilisation of the WRKY-OBE protein complexes, however to be sure that the lower protein levels are not due to the transgene insertion, it could be important that to assess that such reduction the WRKY does not occur in another mutant.
Figure 1C: The structure between WRKYS in different plant kingdom might diverge therefore talking about evolution might be premature. In addition, to further prove this interaction Co-IP in planta is required. The in vitro pull down is important to define an interaction but it doesn't take in accoount the putative competition between other wrkys and obes.
Figure 2C: The pulldown experiment is not clear at all.
First of all there are any bands being pulled down, even the GST on its own which should't really be the case. Arrows are required to indicate the expected molecular weight, as well as a negative control with a protein that does not show interaction, rather than just GST on its own.
Figure 3: I appreciate the authors work in generating sextuple mutants, but perhaps, for the relevance of this manuscript, it would be worth to show the phenotype of double or triple mutant between obes and wrkys, especially if the proteins directly interact.
The authors also compared wrkys with obe mutants in terms of fresh weight as well as plant height. However for the flowering they only used the wrkys. If the interaction is important also for reproductive stages obe mutants should also been analysed.
Heatmaps should also show the expression patterns in the wild type not just between mutants.
Figure 4: ChIPseq experiment: OBE proteins possess a PHD domain that is required to bind mthylated histone tails, therefore do not bind directly DNA. To corroborate that is through WRKY11 I suggest to check some of the OBE1 binding in the quintuple mutant.
Figure 8: The drought experiment needs could also be performed in the obe/wrky double mutants as looking at different obes and wrkys does not give more insights on the interaction. the expression of DREBs should also be measured in control conditions as they already see a change based on the RNAseq data.
It might also be interesting to assess the methylation levels in wrkys and obes mutants

Referee #2:

In this study, the authors found that the group IId WRKY transcription factors interact with histone-binding OBE proteins and form WRKY-OBE complexes in Arabidopsis thaliana. The coiled-coil motif of WRKY transcription factors is responsible for the binding

to OBE proteins and transcriptional repression. In addition, the PHD finger of OBE proteins is responsible for the binding to the histone proteins and interacting with WRRY transcription factors. WRKY-OBE complexes repress the transcription of stress-responsive genes and are required for maintaining normal plant growth. It was suggested that the WRKY-OBE complexes repress the transcription of stress-responsive genes under non-stress conditions. This study provides insight into the mechanism underlying the regulation of the function and activity of WRKY transcription factors in plant growth and stress responses. The experiment in this study was carefully designed and the results were well documented. I have the following suggestions.

Major points:

1. The authors showed that group IId WRKY transcription factors interact with OBE proteins and form WRKY-OBE complexes. Furthermore, the PHD finger of OBE proteins bind to histones. However, it is unclear how WRKY-OBE interaction is involved gene repression. The authors may want to check the histone modification changes of WRKY and OBE targeted genes to analyzed whether WRKYs and OBEs regulate gene expression through histone modifications.

2. By EMSA, the authors showed that the binding of OBE1 to DNA containing W-boxes is dependent on WRKY11 in vitro (Fig. 5D-5F). It is important to show whether the binding of OBE1 or WRKY11 to target genes is also dependent on WRKYs or OBEs in vivo, since in vitro and in vivo data could be different. I suggest that the authors can also carry our ChIP-qPCR assays to analyze whether the binding of OBE1 or WRKY11 in vivo using wrky or obe- mutants.

Minor points:

1. The authors identified the OBE-interacting domain (OID) that is important for the interaction between the group IId WRKY proteins and OBE proteins and for the selection of WRKY domain-binding loci. I am wondering whether the OID domain can also be found in other groups of WRKY proteins.

2. Page 4, line 141: "To identify the proteins that interact with group IId WRKY transcription factors, we performed affinity purification followed by mass spectrometry (AP-MS) using WRKY7-Flag and WRKY11-Flag transgenic plants that express native promoter-driven transgenes.".

Please indicate why WRKY7 and WRKY11 were selected in this study.

3. Page 5, line 155: "By introducing the WRKY11-Flag transgene into the obe1/2 mutant and wild-type backgrounds, we found that, although the transcript level of WRKY11-Flag was similar between the obe1/2 mutant and the wild type, the protein level of WRKY11-Flag was markedly reduced in the obe1/2 mutant (Figure 1B)".

I cannot find the data showing the transcript level of WRKY11-Flag. Please also indicate which promoter was used to drive the WRKY11-Flag transgene.

4. Page 5, line 178: "and the results indicated that the tested WRKY transcription factors interact with OBE1-4 but not with each other (Figure 2A, 2B, and Supplemental Figure 5).".

Please explain why the group IId WRKY transcription factors were co-purified with WRKY7 and WRKY11 in AP-MS, but they did not interact with each other in yeast two-hybrid assays.

5. Fig. 2a

The authors may want to use different color to mark OID, WRKY, PHD and coiled-coil domains.

6. Fig. 3

Fig. 3A-3B have wrky-qm/15+/-, but lack wrky-qm. However, Fig. 3C-3F have wrky-qm, but lack wrky-qm/15+/-. I suggest that the authors also add wrky-qm to Fig. 3A-3B and wrky-qm/15+/- to Fig. 3C-3F.

7. It has been reported that some WRKY proteins regulate gene expression through histone modifications (eg., Plant Physiol. 190: 532-547.). The authors need to cite these references and discuss the possibility whether the group IId WRKY proteins can affect gene expression through histone modifications.

Referee #3:

In this manuscript by Du et al., the authors report that subgroup IId WRKY transcription factors form a complex with OBEs and regulate the expression of stress-responsive genes. The authors provide strong evidence that OBEs interact with WRKYs

through the N-terminal coiled-coil domains, and the interaction is essential for WRKY-mediated transcriptional repression. The authors also show that binding OBEs on the H3K4me2/3 is required for the target selection of WRKYs. In addition, the obe and wrky high-order mutants show very similar and dramatic phenotypes in both retarded growth and drought tolerance. Thus, the manuscript uncovers a highly conserved transcription factor/histone-binding protein complex that might be essential for regulating stress-responsive genes. In my opinion, this manuscript could be published on EMBO J, after addressing a few issues.

Major issues:
1. In Figure 3J, it is interesting that the stress-responsive TF and marker gene, DREB1s and RD29A, are only highly expressed in wrky-qm but not in obe1/2 or qm/15+/-. However, Figure 8 shows that DREB1s are highly expressed in both wrky-qm and obe1/3. What is the reason for this inconsistency? Why did the authors use obe1/3 in RNA-seq and another allele, obe1/2, in phenotypic and qRT-PCR assays? It appears that different obe double mutants have different expressions of stress-responsive genes.

2. In Figures 6 and 7, the authors show strong evidence that OBE-mediated H3K4me2/3 activity is essential for the target selection of WRKY11. It would be more convincing if the authors also compared the H3K4me2/3 regions (perhaps from published results), WRKY11 target sites, and OEB1 binding sites at the genomic level. This data could further support the proposed model.

3. In Figure 8, the authors compare the drought survival phenotype of different wrky and obe mutants. As mentioned above, the obe double mutant used in this figure is inconsistent with the one used in RNA-seq. Please also include obe1/2 in the phenotypic and q-PCR assays. In addition, the drought-tolerant phenotype of obe1/3 and wrky-qm is impressive, and please also perform the water loss assay to strengthen this piece of data.

4. The authors show two biological processes: 1) most OBEs and IId WRKYs show less expression in drought-treated plants, and OBEs/WRKYs can bind to the promoters of stress-induced genes (DREB1s), 2) stress-responsive genes are up-regulated in drought-treated plants, and then propose a model that OBE-WRKY complex balances plant growth and stress tolerance. However, it is not conclusive whether the expression of stress-responsive genes depends on the OBE-WRKY complex. I suggest an additional experiment to dissect this notion. The authors could perform a time-course (for example, 0, 3h, 6h, 12h, 24h, 48h) q-PCR assay of OBE, WRKY, DREB1, and RD29B, to see the time-dependent correlation between the decrease of OBE/WRKY expression and the increase of stress-responsive genes, which might further support the model.
Minor issues:

Minor issues:
1. In the last paragraph of the Introduction, the authors wrote, "the conserved N-terminal motif of the group IId WRKY proteins, which was previously thought to interact with calmodulin based on in vitro assays (Park et al., 2005), is responsible for interacting with the histone-binding OBE proteins but not with calmodulin in Arabidopsis plants". However, as no data showed that it does not interact with the calmodulin in this manuscript, the authors cannot exclude the possibility that this motif also interacts with calmodulin. Please modify.
In addition, the authors gave a new name, OID, to this motif. However, besides interacting with OBEs, this motif is also essential for WRKY-WRKY interaction (this study) and might interact with other proteins like calmodulin. I suggested the authors keep the coiled-coil domain rather than give a new name to it.

2. Though the authors showed that OBEs interact with IId subgroup WRKYs, it is unclear whether the OBEs also interact with WRKYs in other subgroups. This data would help us understand the specificity of this OBE-WRKY complex.

3. Figure 7E, the authors should also test the combination of OBE1+WRKY11, and OEB1+WRKY11-OIDΔ.

Referee #1:

The manuscript from Du and collaborators investigates the interaction between WRKY transcription factors and histone binding proteins OBEs and its relevance in plant growth.

The work is interesting with lots of different datasets however it doesn't fully clarify the role of this interaction in plants.

Response: Thanks very much for the positive and constructive comments. All the concerns have been point-by-point addressed in the revised manuscript.

More in details:

Figure 1B: this experiment gives a hint on the stabilisation of the WRKY-OBE protein complexes, however to be sure that the lower protein levels are not due to the transgene insertion, it could be important that to assess that such reduction the WRKY does not occur in another mutant.

Response: Due to the severe developmental defects of *obe1/2* double mutant, we initially transformed the *WRKY11-Flag* transgene into *obe1+/-;obe2-/-* mutant plants without clear developmental defects and subsequently identified *WRKY11-Flag* transgenic plants with the *obe1/2* double mutant background in the progeny of self-bred *WRKY11-Flag* transgenic plants. To be sure that the lower WRKY11-Flag protein level was not due to the transgene insertion, we identified *WRKY11-Flag* transgenic plants with both the *obe1/2* double mutant and *obe2* single mutant backgrounds in the progeny of self-bred *WRKY11-Flag* transgenic plants. As expected, although the transcript level of *WRKY11-Flag* was similar between the *obe1/2* double mutant and the *obe2* single mutant, the protein level of WRKY11-Flag was markedly reduced in the *obe1/2* double mutant relative to the *obe1* single mutant (Figure 1B), suggesting that the reduced protein level of WRKY11-Flag is due to the loss of OBE1 and OBE2 in Arabidopsis plants.

Figure 1C: The structure between WRKYS in different plant kingdom might diverge therefore talking about evolution might be premature. In addition, to further prove this interaction Co-IP in planta is required. The in vitro pull down is important to define an interaction but it doesn't take in account the putative competition between other wrkys and obes.

Response: As suggested, we tuned down the statement related to the evolution of WRKY-OBE interaction. We agree with you that the specificity of WRKY-OBE interactions needs to be validated in other plants. Therefore, we performed a pull down assay to determine whether the purified WRKY IId transcription factor OsWRKY51 specifically interacts with OBE proteins in the total protein extract of rice seedlings. The pull down assay indicated that the OBE proteins OsOBE1 and OsOBE2 were co-purified with OsWRKY51, supporting the idea that the WRKY IId transcription factors also form a WRKY-OBE complex in other plants (Dataset EV1).

Figure 2C: The pulldown experiment is not clear at all.

First of all there are any bands being pulled down, even the GST on its own which should't really be the case. Arrows are required to indicate the expected molecular weight, as well as a negative control with a protein that does not show interaction, rather than just GST on its own.

Response: To confirm the pull-down result, we performed additional pull down assays using the MBP protein as a negative control. As shown in Figure 2C, a series of GST tagged proteins cannot interact with MBP protein, while the MBP tagged WRKY11-1 protein can interact with full-length OBE1, OBE1-2 and OBE1-3; the MBP tagged OBE2-4 protein can interact with full-length of OBE1 and OBE1-3, which is consistent with the previous result (Appendix Fig S7).

Figure 3: I appreciate the authors work in generating sextuple mutants, but perhaps, for the relevance of this manuscript, it would be worth to show the phenotype of double or triple mutant between obes and wrkys, especially if the proteins directly interact.

Response: As suggested, we generated the *obe1/3/wrky15* and *obe1/3/wrky21* triple mutants by CRISPR-mediated mutagenesis of *WRKY15* and *WRKY21* in the *obe1/3* double mutant background. Phenotypical analysis indicated that the triple mutants show similar phenotype with the *obe1/3* double mutant (Appendix Fig S12), supporting the concept that the WRKY and OBE proteins function in the same protein complex.

The authors also compared wrkys with obe mutants in terms of fresh weight as well as plant height. However for the flowering they only used the wrkys. If the interaction is important also for reproductive stages obe mutants should also been analysed.

Response: As suggested, we analyzed the flowering-time phenotype in *obe1*, *obe2*, *obe3* and *obe4* single mutants and in *obe1/3* and *obe1/4* double mutants, and found that the *obe3* single mutant and the *obe1/3* double mutant exhibited a significant late-flowering phenotype (Appendix Fig S11), suggesting that the flowering-time phenotype is co-regulated by both the WRKY and OBE components in the WRKY-OBE complex.

Heatmaps should also show the expression patterns in the wild type not just between mutants.

Response: In Figure 3H, the heatmaps showed the expression changes in indicated mutants relative to the wild type, and the color bar scale referred to $\log_2$(fold change of RPKM between mutants and wild type). The heatmaps clearly showed that the expression levels of numerous genes are co-regulated in the *obe* and *wrky* mutants (Figure 3H). As suggested, we showed our RNA-seq data by box plots in both the mutants and the wild type (Appendix Fig S14), further conforming that gene expression is co-regulated in the *obe* and *wrky* mutants relative to the wild type. Moreover, Dataset EV2 indicated the RPKM of RNA-seq data for differently expressed genes in both the mutants and the wild type.

Figure 4: ChIPseq experiment: OBE proteins possess a PHD domain that is required to bind methylated histone tails, therefore do not bind directly DNA. To corroborate that is through WRKY11 I suggest to check some of the OBE1 binding in the quintuple mutant.

Response: As suggested, we performed ChIP-PCR for OBE1-Flag in the wild type and *wrky-qm* mutant backgrounds to determine the effect of *wrky-qm* on the association of OBE1 with chromatin. The result indicated that the association of OBE1 with target genes was significantly reduced in the *wrky-qm* mutant relative to the wild type (Figure 5G, 5H), suggesting that the association of OBE1 with target genes depends on the DNA binding ability of WRKYs.

Figure 8: The drought experiment needs could also be performed in the obe/wrky double mutants as looking at different obes and wrkys does not give more insights on the interaction. The expression of DREBs should also be measured in control conditions as they already see a change based on the RNAseq data.

Response: As suggested, we created the *obe1/3/wrky15* and *obe1/3/wrky21* triple mutants by CRISPR-mediated mutagenesis of *WRKY15* and *WRRKY21* in the *obe1/3* double mutant background and found that the triple mutants showed similar morphological phenotypes to the *obe1/3* double mutant (Appendix Fig S12). This genetic analysis supports the notion that the WRKY and OBE proteins function in the same protein complex. The similar morphological phenotypes observed in the *obe1/3* double mutant and the *obe1/3/wrky15* or *obe1/3/wrky21* triple mutants (Appendix Fig S12) suggest that the triple mutants may also exhibit a comparable drought tolerance phenotype to the *obe1/3* double mutant. Our RNA-seq data indicated that the expression levels of most *WRKY IId* and *OBE* genes were significantly reduced by the drought treatment (Figure 8F). As suggested, we also showed the expression levels of *DREBs* under both control and drought conditions (Figure 8F), indicating that the expression levels of *DREB1A/1B/1C* were substantially induced by the drought treatment.

It might also be interesting to assess the methylation levels in wrkys and obes mutants.

Response: As suggested, we assessed the histone H3K4me2, H3K4me3 and H3K27me3 levels in the wild type and *obe1/2*, *wrky-qm/15*$^{+/-}$ and *wrky-qm* mutants by immunoblotting, and found that the H3K4me2, H3K4me3 and H3K27me3 levels were not affected at the whole-genome level (Appendix Fig S17C). Given that the PHD domain of OBE1 is capable of binding to H3K4me3, we determined whether the WRKY-OBE complex tends to occupy H3K4me3-enriched genes. By analyzing previous H3K4me3 and H3K27me3 ChIP-seq data (Shang et al., 2021; Zhao et al., 2022), we found that the H3K4me3 levels are significantly higher in WRKY11- and OBE1-occupied genes than in random genes, while the H3K27me3 levels are significantly lower in WRKY11- and OBE1-occupied genes than in random genes (Appendix Fig S17A), supporting

the inference that the binding of OBE1 to H3K4me3 is involved in the association of the WRKY-OBE complex with chromatin.

Referee #2:

In this study, the authors found that the group IId WRKY transcription factors interact with histone-binding OBE proteins and form WRKY-OBE complexes in Arabidopsis thaliana. The coiled-coil motif of WRKY transcription factors is responsible for the binding to OBE proteins and transcriptional repression. In addition, the PHD finger of OBE proteins is responsible for the binding to the histone proteins and interacting with WRRY transcription factors. WRKY-OBE complexes repress the transcription of stress-responsive genes and are required for maintaining normal plant growth. It was suggested that the WRKY-OBE complexes repress the transcription of stress-responsive genes under non-stress conditions. This study provides insight into the mechanism underlying the regulation of the function and activity of WRKY transcription factors in plant growth and stress responses. The experiment in this study was carefully designed and the results were well documented. I have the following suggestions.

Response: Thanks for the positive and constructive comments. All the concerns have been point-by-point addressed in the revised manuscript.

Major points:

1. The authors showed that group IId WRKY transcription factors interact with OBE proteins and form WRKY-OBE complexes. Furthermore, the PHD finger of OBE proteins bind to histones. However, it is unclear how WRKY-OBE interaction is involved gene repression. The authors may want to check the histone modification changes of WRKY and OBE targeted genes to analyze whether WRKYs and OBEs regulate gene expression through histone modifications.

Response: Since we did not identify that the WRKY-OBE complex interacts with any histone modifiers, we have no evidences for supporting the notion that the WRKY-OBE complex is involved in catalyzing histone modifications. By immunoblotting, we found that the H3K4me3 and H3K27me3 levels were not significantly affected in the *wrky-qm*, *wrky-qm/15*$^{+/-}$, or *obe1/2*

mutants relative to the wild type (Appendix Fig S17C). To determine how the WRKY-OBE interaction is involved in transcriptional repression, we performed a complementation test to investigate whether the deletion of the coiled-coil domain in WRKY11 affects its function in Arabidopsis plants and found that the deletion disrupts the WRKY-mediated transcriptional repression at WRKY target genes in Arabidopsis plants (Figure 7F-7I). Furthermore, by performing additional reporter assays in Arabidopsis protoplasts, we found that OBE1 substantially enhances the WRKY11-mediated transcriptional repression of the luciferase reporter gene, and that the deletion of the coiled-coil domain in WRKY11 disrupts the effect of OBE1 on the WRKY11-mediated transcription repression (Figure 7E). These analyses strongly suggest that the WRKY-OBE interaction is involved in WRKY-mediated transcriptional repression. By analyzing previous H3K4me3 ChIP-seq data (Shang et al., 2021), we found that both WRKY11 and OBE1 target genes are enriched with H3K4me3 in Arabidopsis plants (Appendix Fig S17A-B), which is consistent with the finding that OBE1 is capable of binding to H3K4me3 as determined by *in vitro* binding assays (Figure7A-C).

2. By EMSA, the authors showed that the binding of OBE1 to DNA containing W-boxes is dependent on WRKY11 in vitro (Fig. 5D-5F). It is important to show whether the binding of OBE1 or WRKY11 to target genes is also dependent on WRKYs or OBEs in vivo, since in vitro and in vivo data could be different. I suggest that the authors can also carry our ChIP-qPCR assays to analyze whether the binding of OBE1 or WRKY11 in vivo using wrky or obe- mutants.

Response: As suggested, we performed ChIP-PCR for OBE1-Flag in the wild-type and *wrky-qm* mutant backgrounds to determine whether WRKYs are involved in the binding of OBE1 to its target genes *in vivo*. As shown in Figure 5G and 5H, the binding of OBE1 to its target genes was substantially reduced in the *wrky-qm* mutant compared to the wild type. These results support the notion that the binding of OBE1 to its target genes is dependent on WRKYs *in vitro* and *in vivo*. Considering that the protein level was markedly reduced in the *obe1/2* mutant compared to the wild type (Figure 1B), the OBE proteins are likely required for maintenance of the WRKY protein stability. Therefore, it is not possible to obtain WRKY11-Flag transgenic plants with a comparable protein level of *WRKY11-Flag* between the *obe1/2* and wild-type backgrounds for ChIP-PCR

analysis.

Minor points:

1. The authors identified the OBE-interacting domain (OID) that is important for the interaction between the group IId WRKY proteins and OBE proteins and for the selection of WRKY domain-binding loci. I am wondering whether the OID domain can also be found in other groups of WRKY proteins.

Response: The OID domain (which was named coiled-coil domain in the revised version) was conserved in the group IId WRKY proteins and cannot be found in other groups of WRKY proteins. We clearly indicated the statement in the revised manuscript.

2. Page 4, line 141: "To identify the proteins that interact with group IId WRKY transcription factors, we performed affinity purification followed by mass spectrometry (AP-MS) using WRKY7-Flag and WRKY11-Flag transgenic plants that express native promoter-driven transgenes". Please indicate why WRKY7 and WRKY11 were selected in this study.

Response: Within the members of group IId WRKY transcription factors, WRKY7 and WRKY11 belong to different subgroups (Appendix Fig S2). Therefore, WRKY7 and WRKY11 were selected as representatives of group IId WRKY transcription factors. We indicated the statement in the revised manuscript.

3. Page 5, line 155: "By introducing the WRKY11-Flag transgene into the obe1/2 mutant and wild-type backgrounds, we found that, although the transcript level of WRKY11-Flag was similar between the obe1/2 mutant and the wild type, the protein level of WRKY11-Flag was markedly reduced in the obe1/2 mutant (Figure 1B)". I cannot find the data showing the transcript level of WRKY11-Flag. Please also indicate which promoter was used to drive the WRKY11-Flag transgene.

Response: The bar chart shown in Figure 1B showed the transcript levels of *WRKY11-Flag* in the

obe1/2 mutant and wild-type backgrounds (Figure 1B). To avoid misunderstanding, we clearly labelled it in the revised Figure.

4. Page 5, line 178: "and the results indicated that the tested WRKY transcription factors interact with OBE1-4 but not with each other (Figure 2A, 2B, and Supplemental Figure 5)". Please explain why the group IId WRKY transcription factors were co-purified with WRKY7 and WRKY11 in AP-MS, but they did not interact with each other in yeast two-hybrid assays.

Response: Although the group IId WRKY transcription factors did not interact with each other in yeast two-hybrid assays, In the WRKY-OBE complex, two copies of OBE proteins directly interact to form a dimer, and the OBE dimer functions as a bridge connecting two copies of WRKY proteins, thus revealing a previously uncharacterized mechanism underlying the dimerization of WRKY proteins.

5. Fig. 2a

The authors may want to use different color to mark OID, WRKY, PHD and coiled-coil domains.

Response: As suggested, we used different colors to mark conserved domains of WRKY and OBE proteins in Figure 2A and 2F.

6. Fig. 3

Fig. 3A-3B have wrky-qm/15+/-, but lack wrky-qm. However, Fig. 3C-3F have wrky-qm, but lack wrky-qm/15+/-. I suggest that the authors also add wrky-qm to Fig. 3A-3B and wrky-qm/15+/- to Fig. 3C-3F.

Response: As suggested, we added *wrky-qm* to the revised Fig. 3A-3B. The morphological phenotypes of adult plants were shown in Fig. 3C-3F. Due to severe developmental defects of *wrky-qm/15*$^{+/-}$, *obe1/2* and *obe3/4* mutants, the adult plants of these mutants were not available.

7. It has been reported that some WRKY proteins regulate gene expression through histone

modifications (eg., Plant Physiol. 190: 532-547.). The authors need to cite these references and discuss the possibility whether the group IId WRKY proteins can affect gene expression through histone modifications.

Response：As suggested, we cited two references (Kim et al., 2008; Hung et al., 2022) and showed the possibility that WRKY transcription factors can affect transcription through histone modifications in the discussion part.

Referee #3:

In this manuscript by Du et al., the authors report that subgroup IId WRKY transcription factors form a complex with OBEs and regulate the expression of stress-responsive genes. The authors provide strong evidence that OBEs interact with WRKYs through the N-terminal coiled-coil domains, and the interaction is essential for WRKY-mediated transcriptional repression. The authors also show that binding OBEs on the H3K4me2/3 is required for the target selection of WRKYs. In addition, the obe and wrky high-order mutants show very similar and dramatic phenotypes in both retarded growth and drought tolerance. Thus, the manuscript uncovers a highly conserved transcription factor/histone-binding protein complex that might be essential for regulating stress-responsive genes. In my opinion, this manuscript could be published on EMBO J, after addressing a few issues.

Response: Thanks for the positive and constructive comments. All the concerns have been point-by-point addressed in the revised manuscript.

Major issues:

1. In Figure 3J, it is interesting that the stress-responsive TF and marker gene, DREB1s and RD29A, are only highly expressed in wrky-qm but not in obe1/2 or qm/15+/-. However, Figure 8 shows that DREB1s are highly expressed in both wrky-qm and obe1/3. What is the reason for this inconsistency? Why did the authors use obe1/3 in RNA-seq and another allele, obe1/2, in phenotypic and qRT-PCR assays? It appears that different obe double mutants have different expressions of stress-responsive genes.

Response: As suggested, we used *obe1/3* in phenotypic and qRT-PCR analyses (Figure8A-D; Appendix Fig S11). Our RNA-seq analysis identified numerous upregulated DEGs (including *DREBs*) in *wrky-qm* that were not upregulated in *obe1/2* or *wrky-qm/15*$^{+/-}$ mutants (Figure 3G). We predicted that the upregulated DEGs identified in *wrky-qm* were exclusively expressed in well-developed roots and leaves, and that the failure in identifying these upregulated DEGs in *obe1/2* and *wrky-qm/15*$^{+/-}$ is caused by the absence of well-developed roots and leaves in these mutants. Supporting the prediction, the expression levels of *DREB1s* were upregulated in the *wrky-qm* and *obe1/3* mutants with weak developmental defects but not in the *wrky-qm/15*$^{+/-}$ and *obe1/2* mutants with serious developmental defects (Figure 3G and Figure8D). In the OBE protein family, OBE1 and OBE2 belong to one subfamily and OBE3 and OBE4 belong to another subfamily. It is possible that the OBE proteins within the same subfamily are more redundant than the OBE proteins from different subfamilies. Therefore, it is reasonable that the *obe1/2* and *obe3/4* mutants show more serious developmental defects than the *obe1/3* mutant.

2. In Figures 6 and 7, the authors show strong evidence that OBE-mediated H3K4me2/3 activity is essential for the target selection of WRKY11. It would be more convincing if the authors also compared the H3K4me2/3 regions (perhaps from published results), WRKY11 target sites, and OBE1 binding sites at the genomic level. This data could further support the proposed model.

Response: As suggested, we assessed the H3K4me3 levels of WRKY11 and OBE1 target genes, indicating that both WRKY11 and OBE1 target genes showed a significantly higher level of H3K4me3 than random genes (Appendix Fig S17A). Moreover, we determined the overlaps of H3K4me3-enriched genes, WRKY11 target genes and OBE1 target genes, and found that both WRKY11 and OBE1 target genes are significantly overlapped with H3K4me3-enriched genes (Appendix Fig S17B). These analyses support the notion that OBE-mediated recognition of H3K4me2/3 is involved in the target selection of the WRKY-OBE complex. These results were added to the revised manuscript.

3. In Figure 8, the authors compare the drought survival phenotype of different wrky and obe

mutants. As mentioned above, the obe double mutant used in this figure is inconsistent with the one used in RNA-seq. Please also include obe1/2 in the phenotypic and q-PCR assays. In addition, the drought-tolerant phenotype of obe1/3 and wrky-qm is impressive, and please also perform the water loss assay to strengthen this piece of data.

Response: As indicated above, the failure in identifying the upregulation of stress-responsive genes in the *obe1/2* and *wrky-qm/15*$^{+/-}$ mutant is caused by the absence of well-developed roots and leaves in these mutants. Therefore, we used the *obe1/3* and *wrky-qm* mutants with weak developmental defects for analysis of drought stress tolerance. According to your suggestion, we performed the water loss assay in wild type, *obe1/3* and *wrky-qm*. The result indicated that the water loss rate was lower in the *obe1/3* and *wrky-qm* mutant compared with the wild type (Figure 8C), which is consistent with the increased drought-tolerance phenotype of these mutants.

4. The authors show two biological processes: 1) most OBEs and IId WRKYs show less expression in drought-treated plants, and OBEs/WRKYs can bind to the promoters of stress-induced genes (DREB1s), 2) stress-responsive genes are up-regulated in drought-treated plants, and then propose a model that OBE-WRKY complex balances plant growth and stress tolerance. However, it is not conclusive whether the expression of stress-responsive genes depends on the OBE-WRKY complex. I suggest an additional experiment to dissect this notion. The authors could perform a time-course (for example, 0, 3h, 6h, 12h, 24h, 48h) q-PCR assay of OBE, WRKY, DREB1, and RD29B, to see the time-dependent correlation between the decrease of OBE/WRKY expression and the increase of stress-responsive genes, which might further support the model.

Response: The expression levels of *WRKY* and *OBE* genes were reduced by long-term drought treatments (shown in this study) but not by short-term drought treatments (shown by online RNA-seq and microarray data in previous studies).Therefore, the Arabidopsis Col-0 plants were grown in soil under either well-watered or long-term drought-treated conditions (for 0, 5, 8, 11, 14, 17, or 20 days), and then the expression levels of *WRKY17* and *DREB1A* were determined by quantitative RT-PCR. The results indicated that the expression level of *WRKY17* was reduced by

the drought treatment in a time-dependent manner, whereas the expression of *DREB1A* was induced over time (Appendix Fig S18). In combination with the finding that the WRKY-OBE complex is involved in the transcriptional repression of *DREB1s*, our study supports the notion that the drought-induced transcription of stress-responsive genes is partially caused by the reduced expression of *WRKY* and *OBE* genes.

Minor issues:

1. In the last paragraph of the Introduction, the authors wrote, "the conserved N-terminal motif of the group IId WRKY proteins, which was previously thought to interact with calmodulin based on in vitro assays (Park et al., 2005), is responsible for interacting with the histone-binding OBE proteins but not with calmodulin in Arabidopsis plants". However, as no data showed that it does not interact with the calmodulin in this manuscript, the authors cannot exclude the possibility that this motif also interacts with calmodulin. Please modify.

Response: We agree with the reviewer that we cannot exclude the possibility that the conserved N-terminal motif of the group IId WRKY proteins also interacts with calmodulin even though we did not identify the interaction by AP-MS in this study. As suggested, we modified it in our revised manuscript.

In addition, the authors gave a new name, OID, to this motif. However, besides interacting with OBEs, this motif is also essential for WRKY-WRKY interaction (this study) and might interact with other proteins like calmodulin. I suggested the authors keep the coiled-coil domain rather than give a new name to it.

Response: According to the suggestion, we termed it the coiled-coil domain instead of the OBE-interaction domain in the revised manuscript.

2. Though the authors showed that OBEs interact with IId subgroup WRKYs, it is unclear whether the OBEs also interact with WRKYs in other subgroups. This data would help us understand the specificity of this OBE-WRKY complex.

Response: As suggested, we chose WRKY40, WRKY36, WRKY12 and WRKY14 as the representatives of IIa, IIb, IIc and IIe WRKY subgroups for OBE interaction analysis. As shown in our Y2H result (Appendix Fig S6), the OBEs interact with IId subgroup WRKYs but not with other subgroups of WRKYs, suggesting that the OBE proteins specifically form a complex with group IId WRKYs.

3. Figure 7E, the authors should also test the combination of OBE1+WRKY11, and OEB1+WRKY11-OIDΔ.

Response: As suggested, we test the OBE1+WRKY11 and OEB1+WRKY11-CCΔ combinations in Figure7E. The result indicated that OBE1 markedly enhances WRKY11-mediated transcriptional repression, and showed that the effect of OBE1 on transcriptional repression is disrupted by the deletion of the coiled-coil domain in WRKY11, suggesting that the interaction of OBEs with WRKYs contributes to WRKYs-mediated transcriptional repression.

Dear Dr He,

We have now received re-review reports for the revised version of your manuscript from two of the three referees, which I have attached below. As you will see, you have addressed their concerns satisfactorily. I have looked through referee 1's comments on the revised version; I find your responses to them reasonable and comprehensive, and am happy to proceed towards publication (on the proviso that Referee 1 doesn't come back with over-riding technical concerns). I would, however, like you to check the new labelling of figure 1B - should one of these panels be labelled obe1? Please also consider accompanying figure 1A with a Western blot to illustrate the co-IP shown. This could be from one of your tagged lines, and may help the readers' orientate themselves in the paper.
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Referee #2:

The authors have adequately addressed my concerns in the revised manuscript.

Referee #3:

The authors did a great job to revise and manuscript and answer the reviewers' comments. I have no more concern on this revision.

All editorial and formatting issues were resolved by the authors.

2nd Revision - Editorial Decision **13th Jul 2023**

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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.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	RNA-seq and ChIP-seq data are deposited to Gene Expression Omnibus (GEO) database. Mass spectrometry data are provided in Appendix tables.
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Yes	Reference