

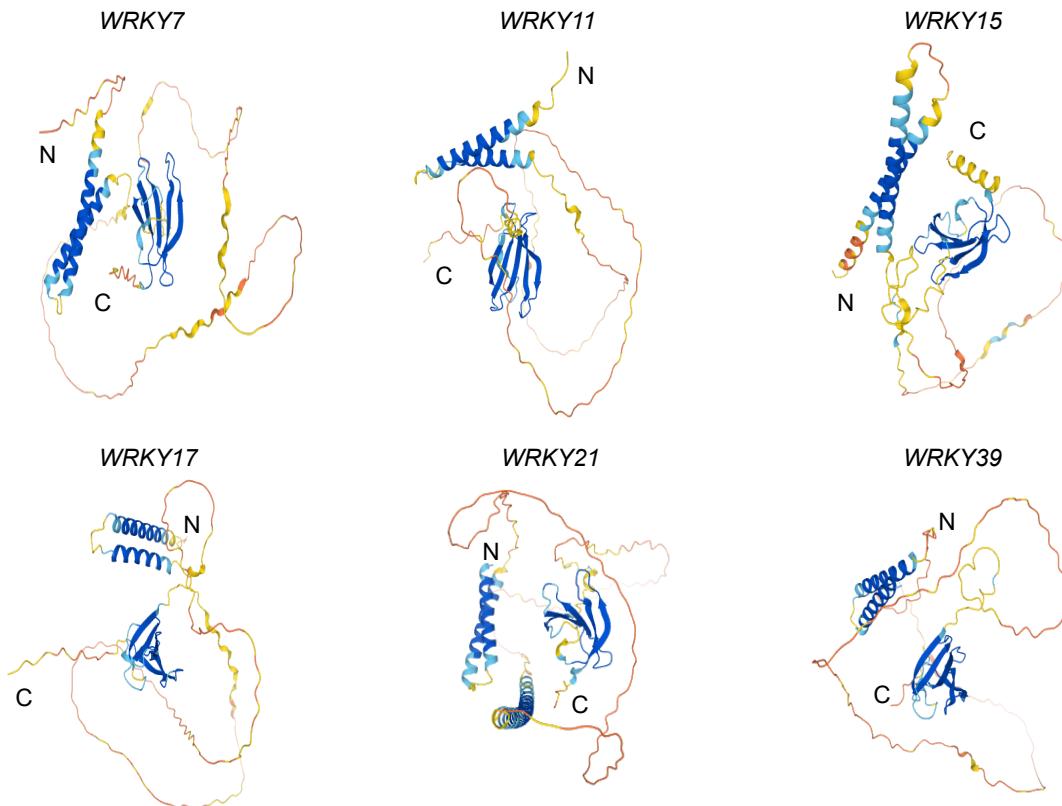
WRKY transcription factors and OBERON histone-binding proteins form complexes to balance plant growth and stress tolerance

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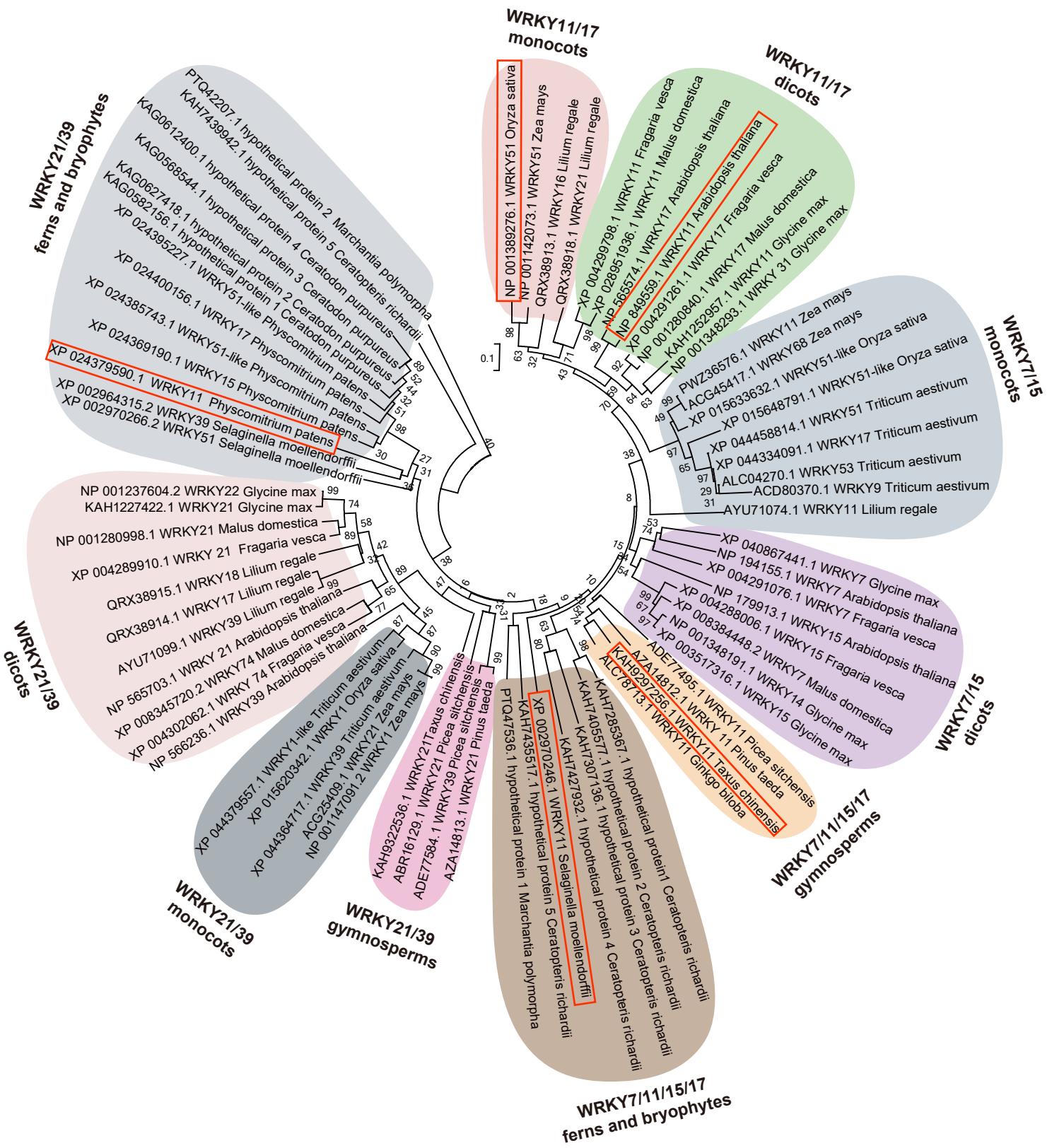
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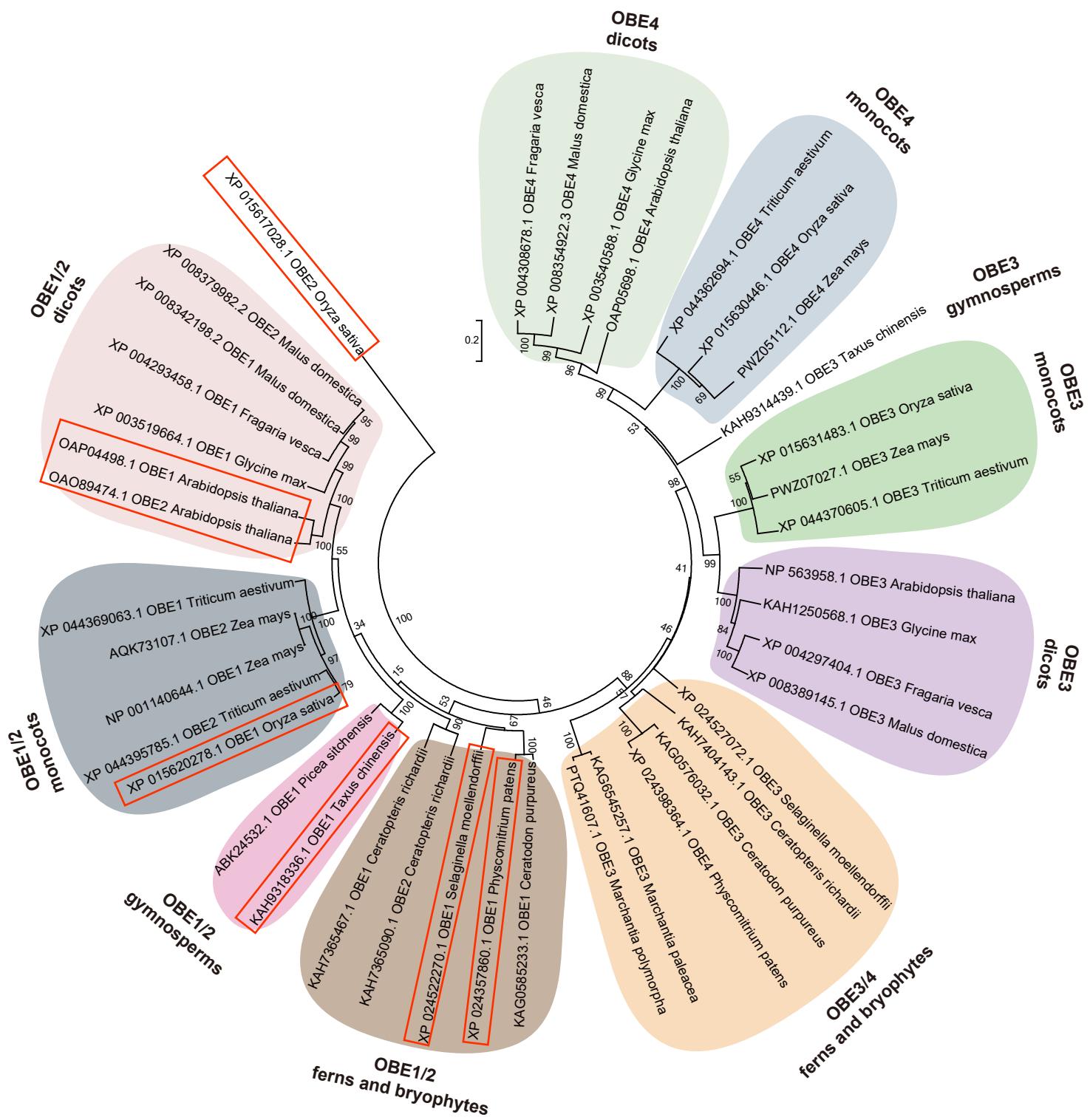
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AtWRKY7	MTVLEMMSSYGGGGGDGFFPAIAAAAKMEDITALR.EAASAGIHGVEEFLKIGQSQQPTKES.QTEITATJDV..ANNSFEEVISLLGRSRTGHARFR	96	
AtWRKY11MAVDLMPFKIDDQTAIGEAAASQGLQSMEH...IRVLNRPEQQHNVDCSEIDDF..IVSKFPRIVISLLNRT...GHARFR	75	
AtWRKY15	MAVELMLTRNYIISVGVGADSFVQEAAAASGLKSIENF.IGLMSRDSFNQDQPSSSSASASASAAA.DLESARN.TADAWSKFKEVISLLDRTTGHARFR	98	
AtWRKY17MTWDIRMLPQMEDQTAIGEAAASQGLQSMEH...IRVLNRPEER.NVDCSEIDDF..IVSKFPRIVISLLNRS..GHARFR	74	
AtWRKY21MEEIEGTNRAAVESCHRVINL..IHSQSQDHVGF.EKNLVSEIRE.AVIRFPRVGSLSSS..VGHARFR	65	
AtWRKY39MEEVEAARNRSAIESCHGVINL..ISQRTSDP.....KSITVETGE..VWSKFRVAVSLLTG..LGHGKER	60	
		coiled-coil domain	
AtWRKY7	AFP....STQTPFKQTFVVEEEVEEKKPETSSVLTK..QKTEQYHGGGSASFVRYCPTPIHRRPPLS	158	
AtWRKY11	GPV....HS.....TSSAASQKLQSQIVKNTQPE.....APIVRTTTNHF.....	111	
AtWRKY15	AFV....HVISP.....PPSSVPPP...VKVITPA.....PTQISAPAPVS	120	
AtWRKY17	GPV....HS.....SQSLILDPCQORTIEVEVSSSSQKTPVLRSGFQELSRLQPSDSLTLGTRSFSLNSNAKAPFLQLQLNQQTMPFS	106	
AtWRKY21	RAKKLQSHF.....EQHIFILESPICCGNDLSDGYTDQVLAPEPLQMVFASAVYNEMEPHKHQILGHPSLMLSHKMCVDKSFLELKPP	145	
AtWRKY39	STNKFRSSF.....EQHIFILESPICCGNDLSDGYTDQVLAPEPLQMVFASAVYNEMEPHKHQILGHPSLMLSHKMCVDKSFLELKPP	140	
		WRKY domain	
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AtWRKY17FVGQANQCSVILDFTRPSVFGAKTKSSEVVEFAKESSFSVSVNSNSFMSSA...ITGDGSVSKGSSIFL	169	
AtWRKY21NYPTFLFVQQQQQQQQQQQQQQQQQQQQQQFHERLQAHHLHQQQQLQKHQAELMLRKCNNGGSLSFDNSSCTFTMSS...RSFVSSLSIDGSVAN	241	
AtWRKY39	FRAPY.....QLIHNHQIAYSRNSGVNLKEDGGSSCYTFSVNSGSRSFVSSLMDASVTD	198	
AtWRKY7	FTNPESQLSGSRGKPPLSSASILKRRCNS.....SP.SSCHOSKRRSRVKEVIRVPVSKMPLIESIEFSWRKYGQKPIKGSEPRGYYKC	306	
AtWRKY11	ASAPFLQPVNSSGKPFPLAGHPYRKRCLEHEHSESFSGKVGSA.YGHCCHOSKRRSRNMRRTVVRPAISKIALIPEDEYSWRKYGQKPIKGSEPRGYYKC	271	
AtWRKY15	.TTVSSFSKSTRKCNSENLLTGCAS.....ASSSGCHOSKRRIKQRIIRVPVPIASKMSPVPEIDYSWRKYGQKPIKGSEPRGYYKC	265	
AtWRKY17	APAPAPVPTSSGKPFPLSGLPYRKRCFEHDHSEGFSGKISGSG.NGKCHOSKRRSRNMRRTVVRPAISKIALIPEDEYSWRKYGQKPIKGSEPRGYYKC	268	
AtWRKY21	IEGKNSNFHEGVESSTDQNSLHSKRRKCPLIKGDHEGSLKCGSSSR..CHOSKRRKHRVRSIRVPVPIASKVADIPEDIDYSWRKYGQKPIKGSEPRGYYKC	338	
AtWRKY39	YDR.NSFHTLGLSRGSDDQHTRKMS.....GSLKCGSRSK..CHOSKRRKHRVRSIKVPAISKVADIPEDIEYSWRKYGQKPIKGSEPRGYYKC	287	
		WRKY domain	
AtWRKY7	SEVRGCPARKHVERALDDAMLIVTYEDHNEAL.VLETTTMNHDTL.....	353	
AtWRKY11	SEVRGCPARKHVERALDDPMLIVTYEGEPRHQ.S....AMQENISSSGINDLVFA	325	
AtWRKY15	SEVRGCPARKHVERAADDSSMLIVTYEGDHNESL.SAADLAGAAVADLILESS.....	317	
AtWRKY17	SEVRGCPARKHVERALDDSTMMLIVTYEGEPRHQ.S...TMQEHVTEPSVSG.LVFGS	320	
AtWRKY21	SEMRGCPARKHVERCLDEDAMLIVTYEGEPRHQ.K.L...PSQAIT.....	380	
AtWRKY39	SEVRGCPARKHVERCIEDTSMLIVTYEGEPRHQ.SSQSAAH.....	330	

B

Appendix Figure S1. Alignment and structure prediction of group IIId WRKY transcription factors in *Arabidopsis*. (A) Alignment of group IIId WRKY transcription factors in *Arabidopsis*. All group IIId WRKY transcription factors, including WRKY7, WRKY11, WRKY15, WRKY17, WRKY21, and WRKY39, were subjected to sequence alignment. The conserved CC and WRKY domains are labeled. Alignment was performed using DNAMAN. (B) Predicted structures of group IIId WRKY transcription factors. Structure was predicted by AlphaFold.

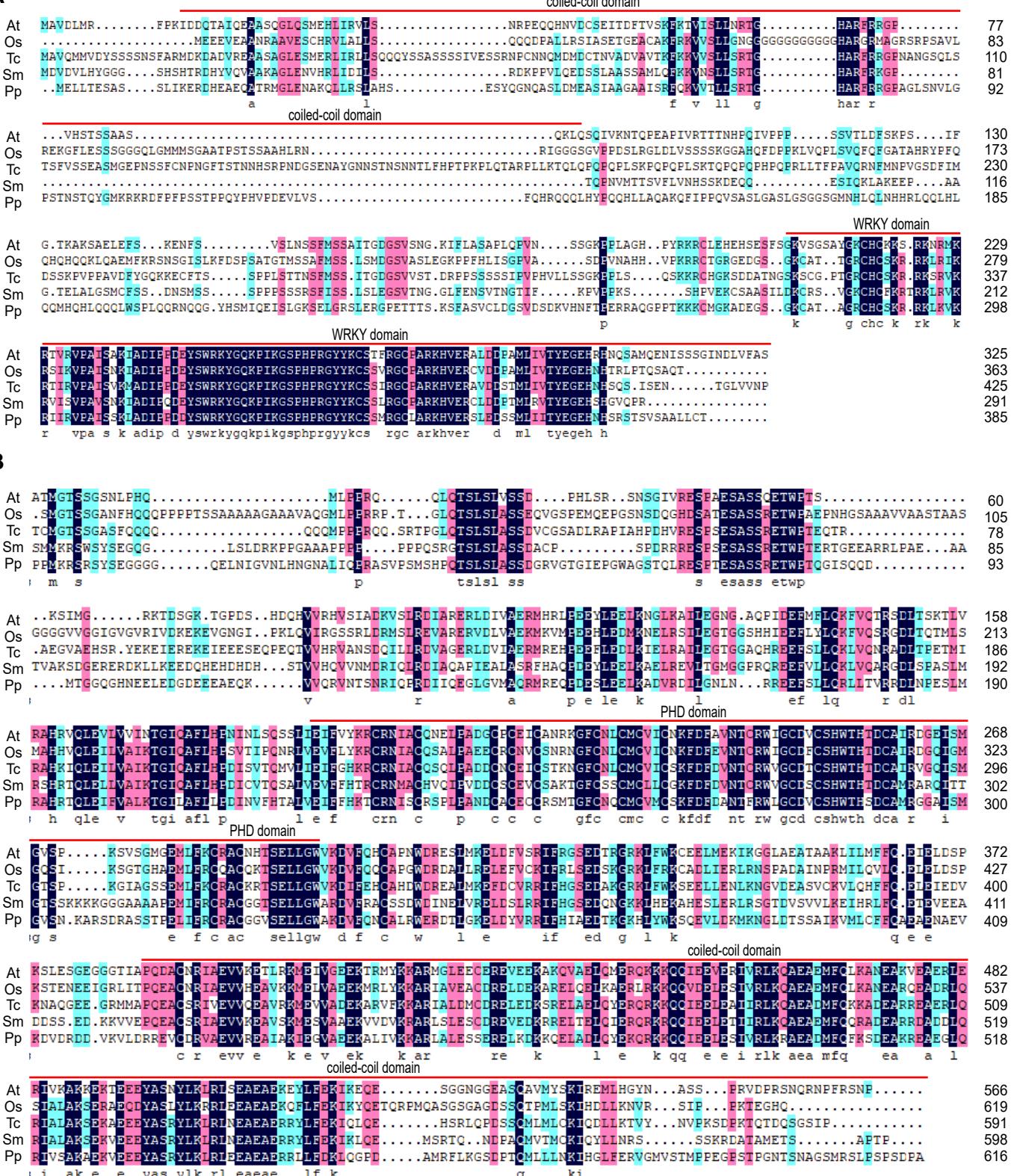


Appendix Figure S2. Phylogenetic relationship of group IIId WRKY transcription factors in plants. Group IIId WRKY transcription factors in bryophytes, ferns, gymnosperms, monocots and dicots were subjected to phylogenetic analysis. Group IIId WRKY transcription factors in other plants were obtained by BLAST using the *Arabidopsis* group IIId WRKY proteins. Accession numbers are from the NCBI database. The neighbor-joining tree (bootstrap method 1000) was drawn with MEGA5 software. The proteins labeled by red boxes were expressed in bacteria for the detection of WRKY-OBE interaction.

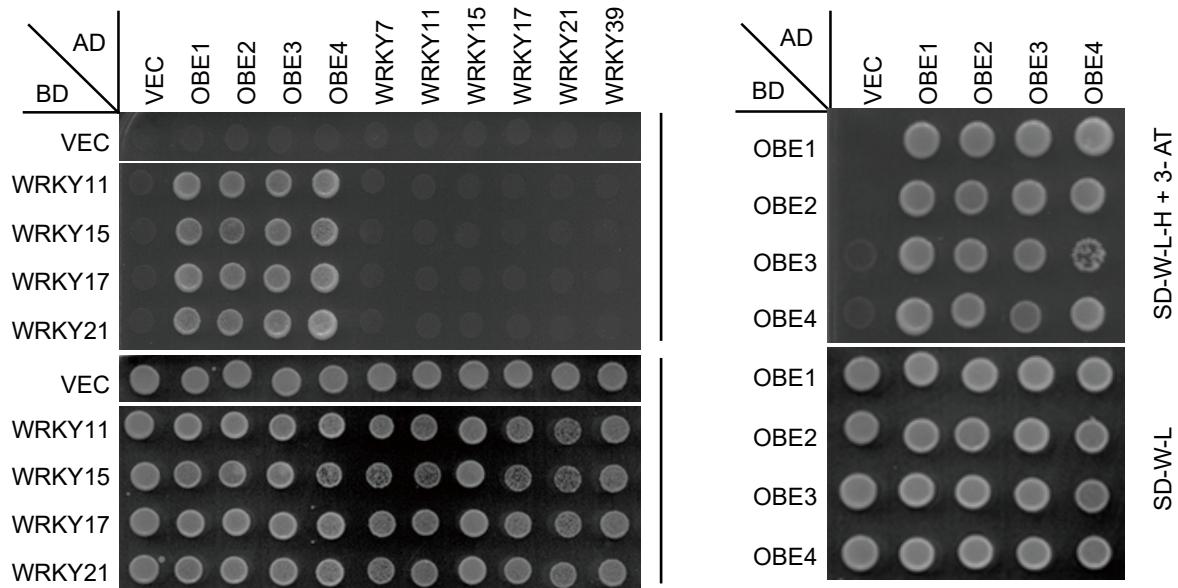


Appendix Figure S3. Phylogenetic relationship of OBE proteins in plants. OBE orthologs in bryophytes, ferns, gymnosperms, monocots and dicots were subjected to phylogenetic analysis. OBE orthologs in other plants were obtained by BLAST using the *Arabidopsis* OBE proteins. Accession numbers are from the NCBI database. The neighbor-joining tree (bootstrap method 1000) was drawn with MEGA5 software. The proteins labeled by red boxes were expressed in bacteria for the detection of WRKY-OBE interaction and for the histone peptide pull-down assay.

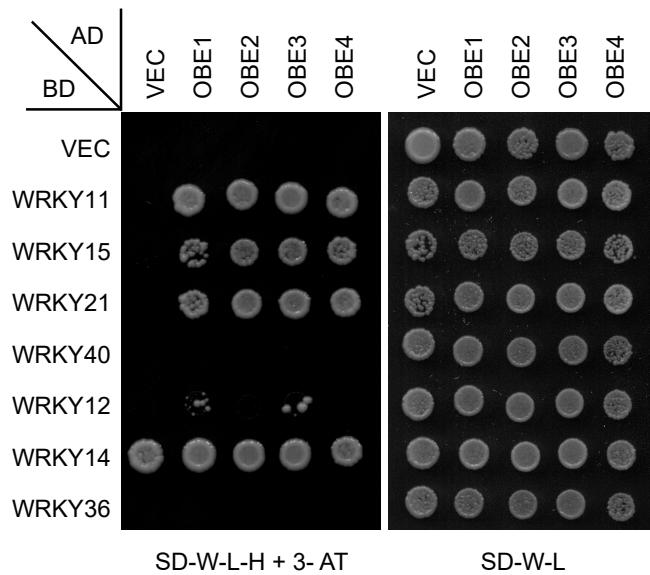
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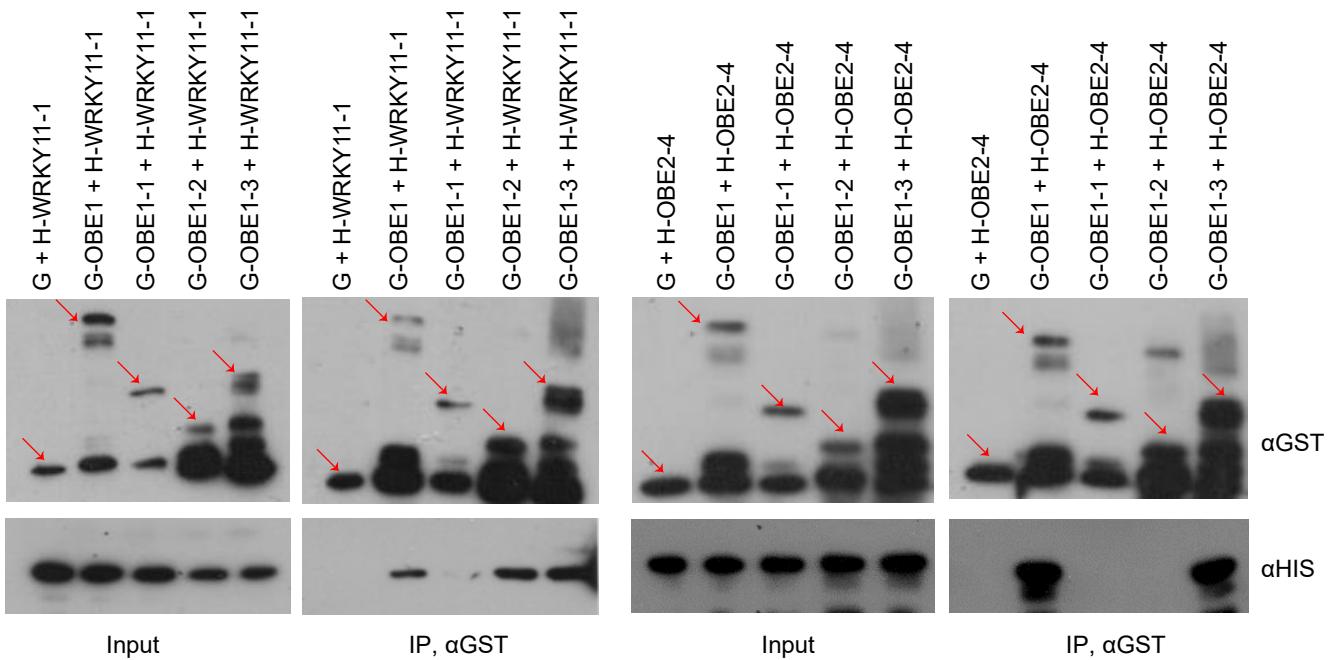
Appendix Figure S4. Alignment of WRKY11 and OBE1 and their orthologs in plants. (A) Alignment of WRKY11 and its orthologs in *Physcomitrium patens* (Pp), *Selaginella moellendorffii* (Sm), *Taxus chinensis* (Tc), and *Oryza sativa* (Os) in plants. The conserved CC and WRKY domains are shown. (B) Alignment of OBE1 and its orthologs in *Physcomitrium patens* (Pp), *Selaginella moellendorffii* (Sm), *Taxus chinensis* (Tc), and *Oryza sativa* (Os) in plants. The conserved PHD and CC domains are shown. The alignment is performed using DNAMAN.



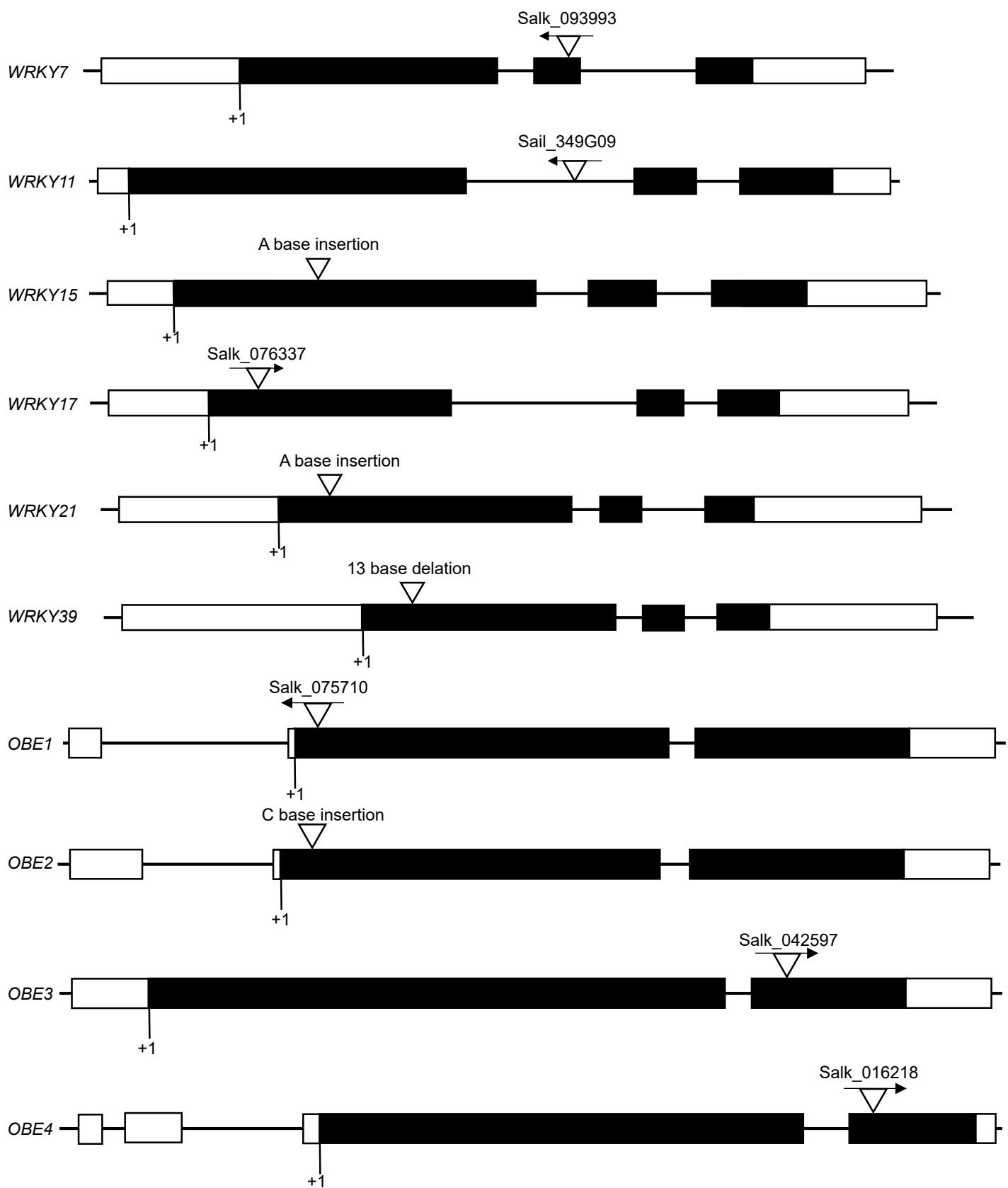
Appendix Figure S5. Determination of pairwise interactions among the WRKY-OBE complex components by Y2H assays. Yeast strains expressing the indicated GAL4-AD and GAL4-BD fused proteins were grown on SD medium lacking Trp and Leu (SD-W-L) and SD medium lacking Trp, Leu, and His (SD-W-L-H) supplemented with 3 mM 3-AT. Yeast strains harboring empty GAL4-AD or GAL4-BD vectors (VEC) were used as negative controls.



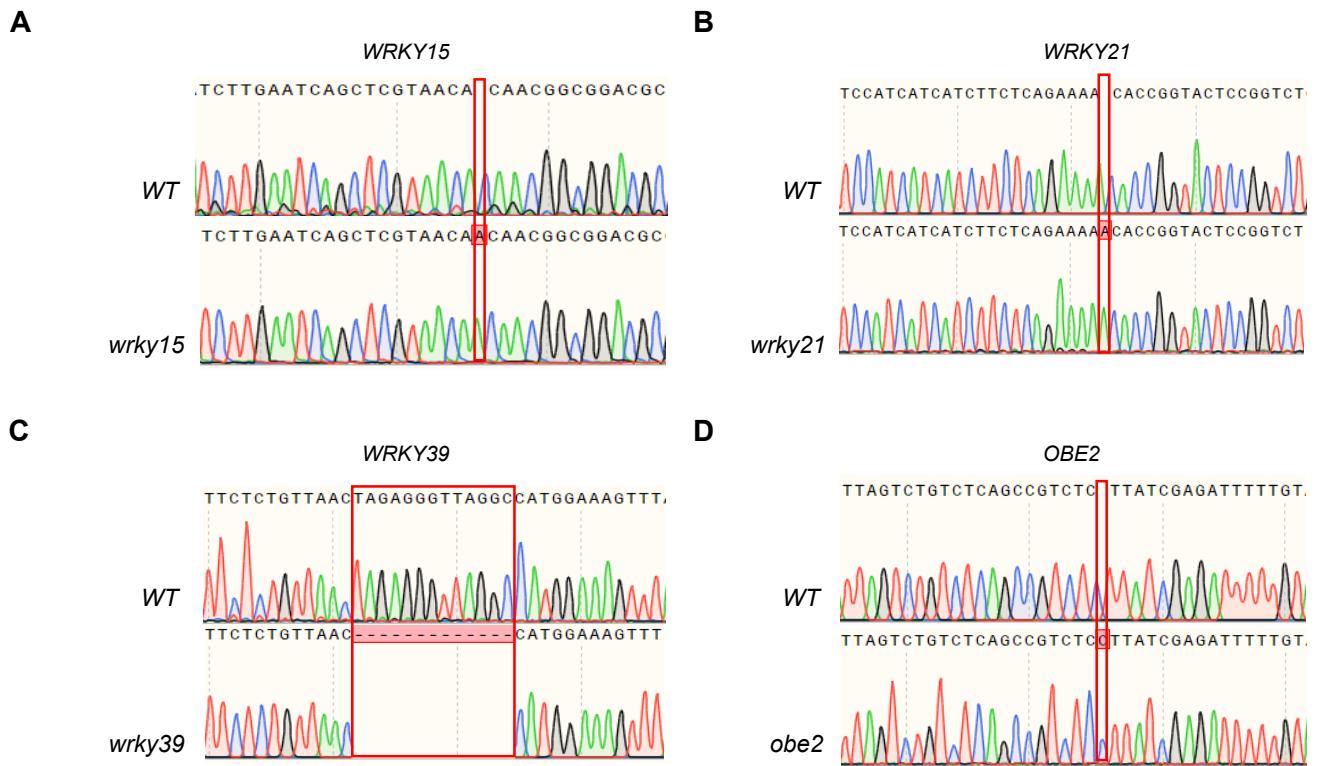
Appendix Figure S6. Determination of the interactions between OBE proteins and different groups of WRKY transcription factors by Y2H assays. Yeast strains expressing the indicated GAL4-AD and GAL4-BD fused proteins were grown on SD medium lacking Trp and Leu (SD-W-L) and SD medium lacking Trp, Leu, and His (SD-W-L-H) supplemented with 3 mM 3-AT. Yeast strains harboring empty GAL4-AD or GAL4-BD vectors (VEC) were used as negative controls.



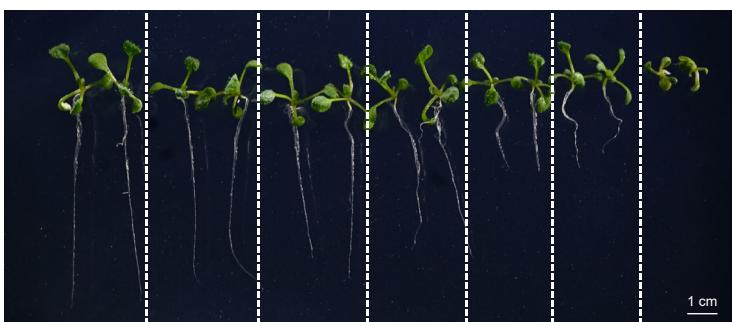
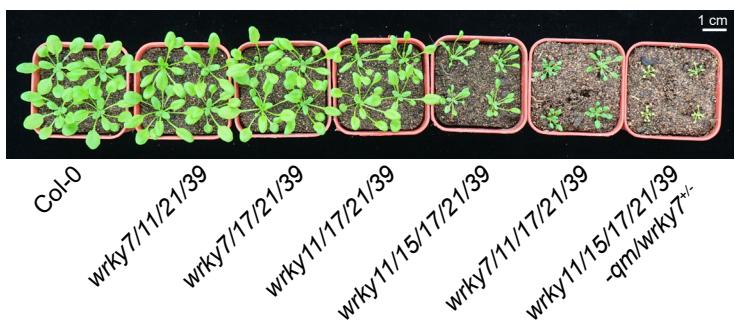
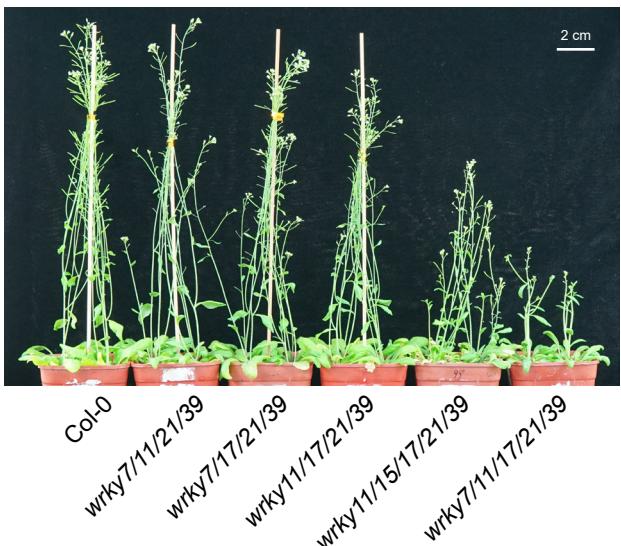
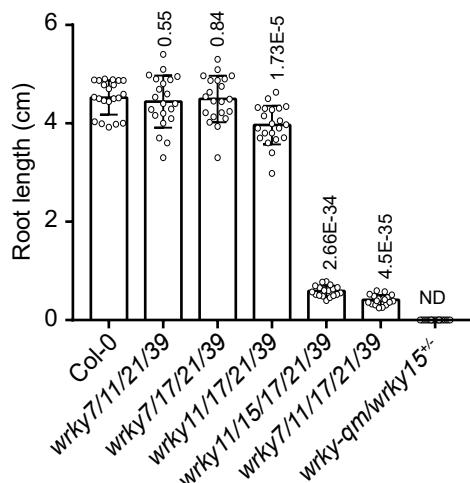
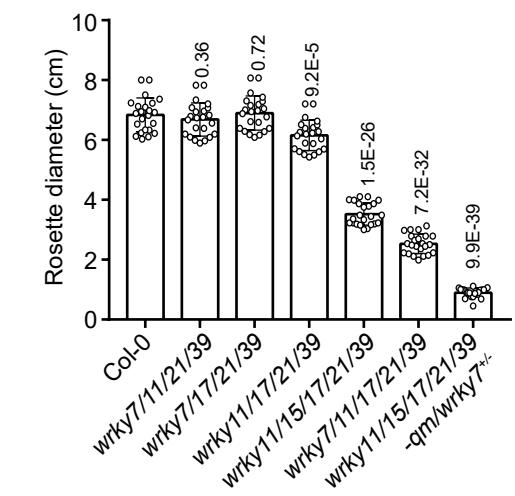
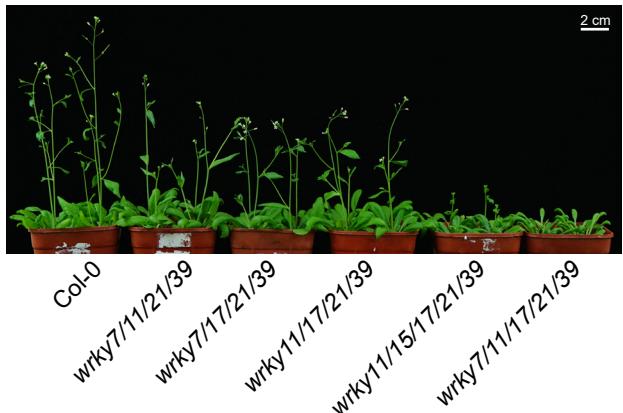
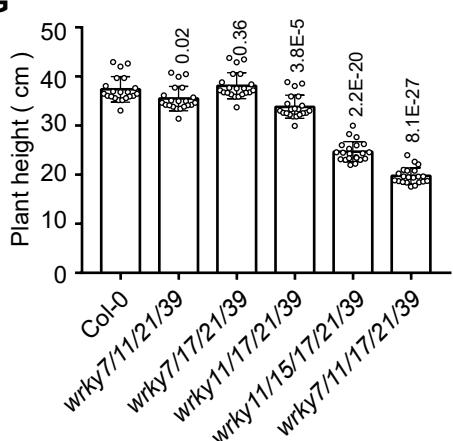
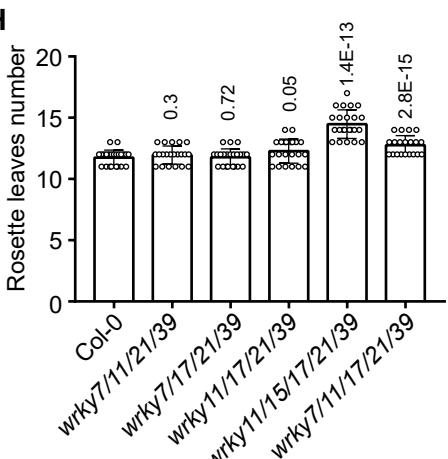
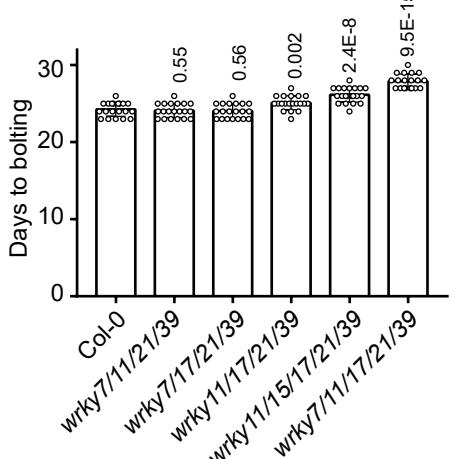
Appendix Figure S7. Determination of the protein-protein interactions in the WRKY-OBE complex by pull-down assays.
 Full-length and/or truncated versions of GST- or His-tagged WRKY11, OBE1, and OBE2 proteins were expressed and purified in bacteria, and then subjected to pull-down assays. GST- and His-tagged proteins were subjected to the pull-down assay. G, GST; H, His. Arrow represented the indicated proteins.



Appendix Figure S8. Schematic representation of wrky and obe mutations. The wrky7, wrky11, wrky15, wrky17, wrky21, wrky39, obe1, obe2, obe3, and obe4 mutations used in this study are shown. Boxes and lines are exons and introns, respectively. Blank boxes are 5'-UTR or 3'-UTR, and black boxes are protein-coding regions. The positions and directions of T-DNA insertions and point mutations generated by CRISPR-Cas9 are shown.

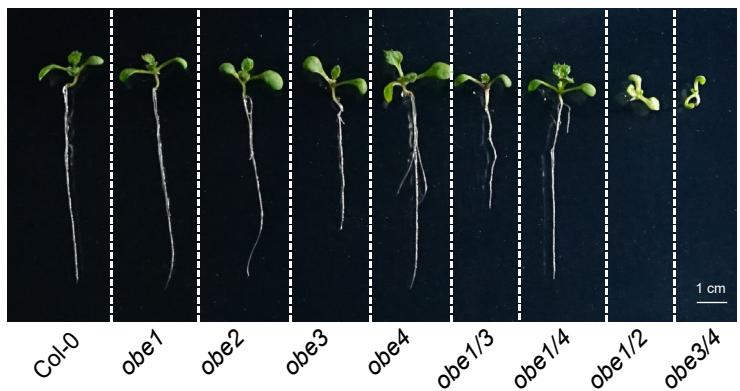


Appendix Figure S9. Validation of CRISPR-Cas9-induced *wrky15*, *wrky21*, *wrky39* and *obe2* mutations by Sanger sequencing. (A) The genomic sequences of mutated *WRKY15*. (B) The genomic sequences of mutated *WRKY21*. (C) The genomic sequences of mutated *WRKY39*. (D) The genomic sequences of mutated *OBE2*. All mutated sequences are aligned with the corresponding wild-type sequences.

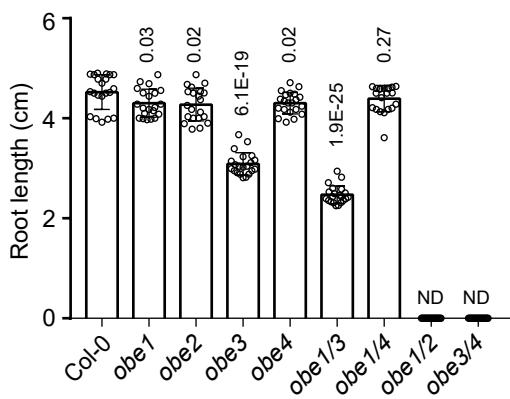
A**C****E****B****D****F****G****H****I**

Appendix Figure S10. Morphological phenotypes of wrky mutants. (A) Morphological phenotypes of 12-day-old plants. (B) Statistical analyses of the root length of 12-day-old plants. (C) Morphological phenotypes of 20-day-old plants. (D) Statistical analyses of the rosette diameter of 20-day-old plants. (E) Morphological phenotypes of adult plants. (F) Morphological phenotypes of 30-day-old plants. (G) Statistical analyses of the plant height of adult plants. (H) Statistical analyses of the number of rosette leaves from bolting plants and statistical analyses of days to bolting. Values are means \pm SD ($n \geq 20$) in (B), (D), (G) and (H). P values were determined using two-tailed Student's t-test and are indicated above columns. ND (no data) shown in (B) indicates that wrky-qm/15^{+/} mutant seedlings lacked visible roots.

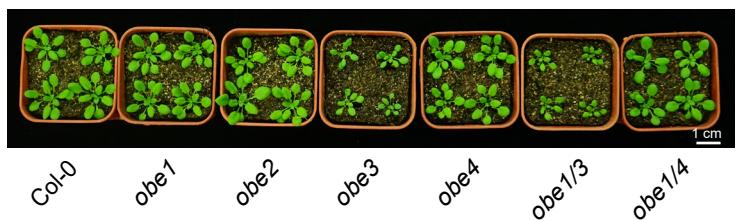
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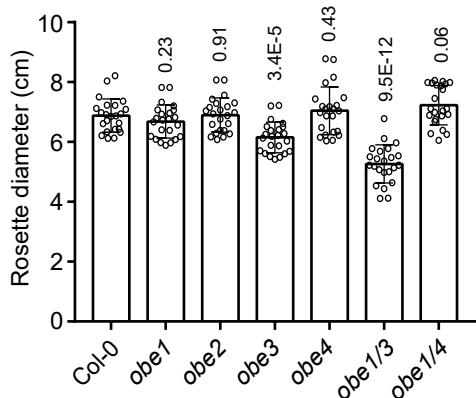
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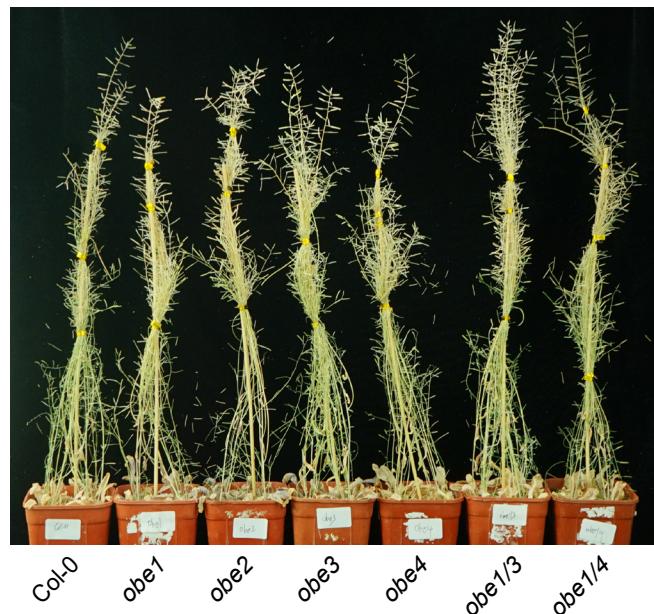
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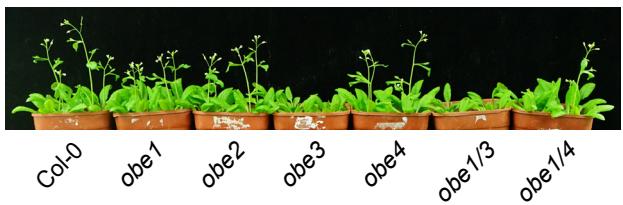
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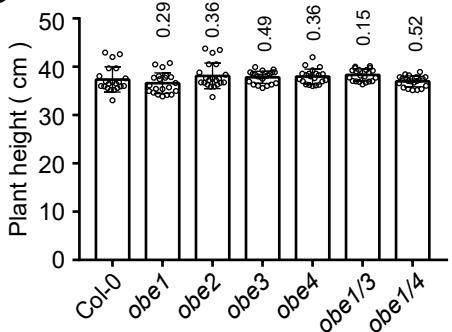
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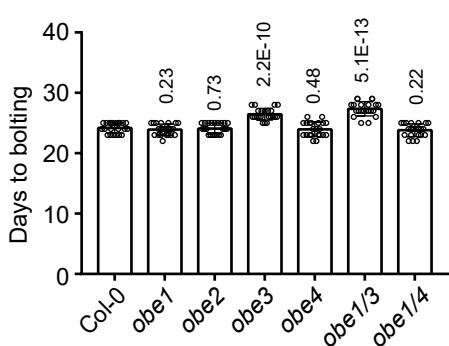
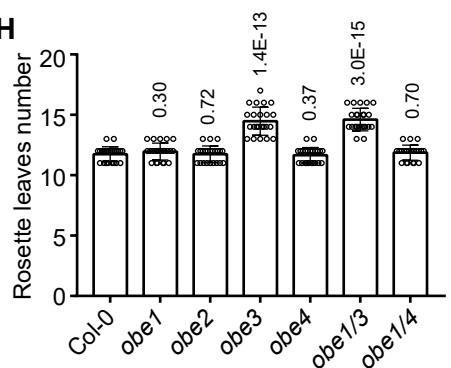
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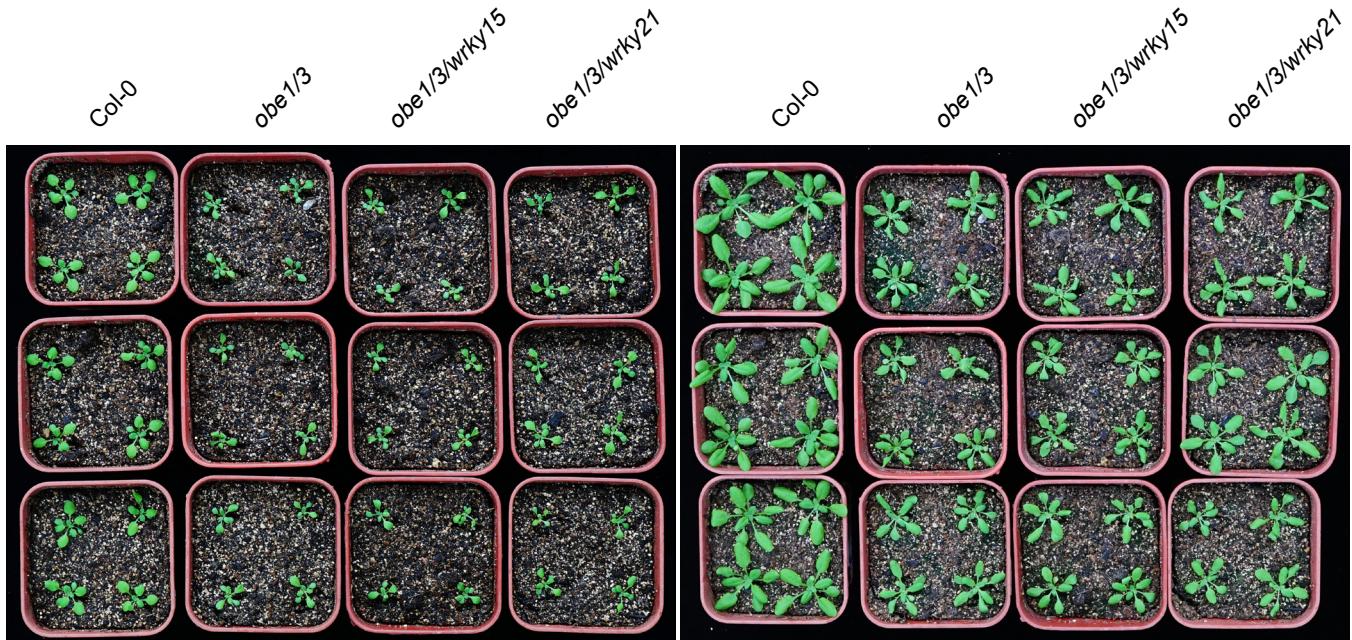
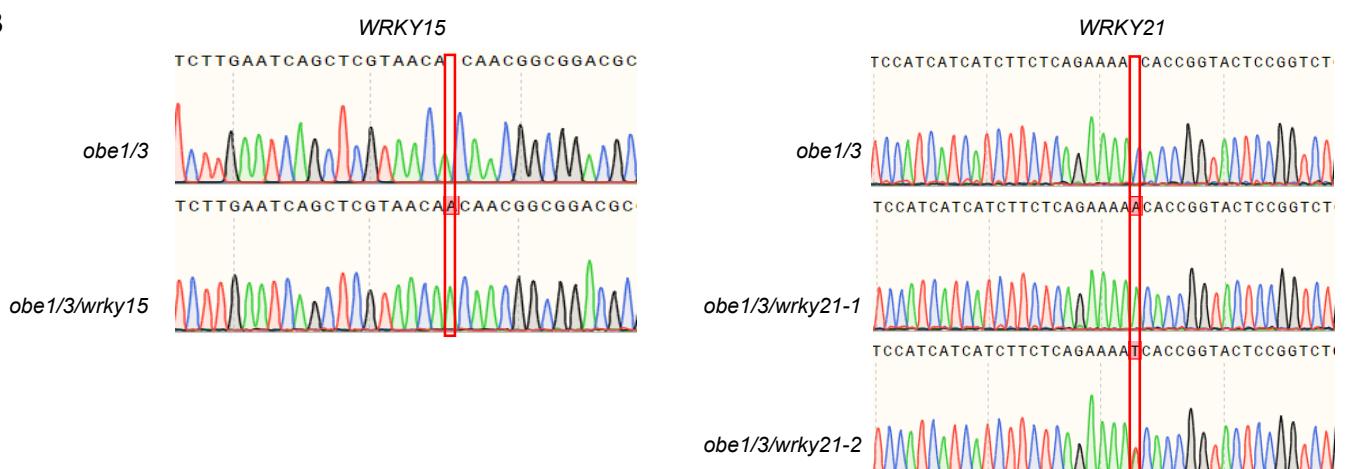
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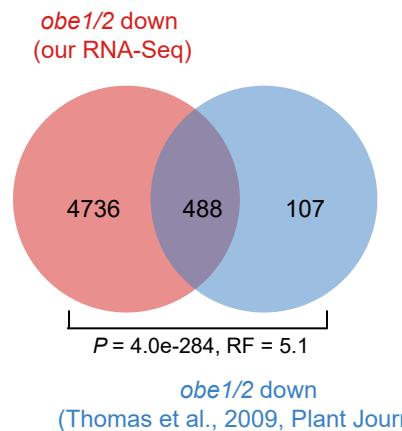
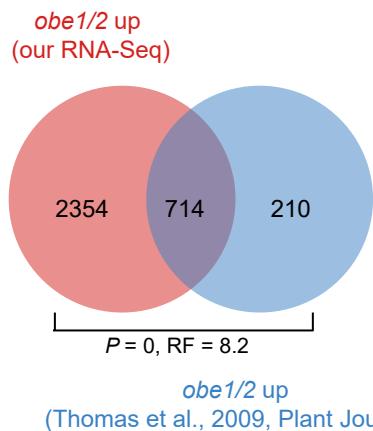
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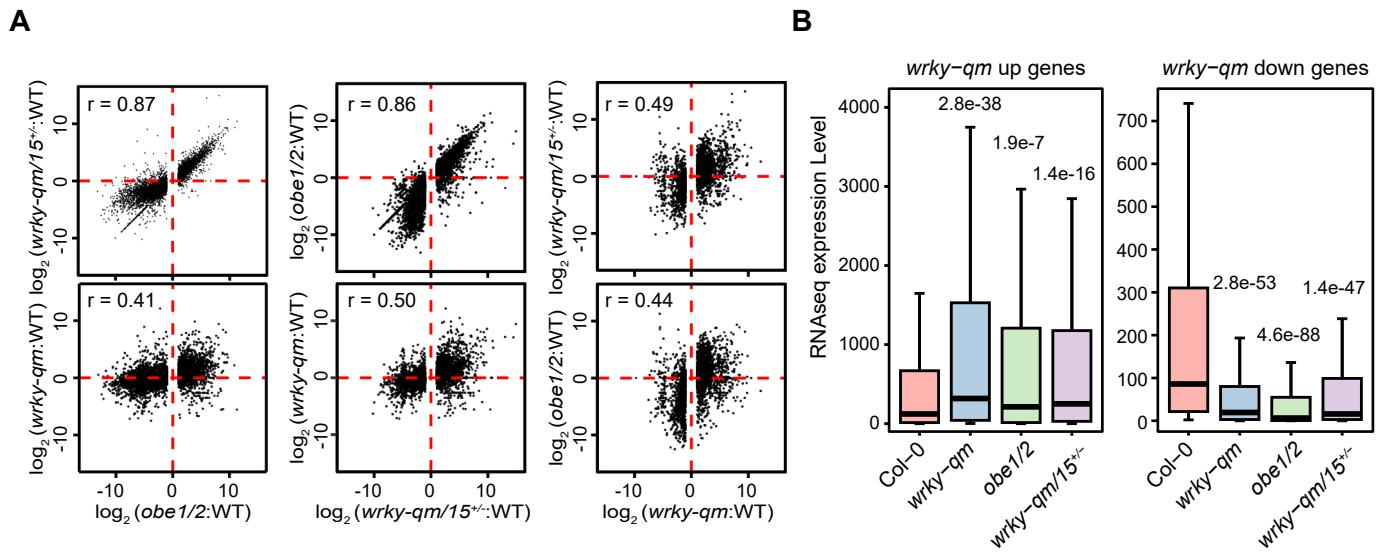
Appendix Figure S11. Morphological phenotypes of *obe* mutants. (A) Morphological phenotypes of 12-day-old plants. (B) Statistical analyses of the root length of 12-day-old plants. (C) Morphological phenotypes of 20-day-old plants. (D) Statistical analyses of the rosette diameter of 20-day-old plants. (E) Morphological phenotypes of adult plants. (F) Morphological phenotypes of 30-day-old plants. (G) Statistical analyses of the plant height of adult plants. (H) Statistical analyses of the number of rosette leaves from bolting plants and the days to bolting. Values are means \pm SD ($n \geq 20$) in (B), (D), (G) and (H). P values were determined using two-tailed Student's t-test and are indicated above columns. ND (no data) shown in (B) indicates that *obe1/2* and *obe3/4* mutant seedlings lacked visible roots.

A**B**

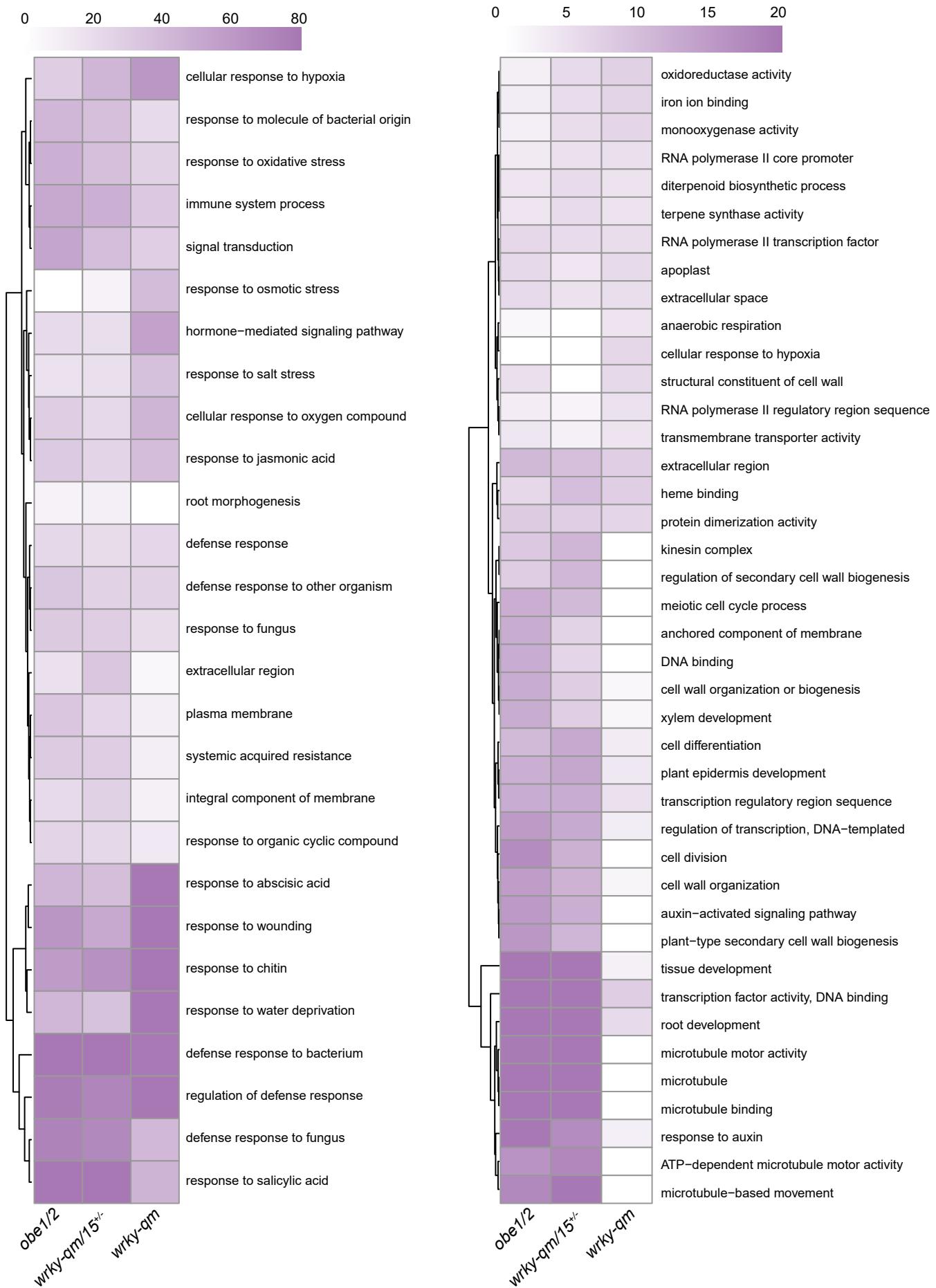
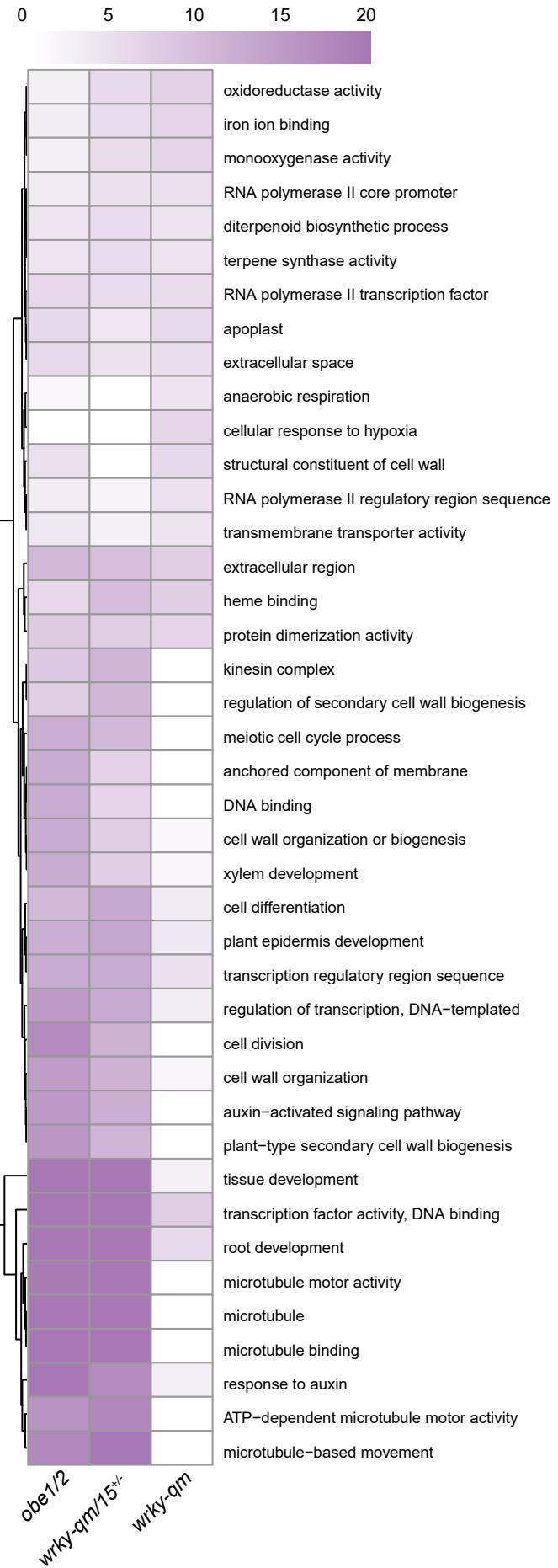
Appendix Figure S12. Morphological phenotypes of *obe1/3/wrky15* and *obe1/3/wrky21* triple mutants. (A) Morphological phenotypes of 14-day-old plants (left) and 21-day-old plants (right). (B) The mutated WRKY15 sequence in the *obe1/3/wrky15* triple mutant was aligned with the wild-type WRKY15 sequence in the *obe1/3* double mutant on the left, and the mutated WRKY21 sequences in the *obe1/3/wrky21-1* and *obe1/3/wrky21-2* triple mutants were aligned with the wild-type WRKY21 sequence in the *obe1/3* double mutant on the right.



Appendix Figure S13. Venn diagrams showing the overlap between *obe1/2*-regulated differentially expressed genes identified in the current study and the previously published study. Up- and down-regulated DEGs were independently compared between the current study and the previously published study. P values determined by the hypergeometric test (one-tailed) indicate the significant of overlapping. RF (representation factor) represents the number of observed overlapping genes divided by the number of expected overlapping genes drawn from two independent groups.



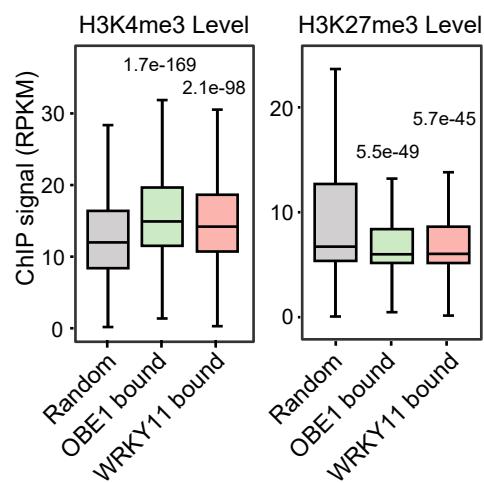
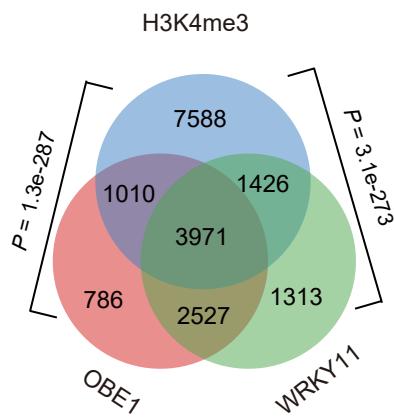
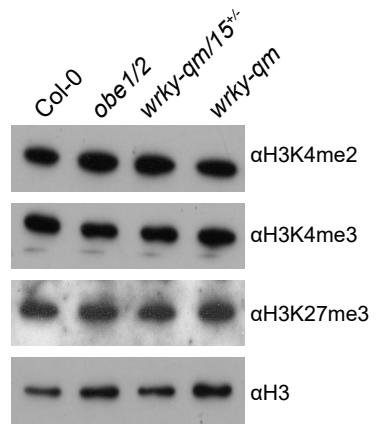
Appendix Figure S14. WRKY and OBE proteins co-regulate gene expression. (A) Scatter plots showing the correlation of the expression changes between the *obe1/2*, *wrky-qm15^{+/}* and *wrky-qm* mutants. The Pearson correlation coefficient (*r*) is shown. (B) Box plots showing the gene expression levels of *wrky-qm*-affected up- and down-regulated genes in the wild type, *wrky-qm*, *obe1/2* and *wrky-qm15^{+/}* mutants. In boxplots, center lines and box edges are medians and the interquartile range (IQR), respectively. Whiskers extend within 1.5 times the IQR. *P* values were determined by two-tailed Mann Whitney *U* test (paired) for nonnormally distributed data.

A**B**

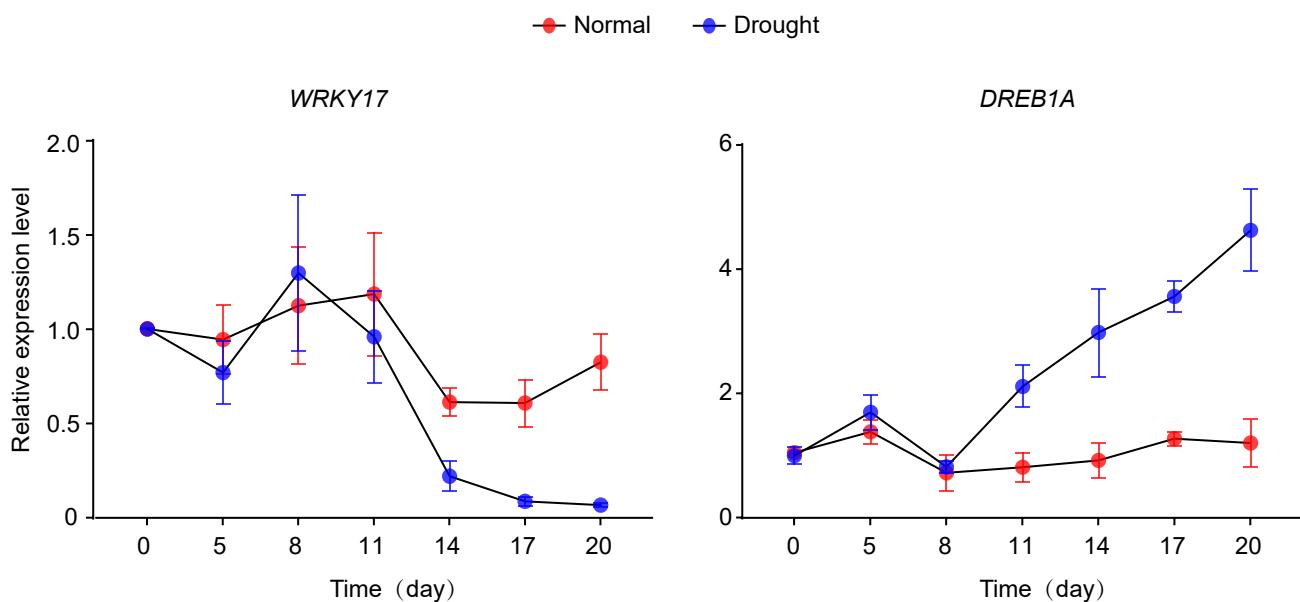
Appendix Figure S15. GO analysis of DEGs in *obe1/2*, *wrky-qm/15^{+/−}* and *wrky-qm*. (A) GO analysis of up-regulated DEGs in indicated mutants relative to the wild type. (B) GO analysis of down-regulated DEGs in indicated mutants relative to the wild type. GO analysis was performed using the DAVID website (<https://david.ncifcrf.gov/tools.jsp>). The number of DEGs for each GO term is represented by the color density.

Rank	Motif	P-value	Best Match/Details
1		1e-1089	WRKY23/MA1080.1/Jaspar(0.989) More Information Similar Motifs Found
2		1e-315	BMAL1(bHLH)/Liver-Bmal1-ChIP-Seq(GSE39860)/Homer(0.988) More Information Similar Motifs Found
3		1e-289	Osl_08196/MA1050.1/Jaspar(0.956) More Information Similar Motifs Found
4		1e-205	WRKY50/MA1317.1/Jaspar(0.838) More Information Similar Motifs Found
5		1e-168	CAMTA1(CAMTA)/col-CAMTA1-DAP-Seq(GSE60143)/Homer(0.929) More Information Similar Motifs Found
6		1e-167	Trl/MA0205.2/Jaspar(0.918) More Information Similar Motifs Found
7		1e-88	ZIM(C2C2gata)/col-ZIM-DAP-Seq(GSE60143)/Homer(0.824) More Information Similar Motifs Found
8		1e-68	AT3G10030(Trihelix)/colamp-AT3G10030-DAP-Seq(GSE60143)/Homer(0.921) More Information Similar Motifs Found
9		1e-65	TGA2(bZIP)/colamp-TGA2-DAP-Seq(GSE60143)/Homer(0.740) More Information Similar Motifs Found
10		1e-62	QKR58E-1(KH)/Drosophila_melanogaster-RNCMPT00142-PBM/HughesRNA(0.906) More Information Similar Motifs Found

Appendix Figure S16. Identification of significantly enriched motifs in genomic region co-occupied by WRKY11 and OBE1. The motif was identified using the Homer *de novo* motif analysis. The identified motifs were ranked according to their enrichment, motif enrichment is calculated using a modified version of the cumulative hypergeometric distribution.

A**B****C**

Appendix Figure S17. Determination of the histone modification levels of WRKY11- and OBE1-bound genes and the effect of *wrky* and *obe* mutations on histone modifications. (A) Box plots showing the H3K4me3 and H3K27me3 levels of WRKY11- and OBE1-bound genes and randomly selected genes. In boxplots, center lines and box edges are medians and the interquartile range (IQR), respectively. Whiskers extend within 1.5 times the IQR. P values were determined by two-tailed Mann Whitney U test (unpaired) for nonnormally distributed data. (B) Venn diagram showing the overlap between WRKY11, OBE1 and H3K4me3-enriched genes. P values determined by the hypergeometric test (one-tailed) indicate the significance of overlapping. (C) Global H3K4me2/3 and H3K27me3 levels detected by immunoblotting in the wild type (Col-0), *obe1/2*, *wrky-qm/15^{-/-}*, and *wrky-qm* mutants. The histone H3 level was determined as a loading control.



Appendix Figure S18. The expression levels of *WRKY17* and *DREB1A* under non-stress and drought stress conditions as determined by quantitative RT-PCR. Arabidopsis Col-0 plants were grown in soil under long-day conditions (16 h light/8 h dark) at 22°C and were either well-watered or subjected to drought treatment for 0, 5, 8, 11, 14, 17, or 20 days. Values are means \pm SD of three biological replicates.