Appendix

Chromatin mechanism of DELLA-mediated gene repression and GA-induced gene activation in rice

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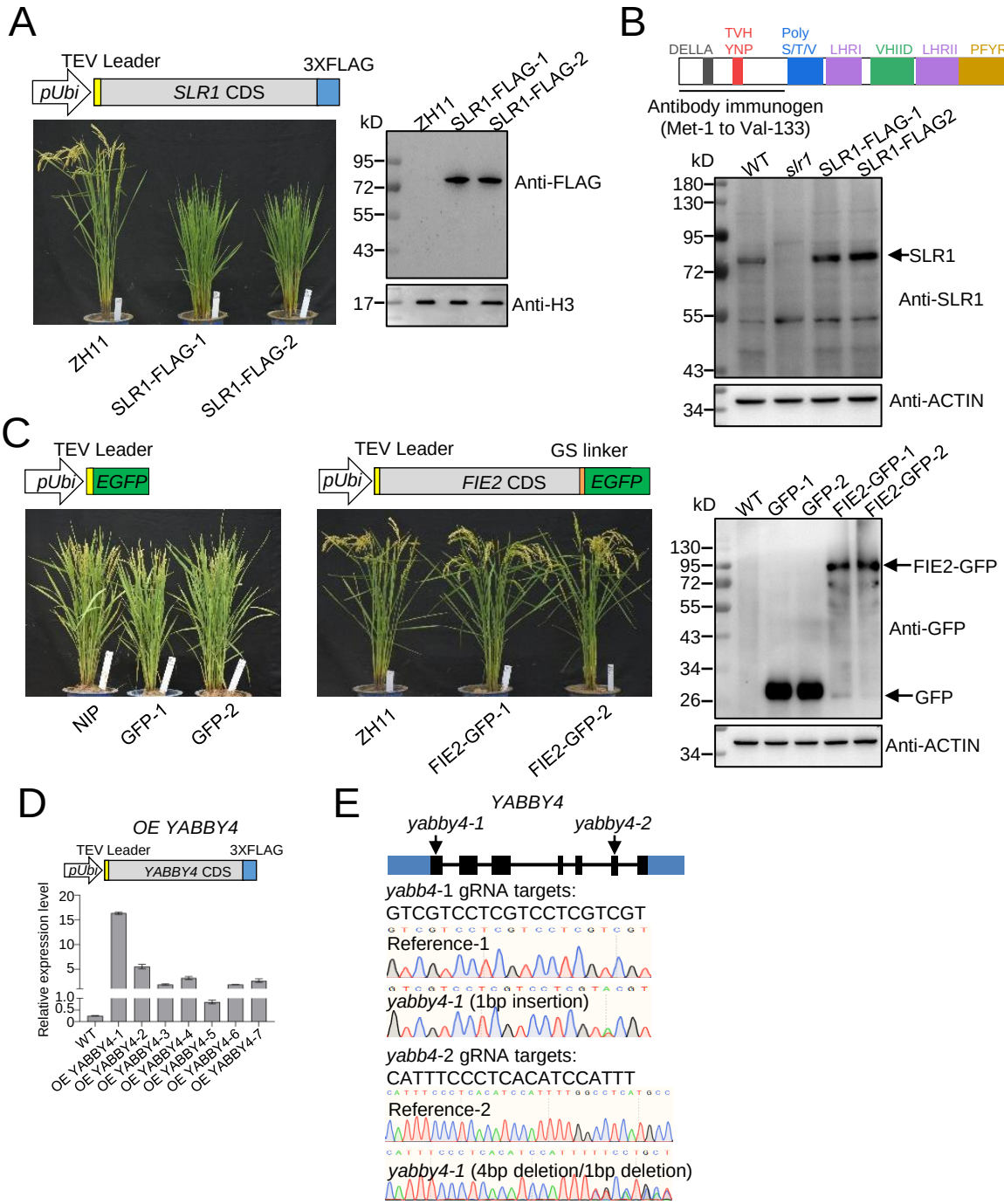
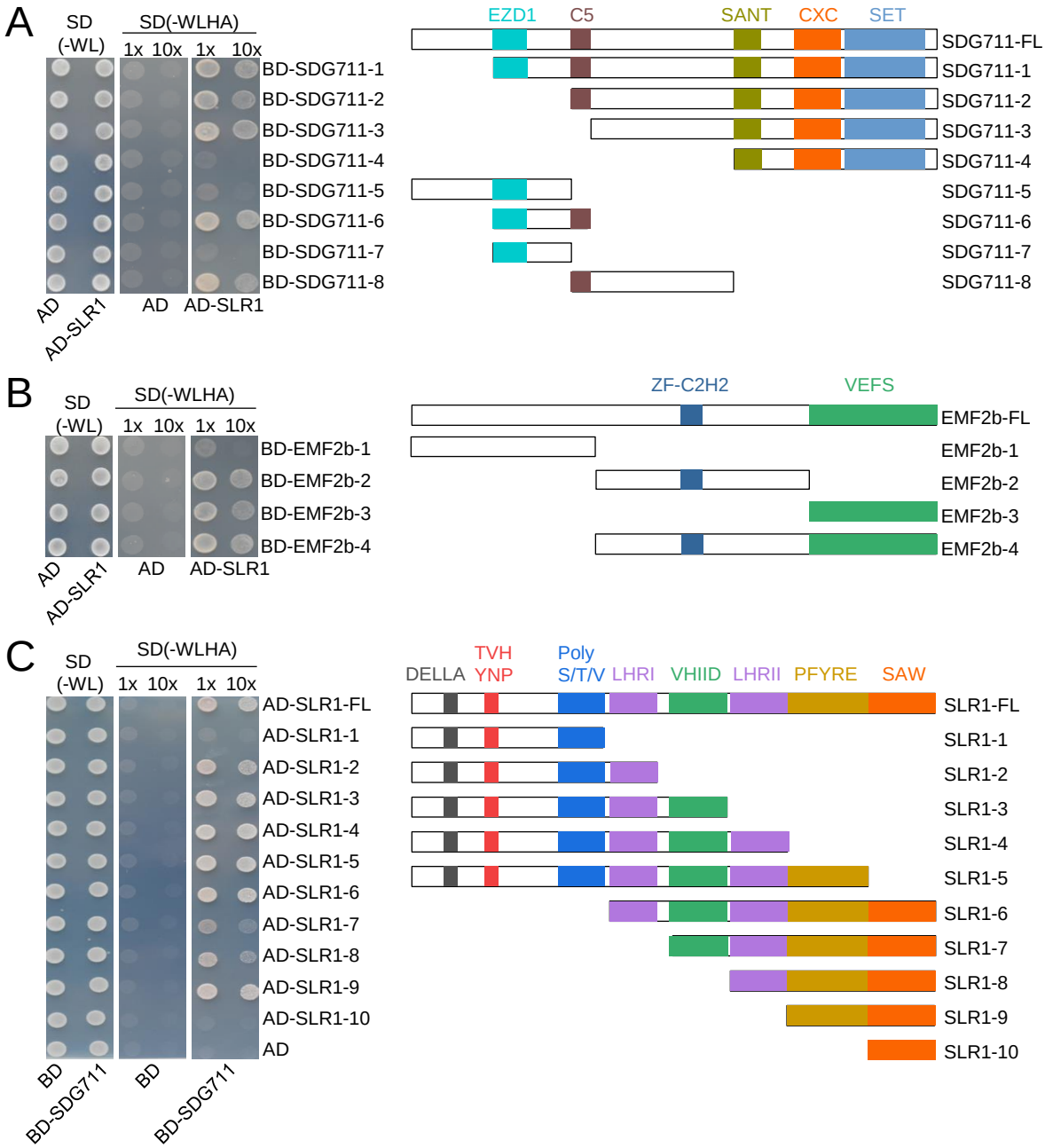
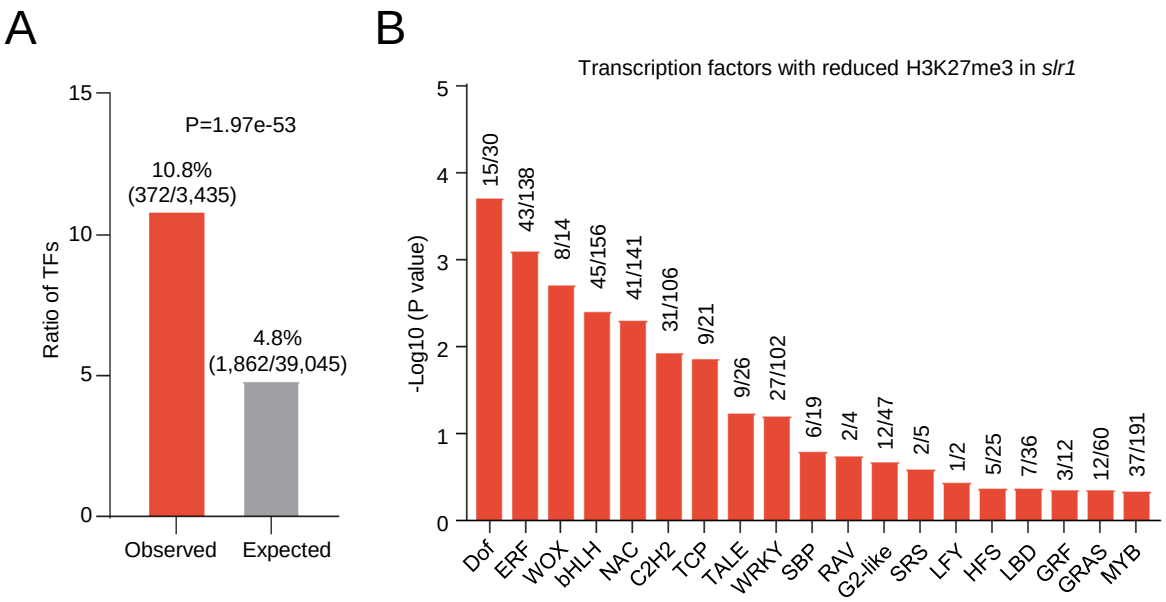


Appendix Figure S1. Production of transgenic plants and SLR1 antibody.
A. Production of SLR1-FLAG plants. The vector, morphology (at mature stage) and protein levels (by immunoblots) of the transgenic plants (2 independent lines) are shown. SLR1-FLAG plants at T2 generations were used in this study. **B.** Production of SLR1 antibody. The N-terminal domain (Met-1 to Val-133) of SLR1 was used as the epitope for SLR1 antiserum production in rabbits. Western blot analysis of total proteins extracted from seedlings of 12-day-old wild type, *slr1* mutant, and the *SLR1-FLAG* lines using the produced SLR1 antibody. ACTIN was used as internal control. Details of antibody preparation are described in Methods section. **C.** Production of FIE2-EGFP plants. The FIE2-GFP and GFP empty vector plant morphology and Western blot analysis of FIE2-GFP and GFP levels (with anti-GFP) are shown. **D.** Production and characterization of *OsYABBY4* overexpression lines in the ZH11 background. *OsYABBY4* transcript levels in the transgenic lines were tested by RT-qPCR. *OE YABBY4-1* and *OE YABBY4-2* were selected for analysis in this study. **E.** Creation of rice *yabby4* mutants by gene editing.



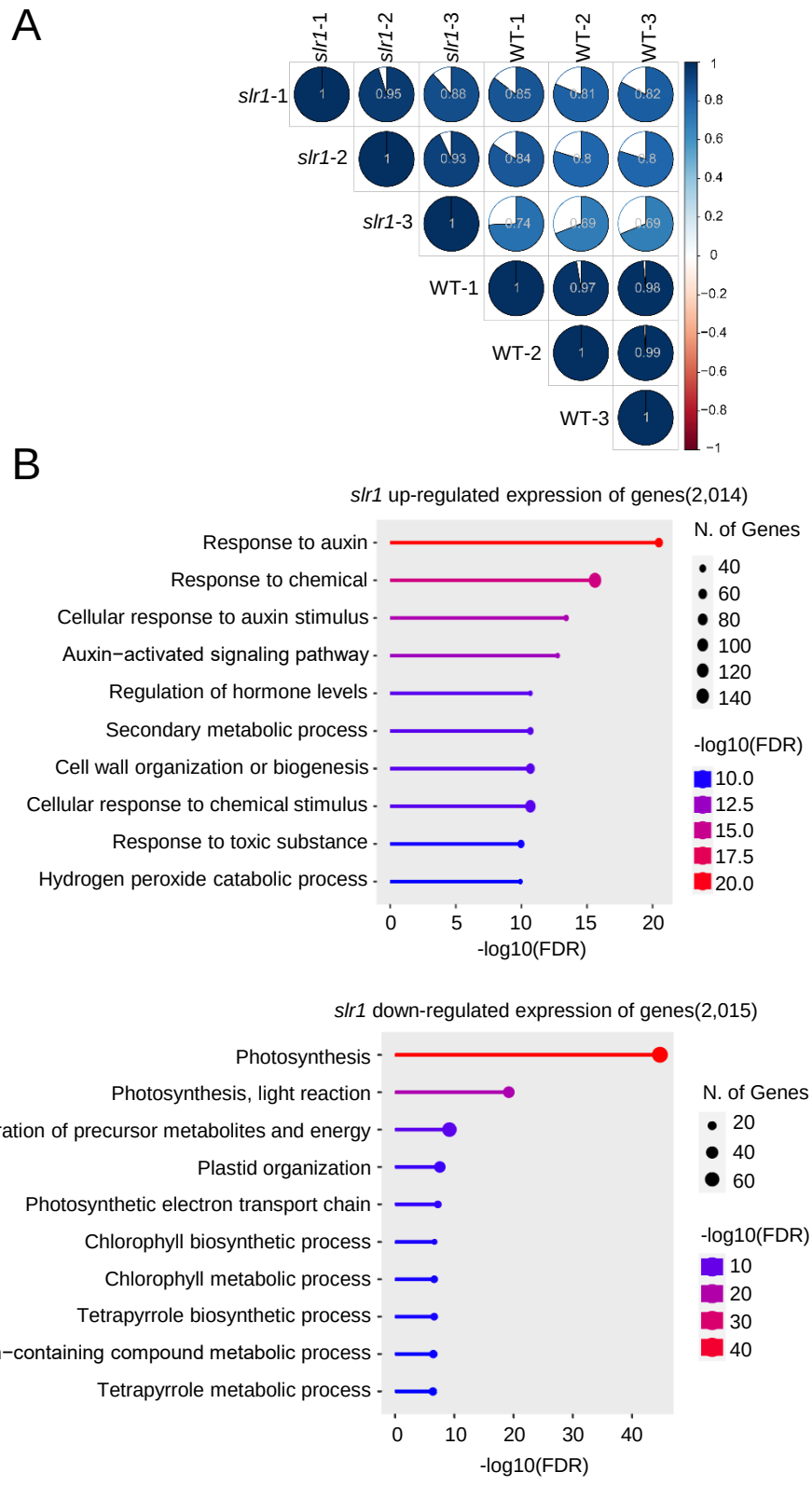
Appendix Figure S2. Y2H assay of interaction between the truncated regions of SLR1 and PRC2 proteins.

A. Tests of SDG711 fragments for interaction with SLR1. Full length SDG711 (amino acids [aa] 1-896), SDG711-1 (aa 121-896), SDG711-2 (aa 249-896) , SDG711-3 (aa 324-896), SDG711-4 (aa 523-896), SDG711-5 (aa 1-248), SDG711-6 (aa 121-323),SDG711-7 (aa 121-248), SDG711-8 (aa 249-522) were fused to the GAL4 BD (binding domain) as indicated. Yeast cells were spotted onto a stringent selection medium lacking Trp, Leu, His, and ADE (-WLHA) or a nonselective medium lacking Trp and Leu (-WL) as control. Right: Structures of SDG711. **B.** Tests of EMF2b fragments for interaction with SLR1. EMF2b-FL (aa 1-604), EMF2b-1 (aa 1-207), EMF2b-2 (aa 208-452), EMF2b-3 (aa 453-604), EMF2b-4 (aa 208-604) were fused to the GAL4 BD. Right: Structures of EMF2b. **C**. Tests of SLR1 fragments for interaction with SDG711. Full length SLR1 (amino acids [aa] 1-625), SLR1-1 (aa 1-231), SLR1-2 (aa 1-297) , SLR1-3 (aa 1-381), SLR1-4 (aa 1-456), SLR1-5 (aa 1-550), SLR1-6 (aa 237-625),SLR1-7 (aa 310-625), SLR1-8 (aa 383-625) , SLR1-9 (aa 456-625) , SLR1-10 (aa 550-625) were fused to the GAL4 AD (activation domain). Right: Structures of SLR1.

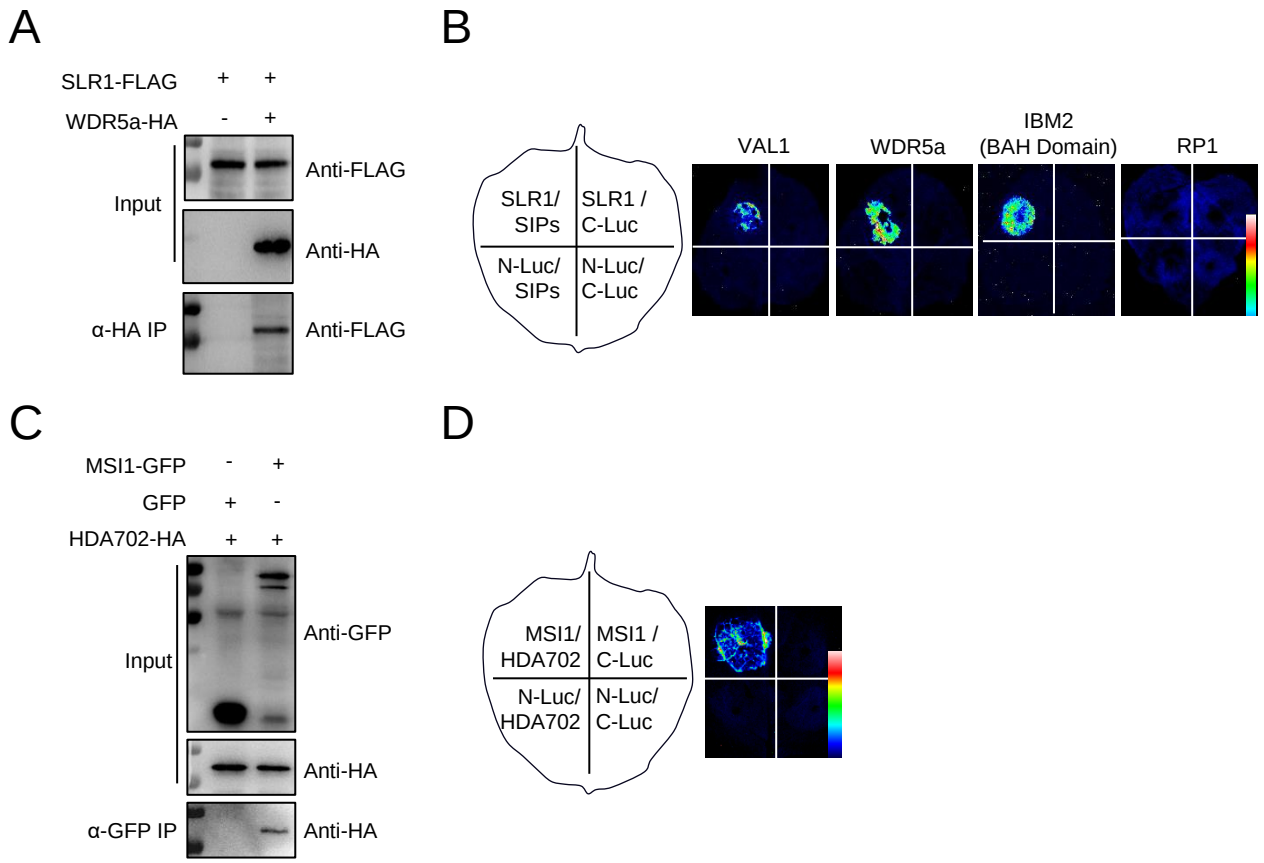


Appendix Figure S3. Genes with reduced H3K27me3 in *slr1* are enriched for transcription factors.

A. Relative enrichment of transcription factors in genes with reduced H3K27me3 in *slr1.* The frequency of transcription factor genes among H3K27me3-reduced genes were compared with the expected genomic frequency. P-values were calculated using Fisher's test. **B.** Relative enrichments of transcription factor families among H3K27me3-reduced genes in *slr1*. Detected gene number over the total gene number is indicated for each family.

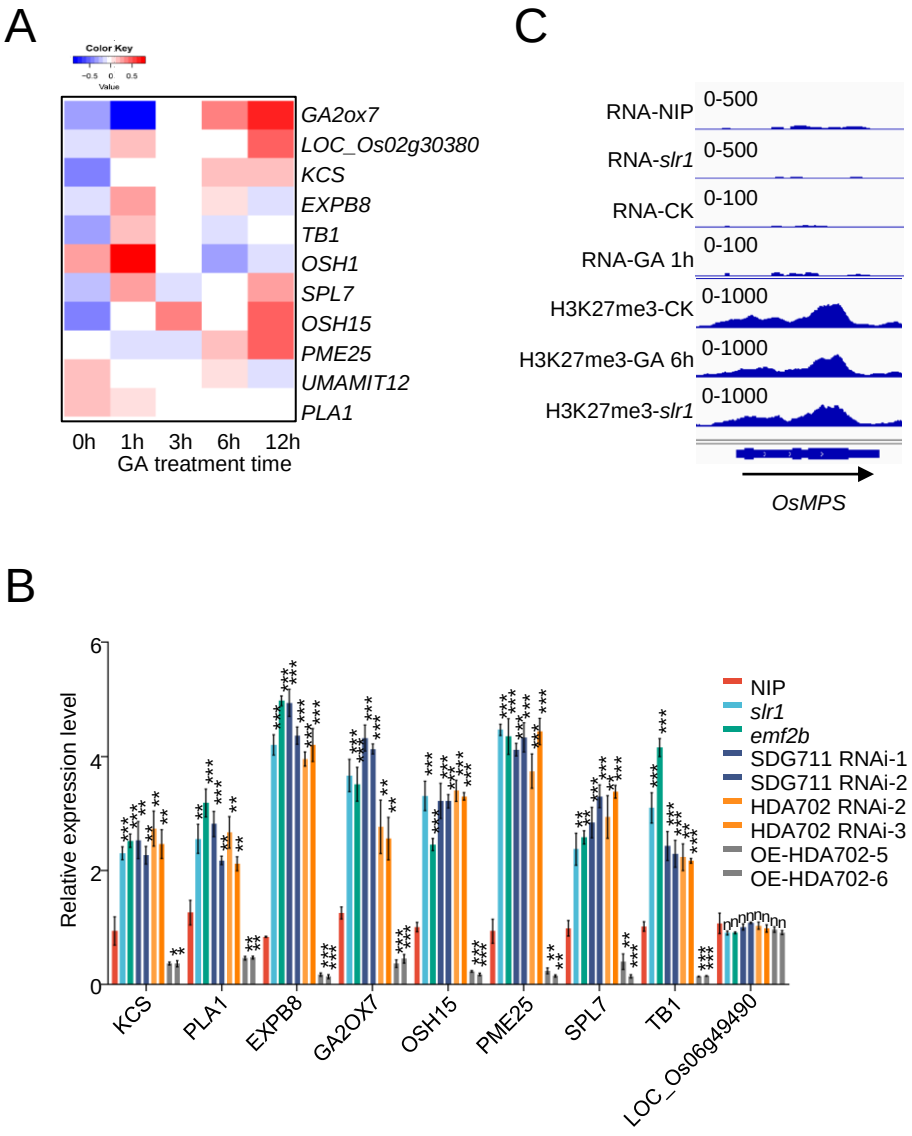


Appendix Figure S4. RNA-seq analysis of *slr1* and wild type.
A. Pearson correlation coefficients of RNA-seq data sets between replicates. **B.** GO enrichment of up-regulated and down-regulated genes in *slr1.*



Appendix Figure S5. Confirmation of interaction between SLR1 and chromatin proteins revealed by IP-MS.

A. Co-immunoprecipitation assays of SLR1 and WDR5a interaction in rice protoplasts. *35Spro:SLR1–FLAG* and *35Spro:WDR5a–HA* were co-transfected into rice protoplasts, followed by immunoprecipitation with anti–HA and immunoblots with anti-FLAG. **B.** Split luciferase complementary assays of interaction between SLR1 and VAL1, WDR5a or IMB2. cLUC- tagged SIP proteins (SIP:SLR1 interacting proteins) or cLUC alone were co-transformed into tobacco leaf cells with nLUC-tagged SLR1 or nLUC alone as schematized. RP1, a ribosomal protein, was used as a negative control. **C.** Tests of in vivo interaction between SLR1 and HDA702. *35Spro:MSI1–GFP* and *35Spro:HDA702–HA* were co-transfected into rice protoplasts, followed by immunoprecipitation with anti–HA and immunoblots with anti-GFP. GFP empty vector was transfected as a negative control. **D.** Split luciferase complementary assays of HDA702 interaction with MSI1. cLUC -tagged HDA702 or cLUC alone was co-transformed into tobacco leaf cells with nLUC-tagged MSI1 or nLUC alone, as schematized.



Appendix Figure S6. **Genes co-regulated by PRC2 and HDA702 are GA-responsive.**
A. GA-induced transcript levels of genes selected from the 397 genes in Figure 3D, data are from RiceXPro (https://ricexpro.dna.affrc.go.jp/) website. **B.** RT-qPCR validation of expression of the 8 GA-induced genes (with a non-induced control) in WT, *slr1*, *SDG711 RNAi*, *emf2b*, *HDA702 RNAi* and *OE* plants. Student's two-tailed t test (*** p<0.001, **, p<0.01, *, p<0.05; n, not significant) was used. **C**. Integrative Genomics Viewer images of RNA-seq and ChIP-seq signals at the *OsMPS* locus.

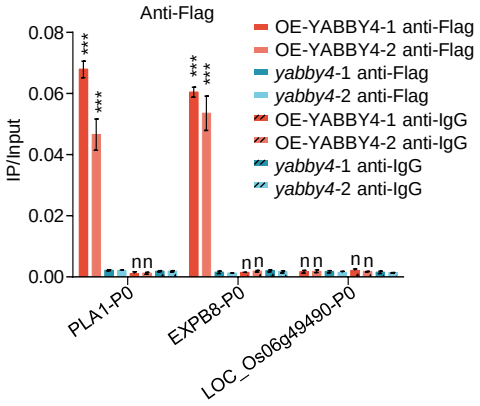
A

TF loucs	TF name	TF family	Gene # (background)	Target # (background)	Gene # (input)	Target # (Input)	p-value
LOC_Os01g51610	*OsLFL1*	B3 DNA binding domain	55986	2365	397	40	1.67E-07
LOC_Os12g39400	*OsDLN263*	C2H2 zinc finger protein	55986	893	397	15	7.55E-04
LOC_Os09g34060	*OsbZIP75*	bZIP	55986	560	397	11	7.56E-04
LOC_Os02g42950	*OsYAB4*	bZIP (YABBY domain)	55986	1526	397	20	3.30E-03
LOC_Os01g16810	*OsCSA*	MYB family	55986	372	397	7	5.43E-03
LOC_Os07g43030		calmodulin-binding	55986	303	397	6	6.23E-03
LOC_Os04g39470	*OsMYB80*	MYB family	55986	1070	397	14	1.03E-02
LOC_Os08g33940		MYB family	55986	1483	397	18	1.04E-02
LOC_Os12g33070	*OsMYB46*	MYB family	55986	435	397	7	1.31E-02
LOC_Os08g44820	*OsNTL5*	NAC	55986	712	397	10	1.37E-02
LOC_Os03g07450	*OsHox21*	homeobox associated	55986	1637	397	19	1.40E-02
LOC_Os02g42970	*ONAC052*	NAC domain	55986	443	397	7	1.45E-02
LOC_Os01g59660	*OsGAMYB*	MYB family	55986	360	397	6	1.50E-02
LOC_Os08g33150		MYB family	55986	832	397	11	1.68E-02
LOC_Os03g21800	*OsMYB52*	MYB family	55986	546	397	8	1.69E-02
LOC_Os02g42870	*OsMYB38*	MYB family	55986	1045	397	13	1.83E-02
LOC_Os03g20780	*OsEIL1*	EIN3	55986	761	397	10	2.12E-02
LOC_Os03g20790	*OsbZIP30*	bZIP	55986	761	397	10	2.12E-02
LOC_Os12g06520	*OsbZIP84*	bZIP	55986	579	397	8	2.36E-02
LOC_Os04g21950	*OsMYB80*	MYB family	55986	1082	397	13	2.38E-02
LOC_Os03g51110	*OsWRKY3*	WRKY3	55986	228	397	4	2.39E-02
LOC_Os03g55080	*OsWRKY51*	WRKY51	55986	681	397	9	2.48E-02
LOC_Os07g02060	*OsWRKY29*	WRKY29	55986	681	397	9	2.48E-02
LOC_Os01g66120	*OsNAC6*	NAC	55986	598	397	8	2.82E-02
LOC_Os02g16540	*OsWRKY39*	WRKY39	55986	1007	397	12	2.91E-02

B

YABBY	SLR1 target gene	Position to ATG	Strand	p-value	Matched sequence
YABBY4	*PLA1*	-1829 to -1838	+	2.70E-05	TTATAATAAT
YABBY4	*PLA1*	-2086 to -2095	+	3.12E-05	TAATCATAAA
YABBY4	*EXPB8*	-1114 to -1123	+	4.38E-05	CAATAATAAG
YABBY4	*EXPB8*	-184 to -193	-	5.59E-05	AAATAATAAT
YABBY4	*GA2OX7*	-1089 to -1098	-	2.70E-05	TTATAATAAT
YABBY4	*GA2OX7*	-1090 to -1099	+	6.33E-05	TTATTATAAT
YABBY4	*GA2OX7*	-906 to -915	+	7.97E-05	CAATGATAAA
DL(OsYABBY)	*GA2OX7*	-907 to -914	+	8.59E-05	AATGATAA
DL(OsYABBY)	*GA2OX7*	-1097 to -1104	+	8.59E-05	AATGATAA
YABBY4	*KCS*	-2422 to -2431	+	7.38E-06	TAATAATAAT
YABBY4	*KCS*	-2955 to -2964	+	2.15E-05	TAATTATAAT

C



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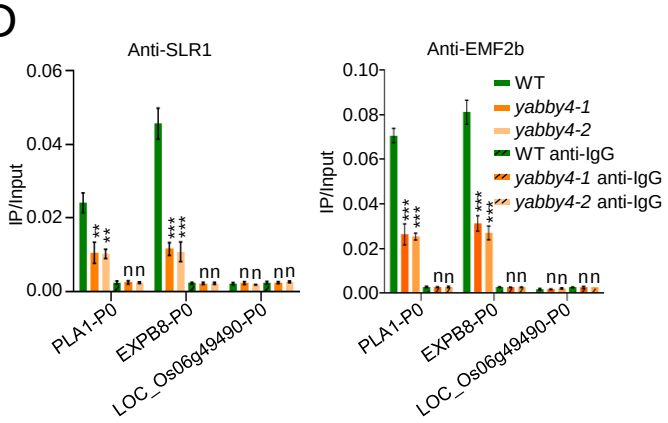
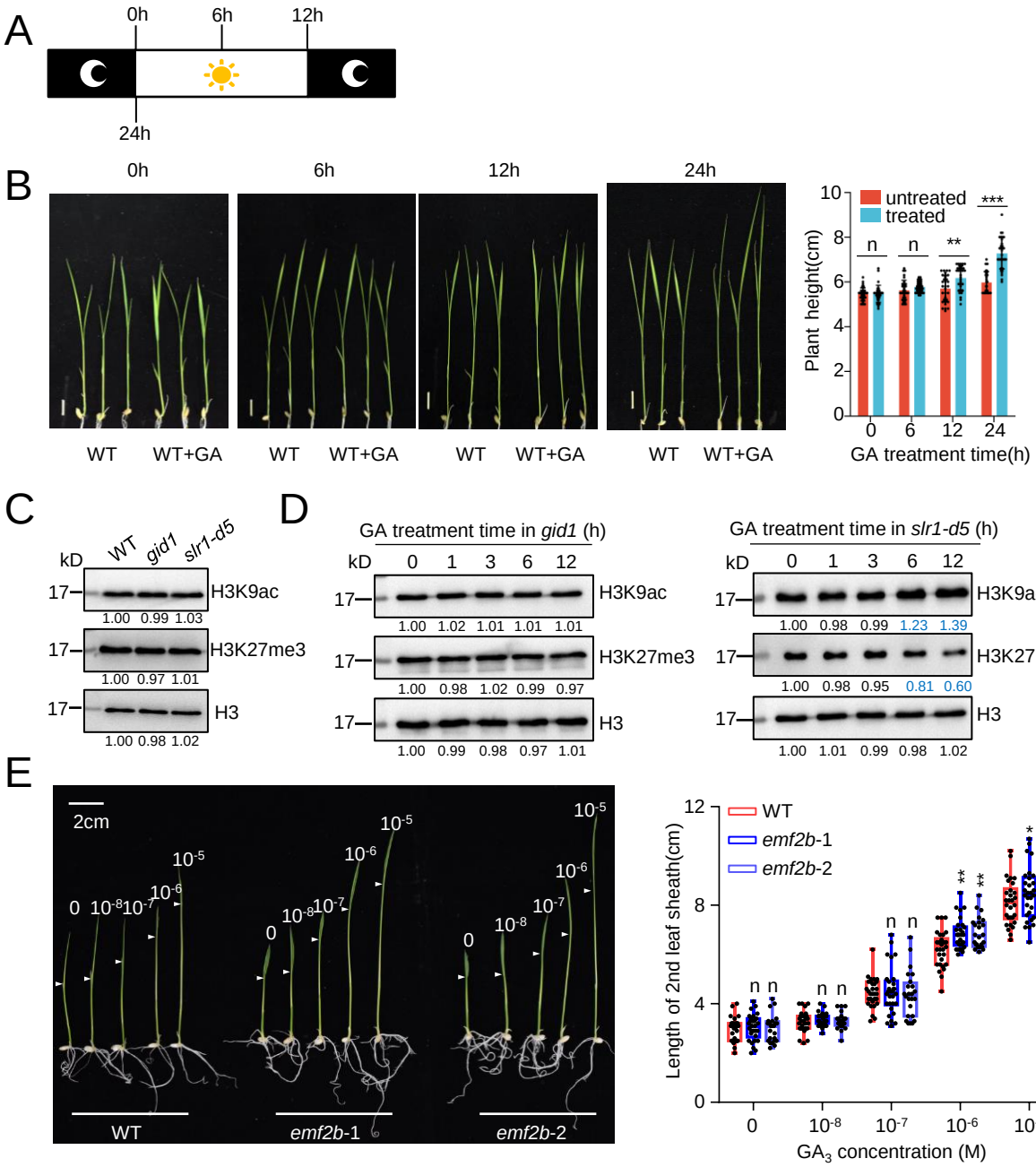


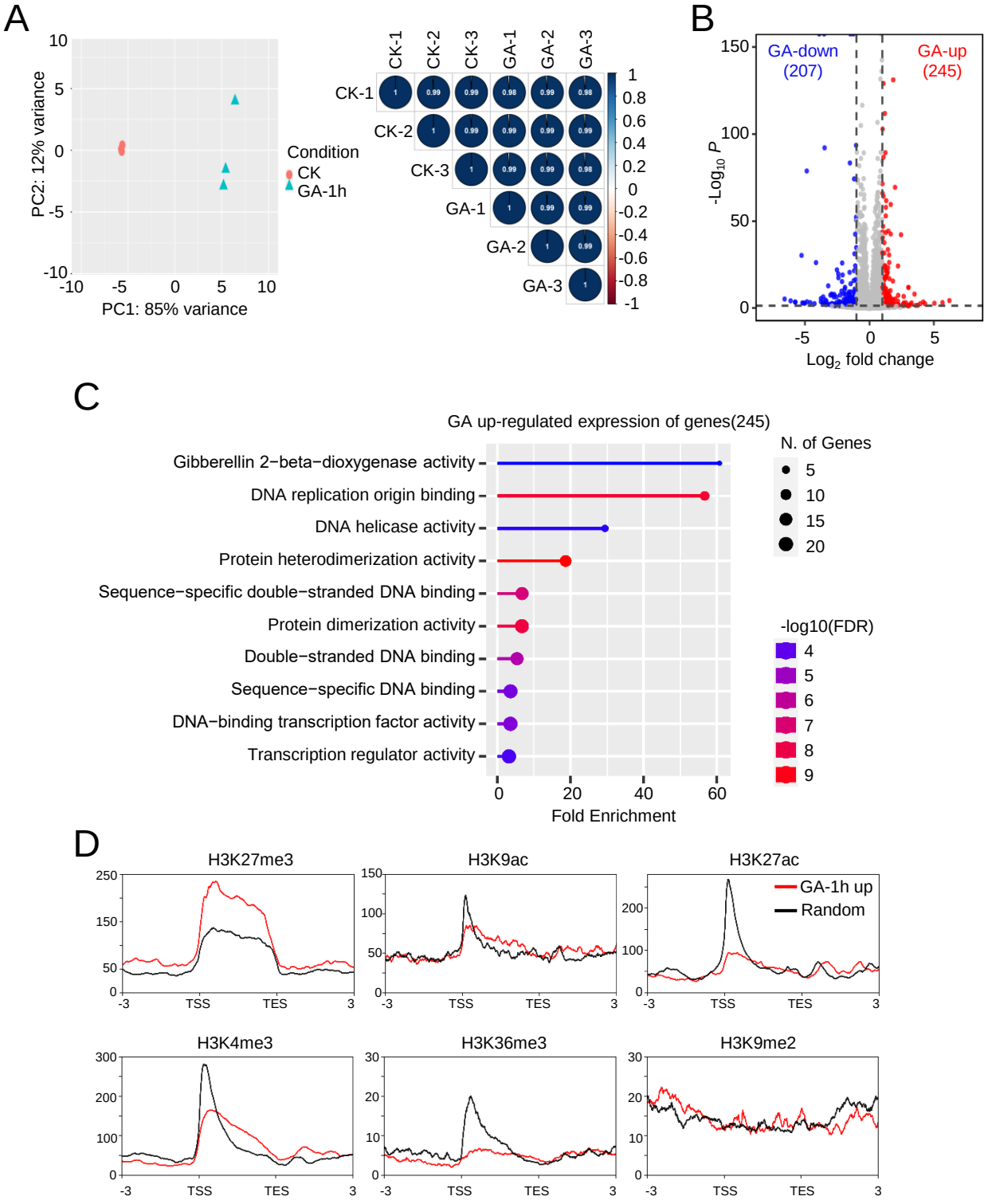
Appendix Figure S7. DNA-binding transcription factors involved in SLR1-PRC2 targeting to downstream genes

A. Computational identification of transcription factors potentially binding to SLR1-PRC2 targets in rice. **B.** YAB4-binding sites in 4 SLR1 downstream genes**. C.** Anti-Flag ChIP-qPCR detection of YAB4 occupancy in SLR1 target genes (*PLA* and *EXPB8*) in *OE-YABBY4* and *yabby4* plants. **D**. ChIP-qPCR detection of SLR1 and EMF2b occupancy at two SLR1 target genes in wild type and *yabby4* mutants. Os06g49490 is a negative control gene. IgG antibody was used as negative control . Student's two-tailed t test was used in **C** and **D** (*** p<0.001, **, p<0.01, *, p<0.05; n, not significant).

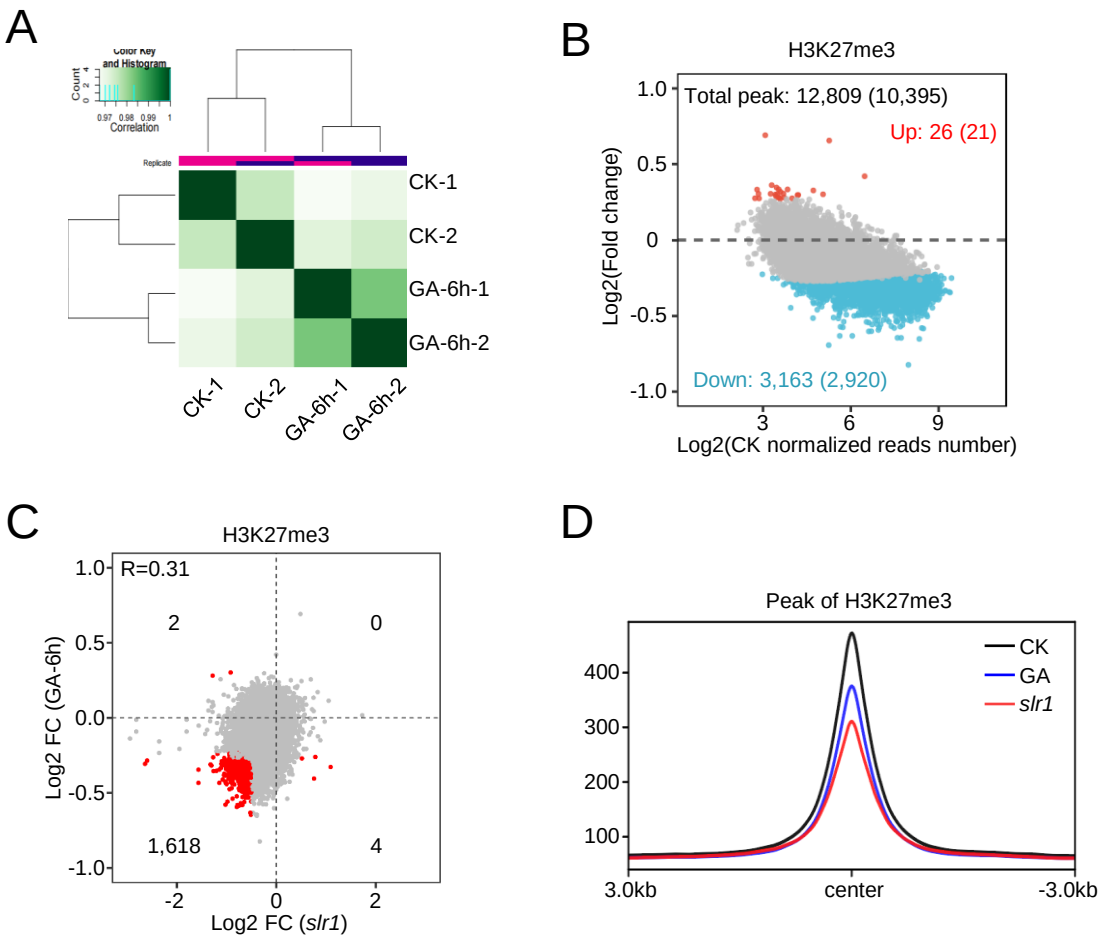


Appendix Figure S8. GA treatment and sampling.

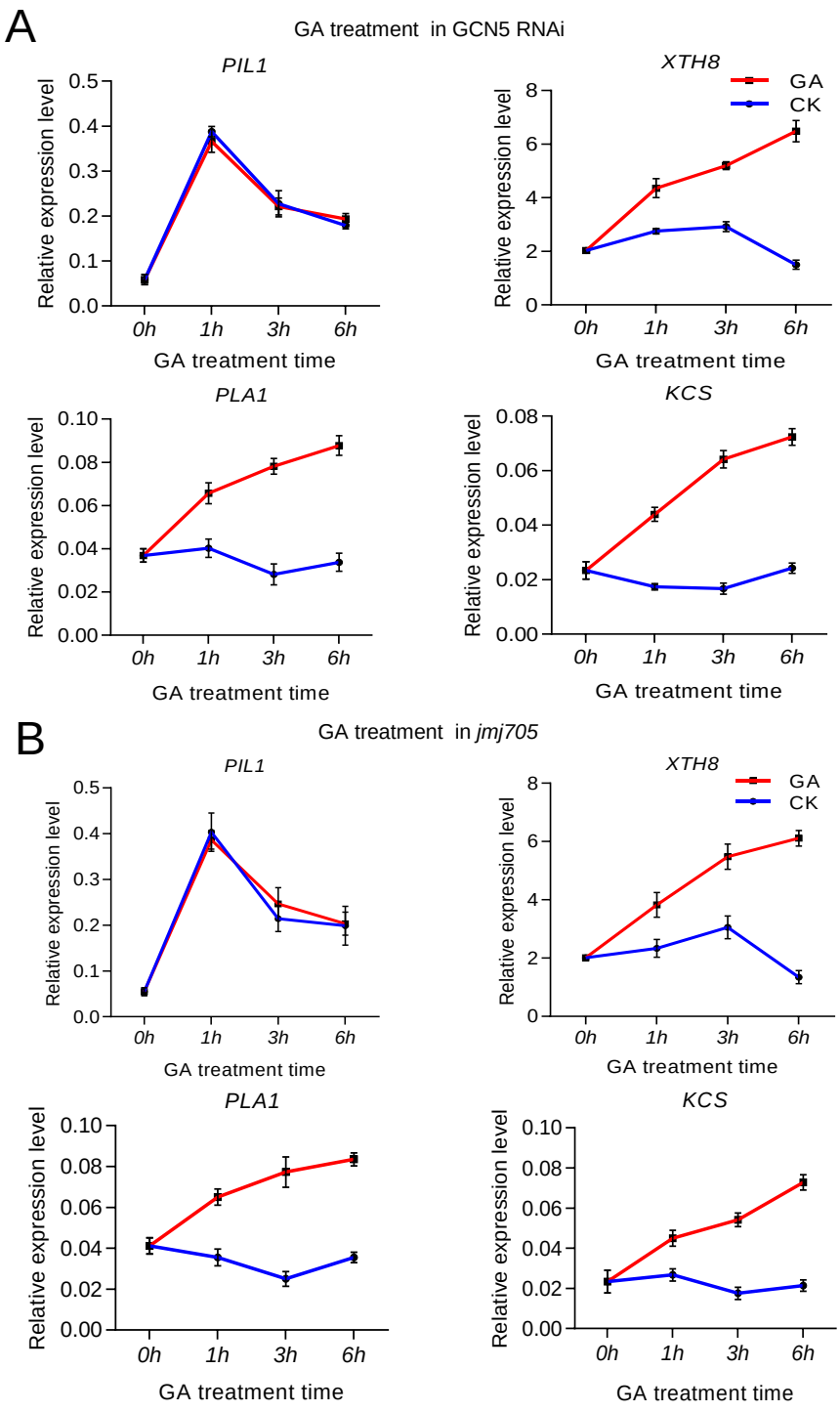
A. Schematic representation of GA treatment and sampling. **B.** Photographs of wild-type seedlings taken at 0h, 6h, 12h and 24h after 100 μM GA treatment or without treatment. The bar graphs show plant height statistics at different time points with or without GA treatment. Data in each from 32 seedlings. **C.** Histone modification levels in *gid1* and *slr1-d5* compared with WT plants. **D.** Overall histone modification levels in *gid1* and *slr1-d5* plants treated with GA at different time points. **E.** *emf2b* RNAi plants show increased GA- response. Measurement of the length of 2nd leaf sheaths of 7-day-old *emf2b* RNAi seedlings in the presence of exogenous GA3 (0, 10^{-8}, 10^{-7},10^{-6} or 10^{-5}M). Error bars represent SD (n=30). Statistical significance was calculated by Student's t test in **B** and **E**. * p<0.05, ** p<0.01, *** p<0.001, n stands for no significant difference.



Appendix Figure S9. RNA-seq analysis of rice seedlings treated with or without GA for 1h. **A.** Repeatability of RNA-seq data revealed by PCA (principal component analysis) (left) and correlation coefficients (right). **B.** Average read count plots of gene expression induced after 1 h GA treatment. The x axis represents log2 FPKM fold change (treated /untreated) and the y axis represents average gene expression level (FPKM). Significant (P<0.05 and 2-fold change) up-regulated and down-regulated gene numbers are indicated. **C.** GO enrichment analysis of the up-regulated genes. **D.** Histone modification profiles of the up-regulated genes in wild type background.



Appendix Figure S10. Detection genome-wide H3K27me3 levels after GA treatment.
A. Pearson correlation coefficient between replicates of H3K27me3 ChIP-seq datasets after GA treatment. **B.** Scattering plot of H3K27me3 levels between GA-treated (for 6 h) and untreated plants. GA-induced up- and down-regulated peaks (genes) are indicated. **C.** Scatterplot of H3K27me3 changes in GA-treated versus untreated seedlings and in *slr1* versus wild type seedlings. The1,618 genes (red dots) showed significant decreases of H3K27me3 in both the GA-treated wild type and the *slr1* mutant seedlings. **D**. Metagene plots of H3K27me3 levels of the ChIP-seq peaks of *slr1* (red line) and GA-treated (blue line) compared to untreated WT (black line) plants.



Appendix Figure S11. Time course of GA-induced gene expression in GCN5 RNAi and *jmj705* mutant plants.

RT-qPCR validation of expression of the 4 genes at different time points of GA treatment of GCN5 RNAi (**A**) and *jmj705* mutant (**B**) plants. Values are means of three biological replicates and error bars represent -/+ SD.