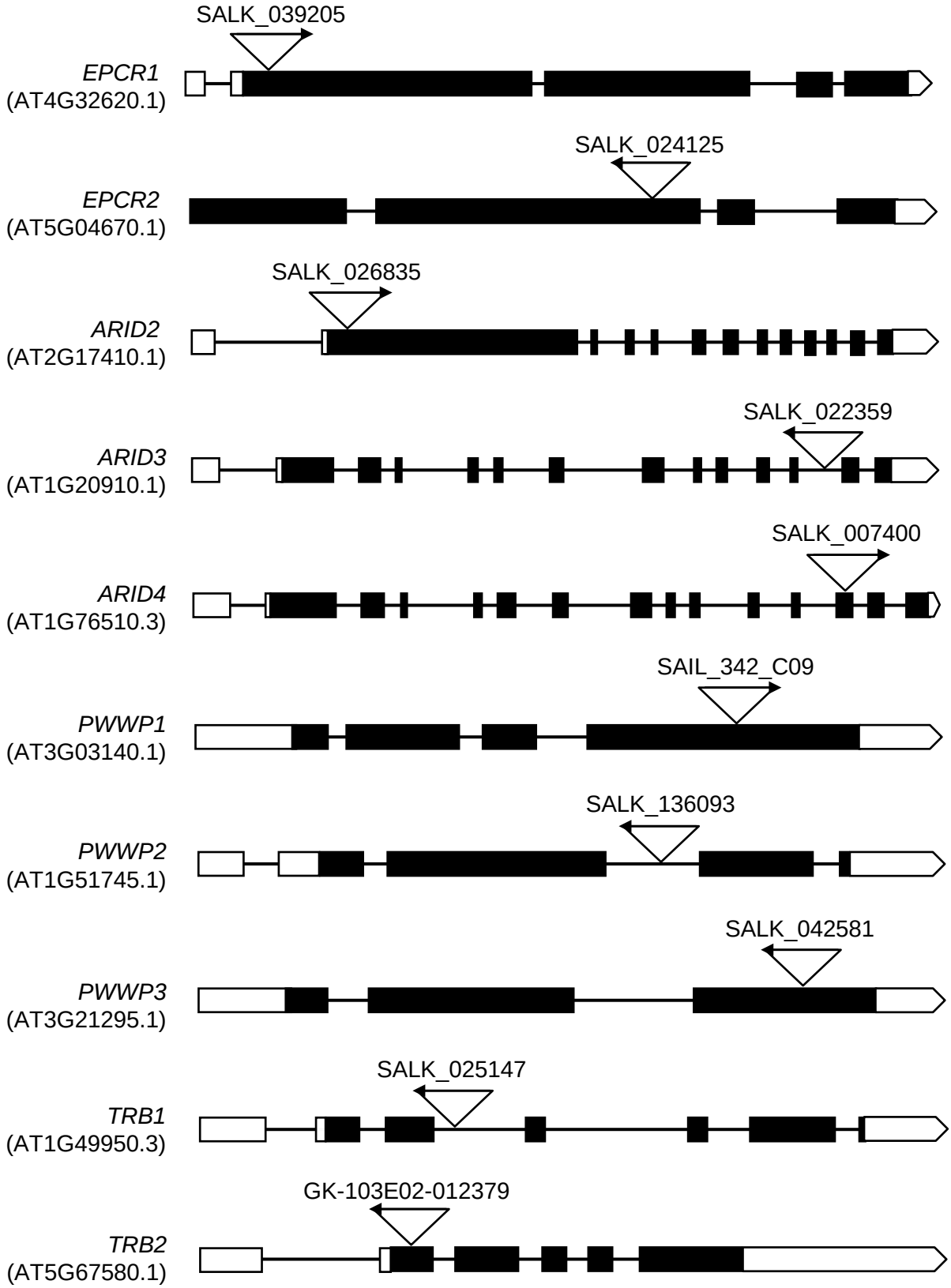
The PEAT protein complexes are required for histone deacetylation and heterochromatin silencing

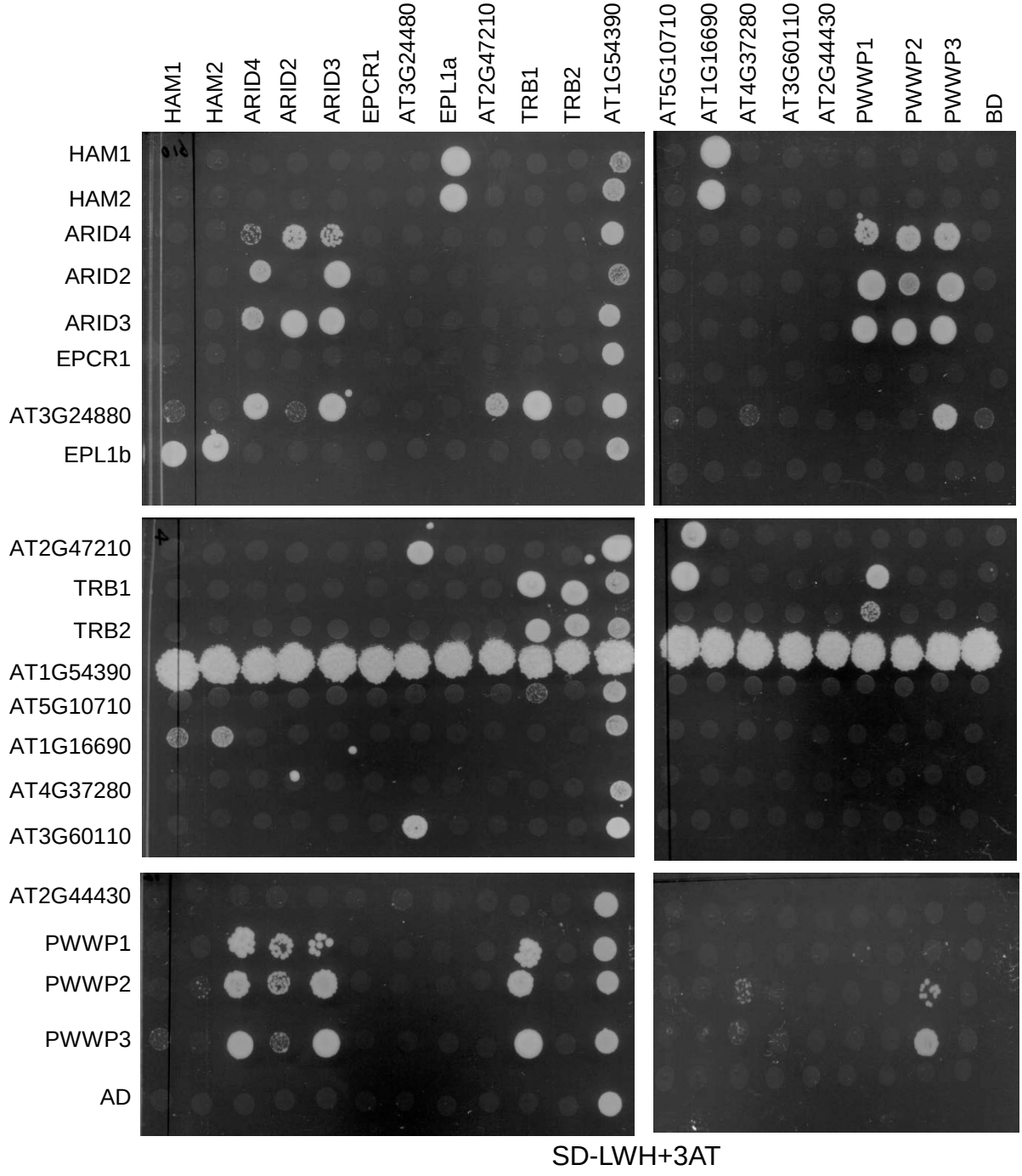
Appendix table of contents:

Appendix Figure S1



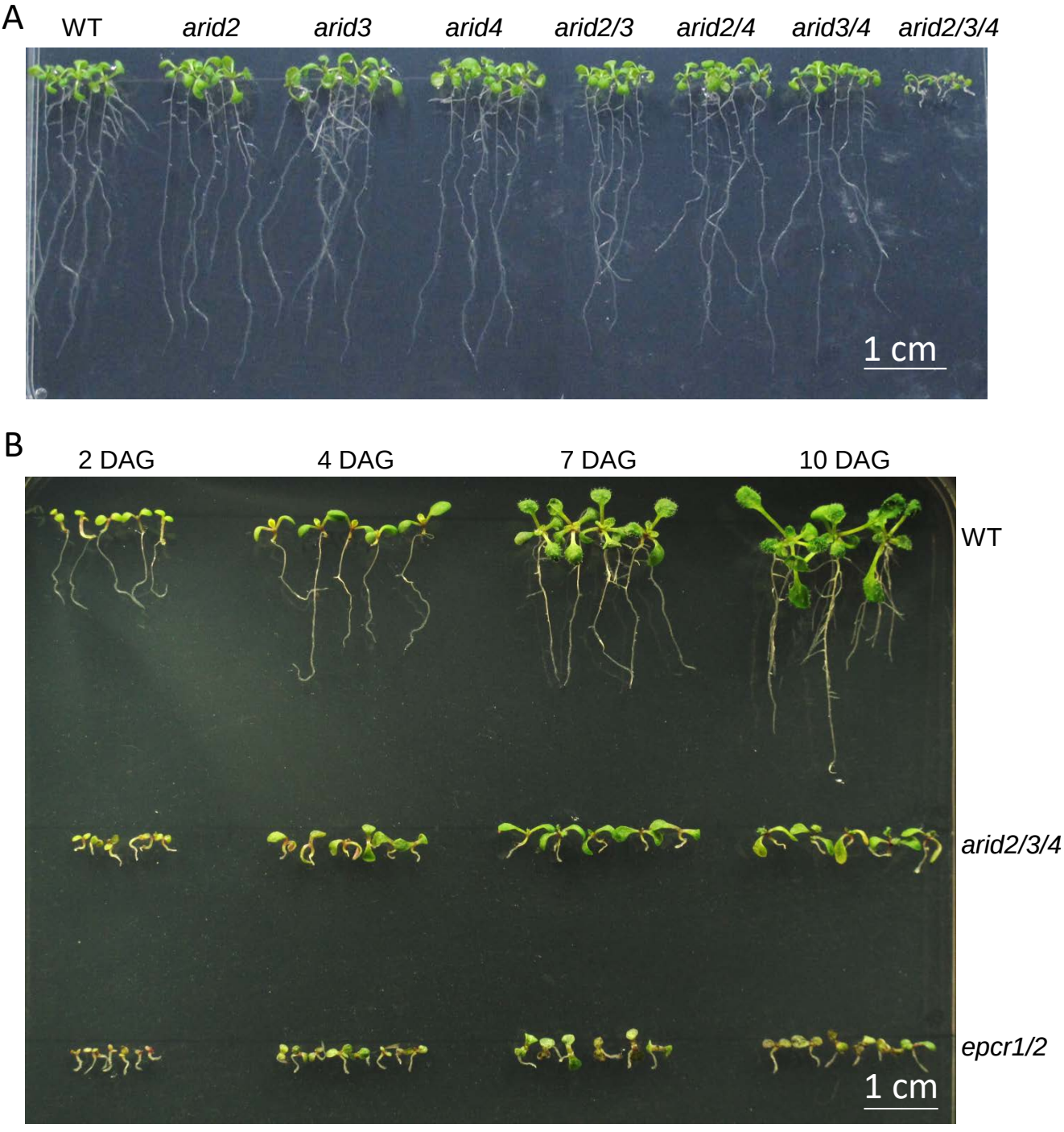
Appendix Figure S1. Schematic representation of *epcr1/2*, *arid2/3/4*, *pwwp1/2/3*, and *trb1/2* mutations. Boxes and lines are exons and introns, respectively. Blank boxes are 5'-UTR or 3'-UTR, and black boxes are protein-coding regions. Positions and directions of T-DNA insertions used in this study are shown.

Appendix Figure S2



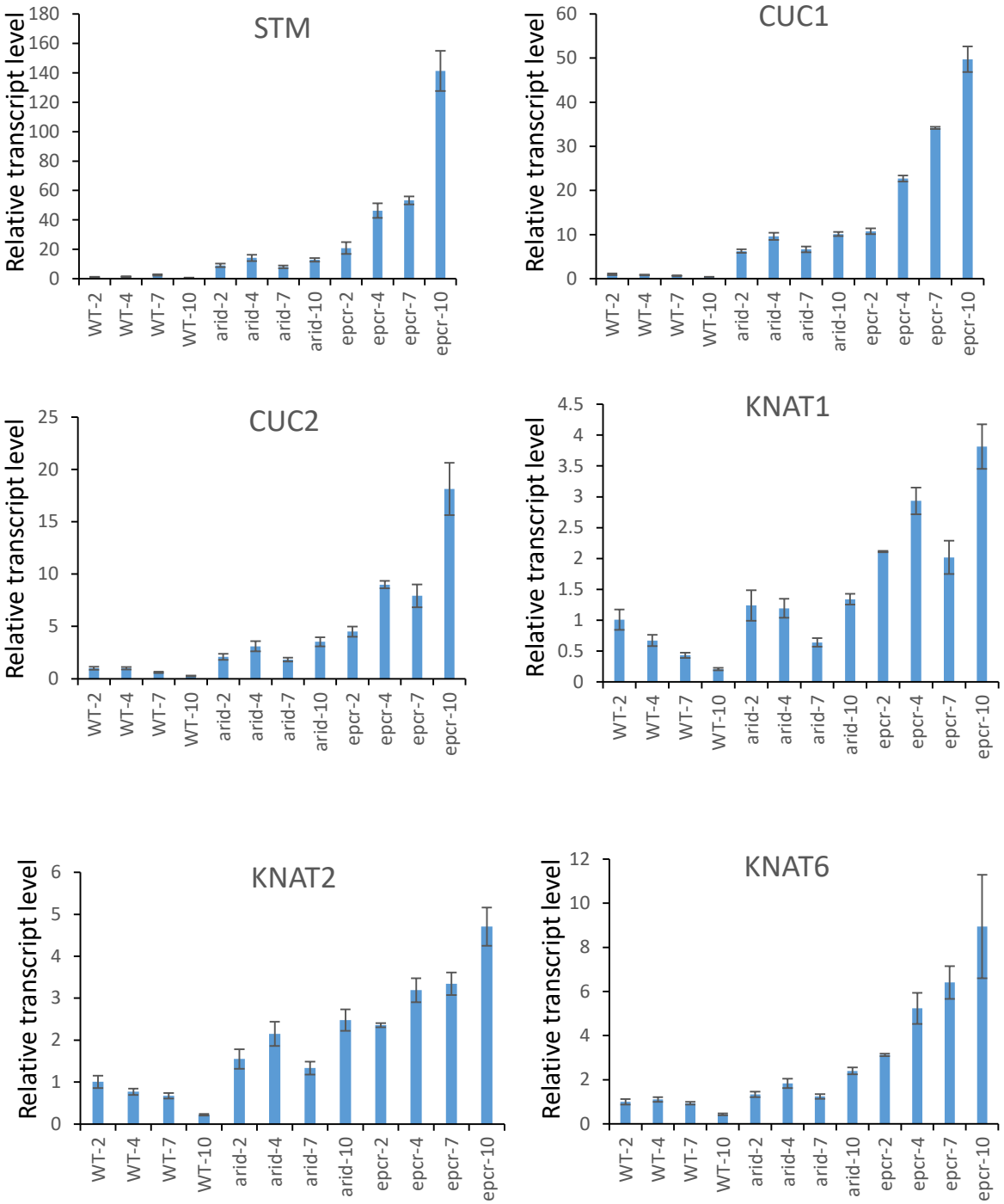
Appendix Figure S2 . Determination of the interaction of EPCR, ARID, and PWWP proteins by yeast two-hybrid assays. Full-length cDNA sequences of indicated genes were cloned into GAL4-BD and GAL4-AD vectors for yeast two-hybrid assay. The GAL4-BD and GAL4-AD vectors were separately transformed into two different mating types of yeast strains. The yeast strains carrying both the GAL4-BD and GAL4-AD vectors were generated by mating and then subjected to yeast two-hybrid assays. SD-LWH, synthetic Drop-out medium minus Leu, Trp, and His supplemented with 3-AT (3-Amino-1,2,4-triazole).

Appendix Figure S3



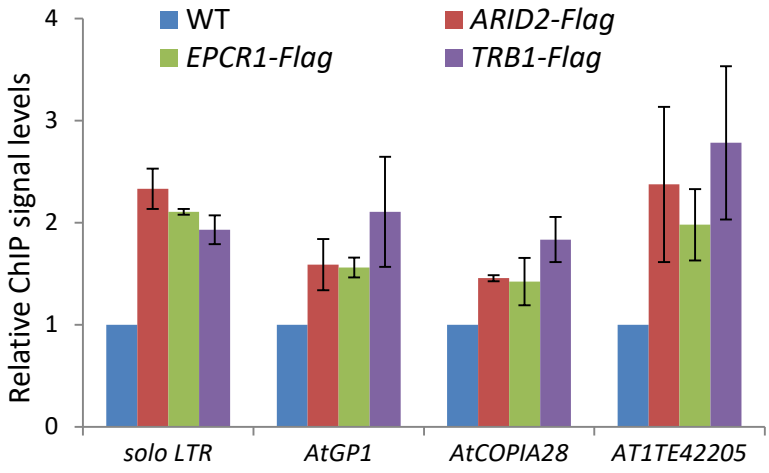
Appendix Figure S3. Morphological phenotypes of mutants of ARID and EPCR proteins. (A) Morphological phenotypes of the mutants of ARID2, ARID3, and ARID4. Arabidopsis seedlings of the indicated genotypes were grown on a vertical MS medium plate for ten days after germination. (B) Morphological images of the wild type and the *arid2/3/4* and *epcr1/2* mutant seedlings grown for 2, 4, 7, and 10 days after germination (DAG). The seedlings were grown on MS medium plates for indicated time and then transferred to a new MS medium plate for imaging.

Appendix Figure S4



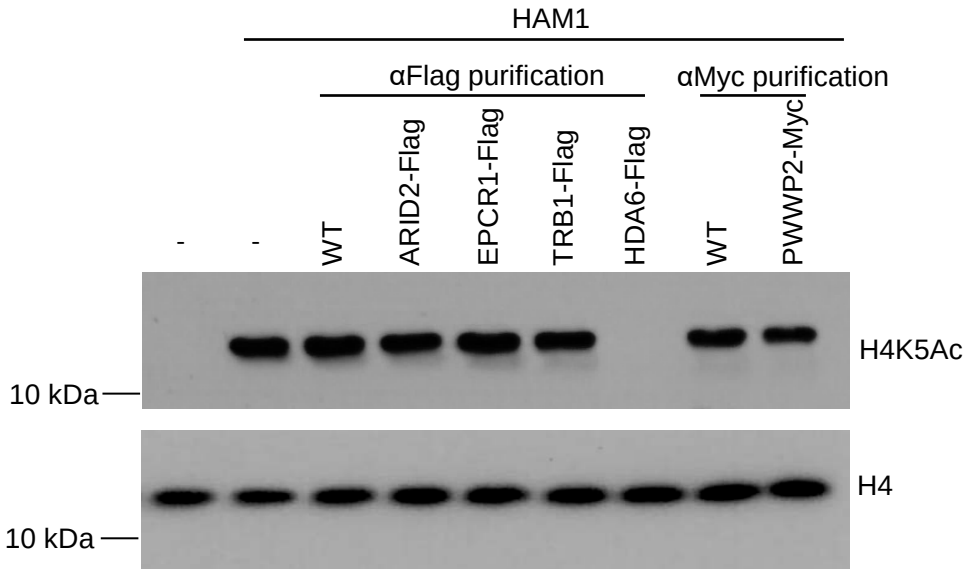
Appendix Figure S4. Transcript levels of development-related genes in the wild type and the *arid2/3/4* and *epcr1/2* mutant seedlings at different development stages. The transcript levels of the development-related genes *STM*, *CUC1*, *CUC2*, *KNAT1*, *KNAT2*, and *KNAT6* in the wild type and the *arid2/3/4*, and *epcr1/2* mutant seedlings grown for 2, 4, 7, and 10 days after germination. Shown are transcript levels of indicated loci, as determined by qPCR analysis. Error bars represent SD from two independent biological replicates.

Appendix Figure S5



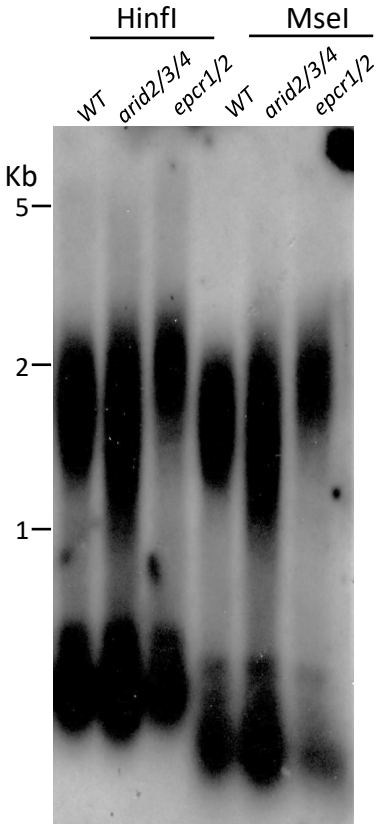
Appendix Figure S5. Enrichment of ARID2, EPCR1, and TRB1 on their target genomic loci. *ARID2-Flag*, *EPCR1-Flag*, and *TRB1-Flag* transgenic seedlings were subjected to the chromatin immunoprecipitation (ChIP) experiment by anti-Flag antibody. The wild type seedlings were used as a negative control. The ChIP signals on indicated loci were normalized by *ACT2*. Error bars represent standard deviation of two biological replicates.

Appendix Figure S6



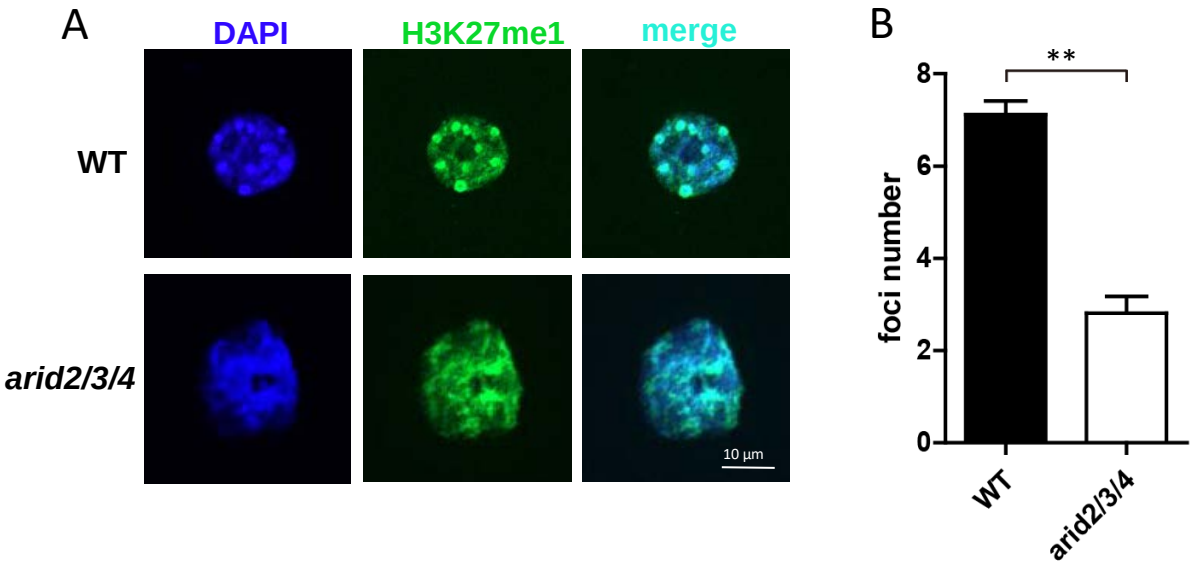
Appendix Figure S6. Histone deacetylation assay for ARID2-Flag, EPCR1-Flag, TRB1-Flag, and PWWP2-Myc. Histone deacetylation assay. The histone H4 peptide was acetylated by the histone acetyltransferase HAM1. The acetylated H4 peptide was then used for the histone deacetylation assay. The possible histone deacetylation activity was detected for the ARID2-Flag, EPCR1-Flag, TRB1-Flag, and PWWP2-Myc proteins purified from the corresponding transgenic plants. The histone deacetylase HDA6-Flag purified from its transgenic plants was used as a positive control. The background proteins isolated by αFlag and αMyc antibodies from the wild type plants (WT) were used as negative controls.

Appendix Figure S7



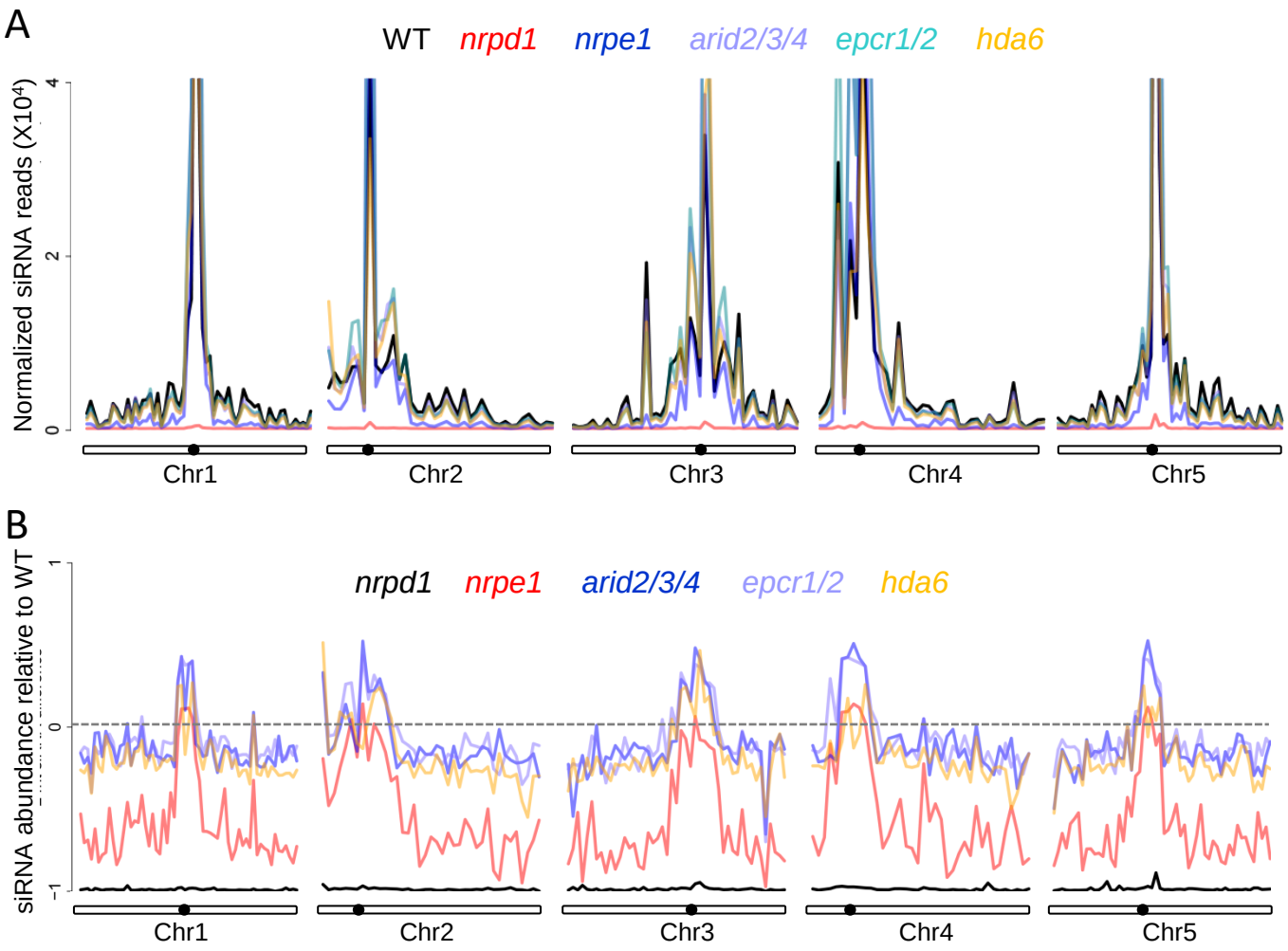
Appendix Figure S7. Telomere alteration in the wild type and the *arid2/3/4* and *epcr1/2* mutants as determined by Terminal Restriction Fragmentation (TRF) analysis. For Genomic DNA isolated from the seedlings of the wild type and the mutants was digested with HinfI and MseI. The telomere length was examined by Southern blotting with the telomeric repeat probe.

Appendix Figure S8



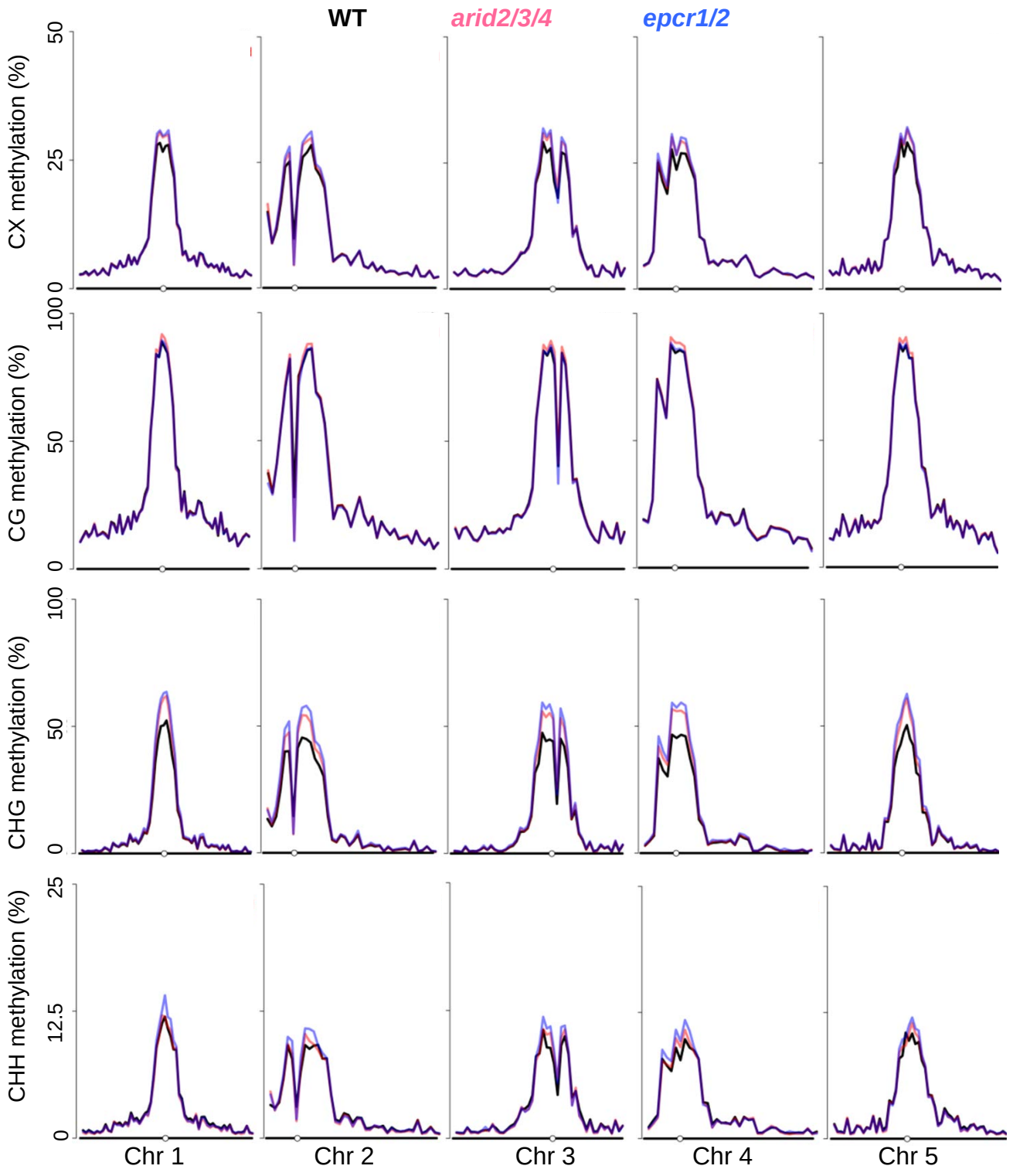
Appendix Figure S8. Determination of the effect of the *arid2/3/4* mutation on heterochromatin condensation.(A) The representative nuclei of the wild type and the *arid2/3/4* mutant as marked by DAPI staining and H3K27me1 immunostaining. (B) The numbers of condensed foci in the nuclei of the wild type and the *arid2/3/4* mutant. The result was obtained from at least 50 nuclei of the wild type and the *arid2/3/4* mutant. Asterisks indicates statistical significance (P<0.01) as determined by Student's t-test.

Appendix Figure S9



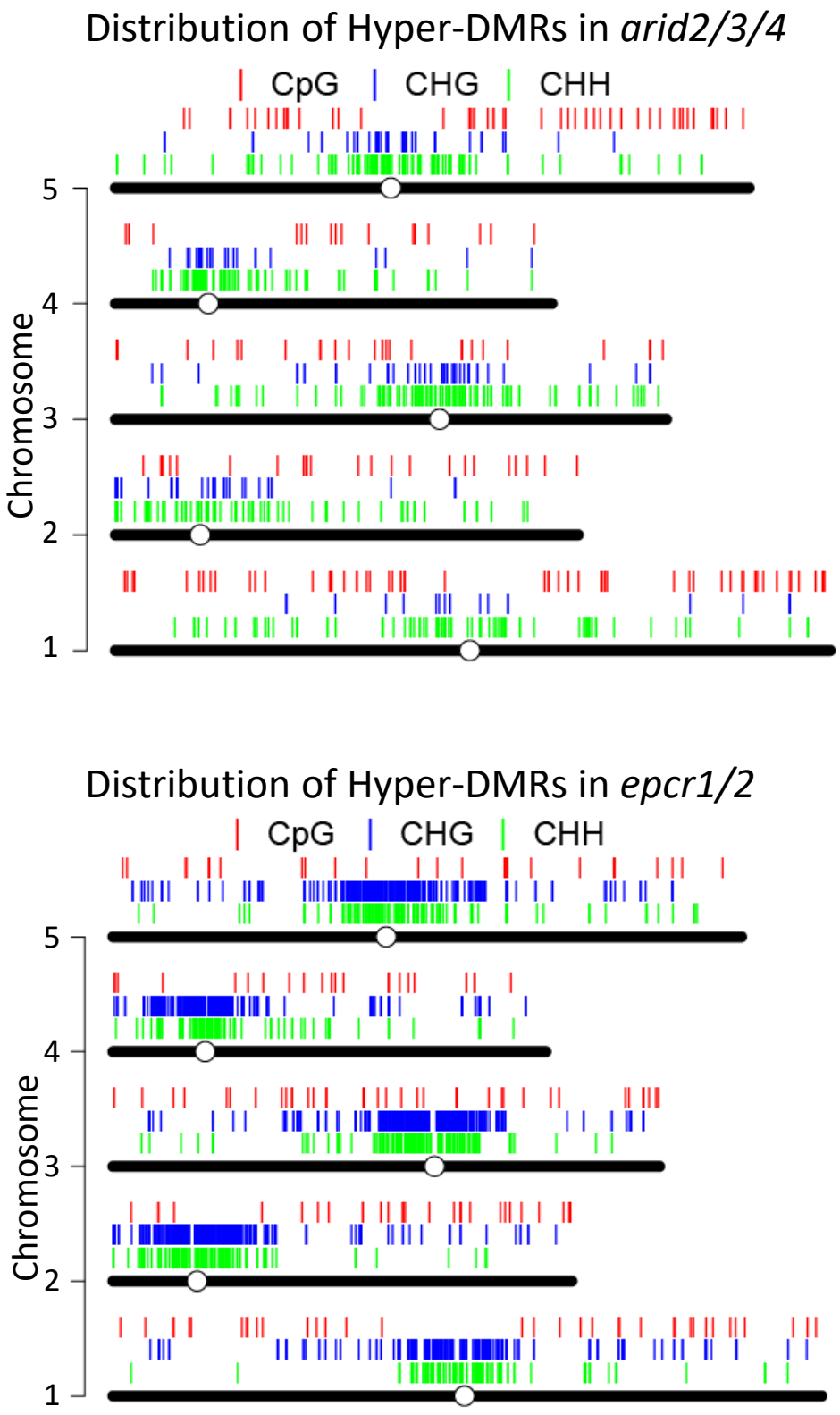
Appendix Figure S9. Distribution of Pol IV-dependent siRNAs in the wild type, *epcr1/2*, *arid2/3/4*, *nrpd1*, *nrpe1*, and *hda6* mutants on chromosomes. (A) Distribution of Pol IV-dependent 24-nt siRNAs across five Arabidopsis chromosomes in the wild type and indicated mutants. Normalized 24-nt siRNA reads per 10 million in 500-bp genomic regions are indicated by the Y coordinate. The five Arabidopsis chromosomes are shown on the X coordinate. (B) Abundance of Pol IV-dependent siRNA reads in mutants relative to wild type was indicated by (Mut-WT)/(Mut+WT) on chromosome 1 to 5 of Arabidopsis.

Appendix Figure S10



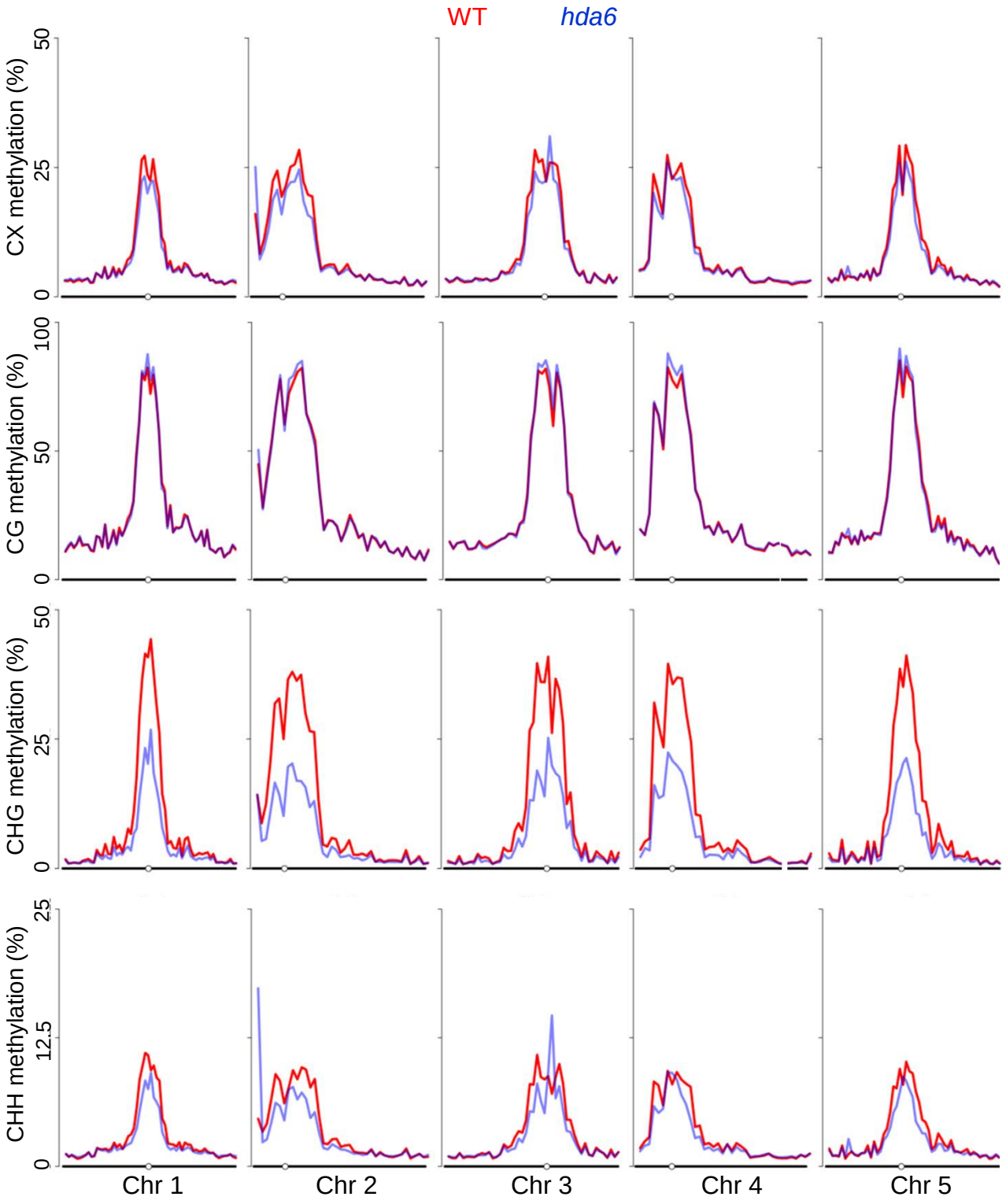
Appendix Figure S10. Metaplot of CX, CG, CHG, and CHH methylation on chromosomes of Arabidopsis in wild type, *arid2/3/4*, and *epcr1/2*. X indicates any nucleotide, whereas H indicates C, T, or A but not G.

Appendix Figure S11



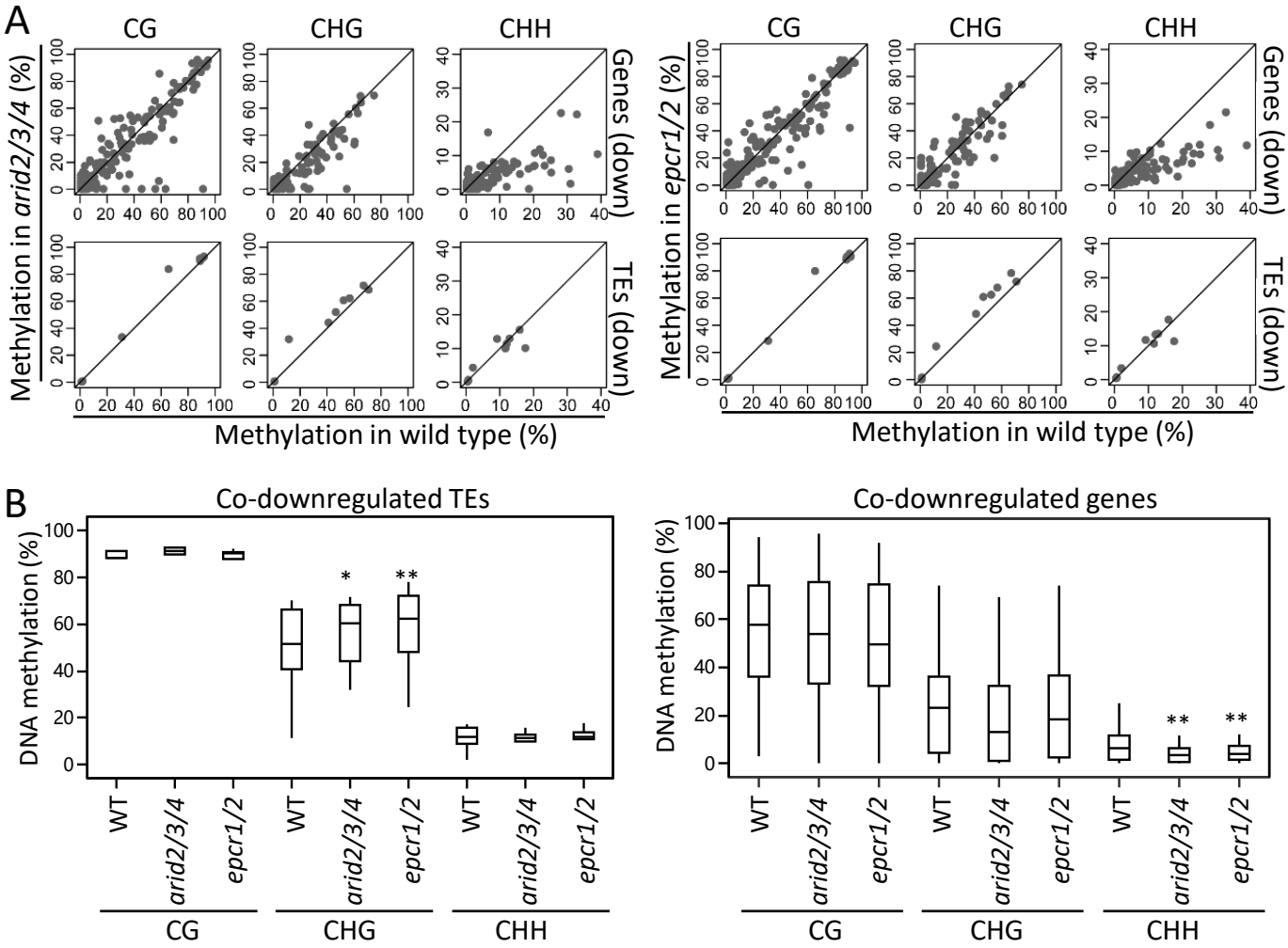
Appendix Figure S11. Distribution of *arid2/3/4* and *epcr1/2* hyper-DMRs of CG, CHG, and CHH sites on chromosomes. Hyper-DMRs of CG, CHG, and CHH sites were separately identified in the *arid2/3/4* and *epcr1/2* mutants as compared to the wild type.

Appendix Figure S12



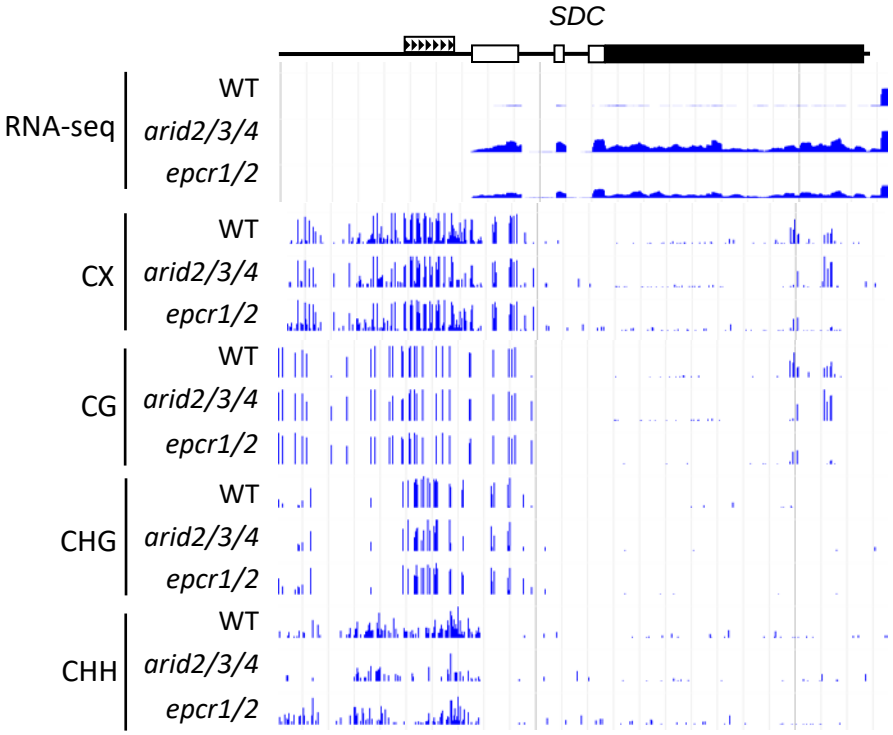
Appendix Figure S12. Metaplot of CX, CG, CHG, and CHH methylation on chromosomes of Arabidopsis in wild type and *hda6*. X indicates any nucleotide, whereas H indicates C, T, or A but not G.

Appendix Figure S13



Appendix Figure S13. Effect of *arid2/3/4* and *epcr1/2* on DNA methylation of co-downregulated TEs and genes. (A) Scatter plots showing the effect of the *epcr1/2* and *arid2/3/4* mutations on CG, CHG, and CHH methylation at the co-downregulated TEs and genes in the *epcr1/2* and *arid2/3/4* mutants. TE methylation refers to methylation of the bodies of the co-downregulated TEs. Gene methylation refers to methylation of the 1-kb promoters of the co-downregulated genes. (B) Box plots of CG, CHG, and CHH methylation in the wild type, *arid2/3/4*, and *epcr1/2* mutants at the co-downregulated TEs and genes. TE methylation refers to DNA methylation levels of differentially expressed TEs. Gene methylation refers to DNA methylation levels of the 1-kb promoters of the co-downregulated genes. As determined by paired student t test, CHG methylation of co-downregulated TEs is significantly increased in *arid2/3/4* ($P=0.038$) and *epcr1/2* ($P=8.0\times10^{-4}$); CHH methylation of co-downregulated genes is significantly decreased in *arid2/3/4* ($P=2.2\times10^{-8}$) and *epcr1/2* ($P=2.6\times10^{-8}$). "*" and "**" indicates $P<0.05$ and $p<0.01$, respectively.

Appendix Figure S14



Appendix Figure S14. Genome browser snapshots showing RNA transcript and DNA methylation levels of *SDC* in wild type, *arid2/3/4*, and *epcr1/2*. In the schematic representation of *SDC*, filled and blank boxes represent exons and 5'-UTR, respectively. Tandem repeats in the promoter region of *SDC* are indicated.