

Supplementary Materials for:

ABA negatively regulates the Polycomb-mediated H3K27me3 through the PHD-finger protein, VIL1

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Fig. S1 Differentially expressed genes in *vil1* mutants.

Fig. S2 VIL1 regulates ABA response in *Arabidopsis*.

Fig. S3 VIL1 represses *ABI3* and *ABI4* expression during germination.

Fig. S4 The H3K27me3 levels at *ABI5* locus during seed germination.

Fig. S5 VIL1 expressions are not regulated by ABA treatment.

Fig. S6 CLF and SWN directly bind to *ABI3* and *ABI4* but not to *ABI5*.

Fig. S7 VIL1 functions together with PRC2 components to regulate the ABA response.

Fig. S8 Seed phenotype of Col-0, *vil1*, *abi3*, and *vil1 abi3* double mutants in *Arabidopsis*.

Fig. S9 Water loss assay in *Arabidopsis*.

Fig. S10 Working model showing that VIL1-PRC2 regulates seed germination in *Arabidopsis*.

Table S1. Differentially expressed gene in *vil1*. (Separate Excel File)

Table S2. Primers used in this study. (Included in this file)

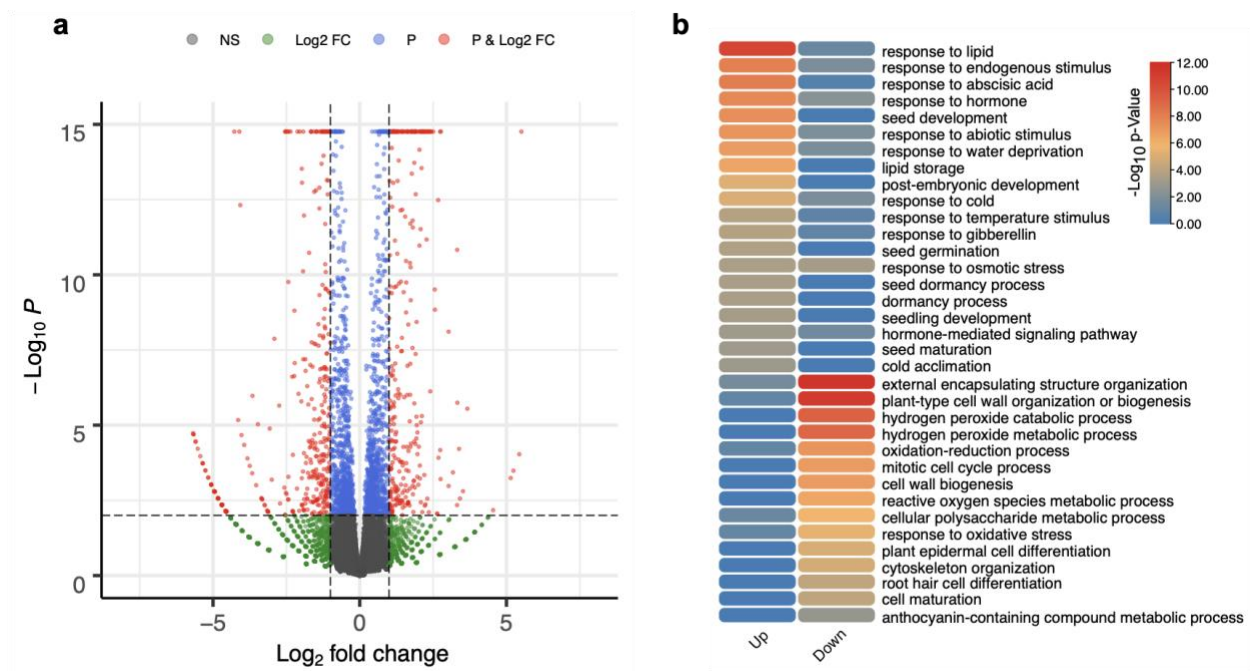


Fig. S1 Differentially expressed genes in *vil1* mutants. (a) Volcano plot displaying significantly up-regulated and down-regulated genes in *vil1* mutants compared to WT (*Col-0*). The y-axis is the $-\text{Log}_{10}$ of the P-value for the significance of the differential expression. Significant differentially expressed genes (DEGs) are indicated as red dots ($P\text{-Value} < 0.01$, $|\text{Log}_2 \text{FC}| \geq 0.7$). ns, not significant. (b) Heat map showing the gene ontology categories of DEGs from *vil1* mutants. Up, genes up regulated in *vil1* when compared with WT; Down, genes down regulated in *vil1* when compared with WT.

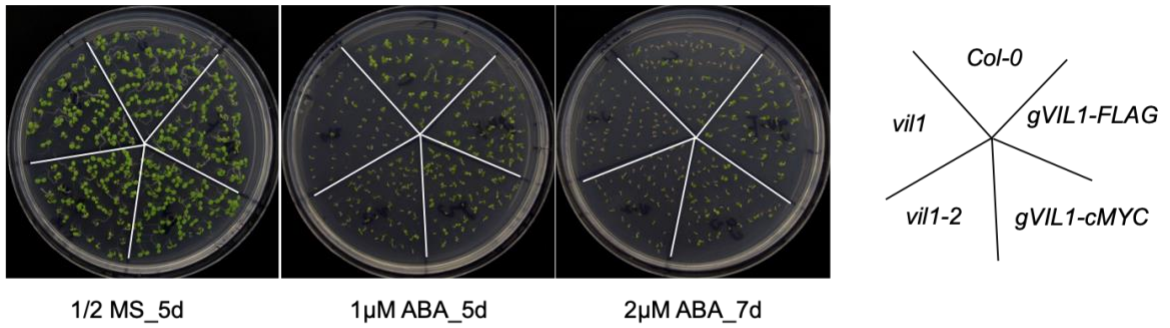
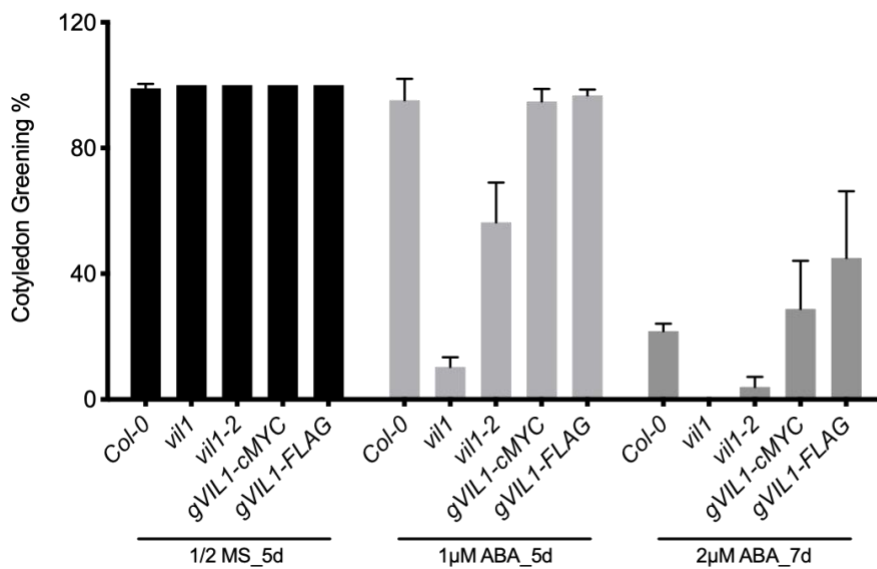
a**b**

Fig. S2 VIL1 regulates ABA response in *Arabidopsis*. (a) Germination phenotypes of WT (Col-0), *vil1* (*vil1-1*), *vil1-2*, and complementation lines (*gVIL1-cMYC* and *gVIL1-FLAG*) in the presence of 1 μ M or 2 μ M of ABA. (b) The percentage of germinated embryos that developed green cotyledons in the presence of 1 μ M or 2 μ M of ABA in the Col-0, *vil1*, and *gVIL1*. Values are mean \pm s. d. ($n = 2$).

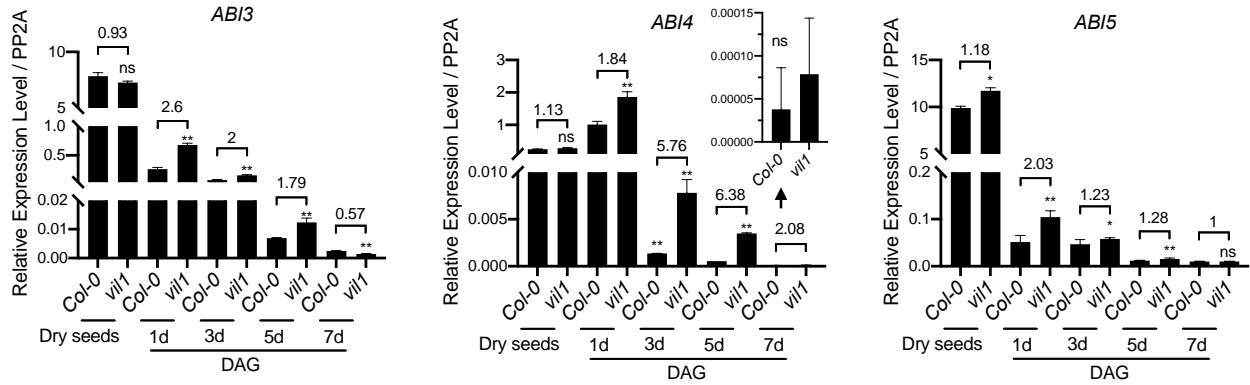


Fig. S3 VIL1 represses *ABI3* and *ABI4* expression during germination. For dry seeds of Col-0 and *vil1*, seeds were sterilized and imbibed in water for 30 mins and then harvested for RNA extraction. For germinated seeds, Col-0 and *vil1* seeds were first sterilized and stratified at 4°C for 3 days and then germinated at 22°C under 16h light /8h dark condition. Numbers over the column indicates fold changes between distinct groups. Transcript levels were normalized to *PP2A* and error bars: \pm s. d. ($n = 3$). * $P < 0.05$, ** $P < 0.01$, Significant difference using Student's *t*-test. ns, not significant.

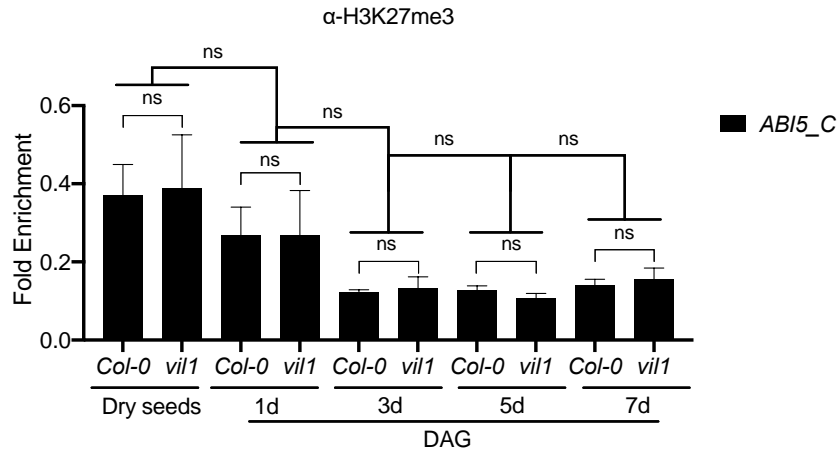


Fig. S4 The H3K27me3 levels at *ABI5* locus during seed germination. CHIP-qPCR analysis of H3K27me3 levels at *ABI5* at different germination stages of the *Col-0* and *vil1-1*. Refer to Figure 3C and 3D for comparison and the H3K27me3 levels at *ABI5* are below background levels. The location of primers used illustrated in Figure 2B. The value was normalized to *TA3* and error bars: \pm s. d. ($n = 3$). ns no significant differences between distinct groups. Significant difference using Student's *t*-test.

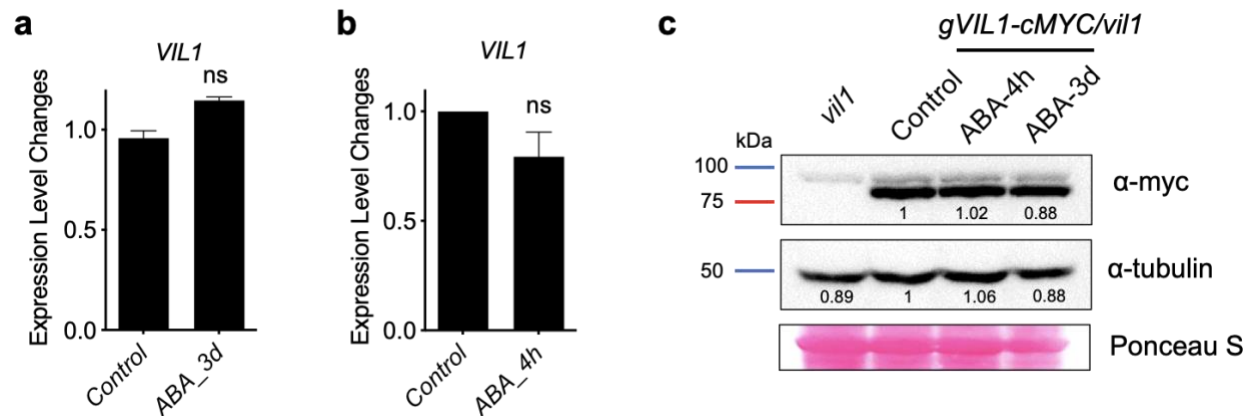


Fig. S5 VIL1 expressions are not regulated by ABA treatment. (a) Relative expression of *VIL1* in Col-0 seedlings treated with or without ABA. Stratified Col-0 seeds were germinated with (ABA_3d) or without 0.5 μ M ABA (Control) for 3 days. (b) Relative expression of *VIL1* in Col-0 seedlings treated with or without ABA. 3-day-old Col-0 seedlings were treated with (ABA_4h) or without (Control) 50 μ M ABA for 4 hours. Transcript levels were normalized to *PP2A* and error bars: \pm s. d. ($n = 3$). ns indicate there is no significant differences of distinct groups. Significant difference using Student's *t*-test. (c) VIL1 protein levels under short-term (ABA_4h) or long-term (ABA_3d) ABA treatments. An anti-myc antibody and an anti-Tubulin antibody were used for the analysis. Ponceau S staining was shown to visualize protein loading. Proteins were quantified by image J software.

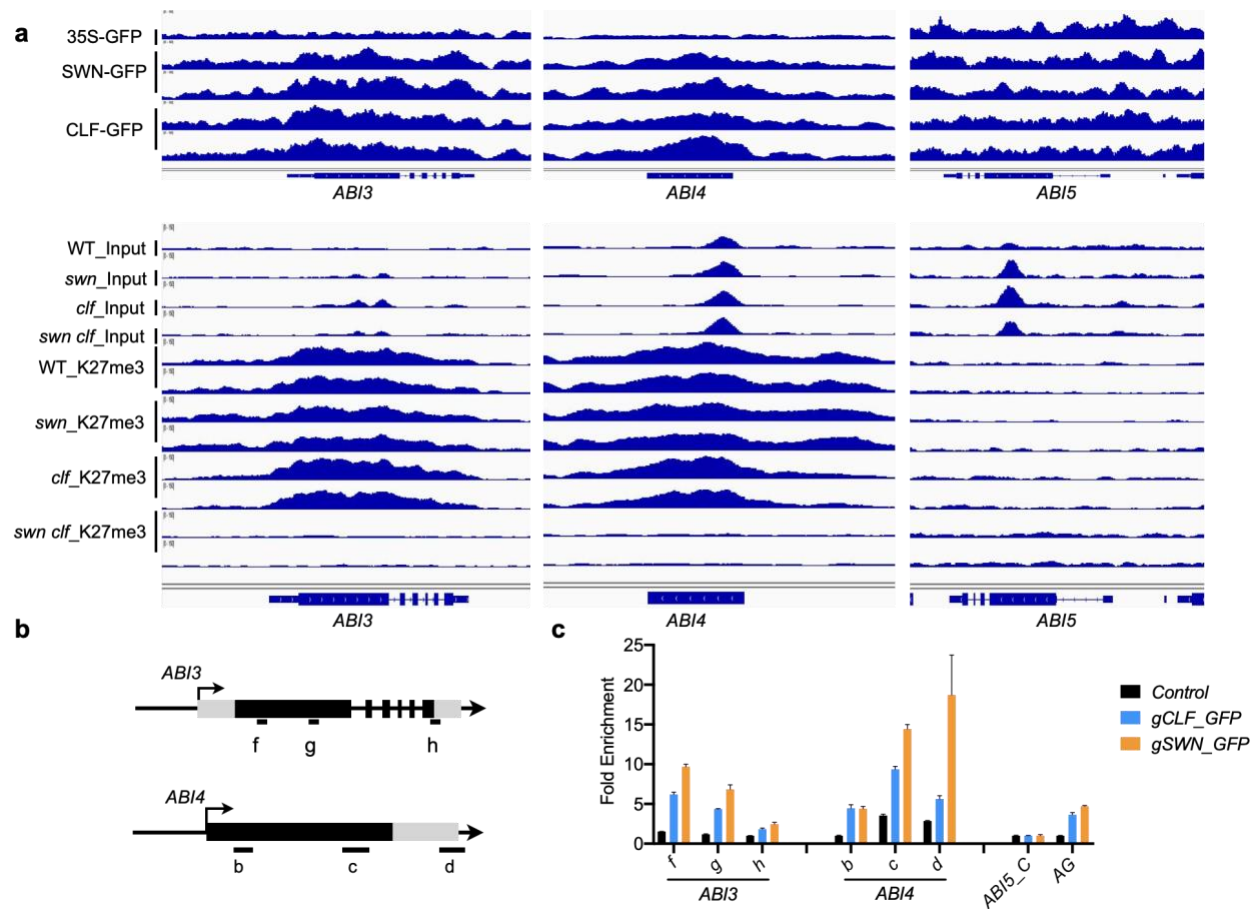


Fig. S6 CLF and SWN directly bind to *ABI3* and *ABI4* but not to *ABI5*. (a) Genome-browser views of selected genes showing the occupancy of CLF and SWN, and the distribution of H3K27me3 in WT (Col-0), *swn-4* (*swn*), *clf-29* (*clf*), and *swn-4 clf 29* (*swn clf*). (b) Schematic diagram showing the genome regions of *ABI3* and *ABI4*. Exons are represented by black boxes, while black lines between exons represent introns. DNA fragments amplified in ChIP assays are labeled beneath the genomic regions. Black arrows with vertical lines indicate transcription start sites and direction of arrows indicate the orientation of transcription. (c) Analysis of CLF and SWN binding to *ABI3* and *ABI4* by ChIP-qPCR. 3-day-old seedlings of *gCLF_GFP*, *gSWN_GFP*, and control (*35S-GFP*) are used in ChIP assay. *ABI3* and *ABI4* fragments immunoprecipitated by anti-GFP were quantified by qPCR and normalized to *TA3*. Relative levels in *gCLF_GFP* and *gSWN_GFP* over control are presented, and error bars: \pm s. d. ($n = 3$). *ABI5* and *AGAMOUS* (AG) were used as negative and positive controls, respectively.

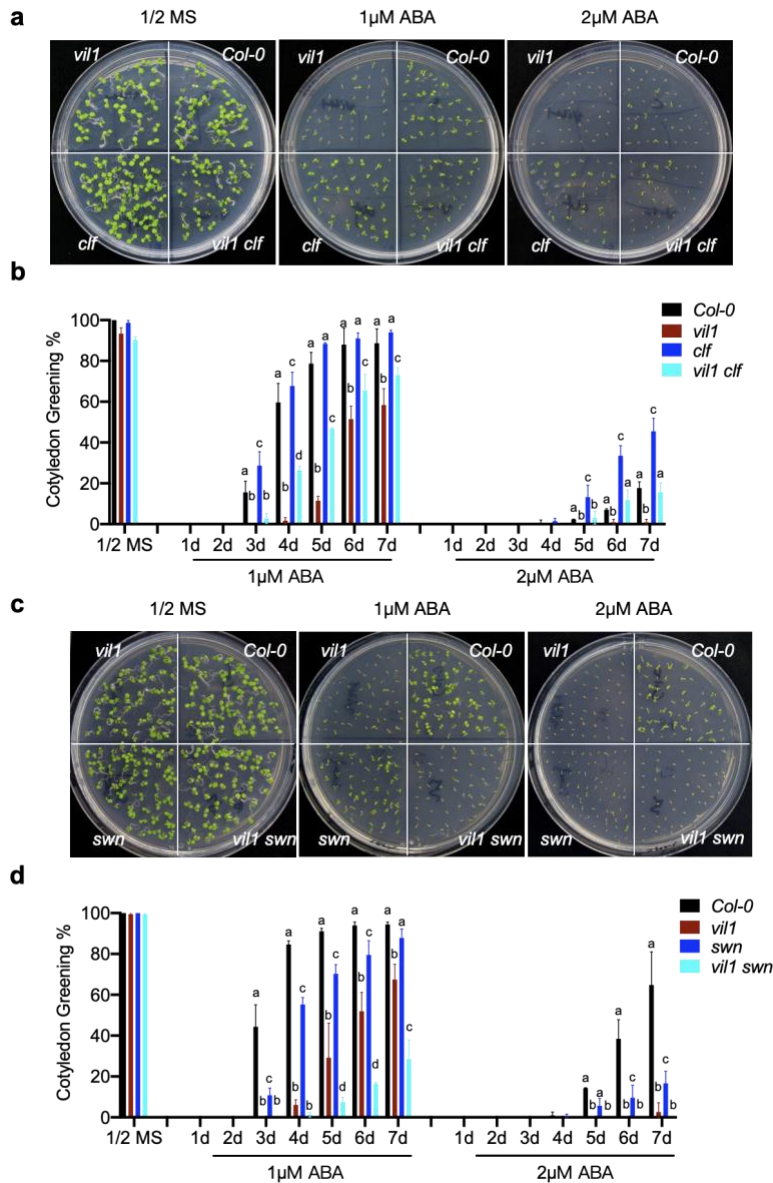


Fig. S7 *VIL1* functions together with PRC2 components to regulate the ABA response. (a) Germination phenotype of WT (*Col-0*), *vil1*, *clf*, and *vil1 clf* in the presence of 1 μ M or 2 μ M ABA. (b) The percentage of germinated seeds of indicated genotypes that developed green cotyledons in the presence of 1 μ M or 2 μ M ABA. Values are mean \pm s. d. ($n = 3$). One-way ANOVA Tukey's multiple comparison test was conducted; letters indicate $P < 0.05$ of distinct groups. (c) Germination phenotype of WT (*Col-0*), *vil1*, *swn-3*, and *vil1 swn-3* in the presence of 1 μ M or 2 μ M ABA. (d) The percentage of germinated seeds of indicated genotypes that developed green cotyledons in the presence of 1 μ M or 2 μ M ABA. Values are mean \pm s. d. ($n = 3$). One-way ANOVA Tukey's multiple comparison test was conducted; letters indicate $P < 0.05$ of distinct groups.

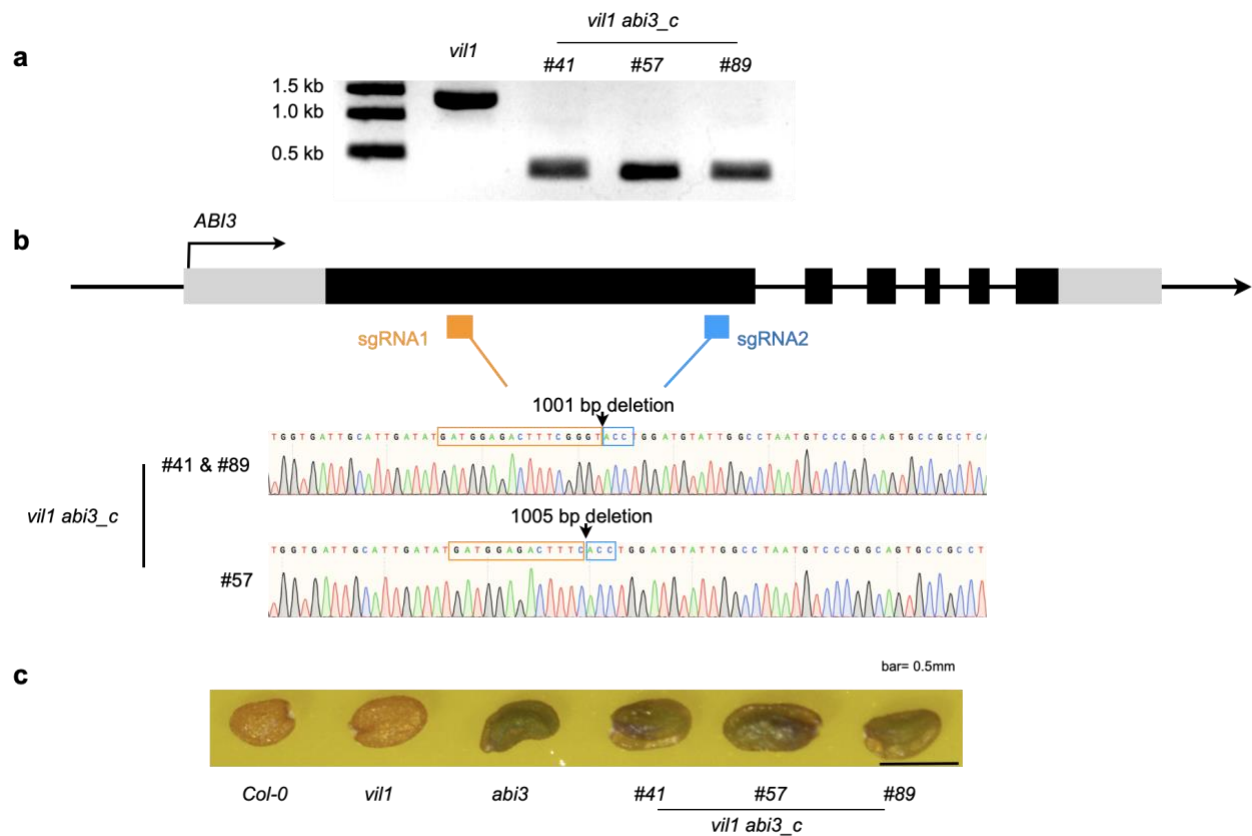


Fig. S8 Seed phenotype of Col-0, *vil1*, *abi3*, and *vil1 abi3* double mutants in *Arabidopsis*. (a) Deletions of the *ABI3* gene in *vil1 abi3_c*. (b) Schematic diagram showing the deletions of the *ABI3* gene caused by the CRISPR/Cas9 in *vil1* mutants. Exons are represented by black boxes, while black lines between exons represent introns. Black arrow with a vertical line indicates the transcription start site and the direction of arrow indicates the orientation of transcription. The locations of the two sgRNAs are labeled as orange and blue boxes, respectively. The Sanger sequencing results of each independent line are shown; the orange and blue boxes indicate the locations of the sgRNA1 and sgRNA2 on the *ABI3* locus, respectively. Arrows indicate the deletion sites of the *ABI3* in each line. (c) Seed phenotype of indicated genotypes.

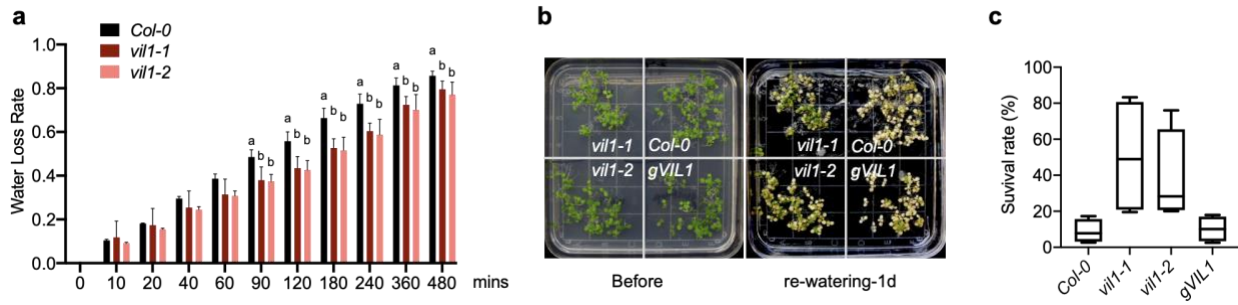


Fig. S9 Water loss assay in *Arabidopsis*. (a) Plants of Col-0, *vil1-1* and *vil1-2* are grown in long days. Leaves with similar developmental stages were detached and weighted at the indicated time. Water loss represents proportion of total weight lost compared with initial weight. Data Values are mean \pm s. d. from three independent sets. One-way ANOVA Tukey's multiple comparison test was conducted; letters indicate $P < 0.05$ of distinct groups. (b) Plant phenotypes for indicated genotypes before and after dehydration treatment. (c) Survival rate of 7-day-old Col-0, *vil1-1*, *vil1-2* and *gVIL1* seedlings after 5-day dehydration treatment. Horizontal lines indicate mean values \pm s. d. ($n = 6$; biological replicates).

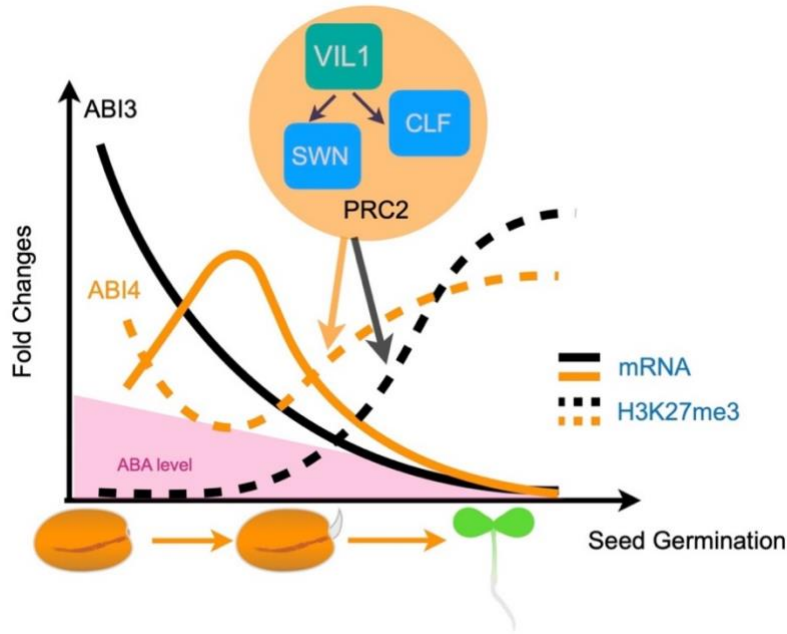


Fig. S10 Working model showing that VIL1-PRC2 regulates seed germination in *Arabidopsis*. When germination begins, the endogenous ABA content in seeds rapidly declines at the early phase of germination (within 6-12 h) which causes the downregulation of the *ABI3*. The repression of *ABI3* and *ABI4* occurs during germination and the VIL1 facilitates the H3K27me3 mark to the gene body of *ABI3* and *ABI4* to form a repressive chromatin state, which helps maintain suppressed expression of both *ABI3* and *ABI4*.

Table S1. Primers used in this study.

For ChIP-qPCR	
ABI3_a_F	ACGCATAGTAAAACAAAGTTCACA
ABI3_a_R	TGTTGGGATCGAAAAACCCT
ABI3_b_F	TGATGTTTCGTGCATGATGGT
ABI3_b_R	TGTACGTCGAGATGGCATGT
ABI3_c_F	AGTGTTTAAGAACCACCGCTTG
ABI3_c_R	TCCTCGTGCCGCTAGTATCT
ABI3_d_F	ACTGGAACACATGGGCTCTC
ABI3_d_R	ACTCCAACAAGAAGACTCTCTCT
ABI3_e_F	TCCTTGCCTCCTTACTCACA
ABI3_e_R	CGCCACATGCAAGCTTTTCA
ABI3_f_F	CAGCCTCTTCTTCTTCGGCA
ABI3_f_R	GCACCAGAAGAGTCGTCACA
ABI3_g_F	GTTCTCCACCACCACAACA
ABI3_g_R	GCCATGACGGTGGAGATTCA
ABI3_h_F	TGGTCGCTTCACCAACTTCT
ABI3_h_R	ATCCCATGCATGCACGAGAA
ABI4_a_F	TGGGTAATTCATGCCACGCT
ABI4_a_R	GCATCGTTTAAAGTTGGCAAGC
ABI4_b_F	TCCAAGTTCCGTTACCGTGG
ABI4_b_R	TGCGAAAGTACCAAGCCACT
ABI4_c_F	TAGGGCAGGAACAAGGAGGA
ABI4_c_R	AACCCGGATCCAGACCCATA
ABI4_d_F	CGGAGGAGATGGTGGAAAGT
ABI4_d_R	ACCTCTGAAACTCGAACAACCA
ABI5_C_F	AATTCTCCGGCGGCTTTT
ABI5_C_R	CCGGTGGCTTTGTGTTCC
AG_ChIP_F	CCCATCTCTTCACCAGCACA
AG_ChIP_R	GGGGGAGAAGAACAAGGGG
RD29A_1F	CGAATGAGAAGGATGTGCCG
RD29A_1R	TCGGTCCATGTCTATTGGCC
RD29A_2F	TGTTCCAGCATCGGAGGAAA
RD29A_2R	GGTGCATCGTGTCCGTAAGA

RD29A_3F	GGCGGGAAGTGTGATGAGA
RD29A_3R	TTCCCCTCGTTGCTCCTCTA
TA3-ChIP_F	TGGAATCTCAGGGTCAAGG
TA3-ChIP_R	CCTTCTGAGGTGAGGGACA

For RT-qPCR

ABI3_qF	TCGGAAGGATCGTTTTGCCA
ABI3_qR	CCAAACACGAGAGGTTCCGA
ABI4_qF	GTCCAGATGGGACAATCCAACACC
ABI4_qR	CCCTAACGCCACCTCATGATGAAAC
ABI5_qF	CAGCTGCAGGTTACATTCTG
ABI5_qR	CACCCTCGCCTCCATTGTTAT
RD29A_qF	TGTGGCGGAGAACTGACAA
RD29A_qR	TTCACTTCCTCTTCCACCGC
VIL1_qF	GGAGCGTGAAGGCCACATTA
VIL1_qR	AAATGCGTCGACAAGTTGGC
PP2A_qF	TAACGTGGCCAAAATGATGC
PP2A_qR	GTTCTCCACAACCGCTTGGT

For *abi3-c* constructs and genotyping

ABI3_DT1_BsF	ATATATGGTCTCGATTGATGGAGACTTTCGGGTACAGTT
ABI3_DT1_F0	TGATGGAGACTTTCGGGTACAGTTTTAGAGCTAGAAATAGC
ABI3_DT2_R0	AACGGTCCCTCCCGTGGCGGTTCAATCTCTTAGTCGACTCTAC
ABI3_DT2_BsR	ATTATTGGTCTCGAAACGGTCCCTCCCGTGGCGGTTCAA
ABI3_CRIS_F	CAGCCTCTTCTTCTTCGGCA
ABI3_CRIS_R	CGATCTGGTACCACCTGCTG
