Article title: CDK8 is associated with RAP2.6 and SnRK2.6 and positively modulates abscisic acid signaling and drought response in Arabidopsis
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Article acceptance date: 20 June 2020

The following Supporting Information is available for this article:

**Fig. S1** Root lengths of WT and two lines of cdk8 mutants transferred to MS and MS+ABA (50 µM) medium at post-germination stage.

![Graph showing root lengths](image1)

**Fig. S2** Increased expression of CDK8 by ABA treatments.

![Graph showing CDK8 expression](image2)
**Fig. S3** The expression of CDK8 in 35S:CDK8 transgenic lines.

**Fig. S4** The requirement of CDK8 kinase activity for the ABA response.
**Fig. S5** BiFC assays showing no interactions between SnRK2.6/CDK8 and RAP2.6 C-half (121-190 amino acid).

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Fig. S7 Analysis of CDK8-regulated genes under mock conditions.

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Fig. S10 rap2.6 mutants did not show altered response to ABA.
**Fig. S11** Transcription, protein level and subcellular localization of RAP2.6 in *RAP2.6* over-expression lines.

**Fig. S1** Root lengths of WT and two lines of *cdk8* mutants transferred to MS and MS+ABA (50 µM) medium at post-germination stage. Values are means ± SD (n=10). Different letters indicate the statistical differences (P < 0.05, two-way-ANOVA statistical analysis).

**Fig. S2** Increased expression of *CDK8* by ABA treatments. Seven-day-old seedlings of wild type were treated 50 µM ABA for different hours at room temperature. The *CDK8* expression was normalized to *ACTIN2*. Values are means ± SD (n=3).

**Fig. S3** The expression of *CDK8* in 35S:CDK8 transgenic lines. (a) The expression levels of CDK8 in WT and two independent 35S:CDK8 transgenic lines. RNA was extracted from seven-day-old seedlings of wild type and CDK8 over-expression lines. The CDK8 expression was normalized to *ACTIN2*. Values are means ± SD (n=3). (b) The CDK8 protein expression in 35S:CDK8 transgenic lines detected by Western blots. CDK8-HA fusion proteins were detected by anti-HA antibody.

**Fig. S4** The requirement of CDK8 kinase activity for the ABA response. (a) Phenotypes of WT, 35S:CDK8-MYC and 35S:CDK8D176A-MYC transgenic lines grown on MS and MS+ABA (50
μM) medium. (b) Root length of indicated seedlings grown on MS and MS+ABA (50 μM) media. Values are means ± SD (n=10). Different letters indicate the statistical differences (P < 0.05, two-way-ANOVA statistical analysis). (c) The cuticle permeability of WT, 35S:CDK8-MYC and 35S:CDK8D176A-MYC transgenic lines determined by Toluidine-Blue staining.

**Fig. S5** BiFC assays showing no interactions between SnRK2.6/CDK8 and RAP2.6 C-half (121-190 amino acid). (a) The co-expression of RAP2.6-C-NYFP and CDK8-CYFP did not lead to YFP signal in tobacco leaves. Scale bars = 40 μm. (b) The co-expression of RAP2.6-C-CYFP and SnRK2.6-NYFP did not lead to YFP signal in tobacco leaves. (c) The co-expression of RAP2.6-CYFP and SnRK2.6-NYFP resulted in YFP signal.

**Fig. S6** No obvious phosphorylation was detected between CDK8 and SnRK2.6 in vitro. (a) The purified recombinant proteins MBP-CDK8 and its kinase dead GST-SnRK2.6 G33R were incubated in kinase reaction buffer at room temperature for 30 min. The samples were then separated by SDS-PAGE. The autoradiogram and CBB staining of the protein gel were shown. (b) GST-SnRK2.6, GST1-SnRK2.6 G33R (kinase dead), GST-CDK8-KD (kinase dead) were incubated as indicated in kinase reaction buffer at room temperature for 30 min. The samples were then separated by SDS-PAGE. The autoradiogram and CBB staining of the protein gel were shown.

**Fig. S7** Analysis of CDK8-regulated genes under mock conditions. (a) heat map for the CDK8-regulated genes under mock conditions. (b) GO enrichment of CDK8-regulated genes under mock conditions (fold change >2, p-value < 0.05).

**Fig. S8** The enrichment of CDK8 at the promoter region of RAP2.6 under mock condition. The association of CDK8 with the indicated promoter regions in WT was set at 1. Values are means ± SD (n=3).

**Fig. S9** EMSA assays showing no direct binding between CDK8 and DRE, GCC-motif and COR15A promoter sequences.

**Fig. S10** rap2.6 mutants did not show altered response to ABA. (a) Verification of rap2.6 T-DNA insertion mutants by genomic PCR. LP, T-DNA left border genomic primer, RP, T-DNA right border primer. (b) Expression of RAP2.6 in WT and rap2.6 mutants. The RAP2.6 expression was normalized to ACTIN2. The expression of RAP2.6 in WT seedlings was set to 1. Values are means ± SD (n=3). *P < 0.05 (Student’s t-test). (c) The phenotype of WT and rap2.6 mutants in
response to ABA. (d) Root length of WT and 
rap2.6 mutants in Fig c. Values are means ± SD 
(n=10).

**Fig. S11** Transcription, protein level and subcellular localization of RAP2.6 in RAP2.6 over-
expression lines. (a) The transcription of RAP2.6 in the WT and two independent RAP2.6 over-
expression lines. Gene expression was normalized against the reference gene ACTIN2. The 
expression in the WT was set at 1. The error bars indicate SD (n=3). Asterisks indicate 
significantly different from the WT (Student’s t-test, P< 0.01). (b) The detection of RAP2.6-GFP 
in RAP2.6 over-expression lines. The total protein was extracted from WT and transgenic plants. 
The GFP tagged RAP2.6 was detected by Western Blot with anti-GFP antibody (Roche). (c) The 
subcellular localization of RAP2.6-GFP in transgenic plants. Scale bars =10 µm.

**Table S1** Primers used in this study.

**Table S2** List of CDK8-regulated DE genes identified under mock conditions.

**Table S3** List of ABA-responsive genes identified in the wild type.

**Table S4** List of ABA-responsive genes regulated by CDK8.