Title: Mutation of Mediator subunit *CDK8* counteracts the stunted growth and salicylic acid hyper-accumulation phenotypes of an Arabidopsis *MED5* mutant

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Summary

- The Mediator complex functions as a hub for transcriptional regulation. MED5, an Arabidopsis Mediator tail subunit, is required for maintaining phenylpropanoid homeostasis. A semi-dominant mutation (*ref4-3*) that causes a single amino acid substitution in MED5b functions as a strong suppressor of the pathway, leading to decreased soluble phenylpropanoid accumulation, reduced lignin content and dwarfism. In contrast, loss of MED5 results in increased levels of phenylpropanoids.
- We used a reverse genetic approach to identify suppressors of *ref4-3* and found that *ref4-3* requires CDK8, a kinase module subunit of Mediator, to repress plant growth. The genetic interaction between *MED5* and *CDK8* was further characterized using mRNA-sequencing (RNA-seq) and metabolite analysis.
- Growth inhibition and suppression of phenylpropanoid metabolism can be genetically separated in *ref4-3* by elimination of CDK8 kinase activity; however, the stunted growth of *ref4-3* is not dependent on the phosphorylation event introduced by the G383S mutation. In addition, rather than perturbation of lignin biosynthesis, mis-regulation of *DJC66*, a gene encoding a DNAJ protein, is involved in the dwarfism of the *med5* mutants.
- Together, our study reveals genetic interactions between Mediator tail and kinase module subunits and enhances our understanding of dwarfing in phenylpropanoid pathway mutants.

Keywords: plant Mediator, phenylpropanoids, dwarfism, CDK8 kinase activity, salicylic acid, *Arabidopsis thaliana*

Introduction

The multiprotein Mediator complex comprises about 30 subunits and serves as an integrative hub for transcription regulation in eukaryotic systems (Malik & Roeder, 2010). The core Mediator complex has been subdivided into head, middle and tail domains and functions as a bridge between transcription factors and the basal transcription machinery (Asturias *et al*., 1999; Tsai *et al*., 2014). The CDK8 kinase module, which reversibly associates with the core Mediator complex, differentially regulates Mediator's activity as either a co-activator or co-repressor (Hengartner *et al*., 1998; Borggrefe *et al*., 2002). Recently, cryomicroscopy (cryo-EM) structures of yeast and human Mediator revealed that the association between the kinase module and the core Mediator complex is predominantly achieved through the interaction between MED13 (kinase module) and MED19 (middle module) (Tsai *et al*., 2013; Tsai *et al*., 2014). The high-resolution cryo-EM maps not only demonstrate the interfaces between different modules of Mediator which are critical for proper transcriptional regulation (Nozawa *et al*., 2017; Tsai *et al*., 2017), but suggest an overall conserved structure of Mediator across different eukaryotic systems as well.

As in other eukaryotes (Conaway & Conaway, 2011), the Mediator complex plays a role in many aspects of plant life, including growth, development and responses to stress (Dolan & Chapple, 2017). Despite the critical nature of the complex overall, disruption of some Mediator subunit (MED) genes is not lethal in plants, and in many cases leads to distinctive phenotypes (Yang *et al*., 2016; Dolan & Chapple, 2017). The ability to knock out specific subunits and study the resulting phenotypes suggests that plants can be valuable eukaryotic systems to mechanistically characterize Mediator and its involvement in plant-specific biological processes.

In addition to its role in growth and development, recent studies have demonstrated that Mediator is required for the normal regulation of secondary metabolism in Arabidopsis. Specifically, MED5a and MED5b, two MED tail subunits, are required to maintain phenylpropanoid homeostasis (Bonawitz *et al*., 2012). Three *reduced epidermal fluorescence 4* mutants (*ref4-1, ref4-2* and *ref4-3*) characterized by single amino acid substitutions in MED5b (D647N for *ref4-1* and *ref4-2* and G383S for *ref4-3*) were isolated as strong repressors of the phenylpropanoid pathway, indicated by decreased soluble phenylpropanoid metabolite accumulation, reduced lignin content and dwarfism (Stout *et al*., 2008). In contrast, disruption of *MED5a* and *MED5b* (*med5a/5b*) results in the hyper-accumulation of phenylpropanoids (Bonawitz *et al*., 2012), indicating that MED5 plays a widespread role in homeostatic repression of phenylpropanoid biosynthesis.

A *ref4-3* suppressor screen identified three tail subunits of Mediator, MED2, MED16 and MED23, that are required for the repressive action of *ref4-3* upon phenylpropanoid metabolism and plant growth (Stout *et al*., 2008; Dolan *et al*., 2017). Disruption of either MED16 or MED23 restores soluble phenylpropanoid accumulation and growth in *ref4-3* background, whereas loss of MED2 rescues only the dwarfism of *ref4-3* (Dolan *et al*., 2017). Transcriptome analysis of *ref4-3* revealed that genes encoding the enzymes in the phenylpropanoid pathway display only modest changes in expression. In contrast, negative regulators of phenylpropanoid metabolism are up-regulated compared to wild type to an extent that is positively correlated with the level of soluble phenylpropanoid restoration in each of the suppressors (Dolan *et al*., 2017).

In the original *ref4-3* suppressor screen, we isolated multiple alleles of *med23* and *med16*, but only a single allele of *med2*, suggesting that the screen might not have been saturated. In addition to the tail module subunits, the dissociable CDK8 kinase module can regulate the activity of the core Mediator complex during transcription. Although CDK8 is generally recognized as a negative regulator of transcription in yeast (Kuchin *et al*., 1995; Rickert *et al*., 1999; Gonzalez *et al*., 2014), studies in mammalian systems indicate that CDK8 contributes to both transcriptional activation and repression (Knuesel *et al*., 2009; Nemet *et al*., 2014). Investigations in Arabidopsis revealed that CDK8 is necessary for floral organ development (Wang & Chen, 2004), mitochondrial retrograde signaling (Ng *et al*., 2013), pathogen defense (Zhu *et al*., 2014) and auxin signaling (Ito *et al*., 2016). Considering that CDK8 can activate down-stream gene targets in a Mediator-dependent fashion, and in *ref4-3*, negative regulators of the phenylpropanoid pathway show elevated steady state mRNA levels, we tested the hypothesis that CDK8 is required for *ref4-3* to repress phenylpropanoid metabolism and plant growth.

Here, we report that *MED5* genetically interacts with *CDK8* in Arabidopsis. Our data indicate that CDK8, and specifically its kinase activity, is required for *ref4-3* to repress plant growth. In contrast, the lignin content of *ref4-3 cdk8-1* remained low compared to wild type, indicating that low lignin content is not the cause of dwarfing in *ref4-3.* Although the phytohormone salicylic acid (SA) is hyper-accumulated in *ref4-3* and this phenotype can be suppressed by elimination of CDK8 kinase activity, blocking SA biosynthesis is not sufficient to rescue the stunted growth of *ref4-3*. In contrast, disruption of a gene encoding a plastid-targeted DNAJ protein that is upregulated in *ref4-3* partially suppresses this growth phenotype. Together, our data demonstrate that growth inhibition, suppression of phenylpropanoid metabolism and hyper-accumulation of SA can be genetically separated in *ref4-3* mutants, and that chloroplast localized chaperones might play an unexpected role in regulating plant growth.

Materials and Methods

Plant Material and Growth Conditions

Arabidopsis thaliana ecotype Columbia-0 (Col-0) was the wild type in this study. Plants were cultivated at a temperature of 23°C, under a long-day photoperiod (16 hr light/8 hr dark) with a light intensity of 100 $\mu E\ m^{-2}\ s^{-1}$. Homozygous mutants used in this study were isolated based on previous reports, with the corresponding accession numbers and primers listed in Supporting Information Table S1 (Cao *et al*., 1997; Kim *et al*., 2007; Bonawitz *et al*., 2012; Zhu *et al*., 2014).

Transgenic Plants

Plant binary vectors (pBA-myc) that carry either *CDK8* or *CDK8*^{D176A} driven by the 35S promoter were transformed into *ref4-3 cdk8-1* mutants by *Agrobacterium tumefaciens*-mediated transformation (Clough & Bent, 1998; Zhu *et al*., 2014). Similarly, a series of *MED5b*^{G383*} ('*' represents amino acids G, S, T, D, E, A and V) constructs were first cloned into pCC0996, a binary vector in which transgene expression is driven by the Arabidopsis *C4H* promoter (Bonawitz *et al*., 2012), and transformed into *med5* mutants. Transgenic lines were selected based on their resistance to Basta. The homozygous lines identified in the T3 generation were used for phenotypic characterization. To determine the CDK8 protein levels in the selected transgenic lines, 0.5 g of two-week-old seedlings were harvested and prepared for crude protein extracts in 1 mL Tris-HCl buffer (150 mM, pH 8.0). After centrifugation, 50 µL lysate from each sample was loaded on 10% SDS-page gel and protein gel blotting was performed using anti-MYC antibody (1:1000 dilution, Sigma M4439).

Lignin analysis

Total lignin content was quantified using extractive free cell walls by thioglycolic acid (TGA) lignin analysis, as described previously (Li *et al*., 2015).

HPLC analysis of secondary metabolites

Sinapoylmalate content of three-week-old whole rosettes was quantified by HPLC as previously reported (Dolan *et al*., 2017).

High-throughput mRNA sequencing

Samples of wild-type, *ref4-1*, *ref4-3*, *cdk8-1* and *ref4-3 cdk8-1* three-week-old rosettes were harvested in triplicate with a randomized design. Each sample contained five whole rosettes of the same genotype from five individual pots. RNA extraction and whole-transcriptome sequencing were performed as previously described (Dolan *et al*., 2017). The RNA-seq data of this study have been deposited in NCBI's Gene Expression Omnibus (Edgar *et al*., 2002) with accession number GSE111290. The previous RNA-seq data of *ref4-3* and its suppressors have

been deposited in Gene Expression Omnibus with accession number GSE95574 (Dolan *et al*., 2017).

Differential expression analysis

Count matrices for individual samples were generated for each gene using HTSeq-count (Anders *et al*., 2015). Differential expression analysis was performed based on the result of expressed genes with greater than one count per million (CPM) reads in at least three samples. The filtered data was then subjected to edgeR (v3.12.1) analysis using generalized linear model (GLM) approach (McCarthy *et al*., 2012), which was performed using statistical program R (v3.4.1). Significance testing was performed and adjusted using Benjamini-Hochberg method, reported as False Discovery Rate (FDR) with a cut-off at FDR < 0.05. Venn diagrams were created with the online tool Venny (v 2.1, http://bioinfogp.cnb.csic.es/tools/venny/) and GO term analysis was performed using the DAVID Bioinformatics Resource (v.6.8, https://david.ncifcrf.gov/) (Huang *et al*., 2008).

Determination of salicylic acid levels

SA extraction and detection was performed as previously described (Rozhon *et al*., 2005). Both free and total SA were quantified by HPLC using 2-methoxybenzoic acid as an internal standard.

Results

Loss of CDK8 rescues stunted growth in *ref4* mutants

To test whether *ref4-3* requires CDK8 to repress plant growth, we generated *ref4-3 cdk8-1* double mutants by crossing *cdk8* T-DNA insertion mutants (SALK_138675, *cdk8-1* and SALK_016169, *cdk8-2*) with *ref4-3*. Although *ref4-3* mutants exhibit a dwarf phenotype, both *ref4-3 cdk8-1* and *ref4-3 cdk8-2* are nearly normal in stature and rosette diameter (Figs 1, S1a). Similarly, loss of CDK8 restores the growth defect in *ref4-1*, another allele identified from the previous *ref* screen (Ruegger & Chapple, 2001) (Fig. S1b). Based on these results, we conclude that there is a genetic interaction between *CDK8* and *MED5* in Arabidopsis, and that CDK8 is required for *ref4-1* and *ref4-3* to repress plant growth.

We then tested whether the growth repression in *ref4-3* is dependent on the kinase activity of CDK8 using a transgene encoding a D176A kinase-dead version of the protein (Zhu *et al*., 2014; Kong & Chang, 2018) to generate *ref4-3 cdk8-1 35S:CDK8-MYC* (*ref4-3 CDK8*) and *ref4-3 cdk8-1 35S:CDK8*^{D176A}*-MYC* (*ref4-3 CDK8*^{D176A}) transgenic plants. After five weeks on soil, *ref4-3 CDK8* displayed stunted growth comparable to *ref4-3*, whereas *ref4-3 CDK8*^{D176A} looked identical to *ref4-3 cdk8-1* (Fig. 2a). An anti-MYC blot revealed that CDK8 was expressed at similar levels in *ref4-*

194 *3 CDK8* and *ref4-3 CDK8*^{D176A} (Fig. S2) indicating that these phenotypes were not the result of
195 different levels of transgene expression. The distinct growth phenotype between *ref4-3 CDK8* and
196 *ref4-3 CDK8*^{D176A} indicates that it is the kinase activity of CDK8 that is essential for growth
197 repression in *ref4-3*.
198 In addition to CDK8, the Arabidopsis Mediator kinase module consists of C-type cyclin (cycC),
199 MED12 and MED13. MED12 is necessary for CDK8 to demonstrate kinase activity (Knuesel *et*
200 *al*., 2009). To independently evaluate whether CDK8 kinase activity is required for *ref4-3*
201 phenotypes, we used a T-DNA insertion line in *MED12* (SALK_108241, *med12*) to generate *ref4-*
202 *3 med12* double mutants. As with CDK8, we found that loss of MED12 is sufficient to rescue the
203 stunted growth of *ref4-3* in terms of rosette size (Fig. 2b). Notably, the resulting double mutants
204 also exhibited a late flowering phenotype that was not observed in either *ref4-3* or *med12* single
205 mutants under the same growth condition, indicating that the flowering phenotype is a result of a
206 synthetic interaction between *ref4-3* and *med12*.
207 The restored growth of *ref4-3 cdk8-1* raised the question of whether CDK8 is required for growth
208 repression in other lignin-deficient mutants. Among the *ref* mutants, *ref8-1*, a mutant deficient in
209 the gene encoding the phenylpropanoid biosynthetic enzyme *p*-coumaroyl shikimate 3'-
210 hydroxylase, is similar to *ref4-3* in that its phenylpropanoid metabolism is repressed and it exhibits
211 stunted growth (Bonawitz *et al*., 2014). Moreover, loss of MED5 restores the stunted growth and
212 transcriptional reprogramming of *ref8-1*, suggesting that the dwarfism of this other lignin-deficient
213 mutant requires intact Mediator (Bonawitz *et al*., 2014). To elucidate the relationship between
214 CDK8 and the stunted growth of *ref8*, we generated *ref8-1 cdk8-1* mutants. Little, if any, growth
215 restoration was observed in the resulting double mutants compared to *ref8-1* at multiple growth
216 stages (Fig. S3). Thus, our observations indicate that *ref8-1* leads to growth repression largely
217 independent of CDK8.
218
219 **CDK8 is not required for down-regulation of phenylpropanoids in *ref4-3***
220 Given that *ref4-3* plays a repressive role in phenylpropanoid metabolism that can be
221 suppressed by several Mediator tail subunits (Dolan *et al*., 2017), we next determined whether
222 loss of *CDK8* also suppresses the phenylpropanoid deficient phenotype of *ref4-3*. Because
223 sinapoylmalate is localized in the upper epidermis, it can be readily visualized *in vivo* under
224 ultraviolet (UV) light. As expected, *ref4-3* displayed a characteristic *ref* phenotype compared to
225 wild type and *cdk8-1* (Fig. 3a), indicating a decreased level of sinapoylmalate in the mutant. *ref4-*
226 *3 cdk8-1* was similarly red under UV light even though the growth phenotype of *ref4-3* had been
227 reversed (Fig. 3a). The *ref* phenotype was also observed in *ref4-3 cdk8-2* and *ref4-1 cdk8-1* (Fig.

S4a,b). High performance liquid chromatography (HPLC) analysis confirmed these observations (Fig. 3b), indicating that CDK8 is not required for *ref4-3* to repress sinapoylmalate biosynthesis. Similarly, in *ref4-3*, total lignin content was reduced 40% compared to wild-type and *cdk8-1* plants and in spite of the strong growth restoration seen in *ref4-3 cdk8-1*, their total lignin content remained low (Fig. 3c,d). Consistent with the dispensable role of CDK8 in down-regulation of phenylpropanoid metabolism in *ref4-3*, expression of either wild-type or kinase-dead *CDK8* in *ref4-3 cdk8-1* background did not cause any difference in sinapoylmalate accumulation compared to *ref4-3 cdk8-1* (Fig. S4c). Taken together with the observation that loss of CDK8 largely rescues the stunted growth of *ref4-3*, these findings indicate that the dwarfism of *ref4-3* is independent of its restricted phenylpropanoid metabolism.

The stunted growth of *ref4-3* is not dependent on the phosphorylation event introduced by the G383S mutation

Because the G383S mutation in the *ref4-3* allele introduces a potential phosphorylation site, we wondered if the defects in growth and phenylpropanoid metabolism observed in *ref4-3* plants could result from ectopic/hyper-phosphorylation of MED5 by one or more kinases, possibly including CDK8 (Stout *et al*., 2008). We also considered whether the increased side-chain size of S383 in *ref4-3* could itself lead to these phenotypes, independent of phosphorylation status. To distinguish between these possibilities, we generated a series of *med5* constructs in which various site-directed mutants were expressed under the control of the *CINNAMATE 4-HYDROXYLASE* (*C4H*) promoter (Bonawitz *et al*., 2012) such that the transgenes would be expressed in cells involved in phenylpropanoid metabolism. *MED5b* transgene was expressed at similar level across all different transgenic lines, none of which was less than the expression of *MED5b* in wild-type plants (Fig. S5). We then assayed transgenic *med5a/5b* double mutant plants carrying these constructs for sinapoylmalate and lignin content. MED5a and MED5b share semi-redundant function in repression of phenylpropanoid metabolism and *med5a/5b* double mutants have increased sinapoylmalate and lignin content compared to wild type (Bonawitz *et al*., 2012); thus, expression of *C4H:MED5b* constructs with wild-type function should restore levels to that of the single *med5a* mutant alone. Indeed, the control *MED5b*^{G383G} transgenic displayed normal growth and accumulated sinapoylmalate and lignin content similar to that in *med5a* (Fig. 4). In contrast, the *ref4-3* mimic, *MED5b*^{G383S}, showed similar dwarf phenotypes as compared with *ref4-3* and accumulated less phenylpropanoids compared to *med5a* (Fig. 4). Expression of *MED5b*^{G383T} containing an alternative phosphorylation site, or the phospho-mimics *MED5b*^{G383D} and *MED5b*^{G383E}, also resulted in dwarfing and reduced phenylpropanoid levels (Fig. 4). In contrast,

expression of the non-phosphorylatable *MED5b*^{G383A} and *MED5b*^{G383V} had differing effects on plant growth and phenylpropanoid metabolism; *MED5b*^{G383A} was slightly dwarf and showed similar sinapoylmalate levels compared to the *MED5b*^{G383G} control, but *MED5b*^{G383V} plants were more stunted and showed sinapoylmalate levels that were intermediate between the *MED5b*^{G383G} control and *MED5b*^{G383S} (Fig. 4a,b). Because neither A nor V can be phosphorylated, but these showed different plant growth phenotypes and sinapoylmalate levels, we conclude that the G383 residue is important to the function of MED5b, and the increased side-chain size at position 383 caused by substitution of S for G is likely responsible for the plant growth and phenylpropanoid phenotypes associated with *ref4-3*. In contrast to sinapoylmalate, lignin levels were reduced in both *MED5b*^{G383A} and *MED5b*^{G383V} (Fig. 4c). Thus, the reduced phenylpropanoid accumulation in *ref4-3* is likely independent of the phosphorylation event introduced by the G383S mutation. We note that although the D647N mutation in *ref4-1* does not introduce a novel phosphorylation in MED5b, *ref4-1* mutants can also be rescued by loss of CDK8, further suggesting that the *ref4* phenotypes are not dependent upon CDK8-mediated phosphorylation of MED5b.

Disruption of *CDK8* partially rescues the transcriptional reprogramming of the *ref4-3* mutants

Our observations on *ref4-3 cdk8-1* indicate that the phenotypes of *ref4-3* plants can be genetically separated, and that dwarfism in these plants may result from aberrant gene expression in biological processes other than lignin biosynthesis. we performed messenger RNA sequencing (RNA-seq) using three-week-old rosettes of *ref4-3 cdk8-1* together with wild type, *cdk8-1*, *ref4-1* and *ref4-3*. Principle component analysis (PCA) revealed a clear clustering of samples by genotype (Fig. S6). We next determined the differentially expressed gene set in each mutant compared to wild type, and performed a gene ontology (GO) term analysis focused on biological processes. Compared to *ref4-3*, *ref4-1* is a weaker allele in terms of reduced phenylpropanoid accumulation and stunted growth (Fig. S7a). In *ref4-1*, 2927 genes were differentially expressed (Fig. S7b). More substantial gene expression changes were observed in *ref4-3*, which included 7770 mis-regulated genes, representing more than one-third of the expressed genes (count per million (CPM) reads > 1 in 3 or more samples) (Fig. S7b). We noticed that over 90% of the differentially expressed genes in *ref4-1* were also mis-regulated in *ref4-3* but with larger fold change (Fig. S7b,c). This finding not only suggests that the point mutations in *ref4-1* and *ref4-3* lead to a widespread transcriptional reprogramming by similar mechanisms, but also indicates that our RNA-seq analysis captures subtle differences in gene expression between alleles. Consistent with our previous observation (Dolan *et al*., 2017), GO term analysis of the up-

regulated genes in *ref4-3* showed an enrichment of genes involved in different stress responses and transcription regulation (Table S2). Up-regulated genes in *ref4-1* were also enriched for stress responses except for response to UV-B and salt stress, and transcriptional regulation (Table S2). In contrast to the up-regulated genes, genes that were down-regulated in *ref4-1* and *ref4-3* were enriched for those involved in photosynthesis (Table S3). In *ref4-3*, genes related to ribosome biogenesis and cytokinin response were also enriched among the down-regulated genes (Table S3).

We identified 4053 genes that were mis-regulated in *cdk8-1*, 60% of which displayed reduced expression compared to wild type (Fig. S8). Among the genes up-regulated in *cdk8-1*, only a limited number of GO terms were enriched, namely response to light, photosynthesis, and microtubule-based movement (Table S2). In contrast, transcripts related to defense response were significantly over-represented among the down-regulated genes (Table S3), which is consistent with the reported function of CDK8 in biotic stress responses (Zhu *et al*., 2014).

Although *ref4-3 cdk8-1* displays wild-type growth, a significant number of genes remained mis-regulated in the double mutant, including 3767 up-regulated genes and 4537 down-regulated genes when compared to wild type. Transcripts associated with response to water deprivation and abscisic acid (ABA) were the most significantly enriched among up-regulated genes (Table S2), whereas the down-regulated genes were enriched for those involved in defense responses (Table S3). Consistent with their *ref* phenotypes, many phenylpropanoid biosynthetic genes were down-regulated in *ref4-3*, and most of them were not rescued in *ref4-3 cdk8-1* (Fig. S9).

Our phenotypic analysis revealed that *ref4-3* requires CDK8 to repress plant growth but not phenylpropanoid metabolism. To identify the genes that are associated with the dwarf phenotype of *ref4-3*, we focused on the genes that displayed altered expression in *ref4-3* compared to wild type, but whose expression was rescued in *ref4-3 cdk8-1* (Fig. 5a). In total, 73 genes were significantly down-regulated in *ref4-3* compared to wild type and displayed at least a partially restored expression in the absence of CDK8 (FDR < 0.05, absolute value of $\log_2$FC > 1) (Fig. 5b, Table S4). GO term analysis revealed that within this gene set, genes associated with regulation of organ growth, photosynthesis and auxin-related signaling pathway were over-represented (Table S5). In contrast, 378 genes were significantly up-regulated in *ref4-3* compared to wild type, the abnormal expression of which was at least partially alleviated in *ref4-3 cdk8-1* (Fig. 5c, Table S6). The most significantly enriched GO categories within this gene set included suberin biosynthesis, lipid transport and defense responses (Table S7).

SA biosynthesis and signaling are activated in *ref4-3* but not in *ref4-3 cdk8-1*

GO term analysis revealed that genes involved in defense responses, especially those respond to SA, were up-regulated in *ref4-3* in a CDK8-dependent manner (Table S7). Previous studies proposed that hyper-activated SA biosynthesis and signaling leads to dwarfism of hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl transferase (HCT)-RNA interference (RNAi) transgenics in both Arabidopsis and alfalfa (Gallego-Giraldo *et al*., 2011a,b). Thus, we wondered if the aberrant activation of SA signaling could be the cause of dwarfism in *ref4-3*. In *ref4-3*, genes encoding proteins involved in SA biosynthesis and storage (Fig. 6a) (Ward *et al*., 1991; Yang *et al*., 1997; Dempsey *et al*., 2011) were significantly increased in expression compared to wild type and *cdk8-1* (Fig. 6b). SA signaling marker genes were also up-regulated in *ref4-3* (Fig. 6c). Loss of CDK8 in *ref4-3* eliminated the up-regulation of SA biosynthetic and signaling genes (Fig. 6b,c), indicating that *ref4-3* requires CDK8 to activate SA signaling. Consistent with these observations, there was an enhanced accumulation of both free SA and SA conjugates in *ref4-3*, which was blocked by loss of *CDK8* (Fig. 6d,e). Moreover, both free SA and SA conjugates were hyper-accumulated in *ref4-3 CDK8*, whereas *ref4-3 CDK8*^{D176A} plants accumulated wild-type level of SA (Fig. 6d,e), indicating that over-accumulation of SA in *ref4-3* is dependent on the kinase activity of CDK8. Taken together, our RNA-seq analysis and SA measurement demonstrate that CDK8 and its kinase activity is necessary for the hyper-accumulation of SA in *ref4-3*.

To test whether SA accumulation in *ref4-3* leads to the growth defects in these plants, we crossed *ref4-3* with *salicylic acid induction deficient 2* (*sid2-4*), a mutant defective in isochorismate synthase 1. We found that the growth phenotype of *ref4-3 sid2-4* was unchanged relative to *ref4-3* (Fig. 6f), even though HPLC analyses revealed that both free SA and total SA levels were reduced to below wild-type levels in *ref4-3 sid2-4* (Fig. 6g). We also used a mutant line with disruption in *NPR1* (CS_3726, *npr1-1*) (Cao *et al*., 1997), an essential regulator of SA signaling, to generate a *ref4-3 npr1-1* double mutant. Whereas *npr1-1* mutants displayed wild-type growth, *ref4-3 npr1-1* was indistinguishable from *ref4-3* (Fig. S10). These data indicate that SA accumulation is not the cause of dwarfing in the mutant.

Enhanced auxin accumulation is not sufficient to restore the stunted growth of *ref4-3*

Our GO term analysis suggested that auxin signaling is perturbed in *ref4-3* and rescued in *ref4-3 cdk8-1* (Table S5). Multiple genes involved in auxin signaling, including indole-3-acetic acid (IAA) induced genes such as *IAA1*, *IAA7* and *IAA29*, as well as small auxin up RNA (SAUR) genes including *SAUR20*, *SAUR22* and *AT5G18010* were down-regulated in *ref4-3* compared to wild type (Fig. 7a). In contrast, disruption of *CDK8* resulted in up-regulation of all the genes mentioned above, which is consistent with a previous finding that CDK8 kinase module plays a

repressive role in auxin transcriptional responses (Ito *et al*., 2016). Except for *IAA29,* which was up-regulated in *ref4-3 cdk8-1* compared to wild type, the other five genes displayed wild-type expression in the absence of CDK8 in the *ref4-3* background, suggesting that *ref4-3* represses auxin signaling in a CDK8-dependent fashion.

Given that auxin plays a critical role in plant growth (Teale *et al*., 2006), we next aimed to determine whether repressed auxin signaling contributes to the dwarfism of *ref4-3*. A previous study demonstrated that YUCCA6 (YUC6) functions in tryptophan-dependent auxin biosynthesis, and the dominant mutant *yuc6-1D* is sufficient to cause hyperaccumulation of auxin (Kim *et al*., 2007). We therefore constructed a *ref4-3 yuc6-1D* double mutant and evaluated its growth phenotype. Although introduction of *yuc6-1D* into *ref4-3* led to activation of the auxin-responsive gene *At4g02520* (Fig. 7b) (Smith *et al*., 2003) as well as high-auxin developmental phenotypes including elongated petioles and narrow leaves (Fig. 7c), *ref4-3 yuc6-1D* was as dwarf as *ref4-3* (Fig. 7d), indicating that repressed auxin signaling in *ref4-3* is probably not the leading cause for its stunted growth.

Disruption of a DNA J PROTEIN C66 (DJC66) partially restores the growth deficiency of *ref4-3*

Among the genes that showed greatest mis-regulation in *ref4-3*, *DJC66*, a gene encoding a small J-domain containing protein, was up-regulated more than 23-fold in *ref4-3* compared to wild type, and its expression was partially rescued in all *ref4-3* suppressors including *ref4-3 cdk8-1* (Fig. 8a,b). While DJC66 has not been functionally characterized, it was proposed to be critical for leaf growth because of its interaction with anaphase-promoting complex subunit 8 (APC8) (Arabidopsis Interactome Mapping Consortium, 2011; Schulz *et al*., 2014), a protein involved in cell cycle progression (Eloy *et al*., 2015). To test whether DJC66 is required for the dwarfism of *ref4-3*, we crossed *ref4-3* to a T-DNA insertion line in *DJC66* (SALK_149745C, *djc66*) and generated *ref4-3 djc66* double mutants. Compared to *ref4-3*, *ref4-3 djc66* displayed modest but significant growth restoration (Fig. 8c,d,e,f), suggesting that the stunted growth of *ref4-3* is partially dependent on DJC66. Moreover, like *ref4-3*, *ref4-3 djc66* had lower levels of sinapoylmalate and lignin compared to wild type, indicating that the protein is not involved in the suppression of phenylpropanoid metabolism and may function specifically in the dwarfing phenotype of the mutant (Fig. 8g,h). Taken together, our data reveal that DJC66 is a novel suppressor that partially suppresses the stunted growth of *ref4-3*. Further, unlike all previous *ref4-3* suppressors, DJC66 presumably functions independent of Mediator's role in transcriptional regulation because it is localized to the plastid, rather than the nucleus (Chiu *et al*., 2013).

Discussion

In this study, we used the Arabidopsis *ref4-3* mutant to examine the function of MED5 in the context of Mediator. *ref4-3* carries a missense mutation in *MED5b*, and exhibits dwarfism and reduced phenylpropanoids (Stout *et al*., 2008; Bonawitz *et al*., 2012). Loss of MED5 leads to increased phenylpropanoid accumulation in an otherwise wild-type genetic background (Bonawitz *et al*., 2012), and disruption of *MED5a* and *MED5b* can restore the phenylpropanoid-deficient phenotypes of other *ref* mutants (Anderson *et al*., 2015; Kim *et al*., 2015). Thus, our data suggest that the proteins encoded by semi-dominant *ref4* alleles mimic the action of wild-type MED5 in homeostatic repression of phenylpropanoid biosynthesis, and thus provide genetic tools that are complementary to biochemical approaches to investigate the interaction between MED5 and other transcriptional regulators.

We previously reported that loss of Mediator tail module subunits MED2, MED16 or MED23 relieves the growth defects of *ref4-3* (Dolan *et al*., 2017). Here, we show that loss of CDK8, a kinase module subunit, has a similar effect. Unlike disruption of MED16 or MED23, loss of CDK8 does not restore the restricted lignin biosynthesis in *ref4-3*, which again demonstrates that the stunted growth and reduced lignin content of *ref4-3* can be genetically disentangled as was found for MED2 (Dolan *et al*., 2017).

The identification of CDK8 as a novel *ref4-3* suppressor also provides new evidence for the functional/genetic, and potentially physical, interaction between the tail and kinase modules of Mediator. Arabidopsis CDK8 functions together with MED25 to activate the pathogen defense marker gene *PDF1.2* (Zhu *et al*., 2014), and physical interaction between MED5 and the kinase module has been suggested by several studies in mammalian cells (Ito *et al*., 2002; Kneusel *et al*., 2009). Our study suggests that the interaction between MED5 and CDK8 may be preserved in the Arabidopsis Mediator complex. Alternatively, the genetic interaction between CDK8 and MED5 may reflect a functional but indirect interaction between these two subunits. Recent cryo-EM structures of yeast Mediator complex revealed that the CDK8 kinase module can reversibly associate with the head and middle module through the interaction between MED13 (kinase module) and MED19 (middle module) (Tsai *et al*., 2013), whereas MED5, embedded in the tail, is located distal to those two modules (Tsai *et al*., 2014). Given that the overall structure of Mediator is conserved in eukaryotic systems (Tsai *et al*., 2014), the available high-resolution map of yeast Mediator (Tsai *et al*., 2013; Tsai *et al*., 2014) suggests that MED5 and CDK8 do not physically interact with each other. Nevertheless, the potential physical interaction between different Mediator subunits in plants still needs to be evaluated by future studies.

Although some genetic studies have shown that CDK8 has functions independent of its kinase activity (Zhu *et al*., 2014), our data demonstrate that CDK8 kinase activity is required for both growth deficiency and increased SA accumulation in *ref4-3*, consistent with the critical role of CDK8 with intact kinase activity in retrograde signaling and stress response (Ng *et al*., 2013; Zhu *et al*., 2014). Because CDK8 is dispensable for normal phenylpropanoid accumulation whereas MED5 is critical for this process, it is unlikely that wild-type MED5 is a general substrate of CDK8, and that the phosphorylation of MED5 by CDK8 is required for phenylpropanoid homeostasis.

The interacting partners and/or substrates of Arabidopsis CDK8 remain to be identified, but in other eukaryotes include the C-terminal domain of Pol II, histone proteins, individual Mediator tail subunits including MED2 and MED3 (Hallberg *et al*., 2004; Gonzalez *et al*., 2014) and various transcription factors (Rzymski *et al*., 2015; Poss *et al*., 2016). Notably, a recent study in common wheat revealed that CDK8 can phosphorylate the transcription factor wax inducer 1 (TaWIN1), which thereby activates TaWIN1-targeted genes and promotes very-long-chain aldehyde biosynthesis (Kong & Chang, 2018). The identification of TaWIN1 as a target of CDK8 suggests that besides substrates of CDK8 common to all eukaryotes, CDK8 may phosphorylate plant-specific transcription factors, possibly including those that are necessary for growth inhibition of *ref4-3*.

Many plant hormones including SA and auxin play critical roles in the cross-talk between growth and immunity (Kazan & Manners, 2009; Huot *et al*., 2014). Although the stunted *ref4-3* mutant hyper-accumulates SA, our data suggest that the SA content of the mutant is unrelated to its dwarfism. Similarly, dwarfism of *ref8-1*, another lignin-deficient mutant, is also independent of its SA accumulation (Bonawitz *et al*., 2014). Thus, hyper-accumulation of SA is not a universal mechanism underpinning dwarfism in lignin-deficient mutants. Moreover, our data further show that the repressed growth in *ref4-3* is likely independent of auxin signaling. Together, we conclude that perturbation of hormone signaling is not the underlying cause for dwarfism associated with lignin deficiency.

Although *ref4-3* and *ref8-1* show multiple similarities including repressed phenylpropanoid metabolism, significant changes in their transcriptome and growth deficiency independent of SA, CDK8 is a suppressor of *ref4-3*, but not of *ref8-1*. In fact, while multiple MED subunits were identified as suppressors of *ref4-3* (Dolan *et al*., 2017), MED5 is the only characterized suppressor that can fully restore the growth of *ref8-1* (Bonawitz *et al*., 2014). The difference between *ref4-3* and *ref8-1,* as well as previously identified low-lignin mutants, indicates that multiple mechanisms exist for dwarfing in plants that co-occur with perturbed phenylpropanoid

metabolic phenotypes. Specifically, the dwarfism of *ref4-3* may result from abnormal transcriptional reprogramming achieved by mutated MED5b itself, whereas the stunted growth of *ref8-1* is due to restricted flux through phenylpropanoid pathway or an abnormal response triggered by over-accumulation of phenylpropanoid pathway intermediates that requires wild-type MED5 for perception.

Our study raises the possibility that an alternative mechanism involving chaperone pathways might be involved in *ref4-3* associated dwarfism. We identified *DJC66*, encoding a co-chaperone DnaJ protein, as a highly-upregulated gene in *ref4*-3 that was partially rescued by loss of CDK8. Similar to elimination of CDK8, loss of DJC66 suppresses the stunted growth of *ref4-3* but does not affect phenylpropanoid biosynthesis. DJC66 interacts with the anaphase-promoting complex subunit APC8, suggesting its potential role in cell cycle regulation and plant growth (Schulz *et al*., 2014; Eloy *et al*., 2015). In addition, DJC66 can be targeted to chloroplasts, and its expression is significantly induced under heat and cold stresses (Chiu *et al*., 2013). Given that CDK8 is essential for retrograde signaling and general abiotic stress responses (Ng *et al*., 2013), it is likely that DJC66 functions downstream of CDK8 in growth repression of *ref4-3* (Fig. 9). Moreover, the partial growth restoration in *ref4-3 djc66* and the fact that DJC66 is only one of the DnaJ cochaperones (Chiu *et al*., 2013) suggest that other DnaJ proteins may share redundant function with DJC66 and contribute to the stunted growth of *ref4-3* as well.

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Author Contributions

X.M., V.M.W. and C.C. designed the research project; X.M., J.I. K., M.T.W. and A.K.H. performed the experiments; X.M., M.T.W., V.M.W. and C.C. analyzed the data; X.M., V.M.W. and C.C. wrote the manuscript.

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Supporting Information

Fig. S1 CDK8 is required for growth repression in *ref4* mutants.

Fig. S2 Wild-type and kinase-dead CDK8 are expressed at similar levels in the *ref4-3 cdk8-1* background.

Fig. S3 CDK8 is not necessary for *ref8-1* to repress plant growth.

Fig. S4 CDK8 is dispensable for reduced phenylpropanoid accumulation in *ref4* mutants.

Fig. S5 *MED5b* transgene is expressed at similar level in the selected transgenic mutants, all of which are comparable or more than expression of *MED5b* in wild type.

Fig. S6 Principle component analysis (PCA) of the RNA-seq samples.

Fig. S7 Transcriptional reprogramming in *ref4* mutants reflects the severity of alleles.

Fig. S8 Comparison between wild type and *cdk8* mutants.

Fig. S9 Phenylpropanoid biosynthetic genes are generally repressed in *ref4-3* and *ref4-3 cdk8-1.*

Fig. S10 The stunted growth of *ref4-3* is independent of NPR1.

Table S1. Primers used in this study.

Table S2. GO term analysis of the genes that are up-regulated in *ref4-1*, *ref4-3*, *cdk8-1* and *ref4-3 cdk8-1* compared to wild type respectively.

Table S3. GO term analysis of the genes that are down-regulated in *ref4-1*, *ref4-3*, *cdk8-1* and *ref4-3 cdk8-1* compared to wild type respectively.

Table S4. A full list of the genes that are down-regulated in *ref4-3* and with restored expression in *ref4-3 cdk8-1* (FDR < 0.05, absolute value of $\log_2$FC > 1).

Table S5. Gene ontology analysis for the genes that are down-regulated in *ref4-3* and that have restored expression in *ref4-3 cdk8-1*.

Table S6. A full list of the genes that are up-regulated in *ref4-3* and with restored expression in *ref4-3 cdk8-1* (FDR < 0.05, absolute value of $\log_2$FC > 1).

Table S7. Gene ontology analysis for the genes that are up-regulated in *ref4-3* and that have restored expression in *ref4-3 cdk8-1*.

Figure legends

Figure 1. CDK8 is required for *ref4-3* to repress plant growth.

(a-b) Representative photographs of *ref4-3 cdk8-1* compared to wild-type *Arabidopsis thaliana* (Columbia-0, Col-0), *ref4-3* and *cdk8-1*. *cdk8-1* is a T-DNA insertion line of CDK8, a subunit of the Mediator kinase module. Soil-grown plants were compared three weeks (a) or six weeks (b) after planting.

(c-d) Height (c) and rosette diameter (d) measurement of *ref4-3 cdk8-1* together with wild type, *ref4-3* and *cdk8-1* after growth on soil for six weeks. Data represent mean ± standard deviation (SD) (n=10). The means were compared by one-way ANOVA, and statistically significant differences ($p < 0.05$) were identified by Tukey's test and are indicated by a to c to represent difference between groups.

Figure 2. Elimination of CDK8 kinase activity is sufficient to suppress the dwarfism of *ref4-3*.

(a) Five-week-old soil-grown *Arabidopsis thaliana* transgenic lines overexpressing *CDK8* in a *ref4-3 cdk8-1* background (*ref4-3 cdk8-1 CDK8*) together wild type, *ref4-3*, *cdk8-1* and *ref4-3 cdk8-1* respectively. $CDK8^{D176A}$ indicates a kinase-dead version of CDK8 which carries a D to A mutation at residue 176.

(b) Five-week-old soil-grown *ref4-3 med12* compared to wild type, *ref4-3* and *med12* respectively.

Figure 3. *ref4-3* represses phenylpropanoid metabolism independent of CDK8.

(a) Representative photograph of wild type, *ref4-3, cdk8-1* and *ref4-3 cdk8-1* under ultraviolet (UV) light. Plants were compared three weeks after planting.

(b) Sinapoylmalate content of three-week-old plants from each genotype determined by high-performance liquid chromatography (HPLC).

(c) Total lignin content in seven-week-old stem tissues quantified by thiolglycolic acid (TGA) lignin analysis.

(d) Lignin monomer composition in seven-week-old stem tissues determined by the derivatization followed by reductive cleavage (DFRC) method. The p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignin subunit contents were quantified and normalized to the weight of dried cell wall samples.

For panels b-d, data represent mean ± SD (n=3). The means were compared by one-way ANOVA, and statistically significant differences ($p < 0.05$) were identified by Tukey's test and are indicated by a to b.

Figure 4. The stunted growth and reduced phenylpropanoids of *ref4-3* is not dependent on the phosphorylation event introduced by the G383S mutation.

(a) Representative photograph of wild type, *ref4-3*, *med5a/5b*, *med5a* and *C4H* promoter-driven site-directed *MED5b* mutants at G383 site in *med5a/5b* background (*med5a/5b C4H: MED5b*^{G383*}). Plants were compared six weeks after planting. '*' represents the amino acid substitution including G, S, T, D, E, A and V.

(b) Quantification of sinapoylmalate content in three-week-old wild type, *ref4-3*, *med5a/5b*, *med5a* and *MED5b*^{G383*} mutants.

(c) Quantification of lignin content in seven-week-old wild type, *ref4-3*, *med5a/5b*, *med5a* and *MED5b*^{G383*} transgenics.

For panels B-C, data represent the mean ± SD (n = 3). ‡ and * indicate $p < 0.05$ (Dunnett's test) when compared to *MED5b*^{G383G} and *MED5b*^{G383S}, respectively.

Figure 5. Disruption of *CDK8* rescues gene expression changes in the *ref4-3* mutant.

(a) idealized histograms demonstrating the criteria for growth-related gene targets of interest. The potential gene targets should either be down-regulated in *ref4-3* compared to wild type with at least partial restoration of expression in *ref4-3 cdk8-1* compared to *ref4-3* (left), or up-regulated in *ref4-3* compared to wild type and at least partially repressed in *ref4-3 cdk8-1* compared to *ref4-3* (right). '*' represents the significant difference of gene expression in two genotypes (FDR <0.05, absolute value of $\log_2$FC > 1).

(b) The number of genes with significantly decreased expression in *ref4-3* compared to wild type is represented by the left Venn diagram, while the number of genes with significantly decreased expression in *ref4-3* compared to *ref4-3 cdk8-1* is represented by the right Venn diagram (FDR < 0.05, absolute value of $\log_2$FC > 1).

(c) The number of genes with significantly increased expression in *ref4-3* compared to wild type is represented by the left Venn diagram, while the number of genes with significantly decreased expression in *ref4-3* compared to *ref4-3 cdk8-1* is represented by the right Venn diagram (FDR < 0.05, absolute value of $\log_2$FC > 1).

For panels b-c, the overlapping region represents the genes that fit the criteria in the left histogram and the right one in (a) respectively.

Figure 6. Hyper-accumulation of SA in *ref4-3* is dependent on CDK8, but it is not the major cause of dwarfing in *ref4-3*.

(a) The SA biosynthesis and signaling pathways. SA is synthesized from the precursor chorismate via isochorismate synthase 1 (ICS1). Phenylalanine ammonia lyase (PAL) catalyzes the first step in a less significant SA biosynthetic pathway which has yet to be fully elucidated. SA can either be converted to its glucoside form for storage by UDP-glucose dependent glucosyltransferases (UGT) UGT74F1 or UGT74F2, or serve as signal molecules for plant development and stress responses. The *pathogenesis-related* (*PR*) genes including *PR1*, *PR2* and *PR5* are marker genes for SA signaling.

(b-c) Expression level (fragments per kilobase per million, FPKM) of SA biosynthetic genes (b) and SA signaling marker genes (c) in wild type, *ref4-3*, *cdk8-1* and *ref4-3 cdk8-1*, determined by RNA-seq analysis. Data represent mean ± SD (n=3). '*' indicates FDR < 0.05 compared to wild type.

(d-e) Free SA (d) and total SA (e) in wild type, *ref4-3*, *cdk8-1* and *ref4-3 cdk8-1, ref4-3 cdk8-1 CDK8* and *ref4-3 cdk8-1 CDK8* ^{D176A} quantified by HPLC using fluorescence detection. Rosettes from three-week-old plants were used to perform quantification. Data represent mean ± SD (n=3). The means were compared by one-way ANOVA, and statistically significant differences ($p < 0.05$) were identified by Tukey's test and are indicated by a to c.

(f) Five-week-old soil-grown *ref4-3 sid2-4* compared to wild type, *ref4-3* and *sid2-4* respectively.

(g) Free SA and total SA in wild-type, *ref4-3*, *sid2-4* and *ref4-3 sid2-4* quantified by HPLC using fluorescence detection. Rosettes from three-week-old plants were used to perform quantification. Data represent mean ± SD (n=3). '*' indicates $p < 0.05$ compared to wild type according to Student's t-test.

Figure 7. Enhanced auxin accumulation does not restore the stunted growth of *ref4-3*.

(a) Expression level (FPKM) of major auxin signaling genes in wild type, *ref4-3*, *cdk8-1* and *ref4-3 cdk8-1*, determined by RNA-seq analysis. Data represent mean ± SD (n=3). '*' indicates FDR < 0.05 compared to wild type using EdgeR analysis.

(b) Expression of *At4g02520* normalized to the reference gene *At1g13220* in wild type, *ref4-3*, *yuc6-1D* and *ref4-3 yuc6-1D*, determined by quantitative PCR analysis. Data represent mean ± SD (n=3). The expression of *At4g02520* is not detectable (n.d.) in wild type and *ref4-3.*

(c-d) Representative photographs of three-week-old (c) and five-week-old (d) soil-grown *ref4-3 yuc6-1D* compared to wild type, *ref4-3* and *yuc6-1D*.

Figure 8. Disruption of DJC66 partially restores the dwarfism of *ref4-3*.

(a) Expression level (FPKM) of *DJC66* in wild type, *ref4-3*, *cdk8-1* and *ref4-3 cdk8-1.* Data represent mean ± SD (n=3). Statistically significant differences (FDR < 0.05) were indicated by a to c.

(b) Expression level (FPKM) of *DJC66* in wild type, *ref4-3*, *med* T-DNA lines and *med ref4-3* double mutants determined by a previous RNA-seq analysis (Dolan et al., 2017). Data represent mean ± SD (n = 3). ‡ and * indicate $p < 0.05$ (EdgeR analysis) when compared to Col-0 and *ref4-3*, respectively.

(c-d) Three-week-old (c) and six-week-old (d) soil-grown *ref4-3 djc66* together with wild type, *ref4-3* and *djc66*.

(e-f) Height (e) and rosette diameter (f) measurement of *ref4-3 djc66* together with wild type, *ref4-3* and *djc66* after growth on soil for three weeks and six weeks respectively.

(g) Sinapoylmalate content of three-week-old wild-type, *ref4-3*, *djc66* and *ref4-3 djc66* plants determined by HPLC.

(h) Total lignin content in six-week-old stem tissues quantified by TGA lignin analysis.

For panel e-f, Data represent mean ± SD (n=10). For panel g-h, Data represent mean ± SD (n=3). The means were compared by one-way ANOVA, and statistically significant differences ($p < 0.05$) were identified by Tukey's test and are indicated by a to c.

Figure 9. A model of the genetic interaction between *CDK8* and *ref4-3*.

The findings of this study are summarized into the genetic interaction between *ref4-3* and *CDK8* in the Mediator complex (white background), mis-regulated gene targets (light grey background) and the resulting phenotypes (dark grey background). Particularly, *ref4-3* requires CDK8 with intact kinase activity (indicated by $CDK8^{ATP}$) to activate genes involved in SA biosynthesis and therefore leads to enhanced SA signaling. The kinase activity of CDK8 is required for growth repression of *ref4-3*; however, elimination of the kinase activity of CDK8 does not rescue the down-regulated phenylpropanoid metabolism in *ref4-3*. *DJC66* is one of the targets that are related to the dwarfism of *ref4-3*, which could be downstream of CDK8.