Silencing **SlMED18**, tomato Mediator subunit 18 gene, restricts internode elongation and leaf expansion

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Mediator complex, a conserved multi-protein, is necessary for controlling RNA polymerase II (Pol II) transcription in eukaryotes. Given little is known about them in tomato, a tomato Mediator subunit 18 gene was isolated and named **SlMED18**. To further explore the function of **SlMED18**, the transgenic tomato plants targeting **SlMED18** by RNAi-mediated gene silencing were generated. The **SlMED18**-RNAi lines exhibited multiple developmental defects, including smaller size and slower growth rate of plant and significantly smaller compound leaves. The contents of endogenous bioactive GA3 in **SlMED18** silenced lines were slightly less than that in wild type. Furthermore, qRT-PCR analysis indicated that expression of gibberellins biosynthesis genes such as **SlGACPS** and **SlGA20ox2**, auxin transport genes (**PIN1**, **PIN4**, **LAX1** and **LAX2**) and several key regulators, **KNOX1**, **KNOX2**, **PHAN** and **LANCEOLATE** (LA), which involved in the leaf morphogenesis were significantly down-regulated in **SlMED18**-RNAi lines. These results illustrated that **SlMED18** plays an essential role in regulating plant internode elongation and leaf expansion in tomato plants and it acts as a key positive regulator of gibberellins biosynthesis and signal transduction as well as auxin proper transport signalling. These findings are the basis for understanding the function of the individual Mediator subunits in tomato.

Plant growth and development, including vegetative growth and reproductive growth, is a multiphase process that requires a tight coordination among molecular, biochemical and structural elements. The vegetative growth includes the development of root, stem and leaf, the reproductive growth includes flower, fruit and seed development and the vegetative growth is necessary for providing the essential nutrients for the reproductive growth. Plants usually grow vegetatively at early developmental stage then translate to the reproductive growth stage. In the vegetative growth, the roots provide water and other nutrients to the plant, and the growth of the stem provides the possibility for the plant to produce more leaves, while the development and growth of the leaves provide more nutrients and energy for the reproductive growth of the plant.

The regulation of plant gene expression is necessary for the normal growth and development of plants, which is a complex and accurate network system that generally includes both transcription and translation of a series of genes. Transcription in eukaryotic organism is an intricate and extremely orchestrated process, through RNA Pol II assisted by a number of transcriptional regulators. The transcriptional regulators include transcriptional activators, various general transcription factors (GTFs), and a series of transcription cofactors. Several transcription factor families, including MADS-box, GRAS, and MYB have been characterized for their regulatory roles in plant vegetative development. In addition, Mediator, a multi-subunit complex, is one cofactor that promotes transcription initiation as the bridges between transcription activators bounding to regulatory upstream promoter or enhances DNA elements and the RNA Pol II general transcriptional machinery at the essential promoter. Nowadays, Mediator complex has emerged as possibly the most essential section for regulating Pol II transcription in eukaryotes.

Mediator complex was first identified in **Saccharomyces cerevisiae** and structural studies showed that the 21 yeast Mediator subunits have been divided into four submodules, the head, middle, tail, and kinase modules.
Moreover, the modular architecture and subunit composition of Mediator complex is evolutionarily conserved in eukaryotes. In plants, more than 30 different Mediator subunits have been shown that are part of the Mediator complex in different organisms. In the past, the studies of Mediator complex have been focused on yeasts and metazoans. Until 2007, more than a decade after its discovery in S. cerevisiae, Mediator complex was successfully purified from plants. Since then, some plants Mediators and the physiological functions of several subunits began to be revealed. The discovery of the Mediator complex in Arabidopsis thaliana indicated the Mediator complex subunits possess their own specific function within the whole complex, and already, a majority of Mediator complex subunits have been reported to have important regulatory roles. In Arabidopsis thaliana, AtMED12 and AtMED13, referred to as GRAND CENTRAL (GCT) and CENTER CITY (CCT), effect various aspects of plant development including embryo pattern formation, developmental timing and flowering and floral morphogenesis. The genetic and physiological study suggested that AtMED25/PHYTOCHROME AND FLOWERING TIME 1 (PFT1) are implicated in regulating IA-triggered gene expression and effect flowering under suboptimal light conditions. AtMED14, which was originally described as STRUWWELPETER (SWP), affects cell proliferation and shoot meristem development. AtMED20a, AtMED18, AtMED8, and AtMED17 were known to implicated in non-coding RNA production. AtMED14, AtMED15, and AtMED16 have recently been speculated to be involved in defense signalling. In another study, Arabidopsis Med32 played a role in root development and senescence. Although there are some studies on the Mediator function in model plant which made some meaningful progress, the roles of plant mediator in RNA transcription remain to be further elucidated on account of the lack of relevant mutant gene material.

MED18 (Mediator subunit 18 gene) is one of the Mediator complex subunits genes. Structural studies illustrated that MED18 is located in the movable jaw of the head module structure and resembles the head of a crocodile with one limb. Recently several studies about MED18 in Arabidopsis thaliana have been reported that AtMED18 was involved in regulating multiple plant physiological processes, including plant immunity, abscisic acid (ABA) responses, flowering time and floral organ identity through interactions with distinct transcription factors.

Solanum lycopersicum is a kind of widely grown economic crop, which not only has high agricultural economic value but also is an important model plant for studying plants development. To date, although Mediator complex plays a critical role in promoting the transcription of genes, little is known about them in tomato. In this study, a member of mediator family, named Mediator subunit 18 (SIMED18), was isolated from tomato. To further explore the function of SIMED18 in plant growth and development, we created transgenic tomato plants using S. lycopersicon Mill. cv. Ailsa Craig as wild type tomato by RNA interference. Down-regulation of SIMED18 restricted internode elongation and leaf expansion, producing dwarf plants and smaller leaves than wild type. The molecular and cellular levels investigation of SIMED18-RNAi lines indicated that SIMED18 plays an important role in regulating the development of leaf and stem. This research enhanced our knowledge about the roles of SIMED18 in tomato developmental processes.

**Results**

**SIMED18 isolation and expression pattern analysis.** In this study, a Solanum lycopersicum Mediator of RNA polymerase II transcription subunit 18 gene, named SIMED18, was isolated from wild type tomato leaves with specific primers (Supplementary Table S1) based on a cDNA clone (GenBank accession no. XM_010323502.2). Sequencing validation indicated that the correct gene sequence was obtained and sequence analysis showed that SIMED18 contains an open reading frame (ORF) of 651 bp and encodes 216 amino acid residues.

In addition, keyword searches and BLAST searches were performed against the Arabidopsis thaliana Mediator complex subunit gene sequences. To dissect the evolutionary relationships of MED18, the MED18 sequences of tomato, Zea mays(Zm), Solanum tuberosum (St), S. cerevisiae, Human (Hs), the dicot model plant Arabidopsis thaliana (At) and the monocot model plant Oryza sativa (Os) were used to conduct multiple sequence alignment and an original tree was constructed. Phylogenetic analysis implied that sequences are weakly conserved between S. cerevisiae, Human (Hs) and plants, but they are conserved across the plant kingdom (Fig. 1A,B).

As we all know that the tissue specificity of gene expression may be correlated with specific biological functions. So the real-time PCR analysis was conducted to clarify the expression profile of SIMED18 in various tissues, which consist of roots, stems, leaves, flowers and fruits at different developmental stages (Fig. 1C). The results showed that SIMED18 expressed abundantly in all the organizations we examined, which was consistent with pre-expression pattern (Supplementary Fig. S1). The bar graph of expression profile in various tomato tissues was obtained from Tomato lab website. These results indicated that SIMED18 may have essential roles in multiple tomato plant growth and development process.

**Repression of SIMED18 causes plant developmental defects.** To further investigate the biological functions of SIMED18 in tomato, an RNAi expression vector targeting SIMED18 gene was created and transformed into tomato (Solanum lycopersicum 'Ailsa Craig' AC''). via Agrobacterium tumefaciens mediated transformation. 25 independent transgenic lines were confirmed by PCR using primers of NPT II (Supplementary Table S1), then their total RNAs were extracted from young leaves to investigate the relative expression of SIMED18, respectively. qRT-PCR data showed that the three transgenic lines (RNAi 5, RNAi 15 and RNAi 23), compared with the control, displayed 90%, 93% and 96% reduction in SIMED18 mRNA levels, respectively (Fig. 2A). Later these three transgenic lines were chosen and used for subsequent experiments. Compared with wild type plants, the SIMED18-RNAi lines displayed shorter and thinner stem with significantly smaller compound leaf (Fig. 2B–D and Supplementary Fig. S2A–C). The height of 60-days-old wild type plant was about 22.5 cm, while the three transgenic lines (RNAi 5, RNAi 15 and RNAi 23) exhibited a lower growth rate and had 9.1, 8.8 and 8.0 cm plant height. Thus, the RNAi 5, RNAi 15 and RNAi 23 plants were 59.56%, 60.9% and 64.34%
shorter than the control, respectively (Fig. 2E). Furthermore, the internode lengths of 60-days-old wild type and SlMED18-RNAi plant from the first to the fifth node were measured, the result showed that every corresponding internode in SlMED18-RNAi transgenic tomato plants was reduced remarkably compared with the wild type (Fig. 2F). Morphology observation displayed that the stems of SlMED18-RNAi lines were thinner than the wild type (Fig. S2A,C), and the leaves were significantly smaller (Fig. 3A,B). The length, width as well as area of mature compound leaves was significantly less than wild type (Fig. 3C,D).

Transgenic plants have smaller leaf cells. Given the differences of leaves between transgenic lines and the wild type, an anatomical investigation of sections of leaf was performed to investigate whether this phenotype was associated with a decrease in the cell size or in the cell number. The cellular analysis showed that the size of leaf cell in the SlMED18-RNAi lines was dissimilar from those in the wild type at the same stage of tomato plant development (Fig. 4A–B). The cellular data indicate that cell size of the SlMED18-RNAi leaf was significantly smaller and the cell number of leaf vein was notably less, compared with those in the wild type (Fig. 4C and D). The results suggested that the smaller and narrower leaves might due to both the reduction of leaves cell size and cell number.

To explore the molecular mechanism of smaller leaf of transgenic plants, some genes associated with cell expansion and cell cycle genes, such as SlCycA3;1, SlCycB1;1, SlCycD2;1, SIXTH2, SIPRE1, SIPRE2, SIPRE3, SIPRE4 and SIPRE5 were examined in leaf. As shown in Fig. 4E, the representative cell cycle regulatory genes, SlCycA3;1, SlCycB1;1 and SlCycD2;1 were down-regulated in SlMED18-RNAi lines. Moreover, the transcript levels of SIPRE1, SIPRE2, SIPRE3, SIPRE4 and SIPRE5, which involved in cell division and expansion 33, were markedly decreased in SlMED18-RNAi lines (Fig. 4F).
SlMED18-RNAi plants have decreased endogenous bioactive Gibberellins (GAs) contents. It has been reported that the plant hormone GAs is involved in internode elongation as well as other plant developmental processes. To date, a lot of genes encoding GAs biosynthetic or signalling transduction genes have been characterized as being associated with this process\(^{34,35}\). As the SlMED18-RNAi plants were dramatically

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**Figure 2.** Repression of SlMED18 causes plant developmental defects. (A) The expression levels of SlMED18 in three SlMED18-RNAi transgenic lines (RNAi-5, RNAi-15 and RNAi-23) and wild type plants. We collected young leaves and stems of the plants as materials detected by real-time qPCR and normalized the expression data of wild type plants to 1. (B, C) The SlMED18-RNAi plants (RNAi-5, RNAi-15 and RNAi-23) were exhibited multiple developmental defects. 30-day old plants rooted on the MS culture medium in culture bottle (B) and then transplanted them into pots to cultivate 30 days (C). (D) Phenotypes of leaves in SlMED18-RNAi lines. The compound leaves were collected from 60-day-old wild type and SlMED18-RNAi lines at the same nodes. (E) The growth rate of wild type and SlMED18-RNAi plants. We measured the height of plants in 0-day old, 30-day old, 45-day old and 60 day old. (F) Internode length of the first to the fifth node from 60-days-old wild type and SlMED18-RNAi lines.

**Figure 3.** Altered leaf morphology in SlMED18-RNAi tomato plants. The compound leaves (A) and the main leaflets (B) were collected from the same node (the fifth node) of 60-days old wild type and SlMED18-RNAi tomato plants. The maximum length, maximum width (C), and the area of leaves (D) compared between wild type and SlMED18-RNAi tomato plants. Error bar indicates means ± SD (n = 8). *P < 0.05 and **P < 0.01 according to t-test.
shorter than the wild type (Fig. 2C), the contents of endogenous bioactive GA$_3$ in the stems of wild type and SlMED18-RNAi lines were measured. The results showed that endogenous GA$_3$ in transgenic plants were significantly less than that of wild type (Fig. 5A), indicating that the dwarfism of SlMED18-RNAi plants is due to the deficiency of endogenous GAs. Therefore, we further detected some genes involved in the GAs biosynthesis and signal transduction. The transcript accumulation of two genes, tomato ent-copalyl diphosphate synthase (SlCPS) and tomato ent-kaurenioic acid oxidase (SlKAO), which involved in the GA biosynthetic pathway were

Figure 4. Anatomical analyses of leaf between wild-type and the SlMED18-RNAi lines. (A and B) The lead of wild type (A) and SlMED18-RNAi plant (B) were prepared transverse sections. Bars = 200μm. (C and D) The estimated cell area (μm$^2$) (C) and cell number of leaf vein (D) were measured and compared between transgenic and wild type at the same position in both cases. Data are shown as the mean ± SE. (E and F) Expression of several genes associated with cell expansion and cell cycle genes (E) SlCycA3;1, SlCycB1;1, SlCycD2;1, SIXTH2; (F) SIPRE1, SIPRE2, SIPRE3, SIPRE4 in leaf.
markedly reduced in stems and leaves of SlMED18-RNAi lines (Fig. 6A,B). In many plant species, GA20oxs are the core GA biosynthetic enzymes that determine the GAs concentration and GA3oxs catalyze the final step to produce bioactive GAs (GA1, GA3, and GA4)46. In our study, SIGA20ox1 and SIGA3ox2 were dramatically down-regulated in leaves and stems of SlMED18-RNAi lines compared with the wild type (Fig. 6C,D). To further assess whether SlMED18 affects GA signal transduction factors, we also determined the expression level of SIGAST1 (tomato gibberellin-stimulated transcripts 1), a tomato GA-responsive downstream gene, as well as a GA receptor SIGID1-A (tomato GA INSENSITIVE DWARF1-A)37-39. The results displayed that SIGAST1 and SIGID1-A were significantly down-regulated in leaves of SlMED18-RNAi plants but not drastically decreased in stems of transgenic plants (Fig. 6E,F). These results denoted that SlMED18 affects GA biosynthesis and signal transduction.

Silencing of SlMED18 alters the expression of auxin transport and response genes. In our study, we compare the expression level of three genes ToFZY1, ToFZY4 and ToFZY5, which contributed to the localized biosynthesis of IAA, between SlMED18-RNAi plants and wild type40. In transgenic plant, ToFZY1, ToFZY4 and ToFZY5 were down-regulated. However, the contents of IAA in the stems of SlMED18-RNAi lines showed no significant change compared to wild type (Fig. 5B). The spatiotemporal localization of auxin acts as an essential regulator of leaves organogenesis and stems growth. The polar auxin transport mediated by PIN and AUX/LAX proteins is a major mechanism that regulates auxin distribution, which controls cellular auxin efflux and influx respectively41. So we further detected the expression of auxin transport and response genes in SlMED18-RNAi plants and wild type. In this study, two PIN genes, PIN1 and PIN4, and two LAX genes, LAX1 and LAX2, were examined in stems and leaves of the transgenic lines and the wild type, respectively. The results demonstrated that these four genes were significantly down-regulated in the stems and leaves of the transgenic lines (Fig. 7D–G). TIR1 is reported to be an auxin receptor that mediates rapid degradation of Aux/IAA proteins and consequently changes the expression of auxin-regulated genes42. In our study, the expression level of SITIR1 was markedly decreased in leaves of transgenic lines but no significant changes were observed in stems of the transgenic lines compared with the wild type (Fig. 7H). These results may explain the no significant change of IAA content that due to the severe inhibition of auxin transport and the IAA were accumulated at the apices of the stem. We also examined the expression of two genes of Aux/IAA family, SlIAA3 and SlIAA14, which are known to be implicated in modified auxin response. The results displayed that the transcripts of SlIAA3 were remarkably reduced in leaves and stems of transgenic lines and SlIAA14 was significantly down-regulated in leaves but not in stems of transgenic lines (Fig. 7I,J). SIARF19, a transcriptional activator of early auxin response gene, was also significantly reduced in SlMED18-RNAi lines (Fig. 7K). The relative expression level of auxin response gene SIARF8, which may control the free IAA level in a negative feedback fashion43, was up-regulated in the SlMED18-RNAi lines compared with wild type (Fig. 7L). As a result, our study suggested that repression of SlMED18 may affect auxin regulation by regulating the expression of polar auxin transport genes and auxin responsive genes.

Suppression of SlMED18 results in down-regulated expression of leaf morphogenesis related genes. Given that the SlMED18-RNAi transgenic lines had significantly smaller compound leaves with narrower lamina compared with wild type lines. (Fig. 3A and B), we detected the expression of four genes, KNOX1, KNOX2, PHAN and LANCEOLATE (LA), which are associated with leaf morphology and development by qRT-PCR in the leaves of 90-day old wild type and SlMED18-RNAi plants. The results showed that the transcription levels of these genes were sharply decreased in the SlMED18-RNAi lines compared with the wild type (Fig. 8A–D). These results suggested that SlMED18 may affect leaf morphology development through regulating the expression of these key regulators.

Discussion
Mediator complex is a central part of the transcriptional machinery in all eukaryotes. Since the first purification of Mediator complex from yeast, subsequent studies of yeast Mediator subunit mutants expression profiling exposed that some specific sets of genes can be directly regulated by Mediator44. In plants, the research of Mediator is
not lagging so far. Over the past few years, a series of reports have emerged revealing the Mediator subunits are essential in regulating diverse processes like embryo pattern formation, developmental timing, flowering and response to multiple different biotic and abiotic stresses\(^\text{15,45,46}\). However, the information about function of the Mediator subunits on plant developmental processes is lacking and the studies on Mediator subunits among plant kingdom only focus on *Arabidopsis thaliana* and *Oryza sativa*. To date, there is little research about the function of Mediator in tomato, which is one of the important model plants. 

*MED18* is part of the head module of Mediator complex, and some research identified that *MED18* plays essential roles in multiple biological functions, including plant responses to microbial infection and regulating flowering and germination by environmental signals in *Arabidopsis*\(^\text{28,29}\). Here, phylogenetic analysis among *MED18* in tomato, *S. cerevisiae* (*Sc*), *Human* (*Hs*) and several typical model plants showed that Mediators despite have low sequence homology between *S. cerevisiae* (*Sc*), *Human* (*Hs*) and plants, but they conserved across plant kingdoms (Fig. 1A,B). In addition, the *MED18* was isolated from tomato and expression profile exhibited that *SlMED18* was extensively expressed in all of the examined tissues, illustrating the *SlMED18* may have effect on different plant physiological processes. Unlike the report in Arabidopsis mutant\(^\text{29}\), silencing of *SlMED18* in tomato caused severe plant developmental defects, including smaller size and slower growth rate of the plant with significantly smaller compound leaves and narrower lamina and smooth margins (Fig. 2B–D and Supplementary Fig. S2A–C). The result suggested that *SlMED18* plays important roles in the plant developmental processes.

In *SIMED18*-RNAi lines, the plant height, width and length of compound leaves and leaf area were measured. The date explicated that the stem and leaf developmental result in a strong reduction in plant growth of...
whole plant (Figs 2E,F and 3C,D). Moreover, the anatomical characterization of leaf from SlMED18 silenced plants and wild type was performed and we found that the size and number of leaf cell in SlMED18-RNAi lines were drastically decreased (Fig. 4C,D). The genes associated with cell expansion and cell cycle genes, including SlCycA3;1, SlCycB1;1, SlCycD2;1, SlXTH2, SlPRE1, SlPRE2, SlPRE3, SlPRE4 and SlPRE5 (Fig. 4E,F) were remarkably up-regulated in SlMED18-RNAi lines. These results may partially explain the smaller compound leaves in the transgenic lines.

Plant hormones play the crucial roles in regulating plant growth and development as well as plant responses to various biotic and abiotic stress responses. Among the seven different kinds of plant hormones, gibberellin (GAs) and auxin are both key signals in plant developmental and often act synergistically. Some research of dwarf mutants and analysis of their GAs contents made sure that bioactive GAs is associated with the regulation of stem growth in plants47. In tomato, gibberellin-responsive mutants (gib-l, gib-2, and gib-3) were identified by reduced plant height due to shorter internodes and their leaves are smaller, darker green, and structure differently as compared to wild type48. In our study, the endogenous GA3 content of transgenic lines is less than wild

Figure 7. (A–C) The expression levels of three genes ToFZY1, ToFZY4 and ToFZY5, which contributed to the localized biosynthesis of IAA. (D–G) Expression of several key genes of auxin transport and auxin-responsive in SIMEDI8-RNAi transgenic lines. The relative mRNA level of major polar auxin transport genes SIPIN1 (D), SIPIN4 (E), SILAX1 (F) and SILAX4 (G) in leaf and stem, respectively. (H) Transcript accumulation of TIR1, an auxin receptor. (I–L) The auxin-responsive genes (SIIAA3, SIIAA14, SIARF19 and SIARF8) in leaf and stem tissues were investigated by qRT-PCR. The expression in each sample was used for standardization, and the relative expression of each gene in the wild-type was set to 1.0. Each value represents the mean ± SD of three replicates (n = 8). Asterisks indicate a significant difference (*P < 0.05 and **P < 0.01) between wild type and transgenic lines, according to t-test.
type (Fig. 5), demonstrating that the dwarf plant and smaller leaves phenotype of SlMED18-RANi lines may be ascribed to the decreased levels of endogenous GAs. As we all know, the content of endogenous GAs is regulated by the expression of several key genes involved in GAs biosynthesis and signal transduction such as CPS, KAO, SlGA20oxs and SlGA3ox. In previous reports, a number of examples explicated that these genes were involved in alteration of GAs level and lead to a developmental defect plant. Loss-of-function CPS mutants of several plant species, such as Oryza sativa and Arabidopsis thaliana, resulted in severely impaired plant growth 49,50. In Arabidopsis thaliana, kao1 kao2 double mutant shows typical non-germinating GA-dwarf phenotypes 51. Besides, in tomato, up-regulation of SlGA20oxs, and SlGA3ox can lead to higher levels of bioactive GAs and constitutive co-suppression of the SlGA20ox1 gene cause serious defects in vegetative and reproductive development. In our study, the expression of SlCPS, SlKAO, SlGA20oxs and SlGA3ox can lead to higher levels of bioactive GAs and constitutive co-suppression of the SlGA20ox1 gene cause serious defects in vegetative and reproductive development. However, biosynthesis of endogenous bioactive gibberellin (GA) is a complicated process, including the regulated at transcription level, the post-transcriptional level, translation level and post-translational level of key GA biosynthesis enzyme genes. Especially, in eukaryotic, many proteins undergo extensive post-translational modifications such as methylation, acetylation, phosphorylation, glycosylation, and ubiquitination can affect the protein content. For example, ubiquitination has been shown to be involved in the regulation of protein degradation and gene expression. It may explain the reduction in gibberellins contents present in the transgenic line compared with the wild type was not as significant as the expression levels of GA related genes. In addition, the expression levels of a GA response gene SlGAST1 37 and SlGID1-A were also notably reduced in the leaves of SlMED18-RNAi stems (Fig. 6E,F), probably because of the feedback of GA deficiency.

The plant hormone auxin has a key role in many developmental processes, and it is known that the level of auxin throughout whole plant tissues is tightly controlled the coordination of plant growth and development 52. The multidimensional effects of auxin need to coordinate regulation of auxin influx and efflux carriers, guiding the auxin transport in a polar way that together form a polar auxin transport (PAT) network 53. Unlike other acknowledged plant hormones, auxin can be actively transported in a directional fashion in order to affect auxin concentrations during phototropic and gravitropic response. Two largest participators of directional transport in the PAT system are the PIN-FORMED (PIN) auxin transporters, which act as auxin efflux carriers, and the AUX1 (AUXIN RESISTANT1) and its close homologues LAX (LIKE AUXIN RESISTANT) that take participate in auxin influx as the influx carrier. The PIN1, PIN4, LAX1, LAX4 genes were dramatically declined (Fig. 7D–G) and several auxin responsive genes were also changed in SlMED18 RNAi lines (Fig. 7H–L). The results collectively
suggested that MED18 takes part in the regulation of auxin distribution, possibly through controlling the activity of polar auxin transport genes.

Gibberellin (GA) and auxin are both key signals in plant growth and are often observed to act synergistically54. However, much of our knowledge on GAs and auxin has come from studying them independently. Recently, some studies proposed a new idea that there is crosstalk between GA signalling and auxin transport. There are some reports that the auxin can induce the transcription of several GA biosynthesis genes such as in Arabidopsis, after decapitation the apical source of auxins, the active GA levels in stems are lower than those in stems of intact plants95. In contrast, GA biosynthesis and signalling has a positive impact on proper auxin transport96. In Arabidopsis GA mutants, the GA pathway cross-talk with PIN protein–dependent auxin transport pathway lead to the auxin transport impairment in mutants, which causative for defect of cotyledon differentiation and root gravitropic responses97.

Several genes, including class 1 KNOTTED homeodomain genes (KNOX), PHANTASTICA gene PHAN and CIN-TCP transcription factor LANCEOLATE (LA), have been characterized for their regulatory roles in altering leaf shape and development. PHAN is required for normal meristem function58. The KNOX gene in tomato was involved in organ leaf formation, and the tomato LANCEOLATE (LA) gene was shown to promote leaf differentiation59,60. Silencing SIMED18 in tomato altered the expression of these genes (Fig. 8A–D), suggesting that SIMED18 regulates the leaf development. Interestingly, recently these genes were exhibited to act in part by adjusting gibberellin and auxin levels. KNOX proteins partly linked to GAs pathways and have a positive impact on auxin signalling60. It is reported that expression of the SIGA20ox1 is up-regulated in gain-of-function LA mutants, illustrating that LA activity is partly mediated by positive regulation of the GAs response, probably by regulation of endogenous bioactive GAs levels60. In addition, in leaf primordial of harboring the dominant LA mutant the auxin signal is very weak, implying the specification of marginal out-growths, controlled by LA. Thus, narrower lamina of SIMED18-RNAi lines probably not only accounts for the alteration of the key genes for leaf development but also is induced by modulation of GA and auxin signalling. Additionally, we also found the repression of SIMED18 altered inflorescences architecture (Supplementary Fig. A and B), whether this is due to the direct affection on transcriptional regulators or indirect result of hormone signalling pathways, future biochemical analyses will need to identify.

Silencing SIMED18 in tomato caused multiple plant developmental defects, including smaller size and slower growth rate of plant and significantly smaller compound leaves, further we found that SIMED18 affected a set of transcription factors to regulate diverse physiological and cellular processes through impacting intra and extra-cellular signals. Furthermore, understanding the function of this Mediator subunit in tomato may provide information for entire tomato Mediator complexes.

Materials and Methods

Plant materials and growth conditions. In this article, We planted the tomato (Solanum lycopersicum, 'Ailsa Craig' AC+) as wild type that together with transgenic cultures were grown in controlled greenhouse conditions (16-hour-day (25 °C)/8-hour-night (18 °C)cycles, 80% humidity and a 250 μmol m⁻² s⁻¹ light intensity) and managed them routinely. The sample of roots, stems, young leaves, mature leaves, senescent leaves, flowers, sepalas and fruits of five periods were collected from tomato for SIMED18 organ-specific expression profiling.

Transgenic tomato plants that came from tissue culture in first generation (T0) were used in our experiment because the transgenic plants were sterility. For analysis of gene expression, the fifth leaf from top and the young stem that at the corresponding developmental stage from the 90-day-old plant were collected. Plant samples that we used were frozen in liquid nitrogen immediately then stored at -80 °C.

Phylogenetic Analysis. The cDNA and protein sequence date of tomato mediator of RNA polymerase II transcription subunit 18 was found in NCBI (http://www.ncbi.nlm.nih.gov/) and Sol Genomics Network (SGN, http://solgenomics.net/). MED18 in Solanum lycopersicum (Sl), Arabidopsis thaliana (At), Oryza sativa (Os), Solanum tuberosum (St), Zea mays (Zm), Saccharomyces. cerevisiae (Sc) and Human (Hs) were obtained from the NCBI databases. GenBank accession numbers for multiple sequence alignment and phylogenetic analysis are as follows: SIMED18 (XP_010321804), AtMED18 (NP_565534), OsMED18 (XP_015625319), SlMED18 (XP_006364546), ScMED18 (NP_011618) and HsMED18 (NP_001120822). Multiple sequence alignment of MED18 in tomato with other species was conducted by DNAMAN 6.0 programs. Alignments of amino acid sequences of MED18 subunits proteins were made using Clustal X2.16. A phylogenetic tree of Mediator of RNA polymerase II transcription subunit 18 protein sequence in 7 different species was constructed using MEGA7 (http://megasoftware.net/) and the phylogenetic analysis was inferred using the Neighbor-Joining method.

Total RNA extraction and quantitative real-time PCR analysis. Using RNAiso Plus (Takara) in accordance with the instructions, we extracted Total RNA from wild type and MED18-RNAi plant tissues. cDNA was synthesized with oligo(dT)₁₈ as primer by RNA that reverse-transcribed using M-MLV reverse transcriptase (Promega). In addition, the synthesized cDNA need to fold dilute three times with nuclease-free water for quantitative real-time PCR analysis. Additionally, the qRT-PCR analysis was conducted with the GoTaq qPCR Master Mix (Promega), 1.0μL mixture primers, 1.0μL cDNA, 3.0μL ddH₂O by CFX96™ Real-Time System (Bio-Rad, USA). We performed the NRT (no reverse transcription control) and NTC (no template control) to eliminate the effect by genomic DNA and the environment factor. The organ-specific expression analysis was detected using the SICAC gene (accession number: SGN-U314153) which showed stable expression across diverse tissues, as internal standard88. The 2−ΔΔCt method was used to performed relative gene expression levels analysis89. In addition, each sample was repeated three times and standard curves were run at the same time. All primers we used were shown in Supplementary Table S1 that designed by Primer premier 6.24 software (http://www.premierbiosoft.com/crm/ jsp/com/pbi/crmclientside/ProductList.jsp).
Expression analysis prediction of SIMED18.  SIMED18 expression prediction atlas of tomato tissues were obtained using Tomato lab website (http://tomatolab.cshl.edu/~lippmanlab2/allexp_query.html). In this website, the tomato genome sequence formed Tomato Genome Consortium and searched by gene ID came from Sol Genomics Network (http://solgenomics.net/)[4,6]

SIMED18-RNAi vector construction and plant transformation.  To generate MED18-RNAi lines, an RNAi vector was constructed. Primers of SIMED18-RNAi were showed in Supplementary Table S1, tagged with XhoI, Xbal and HindIII, KpnI restriction sites at the 5’end respectively, were used to amplify a 343-bp specific fragment of SIMED18 cDNA. We used cloning vector pHANNIBAL as the original vector, digested the above amplified fragment of SIMED18 products that with HindIII/Xbal and KpnI/XhoI, inserted into the pHANNIBAL plasmid at the HindIII/Xbal restriction site in the sense orientation while at the KpnI/XhoI restriction site in the antisense orientation. Lastly, a double-stranded RNA expression unit, covered the calf mosaic virus (CaMV) 35S promoter, was purified and then integrated into a binary vector pBIN19 with Saci and Xbal restriction sites. After SIMED18-RNAi Vector constructed, the generated binary plasmids, confirmed by restriction digest analysis and sequencing validation, were transferred into the Agrobacterium tumefactions LBA4404 strain, then the Agrobacterium tumefactions-mediated transformation was carried out and the SIMED18-RNAi lines were obtained[7]. The transgenic plants rooted on MS solid medium containing kanamycin for selecting the positive transgenic lines and the primers NPTII-F/-R were used to detect the MED18-RNAi plants (Supplementary Table S1) and the positive transgenic lines were used for further study.

Quantification of phenotypes and statistical analysis.  To study the differences between the transgenic plants and wild type, in addition to the height and internode length were measured; the length, width and area of mature leaf (4–5 circles from top) were also calculated. The plant height was measured after cutting shoot rooted on the MS culture medium for 30 days and 30 days after transplanting them into pots, respectively. The other dates were measured from 60-days old plants planted into pots. Besides, the image-analyzing program Image J (http://rsb.info.nih.gov/ij/) was adapted to measure the length, width and area of compound leaves. Averages and standard errors were calculated from eight different plants.

Anatomical analyses of the leaf.  Sectioning of leaf was carried out by hand on mature fresh tissues of 90-day-old plants. Fine cut, with the help of a sharp razor blade and instantly fixed by 70% ethanol/acetic acid/40% formaldehyde (18:1:1, by volume; FAA), dehydrated in gradient ethanol–water series, then fixation, sectioning and dew axing. Cut samples along the middle to prepare the transverse sections of leaves. Finally, we visualized anatomical structure of leaf under a microscope (OLYMPUS IX71) and photographed. The areas of cells and average cell number per area (10000 μm²) were quantified by Image J software (http://rsbweb.nih.gov/ij/).

Quantification of endogenous bioactive gibberellin contents.  Collected apical shoots stem and frozen in liquid nitrogen then used for GA determination immediately. We used gibberellin (GA₃) kit (GA-4-Y Comin Biotechnology Co., Ltd., China) and IAA (IAA-4-C Comin Biotechnology Co., Ltd., China) to extract and purify GA₃ and IAA. The HPLC (High Performance Liquid Chromatography) was used to measure the concentration of endogenous bioactive gibberellin (GA₃) and IAA in apical shoots stem of wild type and MED18-RNAi lines. Moreover, the experimental operation is strictly related to gibberellin (GA₃) kit instruction manual.

Statistical analysis.  Data were displayed as mean ± standard deviation. Significant differences between SIMED18-lines and wild type were analyzed by the t-test (** P < 0.01 and * P < 0.05). Considering the biological significance of the differential expression, we used the mean value of the standard deviation (SD) with three biological repeats to represent the measured value.

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**Author Contributions**

G.C. and Z.H. designed and managed the research work and improved the manuscript. J.Z., X.Y., J.G., H.L. and J.Z. performed the experiments. Y.W. performed the experiments and wrote the manuscript.

**Additional Information**

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