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Silencing *SlELP2L*, a tomato Elongator complex protein 2-like gene, inhibits leaf growth, accelerates leaf, sepal senescence, and produces dark-green fruit

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The multi-subunit complex Elongator interacts with elongating RNA polymerase II (RNAPII) and is thought to facilitate transcription through histone acetylation. Elongator is highly conserved in eukaryotes, yet has multiple kingdom-specific functions in diverse organisms. Recent genetic studies performed in *Arabidopsis* have demonstrated that Elongator functions in plant growth and development, and in response to biotic and abiotic stress. However, little is known about its roles in other plant species. Here, we study the function of an Elongator complex protein 2-like gene in tomato, here designated as *SlELP2L*, through RNAi-mediated gene silencing. Silencing *SlELP2L* in tomato inhibits leaf growth, accelerates leaf and sepal senescence, and produces dark-green fruit with reduced GA and IAA contents in leaves, and increased chlorophyll accumulation in pericarps. Gene expression analysis indicated that *SlELP2L*-silenced plants had reduced transcript levels of ethylene- and ripening-related genes during fruit ripening with slightly decreased carotenoid content in fruits, while the expression of DNA methyltransferase genes was up-regulated, indicating that SlELP2L may modulate DNA methylation in tomato. Besides, silencing *SlELP2L* increases ABA sensitivity in inhibiting seedling growth. These results suggest that *SlELP2L* plays important roles in regulating plant growth and development, as well as in response to ABA in tomato.

The key enzyme for transcription of protein-encoding genes in eukaryotes is RNA polymerase II (RNAPII). The histone acetyltransferase (HAT) Elongator copurifies with RNAPII during transcriptional elongation, presumably by rendering the DNA more accessible for the passage of the polymerase[1,2]. Except for its important role in transcriptional elongation through the histone and α-tubulin acetylation[3–5], there are a variety of mechanisms believed to be involved, including cytoskeleton organization, exocytosis, and tRNA modification[6–9]. Elongator is composed of two subcomplexes: the core subcomplex with ELP1-3, and the accessory subcomplex with ELP4-6[10]. ELP1 contains a nuclear localization sequence which is essential for Elongator function[11,12], while ELP2 is dispensable for the integrity of the holo-Elongator complex because the complex lacking ELP2 even retains the ability to acetylate histones *in vitro*[13]. ELP3 is the catalytic subunit of the Elongator complex and encodes a histone acetyltransferase[3]. ELP3 also contains an Fe4S4 cluster which is characterized as S-adenosylmethionine (SAM) binding domain, and is likely to carry out demethylation of histone residues[14,15]. In addition, Elp4-6 subcomplex forms a hexameric ring-shaped structure both in vitro and in vivo that is important for histone H3 and tRNA binding[16,17].

The subunit structures of Elongator are highly conserved among yeast, human and plants. However, their functions are diverse in eukaryotic organisms[1,18]. For example, mutations in yeast Elongator subunits lead to resistance to the zymocin γ-toxin subunit and sensitivity to salt, caffeine, and temperature[19]. Mutations in the IKBKAP (human ELP1) result in abnormal neuron development and cause human severe hereditary familial dysautonomia disease[20]. Presently limited genetic studies performed in *Arabidopsis* have emerged indicating that Elongator functions in plant growth and development, and in responses to biotic and abiotic stress. At the macroscopic level, Elongator mutants in *Arabidopsis* display a pleiotropic phenotype, including reduced primary

root growth, narrow and elongated leaves, aberrant inflorescence architecture, reduced germination frequency, delayed seedling growth, and hypersensitivity to abscisic acid (ABA)[21,22]. Disruption of each Elongator subunit (ELP1–ELP6) results in similar phenotypes, and double or triple mutants resemble single mutants in *Arabidopsis*, suggesting that plant Elongator subunits work as a complete complex and share common functions[21,23]. For example, all the four Elongator mutants (*abo1*/*elo2*/*elp1*, *elp2*, *elo3*/*elp3*, and *elo1*/*elp4*) have narrow leaves, reduced root growth, ABA hypersensitivity, and an increased accumulation of anthocyanins[23]. Nelissen, et al.[18] showed that Elongator likely controls plant development through regulating auxin-responsive genes, as a large number of auxin-responsive genes are differentially expressed in *elp* plants. Abnormal development may also derive from the upregulated jasmonic acid (JA) and ethylene (ET) signaling pathways observed in *elongata* (*elo*) mutants. Besides their critical functions in plant growth and development, Elongator has also been implicated in the response to biotic and abiotic stress. Disruption of both core and accessory subcomplexes results in the hyperinhibition by ABA of germination and seedling growth. Besides, the *abo1*/*elo2*/*elp1*, *elp2*, *elo3*/*elp3*, and *elo1*/*elp4* mutants are all more resistant to oxidative stress than the wild-type[23], suggesting that Elongator plays crucial roles in regulating plant responses to ABA, oxidative stress resistance, and anthocyanin biosynthesis in *Arabidopsis*. Consistently, *abo1*/*elo2*/*elp1* mutant exhibits a drought-resistant phenotype[24]. In summary, *Arabidopsis* Elongator appears to be essential for maintaining hormonal balance, as disruption of Elongator shifts hormonal signaling, resulting in pleiotropic growth and defense phenotypes.

Although some functional studies of Elongator have been performed in *Arabidopsis*, the biological, biochemical, and molecular function of Elongator still need further elucidation, and little is known about its roles in other plant species. Tomato (*Solanum lycopersicum*) is economically one of the most important crop plants. Unlike *Arabidopsis*, the tomato plant yields fleshy fruits and serves as another excellent model plant. In our present study, a tomato Elongator subunit 2-like gene, here designated as *SlELP2L*, was isolated and its expression profiles under various phytohormones, and the tissue-specific expression were investigated. Silencing *SlELP2L* in tomato leads to reduced leaf growth, accelerated senescence of leaves and sepals, and dark-green fruits with increased chlorophyll content and reduced carotenoid accumulation, and down-regulation of ethylene- and ripening-associated genes, while the expression of DNA methyltransferase genes was up-regulated. Besides, the transgenic plants are hypersensitive to ABA during seedling growth. These results suggest that *SlELP2L* plays an important role in tomato growth and development, as well as in ABA signaling. Our results also suggest that *SlELP2L* in tomato plays a distinct role compared with in *Arabidopsis*, indicating that Elongator has functional diversity in different species.

Results

Cloning and tissue expression pattern analysis of *SlELP2L* gene. In order to explore the potential functions of Elongator in tomato, we isolated a putative ELP gene (Elongator complex protein 2-like) from wild-type tomato fruits based on a cDNA clone (GenBank accession no. XM_004240442), here we named it *SlELP2L*. Nucleotide sequence analysis revealed that *SlELP2L* was consistent with the cDNA clone, and contained an open reading frame (ORF) of 2190 nucleotides and encoded 729 amino acid residues with an estimated molecular mass of 80.4 kDa. The search of conserved structure domains showed that the location of 531–671 of SlELP2L protein are WD-repeats region, and its theoretical isoelectric point is 6.2. The sequence alignment conducted by DNAMAN 5.2.2 program showed that tomato SlELP2L shared 64.2% identity with the *Arabidopsis* ELP2 (AT1G49540) protein at the amino acid level.

To extend our understanding of the role of *SlELP2L* in tomato growth and development, we examined its expression patterns in a wide range of tomato organs, including vegetative tissues such as young, mature and senescent leaves, stems and roots, and reproductive tissues such as sepals, flowers and fruits at different stages of development. Quantitative RT-PCR analysis showed that *SlELP2L* was constitutively expressed at high levels in most of organs we examined, but was expressed at relatively low levels in flowers (Fig. 1). Previous reports have demonstrated that a number of Elongators are involved in regulating the essential steps in *Arabidopsis* growth and development[25]. The observation of high and constant *SlELP2L* expression in tomato suggests that *SlELP2L* may have a specialized function in growth or reproductive development of tomato plant.

Expression profiles of *SlELP2L* gene under exogenous hormone treatments. In plants, growth and form are determined by the temporal and spatial regulation of cell division and cell expansion in which plant hormones play a crucial role. To determine whether *SlELP2L* is involved in phytohormone response, WT tomato seedlings were treated with different hormones. Quantitative RT-PCR analysis showed that the transcripts of *SlELP2L* mRNA remained unchanged at the beginning, while increased slightly at 24 h after ABA treatment (Fig. 2A). Whereas the expression of *SlELP2L* was inhibited by gibberellin acid (GA_3) treatment (Fig. 2B). In addition, Fig. 2C showed that *SlELP2L* transcripts were also induced by ACC (the ethylene precursor) treatment. So far, many cis-elements have been reported for their critical roles in determining the hormone-responsive expression profiles of plant genes. Statistical analysis showed that there are multiple hormone-related cis-elements enriched in the promoter of *SlELP2L* gene, including ABA, GA and ethylene (Table S1). The existence of these cis-elements indicates that they likely have potential functions, but this is not always the case (For example, although there are GA-responsive cis-elements in *SlELP2L* promoter region, it is not induced by GA). The results suggest that *SlELP2L* may play a role in ABA and ACC response and signal transduction.

Silencing *SlELP2L* inhibits leaf growth and accelerates leaf senescence. To explore the physiological role of *SlELP2L* in greater depth, we obtained 11 independent *SlELP2L*-silenced lines by RNAi. To verify the repression of *SlELP2L* in the six transgenic lines which exhibited

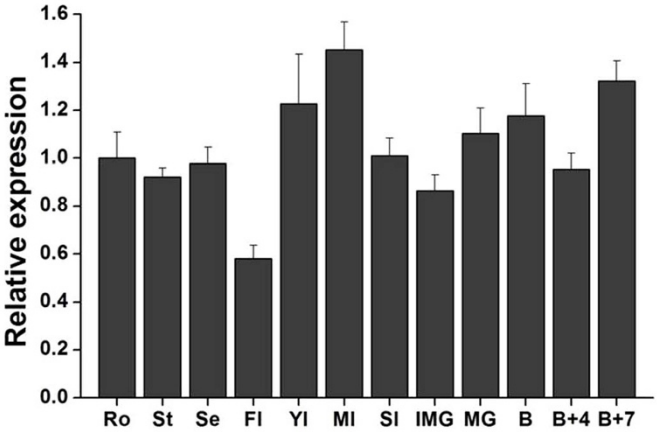

Figure 1 | Expression patterns of *SlELP2L* gene in different tissues and during fruit development in wild-type tomato. Ro, roots; St, stems; Se, sepals; Fl, flowers; Yl, young leaves; Ml, mature leaves; Sl, senescent leaves; IMG, immature green; MG, mature green; B, breaker; B+4, 4 day after breaker stage; B+7, 7 day after breaker stage. The expression data of root were normalized to 1. Each value represents the mean ± SD of three replicates.

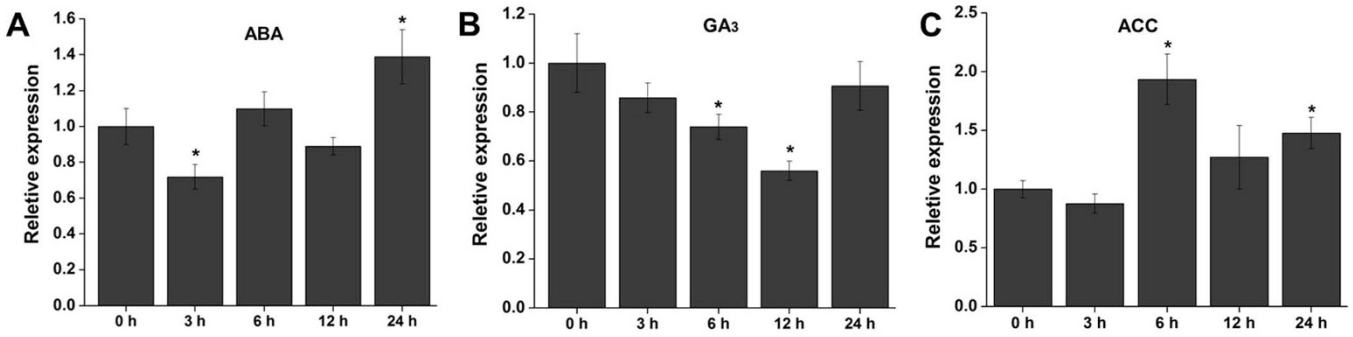

Figure 2 | Quantitative RT-PCR analysis of the expression of *SlELP2L* gene in leaves under ABA (A), GA_3 (B) and ACC (C) treatments . The relative expression levels were normalized to 1 in unstressed plant (0 h). Each value represents the mean ± SD of three replicates. The asterisks indicate statistically significant differences between the unstressed plants and treated plants ($P < 0.05$).

distinguishable alterations from WT tomato, total RNA was isolated from young leaves in WT and transgenic plants. Quantitative RT-PCR analysis showed that the accumulation of *SlELP2L* transcripts in lines 1 and 8 was significantly reduced to roughly 6–8% of control levels, while only about 70–85% of transcript accumulation was repressed in the other lines (Fig. 3A). Thus lines 1 and 8 were selected for further characterization. To verify specific repression of *SlELP2L*, the expression of *SlELP1L* (XM_004239729), *SlELP3L* (XM_004236311), *SlELP4L* (XM_004250120) and *SlELP6L* (XM_004251673) was also monitored. Fig. S1 showed that the expression of these four genes was generally higher or remained unchanged in *SlELP2L*-RNAi lines except *SlELP1L* expression was slightly lower at breaker stage in transgenic fruits than that of WT, suggesting the presence of a regulatory relationship between these genes. The results also indicate that *SlELP2L* mRNA was specifically targeted by the *SlELP2L* RNAi transgene RNA.

Then the seeds of WT and the two T1 *SlELP2L*-silenced transgenic lines were germinated and cultivated concurrently in the greenhouse. The germination rate of transgenic tomato seeds was similar to that of WT tomato under normal conditions (data not shown). About seven weeks later, *SlELP2L*-silenced tomato plants showed a visible pleiotropic phenotype in leaves, such as reduced leaf rachis length and rapid senescence of tomato leaves (Fig. 3B). The compound leaves were collected from WT and *SlELP2L*-silenced tomato plants at the same nodes. Fig. 3C showed that the leaves from WT plants had longer leaf rachis length than that of *SlELP2L*-silenced tomato plants. This dwarf phenotype is usually observed in gibberellin disordered plants. GA has a crucial role in regulating internode length and leaf size, and the latter is more sensitive to GA content modification in tomato[26]. Besides, indole-3-acetic acid (IAA), the main auxin in higher plants, also has profound effects on plant growth and development, including embryogenesis, seedling growth, and flower development[27]. In order to explore the underlying reasons for the smaller shapes of transgenic plants, endogenous GA and IAA contents were determined in the leaves of WT and *SlELP2L*-silenced plants using an enzyme-linked immunosorbent assay (ELISA). The results showed that these two plant hormone contents were significantly decreased in transgenic plants (Fig. 3D, E).

Besides, about three months later, the lower leaves of WT plants remained green, while the leaves of *SlELP2L*-silenced tomato plants showed yellowing and senescence (Fig. 3F). To gain further information on the accelerated senescence phenotype observed in transgenic plants, the transcription of small subunit of ribulose-1, 5-biphosphate carboxylase-oxygenase (Rubisco) gene (*Rbcs-2*) was determined in tomato leaves at different stages of senescence. Rubisco initiates the pathways of photosynthetic carbon reduction and photorespiratory carbon oxidation, plays a decisive impact on net photosynthetic rate[28]. Fig. 3G showed that *Rbcs-2* mRNA was highly expressed in fully expanded mature leaves of WT plants, then declined gradually during leaf senescence. Whereas *Rbcs-2* expression in *SlELP2L*-silenced lines was markedly decreased at all three stages detected compared with that of WT, indicating that the photosynthetic rate may also be reduced in *SlELP2L*-silenced lines. In addition, Elongator complex has been reported to have a functional connection with the Urm1p, an ubiquitin-like protein (UBLs), suggesting a potential relationship between Elongator and protein ubiquitination process[29]. RCHY1 (ring finger and CHY zinc finger domain-containing 1), as a member of the RING finger family of E3 ubiquitin ligases can facilitate protein degradation via the ubiquitin-proteasome pathway[30]. Quantitative RT-PCR analysis showed that *RCHY1* expression was increased significantly in the early senescence of leaves (less than 10% yellowing) of *SlELP2L*-silenced lines (Fig. 3H), indicating that the increase of protein ubiquitination may trigger a rapid senescence of *SlELP2L*-silenced transgenic tomato leaves.

Silencing *SlELP2L* produces dark-green fruits and accelerates sepal senescence. Visible alterations were displayed during the process of tomato fruit development and ripening, it is interesting to note that *SlELP2L*-silenced transgenic fruits showed darker green phenotype from IMG to breaker stage compared with WT (Fig. 4A–C). However, no significant difference was observed at B+4 and B+7 stages. In view of the color changes, the time from anthesis to breaker stage was measured, we found that there is no significant difference in the ripening time between WT and *SlELP2L*-silenced fruits (Table 1). Besides, another striking phenotype was observed in *SlELP2L*-RNAi fruits. The sepals of transgenic fruits began to lose their chlorophyll apparently and accelerated senescence was observed from B+4 stage, while WT sepals were still green (Fig. 4A, C). We further measured the time from breaker stage to sepal senescence (characterized as the first appearance of yellowing in sepals), and observed that the senescence time of *SlELP2L*-silenced sepals was about six days in advance compared with that of WT (Table 1), indicating that the silencing of *SlELP2L* in tomato leads to accelerated sepal senescence.

The typical features of green-to-red color change in ripening tomato fruit is primarily because of chlorophyll degradation and carotenoid accumulation[31]. To determine whether or not the dark-green phenotype represented a change in total chlorophyll and carotenoid contents between WT and *SlELP2L*-silenced fruits, total chlorophyll and carotenoid were extracted from pericarps. The results showed that *SlELP2L*-silenced fruits had higher levels of chlorophyll content compared with the WT fruits at mature green (MG) stage as expected (Fig. 5A), which is responsible for the dark-green coloration of transgenic fruits. The total carotenoid content in B+4 stage fruits was also determined. Fig. 5B showed that *SlELP2L*-silenced fruits exhibited a slightly reduced carotenoid concentration, this slight reduction in transgenic fruits was not reflected apparently

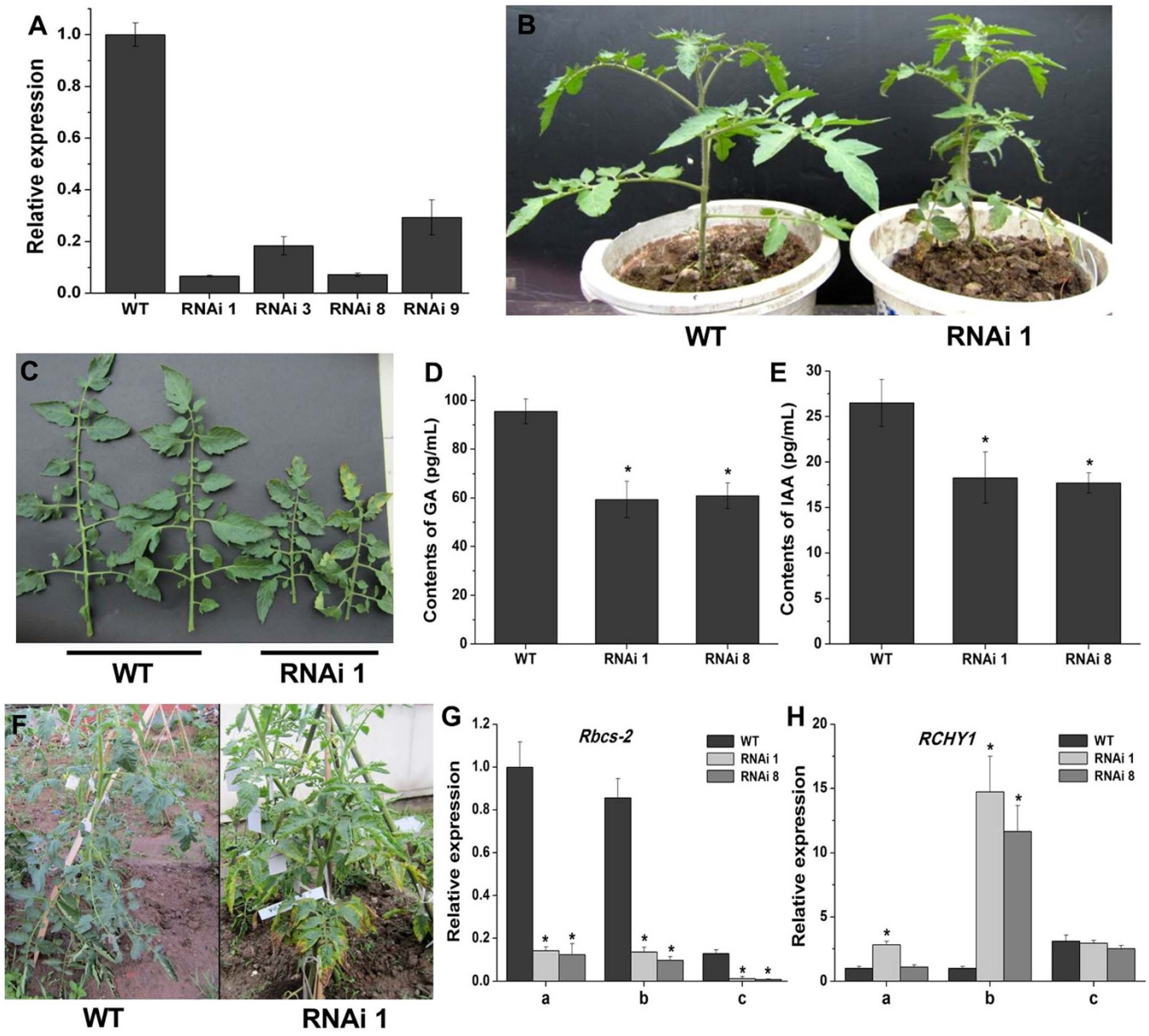

Figure 3 | *SlELP2L* gene expression analysis and growth phenotype of *SlELP2L* transgenic plants. (A). Relative expression profiles of *SlELP2L* between WT and *SlELP2L*-RNAi lines. The expression data of WT plants were normalized to 1. Each value represents the mean ± SD of three replicates. (B). Growth characteristics of WT and transgenic tomato plants. Seeds of WT and T1 generation *SlELP2L*-RNAi tomato were sowed for one week, then transplanted into pots, picture was taken for the representative plants after seven weeks. (C). Leaves obtained from the same node of WT and *SlELP2L*-RNAi plants. D and E. Comparisons of GA content (D) and IAA content (E) in the leaves of WT and transgenic plants. Each value represents the mean ± SD of three replicates. Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines. (F). Growth characteristics of WT and transgenic tomato plants grown for three months in the greenhouse. G and H. Relative expression profiles of *Rbcs-2* (G) and *RCHY1* (H) in the leaves of different stages of senescence between WT and *SlELP2L*-RNAi plants. a, mature leaves (no visible senescence); b, early senescing leaves (<10% yellowing); c, senescent leaves (about 30% yellowing). The expression data of mature leaves were normalized to 1. Each value represents the mean ± SD of three replicates. Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines.

in the phenotype (Fig. 4A, C). *SlELP2L* thus may also affect the accumulation of pigments in fruits.

Ethylene- and ripening-related genes were down-regulated in *SlELP2L*-silenced fruits. The synthesis, perception, and signal transduction of ethylene is essential for the coordination and completion of fruit ripening[32], and ethylene also regulates the accumulation of carotenoid during ripening[33]. Currently, two systems of ethylene regulation have been proposed to operate in climacteric fruit. System-1 is responsible for producing the basal levels of ethylene detectable in all tissues. Whereas system-2 is autostimulatory during the climacteric fruit ripening and petal senescence, and requires the induction of both of *ACS* (ACC synthase) and *ACO* (ACC oxidase) genes, which catalyze the rate limiting and final steps in ethylene biosynthesis, respectively[34]. The dark-green fruits and slightly reduced content of carotenoid in ripe fruit were observed in transgenic tomato (Fig. 4, 5). To further characterize the potential molecular mechanisms of *SlELP2L* in fruit ripening, the transcript levels of ethylene-related genes were examined from MG to B+7 stage between WT and transgenic tomato fruits. Fig. 6 showed that the expression of *ACS2* was inhibited by about half mainly at breaker and B+4 stages, while

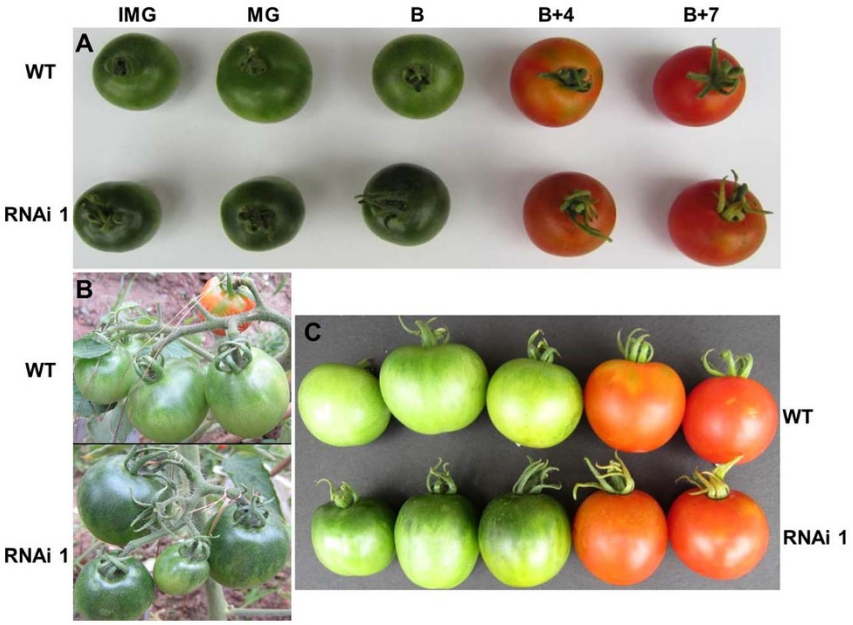

Figure 4 | Phenotypes of fruits and sepals in WT and *SlELP2L*-RNAi lines. (A). Representative fruits and sepals from WT and transgenic tomato. IMG, immature green; MG, mature green; B, breaker; B+4, 4 day after breaker stage; B+7, 7 day after breaker stage. (B). Color characterization of WT and *SlELP2L*-RNAi fruits on the plants. (C). Phenotypic characteristics of WT and *SlELP2L* transgenic fruits corresponding with Fig. 4A.

ACS4 was down-regulated by about two thirds at almost all of the stages tested in *SlELP2L*-silenced lines. *ACO1* was decreased by about two thirds mainly at B+4 stage, and the most significant repression (about 75–85%) of *ACO3* expression was observed at breaker and B+7 stages. Furthermore, the expression of three known ethylene-regulated ripening-related genes was also detected between WT and transgenic fruits. The expression of *RIN*, which encodes a MADS-box protein necessary for the onset and completion of fruit ripening[35], was reduced by about one third in *SlELP2L*-silenced fruits mainly at B+4 and B+7 stages compared with that of WT fruits (Fig. 6). *NOR*, a NAC transcription factor gene necessary for fruit ripening[36], its expression was inhibited by about half at B+4 stage. For *E8*, which responds specifically to ethylene[37], its transcript was decreased by about half in *SlELP2L*-silenced fruits at almost all of the stages detected (Fig. 6). These results suggest that the silencing of *SlELP2L* may suppress the expression of these ethylene- and ripening-related genes which are normally induced during ripening.

Given that fruit ripening is correlated with the occur of cell wall alterations[38], thus the expression of four ripening-related cell wall metabolism genes, *PG* (polygalacturonase)[39], *PE1* (pectineaterase)[40], *CEL2* (endo-beta-1,4-glucanase)[41], and *XTH5* (Xyloglucan endo-transglucosylase/hydrolase)[42] was also detected between WT and transgenic tomato fruits from breaker to B+7 stage. Fig. 7 showed that the expression of *PG* was inhibited by about two thirds at B+4 stage, while *PE1* expression was decreased by about half at breaker stage. For *CEL2*, its expression was down-regulated significantly at all of the stages tested in *SlELP2L*-silenced lines, while 50–70% inhibition of *XTH5* expression was observed at B+4 and B+7 stages. Consistent with the down-regulated expression of ethylene- and ripening-related genes, these results further suggest that *SlELP2L* may affect the process of tomato fruit ripening.

Expression analysis of tomato DNA methyltransferase genes. In eukaryotic nuclei, DNA is tightly wrapped around octamers of histones to form the chromatin, and chromatin organisation is crucial for the control of gene expression and other nuclear processes. Changes in chromatin structure are determined by protein complexes involved in the ATP-dependent repositioning of nucleosomes, in histone posttranslational modifications, and in the methylation of cytosine residues[43,44]. In *Arabidopsis*, ELP2 has been shown to be required for pathogen-induced dynamic DNA methylation changes in defense related genes. The *elp2* mutation alters (increases or decreases) methylation levels of specific methylcytosines[45]. To investigate whether SlELP2L also modulates DNA methylation in tomato, the transcript levels of genes encoding DNA methyltransferases were examined from MG to B+7 stage between WT and transgenic lines. Consistent with the results reported previously[45], the expression of *SlDRM7*, *SlDRM8*, and *SlMET1* genes[43] was higher in *SlELP2L*-silenced lines than in the WT (Fig. 8), indicating increased methylation levels. Among which, the expression of *SlDRM7* and *SlDRM8* in *SlELP2L*-silenced lines was about twice as WT. *SlMET1* expression was only slightly up-regulated in transgenic lines, while there is no obvious change was detected in *SlDRM5* expression. These results indicate that SlELP2L may regulate gene expression through DNA methylation.

***SlELP2L*-silenced tomato was hypersensitive to ABA in seedling growth.** Previous reports showed that Elongator mutants are

Table 1 | Days from anthesis to breaker of fruits and days from breaker of fruits to sepals show senescence between wild-type and *SlELP2L*-RNAi fruits. Values represent the means ± SD in days for at least 15 fruits of each line

WT/RNAi fruits	Days from anthesis to breaker of fruits	Days from breaker of fruits to sepals show senescence
WT	42 ± 3.2	11 ± 2.1
RNAi 1	42 ± 2.1	4 ± 1.3
RNAi 8	41 ± 4.2	5 ± 1.1

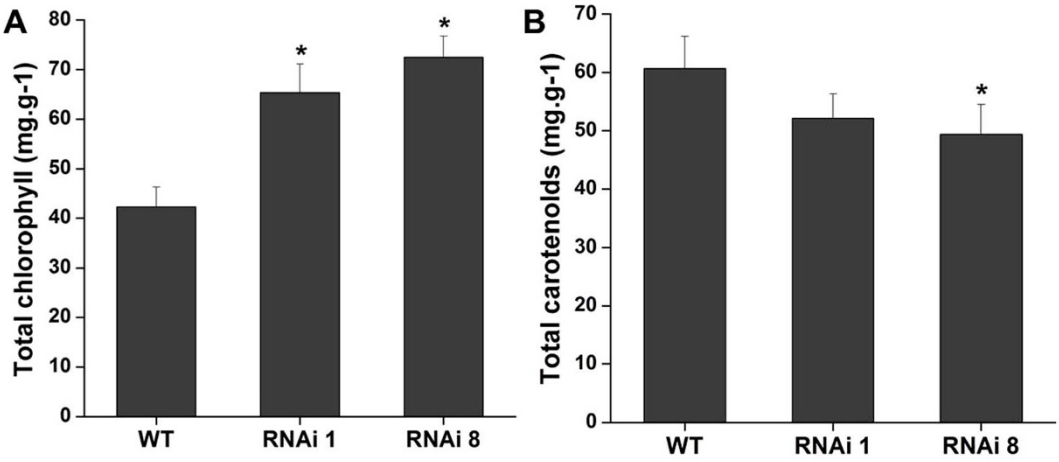

Figure 5 | Chlorophyll and carotenoid accumulation between WT and *SlELP2L*-RNAi fruits in pericarps. (A). Pericarp chlorophyll content in mature green (MG) fruits. (B). Total carotenoid accumulation in red ripe fruits of B+4 (4 day after breaker stage). Biological replicates (2–3 fruits per fruit ripening stage) were performed in triplicate, and the data are presented as mean ± SD. Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines.

hypersensitive to ABA, suggesting that they are involved in ABA signaling[23,24]. We showed that the transcripts of *SlELP2L* mRNA were induced slightly by ABA treatment (Fig. 2A). To further investigate the effect of ABA on tomato seedling growth, WT and transgenic seeds of consistent germination were transferred to MS medium containing 0 and 10 μM ABA for 7 days. As shown in Fig. 9A, no discernible difference in the length of shoot and root between WT and *SlELP2L*-RNAi seedlings was observed when grown in the control medium. Addition of 10 μM ABA to the incubation medium, the seedling growth was inhibited in both WT and transgenic tomato, while stronger inhibition was observed in transgenic seedlings (Fig. 9A). Then the length of roots was measured. Fig. 9B showed that the root length of transgenic seedlings was reduced more significantly than that of WT under ABA treatment, indicating that the silencing of *SlELP2L* causes a obvious increase in ABA sensitivity during seedling growth. We further detected the content of endogenous ABA in the leaves of WT and *SlELP2L*-silenced plants using an ELISA. The results show that ABA content was increased in transgenic plants (Fig. 9C).

Discussion

The multi-subunit complex Elongator has been reported to be involved in diverse biological processes in yeast, human and *Arabidopsis*, including exocytosis, cell proliferation, cell differentiation, embryogenesis, and response to biotic and abiotic stress[4,8,19,21,25,46]. The *Arabidopsis* Elongator mutants have narrow and elongated leaves, decreased primary root growth, disorganized shoot apical meristem, aberrant inflorescence and flower architecture, reduced germination frequency and delayed seedling growth[21,22,25]. Leaf growth is a complex developmental process controlled by genetic and environmental factors, and is determined by a proliferation, expansion and maturation phase[47]. Leaves of *Arabidopsis elo* mutants have larger and fewer cells, suggesting that the cell division rate of these leaves is reduced, which may be responsible for the reduced plant growth[21]. Mutations in Elongator subunits also result in aberrant DNA replication and increased DNA damage in plant cell cycle progression, and alter cell division affecting leaf polarity formation[48]. Here, we explored the function of tomato *SlELP2L* by RNAi-mediated gene silencing. Similar to the reports

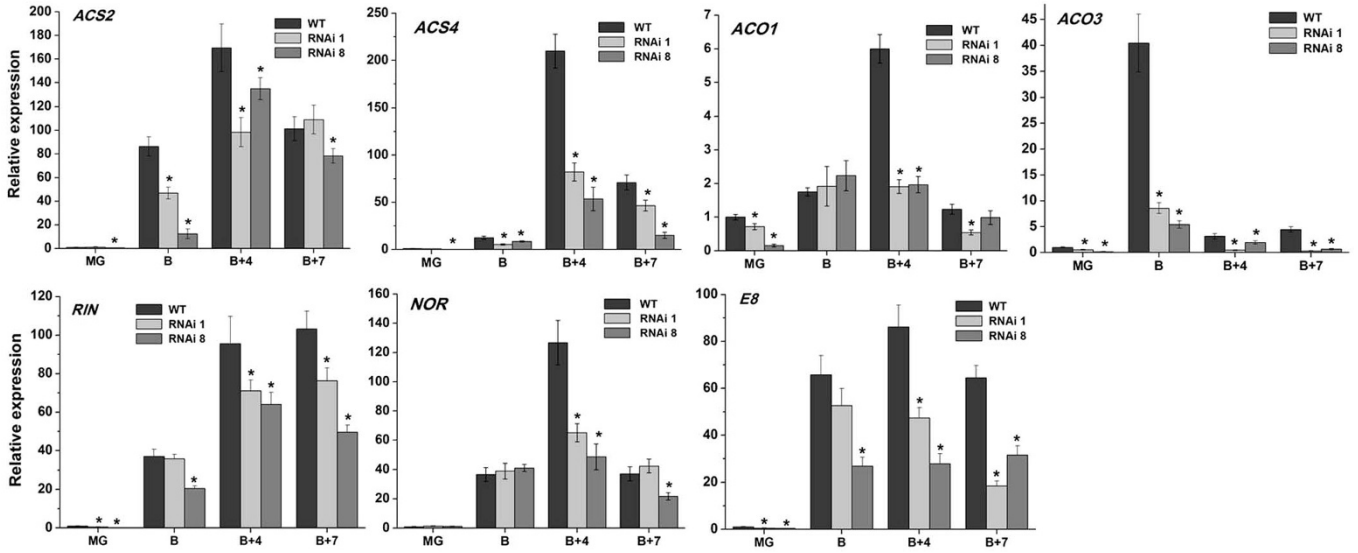

Figure 6 | Relative expression profiles of ethylene-related and ripening-related genes in pericarps between WT and *SlELP2L*-RNAi fruits. MG, mature green; B, breaker; B+4, 4 day after breaker stage; B+7, 7 day after breaker stage. The expression data of mature green (MG) fruit were normalized to 1. Each value represents the mean ± SD of three replicates. Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines.

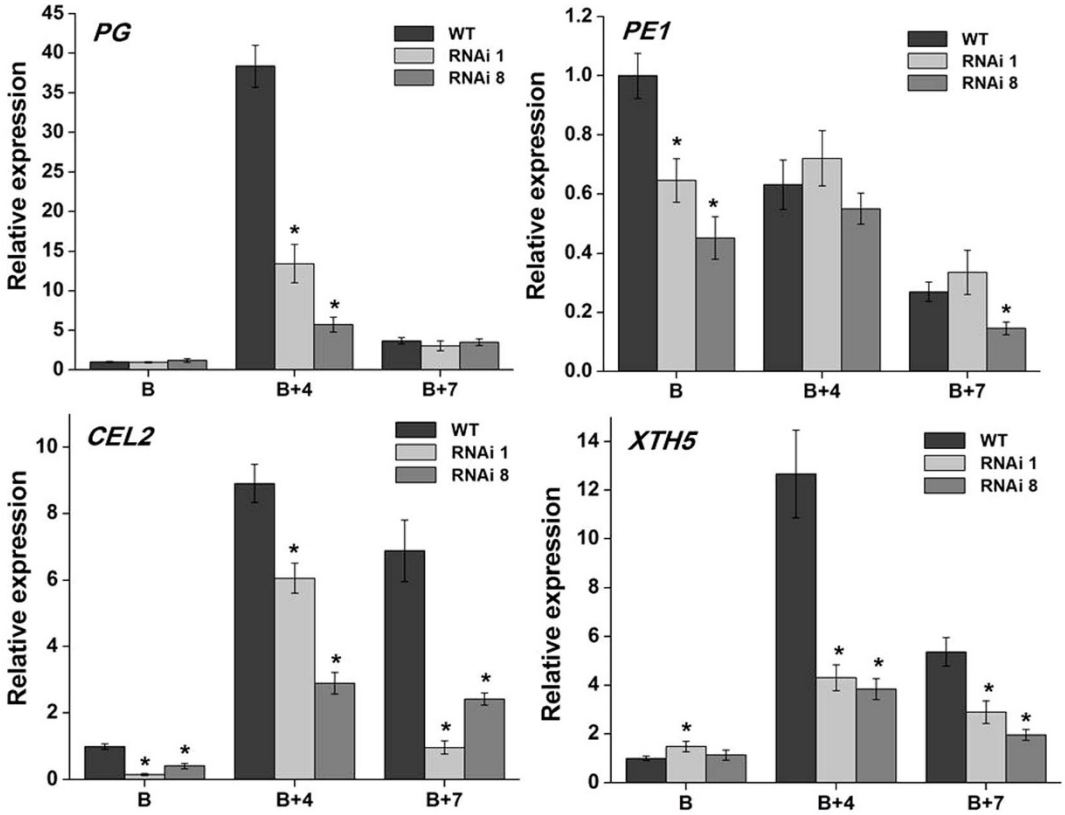

Figure 7 | Relative expression profiles of cell wall metabolism genes in pericarps between WT and *SlELP2L*-RNAi fruits. B, breaker; B+4, 4 day after breaker stage; B+7, 7 day after breaker stage. The expression data of breaker fruits were normalized to 1. Each value represents the mean ± SD of three replicates. Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines.

in *Arabidopsis*, the silencing of *SlELP2L* in tomato also causes pleiotropic phenotypes, such as delayed seedling growth, rapidly senescing leaves and sepals, dark-green fruits, and hypersensitivity to ABA (Fig. 3, Fig. 4, Fig. 9), suggesting *SlELP2L* plays an important role in diverse developmental processes. It was reported that Elongator modulated crosstalks of several hormones like auxin, jasmonic acid, ethylene and ABA, appearing to be essential to maintain hormonal balance, as disruption of Elongator shifts hormonal signaling, resulting in pleiotropic growth and defense phenotypes[25]. In our present study, reduced GA and IAA contents were detected in *SlELP2L*-RNAi plants (Fig. 3). GA has a crucial role in regulating internode length and leaf size[26], and IAA has profound effects on plant growth and development, including seedling growth[27]. Thus possible hormonal misregulation might contribute to the pleiotropic phenotypes of *SlELP2L*-silenced plants.

RNAi repression of *SlELP2L* resulted in elevated mRNA accumulation of *SlELP1L*, *SlELP3L*, *SlELP4L* and *SlELP6L* (Fig. S1). Thus *SlELP2L* and the other tomato Elongator subunits expression appears a certain degree of functional compensation. This is consistent with that the disruption of each Elongator subunit (ELP1–ELP6) results in similar phenotypes in *Arabidopsis*, suggesting that plant Elongator subunits work as a complete complex and share common functions[21,23]. Although *SlELP2L* is highly homologous to *Arabidopsis ELP2*, distinct phenotypes were observed between *Arabidopsis elp2* mutant and *SlELP2L*-silenced tomato plants, such as reduced leaf rachis length, rapid senescence of leaves and sepals, and dark-green fruits (Fig. 3, Fig. 4), indicating the diverse functions of Elongator in different organisms. Similar alteration of fruit coloration phenotype was observed in the light-hypersensitive mutants dark green (*dg*) and high pigment 1 and 2 (*hp1* and *hp2*) in tomato characterized by their exaggerated light responsiveness. *dg* is an alternative allele at the *HP-2* locus, identified as the tomato *DET1* gene which involves in the light signal transduction and morphogenesis[49,50]. DET1 was also reported to bind the amino-terminal tail of histone H2B[51]. Besides, *Arabidopsis* histone acetyltransferase TAF1/HAF2, GCN5 and histone deacetylase HD1/HDA19 are necessary for the light regulation of plant development and gene expression[52]. These evidences establish a potential connection between histone modification and light signal transduction in plants. Furthermore, Elongator plays a vital role in transcriptional elongation via histone and α-tubulin acetylation[3–5]. Therefore, the silencing of *SlELP2L* in tomato may lead to abnormal histone modification, and thus affects the light signaling pathway, which further affects fruit development and coloration, and results in dark-green fruits.

Elongator was first isolated together with RNAPII, and the studies in yeast and humans suggest that one of Elongator's functions is to facilitate mRNA transcription elongation during transcription by RNA polymerase II[2,23,53], thus the mutations in Elongator would decrease the transcript levels of their target mRNAs. In yeast and *Arabidopsis*, Elongator subunit deletions or mutations lead to both up- and down-regulated gene expression[23,54]. In this study, we found that repressed *SlELP2L* reduces the expression of *Rbcs-2* (Fig. 3G), ethylene-related genes (*ACS2*, *ACS4*, *ACO1* and *ACO3*), and ripening-related genes (*E8*, *NOR* and *RIN*) (Fig. 6). Besides, the expression of ripening-related cell wall metabolism genes, *PG*, *PE1*, *CEL2* and *XTH5* was also down-regulated (Fig. 7). We also found, however, that the expression of *RCHY1* was upregulated in *SlELP2L*-RNAi plants (Fig. 3H). To date it has been well documented that ethylene plays a key regulatory role in fruit ripening[55], and most ripening-deficient mutants fruit characterized are the consequence of defects in carotenoid biosynthesis[56]. In addition, reduced expression of *SlELP2L* also results in reduced carotenoid content (Fig. 5B). Because *SlELP2L* may be involved in the mRNA elongation process, *SlELP2L* may not directly participate in the regulation of tomato

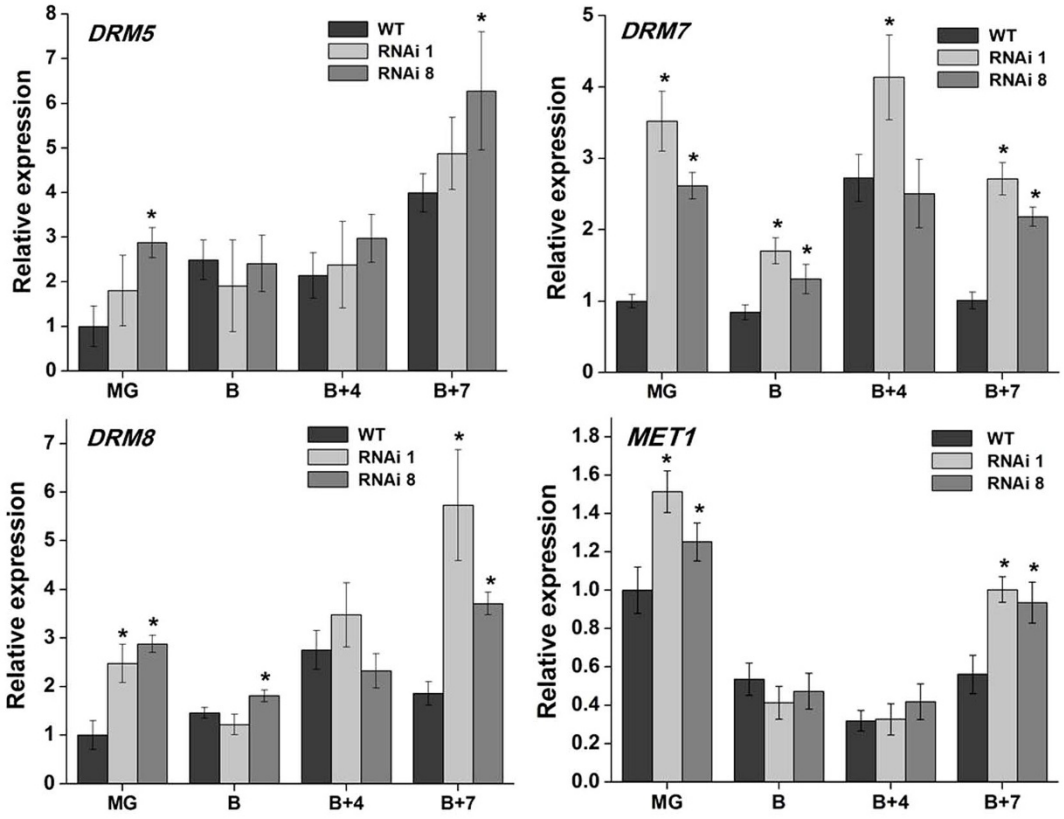

Figure 8 | Relative expression profiles of DNA methyltransferase genes in pericarps between WT and *SlELP2L*-RNAi fruits. MG, mature green; B, breaker; B+4, 4 day after breaker stage; B+7, 7 day after breaker stage. The expression data of mature green (MG) fruits were normalized to 1. Each value represents the mean ± SD of three replicates. Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines.

growth and development, but by regulating another gene (s) that plays a role in plant growth and development. Besides, the higher transcript levels of DNA methyltransferase genes, *SlDRM7*, *SlDRM8* and *SlMET1*, were observed in *SlELP2L*-silenced lines than that of WT (Fig. 8), suggesting that SlELP2L may regulate gene expression through DNA methylation, thereby affecting the expression of related genes. Together, these results indicate that SlELP2L may modulate DNA methylation in tomato, and the silencing of *SlELP2L* in tomato affects fruit ripening possible via regulating the expression of ethylene- and ripening-related genes. Besides, consistent with the role of *Rbcs-2* and *RCHY1*, the *SlELP2L*-RNAi plants expressed reduced *Rbcs-2* and improved *RCHY1* may partially explain the phenotypes of accelerated senescence of leaves and sepals.

Recent studies have suggested that mRNA metabolism is closely related to ABA signal transduction. For example, an *ABH1* (ABA-hypersensitive 1) mutation encodes an mRNA capbinding protein which, together with CBP20, forms a heterodimeric nuclear cap-binding complex. Mutations in either *ABH1* or *CBP20* render plants hypersensitive to ABA in seed germination and seedling growth[57,58]. Other important components, such as transcription factors and those in the ABA signaling pathway can also affect the sensitivity to ABA[59,60]. The plant hormone ABA is involved in regulating many

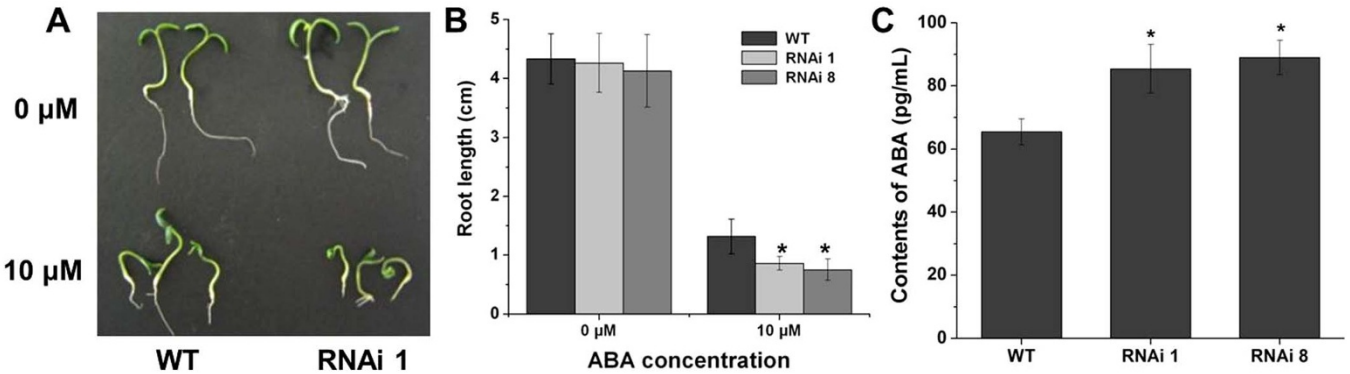

Figure 9 | Seedling growth of *SlELP2L*-RNAi lines was hypersensitive to ABA. A and B. Representative growth performance (A) and root length (B) of WT and transgenic seedlings. WT and *SlELP2L* transgenic tomato seeds were surface-sterilized and germinated in moist and disinfectant filter papers for three days, then the seeds of consistent germination (n = 20 each) were transferred to MS medium containing 0 and 10 μM ABA. Seedlings were photographed seven days after transfer. (C). ABA content in the leaves of WT and *SlELP2L* transgenic plants. Each value represents the mean ± SD of three replicates. Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines.

aspects of plant growth and development, such as seed dormancy, seed germination, seedling growth, and plays important roles in plant responses to environmental stresses. ABA also has a role in inducing leaf abscission, flower and leaf senescence[23]. Interestingly, we found that *SlELP2L*-RNAi plants had greater sensitivity to ABA than the WT plants during seedling growth (Fig. 9). Similarly, it has been shown that all the four Elongator mutants (*abo1*/*elo2*/*elp1*, *elp2*, *elo3*/*elp3*, and *elo1*/*elp4*) in *Arabidopsis* exhibit ABA hypersensitivity in seed germination and seedling growth, suggesting important roles for Elongator in ABA signaling[23]. Transcriptional elongation mediated by RNA polymerase II is a critical process in gene regulation, and is highly regulated in eukaryotes by numerous factors in mRNA biogenesis and maturation[61]. Together, these results suggest that plant responses to hormone ABA can be regulated by different molecular mechanisms and at different stages during mRNA processing, including mRNA metabolism, transcriptional initiation, transcriptional elongation and protein modifications[23,59,60,62].

In conclusion, this study concerned the morphological, physiological and molecular features of *SlELP2L*-RNAi transgenic tomato. Presently, the functional studies of Elongator in plants are mainly limited in the model plant *Arabidopsis*, and the specific biological functions of most plant Elongator still remain to be elucidated. Clearly, understanding the role of *SlELP2L* will not only extend knowledge of the biological function of Elongator, but also provide insight into exploring further the significance of Elongator in regulating plant growth and development, as well as in responses to ABA. Besides, previous reports indicated that mutants of different subunits of Elongator complex exhibit similar phenotypes in yeast, humans, and plants[18,54,63], while whether other subunits of tomato Elongator complex have similar functions with *SlELP2L* still remain to be further elucidated.

Methods

Plant materials and growth conditions. The seeds of wild-type (WT) tomato (*Solanum lycopersicum* Mill. cv. Ailsa Craig) and *SlELP2L*-RNAi transgenic tomato (T1) were germinated, and the seedlings were grown in a greenhouse under following conditions: 16/8 h day/night cycle, 25/18°C day/night temperature and 80% humidity. All the plants were watered daily. For organ-specific expression profiling of *SlELP2L*, the tissues of roots, stems, leaves, sepals, flowers, and fruits of different period from tomato plant with ripe fruits were collected. Flowers were tagged at anthesis and fruit development recorded as days post-anthesis (DPA). Immature green (IMG) fruits were defined as 28 DPA. Mature green (MG) fruits were defined as 35 DPA and characterized as full fruit expansion and shiny with no obvious mature-related color change. Breaker (B) were defined as fruit emerging the first clearly orange. Subsequent ripening stages were defined in days post-breaker: B+4 (4 day after breaker), and B+7 (7 day after breaker). All plant materials for total RNA extracting were taken in the same time each day, and frozen in liquid nitrogen and stored at −80°C until needed.

Plant hormone treatments. To verify the expression profiles of *SlELP2L* under phytohormone treatments, the WT tomato seeds were germinated and the seedlings were grown in greenhouses. Then three-week old WT tomato seedlings were treated with 100 μM ABA, 50 μM GA_3, and 100 μM ACC, respectively[64]. Plants were enclosed in plastic immediately following spraying and left for 3, 6, 12 and 24 h. Individual seedlings were used in each treatment with three biological replicates. Unstressed seedlings were used as control. All the samples were collected at certain time points and frozen in liquid nitrogen for Quantitative RT-PCR analysis.

ABA sensitivity experiment was performed as follows: WT and transgenic tomato seeds were germinated according to the above procedure, then the seeds of consistent germination were selected and transferred to MS medium containing 0 and 10 μM ABA. Culture vessels containing the seeds were incubated in a growth chamber for 7 d, then seedlings were taken picture and root length was measured.

***SlELP2L* isolation and sequence analysis.** The primers for cloning the coding region of *SlELP2L* were as follows: Forward, 5' TACACTGGCTGGTCATAAAG 3' and Reverse, 5' GACAAGGCTAAAGCAAACAT 3'. The PCR procedure was incubated at 94°C for 5 min followed by 35 cycles of 30 s at 94°C, 30 s at 56°C and 2 min at 72°C, and a final extension at 72°C for 10 min. The amplified products were subcloned into pMD18-T vector (Takara, Dalian, China), and confirmed by sequencing. The translation of *SlELP2L* gene using the ExPASy translate tool, grand average of hydropathicity (GRAVY) and theoretical molecular weight (Mw) were calculated using the ExPASy ProtParam tool (http://www.expasy.org/). Sequence alignment was conducted using the DNAMAN 5.2.2 program. Conserved structure domains were annotated according to ScanProsite (http://prosite.expasy.org/scanprosite/). To analyze putative cis-elements in the promoter region of *SlELP2L* gene, promoter sequence (1000 bp regions upstream the 5′ end of the predicted ORF) of *SlELP2L* gene was extracted from SGN database and searched against the promoter database PLACE (http://www.dna.affrc.go.jp/PLACE/index.html)[65].

Quantitative RT-PCR analysis. Total RNA was isolated using Trizol (Invitrogen, Shanghai, China) according to the manufacturer's instructions and was treated with the Dnase-I (Promega, Beijing, China) for 30 min at 25°C. The first strand cDNA synthesis was performed using M-MLV reverse transcriptase (Promega, Beijing, China) with oligo $(dT)_{18}$ primer. Quantitative RT-PCR was carried out using the CFX96™ Real-Time System (Bio-Rad, USA). All reactions were performed using the SsoFast™ EvaGreen® Supermix (TaKaRa, Dalian, China) according to the manufacturer's recommended protocol. Quantitative RT-PCR reactions were performed using a two-step method: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, and 60°C for 30 s. For each gene analysis, NTC (no template control) were also performed. Tomato *CAC* gene was selected as internal standard for organ-specific expression studies[66], and tomato *EFα1* gene was used as internal control under hormone treatments[67]. The analysis of relative gene expression levels were detected using the $2^{-\Delta\Delta C}{}_T$ method[68]. All Quantitative RT-PCR experiments were taken in three biological replicates and each reaction was run in triplicate. Gene-specific primers used for quantitative RT-PCR are listed in Table S2 and a standard curve by serial dilution was analyzed for each specific gene using wild-type tomato cDNA.

RNAi vector construction and plant transformation. A 313 bp *SlELP2L*-specific DNA fragment was amplified from wild-type fruit cDNA using the following primers: Forward, 5' CGGTGGTACCAAGCTTGGAAATCCGTTGGTCGTT 3' with *Kpn* I & *Hin*d III restriction sites, and Reverse, 5' CCGCTCGAGTCTAGATTGAAAG-GTGGAAGTGTC 3' with *Xho* I & *Xba* I restriction sites, respectively. The PCR products were divided into two groups: one group was digested with *Hin*d III & *Xba* I, linked into the pHANNIBAL plasmid at *Hin*d III & *Xba* I restriction sites in the sense orientation, then another group was digested with *Kpn* I & *Xho* I and linked into the above plasmid digested with the same restriction sites in the antisense orientation. After that, the double-stranded (ds) RNA expression unit containing the cauliflower mosaic virus (CaMV) 35S promoter and terminator, was digested with *Sac* I & *Spe* I, and linked into the plant binary vector pBIN 19 to form *SlELP2L*-RNAi vector. Subsequently, this resulted vector was transformed into WT tomato cotyledon explants via Agrobacterium strain LBA4404[69]. Positive transgenic lines were screened for kanamycin (50 mg L^{-1}) resistance. Genomic DNA of transgenic tomato plants was extracted according to the manufacturer's protocol of Genomic DNA Extraction Kit (Invitrogen, Shanghai, China) and was used for detection of the presence of T-DNA by PCR with primers NPTII-F (5' GACAATCGGCTGCTCTGA 3') and NPTII-R (5' AACTCCAGCATGAGATCC 3'). T1 seeds from the selected transgenic lines were germinated on a medium with kanamycin (50 mg L^{-1}) and positive lines were selected for further study.

Extraction and quantification of plant phytohormones. The total endogenous contents of ABA, IAA and GA in the leaves of wild-type and transgenic plants were determined using one-step double-antibody sandwich method enzyme-linked immunosorbent assay (ELISA). For each test, a 0.5 g sample was ground in anice-cooled mortar in 5 mL 80% (v/v) methanolextraction medium, incubated at 4°C for 4 h, then the extracts were centrifuged at 4,000 rpm for 15 min at 4°C, and supernatant was analyzed by ELISA. All of the ELISA experiments were carried out by using the same procedure according to the manufacturer's instructions (http://www.wksubio.com/product/5794959.html). Contents for three kinds of hormones were all measured at 450 nm using a Bio-Rad680 microplate reader. The final results are means of three replicates. The correlation coefficients (R^2) of ABA, IAA and GA standard curves were 0.992, 0.995 and 0.991, respectively. The concentration of each sample was calculated from the linear regression equations.

Fruit chlorophyll and carotenoid determination. Tomato chlorophyll and carotenoid were extracted from pericarp tissues using a modified protocol from Forth and Pyke[70]. A section of fruit pericarp was cut from an area above a locule in a 5 mm wide strip around the equator of fruits, weighed and ground into powder in a mortar with liquid nitrogen, then this powder was transferred into a 50 mL centrifuge tube and extracted with 10 mL of hexane : acetone (60 : 40, v/v). The extract was centrifuged at 4000 rpm for 5 min and the supernatant was carefully transferred to a fresh tube. The cell debris was repeatedly extracted with fresh solvent until colorless and the pooled supernatants volume was measured. The optical absorbance of the supernatants was immediately measured in a lambda 900 scanning spectrophotometer (PerkinElmer). The amount of pericarp chlorophyll and carotenoid contents was calculated by the following equation: total chlorophyll mg mL^{-1} = 8.02 (OD643) + 20.2 (OD647) and total carotenoid mg mL^{-1} = (OD450)/0.25. Individual tissue samples were taken from 3–4 fruits for each ripening stage in triplicate.

Measurement of plant leaf area. The digital image processing method was adopted to measure plant leaf area. The photos of plant leaf were taken, then Photoshop CS5 software was used to count pixels and determine the unit pixel area. The leaf area was calculated by the following equation: Leaf area = number of leaf pixels/the number of pixels per unit area contained.

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Author contributions

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

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