SLGT11 Controls Floral Organ Patterning and Floral Meristem Termination in Tomato

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Abstract

**Background:** Flower development affects fruit production directly in tomato. Despite the framework mediated by ABC genes have been established in Arabidopsis, the spatiotemporal precision of floral development in tomato has not been well examined.

**Results:** Here, we analyzed a novel tomato mutant in which the normal development of stamens and carpels failed, resulting in ectopic formation of floral and shoot apical meristem in the fourth whorl position, which later developed into stem- and leaf-like structures. Using bulked segregant analysis (BSA), we assigned the causal mutation to the gene *SLGT11* that encodes a transcription factor belonging to Trihelix gene family. Further RNAi silencing of *SLGT11* verified the defective phenotypes of *slf* mutant. The failed termination of floral meristem and the occurrence of floral reversion in *slf* mutant indicate that *SLGT11* functions as a non-canonical C type gene. Furthermore, we found that the defects in *slf* were substantially enhanced at higher temperature, with petals transformed into sepals, all stamens disappeared, and increased frequency of ectopic floral meristem.

**Conclusions:** Together with the spatiotemporal expression pattern, we suggest that *SLGT11* functions in floral organ patterning and termination of floral meristem identity in tomato.

**Introduction**

Flowers are reproductive organ of angiosperms which play an important role in reproduction. The initiation and termination of floral meristem, are precise steps to make sure the successful development of the flower, in which a set of transcription factors are spatiotemporally coordinated. In Arabidopsis, the activity of floral meristem is maintained through the WUSCHEL-CLAVATA (WUS-CLV) signaling pathway until carpel primordial is initiated [1]. In later stage of flower development (starting from stage), AGAMOUS (AG) represses WUS through activating KNUCKLES (KNU) directly in floral meristem to ensure the correct progression of further floral development [2, 3]. A large number of regulators such as PAN, ULT1, RBL, SQN, SUP, SEP3, ARF3, MIF and CRC, participate in floral regulation by direct or indirect regulation of WUS activity [4-13]. These regulators form the complex network interplaying with plant hormones during the floral development. It was found that ARF3 is regulated by auxin, AG and AP2 in developing flowers, which represses cytokinin activity to inhibit
WUS [14]. The mechanisms of floral development could also be conserved among species. For example, it was found KNU interacts with MIF to regulate WUS expression and this mechanism is conservative between Arabidopsis and tomato [12].

Flower reversion is an unusual process in which the committed flower development is reverted back to vegetative growth, resulting in outgrowth of leaf or inflorescence structures emerged from the first flower [1]. This phenomenon is often related to varied environmental conditions, such as temperature and photoperiod. For example, such floral reversion was observed in Ify-6 and ag-1 mutants of Arabidopsis grown in short days [15]. Besides over expression of AGL24 also causes ectopic inflorescence development [16]. Floral reversion was also observed in the natural allopolyploid Arabidopsis suecica, in which abnormal expression of floral genes, including AGL24, AP1, SVP and SOC1 was detected [17, 18]. Unlike Arabidopsis, LFY, TFL1 and AG in Impatiens balsamina seemed not to be involved in terminal flowering and floral determinacy [19]. In Petunia hybrida, co-suppression of FBP2, a homolog of Arabidopsis SEPALLATA-like gene, led to new inflorescences growing from axils of carpels [20]. In tomato, down-regulation of TM29 resulted in ectopic leafy stems and flowers formed in fruits [21].

A typical eudicot flower, such as Arabidopsis and tomato, consist of four different organs arranged in four whorls at the tip of floral shoot. Based on genetics studies in model plants including Arabidopsis[22, 23], Antirrhinum majus [24] and Petunia hybrid [25] an elegant model involving ABCDE class genes, namely homeotic genes, has been proposed to explain the organ patterning in floral meristem [22, 23, 25]. In Arabidopsis, B class genes AP3 and PI can form protein complex with C class gene AG and E class gene SEPs to promote stamen development. The carpel formation is regulated by both C class gene AG and E class gene SEPs [26, 27]. Compared to Arabidopsis, tomato genome has more homologous ABCDE genes. In tomato, there are four B class homologous genes (TAP3, TM6, TPI and TPIB) [28-30], two C class homologous genes (TAG1 and TAGL1) [31, 32] and six E class homologous genes (TM5, TM29, J2, EJ2, RIN and Solyc04g005320) [33]. Despite the potential functional redundancy of these homologous genes in tomato, some tomato mutants have been identified including tap3 [30], 7B-1 [34] and tm6 [35] that affect floral development.
Development of stamen and carpel has drawn particular attention as the regulation of these two floral parts is important for breeding. In tomato, stamenless mutants were identified to have mutations in B class genes, affecting stamen and carpel development [36-38]. In other species, stamenless1 (sl1) mutant exhibiting homeotic conversions of lodicules and stamens to palea/lemma like organs and carpels, which were found to resemble the B-class gene spw1 mutant in rice [39]. Recently a mutant of AG gene in Medicago truncatula named AGAMOUS-LIKE FLOWER (AGLF) has been reported. In aglf mutant, the stamens and carpel in the inner whorl were replaced by extra whorls of petals and sepals, resembling the floral phenotype of ag-1 mutant in Arabidopsis [40, 41]. Further evidence supported that AGLF gene transcriptionally activated MtAGs to control floral organ identity. Molecular and genetic analyses also revealed that AGLF regulated the transcription of MtTFL1 to ensure the progression of the correct inflorescence development and inhibit the reversion to floral primordium [42]. But interestingly, the mutation in its Arabidopsis homologous gene AtAGLF (At5g51800) did not affect the development of floral organs [40], indicating that the function of AGLF is not entirely conserved among different species.

Normal floral development is closely related to fruit quality. In searching for genes regulating flower development, we identified a tomato recessive mutant with the mutation in the gene SLGT11 that encodes a transcription factor belonging to Trihelix gene family. We found loss-function of SLGT11 resulted in ectopic formation of stem- and leaf-like structures in the fourth whorl position. The failed termination of floral meristem and the occurrence of floral reversion in slf mutant indicate that SLGT11 functions as a non-canonical C type gene. In addition, high temperature seemed to enhance phenotypes in slf, with petals transformed into sepals, all stamens were disappeared, and increased frequency of ectopic floral meristem. Spatiotemporal expression analysis showed that SLGT11 was expressed throughout the FM in the early stage and its expression became more specific to the primordium position that corresponds to stamen and carpel in later stage of flower development. Together our results suggest that SLGT11 functions in floral organ patterning and termination of floral meristem identity in tomato.

Result
Identification of the *slf* mutants with the defects in floral organ identity and fruit

In order to further study the mechanism regulating organ identity in floral meristem in tomato, we screened tomato EMS-mutant library[43] and identified a mutant (TOMJPG2637-1) with defects in stamen and pistil development (*Fig. 1a-c,g*). Whose stamens and carpel were unable to complete their normal development, and the floral meristem cannot be terminated normally, which reverse into vegetative organ. Compared with wild type (WT), the number of stamen was decreased significantly or even disappeared from the third whorl. 98.97% of the WT flowers had 6 stamens while 77.05% of the mutant flowers had no stamens (*Fig. 1d*). The average stamen number decreased to 0.37 and the carpel number increased to 7.08 in the mutant. Contrary to the reduced stamen, the number of carpel increased significantly in the mutant (*Fig. 1e*). To verify these phenotypical defects, we performed anatomic observation of paraffin sections of the flower buds of the WT and the mutant. We found in the transverse sections that the number of stamens and ovules in mutants were both decreased, while the locules became increased (*Fig. 1b*). In the longitudinal sections, it was observed that the pistil-like structures formed in the third whorl position where stamens form (*Fig. 1b*). This fused structure was later formed radial cracks on the fruit surface, which greatly reduced the fruit quality (*Fig. 1c*). As the phenotype is similar to previously reported stamenless mutants, we thus named the mutant *slf*.

The fused ovary in *slf* developed into a parthenocarpic fruit without pollination (*Fig. 1c, d, Fig. S1c, d*). As a result, we attempted to cross-pollination using WT pollen grains in *slf* mutant was mostly failed with only a small amount of seeds produced, despite the normal development of the ovule in *slf* (*Fig. S1e, g*). Interestingly, pollen grains in *slf* seemed to be vital, indicating ovary and stamen in *slf* mutant were sterile. Thus the abnormal structure of stamen and pistil made *slf* a semi-sterile mutant. Despite the defective third whorl, the average number of sepals and petals in *slf* mutant was the same as that of the WT (*Fig. 1e*). In later stages of fruit development, *slf* mutant produced ectopic leaves and flowers which emerged from the initially formed fruit set (*Fig. 1g*), an indication of loss of FM termination. The anatomic observation of the early flower buds showed that the interminated meristem grew abnormally from the placenta at the center of the ovary (*Fig. 1g*).
Candidate gene **SLGT11** encodes a regulator involved in floral identity

To identify causal genes for the developmental defects in *slf* mutant, we first conducted a genetic analysis by crossing the mutant to the WT. In F2 segregated population, we found 28 progenies with *slf* phenotypes and 92 progenies resembling WT. The phenotypic characterization of 120 plants revealed a segregation ratio as WT:*slf* mutant (92:28) that is close to 3:1 Mendelian segregation rule, indicating that the phenotypes in *slf* mutant were caused by a recessive mutation at a single locus. Through bulked segregant analysis sequencing (BSA-Seq), we identified a signal peak in chromosome 3 (Fig. 2a). Further SNP analysis assigned the causal mutation to the gene *Solyc03g006900* that encodes a nucleus-localized transcription factor containing a putative GT1 DNA-binding domain and a PKc kinase domain (Fig. S3), belonged to Trihelix transcription factor family. An A to T transversion in the position 2195bp was identified, forming termination codon TAG that caused an early termination (Fig. 2b). Further sequencing analysis verified that this base transversion occurred in all 28 F2 progenies with *slf* phenotypes (Fig. 2c). Consistent with the presence of DNA-binding domain, SLGT11-GFP located in the nucleus shown by the transient expression assay in tobacco leaves (Fig. 2d).

To further functionally verify the **SLGT11** function, we transformed WT with an RNA interference (RNAi) plasmid targeting the C-terminal in pK7GWIG2 (II). The phenotypes of five independent transgenic RNAi lines were consistent with the mutant *slf*. qRT-PCR verified the significant reduction with different level of **SLGT11** expression in RNAi lines (Fig. 2f). The observed phenotypes including decreased number of stamens and fused carpel indicated that **SLGT11** was the gene causing the developmental defects of stamen and carpel in *slf* (Fig. 2e). In addition, abnormal fruits were also found in transgenic lines #1 and #6, indicating that **SLGT11** plays an important role both in regulating the development of flower and maintaining floral identity (Fig. 2g).

The phylogenetic analysis showed that **SLGT11** homologous genes in *solanaceae* were all grouped into the same cluster, while Arabidopsis homologous gene *At5g51800* belonged to a far related cluster (Fig. S2). Consistent with this phylogenetic distance, *At5g51800* mutation did not cause the similar floral phenotype [42], indicating the function of this gene is not completely conserved in
different species. Comparative analysis of the amino acid sequence of SLGT11 in *solanaceae* showed that the N-terminal GT1 domain and the C-terminal PKC kinase domain are highly conserved (Fig. S3).

Spatial and temporal expression pattern of SLGT11 in tomato

To examine the expression pattern of *SLGT11*, we performed qRT-PCR in different tomato tissues including the root, hypocotyls, cotyledon, stem, leaf, flower and fruit. The expression of *SLGT11* was highly enriched in the flower (Fig. 3a). Further RNA was extracted from different parts of flower at anthesis for qRT-PCR and found *SLGT11* was mostly expressed in stamen and had a low expression in sepal, petal and carpel, implying that *SLGT11* could be important for stamen development (Fig. 3b). Furthermore, we analyzed the temporal expression pattern of *SLGT11* during flower development. qRT-PCR showed that *SLGT11* expression was time-specific, with a high expression level from 6 days to 2 days before flowering (at stage 12-18) (Fig. 3c).

To verify the spatiotemporal expression pattern of SLGT11, we constructed GUS reporter driven by SLGT11 promoter and transformed it into WT tomato (Fig. 3d). GUS staining showed that SLGT11 was expressed throughout the FM in the early stage and the expression became more specific to the primordium position that corresponds to stamen and carpel in later stage of flower development, and the vascular bundles of the middle columns of the ovary (Fig. 3e). The expression of AGLF, which was the homologous genes of *SLGT11* in tomato, was observed in the center of the FM at the early stages and restrained in the stamen and carpel primordia when floral organ primordia completed differentiation at the late stages[40]. The expression pattern of *SLGT11* in the inner two whorls of floral organs was similar to AGLF, implying that *SLGT11* is probably involved in the regulation of the stamen and carpel development similar to *AGLF* in tomato.

Stamen defects occur at the early stage

To investigate how slf affects stamen and carpel at different flower developmental stages, we analyzed the FM in WT, slf mutant and SLGT11 RNAi lines with scanning electron microscopy (SEM) (Fig. 4a-o). The FM development in slf mutant and SLGT11 RNAi lines was the same as WT until stage 3 when sepal primordia and petal primordia were initiated (Fig. 4a-f). At stage 5, the
differences between WT and slf mutant or SLGT11 RNAi lines became more prominent. In WT, six stamen primordia and one carpel primordia with four locules were initiated in the third and fourth whorl respectively (Fig. 4g). In contrast, the stamen and carpel primordia in slf mutant and SLGT11 RNAi lines were initiated in disorder (Fig. 4h, j). The defective FM became more severe in slf mutant and SLGT11 RNAi lines at stages 6 and 7, with carpel fused together in WT (Fig. 4j, m). While in the mutant, most of the stamen transform into carpel and fused with the original carpel, expanding the area of the carpel (Fig. 4k, l, n and o). As the flower matured, ovary and style were visible in the WT, while the ovary in the mutants became irregular with multiple locules and styles. Combined with the spatiotemporal expression result, we concluded that SLGT11 plays an essential role in the early development of flower organs.

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**High temperature inhibited SLGT11 and TM29 expression**

Growing in summer when the temperature in green house was higher than the standard condition, the
stamens were hardly visible in slf mutants and the defective flowers with ectopic floral meristem became increased (not show). Because temperature can play a role in the development of stamen and carpel in male-sterile *stamenless-2* [38], meanwhile, the floral reversion is relate to temperature, we tested if the *SLGT11* function is also influenced by temperature. To that end, we germinated WT and slf mutant seeds at 25 °C for 4 weeks, followed by the growth in a heated incubator (37 °C daytime/28 °C at night) for 20 days. The slf mutants grown in high temperature produced flowers with carpelloid structures in the stamen position of the third whorl (*Fig. 6d*). This structure made the third whorl resemble carpel more than stamens. Meanwhile, the number of carpel was also increased significantly. In addition, the petals seemed to partially acquire sepal identity by forming greenish petals with sepal structure (*Fig. 6f*). Furthermore, we observed a number of floral meristems were produced at the center of the mature flowers (*Fig. 6d*). Despite the carpelloid stamens and ectopic floral meristem were occasionally produced in slf flowers at lower temperature (25 °C daytime/28 °C night), the occurrence became significantly more frequent under the high temperature (not show).

To further dissect the influence of high temperature on *SLGT11*, we performed qRT-PCR to analyze the potential transcriptional change. We germinated WT and slf mutant seeds at 25 °C for 4 weeks, followed by different temperature treatments. The floral buds at early stages were collected for RNA extraction and qRT-PCR. Our results showed that *SLGT11* expression was inhibited by the high temperature (*Fig. 6g*). To further verify this, we examined the expression levels of BCE genes at 3 h, 7 h and 24 h after high temperature treatment. Our results showed that *TM29* was significantly down-regulated, which was consistent with its transcriptional change in slf mutants (*Fig. 6h, i*). However, Yeast-one-hybrid assay failed to detect the direct binding of *TM29* promoter region by *SLGT11*, indicating the *SLGT11* mediated regulation could be multi-level and highly complex.

**Discussion**

Floral development is strictly controlled by complex regulatory networks to ensure successful reproduction in plants [44]. In natural conditions, the transition from vegetative to reproductive growth is irreversible so the correct tissue patterning can be guaranteed during the floral development [45]. Here we identified a recessive mutant of *SLGT11* gene which has the phenotypes
resembling some previously characterized mutants. *Sl*, a male sterile mutant was first reported in 1954 in which stamens degenerated and fused with the style [37]. This defect in floral meristem finally caused longitudinal cracks on the surface of the parthenocarpic fruit. *Stamenless* mutant was characterized as reduced and stunted stamens which led to the transformation of stamen into carpel structure [38, 39]. The similar defects in *slf* mutant suggest that SLGT11 could function in the same pathway as these previously reported genes.

However, *slf* also showed a reversion of floral development to vegetative organs, indicating that meristem termination in flowers became defective. This reversion phenotype is not necessarily associated with the defect of fused stamen and carpel, as evidenced by a number of previously characterized mutants including *TAP3*, *TPIB* and TM6. These tomato homeotic protein mutants cause severe floral organ patterning. In *Arabidopsis*, a weak allele *ag-4*, the carpel was transformed into sepal while stamens and carpels in a strong allele *ag-6* were entirely transformed into petals and sepals [46]. Despite new flowers formed in whorl four of *ag-2* flowers [47], no leaves were seen, indicating this defect represents only the aberrant termination of flower meristem. A direct homologous gene of *AG* in tomato is *TAG1*. In line with the conserved function of *AG*, *tag1* showed a transformation of stamens into sepals [32]. However, the reversion of floral meristem back to vegetative development was neither reported in *Arabidopsis* *ag* mutant, nor in tomato *tag1* mutant in nature condition. However, *slf*, we identified here exhibited an inhibited termination of flower meristem and the floral development was reversed into vegetative organs.

Interestingly the homologous gene of *SLGT11* in *Medicago truncatula* seemed to only function as C type gene as the phenotype of the *Medicago* mutant caused the transformation of stamens and carpels into petals and stamens [40, 42]. This report has not described the reversal phenotype of floral organs, indicating that *SLGT11* function may have experienced sub-functionalization in different species. In *Arabidopsis*, this reversal phenomenon was observed in the triple mutant of class E gene *sep1/2/3* [10]. When the function of these three E genes were lost, all floral organs transformed into sepals and blades. Such phenomenon was even more pronounced in tomato. After *SEPALLATA* homologous gene *TM29* was silenced in tomato, the development of inner three floral organs was
affected, and the aberrant stems with flowers and leaves were initiated from the fruits [21].

Based on the defects in stamen and carpel, as well as the reversal phenotype in slf mutant, we hypothesize that SLGT11 regulates the stamen and carpel through AG, and also maintains the flower identity together with TM29. In our observation, we found the phenotype of slf was stronger than that of TM29 and TAG1. In addition, both TM29 and TAG1 were significantly down-regulated in slf mutant, indicating that SLGT11 could function in the upstream of TM29 and TAG1. The high temperature treatment enhanced slf phenotypes by increasing floral organ reversal rate, promoting petals to turn green and making sepals appear in the fourth whorl. These phenotypes indicated that the expression of class A genes could spread to the position of the fourth whorl, which is consistent with the phenotype of class C gene deletion. Our qRT-PCR also showed that TM29 was significantly down-regulated at high temperature, supporting the hypothesis that SLGT11 could regulate TAG1 and TM29.

SLGT11 expressed extensively in the early stage of flower, but its expression gradually became concentrated in stamens and the vascular bundles of the middle columns of the ovary. It was speculated that SLGT11 plays roles in the initiation of each whorl of flower organs, especially the initiation of stamens. It has been reported that the expression of BCE gene which affects stamen development overlaps with SLGT11 expression domain. Class B genes including TAP3, TM6 and TPI were all shown to have expression in stamen position [29, 30]. The C gene TAG1 is also mainly expressed in the stamens and carpel during tomato flower development [32]. Compared with the class B and C genes, the expression of TM29 in early stage was more extensive, including vascular bundles. But during the later stage of floral development, TM29 expression is mainly concentrated in stamens and carpels [21], which overlaps with the expression region of SLGT11, and the SLGT11 also express in vascular bundles, which could be the origin of the abnormal stem. Compared with the WT, the expression of TAP3, TPI, TPIB and TM29 in slf mutant was all down-regulated, suggesting SLGT11 could regulate the BCE gene expression to promote stamen development. Therefore, SLGT11 could be one of regulators in addition to the ABC model genes that regulate floral organ development.

In tomato, the regulation of stamen development seems to be more complicated than that in
Arabidopsis. When the class B genes including AP3 or PI in Arabidopsis were mutated, the petals turned into sepals, and the stamens were missing or turned into carpels [26]. In tomato, there are four homologous class B genes: TAP3, TM6, TPI and TPIB. Despite similar phenotypes were observed when TAP3 and TPIB were mutated, mutations in TM6 or TPI only caused stamen transformation into carpel without affecting petal and carpel [30]. Since no homologous shift phenomenon was observed in sif mutant, it is difficult to fully explain SLGT11 function in floral development by including SLGT11 into any class of genes in the classical ABCE model. It seemed that SLGT11 had a wider range of functions. It is possible that SLGT11 not only regulates one type of ABCE genes, but also affects ABCE genes together.

Floral development is strictly controlled by complex regulatory networks to ensure successful reproduction in plants [44]. In natural conditions, the transition from vegetative to reproductive growth is irreversible so the correct tissue patterning can be guaranteed during the floral development [45]. Here we identified a recessive mutant of SLGT11 gene which has the phenotypes resembling some previously characterized mutants. Sl, a male sterile mutant was first reported in 1954 in which stamens degenerated and fused with the style [37]. This defect in floral meristem finally caused longitudinal cracks on the surface of the parthenocarpic fruit. Stamenless mutant was characterized as reduced and stunted stamens which led to the transformation of stamen into carpel structure [38, 39]. The similar defects in sif mutant suggest that SLGT11 could function in the same pathway as these previously reported genes.

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Conclusions
The results obtained by this study indicate that the disruption of a tomato novel Trihelix gene SLGT11 activity results in the loss of floral organ identity and the reversion of the flower to vegetative development during the floral development. Together with the spatiotemporal expression pattern, we suggest that SLGT11 is required for reproductive organ development and that SLGT11 function
diverged from that of the Arabidopsis homologous gene. The present study provides new knowledge on the function of Trihelix gene SLGT11 in floral development.

Materials And Methods

**Plant material and growth conditions**

All plants used in this study were in tomato (*Solanum lycopersicum L.*) accession Micro-tom background. Seeds of *stamenless like flower* (*slf*) mutant (TOMJPG2637-1) were obtained from the Tomato Mutants Archive (http://tomatoma.nbrp.jp/) by mutagenesis with gamma irradiation. Since the *slf* mutant was partial sterility, seeds from heterozygous plants were used for generated homozygous individuals.

Seeds were pre-germinated on moistened filter paper at 28 °C in complete darkness. Plants were grown under long-day conditions (16-h light/8-h dark) in a greenhouse with a relative humidity of 60%. Daytime and nighttime temperatures were 26 °C and 22 °C, respectively. All plants received regular watering and fertilizer treatments.

**Phenotype characterization**

For analyses the defects of floral organ, we counted the number of floral organ at least 20 flowers on each replicate plant at anthesis. For analyses the number of stamen in each flower, flowers at anthesis were collected and statistics the ratio. For analyses the ectopic floral meristem, flowers before anthesis were removed sepal and petal to show floral meristem. Immediately after dissection, morphology of ectopic floral meristem was imaged using Nikon SMZ18 stereomicroscope.

**Histological analysis**

To determine morphological and developmental characteristics, fresh floral organs were dissected and examined by Nikon SMZ18 stereomicroscope. For the toluidine blue staining assay[48], the flower buds from the six-week-old wild-type and *slf* plants were harvested and treated in FAA (3.7% formaldehyde, 5% acetic acid, 50% ethanol) under vacuum conditions for 30 min, respectively. These samples were kept overnight at room temperature under nature conditions and dehydrated in a graded ethanol and tertbutanol series. Embedded in a paraffin solution containing 50% tertbutanol for 4 h, place the samples over night in pure paraffin (Sigma-Aldrich).
Sections (10 μm thick) were cut with a Leica RM2255 microtome, then bake the sections at 45 °C overnight, further paraffin was removed from the dewaxing agent and tissue parts were washed in pure water carefully, thus stained for 1 min in 0.25% toluidine blue-O (Sigma-Aldrich, U.S.A), then observed and photographed with a Nikon SMZ18 stereomicroscope.

**Scanning electron microscopy**

Flowers at the early stage analysis by scanning electron microscopy (SEM) were conducted as follows [43]: the sepals and petals were separated from fresh floral organs under stereomicroscope. These samples were observed using a TM3030 PLUS scanning electronic microscopy under a quanta 250 FEG scanning electron microscope at an accelerating voltage of 5 kV.

**Subcellular localization**

The 35Spro:SLGT11-GFP and the empty vector pHellsgate 8 (35Spro: GFP) as control were transformed into agrobacterium GV3101 and injected into *Nicotiana benthamiana* subcuticle, respectively. The plants with infiltrated leaves were incubated at 25 °C in the dark for 24 h and then exposed to light for 12 h before GFP signals were observed by confocal microscopy (LSM 880, Germany Carl Zeiss). The primers were listed in the S1 Table.

**Bulked segregant analysis (BSA)**

Bulked segregant analysis was performed according to Chang et al [49]. The slf homozygous plants as female parent are crossed to the WT and then selfed to generate F2 mapping population. For BSA-seq, we extracted genomic DNA from 28 slf mutant individuals in the F2 mapping population and 30 WT individuals using CTAB [50], respectively. All DNA quality and concentration were checked to ensure equal representation of individuals and then mixed to construct two bulks (slf bulk; WT bulk). The slf bulk and WT bulk were sequenced to a depth of 28× and 30× coverage of the tomato genome by HiseqXten-PE150 (Novogene), respectively. Trimmed sequences are mapped onto the tomato reference genome (Heinz 1706 cultivar) and mutation variants are filtered. Analysis of the allelic variant frequencies in the pools leads to the identification of the causal mutation with 100% frequency in the slf bulk. The genes with the expected allelic frequency tend to be 1 were performed mutation identification and transgenic verification. Then, the candidate genes were cloned and
sequenced to verify the mutant site. The primers were listed in the S1 Table.

**SLGT11 RNAi gene constructs**

To generate an SLGT11 RNA interference line, a 253 bp-sequence near the 3’ end of the PKC kinase-like domain selected by Sol Genomics Network vigs tool (https://vigs.solgenomics.net/) was amplified from the wild type cDNA cloned into entry vector PDONR221, then, cloned into the binary vector pK7GW1WG2 (II) by the gateway system. The binary plasmids were transformed into agrobacterium C58 strain for further using in genetic transformation experiments to generate SLGT11 RNAi transgenic lines.

**Plant genetic transformation**

Agrobacterium-mediated transformations of tomato were performed according to Brooks et al [51]. In brief, cotyledon segments from 6 to 8 d old seedlings were precultured for 1 d followed by inoculation with agrobacterium strain C58 containing the RNAi construct of SLGT11. Following a 2 d cocultivation, the cotyledon segments were transferred to a selective regeneration medium that contained kanamycin. Subcultures were performed every 15 days until these seedlings grown three true leaves. These seedlings were transferred to a selective rooting medium that also contained kanamycin. Only well-rooted plants were transferred to the greenhouse.

**Phylogenetic and sequence analyses**

Sequences of tomato and other species of SLGT11 family members were obtained from the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and aligned using the ClustalW function in MEGA5. Phylogenetic trees for proteins with 1,000 bootstrap replicates were constructed using the maximum likelihood method in MEGA5 [40].

**Imaging, microscopy and GUS staining**

To produce the pSLGT11::GUS construct, 3 kb of genomic sequence comprising the SLGT11 upstream region, cloned into PGWB432 binary vector by the infusion cloning method. Transgenic plants for this construct show specific GUS reporter expression. Whole-floral primordium and flowers at different stages were stained by GUS solution in 37 °C for 10 h after the fixation in cold 90% acetone for 20 min. These samples were dehydrated in a solution (ethanol: acetic acid glacial, in proportions 4:1 by
volume) about 6 h until clear and washed briefly by different concentration ethanol. We proceed as follows: 40% ethanol for 15 min, 20% ethanol for 15 min, 10% ethanol for 15 min, and embedded in 5% agar. The samples were sectioned by Vibrating slicer (Leica, Germany), thus observed the expression pattern of SLGT11 by a Nikon SMZ18 stereomicroscope [52].

**Quantitative real-time PCR analysis**

Gene expression analyses were performed by quantitative real time PCR (qRT-PCR) on wild type and *slf* flowers around stage 5 [48, 53]. Total RNA was isolated using the Eastep Super Total RNA Extraction Kit (shanghai promega) according to the manufacturer’s recommendations. Subsequently, HiScript II 1st Strand cDNA Synthesis Kit (+gDNA wiper, Vazyme) was used to synthesize first strand cDNA. ChamQ Universal SYBR qPCR Master Mix kit (Vazyme) was used to perform qRT-PCR reactions in a 7300 Real-Time PCR System (CFX Connect, BIO-RAD), according to the manufacturer’s instructions. An actin gene was used as a constitutive control. The relative expressions of genes were calculated using the $2^{\Delta\Delta C_{t}}$ method. All analyses were performed in three biological replicates and two technical replicates. All primer sequences can be found in Table S1.

**Yeast-one-hybrid assay**

Yeast-one-hybrid assay was performed using the Matchmaker EYG48 Yeast-one-hybrid system (Clontech) as described in yeast manual. The coding sequence of SLGT11 for effector protein was cloned into the PJG4-5 vector, and the promoter sequence of TAG1 was cloned into the reporter vector Placzy. Both the vectors were transformed into the EYG48 yeast strain. Diploid yeast cells were grown and selected on dropout medium without leucine and tryptophan. To assay protein-promoter interactions, clones were grown on two-dropout medium without leucine and tryptophan, but with x-gal, for 2 d at 30 °C. The empty vectors are as control. All primer sequences used for cloning can be found in S1 table.

**Declarations**

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**Author contributions**

S.W., S.Q. designed the research; L.Y. performed the experiments; L.Y., S.Q., H.L. and S.W. analyzed the data; L.Y., A.T. and S.W. wrote the paper.

**Competing interests**

The authors declare no competing financial interests.

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The slf mutant shows deficiencies in identification of floral organs. (a) The flower, stamens and ovaries of WT and slf. (b) Transverse and longitudinal section of the WT and slf flower, stained with toluidine blue at developmental stage 18. Red and black numeric show number of floral stamens and locules, red asterisk indicates the conversion of the stamens into carpel in third whorl. (c) The fruit of WT and slf, the green triangles indicate rough radial
lines caused by formation of vestigial stamens. (d) Percentage of flowers with 0-6 stamens for WT and slf genotype. The numbers of sepal, petal, stamen and carpel in WT and slf. The data represent means ±SD (n=296). (f) A schematic diagram of WT and slf floral organs. The WT flower consists of four whorls: sepal (green), petal (orange), stamen (yellow), and carpel (purple). A slf flower is consist of sepals in the 1st whorl, petals in the 2nd, stunted stamens in the 3rd whorl or disappeared and develop into carpel, and irregular ovary in the 4th whorl. (g) Ectopic floral meristem emerge in the flower, and ectopic shoot produce flowers and leaf-like structures in the fruit, longitudinal sections of the slf flowers show ectopic floral meristem stained with toluidine blue at the floral developmental stage 14 d. emf: ectopic floral meristem; es: ectopic stem; ef: ectopic flower; el: ectopic foliage; ec: ectopic carpel. Scale bars: (a, b, g) 1 mm;
Fine-mapping of slf and functional verification of candidate gene SLGT11. (a) SLGT11 is located on chromosome 3. Red triangle indicates a signal peak in chromosome 3. (b,c) A to T transversion in the position 2195 bp and forming termination codon TAG. (d) Nuclear localization of the SLGT11-GFP fusion protein in tobacco mesophyll cells. (e) Genotypes are SLGT11 RNAi transgenic lines (# 1, # 2 and # 6) and WT. (f) Relative quantitative RT-PCR expression analysis of the SLGT11 gene in WT and SLGT11 RNAi lines # 1, # 2, and # 6. SLACTIN was used as internal control. Error bars represent the SD from three biological replicates. (g) The fruit of WT and SLGT11 RNAi lines # 1 and # 6 show the radial cracks.
and ectopic stems. #1: SLGT11-RNAi-1; #2: SLGT11-RNAi-2; #6: SLGT11-RNAi-6; Scale bars:

(e) 1 mm; (g) 1 cm.
Figure 3
Spatial-temporal expression pattern of SLGT11. (a) qRT-PCR analysis of SLGT11 gene in different tissue. (b) qRT-PCR analysis of SLGT11 gene in different floral organs. (c) qRT-PCR analysis of SLGT11 gene at different flower developmental stage. SLACTIN was used as internal control. Error bars represent the SD from three biological replicates. (d) The diagram of carrier construction of the GUS protein expression driven by SLGT11 promoter. (e) GUS activity is throughout the development of the flowers in sepal, petal, stamens and carpel primoria with strong expression in the stamens and vascular bundles in the ovary. (f) A cross section of the stamens and ovary at stage 12 and 14 showing GUS activity in anther and vascular bundle. H: hypocotyl; C: cotyledon; S: stem; L: leaf; Fl: flower; Fr: fruit; st: stage. Scale bars: (c and e) 1 mm; (f) 100 μm.
Figure 4

The difference among WT, slf and SLGT11-RNAi6 at early floral development stage. SEM analysis of floral meristem in WT (a, d, g, j and m), slf (b, e, h, k and n) and SLGT11-RNAi-6 (c, f, i, l and o); Green stars indicate sepal primordia, orange stars indicate petal primordia, yellow stars indicate stamen primordia, pink stars indicate carpel primordia, blue stars indicate unusual primordia of the carpel; red circle indicates abnormal primordia of the carpel; Scale bars: 100 μm; se, sepal; pe, petal; st, stamen; ca, carpel.e, sepal; pe, petal; st, stamen; ca, carpel.
Expression levels of BCE gene in WT and slf. Expression levels of TAP3, TPIB, TPI, TM6, TAG1 and TM29 in WT and slf flowers by qRT-PCR, SLACTIN was used as internal control.
High temperature treatment impacts on the floral identity on slf mutant and WT. (a) The flowers of WT treated with high temperature with (e) normal sepals and petals, and (c) spreading stamens; (b) The flowers of slf mutant treated with high temperature showing (f) greenish petals and (d) ectopic floral meristem (efm) in the fourth floral whorl organs. (g) qRT-PCR expression analysis of SLGT11 in WT floral buds treated with 25 °C (WT-25) and 37 °C (WT-37). (h) qRT-PCR expression analysis of TM29 in WT floral buds treated with 25 °C (WT-25) and 37 °C (WT-37). (i) qRT-PCR expression analysis of TM29 in WT and slf mutant floral buds treated with 25 °C (WT-25 and slf-25) and 37 °C (WT-37 and slf-25) after 3 h, 7 h, 24 h, respectively. SLACTIN was used as internal control. Error bars represent the SD from three biological replicates. efm, ectopic floral meristem ; se, sepal; pe, petal; Scale bars: (c)
1 cm; (d) left 250μm, right 1mm; (f) 1mm.

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