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Long-distance control of potato storage organ formation by SELF PRUNING 3D and FLOWERING LOCUS T-like 1

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ABSTRACT

Plants program their meristem-associated developmental switches for timely adaptation to a changing environment. Potato (*Solanum tuberosum* L.) tubers differentiate from specialized belowground branches or stolons through radial expansion of their terminal ends. During this process, the stolon apex and closest axillary buds enter a dormancy state that leads to tuber eyes, which are reactivated the following spring and generate a clonally identical plant. The potato FLOWERING LOCUS T homolog SELF-PRUNING 6A (StSP6A) was previously identified as the major tuber-inducing signal that integrates day-length cues to control the storage switch. However, whether some other long-range signals also act as tuber organogenesis stimuli remains unknown. Here, we show that the florigen *SELF PRUNING 3D* (*StSP3D*) and *FLOWERING LOCUS T-like 1* (*StFTL1*) genes are activated by short days, analogously to *StSP6A*. Overexpression of *StSP3D* or *StFTL1* promotes tuber formation under non-inductive long days, and the tuber-inducing activity of these proteins is graft transmissible. Using the non-tuber-bearing wild species *Solanum etuberosum*, a natural *SP6A* null mutant, we show that leaf-expressed *SP6A* is dispensable for StSP3D long-range activity. StSP3D and StFTL1 mediate secondary activation of *StSP6A* in stolon tips, leading to amplification of this tuberigen signal. StSP3D and StFTL1 were observed to bind the same protein partners as StSP6A, suggesting that they can also form transcriptionally active complexes. Together, our findings show that additional mobile tuber-inducing signals are regulated by the photoperiodic pathway.

Key words: potato, *Solanum tuberosum* L., *FLOWERING LOCUS T*, StSP3D, tuberization, tuber-inducing signal, long-distance signaling

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INTRODUCTION

Plant organogenesis shapes the morphology of adult plants and is central to vegetative propagation and flowering transition. It is finely controlled and exquisitely sensitive to environmental changes. Potato is a model system for belowground storage organ formation. Potato plants usually form tubers from specialized underground branches or stolons, which initiate from axillary buds at the base of the main stem. Shoot axillary meristems of potato have the potential to remain in a dormant state or to give rise directly to leafy shoots, stolons, and even sessile tubers. Upon inductive conditions such as short days, potato tubers develop from underground stolons by radial expansion of their terminal ends. Newly formed tubers are the economically important parts of the plant. Thus, factors that cause the tip of a stolon to change its developmental program and turn into the very specialized stem/tuber have been widely studied (Zierer et al., 2021).

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Potato adjusts tuber formation to day-length conditions. Plants grown in short days (SDs) tuberize earlier than those kept in long-day (LD) conditions. However, in *S. tuberosum* ssp. *andigena*, short photoperiods are strictly required for tuber formation, making this species an attractive model for studying photoperiodic tuberization signals (Navarro et al., 2011; Kloosterman et al., 2013; Abelenda et al., 2016). Inductive SD conditions, specifically long nights, are perceived in the leaves, where a tuberization stimulus is produced and then transported to underground stolons to initiate tuber formation (Gregory, 1956; Kumar and Wareing, 1973; Chailakhyan et al., 1981). Elegant grafting studies have shown that flowering tobacco shoots induced tuberization when grafted onto potato stocks, indicating that the floral stimulus may act as a tuber-inducing signal (Chailakhyan et al., 1981). Currently, research in tomato, *Arabidopsis*, and rice has documented that the molecular nature of this florigenic signal corresponds to the FLOWERING LOCUS T (FT) protein (Weigel et al., 2000; Lifschitz et al., 2006; Corbesier et al., 2007; Tamaki et al., 2007), which belongs to the family of PHOSPHATIDYLETHANOLAMINE BINDING PROTEINS (PEBPs). In potato, overexpression of the *FT-like* gene *StSP6A* promotes tuber formation in non-inductive LDs, showing that StSP6A acts as a major component of the tuberigen (Navarro et al., 2011), and *StBEL5* mRNA, miR156, and miRNA172 mobile molecules also function as tuber-promoting or tuber-inducing signals (Banerjee et al., 2006; Martin et al., 2009; Bhogale et al., 2014).

Accumulated evidence has shown that the expression of *StSP6A* is under the control of a conserved CDF/PHYTOCHROME–CONSTANS–FT flowering molecular module. Diurnal oscillation of the *StCOL1* gene is partly regulated by the StCDF1–FKFI–GI complex (Kloosterman et al., 2013). Unlike in *Arabidopsis*, the light receptors PHYTOCHROME B (PHYB) and PHYTOCHROME F (PHYF) are essential for StCOL1 protein stabilization under LD conditions in potato (Abelenda et al., 2016; Zhou et al., 2019). Accumulation of StCOL1 activates the expression of another *FT-like* gene, *SELF PRUNING 5G* (*StSP5G*), which negatively regulates *StSP6A* expression in leaves to suppress tuber formation (Abelenda et al., 2016). In SDs, the StCOL1 protein is not stabilized, enabling the activation of *StSP6A* in leaves and its movement to stolons. There, StSP6A forms an autoregulatory loop to amplify the systemic signal and reprograms the expression of genes such as *StGA2ox1*, *StMADS1* (*AGL8*), and *StMADS13* (*AGL8-like*) (Navarro et al., 2011). Furthermore, the mRNA level of *StSP6A* is regulated by the small RNA *suppressing expression of SP6A* (*SES*) in leaves in response to high temperature (Lehretz et al., 2019; Park et al., 2022), by the StBEL5/POTH1 transcriptional complex in both leaves and stolons (Sharma et al., 2016), and by the interaction of StSP6A with the StTOC1 (TIMING OF CAB EXPRESSION 1) transcription factor in a feedback mechanism (Morris et al., 2019). Studies of StSP6A signaling mechanisms have revealed the functional conservation of storage organ and flowering transitions. StSP6A has been shown to form a tuberigen activation complex (TAC) comprised of StFDL1 and 14-3-3 proteins to promote tuberization (Teo et al., 2017). In addition, StSP6A binds and inactivates SWEET proteins to control source-sink balance (Abelenda et al., 2019). We have previously shown that StABI5 like 1 (StABL1) also interacts with StFT-like proteins to form alternative TACs (aTACs) that promote flowering and tuberization (Jing et al., 2022b). Conversely, TFL1/CEN competes with StSP6A in the TAC to antagonize its tuber-inducing activity (Zhang et al., 2020). Recently, it was reported that the tuber-inducing activity of StSP6A in aerial nodes is spatially blocked by a conserved FT–BRANCHED1b (StBRC1b) genetic module that helps to restrict tuberization belowground (Nicolas et al., 2022). To date, most studies have focused on StSP6A. However, it remains to be determined whether other long-range signals derived from leaves also function as tuber-inducing stimuli to trigger the tuber organogenesis developmental switch.

In potato, the transition to flowering is also dependent on mobile FT-like florigen proteins, and it has been established that SELF PRUNING 3D (StSP3D) is the major player (Navarro et al., 2011; Teo et al., 2017). The *FT-like* gene family has undergone preferential duplication and sub-functionalization in *Solanaceae* (Abelenda et al., 2014), and earlier evidence has shown that potato floral and tuber transitions are controlled by two different *FT-like* paralogs, florigen StSP3D/SFT and tuberigen StSP6A (Navarro et al., 2011; Teo et al., 2017), which respond to different environmental cues. Flowering transition in *S. tuberosum* can occur independently of photoperiod (Seibert et al., 2020), although LDs combined with high light irradiance accelerate flower development, whereas SDs or LDs and low irradiance significantly decrease flowering. Notably, genetic knockdown of *PHYF*, *StCOL1*, or *StSP5G* led to rapid flowering and tuber formation under LD conditions (Abelenda et al., 2016; Zhou et al., 2019), suggesting that these tuberization pathway repressors also repress flowering. However, further studies are needed to determine whether potato flowering and tuber formation are controlled by independent FT-like proteins.

Here, we provide evidence showing that expression of florigen *StSP3D* and *FLOWERING LOCUS T-like 1* (*StFTL1*) is activated by SDs analogously to *StSP6A*. Overexpression of *StSP3D* or *StFTL1* is shown to promote tuber formation in non-inductive LDs, and we observe that these proteins, along with their tuber-inducing effects, are graft transmissible. Leaf-expressed *SP6A* is dispensable for the promotion of tuberization by SP3D, whereas both FT-like StSP3D and StFTL1 proteins are involved in the regulation of *StSP3D*, *StSP6A*, *StSP5G*, and other tuberization-related genes in stolon tips, possibly via interaction with the same StSP6A-binding proteins. Thus, our study describes a previously unidentified tuber-inducing activity of the StSP3D and StFTL1 proteins, in addition to their activity as florigens, and provides new insights into potato photoperiodic tuberization.

RESULTS

Identification and expression analysis of potato *FT-like* genes

Previously, Navarro et al. (2011) identified four *FT-like* genes in potato, *StSP3D*, *StSP5G* (*StSP5G-A*), *StSP5G-like*, and *StSP6A*. Using tomato FT-like amino acid sequences as protein BLAST queries of the potato genomics resource (Spud DB, http://spuddb.uga.edu/), we identified two additional *FT-like* family members in the potato genome (Figure 1A), *StSP5G-B*, which was named after *StSP5G-A* (Abelenda et al., 2016), and *FLOWERING LOCUS T-Like 1* (*StFTL1*), which was named after

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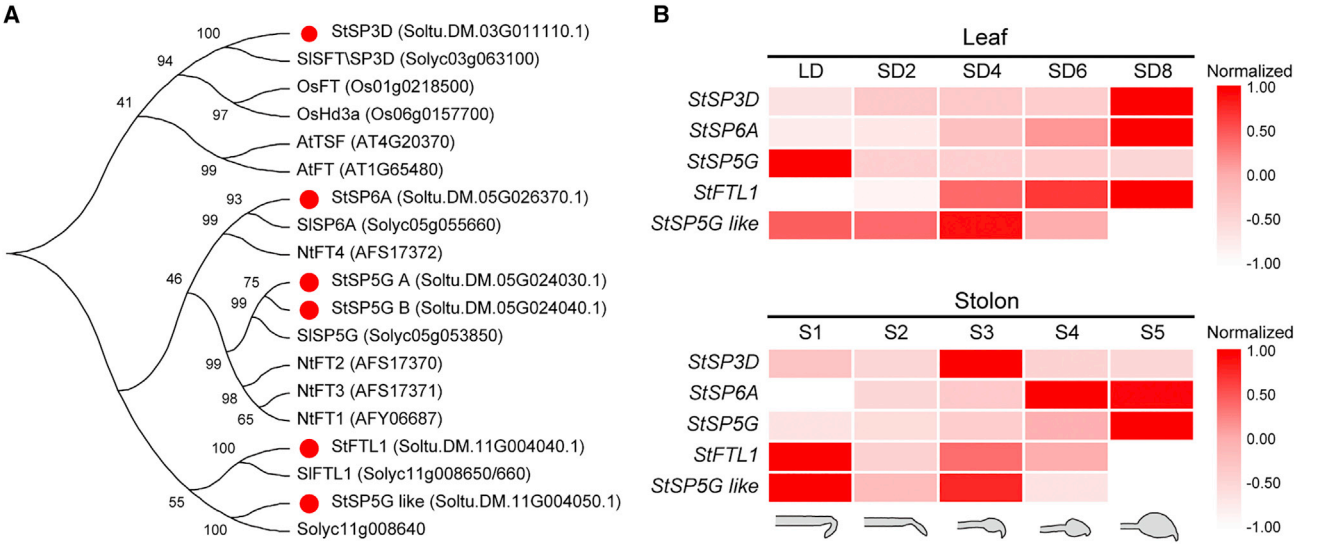

Figure 1. Identification and expression analysis of potato *FT-like* genes.

(A) Phylogeny of the *StFT-like* genes and homologs from *Solanum lycopersicum*, *Oryza sativa*, *Arabidopsis thaliana*, and *Nicotiana benthamiana*. Potato *FT-like* genes are indicated by red circles. In the neighbor-joining tree (MEGA X, Poisson model), numbers above the branches indicate the percent support for nodes defining the families in distance bootstrap analyses (1000 replicates).

(B) Heatmap showing the normalized expression levels of *StFT-like* genes in E109 leaves and stolons. LD, long day. SD2, SD4, SD6, and SD8 indicate leaves sampled at 2, 4, 6, and 8 days after transfer to short-day conditions. S1–S5 indicate stolon tips at different developmental stages. Samples were collected at 8 days after transfer to short days. S1, stolon with apical hook; S2, stolon with straight apical hook; S3, tuber initiation stage; S4, tuber setting stage; S5, tuber bulking stage.

its ortholog in tomato (Song et al., 2020). *StSP5G-A* and *StSP5G-B* are organized in tandem on chromosome 5, and *StSP5G-like* and *StFTL1* are arranged in tandem on chromosome 11. The phylogeny of these genes grouped *StSP3D*, *StSP6A*, *StSP5G-A*, *StSP5G-B*, *StFTL1*, and *StSP5G-like* into three sub-clades. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) data showed that the expression of *StSP3D*, *StSP6A*, and *StFTL1* was induced in leaves in response to SDs, although *StSP3D* activation was lower than that of *StSP6A* or *StFTL1* (Figure 1B). Expression of *StSP5G* and *StSP5G-like*, on the other hand, gradually decreased under SDs. Because *StSP5G-A* and *StSP5G-B* share almost identical coding regions, we amplified and quantified both *StSP5G-A* and *StSP5G-B* transcripts by qRT-PCR. In stolons, *StSP6A* and *StSP5G* transcripts accumulated as the tubers initiated bulking, whereas *StSP5G-like* and *StFTL1* exhibited the opposite expression pattern. *StSP3D* expression was also slightly induced upon tuber initiation. Previously, we showed that silencing of *PHYF* (*PHYFi*) promotes day-neutral tuberization by activating *StSP6A* (Zhou et al., 2019). Interestingly, the expression of *StSP3D* was also upregulated in leaves and swelling stolons of *PHYFi* lines (Supplemental Figure 1A and 1B), in contrast to *StFTL1*, which was downregulated in these plants. Collectively, these data showed that *StSP3D* and *StFTL1* are SD activated similarly to *StSP6A*, suggesting a possible role of these proteins as long-distance tuber-inducing signals.

Graft-transmissible induction of potato tuberization by StSP3D

To test whether StSP3D could induce tuber formation, we generated stable transgenic plants expressing a CaMV 35S promoter-driven *StSP3D* overexpression construct in the SD potato E109 background (Supplemental Figure 2A and 2B). We found that *StSP3D*-overexpressing (*StSP3Dox*) plants started to tuberize under non-inductive LD conditions 1 month after planting, whereas no tubers were formed in control E109 plants (Figure 2A). After 10 weeks of cultivation under LDs, one to four mature tubers could be harvested from these transgenic plants, whereas no tubers were formed in control E109 plants (Figure 2B). Likewise, *StSP3Dox* plants tuberized under natural LD conditions (Supplemental Figure 3). These results suggest that StSP3D also exhibits tuber-inducing activity in potato. We observed that the stolon apical meristem of *StSP3Dox* plants initiated floral buds (Figure 2C), similar to the phenotype observed in *StSP6A*-overexpressing plants (Navarro et al., 2011; Lehretz et al., 2019), and that expression of *StSP3D* led to early flowering (Supplemental Figure 4), indicating the conserved florigenic activity of StSP3D. Moreover, unlike E109 tubers, which formed etiolated shoots from apical buds after tuber dormancy release, most sprouts of the *StSP3Dox* tubers generated secondary tubers directly (Figure 2D). Normal sprouts of *StSP3Dox* tubers formed new tubers directly when buried in soil (Figure 2E), unlike sprouts of E109, which developed as upward-growing shoots, suggesting that sprout meristem identity is reprogrammed in *StSP3Dox* tubers.

To examine the potential role of StSP3D in regulation of the remaining *FT-like* genes under LDs, the diurnal oscillation of *StSP5G* and *StSP6A* in leaves was analyzed in E109 and *StSP3Dox* plants. Expression of these genes exhibited no significant differences between E109 and *StSP3Dox* leaves, although *StMADS1*, which acts downstream of StSP6A (Navarro et al., 2011), was significantly upregulated in *StSP3Dox* plants (Figure 2F), suggesting that StSP3D may directly travel to stolons for tuber induction. Indeed,

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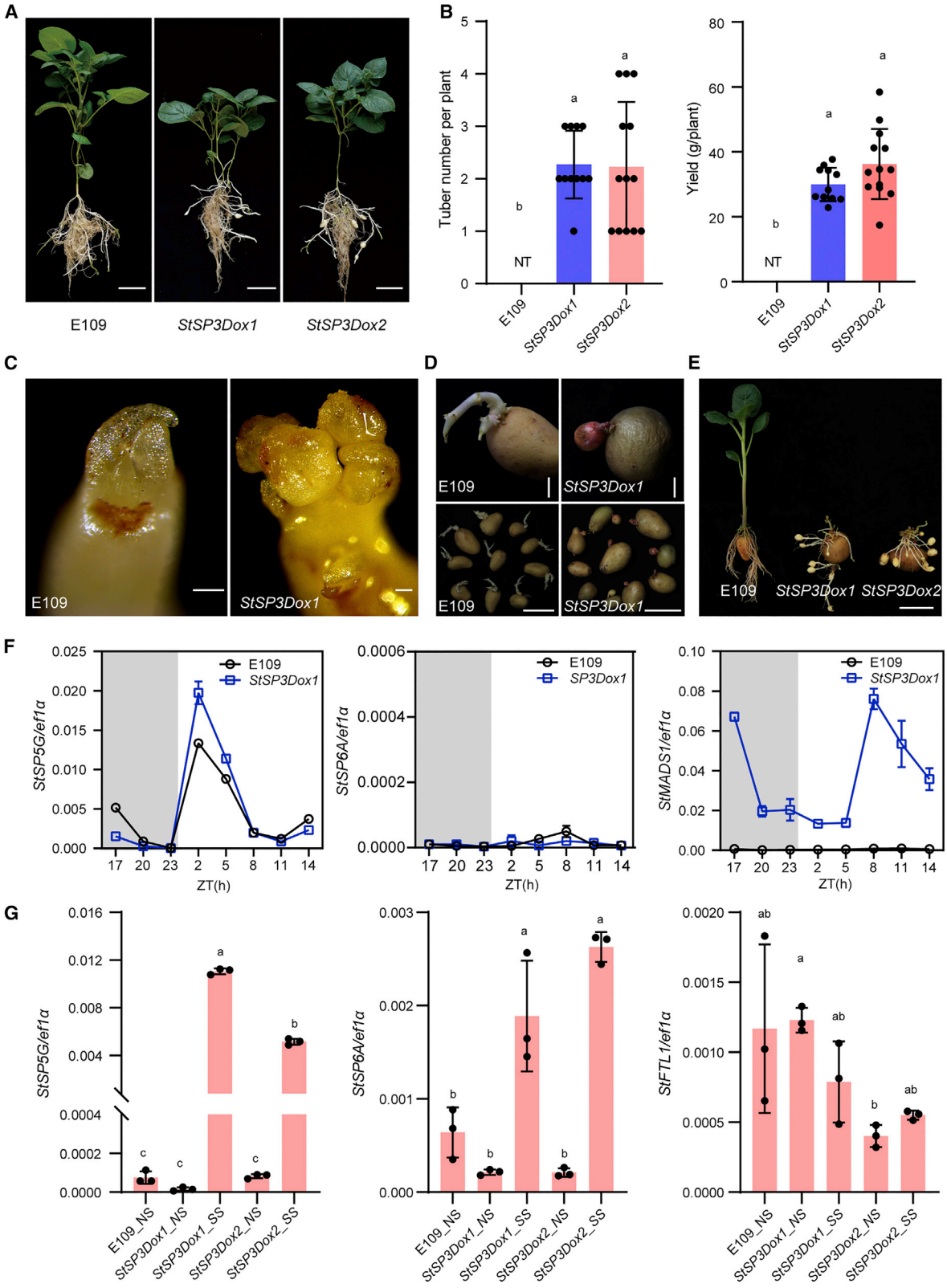

Figure 2. Overexpression of *StSP3D* induces tuber formation under non-inducing LD conditions.

(A) Tuberization phenotype of *StSP3D*-overexpressing plants (*StSP3Dox*) under LD conditions. E109 plants do not tuberize. Thirty-five days after planting. NT, no tuber. Scale bars, 5 cm.

(B) Tuber number and tuber yield of *StSP3Dox* plants. Twelve weeks under long-day conditions. NT, no tubers. Data are shown as mean ± SD, n ≥ 11. The lowercase letters above the bars indicate significant differences among means (one-way ANOVA, followed by Tukey's multiple comparison test, $p < 0.05$).

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when we analyzed *FT-like* gene expression in stolons of E109 and *StSP3Dox* plants, we observed that *StSP5G* and *StSP6A* mRNA levels were significantly induced in swelling stolons of *StSP3Dox* plants (Figure 2G), which suggests that StSP3D may be involved in the secondary activation of *StSP5G* and *StSP6A* in swelling stolons.

To further investigate whether StSP3D is a graft-transmissible tuber-inducing signal, we used LD-grown *StSP3Dox* plants as scions or stocks in grafting studies with E109 plants (scion/stock, E109/*StSP3Dox1* and *StSP3Dox1*/E109) to analyze the tuberization response of these graft combinations. Self-grafted combinations (E109/E109 and *StSP3Dox1*/*StSP3Dox1*) served as controls. When *StSP3Dox1* plants were used as either the scion or stock, the grafted plants tuberized under LDs, whereas the E109/E109 combination produced almost no tubers (Figure 3A and 3B), suggesting that expression of *StSP3D* in aerial parts or stolons is sufficient for the stolon-to-tuber transition under LD conditions. We next analyzed the levels of *StSP3D-HA* transcript and StSP3D-HA protein in stolons and leaves of *StSP3Dox1*/E109 and E109/*StSP3Dox1* heterografts. StSP3D-HA was detectable in stolons of *StSP3Dox1*/E109 plants but not in leaves of E109/*StSP3Dox1* plants (Figure 3C). *StSP3D-HA* transcripts were not detected in E109 stocks (Figure 3D), showing that the StSP3D-HA protein travels from scion to stock. Long-distance control by StSP3D was also confirmed using LD-grown *StSP3Dox* plants as scions or stocks in grafting studies with *S. etuberosum* plants (*StSP3Dox1*/*S. etuberosum* and *S. etuberosum*/*StSP3Dox1*), followed by harvest of stems above the grafts and belowground stems/rhizomes for StSP3D-HA detection. These studies also showed that StSP3D is graft transmissible, although the *StSP3Dox1*/*S. etuberosum* heterografts did not form any tubers (Supplemental Figure 5), both here and in a previous study (Plantenga et al., 2018). Taken together, these results support the identification of StSP3D as a graft-transmissible tuber-inducing signal.

Graft-transmissible induction of potato tuber formation by StFTL1

The fact that the expression of *StFTL1* is also activated under SD conditions prompted us to determine whether *StFTL1* could also promote potato tuber formation. To this end, we created stable 35S promoter-driven *StFTL1* overexpression (*StFTL1ox*) lines in the E109 background (Supplemental Figure 2C and 2D). Notably, *StFTL1ox* plants also tuberized under non-inductive LDs (Figures 4A and 4B). As observed for *StSP3D* overexpression, overexpression of *StFTL1* did not direct the diurnal oscillation of *StSP3D* and *StSP6A* in leaves, and the expression of *StMADS1* was strongly upregulated in leaves of *StFTL1ox* plants (Figure 4C). Expression of *StSP5G* and *StSP6A* was also significantly upregulated in swelling stolons (Figure 4D), suggesting that StFTL1 moves to the stolons for tuber induction. Additional grafting experiments were therefore performed to assess the mobility of this protein. The grafted plants tuberized under LD conditions independently of the use of *StFTL1ox* plants as scions or rootstocks (Figures 4E and 4F). We next detected the StFTL1-HA protein in stolons and leaves of *StFTL1ox26*/E109 and E109/*StFTL1ox26* heterografts. StFTL1-HA was detectable in stolons of *StFTL1ox26*/E109 plants but not in leaves of E109/*StFTL1ox26* plants (Figure 4G), indicating that StFTL1-HA can travel at least from scion to stock and can also function as a long-range tuberization inducer.

SP3D induces tuber formation independently of leaf-expressed SP6A

Our expression analyses showed that overexpression of *StSP3D* or *StFTL1* induced tuber formation but did not stimulate *StSP6A* expression in leaves. To verify that tuber induction by SP3D was independent of leaf-expressed SP6A, we used the non-tuber-bearing wild species *S. etuberosum* for grafting experiments. We amplified the *SeSP6A* gene by homology-based cloning and found that it is naturally inactivated by a deletion of the whole fourth exon (Figure 5A and Supplemental Figure 6), leading to the loss of nearly half of the full-length protein sequence. Thus, *S. etuberosum* is a natural *SP6A* null mutant. Notably, *S. etuberosum* strictly requires LD and high light for flowering, and it is more closely related to potato than tobacco. As observed in earlier tobacco-grafting experiments, when LD-grown *S. etuberosum* scions were grafted onto potato E109 rootstocks, the grafted plants flowered and tuberized. However, none of the grafted plants transitioned to flowering or tuberization when *S. etuberosum* scions were grown under SD conditions (Figures 5B and 5C). We next created stable transgenic *S. etuberosum* lines that expressed a CaMV 35S promoter-driven *StSP3D* overexpression construct (Supplemental Figure 2E and 2F). The *StSP3Dox S. etuberosum* lines flowered much earlier than the wild type (Supplemental Figure 7A and 7B), but they did not produce any tubers, owing to a lack of tuberization competence (Supplemental Figure 8). When these *StSP3Dox26* plants were used as donor scions onto E109, the rootstocks tuberized even under SD conditions (Figures 5B and 5C), indicating that SP3D induces tuber formation independently of leaf-expressed *SP6A*. In addition, the fact that *S. etuberosum*/E109 grafts tuberized under LD conditions but not under SD conditions indicates that an SP6A-independent mobile signal accounts for day length control of tuberization in these plants.

We next tested whether endogenous *SeSP3D* was involved in tuber induction of *S. etuberosum*/E109 heterografts. To this end, we

(C) Flowering induction in *StSP3Dox* stolons under LD conditions. Scale bars, 100 μm.
(D) Phenotypes of E109 and *StSP3Dox1* tubers after dormancy release. Most *StSP3D*-overexpressing tuber sprouts formed secondary tubers. Scale bars, 1 cm (upper panels), 5 cm (lower panels).
(E) Phenotypes of tuber-derived E109 and *StSP3Dox* plants at three weeks after planting of sprouting E109 and *StSP3Dox* transgenic tubers. Scale bar, 5 cm.
(F) qRT-PCR analysis of *StSP5G*, *StSP6A*, and *StMADS1* gene expression in leaves of E109 and *StSP3Dox* plants grown under LD conditions (5 weeks). Light (70 μmol m^{-2} s^{-1}) and dark conditions are indicated by white and gray backgrounds, respectively. Data are shown as mean ± SD of three independent biological replicates.
(G) Expression analysis of *StSP5G*, *StSP6A*, and *StFTL1* in non-swelling stolons (NS) or swelling stolons (SS) of E109 and *StSP3Dox* plants after 35 days of growth under LD conditions. Data are shown as mean ± SD of three independent biological replicates. The lowercase letters above the bars indicate significant differences among means (one-way ANOVA, followed by Tukey's multiple comparison test, $p < 0.05$).

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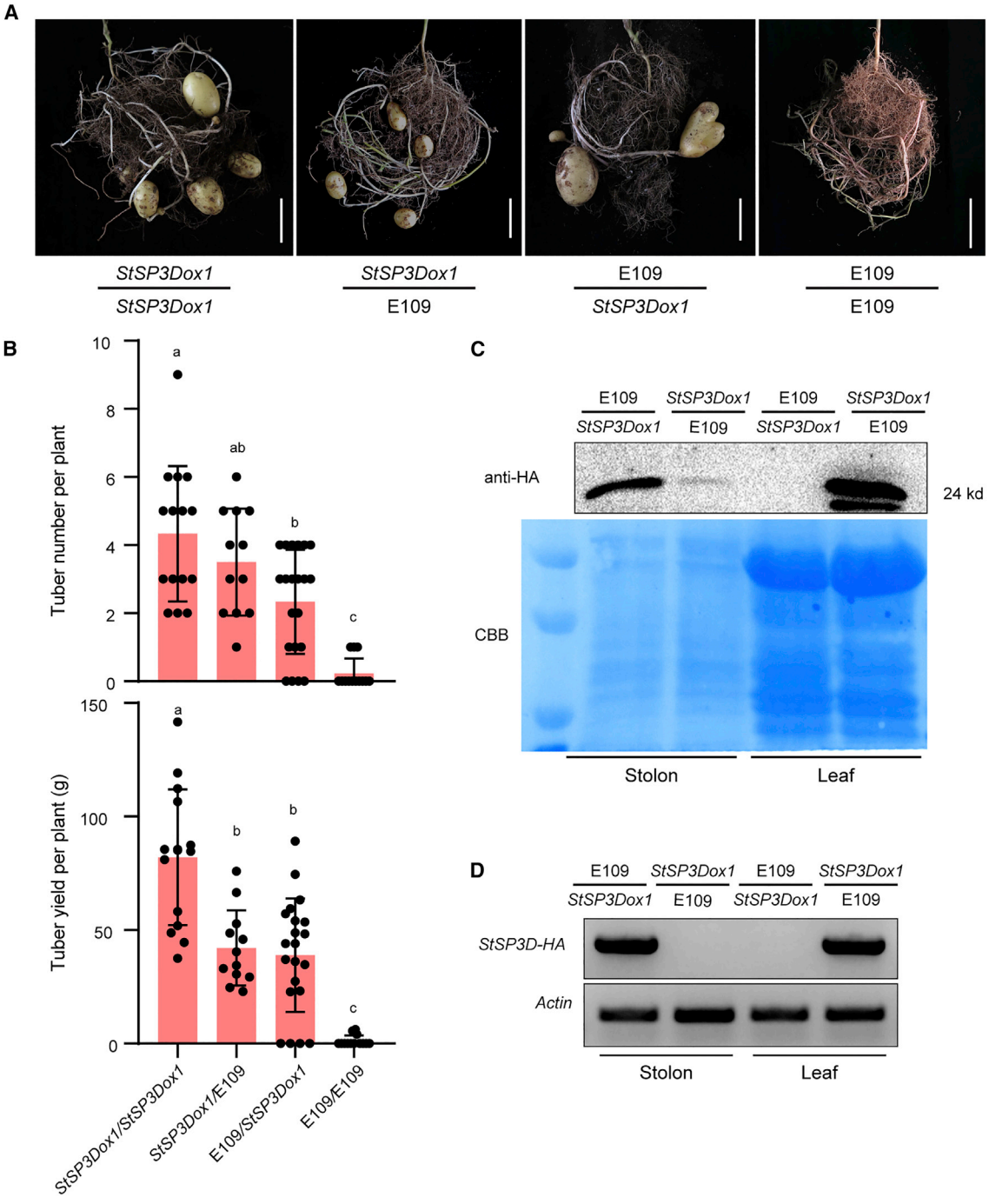

Figure 3. Graft-transmissible induction of potato tuber formation by StSP3D.

(A) Tuber formation of grafted plants under long day (LD) conditions 12 weeks after grafting. Scale bars, 5 cm.

(B) Tuber number and tuber yield of grafts between *StSP3Dox* and E109 (wild-type) plants grown for 12 weeks under long-day conditions. Data are shown as mean ± SD, n ≥ 12. The lowercase letters above the bars indicate significant differences among means (one-way ANOVA, followed by Tukey's multiple comparison test, $p < 0.05$).

(C) StSP3D-HA protein was detected using an anti-HA antibody in leaves and stolons of the grafted plants, 1 month after grafting. Coomassie brilliant blue (CBB) staining was used as a loading control.

(D) *StSP3D-HA* transcript levels were analyzed by RT-PCR in leaves and stolons, 1 month after grafting. Actin was used as a control.

knocked out the *SeSP3D* gene using CRISPR/Cas9 technology. We generated several knockout homozygous mutants (Figure 5D and Supplemental Figure 2G and 2H) that failed to show the flowering transition under normal growth conditions (Supplemental Figure 7C). When shoots of *CR-SeSP3D* lines or wild-type *S. etuberosum* were grafted onto E109 rootstocks, the *CR-SeSP3D*/E109 grafts exhibited significantly delayed tuber formation under LD conditions compared with *S. etuberosum*/E109

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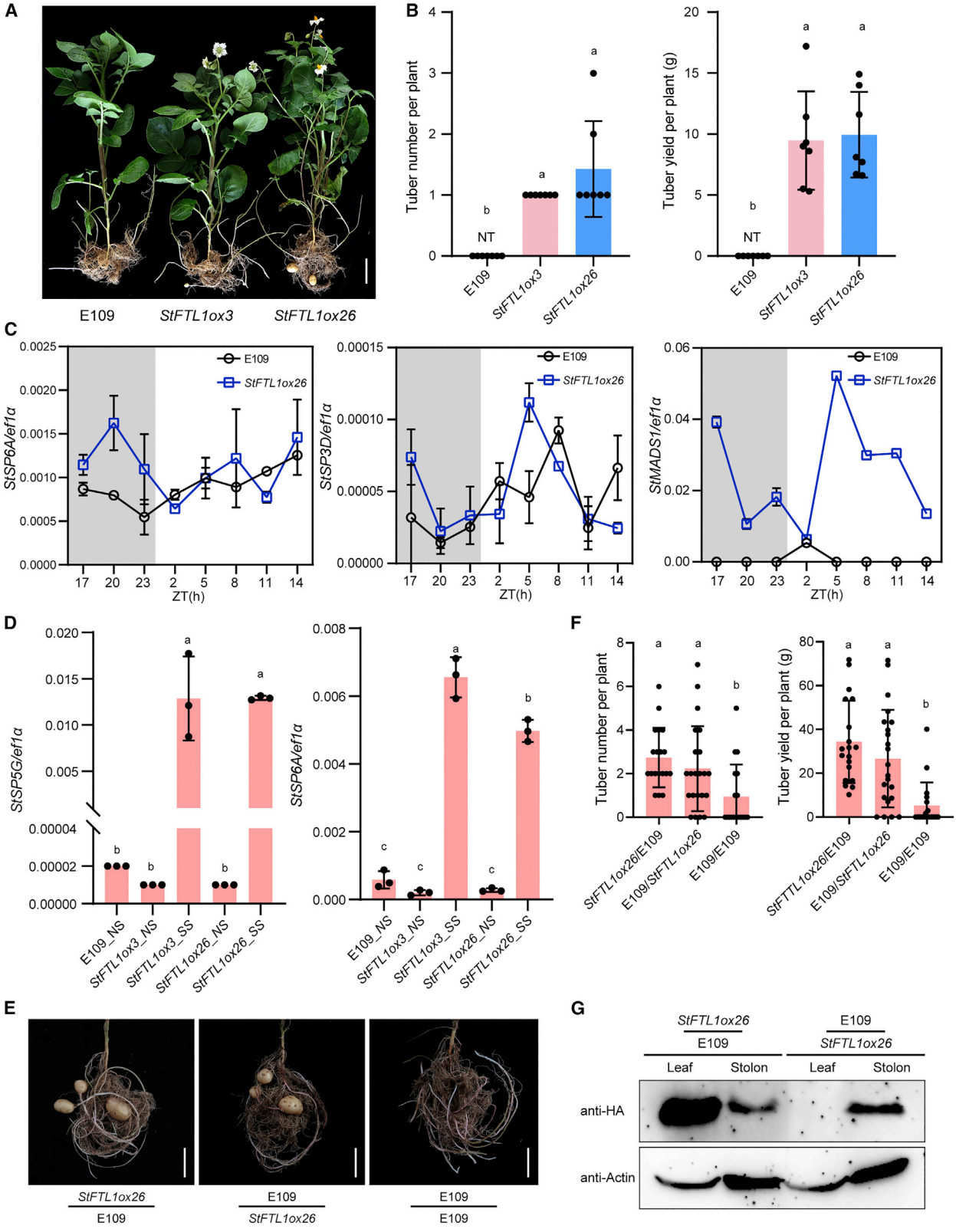

Figure 4. Expression of *StFTL1* promotes tuber formation under non-inducing LD conditions.

(A) LD tuberization phenotype of *StFTL1*-overexpressing plants (*StFTL1ox*). E109 plants do not tuberize. Forty days under long-day (LD) conditions. Scale bar, 5 cm.

(B) Tuber number and tuber yield of *StFTL1ox* plants. NT, no tubers. Ten weeks under long-day conditions. Data are shown as mean ± SD, n = 7 each. The lowercase letters above the bars indicate significant differences among means (one-way ANOVA, followed by Tukey's multiple comparison test, $p < 0.05$).

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grafted plants (Figures 5E and 5F), suggesting that endogenous *SeSP3D* is indeed necessary for tuber induction independent of leaf-expressed SP6A. Taken together, these results suggest that leaf-expressed SP6A is dispensable for SP3D induction of tuber formation. In addition, we used the *sft* mutant in the cultivated tomato (*S. lycopersicum*) Ailsa Craig (AC57) background (*SP6A* inactivated, Sato et al., 2012) as a scion for grafting onto E109 rootstocks (Supplemental Figure 9) and observed that both AC57/E109 and *sft*/E109 grafts tuberized under LD conditions. Thus, it will be interesting in future studies to test whether SD-activated *SlFTL1* is responsible for tuber induction of the potato rootstock plants.

Transcriptomic regulatory patterns of *SP3Dox* and *StFTL1ox* in non-swelling and swelling stolons

To gain a better understanding of the molecular mechanisms of tuber formation under non-inductive LD conditions, we analyzed the transcriptional profiles of *StSP3Dox1* and *StFTL1ox26* lines during tuberization by RNA sequencing. To this end, we compared non-swelling stolon tips (NS) and swelling stolon tips (SS) of the transgenic lines to non-swelling stolon tips of E109 (Figure 6A). A total of 27 880 expressed genes (genes with low expression were removed) were identified among the 52 953 annotated protein-coding genes (Supplemental Data 1). Principal component analyses (PCA) of global relative relationships among the various samples showed a clear separation among the three organs (Supplemental Figure 10), consistent with them being quite distinct. However, a strong correlation was observed for *StSP3Dox*-induced and *StFTL1ox*-induced transcriptomes (Figure 6B). Indeed, common differentially expressed genes (DEGs) obtained from the four comparisons (Figure 6A) showed that most *StSP3D*-responsive genes were also coregulated by *StFTL1* (Figure 6C).

To identify coregulated genes of interest, we defined DEGs (Supplemental Data 2) specific to the non-swelling stage (NS DEGs), DEGs shared by non-swelling and swelling stages (NS and SS DEGs), and DEGs specific to the swelling stage (SS DEGs). Gene ontology (GO) enrichment analysis of up- and downregulated DEGs revealed that the GO terms response to abiotic stimulus and transcription regulator activity were significantly enriched among the DEGs upregulated in NS (Figure 6D), whereas structural molecule activity, cytoskeleton, and cell cycle were enriched among the upregulated SS DEGs. By contrast, the GO terms cell communication, response to light stimulus, and circadian rhythm were enriched among the downregulated NS or SS DEGs. Notably, the StSP6A-regulated *AGAMOUS like* genes *StMADS1* and *StMADS13* were induced before stolon swelling, whereas *StSP6A*, *StSP5G-B*, and *StGA2ox1* were significantly upregulated only in swelling stolons (Figure 6B, 6E, and 6F), showing that induction of *StMADS1* and *StMADS13* by StSP3D and StFTL1 occurs prior to that of *StSP6A* and *StSP5G-B*.

We next studied the expression patterns of genes with high fold changes in more detail (Figure 6E). Genes encoding the flowering-related *LEAFY* (*LFY*) and *SQUAMOSA promoter-binding protein-like 5* (*SPL5*) transcription factors (Lee et al., 2008; Wang et al., 2009) were upregulated only in the non-swelling stage, whereas *phytochrome interacting factor* 3 (*PIF3*) and *jasmonate-zim-domain protein* (*JAZ-domain protein*) were strongly upregulated in the swelling stage. In addition, *ETHYLENE INSENSITIVE3* (*EIN3*) was downregulated at the non-swelling and swelling stages. This protein is a key regulator of apical hook development, and *ein3 eil1* double mutants are defective in apical hook development (An et al., 2012; Zhang et al., 2014). Opening of the stolon apical hook is one of the earliest phenotypic indications of the stolon-to-tuber transition, and *StSP6A RNAi* plants show an increased apical hook angle, which is reduced in *SUC2:StSP6A* lines (Abelenda et al., 2019). Thus, downregulation of the *E1N3* family protein by StSP3D and StFTL1 is also likely to have a role in inducing the opening of the stolon apical hook.

StSP3D and StFTL1 interact with StSP6A-binding proteins

In *Arabidopsis* and tomato, FT has been shown to be transported via the phloem to the shoot apical meristem, where it interacts via 14-3-3 proteins with the bZIP transcription factor FD to form a floral activator complex (FAC) that activates the downstream *MADS-box* genes to trigger floral development (Abe et al., 2005; Wigge et al., 2005; Jung et al., 2012; Lifschitz et al., 2006). Since StSP6A was established as a main component of tuberigen in potato, several StSP6A-binding proteins that regulate tuber development have been identified (Abelenda et al., 2019; Jing et al., 2022b; Nicolas et al., 2022; Teo et al., 2017). We selected the components of the TAC (Teo et al., 2017) and alternative aTAC (Jing et al., 2022b) to test whether StSP3D and StFTL1 interact with these StSP6A partners. First, we investigated the interactions of StSP3D/StFTL1 with StFDL1, StABL1, and a 14-3-3 isoform (St14f) in yeast (Supplemental Figure 11A). Like StSP6A, both StSP3D and StFTL1 interacted with St14f, but only StSP6A showed a strong interaction with StFDL1 in yeast. Both StSP3D and StSP6A interacted with StABL1, but StFTL1 did not. Bimolecular fluorescence complementation (BiFC) assays in tobacco leaf cells showed results similar to those reported for StSP6A: StSP3D and StFTL1 were able to

(C) Diurnal oscillations of *StSP6A*, *StSP3D*, and *StMADS1* in E109 (wild-type) and *StFTL1ox* plants under LD conditions (4 weeks). Light (250 μmol m^{-2} s^{-1}) and dark conditions are indicated by white and gray backgrounds, respectively. Data are shown as mean ± SD of three independent biological replicates.

(D) Expression analysis of *StSP5G* and *StSP6A* in non-swelling stolons (NS) or swelling stolons (SS) of E109 and *StFTL1ox* plants grown under LD conditions (5 weeks). Data are shown as mean ± SD, n = 8 each. The lowercase letters above the bars indicate significant differences among means (one-way ANOVA, followed by Tukey's multiple comparison test, $p < 0.05$).

(E) Tuber formation of grafts under LD conditions (12 weeks). Scale bars, 5 cm.

(F) Tuber number and tuber yield of grafts between *StFTL1ox* and E109 (wild-type) plants grown under LD conditions for 12 weeks. Data are shown as mean ± SD, n ≥ 12. The lowercase letters above the bars indicate significant differences among means (one-way ANOVA, followed by Tukey's multiple comparison test, $p < 0.05$).

(G) StFTL1-HA protein was detected using an anti-HA antibody in leaves and stolons, 1 month after grafting. Anti-actin was used as a loading control.

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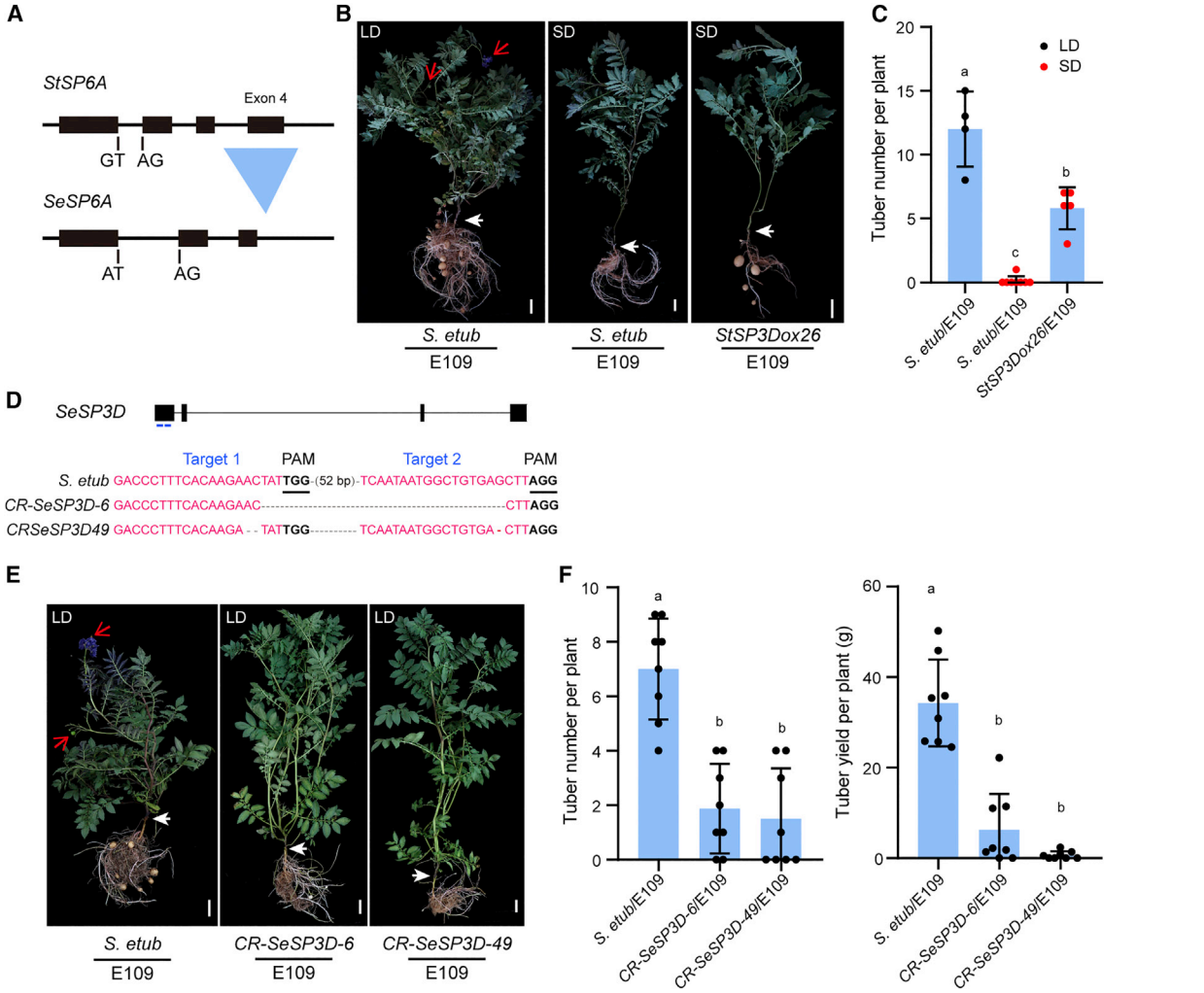

Figure 5. SP3D induces tuber formation independently of leaf-expressed SP6A.

(A) Gene architecture of *SP6A* in potato and *S. etuberosum*. The "G" to "A" mutation in the GT-AG sequence in the first exon–intron junction of *SeSP6A* and the deletion of the whole fourth exon are shown.

(B) Tuberization phenotypes of E109 stocks grafted with *StSP3D*-overexpressing (*StSP3Dox26*) and wild-type *S. etuberosum* scions under LD and SD conditions (8 weeks after grafting). Red arrows indicate fruit or open flowers; white arrows indicate graft unions. Scale bars, 5 cm.

(C) Tuber numbers of 12-week-old E109 stocks grafted with *StSP3D*-overexpressing *S. etuberosum* scions (*StSP3Dox26*) shown in **(B)** under LD and SD conditions. Black dot, LD; red dot, SD. Data are shown as mean ± SD, n ≥ 4. The lowercase letters above the bars indicate significant differences among means (one-way ANOVA, followed by Tukey's multiple comparison test, $p < 0.05$).

(D) The first exon of *SeSP3D* was targeted by CRISPR/Cas9 using two single-guide RNAs (sgRNA). Blue lines, sgRNAs. Sequences of wild-type and *CR-SeSP3D* alleles (*CR-SeSP3D-6* and *CR-SeSP3D-49*) are presented. sgRNA targets and protospacer-adjacent motif (PAM) sequences are indicated by blue and dark lines, respectively. Deletions are indicated by dark dashes.

(E) Tuberization phenotypes of E109 stocks grafted with *CR-SeSP3D S. etuberosum* scions (*CR-SeSP3D-6* and *CR-SeSP3D-49*) under LD conditions (8 weeks). Red arrows indicate fruit or open flowers; white arrows indicate graft unions. Scale bars, 5 cm.

(F) Tuber number and tuber yield of grafts between *CR-SeSP3D/S. etuberosum* scions and E109 (wild-type) plants after 8 weeks of growth under LD conditions. Data are shown as mean ± SD, n = 8 each. The lowercase letters above the bars indicate significant differences among means (one-way ANOVA, followed by Tukey's multiple comparison test, $p < 0.05$).

interact with St14f, StFDL1, and StABL1. The BiFC signal for the StSP3D/StFTL1–St14f interaction was predominantly observed in the cytoplasm (Supplemental Figure 11B), whereas the interaction of StSP6A/StSP3D/StFTL1 and StFDL1/StABL1 was observed in the nucleus. Thus, mobile StSP3D and StFTL1 proteins probably promote tuberization in stolon tips in a manner similar to that of long-distance transported StSP6A.

DISCUSSION

Potato, a model for geophytic species (Khosa et al., 2021), has evolved an additional reproductive strategy to survive and propagate under extreme climate conditions through the formation of vegetative storage organs called tubers. Potato is cultivated worldwide for these starch-rich organs, which are used as a staple food in many countries. The adaptation to LD

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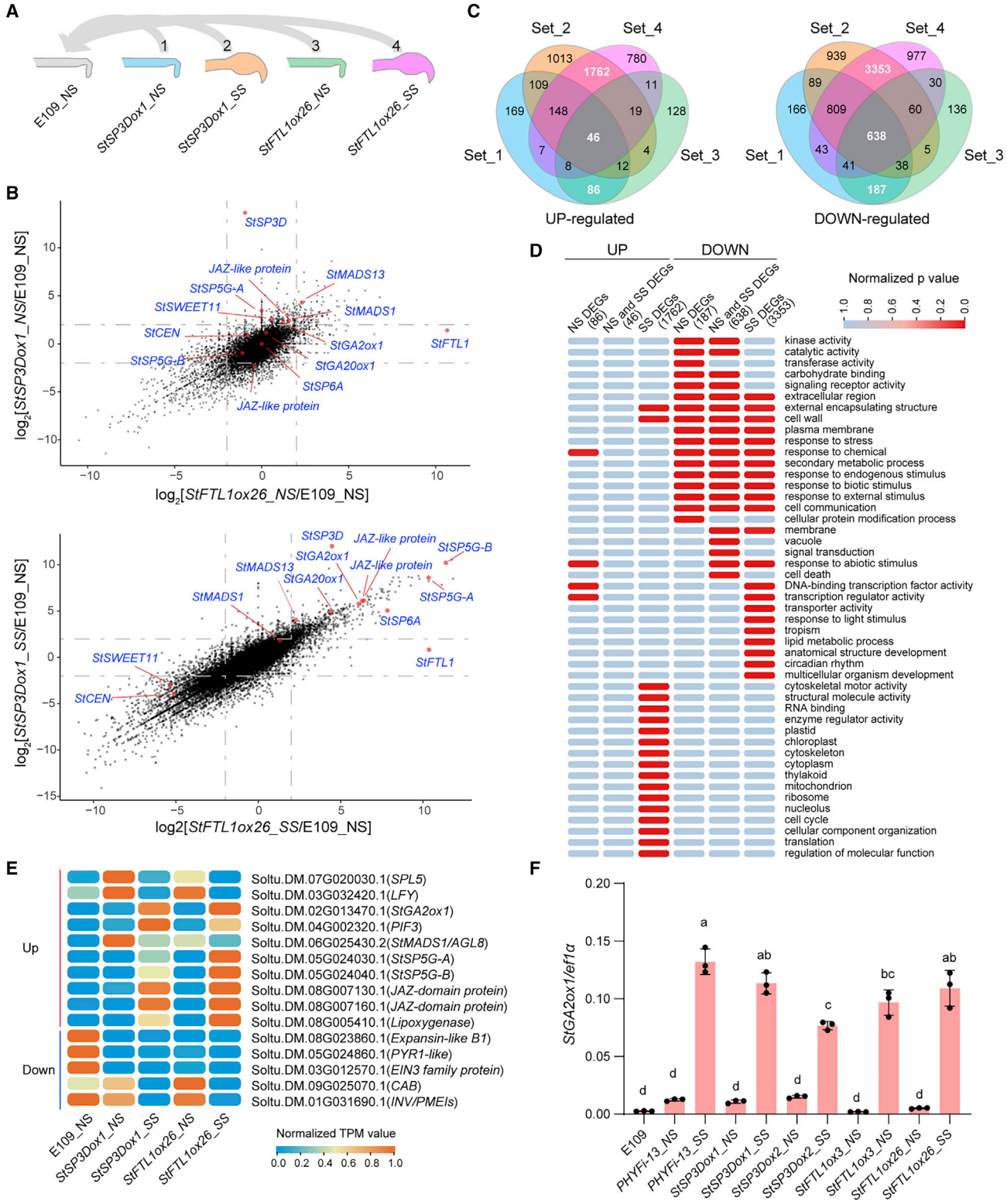

Figure 6. Transcriptome analysis at different tuber developmental stages.

(A) Diagram showing the non-swelling and swelling stolon tips used for RNA-seq. The comparisons performed are numbered. E109_NS, E109 non-swelling stolons; *StFTL1ox26_NS*, *StFTL1ox26* non-swelling stolons; *StFTL1ox26_SS*, *StFTL1ox26* swelling stolons; *StSP3Dox1_NS*, *StSP3Dox1* non-swelling stolons; *StSP3Dox1_SS*, *StSP3Dox1* swelling stolons.

(B) Identification of genes with *StSP3Dox*- and *StFTL1ox*-dependent expression changes. *x* axis, log2 [ratio of gene expression in *StFTL1ox26_NS/SS* and E109_NS]; *y* axis, log2 [ratio of gene expression in *StSP3Dox1_NS/SS* and E109_NS]. Genes related to tuberization are indicated in color.

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tuberization from the SD-dependent progenitor *S. tuberosum* Group Andigena greatly contributed to the geographic expansion of potato (Gutaker et al., 2019). Day length is detected by the leaves, which produce a mobile stimulus that is transported to the shoot apex or underground stolons to induce flowering or tuber formation. Given the importance of tubers as a food source, identification of StSP6A in the past decade has promoted extensive research aimed at uncovering the mechanisms that underlie storage organ formation.

In the present study, we have shown that overexpression of SD-activated *StSP3D* and *StFTL1* genes promotes photoperiod-independent tuberization, and this effect is graft transmissible, suggesting that StSP3D and StFTL1 also function as long-range tuber-inducing signals. Previously, characterization of *StSP3D* showed that *StSP3D*-silenced plants tuberized at the same time as untransformed wild-type plants under SD conditions, but they exhibited a clear delay in floral transition under LD conditions (Navarro et al., 2011; Teo et al., 2017). In fact, *StSP3D*, *StFTL1*, and *StSP6A* transcripts are all SD-activated in leaves, although the activation of *StSP3D* expression is weaker than that of *StFTL1* and *StSP6A*. Thus, a partially overlapping tuber-inducing role of *StSP3D*, *StFTL1*, and *StSP6A* in leaves under SD conditions cannot be fully ruled out. Consistent with this notion, we found that *SeSP6A* of the wild *Solanum* species *S. etuberosum* is naturally inactivated, as also reported recently (Tang et al., 2022). However, when *S. etuberosum* plants were used as scion donors, they were still able to induce tuber formation in potato rootstock under LD conditions. Modern potato cultivars are facultative SD plants, but tuberization is still accelerated under SD conditions (Gutaker et al., 2019). Furthermore, our finding that overexpression of *StSP3D* in *S. etuberosum* promoted tuberization of E109 stock plants under SDs suggests that leaf-expressed SP6A is dispensable for SP3D induction of tuber formation. In addition, our use of CRISPR/Cas9 to engineer *SP3D* mutations in the *S. etuberosum* background (*SeSP6A* inactivated) showed that SP3D actually acts as a mobile tuber-inducing signal independently of leaf *SP6A* expression . However, it is be possible that StSP6A is the normal "real" tuberigen in potato but that other FT homologs (StSP3D and StFTL1) have, for evolutionary reasons, high similarity to StSP6A and thus share some of its tuber-inducing abilities, perhaps serving as a safety net or fallback system to ensure proper tuberization. We thus hypothesize that all SD-activated *FT-like* genes may contribute to the robustness of SD-responsive tuberization, thus enabling potato to adapt this developmental response to different latitudes. Similarly, *GmFT2a* and *GmFT5a* together regulate flowering time in soybean, and the effect of *GmFT2a* is more prominent than that of *GmFT5a* under SD conditions, whereas *GmFT5a*, not *GmFT2a*, has been found to be essential for soybean adaption to high latitude regions (Cai et al., 2020).

Overexpression of either *StSP3D*, *StSP6A*, or *StFTL1* promotes tuber formation under non-inductive LD conditions. However, we found that mutation of *SeSP3D* in *S. etuberosum* (*SP6A* inactivated) did not fully block tuberization of *S. etuberosum*/E109 grafts under LD conditions. Likewise, the tomato *sft* mutant (*SP6A* inactivated (Sato et al., 2012)) scion can induce tuber formation in E109 rootstocks (Supplemental Figure 9). Thus, another study using intact potato CRISPR mutants will be required in the future to assess whether these tuber-inducing *FT* homologs genes are redundant and essential for potato tuberization. In addition, the expression either of *StSP3D*, *StSP6A*, or *StFTL1* can induce *StSP6A* expression in stolons, and thus, are involved in the positive feedback loop enhancing *StSP6A* expression in stolons (Navarro et al., 2011). Tuberization onset was previously reported to be delayed in *Hd3a*/*StSP6A RNAi* grafts relative to *Hd3a*/wild-type grafted controls (Navarro et al., 2011), and thus, the lack of *StSP6A* may interfere with the tuber-inducing activity of the phloem-transported StSP3D and StFTL1 proteins, leading to developmental epistasis (Hendelman et al., 2021). However, what exactly makes StSP6A unique leaves room for future research. Interestingly, we noticed that *StSP3D* expression was also weakly induced in wild-type stolon tips at tuber initiation (Figure 1B) and in *PHYF*-silenced (Figure 1D) and *StFTL1ox* lines (Figure 6B). This secondary *SP3D* activation has been also described in the tomato shoot apical meristem (SAM) (Meir et al., 2021), and thus, it is possible that activation of this gene in the stolon tip by phloem-transported tuber-forming FT-like signals acts in part redundantly with the activation of *StSP6A*.

Members of the *PEBP* gene family have undergone preferential duplication and subfunctionalization in *Solanaceae* (Abelenda et al., 2014; Navarro et al., 2015). However, it seems that they still act in concert to modulate the induction of day length-dependent flowering/tuberization. In tomato, disruption of *SlSP5G* and *SlFTL1* in cultivated tomato results in day-neutral flowering and leads to upregulated *SlSP3D*/*SFT* expression under LD or SD conditions (Song et al., 2020). Here, expression of both *StSP3D* and *StSP6A* was upregulated in *PHYFi* lines, whereas that of *StFTL1* was suppressed, possibly due to the feedback inhibition of increased StSP6A and StSP3D expression. In this regard, the mRNA level of the endogenous *StSP6A* transcript is decreased in *StSP6A*^{cop}-overexpressing transgenic plants (Lehretz et al., 2019). On the contrary, from the *StFTL1* expression pattern, it is evident that this gene has a different function in stolons than *StSP3D* or *StSP6A*. Furthermore, *StFTL1* seemed to respond specifically to SD conditions, at least in the genotype used in this study. Consistent with this observation, *SlFTL1*, the ortholog of *StFTL1* in tomato, has been reported to respond specifically to SD conditions, with its loss-of-function leading to impaired SD sensitivity (Song et al.,

(C) Venn diagram of the numbers of differentially expressed genes (DEGs) in the comparisons shown in **(A)**. Differentially expressed genes were identified using DESeq2 (adjusted $p \leq 0.05$, Log2Fold change ≤ -1 or ≥ 1).

(D) Gene Ontology (GO) enrichment analyses for the upregulated and downregulated DEGs shown in **(C)**. DEGs specific to non-swelling stages are defined as NS DEGs, DEGs shared by non-swelling and swelling stages are defined as NS and SS DEGs, and DEGs specific to swelling stages are defined as SS DEGs. Light blue, not significantly enriched (assigned $p = 1$). Red, significantly enriched (adjusted $p < 0.05$). UP and DOWN represent genes significantly upregulated or downregulated, respectively. The number represents the number of DEGs shown in **(C)**.

(E) Expression patterns of genes with high fold changes.

(F) qRT-PCR analysis of *StGA2ox1* expression in E109, *PHYFi-13_NS*, *PHYFi-13_SS*, *StSP3Dox_NS*, *StSP3Dox_SS*, *StFTL1ox_NS*, and *StFTL1ox_SS* lines. Data are shown as mean ± SD, n = 3 each. The lowercase letters above the bars indicate significant differences among means (one-way ANOVA, followed by Tukey's multiple comparison test, $p < 0.05$).

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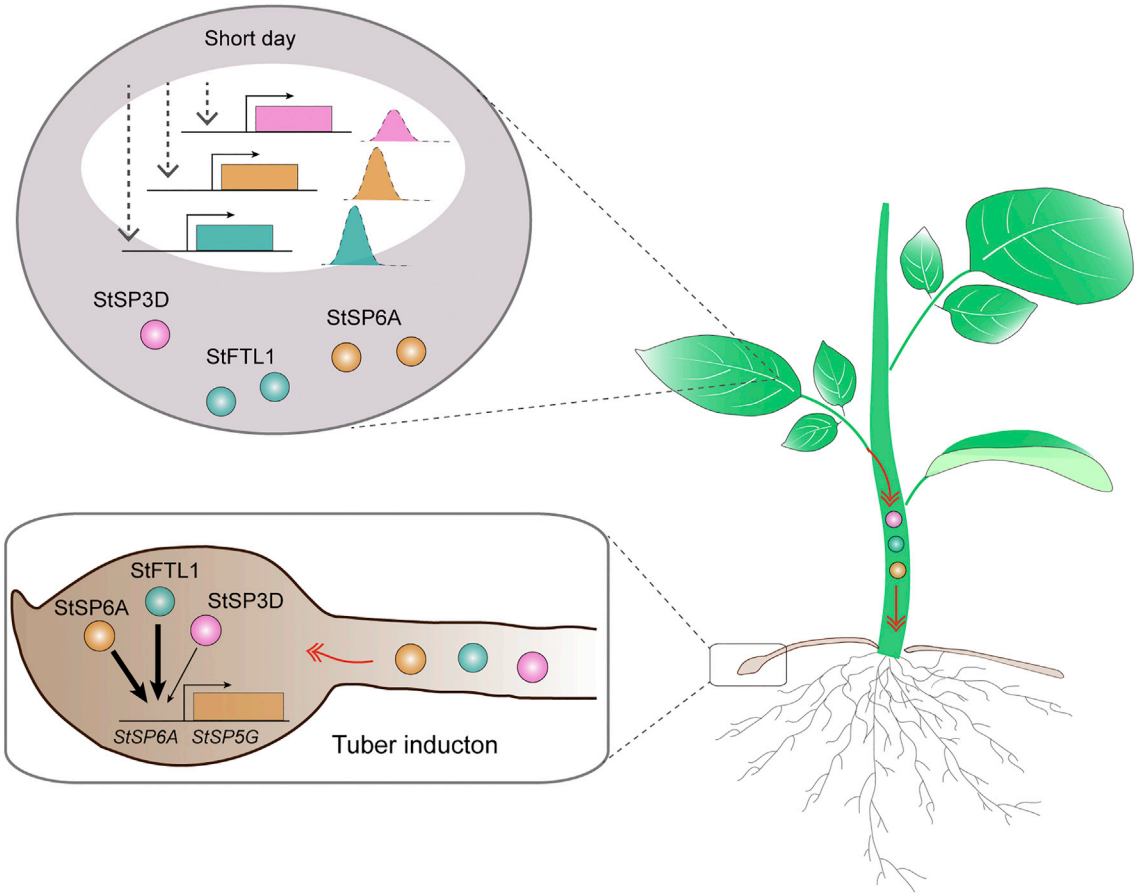

Figure 7. Proposed model for photoperiodic induction of tuberization by FT-like mobile proteins.
Under short-day conditions, *StSP6A*, *StSP3D*, and *StFTL1* are induced and their proteins synthesized in leaves. These mobile signals are transported to the stolons, where they participate in secondary activation of *StSP6A* and expression regulation of other tuberization-related genes to induce tuber formation.

2020). Furthermore, we observed that the expression of potato *FT-like* genes is regulated in an organ-specific manner. Similar to the *StSP5G-StSP6A* module, the tandemly arranged *StSP5G-like* and *StFTL1* paralogs show inverse expression patterns in leaves, but are coregulated in stolon tips (Figure 1B), suggesting that these potato FT-like proteins form different interaction/transcriptional regulatory complexes in leaves and stolons. Furthermore, by RNA-seq analysis, we showed that StSP3D and StFTL1 suppress the expression of the newly identified *EIN3 family protein* factor in non-swelling and swelling stolons, which possibly contributes to the opening of the stolon apical hook (Abelenda et al., 2019).These identified NS DEGs and SS-specific DEGs are possible targets for FT-mediated signaling and the initiation of tuber development.

Finally, *SlSP3D/SFT*, the tomato ortholog of *StSP3D*, drives enhanced heterosis yield via the dosage-dependent inhibition of *sp*-mediated meristem termination (Krieger et al., 2010; Jiang et al., 2013). Thus, it will be important to examine in future studies whether different allelic combinations of these tuber-inducing *FT-like* genes drive similar heterosis in potato. In summary, our results uncover the function of SD-induced StSP3D and StFTL1 as long-range tuber-inducing signals in potato. Furthermore, StSP3D and StFTL1-induced tuber formation may partly interact with StFDL1 and StABL1 in stolon tips (Figure 7), which are phloem-transported from leaves to stolons, where they activate the expression of *StSP6A* to initiate tuber formation. Knockdown of *StSP3D* delays floral transition under LD conditions, relative transport of this protein to the apical meristem and stolons, as well as the dose-dependent effects on flowering and tuberization, presumably mediating the pleiotropic effects of this *FT* ortholog. In this way, the control of flowering, tuber induction, and meristem termination by these *FT-like* genes makes them the prime targets for potato genetic improvement.

METHODS

Plant material, growth conditions, and phenotyping

The tetraploid potato cultivar E-potato-109 (E109), which does not form tubers under LD conditions as described previously (Zhou et al., 2019), and the non-tuber-bearing wild species *Solanum etuberosum* were used in the present study. Plants were propagated *in vitro* on MS medium supplemented with 3% sucrose (m/v) using a single stem node under LD conditions (16 h light/8 h dark) at 20°C. For phenotyping of transformed lines and wild-type plants in the growth room, 3-week-old *in vitro* potato plants were transplanted into pots with a diameter of 10 cm (one plant per pot) and grown under LD conditions (16 h light/8 h dark) with light/dark temperatures of 23°C/20°C. Each experiment was performed with three replicates of eight pot-grown plants. For

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time-course gene expression analyses, plants were grown in the growth room until they reached the 10-leaf stage (5-week-old plants). The fifth leaf from the shoot apex was sampled. Three plants were sampled at 3-h intervals for 24 h, and samples were directly frozen in liquid nitrogen and stored at −80°C until use. For gene expression analysis of stolons, 0.5–1-cm non-swelling stolon (NS) tips and swelling stolon (SS) tips were sampled, directly frozen in liquid nitrogen, and stored at −80°C until use. All plants were grown under a normal light intensity (250 μmol m^{-2} s^{-1}), except for *StSP3Dox plants used for* time-course gene expression analysis (70 μmol m^{-2} s^{-1}), as these transgenic plants exhibited much slower shoot growth. For phenotyping in the net house, 4-week-old plants grown in the growth room or sprouting tubers were planted in pots with a diameter of 25 cm (one plant per pot). The final harvest was performed at 12 weeks after planting.

Cloning and vector construction

All genes in this study were amplified by polymerase chain reaction (PCR) using Phanta Super-Fidelity DNA Polymerase (Vazyme) according to the manufacturer's instructions. Primers used for amplification are shown in Supplemental Table 1. For TA cloning, the PCR products were cloned using the ClonExpress Ultra One Step Cloning Kit (https://www.vazyme.com/, C115-01). To create 35S-promoter-driven *StSP3D*-overexpression (*StSP3Dox*) and *StFTL1*-overexpression (*StFTL1ox*) transgenic plants, the coding sequences of *StSP3D* and *StFTL1* were amplified from complementary DNA (cDNA) of E109 plants, inserted into pH7LIC8.0-ccdB-C-HA, and digested with *Stu*I to generate *35S:StSP3D-3×HA* and *35S:FTL1-3×HA* using Exnase II (Vazyme) according to the manufacturer's recommendations. The vectors were introduced into *Agrobacterium tumefaciens* strain GV3101 and transformed into E109, *Arabidopsis* Col-0, or *Solanum etuberosum.*

To create knockout mutants, *SeSP3D* mutagenesis by CRISPR-Cas9 was performed by transforming plants with the PJCV55 vector (Jing et al., 2022a), which contains two reported guide RNAs (Song et al., 2020). For CRISPR/Cas9 vector construction, mutagenesis was confirmed by TA cloning, sequencing, and restriction enzyme digestion.

Transgenic plant generation

For potato genetic transformation, plasmids were introduced into *Agrobacterium tumefaciens* strain GV3101, which was then used for transformation of the E109 line as described previously (Jing et al., 2022b). For *Solanum etuberosum* transformation, healthy fully expanded leaves were collected from 4-week-old *in vitro* plantlets, and 2 or 3 cuts were made along the middle rib with a razor blade. Leaves were placed upside down in co-cultivation medium (MS medium with 2.3 g/L Phytagel supplemented with 30 g/L sucrose, 2 mg/L 6-BA, 0.2 mg/L NAA, and 0.05 mg/L GA_3) for two days under dark conditions at 25°C. *A. tumefaciens* GV3101 with the recombinant T-DNA vector was cultured at 28°C in 10 mL of LB medium with antibiotics overnight, and a 1-mL culture was further cultured at 28°C in 50 mL of LB medium with antibiotics until reaching an OD_{600nm} of 0.6–0.8. Bacterial cells were then collected by centrifugation at 4000 *g* for 8 min and resuspended in 15 mL of liquid MS medium with 30 g/L sucrose. The *A. tumefaciens* suspension was incubated with pre-treated leaf explants for 10 min. After inoculation, explants were dried on sterile filter paper and then transferred to co-cultivation medium for two days under dark conditions at 25°C. After 48 h of incubation in the dark at 25°C, the explants were transferred to callus and shoot induction medium (MS medium with 2.3 g/L Phytagel supplemented with 30 g/L sucrose, 2 mg/L 6-BA, 0.2 mg/L NAA, 0.05 mg/L GA_3, 50 mg/L kanamycin, and 200 mg/L Timentin) and grown under LD conditions at 22°C for 4 weeks, during which time explants were transferred to fresh callus and shoot induction medium every two weeks. When the regenerated shoots reached 2–3 cm in length, they were transferred to rooting medium (MS medium with 2.3 g/L Phytagel supplemented with 50 mg/L kanamycin and 200 mg/L Timentin).

RNA extraction and qRT-PCR

Total RNA was extracted from frozen samples using the Total RNApure Kit (ZOMANBIO, http://zomanbio.com). Reverse transcription into complementary DNA was performed using the 5× All-in-One RT MasterMix reverse transcription kit (ABM, http://www.abmgood.com). The quality of the cDNA was evaluated using actin primers within 2 adjacent exons. Quantitative RT-PCR was performed on the LightCycler 480 II system (Roche, Switzerland) with EvaGreen 2× qPCR MasterMix (ABM). The potato *Ef1α* gene was used as the control gene for expression normalization (Nicot et al., 2005). Gene expression levels were calculated by the $2^{-\Delta Cq}$ method. All primer sequences for qRT-PCR analysis are described in Supplemental Table 1.

Grafting

For grafting experiments, 3-week-old plants grown under LD conditions were used, and scions were prepared by cutting each stem below the second or third leaf from the apex using a new razor blade. Samples were immediately placed in water to prevent formation of air bubbles in the xylem and subsequent dehydration. Stocks were prepared similarly by cutting the stem at about 5 cm above the soil and removing the lower leaves. A drop of water was applied to the cut stem to prevent water loss. The scion to be grafted was then placed on top of the stock, and the two sections were held in place by binding them together with a grafting clip. Grafts were covered for at least one week with a plastic cup humidified by spraying the inside with water. Cups were sprayed with water for the first 2–3 days and removed one week later. Grafts were grown for another week in the growth room under LD conditions and then transferred to larger pots with a diameter of 20 cm (one plant per pot) for tuberization under LD or SD conditions. Tuber number and tuber yield were quantified 8–12 weeks after grafting.

Western blot analysis

Protein extraction from potato was performed as described previously (Abelenda et al., 2016). The potato protein samples and immunoprecipitates were separated on a 10% SDS-PAGE gel and transferred to a PVDF membrane, and the membrane was blocked in 5% milk in 1× TBS (150 mM NaCl, 10 mM Tris–HCl [pH 7.4]) with 0.05% (v/v) Tween-20 before hybridization with the primary antibodies anti-HA-tag mAb (MBL, M180-3) and anti-actin (Abmart, 26F7) at 1:3000 dilutions. The membrane was washed with 1× TBST before addition of the secondary antibody at a 1:3000 dilution (anti-IgG (H + L chain) (mouse) pAb-HRP, MBL). ECL detection was performed according to the manufacturer's recommendations.

RNA sequencing

Stolons at the non-swelling stage of E109 (wild-type) plants and the non-swelling and swelling stages (stage 3) of *StSP3Dox1* and *StFTL1ox26* plants were sampled for RNA sequencing. Stolon samples were collected after 35 days of growth under LD conditions. Total RNA was extracted from three independent replicates for each genotype. RNA quality and concentration were determined using a NanoDrop OneC spectrophotometer (Thermo Fisher Scientific). Two micrograms of total RNA was used for stranded RNA sequencing library preparation using the KC Stranded mRNA Library Prep Kit for Illumina (DR08402, Wuhan Seqhealth, China) according to the manufacturer's instructions. PCR products corresponding to 200–500 bp were enriched, quantified, and sequenced on the HiSeq X 10 system (Illumina). The raw data were cleaned (with adaptor and low-quality reads filtered out) using Trimmomatic (version 0.36) (Bolger et al., 2014). On average, more than 43.3 million reads with a mean Q30 of 92.6% were generated per sample. The clean reads were mapped to the *Solanum tuberosum* genome (version 6.1, http://spuddb.uga.edu/) using the transcript quantification tool Salmon (version 1.4.0) with validateMappings (Patro et al., 2017). Reads and transcripts per kilobase of exon model per million mapped reads (TPMs) were calculated. Genes with low expression (total counts in fifteen samples ≤10) were removed from further analysis. DEGs were identified using

DESeq2 (Love et al., 2014) (adjusted $p < 0.05$, fold change >2). Gene Ontology (GO) enrichment, Venn diagram construction, and heatmap analysis of the DEGs were performed using TBtools (version 1.0.98) (Chen et al., 2020).

Yeast two-hybrid assay

The full-length coding sequences of *StFTL1* and *StFDL1* were cloned into the *Eco*RI and *Sal*I sites of pGBKT7 using the primers listed in (Supplemental Table 1). The full-length coding sequence of *StFTL1* was amplified from E109 and cloned into the *Eco*RI and *Bam*HI sites of pGADT7. Other vectors were constructed previously (Jing et al., 2022b). Their pairwise combinations or the corresponding empty vectors were co-transformed into yeast strain AH109 using the BD Matchmaker Screening Kit according to the manufacturer's instructions.

BiFC

The full-length coding sequences of *StFTL1* and *StFDL1* were amplified using specific primers (Supplemental Table 1) and then cloned into the NYFP and CYFP vectors via restriction digestion with *Bam*HI and *Sal*I. Other vectors were constructed previously (Jing et al., 2022b). These constructs were transformed into *A. tumefaciens* strain GV3101 by electroporation, and the transformed strain was subsequently infiltrated into leaves of 1-month-old *N. benthamiana* plants. YFP fluorescence was observed 48 h after infiltration using a confocal laser scanning microscope (CLSM; TCS-SPE; Leica, http://www.leica.com) according to the manufacturer's instructions.

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Plant Communications Online*.

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AUTHOR CONTRIBUTIONS

S.J., P.J., X.S., L.Y., E.W., and J.Q. conducted the experiments. S.J. and B.S. designed the experiments. S.J., B.S., S.P., and F.Z. wrote the paper.

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