FT control of flowering and storage organ formation in potato

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Seasonal fluctuations in day length regulate important aspects of plant development such as flowering transition or, in potato, the formation of tubers. Day length is perceived by the leaves, which produce a mobile signal transported to the shoot apex or underground stems to induce flowering/tuberisation transitions. Work in *Arabidopsis,* tomato, as well as in rice identified the mobile FLOWERING LOCUS T (FT) protein as a main component of the long-range *florigen* signal[1-3]. Here, we show that expression of the FT rice *Hd3a* gene induces strict SD potato types[4] to tuberise in long days. Tuber induction is graft-transmissible and the Hd3a-GFP protein is detected in the stolons of grafted plants, transport of the fusion protein thus correlating with tuber formation. We provide evidence showing that potato floral and tuberisation transition is controlled by two different *FT*-like paralogs (*StSP3D* and *StSP6A*) that respond to independent environmental cues and show that an autorelay mechanism involving CONSTANS modulates expression of the tuberisation control *StSP6A* gene.

Potato, the third largest global food crop after wheat and rice, is cultivated for its underground storage stems or tubers, rich in starch and other nutrients. Short days (SD) and cool temperatures promote tuber formation, ensuring that differentiation of these vegetative propagation organs precedes winter. While cultivated potatoes derive from *Chilean* landraces more adapted to long-day (LD) conditions, *Andean* types such as *S. tuberosum* group *Andigena* tuberise only in SDs[5]. These plants require night periods above a critical length, a pulse of light during the night (*night break*) repressing tuberisation, as seen in strict SD flowering plants[6,7].

Day-length is perceived in expanded leaves, which synthesize a mobile signal or *tuberigen*, transported to the underground stems to induce tuber formation[4]. This long-distance signal shares several features with the mobile *florigen*, different evidences suggesting that related photoperiodic pathways may control synthesis of both signals[8]. In *Arabidopsis thaliana*, activation of the *FLOWERING LOCUS T* (*FT*) gene by CONSTANS (CO), mediates floral transition under LDs[9,10]. Transport of the FT protein from the leaf to the shoot apical meristem has been demonstrated in diverse ways[11-13], it being broadly accepted that this phloem mobile protein functions as the *florigen*. Closely related genes mediate also flowering control in rice (*Oryza sativa*) although in this SD plant, the CO ortholog *Heading date 1* (Hd1)[14] activates expression of the FT-like *Hd3a* gene under SDs, but represses its expression in LD conditions[15,16].

A CO-dependent pathway is also thought to mediate SD-tuberisation, as expression of *Arabidopsis CO* in *Andigena* plants delays tuber formation in SDs[17]. High light irradiance, however, induces *Andigena* potatoes to

flower in LDs, although these conditions are restrictive for tuberisation[8]. This LD flowering response seemed to argue against a role of FT in tuberisation, implicating an additional CO target as the mobile *tuberigen*. Here, we show that ectopic expression of the rice *Hd3a* gene induces *Andigena* plants to flower and tuberise under non-inductive LDs, demonstrating the potential of FT to act as the mobile *tuberigen*. We show, in addition, that flowering and SD-tuberisation responses are regulated by two members of the potato *FT*-like gene family that respond to different environmental cues.

To assess a role for FT in tuberisation, we transformed *Andigena* plants with the *rolC*::Hd3a-GFP construct, which in rice promotes floral transition in LDs[18]. Lines expressing this construct were induced to flower (Fig. 1c) and were able to tuberise in non-inductive LDs (Fig. 1a). When grafted to wild-type (wt) plants, these lines induced the wt controls to tuberise in LDs, independently of whether they were used as donors (*Hd3a* onto wt) or as stocks (wt onto *Hd3a*), while none of the control grafts (wt onto wt, Fig. 1d) tuberised in non-inductive LDs. We detected the Hd3a-GFP protein but not its transcript in the stolons of grafted wt stocks (Supplementary Fig. 1c and 1a), demonstrating that the protein but not the RNA can move across the graft junction and function as a powerful tuberisation inducer.

Studies in tomato identified six members of the *SELF-PRUNING* (*SP*) gene family[19] and sequencing of two diploid potato (RH and DM) genomes recently leaded to the identification of three additional *FT*/TFL family members[20]. Phylogeny of these genes grouped the *StSP6A*, *StSP5G,*

StSP5G-like and *StSP3D* homologues into the same clade as the Arabidopsis *FT*, tomato *SFT* and rice *Hd3a* genes (Supplementary Fig. 2). RT-qPCR revealed that these transcripts are expressed in leaves or stolons, whereas transcripts for SP/TFL1 and MFT members are more ubiquitously distributed (Fig. 1e). Interestingly, *StSP6A* gene expression strongly correlates with tuberisation, high levels of expression being observed in leaves and stolons of SD-induced plants and in antisense *phyB*-lines, with constitutive tuberisation[21] (Fig. 1f; see Supporting text 1). This expression profile suggests a role of this gene in tuberisation control, *StSP6A*ox lines being able to tuberise under non-inductive LDs (Fig. 2a, b) and induced to flower, although their flowering phenotype is less severe than in *Hd3a* lines (Supplementary Fig. 3a, b; see Supporting text 2). *StSP6A* silencing, in turn, strongly delays tuber formation in SDs (Fig. 2c, d), pointing to an essential role of this FT-like protein in tuberisation promotion. *StSP6A* expression analyses in commercial cultivars with early (*Jaerla*), late (*Baraka*) and intermediate (*Kennebec*) tuberisation periods, on the other hand, shows that accumulation of this transcript in leaves correlates with the tuberisation time of these cultivars (Supplementary Fig. 6), pointing to a tuberisation control role of this *FT* paralog even in non-photoperiodic cultivars.

Day-neutral tomato flowering is regulated by the FT-homologue SINGLE FLOWER TRUSS (SFT)/ *SP3D* gene[2]. This gene has been reported to be regulated independently of *CO* and day length[22, 23], its potato ortholog being a likely candidate to play a role in light irradiance-dependent flowering. *StSP3D*-silenced lines were actually found to be late flowering (Fig 2e-h), although in SD conditions tuberised at the same time as

untransformed controls (Supplementary Fig. 7; see Supporting text 2), hence supporting a major role for this gene in flowering but not in tuberisation transition. Remarkably, in modern tomato cultivars *SP6A* was reported to have an extra T residue (position 421) that leads to a premature stop codon, implying a non-functional role for this gene[19]. *StSP3D*, in turn, retains a weak SD activation response in *Andigena*, although transcript levels are lower than those of *StSP6A* (Fig. 1e). Together, these observations suggest that *Solanaceae* members of the *FT*-like family diversified such that *SP3D*/*SFT* lost regulatory control by CO[22,23] and gained response to other environmental cues, playing a prevalent role in day neutral flowering control. *SP6A* function was lost in modern tomato cultivars but in potato plays a major role in tuberisation, day length-dependent activation of this gene mediating strict SD tuberisation of *Andigena* species. Thus, roles of these two FT paralogs in flowering and tuberisation control has evolved, at least in part, through changes in their expression profiles, both genes encoding for functionally similar proteins, as indicated by the finding that *StSP6A* expression in *Arabidopsis* rescues the late-flowering phenotype of *co-1* and *ft-1* mutants (Supplementary Fig. 8).

Interestingly, two other *FT*-clade members, *StSP5G* and *StSP5G*-like (see Supplementary Table 2), are expressed under non-inductive LD conditions (Fig. 1e). This expression profile suggests an antagonistic function of *StSP6A* and these two FT paralogs (see Supporting text 3) as recently reported for the sugar beet *BvFT1* and *BvFT2* genes[24]. Further studies will be required to assess whether these *FT*-members exert an inhibitory role on tuberisation.

StSP6A is expressed not only in leaves but also in stolons of tuberising plants (Fig. 1f). Expression in these organs is delayed with respect to the leaves, suggesting an auto-regulatory loop by the transported protein. In support of this relay mechanism, increased levels of expression of the endogenous *StSP6A* transcript are observed in *Hd3a* lines (Supplementary Fig. 10) and wt stocks grafted to these plants (Supplementary Fig. 10a). Thus, in contrast to the *Arabidopsis FT* or rice *Hd3a* genes, *StSP6A* is regulated by a relay mechanism that sustains synthesis of the inducing signal in stolons. In this regard, a local balance between the SP floral repressor and the tomato SFT *florigen* signal has been shown to contribute to the differential flowering response of primary and secondary shoot meristems[25]. Hence, it is possible that higher SP levels in stolons confer a reduced sensitivity to FT, which may explain why floral transition is activated without tuber formation, while *Hd3a* over-expression activates both developmental processes.

To rule out that tuberisation of grafted stock plants is mostly mediated by this relay mechanism, we grafted *Hd3a* grafts onto *StSP6A*-RNAi stocks to test if inhibition of *StSP6A* expression blocks Hd3a signaling. As shown in Fig. 3a, activation of the endogenous *StSP6A* gene is strongly reduced in *StSP6A*-RNAi compared to the wt stocks. Tuberisation onset is delayed and tuber yield reduced in RNAi lines compared to the *Hd3a*/wt grafted controls (Fig. 3b) due to impaired signal amplification, but this does not preclude tuberisation of the RNAi stocks, highlighting induction by the Hd3a protein.

Finally, we tested whether this regulatory relay requires CO function, by grafting *StSP6A*ox plants into *StCO*ox stocks and analysing endogenous

StSP6A gene expression (Fig. 3c, d). Likewise the rice Hd1 protein[15,16], StCO represses *StSP6A* gene expression in LDs and transfer to SD conditions relieves this repression (see Supporting text 4). In line with this function, activation of the *StSP6A* gene is largely repressed in stolons of the *StSP6A*ox/*StCO*ox grafted plants (Fig. 3c) with respect to *StSP6A*ox/wt grafts used as controls, hence implying a role of StCO in the auto-regulatory loop that drives *StSP6A* expression in stolons. Moreover, *StSP6A* accumulates only to basal levels in the stolons of *StCO*ox/wt and wt/wt grafts used as negative controls (*StSP6A* is not expressed in LDs in *StCO*ox or wt scions), which implies that expression in these organs requires the mobile protein produced in the leaves.

In summary, our data provide the molecular basis for a long standing physiological observation, whereby flowering tobacco plants grafted onto non-induced potato stocks induce tuberisation of the stock plants, irrespective of the photoperiodic requirement of the donor plant[26]. An additional issue is how flowering and tuberisation transition is differentially triggered in response to the mobile FT signal. In *Arabidopsis*, FT interacts with FD, to activate expression of floral meristem identity genes[27,28]. We observed that *Hd3a* stolons initiate floral buds from the apical meristem (Fig. 4a-c) at the same time that they differentiate tubers from the sub-apical region. Using transgenic lines expressing the StSP6A protein under control of an ethanol inducible promoter, tuber-specific transcripts were observed within 4 h of induction in the stolons (Fig. 4d, e; see Supporting text 5). This very rapid response precludes transport of any mobile signal from the leaves and thus supports the notion that the StSP6A protein is the mobile *tuberigen* transported to belowground organs. Thus, it is possible

that StSP6A interacts in stolon subapical cells with an as yet unknown transcriptional regulator or that FD-StSP6A differentially activate a set of target genes specific to these cells, such as *StGA2ox1* (Fig. 4d). An exciting challenge for the future is to identify such stolon-specific StSP6A target genes and/or StSP6A-interacting partners, to establish how formation of these storage organs is regulated.

Identification of FT as a switch for tuberisation supports the notion that FT function extends beyond flowering induction. Other studies have implicated FT in seasonal control of growth cessation in poplar trees[29] and meristem growth termination in tomato[25]. Thus, FT is emerging as a key mobile signal controlling not only flowering, but also a number of other meristem-associated transitions.

Methods Summary

Plant materials and growth conditions. *Andigena* 7540 wt plants, antisense phytochrome B (*phyB*)[21] and *At*CO over-expression lines (*AtCO*ox)[17] were grown in the greenhouse under LDs. Growth chambers were used for controlled SD (8 h light /16 h dark) and SD+NB (SD plus a 30 min pulse of light in the middle of the night) treatments. Transgenic A*ndigena* plants were generated by *Agrobacterium*-mediated transformation of leaf explants. Plants were grafted as previously described[17] and cultivated under LDs to analyse their tuberisation response.

Plasmid constructs. *StSP6A* was cloned into the pBinAR binary vector to generate *StSP6A* over-expressing lines. *StSP6A*-, *StSP3D*- and *StCO*-RNAi

constructs were generated by inserting the 3'-non-conserved regions of these genes in opposite orientations into the pBIN19RNAi vector. The pGWB2 vector was used for *StCO* over-expression. *StSP6A* ethanol inducible lines were generated by transformation with the pBinSRNA-GW plasmid containing the *StSP6A* coding region. Primer sets used for these constructs are listed in Supplementary Table 3.

Real-time RT-PCR analyses. First strand cDNA was synthesized from 2 µg total RNA and 1 µl of the reaction used for real time gene expression analysis with the SYBR Green PCR master mix (Applied Biosystems). The *actin8* gene was used for normalization. Identical procedures were used for semi-quantitative RT-PCR, except that amplification was conducted in a Peltier Thermal Cycler (PTC-200, MJ Research). For sets of specific primers and product lengths see Supplementary Table 3.

Full Methods and any associated references are available in the online version of the manuscript at XXXXXXXXXX

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Supplementary Information is linked to the online version of the paper at www.XXXXXX.XXXXX

Ackowledgments We thank Javier Paz-Ares, Glenn Bryan and Christian Bachem for their comments on the manuscript. We also thank Shuji Yokoi for his help in this work. This research was supported by grants from the Spanish MCyT and the EU-SOL European Union Integrated Project. Support by the JSPS and CSIC under the Japan-Spain research cooperative program

and Grants-in-Aid for Scientific Research on Priority Areas of the MEXT of Japan is also acknowledged. C.N. was recipient of an I3P post-doctoral contract from the CSIC.

Author Contributions C.N., J.A.A., E.C.-O., C.A.C., J.S. and S.P. performed experiments and analysed the results. S.T. and K.S. provided the rice Hd3a construct and performed the Hd3a protein mobility studies. S.P. designed the experiments and wrote the manuscript.

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Figure legends

Figure 1: Phenotype of *andigena rolC*::Hd3a-GFP lines and expression profiles of the potato *FT*/*TFL1*-like genes. a, *Hd3a* lines (centre and right) tuberise under LDs. Left: wt control. **b, c,** Early-flowering *Hd3a* plants (**c**) relative to wt (**b**). **d,** Diagram showing tuber induction in LDs of grafted wt plants with *Hd3a* donors (right) or stocks (centre). wt control grafts do not tuberise (left). Red lines indicate the graft junction. **e,** Relative levels of expression of the potato *FT*/*TFL1*-like genes. *11707*: *PGSC0003DMG200011707*; *16180*: *PGSC0003DMG200016180*; *07111*: *PGSC0003DMG200007111*; *14322*: *PGSC0003DMG200014322*. **f,** Semi-quantitative RT-PCR analysis of *StSP6A* expression in leaves and stolons of plants with different tuberisation states (indicated on top).

Figure 2: Function of the *StSP6A* and *StSP3D* genes in SD-dependent tuberisation and day-neutral flowering control. *StSP6A* expression in

leaves of *StSP6Aox* lines (**a**) and tuber induction in these lines (**b**) in LDs. *StSP6A* silencing in leaves of *StSP6A*-RNAi (*SP6A*i) lines (**c**) correlates with late tuberisation in SDs (**d**). **e,** Relative *StSP3D* expression in *StSP3D*-RNAi (*SP3D*i) lines. Error bars indicate s.d. (n>3). **f,** Late flowering of *StSP3D* silenced lines. **g, h,** SEM images of the *StSP3D*-RNAi apexes. **g,** vegetative apex in L26. **h,** inflorescence apex in L32. Scale bars: 200 µm.

Figure 3: Auto-regulatory loop for *StSP6A* expression. a, Levels of *StSP6A* transcript in transgenic (left) and grafted plants (right). *Hd3a* scions were grafted onto wt or *StSP6A*-RNAi (*SP6A*i) lines. **b,** Tuber induction in grafted plants, scored when 100% of the *Hd3a*/wt grafts were tuberising. **c,** Relative expression of the endogenous *StSP6A* transcript in *CO*ox and *SP6A*ox plants (left) and in grafts with wt and *CO*ox stocks (right). **d,** Tuber induction of the grafted plants grown under non-inductive LDs. **e,** Diagram showing *StSP6A* auto-regulation. Error bars indicate the s.d. of two experimental replicates (n>8). See Supplementary Fig. 12 for each replicate result.

Figure 4: Floral phenotype of *Hd3a* stolons and proposed model for flower and tuber induction. a-c, Flower induction in *Hd3a* stolons. SEM images of wt (**a**) and *Hd3a* stolon apical meristems (**b**, **c**). Scale bars: 100µm. **d,** Local StSP6A-induction activates *StGA2ox1* gene expression in the stolon. **e,** Activation of other genes reported to be early induced during tuber development. Color bars represent fold change in gene expression in Alc-*StSP6A* stolons relative to Alc-*uiD* controls (black bars). Error bars represent the s.d. of two experimental replicates (n>10). **f,** Model for regulation of flowering and tuberisation transition. See Supporting text 6, for a detailed explanation.

Supporting text

1. Expression of the *StSP6A* gene fully correlates with tuberisation induction. Expression analyses of the *StSP6A* FT-homologue showed that this gene is strongly up-regulated in leaves and stolons of SD-induced plants (Fig. 1f). Accumulation of this transcript is observed as early as 2-4 days after transfer to SD inductive conditions (Fig. 1f) and thus precedes tuber formation. Interruption of the long nights with a 30 min *night break* inhibits tuberisation and prevents *StSP6A* accumulation both in the leaves and stolons. Moreover, high levels of the *StSP6A* mRNA are detected in leaf or stolon tissues of LD-grown antisense *phyB* plants, with a defect in photoperiodic tuberisation control[21]. This transcript is not detected in lines over-expressing the *Arabidopsis* CO protein (*AtCO*ox) after 8d cultivation in SDs. These plants are strongly delayed in tuberisation[17] and need longer than 30 inductive days to tuberise.

2. Roles of the *StSP6A* and *StSP3D* genes in SD tuberisation induction and in day length-independent floral promotion. To assess a role of *StSP6A* in tuberisation control, we generated transgenic *Andigena* lines that over-expressed or were silenced for expression of this gene. Transgenic *StSP6A*ox lines tuberised under non-inductive LDs (Fig 2a, b) and were induced to flower, although their flowering phenotype was less severe than that of *Hd3a* lines (Supplementary Fig. 3). A good correlation was observed between the tuberisation response of these plants and *StSP6A* transcript levels (Fig. 2a, b), consistent with a principal role of this gene in tuberisation control. RNA profiling analyses of the stolons revealed up-regulated levels of expression of tuber-specific transcripts in LDs (Supplementary Fig. 4), thus confirming induction of these plants. *StSP6A*ox scions grafted to wt stocks induced the stocks to tuberise (Fig. 3c, d), supporting movement of the StSP6A inductive signal through the graft junction, as seen for *Hd3a* plants. Transgenic *StSP6A*-RNAi (*SP6A*i) lines, in opposite, exhibited strongly delayed tuberisation in SDs (Fig. 2c, d). RNA profiling studies of the RNAi stolons showed that tuber-specific genes are not yet up-regulated after 6 inductive days (Supplementary Fig. 4). While non-transgenic plants tuberise after 6-8 days under SDs, *SP6A*i plants required on average more than 3 weeks prior to tuberise. However, when

cultivated under high irradiance conditions, they flowered at the same time as the untransformed controls, evidencing that *StSP6A* repression does not affect floral transition. By RT-PCR studies we confirmed that expression of the *StSP3D* and *StSP5G* genes was not affected in these plants (Supplementary Fig. 5a, b), silencing therefore being specific to the *StSP6A* gene.

To test whether *StSP3D* is essential for floral induction, we down-regulated expression of this gene by using an RNAi construct. Silenced lines showed a late flowering response, with 20% of the less severely repressed lines succeeding to flower (line 32, Fig. 2e, f). Flowering was suppressed in the strongest silenced lines. Scanning micrographs confirmed that the silenced plants were in a vegetative stage or had just started floral transition (Fig. 2g, h), whereas fully open flowers were observed in the controls. These plants, however, tuberised in SDs at the same time as the untransformed controls and produced an equivalent number of tubers (Supplementary Fig. 7), which demonstrates that expression of this gene is not required for normal tuber development. RT-PCR studies confirmed that *StSP6A* gene expression is not affected in these plants (Supplementary Fig. 5c), *StSP3D* playing a major role in flowering but not in day length tuberisation control.

3. Possible *StSP6A*-antagonistic function of the *StSP5G* and *StSP5G*-like *FT*-paralogs. Expression analyses of the two additional *FT*-like members, *StSP5G* and *StSP5G*-like (*PGSC0003DMG200016180*) showed that transcripts for these genes are elevated in leaves of LD-grown plants (Figure 1e). The *StSP5G* transcript, in addition, accumulates to high levels in the stolons of SD induced plants, although expression is these organs is delayed with respect to the *StSP6A* gene. These expression profiles are suggestive of a negative role of these *FT*-like paralogs in day length control of tuberisation. In line with such function, recent studies in sugar beet showed that in this LD vernalization-requiring species, flowering time is controlled by the interplay of an antagonistic pair of mutually exclusive FT-homologs that respectively promote or suppress flowering[24]. *BvFT2* is essential for flowering, while *BvFT1* prevents bolting in non-vernalized plants. This gene is down-regulated in LDs, in annual and vernalized-biennial plants, decreased levels of expression being required for *BvFT2*

expression and floral transition. Interestingly, BvFT1 carries three amino acid substitutions in an external loop formed by the fourth exon of the protein and both StSP5G and StSP5G-like homologs differ from the StSP6A and StSP3D proteins in this region (Supplementary Fig. 9).

4. Potato StCO represses *StSP6A* gene expression in LDs. In potato, CO is encoded by three tandem duplicated genes but only one of these gene copies is expressed to relatively high levels. This transcript oscillates with a diurnal rhythm of expression that peaks at dawn[8]. Several lines of evidence suggest that potato StCO represses *StSP6A* gene expression in LDs and that transfer to SD conditions relieves this repression. In line with this activity, leaves of *StCO*-RNAi (*CO*i) lines were found to express high levels of the *StSP6A* transcript in LDs, and to tuberise in these non-inductive conditions (Supplementary Fig. 11d-f). *StSP6A* gene expression, by contrary, is repressed in *StCO* over-expressing (*CO*ox) plants, although expression is fully restored after 6 days of transfer to SD conditions (Supplementary Fig. 11a-c), hence pointing to a day length dependent switch in StCO repressor activity as reported for the rice Hd1 protein[15,16].

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induction (4 hours) of this transcript, on the other hand, excludes transport of the protein from the leaves or the transport of any further downstream mobile signal, which demonstrates that StSP6A is active in stolon cells and likely corresponds to a mobile protein.

6. Model for StSP6A and StSP3D regulation of tuberisation and floral transition. *StSP6A* is repressed by StCO in LDs, the light receptor phyB being somehow involved in the modulation of this repressor activity. Transfer to SDs induces a switch in StCO repressor function and activates *StSP6A* gene expression in the leaves. During transport this signal is amplified by an auto-relay mechanism partially mediated by StCO. *StSP6A* activation in leaves and stolons promotes tuber formation. Although StCO weakly induces *StSP3D* gene expression in SDs, this gene mainly responds to cues such as high light irradiance. The StSP3D protein promotes flowering in the shoot apex.

Full Methods

Plant materials and growth conditions. A*ndigena* 7540 wt lines and the antisense phytochrome B (*phyB*)[21] and *AtCO*-overexpresser (*AtCO*ox)[17] lines were grown in the greenhouse under LDs. Growth chambers were used for controlled SD (8 h light /16 h dark), SD+NB (SD plus a 30 min pulse of light given 8h after beginning of the dark period) and LD (16 h light/8 h dark) treatments.

Transgenic *Andigena* plants carrying the *rolC*::*Hd3a-GFP*[18], *StSP6A* over-expression, *StSP6A*-RNAi, *StSP3D*-RNAi, *StCO*ox and *StCO*-RNAi constructs were generated by *Agrobacterium*-mediated transformation of leaf explants as described[17]. Different graft combinations were obtained as previously described[17] and their tuberisation phenotype was scored under LDs. Tuber formation in *StSP6A*-RNAi and *StSP3D*-RNAi lines was analyzed by growing the plants under LD greenhouse conditions until a 10-leaf stage, before transferring them to SDs. For flowering studies, plants were grown under high irradiance (>200 μE/s m^{-2}) LD conditions. At least nine replicates of each line were used for these studies.

Arabidopsis constans-1 (*co-1*) and *flowering locus t-1* (*ft-1*) mutants in the *Columbia* (Col-0) and *Landsberg erecta* (Ler) backgrounds were transformed using the floral dip method. Flowering time was measured as the number of rosette leaves at floral initiation under LDs, in at least 10 individuals.

Plasmid constructs. The *rolC::Hd3a-GFP* construct has been described elsewhere[18]. Transcripts corresponding to the potato *StSP6A*, *StSP3D* and *StCO* genes were amplified using primers designed on the tomato sequences (AY186737, AY186735 and AY490253, respectively). The *StSP6A* over-expression construct was generated by amplifying the protein coding

region and subsequent insertion of the PCR product into the *Sma*I site of the pBinAR binary vector, between the *35S* promoter and *ocs* terminator. To silence the *StSP6A*, *StSP3D* and *StCO* transcripts, *Andigena* plants were transformed with RNA interference constructs designed on the non-conserved region of the genes. The amplification products were cloned into the pENTR™/D-TOPO plasmid (Invitrogen) and inserted in opposite orientations by recombination with the LR clonase™ II enzyme (Gateway Technology, Invitrogen) into the pBIN19RNAi destination vector. The pBIN19RNAi interference vector was generated by partial *Xba*I/*Hind*III digestion of the pH7GWIWG2(II) plasmid (http://gateway.psb.ugent.be/) and insertion of the Gateway cassette fragment into the same restriction sites of the pBIN19 binary vector. The *StCO* over-expression construct was generated by amplification and cloning the protein coding region into the pENTR™/D-TOPO plasmid (Invitrogen) and further insertion by recombination into the pGWB2 destination vector[33]. To generate the *StSP6A* ethanol inducible lines, the pBinSRNA-GW destination vector was created by insertion of the blunt ended pAlcA-R1-R2-t35S cassette from the AlcAP-GW pGreen vector (gift of Patrick Laufs; INRA, Versailles, France) generated by *XbaI* digestion, into the blunted *HindIII* sites of the binary vector pBinSRNACatN. The *StSP6A* coding region was then introduced in this destination vector by LR clonase recombination. A list of the primer sets used to generate these constructs is shown in Supplementary Table 3.

Real-time and semi-quantitative RT-PCR analyses. Expression of potato *FT*/*TFL1*-like family members was quantified by real time PCR. First strand cDNA was synthesized from 2 µg total RNA and 1 µl of the reaction used for real time gene expression analysis with the SYBR Green PCR master mix (Applied Biosystems). qPCR was performed using the Power SYBR® Green PCR Master Mix (Applied Biosystems, Inc, U.S.) on an

ABI7500 Real-Time PCR System (Applied Biosystems, Inc, U.S.) following the manufacturer's instructions. Primer pairs were specifically designed for each gene using Primer Express® 3.0 software (Applied Biosystems, Inc, U.S.) and probed for high efficiency amplification at standard qPCR conditions. All reactions were carried out at least in two independent biological replicates. The Pfaffl[34] method was used for relative quantification of gene expression. Direct $2^{-\Delta CT}$, where ΔC_T=(C_TTarget gene-C_TActin)[35], was used to generate the tissue specific heat map in Fig. 1e. The comparative critical threshold ($2^{-\Delta\Delta CT}$) method[36] was used to analyze relative *Hd3a* and *StSP6A* expression levels in Supplementary Figures 1 and 10.

Identical procedures were used for semi-quantitative RT-PCR, except that amplification was conducted in a Peltier Thermal Cycler (PTC-200, MJ Research) and 1/10 of the cDNA reaction was used for actin amplification. For sets of specific primers and product lengths see Supplementary Table 3.

Microarray sample hybridization and analysis. Stolons of wt plants were collected in LDs and 2, 6 and 8 days after transfer the plants to SDs. Time-course profiling analyses of SD tuberisation induction was performed as described[32].

Arrays of *StSP6A* over-expression versus wt (sampled in LDs) and *StSP6A*-RNAi stolons versus wt (sampled in SDs, day 6) were performed with samples from three independent lines. Samples were hybridized against POCI (Potato Oligo Chip Iniciative) microarrays and background correction and normalization were performed using LIMMA[37,38]. The obtained data was statistically checked (False Discovery Rate, FDR<0.05) and genes with a log ratio change of +2 or -2 for *StSP6A*-RNAi and +1.8 or -1.8 for *StSP6A*ox plants were selected. Hierarchical cluster of genes present in both experiments was calculated, compared with the SD profile, and represented

using the TIGR MeV free software (http://www.tm4.org/mev/). Genes found to be differentially expressed are listed in Supplementary Table 1.

Immunoblot analysis. Total protein extracts from stolon tissues were obtained in lysis buffer (50mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Na deoxycholate, 0.5% Triton X-100, 1mM PMSF and protease inhibitors). For analysis of protein graft transmissibility, total extracts were overnight incubated with 10 µl of an anti-GFP affinity matrix (MBL). After extensive washing, unbound and bound proteins were separated on 10% SDS-PAGE, blotted onto nitrocellulose and probed with an anti-GFP antibody (Roche). Immuno-reactive proteins were detected with the SuperSignal® West Pico Chemiluminescence kit (Pierce).

Microscopy. GFP fluorescence was observed on longitudinal stolon sections (150 µm) obtained with a vibratome (PELCO 101). Fluorescence was excited with a 488 nm Argon laser and emission images were collected in the 500-600 nm range using a Leica TCS SP5 spectral confocal microscope.

For scanning electron microscopy, potato stolons and apical meristems were frozen in an Oxford CT 1500 cryosystem (Oxford Instruments), sublimated under vacuum and observed in a DIOL JSM 5410 electronic microscope operating at 10 kV.

Phylogenetic analysis. Full length protein sequences, except sequences of potato FT/TFL1-like family members, were obtained from GenBank. Sequences of potato FT/TFL1-like family members were obtained from the Potato Genome Sequencing Consortium Data Release (http://potatogenomics.plantbiology.msu.edu/)[20] by TBLASTX using as a query previously described tomato sequences[19] (*SlSP*, No. U84140; *SlSP6A*, No. AY186737; *SlSP3D* No. AY186735; *SlSP9D* No. AY186738; *SlSP2I* No. AY186734 and *SlSP5G* No. AY186736). Best genome matches described in

Supplementary Table 2 were downloaded and ORFs were predicted using FGENESH (http://linux1.softberry.com/berry.phtml) and ORF finder (http://www.ncbi.nlm.nih.gov/projects/gorf/). The potato *SlSP2I* counterpart was not found in the potato genome. Phylogenetic analyses were conducted in MEGA4[39]. First, all sequences were aligned with the COBALT program (http://www.ncbi.nlm.nih.gov/tools/cobalt/) and then the phylogeny reconstruction was inferred using the Neighbor Joining method[40]. All ambiguous positions were removed for each sequence pair. The evolutionary distances were computed using the Poisson correction method and the bootstrap consensus tree was inferred from 1000 replicates. The accession numbers for the corresponding genes are indicated in the tree (Supplementary Fig. 2).

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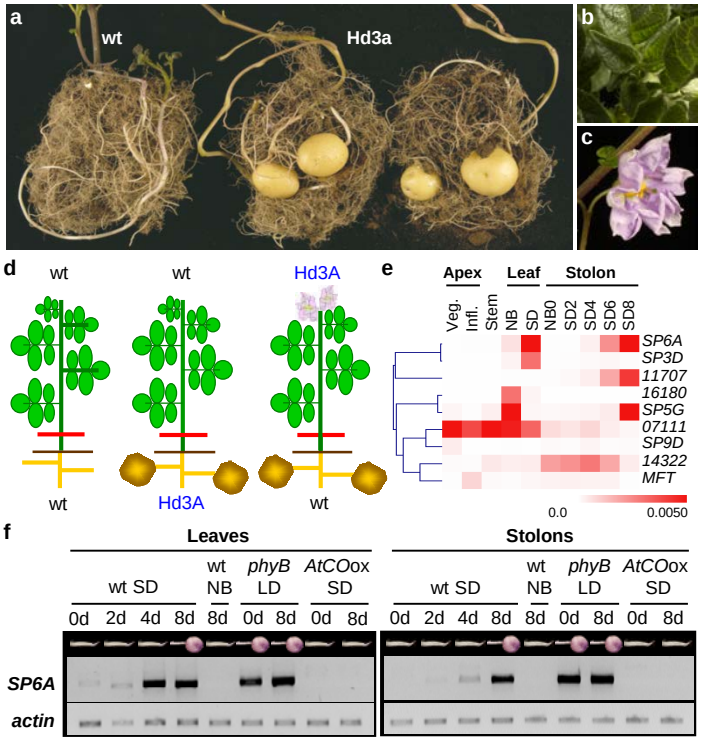
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Infl.
Stem
NB
SD
NB0
SD2
SD4
SD6
SD8
SP6A
SP3D
11707
16180
SP5G
07111
SP9D
14322
MFT
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0.0050
f
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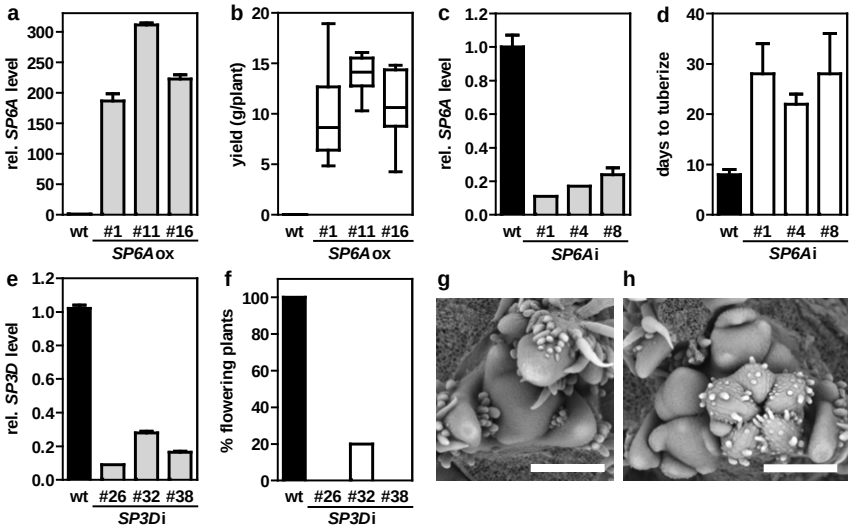
SP6A
actin

Figure 1.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250
200
150
100
50
0
wt #1 #11 #16
SP6Aox
b
yield (g/plant)
20
15
10
5
0
wt #1 #11 #16
SP6Aox
c
rel. SP6A level
1.2
1.0
0.8
0.6
0.4
0.2
0.0
wt #1 #4 #8
SP6Ai
d
days to tuberize
40
30
20
10
0
wt #1 #4 #8
SP6Ai
e
rel. SP3D level
1.2
1.0
0.8
0.6
0.4
0.2
0.0
wt #26 #32 #38
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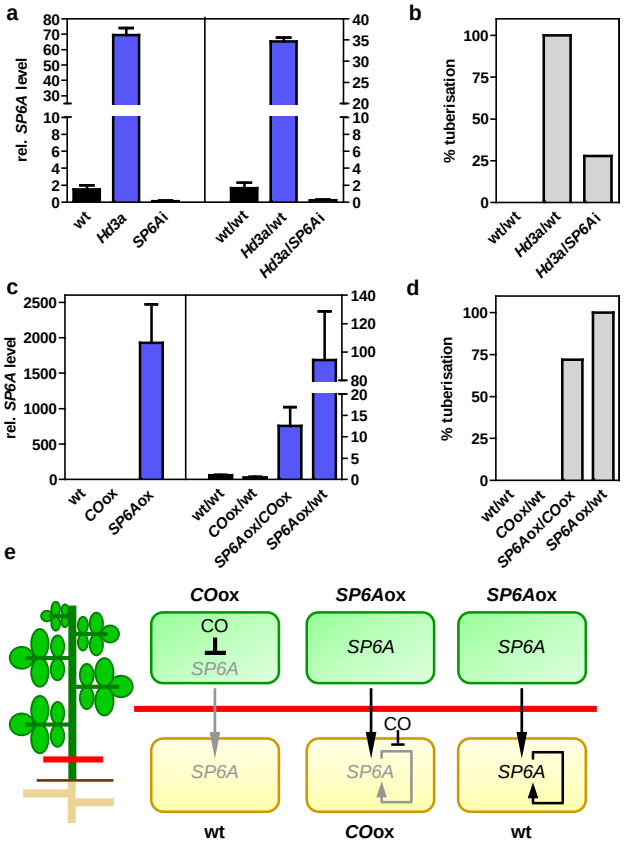
100
80
60
40
20
0
wt #26 #32 #38
SP3Di
g
h

Figure 2.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wt
Hd3a
SP6Ai
wt/wt
Hd3a/wt
Hd3a/SP6Ai
b
% tuberisation
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Hd3a/wt
Hd3a/SP6Ai
c
rel. SP6A level
wt
COox
SP6Aox
wt/wt
COox/wt
SP6Aox/COox
SP6Aox/wt
d
% tuberisation
wt/wt
COox/wt
SP6Aox/COox
SP6Aox/wt
e
COox
SP6Aox
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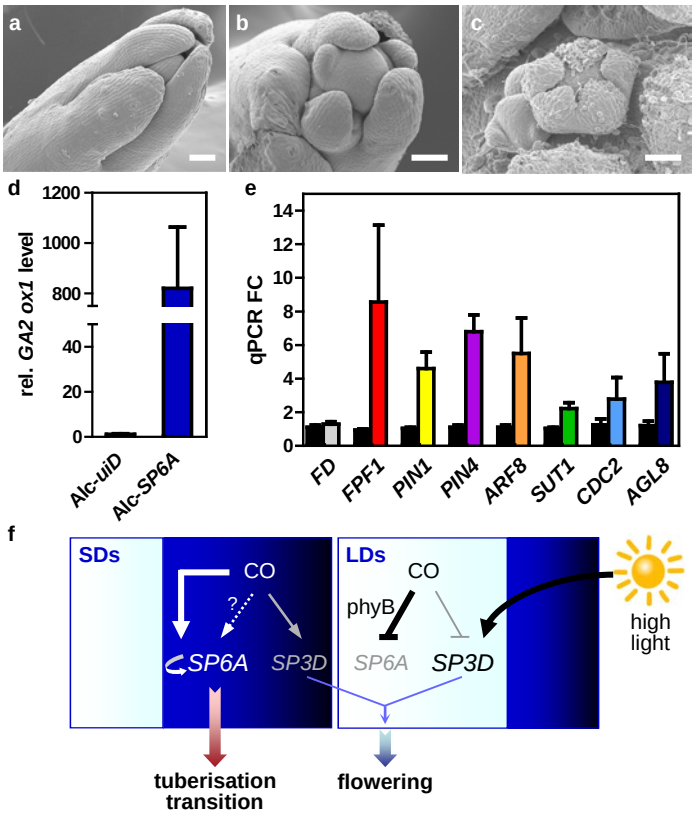
SP6A
SP6A
SP6A
CO
SP6A
SP6A
SP6A
wt
COox
wt

Figure 3.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d
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1000
800
40
20
0
Alc-uiD
Alc-SP6A
e
qPCR FC
14
12
10
8
6
4
2
0
FD
FPF1
PIN1
PIN4
ARF8
SUT1
CDC2
AGL8
f
SDs
CO
?
SP6A
SP3D
LDs
CO
phyB
SP6A
SP3D
high light
tuberisation transition
flowering

Figure 4.

Supplementary information for

Control of flowering and storage organ formation in potato by FLOWERING LOCUS T

Cristina Navarro, José A. Abelenda, Eduard Cruz-Oró, Carlos A. Cuéllar, Shojiro Tamaki, Javier Silva, Ko Shimamoto and Salomé Prat

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Supplementary Text 1. Expression of the St*SP6A* gene fully correlates with tuberisation induction. Expression analyses of the St*SP6A* FT-homologue showed that this gene is strongly up-regulated in leaves and stolons of short day induced plants (Fig. 1f). Accumulation of this transcript is observed as early as 2-4 days after transfer to short day inductive conditions (Fig. 1f) and thus precedes tuber formation. Interruption of the long nights with a 30 min night break inhibits tuberisation and prevents St*SP6A* accumulation both in the leaves and stolons. Moreover, high levels of the St*SP6A* mRNA are detected in leaf or stolon tissues of long day grown antisense *phyB* plants, with a defect in photoperiodic tuberisation control[1]. This transcript is not detected in lines over-expressing the *Arabidopsis* CO protein (At*CO*ox) after 8 days cultivation in short days. These plants are strongly delayed in tuberisation[2] and need longer than 30 inductive days to tuberize.

Supplementary Text 2. Roles of the St*SP6A* and St*SP3D* genes in short day tuberization induction and in day length-independent floral promotion. To assess a role of St*SP6A* in tuberization control, we generated transgenic Andigena lines that over-expressed or were silenced for expression of this gene. Transgenic St*SP6A*ox lines tuberized under non-inductive long days (Fig 2a, b) and were induced to flower, although their flowering phenotype was less severe than that of *Hd3a* lines (Supplementary Fig. 3). A good correlation was observed between the tuberisation response of these plants and St*SP6A* transcript levels (Fig. 2a, b), consistent with a principal role of this gene in tuberization control. RNA profiling analyses of the stolons revealed up-regulated levels of expression of tuber-specific transcripts in long days (Supplementary Fig. 4), thus confirming induction of these plants. St*SP6A*ox scions grafted to wild-type stocks induced the stocks to tuberize (Fig. 3c, d), supporting movement of the StSP6A inductive signal through the graft junction, as seen for *Hd3a* plants. Transgenic St*SP6A*-RNAi (*SP6A*i) lines, in opposite, exhibited strongly delayed tuberization in shot days (Fig. 2c, d). RNA profiling studies of the RNAi stolons showed that tuber-specific genes are not yet up-regulated after 6 inductive days (Supplementary Fig. 4). While non-transgenic plants tuberize after 6-8 days under short days, *SP6A*i plants required on average more than 3 weeks prior to tuberize. However, when cultivated under high irradiance conditions, they flowered at the same time as the untransformed controls, evidencing that St*SP6A* repression does not affect floral transition. By RT-PCR studies we confirmed that expression of the St*SP3D* and St*SP5G* genes was not affected in these plants (Supplementary Fig. 5a, b), silencing therefore being specific to the St*SP6A* gene.

To test whether St*SP3D* is essential for floral induction, we down-regulated expression of this gene by using an RNAi construct. Silenced lines showed a late flowering response, with 20% of the less severely repressed lines succeeding to flower (line 32, Fig. 2e, f). Flowering was suppressed in the strongest silenced lines. Scanning micrographs confirmed that the silenced plants were in a vegetative stage or had just started floral transition (Fig. 2g, h), whereas fully open flowers were observed in the controls. These plants, however, tuberized in short days at the same time as the untransformed controls and produced an equivalent number of tubers (Supplementary Fig. 7), which demonstrates that expression of this gene is not required for normal tuber development. RT-PCR studies confirmed that St*SP6A* gene expression is not affected in these plants (Supplementary Fig. 5c), St*SP3D* playing a major role in flowering but not in day length tuberization control.

Supplementary Text 3. Possible St*SP6A*-antagonistic function of the St*SP5G* and St*SP5G*-like *FT*-paralogs. Expression analyses of the two additional *FT*-like members, St*SP5G* and St*SP5G*-like (*PGSC0003DMG400016180*) showed that transcripts for these genes are elevated in leaves of long day grown plants (Figure 1e). The St*SP5G* transcript, in addition, accumulates to high levels in the stolons of short day induced plants, although expression in these organs is delayed with respect to the St*SP6A* gene. These expression profiles are suggestive of a negative role of these *FT*-like paralogs in day length control of tuberization. In line with such function, recent studies in sugar beet showed that in this long day vernalization-requiring species, flowering time is controlled by the interplay of an antagonistic pair of mutually exclusive FT-homologs that respectively promote or suppress flowering[3]. Bv*FT2* is essential for flowering, while Bv*FT1* prevents bolting in non-vernalized plants. This gene is down-regulated in long days, in annual and vernalized-biennial plants, decreased levels of expression being required for Bv*FT2* expression and floral transition. Interestingly, BvFT1 carries three amino acid substitutions in an external loop formed by the fourth exon of the protein and both StSP5G and StSP5G-like homologs differ from the StSP6A and StSP3D proteins in this region (Supplementary Fig. 9).

Supplementary Text 4. Potato StCO represses St*SP6A* gene expression in long days. In potato, CO is encoded by three tandem duplicated genes but only one of these gene copies is expressed to relatively high levels. This transcript oscillates with a diurnal rhythm of expression that peaks at dawn[4]. Several lines of evidence suggest that potato StCO represses St*SP6A* gene expression in long days and that

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Supplementary Text 5. Local induction of the StSP6A protein in stolons activates tuber-specific gene expression. To assay if StSP6A promotes tuberisation transition when locally expressed in stolons, we generated transgenic lines in which expression of this protein is driven by the AlcA/AlcR ethanol inducible system. StSP6A expression was locally activated by submerging the belowground part of the plant in a 0.5% ethanol solution and gene expression analyzed after 4 hours of induction. As seen in Figure 4d, a strong up-regulation of the tuber-specific St*GA2ox1* transcript[7] is observed in the stolons of these plants but not in transgenic controls expressing a fragment of the GUS gene under control of the same AlcA/AlcR inducible system (Alc-*uiD*). Likewise, many other genes reported to be induced during early stages of tuber-development[8] are found to be up-regulated in Alc-St*SP6A* stolons*,* although to lower levels than the St*GA2ox1* gene (Figure 4e). Relative St*GA2ox1* transcript levels in ethanol induced stolons actually were similar to those observed in swelling stolons, after 6 days of transfer to short day conditions (Supplementary Fig. 13), consistent with a function of this GA catabolic enzyme as a direct target of the StSP6A protein. Rapid induction (4 hours) of this transcript, on the other hand, excludes transport of the protein from the leaves or the transport of any further downstream mobile signal, which demonstrates that StSP6A is active in stolon cells and likely corresponds to a mobile protein.

Supplementary Text 6. Model for StSP6A and StSP3D regulation of tuberisation and floral transition. St*SP6A* is repressed by StCO inlong days, the light receptor phyB being somehow involved in the modulation of this repressor activity. Transfer to short days induces a switch in StCO repressor function and activates St*SP6A* gene expression in the leaves. During transport this signal is amplified by an auto-relay mechanism partially mediated by StCO. St*SP6A* activation in leaves and stolons promotes tuber formation. Although StCO weakly induces St*SP3D* gene expression in

short days, this gene mainly responds to cues such as high light irradiance. The StSP3D protein promotes flowering in the shoot apex.

Suplementary Information References

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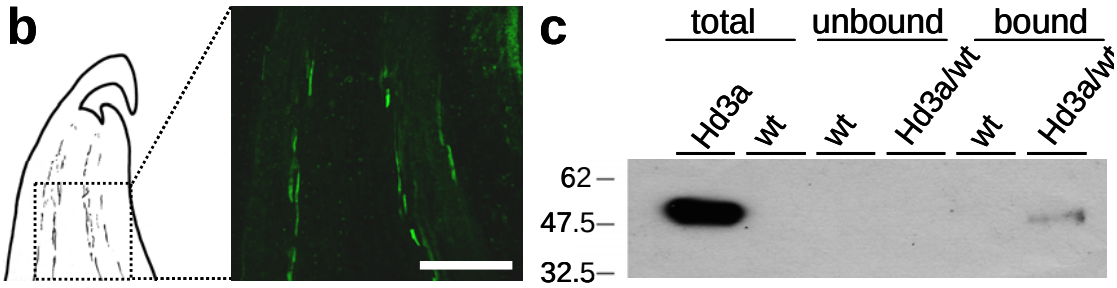
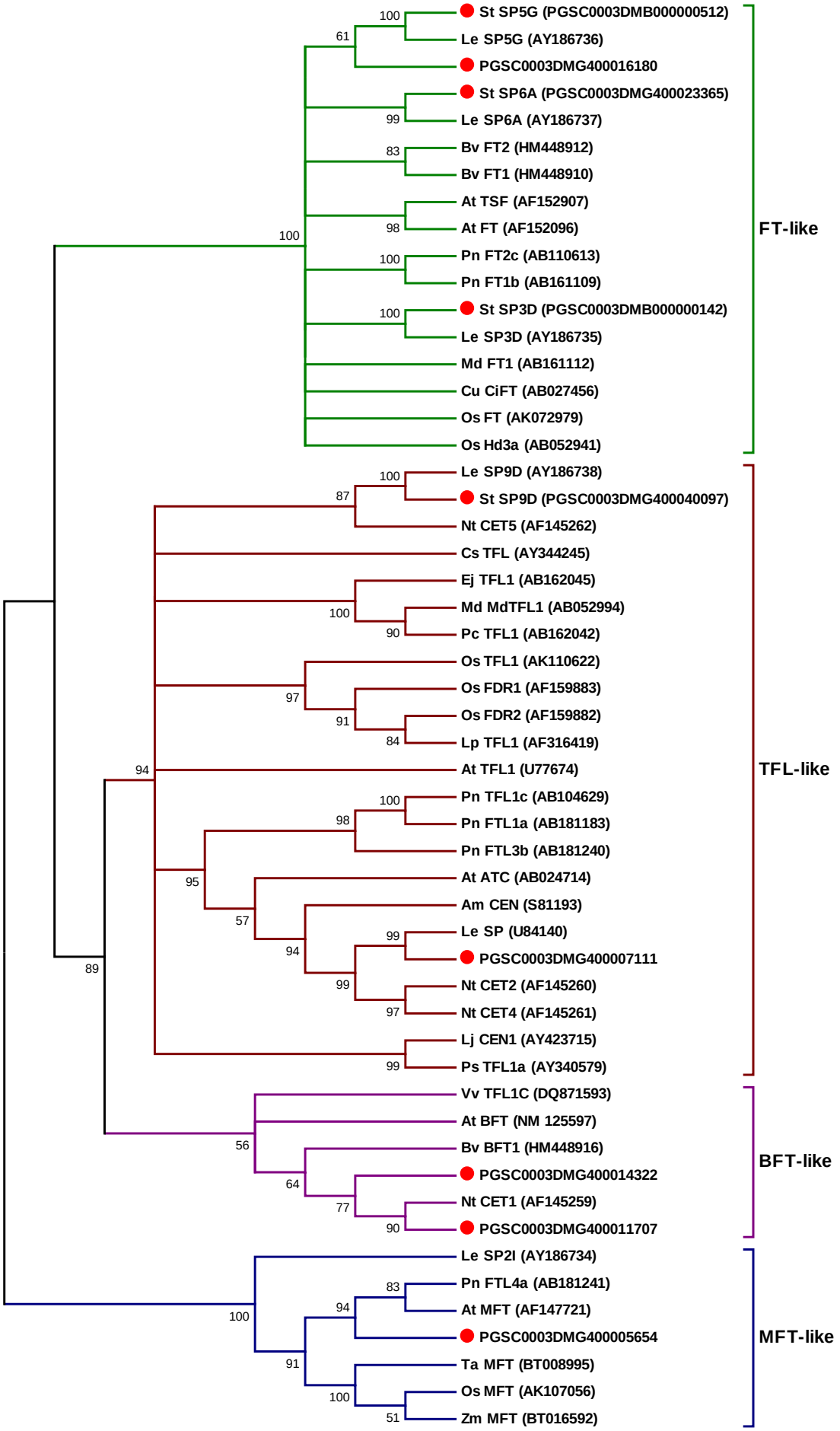
9 Chincinska, IA *et al*. Sucrose transporter StSUT4 from potato affects flowering, tuberization, and shade avoidance response. *Plant Physiol.* **146**, 515-28 (2008).

a

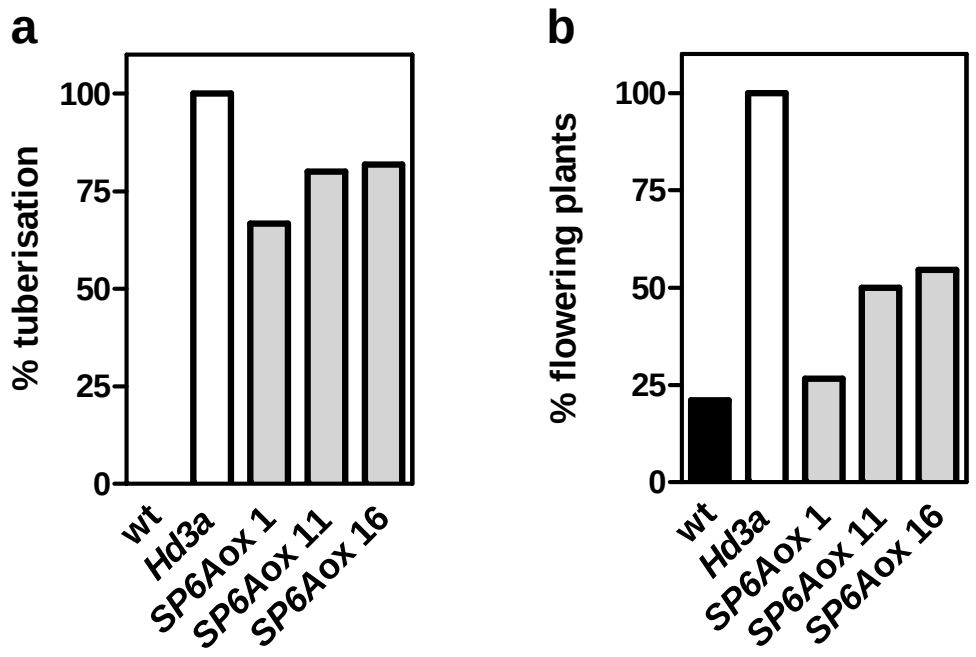
	WT	Hd3a/WT	WT/Hd3a
apex	1 ± 1	234991 ± 9767	n.d.
leaf	n.d.	202286 ± 1402	n.d.
upper stem	4 ± 1	88853 ± 5842	1 ± 0
lower stem	5 ± 2	2 ± 2	50375 ± 1222
stolon	5 ± 1	n.d.	127138 ± 881



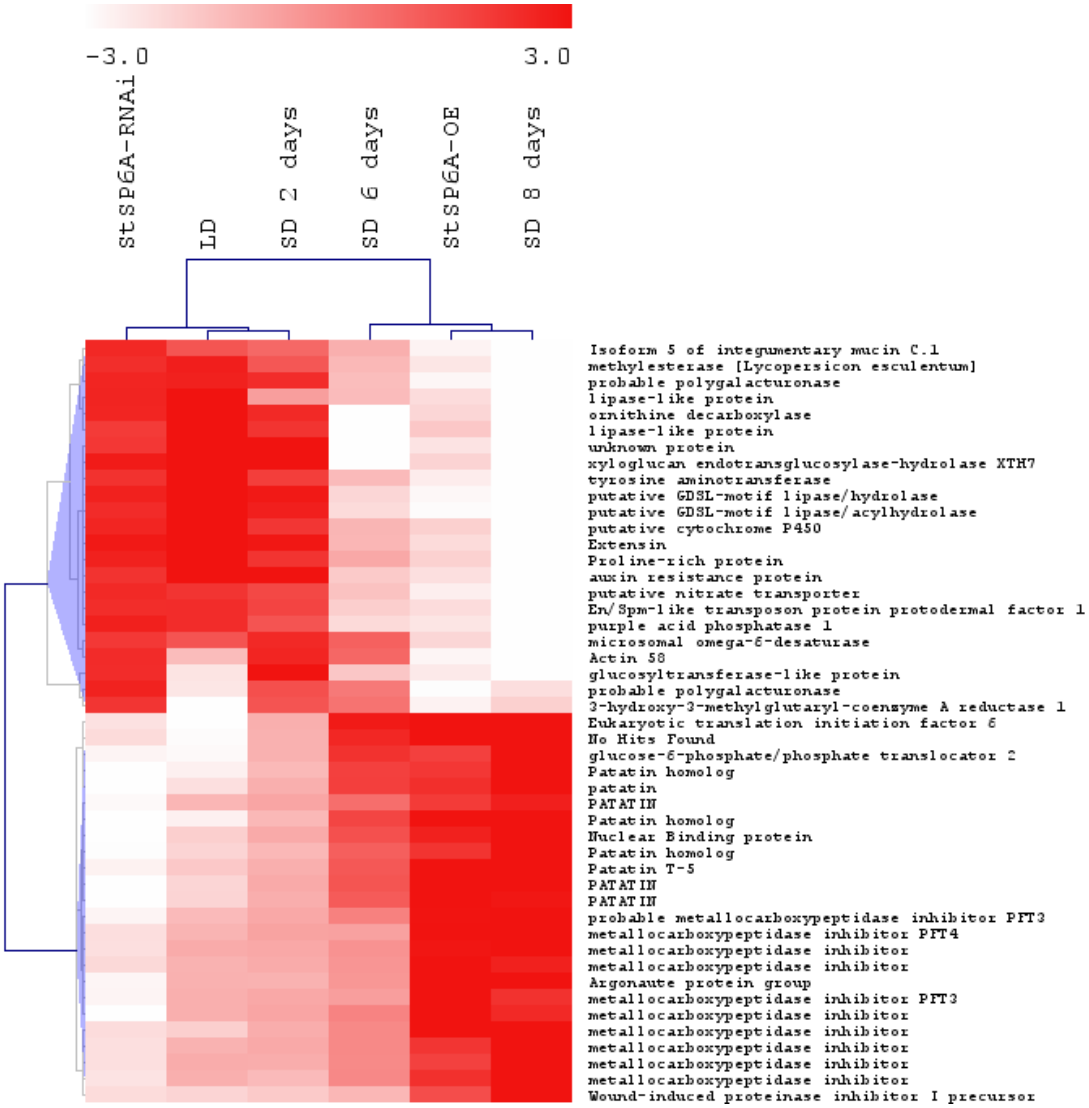
Supplementary Figure 1. RNA and protein analyses of Andigena *rolC*::Hd3a-GFP lines. a, RT-qPCR amplification of the Hd3a transcript in grafted plants. Highlighted in blue are the transgenic *Hd3a* tissues. Data are means +/- standard deviation (n=3). **b,** Confocal microscopy of transgenic *Hd3a* stolons. Hd3a-GFP fluorescence is detected in phloem companion cells. Scale bar: 250μm. **c,** Western blot detection of the Hd3a-GFP protein in the stolon tips of grafted wild-type stocks (*Hd3a*/wt). Protein extracts of transgenic stolons (*Hd3a*) were loaded for comparison and wild-type stolons (wt) were used as negative controls. Extracts were concentrated by affinity binding to an anti-GFP agarose matrix (bound). The unbound fraction (unbound) was loaded to test for binding efficiency.


St SP5G (PGSC0003DMB000000512)
Le SP5G (AY186736)
PGSC0003DMG400016180
St SP6A (PGSC0003DMG400023365)
Le SP6A (AY186737)
Bv FT2 (HM448912)
Bv FT1 (HM448910)
At TSF (AF152907)
At FT (AF152096)
Pn FT2c (AB110613)
Pn FT1b (AB161109)
St SP3D (PGSC0003DMB000000142)
Le SP3D (AY186735)
Md FT1 (AB161112)
Cu CiFT (AB027456)
Os FT (AK072979)
Os Hd3a (AB052941)
FT-like
Le SP9D (AY186738)
St SP9D (PGSC0003DMG400040097)
Nt CET5 (AF145262)
Cs TFL (AY344245)
Ej TFL1 (AB162045)
Md MdTFL1 (AB052994)
Pc TFL1 (AB162042)
Os TFL1 (AK110622)
Os FDR1 (AF159883)
Os FDR2 (AF159882)
Lp TFL1 (AF316419)
At TFL1 (U77674)
Pn TFL1c (AB104629)
Pn FTL1a (AB181183)
Pn FTL3b (AB181240)
At ATC (AB024714)
Am CEN (S81193)
Le SP (U84140)
PGSC0003DMG400007111
Nt CET2 (AF145260)
Nt CET4 (AF145261)
Lj CEN1 (AY423715)
Ps TFL1a (AY340579)
TFL-like
Vv TFL1C (DQ871593)
At BFT (NM 125597)
Bv BFT1 (HM448916)
PGSC0003DMG400014322
Nt CET1 (AF145259)
PGSC0003DMG400011707
BFT-like
Le SP2I (AY186734)
Pn FTL4a (AB181241)
At MFT (AF147721)
PGSC0003DMG400005654
Ta MFT (BT008995)
Os MFT (AK107056)
Zm MFT (BT016592)
MFT-like

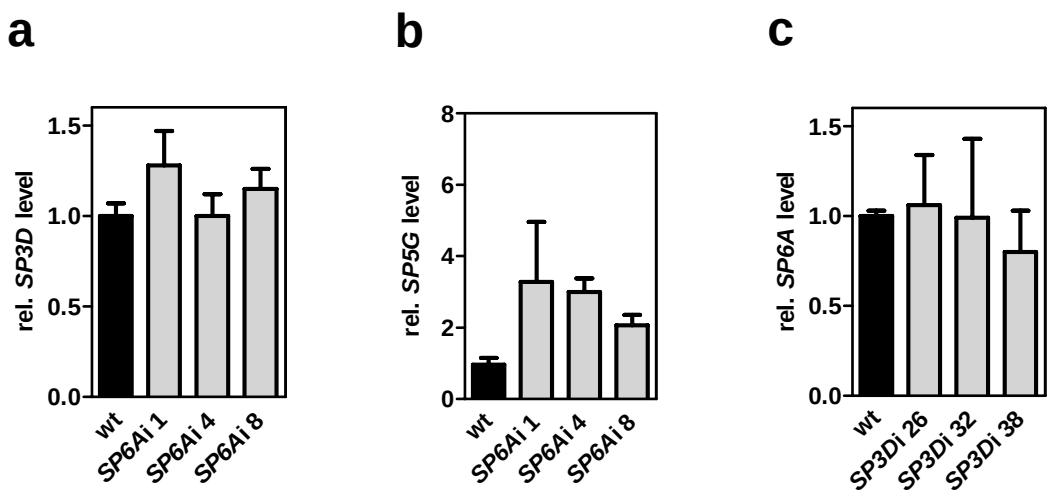
Supplementary Figure 2. The potato FT/TFL1-like gene family. Neighbor-Joining phylogenetic tree of *FT/TFL1*-like genes from different species. FT, TFL1, BFT and MFT clades are highlighted in green, brown, purple and blue, respectively. Genes from potato are marked with a red dot. Am: *Antirrhinum majus*, At: *Arabidopsis thaliana*, Bv: *Beta vulgaris*, Cs: *Citrus sinensis*, Cu: *Citrus unshiu*, Ej: *Eriobotrya japonica*, Le: *Solanum lycopersicum*, Lj: *Lotus japonicus*, Lp: *Lolium perenne*, Pn: *Populus nigra*, Md: *Malus x domestica*, Nt: *Nicotiana tabacum*, Os: *Oryza sativa*, Pc: *Pyrus communis*, Ps: *Pisum sativum*, St: *Solanum tuberosum*, Ta: *Triticum aestivum*, Vv: *Vitis vinifera*, Zm, *Zea mays*.



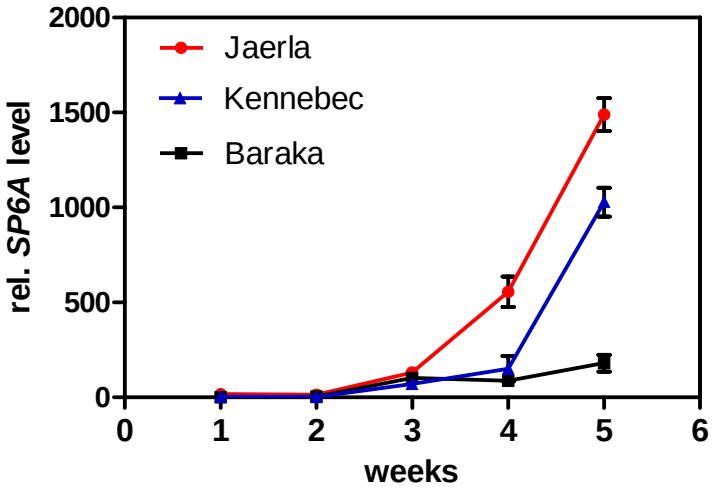
Supplementary Figure 3. Long day tuberization and flowering phenotypes of *Hd3a* and St*SP6A* over-expressing plants (*SP6A*ox). a, Tuberization of *Hd3a* and St*SP6A* over-expressing plants (*SP6A*ox) in long days scored when 100% of the *Hd3a* plants had initiated tuberization. **b,** Percentage of flowering plants, scored when all the *Hd3a* plants were flowering. n=27 in wt and n=15 in *Hd3a* and St*SP6A*ox lines.



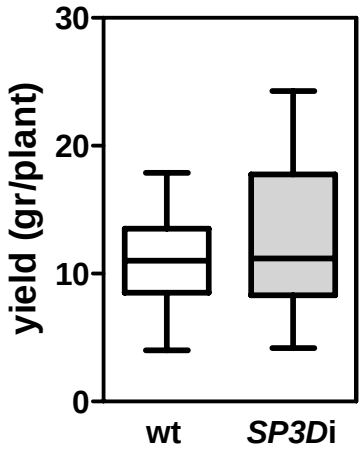
Supplementary Figure 4. Hierarchical clustering of genes differentially expressed in stolons of St*SP6A* over-expression and RNAi plants. Heat map representation of relative expression profiles of St*SP6A*ox and St*SP6A*-RNAi stolons, analyzed under non inductive long days (LD) and inductive short days (SD 6 days), respectively. Gene expression in non-induced wild-type stolons and time course regulation of these genes after 2 days, 6 days and 8 days of transfer to short days is also shown for comparison. Note that the expression profiles of short days St*SP6*-RNAi stolons group with non-induced/2 days induced wild-type stolons, while those of long day St*SP6A*ox stolons group with 6 days and 8 days induced wild-type stolons. Gene description and numeric values are provided in Supplementary Table 1.



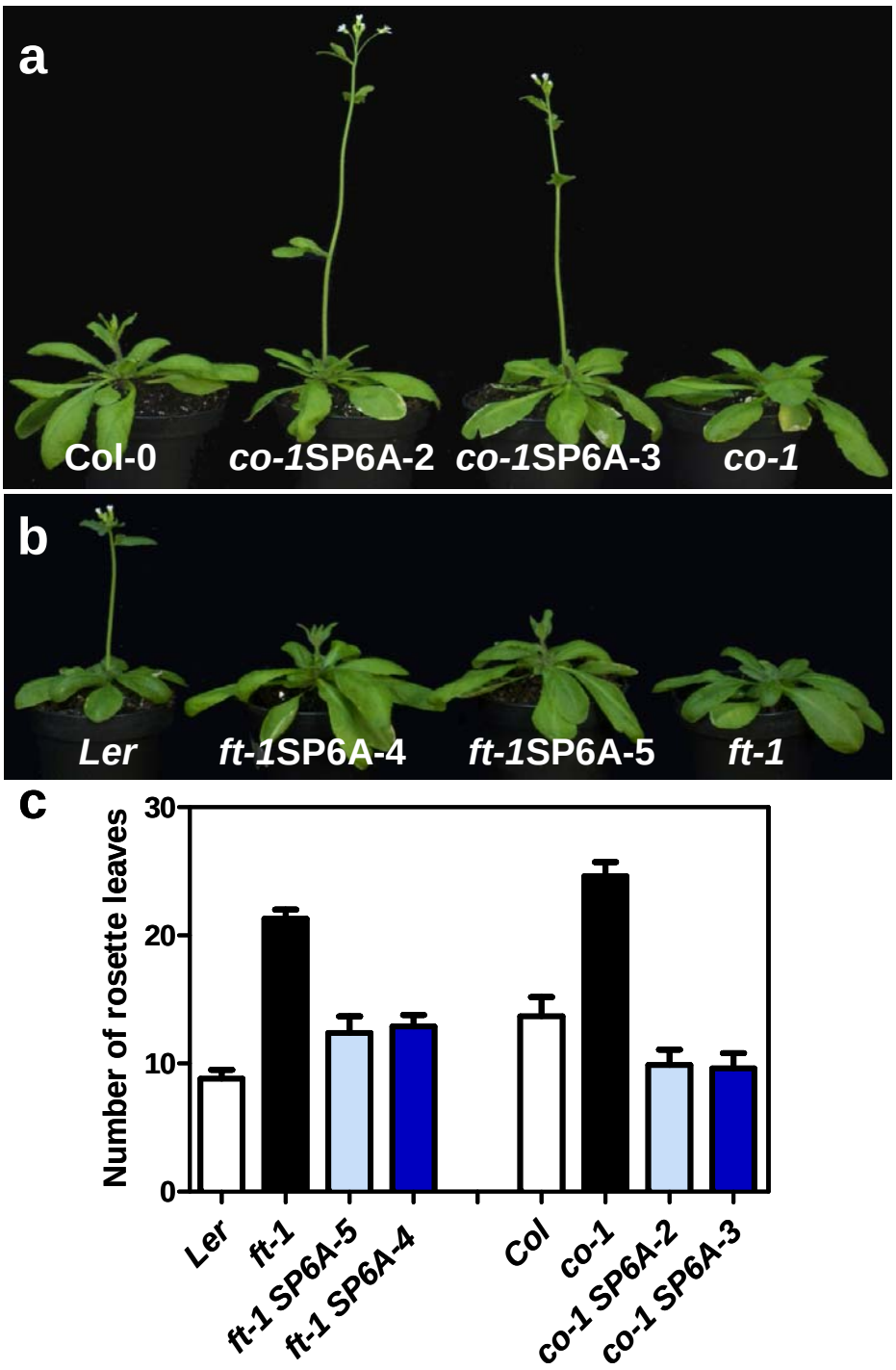
Supplementary Figure 5. Expression of the St*SP3D* and St*SP5G* transcripts in St*SP6A*-RNAi (*SP6A*i) lines (a-b) and St*SP6A* transcript levels in St*SP3D*-RNAi (*SP3D*i) lines (c). Transcript levels were quantified by RT-qPCR analyses of the transgenic leaves sampled after 6 days of transfer to short day inductive conditions. Error bars indicate standard deviation (n=3).



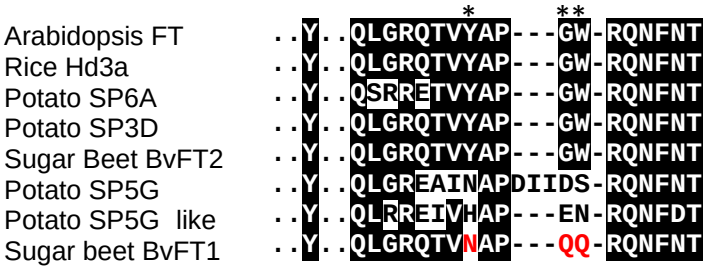
Supplementary Figure 6. St*SP6A* expression in commercial varieties. Time course of St*SP6A* relative expression in three commercial potato varieties: *Jaerla* (early-maturing), *Kennebec* (intermediate) and *Baraka* (late-maturing). Error bars represent standard deviation (n=3).



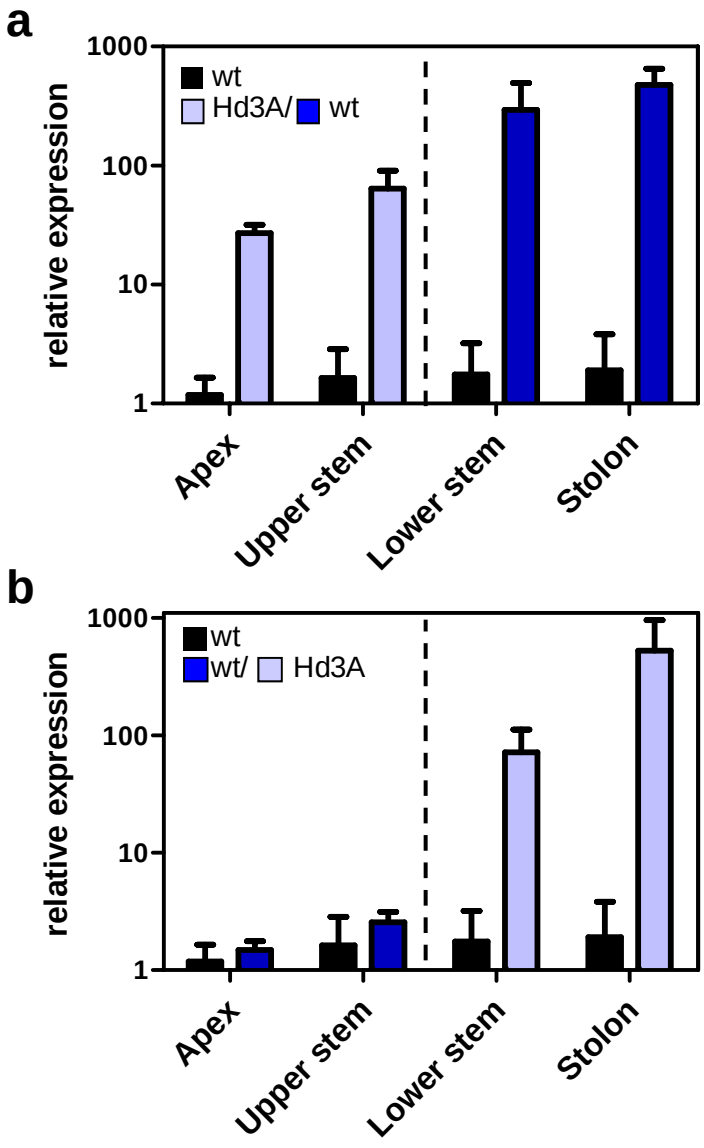
Supplementary Figure 7. Tuber yield per plant in St*SP3D*-RNAi lines. Box-whisker plots of tuber yield (gr/plant) distributions of wild-type and St*SP3D*-RNAi (*SP3D*i) plants under short day conditions (n=30, wt and n=24, *SP3D*i). A t-test analysis (two tailed p-value 0.1484) showed no significant difference between these populations.



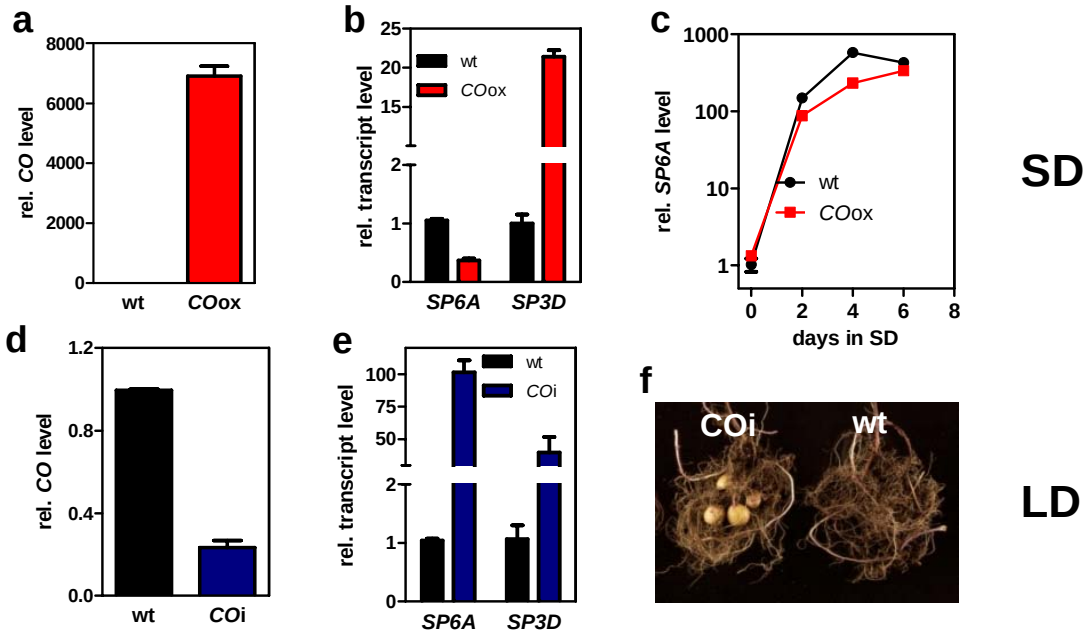
Supplementary Figure 8. Complementation of the late flowering phenotype of the *co-1* and *ft-1 Arabidopsis* mutants by the St*SP6A* gene. a, b, Lines harboring the potato *StSP6A* transgene bolted earlier than the corresponding *co-1* **(a)** and *ft-1* **(b)** mutants. **c,** Flowering time of the transgenic lines estimated as the number of rosette leaves at flowering. Error bars indicate standard deviation (n=10).



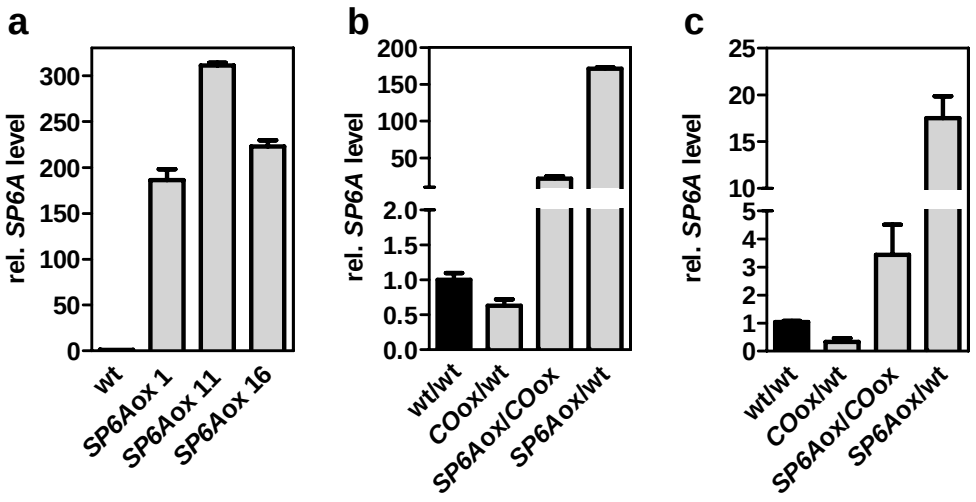
Supplementary Figure 9. StSP6A, StSP5G and PGSC0003DMG200016180 (SP5G-like) amino acid sequence alignment with other FT-like proteins. Partial alignment showing amino acid exchanges in the segment region B of the fourth exon. Black stars in the upper part and red letters in the sugar beet BvFT1 sequence indicate the amino acid residues found to be implicated in flowering repression in sugar beet[3].



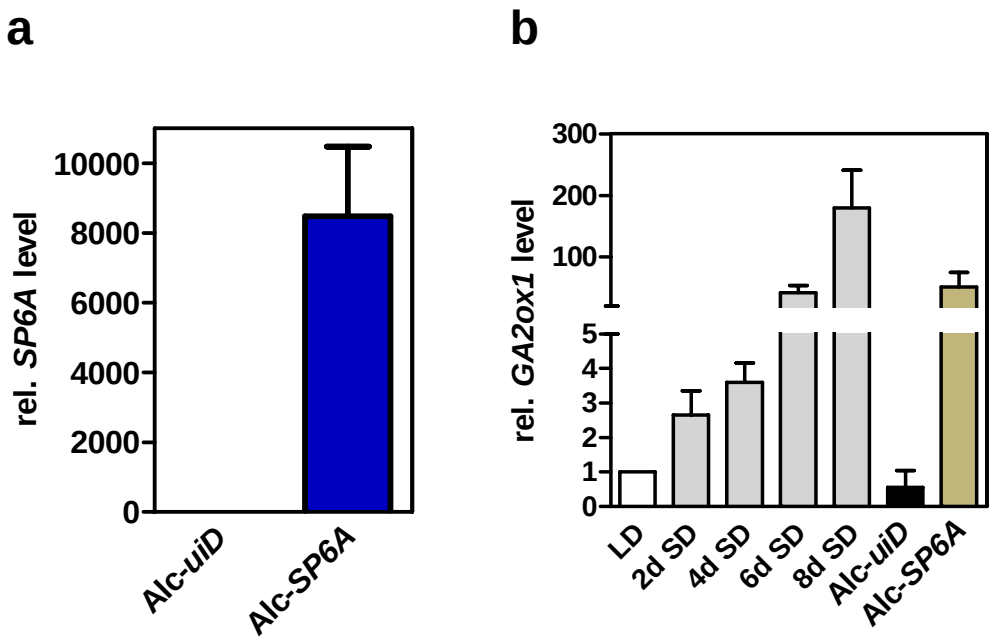
Supplementary Figure 10. St*SP6A* expression in *Hd3A* grafted plants. RT-qPCR was used to analyze relative St*SP6A* expression in different organs of grafted plants grown under long days. **a,** *Hd3a* scions grafted onto wild-type stocks. **b,** Wild-type scions grafted onto *Hd3a* stocks. St*SP6A* transcript levels in wild-type (dark blue bars) and *Hd3a* tissues (light blue bars) were estimated as the $2^{-\Delta\Delta C_T}$ value relative to the mean values obtained in wild-type control tissues. Values are represented in a logarithmic scale. The dashed line indicates the graft junction. Error bars indicate standard deviation of three independent experiments.



Supplementary Figure 11. St*CO* acts as a repressor of tuberization under long days. a, St*CO* transcript levels in leaves of St*CO* over-expressing lines (*CO*ox). **b,** Relative St*SP6A* and St*SP3D* expression in leaves of wild-type and transgenic St*CO*ox, after 4 inductive days. **c,** Short days (SD) kinetics of St*SP6A* regulation in St*CO*ox leaves. Values are represented in a logarithmic scale. **d,** St*CO* transcript reduction in leaves of RNAi plants (*CO*i). **e,** St*SP6A* and St*SP3D* regulation in leaves of St*CO*-RNAi plants under long days (LD). Error bars indicate standard deviation of three biological replicates. **f,** Tuberization of St*CO*-RNAi plants (left) under non-inductive long days. Wild-type controls (right) do not tuberize.



Supplementary Figure 12. St*CO* repression of the relay mechanism is conserved in two different St*SP6A*ox lines. St*SP6A* expression in leaves of different St*SP6A*ox lines (**a**). Relative St*SP6A* expression in grafting experiments with two different St*SP6A*ox lines, line 11 (**b**) and line 16 (**c**). Error bars indicate standard deviation (n=3).



Supplementary Figure 13. St*GA2ox1* response to local St*SP6A* induction. a, St*SP6A* expression in Alc-*SP6A* stolons relative to Alc-*uiD* controls, after four hours of ethanol induction. **b,** St*GA2ox1* expression in wild-type Andigena stolons after transfer to short day inductive conditions, compared to St*GA2ox1* in long days (LD) grown Alc-*SP6A* lines after four hours of St*SP6A* induction with ethanol. Error bars indicate standard deviation of two independently treated samples. This experiment was repeated in different transgenic lines with similar results.

Supplementary Table 1. Description and numeric values of the set of genes represented in Supplementary Figure 4. Gene expression is represented as the relative log ratio.

Photoperiod	SD	LD	LD	SD 2 days	SD 6 days	SD 8 days			
Genotype	StSP6Ai	StSP6Aox	WT	WT	WT	WT	Accessions	GeneName	Description
	-2.68	3.82	-1.62	-1.00	1.25	3.44	NA|00000	ACDA01685E05.T3m.scf	Patatin T-5
	-2.19	3.06	-1.11	-0.66	-0.61	3.07	NA|00000	bf_arrayxxx_0049e03.t7m.scf	probable metallocarboxypeptidase inhibitor PFT4 - potato dbj_BAA21500.1_ metallocarboxypeptidase inhibitor [Solanum tuberosum]
	-2.75	2.99	-1.26	-0.79	0.22	3.21	NA|00000	bf_arrayxxx_0053a08.t7m.scf	probable metallocarboxypeptidase inhibitor PFT3 - potato dbj_BAA21495.1_ metallocarboxypeptidase inhibitor [Solanum tuberosum]
	-2.11	1.52	-1.94	-1.68	-1.18	4.30	sp|P08454	bf_cswcxxxx_0007b05.t3m.scf	Wound-induced proteinase inhibitor I precursor (Chymotrypsin inhibitor I D subunit) gb_AAA69781.1_ proteinase inhibitor I
	2.48	-2.59	2.16	1.68	-1.46	-8.93	gb|BAD86972	bf_mxlfxxxx_0014b09.t3m.scf	putative nitrate transporter [Oryza sativa (japonica cultivar-group)] dbj_BAB56042.1_ putative nitrate transporter [Oryza sativa (japonica cul
	-2.75	1.87	-2.87	-1.03	2.23	3.24	gb|AAO19451	MICRO.1076.C1	glucose-6-phosphate/phosphate translocator 2 [Solanum tuberosum]
	2.57	-1.81	3.00	2.11	-1.14	-7.98	gb|AAM61354	MICRO.13031.C1	putative cytochrome P450 [Arabidopsis thaliana] gb_AAD30263.1
	2.21	-2.21	3.72	3.84	-1.66	-11.46	gb|CAH59413	MICRO.13184.C1	auxin resistance protein [Plantago major]
	2.68	-2.35	2.38	1.36	-2.07	-7.35	gb|AAT37529	MICRO.15077.C1	purple acid phosphatase 1 [Solanum tuberosum]
	-3.43	2.30	-2.22	-0.97	1.88	4.47	gb|AAZ75960	MICRO.1520.C14	patatin [Solanum tuberosum] pir__S51596 patatin precursor non-sucrose-inducible - Solanum brevidens gb_AAA66198.1_ patatin precursc
	-2.88	2.01	-1.16	-0.74	0.69	2.71	gb|AAZ75962	MICRO.1520.C23	patatin [Solanum tuberosum] pir__A26017 patatin T5 precursor
	-3.66	4.15	-1.95	-0.85	1.35	3.15	pir|A29810	MICRO.1520.C3	patatin [Solanum tuberosum] sp_P07745_PAT0_SOLTU PATATIN PRECURSOR (POTATO TUBER PROTEIN)
	-4.24	4.42	-1.93	-0.93	1.17	2.91	gb|AAZ75958	MICRO.1520.C9	patatin [Solanum tuberosum] pir__S51596 patatin precursor non-sucrose-inducible - Solanum brevidens gb_AAA66198.1_ patatin precursc
	2.49	-2.72	1.34	0.84	-0.98	-3.80	NA|00000	MICRO.15952.C1	Isoform 5 of integumentary mucin C.1
	2.87	-2.09	3.98	2.91	-1.16	-12.00	gb|BAC43682	MICRO.15966.C1	At1g26948 [Arabidopsis thaliana] ref_NP_849712.1_ expressed Pollen protein. Allergen and extensin family
	2.67	-1.84	3.65	2.18	-0.80	-10.36	gb|AAD24597	MICRO.17678.C1	ref_NP_179254.2_ proline-rich family protein [Arabidopsis thaliana]
	2.09	-2.30	8.72	10.31	-5.83	-20.66	gb|AAV59327	MICRO.18299.C1	unknown protein [Oryza sativa (japonica cultivar-group)] ref_XP_476200.1_ unknown protein [Oryza sativa (japonica cultivar-group)]
	-2.75	3.38	-1.00	-0.80	-0.53	2.24	gb|BAA21495	MICRO.2033.C1	probable metallocarboxypeptidase inhibitor PFT3 - potato dbj_BAA21495.1_ metallocarboxypeptidase inhibitor [Solanum tuberosum]
	-2.21	2.13	-1.05	-0.83	-0.01	3.04	NA|00000	MICRO.2033.C11	metallocarboxypeptidase inhibitor
	-3.07	3.69	-1.00	-0.73	0.13	2.46	gb|BAA21493	MICRO.2033.C2	metallocarboxypeptidase inhibitor [Solanum tuberosum]
	-2.31	2.29	-0.96	-1.26	0.03	4.02	gb|AAZ94194	MICRO.2033.C6	metallocarboxypeptidase inhibitor [Solanum tuberosum]
	2.35	-2.14	2.38	1.76	-1.77	-8.56	gb|AAD21725	MICRO.2406.C1	[Arabidopsis thaliana] gb_AAD33868.1_ protodermal factor 1 [Arabidopsis thaliana]
	-2.10	3.60	-2.91	-1.03	2.51	3.86	NA|00000	MICRO.3274.C2	No Hits Found
	-2.27	4.23	-3.22	-0.98	2.84	4.30	NA|00000	MICRO.3274.C4	Eukaryotic translation initiation factor 6 (EIF-6)-like protein; n=1; Arabidopsis thaliana
	2.66	-2.82	4.88	2.87	-1.97	-10.71	gb|AAM65485	MICRO.4133.C1	putative GDSL-motif lipase/hydrolase [Arabidopsis thaliana]
	2.58	-2.14	3.37	-0.54	-1.30	-3.76	gb|CAB78857	MICRO.4160.C1	lipase-like protein [Arabidopsis thaliana] emb_CAA16735.1
	2.01	-1.60	5.74	2.20	-3.19	-6.18	gb|CAB78857	MICRO.4160.C6	lipase-like protein [Arabidopsis thaliana] emb_CAA16735.1_ lipase-like protein
	2.39	-2.92	5.05	2.71	-2.10	-10.42	gb|AAM64916	MICRO.4199.C1	putative GDSL-motif lipase/acylhydrolase [Arabidopsis thaliana] gb_AAO50514.1
	2.83	-1.90	4.89	5.14	-3.28	-9.85	gb|AAS46243	MICRO.4590.C1	xyloglucan endotransglucosylase-hydrolase XTH7 [Lycopersicon esculentum]
	2.56	-1.98	5.33	2.46	-3.20	-5.43	gb|AAB82301	MICRO.561.C1	ornithine decarboxylase [Lycopersicon esculentum] gb_AAB82301.2_ ornithine decarboxylase [Lycopersicon esculentum]
	2.32	-2.37	2.73	1.26	-1.19	-5.34	gb|AAV87156	MICRO.6179.C1	methylesterase [Lycopersicon esculentum]
	2.15	-2.71	-3.36	1.37	0.56	-1.81	pir|S59944	MICRO.6420.C1	3-hydroxy-3-methylglutaryl-coenzyme A reductase 1 (HMG-CoA reductase 1) (HMGR1)
	2.37	-2.48	-2.34	3.16	-1.57	-4.00	gb|AAM13292	MICRO.6450.C1	glucosyltransferase-like protein [Arabidopsis thaliana] gb_AAM13292.1
	2.39	-2.78	-1.32	2.56	0.87	-12.15	gb|CAA39278	MICRO.6793.C1	actin [Solanum tuberosum] pir__S20094 actin 58 - potato sp_P30167_ACT3_SOLTU Actin 58
	2.06	-1.98	1.36	2.46	1.02	-12.57	gb|CAI48076	MICRO.8531.C1	microsomal omega-6-desaturase [Nicotiana tabacum]
	2.57	-2.81	2.65	2.38	-1.33	-5.86	gb|AAB39556	MICRO.8715.C1	probable polygalacturonase (EC 3.2.1.15) 1 - tomato gb_AAB39556.1_ AROGP2
	2.63	-2.97	-2.38	1.49	0.41	-2.18	gb|AAB39557	POABU26TP	POABU26TP_501-probable polygalacturonase (EC 3.2.1.15) 1 - tomato gb_AAB39557.1_ AROGP3
	2.23	-2.55	4.89	1.92	-1.26	-10.59	gb|CAD30341	SDBN002A15u.scf	SDBN002A15u.scf_448-tyrosine aminotransferase [Solenostemon scutellarioides]
	-2.15	3.29	-1.78	-0.91	-0.01	4.16	NA|00000	TBSK00763FH07.t3m.scf	metallocarboxypeptidase inhibitor
	-2.22	1.84	-0.89	-0.92	-0.02	3.11	NA|00000	TBSK01466FC02.t3m.scf	metallocarboxypeptidase inhibitor [Solanum tuberosum]
	-3.38	2.67	-1.79	-0.85	1.47	3.63	NA|00000	TBSK02196FG12.t3m.scf	Nuclear Binding protein DMG200020488
	-2.05	3.06	-1.07	-0.86	-0.30	2.67	gb|AAZ94194	TBSK03109FD01.t3m.scf	metallocarboxypeptidase inhibitor [Solanum tuberosum]
	-2.22	2.95	-0.87	-0.81	-0.23	3.07	NA|00000	TBSK03444FG12.t3m.scf	metallocarboxypeptidase inhibitor [Solanum tuberosum]
	-3.49	2.10	-2.63	-1.22	1.86	4.45	NA|00000	TBSK03752FA08.t3m.scf	Patatin homolog
	-2.77	3.60	-1.03	-1.07	-0.36	3.04	NA|00000	TBSK04121FH05.t3m.scf	Argonaute protein group
	-2.96	2.18	-1.94	-1.19	1.08	3.20	NA|00000	TBSK04176FD12.t3m.scf	Patatin homolog
	-3.88	3.61	-2.63	-1.21	1.75	3.98	NA|00000	TBSK04996FA04.t3m.scf	Patatin homolog

Supplementary Table 2. FT/TFL1 family members identified in the Potato Genome (http://potatogenomics.plantbiology.msu.edu/).

Locus	Protein	Transcript	Chr.	Start poss.	End poss.	Superscaffold	Scaffold	comments
PGSC0003DMG400016180	DMP200028269	DMT400041726	11	4756770	5999682	PGSC0003DMB000000152	PGSC0003DMS000002042	SP5G like
PGSC0003DMG400014322	DMP200025227	DMT400037143	3	22117937	22761173	PGSC0003DMB000000154	PGSC0003DMS000001644	CEN 1
PGSC0003DMG400007111	DMP200012606	DMT400018307	6	61340731	61409304	PGSC0003DMB000000091	PGSC0003DMS000002691	SELF-PRUNING
PGSC0003DMG400023365	DMP200040404	DMT400060057	5	53183226	53934257	PGSC0003DMB000000072	PGSC0003DMS000000201	SP6A
PGSC0003DMG400011707	DMP200020755	DMT400030575	1	37852084	37877323	PGSC0003DMB000000560	PGSC0003DMS000002631	CEN 1
PGSC0003DMG400015751	DMP200027622	DMT400040735	5	66063	69181	PGSC0003DMB000000512	PGSC0003DMS000002164	SP5G
PGSC0003DMG400040097	DMP200062201	DMT400090526	9	7462255	7564753	PGSC0003DMB000000021	PGSC0003DMS000000876	SP9D
PGSC0003DMG400005654	DMP200009953	DMT400014409	3	51272594	52284276	PGSC0003DMB000000026	PGSC0003DMS000001550	MOTHER of FT
No ID	No ID	No ID	3	1063000	1068600	PGSC0003DMB000000142	PGSC0003DMS000000135	SP3D

Supplementary Table 3. Primer sets used in this study. St*GA2 oxidase 1* and St*SUT1* primers have been described elsewhere[7,9].

PRIMER NAME	SEQUENCE (5' to 3')	TARGET GENE	AMPLICON LENGHT	USAGE
Hd3a:GFP for	GGAAAAGCTTGCCTATGTGG and	*Hd3a*	72 bp	qPCR
Hd3a:GFP rev	CTGCTCCTGGCAGTTTCAA	*Hd3a*	72 bp	qPCR
actin for	GGAAAAGCTTGCCTATGTGG	St*actin8*	59 bp	qPCR
actin rev	CTGCTCCTGGCAGTTTCAA	St*actin8*	59 bp	qPCR
SP6A for	GACGATCTTCGCAACTTTTACA	St*SP6A*	74 bp	qPCR
SP6A rev	CCTCAAGTTAGGGTCGCTTG	St*SP6A*	74 bp	qPCR
SP3D for	GGACCCAGATGCTCCAAGTC	St*SP3D*	96 bp	qPCR
SP3D rev	CTTGCCAAAACTTGAACCTG	St*SP3D*	96bp	qPCR
q5G_Afor	GGTGTGTAGACTTTGGTGTGGTTT	St*SP5G*	64 bp	qPCR
q5G_Arev	GGCCTCAAGGCACATCCAT	St*SP5G*	64 bp	qPCR
CO_q_For	GTAGCAACAATTGGGCAAGGG	St*CO*	64 bp	qPCR
CO_q_Rev	AGTAAACGGTACATGTTGCGGA	St*CO*	64 bp	qPCR
PGSC0003DMT200041726 for	CCAACTCCGAGCAATCCTTA	*PGSC0003DMG400016180*	63 bp	qPCR
PGSC0003DMT200041726 rev	TGCTGGGATATCAGTGACCA	*PGSC0003DMG400016180*	63 bp	qPCR
PGSC0003DMP200020755 for	TGCCTCTTGTCATTGCTTCTAA	*PGSC0003DMG400011707*	110 bp	qPCR
PGSC0003DMP200020755 rev	AGGATCACTAGGACCTGGAACA	*PGSC0003DMG400011707*	110 bp	qPCR
PGSC0003DMP200025226 for	ACCATCAACAAGGGATCAATTC	*PGSC0003DMG400014322*	100 bp	qPCR
PGSC0003DMP200025226 rev	AGTTTCTCTCTGGGCATTGAAA	*PGSC0003DMG400014322*	100 bp	qPCR
PGSC0003DMP200012606 for	TCCAGATGTTCCTGGTCCTAGT	*PGSC0003DMG400007111*	86 bp	qPCR
PGSC0003DMP200012606 rev	AGCAATCTGTAGTGCCTGGAAT	*PGSC0003DMG400007111*	86 bp	qPCR
PGSC0003DMP200062201 for	AGGATCTGTCATGACCAGTGTG	*PGSC0003DMG400040097*	89 bp	qPCR
PGSC0003DMP200062201 rev	TTTTCCCTTCTGCTGTTTCTTC	*PGSC0003DMG400040097*	89 bp	qPCR
qMFT_for	GGAATTTTGCAGCCACCAGTAG	*PGSC0003DMG400005654*	125 bp	qPCR
qMFT_rev	TTTCGATTTGCTGGTTCCTTGT	*PGSC0003DMG400005654*	125 bp	qPCR

SP3D_RNAi for	CACCGTTCAGACAATTAGGTCGACA	St*SP3D*	310 bp	RNAi construct
SP3D_RNAi rev	AAGTAGTAGAGATTGGTGGTT	St*SP3D*	310 bp	RNAi construct
SP6A_RNAi for	CACCTAGAATAAAGTCTATATTGAC	St*SP6A*	257 bp	RNAi construct
SP6A_RNAi rev	ATCATATGCT AACCAATATACTC	St*SP6A*	257 bp	RNAi construct
Co_RNAi for	CACCGTTAGCGGTGGTTCTATGAT	St*CO*	1052 bp	RNAi construct
Co_RNAi rev	AGAACTCTTTTTACATATACAACA	St*CO*	1052 bp	RNAi construct
Col1_for	CACCATGTTGAAAAAAGAGAAGAGTG	St*CO*	1239 bp	OE construct
Col1_rev	GAATGAAGGGACAATTCCATAAC	St*CO*	1239 bp	OE construct
SP6A-ATG	ATCATGCCTAGAGTTGATCCATTG	St*SP6A*	522 bp	OE construct
SP6A-TAA	TTTTTATGCGCGACGTCCTCCAGTAC	St*SP6A*	522 bp	OE construct
SP6A-SQ for	CCATTGATAGTTGGTCGTGTG	St*SP6A*	458 bp	semi-quantitative PCR
SP6A-SQ rev	ACAGCTGCAACAGGCAATCC	St*SP6A*	458 bp	semi-quantitative PCR
ActinF-SQ	CCTTGTATGCTAGTGGTCG	St*actin8*	251 bp	semi-quantitative PCR
ActinR-SQ	GCTCATAGTCAAGAGCCAC	St*actin8*	251 bp	semi-quantitative PCR
StGA2ox1 forward[7]	AGGCACAGAGTGATCGCAGAT	St*GA2 oxidase 1*	65 bp	qPCR
StGA2ox1 reverse[7]	TGGTGGCCCTCCAAAGTAAA	St*GA2 oxidase 1*	65 bp	qPCR
AGL8_q_for	AGCAAAACAACCAGCTTTCCAA	St*AGL8*	73 bp	qPCR
AGL8_q_rev	TGATCCCACTGATTTTGCTGTG	St*AGL8*	73 bp	qPCR
PIN1For	CGTGTTCGGTGGCAATGAT	StPIN1 like protein	56 bp	qPCR
PIN1Rev	CTCCATCGGATCGACCTGAT	StPIN1 like protein	56 bp	qPCR
PIN4For	GTTTCATTGCGGCGGATTC	StPIN4 like protein	60 bp	qPCR
PIN4Rev	CCCATAGCGAAAGAACAACCA	StPIN4 like protein	60 bp	qPCR
AuxTF_For	CAGCCTAAGCGGCATCTTCT	St*ARF8*	59 bp	qPCR
AuxTF_Rev	AGCCTTTTGGCGCTAACAAA	St*ARF8*	59 bp	qPCR
LC-SUT1_for[9]	TTCCATAGCTGCTGGTGTTC	St*SUT1*	127 bp	qPCR
LC-SUT1_rev[9]	TACCAGAAATGGGTCCACAA	St*SUT1*	127 bp	qPCR
FD forward	GGAGACGGAAGTGGCACATT	St*FD*	55 bp	qPCR

FD reverse	TAACTGCAGCTAAGCGTAACTGTTG	St*FD*	55 bp	qPCR
FPF_q_rev	CTAGTTGAGAACGCCGGTGACT	St*FPF1*	127 bp	qPCR
FPF_q_for	CCCATCCAAGAGAGTACAAATTCC	St*FPF1*	127 bp	qPCR
CDC2_q_for	ATGTCGCCAGTGTAGGGAATGT	St*CDC2*	142 bp	qPCR
CDC2_q_rev	TTTCCCTTCTTGGATGCTCTCA	St*CDC2*	142 bp	qPCR
SP6A-Topo-For	CACCATGCCTAGAGTTGATCCATTG	St*SP6A*	522 bp	AlcA-SP6A Cloning
SP6A-Topo-Rev	GTACTGGAGGACGTCGCGCATAA	St*SP6A*	522 bp	AlcA-SP6A Cloning