New Phytologist

***New Phytologist* Supporting Information**

Article title: Impaired KIN10 function restores developmental defects in the *Arabidopsis trehalose 6- phosphate synthase1* (*tps1*) mutant

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The following Supporting Information is available for this article:

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Fig. S2 Flowering time of F1 plants from complementation crosses among *kin10* and *snf4* alleles.

Fig. S3 Flowering time of suppressor mutants in SD and double mutants in LD lacking the *TPS1* inducible construct.

Fig. S4 The *kin10* T-DNA line (*snrk1α-3*) rescues *tps1-2* flowering.

Fig. S5 Relative expression of *TSF* in whole rosettes of 14- to 34-day-old plants.

Fig. S6 RNA-seq data from Col-0, *tps1-2 GVG::TPS1, kin10-5 tps1-2 GVG::TPS1* and *snf4-1 tps1-2 GVG::TPS1* apices.

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Table S1 List of oligonucleotides used in this study.

Table S2 Number of identified SNPs in individual EMS suppressor lines.

Table S3 Unique SNPs identified in all sequenced EMS suppressor lines.

Table S4 EMS suppressor lines bearing non-synonymous mutations in *KIN10* and *SNF4*.

Table S5 Differentially expressed genes in a) *tps1-2 GVG::TPS1* vs Col-0 b) the suppressor mutants and *tps1-2 GVG::TPS1*.

Methods S1 Genotyping of *kin10* and *snf4* mutations.

Methods S2 Mapping of EMS-induced mutations by high-throughput sequencing.

Methods S3 RT-qPCR information.

Methods S4 RNA-Seq data analyses.

Methods S5 *SOC1* RNA *in situ* hybridization**.**

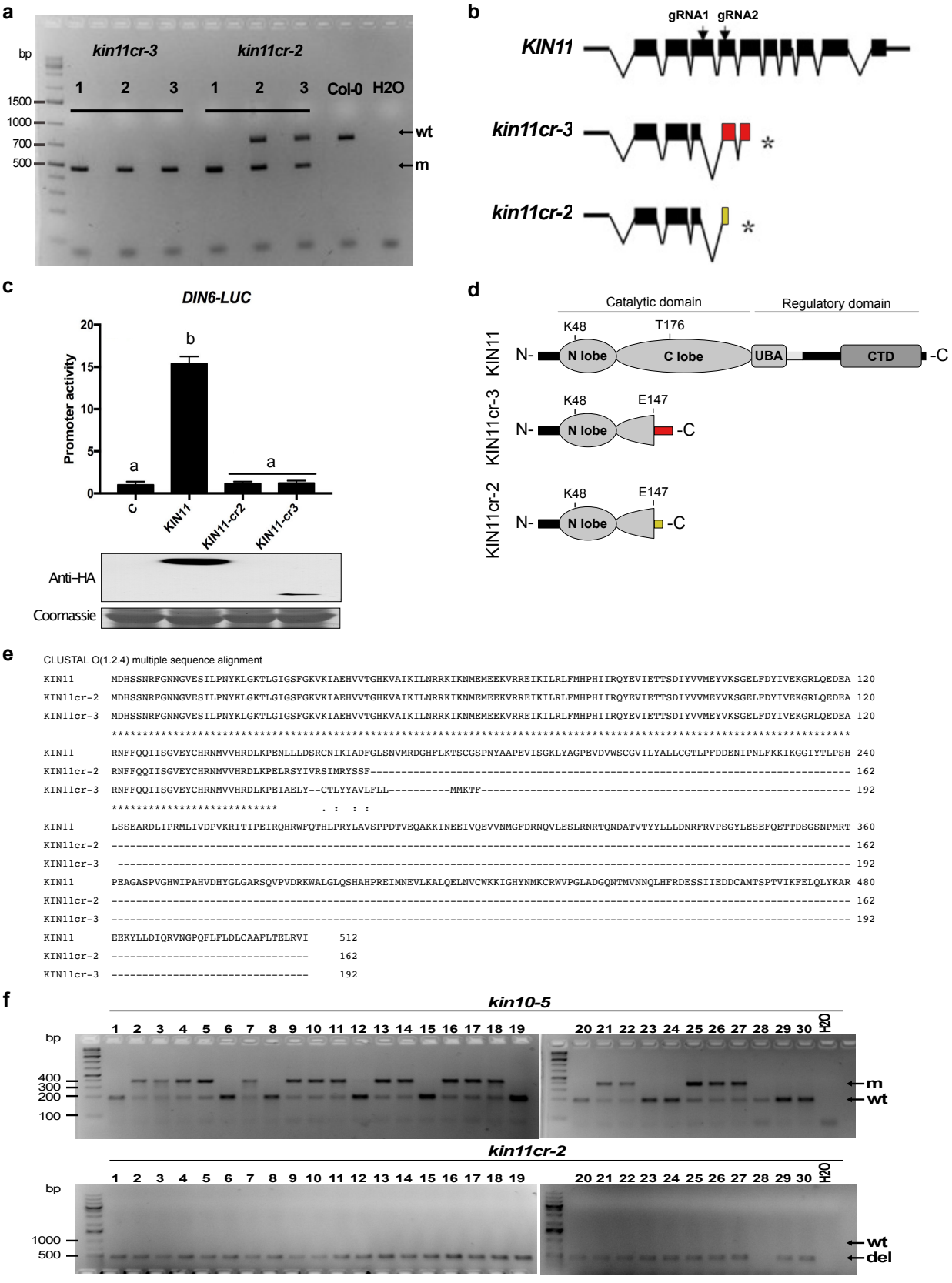
a
bp
kin11cr-3
kin11cr-2
1 2 3 1 2 3 Col-0 H2O
1500
1000
700
500
wt
m
b
gRNA1 gRNA2
KIN11
kin11cr-3
kin11cr-2
c
DIN6-LUC
Promoter activity
20
15
10
5
0
a
b
a
c
KIN11
KIN11-cr2
KIN11-cr3
Anti–HA
Coomassie
d
Catalytic domain
Regulatory domain
K48
T176
KIN11
N-
N lobe
C lobe
UBA
CTD
-C
K48
E147
KIN11cr-3
N-
N lobe
-C
K48
E147
KIN11cr-2
N-
N lobe
-C
e
CLUSTAL O(1.2.4) multiple sequence alignment
KIN11 MDHSSNRFGNNGVESILPNYKLGKTLGIGSFGKVKIAEHVVTGHKVAIKILNRRKIKNMEMEEKVRREIKILRLFMHPHIIRQYEVIETTSDIYVVMEYVKSGELFDYIVEKGRLQEDEA 120
KIN11cr-2 MDHSSNRFGNNGVESILPNYKLGKTLGIGSFGKVKIAEHVVTGHKVAIKILNRRKIKNMEMEEKVRREIKILRLFMHPHIIRQYEVIETTSDIYVVMEYVKSGELFDYIVEKGRLQEDEA 120
KIN11cr-3 MDHSSNRFGNNGVESILPNYKLGKTLGIGSFGKVKIAEHVVTGHKVAIKILNRRKIKNMEMEEKVRREIKILRLFMHPHIIRQYEVIETTSDIYVVMEYVKSGELFDYIVEKGRLQEDEA 120
KIN11 RNFFQQIISGVEYCHRNMVVHRDLKPENLLLDSRCNIKIADFGLSNVMRDGHFLKTSCGSPNYAAPEVISGKLYAGPEVDVWSCGVILYALLCGTLPFDDENIPNLFKKIKGGIYTLPSH 240
KIN11cr-2 RNFFQQIISGVEYCHRNMVVHRDLKPELRSYIVRSIMRYSSF---------- 162
KIN11cr-3 RNFFQQIISGVEYCHRNMVVHRDLKPEIAELY--CTLYYAVLFLL----------MMKTF---------- 192
KIN11 LSSEARDLIPRMLIVDPVKRITIPEIRQHRWFQTHLPRYLAVSPPDTVEQAKKINEEIVQEVVNMGFDRNQVLESLRNRTQNDATVTYYLLLDNRFRVPSGYLESEFQETTDSGSNPMRT 360
KIN11cr-2 ---------- 162
KIN11cr-3 ---------- 192
KIN11 PEAGASPVGHWIPAHVDHYGLGARSQVPVDRKWALGLQSHAHPREIMNEVLKALQELNVCWKKIGHYNMKCRWVPGLADGQNTMVNNQLHFRDESSIIEDDCAMTSPTVIKFELQLYKAR 480
KIN11cr-2 ---------- 162
KIN11cr-3 ---------- 192
KIN11 EEKYLLDIQRVNGPQFLFLDLCAAFLTELRVI 512
KIN11cr-2 ---------- 162
KIN11cr-3 ---------- 192
f
kin10-5
bp
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 H2O
400
300
200
100
m
wt
kin11cr-2
bp
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 H2O
1000
500
wt
del

Fig. S1 ***Arabidopsis thaliana KIN11*** **CRISPR/Cas9 mutations.** a. Confirmation of two independent CRISPR/Cas9-induced deletions in *KIN11*, *kin11cr-3* and *kin11cr-2*. wt: Wildtype (Col-0) PCR amplicon and m: *kin11cr* mutant PCR amplicon. b. Schematic representation of *KIN11* main transcript and truncated transcripts *in kin11cr* mutants. Black boxes are exons and zig-zag lines indicate introns. Red and yellow boxes are coding out-of-frame sequences specific for *kin11cr-3* and *kin11cr-2,* respectively*.* Arrows indicate the position of guide RNAs (gRNAs) and stars the premature termination of the frame-shifted transcripts in *kin11cr* mutants caused by the induced deletions. In the *kin11cr-2* mutant, the Cas9 cuts occurred three nucleotides 5' of the PAM sequences, as expected, while in *kin11cr-3* the gRNA1 cut four nucleotides 5' of the PAM sequence and the gRNA2 three nucleotides after the PAM sequence resulting in a different frameshift. c. *DIN6* promoter activity in *A. thaliana* leaf protoplasts upon transient expression of wild type and mutant KIN11 protein versions 6h after transfection. Values are averages with standard deviations, n=4, ANOVA Tukey's multiple comparisons test applied, and letters represent statistical differences among samples, $P < 0.001$, error bars represent SD. Protein expression was assessed by immunoblot analysis with anti-HA antibodies. d. Schematic representation of KIN11, and the predicted KIN11cr-2 and KIN11cr-3 proteins. KIN11 protein consisting of a catalytic domain formed by the N-lobe and C-lobe, which contain the conserved K48 residue important for phospho-transfer and the T-loop with indicated conserved threonine (T176) residue; a ubiquitin-associated (UBA) domain and linker sequence; and a C-terminal domain (CTD), essential for complex formation and other protein-protein interactions. Red and yellow boxes indicate protein sequences specific for KIN11cr-3 and KIN11cr-2*,* respectively, which are caused by the frameshift mutations and are not present in KIN11*.* e. Protein alignment of KIN11, KIN11cr-2 and KIN11cr-3 using ClustalO. f. Genotyping of F3 *kin10-5* x *kin11cr-2* plants. *kin11cr-2* is in homozygosity from F2 and *kin10-5* is segregating, m: BsuRI does not cut when *kin10-5* mutation is present, del: PCR amplification for the *kin11cr-2* mutant.

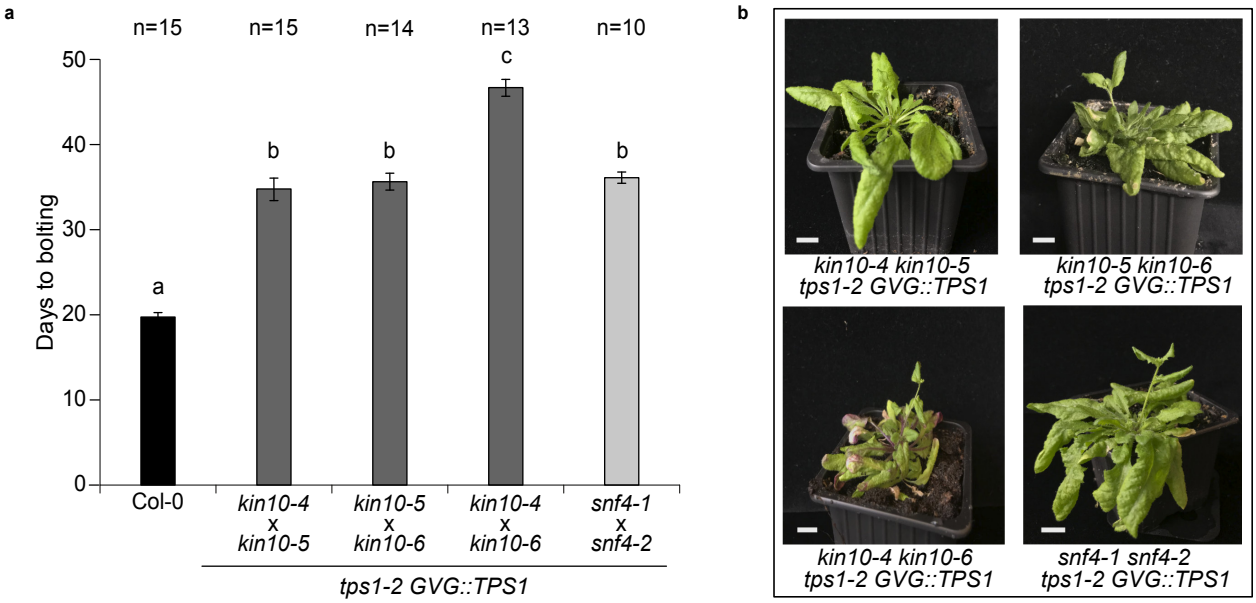

Fig. S2 Flowering time of F1 plants from complementation crosses among *Arabidopsis thaliana kin10* and *snf4* alleles. a. *kin10* and *snf4* mutants bearing two different mutant alleles can rescue the flowering time of *tps1-2 GVG::TPS1* mutant. Plants grown under long day (LD) conditions. ANOVA Tukey's multiple comparisons test applied, and letters represent statistical differences among genotypes, $P < 0.001$, error bars represent SD. n: number of individuals. b. Representative pictures of the F1 plants after bolting. Scale bar: 1cm.

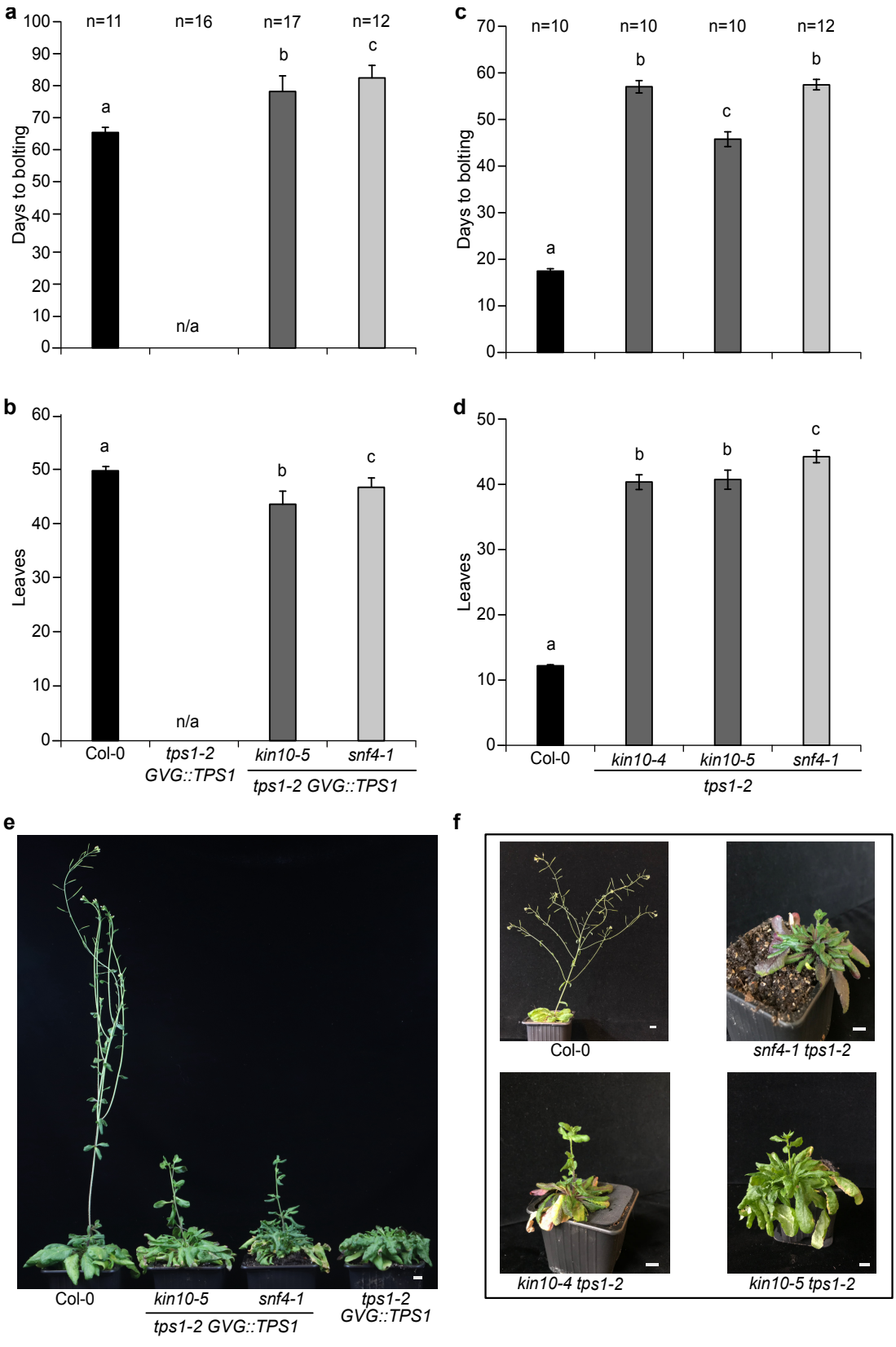
a
n=11
n=16
n=17
n=12
Days to bolting
n/a
b
c
c
n=10
n=10
n=10
n=12
b
Leaves
Col-0
tps1-2 GVG::TPS1
kin10-5
snf4-1
tps1-2 GVG::TPS1
kin10-4
kin10-5
snf4-1
tps1-2
e
f
Col-0
kin10-5
snf4-1
tps1-2 GVG::TPS1
tps1-2 GVG::TPS1
snf4-1 tps1-2
kin10-4 tps1-2
kin10-5 tps1-2

Fig. S3 Flowering time of suppressor mutants in SD and double mutants in LD lacking the *TPS1* inducible construct in *Arabidopsis thaliana*. a-b. Suppressor mutants were grown under short day (SD) conditions without dexamethasone application. c-d. Double *kin10 tps1-2* and *snf4 tps1-2* lacking the *GVG::TPS1* construct. Plants grown under LD conditions. n: number of individuals, n/a: not applicable because plants do not flower. ANOVA Tukey's multiple comparisons test applied, and letters represent statistical differences among genotypes, $P < 0.001$, error bars represent SD e. Representative picture of the suppressor mutants grown in SD without dexamethasone application. Scale bar: 1cm. f. Representative pictures of the double *kin10 tps1-2* and *snf4 tps1-2* plants grown in LD. Scale bar: 1cm.

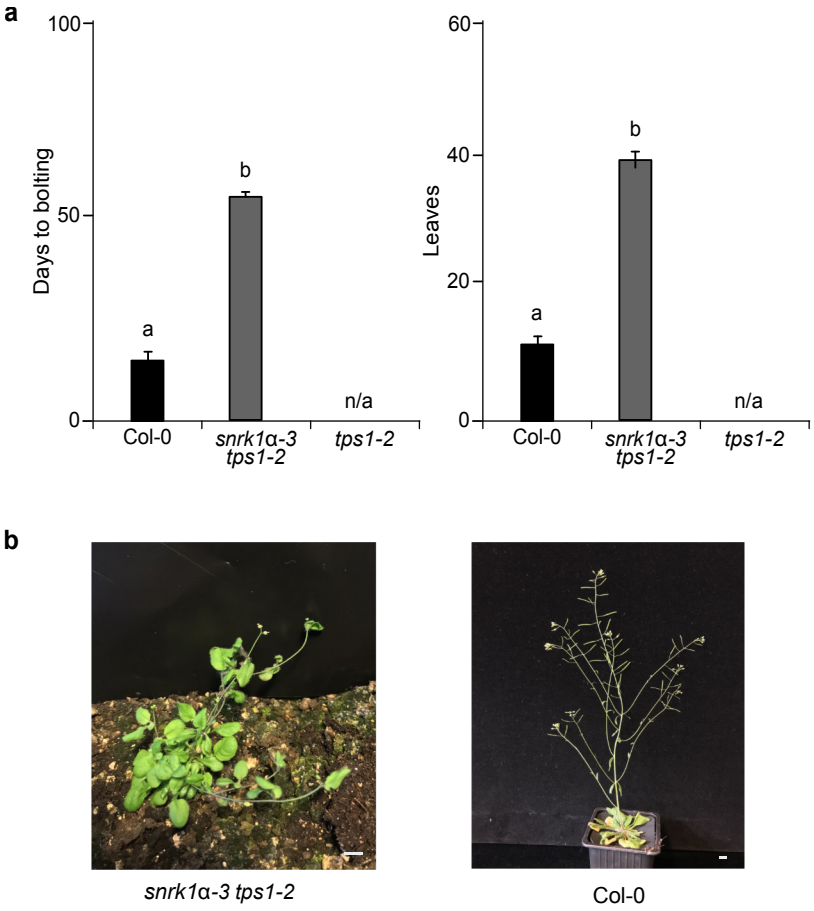

Fig. S4 The *kin10* T-DNA line (*snrk1α-3*) rescues *tps1-2* flowering in *Arabidopsis thaliana*. a. Double *snrk1α1-3 tps1-2* mutants lacking the *GVG::TPS1* construct are able to flower in LD. n/a: not applicable because plants do not flower. ANOVA Tukey's multiple comparisons test applied, and letters represent statistical differences among genotypes, *P* <0.001. b. Representative pictures of *snrk1α-3* tps1-2 and Col-0 plants. Scale bar: 1cm.

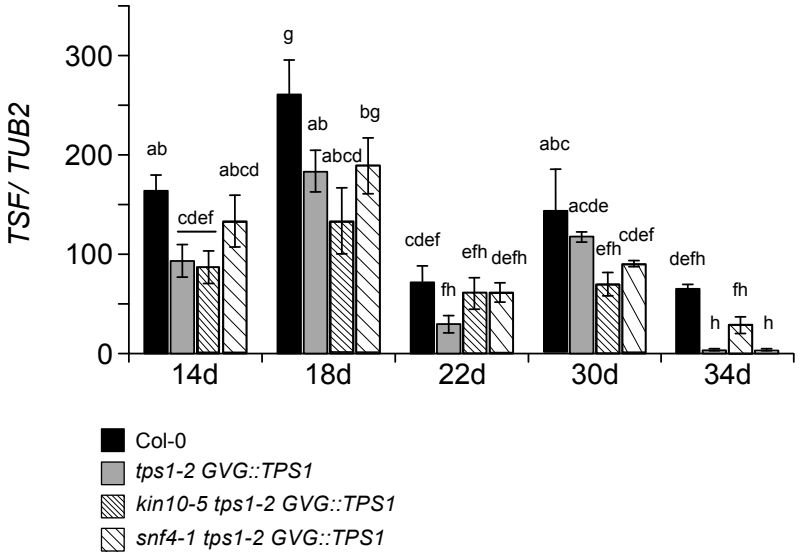

Fig. S5 Relative expression of *TSF* in whole rosettes of 14- to 34-day-old *Arabidopsis thaliana* plants. *TSF* expression in apices of 14 to 34-day-old plants in LD. Error bars: SD of three biological replicates. ANOVA Tukey's multiple comparisons test applied, and letters represent statistical differences, $P < 0.001$.

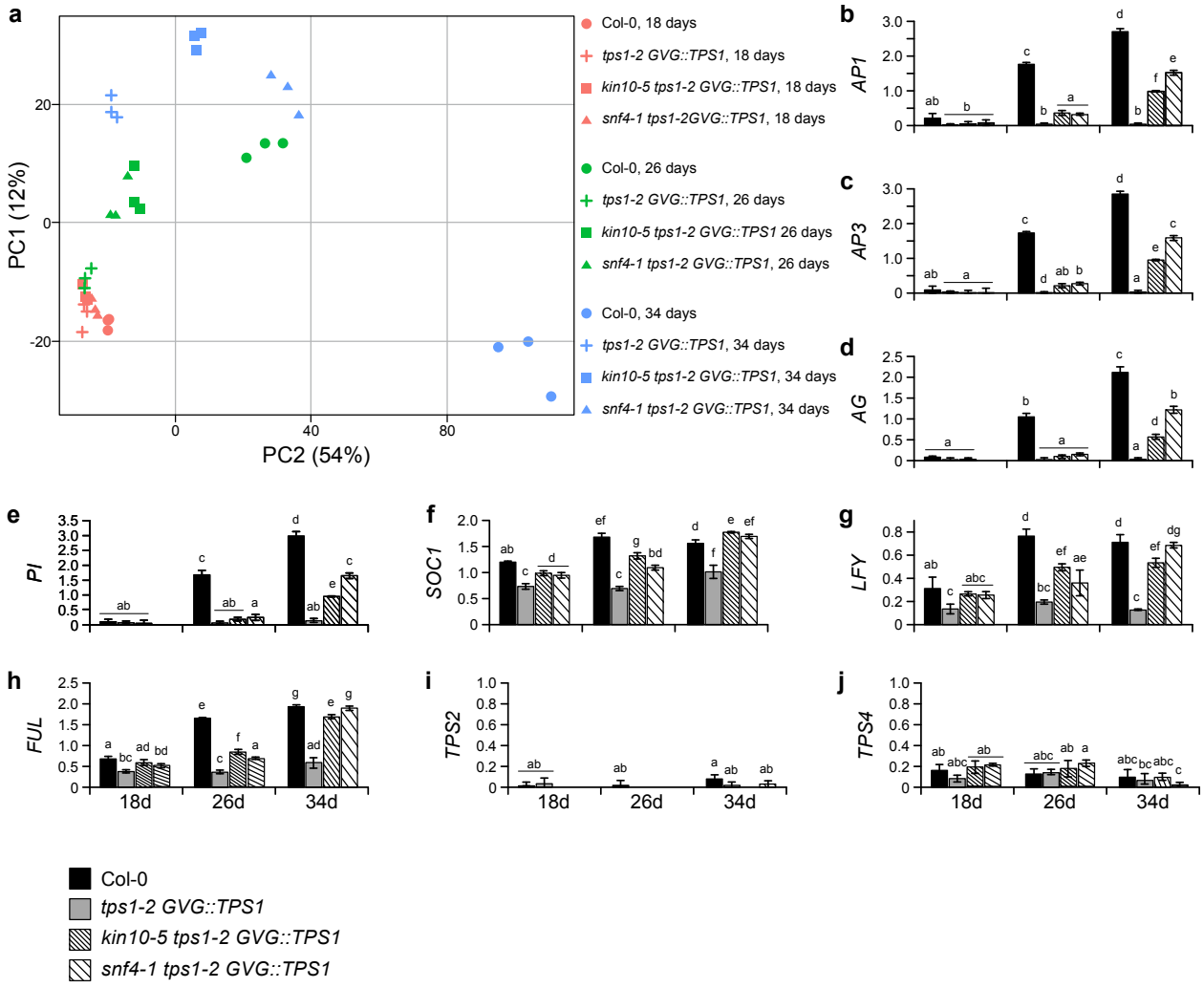

Fig. S6 RNA-seq data from *Arabidopsis thaliana* Col-0, *tps1-2 GVG::TPS1, kin10-5 tps1-2 GVG::TPS1* and *snf4-1 tps1-2 GVG::TPS1* apices. a. Principal component analysis (PCA) plot, b-h. Gene expression of selected flowering time and flower development genes. i-j. Gene expression of *TPS2* and *TPS4*. Y-axis shows vst (variance stabilising transformation) expression estimates. Error bars represent the SD of mean values from three biological replicates. ANOVA Tukey's multiple comparisons test applied, and letters represent statistical differences, $P < 0.001$.

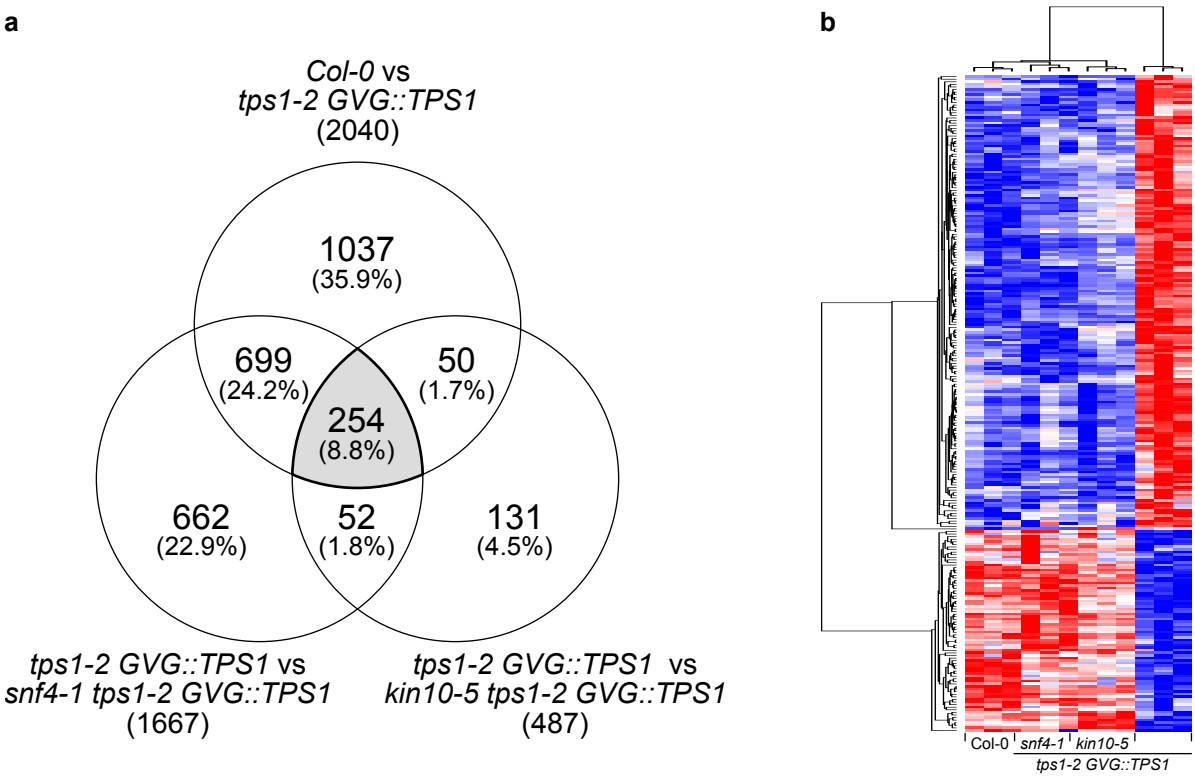

Fig. S7 Analysis of significantly differentially expressed genes in 18-day-old *Arabidopsis thaliana* plants. a. Venn diagram of significantly differentially expressed genes. Of the 2040 genes significantly differentially expressed between Col-0 and *tps1-2 GVG::TPS1* plants, 254 are also significantly differentially expressed in *kin10-5 tps1-2 GVG::TPS1* and *snf4-1 tps1-2 GVG::TPS1* when compared to *tps1-2 GVG::TPS1*. b. Hierarchical cluster analysis of 254 differentially expressed genes and 4 genotypes (3 replicates each). Heatmap and cluster analysis were performed using Morpheus (https://software.broadinstitute.org/morpheus).

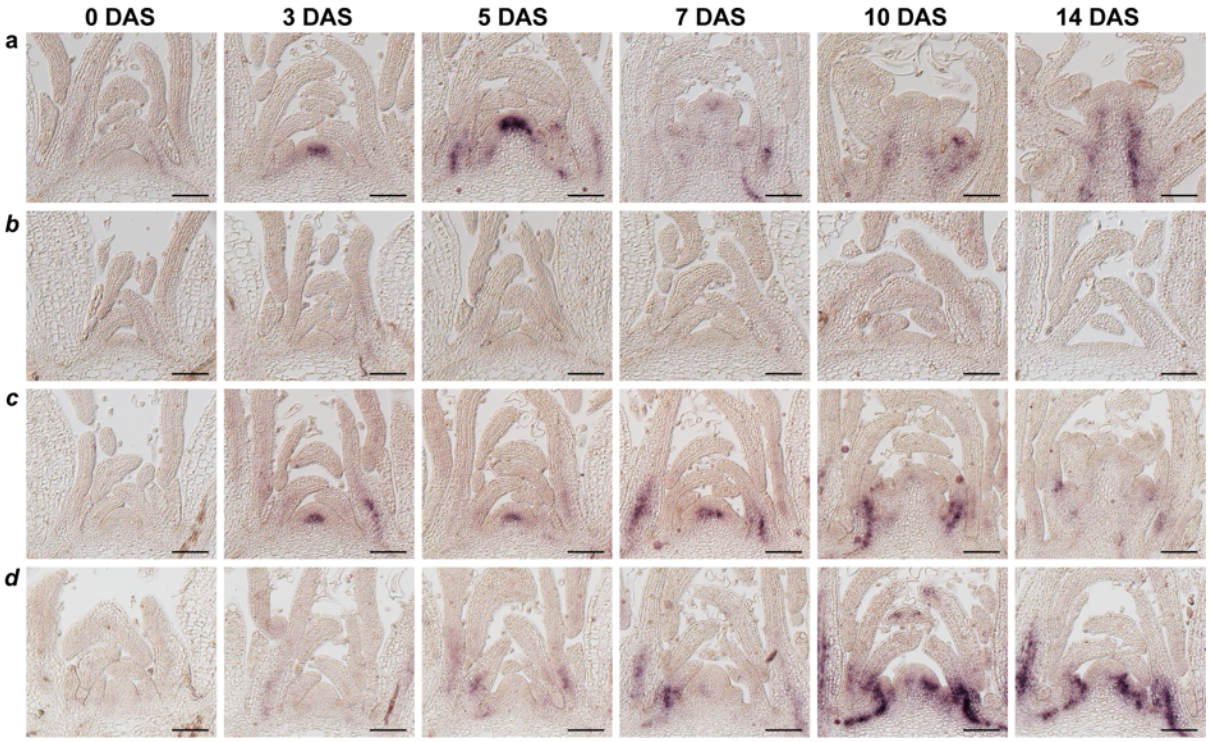

Fig. S8 Expression of *SOC1* in *Arabidopsis thaliana* SAM detected by RNA *in situ* hybridisation. *SOC1* expression in SAMs of two-week-old plants grown in short days for 30 days (0) and then transferred to and grown in long days for 3, 5, 7, 10, and 14 days. a. Col-0, b. *tps1-2 GVG::TPS1*, c. *snf4-1 tps1-2 GVG::TPS1*, d. *kin10-5 tps1-2 GVG::TPS1*. Scale bar, 100µm

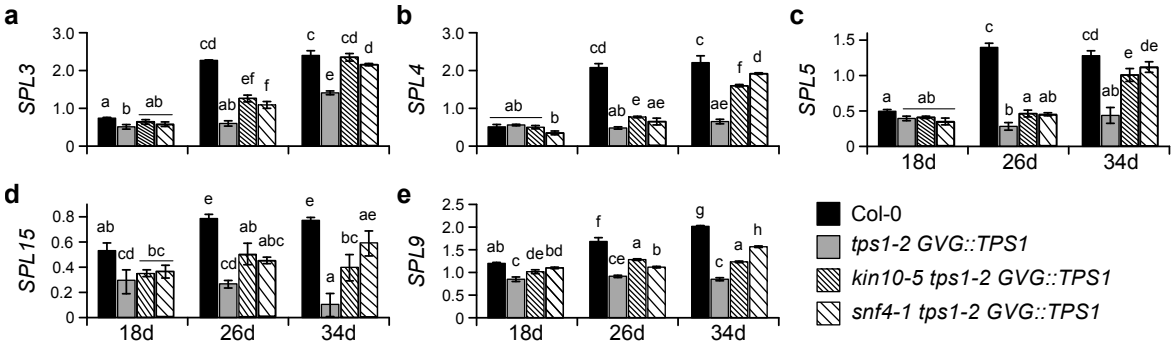

Fig. S9 Expression of *SPL* genes in *Arabidopsis thaliana* apices obtained by RNA-seq. a-e Expression of *SPL* genes in apices obtained by RNA-seq. Error bars represent the SD of mean values from three biological replicates. ANOVA Tukey's multiple comparisons test applied, and letters represent statistical differences, $P < 0.001$. Y-axis shows vst (variance stabilising transformation) expression.

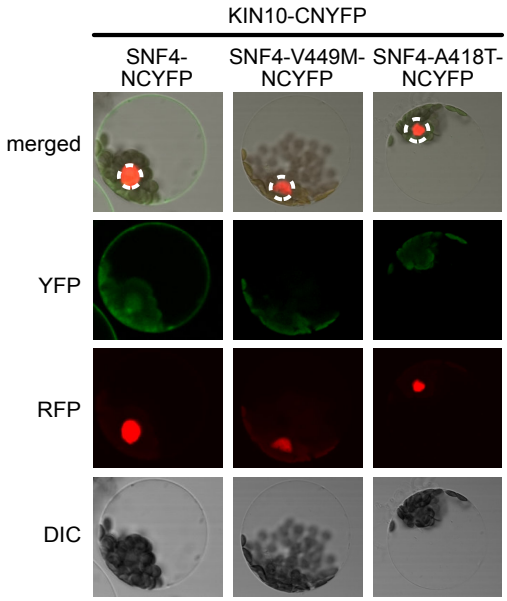

Fig. S10 SNF4 protein mutant versions are no longer participating in SnRK1 heterotrimeric complexes. BiFC assay of the interaction between KIN10 and SNF4 or SNF4-V449M/ SNF4-A418T upon transient co-expression of indicated HA-tagged split-YFP constructs and SNF4, 16h after transfection of *Arabidopsis thaliana* protoplasts. An SCF30-RFP nuclear marker was co-expressed which produces red fluorescence in the nucleus. Dashed circles indicate the nucleus. Top to bottom: merged; YFP (construct); RFP (SCF30-RFP nuclear marker and chlorophyll autofluorescence); DIC: differential interference contrast picture.

Methods S1 Genotyping of *kin10* and *snf4* mutations.

All newly characterized *kin10* and *snf4* mutations were confirmed by genotyping using the primer sets 438-627, 438-630, 438-628, 440-632, and 440-441 (Table S1) for *kin10-4, kin10-5*, *kin10-6*, *snf4-1,* and *snf4-2,* respectively. The resulted PCR products were digested with *Xba*I (*kin10-4*), *Bsu*RI (*kin10-5*), *Xho*I (*kin10-5)*, *ScaI* (*snf4-1*) and *Bfo*I (*snf4-2*). PCR products were amplified with DreamTaq (Thermo Scientific) and all enzymatic digestions (Thermo Scientific) were performed at 37°C for 2-3h.

Methods S2 Mapping of EMS-induced mutations by high-throughput sequencing

Genomic DNA was extracted from 64 suppressor mutant lines, pooled suppressor mutants of the #160-1 BC1F2 population (Table S2; Table S3), and non-mutagenized *tps1-2 GVG::TPS1* (control) using the DNeasy Plant Mini Kit (Qiagen). Libraries were generated from 1μg genomic DNA using the Illumina TruSeq library preparation kit and 96-bp paired-end sequencing was performed using an Illumina Genome Analyzer. Reads were aligned to the wild-type Col-0 reference genome using GenomeMapper. Variations between mutants and reference were identified using SHORE consensus after correcting the paired-end alignments. SNPs present in *tps1-2 GVG:TPS1* were removed and only EMS-induced SNPs were retained. Allele frequency was calculated as the ratio of reads of mutant alleles divided by all the reads at that specific locus. SNPs with an allele frequency lower than 25% were discarded. The Arabidopsis Information Resource 10 (TAIR) genome annotation was used to identify the effect of sequence change in the mutated genes.

Methods S3 RT-qPCR

TPS1, *DIN6* and *SEN5* expression were analysed using total RNA isolated from 14-day-old whole seedlings. *FT* expression was analysed in rosette leaves collected at Zeitgeber time (ZT) 15-16 for each time point. For *miR156, SPL3, AP1* expression analysis, 20-30 apices were collected per

biological replication at ZT 15-16 for each time point. RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) or by phenol/chloroform extraction using TRIzol® Reagent (Invitrogen), followed by sodium acetate/glycogen-assisted ethanol precipitation. Extracted RNA was treated with RNase-free DNase I (Thermo Scientific). cDNA was synthesized from 1-2 μg total RNA (RevertAid First Strand cDNA Synthesis Kit, Thermo Scientific) with oligo-dT or, in the case of mature miR156 expression analyses, a 1:1 mixture of the miR156-specific loop primer (2166; Table S1) and oligo-dT. qPCR was performed using a LightCycler 480 SYBR Green I Master (Roche Life Science) reaction mix in Bio-Rad CFX96 or CFX384 machines with the following cycling conditions: 10 min at 95°C, 42 cycles of 20 s at 95°C, 10 s at 55°C, and 30 s at 72°C. *TUBULIN2* was used as an internal standard. For each sample, three independent biological replicates with two to three technical replicates each were used. All values were normalized and displayed against the lowest expression value in every experiment. The sequences of the primers used for RT-qPCR are listed in Table S1.

Methods S4 RNA-Seq data analyses

RNA was isolated from apices of 18, 26 and 34-day-old plants grown under LD conditions, as described above. RNA-seq library preparation and sequencing were carried out by Novogene.

The data pre-processing was performed following the guidelines described here: http://www.epigenesys.eu/en/protocols/bio-informatics/1283-guidelines-for-rna-seq-data-analysis. Briefly, the quality of the raw sequence data was assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), v0.11.4. Residual ribosomal RNA (rRNA) contamination was assessed and filtered using SortMeRNA v2.1, (Kopylova *et al.*, 2012) settings --log--paired_in--fastx--sam--num_alignments1) using the rRNA sequences provided with SortMeRNA (rfam-5s-database-id98.fasta, rfam-5.8s-database-id98.fasta, silva-arc-16s-database-id95.fasta, silva-bac-16s-database-id85.fasta, silva-euk-18s-database-id95.fasta, silva-arc-23s-database-id98.fasta, silva-bac-23s-database-id98.fasta and silva-euk-28s-database-id98.fasta). Data were then filtered to remove adapters and trimmed for quality using Trimmomatic v0.39, (Bolger *et al.*, 2014) settings TruSeq3-PE-2.fa:2:30:10 SLIDINGWINDOW:5:20

MINLEN:50). After both filtering steps, FastQC was run again to ensure that no technical artefacts were introduced. Read counts were obtained using salmon v0.14.1, (Patro *et al.*, 2017) with non-default parameters --gcBias–seqBias and using the ARAPORT11 cDNA sequences as a reference (retrieved from the TAIR resource), (Berardini *et al.*, 2015; Cheng *et al.*, 2017). The salmon abundance values were imported into R (v3.6.2;R Core Team 2019-). https://www.R-project.org/.) using the Bioconductor (v3.10), (Gentleman *et al.*, 2004) tximport package (v.1.12.3), (Soneson *et al.*, 2015). For the data quality assessment (QA) and visualisation, the read counts were normalised using a variance stabilising transformation as implemented in DESeq2. The biological relevance of the data - e.g. biological replicates similarity - was assessed by Principal Component Analysis (PCA) and other visualisations using custom R scripts, available at https://github.com/nicolasDelhomme/tps1-kin10-snf4. Statistical analysis of gene and transcript differential expression (DE) between conditions was performed in R using the Bioconductor DESeq2 package (v1.26.0), (Love *et al.*, 2014), with the following model: ~MGenotype*MDay to account for both the genotype and the day of harvesting. FDR adjusted p-values were used to assess significance; a common threshold of 1% was used throughout. All the expression results were generated in R, using custom scripts.

Berardini TZ, Reiser L, Li D, Mezheritsky Y, Muller R, Strait E, Huala E. 2015. The Arabidopsis information resource: Making and mining the "gold standard" annotated reference plant genome. *Genesis* **53**(8): 474-485.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**(15): 2114-2120.

Cheng CY, Krishnakumar V, Chan AP, Thibaud-Nissen F, Schobel S, Town CD. 2017. Araport11: a complete reannotation of the *Arabidopsis thaliana* reference genome. *Plant J* **89**(4): 789-804.

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, et al. 2004. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* **5**(10): R80.

Kopylova E, Noe L, Touzet H. 2012. SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics* **28**(24): 3211-3217.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**(12): 550.

Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. 2017. Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods* **14**(4): 417-419.

Soneson C, Love MI, Robinson MD. 2015. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Res* **4**: 1521.

Methods S5 *SOC1 RNA in situ hybridization*

Col-0, *tps1-2 GVG::TPS1*, *snf4 tps1-2 GVG::TPS1*, and *kin10 tps1-2 GVG::TPS1* plants were grown in short day (SD) conditions (8h light/16h dark; 160µmol m^{-2} s^{-1}, 22°C) for 30 days and transferred to long day (LD) (16h light/8h dark; 160µmol m^{-2} s^{-1}, 22°C) for 3, 5, 7, 10, and 14 days. Shoot apices were harvested at the end of the day and processed as described before. A specific *SOC1* antisense probe was used to hybridize longitudinal, consecutive sections through shoot apices (thickness: 8µm) of at least three individual plants per time point and genotype. The method, machines and microscopes used were described before. Representative middle sections were chosen for Fig. S9.