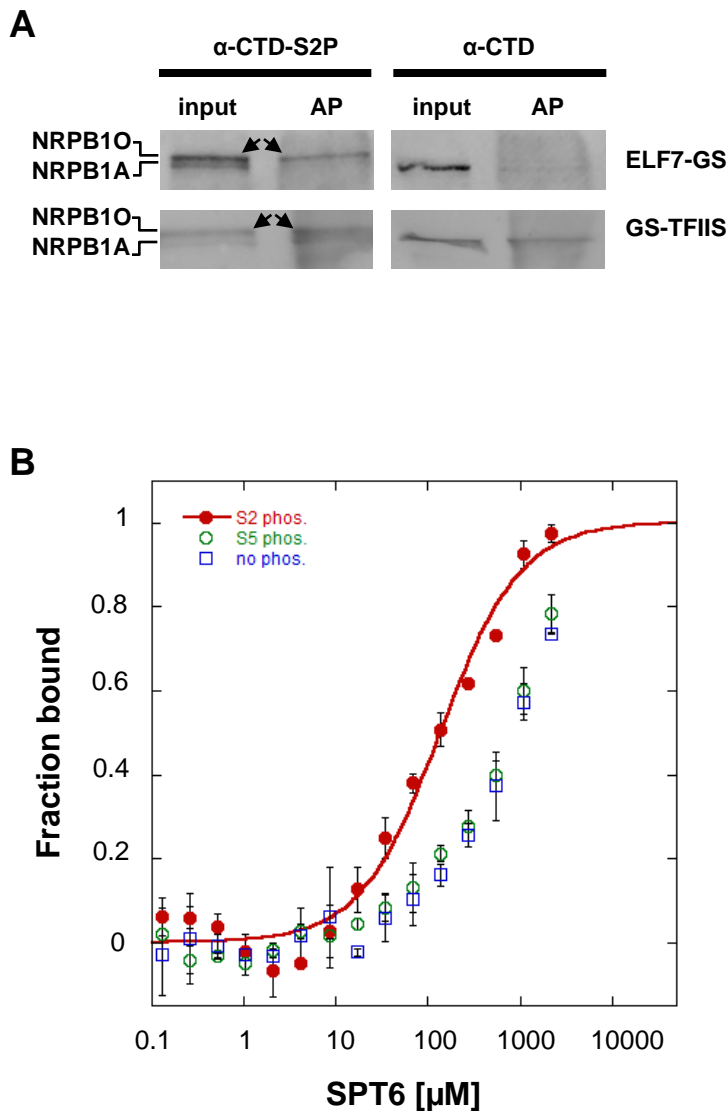
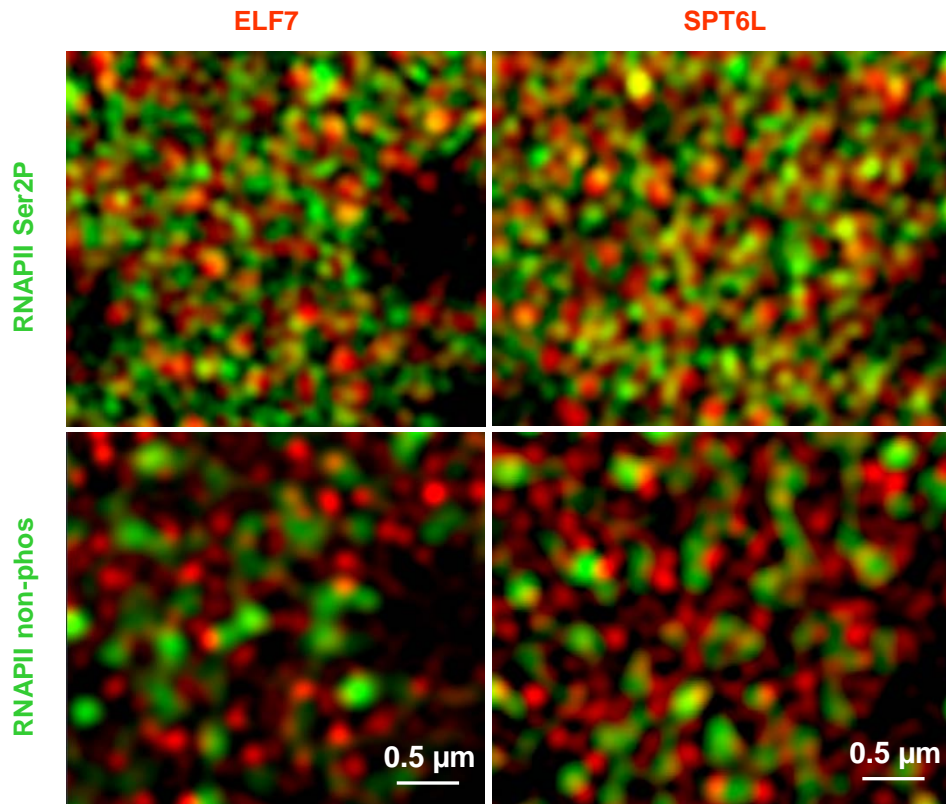


**Supplemental Figure 1.** Expression of *SPT6L* and *SPT6*. (Supports Figure 1.)

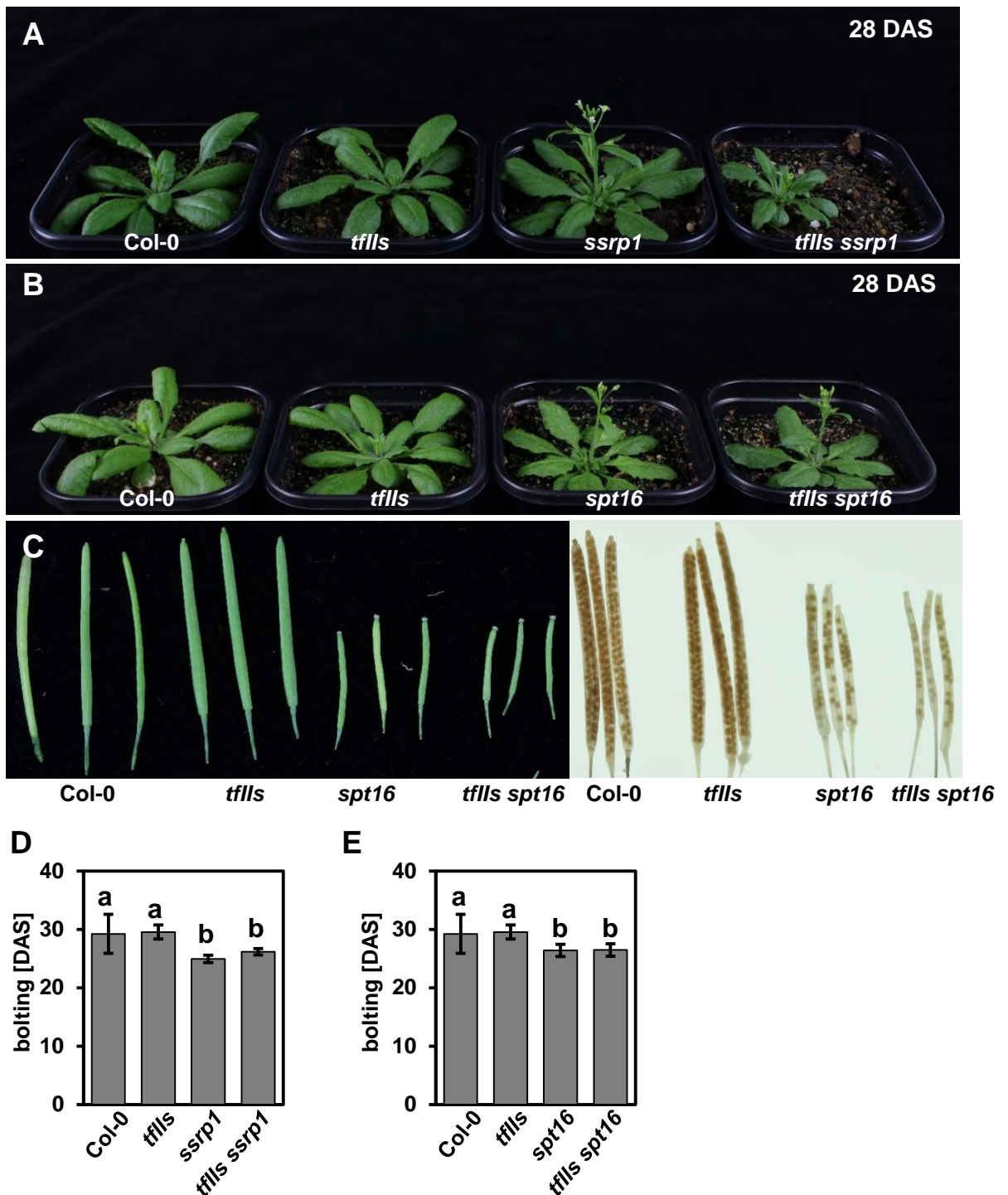
Transcript levels of *SPT6L* (At1g65440) and *SPT6* (At1g63210) were examined by RT-PCR (+RT) with RNA from 16 DAS Col-0 seedlings (**A**) and PSB-D cells (**B**). This experiment indicates that in line with publicly available microarray data (<http://www.arabidopsis.org/>) only *SPT6L* is expressed in seedlings, while the transcripts of both *SPT6* and *SPT6L* are detected in PSB-D cells. As controls the *ACT2* transcript was analysed comparatively as well as samples without reverse transcription (-RT) (right panels in **A,B**). Moreover, the functionality of the primer combinations was tested in PCR reactions using genomic DNA as a template (**C**).



**Supplemental Figure 2.** Enrichment of Ser2-phosphorylated RNAPII in TEF affinity purifications and interaction of SPT6L with Ser2-phosphorylated RNAPII-CTD repeats. (Supports Figure 1 and 2.) (A) Immunoblot analysis of affinity purifications (AP) vs. input of ELF7-GS and GS-TFIIS with antibodies directed against Ser2-phosphorylated RNAPII-CTD (left) and against non-phosphorylated RNAPII-CTD (right). The hypophosphorylated largest subunit NRPB1A has a slightly higher electrophoretic mobility than the hyperphosphorylated NRPB1O (indicated by arrows). (B) MST analysis of the interaction of GST-SPT6L(Phe1218-Asp1412) with RNAPII-CTD repeat peptides. A constant amount of the GST-SPT6L protein was incubated with increasing concentrations of FITC-labelled CTD repeat peptides that were either non-phosphorylated (blue), or phosphorylated at Ser2 (red) or Ser5 (green). The error bars represent the standard deviation of three technical experiments (independently prepared reagent mixtures followed by MST measurements). Binding affinity ( $K_D$ ) was calculated by fitting the data points (red line) using the  $K_D$  Fit derived from the law of mass action (0 = unbound, 1= bound) SPT6 showed an affinity of  $134.8 \pm 26.6 \mu\text{M}$  ( $R^2 = 0.980$ ). For S5 and non-phosphorylated weaker binding is indicated.

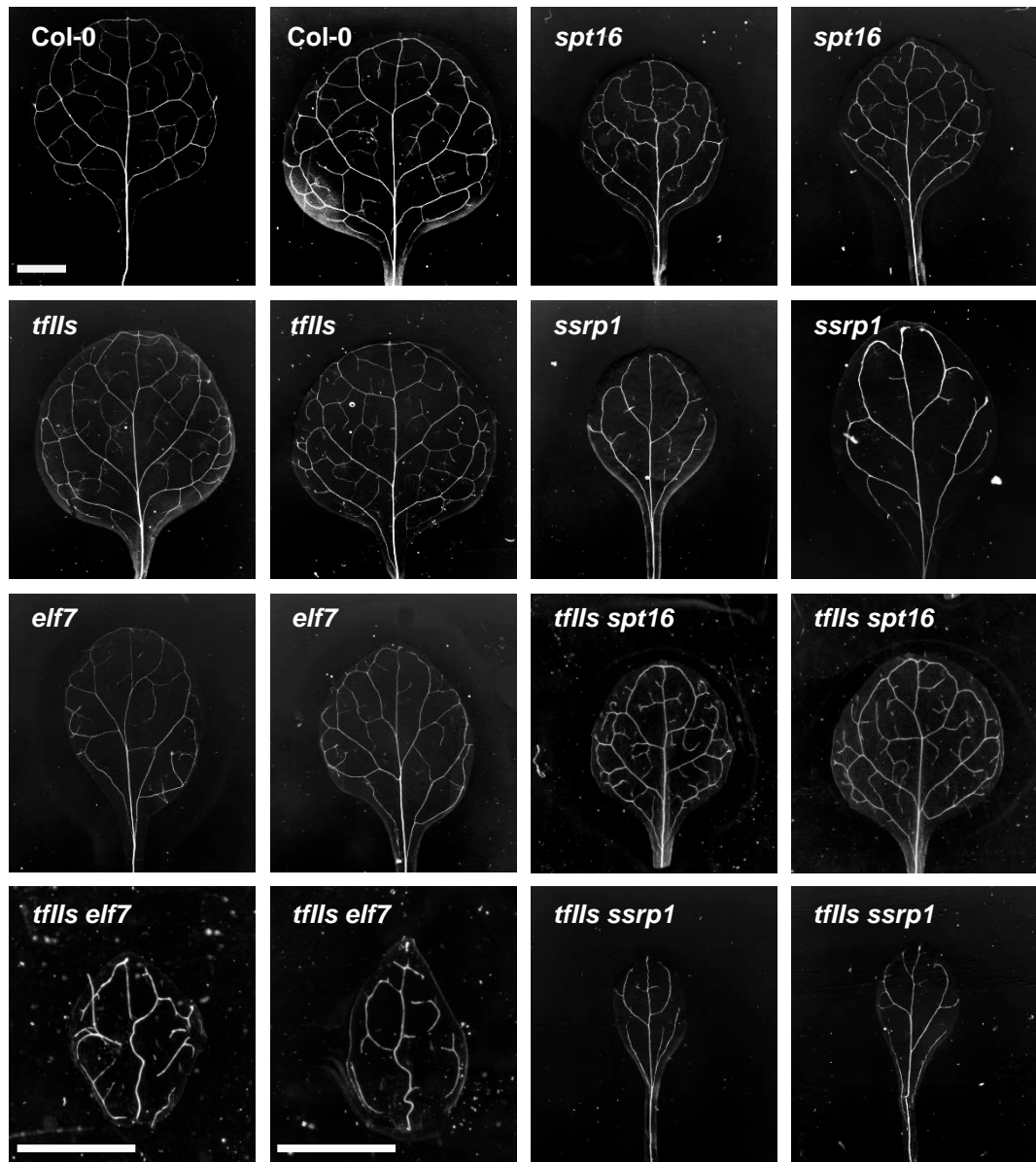


**Supplemental Figure 3.** ELF7 and SPT6L co-localise with RNAPII in euchromatin. (Supports Figure 3.) Co-localisation analysis of ELF7 and SPT6L with RNAPII-Ser2P and non-phosphorylated RNAPII within euchromatic regions of flow-sorted 8C nuclei of leaf cells visualised by SIM. For comparison, in the merges the same colour is used each for both forms of RNAPII (green) and both TEFs (ELF7, SPT6L; red). In the merge of the TEF signals with those of non-phosphorylated RNAPII more clearly red areas (representing TEFs, but no RNAPII) are visible, while in the merge with RNAPII-S2P more prominently yellow/orange areas (representing TEFs and RNAPII) are visible.



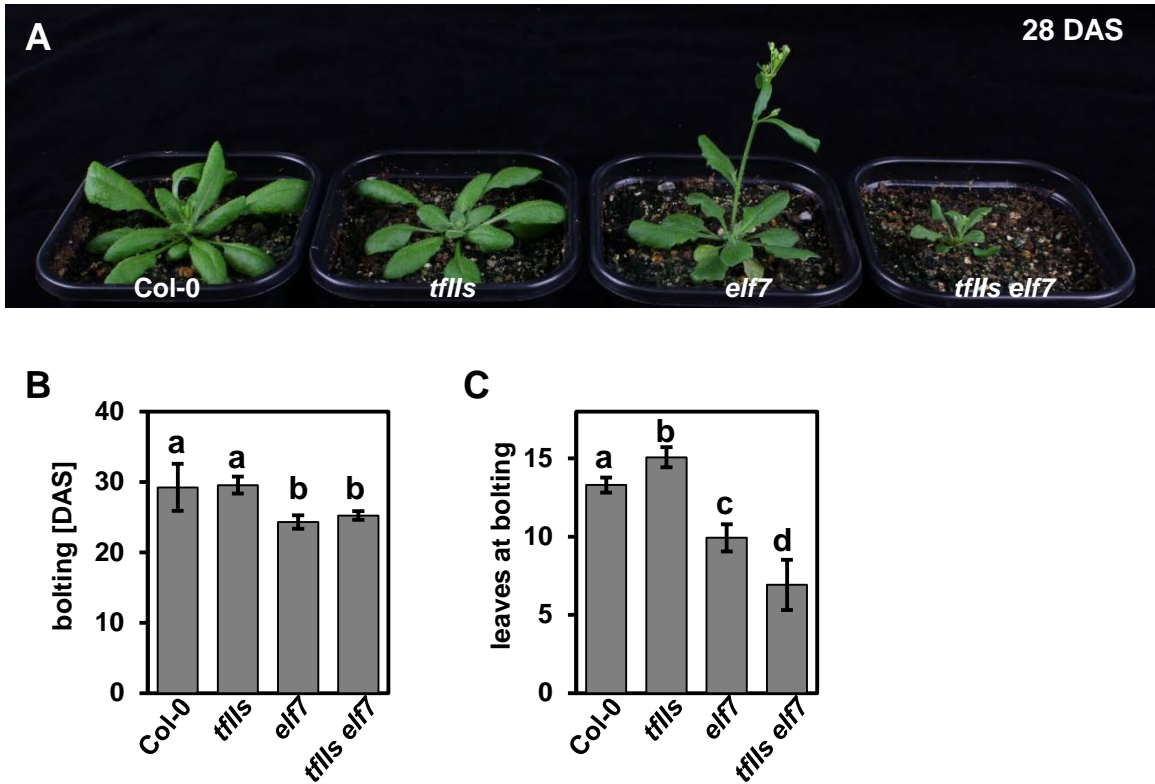
**Supplemental Figure 4.** Analysis of *tflls sssrp1* and *tflls spt16* double-mutants in comparison to the respective single-mutants and the Col-0 wild type. (Supports Figure 4.)

Representative images of the plants at 28 days after stratification (DAS) under long-day conditions are shown (A,B). Freshly harvested siliques (left) and cleared siliques (right) of *tflls spt16* and controls are shown in (C) – *tflls sssrp1* plants are sterile, producing no seeds. The time of bolting (D,E) was determined (n=13). Data were analysed by two-way ANOVA and error bars indicate standard deviation calculated from the measurements of 13 individual plants for each line. The letters above the histogram bars indicate the outcome of a multi comparisons Tukey's test ( $p < 0.05$ ).



**Supplemental Figure 5.** Leaf vein patterning of Col-0, single- and double mutants. (Supports Figure 4.)

Vein pattern of cleared leaves of the indicated plant lines. Representative first leaves (left) and second leaves (right) of 21 d old plants are shown. All images are of the same magnification except for *tflls elf7*. Size bars indicate 1 mm.

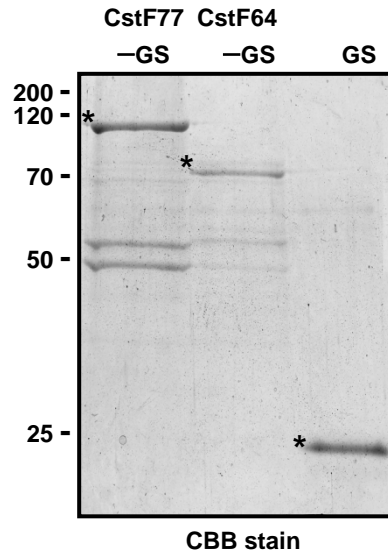


**Supplemental Figure 6.** Analysis of *tflls elf7* double-mutants in comparison to the respective single-mutants and the Col-0 wild type. (Supports Figure 4.)

Representative images of the plants at 28 days after stratification (DAS) under long-day conditions are shown (A). The time of bolting in DAS (B) and the number of leaves at bolting (C) were determined (n=13). Data were analysed by two-way ANOVA and error bars indicate standard deviation calculated from the measurements of 13 individual plants for each line. The letters above the histogram bars indicate the outcome of a multi comparisons Tukey's test ( $p < 0.05$ ).



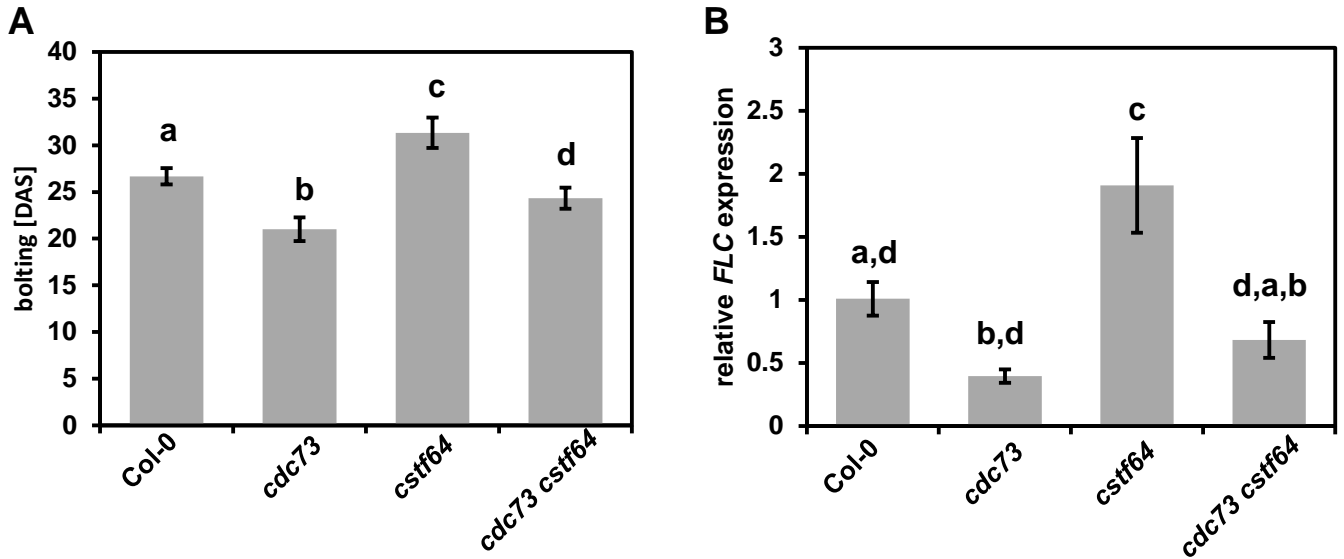
**Supplemental Figure 7.** Phenotype of *spt16 elf7* double-mutant plants in comparison to the respective single-mutants and Col-0. (Supports Figure 4.) Representative image plants at 28 days after stratification (DAS) under long-day conditions. For the cross of the *ssrp1* and *elf7* mutants, no homozygous double-mutants were obtained. The proportion of 18.4% non-germinating seeds observed with the segregating population (*ELF7*<sup>+/-</sup> *SSRP1*<sup>-/-</sup>, n = 49) indicates that this double-mutant combination is lethal.



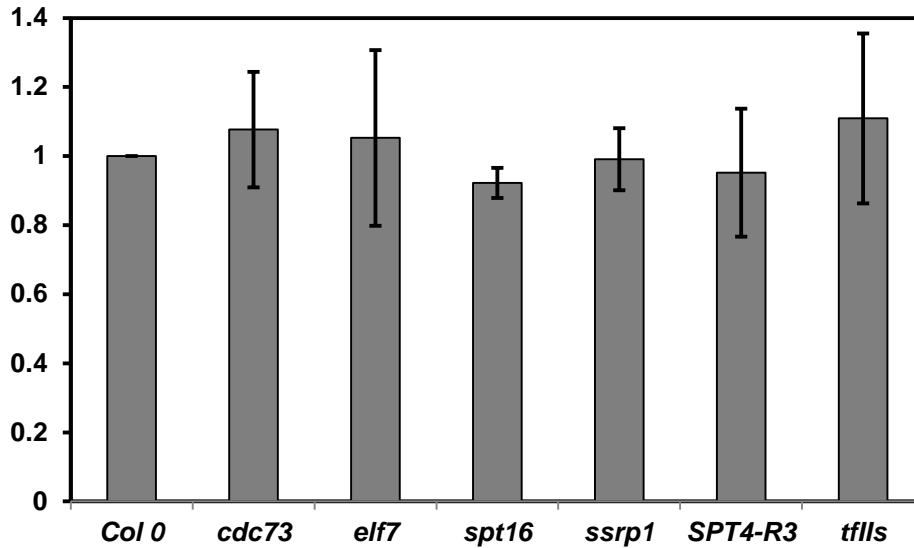
**Supplemental Figure 8.** Isolation of the CstF complex. (Supports Figure 1 and 2.)

Eluates of affinity purifications from cells expressing CstF77-GS, CstF64-GS or unfused GS after SDS-PAGE and Coomassie-staining of the gel. The unfused GS-tag and GS-fusion proteins are indicated by asterisks. The migration positions of molecular weight marker (in kDa) are indicated on the left.





**Supplemental Figure 9.** Early bolting phenotype of *cdc73 cstf64* double-mutant plants. (Supports Figure 5.) (A) Bolting time in DAS of the indicated genotypes (n=15) under LD conditions. (B) *FLC* transcript levels of the different genotypes quantified by qRT-PCR from three biological replicates (independently pooled collections of tissues) and at least two technical replicates each. Data were analysed by two-way ANOVA and error bars indicate standard deviation. The letters above the histogram bars indicate the outcome of a multi comparisons Tukey's test ( $p < 0.05$ ), and for the bars (in B) that are indicated with more than one letter above the bar, the bars sharing the same letter are not significantly different.



**Supplemental Figure 10.** 35S pre-rRNA levels in mutants deficient in different TEFs. (Supports Figure 6.) Quantification of RNA gel blot analyses of total RNA isolated from Col-0, the indicated T-DNA insertion mutants and SPT4-R3, which is a plant line expressing anti-sense RNA directed against *SPT4* that contains strongly reduced amounts of *SPT4-1* and *SPT4-2* transcripts (Dürr et al., 2014). The blots were hybridised with radio-labelled probes detecting the 35S pre-rRNA (ITS1) and *UBQ5*. The data depicted in the histogram represent the amount of the 35S pre-rRNA signal relative to the *UBQ5* signal (quantified using a phosphorimager) and they correspond to mean values of three experiments. Error bars represent standard deviation and analysis of the data by one-way ANOVA revealed that the datasets are not significantly different from that of Col-0.

**Supplemental Table 1.** Degree of colocalisation of ELF7 and SPT6L with RNAPII (phosphorylated at Ser2 or non-phosphorylated)

	No. nuclei	%		No. nuclei	%
<b>Voxel intensities</b>					
RNAPII non-phos	13	86.0	RNAPII non-phos	13	94.5
ELF7	13	64.0	SPT6L	13	90.9
RNAPII Ser2P	13	100	RNAPII Ser2P	13	100
<b>Co-localisation</b>					
RNAPII non-phos colocalising to ELF7	13	52.1	RNAPII non-phos colocalising to SPT6L	13	59.0
ELF7 colocalising to RNAPII non-phos	13	55.5	SPT6L colocalizing to RNAPII non-phos	13	60.0
RNAPII non-phos colocalising to RNAPII Ser2P	13	53.2	RNAPII non-phos colocalising to RNAPII Ser2P	13	55.8
RNAPII Ser2-P colocalising to RNAPII non-phos	13	59.3	RNAPII Ser2P colocalising to RNAPII non-phos	13	60.7
ELF7 colocalising to RNAPII Ser2P	13	62.8	SPT6L colocalising to RNAPII Ser2P	13	67.2
RNAPII Ser2P colocalising to ELF7	13	64.7	RNAPII Ser2P colocalising to SPT6L	13	74.8

*Voxel intensities calculated by Imaris 8.0*

**Supplemental Table 2.** Overview of phenotypes observed for single- and double mutants defective in various TEFs.

trait <sup>1</sup> \ genotype	Col-0	<i>tflls</i>	<i>ssrp1</i>	<i>spt16</i>	<i>elf7</i>	<i>tflls</i> <i>ssrp1</i>	<i>tflls</i> <i>spt16</i>	<i>tflls</i> <i>elf7</i>	<i>ssrp1</i> <i>elf7</i>	<i>spt16</i> <i>elf7</i>
viability	✓	✓	✓	✓	✓	✓	✓	✓	-	✓
rosette ø	++++	++++	+++	+++	+++	++	+++	+	-	+
inflorescence No.	+	+	++	++	++	+++	++	++++	-	n.d.
bolting [DAS] <sup>2</sup>	+++	+++	++	++	++	++	++	++	-	n.d.
bolting [leaves] <sup>3</sup>	+++	++++	+++	+++	++	n.d.	n.d.	+	-	n.d.
leaf veins	++++	++++	++	+++	+++	++	+++	+	-	n.d.
seed set	+++	+++	+	+	++	-	+	-	-	-

<sup>1</sup>Quantitative differences between the different genotypes regarding the traits analysed in this report are summarised here.

<sup>2</sup>The bolting time [DAS] relative to Col-0 (+++ ) is reduced in some genotypes (++) .

<sup>3</sup>The leaf number at bolting is increased (++++ ) or reduced (++,+ ) relative to Col-0 (+++ ) in some genotypes.

**Supplemental Table 3.** Oligonucleotide primers used in this study and construction of plasmids

	use	plasmid	restr. site
AAGGATCCATGGCGGCTGCGGCTTTTGGGCA	Insertion of <i>CDKC2</i> CDS in pCambia2300-GS	pCambia 2300- CDKC2-GS	BamHI
GCGGAGCTCCGGTTGCCATCCATATTGTTGGT	Insertion of <i>CDKC2</i> CDS in pCambia2300-GS	pCambia 2300- CDKC2-GS	SacI
TATAGTCGACATGGATACGAGGTTTCCGTTTC	Insertion of <i>NRPB1</i> CDS in pCambia2300-GS	pCambia 2300- NRPB1-GS	Sall
CGCGTCTAGAAGGGTTGCCTTTATCATCCTTAC	Insertion of <i>NRPB1</i> CDS in pCambia2300-GS	pCambia 2300- NRPB1-GS	XbaI
CGGAGCTCCCTGTGCTTGCCTTTAGATGGT	Insertion of <i>SPT16</i> CDS in pCambia2300-GS	pCambia 2300- SPT16-GS	SacI
GCTCTAGAATGGATCCATTATCAGT	Insertion of <i>CDC73</i> CDS in pCambia 2300-GS	pCambia 2300-CDC73-GS	XbaI
TCCCCGGGCCAGAATGCGACCG	Insertion of <i>CDC73</i> CDS in pCambia 2300-GS	pCambia 2300-CDC73-GS	SmaI
GCTCTAGAATGGCTTCATCATCA	Insertion of <i>CSTF64</i> CDS in pCambia 2300-GS	pCambia 2300-CSTF64-GS	XbaI
TCCCCGGGCCTGAAGGCTGCATCAT	Insertion of <i>CSTF64</i> CDS in pCambia 2300-GS	pCambia 2300-CSTF64-GS	SmaI
GCTCTAGAATGGCTGATAAGTAC	Insertion of <i>CSTF77</i> CDS in pCambia 2300-GS	pCambia 2300-CSTF77-GS	XbaI
TCCCCGGGCCGCCAGTGTACCAGA	Insertion of <i>CSTF77</i> CDS in pCambia 2300-GS	pCambia 2300-CSTF77-GS	SmaI
GCTCTAGAATGGCGTCGTACCG	Insertion of <i>ELF7</i> CDS in pCambia 2300-GS	pCambia 2300-ELF7-GS	XbaI
TCCCCGGGCCTTCAGAATAATC	Insertion of <i>ELF7</i> CDS in pCambia 2300-GS	pCambia 2300-ELF7-GS	SmaI
GCTCTAGAATGGCAGACTCTCGGAATGGTAAT	Insertion of <i>SPT16</i> CDS in pCambia2300-GS	pCambia 2300-SPT16-GS	XbaI
AATTGCGGCCGCATATGGCGGACGGCCACTCC	Insertion of <i>SSRP1</i> CDS in pCambia2300-GS	pCambia 2300-SSRP1-GS	NotI
CGGAGCTCGTTACTATCGGAATCGTTTCCTGAGT	Insertion of <i>SSRP1</i> CDS in pCambia2300-GS	pCambia 2300-SSRP1-GS	SacI
TTGGGCCCAACAATGGGAAGCGCACCAGCTCAGATTC	Insertion of <i>SPT4-2</i> CDS in pCambia2300-GS	pCambia2300-SPT4-2-GS	SmaI
TTGAGCTCTCACATGCGTTTTCGGCAGAACAT	Insertion of <i>SPT4-2</i> CDS in pCambia2300-GS	pCambia2300-SPT4-2-GS	SacI
AATTCTCGAGTTTGTGAAAAGCCCATCAAACCTTGG	Insertion of <i>TFIIS</i> promoter in pGreenII0179-GS	pGreenII0179:TFIISp::GS-TFIIS	XhoI
AACTCGAGACGTTCCGACAATCCCTAGCTCA	Insertion of <i>TFIIS</i> promoter in pGreenII0179-GS	pGreenII0179:TFIISp::GS-TFIIS	XhoI
CGGGATCCATGGAGAGTGATTTGATTGATTTG	Insertion of <i>TFIIS</i> CDS in pGreenII0179-GS	pGreenII0179:TFIISp::GS-TFIIS	BamHI
AAGAATTCCTCAACAGAACTCCAGTGGTTG	Insertion of <i>TFIIS</i> CDS in pGreenII0179-GS	pGreenII0179:TFIISp::GS-TFIIS	EcoRI
CGGGATCCCAAAGTCTCGGATGATT	Insertion of <i>SPT6</i> CDS (aa1218-1412) in pGex5x-1	pGex5x-1-SPT6_partial	BamHI
ACGCGTCGACTCAGTCATCAATATGCCT	Insertion of <i>SPT6</i> CDS (aa1218-1412) in pGex5x-1	pGex5x-1-SPT6_partial	Sall
CGGGATCCGATTCTGAATCTTTGGC	Insertion of <i>ELF7</i> CDS (aa401-589) in pQE9	pQE9-ELF7_partial	BamHI
CCCAAGCTTTCATTCAGAATAATCATC	Insertion of <i>ELF7</i> CDS (aa401-589) in pQE9	pQE9-ELF7_partial	HindIII
AAGGATCCCTATCACCAGCTAGCCCTAC	Insertion of <i>NRPB1</i> CDS (aa1746-1839) in pQE9	pQE9-NRPB1_partial	BamHI
ATATAAGCTTTCAAGGGTTGCCTTTATCATCC	Insertion of <i>NRPB1</i> CDS (aa1746-1839) in pQE9	pQE9-NRPB1_partial	HindIII
CGGGATCCGTATTAGATTCTGGGTTA	Insertion of <i>SPT6</i> CDS (aa1121-1430) in pQE9	pQE9-SPT6_partial	BamHI
ACGCGTCGACCTACATCGGTACCTTGGCAGC	Insertion of <i>SPT6</i> CDS (aa1121-1430) in pQE9	pQE9-SPT6_partial	Sall
ATGGATCCATGGAGAGTGATTTGATTGATT	Insertion of <i>TFIIS</i> CDS in pQE9	pQE9-TFIIS	BamHI
ATATCTGCAGTCAACAGAACTTCCAGTGGT	Insertion of <i>TFIIS</i> CDS in pQE9	pQE9-TFIIS	PstI
AGCCAAGAAGACCGAACTCA	qRT-PCR <i>FLC</i>		
TTTGTCCAGCAGGTGACATC	qRT-PCR <i>FLC</i>		
GTTGTAACAAGATGGATGCCA	qRT-PCR <i>EF1</i>		

GGACAAATGGGATTTTGTGACG	qRT-PCR <i>EF1</i>		
GTGGAAGAGCTGCCGTTGAATT	qRT-PCR <i>SPT6</i>		
GCACAATCCTCCCTTCACCAAT	qRT-PCR <i>SPT6</i>		
GTTGTTGGGGACCTGGGAAG	qRT-PCR <i>SPT6L</i>		
ATCCATCCCATGTCCGACATC	qRT-PCR <i>SPT6L</i>		
GCTGGAATCCACGAGACAAC	qRT-PCR Actin 2		
AAGCCTTTGATCTTGAGAGCTT	qRT-PCR Actin 2		
GGTAACATTGTGCTCAGTGGTGG	ChIP qPCR, Actin2		
GGTGCAACGACCTTAATCTTCAT	ChIP qPCR, Actin2		
AGATGGTTTTCGGGGAGGAAAATCAC	ChIP qPCR, At3g02260		
GCTTCTCTTCGACAGGCCAGAG	ChIP qPCR, At3g02260		
GGATGCGATCATACCAGCACTAATG	ChIP qPCR, 18S rDNA -1		
GAGGGATGCAACACGAGGACTTC	ChIP qPCR, 18S rDNA-1		
CGGGGGCATTTCGTATTTTCATAGTCA	ChIP qPCR, 18S rDNA-2		
GACTAGGACGGTATCTGATCGTCTTC	ChIP qPCR, 18S rDNA-2		
TAGGGTTCTTAGTTGATCTTGATTGAGCTC	ChIP qPCR, TA3		
TTTGCTCTCAAACCTCTCAATTGAAGTTT	ChIP qPCR, TA3		
TTTCGAGACGGCGACACCAATG	ChIP qPCR, U6-1		
GAGAGTAGCTGATCCTAAATGCTATC	ChIP qPCR, U6-1		
AAACGATGCGTTGGGATAGGTC	ChIP qPCR, TA2		
ATACTCTCCACTTCCCCTTTTTCTTTTTA	ChIP qPCR, TA2		
ACTGGCTCATCGTTCTCCATT	ChIP qPCR, 7SL-1		
ATTTGTTTTGATTGCTCGGTTT	ChIP qPCR, 7SL-1		
ACTTTCCATTTCGGAGTTTTTGT	ChIP qPCR, U6-26		
GCCTGCTTCTCTTTTCAGATT	ChIP qPCR, U6-26		
CGA CCC TCC CCT AAA TCA CTC CA	ChIP qPCR, 3'ETS		
ACT TGG CGA GGT CCG GAA TCT TAC	ChIP qPCR, 3'ETS		
GAAGGCGAAGATCCAAGACAAGGAA	ChIP qPCR, UBQ5		
GGAGGACGAGATGAAGCGTTCGA	ChIP qPCR, UBQ5		
ATCGACGACGAATACGAAAG	Expression of <i>CSTF64</i> (SALK_088877)		
CTAAGTTGTTCTCGGTCCG	Expression of <i>CSTF64</i> (SALK_088877)		
TCGTTTCCTGAAGACGGACC	Expression of <i>CSTF77</i> (SALK_104082)		
CAAAGCTCTCTTGCCAAAG	Expression of <i>CSTF77</i> (SALK_104082)		
GCTCTAGAATGGATCCATTATCAGT	Expression of <i>CDC73</i> (SALK_008357)		
TTGTTCTGCTTCTGACTCTG	Expression of <i>CDC73</i> (SALK_008357)		
ATCGACGACGAATACGAAAG	Genotyping, T-DNA insertion <i>cstf64-3</i> (SALK_088877)		
CTAAGTTGTTCTCGGTCCG	Genotyping, T-DNA insertion <i>cstf64-3</i> (SALK_088877)		
TCGTTTCCTGAAGACGGACC	Genotyping, T-DNA insertion <i>cstf77-2</i> (SALK_104082)		
CAAAGCTCTCTTGCCAAAG	Genotyping, T-DNA insertion <i>cstf77-2</i> (SALK_104082)		

GCTCTAGAATGGATCCATTATCAGT	Genotyping, T-DNA insertion <i>cdc73-2</i> (SALK_008357)		
TTCGTTCTGCTTCTGACTCTG	Genotyping, T-DNA insertion <i>cdc73-2</i> (SALK_008357)		
ATCCTCTGGAATGTTGATAGT	Genotyping, T-DNA insertion <i>tflls-1</i> (SALK_056755)		
TTTCCTCTTGTCACCTTGCCAT	Genotyping, T-DNA insertion <i>tflls-1</i> (SALK_056755)		
ATTTTGCCGATTTTCGGAAC	Genotyping, T-DNA insertion SALK LBb1.3 ( <i>tflls-1</i> )		
CCCTCATCTTACGCGTATCAGA	Genotyping, T-DNA insertion <i>ssrp1-2</i> (SALK_001283)		
AATTAAGCTTAGTTACTATCGGAATCGTTTCCT	Genotyping, T-DNA insertion <i>ssrp1-2</i> (SALK_001283)		
CTATCTCTGCATTGCCTCTTAGC	Genotyping, T-DNA insertion <i>spt16-1</i> (SAIL_392_G06)		
TACTTGTCTAACGCAGCGAAATC	Genotyping, T-DNA insertion <i>spt16-1</i> (SAIL_392_G06)		
GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC	Genotyping, T-DNA insertion SAIL LB ( <i>SPT16</i> )		
TTGGACCCTTCAATTCGTGATG	Genotyping, T-DNA insertion <i>elf7-2/-3</i> (SALK_046605/_019433)		
CCTGGCCCTTTTCTTCCTCA	Genotyping, T-DNA insertion <i>elf7-2/-3</i> (SALK_046605/_019433)		
GTTGCCCGTCTCACTGGTGA	Genotyping, T-DNA insertion SALK LBb1.3 ( <i>elf7-2, elf7-3, ssrp1-2</i> )		
TCGATACCTGTCCAAAACAG	RNA gel blot probe for pre-rRNA <i>ITS1</i>		
AGACTTCAGTTCGCAGC	RNA gel blot probe for pre-rRNA <i>ITS1</i>		
CTCACACGAATAAGGTACAAAGTTC	RNA gel blot probe <i>FLC</i> antisense		
GAAGGCGAAGATCCAAGACAAGGAA	RNA gel blot probe for <i>UBQ5</i>		
GGAGGACGAGATGAAGCGTCGA	RNA gel blot probe for <i>UBQ5</i>		