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Supplementary Information for

Beyond flowering: The genetic and molecular basis for the vegetative functions of florigen in tomato

Akiva Shalit-Kaneh*[1], Tamar Eviatar–Ribak*[1], Guy Horev*[2], Naomi Suss[1], Roni Aloni[3], Yuval Eshed[4], Eliezer Lifschitz [1,5]

Eliezer Lifschitz

Email lifs@technion.ac.il

This PDF file includes:

Tables S1 to S2

Figs. S1, S2, S4, S5, S5-2

Materials and Methods details

Captions for Databases S1 to S6

References for SI reference citations

Other supplementary materials for this manuscript include the following:

Datasets S1 to S6

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Table S1: Expression profile of MADS genes in Fig. 1

	Name	Description	WT	*sft*	*pSFT:SFT*
Solyc02g065730	AP67	AGL8, FUL | AGL8 (agamous-like 8)	22	9	156
Solyc06g069430	Full 1	AGL8, FUL | AGL8 (agamous-like 8)	122	34	1722
Solyc03g114830	Full 2	AGL8, FUL | AGL8 (agamous-like 8)	56	15	587
Solyc11g010570	JOINTLESS	SVP, AGL22 | SVP (SHORT VEGETATIVE PH.	206	306	187
Solyc05g056620	MC		2	2	10
Solyc05g012020	RIN	AP1, AGL7 | AP1 (APETALA1)	75	36	68
Solyc04g005320	sep04	SEP1, AGL2 | SEP1 (SEPALLATA1)	0	2	2
Solyc02g089210	TDR4/FUL-like/	AP1, AGL7 | AP1 (APETALA1)	1	1	40
Solyc01g092950	TM3/JIP56	AGL20, SOC1 | AGL20 (AGAMOUS-LIKE 20)	1840	1693	1711
Solyc01g092960	TM3/JIP56	AGL20, SOC1 | AGL20 (AGAMOUS-LIKE 20)	526	551	804
Solyc05g015750	TM5	SEP3, AGL9 | SEP3 (SEPALLATA3)	4	0	2
Solyc03g019710	TM8	SHP2, AGL5 | SHP2 (SHATTERPROOF 2)	2	0	87
Solyc00g179240	#N/A	AGL20, SOC1 | AGL20 (AGAMOUS-LIKE 20)	189	179	203
Solyc01g066500	#N/A	MADS-box protein (AGL40)	7	3	14
Solyc01g066730	#N/A	MADS-box protein (AGL60)	36	43	63
Solyc01g087990	#N/A	AGL15 | AGL15 (AGAMOUS-LIKE 15)	43	52	21
Solyc01g103550	#N/A	MADS-box protein (AGL47)	2	4	4
Solyc01g105800	#N/A	SVP, AGL22 | SVP (SHORT VEGETATIVE PH.	663	698	484
Solyc01g106170	#N/A	AGL21 | AGL21	1	1	12
Solyc02g063500	#N/A	NA)	7	3	1
Solyc02g071730	#N/A	AG | AG (AGAMOUS)	0	4	1
Solyc02g084630	#N/A	AP3, ATAP3 | AP3 (APETALA 3)	450	454	397
Solyc02g089200	#N/A	SEP2, AGL4 | SEP2 (SEPALLATA 2)	0	2	34
Solyc02g091550	#N/A	AGL21 | AGL21	8	5	3
Solyc03g006830	#N/A	AGL42 | AGL42 (AGAMOUS LIKE 42)	10	4	5
Solyc03g114840	#N/A	SEP4, AGL3 | SEP4 (SEPALLATA 4)	7	7	1
Solyc03g115910	#N/A	AGL65 | AGL65 (AGAMOUS-LIKE 65)	7	7	5
Solyc03g119680	#N/A	AGL61, DIA | AGL61 (AGAMOUS-LIKE 61)	5	8	6
Solyc04g016070	#N/A	NA)	3	5	2
Solyc04g076680	#N/A	ANR1, AGL44 | AGL44 (AGAMOUS-LIKE 44)	73	84	49
Solyc04g076700	#N/A	AGL16 | AGL16 (AGAMOUS-LIKE 16)	101	87	50
Solyc05g015720	#N/A	MAF1, FLM, AGL27 | MAF1 (MADS AFFECTI	115	121	53
Solyc05g015730	#N/A	FLC, FLF, AGL25 | FLC (FLOWERING LOCUS	42	51	24
Solyc07g052700	#N/A	AGL66 | AGL66 (AGAMOUS-LIKE 66)	1129	1433	943
Solyc07g052720	#N/A	NA)	568	595	484
Solyc08g067230	#N/A	PI | PI (PISTILLATA)	0	0	0
Solyc08g080100	#N/A	AGL19, GL19 | AGL19 (AGAMOUS-LIKE 19)	124	166	143
Solyc11g030380	#N/A	unknown protein	1	3	651
Solyc11g030390	#N/A	unknown protein	591	656	593
Solyc11g032100	#N/A	AGL12, XAL1 | AGL12 (AGAMOUS-LIKE 12)	344	323	220
Solyc11g045310	#N/A	AGL30 | DNA binding / transcription factor	296	269	320
Solyc12g016150	#N/A	AGL96 | AGL96 (AGAMOUS-LIKE 96)	0	0	0
Solyc12g016180	#N/A	PHE2, AGL38 | AGL38 (AGAMOUS-LIKE 38)	0	0	0
Solyc12g038510	#N/A	SEP4, AGL3 | SEP4 (SEPALLATA 4)	0	0	0
Solyc12g056460	#N/A	AGL20, SOC1 | AGL20 (AGAMOUS-LIKE 20)	351	428	187
Solyc12g087830	#N/A	MAF3, FCL3, AGL70 | MAF3 (MADS AFFECTI	1068	277	1140
Solyc12g088080	#N/A	ANR1, AGL44 | AGL44 (AGAMOUS-LIKE 44)	0	1	1
Solyc12g088090	#N/A	AGL16 | AGL16 (AGAMOUS-LIKE 16)	0	0	1
Solyc01g097850	#N/A	AGL62 | AGL62 (Agamous-like 62)	14	18	15
Solyc01g106700	#N/A	AGL62 | AGL62 (Agamous-like 62)	92	77	98
Solyc01g106710	#N/A	AGL62 | AGL62 (Agamous-like 62)	2	1	7
Solyc04g064860	#N/A	AGL62 | AGL62 (Agamous-like 62)	52	52	35

Table S2: Expression profile of MADS genes in Fig. 2

	Name	Description	WT//WT	*sft//sft*	*35S:SFT //sft*	*35S:SFT //WT*	*35S:SFT //sp*
Solyc02g065730	AP67	AGL8, FUL | AGL8 (agamous-like 8)	8	4	27	22	37
Solyc06g069430	Full 1	AGL8, FUL | AGL8 (agamous-like 8)	51	15	132	286	374
Solyc03g114830	Full 2	AGL8, FUL | AGL8 (agamous-like 8)	82	26	306	354	416
Solyc11g010570	JOINTLESS	SVP, AGL22 | SVP (SHORT VEGETATIVE PH	196	228	201	201	336
Solyc05g056620	MC		3	5	4	2	53
Solyc05g012020	RIN	AP1, AGL7 | AP1 (APETALA1)	60	80	84	70	95
Solyc04g005320	sep04	SEP1, AGL2 | SEP1 (SEPALLATA1)	1	5	2	8	34
Solyc02g089210	TDR4/FUL-like/	AP1, AGL7 | AP1 (APETALA1)	2	0	3	6	13
Solyc01g092950	TM3/JIP56	AGL20, SOC1 | AGL20 (AGAMOUS-LIKE 20)	2093	1959	2575	2150	2144
Solyc01g092960	TM3/JIP56	AGL20, SOC1 | AGL20 (AGAMOUS-LIKE 20)	654	569	870	855	735
Solyc05g015750	TM5	SEP3, AGL9 | SEP3 (SEPALLATA3)	5	2	6	5	5
Solyc03g019710	TM8	SHP2, AGL5 | SHP2 (SHATTERPROOF 2)	34	6	112	438	258
Solyc00g179240	#N/A	AGL20, SOC1 | AGL20 (AGAMOUS-LIKE 20)	254	259	318	288	262
Solyc01g066500	#N/A	MADS-box protein (AGL40)	4	3	6	3	2
Solyc01g066730	#N/A	MADS-box protein (AGL60)	38	37	41	48	61
Solyc01g087990	#N/A	AGL15 | AGL15 (AGAMOUS-LIKE 15)	24	39	24	28	30
Solyc01g103550	#N/A	MADS-box protein (AGL47)	5	4	9	4	4
Solyc01g105800	#N/A	SVP, AGL22 | SVP (SHORT VEGETATIVE PH	1229	1217	1169	1185	1058
Solyc01g106170	#N/A	AGL21 | AGL21	2	1	1	4	7
Solyc02g063500	#N/A	NA)	6	3	5	5	4
Solyc02g071730	#N/A	AG | AG (AGAMOUS)	3	1	3	2	1
Solyc02g084630	#N/A	AP3, ATAP3 | AP3 (APETALA 3)	488	509	489	367	415
Solyc02g089200	#N/A	SEP2, AGL4 | SEP2 (SEPALLATA 2)	5	3	2	3	4
Solyc02g091550	#N/A	AGL21 | AGL21	5	2	6	3	6
Solyc03g006830	#N/A	AGL42 | AGL42 (AGAMOUS LIKE 42)	81	138	162	116	47
Solyc03g114840	#N/A	SEP4, AGL3 | SEP4 (SEPALLATA 4)	1	4	2	2	1
Solyc03g115910	#N/A	AGL65 | AGL65 (AGAMOUS-LIKE 65)	7	4	5	5	7
Solyc03g119680	#N/A	AGL61, DIA | AGL61 (AGAMOUS-LIKE 61)	7	8	8	15	13
Solyc04g016070	#N/A	NA)	1	5	3	1	5
Solyc04g076680	#N/A	ANR1, AGL44 | AGL44 (AGAMOUS-LIKE 44)	127	115	100	103	93
Solyc04g076700	#N/A	AGL16 | AGL16 (AGAMOUS-LIKE 16)	91	78	74	87	93
Solyc05g015720	#N/A	MAF1, FLM, AGL27 | MAF1 (MADS AFFECTI	128	116	104	105	125
Solyc05g015730	#N/A	FLC, FLF, AGL25 | FLC (FLOWERING LOCUS	61	53	48	60	55
Solyc07g052700	#N/A	AGL66 | AGL66 (AGAMOUS-LIKE 66)	732	1027	974	977	994
Solyc07g052720	#N/A	NA)	327	425	405	390	475
Solyc08g067230	#N/A	PI | PI (PISTILLATA)	1	1	1	2	3
Solyc08g080100	#N/A	AGL19, GL19 | AGL19 (AGAMOUS-LIKE 19)	264	293	308	191	213
Solyc11g030380	#N/A	unknown protein	2	2	4	1	28
Solyc11g030390	#N/A	unknown protein	801	713	683	822	798
Solyc11g032100	#N/A	AGL12, XAL1 | AGL12 (AGAMOUS-LIKE 12)	488	486	477	414	485
Solyc11g045310	#N/A	AGL30 | DNA binding / transcription factor	369	342	333	371	321
Solyc12g016150	#N/A	AGL96 | AGL96 (AGAMOUS-LIKE 96)	0	0	0	0	0
Solyc12g016180	#N/A	PHE2, AGL38 | AGL38 (AGAMOUS-LIKE 38)	0	0	0	0	0
Solyc12g038510	#N/A	SEP4, AGL3 | SEP4 (SEPALLATA 4)	0	0	0	0	1
Solyc12g056460	#N/A	AGL20, SOC1 | AGL20 (AGAMOUS-LIKE 20)	366	503	509	267	483
Solyc12g087830	#N/A	MAF3, FCL3, AGL70 | MAF3 (MADS AFFECTI	1289	1274	1353	1316	398
Solyc12g088080	#N/A	ANR1, AGL44 | AGL44 (AGAMOUS-LIKE 44)	2	2	1	1	0
Solyc12g088090	#N/A	AGL16 | AGL16 (AGAMOUS-LIKE 16)	1	1	0	2	0
Solyc01g097850	#N/A	AGL62 | AGL62 (Agamous-like 62)	16	22	21	20	13
Solyc01g106700	#N/A	AGL62 | AGL62 (Agamous-like 62)	71	52	55	59	75
Solyc01g106710	#N/A	AGL62 | AGL62 (Agamous-like 62)	0	1	0	1	0
Solyc04g064860	#N/A	AGL62 | AGL62 (Agamous-like 62)	51	50	43	38	41

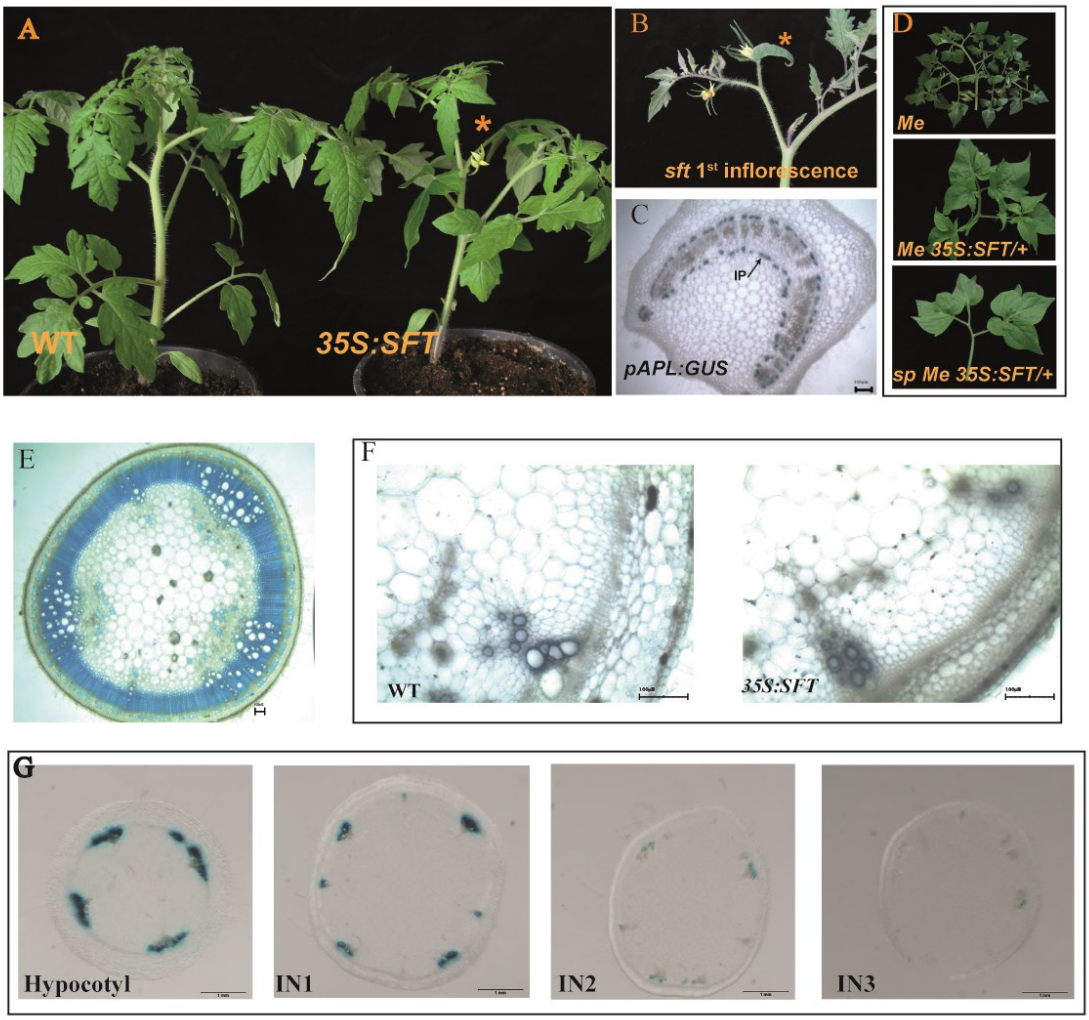
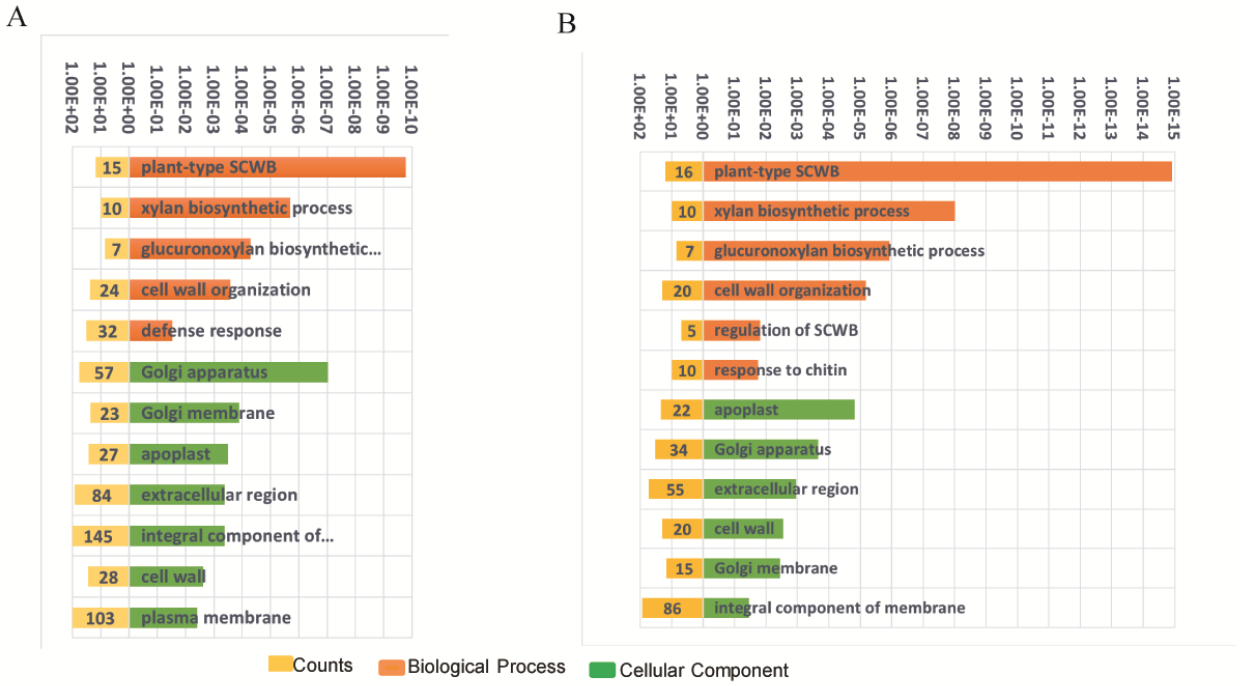
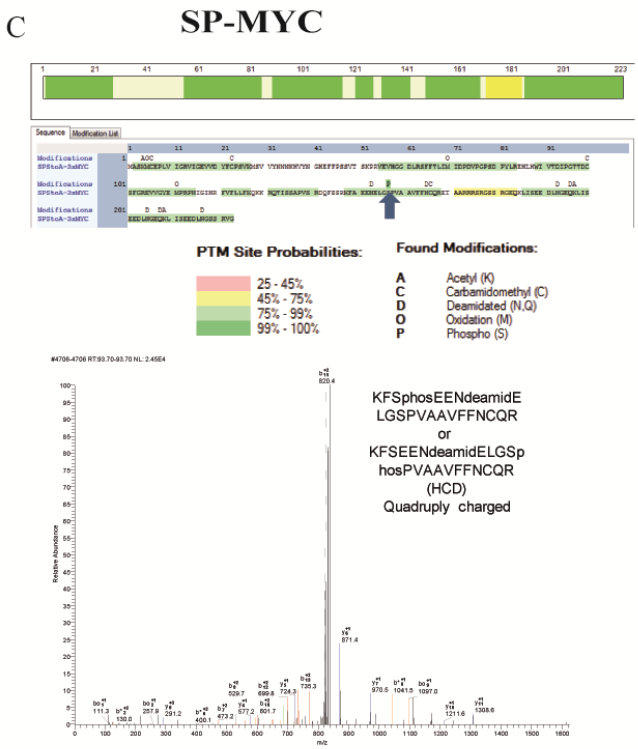
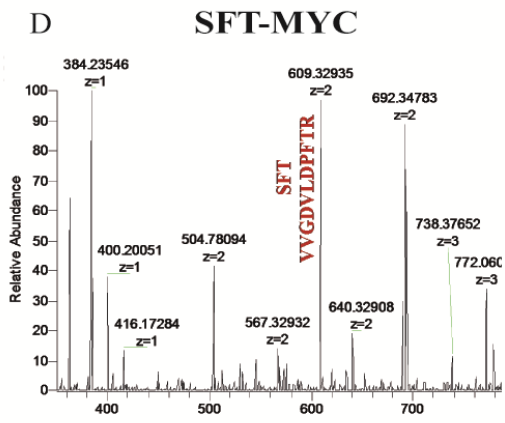


Fig. S1 Florigen stimulates SCWB to restrict lateral expansion of tomato stems

A) *35S:SFT* induces premature flowering (left). B) single flower truss from *sft* plants. * A flower. Note the extended adaxial sepal in *sft*. In sympodial plants, the primary apical bud is terminated by differentiating organs, typically inflorescences. Subsequently the upper most axillary bud, called sympodial, is released and eventually displaces the terminal inflorescence sideways. The reiteration of this process results in a compound sympodial shoot. In tomato, in correlation with the integral light dose, the primary shoot is terminated after 7-12 leaves. The first and subsequent sympodial units are terminated by an inflorescence after forming just 3 leaves. Therefore, flowering is synonymous with termination and termination of the vegetative apices, primary or sympodial, is required for the activation of the next sympodial cycle. In *sft* mutant plants, primary termination is significantly delayed and the sympodial mechanism is disrupted. The apex is terminated by a vegetative inflorescence and the next sympodial bud is arrested. It is not clear which of these two morphogenetic events is primarily critical. In *sp*, the flowering time of the primary shoot is normal but sympodial units become progressively shorter until the compound shoot is terminated by two sequential inflorescences. (C) A reference cross-section of tomato leaf petiole showing the expression of the p*TAPL*:*GUS* reporter gene (1) in both phloem layers. (D) Florigen is a potent suppressor of the *KNOTTED2*-induced leaf ramification. Top- The hyper-compound leaf of *Mouse ears (Me)* plants, a *GOF* allele of *TKN2* (2). Middle- *Me 35S:SFT/+* . Bottom- *sp Me 35S:SFT/+*. Note the gradual simplification with increasing *SFT/SP* ratios. E). A cross section of a mature *35S:SFT* plant stem. TBO staining for lignin. F) Cross sections of stems from 20 day old WT and *35S: SFT* plants. The *35S: SFT* plants developed stage 10 inflorescences while only floral primordia differentiated in WT plants. No secondary growth was observed at that time in the IF regions of both phenotypes. Bars 100mM G) Expression of the *pTCesA:GUS* reporter gene in hypocotyl and sequential stem internodes of a 3 week old WT plant. GUS staining.



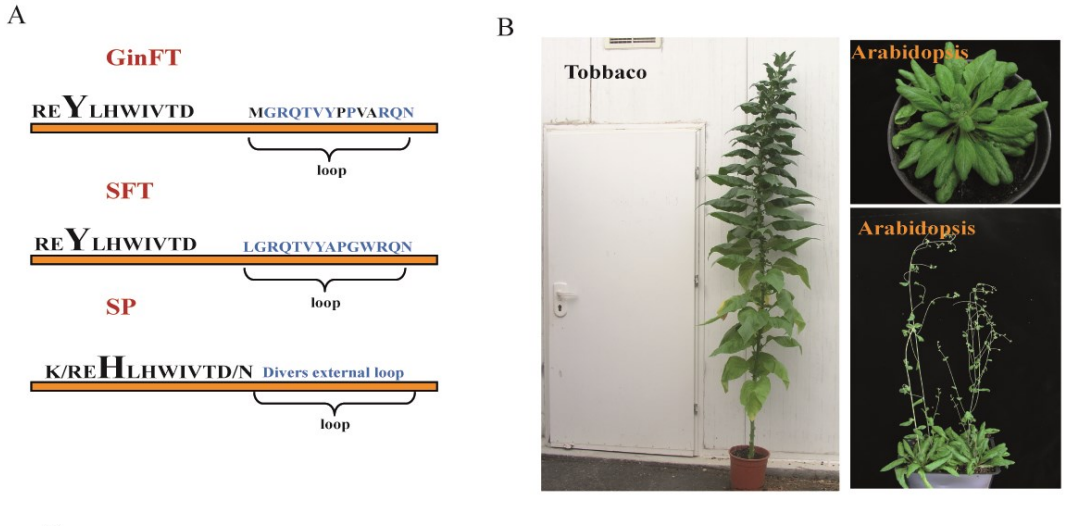




E Quantification of SFT-3xMYC

	ng of SFT-3xMYC in gr tissue	ng SFT-MYC per ug protein
L4 5cm	145	0.044
L3 9cm	91	0.077
L1-2 7-11cm	64	0.072
Stem Young	27	0.130
Stem Mature	14	0.114

Fig S2 Graft-transmissible-florigen enhances the SCWB network in sft and WT recipients A) Functional classifications (Biological Processes and Cellular Components) of the 593 m-florigen-responsive genes (figure 2D) in the grafting experiment. The 12 top categories are shown. B) Functional characterization of the 365 genes of cohort DU of PG1 (figure 2E). C) Serine 157 of affinity enriched SP-MYC is Phosphorylated, while the affinity enriched mobile SFT-MYC protein is free of major post translation modification (D). E) A rough quantification of Florigen/SFT-3xMYC in receptor tissues was made by comparing band intensity of equal volumes of protein extract from equal sample weight to a known amount of MYC tagged control protein.



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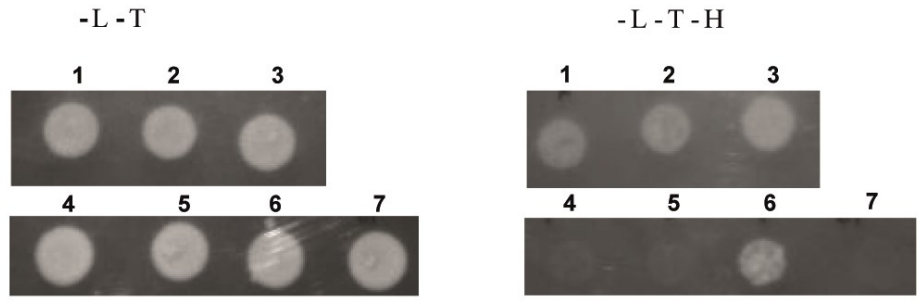

BD	AD						
	1	2	3	4	5	6	7
	SPGB	At. FD	At. SFD	SIP4	SPAK	CaMBP	Activation
GinFT	+++	+++	+++	-	-	+++	-

Fig. S4 The gymnosperm *GinFT* gene antagonizes florigen, suppresses SCWB and prolongs vascular maturation A) Amino acids sequence alignment of the GinFT, SFT and SP polypeptides. In tomato GinFT carries Y88, which is conserved in the FT (SFT) clade, but features a divergent external loop a typical to florigen antagonists of the TFL (SP) clade of CETS proteins. B) Overexpression of *GinFT* delays flowering in Arabidopsis and tobacco. Left -A transgenic *35S:GinFT* tobacco plant. Such plants form more than 80 leaves (vs. 24 of WT) before terminating with a smaller but normal inflorescence. Right - late flowering and vegetative inflorescence in arabidopsis *35S:GinFT* plants. C) GinFT interacts with the tomato and Arabidopsis FD transcription factors. These results were extracted from an experiment performed beforehand for other purposes and were also summarized in a table. The original composite plates are available upon request. The numbers of the colonies refer to those in the table above. The term florigen "antagonist" refers to members of the CETS (for *CEN TFL SP)* family that suppress flowering when overexpressed, display different phenotypes when inactivated and are characterized molecularly by divergent external loops in exon 4th. Overexpression of the four florigen antagonists in tomato delay flowering by 3-5 leaves but do not completely suppress it and are not as extreme as *SFT* LOF alleles. Moreover, flowering suppression by florigen antagonists is only partially rescued by high *SFT*. Five flowering suppressors have been partially characterized in tomato and it seems that each operates a unique antagonistic system: Recessive *SP* alleles only accelerate the sympodial flowering while *sp5g* accelerates both flowering system and in *sp* dependent manner. High *CET1* and *SP9D* delay flowering but their inactivation does not enhance it. Moreover, each of the antagonist genes displays a unique expression pattern and expression level in tomato stems. The extreme impact of the alien *GinFT* reveals yet another way of antagonizing florigen.

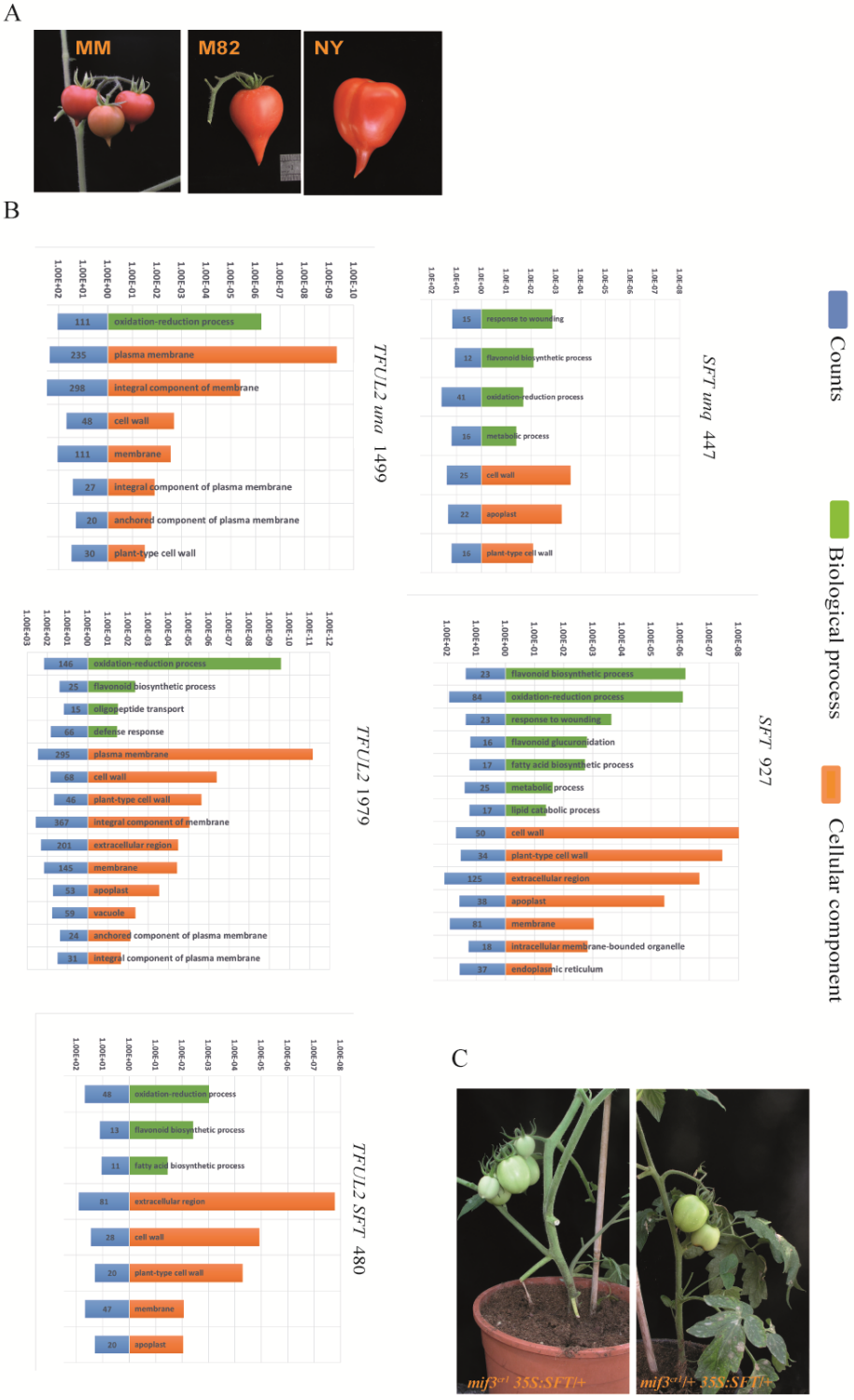

Fig. S5 *TFUL2, TMIF2* and *TMIF3* induce premature, florigen-independent, SCW deposition
A) Abundant expression of *TFUL2* induces pointed beaked fruits in three backgrounds. Similarly, overexpression of four other *TFUL* like genes; *AtAP1*, *MACROCALYX, TFUL1* and *AP67* induced slender stems and beaked fruits in all three backgrounds. B) A detailed functional analysis of *SFT* and *TFUL2* regulated genes as classified in the Venn diagram in figure 5J. GO terms of *TFUL2*-specific regulated genes (*TFUL2 u*nq), all *TFUL2* regulated genes (*TFUL2*), all *SFT* regulated genes *(SFT), SFT* specific regulated genes (*SFT* unq), and genes regulated by both *SFT* and *TFUL2 (FUL2 SFT)*. The numbers of DEG for each analysis are indicated. C) The effect of high *SFT* on stem sizes of mature *mif3*^{cr1} plants. Fruit bearing stems of *mif3*^{cr1} *35S:SFT/+* and *mif3*^{cr1}*/+ 35S:SFT/+* plants. Note the normal girth of *mif3*^{cr1} *35S:SFT/+* stems.

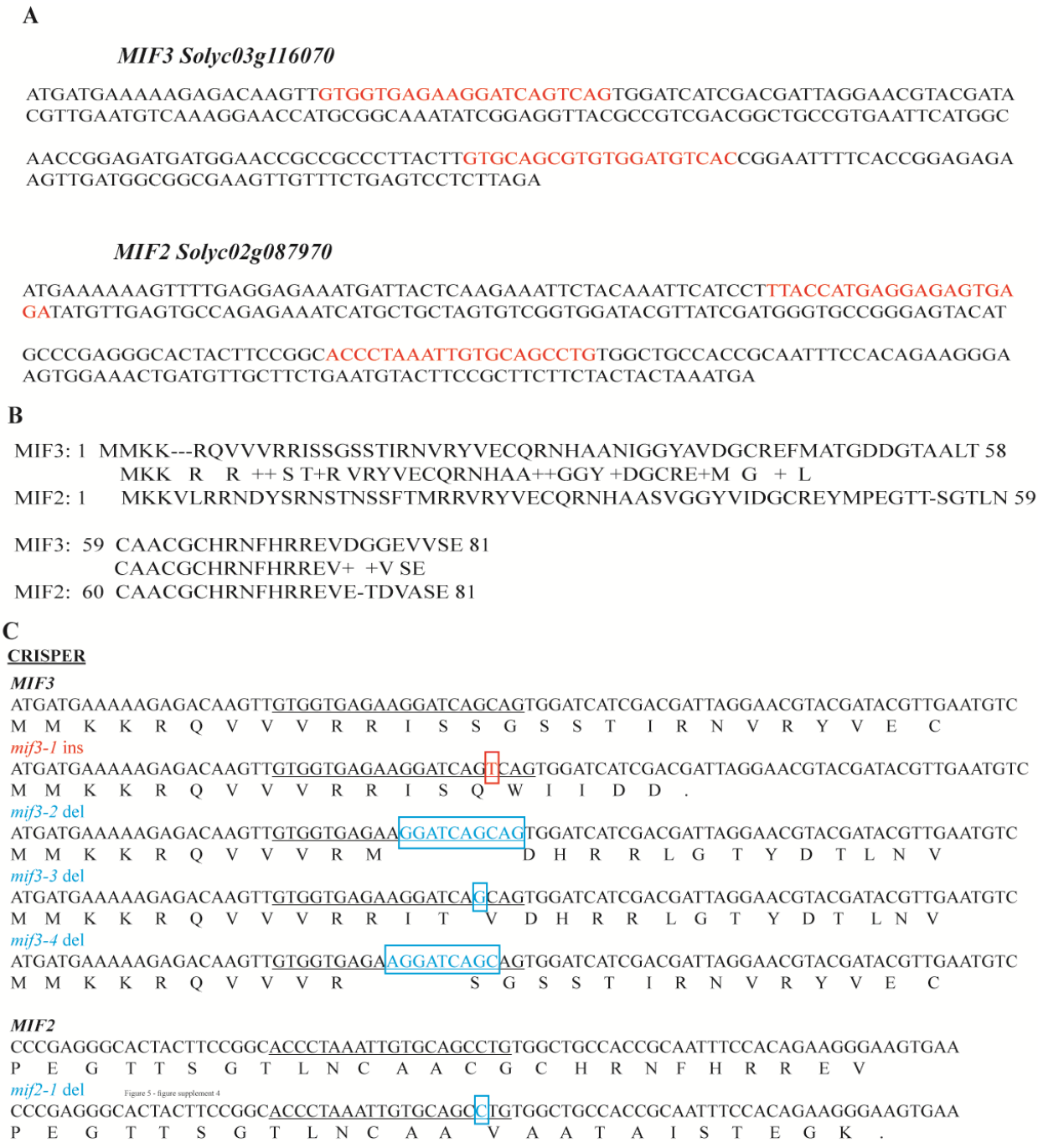

Fig. S5-2 Genome editing of the *MIF2* and *MIF 3* genes

(**A**) RNA guides (Red) for the tomato *MIF2* and *MIF3* genes. (**B**) Amino acid alignment for MIF2 and MIF3 polypeptides. (**C**) CRISPR – edited alleles of *MIF3* Top and *MIF2*, Bottom. Insertions, Red and Deletions, Blue

Materials and Methods details

Cloning procedures

SFT was amplified from cDNA with the appropriate primers containing EcoRI at the 5' and SmaI at the 3' end (as listed in Table 1 below) and cloned into p1638 3xMYC. An XhoI – *SFT* 3xMYC- HindIII fragment was subcloned into the pART7 intermediary vector, containing the 35S promoter and the OCS terminator at the XhoI/HindIII site. The final construct was then transferred as a NotI fragment into the ART27 binary vector for transformation. *SP* and *SP5G* were amplified from cDNA with the appropriate primers containing XhoI or SalI at the 5' and SmaI at the 3' end (see Table 1). *CrFT* was synthesized by GenScript and the *GinFT* cDNA was gift from E. Brenner at NYU. Both were then amplified as the others. The *SFT* in the pART27-35S::*SFT*3XMYC::OCS was then substituted by the appropriate gene using the XhoI and SmaI restriction enzymes. The XhoI-*SFT*-Flag-HindIII fragment was synthesized and cloned into pUC57 by GENEWIZ Inc. A XhoI-SFT-Flag-HindII-SmaI fragment from pUC57 was used to substitute *FT* in the pK2GW7-pHS::*FT*::35S terminator (a gift from Ove Nillson), or cloned into pART7 and pART27 to form pArt27:35S::*SFT*-Flag::OCS. The *SFT* gene was then substituted with the *TMIF2* gene that was amplified from a former construct including 43bp upstream to atg. *TFUL2* was cloned from cDNA with the appropriate primers and subcloned into pBS. A SalI-BamHI fragment was cloned into pART7 and then as a Not I fragment into pART27. pPZP212-*pSFT::SFT*::NOS was cloned by subcloning the 2200bp of p*SFT* and *SFT*::NOS from pPZP111-p*SFT*::GUS::NOS and pCGN1548-35S::*SFT*::NOS, respectively, into pBS. The *pSFT::TFT*::NOS fragment was then cloned to the binary vector pPZP212. The 2800pb of the tomato p*APL* was subcloned into pBS in two fragments: 542bp of the promotor 3' end was synthesized by GENEWIZ Inc. with StyI at the 5'. The rest was amplified from genomic DNA with appropriate primers (see Table 1). The entire promotor was then cloned into the pART27-GUS::OCS cassette that was constructed from pPZP111-p*SFT*::GUS::NOS, pART7 and pART27. Tomato p*CEAS4* was amplified from genomic DNA with appropriate primers (see Table 1) and subcloned into pBS. The promotor was then cloned into the pART27-GUS::OCS cassette.

Table 1: Primers for cloning

Gene name	Gene ID	Forward primer	Reverse primer	Res. enzyme
SFT	Solyc03g063100	GGAATTCCTCGAGATGCCTAGAGAACGTGATCC	TTTCCCGGGAATCAGCAGATCTTCTACGTC	EcoRI, xhoI,SmaI
SP	Solyc06g074350	CTCGAGAATGGCTTCCAAAATGTGTGAACCCC	TTTCCCGGGAACGCCTTCTAGCGGCA	XhoI, SmaI
SP5G	Solyc05g005380	AAAGTCGACATGCCTAGAGATCCTTTA	TTTCCCGGGATAGGCGACGACCACCG	SalI, SmaI
CrFT	GenBank: EX927041	AAAGTCGACATGCCTAGATCAGTTGATC	TCCCGGGAAACTGAAGAAGCTGAAAGTC	SalI, SmaI
GINFT	GenBank: EX932314	aaaGTCGACATGACGAGTAGATTCA	TTTCCCGGGAACGCCTTCGCCCTGC	SalI, SmaI
FUL2	Solyc03g114130	GAGTCGACATGGGTAGAGGAAGAGTA	GTTTAACCGTTGAGATGGCGA	SalI, blunt
MIF2	Solyc02g087970	GAGGACACGCTCGAGGAATTC*	GTAGTAGAAGAAGCGGACAGTA	XhoI. blunt
APL	Solyc12g017370	CTACTCAGTACCCGTGTTTTGT	TTGAGTCCCCTTGGCCCTTGA	Blunt, StyI
CESA4	Solyc09g072820	CATGTTAAAGTAGGCTTAGGAATTCCA	GGGGTTAGATGGATATTGGCAGTAAG	Blunt

Construction of CRISPR/Cas9-induced mutant plants

Generation of transgenic plants for CRISPR/Cas9 mutagenesis was performed as per a published protocol (3). Vectors were assembled using the Golden Gate cloning system (4). The sgRNAs were cloned downstream of the *At* U6 promoter; sgRNA sequences are shown in Fig. S5-2. Binary vectors were transformed into M82 and transgenics were genotyped for induced lesions using forward and reverse primers flanking the sgRNA target sites. Next-generation plants carrying mutant alleles and lacking the transgene were used for detailed analyses.

Sample preparation for NGS analysis

Five, seemingly homogeneous plants were selected from a larger population to represent the natural variation among greenhouse tomato plants. Independent stem samples consisted of 5 mid-segments of internodes harvested in duplicates 2 h after dawn. Total RNA was extracted using the RNeasy Mini Kit (Qiagen), and treated with DNase I using the RNase-Free DNase set (Qiagen). RNA samples were processed for sequencing, quality control, and differential expression analysis at the Technion Genome Center. Libraries of single 50 bp reads were prepared using the Ilumina TruSeq RNA library preparation kit v2. The barcoded libraries were sequenced on Ilumina Hiseq 2500, six samples per lane (~30,000,000 reads). If an experiment included more than 6 samples, each sample was divided equally, in all the lanes. Library quality control was conducted using FASTQC, version 0.11.5. Sequences were trimmed by quality using trim galore and aligned to the tomato ITAG version 2.5 genome (ftp://ftp.sgn.cornell.edu/genomes/Solanum_lycopersicum/annotation/ITAG2.5_release), using Tophat2, version 2.1.0 (uses Bowtie2 version 2.2.6). Raw gene expression levels were counted by HTseq-count, version 0.6.1 and then normalized by DESeq2 R package, version 1.14.1. Data analysis, gene annotations, and the corresponding A.t. codes, if available, were obtained from the SGN ITAG 2.5 release. All Downstream analyses were conducted in R with DESeq2 (5) as follows: raw data were filtered by independent filtering except when otherwise specified. Count normalization was performed by DESeq2 default. To identify differentially expressed genes (DE/DEG) differing between two conditions (contrast) in the various comparisons described below, we used the Wald test, as implemented in DESeq2. We used padj for adjusted p-value, FC for expression fold change and |FC| for the absolute value of FC. All RNA-sequencing data was deposited to the Gene expression Omnibus (GEO) with accession number GSE132280.

NGS statistical analysis

Fig.1

One factor design (genotype) was used, with three levels (WT, *sft*^{7187} and *pSFT:SFT*). Three contrasts were tested: WT vs. *pSFT:SFT*, *sft*^{7187} vs. *pSFT:SFT* and *sft*^{7187} vs. WT. Genes that significantly differed between conditions were defined as those with padj<0.1 and |FC>2|.

Fig. 2

Graft – A two-factor design was used, with genotype (WT, *sft, sp*) and induction (homograft, heterograft) as main factors. Genes that significantly differed between homograft and heterograft were defined as those with padj<0.1.

Heat map – The replicate means of the 593 genes showing significant differences between homografts and heterografts (see supplemental table 1 for the normalized counts of the 593 genes). Pearson's correlation as distance matrix and complete agglomeration were used to construct a tree. The tree was then cut into eight clusters and a heatmap was plotted.

Punnett grid (PG) - All 20,286 expressed genes (with normalized expression value >20 in at least one genotype) of the recipients WT//WT*, sft//sft and SFT//sft* were sorted into 9 cohorts based solely on their expression trajectories. The expressed genes in each tripartite comparison were defined first by two bipartite genotypic comparisons WT//WT vs *sft//sft* and *sft//sft vs SFT//sft* and subsequently by 3 possible response categories: Up (U), Down (D) and No change (N), demanding a modest |FC≥1.5| as the cutoff.

Cohort DU - genes downregulated in *sft* of the *sft//sft* homograft but upregulated at least 1.5 fold in comparison to *sft* of the *35S SFT//sft* heterograft- is enriched for the florigen–regulated genes analysed in Fig. 2C (101/593, $p< 5.4*10^{-70}$ hypergeometric) and for SCWB genes (54/ 365, $p< 4.4*10^{-26}$).

Fig. 3

Heat induced *FT* –one factor design was used to test the contrast between two factors: heat induced FT and all other conditions (*FT*, heat shock and no added genotype treatment) and genes that significantly differed between conditions were defined as those with padj<0.1 and |FC>1.8|.

Fig. 4 and 5

GinFT and *TFUL2* – two-factor design with genotype and developmental stage as main factors and their interaction. To compute the model, the log-ratio test was used and differential expression was calculated using the Wald test with the specific contrasts. Genes that significantly differed between conditions were defined as those with padj<0.1 and |FC>2|. GO enrichment –DAVID enrichment of biological processes (BP) was applied to all DEGs with known Arabidopsis homologues. Overlap between gene lists – where appropriate, the significance of overlap between lists of genes was tested using the hypergeometric tests.

qRT-PCR experiments

Total RNA was extracted using the Plant RNA mini kit (Biomiga) and treated with the RNase-Free DNase Set (QIAGEN), according to the manufacturer's instructions. 500 ng to 1 µg of total RNA was used for cDNA synthesis using the qScript cDNA Synthesis Kit (Quanta). All primer sequences are presented in Table 2 below.

Table 2: Primers for RT-PCR

Gene name	Gene ID	Forward primer	Reverse primer
SFT	Solyc03g063100	CCAAGTCCGAGTGATCCAAAT	GTTGAAATTCTGACGCCATCC
FT	AT1G65480	AGTCCTAGCAACCCTCACCT	CCTGCAGTGGGACTTGGATT
Ful2	Solyc03g114830	CTCTGTGCTTTGCGATGCTG	TTCCAAAGTCCAGCTACCCG
CET1	Solyc03g026050	CCCTGTTGCTGCTGTTTATTTCA	CTGGGGCAGTACTACTTTTGTCT
Ful1	Solyc06g069430	GAGGGAGAAAGAGGTGGCAC	ACCTCCTTCCACTTCCCCAT
ZF 36 C3H	Solyc05g008670	TCAACACAGCGCAAAGGGTA	GACATGCCCCTGTTTCTTGC
MYB55	Solyc05g015750	GAGCTGCAGACTTAGGTGGATTA	CAATCTGAGACCACTTGTTCCCT
MYB103	Solyc08g081500	GGCTATGGCTGTTGGAGTGA	CAGCTCCATGTAGACTTATAATC

Histological procedures

Sections of tomato stems, cut by hand, were cleared with 5:1 ethanol acetic acid, stained in 0.05% TBO in 20% $CaCl_2$ for 2 min, washed in 10% ethanol and kept in 20% $CaCl_2$. Images were acquired either by the Nikon eclips e600 microscope equipped with Invenio 3SII camera and Delltapix insight software, or with an Olympus SZX16 binocular microscope equipped with a cooled color CCD camera (Olympus (DP72) and CellSens software (Olympus).

GUS staining was carried out as previously described (6). Briefly, handmade sections of tomato stems were fixed in 90% acetone for 20 min at 4^{o}C, equilibrated in GUS buffer (5mM $K_3Fe(CN)_6$, 5mM $K_4Fe(CN)_6$, 100mM PO_4 buffer, 1% Triton, 1mM EDTA) for 30 min and stained in GUS buffer contained 25mg/ml X-gluc, at 37^{0}C, for 2-18 h.

Western blot analysis

Protein sample concentrations were assessed via the Bradford assay. Equal amounts of protein were prepared for loading with sample buffer (0.1mM Tris-HCl pH 6.8, 2% SDS, 4% glycerol, 400mM dithiothreitol, 0.01% bromophenol blue), heated for 5 min at 100 °C and centrifuged for 1 min. The supernatant separated on a 12.5% SDS-PAGE gel (1 h, in 25mM Tris-HCl, 192 mM glycine buffer, 0.1% SDS). Proteins were transferred to a nitrocellulose membrane (Protran) via wet transfer at 4 °C in a 25mM Tris-HCl, 192 mM glycine 20% methanol and 0.03% SDS buffer. Membranes were blocked with 1% skim milk, for 1 h, at RT, and incubated with a primary antibody overnight, at 4 °C. Membranes were then washed 3 times with TBST (20mM Tris-HCl pH7.6, 150mM NaCl, 0.1% Tween-20) and incubated for 1 h, at RT, with the secondary antibody conjugated to horseradish peroxidase. Then, membranes were washed 3 times with TBST and an ECL reaction was performed. The protein signal was visualized with a Biorad-Chemidoc analyzer.

Enrichment for the mobile MYC-tagged CETS proteins

Leaves or stems from recipient grafts were ground in liquid nitrogen and stored at -80 °C. For SFT-3XMYC, the tissue powder was extracted in 5 volumes of Mg/NP40 extraction buffer (7), mixed with 4 volumes of 90% cold acetone, and stored at -20ºC for 1h. The acetone mix was briefly precipitated, the pellet was resuspended in HEPES-Sorbitol buffer, and then precipitated again at 100,000 g for 1h. The final supernatant was subjected to ammonium sulfate (AS) fractionation as detailed in the Supplemental Methods. Enrichment for other CETS proteins was performed without the prior acetone purification. Tissue powder was homogenized in Mg/NP40 (7) at a 1:5 w/v tissue:buffer ratio. [0.5M Tris-HCl, pH 8.3, 2% v/v NP-40, 20mM MgCl2, 2% v/v β-mercaptoethanol, 1mM phenylmethylsulfonylfluoride (PMSF) and 1% w/v polyvinylpolypyrrolidone (PVPP)], for 15 min 12,000 g and subjected to AS fractionation.

Purification of CETS proteins for MS analysis

Plant tissue (10 g) was extracted with Mg/NP40 followed by AS fractionation, as above, with the exception of SP-3xMYC which was subjected to a 20-50% fractionation. The pellets were suspended in 2 ml 50mM Tris-HCl, pH8, 150mM NaCl, filtered through a 0.45 μm filter (Minisart Sartorius®), and then purified on anti-MYC columns. Anti-MYC column preparation. General Reference Protein A beads, for a 50 μl bed volume, were briefly centrifuged at 3000 rpm and pellets were suspended in 0.5ml NaBr buffer [50mM NaBr pH 9, 3M NaCl]. anti-MYC (Hybridoma purified, see suppl M&M; 50 μg) in 0.5 ml NaBr buffer were combined with the beads in a test tube and rotated for 1 h, at RT. After centrifugation at 10,000g for 30 sec, beads were washed with NaBr buffer, centrifuged, and resuspended in 2 ml 200 mM NaBr pH 9, 3M NaCl. Dimethyl pimelimidate (DMP) was added to bring the same to a 20 mM concentration for crosslinking and the test tube was rotated for 30 min at RT. After centrifugation and resuspension in 0.2M ethanolamine, pH 8, the test tube was rotated for 2 h, at RT. The test tube was then centrifuged and beads resuspended in PBS, pH 7.4, the crosslinked beads were placed in a tip column (TopTip Empty Glygen®) and stored at with 0.02% Na-azide, at 4 ºC until use.

Immunoaffinity purification of MYC-tagged proteins for MS

An anti-MYC column was washed with 1 column volume of acetic acid, pH 3, 10 column volumes of Tris-HCl 100mM, pH 8 and 2 column volumes of 50 mM Tris-HCl, pH8, 150 mM NaCl. Samples (2ml) were loaded, washed with 3 volumes of 50 mM Tris-HCl, pH 8, 150 mM NaCl and eluted in 10 fractions of 50 μl acetic acid 0.1 N, pH 3, 8 μl 1M Tris-HCl, pH9 were added per fraction for neutralization. Samples of the fractions were Western blotted and strongly reacting fractions were used for mass spectrometry analysis.

LC-MS/MS

Samples were either trypsinized or chymotrypsinized and peptides were analyzed by LC-MS/MS, performed with an OrbitrapXL mass spectrometer (Thermo). The data were analyzed using the Sequest 3.31 software vs the Solanum lycopersicon section of the NCBI-NR database and vs. the specific protein sequences. SP-3xMYC results were further validated via higher-energy collisional dissociation (HCD) fragmentation. All analyses were carried out at the Smoler Proteomics Center, Department of Biology, Technion - Israel Institute of Technology Haifa Israel.

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Captions for Dataset Files

Dataset 1: WT vs p *SFT-SFT* (Fig. 1)

Dataset 2: Graft experiment (Fig. 2)

Dataset 3: Cell wall genes (Fig. 2)

Dataset 4: Heat Shock experiment (Fig. 3)

Dataset 5: *GINFT* (Fig. 4)

Dataset 6: *TFUL2-SFT* Venn (Fig. 5)

Dataset 1: WT vs p *SFT-SFT*

The 981 genes showing |FC>2| *P* value < 0.1 between *WT* and *pSFT-SFT* are listed. columns B-D show the sum of mean normalized DESeq2 expression values for WT, *sft* mutant and *pSFT-SFT* overexpression. Column E) TF- Transcription Factor, F) CW- cell wall, G) PCW/ SCW - primary or secondary cell wall, H+I) description, J) AT code - putative *Arabidopsis thaliana* homolog. The remaining sheets show only TF and only SCW.

Dataset 2: Grafts experiment

In the first tab, **Graft,** 593 genes showing P value < 0.1 are listed. B-F columns showing the sum of mean normalized DESeq2 expression values for *WT//WT, sft//sft, 35S:SFT//sft, 35S:SFT//WT, 35S:SFT//SP*.

Column G) TF- Transcription Factor, H) CW- cell wall, I) PCW/ SCW - primary or secondary cell wall, J+K) description, L) AT code - putative *Arabidopsis thaliana* homolog, M) cluster - the cluster in the heat map. In the second tab, **DU,** 365 genes downregulated in *sft//sft* homograft but upregulated at least 1.5 fold in comparison to *35S SFT//sft* heterograft are listed (Fig. 2E). B-F column showing the sum of mean normalized DESeq2 expression values for *WT//WT, sft//sft, 35S:SFT//sft, 35S:SFT//WT, 35S:SFT//SP*. Column G) TF- Transcription Factor, H) CW- cell wall, I) PCW/ SCW - primary or secondary cell wall, J+K) description, L) AT code - putative *Arabidopsis thaliana* homolog.

Dataset 3: Cell wall genes

List of 2675 genes that were defined as cell wall genes as described in M&M. Column B) TF- Transcription Factor, C) PCW / SCW - primary or secondary cell wall, D+E) description, F) AT code - putative *Arabidopsis thaliana* homolog, G) Source - the reference, H) specific description, I) Express in stem - indicates if the gene is expressed (at least over 1 read) in our data.

Dataset 4: Heat Shock experiment

354 genes showing *P* value < 0.1 and |FC>1.8| are listed. B-E columns showing the sum of mean normalized DESeq2 expression values for treated *sft pHS:FT*, untreated *sft pHS:FT,* treated *sft* and untreated *sft.* Column F) TF- Transcription Factor, G) CW- cell wall, H) PCW/ SCW - primary or secondary cell wall, I+J) description, K) AT code - putative *Arabidopsis thaliana* homolog, L) Punnet – the position in PG1.

Dataset 5: *GinFT*

1122 genes showing |FC>2| and *P* value < 0.1 are listed. Columns B-C show the sum of mean normalized DESeq2 expression values between WT and *35S:GinFT*. Column D) TF- Transcription Factor, E) CW- cell wall, F) PCW/ SCW - primary or secondary cell wall, G+H) description, I) AT code - putative *Arabidopsis thaliana* homolog.

Dataset 6: *TFUL2- SFT* Venn

Differentially expressed genes between *WT* and *35S:TFUL2 or WT* and *35S:SFT* showing |FC>2| and *P* value < 0.1 are listed. Columns B-D show the sum of mean normalized DESeq2 expression values for WT, *35S:TFUL2* and *35S:SFT* . Column E) TF- Transcription Factor, F) CW- cell wall, G) PCW/ SCW - primary or secondary cell wall, H+I) description, J) AT code - putative *Arabidopsis thaliana* homolog. Tab descriptions according to CW Venn: ***SFT* 927 -** DEG between WT and *35S:SFT* (class I+II+III+VI), **TFUL2 1979** - DEG between WT and *35S:TFUL2* (class III+IV+V+VI). ***SFT*_ung_447** - DEG between WT and *35S:SFT* but not between WT and *35S:TFUL2* (*SFT* unique class I+II). ***TFUL2*_unq_1499 -** DEG between WT and *35S:TFUL2* but not between WT and *35S:SFT (TFUL2* unique class IV+V). ***SFT_TFUL2*_480 -** genes regulated by both *SFT* and *TFUL2* (class III+VI).