

Figure S1

Fig. S1. Alignment of 5'-UTR and -842 bp upstream region of Santa Rosa (SR) and Sweet Miriam (SM) *ACSI* gene. Transcription start site is marked by +1. First ATG is at position +295.

SR	ATAAGAGTGACAATTTCCCTTAAAGTCAAATAGACAATCATCACGTTTAGTTGGTGCTAT	-782
SM	ATAAGAGTGACAATTTCCCTTAAAGTCAAATAGACAATCATCACGTTTAGTTGGTGCTAT	

SR	ACTACTTTTGGGTGTACATGTTTATTTCCGGATCTAAATTTTGAAATTTGTACATCCCT	-722
SM	ACTACTTTTGGGTGTACATGTTTATTTCCGGATCTAAATTTTGAAATTTGTACATCCCT	

SR	ATAGAGTAACAAGAGGCAAAACCCCAACAGACTGCGTTTAAATTCAGTAGCTTTTGGG	-662
SM	ATAGAGTAACAAGAGGCAAAACCCCAACAGACTGCGTTTAAATTCAGTAGCTTTTGGG	

SR	ACCGCGTGAAGAAGCGATTTTGAAATGTGGCTTAATTAAGTCTTCCCAACTATACGGG	-602
SM	ACCGCGTGAAGAAGCGATTTTGAAATGTGGCTTAATTAAGTCTTCCCAACTATACGGG	

SR	AAGTAAATGCATTCCCTTCATAACTTTTAGAAATGGAATAAATGTGATGCAAACTGAGCT	-542
SM	AAGTAAATGCATTCCC-TCATAACTTTTAGAAATGGAATAAATGTGATGCAAACTGAGCT	

SR	CATTACGCGGAACCAAAAATAGGAAAATTTGTGGAACACTTTGAACTAAAAC TTGGA	-482
SM	CATTACGCGGAACCAAAA-ATAGGAAAATTTGTGGAACACTTTGAACTAAAAC TTGGA	

SR	GTCTCACAAGAAACCGTGGAGGAAAAGGAAACCTAGAGGAAAATTTAGTGCCCTTTAGA	-422
SM	GTCTCACAAGAAACCGTGGAGGAAAAGGAAACCTAGAGGAAAATTTAGTGCCCTTTAGA	

SR	TTTGTAGACTTTCCTACATGTGTGAGTCTTGTGCATTAACTACTATTGGTGCATAAGTCC	-362
SM	TTTGTAGACTTTCCTACATGTGTGAGTCTTGTGCATTAACTACTATTGGTGCATAAGTCC	

SR	AAATACTGGCCAAATAAAAATGTTCTCATGTGTGCTTCAAGCTAGTCAACACCGAAAGC	-302
SM	AAATACTGGCCAAATAAAAATGTTCTCATGTGTGCTTCAAGCTAGTCAACACCGAAAGC	

SR	TTTCTTCTCTCATGGTGCTTTCCGGGAAGCTTCATTGCCAGGAAGAACACAACCAACC	-242
SM	TTTCTTCTCTCATGGTGCTTTCCGGGAAGCTTCATTGCCAGGAAGAACACAACCAACC	

SR	ACACATCAATTGAAAGAAGAAAAAATATTAATACTAATTCACTAAATATGTGTGTGC	-182
SM	ACACATCAATTGAAAGAAGAAAAAATATTAATACTAATTCACTAAATATGTGTGTGC	

SR	TAAGGCGTTAAAAATAAGGTCCGATTGCGATTTGAAAAAGATAAATCATCAATATTTA	-122
SM	TAAGGCGTTAAAAATAAGGTCCGATTGCGATTTGAAAAAGATAAATCATCAATATTTA	

SR	TTGTGTGGAACAATAACACCAATGATATATGTAACTGAAACGAAGTTCATGGAAGTTC	-62
SM	TTGTGTGGAACAATAACACCAATGATATATGTAACTGAAACGAAGTTCATGGAAGTTC	

SR	ACGGAAAAATAGTGTGGACCACCAACAGGCACACTACTGGGTACACGTGGATATGGAT	-2
SM	ACGGAAAAATAGTGTGGACCACCAACAGGCACACTACTGGGTACACGTGGATATGGAT	

	+1	
SR	CGATAT CAACGTACAACACGCAAGCAATTACGCCATTGACCAATATTAACACAATTAA	+59
SM	CGATAT CAACGTACAACACGCAAGCAATTACGCCATTGACCAATATTAACACAATTAA	

SR	TTCTGTTATTTCTATTCTGGGAGTCGGTTTTTCCAAAGTTGAAC TTGAAAAGAAAA	+119
SM	TTCTGTTATTTCTATTCTGGGAGTCGGTTTTTCCAAAGTTGAAC TTGAAAAGAAAA	

SR	AAAAACCTTTGAAAAC TTTGCTTCCCTCCTGCTTGCCAATCTGCCATCGCCTATAAAT	+179
SM	AAAAACCTTTGAAAAC TTTGCTTCCCTCCTGCTTGCCAATCTGCCATCGCCTATAAAT	

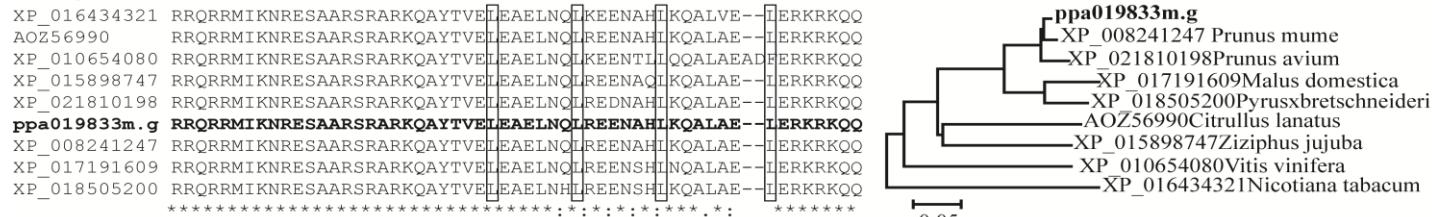
SR	TCCCACTACTATCTCACCATTGCCCCACAATTGATAGCTTGCTTGCTTGCAAACTCA	+239
SM	TCCCACTACTATCTCACCATTGCCCCACAATTGATAGCTTGCTTGCTTGCAAACTCA	

SR	CTAAAAATTCACATAATATTCTTGTGTGTGAATATTCCTCGGAACCAAGAAAT ATG	+297
SM	CTAAAAATTCACATAATATTCTTGTGTGTGAATATTCCTCGGAACCAAGAAAT ATG	

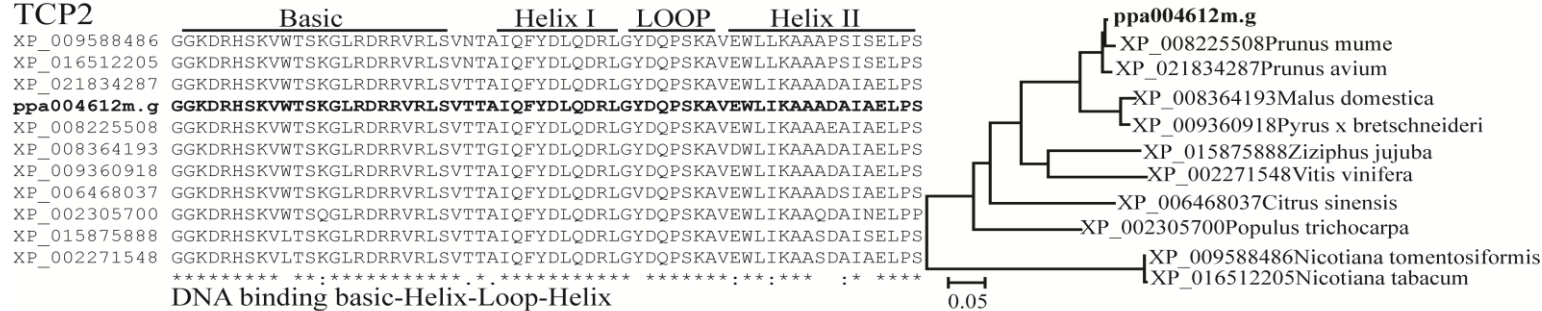
Fig S2. Amino acid sequence alignment and phylogenetic analyses of ABI5, TCP2 and GL2. The conserved domains were aligned by Clustal. The leucine residues in the zipper region are boxed for ABI5. Conserved helices are labeled for TCP2 and GL2 (Martín-Trillo and Cubas., 2010; Rerie et al., 1994). Species names are followed by their NCBI ID. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model. Initial tree(s) for the heuristic search are obtained automatically as the following. When the number of common sites is < 100 than one fourth of the total number of sites, the maximum parsimony method is used; otherwise BIONJ method with MCL distance matrix is used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis for ABI5 involved 9 amino acid sequences, 11 sequences for TCP2, 10 sequences for GL2. All positions containing gaps and missing data are eliminated. A total number of 384 positions in the final dataset are for ABI5, 379 positions for TCP2, and 725 positions for GL2. Evolutionary analyses is conducted in MEGA5 (Tamura et al., 2011).

Figure S2

ABI5



TCP2



GL2

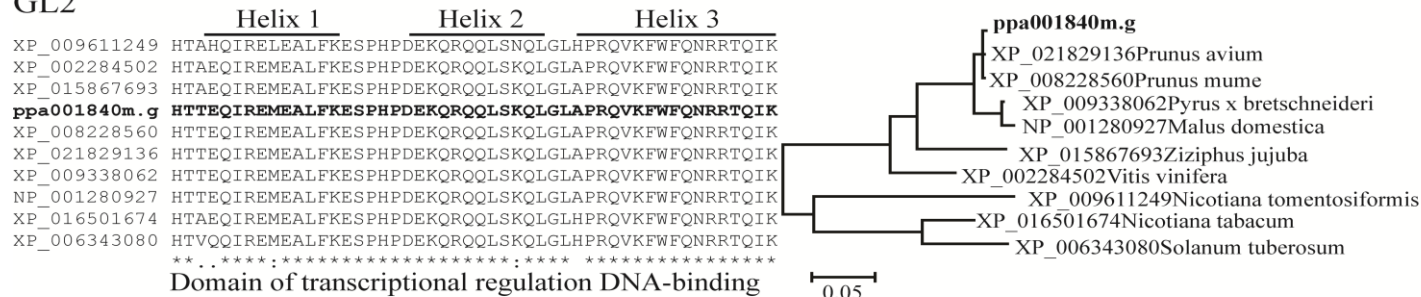


Table S1. Synthetic promoters containing sequences of *cis*-element core sequences and corresponding mutated sequences for one-by-one bait/prey interactions

Target <i>Prunus persica</i> gene IDs	Name of bait constructions	Synthesized sequence (5'-3')	# of tandem repeats
ppa019833m	<i>PsABI5-binding site (bs)</i>	ATC <u>ACG</u> TTTAGTACATGTTTATCTACATG TGTGATCTCATGTGTGCAAATATGTGTGT GTGTAC <u>ACG</u> TGGATAC <u>ACG</u> TGGATATGT AC <u>ACG</u> TGGATAC <u>ACG</u> TGGAT	3×
	<i>PsABI5-mutated-binding site (bs)</i>	ATCTGATTTAGTAGTTGTTTATCTAGTTG ACTGATCTCATGACTGCAAATATGACTGT GACTACTGCTGGATACTACTGGATATGTA CTGCTGGATACTGCTGGAT	Mutant ,3×
ppa004612m	<i>PstCP2-binding site (bs)</i>	CAAGGACCAATAGTTGGTGCTATACTCAT GGTGCTTTCCGTGTGGACCACCA	4×
	<i>PstCP2-mutated binding site (bs)</i>	CAAGCTGCAATAGTTGCACCTATACTCAT GCACCTTTCCGTGTGCTGCACCA	Mutant ,4×
ppa001840m	<i>PsGL2-binding site (bs)</i>	GCTCATTAACGCGGGTGCAATTAATACTACT	6×
	<i>PsGL2-mutated binding site (bs)</i>	GCTCAAATACGCGGGTGCAAATACTACT	Mutant ,6×

Table S2. Primers used for qPCR analyses

Primer Name	Sequence (5'-3')	<i>Prunus persica</i> gene IDs
PsABI5-F	AGCCAGGAAACAGGCATACACA	ppa019833m.g
PsABI5-R	TAGCTTTCTGGGCCCTGCTC	ppa019833m.g
PsTCP2-F	CGGTCCGAGATTCGGGTGAA	ppa004612m.g
PsTCP2-R	GCTGGTGGTGGTGAGCAATG	ppa004612m.g
PsGL2-F	ACAGGCACACCACTGAGCAA	ppa001840m.g
PsGL2-R	GGCGCTCTTGTATGGCCTTG	ppa001840m.g
MON-F	GGGAACCTTATATTGGCGTAGG	
MON-R	CTGCTGACGAGGACTACTTATTG	

Table S3. *cis*-elements in P_{PsACS1} as predicted by PlantCARE

<i>cis</i> -elements	Sequences	Position/Strand	Function
5'UTR Py-rich stretch	TTTCTTCTCT	549/+,621/-	cis-acting element conferring high transcription levels
AAGAA-motif	GAAAGAA	620/+	/
ABRE	CACGTG	834/-	cis-acting element involved in the abscisic acid responsiveness
ACE	ACGTGGA	835/+	cis-acting element involved in light responsiveness
ARE	TGGTTT	320/-	cis-acting regulatory element essential for the anaerobic induction
Box 4	ATTAAT	637/-	light responsiveness
Box I	TTTCAAA	109,955,350, 976, 207,700/-	light responsive element
Box-W1	TTGACC	885/+	fungal elicitor responsive element
CAAT-box	CCAAT/CAAT/CAA TT/CAAAT/	6,7,19,35,43,488,499, 615,720,739,748,873, 889,890,901,1006,10 07,1058/+;115,337,41 0,411,473,584,616,61 7,693,699,728,884,10 48,1059,1060/-	common cis-acting element in promoter and enhancer regions
CATT-motif	GCAATC	257/+	part of a light responsive element
ERE	ATTTCAAA	109,207/-	ethylene-responsive element
G-Box	CACGTT/TAAACG TG	50/+,834/-; 50/-	cis-acting regulatory element involved in light responsiveness
GARE-motif	TCTGTTG	154/-	gibberellin-responsive element
GT1-motif	GTGTGTGAA	1115/+	light responsive element
I-box	GATATGG	840/+	part of a light responsive element
LTR	CCGAAA	541/+	cis-acting element involved in low-temperature responsiveness
MNF1	GTGCCC(A/T)(A/T)	417/+	light responsive element
Sp1	CC(G/A)CCC	149/+,927/-	light responsive element
TATA-box	TATA/TTTTA/TAAT A/ATATAT/TATAAA T/	66,128,241,273,639,1 020,1022,1102,1105/ +;225,358,504,636,67 7,754,755,893,1090/-	core promoter element around -30 of transcription start
TATCCAT/C-motif	TATCCAT	847/-	/
W box	TTGACC	885/+	/
chs-Unit 1 ml	ACCTACCACAC	602/+	part of a light responsive element

Table S4. List of transcription factors (TF) and their number (#) in the various libraries, SR S2, SR S4, SM S2 and SM S4, along with their *Prunus persica* protein ID, and annotations in *P. persica* and *Arabidopsis*. TFs in bold were predicated by PlantPan as having binding sites in the promoter of *ACS1*.

SR S2		SR S4		SM S2		SM S4		<i>Prunus persica</i> protein ID	<i>Prunus persica</i> annotation	<i>Arabidopsis</i> annotation
#	NCBI ID	#	NCBI ID	#	NCBI ID	#	NCBI ID			
1	XM_020567027.1	1	XM_020567027.1					ppa002612m.g	Prunus persica AP2-like ethylene-responsive transcription factor ANT	DRG [Arabidopsis thaliana]
1	XM_020567870.1							ppa001612m.g	Prunus persica transcriptional corepressor LEUNIG (LOC18770397), transcript	AP2/EREBP transcription factor [Arabidopsis thaliana]
		1	XM_020567847.1					ppa019833m.g	Prunus persica protein ABSCISIC ACID-INSENSITIVE 5	Basic-leucine zipper (bZIP) transcription factor family protein [Arabidopsis]
		1	XM_007201172.2					ppa013219m.g	Prunus persica CLAVATA3/ESR (CLE)-related protein 41 (LOC18766746),	no hits
1	XM_007202246.2			1	XM_020568507.1			ppa008024m.g	Prunus persica G-box-binding factor 1 (LOC18771192), transcript variant X1,	G-box binding factor 1 [Arabidopsis lyrata subsp. lyrata]
1	XM_007202528.2							ppa011596m.g	Prunus persica high mobility group B protein 2 (LOC18770435), mRNA	NFD2 [Arabidopsis thaliana]
						1	XM_007205702.2	ppa010331m.g	Prunus persica 14-3-3-like protein GF14 kappa (LOC18772503), mRNA	general regulatory factor 8 [Arabidopsis thaliana]
		1	XM_007207945.2					ppa003727m.g	Prunus persica chromatin assembly factor 1 subunit FAS2 (LOC18773563), mRNA	Transducin/WD40 repeat-like superfamily protein [Arabidopsis thaliana]
		1	XM_007209140.2			1	XM_007209140.2	ppa006484m.g	Prunus persica bZIP transcription factor 16 (LOC18775920), mRNA	hypothetical protein ARALYDRAFT_473396 [Arabidopsis]
						2	XM_007209393.2	ppa009757m.g	Prunus persica transcription factor bHLH79 (LOC18776453), transcript	transcription factor BIG PETAL P (BPE) [Arabidopsis thaliana]
				1	XM_007211775.2	1	XM_007211775.2	ppa004612m.g	Prunus persica transcription factor TCP2 (LOC18779022), transcript	putative basic helix-loop-helix DNA binding protein TCP2, partial
7	XM_007217098.2							ppa001840m.g	Prunus persica homeobox-leucine zipper protein GLABRA 2	same
2	XM_007218446.2	2	XM_007218446.2					ppa012424m.g	Prunus persica high mobility group B protein 1 (LOC18785344), mRNA	NFD2 [Arabidopsis thaliana]
1	XM_007225154.2							ppa001977m.g	Prunus persica protein CHROMATIN REMODELING 19 (LOC18792817),	SNF2 domain-containing protein / helicase domain-containing protein [Arabidopsis]
1	XM_007225901.2							ppa012509m.g	Prunus persica 50S ribosomal protein L18, chloroplastic (LOC18791554), mRNA	Z-box binding factor 3 [Arabidopsis thaliana]

						1	XM_020554291.1	ppa001493m.g	Prunus persica calmodulin-binding transcription activator 5 (LOC18792253),	
		1	XM_020561047.1					ppa018846m.g	Prunus persica glycosyltransferase family 92 protein RCOM_0530710	zinc finger family protein [Arabidopsis lyrata subsp. lyrata]
		1	XM_020564500.1					ppa014801m.g	PREDICTED: Prunus persica zinc finger MYM-type protein 1-like	no hits
2	XM_020565363.1	4	XM_020565363.1	1	XM_020565363.1			no	Prunus persica ribonuclease P protein subunit p25-like protein (LOC18789816),	Alba DNA/RNA-binding protein [Arabidopsis thaliana]
2	XM_020565367.1			1	XM_020565367.1			no	Prunus persica ribonuclease P protein subunit p25-like protein (LOC18789816),	Alba DNA/RNA-binding protein [Arabidopsis thaliana]
						1	XM_020567936.1	ppa012912m.g	Prunus persica calmodulin-7 (LOC18770340), mRNA	Z-box binding factor 3 [Arabidopsis thaliana]
				1	XM_007209089.2			ppa005912m.g	Prunus persica AP-2 complex subunit mu (LOC18775895), mRNA	
				1	XM_020566022.1			ppa000612m.g	Prunus persica calmodulin-binding transcription activator 3 (LOC18773983),	Calmodulin-binding transcription activator 3 [Arabidopsis thaliana]
				1	XM_007207935.2			ppa014789m.g	Prunus persica scarecrow-like protein 30 (LOC18775211), mRNA	GRAS family transcription factor [Arabidopsis thaliana]
				1	XM_020566232.1			ppa002392m.g	Prunus persica scarecrow-like protein 14 (LOC18773542), mRNA	GRAS family transcription factor [Arabidopsis thaliana]
14	XR_002272096.1	4	XR_002272096.1	3	XR_002272096.1	1	XR_002272096.1	ppa011892m.g	Prunus persica uncharacterized LOC18790550 (LOC18790550),	signal transducer/transcription protein, putative (DUF1685) [Arabidopsis thaliana]