

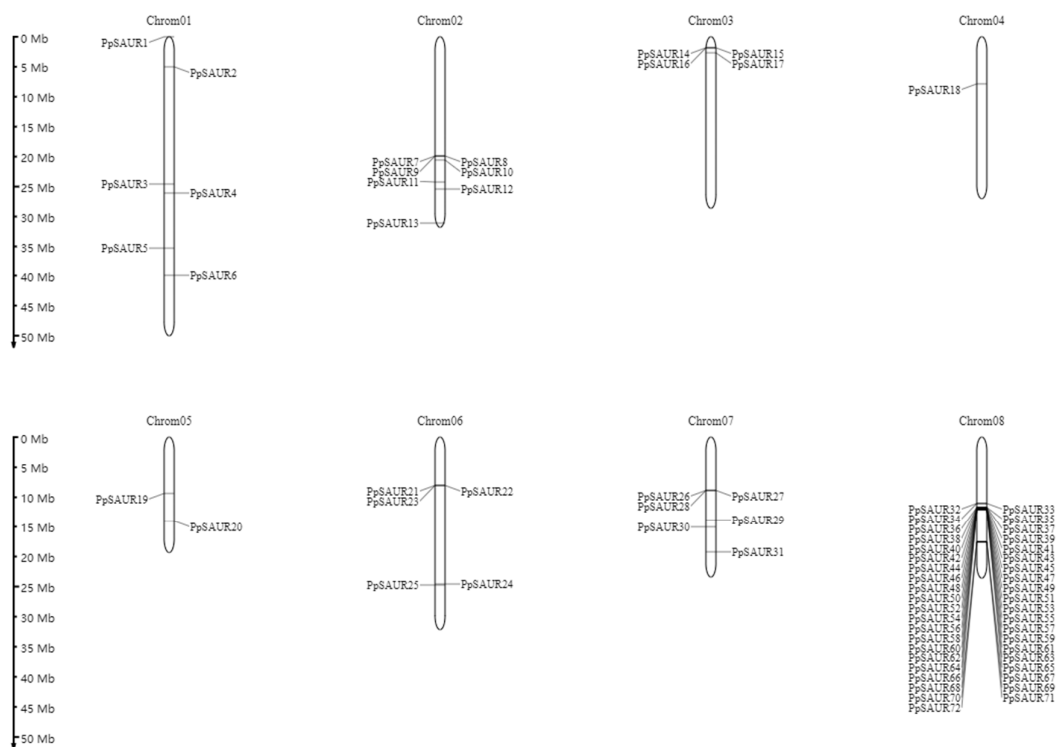
## Supplemental File

# **Auxin Responsive Gene, PpSAUR43, is Involved in the Ripening and Softening of Peach Fruit**

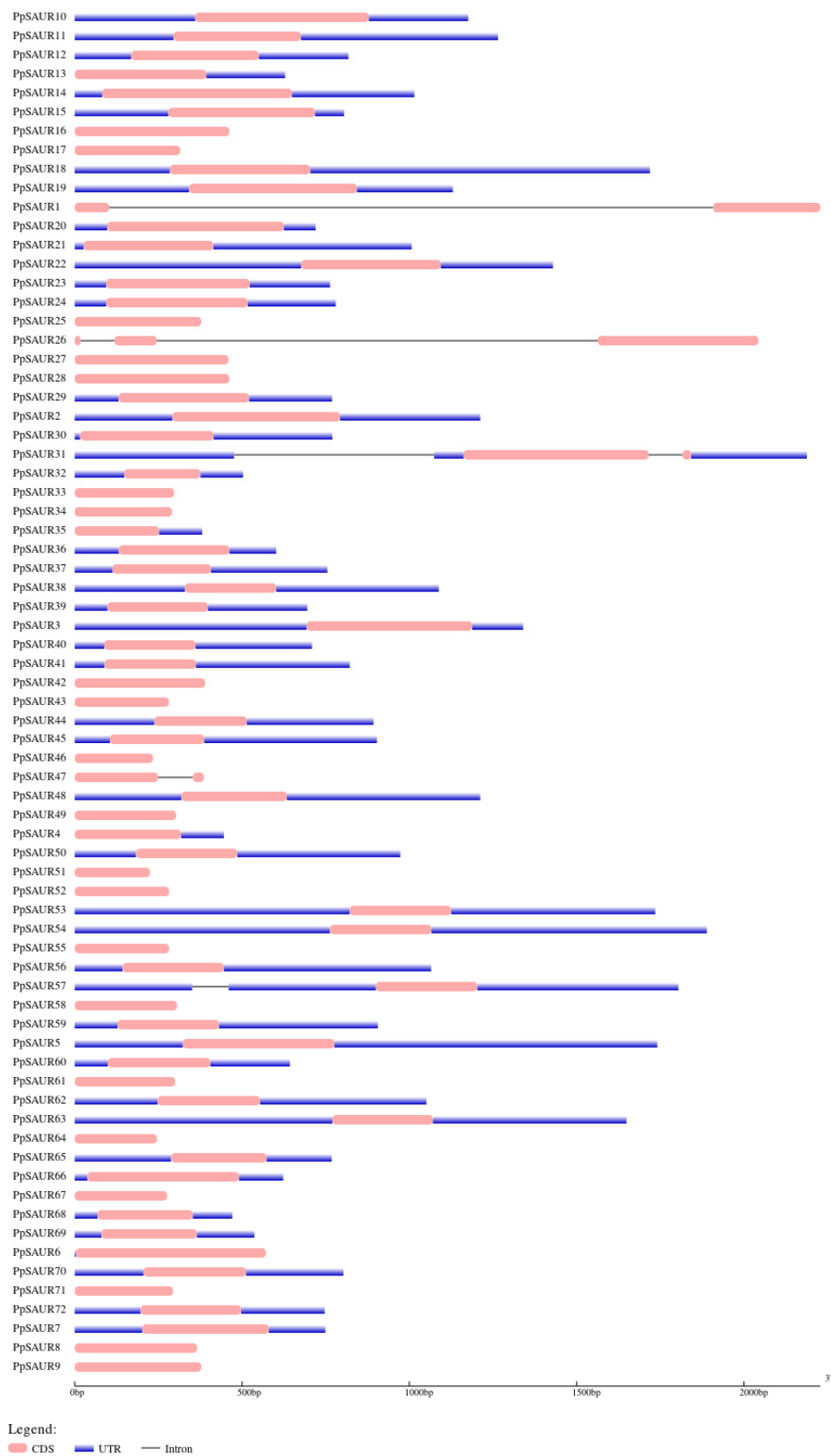
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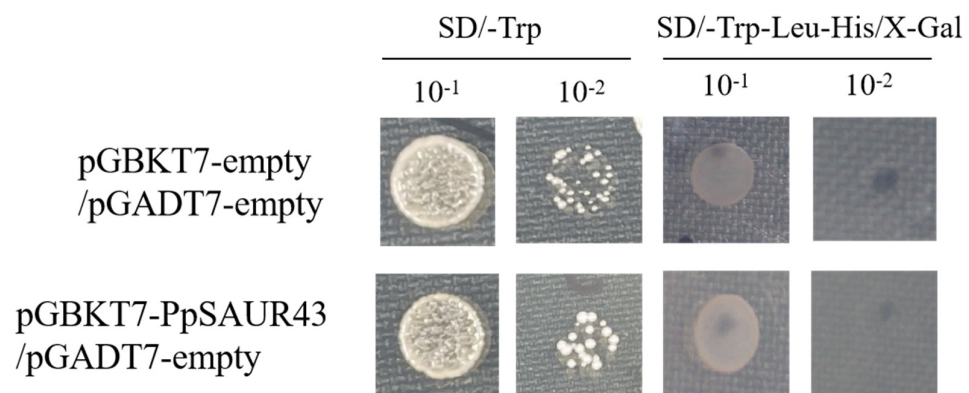
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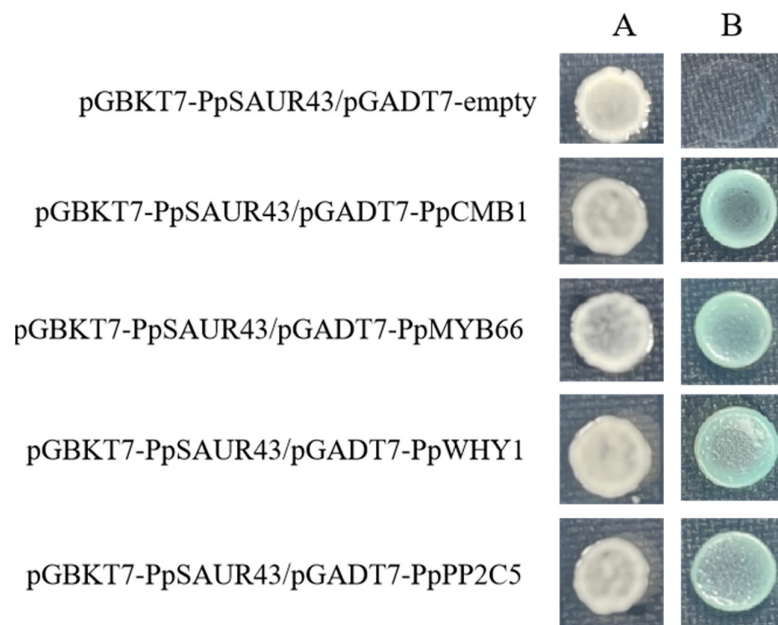
**Figure S1.** Chromosomal localization of SAUR family members.



**Figure S2.** Gene structure of SAUR family members.

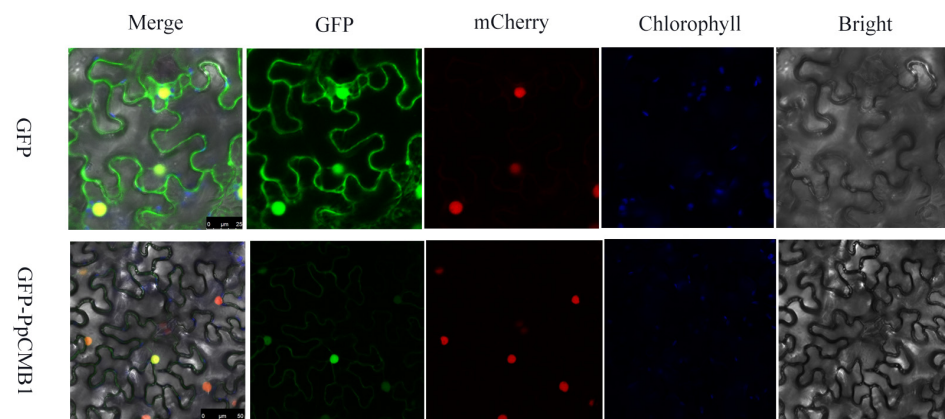


**Figure S3.** Transcriptional activation identification of the full length of PpSAUR43.



**Figure S4.** The interacting confirmation between PpSAUR43 and PpCMB1, PpMY66, PpWHY1 and PpPP2C5 in yeast two-hybrid system.

A. SD/-Trp/-Leu; B.SD/-Ade/-His/-Leu/-Trp/X- $\alpha$ -gal.



**Figure S5.** Subcellular localization of GFP constructs of PpCMB1 proteins.

**Table S1.** Primers for cloning and RT-qPCR in this study

Genes	Primer	Sequence (5'-3')	
<b>Overexpression and subcellular localization analysis</b>			
<i>PpSAUR43</i>	PF	ACGAGCTCGGTACCATGGGGTTCCGGTTGCCAG	
	PR	CATGGTGTCTGACTCTAGATACACGTAAACGGGAAGTGAGATC	
<b>Subcellular localization analysis</b>			
<i>PpCMB1</i>	PF	ACGAGCTCGGTACCATGGGAAGAGGTAGAGTTGAGCT	
	PR	CATGGTGTCTGACTCTAGAAAGCATCCACCCAGGAATGAATC	
<b>Bimolecular fluorescence complementation (BIFC)</b>			
<i>PpSAUR43</i>	PF	GGGACTCTAGAGGATCTCGAGATGGGGTTCCGGTTGCCAG	
	PR	ATCGTATGGGTACATGGTACCTACACGTAAACGGGAAGTGAGATC	
<i>PpCMB1</i>	PF	TCCGTCGACCTCGAGGGTACCATGGGAAGAGGTAGAGTTGAGCT	
	PR	CTCCTACCCGGGAGCGGTACCAAGCATCCACCCAGGAATGAATC	
<b>Yeast two-hybrid assay (Y2H)</b>			
<i>PpSAUR43</i>	PF	ATGGCCATGGAGGCCGAATTCATGGGGTTCCGGTTGCCAG	
	PR	CCGCTGCAGGTCGACGGATCCTACACGTAAACGGGAAGTGAGATC	
<i>PpCMB1</i>	PF	GGAGGCCAGTGAATTCATGGGAAGAGGTAGAGTTGAGCT	
	PR	CGAGCTCGATGGATCCAAGCATCCACCCAGGAATGAATC	
<b>Quantitative real-time polymerase chain reaction (RT-qPCR)</b>			<b>Amplicon Length (bp)</b>
<i>PpSAUR43</i>	PF	GCAGTGTATGTTGGGGAGAGCCAG	134
	PR	GTCTTCACTGCAGGGGATGGTG	
<i>PpCMB1</i>	PF	GAGGAGGAAGCTGGAAGAAAC	107
	PR	GGCTTGAGGGTCGGCATAGTTTAC	
<i>PpACO1</i>	PF	TGATGGCACCAGAATGTCAATAGC	183
	PR	CCACTGCTTTCATGGCTTCAAATC	
<i>PpPG</i>	PF	AACCAGATGGCAAGACTGACTCAA	140
	PR	AGCAATCCACTCACCACAATGTTG	
<i>PpACS1</i>	PF	GCAACTTCCACAGTCTCGTCATCC	158
	PR	GGATGAGGAGCAATAGCAAGGC	
<i>PpILR1</i>	PF	TCTTCCAATAGGAGCAGCACT	180
	PR	CATTTGTGCTTTAGTAAGACGG	
<i>PpPIN1</i>	PF	ATGATGGCGGCTGGGAGGAACT	147
	PR	TTGCTGGCCGCCGTGGTAAA	
<i>PpGH3.1</i>	PF	AAGAGCGGCACGTTTGAGGAGTT	91
	PR	CAATGCGGTAAAGATGGGCTAAAA	
<i>PpTUA</i>	PF	TTCTCTCTACTCATTCCCTCCTTG	100
	PR	GATTGGTGTATGTTGGT CTCTCG	

PF= Primer forward, PR= Primer reverse.

Accession numbers: The sequence involved in this article can be found in the Genome Database for Rosaceae (GDR, <http://www.rosaceae.org>). And the sequence accession numbers are as follows: *PpSAUR43* (Prupe.8G079500); *PpCMB1* (Prupe.5G208400); *PpACO1* (Prupe.3G20990); *PpPG* (Prupe.6G202500); *PpACS1* (Prupe.2G176900); *PpILR1* (Prupe.7G100000); *PpPIN1* (Prupe.5G233100); *PpGH3.1* (Prupe.8G137900); *PpTUA* (Prupe.6G004100).

**Table S2.** Result of partial candidate positive clones by yeast two-hybrid (Y2H) library assay in peach

<b>Accession</b>	<b>Encoding protein</b>	<b>Function classification</b>
XM_020568055.1	FT-interacting protein 1	An essential regulator required for FT protein transport
XM_007223496.2	early nodulin-like protein 1	Electron carrier activity, binding of copper ions, associated with anthocyanin synthesis, drought stress
XM_007209877.2	protein ASPARTIC PROTEASE IN GUARD CELL 1	Seed dormancy
XM_007208651.2	U-box domain-containing protein 33	Drought stress
XM_007212858.2	probable transcription repressor OFP9	Transcription
XM_007209443.2	MADS-box protein CMB1	Transcription
XM_020568009.1	sucrose synthase	Sucrose synthesis
XM_007209944.2	transcription factor WER	Transcription
XM_007222413.2	single-stranded DNA-binding protein WHY1	Plant leaf senescence
XM_007208244.2	ETHYLENE INSENSITIVE 3-like 3 protein	Transcription factors in the ethylene signaling
XM_007201010.2	probable protein phosphatase 2C 60	Posttranslational modification
XM_007205078.2	E3 ubiquitin-protein ligase Haka	Posttranslational modification, protein turnover, chaperones
XM_020558191.1	E3 ubiquitin-protein ligase UPL2	Posttranslational modification, protein turnover, chaperones
XM_007204998.2	probable protein phosphatase 2C 6	Posttranslational modification
XM_007201478.2	phytochrome A-associated F-box protein	Signal transduction mechanisms
XM_020562584.1	NAC domain-containing protein 72	Transcription
XM_007201725.2	F-box protein SKP2A	Regulates the proteolysis of cell cycle transcription factors
XM_007220940.2	zinc finger CCCH domain-containing protein 11	Transcription
XM_034366039.1	probable protein phosphatase 2C 5	Posttranslational modification
XM_007225246.2	linoleate 9S-lipoxygenase 5	Function unknown
XM_007226905.2	auxin efflux carrier component 3	Auxin transport
XM_007213751.2	Anthocyanidin reductase	Anthocyanin synthesis
XM_020561985.1	probable WRKY transcription factor 31	Transcription



**Table S3.** Cis-acting regulatory elements were predicted in the promoter regions of *PpSAUR43*, *PpCAMB1* related to fruit development and ripening in peach.

Genes	cis-element	Sequence	Probable function
<i>PpCAMB1</i>	ERE	ATTTTAAA	ethylene responsive element
	AuxRR-core	GGTCCAT	cis-acting regulatory element involved in auxin responsiveness
	TGA-element	AACGAC	auxin-responsive element
	ABRE	ACGTG CACGTG CGCACGTGTC	abscisic acid-responsive element
	CCAAT-box	CAACGG	MYBHv1 binding site
	G-Box	CACGTG CACGTT ACACGTGT CAGACGTGGCA	cis-acting regulatory element involved in light responsiveness
	TGACG-motif CGTCA-motif	TGACG CGTCA	cis-acting regulatory element involved in the MeJA-responsiveness
	GARE-motif P-Box TATC-box	TCTGTTG CCTTTTG TATCCCA	gibberellin-responsive element
	TCA-element	CCATCTTTTT	cis-acting element involved in salicylic acid responsiveness
<i>PpSAUR43</i>	AuxRR-core	GGTCCAT	cis-acting regulatory element involved in auxin responsiveness
	ERE	ATTCATA	ethylene responsive element
	G-box	TACGTG	cis-acting regulatory element involved in light responsiveness