

Supplementary Data

FaMYB44.2 negatively regulates sucrose accumulation in strawberry receptacles through interplay with FaMYB10

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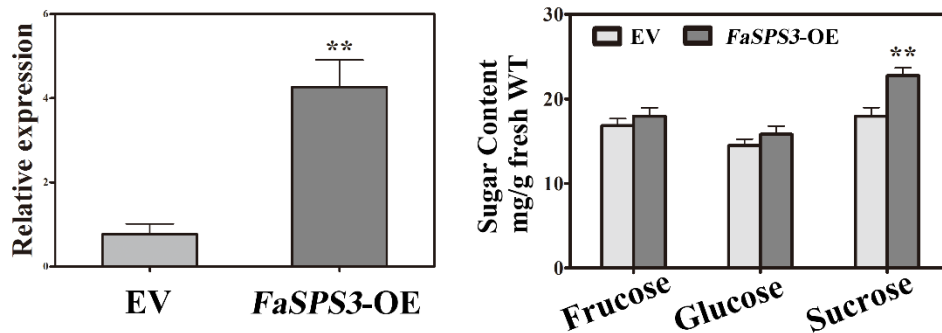
Table S5. Probes used for the EMSA.

Table S6. Sequences and primers used for microRNA expression analysis by qRT-PCR.

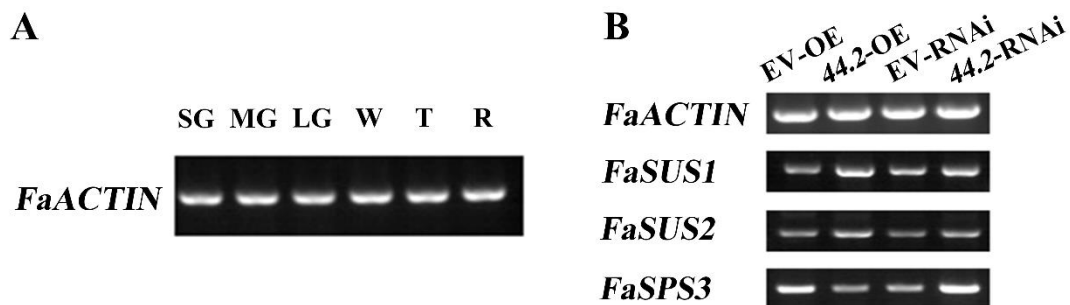
Table S7. Primers used for gene expression analysis by RT-PCR.

Table S8. *FaSUS*, *FaSPS* and *FabHLH33* expression at different developmental and ripening stages according to RNA-sequence data.

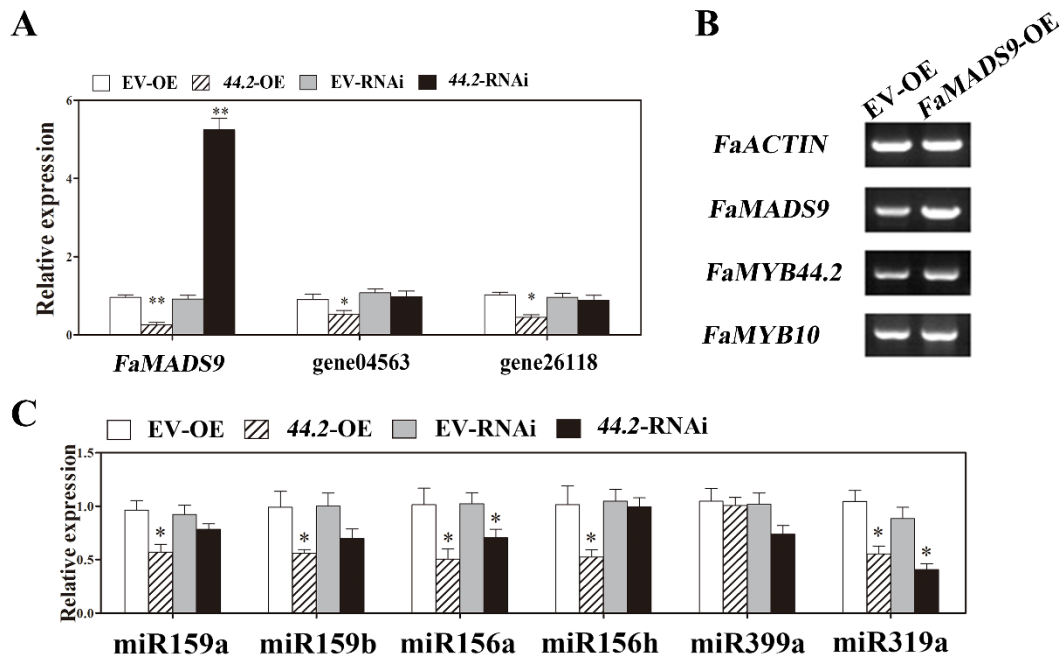
Table S9. Quantitative analysis of *FaMYB10* and *FaMYB44.2* expression levels by qRT-PCR.



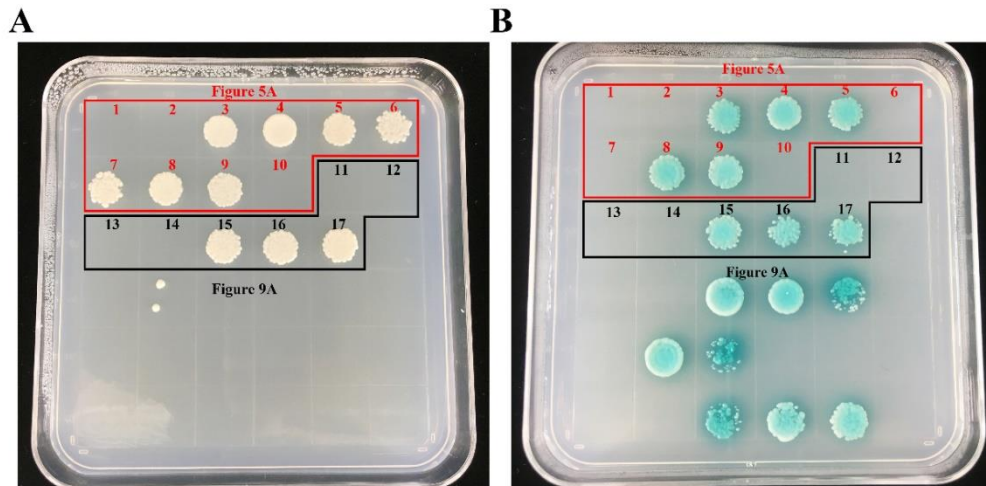
Supplementary Figure S1. The effects of *FaSPS3*-OE on sucrose accumulation in strawberry fruits. *FaSPS3* was overexpressed in white strawberry fruits using pBI121 vector, and the main sugar contents were analyzed by HPLC assay. Values are means \pm SD of three biological replicates and normalized using *FaACTIN* as an internal control. The Δ CT of EV sample was chosen as the calibrator. Statistically significant were tested by Student's t test: *P < 0.05 and **P < 0.01. Thirty fruits were pooled as one sample and three biological replicates were performed.



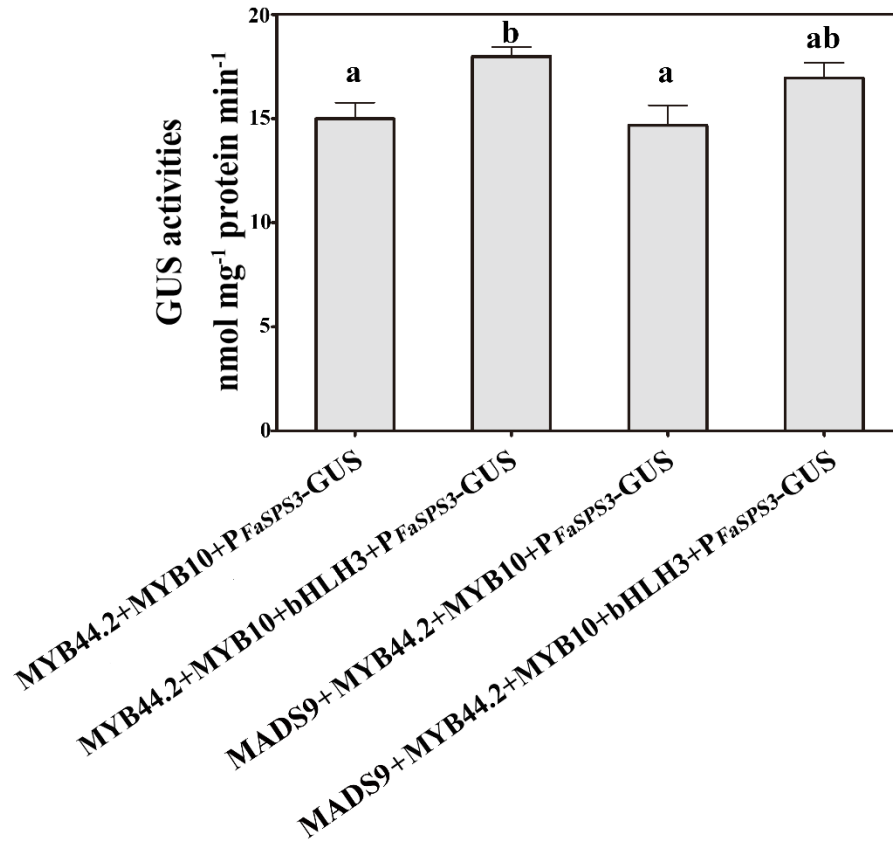
Supplementary Figure S2. Detect the expression levels of *FaACTIN*, *FaSUS* and *FaSPS3* in strawberry fruits. **A**, The expression pattern of *FaACTIN* during strawberry fruit ripening. SG, small green fruit; MG, middle green fruit; LG, large green fruit; W, white fruit; T, turning fruit and R, red fruit. **B**, The expression levels of *FaSUS1*, *FaSUS2* and *FaSPS3* in *FaMYB44.2*-OE and *FaMYB44.2*-RNAi fruits.



Supplementary Figure S3. The expression levels of *miRNA* and *MADS* in transgenic *FaMYB44.2* and *FaMADS9* fruits. **A**, The expression levels of *FaMADS9* and its homologues in *FaMYB44.2*-OE and *FaMYB44.2*-RNAi fruits (Feng, 2015). **B**, *FaMADS9* was overexpressed in white strawberry fruits using pSPYCE vector, and the expression of *FaMADS9*, *FaMYB44.2* and *FaMYB10* was analyzed with RT-PCR. **C**, The expression levels of *miRNA 159*, *miRNA156*, *miRNA399* and *miRNA319* (Liu and Carroll, 2010; Csukasi *et al.*, 2012; Ren and Tang, 2012; Yang *et al.*, 2013; Qian *et al.*, 2017) in *FaMYB44.2*-OE and *FaMYB44.2*-RNAi fruits. The primers for RT-PCR were listed in Table S7.



Supplementary Figure S4. Identify the interaction proteins of FaMYB44.2 and FaMYB10 by yeast two-hybrid assay. A, $-Leu/-Trp/-His$ (SD-3) of Fig. 5A. 1, pGAD+pGBK; 2, pGAD+BD-FaMYB44.2; 3, AD-FaMYB44.1+BD-FaMYB44.2; 4, AD-FaMYB44.2+BD-FaMYB44.2; 5, AD-FaMYB44.3+BD-FaMYB44.2; 6, AD-FabHLH3+BD-FaMYB44.2; 7, AD-FabHLH33+BD-FaMYB44.2; 8, AD-FaTTG1+BD-FaMYB44.2; 9, AD-FaMYB1+BD-FaMYB44.2; 10, AD-FaMYB10+BD-FaMYB44.2. $-Leu/-Trp/-His$ (SD-3) of Fig. 9A. 11, pGAD+pGBK; 12, pGAD+BD-FabHLH3; 13, pGAD+BD-FabHLH33; 14, pGAD+BD-FaTTG1; 15, AD-FaMYB10+BD-FabHLH3; 16, AD-FaMYB10+BD-FabHLH33; 17, AD-FaMYB10+BD-FaTTG1. B, $-Leu/-Trp/-His/-adenine$ (SD-4) of Fig. 5A and Fig. 9A. The panel was in the same order as shown in Fig. S4A.



Supplementary Figure S5. Detecting the effects of FaMDS9 on FaMYB44.2/FaMYB10-mediated P_{FaSPS3} activity.

Table S1. The full-length protein sequences for MYB44 and MYB1 genes. Please see the details in the attached Excel file.

Table S2. Primers used for vector construction. The restriction enzyme sites in the primer sequences are underlined.

Primer name	Enzyme sites	Forward primer (5'-3')	Reverse primer (5'-3')
<i>FaMYB44.2</i> -OE	Gateway adapters	<u>AAAAAGCAGGCT</u> ATGGCTTCTTCTCA ACCAAGA	AGAAAGCTGGGICTACTCCACC TGCTAATCCCAA
<i>FaMYB44.2</i> -RNAi-P	AscI/SwaI	<u>GGCGGCCAT</u> CACCCCTTCTCTCTC AGAACAA	<u>ATTTAAATT</u> ACGCCGGCAGAC TAGAATCGCTC
<i>FaMYB44.2</i> -RNAi-N	PacI/AvrII	<u>TTAATTAAT</u> CACCCCTTCTCTCTCA GAACAAG	<u>CCTAGGGT</u> ACGCCGGCAGACT AGAATCGC
<i>FaSPS3</i> -OE	BamHI/SacI	<u>GGATCCATG</u> CCGAGGGTACCAGAAC	<u>GAGTCCT</u> AGTTCTTGACAACCT CTAATTTCTC
pGEX-4T-1-MYB44.2	EcoRI/XhoI	<u>GAATTCATG</u> GCTTCTTCAACCAAG AAAGC	<u>CTCGAGCT</u> ACTCCACCTTGCTAA TCCCAATC

Y2H-AD-FaMYB44.1	EcoRI/BamHI	<u>GAATTC</u> ATGGCTATGAATCGGAAGGA	<u>GGATCC</u> TCACTCAATTCTGCTGATC
Y2H-AD-FaMYB44.2	NdeI/EcoRI	CATATGATGGCTTCTTCTCAACCAG	<u>GAATTC</u> CTACTCCACCTTGCTAATCC
Y2H-BD-FaMYB44.2	NdeI/EcoRI	CATATGATGGCTTCTTCTCAACCAG	<u>GAATTC</u> CTACTCCACCTTGCTAATCC
Y2H-AD-FaMYB44.3	NdeI/EcoRI	CATATGATGGATTGCGAATCGGTTCAAG CAGA	<u>GAATTC</u> TACGAGAGCTTGGTATACCAAGG
Y2H-AD-FabHLH3	EcoRI/BamHI	<u>GAATTC</u> ATGGGTGAGAAGCTTTGGGT GAAT	<u>GGATCC</u> TTACACGGATGATTTTGAAGAGC
Y2H-BD-FabHLH3	EcoRI/BamHI	<u>GAATTC</u> ATGGGTGAGAAGCTTTGGGT GAAT	<u>GGATCC</u> TTACACGGATGATTTTGAAGAGC
Y2H-AD-FabHLH33	NdeI/EcoRI	CATATGATGCGGATAATTGAAAACCC ATGCT	<u>GAATTC</u> TAACTTACCAGCAATTTTCCAA
Y2H-BD-FabHLH33	NdeI/EcoRI	CATATGATGCGGATAATTGAAAACCC ATGCT	<u>GAATTC</u> TAACTTACCAGCAATTTTCCAA
Y2H-AD-FaTTG1	NdeI/XmaI	CATATGATGGAGAATTCGACCCTCGA ATCCCACC	<u>CCCGGG</u> TCAAACCTTCAAGAGCTGCATCTTGTT
Y2H-BD-FaTTG1	NdeI/XmaI	CATATGATGGAGAATTCGACCCTCGA ATCCCACC	<u>CCCGGG</u> TCAAACCTTCAAGAGCTGCATCTTGTT
Y2H-AD-FaMYB1	NdeI/XmaI	CATATGATGAGGAAGCCCTGCTGCGA GA	<u>CCCGGG</u> TAAAGCAACTTGAGGATCAGCCA
Y2H-AD-FaMYB10	EcoRI/BamHI	<u>GAATTC</u> ATGGAGGGTTATTTCCGGTGTG	<u>GGATCC</u> TACATCGTAGGAGATGTTGACTAGAT
PMDC83-FaMYB44.2	SpeI/AscI	<u>ACTAGTAT</u> GGCTTCTTCTCAACCAAG AAAGC	<u>GGCGGC</u> CTCTCCACCTTGCTAATCCCAATC
SPYCE-FaMYB44.1	SpeI/XhoI	<u>ACTAGTAT</u> GGCTATGAATCGGAAGGA AAT	<u>CTCGAG</u> CTCAATTCTGCTGATCCCAATTC
SPYNE-FaMYB44.2	SpeI/SalI	<u>ACTAGTAT</u> GGCTTCTTCTCAACCAAG AA	<u>GTCGAC</u> CTCCACCTTGCTAATCCCAATCC
SPYCE-FaMYB44.2	SpeI/SalI	<u>ACTAGTAT</u> GGCTTCTTCTCAACCAAG AA	<u>GTCGAC</u> CTCCACCTTGCTAATCCCAATCC
SPYCE-FaMYB44.3	SpeI/XhoI	<u>ACTAGTAT</u> GGATTGCGAATCGGTTCAAG CAGA	<u>CTCGAG</u> CGAGAGCTTGGGTATACCAAGGTGC
SPYNE-FaMYB10	BamHI/SmaI	<u>GGATCC</u> ATGGAGGGTTATTTCCGGTGTG AGA	<u>CCCGGG</u> GAGATGTTGACTAGATCT
SPYCE-FaMYB10	BamHI/SmaI	<u>GGATCC</u> ATGGAGGGTTATTTCCGGTGTG AGA	<u>CCCGGG</u> GAGATGTTGACTAGATCT
SPYCE-FaMYB1	BamHI/SmaI	<u>GGATCC</u> ATGAGGAAGCCCTGCTGCGA GA	<u>CCCGGG</u> GAGCAACTTGAGGATCAGCCATTC
SPYCE-FabHLH3	SpeI/XhoI	<u>ACTAGTAT</u> GGGTGAGAAGCTTTGGGT GAAT	<u>CTCGAG</u> CACGGATGATTTTGAAAGAGCAGC
SPYCE-FabHLH33	BamHI/SmaI	<u>GGATCC</u> ATGCGGATAATTGAAAACCC ATGCT	<u>CCCGGG</u> GACACTTACCAGCAATTTCCAAAGC

SPYCE-FaTTG1	BamHI/SmaI	<u>GGATCCATGGAGAATTCGACCCTCGA</u> ATCC	<u>CCCGGGAACCTTCAAGAGCTGC</u> ATCTTGTT
SPYCE-FaMADS9	BamHI/SmaI	<u>GGATCCATGGGGAGGGGAAGAGTGGA</u> GC	<u>CCCGGGGAGCATCCAACCAGGA</u> ATGAAA
nLUC-FaMYB44.2	SacI/SalI	<u>GAGCTCATGGCTTCTTCTTCAACCAAG</u> A	<u>GTCGACCTCCACCTTGCTAATCC</u> CAATCC
cLUC-FaMYB44.2	Sall/PstI	<u>GTCGACATGGCTTCTTCTTCAACCAAG</u> AAAG	<u>CTGCAGCTACTCCACCTTGCTAA</u> TCCCAATC
cLUC-FabHLH3	KpnI/BamHI	<u>GGTACCATGGGTGAGAAGCTTTGGGT</u> GAAT	<u>GGATCCTTACACGGATGATTTTG</u> AAAGAGC
cLUC-FaTTG1	KpnI/BamHI	<u>GGTACCATGGAGAATTCGACCCTCGA</u> ATC	<u>GGATCCTCAAACCTTCAAGAGC</u> TGCATC

Table S3 Primers used for gene expression analysis by qRT-PCR.

Gene	Full Name	Gene ID	Forward primer (5'-3')	Reverse primer (5'-3')	Note (related ripening parameter)
<i>FaMYB44.1</i>	MYB domain protein 44.1	gene24027	ATCTCCAACCT CGCTGTCC	GCCTGTTCTGAG TCGACTC	---
<i>FaMYB44.2</i>	MYB domain protein 44.2	gene00185	CTCATTCGCAAG CAGCCTTC	GCTGAGTGATGTC GGTGGAT	Sugar and acid accumulation
<i>FaMYB44.3</i>	MYB domain protein 44.3	gene26289	GCGCATTCGAA ATACGGCAA	CATGGACGAGTA CTTCCGCT	---
<i>FaSUS1</i>	Sucrose synthase 1	gene12940	CCCTGATTCTGA CCTTTACTGG	GATGATGAAGTC GGTGTGGTT	Sugar metabolism
<i>FaSUS2</i>	Sucrose synthase 2	gene11077	TTGCTGAGGAT GTTGCAGGT	AACAACGAGGCG ACGAGATT	Sugar metabolism
<i>FaSPS1</i>	Sucrose- phosphate synthase 1	gene11606	GAGCCTTGAAT GTCCCAATGT	ATCTCCTGTCTGG TGCTGGTT	Sugar metabolism
<i>FaSPS2</i>	Sucrose- phosphate synthase 2	gene22863	ACTGTGCAAAG TTGGCGTCTT	GATCATCGCAATC CACGGCTA	Sugar metabolism
<i>FaSPS3</i>	Sucrose- phosphate synthase 3	gene31122	CGTAGATTGGA GTTATGGAGAG C	CGAATGATGTAA GAACCACTGC	Sugar metabolism
<i>FaSUTI</i>	Sucrose transport protein 1	gene26850	GAGTGTTTGTGT TTGGGTTTTG	CGATGGTCCTTTC CAGTGA	Sucrose transporter
<i>FaNI</i>	Neutral invertase	gene05019	CGACCTCGGTG GATAGTCAG	CCACATCACCATT CCCCGTC	Sugar metabolism
<i>FaHXKI</i>	Hexokinase 1	gene11313	AGCATTGGATG GTGGCTTGT	TATGTGCTCGGCG ACATCAT	Sugar metabolism and signaling

<i>FaHXK2</i>	Hexokinase 2	gene08515	GCTGTCACCGA GCTTCTAGG	CAGCCCCTATACC AGACCCA	Sugar metabolism and signaling
<i>FaTPS7</i>	Trehalose phosphate synthase 7	gene29717	CTGCTGTGAGG GATGGGATG	CTTGGGCCCACTG GATTCTG	Sugar metabolism and signaling
<i>FaFRK4</i>	Fructokinase 4	gene21673	GCCTTGCTCTCC TATGACCC	TGACCTTGATCAC GTCTGCC	Sugar metabolism and signaling
<i>FaCS1</i>	Citrate synthase 1	gene00583	ATCCTGATGCC AATCCTGCC	TGGTGCCTCCCA AGAATCC	Citric acid accumulation
<i>FaCS2</i>	Citrate synthase 2	gene05118	CATCCCACTGC CGATGTCTT	GACTCTGATGGTT GGCTGCT	Citric acid accumulation
<i>FaVHA-A</i>	V-type proton ATPase catalytic subunit A	gene05361	AGAGCGAGTTT GGCTACGTC	CATTTCCGACACG AACCAGC	Acid metabolism
<i>FaMDH1</i>	Malate dehydrogenase 1	gene05605	GGTGTCAAGA AAGCCGGGA	CCCAGAACCTCTG CCACAAA	Malic acid metabolism
<i>FaMDH2</i>	Malate dehydrogenase 2	gene11464	TGTGACCACAT CCGGGATTG	AGCTGGTACACCG TATGAGC	Malic acid metabolism
<i>FaGAD</i>	Glutamate decarboxylase	gene09305	GCCTACACTAT GCCAGCCAA	GAGTCTCTCTGCC AAGCTCC	Acid metabolism
<i>FaNADP- ME</i>	NADP- dependent malic enzyme	gene11286	TGGTGACCTTG GTTGTCAGG	GGCAAACATGTTG AGGGACG	Malic acid metabolism
<i>FaAco</i>	Aconitate hydratase	gene23775	CCTTACAGCTT CGCTCTCA	ATGACTTTCCGGA GCCATGG	Acid metabolism
<i>FaVHP</i>	Vacuolar membrane proton pump	gene30771	GTGGGAAGTGC TGCTCTGAA	AGCATAGTCAGG CTTGGCAG	Acid metabolism
<i>FaAMY3</i>	Alpha-amylase 3	gene04016	TAGCTGCGACG TTTGAGTCC	CCCGTGATCCAAC CTCACAT	Starch metabolism
<i>FaBAM1</i>	Beta-amylase 1	gene28316	GGGACATGGTC ATCCCTGG	GTGCTACCCATT CTGGCTT	Starch metabolism
<i>FaFUM1</i>	Fumarate hydratase 1	gene11092	GGACGTACTCA CACGCAAGA	TGCACAAGACTCG CTCGATT	Starch metabolism
<i>FaADSS</i>	Adenylosuccin ate synthase	gene03239	GGTGTGTGGT GCATCTTCC	GGTGAGCACGATC AGAGACC	Starch metabolism
<i>FaQR</i>	Quinone oxidoreductase	gene28406	CACTGACTCTCC CCTACCTACA	ATACACTTCATCC CCCACCTTA	Aroma production
<i>FaEXPI</i>	Expansin 1	gene18424	AGGACGGAGTT GGATTGC	TGAGCGTGAGCGT GAAG	Softness

FaEXP3	Expansin 3	gene04435	TCACTGCCACTA ACTTCTGC	TTATGCCTCTGC TCTCCT	Softness
FaXYLI	Xylosidase 1	gene05164	ATGGAAGCCT ACTTGTGCTG	CTGGTGAATGTT GTTGGTCGT	Softness
FaCHS	Chalcone synthase	gene26825	GCCTTTGTTTGG CTGGCT	CCCAGGAACATCT TTGAGGA	Anthocyanin accumulation
FaCHI	Chalcone-- flavonone isomerase	gene23367	AGCGAAAGCCA TTGAAAAGT	CATTTGGTGATTG TGTGAAGAG	Anthocyanin accumulation
FaPAL	Phenylalanine ammonia-lyase	gene23261	CTTCTGTGGTGC TGTTGATG	AGGGTGGTGCTTC AGTTTATGT	Flavonoid metabolism
FaMADS9	MADS-box transcription factor 9	gene04229	ACGCTGAGGTT GCTCTCATC	AGGACTCTAGCTG ACGCTCA	Ripening-related transcription factor
FaSHP	SHATTERPR OOF-like MADS-box transcription factor	gene24494 gene04563	TGATCCTGAGA GCTCTCCCA GAGAAGGAACG GGTTGCTGA	GCGCTTGCAGAAT GTGACTT ACTCATAGAGCTT GCCACGG	Ripening-related transcription factor
	MADS-box transcription factor	gene26118	GTGCTCCACTTC ATCTGGCA	TGCTGAAAGAAA CCTTGCGAC	
FaMYB1	MYB domain protein 1	gene09407	TAGCTGGAAGA CTGCCTGGA	GGACGAAGAGTA GTGCCTGT	Anthocyanin accumulation
FaMYB10	MYB domain protein 10	gene31413	CAACAGCACCA CCACAGACT	GCTTGCCGATTGT ACCGTAT	Anthocyanin accumulation
FaEOBII	Emission of benzenoid II	gene28435	GCAGATCAGGC AAGCACAAG	GAGGCTGAACCA GAGTTGCT	Aroma production
FaGAMYB	Transcription factor GAMYB	gene00431	ATCCAATGTGAA ACCTGAACCA	CCAGCCAATCTGA ACCAAGTAA	Anthocyanin accumulation
FabHLH3	Basic helix- loop-helix 3	gene08231	CAGGAGTTGTG GAGCTTGGT	GCCTGGACAGAG CTAGGAAC	Ripening-related transcription factor
FabHLH33	Basic helix- loop-helix 33	gene19321	TGACAAGGCAT CGATCCTCG	GTGCTTCCACAGT GTCCATG	Ripening-related transcription factor
FaTTG1	Transparent testa glabra 1	gene12450	GGACTTGAGGT ACATGGCGA	ATTCACACTCCCT CTGTGCC	Ripening-related transcription factor
FaCOII	Coronatine- insensitive protein 1	gene20913	GAGGAGATTGC GGAGTCGTT	CGGAGCACTTGTC CAGCTTA	JA receptor
FaJAZI	Jasmonate- zim-domain protein 1	gene12541	AACCTCGTCGA CATGCAGTT	ATTGAAGCCTCT GGGAGCC	JA signaling

<i>FaJAZ2</i>	Jasmonate-zim-domain protein 2	gene00583	AGACATGCAAC CTCCTCAGC	GATCTCCTGGCC TTCTCCG	JA signaling
<i>FaJAZ8</i>	Jasmonate-zim-domain protein 8	gene30624	ATTGGAGCTCC GCCTTAAT	GAAGATCGTCAAT GGCTGCT	JA signaling
<i>FaMYC2</i>	Transcription factor MYC2	gene10501	CTCTCCGAGCT GTTGTTCCA	TCTGACTCCGTCG TTTGACG	JA signaling
<i>FaOPR3</i>	Oxophytodien oate reductase 3	gene12480	GGAGCCGTTATT TTCTGTCAGC	TCAAAACCTGCTC GAATGGC	JA biosynthesis
<i>FaARF6A</i>	Auxin response factor 6A	gene22728	TGAAGTGCAAC AAATGGGAA	CGGCTTACGTAGT CGTCACA	Auxin signaling
<i>FaARF6B</i>	Auxin response factor 6B	gene30394	TGGCTTGCAGA CAGACATGT	GCAGAAGGGACG TAGGTAGC	Auxin signaling
<i>FaPIN8</i>	Auxin efflux carrier component 8	gene16792	ACCCTCCCACTC TTCACCTT	GCACAACCACGAT GATGAGC	Auxin transporter
<i>FaMRLK47</i>	FERONIA/FE R-like receptor kinase 47	gene13568	GCTTAGGTCTTG GTATGATGA	TAGTTGAGGTTGA TGTGTATTTC	Ripening-related protein kinase
<i>FaPYL1</i>	Pyrabactin resistance like 1	gene10508	GCTTCGTAATGA GCGTGGGA	CTCTCCTCGTCCA GCAAGTC	ABA receptor
<i>FaPYL8</i>	Pyrabactin resistance like 8	gene13100	GGAGGAGGTGC GAGTGAGTA	TGAACAGGCGCTT TGATGTG	ABA receptor
<i>FaPYL9</i>	Pyrabactin resistance like 9	gene19210	ACTGGAGCTTC TGGATGACG	ACCTCAGGATGGA CGGTGAT	ABA receptor
<i>FaSnRK2.6</i>	SNF1-related protein kinase2.6	gene31902	GCTACACTCGC AACCAAAATC	ACCCCAAGAC CAGACATC	Ripening-related protein kinase
<i>FaNCED1</i>	9-cis-epoxycarotenoid dioxygenase 1	gene31335	ACTGCTTCTGCT TCCATCTCT	AGACACTCGTCGC ATTCATT	ABA biosynthesis
<i>FaNCED2</i>	9-cis-epoxycarotenoid dioxygenase 2	gene30616	GACACCTTTTGC TTCCACTTG	GGATTTCGGACAA CACACTCT	ABA biosynthesis

<i>FaNCED3</i>	9-cis- epoxycaroteno id dioxygenase 3	gene24386	CAAACCTATCA CCCTTCTCAC	TGGGCAACTTCTG CTTCTTT	ABA biosynthesis
<i>FaACTIN</i>	Actin protein	gene22626	GCCAACCGTGA GAAGATG	TCCAGAGTCAAG AACAATACCAG	---

Table S4. Primers used to construct the vectors for the GUS activity assay. The enzyme sites in the primer sequences are underlined.

Primer name	Enzyme sites	Forward primer (5'-3')	Reverse primer (5'-3')
PBI121-FaMYB10	XbaI/SacI	<u>TCTAGA</u> ATGGAGGGTTATTTCCGGTGTG	<u>GAGCTC</u> GGATCCTCATACTAGTAG GAGATGTTGACTAGAT
1304- <i>P_{FaSP53}</i>	NcoI/BglII	<u>CCATGGTT</u> AGCGTCTACTCTCCACAGT TTCCTGCC	<u>AGATCT</u> CGTCGGAGCTCGAATTC AGACCAAATC
1304-FaMYB44.2	EcoRI/XhoI	<u>GAATTC</u> ATGGCTTCTTCTCAACCAAG AAAGC	<u>CTCGAG</u> CTACTCCACCTTGCTAA TCCCAATCC
1304-FabHLH3-S	PstI/BamHI	<u>CTGCAGAT</u> GGGTGAGAAGCTTTGGGT GAATGA	<u>GGATCCTT</u> ACACGGATGATTTTG AAAGAGCAG
1304-FabHLH3-D	PstI/SalI	<u>CTGCAGAT</u> GGGTGAGAAGCTTTGGGT GAATGA	<u>GTCGACTT</u> ACACGGATGATTTTG AAAGAGCAG
1304-FaTTG1-S	HindIII/XbaI	<u>AAGCTT</u> ATGGAGAATTCGACCCTCGAA TCC	<u>TCTAGAT</u> CAAACCTTCAAGAGCT GCATC
1304-FaTTG1-D	BamHI/KpnI	<u>GGATCC</u> ATGGAGAATTCGACCCTCGAA TCC	<u>GGTACCT</u> CAAACCTTCAAGAGC TGCATC

Table S5. Probes used for the EMSA. The sequences of cis-elements are underlined.

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')
<i>P_{FaSP53}</i> -MBS	ATTGCATTTTAGTTTT <u>CAACTG</u> TTTTACGT GTTTAGTCAG	CTGACTAAACACGTAAAACAGTTGAAAACCTA AAATGCAAT
<i>P_{FaSP53}</i> -CGTCA-motif	ATCAATTTTCTTTGGAAT <u>GACGAGT</u> GATG GTAAACTAAA	TTTAGTTTACCATACT <u>CGTCA</u> TTCCAAAGA AAATTGAT
<i>P_{FaSP53}</i> -TGA-element	TTAAACTAGGGTGGAAC <u>GACAG</u> AGATA AGGCGTTTACG	CGTAAACGCCTTATCTCT <u>GTCGTT</u> TCCACCCT AGTTTAA
<i>P_{FaCS}</i> -MBS	TGTGGTGAACAT <u>AACTG</u> CTTTCTTAATTGA TTACTTTAAA	TTTAAAGTAATCAATTAAGAAAG <u>CAGTT</u> ATG TTCACCACA
<i>P_{FaSUS1}</i> -HSE+TC-rich	GTGGGTAAGAGAAAATTCATAGTCTTCTG GGAAGAAAATATAGTATATAT	ATATATACTATATTTTCTTCCAGAAAGACTAT <u>GAATTTTCTCTT</u> ACCCAC
<i>P_{FaSUS1}</i> -TC-rich	CCAAGTCTTGCATTTACTCCAATTTCTGTT TCGCGCTTCT	AGAAGCGCGAAAACAGAAAT <u>TGGAGTAAAT</u> GC AAAGACTTGG

P_{FaSUS1}-TATCCAT	TATACAGAAAAGGAGAGAT <u>TCCATGGATG</u> GACATGCAAGA	TCTTGCATGTCCATCCATGGATACTCTCCTTT TCTGTATA
P_{FaHXX2}-G-box	AATAAAAACCA <u>ACGTGTTGATCTCATACT</u> TCATTTTCGTAGATCT <u>CCACGTAATAACTG</u>	CAGTTATT <u>ACGTGGAGATCTACGAAAATGAA</u> GTATGAGATCAAC <u>ACGTTGGTTTTTATT</u>
P_{FaHXX2}-ABRE	TTCGTAGATCTCC <u>ACGTAATAACTGTAACC</u>	GGTTACAGTTATT <u>ACGTGGAGATCTACGAA</u>
P_{FaHXX2}-MBS	TCCACGTAATA <u>ACTGTAACCGTACGTC</u> AA CCA <u>ACTCAGCA</u>	TGCTGAGTTGGTTGACGTACGGTT <u>ACAGTTA</u> TTACGTGGA
P_{FaHXX2}-CGTCA-motif	TAGTAGCTGCGCTTCC <u>GTTCATGCTCCATGG</u> ACCAAT <u>GACGATCGTAACT</u>	AGTTTACGAT <u>CGTCATTGGTCCATGGAGCAT</u> <u>GACGGAAGCGCAGCTACTA</u>
P_{FaHXX2}-TATCCAT	TCACTATGTCAAATGAAACT <u>TATCCATGGG</u> CGGCAACTCGA	TCGAGTTGCCGCCAT <u>TGGATAGTTTCATTG</u> A CATAGTGA
P_{FaMDH1}-MBS	TTCTTGCTTTCTGAAACTGATA <u>ACTGATTC</u> AAATTGTGAT	ATCACAATTTGAAT <u>CAGTTATCAGTTTCAGA</u> AAGCAAGAA
P_{FaMDH1}-TATCCAT	TTTATGTCTGATAACGTGGTT <u>TATCCATGA</u> CATTGTATTT	AAATACAATGTCA <u>TGGATAAACCACGTTATC</u> AGACATAAA

Table S6. Sequences and primers used for microRNA expression analysis by qRT-PCR.

miRNA family	miRNA name	Forward primer (5'-3')	Sequence
miR159	fve-miR159a	ATTGGATTGAAGGGAGCTCTC	AUUGGAUUGAAGGGAGCUCUC
miR159b	fve-miR159b	CGTTGGATTGAAGGGAGCTCTA	UUUGGAUUGAAGGGAGCUCUA
miR828	fve-miR828	GCCTCTTGCTTAAATGAGTATTC CA	UCUUGCUUAAAUGAGUAUUC
miR156	fve-miR156a	GCTTGACAGAAGAGAGTGAGCA C	UUGACAGAAGAGAGUGAGCAC
miR156	fve-miR156h	GCCTGACAGAAGAGAGTGAGCT C	UGACAGAAGAGAGUGAGCUC
miR399	fve-miR399a	TGCCAAAGGAGAGTTGCCCT	UGCCAAAGGAGAGUUGCCCUG
miR319	fve-miR319a	CTTGACTGAAGGGTGCTCCTT	CUUGGACUGAAGGGUGCUCCU
U6		GGGGACATCCGATAAAATT	

Table S7. Primers used for gene expression analysis by RT-PCR.

Gene	Full Name	Gene ID	Forward primer (5'-3')	Reverse primer (5'-3')
<i>FaSUS1</i>	Sucrose synthase 1	gene12940	TCCGATATTCCTCGTGTTC	TGATGTTCTTTACACGGTCCA
<i>FaSUS2</i>	Sucrose synthase 2	gene11077	GAATGATCCATCAAAGCCC	AGACATACTTCCAGAAACCGTA
<i>FaSPS3</i>	Sucrose-phosphate synthase 3	gene31122	CCGCATGGTGGTAATTCCTC	CCCTTTGTCTTTGCTGCGAGA
<i>FaMYB44.2</i>	MYB domain protein 44.2	gene00185	TCAAGCGCAAATTCTCGTCC ATGTCC	GAACTGCTTATCAATCCCCACCTC ACT

<i>FaMADS9</i>	MADS-box transcription factor 9	gene04229	ATGGGGAGGGGAAGAGTGG AGC	GAGCATCCAACCAGGAATGAAA
<i>FaMYB10</i>	MYB domain protein 10	gene31413	ATGGAGGGTATTTCGGTGT G	TCATACGTAGGAGATGTTGACTA GAT
<i>FaACTIN</i>	Actin protein	gene22626	TGGGTTTGCTGGAGATGAT	CAGTTAGGAGAACTGGGTGC

Table S8. *FaSUS*, *FaSPS* and *FabHLH33* expression at different developmental and ripening stages according to RNA-sequence data. The RNA-seq was performed with diploid strawberry (*Fragaria × ananassa* ‘Benihoppe’). RPKM (Reads Per Kilobase Million) value was used to present the gene expression levels, while the changed fold of genes was calculated using the log₂ ratio of RPKM value between two different samples. Gene ID of *FaSUS*s and *FaSPS*s were obtained using the protein sequences of *AtSUS* and *AtSPS* as queries BLAST against the strawberry genome database.

Gene Description		Expression Level (RPKM)			Fold Change (Log ₂ *)	
Gene Name	Gene ID	Green fruit	White fruit	Red fruit	White/Green *	Red/White *
Sucrose synthase 1	gene12940	213.37	146.73	48.83	-0.54	-1.58
Sucrose synthase 2	gene11077	37.68	44.91	74.31	0.25	0.72
Sucrose synthase 3	gene07050	1.75	0.76	0.05	-1.19	-3.89
Sucrose synthase 4	gene11429	5.89	6.14	3.73	0.06	-0.71
Sucrose synthase 5	gene31666	6.64	5.36	0.87	-0.30	-2.62
Sucrose-phosphate synthase 1	gene11606	0.65	0.56	4.68	-0.22	3.06
Sucrose-phosphate synthase 2	gene22863	7.89	14.38	15.23	0.86	0.08
Sucrose-phosphate synthase 3	gene31122	52.34	91.67	156.54	0.81	0.77
Sucrose-phosphate synthase 4	gene06523	1.62	1.43	0.40	-0.17	-1.83
Sucrose-phosphate synthase 5	gene31164	19.03	11.61	9.41	-0.71	-0.30
Sucrose-phosphate synthase 6	gene04429	11.54	12.67	7.65	0.13	-0.72
Sucrose-phosphate synthase 7	gene03841	0.13	0.04	0.17	-1.69	2.04
Sucrose-phosphate synthase 8	gene04408	9.34	10.96	14.37	0.23	0.39

FabHLH33	gene19321	0.10	0.18	0.88	0.75	0.07
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Table S9. Quantitative analysis of *FaMYB10* and *FaMYB44.2* expression levels by qRT-PCR. The $2^{-\Delta\Delta CT}$ method was used to calculate transcript levels (Schmittgen and Livak, 2008). Transcript values were normalized using *FaACTIN* as an internal control. The ΔCT of small green fruit was chosen as the calibrator (Livak & Schmittgen, 2001). The ratio of gene expression levels in different fruit stages between *FaMYB10* and *FaMYB44.2* was calculated by transcript values. Three biological replicates were repeated.

Fruit Stage	Ratio of gene expression levels
Green fruit	<i>FaMYB10</i> : <i>FaMYB44.2</i> = 0.16
White fruit	<i>FaMYB10</i> : <i>FaMYB44.2</i> = 4.67
Turning fruit	<i>FaMYB10</i> : <i>FaMYB44.2</i> = 6.28
Red fruit	<i>FaMYB10</i> : <i>FaMYB44.2</i> = 13.22