Supplemental Figure S1. The sugar components analysis in fruit ripening stage of F2 hybrid progenies in 2018 and 2019 year. The X-axis shows sugar content (mg \cdot g⁻¹ FW, fresh weight).



35.40

B20

C7 AG 10 15 50 55 55 00 00 57 10 15 75



0

Supplemental Figure S2. Phenotypes of fruit development of different lines. Numbers indicate days after full blooms (d). H1-H3 and L1-L3 represent different lines. The fruit images were digitally extracted for comparison. Bars = 3 cm.



Supplemental Figure S3. Evolutionary tree analysis of SWEET III subfamily in *Arabidopsis Thaliana* and apple. Scale bar = 0.2 substitutions/site. (*Arabidopsis thaliana* sequences are available on the https://www.arabidopsis.org/website; login number is as follows: AtSWEET9: AT2G39060.1; AtSWEET10: AT5G50790.1; AtSWEET11: AT3G48740.1; AtSWEET12: AT5G23660.1; AtSWEET13: AT5G50800.1; AtSWEET14: AT4G25010.1; AtSWEET15: AT5G13170.1).



Supplemental Figure S4. RT-qPCR analysis of SWEET III subfamily expression in different sugar content lines. The samples used were fruits that ripened 130 days after full bloom. Error bars represent the \pm SD of three independent biological replicates.



Supplemental Figure S5. Expression levels of *MdSWEET9b/10a/15a/15b* in 30 hybrid progenies. Different lines marked on the X-axis. Error bars represent the \pm SD of three independent biological replicates.



Supplemental Figure S6. Analysis of the relationship between *MdSWEET9b* expression level and the contents of sugar components (sucrose, fructose, glucose and sorbitol) in 30 hybrid progenies. Analyze and draw using Microsoft Excel. All progeny samples were mature fruits. Note: FW means fresh weight.



Supplemental Figure S7. Cell-specific localization of MdSWEET9b transcripts analyzed by *in situ* hybridization. (A). The cross-sections of ripe apple fruits were hybridized with *MdSWEET9b* specific anti-sense probes. (B). The sense probe was used as a control. The vascular bundle and surrounding parenchyma cells were outlined by dotted lines. The location containing the target gene was stained blue-purple. The mRNA signal mainly concentrated in the vascular bundle sieve elements (SE) and its surrounding parenchyma cells (VC), and pulp cells (PC) also showed weak signal. This result was repeated twice independently, which was the same as the result in Figure 3A.



Supplemental Figure S8. Functional validation of MdSWEET9b in yeast mutants EBY.VW4000 lacking hexose transport. Yeast cells with vector as a negative control. The numbers under the panel indicate the dilution fold.



Supplemental Figure S9. Phenotypes of *MdSWEET9b* and *MdWRKY9* transgenic calli. (A). Phenotype of *MdSWEET9b* overexpression calli (OE-S9b-2/4/6) and CRISPR/Cas9 knockdown calli (S9b-Cas9-3/5/7). (B). Phenotype of *MdWRKY9* overexpression calli (W9-OE-2/4/6) and CRISPR/Cas9 knockdown calli (W9-Cas9-1/2/3).



Supplemental Figure S10. Content analysis of sugar components in *MdWRKY9* transgenic calli. Fructose (A), glucose (B) and sorbitol (C) contents in *MdWRKY9* transgenic calli (*MdWRKY9* overexpression calli: W9-OE-2/4/6 and CRISPR/Cas9 knockdown calli: W9-Cas9-1/2/3). FW, fresh weight. Error bars represent the \pm SD of three independent biological replicates. Asterisks indicate statistical significance by SPSS statistical 22 software. (***P* <0.01, **P* <0.05, Student's *t*-test).



Supplemental Figure S11. The expression levels of *MdSWEET9b* and *MdWRKY9* in *MdWRKY9* and *MdSWEET9b* single and co-transferable calli. (A). The expression levels of *MdSWEET9b* gene. (B). The expression levels of *MdWRKY9* gene. Error bars represent the \pm SD of three independent biological replicates. Asterisks indicate statistical significance by SPSS statistical 22 software. (***P* <0.01, *P <0.05, Student's *t*-test).



Supplemental Figure S12. Content analysis of sugar components in *MdWRKY9* and *MdSWEET9b* single and co-transferable calli. The content of Fructose (A), glucose (B) and sorbitol (C) in calli. FW, fresh weight. Error bars represent the \pm SD of three independent biological replicates. Asterisks indicate statistical significance by SPSS statistical 22 software (***P* <0.01, **P* <0.05, Student's *t*-test).



Supplemental Figure S13. The promoter and CDS sequences of *MdWRKY9* in differential lines. (A). Promoter sequencing results of *MdWRKY9* in different lines. (B). CDS sequencing results of *MdWRKY9* in different lines. Sequences were aligned using DNAMAN, the dark region is the target sequence.



Supplemental Figure S14. Images of fruit starch staining treated with ABA and ABA inhibitor FLU. The fruits were dyed in starch potassium iodide solution for 1 min. The fruit images were digitally extracted for comparison. Bars = 2 cm. H1-H3 and L1-L3 represent different lines. The treatment condition: CK: water solution, 0.5 mM ABA: 0.5 mmol·L⁻¹ abscisic acid solution, 50 μ M FLU: 50 μ mol·L⁻¹ fluridone solution.



Supplemental Figure S15. The interaction between ABA signal transduction related proteins (MdSnRK2A/2E/2I, MdbZIP23/46, MdPP2C51/6/56) and MdWRKY9 were verified by Y2H assay.



Supplemental Figure S16. Analysis of the expression level of *MdbZIP23* and *MdbZIP46* in different sugar content lines at development stage. Numbers indicate days after full blooms (d). H1-H3 and L1-L3 represent different lines. Error bars represent the averages of three biological replicates \pm SD. Different letters represent differences in the fruit development process. Significance was defined at *P* < 0.05 (Student's *t*-test).



Supplemental Figure S17. The EMSA experiment was used to detect the binding of MdbZIP23 and MdbZIP46 to G-box1 and G-box2 of *MdWRKY9* promoter. The '+' and '-' indicate the presence and absence of the indicated probe or protein, respectively.



Supplemental Table S1. Correlation analysis of total sugar and sugar components in F_2 hybrid progenies of 2018 and 2019 year. Asterisks indicate statistical significance (**P < 0.01, *P < 0.05, Student's *t*-test).

Year	Phenotype	Total sugar	Fructose	Glucose	Sucrose	Sorbitol
2018	Total sugar	1	.765**	.614**	.859**	.448*
	Fructose	.765**	1	$.055^{*}$	$.374^{*}$.053
	Glucose	.614**	$.055^{*}$	1	.736**	.363*
	Sucrose	.859**	.374*	.736**	1	$.530^{*}$
	Sorbitol	$.448^{*}$.053	.363*	.530*	1
2019	Total sugar	1	.803**	.393*	.813**	.616**
	Fructose	.803**	1	.032	.492*	.391*
	Glucose	.393*	.032	1	.095	.159
	Sucrose	.813**	$.492^{*}$.095	1	$.487^{*}$
	Sorbitol	.616**	.391*	.159	$.487^{*}$	1

Role	Name	primer (5'-3')		
	MdSWEET9a-F	GTGGTTCTTCTACGGACTA		
	MdSWEET9a-R	CTCAACTTCTTCATCTCCATAG		
	MdSWEET9b-F	TGTGGTTCTTCTACGGATT		
	MdSWEET9b-R	AACTTCTTCATCTCCTGAGT		
	MdSWEET10a-F	ACTGGGCTTCTTCTTAGG		
	MdSWEET10a-R	ATGTCACTTGTAATGTCCAC		
	MdSWEET10b-F	TTCAAATGGCGGTTTACG		
	MdSWEET10b-R	GACTCAGTTCTGGACACA		
	MdSWEET11-F	TGTTATGGCTTACTCCTCAA		
	MdSWEET11-R	CTTGTACCTCCACCTCAG		
	MdSWEET14-F	CTTTGGGTTGGTTCAGATG		
	MdSWEET14-R	GCTCAGGAGTAGTAGTCAA		
	MdSWEET15a-F	CTTCAGATGCTGCTGTATG		
	MdSWEET15a-R	CGTCATCATTGCGATTGT		
	MdSWEET15b-F	CTTCAGATGCTGCTGTATG		
	MdSWEET15b-R	GGTGTCATTGTTGCGATT		
	MdZEP-F	TGTGCTATTGACGGAGAG		
	MdZEP-R	GCGAGCGTATTGTTATTGA		
DT «DCD	MdNCED1-F	CTCCGAGAATCTGAACCT		
RI-qPCK	MdNCED1-R	TCGTCACCGTAGATATGC		
	MdNCED2-F	ACGACAAGACCAAGATGT		
	MdNCED2-R	ATCCAATCACAACGACCT		
	MdAAO-F	TCCAGCAAGAGTAAGCAT		
	MdAAO-R	TCCAGCAAGAGTAAGCAT		
	MdCYP707A2-F	CTCATCCTCCTCCATCAC		
	MdCYP707A2-R	AGTTGCCTTCTCGTTTCT		
	MdCYP707A4-F	ATTCTTCTCAGACCCTCAAA		
	MdCYP707A4-R	CAACTTGGCAAGCTCATT		
	MdSRK21-F	TGAGGAGGCTACTATACCA		
	MdSRK21-R	CGCTGCTATCAACATCAAG		
	MdSRK2E-F	AACTTCCGCAAGACAATAC		
	MdSRK2E-R	TTCGTGGTTCCTGATCTC		
	MdSRK2A-F	CTCTCTCGCATCTTTGTTG		
	MdSRK2A-R	GCTTCCTCCACTATCTTCA		
	MdbZIP46-F	TAGCAGTGTTGGTGTGAT		
	MdbZIP46-R	GCTGAGAAGTGTTGTAAGTC		
	MdbZIP23-F	TAATGTTGGTGGAGTGAGAA		
	MdbZIP23-R	AGCGGTGTTAATAGAAGGT		

Supplemental Table S2. Primers used in this study.

	MdPP2C51-F	GGCCGTATGTGATATCAAAACC
	MdPP2C51-R	GTCGACGACAATCACACTAATG
	MdPP2C6-F	CAAGACCCAAATGACTTAACCG
	MdPP2C6-R	TTTCGCAATTTCTAGCTCTTCG
	MdPP2C56-F	AGATCTCACCCGTTCATTTCTT
	MdPP2C56-R	GCATTCTCTCAGCGCAAAATTT
	MdPYL2-F	ATGAGTTCGTTAATTGCGATGG
	MdPYL2-R	GTTCAACTTGATCACAGTGTCC
	MdActin-F	TGACCGAATGAGCAAGGAAATTACT
	MdActin-F	TACTCAGCTTTGGCAATCCACATC
	MdSWEET9b (PRI101-GFP)-F	ttgatacatatgcccgtcgacATGCGGTTCTTGAATACTGAACAA (Sal I)
Location	MdSWEET9b (PRI101-GFP)-R	tcagaattcggtacccccgggTACGTGTTCATCGTTTGCTTCG (Sma I)
and transgene	MdWRKY9 (PRI101-GFP)-F	ttgatacatatgcccgtcgacATGACTTCTTCCTTCACGAACCTC (Sal I)
	MdWRKY9 (PRI101-GFP)-R	tcagaattcggtacccccgggGAACTCAGAAAATCCATAACTTCCTTC (Sma I)
In situ hybridization	MdSWEET9b-sense	AATTAACCCTCACTAAAGGGctcataatgcttagaggagcagcaaa (5'Dig, T3)
	MdSWEET9b-antisense	TAATACGACTCACTATAGGGaagtgcgactttattgttgt (5'Dig, T7)
Transformation of	MdSWEET9b (pYES-DEST2)-F	ACCGGTATGCGGTTCTTGAATACTGAACA (Age I)
yeast mutants	MdSWEET9b (pYES-DEST2)-R	GTTTAAACTACGTGTTCATCGTTTGCTTC (Pem I)
	proSWEET9b (pABAi)-F	gaaaagettgaattcgageteGCATGTTTAAGCCTTATAACATGTTTT (Sac I)
	proSWEET9b (pABAi)-R	agcacatgcctcgaggtcgacCGACACTGCAAAAAGCTTAAGGA (Sal I)
	proSWEET9b (pHIS2)-F	gactcactatagggcgaattcGCATGTTTAAGCCTTATAACATGTTTT (EcoR I)
	proSWEET9b (pHIS2)-R	gattcgcgaacgcgtgagctcCGACACTGCAAAAAGCTTAAGGA (Sac I)
	MdWRKY9 (pGADT7)-F	gtaccagattacgctcatatgATGACTTCTTCCTTCACGAACCTC (Nde I)
	MdWRKY9 (pGADT7)-R	cagetegagetegatggatecGAACTCAGAAAATCCATAACTTCCTTC (BamH I)
	MdSnRK2L (pGADT7)-F	gtaccagattacgctcatatgATGGATCGGCAGGCAATGA (Nde I)
	MdSnRK2L (pGADT7)-R	cagetegagetegatggatecAATTGCGTAGACTATTTCCCCG (BamH I)
	MdSnRK2E (pGADT7)-F	gtaccagattacgctcatatgATGAATCGGTCTGTGCTCACAGT (Nde I)
	MdSnRK2E (pGADT7)-R	cagetegagetegatggatecAATTGCATAGACTATCTCTCCGCTG (BamH I)
V1U and V2U	MdSnRK2A (pGADT7)-F	gtaccagattacgctcatatgATGGAGAAGTACGAGCTTGTTAAGG (Nde I)
1111 and 1211	MdSnRK2A (pGADT7)-R	cagctcgagctcgatggatccGCTGAAGCGCACTTCTCCAC (BamH I)
	MdbZIP46 (pGADT7)-F	gtaccagattacgctcatatgATGGGAAGCAACATGAACTTCAA (Nde I)
	MdbZIP46 (pGADT7)-R	cagctcgagctcgatggatccCCATTCTGCCGCATGAATCT (BamH I)
	MdbZIP23 (pGADT7)-F	gtaccagattacgctcatatgATGGGGTCTAATTTCAACTTCAAGA (Nde I)
	MdbZIP23 (pGADT7)-R	cagctcgagctcgatggatccCCAAGGGCCTGTCAATGTTC (BamH I)
	MdPP2C56 (pGADT7)-F	gtaccagattacgctcatatgATGGACTCCACAGAGGTCGAGC (Nde I)
	MdPP2C56 (pGADT7)-R	cagetegagetegatggatecTAGCCTTTGCTGGCGTTTTC (BamH I)
	MdPP2C51 (pGADT7)-F	gtaccagattacgctcatatgATGAATGAAAAGATGAGACGAAAGAG (Nde I)
	MdPP2C51 (pGADT7)-R	cagetegagetegatggatecGCTGGTTTTTGTGAGGTCGACG (BamH I)
	MdPP2C6 (pGADT7)-F	gtaccagattacgctcatatgATGGAGGATTTAGCTCAGGCAG (Nde I)
	MdPP2C6 (pGADT7)-R	cagetegagetegatggatecGGGTTTTTTCTTAAACTTCCTTGTG (BamH I)

tcagaggaggacctgcatatgATGACTTCTTCCTTCACGAACCTC (Nde I) ccgctgcaggtcgacggatccTCAGAACTCAGAAAATCCATAACTTCC (BamH I) tcagaggaggacctgcatatgATGACTTCTTCCTTCACGAACCTC (Nde I) ccgctgcaggtcgacggatccATGCAAGTAATCGGACTTGGGA (BamH I) tcagaggaggacctgcatatgATGCATTCTACTCAATCTTCGCAA (Nde I) ccgctgcaggtcgacggatccGTTTTGTTCAAACTCATCCTCTCCC (BamH I) tcagaggaggacctgcatatgATGTCTCCAATAAGTAATTCAGGAGGA (Nde I) ccgctgcaggtcgacggatccGCTCCCACGAGCTGCGGG (BamH I) tcagaggaggacctgcatatgATGGCACTCAGAACTCATCCTCCC (BamH I) tcagaggaggacctgcatatgATGGGCAATTACAGCAATGCA (Nde I) ccgctgcaggtcgacggatccTCAGAACTCAGAAAATCCATAACTTCC (BamH I) tcagaggaggacctgcatatgATGTCTACTCAAACTCCATAACTTCC (BamH I) ccgctgcaggtcgacggatccGAACTCAGAAAATCCATAACTTCC (BamH I)

cgctctagaactagtggatccATGACTTCTTCCTTCACGAACCTC (BamH I) gtcgacggtatcgataagcttGAACTCAGAAAATCCATAACTTCCTTC (Hind III) cgctctagaactagtggatccATGGGGTCTAATTTCAACTTCAAGA (BamH I) gtcgacggtatcgataagcttCCAAGGGCCTGTCAATGTTC (Hind III) cgctctagaactagtggatccATGGGAAGCAACATGAACTTCAA (BamH I) gtcgacggtatcgataagcttCCATTCTGCCGCATGAATCT (Hind III) gggccccccctcgaggtcgacTTCCAATCAATTAAGTGAACCACAT (Sal I) cgctctagaactagtggatccTCGTTGGATCGGATGAATTGA (BamH I) gggccccccctcgaggtcgacCATACGAAACATCTTTAAGTATATCCAAA (Sal I) cgctctagaactagtggatccGTTTTTGGCTTCTGTGAGAGAGAA (BamH I) gccatggctgatatcggatccATGACTTCTTCCTTCACGAACCTC (BamH I) ttgtcgacggagctcgaattcGAACTCAGAAAATCCATAACTTCCTTC (EcoR I) gatctggttccgcgtggatccATGGGAAGCAACATGAACTTCAA (BamH I) ctcgagtcgacccgggaattcCCATTCTGCCGCATGAATCT (EcoR I) gatctggttccgcgtggatccATGGGGTCTAATTTCAACTTCAAGA (BamH I) ctcgagtcgacccgggaattcCCAAGGGCCTGTCAATGTTC (EcoR I) ATAAGTACAAATTTCGACTTTTGACCTAATTTGCGATGTCATTTAA (3`Biotin) TTAAATGACATCGCAAATTAGGTCAAAAGTCGAAATTTGTACTTAT (3`Biotin) ATAAGTACAAATTTCGACTTTGACCTAATTTGCGATGTCATTTAA TTAAATGACATCGCAAATTAGGTCAAAAGTCGAAATTTGTACTTAT ATAAGTACAAATTTCGACTTCTGAACTAATTTGCGATGTCATTTAA(3`Biotin) TTAAATGACATCGCAAATTAGTTCAGAAGTCGAAATTTGTACTTAT(3`Biotin) CCGCCACCTACCATTACGTCTACGTGGGCACTCTCCGCAGTCAAGT (3`Biotin) ACTTGACTGCGGAGAGTGCCCACGTAGACGTAATGGTAGGTGGCGG (3`Biotin) ATCTTATTTGAAAAAGGAGTCACGTGTGTTATTCAGCTAACGAAGT (3`Biotin) ACTTCGTTAGCTGAATAACACACGTGACTCCTTTTTCAAATAAGAT (3`Biotin)

ATCTTATTTGAAAAAGGAGT<mark>CACGTG</mark>TGTTATTCAGCTAACGAAGT ACTTCGTTAGCTGAATAACA<mark>CACGTG</mark>ACTCCTTTTTCAAATAAGAT

MdWRKY9-FL (pGBKT7)-F MdWRKY9-Fl (pGBKT7)-F MdWRKY9-Fl (pGBKT7)-F MdWRKY9-F2 (pGBKT7)-F MdWRKY9-F2 (pGBKT7)-F MdWRKY9-F3 (pGBKT7)-F MdWRKY9-F3 (pGBKT7)-F MdWRKY9-F4 (pGBKT7)-F MdWRKY9-F4 (pGBKT7)-F MdWRKY9-F5 (pGBKT7)-F

MdWRKY9 (pGreenII62-SK)-F MdWRKY9 (pGreenII62-SK)-R MdbZIP23 (pGreenII62-SK)-F MdbZIP23 (pGreenII62-SK)-R MdbZIP46 (pGreenII62-SK)-F MdbZIP46 (pGreenII62-SK)-R proMdSWEET9b (pGreenII0800-Luc)-F proMdWRKY9 (pGreenII0800-Luc)-F proMdWRKY9 (pGreenII0800-Luc)-R

MdWRKY9 (PET32a)-F

MdWRKY9 (PET32a)-R MdbZIP46 (PGEX4T-1)-F MdbZIP46 (PGEX4T-1)-R MdbZIP23 (PGEX4T-1)-F MdbZIP23 (PGEX4T-1)-R MdSWEET9b-W-box (hot probe)-F MdSWEET9b-W-box (hot probe)-R MdSWEET9b-W-box (cold probe)-F MdSWEET9b-W-box (cold probe)-R MdSWEET9b-W-box (mutant probe)-F MdSWEET9b-W-box (mutant probe)-R MdWRKY9-G-box1 (hot probe)-F MdWRKY9-G-box1 (hot probe)-R MdWRKY9-G-box2 (hot probe)-F MdWRKY9-G-box2 (hot probe)-R MdWRKY9-G-box2 (cold probe)-F MdWRKY9-G-box2 (cold probe)-R

LUC

EMSA

ATCTTATTTGAAAAAGGAGTTACATGTGTTATTCAGCTAACGAAGT (3'Biotin)

MdWRKY9-G-box2 (mutant probe)-F MdWRKY9-G-box2 (mutant probe)-R

ACTTCGTTAGCTGAATAACACATGTAACTCCTTTTTCAAATAAGAT (3°Biotin)

CHID DCD	proMdSWEET9b (Contains W-box site)-F	TTCCAATCAATTAAGTGAACCAC		
Chip-PCR	proMdSWEET9b (Contains W-box site)-R	GTTTTGCCAATTTAAGAAATCA		
	MdWRKY9 (pUC-SPYCE)-F	ggatccATGACTTCTTCCTTCACGAACCTC (BamH I)		
	MdWRKY9 (pUC-SPYCE)-R	gtcgacGAACTCAGAAAATCCATAACTTCCTTC (Sal I)		
PUPC	MdbZIP23 (pUC-SPYNE)-F	ggatccATGGGGTCTAATTTCAACTTCAAGA (BamH I)		
BIFC	MdbZIP2 3(pUC-SPYNE)-R	gtcgacCCAAGGGCCTGTCAATGTTC (Sal I)		
	MdbZIP46 (pUC-SPYNE)-F	ggatccATGGGAAGCAACATGAACTTCAA (BamH I)		
	MdbZIP46 (pUC-SPYNE)-R	gtcgacCCATTCTGCCGCATGAATCT (Sal I)		
	MdSWEET9b-DT1-BsF	ATATATGGTCTCGATTGgccaccatgtggttcttctaGTT		
	MdSWEET9b-DT1-F0	TGgccaccatgtggttcttctaGTTTTAGAGCTAGAAATAGC		
	MdSWEET9b-DT2-R0	AACgcgcaccaactttggacgatCAATCTCTTAGTCGACTCTAC		
Used for	MdSWEET9b-DT2-BsR	ATTATTGGTCTCGAAACgcgcaccaactttggacgatCAA		
CRISPR/ Cas9	MdWRKY9-DT1-BsF	ATATATGGTCTCGATTGgtatgggcagaaacaagtgaGTT		
	MdWRKY9-DT1-F0	TGgtatgggcagaaacaagtgaGTTTTAGAGCTAGAAATAGC		
	MdWRKY9-DT2-R0	AACgatggactcaactttcggatCAATCTCTTAGTCGACTCTAC		
	MdWRKY9-DT2-BsR	ATTATTGGTCTCGAAACgatggactcaactttcggatCAA		

Gene ID	Annotated Function
XM_029107854	F-box protein
XM_008355635	glutamine synthetase cytosolic isozyme
XM_008378539	acyl-CoA-binding domain-containing protein 1-like
XM_008341717	hypersensitive-induced response protein 2
XM_008364737	NADP-dependent malic enzyme
XM_008352628	ATP synthase subunit beta
XM_029099839	ABC transporter G family member 3
XM_029093882	thioredoxin reductase NTRB-like
XM_008395385	(S)-coclaurine N-methyltransferase
XM_029087627	pyrophosphatefructose 6-phosphate 1-phosphotransferase subunit beta
XM_008364900	NAD(P)H dehydrogenase (quinone) FQR1-like 2
NM_001294127	WRKY transcription factor 9
XM_008343854	LOB domain-containing protein 41
XM_008391704	pyruvate dehydrogenase E1 component subunit beta-1

Supplemental Table S3. List of genes identified from yeast one-hybrid screen using *MdSWEET9b* promoter as bait.