

Supplementary information

A tomato MADS-box protein, SICMB1, regulates ethylene biosynthesis and carotenoid accumulation during fruit ripening

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Supplementary Table S1. Specific primer sequences used for *SICMB1* gene amplification and cloning procedures.

Table S1. Details of primers for qPCR amplification

Primer Name	Primer Sequence (5'-3')	Application
<i>SICMB1-i</i>	CGGGGTACCAAGCTTACTCTGTAGCACATTGGCATATCAC	To establish <i>SICMB1</i> RNAi lines; add <i>KpnI</i> +
	CCGCTCGAGTCTAGAAAGATTAGTATGAATATCACGCACAAGTC	<i>Hind</i> III and <i>Xho</i> I + <i>Xba</i> I site underlined, respectively
<i>SICMB1</i> -Full	TACATAGCCTTTTTTCTTTTCCT TGAATATCACGCACAAGTCG	Full-length amplification of
<i>NPT II</i>	CTCAGAAGAAGCTCGTCAAGAAGG GACTGGGCACAACAGACAATC	Positive transgenic plants
<i>SICMB1</i> (Y2H)	CCGGAATTCATGGGAAGAGGTAAGGTAGAATTGA CGCGGATCCTCAGATTCTGAATTCGCCCCT	
<i>RIN</i> (Y2H)	CCGGAATTCATGGGTAGAGGGAAAGTAGA CGCGGATCCTCATAGATGTTTATTCAT	
<i>SIMADS1</i> (Y2H)	CCGGAATTCATGGGAAGAGGAAGAGTTG CGCGGATCCTTAAAGCATCCATCCATGAATA	Construction of yeast two -hybrid vector
<i>AP2a</i> (Y2H)	CCGGAATTCATGTGGAATTTAAATGATTCCCC CGCGGATCCTCAAGGTCTCATAAAATAATGATGGA	
<i>TAGL1</i> (Y2H)	CCGGAATTCATGGTTTTTCTATTAATCAGG CGCGGATCCTCAGACAAGCTGGAGAGGAG	

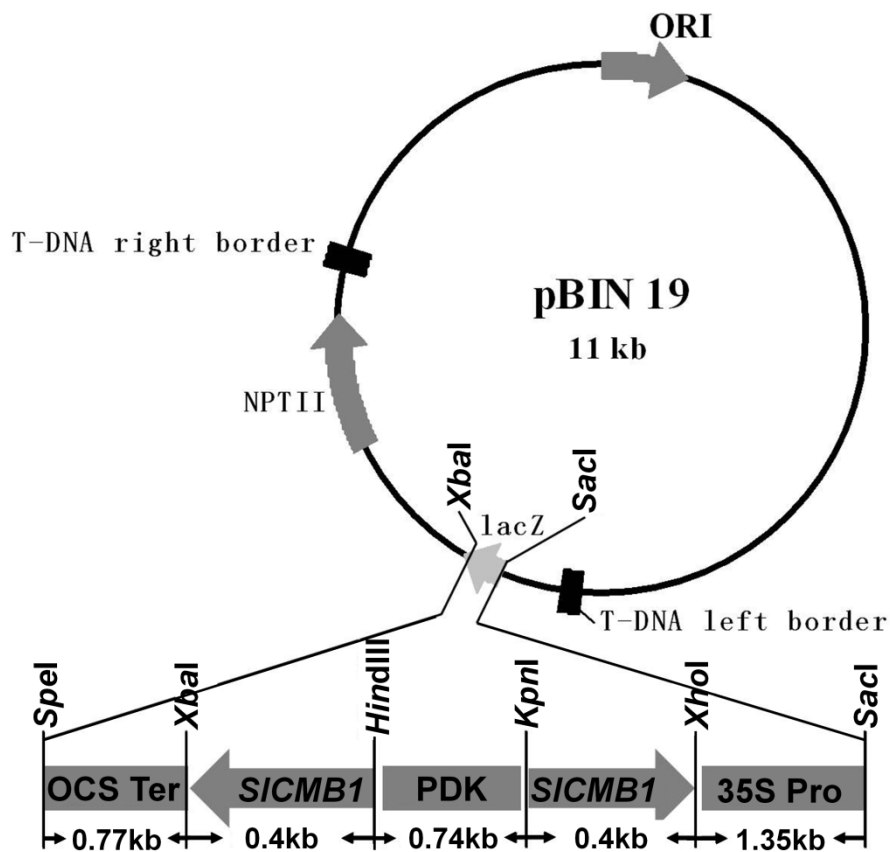
Supplementary Table S2. Specific primer sequences used for qRT-PCR analysis

Primer Name	Primer Sequence (5'–3')	Product (bp)
<i>SICAC</i>	CCTCCGTTGTGATGTAACCTGG ATTGGTGAAAAGTAACATCATCG	173 bp
<i>q-SICMB1</i>	TGAGCGTCAACTGGATTCATCTT CCCTCTGACTGAGCAGGTTGTT	213 bp
<i>ACO1</i>	ACAAACAGACGGGACACGAA CTCTTTGGCTTGAAACTTGA	181 bp
<i>ACO3</i>	CAAGCAAGTTTATCCGAAAT CATTAGCTTCCATAGCCTTC	113 bp
<i>ACS2</i>	GAAAGAGTTGTTATGGCTGGTG GCTGGGTAGTATGGTGAAGGT	107 bp
<i>ACS4</i>	GCTCGGAGGTAGGATGGTTTC GTCCCTCTCCATTGTGCTTGT	151 bp
<i>E4</i>	AGGGTAACAACAGCAGTAGCA CCCAACCTCCGTCTTCAC	167 bp
<i>E8</i>	GGCACCATTCAACATACCG CTTTCACCGAAGAAGCACG	242 bp
<i>ERF1</i>	TTTTAGTATCGGATGGACG GGCGGAGAAACAGAAGTA	102 bp
<i>PSYI</i>	AGAGGTGGTGGAAAGCAA TCTCGGGAGTCATTAGCAT	298 bp
<i>PDS</i>	GCTTTACCCGCTCCTTTA ACCTTGCTTTCTCATCCA	174 bp
<i>LCYB</i>	TTGACTTAGAACCTCGTTATTGG AACAGTTCCCTTTGTCAATTATCTC	137 bp
<i>LCYE</i>	GCCACAGGTTATTCAAGTCGTCA CCAGTCCAAATAGGAAAAACGAT	196 bp
<i>CYCB</i>	CGACGTGATCATTATCGGAGC GTGGTGAAGGGTCAACACAACA	98 bp
<i>RIN</i>	CATCATGGCATTGTGGTGAGC AATTCAAAGCATCCATCCAGGTAC	194 bp
<i>TAGL1</i>	CGCAATAACTCCCTGCCTGTA GAAGATGAAGAGCCTTGACCC	143 bp
<i>FUL1</i>	AAAATCAGTGGGAAATCAACTCATC CCTTGCTGCTGTGAAGAACTACC	139 bp
<i>FUL2</i>	CCGTGGGAGCAACAGAGTCAT GGAGGCATCACAGAAGCACTG	167 bp
<i>LoxC</i>	TTTACTCCGCCCTACACGC CCTGAAAGATCGACACCCA	121 bp
<i>PE</i>	GCTTGCGTCTTTGACAACTCAGG GTGCCACCACTGCATTGCTAT	137 bp

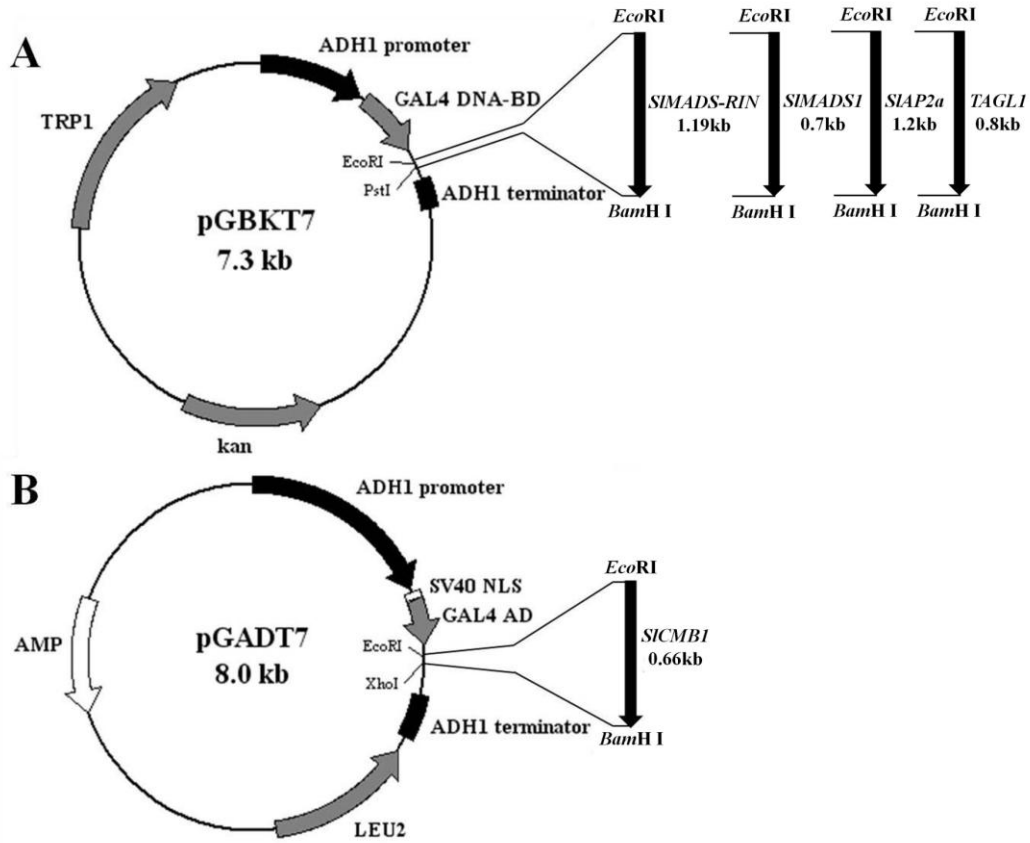
Supplementary Figures

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-3500 AAAIATTAAGTTCATATGTTAGTGAGAATGAATGATAAGAATGGTATTATTAGGAATTTTGACAAAATCCATCATGTAAACATTTTATATTAATATCAATT
-3391 ACAAGGAAGAAITCTTGCCATATAATTAGTGTGGTCTTACTTTGTATTGGGAGAGACAAAATAATGTAAGAAAAGATGAACAACCTTATATTGAGGTAGCGTAGT
-3286 TAAAGAGAGAGTTGAGATAAAGAAAAGTATTTTAAAGTCTTAACTATGTATATTGCAATATAAAAAGAGAGTAATTATATATAAAGAAGGTATCTGTTTGGGAT
-3179 CTTTGGACTCTTAACTAAATTCGAAGTTGTTGAGTTGTACAACGTTGTAGGTTATTGTATTCGGAAGGGAACAAGTCAAGAGTTATACCGCTACATCGGTATA
-3073 GATATGTCGCAAAGTGAGCTTAAATCTTTAAAGTGAGCGAGATATCCGCGCTAAGTCTAATAATTTGTTIATCAITTTTGTCAITTTTAACTGTAATAT
-2965 ATTATATTGTTGATATAGTACTAACAGATCTTGCTATGATATCATGTAAATTAGATCTTTTATCGAACTATAACCTCAAATGCTAGGTCAAATAAAAAGAATTA
-2857 ATGCTAAAGCTTATAAAGAAGTCCAACGATCTTATTACTCTATTGGTTGAGTAGGGTGATAAGGACCCTAATTAITGGATCTTCATGGATTACAATAATAG
-2750 TCAAGCAAAGCAAAAAGAAAACATACACAATTAAGTGAAGGGGAGAAAGATTTTTTTTTGGTTTCTCACTCGGTGCCAAAATCATATTGAAGTCTCGACT
-2646 AATTCGGAAGAGCTCTGAAGGGCTCAITTTGGGGGGGGTCTTCCAACCATGATTTCTCCATACCCAAGACTTGAACCTCAAGACCTCTGATTAACCATAAA
-2542 CCAITGTGATAGAAGAGAGTTTATTTAAAAATGAATCATTATATATTGCTTAGTTTTTATTTTGTATGATGAAGAAAAGTAGAAGGAAACCATATTATCTT
-2434 TATTAACCACATATATTTTTCATTATATAAAATCTTTTTCATTATATAAAATAATTTTCAATTTTGGAAATAAATATTAGTCTGTGAAGAGTACCATATAT
-2323 ACAATGCAAAAAGAACTTTATCTAATATGTCATTTCTTATTCAAGATGTAATTGTAGATTTCGACCAAAAAGAGATAAGAGAATAGATAAATCCAATATA
-2217 CTTAGCTCCATCTTTTAGCAGAGATATACCCATCTCCA AAAAGATGGCAATATGTTTACCATAAATAAATAAATATATATAGGTCAATTTGATGGAATG
-2109 TATATAAGACTATGAAAATTAATATAAAATAGGCATTATCTAATTTTATAITTTTATTTTATGAAATGAGTGTACTAATTGATATTATGAATTGTGATATTCAAC
-1999 TTAGGTATTGAAATCAATCCCATACACTATGTCTTTTATCTTTTGAATCAATTTTCTCTCATTAATATTGTTGTAATCAAGATTGATTAATACGTTAACGT
-1889 ATTTAGTTTATGTGATTACAACTTGAITTTAAATATTGAAAGTGAATATTGTTGATATGATGGAGTAACATGATGACACATCTTAGGTATCTAAT
-1780 ATCAAAATATCCAACCTATGTAACAGAAACATGTGGTATGTCCTCAA AAAAGATACATCAAGGGGGAGTGTGCTATGCTTGTTTAAGGAAAAACAACAACATTA
-1676 AGGTGTTATACATTTTATAGCACCAAAAATCTTGTAAAACCTTGATTGCTAAATACTTTTCGACTTGAATATATGTTGACAGTGAAAAAGATATTCACG
-1569 ATCGAATATTGTTTCAGTAATTTATGATGAATTTAAATGATAGTGTAGTAAGATATACTTACCTATACTATATTTGATGATGAAAAGATATTATAATGCTAGT
-1459 ATGATGTAATTTGTGAAAAGTCCGCTCGATGGTTAAGAATTGGTATATTTTAAATATGATCTGAACAATTTTTTCTTATGAGTTCGCTCTTACATGATATT
-1350 AATACACGATTCATCCATCTTGCTTTTGTGATGTAGATCTTCAATAAAATATCCACGCATATATGCCAGTCATAGCTAGAAAAATATGATCTCTTTAA
-1242 TATAATTAGATATTTTTTTCGTCAATTAACCTTTTGAATTTTATAGATATGATATTCCAATATAAGGGCTAATAATGATCTCCATATAAAGGCTTTTCAAAC
-1132 ACTCCCAATCCAGTAAGGGATACA AATACCTACAACCTTTAATATATGCTTACAATTTCCATTAATAATGGAATGTTGGGAAAACCTTTTATTTTAGTAGTTT
-1025 ATAAAATAATGAGAATTGGAACTTTTAAATAAATGTAATTACAACCGTTTCTGATACTTTATTTACGAATCGTATTACTCATTTAAAAAATGATATAAAATAT
-917 ATAAATAAATTTTCAATAATTTAATAGTTTCAACAATATGTTGTTTCCAATAAATAATATTTTTTATAATCTAATTACTATTTAAATAAATTTAATTTTG
-806 ATTCCAATCAATCTAACTTCAAAATCTTTAACTTATGCTTCCAATTAGTACTACACAACCTTATACCTAAAAAATTTCAAACTTATGCTCTCTTTAATTA
-698 TTTTGAATTTGATACAAAATAATACCACCCAAAATAGGGGCAAAAACCTTGATATTAAGGGTTAGTTGGTGAGAAACCAAAAATAATAATGTTAATAGTATT
-592 GACTGTCTCTTTTTTTGGCCCAAACATCAAAACACATTTTACTTATATTAATTATAGAGAGACAAGGGACACAGGCAGAGAAATAACA AAAAGCAGTTTG
-487 CAGAATTACCAAAAATGCAATATTTAAAAGGGTCTAAAAGTAATATTAATAAGGATCATTGATCGTGAATAAATTTTCAAGAATTAATTAATCTCAAATAAG
-380 TTATTTTATTTGCTATACATATGAGATAACATATCCGTTATTAATAATAAATGGGGTAGGAGAAATAATCTAAGAATAACTACCTAAGCTTCAACTAACAC
-272 ACGATAAATTTATCTGAAATTTATATCTTATACGTGATAGATAAGAAAATAAGATACAATGTTGTAATATTAGGAAACCCCATGGCCCAACAAGCTATTAT
-164 TATTACCCTAAGAAACAAAAGGACAGAAAAAGAACCAAAACCATACTACTATCAATATCTTATATACATAGCCTTTTTTCTTTCTTTTATCAAAACATGA
-58 CTAGATTTGAGATTATAATATAAGATTTTGATACTATATTTATAATAATATTATAATGGGAAGAGGTAAGGTAGAATTGAAGAGAATAGAAAATAA
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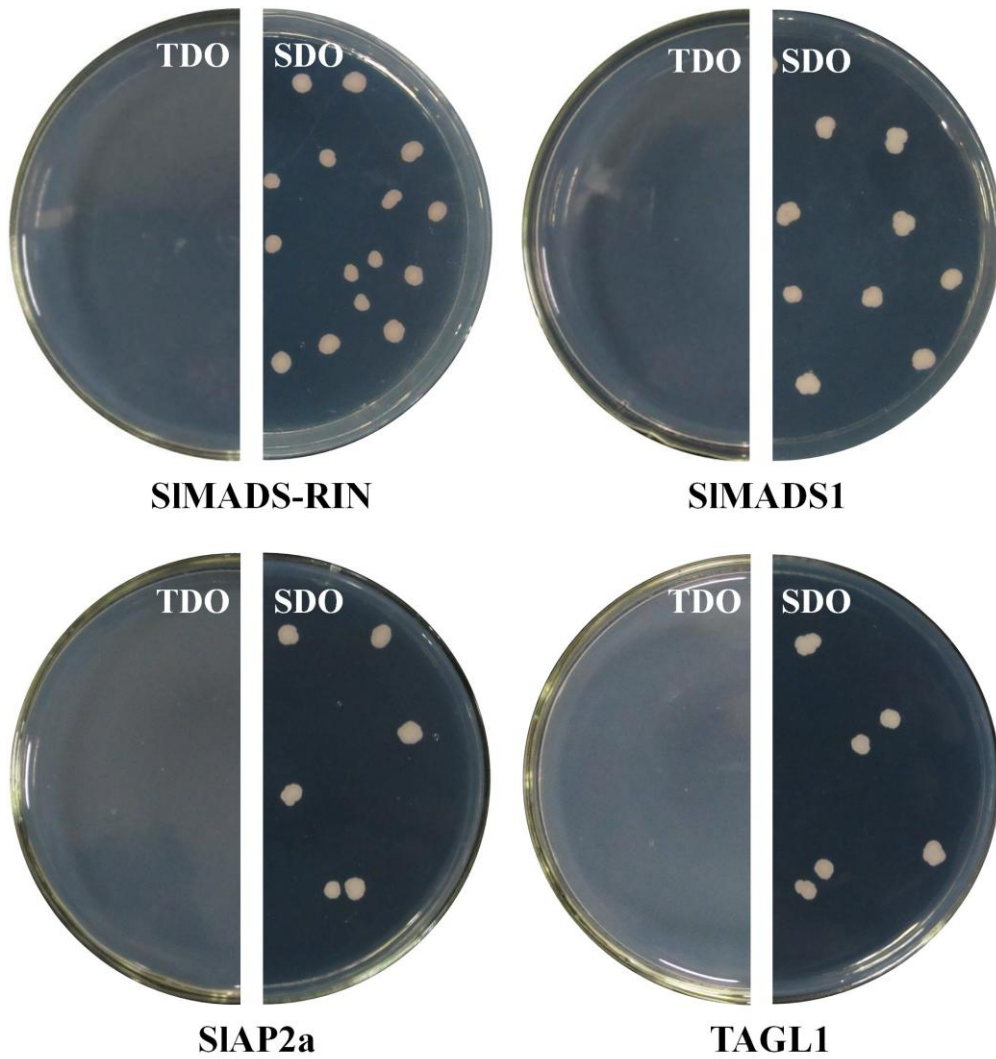
Supplementary Fig. S1. Promoter analysis of *SICMB1* gene. Promoter sequence (3500 bp regions upstream the 5' end of the predicted ORF) of *SICMB1* gene was extracted from SGN database and searched against the promoter database plant CARE (<http://bioinformatics.Psb.ugent.be/webtools/plantcare/html/>), ERE motif (ATTTCAAA) are the ethylene-responsive element in *SICMB1* promoter region.



Supplementary Fig. S2. Hairpin construct of the *SICMB1* gene for double-stranded RNAi vector. The *SICMB1* gene-specific sequence in the antisense and sense orientations were linked with a PDK gene fragment and as a transcriptional unit for hairpin RNA expression which promoted by the CaMV 35S promoter and terminated by the OCS terminator. Among which, *SpeI* and *XbaI* are isocaudamers.



Supplementary Fig. S4. Construct of SICMB1 and RIN gene for yeast two-hybrid vector. (A) The ORFs of *SIMADS-RIN*, *SIMADS1*, *SIAP2a*, *TAGL1* were cloned into pGBKT7 bait vector to obtain the vector pGBKT7-*SIMADS-RIN*, pGBKT7-*SIMADS1*, pGBKT7-*AP2a* and pGBKT7-*TAGL1*, respectively. (B) The ORF of *SICMB1* was cloned into pGADT7 prey vector to obtain the vector pGADT7-*SICMB1*.



Supplementary Fig. S5. Yeast two-hybrid assay for SICMB1 and SIMADS-RIN, SIMADS1, SIAP2a and TAGL1. TDO, SD medium without Trp, His, Ade (autoactivation assay); SDO, SD medium without Trp (autoactivation assay).