Supplementary information

A tomato MADS-box protein, SlCMB1, regulates ethylene biosynthesis and carotenoid accumulation during fruit ripening

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Supplementary Table S1. Specific primer sequences used for *SlCMB1* gene amplification and cloning procedures.

Table S1. Details of primers for qPCR amplification

Primer Name	Primer Sequence (5'–3')	Application
SlCMB1-i	CGGGGTACCAAGCTTACTCTGTAGCACATTGGCATATCAC	To establish *SlCMB1* RNAi lines; add *Kpn*I+ *Hind* III and *Xho*I +*Xba*I site underlined, respectively
	CCGCTCGAGTCTAGAAGATTAGTATGAATATCACGCACAAGTC	
SlCMB1-Full	TACATAGCCTTTTTTCTTTTCCT	Full-length amplification of Positive transgenic plants
	TGAATATCACGCACAAGTCG	
NPT II	CTCAGAAGAACTCGTCAAGAAGG	
	GACTGGGCACAACAGACAATC	
SlCMB1(Y2H)	CCGGAATTCATGGGAAGAGGTAAGGTAGAATTGA	Construction of yeast two -hybrid vector
	CGCGGATCCTCAGATTCTGAATTCGCCCCT	
RIN(Y2H)	CCGGAATTCATGGGTAGAGGGAAAGTAGA	
	CGCGGATCCTCATAGATGTTTATTCAT	
SlMADS1(Y2H)	CGCGGATCCTTAAAGCATCCATCCATGAATA	
	CCGGAATTCATGGGAAGAGGAAGAGTTG	
AP2a(Y2H)	CGCGGATCCTTAAAGCATCCATCCATGAATA	
	CCGGAATTCATGTGGAATTTAAATGATTCCCC	
TAGL1(Y2H)	CGCGGATCCTCAAGGTCTCATAAAATAATGATGGA	
	CCGGAATTCATGGTTTTTCCTATTAATCAGG	
	CGCGGATCCTCAGACAAGCTGGAGAGGAG	

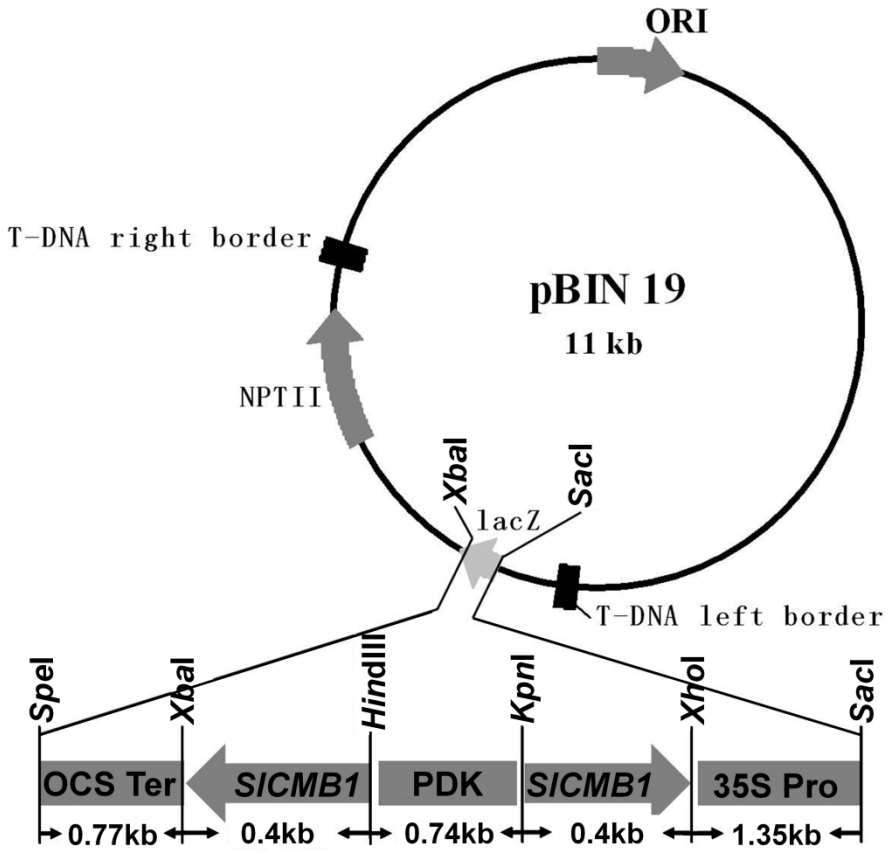
Supplementary Table S2. Specific primer sequences used for qRT-PCR analysis

Table S2. Details of primers for qPCR amplification

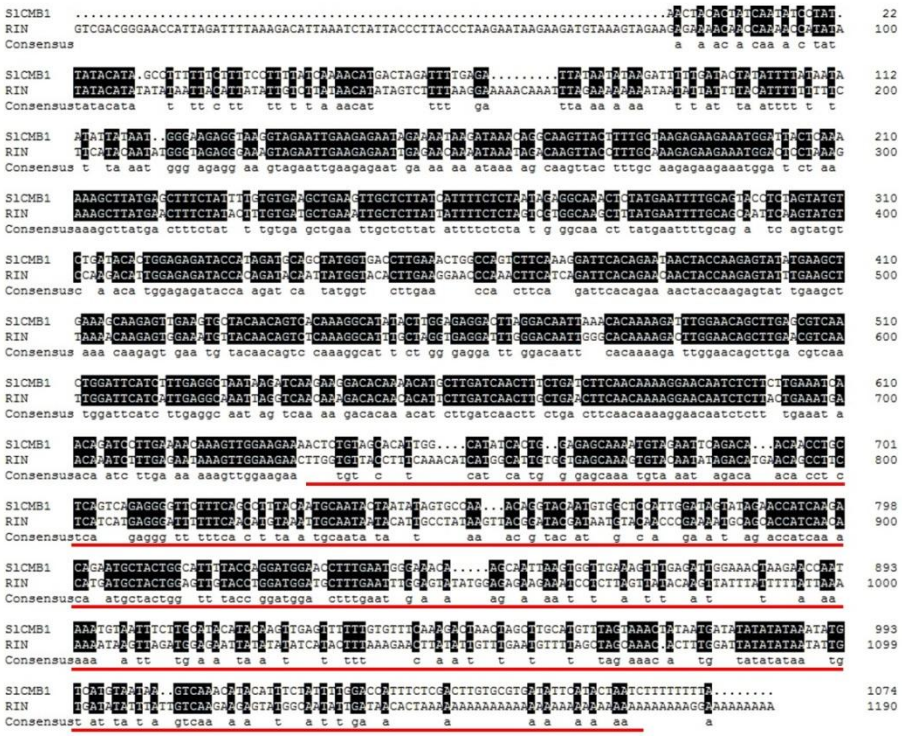
Primer Name	Primer Sequence (5'–3')	Product (bp)
SlCAC	CCTCCGTTGTGATGTAACTGG ATTGGTGGAAAGTAACATCATCG	173 bp
q-SlCMB1	TGAGCGTCAACTGGATTCATCTT CCCTCTGACTGAGCAGGTTGTT	213 bp
ACO1	ACAAACAGACGGGACACGAA CTCTTTGGCTTGAAACTTGA	181 bp
ACO3	CAAGCAAGTTTATCCGAAAT CATTAGCTTCCATAGCCTTC	113 bp
ACS2	GAAAGAGTTGTTATGGCTGGTG GCTGGGTAGTATGGTGAAGGT	107 bp
ACS4	GCTCGGAGGTAGGATGGTTTC GTTCCTCTTCCATTGTGCTTGT	151 bp
E4	AGGGTAACAACAGCAGTAGCA CCCAACCTCCGTCTTCAC	167 bp
E8	GGCACCATTCAACATACCG CTTTCACCGAAGAAGCACG	242 bp
ERF1	TTTTAGTATCGGATGGACG GGCGGAGAAACAGAAGTA	102 bp
PSY1	AGAGGTGGTGGAAAGCAA TCTCGGGAGTCATTAGCAT	298 bp
PDS	GCTTTACCCGCTCCTTTA ACCTTGCTTTCTCATCCA	174 bp
LCYB	TTGACTTAGAACCTCGTTATTGG AACAGTTCCCTTTGTCATTATCTC	137 bp
LCYE	GCCACAGGTTATTCAGTCGTCA CCAGTCCAAATAGGAAAAACGAT	196 bp
CYCB	CGACGTGATCATTATCGGAGC GTGGTGAAGGGTCAACACAACA	98 bp
RIN	CATCATGGCATTGTGGTGAGC AATTCAAAGCATCCATCCAGGTAC	194 bp
TAGL1	CGCAATAACTCCCTGCCTGTA GAAGATGAAGAGCCTTGACCC	143 bp
FUL1	AAAATCAGTGGGAAATCAACTCATC CCTTGCTGCTGTGAAGAACTACC	139 bp
FUL2	CCGTGGGAGCAACAGAGTCAT GGAGGCATCACAGAAGCACTG	167 bp
LoxC	TTTACTCCGCCCTACACGC CCTGAAAGATCGACACCCA	121 bp
PE	GCTTGCGTCTTTGACAACTCAGG GTGCCACCACTGCATTCGCTAT	137 bp

Supplementary Figures

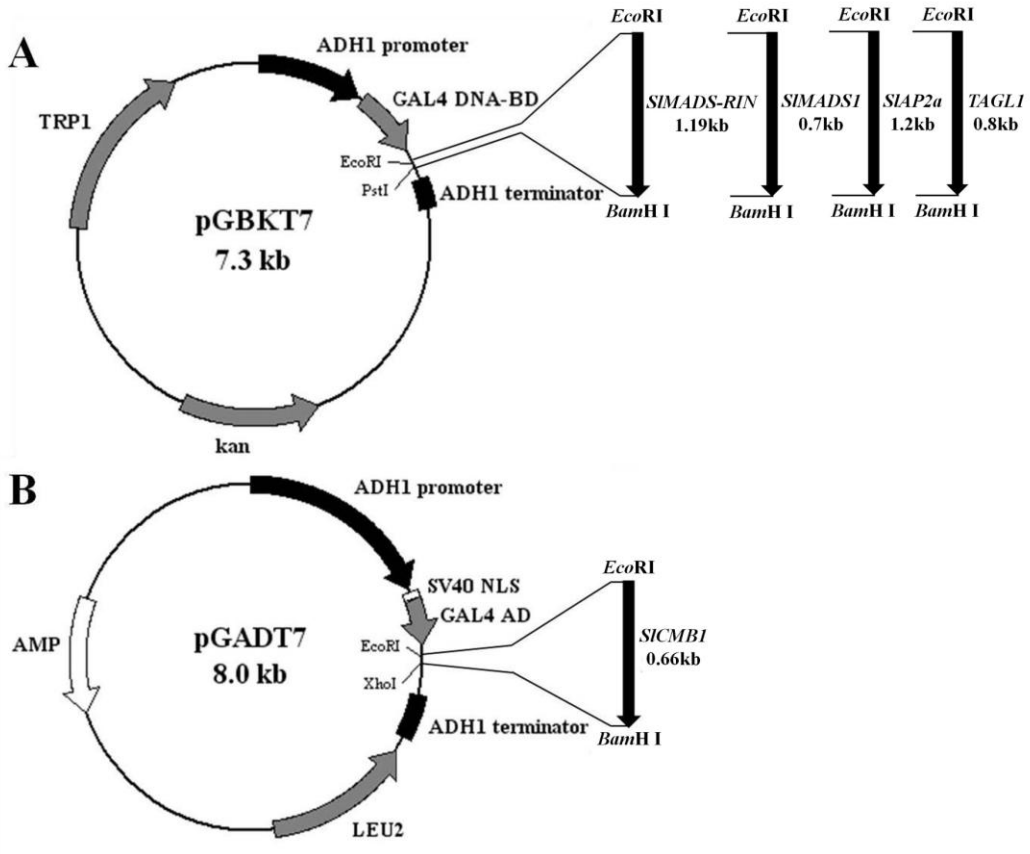
Supplementary Fig. S1. Promoter analysis of *SlCMB1* gene. Promoter sequence (3500 bp regions upstream the 5' end of the predicted ORF) of *SlCMB1* gene was extracted from SGN database and searched against the promoter database plant CARE (http://bioinformatics. Psb.ugent.be/webtools/plantcare/html/), ERE motif (ATTTCAAA) are the ethylene-responsive element in *SlCMB1* promoter region.



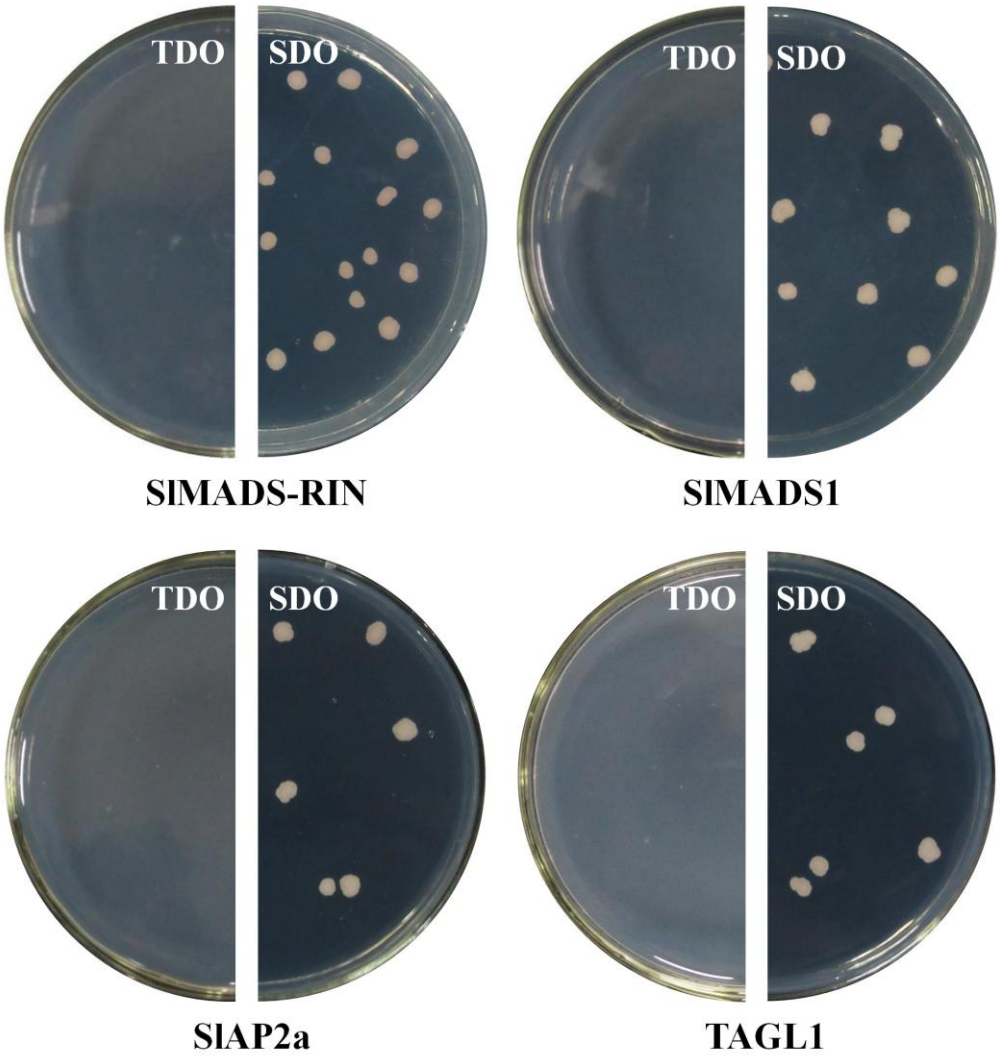
Supplementary Fig. S2. Hairpin construct of the *SlCMB1* gene for double-stranded RNAi vector. The *SlCMB1* gene-specific sequence in the antisense and sense orientations were linked with a PDK gene fragment and as a transcriptional unit for hairpin RNA expression which premoted by the CaMV 35S promoter and terminated by the OCS terminator. Among which, *Spe*I and *Xba*I are isocaudamers.



Supplementary Fig. S3. Multiple sequence alignment of *SlCMB1* and *RIN*. The 426 bp 3' specific fragment of *SlCMB1* used in this study is indicated by the red line.



Supplementary Fig. S4. Construct of SlCMB1 and RIN gene for yeast two-hybrid vector. (A) The ORFs of *SlMADS-RIN*, *SlMADS1*, *SlAP2a*, *TAGL1* were cloned into pGBKT7 bait vector to obtain the vector pGBKT7-*SlMADS-RIN*, pGBKT7-*SlMADS1*, pGBKT7-*AP2a* and pGBKT7-*TAGL1*, respectively. (B) The ORF of *SlCMB1* was cloned into pGADT7 prey vector to obtain the vector pGADT7-*SlCMB1*.



Supplementary Fig. S5. Yeast two-hybrid assay for SlCMB1 and SlMADS-RIN, SlMADS1, SlAP2a and TAGL1. TDO, SD medium without Trp, His, Ade (autoactivation assay); SDO, SD medium without Trp (autoactivation assay).