SmMYB36, a Novel R2R3-MYB Transcription Factor, Enhances Tanshinone Accumulation and Decreases Phenolic Acid Content in *Salvia miltiorrhiza* Hairy Roots

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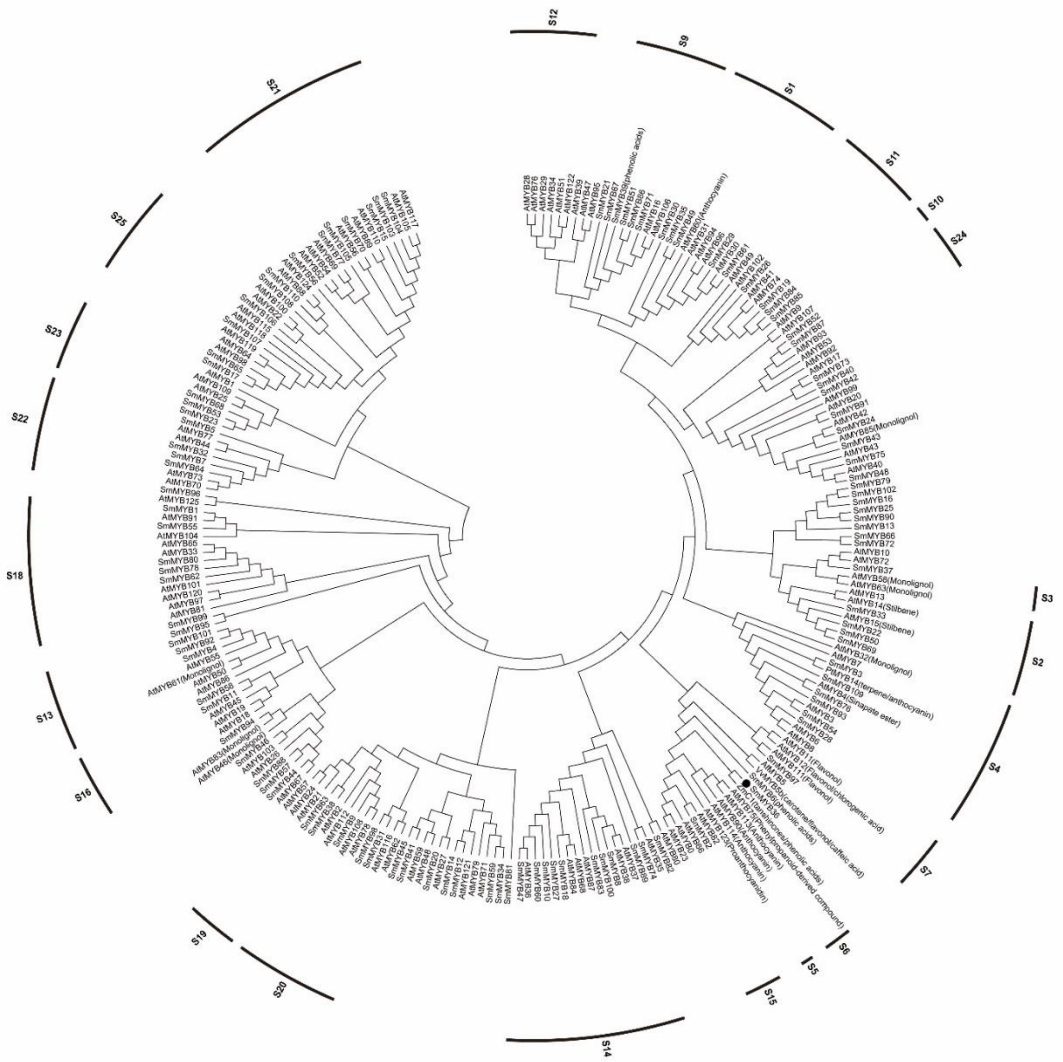
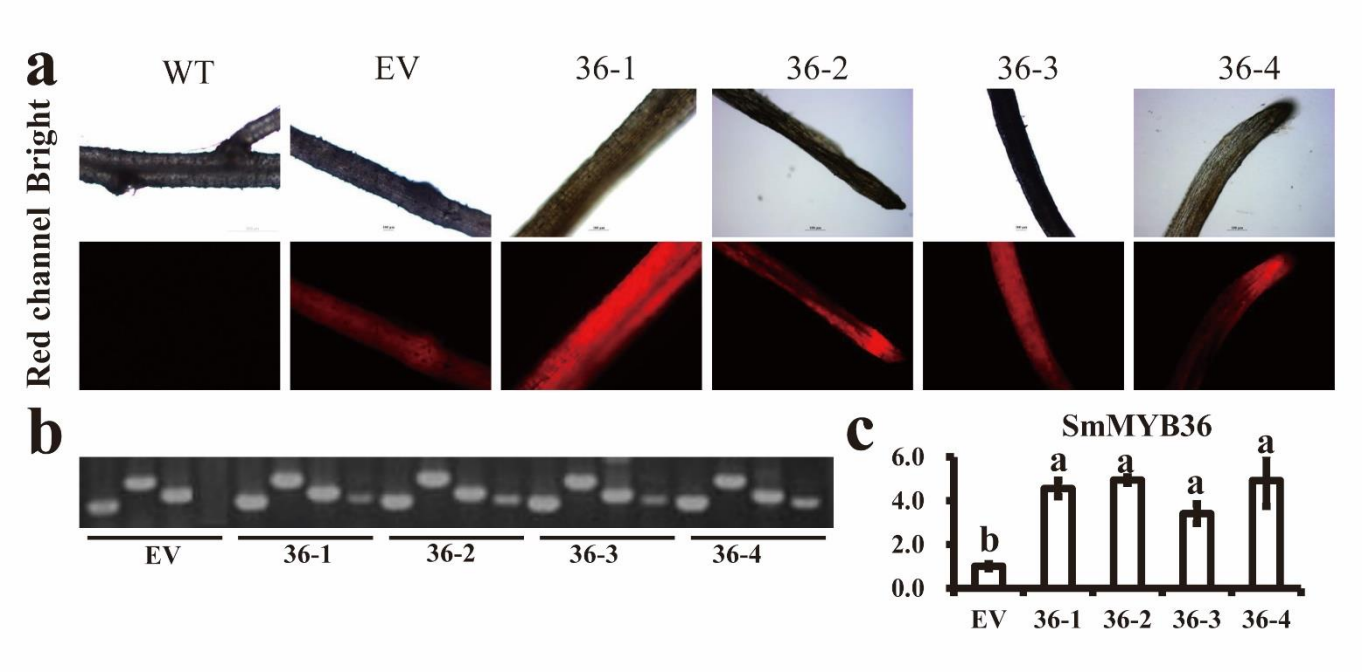
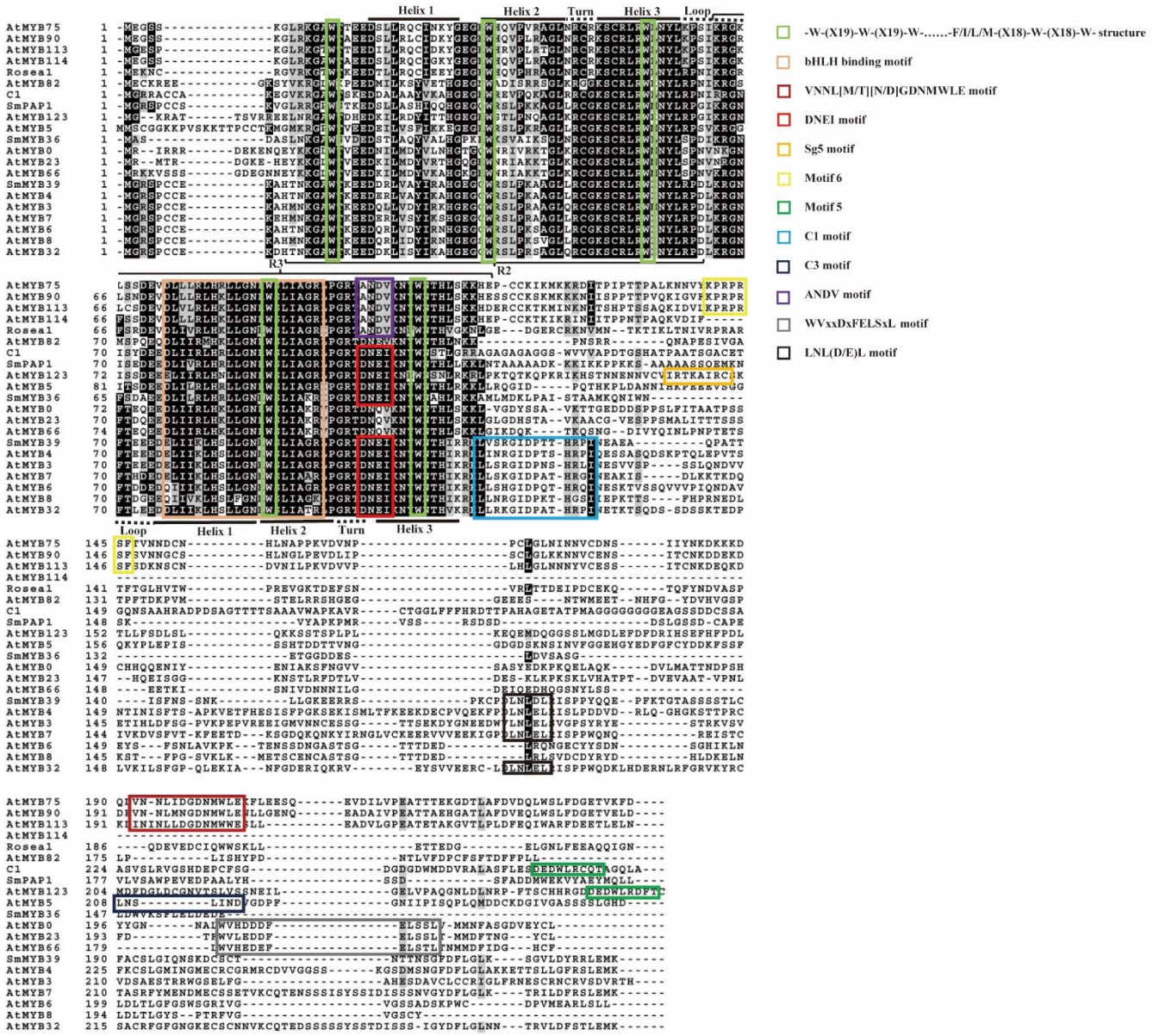


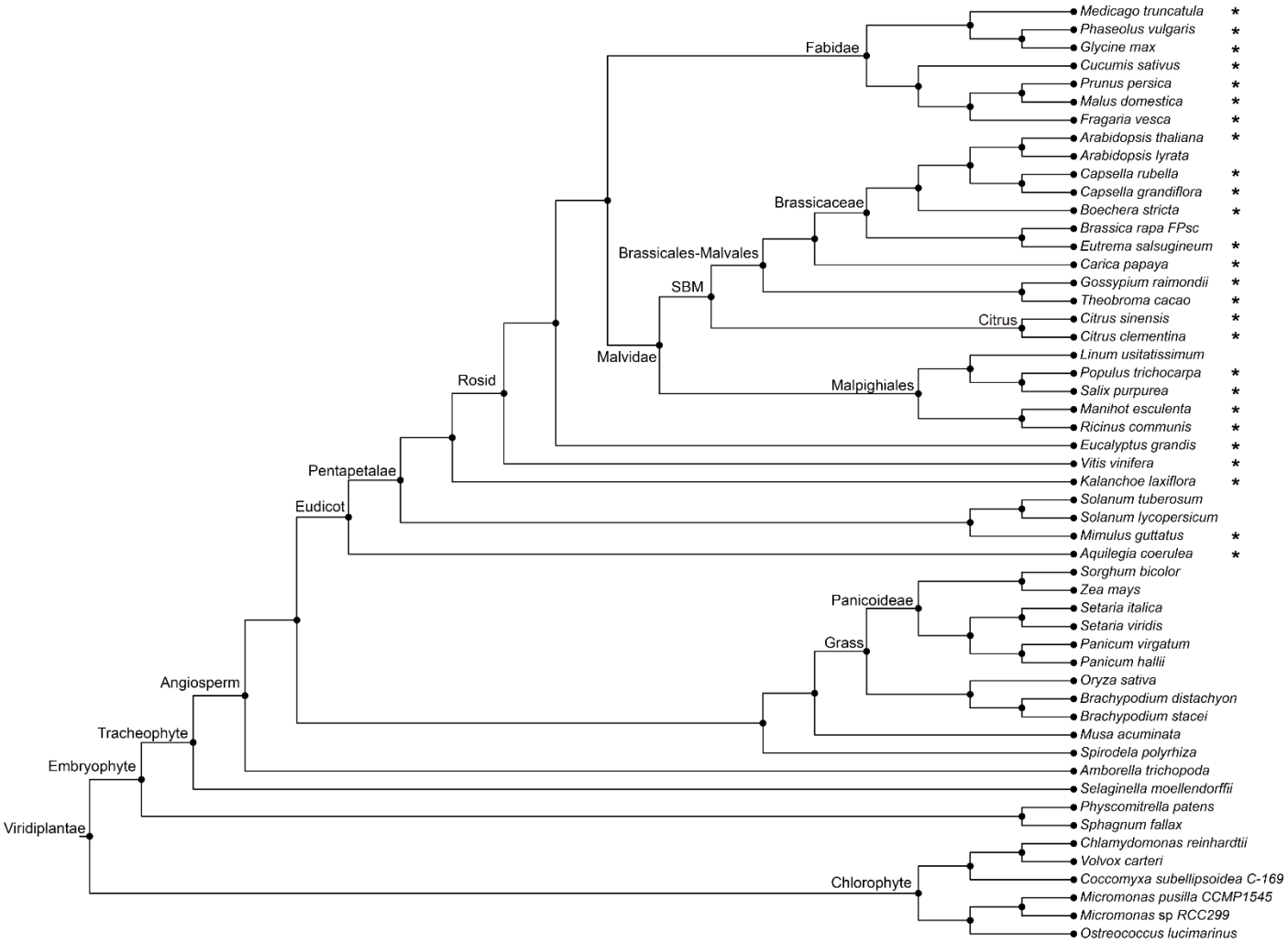
Supplementary Fig. S1. The phylogenetic tree for predicting the function of SmMYB36. The phylogenetic tree was constructed by maximum likelihood method of MEGA 6.06 based on the multiple sequence alignment using ClustalW method. The amino acid sequences of SmMYB36, ZmC1, VvMYB5b, PtMYB14 and all R2R3-MYBs of *Arabidopsis thaliana* and *Salvia miltiorrhiza* were used to construct the tree. SmMYB36 gathers together with these R2R3-MYBs whose function is regulating terpene or phenylpropanoid-derived compound biosynthesis.



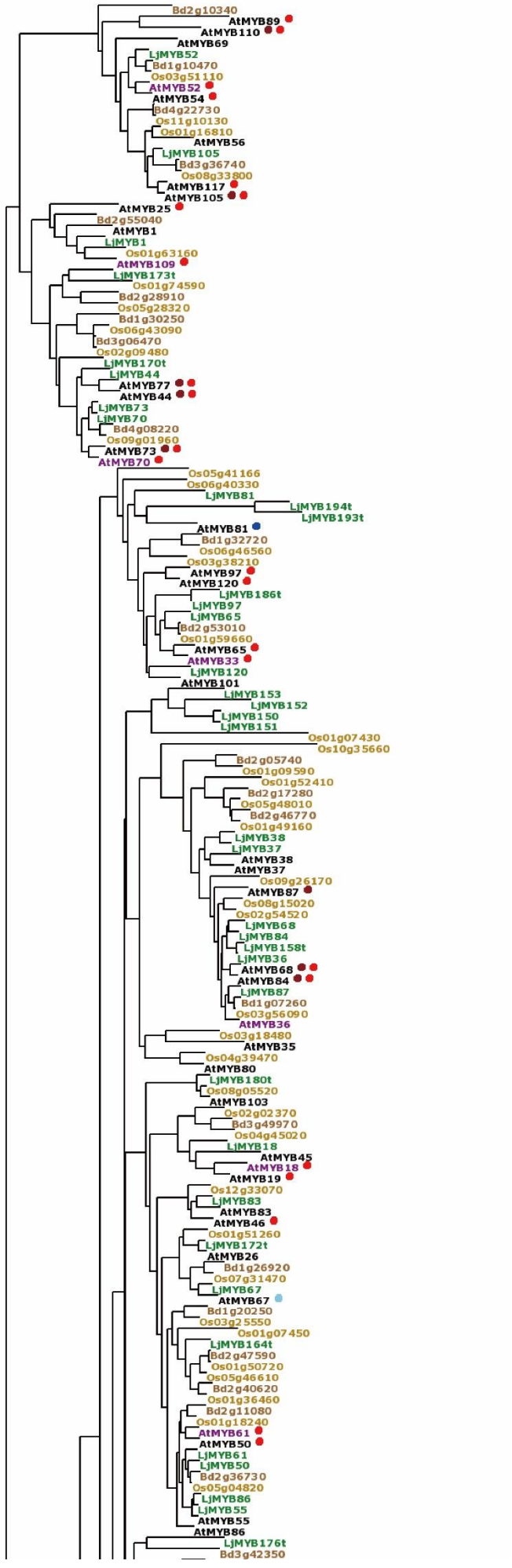
Supplementary Figure S2. (a) Bright field (upper lane) and red fluorescent field (bottom lane) of hairy roots of *Salvia miltiorrhiza*. Fluorescence was observed using a fluorescent microscope after harvest. (b) PCR identifications of hairy roots using *rolB*, *rolC*, npt and *SmMYB36* specific primers. The pictures show WT (infected by *Agrobacterium rhizogenes* strain ATCC15834), EV (infected by *Agrobacterium rhizogenes* strain ATCC15834 containing plasmid pK7FWG2R-EV), line 36-1, line 36-2, line 36-3, line 36-4 (infected by *Agrobacterium rhizogenes* strain ATCC15834 containing plasmid pK7FWG2R-SmMYB36) hairy roots from left to right. (c) Relative expression level of *SmMYB36* in transgenic hairy roots.



Supplementary Figure S3. The multiple sequence alignment and motif analysis of R2R3-MYB transcription factors.



Supplementary Figure S4. The species phylogenetic tree which was slightly modified from the tree representation of the species in Phytozome v11.0. The species were emphasized (*) which contains the predicted orthologous genes of SmMYB36.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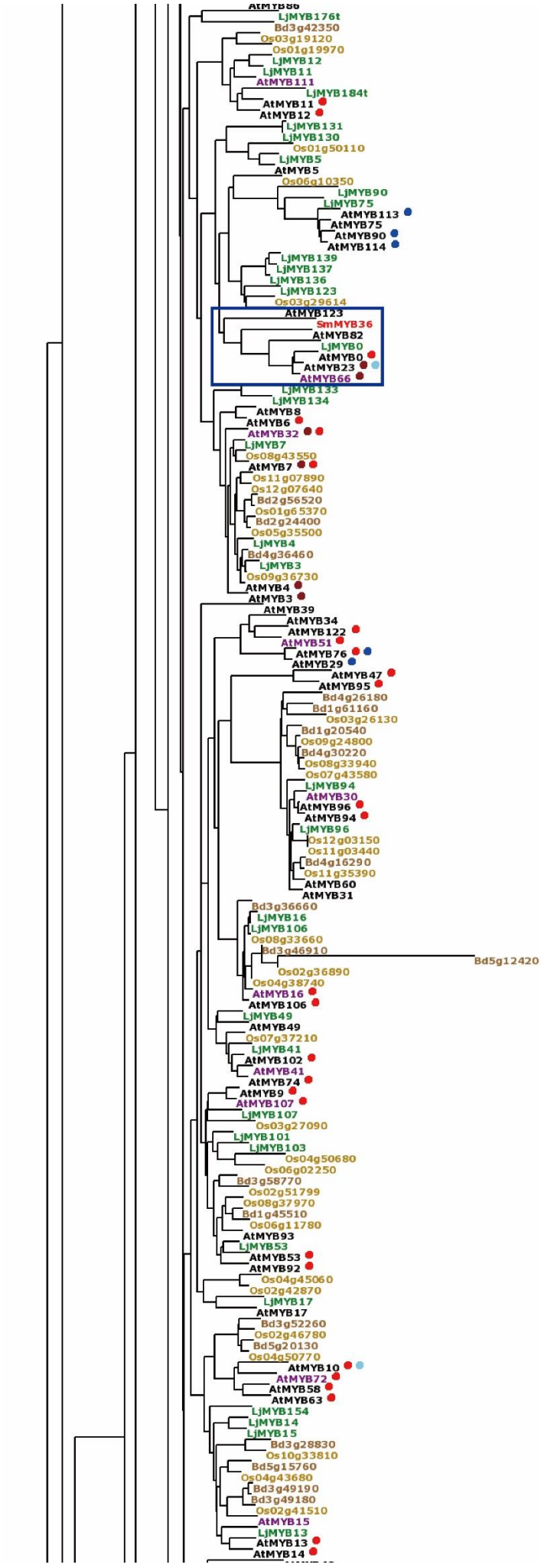
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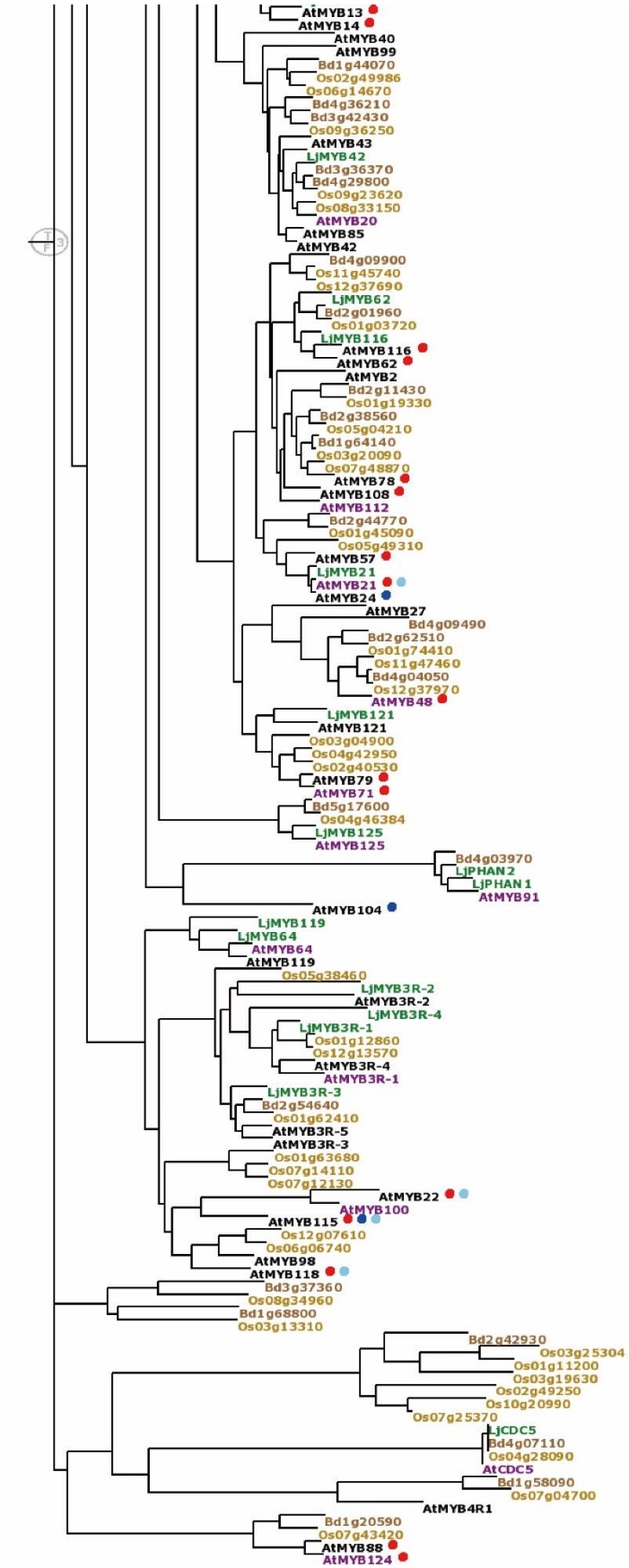
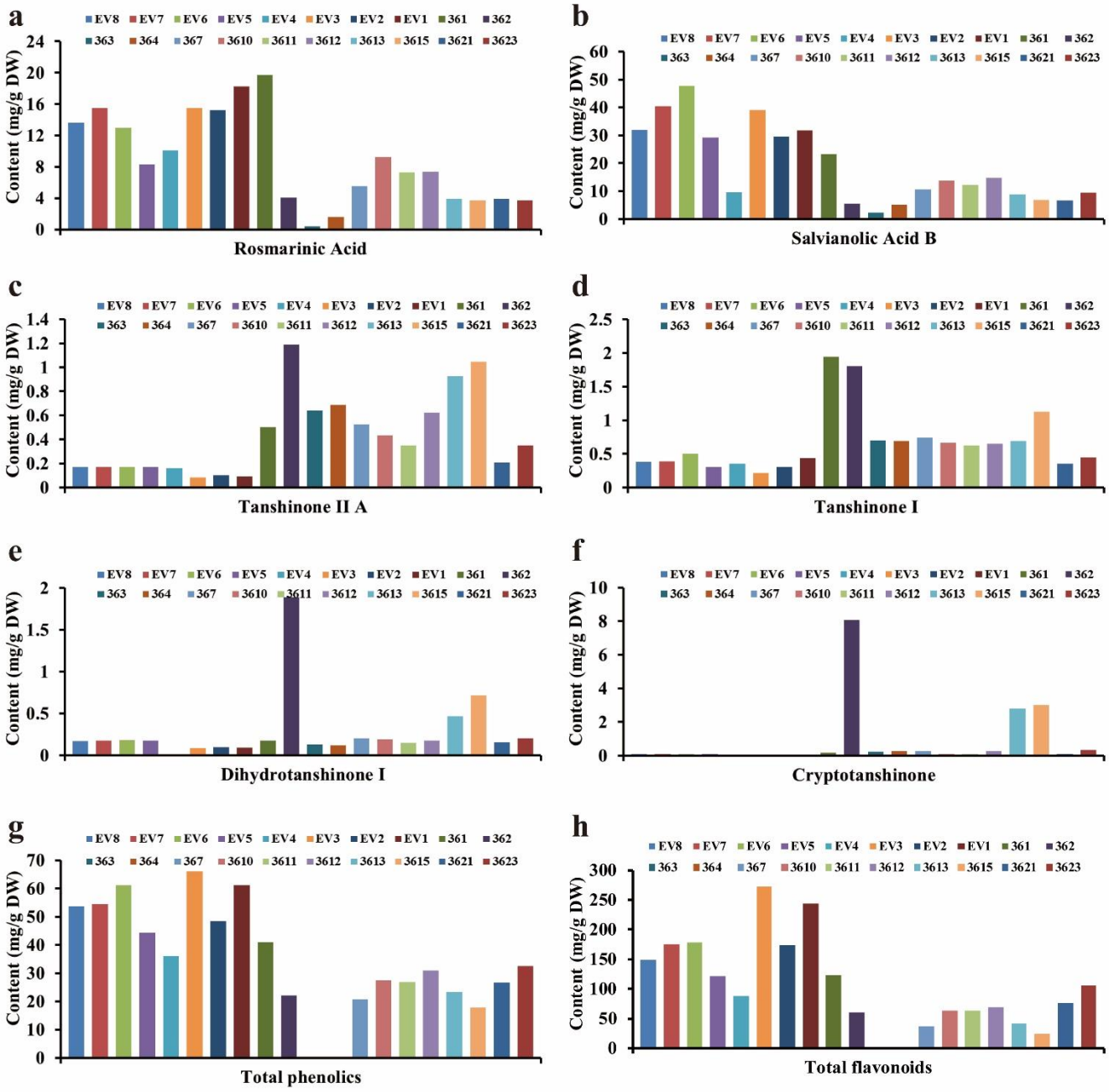
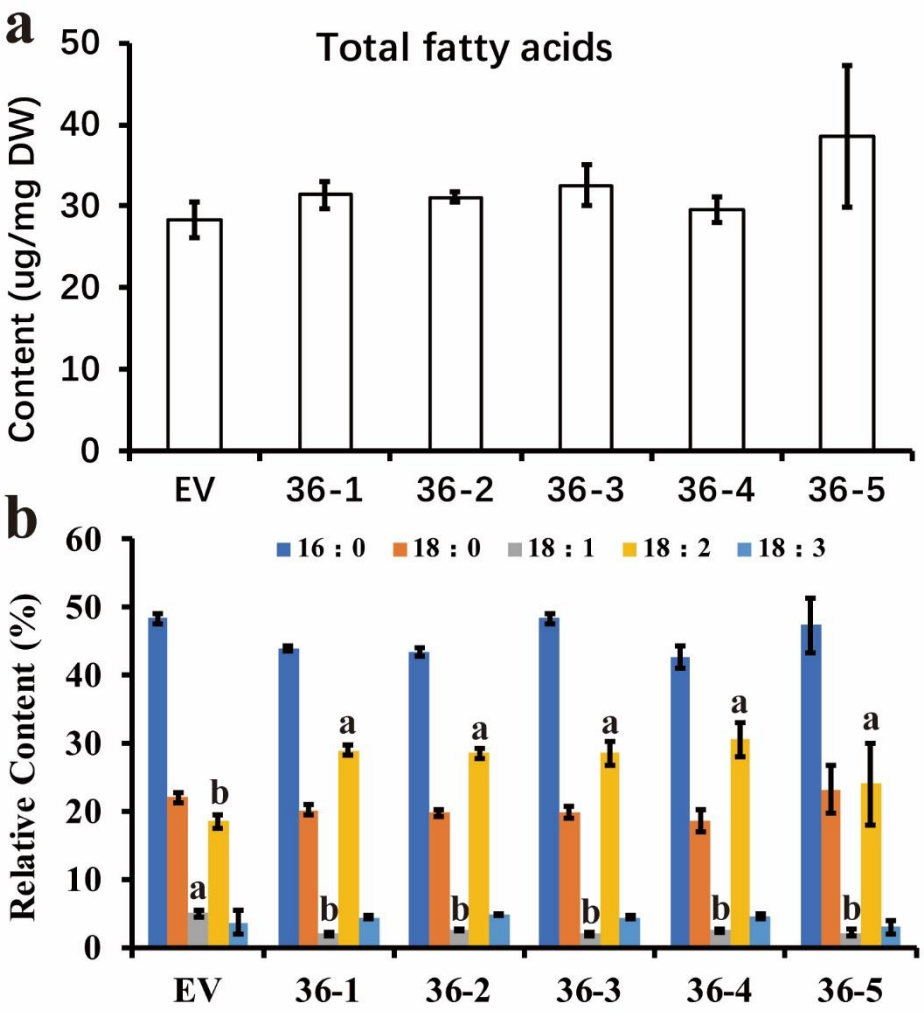


Supplementary Figure S5. The phylogenetic tree of R2R3-MYB transcription factors based on IT3F. The phylogenetic tree was constructed by submitting the amino acid sequence of SmMYB36 to the R2R3-MYB protein family of *Arabidopsis thaliana*, *Oryza sativa L.*, *Brachypodium distachyon* and *Lotus japonicas* in IT3F website (http://jicbio.nbi.ac.uk/IT3F/). The red text represents the query sequence (SmMYB36) and the R2R3-MYBs of *A.thaliana* were outlined by blue box which have the closest relationships with SmMYB36.



Supplementary Fig. S6. The preliminary experiment results of twelve *SmMYB36*-overexpressing lines (without replicates) and eight empty-vector control lines (without replicates). (a) The content of rosmarinic acid in hairy roots. (b) The content of salvianolic acid B in hairy roots. (c) The content of tanshinone II A in hairy roots. (d) The content of tanshinone I in hairy roots. (e) The content of dihydrotanshinone I in hairy roots. (f) The content of cryptotanshinone in hairy roots. (g) The content of total phenolics in hairy roots. The content of line 363 and 364 was not detected. (h) The content of total flavonoids in hairy roots. The content of line 363 and 364 was not detected.



Supplementary Fig. S7. (a) The content of total fatty acids in hairy roots of *S. miltiorrhiza*. (b) The relative contents of palmitic acid (C16:0), stearic acid (18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) in hairy roots of *S. miltiorrhiza*. The GC analysis have three biological repeats of transgenic lines and each biological repeat has three technological repeats. The metabolite contents were shown by their means ± SD.

Supplementary Table S1. Primers and probes used in the research

Primer Name	Primer Sequence（5' to 3'）	Description
SmMYB36 F1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATG GCGAGTGATGCATCTCT	The primers were used to generate the pDONR207-SmMYB36 entry vector.
SmMYB36 R1	GGGGACCACTTTGTACAAGAAAGCTGGGTATCATT CATCCTCGTCGAGTTC	
SmMYB36 F2	CCGCTCGAGATGGCGAGTGATGCATCT	The primers were used to generate the pA7-GFP-SmMYB36 vector.
SmMYB36 R2	GGACTAGTGATTCATCCTCGTCGAGTTCAAGAAA	
SmMYB36 F3	ATTTGGAGAGGACTCCGGTATT	The primers were used to identify positive hairy roots.
SmMYB36 R3	AATCCAACCCAGAGGCAGAG	
SmMYB36 F4	CCTCAACAGGTGCGGTAAGAGTT	The primers were used to quantitative analysis of SmMYB36.
SmMYB36 R4	TTCTTCCTCAAGTGAGCATTCCAGT	
rolB F	GCTCTTGCAGTGCTAGATTT	The primers were used to confirm transgenic hairy roots.
rolB R	GAAGGTGCAAGCTACCTCTC	
rolC F	CTCCTGACATCAAACTCGTC	The primers were used to confirm transgenic hairy roots.
rolC R	TGCTTCGAGTTATGGGTACA	
NPT F	ACGTTGTCACTGAAGCGGGAAGG	The primers were used to confirm transgenic hairy roots.
NPT R	GGCGATACCGTAAAGCACGAGGAA	
SmMYB36 F5	GTTATTTCATTTGGAGAGGACTCCG	The primers were exogenous SmMYB36 specific primers and used to confirm transgenic hairy roots.
SmMYB36 R5	CCAGTAGTTCTTGATCTCGTTGTC	
SmMYB36 F6	CGCGGATCCATGGCGAGTGATGCATCT	The primers were used to generate the pET32a-SmMYB36 vector
SmMYB36 R6	CCGCTCGAGTTCATCCTCGTCGAGTTCAAG	
C4H1-MBS1-F	TCTTTAAAACTC**CAACTG**ATATGATGTTCG	The primers were used to generate the specific C4H1-MBS1 probe of *C4H1* promoter for EMSA.
C4H1-MBS1-R	CGAACATCATAT**CAGTTG**GAGTTTTAAAGA	
C4H1-MBS3-F	GTTTAAAAGACA**TAACTG**AGTTATCGAACA	The primers were used to generate the specific C4H1-MBS3 probe of *C4H1* promoter for EMSA.
C4H1-MBS3-R	TGTTCGATAACT**CAGTTA**TGTCTTTTAAAC	
4CL2-MRE-F	TTTGTAGAGTTG**AACCTAA**AGATGATGGGTG	The primers were used to generate the specific 4CL2-MRE probe of *4CL2* promoter for EMSA.
4CL2-MRE-R	CACCCATCATCT**TTAGGTT**CAACTCTACAAA	
HPPR-MRE-1-F	TTTTGTGGGACA**AACCTAA**AAAGAAACTGAA	The primers were used to generate the specific HPPR-MRE probe of *HPPR* promoter for EMSA.
HPPR-MRE-1-R	TTCAGTTTCTTT**TTAGGTT**TGTCCCACAAAA	
HPPR-MRE-2-F	AATGATTGATTT**AACCTAA**TCATTGTGAAACC	The primers were used to generate the specific

		HPPR-MRE probe of *HPPR* promoter for EMSA.
HPPR-MRE-2-R	GGTTTCACAATGA**TTAGGTT**AAATCAATCATT	
DFR-MBS3-F	AGCTAGCTAAT**TAACTG**AAAAAACAATATT	The primers were used to generate the specific DFR-MBS3 probe of *DFR* promoter for EMSA.
DFR-MBS3-R	AATATTGTTTTTT**CAGTTA**ATTAGCTAGCT	
DXR-MRE-F	CCCCTTTTACAA**AACCTAA**TTTCCCCCCAT	The primers were used to generate the specific DXR-MRE probe of *DXR* promoter for EMSA.
DXR-MRE-R	ATGGGGGGAAA**TTAGGTT**TTGTAAAAGGGG	
MCT-MBS2-1-F	AGGAAACCCTCA**CGGTCA**CCTTAACATTCA	The primers were used to generate the specific MCT-MBS2 probe of *MCT* promoter for EMSA.
MCT-MBS2-1-R	TGAATGTTAAGG**TGACCG**TGAGGGTTTCCT	
MCT-MBS2-2-F	AAAACAGGCATC**CGGTCA**GTAAGTCCGCAT	The primers were used to generate the specific MCT-MBS2 probe of *MCT* promoter for EMSA.
MCT-MBS2-2-R	ATGCGGACTTAC**TGACCG**GATGCCTGTTTT	
MCT-MBS3-1-F	TATCATCTTCGG**TAACTG**ATACTCAAGGAT	The primers were used to generate the specific MCT-MBS3 probe of *MCT* promoter for EMSA.
MCT-MBS3-1-R	ATCCTTGAGTAT**CAGTTA**CCGAAGATGATA	
MCT-MBS3-2-F	AACTAGATGAAT**TAACTG**GTAGCAATTACC	The primers were used to generate the specific MCT-MBS3 probe of *MCT* promoter for EMSA.
MCT-MBS3-2-R	GGTAATTGCTAC**CAGTTA**ATTCATCTAGTT	
CMK-MBS1-F	TGAAGAATGGTG**CAACTG**CAATCAGTAAAA	The primers were used to generate the specific CMK-MBS1 probe of *CMK* promoter for EMSA.
CMK-MBS1-R	TTTTACTGATTG**CAGTTG**CACCATTCTTCA	
CMK-MBS3-F	CTCCTAATTATA**TAACTG**TCTCTGTATTAT	The primers were used to generate the specific CMK-MBS3 probe of *CMK* promoter for EMSA.
CMK-MBS3-R	ATAATACAGAGA**CAGTTA**TATAATTAGGAG	
CMK-MRE-1-F	TCCACACTACAA**AACCTAA**CCCTAACCGTA	The primers were used to generate the specific CMK-MRE probe of *CMK* promoter for EMSA.
CMK-MRE-1-R	TACGGTTAGGG**TTAGGTT**TTGTAGTGTGGA	
CMK-MRE-2-F	AGAATGGAATTT**AACCTAA**ATTAGTGTTCA	The primers were used to generate the specific CMK-MRE probe of *CMK* promoter for EMSA.
CMK-MRE-2-R	TGAACACTAAT**TTAGGTT**AAATTCCATTCT	
GGPPS1-MBS2-F	TTAAAATCGTTA**CGGTCA**TACCACGGAAAC	The primers were used to generate the specific GGPPS1-MBS2 probe of *GGPPS1* promoter for EMSA.
GGPPS1-MBS2-R	GTTTCCGTGGTA**TGACCG**TAACGATTTTAA	
GGPPS1-MRE-F	TTTTTCGATAGT**AACCTAA**TTTTCTGTAGTG	The primers were used to generate the specific GGPPS1-MRE probe of *GGPPS1* promoter for EMSA.
GGPPS1-MRE-R	CACTACAGAAAA**TTAGGTT**ACTATCGAAAAA	

IPPI-MBS1-F	CCGCCGCCGCCA**CAACTG**TAATGGTGCTTT	The primers were used to generate the specific IPPI-MBS1 probe of *IPPI* promoter for EMSA.
IPPI-MBS1-R	AAAGCACCATTA**CAGTTG**TGGCGGCGGCGG	
HMGS1-MBS3-F	GTGTGTGCTGTT**TAACTG**ATCCGCTATTTC	The primers were used to generate the specific HMGS1-MBS3 probe of *HMGS1* promoter for EMSA.
HMGS1-MBS3-R	GAAATAGCGGAT**CAGTTA**AACAGCACACAC	
MBSⅠ-1-F	**AAAAAACCGTTA**	The primers were used to generate the core MBSⅠ probe for EMSA.
MBSⅠ-1-R	**TAACGGTTTTTT**	
MBSⅠ-2-F	**AAAAAACGGTTA**	The primers were used to generate the core MBSⅠ probe for EMSA.
MBSⅠ-2-R	**TAACCGTTTTTT**	
MBSⅡ-F	**AAAAGTTAGTTA**	The primers were used to generate the core MBSⅡ probe for EMSA.
MBSⅡ-R	**TAACTAACTTTT**	
MBSF1	**CAACTG**	The primers were used to generate the core MBS1 probe for EMSA.
MBSR1	**CAGTTG**	
MBSF2	**CGGTCA**	The primers were used to generate the core MBS2 probe for EMSA.
MBSR2	**TGACCG**	
MBSF3	**TAACTG**	The primers were used to generate the core MBS3 probe for EMSA.
MBSR3	**CAGTTA**	
MREF	**AACCTAA**	The primers were used to generate the core MRE probe for EMSA.
MRER	**TTAGGTT**	
6CKF	GATTCG	The primers were used to generate the control probe for the core probe (MBS1) for EMSA.
6CKR	CGAATC	
7CKF	AGAAAGC	The primers were used to generate the control probe for the core probe (MRE) for EMSA.
7CKR	GCTTTCT	
12CKF	AGTTTTCTTGAA	The primers were used to generate the control probe for three core probes (MBSⅠ-1, MBSⅠ-2, MBSⅡ) for EMSA.
12CKR	TTCAAGAAAACT	

Note: The **bold** bases represent the MYB-related core elements (MBS1, CAACTG; MBS2, CGGTCA; MBS3, TAACTG; MRE, AACCTAA; MBSⅠ, AAAAAAC(C/G)GTTA; MBSⅡ, AAAAGTTAGTTA).

Supplementary Table S2. The conserved motifs of subgroup 4, 5, 6 and 15

Amino Acid Sequence	Motif Name	Subgroup	Reference
[D/E]Lx2[R/K]x3Lx6Lx3R	bHLH binding motif	4, 5, 6, 15	[1,2]
Lx3GIDPxTHRPI	C1 motif	4	[3]
LNL[E/D]L	C3/EAR/ERF motif	4	[2,4]
DNEI	DNEI motif	5	[5]
IRTKA[I/L]RC	Sg5 motif	5	[6]
DEDWLRxxT	Motif 5	5	[7]
KPRPR[S/T]F	Motif 6	6	[8]
VNNL[M/T][N/D]GDNMWLE	-	6	[9]
ANDV	ANDV motif	6	[10]
WVxxDxFELSxL	-	15	[7]
-W-(X_{19})-W-(X_{19})-W-……-F/I/L/M-(X_{18})-W-(X_{18})-W-	-	4, 5, 6, 15	[8]

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Supplementary Table S3. The promoter sequences of pathway genes which contains MYB-related elements

Gene Name	Promoter Sequence
SmC4H1	TGTGAGTTAATGTTTTACCTTGGAATAAAAACAGCTACTTTTTTAAATAATAAACTCTCT GCCTGAATAAAGATTTGTCGAATATCCACACTTTTCTTCTTCAAAATAAGCACCTTGTTT TGCTTTTGACTTTTTAAATTTGCATCGCAGTTTGAAAGAAGAAAAATAGACTGTTAGCT AGAAAGTGAACTTAACTTTACAAAAATACTTGGAGGAAATGTCAGCGGAATTAGTTTA TCTCTTAAAATAACATTTATACTACTCCATTTTTTATTTATTTTTTGCTATTCCTACTTATG ACGAAAATAGAAAAGTTAATTGTTATTTGAAGATGTGTTTGGTATAGATTTAATGTCGTA ATTCAGTTGCATTGAGTACTTTTGACTAAGTTTTGTTGAATAAATCCGACACATAAGATA TTGACGATGTTCTAGATTGGGCTAATTGTCCTAAATAGGAACTAGTACCCTAATTTGTAA TTACAATACAATTATCAAAATATTCAAATTATAGACCATCTATTATTTTCACAATTACACC ATTCCAAATTTTTTAAACAGACAAGACAAGCTATACTAAATTGGAAACTATTAACTAAA AGATTGAATTATAATTACATTGTTTTGTGATTACAATTTATGATTAGTCTATGTTTTAAATA GTTGGCCCTTTCAAAAATGACGATCTCAACTACAAACTTGTCAAAAATCATGAGGTGG TTTAGACATATATAGAAGGAGCATTTTACATATCAAAATTCATGAGAGTGGCTCCAGAC ATAAATTTGAAAACAATTAAATACC**AACCTAA**TTATAAATGTGTAATTAAATATAACTTC AAATCTTGACTGGTTTAATCGTGAGAAGATTTCACAAACGTTGTGCTAGTATATGATATT GACTTAATATGTCTAGCGAACGACTAATTAAACTCAATTTTTTCAAATTGTTAATTTTATT ACTTATTGGCGTTTACCACGATTGATTATATGCATGATTGAGTATTTTTATTCTAAAGAGT AGGATTTCTCCAATTTTACTTTTTCTATGGGATAAGAATCAAATAAAATTAGGTTGAATG ATAAAAATAATAAAGTATCTTAAAATATTTCGAAGTTTCAATCCATTAAAATATAAGACA TAGAGTCTATGTGTCTATGAAATGAACGACTTAAGAACGAGTAATAAATAATTAGATAA CTTTCTTACAAATCCATCTCCAACTACACGATACATTAAGTCCAATTTCGTGAGTAAATG TATATCTCGGTAAGAGACAAAAAGTTTTTTTAATTTTAAAAATCAAATCTAGGTCNCTTT TTATAGTGTTTGTGGCATTGTACTCGTGGTGCGGTAGTTGTCACAAATTTGTAAGGCGG GGAAGCACCGAATTTTTTTTTATCAAGAAATACACGTTTTCATTGAGAGTTAGTTGTAG CACAAGCAATCCTACAACGTTTTCACAAAATATATCCATTATATAATACTCCCTTCGTCC ACCAAAGATATGCCACAATTTTCTTTTTCGTCCGTTCACAAAAAATATATCACATTCATT TTTAGTAATAGAGTCTACACCATTTCATTCACATTTAAAATGAGATCCTTACTCCACTAC CAACTTCACTCACATCTTATTAAAACTCGTGCCGAAAGTAAAGTGTCATATTTTTTGTG GACGGAGGGAGTAGGTTATATTTTTCAAAGCCTCCAGCCATTCTATATCTGATGTGTCGT TCTCAATAATCTTCATTCTCTTTTTTCTAATTTCTACAATTATTCATTTTTAATAATTATTAA ATATACATATATTATTTAAAAACTTTCATTTTCAGTGTTCTTTTAGTTTTGTAATTCACTTT TTGGAAAATGATTTCTTTAATTGTTATTATTCTAATTATTCATTAATATTTATCAATCAAAT AATTAATTAGCACGTTAATATTAATTACATTAATAAATTTTTATTTTCTATACTATTTTTTCA CCTATAAATTATAGAAATTTGCAACCAATTTCATCCACATTAGTTCTTTATGCATCTTCAT TCGCTTTTCCTATCCAGCGACTCAAAATGCTATAATATATTGTATTATGTGGTAGCAAATT AAGATGATTCTAAAAGTCTCAAGAGAAAAAAGAATTTTTTTAAAAATAAGAAATCAAG GATTCTTATTGAATCAGAAGCTTTCTCTCAACTTTTTTTTTTTTTTTTTTTGAGAGTGAA GCAGAAACTCTTAGTTGCTTACATTGTAAAACGAAGTACTCCTTCCGTCTCAAGAAAAT TGCGTATTTCTTTTTGAGTTATCCCAACGAAAGTGGTGCATTTTTTTATAGCAAAAATTA ATAAATTTAAAAACTTAATTGACAAAACTAAATGCACCCTTATTTTTTTTAAATTAAATT AATTAAACACACACTCTCTCTCTTCTATCTTCCCCATGTCTCCTAAAACAATCCATTTGG ATGAAAGAACAAAAGCAGCCCACCACCGTGGCCGCTGCCGCCCTCATCCTCTGGCGC CTTCCCCTGCCGTGCTGGAGCCGCGAGTTGACGTCGTCTTCCCCCGCCGTGCTAGGGT TTCGATTTGGGGTGGGTGCAGGGAGCAGTTGGGGATTTGGGGCTTGGCGATGCCAGCG GCCTCAGCTACAGGTCACGGACGAGAAAGGTTAGAAAAGCAGATAAGGTGGTTTTTG CGGCGATTTATCAAAAAATCGAAAGAGAAGAAATTATGAAGTTTAAAAAGTTAGGGAT TTTGTTTATATGTGTTCGATTTGAGAGATTGTCATTTAAAGTAGCGGTGGCTGAAAGTG

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Note: The **bold** bases represent the MYB-related core elements (MBS1, CAACTG; MBS2, CGGTCA; MBS3, TAACTG; MRE, AACCTAA; MBS Ⅰ, AAAAAAC(C/G)GTTA; MBS Ⅱ, AAAAGTTAGTTA). The underlined bases were synthesized for EMSA.

Supplementary Table S4. The homologous genes of SmMYB36 in different species

Organism	Defline
Physcomitrella patens	Pp3c1_4970V3.2
Sphagnum fallax	Sphfalx0111s0046.1
Selaginella moellendorffii	109587
Amborella trichopoda	evm_27.model.AmTr_v1.0_scaffold00024.222
Musa acuminata	GSMUA_Achr11T23500_001
Spirodela polyrhiza	Spipo0G0167800
Brachypodium distachyon	Bradi1g60106.1
Brachypodium stacei	Brast02G211100.1
Oryza sativa	LOC_Os01g50110.1
Panicum hallii	Pahal.D02873.1
Panicum virgatum	Pavir.J00444.1
Setaria italica	Seita.4G086300.1
Setaria viridis	Sevir.7G295300.1
Sorghum bicolor	Sobic.001G340900.1
Zea mays	GRMZM2G000818_T01
Aquilegia coerulea	**Aquca_011_00570.1**
Kalanchoe laxiflora	**Kalax.0006s0265.1**
Mimulus guttatus	**Migut.A01129.1**
Solanum lycopersicum	Solyc10g055410.1.1
Solanum tuberosum	PGSC0003DMT400034372
Eucalyptus grandis	**Eucgr.D02099.1**
Vitis vinifera	**GSVIVT01006275001**
Linum usitatissimum	Lus10033438
Manihot esculenta	**Manes.02G041300.1**
Populus trichocarpa	**Potri.006G221200.1**
Ricinus communis	**28226.m000839**
Salix purpurea	**SapurV1A.1360s0110.1**
Citrus sinensis	**orange1.1g028843m**
Citrus clementina	**Ciclev10009521m**
Citrus clementina	**Ciclev10026498m**
Carica papaya	**evm.model.supercontig_46.144**
Gossypium raimondii	**Gorai.004G196800.1**
Theobroma cacao	**Thecc1EG015933t1**
Arabidopsis lyrata	488320
Arabidopsis thaliana	**AT5G40330.1**
Boechera stricta	**Bostr.1460s0063.1**
Brassica rapa	**Brara.J01999.1**
Capsella grandiflora	**Cagra.3166s0078.1**
Capsella rubella	**Carubv10001990m**
Eutrema salsugineum	**Thhalv10014715m**
Cucumis sativus	**Cucsa.340790.2**
Fragaria vesca	**mrna07418.1-v1.0-hybrid**
Glycine max	**Glyma.05G061900.1**
Malus domestica	**MDP0000226215**
Medicago truncatula	**Medtr4g100720.1**
Phaseolus vulgaris	**Phvul.003G222400.1**

Prunus persica	**Prupe.5G065500.1**
Chlamydomonas reinhardtii	Cre16.g677382.t1.1
Volvox carteri	Vocar.0011s0012.1
Coccomyxa subellipsoidea	12896
Micromonas pusilla	39026
Micromonas sp	73784
Ostreococcus lucimarinus	8749

Notes: The analysis was based on the Phytozome database (https://phytozome.jgi.doe.gov/pz/portal.html) and NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome). The **bold** represents the predicted orthologous genes of SmMYB36.