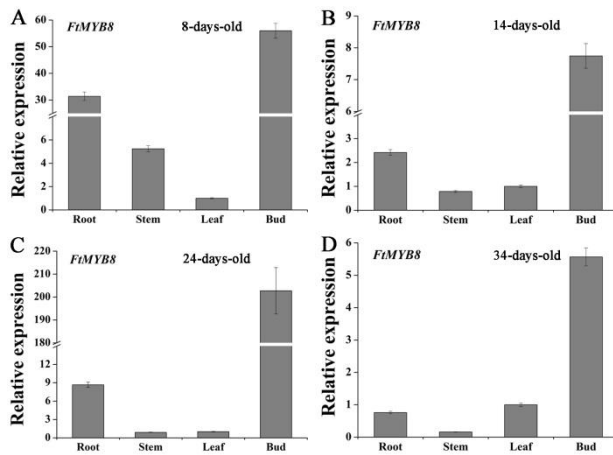


***FtMYB8* from Tartary buckwheat inhibits both anthocyanin/proanthocyanidin accumulation and marginal trichome initiation**

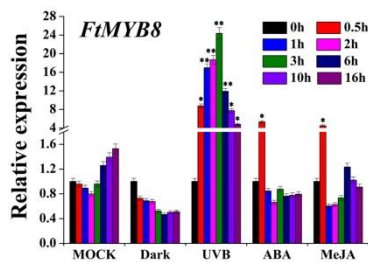
Yunji Huang<sup>#</sup>, Qi Wu<sup>#</sup>, Shuang Wang, Jiaqi Shi, Qixin Dong, Panfeng Yao, Guannan Shi, Shuangxiu Xu, Renyu Deng, Chenglei Li, Hui Chen, Haixia Zhao<sup>\*</sup>

**Additional file**



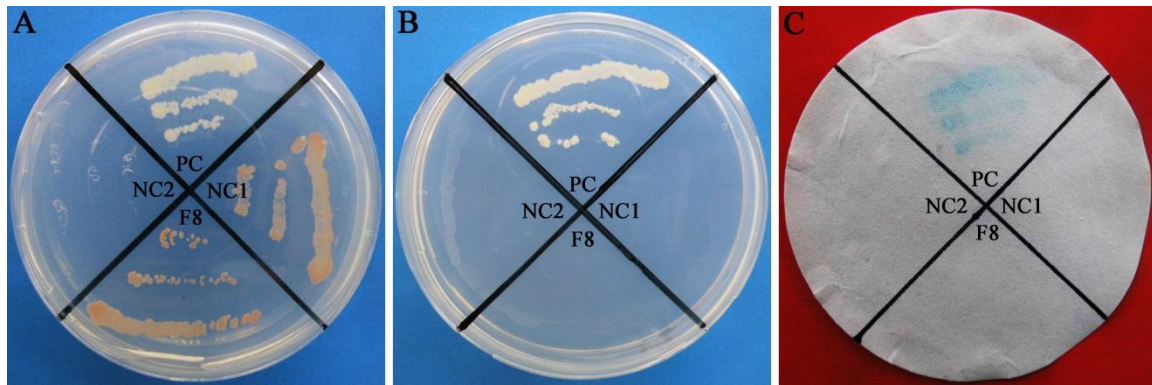
**Figure S1.** Expression of *FtMYB8* during Tartary buckwheat development.

The relative expression of the *FtMYB8* gene was evaluated by the  $2^{-\Delta\Delta CT}$  method. *FtH3* was used as a reference gene. The accumulation of *FtMYB8* mRNA in the leaves was defined as "1" at each developmental stage. Means were calculated from three repeats, and error bars reflect  $\pm$ SDs.

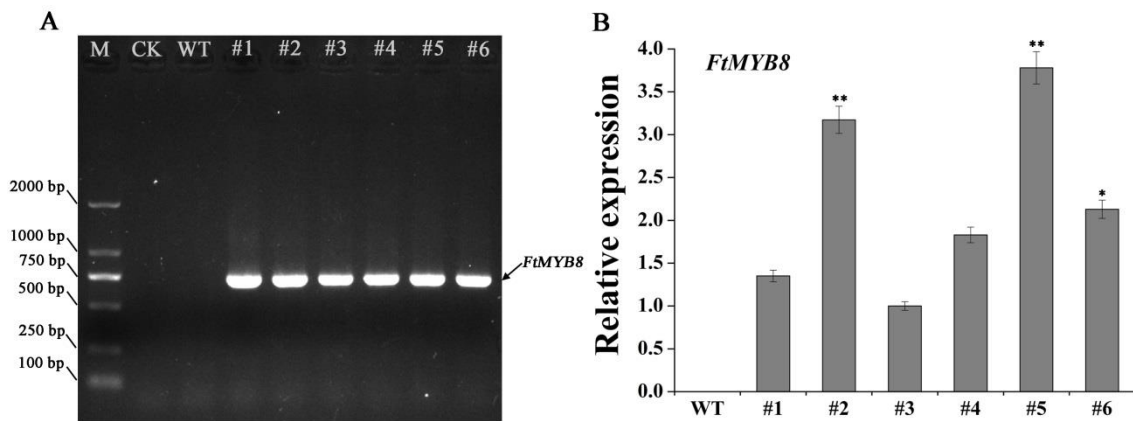


**Figure S2.** Relative expression of *FtMYB8* in Tartary buckwheat seedlings under the influence of environmental factors and hormone treatments.

The relative expression of the *FtMYB8* gene was evaluated by the  $2^{-\Delta\Delta CT}$  method. *FtH3* was used as a reference gene. The level of *FtMYB8* mRNA at 0 h was set to "1". Means were calculated from three repeats, and error bars reflect  $\pm$ SDs. \*\*P < 0.01; \*P < 0.05.

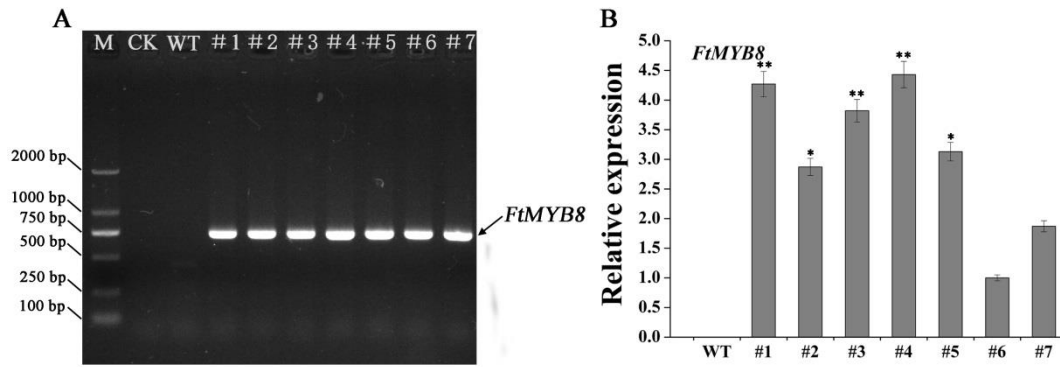


**Figure S3.** Transactivation assay of FtMYB8 in AH109 cells. (A-B) The transformed cells were selected on SD/-Trp and SD/-His-Trp media. (C)  $\beta$ -Galactosidase activity assay. PC: pBridge-*FtbHLLH2*; NC1: empty plasmid; NC2: AH109 cells; F8: pBridge-*FtMYB8*.



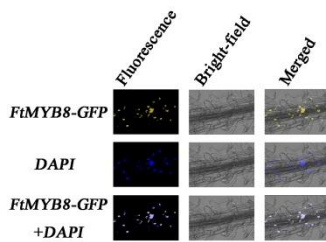
**Figure S4.** Generation of transgenic tobacco plants.

(A) PCR amplification of transgenic tobacco. Lane M, DNA marker 2000; lane CK, water, as a negative control; lane WT, wild-type tobacco; lanes #1 to #6, transgenic tobacco. (B) Detection of *FtMYB8* gene expression in transgenic tobacco and WT tobacco by qPCR. The tobacco  $\beta$ -actin gene served as an internal control. Error bars represent  $\pm$ SDs. \*\* $P < 0.01$ ; \* $P < 0.05$ .



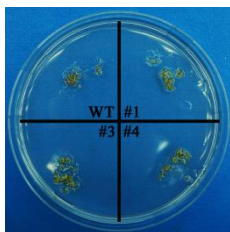
**Figure S5.** Generation of transgenic Arabidopsis plants.

(A) PCR amplification of transgenic Arabidopsis. Lane M, DNA marker 2000; lane WT, wild-type Arabidopsis; lane CK, water, as a negative control; lanes #1 to #7, transgenic Arabidopsis. (B) Detection of *FtMYB8* gene expression in transgenic Arabidopsis and WT Arabidopsis by qPCR. Arabidopsis *Atactin2* served as a reference gene. Error bars represent  $\pm$ SDs. \*\* $P < 0.01$ ; \* $P < 0.05$ .



**Figure S6.** Subcellular localization of FtMYB8.

Root of *FtMYB8*-overexpressing Arabidopsis under a fluorescence microscope. DAPI was used as a nuclear localization marker.



**Figure S7.** Seed germination of WT and transgenic lines.

WT and transgenic seeds germinated on MS medium; photos were taken after three days of germination.

**Table S1.** Sequence of the *P<sub>F1MYB8</sub>* promoter

Gene	Sequence (5'→3')
<i>P<sub>F1MYB8</sub></i>	TAGGAAGGTATGACAGTTTGATTGGTTTATTTAGTTTTGCCTTCTAAATTTACACTTTATTCTAA TTACTAGTTTACTTTAAAAACATATTGTATTTTACCTAAAAAGAACTTGATTTATCTATTACTCA ATGAGTACGAACACAAAAAAGATATGAGTGGATGATGGATCAGATGAAAATTTTAGGGGAT GGATCGAGTGATGTGAGTGGTTTATCCCCGTCAATCGACCCGTTGCCGACCTATATAAGATT AAGCTAATACATCATAGCTAATTATATCCTTGTCATTTGTAAGCTTGAATGCAAGATGCATAG AGGTTATGAAAGCTTGTGGGCGTTCAATCAACATTATAGTTTAAGGTTTTTATATGTGGTAATC TCTTATTGAATAACATATTTTATCAGGTGTCATTTCTATTAATCAAAGTTAAATTAATATTTTT CTTTCAAAAAATACAAATATAAACTTATTGAAGGTAGGCTCATATTAATAACAACCTCAATTTGA AAGTCAATCAACAAAATCCCTAACTTGGTTGAACTTACACCAATAAAAGTTACCATTAGAAA TGTGGAAATATACATTTTAAAACTACAAAATTTGGAAAAAAAACCATAAAATAATCTAAA AATGGATTAAGTGAAATCCAAGTTCAATAAATGTTTGTTCATATATATACTTTTCTGACATTGCA TTAGTATAGTTGTTAAATTAGAAATGTTATTGTCACATAAATTTCTAACTGACAACGATGTC TCCTTTTATTTAATATTGAAAAACGTTTAAAGGAAATAAAAATACTTTTTATTATTTAAAAAAA TGTTTCATAGAGCATGGTAGGTTGTGCGGGTAAGTAAGAATGGCGGGATCTGCATGCGGAGGC AAAAAGAAAACGACTGCTACTTAACGGTGTCAATCCCTTCACGGTAAACAACCAGTCAGCG TTGTACGTTTTACGGAATCAATGCACCATTCTAACGGACTTAACCAAATAGTTTAAACAAAAC CAACTAACCAAAGGTTAAATTTGTAAACGGATTTTTTTTTTAAAAAATCATCAAATATTCAATTA TTAACAGATTACTACATACAGTTATAAGTTATAACTTAGACAGATAACAAGTTGTGCTGTCCTT ACATGTCCATCATTATTAATATTAATATAAGTTAGTTGTCATATAAATTTGGCTAAAGATATTATTT TGTGGAAACAATCTAATATTAATAATGAATATTATAAACAATTTGATGGGTTAATTTTTCTATT TTCAATTGGTTTTAGTATAAAAATTTTTTTGAACATACGATGTACTTTTATCACATGATATGTTT GAAATCGGACCTACCATAATTTAAAGATCTATTTTTTCATTGTAAAGTCAAACCTTAAAAAGTCT TCACAAAGAAATGTATTTTTTTAATTATATTATTTTTCTTTTAAACTGAGATGCTAAATAAAC ATTTTTTTTCTTCATAATTTTCAGTTCTAGTTTTTAAAGCCTAAAACCTGAAAAGATAGTAAGT AGTGCCTCATCCGGAATGAGTACATGTTTTGTTTGATCATTTTCATCTAGTTTAGAATTTAAT CTAGTTGGTGCCGAGCTAAAGTTTTTCCAGCTTCTAAATGTGTTTATAATCGACTTATTTAGTC AAGTGAATGGGTACAAGCTAATTTTTTTTACGAGTTGATCTCAAATAGGCTGATTCATCACTA CCGATTGAATATTGACTTGTCTAGTCTTCTTAGGATTTCAAGAATGTTCTAGAACTACCCAA ACTTTGGTATACTCGTGATTAGCTCGTTTGGAAATCGGGTTGATGTCACAAAGTTTAAATTTAA CCGAACATACAAATAAATATATTTCATAATTCATCTTGTTTACTCAAGTGATCGTGTCCGAGTCT GAATTGAGTTTTTCTTCGAGTTGAGCTCGGATAACTTGGTTTTTGGTAACAAATAGAACC TGCGAGCATTCTATTGTACGTAATTGTAGAAGTCCTATGTTAGGTAAAGTGTCGAACAAGACA TTACTATGCCGTATAGTATAATAAAGAAGTTCTTCAATTCCTGGCGCTATTTGCTTGCGTATG CGTGACCCACCTAAATAGTCTCTCCTCAAACCGCCGGTCCAAGTCAACTCATCTTCGATACTC TTTAAGTATTTCTTAAGAACTTGATTTTTATATATATGTGCAGCAGCTTCTGCCAGCTTTTAAACG CCGTAATCTATCCCGTGATCGATTTTCTTCTTTTTGTTGTTTCTGATCGACTGAGCTTGCCGG ATCAAACCTCGATAACGCGA

**Table S2.** Predicted cis-elements of the *P<sub>F1MYB8</sub>* promoter

Name of the element	Sequence	Function of element	Number
ABRE	GCAACGTGTC	cis-acting element involved in the abscisic acid	1

		responsiveness	
AE-box	AGAAACAA	part of a module for light response	1
ARE	AAACCA	cis-acting regulatory element essential for the anaerobic induction	6
AT-rich element	ATAGAAATCAA	binding site of AT-rich DNA binding protein (ATBP-1)	1
ATC-motif	AGTAATCT	part of a conserved DNA module involved in light responsiveness	1
Box 4	ATTAAT	part of a conserved DNA module involved in light responsiveness	4
CAAT-box	CAAT	common cis-acting element in promoter and enhancer regions	41
CCAAT-box	CAACGG	MYBHv1 binding site	1
CGTCA-motif	CGTCA	cis-acting regulatory element involved in the MeJA-responsiveness	1
GA-motif	ATAGATAA	part of a light responsive element	1
GATT-motif	CTCCTGATTAGC	part of a light responsive element	1
GT1-motif	GGTTAA	light responsive element	4
O2-site	GATGACATGG	cis-acting regulatory element involved in zein metabolism regulation	1
TATA-box	TATA	core promoter element around -30 of transcription start	60
TCT-motif	TCTTAC	part of a light responsive element	1
TGACG-motif	TGACG	cis-acting regulatory element involved in the MeJA-responsiveness	1
circadian	CAAAGATATC	cis-acting regulatory element involved in circadian control	1

**Table S3.** cDNA sequences of *FtGL3*, *FtEGL3*, *FtTT8* and *FtTTG1* genes from Tartary buckwheat.

Gene	cDNA sequence (5'→3')
<i>FtGL3</i>	ATGGAGAAAGTTTCACAAAATCTAAGAAAACAGCTTGCAATTGCTGTAAGAAGCATTCAAT GGAGTTACTCAATTTTCTGGTCAATTCCTCTTCTGAACAAGGTGTTTTGGAATGGAGTGAA GGGTTTTACAATGGAGATATCAAAACAAGAAAACTATACAAGCTGTTGAATGGGATGTAG ACCAAATGAGCTTGAAAAGAAGTGAACAATTAAGAGAGCTTTATGAATCTCTCTTAACTGT

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CAGCTTTACAAAATGGCGAGCCTATATGGCTATGCAATGCTCACCAAGCGGATAGCAGATTG  
TTCAGTCGCTCTTCTCGCTAAGAGTGCTTCAATTCAGACTGTAGTATGCTTTCTTTCTTA  
GGAGGCGTAGTTGAGCTCGGCGCTACCGATTTGGTTTTAGAGGATCATTATTGATCAAATA  
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ACAATGTGAAGAGTTGAGATTGGTAGAGTGTTTCGAATAACAGCTTGATTATCTAATGGAGG  
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*FiEGL3*

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*FtT8*

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*FtTG1*

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TGACTGGATTTCCATCGCCTTTGCCAATAAATTGCAGATGCTGAAAGTTTAA

**Table S4.** Sequences of all primers used in this study

Primer name	Forward primer (5'→3')	Reverse primer (5'→3')
<i>FtMYB8</i>	ATGAGGAAGCCGTGTTGCGAGA	TCATCTGAATAGGTTAAGAGTAGGA
<i>PfMYB8</i>	TAGGAAGGTATGACAGTTTGATTG	TGCTTGACCAAGCTCCCTTG
<i>PBG-FtMYB8</i>	TGACTGTATCGCCGGAATTCATGAG GAAGCCGTGTTGCGA	TAGCTTGGCTGCAGGTCGACTCATCT GAATAGGTTAAGAGTAG
<i>PYFP-FtMYB8</i>	GACGAGCTCGGTACCATGAGGAAG CCGTGTTGCGAG	GCTCACCATGTGACTCTGAATAGGTT AAGAGTAGG
<i>PBI-PfMYB8</i>	TGATTACGCCAAGCTTTAGGAAGG TATGACAGTTTGATTG	ATCCTCTAGAGTCGACTCGCGTTATCG AGTTTGATCCGGC
<i>PGAD-FtMYB8</i>	TGAATTCACCCGGT ATGAGGAAGCCGTGTTGCGAGA	GAGCTCGATGGATCC TCATCTGAATAGGTTAAGAGTAG
<i>PGBK-AiTT8</i>	ATGGAGGCCGAATTCATGGATGAA TCAAGTATTATTC	CCGCTGCAGGTCGACCTATAGATTAGT ATCATGTATT
<i>PGBK-AiGL3</i>	ATGGAGGCCGAATTCATGGCTACC GGACAAAACAG	CCGCTGCAGGTCGACTCAACAGATCC ATGCAACCC
<i>PGBK-AiEGL3</i>	ATGGAGGCCGAATTCATGGCAACC GGAGAAAACAG	CCGCTGCAGGTCGACTTAACATATCCA TGCAACCC
<i>PGBK-AiTTG1</i>	ATGGAGGCCGAATTCATGGATAATT	CCGCTGCAGGTCGACTCAAACCTCTAA



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	CAGCTCCAGA	GGAGCTGCA
<i>PGBK-FtTT8</i>	ATGGAGGCCGAATTCATGGCGGCA	CCGCTGCAGGTTCGACCTAGAAGTCAT
	CCACCGCCAGG	CAGAGTGCG
<i>PGBK-FtGL3</i>	ATGGAGGCCGAATTCATGGAGAAA	CCGCTGCAGGTTCGACTCAACTTCTTA
	GTTTCACAAAATC	TCACGACTCTT
<i>PGBK-FtEGL3</i>	ATGGAGGCCGAATTCATGGAAACT	CCGCTGCAGGTTCGACTCAAGCTTTCA
	GGGCTCCAAAA	TTAGAACTCTC
<i>PGBK-FtTTG1</i>	ATGGAGGCCGAATTCATGGAGAAT	CCGCTGCAGGTTCGACTTAAACTTTCA
	TCGACTCAGGA	GCATCTGCA
<i>FtH3</i>	AATTCGCAAGTACCAGAAG	CCAACAAGGTATGCCTCAGC
<i>qFtMYB8</i>	TTGATTGCGGGGAGGTTACC	TCGGTTATCTGCGAGACGTG
<i>qFtPinG0505906200.01</i>	GCGTTCTATTCCTAAAGCTGCC	CTTCCCGCAATCAAAGACCA
<i>qFtPinG0606379100.01</i>	AAGCATGGAGAGGGGTGTTG	TCCGTTCTTCCTGGTAGCCT
<i>qFtPinG0100390100.01</i>	TCACAAACTCCTCGGGCTCA	TGGCATGGCAATGGTTAGATC
<i>AtActin2</i>	CTGGAATGGTGAAGGCTGGTT	CGATTGGATACTTCAGAGTGAGGAT
<i>Ntβ-actin</i>	TATCCAGAAAGAGTCAACCCGTCA	TAGACAAAGCACATCACGACCACAA
	CCT	CC
<i>GUS</i>	ATGCGGACTTACGTGGCAAAGGAT	GCCGACAGCAGCAGTTTCATCAATCA
	TCGA	CC
<i>NtCHS</i>	ACAAGACATAGTGGTGGTTGAAGT	GAGCCCGAGTAGCTTAGTGAGTTGGT
	GCC	AG
<i>NtCHI</i>	GTGCTCTTCCTTTTCTCGCCGC	ATTTTCTGCCACCTTCTCTGAGTATTG
<i>NtF3'H</i>	GGTGGTTGTGGCGTCTGCGTCG	AAGCCTTGGCAGAGAAAAGATGAAC
		AGA
<i>NtFLS</i>	ACAGGCAATAGCGTCAATAACAA	GCTAGCATCCGATATGAGTTTCACTA
	AATG	CA
<i>NtDFR</i>	GCTAAAGCAAACACAGTGAAGAG	CATCCATCCTGTCATCTTCTTAGCAT
	GC	A
<i>NtANS</i>	AGTATGCTAATGACCAACCCTCTG	TTGTAACCCAGGCACCATATTGTG
<i>AtCHS</i>	ACGGACATTTGAGGGAAGTTGG	AGGGTGGGCTATCCAGAAGAGG

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<i>AtCHI</i>	GCGGTTCTGGAATCTATCATCG	TCGTCCTTGTTCCTTCATCATTAGC
<i>AtF3'H</i>	TCGTGGTCGCCGCTTCTAA	ATCGGTGTCCGTAAGGTGCA
<i>AtFLS</i>	ACCGTTTGCTTTCAAGGATTACAGT TAC	AAATGATCACCGATTTTTTTTCCGTG
<i>AtDFR</i>	GCCAAACGCCAAGACGCTA	CATTCACTGTCGGCTTTATCACTTC
<i>AtANS</i>	AGGTTAGGATTTCTTGGGCTGTG	CCGTGGAGGAAACTTAGCCG
<i>AtBAN</i>	GCACGGGAAACTTAGCCTCT	GATCGGAGTTGCGACATGGA
<i>AtTT12</i>	TGGTACTCGGGTCCAATCCT	CCAAGCAAGCCGAAATCCAG
<i>AtTT19</i>	CATCTTCTTCGTCAGCCATTTGGT	CGTCACATTTCTCGCCTAACCT
<i>AtAHA10</i>	CCGAGGATTTGGACAAGCCA	GACCTAGACTTTGACTATTAGC

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