

Supplementary Table S1 PCR primers and an RNA adapter (5' to 3')

RACE PCR		
poly(T)-adaptor primer	GGCGACCCCTCGACTAGATGCGGCCGCTTTTTTTTTTTTTT	
3'RACE, 1st PCR	TTCTCCACATGGGAAAGC	GGCGACCCCTCGACTAGATG
3'RACE, 2nd (nested) PCR	TCCATCTATGGTGCAGAAGC	TCGACTAGATGCGGCCGC
5'RACE, 1st PCR	GGCGACCCCTCGACTAGATG	CTCTGCTCATACACAACATCAC
5'RACE, 2nd (nested) PCR	TCGACTAGATGCGGCCGC	TGTCTCGATATGGAGCTGGA
5'RACE, 3rd (nested) PCR	TCGACTAGATGCGGCCGC	TCAGGCAAGCATAGGTTACC
RLM-RACE PCR		
RNA adapter	GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGAUGAAA	
reverse transcription	TGTTTACATGGACCTTGCTAT	
1st PCR	GCTGATGGCGATGAATGAACACTG	TATCAGAAAGCAAGCCGTCC
2nd (nested) PCR	TGAACACTGCGTTTGCTGGATG	CGAATCCATTTGGTCATTCAT
35S-p::pre-MIR828 construct (red letters indicate restriction sites)		
	GAC TCTAG ATTTTCCTCCACATGGGAAAG	TT CAGCTC TCTTCTTCTCCACAGAATG
stem-loop pulsed PCR		
reverse transcription	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACTGGAAT	
end-point PCR	TCGCG TCTTGCTCAAATGAGT	GTGCAGGGTCCGAGGT
U6	CGGGGACATCCGATAAAATTGGAACG (U6af)	CGATTTGTGCGTGTGCATCCTTGC (U6br)
qRT-PCR in tobacco (agroinfiltration)		
<i>MYB12</i>	CTCTTGGGAAACAGGTGGTC	CAGCTTCACCCTGTTTCTTCATCCTCAACC
<i>NtDFR</i>	TGAGTTTAAAGGCATCGATAAGGA	GAATTGAAACCCCATATCCGTC
<i>iGUS</i>	TGGATCCCTACAGGTTACAG	GAATATCTGCATCGGCGAAC
<i>NtUBQ</i>	AAGATTCAGGACAAGGAAGGCA	AGCTGCTTACCTGCGAAAATCA
<i>Rluc</i>	ATCATGGGATGAATGGCCTG	GCAACATGGTTTCCACGAAG
qRT-PCR in lilies		
<i>Pri-MIR828</i>	CCTCCAGCTCCATATCGAGAC	CTCTGCTCATACACAACATCAC
<i>MYB12</i>	GGGTGAAGCTGAACCAAAAA	CGAATCCATTTGGTCATTCAT
<i>CHSa</i>	GCGAAGCTGGGACTGCAGAAGG	CAAGACCACCGTTTCCACGGTT
<i>CHSb</i>	CTGAAGCTGGCGCTGGACAAAAAG	GGTAGTGATCGGAATGCTGTGAAGA
<i>F3H</i>	GGTGCCTTTGTCGTCAATCT	AACTTCGGTGGGCTTCTTCG
<i>DFR</i>	AATGGTTGCACCGGTGTGTT	GCACGTTACAGTTCACGA
<i>ANS</i>	GGTGGTGACCAAGATGCTGT	CCAATGTGGACGAGAAGGGA
<i>ACTIN</i>	GGAGTGAGCCACACAGTTCC	ATAGCTCTTCTCCACAGAG
MYB12 mRNA-cleavage products (Figure 5)		
	GTTTGCTGGATGAAATTGAGC	CGAATCCATTTGGTCATTCAT

Supplementary Table S2 Putative targets of miR828 estimated using psRNATarget: A Plant Small RNA Target Analysis Server¹. R2R3-MYB genes are shown in red.

Accession # ²	Target annotation	Blast top hit	Expectation ³	UPE ⁴	Target aligned fragment	Inhibition
c24386_g1_i2	MYB15like (subgroup 6 R2R3-MYB)	LC218141.1 [Lilium hybrid division I (Asiatic hybrid lilies)]	2	9.996	UGGAACUCUCAUUUGAGCAAGA	Cleavage
c22900_g1_i4	MYB12 (subgroup 6 R2R3-MYB)	AB534586.1 [Lilium hybrid division I (Asiatic hybrid lilies)]	2.5	11.116	UGGAAUUCUCACUUGAGCAAGA	Translation
c20211_g1_i1	calcineurin-binding protein 1-like	XP_017701940.1 [Phoenix dactylifera]	2.5	21.912	CACGAUGGUCAUUUGAGCAAGG	Cleavage
c24386_g2_i1	MYB16 (subgroup 6 R2R3-MYB)	LC218139.1 [Lilium hybrid division I (Asiatic hybrid lilies)]	3	17.361	UGGAACGCUCACUUGAGCAAGA	Translation
c28082_g1_i1	ADP-ribosylation factor GTPase-activating protein	XP_010926864.1 [Elaeis guineensis]	3	17.961	CAAGAUCUUCAUUUUGAGCAAGC	Cleavage
c29023_g1_i1	ATP-dependent DNA helicase homolog RECG, chloroplastic	XP_010936813.1 [Elaeis guineensis]	3.5	18.032	UCGAACAUUUAGUUGAGCAAGA	Translation
c28053_g1_i1	putrescine-binding periplasmic protein-like protein	NP_174426.2 [Arabidopsis thaliana]	4	14.292	UGGAAGGCUUUAUUUGUCAGGA	Cleavage
c12438_g1_i3	myb-related protein Zm1-like (subgroup 2 R2R3-MYB)	XP_020251095.1 [Asparagus officinalis]	4	20.706	UGGAAUACCCAUUUGAAGAAGA	Cleavage
	MYB19S (subgroup 6 R2R3-MYB) ⁵	LC519097 [Lilium hybrid division I (Asiatic hybrid lilies)]	4	9.863	UGGAAUUCACACUUGAGUAAGA	Translation
c30057_g1_i5	cullin-associated NEDD8-dissociated protein-like	XP_008795267.1 [Phoenix dactylifera]	4	14.624	CUGAAUGCACAUUUUGGGGAGA	Cleavage
c27842_g1_i1	nodulin homeobox-like	XP_008805054.1 [Phoenix dactylifera]	4	15.802	CCGAAAGUUCAUUUUGCCAAGA	Cleavage
c28282_g1_i1	hypothetical protein	RWR81552.1 [Cinnamomum micranthum f. kanehirae]	4	20.989	CCUUAUGCUCUUGAGCAAGC	Translation
c30149_g1_i1	E3 ubiquitin ligase PQT3-like	XP_010917745.1 [Elaeis guineensis]	4	14.679	UGCAAGAUCCAUUUGAGCAACA	Cleavage
c23173_g1_i1	fumarylacetoacetase	XP_008776647.1 [Phoenix dactylifera]	4	17.310	GACAAUGCUAUUUGAGGGAGA	Cleavage
c29559_g1_i1	uncharacterized protein	XP_010912273.1 [Elaeis guineensis]	4	23.804	UAUAAUUAUUAUUGAGGAGGA	Translation

¹ <http://plantgrn.noble.org/psRNATarget/>

² Lollypop tepal transcriptome (Suzuki et al. 2016)

³ Expectations lower than 4 are shown

⁴ UPE: Target accessibility-maximum energy to unpair the target site (Less energy means more possibility that microRNA is able to cleave target mRNA)

⁵ Yamagishi, 2020b

Supplementary Table S3 Overview of the small RNA-seq

Trimming summary

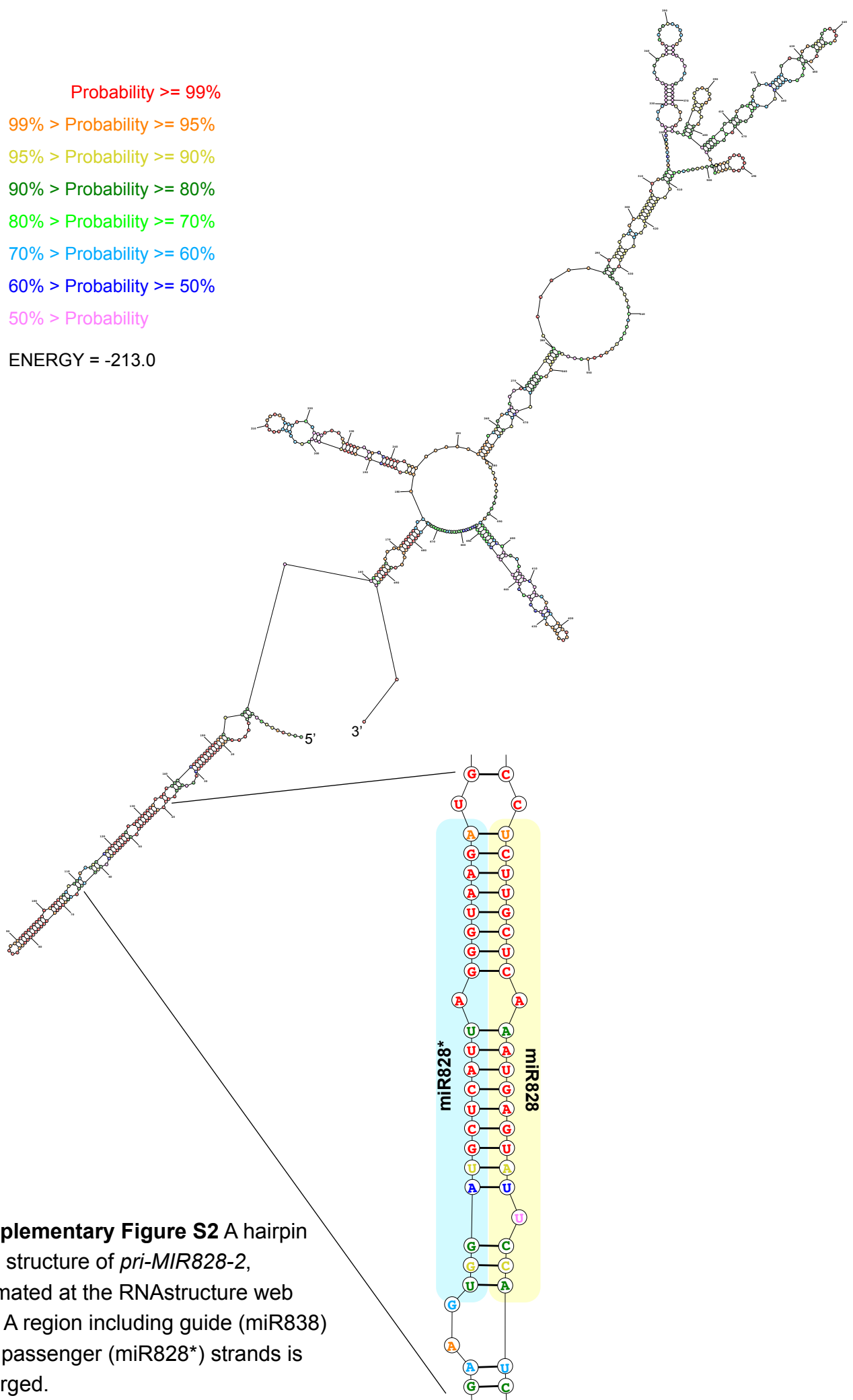
Sample Name	# Reads	# Reads Pairs	# Excluded Read Pairs	Matched		Mismatched	
				# Pairs	% Pairs	# Pairs	% Pairs
Lower half	43,917,562	21,958,781	2,454,028	18,887,232	86.01	617,521	2.81
Upper half	43,401,220	21,700,610	2,066,021	18,997,636	87.54	636,953	2.94

Mapping summary

Sample Name	# Input Reads	# Total Mapped Reads	% of Total Mapped Reads
Lower half	18,887,232	874	0.0046
Upper half	18,997,636	75	0.0004

		miR828
pri-MIR828-1	GAGCATCATCTCCATAATTTTCCTCCCACATGGGAAAGCC	TCTTGCTCAAATGAGTATTCCA
pri-MIR828-2	GAGCATCATCTCCATAATTTTCCTCCCACATGGGAAAGCC	TCTTGCTCAAATGAGTATTCCA
		miR828*
pri-MIR828-1	GTGCAGAAGCTTTGGGTCCTCTCGGAGCTTCATGCATTGCAGAAG	TGGATGCTCATTAGGGTAAGATG
pri-MIR828-2	GTGCAGAAGCTTTGGGTCCTCTCGGAGCTTCATGCATTGCAGAAG	TGGATGCTCATTAGGGTAAGATG
pri-MIR828-1	CATTCTGTGGGAGGAAGAAGAGATATTCAAATGG	TGGCCAACAGGTTCTCAGCAGCATAA
pri-MIR828-2	CATTCTGTGGGAGGAAGAAGAGATATTCAAATGG	TGGCCAACAGGTTCTCAGCAGCATAA
pri-MIR828-1	ATTTCTCCGCTTATTTTGGTAGCCTATGCTTGCCTGAGTGCCCTCTGGTACGAGTGAGC	CCCATC
pri-MIR828-2	ATTTCTCCGCTTATTTTGGTAGCCTATGCTTGCCTGAGTGCCCTCTGGTACGAGTGAGC	CCCATC
pri-MIR828-1	CCCTCCATGCAAAATCCCTCCCCGACTTATCATATACCTTCATCTTGTTAATTGTTTTCTGTGTCTCC	
pri-MIR828-2	CCCTCCATGCAAAATCCCTCCCCGACTTATCATATACCTTCATCTTGTTAATTGTTTTCTGTGTCTCC	
pri-MIR828-1	TCCTCCAGCTCCATATCGAGACAATCACACAGCCC	CACCTGCGCCGACACTGAGGCAAGCAGTGCCG
pri-MIR828-2	TCCTCCAGCTCCATATCGAGACAATCACACAGCCC	CACCTGCGCCGACACTGAGGCAAGCAGTGCCG
pri-MIR828-1	CCCTCCAGCTACTCAGCATCTCCTTAAACCTTCAAAGTTTCTGGTGTGTGCA	TGGGCCCGAGAGTCG
pri-MIR828-2	CCCTCCAGCTACTCAGCATCTCCTTAAACCTTCAAAGTTTCTGGTGTGTGCA	TGGGCCCGAGAGTCG
pri-MIR828-1	GCAATTGCACCTAAACCTGCAAAGTACCTGTGATGTTGTGTATGAGCAGAGGGGCGGGTAT	CTACACC
pri-MIR828-2	GCAATTGCACCTAAACCTGCAAAGTACCTGTGATGTTGTGTATGAGCAGAGGGGCGGGTAT	CTACACC
pri-MIR828-1	TCCAAAAAGTCTATGGCCAGGGGGCGGCAACTGTACCTACAT	CTAAAAAAGATTAAATTAATTAAT
pri-MIR828-2	TCCAAAAAGTCTATGGCCAGGGGGCGGCAACTGTACCTACAT	CTAAAAAAGATTAAATTAATTAAT
pri-MIR828-1	TAAAAAGACCTTCTGGTCTTAGT	
pri-MIR828-2	TAAAAAGACCTTCTGGTCTTAGT	
pri-MIR828-1	----- AAAAAAAAAAAAAAAAAA	
pri-MIR828-2	T TACTGTCTTGAATACCAAAAAAAAAAAAAAAAAA	

Supplementary Figure S1 Nucleotide sequence alignment of *pri-MIR828* in Lollypop. Guide (miR828) and passenger (miR828*) strand sequences are shown in red background.



Supplementary Figure S2 A hairpin loop structure of *pri-MIR828-2*, estimated at the RNAstructure web site. A region including guide (miR838) and passenger (miR828*) strands is enlarged.

A

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TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
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B

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TGAACACTGCGTTTGCTGGATGAAA-----TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA-----TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA-----TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA-----AGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA-----TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAACTCACTTGAAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA-----TTGGGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA-----AGCAAGAGAAAAAGTGAATGTCGAACA...
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C

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TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA---AGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
TGAACACTGCGTTTGCTGGATGAAA TTGAGCAAGAGAAAAAGTGAATGTCGAACA...
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Supplementary Figure S3 Sequences of the RLM-RACE PCR products of *MYB12*. Nucleotides corresponding to the RNA adapter are shown in blue letters and those corresponding to *MYB12* sequences are shown in black letters. A: RNA was isolated from tobacco leaves infiltrated with the 35S-p::MIR828 and 35S-p::MYB12 constructs. B: RNA was derived from tobacco leaves infiltrated with the 35S-p::MIR828, 35S-p::MYB12 and other constructs. C: RNA was isolated from the lower halves of Lollypop tepals at stage 4. The results shown in A, B, and C are summarized in Figure 2B, 2D, and 3B, respectively.

		microRNA828 in Arabidopsis	3'- ACCTTATGAGTAAA T C G T T C T -5'			Expectation	UPE	Inhibition
		microRNA828 in other plants	3'- ACCTTATGAGTAAACTCGTTCT -5'					
Monocots	Lily	MYB12	5'- ATATTAAGAACTAC	TGGAATTCTCACTTGAGCAAGAGAAAAGTGAATGTC-3'	(2)	2.5	11.1	Translation
		MYB19S	5'- ATATAAAAAAATACT	TGGAATTCTCACTTGAGTAAGAGAAAAGTGAATGTT-3'	(3)	4	9.9	Translation
		MYB15like	5'- ACGTAAAGAACCACT	TGGAACCTCTCATTTGAGCAAGAGAACTCATTTCCGGA-3'	(2)	2	10.0	Cleavage
		MYB16	5'- ACGTGAAGAACTACT	TGGAACGCTCACTTGAGCAAGAGAAAGTTGTAGCTCGA-3'	(2)	3	17.4	Translation
	Anthurium	AaMYB2	5'- ACATCAAGAACTATT	TGGAACCTCGTACTTGGGCAAGAGAGGTAGTTTCCCAG-3'	(5)			
	Oil palm	EgVIR	5'- ACATCAAGAACTACT	TGGAACACTCACTTGGGCAAGAGAAAGTAG- - - - -3'	(3)	4.5	13.2	Translation
	Onion	AcMYB1	5'- ACATAAAAAAATACT	TGGAACACTCACTTGAGCAAGAGAGAAAGT- - - - -TA-3'	(3)	4	13.9	Translation
	Phalaenopsis	PeMYB2	5'- AAAATAAGAACTACT	TGGAACACCAACCTTGCGCAAGAGAGACCAACCTCAC- - -3'	(7)			
		PeMYB12	5'- AGATAAAAAATTACT	TGGAACACCAACCTTAGGTAAAAAGCTTCGC- - - - AA-3'	(7)			
		PeMYB11	5'- AGATCAAGAACTATT	TGGAACACGACTCTATCGAAGAGAGATTCAAACAA- - -3'	(9)			
Eudicots	Arabidopsis	AtPAP1	5'- ACGTCAAGAAATTACT	TGGAACACTCATCTGAGTAAGAGAACAA- - -TGAACC- -3'	(2)	3	16.7	Translation
		AtPAP2	5'- ATGTCAAAAAATTACT	TGGAACACCCCATCTGAGTAAGAGAACAA- - -TGAGTCT-3'	(4)			
		AtMYB113	5'- ATGTCAAGAAATTACT	TGGAACACTCATTTGAGTAAGAGAGCA- - -CGATGAA-3'	(1)	1.5	17.5	Cleavage
		AtMYB114	5'- ATGTCAAGAACTACT	TGGAACACCCCATCTGAGTAAGAGAGCA- - -TGAACC- -3'	(3)	4	16.1	Translation
	Petunia	PhAN4	5'- ATGTGAAAAAATACT	TGGAACACTAACTTCTAAGGAGGTCAAAGTTTGGCT-3'	(8)			
		PhDPL	5'- ATGTGAAAAAATACT	TGGAATACTCAACTTCTAAGGAGGTCAAACTTTGCT-3'	(6)			
		PhPHZ	5'- ATGTCAAAAAATTACT	TGGAACACACACCTGCAGAGGAGAGTTAA- - -TTGCT-3'	(7)			
		PhAN2	5'- ATGTCAAAAAATACT	TGGAACACCCCACTTCGAAAGAGTTAATTGCTCCT-3'	(7)			
	Snapdragon	AmROSEA1	5'- ACGTGAAGAACTTTT	TGGAATACTCATGTGGGGAAGAGATTTAG- - - - GC-3'	(2)	3.5	14.4	Translation
	Apple	MdMYB10a	5'- CTGTGAAAAAATTACT	TGGAACACTCGATTGCGGATCGATTTC- - - - -3'	(6)			
	Grape	VIMYBA1-1	5'- ATGTCAAGAACTATT	TGGCATGGTCAACCATTTGAAAAAGAGAGG- - - - TT-3'	(10)			
		VIMYBB1-1	5'- AGATAAAAAAATGTCT	TGGCACACACCACTGAGAGAGAGGCTCAAGAAAAAA-3'	(7)			
	Salvia	SmMYB36	5'- AGATCAAGAACTACT	TGGAATTGCTCACTTGAGGAAGAGAAAGC- - -CATGTTG-3'	(2)	3.5	14.1	Translation

R3 repeat

Supplementary Figure S4 Putative miR828 target sites in subgroup 5 members (*Phalaenopsis*) and subgroup 6 members (other species) of R2R3-MYB sequences regulating anthocyanin biosynthesis in monocots and eudicots. Number of mismatched nucleotides is shown in parentheses. Expectation, UPE, and Inhibition were estimated using "psRNATarget: A Plant Small RNA Target Analysis Server" (see Supplementary Table S2). Expectations lower than 5 are shown. The R3 repeat region is represented by a blue bar.

Accession numbers (in parentheses) are AaMYB2 (KU726561) in *Anthurium andraeanum*, Virescens (EgVIR, KJ789862) in *Elaeis guineensis*, AcMYB1 (KX785130) in *Allium cepa*, PeMYB12 (AIS35929), PeMYB2 (AIS35919), and PeMYB11 (AIS35928) in *Phalaenopsis equestris*, AtPAP1 (NM_104541), AtPAP2 (NM_105310), AtMYB113 (NM_105308), and AtMYB114 (NM_105309) in *Arabidopsis thaliana*, PhAN2 (AF146702), PhAN4 (HQ428105), PhDPL (HQ428109), and PhPHZ (HQ428103) in *Petunia hybrida*, AmROSEA1 (DQ275529) in *Antirrhinum majus*, MdMYB10a (DQ267896) in *Malus × domestica*, VIMYBA1-1 (AB073010) and VIMYBB1-1 (AB073016) in *Vitis labrusca* × *V. vinifera*, and SmMYB36 (KF059390) in *Salvia miltiorrhiza*.