Supplementary Material

Functional and transcriptional regulation of the anthocyanidin acyl modifier gene *Gs5AT* of *Gentiana sino-ornata*

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Supplementary Material

Supplementary Fig. S1 Open reading frame and predicted amino acid sequence of *Gs5AT*

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        10        20        30        40        50        60        70        80        90       100       110       120
  1  ATGGAGCAAATCCAAATGATGAAAGTTCTTGAAAAATGCCAAGTTACACCACCCTCTGACACAACAGATGTCGAGTTATCGCTACCGATAACATTCTTCGATATCCCCTGGTTGCATTTA
  1   M  E  Q  I  Q  M  M  K  V  L  E  K  C  Q  V  T  P  P  S  D  T  T  D  V  E  L  S  L  P  I  T  F  F  D  I  P  W  L  H  L

       130       140       150       160       170       180       190       200       210       220       230       240
121  TATAAGATGTCGTCTCTTTTGTTTTACGATTTTCCTTACCCGAAAACGCATTTCTTGGACACAATTATCCCTAATCTCAAGGCCTCTTTGTCTCTCACTCTAAAACACTACCTCCCACTT
 41   Y  K  M  S  S  L  L  F  Y  D  F  P  Y  P  K  T  H  F  L  D  T  I  I  P  N  L  K  A  S  L  S  L  T  L  K  H  Y  L  P  L

       250       260       270       280       290       300       310       320       330       340       350       360
241  TGTGGCAATTTGTTAATGCCCATCAAATCAGGCAAAAAGCCAAAGTTTCATTACTCGCGTGGTGACGGAGACTCGATGGCTTTGGTCTTCGTGGAGTCTGATCAGGATTTTGACTACCTT
 81   C  G  N  L  L  M  P  I  K  S  G  K  K  P  K  F  H  Y  S  R  G  D  G  D  S  M  A  L  V  F  V  E  S  D  Q  D  F  D  Y  L

       370       380       390       400       410       420       430       440       450       460       470       480
361  AAAGGTCATCATCACACGCGTGATTCCAATGATTTGCACGCGCTTCTTTATGTTATGCCACGGACCGTAAGGATCACGCAAGAATATAAAGTGATCCCGCTCGTAGCTGTGCAAGTAACG
121   K  G  H  H  H  T  R  D  S  N  D  L  H  A  L  L  Y  V  M  P  R  T  V  R  I  T  Q  E  Y  K  V  I  P  L  V  A  V  Q  V  T

       490       500       510       520       530       540       550       560       570       580       590       600
481  GTCTTTCCTAACCATGGCTTAGCCGTGGCTCTGACGACACATCATTCAATTGGAGATGGAAGAAGTTTTGCGAAGTCATGCATGCTTGGGCCTATATGAACAAATTTGGAAAAGACGCG
161   V  F  P  N  H  G  L  A  V  A  L  T  T  H  H  S  I  G  D  G  R  S  F  A  K  F  M  H  A  W  A  Y  M  N  K  F  G  K  D  A

       610       620       630       640       650       660       670       680       690       700       710       720
601  GACTTATCGTGTAGAAATCTTATCCCGTCTTTTGATAGATCATCGATAATCAAAGATCCGAATGGCCTAGAGGGAAATATTTTGGAACGAAATGCAAGACGTTCTTGAATTGTTCTCCCGA
201   D  L  S  C  R  N  L  I  P  S  F  D  R  S  S  I  I  K  D  P  N  G  L  E  E  I  F  W  N  E  M  Q  D  V  L  E  L  F  S  R

       730       740       750       760       770       780       790       800       810       820       830       840
721  TTTGGAAGCAAACCCCCTCGATTCAACAAGGTACGAGCCCACGTATGTCCTATCCCTTGTTGAAATCGAGGAGGCTAAAGAACAGAGTACTGAATCTCAGAGGATCCGGACCAGCAATACGC
241   F  G  S  K  P  P  R  F  N  K  V  R  A  T  Y  V  L  S  L  V  E  I  E  R  L  K  N  R  V  L  N  L  R  G  S  G  P  A  I  R

       850       860       870       880       890       900       910       920       930       940       950       960
841  GTAACAACATTCACAGTGACATGCGGATACGTATGGACATGCATGGTCAAATCAAAAGATGCCATATCAGAGGAATCACCAAACGACGAAAATGAGCTCGAATACTTCAGTTTTACAGCA
281   V  T  T  F  T  V  T  C  G  Y  V  W  T  C  M  V  K  S  K  D  A  I  S  E  E  S  P  N  D  E  N  E  L  E  Y  F  S  F  T  A

       970       980       990      1000      1010      1020      1030      1040      1050      1060      1070      1080
961  GATTGCCGAGGGCTTCTGACGCCCCCGTGTCCACCTAACTACTTCGGGAATTGCATTGCGCCGTGCCTTGCAAAAGCAACACATAAAGAGTTACTTGGAAGTGAAGGGCTTCTTGTTGCA
321   D  C  R  G  L  L  T  P  P  C  P  P  N  Y  F  G  N  C  I  A  P  C  L  A  K  A  T  H  K  E  L  L  G  S  E  G  L  L  V  A

      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
1081 GTTGCAGCTATCGGGGATGCCATCGAGAAAAGGGTGCAGAACAAAGAAGGTGTTCTTGCAGATGCGAAAACATGGTTATCAGAATCTAAAGGAATCCCTTCAAATAGACTGCTCGGGATT
361   V  A  A  I  G  D  A  I  E  K  R  V  Q  N  K  E  G  V  L  A  D  A  K  T  W  L  S  E  S  K  G  I  P  S  N  R  L  L  G  I

      1210      1220      1230      1240      1250      1260      1270      1280      1290      1300      1310      1320
1201 AGCGGATCTCCTAAGTTCGATTCGTATGGTGTAGATTTCGGATGGGGAAAGCCAGCAAAATTTGAAAAGGCTTCTATTGATTATGCAGAATTGATTTCTCTGATTGAGTCTAGGGAGTTT
401   S  G  S  P  K  F  D  S  Y  G  V  D  F  G  W  G  K  P  A  K  F  E  K  A  S  I  D  Y  A  E  L  I  S  L  I  E  S  R  E  F

      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420
1321 GATAAAGGTGTGGAGGTTGGAGTGTCATTCCCTAAGATTCATATGGATGCATTTTCAAGAATCTTTGAACAAGGATTATTGCCATTAGTCAAGTCTTTTTAA
441   D  K  G  V  E  V  G  V  S  F  P  K  I  H  M  D  A  F  S  R  I  F  E  Q  G  L  L  P  L  V  K  S  F  *
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Supplementary Tables S1 and S2 show the reaction systems for three rounds of thermal asymmetric PCR. Take 5μL of each round of reaction solution, perform electrophoresis for 25 minutes using 2% agarose gel at 220V and 135A, stain with GelGreen nucleic acid stain, observe the results in a gel imager, recover the third-round PCR products that meet the primer length difference, and sequence them using Gs5ATP3 as the primer.

Supplementary Table S1 PCR reaction procedures and conditions for *Gs5AT* promoter cloning

The first round of PCR reaction	
94°C 1min; 98°C 1min;	
94°C 30s;	
65°C 1min;	15 cycles
72°C 3min;	
94°C 30s; 25°C 3min; 72°C 3min;	
94°C 30s; 65°C 1min; 72°C 3min;	
94°C 30s; 65°C 1min; 72°C 3min;	15 cycles
94°C 30s; 25°C 1min; 72°C 3min;	
72°C 10min;	

94°C 30s; 65°C 1min; 72°C 3min;	
94°C 30s; 65°C 1min; 72°C 3min;	15 cycles
94°C 30s; 25°C 1min; 72°C 3min;	
72°C 10min;	

The third round of PCR reaction

94°C 30s; 65°C 1min; 72°C 3min;	
94°C 30s; 65°C 1min; 72°C 3min;	15 cycles
94°C 30s; 25°C 1min; 72°C 3min;	
72°C 10min;	

Supplementary Table S2 PCR reaction system for Gs5AT promoter cloning

| 1st PCR Reagents	Dosages/μL	2nd PCR Reagents	Dosages/μL	3rd PCR Reagents	Dosages/μL
gDNA	1	1st PCR Reaction solution(100-fold diluted solution)	1	2nd PCR Reaction solution(100-fold dilute	1
Dntp mixture (2.5Mm each)	8	Dntp mixture (2.5Mm each)	8	Dntp mixture (2.5Mm each)	8
10*La PCR Buffer II(Mg^{2+} pius)	5	10*La PCR Buffer II(Mg2+ pius)	5	10*La PCR Buffer II(Mg2+ pius)	5
Takara Taq	0.5	Takara Taq	0.5	Takara Taq	0.5
Ap(100 pmol/μL)	1	Ap(100 pmol/μL)	1	Ap(100 pmol/μL)	1
Gs5ATP1 (10 pmol/μL)	1	Gs5ATP2 (10 pmol/μL)	1	Gs5ATP3 (10 pmol/μL)	1
DDH$_2$O	33.5	DDH2O	33.5	DDH2O	33.5
Total	50	Total	50	Total	50

Supplementary Fig. S2 Promoter sequence of *Gs5AT*

Note: the sequence in the dotted box is TATA-box, the underlined sequence is CAAT-box, and the font bold is Gs5AT initiation codon

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AAAAAAAAAAAAATAAGAATAGTATGATATGTCACATCATACCATTATATGTTTTGTATAC
ACCATTAGTAGTGATCTCATCTTGCAAAAGCTCATTAGTAGTGATCTCATCTTGTAAAAT
ACTAGCAATTATTCGATGCATTGTGCTTTTAAACTCTAAAATCTAAGCTAATTAAAAACT
ATTTGTTACATTGCATAAAAAAATCAGAACCTAAAAGCTAAATGATGATACGGATGCCA
CGATCCCTTTATCAACAATCAACTCGTCGGAGACTAAGTCAAACCACCAGTCATGGCCT
CGCCAGCGATGACGTCGACGCCCGGGGGCCGCCCTCCACGACGTCGCCAATAGCTTAC
ACATCAGGGGGGGCACTCAATGGCAACAGCTCGCCGACACCAGCTCGCCAACATCAG
TTTTCCATGCTTTACTACCTTTTATATCAAGAACAACACTTGTAATATCATAGAGATAATC
CTAAATTTACATTGTCAGGGCGTCGCCCTATTAGCCGTCTGGCGGGGCGTCGCTGCCAC
GTGTCTCTTTTCTAGGGCATCCCTCTCCCTATAAATACCCCTATGTGTAATAGCTATAAAT
CTAACTTATCTTGGGTGAAATATACTATTCTTGCTTTTGGTCTCTTGTGAGTGTTTCCTTC
TCTCTTTGTGTTTACTATCACCACCGCCGGAATACTCACCCGTACGGCGTCGCCACCGT
CCTCCACCATCTTGCTGCACCACCAAAAGCCAAGATATCCTCCAAGTGATCTCTTGATC
ACCACCGTGGTAGTATTATACGCTATCAAGTGGCTCCGTTACCGGGAAAGTACTACTTT
CAGCAAGCTTCTTCCTTCTTCTCCGGCGAAAACAGGAGGTTTTTGTCCTCTGCTCAGC
GTCGCCGACGAGGACTTTCTCTTACACCGCCGTGGGTCGTACGTTTTCTTCACCAGCAT
CAAGCTTCACCAACGGGATTACTTTTATTTCTCCACTGATTGCTACCTCCAGGTTCCGTT
TTCTCCATACTTAAGCTCTTTTCTTTGATTTCCTAACTGCTACTTGCGTTCTTGAGCTTG
GTACAACTCCGACCACCGCATTTTTAGGCAGCATTTCATCTCTTGGTTGTTCATTCTGCT
GAGCATCGTTGGAAGTTGTACTATTCGGGGTTTTGTTGTCATCAAAATCGTACTACTTC
GCTTGAGTAGCTACCTGCGACCACCGTCTCCATCTTATCACGGTAGCCGGATTTCTATCC
CGCCGTGATTTTCTGGGTTTTTTGGGTATCATTCGTACTGATTTCGCCGGAATCTGGGTT
TCTACGGCTATTTCTGGGTCTGATCTCGCCGGAATCGTCATTCTTGTGCCGCAAATACTC
GATTCGGCGCCGGAGTTTGTACTATTTGTCCTCAGCCACTTGTGTTGCGCCATGTGTCC
TGCCTTCACACGCTTTCTGCATATTCTGCTAGTGGTGCACCCGCTGTACTATTGTCAGAA
GTTTGTTATTACGATCTCCAATCACGGTTTGACACATGTCCTGTCCTTTGATACTATCTG
AAAGTTTGTTGCTCTGACATCATGACTGGACCACCTCCAATTCACCCTCAGCAATTCAC
CTCGCACCAAGATGCCCTTGTCATTCAACCAGAAGAAAATCCAACTGCCAGTATTGAA
GCTCGCGACCTGATCGAGCTTCGTAAGAAAAACCCCCCAACAAAATCTTGATTCAAAA
GTACACAGTTTTTTGCTATCATCATTAGTCCATTATG
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Supplementary Fig. S3 Distribution of predicted light response elements, MYBCORE, MYCCORE in promoter sequence of *Gs5AT*
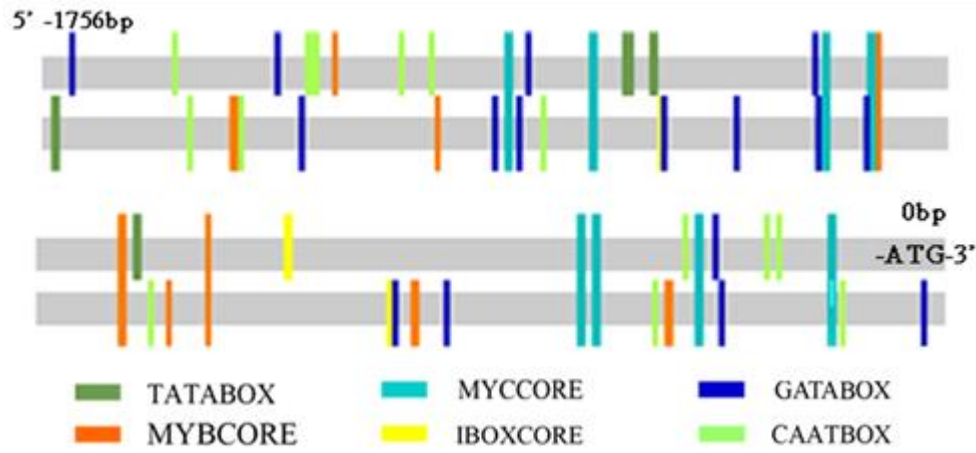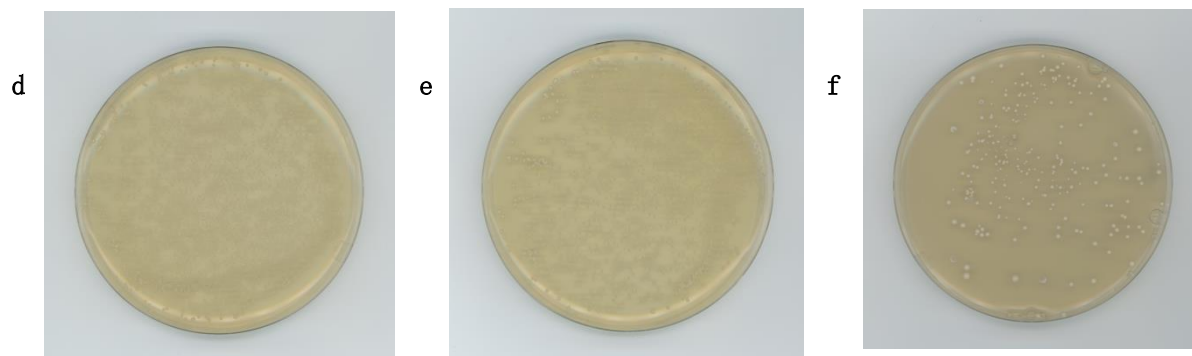

Supplementary Fig. S4 The Y1HGold[p53-AbAi] / Y1HGold[GS Promoter-pAbAi] on SD/-Ura media agar plates
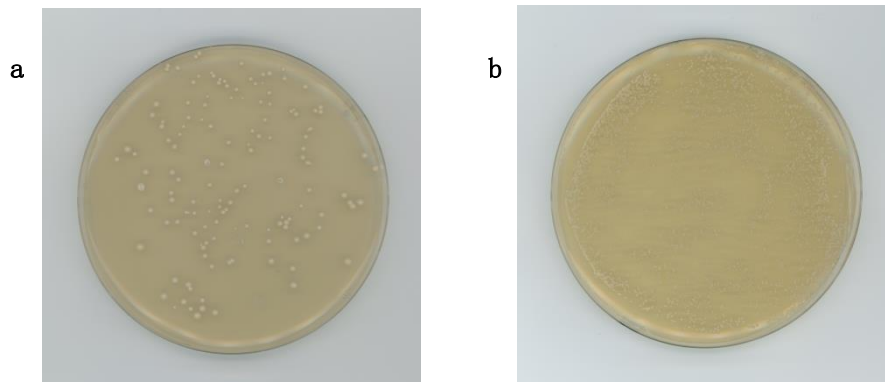
Note:A:Y1HGold[p53-AbAi];B:Y1HGold[GS Promoter-pAbAi]

Supplementary Fig. S5 The bait-reporter Y1HGold strains were cultured on SD/-Leu-Ura agar plates

Supplementary Table S3 Primer sets used for amplifying and sequencing

Primer	Sequence
AP1	Genome Walking Kit provided, sequence unknown
AP2	Genome Walking Kit provided, sequence unknown
AP3	Genome Walking Kit provided, sequence unknown
AP4	Genome Walking Kit provided, sequence unknown
SP1	TATAGGCCCAAGCATGCATGAACT
SP2	AGACCGTTACTTGCACAGCTACGA
SP3	TGGAATCACGCGTGTGATGATGAC
SP4	GTAGTACTTTCCCGGTAACG
SP5	TGAGATCACTACTAATGAGC
SP6	AATGGACTAATGATGATAGC
pMiniT 2.0 vector Cloning Analysis Forward Primer	ACCTGCCAACCAAAGCGAGAAC
pMiniT 2.0 vector Cloning Analysis Reverse Primer	TCAGGGTTATTGTCTCATGAGCG
GS Promoter F	gaaaagcttgaattcgagctGAATAGTATGATATGTCACATCATAC
GS Promoter R	atacagagcacatgcctcgaAATGGACTAATGATGATAGC
pAbAi Seq F	TGTTCGGAGATTACCGAATC

Supplementary Table S4 Species name in phylogenetic tree and GenBank registration number

	Species name	GenBank registration numbers
flavonoid/anthocyanin acyltransferase	*Glandularia x hybrida*	VH3MAT (AAS 77402)
	Lamium purpureum	LP3MAT (AAS 77404)
	Nicotiana tabacum	NtMAT (BAD93691)
	Perilla frutescens	Pf5MaT(AAL50565)
	Dahlia pinnata	DV3MAT(AAO12206)
	Chrysanthemum x morifolium	DM3MAT1 (AAQ63615)
	Chrysanthemum x morifolium	Dm3MaT2 (AAQ63616)
BAHD acyltransferase	*Zea mays*	Glossy (CAA61258.1)
	Arabidopsis thaliana	CER (AAM64817)
	Hordeum vulgare	ACT (AAO73071.1)
	Fragaria vesca	VAAT (CAC09062.1)
	Fragaria x ananassa	SAAT (AAG13130.1)
	Rosa hybrid cultivar	RhAAT (AAW31948.1)
	Rauvolfia serpentina	Vinorine synthase (CAD89104.2)
	Catharanthus roseus	DAT (AAC99311.1)
	Nicotiana tabacum	NtEBT (AAN09798)
	Petunia x hybrida	BPBT (AAU06226.1)
	Cucumis melo	CmAAT1 (AAW51125)
	Clarkia breweri	CbBEBT (AAN09796)
	Vitis labrusca	AMAT (AAW22989)
	Cucumis melo	CmAAT2 (AAL77060)
	Malus domestica	MpAATt (AAU14879)
	Taxus canadensis	DBNTBT (AAM75818)
	Taxus cuspidata	DBAT (AAF27621)
	Taxus cuspidata	BAPT (AAL92459)
bHLH	*Arabidopsis thaliana*	GL3(AT5G41315)
	Arabidopsis thaliana	EGL3(AT1G63650)
	Arabidopsis thaliana	NP_001329692(AT4G00480)
	Lilium hybrid division I	LHBHLH1(BAE20057)
	Zea mays	B1(AGO65322)
	Gentiana triflora	GTBHLH1(BAH03387)
	Arabidopsis thaliana	TT8 (AT4G09820)
	Dahlia pinnata	DVLVS(BAM84241)
	Arabidopsis thaliana	AAC98450
	Arabidopsis thaliana	AAC64222
	Arabidopsis thaliana	At3g26744
	Arabidopsis thaliana	AT5G65640
	Arabidopsis thaliana	AED91564
	Arabidopsis lyrata subsp. petraea	AHM26490

Elaeis guineensis	XP_010906815
Hordeum vulgare subsp. vulgare	BAJ92170
Oryza sativa Japonica Group	XP_015618080
Zea mays	PIL5 (ONM07085)

Supplementary Table S5 Primer sets used for amplifying and sequencing

Primer name	sequence（5'-3'）
Gs5AT-F	CTAGAAGGCCTCCATGGGGATCCCCAAACGACGAAAATGAGC
Gs5AT-R	GCCTCGAGACGCGTGAGCTCCCATCCGAAATCTACACCATA
PTRV2-seqE	CACATATTCGCACGTATGAAGT
Gsbhlh7-F	TCTAGAAGGCCTCCATGGGGATCCGCCAAGCAAAAGACAGCC
Gsbhlh7-R	GGGCCTCGAGACGCGTGAGCTCGTGTTCCTCCTTGCTCGTATT
PTRV2-seqS	AACAAAGTCCGTTCCCCTAT