

Figure S1 Establishment of *BcWRKY33A*-overexpressed and silenced lines.

(a) The relative expression of *BcWRKY33A* in WT and 35S:*BcWRKY33A* lines. N/A represents no expression. (b) The relative expression of *BcWRKY33A* in pTY and pTY-BcWRKY33A. Error bars represent standard deviation (\pm SD). The data are the mean \pm SD of three biological replicates. Asterisks indicate significant differences from the control (** $p < 0.01$, *** $p < 0.001$) using Student's *t*-test.

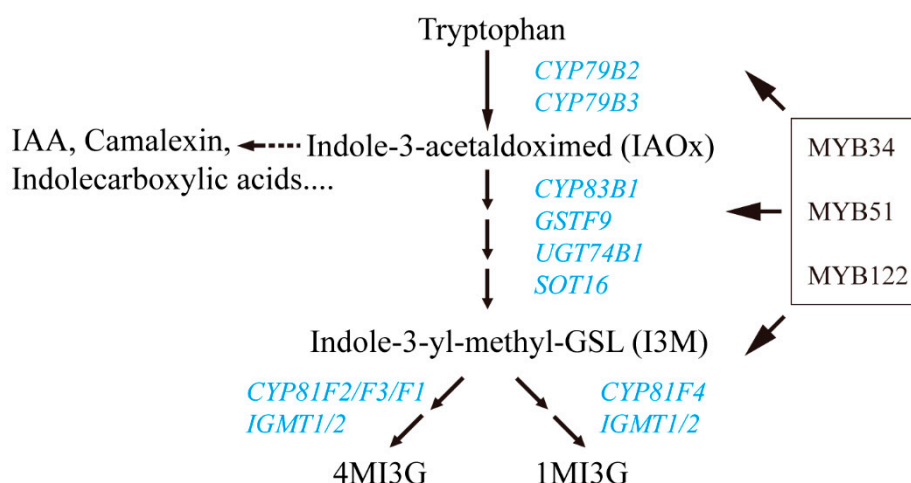


Figure S2 Schematic plot of the IGSs biosynthetic pathway.

Figure S2 is adapted from a previous study (Frerigmann & Gigolashvili, 2014), in *Arabidopsis*, IAOx was not only needed for the formation of IGSs, but also the precursor of indole-3-acetic acid (IAA), camalexin and indole-carboxylic acids, etc. (indicated by dotted arrow). Enzymatic steps are indicated by black arrows. 4MI3G, 4-methoxyindole-3-ylmethyl glucosinolate; 1MI3G, 1-methoxyindole-3-ylmethyl glucosinolate.

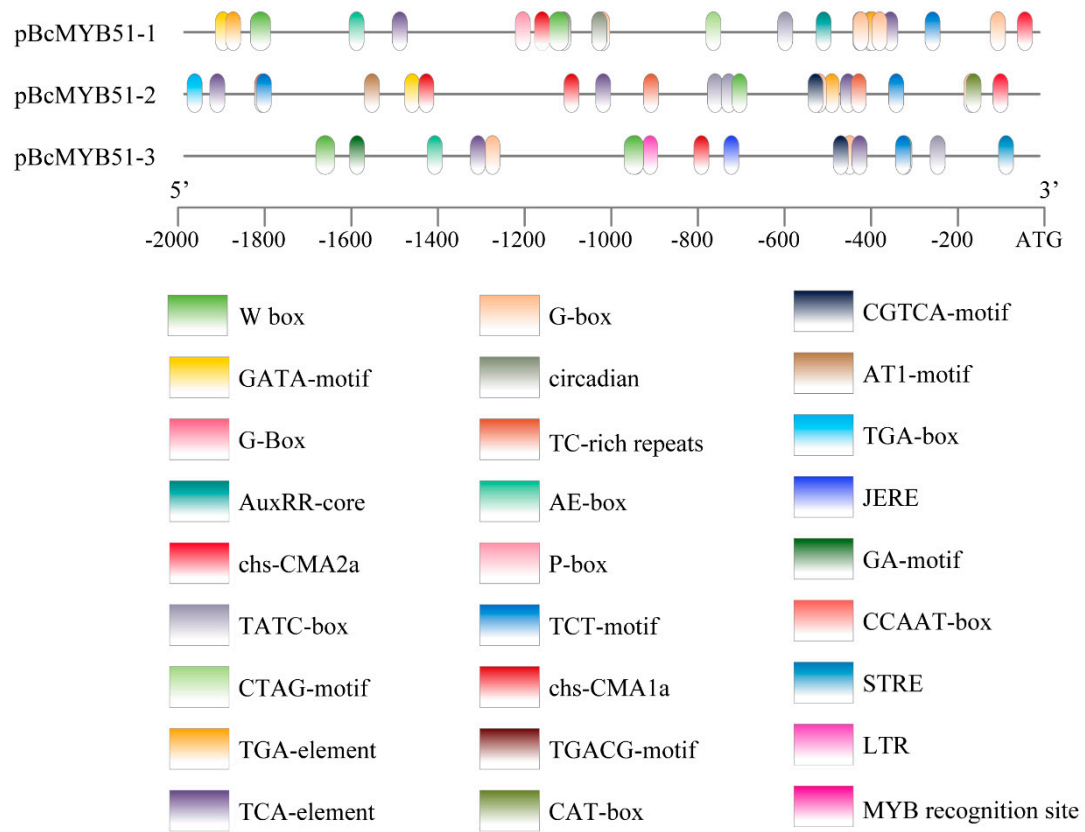


Figure S3 The *cis*-elements distribution in the promoters of *BcMYB51*s.

pBcMYB51-1, pBcMYB51-2, pBcMYB51-3 represent the promoter of *BcMYB51*-1, *BcMYB51*-2, *BcMYB51*-3, respectively.

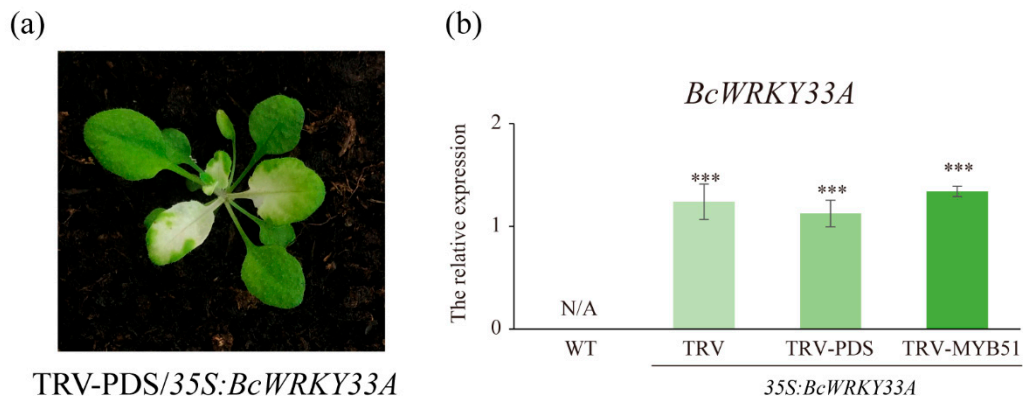


Figure S4 The efficient TRV-based VIGS system.

(a) The phenotype of *TRV-PDS/35S:BcWRKY33A* lines. (b) The relative expression level of *BcWRKY33A* in WT, *TRV/35S:BcWRKY33A*, *TRV-PDS/35S:BcWRKY33A*, *TRV-MYB51/35S:BcWRKY33A* lines. N/A represents no expression. Error bars represent SD. Data are means \pm SD of four biological replicates. Asterisks indicate significant differences from the WT (***) $p < 0.001$ using Student's *t*-test.

Table S1 The function prediction of *cis*-elements in promoters of *BcMYB51s*.

<i>cis</i>-elements	function prediction
AE-box	part of a module for light response
AT1-motif	part of a light responsive module
AuxRR-core	cis-acting regulatory element involved in auxin responsiveness
CAT-box	cis-acting regulatory element related to meristem expression
CCAAT-box	CCAAT-box
CGTCA-motif	cis-acting regulatory element involved in the MeJA-responsiveness
chs-CMA1a	part of a light responsive element
chs-CMA2a	part of a light responsive element
circadian	cis-acting regulatory element involved in circadian control
CTAG-motif	CTAG-motif
GA-motif	part of a light responsive element
GATA-motif	part of a light responsive element
G-Box	cis-acting regulatory element involved in light responsiveness
JERE	JERE
LTR	cis-acting element involved in low-temperature responsiveness
MYB recognition site	MYB recognition site
P-box	gibberellin-responsive element
STRE	STRE
TATC-box	cis-acting element involved in gibberellin-responsiveness
TCA-element	cis-acting element involved in salicylic acid responsiveness
TC-rich repeats	cis-acting element involved in defense and stress responsiveness
TCT-motif	part of a light responsive element
TGA-box	part of an auxin-responsive element
TGACG-motif	cis-acting regulatory element involved in the MeJA-responsiveness
TGA-element	auxin-responsive element
W box	W box

Table S2 The sequence of probes used in EMSA

Name of probes	Sequence	Note
Probe 1	ATG <u>TTGACTAGTT</u> ATG <u>TTGACTAGTT</u>	biotin labeled
Competitor 1	ATG <u>TTGACTAGTT</u> ATG <u>TTGACTAGTT</u>	no labeled
Probe 2	<u>AGAAAATTCAACGGAGAAAATTCAACGGAGAAAATTCAACGG</u>	biotin labeled
Competitor 2	<u>AGAAAATTCAACGGAGAAAATTCAACGGAGAAAATTCAACGG</u>	no labeled

Table S3 Primers used in this study

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Primer Name	Sequences (5'-3')	Function
BcMYB51-1F	CAACCCCATCAAGAATCAAG	Cloning
BcMYB51-1R	TCATGCAAAATAGTTATCAAC	
pBcMYB51-1F	GAACAGATTAAAAACAGAATG	
pBcMYB51-1R	TGTCCGCACCATTCTTGATTC	
BcMYB51-2F	ATGGTGCGGACACCATGTTGCAA	
BcMYB51-2R	TGTGGGATTTCGTCAGAAGATTCACGC	
pBcMYB51-2F	GACGTAAACAAAATTGTGAAG	
pBcMYB51-2R	TGTCCGCACCATTCTTGATTC	
BcMYB51-3F	TCGAATCACTAGCTCAAAAAAGCTGAGA	
BcMYB51-3R	TCTTCAAACTGTCTTGATGCTC	
pBcMYB51-3F	GATTTAGTTGTCTTCTTTTAGCAT	
pBcMYB51-3R	TGTCCGCACCATTCTTGATTC	
PDS-F	TTCTGCGGCGAATTGCTTATCAAAACG	
PDS-R	AGAAACTCTTAACCGTGCCATCGTCATTGAG	
MYB51-F	AGCTCGTGGACTACCAGGAA	
MYB51-R	TTGACGTTTCATAGACCGGCG	
pRI101-BcMYB51-1F	TCTTCACTGTTGATACATATGATGGTGCGGACACCG	Subcellular localization
pRI101-BcMYB51-1R	GCTCACCATGGATCCGGTACCTGCAAAATAGTTATC	
pRI101-BcMYB51-2F	TCTTCACTGTTGATACATATGATGGTGCGGACACCA	
pRI101-BcMYB51-2R	GCTCACCATGGATCCGGTACCTGTGGGATTCGTCAG	
pRI101-BcMYB51-3F	TCTTCACTGTTGATACATATGATGGTGCGGACACCA	
pRI101-BcMYB51-3R	CATGGATCCGGTACCTGCAAAATAGTTATCAATA	
pABAi-pBcMYB51-1F	GAAAAGCTTGAATTCGAGCTCGAACAGATTAATAAAC	Yeast-one-hybrid assay
pABAi-pBcMYB51-1R	AGCACATGCCTCGAGGTCGACTTGAGGATGAGGCAC	
pABAi-pBcMYB51-2F	GAAAAGCTTGAATTCGAGCTCGACGTAAACAAAATT	
pABAi-pBcMYB51-2R	AGCACATGCCTCGAGGTCGACTGTGGTGGAGGCACC	
pABAi-pBcMYB51-3F	GAAAAGCTTGAATTCGAGCTCGATTAGTTGTCTTC	
pABAi-pBcMYB51-3R	AGCACATGCCTCGAGGTCGACTTGAGGAAGAGGCAC	
nBcWRKY33A-AD-F	GCCATGGAGGCCAGTGAATTCATGGCTGCTTCTTCT	
nBcWRKY33A-AD-R	CAGCTCGAGCTCGATGGATCCTCACCTTCCATTGTA	
cBcWRKY33A-AD-F	GCCATGGAGGCCAGTGAATTCATGGAGCAAAGGAAA	Dual-luciferase reporter assay
cBcWRKY33A-AD-R	CAGCTCGAGCTCGATGGATCCTCAGGACAAAAACGA	
p0800-pBcMYB51-3F	CTTGATATCGAATTCCTGCAGGATTTAGTTGTCTTC	
p0800-pBcMYB51-3R	CGCTCTAGAACTAGTGGATCCTTGAGGAAGAGGCAC	
pRI101-BcWRKY33A-F	TCTTCACTGTTGATACATATGATGGCTGCTTCTTCT	
pRI101-BcWRKY33A-R	GCTCACCATGGATCCGGTACCGGACAAAAACGAATC	
pTRV2-PDS-F	GTGAGTAAGGTTACCGAATTCCTCTGCGGCGAATTT	Virus-induced gene silencing
pTRV2-PDS-R	TCCCCATGGAGGCCTTCTAGAAGAACTCTTAACCG	
pTRV2-MYB51-F	GTGAGTAAGGTTACCGAATTCAGCTCGTGGACTACC	
pTRV2-MYB51-R	TCCCCATGGAGGCCTTCTAGATTGACGTTTCATAGAC	

Table S4 Recipes of V8 and SMB medium

V8 agar medium
72 ml V8 juice
4 g Agar
0.4 g CaCO ₃
Add ddH ₂ O to 200 ml
Autoclave at 121 °C for 15 min
SMB medium
0.25 g Peptone
1 g Maltose
Add ddH ₂ O to 25 ml
Final pH (at 25 °C): 5.6 ± 0.2
Autoclave at 121 °C for 15 min

Frerigmann, H., & Gigolashvili, T. (2014). MYB34, MYB51, and MYB122 distinctly regulate indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. *Molecular Plant*, 7(5), 814-828.