## **Supplemental Data**



Supplementary Figure S1. Comparison of seed GSL content between NIL(ZY50) and NIL(G120). A two-tailed Student's *t*-test between NIL(ZY50) and NIL(G120) was used to calculate the *P* value. The data represent the mean  $\pm$  SD ( $n \ge 10$ ), \*\*\**P* < 0.001. 2018 and 2019 represents plants in 2017-2018 and 2018-2019 growing season, respectively. GSL, glucosinolate; NIL, near-isogenic lines.







Supplementary Figure S2. Comparison of coding sequences and amino acid sequences of candidate genes between parental lines. (A - C) Comparison of coding sequences and amino acid sequences of *BnaC02G0527300ZS*, *BnaC02G0527500ZS* and *BnaC02G0527600ZS*, respectively, between parental lines.



Supplementary Figure S3. Integrative genomics viewer (IGV) displays the transcripts of *BnaC2.MYB28*<sup>ZY50</sup> and *BnaA2.MYB28*<sup>ZY50</sup>. (A) Part of the alignment tracks of RNA-seq reads in the region of *BnaC2.MYB28*. (B) Part of the alignment tracks of RNA-seq reads in the region of *BnaA2.MYB28*. Kb, kilobase.



Supplementary Figure S4. SGC of *BnaC2.MYB28*<sup>G120</sup>-sgRNA plants in the T<sub>1</sub> generation. A two-tailed Student's *t* test between G120 and *BnaC2.MYB28*<sup>G120</sup>-sgRNA plants was used to calculate the *P* value. The data represent the means  $\pm$  SD ( $n \ge 8$ ); asterisks indicate significant differences (*t*-test; \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001). GSL, glucosinolate.

BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	GCATATTGAATTATGCAGTATC <mark>A</mark> ACTATATATTTATATTTTCTTG <mark>ATGTATATATATATAACATGAAAAAATAAGCCATACA GCATATTGAATTATGCAGTATOG<mark>ACTATATATTTTATATTCTTG</mark><mark>ATATATAT</mark><mark>ATAAGCCATACA</mark> GCATATTGAATTATGCAGTATC ACTATATATTTATATTCTTG ATATATAT ATAAGCCATACA</mark>	:	80 63
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	ATTATGTTTTG <mark>CAGTTTTT</mark> AACAAAATTTGGTT <mark>T</mark> TTGGGTAAATCAAATCATCTTATTTTTTCATGATATCATAAGAAT ATTATGTTTTGAACAAAATTTGGTTC TTGGGTAAATCAAAATCTTAG-TTTTTCATGATATCATAAGAAT ATTATGTTTTG AACAAAATTTGGTT TTGGGTAAATCAAATC	:	160 134
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	AACTACCTACTATATACATTTTAATATAAACGATATATAGCATAGTACTTTTTATTATAGCATGTTTAAGTTAATTATCTA AACCACCTACTATCATTTTAATATAAACGATATGT AAC ACCTAC TATCATTTTAATATAAACGATAT T GCATAGTACTTT	:	240 182
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	::	TTTATTAGTACTGATGTACCATAAAATACCTGGTAGTTAATTGAATATAATTATATTACTCATTTCTGGGTCACCATC	:	320
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	::	AACTAGCAAGTAACACTATGAAATTTATTCTTGTTTGCATTTCTTTTTTCTTTTTTAAATTAAAATCATTAAGC	:	400
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	ATTAACTCAACCTTAAAAACATGATATTTTTCGTGATGTTGAGTTAGTAATTTTATAGAAGTAATAACTTAAAATTGCAATT	:	480
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	CAATCATAACACATAATAACTCAAATTTAAAATCAAAAATTTTAAATTAATACATGAGTGGTCATTTTTGAGTTACAGCC	:	560 -
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>2Y50</sup> -Promoter	:	АТАААТСАААСТААААААСТСТААСТТАGGTCACCAATCAAAGCTTTAAACCGAAATTTTAAACATACAT	:	640
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	CGTCATTAGTGCTAATCCTTCTTTTTGTGCTAGAAATGCTTCAAAAATAATGTATGGCAAAATGTAGTAGTAATATATACA TCATTAGTGCTAATCCTTCTTTTTGTGCTAGAAATGCTTGAAAATAAAT	:	720 260
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	GCATCAA <mark>ATTGCAAATATTTTTCA</mark> TGTTAGA <mark>G</mark> AATAATTGCGAGCTAGTTTAAACAAATTGATCTATTTAAATAATTAAT	:	800 340
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	AATTCGCGTACCAAAATATCTATCATAATCAACATTGTCAGCATGTACGTGTGTATATATA	:	880 420
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	CTCACCACCATCACACATTCATTGCTCTTCTCACAAGTTTCTGTAGTCTGATCTTATAGTGTTATAAAAATACATATA CTCACCACCATCACACAATTCATTGCTCTTCTTCACAAGTTTCTTTAGTCTAAACATACGTGTTATAAAAATAC CTCACCACCATCACAAATTCATTGCTCTTCTTCACAAGTTTCT TAGTCT ATCTTATAGTGTTATAAAAATAC	:	960 495
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	АТАТТТСААGGATATATAGAA <mark>G</mark> GCAAAAA <mark>C</mark> TCAAAGTCTACTTGAAAAACATGAAAACACCTAGCAGCTCTGTGGGTAAG ATATTTCAAGGATATATAGAA <mark>A</mark> GCAAAAATTCAAAGTCTACTTGAAAAACATGAAAACACCTAGCAGCTCTGTGGGCAAG ATATTTCAAGGATATATAGAA GCAAAAA TCAAAGTCTACTTGAAAAACATGAAAACACCTAGCAGCTCTGTGGG AAG	:	1040 575
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	ACCCAAGAGCGCTTCTCGATTAGTCTCATATTCAGATGTATCAGAGTTCTCATTAACAGATCTATTTCTTTC	:	1120 655
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	TTAGAA ATTTCCTTTCTGATTTTAGTTTCCTCAAGTATATTTTTCTCAATATAGTATTTCCTTTGGTA   TTAGAA ATTTCCTTTCTGATTTTAGTTTCC   CAATATAGTATTTCCTTTCGGTGTTTGGACC CAATATAGTATTTCCTTTGGTGTGTGACC   TTAGAA ATTTCCTTTCTGATTTTAGTTTCC   CAATATAGTATTTCCTTTGGTTTGAACC CAATATAGTATTTCCTTTGGT	:	1200 719
BnaC2.MYB28 <sup>G120</sup> -Promoter BnaC2.MYB28 <sup>ZY50</sup> -Promoter	:	TTTTACACATTAGTGTTCATCTATATATATCG <mark>G</mark> AGAAA : 1238 TTTAACACATTAGTGTTCATATATATATATCGGGAGAAA : 758 TTT ACACATTAGTGTTCAT TATATATATCG GAGAA		

Supplementary Figure S5. Comparison of the promoter sequences of *BnaC2.MYB28* between the parental lines.



Supplementary Figure S6. Subcellular localization of BnaC2.MYB28 in *Nicotiana benthamiana* leaves. The nuclear protein OsGHD7 fused with Turquoise2 was used as a nuclear marker. Bars =  $50 \mu m$ .



В	·	SD/-Trp	-Leu	SD/-T	rp-Leu-H	is-Ade	С	S	D/-Trp-L	eu	SD/-Trp	-Leu-His	s-Ade	
	10 <sup>0</sup>	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>0</sup>	10 <sup>1</sup>	10 <sup>2</sup>		10 <sup>0</sup>	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>0</sup>	10 <sup>1</sup>	10 <sup>2</sup>	
BD-MYB28-GV Al		•	۲		۲	:50	BD-MYB28-ZW AD	۲		魏	•			
BD-MYB28-G Al		۲	2	•	۲	٢	BD-MYB28-Z1 AD			1			1	
BD-MYB28-G Al		۲	۲				BD-MYB28-Z2 AD	0	*	:**3		۲	di	
BD-MYB28-G Al	3 D	۲		۲			BD-MYB28-Z3 AD		۲	-	•			
BD-MYB28-G Al	4	0	12		۲	200 200 200 200	BD-MYB28-Z4 AD		0	-	۲	۲	\$10	
BD-MYB28-G Al	5	٩	199				BD-MYB28-Z5 AD		٩	1.2	•	۲	100	
BD-5 AD-Rec	3	۲				şiş-	BD-53 AD-RecT		۲	-		۲	43	
BD-lar AD-Rec	n 🔘	۲	₩.	0			BD-lam AD-RecT	۲	۲		0			
			213			٥								
	10 <sup>0</sup>	10 <sup>1</sup>	10 <sup>2</sup>	5D/-11	10 <sup>1</sup>	-Ade 10 <sup>2</sup>								
D BD-MYB28-G2 AD-MYB28-GW	۲	4	2.95		۲	0	E				72			
BD-MYB28-G2 AD	•						cLUC-MYB28-GW cLL	JC-MYB	28-GW (	LUC-M	(B28-ZW	cLUC-M	MYB28-ZW	/
AD-MYB28-GW BD			1 <sup>1</sup> 0,				Carlos -				7			High
BD-MYB28-Z3 AD-MYB28-ZW	$\bigcirc$					8 <sup>9</sup>					P			g.i
BD-MYB28-Z3 AD	0	٢								477				
AD-MYB28-Z3 BD	$\bigcirc$	@-	48					1			Contraction of the second			Low
BD-53 AD-RecT			2000				MY	B28-G2- cLUC	nLUC			MYB28 c	8-Z3-nLUC LUC	
BD-lam AD-RecT	0	(9)	2.2											

**Supplementary Figure S7. Functional regions of BnaC2.MYB28.** (A) Schematic diagram showing the various fragments of BnaC2.MYB28. MYB28-GW and MYB28-ZW indicate the whole length of BnaC2.MYB28<sup>G120</sup> and BnaC2.MYB28<sup>ZY50</sup> protein, respectively. Transcriptional self-activation test of BnaC2.MYB28<sup>G120</sup> (B) and BnaC2.MYB28<sup>ZY50</sup> (C) in yeast-two-hybrid assays. BnaC2.MYB28 forms

homodimers in yeast-two-hybrid (D) and SLC analysis (E). BD-53/AD-RecT was the positive control, and BD-Lam/AD-RecT was the negative control. AD, pGADT7/activation domain; BD, pGBKT7/binding domain; Ade, adenine; His, histidine; Lam, human lamin C; Leu, leucine; RecT, recombination of the SV40 large T antigen; SD, synthetic defined medium; Trp, tryptophan.



Supplementary Figure S8. RNA-seq analysis between parental lines and *BnaC2.MYB28* overexpressing lines. (A) Volcano plot of gene expression between G120 and its *BnaC2.MYB28* overexpressing line G120-OE (fold change (FC)  $\ge 2$ ; *P* value < 0.05). (B) GO analysis of differentially expressed genes between G120 and G120-OE. (C) Volcano plot of gene expression between ZY50 and its *BnaC2.MYB28* overexpressing line ZY50-OE (fold change (FC)  $\ge 2$ ; *P* value < 0.05). (D) GO analysis of differentially expressed genes between ZY50 and ZY50-OE.



Supplementary Figure S9. RT-qPCR confirmation of the differentially expressed genes involved in GSL biosynthesis obtained by RNA-seq. Error bars represent standard errors from three independent RNA samples. A two-tailed Student's *t*-test was used to generate the *P* values. \*\*P < 0.01.



Supplementary Figure S10. Co-selection analysis of *BnaC2.MYB28* and *BnaC2.GTR2* in natural population. (A) LD analysis between *BnaC2.MYB28* and *BnaC2.GTR2*. Kb, kilobase. (B) Two major haplotypes were identified in the *BnaC2.GTR2* gene regions. bp, base pair. (C) SGC of the two haplotypes. The horizontal and vertical lines represent the mean value and standard deviation, respectively. Each colored dot represents an individual plant. Data are shown as mean  $\pm$  SD ( $n \ge 117$ ). A two-tailed Student's *t*-test was used to generate the *P* values. (D) Accession statistics of *BnaC2.MYB28* and *BnaC2.GTR2* haplotypes.

Transgenic line	$Mean \pm SD$		No. of	P-Value <sup>a</sup>	
	Negative	Positive	Negative	Positive	
ZG-4	$17.5\pm1.8$	$24.6\pm2.1$	14	28	9.07E-14
ZG-8	$18.3\pm2.1$	$23.2\pm1.7$	11	35	9.35E-10
ZG-13	$18.9\pm2.5$	$25.3\pm2.7$	10	29	1.01E-07

Table S1. SGC in *BnaC2.MYB28*<sup>G120</sup>-transgenic T<sub>1</sub> lines.

Data are shown as mean  $\pm$  SD ( $n \ge 10$ ). *P* values were generated by a two-tailed Student's *t*-test. SD, standard deviation.

<sup>a</sup> indicates the *P* value calculated between negative and positive plants.

Transgenic lineMean $\pm$ SDNo. of plants <i>P</i> -Value <sup>a</sup> G120 $32.5 \pm 2.7$ 28GCR-52-2 $23.9 \pm 3.2$ 26 $8.51E-15$ GCR-52-3 $21.7 \pm 2.5$ 39 $1.70E-25$ GCR-52-6 $24.1 \pm 3.3$ 43 $3.51E-17$ GCR-403-16 $14.1 \pm 2.4$ 32 $1.84E-35$ GCR-403-17 $16.5 \pm 2.0$ 40 $1.08E-38$ GCR-403-35 $15.6 \pm 2.4$ 43 $2.94E-39$					
G120 $32.5 \pm 2.7$ 28GCR-52-2 $23.9 \pm 3.2$ 26 $8.51E-15$ GCR-52-3 $21.7 \pm 2.5$ 39 $1.70E-25$ GCR-52-6 $24.1 \pm 3.3$ 43 $3.51E-17$ GCR-403-16 $14.1 \pm 2.4$ 32 $1.84E-35$ GCR-403-17 $16.5 \pm 2.0$ 40 $1.08E-38$ GCR-403-35 $15.6 \pm 2.4$ 43 $2.94E-39$	Transgenic line	$Mean \pm SD$	No. of plants	P-Value <sup>a</sup>	
GCR-52-2 $23.9 \pm 3.2$ $26$ $8.51E-15$ GCR-52-3 $21.7 \pm 2.5$ $39$ $1.70E-25$ GCR-52-6 $24.1 \pm 3.3$ $43$ $3.51E-17$ GCR-403-16 $14.1 \pm 2.4$ $32$ $1.84E-35$ GCR-403-17 $16.5 \pm 2.0$ $40$ $1.08E-38$ GCR-403-35 $15.6 \pm 2.4$ $43$ $2.94E-39$	G120	$32.5\pm2.7$	28		
GCR-52-3 $21.7 \pm 2.5$ $39$ $1.70E-25$ GCR-52-6 $24.1 \pm 3.3$ $43$ $3.51E-17$ GCR-403-16 $14.1 \pm 2.4$ $32$ $1.84E-35$ GCR-403-17 $16.5 \pm 2.0$ $40$ $1.08E-38$ GCR-403-35 $15.6 \pm 2.4$ $43$ $2.94E-39$	GCR-52-2	$23.9\pm3.2$	26	8.51E-15	
GCR-52-6 $24.1 \pm 3.3$ $43$ $3.51E-17$ GCR-403-16 $14.1 \pm 2.4$ $32$ $1.84E-35$ GCR-403-17 $16.5 \pm 2.0$ $40$ $1.08E-38$ GCR-403-35 $15.6 \pm 2.4$ $43$ $2.94E-39$	GCR-52-3	$21.7\pm2.5$	39	1.70E-25	
GCR-403-16 $14.1 \pm 2.4$ $32$ $1.84E-35$ GCR-403-17 $16.5 \pm 2.0$ $40$ $1.08E-38$ GCR-403-35 $15.6 \pm 2.4$ $43$ $2.94E-39$	GCR-52-6	$24.1\pm3.3$	43	3.51E-17	
GCR-403-1716.5 ± 2.0401.08E-38GCR-403-3515.6 ± 2.4432.94E-39	GCR-403-16	$14.1\pm2.4$	32	1.84E-35	
GCR-403-35 15.6 ± 2.4 43 2.94E-39	GCR-403-17	$16.5\pm2.0$	40	1.08E-38	
	GCR-403-35	$15.6\pm2.4$	43	2.94E-39	

Table S2. SGC in *BnaC2.MYB28*-CRISPR T<sub>2</sub> lines.

Data are shown as mean  $\pm$  SD ( $n \ge 26$ ). *P* values were generated by a two-tailed Student's *t*-test. SD, standard deviation

<sup>a</sup> indicates the *P* value calculated between transgenic lines and G120 plants.

		, · · · ·			
Transgenic line	$Mean \pm SD$		No. of	P-Value <sup>a</sup>	
_	Negative	Positive	Negative	Positive	
GZ-2	$32.6\pm1.4$	$40.6\pm2.3$	11	36	6.11E-14
GZ-5	$30.7\pm2.1$	$37.2 \pm 2.1$	13	27	2.38E-11
GZ-6	$32.2\pm2.3$	$44.3\pm3.2$	10	23	5.61E-12

Table S3. SGC in *BnaC2.MYB28*<sup>ZY50</sup>-transgenic T<sub>1</sub> lines.

Data are shown as mean  $\pm$  SD ( $n \ge 10$ ). *P* values were generated by a two-tailed Student's *t*-test. SD, standard deviation.

<sup>a</sup> indicates the *P* value calculated between negative and positive plants.

Transgenic line	Mean ± SD		No. of	plants	P-Value <sup>a</sup>
	Negative	Positive	Negative	Positive	
ZOE-22	$22.3\pm3.6$	$47.9\pm20.0$	9	6	0.002
ZOE-25	$19.8 \pm 1.1$	$161.3\pm15.8$	14	46	2.04E-39

Table S4. SGC in two p35S::*BnaC2.MYB28*<sup>ZY50</sup>-transgenic T<sub>1</sub> lines.

Data are shown as mean  $\pm$  SD ( $n \ge 6$ ). *P* values were generated by a two-tailed Student's *t*-test. SD, standard deviation.

<sup>a</sup> indicates the *P* value calculated between negative and positive plants.

Table S5. See in KIVAI and over expressing of $Dhuc_2.WIID_20$ - "I ansgeme 10 miles	Table S5. SGC in RNAi a	nd overexpressing	of BnaC2.MYB28G120	transgenic T <sub>0</sub> lines.
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Transgenic line	Mean $\pm$ SD		No. of	plants	P-Value <sup>a</sup>
	Negative	Positive	Negative	Positive	
G120-RNAi	$33.4\pm3.1$	$23.7\pm2.0$	17	6	4.22E-07
G120-OE	$32.3{\pm}~5.0$	$128.7\pm35.9$	40	16	4.09E-23

Data are shown as mean  $\pm$  SD ( $n \ge 6$ ). P values were generated by a two-tailed Student's t-test. SD, standard deviation.

<sup>a</sup> indicates the *P* value calculated between negative and positive plants.

		Arabidopsis				
Gene ID (ZS11)	Gene name	locus ID	Description			
BnaC03G0321400ZS	AOP1	AT4G03070	Encodes a possible 2-oxoglutarate-dependent dioxygenase that is involved in glucosinolate biosynthesis.			
BnaA02G0257300ZS	AOP3	AT4G03050	Encodes a 2-oxoglutarate-dependent dioxygenase which is involved in glucosinolate biosynthesis.			
BnaA03G0259200ZS						
BnaA09G0244600ZS			Including the terms of a f 2 lasts and a f strength at the terms of the second strength of the second strengt ot the second strength of t			
BnaC03G0308400ZS	BAT5	AT4G12030	and the cytosol			
BnaC03G0308500ZS			and the cytosol.			
BnaC09G0288000ZS						
BnaA03G0359600ZS			Involved in the methicaning chain elongation nothway that			
BnaA05G0362200ZS	BC ATA	AT3G10710	leads to the ultimate biosynthesis of methionine-derived			
BnaC03G0438900ZS	DCAIT	A15017/10	ieaus to the ultimate biosynthesis of methionine-derive			
BnaC05G0396400ZS			gracosmonaes.			
BnaA08G0192100ZS	CYP79B1	AT4G39950	Belongs to cytochrome P450 and is involved in tryptophan metabolism.			
BnaA04G0074700ZS	CYP83A1	AT4G13770	Encodes a cytochrome p450 enzyme that catalyzes the initial conversion of aldoximes to thiohydroximates in the synthesis			
BnaC04G0359300ZS			of glucosinolates not derived from tryptophan.			
BnaA09G0155300ZS	FMO co ave	AT1G65860	Belongs to the flavin-monooxygenase (FMO) family			
BnaC09G0170500ZS	1 1010 65-031	AI1005000	Belongs to the navin-monooxygenase (1 WO) family.			
BnaA09G0155200ZS	FMO GS-0X2	AT1G62540	Belongs to the flavin-monooxygenase (FMO) family.			
BnaC03G0090500ZS	GS-OH	AT2G25450	Encodes a 2-oxoacid-dependent dioxygenase involved in the production of 2-hydroxybut-3-enyl glucosinolate.			
BnaA02G0052200ZS		ATEC 1 4200	Involved in glucosinolate biosynthesis, in methionine chain			
BnaC02G0060000ZS	IPMDHI	AI3G14200	elongation.			
BnaA05G0036500ZS	IPMI2	AT2G43100	Together with IPMI SSU3 participates in the Met chain elongation pathway.			
BnaA06G0128100ZS	00717	1771 (710 500	Encodes a desulfoglucosinolate sulfotransferase, involved in			
BnaC05G0156200ZS	SOTT/	ATTG18590	the final step of glucosinolate core structure biosynthesis.			
BnaC06G0404300ZS	SOT18	AT1G74090	Encodes a desulfoglucosinolate sulfotransferase, involved in the final step of glucosinolate core structure biosynthesis			
			Encodes a C-S lyase involved in converting			
BnaA07G0001300ZS	SUR I	AT2G20610	S-alkylthiohydroximate to thiohydroximate in glucosinolate			
BnaC07G0005900ZS			biosynthesis.			
BnaA09G0448300ZS	UTG74R1	AT1G24100	Encodes a UDP-glucose: thiohydroximate			
BnaC05G0210800ZS	010/701	111027100	S-glucosyltransferase, involved in glucosinolate biosynthesis.			

Table S6. Overlapping genes involved in GSL biosynthesis of ChIP-seq and RNA-seq.

Table S7. Summary of primers used in this study.

Primer name	Sequence 5'-3'	Purpose
SSA2-102F	AGCAAAAGCACCTCATTAGC	Fine mapping (co-segregation marker of qGSL-C2)
SSA2-102R	TCCCAGCGTGAATTGAACCTA	
SSA2-146F	GGTTAATCCACCATGATGTC	Fine mapping (flanking marker of <i>qGSL-C2</i> )
SSA2-146R	GCTAAGATTGTTAGAGCGTTCC	
SSA2-157F	ACTCTCTAGCCACCAAGTCCA	Fine mapping (flanking marker of <i>qGSL-C2</i> )
SSA2-157R	ACACCGAAAAACTTGCGACTGCT	
G120-pM999F	GGAGAGGACAGGGTA <u>CCCGGG</u> ATGTCAAGAAAGCCATGTTGT	BnaC2.MYB28 <sup>G120</sup> -GFP construction
G120-pM999R	GGCAGCGGCAGCAGCC <u>GGATCC</u> TATGAAATCATAGTCCGTGTCA	
ZY50-pM999F	GGAGAGGACAGGGTA <u>CCCGGG</u> ATGTCAAGAAAGCCATGTTGT	BnaC2.MYB28 <sup>ZY50</sup> -GFP construction
ZY50-pM999R	GGCAGCGGCAGCAGCC <u>GGATCC</u> TATGATTTGCTTATCGAAGAAATC	
qBCAT4-F	GGATAACATTAGCAGAGGCGA	RT-qPCR analysis of BnaBCAT4 expression
qBCAT4-R	TTCCTCAACCTTGTAGCCGA	
qIPMDH1-F	TTCCATCTGCTAGTCTCGGT	RT-qPCR analysis of <i>BnaIPMDH1</i> expression
qIPMDH1-R	CATCCCACCAGTTTATTTCCAG	
qCYP79F1-F	ATTTCATTCCCAAAGGTAGCCAC	RT-qPCR analysis of BnaCYP79F1 expression
qCYP79F1-R	AGAAAGCTCCTTCGAGATTCC	
qCYP83A1-F	AGATCAACCTTTCGCCTCCAAG	RT-qPCR analysis of <i>BnaCYP83A1</i> expression
qCYP83A1-R	ACTTCATCAAATACGTCATTCCCCA	
qBnACTIN7-L	CCCTGGAATTGCTGACCGTA	RT-qPCR control for <i>B. napus</i>
qBnACTIN7-R	TGGAAAGTGCTGAGGGATGC	
qBnaC2.MYB28-F	CATCATGCTCCATGCTGCTC	RT-qPCR analysis of BnaC2.MYB28 expression
qBnaC2.MYB28-R	AGAAGCTAGTGGCTTGTGAGTCAC	
G120- DT1-BsF	ATATAT <u>GGTCTC</u> GATTGGTGTGGTGATCTCGGAAACGTT	CRISPR/Cas9 construction of BnaC2.MYB28G120
G120-F0	TGGTGTGGTGATCTCGGAAACGTTTTAGAGCTAGAAATAGC	

G120-DT2-R0	AACGCCCTCGAAGTTTCCTATCCAATCTCTTAGTCGACTCTAC	CRISPR/Cas9 construction of <i>BnaC2.MYB28</i> G120
G120-DT2-BsR	ATTATT <u>GGTCTC</u> GAAACGCCCTCGAAGTTTCCTATCCAA	
LUC-ZY50F	CTATAGGGCGAATTG <u>GGTACC</u> ATATGTGAGGGGTGGTAAGAGACG	Promoter activity analysis of <i>BnaC2.MYB28</i> <sup>ZY50</sup>
LUC-ZY50R	CGCTCTAGAACTAGT <u>GGATCC</u> TTTCTCCGATATATATATATGAACAC	
LUC-G120F	CTATAGGGCGAATTG <u>GGTACC</u> CCGGATATCCATAAACCTTCG	Promoter activity analysis of <i>BnaC2.MYB28</i> <sup>G120</sup>
LUC-G120R	CGCTCTAGAACTAGT <u>GGATCC</u> TTTCTCCGATATATATAGATGAAC	
35S-G120F	CAGGTCGACTCTAGA <u>GGATCC</u> ATGTCAAGAAAGCCATGTTGTGT	35S::BnaC2.MYB28G120 construction
35S-G120R	CGAGAATTCGAGCTC <u>GGTACC</u> TCATATGAAATCATAGTCCGTGTCA	
35S-ZY50F	CAGGTCGACTCTAGA <u>GGATCC</u> ATGTCAAGAAAGCCATGTTGTGT	35S::BnaC2.MYB28 <sup>ZY50</sup> construction
35S-ZY50R	CGAGAATTCGAGCTC <u>GGTACC</u> TCATATGATTTGCTTATCGAAGAAATC	
HB-G120F	TATGACCATGATTAC <u>GAATTC</u> CCGGATATCCATAAACCTTCGAAG	Functional analysis for BnaC2.MYB28G120
HB-G120R	CAGGTCGACTCTAGA <u>GGATCC</u> GACGATAAGAACCTTTGCGAGATC	
HB-ZY50F	TATGACCATGATTAC <u>GAATTC</u> ATATGTGAGGGGTGGTAAGAGACG	Functional analysis for BnaC2.MYB28ZY50
HB-ZY50R	CAGGTCGACTCTAGA <u>GGATCC</u> CGGCTCACACTTCCGATTATCTG	
MR-G120F1	TTACAATTACCATGG <u>GGCGCGCC</u> CCAATGGCTTCCCTGAG	RNAi construction of BnaC2.MYB28G120
MR-G120R1	AAGAAATTCTTACACATTTAAATTACTCGACATTCGTATTGTGC	
MR-G120F2	AATTTGCAGGTATTT <u>GGATCC</u> TACTCGACATTCGTATTGTGC	RNAi construction of <i>BnaC2.MYB28</i> G120
MR-G120R2	ACTCTAGGGACTAGT <u>CCCGGG</u> CCAATGGCTTCCCTGAG	
CR-gF1	CATGTCTCTTCCTCTTTCCT	Detection primer of target 1 for CRISPR construction
CR-gR1	AGCCTTGATGTAGAGCTCGA	
CR-gF2	CTAGATGCATCTAGTTCCGA	Detection primer of target 2 for CRISPR construction
CR-gR2	AACCTCTCTGAACTGGTGTG	