Supplemental Information Appendix

Arabidopsis TSO1 and MYB3R1 form a regulatory module to coordinate cell proliferation with differentiation in shoot and root

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Short title: A cell cycle regulatory module in plant meristems

Supplemental Figures

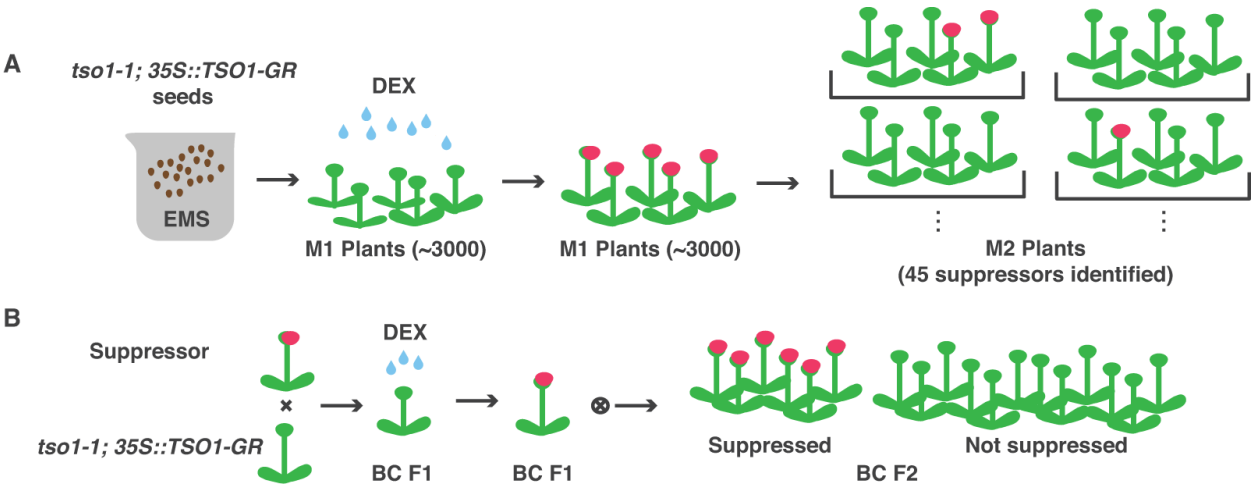

Figure S1. Scheme of the suppressor screen and constructing the mapping population.
(**A**) *tso1-1; 35S::TSO1-GR* seeds were treated with EMS. Seeds were then germinated on soil. Since these M1 *tso1-1; 35S::TSO1-GR* plants were completely sterile, they were sprayed with DEX at bolting to give rise to M2 seeds. M2 plants derived from ~3000 M1 families were grown and screened for their ability to set seeds in the absence of DEX treatment. (**B**) Individual suppressor was back crossed with its wild type parent *tso1-1; 35S::TSO1-GR*. F1 cross progeny was supplied with DEX to set F2 seeds. F2 plants were scored and separated into suppressed and unsuppressed mapping populations. F2 plants showing suppressed phenotype were pooled and their DNA sequenced for identifying linked SNPs to the suppressor mutation. BC: Backcross. Red shoot apex denotes fertile flowers.

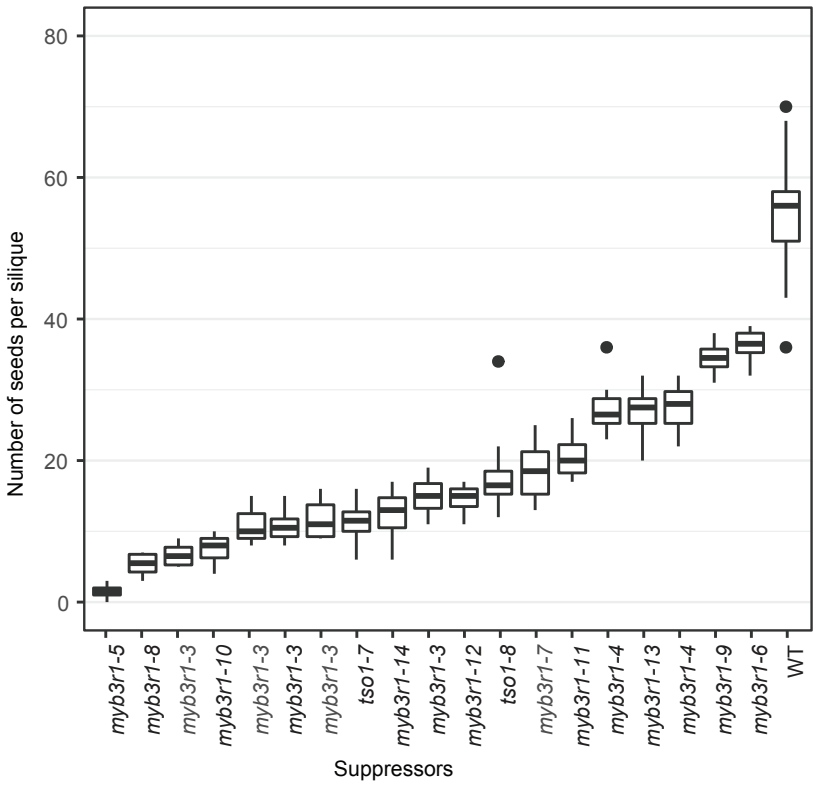

Figure S2. Quantification of suppression based on the number of seeds produced per silique.

Box plot of seeds per silique from WT (L*er*) and 19 suppressors (17 new *myb3r1* alleles and 2 new *tso1* alleles). Multiple identical mutations were isolated and were denoted identical allele number. Background mutations may contribute to the extent of the suppression. Data ranked and ordered by median. Around 10 siliques were sampled from representative M3 suppressor plants.

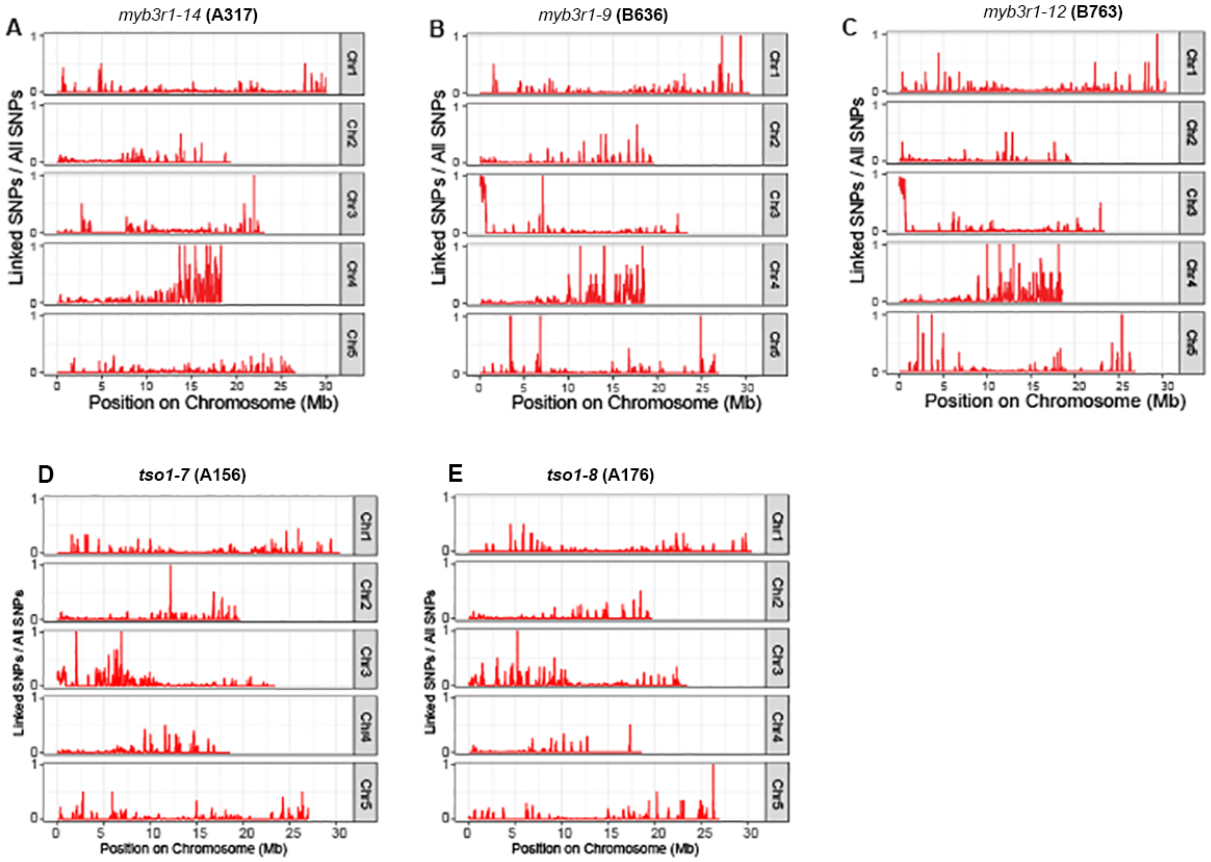

Figure S3. Genome wide SNP mapping of five *tso1-1* suppressors identified two distinct loci.

(**A**-**C**) Genome wide SNP mapping of F2 mapping populations for suppressor A317 (**A**), B636 (**B**), and B763 (**C**). Linked SNP peak in each case resides at the lower arm of *Arabidopsis* chromosome 4. In each of the three cases, a mutation was subsequently found in *MYB3R1*. (**D**-**E**) SNP mapping of F2 mapping population for suppressor A156 (**D**) and A176 (**E**). Both mapped to the top arm of chromosome 3. In each case, a mutation was found in the *TSO1* gene. Y-axis indicates the ratio of linked SNPs to all SNPs detected in a 100,000bp sliding window. X-axis denotes position on the chromosome.

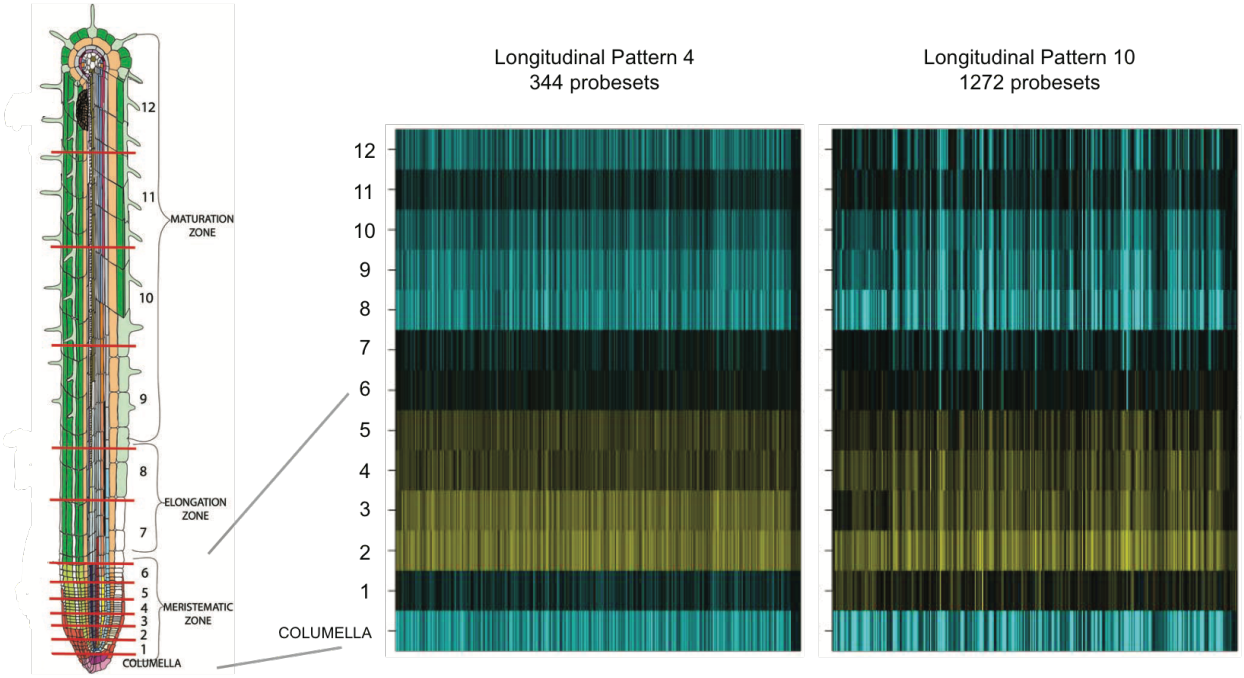

Figure S4. TSO1 RNA expression in roots.
Heatmap of two clusters of genes (cluster 4 and 10) showing highest expression in the meristematic zone (especially section 2 of the meristematic zone). TSO1 belongs to cluster 4 and 10. Series cross sections of the root were collected and subjected to transcriptome profiling using ATH1 DNA microarray[1]. Genes showing distinct expression pattern along the longitudinal axis of the root are clustered by fuzzy k-means clustering. The data and the root schematic drawing are from Brady et al. (2007)[1].

Figure S5. The effect of *35S::MYB3R1* and *gMYb3R1* transgenes in different backgrounds.
(**A**) Phenotypically wild type (WT) inflorescence of a *tso1-3/+* plant.
(**B**) A *35S::MYB3R1; tso1-3/+* transgenic plant showing a wild type phenotype.
(**C**) A *tso1-1; myb3r1-9* double mutant inflorescence showing a wild type phenotype.
(**D**) Inflorescence of a *tso1-1; myb3r1-9* double mutant showing the suppressed phenotype even in the presence of *35S::MYB3R1*, indicating that *35S::MYB3R1* failed to rescue *myb3r1-9*.
(**E**) Inflorescence of a *tso1-1; myb3r1-9* double mutant showing the *tso1-1* mutant phenotype in the presence of the *gMYB3R1* transgene. This indicates that the *gMYB3R1* transgene complemented the *myb3r1-9* mutation.

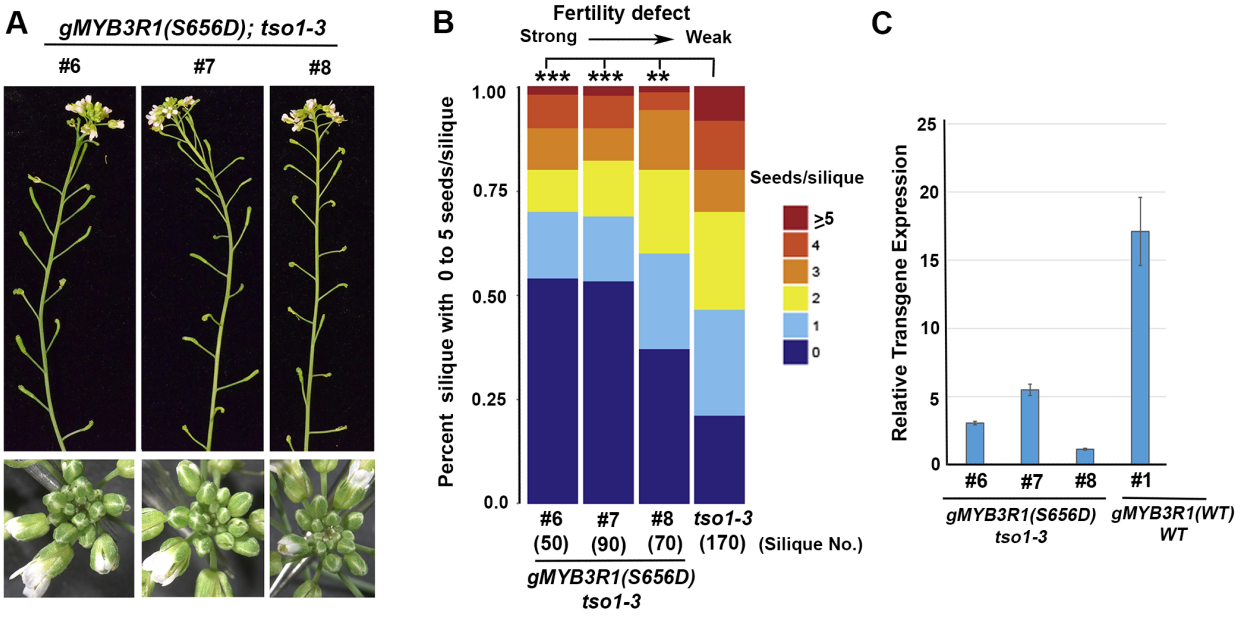

Figure S6. Correlation between *gMYB3R1(S656D)* transgene expression and phenotype severity.

(**A**) Inflorescence shoots showing the fertility defect of three different *gMYB3R1(S656D); tso1-3* transgenic lines.

(**B**) Quantitative measurement of the fertility defect (seeds per silique) in the three *gMYB3R1(S656D); tso1-3* transgenic lines in comparison to *tso1-3*. Total number of siliques analyzed per line (at 10 siliques per plant) is indicated beneath each column in parentheses. Enhanced sterility exhibited by each the three transgenic lines when compared with *tso1-3* is statistically significant, which is indicated by two asterisks ($p<=0.01$) or three asterisks ($p<=0.001$). The statistic test used is two-tailed two-sample t.test.

(**C**) qRT-PCR results showing relative transgene expression level in each of the three *gMYB3R1(S656D); tso1-3* transgenic lines. In addition, the wild type *gMYB3R1(WT)* transgene (line #1) did not cause any phenotypes even though it was expressed at a high level.

Supplemental References

1 Brady et al. (2007) A high-resolution root spatiotemporal map reveals dominant expression patterns. *Science* 318(5851):801–806.

Table S1. Summary of *tso1-1* suppressors						
Suppressor	**Gene**	**Allele designation**	**Evidence**	**Base change**	Amino acid change	Notation
A156	TSO1	tso1-7	Mapping	G-A splicing donor site of the 8th intron		
A176	TSO1	tso1-8	Mapping	G-A splicing acceptor site of the 1st intron		
B378	TSO1	tso1-9	Complementation test			
A144	Chromosome 1		Mapping			
A317	MYB3R1	*myb3r1-14*	Mapping	C-T / CGA-TGA	Arg-stop	R652X
P29	MYB3R1	*myb3r1-14*	Complementation test	C-T / CGA-TGA	Arg-stop	R652X
B636	MYB3R1	*myb3r1-9*	Mapping	G-A / GGG-GAG	Gly-Glu	G119E
B763	MYB3R1	*myb3r1-12*	Mapping	G-A / TGG-TGA	Trp-stop	W143X
B12	MYB3R1	*myb3r1-5*	PCR_sequencing	G-A / GGa-AGA	Gly-Arg	G88R
B845	MYB3R1	*myb3r1-10*	PCR_sequencing	G-A / CGC-CAC	Arg-His	R120H
P17	MYB3R1	*myb3r1-2*	PCR_sequencing	C-T / CAA-TAA	Gln-stop	Q37X
B846	MYB3R1	*myb3r1-4*	PCR_sequencing	C-T / GCT-GTT	Ala-Val	A62V
P15	MYB3R1	*myb3r1-4*	PCR_sequencing	C-T / GCT-GTT	Ala-Val	A62V
P19	MYB3R1	*myb3r1-4*	PCR_sequencing	C-T / GCT-GTT	Ala-Val	A62V
P32	MYB3R1	*myb3r1-4*	PCR_sequencing	C-T / GCT-GTT	Ala-Val	A62V
P34	MYB3R1	*myb3r1-4*	PCR_sequencing	C-T / GCT-GTT	Ala-Val	A62V
B341	MYB3R1	*myb3r1-4*	Complementation test/PCR_sequencing	C-T / GCT-GTT	Ala-Val	A62V
B667	MYB3R1	*myb3r1-11*	Complementation test/PCR_sequencing	G-A / TGT-TAT	Cys-Tyr	C125Y
B759	MYB3R1	*myb3r1-6*	Complementation test/PCR_sequencing	G-A / TGG-TGA	Trp-stop	W90X
P11	MYB3R1	*myb3r1-8*	Complementation test/PCR_sequencing	G-A / GAT-AAT	Asp-Asn	D95N
B741	MYB3R1	*myb3r1-13*	Complementation test/PCR_sequencing	G-A / GAG-AAG	Glu-Lys	E146K

P3	MYB3R1	*myb3r1-7*	Complementation test/PCR_sequencing	G-A splicing donor site of the 5th intron and the 6th exon/AAGAGgtgatttt/ttttgaagGAGGATA	premature stop codon	
P7	MYB3R1	*myb3r1-3*	Complementation test/PCR_sequencing	G-A splicing acceptor site of the 3rd intron/gatcagGACGA	premature stop codon	
P8	MYB3R1	*myb3r1-3*	Complementation test/PCR_sequencing	G-A splicing acceptor site of the 3rd intron/gatcagGACGA	premature stop codon	
P9	MYB3R1	*myb3r1-3*	Complementation test/PCR_sequencing	G-A splicing acceptor site of the 3rd intron/gatcagGACGA	premature stop codon	
P10	MYB3R1	*myb3r1-3*	Complementation test/PCR_sequencing	G-A splicing acceptor site of the 3rd intron/gatcagGACGA	premature stop codon	
P12	MYB3R1	*myb3r1-3*	Complementation test/PCR_sequencing	G-A splicing acceptor site of the 3rd intron/gatcagGACGA	premature stop codon	
A40	MYB3R1		Complementation test			
B447	MYB3R1		Complementation test			
B478	MYB3R1		Complementation test			
B783	MYB3R1		Complementation test			
P18	MYB3R1		Complementation test			
P20	MYB3R1		Complementation test			
P21	MYB3R1		Complementation test			
P22	MYB3R1		Complementation test			
P30	MYB3R1		Complementation test			
B701	Not determined					
P13	Not determined					
P14	Not determined					
P4	Not determined					
P6	Not determined					
B847	Not determined					
P5	Not determined					
P16	Not determined					

Table S2. Summary of transgenic studies using *35S::MYB3R1* and *gMYB3R1*

Background	**Transgene →**	***35S::MYB3R1***	***gMYB3R1^***	***gMYB3R1 (S656D)^***	***gMYB3R1 (S709D)^***
WT*	Transgenic phenotype	Same as WT	Same as WT	Same as WT	Same as WT
	No. lines analyzed[#]	12	10	8	2
tso1-3	Transgenic phenotype	Same as *tso1-3*	Same as *tso1-3*	Stronger than *tso1-3*	Same as *tso1-3*
	No. lines analyzed[#]	10	2	3	4
tso1-1; myb3r1-9 (B636)	Transgenic phenotype	Failed to rescue *myb3r1-9*	Rescued *myb3r1-9*	NA	NA
	No. lines analyzed[#]	4	2		

* Phenotypically wild type plants either heterozygous for *tso1* or +/+.
^ These *gMYB3R1* transgenes are all tagged by GFP
# At least 20 T1 transgenic lines were generated for each construct. These transgenic lines showed the phenotype indicated in the table. The indicated number of lines refers to the number of lines further analyzed and documented.

Table S3. Primers used in this study

Primer name	Sequence	Contruct	Purpose
TSO1-GR.F	cgcggatcctATGgacaaatcccagaagaatc	35S::TSO1-GR	Induciable TSO1-GR system
TSO1-GR.R	cgcggatccttaCActgatttgggttgagaga	35S::TSO1-GR	
TSO1.geno.F	ATAAGCTTCACCTTGCTGTTCCAAGACAC		TSO1 translational reporter
TSO1.geno.R	CTGATTTGGGTTGAGAGAAGGAAATGC		
BamHI-TSO1-F	CTCGGATCCATGGACAAATCCCAGAAG		PHB-FLAG-TSO1 vector for Co-IP
Spe1-TSO1-R	CTGACTAGTCACTGATTTGGGTTGAGA		
TSO1.3xGFP.n.R	TGATTTGGGTTGAGAGAAGGAAATGC		TSO1-NLS-3xeGFP vector
AtU6P-F	TGGGTCGACGTAAAGCCTGTAGAAGAGGT	MYB3R1.CRISPR4	MYB3R1 CRIPSR construct targeting the 4th exon
U6P-MYB3R1-4E-R	GCTTGTGAAAGGACCGTCAATCACTACTTCGACTCTAGC	MYB3R1.CRISPR4	
U6T-MYB3R1-4E-F	GTCCTTTCACAAGCTCTGTTTTAGAGCTAGAAATAGCAA	MYB3R1.CRISPR4	
AtU6T-R	ATTGGTACCAAAAATTATATCCTGTGGTCGTATATTA	MYB3R1.CRISPR4	
pMYB3R1.F	AACTGCAGTATTAGCCAATGAAGGTGGACTAGC	pMYB3R1:GUS	MYB3R1 promoter
pMYB3R1.R	AAGTCGACAATTAAGACGCTGAGAATCCAGATG	pMYB3R1:GUS	
pMYB3R4.F	AACTGCAGAGTCGGTAACATTCTGCCAGAGATG	pMYB3R4:GUS	MYB3R4 promoter
pMYB3R4.R	AAGTCGACGAGCTTCAGAAATGGAAGTGGTTC	pMYB3R4:GUS	
MYB3R1 .F	ATGAAGCGTGAGATGAAAGCAC	35S::YFP-MYB3R1	YFP-MYB3R1 vector of Co-IP
MYB3R1 .R	CTATCTGCAACTCTTCAAAAGGTAAGATG		
S656D.F	AGACTTGCTTGACCCTGTGCTTGATAG	35S::MYB3R1-S656D	S656D phospho mimiking
S656D.R	CGATGCCGCTTCTTTAAG		
S709D.F	TATATGTGCCGACCCTTCCATAGC	35S::MYB3R1-S709D	S709D phospho mimiking
S709D.R	TTTTTATCTTCAGGAGACTCTG		
MYB3R4.CRIPSR.F	ATTGTCGAGGCCAATGGACAGCTG	MYB3R4.2nd.exon. CRIPSR	MYB3R4 CRISPR construct targeting the 2nd exon
MYB3R4.CRIPSR.R	AAACCAGCTGTCCATTGGCCTCGA		
MYB3R1.3xGFP.n.R	CTGCAACTCTTCAAAAGGTAAGATGAT	gTSO1-NLS-3xeGFP	gTSO1-NLS-3xeGFP translational reporter
qPCR.GFP.R	CTCCAGTGAAAAGTTCTTCTCC		qPCR for gMYB3R1-GFP transgene
qPCR.MYB3R1.F	TGAGTGAACACTCAGCTACGG		qPCR for gMYB3R1-GFP transgene
AtUBC9-F	CAGTGGAGTCCTGCTCTCACAA		qPCR reference gene control
AtUBC9-R	CATCTGGGTTTGGATCCGTTA		qPCR reference gene control

Table S4. Summary of Illumina DNA-seq data

	Number of plants pooled	Reads in fastq	Mapped reads	overall alignment rate	aligned exactly 1 time	aligned >1 times
A176NS	50	37,881,321	35,463,736	93.62%	24532907 (64.76%)	10930829 (28.86%)
A176S	34	36,524,977	34,251,910	93.78%	24009642 (65.73%)	10242268 (28.04%)
A317NS	50	36,897,865	34,419,918	93.28%	24392999 (66.11%)	10026919 (27.17%)
A317S	30	43,189,889	40,344,914	93.41%	28756191 (66.58%)	11588723 (26.83%)
TSO1-GR	3	24,660,032	22,232,665	90.16%	14319121 (58.07%)	7913544 (32.09%)
A144S	50	46,538,146	43,500,250	93.47%	31742725 (68.21%)	11757525 (25.26%)
B636S	50	42,847,379	39,978,013	93.30%	28047532 (65.46%)	11930481 (27.84%)
B763S	50	41,198,897	38,654,995	93.83%	26643726 (64.67%)	12011269 (29.15%)
A156S	50	43,989,567	41,090,565	93.41%	30646817 (69.67%)	10443748 (23.74%)
NS: Non-suppressed F2 progenies						
S: Suppressed F2 progenies						