Supplemental Figures and Table

Enhancing microRNA167A expression in seed decreases the α-linolenic acid content and increases seed size in *Camelina sativa*

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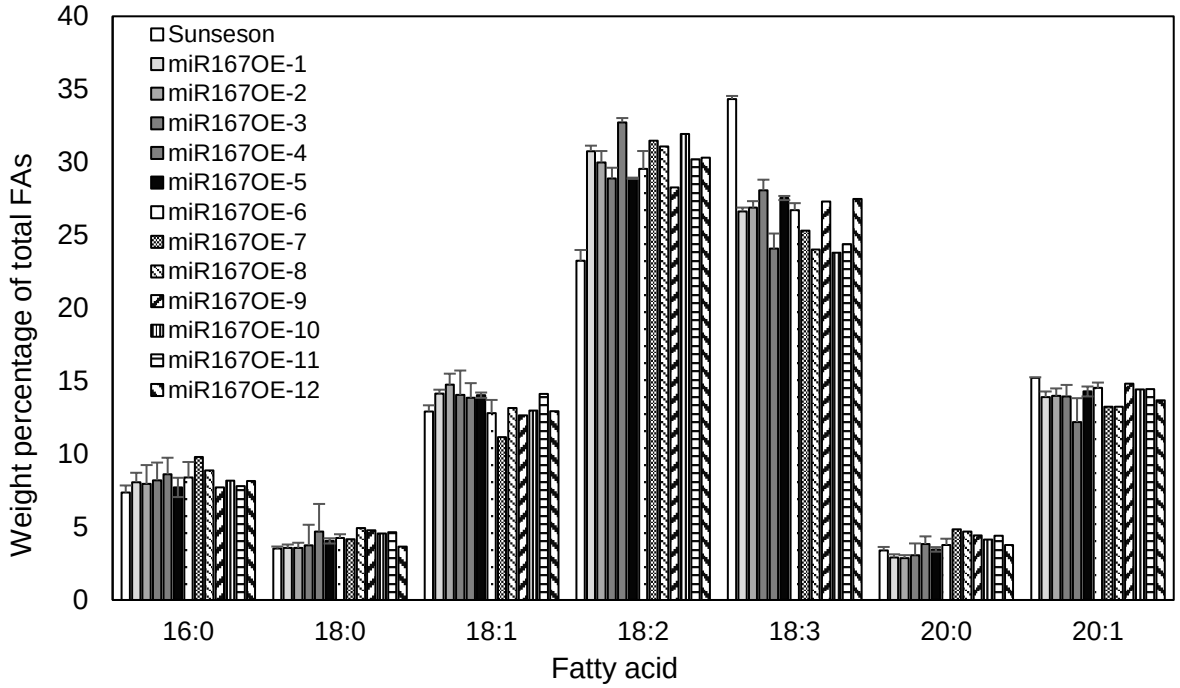


Figure S1. Comparison of seed fatty acid composition in Suneson and miR167OE T1 plants. Fatty acid composition was determined by GC in single seeds from wild-type control (Suneson) and twelve independent miRNA167A overexpressing transgenic lines. For miR167OE lines, only individual seeds (n>3) showing changed fatty acids were included.

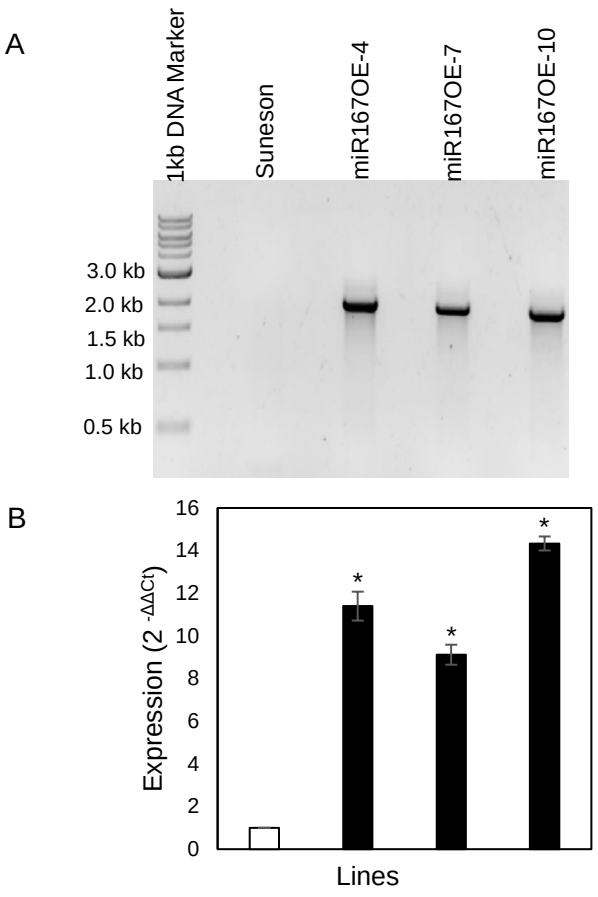


Figure S2. Stable insertion of pGDP-miR167A plasmids and expression of miR167 in camelina. (**A**) PCR with genomic DNA from Suneson and T3 miR167OE lines. (**B**) Expression level of miR167 of non-transgenic control (Suneson) and miR167OE lines. Expression level of miR167 in both lines were normalized by the expression of actin7 from both lines. Asterisks mark significant changes ($P < 0.5$).

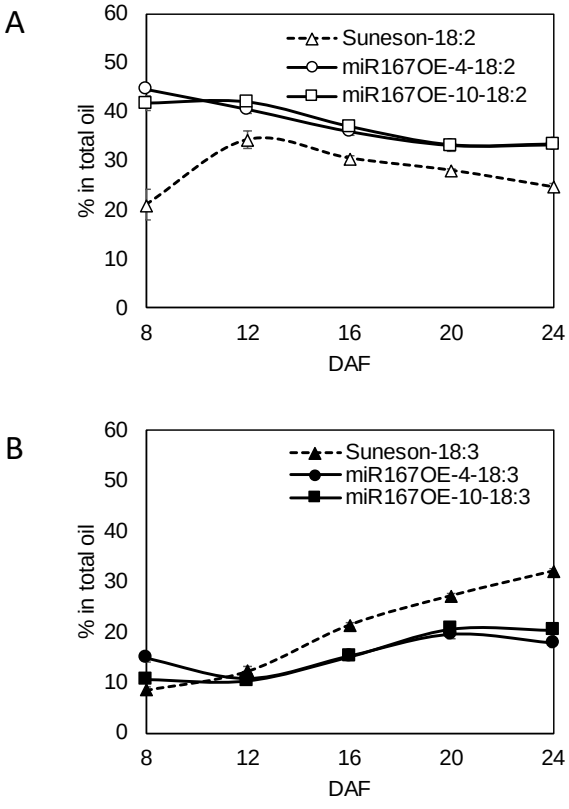
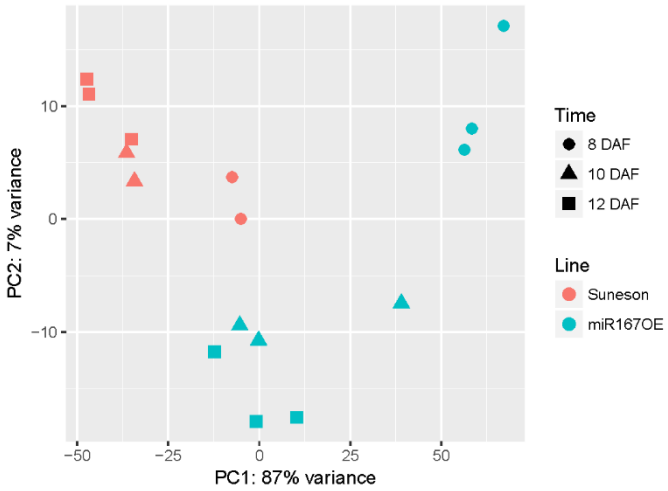


Figure S3. The effect of miR167A overexpression on α-linolenic acid formation in camelina seeds.

The change of 18:2 (A) and 18:3 (B) in Suneson and miR167OE lines during seed development; DAF (Days After Flowering). Data are the average with S.E. (error bars) of three replicas.

A



B

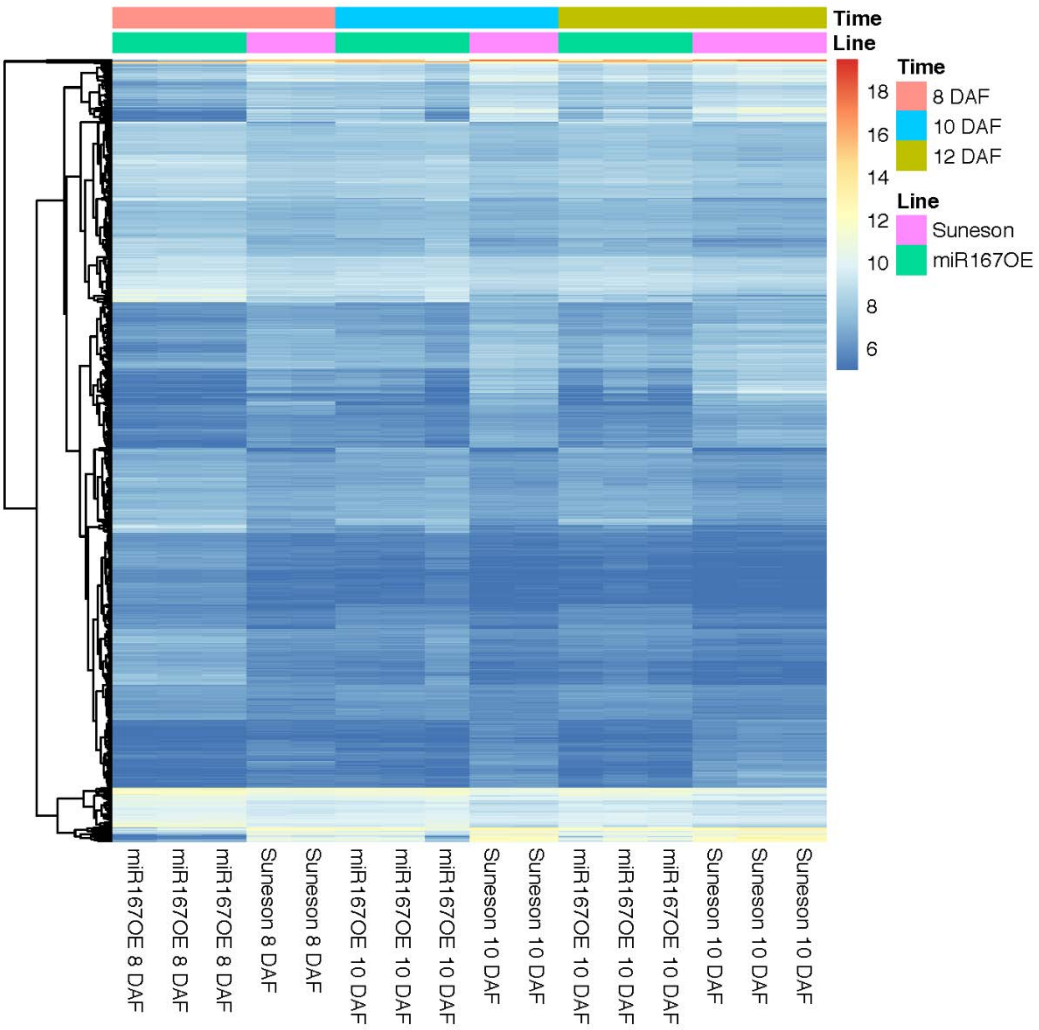


Figure S4. (A) Principal component analysis of RNAseq libraries using variance stabilizing transformation of read count data. (B) Heatmap shows expression levels of overall differentially expressed genes. Columns are RNA-seq libraries and rows are genes clustered using euclidean distance as implemented in the pheatmap R package and function (Kolde 2018). Expression levels are based on variance stabilizing transformation of read count data.

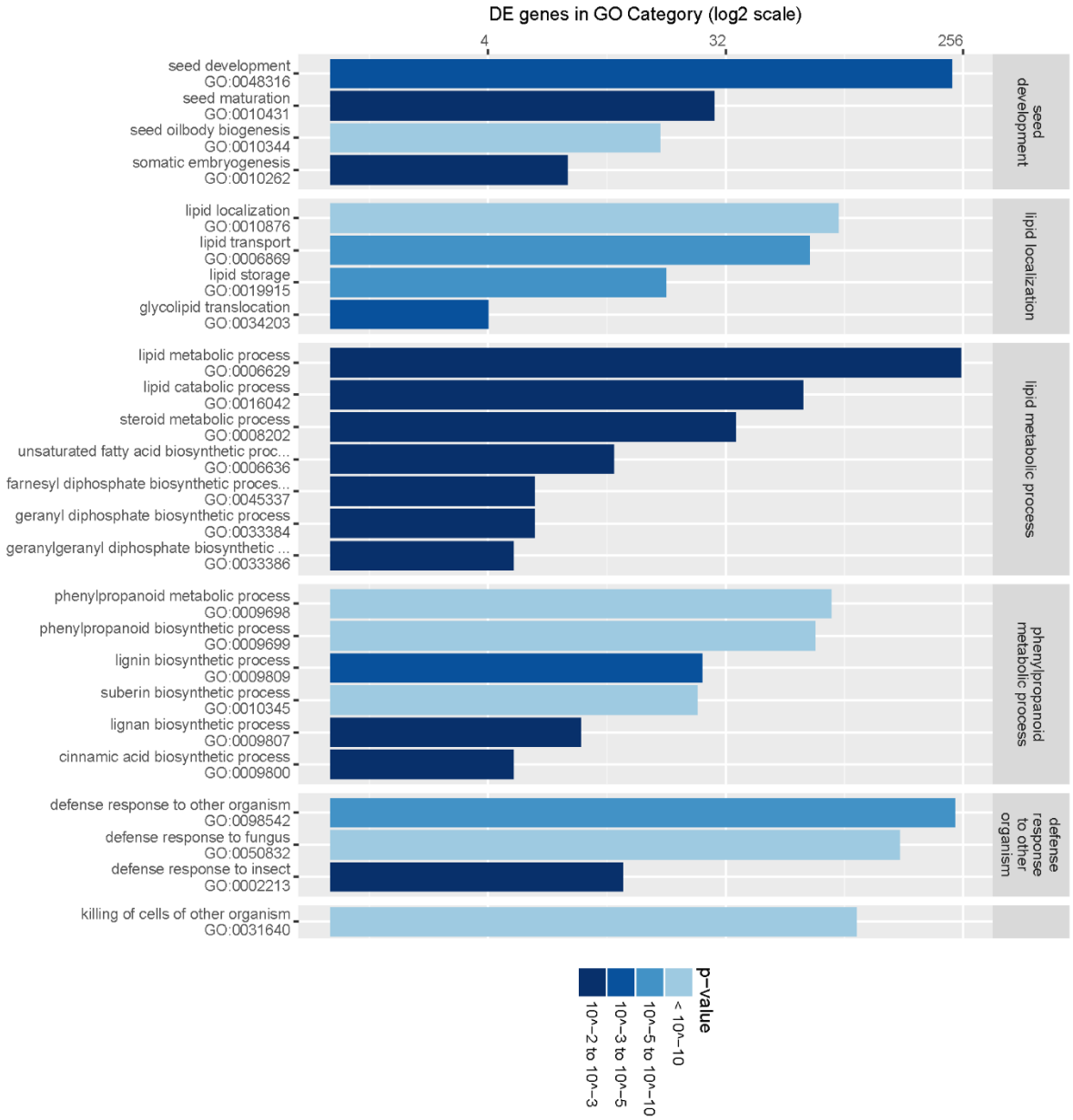


Figure S5. Enriched GO terms of DE genes

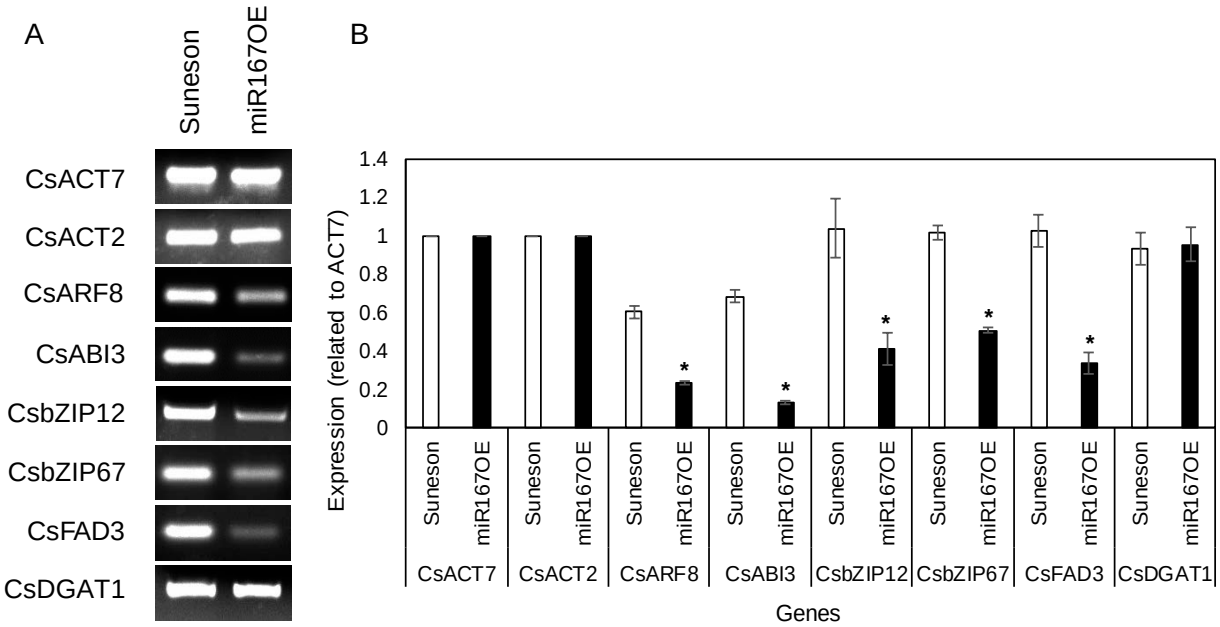


Figure S6. Verification of RNA-seq results of selected genes by semi-quantitative RT- PCR. (A) Gel images of Semi-qRT-PCR using gene specific primers. RNA samples were isolated from Suneson and T3 homozygous miR167OE. (B) Quantification of gene expression using ImageJ. Data are representative of results from three independent experiments. Values are normalized relative to the value of CsACT7 (Actin 7). Significant changes from Suneson are marked with asterisks ($P < 0.5$).

CsACT2 (Actin 2), an additional control.
CsARF8 (Auxin Response Factor 8), CsABI3 (Abscisic acid Insensitive 3), CsbZIP12 (basic leucine Zipper TF 12), CsbZIP67 (basic leucine Zipper TF 67) and CsFAD3 (Fatty Acid Desaturase 3) showed reduced transcripts in miR167OE compared to Suneson, while CsDGAT1 (Diacylglycerol acyltransferase 1) was not significantly changed by RNA-seq analysis.

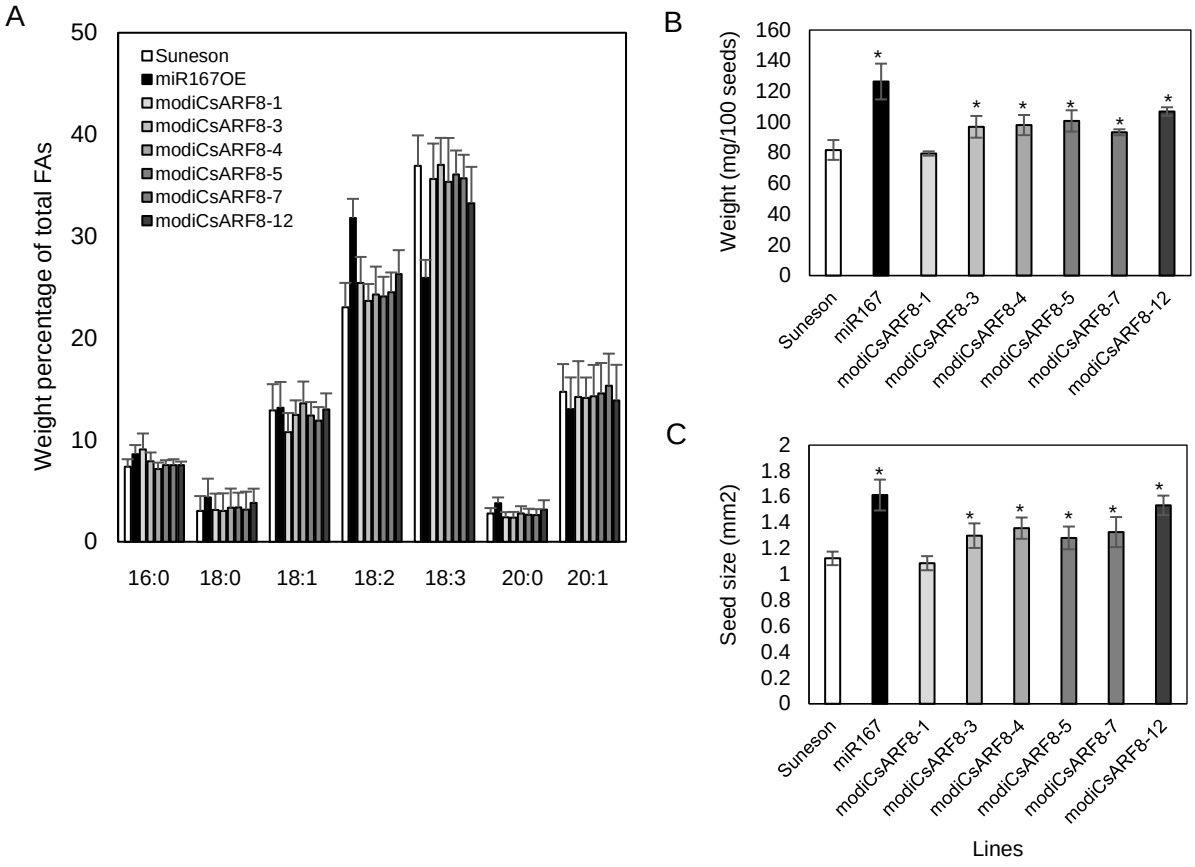


Figure S7. Seed traits of miR167OE expressing the modified CsARF8 target. (A) Fatty acid composition, (B) Seed weight, and (C) Seed size of independent transgenic lines.

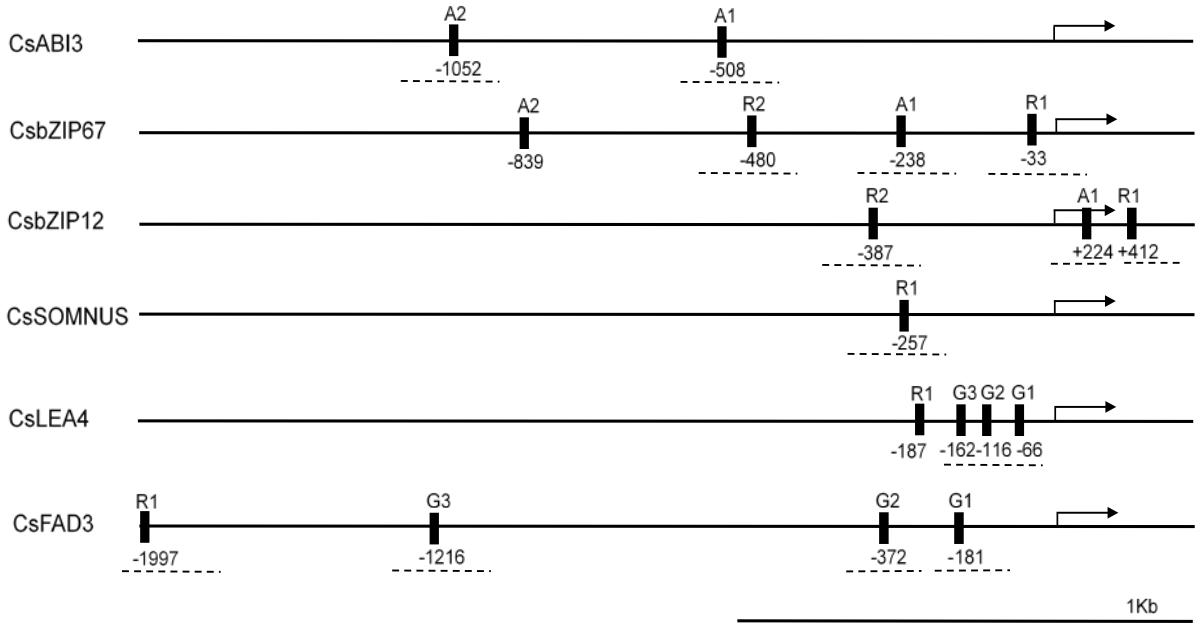


Figure S8. Schematic illustration of the position of putative *cis*-elements in gene promoters. Positions correspond to the start of the elements with the distance upstream of the transcriptional start site marked in base pairs. Arrows represent the transcriptional start site. A, R, and G stand for Auxin response element (A, TGTCTC), RY element (R, CATGCA), and G-box element (G, ACGT to AAGG), respectively. Dashed lines represent of PCR amplicons.

Table S3. List of primers used in this study

Gene	RT-PCR	
	Forward	Reverse
CsACT7	TTGCTATTCAGGCCGTTCTT	CCCTCGTAGATTGGCACAGT
CsARF8	CACTAGACATCTCCCGATTCAG	CAACGAATACAAGCTGCCAG
CsABI3	ACTCCGACGTTAAATGTGGC	GTTCCTCTGCGACTTGTTTTG
CsbZIP67	GAATCTGCGGCTCGGTC	TCGGTAAGGTTGTGCACTTC
CsbZIP12	GAGATCAAGGTTGAAAGGTTAGAAG	AAGCAGAGTTTGTTCGCC
CsbZIP2	ATCGACGGCTGTGGTTTC	CCACCCCAATGATTAACACCA
CsFAD2	TTGCCTCACTACGATTCATCC	TCCATAGTCTCTGTCTACGGTAG
CsFAD3	TGTCATTCATCTTCGGTCCA	CGTCCAACCACATCACAAAG
CsFAE1	TTCCCATCTCCAACACAACC	ACTAACTTTGAGATGCGGTGG
Gene	Cloning	
	Forward	Reverse
CsARF8	GTCGACATGAAGCTGTCAACATCT	CTCGAGGAGGAGATGGGTGGGGTTTT
CsABI3	TCTAGAATGAAAAGCTTGCATG	GGATCCTTTAACAGTTTGAGAA
CsbZIP67	TCTAGAATGTCTGCTGTTTTTG	GGATCCACCCCACCCGGCGCTAG
CsbZIP2	TCTAGAATGGCGTCATCAAGCA	GGATCCATACATATTGATAT
CsbZIP12	TCTAGAATGGGTTCTATTAGAG	GGATCCGAGAGAAGCAGAGTTT
CsFAD3 promoter	AAGCTTCCTAAAGATATGTACC	GTCGACCTCCGGAGAAAGAGAGAG
Gene	ChIP	
	Forward	Reverse
CsABI3-A1	CGTTTGCTTTTTGTCTTCTT	TATGTTCGTGAACTCTTTTT
CsABI3-A2	TATGCTACTACTCTAATGTTA	GCTTTCCACCTTCTATGTGC
CsbZIP67-A1	GAGTGAAAAAATAAGATAAC	GTAGTAGTGAAGTAGAAGAG
CsbZIP67-R1	CTCTTCTACTTCACTACTAC	GTGTCAGTGGAAACTGATAC
CsbZIP67-R2	TTTACGCCAATACATCTAAA	ATTCTACACGTTTACAATAC
CsPAT1-A1	AAGATTAGAGATAGTGAATG	ACGATTATACCACTCAAACA
CsHSF4-A1	GCCTTTTATCATCCGACACT	TATATTGTTTCAACTTTGGG
CsbZIP2-A1	GAACTTCAAGGAAAGGCGAG	ATCAGAATTGGGAGAAAAAG
CsbZIP2-A2	TCCTCTACTGGTTTTACAACG	AAGGACAGATCTTTATGTTT
CsbZIP12-A1	TGTAACAGTTTCTAAGGCAA	AACTTGGGTTCTCTGTGTCC
CsbZIP12-R1	AAAGAGAAAAGGGAAACAGAA	GAAAGGGGGAGGGGGACAAA
CsbZIP12-R2	CAAGTTTAGTTATTATCTGCA	ACCACCTAGAAAATGAGAAG
SOMNUS-R1	GAGAGAAAGAGATGATGATGA	GTGGAAGGGAGTCGTGTGAGA
LEA4	TCGAAGCCTAAACCAATCC	ATGGCCACGTGTTATAACTC
FAD3-G1	TATATTTTCAAACTTACACG	TAGACAAATGAGAGAATAAC
FAD3-G2	ATACTCGTTGTCTTGTCAGT	CGTGTAAGTTTGAAAATATA
FAD3-G3	GTCTTCCCTATGTTTCTGA	TCACATGAATATCGACCTTG
FAD3-R1	CCCATTGATGTCGTGCAAA	TATAGCCTATATGACTGATT