Supplemental Data. Lee et al. (2015)

## **Supplemental Figures**



#### Supplemental Figure S1. Temporal expression of MYB96

Wild-type seeds were germinated and grown at 23°C under long day conditions (LDs). Whole plant materials were harvested at indicated time points. Transcript accumulation was analyzed by quantitative real-time RT-PCR (qRT-PCR). The *EUKARYOTIC TRANSLATION INITIATION FACTOR 4A1 (eIF4a)* gene (At3g13920) was used as an internal control. Biological triplicates were averaged. Bars indicate standard error of the mean. DAC, days after cold imbibition.



## Supplemental Figure S2. Expression of MYB96 in embryo after cold imbibition

The *pMYB96:GUS* transgenic seeds, in which a promoter sequence covering an approximately 2-kb region upstream of the *MYB96* transcription start site was transcriptionally fused to a  $\beta$ -glucuronidase (GUS)-coding sequence, were subject to GUS staining after cold imbibition. Scale bars = 1  $\mu$ m.



# Supplemental Figure S3. ABA induction of MYB96 in embryo

Stratified *pMYB96:GUS* transgenic seeds were plated on Murashige and Skoog (MS)-medium supplemented with or without 1  $\mu$ M abscisic acid (ABA) and incubated for 2 days. The seeds were subject to GUS staining. Scale bars = 1  $\mu$ m.



**Supplemental Figure S4. Expression of** *MYB96* in quadruple-DELLA and GA-deficient mutants Ten-day-old quadruple-DELLA (*QD della*; *gai-t6 rga-t2 rgl1-1 rgl2-1*) and gibberellin (GA)-deficient *ga1-3* mutant seedlings grown at 23°C under LD conditions were used to determine transcript levels of *MYB96*. Transcript accumulation was analyzed by qRT-PCR. The *eIF4a* gene (At3g13920) was used as an internal control. Biological triplicates were averaged. Bars indicate standard error of the mean.



Supplemental Figure S5. Cotyledon opening of *myb96-ox* and *myb96-1* in the presence of ABA Cotyledon opening of the indicated genotypes grown on different concentrations of ABA was quantified after the end of stratification. Fully opened cotyledon was used as a morphological marker. At least 50 seeds per genotype were measured in each replicate. Biological triplicates were averaged. Bars indicate standard error of the mean. Statistically significant differences between the wild-type and mutants are indicated by asterisks (Student's *t*-test, \*P<0.05).



### Supplemental Figure S6. ABA dose-response assays

Wild-type, *myb96-ox*, and *myb96-1* seeds were germinated on different concentrations of ABA. Fully opened cotyledons were counted 84 h after cold stratification. Biological triplicates were averaged. Bars indicate standard error of the mean. Statistically significant differences between the wild-type and mutants are indicated by asterisks (Student's *t*-test, \*P<0.05).



Supplemental Figure S7. Expression of *ABI4* and *BLH1* in *myb96-ox* mutant in the presence of ABA

Germinating seeds were plated on MS-medium supplemented with or without 5  $\mu$ M ABA and incubated for 24 h. Transcript accumulation was analyzed by qRT-PCR. Biological triplicates were averaged. Bars indicate standard error of the mean.



#### Supplemental Figure S8. ABI4 expression in dry seeds and during seed germination

Wild-type dry seeds were stratified and transferred to LD conditions at 22°C for germination. Transcript accumulation was analyzed by qRT-PCR. The *eIF4a* gene was used as an internal control. Biological triplicates were averaged. Bars indicate the standard error of the mean. DAC, days after cold stratification.



#### Supplemental Figure S9. Spatial expression of MYB96 and ABI4

Eight-week-old plants were used to analyze tissue-specific expression of *MYB96* and *ABI4*. Transcript accumulation was analyzed by qRT-PCR. The *eIF4a* gene (At3g13920) was used as an internal control. Biological triplicates were averaged. Bars indicate the standard error of the mean. The y-axis is presented on a logarithmic scale for better comparison of fold changes. St, stems; Fl, flowers; Si, siliques; CL, cauline leaves; RL, rosette leaves; Ro, roots.



# Supplemental Figure S10. Chromatin immunoprecipitation using 35S:96BD-MYC transgenic plants

Total protein extracts from 35S:96BD-MYC transgenic plants grown for 2 weeks under LD were immunoprecipitated with an anti-MYC antibody. Fragmented genomic DNA was eluted from the protein-DNA complexes and subjected to quantitative PCR analysis. Biological triplicates were averaged and statistical significance of the measurements was determined using a Student's *t*-test (\*P < 0.05). Bars indicate the standard error of the mean. The measurement values in pBA002 were set to 1 after normalization against *eIF4a* for quantitative PCR analysis.



Supplemental Figure S11. Lipid breakdown in wild-type, *myb96-ox*, and *myb96-1* seeds during seed germination in the absence of ABA

Seeds were germinated and incubated at 23°C under LD conditions for 3 days. Lipid breakdown was determined by measuring the abundance of total fatty acid (A) and eicosenoic acid (20:1) (B). Fatty acid content was expressed as the percentage of the amount present in dry seeds of the indicated genotypes. At least 30 seeds per genotype were measured in each replicate. Biological triplicates were averaged. Bars indicate the standard error of the mean.

Primer	Usage	Sequence		
		F	R	
eIF4a	qRT-PCR	TGACCACACAGTCTCTGCAA	ACCAGGGAGACTTGTTGGAC	
MYB96	qRT-PCR	TGCAGTCTCGGAAGAAGGTG	CATCTCGTGGCTTTGCTCAT	
ABI1	qRT-PCR	CGTCTCACATCTTCGTCGCT	TCAATCCTCGCAGCTTCATC	
ABI2	qRT-PCR	GGCTCGGAAACGGATTTTAC	GCAAAGCCATCTTCGACAAA	
ABI3	qRT-PCR	GATTGAATCAGCGGCAAGAA	GTTGTTGTGGTGGTGGAGGA	
ABI4	qRT-PCR	ATCCTCAATCCGATTCCACC	ATTTGCCCCAGCTTCTTTGT	
ABI5	qRT-PCR	GGCGCAAGCGAGACATAAT	CCCTCGCCTCCATTGTTATT	
ACBP1	qRT-PCR	AACCACACGACTCAATCGGA	TTTCGACACCTTCCCAATCA	
AHK1	qRT-PCR	TTCAGCGTCCAGTCTTACGG	GCAAACAGAGCCCATGTCAC	
BLH1	qRT-PCR	AGACCTCAACGTGGTCTCCC	GGATCCCATGTTCTTTGCCT	
FY	qRT-PCR	TGACGGCTCCATTTGTCATT	CCATGCAAGATCCCAAACAC	
RHA2a	qRT-PCR	CTCTCTCTCCTCGCCGTCTT	CTGATCGGCGAGAACGATTA	
SnRK2.2	qRT-PCR	TAATGCCGGACGGTTTAGTG	TATTTTCAAACGAGGTGCCG	
SnRK2.3	qRT-PCR	CATTTTGACGCCGACTCATC	TTCAGGTCACGATGGCAAAT	

## Supplemental Table S1. Primers used in qRT-PCR

qRT-PCR primers were designed using the Primer Express Software installed into the Applied Biosystems 7500 Real-Time PCR System. The sizes of PCR products ranged from 80 to 300 nucleotides in length. F, forward primer; R, reverse primer.

# Supplemental Table S2. Primers used in ChIP assays

Primer	Usage	Sequence		
		F	R	
eIF4a ABI4(A) ABI4(B) ABI4(C)	ChIP ChIP ChIP ChIP	TGACCACAGTCTCTGCAA CAACATGCGAGTATTTCTCAC CCAGAAATATGATTCTAGTTTTTACTTATGTC TTTCAAAATTCCTTTTCTTATAAAAAATG	ACCAGGGAGACTTGTTGGAC GAGAAAAATAGTGGAGAGGACG CTCTATTTTAGAGGTGACCATTGG TTAGTCCACTTAACACCATTCTTGG	

F, forward primer; R, reverse primer.