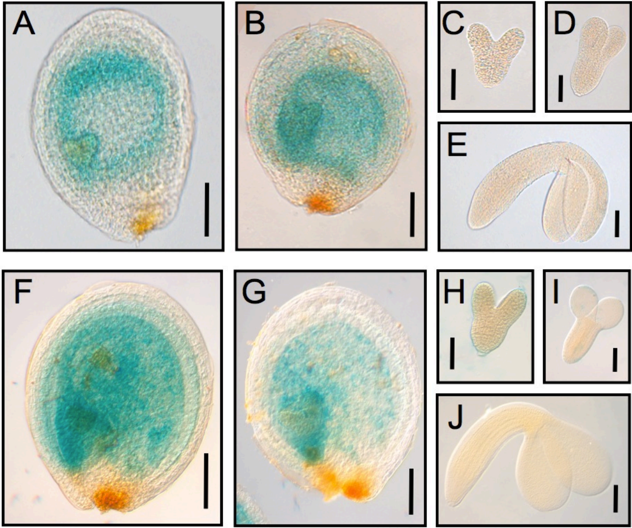
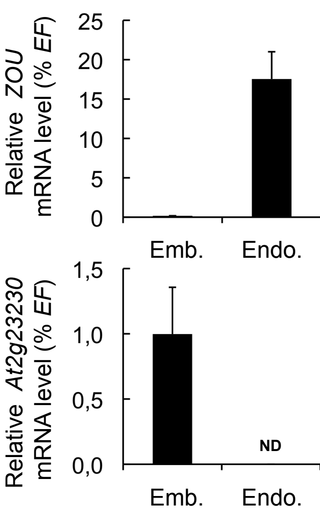
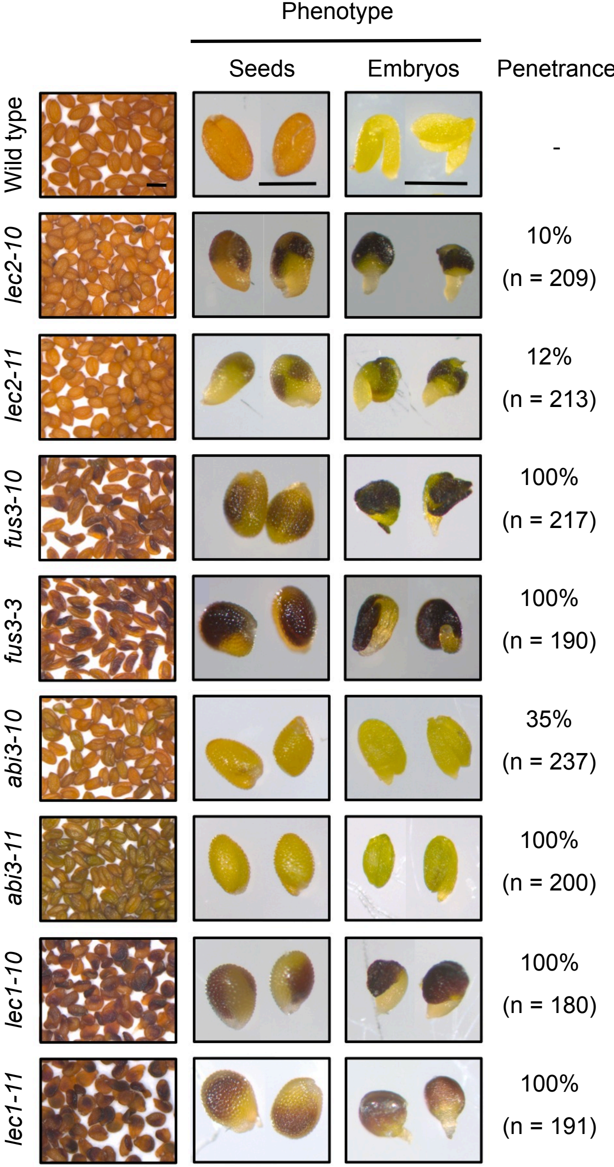


Supplemental Figure 1. Complementary results for the characterization of the expression pattern of *MYB118.*

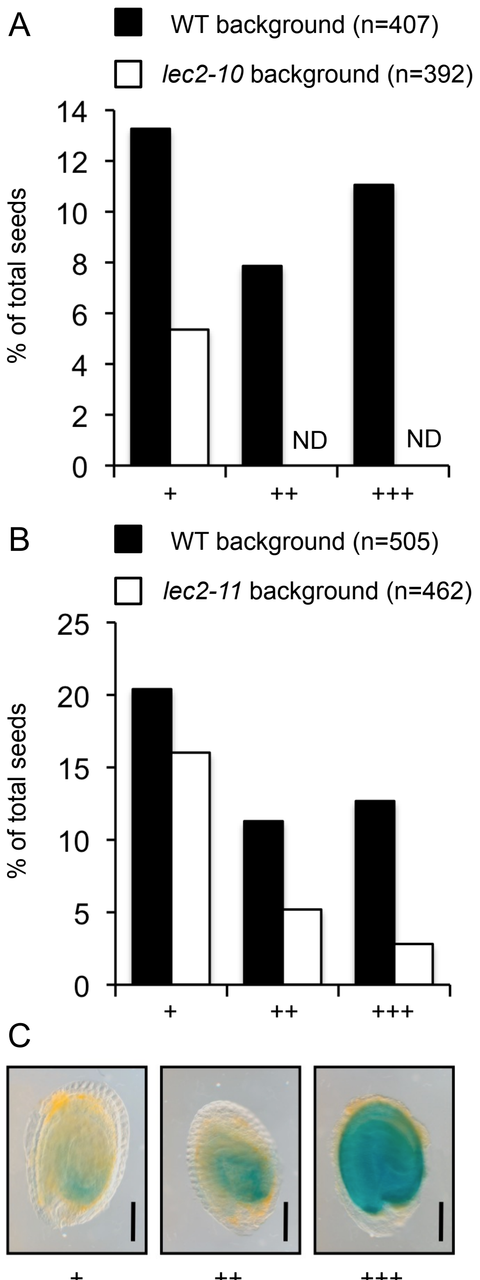
Pattern of activity of the *ProMYB118(-970bp):uidA* cassette (A-E) and of the *ProMYB118:uidA* cassette by Wang et al. (2008) (F-J) in developing seeds observed 6 (A and F) or 8 DAA (B and G), and in early maturing embryos observed 8 (C and H), 10 (D and I), or 12 DAA (E and J). For histochemical detection of GUS activity, tissues were incubated overnight in a buffer containing 0.2 mM each of potassium ferrocyanide and potassium ferricyanide. Seeds and embryos were observed using Nomarski optics. Bars = 100 µm. DAA, days after anthesis.



Supplemental Figure 2. Purity of the different seed fractions dissected.
qRT-PCR analysis of endosperm-specific *ZHOUPI* (*ZOU*) and embryo-specific At2g23230 gene transcript levels with cDNA prepared from embryo and endosperm fractions isolated from seeds aged 12 days after anthesis. Values are the means and SE of three replicates carried out on cDNA dilutions obtained from three independent mRNA extractions. ND, not detected.

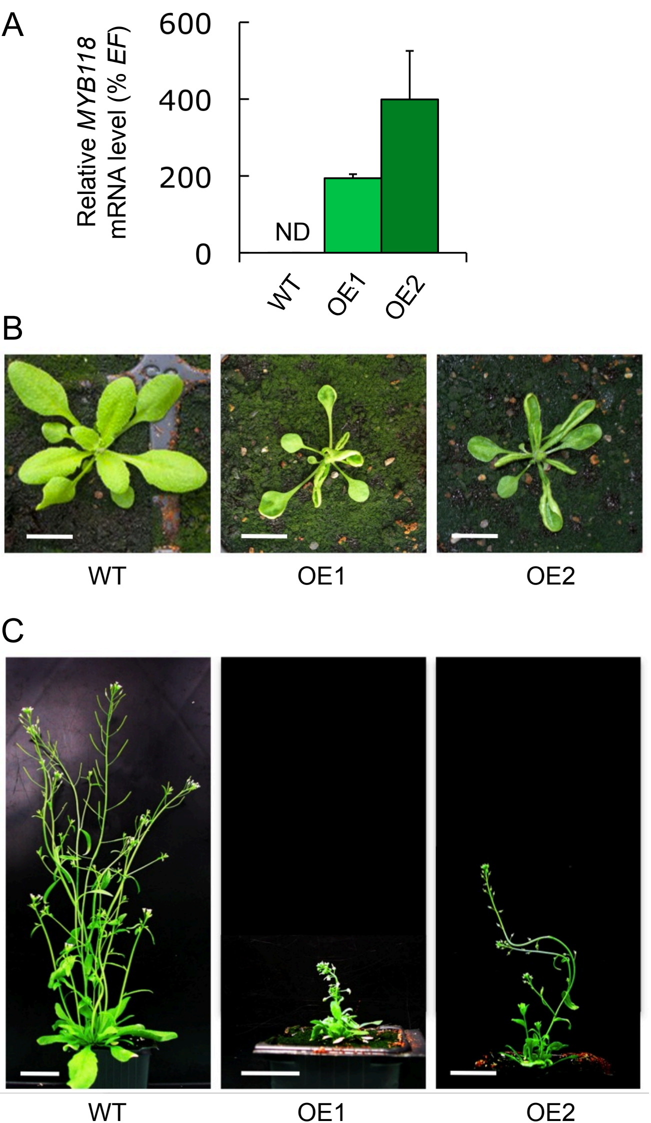


Supplemental Figure 3. Phenotypes of mutant seeds and embryos affected in master regulators of the maturation program.
Batches of mature dry seeds were observed. Magnification of representative mutant seeds and excised embryos are presented. The penetrance of the corresponding phenotypes is also indicated. Bars = 500 µm.

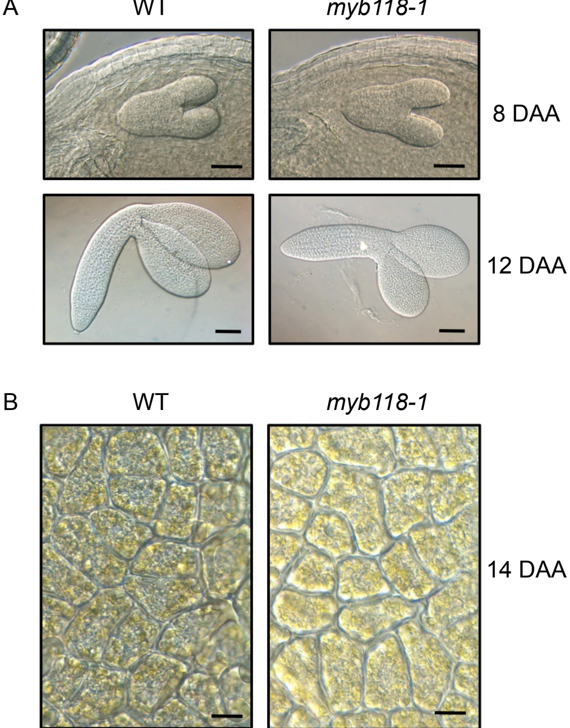


Supplemental Figure 4. Complementary results for the characterization of the regulation of *MYB118* by LEC2.

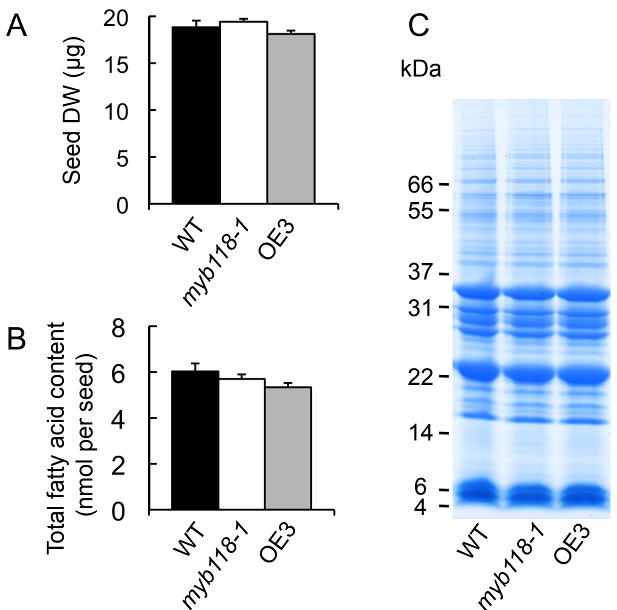
Patterns of activity of the *ProMYB118(-2.5 kb):uidA* cassette introduced in *lec2-10* (A) and in *lec2-11* (B) mutant backgrounds were compared to the pattern observed in a wild-type context. Plant material used for this study consisted of *lec2* homozygous lines hemizygous for the *ProMYB118(-2.5 kb):uidA* cassette. Control plants consisted of Col-0 lines also harbouring the *ProMYB118(-2.5 kb):uidA* cassette in the hemizygous state. Segregating seeds from these different lines were harvested at the maturation stage and incubated overnight in a buffer containing 0.2 mM each of potassium ferrocyanide and potassium ferricyanide. Seeds were observed to score for GUS-positive individuals and to evaluate the intensity of the corresponding GUS staining (from + to +++, as exemplified in C). Bars = 250 µm.



Supplemental Figure 5. Characterization of *MYB118* overexpressing lines.
(A) qRT-PCR analysis of *MYB118* transcript abundance in cDNA prepared from two-week-old rosette leaves. Values are the means and SE of three to four replicates carried out on cDNA dilutions obtained from three independent mRNA extractions. ND, not detected.
(B) Observation of two-week-old rosettes. Bars = 2 cm.
(C) Observation of four-week-old plants. Bars = 2 cm.
WT, wild type (Col-0).



Supplemental Figure 6. Microscopic observation of *myb118-1* seed development.
(A) Observation of seed development. Whole mounts of early developing seeds (8 DAA) and of maturing embryos (12 DAA) were observed with Nomarski optics. Bars = 50 µm.
(B) Observation of maturing endosperm. Whole mounts of peeled endosperms (14 DAA) were observed with Nomarski optics. Bars = 20 µm.
DAA, days after anthesis; WT, wild type.



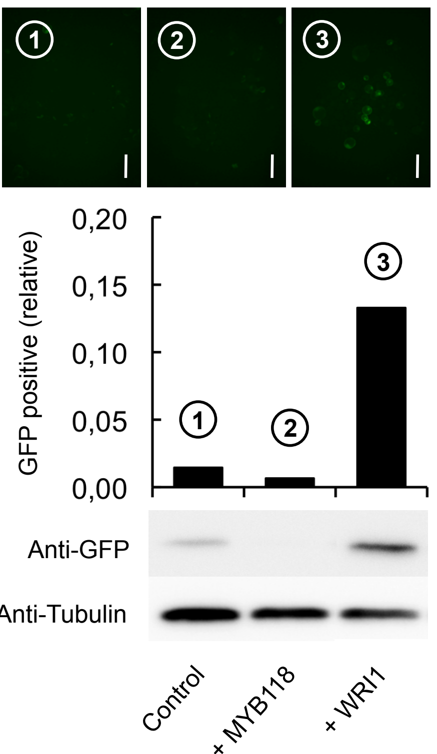
Supplemental Figure 7. Characterization of *myb118-1* and OE3 seeds.

(A) Seed DW. Values are the means and SE of five replicates carried out on batches of 20 individuals from five distinct plants.

(B) Total fatty acid content. Values are the means and SE of five replicates carried out on batches of 20 individuals from five distinct plants.

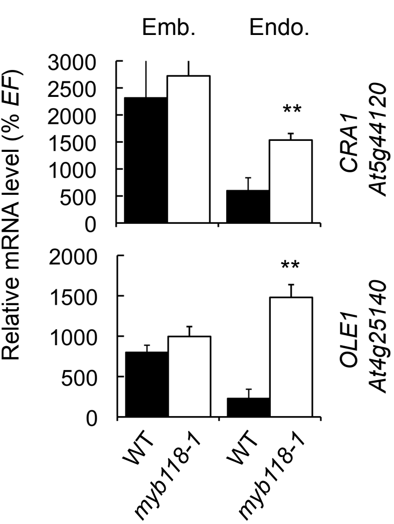
(C) Storage protein content. A representative gel stained with Coomassie Blue G250 is presented. Each lane contains an amount of proteins equivalent to four seeds.

DW, dry weight; WT, wild type (Col-0).



Supplemental Figure 8. Negative control for transcriptional activation studies in protoplasts of *Arabidopsis*.

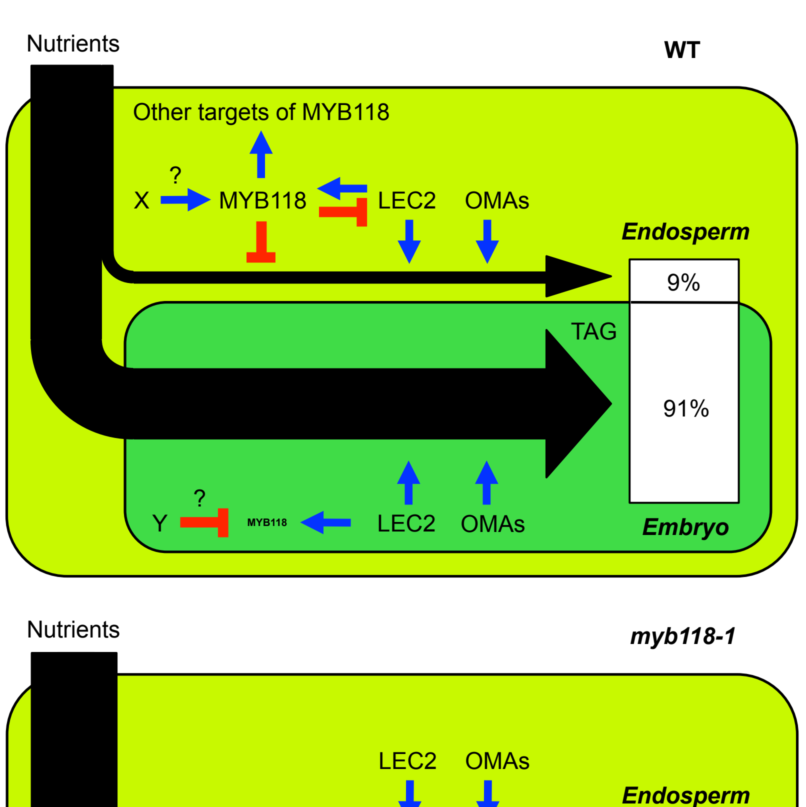
Protoplasts were transformed with the *ProBCCP2:GFP* reporter construct alone or in combination with a vector allowing the expression of MYB118 or WRI1 (positive control). Whole mounts of protoplasts were observed 12 h after transformation and the relative proportion of GFP-expressing protoplasts was calculated (between 800 and 1000 protoplasts were scored). Immunoblots of a batch of 0.5 ml of transformed protoplasts for each combination of constructs assayed and using primary antibodies raised against GFP (anti-GFP serum) or alpha-tubulin (anti-Tubulin serum) are presented. Bars = 50 µm. .

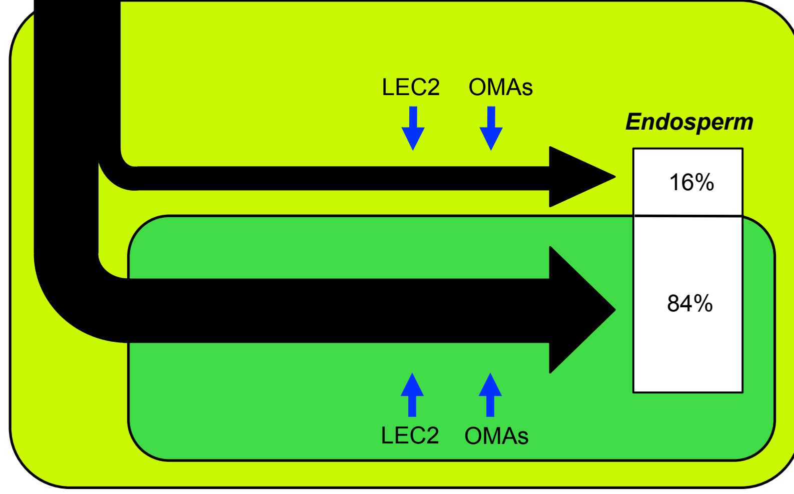


Supplemental Figure 9. Complementary results for the study of the expression pattern of maturation-related genes in the *myb118-1* mutant background.

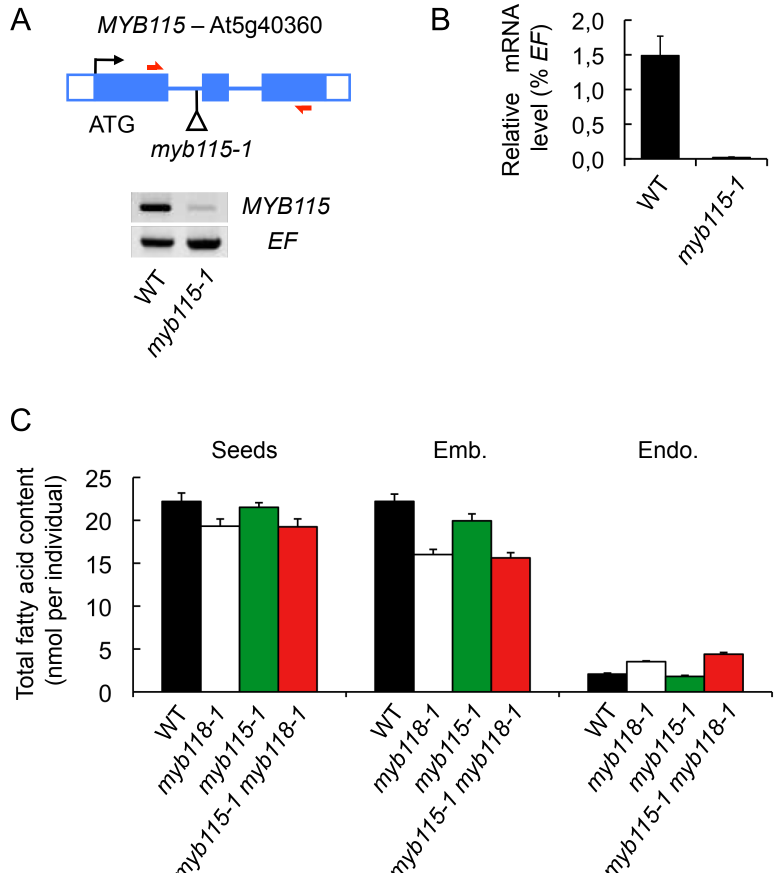
qRT-PCR analysis of gene transcript abundance in cDNA prepared from embryo and endosperm fractions isolated from seeds harvested 10 days after anthesis. Values are the means and SE of three replicates carried out on cDNA dilutions obtained from three independent mRNA extractions.

Emb., embryo; Endo., endosperm; WT, wild type (Col-0). **, Significant difference from WT according to *t*-test, P < 0.01.

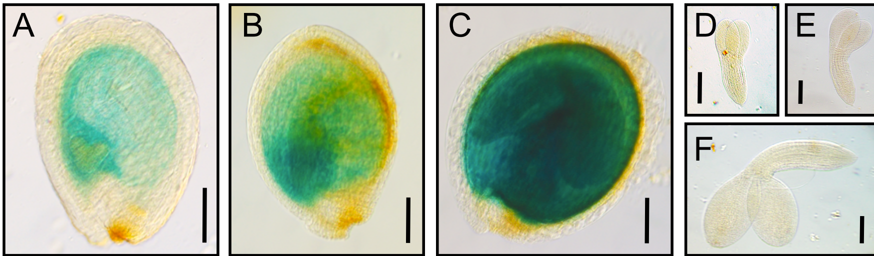




Supplemental Figure 10. A model for the transcriptional regulation of seed maturation in *Arabidopsis*.
Nutrients imported from the parent plant (black arrow) are shared between the embryo and the endosperm to produce storage compounds like oil (white box). LEC2 and other master activators (OMAs) of the maturation program promote storage compound biosynthesis in the two zygotic tissues. In the endosperm, MYB118 represses their biosynthesis. The relative proportions of oil stored in the endosperm and in the embryo of dry seeds are presented in various genetic backgrounds. X and Y are unknown transcriptional regulators of *MYB118*.



Supplemental Figure 11. Characterization of *myb115-1 and myb115-1 myb118-1* mutants.
(A) Molecular characterization of *myb115-1*. Structure of the *MYB115* gene showing the position of the T-DNA insertion in *myb115-1* is presented. Confirmed flanking sequence tag is anchored in the gene structure and represented by a vertical bar. Closed boxes represent exons and open boxes untranslated regions (UTR). Accumulation of *MYB115* mRNA in wild-type and transgenic backgrounds was studied by RT-PCR on developing seeds harvested 10 DAA. *EF1αA4* (*EF*) gene expression was used as a constitutive control. Primers used for this study are indicated as red arrows (see Supplemental Table 5 online).
(B) Accumulation of *MYB115* mRNA in wild-type and *myb115-1* seeds was quantified 10 days after anthesis by qRT-PCR and presented as the percentage of the constitutive *EF1αA4* (*EF*) gene expression. Values are the means and SE of three to six replicates carried out on three independent cDNA preparations obtained from batches of seeds dissected from four to five siliques. The three silique sets were harvested on distinct individuals.
(C) Total fatty acid content of mature seeds (Seeds), embryo (Emb.) and endosperm fractions (Endo.) dissected from mature seeds. Values are the means and SE of five replicates carried out on batches of 20 individuals from five distinct plants.



Supplemental Figure 12. Characterization of the expression pattern of *ODD*.

Pattern of activity of the *ProODD:uidA* cassette in developing seeds observed 6 (A), 8 (B), or 10 DAA (C), and in early maturing embryos observed 8 (D), 10 (E), or 12 DAA (F). For histochemical detection of GUS activity, tissues were incubated overnight in a buffer containing 2 mM each of potassium ferrocyanide and potassium ferricyanide. Seeds and embryos were observed using Nomarski optics. Bars = 100 µm. DAA, days after anthesis.

Supplemental Table 1. Selected genes activated by MYB118 as identified by microarray analyses

	Gene	AGI	Log ratio	
			WT/*myb118-1*	WT/OE3
Auxin metabolism and signaling	*SAUR-like*	At2g45210	0.90	1.24
	ARF11	At2g46530	1.35	1.28
	NPH3	At2g47860	1.11	1.39
GA metabolism and signaling	*GA1*	At4g02780	1.23	1.19
	GA3	At5g25900	1.69	1.46
Cytokinin metabolism and signaling	*ARR15*	At1g74890	1.94	2.13
	CKX3	At5g56970	1.75	1.73
Ethylen metabolism and signaling	*AtERF8*	At1g53170	0.97	1.28
ABA metabolism and signaling	*AAO2*	At3g43600	1.00	1.25
	HB7	At5g53730	1.03	1.27
Growth and development	*WOX9*	At2g33880	1.02	1.16
	EMB1353	At4g30090	1.99	1.87
	PSY1	At5g58650	0.84	1.23
Biotic and abiotic stresses	*CYP86C3*	At1g13140	2.29	2.37
	RLK1	At1g48480	1.16	1.09
	PER17	At2g22420	1.84	1.88

Supplemental Table 2. Selected genes repressed by MYB118 as identified by microarray analyses

	Gene	AGI	Log ratio	
			myb118-1/WT	OE3/WT
Master regulator	*LEC2*	At1g28300	1.59	1.42
Seed storage proteins	*At2S1*	At4g27140	1.81	1.78
	At2S-like/At2S5	At5g54740	1.80	1.88
	-	At1g03890	1.79	2.17
Oleosins	*SM3*	At2g25890	1.68	1.82
	GRP19	At5g07550	3.69	3.40
	-	At5g56100	1.02	1.29
Late embryogenesis abundant (LEA)	-	At2g03850	3.60	3.89
	-	At4g21020	1.81	1.85
	-	At5g53730	2.90	3.13

Supplemental Table 3. Primers used for molecular characterization of T-DNA insertions

Mutant	Border 1		Border 2	
	Forward primer (gene sequence, 5'→3')	Reverse primer (T-DNA, 5'→3')	Forward primer (T-DNA, 5'→3')	Reverse primer (gene sequence, 5'→3')
abi3-10	AAACTATCCGCCACAGC	CGGAACCACCATCAAACAG	(the border could not be amplified)	
abi3-11	GATATGATGGAGACTTTCGG	CGGAACCACCATCAAACAG	(the border could not be amplified)	
fus3-10	AGCTCTCTCCGACGTATG	CCCATTTGGACGTGAATGTAG	CCCATTTGGACGTGAATGTAG	CGATGCTTTTCTTGCTTG
lec1-10	(the border could not be amplified)		CGGAACCACCATCAAACAG	CGATACCATTGTTCTTGTCAC
lec1-11	(the border could not be amplified)		CGGAACCACCATCAAACAG	TGAAACCCAATACCATTTC
lec2-10	(the border could not be amplified)		CGGAACCACCATCAAACAG	ATAGTGATCGTTTAGATTTCCG
lec2-11	CAAGAGAGAGGTGGTTTTC	GCCTTTTCAGAAATGGATAAAT AGCCTTGCTTCC	(the border could not be amplified)	
myb115-1	(the border could not be amplified)		CGGAACCACCATCAAACAG	ATTTTTCTGAAAACACACATTC
myb118-1	GAAAACGATCAAAACCAAG	CGGAACCACCATCAAACAG	CGGAACCACCATCAAACAG	CTGCACTGTTTTCCAACTC

Supplemental Table 4. Primers used for mutant characterization				
Mutant	AGI		Forward primer (5'→3')	Reverse primer (5'→3')
abi3	At3g24650		GATATGATGGAGACTTTCGG	CAGCTTTAATCATGACCCTC
			TACTCCGACGTCAAATGTG	CAGCTTTAATCATGACCCTC
fus3	At3g26790		CAGCGTCCATTTTAAGC	CGATGCTTTTCTTGCTTG
			CATCGGAAGAAGAAGAAG	AGTCGTCGTATGTCCAC
lec1	At1g21970		GGCAGAGAAACAATGGAAC	TTTGGGTTAAATCATTTGC
			AATCGCAAACGTCATAAG	CGAAGAGCCACCACC
lec2	At1g28300		CAAGAGAGAGGTGGTTTTC	ATAGTGATCGTTTAGATTTCCG
			GGAAGAGAAAATGAGTCG	CTTCCACCACCATATCAC
myb115	At5g40360		GGGCAATGGACTCCTAC	ATTTTTCTGAAAACACACATTC
myb118	At3g27785		GAAAACGATCAAAACCAAG	CTGCACTGTTTTCCAACTC
			GATGGATGGACTGAAGAAG	CAAAGCAATCTCGTTCATC

Supplemental Table 5. Details of reporter constructs comprising concatemers of MYB elements and used for transactivation assays in *Nicotinana benthamiana*

The reporter construct containing four repeats of the MYB element (identified in the promoter sequence of the *ODD* gene) fused to the 35S cauliflower mosaic virus minimal promoter upstream of the *GUS* reporter gene was obtained by annealing of the PCI118F and PCI118R primers followed by amplification of the double-stranded DNA fragment thus obtained by PCR reaction with the proofreading Pfu Ultra DNA polymerase (Stratagene) and using PC118F and PC118R primers, *att*B1 and *att*B2 referring to the corresponding Gateway recombination sequences. The PCR product was introduced by BP recombination into the pDONR207 entry vector (Invitrogen) and transferred into the destination vector pGWB3 (Nagawa et al., 2007).
The reporter construct containing four repeats of the mutated version of the MYB element studied fused to the 35S cauliflower mosaic virus minimal promoter upstream of the *GUS* reporter gene was obtained by annealing of the PCI118FM and PCI118RM primers followed by amplification of the double-stranded DNA fragment thus obtained by PCR reaction with the proofreading Pfu Ultra DNA polymerase (Stratagene) and using PC118F and PC118R primers.

Primer	Sequence (5'→3')
PCI118F	CAAAAAAGCAGGCTTACGTAACGGTCATATTACGTAACGGTCATATTACGTAA CGGTCATATTACGTAACGGTCATATGCAAGACCCTTCC
PCI118R	GGAAGGGTCTTGCATATGACCGTTACGTAATATGACCGTTACGTAATATGAC CGTTACGTAATATGACCGTTACGTAAGCCTGCTTTTTTG
PCI118FM	CAAAAAAGCAGGCTTACGTGGTGATCATATTACGTGGTGATCATATTACGTG GTGATCATATTACGTGGTGATCATATGCAAGACCCTTCC
PCI118RM	GGAAGGGTCTTGCATATGATCACCACGTAATATGATCACCACGTAATATGATC ACCACGTAATATGATCACCACGTAAGCCTGCTTTTTTG
PC118F	*att*B1-TACG
PC118R	*att*B2- GTCCTCTCCAAATGAAATGAACTTCCTTATATAGAGGAAGGGTCTTGCATATG

Supplemental Table 6. Primers used for quantitative RT-PCR

Gene	AGI	Forward primer (5’→3’)	Reverse primer (5’→3’)
-	At2g23230	ATGGTGCATTAGCCGAGCG	CCCTAGCCTTCGGTCCTAACCC
ABI3	At3g24650	QuantiTect Primer Assay (Qiagen)	
ACPT/CPT3	At2g23410	ACTTATGTTGTCCGCGAGGATGG	GCCGTTTAGCTCCCGCC
ARR15	At1g74890	AGTGGGACTAGGGCTCTGC	ATTTCTCTGAGTGCTGAAGACTCTTTG
AT2S1	At4g27140	CCATCTACCGCACCGTCG	CTACGGCCTTGCCTTGCT
AT2S5	At5g54740	ATCCTGCCAGTTCAAGGCAT	ACTCATACAGTACAAACATTTATCTACTATACAAACACG
BCCP2	At5g15530	GACCCGGTGAACCCCCT	GTCAACGCTGACTGGTTTTCCAT
CKX3	At5g56970	GTTGTGGTCAACATGCGGTCC	CGTTTGATAACGTCCCACCGACTG
CRA1	At5g44120	ATCGTAAACGACAATGGTAACAGAGTG	AGACTGAGGTTCGTCCCGC
CRUL	At1g03890	ACCATTTTAGCACACGAGCCG	CCTCCTGTGACGTATAGCACCG
EF1alphaA4	At5g60390	CTGGAGGTTTTGAGGCTGGTAT	CCAAGGGTGAAAGCAAGAAGA
EMB1353	At4g30090	CGATGGGTGGTTCTTGTCTAAACT	AGGTCATAATCCGGGTCGTGT
ERF8	At1g53170	CCCGACCCGGTTGCTCC	GTCGAACGTACCGAGCCAG
FUS3	At1g26790	QuantiTect Primer Assay (Qiagen)	
GRP19	At5g07550	ATGTTTGAGATTATTCAGGCGGTCTT	GCTGGCACGAGAACGGG
KCS7	At1g71160	TGACATTGATTTCGCTGCTC	TGAAAGGTTGTTCCTACAATGC
LEA	At2g03850	AGAAGCGGCGTTGGACT	ATCATCGAGCCATTTGGGGT
LEA	At4g21020	CGCTCTAGTTGGTCTCTCAAAGGC	CCTCCCTCCATTTCTTCGATCCG
LEA	At5g53730	CAAGGGACTGAGCTACCCG	CGGTATGCACCCGAGACCCA
LEC1	At1g21970	QuantiTect Primer Assay (Qiagen)	
LEC2	At1g28300	QuantiTect Primer Assay (Qiagen)	
MYB115	At5g40360	QuantiTect Primer Assay (Qiagen)	
MYB118	At3g27785	QuantiTect Primer Assay (Qiagen)	
ODD	At1g04380	CTCGCTTGACCTCACCAAAACA	CTGCAAGCCACTGGGAGAT
OLE1/S3	At4g25140	AGGCAGATTGCTAAAGCTGCAAC	ACTGTGATGAGAGCCGGG
PER17	At2g22420	ATCTTTCGAAGTCGTTGACGACAT	TGCTGACTCGCTGTTAAACTGTCT
PKp-ß1	At5g52920	AGTCACTATCGTCCTTCCG	CTGTACGATTGCTATTTCCTC
RLK1	At1g48480	ATTGCTATTGGTGCAGCTCGT	AGCTGGGCTAAGCCAAAGT
SM3	At2g25890	TCTGTTCAGCCCGGTGTTGG	CGCTATCCTCATCCTCGCGT
WRI1	At3g54320	Quantitect Primer Assay (Qiagen)	
ZOU/RGE1	At1g49770	CCATCATCATCCTCTTCTCCAACAGC	GGTAAGGCAGGTTCGCAGC