SI APPENDIX

Kim *et al*. The DME demethylase regulates sporophyte gene expression, cell proliferation, differentiation and meristem resurrection

SI Materials and Methods

Seed Germination Analysis. Wild-type and viable homozygous dme-2 seeds were sterilized and sown with a sterilized Pasteur pipette, one by one on a MS plate. MS plates were covered with aluminum foil for dark treatment. After 3 days of dark 4°C treatment, the foil was removed and incubated in a cold room with continuous light. Seed counting was performed in the cold-room to prevent temperature changes. Seed coat ruptured seeds were the imbibed seeds showing a crack on their surface and endosperm ruptured seeds were the ones with a visible radicle.

RAM Visualization. Seeds were sterilized with a 0.5% TritonX100, 75% EtOH solution and sown on square form 0.5X MS media plates (0.5X Mushirage and Sqoog medium, 1% Phytoagar) in a single line. MS plates were placed in cold-dark for 3 days and grown vertically in a long-day (16 hrs light/8 hrs dark) photoperiod under cool white fluorescent light (100 μmole/m2/s) at 22°C with 60% humidity. Root tips were cut using a sharp knife on the medium. Samples were mounted on slide glass with 1ug/ml PI solution. All samples were visualized under 40x water immersion lens, RFP filter, LSM700 (Carl Zeiss).

Observation of Root Hair Phenotypes. Root hair phenotypes were observed under a stereomicroscope (M205 FA;Leica). Root hair length was measured as described by Lee and Cho (1) with modifications. Roots of 3-d-old seedlings were digitally photographed using the stereomicroscope at 40X magnification. The hair length of nine consecutive hairs protruding perpendicularly from each side of the root, for a total of 18 hairs from both sides of the root, was calculated using ImageJ 1.50b software (National Institutes of Health). To assess root hair cell distribution in the root epidermis, the ratio of root hair-containing cells in 10 consecutive epidermal cells from the H (root hair cell) position or from the N (non-root-hair cell) position was estimated from 15 to 20 roots of 4-d-old seedlings for each line.

Stomata Visualization and Normalization. DAG5 seedling cotyledon were cut with a sharp knife and mounted with 1ug/ml PI on a slide glass. Samples were visualized with under 40x water immersion lens, RFP filter, LSM700 (Carl Zeiss). All photographs were in 123.11um^2 size, same magnification. Stomata and precursor cells on each image were identified by their morphology and counted. After accumulation of data, all numbers were normalized by 25000um^2 area for comparison.

Plant Growth and Flowering Resurrection Study. At least 16 plants were sown and transferred the same day and grown in the same environment. After bolting, the main stem was hand-measured with a ruler and documented. All plants were watered even after termination to check the resurrection phenotype. After the second termination, silique numbers were counted and recorded.

Generation of *dme-2/dme-2* homozygous lines
We used the following genetic strategy to isolate a *dme-2* homozygous mutant plant. First, *dme-*

1 homozygous female plants were pollinated with *dme-2/+* heterozygous pollen, to generate *dme-1/dme-2* F1 mutant plants. Next, the *dme-1/dme-2* F1 plants were self-pollinated, and a total of 63 viable F2 plants were obtained with the following genotypes confirmed by molecular analyses (*SI Appendix,* Fig. S2): 25 *dme-1*/*dme-1*, 31 *dme-1/dme-2*, and 7 *dme-2/dme-2*, which displayed F3 seed abortion ratios of 91.5 %, 96.8, and 97.1 %, respectively (Fig. 1B and *SI Appendix,* Table S1). Thus, we generated multiple, independent *dme-2/dme-2* homozygous mutants lines for subsequent phenotypic analyses.

1. S. H. Lee, H. T. Cho, PINOID positively regulates auxin efflux in Arabidopsis root hair cells and tobacco cells. *Plant Cell* **18**, 1604-1616 (2006).
2. T. F. Hsieh *et al.*, Regulation of imprinted gene expression in Arabidopsis endosperm. *Proc Natl Acad Sci U S A* **108**, 1755-1762 (2011).

	dme-1/dme-1			*dme-1/dme-2*			*dme-2/dme-2*	
	Abortion	**Normal**		**Abortion**	**Normal**		**Abortion**	**Normal**
#1	51	4	**#4**	47	3	**#2**	53	2
90.20%	45	10	**93.10%**	49	5	**95.60%**	49	3
	50	4		55	5		56	4
	48	3		51	2		58	1
	194	21		202	15		216	10
#3	57	4	**#7**	49	1	**#5**	49	2
91.00%	46	5	**98.60%**	50	0	**96.60%**	50	2
	55	8		60	0		51	1
	54	4		52	2		46	2
	212	21		211	3		196	7
#6	37	3	**#8**	51	0	**#9**	48	0
88.30%	38	5	**99.50%**	58	0	**96.00%**	33	3
	42	6		51	0		47	1
	41	7		51	1		38	3
	158	21		211	1		166	7
#10	51	6	**#24**	53	0	**#23**	41	1
91.00%	45	2	**97.80%**	40	1	**98.20%**	36	1
	36	5		45	2		49	0
	51	5		39	1		37	1
	183	18		177	4		163	3
#13	41	1	**#25**	51	0	**#28**	44	2
98.50%	51	1	**97.90%**	43	1	**98.40%**	44	0
	49	1		50	2		48	0
	54	0		39	1		46	1
	195	3		183	4		182	3
#27	45	4	**#32**	44	5	**#31**	51	0
91.60%	48	3	**93.40%**	49	6	**98.50%**	44	1
	40	8		54	2		43	0
	41	1		50	1		54	2
	174	16		197	14		192	3
#38	46	6	**#36**	52	1	**#37**	47	3
89.60%	48	9	**97.40%**	46	2	**97.10%**	38	0
	50	5		52	1		48	1
	46	2		39	1		36	1
	190	22		189	5		169	5
Total	1306	122		1370	46		1284	38
Seeds/Silique		51			50.6			47.2
Abortion Ratio		91.5%			96.8%			97.1%

Table S1. Seed abortion ratio in *dme-1* and *dme-2* homozygous plants

	Id	Gene symbol	Short description
Up-regulated in both dme & ros1	AT1G14650	AT1G14650	SWAP(Suppressor-of-White-APricot)/surp domain-containing protein
	AT1G31580	ECS1	ECS1
	AT4G05020	NDB2	NAD(P)H dehydrogenase B2
	AT4G13420	HAK5	high affinity K transporter 5
	AT4G19520	AT4G19520	disease resistance protein (TIR-NBS-LRR class) family
Down-regulated in boh dme & ros1	AT1G44608	AT1G44608	
	AT1G59980	ARL2	ARG1-like 2
	AT1G62760	AT1G62760	Plant invertase/pectin methylesterase inhibitor superfamily protein
	AT1G67105	AT1G67105	other RNA
	AT2G40610	EXPA8	expansin A8
	AT3G43850	AT3G43850	
	AT4G26288	AT4G26288	
	AT5G25120	CYP71B11	cytochrome p450, family 71, subfamily B, polypeptide 11
Up-regulated in both dme & rdd	AT1G64390	GH9C2	glycosyl hydrolase 9C2
	AT1G76180	ERD14	EARLY RESPONSE TO DEHYDRATION 14
	AT2G02100	LCR69	low-molecular-weight cysteine-rich 69
	AT2G40080	ELF4	EARLY FLOWERING 4
	AT3G08770	LTP6	lipid transfer protein 6
	AT3G09440	AT3G09440	Heat shock protein 70 (Hsp 70) family protein
	AT4G36648	AT4G36648	other RNA
	AT5G11740	AGP15	arabinogalactan protein 15
Down-regulated in boh dme & rdd	AT1G29920	CAB2	chlorophyll A/B-binding protein 2
	AT2G05070	LHCB2.2	photosystem II light harvesting complex gene 2.2
	AT2G05100	LHCB2.1	photosystem II light harvesting complex gene 2.1
	AT2G30600	AT2G30600	BTB/POZ domain-containing protein
	AT3G15400	ATA20	anther 20
	AT3G15450	AT3G15450	Aluminium induced protein with YGL and LRDR motifs
	AT3G47340	ASN1	glutamine-dependent asparagine synthase 1
	AT3G59940	AT3G59940	Galactose oxidase/kelch repeat superfamily protein
	AT5G21940	AT5G21940	
	AT5G25350	EBF2	EIN3-binding F box protein 2

Table S2. Common genes that are down- and up-regulated both in *dme-2* and *ros1/rdd* mutants

	Ler (n=9)	*dme-2* (n=11)
	452.99	509.78
	442.46	517.52
	434.40	578.33
	532.50	563.87
	497.29	555.42
	480.46	518.6
	428.78	500.97
	496.68	548.97
	446.77	576.74
		534.81
		530.34
Average	468.04	539.58
Median	452.99	534.81
SD	35.34	26.88
Student t-test		8.31E-05

Table S3. Seed size difference between L*er* wild type and *dme* homozygous seeds. Individual seed length of the major axis was measured under stereo microscope. *$p < 0.001$, student's *t*-test.

		Seed	Seed coat rupture	Endosperm rupture	Total
Cold DAG3	***Ler'***	58(49%)	58(49%)	2(2%)	118
	dme-2	53(63%)	31(37%)	0(0%)	84
Cold DAG4	***Ler***	15(14%)	82(78%)	8(8%)	105
	dme-2	37(37%)	35(35%)	27(27%)	99
Cold DAG7	***Ler***	5(3%)	3(2%)	160(95%)	168
	dme-2	17(11%)	7(5%)	129(84%)	153
		Seed	**Seed coat rupture**	**Germinated**	**Total**
Cold DAG8	***Ler***	3(2%)	0(0%)	174(98%)	177
	dme-2	13(8%)	7(4%)	141(88%)	161

Table S4. Comparison of seed germination between L*er* and *dme-2* mutants in continuous cold and light conditions.

Distribution ratio of wild type and *dme-2* seed germination stage was examined from DAG3 to DAG8. *NG: non-germinating

		Normal	Abnormal	Total
	Ler	16	7	23
DAG2	***dme-1***	13	6	19
	dme-2	8	9	17
	Ler	8	3	11
DAG3	***dme-1***	5	5	10
	dme-2	6	6	12
	Ler	5	7	12
DAG4	***dme-1***	5	8	13
	dme-2	0	10	10
	Ler	6	6	12
DAG5	***dme-1***	2	8	10
	dme-2	0	12	12

Table S5. Primary root count of those displaying abnormally distorted QC.

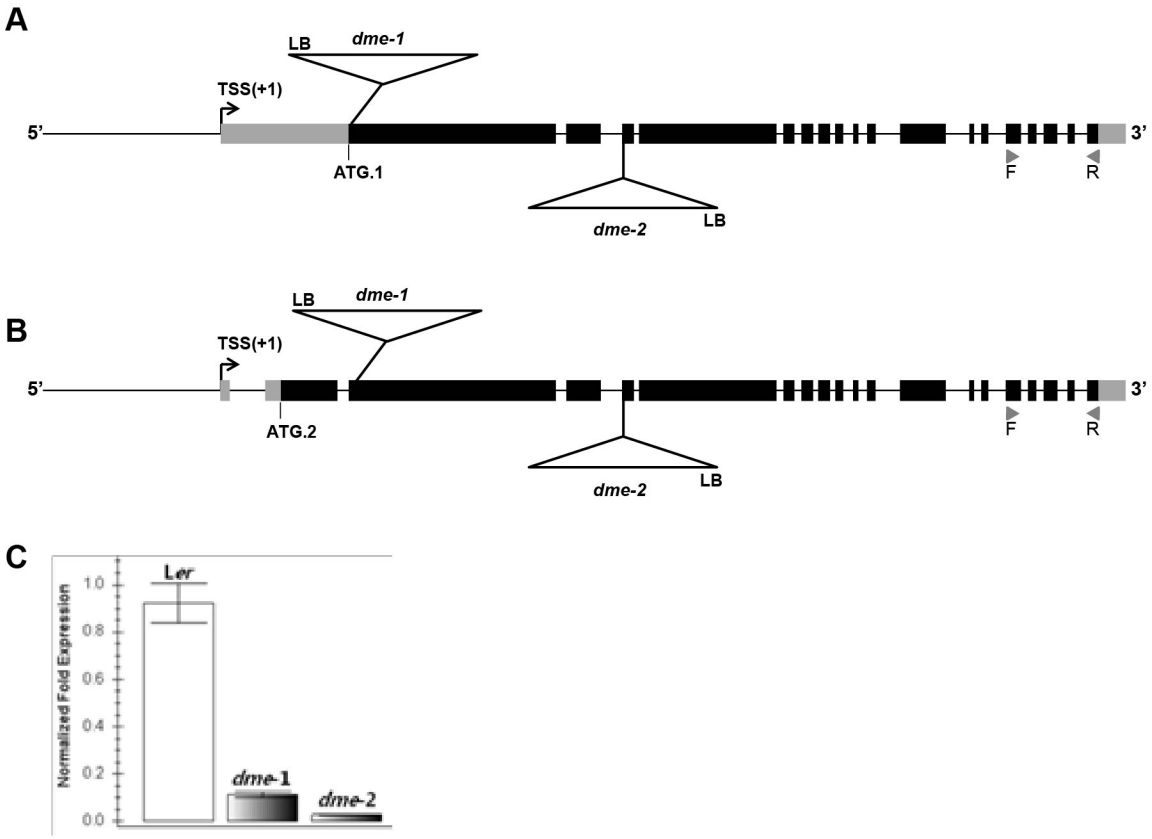

Fig. S1. T-DNA insertion sites of *dme-1* and *dme-2* alleles relative to two DME gene models.

(A) Diagram of T-DNA insertion sites of *dme-1* and *dme-2* alleles relative to DME.1 model (At5g04560.1). *(B)* Diagram of T-DNA insertion sites of *dme-1* and *dme-2* alleles relative to DME.2 model (At5g04560.2). (C) *DME* RNA expression in L*er* wild type, *dme-1* and *dme-2* homozygous plants. qRT-PCR primer set is indicated in the (A) and (B) as arrowheads, F and R.

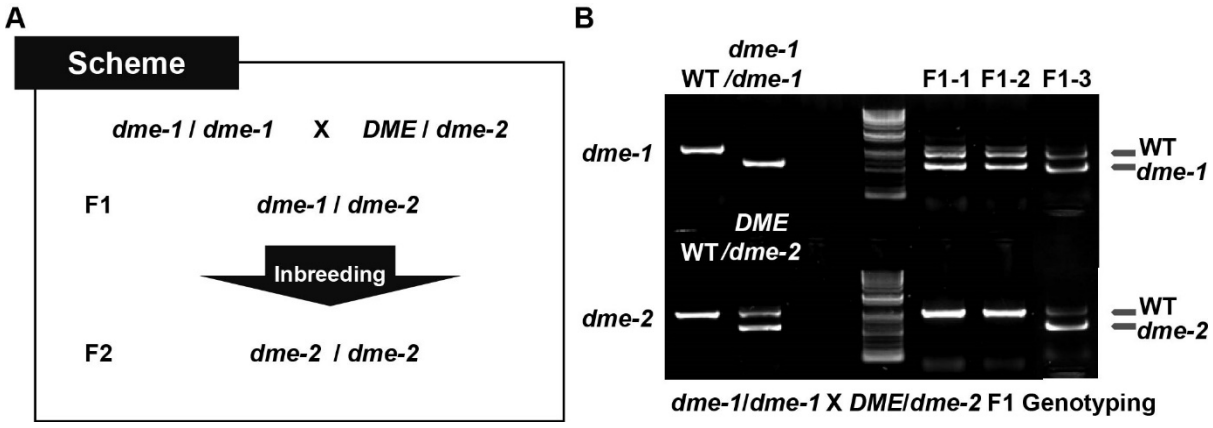

Fig. S2. Generating homozygous *dme-2* mutants. (*A*) The strategy to obtain homozygous *dme-2* mutants using homozygous *dme-1* allele. (*B*) The control genotyping of the *dme-1* and *DME/dme-2* is shown on the left-hand side. From the initial *dme-1/dme-1* (female) cross with *DME/dme-2* (male), three viable plants were obtained and their genotypes are shown on the right-hand side. F1-1 and F1-2 are *dme-1/DME* and F1-3 is *dme-1/dme-2* hybrid.

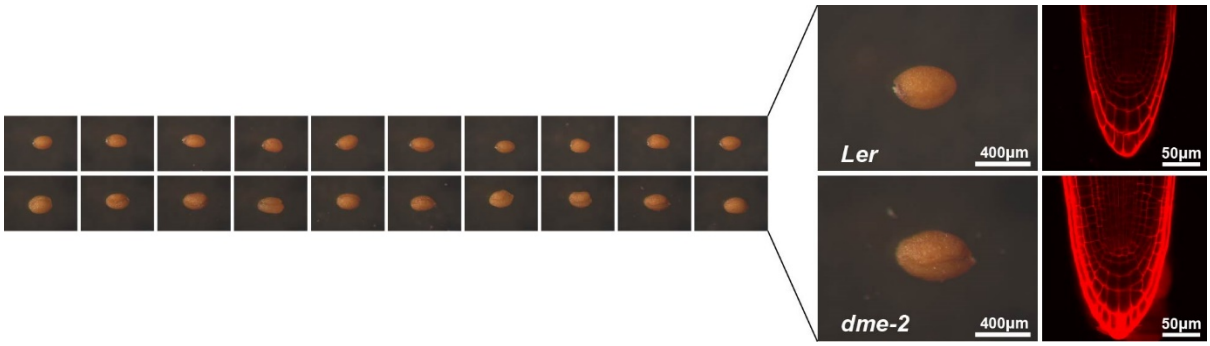

Fig. S3. *dme-2* mutant roots are larger than wild-type roots. Comparison of seeds and root tips. Root tips were stained with PI and observed under a confocal microscope.

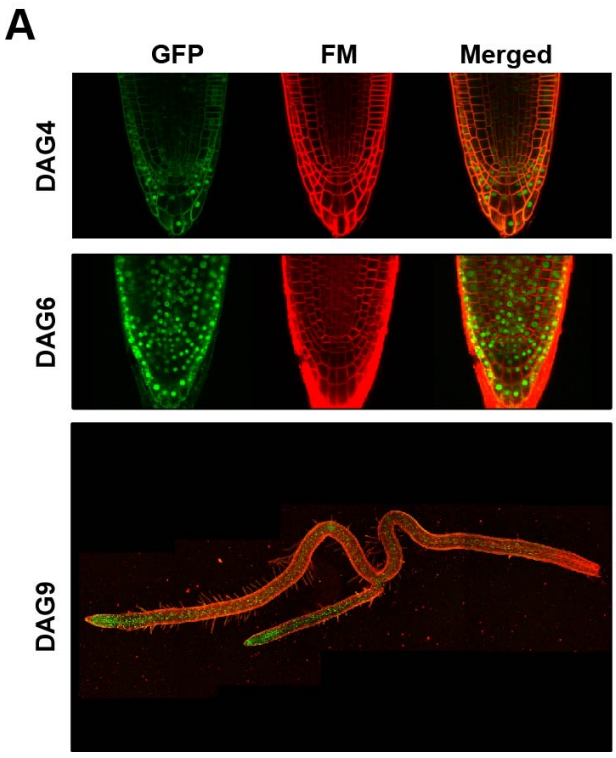

Fig. S4. DME functions in root development. DME::GFP expression in the root apical regions and PI-stained roots are merged.

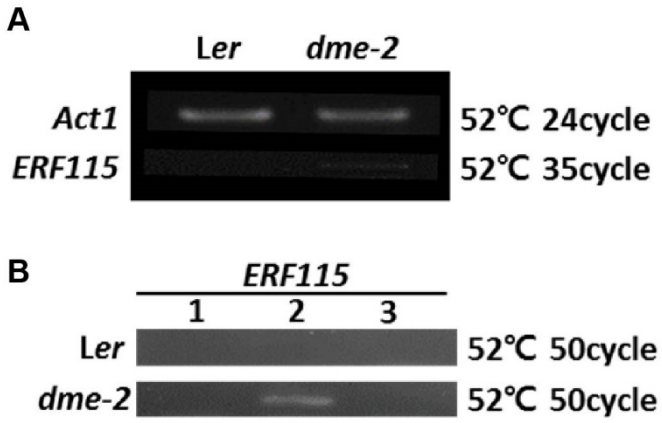

Fig. S5. *ERF115* overexpression was detected in *dme-2* mutant seedlings. (*A*) An *ERF115* band was detected in *dme-2* with 35 PCR cycles. *(B)* A clear *ERF115* band was detected in one *dme-2* sample among three different biological samples of both DAG7 L*er* and *dme-2* seedlings with 50 cycles.

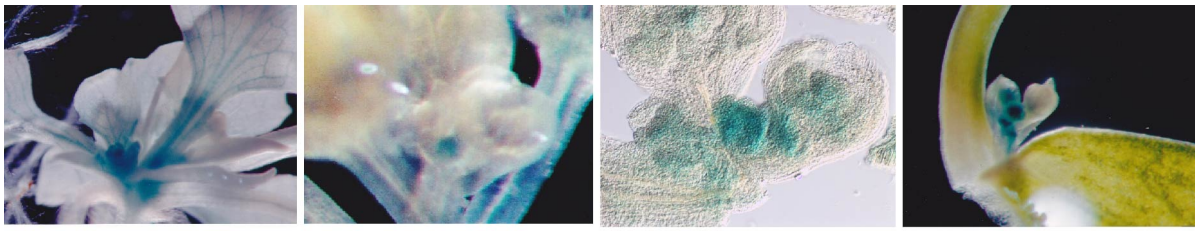

Fig. S6. 2*.3kb DME:GUS* expression in aerial meristematic zone. *DME:GUS* is specifically expressed in meristematic regions such as SAM, inflorescence meristem and floral meristem.

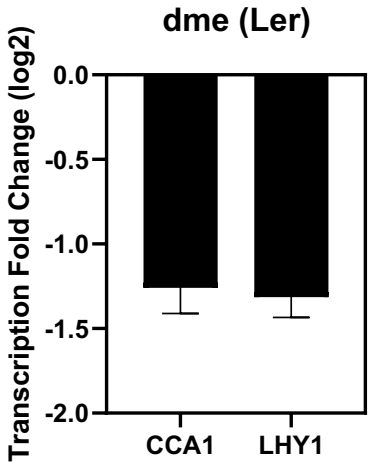

Fig. S7. *CCA1* and *LHY1* expression are significantly reduced in *dme* mutant seedlings compared to wild type (L*er*). Error bar represents standard error of log2 fold change. *p*-value of *CCA1* is 6.17E-16 and *LHY1* is 1.91E-26.