SUPPLEMENTARY INFORMATION

Involvement of a JmjC domain-containing histone demethylase in DRM2-mediated maintenance DNA Methylation.

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SUPPLEMENTARY METHODS

Southern blotting. DNA from young flowers was extracted using a standard CTAB protocol. 1 μg of genomic DNA was digested overnight with MspI. The digestion was run on a 1% agarose gel, transferred to Hybond N+ membranes, blocked and washed according to manufacturer instructions (GE Healthcare). Membranes were probed with a PCR product radiolabeled with alpha ^{32}P-dCTP using the Megaprime DNA Labeling System. *MEA-ISR* PCR product was generated with primers listed in supplementary Table S2.

Bisulfite analysis. DNA from young flowers was extracted using a standard CTAB protocol. We performed sodium bisulfite sequencing using EZ DNA Methylation Gold

(Zymo Research) by following the manufacturer's instructions. Following amplication of bisulfite treated DNA, we cloned the resulting PCR fragments into pCR2.1-TOPO (Invitrogen) and analyzed 15 to 30 clones per sample. The *FWA* transgene was distinguished from the endogene by BglII digestion prior to bisulfite treatement (see *FWA* Transformation methods) and elimination of any clones containing Col-0 polymorphisms from the data set after sequencing. To compare the converted clones to the original unconverted sequence, we used the sequence alignment tool of CLC Workbench™ software. We counted the converted/unconverted cytosines at each site manually and subsequently calculated the percent of methylation. All primers are listed in supplementary Table S2.

***FWA* Transformation.** We performed *FWA* transformation using either an AGL0 *Agrobacterium tumefaciens* strain carrying an empty pCAMBIA3300 vector or a pCAMBIA3300 vector with an engineered version of the L*er* copy of *FWA* in which a BglII site was converted into a EcoRI site. For selection, we plated T1 seeds on MS media containing a 1:12,000 dilution of Finale™.

Protein immunofluorescence analysis. Primary mouse monoclonal anti-Myc (Covance 9E10) and rabbit polyclonal anti-H3K9m2 (Upstate) were used at a 1:200 dilution. Secondary anti-mouse FITC (Abcam) and anti-rabbit Rodamine (Jackson Immuno Research) were used at a 1:200 dilution. DNA was stained using Vectashield mounting medium containing DAPI (Vector Laboratories). Images were captured with the Zeiss AxioImager Z1 microscope with the Hamamatsu Orca-er camera at 100X magnification

and analyzed using the Zeiss Axiovision software. Zeiss FL filter sets used in this study: Zeiss 49 (DAPI), Zeiss 38 (EGFP), and Zeiss 43 (Cy 3).

Chromatin immunoprecipitation (ChIP). Five grams of cross-linked rosette leaves (just before bolting) were ground under liquid nitrogen and resuspended in 30 mL of lysis buffer plus protease inhibitors. Cells were disrupted for 12 minutes with a Dianode Bioruptor (30 seconds on, 30 seconds off; high setting). The chromatin isolated was used for 2 IPs: H3K4m3-containing chromatin was immunoprecipitated with 5 mg of anti-H3K4m3 from Diagenode (pAb-003-050) and H3K4m2-containing chromatin was immunoprecipitated with 5 mg of anti-H3K4m2 from Abcam (AB32356). After reversal of crosslinking overnight, the immunoprecipitated DNA was purified by a regular DNA extraction protocol and analyzed by real-time PCR with TaqMan Probes (*AtSN1*, *FWA*, *ICDH*) or using the Biorad SYBR-Green Supermix (*MEA-ISR*, *ICDH*) on a MX3000. Sequences of primers and probes can be found in supplementary Table S2.

SUPPLEMENTARY TABLES

Supplementary Table 1. List of homozygous T-DNA insertion lines for JmjC-domain containing genes used in this study.

Gene	AGI	Allele	Ecotype
JMJ11	At5g04240	SALK_074694	Col-0
JMJ12	At3g48430	SALK_122006	Col-0
JMJ13	At5g46910	null allele not available	
JMJ14	At4g20400	SALK_135712 (*atjmj14-1*)	Col-0
		SALK_136058 (*atjmj14-2*)	Col-0
JMJ15	At2g34880	null allele not available	

JMJ16	At1g08620	SALK_029530	Col-0
JMJ17	At1g63490	FLAG_076A07	Col-0
JMJ18	At1g30810	null allele not available	
JMJ19	At2g38950	SALK_025269	Col-0
JMJ20	At5g63080	FLAG_316E04	Col-0
JMJ21	At1g78280	SALK_000651	Col-0
JMJ22	At5g06550	SAIL_680_G02	Col-0
JMJ24	At1g09060	GABI_085H03	Col-0
JMJ25	At3g07610	SALK_004652	Col-0
JMJ26	At1g11950	FLAG_484A05	Col-0
JMJ27	At4g00990	SALK_103092	Col-0
JMJ28	At4g21430	WiscDsLox263E02	Col-0
JMJ29	At1g62310	FLAG_390G09	Col-0
JMJ30	At3g20810	GABI_454C10	Col-0
JMJ31	At5g19840	null allele not available	
unnamed	At3g45880	SALK_003313	Col-0

Supplementary Table 2. List of primers and probes used in this study.

MEA-ISR Southern Probe	AAACCTTTCGTAAGCTACAGCCACTTTGTT
MEA-ISR Southern Probe	TCGGATTGGTTCTTCCTACCTCTTTACCTT
MEA-ISR Bisulfite	AAAGTGGTTGTAGTTTATGAAAGGTTTTAT
MEA-ISR Bisulfite	CTTAAAAAATTTTCAACTCATTTTTAAAAAA
FWA Bisulfite	GGTTTTATATTAATATTAAAGAGTTATGGGTYGAAGTTT
FWA Bisulfite	AACCAAAATCATTCTCTAAACAAAATATAAAAAAATC
Ta3 Bisulfite	GAGAATYAGGTTAATAAGAAAGTGAAGTGTT
Ta3 Bisulfite	CCACTRATTCCTRAAACACAACATTTCTRCTRATA
AtSN1 Chop-qPCR	ACTTAATTAGCACTCAAATTAAACAAAATAAGT
AtSN1 Chop-qPCR	TTTAAACATAAGAAGAAGTTCCTTTTTCATCTAC
MEA-ISR ChIP	TTTAGGTATTAGCTCGTTTGGTTTTA
MEA-ISR ChIP	TCCCGCCATTTAACCGTG
FWA ChIP	ATAAAGAGCGGCGCAAGAT
FWA ChIP	CGCTCTAGGGTTTTTGCTTT
FWA ChIP Probe	CAAATAGCACTTGGACCAATGGCG
AtSN1 ChIP	GTTGGCCCAGTGGTAAATCT
AtSN1 ChIP	TGGTGGTTGTACAAGCCTAGTT
AtSN1 ChIP Probe	ATCTCCCAGAGGCGGGACCC
ICDH-IGR ChIP	AGGCCCCATCTCACAAATAC
ICDH-IGR ChIP	GTCGCCAGGTAGATTTGGTT
ICDH-IGR ChIP Probe	TCCGGTTAGACCTTAACGTGGGTCA