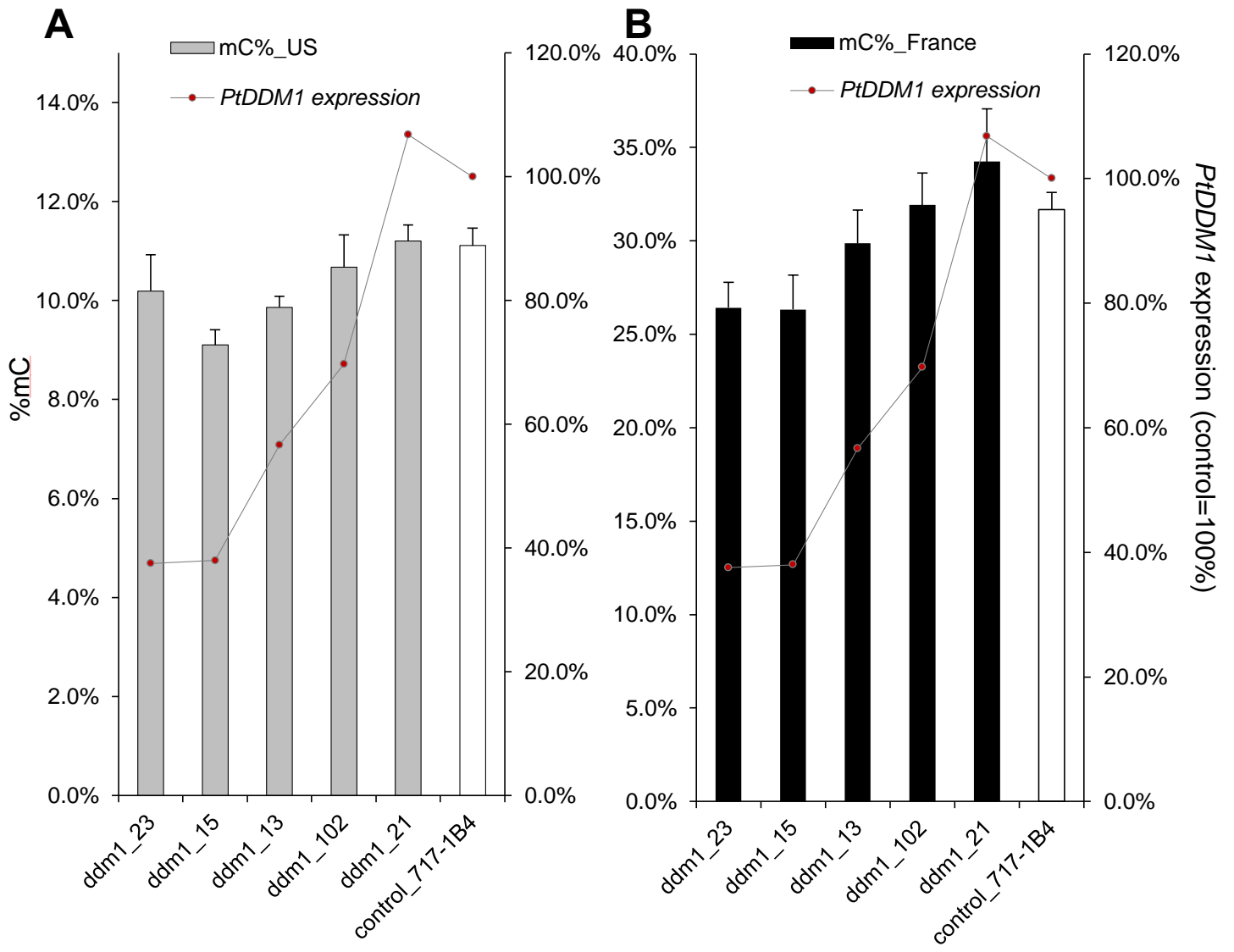


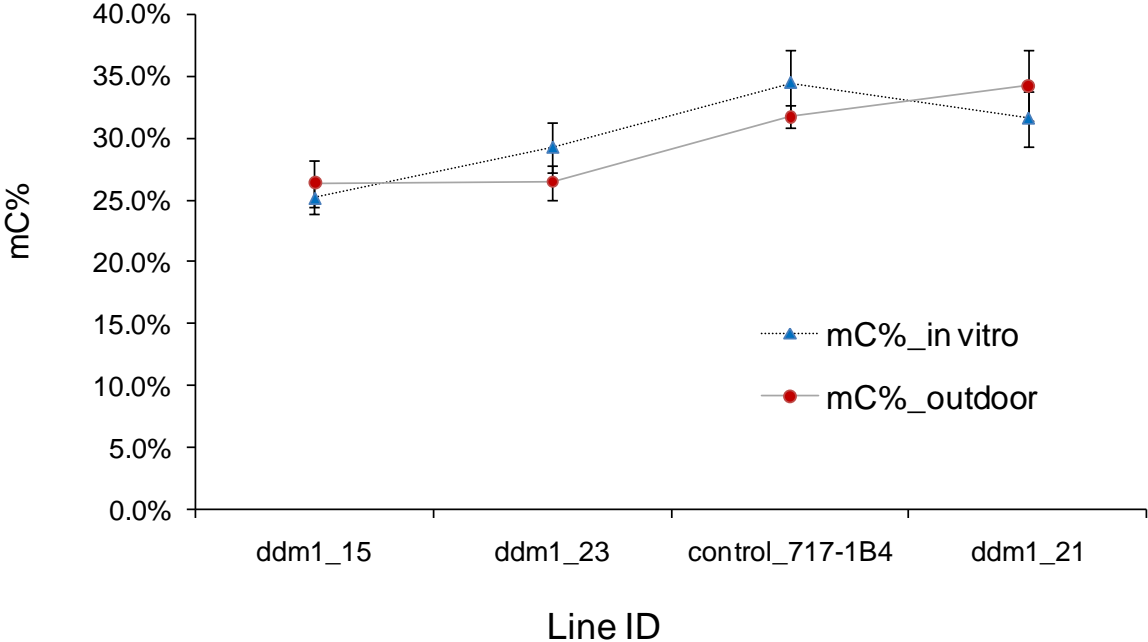
## Supplemental Materials

Zhu et al. – RNAi suppression of DDM1 in poplar

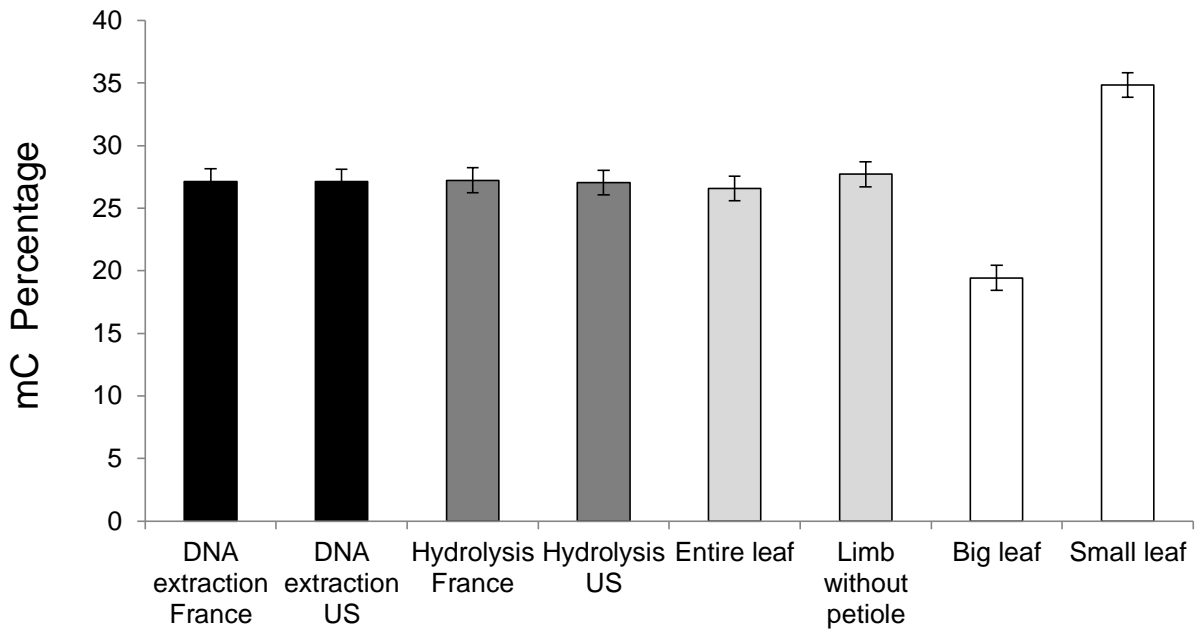
**Supplemental Fig. S1** Total cellular cytosine methylation for selected events determined by HPLC. The association between methylcytosine level (mC%, bars) and *PtDDM1* expression (line) for selected six events where methylation was measured in US (A) and in France (B).



**Supplemental Fig. S2** DNA methylation of *in vitro* leaf and stem material is associated with that of the outdoor leaves.

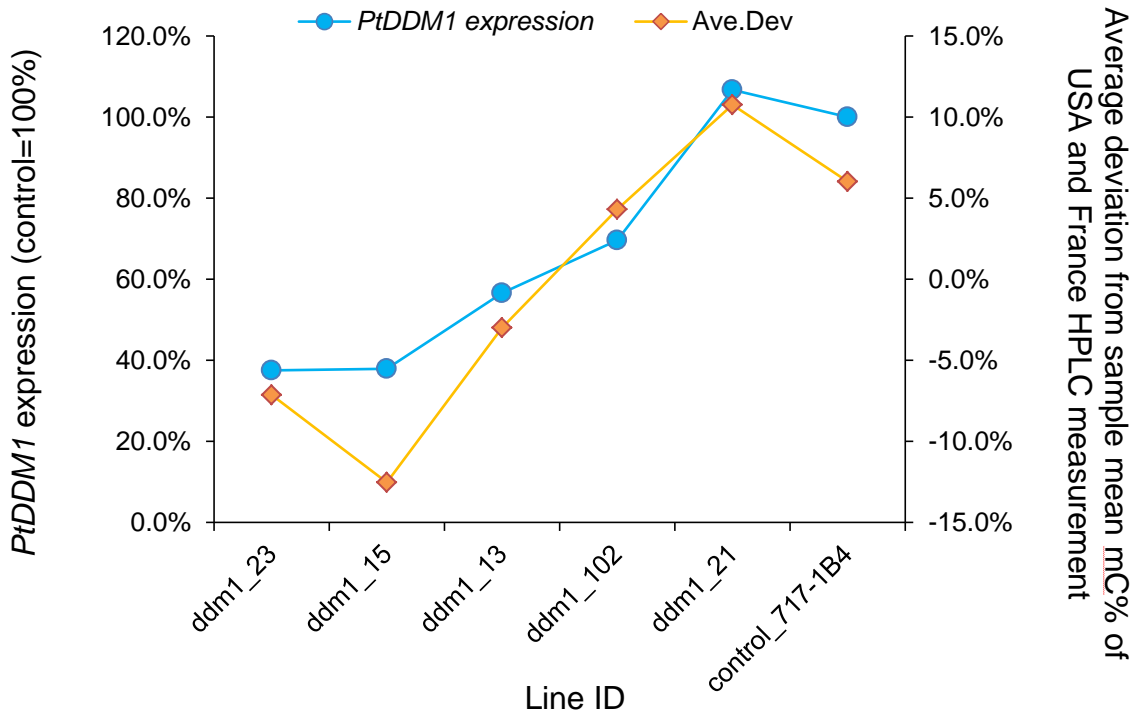


**Supplemental Fig. S3** Comparison between France and US DNA extraction, hydrolysis, and leaf size with respect to DNA methylation (mC%). All bars are means of 8 samples (2 biological samples and 4 technical tests for each). Standard errors were calculated from the 2 biological samples. Student's t-test of "big leaf" versus. "small leaf" samples showed that their difference in methylation percentage was highly statistically significant ( $P < 0.0001$ ).

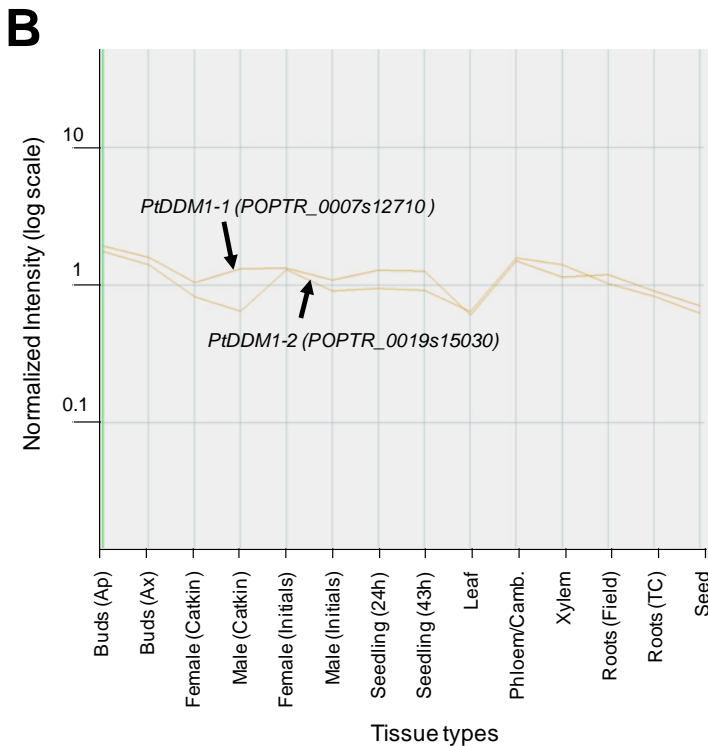


Samples of 717 1B4 non transgenic plants

**Supplemental Fig. S4** Overall association of DNA methylation level (mC%) and gene expression. Deviations from event means for mC% were averaged over US and France estimates.



**Supplemental Fig. S5 A.** *PtDDM1-1* (POPTR\_0007s12710, yellow) and *PtDDM1-2* (POPTR\_0019s15030, orange) are co-expressed in diverse tissues and treatments in poplar expression studies. Experiment ID: GSE12152, organogenesis; GSE13390, developmental tissue series; GSE15595, growth; GSE16420, defense; GSE21171, drought; GSE9673, and interactions with rust fungi (Sjödin et al. 2009). **B.** Expression pattern of *PtDDM1-1*(POPTR\_0007s12710) and *PtDDM1-2* (POPTR\_0019s15030) in different tissue types based on microarray data (Rodgers-Melnick et al. 2012).



## SUPPLEMENTARY TABLES

**Supplemental Table 1** BLAST alignment between *Arabidopsis DDM1* and putative orthologs in *Populus trichocarpa* at genomic level (A), transcript level (B), protein level (C), SNF2\_N domain (D), and HELICc domain (E).

### A. Genomic level

Identity Coverage	<i>DDM1</i> ( <i>A. thaliana</i> )	Putative <i>PtDDM1</i> ortholog-1
Putative <i>PtDDM1</i> ortholog-1	70%	60%
Putative <i>PtDDM1</i> ortholog-2	70%	86%
	49%	61%

### B. Transcript level

Identity Coverage	<i>DDM1</i> ( <i>A. thaliana</i> )	Putative <i>PtDDM1</i> ortholog-1
Putative <i>PtDDM1</i> ortholog-1	71%	97%
Putative <i>PtDDM1</i> ortholog-2	73%	87%
	91%	94%

### C. Protein level

Identity Coverage	<i>DDM1</i> ( <i>A. thaliana</i> )	Putative <i>PtDDM1</i> ortholog-1
Putative <i>PtDDM1</i> ortholog-1	73%	81%
Putative <i>PtDDM1</i> ortholog-2	73%	89%
	78%	83%

### D. SNF2\_N domain

Identity Coverage	<i>DDM1</i> ( <i>A. thaliana</i> )	Putative <i>PtDDM1</i> ortholog-1
Putative <i>PtDDM1</i> ortholog-1	78%	100%
Putative <i>PtDDM1</i> ortholog-2	77%	92%
	100%	100%

E. HELICc domain

Identity Coverage	<i>DDM1</i> ( <i>A. thaliana</i> )	Putative <i>PtDDM1</i> ortholog-1
Putative <i>PtDDM1</i> ortholog-1	89% 100%	
Putative <i>PtDDM1</i> ortholog-2	90% 100%	94% 79%

**Supplemental Table 2** Primers used for construct assembly, PCR confirmation, and qPCR.

PCR target	Genebank accession No.	Primer name	Sequence of primers from 5' to 3'
500 bp from <i>717-1B4 DDM1</i>	NC_008473	DDM1-F01	TGGTTATTGCCCTCTTTCCACTC
		DDM1-R02	CTGAGCCCTTCGCCTTTCTTCTAC
190 bp from <i>PtDDM1</i>	NC_008473	NDDM-F01	CACCGTGGACAATAAGCTC
		NDDM-R01	CCTGAGCCCTTCGCCTTTC
Pcr1, sense <i>PtDDM1</i> sequence	NC_008473	NDDM-R01	CCTGAGCCCTTCGCCTTTC
		OCS-R03	CGTCTCGCATATCTCATTAAAGC
Pcr2, antisense <i>PtDDM1</i> sequence	NC_008473	NDDM-R01	CCTGAGCCCTTCGCCTTTC
		35S pro-F01	TCCAACCACGTCTTCAAAGC
<i>PtDDM1-1</i> transcript position 1748-1855	NC_008473	PtDDM1_C	AAGAGCTTGGTGGACTGGGTA
		PtDDM1-NR01	ACAGGCTTGGTTTGCCCAATTC
Polyubiquitin	BU879229	Ubq L	GTTGATTTTTGCTGGGAAGC
		Ubq R	GATCTTGGCCTTCACGTTGT