Supplementary Information

(Supplementary Methods, Supplementary Table 1, Legends to Supplementary Figures 1 and 2, References, Supplementary Figure 1, Supplementary Figure 2)

MOM1 mediates DNA methylation-independent silencing of repetitive sequences in *Arabidopsis*

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Supplementary Methods

Probes used in gel blot analyses

We amplified the probes to detect 5S rDNA and *106B* repeats by PCR from genomic DNA using 5S-F and 5S-R primers (Kanno et al., 2005) and 106B-F and 106B-R, respectively. Probes to detect 180-bp repeats, *TSI* and *RAN* (for RAS-related nuclear protein; Haizel et al., 1997) were amplified from plasmids carrying two tandemly-repeated 180-bp repeats, *TSI*-A15 and *RAN* fragments, respectively. Probe to detect *MOM1* promoter region was amplified from genomic DNA using primers MOM1-F and MOM1-R; and probe to detect *MOM1* transcript was amplified from cDNA using CD29-F and CLA3-R (Tariq et al., 2002). Probes were labeled with [alpha-^{32}P]dCTP using random hexamer priming (Megaprime DNA labeling system; Amersham).

Chromatin immunoprecipitation PCR conditions

Following chromatin immunoprecipitation with the appropriate antibodies, 5S rDNA, Ta2 (a heterochromatin control), the phosphofructokinase β-subunit gene At4g04040 and *TUBULIN 8* (euchromatin controls) were amplified using primers listed in supplementary table 1 in a 20 µl PCR reaction, starting with 5 min at 95°C and followed by 21-40 (depending on the region being amplified) cycles of 95°C, 60°C (54°C for 5S rDNA) and 72°C (30 s each) with a final elongation of 5 min at 72°C. PCR products were resolved on a 3% agarose gel.

Semi-quantitative RT-PCR conditions

PCR conditions using gene-specific primers were as follow:
5S-210 and *5S-140* transcripts were amplified using RTPCR5S1 and 5SUNIV2 primers for 33 cycles (94°C for 45 s, 51°C for 45 s, 72°C for 30 s);

Ta3 was amplified using primers Ta3 middle-F and Ta3-middle-R (Johnson et al., 2002) for 35 cycles (94°C for 45 s, 55°C for 45 s, 72°C for 1 min);
MULE At1g43280 was amplified using primers At1g43280-F and At1g43280-R for 35 cycles (94°C for 45 s, 55°C for 45 s, 72°C for 1 min);
106B was amplified using primers 106B-F and 106B-R (May et al., 2005) for 35 cycles (94°C for 45 s, 55°C for 45 s, 72°C for 1 min);
MULE At1g40097 was amplified using primers At1g40097-F and At1g40097-R for 35 cycles (94°C for 45 s, 50°C for 45 s, 72°C for 1 min);
Athila-LTR was amplified using primers Athila LTR-F and Athila LTR-R for 35 cycles (94°C for 45 s, 55°C for 45 s, 72°C for 90 s);
FWAtr was amplified using primers FWAtr-F and FWAtr-R (Pontier et al., 2005) for 40 cycles (94°C for 45 s, 60°C for 45 s, 72°C for 1 min);
180-bp repeats were amplified using primers 180(all)-F and 180(all)-R (Johnson et al., 2002) for 30 cycles (94°C for 30 s, 60°C for 30 s, 72°C for 90 s)
ACT2 was amplified using ACT2-F and ACT2-R primers 25 cycles (95°C for 30 s, 55°C for 30 s, 72°C for 30 s).
AtSN1 transcripts were detected using AST15 and ATSN1-F4 primers as described (Herr et al., 2005). All PCRs were ended by a final elongation of 10 min at 72°C. Reverse transcription reactions and cDNA amplification of *MOM1* transcript were performed as previously described (Tariq et al., 2002).

Real-time RT-PCR

Total RNA was extracted using TRI-reagent (Sigma) and aliquots of 3 µg were treated with the RQ1-DNase (Promega) to remove genomic DNA. Absence of genomic DNA contamination in the RNA samples was then controlled by verifying that a PCR (45 cycles) with 180(all)-F and 180(all)-R yielded no amplification. cDNA synthesis was performed on 1 µg total RNA using random hexanucleotides (Roche) as described previously (Mathieu et al., 2003). One microliter cDNA was used in a 25 µl PCR reaction using the Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) and data were collected using a Roche LightCycler. Each real-time RT-PCR run included, as an internal reference, a reaction for *ACTIN2*. The comparative threshold cycle (C_t) method was used to determine relative RNA levels. The primer sets were 106Bq-F and 106Bq-R-1 for *106B*, 5SUNIV2 and RTPCR5S1 for *5S-210*, ACT2-F and ACT2-R for *ACTIN2* (See supplementary table 1).

Detection of minor 5S rRNAs

Reverse transcription reactions were performed as described (Mathieu et al., 2003). 5S cDNA amplification was performed on 50 ng of the cDNA samples using the primers RTPCR5S1 and RTPCR5S2 as described previously (Mathieu et al., 2003). Complete removal of the 5S genomic rDNA in the reverse-transcribed samples was controlled by verifying that a PCR step (40 cycles) using primers 5SUNIV1 and 5SUNIV2 yielded no amplification (Mathieu et al., 2003). PCR products were subcloned in the pGEM-T easy plasmid and sequencing was performed using a CEQ 2000 Dye terminator cycle sequencer (Beckman). Minor 5S rRNA frequencies were compared with Fisher's exact test for a 2x2 contingency table and probabilities were calculated from a one-tailed test.

Supplementary Table 1.

List of primers

Target	Primer	Sequence (5'-3')	Methods
5S rDNA	RTPCR5S1	GGATGCGATCATACCAG	RT-PCR, ChIP, real-time RT-PCR
	RTPCR5S2	GAGGGATGCAMCACSAG	RT-PCR
	5SUNIV1	CTTTTCGGGCNTTTTNGTG	Genomic control
	5SUNIV2	CGAAAAGGTATCACATGCC	RT-PCR, ChIP, real-time RT-PCR
	5S-F	TTGGGCTATATTACGGACCCA	Southern blot probe
	5S-R	GTCCTGCTTCTTTCGTCGGAG	
TUBULIN8	TUB8-F	ATAACCGTTTCAAATTCTCTCTCTC	ChIP
	TUB8-R	TGCAAATCGTTCTCTCCTTG	
Ta2	Ta2-F	AAACGATGCGTTGGGATAGGTC	ChIP
	Ta2-R	ATACTCTCCACTTCCCGTTTTTCTTTTTA	
Ta3	Ta3 middle-F	GATTCTTACTGTAAAGAACATGGCATTGAGAGA	RT-PCR
	Ta3 middle-R	TCCAAATTTCCTGAGGTGCTTGTAACC	
MULE At1g40097	At1g40097-F	GGTTTTGATACCGAATTTTG	RT-PCR
	At1g40097-R	AGCGGAGGAATATACAACTC	
MULE At1g43280	At1g43280-F	GGTTAGGAAAGTGAAGCTTGAG	RT-PCR
	At1g43280-R	CCAGTGAGACAAAGGCATAC	
Athila-LTR	Athila LTR-F	TGTTTCATCCACGTTTCATCTC	RT-PCR
	Athila LTR-R	AGCAATAAGCGCAACTAATCC	
AtSN1	ATSN1-F4	AAAATAAGTGGTGGTTGTACAAGC	RT-PCR
	ATS15	ACCAACGTGCTGTTGGCCCAGTGGTAAATC	
FWAtr	FWAtr-F	TCCCATTCAACATTCATACGAGCGCCGC	RT-PCR
	FWAtr-R	TCTGATATTTGGCTGGAAAAAACAACAATAATC	
ACTIN2	ACT2-F	CTAAGCTCTCAAGATCAAAGGC	RT-PCR
	ACT2-R	AACATTGCAAAGAGTTTCAAGG	
106B repeats	106B-F	TTGATTGATAGATCCCTTCTGGA	RT-PCR
	106B-R	CGAGGATGGGGTAATTGAGT	
	106Bq-F	TCATTATGCTAGGTGGTTGA	Real-time RT-PCR
	106Bq-R-1	GACAACAAGTTCATTAACCA	

180-bp repeats	180(all)-F	ACCATCAAAGCCTTGAGAAGCA	RT-PCR
	180(all)-R	CCGTATGAGTCTTTGTCTTTGTATCTTCT	
MOM1	CD29-F	GCTCCTCTGCAACTTCAGCAATCATC	RT-PCR; Probe for Northern blot
	CLA3-R	TCATCAATTTGTGTTGTGTGATCAGA	
	MOM1-F	CCAAACTGGTAATTAAACGTTTG	Southern blot probe
	MOM1-R	TTACTGACAAGAAGCTTTTGGG	
PFK-β (At4g04040)	B8F	GCCACGAAAACCAAACAGAC	ChIP
	B8R	CCGGAATTTCGATCAATCCT	

Legend to Supplementary Figure 1.

Sequence alignments of 5S-210 transcripts.

(A) Multiple sequence alignment of 5S-210 RT-PCR products from Columbia (Col) wild-type plants with the major 5S rRNA sequence. (B) Multiple sequence alignment of 5S-210 RT-PCR products from *ddm1-2* mutant plants with the major 5S rRNA sequence. Nucleotide positions diverging from the sequence of the major 5S rRNA are highlighted in yellow. Dots highlighted in yellow indicate the position of the chromosome-specific T-stretch (Cloix et al., 2002). Alignments were carried out using the CLUSTALW v.1.8 software (http://searchlauncher.bcm.tmc.edu/multi-align/Options/clustalw.html).

Legend to Supplementary Figure 2.

DNA methylation of *TSI*, *106B* LTR-like repeats and 180-bp satellite repeats.

Genomic DNA was purified from leaves of three-week-old plants and digested with *Hpa*II or *Msp*I. The DNA gel-blot was successively hybridized to *TSI*-A15 (A), *106B* (B) and 180-bp probes (C). WT, wild-type; L*er*, Landsberg *erecta*; Zh, Zürich.

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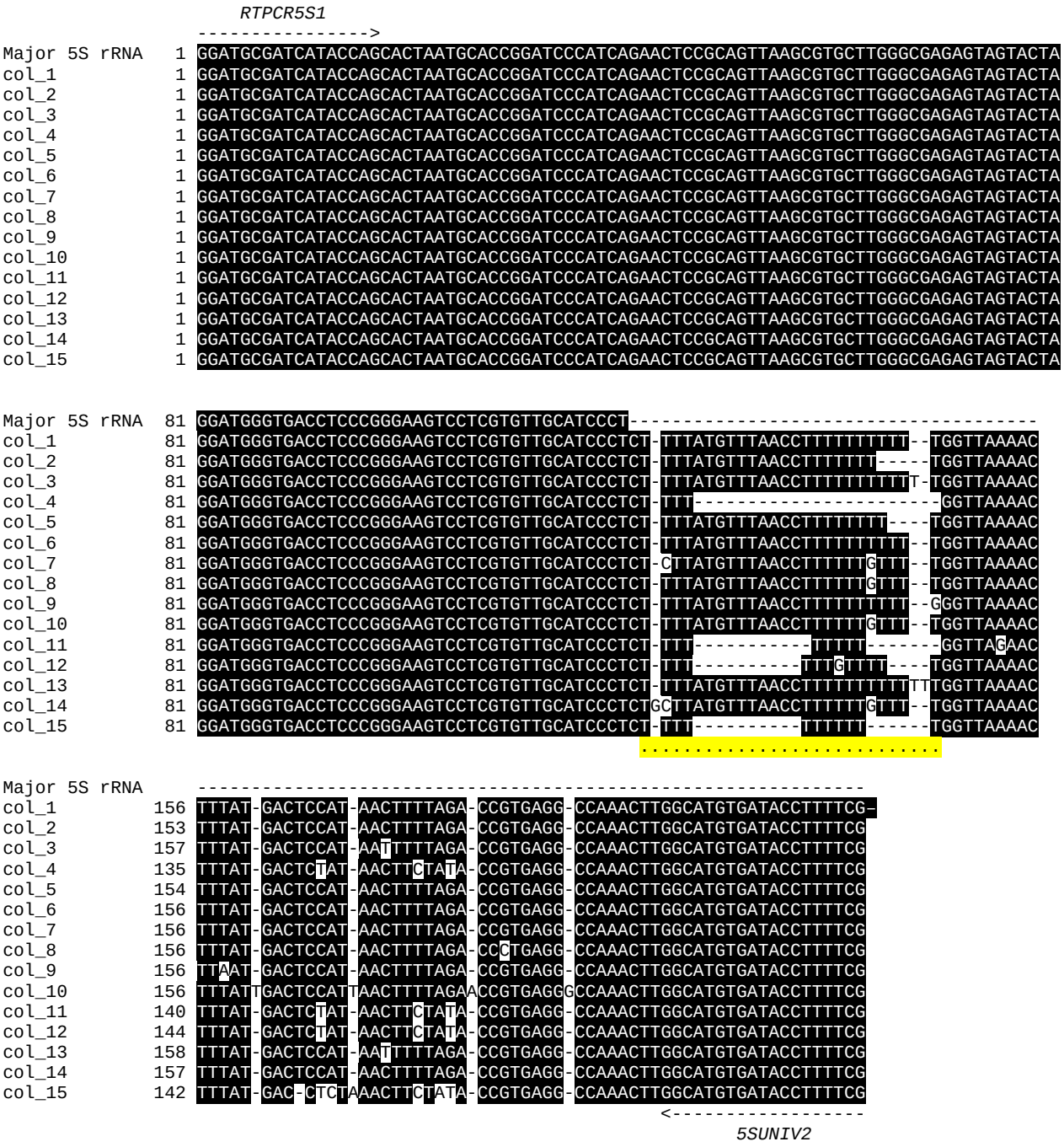
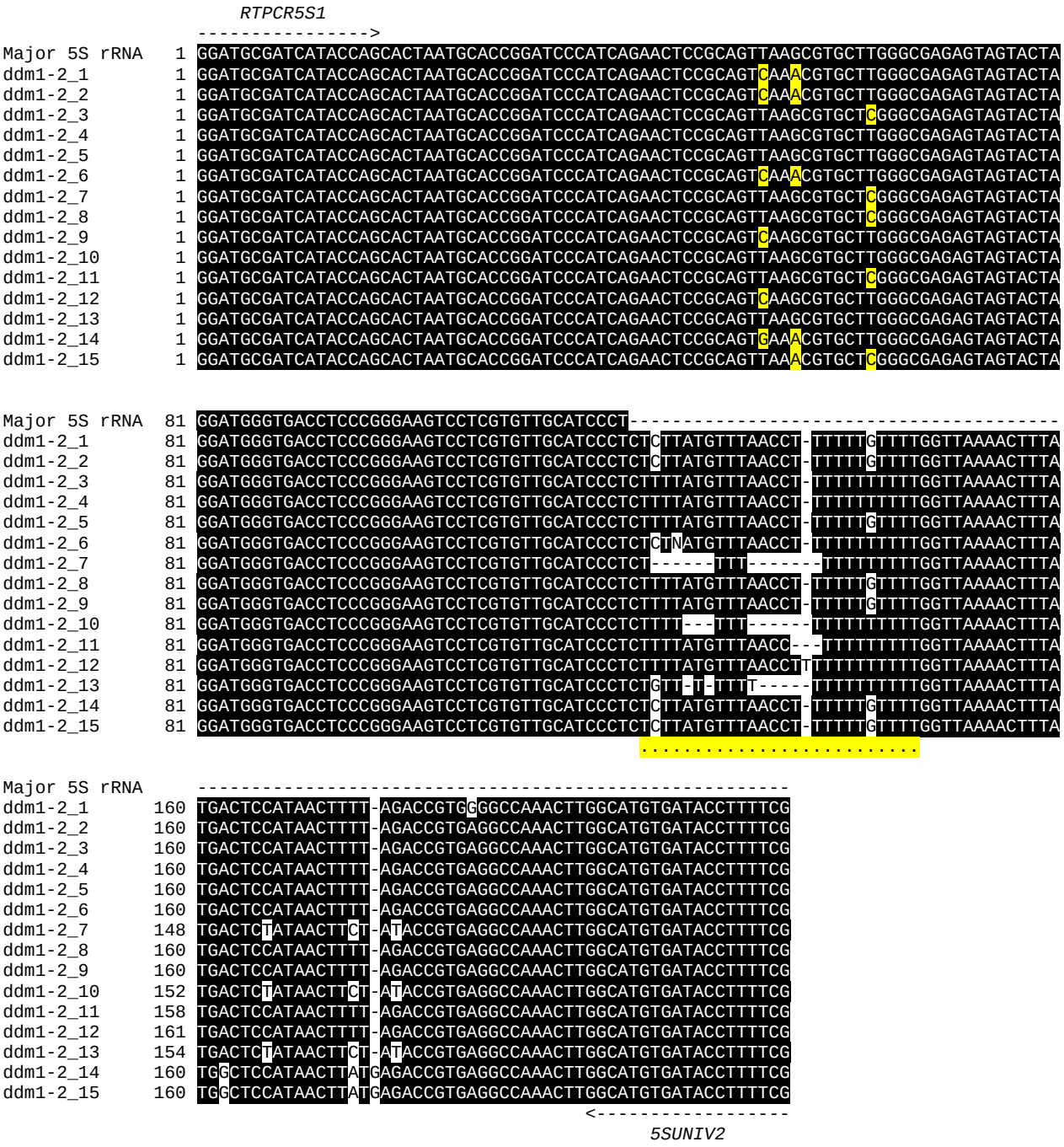
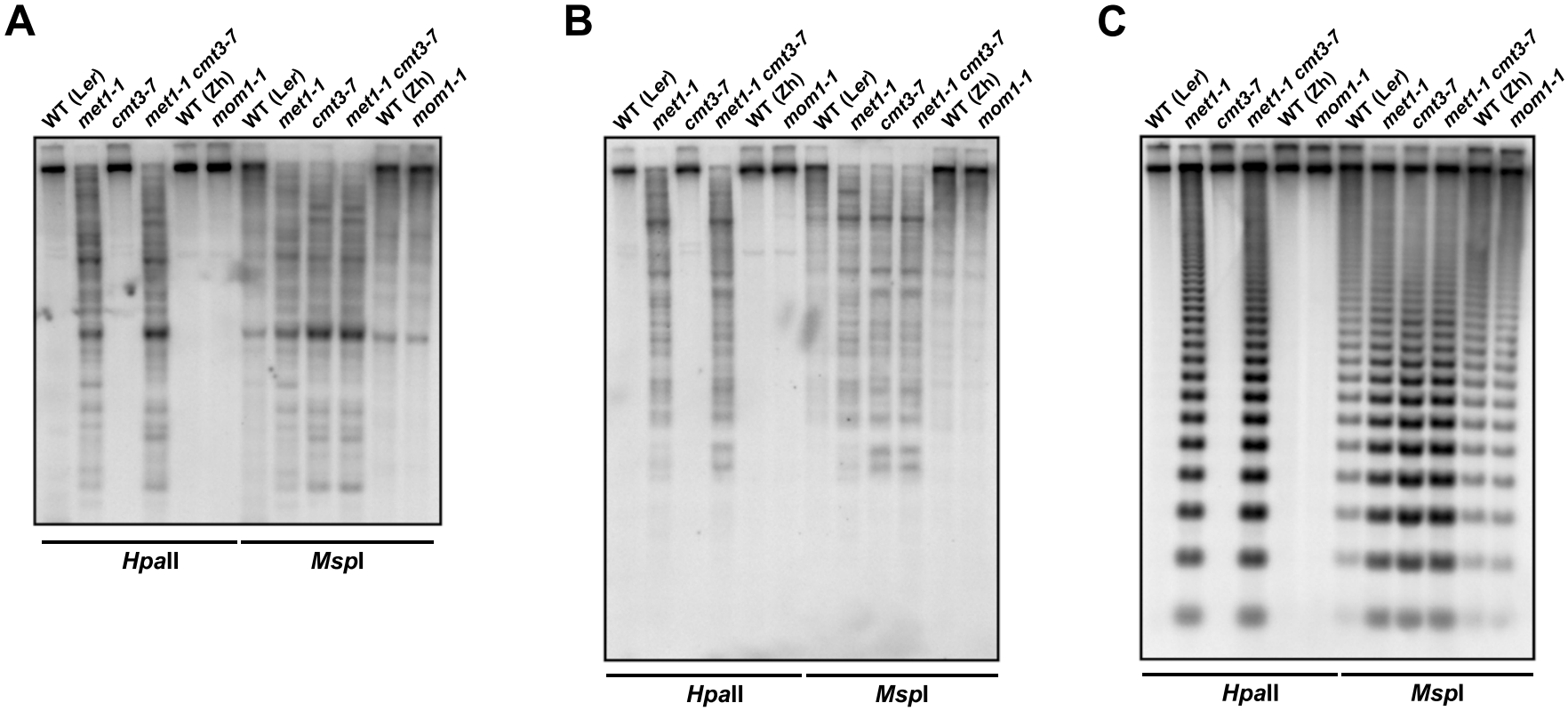
Supplementary Fig 1A



Supplementary Fig 1B





Supplementary Fig 2. Vaillant et al.