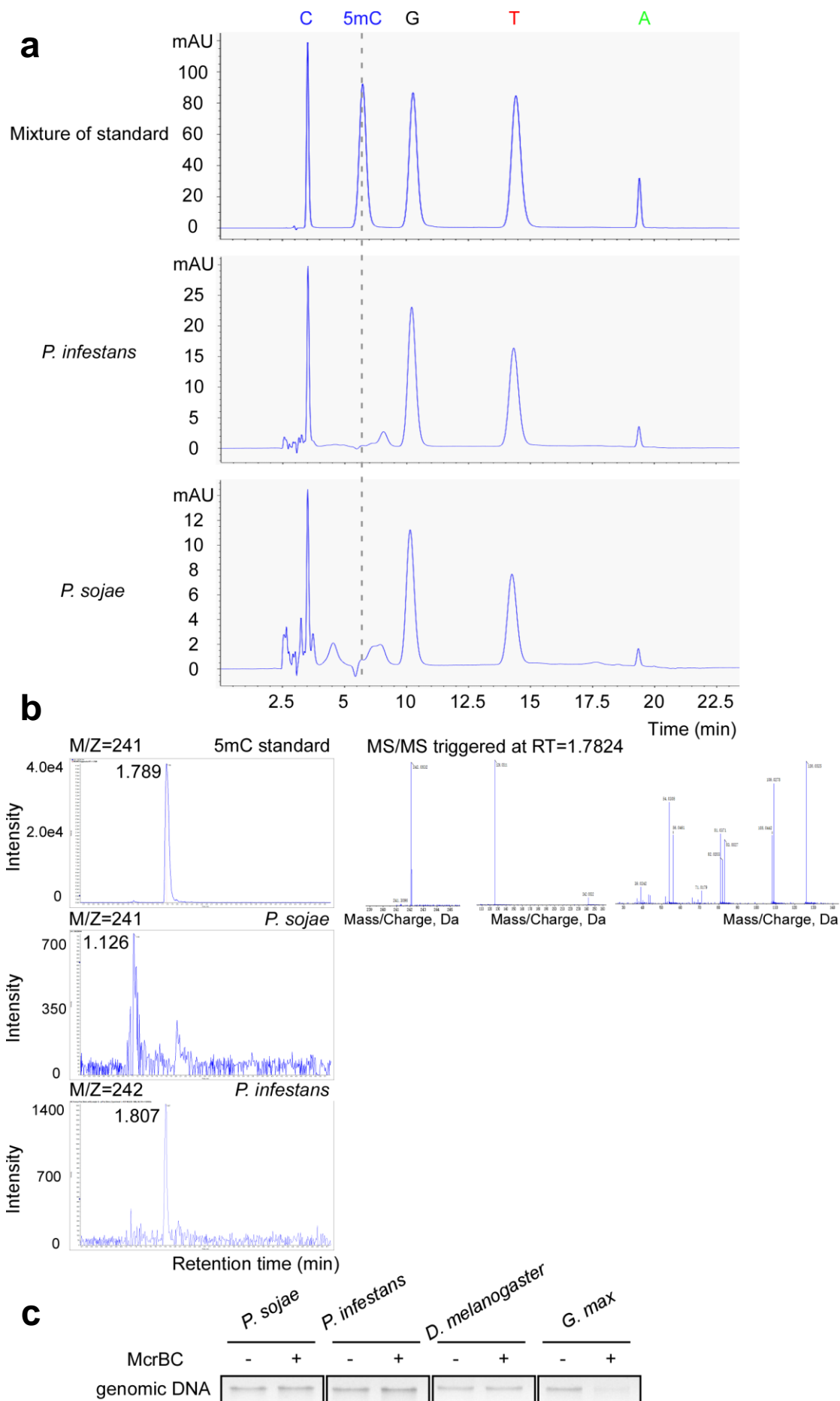


Supplementary Figure 1



Supplementary Figure 1

No detectable 5mC in *P. infestans* and *P. sojae*

(a) Chromatograms from a standard mixture and from samples of hydrolyzed gDNA from *P. infestans* and *P. sojae*. Samples were separated using HPLC with UV detection. The bases A, T, C, G, were detected in all samples, whereas the modified bases 5mC and 6mA occur only in the standard mixture, as shown.

(b) Results from a UPLC-ESI-MS/MS analysis of a control sample of 5mC, and corresponding chromatographic regions from hydrolyzed gDNA from *P. sojae* and *P. infestans*, showing that 5mC cannot be detected in the test samples.

(c) Genomic DNA samples from *P. sojae*, *P. infestans*, *D. melanogaster*, and *G. max* were digested with the 5mC methylation dependent restriction enzyme McrBC. *D. melanogaster* and *G. max* were used as controls. “-” means didn’t add McrBC, “+” means added McrBC.

All the experiments were independently performed with a minimum of three biological replicates, with comparable results.

Supplementary Figure 2

Conservation and gene expression of putative methyltransferases and demethylases in *Phytophthora* species.

(a) Neighbour-Joining tree of N6-adenineMlase domain-containing proteins (DAMTs) in selected oomycete species and their catalytic motifs, shown on the right. More details are in Supplementary Table 2.

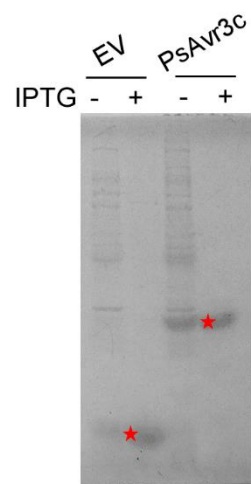
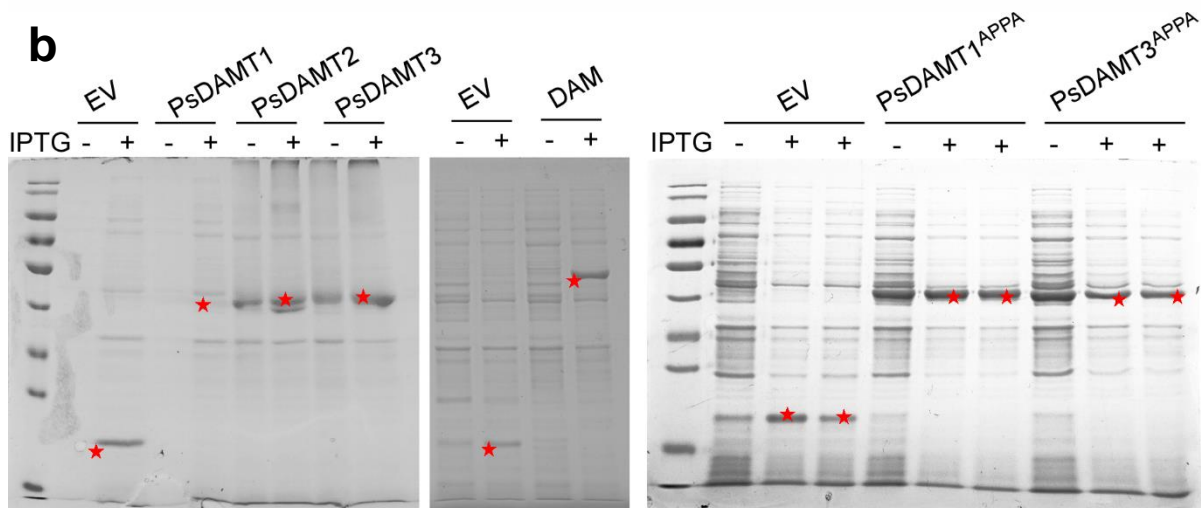
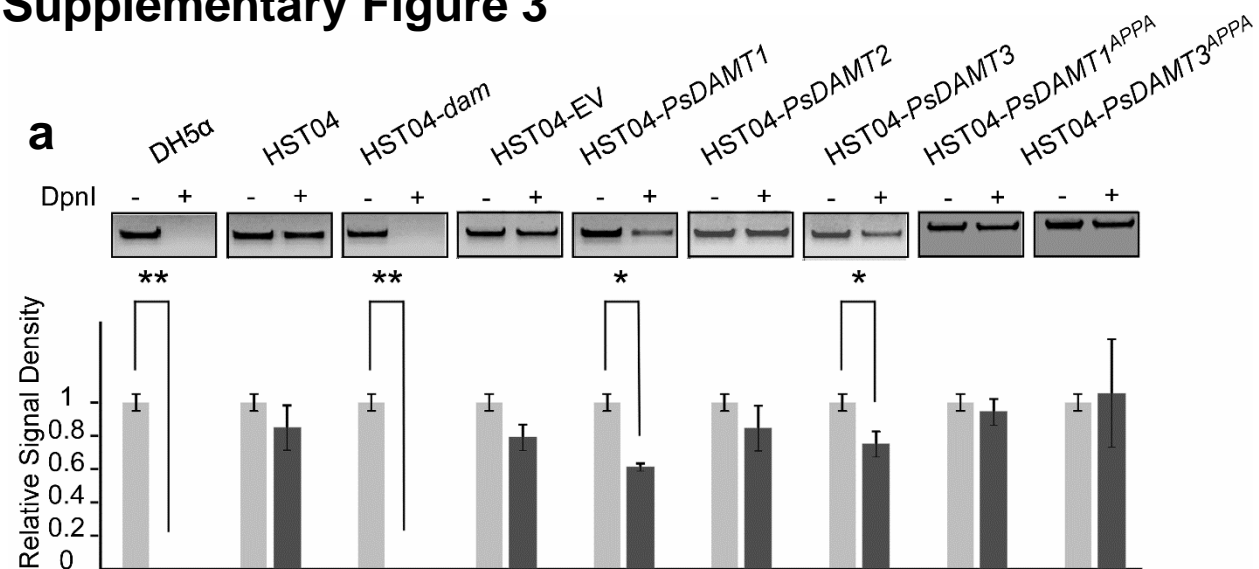
(b) Synteny of the *DAMT3* gene clusters in four *Phytophthora* species. Blue arrows are *PsDAMT3* orthologs. Orthologs marked with blue (*DAMT3* orthologs) and grey (others) lines.

(c) Neighbour-Joining tree of potential DNA and RNA demethylase. Bootstrap percentage >70 was shown.

(d) Expression level of three methyltransferases and seven potential demethylases in different developmental stages of the *P. infestans* strains T30-4, 88069, and 1306 from RNA-seq data. Color codes: high expression (FPKM>1, black), low expression or no expression (FPKM<1, grey). 10 stages: MY for mycelium, SP for sporangium, CL for sporangia undergoing zoosporogenesis, ZO for zoospore, CY for cyst.

(e) Expression level of methyltransferases and potential demethylases in 10 developmental stages of the *P. sojae* P6497 strain from RNA-seq data that were published earlier. GC for germinated cysts, IF1.5h-24h for 1.5 hours to 24 hours post infection.

Supplementary Figure 3



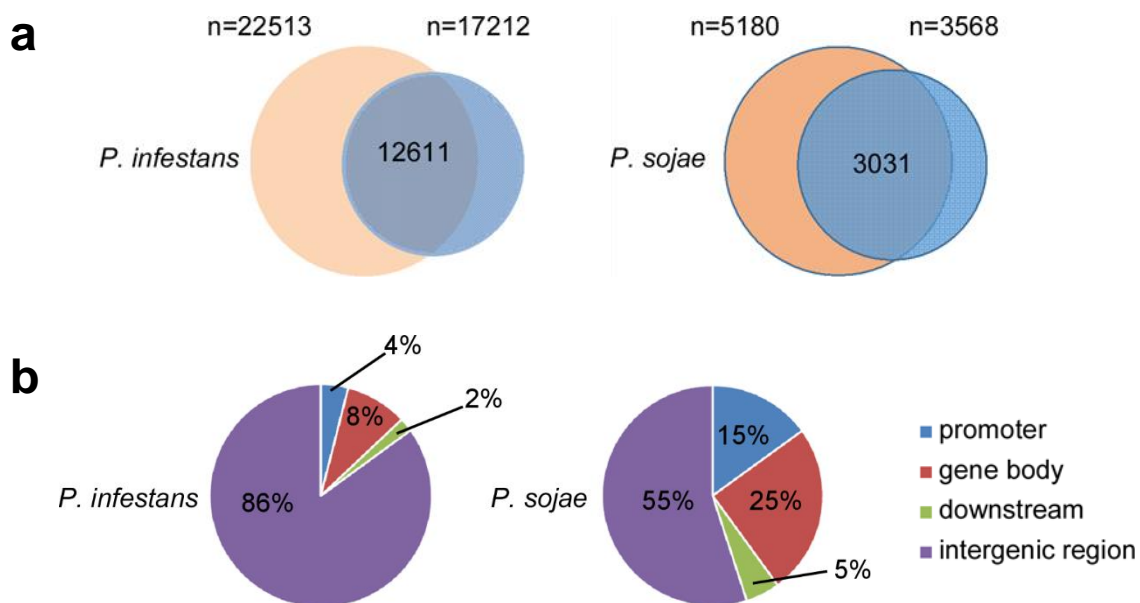
Supplementary Figure 3

Bacteria methyltransferase complementation assay and production of recombinant *P. sojae* methyltransferase

(a) DpnI digestion patterns of gDNA extracted from the *E. coli* strains DH5 α , HST04, and HST04 containing pET-32a-c (+) with native and mutated genes. Both PsDAMT1^{APPA} and PsDAMT3^{APPA} are mutants with catalytic residues substituted with alanine (A). Signal density of the DpnI digestion image was analyzed using software (ImageJ). * and ** represent significant differences ($P < 0.05$ and $P < 0.01$, Students' t test respectively)

(b) SDS-PAGE analysis of pET32a, pET32a-PsDAMT1, pET32a-PsDAMT2, pET32a-PsDAMT3, pET32a-DAM, pET32a-PsDAMT1^{APPA}, pET32a-PsDAMT3^{APPA}, and pET32a-PsAvr3c protein expression. Mutagenesis of PsDAMT1 catalytic motif into APPA abolished the enzyme activity. The recombinant protein bands are indicated by the red asterisks. "-" means didn't added IPTG, "+" means added IPTG

Supplementary Figure 4



Supplementary Figure 4

6mA peak identification and distribution across the genomes of *P. infestans* and *P. sojae*

(a) Venn diagram shows number of unique and overlapping 6mA peaks identified from two MeDIP-seq replicates in *P. infestans* and *P. sojae*.

(b) Pie chart of 6mA enrichment peak distribution among gene features in *P. infestans* and *P. sojae*.

Supplementary Figure 5

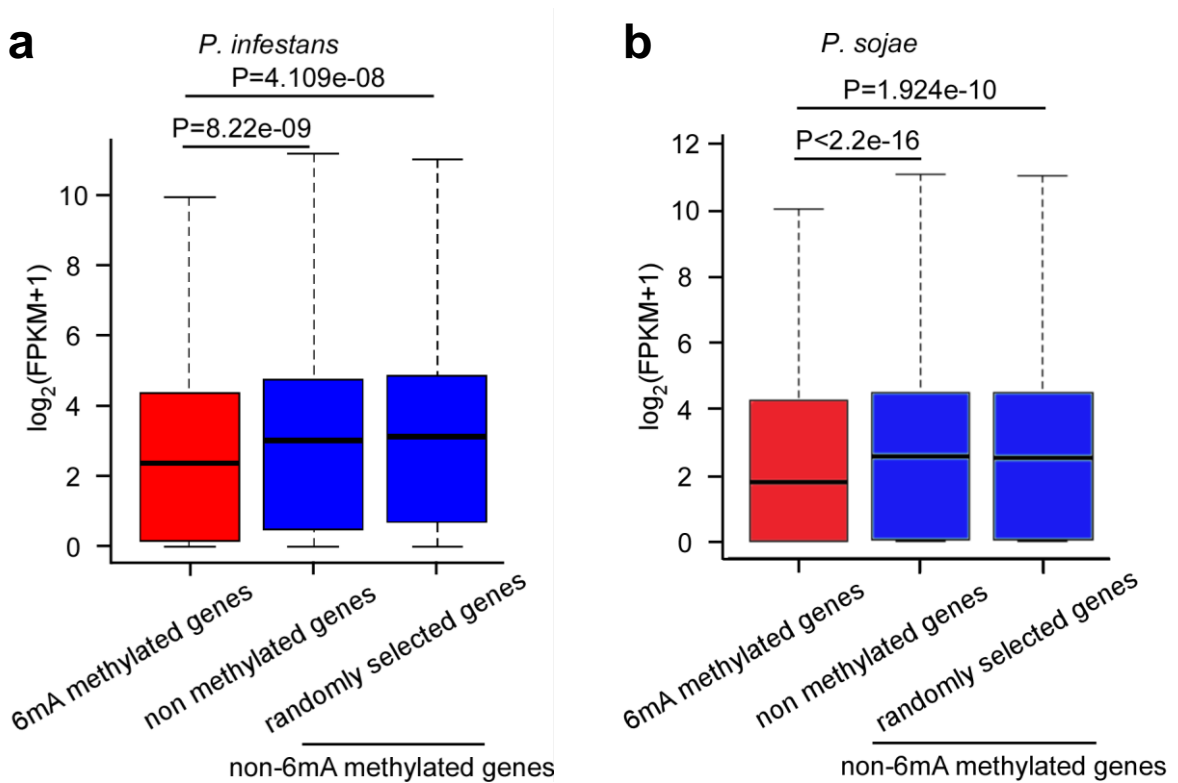
Methylated DNA immuno-precipitation sequencing (MeDIP-seq) read deposition in representative loci and gene ontology (GO) analysis of 6mA methylated genes

(a) Snapshot of 6mA deposition around selected genes in *P. infestans* and *P. sojae*. Y-axis is $\log_2(\text{RPKMIP}/\text{RPKMinput})$

(b) Enrichment of methylated *P. infestans* gene with GO categories. Shown along the x-axis are different GO categories which have P-value of Pearson Chi-square test < 5%. The left y-axis and the right y-axis represent the percent of genes and the number of genes for each GO category, respectively.

(c) Enrichment of methylated *P. sojae* gene with GO categories.

Supplementary Figure 6



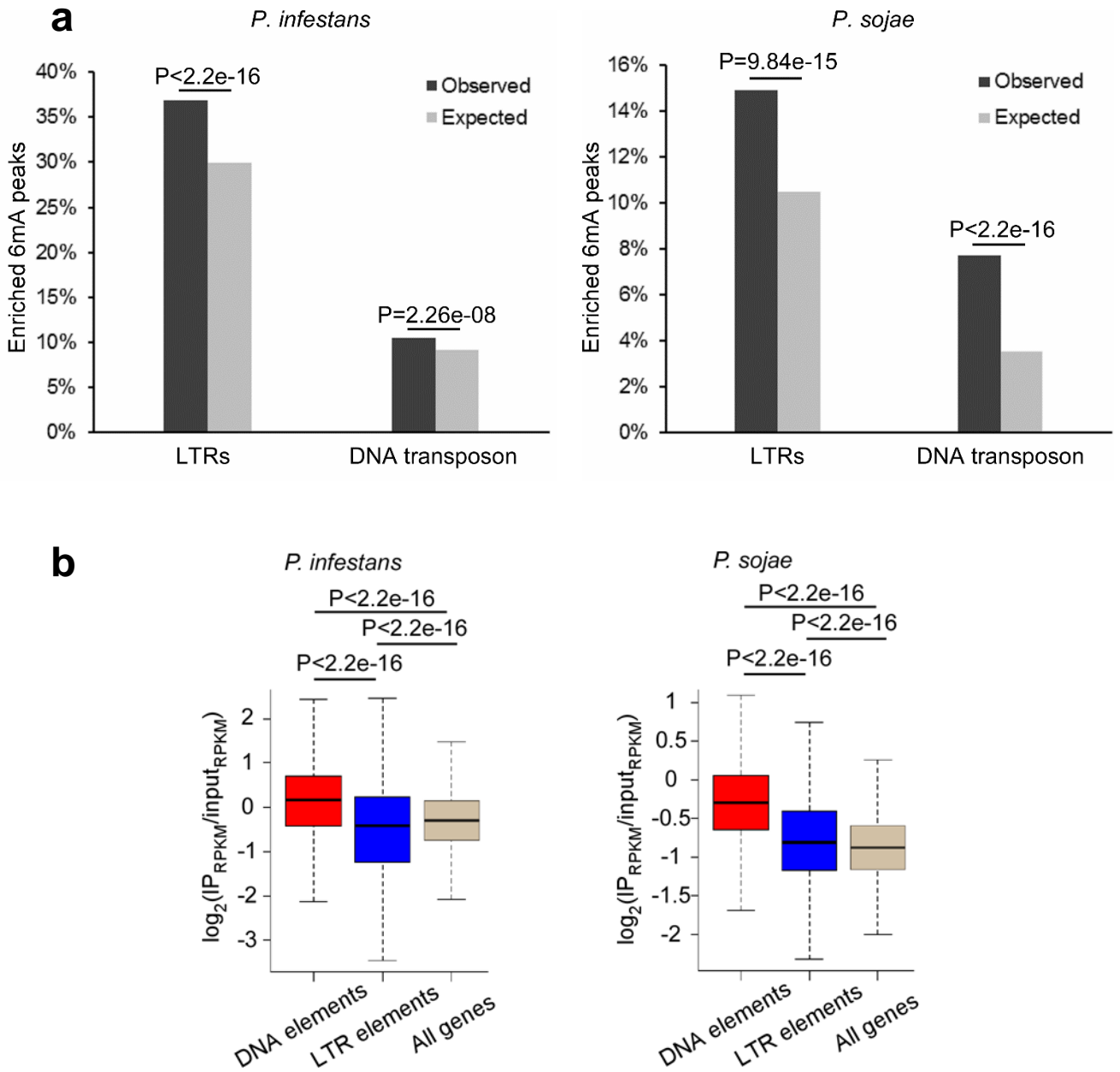
Supplementary Figure 6

Gene expression levels are negatively associated with 6mA methylation levels

(a) In *P. infestans*, 6mA modified genes ($n=1805$) (red), randomly selected non-modified genes ($n=1805$), and all non-modified genes ($n=16374$) (blue) are shown.

(b) In *P. sojae*, 6mA modified genes ($n=1343$), randomly selected non-modified genes ($n=1343$) and all non-modified genes ($n=17853$) are shown. Different groups of genes are shown along the x-axis. The y-axis represents the relative expression level. P values are calculated with the Two-sample Kolmogorov-Smirnov test.

Supplementary Figure 7



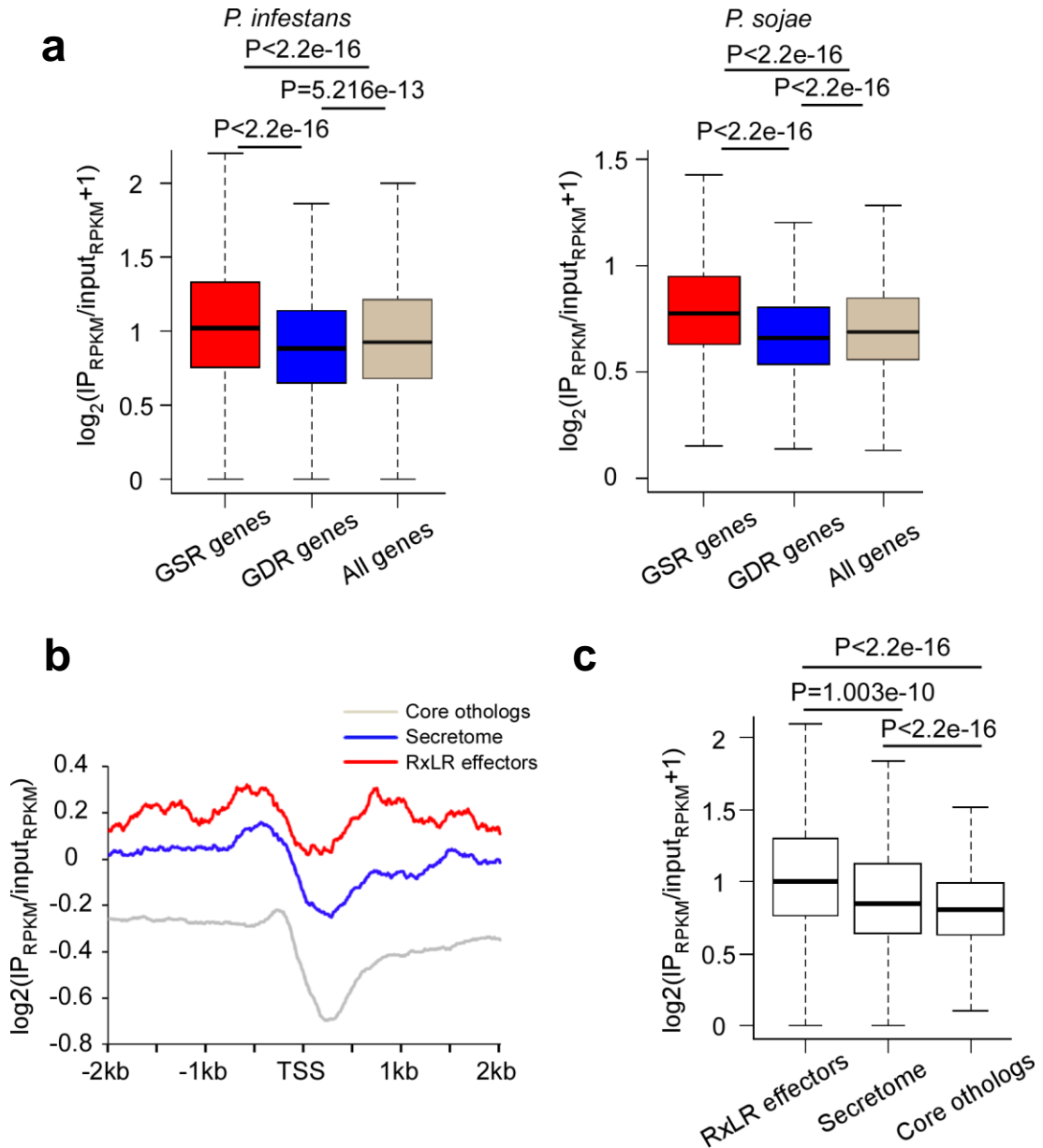
Supplementary Figure 7

6mA predominantly localized in repetitive and transposable elements

(a) 6mA peaks are enriched in LTR elements and DNA elements. Y-axis is a percentage of enriched 6mA peaks. P values were calculated with excel binomial distribution probability.

(b) DNA elements have a higher methylation level. Y-axis is relative methylation level. P values calculated with the Two-sample Kolmogorov-Smirnov test.

Supplementary Figure 8



Supplementary Figure 8

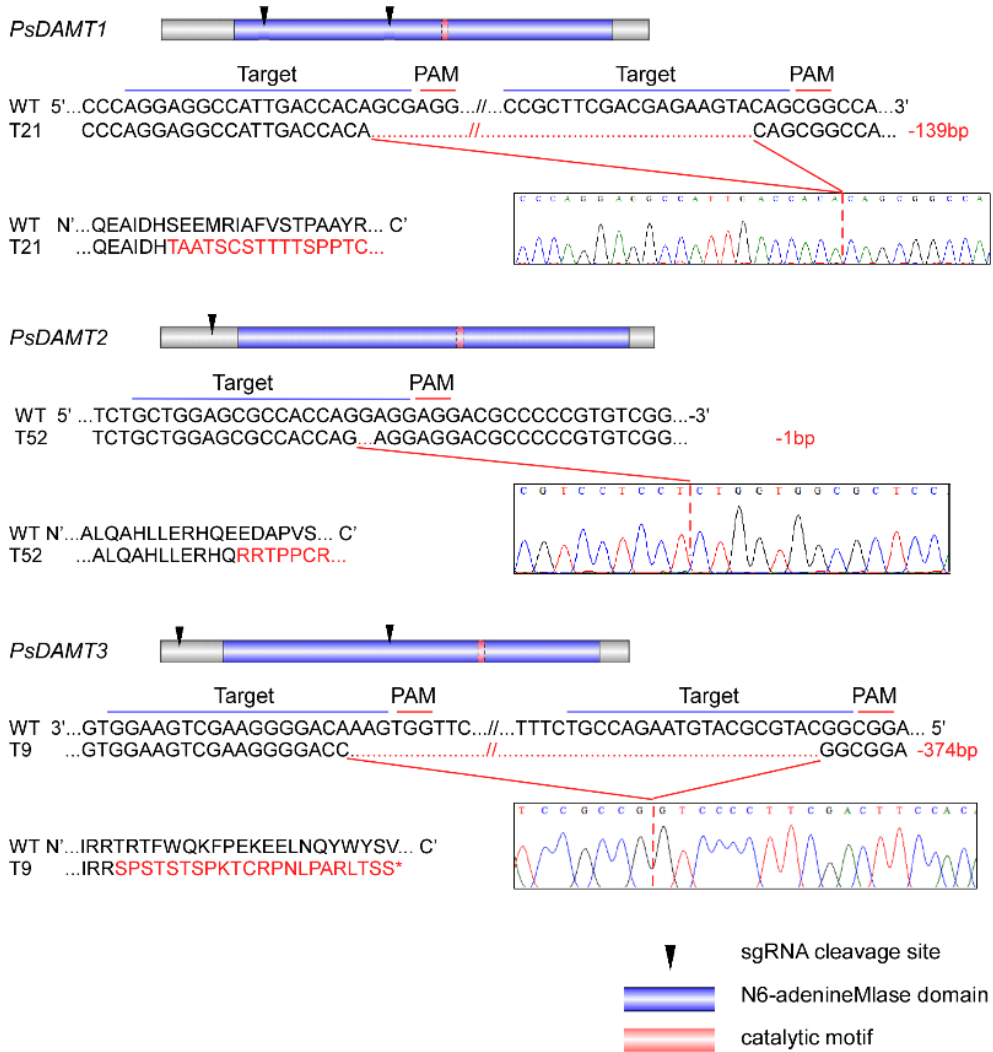
GSR genes, secretome genes and RxLR effector genes have higher 6mA levels

(a) Y-axis is relative 6mA level. P values are calculated with the Two-sample Kolmogorov-Smirnov test.

(b) The relative 6mA levels of core orthologs (n=7113), secretome genes (n=1415), and RxLR effector genes (n=562) around TSS in *P. infestans* are calculated.

(c) The average relative 6mA levels from core orthologs, secretome genes, and RxLR effector genes in *P. infestans* are shown in a Box blot. P values are calculated with the Two-sample Kolmogorov-Smirnov test.

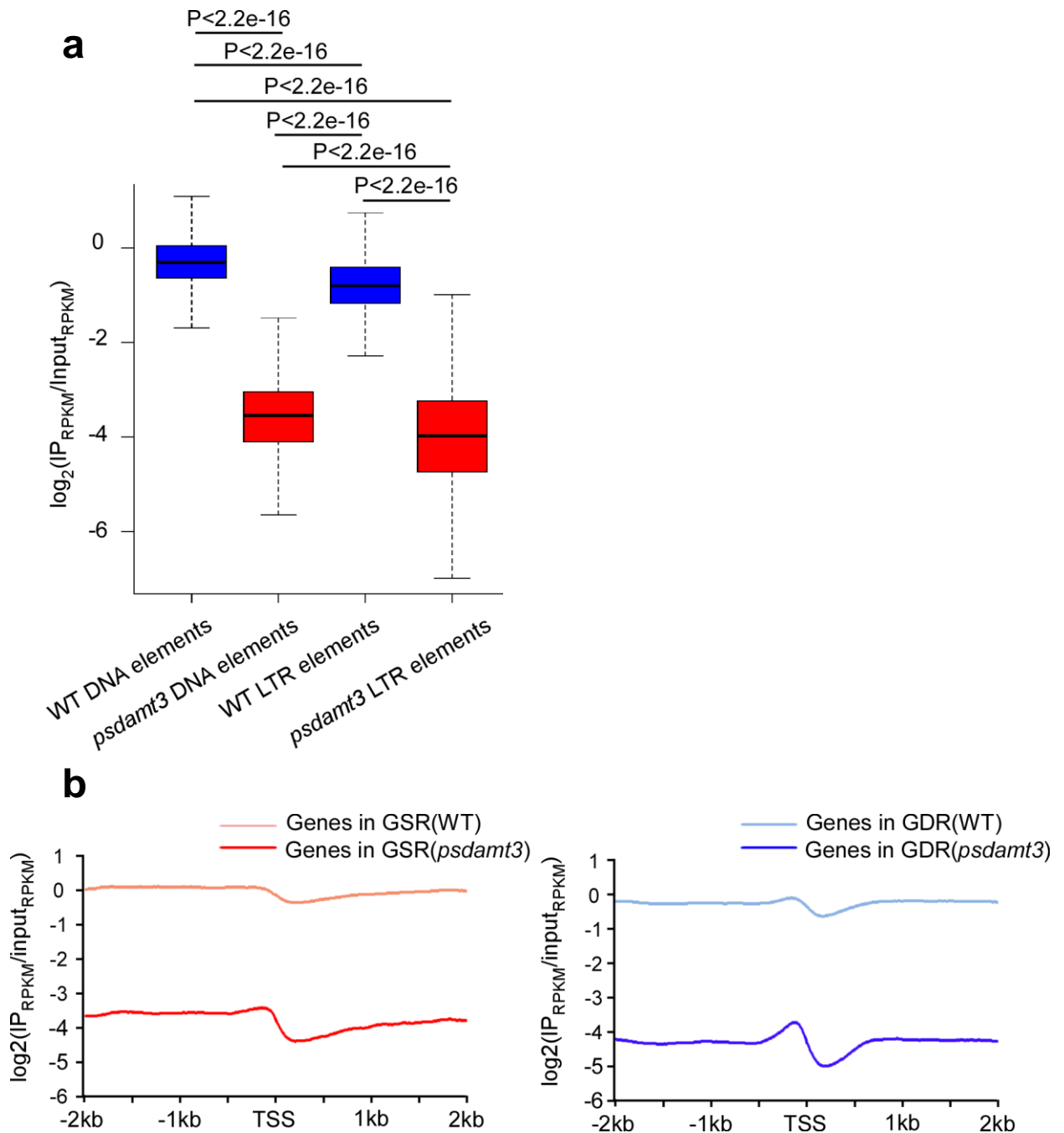
Supplementary Figure 9



Supplementary Figure 9

Schematic representations of *PsDAMTs* knockout mutants
 Representative mutants *psdamt1* (T21), *psdamt2* (T52), and *psdamt3* (T9) that were generated using CRISPR/Cas9 system are illustrated.

Supplementary Figure 10



Supplementary Figure 10

6mA level of *psdamt3* reduced.

(a) In the *P. sojiae psdamt3* knockout mutant, 6mA levels are lower than in WT in DNA elements and LTRs of repeat sequences. P values calculated with the Two-sample Kolmogorov-Smirnov test.

(b) In *P. sojiae psdamt3* knockout mutant, 6mA levels are significantly reduced for genes that are located in both GSR (n=3154) and GDR (n=7240).