Evaluation of N^6-adenine DNA-immunoprecipitation-based genomic profiling in eukaryotes

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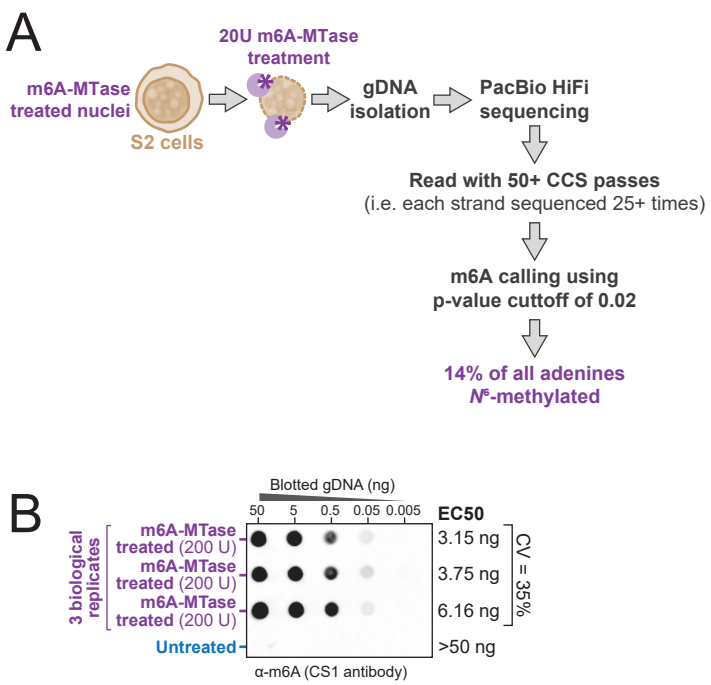
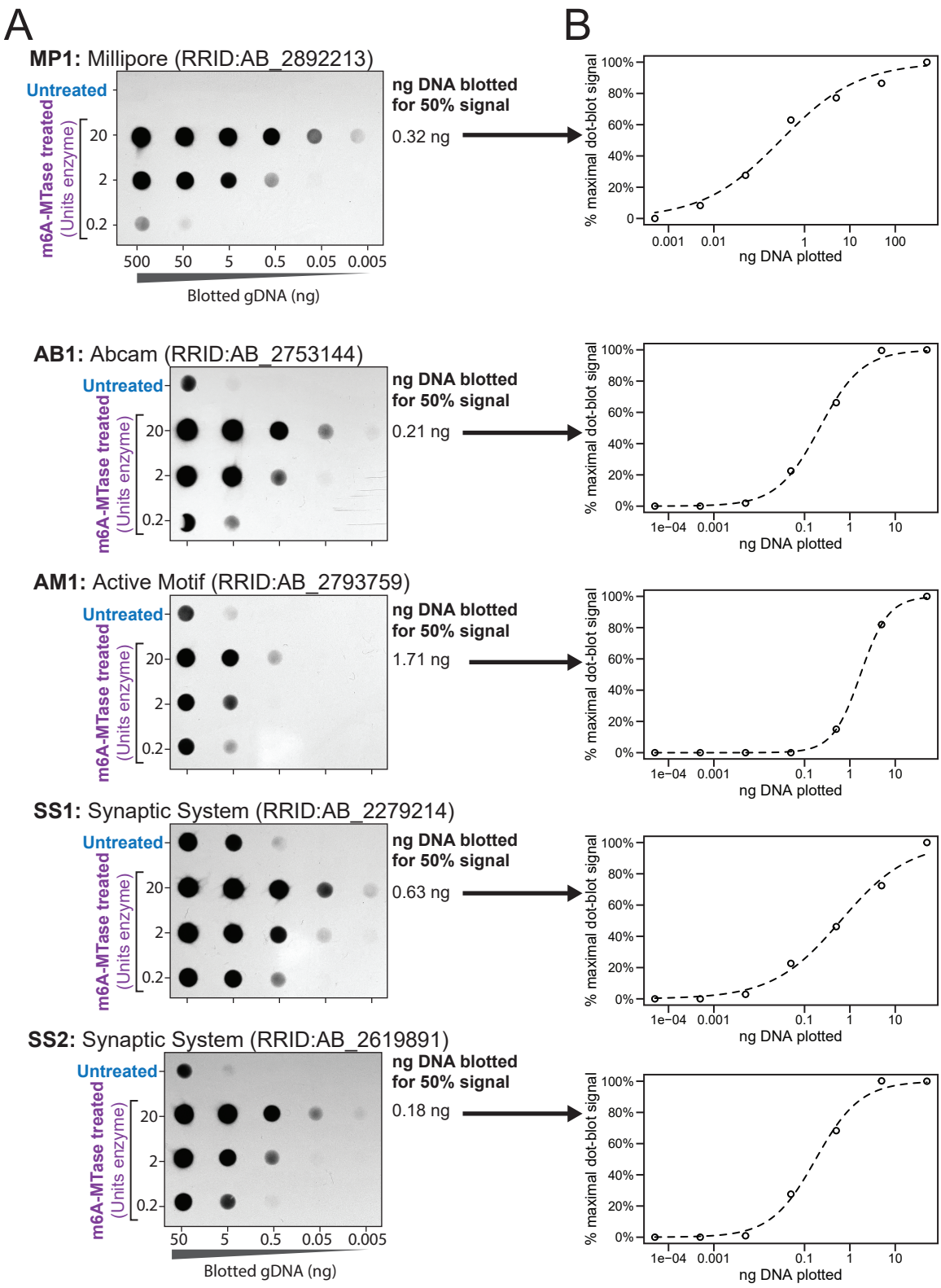
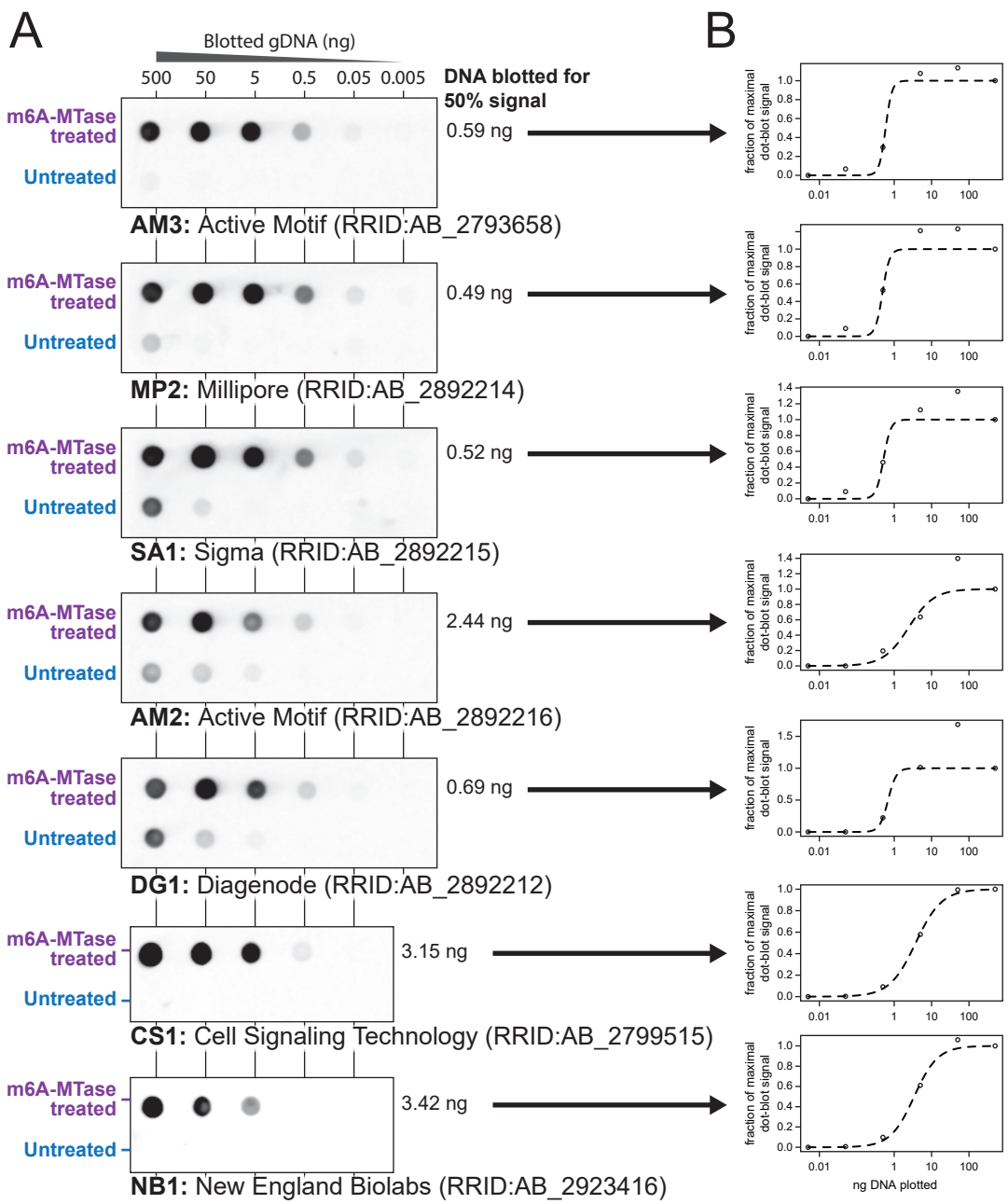


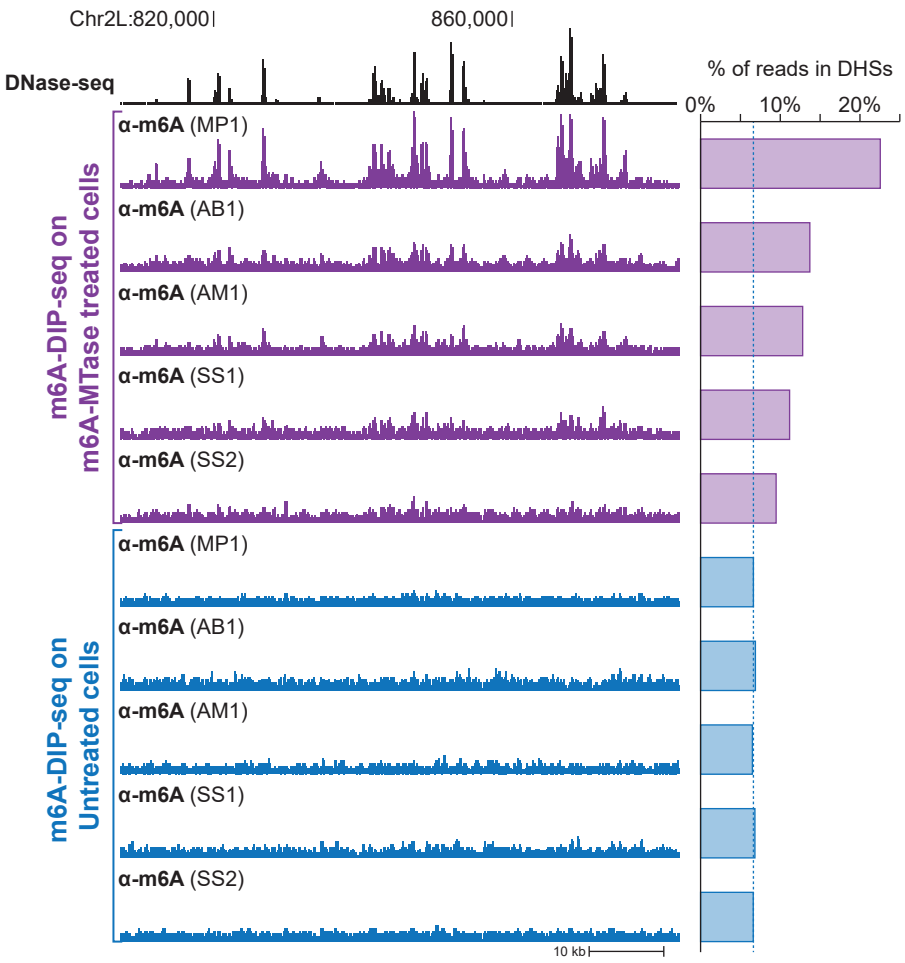
Supplemental Figure S1 | Determining absolute DNA-m6A amount on a highly methyated sample using PacBio single-molecule circular consensus sequencing (CCS).
(A) Schematic demonstrating how Drosophila S2 cell nuclei are treated with 20 units of a non-specific m6A-MTase, followed by genomic DNA isolation, DNA shearing to 1-4kb in length and PacBio circular consensus sequencing using a Sequel I platform. DNA-m6A events were identified on a per-molecule basis using a p-value cut off of 0.02, and analysis was restricted to molecules with 50+ circular passes to ensure robust DNA-m6A identification. Overall, this approach identified that 14% of all adenines are marked by DNA-m6A after treatment of S2 cell nuclei with 20 units of a non-specific m6A-MTase. (B) Variation in m6A quantity between biological replicates. K562 cells treated with 200 units of the Hia5 m6A-MTase, followed by genomic DNA isolation and DNA dot blotting using the CS1 antibody. EC50 calculated using a log linear regression of dot blot intensities, and coresponds to the ng of DNA blotted resulting in 50% reduction in max signal. CV: Coefficient of variation for 1 standard deviation from the mean



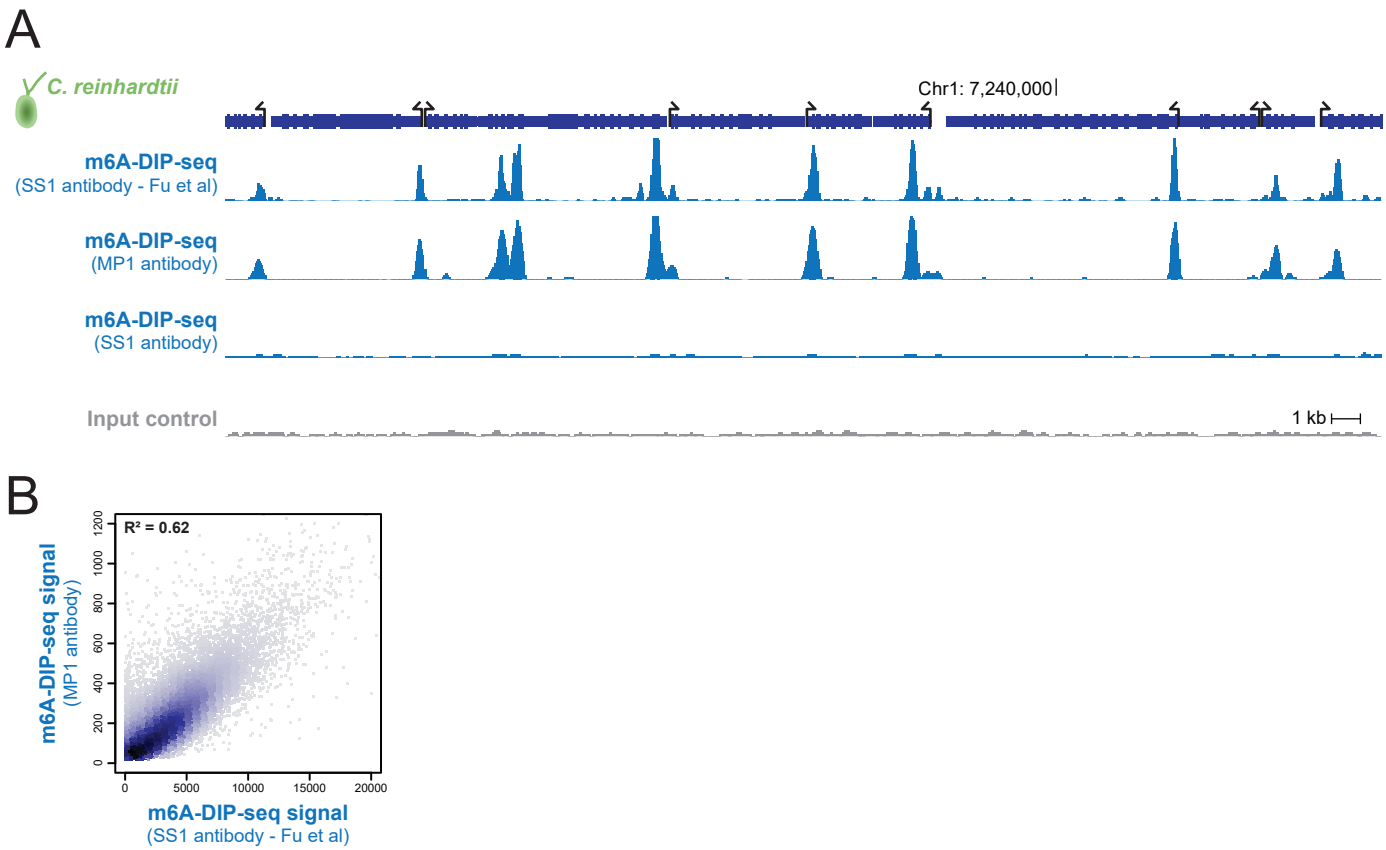
Supplemental Figure S2 | Selectivity of anti-DNA-m6A antibodies towards DNA-m6A.
(A) DNA dot blots using 5 separate anti-DNA-m6A antibodies against genomic DNA from untreated S2 cells, versus genomic DNA from S2 nuclei treated with various amounts of a non-specific DNA m6A-MTase. The top two rows of the AB1, SS1, and AM1 blots are identical to those shown in Figure 1B. To the right are quantification of each dot blot, showing the amount of DNA needed to give 50% of the signal, as calculated using a log-logistic regression. (B) Calculation of DNA dot blot signal intensity for 20 unit treated sample, as well as a four parameter log-logistic fit to these signals (dashed line).



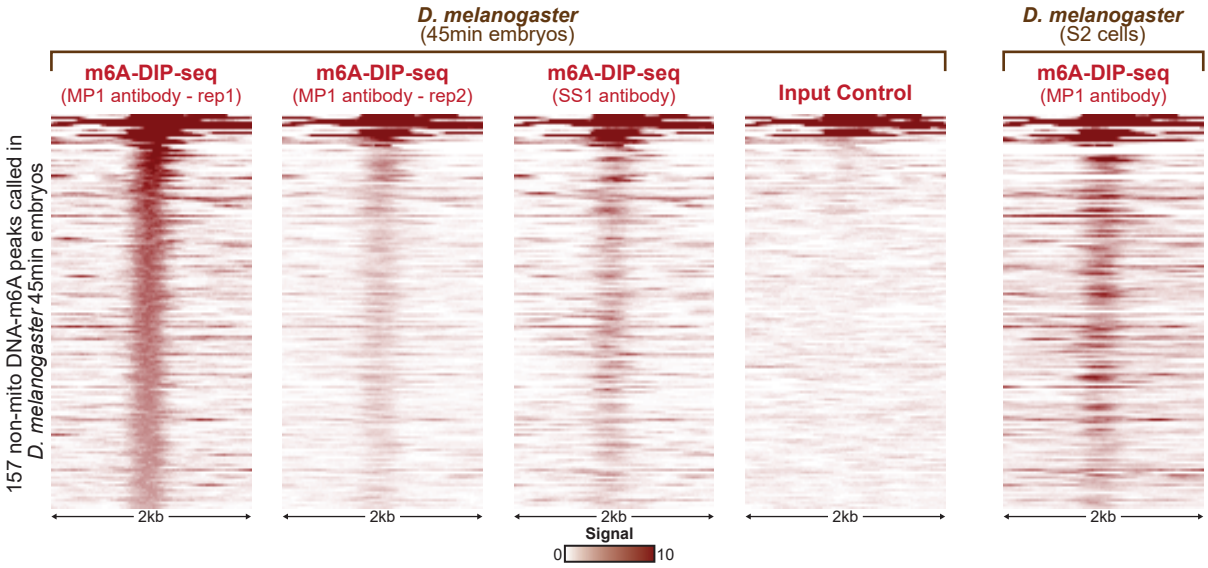
Supplemental Figure S3 | Selectivity of anti-DNA-m6A antibodies towards DNA-m6A.
(A) DNA dot blots using 7 separate anti-DNA-m6A antibodies against genomic DNA from untreated K562 cells, versus genomic DNA from K562 nuclei treated with a non-specific DNA m6A-MTase (400U Hia5 for 10min at 25C). To the right are quantification of each dot blot, showing the amount of DNA needed to give 50% of the signal, as calculated using a log-logistic regression. (B) Calculation of DNA dot blot signal intensity for the treated sample, as well as a four parameter log-logistic fit to these signals (dashed line).



Supplemental Figure S4 | Detection of m6A-modified genomic loci using anti-DNA-m6A antibodies. (left) Genomic locus comparing the relationship between DNase-seq and m6A-DIP-seq signal. m6A-DIP-seq performed using 5 separate antibodies on untreated S2 cell genomic DNA, or genomic DNA from S2 cell nuclei treated with 0.6 U of a non-specific m6A-MTase. Y-axis is identical for all m6A-IP-seq experiments. (right) Percentage of sequencing reads that are localized to DNase I hypersensitive sites for each of the m6A-DIP-seq experiments. Higher values are indicative of higher antibody specificity.

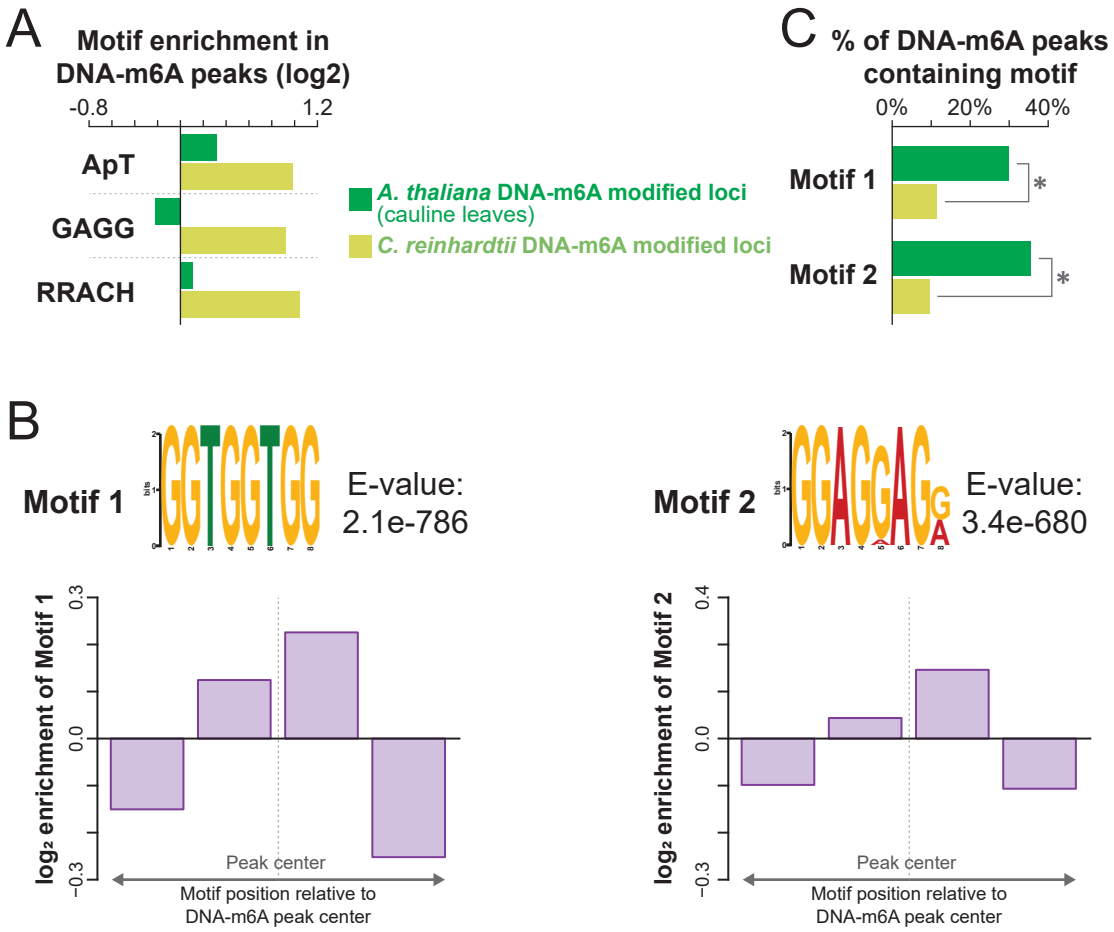


Supplemental Figure S5 | Site-specific DNA-m6A methylation in *C. reinhardtii*.
(A) Genomic locus from *C. reinhardtii* comparing the relationship between m6A-DIP-seq signal collected using either the MP1 or SS1 antibody, m6A-DIP-seq signal previously published using the SS1 antibody, and input control signal. Y-axis is identical for all experiments except the previously published track. (B) Density scatter plot showing the relationship between m6A-DIP-seq signal collected using the MP1 antibody, and m6A-DIP-seq signal previously published using the SS1 antibody.



Supplemental Figure S6 | *D. melanogaster* 45-min embryos lack appreciable cell-selective site-specific DNA-m6A.

Plots showing the signal strength surrounding each of the 157 non-mitochondrial DNA-m6A peaks identified in *D. melanogaster* 45-min embryos. Specifically, displayed is the m6A-DIP-seq signal using two replicates of the MP1 antibody, as well as a single replicate of the SS1 antibody, and input control. Signal from m6A-DIP-seq using the MP1 antibody on S2 cell genomic DNA is also displayed for comparison. The same scale is used for each of the five plots.



Supplemental Figure S7 | Enriched motifs in *A. thaliana* cauline leaf and *C. reinhardtii* DNA-m6A peaks. (A) Motif enrichment relative to shuffled sequences of three previously reported eukaryotic m6A-MTase motifs in DNA-m6A peaks from *C. reinhardtii* and *A. thaliana* cauline leaves. (B) (top) Sequence logo and E-value for the top 2 motifs identified as being enriched in *A. thaliana* cauline leaf DNA-m6A peaks using MEME. (bottom) The distribution of each motif within *A. thaliana* cauline leaf DNA-m6A peaks relative to the size of each peak. Enrichment calculated based on the average density of each motif within *A. thaliana* cauline leaf DNA-m6A peaks. (C) The percentage of *A. thaliana* cauline leaf and *C. reinhardtii* DNA-m6A peaks that contain each of the motifs identified in panel B. * p-value <0.01 (z-test).