TITLE

Diversity of cytosine methylation across the fungal tree of life

AUTHORS

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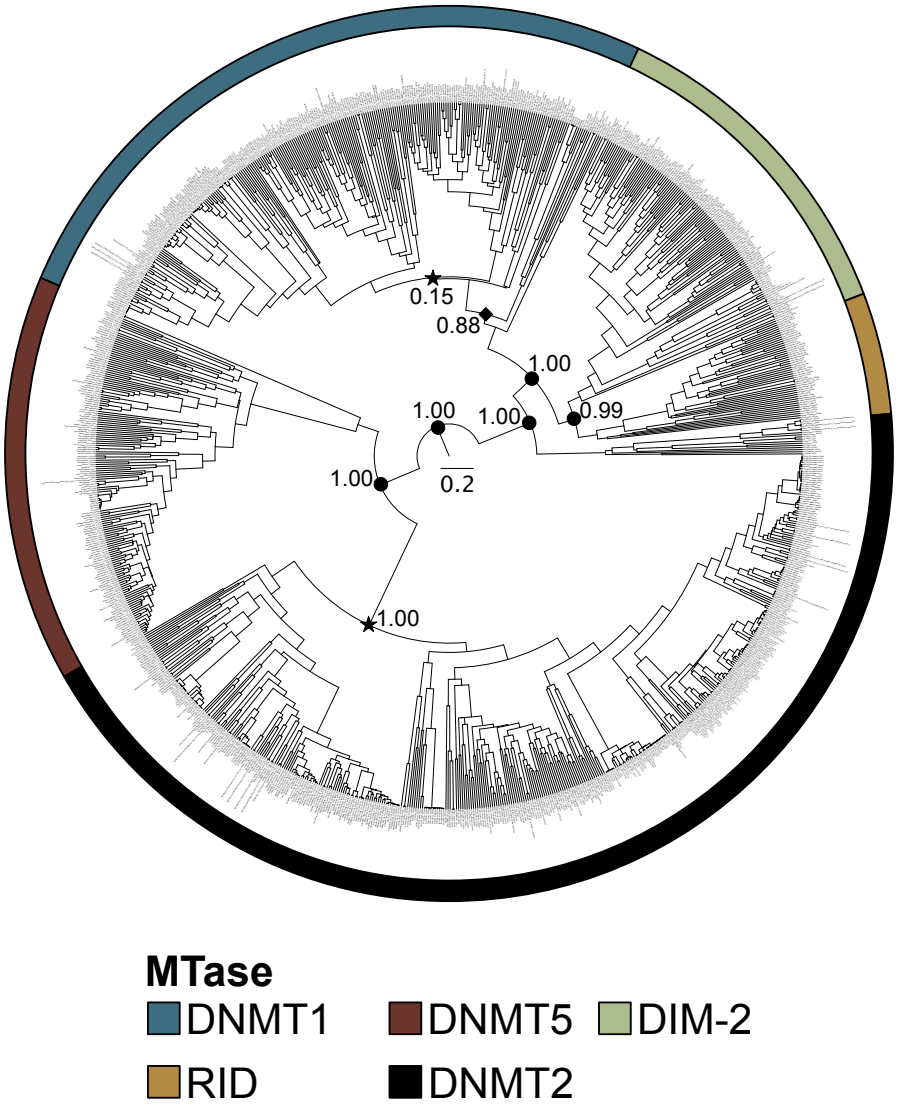
SUPPLEMENTARY INFORTMATION

Supplementary Figure 1

5mC DNA and tRNA MTase phylogeny of fungi.

Evolution of DNA and tRNA methyltransferases across fungi. Values at selected nodes indicate posterior probability. Nodes with a star specify duplications, and the single node with a diamond specifies the clade containing '*DnmtX*' (29). Branch lengths are in units of amino acid substitutions per amino acid site. Phyla abbreviations: Ascomycota (Asco-), Basidiomycota (Basidio-), Blastocladiomycota (Blastocladio-), Chytridiomycota (Chytridio-), Mucoromycota (Mucoro-), and Zoopagomycota (Zoopago-).

Supplementary Fig. 1



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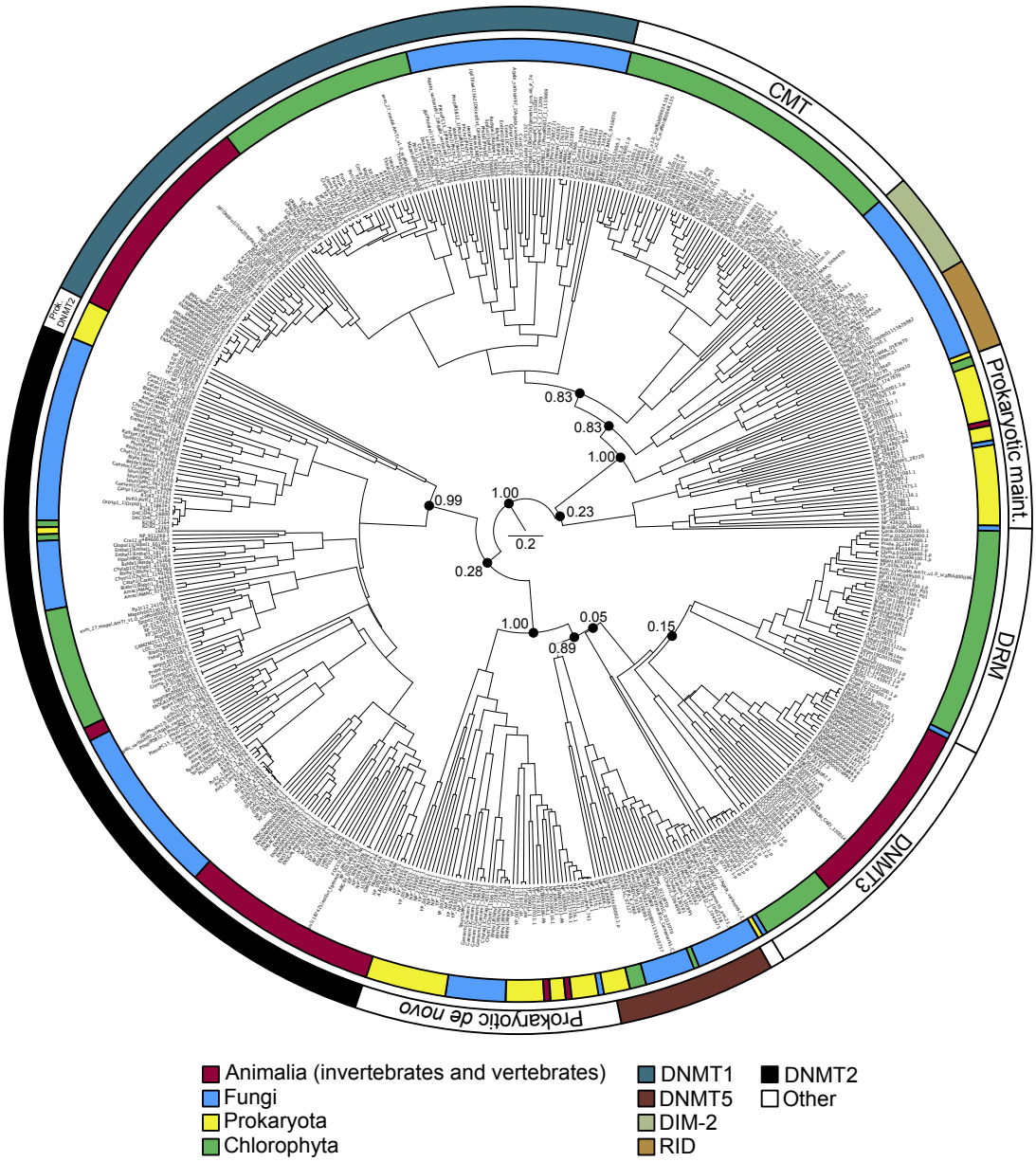
Supplementary Figure 2

5mC DNA and tRNA MTase phylogeny of animalia, chlorophyta, fungi, and prokaryota.

Evolution of DNA and tRNA methyltransferases across the tree of life. Values at selected nodes indicate posterior probability. Branch lengths are in units of amino acid substitutions per amino acid site. Phyla abbreviations: Ascomycota (Asco-), Basidiomycota (Basidio-), Blastocladiomycota (Blastocladio-), Chytridiomycota (Chytridio-), Mucoromycota (Mucoro-), and Zoopagomycota (Zoopago-).

Supplementary Fig. 2



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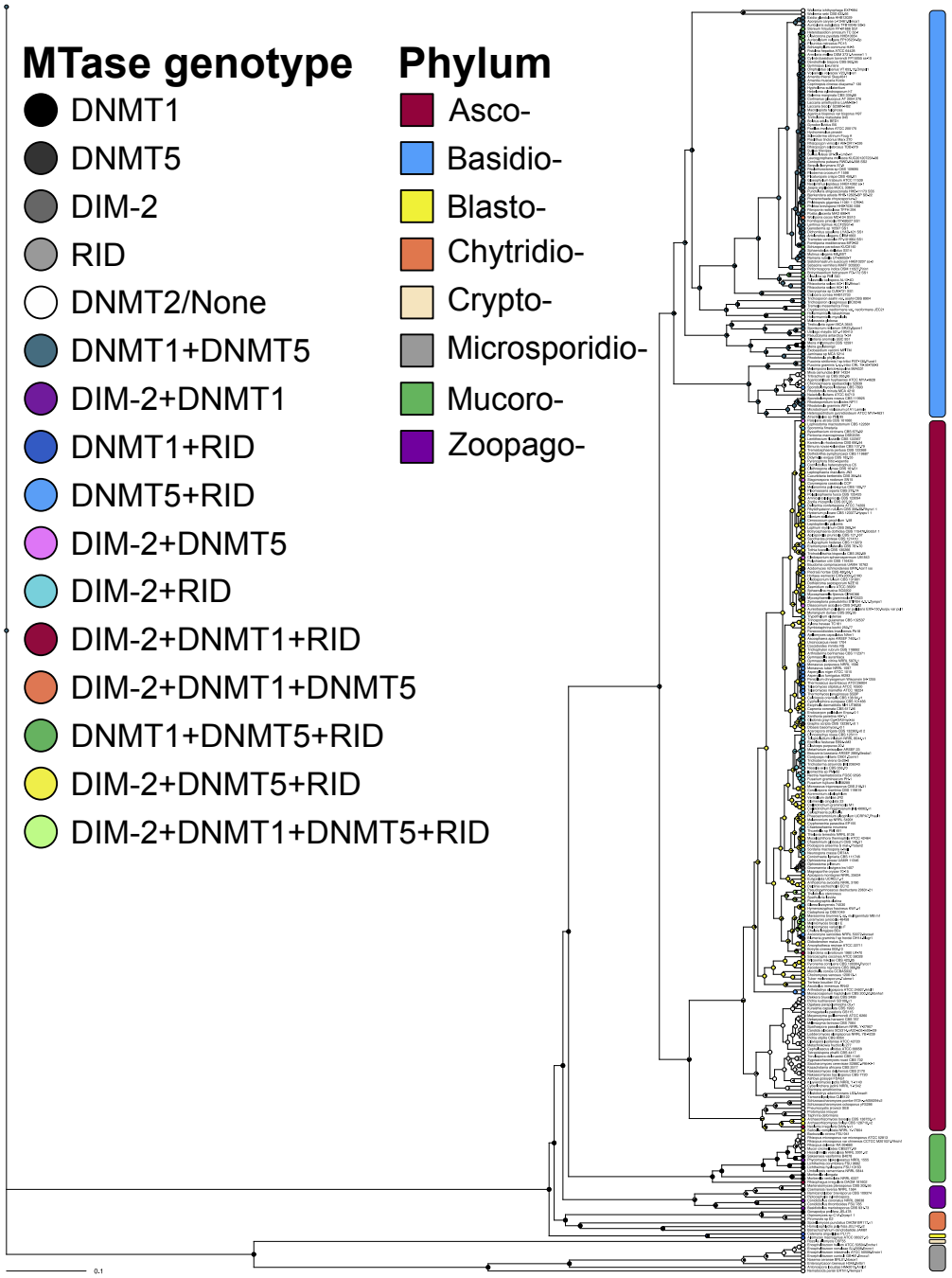
Supplementary Figure 3

Stochastic character mapping of sixteen observed DNA MTase genotypes.

The colored bars indicate phylum of encompassing fungal species. Phyla abbreviations: Ascomycota (Asco-), Basidiomycota (Basidio-), Blastocladiomycota (Blastocladio-), Chytridiomycota (Chytridio-), Mucoromycota (Mucoro-), and Zoopagomycota (Zoopago-). The colored circle adjacent to each species corresponds to the observed genotype. Pie charts at nodes indicate empirical Bayesian posterior probabilities of the genotypes in a common ancestor. Branch lengths are in units of amino acid substitutions per amino acid site.

Supplementary Fig. 3

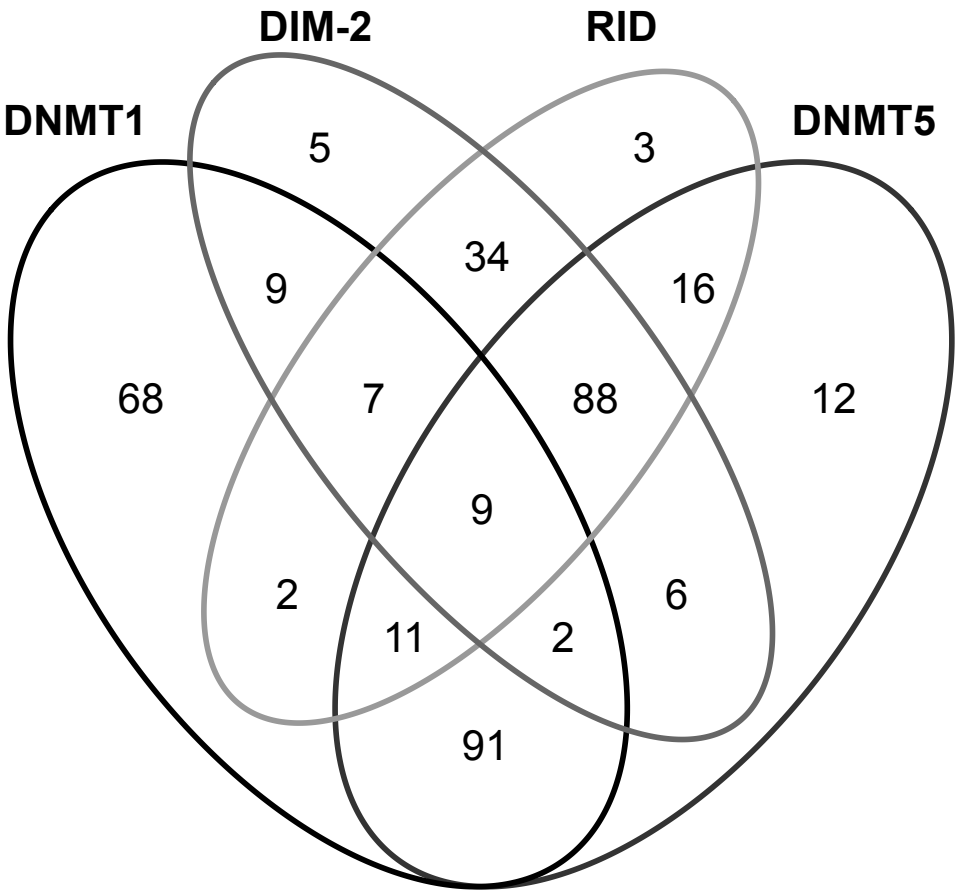


Supplementary Figure 4

Individual and combinations of DNA MTases.

The values within the areas of the Venn diagram indicate the number of fungal species/strains with the corresponding DNA MTase genotype.

Supplementary Fig. 4



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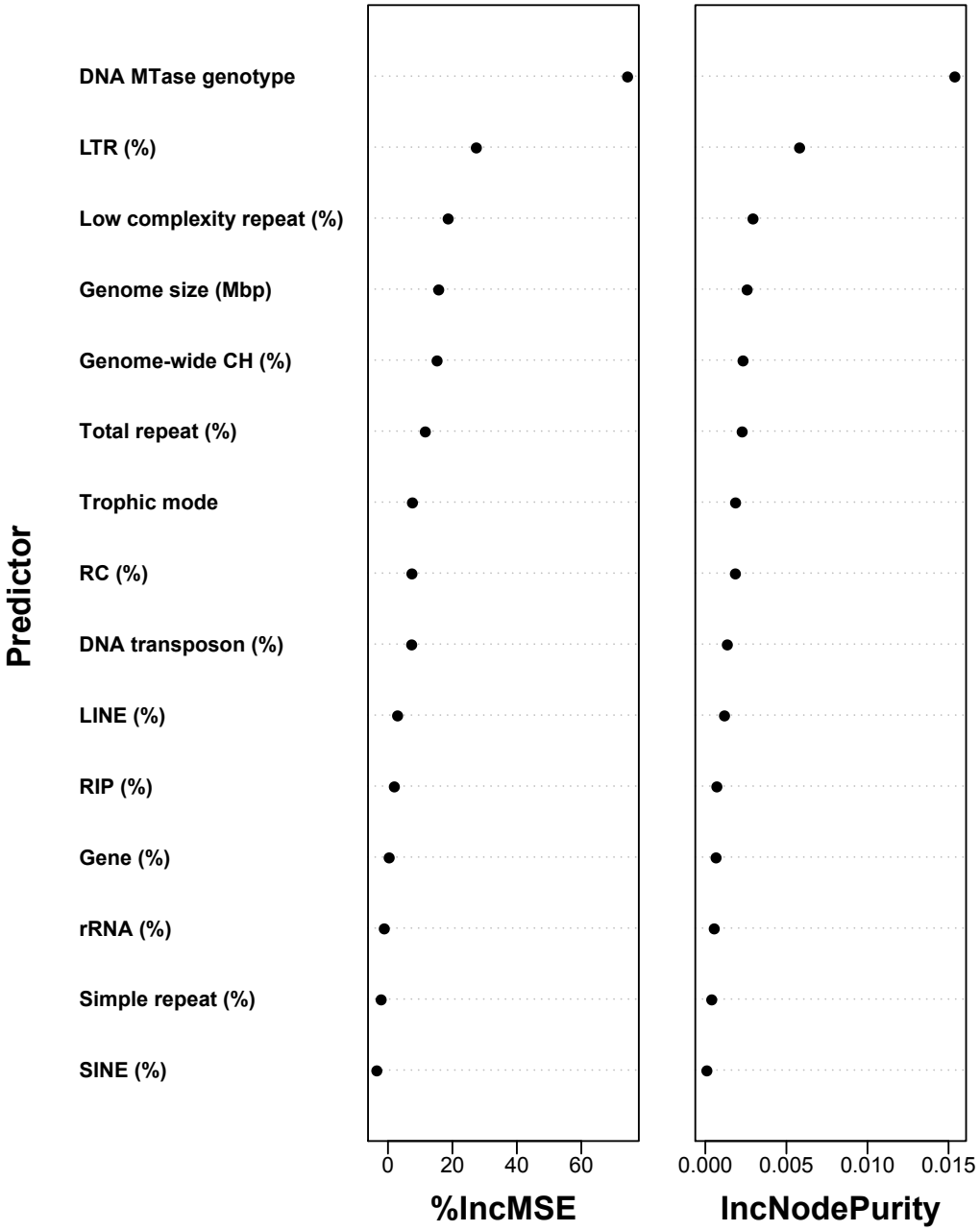
Supplementary Figure 5

Predictors of methylation.

Rank-order from best to worse predictors of genome-wide CG methylation.

Supplementary Fig. 5



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212 **Supplementary Figure 6**

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214 **Explanations for discrepancies between DNA MTase genotype and**
215 **methylation levels.**

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217 (**a**) Tissue-specific expression of DNA MTases in *Bot. cinerea* and *Pse.*
218 *destructans*. Grey-highlighted boxes specify tissues used for methylation
219 analyses. *a* and *b* above bars in *Bot. cinerea* specify RID in-paralogs. Error bars
220 correspond to the standard error of the mean (SEM). (**b**) Methylation in *Lep.*
221 *maculans* 'brassicae' is localized to short, gene-poor regions at the beginnings
222 and ends of supercontigs.

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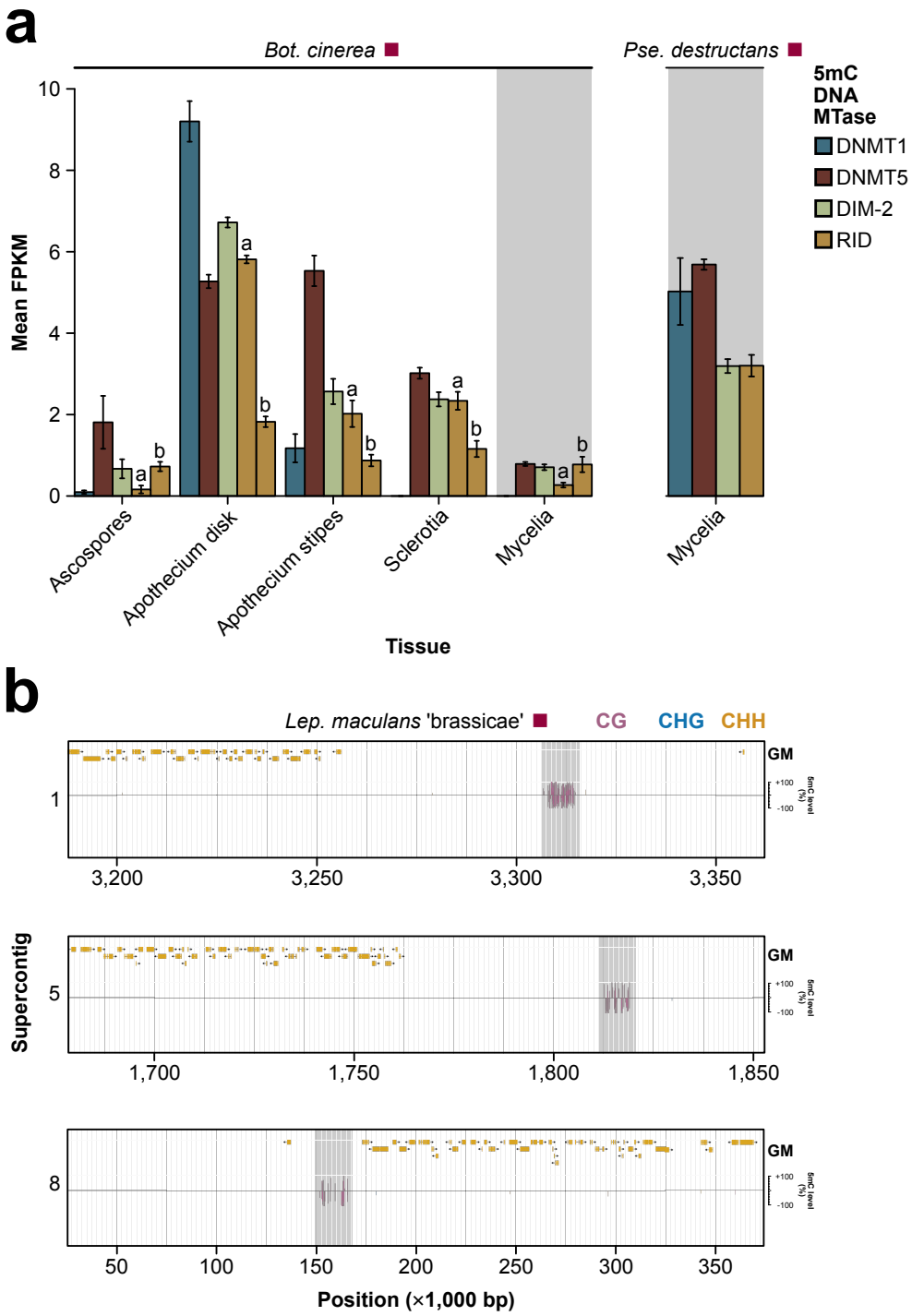
Supplementary Fig. 6



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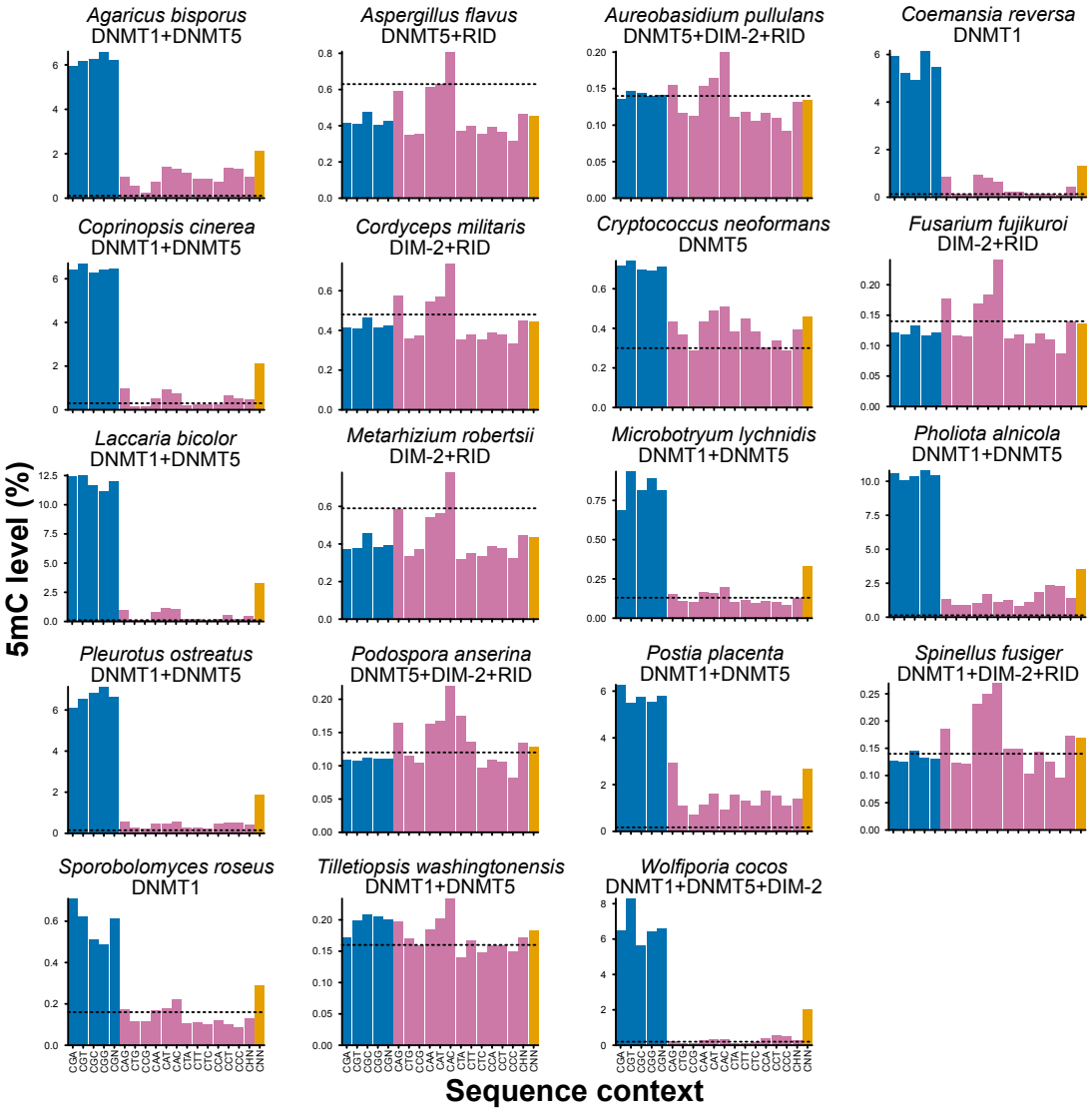
Supplementary Figure 7

Context-specific methylation.

Weighted methylation levels at all sequence contexts found across the genome for fungal species not included in Fig. 2.

Supplementary Fig. 7



Supplementary Figure 8

Genic and repeat and transposon methylation profiles.

Weighted methylation at CG, CH, and CN sites upstream, within, and downstream of all genes for fungal species not included in Fig. 4.

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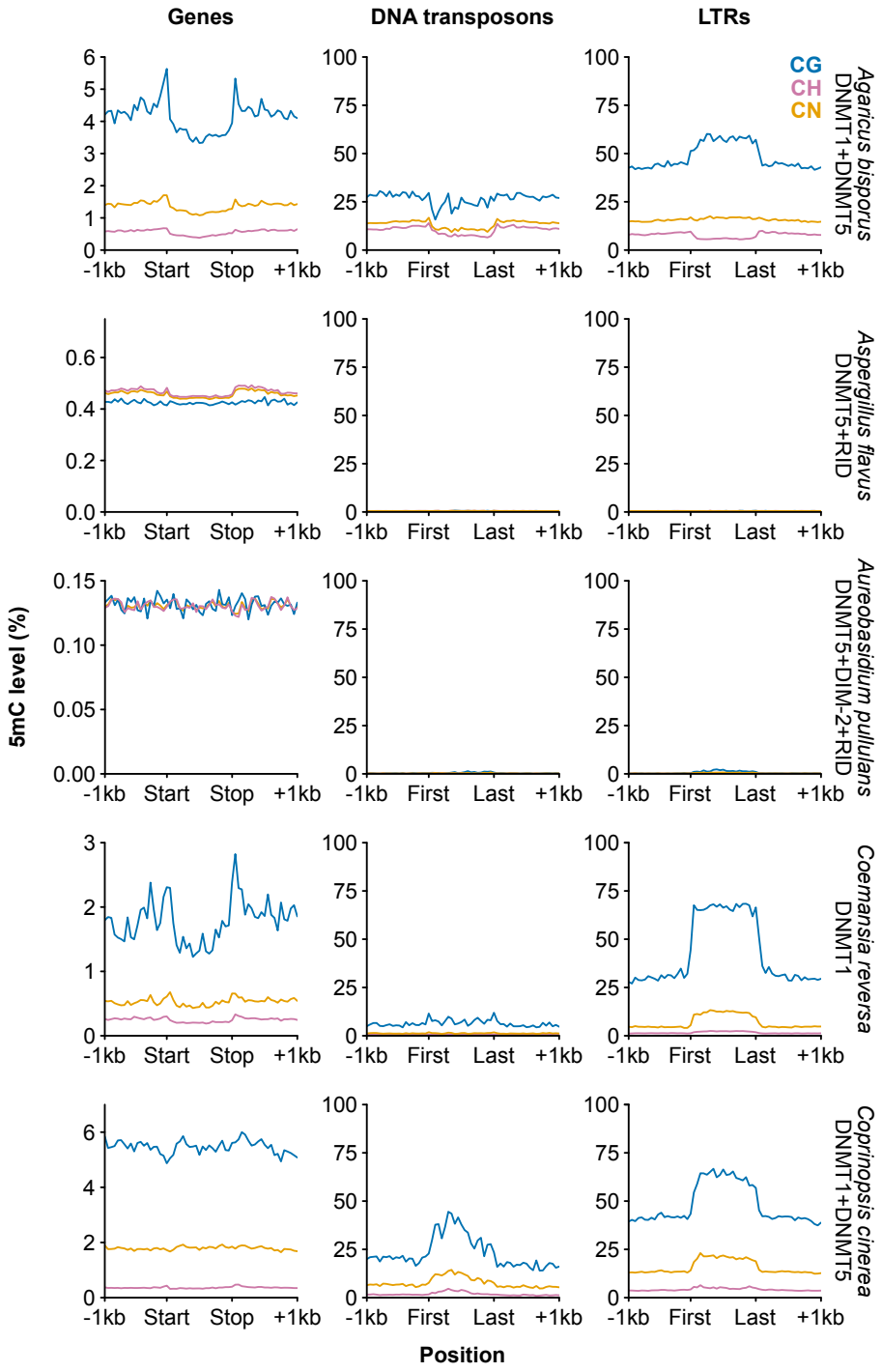
Supplementary Fig. 8



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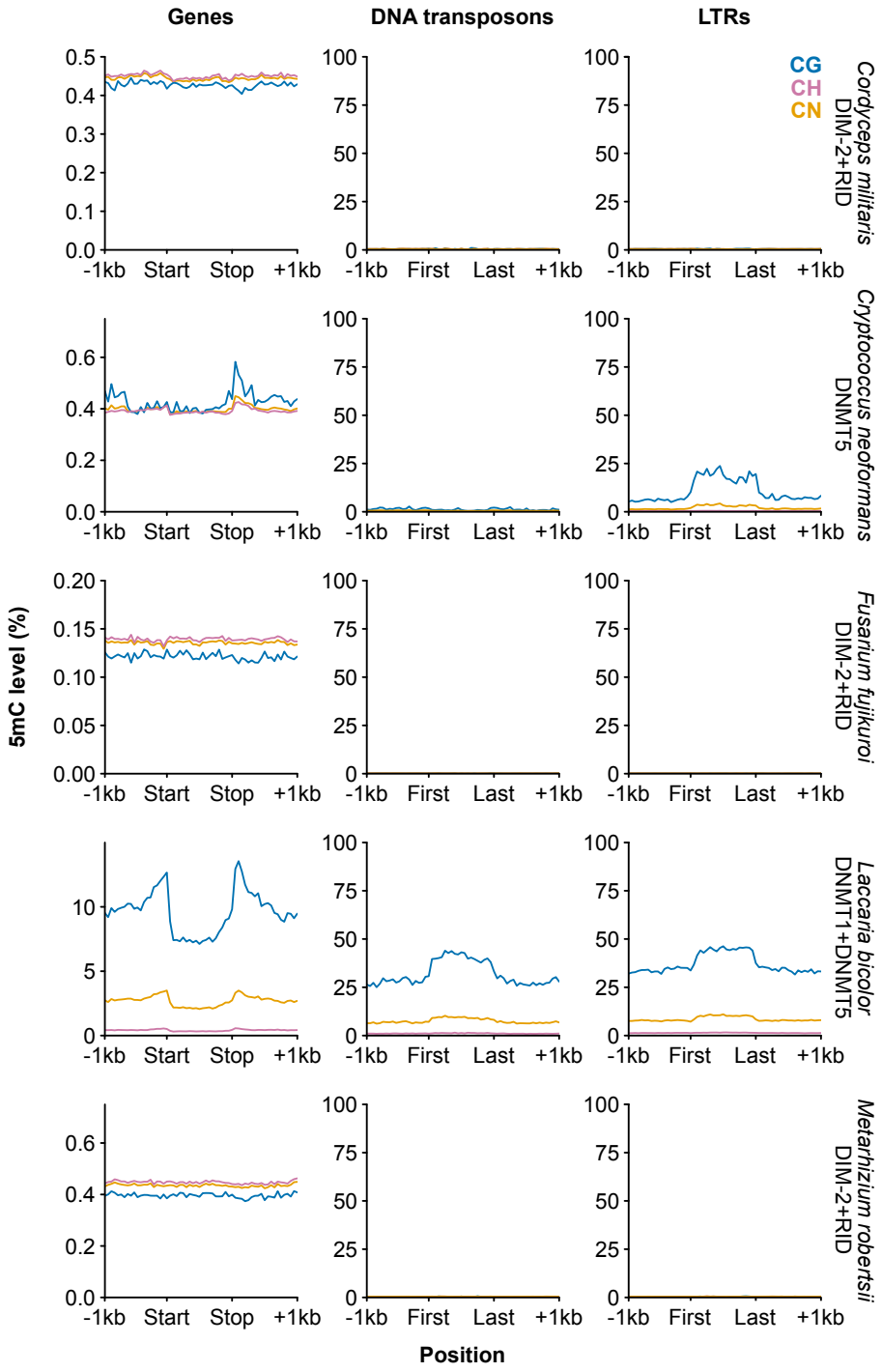
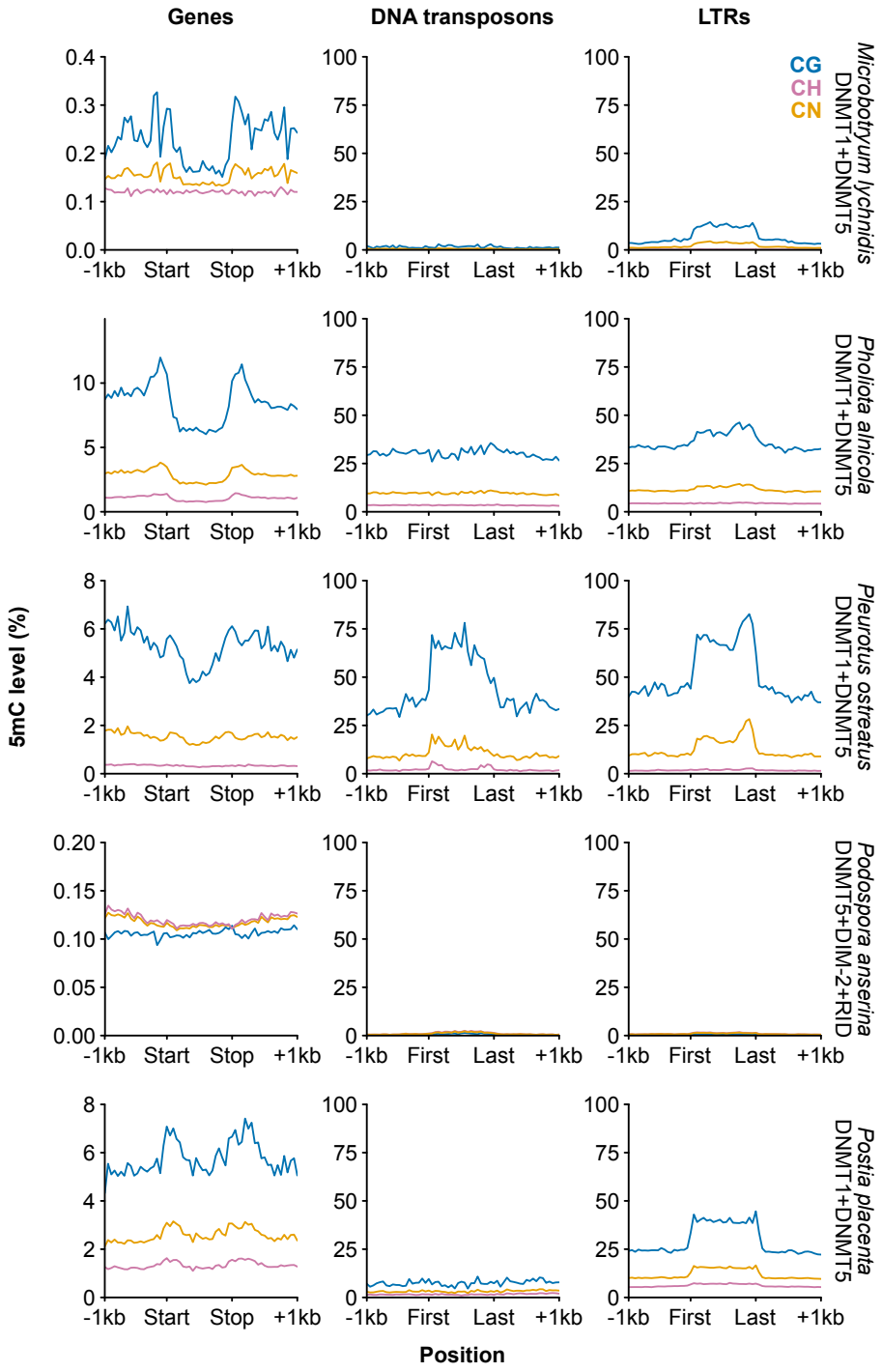
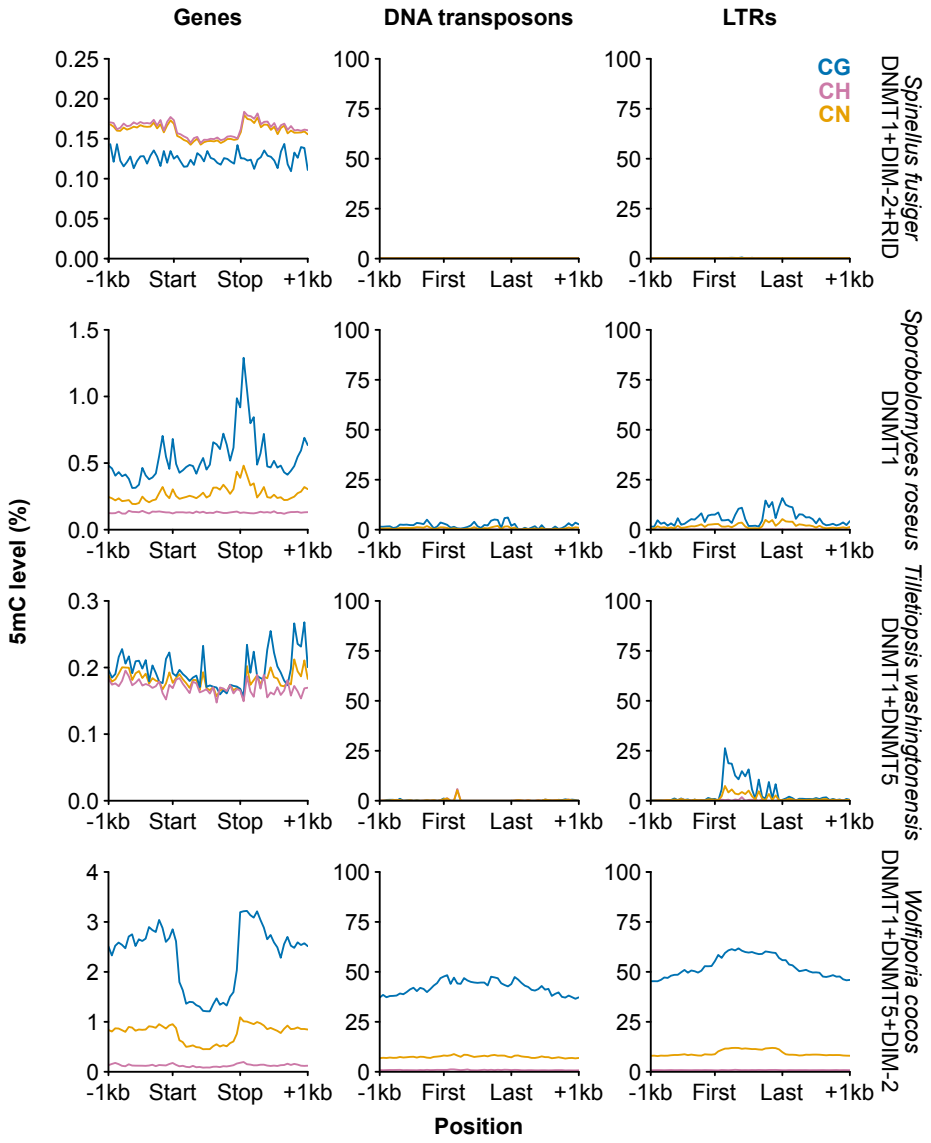
Supplementary Fig. 8



Supplementary Fig. 8



Supplementary Fig. 8



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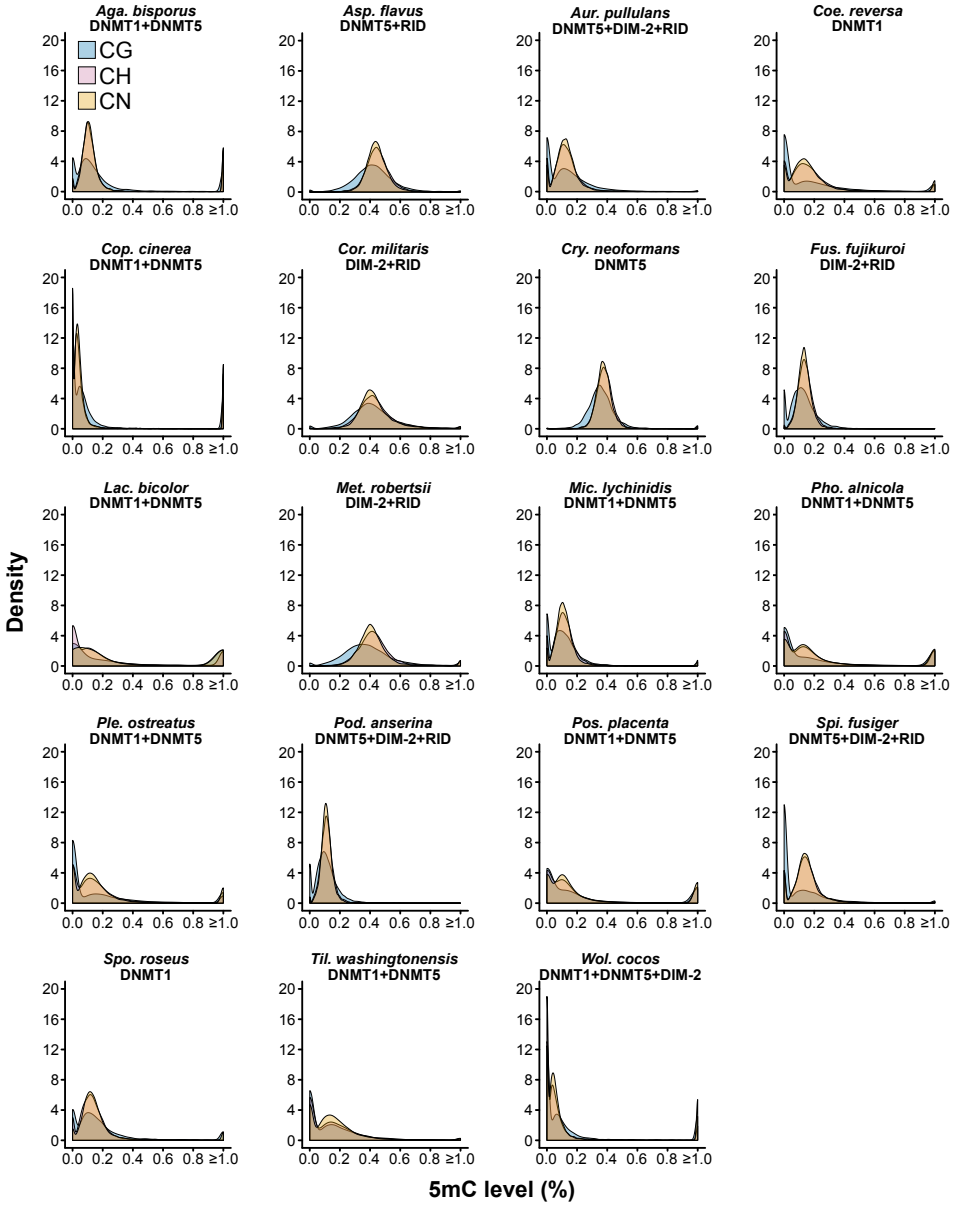
Supplementary Figure 9

Genic 5mC density.

Distribution of 5mC levels for genes for species not used in Fig. 3b.

Supplementary Fig. 9



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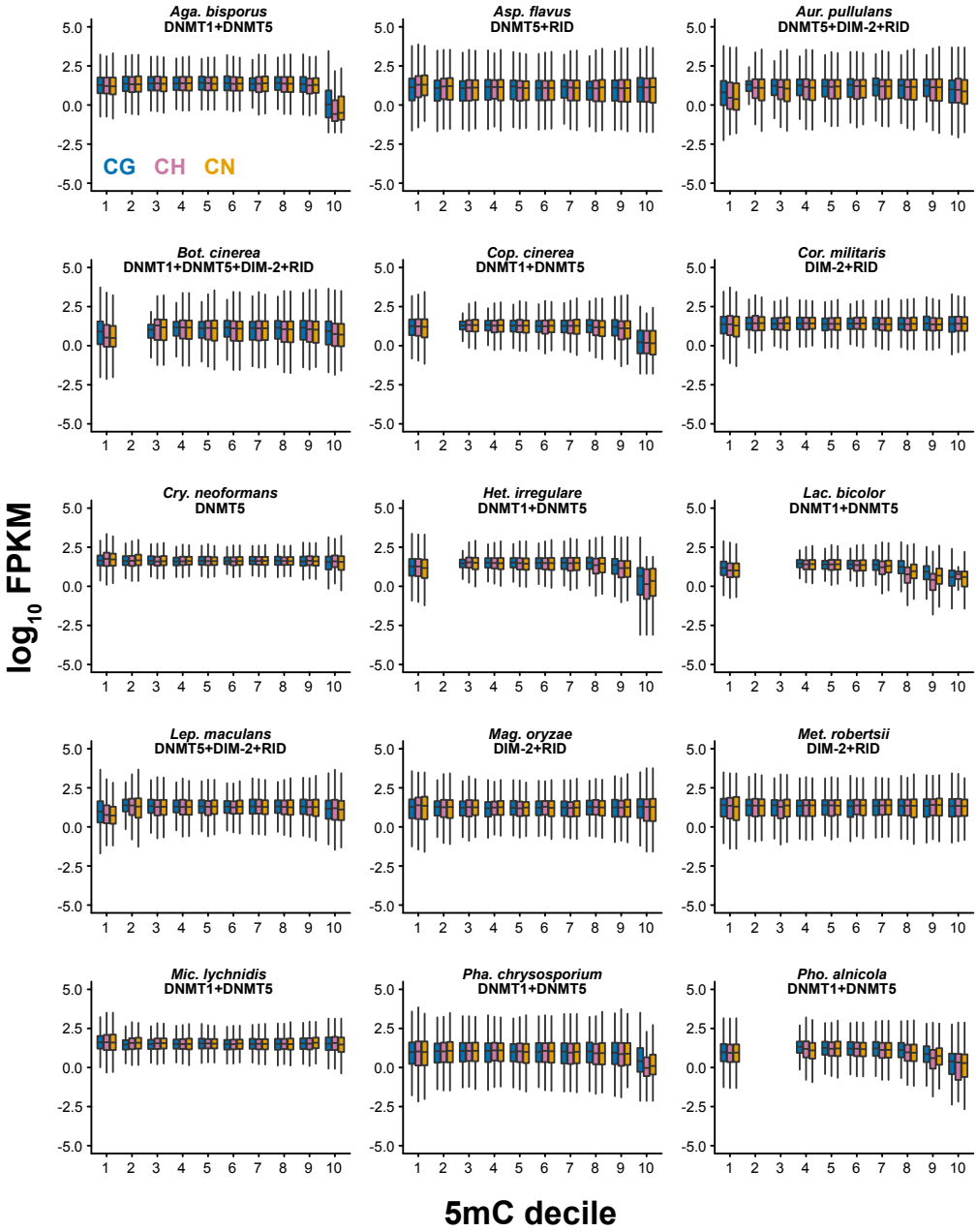
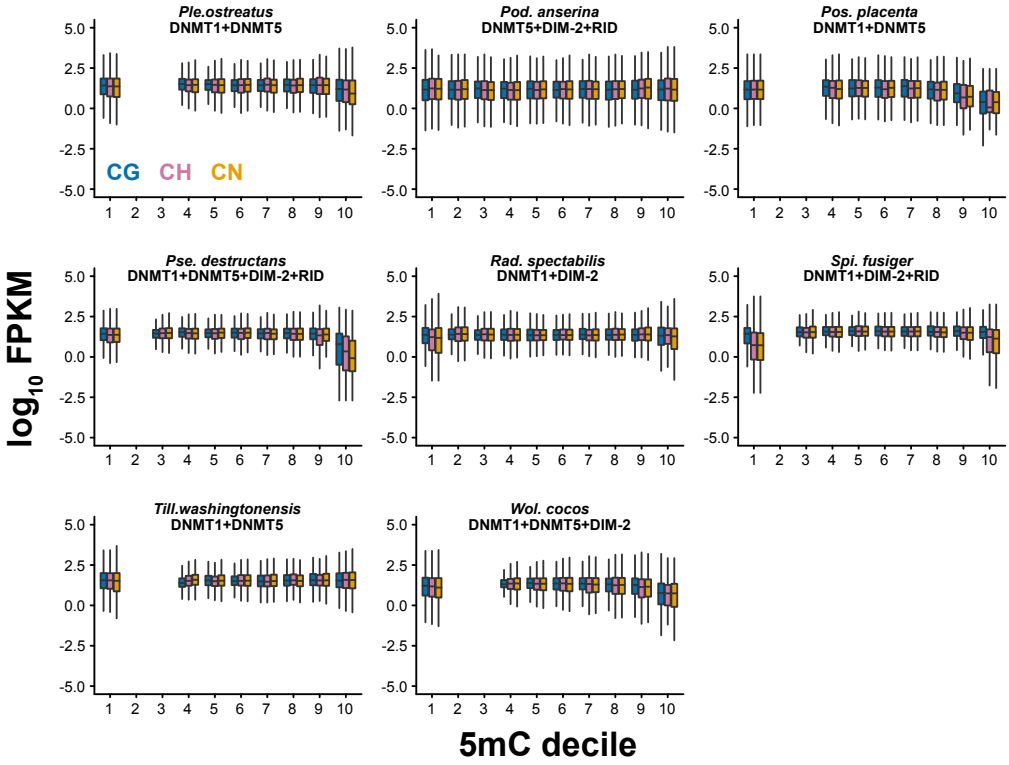
Supplementary Figure 10

Relationship between gene expression (Fragments Per Kilobase of transcript per Million mapped reads [FPKM]) and genic 5mC levels.

Relationship between gene expression measured as FPKM and 5mC levels. Empty deciles correspond to missing genic data for those 5mC levels. Boxplot elements: center line, median; upper and lower "hinges", first and third quartiles (the 25th and 75th percentiles), respectively; whiskers, 1.5× interquartile range.

Supplementary Fig. 10





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411 **Supplementary Figure 11**

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413 **DNA transposon and Long Terminal Repeat (LTR) 5mC density.**

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415 Distribution of 5mC levels for DNA transposons and LTRs for species not used in
416 Fig. 4b and c.

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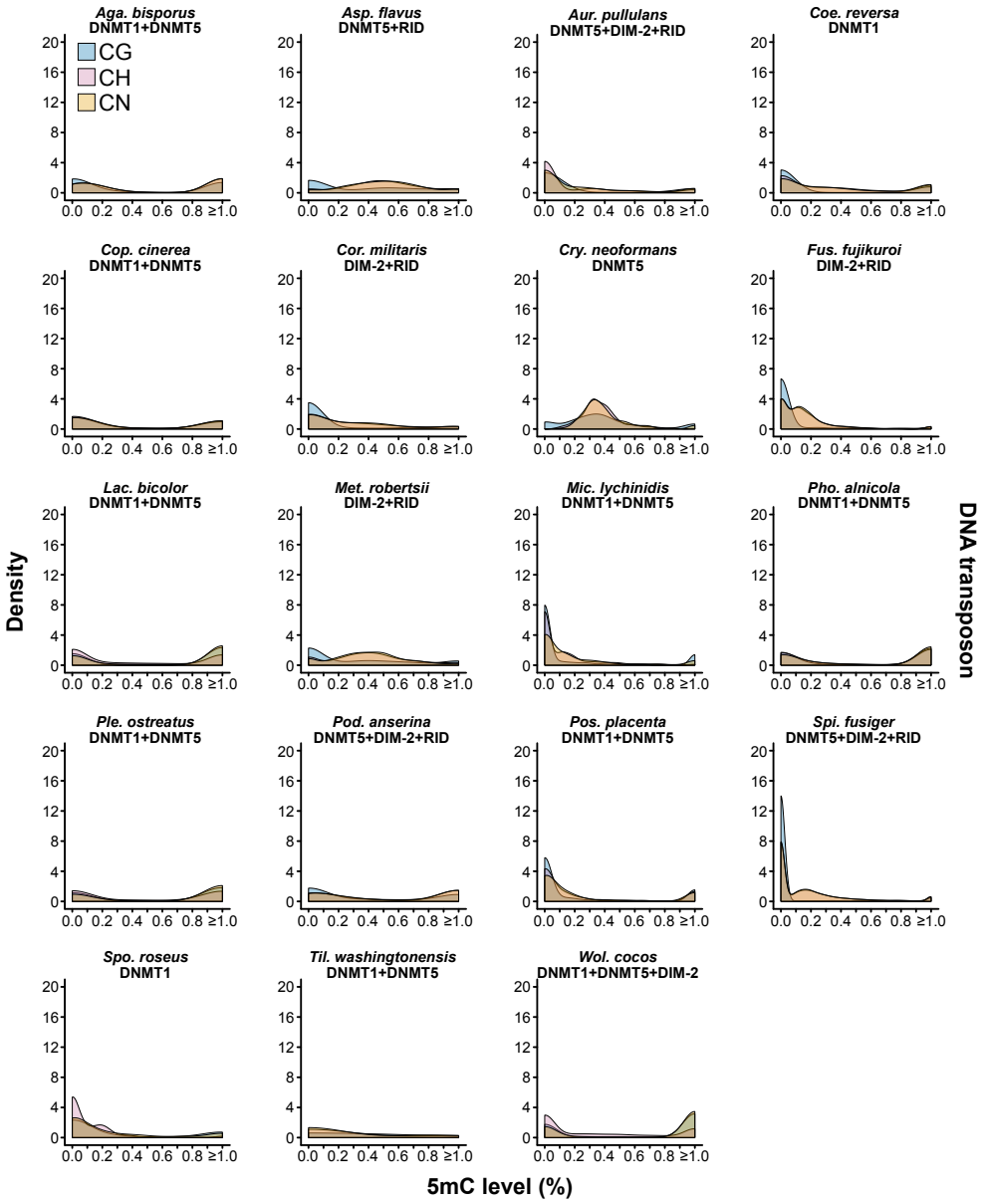
Supplementary Fig. 11

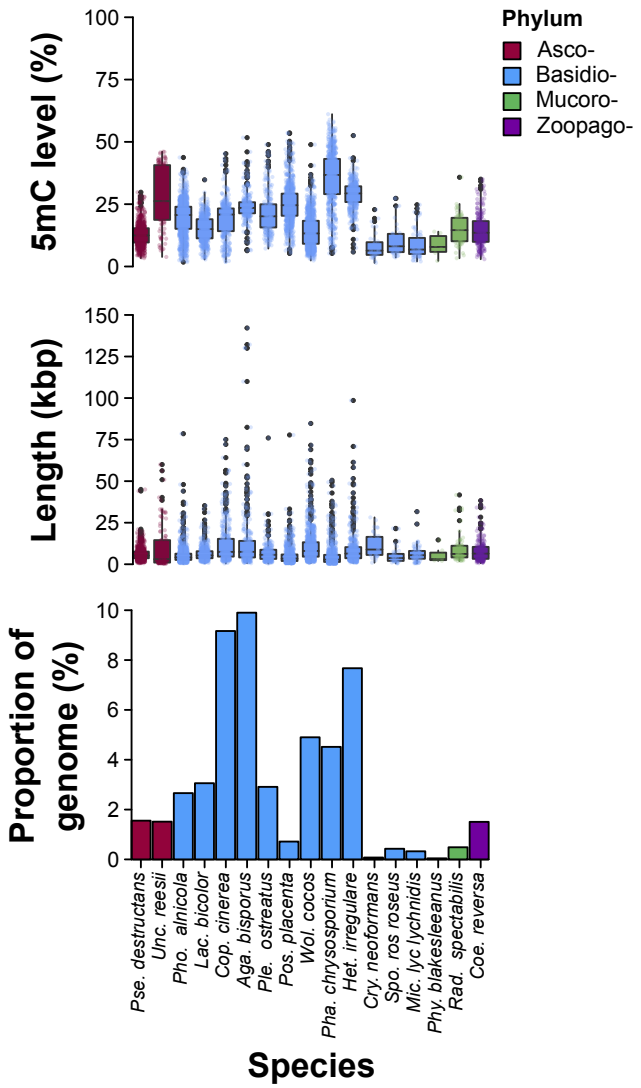


Supplementary Figure 12

Methylated Cytosine Clusters.

Methylation level across all contexts and length distributions of Methylated Cytosine Clusters (MCCs), and proportion of genome occupied by methylated clusters for 17 fungal species. Boxplot elements: center line, median; upper and lower "hinges", first and third quartiles (the 25th and 75th percentiles), respectively; whiskers, 1.5× interquartile range; large points, outliers.

Supplementary Fig. 12



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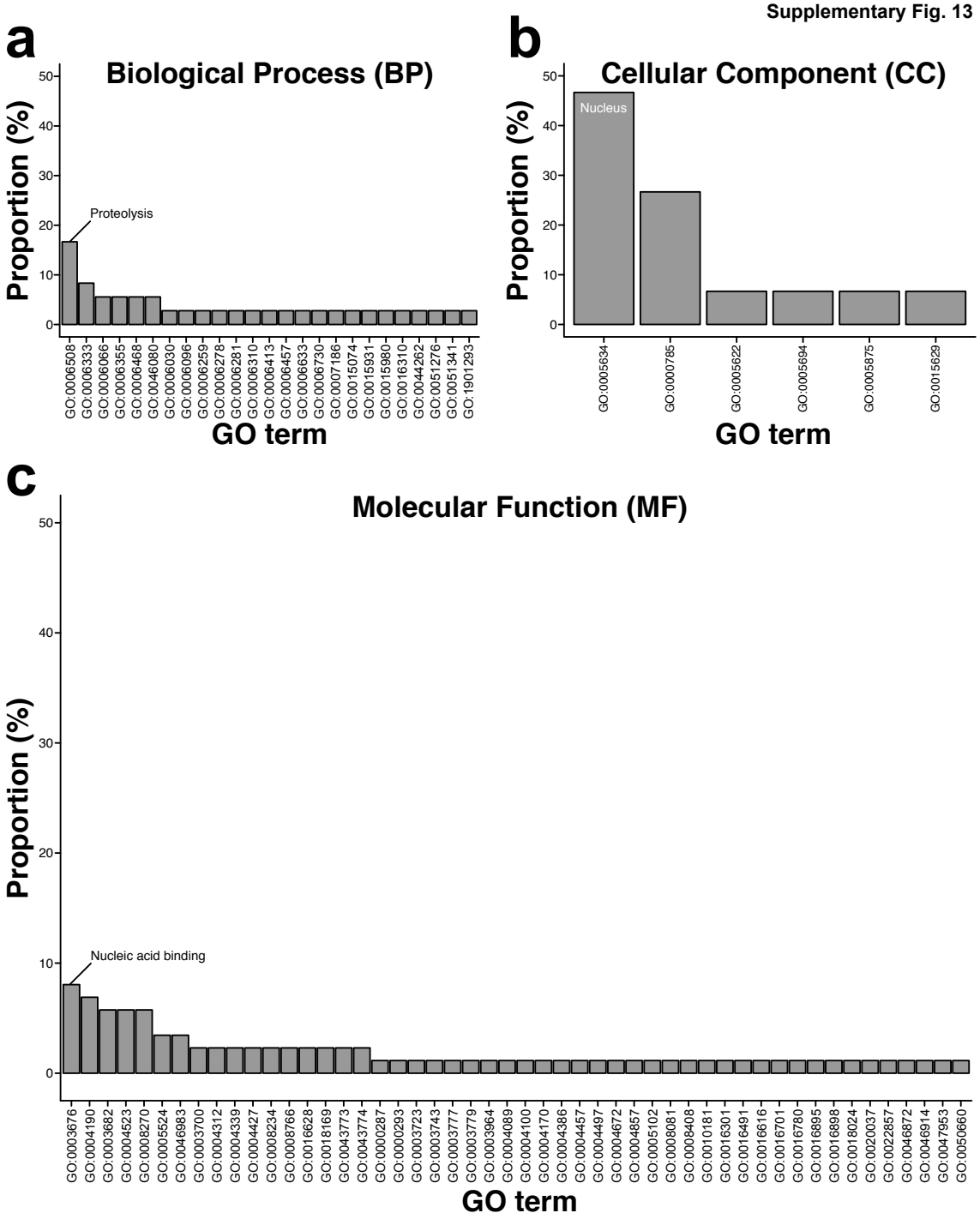
Supplementary Figure 13

Gene Ontology (GO) term enrichment of genes located in MCCs.

Proportion of statistically significant (*P* value <0.05) GO terms for genes located in MCCs.

Supplementary Fig. 13

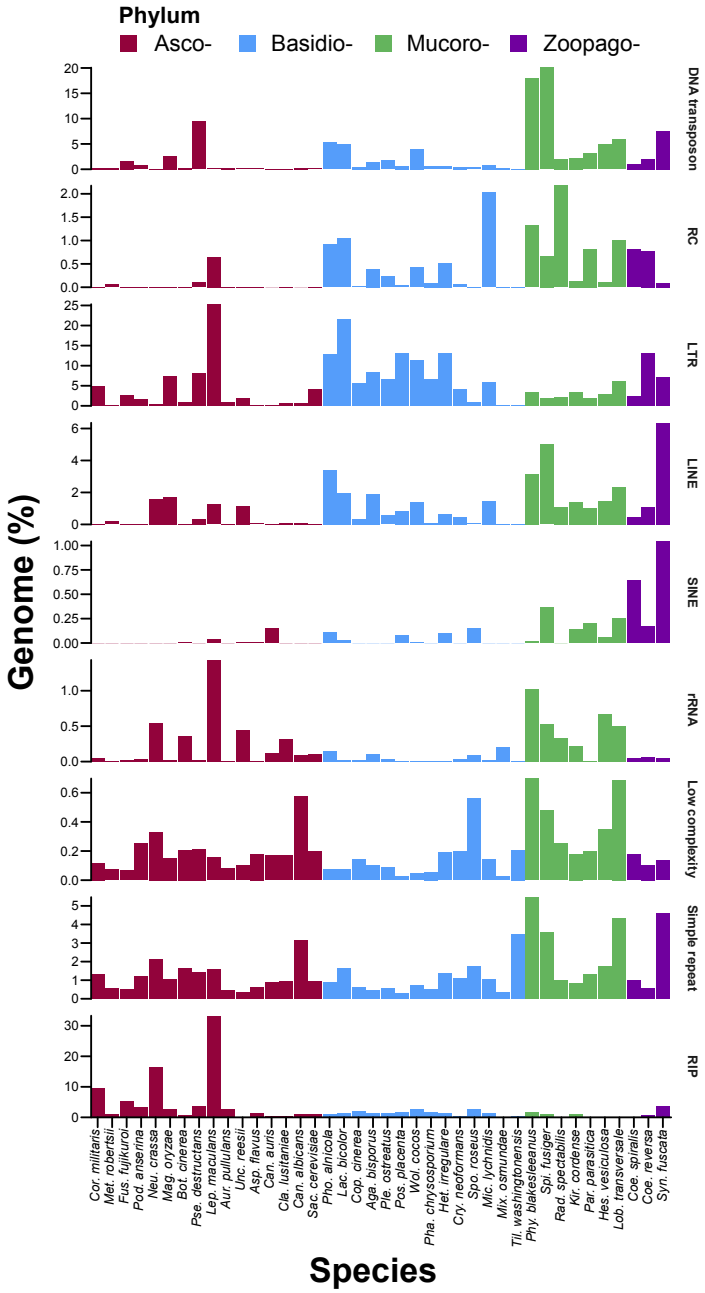


Supplementary Figure 14

Repeat and transposon content.

The genomic proportion of repeats and transposons for 40 fungal species. Repeats were detected and annotated using REPET v2.5. RIP loci were detected and annotated using the RIP product index, the RIP substrate index, and composite RIP index. See **ONLINE METHODS** for further details on repeat and transposon detection and annotation.

Supplementary Fig. 14



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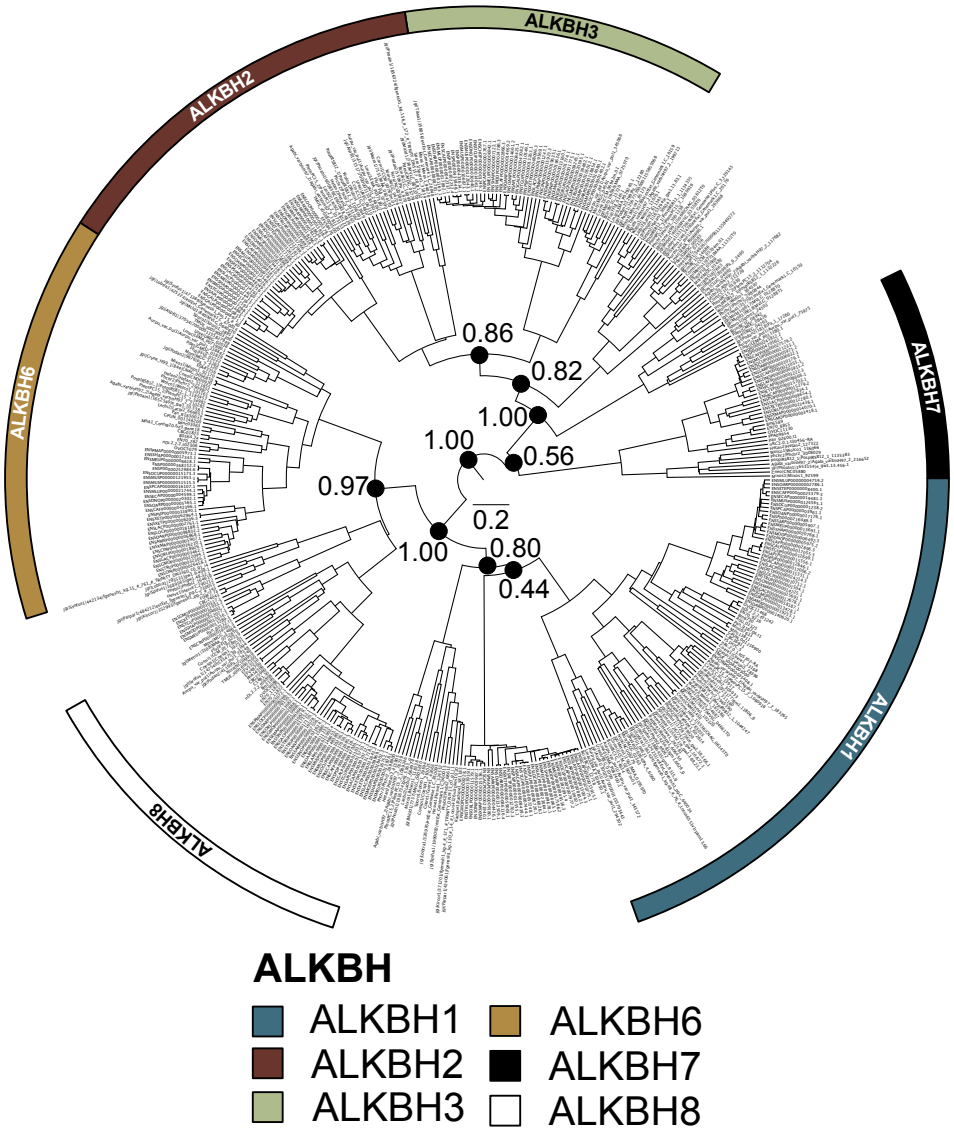
Supplementary Figure 15

ALKBH phylogeny.

Phylogenetic relationships and ALKBHs of Chordata, fungi, and Nematoda. Values at selected nodes indicate posterior probability. Branch lengths are in units of amino acid substitutions per amino acid site. Unlabeled clades represent taxa-specific clades of ALKBHs.

Supplementary Fig. 15



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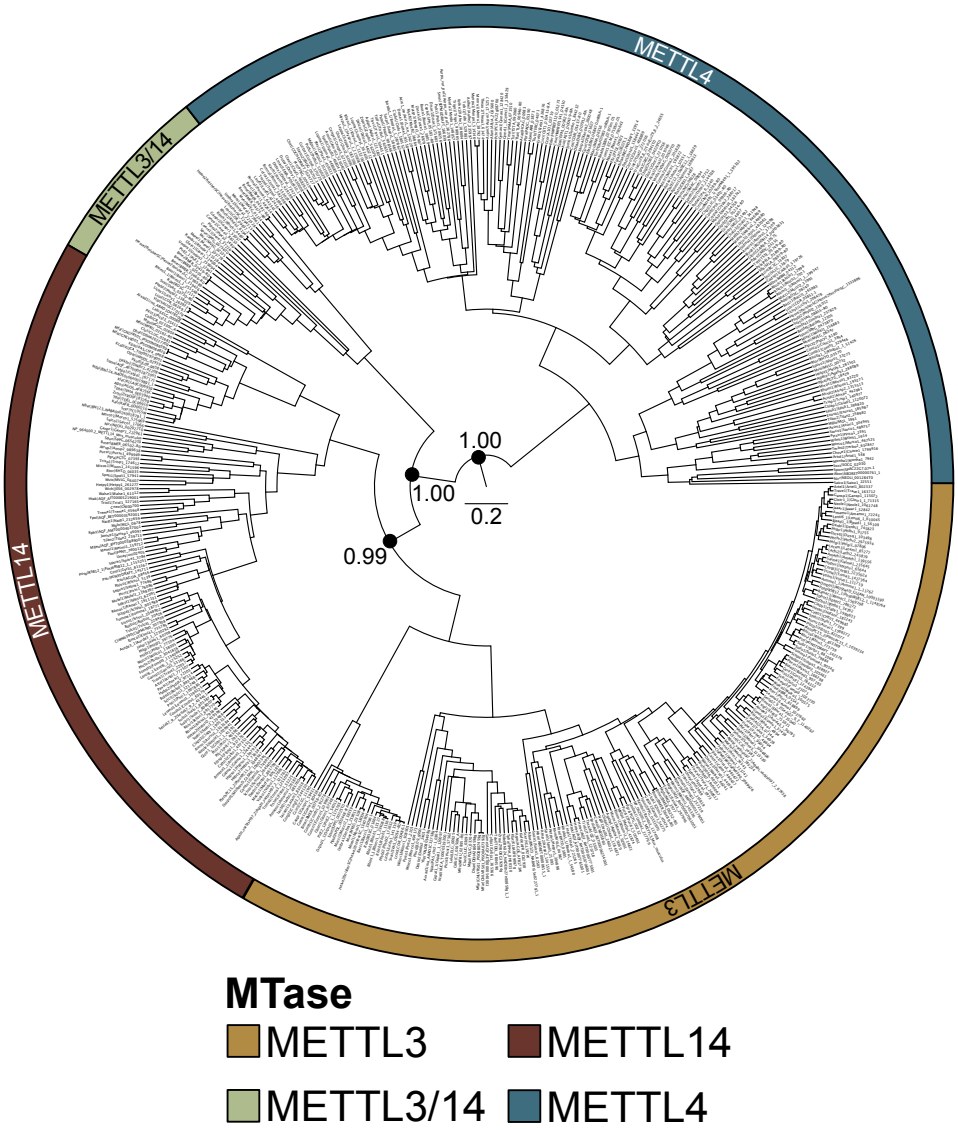
Supplementary Figure 16

METTL phylogeny.

Phylogenetic relationships and METLLs of fungi. Values at selected nodes indicate posterior probability. Branch lengths are in units of amino acid substitutions per amino acid site.

Supplementary Fig. 16



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627 **Supplementary Table 1 | 5mC DNA MTase and the tRNA methyltransferase**
628 **DNMT2 annotations for 528 fungal species investigated in this study.**
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Supplementary Table 2 | WGBS and mapping statistics for 40 fungal species investigated in this study.

Supplementary Table 3 | Number of species per phylum for each observed 5mC MTase genotype.

Supplementary Table 4 | 5mC DNA MTase and the tRNA methyltransferase DNMT2 annotations for a subset of animalia, chlorophyta, fungi, and prokaryota. Protein models correspond to those used in Supplementary Fig. 2.

749 **Supplementary Table 5 | Number of CG-, CH, and CN-enriched genes**
750 **across fungal species investigated.**
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Supplementary Table 6 | ALKBH annotations for Chordata, fungi, and Nematoda investigated in this study. Protein models correspond to those used in Supplementary Fig. 15.

Supplementary Table 7 | Results from Pagel's test for correlated evolution.

Supplementary Table 8 | Results from Phylogenetic Generalized Least Squares (PGLS).

870 **Supplementary Table 9 | Annotated proteins from fungal species**
871 **containing the N-6 DNA Methylase domain (PF02384) as identified by**
872 **Interproscan v5.23-62.0 (45).**
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Supplementary Table 10 | METTL annotations for fungal species investigated in this study. Protein models correspond to those used in Supplementary Fig. 16.

930 **Supplementary Table 11 | Annotated proteins for fungal species containing**
931 **the domain the methyltransferase small domain (i.e., N6AMT1 proteins) as**
932 **identified by Interproscan v5.23-62.0 (45).**