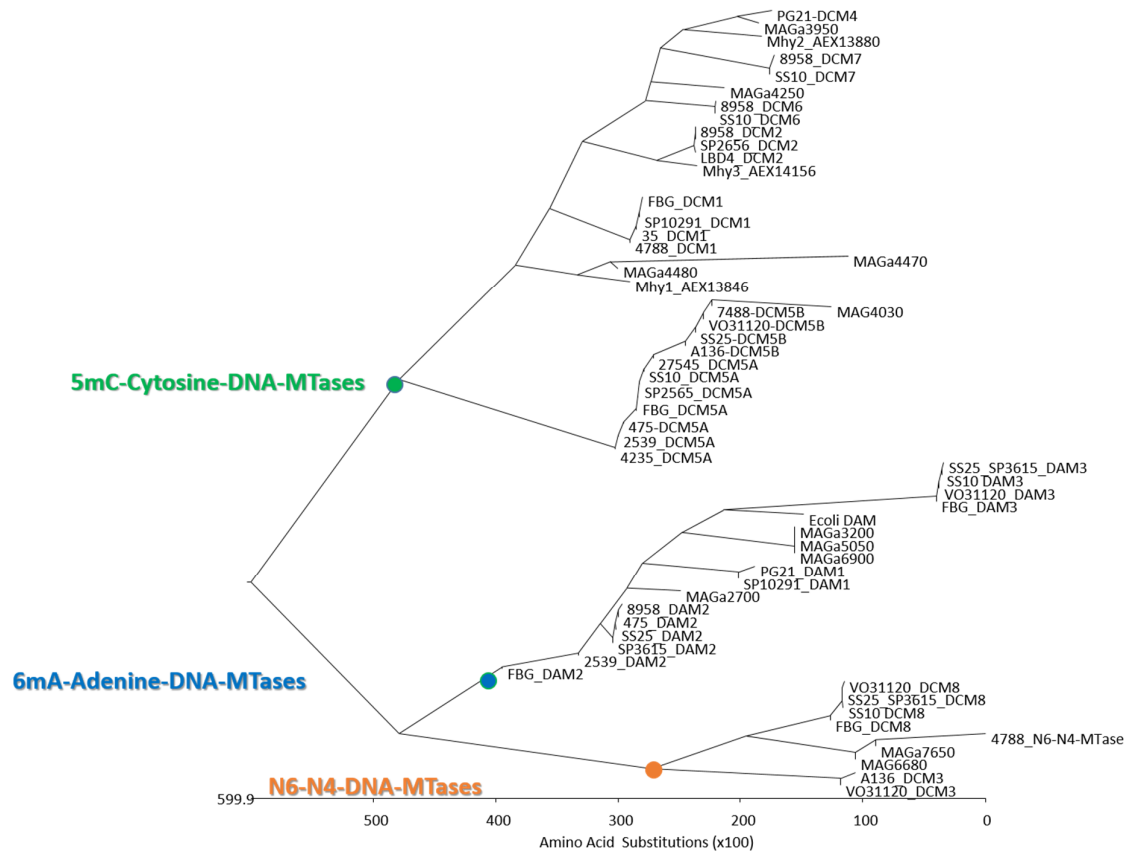
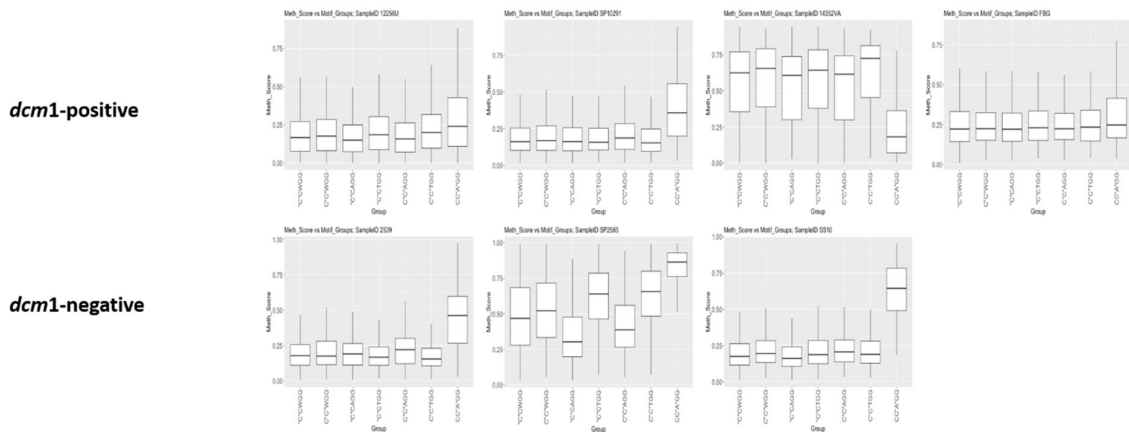


**Figure S1.** Gene composition in gene loci of type II DNA-MTases

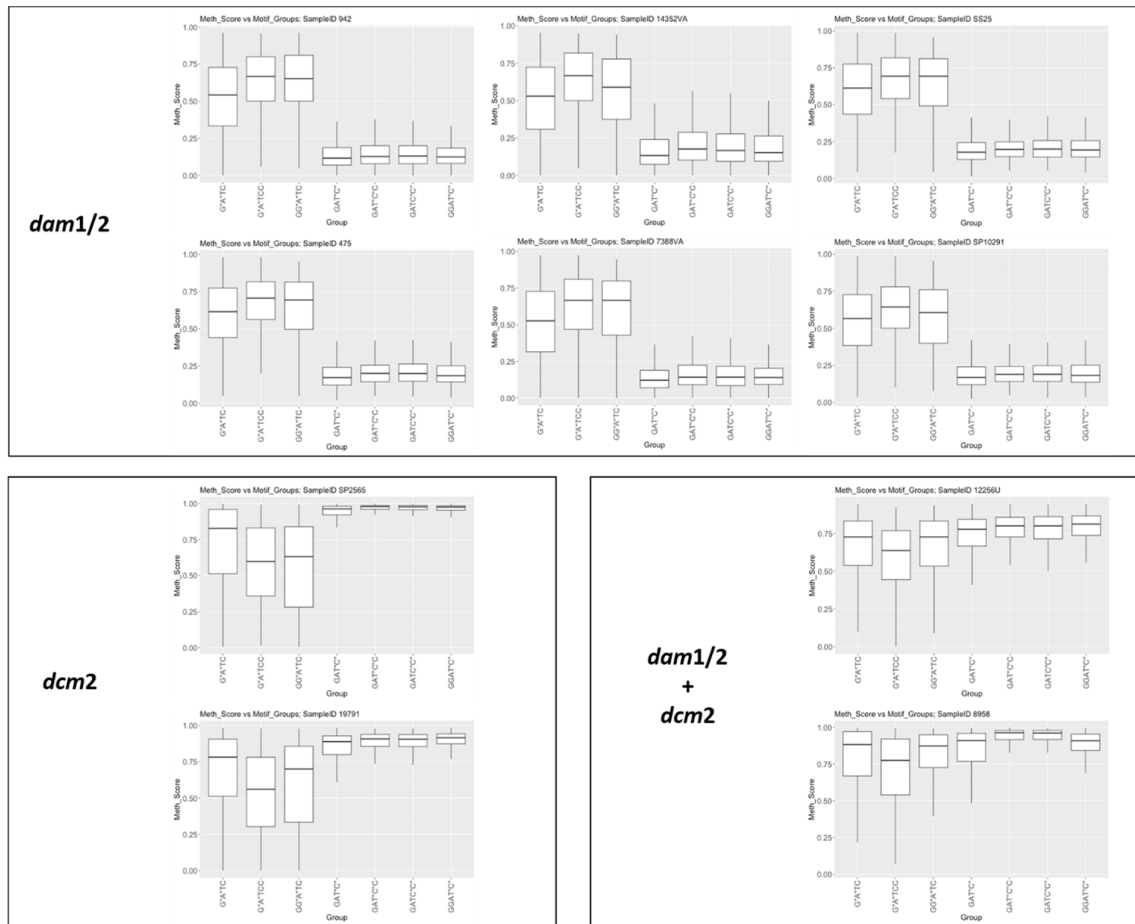
Genes of the different MTase gene loci (*dam1*, *dam2*, *dcm8/dam3* and *dcm1 – dcm6/7*) of the deep sequenced *M. hominis* genomes [1] were presented using Genious. Chromosomal core genes according to type strain PG21 are shaded in blue, ICEHo-I core genes in green, MTase gene regions framed in red and REase gene regions in yellow.



**Figure S2.** Phylogenetic tree of type II DNA MTases of *M. hominis*, *M. agalactiae* and *M. hyorhinis*. In addition to the postulated *M. hominis* MTases already shown in Figure 1, BlastP-derived proteins sequences of other strains of *M. hominis*, *M. agalactiae* [2] and *M. hyorhinis* [3] were aligned by ClustalW for phylogenetic tree construction.



**Figure S3.** Tombo-calculated methylation scores of CCWGG motif variants. Methylation scores of the motifs were calculated for selected *M. hominis* isolates carrying the *dcm1*-gene (*dcm1*-positive) or without *dcm1*-gene (*dcm1*-negative). Position of the calculated 5mC or 6mA nucleotide is marked by “C” or “A”.

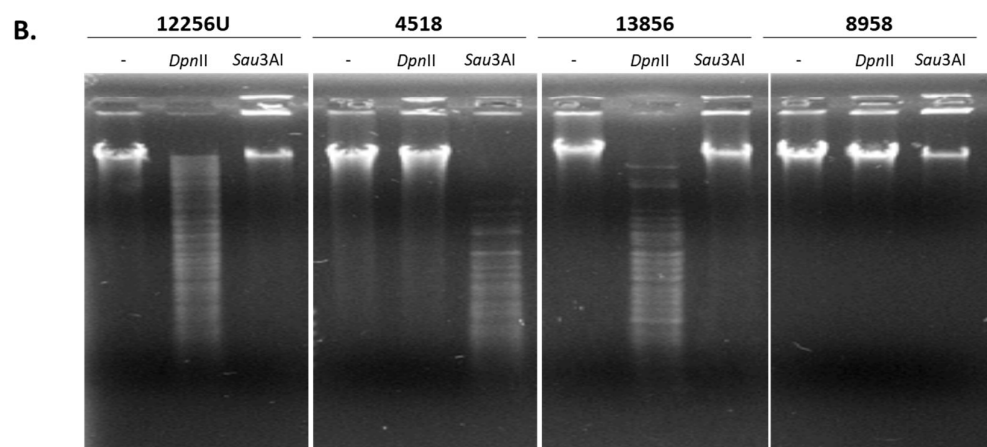


**Figure S4.** Tombo-calculated methylation scores of GATC(C) motif variants  
Methylation scores of the motifs were calculated for selected *M. hominis* isolates carrying *dam1* or *dam2* (**dam1/2**) and *dcm2* genes without *dam1/2* (**dcm2**) or with *dam1/2* (**dam1/2 + dcm2**). Position of the calculated 5mC or 6mA nucleotide is marked by “C” or “A”.

**A.**

| MTase | MTase-Motif        | Restriction enzyme <sup>a</sup>                   |   |   | Number of MTase gene copies / isolate's genome <sup>b</sup> |      |       |      |
|-------|--------------------|---|---|---|---|------|-------|------|
|       |                    | Cut1: <sup>o</sup> X                              | Cut2: <sup>m</sup> X                            | Cut3: <sup>*</sup> X                              | 12256U  | 4518 | 13856 | 8958 |
| DCM2  | GAT <sup>m</sup> C | <i>DpnII</i><br>G <sup>*</sup> AT <sup>o</sup> C  | -   | <i>Sau3AI</i><br>G <sup>o</sup> AT <sup>*</sup> C | 1   | 0    | 1     | 1    |
| DAM1  | G <sup>m</sup> ATC | <i>Sau3AI</i><br>G <sup>o</sup> AT <sup>*</sup> C | <i>DpnI</i><br>G <sup>m</sup> AT <sup>*</sup> C | <i>MboI</i><br>G <sup>*</sup> AT <sup>o</sup> C   | 1   | 1    | 0     | 0    |
| DAM2  |                    |   |   |   | F   | 0    | 1     | 1    |

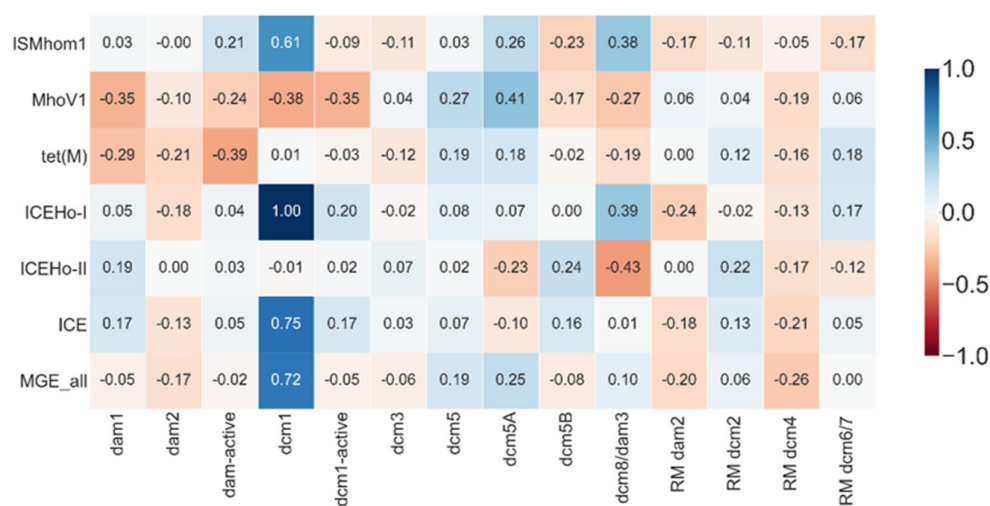
<sup>a</sup> Restriction: independent on methylation of AA X (<sup>o</sup>X), requires methylation of AA X (<sup>m</sup>X) or is inhibited by methylation of AA X (<sup>\*</sup>X); <sup>b</sup> F = fragmented gene, T = truncated gene



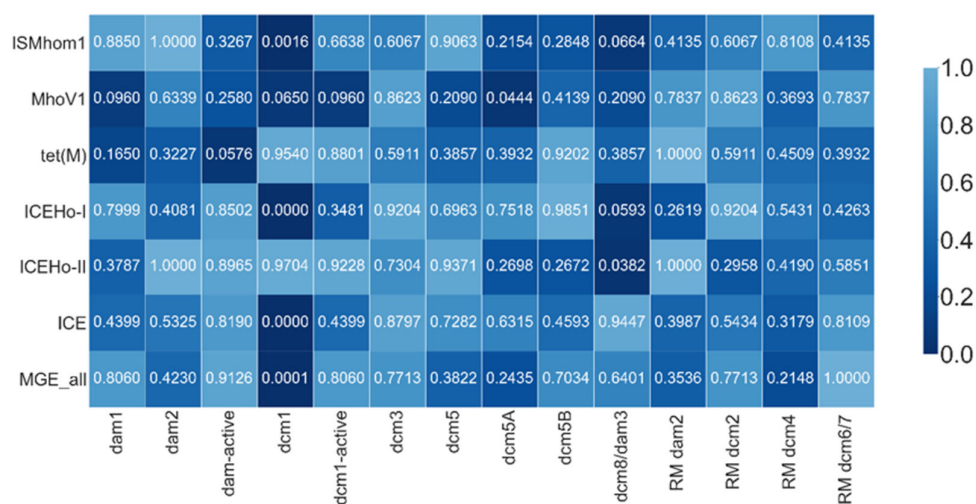
**Figure S5.** Methylation sensitive restriction for proof of DCM2 GAT<sup>m</sup>C-activity

*M. hominis* strains 12256U, 4518, 13856 and 8958 were subjected to methylation sensitive restriction. (A) Table of DCM2, DAM1 and DAM2 gene presence and methylation specificities of restriction enzymes. (B) 1% (w/v) agarose gel of the genomic DNA: unrestricted DNA (-); restricted with *DpnII*, which cuts independent on 5mC methylation, *Sau3AI*, which is inhibited by 5mC methylation of GATC. 4518 serves as a control, as its DNA is digested by *Sau3AI* due to absence of DCM2. DNA of strain 8958 is not cut by *DpnII* due to his DAM2 activity; whereas DNA of strains 12256U and 13856 is cut by *DpnII* indicating absence of 6mA methylation in the GATC motif and respectively, absence of DAM1 or DAM2 methylation activity.

A.



B.



**Figure S6.** Correlation matrices of MTases and MGEs

Presence of MTases was correlated with number of MGEs in 23 *M. hominis* isolates and type strain PG21. Pearson's correlation coefficient (A) and p-values (B) are shown for each analysed correlation. Raw data are shown in Supplementary Table S6.

1. Henrich, B.; Hammerlage, S.; Scharf, S.; Haberhausen, D.; Fürnkranz, U.; Köhrer, K.; Peitzmann, L.; Fiori, P.L.; Spergser, J.; Pfeffer, K., *et al.* Characterisation of mobile genetic elements in mycoplasma hominis with the description of iceho-ii, a variant mycoplasma integrative and conjugative element. *Mobile DNA* **2020**, *11*, 30.
2. Dordet-Frisoni, E.; Vandecasteele, C.; Contarin, R.; Sagné, E.; Baranowski, E.; Klopp, C.; Nouvel, L.-X.; Citti, C. Impacts of mycoplasma agalactiae restriction-modification systems on pan-epigenome dynamics and genome plasticity. *Microbial Genomics* **2022**, *8*.
3. Chernov, A.V.; Reyes, L.; Xu, Z.; Gonzalez, B.; Golovko, G.; Peterson, S.; Perucho, M.; Fofanov, Y.; Strongin, A.Y. Mycoplasma cg- and gatac-specific DNA methyltransferases selectively and efficiently methylate the host genome and alter the epigenetic landscape in human cells. *Epigenetics* **2015**, *10*, 303-318.