**Material and methods**

**Processing of whole-genome bisulfite sequencing data**

The Omics IT and Data Management Core Facility (DKFZ, Heidelberg) processed WGBS data as described earlier 1. Reads were aligned using an updated pipeline published by Wang et al. 2, implemented as a Roddy Workflow (https://github.com/DKFZ-ODCF/AlignmentAndQCWorkflows) in the automated One Touch Pipeline 3. In short, adaptor sequences of raw reads were trimmed using Trimmomatic 4. Next, sequencing reads were in silico bisulfite-converted 5 (C>T for the first read in the pair, G>A for the second). BWA-MEM 5 was used with default parameters to align the converted reads to the in silico bisulfite-converted reference genome hg19, extended with the PhiX and lambda phage sequences. Post alignment, reads were converted back to their original state. Reads with a mapping quality ≥25 and nucleotides with a Phred-scaled quality score ≥25 were considered for PCR duplicate removal per sequencing library using the software Picard 6. Methylation calling and M-bias QC was performed using *MethylDackel* 7 v0.4.0 and the parameters *--OT 6,146,2,144 --OB 7,146,12,150*, according to the M-bias quality control plots. Methylation calls were imported into R 8 v3.6 using the R package methrix 9 v 1.0.05, summarized based on annotated reference indices, and collapsed based on strand information. Furthermore, single nucleotide polymorphisms (SNP) with a minor allele frequency >0.1 were filtered, using the reference provided in the R package BSgenome.Hsapiens.UCSC.hg19 10 v 1.4.0, and initial quality control was performed. For downstream analysis, DNA methylation data were imported into the R package bsseq 11 v1.20.0 and technical replicates were collapsed. To define average CpG island (CpGi) methylation, CpGi annotation was extracted using the R package RnBeads 12 v 2.4.0. For principal component analysis (PCA), methylation levels of the 200,000 most variable methylated CpGs were applied to the R base function *prcomp.*

**Differential methylation analysis**

To define differentially methylated loci, a linear model was applied, considering the epigenotype (HM and non-HM) and sorting quadrant of the respective sample as a covariate. Therefore the function *DMLfit.multiFactorR* with the parameter, *smoothing = TRUE*, from the R package DSS 13 v2.32.0 was applied. The epigenotype HM coefficient was extracted using the function *DMLtest.multiFactor* and differentially methylated regions (DMRs) were defined using the function *callDMR*, based on regions with >3 CpGs, a length of >50 bps, and a Benjamin-Hochberg corrected *P* value <0.05 in at least 50% of all CpGs within that region. Additionally, DMRs were subsetted for an average coverage of ≥5 in at least half of the samples belonging to the HM and non-HM epigenotype respectively and a difference in methylation >0.2. DMRs were annotated using the R package ChIPseeker 14 v1.31.3.900 and TxDb.Hsapiens.UCSC.hg19.knownGene 15 v3.2.2. Hierarchical cluster analysis of DMRs was performed using the R package pheatmap 16 v1.0.12, applying *ward.D2* as the clustering method to *Manhattan* distances.

**Transcription factor motif enrichment**

DNA transcription factor (TF) motif enrichment was performed using the command line tool Homer 17 v4.9.1 and the parameter *-size given*. DMRs were stratified in hypo- and hypermethylated regions, and a set of random regions with equal size, similar CpG frequency (15% tolerance in the deviation of CpG composition were allowed), and a maximum of 20% N’s in the sequence composition, were used as a background. The top 10 most significantly enriched TF motifs in hypo- and hypermethylated DMRs were visualized.

**Locus overlap and enrichment analysis of ChromHMM states**

The R package LOLA 18 v 1.16.0 was used to enrich DMRs with ChromHMM states acquired from Encode. DMRs were stratified in hypo- and hypermethylated regions and enriched to a background containing a set of random regions with equal size, similar CpG frequency (15% tolerance in the deviation of CpG composition was allowed), and a maximum of 20% N’s in the sequence composition.

**Locus plots**

The R package gviz 19 v1.30.3 was used to generate locus plots. For the visualization of WGBS data, methylation calls were smoothed with the R package bsseq 11 v1.20.0, applying the function *BSmooth* with default parameters. 450k array description

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