Whole-Genome Bisulfite Sequencing (WGBS) raw data (reads) were submitted to Bismark(version 0.22.3)1 for mapping and methylation calling, against mouse genome version mm10/GRCm38, discarding duplicate reads. Briefly, the methylation level at a CpG site was the number of reads with that site methylated divided by the total number of reads covering the site.

To ensure comparability of region DNA methylation levels across all samples, only CpGs covered by ≥ 5x in all samples were retained for the computation of DNA methylation levels.

We defined the average methylation for a genomic region as the coverage-weighted mean of the methylation levels of the individual CpGs within the region. Subsequently, we averaged a region’s DNA methylation level over condition samples.

Differentially methylated regions (DMRs) were identified by using an inhouse custom approach (unpublished), built around a two approach methodology, that combines a dynamic fragment strategy to define a methylated region (setting maximal distance between 2 adjacent common cytosines to 100bp and maximal fragment size to 1000bp with minimal CpG number of 10 in one fragment) and a two region weighted t-test on the difference of the average methylation values of each methylated region (p<0.01 & ΔmR > 0.1 - thresholds to select DMRs).

We derived intergenic regions, exons, introns and CGI (CpG Islands) from the RefSeq mouse gene model downloaded from the UCSC browser and we defined promoters as the regions 2000 bp upstream and 500 bp downstream of the same Refseq gene model.

We used GREAT (Genomic Region Enrichment of Annotation Tool)2 to associate DMRs with genes. We limited the associations to genes within 20kb of DMRs and used an FDR threshold of 10−6 and a fold enrichment threshold of 2.

References:

1. Krueger F, Andrews SR. **Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications**.*Bioinformatics*. 2011 Jun 1;27(11):1571-2
2. McLean, C., Bristor, D., Hiller, M. et al. **GREAT improves functional interpretation of cis-regulatory regions.** *Nat Biotechnol* 28, 495–501 (2010). https://doi.org/10.1038/nbt.1630