Library preparation for target-captured bisulfite sequencing

3μg of DNA calculated by Qubit was fragmented by sonication. Subsequently, library preparation was performed with SureSelect^{XT} **Mouse(Rat/Huamn)** Methyl-Seq Reagent Kit (Agilent Technologies). The Methyl-Seq Kit could enrich **109(97/84)** Mb of mouse genomic regions including CpG islands, Gencode promoters, DNase I hypersensitive sites and tissue-specific DMRs. DNA was bisulfite-treated using EZ DNA MethylationGold Kit. Sequencing libraries were assessed by Bioanalyzer and quantified with KAPA Library Quantification Kits. The libraries were then sequenced on HiSeq 2500 (2× 100 bp or 2× 101 bp paired-end reads, Illumina)

DNA methylation data analyses.

General quality control checks were performed with FastQC v0.8.0 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Each dataset was filtered for average base quality score (>20).Filtered datasets were aligned to a reference genome using Bismark v0.7.8 (parameters-X 10000 --non_bs_mm -n 2 -l 50 -e 70 --chunkmbs 1024)41, using Bowtie v0.12.842 as the underlying alignment tool. The reference genome index contained the genome sequence of enterobacteria phage λ (NC_001416.1) in addition to all chromosomes of the mm9 assembly (NCBI 37). Mappings for all datasets generated from the same library were merged, and duplicates removed via the Bismark deduplication tool (deduplicate_bismark_alignment_output.pl). Mapped reads were then separated by genome (mm9 or phage λ) and by source strand (plus or minus). The first four and last one base of each read2 in all read pairs was clipped due to positional methylation bias, and any redundant mapped bases due to overlapping mates from the same read pair were trimmed to avoid bias in quantification of methylation status. Finally, the NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-018-08067-z ARTICLE NATURE COMMUNICATIONS | (2019) 10:305 | https://doi.org/10.1038/s41467-018-08067-z | www.nature.com/naturecommunications 11SAM alignments for multiple libraries from the same animal were merged. Read pairs mapped to phage λ were used as a QC assessment to confirm that the observed bisulfite conversion rate was >99%. Read pairs mapped to the mm9 reference genome were used for downstream analysis

DMC & DMR detection

Using the DSS R package v2.15.0, DMCs were identified by DSS with the call DML function (default parameters), and DMRs were identified with the call DMR function (all other parameters default). DMC and DMR calls were also made via Metilene v.0.2-6 (all other parameters default), with a p value threshold of **0.05 and mean methylation difference of 0.2** for DMCs, with a p value threshold of **0.05 and mean methylation difference of 0.1** for DMRs. All DMR calls from both tools were subject to additional filters, as described below. DMRs were required to contain at least 3 validated CpG sites and have a minimum length for 50bp .