

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- | | | |
|-------------------------------------|-------------------------------------|--|
| ☐ | ☒ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☒ | ☐ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ | Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection no software was used

Data analysis

CRISPR screen: fastx clipper (http://hannonlab.cshl.edu/fastx_toolkit/), bowtie2 (v2.3.3), MAGeCK (<http://liulab.dfci.harvard.edu/Mageck>)
 RNA-seq: FastQC, STAR, Rsubread v2.6.3, edgeR v3.34.1 and limma v3.48.3, msigdb (v7.5.1), clusterprofiler (v4.0.5), and ggplot2 (v3.3.5) R packages. Nextgenmap (v0.5.2), SLAM-DUNK (v0.2.4), REDtools, BEDTools (v2.27.1), and IGV (v2.11.9).
 Reduced Representation Bisulfite Sequencing: Bowtie2 (v2.3.3) within Bismark (v0.22.3), edgeR v3.34.1, SeqMonk (<https://www.bioinformatics.babraham.ac.uk/projects/seqmonk>, version 1.48.0).
 ATAC-seq: FastQC, TrimGalore (v0.4.4), Bowtie2 (v2.3.4.1), Picard suite (v2.23.8), Genrich (<https://github.com/jsh58/Genrich>) (v0.6.0), edgeR (v3.34.1), limma61 (v3.48.3), samtools, IGV (v2.11.9), and Spark.
 ChIP-seq: FastQC (v0.11.5), Cutadapt, BWA-MEM (arXiv:1303.3997v2), deeptools (v3.5.0), MACS2, BEDTools (v2.27), IGV (v2.11.9), and Spark.
 Single molecule tracking: Fiji/ImageJ (v2.1.0/1.53c)
 Whole proteome mass spectrometry: FragPipe
 LC-MS/MS: Maxquant proteomics software package (version 2.0.1.0) and xiSPECV2 tool
 Structural modeling: AlphaFold prediction (AF-P26358-F1-model_v4.pdb), COOT, and PyMOL (<http://www.pymol.org/pymol>).
 Long-read seq: dorado v1.1.1 (1.1.1+e72f1492; dna_r10.4_e8.2_400bps_sup@v5.2.0), samtools (v1.22), bamCoverage (deepTools v3.5), pyGenomeTracks (v3.9)
 Methyl-seq: nf-core (<https://nf-co.re/methylseq/4.1.0>) via Nextflow v24.10.2 and apptainer v1.1.9. Downstream analysis and plotting utilised R v4.4.2 with additional packages GenomicRanges v1.58.0 and ggplot2 v3.5.2 (<https://cran.r-project.org/web/packages/ggplot2/citation.html>).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All high-throughput sequencing data supporting the findings of this study have been deposited in the NCBI Gene Expression Omnibus under primary accession code GSE233730. Proteomics data available on PRIDE, Project accession: PXD053151, Token: oLXZGgMC8SHH

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

An n of at least 3 was used for all experiments, with the exceptions: (i) DNMT1 ChIP-seq (n=1), SUMOylated lysine analysis (n=2), and whole-genome CRISPR screen (n=1). An n of 1 for CRISPR screens are accepted as standard practice as they are resource intensive and have several internal controls in place to confirm the validity of the results. DNMT1 ChIP-seq and SUMOylated lysine methods had an n of 1 and n of 2 respectively as they were technically challenging and resource intensive. (ii) GFP-ChIP-seqs, n=2 per condition/treatment except for UHRF1 KO where n=1 was employed. The combination of UHRF1 knockout and DNMT1 inhibition was extremely toxic to cells, making it difficult to

obtain sufficient material for high-quality ChIP-seq analysis.

No statistical method was used to predetermine sample size.

Data exclusions No data was excluded

Replication Independent orthogonal methods were used where possible to confirm findings. GFP-ChIP-seq datasets independently, orthogonally and repeatedly validated the original DNTM1-ChIP seq data

Randomization Randomization is not relevant as no animals or humans were used in this study

Blinding No blinding was used for this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- n/a Involved in the study
- ☐ ☒ Antibodies
- ☐ ☒ Eukaryotic cell lines
- ☒ ☐ Palaeontology and archaeology
- ☒ ☐ Animals and other organisms
- ☒ ☐ Clinical data
- ☒ ☐ Dual use research of concern
- ☒ ☐ Plants

Methods

- n/a Involved in the study
- ☐ ☒ ChIP-seq
- ☐ ☒ Flow cytometry
- ☒ ☐ MRI-based neuroimaging

Antibodies

Antibodies used

DNMT1 Abcam Mouse ab13537
 DNMT1 Abcam Rabbit ab19905
 SUMO2+3 Abcam Mouse ab81371
 SUMO2+3 Abcam Rabbit ab3742
 PKR Abcam Rabbit ab32506
 MDA5 Abcam Rabbit ab126630
 RanGAP1 Abcam Rabbit ab4784
 PCNA Abcam Mouse ab29
 IgG Abcam Mouse ab91353
 IgG Abcam Rabbit ab171870
 RNase-L Cell Signaling Rabbit 27281S
 RIG-I Cell Signaling Rabbit 3743S
 ADAR1 Santa Cruz Mouse sc-73408
 ACTIN(C-2) Santa Cruz Mouse SC8432
 Tubulin Santa Cruz Mouse SANTSC-8035
 Vinculin Abcam Rabbit ab129002
 HSP60 Santa Cruz Mouse sc-376240
 H3K27ac Abcam Rabbit ab4729
 GAPDH Cell Signaling Rabbit 2118S
 GFP Abcam Rabbit ab290
 5mC Diagenode Mouse C15200081-100
 Histone H3 Abcam Rabbit ab1791
 RPB1 (Clone 4H8) Cell Signaling Mouse 2629
 alpha-tubulin Cell Signaling Mouse 3872S
 beta-actin Cell Signaling Mouse 3700S
 V5-Tag Cell Signaling Mouse 80076S

Validation

<https://www.abcam.com/products/primary-antibodies/dnmt1-antibody-60b12201-ab13537.html> - 195 references
<https://www.abcam.com/products/primary-antibodies/dnmt1-antibody-ab19905.html> - KO validated and 75 references
<https://www.abcam.com/products/primary-antibodies/sumo-2--sumo-3-antibody-8a2-ab81371.html> - 63 references
<https://www.abcam.com/products/primary-antibodies/sumo-2--sumo-3-antibody-ab3742.html> - 91 references
<https://www.abcam.com/products/primary-antibodies/pkr-antibody-y117-ab32506.html> - KO validated and 25 references
<https://www.abcam.com/products/primary-antibodies/mda5-antibody-epr6743-ab126630.html> - KO validated and 8 references
<https://www.abcam.com/products/primary-antibodies/rangap1-antibody-epr3295-ab92360.html> - 11 references
<https://www.abcam.com/products/primary-antibodies/pcna-antibody-pc10-ab29.html> - 662 references
<https://www.cellsignal.com/products/primary-antibodies/rnase-l-d4b4j-rabbit-mab/27281> - 11 citations

<https://www.cellsignal.com/products/primary-antibodies/rig-i-d14g6-rabbit-mab/3743> - 201 citations
<https://www.scbt.com/p/adar1-antibody-15-8-6> - 76 citations
<https://www.scbt.com/p/actin-antibody-c-2> - 2503 citations
<https://www.scbt.com/p/alpha-tubulin-antibody-tu-02> - 1435 citations
<https://www.abcam.com/products/primary-antibodies/vinculin-antibody-epr8185-ab129002.html> - 277 references
<https://www.scbt.com/p/hsp-60-antibody-c-10> - 18 citations
<https://www.abcam.com/products/primary-antibodies/histone-h3-acetyl-k27-antibody-chip-grade-ab4729.html> - 1912 references
<https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118> - 7410 citations
<https://www.abcam.com/en-us/products/primary-antibodies/gfp-antibody-ab290> – 3369 publications. Independently validated – doesn't detect bands of appropriate size in parental cell lines, i.e. only detects exogenously introduced GFP-DNMT1 at the appropriate size. Used in field since 2002.
<https://www.diagenode.com/en/p/5-mc-monoclonal-antibody-33d3-premium-100-ug-50-ul> – Validated by Vendor for MeDIP-seq, Dot blot and IF. >300 references
<https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-antibody-nuclear-marker-and-chip-grade-ab1791> – 4740 publications. Used in field since 2002.
<https://www.cellsignal.com/products/primary-antibodies/rpb1-ctd-4h8-mouse-monoclonal-antibody/2629?srsltid=AfmBOoQvRvgduiS30FKFk79ZO5k5H8jV99ntAwDdIYCcountdtAjMlvVR> – 135 citations
<https://www.cellsignal.com/products/primary-antibodies/alpha-tubulin-dm1a-mouse-monoclonal-antibody/3873?srsltid=AfmBOooxZJ0I2hLUTxK64a-n6vahV7BNHXOSJwhoWDspuTuYxosWM2xl> – 1110 citations
<https://www.cellsignal.com/products/primary-antibodies/beta-actin-8h10d10-mouse-monoclonal-antibody/3700?srsltid=AfmBOorUW-fji9Vide7oWjYejiB26QKcAvPjPeBpun3wi3ORXQaoH5RM> – 5171 citations
https://www.cellsignal.com/products/primary-antibodies/v5-tag-e9h8o-mouse-monoclonal-antibody/80076?srsltid=AfmBOooEVs89-IE-p3V1yFnRijfAUshBiCWus2EWiRjQZ05z1EA7_7 – 33 citations. Independently validated. Doesn't detect bands of appropriate size in parental cell lines, i.e. only detects exogenously introduced V5-GFP-DNMT1 at the appropriate size.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

MV4-11, male, obtained from ATCC
 U937, male, obtained from ATCC
 OCI-AML3, male, obtained from ATCC
 MCF-7 female, obtained from ATCC
 HEK-293ET cells were a gift from Dr. Felix Randow (MRC-LMB, Cambridge, UK).

Authentication

Cell lines were not authenticated

Mycoplasma contamination

All cell lines tested negative for mycoplasma.

Commonly misidentified lines
(See [ICLAC](#) register)

n/a

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE233730>

Files in database submission

R1-gNT1-0nM-d7_S1_R1_001.fastq.gz
 R1-gNT1-0nM-d7_S1_R2_001.fastq.gz
 R1-gNT1-100nM-d7_S2_R1_001.fastq.gz
 R1-gNT1-100nM-d7_S2_R2_001.fastq.gz

R1-gRNF4-1-0nM-d7_S3_R1_001.fastq.gz
 R1-gRNF4-1-0nM-d7_S3_R2_001.fastq.gz
 R1-gRNF4-1-100nM-d7_S4_R1_001.fastq.gz
 R1-gRNF4-1-100nM-d7_S4_R2_001.fastq.gz
 R2-gNT-0nM-d7_S5_R1_001.fastq.gz
 R2-gNT-0nM-d7_S5_R2_001.fastq.gz
 R2-gNT-100nM-d7_S6_R1_001.fastq.gz
 R2-gNT-100nM-d7_S6_R2_001.fastq.gz
 R2-gRNF4-1-0nM-d7_S7_R1_001.fastq.gz
 R2-gRNF4-1-0nM-d7_S7_R2_001.fastq.gz
 R2-gRNF4-1-100nM-d7_S8_R1_001.fastq.gz
 R2-gRNF4-1-100nM-d7_S8_R2_001.fastq.gz
 R3-gNT-0nM-d7_S9_R1_001.fastq.gz
 R3-gNT-0nM-d7_S9_R2_001.fastq.gz
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 R3-gNT-100nM-d7_S10_R2_001.fastq.gz
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 R3-gRNF4-1-0nM-d7_S11_R2_001.fastq.gz
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 R3-gRNF4-1-100nM-d7_S12_R2_001.fastq.gz
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 4KD-DNMT1i_S12_R1_001.fastq.gz
 4NT-DMSO_S9_R1_001.fastq.gz
 4NT-DNMT1i_S11_R1_001.fastq.gz
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 R1_gNT1_100nM_d7_H3GYWDSX2_GGTAGCAT_L002_R1.fastq.gz
 R1_gNT1_100nM_d7_H3GYWDSX2_GGTAGCAT_L002_R2.fastq.gz
 R1_gRNF4_1_0nM_d7_H3GYWDSX2_ATGAGCAT_L002_R1.fastq.gz
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 R1_gRNF4_1_100nM_d7_H3GYWDSX2_CAACTAAT_L002_R1.fastq.gz
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 MCF7-DMSO-Rep2_S6_R1_001.fastq.gz
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 MV411-DMSO-Rep2_S2_R1_001.fastq.gz
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 Input_ChIP_gRNF4_100nm_H7K2TDSX2_TCGACGTC_L001_R2.fastq.gz
 BL1_S15_R1_001.fastq.gz
 BL2_S16_R1_001.fastq.gz
 BL4_S18_R1_001.fastq.gz
 BL5_S19_R1_001.fastq.gz
 BL6_S20_R1_001.fastq.gz
 BL8_S22_R1_001.fastq.gz
 BL9_S23_R1_001.fastq.gz

GFP-ChIP-seq:

DNMT1-CXXC-DMSO-R1_S39_R1_001_trimmed.fq.gz
 DNMT1-CXXC-DMSO-R2_S34_R1_001_trimmed.fq.gz
 DNMT1-CXXC-DNMT1i-R1_S40_R1_001_trimmed.fq.gz
 DNMT1-CXXC-DNMT1i-R2_S35_R1_001_trimmed.fq.gz
 DNMT1-KR-gNT-DMSO-R1_S45_R1_001_trimmed.fq.gz
 DNMT1-KR-gNT-DNMT1i-R1_S49_R1_001_trimmed.fq.gz
 DNMT1-KR-gRNF4-DMSO-R1_S46_R1_001_trimmed.fq.gz
 DNMT1-KR-gRNF4-DNMT1i-R1_S50_R1_001_trimmed.fq.gz
 DNMT1-WT-DMSO-R1_S37_R1_001_trimmed.fq.gz
 DNMT1-WT-DMSO-R2_S51_R1_001_trimmed.fq.gz
 DNMT1-WT-DNMT1i-R1_S38_R1_001_trimmed.fq.gz
 DNMT1-WT-DNMT1i-R2_S33_R1_001_trimmed.fq.gz
 DNMT1-WT-gNT-DMSO-R1_S43_R1_001_trimmed.fq.gz
 DNMT1-WT-gNT-DNMT1i-R1_S47_R1_001_trimmed.fq.gz
 DNMT1-WT-gRNF4-DMSO-R1_S44_R1_001_trimmed.fq.gz
 DNMT1-WT-gRNF4-DNMT1i-R1_S48_R1_001_trimmed.fq.gz
 INPUT-1_S41_R1_001_trimmed.fq.gz
 INPUT-2_S42_R1_001_trimmed.fq.gz
 MV411-parental-anti-GFP-control_S36_R1_001_trimmed.fq.gz

Input-WT-1-DMSO_S102_R1_001_trimmed.fq.gz
 Input-WT-1-DNMT1i_S103_R1_001_trimmed.fq.gz
 MV411-parental-GFP-ChIP_S111_R1_001_trimmed.fq.gz
 NTC-DMSO-GFP-ChIP_S91_R1_001_trimmed.fq.gz
 NTC-DNMT1i-GFP-ChIP_S92_R1_001_trimmed.fq.gz
 RFTS-1-DMSO-GFP-ChIP_S105_R1_001_trimmed.fq.gz
 RFTS-1-DNMT1i-GFP-ChIP_S106_R1_001_trimmed.fq.gz
 RFTS-2-DMSO-GFP-ChIP_S109_R1_001_trimmed.fq.gz
 RFTS-2-DNMT1i-GFP-ChIP_S110_R1_001_trimmed.fq.gz
 UHRF1KO-DMSO-GFP-ChIP_S93_R1_001_trimmed.fq.gz
 UHRF1KO-DNMT1i-GFP-ChIP_S94_R1_001_trimmed.fq.gz
 WT-1-DMSO-GFP-ChIP_S101_R1_001_trimmed.fq.gz
 WT-1-DNMT1i-GFP-ChIP_S104_R1_001_trimmed.fq.gz

CW-DMSO-ChIP-GFP-R1_S177_R1_001_trimmed.fq.gz
 CW-DMSO-ChIP-GFP-R2_S163_R1_001_trimmed.fq.gz
 CW-dTAG-ChIP-GFP-R1_S178_R1_001_trimmed.fq.gz
 CW-dTAG-ChIP-GFP-R2_S164_R1_001_trimmed.fq.gz
 CW-dTAG-DNMT1i-ChIP-GFP-R1_S179_R1_001_trimmed.fq.gz
 CW-dTAG-DNMT1i-ChIP-GFP-R2_S165_R1_001_trimmed.fq.gz
 WT-DMSO-ChIP-GFP-R1_S173_R1_001_trimmed.fq.gz
 WT-DMSO-ChIP-GFP-R2_S180_R1_001_trimmed.fq.gz
 WT-dTAG-ChIP-GFP-R1_S175_R1_001_trimmed.fq.gz
 WT-dTAG-ChIP-GFP-R2_S181_R1_001_trimmed.fq.gz
 WT-dTAG-DNMT1i-ChIP-GFP-R1_S176_R1_001_trimmed.fq.gz
 WT-dTAG-DNMT1i-ChIP-GFP-R2_S182_R1_001_trimmed.fq.gz
 WT-dTAG-DNMT1i-R2-input_S172_R1_001_trimmed.fq.gz
 WT-DMSO-R2-input_S171_R1_001_trimmed.fq.gz

Genome browser session
 (e.g. [UCSC](https://genome.ucsc.edu/s/andreaigillespie/DNMT1i_manuscript_submission))

https://genome.ucsc.edu/s/andreaigillespie/DNMT1i_manuscript_submission

Methodology

Replicates

DNMT1-ChIP-seq (n=1 per rep), GFP-ChIP-seq (n=2 per rep, except for UHRF1 KO where n=1 rep was used)

Sequencing depth

For DNMT1-ChIP-seq:

Total number of reads: 318.4 million reads between all 12 samples

Uniquely mapped reads: 264,719,729 reads between all 12 samples

Read length: 150bp paired-end

For GFP-ChIP-Seq, 100bp SE sequencing performed (Sample | total reads | uniquely aligned reads)

DNMT1-CXXC-DMSO-R1_S39_R1_001_trimmed.fq.gz 44603146 16546325
 DNMT1-CXXC-DMSO-R2_S34_R1_001_trimmed.fq.gz 32506467 15625866
 DNMT1-CXXC-DNMT1i-R1_S40_R1_001_trimmed.fq.gz 45636587 17252656
 DNMT1-CXXC-DNMT1i-R2_S35_R1_001_trimmed.fq.gz 40707409 18249912
 DNMT1-KR-gNT-DMSO-R1_S45_R1_001_trimmed.fq.gz 37594062 17695585
 DNMT1-KR-gNT-DNMT1i-R1_S49_R1_001_trimmed.fq.gz 31722059 13948544
 DNMT1-KR-gRNF4-DMSO-R1_S46_R1_001_trimmed.fq.gz 36687373 17022239
 DNMT1-KR-gRNF4-DNMT1i-R1_S50_R1_001_trimmed.fq.gz 36592595 16632449
 DNMT1-WT-DMSO-R1_S37_R1_001_trimmed.fq.gz 37135143 14872323
 DNMT1-WT-DMSO-R2_S51_R1_001_trimmed.fq.gz 38601877 18621967
 DNMT1-WT-DNMT1i-R1_S38_R1_001_trimmed.fq.gz 39289228 18105935
 DNMT1-WT-DNMT1i-R2_S33_R1_001_trimmed.fq.gz 31511108 15238685
 DNMT1-WT-gNT-DMSO-R1_S43_R1_001_trimmed.fq.gz 40207653 18308897
 DNMT1-WT-gNT-DNMT1i-R1_S47_R1_001_trimmed.fq.gz 38978225 16482392
 DNMT1-WT-gRNF4-DMSO-R1_S44_R1_001_trimmed.fq.gz 29780288 14550145
 DNMT1-WT-gRNF4-DNMT1i-R1_S48_R1_001_trimmed.fq.gz 34415730 15596504
 INPUT-1_S41_R1_001_trimmed.fq.gz 37344498 21892445
 INPUT-2_S42_R1_001_trimmed.fq.gz 37426727 21637060
 MV411-parental-anti-GFP-control_S36_R1_001_trimmed.fq.gz 36211765 12905767

Input-WT-1-DMSO_S102_R1_001_trimmed.fq.gz 21864906 12167083
 Input-WT-1-DNMT1i_S103_R1_001_trimmed.fq.gz 17162212 9434799
 MV411-parental-GFP-ChIP_S111_R1_001_trimmed.fq.gz 19350362 9510532
 NTC-DMSO-GFP-ChIP_S91_R1_001_trimmed.fq.gz 11725582 6063130
 NTC-DNMT1i-GFP-ChIP_S92_R1_001_trimmed.fq.gz 12670513 6227859
 RFTS-1-DMSO-GFP-ChIP_S105_R1_001_trimmed.fq.gz 15432204 7656470
 RFTS-1-DNMT1i-GFP-ChIP_S106_R1_001_trimmed.fq.gz 17357791 7760748
 RFTS-2-DMSO-GFP-ChIP_S109_R1_001_trimmed.fq.gz 15464432 7646790
 RFTS-2-DNMT1i-GFP-ChIP_S110_R1_001_trimmed.fq.gz 21186674 9557967
 UHRF1KO-DMSO-GFP-ChIP_S93_R1_001_trimmed.fq.gz 11655093 6554388

UHRF1KO-DNMT1i-GFP-ChIP_S94_R1_001_trimmed.fq.gz 15214271 7036414
 WT-1-DMSO-GFP-ChIP_S101_R1_001_trimmed.fq.gz 13347796 6814312
 WT-1-DNMT1i-GFP-ChIP_S104_R1_001_trimmed.fq.gz 15045682 6289587

CW-DMSO-ChIP-GFP-R1_S177_R1_001_trimmed.fq.gz 25642048 8326308
 CW-DMSO-ChIP-GFP-R2_S163_R1_001_trimmed.fq.gz 20982677 10079821
 CW-dTAG-ChIP-GFP-R1_S178_R1_001_trimmed.fq.gz 24303855 5897562
 CW-dTAG-ChIP-GFP-R2_S164_R1_001_trimmed.fq.gz 21082699 8915632
 CW-dTAG-DNMT1i-ChIP-GFP-R1_S179_R1_001_trimmed.fq.gz 27040668 7833508
 CW-dTAG-DNMT1i-ChIP-GFP-R2_S165_R1_001_trimmed.fq.gz 20022550 8538682
 WT-DMSO-ChIP-GFP-R1_S173_R1_001_trimmed.fq.gz 26316486 7944041
 WT-DMSO-ChIP-GFP-R2_S180_R1_001_trimmed.fq.gz 18843740 8937640
 WT-dTAG-ChIP-GFP-R1_S175_R1_001_trimmed.fq.gz 17857888 5689396
 WT-dTAG-ChIP-GFP-R2_S181_R1_001_trimmed.fq.gz 16536327 7850205
 WT-dTAG-DNMT1i-ChIP-GFP-R1_S176_R1_001_trimmed.fq.gz 27391677 7113972
 WT-dTAG-DNMT1i-ChIP-GFP-R2_S182_R1_001_trimmed.fq.gz 24689588 9584470
 WT-dTAG-DNMT1i-R2-input_S172_R1_001_trimmed.fq.gz 21621605 11549136
 WT-DMSO-R2-input_S171_R1_001_trimmed.fq.gz 18465216 10165011

Antibodies

DNMT1 Abcam Mouse ab13537 and DNMT1 Abcam Rabbit ab19905 were pooled for DNMT1 ChIP-seq
 H3K27ac Abcam Rabbit ab4729 was used for H2K27ac ChIP-seq
 GFP Abcam Rabbit ab290 used for GFP ChIP-seq

Peak calling parameters

macs2 callpeak -t bam/DNMT1i-ChIP_gNT_0nm_H7K2TDSX2_TACGCTGC.bam -c bam/
 Input_ChIP_gNT_0nm_H7K2TDSX2_CCTAAGAC.bam -f BAMPE -g hs
 macs2 callpeak -t bam/DNMT1i-ChIP_gNT_100nm_H7K2TDSX2_ATGCGCAG.bam -c bam/
 Input_ChIP_gNT_100nm_H7K2TDSX2_CGATCAGT.bam -f BAMPE -g hs
 macs2 callpeak -t bam/DNMT1i-ChIP_gRNF4_0nm_H7K2TDSX2_TAGCGCTC.bam -c bam/
 Input_ChIP_gRNF4_0nm_H7K2TDSX2_TGCAGCTA.bam -f BAMPE -g hs
 macs2 callpeak -t bam/DNMT1i-ChIP_gRNF4_100nm_H7K2TDSX2_ACTGAGCG.bam -c bam/
 Input_ChIP_gRNF4_100nm_H7K2TDSX2_TCGACGTC.bam -f BAMPE -g hs

macs2 callpeak -t bam/H3K27ac_ChIP_gNT_0nm_H7K2TDSX2_ACTCGCTA.bam -c bam/
 Input_ChIP_gNT_0nm_H7K2TDSX2_CCTAAGAC.bam -f BAMPE -g hs
 macs2 callpeak -t bam/H3K27ac_ChIP_gNT_100nm_H7K2TDSX2_GGAGCTAC.bam -c bam/
 Input_ChIP_gNT_100nm_H7K2TDSX2_CGATCAGT.bam -f BAMPE -g hs
 macs2 callpeak -t bam/H3K27ac_ChIP_gRNF4_0nm_H7K2TDSX2_GCGTAGTA.bam -c bam/
 Input_ChIP_gRNF4_0nm_H7K2TDSX2_TGCAGCTA.bam -f BAMPE -g hs
 macs2 callpeak -t bam/H3K27ac_ChIP_gRNF4_100nm_H7K2TDSX2_CGGAGCCT.bam -c bam/
 Input_ChIP_gRNF4_100nm_H7K2TDSX2_TCGACGTC.bam -f BAMPE -g hs

For GFP-ChIP-seq, peak calling parameters --broad -g hs -B -q 0.05)

Data quality

Data quality methods:

DNMT1-ChIP seq:

The default q-value for MACS was implemented in the above commands, which is a cutoff of 0.05. Per sample the number of peaks with q-value <0.05 and fold enrichment >5 are:

DNMT1_gNT_0nm: 5751
 DNMT1_gNT_100nm: 51907
 DNMT1_gRNF4_0nm: 5216
 DNMT1_gRNF4_100nm: 116120
 H3K27ac_gNT_0nm: 31355
 H3K27ac_gNT_100nm: 36817
 H3K27ac_gRNF4_0nm: 28998
 H3K27ac_gRNF4_100nm: 35409

GFP ChIP-seq (peak calling parameters --broad -g hs -B -q 0.05) | number of peaks called:

DNMT1-CXXC-DMSO-R1_S39_R1_001_trimmed.fq.gz 100
 DNMT1-CXXC-DMSO-R2_S34_R1_001_trimmed.fq.gz 27987
 DNMT1-CXXC-DNMT1i-R1_S40_R1_001_trimmed.fq.gz 23
 DNMT1-CXXC-DNMT1i-R2_S35_R1_001_trimmed.fq.gz 42849
 DNMT1-KR-gNT-DMSO-R1_S45_R1_001_trimmed.fq.gz 47121
 DNMT1-KR-gNT-DNMT1i-R1_S49_R1_001_trimmed.fq.gz 60666
 DNMT1-KR-gRNF4-DMSO-R1_S46_R1_001_trimmed.fq.gz 51535
 DNMT1-KR-gRNF4-DNMT1i-R1_S50_R1_001_trimmed.fq.gz 92989
 DNMT1-WT-DMSO-R1_S37_R1_001_trimmed.fq.gz 2210
 DNMT1-WT-DMSO-R2_S51_R1_001_trimmed.fq.gz 51652
 DNMT1-WT-DNMT1i-R1_S38_R1_001_trimmed.fq.gz 4
 DNMT1-WT-DNMT1i-R2_S33_R1_001_trimmed.fq.gz 32903

DNMT1-WT-gNT-DMSO-R1_S43_R1_001_trimmed.fq.gz 49237
 DNMT1-WT-gNT-DNMT1i-R1_S47_R1_001_trimmed.fq.gz 39019
 DNMT1-WT-gRNF4-DMSO-R1_S44_R1_001_trimmed.fq.gz 61438
 DNMT1-WT-gRNF4-DNMT1i-R1_S48_R1_001_trimmed.fq.gz 90165
 INPUT-1_S41_R1_001_trimmed.fq.gz
 INPUT-2_S42_R1_001_trimmed.fq.gz
 MV411-parental-anti-GFP-control_S36_R1_001_trimmed.fq.gz

Input-WT-1-DMSO_S102_R1_001_trimmed.fq.gz
 Input-WT-1-DNMT1i_S103_R1_001_trimmed.fq.gz
 MV411-parental-GFP-ChIP_S111_R1_001_trimmed.fq.gz 426
 NTC-DMSO-GFP-ChIP_S91_R1_001_trimmed.fq.gz 23990
 NTC-DNMT1i-GFP-ChIP_S92_R1_001_trimmed.fq.gz 6194
 RFTS-1-DMSO-GFP-ChIP_S105_R1_001_trimmed.fq.gz 12660
 RFTS-1-DNMT1i-GFP-ChIP_S106_R1_001_trimmed.fq.gz 7861
 RFTS-2-DMSO-GFP-ChIP_S109_R1_001_trimmed.fq.gz 14443
 RFTS-2-DNMT1i-GFP-ChIP_S110_R1_001_trimmed.fq.gz 11495
 UHRF1KO-DMSO-GFP-ChIP_S93_R1_001_trimmed.fq.gz 32954
 UHRF1KO-DNMT1i-GFP-ChIP_S94_R1_001_trimmed.fq.gz 5042
 WT-1-DMSO-GFP-ChIP_S101_R1_001_trimmed.fq.gz 17119
 WT-1-DNMT1i-GFP-ChIP_S104_R1_001_trimmed.fq.gz 4323

CW-DMSO-ChIP-GFP-R1_S177_R1_001_trimmed.fq.gz 5168
 CW-DMSO-ChIP-GFP-R2_S163_R1_001_trimmed.fq.gz 20211
 CW-dTAG-ChIP-GFP-R1_S178_R1_001_trimmed.fq.gz 12401
 CW-dTAG-ChIP-GFP-R2_S164_R1_001_trimmed.fq.gz 33406
 CW-dTAG-DNMT1i-ChIP-GFP-R1_S179_R1_001_trimmed.fq.gz 7783
 CW-dTAG-DNMT1i-ChIP-GFP-R2_S165_R1_001_trimmed.fq.gz 14660
 WT-DMSO-ChIP-GFP-R1_S173_R1_001_trimmed.fq.gz 5457
 WT-DMSO-ChIP-GFP-R2_S180_R1_001_trimmed.fq.gz 21713
 WT-dTAG-ChIP-GFP-R1_S175_R1_001_trimmed.fq.gz 9718
 WT-dTAG-ChIP-GFP-R2_S181_R1_001_trimmed.fq.gz 26188
 WT-dTAG-DNMT1i-ChIP-GFP-R1_S176_R1_001_trimmed.fq.gz 7824
 WT-dTAG-DNMT1i-ChIP-GFP-R2_S182_R1_001_trimmed.fq.gz 15892
 WT-dTAG-DNMT1i-R2-input_S172_R1_001_trimmed.fq.gz
 WT-DMSO-R2-input_S171_R1_001_trimmed.fq.gz

Software

FastQC (v0.11.5), Cutadapt, BWA-MEM (arXiv:1303.3997v2), deeptools (v3.5.0), MACS2, BEDTools (v2.27), IGV (v2.11.9), and Spark.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Apoptosis analysis was prepared as per manufacturers instructions (APC Annexin V Apoptosis Detection Kit with 7-AAD, Cat # 640930)
 Cell cycle analysis cells were fixed with ethanol (70%) overnight at 4oC. Cells were stained by resuspending in PBS containing 0.5% TritonX and DAPI

Instrument

BD FACSymphony™ A5 Cell Analyzer

Software

FlowJo 10.9.0

Cell population abundance

>10,000 events are displayed in all plots and represent >50% of total event collected

Gating strategy

All plots depicted are directly gated from FSC/SSC

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.