STAR METHODS

KEY RESOURSE TABLE

REAGENTS or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Alpha-tubulin (11H10)	Cell Signaling Technology	2125S
DNMT1 (60B1220.1)	Novus Biologicals	NB100-56519
DNMT1 (H-12)	Santa Cruz Biotechnology	sc-271729
GAPDH	Santa Cruz Biotechnology	sc-25778
H3	Abcam	ab1791
Myc tag [Myc.A7]	Abcam	ab18185
O-GlcNAc (RL2)	Abcam	ab2739
OGT	Abcam	ab177941
Bacteria and virus strains		
E. coli NEB 5-alpha	New England Biolabs	C2987H
Chemicals, peptides, recombinant proteins		
DNMT1 recombinant protein	Origene	TP326414
OSMI-4	Selleck Chem	S8910
Thiamet-G	Cayman Chemical	13237
Critical commercial assays		
EpiQuik DNMT Activity/Inhibition ELISA Easy Kit	EpiGentek	P-3139-48

Global DNA Methylation LINE-1 assay	Active Motif	55017
Deposited data		
DNMT1-WT-Ctrl	This paper	GEO: GSE201470
DNMT1-WT-*O*-GlcNAc	This paper	GEO: GSE201470
DNMT1-S878A-Ctrl	This paper	GEO: GSE201470
DNMT1-S878A-*O*-GlcNAc	This paper	GEO: GSE201470
Partially methylated domains of liver cancer	Li et al., 2016	GEO: GSE70091
RNA sequencing	Chang et al., 2014	GEO: GSE49994
Experimental models: Cell lines		
Hep3B	ATCC	HB-8064
HepG2	ATCC	HB-8065
Oligonucleotides		
DNMT1-T158A (Forward): 5'-agccccaggatt CGA aggaaaagcacc-3'	This paper	N/A
DNMT1-T158A (Reverse): 5'-ggtgcttttcct TCG aatcctggggct-3'	This paper	N/A
DNMT1-T616A (Forward): 5'-gacaggggaccc GCG aaagccaccacc-3'	This paper	N/A
DNMT1-T616A (Reverse): 5'-ggtggtggcttt CGC gggtcccctgtc-3'	This paper	N/A
DNMT1-S878A (Forward): 5'-gcgagattcgag GAG cctccaaaaacc-3'	This paper	N/A
DNMT1-S878A (Reverse): 5'-ggtttttggagg CTC ctcgaatctcgc-3'	This paper	N/A
DNMT1-S878D (Forward): 5'-gcgagattcgag GAC cctccaaaaacc-3'	This paper	N/A

DNMT1-S878D (Reverse): 5’-ggtttttggagg GTC ctcgaatctcgc-3’	This paper	N/A
DNMT1-T882A (Forward): 5’-tcccctccaaaa GCC cagccaacagag-3’	This paper	N/A
DNMT1-T882A (Reverse): 5’-ctctgttggctg GGC ttttggagggga-3’	This paper	N/A
Recombinant DNA		
pcDNA3/Myc-DNMT1	Addgene	36939
pcDNA3/Myc-DNMT1-T158A	This paper	N/A
pcDNA3/Myc-DNMT1-T616A	This paper	N/A
pcDNA3/Myc-DNMT1-S878A	This paper	N/A
pcDNA3/Myc-DNMT1-S878D	This paper	N/A
pcDNA3/Myc-DNMT1-T882A	This paper	N/A
Software and algorithms		
bedGraphToBigWig	Kent et al., 2010	https://www.encodeproject.org/software/bedgraphtobigwig/
Graphpad Prism 9 (v9.3.1)	Graphpad	https://www.graphpad.com/scientific-software/prism/
Minimap2 (v2.17) (RRID:SCR_018550)	Li, 2018	https://github.com/lh3/minimap2
Nanopolish (v0.11.1) (RRID:SCR_016157)	Loman et al., 2015	https://github.com/jts/nanopolish
Python (v3.8.2)	Python Core Team	https://www.python.org/

R (v3.4.3)	R Core Team	https://www.r-project.org/
Samtools (v1.10) (RRID:SCR_002105)	Li et al., 2009	

RESOURCE AVAILABILITY

Lead contact

Further information and requests for reagents may be directed to and will be fulfilled by the lead contact, Dustin E. Schones, dschones@coh.org

Material availability

Plasmid generated in this study should be directed to and will be fulfilled by the lead contact, Dustin E. Schones, dschones@coh.org

Data and code availability

PromethION sequencing data have been deposited in the NCBI Gene Expression Omnibus (GEO) and Sequence Read Archive (SRA) under accession number 201470. Further requests for data are available upon correspondence with the Lead Contact.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell culture and plasmid DNA transfection

Hepatocellular carcinoma cell lines (HepG2, and Hep3B) were purchased from ATCC (Manassas, VA, USA). All cell lines were shown to be negative in mycoplasma test using MycoScope (MY01050, Genlantis, San Diego, CA, USA). The following ATCC-specified cell culture media were used: Dulbecco's modified Eagle's medium (DMEM, 11885-084, Grand Island, NY, USA) or high glucose Dulbecco's modified Eagle's medium (DMEM, 11995-065, Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (FBS,

SH30910.03, HyClone, South Logan, UT, USA) and Opti-MEM (1869048, Gibco, Grand Island, NY, USA). All cells were cultured in a 37°C with a 5% CO_2 atmosphere incubator. HepG2 and Hep3B cells were transiently transfected (with the pcDNA3 with or without DNMT1 cDNA) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) and selected with Geneticin (G418, 10131-035, Gibco, Grand Island, NY, USA) according to the manufacturer's instructions. Human DNMT1 plasmid was purchased from Addgene (Watertown, MA, USA).

Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs such as lymphocyte, monocyte or a macrophage were isolated directly from whole blood using Ficoll-Paque (Premium, GE Healthcare, Chicago, IL, USA) density gradient centrifugation. 15ml whole blood was mixed with same volume of phosphate-buffered saline containing 0.1% Fetal bovine serum + 2mM EDTA (PBS solution). Next, the blood mix was placed on top of 15 ml Ficoll and centrifuged at 400g to 200g for 40 min without brake. Next, remove the supernatant and washed three times with PBS solution.

Isolation of B cells and Epstein-Barr virus (EBV) infection for lymphocyte transformation

$CD19^+$ B cells were isolated from PBMCs (peripheral blood mononuclear cells) using Dynabeads $CD19^+$ pan B (11143D, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. 2.5 x 10^8 cells of PBMCs were resuspended in 10ml Isolation buffer (PBS, 0.1% BSA, 2mM EDTA). 250 µl of pre-washed beads were added to PBMCs and incubated for 20 min in 4°C with gentle rotation. For positive isolation of $CD19^+$ B cells, beads and supernatant were separated using magnet, and supernatant was

discarded. Beads were washed three times, and beads bounded with $CD19^+$ B cells were resuspended with 2.5 ml of cell culture medium (80% RPMI1640, 20 % heat-inactivated FBS, Glutamine). $CD19^+$ B cells were released from Dynabeads using DETACHaBEAD (Invitrogen, ca12506D) according to the manufacturer's instruction.

B cells were infected with Epstein-Barr virus (EBV) to transform lymphocyte. 10 ml of B cells were transferred into T75 flask. 1.5ml of stock EBV collected from a B95-8 strain-containing marmoset cell lines and 1ml of Phytohemagglutinin P (PHA-P) were added to flask and incubated in a 37°C with a 5% CO_2 atmosphere incubator. Every 5 to 7 days, 10 ml of cell culture medium was added. Cells were let to grow in CO_2 atmosphere incubator for 30 days until all B cells were transformed to LCL.

METHOD DETAILS

Immunoprecipitation and western blot analysis

Cell lysates were incubated with specific antibodies and lysis buffer for 4 hours. Subsequently, 30 µl of washed Dynabeads (14311D, Thermo Fisher, Waltham, MA, USA) were added to each lysate and incubated overnight at 4°C. Next, the beads were washed five times, and the antigens were eluted twice using 8M Urea buffer (8M Urea, 20mM Tris pH 7.5, and 100mM NaCl) and concentrated. The resulting samples were separated by Mini-PROTEAN TGX (4-20%, 4561093, Bio-Rad Laboratories, Hercules, CA, USA) and transferred onto nitrocellulose membranes (Amersham Hybond, 10600021, GE Healthcare, Chicago, IL, USA) using Trans-Blot SD Semi-dry Transfer Cell system (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were then blocked with 5% skim milk in Tris-buffered saline + Tween-20 (TBS-T; 20 mM Tris, 137 mM NaCl, 0.1% Tween-

20, pH 7.6), incubated overnight at 4°C with a 1:1000 dilution of each antibodies, and subsequently incubated for 1 h with a 1:5000 dilution of a horseradish peroxidase–conjugated goat anti-mouse secondary antibody (ab6789, Abcam, Cambridge, UK) or goat anti-rabbit secondary antibody (ab6721, Abcam, Cambridge, UK). Immunoreactive proteins were detected using SuperSignal West Dura Extended Duration Substrate (34076, Thermo, Rockford, IL, USA) and detected using a ChemiDoc MP Imaging system (Bio-Rad Laboratories, Hercules, CA, USA). The band intensity was densitometrically evaluated using Image Lab software (Version 5.2, Bio-Rad Laboratories, Hercules, CA, USA).

Protein identification using the Thermo Fusion Lumos system LC-MS/MS

LC-MS separation was performed on an Thermo Fusion Lumos system (Thermo Scientific, Waltham, MA, USA). For LC separation, 60-minute LC gradient on EasySpray column (particle sizes: 500 mm, 75 μm) was used for peptide separation. Mass spectra for peptide identification or quantification were acquired using an Orbitrap Lumos mass at 120,000 resolutions. Full MS scan ranges were acquired from 156 to 2000 m/z. MS/MS spectra were acquired at a resolution of 30,000 using HCD at 35% collision energy.

Identify post-translational modifications on DNMT1 using complementary in-solution digestion with three enzymes (chymotrypsin, AspN and LysC). Protein was reduced with DTT and alkylated with iodoacetamide. Aliquots of protein were separately digested with three enzymes and peptides were desalted using a C18 tip.

Raw data files were submitted to Byonic (v2.16.11) for target decoy search against the human protein database (uniprot/swissprot, 2020). Peptide-level confidence threshold was set at 99% (FDR <0.01). The sample was bracketed by *E. coli* QC runs, which were

then correlated to ensure instrument quality. QC passed threshold (≥ 0.9) with an R^2 of 0.98 (correlation value, R=0.99)

Site-directed point mutation

Specific primers for serine (S) and threonine (T) to alanine (A) and aspartic acid (D) mutations of DNMT1 were designed and used to site-directed point mutations in a plasmid vector. A PCR-amplified DNA fragment of pcDNA3-DNMT1 was generated using Q5 Site-Directed Mutagenesis Kit (E0554S, NEB, Ipswich, MA, USA). The primers used in this process are described in KEY RESOURCE TABLE. After PCR, the non-mutated sequences were cleaved using Q5 KLD enzyme (NEB) according to the manufacturer's instructions. The mutated vectors were transformed into E. coli competent cells that were cultured and prepared using a GenElute HP Plasmid Midi kit (NA0200-1KT, Sigma-Aldrich, St. Louis, MO).

DNA methyltransferase activity assay

DNA methyltransferase activities of endogenous DNMT1 and recombinant DNMT1 were measured by EpiQuik DNMT Activity/Inhibition ELISA Easy Kit (P-3139-48, EpiGentek, Farmingdale, NY, USA) according to the manufacturer's instructions. The endogenous DNMT1 (by anti-DNMT1 Ab, 60B1220.1) and recombinant DNMT1 (by anti-Myc, ab18185) were enriched using immunoprecipitation from each cell or tissue lysates. DNMT1s were isolated and normalized by BCA analysis. The activity of 5 ng DNMT1 was analyzed by 450nm ELISA with an optimal wavelength of 655nm.

Global DNA Methylation LINE-1 assay

Global DNA methylation LINE-1 were measured by Active Motif (55017, Carlsbad, CA, USA) according to the manufacturer's instructions. Each Hep3B and myc-DNMT1

overexpressed mutants (DNMT1-WT or DNMT1-S878A) were treated 5mM glucose, or 25mM glucose, and 5-aza (negative control). The activity of 100 ng was analyzed by 450nm ELISA with an optimal wavelength of 655nm.

Agilent 4200 TapeStation

Global DNA methylation were measured by Agilent 4200 TapeStation system (Santa Clara, CA, USA) with the Genomic DNA ScreenTape (5064-5365) and Genomic DNA Reagent (5067-5366) according to the manufacturer's instructions.

Nanopore PromethION sequencing

Reads were aligned using minimap2 (RRID:SCR_018550) with the options -a -x map-ont (Li, 2018). Methylation state of CpGs was called using nanopolish (RRID:SCR_016157) with the options call-methylation -t 100 (Loman et al., 2015). Only loci with greater than 5 x coverage were considered in the analysis. Methylation percentage was averaged across CpG islands.

RNA sequencing data

Hep3B RNA-seq data were obtained from a previous publication (Chang et al., 2014). Fastq files were aligned using HISAT2 version 2.1.0 (RRID:SCR_015530) to the hg19 genome (Siren et al., 2014). Duplications are removed using picard version 2.10.1 (RRID:SCR_006525). Aligned reads were sorted using samtools version 1.10 (RRID:SCR_002105) (Li et al., 2009). UCSC genome browser tracks were established using bedGraphToBigWig (Kent et al., 2010). FPKM was calculated using StringTie version 1.3.4d (RRID:SCR_016323) (Pertea et al., 2015).

For all datasets, Bedgraph files were generated using bedtools version 2.29.0 (RRID:SCR_006646) (Quinlan and Hall, 2010). BigWigs were generated using the UCSCtools bedGraphToBigWig (Kent et al., 2010). Heatmap of global DNA methylation for DNMT1-WT and DNMT1-S878A cells under low or high glucose were generated using a custom script to profile the read coverage at each base and were visualized using pheatmap version 1.0.12 (RRID:SCR_016418). All other heatmaps and aggregate plots of loci that extend were generated using deeptools (RRID:SCR_016366) (Ramirez et al., 2014).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed and graphed using Graphpad Prism 9 (v9.3.1). All statistical tests were performed by three independent experiments assay, and the data are presented as means ± standard deviations. *$p < 0.001$; **$p < 0.0005$; ***$p < 0.0001$ by Student's *t*-test (A and B); n.s., not significant; Data are represented as mean ± SD.