SUPPLEMENTAL INFORMATION

Supplementary Figures

Figure S1. High glucose increases *O*-GlcNAcylation of DNMT1.
(A) Hep3B cells were treated with Thiamet-G (TMG) or OSMI-4 (OSMI). Shown are representative immunoblots of treated Hep3B lysates performed with antibodies targeting *O*-GlcNAc, and GAPDH and bar graphs of relative expression between *O*-GlcNAc compared to control, GAPDH (n = 3, experimental replicates).
(B) Lysates from treated Hep3B with glucose were immunoprecipitated with DNMT1 and immunoprecipitates were immunoblotted with antibody targeting *O*-GlcNAc (n = 3).
(C) HepG2 cells were treated with Thiamet-G or OSMI. Shown are immunoblots of treated HepG2 lysates performed with immunoblots of immunoprecipitates performed with antibodies targeting *O*-GlcNAc.
(D) Shown are immunoblots of B cell and lymphocytes (LCL) lysates performed with immunoblots of immunoprecipitates performed with antibodies targeting *O*-GlcNAc.
(E) HepG2 cells were treated 5mM glucose or sucrose, or 25mM glucose or sucrose. Lysates of HepG2 treated with glucose were immunoprecipitated with DNMT1 and immunoprecipitates were immunoblotted with antibody targeting *O*-GlcNAc.
***$p < 0.0001$ by Student's *t*-test (A-E); Data are represented as mean ± SD from three replicates of each sample.

Figure S2. Myc-DNMT1-WT in Hep3B cells is *O*-GlcNAcylated.
(A) Myc-DNMT1-WT were transfected into Hep3B cells. Shown are immunoblots of treated DNMT1-WT lysates performed with antibodies targeting Myc, DNMT1, Tubulin, H3, and *O*-GlcNAc.
(B) Lysates from treated DNMT1-WT in (A) were immunoprecipitated with Myc antibody. Shown are immunoblots of immunoprecipitates performed with antibodies targeting *O*-GlcNAc and CBB stained gel.
(C) Tandem MS/MS peaks of *O*-GlcNAcylated DNMT1 peptides.

Figure S3. Site specific *O*-GlcNAcylation at DNMT1 sites abrogate the function of methyltransferase and DNA loss of methylation at CpG island under high glucose/TMG conditions.
(A) HepG2 cells were treated 5mM glucose, 25mM glucose with or without Thiamet-G (TMG). Shown are absorbance of DNA methyltransferase activity performed with DNA methyltransferase activity kit. (n = 3, technical replicates from 3 biological replicates for each strain).
(B) Each HepG2 and Myc-DNMT1 overexpressed mutants (DNMT1-WT or DNMT1-S878A) were treated 5mM glucose, or 25mM glucose, or 5-aza (negative control). Shown are absorbance of global DNA methylation of LINE-1 performed with global DNA

methylation LINE-1 kit. (n = 3, technical replicates from 3 biological replicates for each strain).
(C) DNA was extracted from Hep3B and Myc-DNMT1 overexpressed mutants (DNMT1-WT or DNMT1-S878A) were treated 5mM glucose, or 25mM glucose, or 5-aza (negative control) with MspI (negative control) or HpaII. Shown are extracted genomic DNA samples and analyze on the 4200 TapeStation System with the Genomic DNA Screen Tape assay with methylation sensitive enzyme using MspI or HpaII.
*p < 0.001; ***p < 0.0001 by Student's t-test (A and B); n.s., not significant; Data are represented as mean ± SD from three replicates of each sample.

Figure S4. DNA loss of methylation by increased global *O*-GlcNAcylation decreases.
(A) Shown are overall CpGs sites that detected with over 5x coverage DNA methylation analysis using Nanopore technology PromethION sequencer. Each condition is biological replicated.
For (B)-(F), bar graphs represent percentage of global DNA methylation of wild type and DNMT1 mutants (DNMT1-WT or DNMT1-S878A) which treated 5mM glucose, or 25mM glucose with Thiamet-G.
(B) 5'UTR, (C) Promoter, (D) Gene body, (E) 3'UTR, (F) Intergenic regions.
(G) Genome browser screenshot of DNA methylation data at a differentially methylated region by glucose concentration.
(H) Heatmap represent global DNA methylation of wild type and DNMT1 mutants between low FPKM regions and high FPKM regions (DNMT1-WT or DNMT1-S878A) which treated 5mM glucose, or 25mM glucose with Thiamet-G were determined by Nanopolish call methylation. These are defined 'low FPKM' as containing less than 25% of RPKM regions per Mb window, and 'high FPKM' as containing more than 75% of RPKM regions per Mb window.
(I) The distribution of each DNA methylation was divided by DNA replication timing.
(J) Stitched browser plot showing 4 most conserved escapee genes and their methylation state (*MINDY3*, *ZWINT*, *ALG10B*, and *MAP3K7*).

Figure S5. DNA loss of methylation by increased global *O*-GlcNAcylation decreases around the transposable elements (TEs) regions.
(A) Boxplot represents the levels of DNA methylation on the TE regions or non-TE regions of each Myc-DNMT1 overexpressed mutants (DNMT1-WT or DNMT1-S878A) were treated 5mM glucose, or 25mM glucose with Thiamet-G.
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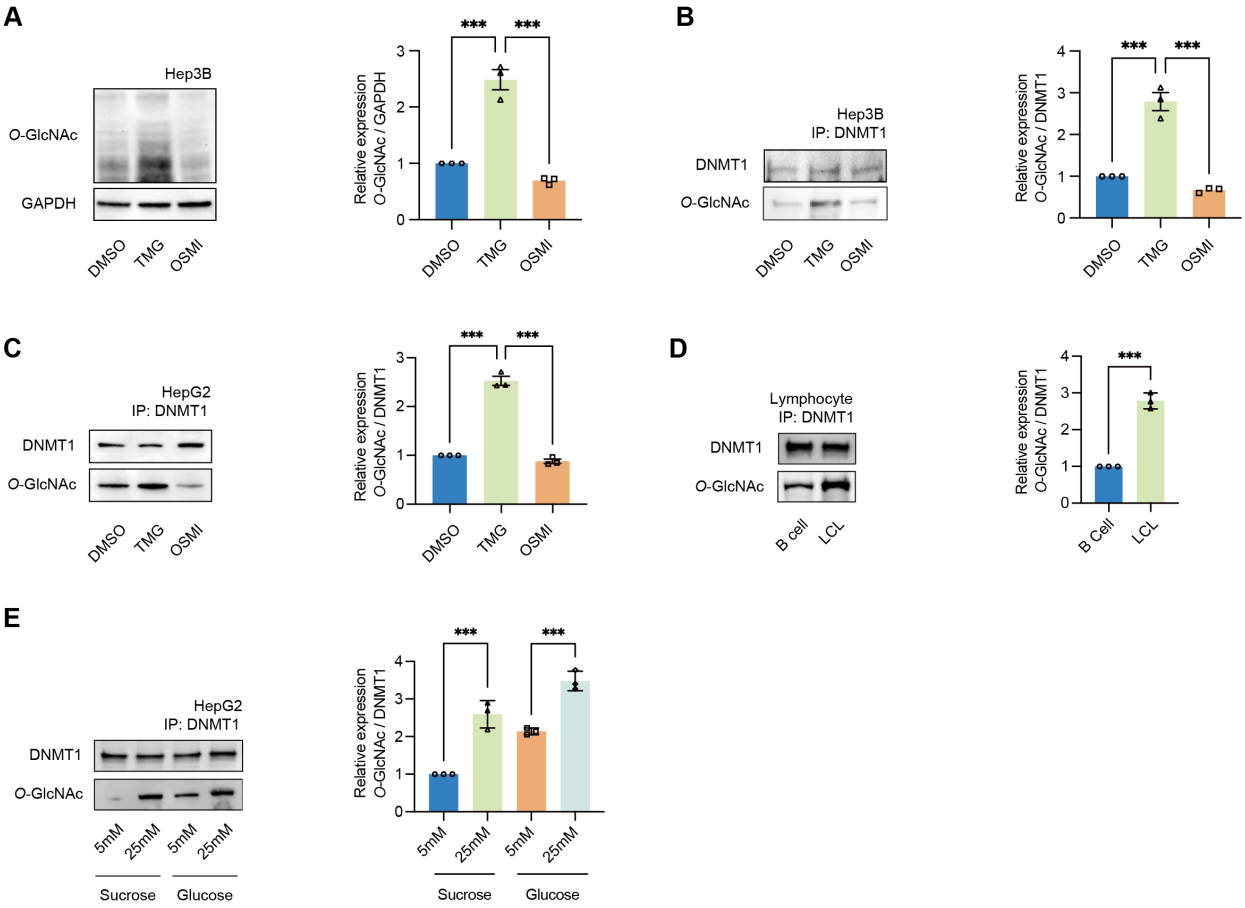
(B) SINE, (C) LINE, (D) LTR regions.

(E) Shown are methylation density around LTR12C regions of each Myc-DNMT1 overexpressed mutants (DNMT1-WT or DNMT1-S878A) were treated 5mM glucose, or 25mM glucose with Thiamet-G.
(F) Genome browser screenshot of DNA methylation data promoter region of *KCNAB1* by glucose concentration.
(G) Boxplot represents the DNA methylation by clades of the human genome (*Homo sapiens* to *Haplorhini*).

Supplementary Tables

Table S1. Prediction of *O*-GlcNAcylated sites within DNMT1 using OGTsite

Table S2. List of total identified proteins



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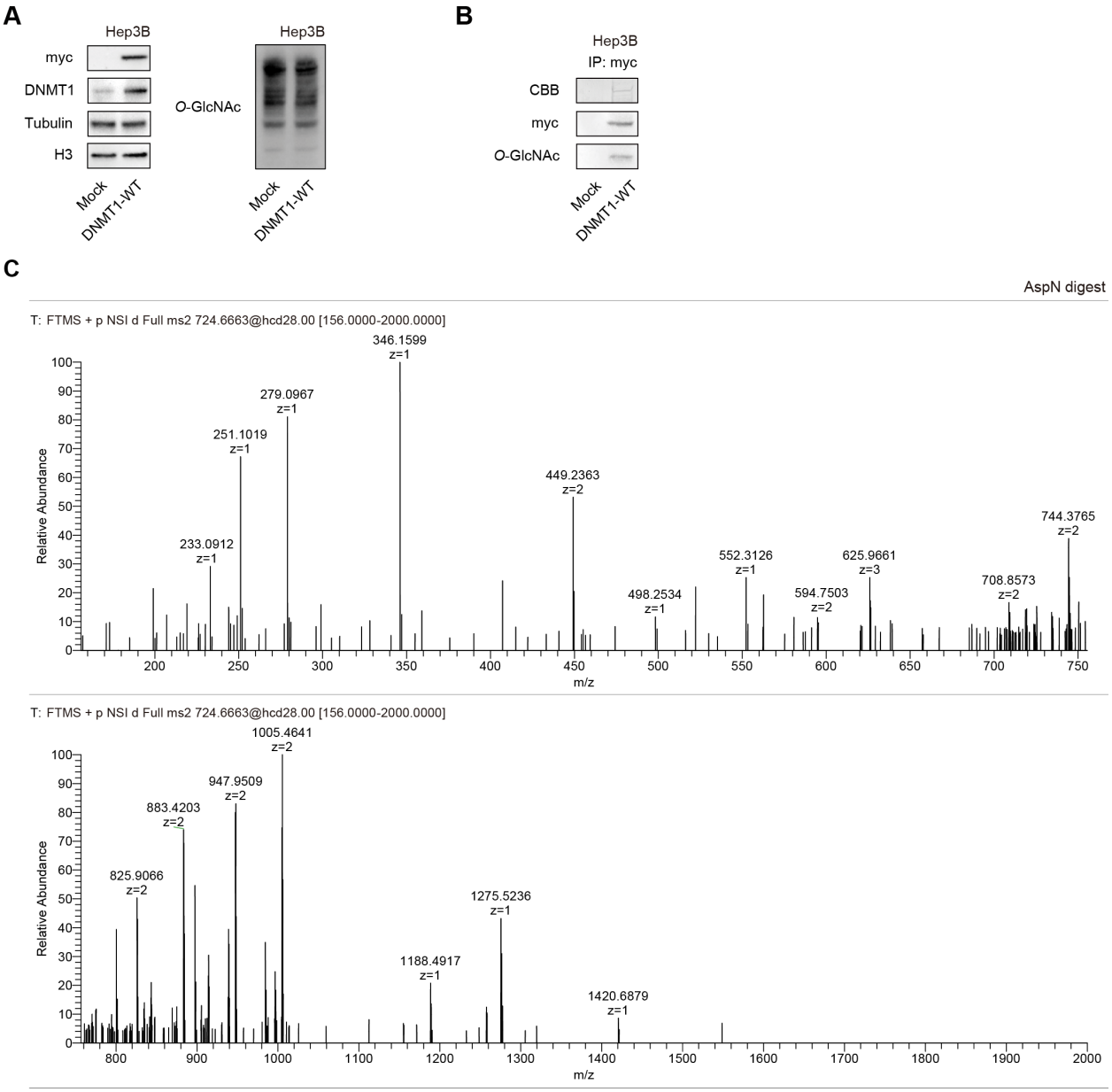

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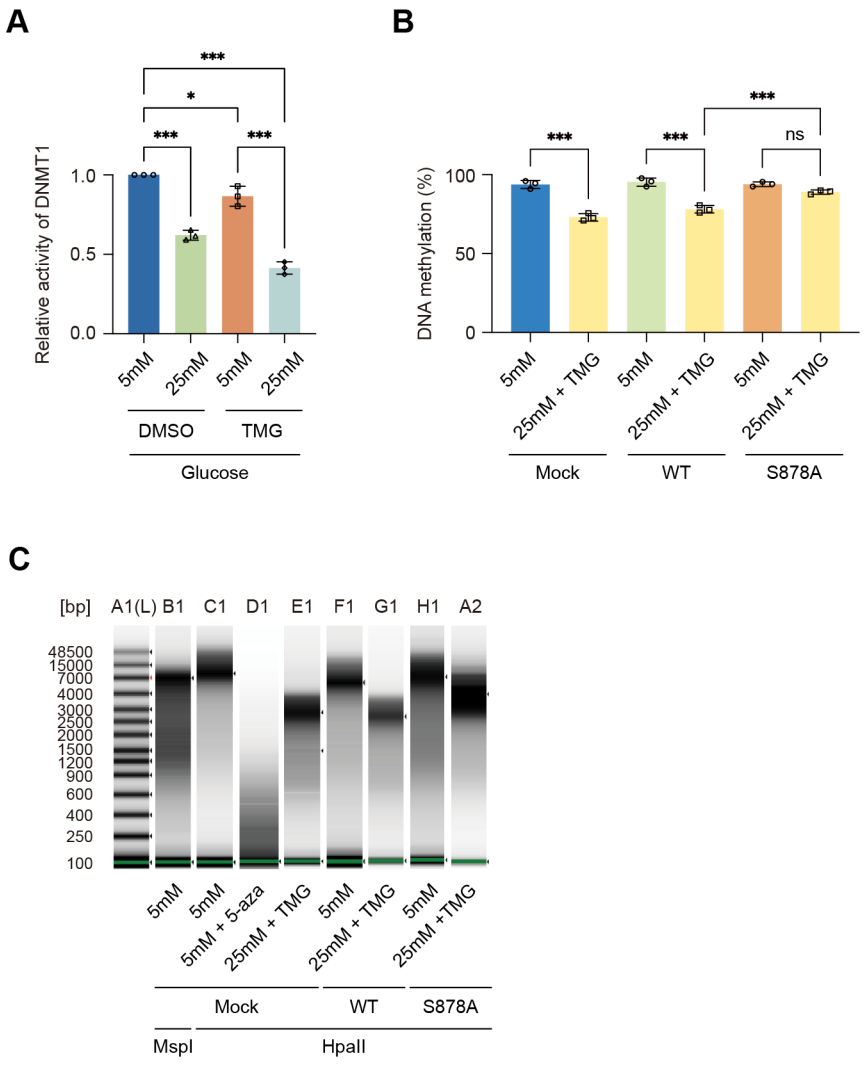

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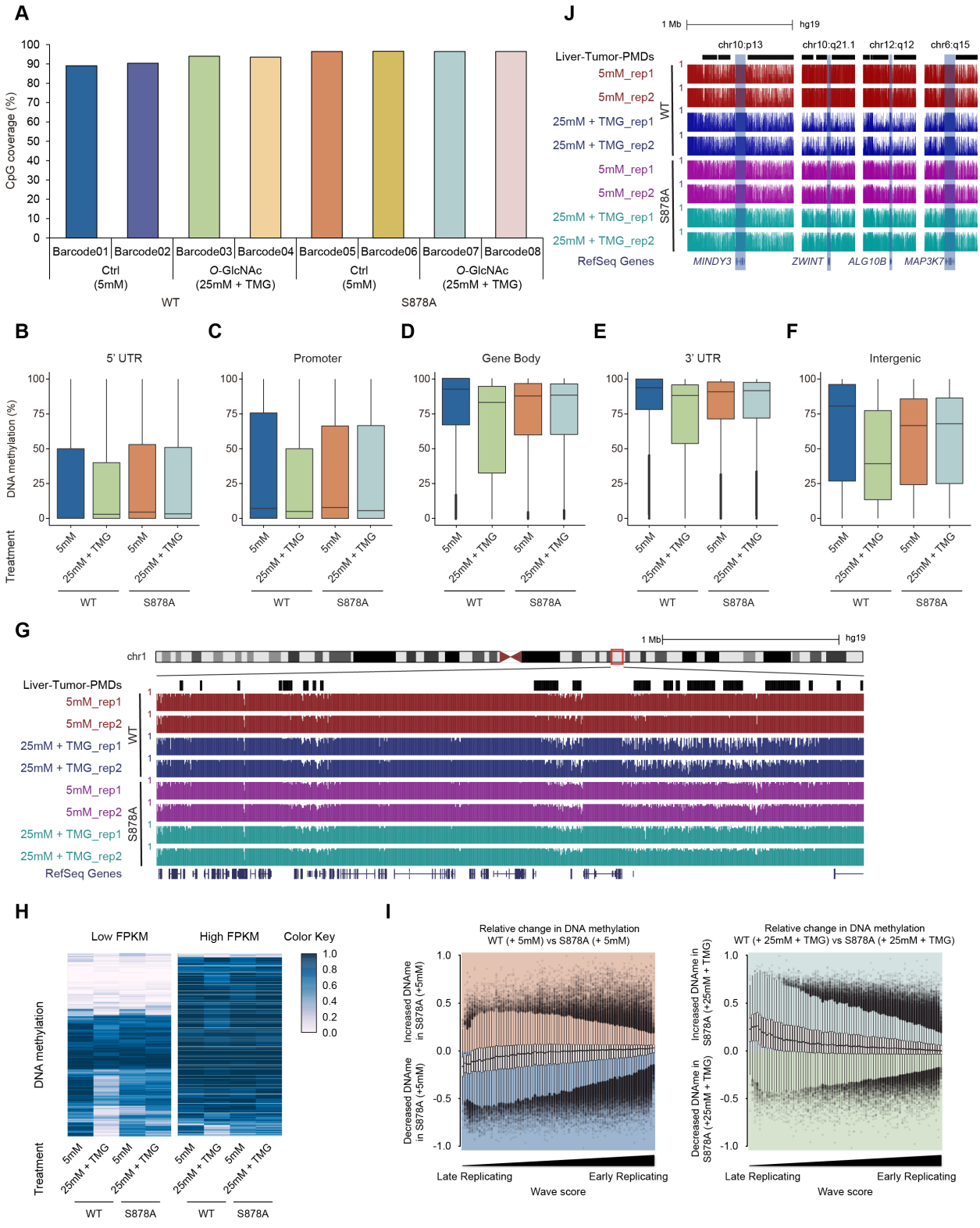
A
CpG coverage (%)
Barcode01
Barcode02
Barcode03
Barcode04
Barcode05
Barcode06
Barcode07
Barcode08
Ctrl (5mM)
O-GlcNAc (25mM + TMG)
Ctrl (5mM)
O-GlcNAc (25mM + TMG)
WT
S878A
J
1 Mb
hg19
chr10:p13
chr10:q21.1
chr12:q12
chr6:q15
Liver-Tumor-PMDs
5mM_rep1
5mM_rep2
25mM + TMG_rep1
25mM + TMG_rep2
WT
S878A
RefSeq Genes
MINDY3
ZWINT
ALG10B
MAP3K7
B
5' UTR
C
Promoter
D
Gene Body
E
3' UTR
F
Intergenic
DNA methylation (%)
Treatment
5mM
25mM + TMG
G
chr1
H
Low FPKM
High FPKM
Color Key
DNA methylation
I
Relative change in DNA methylation
WT (+ 5mM) vs S878A (+ 5mM)
WT (+ 25mM + TMG) vs S878A (+ 25mM + TMG)
Increased DNAme in S878A (+5mM)
Decreased DNAme in S878A (+5mM)
Increased DNAme in S878A (+25mM + TMG)
Decreased DNAme in S878A (+25mM + TMG)
Late Replicating
Early Replicating
Wave score

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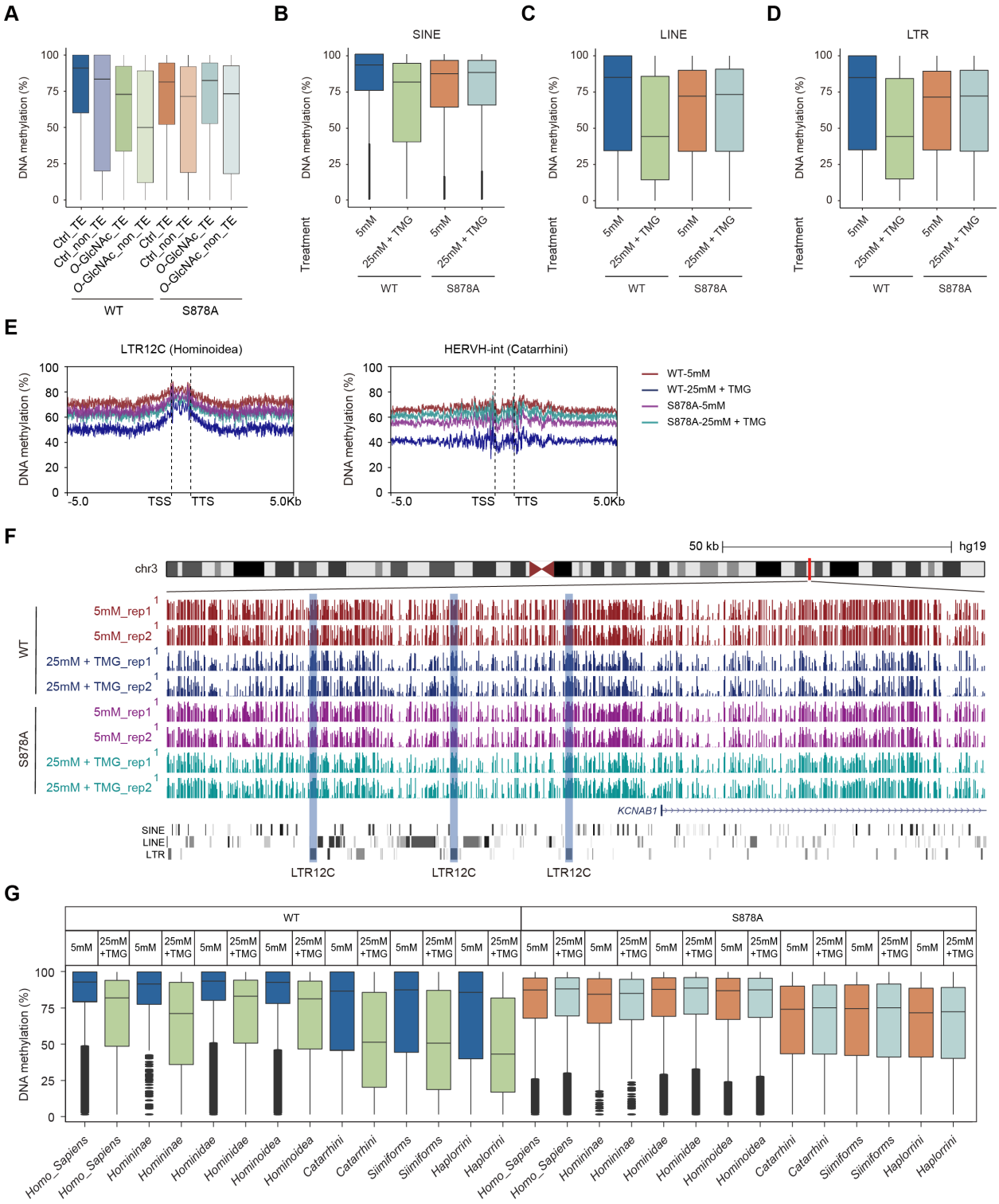

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