Epigenetics Insights

ARTICLE

Open Access

https://doi.org/10.48130/epi-0026-0007

Epigenetics Insights **2026**, 19: e010

Obesity-associated DNMT downregulation is linked to DNA hypomethylation of oncogenic pathways in endometrioid endometrial cancer

Harun Cingoz, Huma Asif and J. Julie Kim*

Department of Obstetrics and Gynecology, Northwestern University, Feinberg School of Medicine, 303 E. Superior Street, 4-117 Chicago, IL 60611, USA

* Correspondence: j-kim4@northwestern.edu (Kim JJ)

Abstract

Obesity plays a crucial role in the development and prognosis of endometrial cancer (EC), yet the molecular mechanisms underlying this association remain incompletely understood. In this retrospective comparative study, we investigated obesity-associated DNA methylation alterations in endometrioid EC using data from 229 patients in The Cancer Genome Atlas (TCGA). Patients were stratified by body mass index (BMI) (lean < 25; obese ≥ 30). Differential methylation analysis using the limma framework (FDR-adjusted $p < 0.05$, $|logFC| > 1$) identified 311 differentially methylated CpG sites (DMCs), 71% of which were hypomethylated in tumors from obese patients. This hypomethylated profile was accompanied by significant downregulation of DNA methyltransferases *DNMT1*, *DNMT3A*, and *DNMT3B*. Stratification by DNMT expression revealed that *DNMT3A*- and *DNMT3B*-low tumors displayed the most extensive methylation changes. CRISPR/Cas9 mediated *DNMT3A* knockout (KO) in HEC1B generated 2,000 DMCs, including 37 shared with obesity associated alterations. Pathway enrichment demonstrated convergence on estrogen response, PI3K/AKT/mTOR, TGF-β signaling, and IL-2/STAT5 signaling. *INPP5F*, a negative regulator of PI3K/AKT signaling implicated in insulin sensitivity, was hypomethylated at the gene body CpG shared between obese tumors and DNMT3A KO cells, showed reduced expression in tumors from obese patients, and was associated with decreased overall survival. These findings suggest that DNMT downregulation may contribute to obesity-associated epigenetic remodeling in EC and could identify candidate methylation-associated genes linking metabolic dysfunction to disease progression.

Keywords: Endometrial cancer, Obesity, Methylation, DNMT

Citation: Cingoz H, Asif H, Kim JJ. 2026. Obesity-associated DNMT downregulation is linked to DNA hypomethylation of oncogenic pathways in endometrioid endometrial cancer. *Epigenetics Insights* 19: e010 https://doi.org/10.48130/epi-0026-0007

Introduction

Obesity, a multifaceted global health issue, is marked by excessive fat accumulation that poses severe health risks. Classified by a body mass index (BMI) ≥ 30, obesity is influenced by a combination of genetic, environmental, lifestyle, and metabolic factors. The global prevalence of obesity in women has more than doubled since 1990. Recent data reveal that there are 172 million individuals with overweight and obesity, which is a concerning increase from past decades in the USA[1]. Projections suggest that by 2030, approximately half of the US adults will fall into the obese category[2].

Endometrial cancer (EC) ranks as the most prevalent gynecological malignancy among women, with an estimated 69,120 new cases anticipated in the United States in 2025, leading to around 13,860 deaths[3,4]. The occurrence of EC continues to climb, increasing by approximately 1% annually[5]. Research has shown a direct correlation between BMI and the risk of developing EC, indicating that a higher BMI substantially heightens this risk[6]. A comprehensive meta-analysis conducted by the American Institute for Cancer Research, encompassing 26 studies, found that a five-unit increase in BMI correlates with a 50% elevated risk of EC[7].

Beyond increasing EC risk[8], obesity is associated with chronic inflammation, insulin resistance, and hyperinsulinemia, which may contribute to the progression of EC[7,9]. Obesity impacts cancers in multiple ways, increasing the likelihood of recurrence, disease progression, and metastasis, making disease management more challenging[10,11]. In EC specifically, obesity is associated with significantly worse outcomes. Calle et al. reported that compared to lean patients, obese patients with a BMI of 30–35 have a 2.5-fold increased risk of disease-specific mortality, while morbidly obese patients with a BMI greater than 40 have a 6.25-fold increased risk[12]. Meta-analyses have further quantified the impact of obesity on EC outcomes. Petrelli et al. showed that obese patients have a 20% increased risk of mortality compared to lean patients (HR 1.20; 95% CI: 1.04–1.38)[13]. Similarly, Kokts-Porietis et al. demonstrated that higher BMI is linked to increased all-cause mortality in Type I (HR 1.22; 95% CI: 1.02–1.46). Notably, BMI was also associated with increased recurrence risk in Type I tumors (HR 1.37; 95% CI: 1.03–1.84), but not in Type II tumors[11]. Obesity can also diminish the efficacy of chemotherapy due to altered drug pharmacokinetics and suboptimal dosing practices[14]. Additionally, obesity can adversely affect the quality of life in cancer survivors; for example, obese survivors of breast cancer face a heightened risk of developing treatment-related lymphedema[15]. Obesity is also associated with increased surgical morbidity, including prolonged hospitalization, wound infection, increased antibiotic use, and venous thromboembolism in EC patients[16].

Emerging evidence suggests that obesity induces epigenetic modifications in DNA. A landmark study by Wahl et al. identified 187 genetic loci where DNA methylation is significantly associated with BMI, with genetic association analyses demonstrating that these methylation alterations are predominantly a consequence rather than a cause of adiposity[17]. Beyond systemic effects, research has highlighted that dietary factors and body weight can alter DNA

https://doi.org/10.48130/epi-0026-0007

methylation patterns in specific tissues, potentially disrupting genes involved in cellular growth and differentiation[18]. Aberrant methylation patterns are frequently observed in cancer, typically involving hypermethylation of tumor suppressor genes and hypomethylation of oncogenes. In EC specifically, hypermethylation of tumor suppressor gene promoters, including *MLH1*, *PTEN*, *RASSF1A*, and *CDH1*, has been extensively documented and contributes to transcriptional silencing of these critical regulatory genes[19–21]. DNA methyltransferases (DNMTs), particularly *DNMT1*, *DNMT3A*, and *DNMT3B*, play central roles in both the establishment and maintenance of DNA methylation patterns. *DNMT1* primarily functions during DNA replication to maintain methylation patterns across cell division, while *DNMT3A* and *DNMT3B* are responsible for *de novo* methylation. Dysregulation of DNMTs has been implicated in cancer development. Studies have linked high DNMT3B levels to elevated global DNA methylation in colon cancer[22], while *DNMT1* knockout leads to genomic instability and hypomethylation in intestinal cancer[23]. In EC, overexpression of DNMTs is associated with increased hypermethylation[24], and notably, both *DNMT1* and *DNMT3B* are overexpressed in endometrioid carcinomas compared to normal endometrium, whereas expression of these enzymes is decreased in uterine serous carcinomas[25].

Despite the well-established association between obesity and EC, the underlying epigenetic mechanisms linking obesity to tumor development and progression remain incompletely understood. Although dysregulation of DNA methyltransferases has been implicated in cancer, the relationship between obesity, DNMT expression, and genome-wide methylation patterns in EC has not been systematically explored. Furthermore, whether reduced DNMT expression contributes to obesity-associated hypomethylation, rather than a secondary consequence, remains unknown. Addressing these gaps is critical for understanding how metabolic dysfunction influences epigenetic regulation in cancer. We therefore hypothesized that obesity is associated with reduced DNMT, leading to widespread DNA hypomethylation and activation of oncogenic pathways that contribute to endometrial tumor progression.

In this study, we analyzed DNA methylation data from 229 endometrioid EC patients in The Cancer Genome Atlas (TCGA) to examine obesity related epigenetic differences. Obese patients exhibited increased tumor hypomethylation, potentially due to reduced expression of *de novo* DNA methyltransferases. Pathway enrichment analysis revealed that hypomethylated genes were enriched in oncogenic signaling cascades implicated in EC progression, including PI3K/AKT/mTOR, Wnt/β-catenin, and early estrogen response pathways. To validate these findings, CRISPR/Cas9 mediated knockout of *DNMT3A* and *DNMT3B* in EC cells was used to identify shared differentially methylated genes that recapitulated the obesity-associated signature. Together, these data support a mechanistic link between reduced DNMT activity and obesity-associated epigenetic alterations in EC.

Materials and methods

Methylation data access and processing

Methylation and clinical data for EC were acquired from The Cancer Genome Atlas (TCGA)[26] utilizing the TCGABiolinks package (v2.18.0)[27]. The methylation profiles were produced with the Illumina Infinium Human Methylation 450 Beadchip (450K array). From a total of 482 tumor samples, cases were filtered based on histological subtype (endometroid type) and body mass index (BMI), resulting in 229 tumor samples (189 from obese patients, BMI ≥ 30, and 40 from lean patients, BMI < 25). We excluded the overweight group (25 < BMI < 30) as it is metabolically and epigenetically heterogeneous, to avoid introducing intermediate phenotypes that could reduce between-group contrast and attenuate differential signals. The lean group contains two underweight individuals. Given this very limited number, we retained these samples within the lean group to preserve statistical power, as excluding them would not meaningfully alter the analysis but would further reduce an already small group. Methylation levels were calculated as beta values, defined as Meth/(UnMeth + Meth), where 'Meth' and 'UnMeth' represent the methylated and unmethylated probe intensities on the Illumina 450K array. We excluded probes associated with SNPs (with a minor allele frequency > 0.05), incomplete data, and cross-reactive probes that mapped to multiple genomic locations to reduce false positives[28]. Probes with greater than 20% missing values across samples were removed. The remaining beta values were subject to quantile normalization (betaqn) using the watermelon package to reduce technical variability. For improved statistical analysis, we transformed the beta values into M values, which are represented as Log2 (beta/1-beta)[29].

Methylation analysis

The Limma R package (v3.66.0) was used to detect differentially methylated CpG sites (DMCs) between tumor samples from obese and lean individuals. A linear modeling framework was applied to M values, and empirical Bayes moderation was used to improve variance estimation. The models were further extended to incorporate clinical covariates, including age at diagnosis and tumor stage, using an additive design. Statistical significance was defined using a false discovery rate (FDR)-adjusted *p*-value threshold of 0.05 and an absolute log fold change greater than 1 ($|logFC| > 1$)[30]. DMCs with higher average methylation levels in tumors from lean patients than in tumors from obese patients were labeled as hypermethylated in lean or hypomethylated in obese samples. The genomic annotation for these methylation probes followed the Illumina protocol, specifically IlluminaHumanMethylation450kanno.ilmn12.hg19. For the DNA methylation analysis comparing DNMT3A/B low-expressing patients to DNMT3A/B high-expressing patients, RNA sequencing counts for DNMT3A/B from all patients were downloaded from TCGA. The raw counts were normalized to $\log_2$ counts per million (CPM) using the edgeR package. Patients were stratified into quartiles based on their DNMT3A and DNMT3B expression levels across the cohort. This approach defines groups relative to the distribution of expression within the dataset. Accordingly, samples in the first quartile (Q1), representing the lowest 25% of expression values, were classified as DNMT3A/B low expression, while samples in the fourth quartile (Q4), representing the highest 25%, were classified as DNMT3A/B high expression. Although this stratification is rank-based, the corresponding expression ranges were also recorded for transparency. For DNMT3A, the Q1 and Q4 thresholds corresponded to expression values of ≤ 5.23 and ≥ 6.02, respectively. For DNMT3B, the corresponding values were ≤ 3.07 (Q1) and ≥ 4.46 (Q4). DNA methylation analysis was then performed between these two groups (Q1 vs. Q4), resulting in balanced group sizes of 76 patients per group.

Correlation analysis

Raw RNA-seq data were obtained from TCGA as RSEM counts generated on Illumina platforms using the TCGABiolinks package (v2.18.0). In the resulting matrix, rows corresponded to gene IDs and columns to individual samples. Genes with low expression were

removed, and the raw counts were normalized to $\log_2$ CPM using the edgeR package. To explore the relationship between methylation changes and gene expression, we selected samples that had both methylation and expression data. Differentially methylated CpG sites were identified as described above based on statistical significance (false discovery rate [FDR] < 0.05) and effect size (|logFC| > 1 in M values). CpG probes were annotated to their corresponding genes using Illumina 450K annotation, and only probes with valid gene annotations were included. For each CpG-gene pair, β values and gene expression levels were extracted across matched samples. Spearman rank correlation analysis was performed using the cor.test function in R to assess the association between methylation and gene expression. Correlations were computed only for CpG-gene pairs with at least three samples containing non-missing paired measurements. The resulting correlation coefficients and *p*-values were recorded, and statistically significant correlations were defined as those with *p*-values < 0.05.

Enrichment analysis

Enrichment analysis was conducted using the Enrichr R package[31], focusing on genes that were hypomethylated and annotated in obese patients. These genes were identified from DNA methylation analysis, applying an FDR-adjusted *p*-value threshold of 0.05 and an absolute log fold change greater than 1. Duplicate genes were removed prior to analysis. The resulting list of unique hypomethylated genes was submitted to Enrichr, and enrichment results were obtained for the MSigDB Hallmark gene sets. Pathways were ranked based on *p*-values, and the top enriched terms were selected for visualization ($p < 0.05$). In a similar manner, we performed enrichment analysis for hypomethylated and annotated genes from DNMT3A/B low patients. These genes were also selected based on the results of DNA methylation analysis, applying the same thresholds.

CRISPR/Cas9 mediated gene knockout

DNMT3A and DNMT3B knockout (KO) HEC1B cell lines were generated using the CRISPR/Cas9 system. Four candidate single-guide RNA (sgRNA) sequences per gene were selected from the Brunello[32] genome-wide library (Addgene #73178) and ordered as oligonucleotides (Integrated DNA Technologies, Iowa, USA). Sequences are as follows: DNMT3A gRNA1_F: CACCGCCGGGAACAG CTTCCCCGCG. DNMT3A gRNA1_R: AAACCGCGGGGAAGCTGTTCC-CGGC. DNMT3A gRNA3_F: CACCGGCGGGCACAAGGGTACCTAC. DNMT3A gRNA3_R: AAACGTAGGTACCCTTGTGCCCGCC. DNMT3B gRNA1_F: CACCGCCATGTGGACGAGTCCCCCG. DNMT3B gRNA1_R: AAACCGGGGGACTCGTCCACATGGC. DNMT3B gRNA3_F: CACCGGG CCTTCCAAGACACCACCA. DNMT3B gRNA3_R: AAACTGGTGGTGTCT TGGAAGGCCC. NT (Non-targeting) F: CACCGACAACTTTACCGAC-CGCGCC. NT (Non-targeting) R: AAACGGCGCGGTCGGTAAAGTTGTC. NT oligos were used as a control. Oligonucleotides were annealed by mixing 10 μM of each strand in annealing buffer (10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA), incubating at 95 °C for 5 min, and cooling gradually to room temperature. The annealed duplexes were diluted 1:200 and ligated into BsmBI-digested p413-Cas9-puromycin (Addgene #52961) using T4 DNA ligase (NEB, #MO202) overnight at 16 °C. Ligation products were transformed into NEB Stable competent *E. coli* (#C3040H). Following selection on LB-ampicillin plates, plasmid DNA was isolated (Qiagen Miniprep, #27206) and validated via Sanger sequencing. Validated constructs were packaged into lentiviruses using HEK293T cells. HEC1B cells were transduced with the resulting viral supernatants in the presence of 10 μg/mL polybrene for 8 h. Two days after infection, cells were selected with 2 μg/mL puromycin. Each sgRNA construct was processed independently until knockout efficiency was evaluated.

Western Blot analysis

To evaluate knockout efficiency, whole-cell lysates were harvested from HEC1B cells using RIPA buffer (Cell Signaling Technology, #9806S) and Halt protease inhibitor mix (Thermo Scientific, #1860932). A Pierce BCA Assay Kit was used to measure protein concentrations (Thermo Scientific, #23227). Samples were mixed with 4x LDS Sample Buffer (Invitrogen, #NP0007), denatured at 95 °C for 10 min, and 40 ug loaded onto NuPAGE 4%–12% Bis-Tris gels (Invitrogen, #NP0335) and run at 100 V for 2 h. Proteins were transferred onto PVDF membranes (Invitrogen, #IB24002) using the iBlot 2 Dry Blotting System. Membranes were blocked for 1 h in 5% milk dissolved in TBS-T (20 mM Tris, 150 mM NaCl, 0.1% Tween 20; pH 7.6) and incubated with primary antibody overnight at 4 °C in 5% milk/TBS-T (1:1,000 dilution) using the following antibodies: anti-DNMT3A (Cell Signaling, #D23G1), anti-DNMT3B (Cell Signaling, #D7070), and anti-GAPDH (Cell Signaling, #14C10). TBS-T was used to wash the membranes three times (10 min each), followed by incubation with an anti-rabbit secondary antibody (1:1,000 dilution; Promega, #W4011) for 1 h. Following three final washes in TBS-T, immunoblots were visualized using SuperSignal West Femto reagent (Thermo Scientific, #34094) on an iBright 1500 imaging system. GAPDH was used as a loading control.

Survival analysis

To determine the clinical relevance of our target genes in a relevant cancer model, we queried the Kaplan–Meier Plotter database[33]. We selected the Uterine Corpus Endometrial Carcinoma (UCEC) dataset ($n = 543$) for analysis. The system filtered patients based on gene expression levels, and survival plots were generated to visualize the relationship between gene expression and Overall Survival (OS). The log-rank test and Hazard Ratio (HR) were computed automatically by the platform to determine the statistical significance of the survival differences between the high and low expression cohorts.

Statistical analysis

All statistical analyses were performed in R (v4.5.2). Differential methylation analysis was conducted using the limma package with empirical Bayes moderation. Multiple testing correction was performed using the Benjamini–Hochberg method to control the false discovery rate. Group comparisons for gene expression were performed using the Wilcoxon rank sum test. Correlation analyses were performed using Spearman rank correlation. Statistical significance was defined as $p < 0.05$.

Results

Patient characteristics of the TCGA endometioid EC cohort

We analyzed the transcriptome and methylation data from the TCGA for 229 endometrioid EC patients. Clinical characteristics of the samples included in the analysis are shown in Table 1. All samples considered were endometrioid adenocarcinoma, with tumor grades ranging from 1 to 3. Among these patients, 189 (82.5%) were classified as obese (BMI > 30), ranging from 30 to 67.9,

https://doi.org/10.48130/epi-0026-0007

Table 1. Clinical characteristics of analyzed tumor samples.

Clinical features	*n*	Lean[1]	Obese[1]
Age (years)	229		
≤ 50		8 (20%)	23 (12%)
50–64		17 (43%)	95 (50%)
≥ 65		15 (38%)	70 (37%)
Age not reported		0 (0%)	1 (0.5%)
Race (self-reported)	229		
Black or African American		3 (7.5%)	40 (21%)
Not reported		2 (5.0%)	7 (3.7%)
Other		4 (10%)	9 (4.8%)
White		31 (78%)	133 (70%)
FIGO_stage	229		
I		27 (68%)	129 (68%)
II		4 (10%)	20 (11%)
III		9 (23%)	34 (18%)
IV		0 (0%)	6 (3.2%)
Tumor grade	229		
Grade 1		3 (7.5%)	36 (19%)
Grade 2		7 (18%)	52 (28%)
Grade 3		16 (40%)	45 (24%)
Not available		14 (35%)	56 (30%)
Recurrence	229		
Yes		3 (7.5%)	22 (12%)
No		21 (53%)	100 (53%)
Not available		16 (40%)	67 (35%)

[1] n (%).

and 40 (17.5%) as lean (BMI < 25), ranging from 17.3 to 25. We excluded individuals with a BMI of 25–30 (overweight category) from the analysis to maintain distinct separation between the normal and obese groups, minimizing overlap and reducing potential noise in the data. In both the obese and lean groups, the majority of patients were over 50 years old. While 70% or more of patients in both groups were white (79% lean, 70% obese), the obese group had a higher percentage of black or African American patients (21%) compared to the lean group (7.5%). The FIGO stage distribution for obese patients was Stage I (68%), Stage II (11%), Stage III (18%), and Stage IV (3.2%). For lean patients, the distribution was: Stage I (68%), Stage II (10%), and Stage III (23%). There were no Stage IV samples for lean patients. While recurrence was observed in 12% of obese patients, it was 7.5% in lean patients.

DNA methylation in EC tumors of obese and lean patients

To investigate obesity-associated DNA methylation alterations, we analyzed TCGA methylation profiles from 229 endometrioid endometrial cancers (grades 1–3) stratified into obese and lean groups using BMI criteria (Fig. 1a). This analysis identified 311 differentially methylated CpG sites (DMCs) between obese and lean patients (Fig. 1b). Strikingly, most of these changes represented loss of methylation in tumors from obese patients, with 71.1% of DMCs hypomethylated and 28.9% hypermethylated. Genomic context analysis showed that hypomethylated CpGs were preferentially enriched in open sea regions (52%) and CpG islands (25%), whereas hypermethylated sites were found predominantly in open sea regions (77%) with only a small fraction mapping to CpG islands (4%) (Fig. 1d). Annotation of these CpGs to the hg19 genome identified 198 differentially methylated genes (DMGs), of which 74.2% were hypomethylated. Examination of the top-ranked genes further highlighted obesity specific patterns. Among the most hypomethylated genes were *THBD*, *FOSB*, *AKAP13*, *SLC45A4*, and *FSTL4*, many of which have roles in angiogenesis, transcriptional regulation, and signal transduction. In contrast, the most hypermethylated genes included *OSTBETA*, *SEZ6L2*, *KCN5I*, and *SLAMF1*, several of which are involved in cell adhesion and developmental signaling (Fig. 1c). Functional enrichment analysis confirmed that hypomethylated genes in tumors from obese patients were strongly associated with oncogenic and hormone responsive pathways, including TGF-β signaling, estrogen response, IL2/STAT5 signaling, androgen response, PI3K/AKT/mTOR signaling, and Wnt/β-catenin signaling (Fig. 1e).

Correlation between gene expression and DNA methylation

We next assessed whether obesity-associated DMGs were linked to transcriptional changes. To explore this, a correlation analysis was done. Our analysis revealed that 147 out of these 198 DMGs showed a significant correlation ranging from −0.69 to 0.82 (median = −0.04) between methylation levels and gene expression. Notably, the majority of these DMGs were hypomethylated, 104 out of 147 (Fig. 1f). We observed that the majority of hypomethylated genes were negatively correlated with gene expression (Fig. 1f), meaning that lower methylation levels were associated with increased expression. Among the 147 DMGs that correlated with gene expression, 43 were hypermethylated. Interestingly, unlike the hypomethylated genes, most of the hypermethylated genes showed a positive correlation with gene expression (Fig. 1f), meaning that despite increased methylation, these genes were also more highly expressed in obese patients.

DNMTs are downregulated in EC tumors of obese patients

Next, to understand the mechanism underlying obesity related changes in the methylation profile, DNA methylation-related enzymes were compared in tumors from obese and lean patients, focusing on the DNA methyltransferases (DNMTs), *DNMT1*, *DNMT3A*, and *DNMT3B*. Our analysis showed a significant reduction in *DNMT1*, *DNMT3A*, and *DNMT3B expression* in tumors from obese patients compared to lean patients (Fig. 2a–c).

Low levels of DNMTs are associated with increased hypomethylation in EC

To further study the correlation between DNMT expression and the methylation profiles of tumors, patients with endometrioid EC were sorted into low and high quartiles based solely on DNMT expression levels, regardless of their BMI. Methylation analysis was conducted using the patients' methylation data from the lowest (bottom 25%) and highest (top 25%) quartiles of DNMT expression (Fig. 3a).

For *DNMT1*, patients with low expression levels exhibited a total of 681 DMCs. Among these, 67.55% were hypomethylated while the remainder were hypermethylated (Fig. 3b). Analysis of *DNMT3A*, one of the *de novo* methyltransferases, revealed a broader effect. Low *DNMT3A* expression was associated with 1,232 DMCs, nearly twice as many as observed for *DNMT1*. Interestingly, although *DNMT3A* produced more overall changes, the balance between hypermethylation and hypomethylation was more even, with 58.52% of the sites showing hypomethylation and 41.48% showing hypermethylation (Fig. 3c). This suggests that altered *DNMT3A* levels influence both the gain and loss of DNA methylation across the genome, rather than strongly favoring one direction. In contrast, *DNMT3B* exhibited

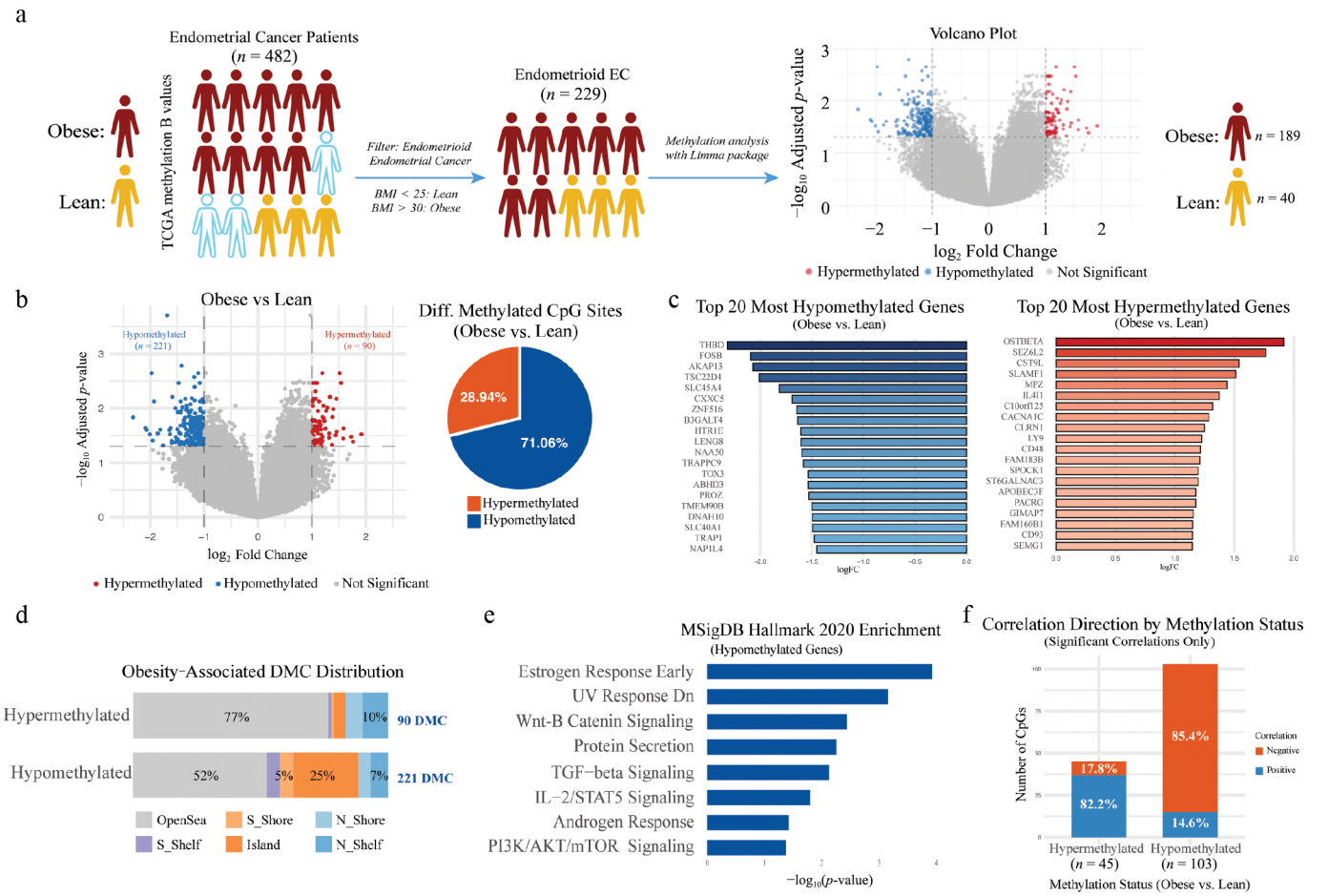

Fig. 1 The study design and obesity-associated DNA methylation features in endometrial cancer. (a) Schematic of study design illustrating the selection of 229 endometrioid endometrial cancer cases from TCGA (482 total tumor samples). (b) Volcano plot displaying DMCs between tumors from obese and lean patients, with hypomethylated sites shown in blue and hypermethylated sites in red (left). Pie chart summarizing the proportions of hypomethylated vs. hypermethylated CpGs among 311 total DMCs (right). (c) Bar plots showing the top 20 most hypomethylated genes (left) and top 20 most hypermethylated genes (right) in tumors from obese patients, ranked by log fold change. (d) Genomic context distribution of obesity-associated DMCs across CpG islands, shores, shelves, and open sea regions for both hypermethylated and hypomethylated sites. (e) MSigDB Hallmark 2020 enrichment analysis of hypomethylated genes in tumors from obese patients. (f) Correlation analysis between methylation status and gene expression among significantly correlated DMGs, showing the proportions of positive vs. negative correlations for hypermethylated and hypomethylated CpGs.

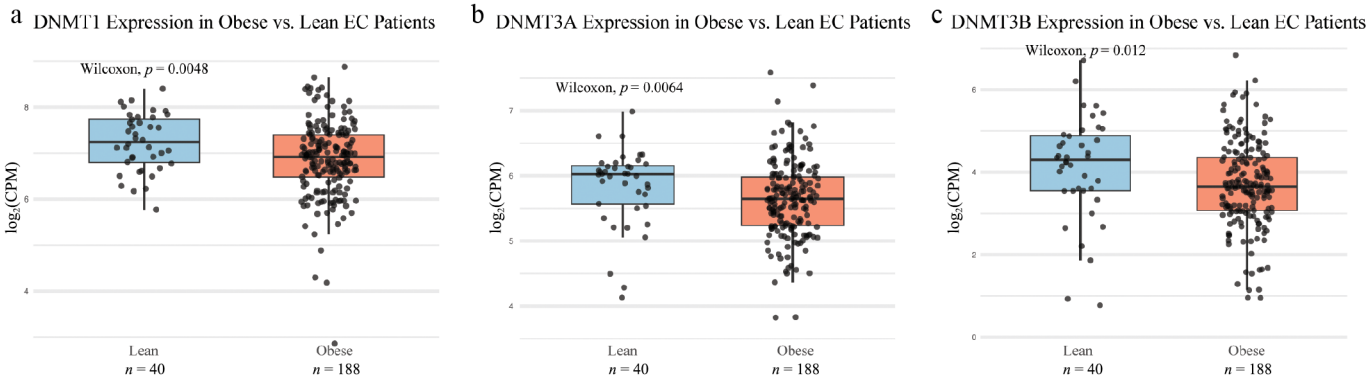

Fig. 2 The expression of DNMTs in obese vs. lean endometrial tumors. Boxplots comparing gene expression levels of (a) DNMT1, (b) DNMT3A, and (c) DNMT3B between lean and obese endometrial cancer patients. Expression values are displayed as $\log_2$ counts per million (CPM). Statistical significance was assessed using the Wilcoxon rank-sum test.

the most striking effect on the methylation landscape. Patients with low *DNMT3B* expression showed 3,080 DMCs, representing by far the largest number among the three DNMTs analyzed. Importantly, 85.49% of these sites were hypomethylated, indicating that *DNMT3B* loss is strongly associated with widespread hypomethylation (Fig. 3d). This pattern highlights *DNMT3B* as a key regulator of global methylation levels in endometrioid endometrial tumors. While *DNMT1* and *DNMT3A* contribute to both hypermethylation and hypomethylation, *DNMT3B* downregulation appears to drive a predominantly hypomethylated state.

https://doi.org/10.48130/epi-0026-0007

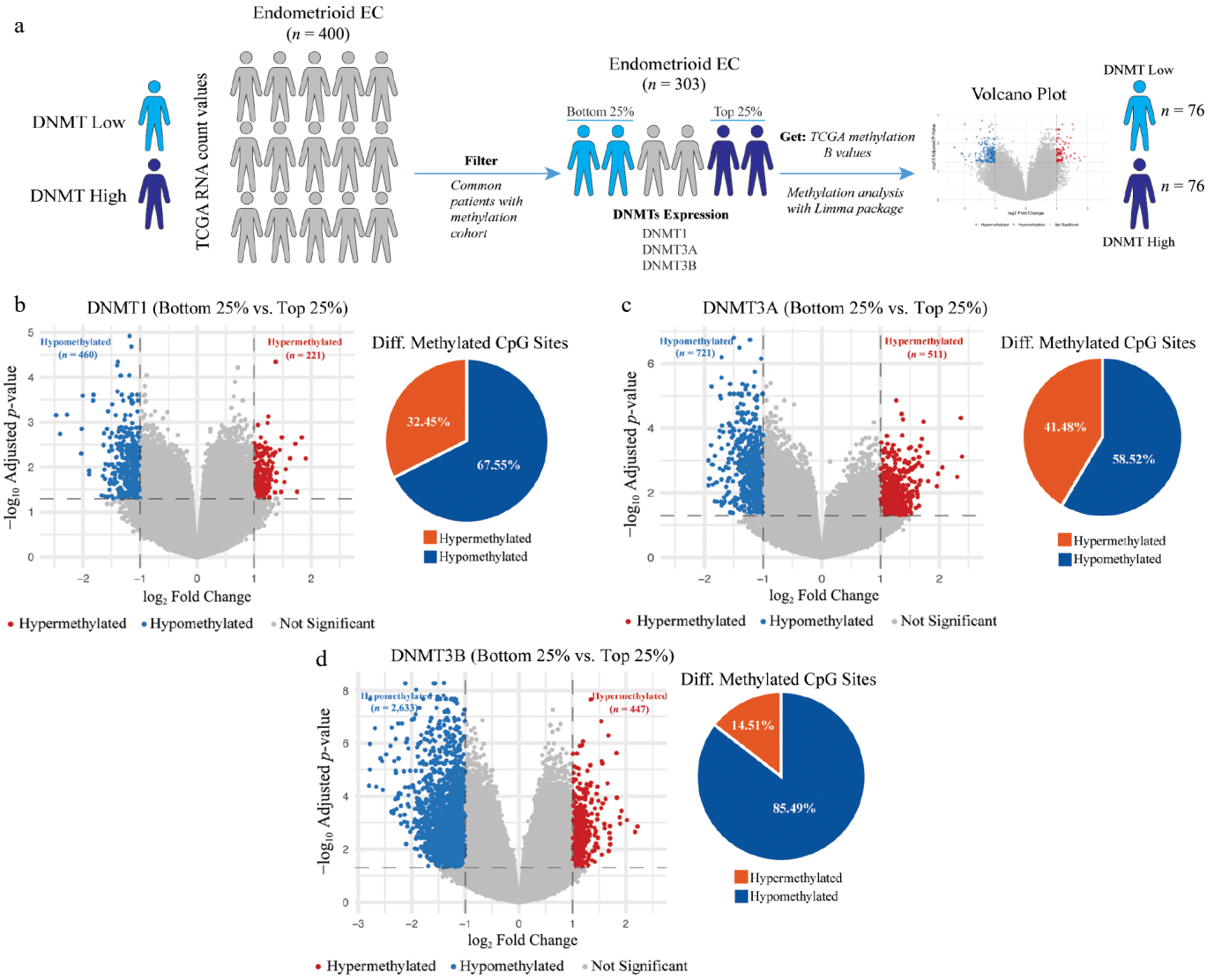

Fig. 3 DNMT-stratified methylation analysis in endometrial cancer. (a) Study design schematic showing stratification of 303 endometrioid EC patients with matched RNA expression and methylation data into DNMT low (bottom 25%) and DNMT high (top 25%) quartiles, followed by differential methylation analysis using the Limma package. (b)–(d) Volcano plots (left) and corresponding pie charts (right) displaying differentially methylated CpG sites between low and high expression groups for (b) DNMT1, (c) DNMT3A, and (d) DNMT3B. Significant hypomethylated sites are shown in blue and hypermethylated sites in red.

We next examined the genomic distribution, gene-level associations, and functional context of *DNMT3A*- and *DNMT3B*-associated DMCs. *DNMT3A*-associated DMCs exhibited different distributions between hypermethylated and hypomethylated sites. Among hypermethylated CpGs, 37% were located within CpG islands, whereas only 4% of hypomethylated CpGs fell in these regions. Instead, *DNMT3A*-associated hypomethylation predominantly occurred in open sea regions (68%) (Fig. 4a [left]). In contrast, *DNMT3B*-associated DMCs displayed an almost opposite pattern. Among the 3,080 *DNMT3B*-associated DMCs, hypermethylated sites were highly enriched in open sea regions (62%) and relatively sparse within CpG islands (13%). Remarkably, 45% of *DNMT3B*-associated hypomethylated CpGs were located in CpG islands (Fig. 4a [right]). Together, these distributional differences demonstrate that *DNMT3A* and *DNMT3B* contribute to distinct methylation programs, with each enzyme influencing different genomic compartments in EC. Gene-level annotation of DMCs revealed that *DNMT3A*- and *DNMT3B*-low tumors exhibit distinct and largely non-overlapping sets of differentially methylated genes, further supporting their divergent epigenomic functions. In *DNMT3A*-low patients, the most hypomethylated genes included *C10orf26*, *GFM1*, *LXN*, *CLSTN1*, *IGF1R*, *EMP2*, *CDK11B*, *ZNF418*, and others involved in growth factor signaling, metabolic regulation, and structural organization. Conversely, the top hypermethylated genes in *DNMT3A*-low tumors included *INPP5A*, *DNMT3A* itself, *GRASP*, *INPP5B*, *PTPRN2*, *CABP7*, *MIR125B1*, *KNDC1*, and *REEP6*, many of which participate in phosphoinositide metabolism, neuronal differentiation, or transcriptional control (Fig. 4b).

DNMT3B-low tumors demonstrated a different methylation profile. The most hypomethylated genes included *SLC12A8*, *MEIS2*, *TSC22D4*, *PLEKHH3*, *NFIC*, *AKAP13*, *DPF1*, *UCKL1*, *CDK2AP1*, and *GRLF1*, representing pathways associated with transcription factor regulation, chromatin remodeling, and cell adhesion. In contrast, hypermethylated targets in *DNMT3B*-low tumors were dominated by *C10orf93*, *ZNF831*, *LMF1*, *BAIAP2L2*, *PDE11A*, *ITIH5*, *ZBTB20*, *SFBMT1*, and *CLN8*, several of which have roles in lipid metabolism, immune signaling, or neuronal development (Fig. 4c). To better understand the biological consequences of *DNMT3A*- and *DNMT3B*-associated

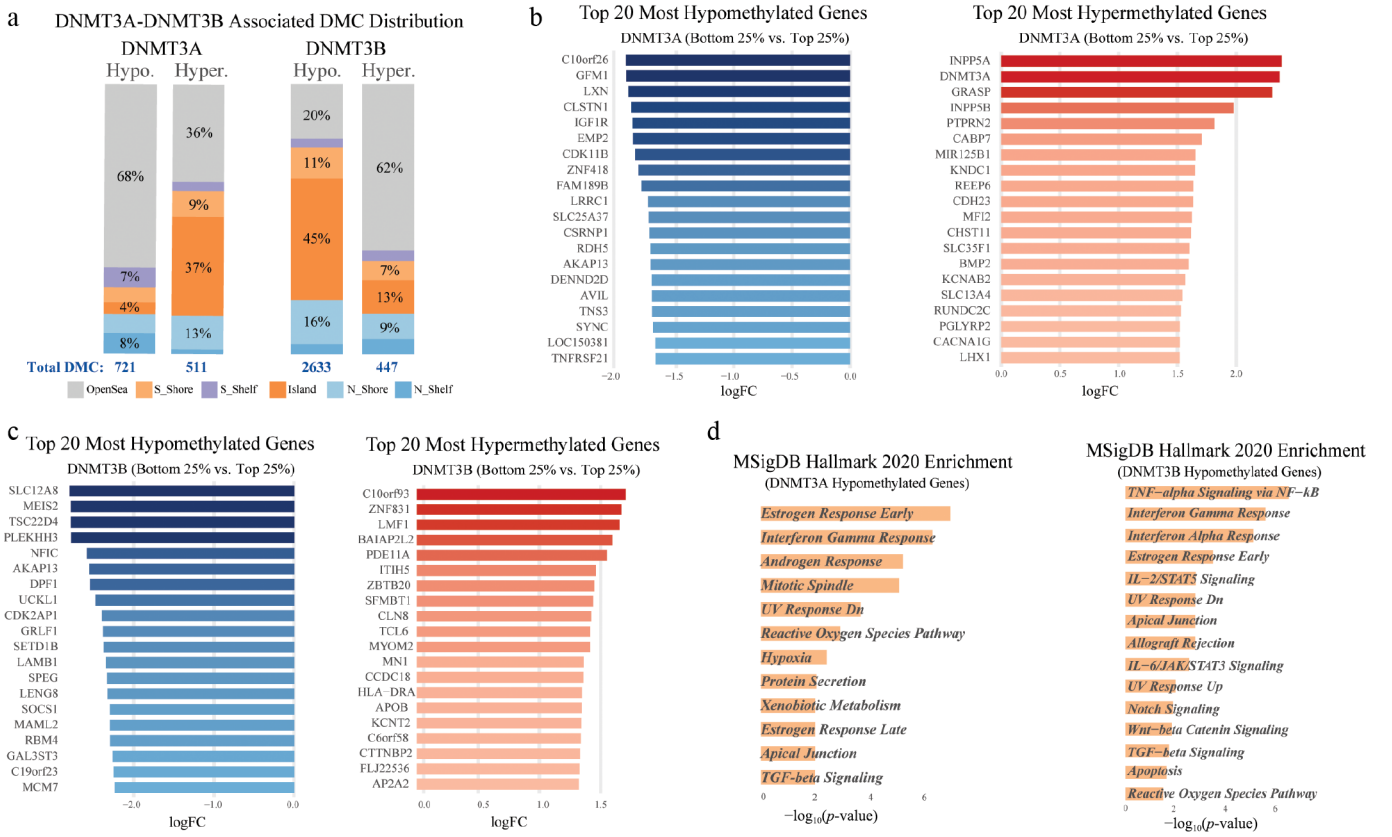

Fig. 4 Genomic distribution and functional annotation of DNMT3A- and DNMT3B-associated methylation changes. (a) Distribution of hyper- and hypomethylated CpGs across genomic in DNMT3A-low vs. DNMT3A-high tumors (left) and DNMT3B-low vs. DNMT3B-high tumors (right). (b) Bar plots showing the top 20 most hypomethylated (left) and hypermethylated (right) genes in DNMT3A-low patients compared to DNMT3A-high patients. (c) Bar plots showing the top 20 most hypomethylated (left) and hypermethylated (right) genes in DNMT3B-low patients compared to DNMT3B-high patients. (d) MSigDB Hallmark 2020 enrichment analysis of hypomethylated genes in DNMT3A low tumors (left) and DNMT3B low tumors (right).

hypomethylation, we performed pathway enrichment analysis using the MSigDB Hallmark 2020 database. Both *DNMT3A*- and *DNMT3B*-low tumors showed enrichment for several shared pathways, including estrogen response, interferon gamma response, TGF-β signaling, and reactive oxygen species pathways. Beyond these common signatures, hypomethylated genes in *DNMT3A*-low tumors were additionally enriched for androgen response, mitotic spindle, and apical junction pathways, suggesting alterations in hormone signaling, cell cycle regulation, and epithelial organization. In contrast, hypomethylated genes in *DNMT3B*-low tumors showed stronger enrichment for immune and cytokine signaling pathways, including TNF-α signaling, IL-2/STAT5, and IL-6/JAK/STAT3 pathways (Fig. 4d).

Shared DMCs and DMGs between obesity-associated and DNMT3A/B-low tumors

Given that *DNMT3A* and *DNMT3B* downregulation was associated with widespread DNA methylation changes in EC, we next asked whether these changes overlap with obesity-driven methylation alterations. Therefore, we compared DMCs and their associated genes, DMGs, which are defined as genes containing or located in proximity to DMCs based on Illumina 450 K array annotation, between these groups. A Venn diagram shows that obesity associated DMCs shared 49 overlapping sites with *DNMT3A*-low tumors and 89 overlapping sites with *DNMT3B*-low tumors, indicating partial convergence between obesity-driven and DNMT-driven methylation changes (Fig. 5a). A similar pattern was observed at the gene level (Fig. 5b), where 54 DMGs overlapped between obesity-associated and *DNMT3A*-low tumors, whereas 75 DMGs overlapped between obesity-associated and *DNMT3B*-low tumors. Pathway enrichment analysis of these shared hypomethylated genes revealed several biologically coherent and reproducible pathways across comparisons (Fig. 5c). Both the obesity-*DNMT3A*-low and obesity-*DNMT3B*-low overlapping gene sets were enriched for estrogen response, TGF-β signaling, and IL-2/STAT5 signaling, suggesting that these pathways represent core biological programs jointly affected by obesity and DNMT dysregulation. In addition, obesity-*DNMT3A*-low shared genes showed enrichment in mitotic spindle and PI3K/AKT/mTOR signaling, whereas Obesity-*DNMT3B*-low shared genes showed enrichment for protein secretion.

DNMT3A and *DNMT3B* knockout in HEC1B cells reveals gene-specific contributions of *de novo* DNA methylation

Because the expression of *DNMT3A* and *DNMT3B* was significantly reduced in tumors from obese patients, we next asked whether loss of *de novo* methyltransferase activity could reproduce a subset of obesity-associated methylation changes in an EC cell line model. To experimentally model DNMT downregulation, we used the HEC1B endometrial cancer cell line and generated CRISPR/Cas9 mediated knockout (KO) lines targeting *DNMT3A* and *DNMT3B*. Multiple guide RNAs were tested for each gene, and efficient gene disruption was achieved using *DNMT3A* gRNA3 and *DNMT3B* gRNA3, whereas other guides did not result in complete knockout (Fig. 6a). For transparency, uncut whole Western blots demonstrating DNMT3A and

https://doi.org/10.48130/epi-0026-0007

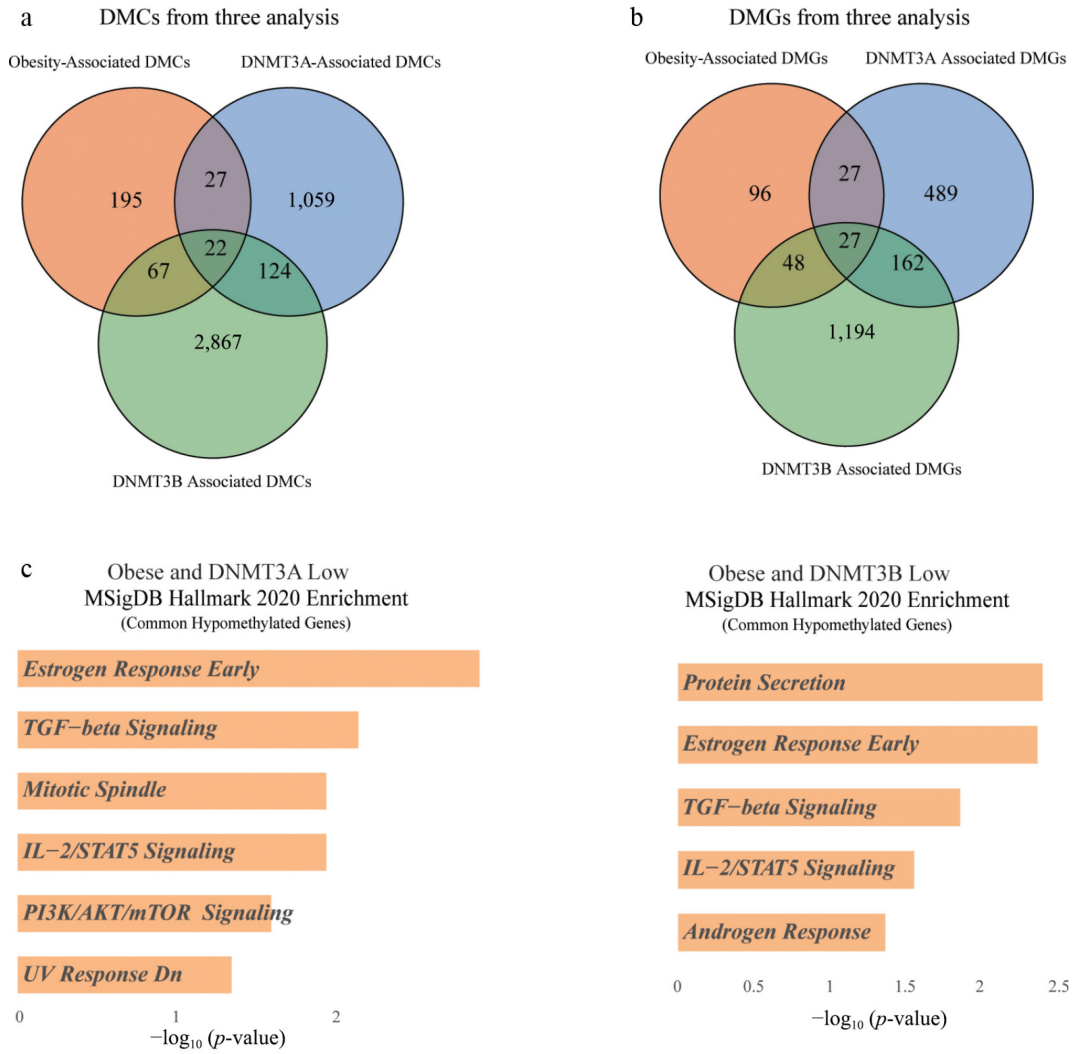

Fig. 5 Intersection of obesity-associated and DNMT3A/B-low-associated methylation changes. (a) Venn diagrams showing overlap of DMCs between obesity-associated DMCs, DNMT3A-associated DMCs and DNMT3B-associated DMCs. (b) Venn diagrams showing overlap of differentially methylated genes (DMGs) between obesity-associated, DNMT3A-associated and DNMT3B-associated DMGs. (c) MSigDB Hallmark 2020 enrichment analysis of common hypomethylated genes shared between obesity-associated and DNMT3A-low signatures (left) and between obesity-associated and DNMT3B-low signatures (right).

DNMT3B protein abundance across all tested guides and non-targeting (NT) controls, along with corresponding GAPDH loading controls, are provided in Supplementary Fig. S1. Following establishment of *DNMT3A* KO and *DNMT3B* KO cells, genome-wide DNA methylation profiling was performed using the Illumina EPIC v2.0 array. To enable direct comparison with TCGA tumors profiled using the 450K array, we restricted downstream analysis to CpG sites common to both platforms. Differential methylation analysis using the limma framework identified 2,000 significant DMCs in *DNMT3A* KO cells relative to non-targeting (NT) controls (FDR < 0.05). Notably, 55.4% of these sites were hypomethylated, consistent with loss of *de novo* methyltransferase activity. In contrast, *DNMT3B* knockout produced only 39 significant DMCs, 36 of which were hypomethylated (FDR < 0.1) (Fig. 6b).

Integration of *DNMT3A* KO methylation signatures with obesity-associated DMCs

To determine whether *DNMT3A* loss affected obesity-associated methylation patterns observed in TCGA patients, we compared DMCs from *DNMT3A* KO HEC1B cells with DMCs identified in tumors from obese vs. lean patients. This analysis revealed 37 CpG sites shared between the two datasets (FDR < 0.1). Additionally, we identified 234 DMCs that were common between *DNMT3A* KO HEC1B cells and *DNMT3A*-low TCGA tumors (Supplementary Fig. S2a). Twelve genes were consistently hypomethylated both in tumors from obese patients and in *DNMT3A* KO cells (Fig. 6c). Importantly, nine of these genes, *FOSL2*, *IGF1R*, *SPRED2*, *INPP5F*, *KCTD9*, *SLC16A3*, *SLC22A5*, *SREBF1*, and *LARP4*, also overlapped with DMCs identified in TCGA patients with low *DNMT3A* expression. Furthermore, among the common differentially methylated genes shared between obesity-associated and *DNMT3A*-low tumors in TCGA, six genes, *INPP5F* (Fig. 6d), *RCOR1*, *KCTD9*, *VWA3B*, *ADAMTSL3*, and *OPRL1*, showed significant expression changes in obese patients (Supplementary Fig. S2b). Notably, the differentially methylated CpG site, cg10053779, annotated to INPP5F, was located within the gene body region, which is consistent with the reduced methylation at cg10053779 in DNMT3A KO cells in the EPIC array (Supplementary Fig. S3).

Among these shared targets, *INPP5F* was selected for closer examination as it is a regulator of phosphoinositide signaling, insulin sensitivity, and the PI3K/AKT pathway. *INPP5F* encodes inositol

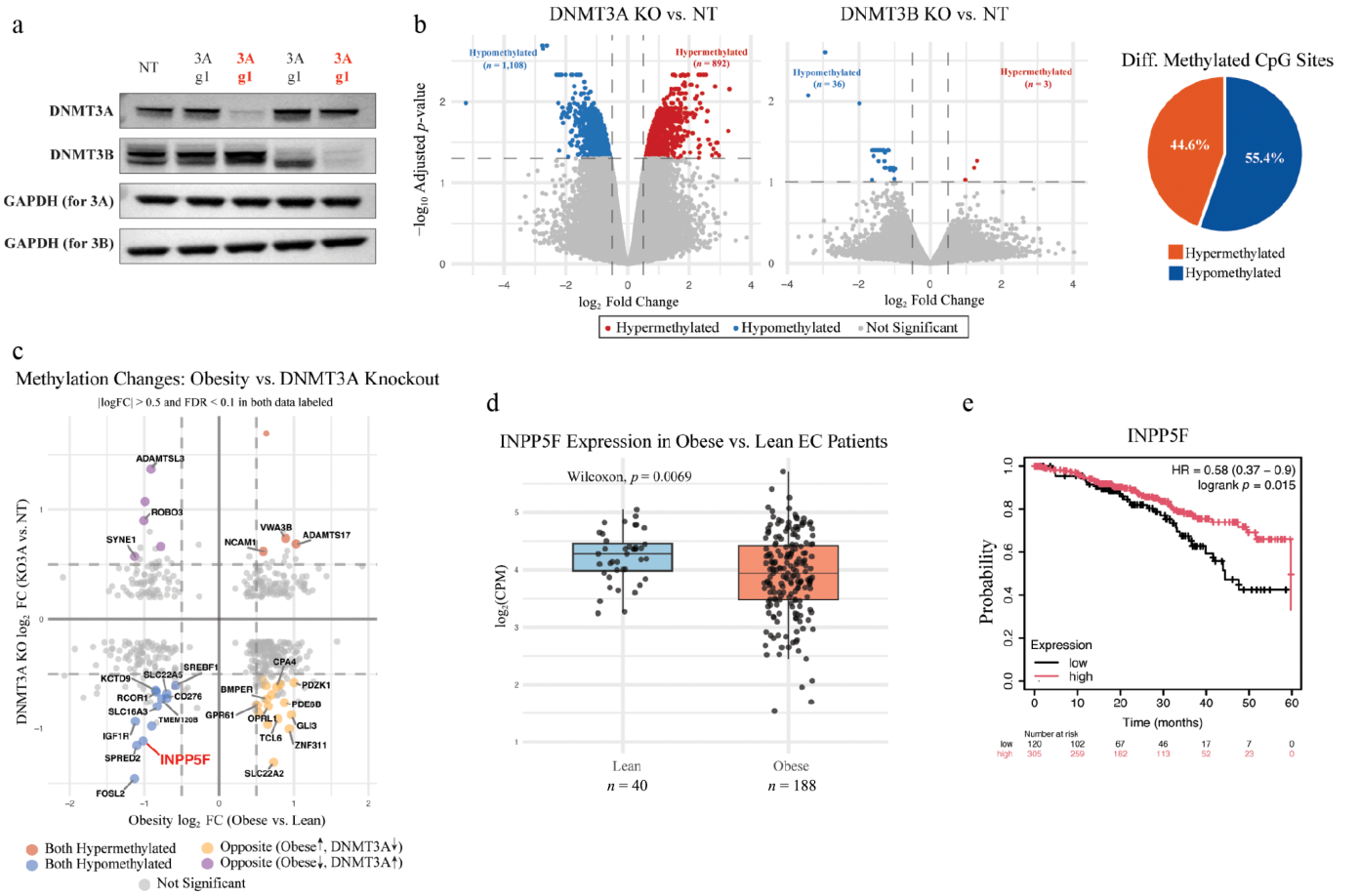

Fig. 6 CRISPR/Cas9 mediated DNMT KO validation and integration with obesity-associated methylation signatures. (a) WB analysis confirming CRISPR/Cas9 mediated KO efficiency in HEC1B endometrial cancer cells. Lanes show non-targeting control (NT) and cells transduced with guide RNAs targeting DNMT3A (g1, g3) or DNMT3B (g1, g3). GAPDH serves as a loading control. (b) Volcano plots showing DMCs in DNMT3A KO (left) and DNMT3B KO (right) cells compared to non-targeting controls, with corresponding pie chart indicating 55.4% hypomethylation and 44.6% hypermethylation in DNMT3A KO cells. (c) Scatter plot integrating obesity-associated methylation changes (*x*-axis: $\log_2$ fold change in tumors from obese vs. lean patients) with DNMT3A knockout-induced changes (*y*-axis: $\log_2$ fold change in DNMT3A KO vs. NT cells). Points represent CpG sites, with colors indicating concordant hypomethylation (blue), concordant hypermethylation (red), or discordant patterns (yellow/green). Significant genes are labeled. (d) Boxplot comparing INPP5F expression between lean and tumors from obese patients (Wilcoxon p = 0.0069). (e) Kaplan–Meier survival curve from the UCEC dataset (n = 543) showing that low INPP5F expression is associated with decreased overall survival (HR = 0.58; 95% CI: 0.37–0.90).

polyphosphate-5-phosphatase F, an enzyme that regulates phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) turnover[34] and acts as a negative regulator of PI3K/AKT signaling[35], functioning broadly as a tumor suppressor in glioblastoma[36]. Beyond its role in tumorigenesis, *INPP5F* is implicated in insulin signaling and type 2 diabetes, conditions strongly associated with obesity. Dysregulated *INPP5F* expression has been linked to impaired insulin sensitivity and metabolic inflammation, suggesting that obesity-associated *INPP5F* hypomethylation may reflect broader metabolic stress within the tumor microenvironment. Consistent with this biology, *INPP5F* was significantly downregulated in tumors from obese patients compared to tumors from lean patients (Fig. 6d). Furthermore, reduced *INPP5F* expression correlated with adverse clinical outcomes. EC patients with low *INPP5F* exhibited decreased overall survival, with a hazard ratio of 0.58 (0.37–0.90) (Fig. 6e). In the DNMT3A KO HEC1B cells, DNMT3A loss was sufficient to alter methylation at locus cg10053779; however, it did not alter INPP5F expression. These findings support INPP5F as a methylation-validated shared target, while suggesting that its transcriptional downregulation in obese tumors likely involves additional obesity- or tumor-context-dependent factors.

Discussion

In this study, we performed a comprehensive analysis of obesity associated DNA methylation alterations in endometrioid EC using TCGA data and validated key findings through CRISPR-based functional experiments. We show that tumors from obese patients exhibit a predominantly hypomethylated epigenome, with more than 70% of differentially methylated CpG sites showing reduced methylation. Importantly, we observe an association between reduced expression of DNA methyltransferases and these alterations, suggesting a potential contribution of DNMT dysregulation to the observed methylation landscape. Obesity-associated and DNMT-low methylation signatures converge on hormone-responsive and oncogenic pathways, indicating that epigenetic dysregulation may represent one mechanism linking obesity and EC pathogenesis.

Our findings provide molecular evidence supporting the well-established clinical observation that obesity worsens EC outcomes. Epidemiological studies have consistently demonstrated that obese EC patients experience significantly poorer survival compared to lean patients, with morbidly obese women facing a 6.25-fold increased

https://doi.org/10.48130/epi-0026-0007

risk of disease-specific-mortality[12]. Meta-analyses have further quantified this relationship, showing that obesity is associated with a 20%–34% increase in all-cause mortality and a 28% increase in recurrence risk among EC survivors[11,13]. The mechanistic basis for these adverse outcomes has remained incompletely understood, and our data suggest that obesity driven epigenetic alterations may contribute to this phenomenon, although additional factors are likely involved.

The hypomethylated epigenetic landscape we observed in tumors from obese patients was characterized by enrichment in pathways directly implicated in EC progression, including estrogen response, PI3K/AKT/mTOR signaling, TGF-β signaling, and Wnt/β-catenin pathways. The PI3K/AKT/mTOR pathway is of particular significance in the context of obesity-associated EC. We have demonstrated the importance of AKT signaling in EC pathogenesis from multiple perspectives, including its role in progestin resistance[37–41]. This signaling cascade serves as a critical node integrating insulin and growth factor signaling with cellular metabolism, proliferation, and survival[42]. Furthermore, our group has shown that increased methylation variability occurs in insulin signaling pathway genes in EC[43]. In the setting of obesity-associated hyperinsulinemia and insulin resistance, constitutive activation of this pathway can drive tumor cell proliferation and survival[44]. The observed hypomethylation of genes within this pathway in tumors from obese patients may represent a contributing epigenetic feature that is consistent with enhanced pathway activity, although direct functional validation in this context is required.

Importantly, while hypomethylation is generally associated with increased gene expression, we also observed cases where hypermethylated CpG sites were linked to higher expression. This can be explained by genomic context, as methylation in gene bodies or distal regulatory regions may be positively associated with transcription, unlike the repressive effect typically seen at promoters. Therefore, the relationship between DNA methylation and gene expression is context-dependent rather than strictly unidirectional.

The relationship between obesity and changes of DNA methylation has been investigated across multiple cancer types, revealing both shared and tissue-specific patterns. In colon cancer, Milner et al. reported that 65% of DMRs were hypomethylated in obese patients compared to lean patients[45]. On the other hand, Hair et al. showed that in 87% of probes analyzed, methylation levels increased with increasing BMI in breast tumors[46]. The predominant hypomethylation observed in our EC cohort may reflect unique aspects of endometrial biology. Endometrioid EC is characterized by estrogen dependence and strong hormone receptor expression. Adipose tissue in obese women serves as a major site of peripheral estrogen synthesis through aromatase-mediated conversion of androgens. Studies have shown that *DNMT3A* and *DNMT3B* expression in human endometrium is regulated by both progesterone and estrogen[47], suggesting that sex steroid hormones directly influence DNA methylation machinery in this tissue. This hormonal regulation of DNMTs may render the endometrium particularly susceptible to obesity induced epigenetic alterations, potentially explaining why hypomethylation predominates in EC. Furthermore, Nagashima et al. demonstrated widespread hypomethylation in epithelial cells from obese women even before cancer develops, with substantial overlap with early-stage EC patterns[48]. Our results extend this concept to established tumors, supporting the notion that obesity-driven epigenetic alterations arise early and persist during tumor progression. Beyond obesity, our group has previously shown that EC tumors from women with high African ancestry also exhibit a predominantly hypomethylated profile compared to those with low African ancestry[49]. Together with the present findings, these data suggest that both obesity and genetic ancestry contribute to the hypomethylated epigenetic landscape characteristic of EC, potentially through distinct but convergent mechanisms.

While our data demonstrate that DNMT downregulation contributes to obesity-associated methylation changes, it is important to recognize that DNMTs explain only a subset of the observed alterations. Of the 311 obesity-associated DMCs we identified, 49 overlapped with *DNMT3A*-low tumors and 89 with *DNMT3B*-low tumors. While these overlaps are biologically meaningful, they represent a fraction of the total obesity-associated methylation changes. Obesity is a modifiable risk factor that impacts cancer biology through multiple interconnected pathways beyond epigenetic modifications. These include chronic inflammation, insulin resistance, altered sex hormone metabolism, oxidative stress, and alterations in the gut microbiome[50]. Each of these obesity-associated perturbations can independently affect cancer cell behavior and may also influence the epigenome through mechanisms distinct from DNMT regulation. The observation that DNMT downregulation accounts for a portion but not all obesity-associated methylation changes is therefore consistent with the multifactorial nature of obesity's impact on cancer biology. While TCGA analyses revealed correlations between methylation changes and DNMT expression levels, the knockout experiments provide supportive functional evidence, particularly for DNMT3A, although the extent of causality and generalizability to tumors *in vivo* remains to be fully established. In contrast, *DNMT3B* knockout generated only 39 DMCs, a surprisingly modest effect given the extensive methylation changes observed in *DNMT3B*-low TCGA tumors. This discrepancy may reflect context-specific methylation patterns, as HEC1B is a cell line. Although cell lines represent valuable models for investigating functional mechanisms, prolonged culture and adaptation to growth conditions may cause epigenetic drift from the original tumor. Nevertheless, the effects of *DNMT3A* and *DNMT3B* knockouts underscore their regulatory function in *de novo* methylation and highlight the importance of cellular contexts in epigenetic outcomes. Because obesity is a modifiable risk factor, interventions targeting weight reduction may impact EC risk and outcomes through multiple mechanisms, with *de novo* methylation being one pathway among many. The convergence between obesity-associated DMCs in TCGA tumors and *DNMT3A* KO-induced DMCs in HEC1B cells provides supportive evidence that *DNMT3A* loss can reproduce a subset of obesity-linked methylation changes in this cell line model. Notably, nine of these genes (*FOSL2*, *IGF1R*, *SPRED2*, *INPP5F*, *KCTD9*, *SLC16A3*, *SLC22A5*, *SREBF1*, and *LARP4*) also overlapped with DMCs from *DNMT3A*-low TCGA tumors, providing multi-level support for a relationship between reduced *DNMT3A* and a subset of obesity associated methylation alterations. Several of these shared targets occupy central positions at the intersection of metabolic regulations and oncogenic signaling. For example, *FOSL2* functions as an oncogenic transcription factor in EC and has been implicated in promoting proliferation, differentiation, and survival[51]. Similarly, *IGF1R* is a key mediator of insulin and growth factor signaling and activates downstream PI3K/AKT and MAPK pathways[52], both of which are critical drivers of endometrioid tumor progression. Among the shared genes, *INPP5F* emerged as an interesting methylation-associated candidate linking metabolic dysregulation to endometrial tumor biology. *INPP5F* encodes inositol polyphosphate-5-phosphatase F, an enzyme that negatively regulates PI3K/AKT signaling through dephosphorylation of phosphatidylinositol-4,5-bisphosphate[35]. Beyond its tumor suppressor function in glioblastoma[36], *INPP5F* has established roles in insulin sensitivity and type 2 diabetes

pathogenesis[35]. In tumors from obese patients, INPP5F showed gene body hypomethylation and reduced expression, and lower INPP5F expression was associated with poorer overall survival. Given that PI3K pathway alterations are among the most frequent molecular events in endometrioid EC, including mutations in PIK3CA, PIK3R1, and PTEN, downregulation of INPP5F may represent an additional non-mutational mechanism of pathway hyperactivation in obese patients. This is particularly relevant given that obesity-associated hyperinsulinemia directly stimulates PI3K/AKT signaling, creating a potential synergy between systemic metabolic dysfunction and tumor-intrinsic epigenetic changes.

This study has several limitations that should be considered when interpreting the findings. We used TCGA data, which represent a retrospective observational dataset. The cohort includes an imbalance in sample size between obese and lean groups, which may influence statistical power and effect size estimation despite the use of limma's variance moderation framework. This is an inherent limitation of studies involving endometrioid endometrial cancer, where lean patient samples are relatively scarce. In addition, to enable a clear comparison between metabolically distinct groups, overweight individuals (BMI 25–29) were excluded from the primary analysis. While this approach reduces phenotypic heterogeneity and enhances contrast between lean and obese groups, it limits the ability to assess methylation changes across the full BMI spectrum. Nevertheless, emerging evidence indicates that obesity-associated epigenetic changes may exhibit a threshold effect rather than a linear dose–response relationship with BMI. Notably, a recent large study found that long-term obesity, but not overweight status, was associated with significantly accelerated epigenetic aging, suggesting that the two BMI categories are not equivalent on the epigenetic level[53]. Further stratification of obesity into subclasses as class I, II, and III was also not performed due to limited sample sizes within each group, which may restrict the evaluation of the dose-dependent effects of obesity. Also, survival analyses were performed using an external tool (KM Plotter) rather than directly modeled within the study dataset due to incomplete annotation, which may introduce variability in interpretation. Finally, although CRISPR/Cas9-mediated DNMT KO experiments provide supportive functional insights, these were conducted in established cell lines, which does not fully recapitulate the tumor microenvironment or *in vivo* epigenetic dynamics.

In summary, obesity is associated with widespread DNA hypomethylation in endometrioid EC, which is accompanied by reduced DNMT expression. Obesity-associated and DNMT-low signatures converge on estrogen-responsive and PI3K/AKT/mTOR pathways, and *INPP5F* emerges as a potential biologically and clinically relevant target. Importantly, DNMT downregulation represents one mechanism among the multiple pathways through which obesity influences EC biology. Given that obesity is a modifiable risk factor, these findings suggest that weight management interventions may impact EC outcomes through multiple mechanisms, including, but not limited to, epigenetic remodeling. Future studies should investigate whether weight loss can reverse obesity associated methylation alterations and whether the identified targets serve as actionable biomarkers for risk stratification or therapeutic intervention.

Conclusions

In conclusion, this study demonstrates that obesity in endometrioid EC is associated with a predominantly hypomethylated epigenome, accompanied by reduced expression of DNA methyltransferases. Our findings suggest that DNMT downregulation contributes, at least in part, to obesity driven epigenetic remodeling of oncogenic pathways including PI3K/AKT/mTOR and estrogen signaling. Integration of patient data with CRISPR-based models provides supportive functional evidence that DNMT3A loss can reproduce selected obesity-associated methylation changes. Overall, these results provide new insight into how obesity influences tumor biology through epigenetic mechanisms and suggest that targeting metabolic–epigenetic interactions may offer novel opportunities for risk stratification and therapeutic intervention in EC.

Ethical statements

The data used in this study were obtained from The Cancer Genome Atlas (TCGA) repository, which provides publicly available and de-identified datasets. Therefore, this study did not require approval from an institutional ethics committee.

Author contributions

The authors confirm their contributions to the paper as follows: conceived and designed the study, performed all experiments and computational analyses, and wrote the manuscript: Cingoz H; contributed initial TCGA data processing and preliminary analyses that informed the study design: Asif H; supervised the project, provided critical guidance and resources, secured funding, and revised the manuscript: Kim JJ. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The raw datasets analyzed in this study are publicly available from TCGA repository and can be accessed at: https://portal.gdc.cancer.gov. All data generated by our group and supporting the findings of this study are available within the paper and its Supplementary Information.

Acknowledgments

Research reported in this publication was supported by the National Institute of Health under award number R01CA243249.

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary information accompanies this paper online at: https://doi.org/10.48130/epi-0026-0007.

Dates

Received 6 March 2026; Revised 16 June 2026; Accepted 26 June 2026; Published online 10 August 2026

References

[1] Ng M, Gakidou E, Lo J, Abate YH, Abbafati C, et al. 2025. Global, regional, and national prevalence of adult overweight and obesity, 1990–2021, with forecasts to 2050: a forecasting study for the Global Burden of Disease Study 2021. *The Lancet* 405:813–838

[2] Ward ZJ, Bleich SN, Cradock AL, Barrett JL, Giles CM, et al. 2019. Projected U.S. state-level prevalence of adult obesity and severe obesity. *New England Journal of Medicine* 381:2440–2450

[3] Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A. 2025. Cancer statistics, 2025. *CA: A Cancer Journal for Clinicians* 75:10–45
[4] Siegel RL, Giaquinto AN, Jemal A. 2024. Cancer statistics, 2024. *CA: A Cancer Journal for Clinicians* 74:12–49
[5] Gu B, Shang X, Yan M, Li X, Wang W, et al. 2021. Variations in incidence and mortality rates of endometrial cancer at the global, regional, and national levels, 1990–2019. *Gynecologic Oncology* 161:573–580
[6] Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. 2008. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *The Lancet* 371:569–578
[7] Onstad MA, Schmandt RE, Lu KH. 2016. Addressing the role of obesity in endometrial cancer risk, prevention, and treatment. *Journal of Clinical Oncology* 34:4225–4230
[8] Nagle CM, Marquart L, Bain CJ, O'Brien S, Lahmann PH, et al. 2013. Impact of weight change and weight cycling on risk of different subtypes of endometrial cancer. *European Journal of Cancer* 49:2717–2726
[9] Tumminia A, Vinciguerra F, Parisi M, Graziano M, Sciacca L, et al. 2019. Adipose tissue, obesity and adiponectin: role in endocrine cancer risk. *International Journal of Molecular Sciences* 20:2863
[10] Pati S, Irfan W, Jameel A, Ahmed S, Shahid RK. 2023. Obesity and cancer: a current overview of epidemiology, pathogenesis, outcomes, and management. *Cancers* 15:485
[11] Kokts-Porietis RL, Elmrayed S, Brenner DR, Friedenreich CM. 2021. Obesity and mortality among endometrial cancer survivors: a systematic review and meta-analysis. *Obesity Reviews* 22:e13337
[12] Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. 2003. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *The New England Journal of Medicine* 348:1625–1638
[13] Petrelli F, Cortellini A, Indini A, Tomasello G, Ghidini M, et al. 2021. Association of obesity with survival outcomes in patients with cancer: a systematic review and meta-analysis. *JAMA Network Open* 4:e213520
[14] Ross KH, Gogineni K, Subhedar PD, Lin JY, McCullough LE. 2019. Obesity and cancer treatment efficacy: existing challenges and opportunities. *Cancer* 125:1588–1592
[15] Helyer LK, Varnic M, Le LW, Leong W, McCready D. 2010. Obesity is a risk factor for developing postoperative lymphedema in breast cancer patients. *The Breast Journal* 16:48–54
[16] Gunderson CC, Java J, Moore KN, Walker JL. 2014. The impact of obesity on surgical staging, complications, and survival with uterine cancer: a Gynecologic Oncology Group LAP2 ancillary data study. *Gynecologic Oncology* 133:23–27
[17] Wahl S, Drong A, Lehne B, Loh M, Scott WR, et al. 2017. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. *Nature* 541:81–86
[18] Keleher MR, Zaidi R, Hicks L, Shah S, Xing X, et al. 2018. A high-fat diet alters genome-wide DNA methylation and gene expression in SM/J mice. *BMC Genomics* 19:888
[19] Esteller M, Catasus L, Matias-Guiu X, Mutter GL, Prat J, et al. 1999. *hMLH1* promoter hypermethylation is an early event in human endometrial tumorigenesis. *The American Journal of Pathology* 155:1767–1772
[20] Salvesen HB, MacDonald N, Ryan A, Jacobs IJ, Lynch ED, et al. 2001. PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. *International Journal of Cancer* 91:22–26
[21] Yang HJ, Liu VWS, Wang Y, Tsang PCK, Ngan HYS. 2006. Differential DNA methylation profiles in gynecological cancers and correlation with clinico-pathological data. *BMC Cancer* 6:212
[22] Nosho K, Shima K, Irahara N, Kure S, Baba Y, et al. 2009. DNMT3B expression might contribute to CpG island methylator phenotype in colorectal cancer. *Clinical Cancer Research* 15:3663–3671
[23] Sheaffer KL, Elliott EN, Kaestner KH. 2016. DNA hypomethylation contributes to genomic instability and intestinal cancer initiation. *Cancer Prevention Research* 9:534–546
[24] Inoue F, Sone K, Toyohara Y, Takahashi Y, Kukita A, et al. 2021. Targeting epigenetic regulators for endometrial cancer therapy: its molecular biology and potential clinical applications. *International Journal of Molecular Sciences* 22:2305
[25] Xiong Y, Dowdy SC, Xue A, Jiang S, Eberhardt NL, et al. 2005. Opposite alterations of DNA methyltransferase gene expression in endometrioid and serous endometrial cancers. *Gynecologic Oncology* 96:601–609
[26] Levine DA. 2013. Integrated genomic characterization of endometrial carcinoma. *Nature* 497:67–73
[27] Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, et al. 2016. *TCGAbiolinks*: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Research* 44:e71
[28] Chen YA, Lemire M, Choufani S, Butcher DT, Grafodatskaya D, et al. 2013. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics* 8:203–209
[29] Du P, Zhang X, Huang CC, Jafari N, Kibbe WA, et al. 2010. Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. *BMC Bioinformatics* 11:587
[30] Law CW, Alhamdoosh M, Su S, Dong X, Tian L, et al. 2016. RNA-seq analysis is easy as 1-2-3 with limma, Glimma and edgeR. *F1000Research* 5:1408
[31] Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, et al. 2016. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research* 44:W90–W97
[32] Sanson KR, Hanna RE, Hegde M, Donovan KF, Strand C, et al. 2018. Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities. *Nature Communications* 9:5416
[33] Győrffy B. 2024. Integrated analysis of public datasets for the discovery and validation of survival-associated genes in solid tumors. *The Innovation* 5:100625
[34] Minagawa T, Ijuin T, Mochizuki Y, Takenawa T. 2001. Identification and characterization of a sac domain-containing phosphoinositide 5-phosphatase. *Journal of Biological Chemistry* 276:22011–22015
[35] Bai D, Zhang Y, Shen M, Sun Y, Xia Q, et al. 2016. Hyperglycemia and hyperlipidemia blunts the Insulin-Inpp5f negative feedback loop in the diabetic heart. *Scientific Reports* 6:22068
[36] Kim HS, Li A, Ahn S, Song H, Zhang W. 2014. Inositol Polyphosphate-5-Phosphatase F (INPP5F) inhibits STAT3 activity and suppresses gliomas tumorigenicity. *Scientific Reports* 4:7330
[37] Winder A, Unno K, Yu Y, Lurain J, Kim JJ. 2017. The allosteric AKT inhibitor, MK2206, decreases tumor growth and invasion in patient derived xenografts of endometrial cancer. *Cancer Biology & Therapy* 18:958–964
[38] Lee, II, Maniar K, Lydon JP, Kim JJ. 2016. Akt regulates progesterone receptor B-dependent transcription and angiogenesis in endometrial cancer cells. *Oncogene* 35:5191–5201
[39] Pant A, Lee, II, Lu Z, Rueda BR, Schink J, et al. 2012. Inhibition of AKT with the orally active allosteric AKT inhibitor, MK-2206, sensitizes endometrial cancer cells to progestin. *PLoS One* 7:e41593
[40] Hoekstra AV, Ward EC, Hardt JL, Lurain JR, Singh DK, et al. 2008. Chemosensitization of endometrial cancer cells through AKT inhibition involves FOXO1. *Gynecologic Oncology* 108:609–618
[41] Ward EC, Hoekstra AV, Blok LJ, Hanifi-Moghaddam P, Lurain JR, et al. 2008. The regulation and function of the forkhead transcription factor, Forkhead box O1, is dependent on the progesterone receptor in endometrial carcinoma. *Endocrinology* 149:1942–1950
[42] Hopkins BD, Goncalves MD, Cantley LC. 2020. Insulin–PI3K signalling: an evolutionarily insulated metabolic driver of cancer. *Nature Reviews Endocrinology* 16:276–283
[43] Asif H, Foley G, Simon M, Roque D, Kim JJ. 2023. Analysis of endometrial carcinoma TCGA reveals differences in DNA methylation in tumors from Black and White women. *Gynecologic Oncology* 170:1–10
[44] Porta C, Paglino C, Mosca A. 2014. Targeting PI3K/Akt/mTOR signaling in cancer. *Frontiers in Oncology* 4:64

[45] Milner JJ, Chen ZF, Grayson J, Shiao SPK. 2022. Obesity-associated differentially methylated regions in colon cancer. *Journal of Personalized Medicine* 12:660

[46] Hair BY, Troester MA, Edmiston SN, Parrish EA, Robinson WR, et al. 2015. Body mass index is associated with gene methylation in estrogen receptor–positive breast tumors. *Cancer Epidemiology, Biomarkers & Prevention* 24:580–586

[47] Yamagata Y, Asada H, Tamura I, Lee L, Maekawa R, et al. 2009. DNA methyltransferase expression in the human endometrium: down-regulation by progesterone and estrogen. *Human Reproduction* 24:1126–1132

[48] Nagashima M, Miwa N, Hirasawa H, Katagiri Y, Takamatsu K, et al. 2019. Genome-wide DNA methylation analysis in obese women predicts an epigenetic signature for future endometrial cancer. *Scientific Reports* 9:6469

[49] Asif H, Kim JJ. 2025. Comparing self-reported race and genetic ancestry for identifying potential differentially methylated sites in endometrial cancer: insights from African ancestry proportions using machine learning models. *Molecular Oncology* 19:3596–3612

[50] Simancas-Racines D, Campuzano-Donoso M, Román-Galeano NM, Zambrano-Villacres R, Memoli P, et al. 2025. Obesity and endometrial cancer: biological mechanisms, nutritional strategies, and clinical perspectives. *Food and Agricultural Immunology* 36:2510961

[51] He L, Jiang Z, Wang J, Han Z. 2025. Mechanism of miR-200b-3p-induced FOSL2 inhibition of endometrial cancer cell proliferation and metastasis. *Scientific Reports* 15:15742

[52] Wang C, Su K, Zhang Y, Zhang W, Zhao Q, et al. 2019. IR-A/IGF-1R-mediated signals promote epithelial-mesenchymal transition of endometrial carcinoma cells by activating PI3K/AKT and ERK pathways. *Cancer Biology & Therapy* 20:295–306

[53] Wen Y, Chen Y, Ma Y, Lu C, Liu Y, et al. 2025. Long-term BMI trajectories and epigenetic age acceleration: the role of genetic risk for obesity. *BMC Medicine* 23:589