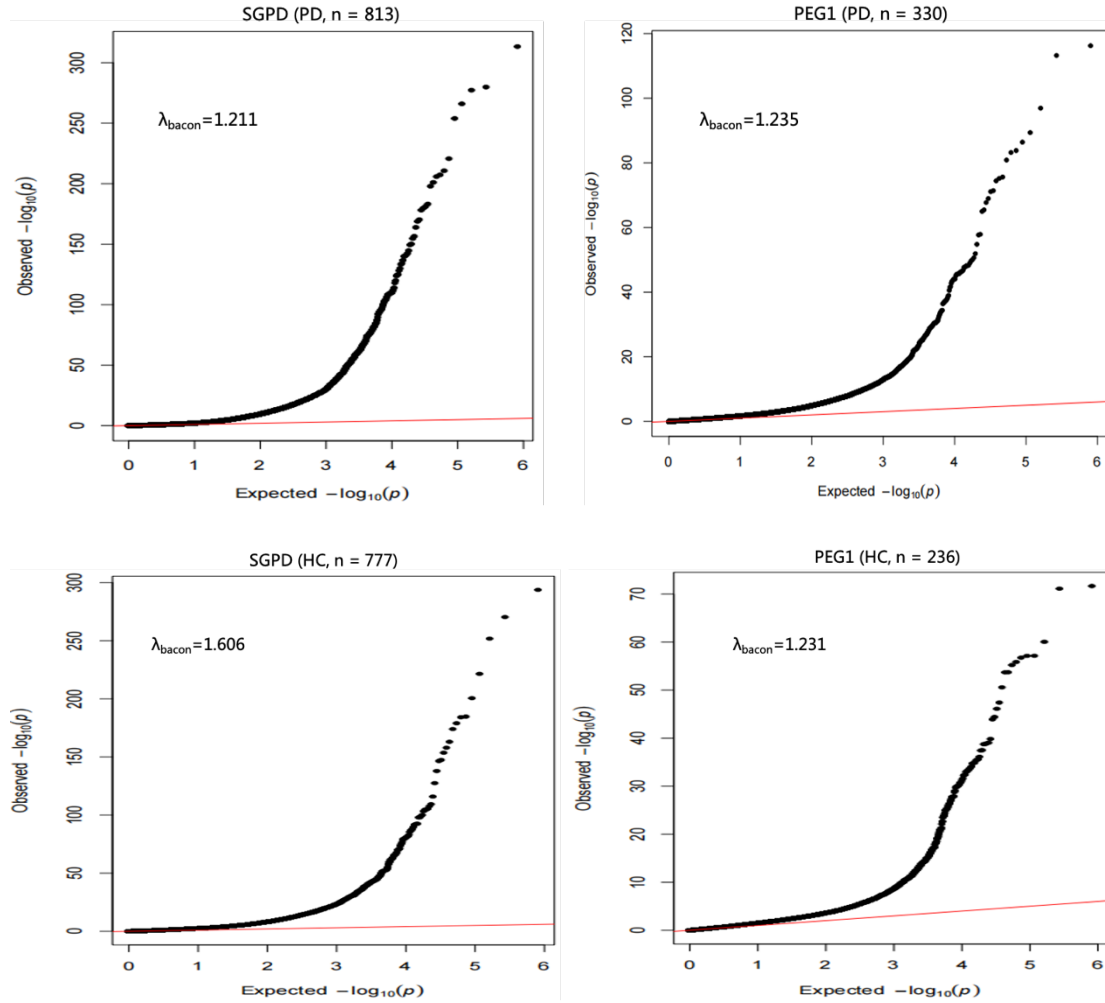


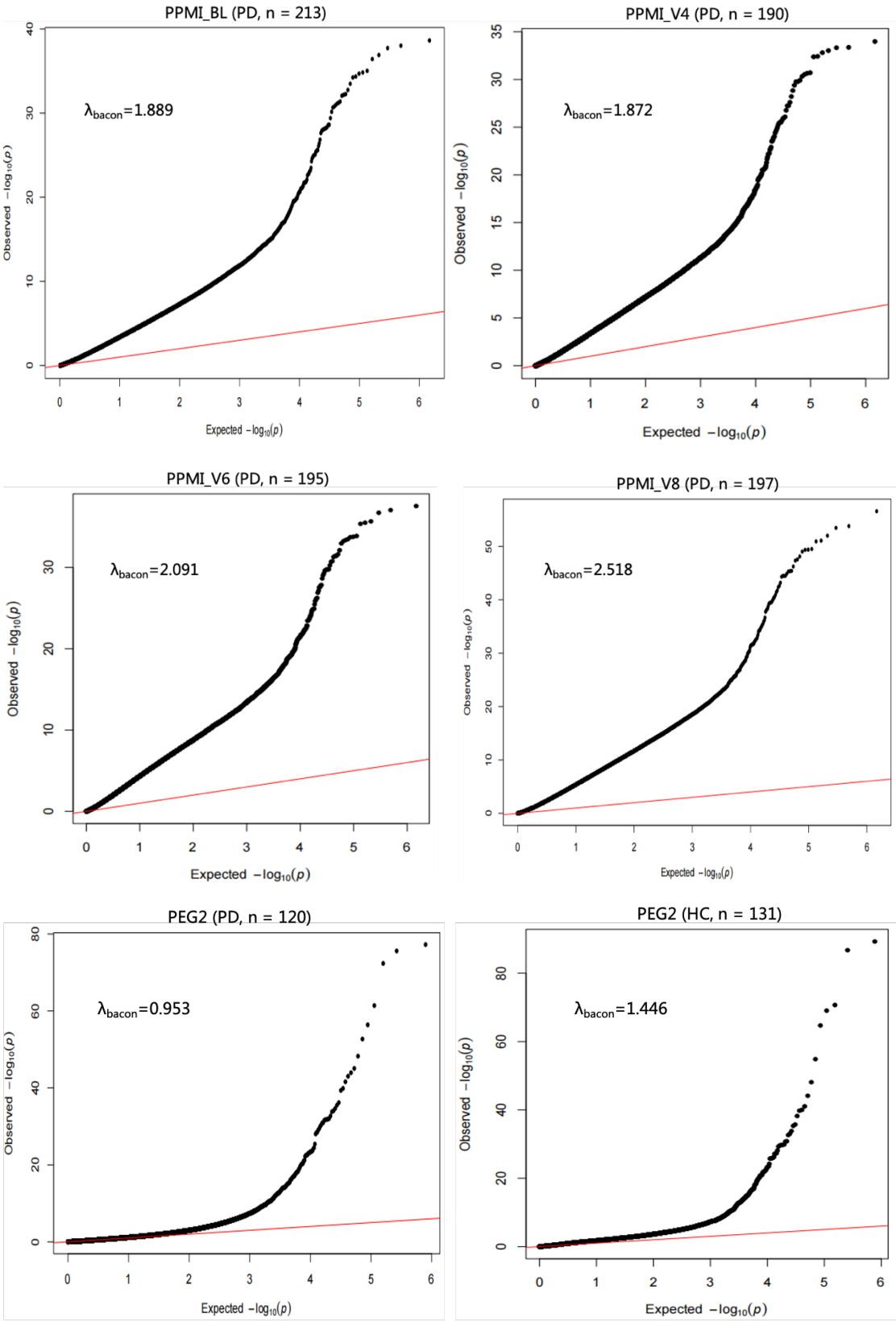
Supplementary Figure 1. The Quantile-quantile (QQ) plots of observed and expected distributions of p-values and λ values from bacon method for study datasets.

The bacon method is a Bayesian approach that utilizes empirical null distribution estimation to control bias and inflation, leading to improved accuracy in epigenome-wide association studies (EWAS).¹

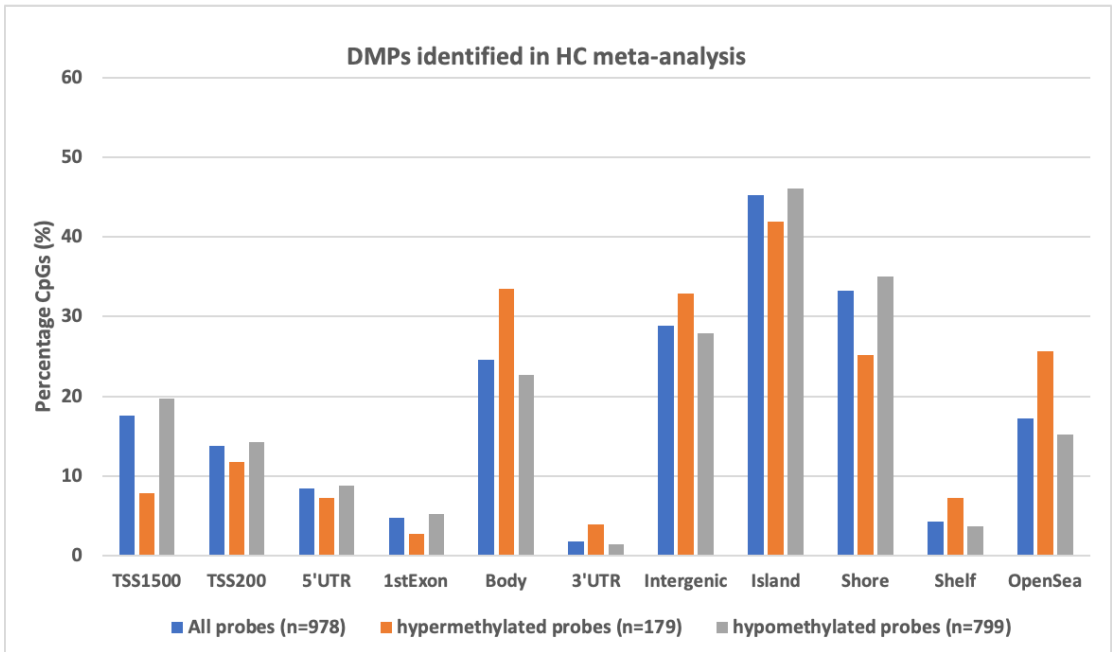
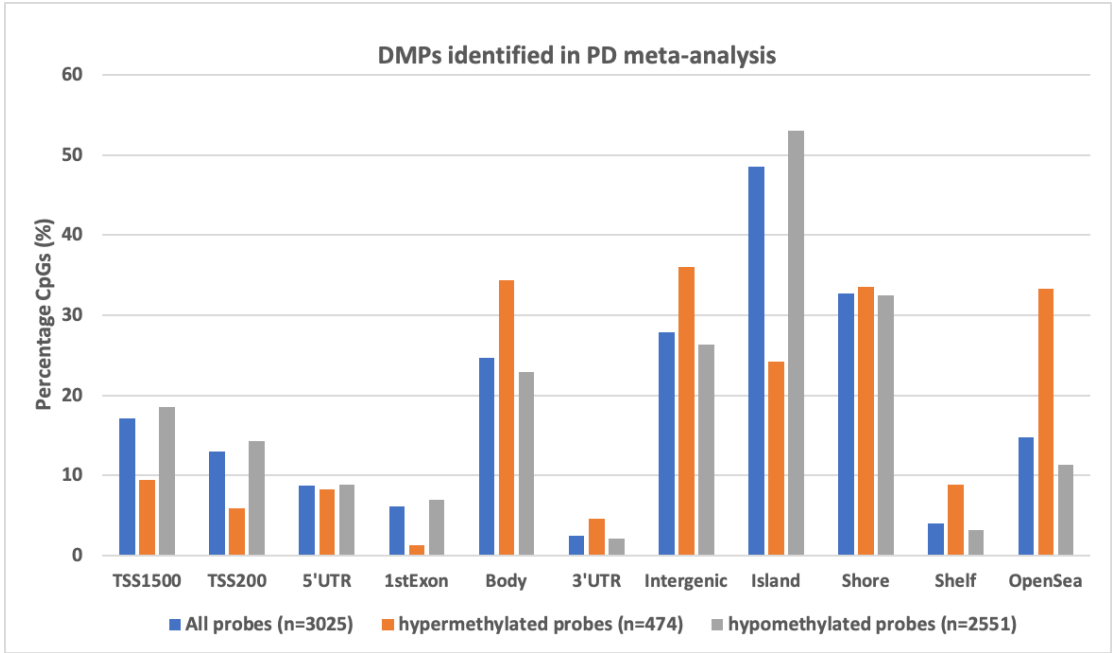
Datasets in the discovery stage:

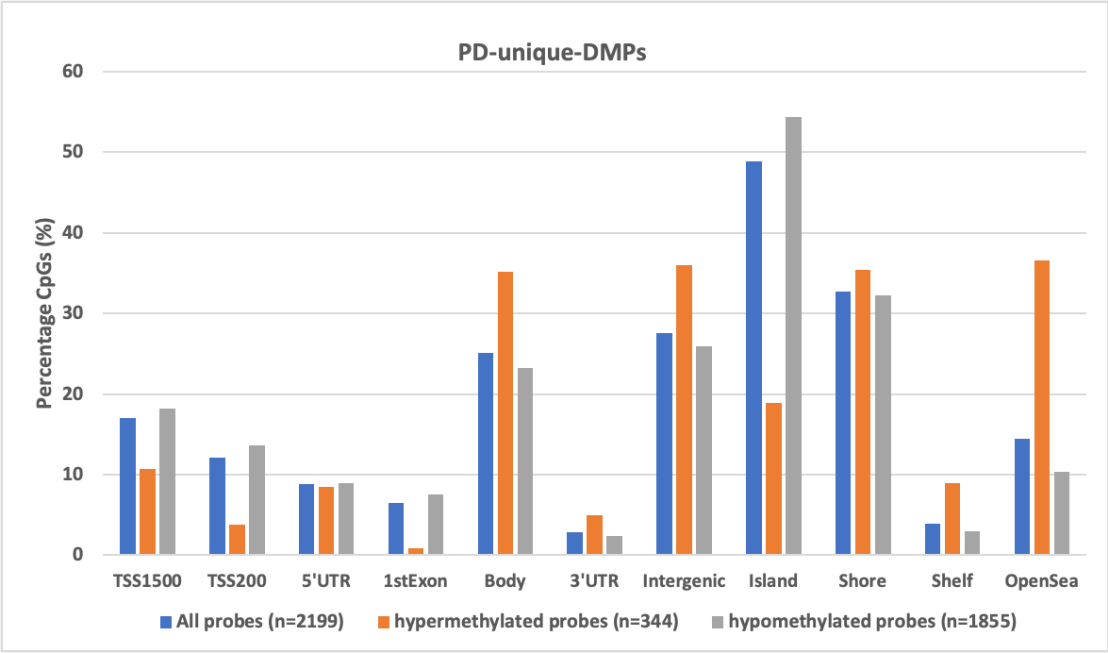


Datasets in the validation stage:



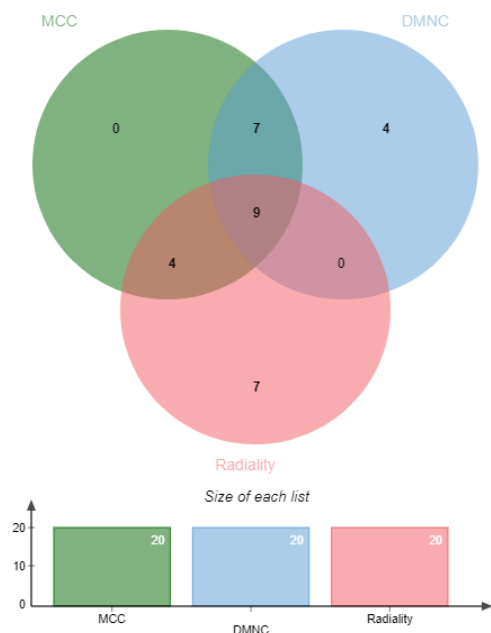
Supplementary Figure 2. Annotation in gene features (TSS1500, TSS200, 5'UTR, 1stExon, body, 3'UTR, intergenic) and CpG features (island, shore, shelf, open sea) of sex-differential methylated positions (DMPs).



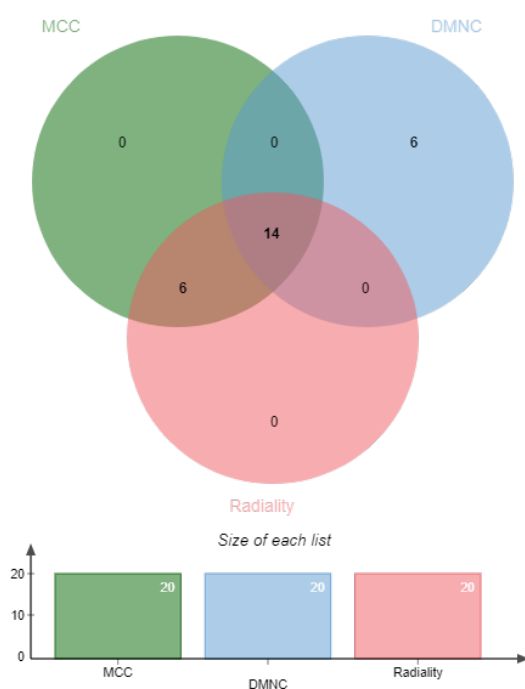


Supplementary Figure 3. Overlap of hub genes identified across three algorithms.

For genes mapped from PD-unique-DMPs, 9 hub genes were consistently identified across three algorithms (MCC, DMNC, and Radiality) using the cytoHubba plugin²: *CACNG2*, *CACNA1C*, *SMAD3*, *BCL2*, *MAPK1*, *RYR2*, *GRIN2D*, *HSP90AA1*, and *PPARG*.



For genes mapped from PD-unique-DMRs, 14 hub genes were consistently identified across three algorithms (MCC, DMNC, and Radiality) using the cytoHubba plugin²: *CCT6A*, *HSPD1*, *PSMA2*, *MRPS22*, *PSMA8*, *HSPE1-MOB4*, *GARS*, *TSFM*, *ETF1*, *MOB4*, *STRN4*, *PINK1*, *SYCP1*, and *MRPL36*.



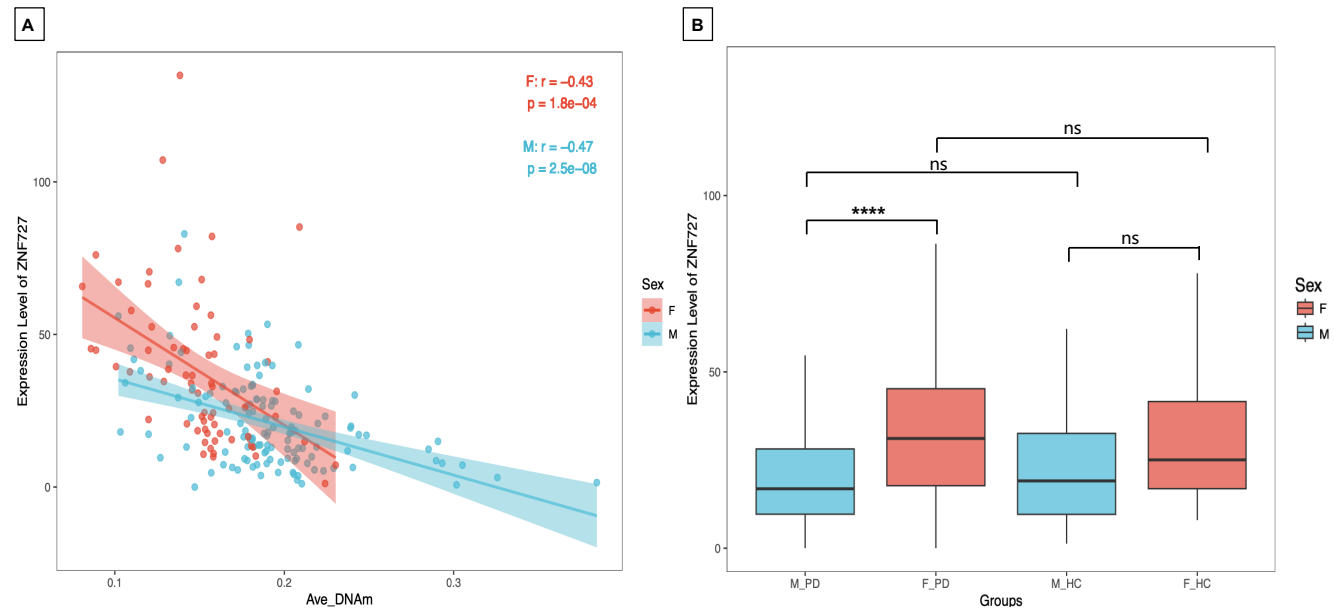
Supplementary Figure 4. DNA methylation and *ZNF727* expression.

A, The relationship between the average DNA methylation level of six cis-eQTMs (cg06098368, cg03124146, cg12479444, cg20067334, cg14285533, cg26911611) and the expression level of *ZNF727* was determined using Pearson correlation coefficient. The data was obtained from 199 PD patients (including 129 males and 70 females) in the PPMI cohort, who had both DNA methylation and transcriptome data available at baseline.

B, *ZNF727* expression levels in male PD patients (n=255), female PD patients (n=138), male HC (n=123), and female HC (n=66) from the PPMI cohort at baseline.

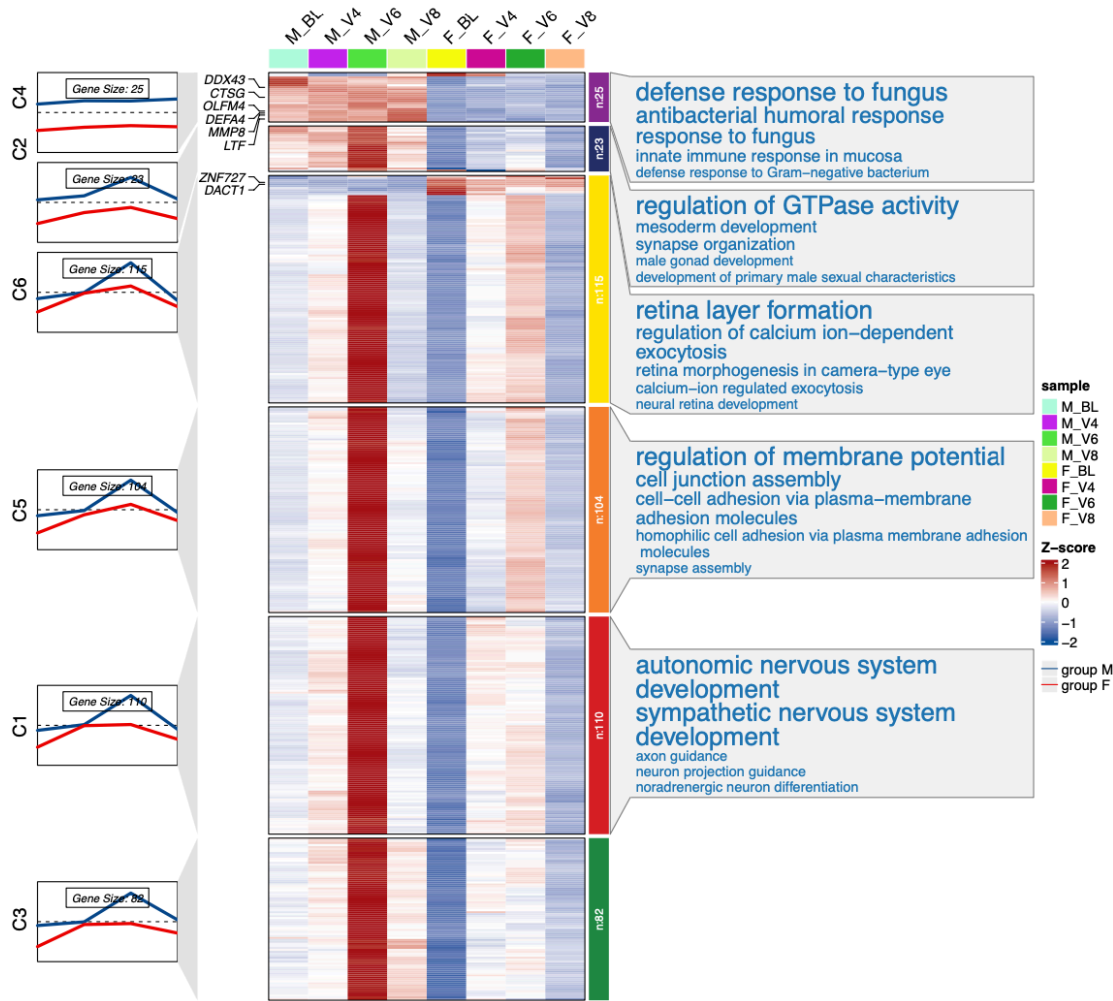
Line within the boxplot represents median. Box represents the 25% and 75% quartile. The definition of differentially expressed genes between males and females was based on the DESeq2 analysis pipeline, considering genes with an adjusted p-value<0.05 and an absolute fold change>1.5 as significantly differentially expressed.

Abbreviations: F, female; HC, healthy control; M, male; PD, Parkinson's disease; ns: not significant; ****, padj<0.0001.



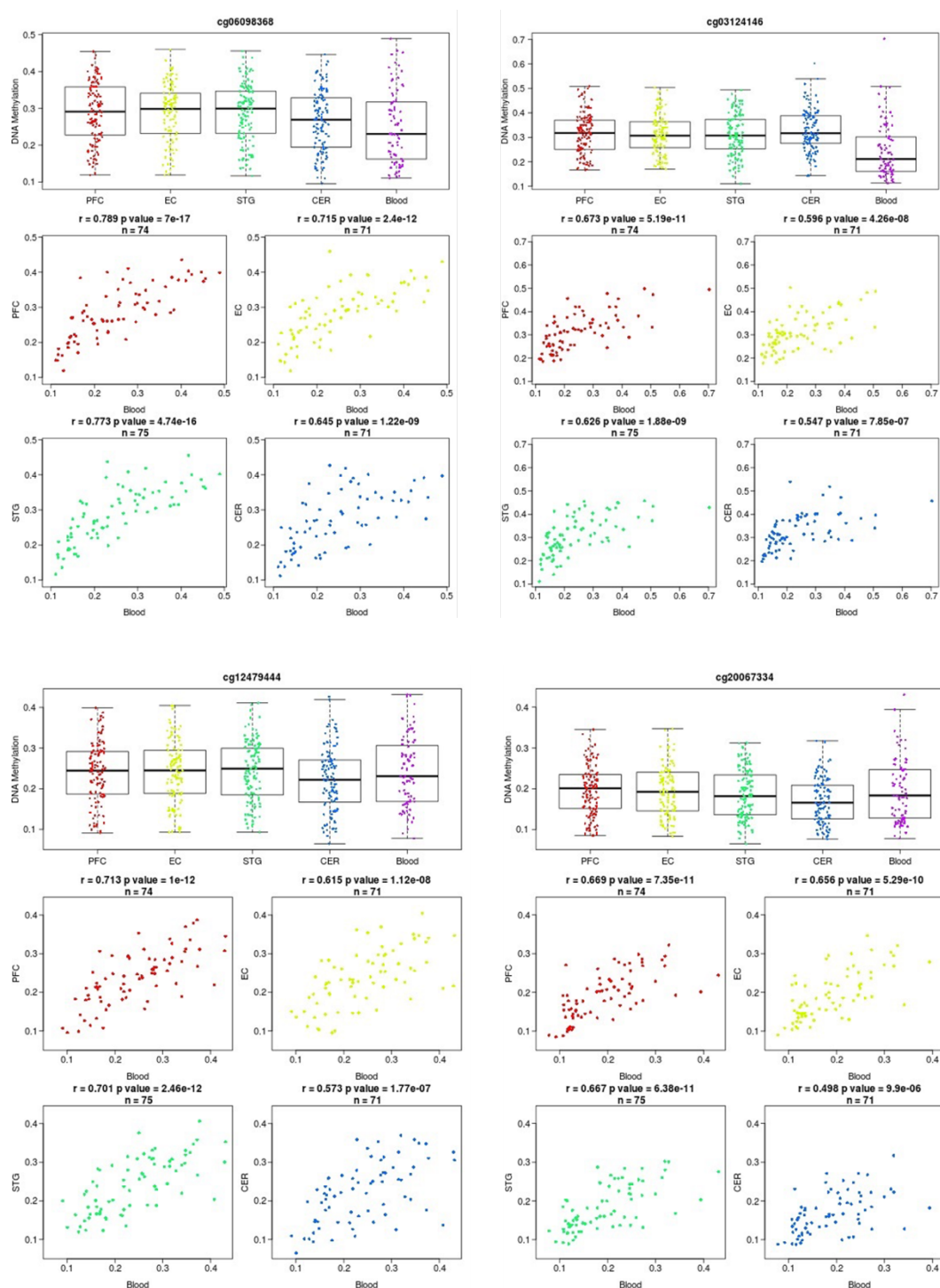
Supplementary Figure 5. Clusters of time series differentially expressed genes (DEGs) in male and female PD patients during follow-ups in the PPMI cohort.

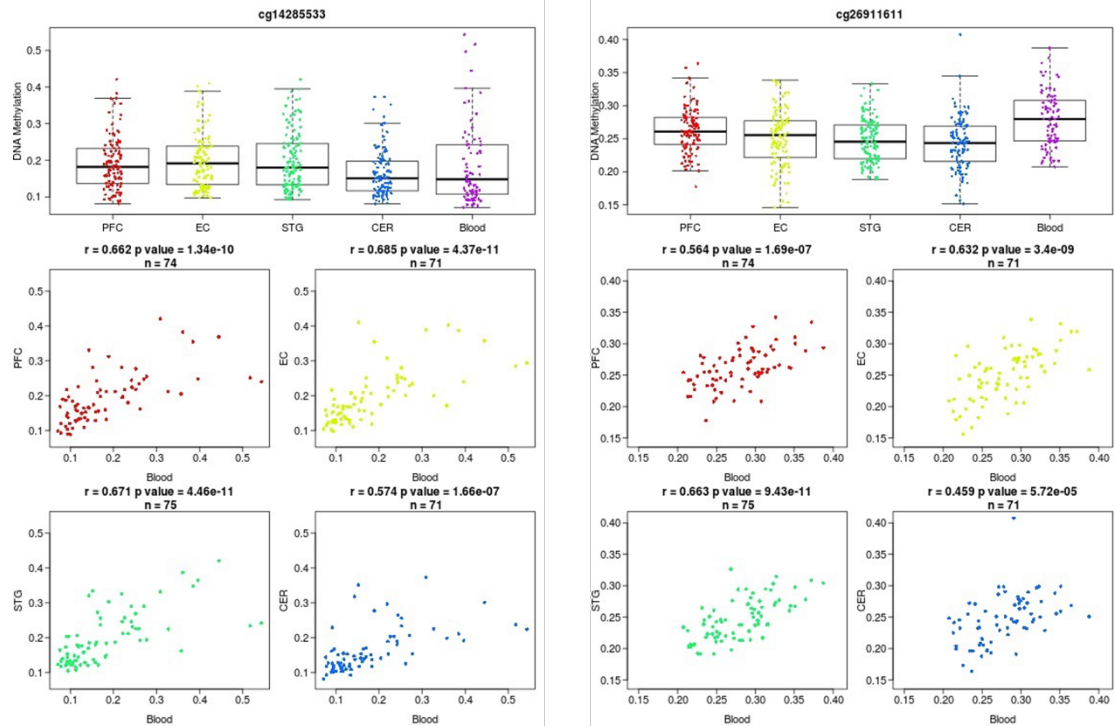
The input data comprised the average normalized counts of significant DEGs (identified at baseline analysis) in male and female PD patients of the PPMI cohort, at baseline (BL), 1-year (V4), 2-year (V6), and 3-year (V8) visits, respectively. Genes with an adjusted $p < 0.05$ (corrected for multiple testing using the Benjamini-Hochberg method) and absolute fold change > 1.5 were considered to be significantly differentially expressed. Clusters (C) were generated using the fuzzy c-means algorithm implemented in the ClusterGVis package.³ The optimal number of clusters ($k=6$) was determined by the elbow method. The dynamic changes for each cluster were visualized using both line plots and heatmaps. Gene enrichment analysis for each cluster was performed using the enrichCluster function, and the top 5 enrichment items were displayed.



Supplementary Figure 6. In silico blood–brain methylation concordance for six ZNF727 cis-eQTMs.

The Blood Brain DNA Methylation Comparison Tool (<http://epigenetics.essex.ac.uk/bloodbrain/>) was developed using data from whole blood and four brain regions [prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG), and cerebellum (CER)] obtained from 122 individuals.⁴ All six cis-eQTMs (cg06098368, cg03124146, cg12479444, cg20067334, cg14285533, cg26911611) exhibited highly significant correlations between whole blood and all four brain regions. eQTMs, quantitative trait methylation sites

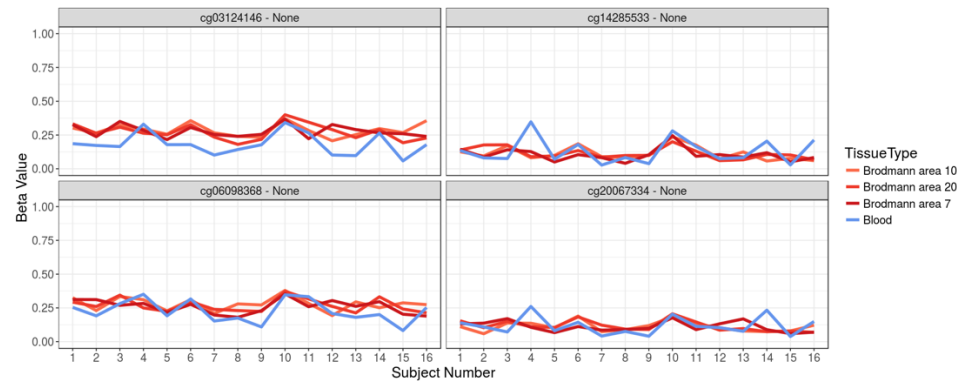




The Blood–Brain Epigenetic Concordance (BECon, <https://redgar598.shinyapps.io/BECon/>) used paired samples from 16 individuals across three brain regions (BA7, BA10, and BA20) and whole blood.⁵ Four out of six cis-eQTMs (cg06098368, cg03124146, cg20067334, cg14285533) were available and validated.

You have selected 4 CpGs associated with 1 genes to look at (4 CpGs have correlation data between blood and brain).

[Download Plots](#)



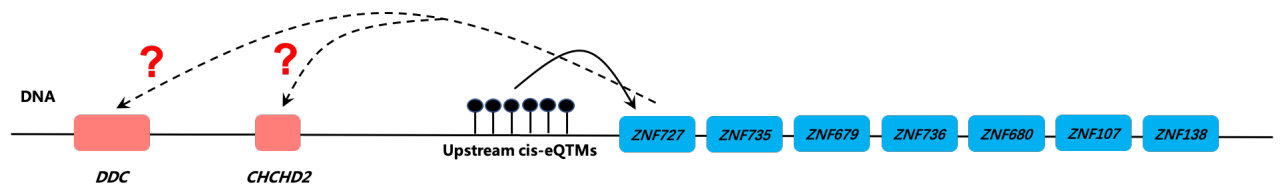
Summary of the Correlations and Variability of Selected CpGs

[Download as a colorful table](#) or a [csv file](#)

Chr	Coor	Gene(s)	Gene Region(s)	BA10 BA20 BA7 Blood				BA10 BA20 BA7			Blood Brain		Correlation or Cell Composition Percentile or Variability Status		
				Variability				Correlation			Cell Composition				
cg03124146	7	63361127	None	intergenic	0.12	0.13	0.11	0.2	0.38	0.44	0.03	0.04	0	90% (Positive)	50-75% (Blood Cell Comp.)
cg14285533	7	63386328	None	intergenic	0.11	0.11	0.09	0.21	0.24	0.35	-0.1	0.06	0	75-90% (Positive)	<50% (Blood Cell Comp.)
														50-75% (Positive)	90% (Brain Cell Comp.)
cg06098368	7	63386616	None	intergenic	0.11	0.12	0.12	0.21	0.36	0.55	0.23	0.03	0	<50% (Positive)	75-90% (Brain Cell Comp.)
														<50% (Negative)	50-75% (Brain Cell Comp.)
cg20067334	7	63387147	None	intergenic	0.09	0.1	0.1	0.18	0.15	0.24	0.12	0.04	0	50-75% (Negative)	<50% (Brain Cell Comp.)
														75-90% (Negative)	Not Variable
														90% (Variable)	Genomic Info
														90% (Blood Cell Comp.)	
														75-90% (Blood Cell Comp.)	

Supplementary Figure 7. Genomic context of *ZNF727*-associated cis-eQTM and nearby PD-related genes.

Six *ZNF727*-associated cis-eQTMs were located in the upstream intergenic region of *ZNF727* and showed inverse methylation-expression associations in the PPMI cohort. Male patients with PD showed higher methylation levels at these CpGs and lower *ZNF727* expression than female patients. *DDC* and *CHCHD2*, two genes implicated in PD biology, are located upstream of *ZNF727*.^{6,7} A series of consecutive zinc finger protein-encoding genes are located downstream of *ZNF727*. Given the transcription factor-related features of KRAB-ZFPs and their potential role in recognizing gene-regulatory elements,^{8,9} this genomic context suggests that *ZNF727* may represent a candidate cis-regulated locus relevant to sex-differential epigenetic regulation in PD. However, the causal inference test did not support a significant DNAm–*ZNF727*–clinical trait mediation pathway in the current dataset. Therefore, this schematic should be interpreted as a hypothesis-generating genomic-context summary rather than evidence of a confirmed causal mechanism.



Supplementary Data legends

This Excel workbook contains 14 sheets supporting the analyses.

Data S1. Quality control information on DNA methylation samples and probes for each dataset contributing to this analysis. Under sample quality control, shown are the numbers of samples remaining after each quality-control procedure. Under probe quality control, shown are the numbers of probes remaining after each quality-control procedure. BL, baseline; F, female; HC, healthy control; M, male; PD, Parkinson's disease; QC, quality control; V4, 1-year visit; V6, 2-year visit; V8, 3-year visit.

Data S2. Significant sex-differentially methylated positions identified in patients with Parkinson's disease in the SGPD dataset. A total of 18,165 significant sex-differentially methylated positions (DMPs) at $FDR < 0.05$ were identified among 813 patients with Parkinson's disease in the SGPD dataset (GSE145361). CpGs hypermethylated in males have positive logFC values. Bacon-correction information is also provided for each CpG.

Data S3. Significant sex-differentially methylated positions identified in patients with Parkinson's disease in the PEG1 dataset. A total of 3,504 significant sex-differentially methylated positions (DMPs) at $FDR < 0.05$ were identified among 330 patients with Parkinson's disease in the PEG1 dataset (GSE111629). CpGs hypermethylated in males have positive logFC values. Bacon-correction information is also provided for each CpG.

Data S4. Significant sex-differentially methylated positions identified in healthy controls in the SGPD dataset. A total of 4,593 significant sex-differentially methylated positions (DMPs) at $FDR < 0.05$ were identified among 777 healthy controls in the SGPD dataset (GSE145361). CpGs hypermethylated in males have positive logFC values. Bacon-correction information is also provided for each CpG.

Data S5. Significant sex-differentially methylated positions identified in healthy controls in the PEG1 dataset. A total of 1,244 significant sex-differentially methylated positions (DMPs) at $FDR < 0.05$ were identified among 236 healthy controls in the PEG1 dataset (GSE111629). CpGs hypermethylated in males have positive logFC values. Bacon-correction information is also provided for each CpG.

Data S6. Sex-differentially methylated positions identified in the meta-analysis of patients with Parkinson's disease. A total of 3,025 significant sex-differentially methylated positions (DMPs) were identified in the meta-analysis of patients with Parkinson's disease (PD), including 2,199 PD-unique DMPs detected only in PD and 826 PD-HC common DMPs detected in both PD and healthy controls. Compared with females, CpGs hypermethylated in males have positive direction values.

Data S7. Sex-differentially methylated positions identified in the meta-analysis of healthy controls. A total of 978 significant sex-differentially methylated positions (DMPs) were identified in the meta-analysis of healthy controls. Compared with females, CpGs hypermethylated in males have positive direction values.

Data S8. Background comparison of PD-unique, PD-HC common, and HC-unique sex-differentially methylated positions.

Data S9. PD-unique sex-differentially methylated regions identified using DMRcate and comb-p. A total of 365 significant sex-differentially methylated regions (DMRs) were identified exclusively between male and female patients with Parkinson's disease, without significant differences between healthy males and females. These DMRs are referred to as PD-unique DMRs and were identified using two complementary methods, DMRcate and comb-p.

Data S10. Gene Ontology enrichment analysis of genes with significant methylation differences between male and female patients with Parkinson's disease.

Data S11. Age-adjusted expression quantitative trait methylation site analysis in patients with Parkinson's disease at baseline with matched DNA methylation and transcriptomic data. Cis-expression quantitative trait methylation sites (cis-eQTM) refer to CpGs associated with expression of genes within 1 Mb, whereas trans-eQTM refer to CpGs associated with expression of distant genes outside this window. The analysis was adjusted for age.

Data S12. Sensitivity analysis of six ZNF727 cis-eQTM additionally adjusted for estimated blood cell proportions.

Data S13. Causal inference test analysis for ZNF727-associated cis-eQTM, ZNF727 expression, and clinical traits in patients with Parkinson's disease. In the causal inference test (CIT) analysis, methylation levels of the six cis-eQTM (cg06098368, cg03124146, cg12479444, cg20067334, cg14285533, and cg26911611) at baseline were specified as instrument variables, ZNF727 expression as the potential mediator, clinical traits as outcomes, and age as the covariate. Clinical traits were analysed as both continuous and binary variables. BL, baseline; GDS, Geriatric Depression Scale; MDS-UPDRS, Movement Disorder Society-Unified Parkinson's Disease Rating Scale; MoCA, Montreal Cognitive Assessment; V4, 1-year visit; V6, 2-year visit; V8, 3-year visit.

Data S14. PD-unique sex-differentially methylated positions identified from saliva samples in the PEG2 dataset. A total of 2,373 PD-unique sex-differentially methylated positions (DMPs) at FDR < 0.05 were identified from saliva samples of 128 patients with PD and 131 healthy controls in the PEG2 dataset (GSE111223). Cross-tissue validation revealed that 491 of these DMPs were consistent with blood-

based PD-unique DMPs, whereas the remaining 1,882 DMPs were specific to saliva. CpGs hypermethylated in males have positive logFC values. Bacon-correction information is also provided for each CpG. HC, healthy control; PD, Parkinson's disease.

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