

# Additional file 1

## The Infinium Methylation EPICv2.0 analysis

The Infinium Methylation EPICv2.0 analysis of 8 pairs of case and control were conducted using ChAMP 2.23.1 in R 4.2.1 and Bioconductor 3.16. Methylation level is represented by the average  $\beta$ -value =  $M / (M + U + \epsilon)$ , where M and U represent the intensity of methylated and unmethylated allele, and ranged from 0 (unmethylated) to 1 (full methylated). In probes quality control, we excluded 2491 probes with poor detection (detection p-value <0.01 in one or more samples), 24173 probes with <3 beads in at least 5% of samples per probe, 3641 non-CpG probes, 31366 SNP-associated probes, 0 multi-hit probes, and 21443 probes located in chromosome X and Y [1,2]. A total of 853941 probes were included for subsequent analysis. We applied the  $\beta$ -mixture quantile intra sample normalization procedure (BMIQ), which provide better normalization for EPIC array data, to adjust type-I and type-II bias [3]. Then, Combat normalization method and RefbaseEWAS method were used for batch effect correction and adjust the heterogeneity correction [166,167,168]. All CpGs were annotated using EPICanno.ilm10b4.hg19, and the differential methylated CpGs position (DMPs) that further adjust for maternal age, pre-pregnancy BMI and gestational age were generated based on the champ.DMP function. The adjusted  $p$  values were calculated using the Benjamini–Hochberg method.

## References

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