

Appendix 1 (1)

1. DNA extraction;
2. Amplification of bacterial 16S rRNA V4 gene region and sequence;
3. Data analysis.

Reference

1. Chao X, Sun T, Wang S, et al. Research of the potential biomarkers in vaginal microbiome for persistent high-risk human papillomavirus infection. *Ann Transl Med* 2020;8:100.

Table S1 Comparison of different methods in the diagnosis CPPS associated with EM/AM

	CPPS due to EM/AM (n)	CPPS without EM/AM (n)	
Training set			
Suspected by method A	30	12	PPN=71.43%; NPV=65.00%; SEN=81.08%; SPE=52.00%
Unsuspected by method A	7	13	
Suspected by method B	15	0	PPN=100.00%; NPV=53.19%; SEN=40.54%; SPE=100.00%
Unsuspected by method B	22	25	
Suspected by method C	33	12	PPN=73.33%; NPV=76.47%; SEN=89.19%; SPE=52.00%
Unsuspected by method C	4	13	
Validation trial			
Suspected by method A	35	32	PPN=52.24%; NPV=71.15%; SEN=70.00%; SPE=53.62%
Unsuspected by method A	15	37	
Suspected by method B	23	6	PPN=79.31%; NPV=70.00%; SEN=46.00%; SPE=100.00%
Unsuspected by method B	27	63	
Suspected by method C	43	34	PPN=55.84%; NPV=83.33%; SEN=86.00%; SPE=50.72%
Unsuspected by method C	7	35	

Method A refers to the relative abundance of *Clostridium disporicum* being over 0.01105% with that of *Lactobacillus reuteri* being under 0.1911349%. Method B refers to the relative abundance of *Clostridium disporicum* being over 0.01105% with that of *Lactobacillus reuteri* being under 0.1911349% and serum CA125 being over 35 U/mL. CPPS, chronic pelvic pain syndrome; EM, endometriosis; AM, adenomyosis.

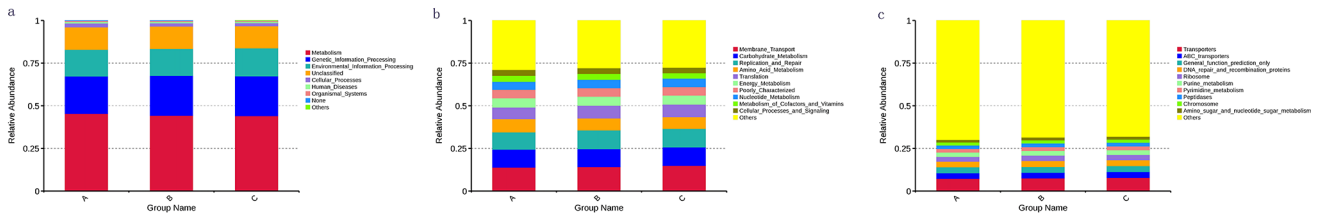


Figure S1 The proportion of predictive functional profiling of the vaginal microbiome in the 3 groups. (A) Bar chart showing the relative proportion of gene function prediction on level 1 of each group. (B) Bar chart showing the relative proportion of gene function prediction on level 2 of each group. (C) Bar chart showing the relative proportion of gene function prediction on level 3 of each group.

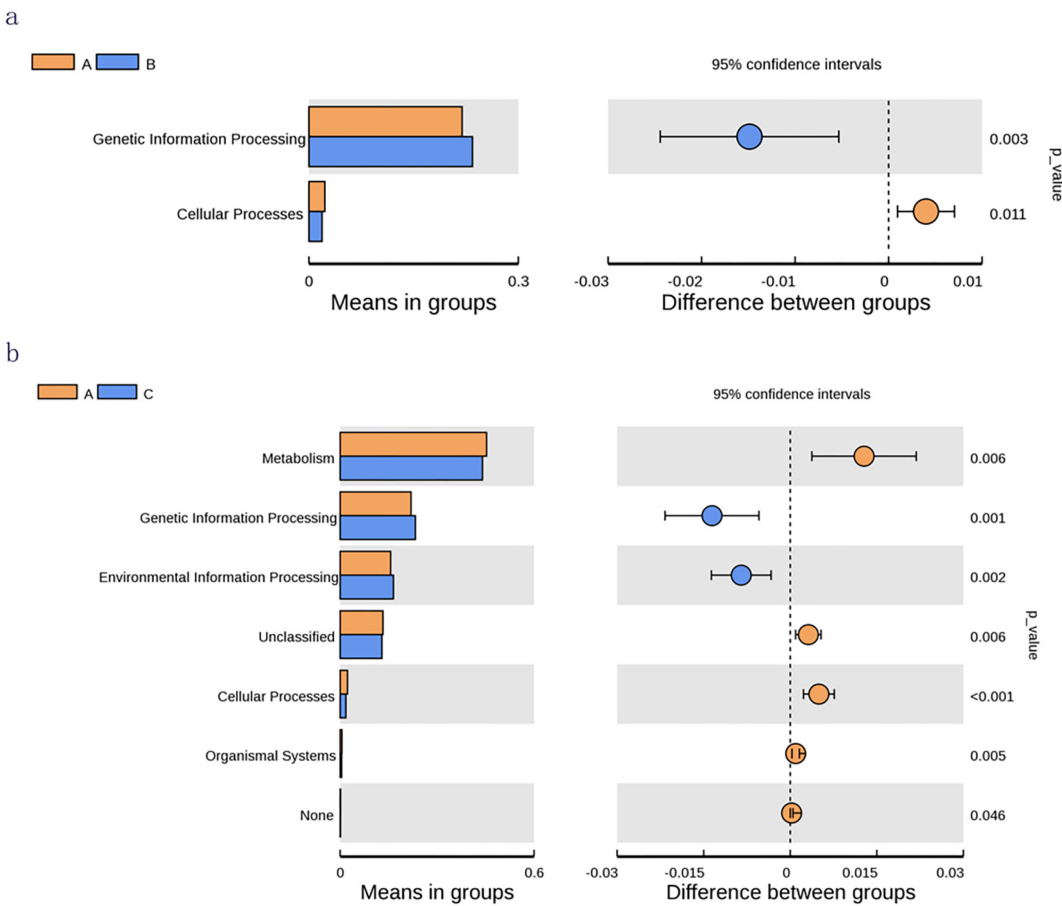


Figure S2 PICRUSt *in silico* functional analyses of the vaginal microbiome of participants in the 3 groups on level 1. Microbial pathways were predicted to be differentially regulated based on microbiomic differences between groups. Upregulated pathways refer to a higher percentage of the mean proportion of expression. P values were calculated by the White's nonparametric t test and corrected for multiple comparisons. (A) A comparison of functional analyses between groups A and B. (B) A comparison of functional analyses between groups A and C.

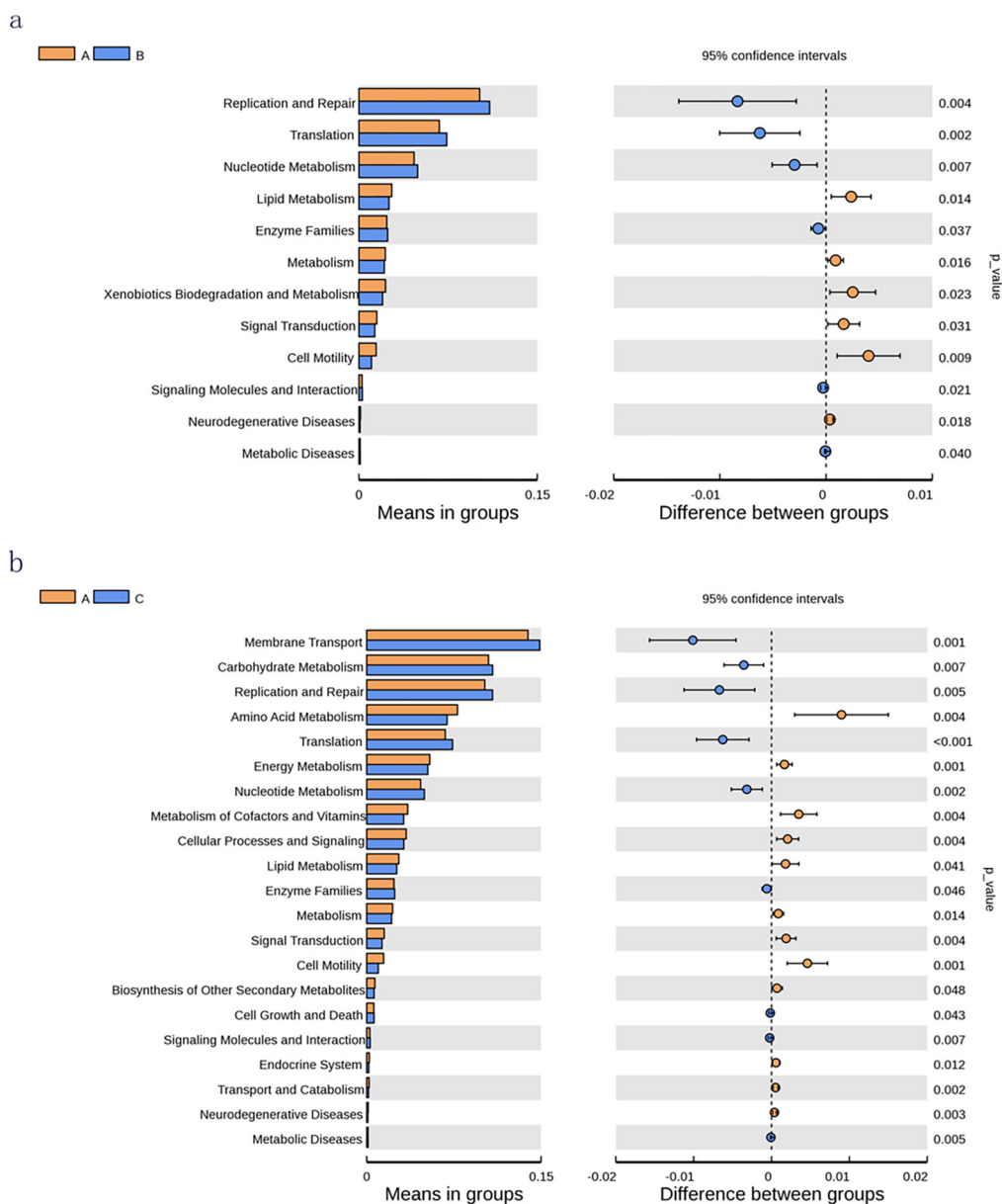


Figure S3 PICRUST *in silico* functional analyses of the vaginal microbiome of participants in the 3 groups on level 2. Microbial pathways were predicted to be differentially regulated based on microbiomic differences between groups. Upregulated pathways refer to a higher percentage of the mean proportion of expression. P values were calculated by the White's nonparametric t test and corrected for multiple comparisons. (A) A comparison of functional analyses between groups A and B. (B) A comparison of functional analyses between groups A and C.

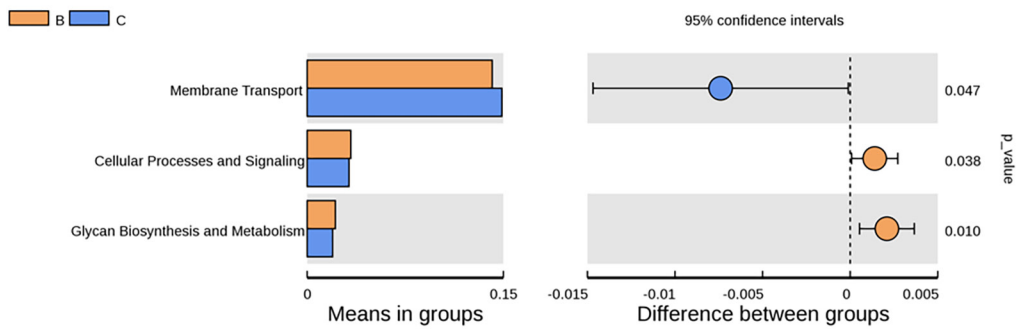


Figure S4 PICRUSt in silico functional analyses of the vaginal microbiome of participants in group B, C on level 3. Microbial pathways were predicted to be differentially regulated based on microbiomic differences between groups. Upregulated pathways refer to a higher percentage of the mean proportion of expression. P values were calculated by the White's nonparametric t test and corrected for multiple comparisons.