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Supplemental information

**Recurrent urinary tract infection and estrogen
shape the taxonomic ecology and function
of the postmenopausal urogenital microbiome**

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Supplemental Table 1

	No UTI History (Group 1)	rUTI History, UTI(-) (Group 2)	rUTI History, UTI(+) (Group 3)	P-value
N (Individuals)	25	25	25	-
Age (years) (CI _{95%})	67 (62-73)	68 (64-77)	76 (70-78)	0.04^A
Race				
<i>African American</i>	1 (4%)	1 (4%)	0 (0%)	
<i>Caucasian</i>	24 (96%)	23 (92%)	22 (88%)	
<i>Hispanic</i>	0 (0%)	1 (4%)	3 (12%)	
<i>Other</i>	0 (0%)	0 (0%)	0 (0%)	0.33 ^B
BMI (CI _{95%})	26.2 (23.7-28.9)	25.3 (23.0-29.1)	27.3 (23.0-29.1)	0.25 ^A
Smoking History				
<i>Never</i>	16 (64%)	17 (68%)	17 (68%)	
<i>Ever</i>	9 (36%)	8 (32%)	8 (32%)	0.94 ^B
EHT				
<i>EHT (-)</i>	10 (40%)	11 (44%)	17 (68%)	
<i>EHT (+)</i>	15 (60%)	14 (56%)	8 (32%)	0.10 ^B
Urine pH (CI _{95%})	6.0 (5.0-7.0)	6.0 (5.0-6.9)	5.5 (5.0-6.3)	0.60 ^A
Urinary Creatinine (µg/ml) (CI _{95%})	644.9 (301.2-1007)	720.7 (419.8-957.1)	771.5 (483.0-1083)	0.69 ^A

^A Kruskal-Wallis test

^B χ^2 test

Table S1. Cohort clinical features, Related to Figure 1 and STAR Methods. Distribution of clinical features among the three groups. Medians and 95% confidence interval are presented for Age, BMI, Urine pH, and Urinary creatinine. P-values generated by Kruskal-Wallis test or χ^2 test. Significant differences between groups have been bolded.

Figure S1

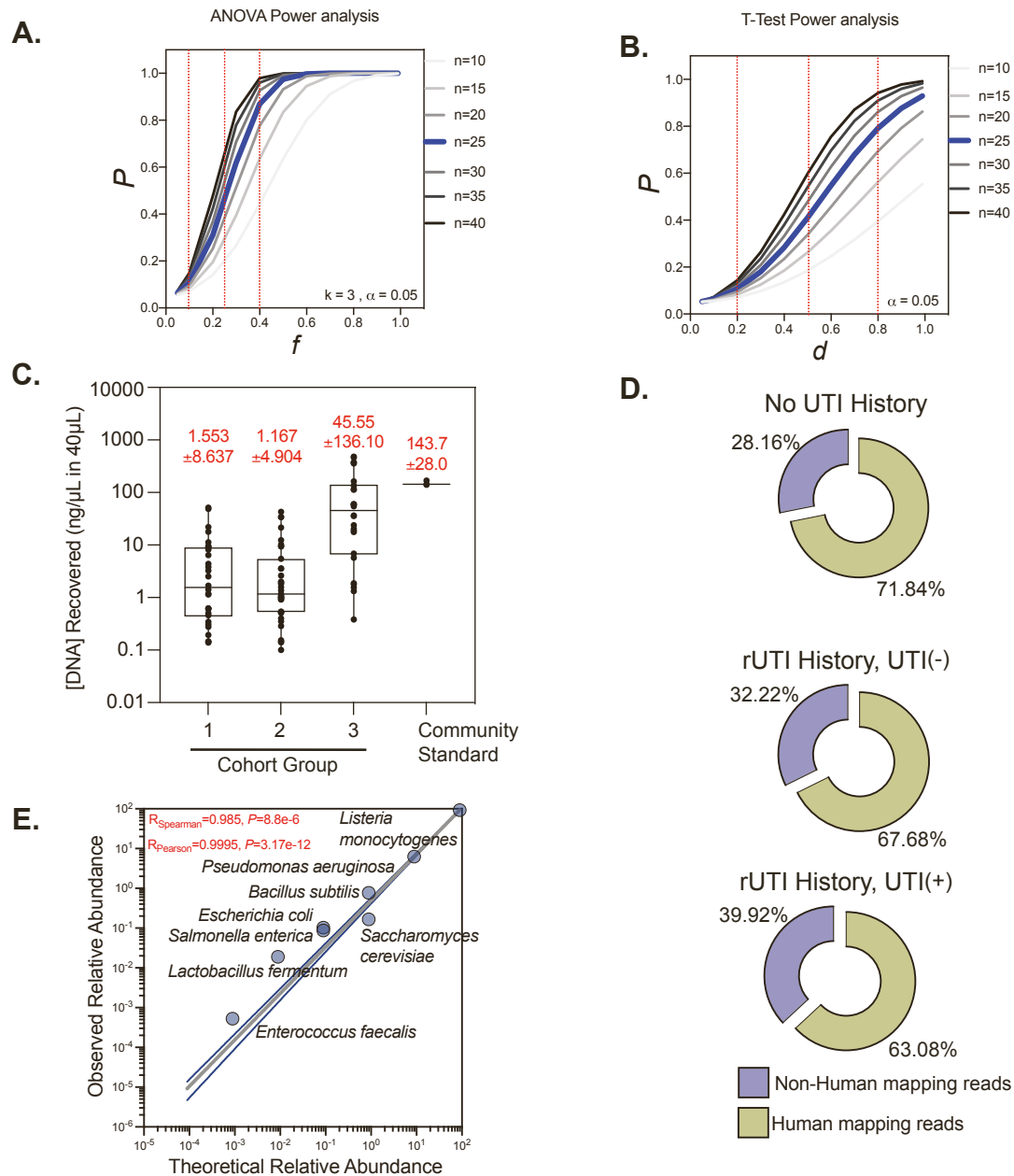


Figure S1. Power analysis and metagenomic dataset characteristics, Related to Figure 1 and STAR Methods. (A) ANOVA power analysis between 3 groups with an alpha of 0.05 for a range of sample sizes per group. Red lines represent small medium and large effect sized. Power curve for chosen sample size is blue. (B) Two-way T-test power analysis a of 0.05 for a range of sample sizes per group. Red lines represent small, medium, and large effect sizes (Cohen's d). Power curve for chosen sample size is blue. (C) Metagenomic DNA yields within the three cohort groups and commercially available community standards (Zymo Research). Bars are drawn from the minimum to maximum of the distribution. Boxes represent the interquartile range. Median is depicted by solid line. (D) Average proportion of human genomic content in the WGMS data among the three cohort groups (E) Correlation of theoretical and observed relative abundance of the Zymo Research Microbial Community standard (Log Distribution). P-value generated by permutation. Group 1). No UTI History: no history of UTI, no active UTI; 2). rUTI History, UTI(-): history of rUTI, no active UTI; 3). rUTI History, UTI(+): history of rUTI, active UTI.

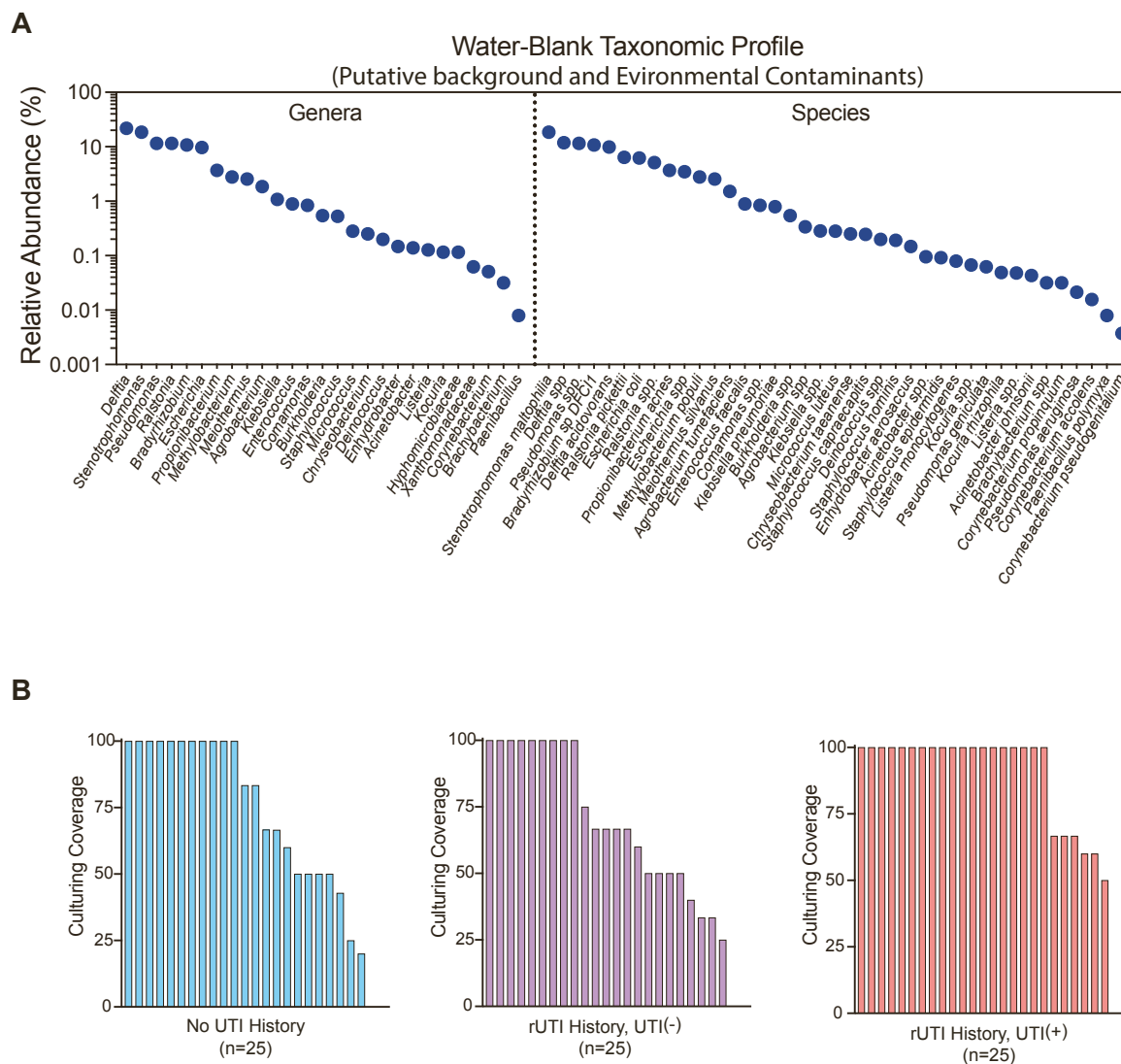


Figure S2. WGMS environmental contaminants and patient-level urine culturing coverage, Related to Figure 1 and STAR Methods. (A) Genera- and species-level taxonomic profile of taxa detected in sequenced water controls. (B) Advanced urine culturing coverage of each patient among the three cohort groups. No UTI History: no history of UTI, no active UTI; rUTI History, UTI(-): history of rUTI, no active UTI; rUTI History, UTI(+): history of rUTI, active UTI.

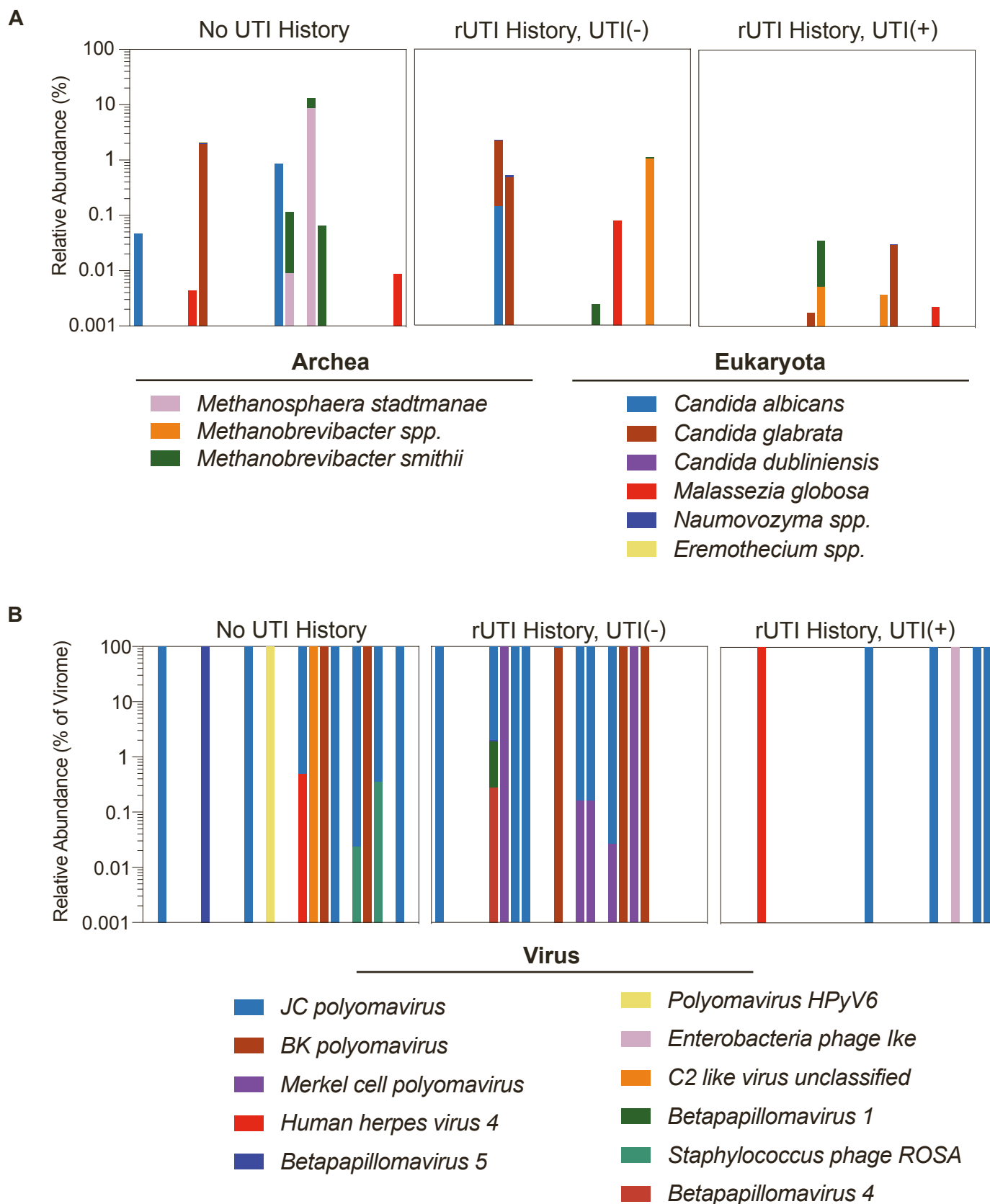
Figure S3

Figure S3. Taxonomic profiles of detected Archea, Eukaryota, and Viral species, Related to Figure 2. (A) Species-level taxonomic profile of Archea and Eukaryota among cohort groups (No UTI History (n=25), rUTI History, UTI(-) (n=25), rUTI History, UTI(+) (n=25)). (B) Species-level taxonomic profile of Viruses among cohort groups (No UTI History (n=25), rUTI History, UTI(-) (n=25), rUTI History, UTI(+) (n=25)). No UTI History: no history of UTI, no active UTI; rUTI History, UTI(-): history of rUTI, no active UTI; rUTI History, UTI(+): history of rUTI, active UTI.

Figure S4

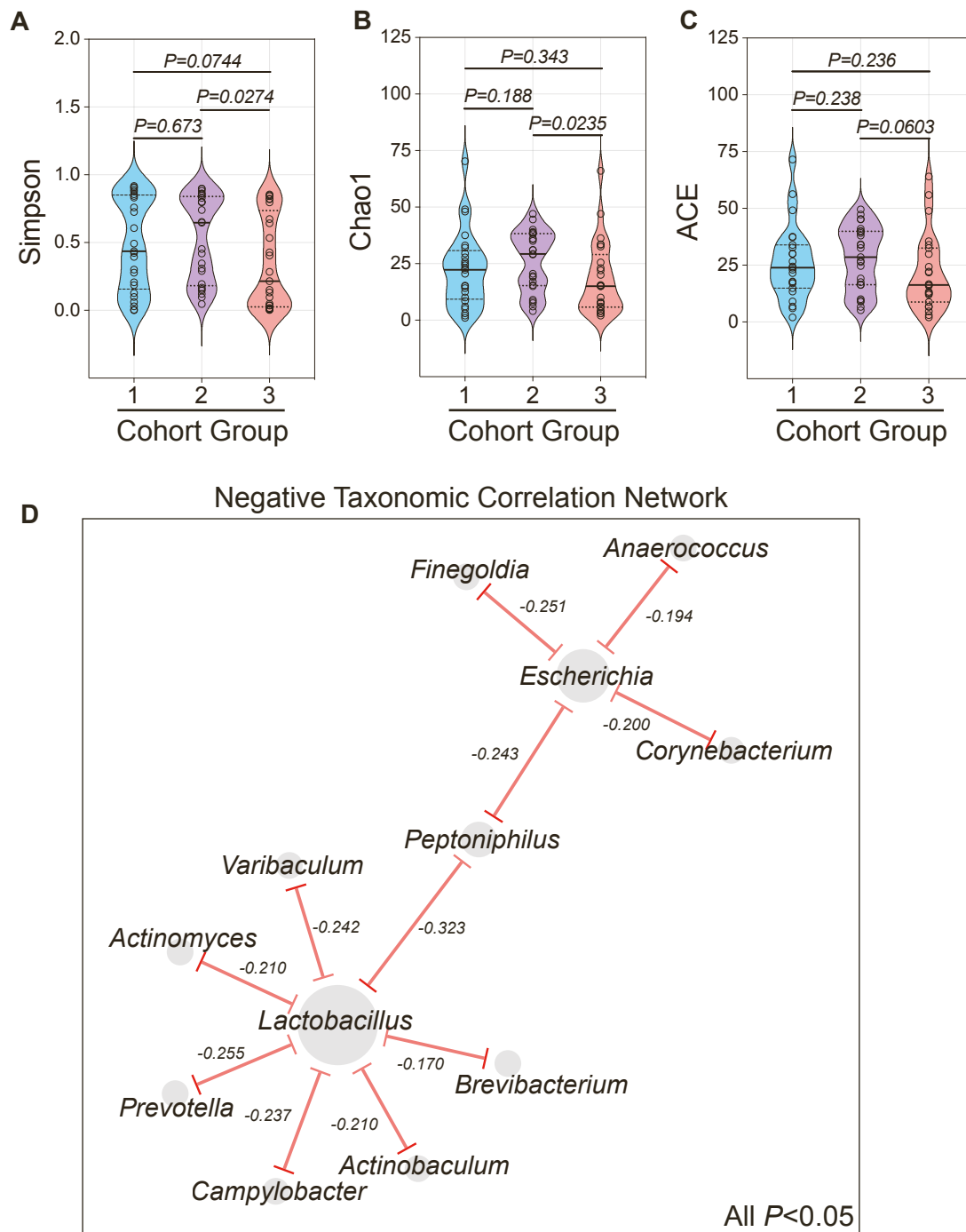


Figure S4. Ecological modeling indices among the cohort groups and negative taxonomic correlation network, Related to Figure 2. (A) Simpson index, (B) Chao1 index, (C) and ACE index comparison between the three cohort groups (No UTI History (n=25), rUTI History, UTI(-) (n=25), rUTI History, UTI(+)) (n=25)). P-value was generated by Kruskal-Wallis test with uncorrected Dunn's multiple correction post hoc. (D) Network analysis of all genera anticorrelation associations with P-value less than 0.05. Nodes represent genera edges are defined by Pearson correlation. Node size is proportional to the degree of the node. Group 1). No UTI History: no history of UTI, no active UTI; 2). rUTI History, UTI(-): history of rUTI, no active UTI; 3). rUTI History, UTI(+): history of rUTI, active UTI.

Figure S5

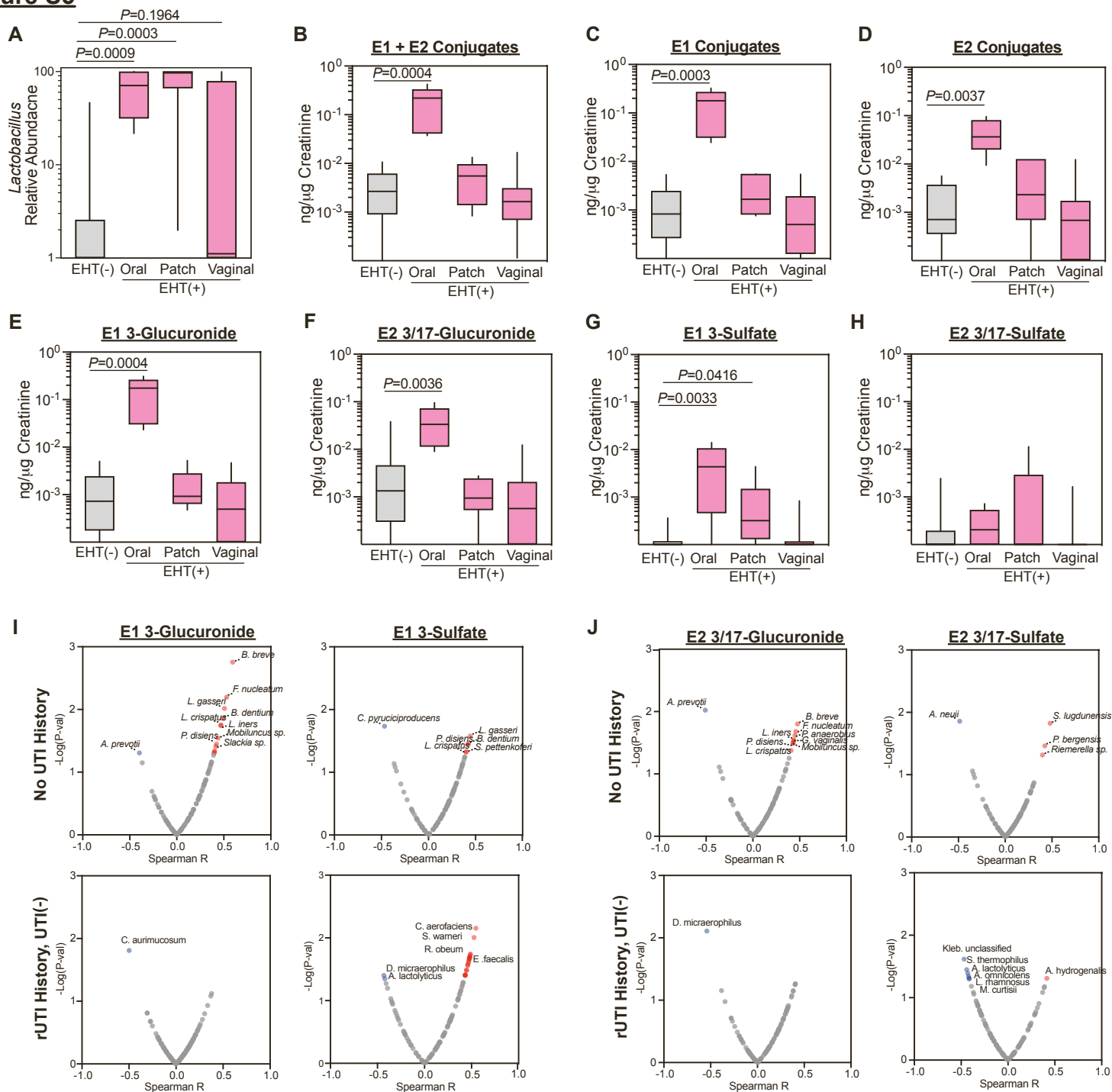


Figure S5. Urinary estrogen conjugate concentrations and taxonomic associations, Related to Figure 5. (A) Comparison of the relative Lactobacillus abundance between EHT(-) (grey, n=21) and EHT(+) (pink, n=29) women separated by EHT modality (Oral, Patch, Vaginal). Creatinine (Cr)-normalized urinary summed estrogen conjugates (E1 + E2) (B), E1 conjugates (C), E2 conjugates (D), E1 3-Glucuronide (E), E2 3/17-Glucuronide (F), E1 3-Sulfate (G), and E2 3/17-Sulfate (H) measured in the urine of EHT(-) (n=21) and EHT(+) women from the No UTI History and rUTI History, UTI(-) groups stratified by EHT modality (Oral (n=6), Patch (n=6), Vaginal (n=17)). Error bars are drawn from minimum to maximum of the data distribution. Boxes represent the interquartile range. Solid lines denote the median. P-value generated by Kruskal-Wallis test with uncorrected Dunn's multiple correction post hoc. (I) Volcano plots depicting correlation of bacterial species with summed Cr-normalized urinary E1 (E) and E2 (J) conjugates by Spearman correlation in No UTI History (Top panels) and rUTI History, UTI(-) (Bottom panels) groups. P-value generated by permutation. Red dots represent significant ($P < 0.05$) positive associations. Blue dots represent significant negative associations. No UTI History: no history of UTI, no active UTI; rUTI History, UTI(-): history of rUTI, no active UTI; rUTI History, UTI(+): history of rUTI, active UTI.