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1 **The bladder microbiome, metabolome, cytokines,**
2 **and phenotypes in patients with systemic lupus erythematosus**

3

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29 **Abstract**

30 **Background and aims**

31 Emerging studies reveal a unique bacterial community in the human bladder, with alteration of
32 composition associated to disease states. Systemic lupus erythematosus (SLE) is a complex
33 autoimmune disease that is characterized by frequent impairment of the kidney. Here, we
34 explored the bladder microbiome, metabolome, and cytokine profiles in SLE patients, as well
35 as correlations between microbiome and metabolome, cytokines, and disease profiles.

36 **Methods and materials**

37 We recruited a cohort of 50 SLE patients and 50 individually matched asymptomatic controls.
38 We used transurethral catheterization to collect urine samples, 16S rRNA gene sequencing to
39 profile bladder microbiomes, and LC-MS/MS to perform untargeted metabolomic profiling.

40 **Results**

41 Compared to controls, SLE patients possessed a unique bladder microbial community and
42 increased alpha diversity. These differences were accompanied by differences in urinary
43 metabolomes, cytokines, and patients' disease profiles. The SLE-enriched genera, including
44 *Bacteroides*, were positively correlated with several SLE-enriched metabolites, including
45 olopatadine. The SLE-depleted genera, such as *Pseudomonas*, were negatively correlated to
46 SLE-depleted cytokines, including IL-8. Alteration of the bladder microbiome was associated
47 with disease profile. For example, the genera *Megamonas* and *Phocaeicola* were negatively
48 correlated with serum complement C3, and *Streptococcus* was positively correlated with IgG.

49 **Conclusions**

50 Our present study reveals associations between the bladder microbiome and the urinary
51 metabolome, cytokines, and disease phenotypes. Our results could help identify biomarkers for
52 SLE.

53

54 **Key words:** bladder microbiome, complement, disease profile, systemic lupus erythematosus,
55 urinary cytokines, urinary metabolome.

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57 **Introduction**

58 Systemic lupus erythematosus (SLE) is a complex autoimmune disease with a chronic
59 relapsing-remitting course that can damage multiple organs and range from mild to
60 life-threatening illness(1). The kidney is one of the most commonly impaired organs, and
61 lupus nephritis (LN) has been reported in approximately 50% of SLE patients(2), 10-30% of
62 LN patients progress to kidney failure that requires kidney replacement therapy(2), and the
63 mortality rate within 5 years of onset directly attributed to kidney disease is 5-25% of patients
64 with proliferative LN(2). Currently, no cure for SLE has been found and the pathogenesis of
65 SLE is currently poorly understood.

66 Alteration of microbial compositions in the gut, oral mucosa or tegument has been
67 reported to be associated with SLE disease manifestations(3-15). Specifically, SLE patients
68 exhibit compositional alterations to the gut microbiome, characterized by lower bacterial
69 diversity(3, 4, 6-8, 14), decreased Firmicutes/Bacteroidetes (F/B) ratio(5, 7, 14, 15), and
70 increased abundance of Lactobacillaceae(6, 8, 11). Similarly, SLE patients exhibit reduced
71 microbial diversity and altered microbial community in their gums and skin(10, 12). However,
72 the association of SLE on microbial communities and metabolic output in the bladder and
73 other urogenital niches has not been studied.

74 Once considered sterile, the bladder is now known to possess microbial communities
75 (bladder microbiome) in individuals with and without urinary tract infections (16).
76 Furthermore, disruption (dysbiosis) of the bladder microbiome is associated with urinary tract
77 disorders, especially urgency urinary incontinence and urinary tract infection(17). However,
78 most studies of bladder microbiome involve only US participants; only a few reports involve
79 Chinese participants(18, 19).

80 Immune response and metabolic output can bridge the gap between the microbiome and SLE
81 phenotypes. Urine is often used to assess metabolic status of the body(20). For example, Yan
82 and co-workers found 23 metabolites dramatically increased in SLE patients compared to
83 healthy controls, including valine, cysteine, and uracil(21). Also, as renal impairment is one of
84 the most serious manifestations of SLE and urine cytokines derived directly from the diseased
85 kidney accumulate in the urine, the level of inflammatory factors in urine may be used as an
86 indicator of chronic inflammation and disease progression. Brugos and co-workers found that

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87 IL-1 and TNF-α were elevated significantly in the urine of patients without renal disease,
88 while IFN-γ was elevated in the urine of LN patients(22). However, the relationship between
89 immunity, metabolism, and microbiome in the bladder of SLE patients is unclear.

90 Given that SLE often affects the kidney, and LN is a common manifestation that leads to
91 irreversible renal impairment, we hypothesize that the bladder microbiomes of individuals
92 with and without SLE differ, and the differences correlate with specific urinary metabolites
93 and cytokines, along with patients' clinical profiles. To test this hypothesis, we analyzed urine
94 obtained by transurethral catheterization from participants with and without SLE. We also
95 compared the gut and vaginal microbiomes of a subset of SLE patients to their bladder
96 microbiome to determine whether these adjacent microbiomes might influence the
97 composition of the bladder microbiome.

98 Methods and materials

99 *Patients and controls recruitment*

100 50 SLE patients, who fulfilled at least 4 of the American College of Rheumatology
101 Criteria for the diagnosis of SLE(23), and 50 sex-, age-, BMI-, and co-morbid
102 disease-matched controls were consecutively recruited from Wuxi Second Affiliated Hospital
103 of Nanjing Medical University (**Fig. S1**). The inclusion and exclusion criteria are described in
104 **File. S1**. Disease activity was measured using SLEDAI score(24). All participants signed their
105 informed consent before sample collection. The study was executed in accordance with the
106 Ethical Committee of the hospital (ref. 201805).

107 *Urine sample collection*

108 Urine samples were collected through a urinary catheter. Before insertion of the catheter,
109 5% iodophor was applied to sterilize the genital and perineal areas. The collected urine was
110 separated into four portions, which were used for detecting or measuring the bladder
111 microbiome, metabolome, creatinine levels, and cytokines. Fecal and vaginal samples were
112 collected before the collection of urine samples. Fecal material was collected in a sterile
113 container by the patient, and 30 mg was immediately placed in a sterile container. Vaginal
114 samples were collected by the nurse using a sterile swab. All urine, feces and vaginal samples
115 were placed in sterile, DNA- and enzyme-free centrifuge tubes, and immediately stored at
116 -80°C until use.

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117 ***DNA extraction and bioinformatics analysis***

118 30 mL urine samples were processed for sequencing as described in **File. S2.** The DNA
119 extraction from the vaginal and fecal samples were the same as urine samples. We used
120 DADA2 (https://github.com/benjjneb/dada2) to process reads derived from 16S rRNA V3-V4
121 region, including quality control (truncQ=8, maxN=0, maxEE=c(2,2)), dereplication, merging
122 forward and reverse reads (trimOverhang=TRUE, minOverlap=5), and chimera removal
123 (method="consensus") to obtain amplicon sequence variants (ASVs). To remove
124 environmental contaminants, we manually removed ASVs whose reads did not exceed 5 times
125 the maximum number of reads in the environmental controls. After decontamination, BLCA
126 was applied to obtain taxonomic identities for the remaining ASVs(25). We only kept taxa
127 with a confidence score above 60 for downstream analysis. Multivariate Association with
128 Linear Modes (MaAslin) framework was used to adjust the effects of confounding factors.

129 ***Urinary metabolome profiling and processing***

130 Urinary metabolome profiling was performed using liquid chromatography tandem mass
131 spectrometry, LC-MS/MS（ExionLC and TripleTOF 5600, SCIEX, Framingham, MA, USA）
132 as previously described (see **File. S3**). In total, 6770 and 6078 peaks were detected in the
133 positive and negative ionization modes, respectively. 6515 (positive mode) and 5970
134 (negative mode) metabolite features remained. The positive-mode and negative-mode features
135 were then annotated using The Kyoto Encyclopedia of Genes and Genomes (KEGG,
136 http://www.kegg.com/) and Human Metabolome Database (HMDB, http:www.hmdb.ca). The
137 result was 1076 annotated metabolites (**Tab. S1**).

138 ***Cytokines and creatinine detection***

139 The Bio-Plex^{TM} 200 System (Bio-Rad) and Bio-Plex Pro Human Cytokine 27-plex assay
140 (Bio-Rad, California, USA) were used to detect urinary cytokines. Creatinine was detected
141 using the human urine Elisa kit of creatinine (Hengyuan Biological Technology Co., Ltd,
142 Shanghai, China).

143 ***Disease profile measurement***

144 Blood samples were collected on the day of urine sample collection. The
145 immunoturbidimetric test was used to assess serum complement (C) and immunoglobulin
146 antibodies (AU5421; Beckman Coulter, USA). Immunoblotting and immunofluorescence

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147 were used to detect serum autoantibodies. Erythrocyte sedimentation rate was determined by
148 the Westergren method (XC-408 ESR Monitor; Mindray, China).
149 Lupus nephritis (LN) was defined as clinical and laboratory manifestations that meet
150 ACR criteria(26). Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K)
151 was used to assess disease severity(24).

152 ***Statistical analysis***

153 For microbiome analysis, Bray-Curtis dissimilarity of microbial communities was
154 calculated using ‘vegdist’ function with “bray” mode, permutational multivariate analysis of
155 variance [PERMANOVA] was calculated using ‘adonis’ function, and Shannon’s H was
156 calculated using ‘diversity’ function with “shannon” mode in R. For metabolome analysis, the
157 concentration of urinary metabolites was adjusted for variability in urine dilution, using Cr as
158 a normalization indicator. For the comparison of the metabolite intensity between SLE and
159 controls, we performed statistical analysis using MetaboAnalyst 5.0
160 (https://www.metaboanalyst.ca). Metabolites with (1) variable importance in the projection
161 (VIP) greater than 1, (2) fold change greater than 2 or less than 0.5 and (3) *P*-value less than
162 0.05, were then log2 transformed and subjected to linear model analysis to control for
163 confounding factors, including nutrient intake (Binary logistic regression model analysis,
164 SPSS 24.0). For cytokine analysis, Cr was used to normalize urinary cytokine concentration.
165 Pearson’s Chi-square or Fisher’s exact tests were used with categorical variables; Student’s *t*
166 test was used on normalized continuous variables and Wilcoxon rank-sum test on non-normal
167 continuous variables. The *P*-value was adjusted for multiple comparisons using the
168 Benjamini–Hochberg (BH) false discovery rate (FDR).
169

170 ## Results

171 ***Demographics***

172 We assessed the bladder microbiome, metabolite profile, and cytokine profile of a total
173 of 50 SLE patients and 50 sex-, age-, BMI-, and comorbid disease-matched asymptomatic
174 controls (Controls) (**Tab. 1**). Both cohorts were 88% (n=44) female, 12% (n=6) male. As
175 expected, the SLE cohorts had lower serum concentrations of C3 and C4, but higher serum
176 concentrations of IgG and uric acid; they also had more urinary white blood cells, red blood

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177 cells and leucocyte esterase (P<0.05 for all comparisons). Of the 50 SLE patients, 38 (76%)
178 had LN (**Tab. S2**). The SLE cohort also had significantly elevated intake of calcium and zinc
179 (**Tab. S3**); these were listed as confounding factors in the downstream analyses.

180 ***Bladder microbiome is altered in SLE patients***

181 To test whether the bladder microbiome differs between SLE patients and asymptomatic
182 controls, we first assessed the microbial community structure using all microbial species
183 present within the bladder microbiome of each sample. Principal Coordinate Analysis (PCoA)
184 of Bray-Curtis dissimilarities revealed differential clustering between the control and SLE
185 cohorts (**Fig. 1A**; R^2=0.128, $P_{(adj)}$=0.001), reflecting a dysbiotic urobiome in SLE patients. To
186 determine whether the bacterial community was affected by medication usage, we separated
187 the SLE patients into subgroups based on their dosages of hydroxychloroquine and
188 prednisone and performed PCoA; we found no differences between/among the subgroups
189 (**Fig. S2A and S2B**; R^2=0.013, $P_{(adj)}$=0.886; R^2=0.020, $P_{(adj)}$=0.428). The disruption of
190 microbial composition between the controls and SLE cohort was highlighted by the
191 observation that species diversity (as measured by the Shannon's H Index) was significantly
192 elevated in the SLE cohort **(Fig. 1B**; $P_{(adj)}$<0.05;), likely due to increased evenness (**Fig. S3A**;
193 $P_{(adj)}$ <0.05;), as there was no significant difference in species richness (**Fig. S3B**).

194 Since we observed a clear difference in diversity, we assessed taxonomic signatures at
195 the phylum level. The relative abundances of the 5 most abundant phyla (>1% relative
196 abundance) differed significantly between the control and SLE cohorts (**Fig. 1C**; $P_{(adj)}$<0.05).
197 The phyla Firmicutes, Proteobacteria, Acidobacteria and Actinobacteria were significantly
198 more abundant in controls, whereas Bacteroidetes was significantly more abundant in the SLE
199 cohort (**Fig. 1D**; $P_{(adj)}$<0.05). The Firmicutes/Bacteroidetes ratio was reduced significantly in
200 SLE patients (**Fig. 1E**; $P_{(adj)}$<0.05), consistent with previous studies of the gut microbiome of
201 SLE patients(5, 27).

202 At the genus level, PCoA based on the Bray-Curtis Dissimilarity Index also revealed
203 differential clustering of bladder microbiomes from controls relative to SLE patients (**Fig.**
204 **S4A**; R^2=0.153, $P_{(adj)}$=0.001). The 15 most abundant genera (>1% relative abundances) are
205 displayed in **Fig. S4B**. Among them, seven genera, (*Staphylococcus*, *Rothia*, *Streptococcus*,
206 *Haemophillus*, *Sphingomonas*, *Gardnerella* and *Pseudomonas*) were significantly more

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207 abundant in controls (**Fig. 2A**; $P_{(adj)}$<0.05), especially *Staphylococcus*, which often
208 predominated. In contrast, 5 genera (*Alistipes*, *Bacteroides*, *Phocaeicola*,
209 *Phascolarctobacterium* and *Megamonas*), were significantly more abundant in the SLE
210 cohort (**Fig. 2B**; $P_{(adj)}$<0.05). However, when we adjusted for the confounding factors, such as
211 nutrient intake and medication usage, using MaAslin analysis, we found that *Alistipes* and
212 *Blautia wexlerae* were affected by calcium intake, and *B. wexlerae* was also affected by
213 prednisone use (**Tab. S4**; $P_{(adj)}$ <0.001); thus, *Alistipes* and *B. wexlerae* were removed from
214 the downstream interaction analysis. The bacterial species >0.5% relative abundances are
215 displayed in **Fig. S5**; 8 species were significantly more abundant in controls, especially *S.*
216 *aureus* (**Fig. 2C**), whereas 11 species were significantly more abundant in the SLE cohorts
217 (**Fig. 2D**). To investigate the potential for the use of urinary microbial profiles to discriminate
218 SLE patients from controls, we performed a backward stepwise selection model to identify
219 bacterial genera/species with optimal model fitting. This model identified 3 genera
220 (*Bacteroides*, *Rothia* and *Sphingomonas)* and 4 species (*Phocaeicola vulgatus*, *Rothia aeria*,
221 *Asticcacaulis excentricus* and *Vicinamibacter silvestris)* that could discriminate controls from
222 SLE with AUC values of 93.16% (**Fig. 2E**) and 98.63% (**Fig. 2F**), respectively.

223 Next, we divided SLE patients into lupus nephritis (LN) and non-LN subgroups (**Tab.**
224 **S5**); the LN and non-LN cohorts matched demographically, except for age. Based on PCoA,
225 the bladder microbiome of these subgroups did not differ (R_2=0.022, $P_{(adj)}$=0.377), but both
226 differed from controls (**Fig. S6A**; R^2=0.072, $P_{(adj)}$=0.002; R^2=0.128, $P_{(adj)}$=0.002, respectively).
227 As measured by Shannon's H Index, microbial diversity also did not differ between these two
228 subgroups, but the SLE patients with LN had more microbial diversity than controls (**Fig.**
229 **S6B**; $P_{(adj)}$<0.05).

230 Finally, as a pilot analysis, we compared the bladder microbiome with the vaginal and
231 gut microbiomes using only the subset (N=15) of SLE patients who provided all 3 sample
232 types. PCoA based on species showed that the SLE microbiome of the bladder differed from
233 those of the vagina and gut (**Fig. S7A**; R^2=0.105, $P_{(adj)}$=0.001). By Bray-Curtis Dissimilarity
234 Index, the SLE bladder microbiome more closely resembled the vaginal microbiome than it
235 did the gut microbiome (**Fig. S7B;** $P_{(adj)}$<0.001, and $P_{(adj)}$<0.01, respectively). However, the
236 predominant species in the bladder microbiome were dissimilar to those of both the gut and

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237 vagina (**Fig. S7C**).

238 ***Urinary metabolome is altered in SLE patients***

239 To test whether the urinary metabolome differs between SLE patients and controls, we
240 performed untargeted metabolomics on the urine samples. Based on Principal Component
241 Analysis (PCA) using all 1076 metabolites detected (**Tab. S1**), the metabolic composition of SLE
242 patients differed significantly from controls (**Fig. 3A;** R^2=0.650, $P_{(adj)}$=0.001). Partial least squares
243 discriminant analysis (PLS-DA) yielded similar results (**Fig. 3B;** P<0.001). Also, we tested the
244 effect of medication usage on the metabolome and found no differences between/among the
245 dosage subgroups (**Fig. S8A and 8B;** R^2=0.004, $P_{(adj)}$=0.420; R^2=0.058, $P_{(adj)}$=0.892). These
246 results suggested that the metabolome differed between controls and SLE patients, and the
247 difference was not due to medication.

248 Of the 1076 annotated metabolites, 120 metabolites were significantly more abundant in the
249 SLE cohort, whereas 124 were significantly less abundant (**Fig. S9A;** P<0.05). Among the top 25
250 most abundant metabolites in the heatmap, 13 were visually less abundant in SLE group (**Fig.
251 S9B**). After adjusting for confounding factors, including nutrient intake, 38 metabolites with
252 variable importance in the projection (VIP) greater than 1 and with fold change less than 0.5 or
253 greater than 2 differed significantly between controls and SLE patients (**Tab. S6-9**, P<0.05, **Fig.
254 3C**). To determine whether these 38 metabolites were affected by medication usage, we compared
255 the metabolites according to dosage, and found no difference (**Tab. S10-11**; $P(adj)$>0.05). Since
256 urinary hydroxychloroquine and desethylchloroquine are metabolites of the medication
257 hydroxychloroquine(28), binary regression analysis was used to determine whether they were
258 affected by hydroxychloroquine intake. It showed that hydroxychloroquine intake was a
259 confounding factor of urinary hydroxychloroquine and desethylchloroquine (**Tab. S12**); thus, they
260 were removed in the downstream analysis. To look for potential biomarkers that could distinguish
261 SLE from controls, classical ROC curve analysis (including logistic regression analysis with
262 selected variables to get the modeling results and compare the performance using the
263 accuracy/performance plots i.e. area under the curve, specificity, and sensitivity) was used to
264 evaluate the performance of single metabolites. From this analysis, 10 metabolites had an AUC
265 value above 0.85, indicating they could be biomarkers of SLE (**Fig. 3D**).

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266 Like the bladder microbiome, the metabolic composition of the LN and non-LN
267 subgroups did not differ, but each differed significantly from the composition of controls (**Fig.**
268 **S10;** Controls vs LN, R^2=0.255, $P_{(adj)}$=0.002; Controls vs nonLN, R^2=0.173, $P_{(adj)}$=0.002; LN
269 vs nonLN, R^2=0.010, $P_{(adj)}$=0.909). However, when we compared the metabolic differences
270 between controls and LN, 427 metabolites were differentially abundant ($P_{(adj)}$<0.05); 185/427
271 had fold change greater than 2 or less than 0.5 and 121/185 metabolites with VIP greater than
272 1 (**Tab. S13-15**). When the control and non-LN cohorts were compared, 239 metabolites were
273 differentially abundant ($P_{(adj)}$<0.05), 132/239 metabolites with fold change greater than 2 or
274 less than 0.5, and 101/132 metabolites with VIP greater than 1 (**Tab. S16-18**).

275 ***Bladder microbiome was associated with urinary metabolome***

276 Bladder microbiome and urinary metabolome correlated robustly across all subjects (**Fig.**
277 **S11,** M^2=0.906, P=0.001). To determine specific associations between the bacterial genera
278 and metabolites, we conducted a Spearman correlation analysis using the abundant bacterial
279 genera (>1% relative abundances) and metabolites that differed between the SLE and control
280 cohorts. Indeed, most of the SLE-enriched genera were positively correlated with most of the
281 SLE-enriched metabolites, and most of the SLE-depleted genera were negatively correlated
282 with most of the SLE-enriched metabolites (**Fig. 4 and Tab. S19;** |r|>0.3, P<0.05). For
283 example, the SLE-enriched genera, such as *Bacteroides*, were positively correlated with
284 SLE-enriched metabolites, such as the lipids and lipid-like molecules, including PC
285 (16:0/16:1(9Z)). Notably, the SLE-enriched genera, including *Bacteroides*, were positively
286 correlated with several SLE-enriched organoheterccocyclic compounds, including olopatadine.

287 ***Urine cytokines were altered in SLE patients***

288 We next tested whether urinary cytokines differed between SLE patients and controls; 26
289 of the 27 cytokines assessed were identified. Among them, 10 cytokines differed in
290 concentration between SLE patients and controls, including 6 cytokines (Eotaxin, G-CSF,
291 IL-8, IL17, IP-10, and MIP-1b) significantly more abundant in SLE patients and 4 cytokines
292 (IL-2, IL-5 IL-12 and IL-13) significantly less abundant in SLE patients (**Fig. 5A;**
293 $P_{(adj)}$<0.05). However, when we compared the cytokines among the controls, LN, and non-LN
294 SLE patients, 12 cytokines, including IL-8, differed significantly between controls and LN
295 SLE patients, and no cytokines differed significantly between controls and non-LN SLE

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296 patients (**Fig. S12;** $P_{(adj)}$<0.05).

297 ***Bladder microbiome was correlated to urine cytokines***

298 To examine the associations between differentially abundant bacterial genera and
299 SLE-linked cytokines, we performed a correlation analysis (**Fig. 5B and Tab. S20**, |r|>0.3,
300 *P*<0.05). Several of the SLE-enriched bacterial genera were positively associated with several
301 SLE-enriched cytokines. In contrast, the SLE-depleted genera, such as *Pseudomonas*, were
302 negatively correlated to SLE-deleted cytokines like IL-8 .

303 ***Bladder microbiome was associated with SLE-linked disease profiles***

304 To look for associations between bladder microbiome and SLE-linked disease profiles,
305 we performed a correlation analysis between the bacterial genera with the patients' disease
306 profiles. The disease profiles were as follows: the duration of SLE and LN, SLEDAI,
307 immunological features, and urinary analysis outcomes that displayed significant differences
308 between controls and SLE cohort or those only be detected in the SLE patients (**Tab. 1**).
309 *Megamonas* and *Phocaeicola* were negatively correlated with serum complement C3,
310 whereas IgG, more abundant in SLE patients, was positively correlated with *Streptococcus*.
311 *Bacteroides* and *Haemophilus* were positively correlated to uric acid (**Fig. 5C and Tab. S21,**
312 |r|>0.3, *P*<0.05).

313

314 **Discussion**

315 This is the first integrated multi-omics analysis of bladder urine. It shows that the bladder
316 microbiome and urinary metabolome profiles of SLE patients differs significantly from those of
317 controls. It also shows that the microbiome profile correlates substantially with the urinary
318 metabolome, urinary cytokines, and disease characteristic profiles.

319 First, we noticed that the bladder microbiome was associated with SLE. Compared with
320 asymptomatic controls, the SLE bladder microbiome was greatly altered with increased microbial
321 diversity and altered abundances of individual taxa at all tested taxonomic levels. Alterations to
322 the microbiomes of SLE patients have been reported for human gut, oral cavity, and skin(3-15);
323 however, the increased diversity of the SLE bladder microbiome was dissimilar to the findings of
324 previous studies of the SLE gut microbiome, in which patients had lower bacterial diversity than
325 controls(3, 4, 8, 11). The increased diversity of the bladder microbiome is quite substantial,

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326 considerably larger than patients with lower urinary tract symptoms(29, 30).

327 The bladder microbiome of asymptomatic controls, most of whom were women from eastern
328 China (Jiangsu province), was most often predominated by *Staphylococcus*. This differs from the
329 *Lactobacillus*-predominant bladder microbiome of asymptomatic controls from the upper
330 midwestern U.S (Chicago area)(31, 32). Different genetic, ethnic, sociocultural, lifestyle, and
331 dietary diversity may contribute to differences in the bladder microbiomes of North American and
332 Chinese women. A large cohort study is needed to verify the differences and investigate the
333 underlying factors that contribute to those differences.

334 The phylum Bacteroidetes, the genera *Bacteroides* and *Alistipes*, and the species *B. uniformis*
335 were most evidently more common in SLE cohorts compared with controls, which resembles a
336 previous study on the SLE gut microbiome(7). The stark contrast in Bacteroidetes between our
337 SLE patients and asymptomatic controls warrants further investigation. *Staphylococcus* and
338 *Pseudomonas* were less common in members of the SLE cohort. A previous bladder microbiome
339 study showed that *Staphylococcus* was associated with urgency urinary incontinence(31). This
340 was an unexpected result as these genera are considered pathogenic in SLE-associated
341 infections(33). This is a striking result that should be investigated.

342 LN is a form of glomerulonephritis that constitutes one of the most severe organ
343 manifestations in SLE patients. Previous human and animal gut microbiome studies demonstrated
344 that LN is associated with microbiome composition(4, 34). Thus, we divided the patients into LN
345 and non-LN subgroups. Although there was no significant difference in bacterial composition or
346 abundance between LN and non-LN patients, microbial diversity differed between LN patients
347 and controls, indicating there might be an interaction between bladder microbiome and
348 inflammation in patients' kidneys. However, the cohort sizes were too small and imbalanced to
349 draw a strong conclusion.

350 The urinary metabolome also differed significantly in our study. Previous studies also showed
351 that the urinary metabolome was altered in SLE and LN patients(35, 36), but those studies
352 analyzed voided urine, which often contains post-urethral contamination(37). We assessed
353 catheterized urine that avoided those contaminants(37), and identified several metabolites altered
354 in SLE patients. For example, organohetercocyclic compounds, including olopatadine, were more
355 abundant in SLE patients.

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356 Like the microbiome and metabolome profiles, bladder cytokines also differed between SLE
357 patients and controls. For example, IL-8 was significantly more abundant in SLE patients,
358 consistent with previous cytokine studies of SLE patients(38, 39). As the kidney is affected by
359 SLE and evaluation of urinary cytokines is reported to predict development of renal flares(40), we
360 compared the urinary cytokines among controls and both LN and non-LN SLE patients. No
361 cytokines differed between controls and non-LN SLE patients. In contrast, 10/12 of the cytokines
362 that differed between controls and LN SLE patients also differed between controls and the entire
363 cohort of SLE patients. The frequently confirmed LN-associated cytokines, such as IL-17(41),
364 IP-10 and MCP-1(42), were only more abundant in the LN group compared to controls. These
365 findings suggest that kidney damage in SLE patients may be responsible for altered bladder
366 cytokine expression.

367 As integration of microbiome and metabolome data have potential for identifying microbial
368 influence on host physiology through production, modification, and/or degradation of bioactive
369 metabolites, we performed correlation analysis on the bladder microbiome and metabolome data.
370 A major finding of the present study is the positive association between *Bacteroides* and
371 organoheterococyclic compounds including olopatadine. Olopatadine is reported to be an
372 antihistamine agent with inhibitory activities against chronic inflammation(43). *Bacteroidetes* also
373 was positively correlated to PC (16:0/16:1(9Z)), which has been reported to have
374 anti-inflammatory properties(44). Thus, the interaction between *Bacteroides* and
375 organoheterococyclic compounds in the bladder might play a potential role in inhibiting
376 inflammation-associated metabolites in SLE.

377 The microbiome plays a vital role in the regulation of host mucosal inflammation. To
378 investigate interactions between bladder microbes and the inflammation response, we performed
379 the integration analysis between the altered bacterial genus and urinary cytokines in patients. The
380 SLE-enriched genus *Bacteroides* was positively correlated to the SLE-enriched cytokines,
381 including IP-10 and IL-17, which are usually used as biomarkers to predict disease severity(41,
382 45). We also noticed that *Pseudomonas* was negatively correlated to IL-8, which can induce
383 chemotaxis in target cells, and cause neutrophils and granulocytes to migrate toward the
384 inflammation site(46). In our study, IL-8 was elevated only in LN patients and not non-LN
385 patients, which fits with these IL-8 functions. Therefore, *Pseudomonas* might play a role in

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386 regulating immunity in bladder microbial community. The correlations between bladder
387 microbiome and urinary cytokines indicate there may be a microbe-inflammation axis that should
388 be explored.

389 It is known that complement deficiency is associated with SLE, predisposing these patients to
390 infection(47). Indeed, the SLE patients in this study had decreased complement C3 and C4 levels
391 and a negative correlation between C3 and SLE-enriched bacterial genera, including *Megamonas*.
392 In contrast, *Megamonas* has been reported to be depleted in the gut of SLE patient and positively
393 correlated to Th17(48). These observations warrant further investigation of *Megamonas* in SLE
394 pathology. In addition, the SLE-depleted genus, *Streptococcus*, was positively correlated to
395 immunoglobulin G (IgG), a major serum immunoglobulin principally responsible for elimination
396 of pathogens and toxic antigens(49). Uric acid accumulation is common in SLE patients(50); it
397 was elevated in our study, and positively correlated with *Bacteroides* and *Haemophilus.* Based on
398 these findings, we hypothesize the possible existence of a host-microbe interaction in the human
399 bladder that contributes to the SLE phenotype.

400 Individuals with SLE are regularly treated with immunosuppressives, which can cause serious
401 adverse effects that severely compromise life quality. Therefore, there is an urgent need to control
402 the disease process. Our present study demonstrated that the bladder microbiome of SLE patients
403 is associated with their urinary metabolites, cytokines, and disease profiles, highlighting plausible
404 disease-specific mechanisms for future investigation. In addition, we noticed a more distinct
405 alteration of the microbiome, metabolome, and cytokines in LN patients compared to non-LN
406 patients. Future mechanistic studies should focus on LN patients.

407 Our study has several limitations. Given frequent infection in SLE patients, it was hard to
408 recruit volunteers who were willing to accept urinary catheterization (which is an invasive
409 procedure); therefore, our sample size was small and entirely recruited at a single center with
410 participants from eastern China. Moreover, as females are more susceptible to SLE, only a few
411 males were recruited to this study. Thus, we could not compare sexual differences. A study with a
412 larger sample size that includes more males and participants from different areas of China should
413 be conducted in the future.

414

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416 We gratefully acknowledge the volunteers who participated in our study.

417

418 **Data accession**

419 Raw data from 16S rRNA sequencing are available in the Sequence Read Archive (SRA) under

420 BioProject ID PRJNA758740 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA758740).

421

422 **Contributions**

423 Conceptualization: Alan J. Wolfe, Longxian Lv, Ninghan Feng

424 Methodology: Fengping Liu, Tianli Ren, Qixiao Zhai,

425 Software: Fengping Liu, Jingjie Du, Ren Yan

426 Validation: Alan J. Wolfe

427 Writing: Alan J. Wolfe, Fengping Liu, Jingjie Du, Aaron W. Miller, Longxian Lv

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429 Funding acquision: Ninghan Feng

430 Project administration: Fengping Liu, Jialing Hu, Lei Hu, Peng Jiang, Jiayi Sheng, Chaoqu Gu,

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435

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589

590 ## Legends

591 **Fig. 1 Bacterial composition, diversity and phylum difference between controls and SLE**
592 **cohort.**

593 A. PCoA based on Bray-Curtis distances at species level showed different microbial compositions
594 between groups of SLE patients and controls. The 95% confidence ellipse is drawn for each group.
595 Permutational multivariate analysis of variance (PERMANOVA) was performed for statistical
596 comparisons of samples in the two cohorts. *P* value was adjusted by the Benjamini and Hochberg
597 false discovery rate (FDR).

598

599 B. Bacterial diversity measured by Shannon index was calculated at the bacterial species level.
600 Wilcoxon rank-sum test was performed and adjusted by Benjamini and Hochberg false discovery
601 rate (FDR). ** indicates $P_{(adj)}<0.01$.

602

603 C. Microbial profile at the phylum level. Only phyla with more than 1% average relative
604 abundances in all samples are shown.

605

606 D. Bacterial phyla that were differentially abundant between controls and SLE patients. *P* value
607 was calculated using Wilcoxon rank-sum test and adjusted by Benjamini and Hochberg false
608 discovery rate (FDR). * and *** indicate $P_{(adj)}<0.05$ and $P_{(adj)}<0.001$, respectively.

609

610 E. Firmicutes/Bacteroidetes ratio differed in controls and SLE patients. *P* value was calculated
611 using Wilcoxon rank-sum test and adjusted by Benjamini and Hochberg false discovery rate
612 (FDR). *** indicates $P_{(adj)}<0.001$.

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613

614 **Fig. 2 Bacterial genera and species are different between controls and SLE cohort.**

615 A. Bacterial genera that were more abundant in controls compared to SLE patients ($P_{(adj)}$<0.05). *P*
616 value was calculated using Wilcoxon rank-sum test and adjusted by Benjamini and Hochberg false
617 discovery rate (FDR).

618

619 B. Bacterial genera that were less abundant in controls compared to SLE patients ($P_{(adj)}$<0.05). *P*
620 value was calculated using Wilcoxon rank-sum test and adjusted by Benjamini and Hochberg false
621 discovery rate (FDR).

622

623 C. Bacterial species that were more abundant in controls compared to SLE patients ($P_{(adj)}$<0.05). *P*
624 value was calculated using Wilcoxon rank-sum test and adjusted by Benjamini and Hochberg false
625 discovery rate (FDR).

626

627 D. Bacterial species that were less abundant in controls compared to SLE patients ($P_{(adj)}$<0.05). *P*
628 value was calculated using Wilcoxon rank-sum test and adjusted by Benjamini and FDR.

629

630 E. ROC curve for bacterial genera. A backward stepwise selection model to identify bacterial
631 genera with optimal model fitting. This model identified 3 genera (*Bacteroides*, *Rothia* and
632 *Sphingomonas)* that could discriminate SLE from controls with AUC values of 93.16%.

633

634 F. ROC curve for bacterial species. A backward stepwise selection model to identify bacterial
635 species with optimal model fitting.

636

637 **Fig. 3 Urinary metabolome differed in SLE patients.**

638 A. Separation of urinary metabolome between patients with SLE and controls, revealed by
639 principal component analysis (PCA). The explained variances are shown in brackets.
640 Permutational multivariate analysis of variance (PERMANOVA) was performed for statistical
641 comparisons of samples in two cohorts. *P* value was adjusted using the Benjamini and Hochberg
642 false discovery rate. The 95% confidence ellipse is drawn for each group.

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643

644 B. Partial Least Square-Discriminant Analysis (PLS-DA) plot. Scores plot between the selected
645 PCs. The explained variances are shown in brackets. PERMANOVA was used to test statistical
646 comparisons of ions in SLE and control cohorts.

647

648 C. The metabolites showing significant difference between the control and SLE cohorts. The
649 metabolites described in the graph met the following criteria: *P(adj)*< 0.05 in Wilcoxon rank-sum
650 test；variable importance in projection (PLS-DA; VIP>1) in Partial Least Square-Discriminant
651 Analysis; and fold change (FC) > 2 or < 0.5.

652

653 D. Receiver operating characteristic curve (ROC) curve for validation of metabolomic
654 classification of control and SLE patients The sensitivity is on the y-axis, and the specificity is on
655 the x-axis. The area-under-the-curve (AUC) is in blue.

656

657 **Fig. 4 Bladder microbiome was associated with metabolites**
658 The heatmap depicted the association between the taxa and metabolites that differ in SLE relative
659 to controls. Spearman correlation analysis was performed on the abundant bacterial genera (>1%
660 relative abundances) and metabolites that differed between the control and SLE cohorts. The
661 correlation of two variables with values of |r|>0.3 and *P*<0.05 are displayed. *, **, and ***
662 indicate *P*<0.05, *P*<0.01 and *P*<0.001, respectively.

663

664 **Fig. 5 Urinary cytokines and profiles in SLE were associated with bladder microbiome.**
665 A. Urinary cytokines increased and decreased in SLE cohort compared to controls. *P* value was
666 calculated using Wilcoxon rank-sum test and adjusted by Benjamini and Hochberg false discovery
667 rate.

668

669 B. Spearman correlation analysis was performed on the most abundant bacterial genera (>1%
670 relative abundances) and cytokines that differed between the controls and SLE cohorts. The
671 correlation of two variables with values of |r|>0.3 and *P*<0.05 are displayed. *, **, and ***
672 indicate *P*<0.05, *P*<0.01 and *P*<0.001, respectively.

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673

674 C. Spearman correlation analysis was performed on the abundant bacterial genera (>1% relative
675 abundances) and disease profiles of SLE patients. The correlation of two variables with values of
676 $|r|>0.3$ and $P<0.05$ are displayed. *, **, and *** indicate $P<0.05$, $P<0.01$ and $P<0.001$,
677 respectively.

678

679

680 **Supplementary Figures**

681 **Fig. S1** Overview of the study design.

682

683 **Fig. S2 Medication usages in patients didn't affect bladder microbiome.**

684 A. PCoA based on Bray-Curtis distances at species level did not show different microbial
685 compositions between SLE patients taking hydroxychloroquine dosages of 0.2 mg/d and those
686 taking 0.4 mg/d. Permutational multivariate analysis of variance (PERMANOVA) was performed
687 for statistical comparisons of samples using different levels of hydroxychloroquine. *P* value was
688 adjusted by Benjamini and Hochberg false discovery rate.

689

690 B. PCoA based on Bray-Curtis distances at species level did not show different microbial
691 compositions among SLE patients taking prednisone of 0 mg/d, 5 mg/d and those taking 10 mg/d.
692 PERMANOVA was performed for statistical comparisons of samples using different levels of
693 prednisone. *P* value was adjusted by Benjamini and Hochberg false discovery rate.

694

695 **Fig. S3 Bacterial evenness and richness of bacterial diversity.**

696 A. Comparison of bacterial species diversity indicator of evenness between control and SLE
697 cohorts. Permutational multivariate analysis of variance (PERMANOVA) was performed for
698 statistical comparisons of samples in two cohorts. *P* value was adjusted by Benjamini and
699 Hochberg false discovery rate. ** indicates $P_{(adj)}<0.01$.

700

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701 B. Comparison of bacterial species diversity indicator of richness between control and SLE
702 cohorts. PERMANOVA was performed for statistical comparisons of samples in two cohorts. *P*
703 value was adjusted by Benjamini and Hochberg false discovery rate.
704
705 **Fig. S4 Bacterial community and composition at bacterial genus level.**
706 A. PCoA based on Bray Curtis distances at the genus level showed different microbial
707 compositions between control and SLE cohorts. Permutational multivariate analysis of variance
708 (PERMANOVA) was performed for statistical comparisons of samples in two cohorts. *P* value
709 was adjusted by Benjamini and Hochberg false discovery rate.
710
711 B. Heatmap of bacterial genera. The 15 abundant genera (>1% relative abundances) were
712 displayed.
713
714 **Fig. S5 Bacterial composition at bacterial species level.**
715 The heatmap displays the bacterial species >0.5% relative abundances.
716
717
718 **Fig. S6 Comparison of bladder microbiome among control, LN and non-LN cohorts.**
719 A. Bacterial community among controls, LN and non-LN SLE patients. PCoA based on Bray
720 Curtis distances at species level was performed. Permutational multivariate analysis of variance
721 (PERMANOVA) was performed for statistical comparisons of samples in two cohorts. *P* value
722 was adjusted by Benjamini and Hochberg false discovery rate.
723
724 B. Bacterial diversity measured by Shannon index was calculated at the bacterial species level.
725 Wilcoxon rank-sum test and adjusted by Benjamini and Hochberg false discovery rate (FDR). **
726 indicates $P_{(adj)}<0.01$.
727
728 **Fig. S7 Comparison of microbiome in bladder, vagina, and gut in SLE patients.**

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729 A. PCoA based on Bray-Curtis distances at species level showed different microbial compositions
730 between the bladder, vagina, and gut. (PERMANOVA) was performed for statistical comparisons
731 of samples in two cohorts. *P* value was adjusted by Benjamini and Hochberg false discovery rate.
732
733 B. Bray-Curtis dissimilarities of the different niches were calculated using the same SLE patient.
734 Wilcoxon rank-sum test and adjusted by Benjamini and Hochberg false discovery rate (FDR). **,
735 *** indicate $P_{(adj)}<0.01$ and $P_{(adj)}<0.001$, respectively.
736
737 C. Microbial profiles of the bladder, gut and vagina at species level. Bacterial species abundance
738 with>0.05 % relative abundances are displayed. On the x axis, the numbers 1 to 15 represent SLE
739 patients with bladder, gut and vaginal samples sequenced.
740
741 **Fig. S8 Medication usages in SLE patients didn't affect urinary metabolome.**
742 A. Principal component analysis (PCA) was used to compare urinary metabolome between SLE
743 patients taking hydroxychloroquine dosages of 0.2 mg/d and those taking 0.4 mg/d. The explained
744 variances are shown in brackets. Permutational multivariate analysis of variance (PERMANOVA)
745 was performed for statistical comparisons of samples in two cohorts. *P* value was adjusted using
746 the Benjamini and Hochberg false discovery rate. The 95% confidence ellipse is drawn for each
747 group.
748
749 B. Principal component analysis (PCA) was used to compare urinary metabolome among SLE
750 patients taking prednisone of 0 mg/d, 5 mg/d, and 10 mg/d. Permutational multivariate analysis of
751 variance (PERMANOVA) was performed for statistical comparisons of samples in two cohorts. *P*
752 value was adjusted using the Benjamini and Hochberg false discovery rate. The 95% confidence
753 ellipse is drawn for each group.
754
755 **Fig. S9 Metabolome comparison between control and SLE cohorts.**
756 A. Volcano plot. Volcano plot of differential metabolites classification of the control and SLE
757 cohorts. Metabolites with FDR < 0.05 obtained by non-parametric tests and fold change (FC) >2
758 were identified as significantly different between the two cohorts. Colored plots indicate upward

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759 trend and downward trend of metabolites, and gray plots indicate that they are not statistically
760 significant.
761
762 B. Clustering result shown as heatmap. Distance was measured using Euclidean, and clustering
763 algorithm was calculated using Ward's method.
764
765 **Fig. S10 Metabolome comparison among control, LN and non-LN SLE cohorts.**
766 Metabolome among controls, LN and non-LN SLE patients were compared using principal
767 component analysis(PCA). Permutational multivariate analysis of variance (PERMANOVA) was
768 performed for statistical comparisons of samples between groups. *P* value was adjusted by
769 Benjamini and Hochberg false discovery rate. The 95% confidence ellipse is drawn for each
770 group.
771
772 **Fig. S11 The relationship between bladder microbiome and urinary metabolome.**
773 Procrustes analysis analyzed the congruence of two-dimensional shapes produced from
774 superimposition of principal component analyses from the datasets of microbiome and
775 metabolome. Euclidian distances of eigenvalues for both the microbiome and metabolome using
776 the Procrustes function in the vegan R package. Longer lines on Procrustes plots indicate more
777 within-subject dissimilarity of the microbiome and metabolome. Significance value shown was
778 calculated using the protest function from the vegan R package.
779
780 **Fig. S12 Urinary cytokines differed in LN SLE patients comparing to controls.**
781 Comparison of urinary cytokines between controls and LN SLE patients. *P* value was calculated
782 using Wilcoxon rank-sum test and adjusted by Benjamini and Hochberg false discovery rate. *, **,
783 *** indicate $P_{(adj)}<0.05$, $P_{(adj)}<0.01$ and $P_{(adj)}<0.001$, respectively.
784
785
786 **Supplementary Tables**
787 **Tab. S1 Annotable metabolites.**

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788 Untargeted urinary metabolite profile was performed on liquid chromatography tandem mass
789 spectrometry.
790
791 **Tab. S2** SLE patient's characteristics.
792 Abbreviations: "-" and "+" represent negative and positive, respectively; ACA, anti-centromere
793 antibodies; AHAs, Antihistone antibodies; AMA-M2, Anti-mitochondrial M2 antibody; ANAs,
794 antinuclear antibodies; Anti-dsDNA, anti-double stranded DNA; Anti-NCS, anti-nucleosome
795 antibodies; Anti-nRNP, anti-nRNP antibodies; Anti-PCNA, antibodies to the proliferating cell
796 nuclear antigen; Anti-PM/Scl, anti-PM/Scl antibodies; Anti-Ro/SSA, anti-Ro/SSA antibodies;
797 Anti-Ro52, anti-Ro52 antibodies; Anti-Scl-70, anti-Scl 70 antibodies; Anti-Sm, anti-Smith
798 antibodies; Anti-SSB, anti-SSB antibodies; ASO, anti-streptolysin O; ESR, erythrocyte
799 sedimentation rate; HCQ, hydroxychloroquin; IgG, immunoglobulin G; Meth, methylprednisolone;
800 MTX, methotrexate; Pred, prednisone; RF, rheumatoid factor
801
802 **Tab. S3 Comparison of nutrient intake between controls and SLE.**
803 Student's *t* test on normalized continuous variables and Wilcoxon rank-sum test was used on
804 un-normalized continuous variables.
805
806 **Tab. S4 Bacterial taxonomy affected by food intake.**
807 MaAsLin (Microbiome Multivariable Associations with Linear Models) was used to adjust
808 confounding factors, food intake, on bacteria showing significant difference at their abundance
809 using Wilcox test (Galaxy Version 1.0.1). *P* value was adjusted using Benjamin Hochberg false
810 discovery rate (FDR).
811
812 **Tab. S5 Demographics of LN and non-LN patients**
813 [a] n, number of subjects;
814 [b] Mean ± SD or n (%);
815 [c] Pearson Chi-square or Fisher's exact test was used with categorical variables; Wilcoxon
816 rank-sum test was used on un-normalized continuous variables.
817 Abbreviations: LN, lupus nephritis; NA, not applicable; SLEDAI, systemic lupus erythematosus

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818 disease activity index

819

820 **Tab. S6 Metabolite comparison between using wilcox rank test (Controls and LN)**

821 Wilcoxon rank sum test was used on the metabolites. *P* value was adjusted using Benjamin

822 Hochberg false discovery rate (FDR).

823

824 **Tab. S7 Metabolites showing Fold change>2 or <0.5 (Controls vs SLE)**

825 For paired fold change analysis, the algorithm first counts the total number of pairs with fold

826 changes that are consistently above/below the specified FC threshold >2 or <0.5 for each variable.

827

828 **Tab. S8 Metabolites with VIP >1 (Controls vs SLE)**

829 VIP was calculated using PLS-DA analysis.

830 Abbreviation: VIP, Variable Importance in Projection

831

832 **Tab. S9 The effects of nutrient intake on metabolites showing significant different between**

833 **controls and SLE**

834 Binary logistic regression model was used.

835 Abbreviations: B, coefficient value; SE, standard error; df, degrees of freedom; 95% CI, 95%

836 confidence interval

837

838 **Tab. S10 Effects of hydroxychloroquine intake on metabolites**

839 Wilcoxon rank-sum test was used on the metabolites. *P* value was adjusted using Benjamin

840 Hochberg false discovery rate (FDR).

841

842 **Tab. S11 Effects of prednisone intake on metabolites**

843 Wilcoxon rank-sum test was used on the metabolites. *P* value was adjusted using Benjamin

844 Hochberg false discovery rate (FDR).

845

846 **Tab. S12 The effects of hydroxychloroquine intake on urinary hydroxychloroquine and**

847 **Desethylchloroquine**

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848 Binary logistic regression model was used.

849 Abbreviations: B, coefficient value; SE, standard error; df, degrees of freedom; 95%CI, 95%

850 confidence interval

851

852 **Tab. S13 Metabolite comparison between controls and LN**

853 Wilcoxon rank-sum test was used on the metabolites. *P* value was adjusted using Benjamin

854 Hochberg false discovery rate (FDR).

855 Abbreviation: LN, lupus nephritis

856

857 **Tab. S14 Metabolite comparison between controls and LN showing Fold change>2 or <0.5**

858 For paired fold change analysis, the algorithm first counts the total number of pairs with fold

859 changes that are consistently above/below the specified FC threshold >2 or <0.5 for each variable.

860

861 **Tab. S15 Metabolites with VIP >1 (Controls vs LN)**

862 VIP was calculated using PLS-DA analysis.

863 Abbreviation: LN, lupus nephritis; VIP, Variable Importance in Projection

864

865 **Tab. S16 Metabolite comparison between controls and non-LN**

866 Wilcoxon rank-sum test was used on the metabolites. *P* value was adjusted using Benjamin

867 Hochberg false discovery rate (FDR).

868 Abbreviation: non-LN, non-lupus nephritis

869

870 **Tab. S17 Metabolites showing Fold change>2 or <0.5 (controls vs non-LN)**

871 For paired fold change analysis, the algorithm first counts the total number of pairs with fold

872 changes that are consistently above/below the specified FC threshold >2 or <0.5 for each variable.

873 Abbreviation: non-LN, non-lupus nephritis

874

875 **Tab. S18 Metabolites with VIP >1 (Controls vs non-LN)**

876 VIP was calculated using PLS-DA analysis.

877 Abbreviation: LN, lupus nephritis; VIP, Variable Importance in Projection

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878

879 **Tab. S19 Correlation between bacterial genus and metabolites that showed significant**
880 **difference between controls and SLE**

881 Spearman correlation evaluated the linear relationship between bacterial genus and metabolite.

882

883 **Tab. S20 Correlation between bacterial genus and cytokines that showed significant**
884 **difference between controls and SLE**

885 Spearman correlation evaluated the linear relationship between bacterial genus and cytokine.

886

887 **Tab. S21 Correlation between bacterial genus and disease profiles in SLE patients**

888 Spearman correlation evaluated the linear relationship between bacterial genus and disease profile.

Tab. 1 Characteristics of controls and SLE patients

Parameters		Value for cohort (n [a]) [b] or statistic		P value [c]
		Controls (n = 50)	SLE (n = 50)	
Female sex, n (%)		44 (88.00)	44 (88.00)	1.000
Age (yrs)		49.22 ± 15.19	45.00 ± 14.61	0.160
Body-mass index (kg/m^2)		23.98 ± 3.23	23.99 ± 2.65	0.992
Duration of SLE (yrs)		NA	7.58 ± 5.97	NA
History of smoking, n (%)		0 (0.00)	0 (0.00)	1.000
History of drinking, n (%)		1 (2.00)	0 (0.00)	1.000
Comorbidity				
	Diabetes, n (%)	5 (10.00)	5 (10.00)	1.000
	Hypertension, n (%)	10 (20.00)	10 (20.00)	1.000
Immunological features				
	Complement 3 (g/L)	1.20 ± 0.64	0.76 ± 0.23	< 0.001
	Complement 4 (g/L)	0.30 ± 0.28	0.16 ± 0.06	< 0.001
	Ig A (g/L)	2.59 ± 0.85	2.60 ± 1.08	0.930
	Ig G (g/L)	10.98 ± 1.88	15.97 ± 7.86	< 0.001
	Ig M (g/L)	1.17 ± 0.51	1.01 ± 0.51	0.105
	ESR（mm/hr）[d]	NA	30.02 ± 22.56	NA
Renal function				
	Serum creatinine (μmol/L)	55.23 ± 10.75	65.79 ± 40.62	0.087
	Blood urea nitrogen (mmol/L)	5.73 ± 5.99	5.46 ± 2.11	0.766
	Serum uric acid (umol/L)	268.04 ± 72.56	318.86 ± 103.66	0.006
	Estimated glomerular filtration rate (mL/min/1.73 m^2)	114.40 ± 20.08	108.84 ± 38.26	0.374
	Urinary creatinine (mg/dL)	1.37 ± 0.19	1.42 ± 0.25	0.268
Urine analysis				
	White blood cells (/uL)	2.22 ± 6.38	9.32 ± 22.40	< 0.001
	Red blood cells (/uL)	0.61 ± 3.03	28.00 ± 131.45	< 0.001
	Nitrites positive, n (%)	1 (2.00)	2 (4.00)	1.000
	Leucocyte esterase, n (%)	0 (0)	12 (24.00)	< 0.001
LN				
	LN, n (%)	NA	38 (76.00)	NA
	Duration of LN (yrs)	NA	5.30 ± 3.60	NA
SLEDAI				
	Score	NA	16.16 ± 24.31	NA
	Low SLEDAI [<8; n(%)]		8 (16.00)	NA
	High SLEDAI [≥8; n(%)]		42 (84.00)	NA

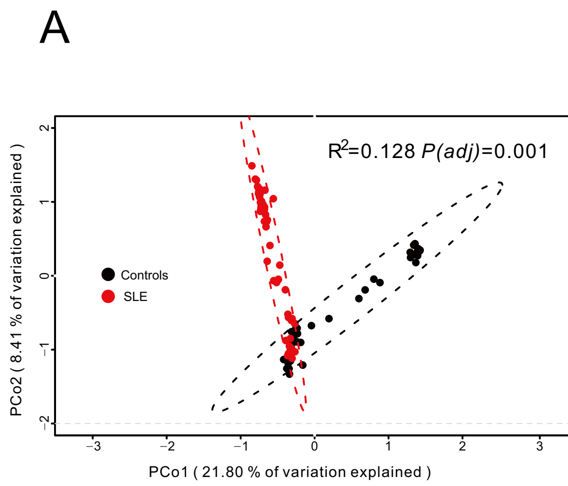
[a] n, number of subjects;

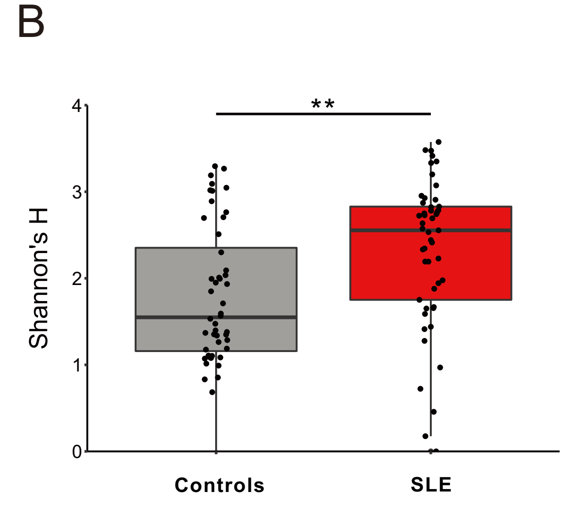
[b] Mean ± SD or n (%);

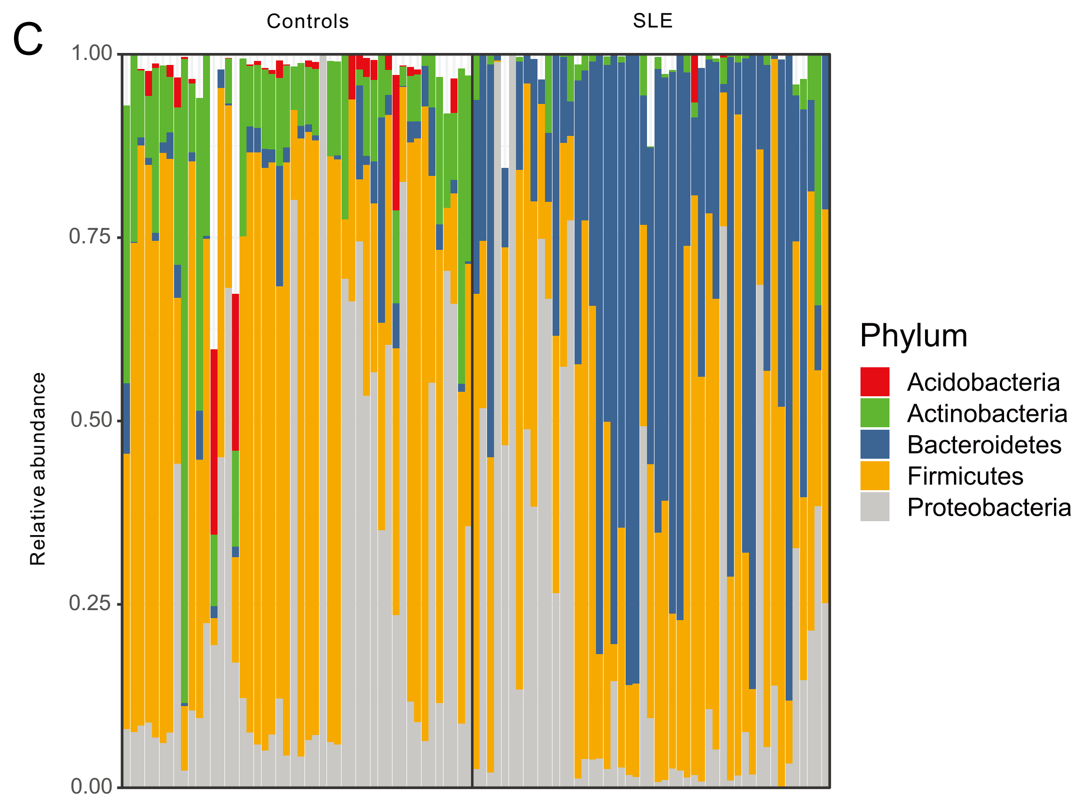
[c] Pearson Chi-square or Fisher's exact test was used with categorical variables; Student's t test on normalized continuous variables and Wilcoxon rank-sum test was used on un-normalized continuous variables.

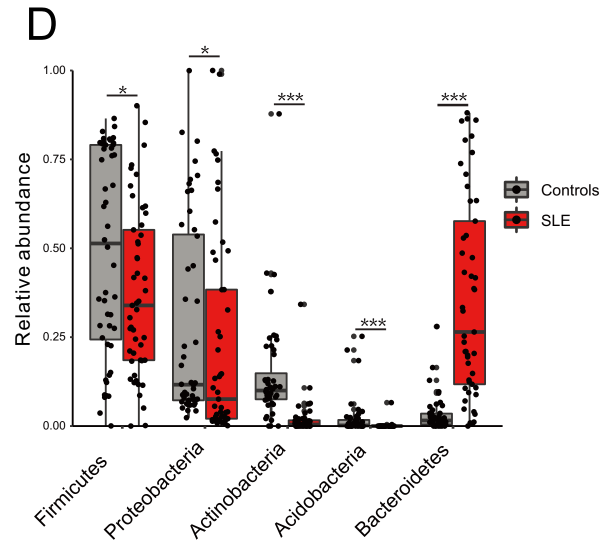
[d] ESR detection threshold was 15 mm/hr.

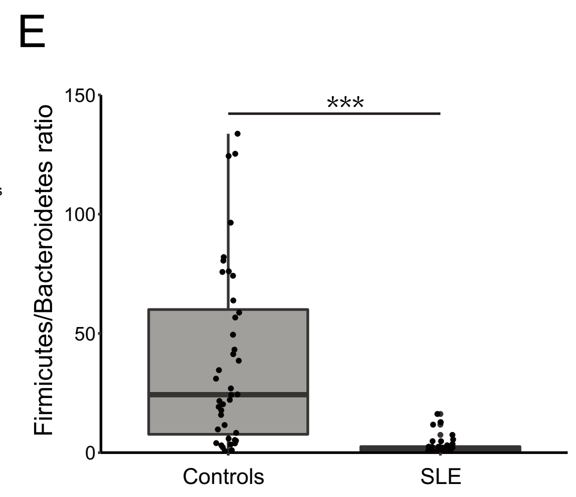
Abbreviations: ESR, erythrocyte sedimentation rate; Ig, immunoglobulin; LN, lupus nephritis; NA, not applicable; SLEDAI, systemic lupus erythematosus disease activity index.

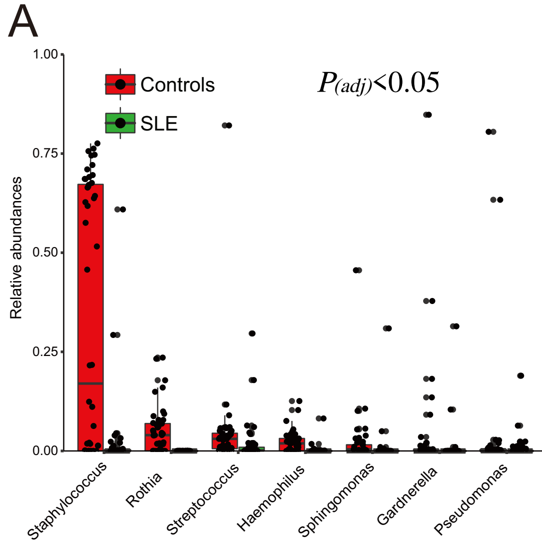

A
R^2=0.128 P(adj)=0.001
Controls
SLE
PCo1 (21.80 % of variation explained)
PCo2 (8.41 % of variation explained)

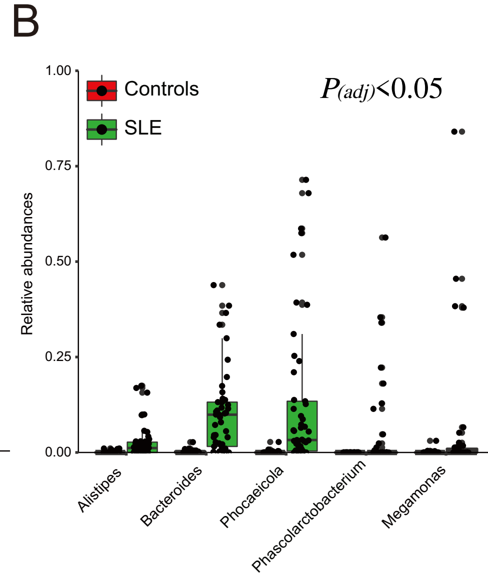

B
**
Shannon's H
Controls
SLE

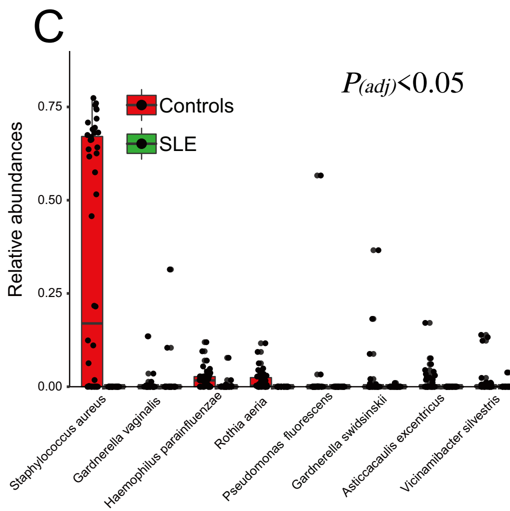

C
Controls
SLE
Relative abundance
Phylum
Acidobacteria
Actinobacteria
Bacteroidetes
Firmicutes
Proteobacteria

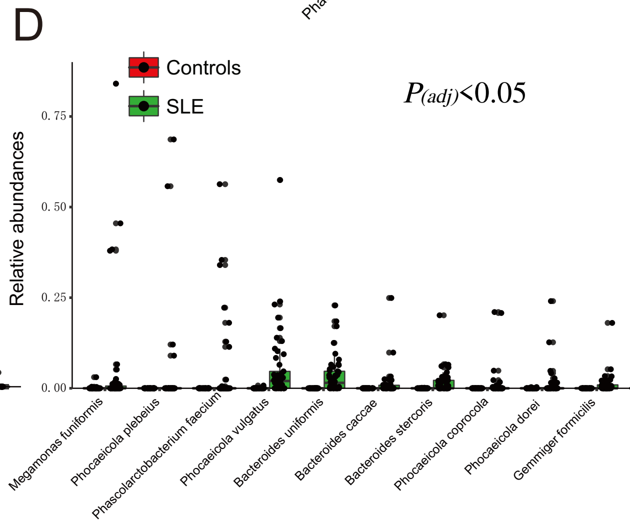

D
Relative abundance
*
*
***
***
***
Controls
SLE
Firmicutes
Proteobacteria
Actinobacteria
Acidobacteria
Bacteroidetes

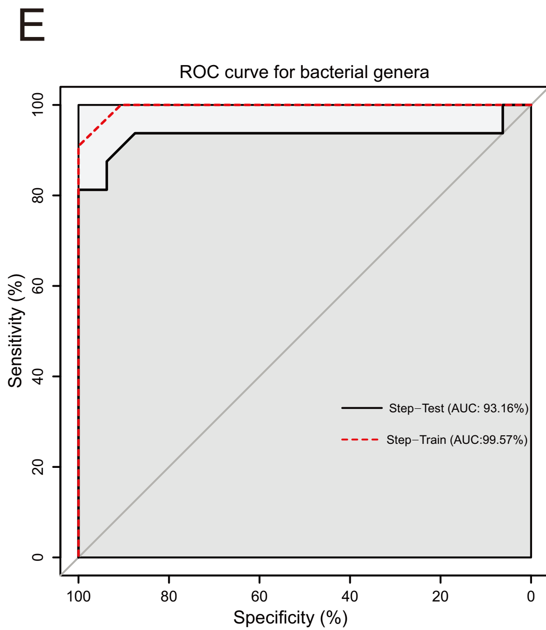

E
***
Firmicutes/Bacteroidetes ratio
Controls
SLE

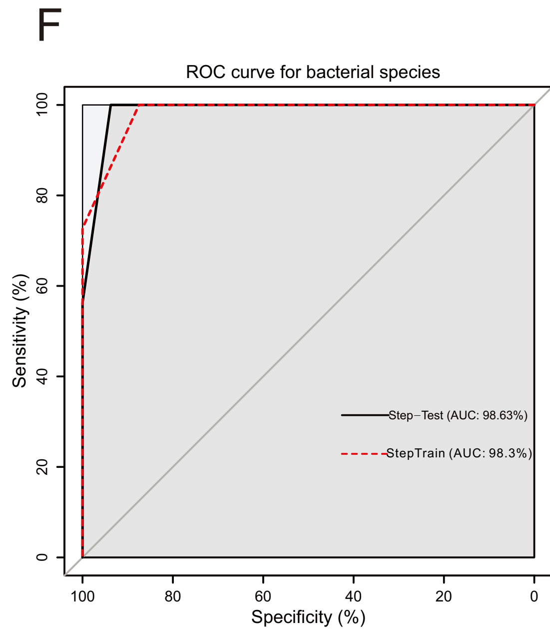

A
Controls
SLE
P(adj)<0.05
Relative abundances
1.00
0.75
0.50
0.25
0.00
Staphylococcus
Rothia
Streptococcus
Haemophilus
Sphingomonas
Gardnerella
Pseudomonas

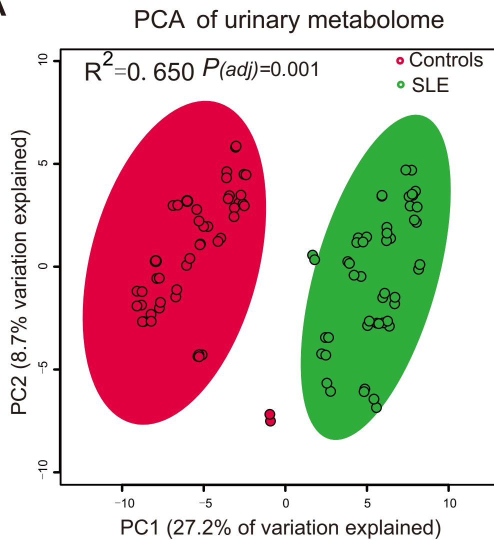

B
Controls
SLE
P(adj)<0.05
Relative abundances
1.00
0.75
0.50
0.25
0.00
Alistipes
Bacteroides
Phocaeicola
Phascolarctobacterium
Megamonas

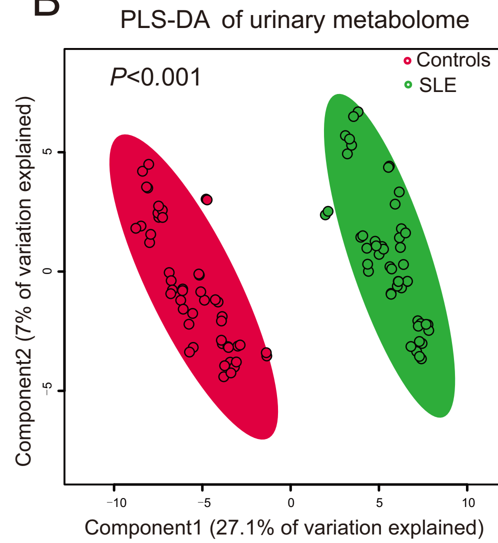

C
Controls
SLE
P(adj)<0.05
Relative abundances
0.75
0.50
0.25
0.00
Staphylococcus aureus
Gardnerella vaginalis
Haemophilus parainfluenzae
Rothia aeria
Pseudomonas fluorescens
Gardnerella swidsinskii
Asticcacaulis excentricus
Vicinamibacter silvestris

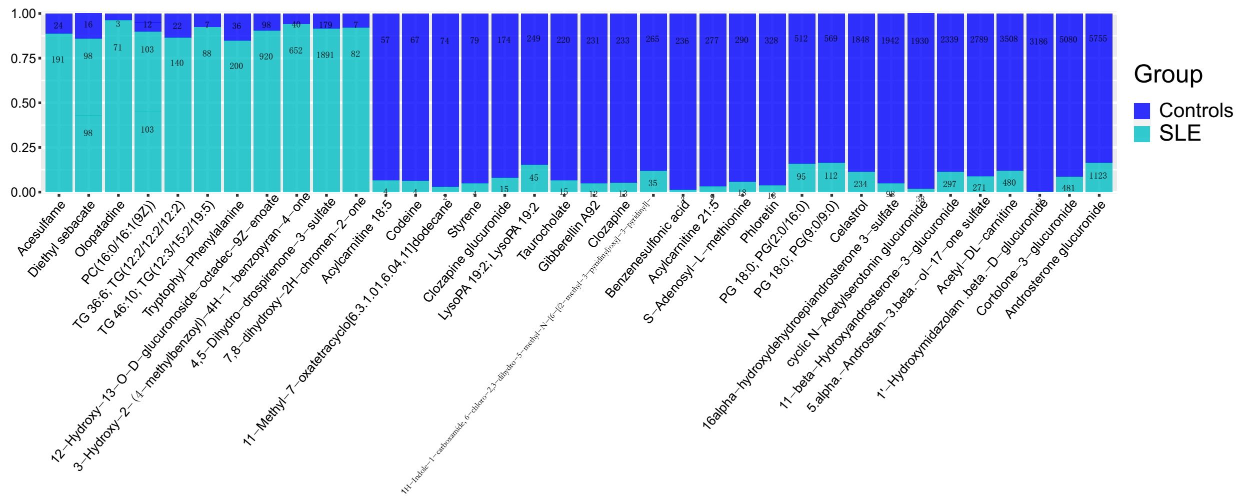

D
Controls
SLE
P(adj)<0.05
Relative abundances
0. 75
0. 50
0. 25
0. 00
Megamonas funiformis
Phocaeicola plebeius
Phascolarctobacterium faecium
Phocaeicola vulgatus
Bacteroides uniformis
Bacteroides caccae
Bacteroides stercoris
Phocaeicola coprocola
Phocaeicola dorei
Gemmiger formicilis

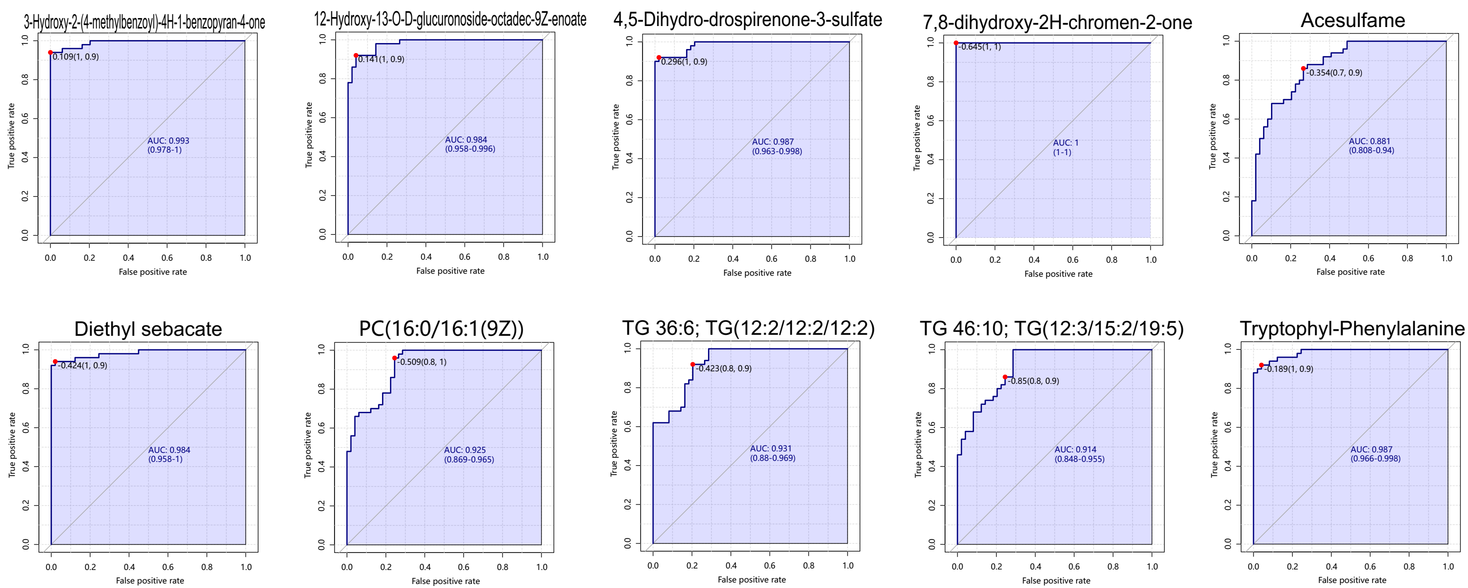

E
ROC curve for bacterial genera
Sensitivity (%)
Specificity (%)
Step−Test (AUC: 93.16%)
Step−Train (AUC:99.57%)


F
ROC curve for bacterial species
Sensitivity (%)
Specificity (%)
Step−Test (AUC: 98.63%)
StepTrain (AUC: 98.3%)


A
PCA of urinary metabolome
R^2=0. 650 P(adj)=0.001
Controls
SLE
PC2 (8.7% variation explained)
PC1 (27.2% of variation explained)


B
PLS-DA of urinary metabolome
P<0.001
Controls
SLE
Component2 (7% of variation explained)
Component1 (27.1% of variation explained)


C
Group
Controls
SLE
Acesulfame
Diethyl sebacate
Olopatadine
PC(16:0/16:1(9Z))
TG 36:6; TG(12:2/12:2/12:2)
TG 46:10; TG(12:3/15:2/19:5)
Tryptophyl-Phenylalanine
12-Hydroxy-13-O-D-glucuronoside-octadec-9Z-enoate
3-Hydroxy-2-(4-methylbenzoyl)-4H-1-benzopyran-4-one
4,5-Dihydro-drospirenone-3-sulfate
7,8-dihydroxy-2H-chromen-2-one
Acylcarnitine 18:5
Codeine
Styrene
Clozapine glucuronide
LysoPA 19:2; LysoPA 19:2
Taurocholate
Gibberellin A92
Clozapine
Benzenesulfonic acid
Acylcarnitine 21:5
S-Adenosyl-L-methionine
Phloretin
PG 18:0; PG(2:0/16:0)
PG 18:0; PG(9:0/9:0)
Celastrol
16alpha-hydroxydehydroepiandrosterone 3-sulfate
cyclic N-Acetylserotonin glucuronide
11-beta-Hydroxyandrosterone-3-glucuronide
5.alpha.-Androstan-3.beta.-ol-17-one sulfate
Acetyl-DL-carnitine
1'-Hydroxymidazolam .beta.-D-glucuronide
Cortolone-3-glucuronide
Androsterone glucuronide
11-Methyl-7-oxatetracyclo[6.3.1.01,6.04,11]dodecane


D
3-Hydroxy-2-(4-methylbenzoyl)-4H-1-benzopyran-4-one
AUC: 0.993 (0.978-1)
12-Hydroxy-13-O-D-glucuronoside-octadec-9Z-enoate
AUC: 0.984 (0.958-0.996)
4,5-Dihydro-drospirenone-3-sulfate
AUC: 0.987 (0.963-0.998)
7,8-dihydroxy-2H-chromen-2-one
AUC: 1 (1-1)
Acesulfame
AUC: 0.881 (0.808-0.94)
Diethyl sebacate
AUC: 0.984 (0.958-1)
PC(16:0/16:1(9Z))
AUC: 0.925 (0.869-0.965)
TG 36:6; TG(12:2/12:2/12:2)
AUC: 0.931 (0.88-0.969)
TG 46:10; TG(12:3/15:2/19:5)
AUC: 0.914 (0.848-0.955)
Tryptophyl-Phenylalanine
AUC: 0.987 (0.966-0.998)
True positive rate
False positive rate

HC enriched

SLE enriched

Metabolite

HC enriched

SLE enriched

Rothia
Sphingomonas
Staphylococcus
Pseudomonas
Haemophilus
Gardnerella
Streptococcus
Bacteroides
Phocaeicola
Megamonas
Phascolarctobacterium

Genus
Acetyl âDL âcarnitine
Clozapine
Taurocholate
Gibberellin A92
S âAdenosyl âL âmethionine
11 âMethyl â7 âoxatetracyclo[6.3.1.01,6.04,11]dodecane
Acylcarnitine 21:5
Benzenesulfonic acid
1' âHydroxymidazolam.beta. âD âglucuronide
Phloretin
Styrene
Acylcarnitine 18:5
Clozapine glucuronide
cyclic N âAcetylserotonin glucuronide
Codeine
PG 18:0; PG(9:0/9:0)
16alpha âhydroxydehydroepiandrosterone 3 âsulfate
5.alpha. âAndrostan â3.beta. âol â17 âone sulfate
Celastrol
LysoPA 19:2; LysoPA 19:2
11 âbeta âhydroxyandrosterone â3 âglucuronide
Androsterone glucuronide
PG 18:0; PG(2:0/16:0)
Cortolone â3 âglucuronide
Acesulfame
PC(16:0/16:1(9Z))
TG 36:6; TG(12:2/12:2/12:2)
TG 46:10; TG(12:3/15:2/19:5)
12 âhydroxy â13 âO âD âglucuronoside âoctadec â9Z âenoate
Diethyl sebacate
3 âhydroxy â2 â(4 âmethylbenzoyl) â4H â1 âbenzopyran â4 âone
7,8 âdihydroxy â2H âchromen â2 âone
4,5 âDihydro âdrospirenone â3 âsulfate
Tryptophyl âPhenylalanine
Olopatadine

Spearman correlation coefficient
0.6
0.4
0.2
0
â0.2
â0.4
â0.6

Genus enriched
HC
SLE

Phylum
Actinobacteria
Firmicutes
Proteobacteria
Bacteroidetes

Metabolite enriched
HC
SLE

Metabolite
Alkaloids and derivatives
Benzenoids
Lipids and lipid âlike molecules
Nucleosides, nucleotides, and analogues
Organic acids and derivatives
Organic oxygen compounds
Organoheterocyclic compounds
Phenylpropanoids and polyketides
Unknown

A

Eotaxin ** | G-CSF ** | IL-8 ** | IL-17 * | IP-10 ** | MIP-1b ** | IL-2 ** | IL-5 ** | IL-12 *** | IL-13 **

Concentration

Controls SLE

B

Bacterial genus

Bacteroides
Phocaeicola
Megamonas
Phascolarctobacterium
Pseudomonas
Sphingomonas
Streptococcus
Rothia
Staphylococcus
Gardnerella
Haemophilus

IL−2 IL−5 IL−12 IL−13 IL−17 Eotaxin IP−10 IL−8 G−CSF MIP−1b

Reprerentative cytokines

Spearman correlation coefficient

SLE enriched
Controls enriched

C

Gardnerella
Pseudomon
Staphylococcus
Sphingomonas
Streptococcus
Megamonas
Phascolarctobacterium
Phocaeicola
Bacteroides
Haemophilus

C3 IgG C4 Urinary red blood cells Urinary white blood cells Disease duration ESR SLEDAI Uric acid

Spearman correlation coefficient