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Outgrowth of *Escherichia* is susceptible to aggravation of systemic lupus erythematosus

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Abstract

Background Systemic lupus erythematosus (SLE) is linked to host gut dysbiosis. Here we performed faecal gut microbiome sequencing to investigate SLE-pathogenic gut microbes and their potential mechanisms.

Methods There were 134 healthy controls (HCs) and 114 SLE cases for 16 S ribosomal RNA (rRNA) sequencing and 97 HCs and 124 SLE cases for shotgun metagenomics. Faecal microbial changes and associations with clinical phenotypes were evaluated, and SLE-associated microbial genera were identified in amplicon analysis. Next, metagenomic sequencing was applied for accurate identification of microbial species and discovery of their metabolic pathways and immunogenic peptides both relevant to SLE. Finally, contribution of specific taxa to disease development was confirmed by oral gavage into lupus-prone MRL/*lpr* mice.

Results SLE patients had gut microbiota richness reduction and composition alteration, particularly lupus nephritis and active patients. *Proteobacteria*/*Bacteroidetes* (P/B) ratio was remarkably up-regulated, and *Escherichia* was identified as the dominantly expanded genus in SLE, followed by metagenomics accurately located *Escherichia coli* and *Escherichia* unclassified species. Significant associations primarily appeared among *Escherichia coli*, metabolic pathways of purine nucleotide salvage or peptidoglycan maturation and SLE disease activity index (SLEDAI), and between multiple epitopes from *Escherichia coli* and disease activity or renal involvement phenotype. Finally, gavage with faecal *Escherichia* revealed that it upregulated lupus-associated serum traits and aggravated glomerular lesions in MRL/*lpr* mice.

Conclusion We characterize a novel SLE exacerbating *Escherichia* outgrowth and suggest its contribution to SLE procession may be partially associated with metabolite changes and cross-reactivity of gut microbiota-associated

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epitopes and host autoantigens. The findings could provide a deeper insight into gut *Escherichia* in the procession of SLE.

Keywords Systemic lupus erythematosus, Shotgun metagenomics, Gut microbiota dysbiosis, *Escherichia*, Gut microbiota-associated epitopes

Background

Systemic lupus erythematosus (SLE) is a prototypical systemic autoimmune disease involving damage of multiple organs due to the breakdown of immune homeostasis [1]. Multiple factors have been implicated in the etiology of SLE, including genetic susceptibility, epigenetic modifications, environmental exposures and hormones, which result in immunologic abnormalities [2]. Human gut microbiota has been demonstrated as an internal environmental factor that prominently influences the homeostasis of immune modulations [3, 4]. The perturbation of intestinal microbiota in abundance and composition have been revealed to be extensively associated with autoimmune diseases such as rheumatic arthritis [5] and type 1 diabetes [6]. With regard to SLE disease, a few small-scale human gut microbiota studies were sequentially performed on the subjects with different disease activities, ethics and sexes [7–11], since *Hevia A et al.* initially uncovered lupus-related gut dysbiosis and reduction of *Firmicutes*/*Bacteroidetes* (F/B) ratio in Caucasian female patients in remission. However, some studies fail to observe the lower F/B ratio in SLE subjects with different disease activities [9, 11]. Moreover, some gut microbiota were found to correlate with SLE disease activity, including typical *Ruminococcus gnavus* [12], *Streptococcus* and *Bifidobacterium* [11]. *Dialister* and *Gemmiger* were reported to correlate with inflammatory cytokines [13]. *H*owever, it remains difficult for us to determine their roles in the pathogenesis of SLE. Some potential mechanisms have been proposed to explain microbiota-mediated abnormality of autoimmunity in mice and humans, including aberrant microbial translocation of *Enterococcus gallinarum* [14], antigen molecular mimicry [15], and imbalanced immune responses induced by microbial metabolites [16]. But the identification of specific gut microbiota and the mechanisms contributing to the procession of SLE disease remain to be elaborated.

Here, we integrated 16 S ribosomal RNA (rRNA) gene sequencing and shotgun metagenomic sequencing to identify the potential pathogenic bacterial species and uncover their contribution to the disease procession of SLE.

Methods

Patient recruitment

114 patients with SLE were recruited for 16 S ribosomal rRNA sequencing at the Department of Rheumatology and Immunology at the Third Affiliated Hospital of Sun Yat-sen University from 2017 to 2019, including 62 non-lupus nephritis (LN) patients and 52 LN patients. All patients (age ≥ 18y) were diagnosed according to the American College of Rheumatology 1997 revised criteria for SLE, and patients with LN were defined as individuals with persistent proteinuria greater than 0.5 g per day or +++ [17]. Participants were excluded if they had antibiotics or cyclophosphamide treatment within the last three months, or comorbid with other rheumatic disorders, autoimmune disorders, pregnancy and cancers. Anonymous clinical information was collected at the time of sampling, including demographics, SLE disease activity index (SLEDAI), and medication history (Mycophenolate Mofetil, Ciclosporin, FK506, Azathioprine). Current disease status was stratified on the basis of SLEDAI-2 K (SLEDAI ≤ 4 or otherwise was defined as 'inactive' or 'active', respectively, as previously described [18]. 134 healthy individuals (age ≥ 18y) were recruited as controls, who self-reported no signs of pregnancy, autoimmune or rheumatic diseases, infections or cancers, and those receiving antibiotic and immune-regulated medicines treatment were excluded. The same inclusion and exclusion criteria was suitable for enrolled 97 healthy controls (HCs) and 124 SLE patients for the metagenomic study, except for 25 SLE patients receiving antibotic treatment and 8 patients receiving Cyclophosphamide treatment in the validation cohort.

The study was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University and all individuals included in the study signed an informed consent form.

Faecal samples collection and processing

Fresh faecal samples (10–50 g per sample) were collected in a sterilized container and kept at 4 °C. Genomic DNA (gDNA) was extracted from 180 to 220 mg faecal sample using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, German) according to the manufacturer's instructions.

16 S rRNA gene sequencing and analysis

For bacterial profiling, V3–V4 variable region of the 16S rRNA gene were selected and amplified by using polymerase chain reaction method as previously described [19]. The primer pairs used in 16S rRNA amplification were 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGAGGCTGCAG-3' (forward) and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACACGGGTATCTAATCC-3' (reverse).

Amplicons were purified with AMPure XP purification beads, followed by additions of sequencing adapters and dual-index barcodes provided in the Nextera XT index kit (Illumina, San Diego, America). The eligible libraries were normalized, pooled and further sequenced on Illumina MiSeq System using MiSeq V3 reagents (Illumina, San Diego, America), yielding 300 bp paired-end sequence reads.

Raw 16 S rRNA gene sequencing reads were filtered based on sequencing quality and then clustered into operational taxonomic units (OTUs) using unoise algorithm with a 97% threshold in the VSEARCH program [20]. Taxonomic classification of OTUs was conducted by the UCLUST algorithm in QIIME (V1.9) [21] based on the EzBioCloud reference database (Version 2017.10) [22]. OTUs with low abundance (<0.01%) were filtered out. Raw OTU table was subjected to scale normalization by scaling the sequencing depth to a fixed value of 20,000 sequences. A phylogeny tree for normalized OTUs was built using the FastTree algorithm in QIIME.

Statistical analysis and data visualization were performed in R (v3.4). Principal Coordination analysis (PCoA) with Bray-Curtis distance was conducted followed by permutational multivariate analysis of variance (PERMANOVA) using 'adonis' function in 'Vegan' package. The F/B ratio and *Proteobacteria*/*Bacteroidetes* (P/B) ratio were calculated using the ratio of the untransformed relative abundance. Relative abundance was subjected to arcsine-squared transformation for visualization convenience. Mann-Whitney test was used to test the difference between groups. False discovery rate (FDR) was used for multiple testing correction of P value. FDR value less than 0.05 was considered significant. Spearman coefficient was used for correlation analysis.

Shotgun metagenomic sequencing and metagenomic analysis

Faecal gDNA was used for library construction using the TruSeq DNA sample preparation kit (Illumina, San Diego, USA). Purified DNA was quantified using the Qubit double-stranded DNA (dsDNA) High-Sensitivity Assay Kit (Invitrogen, California, USA). According to the manufacturer's instructions, the sequence libraries were prepared using the NexteraXT DNA Library Preparation Kit (Illumina, San Diego, USA) followed by size and quality assessment using Agilent 2100. After quantification with the Qubit 3.0 fluorometer, libraries were pooled and sequenced on an Illumina Hiseq Xten sequencing platform using a paired-end 150 bp protocol.

The raw sequencing reads were first carried out quality control using KneadData software. Briefly, the module included removal of low-quality reads and splice sequences using Trimmomatic (trimmomatic-0.36-3, parameters: SLIDINGWINDOW: 4:20 MINLEN:50), followed by mapping with bowtie2 (parameters: --very-sensitive --dovetail) against the host hg38 genome to remove the host sequences. Quality-filtered metagenomes were taxonomically profiled using the MetaPhIAn2 tool [23] with default parameters. Information on the species abundance of microorganisms was obtained, normalized to percentages and used for subsequent analysis. Next, enterotypes analysis was used for analyzing components of intestinal flora in each faecal sample using a clustering approach with phylum level [24]. The bacterial phyla of a sample could be mainly clustered into *Proteobacteria*, *Firmicutes* and *Bacteroidetes*. The Chi-square test was used to analyse the differences of intestinal phenotypes between groups. Besides, the distribution of high-abundance bacteria phylum in the sample sets was analysed using a barplot of R. The P/B ratio was compared between groups using a rank sum test.

For function profiling, we used the HUMAnN2 [25] pipeline mapping reads to functionally annotated organism genomes and used a translated search to align unmapped reads to UniRef90 protein clusters. Furthermore, we attempted to explore the significant differences in the composition and functional enrichment of the gut microbiota according to previous studies [26, 27]. The differentially expressed gut microbial species were selected based on the following criteria using linear discriminant analysis (LDA) effect size (LEfSe) online software [28]: alpha value<=0.05 for the factorial Kruskal-Wallis test among classes and the pairwise Wilcoxon test between subclasses, and the threshold on the logarithmic LDA score for discriminative features>=3; the significant functional pathways of gut microbiota by Kyoto Encyclopedia of Genes and Genome (KEGG) orthology (KO) were selected based on the following criteria: mean relative abundance>0.01%, rank sum test and Deseq2 test, FDR<=0.05, log2foldchange|deseq2.logfoldchange|>=0.58.

The linear relationships among specific bacteria and clinical indices such as SLEDAI and functional pathways were analyzed using correlation analysis using R cor test or linear regression analysis using R lm function. FDR<0.05 was considered statistically significant.

Microbiota-associated epitope (ME) analysis

Gut MEs were predicted as previously described [29]. Briefly, the reference database was constructed by the formatdb function in the local BLAST package version 2.2.28 [30], and Immune Epitope Database (IEDB) 3.0 [31] used as input, which contains sequences information related to epitope peptides. The shotgun metagenome reads without human sequences were blasted against the reference database using Diamond's blast parameters -evalue 0.01 --threads 2 --max-target-seqs 1 --outfmt 6. Finally, the aligned reads were counted and annotated

based on the IEDB as the predicted ME datasets. The criteria for screening differential MEs between groups as follows: mean relative abundance > 0.1%, rank sum test and Deseq2 test, FDR <= 0.05, log2foldchange|deseq2.logfoldchange|>=1. To determine the associations between the candidate MEs and clinical factors such as SLEDAI, a correlation analysis was made using R cor test.

Animal model

9-week-old specific pathogen free (SPF) grade female MRL/*lpr* mice were purchased from Jiangsu Alingfield Biotechnology Co.,Ltd and maintained in an SPF environment. All animal experiments were carried out in accordance with the requirements of the Chinese Institutional Animal Care and Use Committee.

Bacterial solution gavage

Ten untreated patients with high activity were selected, and their faeces were collected in sterile test tubes. The faecal bacteria were isolated and cultured in coated plates. Bacterial DNA was extracted using the Bacterial gDNA Extraction Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. *Escherichia* that had been identified by 16 S rRNA sequencing were finally selected and incubated anaerobically for 24 h at 37 °C in MRS liquid medium, and the supernatant was removed by centrifugation at 4000 rpm for 10 min. The bacterial precipitate was resuspended in saline and made into suspension. Mice were gavaged on alternate days using 10^5 colony-forming unit (CFU)/mL of bacterial fluid for 1 week and bacterial colonisation for 2 weeks as the safe condition of gavage determined by the preliminary study (Data not shown).

Assessment of clinical indices

Body weight was measured every 4 days. Proteinuria level was monitored using proteinuria test strips (Gao'erbao, Guangzhou, CN) at different time points according to the manufacturer's instruction. Clinical score of disease was assessed SLE severity and procession weekly, including behavior and mobility (0 = normal; 1 = small changes; 2 = difficult movement; 3 = immobile), coat (0 = normal; 1 = bristly), alopecia (0 = none; 1 = < 0.5 cm^2; 2 = 0.5–1.0 cm^2; 3 = > 1.0 cm^2), skin lesions (0 = none; 1 = one < 0.5 cm^2; 2 = more than one < 0.5 cm^2; 3 = 0.5–1.0 cm^2; 4 = > 1.0 cm^2), body weight loss (0 = none; 1 = < 10%; 2 = 10–20%; 3 = > 20%) and proteinuria (0 = negative; 1 = 10 mg/dl; 2 = 30 mg/dl; 3 = 100 mg/dl; 4 = 300 mg/dl; and 5 = 2000 mg/dl.) [32]. After colonisation, the mice were euthanised by CO_2 anaesthesia and cervical dislocation. The liver and spleen were removed and weighed separately on filter paper, and the liver (spleen) index was calculated. The liver (spleen) index is the liver (spleen) mass (mg)/body mass (g). Blood were collected from the eyes and centrifuged for 1300 g for 10 min. Serum antinuclear antibody (ANA) and anti-dsDNA antibody were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The whole colon was retained for length measurement and recorded.

Histopathology

Renal tissue samples were obtained at the time of execution, fixed in 10% neutral formalin, dehydrated in conventional gradient alcohol, cleared in xylene, waxed, embedded and then made into 3 μm sections. Paraffin sections were placed in a 60 °C oven for 2 h and then removed. Next, sections were stained with Periodic Acid-Schiff staining kit (Solarbio, Beijing, China) according to the manufacturer's instructions. Briefly, renal sections were dewaxed to water and added with the oxidant for 10 min followed by 5 min of washing in tap water with gentle shaking. The dried sections were applied to Schiff's reagent for 15 min at room temperature. After Schiff's reagent, the sections were washed for 10 min in tap water. Eventually, nuclei were counterstained for 2 min with hematoxylin solution and washed for 10 min in tap water, dehydration, transparency and sealing were performed. Scanning electron microscopy was used to observe pathological changes of the kidneys in the field with 200× and 400× high magnification. The severity of glomerular lesions in the sections was assessed by an experienced pathologist unaware of the study protocol based on glomerular or mesangial cell proliferation, crescent formation and opening of vascular loop.

Statistical analysis

Student's t-test or Mann-Whitney test was used to test the between-group difference. Kruskal-Wallis test was used for analysis of multiple-group ordinal multicategorical data. P value < 0.05 were considered statistically significant.

Results

The profiles of bacterial diversity are changed in SLE patients

To investigate the correlation between gut microbiota and the disease, we performed a microbiome analysis. The full flowchart of the study is shown in Fig. 1. 134 HCs and 114 SLE patients including 52 LN were recruited for 16 S rRNA gene sequencing. The disease activity and creatinine were higher in LN patients compared with non-LN patients (Table 1), consistent with the fact that the disease status of LN patients was more severe. 97 HCs and 124 SLE patients were recruited for shotgun metagenomic sequencing. The discovery cohort consisted of 46 SLE cases and 46 HCs, whose demographics such as sex and age were strictly matched to eliminate possible

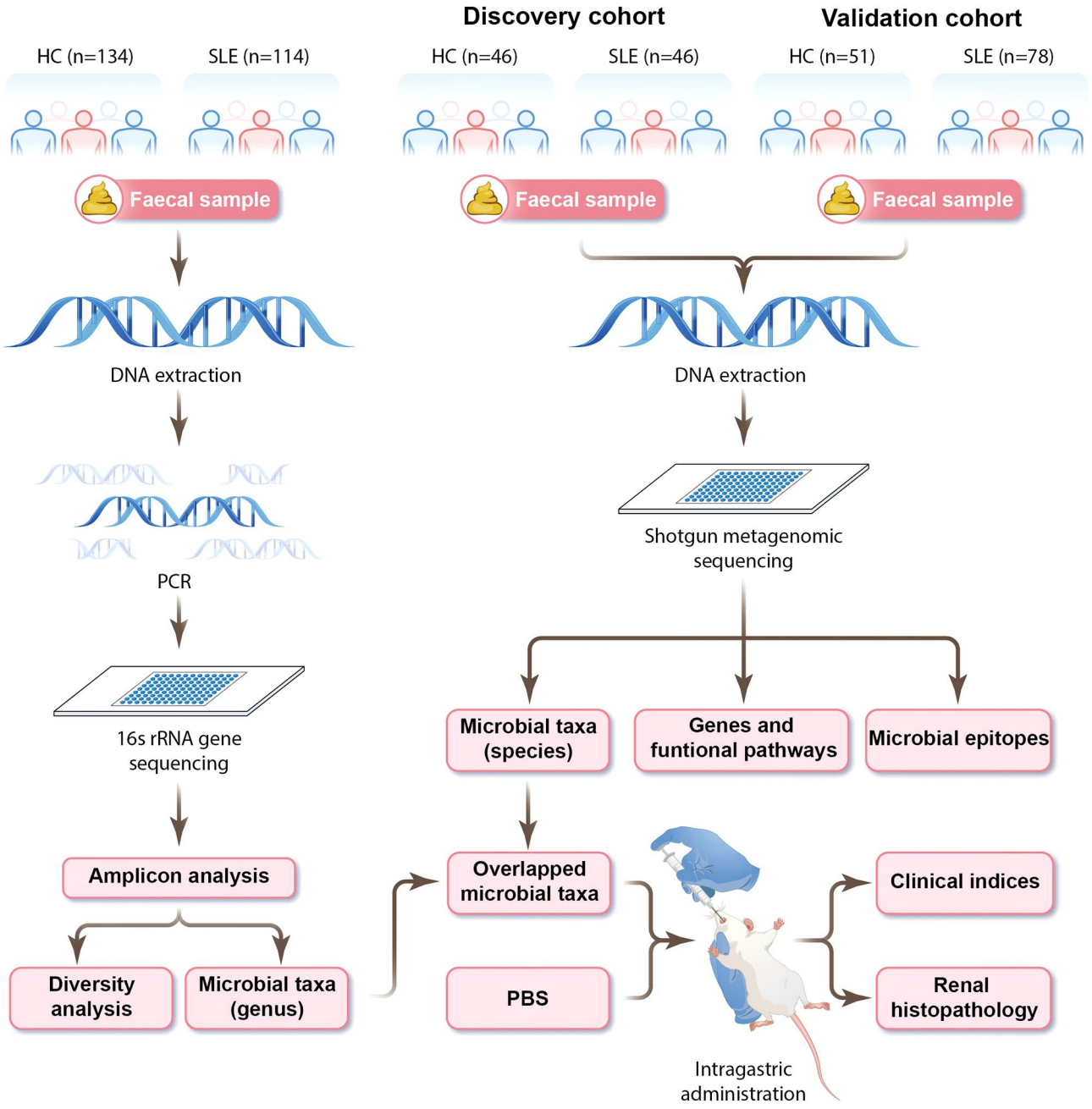

Fig. 1 The full flowchart of the experiments

interference. The remaining 78 SLE cases and 51 HCs constituted the validation cohort (Table 1 and Supplementary Table S1).

To analyze the alteration of bacterial diversity and composition in SLE, alpha and beta diversity were evaluated. Decreased amount of OTUs and Shannon index were observed in SLE patients compared with HCs (Fig. 2A). The reduction of species richness was more pronounced in LN patients than in non-LN patients (Fig. 2B). Moreover, PCoA showed significantly different gut microbial composition between SLE and HC groups (PERMANOVA, $p<0.0001$; Fig. 2C), and between LN and non-LN patients (PERMANOVA, $p=0.037$; Fig. 2D). Similarly, a profound reduction of richness was found in active patients (SLEDAI>4, Fig. 2E). An inverse correlation was observed between microbial richness and SLEDAI score (Fig. 2F), indicating that restriction degree of intestinal microbes was increased as the disease activity. Note that our finding consistently held under different SLEDAI cut-offs for active status (i.e. SLEDAI>8; Supplementary Fig. S1). The microbial richness and composition were insignificantly associated between

Table 1 Characteristics of study subjects at the time of faeces sampling

Characteristic	For 16 S rRNA sequencing				p_1	p_2	For shotgun metagenomic sequencing in the discovery cohort		p_3	For shotgun metagenomic sequencing in the validation cohort		p_4
	HCs	SLE patients					HCs	SLE		HCs	SLE	
		Non-LN	LN	Total								
n	134	62	52	114	-	-	46	46		51	78	
Age in years, mean ± SD /Median(P25-P75)	27.54 ± 9.84	32.74 ± 13.43	30.31 ± 11.58	31.65 ± 12.63	0.007	0.432	25.5(21.5–37)	26(20–37)	0.845	23(21–28)	29(21.75–42.25)	0.006
Sex ratio, n (% of females)	98(73.13)	55(88.71)	46(88.46)	101(88.60)	0.004	1.000	38(82.61)	39(84.78)	1.000	29(56.86)	69(88.46)	9.67×10^{-5}
SLEDAI, Median(P25-P75)	-	4(2–6)	14(4–19)	6(2–13)	-	< 0.001		6.5(4–14)			7.5(4-14.25)	
SLEDAI score, n (%)												
Inactive (≤ 4)	-	32(51.61)	14(26.92)	46(40.35)	-	0.013	-	16(34.78)		-	23(29.49)	
Active (> 4)	-	30(48.39)	38(73.08)	68(59.65)			-	30(65.22)		-	55(70.51)	
Cr, mean ± SD/ Median(P25-P75)	-	58.55 ± 13.32	82.38 ± 44.51	69.31 ± 33.51	-	5.41×10^{-5}	-	60(52.50–79.50)		-	61(50.75–72.50)	
Immunosuppressives usage, n (%)	-	34(54.84)	25(48.08)	59(51.75)	-	0.595	0(0)	0(0)		0(0)	39(50)	

p_1: p value for tests between healthy controls and SLE patients; p_2: p value for tests between Non-LN and LN patients; "-": unavailable to calculate. Immunosuppressive drugs referred to Mycophenolate Mofetil, ciclosporin, FK506, Azathioprine

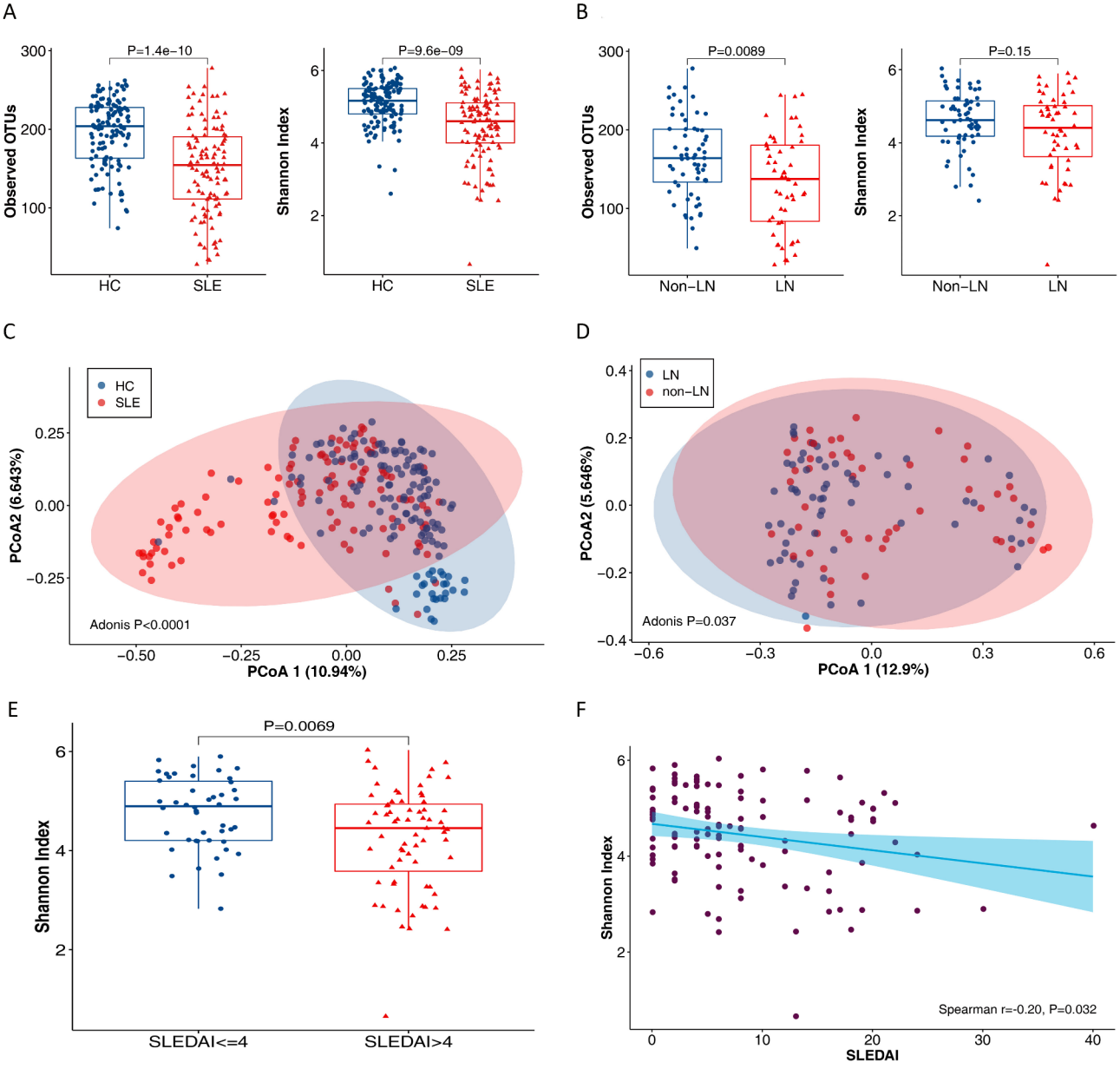

Fig. 2 Obviously reduced diversity and altered gut microbial composition in faecal samples from SLE patients with renal damage and high disease activity. Alpha-diversity of richness (OTUs) and diversity (Shannon index) of faecal microbiota were compared between HCs and SLE (**A**), and between non-LN and LN patients (**B**). PCoA based on Bray-Curtis distance was performed using 'adonis' function in 'Vegan' package and used for the comparison of the difference of bacterial composition between HCs and SLE patients (**C**), and between non-LN patients and LN patients (**D**). (**E**) The Shannon index of gut microbiota was compared between active patients and inactive patients, stratified by SLEDAI-2K. (**F**) The correlation analysis of the observed OTUs and SLEDAI score in patients using Spearman's correlation analysis. HCs, healthy controls; SLE, systemic lupus erythematosus; LN, lupus nephritis; OTUs, operational taxonomic units; PCoA, Principal Coordination analysis; SLEDAI, systemic lupus erythematosus disease activity index. $P < 0.05$ was considered statistically significant

different sexes and between immunosuppressor and non-immunosuppressor treatment, and therefore avoid their potential interferences (Supplementary Figs. S2A-C and S3A, B, respectively). Moreover, the differential observed OTUs and genus of gut bacteria were not influenced by immunosuppressor treatment (Supplementary Fig. S3C, D). In summary, our results illustrated that the community of gut microbiota was restricted and altered in SLE patients, especially in patients with LN or with high disease activity.

Characteristic P/B ratio alteration and *Escherichia* Genus expansion involved in the gut dysbiosis of SLE

To reveal the taxa characteristics of the dysbiotic gut microbiota in SLE, the between-group differences of microbial taxa were analyzed at different biological classifications. First, OTU-level analysis showed that OTUs of *Proteobacteria* phylum were significantly increased, while *Bacteroidetes* phylum-derived OTUs were decreased in SLE patients (Fig. 3A and Supplementary Table S2). Intriguingly, the lower F/B ratio previously reported in SLE did not recur in the current study. Instead, P/B ratio was higher, which was observed as a 2.79-fold increase in all SLE patients than that in the HC group (median ratio

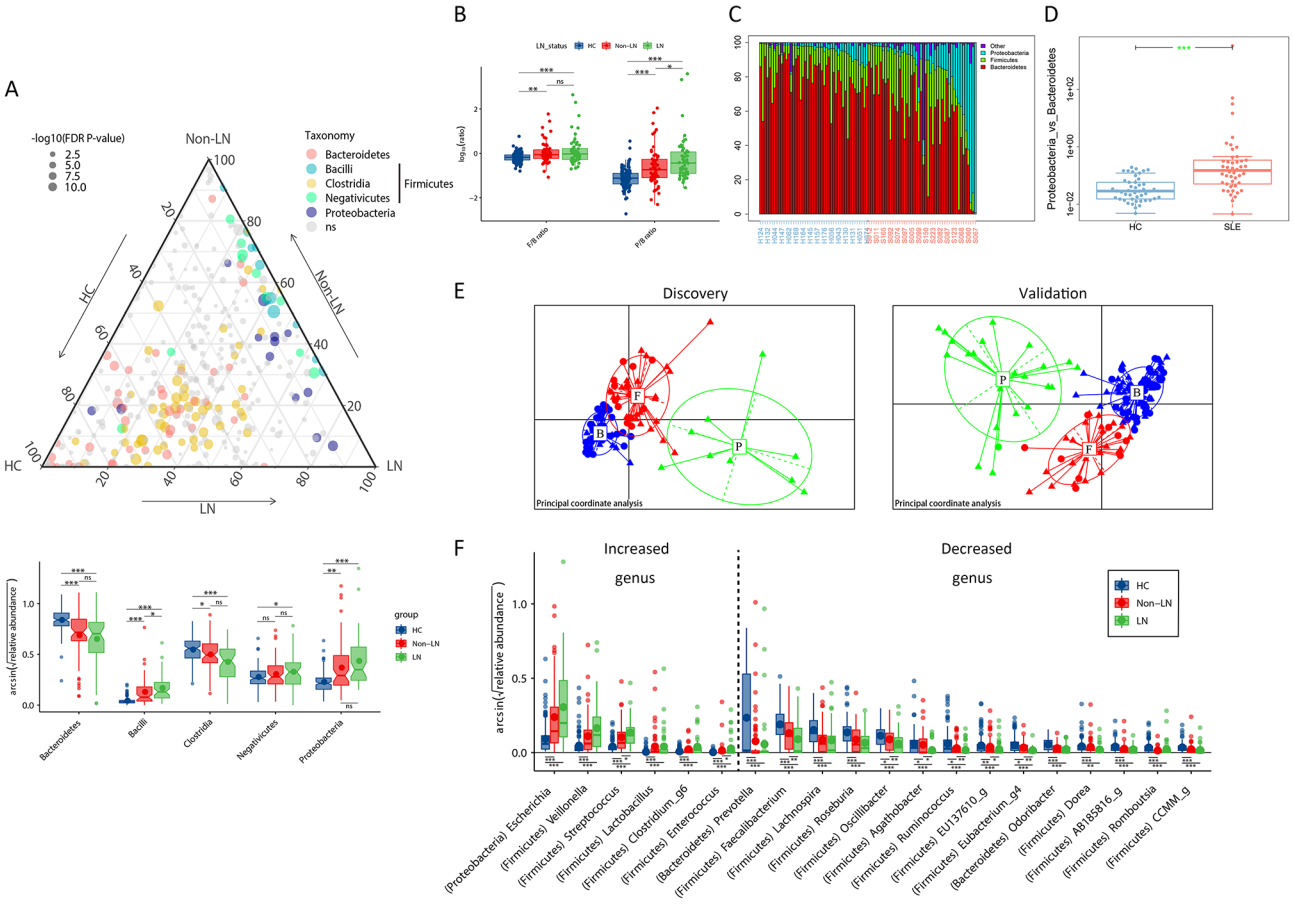

Fig. 3 Upregulation of characteristic P/B ratio and *Escherichia* genus involved in the gut dysbiosis of SLE. (**A**) Ternary plot displays the composition and enrichment of OTUs across the healthy controls (HCs, left corner), non-LN patients (Non-LN, top corner) and LN patients (LN, right corner). The coordination of each OTU is assigned according to its mean relative abundance in each group. The size of each circle represents the degree of significance of each OTU. The color of each circle represents the taxonomy assignment of each OTU. Non-significant OTUs are colored as grey with "ns" taxonomy label. The bottom part shows the comparison of the aggregated abundance of the main phylum or class taxa among HCs, non-LN and LN. Relative abundance was subjected to arcsine-squared transformation for visualization convenience. (**B**) The comparison of P/B ratio and previously reported F/B ratio among HCs, non-LN patients and LN patients. F/B or P/B ratio were calculated using the ratio of the untransformed relative abundance. (**C**) Enterotypes analysis of components of faecal microbiota in HCs and SLE patients using a clustering approach with phylum level. (**D**) The comparison of P/B ratio shown between HCs and SLE patients for shotgun metagenomics. (**E**) PCoA analysis for the distribution of *Firmicutes*, *Bacteroidetes* and *Proteobacteria* phylum of HCs and SLE cases in the discovery cohort and validation cohort. (**F**) The twenty most significant differentially changed bacterial genera were analyzed, followed by ranking of their abundances among HCs, non-LN and LN patients. Differential abundance at the phylum, family or genus level is identified by Wilcoxon rank sum test (FDR, which is correction of p value by Benjamini and Hochberg for multiple comparisons). HCs, healthy controls; LN, lupus nephritis; F: *Firmicutes*; B: *Bacteroidetes*; P: *Proteobacteria*; PCoA, Principal Coordination analysis; FDR, false discovery rate. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, ns, not significant

0.212 vs. 0.076, $p=8.69\times10^{-12}$). Furthermore, P/B ratio was a 1.99-fold increase in LN subjects as compared with that in non-LN subjects (median ratio 0.365 vs. 0.183, $p=0.049$; Fig. 3B), suggests that P/B ratio could be served as a dysbiotic index for SLE and SLE-secondary renal dysfunction. Similarly, shotgun metagenomics observed the finding in SLE by enterotypes analysis and PCoA analysis (Fig. 3C, E). P/B ratio was quantitated to be greatly higher in SLE patients than in HCs (Fig. 3D) as well.

Next, the representative differential taxa were identified for SLE patients and then for those in different subgroups. *Escherichia*, *Veillonella* and *Streptococcus* were identified as the leading increased genera, while *Prevotella* and *Faecalibacterium* were dominantly reduced genera in lupus patients (Supplementary Fig. 4 and Fig. 3F), and LN patients were further observed to tend to have a higher abundance of *Escherichia* genus compared with non-LN patients (Fig. 3F and Supplementary Table S3). The similar changes appeared in those patients with high disease activity (Supplementary Fig. 4B). It is summarized that SLE dysbiosis was characterized by microbial abundance upregulation from *Proteobacteria* phylum and its leading risk contributor *Escherichia* genus.

Shotgun metagenomics reveals the prominent expansion of *Escherichia coli* species among SLE pathogens

In order to distinguish specific species that constituted the differences in intestinal micro-environment between SLE patients and HCs, abundances of bacterial clades between all groups were used for LEfSe pairwise comparision using the Kruskal-Wallis test. 17 bacterial species were differentially expressed both at the discovery and validation phases, of which seven were SLE-enriched. The most upregulated species were *Escherichia coli*, which had the highest LDA score in SLE patients followed by *Escherichia* unclassified species, *Klebsiella pneumoniae*, *Veillonella parvula* and *Ruminococcus gnavus* (Fig. 4A). The abundance of *Escherichia coli* was significantly higher in SLE patients compared to HCs (Fig. 4B).

We further investigate the association between *Escherichia coli* and clinical factors such as SLEDAI in SLE patients. *Escherichia coli* abundance was found to be higher in SLE patients with high SLEDAI than those with low SLEDAI (Fig. 4C) and positively correlated with SLEDAI score (Fig. 4D). Linear regression analysis was used to confirm that the main bacterial species of SLE patients were insignificantly correlated with age, except for *Veillonella parvula* (Supplementary Table S4). In addition, the greatest changes in *Escherichia coli* and *Prevotella_copri* were found to be independent of sex by rank sum test analysis (Supplementary Fig. S5A, B).

Gut *Escherichia coli*-associated metabolic pathways alter in SLE patients

To interpret the biological meaning of gut *Escherichia coli* changes in SLE, we investigated their correlation with metabolic pathways. The results showed that altered gut bacterial composition in SLE patients correlates with variation in gene function. The most statistically significant functional pathways were extensively associated with *Escherichia coli*, including super pathway of purine nucleotide salvage, peptidoglycan (PGN) maturation pathway and unintegrated *Escherichia coli* pathway (Fig. 4E). These metabolic pathways were also positively associated with SLEDAI score in SLE patients (Fig. 4F-H). These results suggest *Escherichia coli* might promote the deterioration of SLE disease by changing their metabolites.

Gut *Escherichia coli*-associated epitopes alter in SLE patients

Previous studies showed that immunogenic MEs predicted by IEDB based on sequence alignment, a database used for prediction of T/B cell antigenic epitopes and ensued potential human antibody and antigenic characteristics analysis, may contribute to gut immunity and/or homeostasis in diseases [29]; thus, in order to reveal the role of *Escherichia*-derived MEs in SLE, the composition and diversity index of MEs were compared between SLE cases and HCs. The results showed the diversity of ME in SLE patients was remarkably increased both at the discovery (Fig. 5A) and validation phase (Fig. 5B). Moreover, the *Proteobacteria* phylum was observed to be positively correlated with MEs diversity (Fig. 5C), which showed the increase of ME diversity may be dominated by *Proteobacteria*. The increased gut ME diversity of SLE patients was positively correlated with SLEDAI score (Fig. 5D). The heatmap showed that the between-group differential MEs were associated with clinical phenotypes, particularly disease activity and renal involvement, including ARIAGINIPDHKHAVIALTSIY, RGQRTKTNARTRKGPRKPIKK, AEIYNKDGNKVDLYGKAVGL, FAGLKYADVGSFDYGRNYGV, FEVGATYYFNKNMSTYVDYI, FKGETQINSDLTGYGQWEYN, GVATYRNSNFFGLVDGLNFA, and LGNGKKAEQWATGLKYDANN epitopes from *Escherichia coli* (Fig. 5E and Supplementary Table S5). Therefore, *Escherichia coli* may utilize self-unique epitopes to disturb the balance of host immune system and thus promote the aggravation of SLE disease.

Escherichia exacerbates SLE manifestations in lupus-prone MRL/*lpr* mice

To determine if *Escherichia* is sufficient to predispose to lupus aggravation, MRL/*lpr* mice were colonized with *Escherichia* (*Escherichia coli* dominated) by oral gavage.

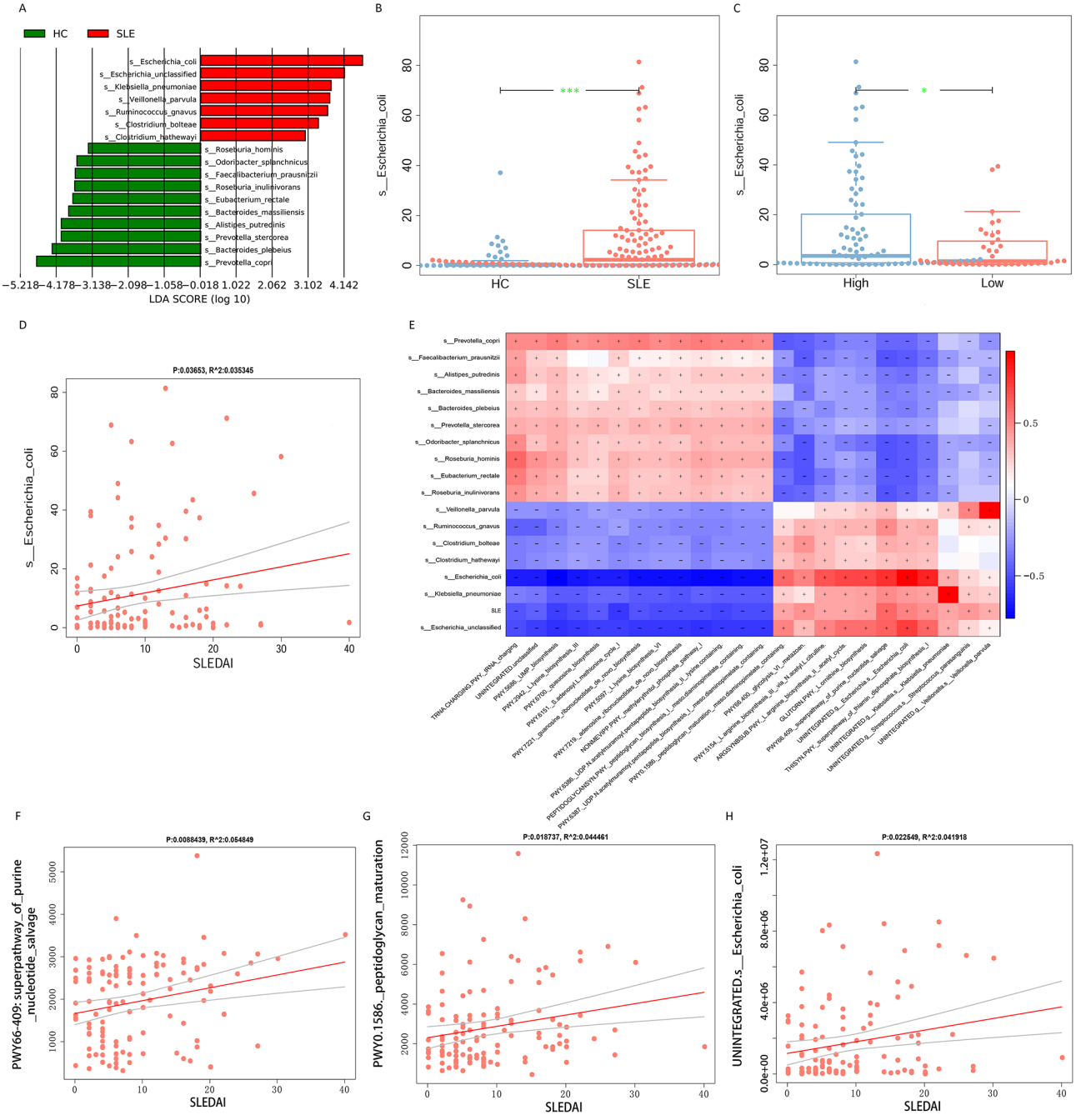

Fig. 4 The most prominently upregulated *Escherichia coli* correlates with disease activity and the associated metabolic pathways changes in patients with SLE. (**A**) The primarily changed bacterial species between HCs and SLE patients by LDA method are plotted for shotgun metagenomics. The abundance alteration of *Escherichia coli* is shown between HCs and patients in (**B**) and between patients with different activities in (**C**). The relationship of *Escherichia coli* abundance and SLEDAI score was analyzed (**D**). (**E**) The relationships of the SLE-altered bacterial species and enriched functional pathways were analyzed using the labeledHeatmap function of the WGCNA of R. (**F**–**H**) The associations of the key pathways about *Escherichia coli* and SLEDAI score were analyzed. HCs, healthy controls; SLE, systemic lupus erythematosus; PCoA, Principal Coordination analysis; LDA: Linear Discriminant Analysis; SLEDAI, systemic lupus erythematosus disease activity index. *$p < 0.05$, ***$p < 0.001$

We isolated and cloned them which had been identified by 16 S rRNA sequencing from faeces of SLE patients and then gavaged into the gut of MRL/*lpr* mice, of which the ears and the tail appeared skin erythema at the lupus onset (Supplementary Fig. S6). The full flowchart of animal experiments in MRL/*lpr* mice is shown in Supplementary Fig. S7. Colonisation for 2 weeks after bacteria gavage, the colon length did not change between MRL/*lpr* mice gavaged with *Escherichia* bacterial solution and PBS, which was not indicative of colitis occurrence

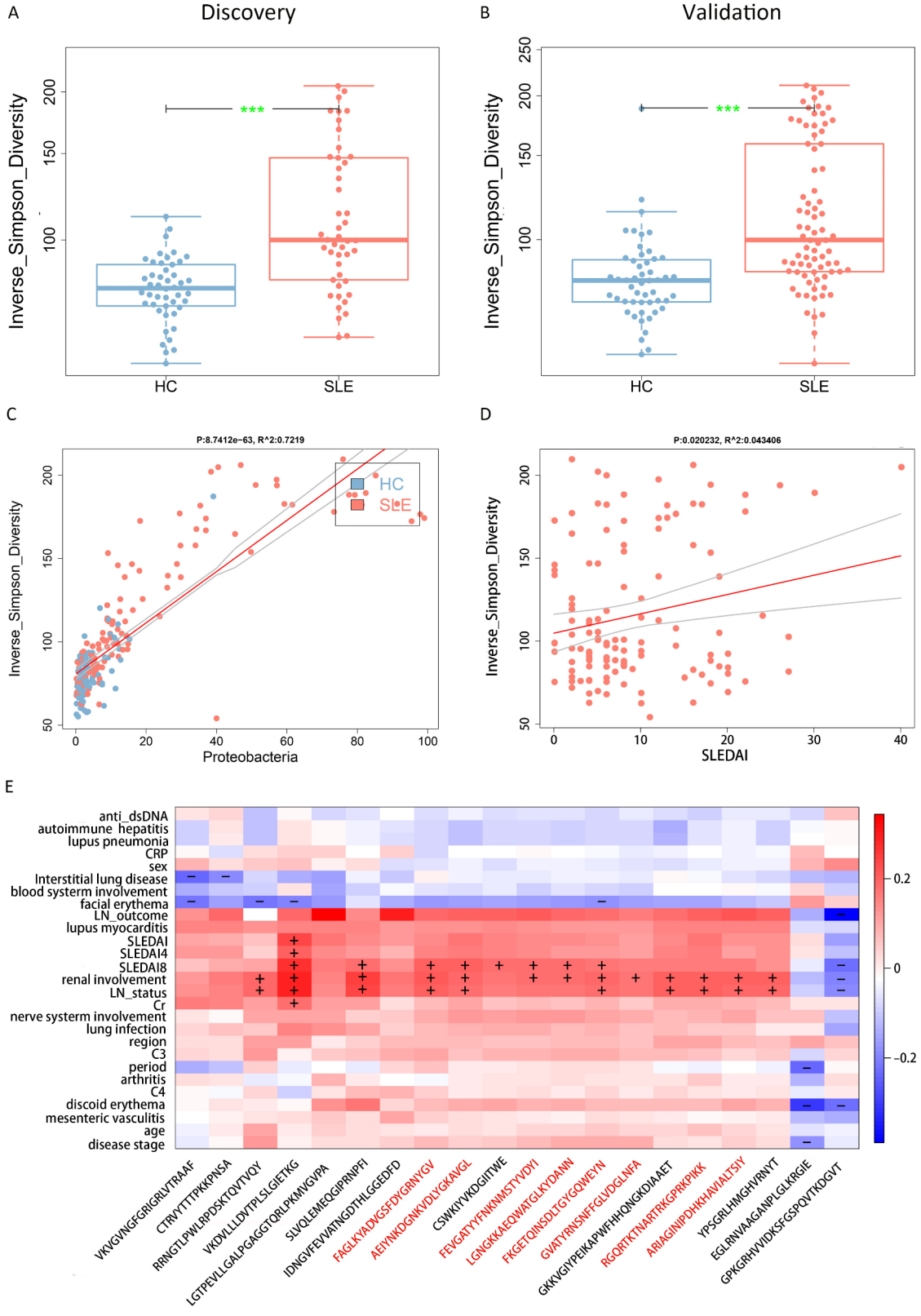

Fig. 5 The gut *Escherichia coli*-associated epitope changes correlate with disease activity in patients with SLE. The diversity of MEs was compared between HCs and SLE patients in the discovery cohort (**A**) and the validation cohort (**B**). The correlation between MEs diversity and the abundance of *Proteobacteria* (**C**) or SLEDAI (**D**) was analyzed in SLE Patients. (**E**) The correlations between the predicted MEs and clinical factors were analyzed. The MEs marked by red font represents the origin of *Escherichia coli*. ***$p < 0.001$. +: positive correlation, -: negative correlation

(Supplementary Fig. S8A), and neither was liver (spleen) index (Supplementary Fig. S8B, C) and body weight (Supplementary Fig. S8D). Mice gavaged with the bacterial solution exhibited higher titers of ANA (Fig. 6A) and a higher anti-dsDNA tendency (Fig. 6B). The level of proteinuria was increased significantly at day 12 and 18 since gavage with bacteria mixture, and then inclined to pick up to a higher level at day 30 (Supplementary Fig. S8E), and the clinical score was higher in bacteria-gavaged mice than PBS- gavaged controls (Fig. 6C).

Microscopically, in MRL/*lpr* mice with bacteria gavage, Periodic Acid-Schiff staining of kidney tissue section displayed that the glomerular volume was slightly increased; mesangial cells were severely proliferated and the corresponding mesangial area was obviously widened; endothelial cell segments were increased, and capillary loops had poor opening. Whereas those mice with PBS gavage only showed moderately proliferated mesangial cells and moderately widened mesangial region of the glomerulus (Fig. 6D). Up to 205 and 20 fibrocellular crescents per section were respectively formed in two mice of gavage group but almost none in the control group (Fig. 6D, F). And glomerular cells were increased in gavage group (Fig. 6E). The statistic analyses are shown in Fig. 6G, H. These data suggest that a *Escherichia*-defined microbiome may be prone to SLE deterioration in the context of a genetically susceptible host. Eventually, the potential mechanisms of *Escherichia coli*-centered gut dysbiosis, contributing to the development of SLE are summarized in Fig. 7.

Discussion

The gut microbiota is increasingly understood as playing fundamental roles in human physiology and health, including maintaining human homeostasis and regulating host immunity [33]. Endogenous gut microbiota in healthy individuals, generally dominated by *Firmicutes* and *Bacteroidetes*, and by relatively lower abundances of *Proteobacteria* and *Actinobacte*ria [34, 35], is essential for nutrient digestion, host metabolism, immune system development, and hormone secretion [36]. An inappropriate immune response destroys intestinal homeostasis and triggers dysbiosis [37]. Therefore, gut microbial stability and richness could serve as a good surrogate representing internal characteristics of an individual [38], and disturbance of gut microbiota, known as dysbiosis, was increasingly recognized to be involved in the procession of human autoimmune diseases [39, 40].

In relation to human SLE, recent studies have consistently concluded that SLE is linked to gut dysbiosis characterized by reduced microbial diversity and altered community composition, irrelevant to different sexes, ethnic populations, and disease activity [9–11, 41]. However, the major disturbed microbial taxa identified in previous studies varied. One possible explanation for the different results is related to the difference in the study subjects, including differences in sexes, disease activity, ethnic population, and the usage of immune-related therapies [42]. More importantly, the single sequencing technology and lack of molecular experiments in previous studies may limit the ability to identify specific bacteria for SLE pathogenesis. To overcome the limitations, we carried out a gut microbiota association study for SLE using 16 S rRNA sequencing on large-scale Chinese cohorts. Our data independently validated the reduced diversity and shaped microbial community in SLE patients as reported by previous studies [9–12, 41], regardless of sexes. Moreover, the degree of dysbiosis was more pronounced in patients with active status (SLEDAI score>4 or >8) and with nephritis, supporting the finding of Azzouz et al. [12]. SLEDAI was a global disease activity measure for SLE patients, and can fully represent the complexity of the manifestation of lupus. It was used as one of the most commonly used disease activity indices in researches [43] and clinical trials [44]. LN is a common and severe manifestation of SLE, is an important contributor to disease related morbidity and mortality [45]. Our results clearly illustrate a close connection between restricted diversity of gut microbiota and disease activity and severity of SLE. Furthermore, the SLE-related dysbiosis in the current study was characterized by the up-regulation of *Proteobacteria* phylum, resulting in a higher P/B ratio instead of the lower F/B ratio as discussed in the literature [7, 8, 10, 41, 46], *which o*ffered an indicator of disease activity for SLE.

To make up for the limitation that 16 S rRNA sequencing hardly identified the species accurately, we used shotgun metagenomic sequencing to identify which species contributed to SLE pathogenesis. We have observed the over represented *Ruminococcus gnavus* in SLE, which was found in other lupus cohort studies [12, 47]. More importantly, we found that *Escherichia coli*, the representative species of *Escherichia* genus, was the significantly highest enriched in SLE patients and positively correlated with SLEDAI, which differs from the result of BD Chen et al., stating the over represented taxa were dominant by *Clostridium species ATCC BAA-442 as well as Atopobium rimae*, *Shuttleworthia satelles*, *Actinomyces massiliensis*, *Bacteroides fragilis*, *and Clostridium leptum* in SLE gut microbiota [48]. We considered that differences of geographic region, components of dietary intake and disease activity might partly contribute to the differences. A previous study showed that *Escherichia coli* promotes intestinal epithelial barrier dysfunction by targeting multiple elements of the barrier such as tight junction proteins [49]. Here, we found super pathway of purine nucleotide salvage and PGN maturation pathway were prominently related pathways of *Escherichia coli*

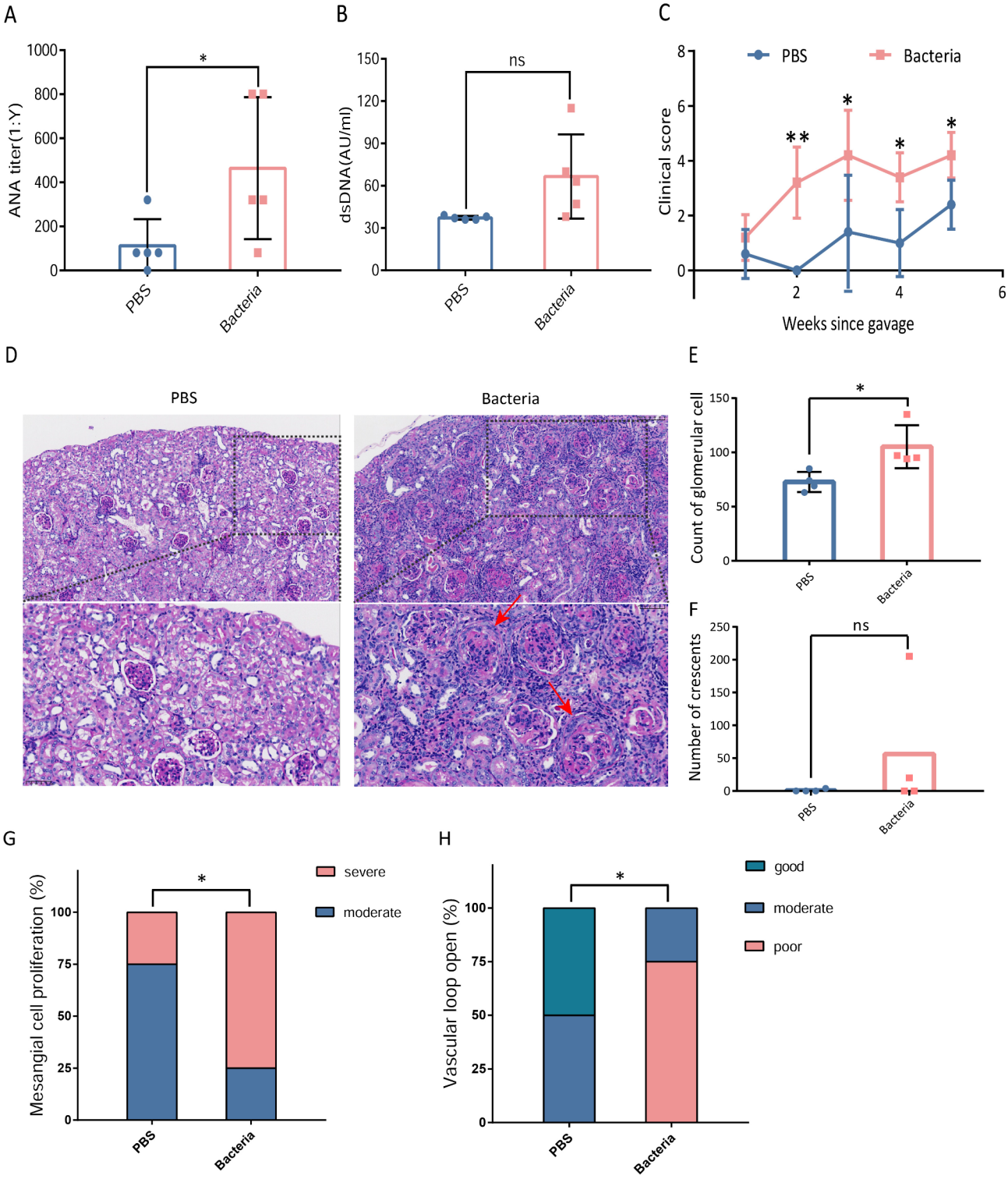

Fig. 6 Gavage with *Escherichia* bacterial solution promotes the increase of lupus-associated traits and renal damage in MRL/*lpr* mice. (**A**–**C**) The titers of ANA and anti-dsDNA, and clinical score were compared between MRL/*lpr* mice gavaged with *Escherichia* bacterial solution and those with PBS. (**D**) The paraffin-embedded renal tissue sections were stained using Periodic Acid-Schiff staining and then scanned by scanning electron microscopy. The upper graph is imaged at 200 times magnification and the bottom one is correspondingly imaged at 400 times magnification. Red arrow points to the area of crescent formation. (**E**-**F**) The number changes of glomerular cells and crescents were analyzed between bacterial solution-gavage group and PBS-gavage group. (**G**-**H**) The degree of renal injury was compared between bacterial solution-gavage group and PBS-gavage group by Kruskal Wallis test. $n = 4–5$. *$p < 0.05$, ** $p < 0.01$, ns, not significant

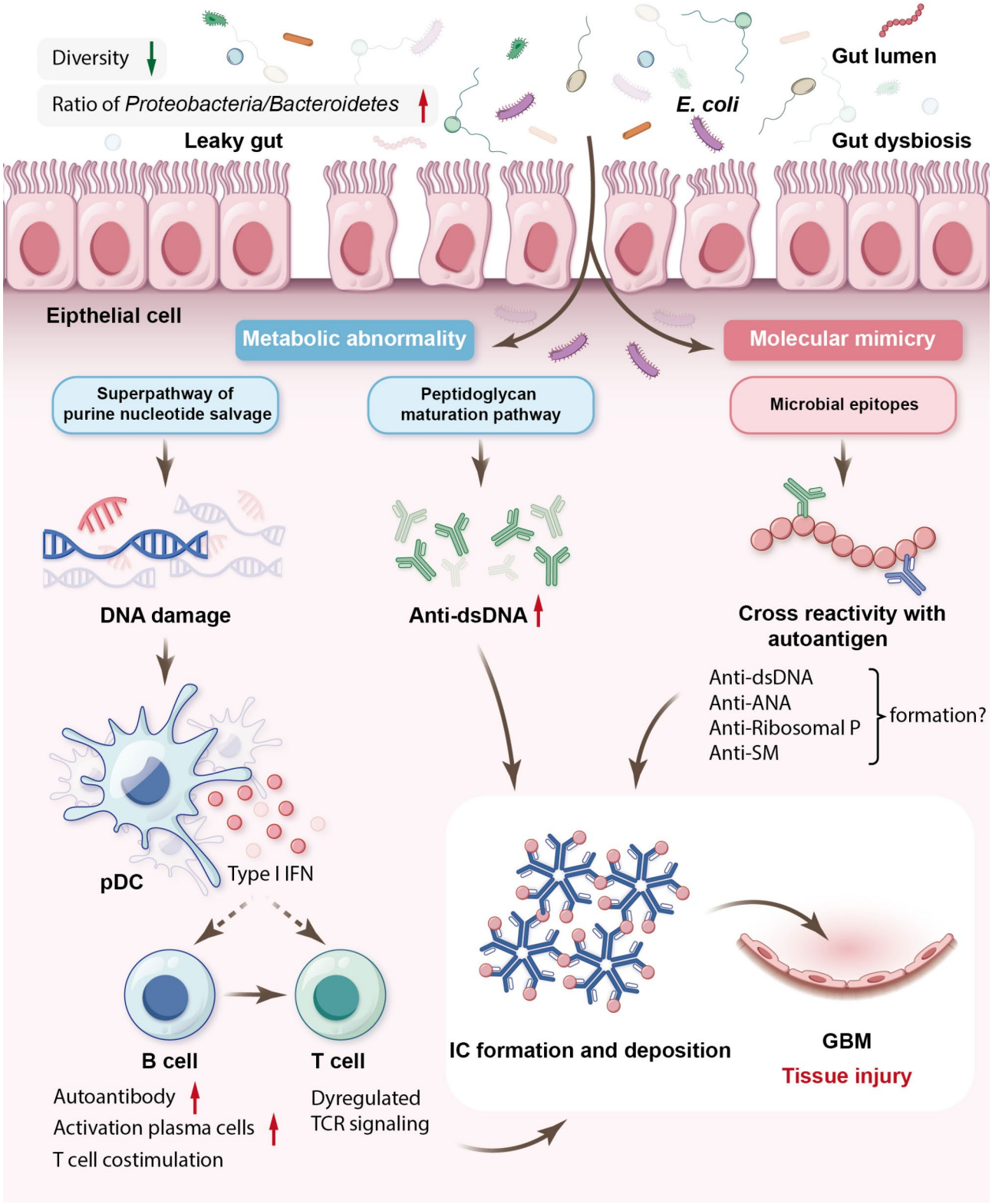

Fig. 7 Schematic illustration for the potential mechanisms of gut dysbiosis characterized by *Escherichia* dominated by *Escherichia coli* outgrowth resulting in the exacerbation of SLE disease. *E.coli*, *Escherichia coli*; dsDNA, double-stranded DNA; ANA, antinuclear antibody; pDC, plasmacytoid dendritic cell; IFN, interferon; TCR: T-cell receptor; IC, immune complexes; GBM, glomerular basement membrane

and positively associated with SLEDAI score. The former pathway is critically important in maintaining cellular functions and integrity. During the degradation of RNA and DNA, nucleotide balance is maintained by de novo synthesis and salvage of nucleosides formed in eukaryotes, in which dysregulation or deficiencies have been implicated in neurodegeneration and DNA damage [50]. As we know, DNA damage contributes to SLE pathophysiology [51]. These pieces of evidence suggest the changes of metabolites in nucleotide balance response to *Escherichia coli* infection may participate in the SLE pathogenesis. The latter pathway is mainly involved in PGN biosynthesis. As a part of the bacterial cell wall, PGN is highly antigenic due to conserved structural molecular motifs unique to a bacterium, which derived from different bacteria can uniquely modulate immune activities [52]. Our study found *Escherichia coli*-associated PGN maturation was positively associated with the disease activity of SLE, which is supported by a previous study demonstrated that *Staphylococcus aureus* PGN induces a sustained anti-dsDNA immunoglobulin G autoantibody response and immune complex-mediated glomerulonephritis in lupus-prone mice [53].

The method for predicting gut MEs in the gut microbiota has previously been applied in gut microbiota research related to schizophrenia [27], autism [29], ankylosing spondylitis (AS) [54], and other conditions. MEs are obtained by blasting human genes-free reads against the IEDB database version 3.0, which contains more than 500,000 epitopes related to human adaptive immunity, are developed by manually collating over 15,000 articles or over 700,000 experiments, and considered there is a specifically positive binding ability to human lekocyte antigen or T/B-cell response [29]. In AS patients, MEs have been validated to elicit antigen-specific immunological responses. The SLE-related MEs we identified are homologous to or share amino acid sequences with epitopes in the IEDB database that have potential immunological responses in the study. The increase of ME diversity was characteristic change in gut microbial composition and associated with increased SLEDAI score in SLE patients. The origin of altered MEs was prominently from *Escherichia coli* and directly correlated with different clinical phenotypes such as SLEDAI. Among them, ARIAGINIPDHKHAVIALTSIY and RGQRTKTNARTRKGPRKPIKK are linear peptidic epitopes studied as part of small ribosomal subunit protein uS13 from *Escherichia coli*, and were observed by Syu WJ et al. to react with ribosomal protein S13-specific monoclonal antibodies in situ [55]; AEIYNKDGNKVDLYGKAVGL, FAGLKYADVGSFDYGRNYGV, FEVGATYYFNKNMSTYVDYI, FKGETQINSDLTGYGQWEYN, GVATYRNSNFFGLVDGLNFA, and LGNGKKAEQWATGLKYDANN epitopes are linear peptidic epitopes studied as part of pore-forming outer membrane proteins from *Escherichia coli*, and they elicited T-cell proliferative responses of mice when the mice were immunized with synthetics of these peptides [56]. These implied that *Escherichia coli* may contribute to accelerate the procession of SLE by the abnormality of immune responses triggered by these MEs. Previous studies considered that *Escherichia* molecules use molecular mimicry to be implicated in the pathogenesis of SLE disease. Specifically, the CpG island of *Escherichia coli* genomic DNA and the bacterial adhesin FimH protein of *Escherichia sp.* are thought as self-antigen to link to SLE-related disease by mimicking host dsDNA and human lysosomal-associated membrane protein, respectively [57]. These pieces of evidence imply that *Escherichia* may aggravate the disease condition of SLE by molecular mimicry against self-antigen.

To confirm in vivo influences of *Escherichia* in the development of SLE, we gavaged the bacterial solution of *Escherichia* to lupus-susceptible MRL*/lpr* mice. To avoid the potentially lethal adverse effects such as acute gastroenteritis and sepsis resulting from large numbers of bacteria entering the digestive tract, we conducted a preliminary experiment to determine the safe condition of gavage in mice. Ultimately, we set the gavage dose at concentrations of 10^5 CFU/mL and took 1 week on alternate days for gavage and then two weeks for colonisation as the safe condition of gavage. In the observation period, the survival of MRL/lpr mice was 100% in both bacteria gavage group and control group (data not shown). Neither body weight nor colon length was significantly changed between the two groups of mice, indicating that the gastrointestinal tract was not affected by the bacteria solution. The bacterial solution-gavaged-mice displayed a worsening illness which was manifested by high titer of ANA, anti-dsDNA and clinical score. Most SLE patients have clinical evidence of renal disease in the course of the disease, usually in the form of abnormal urinalysis, and up to half of SLE patients will eventually develop clinically significant nephropathy [45]. MRL*/lpr* mice have a certain probability of developing LN naturally after the onset of SLE as the disease progresses. In our study, the experimental group exhibited earlier, more frequent and severe manifestations of SLE renal involvement. Overall, these results further verify that the abnormal expansion of gut *Escherichia* is considered as an accelerator of the disease procession. The associated study has reported that transfer of intestinal *Enterococcus gallinarum* through a leaky gut may trigger the activation of the immune system involving up-regulating type I interferon (IFN)-related genes and auto-antibody production to drive autoimmune diseases such as SLE [14].

The current study exists some limitations. First, the study was single-center and needs to have external validation that included large-sample, multiple-center studies.

Second, we did not consider the potential influences of dietary intake, geography, genetic background and psychological states in the enrollment of study populations. Third, functional elucidation is absent for how expanded *Escherichia coli* utilizes self-metabolites and self-epitopes to contribute causally to the development of autoimmunity in SLE procession. Specific antibodies against the screened specific *E. coli*-associated linear peptidic epitopes need to be generated and the ELISA protocols need to be established to measure the levels of the antibodies against these epitopes in serum to continue the subsequent study on the effects of these epitopes on aggravation of SLE and in-depth pathogenic mechanisms. Finally, while *Escherichia* was particularly dominant to exacerbate clinical manifestations, it remains to be investigated whether other gut bacteria taxa perhaps augment or synergise with *Escherichia* in the engagement of downstream signaling pathways in immune abnormality. However, our results remain sufficient to elaborate outspread of gut *Escherichia* deteriorates the manifestations of SLE, and the involvement of metabolites and MEs of *Escherichia coli* in the aggravation of SLE disease, which offer an important basis for further investigation of pathogenesis and new treatment targets in SLE.

Conclusions

In conclusion, we unraveled the outgrowth of *Escherichia* as an important contributor in accelerating SLE procession. The functional changes of *Escherichia coli* may be associated with metabolite alteration and numerous accumulations of self-epitopes, which induce immune response imbalance, and thus contribute to the procession of SLE. Our findings could provide a deeper understanding of the role of gut *Escherichia* in the pathogenesis of SLE.

Abbreviations

SLE	Systemic lupus erythematosus
F/B	Firmicutes/Bacteroidetes
rRNA	Ribosomal RNA
LN	Lupus nephritis
SLEDAI	Systemic lupus erythematosus disease activity index
HCs	Healthy controls
gDNA	Genomic DNA
OTUs	Operational taxonomic units
PCoA	Principal coordination analysis
PERMANOVA	Permutational multivariate analysis of variance
P/B	Proteobacteria/Bacteroidetes
FDR	False discovery rate
dsDNA	Double-stranded DNA
LDA	Linear discriminant analysis
LEfSe	LDA effect size
KEGG	Kyoto Encyclopedia of Genes and Genome
KO	KEGG Orthology
ME	Microbiota-associated epitope
IEDB	Immune epitope database
SPF	Specific pathogen free
CFU	Colony-forming unit
ANA	Antinuclear antibody
ELISA	Enzyme-linked immunosorbent assay
PGN	Peptidoglycan
IFN	Type I interferon

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-024-03413-7.

Supplementary Table S1: Clinical characteristics summary of all enrolled subjects in the metagenomics analysis.

Supplementary Table S2: Comparison of gut microbial taxa between HC and SLE at OUT level.

Supplementary Table S3: Comparison of gut microbial taxa between HC and SLE at genus level.

Supplementary Table S4: The correlation of mainly enriched species and age in metogenomics.

Supplementary Table S5: Main epitopes were shown for SLE patients.

Supplementary Fig. S1: Association between SLEDAI score cut-off (SLEDAI > 8) and gut microbial diversity in all patients. (A) Observed OTUs and Shannon Index for alpha diversity and (B) PCoA analysis based on Bray-Curtis distance for beta diversity. Comparisons were made between patients with SLEDAI < = 8 and SLEDAI > 8 by using Wilcoxon rank-sum test. OTUs, operational taxonomic units; PCoA, Principal coordination analysis.

Supplementary Fig. S2: Effects of sex on gut microbiota. (A) The assessment of bacteria alpha diversity by Shannon index between females and males in HCs and SLE, and lupus subgroups. Beta diversity analyses of PCoA based on Bray-Curtis distance of fecal bacteria composition are made between females and males in HCs (B) and SLE patients (C). HCs, healthy controls; SLE, systemic lupus erythematosus; LN, lupus nephritis; PCoA, Principal coordination analysis. Adonis denotes Permutational Multivariate Analysis of Variance (PERMANOVA).

Supplementary Fig. S3: Effects of immunosuppressor on gut microbiota dysbiosis in SLE patients. (A) Observed OTUs for alpha diversity, (B) PCoA analysis based on Bray-Curtis distance for beta diversity, and (C-D) Volcano plot showing differential abundance test results for (C) OTU level and (D) genus taxa between SLE patients with or without IMSs treatment. Horizontal line represents the log10-transformed FDR-corrected P value of 0.05. Points above the horizontal line mean significant differential abundance. Vertical line represents the log2-transformed mean fold change (IMS over Non-IMS) of value 1. IMS, immunosuppressor; non-IMS, without immunosuppressor; OTUs, operational taxonomic units; PCoA, Principal coordination analysis; P.fdr, false discovery rate corrected p value. Adonis denotes Permutational Multivariate Analysis of Variance (PERMANOVA).

Supplementary Fig. S4: Specific taxa changed in all lupus patients and those with high disease activity. The twenty most significant differentially changed bacterial genera were analyzed between HCs and all SLE patients, and between patients with low activity and with high activity (Wilcoxon rank sum test), followed by ranking of their abundances. HCs, healthy controls; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$.

Supplementary Fig. S5: The effects of sex on the abundance of the most obviously changed microbial species in SLE disease. The abundances of *Escherichia coli* (A) and *Prevotella_copri* (B) were compared between different sexes in HC group and SLE group. not significant, not shown. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Supplementary Fig. S6: MRL/lpr mice. Changes of skin erythema conditions in ears (top) or the tail (bottom) of MRL/*lpr* mice before and after lupus onset.

Supplementary Fig. S7: The flowchart to confirm the contribution of *Escherichia* to the development of SLE disease

Supplementary Fig. S8: Effects of gavage with Escherichia bacterial solution on lupus-associated traits. Colon length (A), liver index (B), spleen

index (C), body weight (D) and the level of proteinuria (E) were compared between MRL/*lpr* mice gavaged with *Escherichia* bacterial solution and controls.*$p < 0.05$, **$p < 0.01$, ns, not significant.

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Not applicable.

Author contributions
LG and XYZ participated in the study design, data collection, data analysis and interpretation, and manuscript writing. JMF, MBW and ZNC participated in clinical data collection, data analysis and manuscript writing. LG, XYZ and YHS participated in animal experiments and data analysis. JQ, ZGC, JLP, YLZ, CPC, PPZ, XHG, QL and XZ participated in participant recruitment and clinical data collection. JLP and YZ participated in data interpretation and manuscript revision. ZML and JRG jointly supervised the study, and conceived, designed, and critically improved the final manuscript. All authors read and approved the final manuscript to be published.

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Data availability
The data supporting the findings of the study are included within the article and its supplementary files. The sequencing data are deposited in the China National Center for Bioinformation (CNCB) with accession code CRA009524 that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University (Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University [2016]2–48) and all individuals included in the study signed an informed consent form. All animal experiments were carried out in accordance with the requirements of the Chinese Institutional Animal Care and Use Committee.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Kaul A, Gordon C, Crow MK, Touma Z, Urowitz MB, van Vollenhoven R, et al. Systemic lupus erythematosus. Nat Rev Dis Primers. 2016;2:16039.
2. Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365:2110–21.
3. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell. 2012;148(6):1258–70.
4. Kamada N, Seo SU, Chen GY, Nunez G. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol. 2013;13(5):321–35.
5. Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med. 2015;21(8):895–905.
6. Marino E, Richards JL, McLeod KH, Stanley D, Yap YA, Knight J, et al. Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. Nat Immunol. 2017;18(5):552–62.
7. Hevia A, Milani C, Lopez P, Cuervo A, Arboleya S, Duranti S, et al. Intestinal dysbiosis associated with systemic lupus erythematosus. mBio. 2014;5(5):e01548–14.
8. Rodriguez-Carrio J, Lopez P, Sanchez B, Gonzalez S, Gueimonde M, Margolles A, et al. Intestinal dysbiosis is Associated with altered short-chain fatty acids and serum-free fatty acids in systemic Lupus Erythematosus. Front Immunol. 2017;8:23.
9. Luo XM, Edwards MR, Mu Q, Yu Y, Vieson MD, Reilly CM, et al. Gut microbiota in human systemic lupus erythematosus and a mouse model of Lupus. Appl Environ Microbiol. 2018;84(4):e02288–17.
10. van der Meulen TA, Harmsen HJM, Vila AV, Kurilshikov A, Liefers SC, Zhernakova A, et al. Shared gut, but distinct oral microbiota composition in primary Sjogren's syndrome and systemic lupus erythematosus. J Autoimmun. 2019;97:77–87.
11. Li Y, Wang HF, Li X, Li HX, Zhang Q, Zhou HW, et al. Disordered intestinal microbes are associated with the activity of systemic Lupus Erythematosus. Clin Sci (Lond). 2019;133(7):821–38.
12. Azzouz D, Omarbekova A, Heguy A, Schwudke D, Gisch N, Rovin BH, et al. Lupus nephritis is linked to disease-activity associated expansions and immunity to a gut commensal. Ann Rheum Dis. 2019;78(7):947–56.
13. Guo M, Wang H, Xu S, Zhuang Y, An J, Su C, et al. Alteration in gut microbiota is associated with dysregulation of cytokines and glucocorticoid therapy in systemic lupus erythematosus. Gut Microbes. 2020;11(6):1758–73.
14. Manfredo Vieira S, Hiltensperger M, Kumar V, Zegarra-Ruiz D, Dehner C, Khan N, et al. Translocation of a gut pathobiont drives autoimmunity in mice and humans. Science. 2018;359(6380):1156–61.
15. Zhao Z, Ren J, Dai C, Kannapell CC, Wang H, Gaskin F, et al. Nature of T cell epitopes in lupus antigens and HLA-DR determines autoantibody initiation and diversification. Ann Rheum Dis. 2019;78(3):380–90.
16. Yin Y, Choi S-C, Xu Z, Perry DJ, Seay H, Croker BP, et al. Normalization of CD4 + T cell metabolism reverses lupus. Sci Transl Med. 2015;7(274):274ra18.
17. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40(9):1725.
18. Grevink ME, Horst G, Limburg PC, Kallenberg CGM, Bijl M. Levels of complement in sera from inactive SLE patients, although decreased, do not influence in vitro uptake of apoptotic cells. J Autoimmun. 2005;24(4):329–36.
19. Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, et al. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome. 2014;2(1):6.
20. Rognes T, Flouri T, Nichols B, Quince C, Mahe F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016;4:e2584.
21. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335–6.
22. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, et al. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol. 2017;67(5):1613–7.
23. Truong DT, Franzosa EA, Tickle TL, Scholz M, Weingart G, Pasolli E, et al. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods. 2015;12(10):902–3.
24. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature. 2011;473(7346):174–80.

25. Franzosa EA, McIver LJ, Rahnavard G, Thompson LR, Schirmer M, Weingart G, et al. Species-level functional profiling of metagenomes and metatranscriptomes. Nat Methods. 2018;15(11):962–8.
26. Wang M, Wan J, Rong H, He F, Wang H, Zhou J, et al. Alterations in Gut Glutamate Metabolism Associated with changes in Gut Microbiota Composition in Children with Autism Spectrum Disorder. mSystems. 2019;4(1):e00321–18.
27. Xu R, Wu B, Liang J, He F, Gu W, Li K, et al. Altered gut microbiota and mucosal immunity in patients with schizophrenia. Brain Behav Immun. 2020;85:120–7.
28. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60.
29. Wang M, Zhou J, He F, Cai C, Wang H, Wang Y, et al. Alteration of gut microbiota-associated epitopes in children with autism spectrum disorders. Brain Behav Immun. 2019;75:192–9.
30. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403–10.
31. Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD, Cantrell JR, et al. The immune epitope database (IEDB) 3.0. Nucleic Acids Res. 2015;43(Database issue):D405–12.
32. Funes SC, Rios M, Gomez-Santander F, Fernandez-Fierro A, Altamirano-Lagos MJ, Rivera-Perez D, et al. Tolerogenic dendritic cell transfer ameliorates systemic lupus erythematosus in mice. Immunology. 2019;158(4):322–39.
33. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. Science. 2016;352(6285):539–44.
34. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science. 2005;308(5728):1635–8.
35. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207–14.
36. Shamriz O, Mizrahi H, Werbner M, Shoenfeld Y, Avni O, Koren O. Microbiota at the crossroads of autoimmunity. Autoimmun Rev. 2016;15(9):859–69.
37. Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015;33(9):496–503.
38. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science. 2009;326(5960):1694–7.
39. Knip M, Siljander H. The role of the intestinal microbiota in type 1 diabetes mellitus. Nat Rev Endocrinol. 2016;12(3):154–67.
40. Van de Wiele T, Van Praet JT, Marzorati M, Drennan MB, Elewaut D. How the microbiota shapes rheumatic diseases. Nat Rev Rheumatol. 2016;12(7):398–411.
41. He Z, Shao T, Li H, Xie Z, Wen C. Alterations of the gut microbiome in Chinese patients with systemic lupus erythematosus. Gut Pathog. 2016;8:64.
42. Ruiz L, Lopez P, Suarez A, Sanchez B, Margolles A. The role of gut microbiota in lupus: what we know in 2018? Expert Rev Clin Immunol. 2018;14(10):787–92.
43. Wang Y, Chen S, Chen S, Du J, Lin J, Qin H, et al. Long noncoding RNA expression profile and association with SLEDAI score in monocyte-derived dendritic cells from patients with systematic lupus erythematosus. Arthritis Res Ther. 2018;20(1):138.
44. van Vollenhoven RF, Kalunian KC, Dörner T, Hahn BH, Tanaka Y, Gordon RM, et al. Phase 3, multicentre, randomised, placebo-controlled study evaluating the efficacy and safety of ustekinumab in patients with systemic lupus erythematosus. Ann Rheum Dis. 2022;81(11):1556–63.
45. Almaani S, Meara A, Rovin BH. Update on Lupus Nephritis. Clin J Am Soc Nephrol. 2017;12(5):825–35.
46. Greiling TM, Dehner C, Chen X, Hughes K, Iñiguez AJ, Boccitto M, et al. Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus. Sci Transl Med. 2018;10(434):eaan2306.
47. Azzouz DF, Chen Z, Izmirly PM, Chen LA, Li Z, Zhang C, et al. Longitudinal gut microbiome analyses and blooms of pathogenic strains during lupus disease flares. Ann Rheum Dis. 2023;82(10):1315–27.
48. Chen B, Jia X, Xu J, Zhao L, Ji J, Wu B, et al. An autoimmunogenic and proinflammatory profile defined by the gut microbiota of patients with untreated systemic lupus erythematosus. Arthritis Rheumatol. 2021;73(2):232–43.
49. Shawki A, McCole DF. Mechanisms of intestinal epithelial barrier dysfunction by Adherent-Invasive Escherichia coli. Cell Mol Gastroenterol Hepatol. 2017;3(1):41–50.
50. Fasullo M, Endres L. Nucleotide salvage deficiencies, DNA damage and neurodegeneration. Int J Mol Sci. 2015;16(5):9431–49.
51. Lo MS, Tsokos GC. Recent developments in systemic lupus erythematosus pathogenesis and applications for therapy. Curr Opin Rheumatol. 2018;30(2):222–8.
52. Griffin ME, Espinosa J, Becker JL, Luo JD, Carroll TS, Jha JK, et al. Enterococcus peptidoglycan remodeling promotes checkpoint inhibitor cancer immunotherapy. Science. 2021;373(6558):1040–6.
53. Ning W, Cheng D, Howe PH, Bian C, Kamen DL, Luo Z, et al. Staphylococcus aureus peptidoglycan (PGN) induces pathogenic autoantibody production via autoreactive B cell receptor clonal selection, implications in systemic lupus erythematosus. J Autoimmun. 2022;131:102860.
54. Yin J, Sternes PR, Wang M, Song J, Morrison M, Li T, et al. Shotgun metagenomics reveals an enrichment of potentially cross-reactive bacterial epitopes in ankylosing spondylitis patients, as well as the effects of TNFi therapy upon microbiome composition. Ann Rheum Dis. 2020;79(1):132–40.
55. Syu WJ. Kahan L.Epitope characterization by modifications of antigens and by mapping on resin-bound peptides. Discriminating epitopes near the C-terminus and N-terminus of Escherichia coli ribosomal protein S13. J Immunol Methods. 1989;118(2):153–60.
56. Williams KM, Bigley EC 3rd, Raybourne RB. Identification of murine B-cell and T-cell epitopes of Escherichia coli outer membrane protein F with synthetic polypeptides. Infect Immun. 2000;68(5):2535–45.
57. Sanchez B, Hevia A, Gonzalez S, Margolles A. Interaction of Intestinal microorganisms with the Human Host in the Framework of Autoimmune diseases. Front Immunol. 2015;6:594.

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