

Supporting Information

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Gut Microbiome Analysis Can Be Used as a Noninvasive Diagnostic Tool and Plays an Essential Role in the Onset of Membranous Nephropathy

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Table S1. The baseline characteristics of 115 UMN and 115 age-and-gender matched HCs. HC, healthy control; UMN, untreated membranous nephropathy; Scr, serum creatinine; ALB, albumin; CHO, cholesterol; TG, Triglyceride; PLA2R, phospholipase A2 receptor, PLA2R; THSD7A, thrombospondin type-1 domain-containing 7A.

	HC n=115	UMN n=115	P value
Gender			1.000
male	60 (52.2%)	61 (53.0%)	
female	55 (47.8%)	54 (47.0%)	
Age(years)	48.0 [40.5;55.0]	51.0 [41.5;56.0]	0.356
Urine protein (g)	0.08 [0.06;0.09]	4.80 [3.17;7.86]	<0.001
Scr (μmol/L)	67.0 [59.0;78.0]	69.0 [56.0;84.0]	0.466
ALB (g/L)	48.0 [46.0;49.4]	25.2 [20.4;30.6]	<0.001
CHO (mmol/L)	4.78 [4.16;5.23]	6.01 [4.93;7.62]	<0.001
TG (mmol/L)	1.16 [0.90;1.48]	1.81 [1.25;2.83]	<0.001
		N=111	
PLAR (RU/mL)			
<14		37 (33.3%)	
>=14		74 (66.7%)	
THSD7A:			
<100		103 (92.8%)	
>=100		8 (7.2%)	

Table S2. The baseline characteristics of discovery cohort (UMN_discovery=72, HC_discovery=72).

	HC_discovery n=72	UMN_discovery n=72	P value
Gender			0.182
male	33 (45.8%)	41 (56.9%)	
female	39 (54.2%)	31 (43.1%)	
Age(years)	48.99±8.98	48.74±11.72	0.739
Urine protein (g)	0.08 (0.06, 0.10)	4.84 (3.37, 8.03)	<0.01
Scr (μmol/L)	65.0 (59.0, 76.8)	68.5 (56.3, 84.0)	<0.01
ALB (g/L)	48.0 (45.5, 49.2)	25.6±6.8	0.309
CHO (mmol/L)	4.69±0.73	6.34±1.84	<0.01
TG (mmol/L)	1.08 (0.89, 1.45)	1.95 (1.32, 3.10)	<0.01
		N=69	
PLAR (RU/mL)			
<14		24 (34.8%)	
>=14		45 (65.2%)	
THSD7A:			
<100		63 (91.3%)	
>=100		6 (8.7%)	

Table S3. The baseline characteristics of validation cohort (UMN_validation=43, HC_discovery=43).

	HC_validation n=43	UMN_validition n=43	P value
Gender			0.129
male	27 (62.8)	20 (46.5%)	

female	16 (37.2)	23 (53.5%)	
Age(years)	48.00 (38.00, 55.00)	49.51±11.83	0.36
Urine protein (g)	0.08 (0.06, 0.09)	5.18±3.21	<0.01
Scr (μmol/L)	69.42±11.85	72.35±22.30	0.935
ALB (g/L)	48.1±9.06	25.5±7.11	<0.01
CHO (mmol/L)	4.71±0.66	5.91 (4.91, 7.68)	<0.01
TG (mmol/L)	1.23±0.39	1.63 (1.16, 2.28)	<0.01
		N=42	
PLAR (RU/mL)			
<14		13 (31.0%)	
>=14		29 (69.0%)	
THSD7A:			
<100		40 (95.0%)	
>=100		2 (5.0%)	

Table S4. Clinical data of 78 UMN, 108 TMN and 100 HCs. TMN, treated membranous nephropathy.

	HC N=100	TMN N=108	UMN N=78	p.overall	p.UMN vs HC	p.UMN vs TMN	p.HC vs TMN
Gender:				0.688	0.799	0.799	0.799
female	41 (41.0%)	48 (44.4%)	37 (47.4%)				
male	59 (59.0%)	60 (55.6%)	41 (52.6%)				

Age(years)	48.0 [42.8;53.0]	50.0 [40.0;55.0]	49.0 [40.2;55.8]	0.966	0.988	0.988	0.988
Urine protein (g)	0.08 [0.06;0.10]	3.72 [1.87;6.24]	4.35 [3.07;6.74]	<0.001	<0.001	0.053	<0.001
Scr (μmol/L)	69.5 [60.8;77.0]	72.5 [61.8;82.0]	68.0 [56.0;85.0]	0.263	0.266	0.611	0.646
ALB (g/L)	47.9 [46.0;49.0]	27.8 [21.0;32.6]	26.7 [23.2;31.6]	<0.001	<0.001	0.809	<0.001
CHO (mmol/L)	4.59 [4.12;5.04]	5.58 [4.66;6.89]	5.92 [4.83;7.50]	<0.001	<0.001	0.344	<0.001
TG (mmol/L)	1.15 [0.89;1.46]	1.89 [1.35;2.80]	1.74 [1.23;2.81]	<0.001	<0.001	0.447	<0.001
PLA (RU/mL)		10.8 [2.00;44.3]	19.1 [3.05;65.9]	0.089			
TSH7A		30.6 [20.8;41.1]	25.1 [16.8;42.5]	0.135			

Table S5. Comparison of clinical data in HPROs and LPROs. HPRO, high urine protein; LPRO, low urine protein.

	HPro N=84	LPro N=171	p.HPro vs LPro
Gender:			0.819
female	28 (33.3%)	61 (35.7%)	
male	56 (66.7%)	110 (64.3%)	
Age(years)	48.5 [36.0;54.2]	47.0 [37.0;55.0]	0.864
Urine protein (g)	6.02 [4.67;7.56]	1.04 [0.58;1.98]	<0.001
Scr (μmol/L)	76.0 [66.1;100]	74.0 [61.0;87.0]	0.122
ALB (g/L)	22.2 [19.0;27.6]	33.9 [29.4;37.1]	<0.001
CHO (mmol/L)	6.25 [5.08;7.14]	4.57 [3.80;5.49]	<0.001

TG (mmol/L)	1.93 [1.45;3.09]	1.58 [1.12;2.25]	<0.001
PLA (RU/mL)	15.8 [2.60;63.1]	2.70 [2.00;12.2]	
TSH7A	30.5 [20.0;49.4]	28.7 [20.0;42.8]	

Table S6: Clinical data of discovery phase in HPROs and LPROs.

	HPro N=56	LPro N=114	p-value
Gender:			0.404
female	17 (30.4%)	42 (36.2%)	
male	39 (69.6%)	72 (63.8%)	
Age(years)	45.64±13.14	48.5 (35.25, 54)	0.585
Urine protein (g)	6.02 (4.63, 7.21)	1.01 (0.58, 1.96)	<0.001
Scr (μmol/L)	76.0 (67.5, 102.8)	71.5 (60.0, 89.0)	0.118
ALB (g/L)	22.1 (18.7, 28.3)	33.1±6.3	<0.001
CHO (mmol/L)	6.33 (5.06, 8.12)	4.63 (3.82, 5.56)	<0.001
TG (mmol/L)	1.79 (1.43, 2.76)	1.69 (1.18, 2.32)	0.053
PLA (RU/mL)	20.8 (2.8, 61.0)	2.8 (2.0, 12.5)	
TSH7A	31.2 (20.1, 53.2)	28.7 (20.6, 42.9)	

Table S7: Clinical data of validation phase in HPROs and LPROs.

	HPro N=28	LPro N=57	p-value
Gender:			0.291
female	11 (28.2%)	19 (25%)	

male	28 (71.8)	57 (75%)	
Age(years)	47.32±13.13	46.28±12.80	0.64
Urine protein (g)	6.01 (4.78, 7.87)	1.15 (0.56, 2.12)	<0.001
Scr (μmol/L)	78.0 (65.5, 102.8)	76.23±17.16	0.575
ALB (g/L)	22.5 (19.3, 27.5)	32.8±6.00	<0.001
CHO (mmol/L)	6.27±2.11	4.45 (3.75, 5.39)	0.001
TG (mmol/L)	2.22 (1.48, 3.79)	1.40 (1.01, 1.98)	0.001
PLA (RU/mL)	11.7 (2.0 80.0)	2.1 (2.0, 12.3)	
TSH7A	29.7 (19.6, 41.8)	28.7 (20.0, 42.8)	

Fig S1

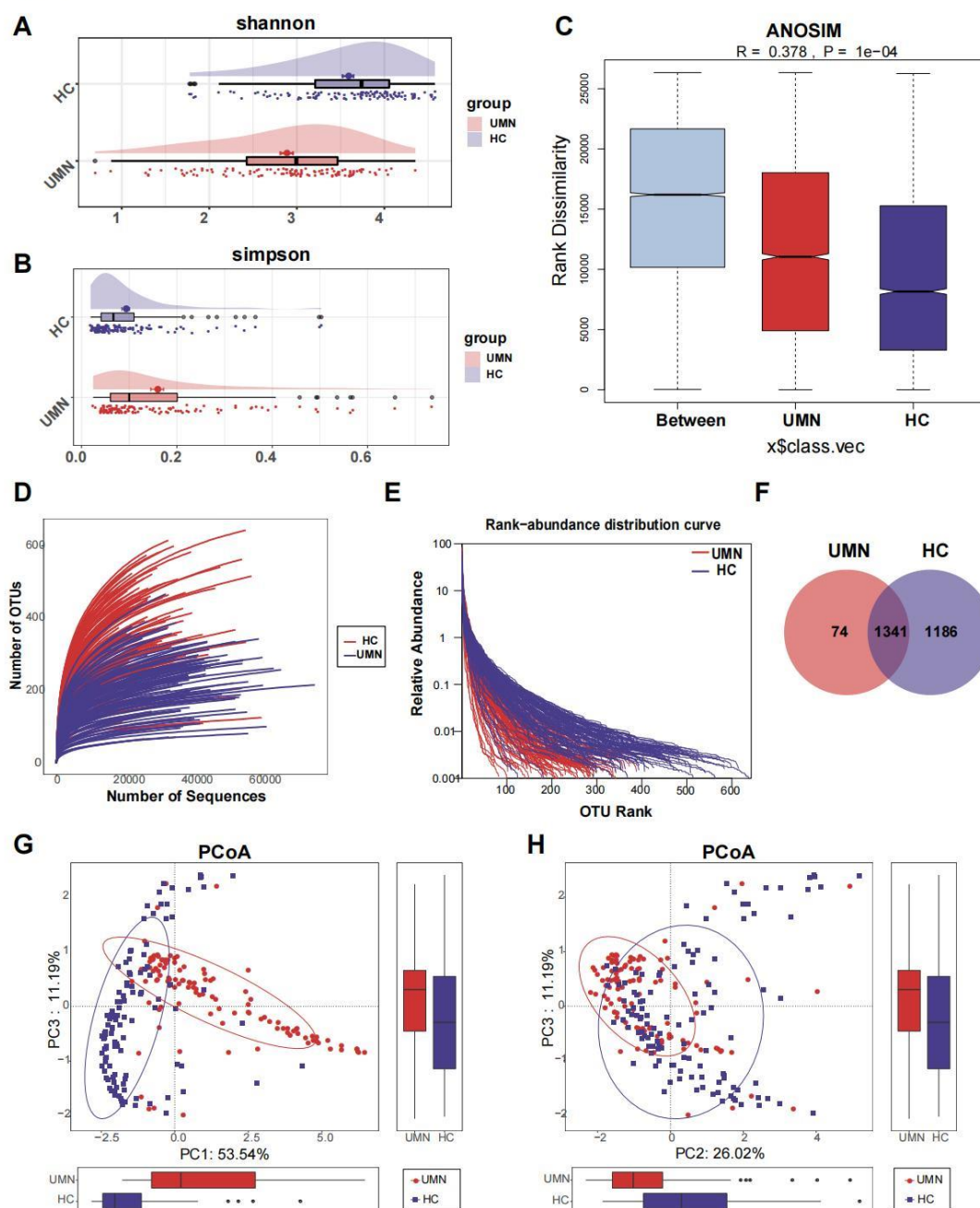


Figure S1. The supplement to diversity of microbiota between UMNs (n=115) and HCs (n=115). (A) Cloudplot showed α -diversity of Shannon index in UMNs and HCs ($p < 0.001$). (B) Cloudplot showed α -diversity of Simpson index in UMNs and HCs ($p < 0.001$). (C) ANOSIM showed the difference between groups was greater than that within groups in UMN group and HC group ($p < 0.0001$). (D) Rarefaction curves showed HCs had more observed OTUs than UMNs in general. (E) The abundance of OTU rank was generally higher in HCs than in UMNs. (F) Venn diagram showed

OTU distribution in UMN and HCs. (G) PCoA analysis showed visualized β -diversity by unweighted UniFrac algorithm along PC1 and PC3. (H) PCoA analysis showed visualized β -diversity by unweighted UniFrac algorithm along PC2 and PC3. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$.

Fig S2

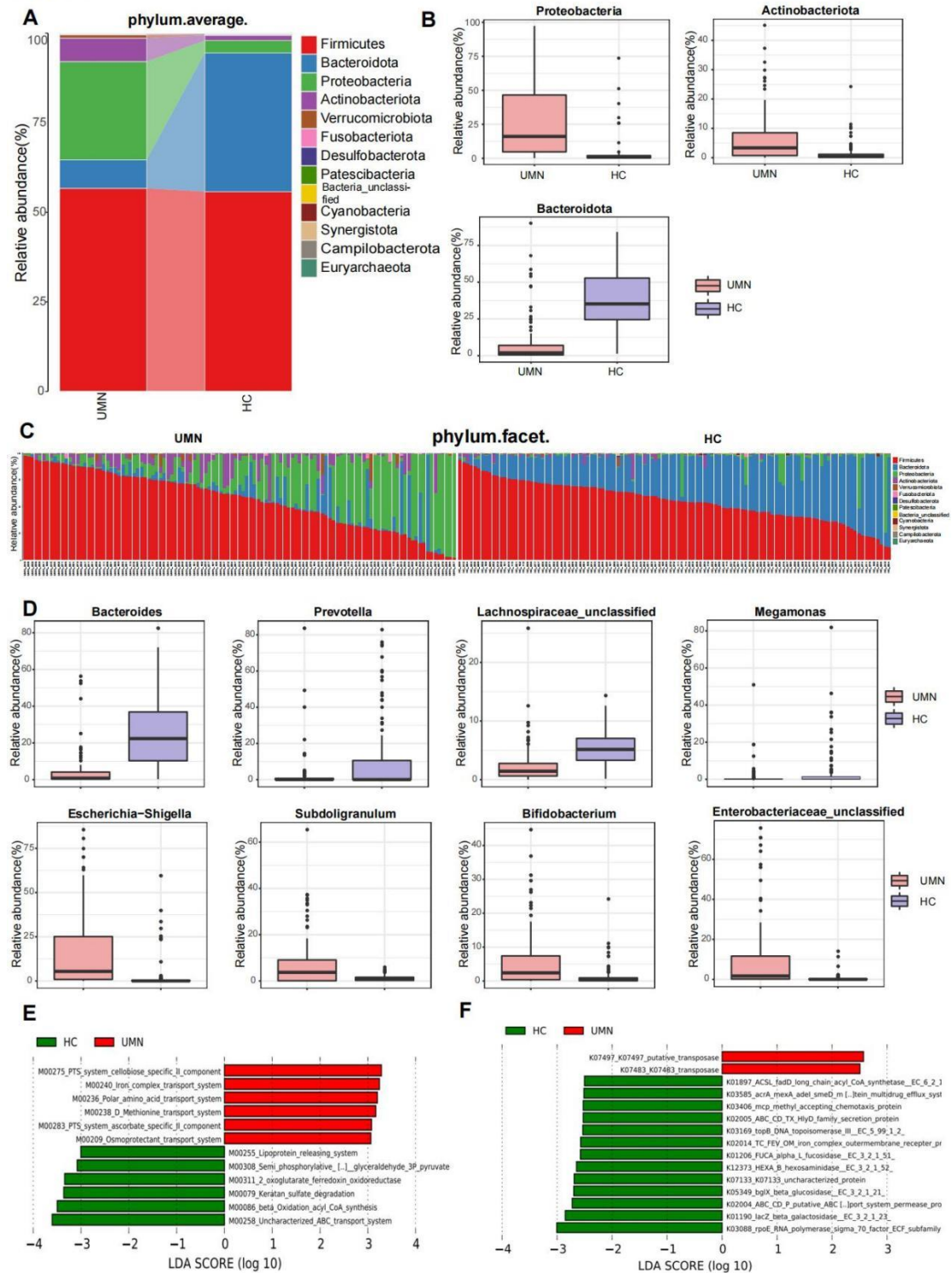


Figure S2. The supplement to characteristics and functional alteration of microbiota between UMN (n=115) and HC (n=115). (A) Average compositions and relative abundances of the bacterial communities in UMN and HC at phylum level. (B)

Barplot showed the difference in relative abundance of *Proteobacteria*, *Actinobacteria*, and *Bacteroidota* at phylum level. (C) Barplot showed relative abundance of gut microbiota of each sample in two groups at phylum level. (D) Eight genera with high abundance and obvious changes in two groups. (E) Predicted metabolic functions with LDA score >3.0 were obtained by PICRUSt on KEGG. (F) Predicted metabolic pathways with LDA score >3.0 were obtained by PICRUSt on KO. PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; KEGG, Kyoto Encyclopedia of Genes and Genomes; KO, KEGG orthologous groups; LDA: Linear Discriminant Analysis.

Fig S3

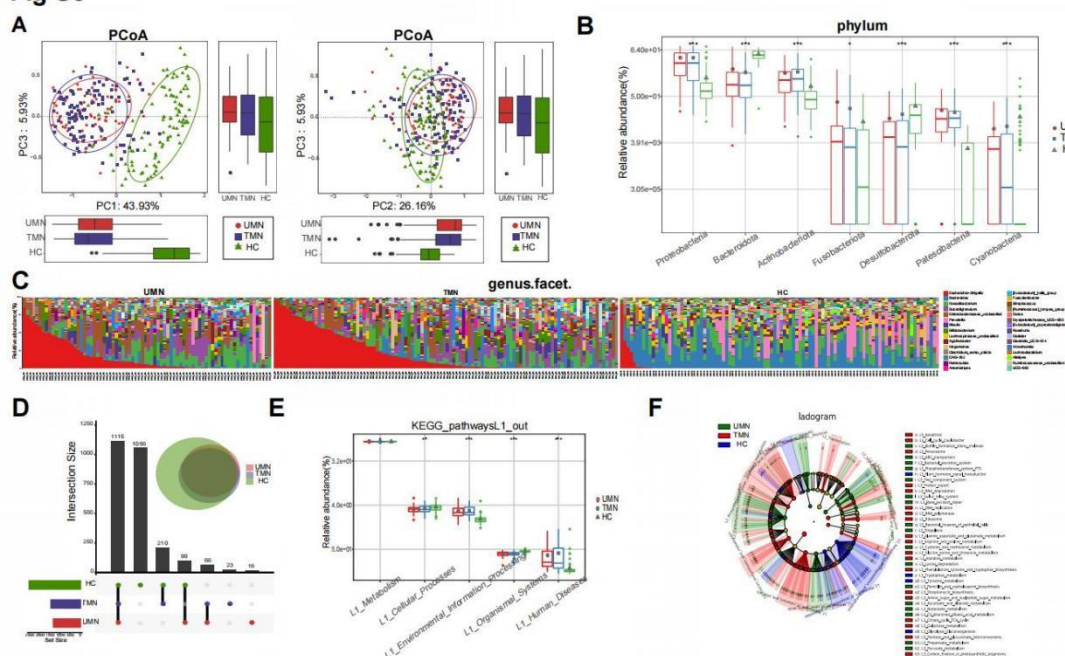


Figure S3. The supplement to characteristics and function alteration of microbiota among UMN (n=78), TMN (n=108) and HCs (n=100). (A) PCoA analysis showed visualized β -diversity by unweighted UniFrac algorithm along PC1 vs. PC3, PC2 vs. PC3. (B) Barplot showed the difference in relative abundance at phylum level by Wilcoxon Rank test among three groups. (C) Barplot showed average compositions and relative abundances of the bacterial communities from three different groups at genus level (Only the 30 genera with the highest relative abundance were listed). (D) Venn diagram showed OTU distribution in three groups. (E) Comparison of predicted metabolic functions obtained by PICRUSt on KEGG at level 1. (F)

Predicted metabolic pathways with LDA score >3.0 were obtained by PICRUSt on KO at level 2 and level 3. * p<0.05; **p<0.01, ***p<0.001.

Fig S4

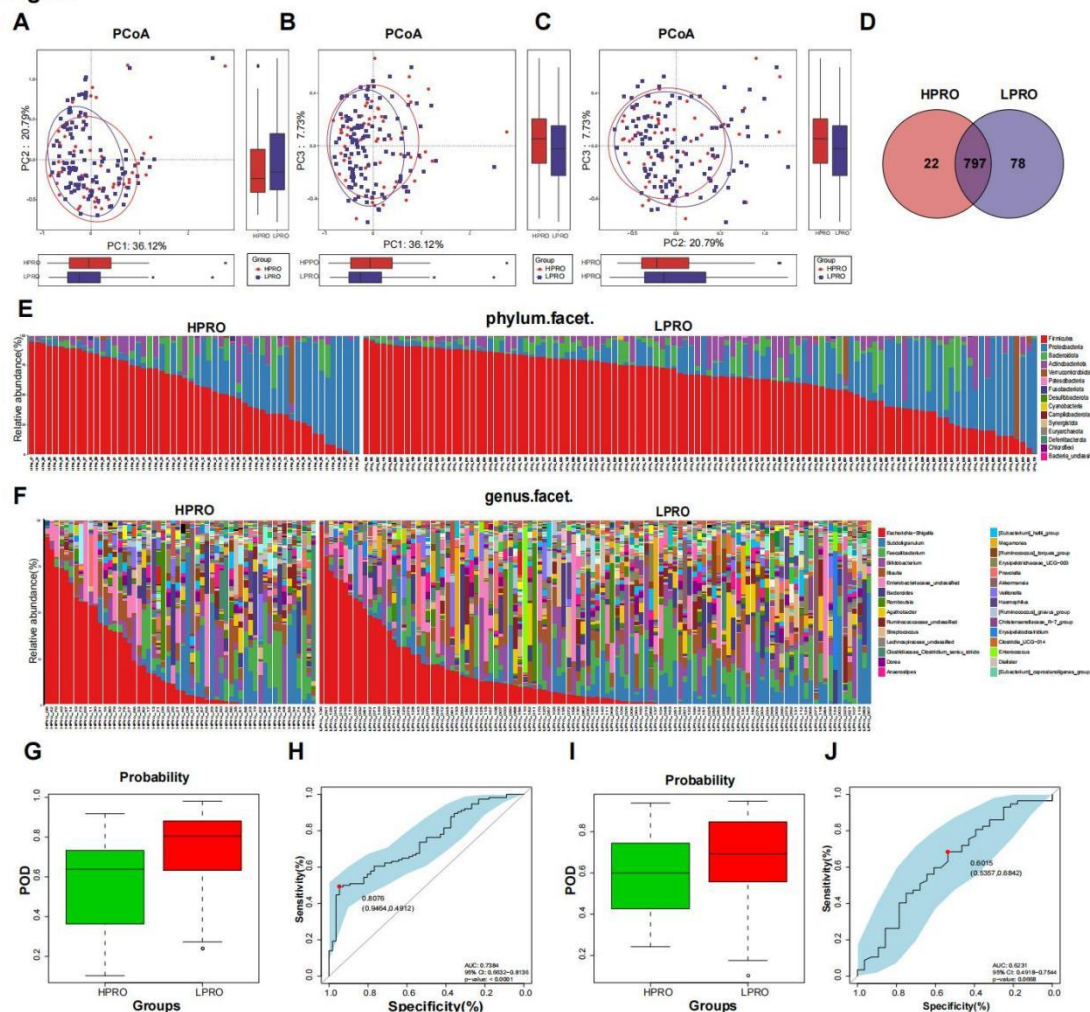


Figure S4. The supplement to characteristics and functional alteration of microbiota between HPROs (n=56) and LPROs (n=114). (A) PCoA analysis showed visualized β -diversity by unweighted UniFrac algorithm along PC1 and PC2. (B) PCoA analysis showed visualized β -diversity by unweighted UniFrac algorithm along PC1 and PC3. (C) PCoA analysis showed visualized β -diversity by unweighted UniFrac algorithm along PC2 and PC3. (D) Venn diagram showed OTU distribution in HPROs and LPROs groups. (E) Barplot showed relative abundance of gut microbiota from HPROs and LPROs groups at phylum level. (F) Relative abundance of gut microbiome in HPRO

and LPROs groups at genus level (Only the 30 genera with the highest relative abundance were listed). (G) New model was constructed for discrimination of HPROs and LPROs. Comparison of POD value based on 8 microbial markers in the training group of the new model. (H) ROC curve based on 8 obtained microbial markers showed discrimination rate of 73.84% in training group. (I) Comparison of POD value based on 8 microbial markers in the test group. (J) ROC curve showed discrimination rate of 62.31% in test group.

Fig S5

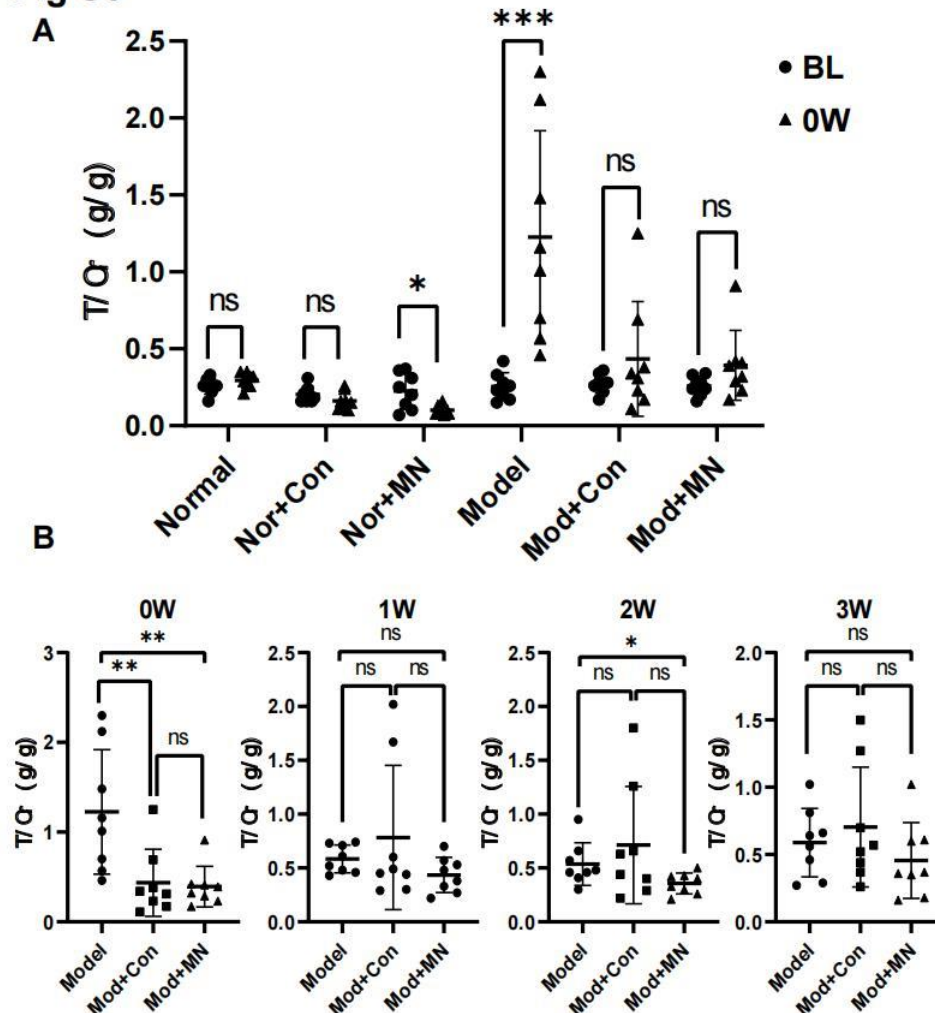


Figure S5. Detailed supplement to the statistical analysis of urinary protein in rat model. (A) Scatter plot showed differences of urinary protein before and after gut microbial cleaning in each group. (B) Scatter plot showed differences of urinary protein from week 0 to week 3 among model, Mod+Con and Mod+MN groups. BL, baseline; T/Cr, Total urinary protein/ urinary creatinine. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, no statistical difference.

Fig S6

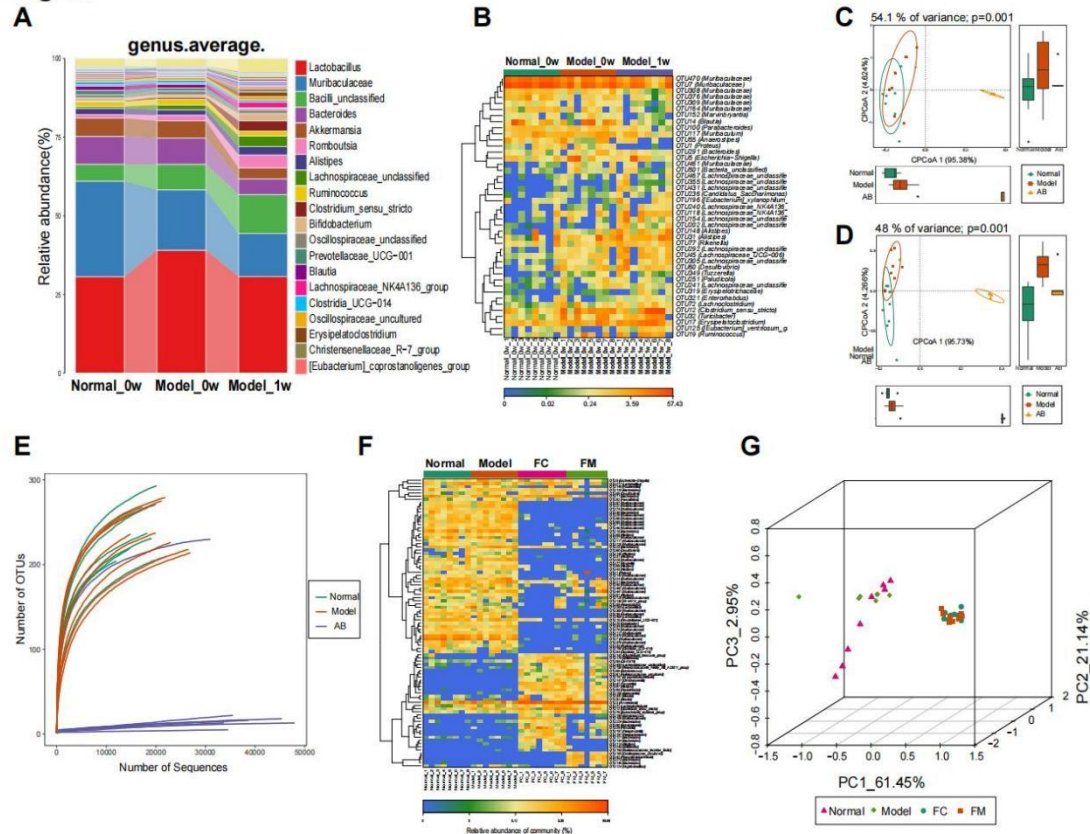


Figure S6. The supplement to characteristics of microbiota in rat model. (A) The relative abundance suggested the alteration of microbial abundance was more obvious at AM than ME at genus level (Only the 20 genera with the highest relative abundance were listed). (B) Heatmap showed the relative abundance of top 1% most

abundant OTUs selected by random forest model in three groups. (C) PCoA analysis of unweighted unifrac showed visualized microbial difference after using antibiotics along PC1 and PC2. (D) PCoA analysis of bray curtis showed visualized microbial difference after using antibiotics along PC1 and PC2. (E) Rarefaction curves showed the decrease of observed OTUs after using antibiotics, suggesting the success of intestine cleaning. (F) Heatmap showed relative abundance of top 1% most abundant OTUs in four groups. (G) Diagram of PCoA suggested different composition of gut microbiome in each group.

Fig S7

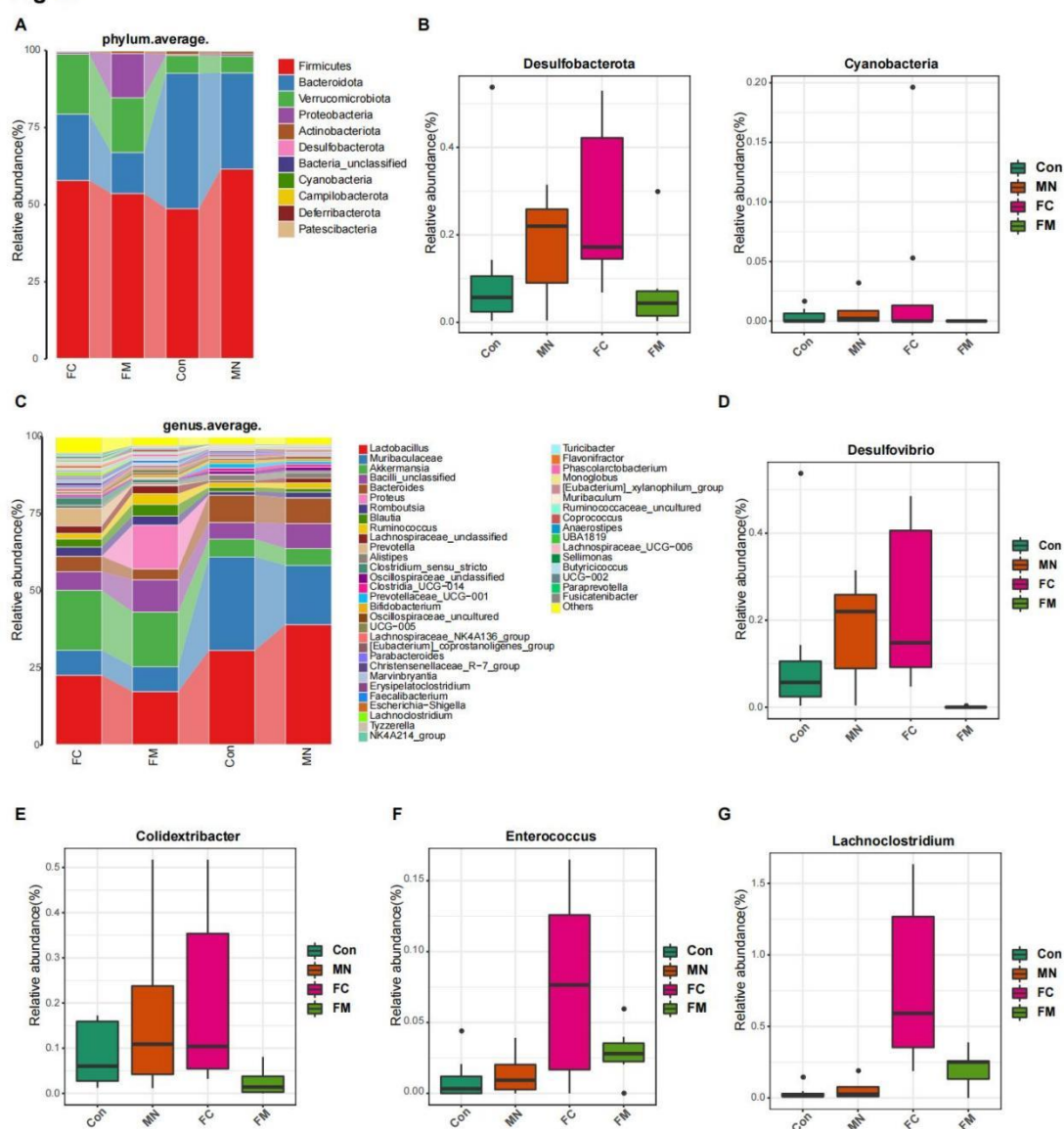


Figure S7. Gut microbiome composition in phylum and genus levels among four different groups. (A) The relative abundance of gut microbiome in phylum level. (B) Comparison of relative abundance of *Desulfobacterota* and *Cyanobacteria* in phylum level among four groups. (C) The relative abundance of gut microbiome in genus level. (D-G) Comparison of relative abundance of *Desulfovibrio* (D), *Colidextribacter* (E), *Enterococcus* (F), and *Lachnoclostridium* (G) in genus level.