ADVANCED SCIENCE
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Supporting Information

for *Adv. Sci.*, DOI: 10.1002/advs. 202001936

Alterations of the Human Gut Microbiome in Chronic Kidney Disease

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Supplementary figures legends

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Authors

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Supplementary figures

Informed consent form and information collection
(Translated from Chinese)

We are from Department of Nephrology, the First Affiliated Hospital of Zhengzhou University. We will free of charge help you monitor your gut microbial community, thereby analyzing whether gut microbiota is dysbiosis and the degree of imbalance. These results will provide auxiliary data for clinical diagnosis and treatment. Now, you just provide samples from the stool and tone coat according to our instruction. The whole process keeps free of charge. These results will be used for scientific research. Thank you for your corporation.

Number:

Diagnosis:

Patient Sign：

Date:

Patient information collection

Name	Gender	Birth date	Height(cm)	Weight (kg)	BMI	Tel
Floor ward	Admission number	Dietary habit (vegetarian diet/meat/Mixture)	Antibiotics use within 2 months	Yoghourt and probiotics	Previously critical Illness	Long-term drug use
Drinking	Alcohol type	Drinking quantity	Time of Duration	Whether or not abstinence	HBV	Etiology

Informed consent form for scientific research

(Translated from Chinese)

Dear participants,

We are from Department of Nephrology, the First Affiliated Hospital of Zhengzhou University. We will free of charge help you monitor your healthy condition and record your clinical information and healthy/disease status or disease progression process. The collected fecal and urea samples from participants in hospital will be used for scientific research. These results and data from the hospital electronic medical records will provide auxiliary data for clinical diagnosis and treatment, and will be used for scientific research. Thank you for your corporation.

Number:	Diagnosis:

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except our research team.

The knowledge that we get from doing this research will be shared with you through community meetings before it is made widely available to the public. Confidential information will not be shared. There will be small meetings in the community and these will be announced. After these meetings, we will publish the results in order that other interested people may learn from our research.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been

answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____

Day/month/year

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ AND

Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. We will free of charge help you monitor your healthy condition and record your clinical information and healthy/disease status or disease progression process.

2. These data from hospital electronic medical records will be used for scientific research.

3. The collected fecal and urea samples will be used for scientific research.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Supplementary Figure

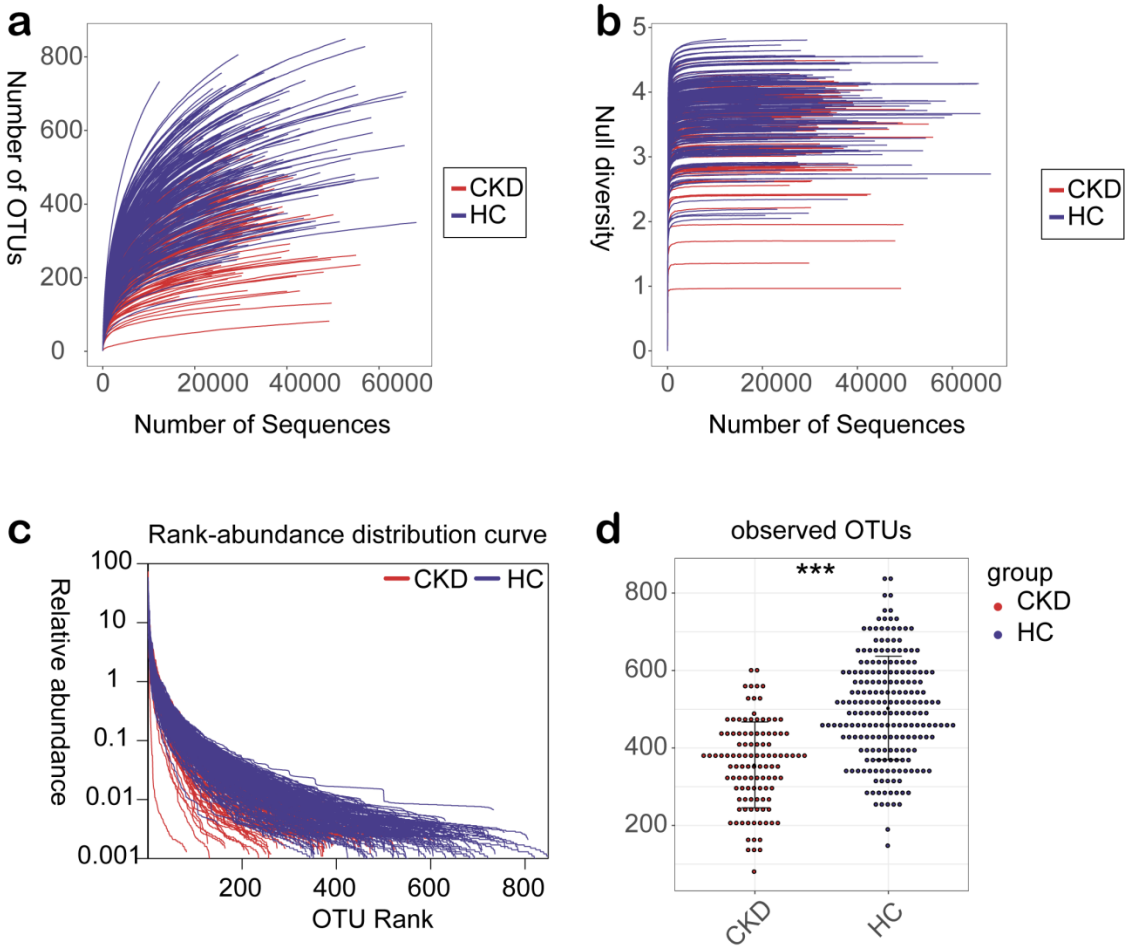


Figure S1. (a) A rarefaction curve between the number of OTUs and the number of sequences in CKD (n=110) and HC (n=210); (b) A shannon-wiener curve between the number of sequences and the null diversity in CKD (n=110) and HC (n=210); (c) A rank-abundance distribution curve for the OTUs of CKD (n=110) and HC (n=210); (d) At the discovery cohort, observed OTUs were significantly decreased in CKD (n=110) versus HC (n=210) (P<0.001). ***, P<0.001. CKD, chronic kidney disease; HC, healthy controls; OTUs, operational taxonomic units.

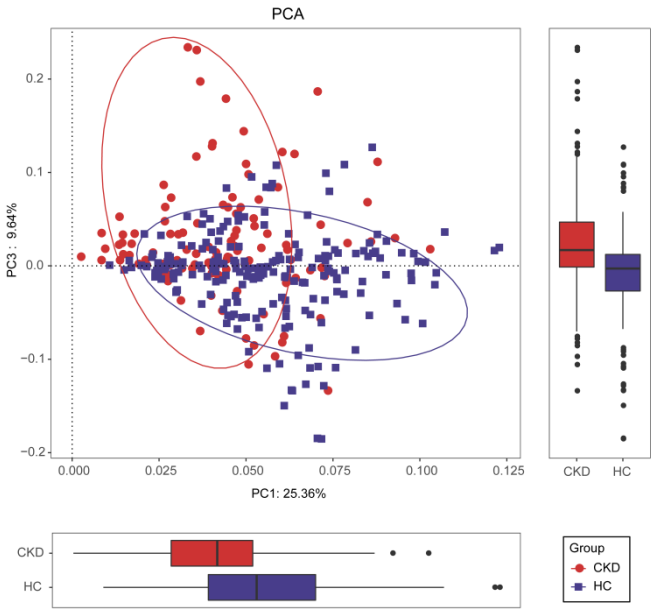


Figure S2. The PCA of gut microbial community for CKD (n=110) and HC (n=210) in the unifrac plot from PC1 and PC3 (25.36% and 9.64%). CKD, chronic kidney disease; HC, healthy controls; PCA, principal component analysis.

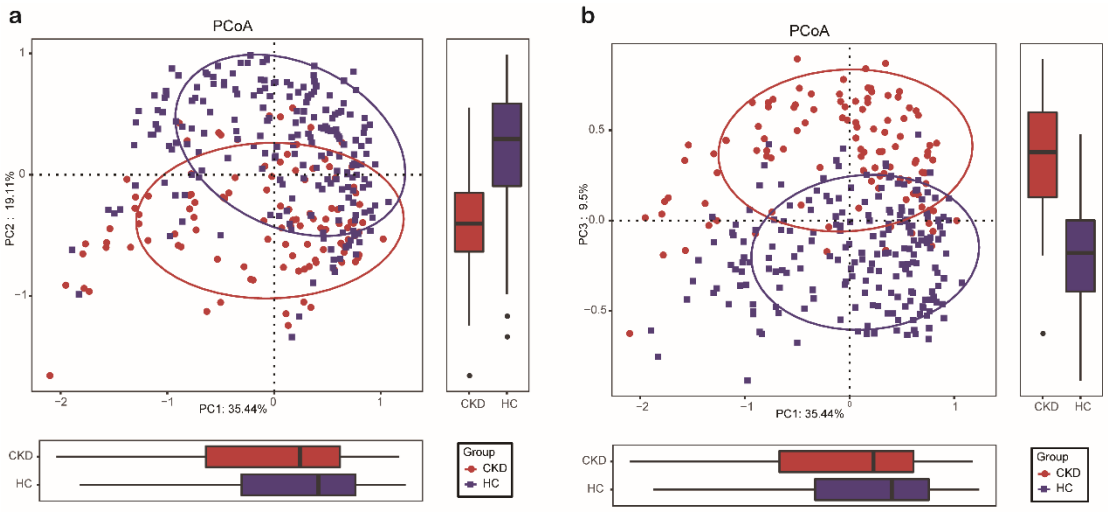


Figure S3. (a) The PCoA of gut microbial community for CKD (n=110) and HC (n=210) in the unweighted Unifrac plot from PC1 and PC2 (35.44% and 19.11%); (b) The PCoA of gut microbial community for CKD (n=110) and HC (n=210) in the unweighted Unifrac plot from PC1 and PC3 (35.44% and 9.5%). CKD, chronic kidney disease; HC, healthy controls; PCoA, principal coordinate analysis.

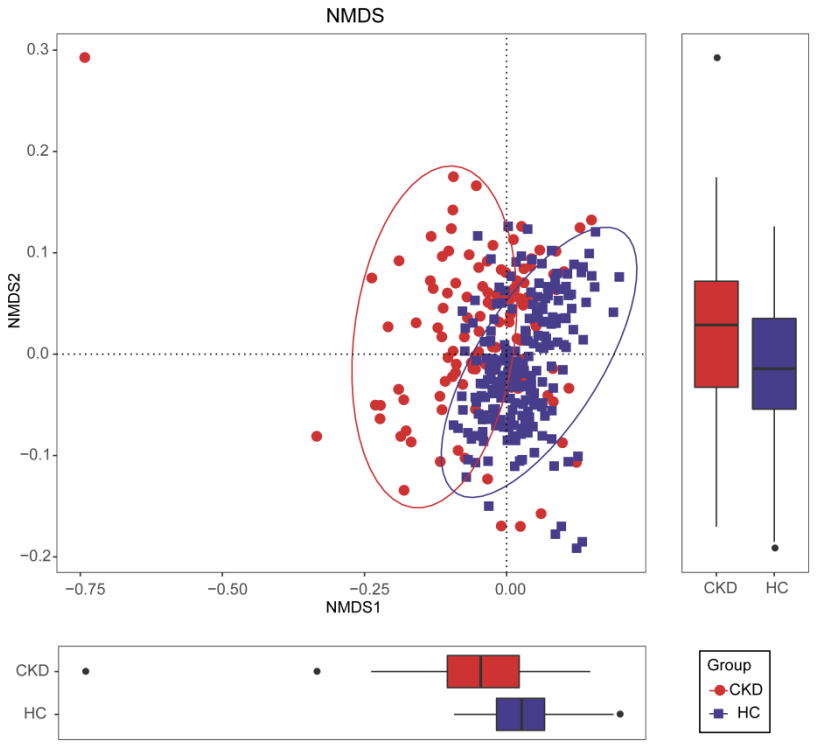


Figure S4. The NMDS analysis of gut microbial community for CKD (n=110) and HC (n=210) in the unweighted Unifrac plot from NMDS1 and NMDS2. CKD, chronic kidney disease; HC, healthy controls; NMDS, non-metric multi-dimensional scaling.

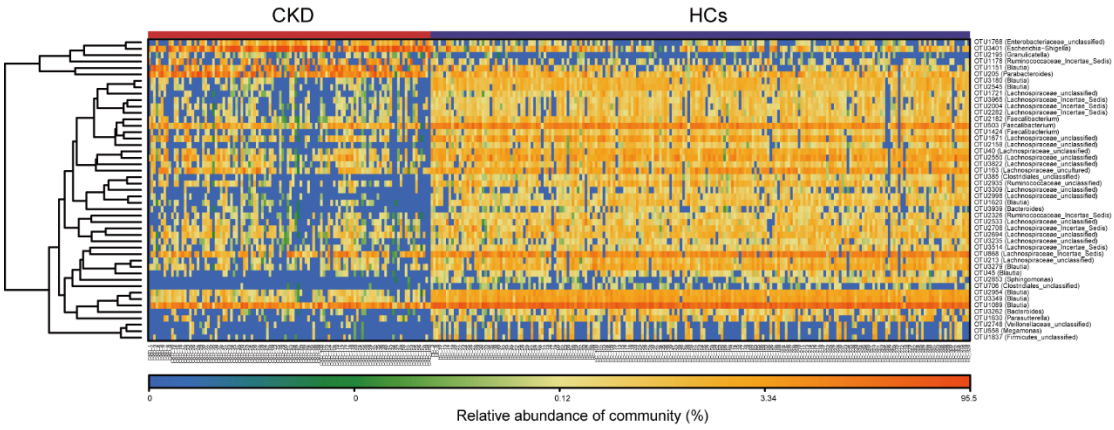


Figure S5. A heatmap showed the relative abundance and distribution of the key 47 OTUs among all samples of the discovery cohort (n=320). Only 6 OTUs were markedly enriched in CKD (n=110), while 41 OTUs were markedly enriched in HC (n=210). CKD, chronic kidney disease; HCs, healthy controls; OTUs, operational taxonomic units.

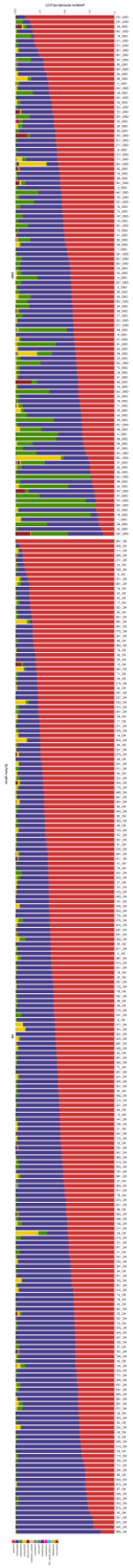


Figure S6. The composition and abundance of bacterial community at the phylum level in each sample of the discovery cohort (n=320). CKD, chronic kidney disease;

HC, healthy controls.

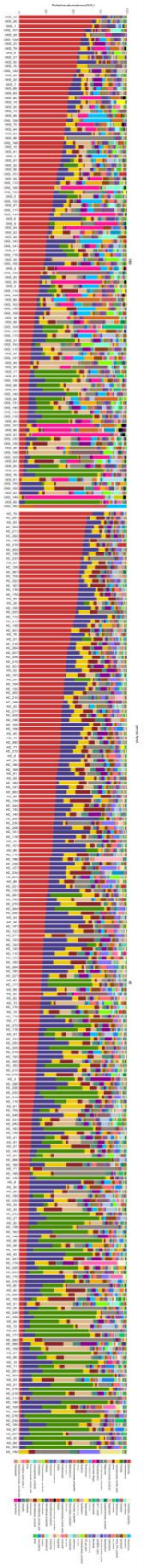


Figure S7. The composition and abundance of bacterial community at the genus level

in each sample of the discovery cohort (n=320). CKD, chronic kidney disease; HC, healthy controls.

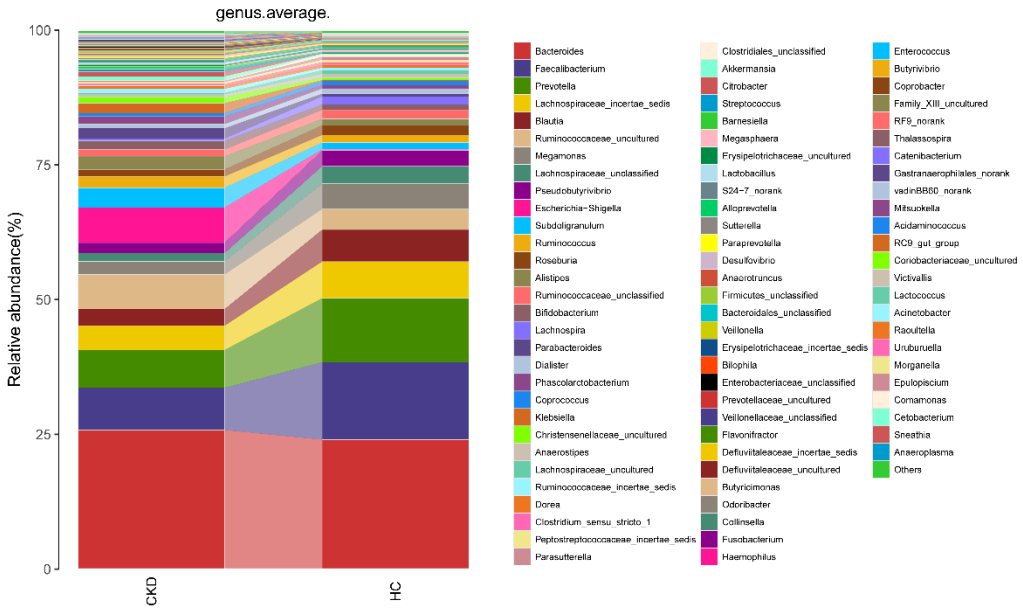


Figure S8. The average composition and relative abundance of the bacterial communities of CKD (n=110) and HC (n=210) at the genus level in the discovery cohort. CKD, chronic kidney disease; HC, healthy controls.

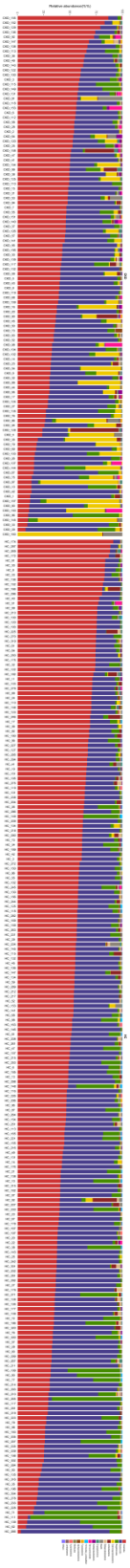


Figure S9. The composition and abundance of bacterial community at the class level in each sample of the discovery cohort (n=320). CKD, chronic kidney disease; HC, healthy controls.

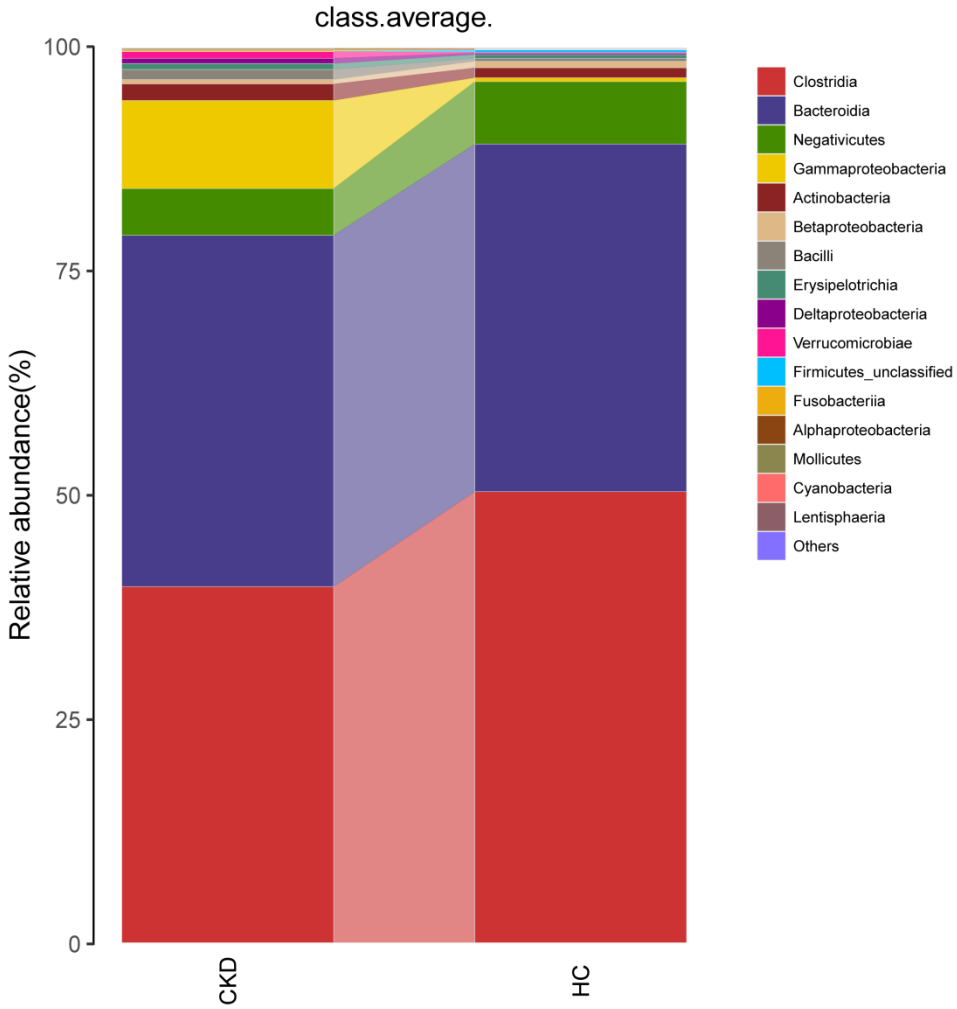


Figure S10. Average compositions and relative abundance of bacterial community in CKD (n=110) and HC (n=210) at the class level. CKD, chronic kidney disease; HC, healthy controls.

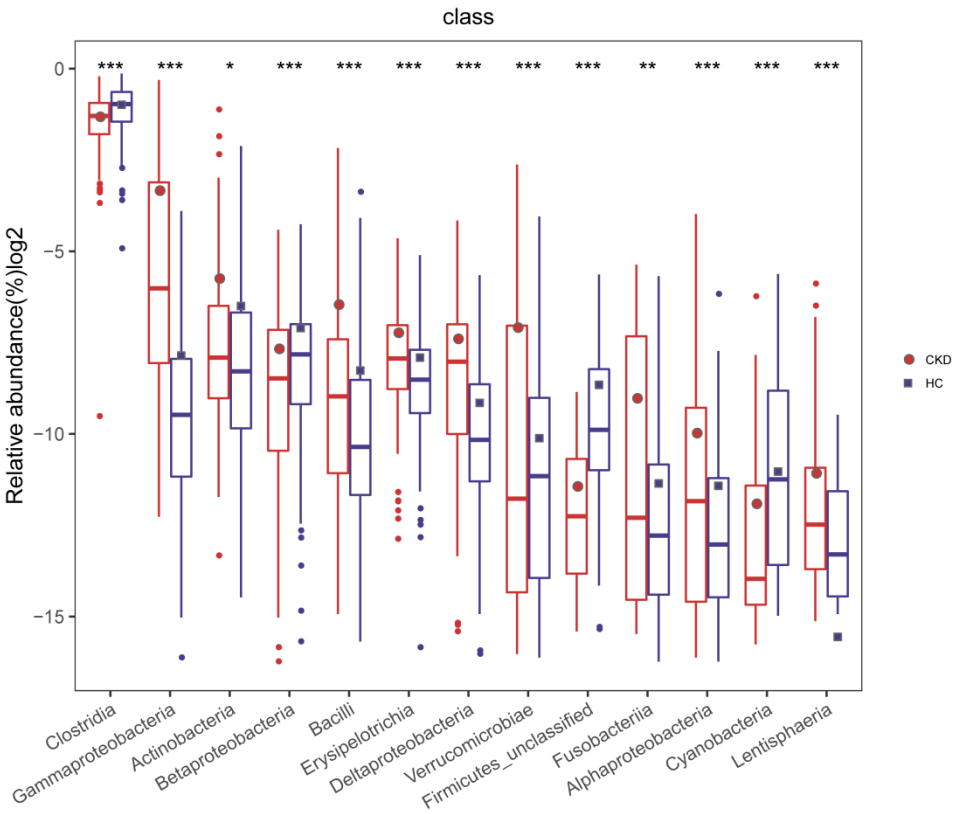


Figure S11. At the class level, 8 bacterial populations were significantly enriched, whereas 5 bacterial populations were significantly reduced in CKD (n=110) versus HC (n=210). *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$. CKD, chronic kidney disease; HC, healthy controls.

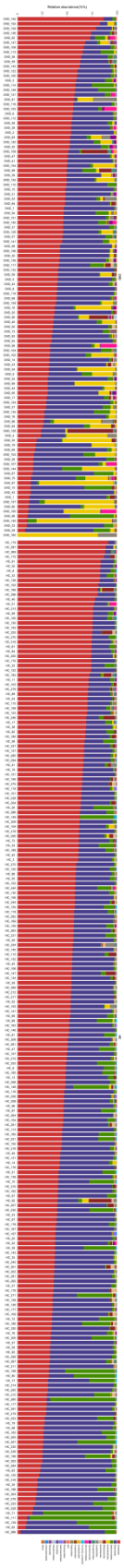


Figure S12. The composition and abundance of bacterial community at the order level in each sample of the discovery cohort (n=320). CKD, chronic kidney disease;

HC, healthy controls.

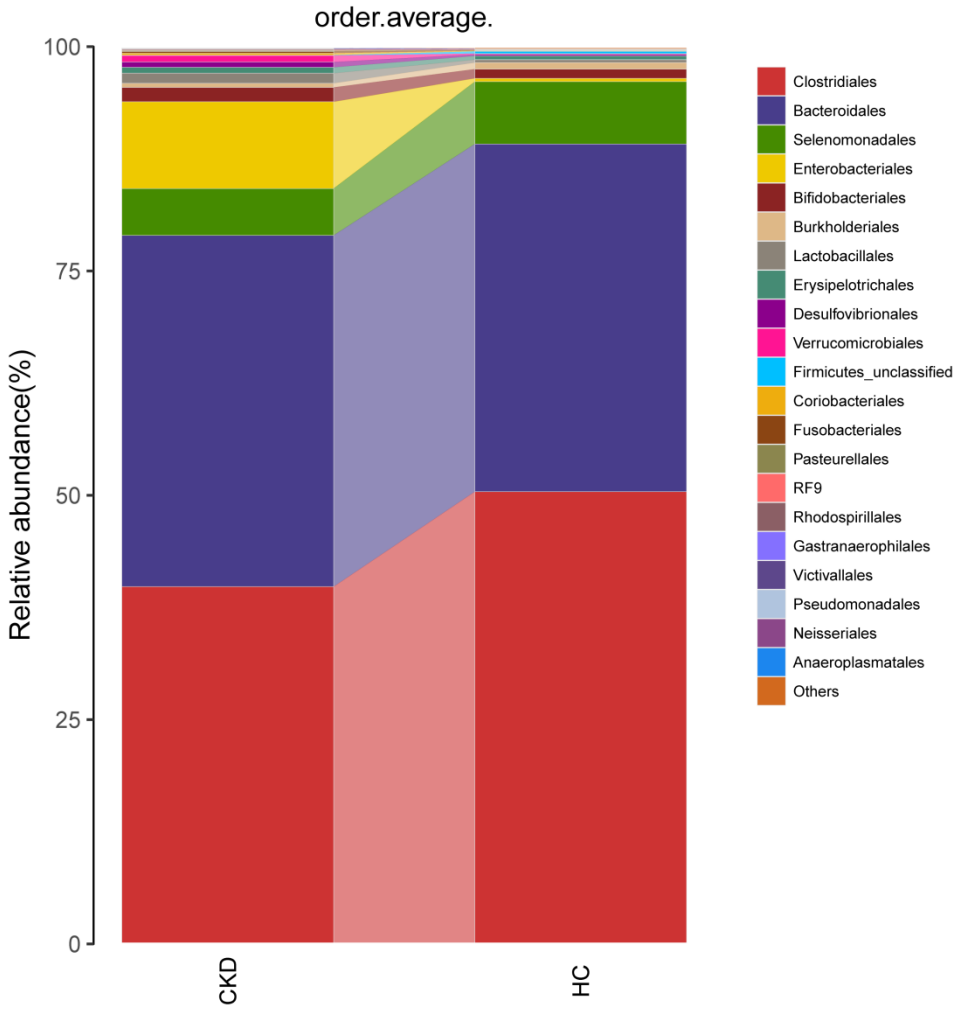


Figure S13. Average compositions and relative abundance of bacterial community in CKD (n=110) and HC (n=210) at the order level. CKD, chronic kidney disease; HC, healthy controls.

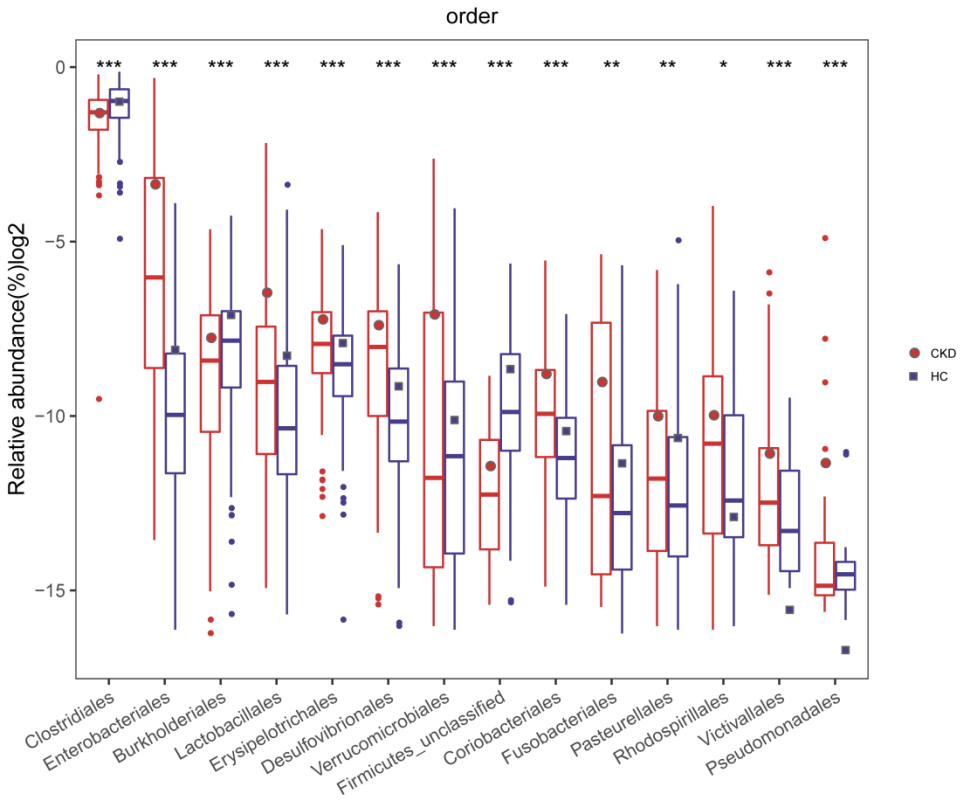


Figure S14. At the order level, 9 bacterial populations were significantly enriched, whereas 5 bacterial populations were significantly reduced in CKD (n=110) versus HC (n=210). *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$. CKD, chronic kidney disease; HC, healthy controls.

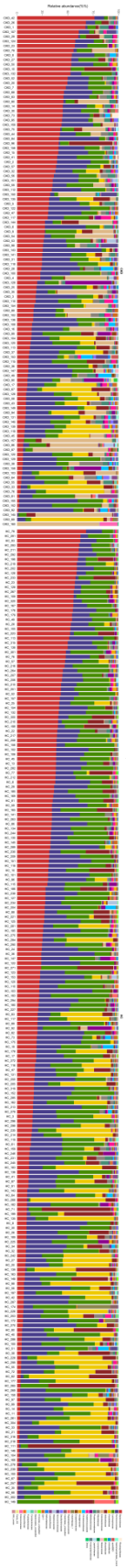


Figure S15. The composition and abundance of bacterial community at the family level in each sample of the discovery cohort (n=320). CKD, chronic kidney disease;

HC, healthy controls.

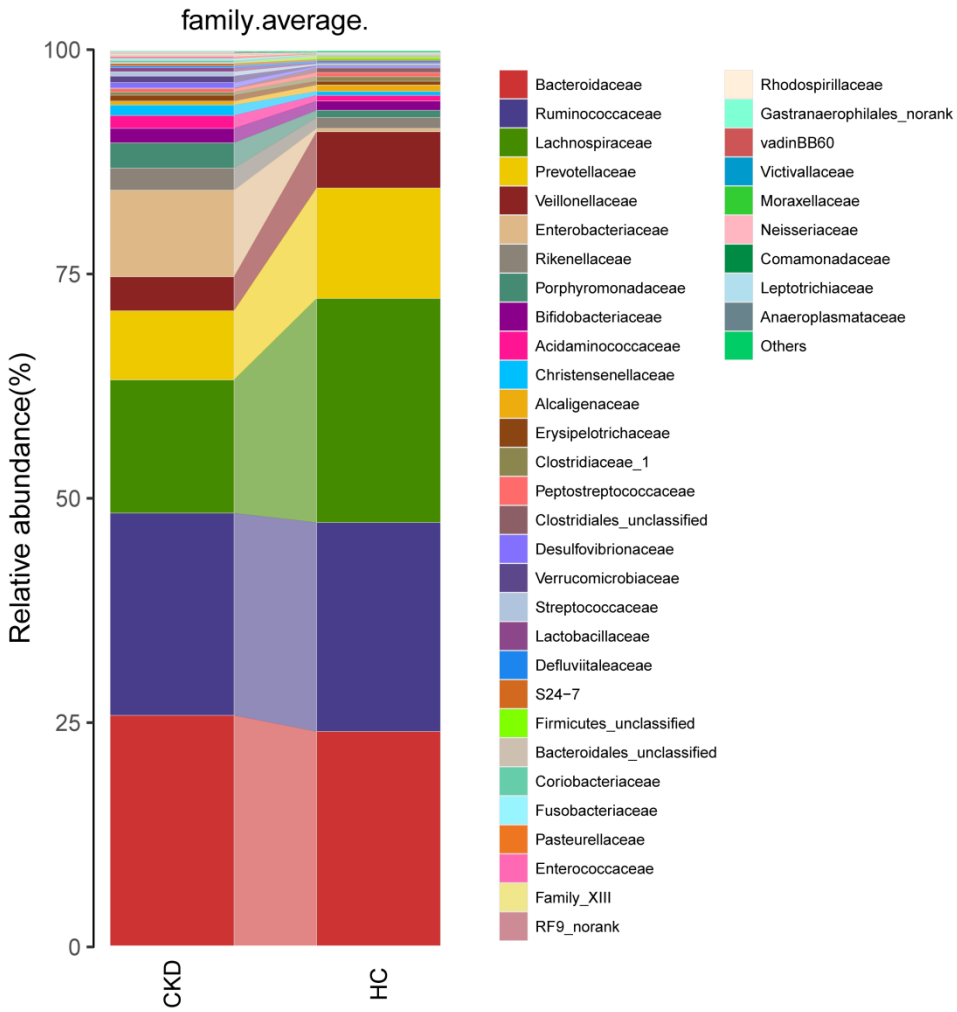


Figure S16. Average compositions and relative abundance of bacterial community in CKD (n=110) and HC (n=210) at the family level. CKD, chronic kidney disease; HC, healthy controls.

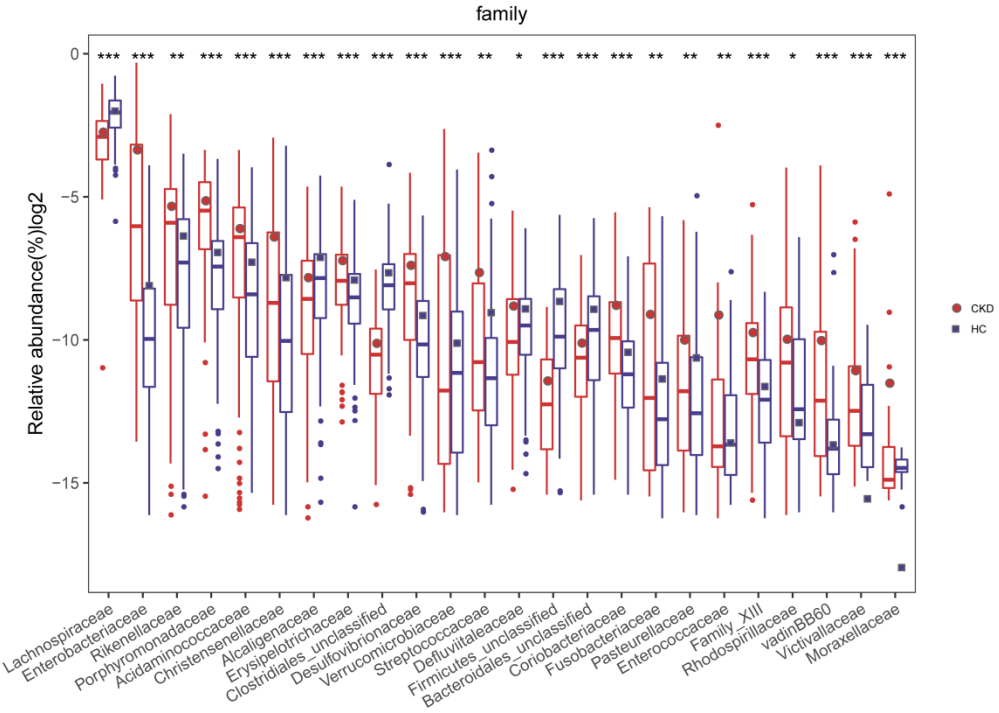
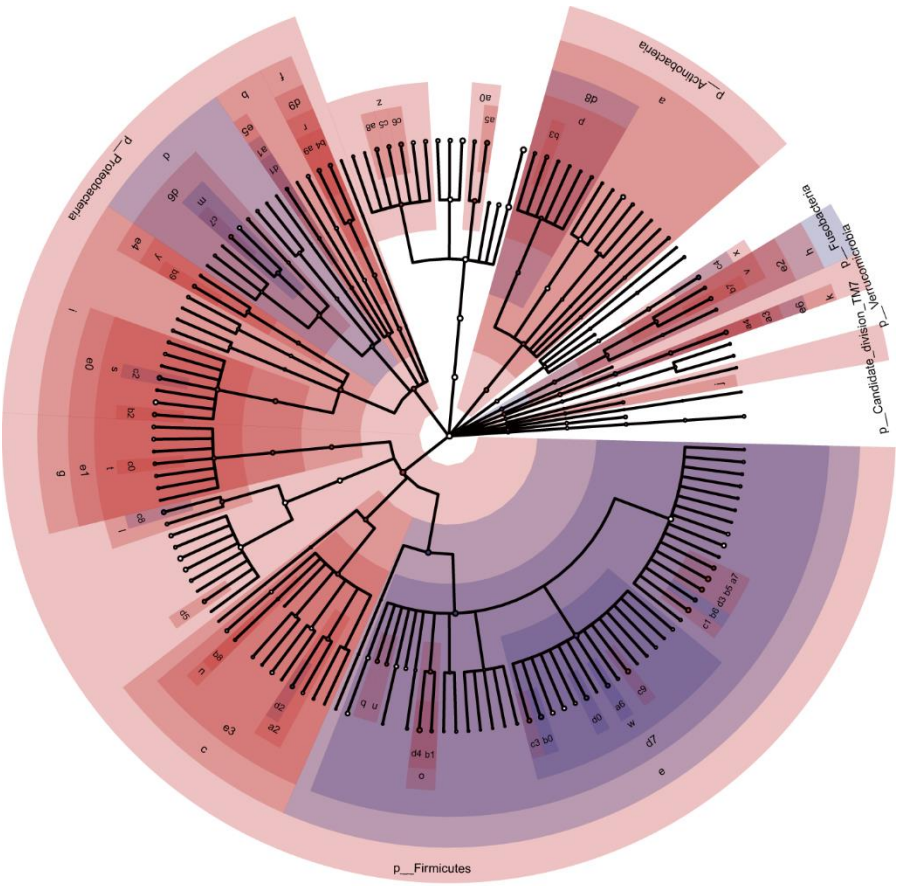


Figure S17. At the family level, 16 bacterial populations were significantly enriched, while 8 bacterial populations were significantly reduced in CKD (n=110) and HC (n=210). *, P<0.05, **, P<0.01, ***, P<0.001. CKD, chronic kidney disease; HC, healthy controls.



Annotation:

a: c__Actinobacteria
b: c__Alphaproteobacteria
c: c__Bacilli
d: c__Betaproteobacteria
e: c__Clostridia
f: c__Deltaproteobacteria
g: c__Erysipelotrichia
h: c__Fusobacteriia
i: c__Gammaproteobacteria
j: c__norank
k: c__Verrucomicrobiae
l: f__Acidaminococcaceae
m: f__Alcaligenaceae
n: f__Carnobacteriaceae
o: f__Christensenellaceae
p: f__Coriobacteriaceae
q: f__Defluviitaleaceae
r: f__Desulfovibrionaceae
s: f__Enterobacteriaceae
t: f__Erysipelotrichaceae
u: f__Family_XIII
v: f__Fusobacteriaceae
w: f__Lachnospiraceae
x: f__norank
y: f__Pasteurellaceae
z: f__Porphyromonadaceae
a0: f__Rikenellaceae
a1: f__Sphingomonadaceae
a2: f__Streptococcaceae
a3: f__Verrucomicrobiaceae
a4: g__Akkermansia
a5: g__Alistipes
a6: g__Anaerostipes
a7: g__Anaerotruncus
a8: g__Barnesiella
a9: g__Bilophila
b0: g__Blautia
b1: g__Christensenella
b2: g__Citrobacter
b3: g__Collinsella
b4: g__Desulfovibrio
b5: g__Faecalibacterium
b6: g__Flavonifractor
b7: g__Fusobacterium
b8: g__Granulicatella
b9: g__Haemophilus
c0: g__Holdemania
c1: g__Incertae_Sedis
c2: g__Klebsiella
c3: g__Lachnospira
c4: g__norank
c5: g__Odoribacter
c6: g__Parabacteroides
c7: g__Parasutterella
c8: g__Phascolarctobacterium
c9: g__Pseudobutyrivibrio
d0: g__Roseburia
d1: g__Sphingomonas
d2: g__Streptococcus
d3: g__Subdoligranulum
d4: g__uncultured
d5: g__Veillonella
d6: o__Burkholderiales
d7: o__Clostridiales
d8: o__Coriobacteriales
d9: o__Desulfovibrionales
e0: o__Enterobacteriales
e1: o__Erysipelotrichales
e2: o__Fusobacteriales
e3: o__Lactobacillales
e4: o__Pasteurellales
e5: o__Sphingomonadales
e6: o__Verrucomicrobiales

Figure S18. The phylogenetic profiles of the specific bacterial taxa and predominant bacteria associated with CKD (n=110) and HC (n=210) using the LEfSe method. CKD, chronic kidney disease; HC, healthy controls; LEfSe, linear discriminant analysis effect size.

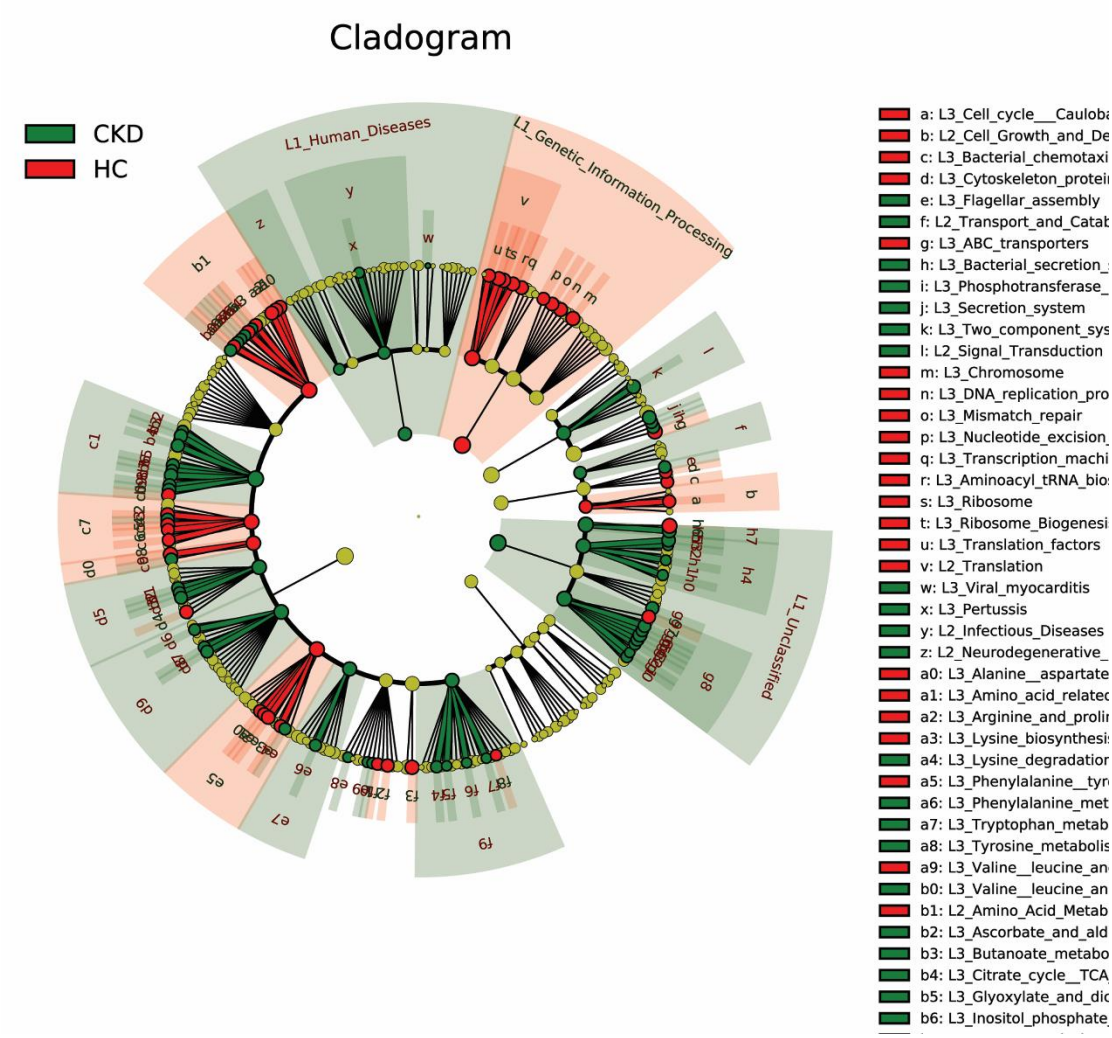


Figure S19. A cladogram showed the gut microbial community function profiles of CKD (n=110) and HC (n=210) and their predominant microbial functions. CKD, chronic kidney disease; HC, healthy controls.

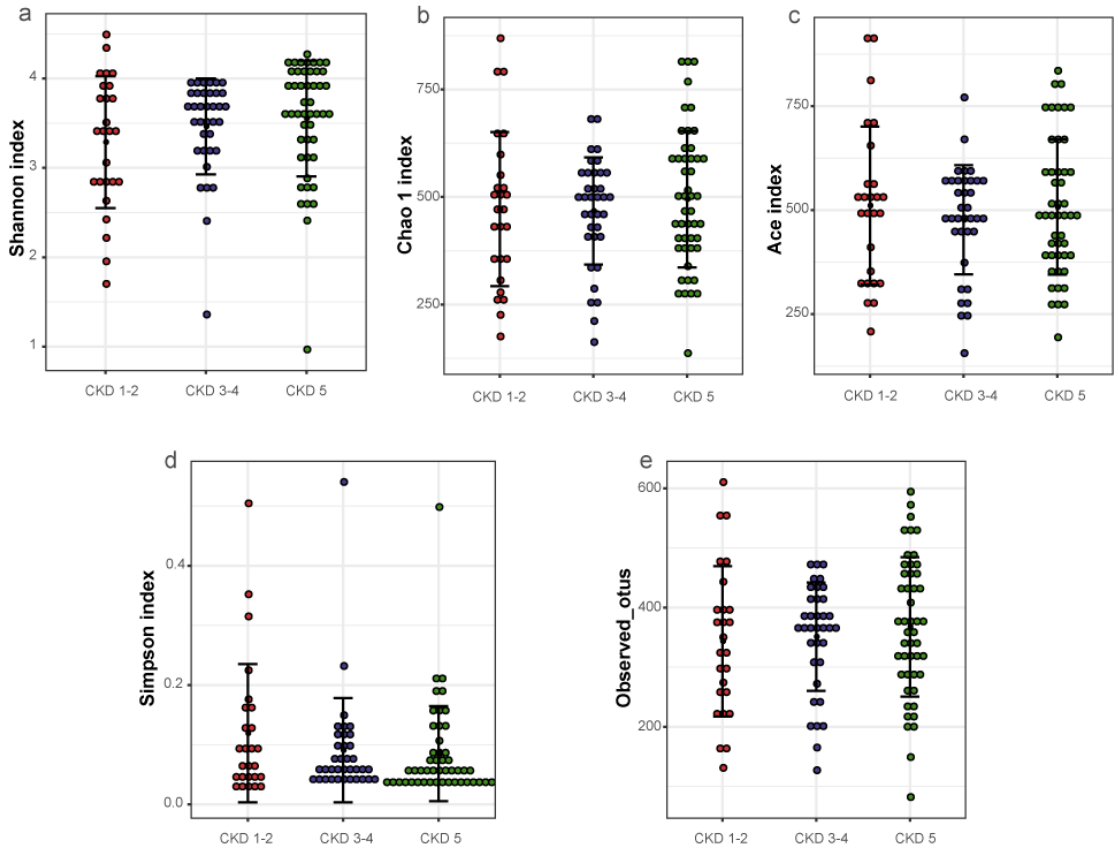


Figure S20. As estimated by the Shannon index (a), Chao 1 index (b), Ace index (c) and Simpson index (d), there was no significant difference in the gut microbial diversity of CKD stage 1-2 (n=26), CKD stage 3-4 (n=36) and CKD stage 5 (n=48) (all $P>0.05$). (e) The observed OTUs of CKD stage 1-2 (n=26), CKD stage 3-4 (n=36) and CKD stage 5 (n=48) was no significant difference ($P>0.05$). CKD, chronic kidney disease; OTUs, operational taxonomic units.

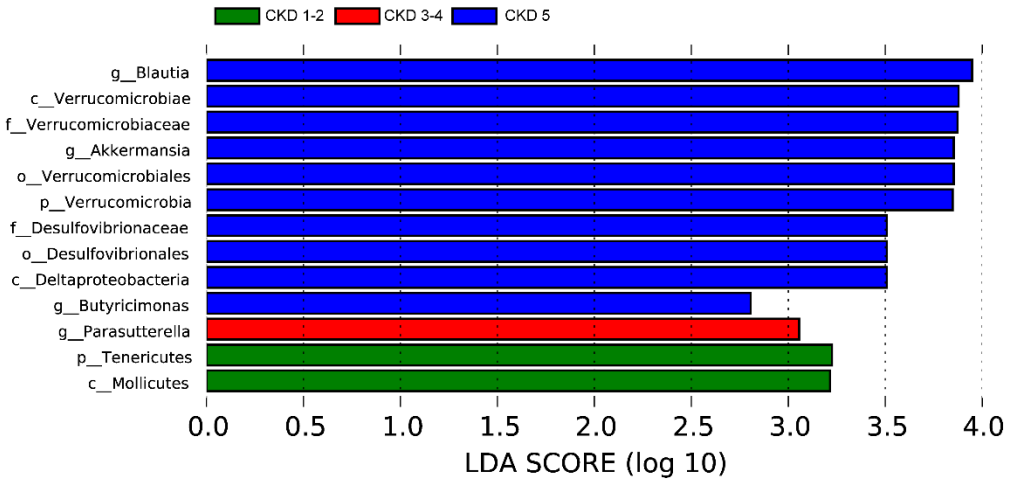


Figure S21. Based on the LDA selection, 2, 1 and 10 gut microbial taxa were enriched in CKD stage 1-2 (n=26), CKD stage 3-4 (n=36) and CKD stage 5 (n=48), respectively (all $P<0.05$). CKD, chronic kidney disease; OTUs, operational taxonomy units; LDA, linear discriminant analysis.