Supplementary materials for

Gut dysbiosis induces the development of preeclampsia through bacterial translocation

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Supplementary materials and methods

Human sample collection

Ethical approval was granted by the Ethics Committee of Nanfang Hospital (NFEC-2017-055). Fecal samples were collected from all enrolled subjects at the hospital and stored at −80°C before further processing. The exclusion criteria of the fecal sampling were as follows: (1) administration of any antibiotic or probiotic treatment one month before sample collection; (2) diseases that may affect microbiome composition such as thyroid disorders, asthma, lipid metabolic disorders, inflammatory bowel disease, irritable bowel syndrome, and celiac disease; (3) chronic hypertension, chronic kidney disease, or other obstetric conditions complicating pregnancy.

Placental samples were randomly obtained from 22 NP and 24 PE patients with severe features during caesarean section in an operating room to ensure sterility. Briefly, following standard obstetrical practice, six 1-cm^3 cuboidal sections were immediately

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obtained from each placenta: three from the maternal side of the placenta and three from the fetal side. The personnel wore facial masks and sterile gloves and used a sterile scalpel and other instruments. Each sample was rinsed in sterile phosphate-buffered saline (PBS), placed in a sterile cryovial, flash-frozen in liquid nitrogen, and stored at −80°C. One maternal-side biopsy sample and one fetal-side sample were selected randomly for mRNA and DNA isolation, respectively. We also collected several types of negative controls as follows: (1) sterile wet swabs that were opened in the sample sampling room, waved in the air and subjected to the same treatment as the placental samples, such as washing with sterile PBS, freezing in cryovials, and storage or transportation of the samples ("Swab"); (2) DNA-free water that was processed with the DNA extraction ("H_2O"); (3) PCR-grade water processed in parallel to the samples during amplification and DNA sequencing acquisition ("H_2O"). These negative controls were strictly performed side-by-side with placental samples at the same time, space, and exposure, with identical reagents, equipment, and personnel. Different sample groups were randomized and not processed separately from collection to sequencing.

Details of animal experimental protocol

C57BL/6 mice were obtained from the experimental animal center of Southern Medical University. All experimental procedures complied with the National Institutes of Health guidelines and ethics approval was obtained from the local Animal Care and Use Committee of the Southern Medical University. Fresh fecal samples were collected from the donors, resuspended in PBS at 0.125 g/mL, and centrifuged to obtain the

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supernatant. Following antibiotic treatment, the recipient mice were randomly divided into three groups and orally inoculated daily for 3 consecutive days and twice each week for 59 days with PBS and the prepared fecal contents mixture from PE or healthy donors. Microbial concentrations of preparations were determined by fluorescent *in situ* hybridization combined with flow cytometry, and mice were administered a dose of approximately 3×10^{10} cells. After overnight mating, we enrolled 10 pregnant mice in each group and other mice were excluded from the study at 45 days post-FMT. Three or four mice were housed in a standard mouse cage before pregnancy. After confirming the pregnancy, the mice were housed individually until the end of the experiment. D*uring* the experiment, two control and two PE-FMT mice delivered before the end of the experiment. The remaining mice in the control (n = 8) PE-FMT (n = 8), and NP-FMT (n = 10) groups were evaluated in subsequent experiments. The gut microbial profiles of recipient mice were analyzed by 16S RNA sequencing after 6 weeks. For BP measurements, SBP was measured via the tail cuff method using a non-invasive BP instrument (Softron Biotechnology, Beijing, China). Urine was collected by massaging the bladder for one time at 6 weeks post-FMT (prior to mating) and 17 days of gestation. Seventeen days after confirming the pregnancy, the mice were anesthetized and sacrificed. The numbers of viable and resorbed pups were counted and recorded; placentas and other tissues were harvested for further analysis.

Total bacterial genomic DNA extraction and sequencing.

Bacterial genomic DNA was extracted using a MinkaGene Stool DNA kit and MinkaGene Tissue DNA kit (Magigene, Guangdong, China) according to the

manufacturer's instructions. After extraction, the 16S rDNA V4 region was amplified by quantitative real-time PCR as described previously[1] and sequenced for fecal samples and placental samples using a HiSeq platform (Illumina, 2×250 bp paired-end) and ISeq platform (Illumina, 2 × 150 bp paired-end), respectively. For the placenta samples, decontam (v1.4.0) and SourceTracker (v1.0.1) were performed to filter the putative contaminants. Based on the bacterial prevalence, R package decontam was employed to determine the suspected contaminated OTUs. Also, SourceTracker was employed to determine the latent contaminant percentage of each OTU based on the bacterial frequency. Then the suspected contaminated OTUs were deleted and the remained OTUs were adjusted by the *Sourcetracker* percentage. The further placental analyses were performed after the decontamination procedures. The placenta samples were handled in an isolated, low-contaminant, controlled environment where surfaces and equipment treated with ultraviolet radiation to minimize and fragment environmental contaminant DNA. Moreover, personnel wore protective clothing and equipment to cover all exposed human surfaces. All placentas and paralleled negative samples were processed separately with any biological tissue sample in the same batch to specifically avoid the introduction of contaminant DNA. The specific primers for quantitative-PCR are shown in Table S5.

Histological procedures

The implantation site (uterus and placenta), kidneys, colon, and ileum were fixed and processed. Briefly, the tissues were fixed, dehydrated, infiltrated, embedded in paraffin, and sliced into 4-μm serial sections. At each implantation site, one set of sections

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containing a central maternal arterial channel was selected for staining. Hematoxylin and eosin (HE) staining was performed and analyzed by microscopy. Immunohistochemistry were performed using primary antibodies for ZO-1, ZO-2, occluding, and claudin-4 (Abcam, Cambridge, UK) as described previously.[2] Sections were examined by a qualified and blinded pathologist to evaluate the degree of pathological changes.

Isolation of lymphocytes

To prepare single-cell suspensions of small intestinal lamina propria lymphocytes (siLPLs), the intestines were gently washed to remove the fecal content and epithelial layers, and then incubated with collagenase IV to isolate lymphocytes. Lymphocytes were passed through a 40-μm mesh, and then further enriched by Percoll density-gradient centrifugation. Splenocyte single-cell suspensions were obtained using 70-μm strainers, followed by erythrocyte lysis and subsequent filtering through a 40-μm mesh.

Quantitative real-time PCR

Quantitative real-time PCR was carried out on an ABI Q5 real-time PCR system with the following cycling protocol: one cycle at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 60 s. Relative expression was calculated using the comparative threshold cycle and expressed relative to control (ΔΔCT method). The levels of 18S RNA were used for data normalization.

Supplementary figures S1-S10

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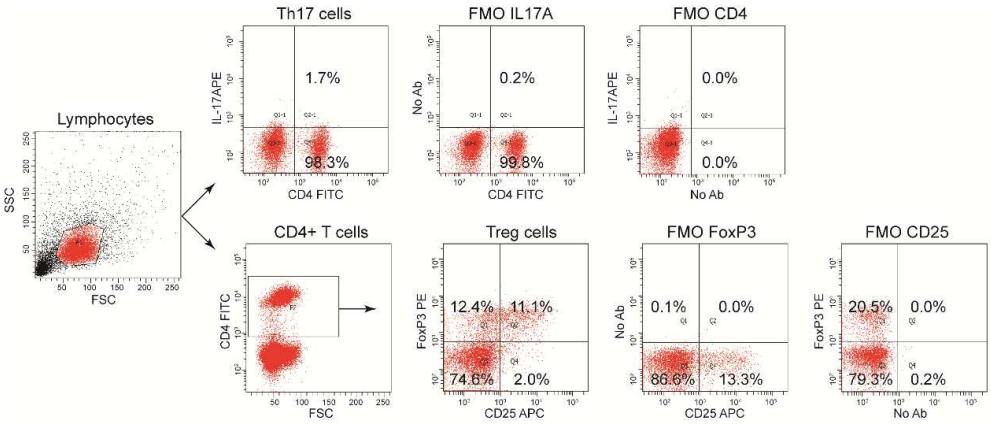

Supplementary figure S1. Gating strategies for regulatory T (Treg) and Helper 17 T (Th17) cells. Treg and Th17 cells were defined as the co-expression of $CD4^+$ $CD25^+$ $Foxp3^+$ and $CD4^+$ $IL\text{-}17A^+$, respectively. Sequential gating was used to quantify the percentage of Treg and Th17 cells among CD4+ T cells. Lymphocyte population was gated from single-cell suspensions according to forward scatter (FSC) characteristics and side scatter (SSC) characteristics. To identify Th17 cells, the lymphocytes were then gated on CD4 and IL-17A double-positive cells based on fluorescence minus one (FMO) controls missing IL-17A and CD4 antibodies. To detect Treg cells, the gated lymphocytes were further characterized by the expression of CD4. Afterward, the cells were gated by co-expression of CD25 and FoxP3. FMO controls for FoxP3 and CD25 were used to determine the quadrant position and fluorescence intensity for subsequent analysis.

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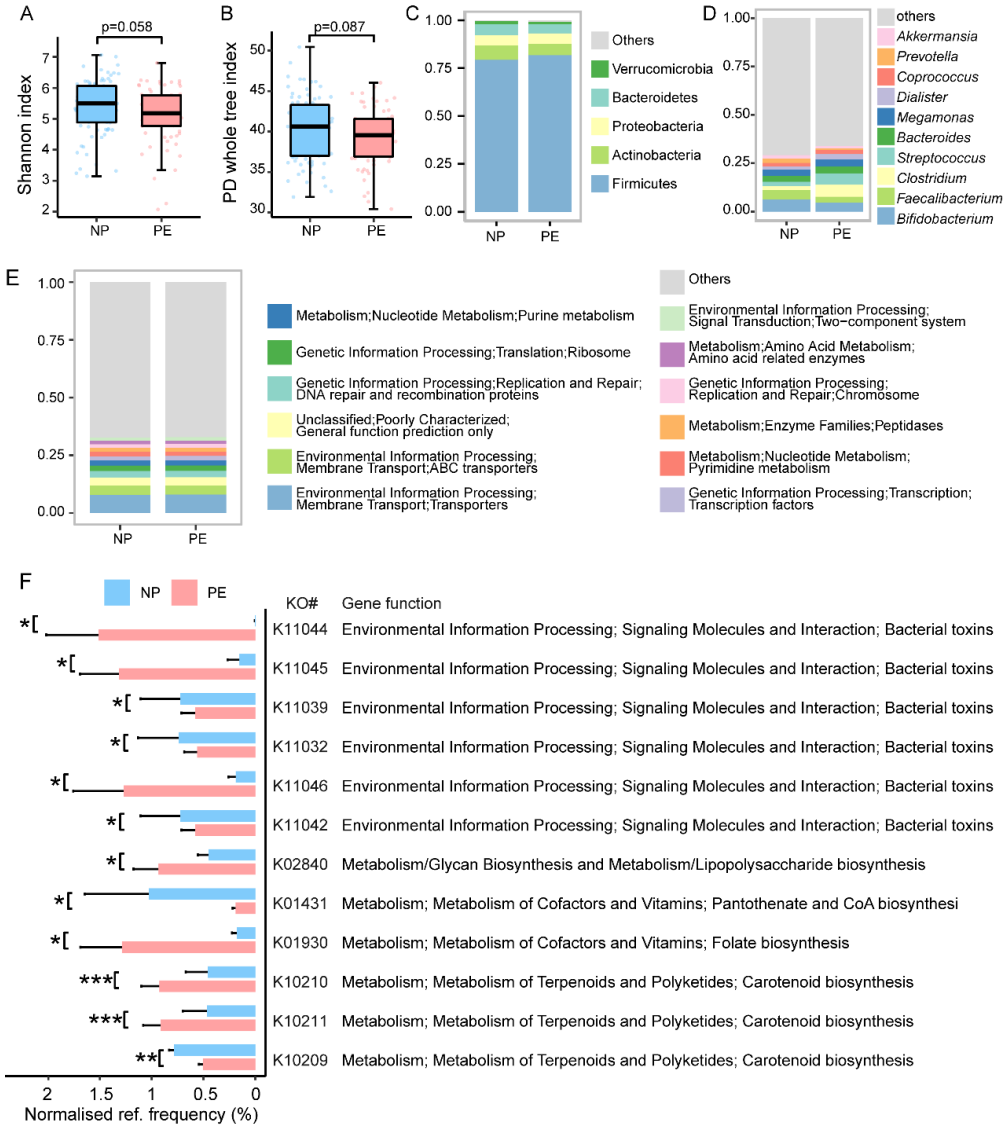
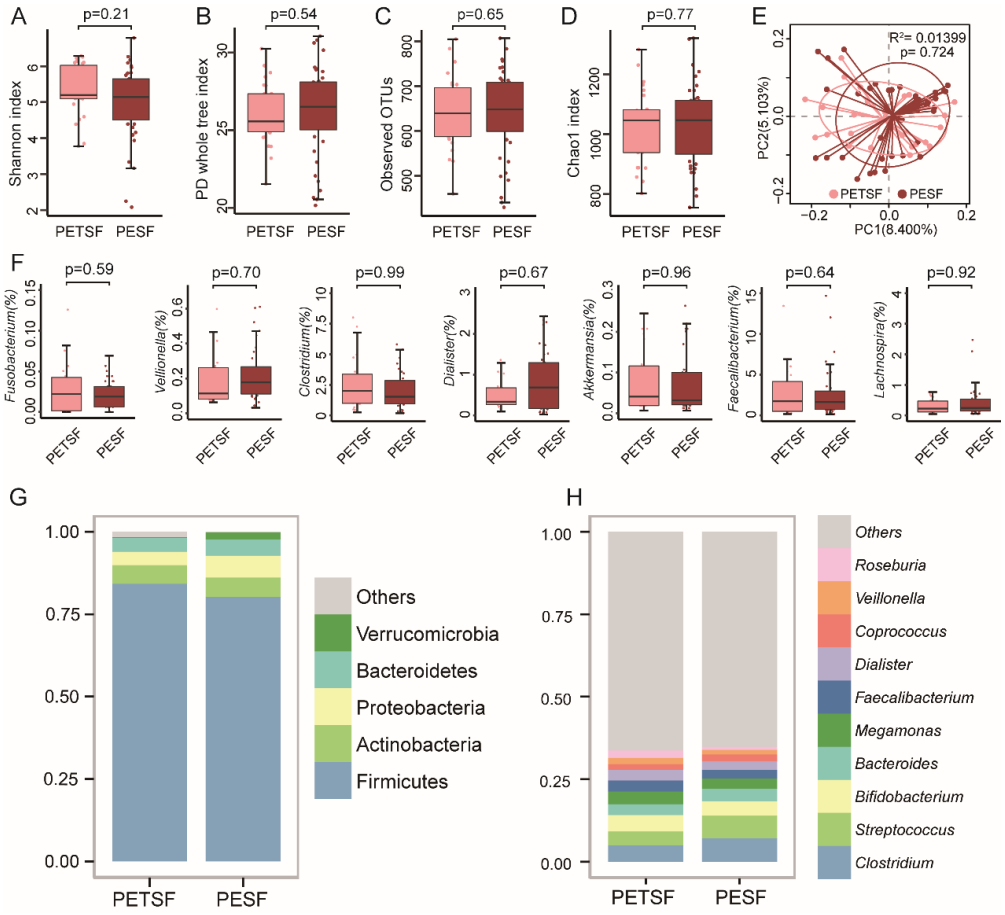
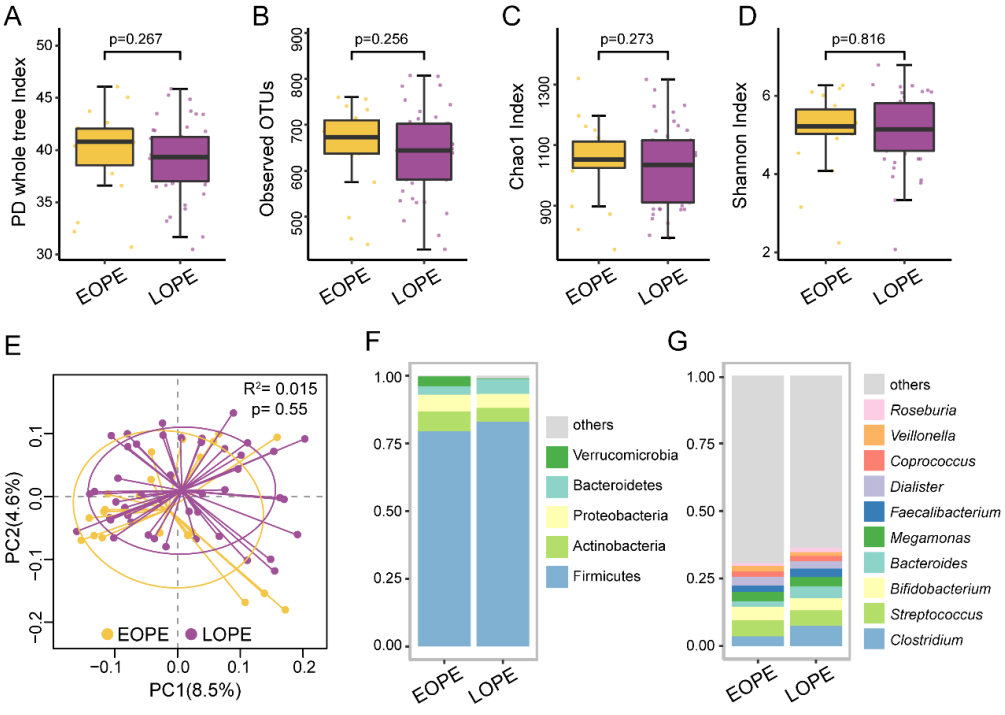


Supplementary figure S2. Differences in bacterial composition between preeclampsia (PE) and normotension (NP) groups. (A, B) Comparison of alpha-diversity indices (Shannon index and PD whole tree index) between PE and NP groups using Wilcoxon rank-sum test. (C, D) Microbial composition at the phylum level and genus level. (E) Stacked bar plot representing the predicted pathways as assessed by PICRUSt. (F) Comparison of underlying disease-correlated KEGG Orthologies (KOs) between PE and NP groups. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$ by

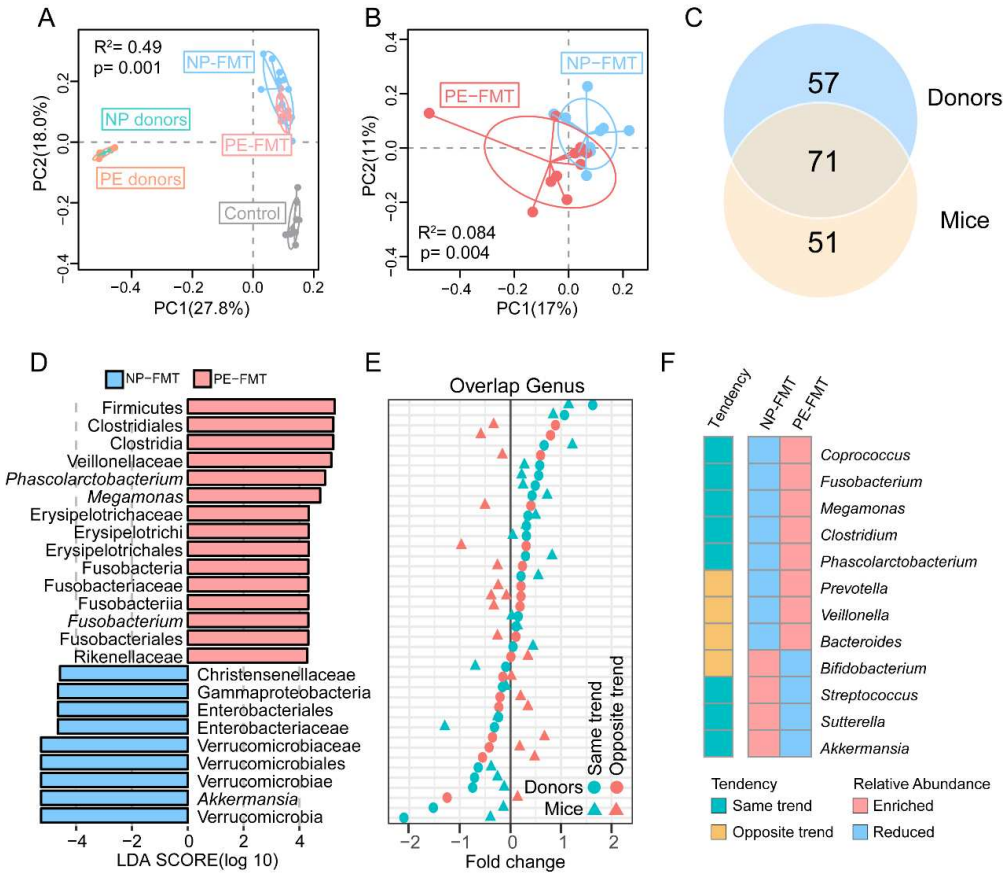
Wilcoxon rank-sum test following the Benjamini and Hochberg method.



Supplementary figure S3. Comparison of gut microbiota between different clinical phenotypes. (A–D) Box plot of alpha-diversity indices comparing preeclampsia without severe feature (PETSF) and preeclampsia with severe feature (PESF) groups using Wilcoxon rank-sum test. (E) Principal coordinate analysis (PCoA) based on unweighted UniFrac distances for bacterial sequences between different clinical phenotypes by PERMANOVA (Adonis). The eigenvalues of axe PC1 and PC2 were 0.74 (8.400%) and 0.43 (5.103%), respectively. (F) Relative abundances of selected genera between PETSF and PESF groups using Wilcoxon rank-sum test. (G, H) Microbial composition at the phylum level and genus level of different clinical phenotypes.



Supplementary figure S4. Profile of gut microbiome composition between early-onset preeclampsia (EOPE) and late-onset preeclampsia (LOPE) groups. (A–D) Comparison of alpha-diversity indices between EOPE and LOPE using Wilcoxon rank sum test. (E) Principal coordinate analysis (PCoA) based on unweighted UniFrac distances for bacterial sequences obtained from fecal samples of EOPE and LOPE patients. The eigenvalues of axe PC1 and PC2 were 0.68 (8.5%) and 0.41 (4.6%), respectively. Difference in beta-diversity were tested by PERMANOVA (Adonis). (F, G) Average relative abundances of predominant taxa at the phylum level and genus level in the EOPE and LOPE groups.



Supplementary figure S5. Post-transplanted intestinal microbial profiles of recipient mice. (A) Principal coordinate analysis (PCoA) plots of human donors and recipient mice based on unweighted UniFrac distance matrices. The eigenvalues of axe PC1 and PC2 were 1.79 (27.8%) and 1.16 (18.0%), respectively. (B) PCoA plots of recipient mice based on unweighted UniFrac distance matrices separate PE-FMT group from the and NP-FMT group. The eigenvalues of axe PC1 and PC2 were 0.41 (17%) and 0.26 (11%), respectively. (C)Venn diagram comparing the shared genera number in the gut microbiome of human donors and recipient mice. (D) Linear discriminant analysis effect size (LEfSe) analysis identified different taxa between NP-FMT and PE FMT groups. The LDA scores (log10) > 4.0 are listed. (E) Concordance of genus variations

between the intestinal microbiota of human donors and recipient mice. The shared genera were compared between donors and recipient mice. Circular points represent genera of human donors' microbiota, and triangle points represent recipient mice's microbiota. Blue points denote genera varying by the same trend, whereas red points denote the opposite trend. (F) Heat map comparing the abundance of altered genera between human donors and recipient mice. Red, more abundant; blue, less abundant. Genera present consistent trend with the variation in the human donors are marked with green points, while those that were inconsistent are marked with yellow points. Difference in beta-diversity were tested by PERMANOVA (Adonis) in (A, B).

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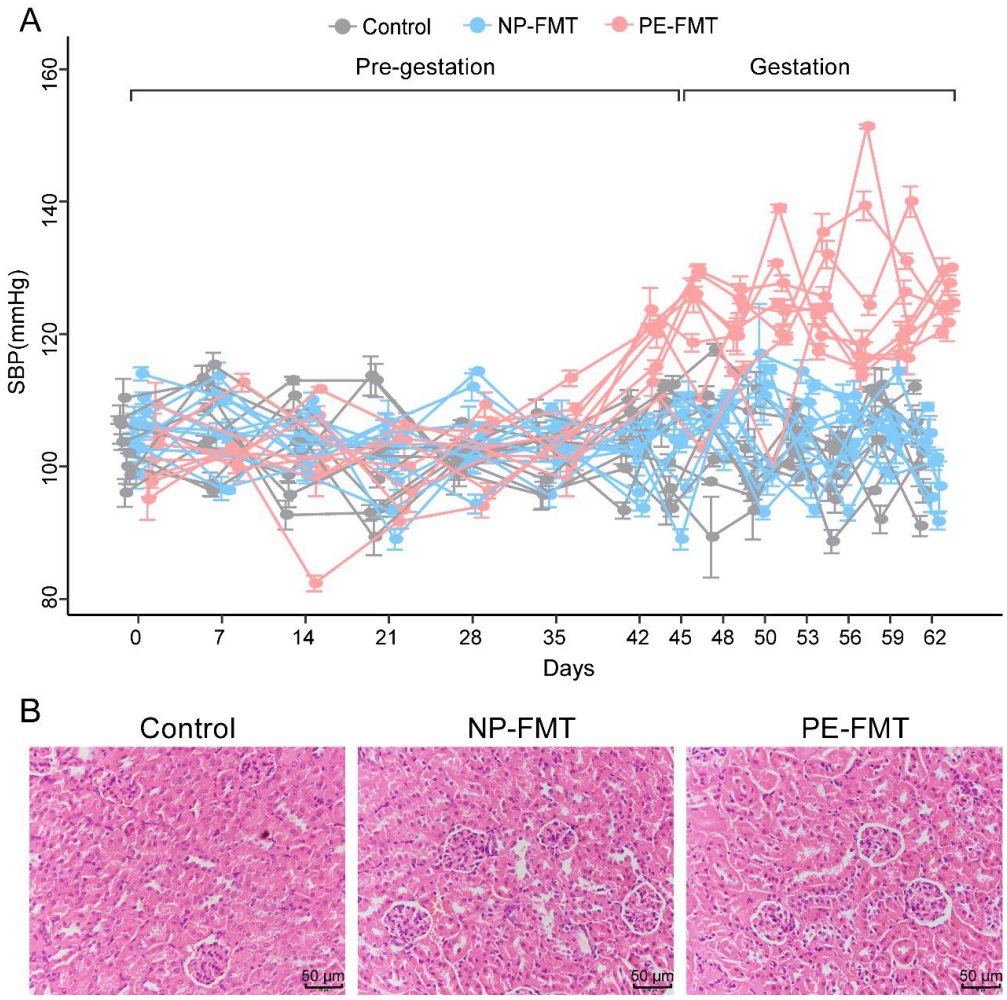
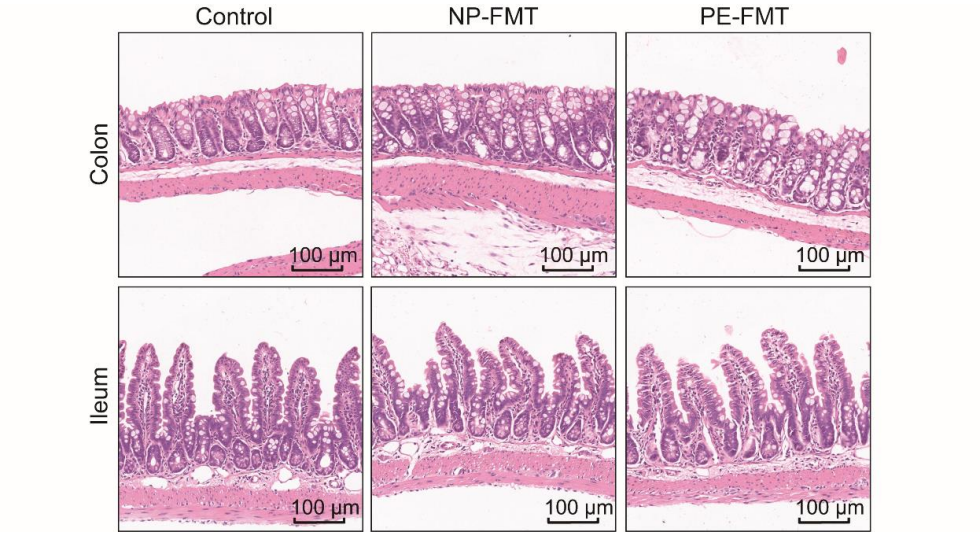


Supplementary figure S6. Systolic blood pressure (SBP) of each mice and kidney morphology of recipient mice. (A) SBP of all mice in the three groups. (B) Representative HE staining of the mouse kidney. Original magnification, ×400; scale bar = 50 μm.



Supplementary figure S7. Intestinal morphology of recipient mice. Representative HE staining of the ileum and colon tissues (original magnification, ×200; scale bar = 100 μm).

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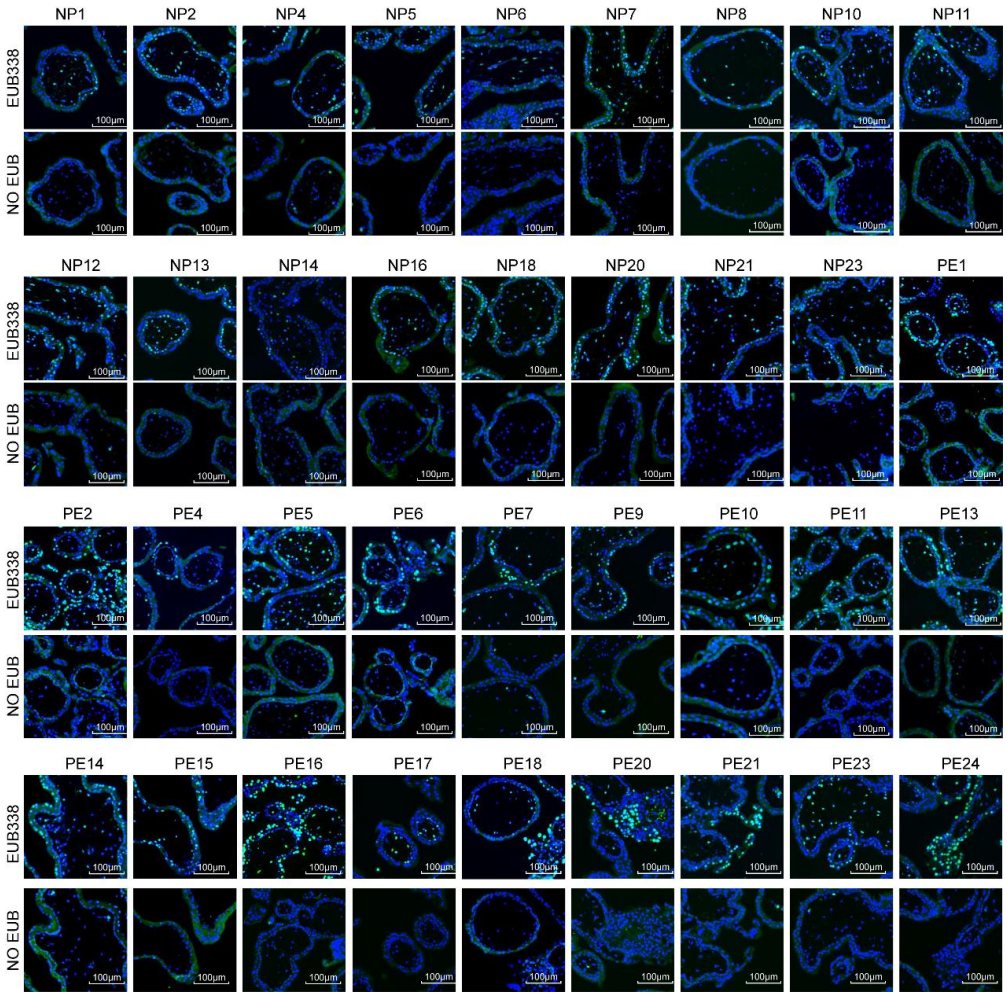
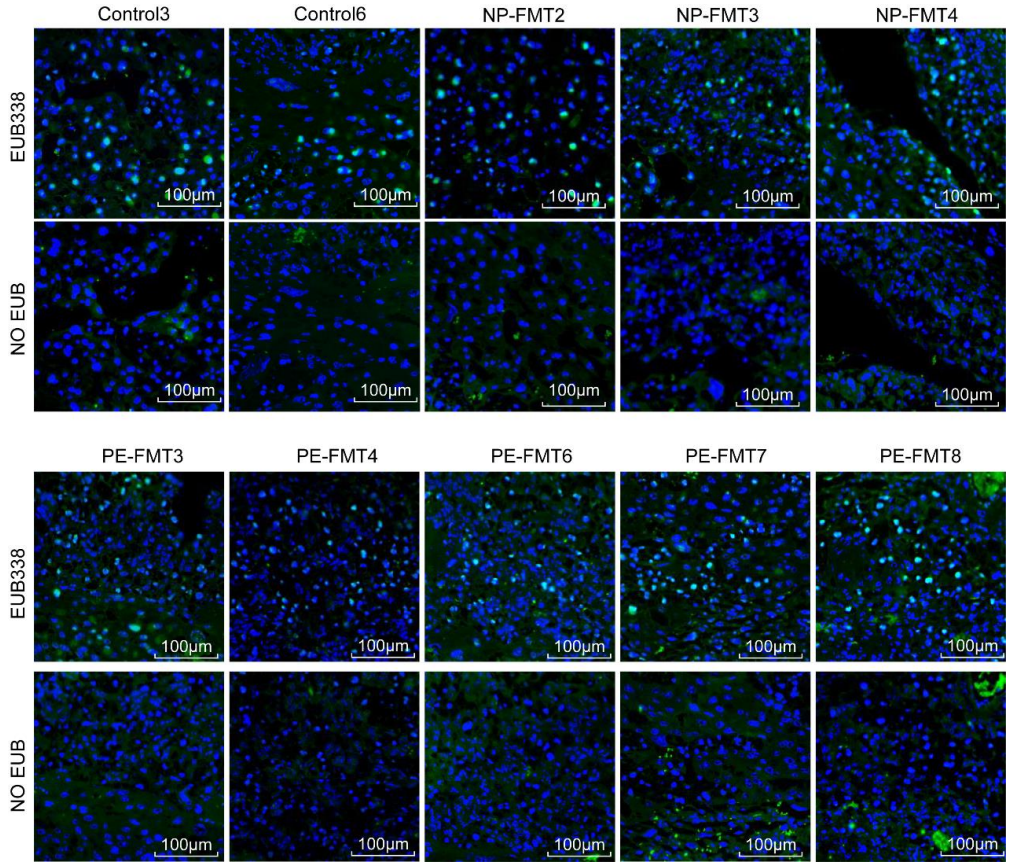
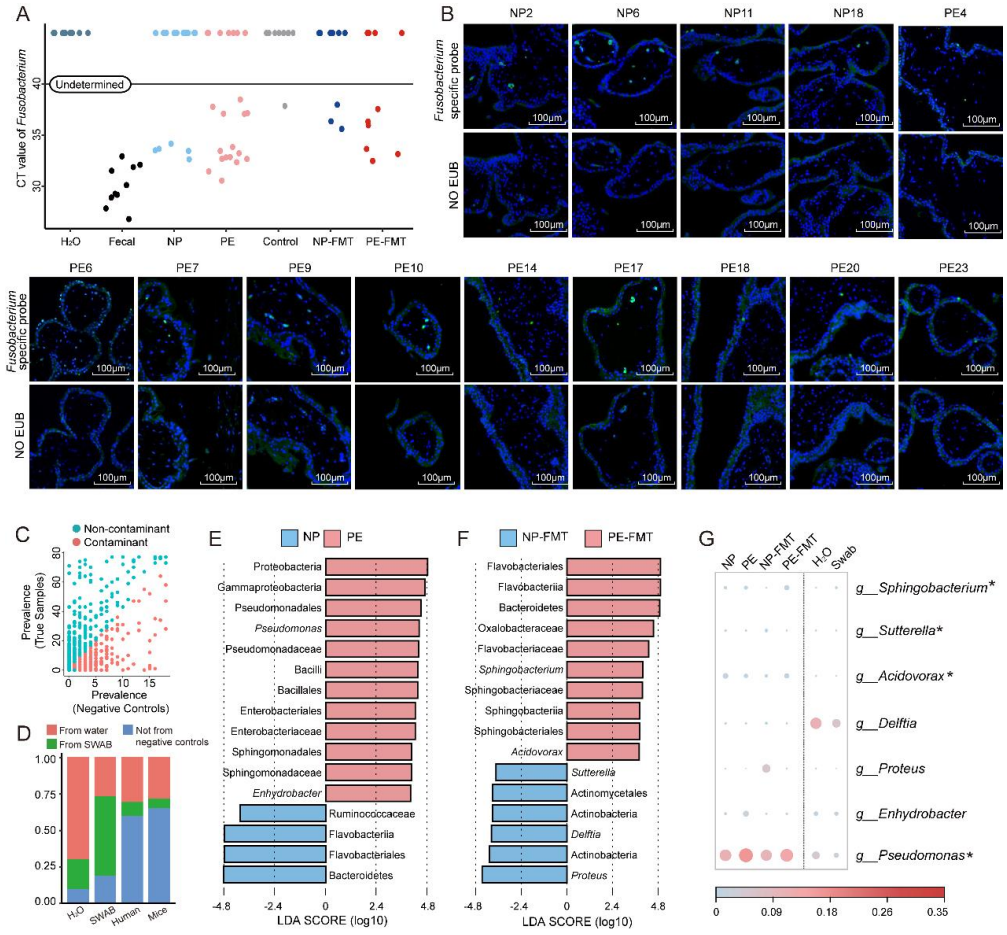


Supplementary figure S8. *In situ* visualized bacterial 16S rRNA signal from normotension (NP) and preeclampsia (PE) placentas. Placental tissue sections were probed with universal eubacterial probe EUB338 (green) and paired with scrambled probe NOEUB. Each image pair is of a separate placenta, with 1 for each of the 18 of 22 NP placentas and 20 of 24 PE placentas examined for which a bacterial 16S rRNA signal was observed. Original magnification, ×200; scale bar = 100 μm.



Supplementary figure S9. *In situ* visualized bacterial 16S rRNA signal from recipient mice. Visualized bacterial signals were observed in the junction layer of the placenta (6 of 8 in PE-FMT placentas, 4 of 8 in NP-FMT placentas, and 3 of 8 placentas of control mice). Each image pair is of a separate placenta, with 1 for each of the placentas examined where bacterial 16S rRNA signal was observed. Original magnification, ×200; scale bar = 100 μm.



Supplementary figure S10. Comparison of bacterial profile between different type of samples. (A) Quantitative-PCR analysis of the presence of genes from *Fusobacterium* in the samples studied Values shown are the cycle of threshold (CT) of each sample. The limit of detection is a CT level of 40 (horizontal line). Samples with no detectable signal are shown above the line. (Statistical comparison between NP and PE placenta, X-squared = 4.6235, $p < 0.05$ by Pearson's Chi-squared test; comparison between PE-FMT, NP-FMT and control placenta, $p = 0.08$ by Fisher's exact test). (B) *In situ* visualized *Fusobacterium* 16S rRNA signal from normotension (NP) and preeclampsia (PE) placentas. Each image pair is of a separate placenta, with 1 for each of the 4 of 22 NP placentas and 10 of 24 PE placentas examined where *Fusobacterium* 16S rRNA

signal was observed. Original magnification, ×200; scale bar = 100 μm. (C) Prevalence plot of OTUs statistically determined to be noncontaminants or contaminants as determined by the decontam isContaminant and isNotContaminant function. A total of 1221 OTUs were found to have statistical support indicating that they represent true OTUs. Red points denote OTUs determined to be contaminants, whereas blue points denote OTUs determined to be non-contaminants. (D) Stacked bar plot representing the source of bacteria identified in each sample using SourceTracker. The proportions of OTUs from the different sources are displayed in colors. Red and green bars represent proportions from DNA-free water during the experiment and sterile swab during placenta sampling respectively, while OTUs not from negative controls are in blue. SourceTracker uses a Bayesian approach to predict the proportion of each sequence or OTU in each sample arising from source environments. (E, F) LEfSe identifies the different taxa between normotension and PE placenta, and NP-FMT and PE-FMT placenta. (G) The abundance of genus identified by LEfSe shown for the placenta sample groups and negative control groups. The asterisk indicates a significantly enriched relative abundance of bacteria in the placenta samples. Wilcoxon rank sum test following Benjamini and Hochberg FDR procedure was performed between bacteria in placentas and negative controls (* indicates $p < 0.05$).

Supplementary tables S1-S6

Supplementary table S1. Characteristics of the study cohort (Mean±SD or N/N (%/%))

Characteristics	NP	PE	P-value	PE-subgroup			
				PETSF	PESF	Early onset	Late onset
N	85	67		27	40	21	46
Age (y)	28.52±4.33	30.16±5.84	P=0.056	29.67±4.95	30.5±6.41	31.14±6.44	29.72±5.56
Gestational age (w)	38.1± 2.5	34.96±3.27	p<0.001	37.4+1.5	33.3+3.1	30.8+1.7	36.9+1.7
Weight (kg)	69.11±8.10	78.07±13.18	p<0.001	79.35±13.15	76.83±13.34	76.46±12.61	78.64±13.49
BMI (kg/m^2)	26.98±2.94	30.52±4.62	p<0.001	30.59±4.12	30.46±5.13	30.23±4.66	30.63±4.66
SBP (mmHg)	120.73±9.64	159.6±11.69	p<0.001	151.89±6.27	164.72±11.7	168.29±12.22	155.57±9.06
DBP (mmHg)	71.71±6.76	100.1±9.51	p<0.001	96.67±6.9	102.47±10.36	103.05±10.65	98.8±8.74
Proteinuria (-,+,++,+++,++++)	62/16/5/2/0	1/11/13/22/20	p<0.001	0/7/5/15/0	1/4/8/7/20	1/3/5/4/8	0/8/8/18/12
Edema (-,+,++,+++)	84/0/1/0	33/17/10/7	p<0.001	16/7/4/0	17/10/6/7	9/4/5/3	24/13/5/4
Neoweight (kg)	3.20±0.54	2.60±0.92	p<0.001	3.18±0.57	2.11±0.88	1.4±0.53	2.94±0.7
AT_III (μg/L)	94.8±12.15	86.77±14.74	p<0.01	89.15±12.46	84.94±16.22	83.53±14.93	88.05±14.65
ALT (U/L)	11.69±4.88	22.79±30.50	p<0.01	13.41±7.52	29.12±37.87	31.86±22.48	18.65±32.92
AST (U/L)	18.12±4.46	28.31±31.47	p<0.01	20±6.1	33.92±39.64	32.43±20.85	26.43±35.33
Cr (μmol/L)	43.33±7.53	55.71±13.90	p<0.001	50.41±10.13	59.47±15.08	59.61±14.58	54.1±13.45
TT (s)	12.63±0.72	13.52±1.67	p<0.001	13.04±1.11	13.86±1.92	13.73±1.54	13.43±1.73
ALB (g/L)	38.76±2.25	34.68±4.24	p<0.001	35.81±2.57	33.88±4.99	33.05±5.55	35.35±3.42
TP (s)	65.94±4.05	61.05±6.26	p<0.001	62.93±4.57	59.71±6.98	58.21±7	62.22±5.6
Hemorrhage (ml)	366.17±103.09	422.37±144.91	p<0.05	463.33±185.14	387.81±88.58	425±110.15	421.63±154.36
HDL(mmol/L)	1.80±0.45	1.75±0.45	p=0.461	1.68±0.42	1.8±0.47	1.77±0.48	1.74±0.44
LDL(mmol/L)	2.60±0.95	2.61±1.19	p=0.953	2.43±1.18	2.74±1.19	2.73±0.89	2.57±1.3
Triglyceride (mmol/L)	3.49±1.20	5.0±3.28	p<0.001	5.01±3.07	4.99±3.45	4.11±1.88	5.36±3.66

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PLT ($\times 10^9$)	221.2±55.3	217.9±74.5	p=0.764	232.3±56.7	207.6±84.2	178.3±67.0	234.2±71.9

NOTE: Differences in characteristics between PE group and NP group were evaluated, using Student's t-test or $\chi 2$ test. Abbreviations: N, sample size; SD, standard deviation; NP, normotensive pregnant women; PE, preeclampsia; PETSF, preeclampsia without severe features; PESF, preeclampsia with severe features; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AT-III, antithrombin III; ALT, alanine aminotransferase; AST, Aspartate aminotransferase; Cr, creatinine; TT, thrombin time; ALB, albumin; TP, total protein; HDL, high density lipoprotein; LDL, low density lipoprotein; PLT, blood platelet.

Supplementary table S2. Characteristics of the donors of placenta (Mean±SD or N/N(%/%))

Characteristics	NP	PE	P-value
N	22	24	
Age (y)	29.05±4.51	30.75±6.22	p=0.29
Gestational age (w)	37.8±2.4	32.6±2.9	<0.001
Weight (kg)	67.4±7.68	73.59±14.1	p=0.12
BMI (kg/m^2)	25.58±2.69	29.03±5.2	p=0.02
SBP (mmHg)	119.82±9.91	162.75±10.23	p<0.001
DBP (mmHg)	70.5±6.95	103.88±10.66	p<0.001
Proteinuria (-,+,++,+++)	19/1/2/0/0	1/2/4/4/13	p<0.001
Edema (-,+,++,+++)	22/0/0/0	12/6/2/4	p<0.001
Neoweight (kg)	3.38±0.49	1.88±0.64	p<0.001
AT_III (μg/L)	94.23±11.61	83.48±15.39	p=0.01
ALT (U/L)	10.73±3.94	22.96±19.15	p<0.01
AST (U/L)	18.09±5.68	27.54±14.72	p<0.01
Cr (μmol/L)	41.27±7.63	59.52±13.72	p<0.001
TT (s)	12.48±0.68	13.72±1.93	p<0.01
ALB (g/L)	38.72±1.99	34.43±4.45	p<0.001
TP (s)	65.96±3.67	60.8±6.61	p<0.01
Hemorrhage (ml)	342.89±75.49	387.22±95.83	p=0.13
HDL(mmol/L)	1.97±0.5	1.85±0.38	p=0.37
LDL(mmol/L)	2.6±1.05	2.83±1.18	p=0.50
Triglyceride (mmol/L)	3.63±1.2	5.11±4.19	p=0.12
PLT (×10^9)	203.9±41.5	223.7±91.6	0.337

NOTE: Differences in characteristics between PE and Normotension group were evaluated, using Student's t-test or $\chi 2$ test. Abbreviations: N, sample size; SD, standard deviation; NP, normotensive pregnant women; PE, preeclampsia; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AT-III, antithrombin III; ALT, alanine aminotransferase; AST, Aspartate aminotransferase; Cr, creatinine; TT, thrombin time; ALB, albumin; TP, total protein; HDL, high density lipoprotein; LDL, low density lipoprotein; PLT, blood platelet.

Supplementary table S3. Characteristics of the donors for fecal microbiota transplantation

Characteristics	NP			PE		
	Health.H042.B	Health.H043.B	Health.H082.B	PIH.P132.B	PIH.P136.B	PIH.P149.B
Age (y)	28	25	25	27	29	26
Gestational age (w)	38.3	38.4	39	39.4	40.2	32
BMI (kg/m^2)	27.82	25.81	34.13	36.11	25.53	34.41
SBP (mmHg)	106	102	111	158	152	183
DBP (mmHg)	60	63	69	101	104	124
Proteinuria (-,+,++,+++)	+	0	0	+++	++	++
Edema (-,+,++,+++)	0	0	0	++	0	++
Neoweight (kg)	3.1	3.4	3.95	3.6	3.1	1.78
AT_III (μg/L)	86	99	89	90	88	70
ALT (U/L)	8	10	8	17	11	43
AST (U/L)	10	15	13	23	22	3
Cr (μmol/L)	43	44	52	67	49	87
TT (s)	13.6	12.5	11.9	13.1	12.7	13.4
ALB (g/L)	36.9	36.9	39.6	33.7	39	26.2
TP (s)	64.9	60.1	68.5	56.5	67.3	53.9
Hemorrhage (ml)	315	315	315	515	1315	315
HDL(mmol/L)	1.53	1.65	1.88	1.56	1.51	3.14
LDL(mmol/L)	1.89	2.46	2.72	1.38	4.96	3.19
PLT (×10^9)	253	192	207	185	213	202

NOTE: Abbreviations: NP, normotensive pregnant women; PE, preeclampsia; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AT-III, antithrombin III; ALT, alanine aminotransferase; AST, Aspartate aminotransferase; Cr, creatinine; TT, thrombin time; ALB, albumin; TP, total protein; HDL, high density lipoprotein; LDL, low density lipoprotein; PLT, blood platelet.

Supplementary table S4. Group and cage number of the mice involved in the study

FMT treatment	group name	NO. of cage	NO. of mice
PBS	control	Cage 1	C49
			C56
		Cage 2	C53
			C15
		Cage 3	C51
			C38
		Cage 5	C13
			C19
fecal supensant mixture from three NP donors	NP-FMT	Cage 6	H9
		Cage 7	H7
			H4
		Cage 8	H10
			H34
			H8
		Cage 9	H26
			H54
		Cage 10	H17
			H23
fecal supensant mixture from three PE donors	PE-FMT	Cage 11	P22
			P37
		Cage 12	P55
			P41
		Cage 13	P30
			P52
		Cage 15	P32
			P63

NOTE: Abbreviations: FMT, fecal microbiota transplantation; NP, normotensive pregnant women; PE, preeclampsia; PBS, phosphate buffer saline.

269 **Supplementary table S5. Primers used in quantitative-PCR analysis**

Gene	Primer	Sequence(5' to 3')
16sRNA	Forward	GTGSTGCAYGGYTGTCGTCA
	Reverse	ACGTCRTCCMCACCTTCCTC
Fusobacterium	Forward	GGATTTATTGGGCGTAAAGC
	Reverse	GGCATTCCTACAAATATCTACGAA
18sRNA	Forward	AGTCCCTGCCCTTTGTACACA
	Reverse	CGATCCGAGGGCCTCACTA
Tjp1	Forward	ACCCGAAACTGATGCTGTGGATAG
	Reverse	AAATGGCCGGGCAGAACTTGTGTA
Tjp2	Forward	TGCAATTCCAAATCCAAACC
	Reverse	GTGATTTTCTTCAACCCGGA
occludin	Forward	CCCAGGCTTCTGGATCTATGT
	Reverse	TCCATCTTTCTTCGGGTTTTCA
claudin4	Forward	TGATTATGGTGCCCGTGTCC
	Reverse	CGAGTAGGGCTTGTCGTTGC
Il6	Forward	TGATGCACTTGCAGAAAACA
	Reverse	ACCAGAGGAAATTTTCAATAGGC
Il1β	Forward	TGTGAAATGCCACCTTTTGA
	Reverse	GGTCAAAGGTTTGGAAGCAG
Ccl3	Forward	ACCATGACACTCTGCAACCA
	Reverse	GTGGAATCTTCCGGCTGTAG
Ccl4	Forward	CATGAAGCTCTGCGTGTCTG
	Reverse	GAAACAGCAGGAAGTGGGAG
Cxcl1	Forward	CCACACTCAAGAATGGTCGC
	Reverse	TCTCCGTTACTTGGGGACAC
Vegf	Forward	TTACTGCTGTACCTCCACC
	Reverse	ACAGGACGGCTTGAAGATG

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272 **Supplementary table S6. Probes used in *In Situ* Hybridization**

Probe name	Sequence(5' to 3')
EUB338I	GCTGCCTCCCGTAGGAGT
EUB338II	GCAGCCACCCGTAGGTGT
EUB338III	GCTGCCACCCGTAGGTGT
NOEUB	ACTCCTACGGGAGGCAGC
Fusobacterium	CTAATGGGACGCAAAGCTCTC

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Reference

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2. Hu J, Luo H, Wang J, *et al*. Enteric dysbiosis-linked gut barrier disruption triggers early renal injury induced by chronic high salt feeding in mice. *Exp Mol Med* 2017;49:e370.