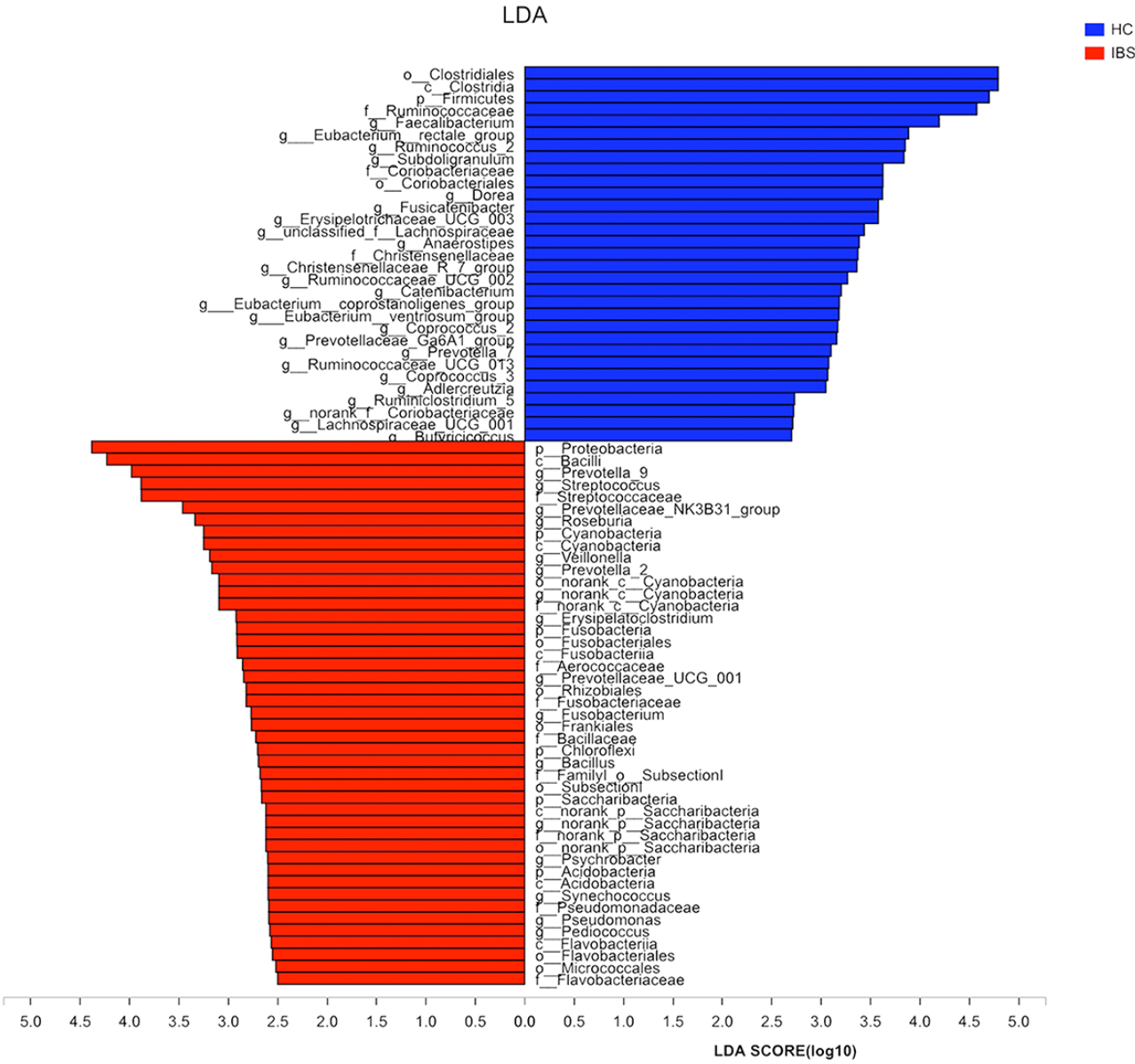
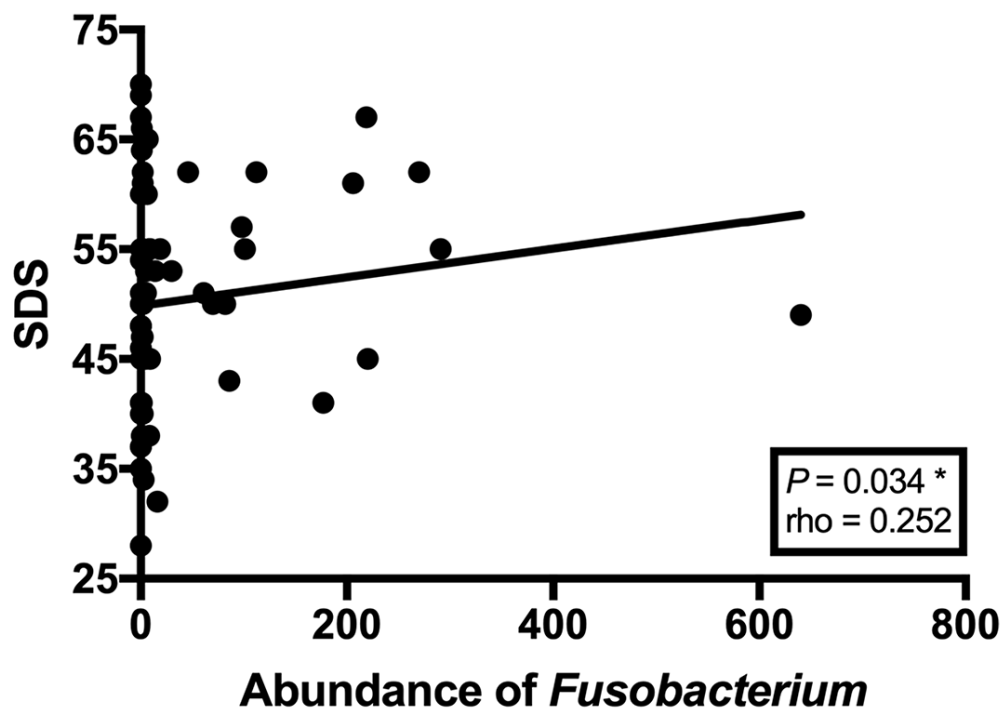


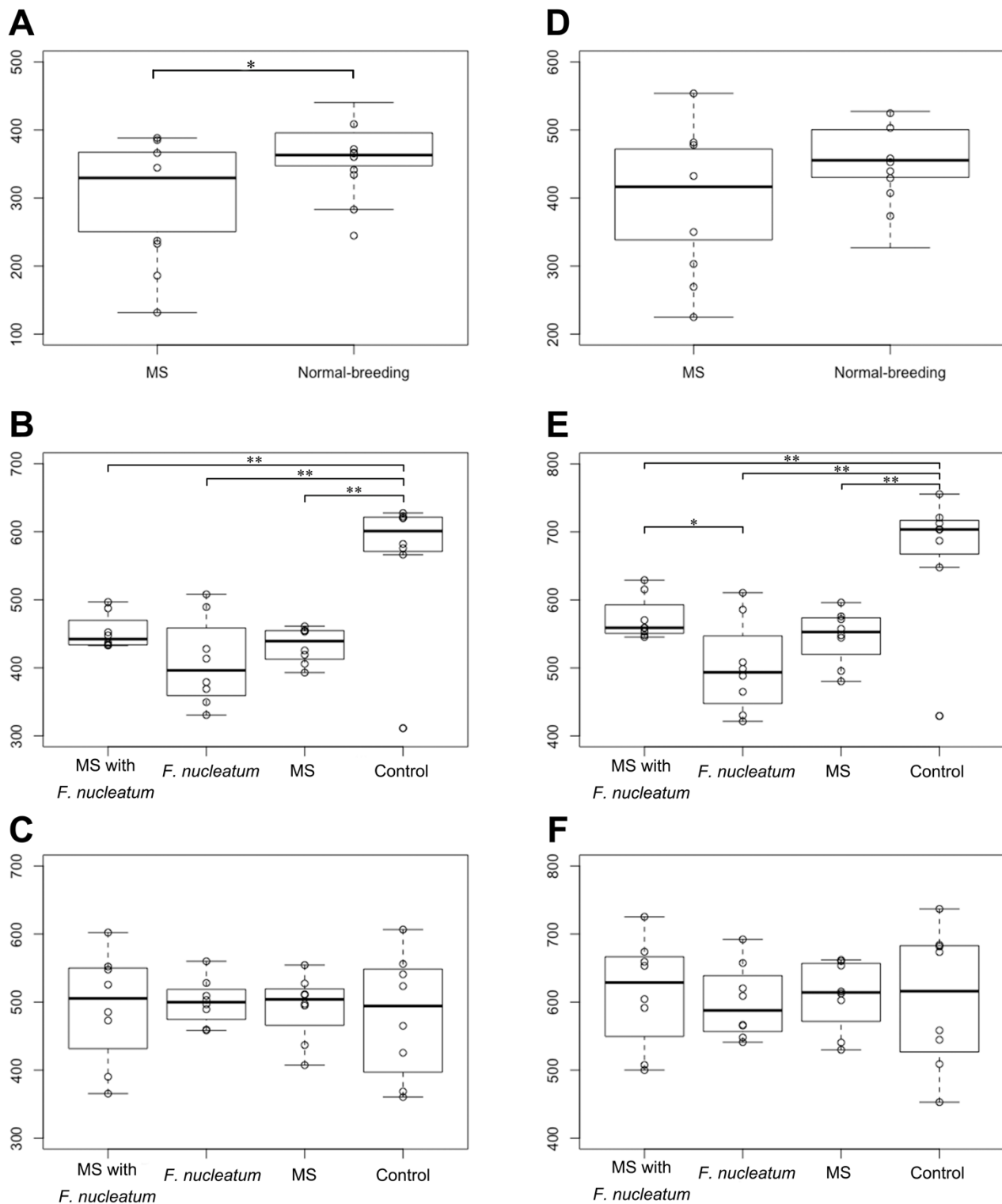
Supplementary Material



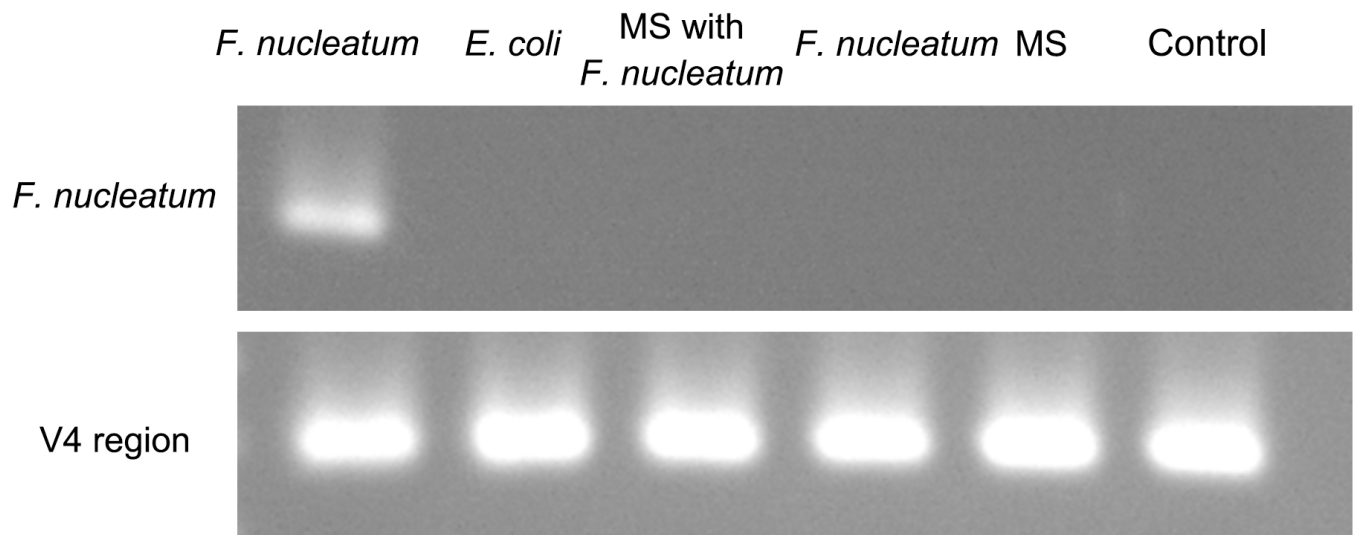
Supplementary Figure 1. Taxonomic difference based on LDA. IBS, IBS-D patients; HC, healthy controls



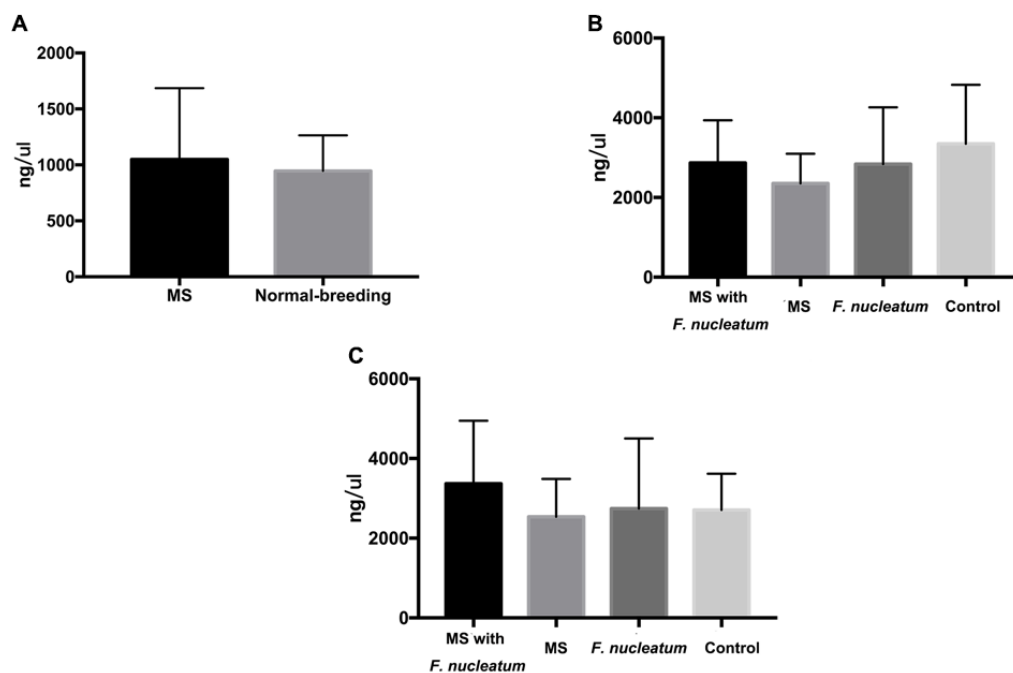
Supplementary Figure 2. The Spearman correlation between abundance of *Fusobacterium* and Self-rating Depression Scale (SDS) in IBS-D patients.



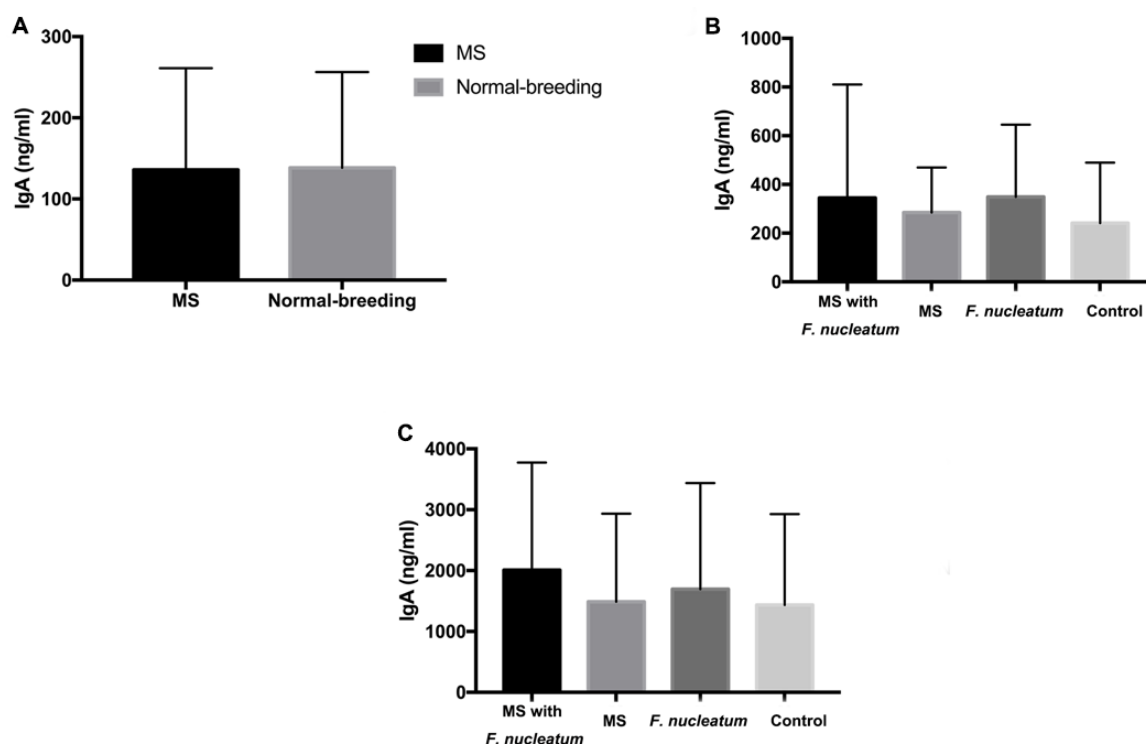
Supplementary Figure 3. The Sobs value at at week3 (A), 8 (B), and 12 (C), and the Chao_1 value at week3 (D), 8 (E), and 12 (F). *: $P < 0.05$, **: $P < 0.01$. MS: maternal separation.



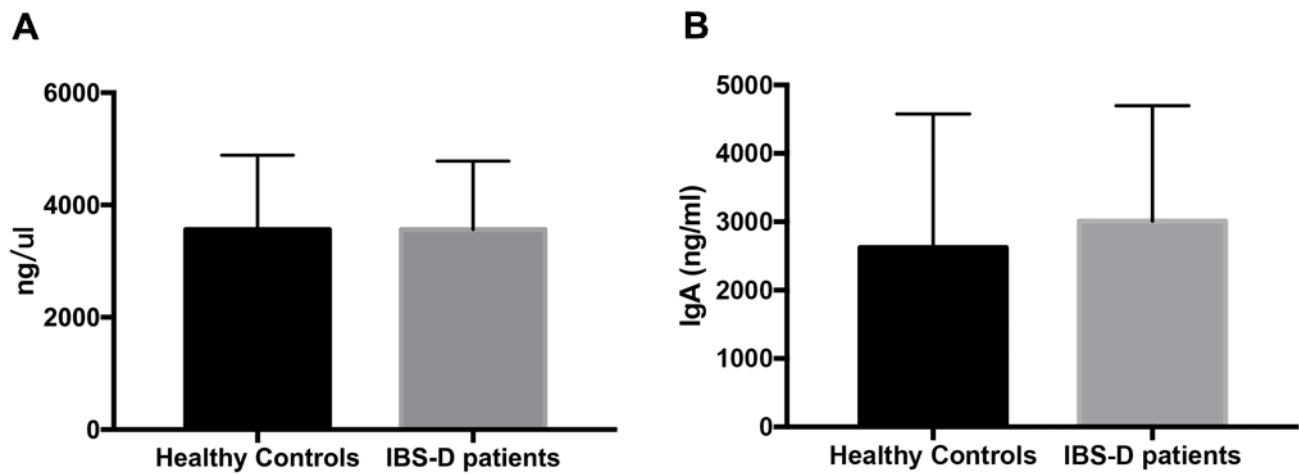
Supplementary Figure 4. Detection of *F. nucleatum*. *F. nucleatum* was detected by polymerase chain reaction in stool of rats in 4 groups, and *F. nucleatum* (ATCC25586) and *E. coli* BL21 (DE3) were used as positive and negative controls, respectively. The V4 region of the bacterial 16S rRNA gene was amplified as internal control. MS: maternal separation.



Supplementary Figure 5. The total protein concentration of rats' FSN was tested by BCA assay at week 3 (A), week 8 (B), week 12 (C). MS: maternal separation.



Supplementary Figure 6. The concentration of IgA in rats' FSN was tested by ELISA kits at week 3 (A), week 8 (B), week 12 (C). MS: maternal separation.



Supplementary Figure 7. The total protein concentration (A) and IgA concentration (B) in FSN of IBS-D patients and healthy controls.

Supplementary Table 1. Taxonomic difference

Taxonomy	number	group	LDA	P value
<i>p__Acidobacteria</i>		IBS-D	2.599 6	0.0003
<i>p__Acidobacteria.c__Acidobacteria</i>		IBS-D	2.599 5	0.0003
<i>p__Actinobacteria.c__Actinobacteria.o__Frankiales</i>		IBS-D	2.768 3	0.0348
<i>p__Actinobacteria.c__Actinobacteria.o__Micrococcales</i>		IBS-D	2.520 5	0.0032
<i>p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Prevotellaceae.g__Prevotella_2</i>		IBS-D	3.168 9	0.0203
<i>p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Prevotellaceae.g__Prevotella_9</i>		IBS-D	3.982 1	0.0163
<i>p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Prevotellaceae.g__Prevotellaceae_NK3B31_group</i>		IBS-D	3.464 7	0.0002
<i>p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Prevotellaceae.g__Prevotellaceae_UCG_001</i>	n = 45	IBS-D	2.847 7	0.0117
<i>p__Bacteroidetes.c__Flavobacteriia</i>		IBS-D	2.567 9	0.0071
<i>p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales</i>		IBS-D	2.555 3	0.0071
<i>p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae</i>		IBS-D	2.502 0	0.0039
<i>p__Chloroflexi</i>		IBS-D	2.705 5	0.0003
<i>p__Cyanobacteria</i>		IBS-D	3.252 4	0.0023
<i>p__Cyanobacteria.c__Cyanobacteria</i>		IBS-D	3.252 4	0.0023

<i>p__Cyanobacteria.c__Cyanobacteria.o__norank_c__Cyanobacteria</i>	IBS-D	3.097 0	0.0036
<i>p__Cyanobacteria.c__Cyanobacteria.o__norank_c__Cyanobacteria.f__norank_c__Cyanobacteria</i>	IBS-D	3.097 0	0.0036
<i>p__Cyanobacteria.c__Cyanobacteria.o__norank_c__Cyanobacteria.f__norank_c__Cyanobacteria.g__norank_c__Cyanobacteria</i>	IBS-D	3.097 0	0.0036
<i>p__Cyanobacteria.c__Cyanobacteria.o__SubsectionI</i>	IBS-D	2.667 7	0.0086
<i>p__Cyanobacteria.c__Cyanobacteria.o__SubsectionI.f__FamilyI_o__SubsectionI</i>	IBS-D	2.680 7	0.0086
<i>p__Cyanobacteria.c__Cyanobacteria.o__SubsectionI.f__FamilyI_o__SubsectionI.g__Synechococcus</i>	IBS-D	2.599 4	0.0119
<i>p__Firmicutes.c__Bacilli</i>	IBS-D	4.232 9	0.0487
<i>p__Firmicutes.c__Bacilli.o__Bacillales.f__Bacillaceae</i>	IBS-D	2.724 1	0.0104
<i>p__Firmicutes.c__Bacilli.o__Bacillales.f__Bacillaceae.g__Bacillus</i>	IBS-D	2.698 1	0.0049
<i>p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Aerococcaceae</i>	IBS-D	2.857 1	0.0187
<i>p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Pediococcus</i>	IBS-D	2.581 9	0.0006
<i>p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Streptococcaceae</i>	IBS-D	3.883 3	0.0452
<i>p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Streptococcaceae.g__Streptococcus</i>	IBS-D	3.885 6	0.0171
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Roseburia</i>	IBS-D	3.338 9	0.0255
<i>p__Firmicutes.c__Erysipelotrichia.o__Erysipelotrichales.f__Erysipelotrichaceae.g__Erysipelatoclostridium</i>	IBS-D	2.923 5	0.0129

<i>p__Firmicutes.c__Negativicutes.o__Selenomonadales.f__Veillonellaceae.g__Veillonella</i>	IBS-D	3.190 6	0.0388
<i>p__Fusobacteria</i>	IBS-D	2.916 8	0.0001
<i>p__Fusobacteria.c__Fusobacteriia</i>	IBS-D	2.911 5	0.0001
<i>p__Fusobacteria.c__Fusobacteriia.o__Fusobacteriales</i>	IBS-D	2.915 6	0.0001
<i>p__Fusobacteria.c__Fusobacteriia.o__Fusobacteriales.f__Fusobacteriaceae</i>	IBS-D	2.820 4	0.0001
<i>p__Fusobacteria.c__Fusobacteriia.o__Fusobacteriales.f__Fusobacteriaceae.g__Fusobacterium</i>	IBS-D	2.772 1	0.0001
<i>p__Proteobacteria</i>	IBS-D	4.384 9	0.0377
<i>p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales</i>	IBS-D	2.820 8	0.0258
<i>p__Proteobacteria.c__Gammaproteobacteria.o__Pseudomonadales.f__Moraxellaceae.g__Psychrobacter</i>	IBS-D	2.604 2	0.0454
<i>p__Proteobacteria.c__Gammaproteobacteria.o__Pseudomonadales.f__Pseudomonadaceae</i>	IBS-D	2.592 9	0.0012
<i>p__Proteobacteria.c__Gammaproteobacteria.o__Pseudomonadales.f__Pseudomonadaceae.g__Pseudomonas</i>	IBS-D	2.592 7	0.0012
<i>p__Saccharibacteria</i>	IBS-D	2.664 4	0.0229
<i>p__Saccharibacteria.c__norank_p__Saccharibacteria</i>	IBS-D	2.622 2	0.0045
<i>p__Saccharibacteria.c__norank_p__Saccharibacteria.o__norank_p__Saccharibacteria</i>	IBS-D	2.622 2	0.0045
<i>p__Saccharibacteria.c__norank_p__Saccharibacteria.o__norank_p__Saccharibacteria.f__norank_p__Saccharibacteria</i>	IBS-D	2.622 2	0.0045

<i>p__Saccharibacteria.c__norank_p__Saccharibacteria.o__norank_p__Saccharibacteria.f__norank_p__Saccharibacteria.g__norank_p__Saccharibacteria</i>	IBS-D	2.622 2	0.0045
<i>p__Actinobacteria.c__Actinobacteria.o__Coriobacteriales</i>	HC	3.626 4	0.0196
<i>p__Actinobacteria.c__Actinobacteria.o__Coriobacteriales.f__Coriobacteriaceae</i>	HC	3.626 4	0.0196
<i>p__Actinobacteria.c__Actinobacteria.o__Coriobacteriales.f__Coriobacteriaceae.g__Adlercreutzia</i>	HC	3.049 6	0.0000
<i>p__Actinobacteria.c__Actinobacteria.o__Coriobacteriales.f__Coriobacteriaceae.g__norank_f__Coriobacteriaceae</i>	HC	2.722 3	0.0005
<i>p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Prevotellaceae.g__Prevotella_7</i>	HC	3.101 7	0.0324
<i>p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Prevotellaceae.g__Prevotellaceae_Ga6A1_group</i>	HC	3.159 8	0.0158
<i>p__Firmicutes</i>	n = 31 HC	4.702 6	0.0113
<i>p__Firmicutes.c__Clostridia</i>	HC	4.793 1	0.0044
<i>p__Firmicutes.c__Clostridia.o__Clostridiales</i>	HC	4.793 9	0.0044
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Christensenellaceae</i>	HC	3.373 8	0.0005
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Christensenellaceae.g__Christensenellaceae_R_7_group</i>	HC	3.364 0	0.0024
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Eubacterium_rectale_group</i>	HC	3.886 5	0.0323
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Eubacterium_ventricosum_group</i>	HC	3.181 4	0.0031

<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiracea</i> <i>e.g__Anaerostipes</i>	HC	3.387 1	0.0121
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiracea</i> <i>e.g__Coproccoccus_2</i>	HC	3.169 8	0.0449
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiracea</i> <i>e.g__Coproccoccus_3</i>	HC	3.067 6	0.0141
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiracea</i> <i>e.g__Dorea</i>	HC	3.622 7	0.0057
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiracea</i> <i>e.g__Fusicatenibacter</i>	HC	3.580 9	0.0005
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiracea</i> <i>e.g__Lachnospiraceae_UCG_001</i>	HC	2.712 9	0.0094
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiracea</i> <i>e.g__unclassified_f__Lachnospiraceae</i>	HC	3.437 9	0.0054
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae</i>	HC	4.577 1	0.0002
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Eubacterium__coprostanoligenes_group</i>	HC	3.185 6	0.0081
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Butyricicoccus</i>	HC	2.703 2	0.0142
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Faecalibacterium</i>	HC	4.195 4	0.0015
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Ruminiclostridium_5</i>	HC	2.732 4	0.0239
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Ruminococcaceae_UCG_002</i>	HC	3.269 1	0.0222
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Ruminococcaceae_UCG_013</i>	HC	3.078 8	0.0008
<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Ruminococcus_2</i>	HC	3.851 9	0.0120

<i>p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Subdoligranulum</i>	HC	3.839 7	0.0097
<i>p__Firmicutes.c__Erysipelotrichia.o__Erysipelotrichales.f__Erysipelotrichaceae.g__Catenibacterium</i>	HC	3.204 7	0.0364
<i>p__Firmicutes.c__Erysipelotrichia.o__Erysipelotrichales.f__Erysipelotrichaceae.g__Erysipelotrichaceae_UCG_003</i>	HC	3.578 7	0.0267

IBS-D, diarrhea predominant-irritable bowel syndrome; HC, healthy controls.

Supplementary Table 2. Adonis and Anosim test on animal treatments and co-housing effect

Time	Methods	Across	Co-housing				
		MS: Normal	MS (A9: B9)		Normal (C7: D5: E7)		
Week 3	Anosim	$P = 0.002(**)$	$P = 0.961$		$P = 0.297$		
	Adonis	$P = 0.003(**)$	$P = 0.905$		$P = 0.293$		
		D: M: F: N	D (A3: A5)	M (A4: B4)	F (C1: D1: E4)	N (C3: D2: E4)	
Week 8	Anosim	$P = 0.001(***)$	$P = 0.655$	$P = 0.336$	$P = 0.239$	$P = 0.577$	
	Adonis	$P = 0.001(***)$	$P = 0.626$	$P = 0.402$	$P = 0.214$	$P = 0.825$	
		D: M: F: N	D (A3: A5)	M (A4: B4)	F (C1: D1: E3)	N (C3: D2: E4)	
Week 12	Anosim	$P = 0.001(***)$	$P = 0.221$	$P = 0.116$	$P = 0.498$	$P = 0.075$	
	Adonis	$P = 0.001(***)$	$P = 0.27$	$P = 0.136$	$P = 0.69$	$P = 0.138$	

** $P < 0.01$, *** $P < 0.001$. MS: maternal separation; Normal: normal-breeding; D: maternal separation with *Fusobacterium nucleatum* gavage; M: maternal separation with normal-saline gavage; F: normal breeding during lactation with *Fusobacterium nucleatum* gavage; N: normal control group with normal-saline gavage.

Supplementary Table 3. Patient characteristics for detection of *F. nucleatum* specific IgA

	IBS-D patients (n = 7)	Healthy controls (n = 5)
Age [mean (SD)]	41.43 (2.48)	36.20 (6.06)
BMI kg/m ² [mean (SD)]	22.66 (1.77)	21.55 (1.18)
SAS [mean (SD)] **	51.86 (5.05)	36.00 (7.52)
SDS [mean (SD)] **	50.00 (10.13)	31.20 (3.90)

IBS-D, diarrhea predominant-irritable bowel syndrome; SD, standard deviation; BMI, Body Mass Index; SAS, Self-rating Anxiety Scale; SDS, Self-rating Depression Scale; **: $P < 0.01$.