Supplementary Information

Irritable bowel syndrome-related differences in the fecal microbiome and metabolome are not affected by short-term improvement after the rifaximin treatment

Natalia Zeber-Lubecka[1#], Maria Kulecka[1#], Filip Ambrozkiewicz[1], Agnieszka Paziewska[1], Krzysztof Goryca[2], Jakub Karczmarski[2], Tymon Rubel[3], Wojciech Wojtowicz[4], Piotr Mlynarz[4], Lukasz Marczak[5], Roman Tomecki[1], Michal Mikula[2], Jerzy Ostrowski[1,2,*]

[1] Department of Gastroenterology, Hepatology and Clinical Oncology, Medical Center for Postgraduate Education; Warsaw, Poland

[2] Department of Genetics, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology; Warsaw, Poland

[3] Institute of Radioelectronics, Warsaw University of Technology, Warsaw, Poland

[4] Department of Bioorganic Chemistry Wroclaw University of Technology, Wroclaw, Poland

[5] Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznań, Poland

#equal contribution

*corresponding author

Jerzy Ostrowski, MD, PhD; Cancer Center-Institute, Roentgena 5, 02-781 Warsaw, Poland,

Tel: +48 225462575, Fax: +48 225462449, E-mail: jostrow@warman.com.pl

Figure S1. *Bacteroidetes/Firmicutes* ratios in healthy controls (HCs) and IBS patients before (IBS) and after (Treatment) treatment.

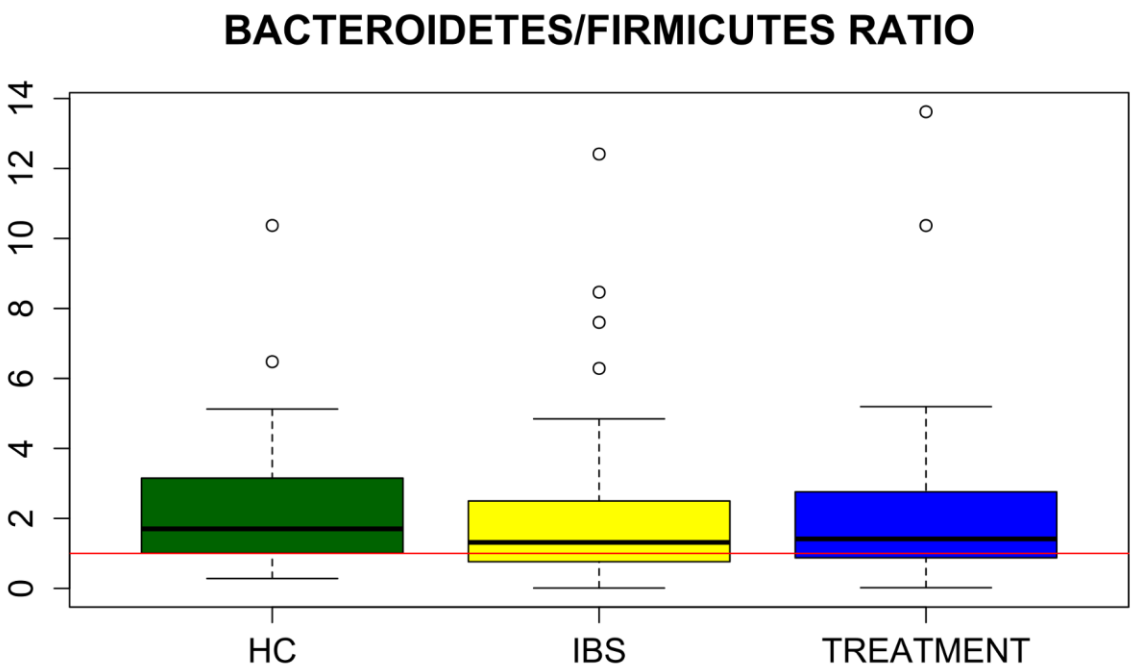


Figure S2. PCA plot of IBS patients (D: Diarrhea subgroup; C: Constipation subgroup; M: Mixed symptoms subgroup) before treatment and healthy controls (HC).

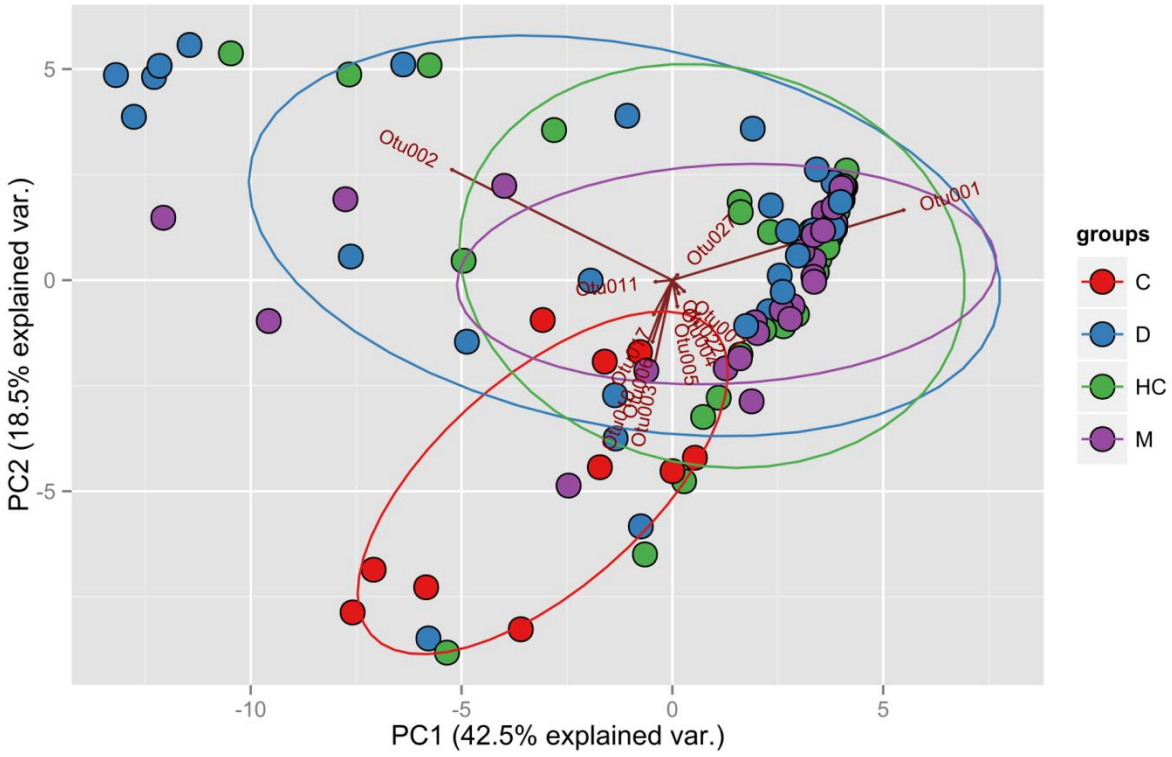
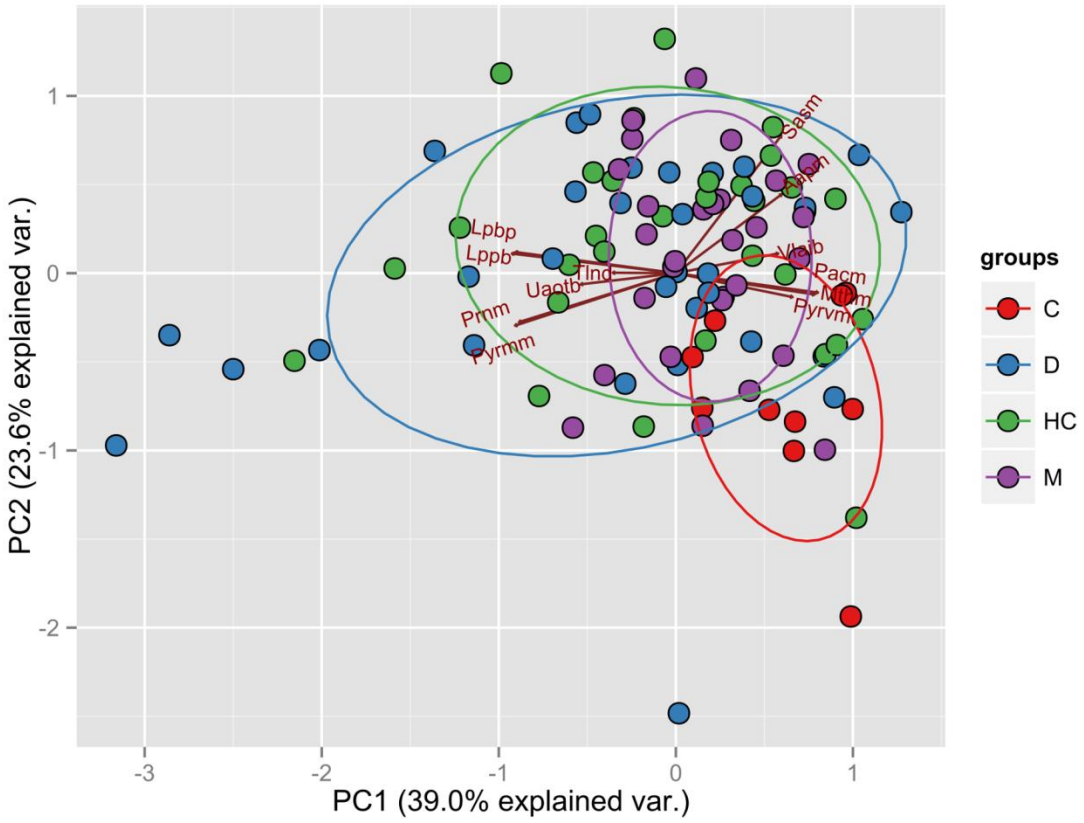
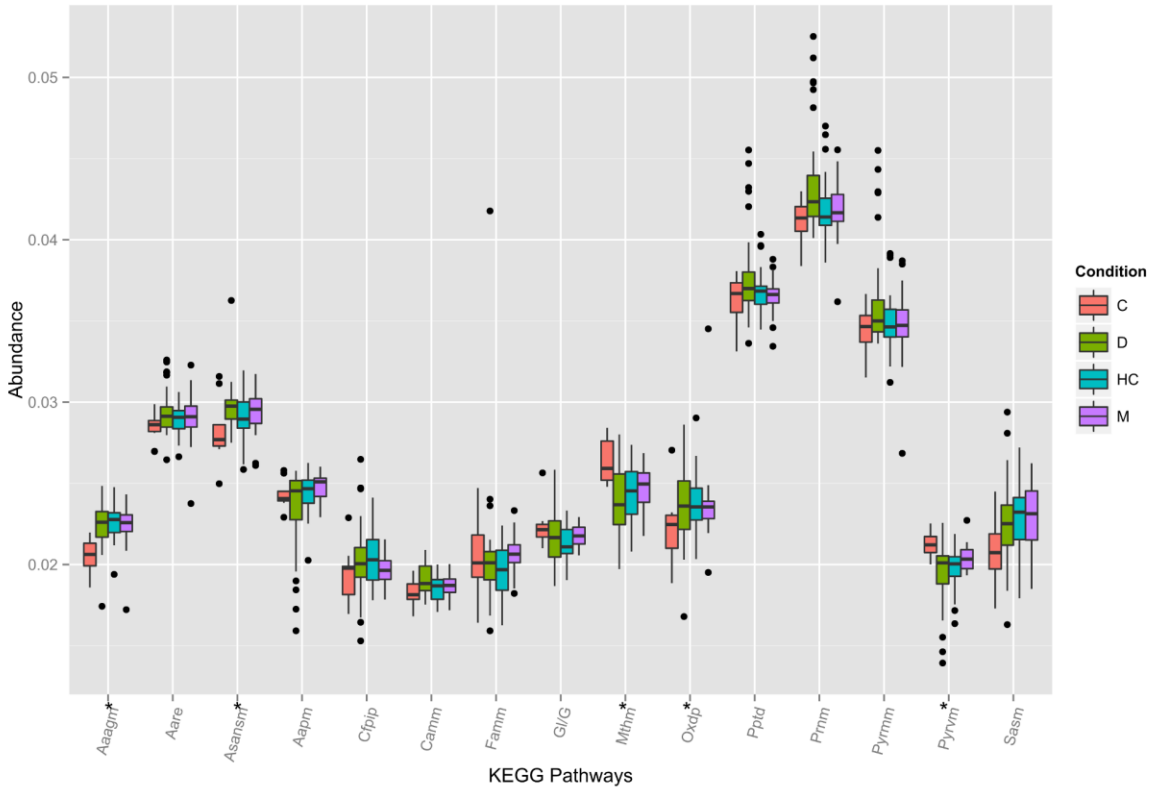


Figure S3. PCA plot of IBS patients before treatment and healthy controls on a functional level, based on KEGG pathway assignment to bacterial taxa. IBS subgroup (D: Diarrhea; C: Constipation; M: Mixed symptoms) and healthy controls (HC).



Pathway	Abbreviation
Arginine and proline metabolism	Aapm
Fructose and mannose metabolism	Famm
Lipopolysaccharide biosynthesis	Lppb
Lipopolysaccharide biosynthesis proteins	Lpbp
Methane metabolism	Mthm
Peptidoglycan biosynthesis	Pptb
Porphyrin and chlorophyll metabolism	Pacm
Purine metabolism	Prnm
Pyrimidine metabolism	Pyrmm
Pyruvate metabolism	Pyrvm
Starch and sucrose metabolism	Sasm
Toluene degradation	Tlnd
Ubiquinone and other terpenoid-quinone biosynthesis	Uaotb
Valine, leucine, and isoleucine biosynthesis	Vlaib

Figure S4. Boxplot of abundances of 15 of the most abundant metabolic pathways, split by IBS type (including healthy controls). IBS subgroup (D: Diarrhea; C: Constipation; M: Mixed symptoms) and healthy controls (HC).



Pathway	Abbreviation
Alanine, aspartate, and glutamate metabolism	Aaagm
Amino acid-related enzymes	Aare
Amino sugar and nucleotide sugar metabolism	Asansm
Arginine and proline metabolism	Aapm
Carbon fixation pathways in prokaryotes	Cfpip
Cysteine and methionine metabolism	Camm
Fructose and mannose metabolism	Famm
Glycolysis/Gluconeogenesis	Gl/G
Methane metabolism	Mthm
Oxidative phosphorylation	Oxdp
Peptidases	Pptd
Purine metabolism	Prnm
Pyrimidine metabolism	Pyrmm
Pyruvate metabolism	Pyrvm
Starch and sucrose metabolism	Sasm

Figure S5. Representative ^{1}HNMR spectra of fecal samples in $CDCl_3$ at 300° K in (A) a healthy control (HC) and (B) a patient with IBS

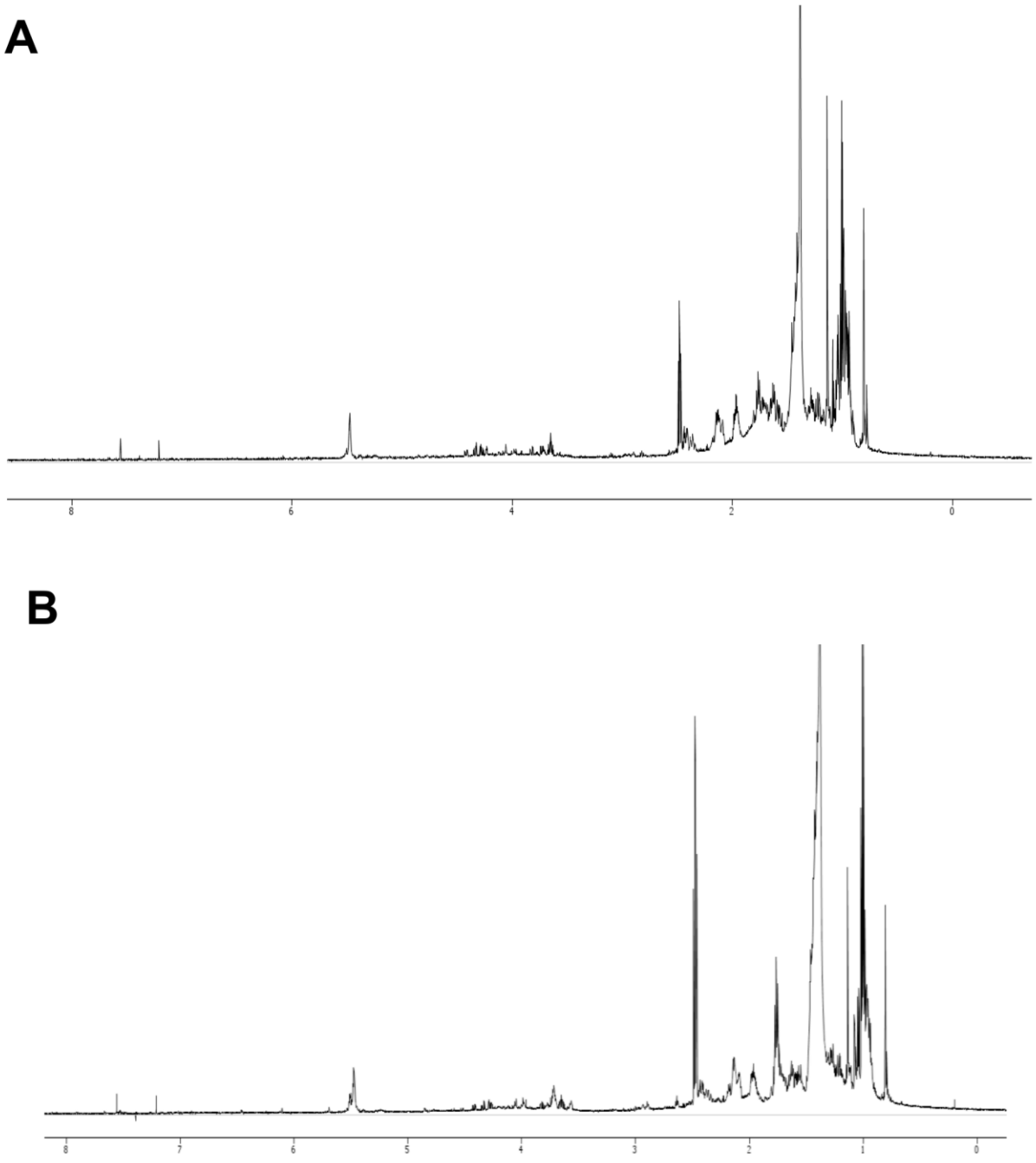


Figure S6. Distribution of the GC-MS measurements of samples from healthy controls (HCs) and IBS patients before treatment (IBS-M, IBS-D, and IBS-C) on the plane spanned by the two first principal components. The PC scores were computed for 13 metabolites exhibiting significant pairwise abundance changes between the studied groups (Table S2).

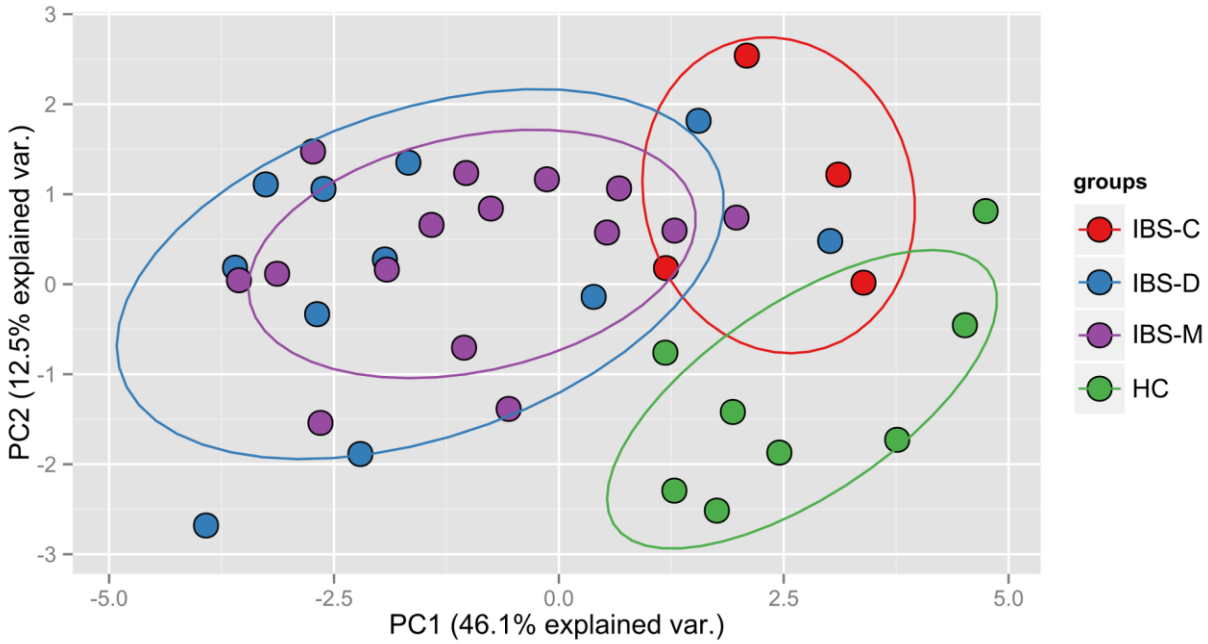


Figure S7. Distribution of the GC-MS measurements of samples from healthy controls (HCs) and IBS patients after treatment (IBS-M II, IBS-D II, and IBS-C II) on the plane spanned by the two first principal components. The PC scores were computed for 13 metabolites exhibiting significant pairwise abundance changes between the studied groups (Table S3).

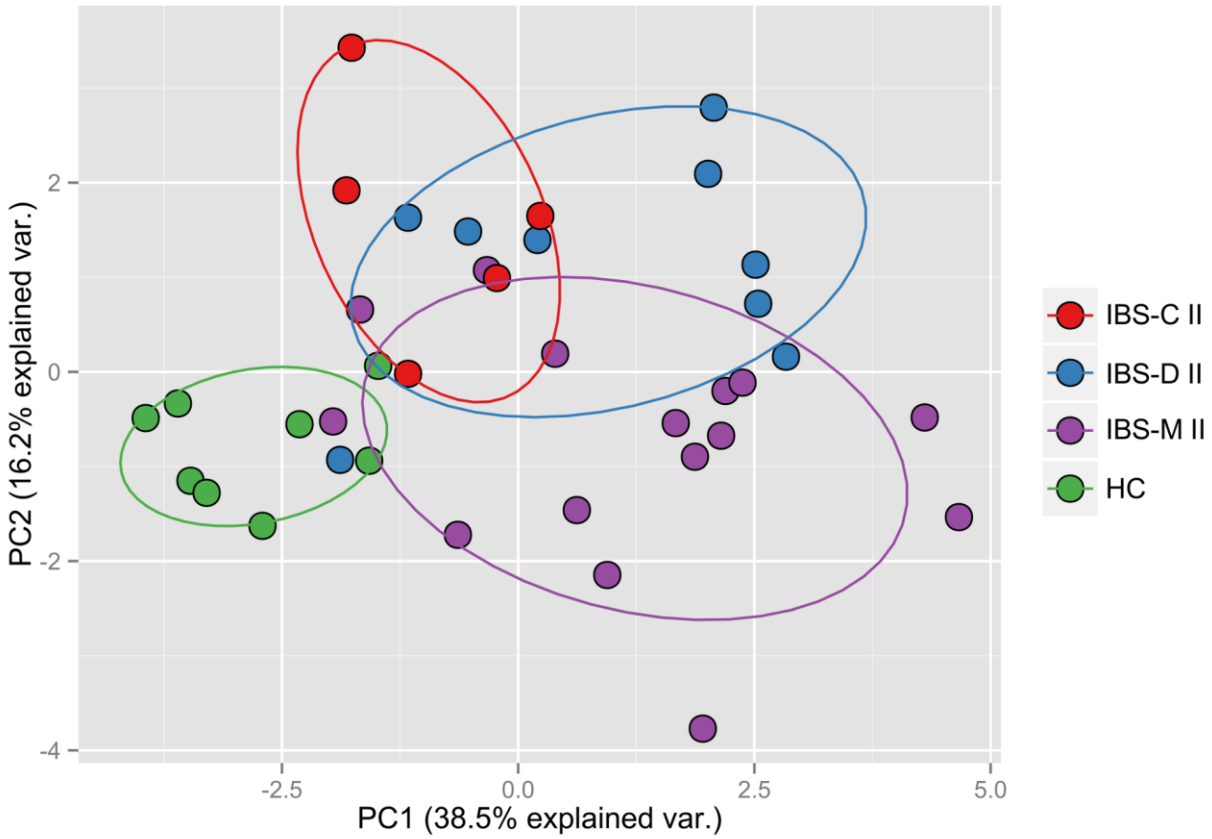


Table S1. Partial least-squares-discriminant analysis models obtained from proton nuclear magnetic resonance-based analysis of fecal samples.

Comparison	Sample number	R^2X(cum)	Q^2(cum)	p value	Number of latent variables
HC vs. IBS I	30	0.497	0.372	0.02	2
HC vs. IBS II	32	0.475	0.109	0.71	2
IBS II_n vs IBS II	26	0.481	-0.0931	1	2
HC vs. IBS-C II	8	0.352	0.468	0.17	1
HC vs. IBS-D II	20	0.509	0.207	0.45	2
HC vs. IBS-M II	20	0.549	0.00579	1	2

HC: healthy control; **IBS-D**: Diarrhea subgroup, before treatment **(DI)**, after treatment with improvement **(DII); IBS-C**: Constipation subgroup, before treatment **(CI)**, after treatment with improvement **(CII); IBS-M**: Mixed symptoms subgroup, before treatment **(MI)**, after treatment with improvement **(MII); IBS II_n**: IBS patients with no improvement; **IBS II**: IBS patients with improvement

Table S2. List of 13 metabolites exhibiting significant (FDR, ≤0.1, and FC, ≥1.5) differences in abundance between healthy controls (HCs) and the three subgroups of IBS patients before treatment (IBS-M, IBS-D, and IBS-C).

	ANOVA	IBS-D vs. HC		IBS-M vs. HC		IBS-C vs. HC		IBS-D vs. IBS-C		IBS-M vs IBS-C		IBS-D vs IBS-M	
	p-value	p-value	FC	p-value	FC	p-value	FC	p-value	FC	p-value	FC	p-value	FC
Tocopherol-τ-tms-derivative	0.0102	0.0029	0.28	0.0213	0.5			0.0024	0.14	0.0016	0.26	0.0399	0.56
1H-Indole, 3-methyl-1-(trimethylsilyl)-	0.0325	0.0004	4.18	0.0053	2.6			0.0186	3.06				
N,O-Bis-(trimethylsilyl)phenylalanine	0.0358	0.0024	18.34	0.0006	6.7								
d-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)-	0.0358	0.0052	13.01	0.0002	7.66								
Pyrimidine, 2,4-bis[(trimethylsilyl)oxy]-	0.0358	0.0017	6.96	0.0012	10.02								
Eicosanoic acid, trimethylsilyl ester	0.0358	0.0109	0.37	0.0035	0.39			0.0337	0.4	0.0063	0.42		
Silane, tetramethyl-	0.0451			0.0018	0.5							0.0318	1.44
2-Desoxy-pentos-3-ulose, bis(methoxime),O,O'-bis(trimethylsilyl)-	0.0558	0.0010	0.38	0.0001	0.4								
E-2-Hydroxymethylcyclopentanol, di(trimethylsilyl) ether	0.0692					0.0212	0.25	0.0041	9.69	0.0074	6.5		
Dodecanoic acid, trimethylsilyl ester	0.0812			0.0024	0.07							0.0244	2.92
l-Alanine, trimethylsilyl ester	0.0812	0.0061	2.75	0.0076	2.92								
n-Pentadecanoic acid, trimethylsilyl ester	0.0812			0.0267	0.5					0.0049	0.36		
Heptadecanoic acid, trimethylsilyl ester	0.0844			0.0335	0.51			0.0328	0.4	0.0030	0.28		

Table S3. List of 13 metabolites exhibiting significant (FDR, ≤0.1, and FC, ≥1.5) differences in abundance changes between healthy controls (HCs) and the three subgroups of IBS patients after treatment (IBS-M II, IBS-D II, and IBS-C II). Positions marked in bold indicate metabolites that also differed significantly before treatment.

Metabolite name	**ANOVA**	**IBS-D II vs. HC**		**IBS-M II vs. HC**		**IBS-C II vs. HC**		**IBS-D II vs. IBS-C**		**IBS-M II vs IBS-C**		**IBS-D II vs IBS-M**	
	p-value	**p-value**	**FC**	**p-value**	**FC**	**p-value**	**FC**	**p-value**	**FC**	**p-value**	**FC**	**p-value**	**FC**
Propanoic acid, 2-(methoxyimino)-, trimethylsilyl ester	0.0011	0.0070	0.7	0.0000	0.4	0.0027	0.6					0.0133	1.5
Nonanoic acid, trimethylsilyl ester	0.0455	0.0013	0.3									0.0021	0.5
Pyrimidine, 2,4-bis[(trimethylsilyl)oxy]-	0.0455			0.0001	5.6	0.0021	6.0						
N,O-Bis-(trimethylsilyl)phenylalanine	0.0455	0.0016	9.1	0.0017	13.0	0.0019	4.3						
Pentasiloxane, dodecamethyl-	0.0455	0.0289	0.7							0.0233	2.2	0.0040	0.5
d-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)-	0.0455	0.0068	13.5	0.0007	10.6	0.0228	3.4						
1H-Indole, 3-methyl-1-(trimethylsilyl)-	0.0455	0.0124	3.8	0.0010	3.7	0.0117	2.6						
2-Desoxy-pentos-3-ulose, bis(methoxime),O,O'-bis(trimethylsilyl)-	0.0455			0.0024	0.5					0.0222	0.3	0.0103	3.0
1,2-Benzenediol bis(trimethylsilyl) ether	0.0455			0.0640	1.6	0.0338	0.7			0.0045	2.3	0.0109	0.6
Cyclopentanecarboxylic acid, 1-amino-, bis(trimethylsilyl) deriv.	0.0455	0.0179	2.9	0.0031	3.1			0.0591	2.8	0.0172	3.1		
Tocopherol-τ-tms-derivative	0.0455	0.0002	0.3					0.0017	0.3				
Heptadecanoic acid, trimethylsilyl ester	0.0491	0.0102	0.3	0.0444	0.6			0.0091	0.3	0.0327	0.6		
n-Pentadecanoic acid, trimethylsilyl ester	0.0617			0.0076	0.4					0.0149	0.4		