

## **Supplementary material**

### **Applied Microbiology and Biotechnology**

#### **Timing for the second fecal microbiota transplantation to maintain the long-term benefit from the first treatment for Crohn's disease**

Pan Li<sup>1,2</sup>, Ting Zhang<sup>1,2</sup>, Yandong Xiao<sup>3,4</sup>, Liang Tian<sup>3</sup>, Bota Cui<sup>1,2</sup>, Guozhong Ji<sup>1,2</sup>, Yang-Yu Liu<sup>3,5</sup>, Faming Zhang<sup>1,2</sup>

<sup>1</sup> Medical Center for Digestive Diseases, the Second Affiliated Hospital of Nanjing Medical University, Nanjing 210011, China.

<sup>2</sup> Key Lab of Holistic Integrative Enterology, Nanjing Medical University, Nanjing 210011, China.

<sup>3</sup> Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115, United States.

<sup>4</sup> Science and Technology on Information Systems Engineering Laboratory, National University of Defense Technology, Changsha 410073, China.

<sup>5</sup> Center for Cancer Systems Biology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115, United States.

Pan Li and Ting Zhang contributed equally to this study.

#### **Corresponding author:**

Faming Zhang, MD, PhD

Professor of Medicine

Director of Intestinal Diseases

The Second Affiliated Hospital of Nanjing Medical University

Tel: +86-25-58509883

E-mail address: [fzhang@njmu.edu.cn](mailto:fzhang@njmu.edu.cn)

ORCID: 0000-0003-4157-1144

Yang-Yu Liu, PhD

Assistant Professor at Harvard Medical School

Associate Scientist at Brigham and Women's Hospital

Tel: +1-617-525-2714

E-mail address: [yyl@channing.harvard.edu](mailto:yyl@channing.harvard.edu)

ORCID: 0000-0003-4184-5379

### **Supplemental Table Legends**

**Supplemental Table S1** Gut microbial compositions in CD patients and donors at the phylum level.

**Supplemental Table S2** Gut microbial compositions in CD patients and donors at the family level.

**Supplemental Table S3** Gut microbial compositions in CD patients and donors at the genus level.

**Supplemental Table S4** Gut microbial compositions in CD patients before and after FMT at the family level.

**Supplemental Table S5** Gut microbial compositions in CD patients before and after FMT at the genus level.

**Supplemental Table S1** Gut microbial compositions in CD patients and donors at the phylum level.

Phylum	Donor (%)	Patient pre-1 <sup>st</sup> -FMT (%)	<i>p</i> -value
<i>Cyanobacteria</i>	0.0283 ± 0.0371	0.0197 ± 0.0591	0.023
<i>Fusobacteria</i>	0.0811 ± 0.1506	9.97 ± 15.33	0.030
<i>Firmicutes</i>	48.33 ± 24.11	27.59 ± 14.35	0.047

**Supplemental Table S2** Gut microbial compositions in CD patients and donors at the family level.

Family	Donor (%)	Patient pre-1 <sup>st</sup> -FMT (%)	<i>p</i> -value	FDR
<i>Ruminococcaceae</i>	23.62 ± 11.36	3.43 ± 2.91	0.0013	0.0825
<i>Clostridiaceae</i>	2.27 ± 3.44	0.46 ± 0.69	0.0243	0.2665
<i>Enterobacteriaceae</i>	1.86 ± 3.26	15.34 ± 24.65	0.0380	0.2687
<i>Coriobacteriaceae</i>	0.12 ± 0.19	0.02 ± 0.03	0.0054	0.1427
<i>Fusobacteriaceae</i>	0.08 ± 0.15	9.95 ± 15.34	0.0302	0.2665
<i>Bifidobacteriaceae</i>	0.06 ± 0.06	0.12 ± 0.35	0.0095	0.2010
<i>Bacteroidales_S24-7</i>	0.04 ± 0.04	0.007 ± 0.006	0.015	0.2265
<i>Comamonadaceae</i>	0.009 ± 0.007	0.002 ± 0.005	0.0175	0.2325
<i>Sphingomonadaceae</i>	0.008 ± 0.008	0.002 ± 0.007	0.0395	0.2791
<i>Pseudomonadaceae</i>	0.006 ± 0.005	0.0005 ± 0.002	0.0030	0.1047
<i>Carnobacteriaceae</i>	0.001 ± 0.002	0.01 ± 0.02	0.0330	0.2665

**Supplemental Table S3** Gut microbial compositions in CD patients and donors at the genus level.

Genus	Donor (%)	Patient pre-1 <sup>st</sup> -FMT (%)	<i>p</i> -value	FDR
<i>Ruminococcus</i>	0.330 ±0.342	2.115 ±5.521	0.0023	0.0752
<i>Anaerostipes</i>	0.378 ±0.731	0.058 ±0.170	0.0124	0.2091
<i>Bifidobacterium</i>	0.060 ±0.055	0.117 ±0.350	0.0095	0.1924
<i>Blautia</i>	1.282 ±1.040	0.875 ±1.980	0.0469	0.3751
<i>Collinsella</i>	0.093 ±0.174	0.004 ±0.009	0.0376	0.3751
<i>Coprococcus</i>	0.281 ±0.352	0.007 ±0.008	0.0007	0.0632
<i>Dorea</i>	0.746 ±0.652	0.431 ±1.080	0.0092	0.1924
<i>Enterobacter</i>	0.001 ±0.002	1.564 ±4.664	0.0418	0.3751
<i>Faecalibacterium</i>	12.590 ±12.067	0.763 ±1.407	0.0013	0.0632
<i>Fusobacterium</i>	0.080 ±0.150	9.947 ±15.322	0.0302	0.3751
<i>Granulicatella</i>	0.001 ±0.002	0.010 ±0.013	0.0330	0.3751
<i>Lachnospira</i>	1.508 ±1.670	0.006 ±0.010	0.0003	0.0632
<i>Pseudomonas</i>	0.006 ±0.005	0.001 ±0.002	0.0030	0.0752
<i>Roseburia</i>	0.694 ±0.474	1.064 ±2.447	0.0458	0.3751
<i>Firmicutes_SMB53</i>	0.530 ±0.850	0.031 ±0.065	0.0029	0.0752
<i>Sphingomonas</i>	0.008 ±0.008	0.002 ±0.007	0.0395	0.3751

**Supplemental Table S4** Gut microbial compositions in CD patients before and after FMT at the family level.

Family	Pre-1 <sup>st</sup> -FMT (%)	3D post-1 <sup>st</sup> -FMT (%)	<sup>a</sup> <i>p</i> -value	FDR	Pre-2 <sup>nd</sup> -FMT (%)	<sup>b</sup> <i>p</i> -value	FDR
<i>Bacteroidaceae</i>	36.70 ± 26.89	33.45 ± 19.07	0.8886	1.000	47.34 ± 17.95	0.4413	0.938
<i>Enterobacteriaceae</i>	15.34 ± 24.65	6.53 ± 8.25	0.4008	0.8603	3.45 ± 4.02	0.3139	0.7272
<i>Veillonellaceae</i>	12.55 ± 12.28	13.97 ± 9.39	0.7794	1.000	9.97 ± 10.60	0.6784	1.000
<i>Fusobacteriaceae</i>	9.95 ± 15.34	11.36 ± 15.82	0.8886	1.000	5.83 ± 8.60	0.6784	1.000
<i>Ruminococcaceae</i>	3.43 ± 2.91	8.13 ± 8.52	0.1614	0.7481	11.45 ± 15.79	0.2604	0.7272
<i>Porphyromonadaceae</i>	2.07 ± 1.95	5.19 ± 5.52	0.208	0.7481	4.21 ± 3.32	0.0209	0.7272
<i>Clostridiaceae</i>	0.46 ± 0.69	0.60 ± 0.69	0.4008	0.8603	0.43 ± 0.30	0.9528	1.000
<i>Aeromonadaceae</i>	0.2 ± 0.56	0.007 ± 0.018	0.0431	0.7481	0.018 ± 0.033	0.735	1.000
<i>Bifidobacteriaceae</i>	0.12 ± 0.35	0.008 ± 0.01	0.0679	0.4696	0.06 ± 0.14	0.7150	1.000
<i>Gemellaceae</i>	0.043 ± 0.072	0.079 ± 0.18	0.753	1.000	0.0019 ± 0.0023	0.0280	0.7272
<i>Desulfovibrionaceae</i>	0.042 ± 0.068	0.11 ± 0.10	0.0180	0.7481	0.054 ± 0.074	0.161	0.7272
<i>Bacteroidales_S24-7</i>	0.007 ± 0.006	0.01 ± 0.03	0.3454	0.7982	0.27 ± 0.58	0.0663	0.7272

<sup>a</sup> *p*-value shows differences in relative abundance between 3D post-1<sup>st</sup>-FMT/pre-1<sup>st</sup>-FMT.

<sup>b</sup> *p*-value shows differences in relative abundance between pre-2<sup>nd</sup>-FMT/pre-1<sup>st</sup>-FMT.

**Supplemental Table S5** Gut microbial compositions in CD patients before and after FMT at the genus level.

Genus	Pre-1 <sup>st</sup> -FMT (%)	3D post-1 <sup>st</sup> -FMT (%)	<sup>a</sup> <i>p</i> -value	FDR	Pre-2 <sup>nd</sup> -FMT (%)	<sup>b</sup> <i>p</i> -value	FDR
<i>Ruminococcus</i>	2.115 ± 5.521	3.016 ± 3.967	0.0357	0.5101	1.841 ± 3.068	0.4413	0.6995
<i>Bilophila</i>	0.042 ± 0.068	0.088 ± 0.078	0.0180	0.5101	0.029 ± 0.059	0.7794	0.8295
<i>Faecalibacterium</i>	0.763 ± 1.407	3.807 ± 7.291	0.2076	0.5101	3.265 ± 4.147	0.0382	0.5562
<i>Gemella</i>	0.022 ± 0.042	0.054 ± 0.128	0.7532	0.8175	0.001 ± 0.002	0.0277	0.5562
<i>Holdemania</i>	0.004 ± 0.008	0.179 ± 0.294	0.0464	0.5101	0.037 ± 0.055	0.0357	0.5562
<i>Parabacteroides</i>	2.069 ± 1.950	5.194 ± 5.521	0.2076	0.5101	4.214 ± 3.321	0.0209	0.5562

<sup>a</sup> *p*-value shows differences in relative abundance between 3D post-1<sup>st</sup>-FMT/pre-1<sup>st</sup>-FMT.

<sup>b</sup> *p*-value shows differences in relative abundance between pre-2<sup>nd</sup>-FMT/pre-1<sup>st</sup>-FMT.