Table S1 Clinical characteristics of patients with constipation

Gender	Male	Female			
	9	11			
Age	18-35	36-50	51-65	66-80	>80
	0	5	11	3	1
Course of the disease (years)	<1	1-5	5.1-10	10.1-20	>20
	2	9	6	1	2

Table S2 Constipation score scale of 20 patients

No.	Treatment time	Frequency of bowel movement	Difficulty: painful evacuation effort	Completeness: feeling incomplete evacuation	Pain: abdominal pain	Time: minutes in lavatory per attempt	Assistance: type of assistance	Failure: unsuccessful attempts for evacuation per 24 h	History: duration of constipation (year)	Total
		0 1-2 times per 1-2 days	0 Never	0 Never	0 Never	0 Less than 5	0 Without assistance	0 Never	0 0	
		1 2 times per week	1 Rarely	1 Rarely	1 Rarely	1 5-10	1 Stimulant laxatives	1 1-3	1 1-5	
		2 once per week	2 Sometimes	2 Sometimes	2 Sometimes	2 10-20		2 3-6	2 5-10	
		3 Less than once per week	3 Usually	3 Usually	3 Usually	3 20-30	2 Digital assistance or enema	3 6-9	3 10-20	
		4 Less than once per month	4 Always	4 Always	4 Always	4 More than 30		4 More than 9	4 More than 20	
1	before	3	3	4	3	3	2	2	3	23
	after	1	1	2	1	3	0	1	3	12
2	before	3	4	2	3	4	2	3	2	23
	after	2	2	0	1	3	0	0	2	10

3	before	3	4	3	4	4	2	3	3	26
	after	2	2	0	1	3	1	0	3	12
4	before	4	4	3	3	3	2	3	4	26
	after	3	2	2	2	2	0	2	4	17
5	before	3	4	4	4	4	1	2	3	25
	after	2	2	1	1	3	0	1	3	13
6	before	3	4	4	2	4	1	2	3	23
	after	1	1	1	0	0	0	0	3	6
7	before	3	4	3	3	4	2	3	4	26
	after	2	2	0	1	2	1	1	4	13
8	before	3	4	4	4	4	1	3	3	26
	after	2	2	1	2	2	0	2	3	14
9	before	3	4	4	4	4	1	3	3	26
	after	2	2	1	1	2	0	0	3	11

10	before	3	4	2	3	3	1	2	4	22
	after	2	1	2	1	3	1	1	4	15
11	before	3	4	4	3	3	2	2	3	24
	after	2	1	1	1	1	1	0	3	10
12	before	3	4	3	2	4	1	3	3	23
	after	2		1	0	2	0	0	3	8
13	before	3	4	3	3	4	1	3	2	23
	after	1	1	0	0	1	1	1	2	7
14	before	3	4	4	2	4	2	2	3	24
	after	2	1	1	0	1	0	1	3	9
15	before	4	4	4	2	3	2	3	3	25
	after	3	2	1	0	2	0	1	3	12
16	before	4	4	4	3	3	2	2	4	26
	after	3	2	1	1	2	0	0	4	13

17	before	2	4	2	3	3	1	2	3	20
	after	1	2	0	1	1	0	0	3	8
18	before	3	3	3	2	4	2	3	3	23
	after	3	1	1	0	1	0	0	3	9
19	before	3	4	3	4	4	2	3	2	25
	after	2	2	1	1	2	0	0	2	10
20	before	3	4	4	2	4	2	3	3	25
	after	2	1	1	0	2	0	0	3	9
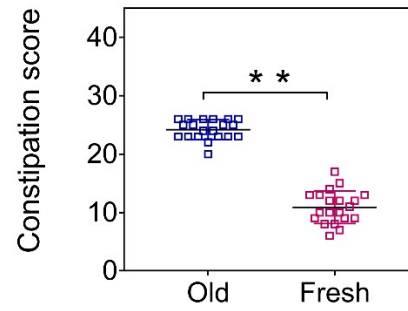
Figure S1 Constipation score were evaluated according to the questionnaire submitted by the patient.
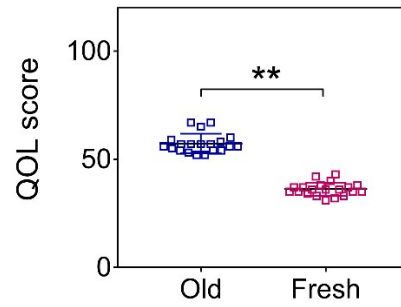
Figure S2 Quality of life (QOL) score were evaluated according to the questionnaire submitted by the patient.
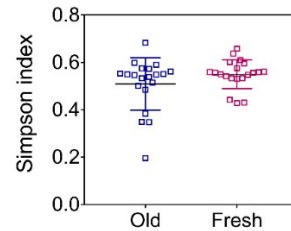
Figure S3 The Simpson index (n=20). Alterations in gut microbial communities between fresh feces and long-term accumulated feces (old feces) in patients with refractory constipation
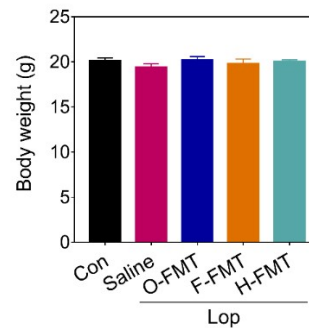

Figure S4 Body weight of mice by transplanting fecal microbiota from the old and fresh feces of refractory constipation patients and healthy volunteers, respectively. FMT, fecal microbiota transplantation; Lop, Loperamide; Con (control) group; Saline group, the mice were gavaged saline; O-FMT group, constipation mice were transplanted with the fecal microbiota from the old feces of refractory constipation patients; F-FMT group, constipation mice were transplanted with the fecal microbiota from the fresh feces of refractory constipation patients; H-FMT group, constipation mice were transplanted with the fecal microbiota from healthy controls. All data are shown as mean ± SD (n=6).
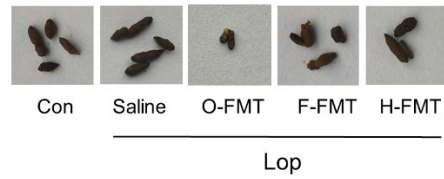
Con   Saline   O-FMT   F-FMT   H-FMT

Lop

Figure S5 The representative images about the appearance of mice feces.

A   Old feces   Fresh feces

B   Fecal moisture content (%)   **

Old feces   Fresh feces

Figure S6 (A) The representative images about the appearance of old feces and fresh feces. (B) The fecal moisture content between fresh and old feces of patients with refractory constipation.