

## **Supplementary materials**

Figure S1 High dose of FOS on obesity and intestinal tract. (A) Serum TC, TG, ALT and AST in HFD and HFD-HF mice. (B) H&E staining of the liver tissues from the HFD and HFD-HF groups (gavage for 4 months). (C) H&E, F4-80 staining and macrophages quantification of the ileum and cecum between the groups. (D) Concentrations of fecal glucose, fructose and FOS in normal pellets of HFD and HFD-HF mice (after gavage for 24 h) or liquid stools of HFD-HF mice (after gavage for 4 h). TC, total cholesterol; TG, triglyceride; AST, aspartate aminotransferase; ALT, alanine aminotransferase; \*p < 0.05, compared between the two groups. Data are represented as the mean ± SEM.



**Figure S2 Excessive intake of FOS affects intestinal and microbiota structure. (A)** Shannon, ACE, Chao and Simpson indices, **(B)** histograms of phylum (left) and class (right) abundance in gut microbiota. HFD, high-fat diet gavage with saline; HFD-LF, high-fat diet gavage with low dose of FOS; HFD-MF, high-fat diet gavage with middle dose of FOS; HFD-HF, high-fat diet gavage with high dose of FOS; ns, not significant. Data are represented as the means ± SEM.



**Figure S3 Role of SCFA in resisting FOS-induced diarrhea.** (**A**) Comparison of fecal bacterial load between HFD and HFD-HF mice at 0, 4, 16 and 24 h after gavage. (**B**) Concentration of acetate, propionate and butyrate in normal pellets of HFD and HFD-HF mice (after gavage for 24 h) or liquid stools of HFD-HF mice (after gavage for 4 h). (**C**) Concentrations of acetate, propionate and butyrate in feces after Mix and FMT treatment. (**D**) Concentration of butyrate in feces after butyrate (100 mM) or β-acid (20 ppm) treatment. (**E**) Diarrhea assessment at 4 h after gavage with 4.5 g/kg FOS combined with NW, β-acid (20 ppm) or butyrate/acetate (100 mM) in drinking water. Mix, mixed antibiotics; FMT, fecal microbiota transplantation; NW, normal water. Data are represented as the mean ± SEM, \**p*< 0.05.



Figure S4 XOS induced idiopathic colitis in obese mice. (A) Diarrhea level, (B) survival rate, (C) relative abundance of *A. muciniphila*, (D) crypt number, (E) histology score, (F) mucus layer thickness and (G) serum PAMPs of mice treated with HFD and HFD-HX. HFD-HX, highfat diet gavage with a high dose of XOS; XOS, xylooligosaccharide. Data are represented as the mean  $\pm$  SEM. \**p*< 0.05, compared between two groups.



Figure S5 FOS/XOS induced idiopathic colitis in healthy mice. (A) Diarrhea level, (B) survival rate, (C) relative abundance of *A. muciniphila*, (D) crypt number, (E) histology score, (F) mucus layer thickness and (G) serum PAMPs of mice treated with CD, CD-HF and CD-HX. CD, chow diet gavage with saline; CD-HF, chow diet gavage with high dose of FOS; CD-HX, chow diet gavage with high dose of XOS. Data are represented as the mean  $\pm$  SEM. \**p*< 0.05, compared between two groups.



Figure S6 Screening probiotics protecting against adverse effects induced by FOS in *in vitro* and *in vivo* experiments. (A) Representative inhibition zone and inhibitory capacity of *L. paracasei* and *P. pentosaceus* on different pathogenic bacteria. (B) FOS degradation curve induced by *L. paracasei* and *P. pentosaceus*. (C) Serum levels of IgA and IgG, (D) relative mRNA expression of β-catenin, Muc2, ZO-1, occludin, (E) IL-1β, IL-4, IL-6 and IL-10 in the

colon after *L. paracasei* and *P. pentosaceus* treatment for 4 months. HFD, high fat diet and gavage with saline; HFD-HF, high fat diet and gavage with high dose of FOS; HFD-HF-L, high fat diet and gavage with high dose of FOS and *L. paracasei*; HFD-HF-P, high fat diet and gavage with high dose of FOS and *P. pentosaceus*; \*p< 0.05; ns: not significant. Data are represented as the mean ± SEM.



**Figure S7 Probiotics improved the composition of intestinal flora. (A)** Ace, Shannon, Chao and Simpson indices, **(B)** histogram of phylum and class abundance, **(C)** histogram of the top 10 key species differences and **(D)** correlation coefficient heatmap of gut microbiota in HFD-HF mice after *L. paracasei* and *P. pentosaceus* treatment for 4 months.

Crown	Food	Information of gavage			Animal
Group	roou	10 am	2 pm	duration	number
HFD	HFD	Saline	-	4 months	10
HFD-LF	HFD	1.2 g/kg FOS	-	4 months	10
HFD-MF	HFD	3.6 g/kg FOS	-	4 months	10
HFD-HF	HFD	7.2 g/kg FOS	-	4 months	10
HFD-HX	HFD	7.2 g/kg XOS	-	4 months	10
HFD'	HFD	Saline	Saline	4 months	10
HFD-HF'	HFD	7.2 g/kg FOS	Saline	4 months	10
HFD-MF×2	HFD	3.6 g/kg FOS	3.6 g/kg FOS	4 months	10
CD	CD	Saline	-	2 months	10
CD-HF	CD	7.2 g/kg FOS	-	2 months	10
CD-HX	CD	7.2 g/kg XOS	-	2 months	10
HFD-HF-L	LIED	7.2 g/kg FOS &		4 months	10
	пгр	$10^8 \text{ CFU/kg L}$	-		10
HFD-HF-P	LIED	7.2 g/kg FOS &		4 months	10
	пгD	10 <sup>8</sup> CFU/kg P	-		10

Table S1 Animal groups and details

Table S2 SCFAs treat animal diarrhea

Group	Food	Drinking (14 days)	Gavage (Last 2 days)	Animal
Oloup			Gavage (Last 2 days)	number
NW	HFD	Normal water	4.5 g/kg FOS	10
β-acid	HFD	β-acid (20 ppm)	4.5 g/kg FOS	10
Butyrate	HFD	Butyrate (100 mM)	4.5 g/kg FOS	10
Acetate	HFD	Acetate (100 mM)	4.5 g/kg FOS	10
Vancomycin	HFD	Vancomycin (0.5 g/L)	4.5 g/kg FOS	10
Neomycin	HFD	Neomycin (0.5 g/L)	4.5 g/kg FOS	10
Mix	HFD	Vancomycin and	$4.5 \alpha/k \alpha EOS$	10
		neomycin (0.5 g/L)	4. <i>3</i> g/kg r05	10

Table S3 Antibiotics and FMT used in HFD-feeding animals (n=10)

Time (day)	1	1-14	15	15-17	17
Treatment	Gavage	Drinking	Gavage	Gavage	Gavage
Concentration	4.5 g/kg FOS	0.5 g/L neomycin and vancomycin	4.5 g/kg FOS	0.4 mL bacteria solution (0.05g feces per mL)	4.5 g/kg FOS

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Origin	Gene symbol	Forward primer	Reverse primer
	β-catenin	TCTCCTTGGCTGGCCTTTCTA	GTCACACAGCCCTGTCAAGA
Mouse	ZO-1	AGGACACCAAAGCATGTGAG	GGCATTCCTGCTGGTTACA
	MUc2	ATGCCCACCTCCTCAAAGAC	GTAGTTTCCGTTGGAACAGTGAA
	Occludin	TCTGCTTCATCGCTTCCTTAG	GTCGGGTTCACTCCCATTA
	IL-1β	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
	IL-4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
	IL-6	AAGTCGGAGGCTTAATTACACATGT	CCATTGCACAACTCTTTTCTCATTC
	IL-10	CTTACTGACTGGCATGAGGATCA	GCAGCTCTAGGAGCATGTGG
	GAPDH	GACGGCCGCATCTTCTTGT	CAGTGCCAGCCTCGTCCCGTACAA
Bacteria	341F and 806R	ACTCCTACGGGAGGCAGCAG	GGACTACHVGGGTWTCTAAT

Table S4 Mouse and bacterial primer sequences for real-time qRT-PCR