

Supporting Information

***Eurotium cristatum*, a potential probiotic fungus from Fuzhuan brick tea, alleviated obesity in mice by modulating gut microbiota**

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Table S1 Summary of pilot dietary intervention of type 2 diabetes or metabolic syndrome using Fuzhuan brick tea¹⁻³.

Index	Before treatment		After treatment		Number of patients	References
	Treatment group	Control group	Treatment group	Control group		
FBG (mmol/L)	8.81±1.87	8.66±1.30	6.47±1.09 ^{*#}	7.31±1.42 [*]	Control group: 20 Treatment group: 20	1
OGTT 2h (mmol/L)	10.58±1.62	10.32±2.09	8.21±1.34 ^{*#}	9.13±1.50 [*]		
HbA1c (%)	7.11±1.00	7.38±1.47	6.01±0.58 ^{*#}	6.42±0.65 [*]		
LDL-C (mmol/L)	3.32±0.37	3.44±0.51	2.49±0.39 ^{*#}	2.77±0.39 [*]		
HDL-C (mmol/L)	0.87±0.13	0.85±0.14	1.17±0.17 ^{*#}	1.06±0.13 [*]		
Blood uric acid (μmol/L)	404±39	415±42	347±45 ^{*#}	381±52 [*]		
FBG (mmol/L)	10.04±2.95		8.06±1.78 [*]		Treatment group: 45	2
TC (mmol/L)	6.7±0.79		5.75±0.78 [*]			
TG (mmol/L)	3.3±1.43		1.86±1.12 ^{**}			
HDL-C (mmol/L)	1.3±0.40		1.34±0.44			
LDL-C (mmol/L)	4.2±0.73		3.71±0.60 [*]			
TC (mmol/L)	6.35±1.23		5.24±1.16 ^{**}		Treatment group: 31	3
TG (mmol/L)	4.02±2.15		3.26±1.88 [*]			
LDL-C (mmol/L)	4.25±1.23		4.17±1.27			
HDL-C (mmol/L)	1.38±0.36		1.48±0.31 [*]			
<div> <div>* <i>P</i><0.05</div> <div>** <i>P</i><0.01</div> </div>						

In reference 1: the patients in the control treatment group were orally given metformin (0.5 g × 2/day) and the traditional Chinese medicine Danshen pill (10 capsules × 3/day); The patients in the treatment group were orally given mulberry black tea 30 g (mulberry leaf 1: 5 Fuzhuan brick tea), as well as metformin (0.5 g × 2/day) and the traditional Chinese medicine Danshen pill (10 capsules × 3/day). The treatments lasted for 12 weeks. * $P<0.05$: there were significant differences before treatment and after treatment after 12 weeks; # $P<0.05$: there were significant differences between the treatment and control groups after 12 weeks.

In reference 2: the patients were given Fuzhuan brick tea (15 g/day) for 1 month. * $P<0.05$, ** $P<0.01$: there were significant differences before treatment and after treatment after 1 months.

In reference 3: the patients were orally given the extract of Fuzhuan brick tea(1L/day) 120 mg/100 mL tea beverage for 30-34 days. * $P<0.05$, ** $P<0.01$: there were significant differences before treatment and after treatment after 30-34 days. FBG, fasting glucose; OGTT, oral glucose tolerance test; TC, total cholesterol; TG, triglyceride; HbA1c, hemoglobin a1c.

References:

1. Liu Z, et al. Clinical research on the effect of mulberry black tea on adjuvant therapy of type 2 diabetes. *Chinese Journal of Guangdong Tea Industry* **4**, 12-15 (2018). (in Chinese)
2. Huang W, et al. Research on the effect of black tea on regulating metabolic syndrome. *Chinese Journal of Capital Food and Medicine* **6**, 29-31 (2016). (in Chinese)
3. Xiao, W. et al. Study on the regulation of blood lipid by Fu Zhuan Tea. *Journal of Tea Science*, **27**, 211 – 214 (2007). (in Chinese)

Table S2 The survival of *E. cristatum* in the feces of C57BL/6J mice of different treatment groups for the first three weeks. a, The presence of *E. cristatum* in the feces of C57BL/6J mice by different treatments after one week (n=5). *E. cristatum* was plated on M40Y agar plates. NCD, normal chow diet group; HFD, high-fat diet group; FBT, normal chow diet with Fuzhuan brick tea; FFBT, normal chow diet with filtered Fuzhuan brick tea; *E. cristatum* (10^4 CFU/day).


























groups	1	2	3	4	5
NCD					
HFD					
FBT					
FFBT					
<i>E. cristatum</i>					

Table S2 b, The presence of *E. cristatum* in the feces of C57BL/6J mice by different treatments after two weeks (n=5). *E. cristatum* was plated on M40Y agar plates. NCD, normal chow diet group; HFD, high-fat diet group; FBT, normal chow diet with Fuzhuan brick tea; FFBT, normal chow diet with filtered Fuzhuan brick tea; *E. cristatum* (10^4 CFU/day).







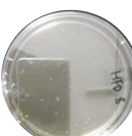

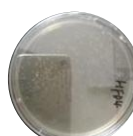


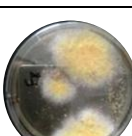


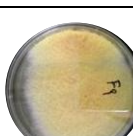
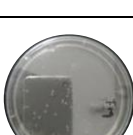





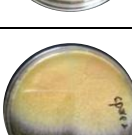

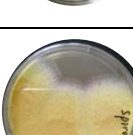

groups	1	2	3	4	5
NCD					
HFD					
FBT					
FFBT					
<i>E. cristatum</i>					

Table S2 c, The presence of *E. cristatum* in the feces of C57BL/6J mice by different treatments after one week (n=5). *E. cristatum* was plated on M40Y agar plates. NCD, normal chow diet group; HFD, high-fat diet group; FBT, normal chow diet with Fuzhuan brick tea; FFBT, normal chow diet with filtered Fuzhuan brick tea; *E. cristatum* (10^4 CFU/day)




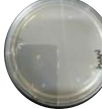



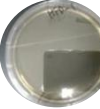




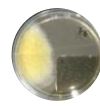
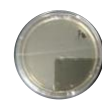
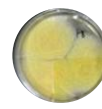
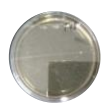



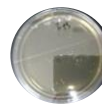





groups	1	2	3	4	5
NCD					
HFD					
FBT					
FFBT					
<i>E. cristatum</i>					

Fig. S1 a, Effects of heating temperature on the survival of *E. cristatum*. 10^4 colony forming units (CFU) of *E. cristatum* were suspended in 1 mL 20% glycerol and heated for 2 min, 5 min, 10 min respectively. b, Effects of heating temperature on the survival of *E. cristatum*. 10^5 colony forming units (CFU) of *E. cristatum* were suspended in 1 mL H₂O and heated for 2 min, 5 min, 10 min respectively. c, Effect of heating temperature (room temperature and 85 °C) of Fuzhuan Brick tea on the survival of *E. cristatum*. The CFU of *E. cristatum* were counted on agar plates containing M40Y medium. There were 3 replicates for every temperature tested

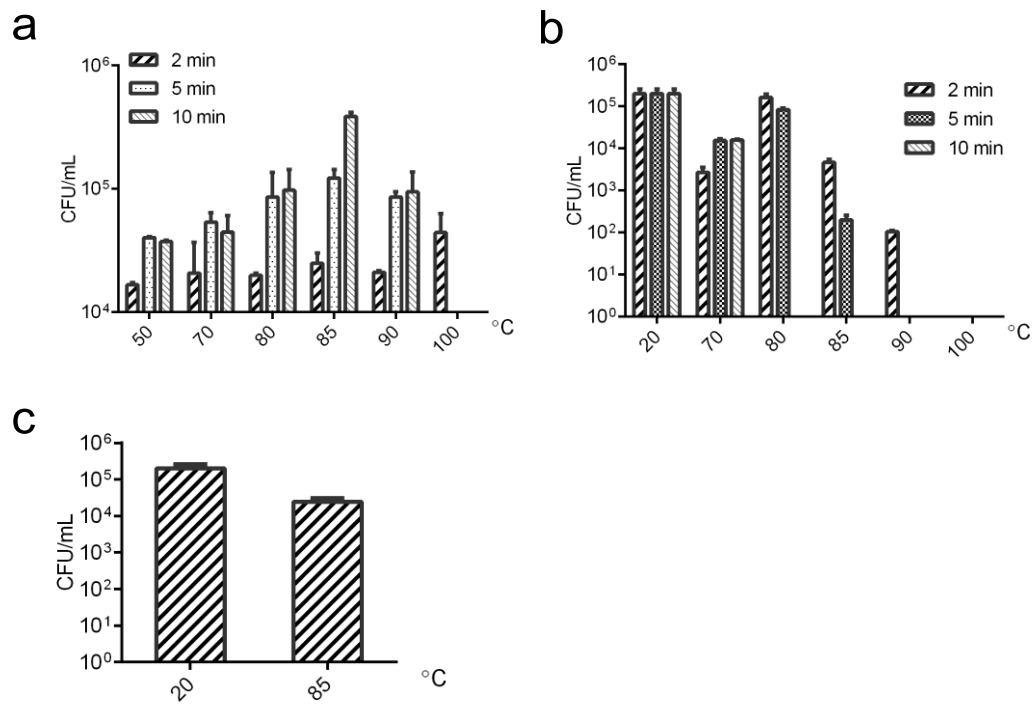


Fig. S2. *E. cristatum* could survive in mouse intestine. a, The timeline for mouse experiments. b, The mice body weights of different groups. NCD, normal chow diet group; FBT, normal chow diet with Fuzhuan brick tea; FFBT, normal chow diet with filtered Fuzhuan brick tea; *E. cristatum* (10^4 CFU/day) and *E. cristatum* (10^3 CFU/day): normal chow diet with the indicated amount of *E. cristatum* in the drinking water. There were 5 mice per group. c, the growth of *E. cristatum* from the feces of NCD group (left) and *E. cristatum* (10^4 CFU/day) mice at the end of 6th week. d, Phylogenetic analysis of the 18S rRNA of *E. cristatum* CB10001 isolated from mice feces using Mega 6 and the Neighbor-Joining method. *Chaetosartorya cremea*: AB002074.1; *Fennellia flavipes*: AB008400.1; *Neosartorya fischeri*: U21299.1; *Eurotium amstelodami*: AB002076.1; *Aspergillus terreus*: AB008409.1; *Aspergillus penicillioides*: AB002078.1; *Aspergillus penicillioides* strain 481: DQ985958.1; *Eurotium cristatum*: JN986762.1.

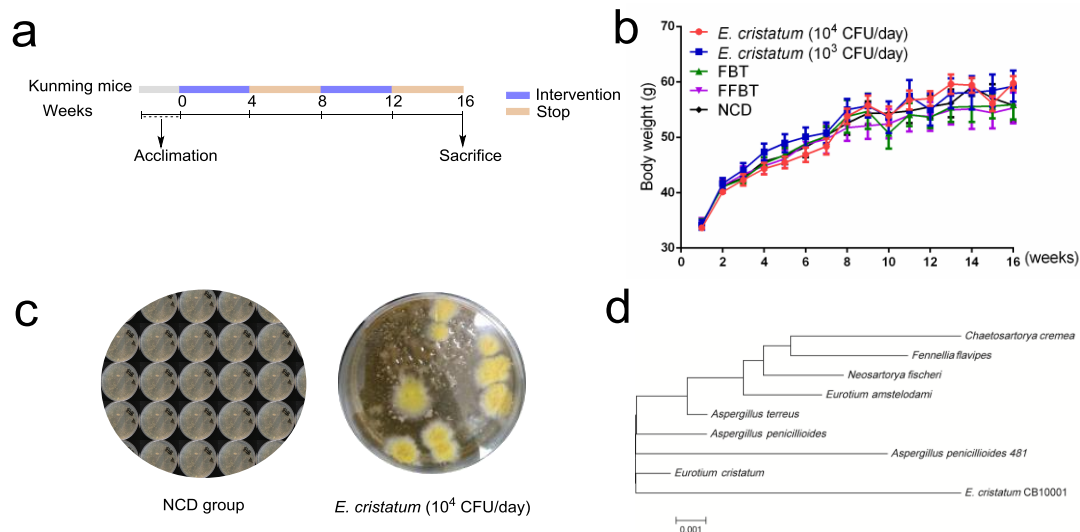


Fig. S3 Diet and water consumption of mice in the first week of treatment for C57BL/6J mice fed with normal chow diet or high-fat diet. a, Diet consumption of each group. b, Water consumption of each group. Data are expressed as means \pm s.e.m (n=5). Graph bars in a and b marked with different letters on top represent statistically significant results ($P<0.05$) based on Newman-Keuls *post hoc* one-way ANOVA analysis, whereas bars labelled with the same letter correspond to results that show no statistically significant differences. In the case whereas two letters are present on top of the bar, each letter should be compared separately with the letters of other bars to determine whether the results show statistically significant differences. NCD, normal chow diet group; HFD, high-fat diet group; FBT, high-fat diet with consumption of Fuzhuan brick tea; FFBT, high-fat diet with the consumption of filtered Fuzhuan brick tea. *E. cristatum*, high fat diet with consumption of *E. cristatum* (10^3 CFU/day). There were 5 mice per group.

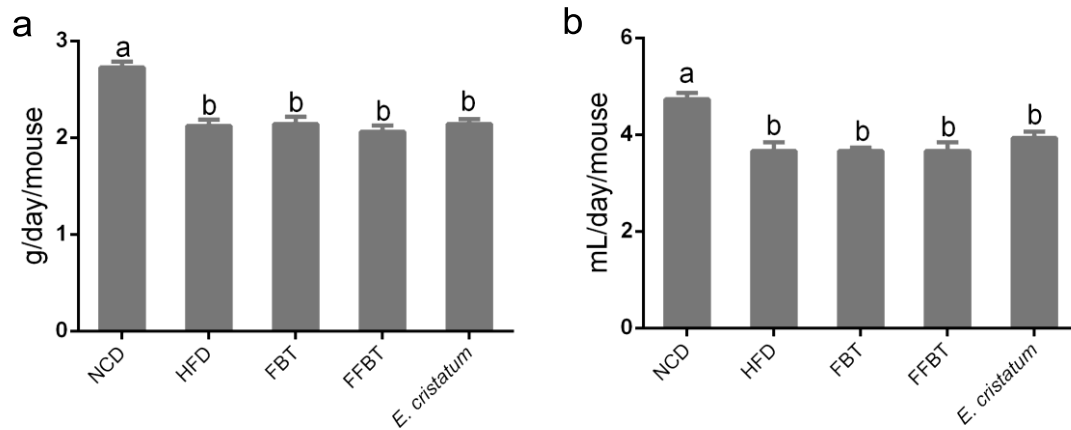


Fig. S4 Alpha diversity analysis of mice microbiota for C57BL/6J mice. a, Rarefaction (chao1) analysis of gut fungi in each treatment group. b, Rarefaction (observed OTUs) analysis of gut fungi in each treatment group. c, Rarefaction (chao1) analysis of gut bacteria in each treatment group. d, Rarefaction (observed OTUs) analysis of gut bacteria in each treatment group. NCD, normal chow diet group; HFD, high-fat diet group; FBT, high-fat diet with consumption of Fuzhuan brick tea; FFBT, high-fat diet with the consumption of filtered Fuzhuan brick tea. *E. cristatum*, high fat diet with consumption of *E. cristatum* (10^3 CFU/day). There were 5 mice per group.

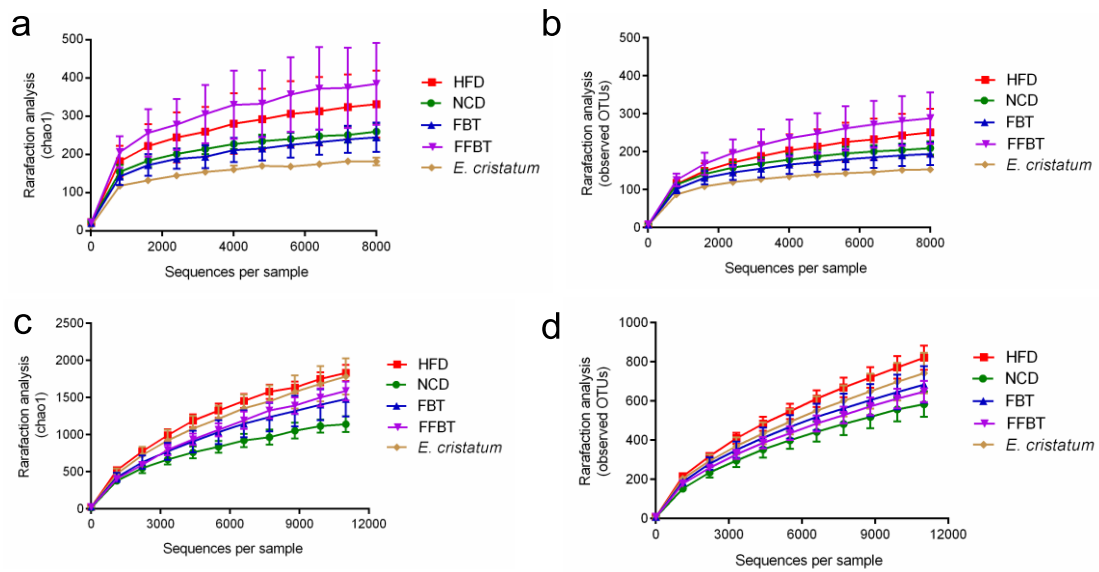


Fig. S5 *E. cristatum*, FBT and FFBT beneficially altered the gut metabolism of high-fat fed mice. PICRUST analysis showed the relative abundance of predicted microbial genes related to metabolism for a, *E. cristatum* and HFD, b, FBT and HFD, c, FFBT and HFD based on Welch's *t* test ($P < 0.05$). The colored circles represent 95% confidence intervals calculated using Welch's inverted method. NCD, normal chow diet group; HFD, high-fat diet group; FBT, high-fat diet with consumption of Fuzhuan brick tea; FFBT, high-fat diet with the consumption of filtered Fuzhuan brick tea. *E. cristatum*, high-fat diet with consumption of *E. cristatum* (10^3 CFU/day). There were 5 mice per group.

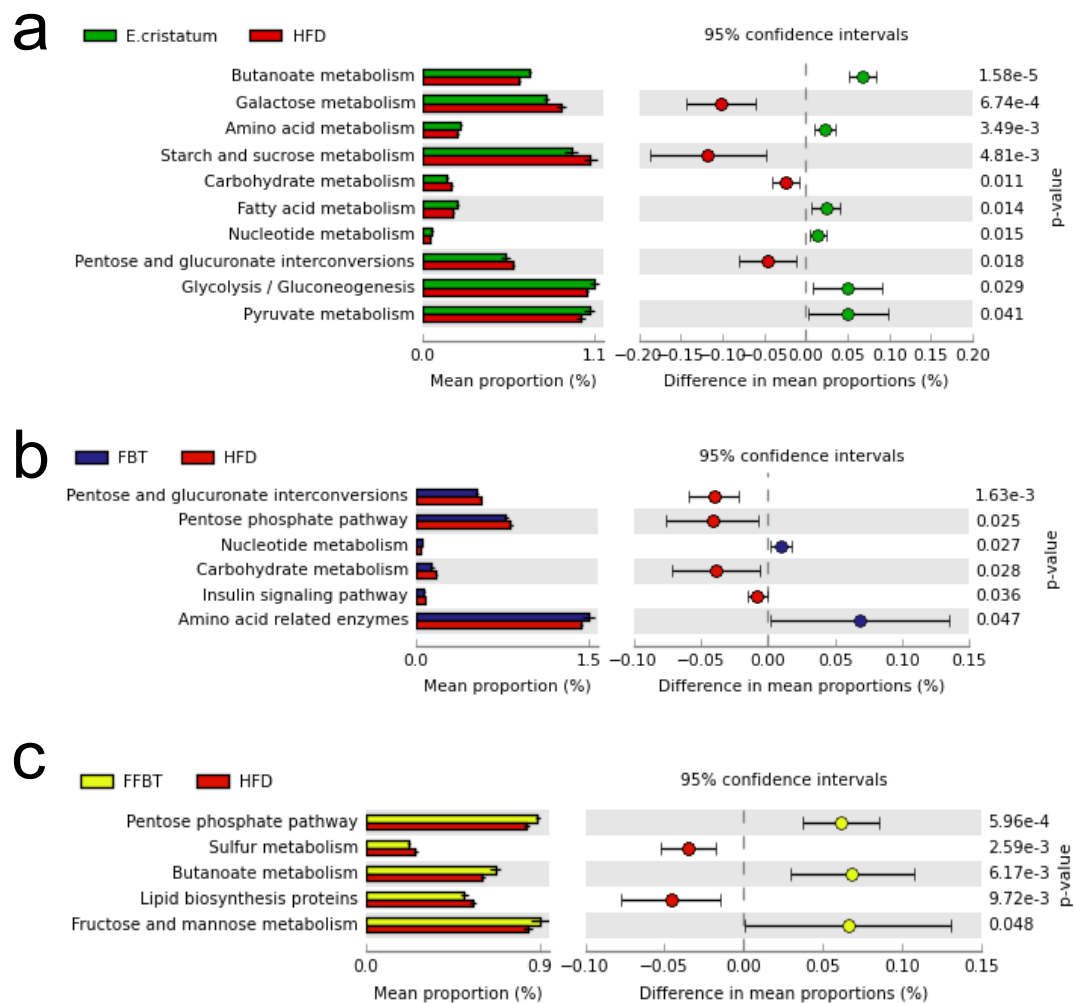


Fig. S6 Phenotypic prediction based on BugBase analysis. BugBase identified that phenotypes associated with a, aerobic. b, anaerobic. c, stress tolerance. d, potentially pathogenic. e, form biofilms. f, gram positive bacteria. g, gram negative bacteria. h. contains mobile elements and f. facultatively anaerobic. Data are expressed as means \pm s.e.m (n=5). Graph bars in a and b marked with different letters on top represent statistically significant results ($P<0.05$) based on Pairwise Mann-Whitney-Wilcoxon Tests.

