

Supplementary Information

Fluoride induced leaky gut and bloom of *Erysipelatoclostridium ramosum* mediate the exacerbation of obesity in high-fat-diet fed mice

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Table S1. The composition of D12492 and D12450J.

Product#	D12492	D12450J
Protein	20% kcal	20% kcal
Carbohydrate	20% kcal	70% kcal
Fat	60% kcal	10% kcal
Total	100%	100%
kcal/g	5.21	3.82

Table S2. Primer sequences used in the RT-qPCR experiment.

Target gene	Primer Sequence (5'-3')
<i>Tnf-α</i>	FW: CTCATGCACCACCATCAAGG
	RV: ACCTGACCACTCTCCCTTTG
<i>Il-1β</i>	FW: AGCTTCAAATCTCGCAGCAG
	RV: TCTCCACAGCCACAATGAGT
<i>Il-6</i>	FW: CTCTGGCGGAGCTATTGAGA
	RV: AAGTCTCCTGCGTGGAGAAA
<i>Tlr4</i>	FW: GCTCTCAGCCATCCACAAAG
	RV: GAGTCGGAAGAGGAAGAGG
<i>Myd88</i>	FW: TTTCCCAGTATCCTGCGGTT
	RV: GCGGAAGAACACAGACAGAC
<i>Zo-1</i>	FW: TGAGTGCGTTTCTCTCCCTT
	RV: CCCTCTGTGTTCTCATGGT
<i>Occludin</i>	FW: AGCACTTAACCTGCCTGGAT
	RV: AGCCTGTGGAAGCAAGAGAT
<i>Claudin-1</i>	FW: AGCTGCCTGTTCCATGTACT
	RV: CTCCCATTGTCTGCTGCTC
<i>Muc1</i>	FW: CACAACCTACCAGCTCTGCAC
	RV: CTGAGCCTGACGTCACTTTG
<i>Gapdh</i>	FW: GGACTTACAGAGGTCCGCTT
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<i>E. ramosum</i>	FW: ACAATGGATGGTGCAGAGGG
	RV: TCAACTCTCTCGTGGTGTGACG

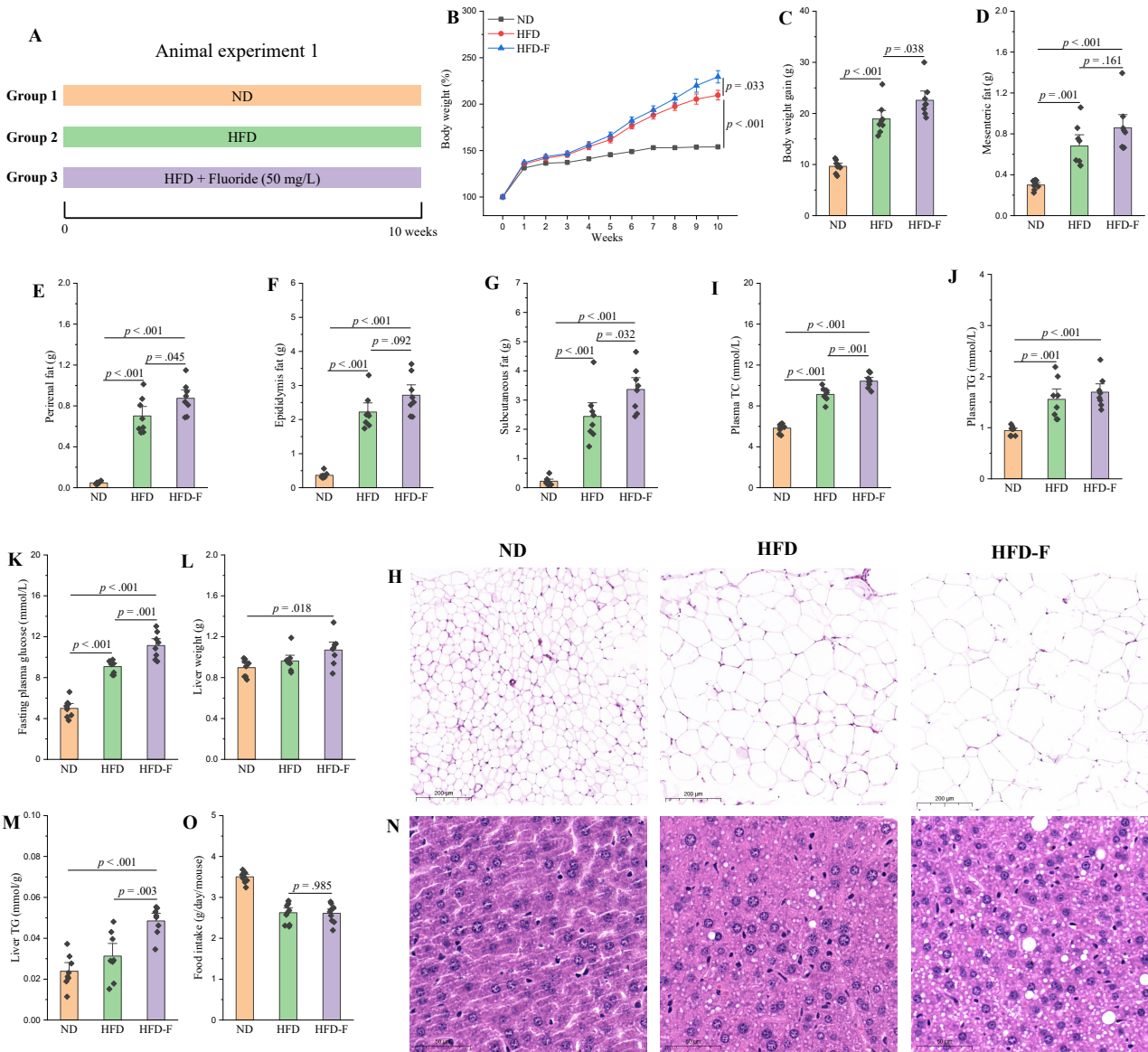


Fig. S1. Fluoride exacerbates the obesity in high-fat diet (HFD) mice. Mice were randomly divided into three groups including normal diet (ND) group, high-fat diet (HFD) group, and HFD plus 50 mg/L of fluoride in drinking water (HFD-F) group (n = 8 per group). **(A)** Scheme of animal experiment 1 over the 10 weeks of dietary intervention. **(B)** Dynamic changes in body weight in mice. **(C)** Body weight gain. **(D)** Mesentery fat. **(E)** Perirenal fat. **(F)** Epididymal fat. **(G)** Subcutaneous fat. **(H)** Representative H&E staining of Epididymal fat. **(I-J)** Plasma levels of TC and TG. **(K)** Fasting plasma glucose. **(L)** Liver weight. **(M)** Liver TG. **(N)** Representative H&E staining of liver. **(O)** Food intake. The results were expressed as means $\pm$ SEM. Statistical significance was carried out by one-way analysis of variance (ANOVA) with Tukey test. A value of $p < 0.05$ was considered to be significant.

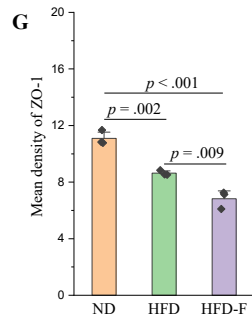
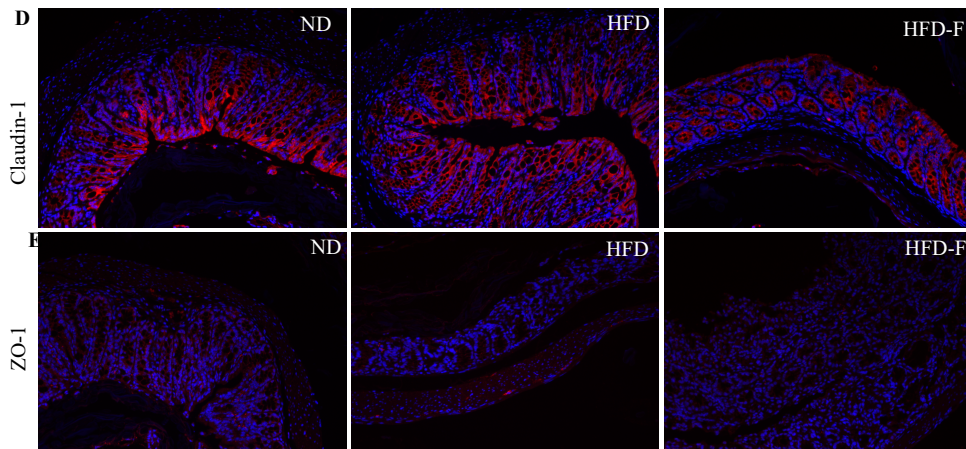
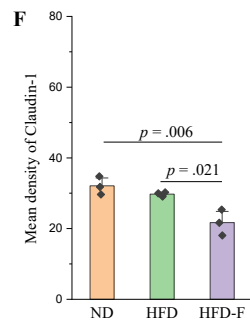
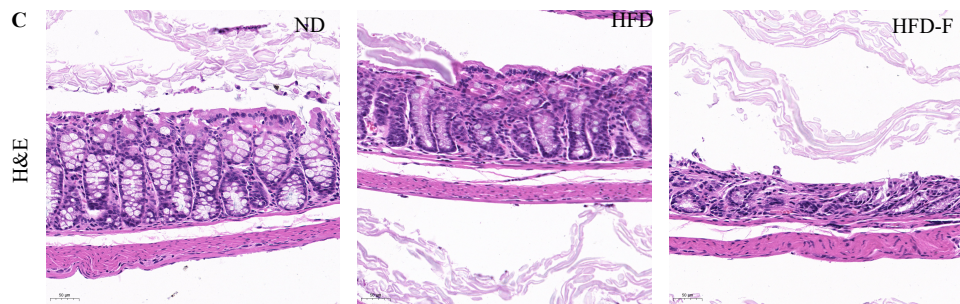
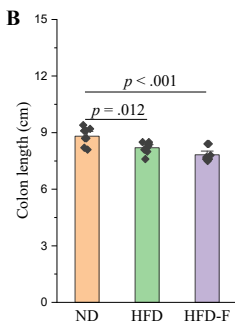


Fig. S2. Fluoride drives the intestinal barrier permeability in HFD mice. (A)

Representative morphology of colon. **(B)** Colon length (n = 8). **(C)** Representative H&E staining of colon. **(D-E)** Representative immunofluorescence images of Claudin-1 and ZO-1 in colon (n = 3). **(F-G)** Mean density of Claudin-1, MUC1 and ZO-1 in immunofluorescence images was evaluated by ImageJ software (n = 3). The results were expressed as means $\pm$ SEM. Statistical significance was carried out by one-way analysis of variance (ANOVA) with Tukey test. A value of $p < 0.05$ was considered to be significant.

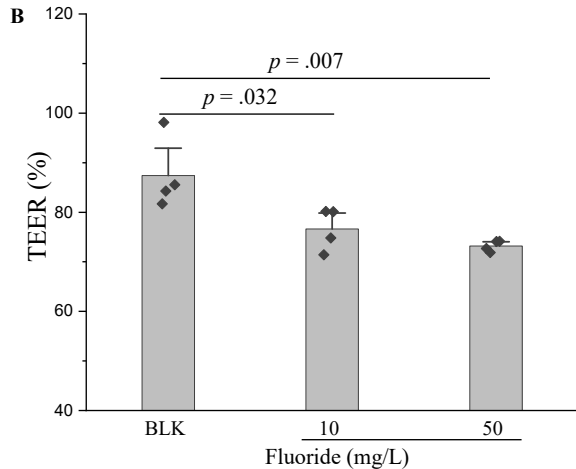
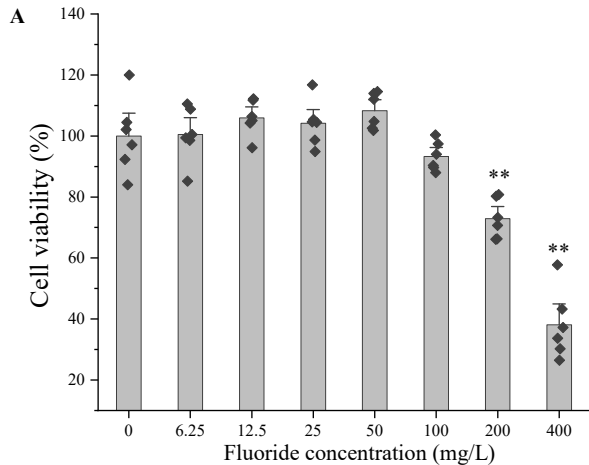


Fig. S3. Fluoride drives Caco-2 cell permeability in vitro. **(A)** The cytotoxicity of fluoride on the Caco-2 cells evaluated by MTT method (n = 6). **(B)** The effect of fluoride on the Caco-2 cell permeability evaluated by transepithelial electrical resistance (TEER) assay (n = 4). The results were expressed as means $\pm$ SEM. Statistical significance was carried out by one-way analysis of variance (ANOVA) with Tukey test. A value of $p < 0.05$ was considered to be significant.

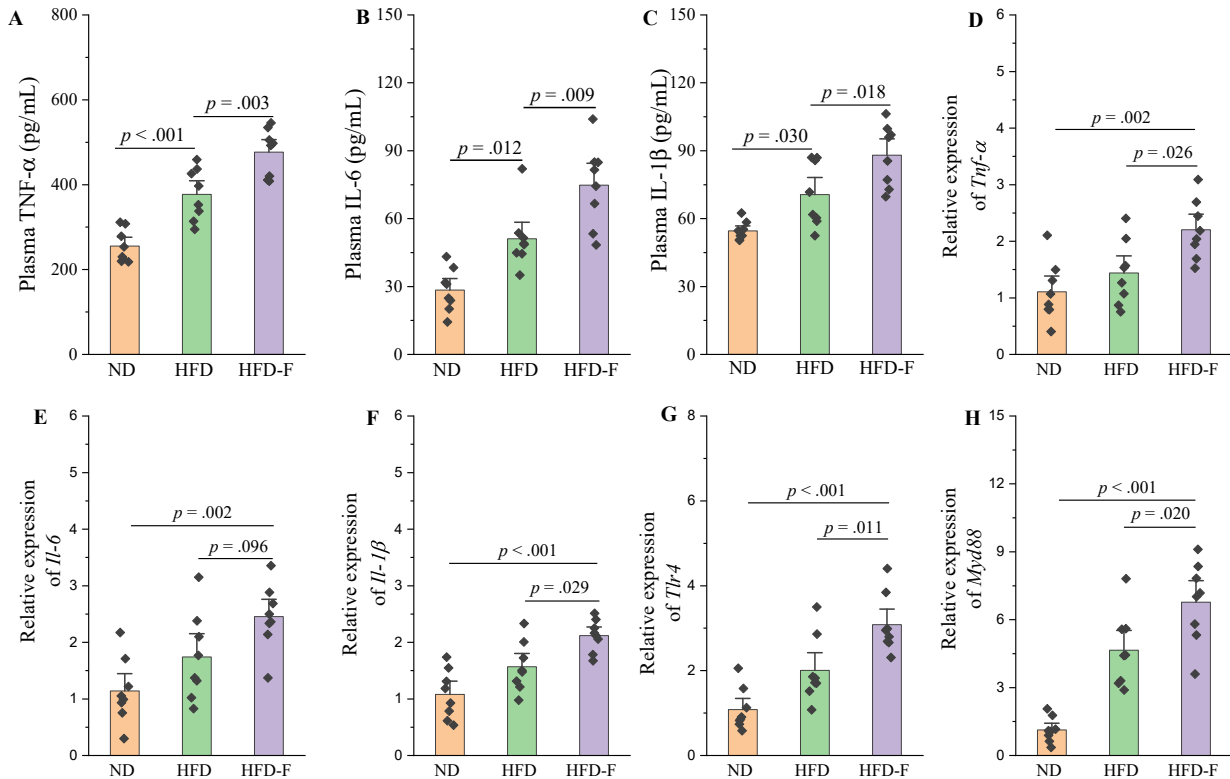


Fig. S4. Fluoride deteriorates the inflammation in HFD mice. (A-C) The plasma levels of TNF- α , IL-6, and IL-1 β (n = 8). **(D-H)** Relative mRNA expression levels of *Tnf- α* , *Il-6*, *Il-1 β* , *Myd88* and *Tlr4* in liver (n = 8). The results were expressed as means $\pm$ SEM. Statistical significance was carried out by one-way analysis of variance (ANOVA) with Tukey test. A value of $p < 0.05$ was considered to be significant.

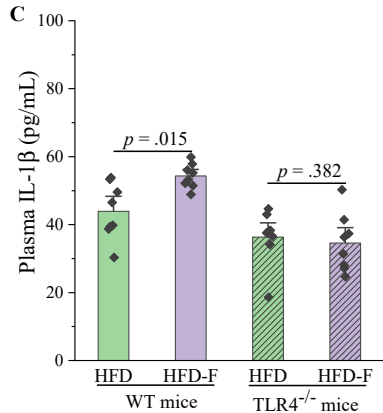
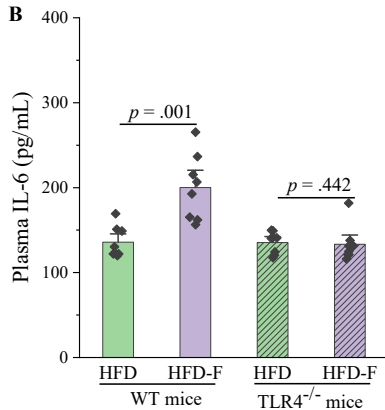
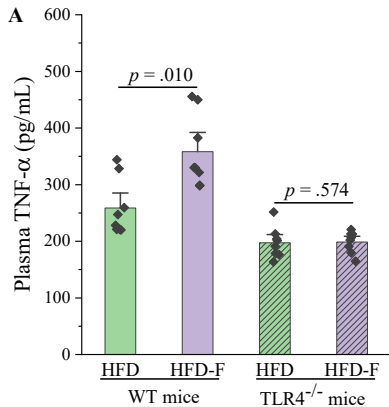


Fig. S5. Fluoride fails to induce the inflammation in Tlr4^{-/-} mice. (A-C) The plasma levels of TNF- α , IL-6, and IL-1 β . The results were expressed as means $\pm$ SEM. Difference in two groups was calculated using the Mann-Whitney test or Kruskal-Wallis test. A value of $p < 0.05$ was considered to be significant.

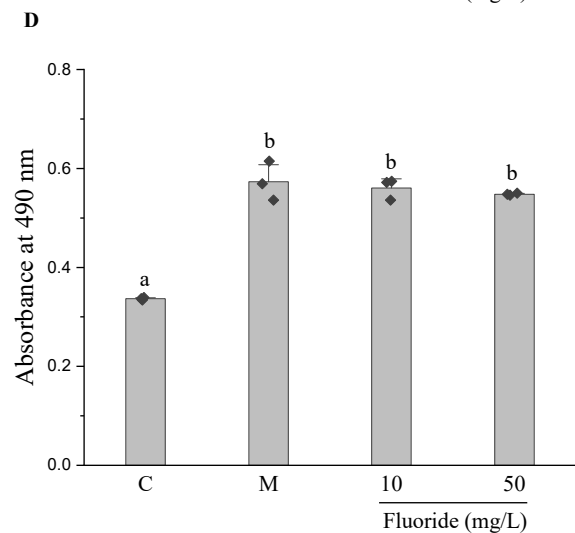
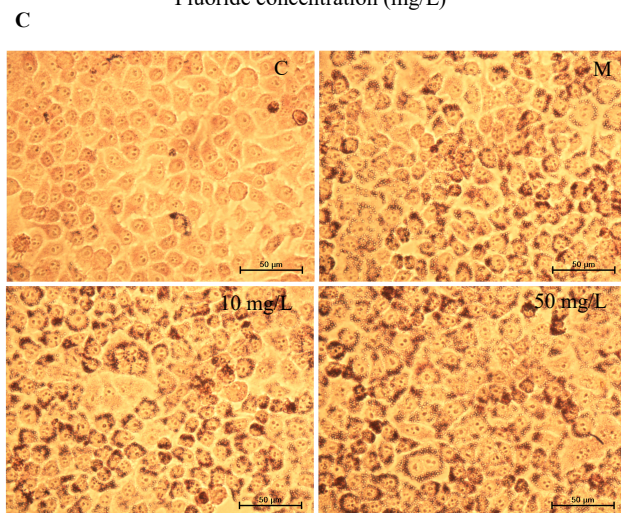
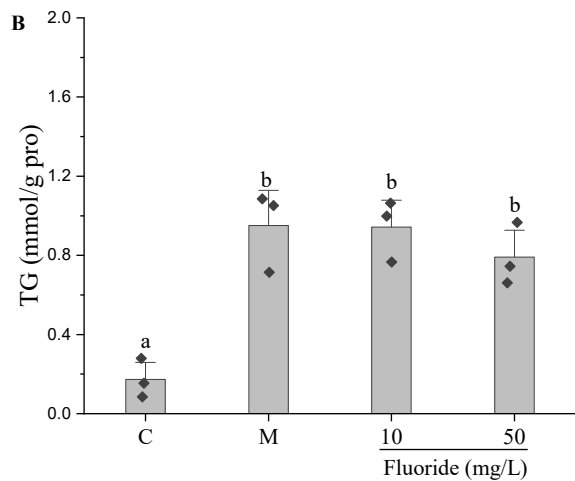
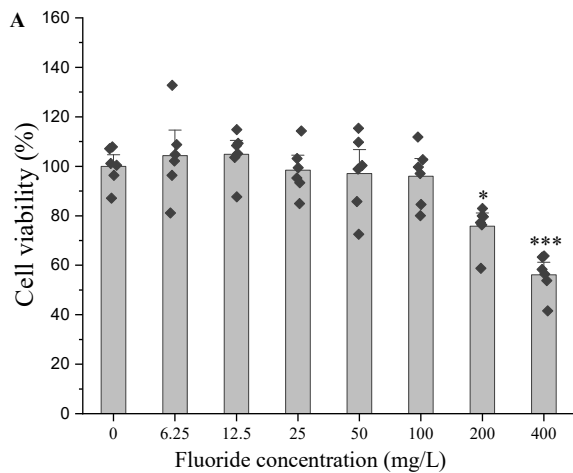


Fig. S6. Fluoride shows limited effect on lipid accumulation using HepG2 cell in vitro. The HepG2 cells were cultivated with oleic acid/palmitic acid (OA/PA) and fluoride (10 and 50 mg/L), C: control group without OA/PA or fluoride, M: model group with OA/PA, fluoride: fluoride group with OA/PA and fluoride. **(A)** The cytotoxicity of fluoride on the HepG2 cell evaluated by MTT method (n = 6). **(B)** The effect of fluoride (10 and 50 mg/L) on the level of TG in OA/PA-induced HepG2 cell (n = 3). **(C)** Representative oil red O staining of HepG2 cell. **(D)** The absorbance at 490 nm of oil red solution washed from HepG2 cell by isopropanol (n = 3). The results were expressed as means $\pm$ SEM. Statistical significance was carried out by one-way analysis of variance (ANOVA) with Tukey test. A value of $p < 0.05$ was considered to be significant.

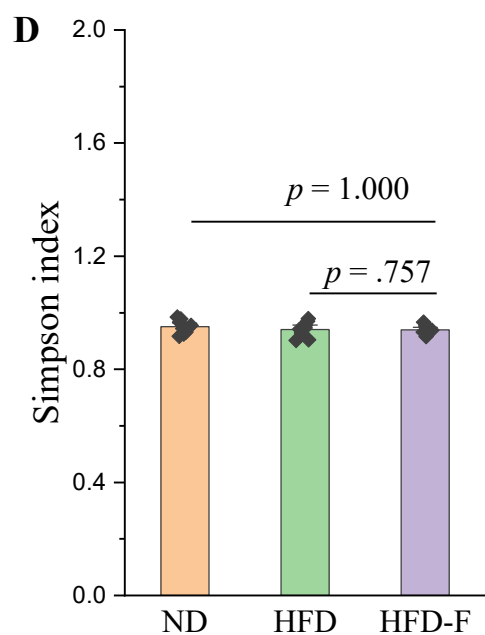
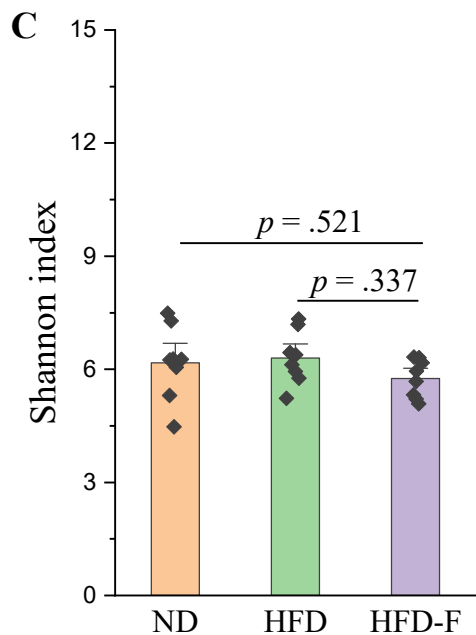
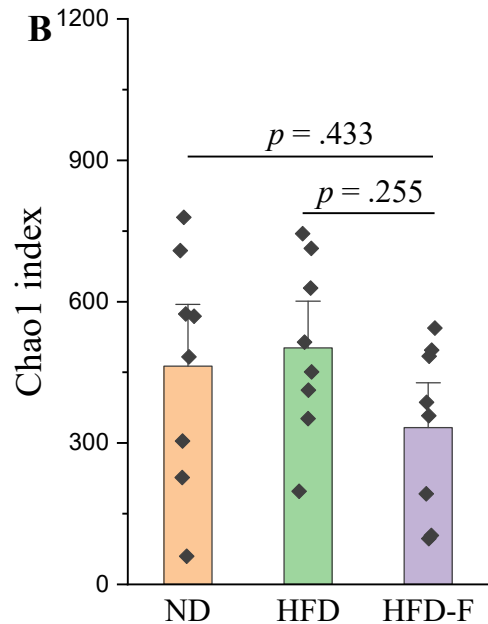
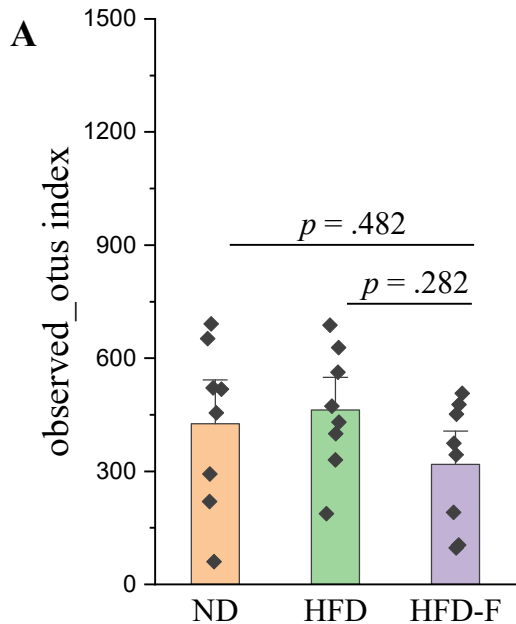


Fig. S7. Fluoride shows limited effect on α -diversity of gut microbiota. (A) observed_otus, **(B)** Chao1, **(C)** Shannon, and **(D)** Simpson indexes. The results were expressed as means $\pm$ SEM. Statistical significance was carried out by one-way analysis of variance (ANOVA) with Tukey test. A value of $p < 0.05$ was considered to be significant.

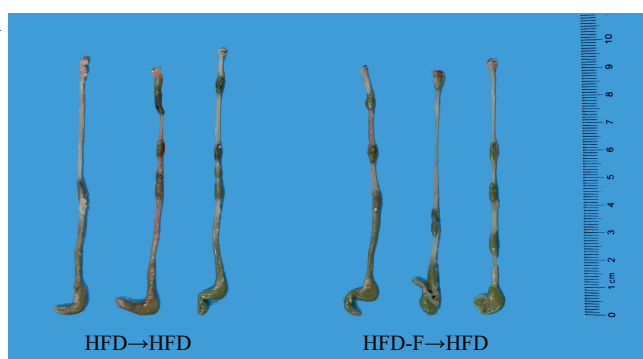
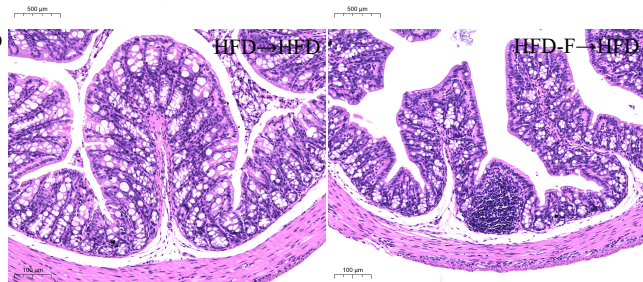
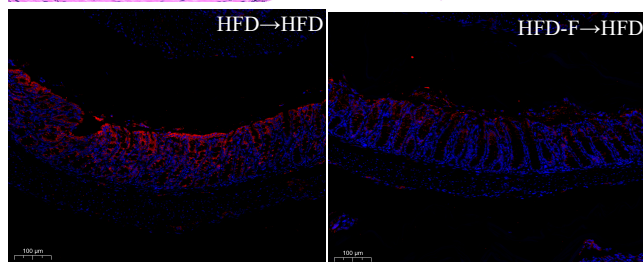
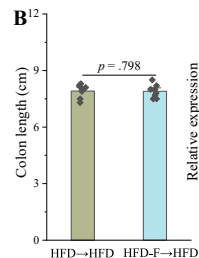
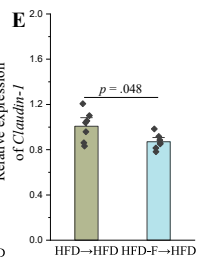
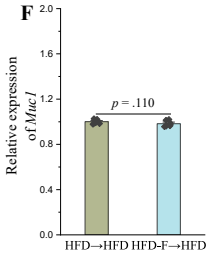
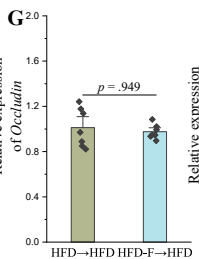
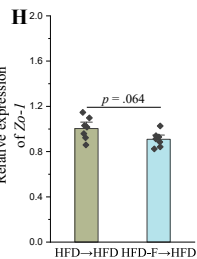
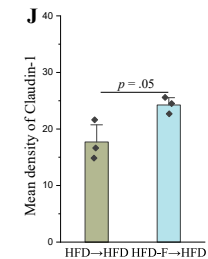
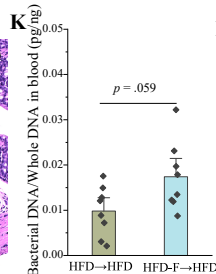
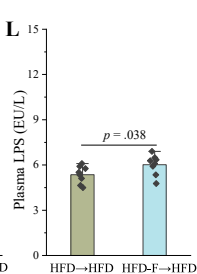
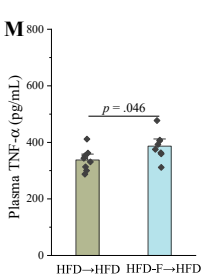
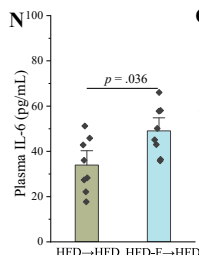
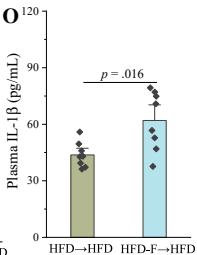
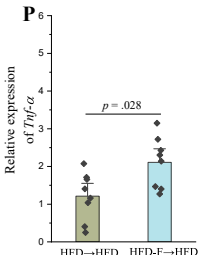
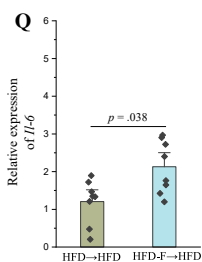
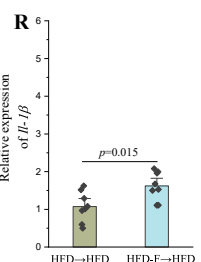
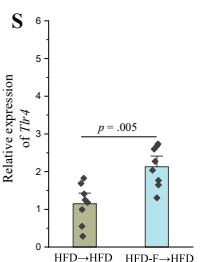
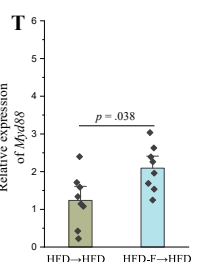
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Fig. S8. Fluoride-induced gut microbiota impairs gut barrier function and promotes the inflammation. (A) Representative morphology of colon. **(B)** Colon length (n = 8). **(C-D)** Representative H&E staining of colon (10× and 40×, respectively). **(E-H)** Relative mRNA expression levels of *Claudin-1*, *Muc1*, *Occludin* and *Zo-1* in colon (n = 7). **(I)** Representative immunofluorescence images of Claudin-1 in colon. **(J)** Mean density of Claudin-1 in immunofluorescence images was evaluated by ImageJ software (n = 3). **(K)** Bacterial DNA/Whole DNA in blood was measured by RT-qPCR (n = 8). **(L)** Plasma LPS (n = 8). **(M-O)** The plasma levels of TNF- α , IL-6, and IL-1 β (n = 8). **(P-T)** Relative mRNA expression levels of *Tnf- α* , *Il-6*, *Il-1 β* , *Myd88* and *Tlr4* in liver (n = 8). The results were expressed as means $\pm$ SEM. Difference in two groups was calculated using the Mann-Whitney test or Kruskal-Wallis test. A value of $p < 0.05$ was considered to be significant.

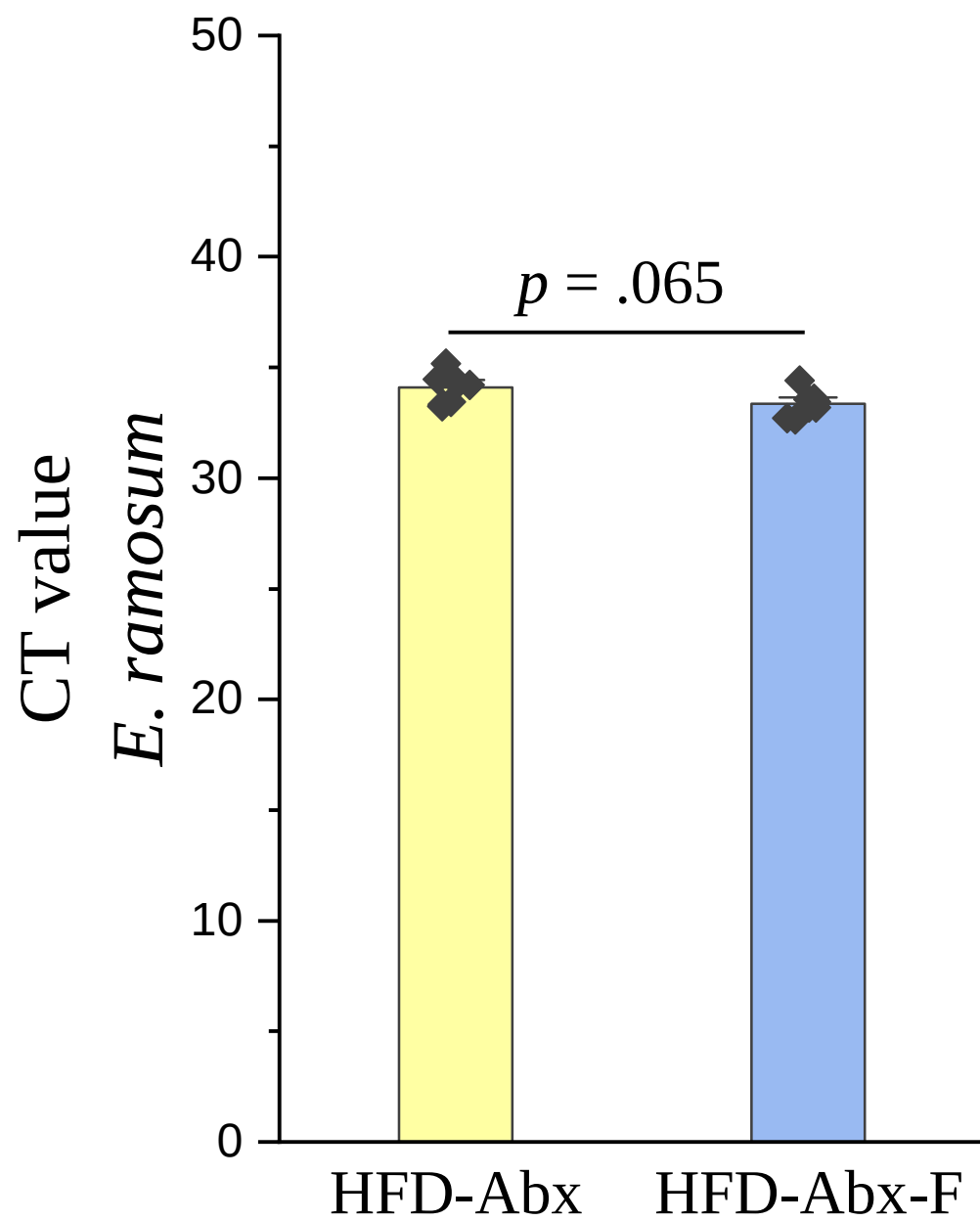


Fig. S9. Level of *E. ramosum* in fecal samples was greatly reduced after depletion of the gut microbiota by antibiotic cocktail (Abx). Level of *E. ramosum* in fecal samples were measured by RT-qPCR using species-specific primers. Higher CT values suggest lower levels of *E. ramosum*. The results were expressed as means $\pm$ SEM. Difference in two groups was calculated using the Mann-Whitney test. A value of $p < 0.05$ was considered to be significant.

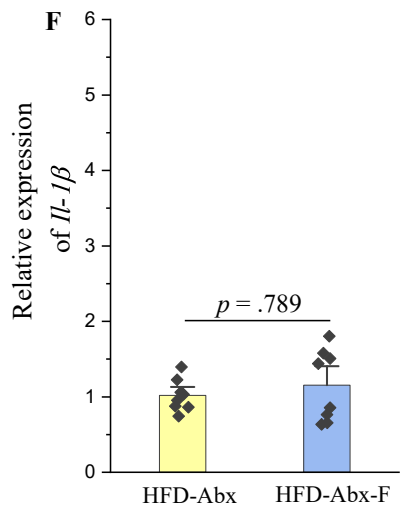
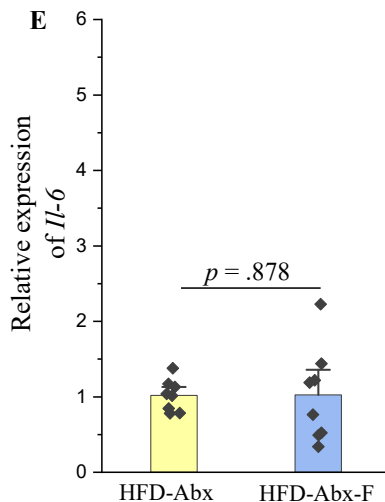
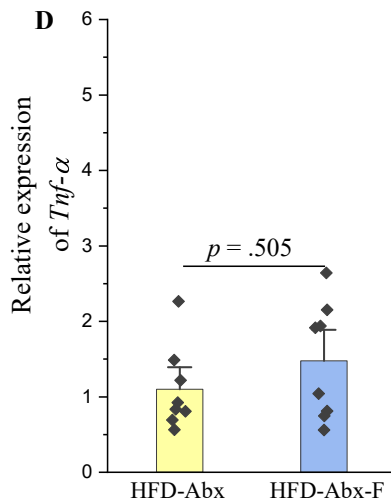
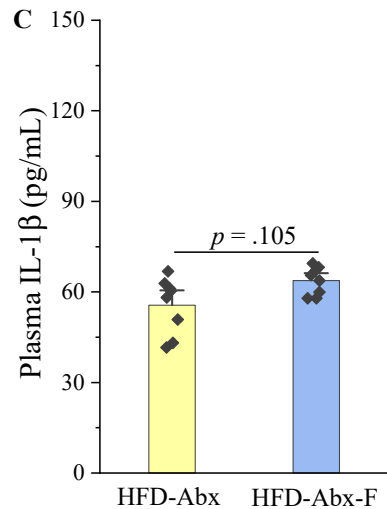
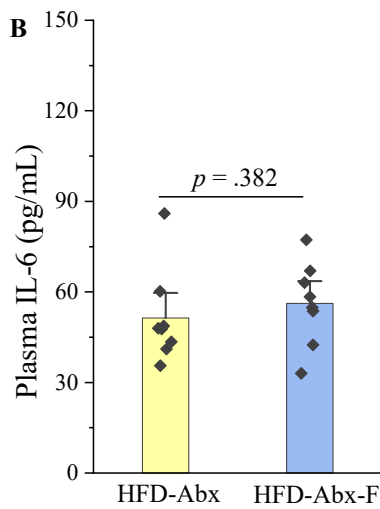
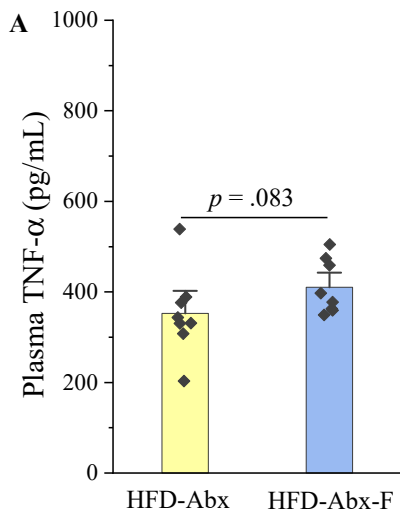


Fig. S10. Fluoride fails to induce the inflammation after depletion of the gut microbiota by Abx. (A-C) The plasma levels of TNF- α , IL-6, and IL-1 β (n = 8). **(D-F)** Relative mRNA expression levels of *Tnf- α* , *Il-6*, and *Il-1 β* in liver (n = 8). The results were expressed as means $\pm$ SEM. Difference in two groups was calculated using the Mann-Whitney test or Kruskal-Wallis test. A value of $p < 0.05$ was considered to be significant.

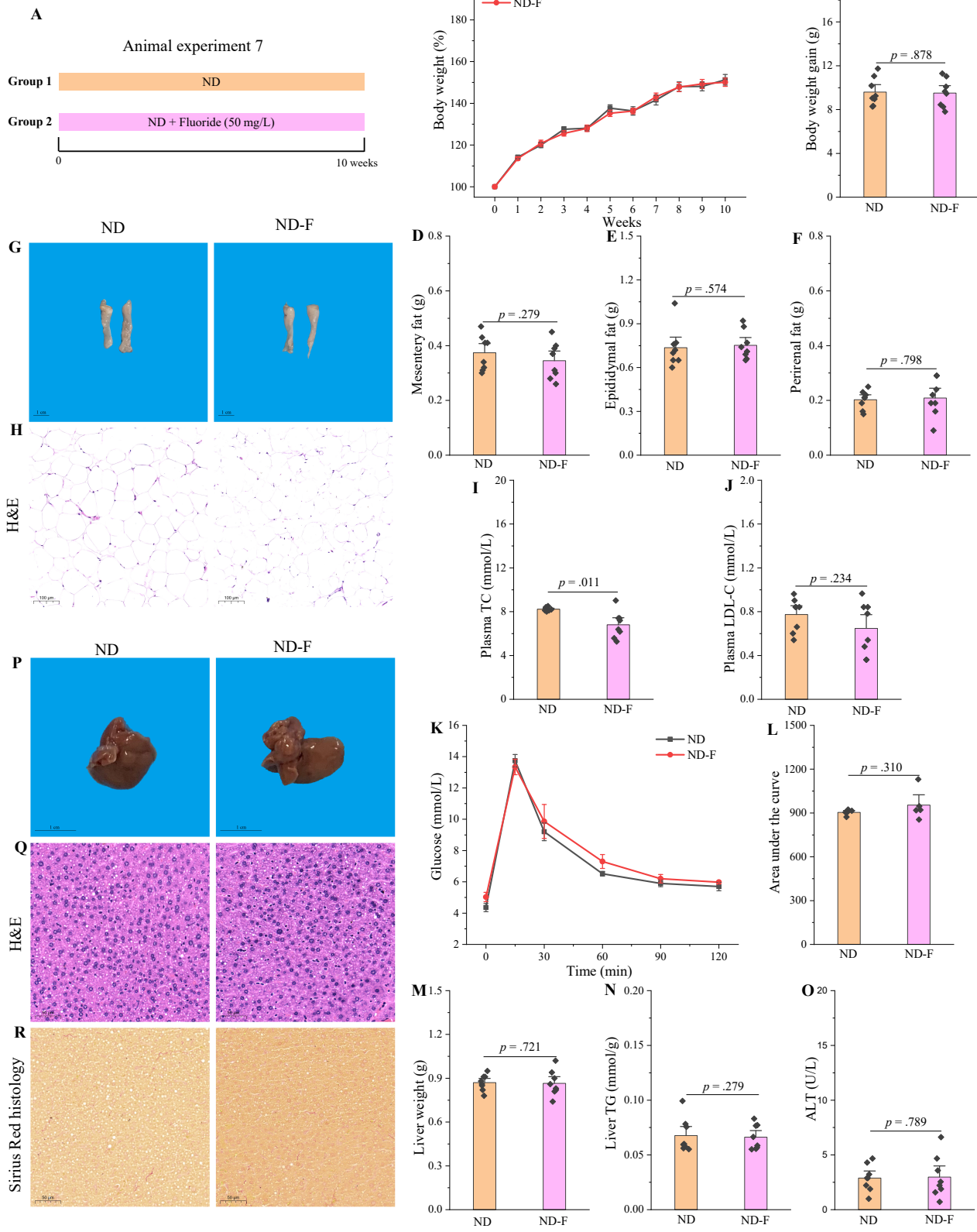


Fig. S11. Fluoride fails to induce the obesity in ND mice. (A) Scheme of animal experiment 7 over the 10 weeks of dietary intervention. Mice were randomly divided into two groups including ND group, and ND plus 50 mg/L of fluoride in drinking water (ND-F) group (n = 8 per group). (B) Dynamic changes in body weight in mice. (C) Body weight gain. (D) Mesentery fat. (E) Epididymal fat. (F) Perirenal fat. (G-H) Representative morphology and H&E staining of Epididymal fat. (I-J) Plasma levels of TC and LDL-C. (K) OGTT was carried out at week 9, mice were fasted overnight and gavaged with a dosage of glucose with 1.5 mg/g body weight (n = 5 per group). (L) Area under the curve (AUC) for OGTT. (M) Liver weight. (N) Liver TG. (O) Plasma ALT. (O-R) Representative morphology, H&E staining and Sirius Red histology of liver. The results were expressed as means $\pm$ SEM. Difference in two groups was calculated using the Mann-Whitney test or Kruskal-Wallis test. A value of $p < 0.05$ was considered to be significant.

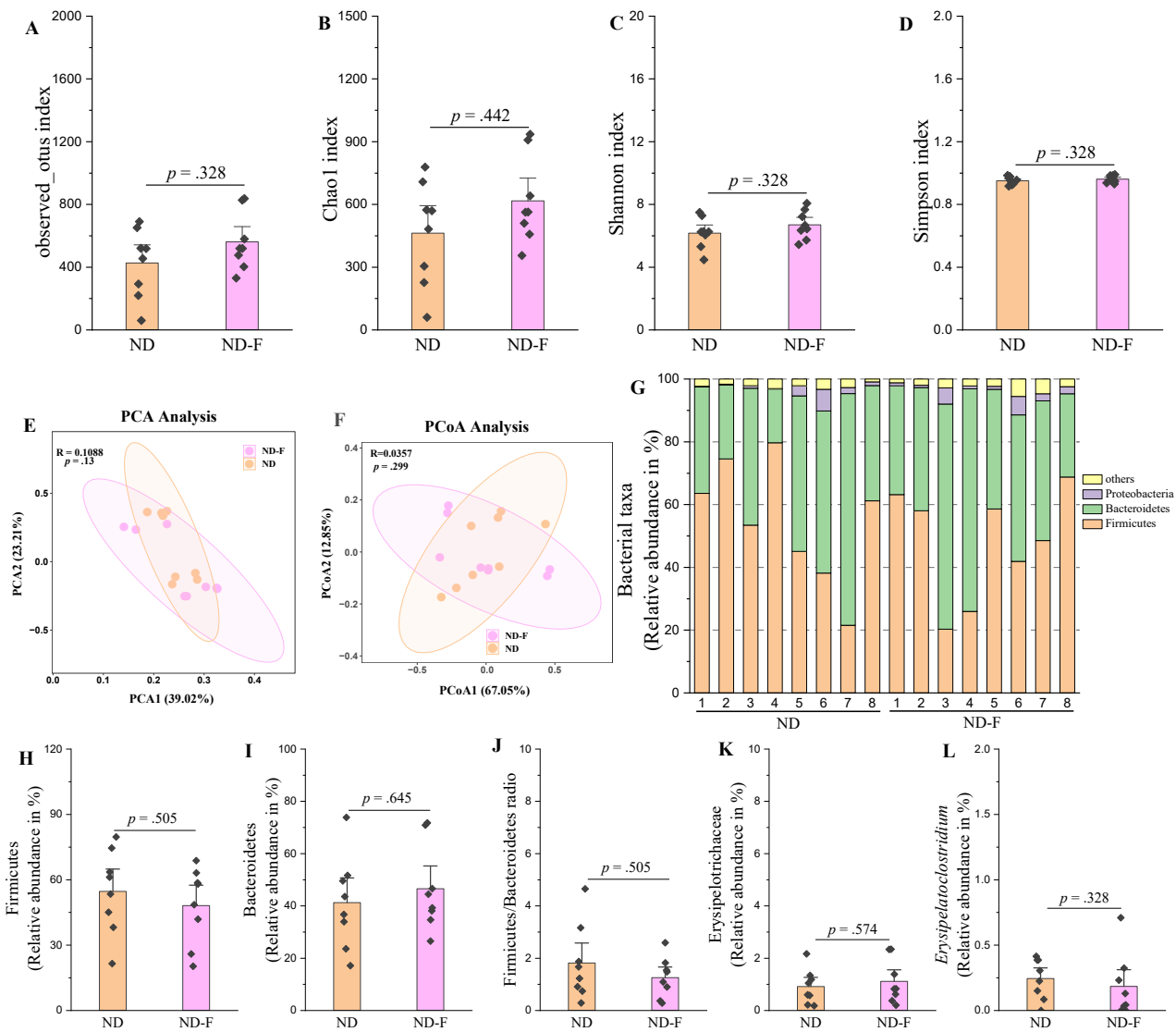


Fig. S12. Fluoride showed limited effect on the gut microbiota of ND mice. Gut microbiota was analyzed by 16S rRNA gene sequencing (n = 8 for each group). **(A-D)** α -diversity of gut microbiota evaluated by **(A)** observed_otus, **(B)** Chao1, **(C)** Shannon, and **(D)** Simpson indexes. **(E)** principal component analysis (PCA) based on the relative abundance of Features of the gut microbiota, **(F)** Principal coordinates analysis (PCoA) of the gut microbiota based on the weighted unifracs distance matrix, **(G)** Bacterial taxonomic profiling at the phylum level of gut microbiota, the relative abundances of **(H)** Firmicutes, **(I)** Bacteroidetes, and **(J)** the ratio of Firmicutes to Bacteroidetes. The relative abundances of **(K)** Erysipelotrichaceae, and **(L)** *Erysipelatoclostridium*. The results were expressed as means $\pm$ SEM. Difference in two groups was calculated using the Mann-Whitney test or Kruskal-Wallis test. A value of $p < 0.05$ was considered to be significant.

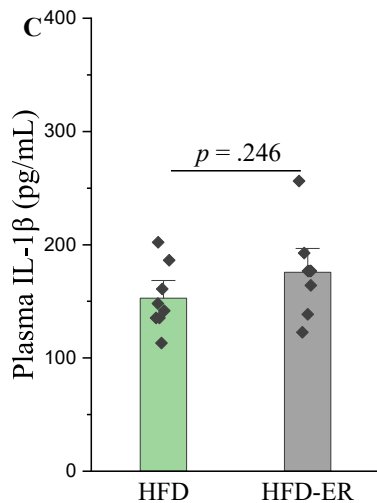
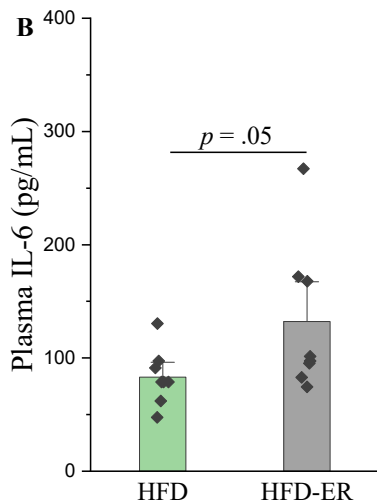
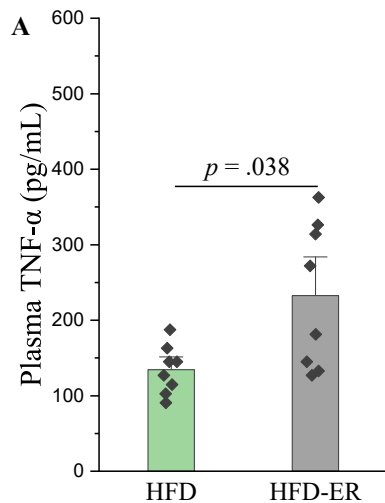


Fig. S13. *E. ramosum* aggravates the inflammation in HFD mice. (A-C) The plasma levels of TNF- α , IL-6, and IL-1 β (n = 8). The results were expressed as means $\pm$ SEM. Difference in two groups was calculated using the Mann-Whitney test or Kruskal-Wallis test. A value of $p < 0.05$ was considered to be significant.

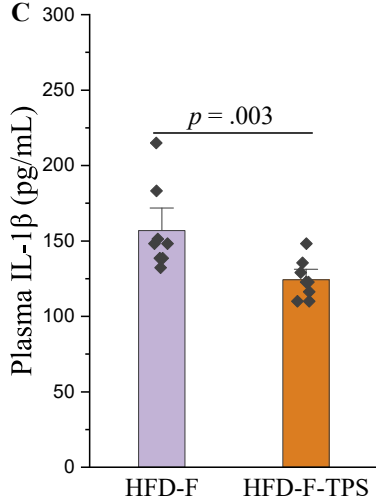
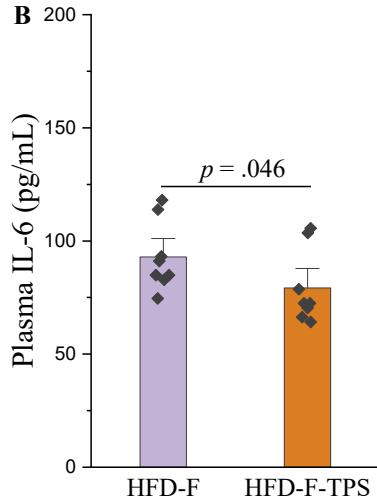
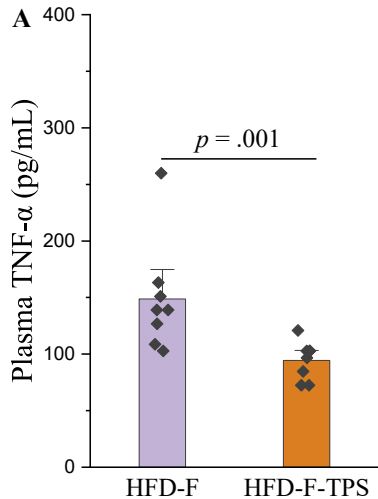


Fig. S14. Fuzhuan brick tea polysaccharides (TPS) abolish the fluoride-induced inflammation in HFD mice. (A-C) The plasma levels of TNF- α , IL-6, and IL-1 β (n = 8). The results were expressed as means $\pm$ SEM. Difference in two groups was calculated using the Mann-Whitney test or Kruskal-Wallis test. A value of $p < 0.05$ was considered to be significant.