

Prevotella Contributing to the Individual Responses of FOLFOX in Colon Cancer

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Research

Keywords: Individualized efficacy, FOLFOX, Colon cancer, Fecal microbiome, Prevotella

DOI: <https://doi.org/10.21203/rs.3.rs-142740/v1>

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Abstract

While FOLFOX has been the preferred chemotherapeutic strategy in colon cancer treatment, the limited response rate has seriously restricted its application in clinic. The underlying mechanisms of the individual response of FOLFOX remain to be elucidated. In this study, pharmacomicrobiomics integrated with pharmacometabolomics was applied to disentangle the key role of specific gut bacteria and microbiota derived metabolites involved. As a result, significant variation of chemotherapy efficacy was observed in tumor bearing mice after FOLFOX administration. 16S rRNA gene sequencing analysis revealed the relative abundance of *Staphylococcus*, *Jeotgalicoccus*, *Sphingomonas* significantly increased in the FOLFOX sensitive group, whereas *Prevotella* was higher in the non sensitive individuals. Meanwhile, verification study on FOLFOX combined with bacteria colonization indicates that *Prevotella* could attenuate the anti cancer efficacy of FOLFOX in vivo. Furthermore, gut derived metabolite 3-Oxocholeic acid (3-Oxo) identified by metabolomics approach was confirmed to associate with *Prevotella* in fecal samples. In addition, preliminary functional exploration suggested that 3-Oxo could reverse the anti cancer effect of FOLFOX and promote malignant progression. Taken together, integrated pharmacomicrobiomics and pharmacometabolomics revealed that *Prevotella* and related metabolite 3-Oxo may be responsible for individualized FOLFOX efficacy, which provides novel predictive biomarkers for FOLFOX precise medicine as well as targets for colon cancer therapy.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

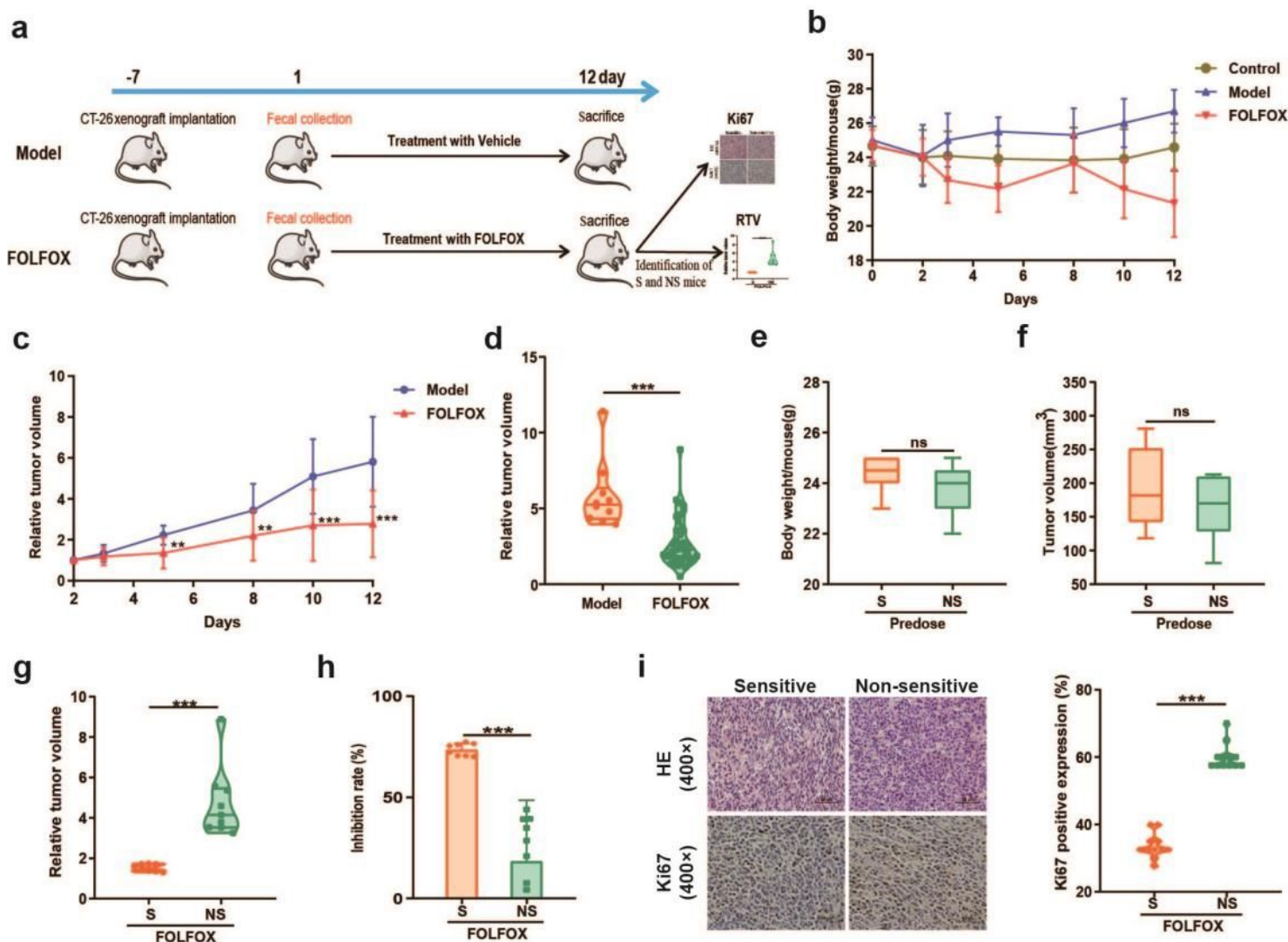


Figure 1

Recognition of S and NS individuals after FOLFOX treatment. a) Schematic diagram of the recognition of individual difference after FOLFOX treatment in CT 26 colon cancer xenograft model. (b) The effects of FOLFOX on the body weight of tumor bearing mice. (c , d) FOLFOX treatment could inhibit the tumor development. There is no obvious difference on body weight (e) and tumor volume (f) in S and NS group before FOLFOX administration. RTV (g), inhibition rate (h) and Ki67 levels (i) of S (n=8) and NS (n=9) group at the end of the experiment (day12). Data was expressed as mean \pm SD. The p values < 0.05 were considered statistically significant, * p < 0.05, **p < 0.01, ***p < 0.001.

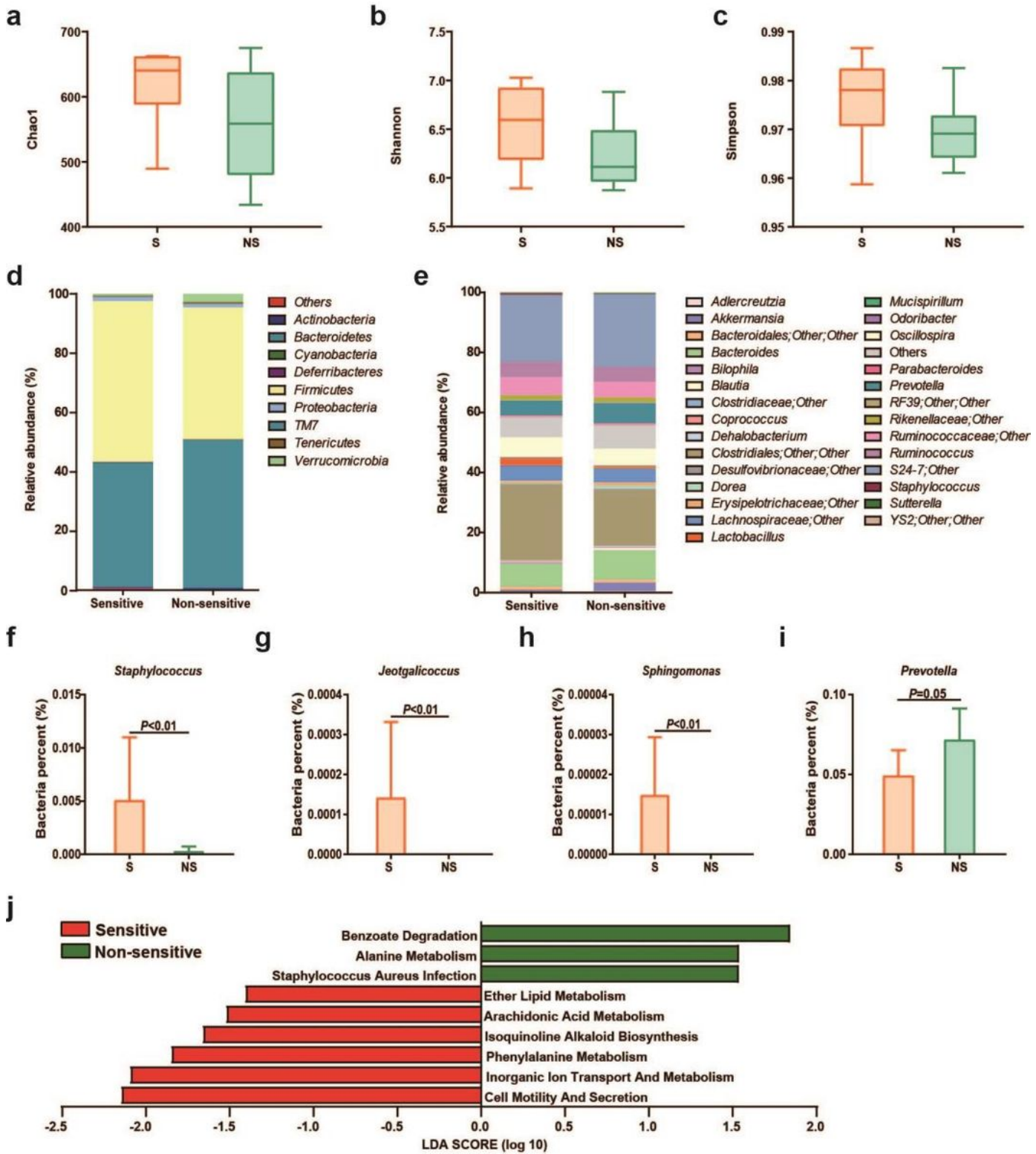


Figure 2

Screening of potential bacterial genus contributing to individualized FOLFOX response . a c The α diversity indexes of Chao1 $p = 0.1729$), Shannon $p =$ and Simpson $p = 0.1177$) of gut microbiota between S and NS groups Taxonomic distribution s of bacteria from S and NS group s at phylum level (d) and genus level (e).(f-i) Comparison of r elative abundance of significantly changed bacterial species

between S and NS mice. (j) Differently abundant of KEGG pathways in S and NS mice were visualized using linear discriminant analysis effect size (LEfSe) analysis

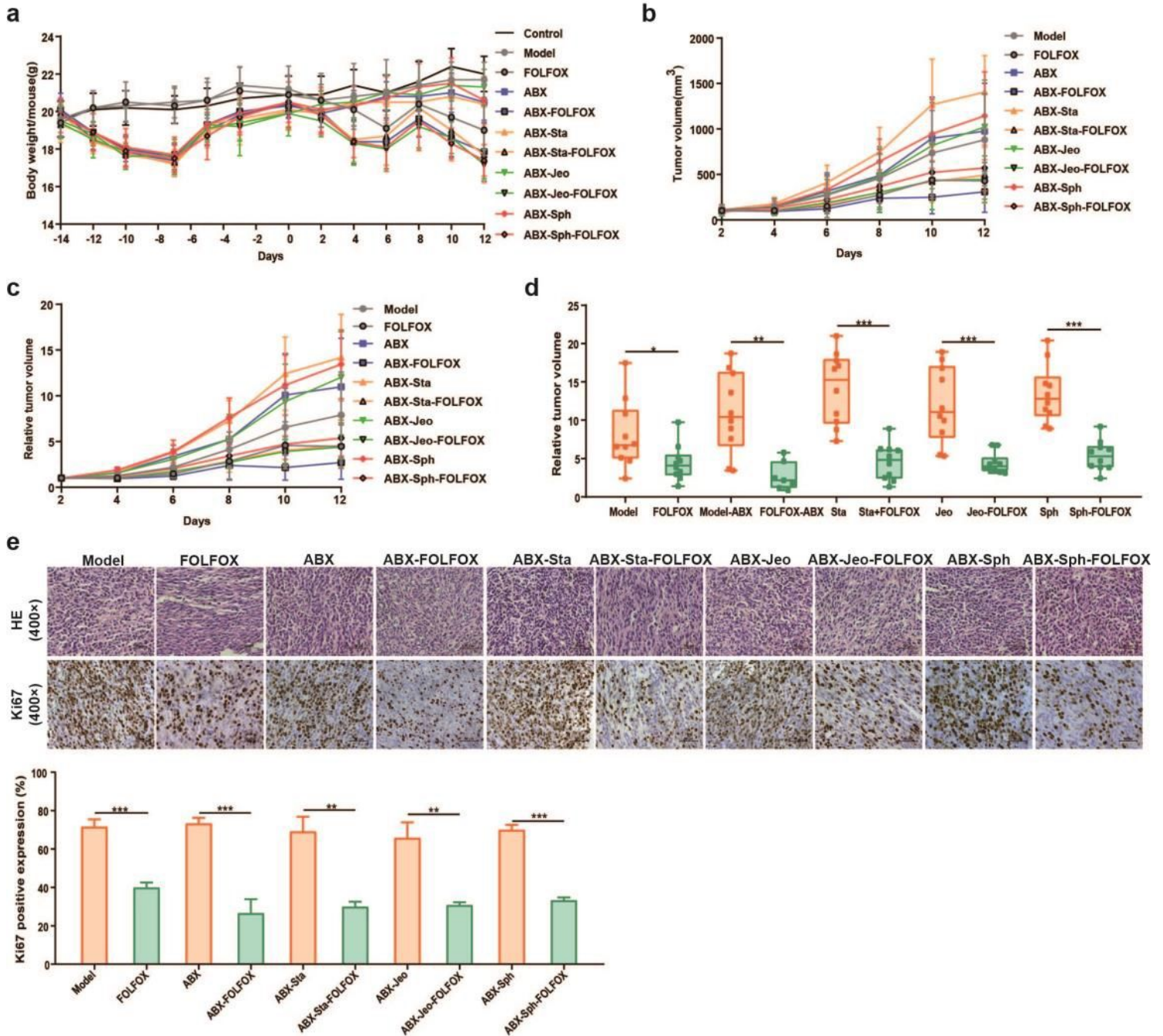


Figure 3

Effects of aerobic bacteria transplantation on FOLFOX efficacy. (a) Change of mice body weight across the experiment. Tumor volume (b) and relative tumor volume (c, d) were measured throughout the experiment. Inhibition rate: Model vs. FOLFOX (45.25%), ABX vs. ABX FOLFOX (75.39%); ABX Sta vs. ABX Sta FOLFOX (67.66%); ABX Jeo vs. ABX Jeo FOLFOX (63.56%); ABX Sph vs. ABX Sph FOLFOX (59.95%). e) Ki67 levels were compared in Model vs. FOLFOX group, ABX vs. ABX FOLFOX group, ABX Sta vs. ABX Sta FOLFOX group, ABX Jeo vs. ABX Jeo FOLFOX group, and ABX Sph vs. ABX Sph

FOLFOX group . Data was expressed as mean \pm SD. The p values < 0.05 were considered statistically significant, * p < 0.05 , ** p < 0.01 , *** p < 0.001 .

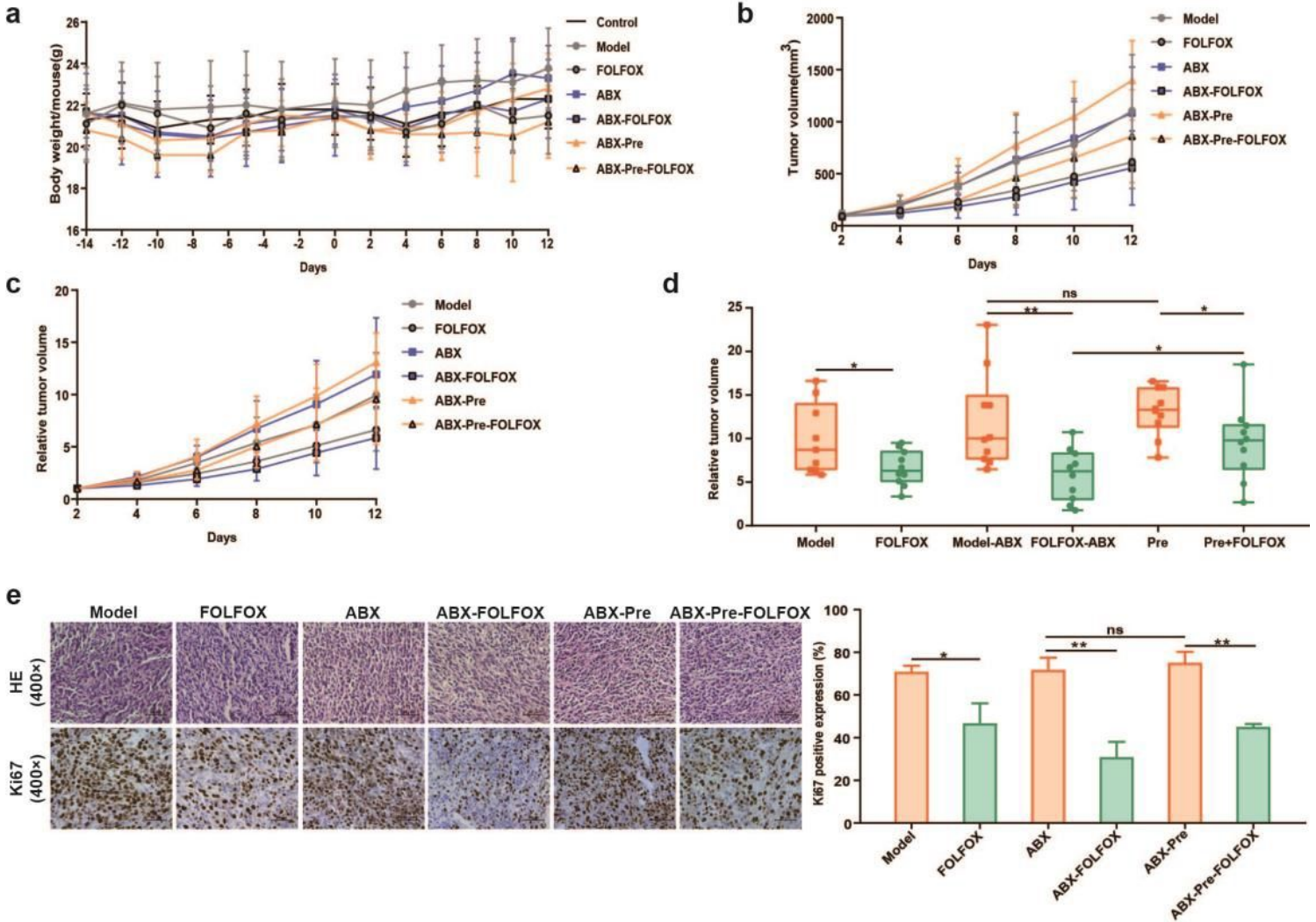


Figure 4

Effects of anaerobic bacteria transplantation on FOLFOX efficacy. (a) Change of mice body weight across the experiment. Tumor volume (b) and relative tumor volume (c, d) were recorded throughout the experiment . Inhibition rate: Model vs. FOLFOX (33.35 ABX vs. ABX FOLFOX (50.88%); ABX Pre vs. ABX Pre FOLFOX (27.04%). e) Ki67 levels were compared in Model vs. FOLFOX group , ABX vs. ABX FOLFOX group , ABX Pre vs. ABX Pre FOLFOX group Data was expressed as mean \pm SD. The p values < 0.05 were considered statistically significant, *p < 0.05 , **p < 0.01 .

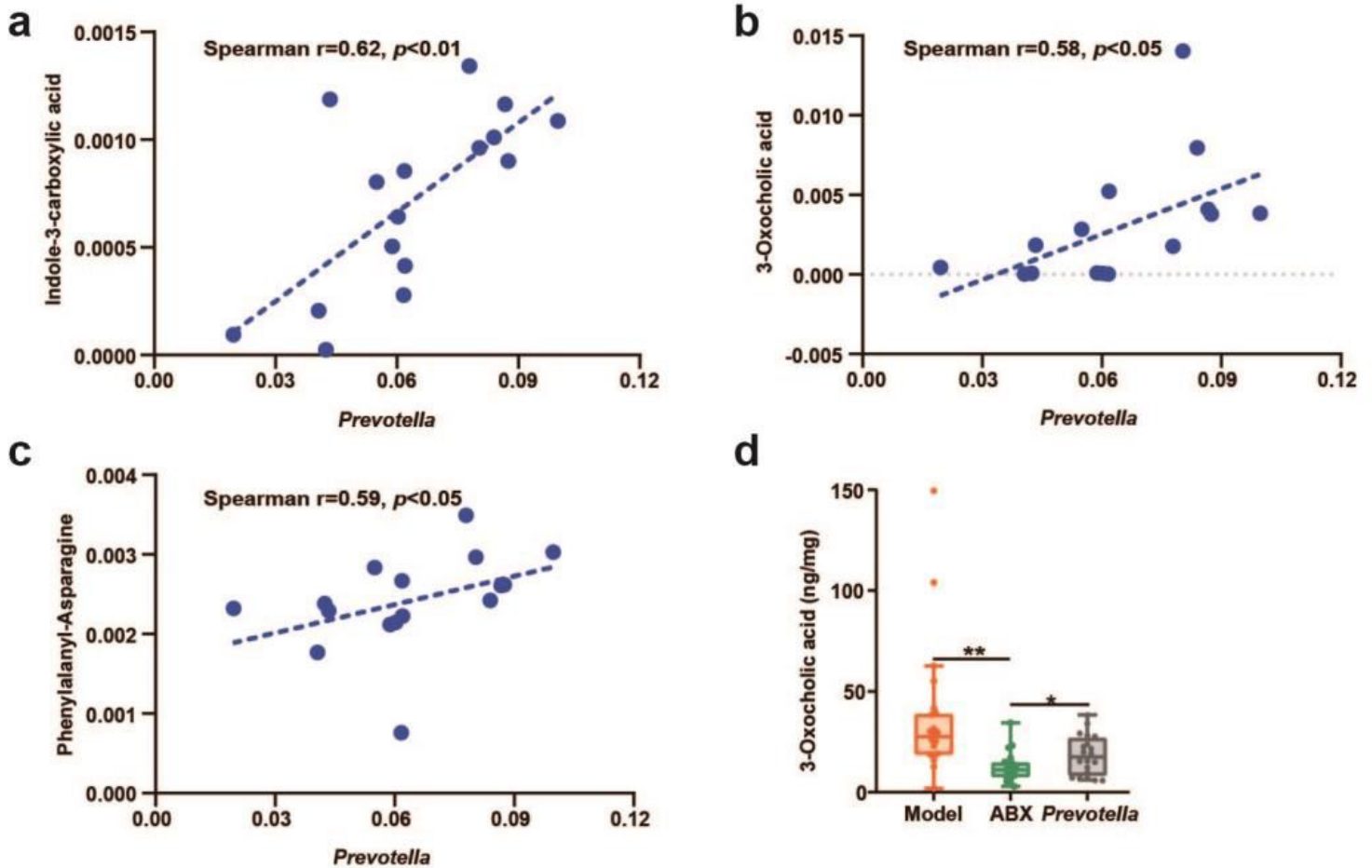


Figure 5

3-Oxo may be the key metabolite responsible for gut microbiota mediated FOLFOX efficacy. Relative abundance of indole 3-carboxylic acid (a), 3-oxochoholic acid b and phenylalanyl-asparaginec was significantly associated with the level of prevotella as measured by the Spearman's correlations analysis. (d) Concentrations of 3-Oxo in fecal samples from Model (n=20), ABX (n=20) and Prevotella (n=20) group, respectively. Data was expressed as mean \pm SD. The p values < 0.05 were considered statistically significant, * $p<0.05$, ** $p<0.01$.

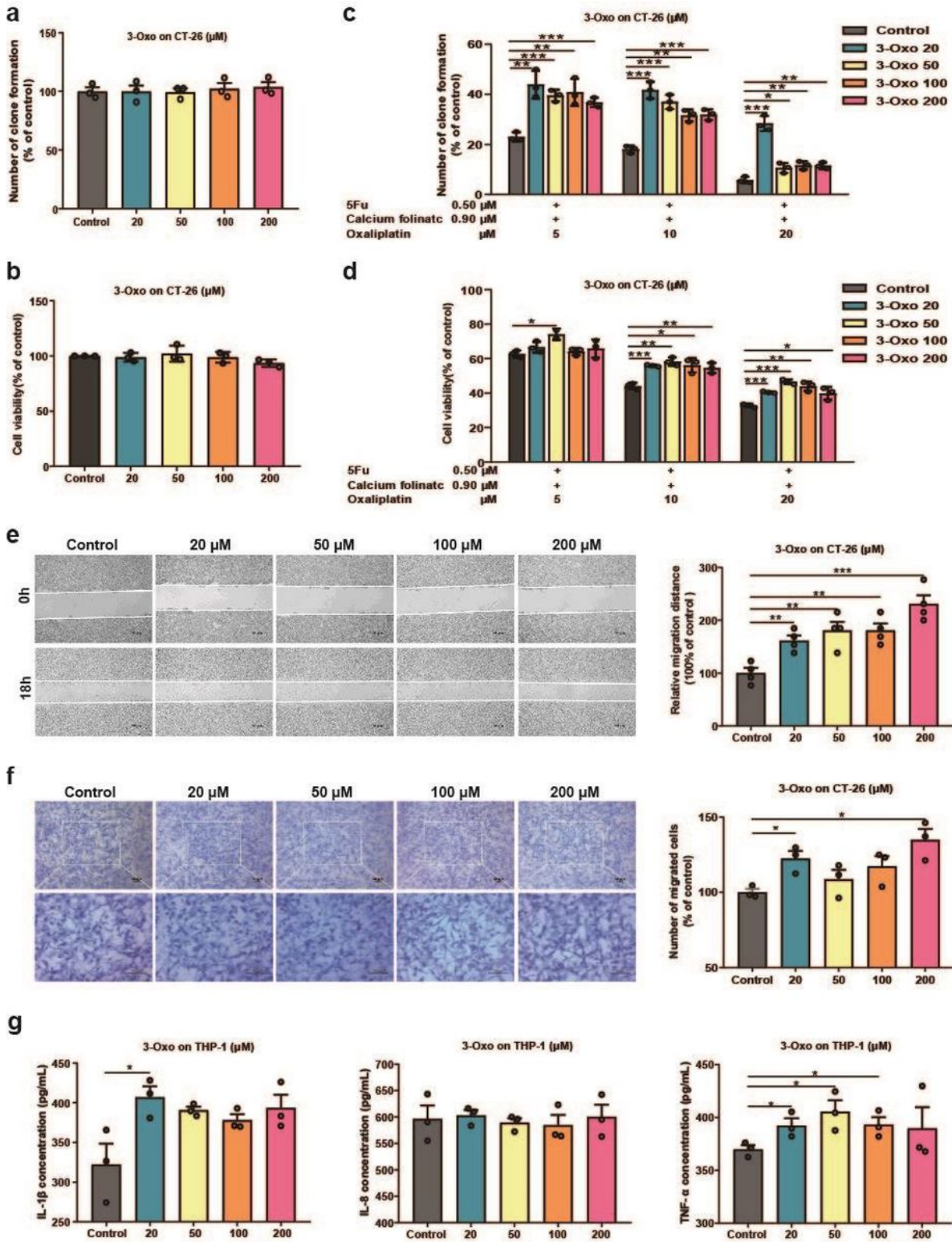


Figure 6

Effects of 3-Oxo on CT 26 migration as well as on the anti cancer effect of FOLFOX. a, b) The effect of 3-Oxo on CT 26 proliferation was evaluated by MTT (72 h) and colon formation assay (7 days). (c, d) 3-Oxo could attenuate the anti proliferation effect of FOLFOX (72h, 7days). (e-f) The effect of 3-Oxo on CT-26 migration was evaluated by wound healing assay and transwell chambers. (g) 3-Oxo could induce the secretion of IL-1 β and TNF- α in macrophages. Data was expressed as mean \pm SD of three

independent experiments. The p values < 0.05 were considered statistically significant, *p<0.05, **p<0.01, ***p<0.001.

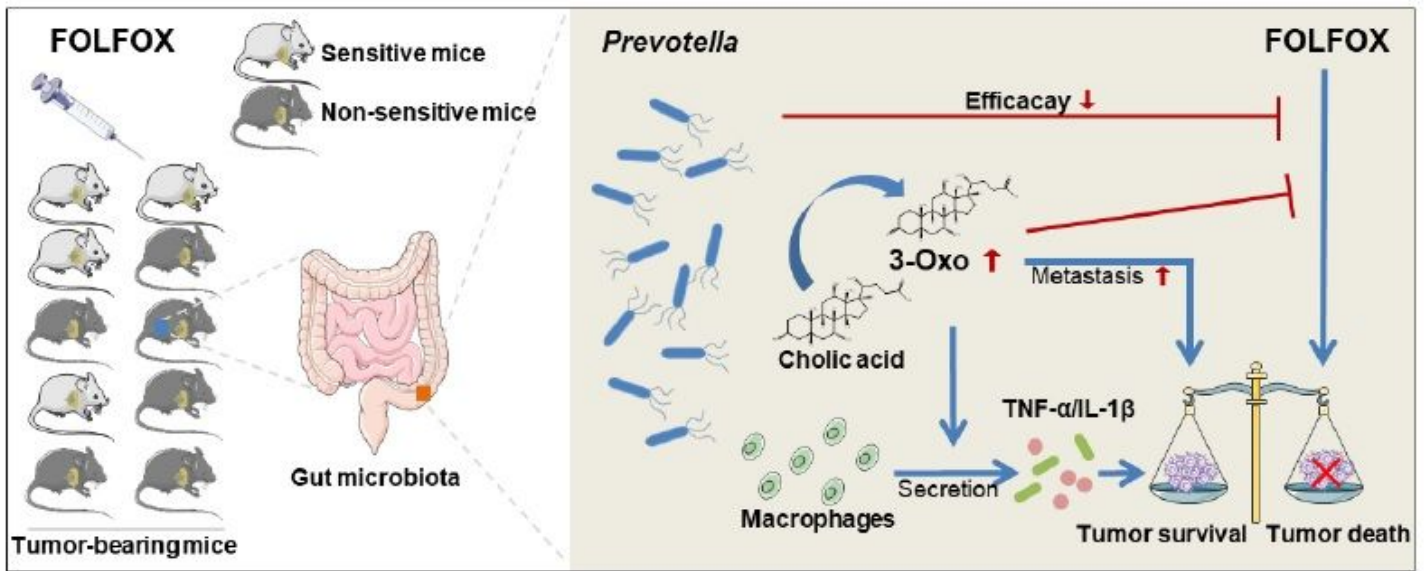


Figure 7

Overview of the current study. Pharmacodynamic evaluation was used to identify individualized S and NS mice after FOLFOX treatment. Pharmacomicrobiomics was used integrated with pharmacometabolomics to identify biomarkers in fecal samples mediating FOLFOX efficacy. *Prevotella* and 3-Oxocholeic acid were finally confirmed for contributing to individualized FOLFOX response.

Supplementary Files

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- [Supplementarymaterial.pdf](#)