1.Method of constructing pseudo-sterile tumor-bearing mice

Following a period of one week for acclimation, the mice were administered a combination of antibiotics via their drinking water for a duration of two weeks, with the aim of eliminating their intestinal flora. In order to preserve the sterility of the mice gut, antibiotics were administered via the drinking water subsequent to the inoculation of tumors in the mice. This measure was used to ensure the maintenance of sterility. The approach employed for reducing the population of microorganisms in mice, namely their intestinal flora, was the administration of an antibiotic solution (ABX). This solution consisted of ampicillin (1 mg/ml), streptomycin (1 mg/ml), colistin mucin (1 mg/ml), and vancomycin (0.25 mg/ml), which were added to the mice's sterile drinking water. The evaluation of mouse flora reduction was conducted using the Columbia Blood Agar medium. This process was repeated on a weekly basis. After a period of two weeks, mice were subjected to subcutaneous inoculation of tumors in order to establish the H-22 tumor mouse model. Following the use of antibiotics to eliminate the mouse colony, antibiotic-homa animal models were established by the subcutaneous injection of tumor cells. Upon the subcutaneous tumors of the mice reaching a volume of approximately 100 mm^3, the mice were subjected to random allocation into six distinct groups, and the treatment starts.

2. **Construction of fecal donor mouse model**

Following a one-week period of acclimatization, mice were subjected to subcutaneous tumor inoculation in order to establish a tumor-bearing model. Once the subcutaneous

tumors reached a volume of approximately 100 mm^3, the mice were randomly assigned to one of two groups: (1) the olive oil gavage group (referred to as Donor-Model, n = 10); and (2) the astaxanthin gavage group (referred to as Donor-AST, n = 10). The mice in the Donor-AST group were gavage by astaxanthin at a dosage of 60 mg/kg per day, whereas the mice in the Donor-Model group were administered 200 μL of olive oil by gavage on a daily basis. Fecal samples were collected from the animals after a period of 14 days of uninterrupted supplementation. Fecal samples were obtained from individual mice between the hours of 15:00 and 17:00 in the afternoon on a daily basis. These samples were then preserved in a sterile phosphate-buffered saline (PBS) solution supplemented with 50% glycerol. Subsequently, the acquired fecal samples were promptly transported to a storage temperature of -80°C. Prior to fecal transplantation, the collected fecal suspension from donor mice was subjected to mixing and subsequent division. Subsequently, for each fecal transplantation procedure, the suspension was randomly picked from the divided suspension and administered through gavage. Throughout the duration of the trial, the mice were provided unrestricted access to food and water, while monitoring the alterations in body weight and tumor volume at three-day intervals.

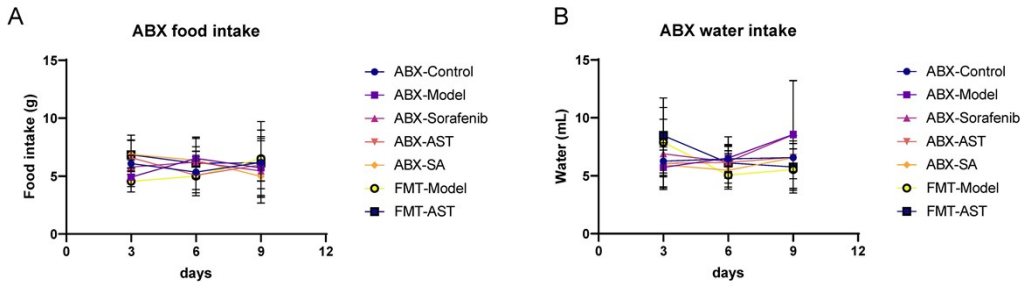

Fig.S1 Food intake (A) and water intake (B) of mice

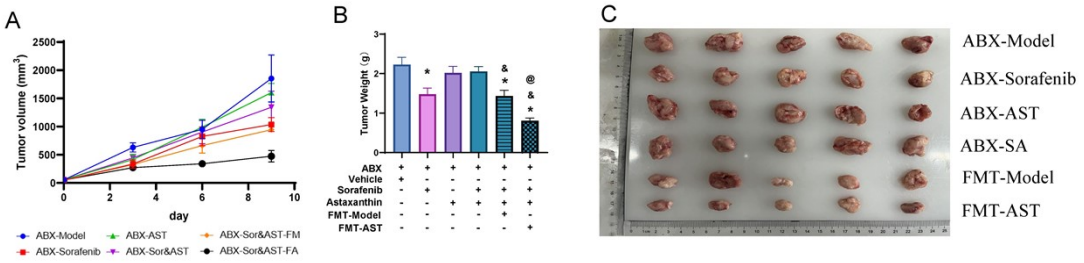

Fig.S2 Antitumor immune response to astaxanthin is dependent on gut microbiota. A: tumor growth curve; B: tumor weight; C: representative images of dissected tumors; *: $P < 0.05$, compared with ABX-Model; #: $P < 0.05$, compared with ABX-Sorafenib; $: $P < 0.05$, compared with ABX-AST； &： $P < 0.05$, compared with ABX-SA； @: $P < 0.05$, compared with FMT-Model.

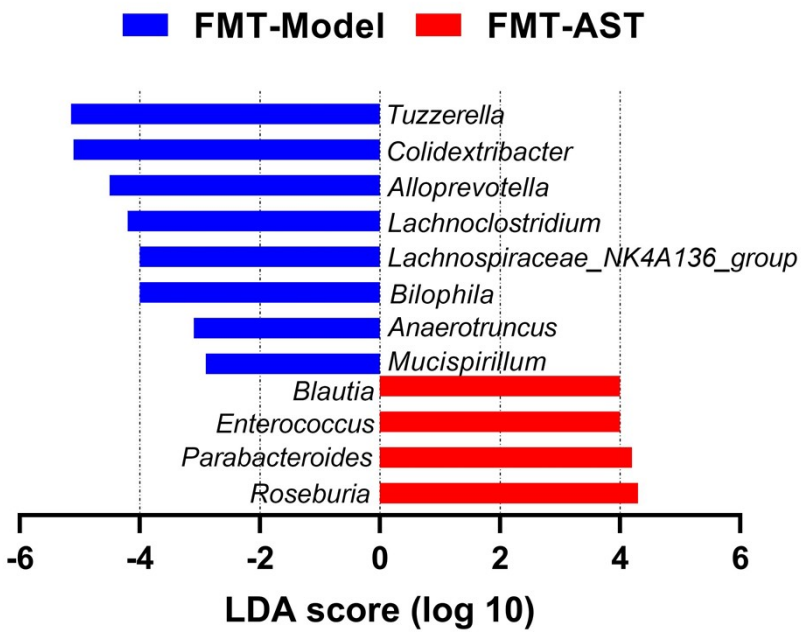

Fig.S3 LEfSe analysis identifies the most differentially abundant taxa in each group.

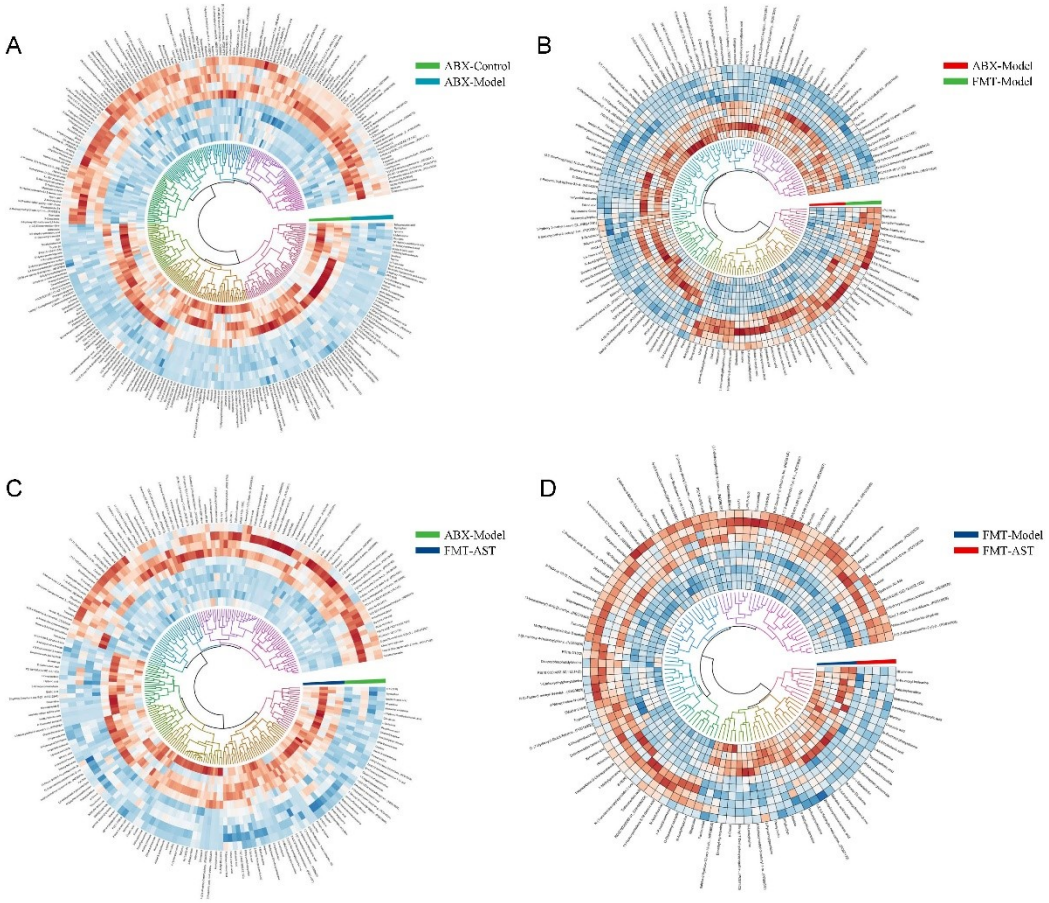

Fig.S4 Heatmap of serum metabolites in mice. A: ABX-Control vs ABX-Model; B: ABX-Model vs FMT-Model; C: ABX-Model vs FMT-AST; D: FMT-Model vs FMT-AST;

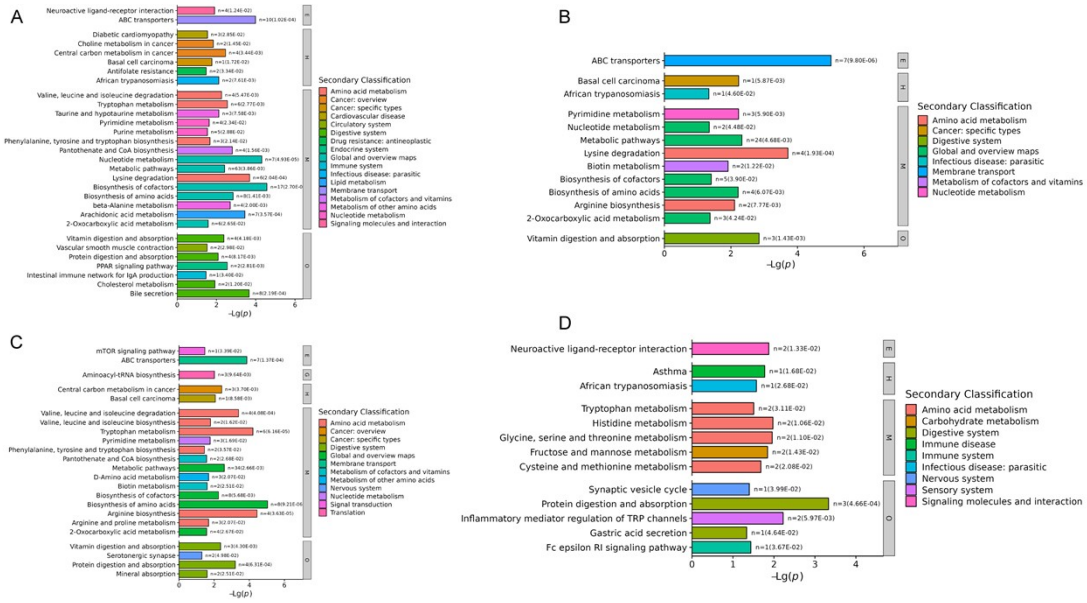

Fig.S5 KEGG pathway of differential metabolites in first classification levels. A: ABX-Control compared with ABX-Model; B: ABX-Model compared with FMT-Model; C: ABX-Model compared with FMT-AST; D: FMT-Model compared with FMT-AST.