Supplementary information

Pretreatment with a heat-killed probiotic modulates the NLRP3 inflammasome and prevents colitis-associated colorectal cancer in mice

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Figure S1. *E. faecalis* enhances ATP- and nigericin-induced IL-1β secretion through upregulation of pro-IL-1β expression. (a and b) THP-1-derived macrophages were pretreated with the indicated amount of *E. faecalis* for 24 h and then stimulated with ATP (for 4 h) or nigericin (for 1 h). (a) Immunoblot analysis of NLRP3 inflammasome molecules in cell supernatants (SN) and cell lysates (CL). The western blot is a representative of three independent experiments. Immunoblot images were quantified with the ImageJ software. (b) ELISA of IL-1β in the supernatant are shown. Symbols: *, *P* < 0.05; and **, *P* < 0.01. All results are presented as the mean ± SD of three independent experiments and were analyzed with the Student’s t test. Abbreviations: procasp-1, p45 precursor of caspase-1; casp-1 p10, active caspase-1 subunits; IL-1β p17, secreted mature IL-1β; and pro-IL-1β, p31 precursor of IL-1β.
Figure S2. *E. faecalis* treatment does not appear to affect pre-existing colitis-associated CRC. Schematic presentation of the mouse model of colitis-associated CRC. Mice were injected intraperitoneally with AOM prior to beginning the first of four cycles of DSS in the drinking water. One cycle was defined as 6 days of DSS followed by 14 days of water. Mice were orally inoculated with *E. faecalis* every day, starting at the end of the third DSS treatment. Eighty days after the start of DSS treatment, all mice were sacrificed; n=11 for the PBS control group and n=10 for the *E. faecalis*-treated group. (b and c) Percent weight change (b) and diarrhea scores (c) were monitored daily for all mice. (d) Representative images of colons. (e) Colon length and tumor number. Scale bars, 1 cm. Symbols: *, P < 0.05; and **, P < 0.01.