Supplementary Methods

Analysis of Fecal Microbiota

C57BL/6J mice were fed with *L. casei, B. breve* (10^9 bacteria each) or placebo daily by oral gavage for 3 months. Then, a few fecal pellets were collected, weighed and suspended in 9 volumes of RNA*later* (Ambion), an RNA stabilization solution, to make a fecal homogenate (100 mg feces/ml). In preparation for RNA and DNA extraction, 200 μl of the fecal homogenate was centrifuged and washed with PBS. The pellet was stored at -80°C (for extraction of RNA) or -30°C (for extraction of DNA). Total RNA and DNA for analysis fecal microflora (Matsuda et al., 2009), DNA for analysis of *L. casei* and *B. breve* (Fujimoto et al., 2008; Fujimoto et al., 2011) were isolated from fecal samples using the method described elsewhere. Briefly, total RNA was isolated using a modified acidic guanidinium thiocyanate-phenol-chloroform extraction method, and DNA was isolated using a modified phenol- chloroform extraction method. The DNA for analysis of *L. casei* and *B. breve* was extracted using a Stool Mini Kit (QIAGEN). Finally, the nucleic acid fraction extracted from 20 mg feces was suspended in 1 ml nuclease-free water (Ambion).

RT-qPCR and qPCR analyses were performed in 384-well optical plates on an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems). RT-qPCR was conducted in a one-step reaction using a QIAGEN OneStep RT-PCR Kit (QIAGEN) for detection and enumeration of the following bacterial targets: *C. difficile*, *C. perfringens*, Total *Lactobacillus* spp. (*L. gasseri* subgroup, *L. brevis*, *L. casei* subgroup, *L. fermentum*, *L. fructivorans*, *L. plantarum* subgroup, *L. reuteri* subgroup, *L. ruminis* subgroup, *L. sakei* subgroup), Enterobacteriaceae, *Enterococcus* spp., *Streptococcus* spp., *Staphylococcus* spp., and *Pseudomonas* spp. as described elswhere (Matsuda et al., 2007; Matsuda et al., 2009; Sakaguchi et al.). qPCR was carried out using rTaq (TaKaRa Bio) for detection and enumeration of the following bacterial targets: *Clostridium coccoides* group, *C. leptum* subgroup, *Bacteroides fragilis* group, Total

Bifidobacterium spp. (*B. adolescentis*, *B. angulatum*, *B. bifidum*, *B. breve*, *B. catenulatum* group, *B. longum*, *B. infantis*, *B. dentium*), *Atopobium* cluster, *Prevotella* spp., *Eubacterium cylindroides* group, *C. ramosum* subgroup, *L. casei* strain Shirota and *B. breve* strain Yakult as described previously (Fujimoto et al., 2008; Fujimoto et al., 2011; Matsuki et al., 2002; Matsuki et al., 2004; Matsuki et al., 1999; Matsuki et al., 1998).

Culture and killing of B. breve

B. breve was inoculated in GAM broth (Nissui Pharmaceutical) supplemented with 1%(w/v) glucose, and cultured for 24 h at 37 °C under anaerobic conditions. The supernatant of the culture medium was obtained by centrifugation at 15,000 rpm for 10 min, and concentrated to 10-fold by Centricon YM-3 (Milipore, USA). For killing of *B. breve*, UV radiation and sonication was used. For UV radiation, 5 ml of *B.breve* suspension was placed to petri dish, and then UV radiation was performed for 30 min by using 200 nm UV lamp. For sonication, total 5 min of sonication was performed with interval at every 1 min. Viability of *B.breve* was checked by MRS agar plate.

Supplementary References

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