Supporting Information

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SI Materials and Methods

Animals and Bacteria. Eight- to 10-wk-old specific pathogen-free BALB/c and C57BL/6 mice were purchased from Taconic Farms. TLR2$^{-/-}$ animals were purchased from Jackson Laboratories. Germ-free C57BL/6 and Rag$^{-/-}$ mice were bred in plastic Trexler isolators, fed autoclaved food and water, and screened weekly by PCR and microbiological plating to ensure sterility. To obtain germ-free C57BL/6 Foxp3-GFP bone-marrow chimeras, germ-free mice were lethally irradiated and reconstituted with RBC-depleted bone marrow from Foxp3-GFP donors by retro-orbital injection. Mice were immediately placed in autoclaved cages and water supplemented with antibiotics (100 μg/mL gentamicin and 10 μg/mL erythromycin) throughout the reconstitution period. Mice were subsequently colonized with strains of *Bacteroides fragilis* NCTC9343 carrying the pFD340 vector to confer erythromycin resistance and verified for proper colonization by plating under aerobic and anaerobic conditions and PCR of fecal samples with primers specific for *B. fragilis* and 16S rRNA bacterial primers. Polysaccharide A (PSA) was purified as previously described for all treatment experiments (1). All animals were cared for by approved Institutional Animal Care and Use Committee guidelines from the California Institute of Technology.

Cytokine Analysis. RNA was collected from indicated cells using TRIzol (Invitrogen). Complementary DNA was made using an iSCRIPT cDNA synthesis kit per manufacturer's instructions (Bio-Rad). Quantitative real-time polymerase chain reactions (qRT-PCR) were performed using IQ SYBR Green Supermix per manufacturer's instructions (Bio-Rad). Reactions were run on the Bio-Rad IQ5 q-PCR machine. Primers are as follows *FOXP3*: F 5′-GCAATAGTTCCTTCCCAGAGTTCT-3′; R 5′-GGATGGCCCATCGGATAAG-3′. *IL-10*: F 5′-CTGGACAACATACTGCTAACCG-3′; R 5′-GGGCATCACTTCTACCAGGTAA-3′. *L32*: F 5′-AAGCGAAACTGGCGGAAAC-3′; R 5′-TAACCGATGTTGGGCATCAG-3′. *IL-17A*: *F* 5′-TTAAGGTTCTCTCCTCTGAA-3′; R 5′-TAGGGAGCTAAATTATCCAA-3′.

Lamina Propria Lymphocyte Extraction and Flow Cytometry. The colon was carefully cleaned of the mesentery and residual fat and cut open longitudinally and subsequently cut into large fragments (1–1.5 cm). Fragments were placed in a 50-mL conical and rinsed well with ice-cold PBS (Invitrogen). Cleaned intestinal fragments were placed in 15 mL of epithelial cell dissociation solution (Ca^+ and Mg^+ free HBSS with 5 mM EDTA and 10 mM Hepes) at 37 °C for 15 min with gentle agitation and subsequently vortexed for 15 s. This step was repeated once more. The fragments were then minced with a razor blade and then placed in a digestion solution [HBSS with 5% FBS, 3 units/mL of Dispase, 0.5mg/mL of Collagenase D and 0.5 mg/mL of DNase I (all from Worthington Biochemical)], digested for 20 min with slow rotation at 37 °C, and then vortexed well. Supernatants were collected by filtering through a 40-μm cell strainer. Digestions were repeated two more times until material was completely digested, Lamina propia lymphocytes (LPLs) were resuspended in 8 mL of 40% Percoll and layered on top of 5 mL of 80% Percoll (GE Healthcare). LPLs were recovered from the interface of the 40 and 80% gradient after centrifugation, washed and used as described. For Foxp3 intracellular staining, 0.5 to 1 × 10^6 cells were first surface stained then permeabilized and fixed in 100 μL of Fixation and Permeabilization buffer (eBiosciences). For IL-10 and IFN-γ intracellular cytokine staining, LPLs were extracted and restimulated with 500 ng/mL of ionomycin and 50 ng/mL of PMA (Calbiochem) in the presence of 0.5 μL of GolgiPlug (BD Biosciences) for 4 to 5 h at 37 °C. Cells were subsequently surface stained and then permeabilized and fixed overnight, as described. Cells were stained with 0.3 μg of either anti-IL-17A or IL-10 for 20 min at 4 °C and washed three times with perm wash. All antibodies were purchased from eBiosciences.

Experimental Colitis. Eight-week-old BALB/c mice were purchased from Taconic Farms. For prophylactic studies, animals were pretreated with 50 μg of PSA every other day for 6 d before administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) and every other day after TNBS induction until necropsy. For curing studies, animals were treated with a single dose of 50 μg of PSA 1 or 2 d following TNBS and every other day until necropsy. For BALB/c mice, 0.75 to 2.5% TNBS (Sigma) in 50% ethanol was rectally delivered using a 3.5-Fr silicone catheter (Instech Solomon). Mice were weighed daily until necropsy 5 to 8 d after TNBS administration. In some cases C57BL/6 mice (or TLR2$^{-/-}$ on a C57BL/6 background) were used (Fig. 3 *E–H*); these mice are more resistant to the development of TNBS colitis; therefore, they were given two 2.5% TNBS doses (administered rectally) separated by 7 d. C57BL/6 mice were followed 5 to 8 d after the second TNBS administration. Colitis scoring for BALB/c (Fig. 3 *A–D* and Fig. 4) was as follows: 0, no signs of inflammation; 1, low level of inflammation; 2, low level of leukocytic infiltration; 3, high level of leukocytic infiltration, high vascular density thickening of colon wall; 4, loss of goblet cells, high vascular density, thickening of colon wall; 5, transmural infiltrations. Mice were also given a score based on the percentage of the colon affected: 0, none; 1, < 15%; 2, 15 to 40%; 3, 40 to 70%; 4, > 70% (colitis scores and percent-tissue affected were added for a maximum score of 9). Colitis scoring for C57BL/6 mice were as follows: 0, no signs of inflammation; 1, low level of inflammation; 2, low level of leukocytic infiltration; 3, high level of leukocytic infiltration, high vascular density thickening of colon wall; 4, transmural infiltrations, loss of goblet cells, high vascular density, thickening of colon wall. Mice were also given a score based on the percentage of the colon affected: 0, none; 1, < 15%; 2, 15 to 40%; 3, 40 to 70%; 4, > 70% (colitis scores and percent-tissue affected were added for a maximum score of 8).

1. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL (2005) An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122(1):107–118.

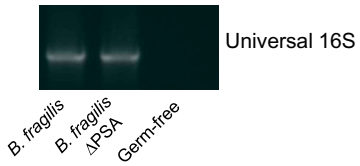

Fig. S1. Verification of the colonization status of mice. DNA was extracted from fecal samples of germ-free mice that were irradiated and reconstituted with bone marrow from Foxp-GFP animals. Universal 16S rDNA primers demonstrate that germ-free mice remained sterile throughout experiment. Microbiological plating was also used to ensure monocolonization of animals.

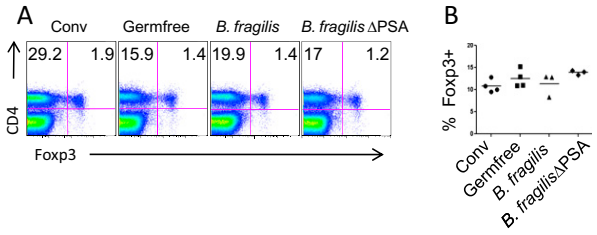

Fig. S2. There is no difference in the percentage of naturally occurring T regulatory cells (Tregs) within the mesenteric lymph nodes (MLNs) based on microbiological status. (*A*) MLNs from conventional, germ-free, *B. fragilis*, and *B. fragilis*ΔPSA-colonized mice were extracted and stained with antibodies to detect CD4 and Foxp3. These data are representative of five independent experiments. (*B*) The percentage of naturally occurring Tregs between conventional, germfree, *B. fragilis*, and *B. fragilis*ΔPSA does not differ within the colon. Lamina propria lymphocytes (LPLs) were extracted from the colons of indicated mice and stained with antibodies to detect CD4 and Foxp3. The numbers represent the percentage of Foxp3-expressing cells within the CD4 compartment. Each symbol represents an individual mouse. Horizontal bars indicate the average between mice. These data are representative of four independent experiments with at least three mice in each group. There is no statistical difference between any of the groups.

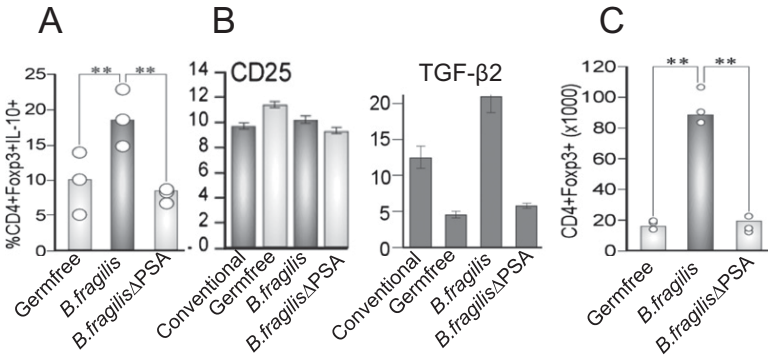

Fig. S3. PSA coordinates inducible Tregs. (*A*) Colonic LPLs were extracted and restimulated with PMA and Ionomycin for 4.5 h in the presence of brefeldin A. Cells were subsequently stained for CD4, Foxp3, and IL-10. Bars indicate the average of three mice from one experiment and circles indicate individual mice. $^{**}P < 0.01$. These data are representative of three independent trials with at least three mice in each group. (*B*) Treg associated gene expression in response to microbial colonization. $CD4^+Foxp3^+$ cells were sorted from the MLN of indicated mice and RNA extracted. Levels of TGF-β2 and CD25 were assayed by qRT-PCR. These data represent three independent experiments. Error bars represent SD of three replicates from the same experiment. (*C*) The absolute numbers of converted Tregs within the MLN. Numbers of cells were counted and percentages of Tregs were determined. Each circle represents an individual mouse. $^{**}P < 0.01$. These results represent two individual trials.

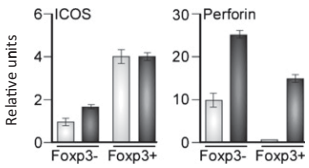

Fig. S4. PSA up-regulates the expression of genes for ICOS and Perforin. Foxp3-GFP mice were orally treated with purified PSA every other day for 6 d. MLNs were extracted and $CD4^+Foxp3^+$ or the $CD4^+Foxp3^-$ T cells were purified by FACS based on ±GFP expression (purity >99%). RNA was extracted and used for qRT-PCR. These data are representative of three independent experiments. Light bars indicated cells derived from PBS treated mice and dark bars from PSA-treated mice. Error bars represent SD from samples run in triplicate from a single experiment. These results represent three independent trials.

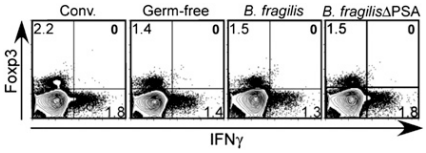

Fig. S5. PSA does not induce IFN-γ in the MLNs. Cells were taken from the MLN and stained with CD4, Foxp3, and IFN-γ. Plots are gated on $CD4^+$ cells and numbers rounded to the nearest tenth. These are data are representative of three independent trials.

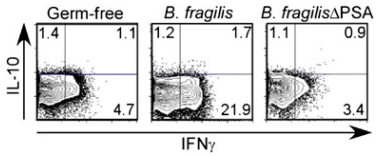

Fig. S6. Colonization with *B. fragilis* induces Th1 cells in the spleen. GF $Rag^{-/-}$ animals were colonized with *B. fragilis* or *B. fragilis*ΔPSA and $CD4^+Foxp3^-$ T cells from Foxp3-GFP mice were adoptively transferred. After 3 wk, spleens were harvested and $CD4^+$ T cells stained for IL-10 and IFN-γ after restimulation with PMA/Ionomycin in the presence of brefeldin A. Plots are gated on $CD4^+Foxp3^-$ (GFP^-) cells. Numbers are rounded to the nearest tenth.

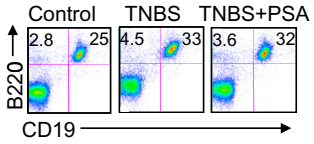

Fig. S7. PSA expands Tregs, but not B cells during experimental colitis. The MLN of TNBS mice were analyzed for the presence of B cells by FC staining for B220 and CD19. These results are representative of three independent experiments.

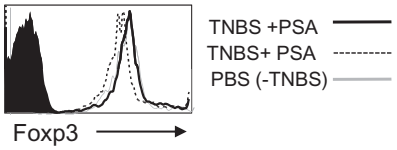

Fig. S8. PSA increases the amount of Foxp3 protein during TNBS. Cells from the MLN were extracted from indicated mice and stained with CD4 and Foxp3. Plots are gated on $CD4^+$ cells. The histogram indicates that PSA treated animals have a higher expression of Foxp3 than cells from TNBS animals. These data are representative of three independent experiments.

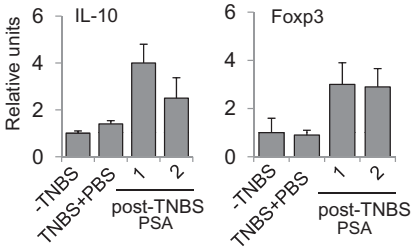

Fig. S9. PSA treatment of animals after induction of TNBS colitis up-regulates IL-10 and Foxp3 expression. BALB/c animals were induced for TNBS colitis and subsequently given PBS 1 d post-TNBS induction (TNBS+PBS) or PSA 1 or 2 d post-TNBS induction (post-TNBS PSA 1 or 2). Five days post-TNBS treatment, RNA was extracted from the colon and IL-10 and Foxp3 gene expression was assayed by qRT-PCR. Error bars represent the SD from samples run in triplicate from one independent trial.

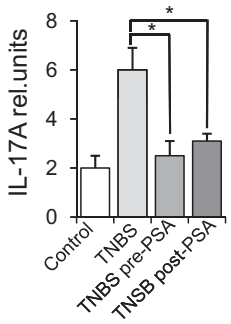

Fig. S10. PSA treatment of colitic animals results in the decreased expression of inflammatory IL-17A. RNA was extracted from the MLN of indicated animals and expression levels of IL-17A were determined by qRT-PCR. Error bars indicate SD from four independent mice. $*P < 0.05$. These data are representative of three independent trials.