Supplemental Text

1. Rich Medium Recipe (per liter) for *Bacteroides* Growth

Twenty grams proteose peptone, 5 g yeast extract, 5 g NaCl, 5 g glucose, 5 g K2HPO4, 0.5 g L-cysteine, 5 mg hemi, and 2.5 μL vitamin K1.

2. Supplemental Experimental Procedures

Isolation of lymphocytes and flow cytometry Small and large intestines were collected and flushed with phosphate buffered saline (PBS) to remove fecal content; fat tissue was then removed. The intestines were inverted and stirred at 500 rpm in PBS containing 3% FBS, 5% DTT, and 1 mM EDTA for 30 min at 37ºC in cups. After removal of epithelial cells, the intestines were washed in PBS by inversion 10 times in 15-ml Falcon tubes, cut into small pieces, and incubated with RPMI 1640 containing 5% FBS, collagenase type II (1.5 mg/ml), and dispase (0.5 mg/ml) (all from GIBCO) for 1 h at 37°C with constant stirring at 500 rpm. The digested tissues were filtered through a strainer (mesh size, 40 μm) and washed three times in PBS containing 2% FBS before being stained for flow cytometry. Lymphocytes from other tissues were processed as previously described (Olszak et al., 2012). The following antibodies were used: phycoerythrin-labeled PBS-57-loaded or -unloaded mCD1d tetramer (NIH Tetramer Core Facility, Atlanta, GA), FITC-labeled CD3 and TCRβ (BD Biosciences), phycoerythrin-Cy7-labeled CD62L and IgG2a isotype control (eBioscience), PerCp-labeled CD69 and IgG isotype control (eBioscience), and APC-labeled CD45RB and IgG2a isotype control (eBioscience). 7-Amino-actinomycin D (7-AAD) viability staining was used to exclude dead cells. To detect apoptotic cells, a TUNEL kit (Invitrogen) was used according to the manufacturer's instructions. Flow cytometry was performed with MACS Quant (Miltenyi Biotec), and data were analyzed with FlowJo software (TreeStar Inc.). Proliferation was measured with a Ki-67 marker (BD Biosciences) and a BrdU kit (Invitrogen) according to the manufacturers' instructions.

Experimental oxazolone colitis model Mice were pre-sensitized by epicutaneous application of 3% oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; Sigma-Aldrich) in 100% ethanol in a volume of 200 μl. Five days later, the animals were re-challenged intrarectally (through a 3.5F catheter) with 1% oxazolone in 50% ethanol (5 μl/g of body weight). For CD1d antibody treatment experiments, 19G11 antibody to CD1d or IgG2b isotype control antibody (0.5 mg/mouse/dose; BioXcell) was administered intraperitoneally to mice three times per week for the first two weeks of life and then once a week for 4 weeks. Body weight, rectal bleeding, and stool consistency were analyzed daily. Tissues obtained at the indicated time points were embedded in paraffin, stained with hematoxylin and eosin, and examined by a pathologist (Dr. Roderick Bronson) in a blinded fashion for evidence of colitis according to five established criteria: hypervascularization, presence of mononuclear cells, epithelial hyperplasia, epithelial injury, and presence of granulocytes.

^{1}H- and ^{1}H-^{1}H correlation spectroscopy (COSY) NMR. The spectrum of GSL-Bf717 was recorded at 298 K in pyridine-d5 with a Varian VNMRS-600 NMR spectrometer. Tetramethylsilane was used as the chemical-shift reference (0 ppm) in NMR experiments.

High-pH anion-exchange chromatography (HPAEC). Purified GSL-Bf717 was hydrolyzed under strongly acidic conditions (1 N HCl, 95°C, overnight) to release the

conjugated sugar head group. The sugar was then further analyzed with HPAEC (Dionex LC20 system with ED50 electrochemical detector and CarboPac PAI column, Thermo Scientific). Hexose standards (glucose, galactose, and mannose) were respectively spiked with the hydrolysate to confirm the conjugated sugar head group.

Colonic RNA purification and real-time reverse transcription PCR (RT-PCR) The protocol followed a published procedure (Olszak et al., 2012). Basically, 0.5-cm samples of tissue from transverse colons were harvested and immediately frozen in liquid nitrogen. RNA samples were prepared by grinding the tissue with a tissue homogenizer and an RNeasy Mini Kit; the complementary DNAs were synthesized with an Omniscript RT Kit (all from Qiagen). Real-time RT-PCR was performed with a SYBR Green Master Mix (Roche) and a CFX96 Real-Time System (Bio-Rad). β-Actin was used to normalize the expression of each sample. The following primer sets were used: *cxcl16*, 5'-CAGATACCGCAGGGTACT TTG-3' and 5'-CTGCAACTGGA ACCTGATAAAGA-3'; *β-actin*, 5'- GATGCTCCC CGGGCTGTATT-3' and 5'- GGG GTACTTCAGGGTCAGGA-3'.

Quantification of tissue and lumen-associated bacteria Six-week old mice were sacrificed to harvest the 1-cm mid section of colons. The colonic contents were scraped out using tweezers for lumen-associated bacteria quantification. The remaining tissues were grinded with a tissue homogenizer and counted for colony forming units (CFU).

Western Blot Independent BFWT and BFΔSPT cultures (4 for each) were grown in rich-medium to OD_{600}=0.8 before 50 μl of each culture was washed two times with phosphate buffered saline and boiled in Laemmli sample buffer (BioRad) with 0.35 M dithiothreitol for 15 min. Total bacterial cell contents were separated by 10% tris-glycine SDS gel and transferred to PVDF membrane. PSA was detected using polyclonal anti-PSA antibody. Known amount of purified PSA was used as a standard and to quantify band signal intensity on the blot. The amount of PSA detected in each sample was then normalized to the numbers of bacteria used.

3. Supplemental Figure Legends

Figure S1. *B. fragilis* sphingolipids modulate the homeostasis of host iNKT cells in the colon but not in other tissues. Related to Figure 1. (A) Growth curves of BFWT and BFΔSPT strains in rich medium. Data are representative of duplicate experiments and are presented as median ± range; n=3. (B) *B. fragilis* mutant strain BFΔSPT did not produce sphingolipids, and the complemented strain C-delta restored sphingolipid production to the level seen in the BFWT strain. Sphingolipids were resistant to mild alkaline hydrolysis, while non-sphingolipids were not. Figure shows thin-layer chromatography of crude lipid extracts with or without 0.02 N NaOH treatment. The chromatography solvent used was chloroform–methanol–acetic acid–water at a ratio of 100:20:12:5. Data are representative of 3 experiments. (C) GF, BFWT and BFΔSPT mice had similar numbers of colonic $CD3^+$ cells. n>3. (D) At 6 weeks of age, numbers of colonic LP iNKT cells in C-delta mice were similar to those in BFWT mice; n≥3. (E–G) Bacterial sphingolipids did not influence iNKT cell numbers in the thymus (E), spleen (F), or Peyer's patches (G). Data in (A) and (C)-(G) were analyzed by the Mann Whitney test.

Figure S2. *B. fragilis* sphingolipids modulate host colitis phenotype via the CD1d-dependent pathway. Related to Figure 2. BFΔSPT mice treated with CD1d antibody

beginning in the neonatal period had reduced iNKT cell counts and were protected from colitis challenge. Total colonic LP iNKT cell numbers (A; n≥3) were lower in α-CD1d-treated mice than in isotype-treated mice at 8 weeks. The α-CD1d-treated mice also lost less weight (B, P value compares data between the two groups at day 4) and had lower cumulative histopathology scores (C) after oxazolone colitis challenge (n=7). Data in (A) and (C) were analyzed by the Mann Whitney test. Data in (B) were confirmed to have normal distribution by the KS normality test with α=0.05, analyzed by the Student's t test and are presented as mean ± SEM.

Figure S3. During neonatal development, BFWT and BFΔSPT mice differ in the proliferation of colonic LP iNKT cells but not in a number of other tested mechanisms. Related to Figure 3. (A) Similar numbers of bacteria resided in the colons of BFWT and BFΔSPT mice; n≥3. P value compares BFWT and BFΔSPT mice. (B) At 6-week of age, the colon tissue-associated and the lumen-associated bacterial numbers in the BFWT and BFΔSPT mice were similar, respectively. (C) At 6–8 weeks of age, GF, BFWT and BFΔSPT mice had similar levels of *cxcl16* mRNA expression in the colon. A.U., arbitrary unit. (D) Colonic iNKT cells in BFWT mice (*red*) and BFΔSPT mice (*blue*) at 6–10 weeks of age expressed CD62L, CD69, and CD45RB at similar levels. Isotype control values (*black*) are also shown. Data were compiled from 3 independent experiments for each group of mice, with 2 or 3 mice per group for each experiment and analysis of 4000–9000 colonic iNKT cells for each group. Numbers indicate mean geometric fluorescence intensity. (E) PSA expression was similar in the *in-vitro* cultures of BFWT and BFΔSPT strains; n=4. (F) Gating of Ki-67 staining on colonic LP iNKT cells in flow cytometry. Data were compiled from 3-5 mice for each group. (G) The proportion of colonic LP iNKT cells expressing Ki-67 was lower in SPF and BFWT mice than in GF and BFΔSPT mice at 8 days of age; n≥3. (H) Gating of BrdU staining on colonic LP iNKT cells in flow cytometry. Data were compiled from 3-5 mice for each group. Within each group, data were processed by subtracting PBS background from BrdU treatment, and then plotted in Fig. 3B. Data in (G) (days 8 and 12) were confirmed to have normal distribution by the KS normality test with α=0.05, analyzed by the Student's t test and are presented as mean ± SEM. Data in (A), (B), (C) and (E) were analyzed by the Mann Whitney test.

Figure S4. GSL-Bf717 does not activate iNKT cells. Related to Figure 5. (A-H) CD1d tetramers added with bacterial sphingolipids did not bind to iNKT cell line 24.7. (I–K) CD1d tetramers added with GSL-Bf717 did not stain liver lymphocytes. Representative FACS plots are shown from 2 independent experiments.

4. Supplemental Tables

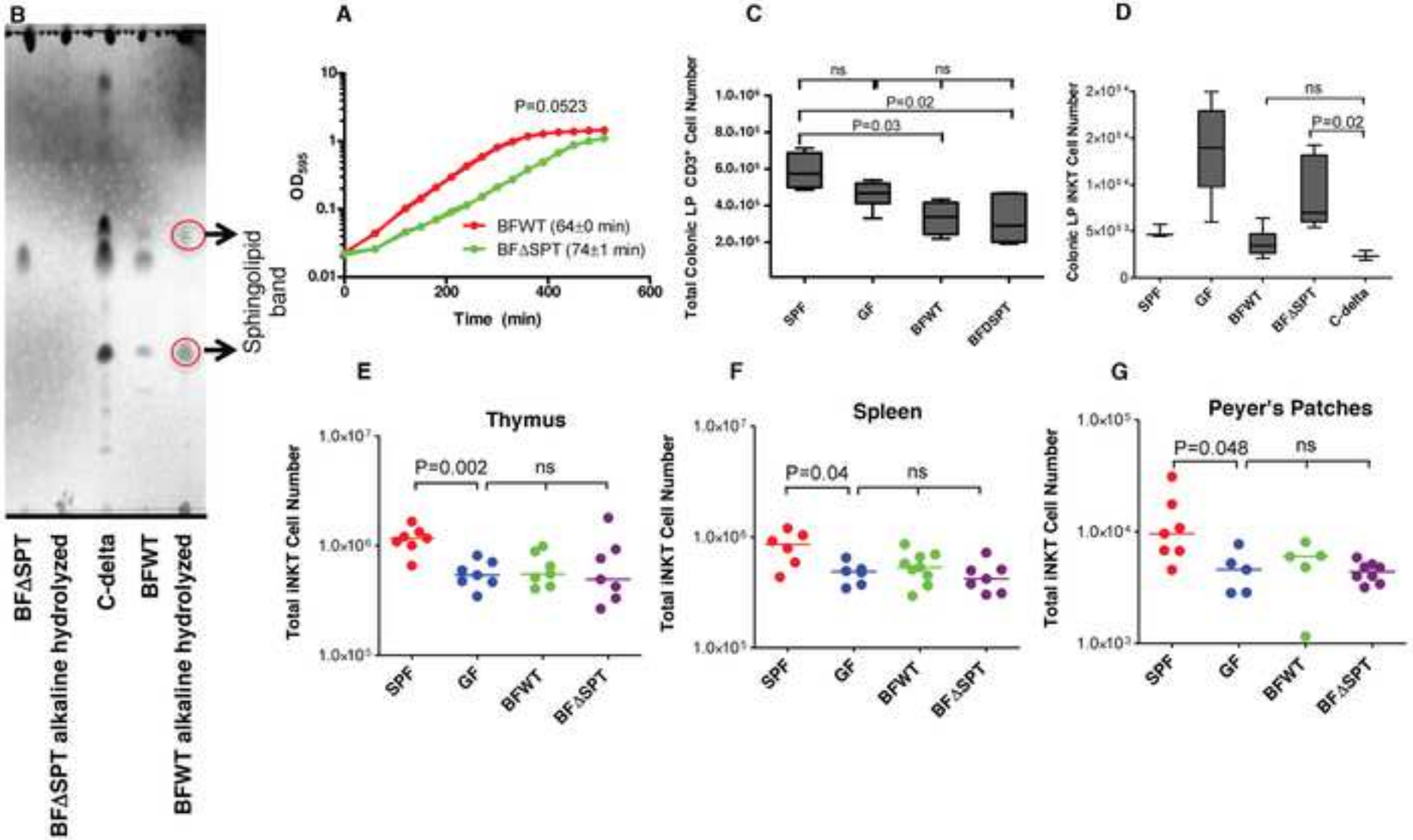
Table S1. Apoptotic populations among colonic iNKT cells are similar and negligible in GF, SPF, BFWT, and BFΔSPT mice.

	Total iNKT Cells Analyzed	Total TUNEL+	%
GF	4765	55	1.2
SPF	1994	30	1.5
BFWT	2551	38	1.5

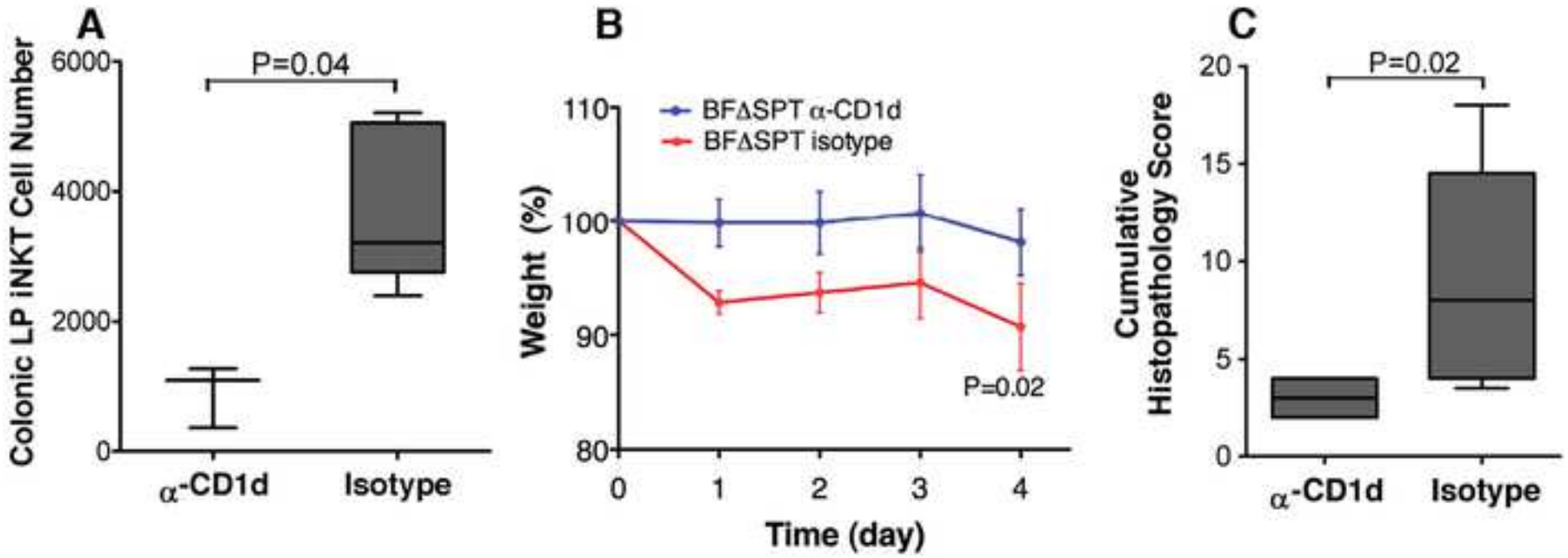
BFΔSPT	9294	81	0.9

Table S2. Statistical analyses.

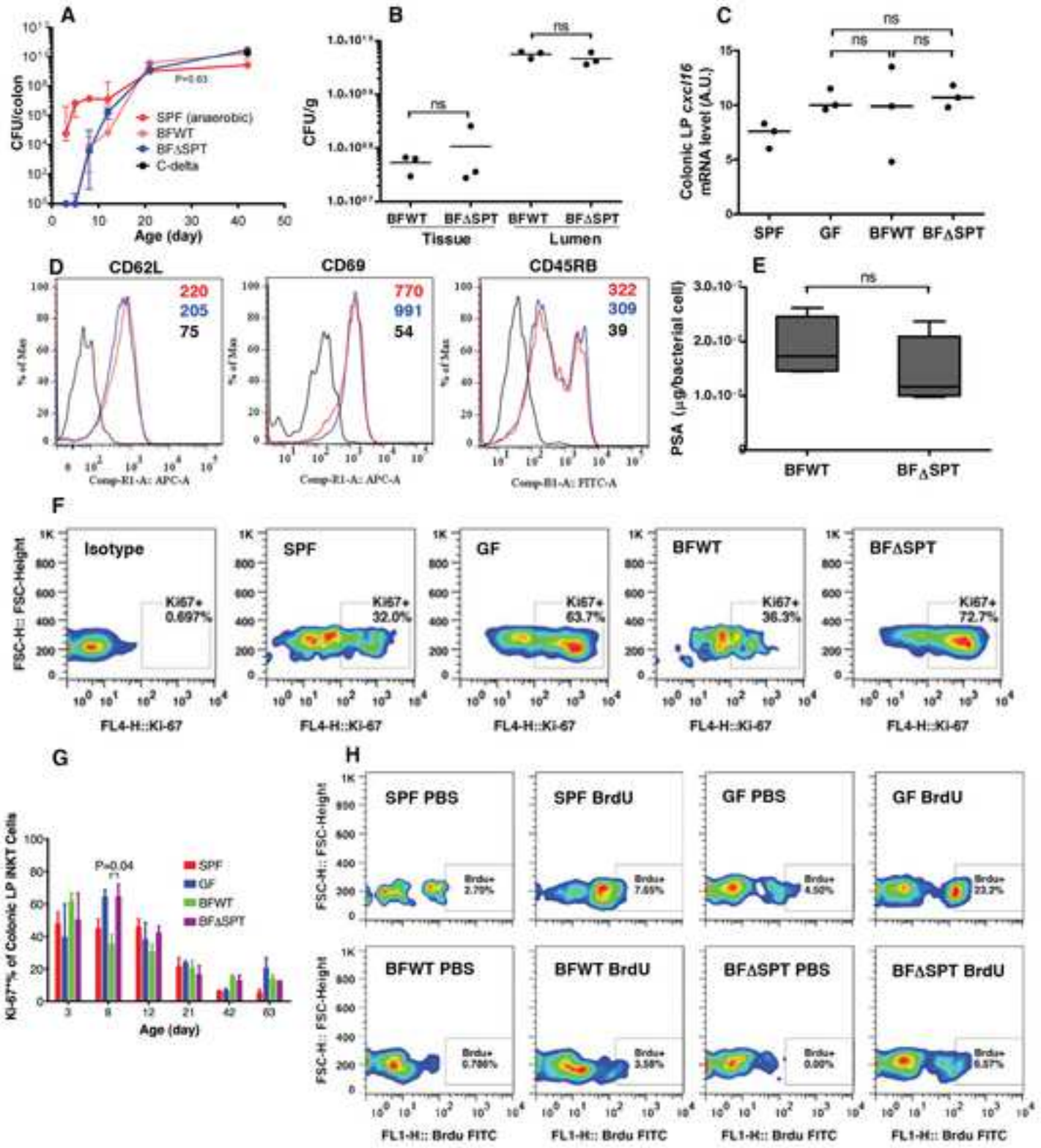
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4