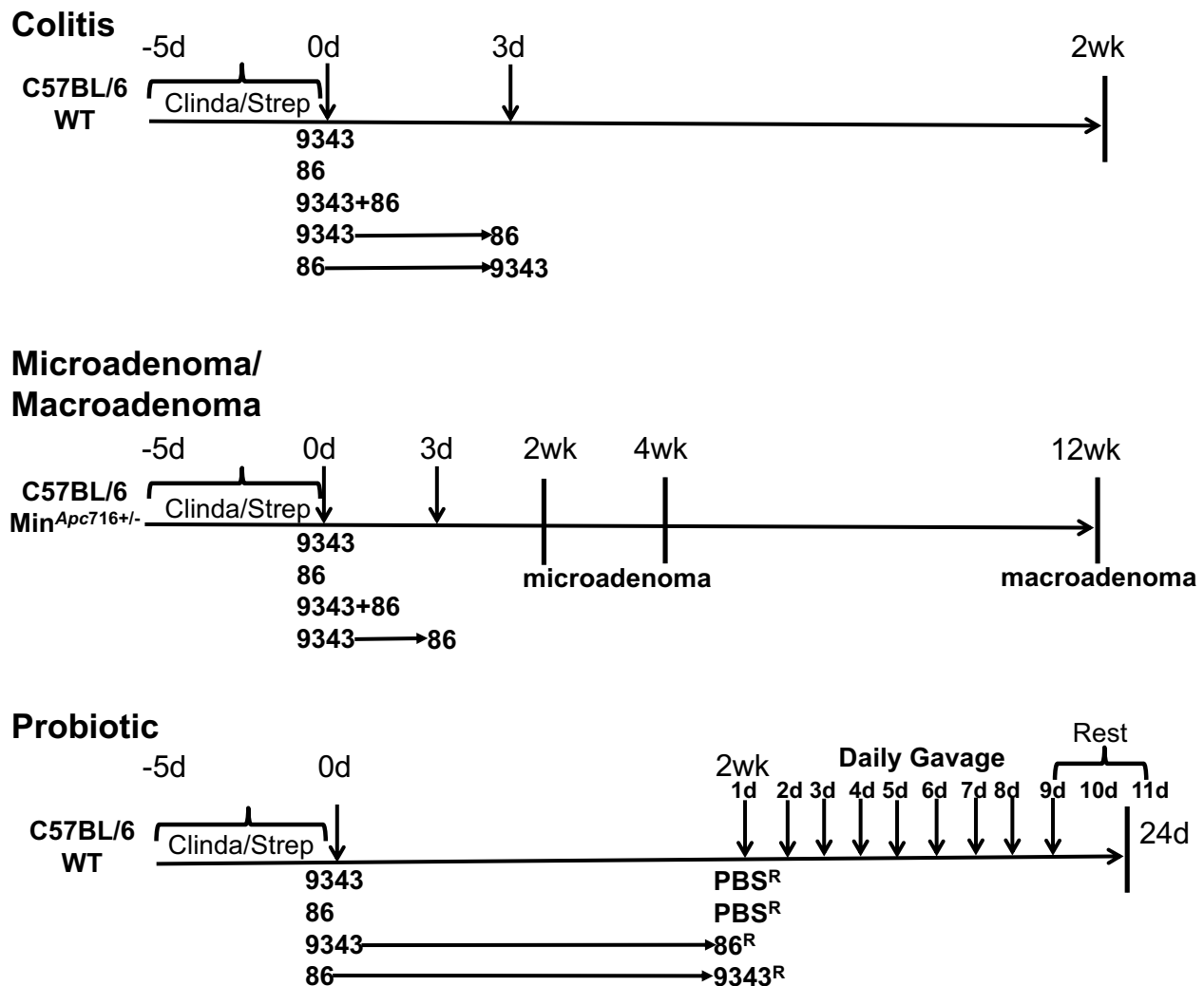
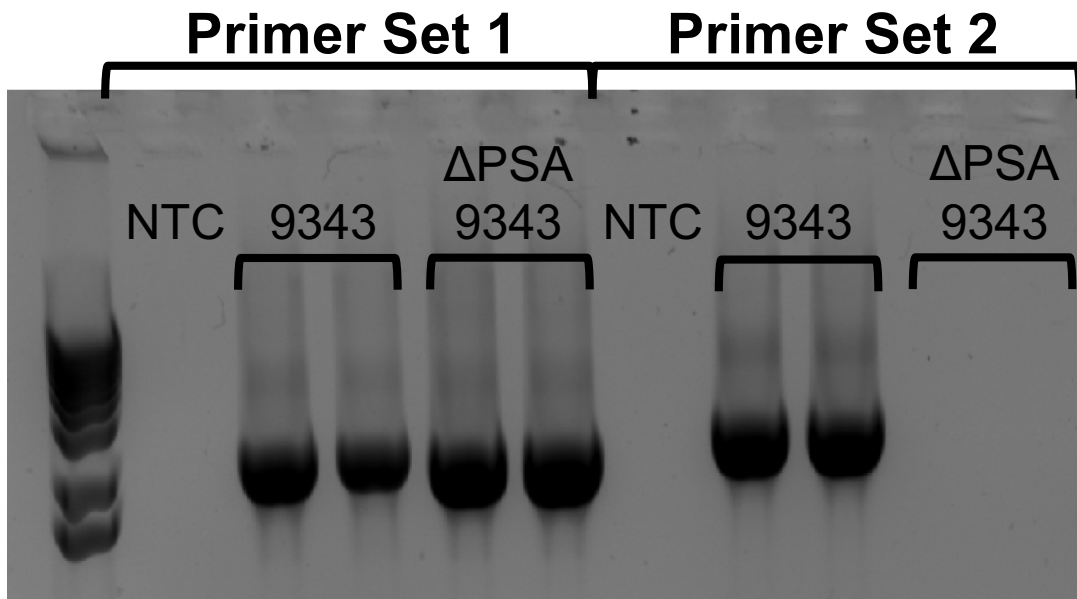


S1 Fig. Schematic of SPF mouse co-infection experiments. Mice received peroral antibiotic water ad lib for 5 to 7 days prior to initial bacterial infection. Sequentially infected mice received challenge strains on day 3. Simultaneously infected mice received both strains on day 0. Fecal colonization of the initial strain (day 0) was confirmed for sequentially treated mice prior to administration of the challenge strain. For colitis experiments, mice were sacrificed 2 weeks post initial strain infection (WT), microadenoma experiments at 2 to 4 weeks (Min), and macroadenoma experiments at 12 weeks (Min). For ‘probiotic’ treatment experiments, mice were colonized with single strains for 2 weeks (86 or 9343). Mice were then gavaged with 9343^{*} (if pre-colonized with 86) or 86^{*} (if pre-colonized with 9343) daily, for 9 days. 9343 or 86 only mice were gavaged with 1X PBS (PBS^{*}). Mice were rested for 2 days after consecutive probiotic treatment and then sacrificed (24 days after initial strain gavage). Superscript^{*} denotes repeated treatments.

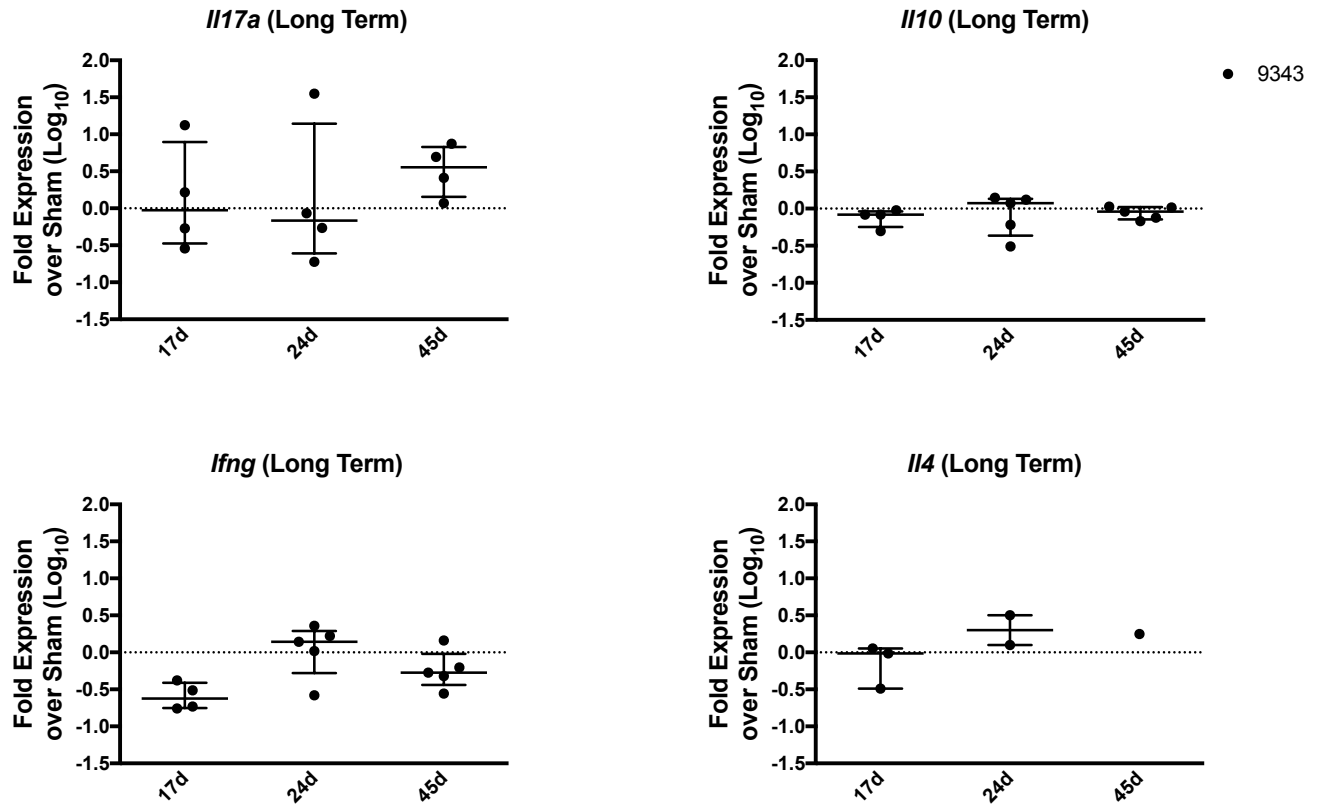


S2 Fig. Confirmation of PSA-competent and PSA-deficient 9343 strains. gDNA was extracted from 9343 and Δ PSA 9343 grown from a single colony. Primer sets 1 (PSA-upstream) and 2 (PSA-internal) were used to confirm the PSA-mutant strain. Δ PSA 9343 was not amplified with primer set 2. NTC = no template control. Primer sets are found in the **S3 Table**.

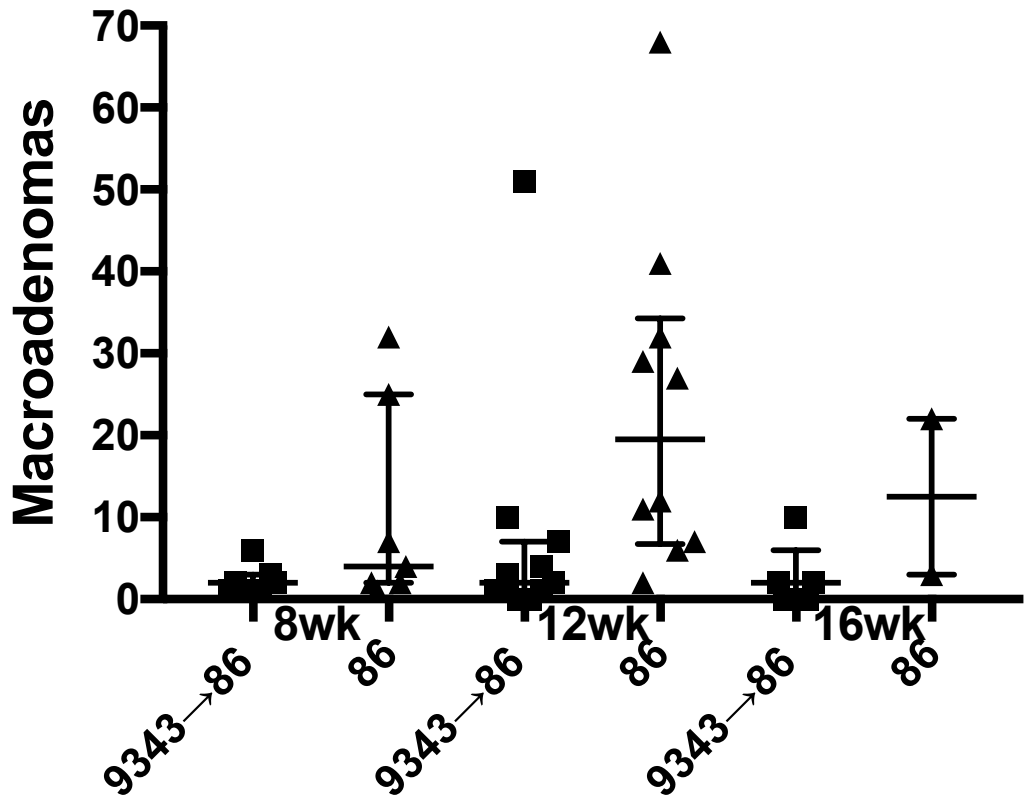


S3 Fig. Cytokine expression determined by colon whole tissue qRT-PCR at 17, 24, and 45 days after 9343

colonization. 9343-colonized SPF WT mice exhibit no changes in total *Il17a*, *Il10*, *Ifng*, and *Il4* expression at early or late time points. Bars indicate median and interquartile range. Data shown are from 1 independent experiment with 4 to 5 mice per time point. Mice were compared to their Sham control littermates.

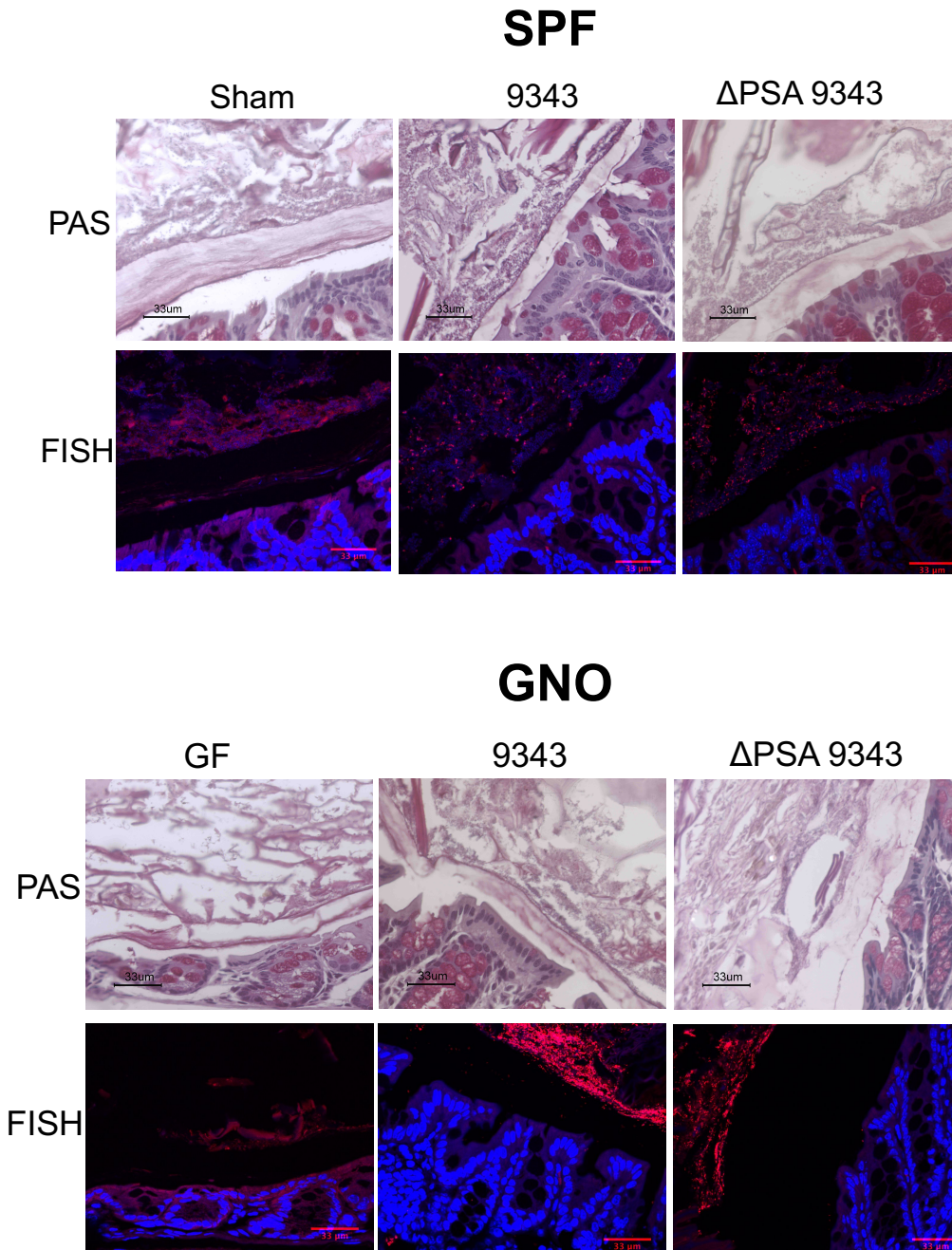


S4 Fig. After strain establishment, 9343 protection against macroadenoma development is durable. Formalin-fixed, methylene blue-stained colons of SPF Min 9343→86 mice and 86 only control mice were observed for macroadenomas at 8, 12, and 16 weeks after initial infection. Data shown at 8 and 16 weeks are from 1 independent experiment (2 to 7 mice per group). Data shown at 12 weeks represents a compilation of 3 independent experiments as shown in **Fig 2B** (10 to 11 mice in total per group). Bars indicate median and interquartile range.



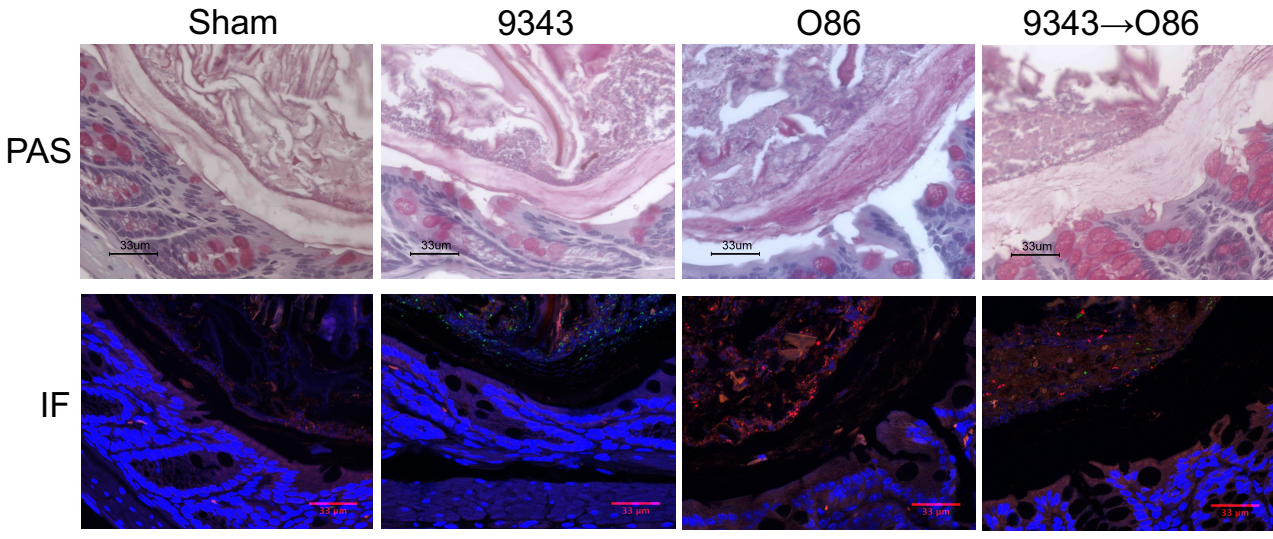
S5 Fig. Periodic acid-Schiff (PAS) stains matched to FISH and IF images. PAS stains for carbohydrate macromolecules, showing the level of mucus preservation retained in presented images. **A.** PAS images in SPF WT and GNO WT mice 2 weeks p.i., parallel to **Fig 3A**. GF = uninfected germ-free control mice. **B.** PAS images in SPF mice at 2 to 4 weeks p.i., parallel to **Fig 4F**. Images are from WT or Min mice, which did not differ.

A.

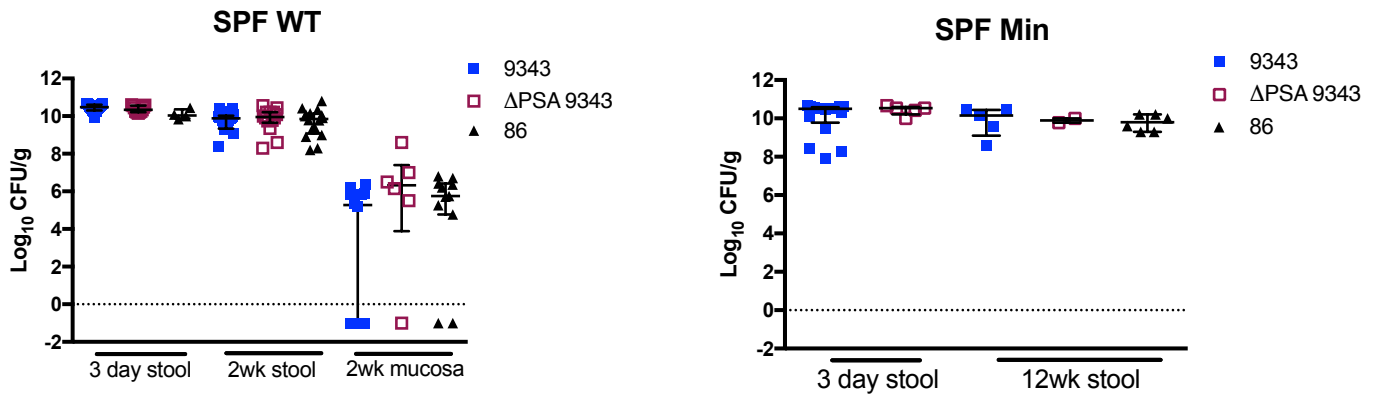


B.

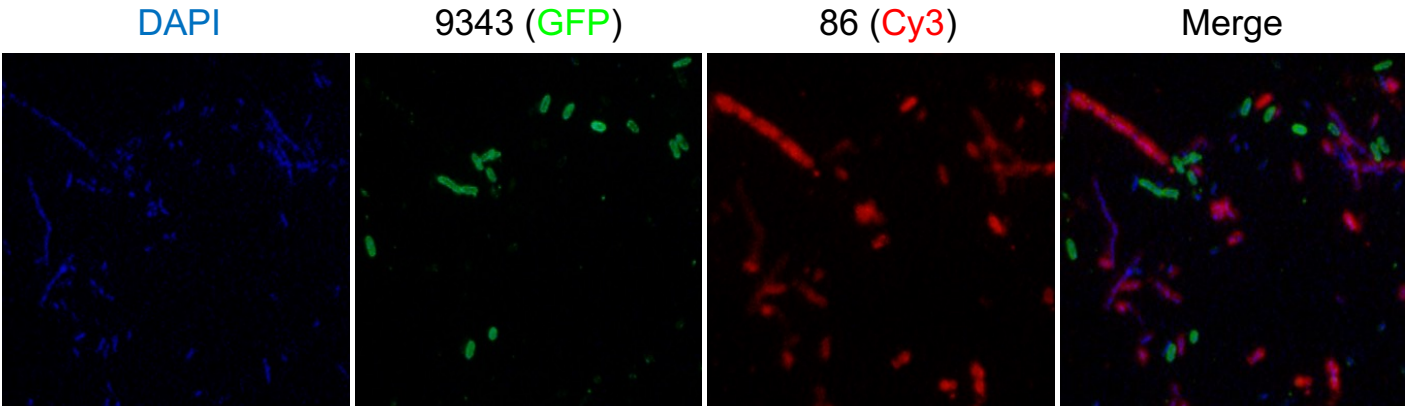
SPF



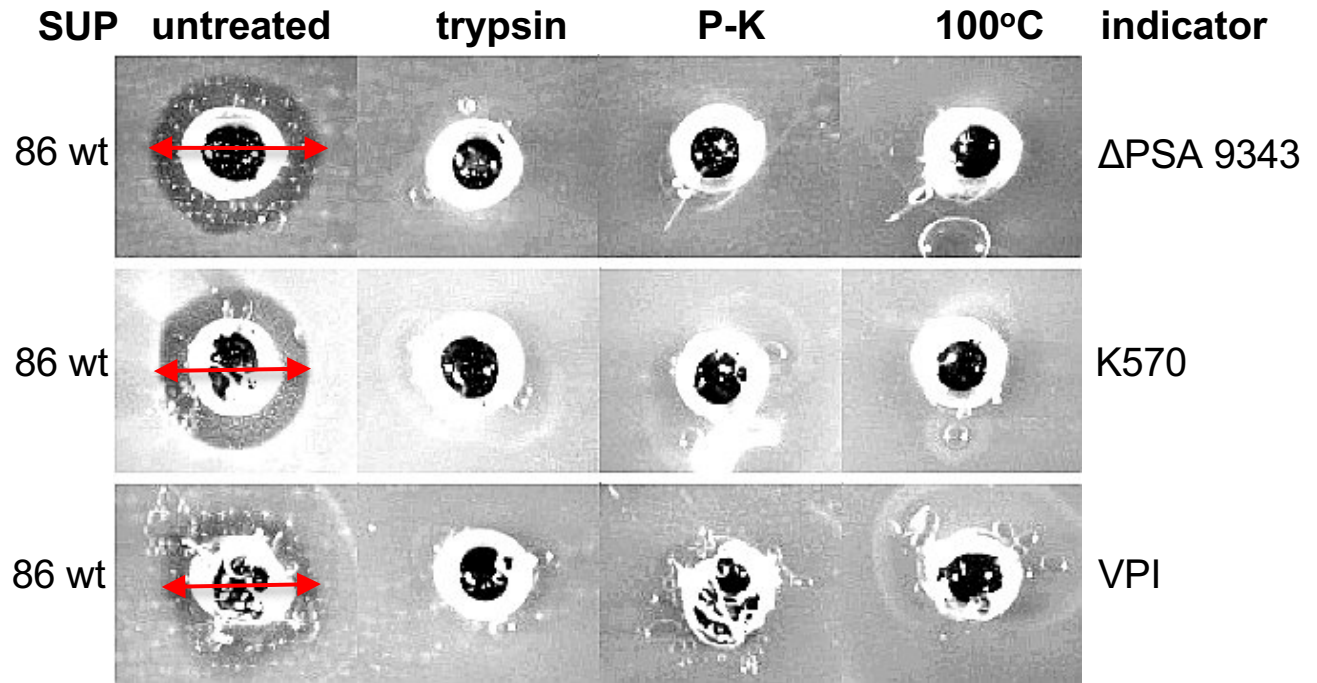
S6 Fig. 9343 and 86 exhibit similar fecal and mucosal colonization burden in mice. SPF WT and SPF Min mice were colonized with various *B. fragilis* strains: 9343, Δ PSA 9343, or 86. In WT mice, stool colonization was evaluated at 3 days and 2 weeks p.i. and mucosal colonization at 2 weeks terminal sacrifice. In Min mice, stool colonization was evaluated at 3 days (9343 and Δ PSA 9343 only) and 12 weeks p.i.. *B. fragilis* colonization burden was similar among all groups. Compiled data showing SPF WT stool: 4 to 19 in total per group; SPF WT mucosa: 6 to 12 in total per group; SPF Min stool: 2 to 17 in total per group.



S7 Fig. *B. fragilis* strain-specific immunofluorescence. Optimization of IF staining was done on formalin-fixed *B. fragilis* 9343 and 86 culture pellets. Image shows IF staining on 9343/86 mixed culture at 400X.

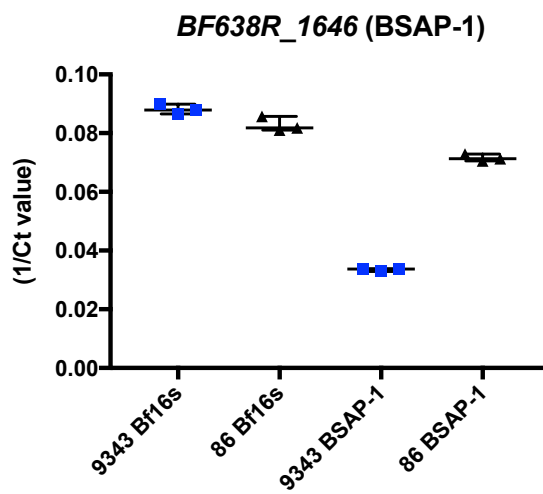


S8 Fig. 86 secretes an intraspecies inhibitory molecule, active on Δ PSA 9343, ETBF Korea 570 (K570) (BFT-3 strain), and ETBF VPI 13784 (VPI) (BFT-1 strain). Agar diffusion assay of various *B. fragilis* indicator strains plated on the agar with 86 wild type (wt) supernatant in wells (See Methods). A zone of clearing (span of red arrow) is induced by the 86 supernatant.

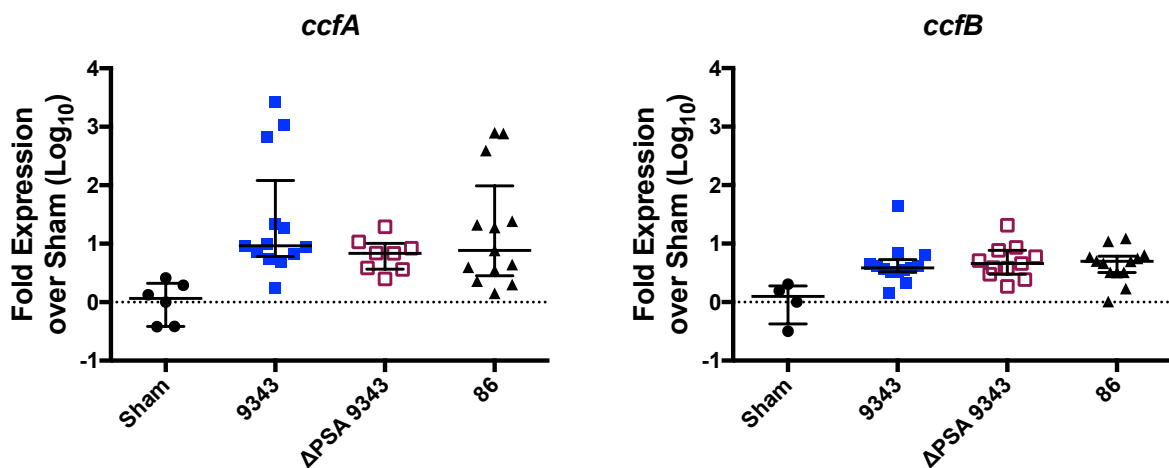


S9 Fig. *B. fragilis* BF638R_1646 (BSAP-1) and *ccf* gene expression. **A.** RNA was extracted from stationary phase cultures of 9343 and 86. cDNA was synthesized and SYBR Green PCR was run with *BF638R_1646* (BSAP-1) (primer set 3) and *B. fragilis 16S* (primer set 8) primers (S3 Table). The inverse raw Ct values are presented. **B.** Distal colon whole tissue RNA was extracted from SPF WT Sham, 9343, or 86-colonized mice (same mice presented in Fig 1). cDNA was synthesized and *B. fragilis ccfA* (sigma factor) and *ccfB* (anti-sigma factor) gene expression was determined via SYBR Green PCR with primer sets 4 and 5 (S3 Table). Samples were normalized to *B. fragilis 16S* and presented as fold expression over sham ($2^{\Delta\Delta Ct}$).

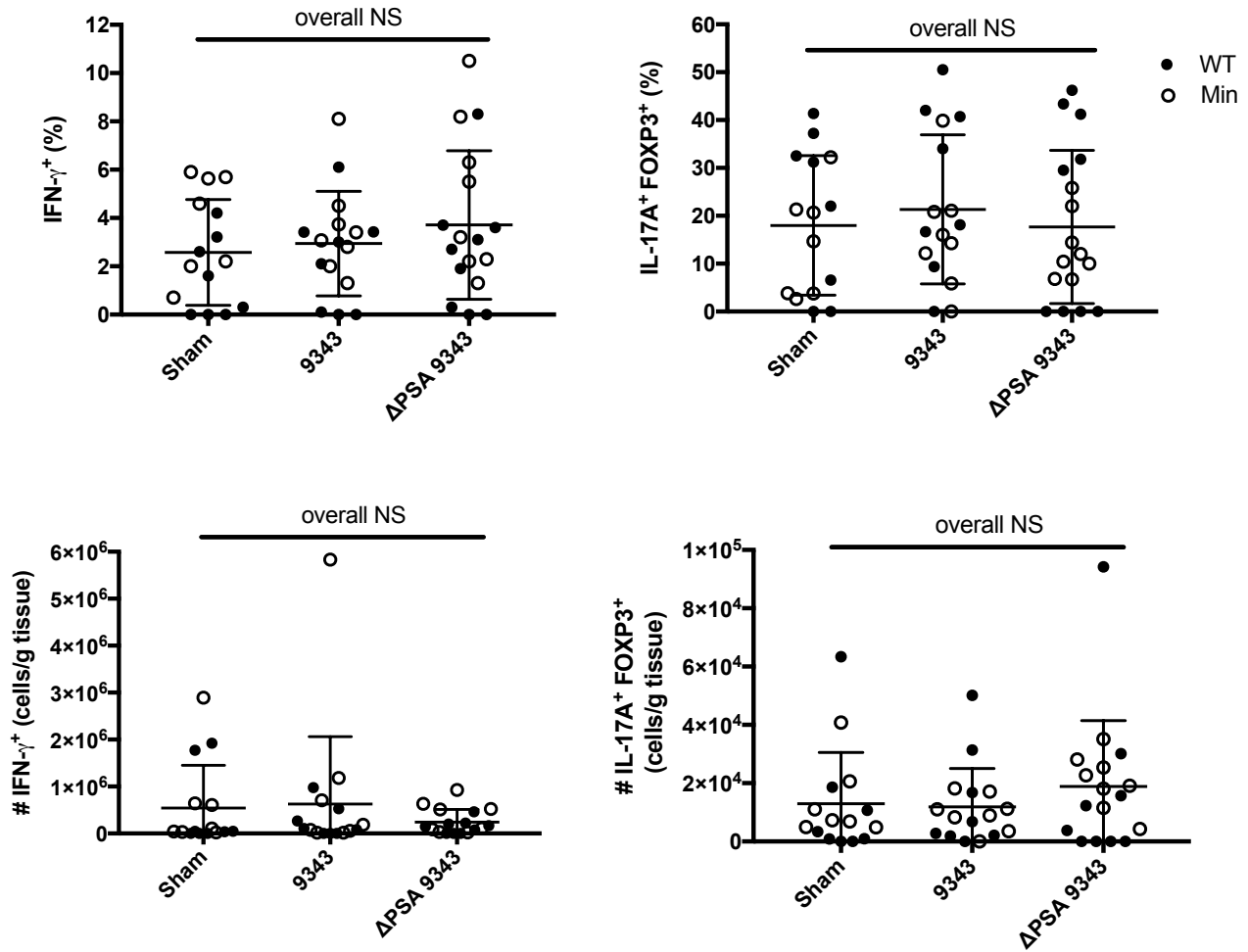
A.



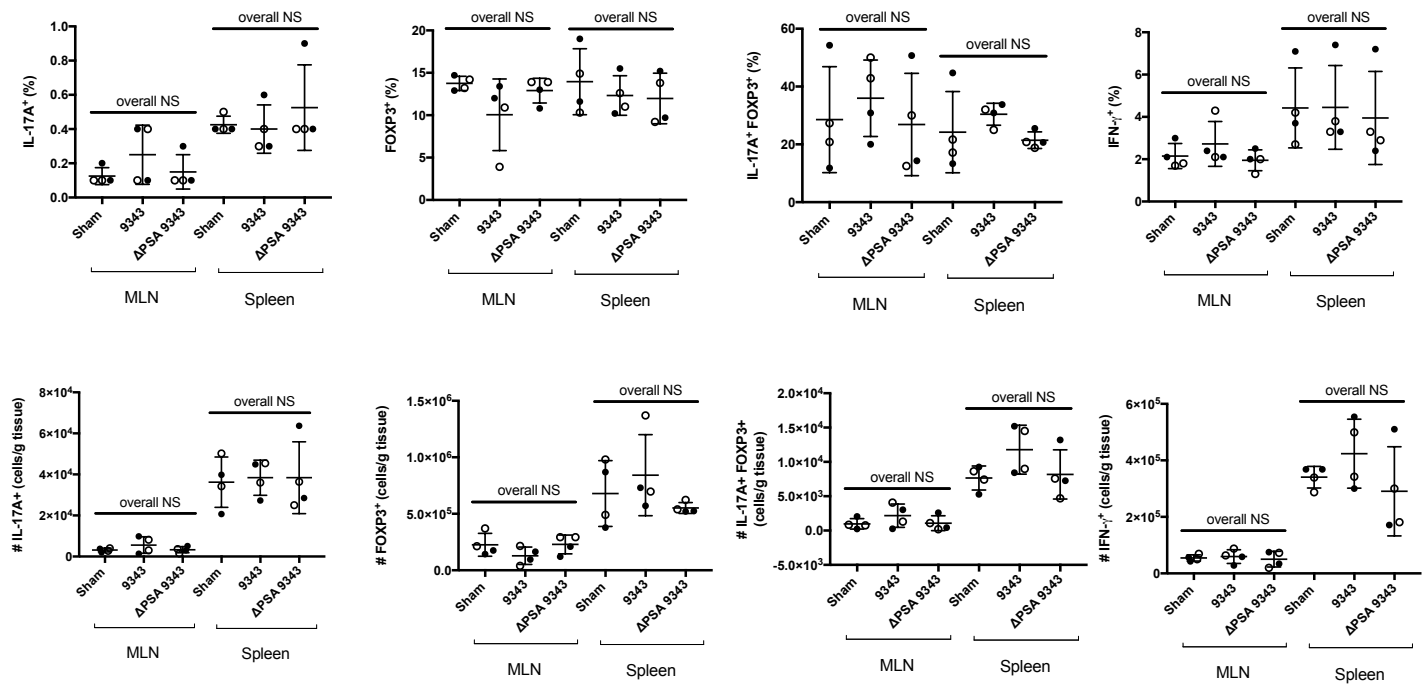
B.



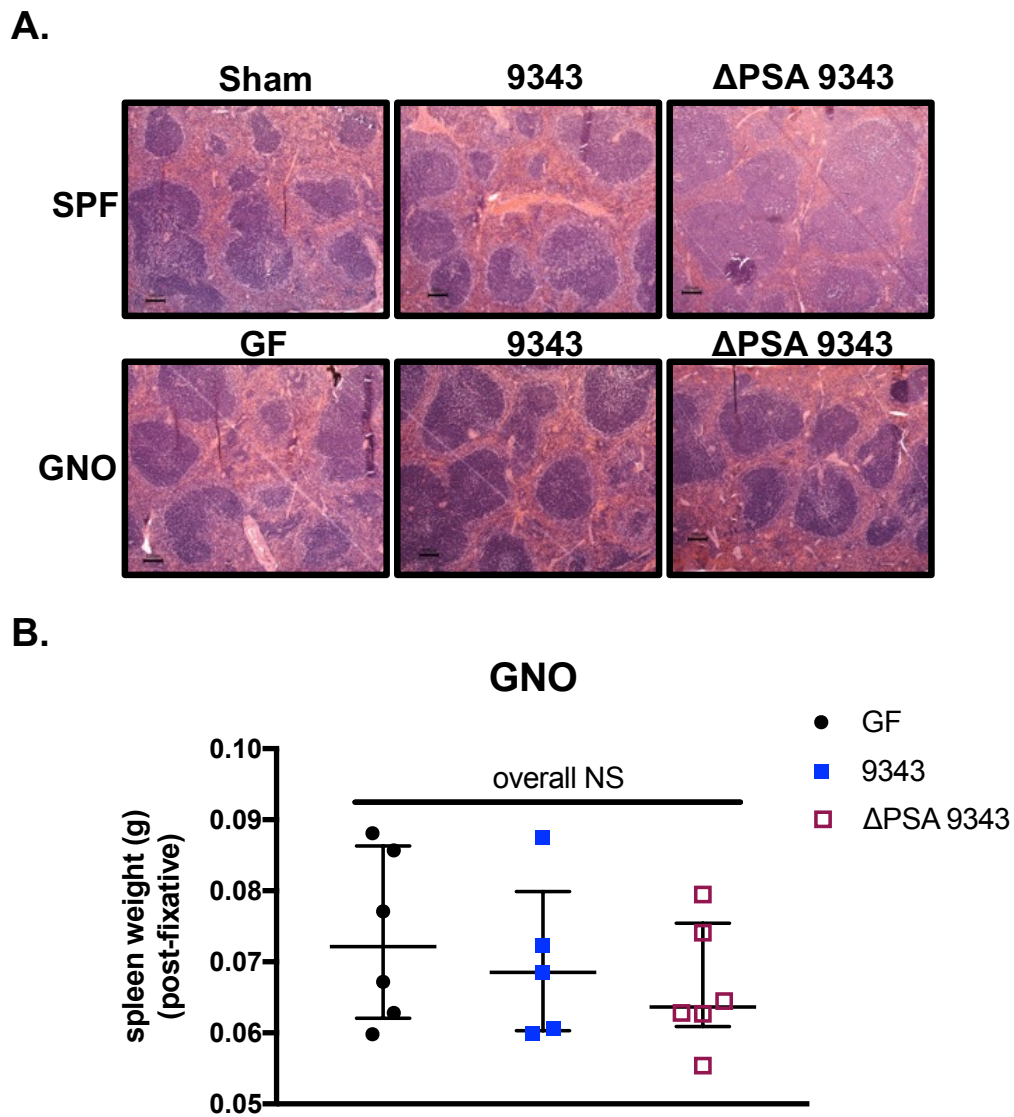
S10 Fig. IFN- γ ⁺ and IL-17A⁺ FOXP3⁺ T cell responses in the colon LP of SPF WT and SPF Min mice colonized with 9343 or Δ PSA 9343. Individual colons were harvested from WT and Min mice of 2 replicate experiments, each 4 to 5 mice per group (shown in **Fig 7A**). Data are presented as percentage, IFN- γ ⁺ of live CD3⁺ lymphocytes and FOXP3⁺ of live CD3⁺ IL-17A⁺ lymphocytes (top row), and as T cell density (# of cells per gram of colon tissue) (bottom row). Multiple group comparisons were conducted using Kruskal-Wallis testing.



S11 Fig. T cell responses in the MLNs and spleens of SPF mice colonized with 9343 or Δ PSA 9343. MLNs and spleens were pooled from WT (closed circles) and Min (open circles) mice of 2 replicate experiments (shown in **Fig 7A**), each 4 to 5 mice pooled per experiment. Data are presented as percentage of live CD3⁺ lymphocytes (top row) and as T cell density (# of cells per gram of colon tissue) (bottom row). No differences between Sham, 9343, or Δ PSA 9343-colonized mice were identified by Kruskal-Wallis testing.

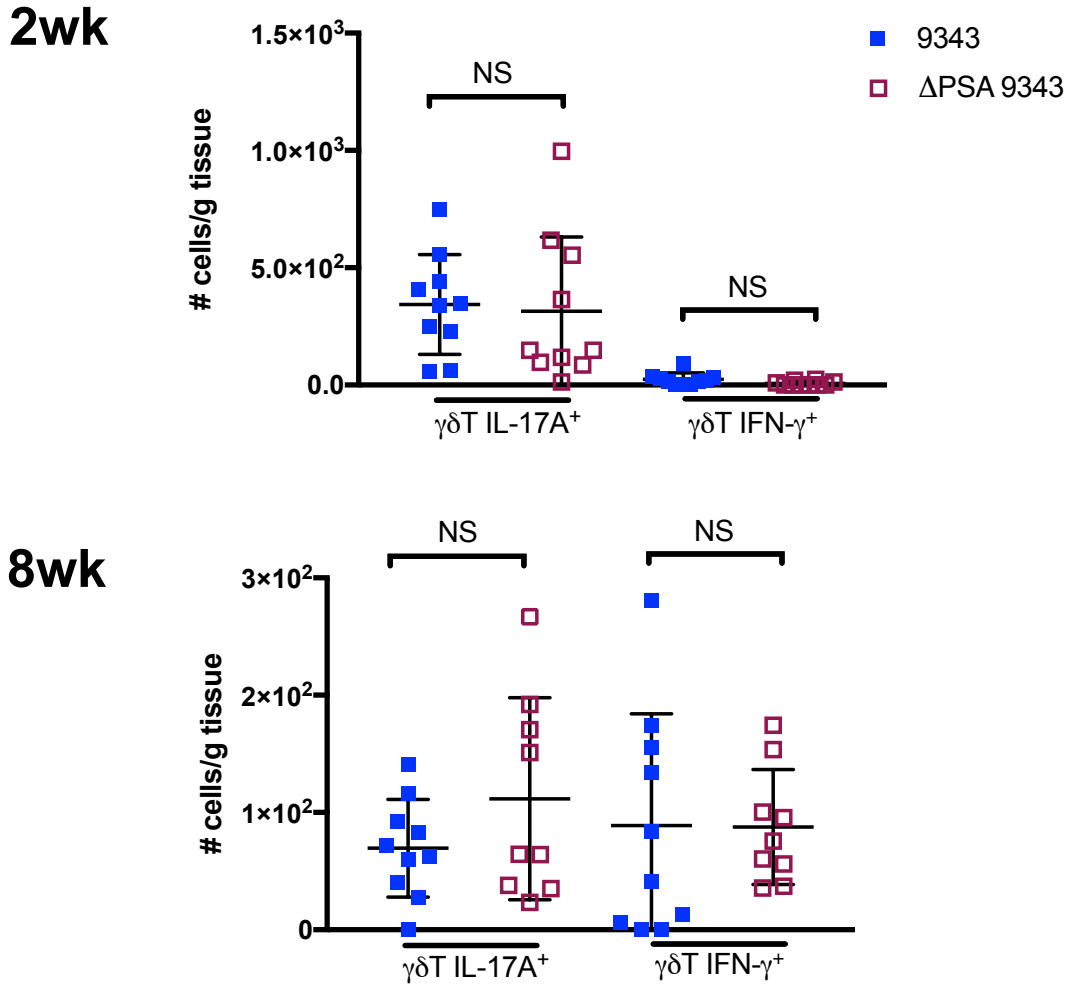


S12 Fig. Splenic morphology of SPF and GNO mice colonized with *B. fragilis*. **A.** Representative H&E stains of formalin-fixed spleens of SPF WT and GNO WT 9343 and Δ PSA 9343-colonized mice 8 weeks p.i.. **B.** After formalin-fixation, spleens from GNO mice were weighed. SPF C57BL/6 control mouse spleen weights consistently range from 0.05 g - 0.1 g. All spleens from monocolonized GNO mice (2 to 8 weeks p.i.) fell within the normal range, with no differences seen among groups. GF = uninfected germ-free control mice. Bars indicate median and interquartile range. Data are representative of 2 independent experiments, each 2 to 3 mice per group. Multiple group comparisons were conducted using Kruskal-Wallis testing.



S13 Fig. IL-17A⁺ and IFN- γ ⁺ $\gamma\delta$ T cell responses in the colon LP of GNO mice colonized with 9343 or Δ PSA 9343.

Individual colons were harvested from GNO WT mice of 2 replicate experiments, each 4 to 5 mice per group (shown in Fig 7B). Data are presented as T cell density (# of cells per gram of colon tissue) 2 weeks (top) or 8 weeks (bottom) p.i..



S1 Table. *B. fragilis* strain quantification in WT mice. Stool copy numbers of 9343 and 86 detected in SPF WT mice by treatment group at 2 weeks. Protocol described in Methods. Results are depicted as percent total (%) of colonized mice out of the total treatment group. Classification: Low (10^1 - 10^5 copies) or High ($\geq 10^5$).

TABLE 1. 9343 and 86 strain quantification (qPCR) in stool of SPF WT mice persistently infected with <i>B. fragilis</i> by different inoculation protocols at 2 weeks					
Percent total (# colonized/total mice)					
Type of Infection	9343 / 86 Co-Colonization			9343 Only	86 Only
	Low / High (10^1 - $<10^5$) / ($\geq 10^5$)	High / Low ($\geq 10^5$) / (10^1 - $<10^5$)	High / High ($\geq 10^5$) / ($\geq 10^5$)	High ($\geq 10^5$)	High ($\geq 10^5$)
9343	0.0% (0/13)	0.0% (0/13)	0.0% (0/13)	100% (13/13)	0.0% (0/13)
9343→86	7.1% (1/14)	50.0% (7/14)	21.4% (3/14)	21.4% (3/14)	0.0% (0/14)
Δ PSA 9343→86	0.0% (0/11)	27.3% (3/11)	0.0% (0/11)	72.7% (8/11)	0.0% (0/11)
86→9343	80.0% (4/5)	0.0% (0/5)	20.0% (1/5)	0.0% (0/5)	0.0% (0/5)
9343+86	22.2% (2/9)	0.0% (0/9)	44.4% (4/9)	0.0% (0/9)	33.3% (3/9)
86	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	100% (12/12)

S2 Table. *B. fragilis* strain quantification in Min mice. Stool copy numbers of 9343 and 86 detected in SPF Min mice by treatment group at 12 to 16 weeks. Protocol described in Methods. Results are depicted as the percent total (%) of colonized mice out of the total treatment group. Classification: Low ($10^1 < 10^5$ copies) or High ($\geq 10^5$ copies).

Table 2. 9343 and 86 strain quantification (qPCR) in stool of SPF Min mice persistently infected with <i>B. fragilis</i> by different inoculation protocols at 12 to 16 weeks					
Percent total (# colonized/total mice)					
Type of Infection	9343 / 86 Co-Colonization			9343 Only	86 Only
	Low / High ($10^1 - <10^5$) / ($\geq 10^5$)	High / Low ($\geq 10^5$) / ($10^1 - <10^5$)	High / High ($\geq 10^5$) / ($\geq 10^5$)	High ($\geq 10^5$)	High ($\geq 10^5$)
9343	0.0% (0/5)	0.0% (0/5)	0.0% (0/5)	100% (5/5)	0.0% (0/5)
9343→86	0.0% (0/15)	66.7% (10/15)	6.7% (1/15)	13.3% (2/15)	13.3% (2/15)
Δ PSA 9343→86	0.0% (0/5)	80.0% (4/5)	0.0% (0/5)	20.0% (1/5)	0.0% (0/5)
9343+86	18.2% (2/11)	9.1% (1/11)	27.3% (3/11)	0.0% (0/11)	45.5% (5/11)
86	0.0% (0/7)	0.0% (0/7)	0.0% (0/7)	0.0% (0/7)	100% (0/7)

S3 Table. Primers and probes used in this study.

Primers and Probes	Sequence 5' to 3'	Target	Reference
1	ACACATATCACTTCCGATGCC	<i>orf1</i> and <i>upaZ</i> (9343 PSA upstream)	[49]
	GTTGACGGAAATGATCGGTATAG		
2	TAACACGATAGGAGTTGCATGG	<i>wzx</i> and <i>wcfN</i> (9343 PSA internal)	[49]
	ACATTGAGAAATACTCGTCCACC		
3	CGACCCTTTCCTCATAATCCTTTCT	<i>B. fragilis</i> 638R_1646 (BSAP-1)	This study
	ATGCTATTCACATTTGCCGCTTG		
4	GGAATTTGCATGACACTTAT	<i>B. fragilis</i> <i>ccfA</i>	[24]
	CTGAGAGGTTTCATCTTCTG		
5	AGTGTCCCCACTTCATCGTC	<i>B. fragilis</i> <i>ccfB</i>	[24]
	TGAAACTTTTGCCGGAGAAT		
6	CTCGGTATGGAGTATGCTCCAG	<i>B. fragilis</i> CTn9343 (<i>bexA</i>)	[50]; This study
	GTATTCCACTGCCCAATATGCCGC		
	Probe: CTACAACAGGAACGGGAGCGACAC		
7	GCGAACTCGGTTTATGCAGT	<i>B. fragilis</i> <i>bft</i>	[18]; This study
	GTTGTAGACATCCCACTGGC		
	Probe: AGCAGAAGGTTATGACGA		
8	TCRGAAGAAAGCTTGCT	<i>B. fragilis</i> 16S	[51]
	CATCCTTTACCGGAATCCT		
	Probe: ACACGTATCCAACCTGCCCTTACTCG		
9	FISH Probe: \5Cy3\GTTTCCACATCATTCCACTG	<i>B. fragilis</i> 16S rRNA	[52]