Supplemental Information

Dysbiosis-induced Secondary Bile Acid Deficiency Promotes Intestinal Inflammation

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Supplementary Figures

Figure S1

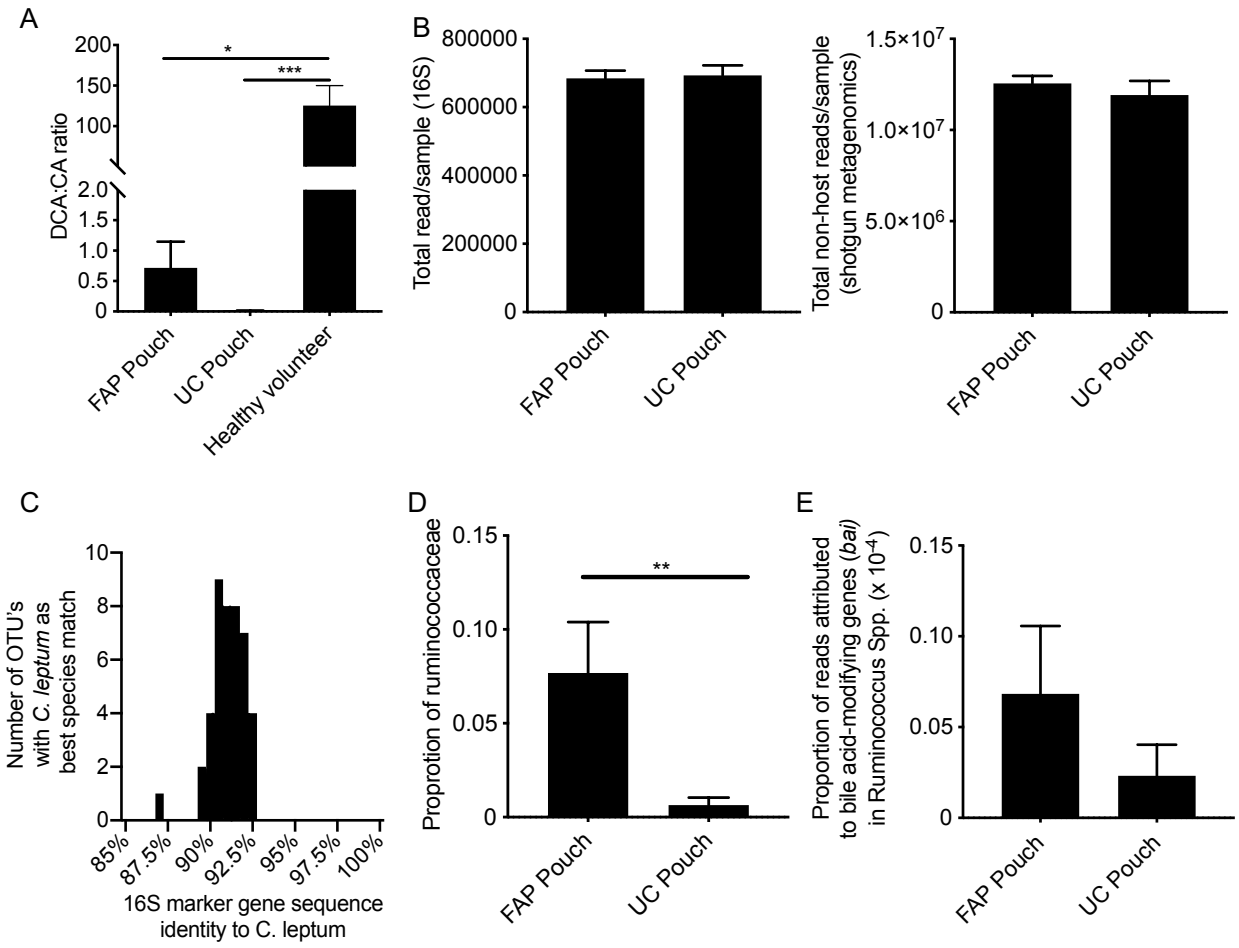

Figure S1, related to Figure 1. Stool from UC pouches have lower SBA:PBA ratio, decreased proportion of Ruminococcaceae, and fewer bile acid modifying genes than non-inflamed controls.

(A) The ratio of DCA to CA in stool samples from UC pouches is lower than in healthy **volunteers** or FAP pouches. (B) The total read/sample of 16S analysis and the total non-host reads/sample from the shotgun metagenomics analysis is not different between FAP Pouch and UC Pouch. (C) A histogram showing the number of OTUs with *C. leptum* as best species match from the 16S marker gene sequence. (D) Proportion of Ruminococcaceae and (E) relative proportions of bile acid modifying genes (*bai*) in Ruminococcus in UC is lower compared to FAP pouches, obtained from shotgun metagenomics analysis. Data represented as mean ± SEM. (A) FAP-Pouch n=7, UC-Pouch n=18 and Healthy control n=30, one-way ANOVA with Tukey's post-hoc. (B) FAP pouch n=5, UC pouch n=11, two-tailed t test (D) FAP pouch n=5, UC pouch n=11 mice, two-tailed t-test, (E) FAP pouch n=4, UC pouch n=7, * P<.05, ** P<.01, *** P<.001, and **** P<.0001, two-tailed t-test.

Figure S2

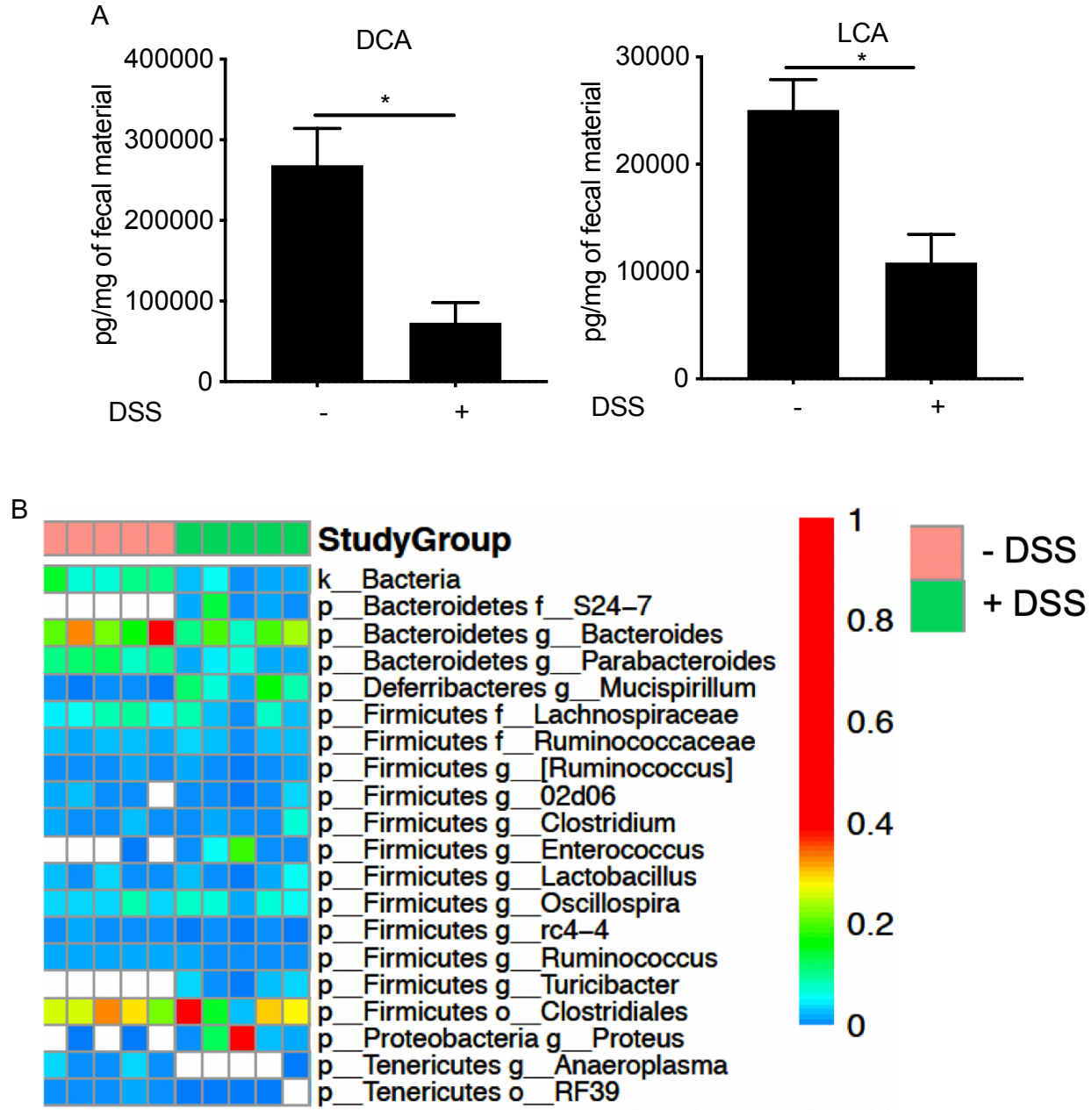

Figure S2, related to Figure 2. Stool DCA and LCA levels are lower in murine DSS colitis compared to healthy controls

(A)The level of DCA and LCA detected in stool collected from the colon of mice with DSS by targeted metabolomic analysis using LC-MS showed decreased DCA and LCA levels than control mice without DSS (-DSS). (B) heat map showing taxa that exceeded 1% abundance in any sample. Each row represents one taxon, typically a genus. White color indicates that taxa were not observed in the sample, dark blue represents taxa that were observed at very low abundance. Abundance values exceeding 40% are colored red, indicating a dominant species. (A) -DSS n=4, +DSS n=5 mice, * P<.05, two-tailed t test, post multiple t-test with Benjamini and Hochberg posttest correction. (B) -DSS n=5, +DSS n=5 mice.

Figure S3

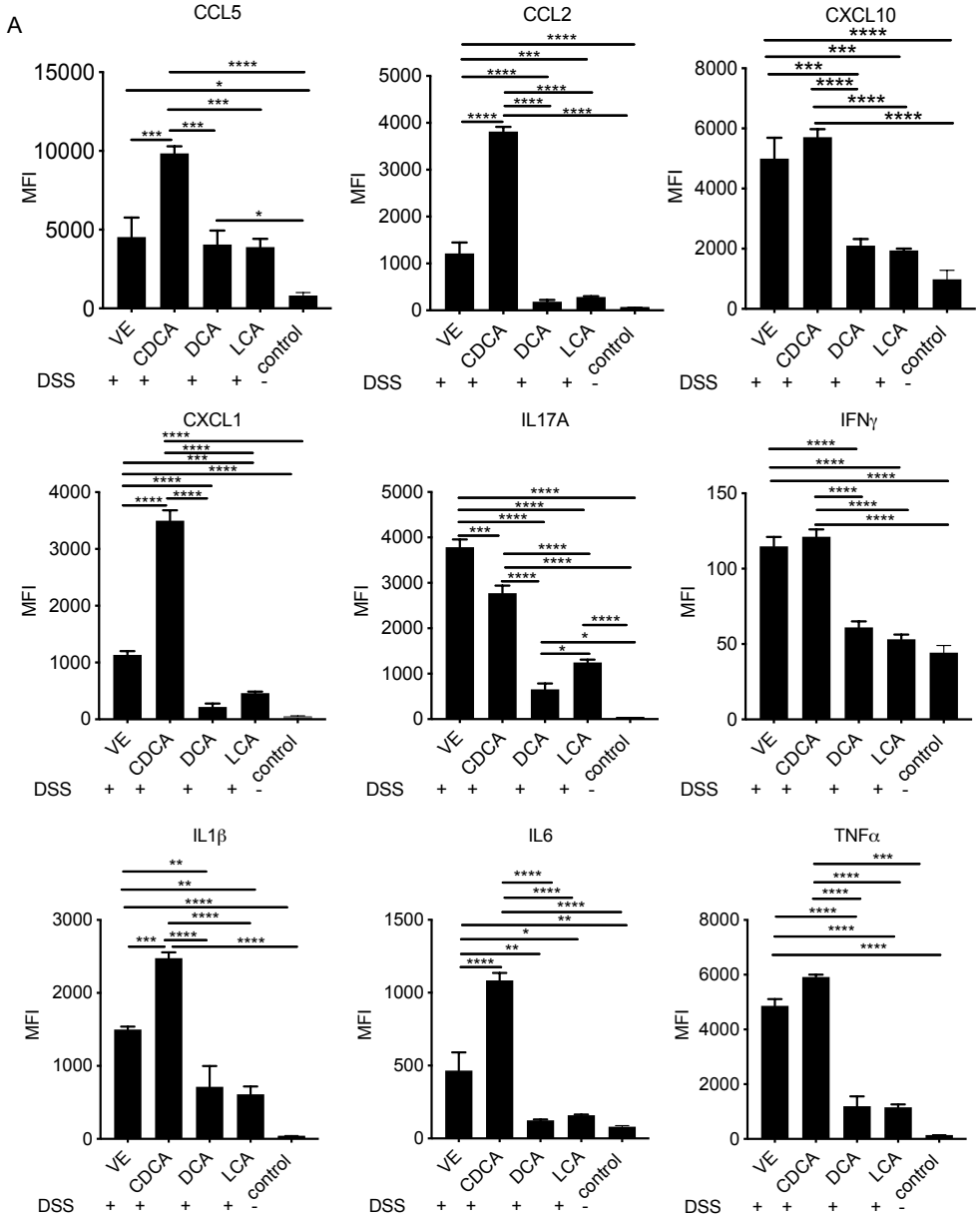

Figure S3, related to Figure 2. Treatment with secondary bile acids promote an anti-inflammatory phenotype

Selected cytokine/chemokine variations in colon tissue homogenate using Luminex from DSS mice treated with LCA and DCA enema (as described in Figure 2) show an anti-inflammatory profile compared to CDCA or VE enema treated mice. Colon homogenate from mice untreated with DSS was used as a control. Data represented as mean ± SEM. (A) All groups n=5, * P<.05, ** P<.01, *** P<.001, and **** P<.0001, one-way ANOVA with Tukey's post-hoc.

Figure S4

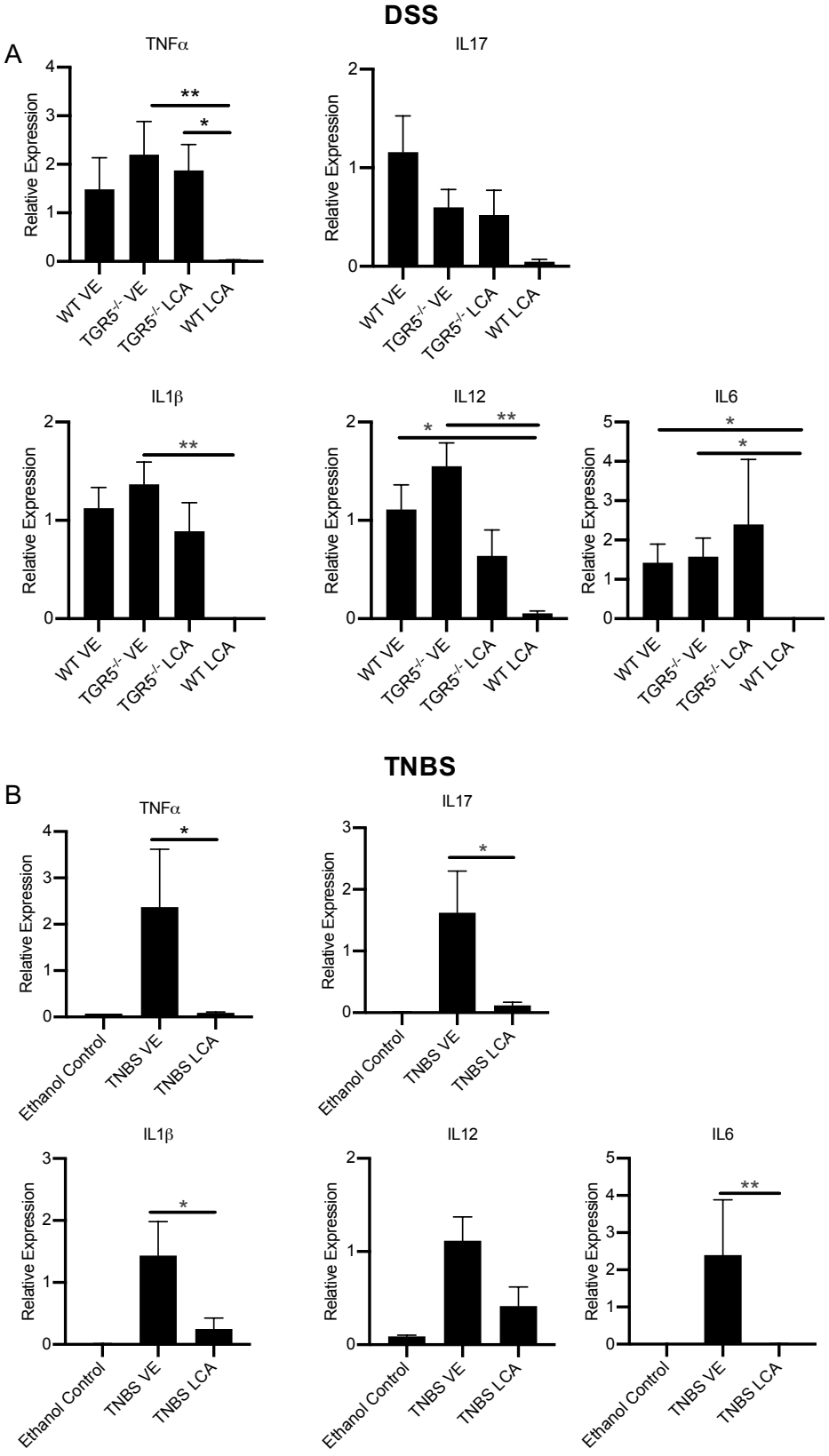

Figure S4, related to Figure 2. LCA treatment dampens the expression of pro-inflammatory cytokines in the gut of WT mice in DSS and TNBS animal colitis model

(A) In DSS-induced colitis, LCA treated WT mice have decreased TNFα, IL17, IL1β IL12 and IL6 expression. (B) In TNBS-induced colitis, LCA treated WT mice have decreased TNFα, IL17, IL1β, IL12 and IL6 expression. (A) WT VE n= 5, WT LCA n= 5, TGR5$^{-/-}$ VE n=9, TGR5$^{-/-}$ LCA n=5, (B) n=5, (A) WT LCA compared to other groups, Kruskal-Wallis test (B) TNBS VE compared to TNBS LCA, * P<.05, ** P<.01, *** P<.001, and **** P<.0001, Mann Whitney's test.

Figure S5

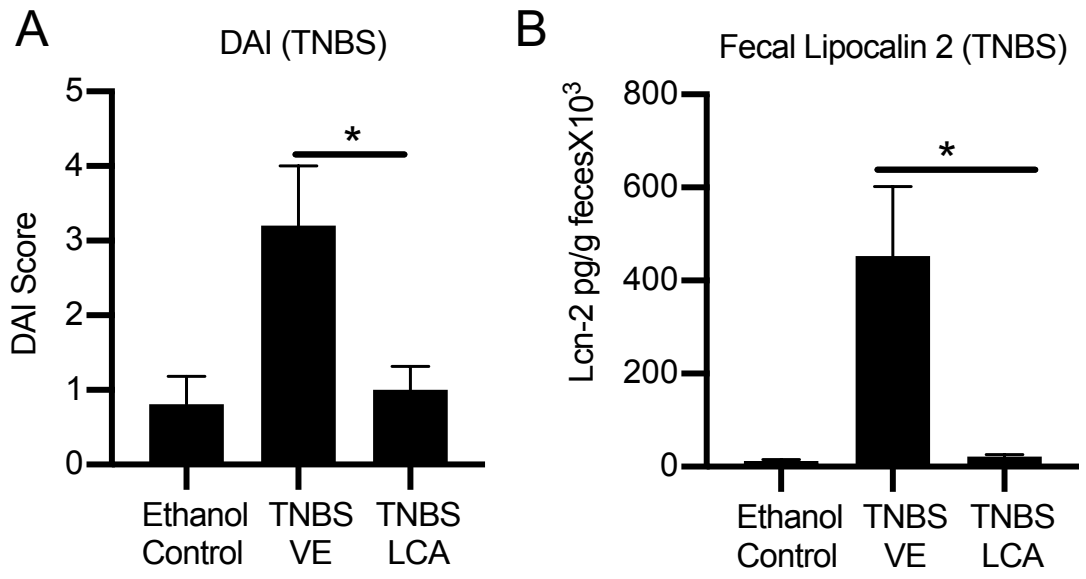

Figure S5, related to Figure 3. Treatment with LCA decreases fecal lipocalin 2 and improves the disease activity index
In TNBS-induced colitis, LCA treated mice have a (A) lower disease activity index (DAI) and (B) lower fecal lipocalin 2 on day 7. Data represented as mean ± SEM. All groups n=5, * P<.05, ** P<.01, *** P<.001, and **** P<.0001, one-way ANOVA with Tukey's post-hoc.

Figure S6

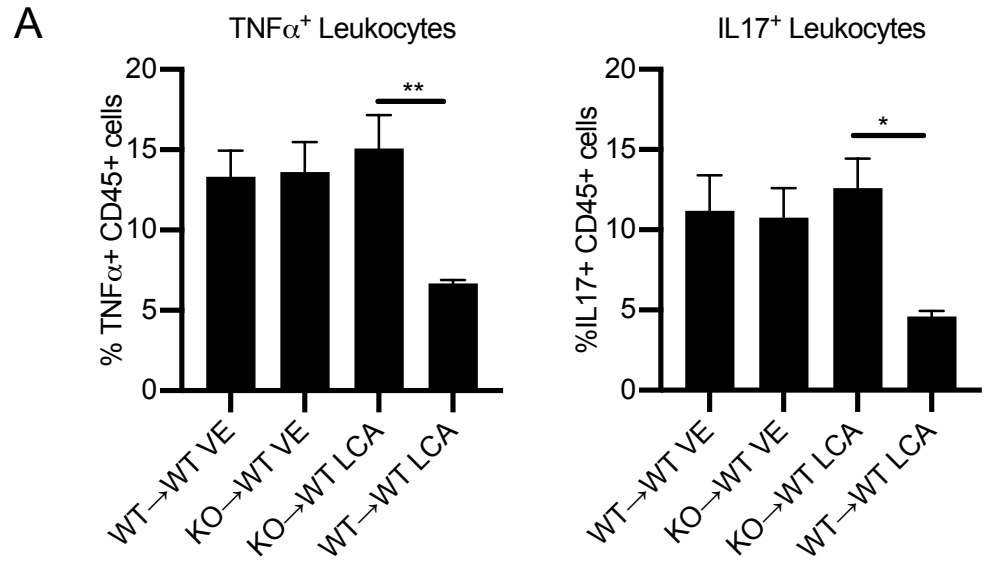

Figure S6, related to Figure 4. LCA treatment effectively reduces pro-inflammatory cytokine producing cells in the colon of WT chimera mice, but not TGR5$^{-/-}$ chimera mice in DSS-induced colitis
Intracellular TNFα^{+} and IL17^{+} colonic leukocytes were higher in KO (TGR5$^{-/-}$)→WT chimera mice treated with LCA compared to WT→WT LCA treated in DSS-induced colitis. All groups n=5, * P<.05, ** P<.01, *** P<.001, and **** P<.0001, one-way ANOVA with Tukey's post-hoc.

Supplementary Tables

Table S1, related to Figure 1| Characteristics of Study Subjects

	FAP Pouch (n=7)	UC Pouch (n=17)
Mean Age (SEM)	37.14 (3.9)	44.78 (5.0)
Female, n (%)	5 (71.4%)	10 (58.8%)
Pouch Age (SEM)	11.7 (2.8)	10.41 (1.9)
History of Pouchitis, n (%)	0	14 (82.4%) ***
Active Pouchitis, n (%)	0	4 (23.5%)
Active Antibiotic Use, n (%)	0	6 (35.3%)
Active Probiotic Use, n (%)	0	5 (29.4%)
Active bile acid binder Use, n (%)	0	0
Active bile acid medication, n (%)	0	0

***P value < .0005 using two-sided Fisher's exact test

Table S2, related to Figure 1| Metabolomic Analysis of Fecal Sample Collected from Patients with UC Pouch and FAP Pouch

	FAP Pouch	UC Pouch		
Bile acids	Mean (SEM)	Mean (SEM)	P value	FDR Adjusted P value
Deoxycholic acid	60957 (26256)	1593 (628.1)	0.002	0.023
Lithocholic acid	30644 (11658.1)	282.9 (73)	0.001	0.020
Chenodeoxycholic acid	71937 (33700.7)	521970 (73782.2)	0.003	0.023
Cholic acid	424471 (167582)	501541.4 (76597)	0.636	0.893
Ursodeoxycholic acid	37621 (18140)	54863.1 (30336)	0.738	0.893
α-Muricholic acid	1 (0.3)	30.8 (10.8)	0.114	0.657
β-Muricholic acid	67.1 (65.8)	51.31 (36.5)	0.827	0.896
ω-Muricholic acid	2017 (1301.8)	4839 (2526.1)	0.511	0.893
Taurocholic acid	171333 (147913)	126128.6 (33089)	0.667	0.893
Taurochenodeoxycholic acid	66485 (55419)	58542.9 (17440)	0.857	0.896
Taurodeoxycholic acid	643 (315)	8510.1 (6071)	0.439	0.893
Taurolithocholic acid	98 (77)	71.4 (39)	0.737	0.893
Taurodehydrocholate	0.1 (0)	0.1 (0)	0.235	0.893
Tauro-ω-Muricholic acid	0.1 (0)	0.2 (0.2)	0.537	0.893
Tauro-α-Muricholic acid	0.1 (0.1)	0.2 (0.1)	0.578	0.893
Tauro-β-Muricholic acid	0.1 (0)	5.1 (5)	0.548	0.893
Tauroursodeoxycholic acid	4043 (1695.5)	1870 (901)	0.243	0.893
Glycocholic acid	272220 (257349)	157322.6 (55851)	0.529	0.893
Glycolithocholic acid	72 (38)	50.4 (22)	0.617	0.893
Glycochenodeoxycholic acid	94329 (83607)	120868.5 (51308)	0.788	0.896
Glycodeoxycholic acid	606 (418)	1487.2 (1054)	0.619	0.893
Glycoursodeoxycholic acid	4558 (3499.4)	4758 (3188.3)	0.972	0.972
Glycohyodeoxycholic acid	94.11 (93.4)	53.4 (23)	0.553	0.893

Multiple t test with Benjamini and Hochberg posttest correction

Table S3, related to Figure 1| Differential Abundance of Common Taxa in UC vs. FAP Control Pouch Microbiota

Taxon	Estimate	P value
Firmicutes Ruminococcaceae	-2.3	0.01*
Firmicutes Veilonella	-2.5	0.10
Fusobacteria Fusobacterium	-4.5	0.24
Proteobacteria Enterobacteriaceae	2.0	0.43
Bacteroidetes Bacteroides	0.4	0.61
Firmicutes Lachnospiraceae	0.3	0.65
Firmicutes Clostridiales	0.5	0.71
Firmicutes Clostridiaceae	0.3	0.78
Bacteroidetes Prevotella	6.9	0.83

Estimate shows increase or decrease of log(proportion) in IBD samples.
*P-value determined using two-sided Fisher's exact test

Table S4, related to Figure 2| Additional Metabolomic Analysis of Fecal Samples Collected from DSS-treated and Untreated Mice

	(-) DSS	(+) DSS		
Bile acid	**Mean (SEM)**	**Mean (SEM)**	**P value**	**FDR Adjusted P value**
Chenodeoxycholic acid	1907 (897)	979 (288)	0.313	0.328
Cholic acid	17861 (9030)	11880 (6265)	0.592	0.503
Ursodeoxycholic acid	4259 (1517)	539 (170)	0.028	0.083
Taurocholic acid	13366 (3116)	5212 (1376)	0.036	0.088
Taurochenodeoxycholic acid	501 (138)	235 (60)	0.099	0.126
Taurodeoxycholic acid	5716 (2587)	312 (74)	0.049	0.088
Taurolithocholic acid	171 (71)	16 (11)	0.045	0.088
Glycocholic acid	26 (15)	123 (83)	0.340	0.337
Glycolithocholic acid	13 (7)	1 (0)	0.086	0.126
Glycochenodeoxycholic acid	2 (0)	14 (8)	0.220	0.262
Glycodeoxycholic acid	99 (45)	21 (8)	0.096	0.126

Multiple t test with Benjamini and Hochberg posttest correction