Supplementary Experimental Model and Subject Details

Mice

For DSS experiments, $Apc^{Min/+}$ and $Apc^{Min/+}Ffar2^{-/-}$ mice were used at approximately 1.5–3 months, and for non-DSS experiments, mice were used between 3.5 and 5 months. All specific pathogen–free mice were housed at the Harvard T. H. Chan School of Public Health. Germ-free mice were maintained in semirigid gnotobiotic isolators under a 12-hour light cycle in the Harvard T. H. Chan Gnotobiotic Center for Mechanistic Microbiome Studies. All mice had access to food and water ad libitum throughout. If needed, germ-free mice were sterilely transferred to isolator cages within the facility. $Ffar2^{fl/fl}$ mice were generated and graciously provided by Brian Layden of University of Illinois at Chicago. $Ffar2^{fl/fl}$ were then bred to $Apc^{Min/+}$ mice and *CD11c-cre* mice[1] that were purchased from the Jackson Laboratory (Bar Harbor, ME; #007567).

FFAR2 Agonist Feeding

FFAR2 agonist (compound 1 in patent no. WO 2011/076732 A1) was discovered and generously provided by Hamid Hoveyda and Graeme Fraser under a Material Transfer Agreement permitting use of the compound (EPICS SA, Gosselies, Belgium).[2,3] This agonist is specific for mouse FFAR2. Mice received the FFAR2 agonist (700 μmol/L) dissolved in drinking water for the indicated duration.

DSS Experiments With $Apc^{Min/+}$ Mice

$Apc^{Min/+}$ mice were treated with 3% weight/volume DSS (MP Biomedicals, Singapore; catalog number 0216011050-50g) in the drinking water for 4 days, followed by regular drinking water for either 6 (for mice killed on day 10) or 17 days (for mice killed on day 21), depending on the experiment. Some mice were treated with the FFAR2 agonist (700 μmol/L) or control deionized drinking water for the remaining 17 days for a total of 3 weeks. Mice treated with α–IL27p28 (Thermo Fisher Scientific, 16-7285-85) or isotype (Thermo Fisher Scientific, 16-4724-82) antibody were injected intraperitoneally with 35 μg in 100 μL sterile PBS every 3 days after 4 days of 3% DSS treatment, for a total of 6 doses. Mice were weighed every day for the first 8 days, followed by every other day until day 21. Then, colons were imaged for tumor counts, and tumors were harvested for flow cytometry.

Dextran Sulfate Sodium Experiments With $Ffar2^{fl/fl}$CD11c-Cre Mice

$Ffar2^{fl/fl}$*CD11c-Cre* and $Ffar2^{fl/fl}$ control mice were treated with 1.5% weight/volume DSS in the drinking water for 5 days. After 5 days, the mice received regular drinking water for 2 days. Control mice received untreated deionized water for 7 days. Mice were weighed every day. On day 7, immune cells from the colon LP were assessed by flow cytometry.

Fluorescein Isothiocyanate–Dextran Feeding

Mice were gavaged with 4 kDa FITC–dextran (Sigma-Aldrich, 46944-500MG-F) (10 mg/20g mouse, 10 mg/100 μL in sterile PBS [Dulbecco's calcium and magnesium free]). Mice had access to food and water ad libitum throughout. After 3 hours, blood was collected into serum separator tubes. Blood was spun for 5 minutes at 5000 revolutions/minute. Serum fluorescence levels were measured (serum samples diluted 1:1 with PBS) with 485-nm excitation and 528-nm emission wavelength. To calculate the serum FITC–dextran concentrations, a calibration curve was generated from FITC–dextran standard controls in PBS and serum (1:1) with final concentrations of 0, 125, 250, 500, 1000, 2000, 4000, and 8000 ng/mL. Prism was used to calculate a linear regression equation from plate reader values. Serum concentration was then calculated from this equation.

Supplementary Method Details

Epithelial Cell Isolation and Quantitative Polymerase Chain Reaction

Complementary DNA was generated by using iScript cDNA synthesis Kit (Bio-Rad, Hercules, CA; 1708891). For *Cdh1* levels, the following primers were used for SYBR-based quantitative PCR (Kapa Biosystems, Charlestown, MA; KK4718): forward, 5′-CAGCCTTCTTTTCGGAAGACT-3′, reverse, 5′-GGTAGACAGCTCCCTATGACTG-3′ as described.[4] Cycle threshold values were compared to β-actin transcript levels by using the following primers: forward, 5′-TACCACCATGTACCCAGGCA-3′, reverse, 5′-CTCAGGAGGAG-CAATGATCTTGAT-3′.

Quantitative Polymerase Chain Reaction Techniques

Tumors were removed from the colon and placed into sterile Eppendorf tubes and rinsed 2× with gentle mechanical disruption in sterile PBS to remove any stool from the lumen. Excess PBS was removed, and tumors were frozen at −80°C. For DNA extraction, tumors were digested in 1 mL lysis buffer (100 mol/L Tris–HCl, pH 8.5; 5 mmol/L EDTA, pH8.0; 0.2% sodium dodecyl sulfate; 200 mmol/L NaCl; 1 mg/mL ProteinaseK), rotating overnight at 55°C. DNA was isolated using a standard phenol:chloroform extraction. For universal 16S quantitative PCR quantification, 80 ng of tumor DNA was analyzed with the following 16S universal primers, as described[5]: forward, 5′-TCCTACGGGAGGCAGCAGT-3′, reverse, 5′-GGACTACCAGGG-TATCTAATCCTGTT-3′. The 16S transcript levels were compared to mouse β-actin transcript levels. For analysis of *il27p28* levels, the following primers were used (Kapa Biosystems, KK4718): forward, 5′-AGCCTGTTGCTGC-TACCCTTGC-3′, reverse, 5′-GTGGACATAGCCCTGAACCTCA-3′ as described.[6] Cycle threshold values were compared to β-actin (primers listed earlier).

Mouse Real-Time Polymerase Chain Reaction Primers

Gene	Forward (5′–3′)	Reverse (5′–3′)
β-Actin	TACCACCATGTACCCAGGCA	CTCAGGAGGAGCAATGATCTTGAT
Cdh1	CAGCCTTCTTTTCGGAAGACT	GGTAGACAGCTCCCTATGACTG
Ffar2	AATTTCCTGGTGTGCTTTGG	ACCAGACCAACTTCTGGGTG
16S	TCCTACGGGAGGCAGCAGT	GGACTACCAGGGTATCTAATCCTGTT
il27p28	AGCCTGTTGCTGCTACCCTTGC	GTGGACATAGCCCTGAACCTCA
CCL20	ACTGTTGCCTCTCGTACATACA	GAGGAGGTTCACAGCCCTTTT
CXCL14	GAAGATGGTTATCGTCACCACC	CGTTCCAGGCATTGTACCACT
CCL5	GCTGCTTTGCCTACCTCTCC	TCGAGTGACAAACACGACTGC
CXCL12	TGCATCAGTGACGGTAAACCA	TTCTTCAGCCGTGCAACAATC
CCL21	GTGATGGAGGGGGTCAGGA	GGGATGGGACAGCCTAAACT
CCL19	GGGGTGCTAATGATGCGGAA	CCTTAGTGTGGTGAACACAACA
CCL4	TTCCTGCTGTTTCTCTTACACCT	CTGTCTGCCTCTTTTGGTCAG
CCL2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Map2k1	AAGGTGGGGGAACTGAAGGAT	CGGATTGCGGGTTTGATCTC
Mapk11	GCGGGATTCTACCGGCAAG	GAGCAGACTGAGCCGTAGG
JAK3	ACACCTCTGATCCCTCAGC	GCGAATGATAAACAGGCAGGATG

Flow Cytometry

Antibodies against mouse (T-cell stain): CD45, CD3, CD4, CD8, and PD-1 (BioLegend; 103105, 100334, 100412, 100714, and 135214, respectively), LAG-3 (eBioscience, San Diego, CA; 46-2231-82), and CD39 (eBioscience, 46-0391-82). For DCs, cells were stained with antibodies against mouse: CD45, CD11b, $CD11c^{+}$, MHCII, CD80 (BioLegend; 103114, 101228, 117306, 107628, and 104714, respectively) and CD103 (eBioscience, 12-1031-82). For DC cytokines, cells were permeabilized and fixed using the Foxp3 Fix/Perm kit (BioLegend, 421403) and stained at room temperature for 45 minutes with the following antibodies against mouse: IL12p40/70 (BD Biosciences, Franklin Lake, NJ; 554479) and IL27p28 (BioLegend, 516906). For intracellular signaling in DCs, sorted MLN DCs were rested for 2 hours in RPMI at 37°C. Cells were unstimulated or stimulated with the FFAR2 agonist (10 mmol/L dissolved in water, pH 7.4) for 30 minutes at 37°C, fixed, permeabilized according to the manufacturer's instructions (BD Cytofix, 554655 and BD Phosflow Perm Buffer II I, 558050 [BD Biosciences]), and stained with PE-conjugated anti-p65 (pS536) (Cell Signaling Technology, Danvers, MA; 5733) for 45 minutes at room temperature. Flow cytometry was conducted using a BD LSRII (BD Biosciences).

16S Sequencing Analysis

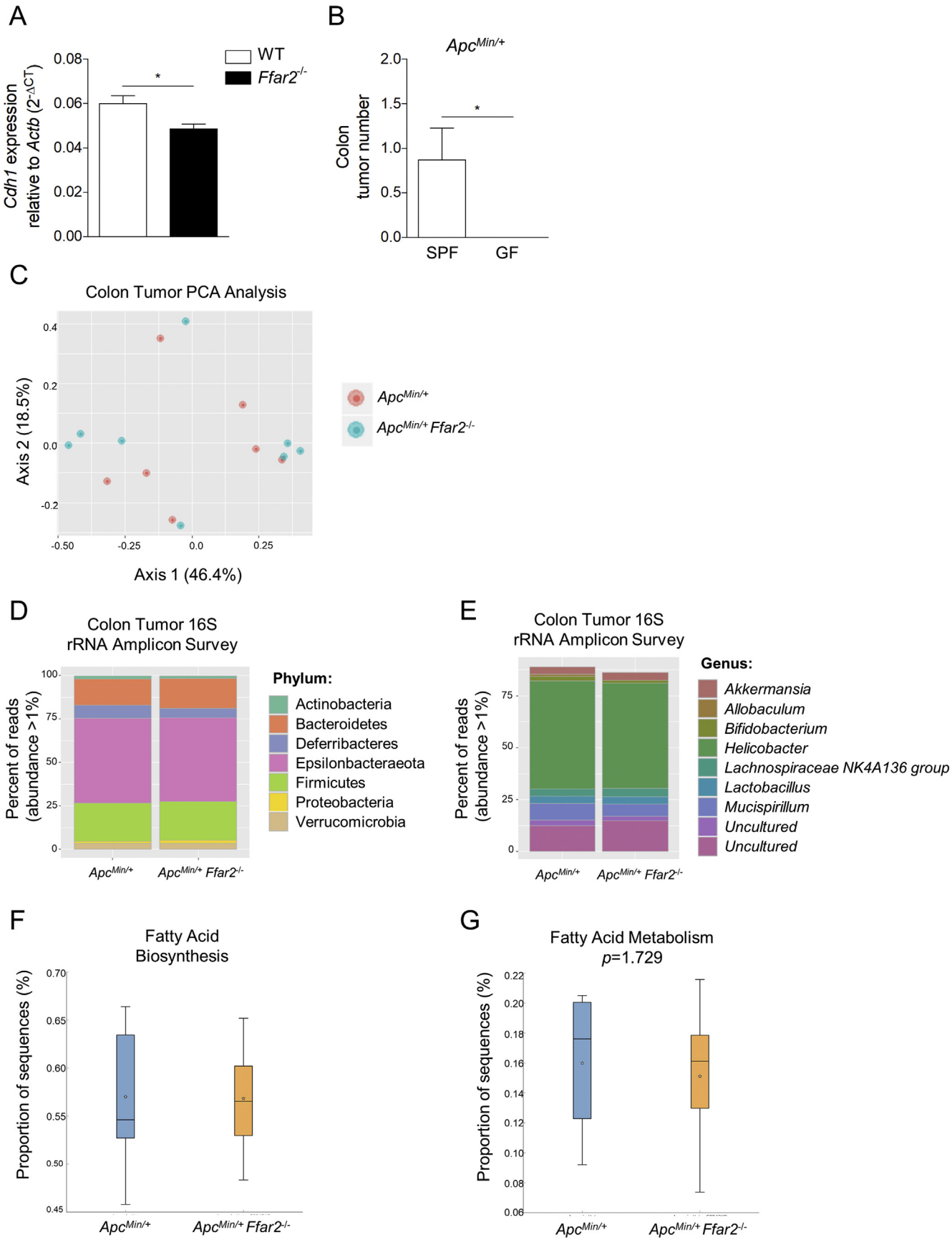
7 $Apc^{Min/+}$ tumor samples and 8 $Apc^{Min/+} \times Ffar2^{-/-}$ tumor samples were selected for 16S amplicon-based taxonomic profiling. The 16S rRNA gene sequencing protocol was adapted from the Earth Microbiome Project. The 16S rRNA V4 region was amplified from the extracted DNA by PCR and sequenced by using the 2 × 250–base pair paired-end reading on a MiSeq instrument (Illumina, San Diego, CA). Analysis of 16S rRNA sequence data was performed using Microbiome Helper scripts.[7] Sequences were clustered into operational taxonomic units (OTUs) at a similarity threshold of 97% by using the sortmerna_sumaclust method of open-reference OTU picking. OTUs were subsequently mapped to a subset of the SILVA database[8] containing only sequences from the V4 region of the 16S rRNA gene to determine taxonomies. To account for variations in sequencing depth, OTU tables were ratified to the lowest sequence depth among samples. Unweighted Unifrac distances were computed with Phyloseq[9] version 1.28. PICRUSt analysis was implemented as described previously.[10]

RNA-Sequencing Data Generation and Analysis

RNA samples showed an average RNA integrity number of 9.0 and libraries were generated using SMART-seq v4 Ultra Low Input RNA kit (Takara Bio, Mountain View, CA; catalog number 640170). Barcoded samples were pooled and sequenced over 4 lanes on a NextSeq 500 instrument (Illumina) to produce 75–base pair paired-end reads. Raw sequencing reads were demultiplexed, and the adapters were trimmed by using Illumina's bcl2fastq2 Conversion software. Duplicate reads were removed based on unique molecular identifier base. Low-quality reads were filtered by using sickle, version 1.33 (https://github.com/najoshi/sickle). Reads were aligned to the National Center for Biotechnology Information GRCm38/mm10 mouse genome by using the STAR aligner, version 2.7,[11] and filtered for uniquely mapped reads. Reads per gene were counted using HTSeq ,version 0.11.1.[12] Differential expression was assessed by DESeq2, version 1.24.0,[13] with default parameters. For pathway analysis (Kyoto Encyclopedia of Genes and Genomes analysis), differentially expressed genes [P value $< .05$ and FPKM ≥ 1]) were used and analyzed by DAVID,[14] Annotated gene ontology biological process was assigned to genes that were altered in DCs. Heatmapping was performed in R (R Core Team, Vienna, Austria) with heatmap package, version 1.0.12.

Supplementary References

1. Stranges PB, Watson J, Cooper CJ, et al. Elimination of antigen-presenting cells and autoreactive T cells by Fas contributes to prevention of autoimmunity. Immunity 2007;26:629–641.
2. Forbes S, Stafford S, Coope G, et al. Selective FFA2 Agonism Appears to Act via Intestinal PYY to Reduce Transit and Food Intake but Does Not Improve Glucose Tolerance in Mouse Models. Diabetes 2015;64:3763–3771.
3. Hoveyda HR, Schils D, Zoute L, Parcq J (2001c). Pyrrolidine or thiazolidine carobxylic acid derivatives, pharmaceutical compositions and methods for use as in treating metabolic disorders as agonists of G-protein coupled receptor 43 (GPR43). International patent WO21011073376. June 23, 2011.
4. Feng Y, Sun X, Yang H, et al. Dissociation of E-cadherin and beta-catenin in a mouse model of total parenteral nutrition: a mechanism for the loss of epithelial cell proliferation and villus atrophy. J. Physiol. (Lond.) 2009;587:641–654.
5. Nadkarni MA, Martin FE, Jacques NA, et al. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology (Reading, Engl.) 2002;148:257–266.
6. Wang R, Han G, Wang J, et al. The pathogenic role of interleukin-27 in autoimmune diabetes. Cell. Mol. Life Sci. 2008;65:3851–3860.
7. Comeau AM, Douglas GM, Langille MGI. Microbiome Helper: a Custom and Streamlined Workflow for Microbiome Research. Eisen J, ed. mSystems 2017;2.e00127–16.
8. Pruesse E, Quast C, Knittel K, et al. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 2007;35:7188–7196.
9. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 2013;8:e61217.
10. Langille MGI, Zaneveld J, Caporaso JG, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 2013;31:814–821.
11. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 2013;29:15–21.
12. Anders S, Pyl PT, Huber W. HTSeq–a Python framework to work with high-throughput sequencing data. Bioinformatics 2015;31:166–169.
13. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550.
14. Huang DW, Sherman BT, Tan Q, et al. The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 2007;8:R183.


A
WT
Ffar2-/-
Cdh1 expression relative to Actb (2-ΔCT)
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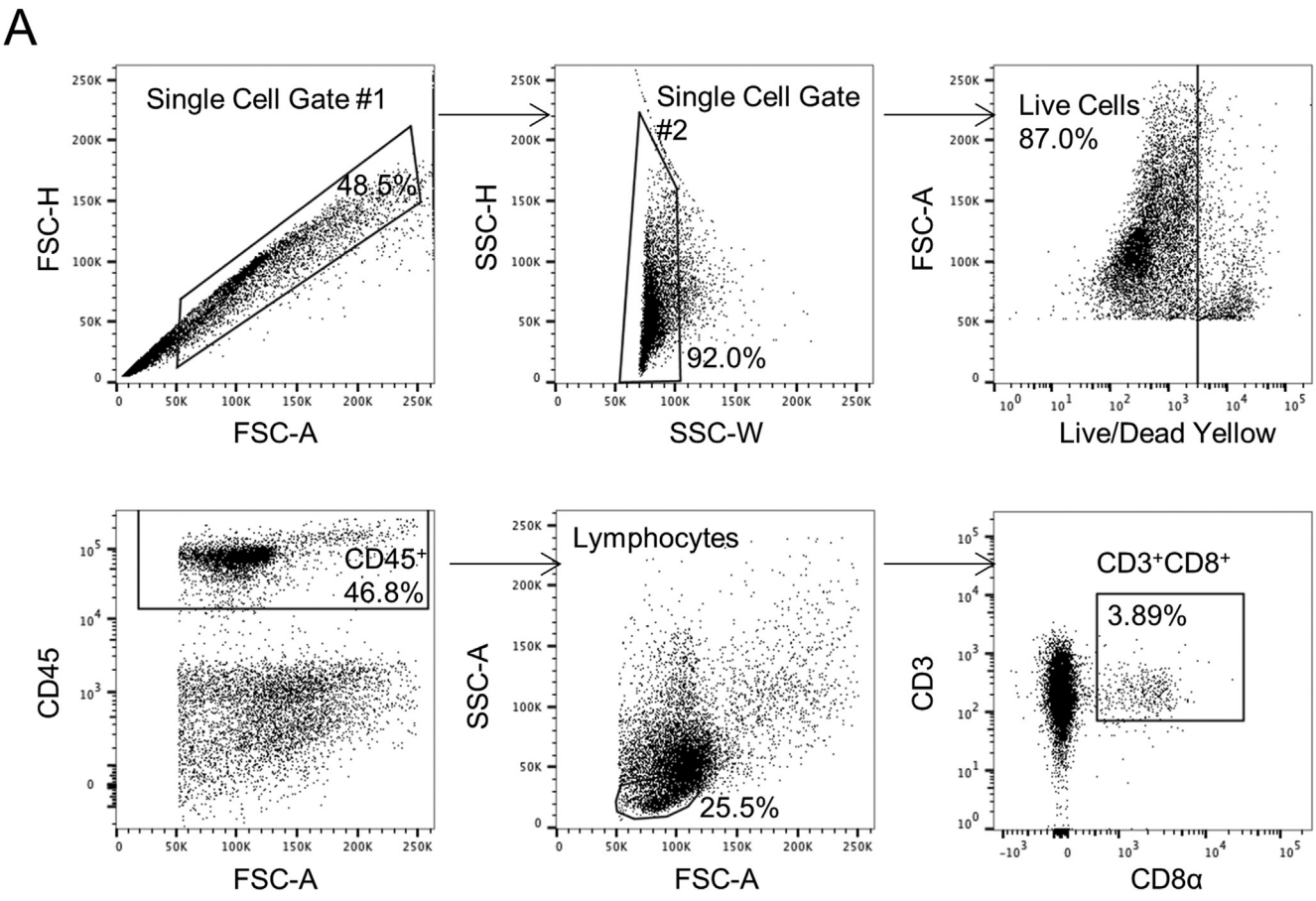
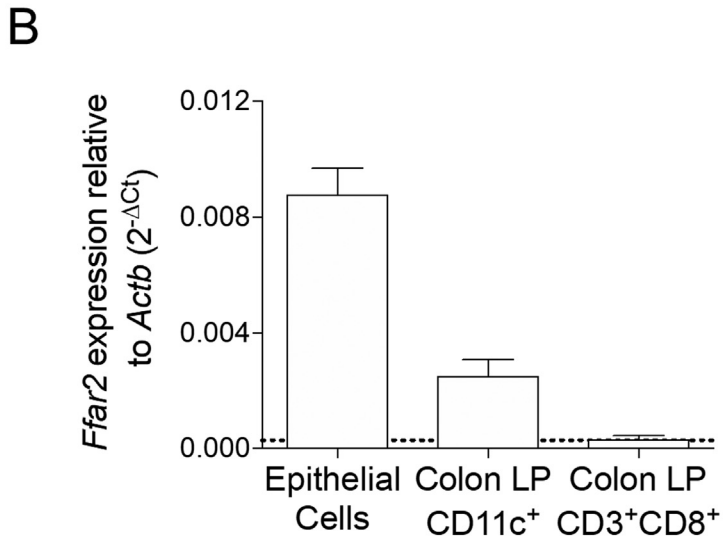
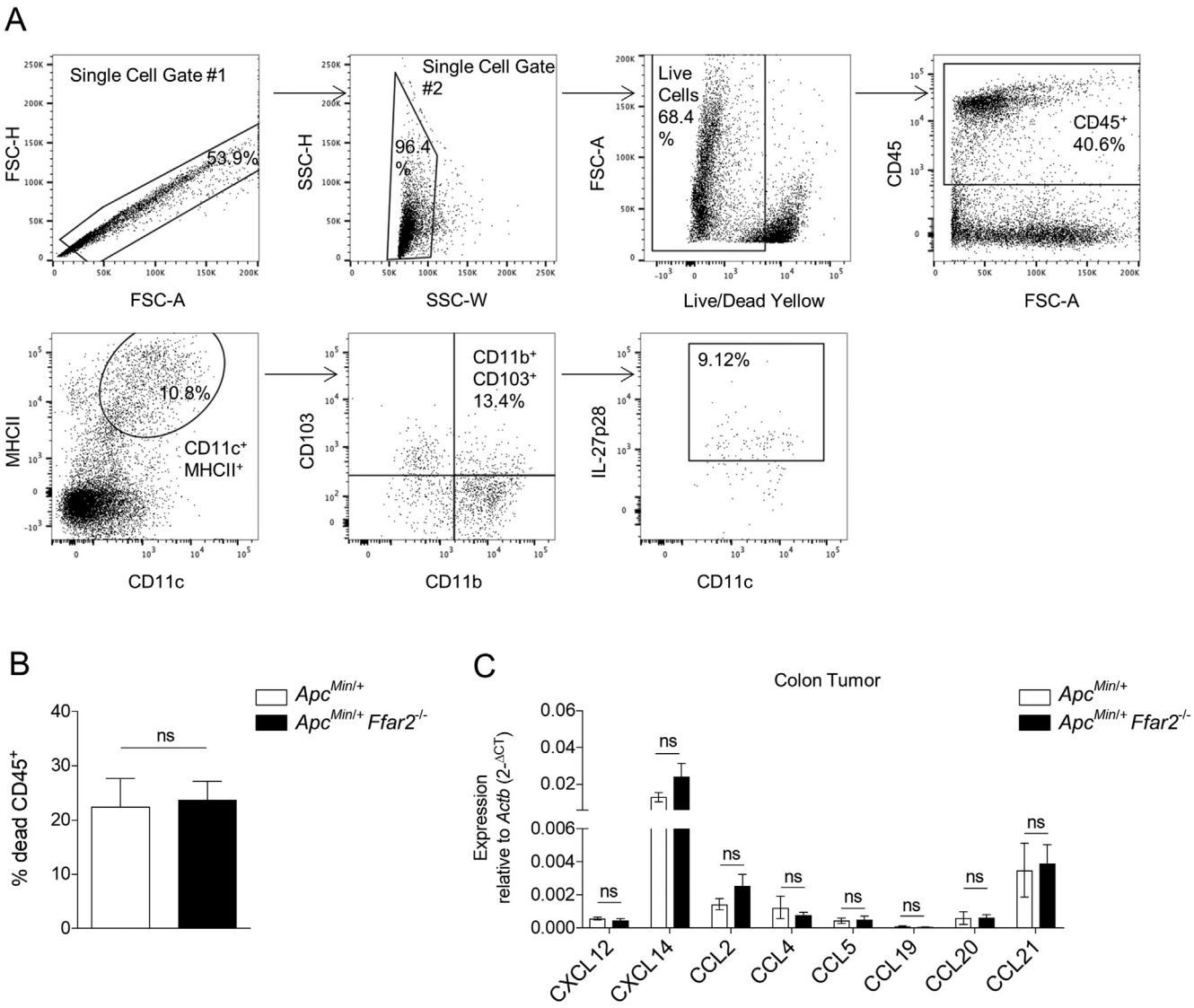
0.06
0.04
0.02
0.00
*
B
ApcMin/+
Colon tumor number
2.0
1.5
1.0
0.5
0.0
*
SPF
GF
C
Colon Tumor PCA Analysis
Axis 2 (18.5%)
0.4
0.2
0.0
-0.2
-0.50
-0.25
0.0
0.25
Axis 1 (46.4%)
ApcMin/+
ApcMin/+ Ffar2-/-
D
Colon Tumor 16S rRNA Amplicon Survey
Percent of reads (abundance >1%)
100
75
50
25
0
ApcMin/+
ApcMin/+ Ffar2-/-
Phylum:
Actinobacteria
Bacteroidetes
Deferribacteres
Epsilonbacteraeota
Firmicutes
Proteobacteria
Verrucomicrobia
E
Colon Tumor 16S rRNA Amplicon Survey
Percent of reads (abundance >1%)
75
50
25
0
ApcMin/+
ApcMin/+ Ffar2-/-
Genus:
Akkermansia
Allobaculum
Bifidobacterium
Helicobacter
Lachnospiraceae NK4A136 group
Lactobacillus
Mucispirillum
Uncultured
Uncultured
F
Fatty Acid Biosynthesis
Proportion of sequences (%)
0.70
0.65
0.60
0.55
0.50
0.45
ApcMin/+
ApcMin/+ Ffar2-/-
G
Fatty Acid Metabolism
p=1.729
Proportion of sequences (%)
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
ApcMin/+
ApcMin/+ Ffar2-/-

Supplementary Figure 1. *Ffar2* deficiency affects epithelial expression of *Cdh1* and overall microbiota abundance. *(A) Cdh1* expression in colon epithelial cells from WT (N = 10) and *Ffar2*$^{-/-}$ (N = 9) mice. (*B*) Colon tumor numbers from specific pathogen–free (SPF) (N = 8) and germ-free (GF) (N = 7) *Apc*$^{Min/+}$ mice. (*C*) Principal coordinate analysis of 16S rRNA gene amplicon data from *Apc*$^{Min/+}$ (N = 7) vs *Apc*$^{Min/+}$ × *Ffar2*$^{-/-}$ (N = 8) colon tumors. (*D*) Phylum-level taxonomic analysis of 16S rRNA gene amplicon data. (*E*) Genus-level taxonomic analysis of 16S rRNA gene amplicon data. (*F*) PICRUSt analysis of fatty acid biosynthesis. (*G*) PICRUSt analysis of fatty acid metabolism. Data from *A* and *B* represent ≥3 independent experiments and are plotted as the mean ± SEM. **P* < .05, Mann-Whitney *U* test. Data from *C*–*G* represent 3 independent experiments, and differences between groups were evaluated by Mann-Whitney *U* tests and were not statistically significant. PCA, Principal Component Analysis.

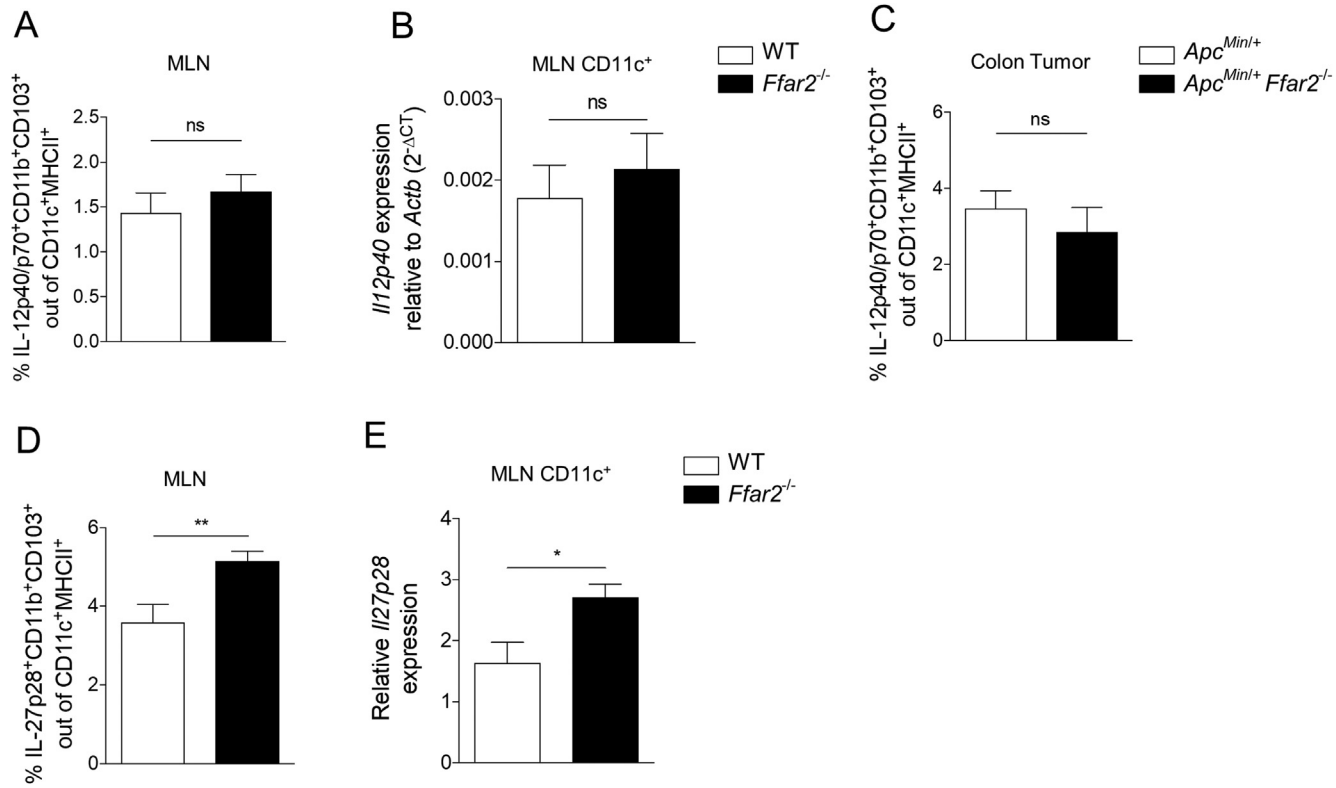




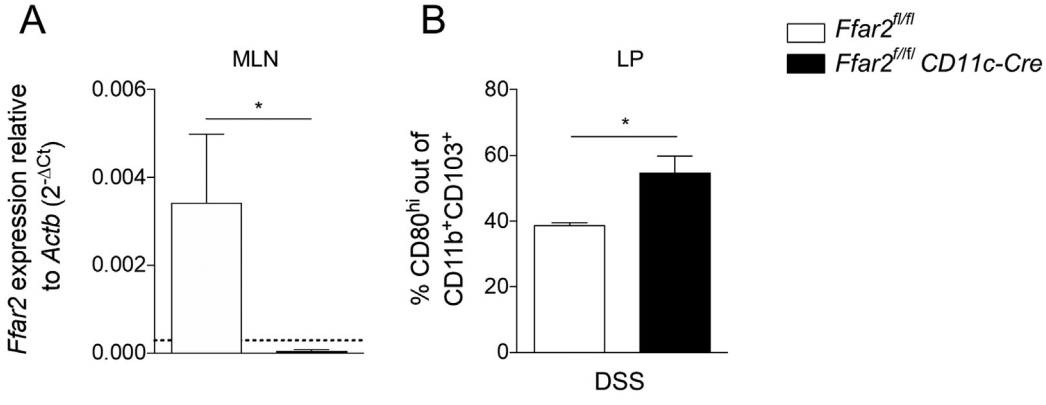
Supplementary Figure 2. Gating strategy for colon tumor $CD8^+$ T cells and *Ffar2* expression in colonic cells. (*A*) Flow cytometry gating strategy for $CD3^+CD8^+$ T cells from $Apc^{Min/+}$ colon tumors. Cells are gated on single cells, live cells, $CD45^+$, lymphocytes, and $CD3^+CD8^+$ cells. (*B*) *Ffar2* expression relative to *Actb* in colon epithelial cells (N = 3), colon LP $CD11c^+$ DCs (N = 4) and $CD3^+CD8^+$ T cells (N = 3) from WT mice. Dotted line indicates limit of detection for *Ffar2* primers. Data from *A* and *B* represent ≥3 independent experiments.



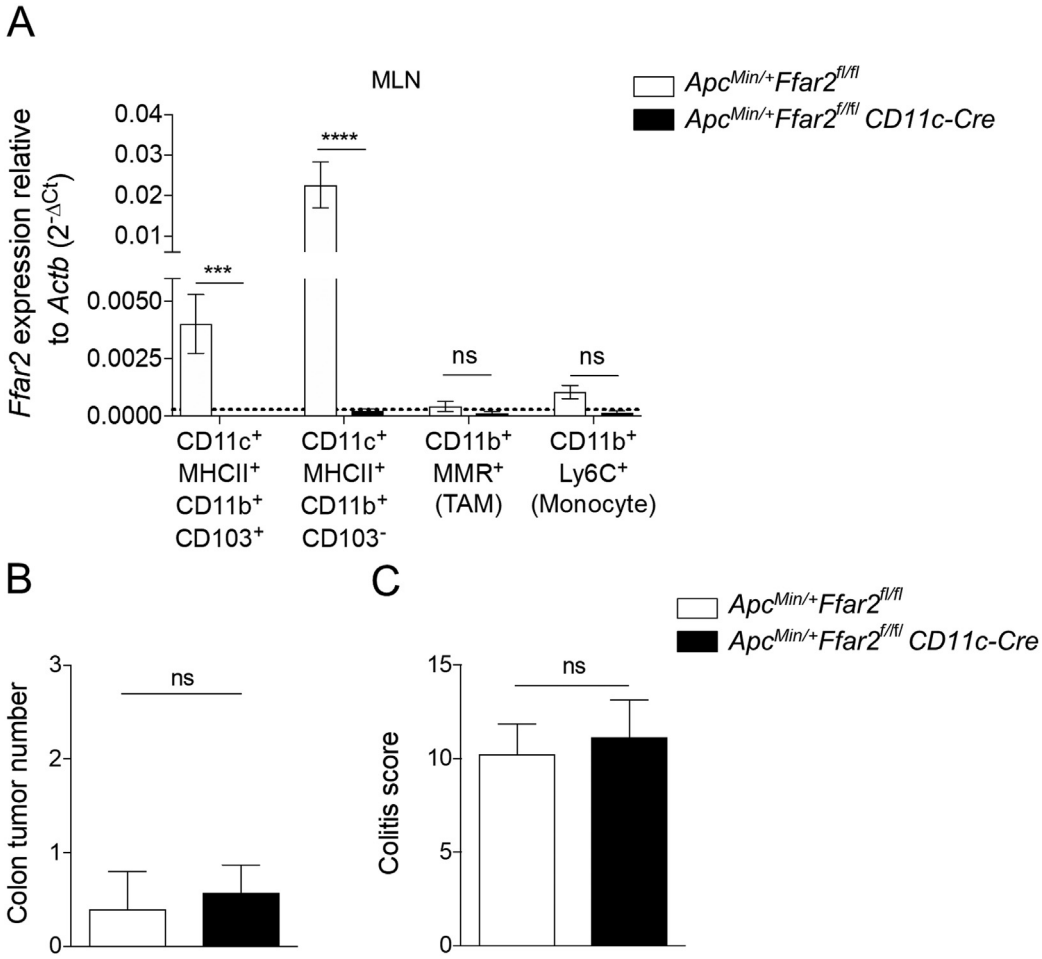
Supplementary Figure 3. Tumor IL-27$^+$ DC gating strategy, CD45$^+$ cell viability, and chemokine expression. (*A*) IL27$^+$ DC flow cytometry gating strategy from *Apc*$^{Min/+}$ tumors. Cells are gated on single live cells, CD45$^+$, CD11c$^+$MHCII$^+$, CD11b$^+$CD103$^+$, and IL-27p28$^+$. (*B*) Dead colon tumor CD45$^+$ cell frequency from *Apc*$^{Min/+}$ (N = 7) vs *Apc*$^{Min/+}$*Ffar2*$^{-/-}$ (N = 12) mice. (*C*) Expression of chemokines in *Apc*$^{Min/+}$ vs *Apc*$^{Min/+}$*Ffar2*$^{-/-}$ colon tumors. Data from *B* and *C* represent ≥3 independent experiments. Data from *B* are plotted as the mean ± SEM. ns, not significant.



Supplementary Figure 4. Ffar2 deficiency affects IL27$^+$ DCs but not IL12$^+$ DCs. (*A*) MLN IL12$^+$ DC frequency in WT (N = 10) vs *Ffar2*$^{-/-}$ (N = 11) mice. (*B*) *Il12p40* expression relative to *Actb* from sorted MLN DCs from WT (N = 6) and *Ffar2*$^{-/-}$ mice (N = 7). (*C*) IL12$^+$ DC frequency in colon tumors from *Apc*$^{Min/+}$ (N = 6) and *Apc*$^{Min/+}$*Ffar2*$^{-/-}$ (N = 7) mice. (*D*) MLN IL27$^+$ DC frequency from WT (N = 10) and *Ffar2*$^{-/-}$ mice (N = 11). (*E*) Relative *il27p28* expression in DCs sorted from WT (N = 4) and *Ffar2*$^{-/-}$ MLNs (N = 5). Data from *A–E* are plotted as mean ± SEM. *$P < .05$, **$P < .01$, Mann–Whitney *U* test. ns, not significant.



Supplementary Figure 5. *Ffar2* expression in MLN DCs and DC activation in colon LP from *Ffar2*$^{fl/fl}$*CD11c-Cre* mice. (*A*) *Ffar2* expression in sorted MLN DCs from *Ffar2*$^{fl/fl}$ (N = 4) and *Ffar2*$^{fl/fl}$*CD11c-Cre* (N = 4) mice. Dotted line indicates the limit of detection for *Ffar2* primers. (*B*) CD80^{hi} cell frequency out of colon LP DCs from DSS-treated *Ffar2*$^{fl/fl}$ (N = 6) and *Ffar2*$^{fl/fl}$*CD11c-Cre* mice (N = 4). Data represent 3 independent experiments. Data are plotted as the mean ± SEM. *$P < .05$, Mann–Whitney *U* test.



Supplementary Figure 6. Conditional deletion of *Ffar2* in DCs does not affect other myeloid cells, tumor number under steady state, and colitis score. (*A*) Ffar2 expression in sorted MLN DCs ($CD11c^+MHCII^+CD11b^+CD103^+$ and $CD11c^+MHCII^+CD11b^+CD103^-$), tumor-associated macrophages (TAM) ($CD11b^+MMR^+$), and monocytes ($CD11b^+Ly6C^+$). Dotted line indicates the limit of detection for *Ffar2* primers. (*B*) Tumor number in $Apc^{Min/+}Ffar2^{fl/fl}$ (N = 5) vs $Apc^{Min/+}Ffar2^{fl/fl}CD11c\text{-}Cre$ (N = 7) mice without DSS treatment. (*C*) Histology-based colitis scores from mice or mice treated with DSS. $Apc^{Min/+}Ffar2^{fl/fl}$ (N = 9) vs $Apc^{Min/+}Ffar2^{fl/fl}CD11c\text{-}Cre$ (N = 7) mice. Data represent ≥3 independent experiments. Data from *A–C* are plotted as the mean ± SEM. ***$P < .001$, ****$P < .0001$, Mann–Whitney *U* test. ns, not significant.