Figure S1 Related to Figure 1
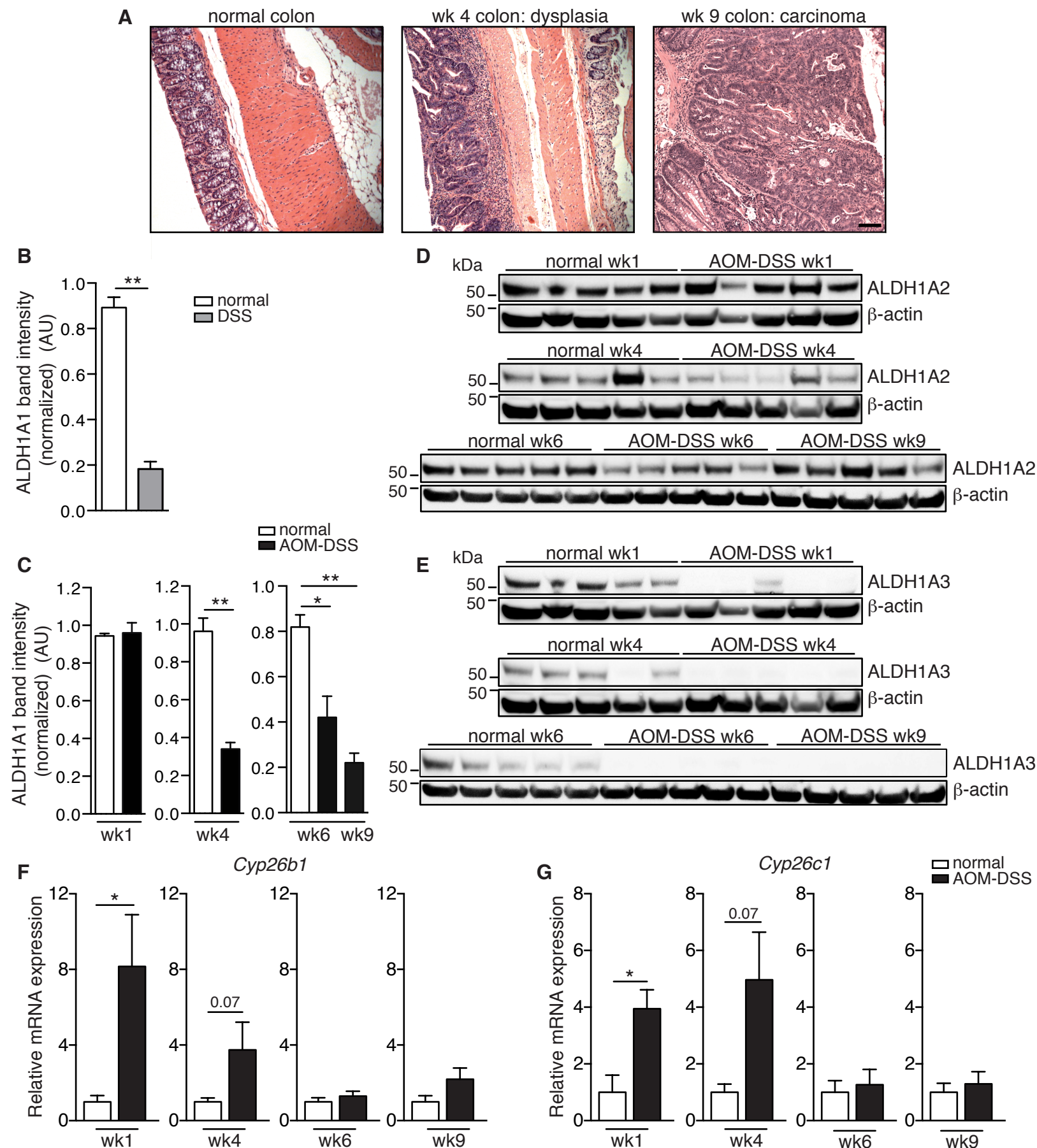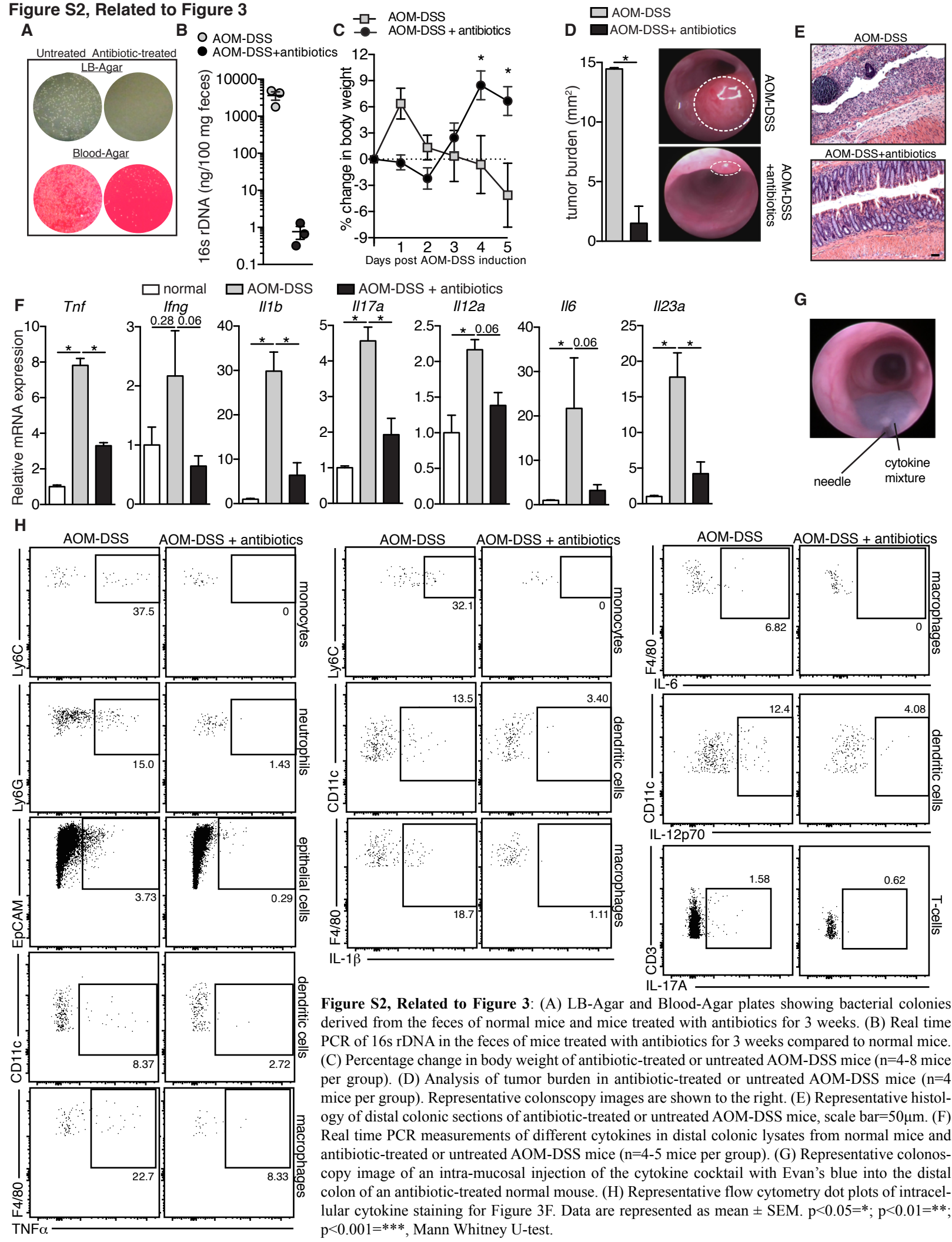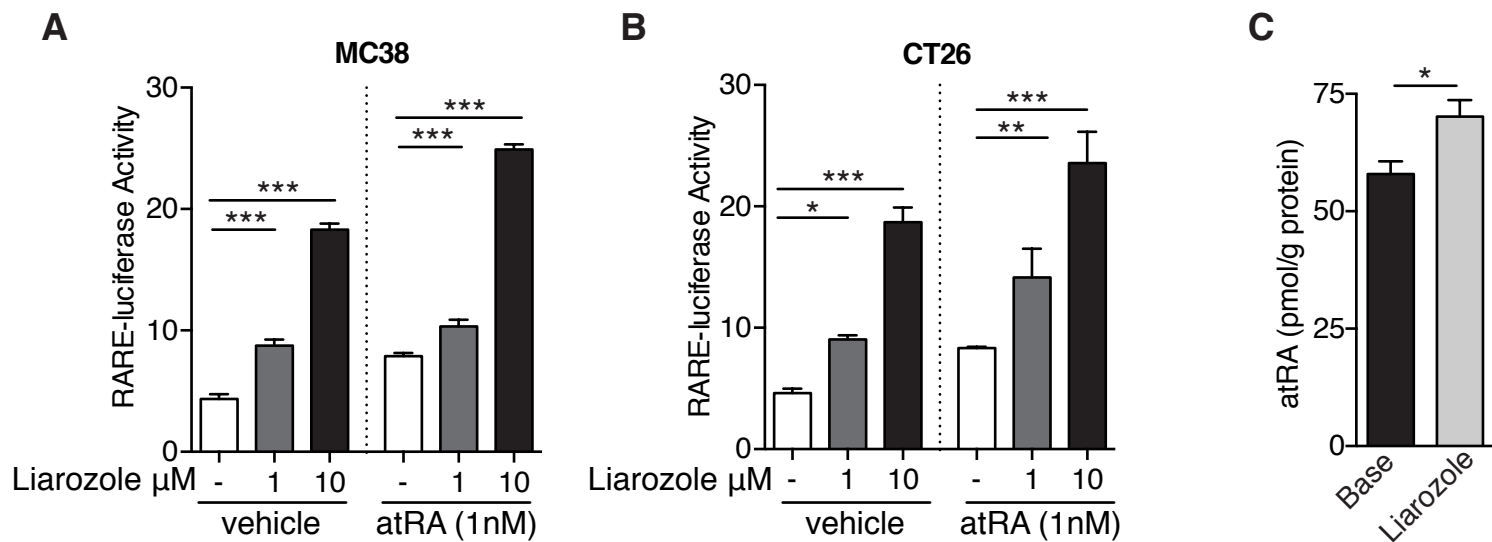

Figure S1, Related to Figure 1: (A) Representative histology of distal colonic sections of AOM-DSS mice at different time points during disease progression (n=5 mice per group). Scale bar=100 µm. (B, C) Immunoblot band intensities quantified for Figure 1 B, C (in arbitrary units, (AU)), normalized to the corresponding band intensities of β-actin and calculated using Image J. (D, E) Immunoblots for ALDH1A2 (D) and ALDH1A3 (E) in distal colonic lysates of mice at different time points after AOM-DSS induction, compared to normal mice (n=5 mice per group). (F, G) qRT-PCR measurements showing fold change in transcript expression of *Cyp26b1* (F) and *Cyp26c1* (G), relative to *Gapdh* expression, in the distal colons of AOM-DSS mice at different time points after disease induction, compared to normal mice. (n=5 mice per group; representative of 2 independent experiments). Results are represented as mean ± SEM. p<0.05=*; p<0.01=**; p<0.01=***, Mann Whitney U-test.

Figure S2, Related to Figure 3


Figure S2, Related to Figure 3: (A) LB-Agar and Blood-Agar plates showing bacterial colonies derived from the feces of normal mice and mice treated with antibiotics for 3 weeks. (B) Real time PCR of 16s rDNA in the feces of mice treated with antibiotics for 3 weeks compared to normal mice. (C) Percentage change in body weight of antibiotic-treated or untreated AOM-DSS mice (n=4-8 mice per group). (D) Analysis of tumor burden in antibiotic-treated or untreated AOM-DSS mice (n=4 mice per group). Representative colonoscopy images are shown to the right. (E) Representative histology of distal colonic sections of antibiotic-treated or untreated AOM-DSS mice, scale bar=50μm. (F) Real time PCR measurements of different cytokines in distal colonic lysates from normal mice and antibiotic-treated or untreated AOM-DSS mice (n=4-5 mice per group). (G) Representative colonoscopy image of an intra-mucosal injection of the cytokine cocktail with Evan's blue into the distal colon of an antibiotic-treated normal mouse. (H) Representative flow cytometry dot plots of intracellular cytokine staining for Figure 3F. Data are represented as mean ± SEM. p<0.05=*; p<0.01=**; p<0.001=***, Mann Whitney U-test.

Figure S3, Related to Figure 4: (A, B) RARE luciferase assay on MC38 (A) and CT26 (B) colon cancer cell lines cultured with increasing amounts of liarozole or vehicle, with or without atRA (1 nM). Data representative of 2 independent experiments. (C) Mass spectrometry measurement of colonic atRA concentrations in AOM-DSS mice orally fed with Liarozole or vehicle (n=6 mice per group). Data are represented as mean ± SEM. $p<0.05$=*; $p<0.01$=**; $p<0.001$=***, One-way Anova (A, B) Mann Whitney U-test (C).

Figure S4, Related to Figure 5
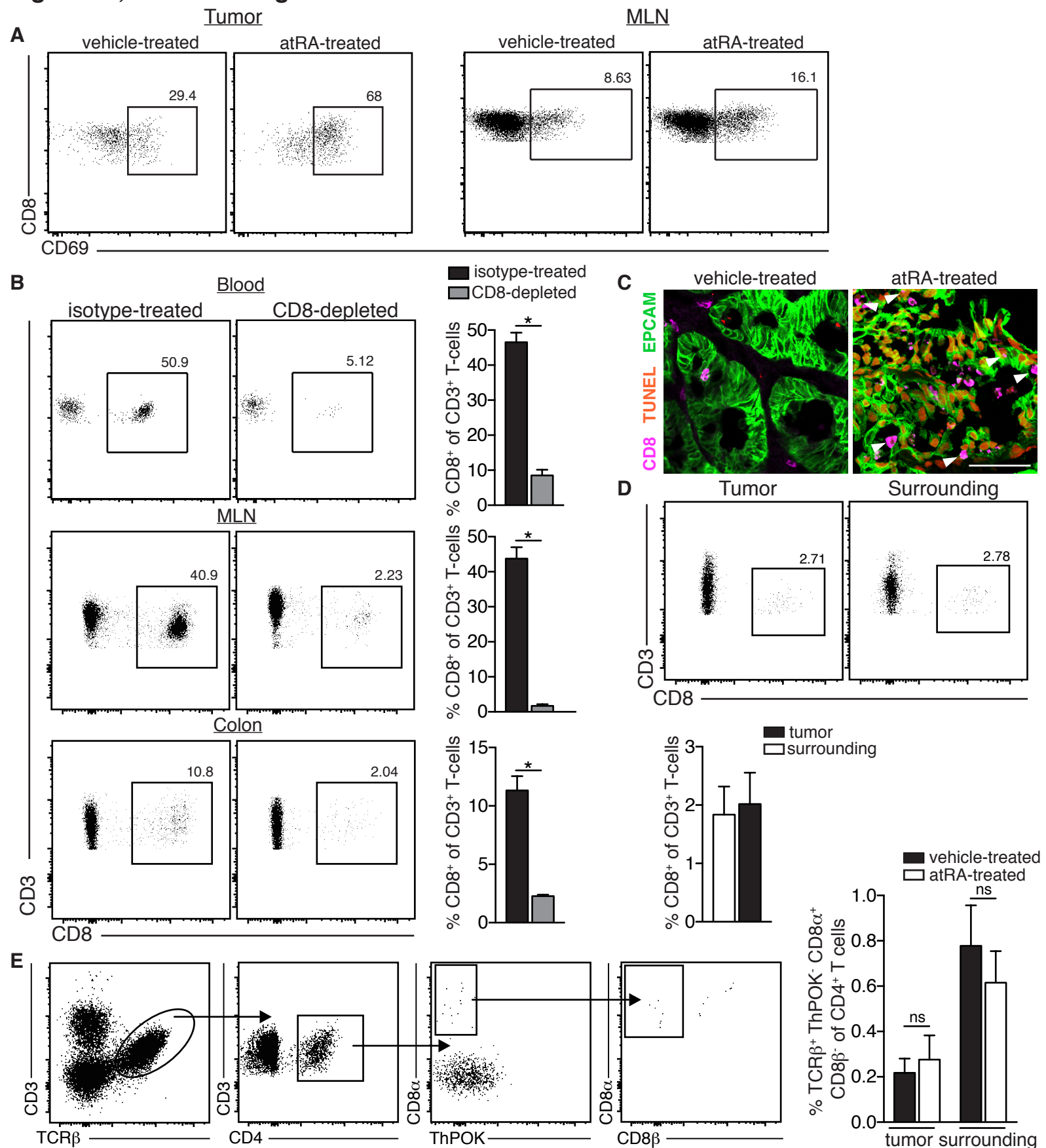
Figure S4, Related to Figure 5: (A) Representative flow cytometry dot plots showing percentages of CD8^{+} T-cells expressing CD69 in colonic tumors and MLNs of vehicle- and atRA-treated AOM-DSS mice. (B) Representative flow cytometry dot plots showing depletion of CD8^{+} T-cells in the blood, MLN and colon of AOM-DSS mice, along with quantifications of frequencies of CD8^{+} T-cells (n=4-5 mice per group). (C) Immunofluorescence image showing EPCAM (green), TUNEL (red) and CD8^{+} T-cells (magenta) in colonic tumor sections from atRA- or vehicle-treated AOM-DSS mice. White arrows depict CD8^{+} T-cells in close proximity to TUNEL^{+} epithelial cells. (D) Representative flow cytometry dot plots showing adoptively transferred CD8^{+} T-cells in tumors and surrounding distal colonic tissue of $Cd8a^{-/-}$ mice induced with AOM-DSS, along with quantification of frequencies of CD8^{+} T-cells (n=10 per group). (E) Representative flow cytometry dot plots and the frequencies of TCRβ^{+} ThPOK^{-} CD8α^{+} CD8β^{-} of CD4^{+} T-cells from the IEL layer of tumors and surrounding distal colonic tissue in atRA- or vehicle-treated mice. Data pooled from 2 independent experiments (n=10 mice per group). Data are represented as mean ± SEM. p<0.05=*; p<0.01=**; p<0.001=***, Mann Whitney U-test.
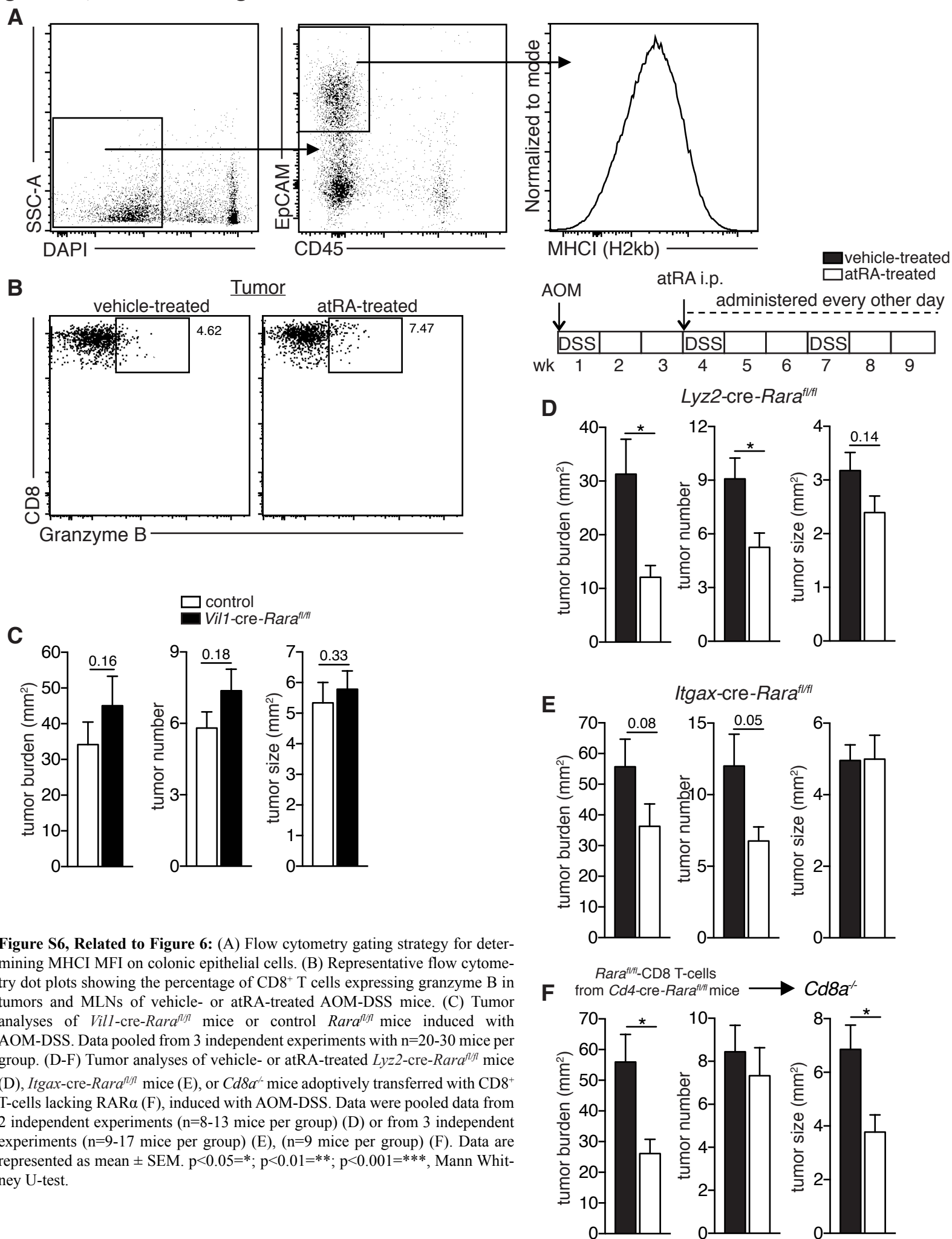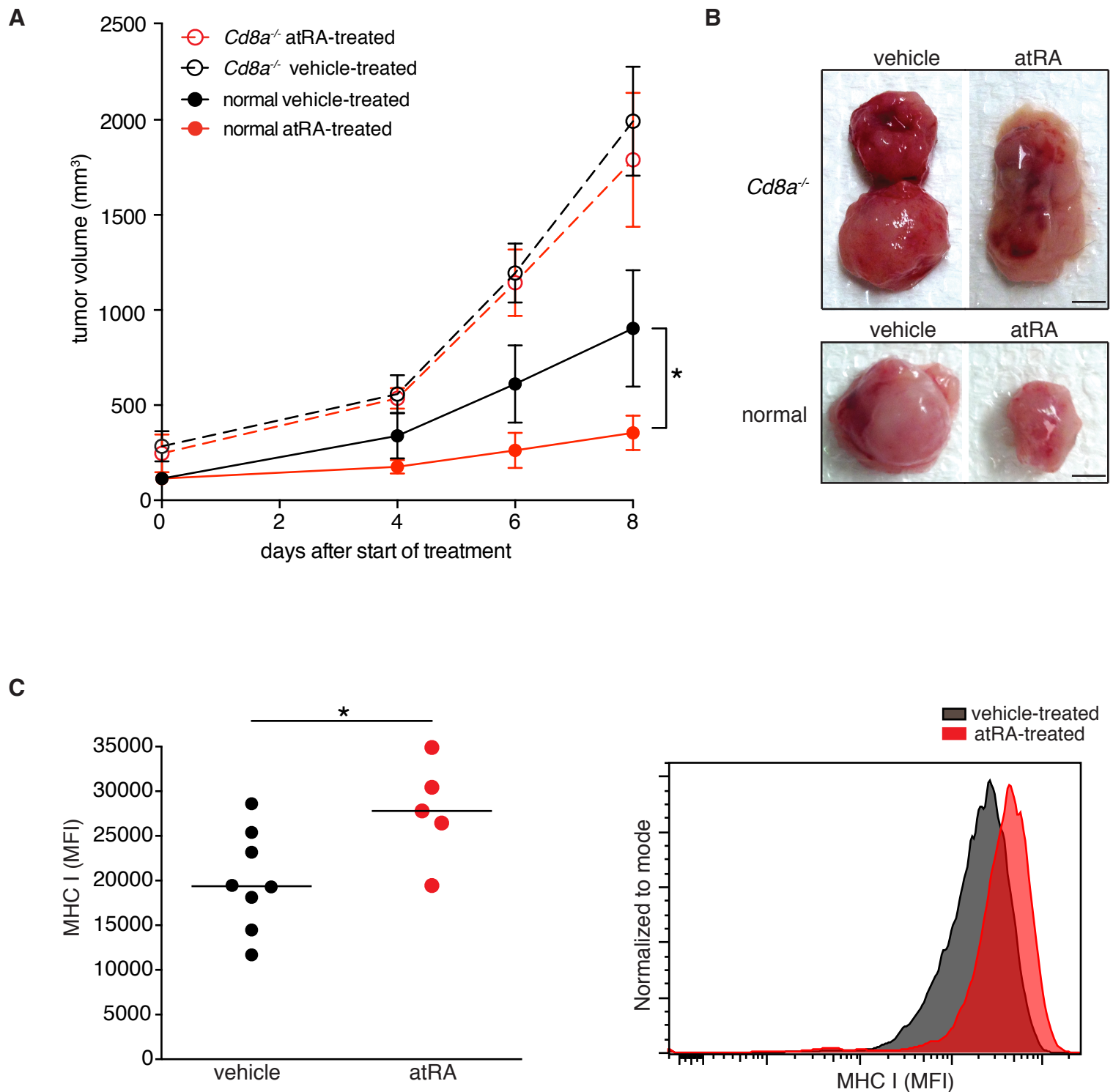
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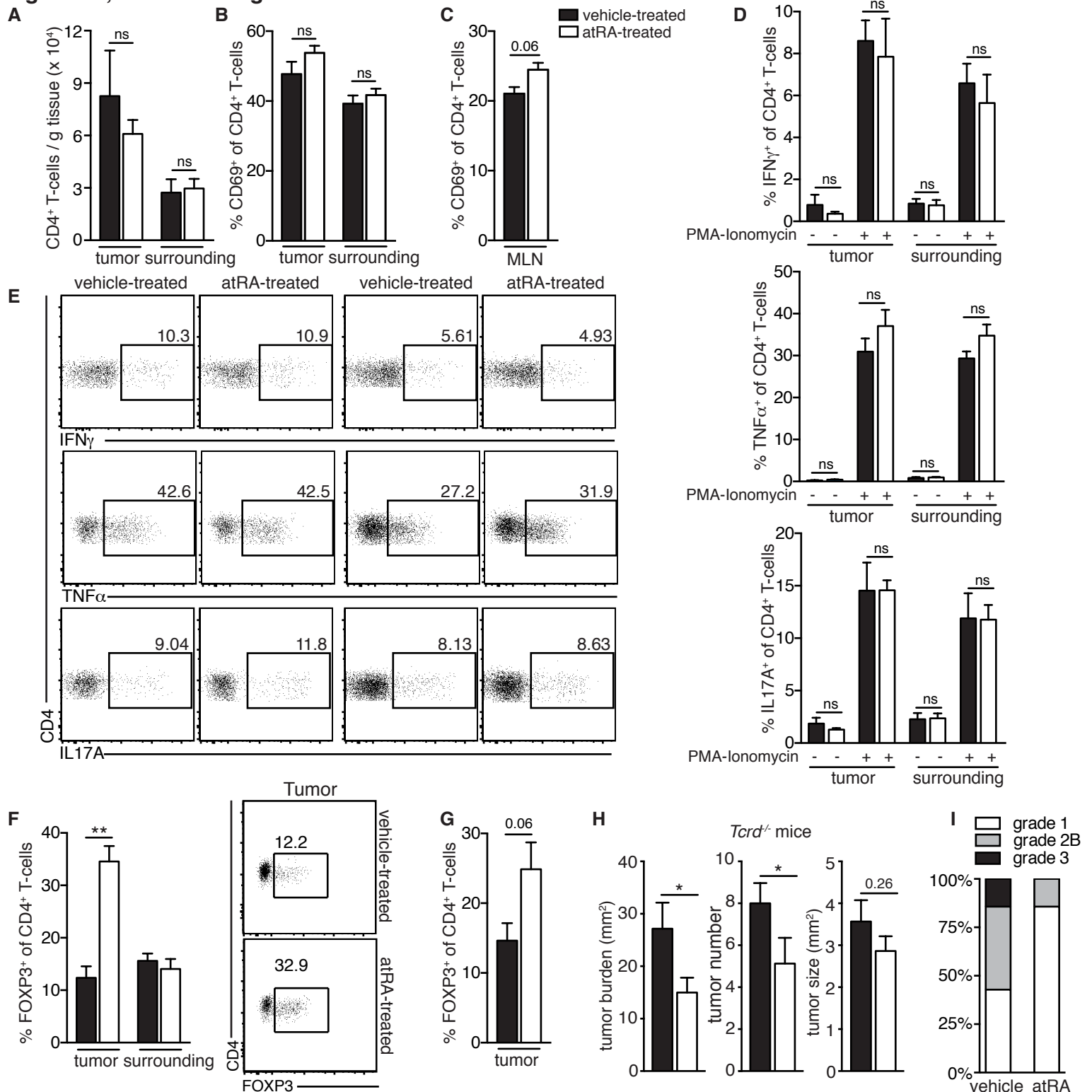
Figure S5, Related to Figure 5: (A-C) Flow cytometry data showing absolute CD4+ T-cell counts (A) and percentages of CD69+ CD4+ T-cells in tumors and surrounding distal colonic tissue (B) and MLNs (C) of vehicle- or atRA-treated AOM-DSS mice. Data were pooled from 2 independent experiments (n=7-8 mice per group) (A), from 3 independent experiments (n=14 mice per group) (B), and from 2 independent experiments (n=8 mice per group) (C). (D-E) Flow cytometry data showing the percentage of CD4+ T-cells expressing different cytokines in vitro following stimulation with PMA-Ionomycin, in tumors and surrounding distal colonic tissue from atRA- or vehicle-treated AOM-DSS mice (D), along with representative flow plots (E).Plots represent pooled data from 2 independent experiments (n=6 mice per group). (F,G) Flow cytometry data showing the frequency of FOXP3+ T-cells in tumors and surrounding distal colonic tissue from atRA- or vehicle-treated AOM-DSS mice (n=5-6 mice per group) (F), along with representative flow plots, and in tumors from $Cd8a^{-/-}$ mice induced with AOM/DSS (n=4 mice per group) (G). (H) Tumor analyses of vehicle- and atRA-treated $Tcrd^{-/-}$ mice mice induced with AOM/DSS. Graphs represent pooled data from 2 independent experiments (n=8 mice per group). (I) Colitis scoring of distal colonic tissues surrounding the tumors from vehicle- and atRA-treated AOM-DSS mice (n=7 mice per group). (J) Representative histology of distal colonic tissues from (I), scale bar=50μm. Data are represented as mean ± SEM. p<0.05=*; p<0.01=**, p<0.01=***, Mann Whitney U-test.

Figure S6, Related to Figure 6

Figure S6, Related to Figure 6: (A) Flow cytometry gating strategy for determining MHCI MFI on colonic epithelial cells. (B) Representative flow cytometry dot plots showing the percentage of CD8+ T cells expressing granzyme B in tumors and MLNs of vehicle- or atRA-treated AOM-DSS mice. (C) Tumor analyses of *Vil1*-cre-*Rara^{fl/fl}* mice or control *Rara^{fl/fl}* mice induced with AOM-DSS. Data pooled from 3 independent experiments with n=20-30 mice per group. (D-F) Tumor analyses of vehicle- or atRA-treated *Lyz2*-cre-*Rara^{fl/fl}* mice (D), *Itgax*-cre-*Rara^{fl/fl}* mice (E), or *Cd8a^{-/-}* mice adoptively transferred with CD8+ T-cells lacking RARα (F), induced with AOM-DSS. Data were pooled data from 2 independent experiments (n=8-13 mice per group) (D) or from 3 independent experiments (n=9-17 mice per group) (E), (n=9 mice per group) (F). Data are represented as mean ± SEM. $p<0.05$=*; $p<0.01$=**; $p<0.001$=***, Mann Whitney U-test.

Figure S7, Related to Figure 6

A

B

C

Figure S7, Related to Figure 6: (A) Data show the growth kinetics of MC38 subcutaneous tumors in atRA- or vehicle-treated normal C57BL/6 and *Cd8a*^{-/-} mice. Data were pooled from 2 independent experiments (n=8 mice per group). Total area under the curves were calculated and analyzed. (B) Representative images of MC38 tumors from atRA- or vehicle-treated normal and *Cd8a*^{-/-} mice. Scale bar = 0.5cm. (C) Plots represent the MHCI MFI on MC38 tumor cells from atRA- or vehicle-treated mice. Also shown are representative histograms of MHCI MFI. Data were pooled from 2 independent experiments (n=5-8 mice per group). Data are represented as mean ± SEM. $p<0.05$=*; $p<0.01$=**; $p<0.001$=***, Mann Whitney U-test.

Supplemental Experimental Procedures

Patient specimens

Formalin-fixed paraffin-embedded blocks containing colonic resections of ulcerative colitis and colorectal cancer (CRC) patients were obtained from the Stanford Tissue Bank and from the Toronto General Hospital (Ontario, Canada) under protocols approved by the Institutional Review Board (IRB, Stanford) and the University Health Network Research Ethics Board (Toronto General Hospital). Final diagnoses of the specimens were confirmed by experienced pathologists from Stanford University and the Toronto General Hospital. The tissue microarray containing sporadic colon cancer core biopsies was obtained from US Biomax, with diagnoses supplied by the company.

Mice

C57BL/6 mice, $Cd8a^{-/-}$, $Vil1$-cre mice, $Lyz2$-cre mice, $Cd4$-cre mice and $Tcrd^{-/-}$ mice, were from The Jackson Laboratory. $Rara^{fl/fl}$ mice were a kind gift from Dr. Yasmine Belkaid (NIAID, Bethesda, MD). All mice were housed in an American Association for the Accreditation of Laboratory Animal Care-accredited animal facility, and maintained in specific pathogen-free conditions on standard rodent chow *ad libitum* unless otherwise stated. Mice were randomly allocated to each experimental group for drug versus vehicle treatment such that the mean weights of all the groups were similar. This was done to nullify any systematic bias before starting the experiment. Animals within each group were randomly allocated to different cages during the treatment period to avoid cage effects. Tumor analysis was done in a blinded fashion wherever possible.

DSS and AOM-DSS mouse models

The DSS and AOM-DSS mouse models of chronic colitis and CAC, respectively, were established using the protocol described by Wirtz et al. (Wirtz et al., 2007), with slight modifications. In brief, for the development of chronic colitis, 8- to 10-week-old mice were given 3% DSS salt (36,000–50,000 Da; MP Biomedicals) in drinking water for 7 days, followed by normal drinking water for 14 days. This cycle was repeated twice. For establishing CAC, mice were given an initial intra-peritoneal (i.p.) injection of AOM at the beginning of the chronic colitis protocol. Dysplastic lesions were found in the distal colon of mice approximately 3 weeks after disease induction, and carcinoma was found after 9 weeks. Mice that did not have any visible tumors at the end of week 9 were excluded from data analyses.

Antibiotic treatment of AOM-DSS mice

Wild-type C57BL/6 mice were given antibiotics dissolved in drinking water before induction with AOM-DSS. The antibiotics used were ampicillin (1g/L), metronidazole (1g/L), vancomycin (0.5 g/L), and neomycin (1g/L) (Sigma-Aldrich). 3% DSS was made in water containing antibiotics. Since *Cyp26a1* expression is increased as early as 1 week after AOM-DSS induction (Figure 1H) and *Aldh1a1* is decreased 3 weeks after disease induction (Figure 1C), distal colons of mice were harvested after week 1 and 3 post-AOM-DSS induction with or without antibiotics for qRT-PCR analysis of *Cyp26a1* and *Aldh1a1* respectively (Figure 3B). For qRT-PCR quantitation of cytokines in the distal colons of mice, antibiotic treatment was provided for 2 weeks prior to disease induction with AOM-DSS and continued throughout disease progression up to the harvest time at the end of week 4 (Figure S2F). For the intracellular flow cytometry determination of cytokine secretion and immune cell frequency quantitation, antibiotic treatment was provided for 2 weeks prior to disease induction with AOM-DSS and continued throughout disease progression up to the harvest time at the end of week 6 (Figure 3F,G)

Bacterial culture on Agar plates

Fecal pellets from antibiotic-treated (3 weeks post treatment) and normal C57BL/6 mice were collected and homogenized in 200 µL PBS. The homogenate was centrifuged at 10,000 rpm for 5 min and 100 µL of the supernatant was cultured on LB Agar and Blood Agar (TSA with 5% Sheep Blood) plates at 37 degrees. 24 hrs later, images of the plates were recorded.

atRA treatment of Caco-2 cell line

$2x10^5$ cells of the Caco-2 (ATCC® HTB-37™) human colon cancer cell line were seeded in a 12-well plate with 1mL Eagle's Minimum Essential Medium (EMEM) supplemented with 20% fetal calf serum. 24 hrs later, the cells were treated with 1µM atRA or DMSO and harvested after 72 hrs for flow cytometry.

Luciferase assay

CT26 or MC38 cells were transfected with a 4:1 ratio of a firefly luciferase reporter plasmid driven by a pGL3-RARE-responsive promoter (Addgene) and a Renilla luciferase reporter plasmid driven by a constitutive TK promoter (Promega). 24 hours later, the 10% FBS (fetal bovine serum)-containing cell culture medium was replaced with 2% FBS-containing medium and 1nM atRA or vehicle along with increasing amounts of Liarozole. 36 hours later, activity of both reporters was measured using the Dual-Luciferase Reporter kit (Promega) and read on a Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek). The RARE luciferase to Renilla luciferase ratio is reported as "RARE-luciferase activity."

Intramucosal colonic injections

11-week-old C57BL/6 wild-type female mice were pretreated with antibiotics for 3 weeks and injected intramucosally in the distal colon with a cytokine mixture containing 100ng each of TNFα, IL-1β, IL-12p70, IL-23, IL-6, IFNγ and IL-17A or vehicle. Evan's blue dye was added to the cytokine mixture and the vehicle to track the site of injection. The injections were guided by the Karl Storz colonoscopy system and performed with a custom-made 30g needle end attached to a syringe via capillary tubing. Injections were done 2 mm from the end of the rectum and were repeated on day 0, day 2 and day 4. Distal colonic tissues—measuring around 5 mm from the end of the rectum and marked by Evan's blue—were collected 4 hours after the last injection for qRT-PCR analyses.

Intestinal organoid culture and cytokine treatment

Tumors were excised from AOM-DSS-treated mice, washed and incubated in 2mM EDTA solution in PBS for 30 minutes on ice. Next, the tumors were minced and incubated in RPMI medium containing 10% FBS with Liberase TL (Roche Applied Sciences) and DNase I (Sigma-Aldrich) for 30min at 37 degrees. The digest was strained through a 70µm filter and centrifuged for 5min. The pellet was resuspended in matrigel containing 1µM Jagged-1 peptide (BD Biosciences) and plated on a non-tissue culture treated plate. After polymerization of the matrigel, DMEM-F12 medium containing 10mM HEPES, 1X glutamine, 10% FBS, 1X N2 (Life Technologies), 1X B27 (Life Technologies), 1mM N-acetylcysteine and 1% Anti-anti (Thermo Fisher Scientific) was added containing the following growth factors: human Rspondin-1 (500ng/mL, from Peprotech), murine EGF (50ng/mL, from Peprotech), murine Noggin (100ng/mL, from Peprotech), 10µM Y-27632 (Sigma-Aldrich) and Wnt3a (100ng/mL, from Peprotech). Organoids were passaged every 3-4 days. One day after passaging the organoids, they were cultured with a cytokine mixture containing 50ng/mL each of TNFα, IL-1β, IL-12p70, IL-23, IL-6, IFNγ in PBS+1% BSA or vehicle alone for 48 hrs and then harvested for qRT-PCR analyses.

Quantitation of all-*trans* retinoic acid expression in mouse colonic tissue

Mouse colon samples were collected under yellow light and flash frozen to prevent atRA degradation. To facilitate homogenization, frozen samples were ground by mortar and pestle. Quantification of retinoids by LC-MS-MS were performed as described (Kane et al., 2008), with an alternate LC separation: Suplex pkb-100 column (Supelco, 2.1 x 250 mm, 5 µm particles) with 3 min of 80% acetonitrile, 20% water, 0.1% formic acid, followed by a linear gradient to 95% acetonitrile, 5% water, 0.1% formic acid over 9 min, held for 4 min, returned by linear gradient to 80-20-0.1 over 1 min, and held 8 min, all at 0.4 mL/min. Results were normalized to either the starting amount of protein in the tissue homogenate or the weight of the tissue.

CD8^{+} T-cell depletion

To deplete CD8^{+} T-cells in AOM-DSS mice, 500µg rat anti-mouse CD8α antibody (clone YTS169.4, BioXCell) was injected i.p. into 10-week-old C57BL/6 female mice once a week, from week 4 to week 9 after disease induction. The control group received the same amount of isotype control antibody (clone LTF2, BioXCell). Flow cytometric analysis showed that CD8^{+} T-cells were decreased by approximately 84% in the blood (assessed at week 9 after AOM-DSS induction), 80% in the colonic lamina propria and 96% in the MLNs (assessed at week 6 after AOM-DSS induction) in the anti-CD8α-treated group compared to the isotype control antibody-treated group (Figure S4B).

Adoptive transfer of CD8^{+} T-cells into *Cd8a*^{-/-} mice

CD8$^+$ T-cells were isolated from the spleen, MLNs and other peripheral lymph nodes from DSS-treated C57BL/6 mice using the EasySep™ Mouse CD8$^+$ T Cell Isolation Kit (STEMCELL Technologies). CD8$^+$ T-cells were adoptively transferred by retro-orbital i.v. injection into 10-week-old $Cd8a^{-/-}$ mice (8x10^6 cells per mouse) 2 weeks after induction with AOM-DSS (Figure S4D). Intraperitoneal injections with atRA or vehicle were administered every other day to recipient mice from the day of adoptive transfer to the end of week 9 post-induction with AOM-DSS.

Immunoblotting
Colons were harvested from normal mice, mice with chronic colitis (week 7 after DSS induction), or from different stages during the development of CAC. Lysates were prepared from the distal half of the colons using RIPA lysis buffer, as the most severe inflammation and dysplastic changes are confined to that region (Araki et al., 2010; Neufert et al., 2007). The following primary antibodies were used for immunoblotting: anti-ALDH1A1 (Protein tech, 15910-1-AP), anti-ALDH1A2 (Abcam, ab75674), anti-ALDH1A3 (Abcam, ab129815) and anti-β-actin (Cell Signaling, 8457S). Primary and secondary HRP-labeled antibodies were used at 1:500 and 1:2000 dilutions respectively. Detection was performed with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific).

Real time quantitative PCR (qRT-PCR)
Total RNA was isolated from distal colons of mice, sorted cell populations from the colon and intestinal organoids using the RNeasy Plus kit (Qiagen). RNA was reverse-transcribed using the High-Capacity Reverse Transcription Kit (Applied Biosystems). Transcript expressions were determined by qRT-PCR using the Fast SYBR Green PCR Master Mix (Applied Biosystems) and the 7900HT real-time PCR instrument (Applied Biosystems). The primers sequences used were as follows: *Aldh1a1*: 5'-GGAAGCTGCAGGGAAAAGCAATC-3' and 5'-TCCGGGATGCTGCGACACA-3', *Cyp26a1*: 5'-GGACTCTACCCACATGTCCTCCAGAA-3' and 5'-ACCGGAGGATTCAATCGCAGG-3', *Cyp26b1*: 5'-TGCCACCCGCGACAA-3' and 5'-GGAACCCTGTAGCAACCAGTGA-3', *Cyp26c1*: 5'-GCTGGGCCGTTTGCGCT-3' and 5'-TCTCATGCGTGTCTCGGATGCTATA-3', *Gapdh*: 5'-ACAGTCCATGCCATCACTGCC-3' and 5'-GCCTGCTTCACCACCTTCTTG-3', *Il6*: 5'-ACCAGAGGAAATTTTCAATAGGC-3' and 5'-TGATGCACTTGCAGAAAACA-3', *Il23a*: 5'-CCAGCGGGACATATGAATCT-3' and 5'-AGGCTCCCCTTTGAAGATGT-3' , I*l17a*: 5'-AGAATTCATGTGGTGGTCCAG-3' and 5'-ACTACCTCAACCGTTCCACG-3', *Ifng*: 5'-TGAACGCTACACACTGCATCTTGG-3' and 5'-CGACTCCTTTTCCGCTTCCTGAG-3' , *Il1b*: 5'-TGTAATGAAAGACGGCACACC-3' and 5'-TCTTCTTTGGGTATTGCTTGG-3', *Tnf*: 5'-CAGCCTCTTCTCATTCCTGC-3' and 5'-GGTCTGGGCCATAGAACTGA-3', *Il12a*: 5'-CTGTGCCTTGGTAGCATCTATG-3' and 5'-GCAGAGTCTCGCCATTATGATTC-3'. qRT-PCR calculations were performed using the comparative C$_T$ method (Schmittgen and Livak, 2008). The data are represented either as fold changes of the treated group compared to the control group or as 2$^{-\Delta CT}$ mRNA transcript abundance (Figure 1 I,J). For the quantification of 16s rDNA in mouse fecal pellets, total fecal DNA was extracted using the REDExtract-N-Amp™ Tissue PCR Kit (Sigma-Aldrich) and purified of inhibitors (Zymo Research). 16s rDNA was quantified using the following primers: 5'-CGGCAACGAGCGCAACCC-3' and 5'-CCATTGTAGCACGTGTGTAGCC-3'. For quantitation of 16s rDNA, we utilized the standard curve method.

Histology and Colitis grading
Distal colonic tissues of normal mice, mice induced with AOM-DSS harvested at the end of week 4 or week 9 after disease induction, distal colonic tissues excised of tumors from AOM-DSS mice treated with or without antibiotics harvested at the end of week 7, or distal colonic tissues excised of tumors from atRA-versus vehicle-treated mice harvested at the end of week 9 were formalin-fixed paraffin embedded and stained with hematoxylin and eosin. Disease diagnosis and colitis grading were determined by an experienced gastrointestinal pathologist at Stanford University who was blinded with respect to treatment. For colitis grading, the scheme from Geboes et al. was used (Geboes et al., 2000).

Immunofluorescence staining
Colonic samples from AOM-DSS mice were washed with PBS and embedded in optimal cutting temperature compound (OCT, Tissue-Tek). The colonic sections were post-fixed in 2% paraformaldehyde

and blocked with 10% goat serum. They were subsequently stained with a primary rabbit anti-ALDH1A1 antibody (Abcam, 15910-1-AP, 1:100) and goat anti-rabbit Alexa Fluor 594 secondary antibody (Life Technologies, 1:200). DAPI was used as a nuclear stain. TUNEL staining on mouse colonic sections was done according to the manufacturer's instructions (Roche Applied Sciences) after staining with primary rat anti-EpCAM antibody or rat anti-EpCAM-biotin antibody (clone G8.8, Biolegend, 1:200) or   rat-anti CD8α antibody (clone 53-6.7, BD Pharmingen). Streptavidin FITC (Life Technologies, 1:1000), goat-anti rat Alexa Fluor 488 or Alexa Fluor 647 were used as secondary stains (Life Technologies, 1:200). Human colonic tissue specimens were acquired from formalin-fixed paraffin-embedded blocks as 5µm-thick sections. Antigen retrieval was achieved using the Diva Decloaker (Biocare Medical). Immunofluorescence staining was performed using the following primary antibodies: rabbit anti-ALDH1A1 (Abcam, 15910-1-AP, 1:100), rabbit anti-CYP26A1 (Sigma-Aldrich, 481-495, 1:250), mouse anti-CD8α (Thermo Scientific, MS-457-S0, 1:100), rabbit anti-CD8α (Abcam, ab93278, 1:100) and mouse anti-granzyme B (Dako, clone GrB-7, 1:50). Goat anti-rabbit Alexa Fluor 594, goat-anti mouse Alexa Fluor 488, donkey anti-rabbit Alexa Fluor 488 and donkey anti-mouse Cy3 (Jackson ImmunoResearch, 1:200) were used as the secondary antibodies (Life Technologies, 1:200). DAPI was used as a nuclear stain. Images were collected using a Zeiss 700 confocal laser-scanning microscope, and analyzed using the Image J software.

Flow cytometry
Tumors, distal colonic tissue surrounding the tumors, and mesenteric lymph nodes (MLNs) were excised from AOM-DSS mice under a dissection microscope. Colonic tissue was then cut into 0.5 cm pieces. Mucous from the colonic pieces was removed by vigorous stirring in HBSS and DTT. Intestinal pieces were subsequently digested with Liberase TL (Roche Applied Sciences) and DNase I (Sigma-Aldrich) for 30min at 37 degrees. For specifically isolating the IEL and LP layer, the colonic pieces were stirred in HBSS and DTT followed by two 20 min washes in IEL buffer containing HBSS, HEPES and EDTA. The remaining intestinal pieces were digested with Liberase TL (Roche Applied Sciences) and DNase I (Sigma-Aldrich) for 30min at 37 degrees. Cells retrieved from the IEL buffer washes were referred to as IEL cells and cells retrieved after enzyme digest steps were called LP cells. MLNs were mashed and filtered through a 70µm strainer. Isolated colonic and MLN cells were resuspended in FACS buffer containing 1% BSA in PBS. After blocking the Fc-receptors with anti-mouse CD16/32 (Biolegend), cells were stained with antibodies against CD45.2 (clone 104, Biolegend), Ly6G (clone RB6-8C5, Biolegend), NK1.1 (clone PK136, Biolegend), Ter-119 (clone Ter-119, Biolegend), CD3 (clone 145-2C11, Biolegend), CD4 (clone RM4-5, Biolegend), CD8α (clone 53-6.7, BD Pharmingen), MHCII (clone M5/114.15.2, eBioscience), MHCI (H-2Kb, clone AF6-88.5.5.3, eBioscience), EpCAM (clone G8.8, eBioscience) and CD69 (clone H1.2F3, BD Pharmingen), Ly6C (clone HK1.4, Biolegend), CD19 (clone 6D5, Biolegend), CD11b (clone M1/70, Biolegend), CD11c (clone N418, Biolegend), TCRβ (clone H57-597, Biolegend), CD8β (clone YTS156.7.7, Biolegend), ThPOK (clone T43-94, BD Pharmingen), TNFα (clone MP6-XT22, Biolegend), IFNγ (clone XMG1.2, Biolegend), IL-6 (clone MP5-20F3, Biolegend), IL-1β pro-form (clone NJTEN3, eBioscience), IL-17A (clone TC11-18H10.1, Biolegend), IL-12(p40/p70) (clone, C17.8, BD Pharmingen), FOXP3 (clone FJK-16s, eBioscience). Live-Dead Aqua (Life Technologies) or DAPI was used as a viability stain. Granzyme B staining (clone NGZB, eBioscience) was done using the intracellular staining kit from eBioscience after stimulating cells for 4 hours in PMA and ionomycin and incubating with brefeldin A. Absolute cell counts were quantified using AccuCount fluorescent particles (Spherotech). MHCI quantification on Caco-2 cells was done using the anti-human HLA-ABC antibody (clone W6/32, Biolegend). For intestinal cell frequency calculations and cell sorts, EpCAM$^+$ was used for gating epithelial cells, CD45$^+$ for immune cells, CD3$^+$ for T cells, CD19$^+$ for B cells, MHCII$^+$CD11b$^+$CD11c$^+$ for macrophage and dendritic cells, CD11b$^+$Ly6G$^+$ for neutrophils and CD11b$^+$Ly6C^{hi} for monocytes. For intracellular flow cytometry cytokine staining assays of CD4$^+$ T cells (Figure S5D,E), *in vitro* stimulation of colonic cells for 4 hours with PMA-ionomycin and brefeldin A was used. For intracellular flow cytometry cytokine staining assays of different colonic cell types from antibiotic-treated versus untreated AOM-DSS mice (Figure 3F), *in vitro* stimulation of colonic cells for 4 hours with fecal extract and brefeldin A was used. Fecal extracts were made by homogenizing fecal pellets pooled from n=5 mice from the antibiotic-treated or untreated AOM-DSS mice group in cell culture medium and centrifuging at 5,000 rpm on a table top centrifuge. Flow cytometric data acquisition was performed on a LSRII flow cytometer (BD Biosciences) and data analysis was done using the FlowJo software.

MC38 tumor model

The MC38 colon cancer cell line was a kind gift from Dr. David Bartlett (University of Pittsburgh). MC38 cells were injected subcutaneously in the right flank of male and female wild-type and $Cd8a^{-/-}$ mice (3×10^5 cells per mouse in 200µL PBS). 200µg atRA (Sigma-Aldrich) dissolved in DMSO (vehicle) or DMSO alone was administered by i.p. injection every other day when the tumor reached approximately 50mm³ in volume. Tumor size was recorded every other day using vernier calipers.

Statistics

Experimental data were analyzed with the Mann Whitney U-test using Prism (GraphPad Software), unless otherwise stated. For MC38 subcutaneous tumor growth kinetics in atRA versus vehicle groups, total area under the curves was calculated and statistical differences between the groups were analyzed using the Mann Whitney U-test. Tissue microarray data were analyzed using the One-way ANOVA test, and correlation analysis was performed using the Pearson's correlation test. Kaplan Meier curves for disease-free survival and overall survival were generated and analyzed with Prism (GraphPad software) using the Log-rank (Mantel Cox) test. Results are represented as mean ± SEM. $p<0.05$=*; $p<0.01$=**; $p<0.001$=***.

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