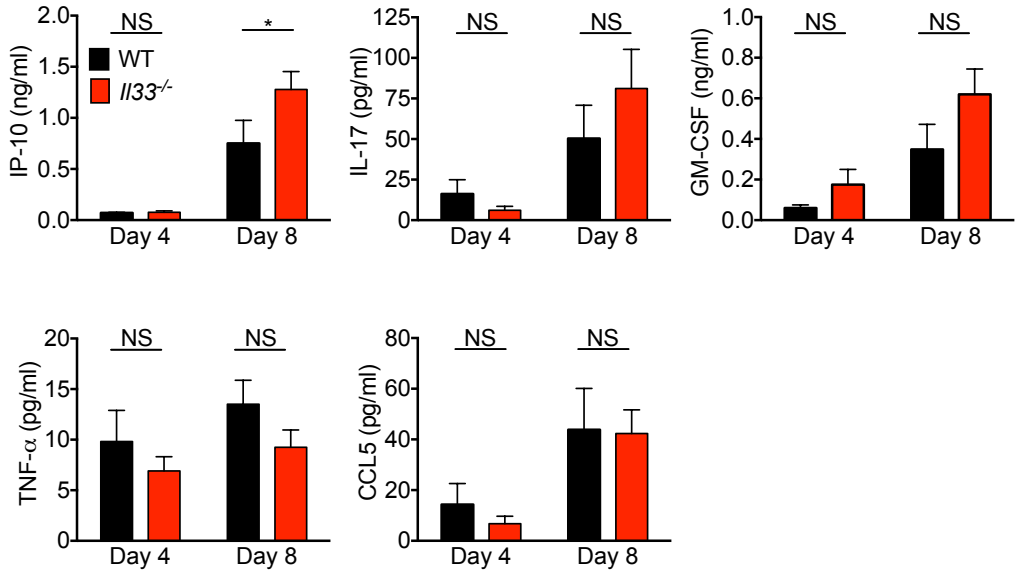
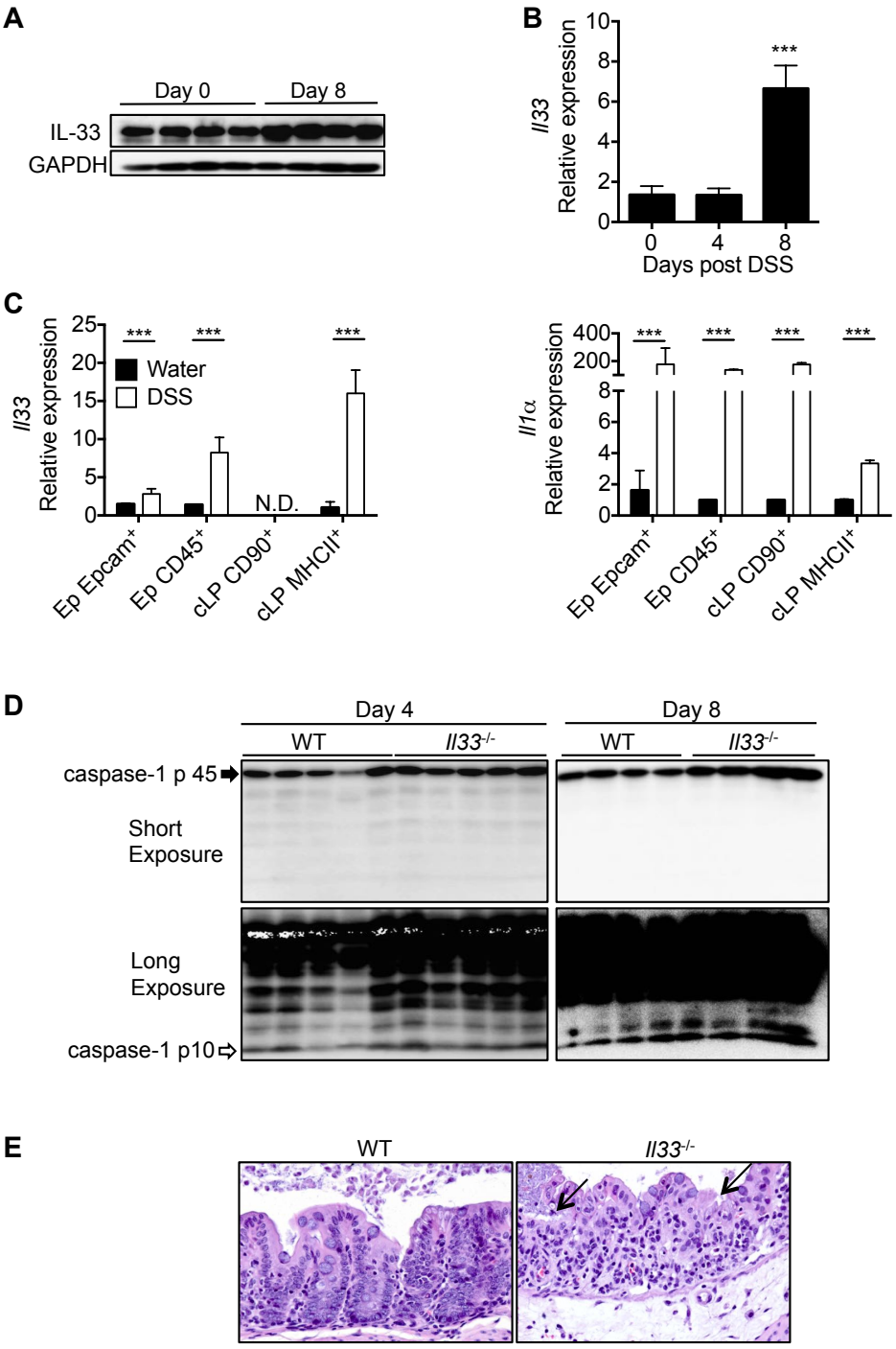
Supplementary Materials

Supplementary figures

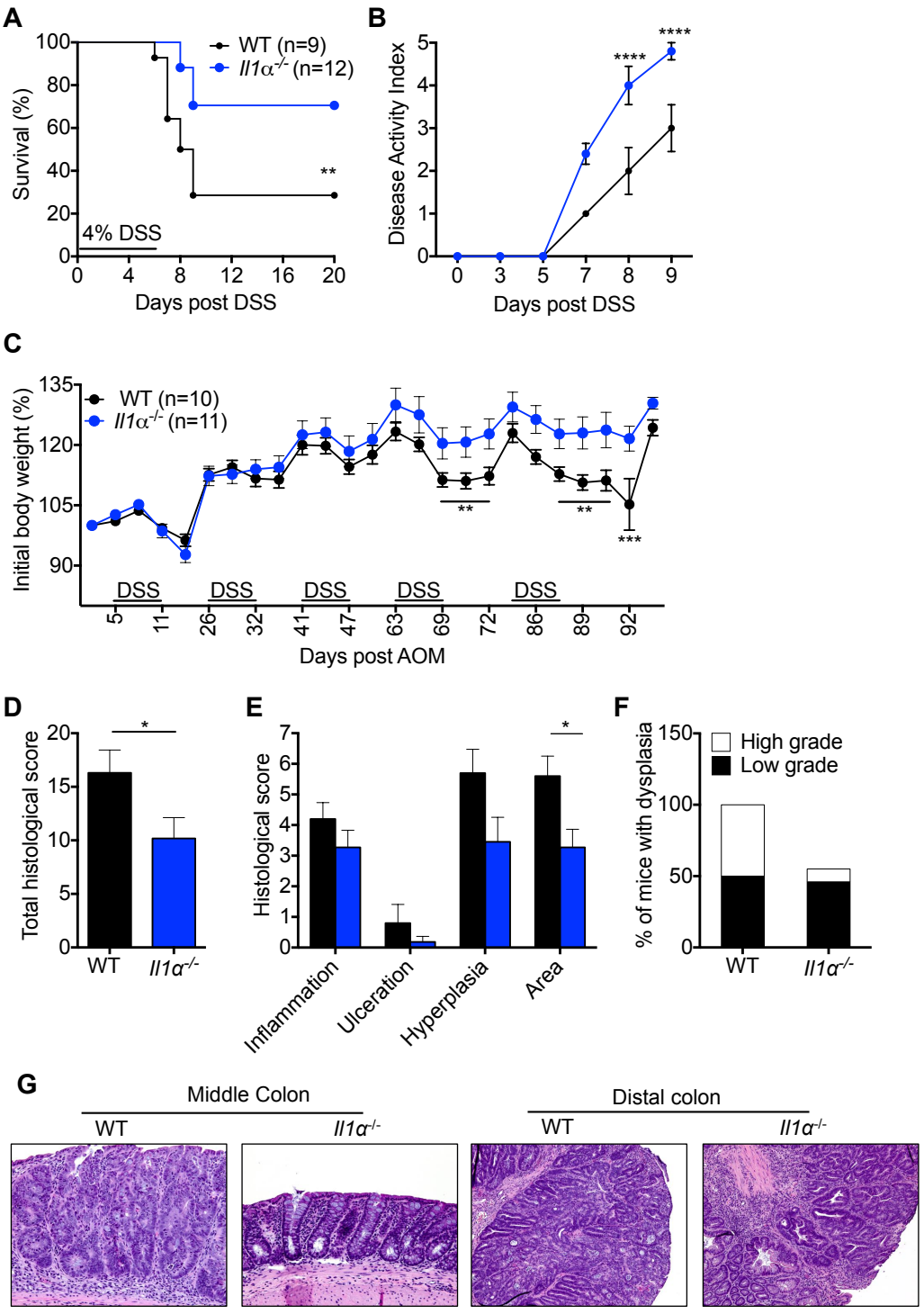


Supplementary figure 1. Modulation in cytokine production in DSS treated *Il33*$^{-/-}$ mice. Quantification of indicated cytokines in the colon explants of WT and *Il33*$^{-/-}$ mice at indicated days post DSS administration. Similar data was obtained with the sera (not shown). Data represent two independent experiments and analyzed by Mann-Whitney U test. Error bars represent mean±S.E.M with 10 mice per group per time point.



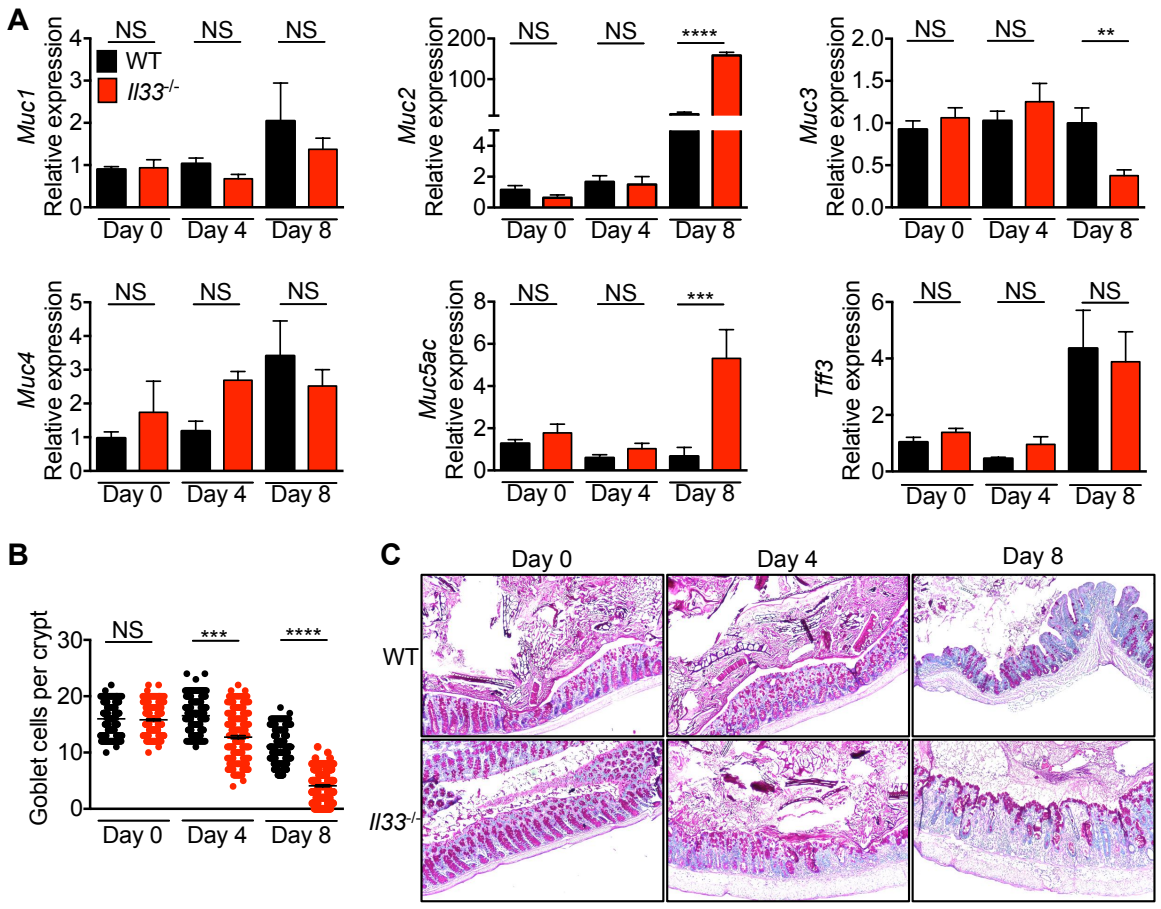
Supplementary figure 2. Levels of IL-33 and IL-1α are increased in the colon after DSS administration. WT and *Il33*$^{-/-}$ mice were treated with DSS in drinking water for 6 days, followed by drinking water for 2 days. **(A)** Western blot analysis for IL-33 in the colon lysates. **(B)** qRT-PCR for *Il33* expression in the colon tissue. **(C)** qRT-PCR analysis of *Il33* and *Il1α* expression from indicated cell populations from the epithelial (Ep) and colonic lamina propria (cLP) fractions of WT mice at day 8. **(D)** Western blot analysis for caspase-1 in colon lysates. **(E)** H&E staining at 40x original magnification of colon sections at day 4 post DSS. Data represent two

independent experiments and analyzed by Mann-Whitney U test. Error bars represent mean±S.E.M with 5 mice per group per time point.

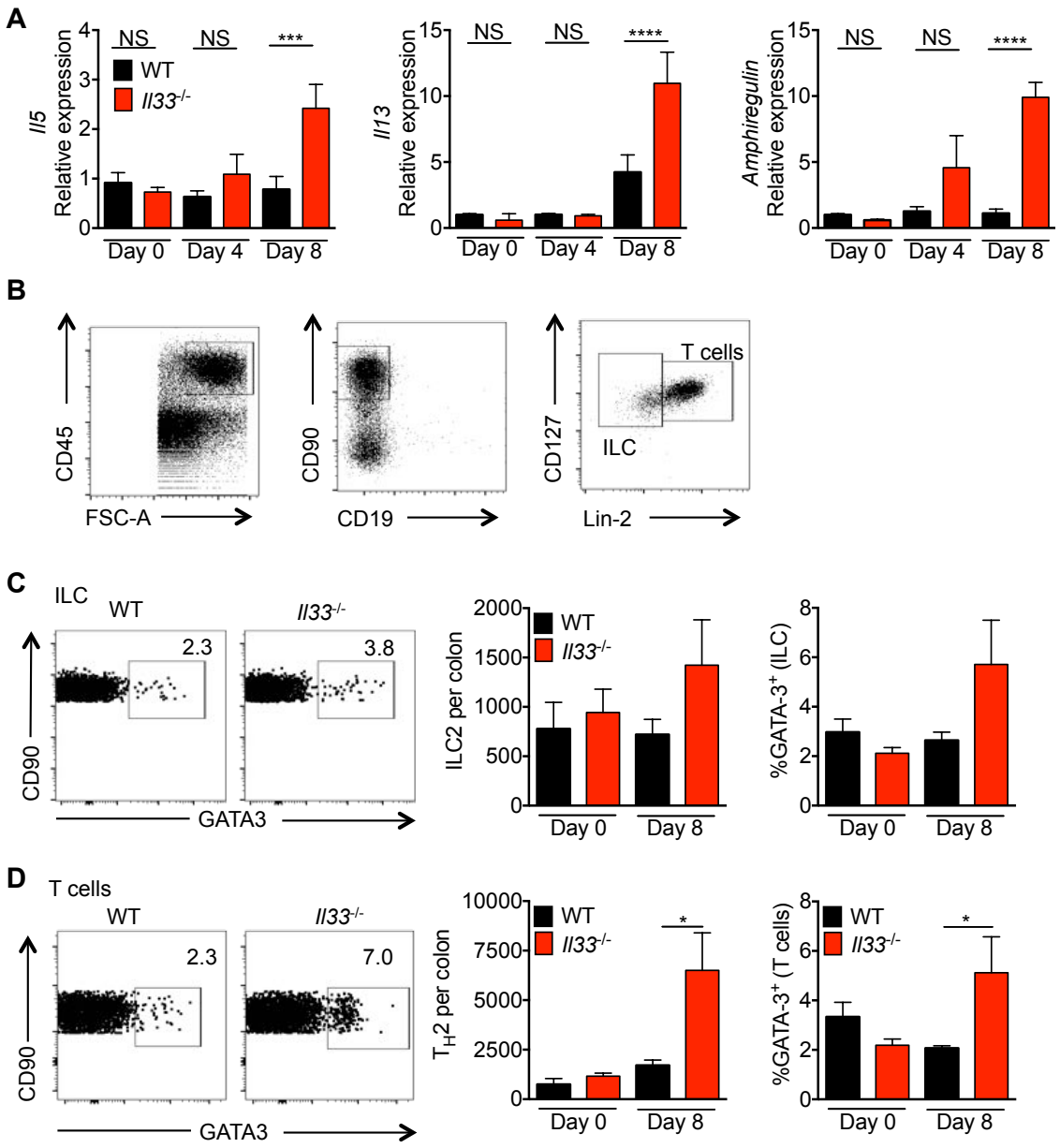


Supplementary figure 3. IL-1α promotes colitis and associated cancer. **(A)** Survival and **(B)** disease Activity Index of WT and *Il1α*$^{-/-}$ mice administered 4% DSS in drinking water. **(C)** Body weight change of WT and *Il1α*$^{-/-}$ mice injected with AOM on day 0 and administered 5 rounds of 3.5% DSS in drinking water. **(D)** and **(E)** Colon histology analysis and **(F)** proportion of mice with low- or high-grade epithelial dysplasia at day 108 post AOM injection. **(G)** H&E staining at 10x original magnification of the distal and middle colon sections at day 108 post AOM injection.

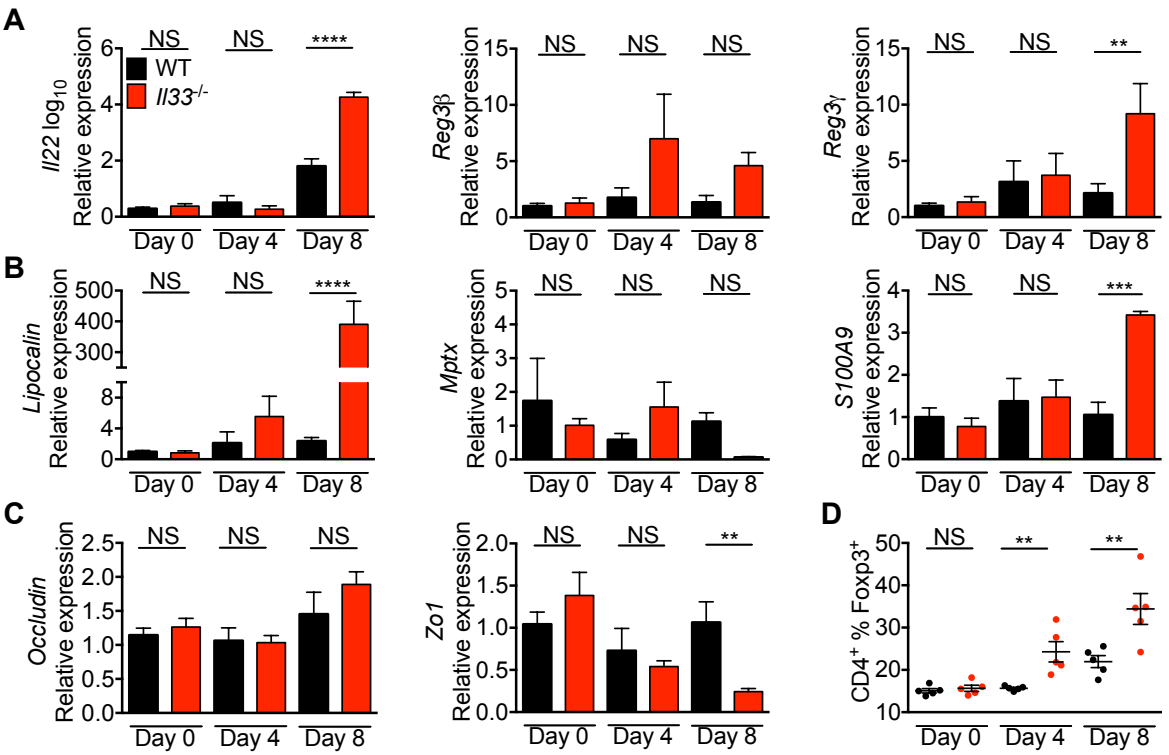
Data represent two independent experiments and analyzed by two-way ANOVA followed by Holm-Sidak post test **(B** and **C)** or Mann-Whitney U test **(D** and **E)**. Error bars represent mean±S.E.M.



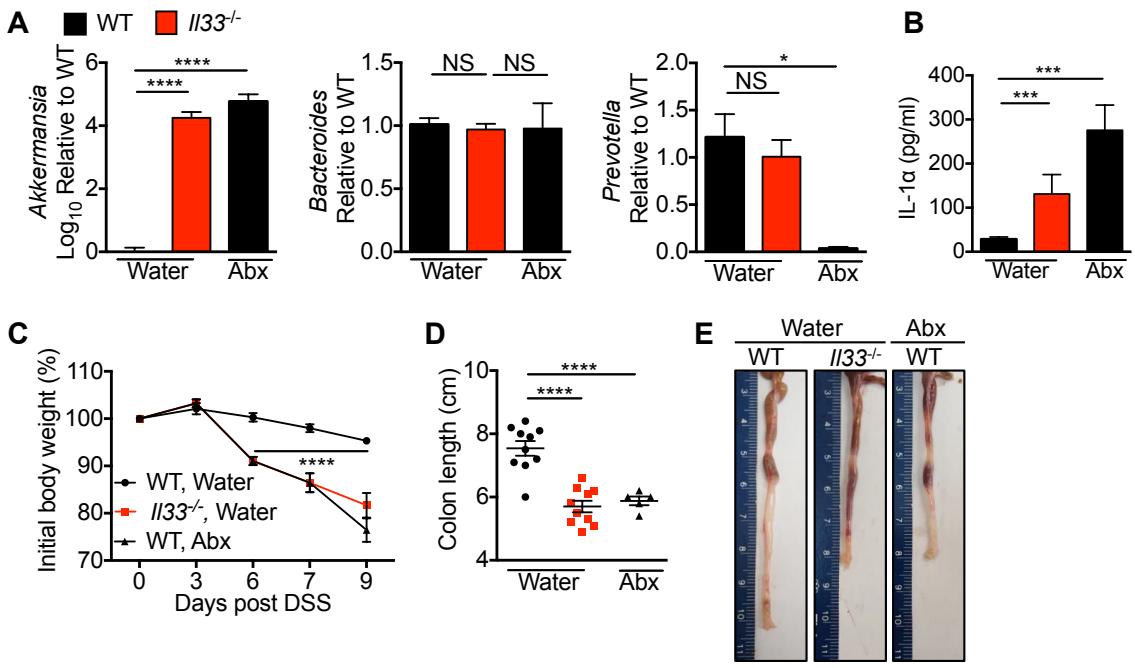
Supplementary figure 4. Modulation of goblet cells during DSS administration in *Il33*$^{-/-}$ mice. **(A)** Quantification of expression of mucins and goblet cell associated genes in the colons of WT and *Il33*$^{-/-}$ mice at indicated days post DSS administration by qRT-PCR. **(B)** Quantification of the number of goblet cells per crypt. **(C)** PAS staining at 10x original magnification of colon sections. Data was analyzed by Kruskal-Wallis test followed by Dunn's post test. Error bars represent mean±S.E.M. N= 8 each for **(A)**, N=5 each for **(B)** and **(C)**. Each dot represents an individual crypt.



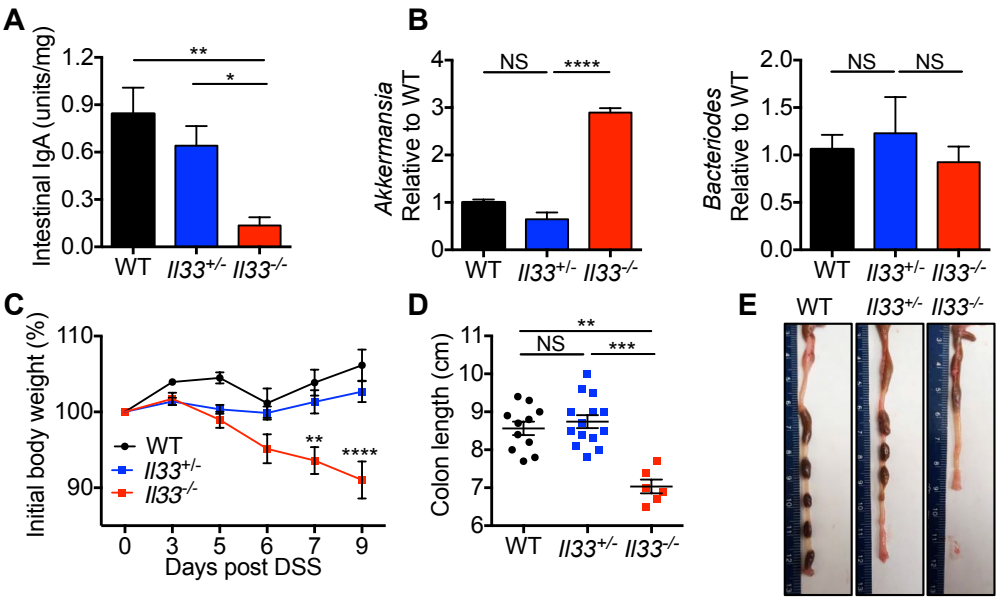
Supplementary figure 5. ILC2 analysis in the colons of WT and *Il33*$^{-/-}$ mice. **(A)** Quantification of expression of type2 cytokines in the colons of WT and *Il33*$^{-/-}$ mice at indicated days post DSS administration by qRT-PCR. **(B)** Gating strategy for ILC2 cells, where Lin^{-} represents CD3^{-}B220^{-}Ly-6C^{-}Ly-6G^{-}CD11b^{-}Ter119^{-}NKp46^{-}. Proportion and total number of **(C)** ILC2s and **(D)** T_H2 cells in the lamina propria. Data represent two independent experiments and analyzed by Kruskal-Wallis test followed by Dunn's post test. Error bars represent mean±S.E.M. with 10 mice per group per time point.



Supplementary figure 6. There is no defect in induction of anti-inflammatory and epithelium healing genes or T regs in the colons of *Il33*$^{-/-}$ mice. **(A-C)** Quantification of expression of indicated genes in the colons of WT and *Il33*$^{-/-}$ deficient mice at indicated days post DSS administration by qRT-PCR. **(D)** Quantification of T regulatory cells gated as CD19^{-} CD3^{+}CD4^{+}Foxp3^{+} cells in the lamina propria of the colon by flow cytometry. Data represent two independent experiments and analyzed by Kruskal-Wallis test followed by Dunn's post test. Error bars represent mean±S.E.M with 5 mice per group per time point.



Supplementary figure 7. Increasing *Akkermansia* level in WT mice increases colitis susceptibility. **(A)** Q-PCR analysis of indicated bacteria from stool samples of WT and *Il33*$^{-/-}$ mice 1-week administration of water or broad-spectrum antibiotic cocktail (125 mg/l ciprofloxacin, 1 g/l bacitracin, 2 g/l streptomycin, 1.5 g/l metronidazole and 172 mg/l gentamycin) in their drinking water. **(B)** IL-1α measurement in colon explants at day 4 post DSS administration **(C)** Body weight loss and **(D)** disease activity index of mice during DSS administration. **(E)** Colon length measurements and **(F)** representative colon images at day 8 post DSS administration. Data represent two independent experiments and analyzed by Kruskal-Wallis test **(A)** **(B)** and **(D)** or two-way ANOVA **(C)** followed by Dunn's post test. Error bars represent mean±S.E.M. and each dot represents an individual mouse. N=10 mice for WT and water, *Il33*$^{-/-}$ and water group, 5 for WT and antibiotics group.



Supplementary figure 8. *Il33*$^{-/-}$ littermates have decreased intestinal IgA, increased level of *Akkermansia* and susceptibility to colitis. (A) IgA measurement by ELISA and **(B)** qRT-PCR analysis of indicated bacteria from stool samples of WT, *Il33*$^{+/-}$ and *Il33*$^{-/-}$ mice 4 weeks after separation. **(C)** Body weight loss of mice during DSS administration. **(D)** Colon length measurements and **(E)** representative colon images at day 8 post DSS administration. Data is analyzed by Kruskal-Wallis test **(A) (B)** and **(D)** or two-way ANOVA **(C)** followed by Dunn's post test. Error bars represent mean±S.E.M. and each dot represents an individual mouse. N=11 mice for WT, 14 for *Il33*$^{+/-}$ and 6 for *Il33*$^{-/-}$ group.

Supplementary Methods

Preparation of single cell suspension from colon.

Single cell suspension was prepared from the colon as described previously (1). Briefly, for removal of epithelial cells, the colon was washed, cut into small pieces, and then the pieces were incubated with calcium- and magnesium-free HBSS supplemented with 5% FBS and 5 mM EDTA (Sigma-Aldrich) at 140 rpm at 25°C for 30 min. The tissues were then incubated with RPMI 1640 containing 10% FBS and 0.5 mg/ml collagenase type IV for 1 hour at 37°C with shaking at 150 rpm. The liberated cells were collected by passage through a 70 μm nylon mesh. The isolated cells from the EDTA (epithelial) and collagenase (lamina propria) treated fractions were separated on a 40/80% discontinuous Percoll gradient (GE Bioscience). The following monoclonal antibodies were used in appropriate combinations: anti-CD3 (clone 145 – 2C11), anti-CD4 (clone RM4-5), anti-CD19 (clone 1D3), anti-IgA (clone RMA-1), anti-Foxp3 (FJK-16s), anti-CD326 (clone G8.8), anti-CD16/CD32 (clone 93), anti-CD127 (clone A7R34), anti-GATA-3 (clone L50-823), anti-NKp46 (clone 29A1.4), anti-CD45.2 (clone 104), anti-CD90.2 (clone 30-H12), anti-MHCII (clone M5/114.152) and anti-mouse Lin cocktail (BioLegend, Catalog #133306). For intracellular cytokine staining, cells were fixed and permeabilized using fixation and permeabilization solution (eBioscience, Catalog # 00-5523).For intracellular cytokine staining, cells were fixed and permeabilized using fixation and permeabilization solution (eBioscience). Intracellular staining for the Foxp3 and GATA-3 transcription factors were performed using the eBioscience Foxp3 staining set according to the manufacturer's recommendations. Flow cytometry data were acquired on LSRII (BD) and were analyzed with FlowJo software (TreeStar).

Western blotting

Proteins were extracted from colon tissues using RIPA lysis buffer supplemented proteinase and phosphatase inhibitors (Roche). Samples were resolved in 12-15% SDS-PAGE and transferred onto PVDF membranes. Blocking was performed in 5% milk for 1 hout and membranes were incubated in primary antibodies overnight at 4°C. Membranes were incubated with HRP-conjugated secondary antibody for 1 hour and proteins were visualized using ECL substrate (ThermoScientific). The primary antibodies were Caspase-1 p10 (1:500 dilution, sc-515, Santa Cruz biotechnology) GAPDH (1:10,00 dilution, clone D16H11, Cell Signaling) and IL-33 (1:1000 dilution, Catalog # AF3626 R&D systems).

Supplementary Table 1. Real time qPCR primer sequences

Gene	Primer Sequences
Gapdh	Forward: 5'-CGTCCCGTAGACAAAATGGT-3'
	Reverse: 5'-TTGATGGCAACAATC TCC AC-3'
β-actin	Forward: 5'-GGCTGTATTCCC CTCCATCG-3'
	Reverse: 5'-CCAGTTGGTAACAATGCCATG T-3'
Il1α	Forward: 5'-AAAATCTCAGATTCACAACTGTTCGT-3'
	Reverse: 5'-TGGCAACTCCTTCAGCAACAC-3'
Il5	Forward: 5'-GCAATGAGACGATGAGGCTT-3'
	Reverse: 5'-CCCACGGACAGTTTGATTCT-3'
Il13	Forward: 5'-TGTGTCTCTCCCTCTGACCC-3'
	Reverse: 5'-CACACTCCATACCATGCTGC-3'
Muc1	Forward: 5'-GCAGTCCTCAGTGGCACCTC-3'
	Reverse: 5'-CACCGTGGGCTACTGGAGAG-3'
Muc2	Forward: 5'-GCTGACGAGTGGTTGGTGAATG-3'
	Reverse: 5'-GATGAGGTGGCAGACAGGAGAC-3'
Muc3	Forward: 5'-CGTGGTCAACTGCGAGAATGG-3'
	Reverse: 5'-CGGCTCTATCTCTACGCTCTCC-3'
Muc4	Forward: 5'-CAGCAGCCAGTGGGGACAG-3'
	Reverse: 5'-CTCAGACACAGCCAGGGAACTC-3'
Il22	Forward: 5'-AGAACGTCTTCCAGGGTGAA-3'
	Reverse: 5'-CAT CGA CAT AAG TCA GCA CCA G-3'
Reg3β	Forward: 5'-ATGGCTCCTACTGCTATGCC-3'
	Reverse: 5'-GTGTCCTCCAGGCCTCTTT-3'
Reg3γ	Forward: 5'-ATGGCTCCTATTGCTATGC-3'
	Reverse: 5'-GATGTCCTGAGGGCCTCTT-3'
Lcn2	Forward: 5'-ACATTTGTTCCAAGCTCCAGGGC-3'
	Reverse: 5'-CATGGCGAACTGGTTGTAGTCCG-3'
Mptx	Forward: 5'-CCTGTTTCTCTCTGTTCTTTCAGG-3'
	Reverse: 5'-GGCCTTCATACACAGAGTGAAG-3'

Areg	Forward: 5'-GCCATTATGCAGCTGCTTTGGAGC-3'
	Reverse: 5'-TGTTTTTCTTGGGCTTAATCACCT-3'
S100A9	Forward: 5'-GGTGGAAGCACAGTTGGCA-3'
	Reverse: 5'-GTGTCCAGGTCCTCCATGATG-3'
Occludin	Forward: 5'-TTGAAAGTCCACCTCCTTACAGA -3'
	Reverse: 5'-CCGGATAAAAAGAGTACGCTGG-3'
Zo1	Forward: 5'-GCCGCTAAGAGCACAGCAA-3'
	Reverse: 5'-GCCCTCCTTTTAACACATCAGA -3'
Tff3	Forward: 5'-CCTGGTTGCTGGGTCCTCTG-3'
	Reverse: 5'-GCCACGGTTGTTACACTGCTC-3'
pIgR	Forward: 5'-AAGAACTGACCAAAGGGAGGA-3'
	Reverse: 5'-AGAGTAACTTCAATTCTGCACCC-3'
Muc5ac	Forward: 5'-CTGTGACATTATCCCATAAGCCC-3'
	Reverse: 5'-AAGGGGTATAGCTGGCCTGA-3'
Eubacteria	Forward: 5'-ACTCCTACGGGAGGCAGCAGT-3'
	Reverse: 5'-ATTACCGCGGCTGCTGGC-3'
Bacteroides	Forward: 5'-GGTTCTGAGAGGAGGTCCC-3'
	Reverse: 5'-CTGCCTCCCGTAGGAGT-3'
Enterobacteriaceae	Forward: 5′-GTGCCAGCMG CCGCGGTAA-3′
	Reverse: 5′-GCCTCAAGGG CACAACCTCC AAG-3′
γ-Proteobacteria	Forward: 5'-TAACGCTTGG GAATCTGCCT RTT-3'
	Reverse: 5′-CATCTRTTAG CGCCAGGCCT TGC-3′
Fungal 18s	Forward: 5'- ATTGGAGGGCAAGTCTGGTG-3'
	Reverse: 5′-CCGATCCCTAGTCGGCATAG-3′
Prevotellacae	Forward: 5'- ATTGGAGGGCAAGTCTGGTG-3'
	Reverse: 5′-CCGATCCCTAGTCGGCATAG-3′
Akkermansia	Forward: 5'-CAGCACGTGAAGGTGGGGAC-3'
	Reverse: 5′-CCTTGCGGTTGGCTTCAGAT-3′
Anaerostipes	Forward: 5'-AAGTCGAACGAAGCACCTTG-3'
	Reverse: 5′-TCCGCCACTCAGTCACAATG-3′
Dorea	Forward: 5'-ACGGTACCTGACTAAGAAGCCC-3'
	Reverse: 5′-CCTCAACGTCAGTCATCGTCC-3′
E. rectale	Forward: 5'-ACTCCTACGGGAGGCAGC-3'
	Reverse: 5′-GCTTCTTAGTCAGGTACCGTCA-3′
Flexispira	Forward: 5'-AATACATGCAAGTCGAACGATGA-3'
	Reverse: 5′-AATCACCGTTTCCAGTGGCT-3′
Clostridia	Forward: 5'-CTCAACTTGGGTGCTGCATTT-3'
	Reverse: 5′-ATTGTAGTACGTGTGTAGCCC-3′
E. coli	Forward: 5'-CATGCCGCGTGTATGAAGAA-3'
	Reverse: 5′-CGGGTAACGTCAATGAGCAAA-3′
Prevotella	Forward: 5'-CACGGTAAACGATGGATGCC-3'
	Reverse: 5′-GGTCGGGTTGCAGACC-3′
Lactobacillus	Forward: 5'-GGAAACAGATGCTAATACCG-3'
	Reverse: 5′-CACCGCTACACATGGAG-3′
MIB	Forward: 5'-CCAGCAGCCGCGGTAATA-3'
	Reverse: 5′-CGCATTCCGCATACTTCTC-3′
SFB	Forward: 5'-GACGCTGAGGCATGAGAGCA-3'
	Reverse: 5′-GACGGCACGGATTGTTATTC-3′
Tm7	Forward: 5'- GCAACTCTTTACGCCCAGT-3'
	Reverse: 5′- GAGAGGATGATCAGCCAG-3′

1. Malik A, Sharma D, St Charles J, Dybas L, and Mansfield L. Contrasting immune responses mediate Campylobacter jejuni-induced colitis and autoimmunity. *Mucosal immunology.* 2013.