Figure S1

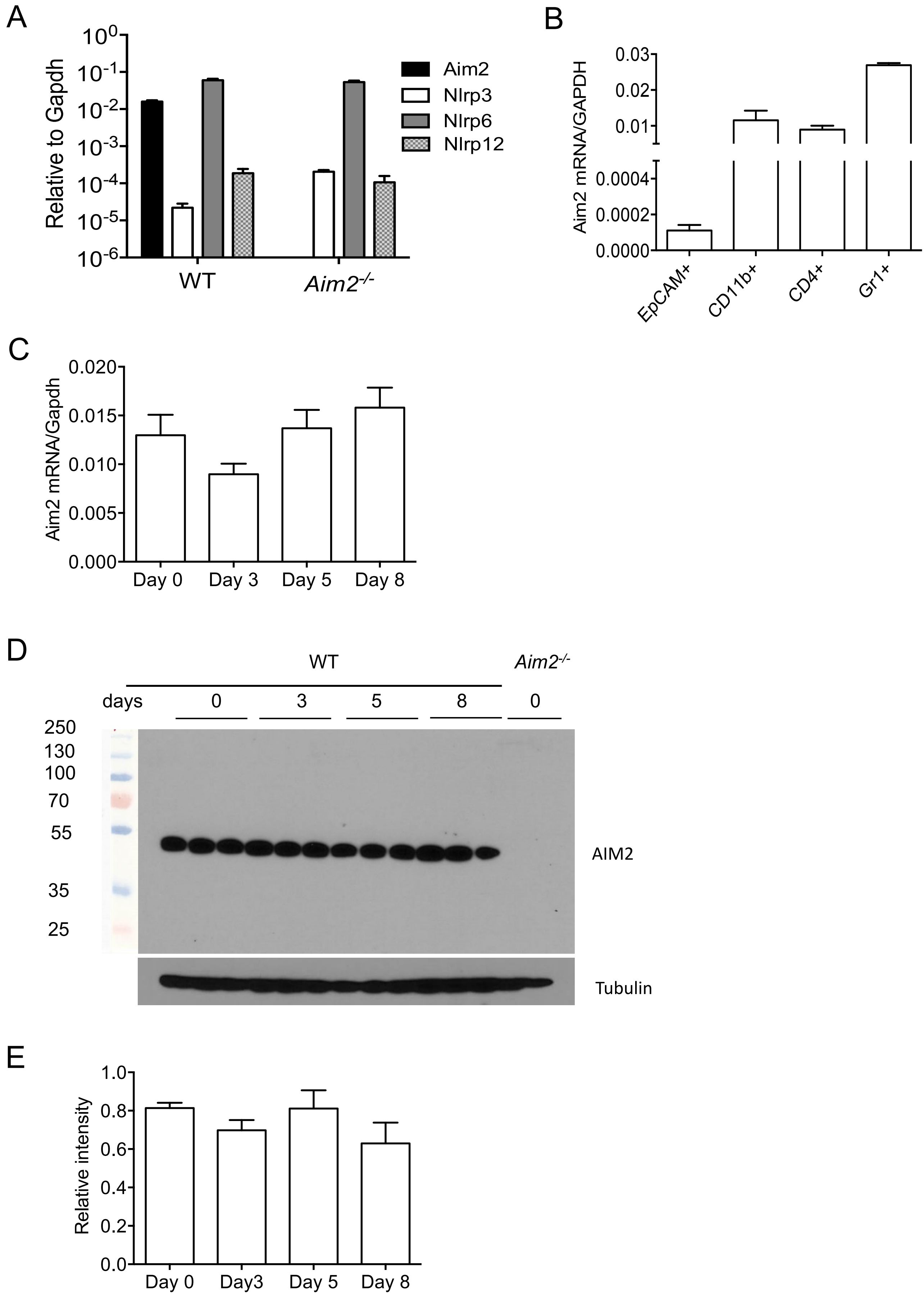
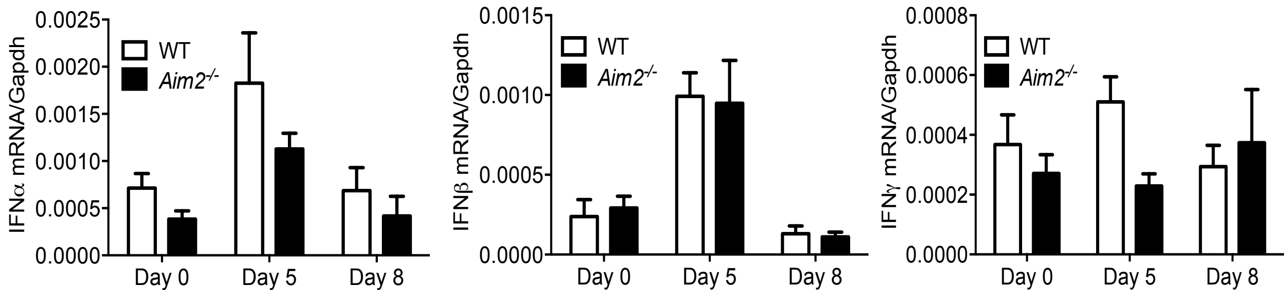


Figure S2

A



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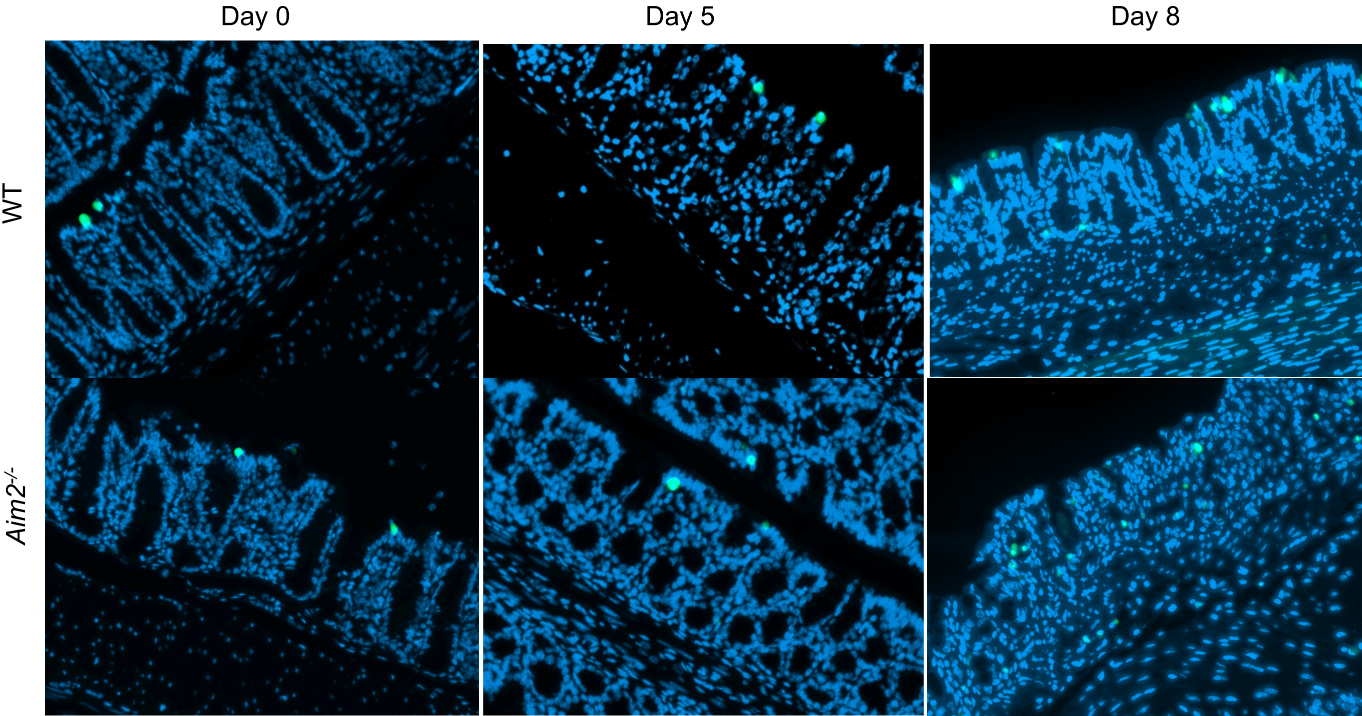


C

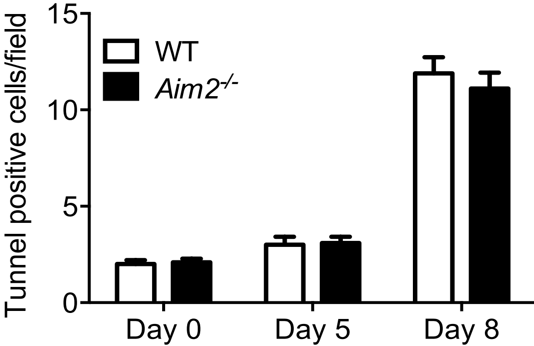


Figure S3

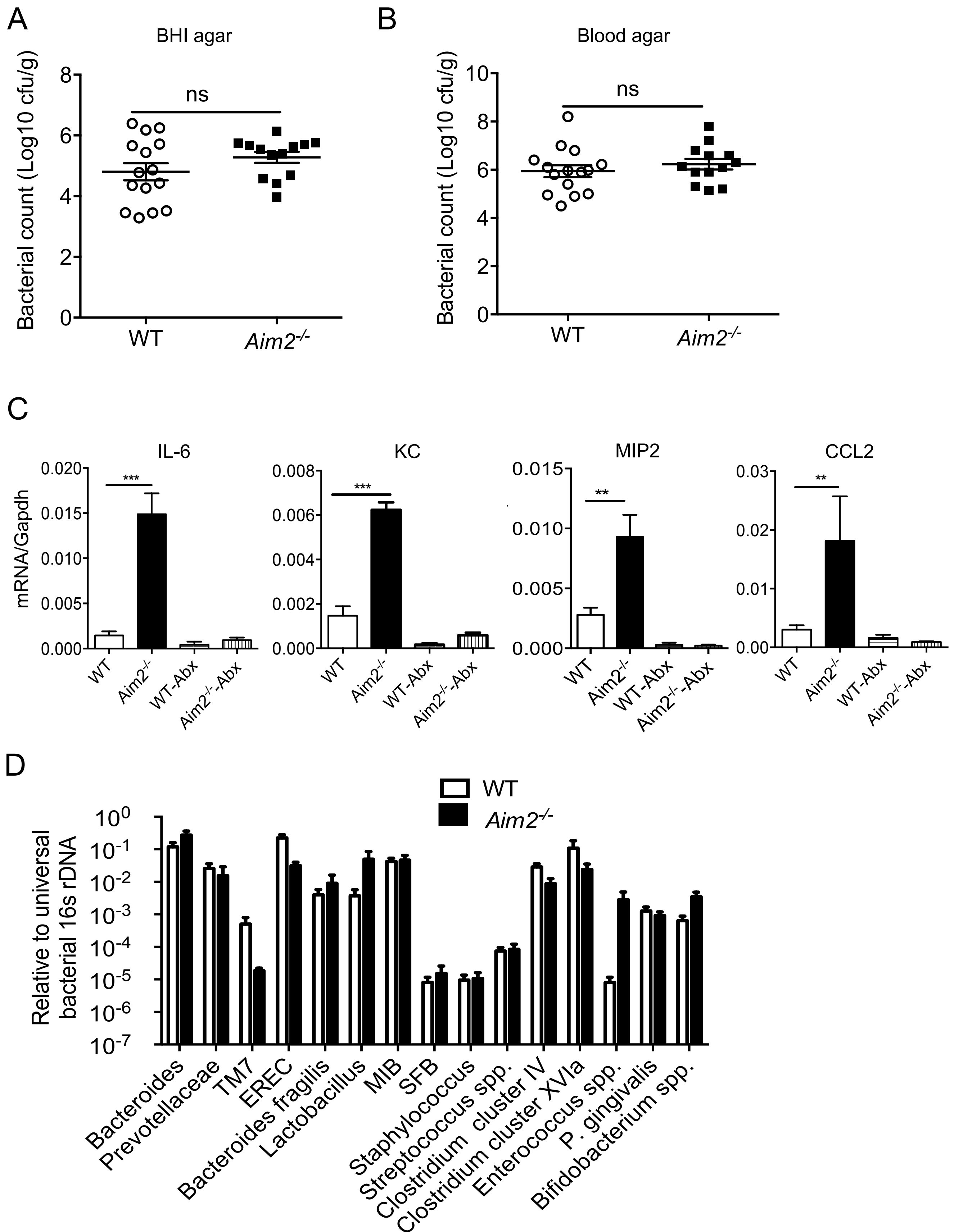


Figure S4

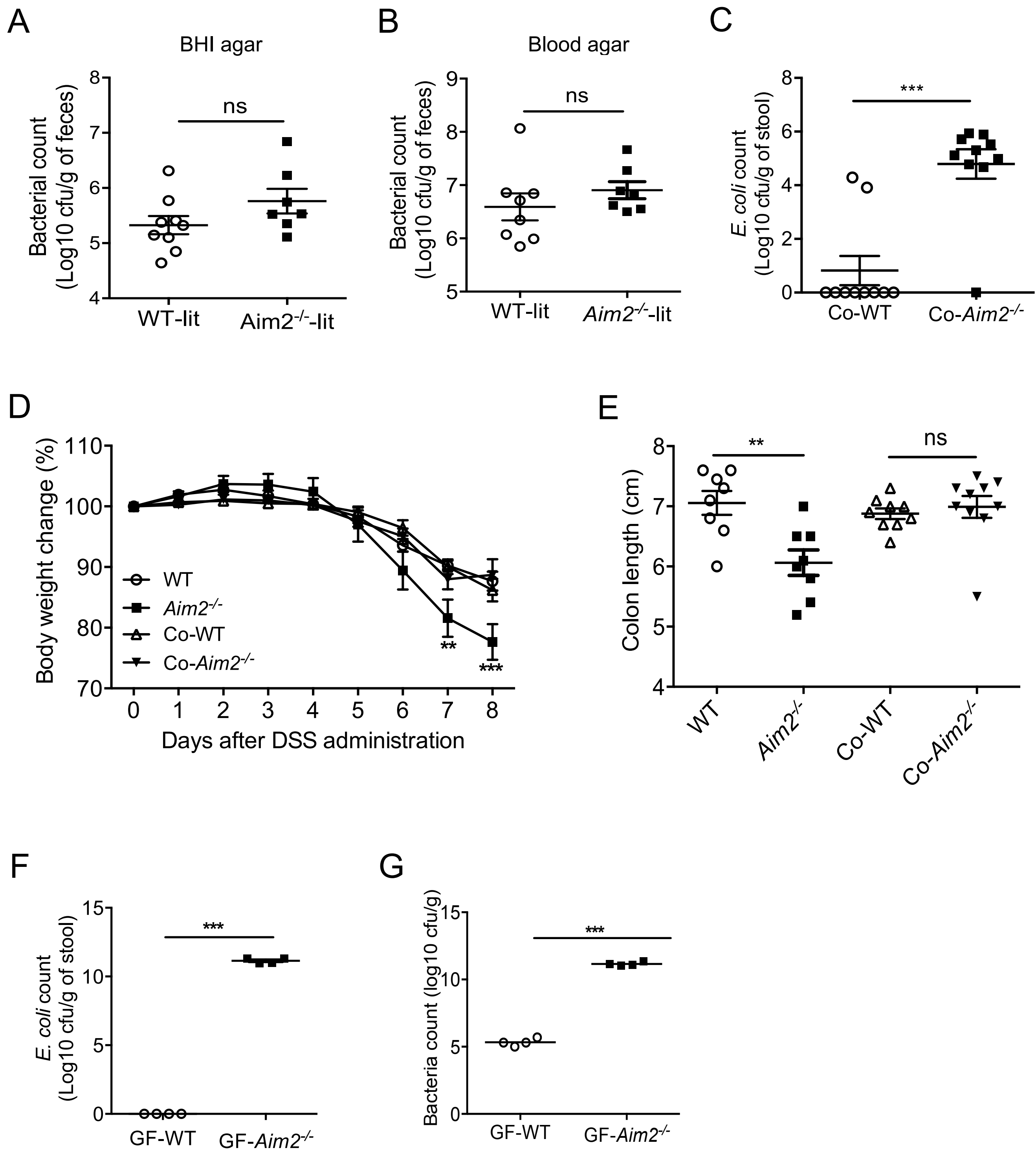
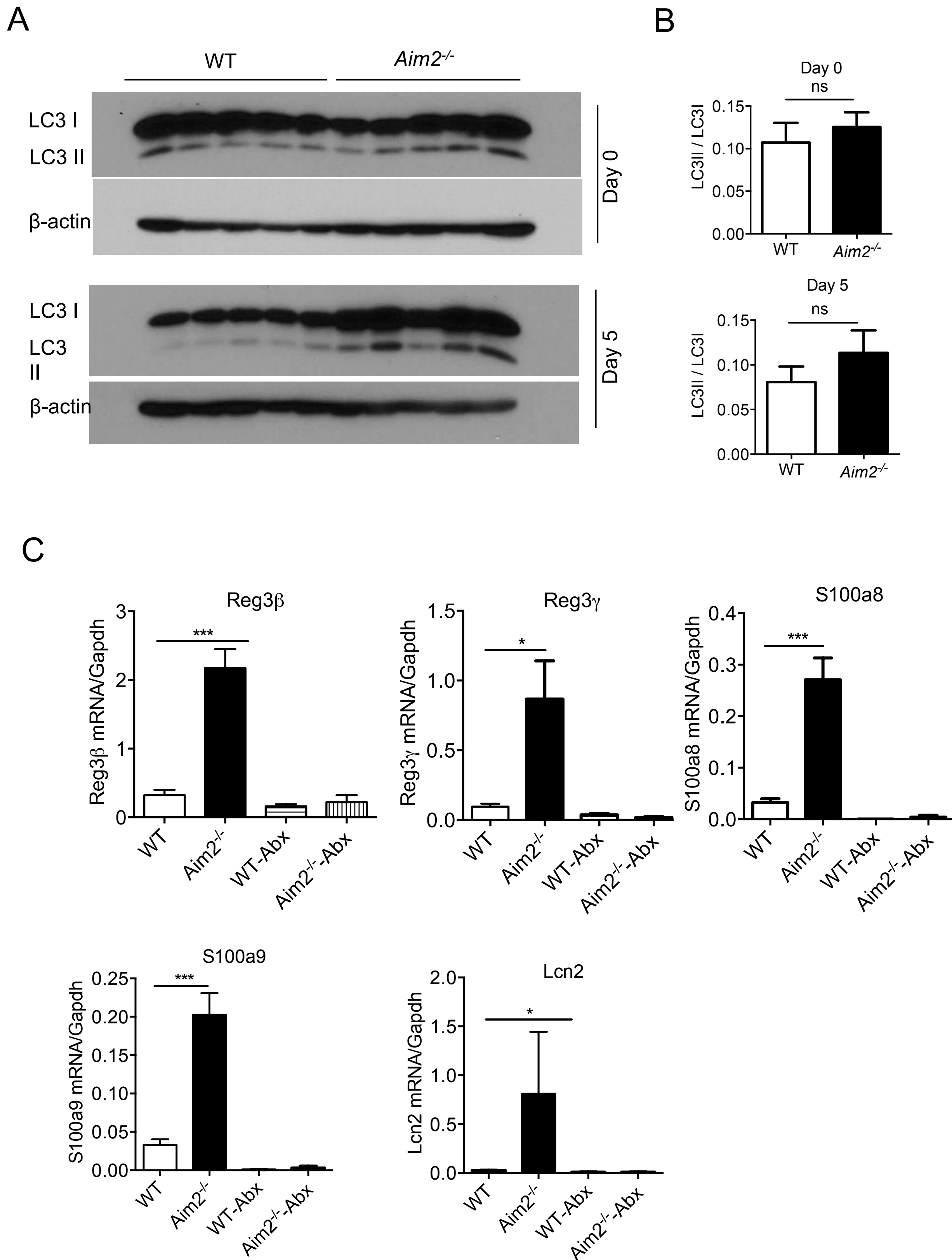


Figure S5



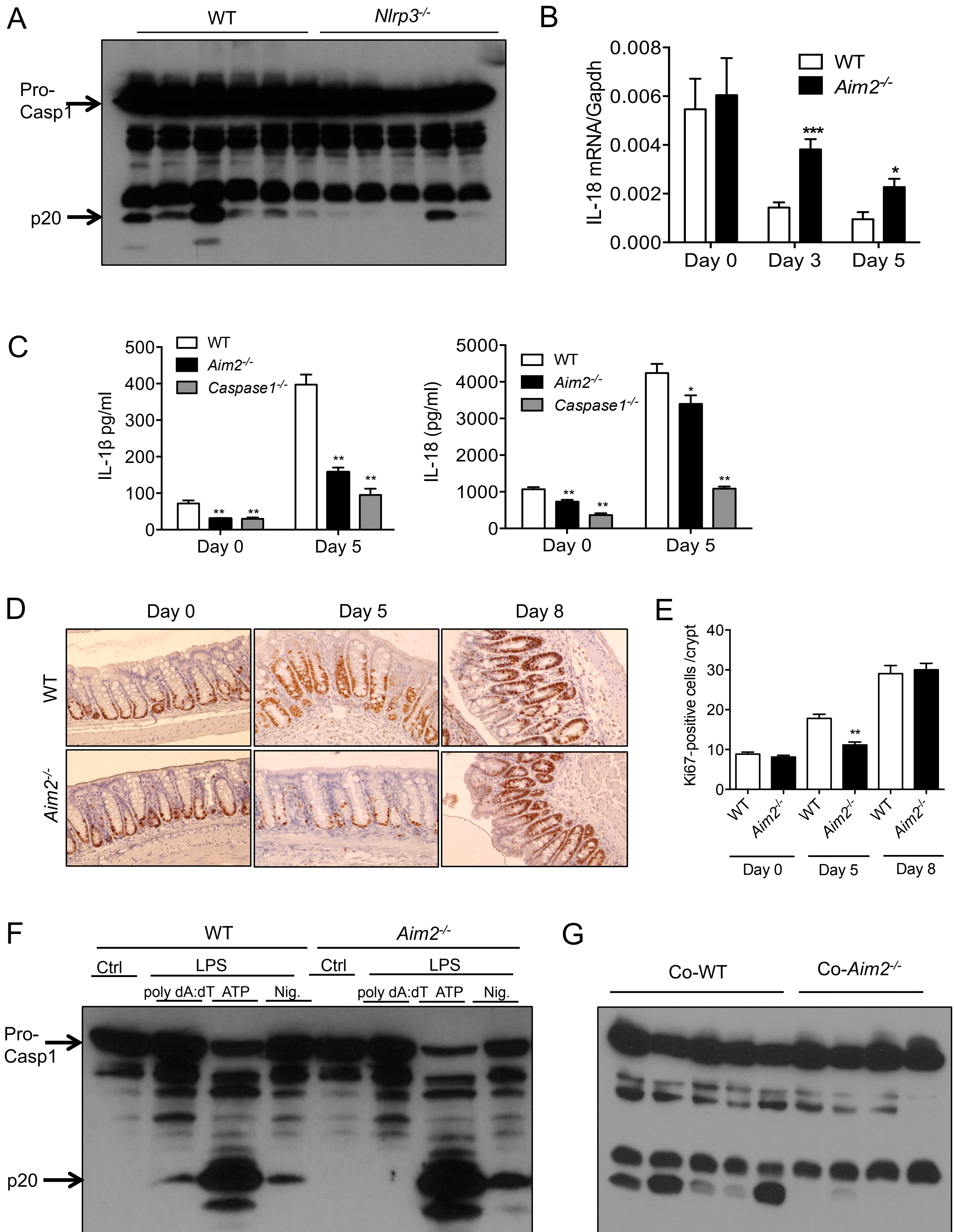
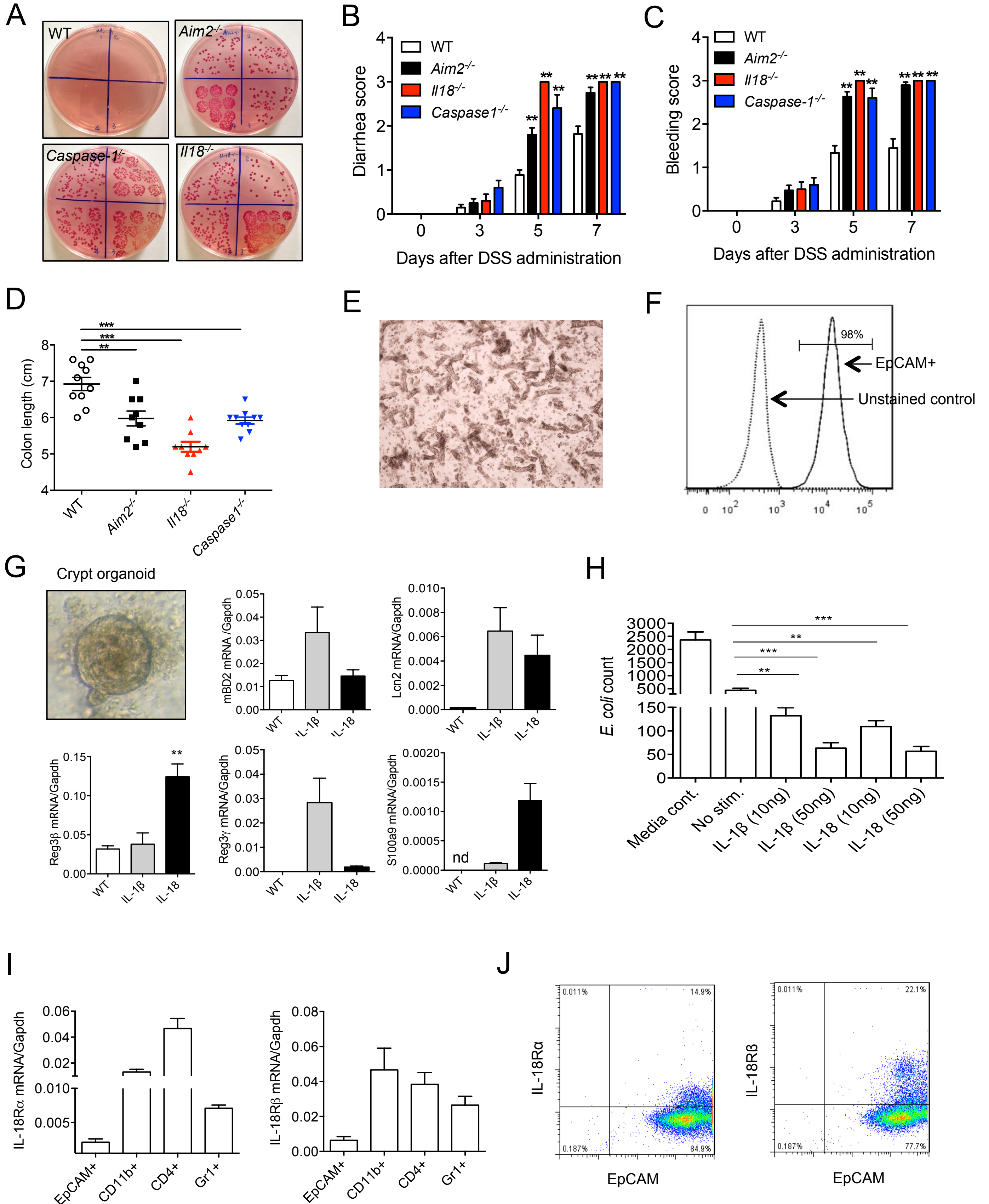


Figure S6
A
WT
Nlrp3-/-
Pro-Casp1
p20
B
IL-18 mRNA/Gapdh
0.008
0.006
0.004
0.002
0.000
WT
Aim2-/-
***
*
Day 0
Day 3
Day 5
C
IL-1β pg/ml
500
400
300
200
100
0
WT
Aim2-/-
Caspase1-/-
**
Day 0
Day 5
IL-18 (pg/ml)
5000
4000
3000
2000
1000
0
WT
Aim2-/-
Caspase1-/-
*
**
Day 0
Day 5
D
Day 0
Day 5
Day 8
WT
Aim2-/-
E
Ki67-positive cells /crypt
40
30
20
10
0
**
WT
Aim2-/-
Day 0
Day 5
Day 8
F
WT
Aim2-/-
Ctrl
LPS
poly dA:dT
ATP
Nig.
Pro-Casp1
p20
G
Co-WT
Co-Aim2-/-

Figure S7



Supplemental Table 1

Genes	Primer sequences
IL-6_F	CAAGAAAGACAAAGCCAGAGTC
IL-6_R	GAAATTGGGGTAGGAAGGAC
KC_F	TGAGCTGCGCTGTCAGTGCCT
KC_R	AGAAGCCAGCGTTCACCAGA
MIP2_F	CAAGAACATCCAGAGCTTGAGTGT
MIP2_R	GCCCTTGAGAGTGGCTATGACTT
CXCL10_F	TCCGGAAGCCTCCCCATCAGCACC
CXCL10_R	TGCAGCGGACCGTCCTTGCGA
CCL2_F	TTGCCGGCTGGAGCATCCACGT
CCL2_R	AGTAGCAGCAGGTGAGTGGGGCG
TNFα_F	TCCCAGGTTCTCTTCAAGGGA
TNFα_R	GGTGAGGAGCACGTAGTCGG
IL-1α_F	GGAGAAGACCAGCCCGTGTTGCT
IL-1α_R	CCGTGCCAGGTGCACCCGACTT
IL-1β_F	GCCTCGTGCTGTCGGACCCATA
IL-1β_R	TGCAGGGTGGGTGTGCCGTCTT
GAPDH_F	TGGCAAAGTGGAGATTGTTGCC
GAPDH_R	AAGATGGTGATGGGCTTCCCG
β-defensin(mBD)1_F	TGGAGCGGAGACAGAATCCT
β-defensin(mBD)1_R	AGTCTTGGACGAAGAACAGATCAA
mBD2_F	TGACCACTGCCACACCAATG
mBD2_R	CCTGGCAGAAGGAGGACAAA
mBD3_F	TCGGTGCATTGGCAACACT
mBD3_R	TGCAGCATTTGAGGAAAGGA
Muc1_F	GCAGTCCTCAGTGGCACCTC
Muc1_R	CACCGTGGGCTACTGGAGAG
Muc2_F	GCTGACGAGTGGTTGGTGAATG
Muc2_R	GATGAGGTGGCAGACAGGAGAC
Muc3_F	CGTGGTCAACTGCGAGAATGG
Muc3_R	CGGCTCTATCTCTACGCTCTCC
Muc4_F	CAGCAGCCAGTGGGGACAG
Muc4_R	CTCAGACACAGCCAGGGAACTC
Reg3β_F	ATGGCTCCTACTGCTATGCC
Reg3β_R	GTGTCCTCCAGGCCTCTT
Reg3γ_F	CAAGGTGAAGTTGCCAAGAA
Reg3γ_R	CCTCTGTTGGGTTCATAGCC
S100a8_F	TGTCCTCAGTTTGTGCAGAATATAAA
S100a8_R	TCACCATCGCAAGGAACTCC
S100a9_F	GGTGGAAGCACAGTTGGCA
S100a9_R	GTGTCCAGGTCCTCCATGATG
Lcn2_F	ACATTTGTTCCAAGGTCCAGGGC
Lcn2_R	CATGGCGAATGGTTGTAGTCCG
IFNα_F	TCTGATGCAGCAGGTGGG
IFNα_R	AGGGCTCTCCAGACTTCTGCTCTG
IFNβ_F	GCCTGGATGGTGGTCCGAGCA
IFNβ_R	TACCAGTCCCAGAGTCCGCCTCT
IFNγ_F	GAAAGACAATCAGGCCATCA
IFNγ_R	TTGCTGTTGCTGAAGAAGGT
IL-18_F	GCCTCAAACCTTCCAAATCA
IL-18_R	TGGATCCATTTCCTCAAAGG
Aim2_F	GATTCAAAGTGCAGGTGCGG
Aim2_R	TCTGAGGGTTAGCTTGAGGAC
Nlrp3_F	TGCAGAAGACTGACGTCTCC
Nlrp3_R	CGTACAGGCAGTAGAACAGTTC
Nlrp6_F	AGCCAGTGCCAGCTGTAGAA
Nlrp6_R	CGAGCATTCCTCTCCTTCAC
Nlrp12_F	GTGAACCTGAGCAATCGTTA
Nlrp12_R	GGCTCAAAGAGGGTCTCC
IL-18R_F	CTCGCCCAGAGTCACTTTTCA
IL-18R_R	AGAAATGTACGTTCCCTCATCCT

Supplemental Table 2

Bacteria	Primer sequences	Reference
Bacteroides F	GGTTCTGAGAGGAGGTCCC	(Matsumoto et al., 2011)
Bacteroides R	CTGCCTCCCGTAGGAGT	
Prevotellaceae F	CCAGCCAAGTAGCGTGCA	(Elinav et al., 2011)
Prevotellaceae R	TGGACCTTCCGTATTACC	
TM7 F	GCAACTCTTTACGCCCAGT	(Elinav et al., 2011)
TM7 R	GAGAGGATGATCAGCCAG	
Eubacterium rectale (EREC) F	ACTCCTACGGGAGGCAGC	(Vaishnava et al., 2011)
Eubacterium rectale (EREC) R	GCTTCTTAGTCAGGTACCGTCA	
Bacteroides fragilis F	ATAGCCTTTCGAAAGRAAGAT	(Matsumoto et al., 2011)
Bacteroides fragilis R	CCAGTATCAACTGCAATTTTA	
Lactobacillus F	AGCAGTAGGGAATCTTCCA	(Vaishnava et al., 2011)
Lactobacillus R	CACCGCTACACATGGAG	
MIB F	CCAGCAGCCGCGGTAATA	(Vaishnava et al., 2011)
MIB R	CGCATTCCGCATACTTCTC	
SFB F	AGGAGGAGTCTGCGGCACATTAGC	(Suzuki et al., 2004)
SFB R	CGCATCCTTTACGCCCAGTTATTC	
Staphylococcus spp. F	TTTGGGCTACACACGTGCTACAATGGACAA	(Matsuda et al., 2009)
Staphylococcus spp. R	AACAACTTTATGGGATTTGCWTGA	
Streptococcus spp. F	AGATGGACCTGCGTTGT	(Dalwai et al., 2007)
Streptococcus spp. R	GCTGCCTCCCGTAGGAGTCT	
Clostridium cluster IV F	GCACAAGCAGTGGAGT	(Matsumoto et al., 2011)
Clostridium cluster IV R	CTTCCTCCGTTTTGTCAA	
Clostridium subcluster XVIa F	AAATGACGGTACCTGACTAA	(Matsumoto et al., 2011)
Clostridium subcluster XVIa R	CTTTGAGTTTCATTCTTGCGAA	
Enterococcus spp. F	CCCTTATTGTTAGTTGCCATCATT	(Matsumoto et al., 2011)
Enterococcus spp. R	ACTCGTTGTACTTCCCATTGT	
P. gingivalis F	CTTGACTTCAGTGGCGGCAG	(Dalwai et al., 2007)
P. gingivalis R	AGGGAAGACGGTTTTCACCA	
Bifidobacterium spp. F	ATTCTGGCTCAGGATGAACGC	(Gueimonde et al., 2004)
Bifidobacterium spp. R	CTGATAGGACGCGACCCCAT	
E. coli F	CATGCCGCGTGTATGAAGAA	(Molloy et al., 2013)
E. coli R	CGGGTAACGTCAATGAGCAAA	
Enterobacteriaceae F	TGCCGTAACTTCGGGAGAAGGCA	(Matsumoto et al., 2011)
Enterobacteriaceae R	TCAAGGCTCAATGTTCAGTGTC	
Eubacteria (Universal) F	ACTCCTACGGGAGGCAGCAGT	(Vaishnava et al., 2011)
Eubacteria (Universal) R	ATTACCGCGGCTGCTGGC	

SUPPLEMENTAL FIGURE LEGENDS

Figure S1 (related to Figure 1). Expression of Aim2 in the colon.

(A) The mRNA expression of *Aim2, Nlrp3, Nlrp6* and *Nlrp12* in the colons of healthy adult WT and *Aim2*$^{-/-}$ mice was detected by real-time PCR. Data represent mean ± SEM. *, p<0.05, ***, p<0.001. (B) Epithelial and lamina propria cells were isolated from WT mice at day 5 post DSS. Cells were immunostained with antibodies for CD11b, CD4, Gr1 and EpCAM, and sorted by flow cytometry. RNA was isolated from each cell population and expression of Aim2 was detected by real-time PCR. (C) RNA isolated from WT mouse colons at different days following colitis induction were analyzed for AIM2 expression. (D) Western blot analysis of AIM2 in colon homogenates collected at different days after DSS administration. (E) Densitometric analysis of AIM2 immunoblot relative to tubulin as shown in D.

Figure S2 (related to Figure 2). AIM2 is not involved in interferon signaling and cell death.

WT and *Aim2*$^{-/-}$ mice were fed with 3% DSS for 5 days, followed by regular drinking water for 3 days. (A) Mice were sacrificed on day 0, 5 and 8 and colon tissues were collected for RNA isolation. The expression of IFNα, IFNβ, and IFNγ in the mouse colons was detected by real-time PCR. Data represent mean ± SEM; n=5-7 mice/group. (B) Formalin fixed colon sections collected at day 8 after DSS were analyzed for cell death using *in situ* TUNEL assay. Samples were counterstained with DAPI and evaluated under fluorescence microscope. Representative pictures at 20x magnification are shown here. (C) TUNEL-positive cells were counted under microscope. Data represent mean ± SEM (n=20 field/section; 3mice/group).

Figure S3 (related to Figure 3). Increased bacterial growth in *Aim2*$^{-/-}$ mice during colitis induces higher cytokines and chemokines.

(A-B) Stools from healthy WT and *Aim2*$^{-/-}$ mice were homogenized in sterile PBS and homogenates were serially diluted and plated on BHI agar plates (A) or Blood agar plates (B). After incubation at 37°C for 24 hours, colonies were counted. Data are shown as cfu per g of fecal material and represented as the mean ± SEM; ns = not significant. (C) WT and *Aim2*$^{-/-}$ mice were fed with a combination of broad-spectrum antibiotics or left un-treated for 4 weeks. Mice were fed with 3 % DSS for 5 days and colons were collected at day 8 after DSS administration. RNA was isolated from colon tissues and quantitative mRNA expression of inflammatory cytokines IL-6, KC, MIP2 and CCL2 were measured by real-time PCR. Data represent mean ± SEM (n=5/group); **, p<0.01, ***, p<0.001. (D) DNA was isolated from feces of WT and *Aim2*$^{-/-}$ mice at day 8 after DSS administration. Bacterial 16S rDNA gene sequences were quantitatively measured by real-time PCR. Data represent mean ± SEM (n=8-10 mice/group).

Figure S4 (related to Figure 4). Altered microbiota in *Aim2*$^{-/-}$ mice promotes colitis susceptibility.

Littermate WT and *Aim2*$^{-/-}$ mice were treated with 3% DSS for 5 days, followed by regular drinking water for 3 days. Feces collected from littermate mice were serially diluted and plated on BHI and Blood agar plates and incubated at 37°C for 24 hours. Colonies were counted and represented as cfu per g of stool. Data represent mean ± SEM; ns = not significant. (C-E) WT and *Aim2*$^{-/-}$ mice (n=8-10 mice/group) were co-housed for 4 weeks. (C) *E. coli* burden in the colon was measured by culturing stool homogenates collected after 4 weeks of co-housing on MacConkey agar. Co-housed or separate-housed WT and *Aim2*$^{-/-}$ mice were fed with 3% DSS

for 5 days, followed by normal drinking water for 3 days. (D) Body weight was monitored daily. (E) Mice were sacrificed on day 8 to measure colon length. Data represent mean ± SEM of a representative experiment, each experiment was repeated 3 times. *, $p<0.05$; **, $p<0.01$. (F-G) Germ-free (GF) WT mice were co-housed with either conventionally raised WT or *Aim2*$^{-/-}$ mice for 3 days. 2 weeks later GF mice were fed with 3% DSS for 5 days. (F) Feces collected at day 7 after DSS administration were diluted and plated on MacConkey agar (F) or BHI agar (G) plates. Number of colonies was counted as Log10 cfu per g of feces. Data represent mean ± SEM. *, $p<0.05$; **, $p<0.01$.

Figure S5 (related to Figure 5). AIM2 doesn't regulate autophagy, but antimicrobial peptide production.

(A) WT and *Aim2*$^{-/-}$ mice were treated with 3% DSS for 5 days, followed by regular drinking water for 3 days. Mice were sacrificed on indicated days and proteins from colons were extracted with RIPA buffer. Autophagy induction was measured by conversion of LC3I to LC3II by Western blot analysis. (B) Densitometric analysis of conversion of LC3II relative to LC3I. (C) WT and *Aim2*$^{-/-}$ mice were treated with a combination of antibiotics or left untreated for 4 weeks and then administered with 3% DSS for 5 days. Colons collected at day 8 after DSS administration were examined for the induction of antimicrobial peptides. Data represent mean ± SEM; n=7-10. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

Figure S6 (related to Figure 6). AIM2 activates the inflammasome in the intestine.

(A) WT and *Nlrp3*$^{-/-}$ mice were treated with 3% DSS for 3 days and then proteins from colons were extracted with RIPA buffer and Caspase-1 activation in the colons of WT or *Nlrp3*$^{-/-}$ mice

were analyzed by Western blotting. Data is representative of three individual experiments. (B) Expression of IL-18 mRNA in colons of WT and *Aim2*$^{-/-}$ mice collected at day 0, 3 and 5 after DSS administration. (C) Whole colons from WT, *Aim2*$^{-/-}$ and *caspase-1*$^{-/-}$ mice at day 0 (n=7/group) and day 5 (n=5/group) after DSS administration were washed and culture *ex vivo* at 37°C for 12h. Secretion of IL-1β and IL-18 in culture supernatant was measured by ELISA. Data represent mean ± SEM; *,p<0.05, **,p<0.001. (D) Formalin fixed colon sections collected at day 0, 5 and 8 after DSS administration were immunostained with Ki67 antibody. Representative images are shown here. (E) Ki67-positive cells per crypt were counted under microscope. Data represent mean ± SEM (n=20 crypt/mouse; 3mice/group); **,p<0.01. (F) BMDM from WT and *Aim2*$^{-/-}$ mice were stimulated with LPS (500 ng/ml) for 5 h followed by treated with ATP (5 mM) or Nigericin (Nig., 1 μM) for 45 min, or transfected with 1 μg synthetic DNA (poly dA:dT) for 4 h. Proteins were extracted and caspase-1 activation was measured by Western blotting. (G) WT and *Aim2*$^{-/-}$ mice were co-housed for 4 weeks and then administered with 3% DSS. Colon tissues collected at day 5 after DSS were analyzed for caspase-1 activation by Western blotting.

Figure S7 (related to Figure 7). The inflammasome regulates colonic microbiota and intestinal inflammation.

(A) Feces from healthy WT, *Aim2*$^{-/-}$, *Caspase-1*$^{-/-}$ and *Il18*$^{-/-}$ mice were diluted in PBS and plated on MacConkey agar. Pictures show growth of *E. coli* on MacConkey agar plat after overnight incubation at 37°C. (B-C) WT, *Aim2*$^{-/-}$, *Caspase-1*$^{-/-}$ and *Il18*$^{-/-}$ mice were treated with 3% DSS for 5 days, followed by regular drinking water for 3 days. Stool consistency (B) and rectal bleeding score (C) were scored daily. (D) Mice were sacrificed at day 8 and the length of the colons was measured. Data represent mean ± SEM; *,p<0.05, **,p<0.001 and ***,p<0.001. (E-F)

Colonic crypts were isolated and immunostained with FITC-labeled EpCAM antibody. Figure E shows isolated epithelial crypts under microscope. Figure F shows flow cytometry analysis of EpCAM-positive cells. (G) Crypts from small intestine were cultured *in vitro* to develop organoids. At day 10, organoids were treated with IL-1β (10 ng) or IL-18 (10 ng) for 4 h. The expression of *mBD2, Reg3β, Reg3γ, S100a8, S100a9* and *Lcn2* was measured by RT-PCR. (H) Colon pieces were cultured *in vitro* in the presence or absence of IL-1β or IL-18 for 16h. The culture supernatants were incubated with *E. coli* (1000 cfu) for 1 h at 37°C followed by plating on MacConkey agar. *E. coli* colonies were counted. (G-H) Data represent mean ± SD of triplicate wells; *, $p<0.05$, **, $p<0.01$; nd = not detected. (I) Intestinal epithelial cells and lamina propria cells were isolated from healthy wild-type mice. Epithelial cells were immunostained with EpCAM antibody and lamina propria cells were immunostained with CD11b, CD4 and Gr1 antibodies, and sorted by flow cytometry. RNA was isolated from each cell population and expression of IL-18Rα and IL-18Rβ was detected by real-time PCR. (J) Colonic lamina propria cells were stained with antibodies for EpCAM, IL-18Rα and IL-18Rβ. Flow cytometry analysis shows EpCAM-positive cells expressing IL-18Rα and IL-18Rβ.

Table S1 (related to Figures 2, 4, 5 and 7). Primer sequences for real-time PCR analyses of cytokines, chemokines and antimicrobial peptides.

Table S2 (related to Figure 3). Primer sequences for real-time PCR analyses of bacterial 16S rRNA genes.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Clinical Scoring of Colitis

Diarrhea scores were determined as follows: 0 = well-formed pellets, 1 = semiformed stools that did not adhere to the anus, 2 = semiformed stools that adhered to the anus, 3 = liquid stools that adhered to the anus. Bleeding scores were determined as follows: 0 = no blood by using hemoccult (Helena Pharmaceuticals), 1 = positive hemoccult, 2 = blood traces in stool visible, 3 = gross rectal bleeding.

Histopathology and Immunohistochemistry

Colon was washed with PBS, fixed in 10% buffered formaldehyde and embedded in paraffin. Tissue sections of whole colon were stained with hematoxylin & eosin (H&E). Histological scoring was performed in a blinded fashion by a pathologist as a combined score of inflammatory cell infiltration. (score 0-3), ulceration (score 0-3), hyperplasia (score 0-3) and area of crypt distortion (score 0-3). For immunohistochemistry, formalin-fixed, paraffin-embedded colon tissues were sectioned, de-paraffinized and hydrated through decreasing concentrations of ethanol. Heat-induced antigen retrieval was performed in 10 mM sodium citrate solution (pH 6.0) for 20 min in a microwave. Slides were blocked with 5% goat serum for 30 min and stained for macrophages using anti-F4/80 (Clone CI:A3-1, Abcam), and Ki67 using rabbit anti-Ki67 (ab16667; Abcam). After overnight incubation at 4°C, slides were washed three times and incubated with secondary HRP-conjugated antibodies (for F4/80 and Ki67) for 1 h at room temperature. PAS staining was performed by commercially available kit using manufacturers

instruction (Sigma, cat# 395B). TUNEL staining was performed by TUNEL assay kit (Roche) following manufacturer's instruction. Images were taken using Zeiss microscope.

Cytokine Assay

Whole colon was homogenized mechanically in RIPA lysis buffer containing complete protease inhibitor mixture (Roche). Colon homogenates were centrifuged at 10000 rpm and the lysates were separated. The concentration of mouse cytokines (IL-1β and IL-6) and chemokine (KC) in colon lysates were determined by ELISA (R&D Systems). IL-18 level was measured by ELISA kit (eBioscience).

Culture and Identification of Bacteria

Stool and colon tissue samples were homogenized in 5 ml PBS. Ten times serial dilutions of the homogenates were plated on BHI and 5% sheep blood agar (Difco) and incubated at 37ºC for 24 - 48 hours. Blood agar plates were cultured in anaerobic condition. For the measurement of *E. coli* and other Enterobacteriace, fecal and colon homogenates were plated on MacConkey agar (Difco). Bacterial colonies grown on blood and MacConkey agar were isolated based on colony morphology and sub-cultured. Single colonies of sub-cultured bacteria were used for identification by MALDI-TOF.

Macrophage Culture and *in vitro* Inflammasome Activation

Bone marrow cells were cultured in L cell-conditioned Iscove's modified Dulbecco's medium supplemented with 10% FBS, 1% non-essential amino acid, and 1% penicillin-streptomycin for six days to differentiate into macrophages. BMDM cells were seeded in 12-well cell culture

plates and were transfected with poly dA:dT (Invivogen) or DNA isolated from mouse feces for four hours using PolyJet transfection reagent (SignaGen). Before DNA transfection, cells were stimulated with LPS (500ng/ml) for 4 h or left untreated. Both the supernatant and cell lysates were collected in RIPA buffer and analyzed for caspase-1 activation by Western blotting.

Induction of Antimicrobial peptides in Intestinal Crypt and Organoid Culture

Small intestines (SI) or colons were collected, washed vigorously in ice-cold PBS, cut longitudinally and incubated in 1mM DTT for 10 min to remove mucin layer. Tissues were then cut into small pieces and incubated with 10 mM EDTA in HBSS buffer containing 100u/ml penicillin/streptomycin and 20μg/ml gentamicin with gentle shaking. For isolation of SI crypts, the villus fraction was removed after 5 min of incubation and replaced with new 10mM EDTA buffer, and incubated additional 15 min at 37°C. For isolation of colonic crypts, colon tissues were incubated for 45 min. Isolated epithelial cells and crypts were passed through 70 μm cell-strainer and the isolated cells were washed several times in ice-cold HBSS by centrifugation at 500 rpm. The colon epithelial crypts were cultured on 24-well plates containing DMEM/F12 medium plus hepes (10mM), glutamax (2mM, Invitrogen) and penicillin-streptomycin (100u/ml, Invitrogen). After 3 h incubation at 37°C, cells were stimulated with IL-1β (10 ng/ml) or IL-18 (10 ng/ml). To develop organoids form SI crypts, the isolated crypts were cultured as described previously (Sato et al., 2009). In brief, 500 to 1000 crypts were mix with matrigel and placed on 96-well culture plate and incubated with DMEM/F12 medium containing hepes (10mM), glutamax (2mM), N2 (1X, Invitrogen), B27 (1X, Invitrogen) Noggin (100ng/ml, Peprotech), EGF (50ng/ml, Peprotech), R-spondin (1ug/ml, Peprotech), N-acetyl cysteine (50ng/ml, Sigma) and penicillin-streptomycin (100u/ml).

Colon Organ Culture and In Vitro *E. coli* Killing Assay

Colons from WT, *Aim2*$^{-/-}$ and *caspase-1*$^{-/-}$ mice were collected and washed in ice cold PBS vigorously. Colons were cut into small pieces and incubated with DMEM/F12 medium containing hepes (10mM), 5% FBS, Penicillin-streptomycin (100u/ml) and gentamicin (20μg/ml) for 12 h at 37°C. Culture supernatants were collected and assayed for IL-1β (R&D systems) and IL-18 (eBioscience) by ELISA.

For *E. coli* killing assay, the colons were cultured in medium containing antibiotics followed by wash three times with pre-warmed DMEM/F12 medium. Colons were then incubated with new media without antibiotics and stimulated with either IL-1β or IL-18 at different concentration (10 and 50ng/ml) for 12 h at 37°C. Culture supernatants were collected, filtered through 0.2 μM filter and incubated with *E. coli* (1000 cfu) for 2 h at 37°C. *E. coli* was also grown in culture media and organ culture supernatant without IL-1β/IL-18 stimulation as controls. The bactericidal activity of IL-1β- or IL-18-stimulated colon organ culture supernatants was measured by plating the culture media on MacConkey agar plate at 37°C.

Preparation of Intestinal Epithelial Cells and Lamina Propria Mononuclear (LPMN) cells

Colon epithelial cells and lamina propria mononuclear cells were collected with little modification as described previously (Weigmann et al., 2007). Colon was incubated with 1 mM DTT/PBS for 10 min on ice and then cut into 5 mm pieces and then incubated twice with 5 mM EDTA in RPMI 1640 supplemented with 100 U/ml penicillin, 100 U/ml streptomycin for 30 min at 37°C. Supernatant containing crypts were harvested and further incubated with 0.3 U/ml Dispase (Roche) for 10 min. After passing through 70μm cell strainer single epithelial cells were collected and used for experiments. The remaining tissues were minced and incubated with 0.5

mg/ml Collagenase D (Roche), 0.5 g/ml DNase I (Roche) and 3 mg/ml Dispase II (Roche) in RPMI 1640 at 37°C for 1 h with continuous shaking. Supernatant were collected, washed and filtered through a 40μm cell strainer. The isolated cells were subjected to density gradient centrifugation through 40%/80% Percoll (GE Healthcare Life Sciences). LPMN cells were harvested from the interface, washed and used for assays.

Flow Cytometry

Isolated colon epithelial cells were incubated with Fc Block™ for 15 min at 4°C and then stained with rat anti-mouse EpCAM (Clone G8.8, Biolegned) or rat IgG2a (Biolegned), followed by incubation with FITC-conjugated rabbit anti-mouse antibody (Southern Biotech). Similarly, isolated LPMN cells were incubated with Fc Block™ for 15 min at 4°C and then stained with FITC-conjugated anti-mouse Gr1 (Clone RB6-8C5, eBioscience), PerCp-Cy5-conjugated anti-mouse CD11b (Clone M1/70, Tonbo Bioscience) and APC-conjugated anti-mouse CD4 (Clone GK1.5, Tonbo Bioscience). Purity of the colon epithelial cells was analyzed by LSR Fortessa and flow sorting was performed using FACS Vantage SE (BD Biosciences, San Jose, CA, USA). For analyses of IL-18R expression, isolated colon epithelial cells were incubated with Fc Block™ for 15 min at 4°C and then stained with APC-conjugated anti-mouse EpCAM (Clone G8.8, Biolegned), PE-conjugated anti-mouse IL-18Rα/CD218a (Clone P3TUNYA, eBioscience) and rabbit anti-mouse CD218b/IL-18Rβ/IL-18RAP (eBioscience), followed by incubation with Alexa Fluor 488-conjugated goat anti-rabbit IgG (Life technologies). The expression of IL-18Rα and IL-18Rβ in EpCAM-positive cells was analyzed by FACSCalibur (BD Biosciences, San Jose, CA, USA).

Bone Marrow Chimera Study

Recipient WT (CD45.1+) and *Aim2*$^{-/-}$ (CD45.2+) mice were exposed with gamma radiation at a dose of 6 Gy. 4 hour later irradiated mice were given a 2nd dose of radiation (5 Gy) to ensure complete depletion of bone marrow cells. Irradiated mice were then injected via tail vein with 10^7 bone marrow cells isolated from the femurs of donor WT or *Aim2*$^{-/-}$ mice. The chimera mice were divided into four groups: CD45.2+ WT bone marrows transferred to CD45.1+ WT recipient (WT>WT); CD45.1+ WT bone marrows transferred to CD45.2+ *Aim2*$^{-/-}$ recipient (WT>*Aim2*$^{-/-}$); CD45.2+ *Aim2*$^{-/-}$ bone marrows transferred to CD45.1+ WT recipient (*Aim2*$^{-/-}$>WT); and CD45.2+ *Aim2*$^{-/-}$ bone marrows transferred to CD45.2+ *Aim2*$^{-/-}$ recipient (*Aim2*$^{-/-}$>*Aim2*$^{-/-}$). 6 weeks after bone marrow transplant, efficiency of reconstitution was analyzed by flow cytometric analyses of expression of CD45.1 and CD45.2 in leukocytes. The average reconstitution efficiency was ~95%. Mice were then fed with 3% DSS for 5 days. Body weight changes were monitored daily for 7 days and mice were sacrificed at day 7 to measure colon length.

Cytokine Infusion Study

Aim2$^{-/-}$ mice were injected with either rIL-1β (0.3μg/mouse) or rIL-18 (0.5μg/mouse) i.p. for 5 consecutive days. Feces collected at day 0, 3 and 6 after cytokine infusion were cultured on MacConkey agar to count *E. coli* burden. At day 6 following cytokine infusion, mice were fed with 3% DSS for 5 days. During DSS-induced administration, IL-1β- and IL-18-treated mice were further injected with either IL-1β (0.3μg/mouse) or IL-18 (0.5μg/mouse) respectively at day 1, 3 and 5 after DSS administration. Clinical features of colitis were monitored daily.

SUPPLEMENTAL REFERENCES

Dalwai, F., Spratt, D.A., and Pratten, J. (2007). Use of quantitative PCR and culture methods to characterize ecological flux in bacterial biofilms. J Clin Microb *45*, 3072-3076.

Elinav, E., Strowig, T., Kau, A.L., Henao-Mejia, J., Thaiss, C.A., Booth, C.J., Peaper, D.R., Bertin, J., Eisenbarth, S.C., Gordon, J.I.*, et al.* (2011). NLRP6 Inflammasome Regulates Colonic Microbial Ecology and Risk for Colitis. Cell *145*, 745-757.

Gueimonde, M., Tolkko, S., Korpimaki, T., and Salminen, S. (2004). New real-time quantitative PCR procedure for quantification of bifidobacteria in human fecal samples. Appl Env Microb *70*, 4165-4169.

Matsuda, K., Tsuji, H., Asahara, T., Matsumoto, K., Takada, T., and Nomoto, K. (2009). Establishment of an analytical system for the human fecal microbiota, based on reverse transcription-quantitative PCR targeting of multicopy rRNA molecules. Appl Env Microb *75*, 1961-1969.

Matsumoto, M., Kurihara, S., Kibe, R., Ashida, H., and Benno, Y. (2011). Longevity in mice is promoted by probiotic-induced suppression of colonic senescence dependent on upregulation of gut bacterial polyamine production. PloS one *6*, e23652.

Molloy, M.J., Grainger, J.R., Bouladoux, N., Hand, T.W., Koo, L.Y., Naik, S., Quinones, M., Dzutsev, A.K., Gao, J.L., Trinchieri, G.*, et al.* (2013). Intraluminal containment of commensal outgrowth in the gut during infection-induced dysbiosis. Cell Host Microbe *14*, 318-328.

Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J.*, et al.* (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature *459*, 262-265.

Suzuki, K., Meek, B., Doi, Y., Muramatsu, M., Chiba, T., Honjo, T., and Fagarasan, S. (2004). Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. Proc Nat Acad Sci U S A *101*, 1981-1986.

Vaishnava, S., Yamamoto, M., Severson, K.M., Ruhn, K.A., Yu, X., Koren, O., Ley, R., Wakeland, E.K., and Hooper, L.V. (2011). The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. Science *334*, 255-258.

Weigmann, B., Tubbe, I., Seidel, D., Nicolaev, A., Becker, C., and Neurath, M.F. (2007). Isolation and subsequent analysis of murine lamina propria mononuclear cells from colonic tissue. Nature Protocols *2*, 2307-2311.