PNAS
www.pnas.org

Supplementary Information for

Protein Kinase p38α signaling in dendritic cells regulates colon inflammation and tumorigenesis

Tingting Zheng, Baohua Zhang, Ce Chen, Jingyu Ma, Deyun Meng, Jian Huang, Ran Hu, Xinguang Liu, Kinya Otsu, Andrew C. Liu, Huabin Li, Zhinan Yin, Gonghua Huang

Gonghua Huang
Email: gonghua.huang@shsmu.edu.cn

This PDF file includes:

Supplementary information text
Figs. S1 to S9
Table S1
References for SI reference citations

www.pnas.org/cgi/doi/10.1073/pnas.1814705115

Supplementary Information Text

Supplementary Materials and Methods

Experimental Animals

Mapk14$^{fl/fl}$, *Il27p28*$^{fl/fl}$ and CD11c-Cre mice have been described previously(1-4). Rosa26-Cre-ER^{T2} mice were kindly provided by Prof. B. Su (Shanghai Jiao Tong University School of Medicine, China). CD4-Cre mice were kindly provided by Prof. H. Wang (Shanghai Jiao Tong University School of Medicine, China). OT-II mice were purchased from The Jackson Laboratory. C57BL/6 mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China). All mice had been backcrossed to C57BL/6 background for at least 8 generations. Age- and Sex-matched mice at 6-10 weeks of age were housed based on their genotypes and used for all experiments. Wild-type control mice were used littermate and, where relevant, included Cre^{+} mice to account for Cre effects (no adverse effects due to Cre expression itself were observed in vitro and in vivo in these studies). All mice were bred and maintained in specific pathogen-free conditions in the Animal Resource Center at Shanghai Jiao Tong University School of Medicine.

Induction of DSS-Induced Colitis

Acute colitis was induced by administration of 3% (w/v) DSS (MW 36,000–50,000, MP Biomedicals) in sterile drinking water for 7 days followed with normal drinking water until the end of the experiment. For IL-10R neutralization experiment, mice were injected intraperitoneally with 200 μg αIL-10R (1B1.3A, Bio X Cell) or isotype control rat IgG1 at day 3 and day 5 upon DSS treatment. The DSS solutions were made freshly every three days and body weight was recorded daily. Stool consistency and rectal bleeding were observed and scored according to a previously described protocol with modification(5). Briefly, stool scores were determined as: 0 = normal stool, 1 = soft stool, 2 = soft stool that adhere to the anus, 3 = diarrhea. Bleeding scores were determined as: 0 = no bleed, 1 = positive hemoccult (Beckman Coulter), 2 = visible blood in stool, 3 = gross rectal bleeding. Stool scores and bleeding scores were added as clinical score.

Intestine inflammation was also evaluated *in situ* by mini-endoscopy before mice were sacrificed. Colon length was measured after mice were sacrificed.

Induction of CACRC

For induction of CACRC, mice were intraperitoneally injected with 10 mg/kg AOM (Sigma-Aldrich). After 5 days, mice were supplied with 2% DSS solution for 5 days followed with normal drinking water for 15 days. The DSS cycle was repeated twice and mice were sacrificed 30 days after the last DSS cycle. Intestine inflammation was also evaluated *in situ* by mini-endoscopy before mice were sacrificed. Colon length, tumor number and tumor size were measured after mice were sacrificed. Tumor load which indicates the sum of tumor diameter per colon was calculated.

Isolation of LP leukocytes (LPLs) and intestinal epithelial cells (IECs)

Colons were dissected, cut longitudinally and washed with ice-cold phosphate-buffered saline (PBS). Colons were then cut into 5 cm pieces and incubated in PBS with 1 mM DTT and 30 mM EDTA for 10 min at 37 ºC with gentle shaking. The wash buffer was collected and saved for isolation of IECs. Colons were further shaken in PBS with 30 mM EDTA for 10 min at 37ºC. The tissues were then cut into about 3 mm pieces and digested in RPMI 1640 medium (Hyclone) with 5% fetal bovine serum (FBS; Gibco), collagenase VIII (125 U/ml) (Sigma-Aldrich) and DNase I (150 μg/ml) (Sigma-Aldrich) at 37ºC for 90 min without shaking. The digested tissues were homogenized by vigorous shaking and passed through a 100 μm cell strainer (BD Falcon) to be spun down. Then the cells were resuspended with 4 ml 40% Percoll (GE Healthcare) and gently layered on 4 ml 80% Percoll to be spun at 2500 rpm with no brake for 20 min at room temperature. The LPLs were harvested from the interphase between 40% and 80% Percoll. The IECs were harvested from the interphase between 20% and 40% Percoll from the saved wash buffer by the same strategy.

Intestinal Permeability Analysis

Intestinal permeability analysis was performed according to previously described (6). Briefly, mice were water starved overnight and then given with 440 mg/kg FITC-dextran

(Sigma-Aldrich) by oral gavage. After 4 hours, blood was collected from anesthetized mice to isolated serum. FITC concentration in serum was determined by spectrophoto fluorometry with 485 nm (20 nm band width) excitation and 528 nm (20 nm band width) emission wavelengths.

Flow Cytometry

For surface marker staining, single cell suspensions were labeled with antibodies in FACS buffer (PBS with 2% FBS) for 30 min on ice. FACS antibodies include: anti-CD11c (N418), anti-MHC II (M5/114.15.2), anti-CD26 (H194-112), anti-CD172a (P84), anti-CD40 (1C10), anti-CD80 (16-10A1), anti-CD86 (GL1), anti-Gr1 (RB6-8C5), anti-CD11b (M1/70), anti-CD90 (53-2.1), anti-CD45 (30-F11), anti-EpCAM (G8.8), anti-CD4 (RM4-5), anti-CD8a (53-6.7), anti-CD19 (1D3), anti-CD25 (PC61.5), anti-CD44 (IM7), anti-CD62L (MEL-14), anti–IL-10 (JES5-16E3), anti–IL-17 (eBio17B7), anti-IFNγ (XMG1.2e), anti-Foxps3 (FJK-165) and anti-RORγt (B2D), 7-AAD and Fixable Viability Dye (FVD) (all from eBioscience); anti-CD64 (X54-5/7.1), anti-XCR1 (ZET), anti-CD3ε (145-2C11), and anti–IL-22 (Poly5164) (all from Biolegend); anti-STAT1 (4a) and anti-STAT3 (4/P-STAT3) (both from BD Phosflow); anti–phospho-p38 (28B10, Cell Signaling Technology). The mixed antibodies of anti-CD4, anti-CD8a, anti-CD3ε, anti-CD19 and anti-CD11b were used as Lineage markers. For intracellular cytokine staining (ICS), cells were pre-stimulated with 1 μM ionomycin (Sigma-Aldrich) and 50 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) in the presence of GolgiStop (BD Biosciences) or GolgiPlug (BD Biosciences) for 5 hours. ICS and transcriptional factor staining were performed with kits from eBioscience according to manufacturer's instructions. Staining of intracellular phosphorylation antibodies, active caspase-3 and BrdU was performed with kits from BD Biosciences according to manufacturer's instructions. Flow cytometry data were acquired on BD LSRFortessa X-20 and analyzed with FlowJo software (Tree Star). 7-AAD$^-$ cells or FVD$^-$ cells were gated as live cells. CD64$^-$CD26$^+$MHCII$^+$CD11c$^+$ cells were gated as DCs; XCR1$^+$CD172a$^-$ DCs were gated as cDC1s; XCR1$^-$CD172a$^+$ DCs were gated as cDC2s; TCRβ$^+$CD4$^+$ cells were gated as CD4$^+$ T cells; lineage$^-$CD90$^+$RORγt$^+$ cells were gated as ILC3s; CD45$^-$EpCAM$^+$ cells were gated as IECs.

Cell Purification and Coculture

Naïve $CD4^+$ T ($CD4^+CD25^-CD44^-CD62L^+$) cells were enriched with CD4 microbeads (Miltenyi Biotec) and sorted with a BD FACSAria III Sorter. Colonic DCs, cDC1s, cDC2s, $CD11c^+$ macrophages and other $CD11c^+$ cells were sorted from colon LPLs directly. For coculture experiment, DCs, cDC1s, cDC2s, $CD11c^+$ macrophages and other $CD11c^+$ cells from wild type and $p38\alpha^{\Delta DC}$ mice were cocultured with naïve $CD4^+$ T cells from OT-II mice in the presence of ovalbumin peptide (0.5 μg/ml) ($OVA_{323-339}$) and LPS (100 ng/ml) (Sigma-Aldrich) with or without αIL-27 (10 μg/ml) (R&D Systems) or isotype. After 6 days, live cells were harvested from coculture cells and furthered stimulated with ionomycin and PMA for ICS, or stimulated with plated-bound αCD3ε (145-2C11; Bio X Cell) for 5 hour mRNA measurement or 24 hour cytokine detection.

Histopathological Analysis

The colon tissues were fixed in formalin and embedded in paraffin according to standard techniques. Longitudinal sections of 6 μm thick were stained with hematoxylin and eosin and analyzed by microscopic examination.

BMDC Culture

BM cells were cultured in RPMI 1640 medium with 10% FBS, GM-CSF (10 ng/ml) (R&D Systems) and IL-4 (5 ng/ml) (R&D Systems) for 7 days for BMDCs. For p38α-deletion in BMDCs of $p38\alpha^{CreER}$ mice, 0.5 μM (Z)-4-Hydroxytamoxifen (4-OHT; Sigma-Aldrich) was added on day 3. BMDCs were stimulated with LPS (100 ng/ml) for Western blot (WB) analysis with or without 30 min pre-treatment of 20 μM SP600125 (Merck Calbiochem) or vehicle. BMDCs were stimulated with LPS (100 ng/ml) for 5 hour RNA analysis with or without 30 min pre-treatment of 20 μM SP600125, 20 μM U0126 (Merck Calbiochem), 20 μM SR 11302 (Tocris) or vehicle.

Western Blot Analysis

Cells were lysed in 1× RIPA buffer (Cell Signaling Technology) containing 2 mM PMSF (Cell Signaling Technology), 1× complete protease inhibitor (Roche) and 1× PhosStop

phosphatase inhibitor (Roche). Protein content was quantified by Pierce BCA Protein Assay (Thermo Scientific). Total protein lysate was separated on SDS-PAGE and transferred to Immobilon®-P Transfer Membrane (Millipore). After blocking for 1 hours, membranes were incubated at 4°C overnight with primary antibodies: p38 (#8690S), phospho-p38 (#4511S), JNK (#9258S), phospho-JNK (#4668S), ERK (#4695S), phospho-ERK (#4370S), MKK4 ((#9152S), phospho-MKK4 (#4514S), MKK7 (#4172S), phospho-MKK7 (#4171S), TAK1 (#5206S), phospho-TAK1 (#4508S), MLK3 (#2817S), c-Jun (#9165S) and phospho-c-Jun (#2361S) from Cell Signaling Technology (all diluted at 1:1000 ratio); phospho-MLK3 (ab191530, 1:500) from Abcam. GAPDH (#10494-1-AP, 1:2000; Proteintech) was used as the loading control. After washing, membranes were incubated with Peroxidase-AffiniPure Goat Anti-Rabbit IgG (Jackson ImmunoResearch). The signal was detected with SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo Scientific). Images have been cropped for presentation.

Enzyme-Linked Immunosorbent Assay (ELISA)

For cytokine detection from colon tissue, pre-weighted colon tissue was homogenized in ice-cold CelLytic MT Cell Lysis Reagent (Sigma-Aldrich) and incubated on ice for 30 min followed with centrifugation at top speed for 15 min at 4°C for supernatant. The colon homogenate supernatant and cell culture supernatant were collected for cytokine measurement. Concentrations of TNFα, IL-1β, IL-6, IFNγ, IL-17A, IL-10 and IL-22 were measured by ELISA with kits from eBioscience according to the manufacturer's instructions.

Quantitative Real-Time PCR (qRT-PCR)

Total RNA of colon tissue and cells was isolated respectively by using the TRIzol reagent (Invitrogen) and RNeasy Mini Kit (Qiagen). cDNA was synthesized with PrimeScript RT Master Mix (Takara) according to the manufacturer's instructions. qRT-PCR was performed with SYBR Green PCR Master Mix (Applied Biosystems) in a Vii7 Real-Time PCR system (Applied Biosystems). Relative mRNA levels were normalized to the

endogenous control gene *Hprt*. Primers used in this paper were listed in *SI Appendix*, Table S1.

Chromatin Immunoprecipitation (CHIP) Assay

CHIP assays were performed with SimpleChIP Enzymatic Chromatin IP Kit (Agarose Beads, Cell Signaling Technology) according to the manufacturer's instructions. In brief, after stimulation with LPS (200 ng/ml) for 4 hours, BMDCs were cross-linked in 1% (w/v) formaldehyde (Sigma-Aldrich) and lysed for nuclei. Then the nuclei were digested with micrococcal nuclease and sonicated with Ultrasonic processor (FS-150N, 30% amplitude, 5 sets of 20 sec pulse) for 150-900 bp chromatin fragments. A portion of sonicated nuclei was saved as input control. Other sonicated nuclei were incubated with 2 μg anti–c-Jun (#9165S, Cell Signaling Technology) or negative control IgG overnight at 4ºC followed with incubation with ChIP-Grade Protein G Agarose Beads for 2 hours at 4ºC. Then chromatin was eluted by incubation for 30 min at 65ºC and reverse cross-link by Proteinase K for 2 hours at 65ºC. Finally, DNA was purified for gene promoter region amplifier by qRT-PCR. The primers for the AP-1 binding site on *Il27p28* promoter (*SI Appendix*, Fig. S9*B*) were listed in *SI Appendix*, Table S1.

Luciferase Reporter Assay

Murine c-Jun cDNA was cloned into pcDNA3.1 expression vector. 852 bp and 313-455 bp upstream of the transcription initiation site of murine *Il27p28* promoter was cloned into pGL3-basic vector to construct -852 and -313-455 p28 luciferase reporters respectively. DC2.4 cells were kindly provided by Prof. Changsheng Du (Tongji University). For luciferase activity detection, 2.5×10^5 DC2.4 cells per well were seeded in a 48-well plate and 16 hours later each well was transfected with mixed plasmids of 500 ng indicated luciferase reporter, 50 ng pRL-TK Renilla and 500 ng or indicated quantity of c-Jun expression plasmid by Lipofectamine 3000 Transfection Reagent (Invitrogen) according to the manufacturer's instructions. After 24 hours, post-transfection cells were stimulated with LPS (200 ng/ml) for another day. Then the cells were lysed for luciferase activity measurement by using the Dual-Luciferase Reporter

Assay System (Promega) and the ratio of firefly luciferase to *Renilla* luciferase was calculated.

Statistics

Statistical analysis was performed with GraphPad Prism 5 and data were presented as mean ± SEM. One-way analysis of variance (ANOVA) with Tukey's posttest and two-way ANOVA with Bonferroni posttest were used for multiple comparisons. Two-tailed Student's *t* test was used when two conditions were compared. *P* values were indicated and $P < 0.05$ was considered significant. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant. "*n*" indicates biological replicates for in vitro experiments and number of mice for in vivo studies.

Study approval

All animal experimental procedures were approved by Institutional Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine.

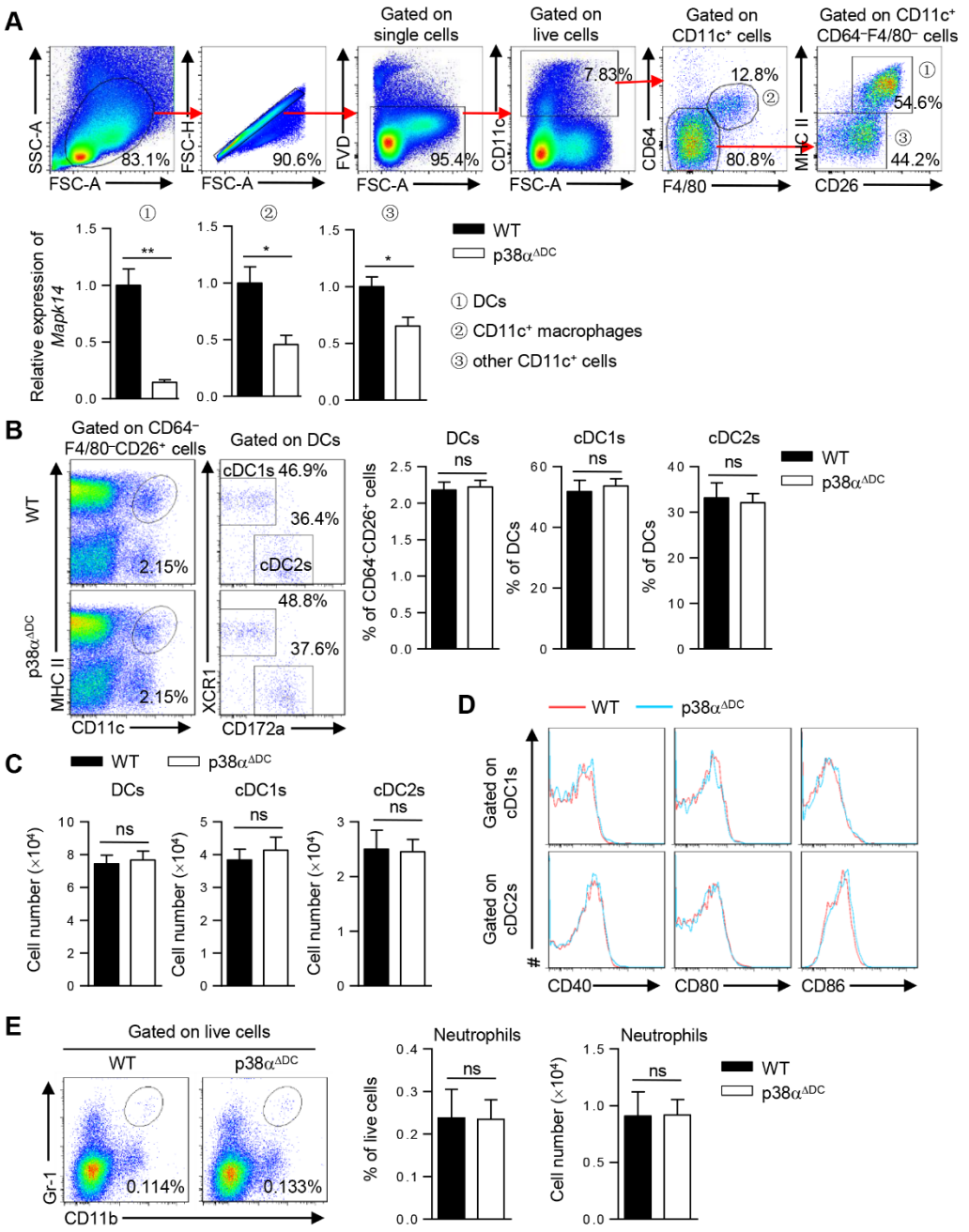

Fig. S1. p38α deficiency in DCs does not affect intestinal homeostasis. (*A*) The gating strategy for sorting CD11c^{+}CD64^{+}F4/80^{+} cells (CD11c^{+} macrophages), CD11c^{+}CD64^{-} F4/80^{-}MHC II^{+}CD26^{+} cells (DCs) and CD11c^{+}CD64^{-}F4/80^{-}MHC II^{-} cells (other CD11c^{+} cells) from colonic LPLs of wild-type (WT) and p38α$^{\Delta DC}$ mice and *Mapk14* expression in the three cell types ($n = 3$). (*B-D*) The percentages (*B*), cell numbers (*C*) and activation status (*D*) of total DCs, cDC1s and cDC2s in colon tissue of naïve WT and p38α$^{\Delta DC}$ mice ($n = 6$). (*E*) The percentages and cell numbers of neutrophils in colon tissue of naïve WT and p38α$^{\Delta DC}$ mice ($n = 6$). Data represent mean ± SEM. Two-tailed Student's *t* test (*A-C* and *E*). Results were replicated ($n = 3$ experiments). *$P < 0.05$; **$P < 0.01$; ns, not significant.

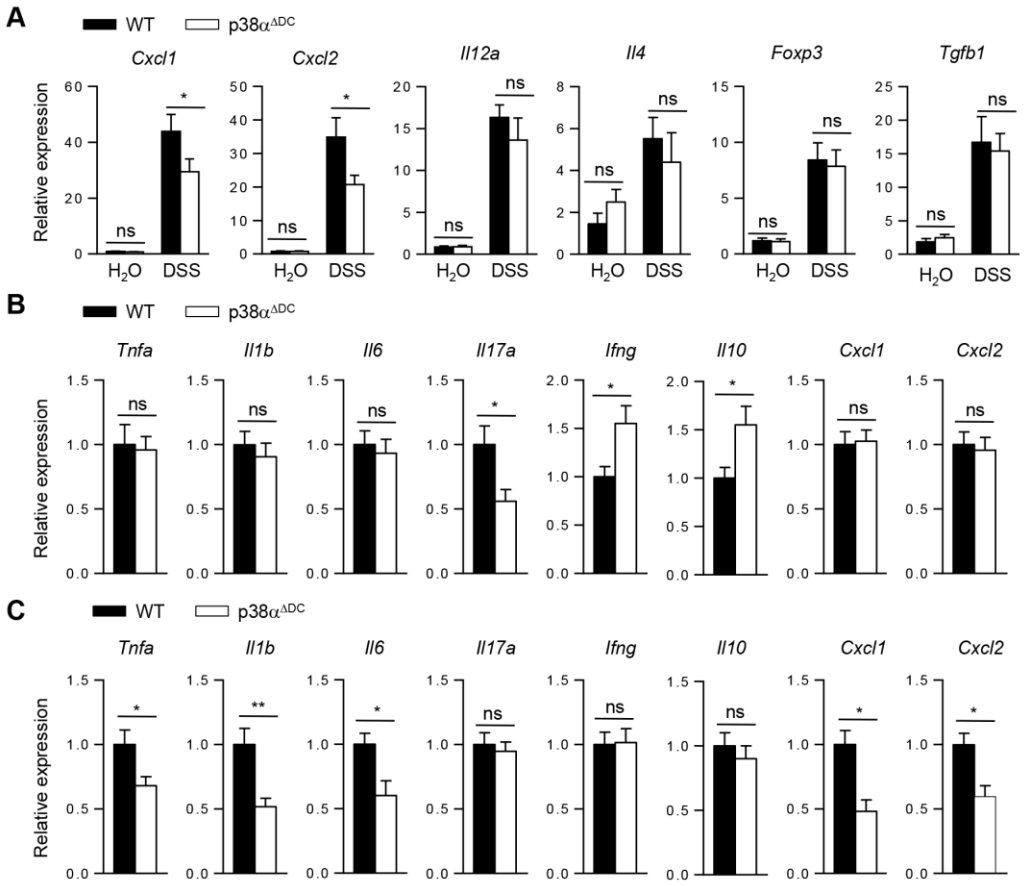

Fig. S2. p38α deficiency in DCs suppresses intestinal inflammatory responses upon DSS treatment. Wild-type (WT) and p38$\alpha^{\Delta DC}$ mice were supplied with 7 d of 3% DSS solution followed with 2 d of normal water or 9-day normal water. (*A*) Relative expression of inflammation-related genes in colon tissue ($n = 5$). (*B* and *C*) $CD4^+$ T cells (*B*) and non-$CD4^+$ cells (*C*) were sorted from LPLs of DSS-treated mice to check inflammation-related gene expression ($n = 5$). Data represent mean ± SEM. Two-way ANOVA with Bonferroni posttest (*A*) and two-tailed Student's *t* test (*B* and *C*). Results were replicated ($n = 3$ experiments). $^*P < 0.05$; $^{**}P < 0.01$; ns, not significant.

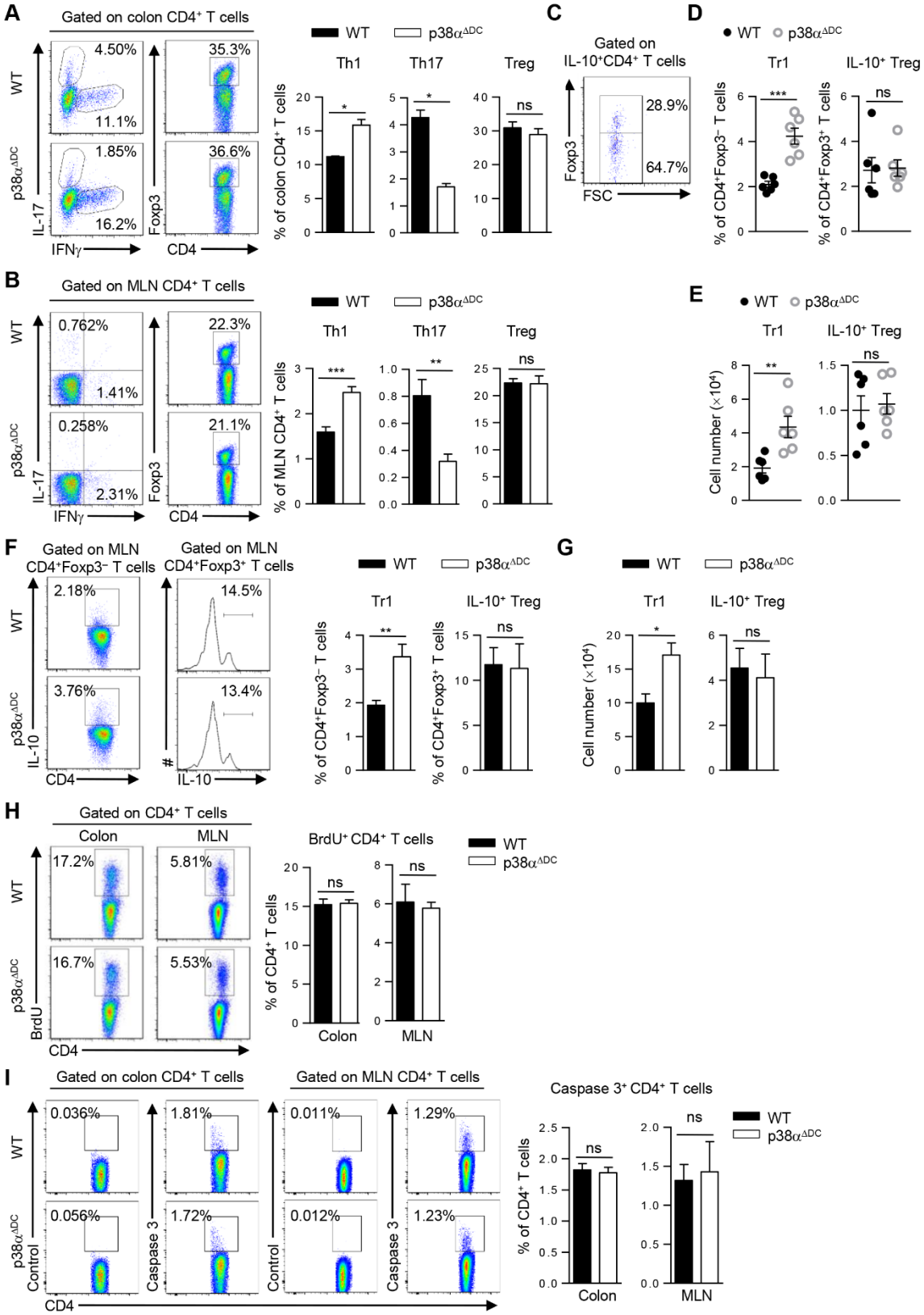
A
Gated on colon CD4+ T cells
WT
p38αΔDC
4.50%
11.1%
1.85%
16.2%
35.3%
36.6%
IL-17
IFNγ
Foxp3
CD4
WT
p38αΔDC
Th1
Th17
Treg
% of colon CD4+ T cells
*
*
ns
B
Gated on MLN CD4+ T cells
0.762%
1.41%
0.258%
2.31%
22.3%
21.1%
***
**
ns
% of MLN CD4+ T cells
C
Gated on IL-10+CD4+ T cells
28.9%
64.7%
Foxp3
FSC
D
Tr1
IL-10+ Treg
% of CD4+Foxp3− T cells
% of CD4+Foxp3+ T cells
***
ns
E
Cell number (×10⁴)
**
ns
F
Gated on MLN CD4+Foxp3− T cells
Gated on MLN CD4+Foxp3+ T cells
2.18%
3.76%
14.5%
13.4%
IL-10
CD4
#
**
ns
G
*
ns
H
Gated on CD4+ T cells
Colon
MLN
17.2%
16.7%
5.81%
5.53%
BrdU
CD4
BrdU+ CD4+ T cells
% of CD4+ T cells
ns
ns
I
Gated on colon CD4+ T cells
Gated on MLN CD4+ T cells
0.036%
0.056%
1.81%
1.72%
0.011%
0.012%
1.29%
1.23%
Control
Caspase 3
CD4
Caspase 3+ CD4+ T cells
ns
ns

Fig. S3. p38α deficiency in DCs suppresses inflammatory responses by promoting Tr1 differentiation without affecting T cell proliferation or survival. Wild-type (WT) and p38$\alpha^{\Delta DC}$ mice were supplied with 7 d of 3% DSS solution followed with 2 d of normal water. (*A* and *B*) The differentiation of Th1 cells, Th17 cells and Treg cells in colon tissue (*A*) and MLNs (*B*) (n = 5). (*C*) The Foxp3 in IL-$10^{+}CD4^{+}$ T cells. (*D* and *E*) The percentages (*D*) and cell numbers (*E*) of Tr1 and IL-10^{+} Treg cells in colon tissue (n = 6). (*F* and *G*) The percentages (*F*) and cell numbers (*G*) of Tr1 and IL-10^{+} Treg cells in MLNs (n = 5). (*H* and *I*) The BrdU (*H*) and active caspase-3 (*I*) staining of $CD4^{+}$ T cells in colon tissue and MLNs (n = 6). Isotype antibody was used as control of active caspase-3 staining. Data represent mean ± SEM. Two-tailed Student's *t* test (*A*, *B* and *D-I*). Results were replicated (n = 2-3 experiments). **P* < 0.05; ***P* < 0.01; ****P* < 0.001; ns, not significant.

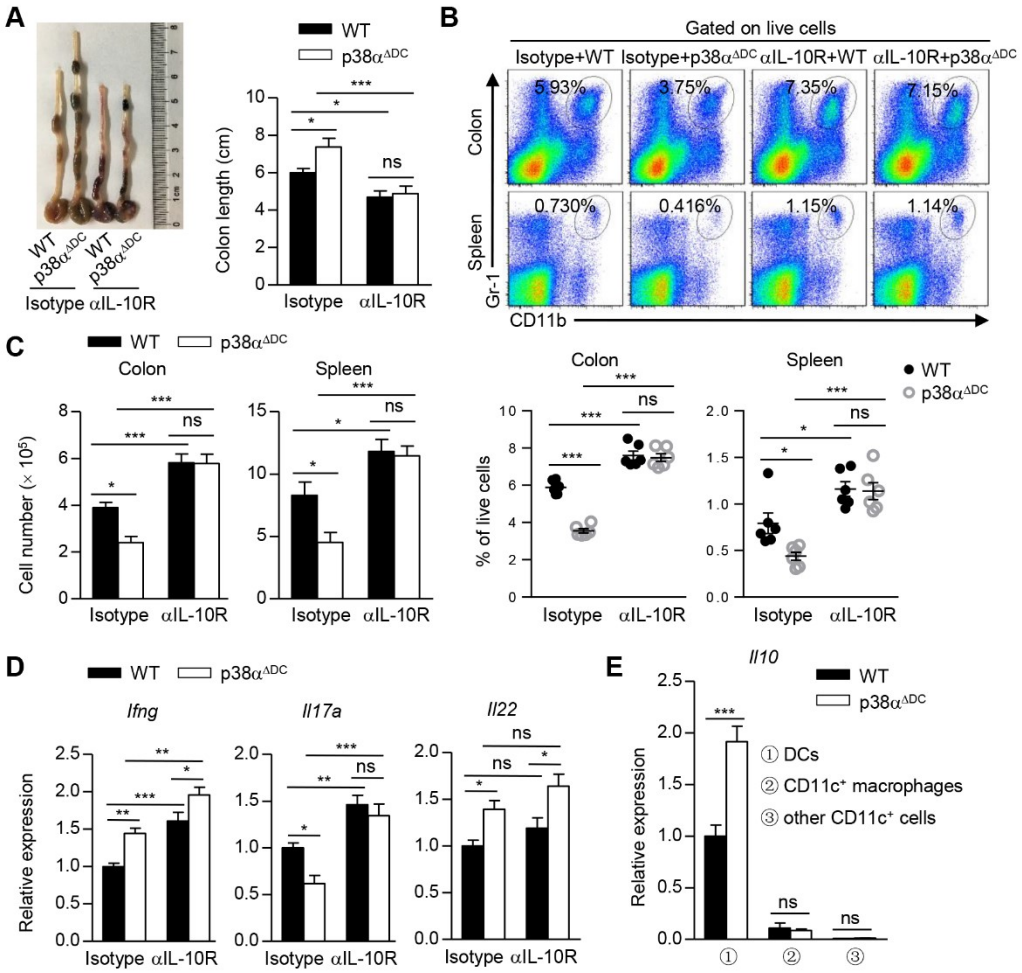

Fig. S4. IL-10R neutralization antibody abrogates the protection effect of p38α deficiency in DCs on DSS-treated mice. (*A-D*) Wild-type (WT) and p38α$^{\Delta DC}$ mice were supplied with 7 d of 2.5% DSS solution followed with 1 d of normal drinking water and injected with αIL-10R or isotype on days 3 and 5 (n = 6). Colon length (*A*), the percentages (*B*) and cell numbers (*C*) of neutrophils in colon tissue and spleen, and the relative expression of inflammation-related genes in colon tissue(*D*) were analyzed. (*E*) Colonic DCs, CD11c$^+$ macrophages and other CD11c$^+$ cells of WT and p38α$^{\Delta DC}$ mice were cocultured with naïve CD4$^+$ T cells for 6 d to check *Il10* expression (n = 3). Data represent mean ± SEM. Two-way ANOVA with Bonferroni posttest (*A-E*). Results were replicated (n = 3 experiments). *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

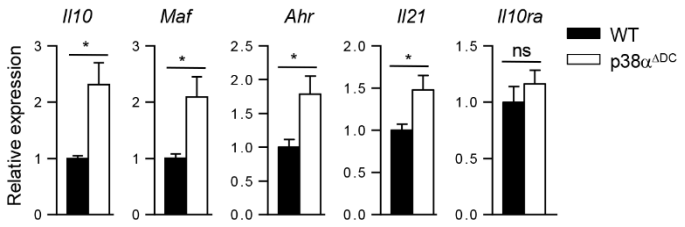

Fig. S5. p38α deficiency in DCs promotes Tr1-related gene expression. Colonic DCs of wild-type (WT) and p38$\alpha^{\Delta DC}$ mice were cocultured with naïve $CD4^+$ T cells for 6 d to check relative expression of Tr1-related genes (n = 5). Data represent mean ± SEM. Two-tailed Student's t test. Results were replicated (n = 3 experiments). *$P < 0.05$; ns, not significant.

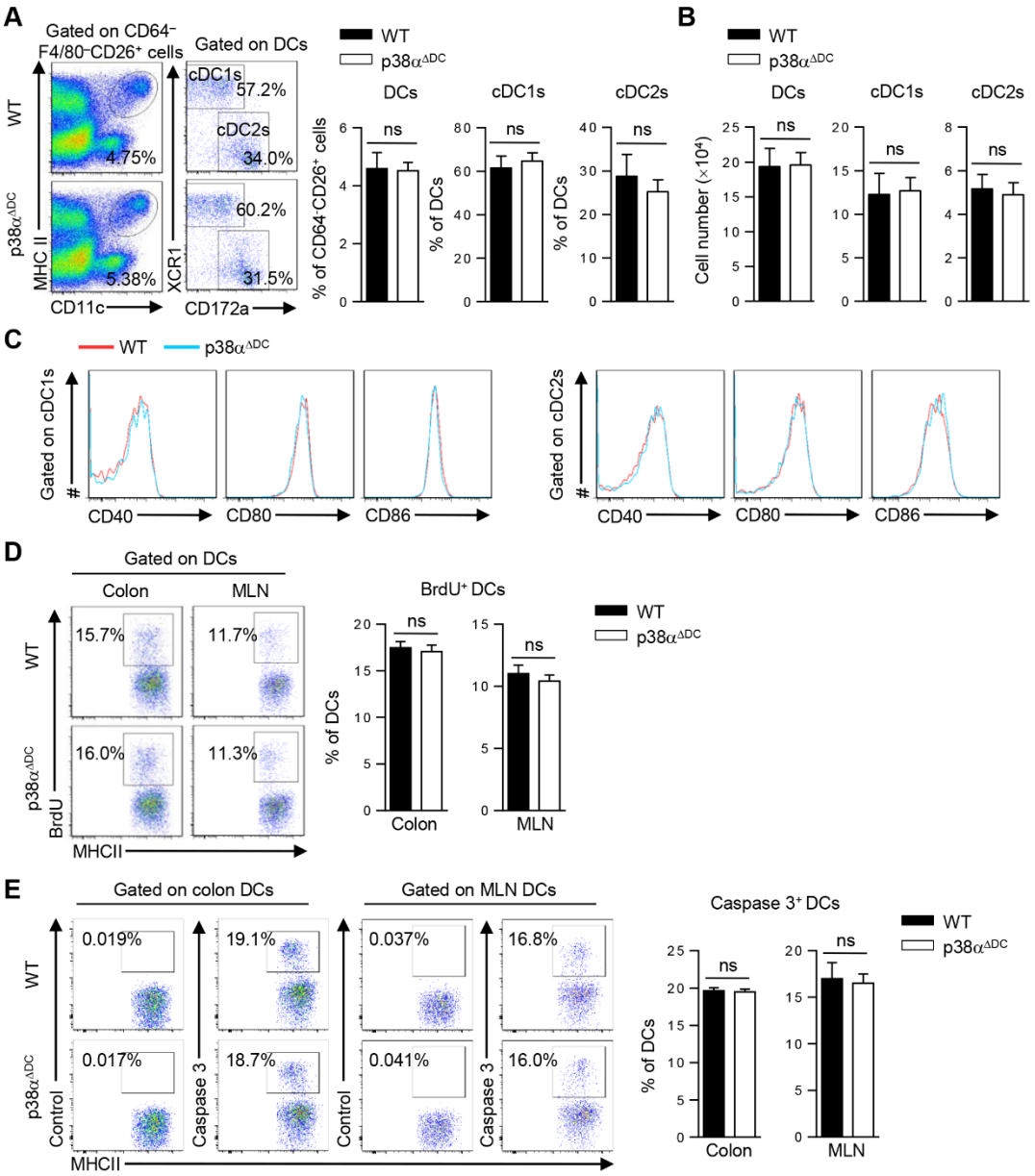

Fig. S6. Deletion of p38α does not affect the development or costimulatory molecule expressions in DCs upon DSS treatment. Wild-type (WT) and p38α$^{\Delta DC}$ mice were supplied with 7 d of 3% DSS solution followed with 2 d of normal water. (*A*-*C*) The percentages (*A*), cell numbers (*B*) and activation status (*C*) of total DCs, cDC1s and cDC2s in colon tissue (n = 5). (*D* and *E*) The BrdU (*D*) and active caspase-3 (*E*) staining of DCs in colon tissue and MLNs (n = 6). Isotype antibody was used as control of active caspase-3 staining. Data represent mean ± SEM. Two-tailed Student's t test (*A*, *B*, *D* and *E*). Results were replicated (n = 2-3 experiments). ns, not significant.

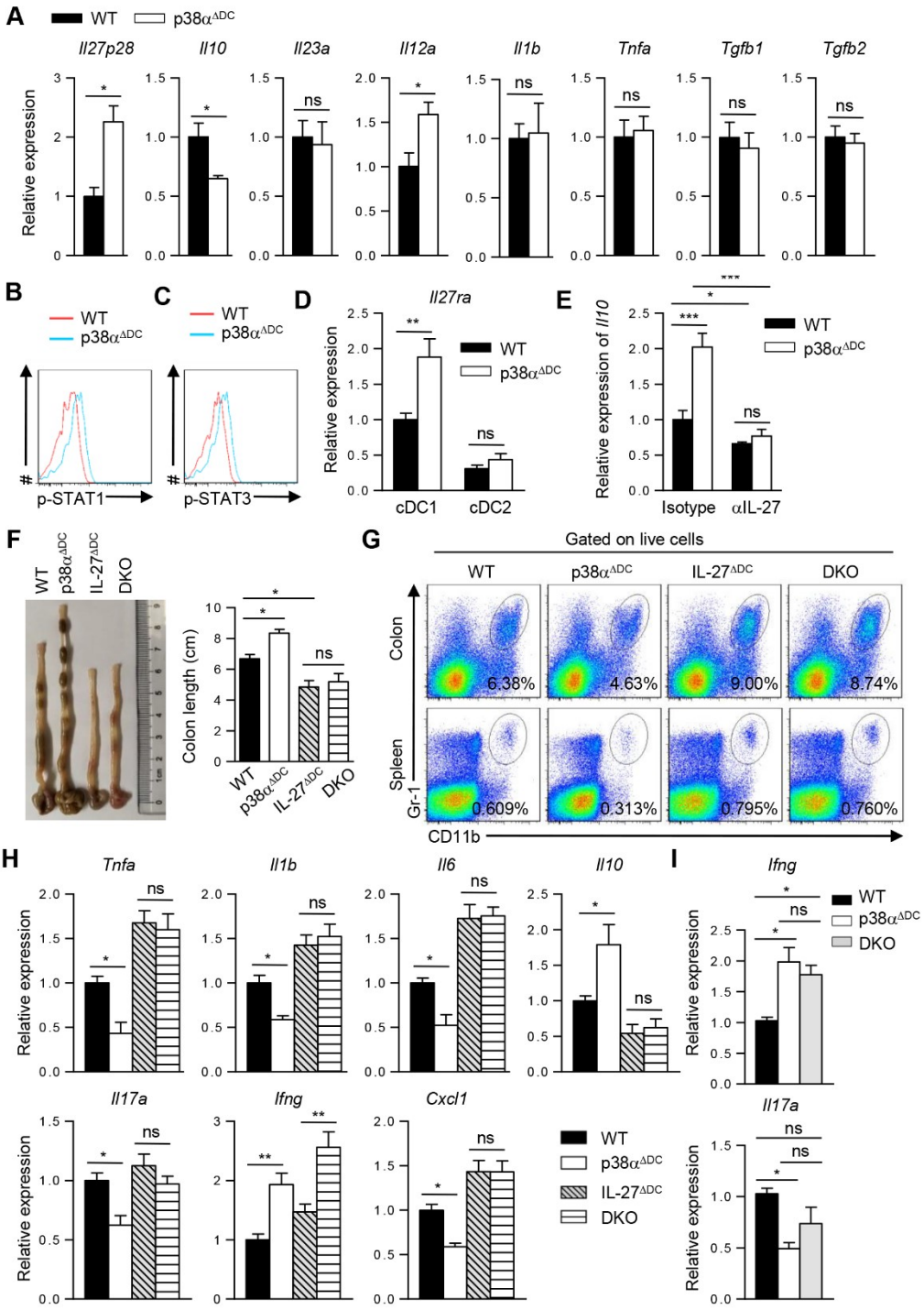
A
WT
p38α^ΔDC
Il27p28
Il10
Il23a
Il12a
Il1b
Tnfa
Tgfb1
Tgfb2
Relative expression
B
C
p-STAT1
p-STAT3
D
Il27ra
cDC1
cDC2
E
Relative expression of Il10
Isotype
αIL-27
F
IL-27^ΔDC
DKO
Colon length (cm)
G
Gated on live cells
IL-27^ΔDC
Colon
Spleen
Gr-1
CD11b
6.38%
4.63%
9.00%
8.74%
0.609%
0.313%
0.795%
0.760%
H
Tnfa
Il1b
Il6
Il10
Il17a
Ifng
Cxcl1
I
Ifng
Il17a
ns

Fig. S7. p38α-dependent IL-27 in DCs regulates the inflammatory responses during DSS-induced colitis. (*A*) Colonic DCs of wild-type (WT) and p38α$^{\Delta DC}$ mice were stimulated with LPS for 5 h to check relative gene expression (n = 3). (*B* and *C*) Colonic cDC1s of WT and p38α$^{\Delta DC}$ mice were cocultured with naïve CD4$^+$ T cells for 48 h to check the p-STAT1 (*B*) and p-STAT3 (*C*) (data pooled from 8 mice). (*D*) Colonic cDC1s and cDC2s of WT and p38α$^{\Delta DC}$ mice were cocultured with naïve CD4$^+$ T cells for 6 d to check *Il27ra* expression (n = 5). (*E*) Colon cDC1s of WT and p38α$^{\Delta DC}$ mice were cocultured with naïve CD4$^+$ T cells for 6 d in the presence of αIL-27 or isotype to check *Il10* expression (n = 4). (*F*-*H*) WT, p38α$^{\Delta DC}$, IL-27$^{\Delta DC}$ and DKO mice were supplied with 7 d of 3% DSS solution followed with 1 d of normal water (n = 6). Colon length (*F*), the neutrophil infiltration in colon tissue and spleen (*G*), and relative expression of inflammation-related genes (*H*) were examined. (*I*) Colonic DCs of WT, p38α$^{\Delta DC}$ and DKO mice were cocultured with naïve CD4$^+$ T cells for 6 d to check gene expression (n = 3). Data represent mean ± SEM. Two-tailed Student's t test (*A*), two-way ANOVA with Bonferroni posttest (*D* and *E*) and one-way ANOVA with Tukey's posttest (*F*, *H* and *I*). Results were replicated (n = 2-3 experiments). *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

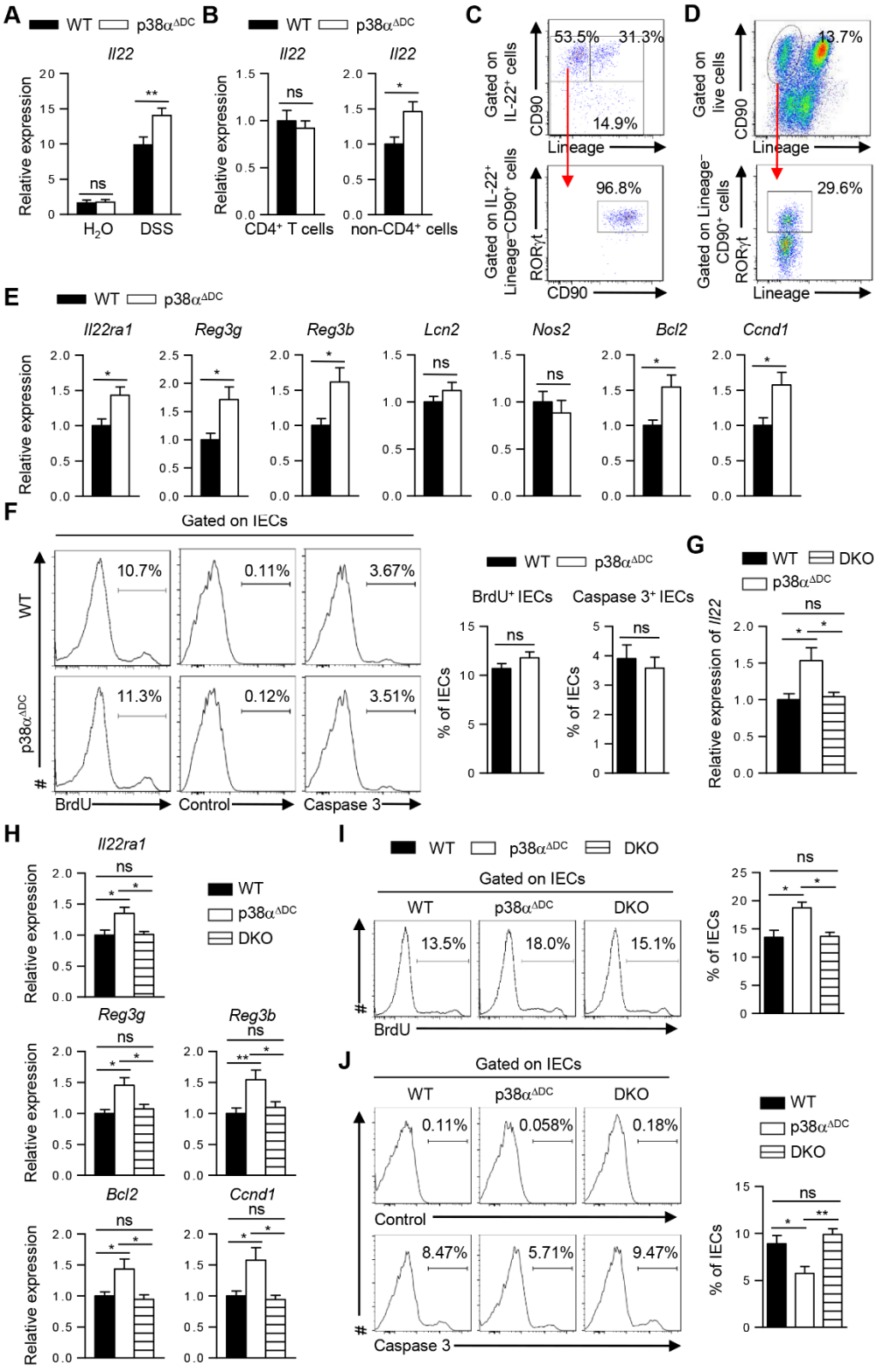
A
WT
p38α^ΔDC
Il22
Relative expression
ns
**
H2O
DSS
B
Il22
ns
*
CD4+ T cells
non-CD4+ cells
C
53.5%
31.3%
14.9%
Gated on IL-22+ cells
CD90
Lineage
96.8%
Gated on Lineage−CD90+ cells
RORγt
D
13.7%
Gated on live cells
29.6%
Gated on Lineage− CD90+ cells
E
Il22ra1
Reg3g
Reg3b
Lcn2
Nos2
Bcl2
Ccnd1
F
Gated on IECs
10.7%
0.11%
3.67%
11.3%
0.12%
3.51%
BrdU
Control
Caspase 3
BrdU+ IECs
Caspase 3+ IECs
% of IECs
G
DKO
Relative expression of Il22
H
I
Gated on IECs
13.5%
18.0%
15.1%
J
0.11%
0.058%
0.18%
8.47%
5.71%
9.47%

Fig. S8. The increased IL-22 production in DSS-treated mice with p38α deletion in DCs is dependent on IL-27. (*A*) Relative *Il22* expression in colon tissue of wild-type (WT) and p38α$^{\Delta DC}$ mice treated with DSS or normal water (n =5). (*B*) Relative *Il22* expression in CD4$^+$ T cells and non-CD4$^+$ cells sorted from LPLs of DSS-treated WT and p38α$^{\Delta DC}$ mice (n = 5). (*C*) The cellular source of IL-22 in colon tissue of DSS-treated mice (n = 5). (*D*) The gating strategy of ILC3s. (*E*) Relative gene expression in IECs of DSS-treated WT and p38α$^{\Delta DC}$ mice (n = 5). (*F*) The BrdU and active caspase-3 staining of IECs in AOM/DSS–treated WT and p38α$^{\Delta DC}$ mice (n = 6). (*G* and *H*) Relative gene expression in colon tissue (*G*) and IECs (*H*) of DSS-treated WT, p38α$^{\Delta DC}$ and DKO mice (n = 6). (*I* and *J*) The BrdU (*I*) and active caspase-3 (*J*) staining of IECs in DSS-treated WT, p38α$^{\Delta DC}$ and DKO mice (n = 6). Isotype antibody was used as control of active caspase-3 staining. Data represent mean ± SEM. Two-way ANOVA with Bonferroni posttest (*A*), two-tailed Student's *t* tests (*B*, *E* and *F*) and one-way ANOVA with Tukey's posttest (*G-J*). Results were replicated (n = 2-3 experiments). *$P < 0.05$; **$P < 0.01$; ns, not significant.

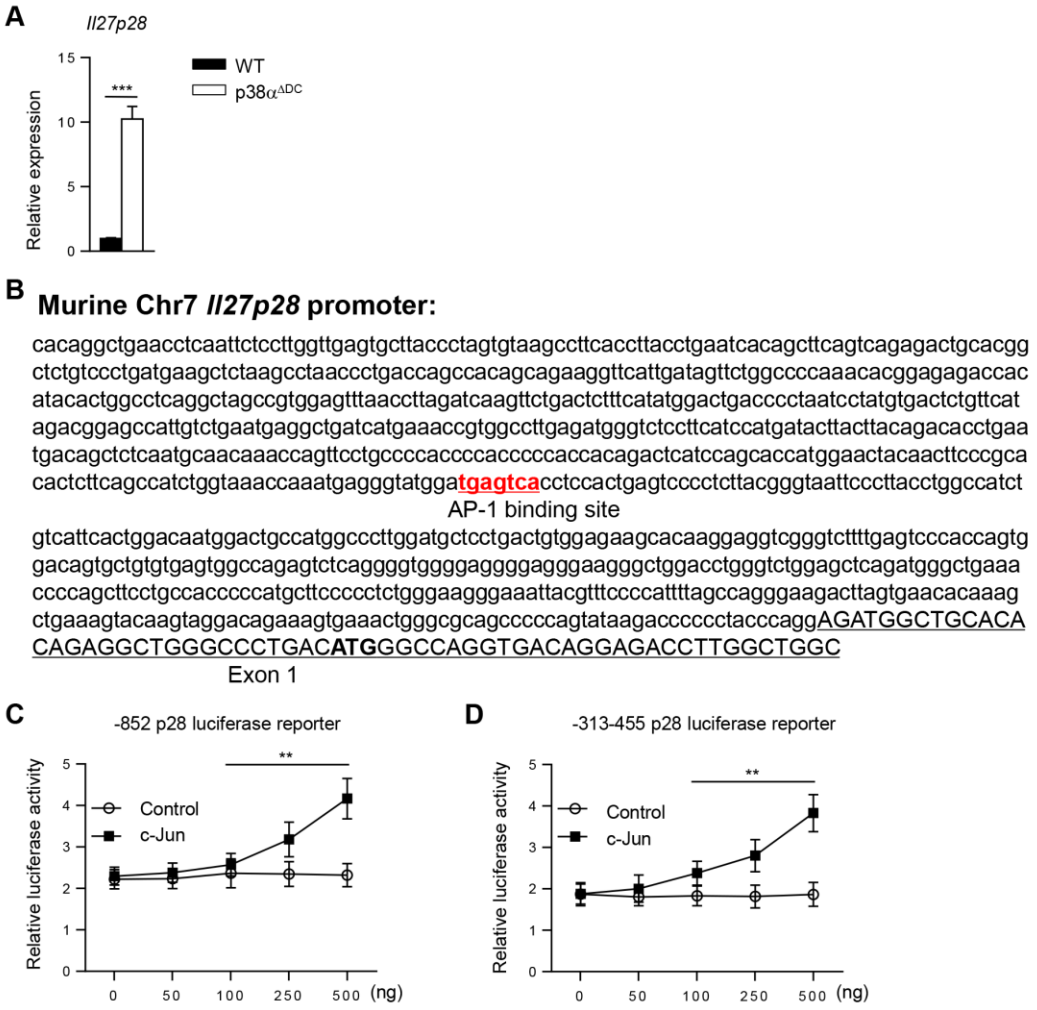

Fig. S9. c-Jun enhances the transcriptional activity of *Il27p28* promoter in DCs. (*A*) *Il27p28* expression in LPS-stimulated BMDCs of wild-type (WT) and p38α$^{\Delta DC}$ mice (n =5). (*B*) An AP-1 binding site on *Il27p28* promoter region. (*C* and *D*) -852 p28 luciferase reporter (*C*) or -313-455 p28 luciferase reporter (*D*) was cotransfected with Renilla and indicated quantity of c-Jun expression plasmid or empty vector (control) into DC2.4 cells to check luciferase activities (n = 3). Data represent mean ± SEM. Two-tailed Student's t tests (*A*) and two-way ANOVA with Bonferroni posttest (*C* and *D*). Results were replicated (n = 3 experiments). $^{**}P < 0.01$; $^{***}P < 0.001$.

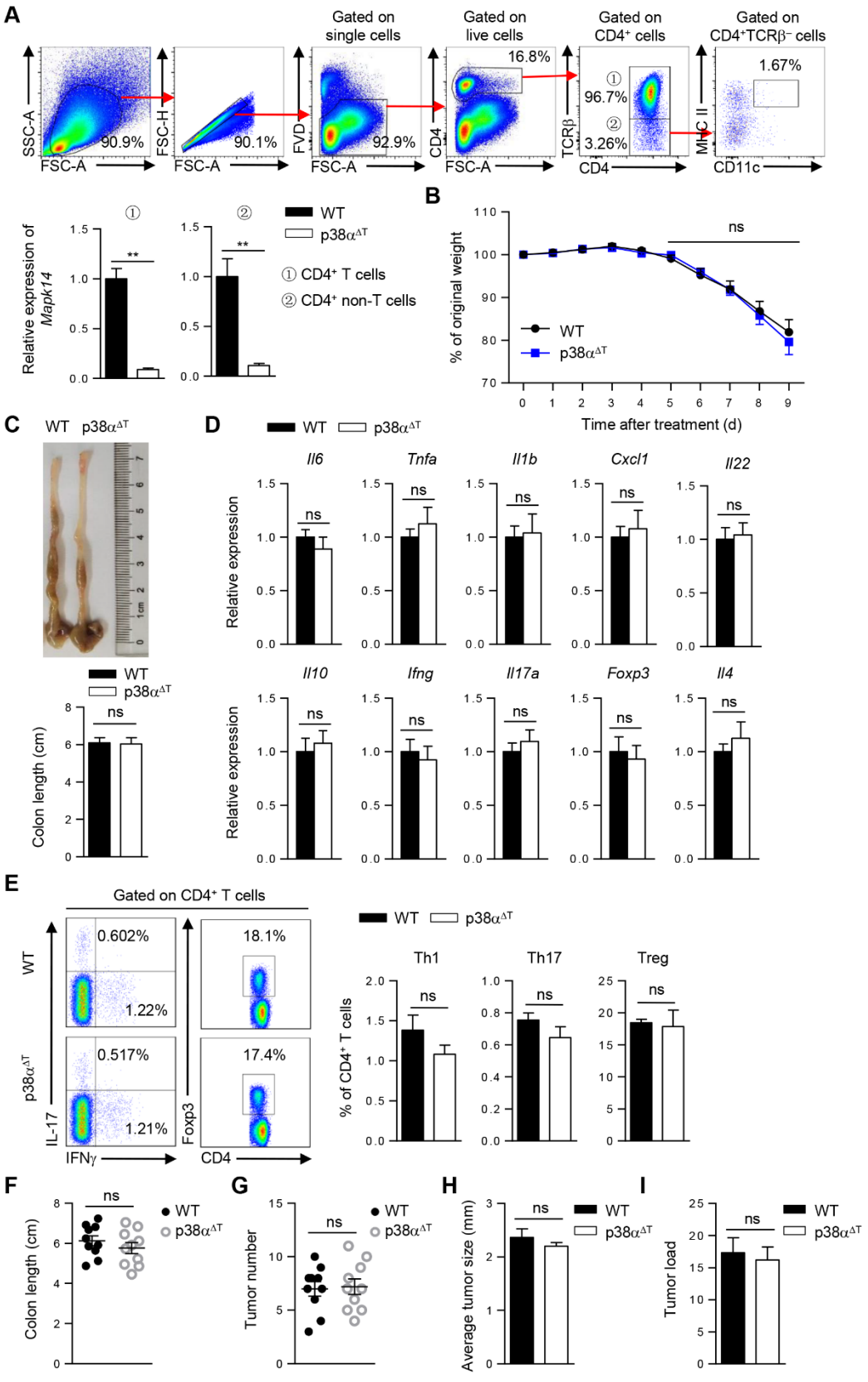
A
Gated on single cells
Gated on live cells
Gated on CD4⁺ cells
Gated on CD4⁺TCRβ⁻ cells
SSC-A
FSC-A
90.9%
FSC-H
90.1%
FVD
92.9%
CD4
16.8%
TCRβ
96.7%
3.26%
MHC II
CD11c
1.67%
B
Relative expression of Mapk14
WT
p38αΔT
① CD4⁺ T cells
② CD4⁺ non-T cells
% of original weight
Time after treatment (d)
ns
C
Colon length (cm)
D
Il6
Tnfa
Il1b
Cxcl1
Il22
Il10
Ifng
Il17a
Foxp3
Il4
Relative expression
E
Gated on CD4⁺ T cells
0.602%
1.22%
18.1%
0.517%
1.21%
17.4%
IL-17
IFNγ
Foxp3
CD4
Th1
Th17
Treg
% of CD4⁺ T cells
F
Colon length (cm)
G
Tumor number
H
Average tumor size (mm)
I
Tumor load

Fig. S10. T-cell–intrinsic p38α signal is not required for DSS-induced acute colitis pathogenesis. (*A*) The gating strategy for sorting $CD4^+TCR\beta^+$ cells ($CD4^+$ T cells) and $CD4^+TCR\beta^-$ cells ($CD4^+$ non-T cells) from LPLs of wild-type (WT) and $p38\alpha^{\Delta T}$ mice and *Mapk14* expression in the two cell types ($n = 3$). (*B*-*E*) WT and $p38\alpha^{\Delta T}$ mice were supplied with 7 d of 3% DSS solution followed with normal water ($n = 5$). Body weight loss (*B*), colon length (*C*), relative expression of inflammation-related genes in colon tissue (*D*) and the differentiation of Th1 cells, Th17 cells and Treg cells in colon tissue (*E*) were analyzed. (*F*-*I*) WT and $p38\alpha^{\Delta T}$ mice were injected with 10 mg/kg AOM on day 0 and then given three cycles of 5 d of 2% DSS solution ($n = 10$). Mice were sacrificed on day 80. Colon length (*F*), tumor numbers (*G*), tumor diameter (*H*) and tumor load (*I*) were examined. Data represent mean ± SEM. Two-tailed Student's *t* test (*A* and *C*-*I*) and two-way ANOVA (*B*). Results were replicated ($n = 2$ experiments). $^{**}P < 0.01$; ns, not significant.

Table S1. Mouse primer sequence for qRT-PCR

Name	Forward	Reverse
Hprt	TCAGTCAACGGGGGACATAAA	GGGGCTGTACTGCTTAACCAG
Mapk14	GAGGTGCCCGAACGATAC	TGGCGTGAATGATGGACT
Tnfa	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
Il1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Il6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
Ifng	GCCACGGCACAGTCATTGA	TGCTGATGGCCTGATTGTCTT
Il17a	TCAGCGTGTCCAAACACTGAG	CGCCAAGGGAGTTAAAGACTT
Il10	CTTACTGACTGGCATGAGGATCA	GCAGCTCTAGGAGCATGTGG
Cxcl1	TGCACCCAAACCGAAGTCAT	TTGTCAGAAGCCAGCGTTCAC
Cxcl2	CCAACCACCAGGCTACAGG	GCGTCACACTCAAGCTCTG
Il12a	CAATCACGCTACCTCCTCTTTT	CAGCAGTGCAGGAATAATGTTTC
Il23a	GCCCCGTATCCAGTGTGA	GCTGCCACTGCTGACTAG
Il4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
Foxp3	CACCTATGCCACCCTTATCCG	CATGCGAGTAAACCAATGGTAGA
Tgfb1	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
Tgfb2	TCGACATGGATCAGTTTATGCG	CCCTGGTACTGTTGTAGATGGA
Maf	AATCCTGGCCTGTTTCACAT	TGACGCCAACATAGGAGGTG
Ahr	GGCTTTCAGCAGTCTGATGTC	CATGAAAGAAGCGTTCTCTGG
Il21	GGACCCTTGTCTGTCTGGTAG	TGTGGAGCTGATAGAAGTTCAGG
Il10ra	CAAACAGTACGGAAACTCAACCT	GGTGATACAGATCCAGGGTGAAC
Il27p28	CTGTTGCTGCTACCCTTGCTT	CACTCCTGGCAATCGAGATTC
Il27ra	ACCAGGAAACCGTTGGAGTAT	GACGGGTCCAGTTGAGCTTG
Il22	ATGAGTTTTTCCCTTATGGGGAC	GCTGGAAGTTGGACACCTCAA
Il22ra1	GCTGGACTCCCTTGTGTGT	CACATGGCCTCAGTCTCAA
Reg3g	ATGGCTCCTATTGCTATGCC	GATGTCCTGAGGGCCTCTT
Reg3b	ATGGCTCCTACTGCTATGCC	GTGTCCTCCAGGCCTCTTT
Lcn2	ACATTTGTTCCAAGCTCCAGGGC	CATGGCGAACTGGTTGTAGTCCG
Nos2	TTGGGTCTTGTTCACTCCACGG	CCTCTTTCAGGTCACTTTGGTAGG
Bcl2	AGTACCTGAACCGGCATCTG	AGGTATGCACCCAGAGTGATG
Ccnd1	CTGCAAATGGAACTGCTTCTGGTGA	AGCAGGAGAGGAAGTTGTTGGGGCT
AP-1 binding site	GAACTACAACTTCCCGCACACTC	AAAGACCCGACCTCCTTGTGCTT

References

1. Huang G, et al. (2012) Signaling via the kinase p38alpha programs dendritic cells to drive TH17 differentiation and autoimmune inflammation. *Nat Immunol* 13:152-161.
2. Zhang S, et al. (2013) High susceptibility to liver injury in IL-27 p28 conditional knockout mice involves intrinsic interferon-gamma dysregulation of CD4+ T cells. *Hepatology* 57:1620-1631.
3. Nishida K, et al. (2004) p38alpha mitogen-activated protein kinase plays a critical role in cardiomyocyte survival but not in cardiac hypertrophic growth in response to pressure overload. *Mol Cell Biol* 24:10611-10620.
4. Caton ML, Smith-Raska MR, Reizis B (2007) Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. *J Exp Med* 204:1653-1664.
5. Wirtz S, Neufert C, Weigmann B, Neurath MF (2007) Chemically induced mouse models of intestinal inflammation. *Nat Protoc* 2:541-546.
6. Gupta J, et al. (2014) Dual function of p38alpha MAPK in colon cancer: suppression of colitis-associated tumor initiation but requirement for cancer cell survival. *Cancer Cell* 25:484-500.