Materials and Methods

CAC model procedure

CAC in mice was induced by AOM-DSS according to the procedure descripted previously[1, 2]. Mice (female and male) at ages of 6-12 weeks were injected intraperitoneally with 12.5 mg/kg AOM (Sigma-Aldrich, MO, USA). After 5 days, 2.5% DSS (MP Biomedicals, CA, USA, MW 36-50 kDa) in drinking water was given for five days. Then, mice were given regular drinking water for 14 days, followed by two additional DSS treatment cycles (5 days of 2.5% DSS and 4 days of 2% DSS).

To determine a microbiota effect on CAC tumorigenesis, sex and age matched mice were co-housed or separately-housed two weeks prior to AOM injection and stayed under the same condition until the CAC experimental model was completed (63 days).

Body weight, the presence of blood and the stool consistency were determined daily or weekly during CAC induction. The clinical score of colitis as described before[3] was assessed by well-trained individuals.

Mice were sacrificed at the end of the model. For survival analysis, mice were monitored for up to 9 months. Colons were removed followed by collection of stools and then flushed with PBS with or without gentamycin (2 mg/ml), and tumors were counted in a blinded fashion and colon photos were taken. Colon length was measured. Portions of the distal colons were fixed in 4% paraformaldehyde or Formalin solution (neutral buffered, 10% (Sigma)) and paraffin embedded for histopathological analysis, and the rest were used for IB and desired analyses.

Microbiota analysis: DNA isolation and bacterial 16S rDNA detection

DNA was isolated from stools of mice using PURELINK MICROBIOME PURIF KIT EA (Invitrogen). Bacterial 16S rDNA was amplified using *Bacteroides* bacteria (Bact B), *Clostridia* bacteria (Clostri B), *Lactobacillaceae* bacteria (Lacto B) and *Segmented Filamentous* bacteria (SFB) primers. PCR was carried out using the program [90 °C for 3 min, 25 cycles x (95 °C for 40 s, 60 °C for 40 s, 60 °C for 4 min), extension at 72 °C for 10 min, hold at 4 °C] followed by electrophoresis and image record[4].

Primer sequences for bacteria groups are as the following:

Bacteroides bacteria:

Fwd: 5' -GGTTCTGAGAGGAAGGTCCC- 3',

Rev: 5' - GCTGCCTCCCGTAGGAGT- 3';

Clostridiales bacteria:

Fwd: 5' -ACTCCTACGGGAGGCAGC- 3',

Rev: 5' - GCTTCTTAGTCAGGTACCGTCAT- 3';

Lactobacillaceae bacteria:

Fwd: 5' - AGCAGTAGGGAATCTTCCA- 3',

Rev: 5' -CACCGCTACACATGGAG- 3';

SFB (*Segmented Filamentous* bacteria:

Fwd: 5' -GACGCTGAGGCATGAGAGCAT- 3',

Rev: 5' - GACGGCACGGATTGTTATTCA- 3'.

Score of colitis

The clinical score of colitis was assessed as described before[3]. Briefly, for stool consistency, 0 point was assigned for well-formed droppings, 2 points for pasty and

semiformed stools, and 3 points for stools adhering to the anus, and 4 points for watery stools. For bleeding, 0 point was assigned for no blood, 2-3 points for blood on stool and anus, and 4 points for gross bleeding. For weight loss, 0 point was assigned for no weight loss, 1 point for weight loss of 1-5% from the baseline, 2 points for weight loss of 6-10%, 3 points for weight loss of 11-20% and 4 points for weight loss of more than 20%.

Colitis histology score was assessed using following criteria:

Water: No colitis; score=0.

DSS challenge: 0: no inflammation; 1>=2 foci or cryptitis or crypt abscesses; 2>2 foci of cryptitis or crypt abscesses; 3: Any cryptitis or crypt abscess plus ulceration/erosion

Immunohistochemistry (IHC) and pathologic review.

Tissue section slides were immunostained separately for Gαi1 (mouse monoclonal (mmAb) IgG antibody), GNAI2 and GNAI3 (rabbit polyclonal IgG antibody, Santa Cruz Biotechnology, CA, Abcam, MA, USA). For mouse colon tissue sections, Gr-1 mmAb (Biolegend, CA, USA) and p-NF-κBp65(S536) and p-STAT3(Y705) rabbit mAb (rmAb, Cell Signaling technology, MA, USA) were used. All antibodies were tested for their specificities by immunoblotting or flow cytometry analysis. Following de-paraffinization in xylene and rehydration, slides were subject to high pH antigen retrieval (10 mmol/L citrate buffer; pH 9.0), followed by 3% hydrogen peroxide, and blocking in 1.5% normal serum. Slides were then incubated with primary antibody for 0.5 hours, followed by incubation with secondary antibody, and detection with avidin-biotin reagent (Vector Laboratories, CA, USA) for 20 minutes and DAB substrate (3,3-diaminobenzidine; Sigma-Aldrich, MO, USA) for 5 minutes. Counterstaining utilizes hematoxylin. The

degree of staining was estimated by experienced pathologists and technicians who were blinded to the study.

Isolation of lamina propria[5]

The colons were collected, opened longitudinally and washed with PBS to remove fecal contents. The colon was cut into 3 pieces and placed into tubes containing 25 ml of Digestion I buffer (1xHBSS with 5% fetal bovine serum (FBS) (Giboco) and 1 mM EDTA (Sigma)). The epithelial cells of the colons were removed by shaking at 150 rpm for 15 minutes at 37 °C followed by gentle scrapping. Then, the colons were further cut into very small pieces and incubated in 30-50 ml of Digestion II buffer (1xHBSS, 20% FBS, 1 mg/ml collagenase D (Roche), 1 mg/ml dispase (Sigma), 40 μg/ml DNase-I (Sigma))/mouse colon for 1 hour at 37°C with shaking at 185 rpm. The cells were filtered through a 100 μm cell strainer and a 40 μm cell strainer sequentially and then washed with 1xHBSS containing 5% FBS and used for desired analyses.

In vitro differentiation of bone marrow-derived myeloid-derived suppressor cells (BMDSCs) and inhibitor treatment.

Bone marrow expansion and BMDMC differentiation were carried out based on our previous study and Cheng et al. with some modification[6, 7]. Bone marrow cells were isolated from femurs and tibias of mice and expanded with hematopoietic medium (IMD, 10% fetal bovine serum (FBS) (Gibco, MA, USA), 1x antibiotics- antimycotics, 1x non-

essential amino acids (HyClone, Utah, USA), and 20 ng/ml of GM-CSF, IL3, IL6 and SCF) (Biolegend, CA, USA) for 5 days[6, 7]. The expanded cells were cultured in MDSC differentiation medium (RMPI1640 supplemented with 10% FBS, 1x antibiotics-antimycotics, 1x non-essential amino acids, and 20 ng/ml of GM-CSF and IL6 for 7 days in the presence or absence of NF-κB/STAT3 inhibitor (BP-1-102, 5 μM) or JAK inhibitor AZD1480 (0.05 or 0.1 μM). Cells were used for flow cytometry analysis and immunoblotting (IB) analysis.

Flow cytometry analysis

Spleens from indicated mice were mashed and red blood cells were lysed by RBC Lysing buffer (Biolegend, CA, USA). About 1-2 x10^6 splenocytes, BMDSCs or LP cells were first incubated with Live/Dead Yellow Dye or Live/Dead Fixable Aqua Dye (Invitrogen or Biolegend, CA, USA) at room temperature (RT) for 10 minutes, washed with PBS, and then incubated with FcR blocking antibodies in FACs staining buffer (0.5% BSA and 0.05% NaN3 sodium azide in PBS) on ice for another 10 minutes. After washing with FACs buffer (0.5% BSA in PBS), cells were incubated on ice or at 4 $^{\circ}$C in the dark with an appropriate combination of the following cell surface staining antibodies in the FACs staining buffer. After 30 minutes incubation, cells were washed with FACs buffer, then either fixed with 4% paraformaldehyde or resuspended with FACs buffer immediately followed by flow cytometry analysis. To perform intracellular or nuclear staining, cells were fixed using Fixation buffer, permeabilized using Intracellular Staining Permeabilization Wash Buffer (Biolegend) and then incubated with antibodies in the Permeabilization Wash Buffer followed by flow cytometry analysis. Flow data was

acquired via LSRFortessa Flow Cytometer (BD, CA, USA) equipped with 405 nm Violet laser, 488 nm blue laser, 561 nm yellow laser, 640 nm red laser and FACS DIVA software version 6.2. All flow analysis was done using FlowJo version 10 (Treestar).

Antibodies used for flow cytometry analysis were purchased from Biolegend or other vendors as indicated: B220-BV510, CD3-PE-Cy5.5 (BD, CA, USA), CD3-AF700 (eBioscience), CD4-PE-Cy5, CD8-PE-Cy5.5, CD11b-PE-Cy7, CD11b-APC-Cy7, CD11c-SB465 (eBioscience), CD11c-PE-Dazzle 594, CD45-BV570, CD45-FITC, CD19-PE-Cy7, Gr-1-PE-Cy5, Gr-1-APC-Cy5, Ly6C-BV570, Ly6C-BV605, Ly6G-BV650, Ly6G-PerCPEF710 (eBioscience), MHC II-SB600 (eBioscience), IL6-APC and p-STAT3(Y705)-APC (BD, CA, USA).

PrimeFlow RNA analysis at the single cell level

The LP was isolated from WT and GNAI1;3DKO mice untreated or treated with 2.5% DSS. Then, *Gnai1*, *Gnai2* and *Gnai3* mRNA expression were analyzed at the single cell level by flow cytometry in combination of *Gnai1*, *Gnai2*, *Gnai3* PrimeFLow probe sets with Live/Dead dye, CD45 and CD11c antibody staining via PrimeFlow RNA Assay Kit (Invitrogen) according to manufacturer's protocols[8, 9]. Cells were analyzed on a LSRFortessa Flow Cytometer. Data were analyzed using FlowJo software.

IL6 neutralization

Purified anti-IL6 InVivoMab (MP5-20F3) and its control IgG isotype (Bio-X-Cell) were diluted in InVivoPure pH 7.0 Dilution Buffer (Bio-X-Cell) followed by

intraperitoneal injection into mice (0.5 mg/mouse). The frequencies and doses of the injection depended on desired experiments.

Cell culture and treatment

WT and KO MEFs were cultured and maintained in DMEM supplemented with 10% FBS and 1x antibiotics- antimycotics. BMDSC culture was described above. Cells were starved overnight and then treated with IL6 (50 ng/ml) in the presence or absence of inhibitors (5z-7-oxozeaenol (5z, Sigma, 2 μM), AZD1480 (AZD, Selleckchem, 3 μM), BP-1-102 (Millipore, 5 μM), MB-120L (Millipore, 30 μM), N-acetyl-L-cysteine (NAC, Sigma, 10 mM), or PKA inhibitor fragment (6-22) amide (Torcris Bioscience, 20 μM)) or left untreated for the indicated time points.

Enzyme-linked immunosorbent assay (ELISA) analysis

Serum or supernatant of the colons cultured for 24 hours were collected and the levels of GM-CSF, IFNγ, IL6, IL-12, IL-17, or TNF were determined by ELISA kits (Biolegend) based on the manufacturers' instructions[7, 10, 11].

Immunoblotting (IB) analysis

The cells were lysed in a buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.1% sodium deoxycholate, 1%TritonX-100, 1.0 mM sodium orthovanadate, 10 mM β-glycerophosphate, 0.5 mM EDTA, 0.5 mM DTT, and Complete Protease Inhibitor Cocktail (Roche Applied Sciences, MO, USA). Equal amounts of total protein from each sample were fractionated by SDS–PAGE and blotted onto polyvinylidene difluoride

(PVDF, Millipore) membrane. The membranes were stripped and re-probed with the indicated primary antibody of interest and then with secondary antibody, followed by detection with Pierce ECL (FisherScientific, MA, USA). IB analysis was performed with following antibodies: GP130, INOS, JAK2, STAT3, phosphor (p)-IκBα(S32), p-JAK2(Y1007/1008), p-NF-κBp65(S536), p-STAT3(Y705) (Cell Signaling, MA, USA), GNAI1, GNAI2, GNAI3, CHUK/IKKB, NF-κB, TAK1 and TRAF6 (Santa Cruz Biotechnology, TX) and β-actin (Sigma-Aldrich, MO, USA).

Immunoprecipitation (IP)

The cells were lysed in a buffer containing 40 mM Tris-HCl (pH 7.5), 120 mM NaCl, 50 mM NaF, 0.3%% CHAPS, 1.0 mM sodium orthovanadate, 10 mM β-glycerophosphate, 10 mM pyrophosphate, 1 mM EDTA, and Complete Protease Inhibitor Cocktail (Roche Applied Sciences, MO, USA). 0.6 to 1 mg of proteins, which were precleared with protein A/G Sepharose (beads) for 1 hour, were incubated with antibodies at 2-4 μg/mg of proteins overnight with shaking, and then incubates with protein A/G beads for another two hours. The beads were washed four times with lysis buffer and once with 1x PBS, and then boiled with laminae buffer, loaded on 10% SDS-PAGE, and the proteins were transferred on a PVDF membrane followed by IB analysis.

RNASeq library preparation and sequencing

RNA was isolated from colonic tumor tissues of WT and GNAI1;3DKO mice challenged with AOM-DSS for 9 weeks. RNA-Seq libraries were prepared in the University of Colorado Denver Genomics and Microarray Core from 200-500 ng of each

sample of total RNA using the TruSeq® Stranded mRNA Sample Preparation Kit (Cat# RS-122-2101, Illumina) according to the manufacturer's instructions. Samples were subjected to poly-A mRNA selection using oligo-dT beads. Purified mRNAs were then fragmented for first strand and second strand cDNA synthesis. Purified double-stranded cDNAs were end repaired, 3' adenylated, adaptor ligated and enriched by limited PCR amplification. Libraries were finally purified using the Agencourt AMPure Beads. An Agilent 2200 TapeStation (DNA 1000 screen tape) was used to evaluate the quality and quantity of the library. RNA sequencing was performed at the University of Colorado Denver Genomics and Microarray Core Facility on an Illumina® HiSeq 2500 using standard protocols for single read 50bp sequencing. The core typically generates ~10 Gb of raw sequencing data per lane, or up to 200M reads per lane. Samples were multiplexed at 6x to provide a minimum of 20M mappable reads/sample. RNA-Seq data was analyzed with TopHat (ver2.1.0) and Cufflinks (ver2.2.0). Sorted by the log2-fold change between each treatment group and control group, the trend of gene expression was recognized for potential biological significance in inflammation, immunology and colon cancer pathways.

Public microarray data analysis

For the gene expression data set GSE 39582 and GMS4107, raw data (CEL files) were downloaded from the NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/). These data were normalized using MAS 5.0 algorithm followed by $\log_2$ transformation[12]. The GSE39582 was to evaluate the potential association between the expression of *Gnai1*, *Gnai2* and *Gnai3* and relapse-free survival

of CRC patients. We classified the CRC patients into different groups using the median expression value of each gene as the cut-off point, and then compared the survival using Kaplan-Meier survival analysis among these groups. The Coxph algorithm is added to the plot shown in Figure 7*G*. The GSE 4107 was used to assess the expression of *Gnai1*, *Gnai2* and *Gnai3* of human healthy colonic mucosa and CRC colonic mucosa by means of Student *t* test. Kaplan-Meier survival analysis was performed using Bioconductor package "survcomp"[13]. All the analyses were performed with the use of R (version 2.15.0, www.r-project.org) and Bioconductor packages[14].

Statistical Analysis

Data are expressed as mean ± S.D or S.E.M. Except analytical methods for body weight, the public DNA microarray database, RNA expression, the RNA Seq data and the survival study, statistical analysis was conducted using Student *t* test. For RNA expression analysis, sensitivities, specificities and ROC/AUC were calculated to evaluate each gene expression. Statistical analyses were performed in GraphPad Prism 6.0 (GraphPad Software, Inc. San Diego, CA) and significant differences were defined as *P* value of < 0.05.

Supplementary Figure legends

Figure S1. (*A*) PCR genotyping analysis for WT and GNAI123cTKO mice. (*B*) IB analysis for expression of GNAI1, GNAI2 and GNAI3 in WT and GNAI123cTKO MDSCs. (*C*) PCR genotyping analysis for $GNAI2^{flox/flox}$ and Vil^{Cre} in GNAI123VilTKO mice.

Figure S2. (*A*) A schematic procedure of the AOM-DSS-induced colon cancer mouse model. (*B*) Body weight change of AOM-DSS-challenged GNAI1;3DKO v.s. WT mice (n=12). (*C*) Colitis severity score assessed on day 25 of the experiment (n=12). (*D*) Average colon length of WT, GNAI1KO, GNAI3KO or GNAI1;3DKO shown in Figure 1*F*. Data are means ± S.D; **P*<0.05 and *P*<0.0001; Student *t* test. (*E*) Schematic procedure of kinetic experiments in the AOM-DSS-induced colon cancer mouse model. (*F*) Mice were euthanized on days 0 and 13, 49 or 93; shown are representative images of the colon for indicated types of mice in the time -point experiments shown in Figure 1*H*. (*G*) Left panel: H&E stains of paraffin-embedded colon sections from WT and GNAI1;3DKO mice shown in Figure 1*H*. Shown are representative images from different time points in the study. Scale bars: 100 μM (upper) and 25 μM (lower). Right panel: Histological analysis.

Figure S3. (*A*) Heatmap analysis of gene expression via RNAseq analysis in the colon tissues of WT, GNAI2KO and GNAI1;3DKO mice challenged with or without AOM-DSS for 63 days. RNA was isolated from mouse colon tumor tissues (n=3) or corresponding normal colon tissues and then subjected to RNAseq. The log2-fold-change

(challenged/unchallenged control) in the gene expression was used. (*B*) Spleens from WT and GNAI1;3DKO mice challenged with AOM-DSS for 93 days. (*C*) IL6 levels in $CD4^+$, $CD8^+$ or $CD11b^+$ DCs from AOM-DSS-treated mice on day 93 were determined by flow cytometry (left) and statistically analyzed (right). *P<0.05; ns= not significant. (*D-F*) Quantitative analysis of B cells ($B220^+CD19^+$), $CD4^+$ T cells and $CD8^+$ T cells in WT, GNAI2KO and GNAI1;3DKO mice challenged with AOM-DSS for 63 days. Data are analyzed as mean ± SD.

Figure S4. (*A-B*) The levels of IL6, GM-CSF and TNF in the serum (*A*) or the supernatant of colon culture (*B*) of AOM-DSS-challenged WT and GNAI1;3DKO mice on day 63 (n=5). (*C*) IHC staining and histological analysis of p-NF-κBp65(S536) and p-STAT3(Y705) positive colonic cells in AOM-DSS-challenged mice (n=4) on day 63. Scale bars: upper, 100 μM; lower, 25 μM. (*D*) GNAI1;3 deficiency augments NF-κB activation by TNF. WT and GNAI1;3DKO MEFs were treated with TNF (20 ng/ml) for the indicated time points. The levels of p-NF-κB and NF-κB were determined by IB using anti-phospho (p)-NF-κBp65(S536) or anti-NF-κB antibodies. (*E*) IB for p-NF-κB, NF-κB and β-actin in WT and GNAI1;3DKO treated by IL6 in the presence or absence of IKKB/NF-κB inhibitor NAC. (*F*) IP/IB analysis for GNAI3's interaction with TAK1. (*G*) IB for INOS, p-NF-κB and NF-κB in WT and GNAI1;3DKO treated by IL6 in the presence or absence of TAK1 inhibitor 5z. (*H*) IP/IB analysis for ChUK/IKKB and TAK1 in indicated MEFs. (*I*) IP/IB analysis for JAK2, TAK1 and TRAF6 in indicated MEFs. (*J* IP/IB analysis for GNAI1, JAK2 and TAK1 in indicated MEFs. (*K*) IP/IB analysis for GNAI3, JAK2, TAK1 and TRAF6 in indicated MEFs. All analyzed data are

means ± SD; *P<0.05 and **P<0.01; ***P<0.001. ND: not detected. Two-tailed Student *t* test.

Figure S5. The IL6-JAK-NF-κB axis is required for GNAI2 and GP130 upregulation

(*A*) IB for GP130, p-STAT3(Y705), p-NF-κBp65(S536) and β-actin in WT cells treated with IL6 in the presence or absence of JAK inhibitor AZD. (*B*) IB for GP130, p-STAT3 and β-actin in WT cells treated with IL6 in the presence or absence of IKKB-NF-κB inhibitor NAC. (*C*) IHC analysis of paraffin-embedded colon tissues of WT and GNAI1;3DKO mice for GNAI2. n=3. (*D*) IB for GNAI2 and NF-κB in WT and GNAI1;3DKO MEFs treated with IL6 in the presence or absence of IKKB-NF-κB inhibitor ML120B. (*E*) IB for GNAI2, p-IκBα(S32), p-JAK2(Y1007/8), p-NF-κBp65(S536), p-STAT3(Y705) and β-actin in WT MEFs treated with IL6 in presence or absence of AZD. (*F*) WT and GNAI1;3 MEFs were treated with IL6 in the presence or absence of PKA inhibitor (PKAi) 6-22. IB analysis for GNAI2, INOS, NF-κB and β-actin. (*G-H*) Quantitative analysis of *Gnai2* mRNA expression in $CD45^+CD11b^+$, $CD45^+$MHC-II^+CD11c^+ and $CD45^+Ly6G^+$ cells in the LP isolated from DSS-treated WT and GANI1;3DKO mice on day 10 (n=6). (*I*) Representative FACS plots of and quantitative analysis of *Gnai1* or *Gnai3* in $CD45^+CD11c^+$ cells in the LP isolated from DSS-treated WT and GNAI1;3DKO mice on day 10 (n=6).

Figure S6. (*A-B*) IL6 and IL-12 levels in the culture supernatant of the colon from WT and GNAI2KO mice treated by DSS on day 9. (*C-G*) IL6, IL-12, TNFα, IFNγ, and IL-17

in the culture supernatant of the colon of WT and GNAI2KO mice challenged with AOM-DSS on day 63. (H) Tumor number of WT (n=5) and GNAI2LckKO (n=5) mice challenged with AOM-DSS on day 63.

Figure S7. (*A*) Representative images of colonic tumors from AOM-DSS-challenged WT, GNAI1;3DKO and GNAI123vilTDKO mice as shown in Figure 6*A*. (*B*) H&E representative images of colonic tumors from indicated AOM-DSS-challenged WT, GNAI1;3DKO and GNAI123vilTDKO mice shown in Figures 6*A*. Scale bars: upper, 100μM; lower, 25 μM. (*C*-*G*) ELSIA for the levels of serum IL6 (*C*) and the levels of IL6 (*D*), TNF (*E*), GM-CSF (*F*), and IL-12 (*G*) in the supernatant of cultured colon tissues from AOM-DSS-challenged WT, GNAI1;3DKO or GNAI123vilTKO mice (n=3). (*H*) Representative images of colonic tumors from AOM-DSS-challenged WT, GNAI1;3DKO and GNAI123cTDKO mice as shown in Figure 6*B*. (*I*) H&E representative images of colonic tumors from indicated AOM-DSS-challenged WT, GNAI1;3DKO and GNAI123cTDKO mice shown in Figures 6*B*. Scale bars: upper, 100μM; lower, 25 μM.

Figure S8. (*A*) IHC analysis for control rabbit IgG and p-NF-κBp65(S536) rabbit mAb in normal colon and CRC colon tumor tissues. (*B*) The 100x image of p-NF-κB-staining of paraffin-embedded colon tissue sections from a CAC subject was used to derive a 400x image shown in Figure 7B. (*C*) A summary of the alteration of GNAI1, GNAI2 and GNAI3 protein expression in T compared to N controls shown in (Figure 7*E*). (*D*) IHC

analysis of paraffin-embedded colon tissue sections from healthy subject (n=4) and CRC patients (n=26). Shown are representative images. Scale bar: 50 μM.

Figure S9. Schematic overview of 'GNAI1;3 suppress GNAI2-mediated CAC tumorigenesis thorough negatively regulating an IL6 signaling platform'.

GNAI1;3DKO mice exhibit markedly increases in CAC tumorigenesis, expansion of MDSCs and $CD4^+$ DCs but reduction of $CD8^+$ DCs and $CD11c^+$ MDSCs; all of which are reversed by additional GNAI2 deletion in CD11c+ cells. CAC tumorigenesis in GNAI1;3DKO mice is also associated with marked increases in GNAI2 and IL6 production and NF-κB activation. Blockade of IL6 largely diminishes MDSC expansion and CAC tumorigenesis stimulated GNAI1;3 deficiency.

Here we demonstrate that IL6 activates NF-κB through GP130, JAK2, TRAF6, TAK1 and CHUK/IKKB (in green) and induces NF-κB-dependent upregulation of CXCR2, GNAI2, GP130, INOS as well as expansion of MDSCs; all of which can be potentiated by GANI1;3 deficiency. GNAI1;3 form a complex with TAK1-TAB1, TRAF6, and JAK2. GNAI1;3 inhibit NF-κB activation by inhibiting TAK1-TAB1 interaction, and interrupting IL6-induced JAK activity-dependent interactions of TAK1 with JAK2 and TRAF6. There is a positive feed back loop, of which IL6 activates GP130, which in turn activates JAK2-TRAF6-TAK1-CHUK/IKKB-NF-κB cascade, then triggers GP130 upregulation. Upregulated GP130 further recruits STAT3 leading to its phosphorylation by JAKs and subsequent nuclear translocation and transcriptional activation (in yellow-orange-pink).

Our data also suggest a relationship between $CD4^+CD11c^+$ DCs, GNAI2-expressing CD11c+ cells and MDSCs in GNAI1;3DKO mice during CAC development. GNAI1;3DKO $CD4^+$ DCs produce high levels of IL6, which in turn induces upregulation of GNAI2 in CD11c+ cells and promotes expansion of MDSCs leading to CAC tumorigenesis.

Therefore, we conclude that GNAI1;3 suppress GNAI2- and IL6-mediated tumorigenesis and MDSC expansion through negatively regulating the IL6 signaling pathway.

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Figure S1

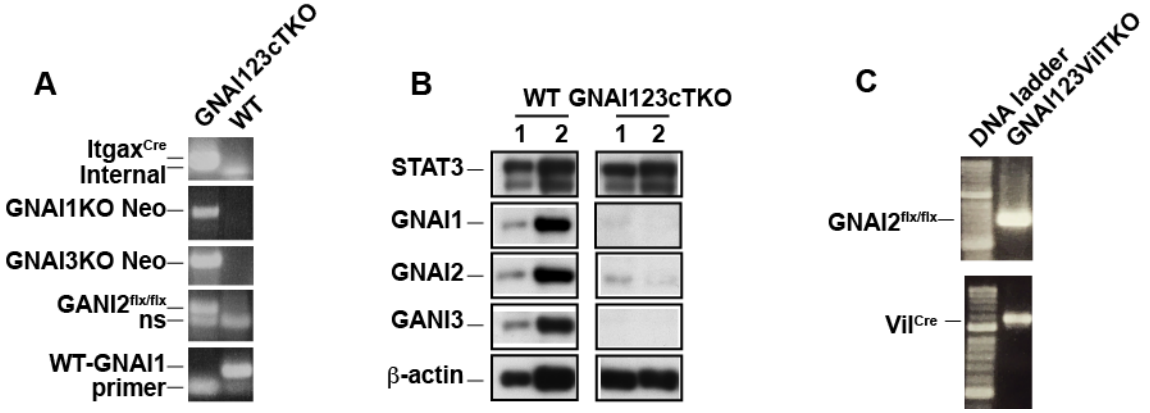
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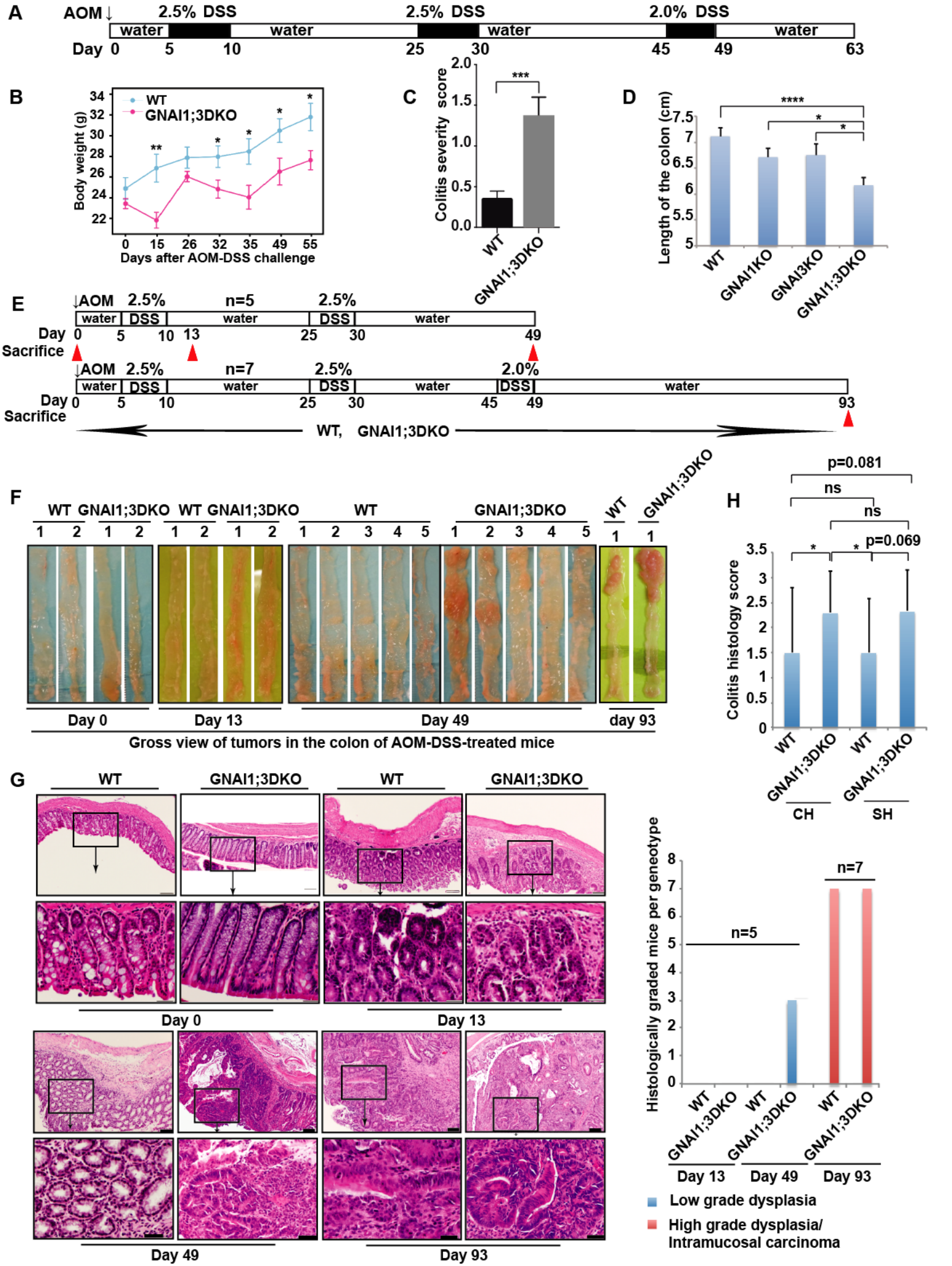
Figure S2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Days after AOM-DSS challenge
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Colitis severity score
WT
GNAI1;3DKO
D
Length of the colon (cm)
WT
GNAI1KO
GNAI3KO
GNAI1;3DKO
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WT, GNAI1;3DKO
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Gross view of tumors in the colon of AOM-DSS-treated mice
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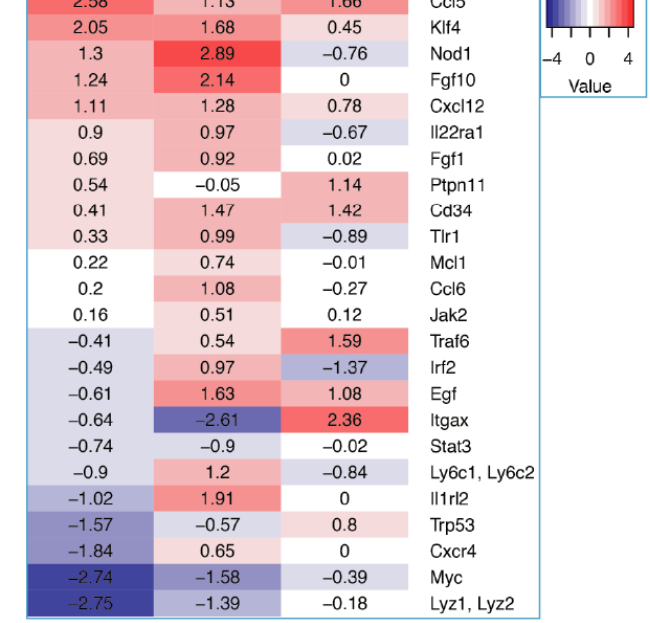
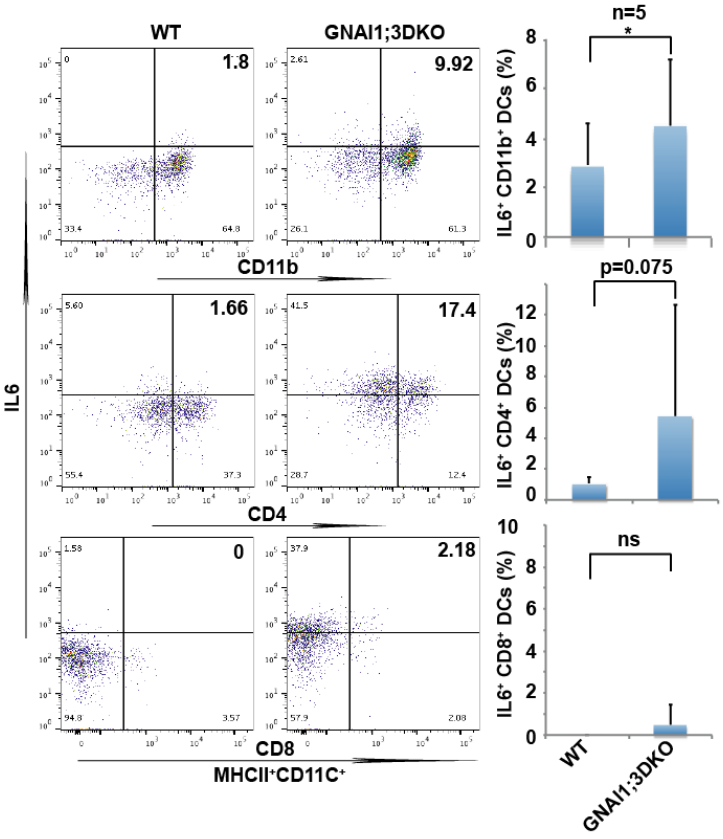
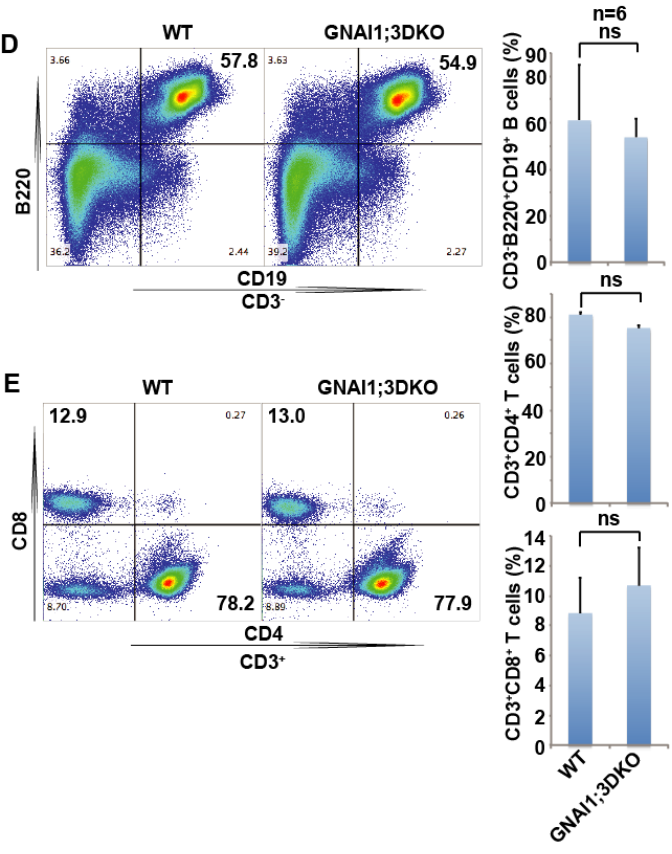
p=0.081
ns
ns
p=0.069
Colitis histology score
WT
GNAI1;3DKO
WT
GNAI1;3DKO
CH
SH
G
WT
GNAI1;3DKO
WT
GNAI1;3DKO
Day 0
Day 13
Day 49
Day 93
Histologically graded mice per geneotype
n=5
n=7
WT
GNAI1;3DKO
WT
GNAI1;3DKO
WT
GNAI1;3DKO
Day 13
Day 49
Day 93
Low grade dysplasia
High grade dysplasia/
Intramucosal carcinoma

Figure S3


A
Log2 folds
WT
GNAI1;3DKO
GNAI2KO
2.77 2.68 4.55 Foxp2
2.58 1.13 1.66 Ccl5
2.05 1.68 0.45 Klf4
1.3 2.89 −0.76 Nod1
1.24 2.14 0 Fgf10
1.11 1.28 0.78 Cxcl12
0.9 0.97 −0.67 Il22ra1
0.69 0.92 0.02 Fgf1
0.54 −0.05 1.14 Ptpn11
0.41 1.47 1.42 Cd34
0.33 0.99 −0.89 Tlr1
0.22 0.74 −0.01 Mcl1
0.2 1.08 −0.27 Ccl6
0.16 0.51 0.12 Jak2
−0.41 0.54 1.59 Traf6
−0.49 0.97 −1.37 Irf2
−0.61 1.63 1.08 Egf
−0.64 −2.61 2.36 Itgax
−0.74 −0.9 −0.02 Stat3
−0.9 1.2 −0.84 Ly6c1, Ly6c2
−1.02 1.91 0 Il1rl2
−1.57 −0.57 0.8 Trp53
−1.84 0.65 0 Cxcr4
−2.74 −1.58 −0.39 Myc
−2.75 −1.39 −0.18 Lyz1, Lyz2
Color Key
−4 0 4
Value


B
WT
GNAI1;3DKO
Spleen from mice challegend with AOM-DSS on day 93


C
WT
GNAI1;3DKO
1.8
9.92
CD11b
IL6
1.66
17.4
CD4
0
2.18
CD8
MHCII+CD11C+
n=5
*
IL6+ CD11b+ DCs (%)
p=0.075
IL6+ CD4+ DCs (%)
ns
IL6+ CD8+ DCs (%)
WT
GNAI1;3DKO


D
WT
GNAI1;3DKO
57.8
54.9
B220
CD19
CD3-
n=6
ns
CD3-B220+CD19+ B cells (%)
E
WT
GNAI1;3DKO
12.9
13.0
78.2
77.9
CD8
CD4
CD3+
ns
CD3+CD4+ T cells (%)
ns
CD3+CD8+ T cells (%)
WT
GNAI1;3DKO

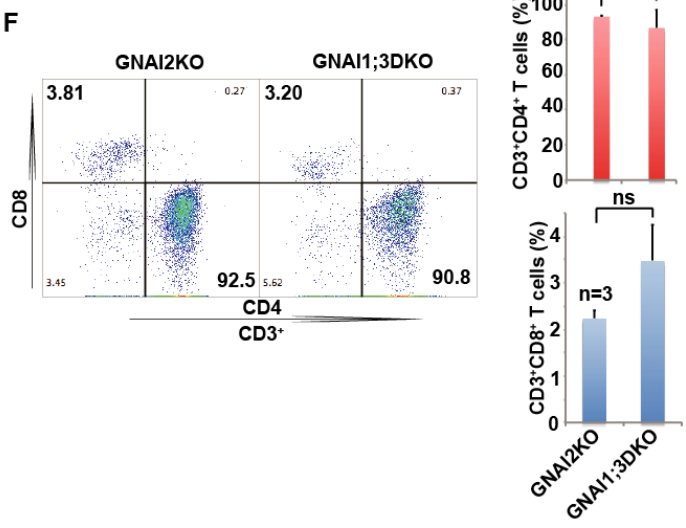
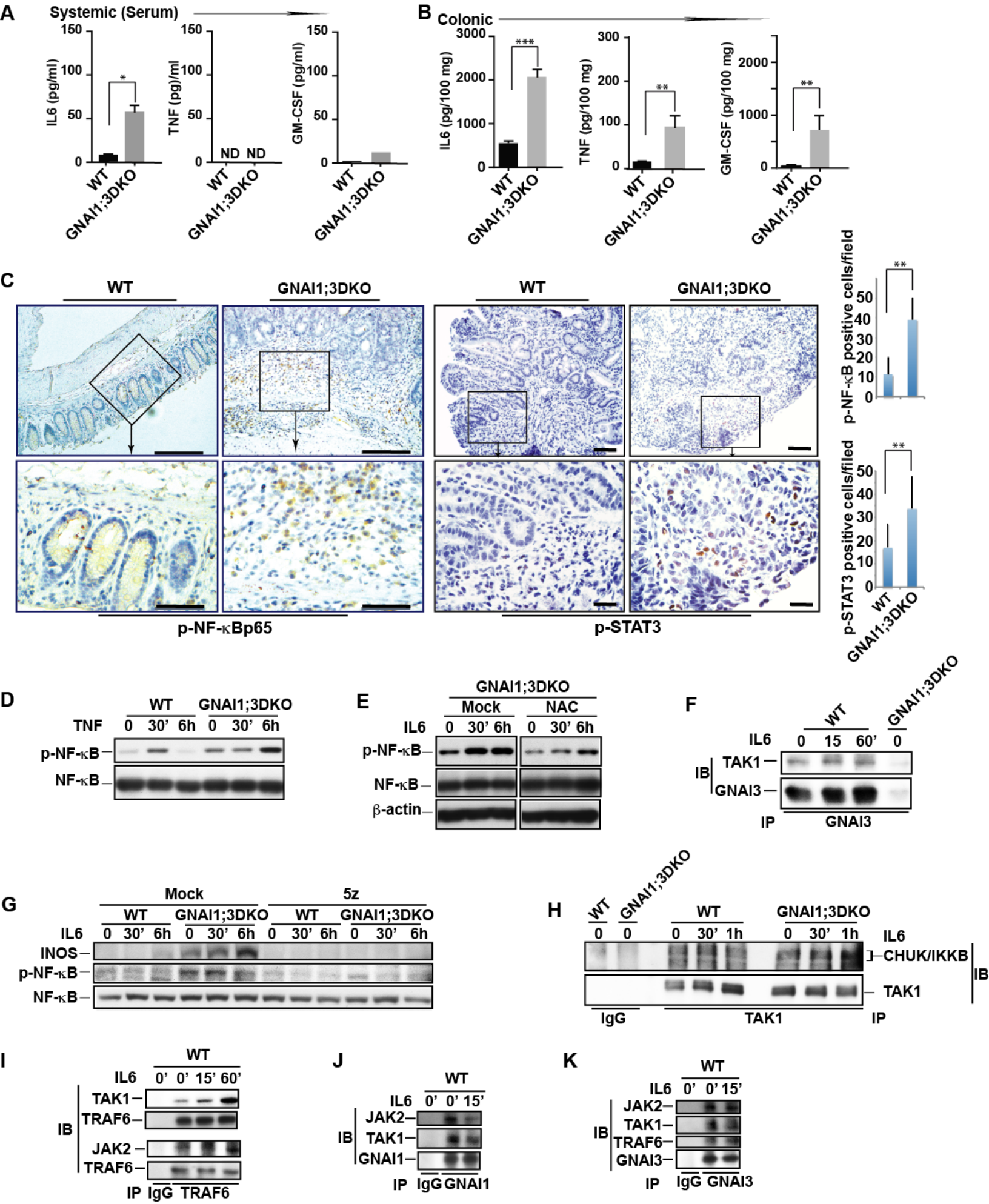

F
GNAI2KO
GNAI1;3DKO
3.81
3.20
92.5
90.8
CD8
CD4
CD3+
n=5
ns
CD3+CD4+ T cells (%)
ns
n=3
CD3+CD8+ T cells (%)
GNAI2KO
GNAI1;3DKO

Figure S4


A
Systemic (Serum)
IL6 (pg/ml)
TNF (pg)/ml
GM-CSF (pg/ml)
ND ND
WT
GNAI1;3DKO
B
Colonic
IL6 (pg/100 mg)
TNF (pg/100 mg)
GM-CSF (pg/100 mg)
C
WT
GNAI1;3DKO
p-NF-κBp65
p-STAT3
p-NF-κB positive cells/field
p-STAT3 positive cells/filed
D
TNF
p-NF-κB
NF-κB
E
Mock
NAC
IL6
β-actin
F
IL6
TAK1
GNAI3
IB
IP
G
Mock
5z
INOS
H
CHUK/IKKB
IgG
TAK1
I
TRAF6
JAK2
J
GNAI1
K

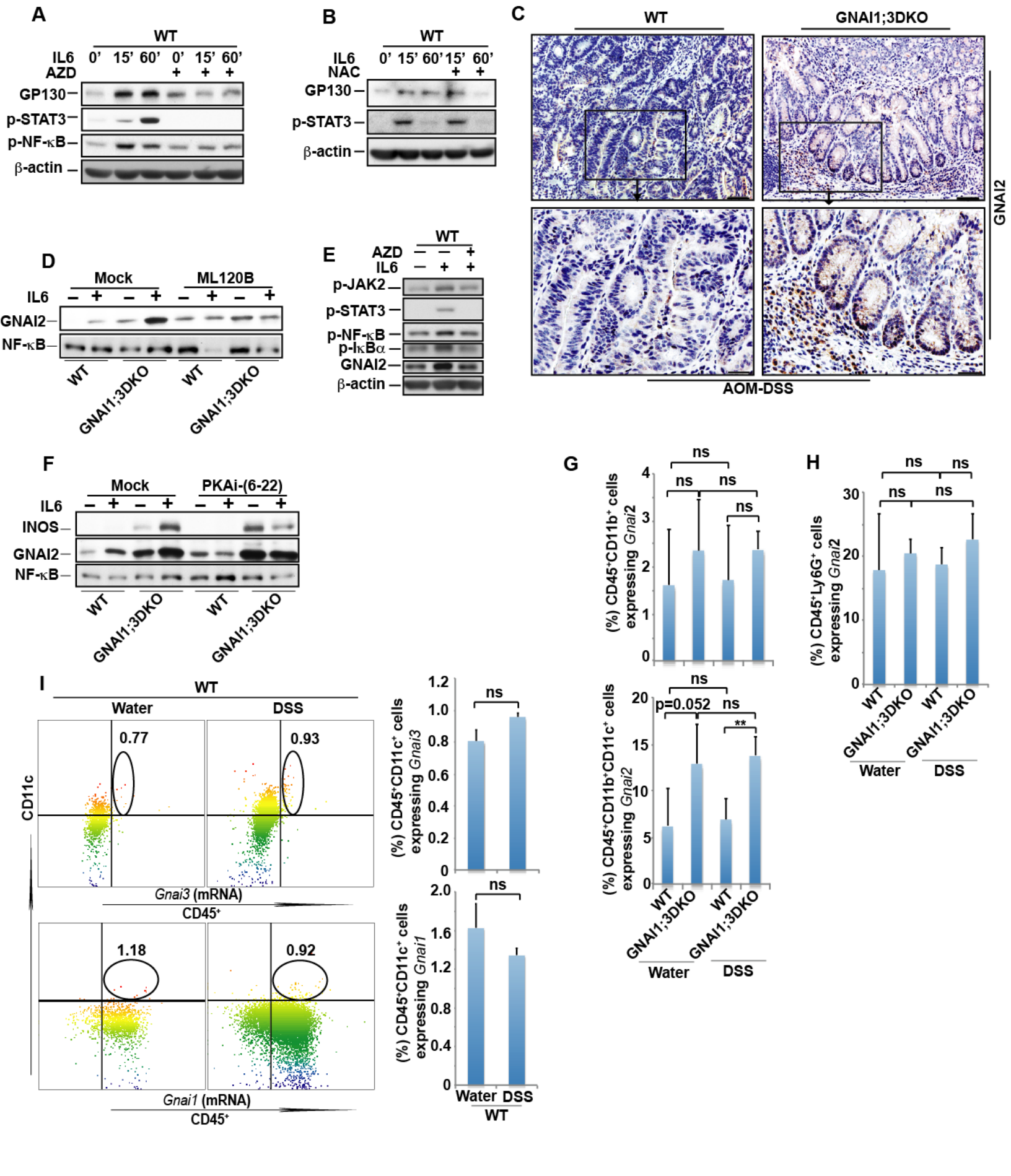
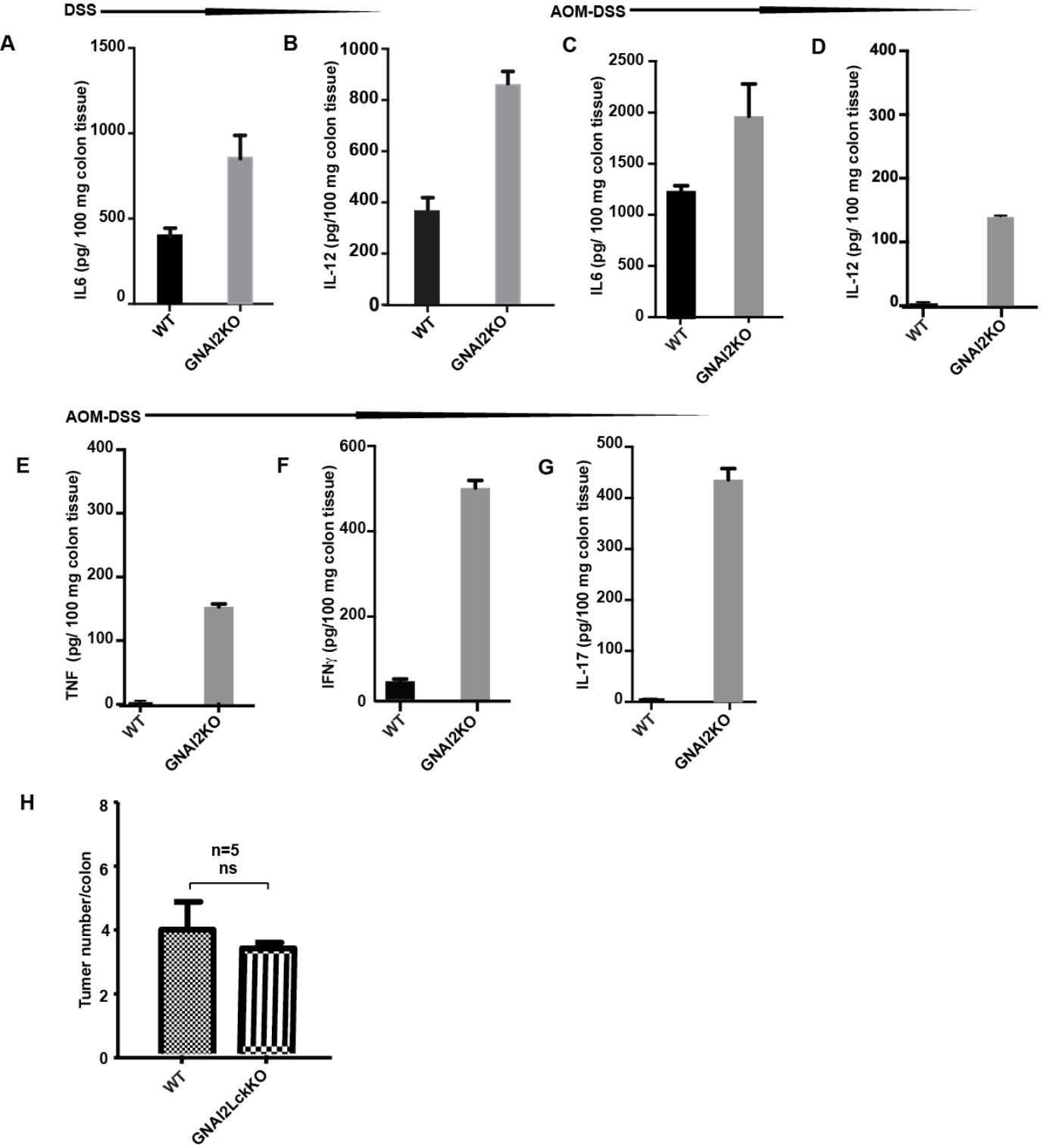

A
WT
IL6 0' 15' 60' 0' 15' 60'
AZD + + +
GP130
p-STAT3
p-NF-κB
β-actin
B
WT
IL6 0' 15' 60' 15' 60'
NAC + +
GP130
p-STAT3
β-actin
C
WT
GNAI1;3DKO
GNAI2
AOM-DSS
D
Mock
ML120B
IL6 – + – + – + – +
GNAI2
NF-κB
WT
GNAI1;3DKO
WT
GNAI1;3DKO
E
WT
AZD – – +
IL6 – + +
p-JAK2
p-STAT3
p-NF-κB
p-IκBα
GNAI2
β-actin
F
Mock
PKAi-(6-22)
IL6 – + – + – + – +
INOS
GNAI2
NF-κB
WT
GNAI1;3DKO
WT
GNAI1;3DKO
G
(%) CD45+CD11b+ cells expressing Gnai2
ns
ns
ns
ns
0
1
2
3
4
(%) CD45+CD11b+CD11c+ cells expressing Gnai2
ns
p=0.052
ns
**
0
5
10
15
20
WT
GNAI1;3DKO
WT
GNAI1;3DKO
Water
DSS
H
(%) CD45+Ly6G+ cells expressing Gnai2
ns
ns
ns
ns
0
10
20
30
WT
GNAI1;3DKO
WT
GNAI1;3DKO
Water
DSS
I
WT
Water
DSS
0.77
0.93
CD11c
Gnai3 (mRNA)
CD45+
1.18
0.92
Gnai1 (mRNA)
CD45+
(%) CD45+CD11c+ cells expressing Gnai3
ns
0
0.2
0.4
0.6
0.8
1.0
1.2
(%) CD45+CD11c+ cells expressing Gnai1
ns
0
0.4
0.8
1.2
1.6
2.0
Water
DSS
WT

Figure S6


DSS
AOM-DSS
A
IL6 (pg/ 100 mg colon tissue)
1500
1000
500
0
WT
GNAI2KO
B
IL-12 (pg/100 mg colon tissue)
1000
800
600
400
200
0
WT
GNAI2KO
C
IL6 (pg/ 100 mg colon tissue)
2500
2000
1500
1000
500
0
WT
GNAI2KO
D
IL-12 (pg/ 100 mg colon tissue)
400
300
200
100
0
WT
GNAI2KO
AOM-DSS
E
TNF (pg/ 100 mg colon tissue)
400
300
200
100
0
WT
GNAI2KO
F
IFNγ (pg/100 mg colon tissue)
600
400
200
0
WT
GNAI2KO
G
IL-17 (pg/100 mg colon tissue)
500
400
300
200
100
0
WT
GNAI2KO
H
Tumer number/colon
8
6
4
2
0
n=5
ns
WT
GNAI2LckKO

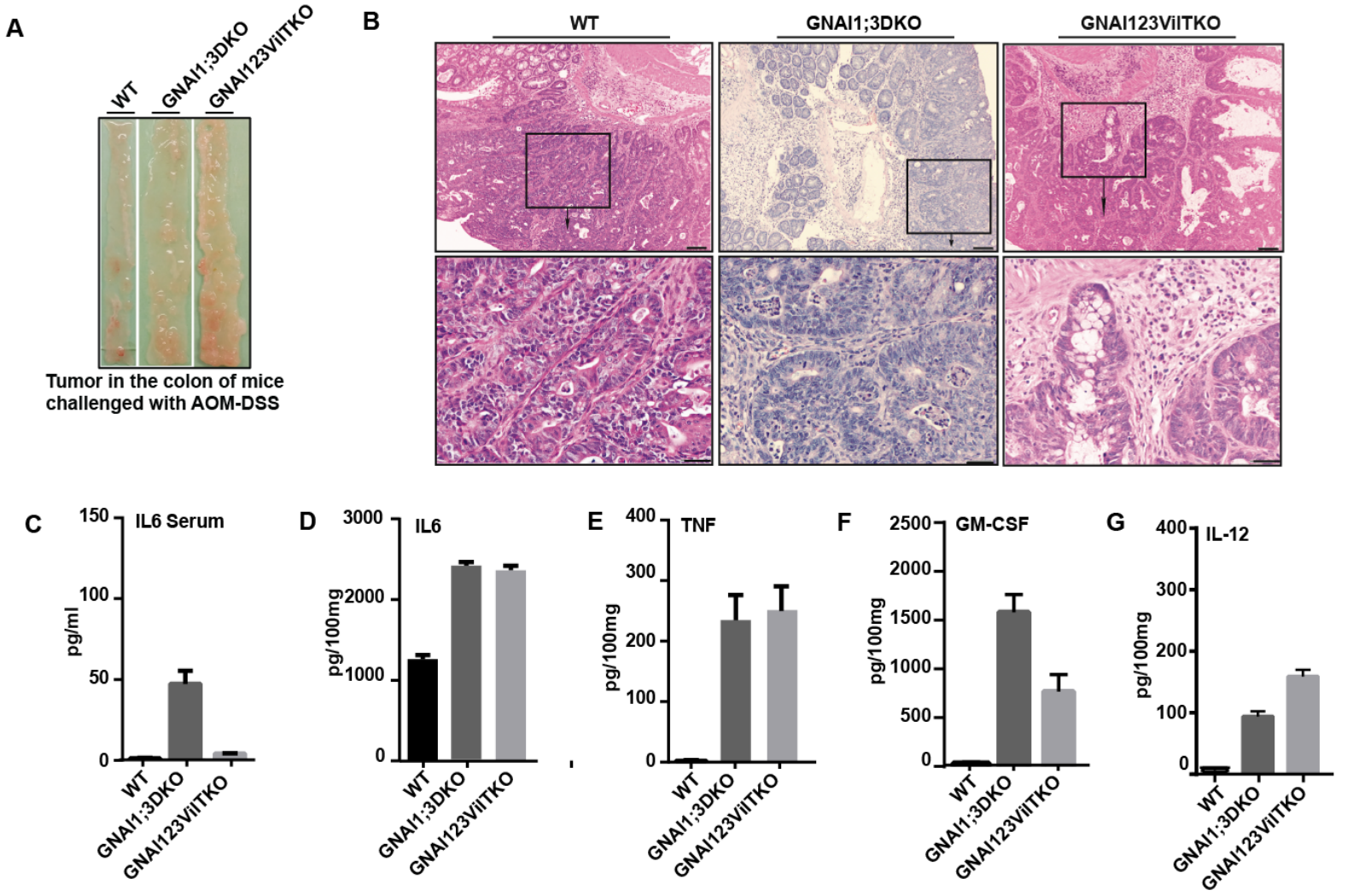

A
WT
GNAI1;3DKO
GNAI123ViiTKO
Tumor in the colon of mice challenged with AOM-DSS
B
WT
GNAI1;3DKO
GNAI123ViiTKO
C
IL6 Serum
pg/ml
150
100
50
0
WT
GNAI1;3DKO
GNAI123ViiTKO
D
IL6
pg/100mg
3000
2000
1000
0
WT
GNAI1;3DKO
GNAI123ViiTKO
E
TNF
pg/100mg
400
300
200
100
0
WT
GNAI1;3DKO
GNAI123ViiTKO
F
GM-CSF
pg/100mg
2500
2000
1500
1000
500
0
WT
GNAI1;3DKO
GNAI123ViiTKO
G
IL-12
pg/100mg
400
300
200
100
0
WT
GNAI1;3DKO
GNAI123ViiTKO
H
WT
GNAI1;3DKO
GNAI123cTKO
1
2
3
Tumor in the colon of mice treated with AOM-DSS
I
WT
GNAI1;3DKO
GNAI123cTKO

Figure S8

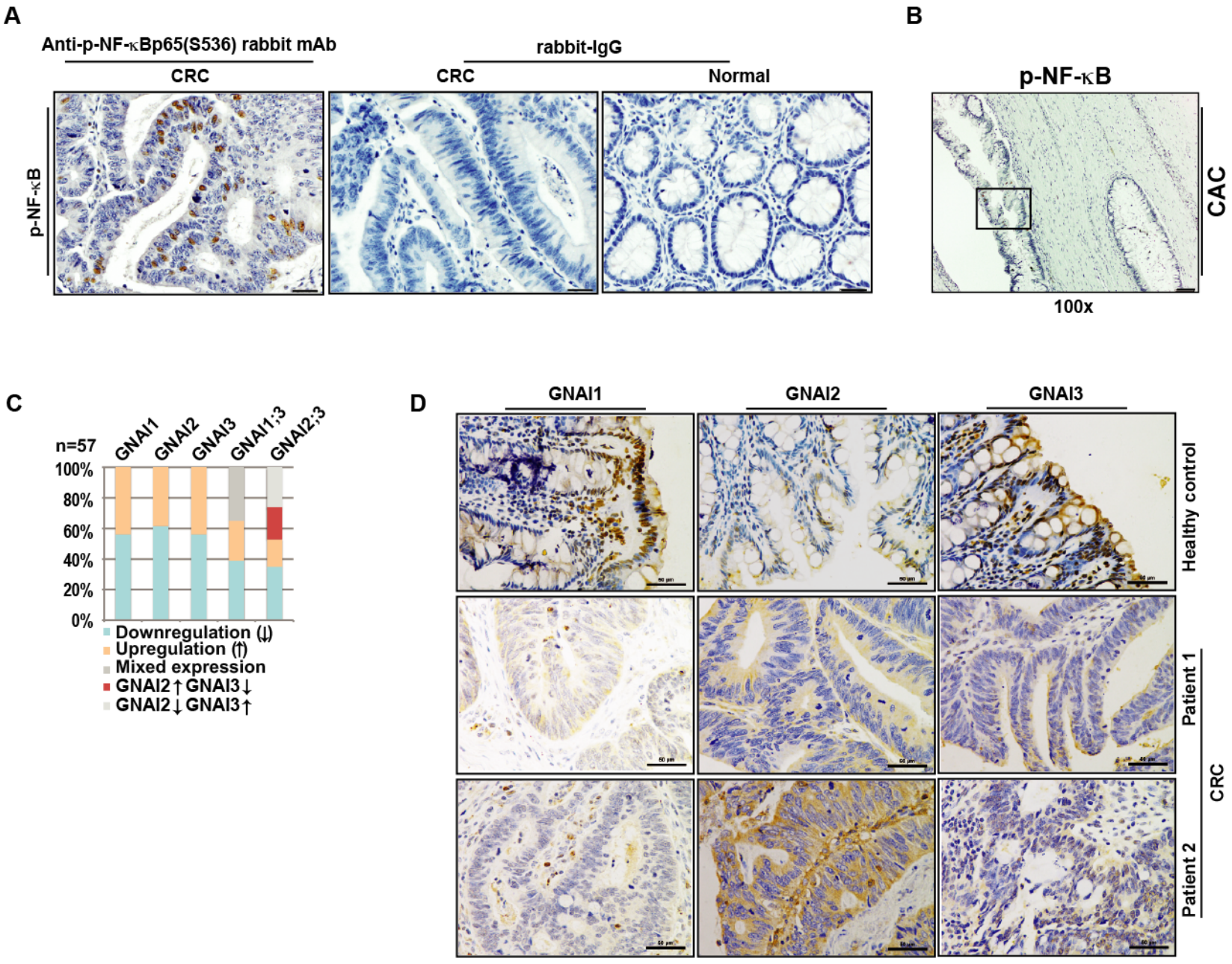
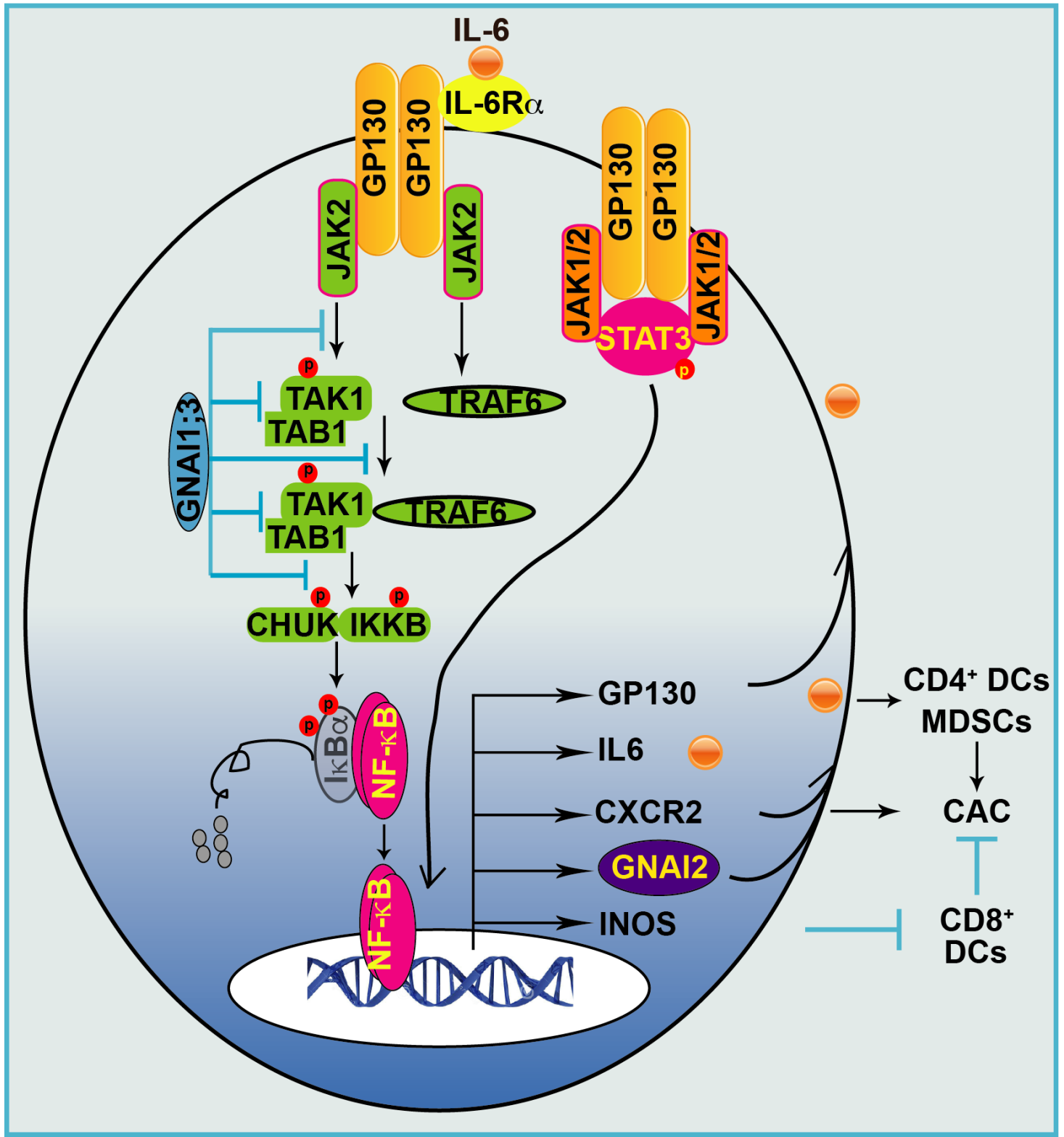

A
Anti-p-NF-κBp65(S536) rabbit mAb
rabbit-IgG
CRC
CRC
Normal
p-NF-κB
B
p-NF-κB
CAC
100x
C
n=57
GNAI1
GNAI2
GNAI3
GNAI1:3
GNAI2:3
100%
80%
60%
40%
20%
0%
Downregulation (↓)
Upregulation (↑)
Mixed expression
GNAI2↑GNAI3↓
GNAI2↓GNAI3↑
D
GNAI1
GNAI2
GNAI3
Healthy control
Patient 1
Patient 2
CRC

Figure S9


IL-6
IL-6Rα
GP130
GP130
JAK2
JAK2
JAK1/2
JAK1/2
STAT3
TAK1
TAB1
TRAF6
GNAI1;3
CHUK
IKKB
IκBα
NF-κB
GP130
IL6
CXCR2
GNAI2
INOS
CD4⁺ DCs
MDSCs
CAC
CD8⁺
DCs