Supplementary Methods

Colon organoid culture

Mouse colon stem cells were cultured in IntestiCult organoid growth medium according to the manufacturer's instructions (06005, STEMCELL Technologies). Colon obtained from wildtype and *Mefv*$^{-/-}$ mice was rinsed with cold PBS, cut into 2-mm segments and washed 20 times with cold PBS. Colonic segments were incubated in Gentle Cell Dissociation Reagent (07174, STEMCELL Technologies), on shaker (350rpm) for 15 min at room temperature, followed by re-suspension in PBS supplemented with 0.1% BSA (A6003, Sigma). Dissociated colonic crypts were filtered through 70-µm strainers and resuspended in DMEM/ F12 medium with 15mM HEPES (36254, STEMCELL Technologies). The cells were re-suspended in Intesticult organoid growth medium and Matrigel (356230, Corning) in a 1:1 ratio and plated in 24-well culture plates (3738, Corning). Intesticult organoid growth medium were added to the cell culture plates to immerse the matrix composed of Intesticult organoid growth medium and Matrigel.

CD40 model of colitis

Age and sex matched WT and *Mefv*$^{-/-}$ mice received intraperitoneal injection of 200µg anti-CD40 antibody (clone FGK4.5, BioXCell) in 1 ml of PBS. The mice were monitored for body weight and colitis assessed by measuring colon length at day 7 post treatment.

Cell Culture and Stimulations

Primary mouse bone marrow–derived macrophages (BMDMs) were cultured as previously described.[38] BMDMs were stimulated with 500 ng/mL ultra-pure LPS from *Salmonella minnesota* R595 (tlrl-smlps, InvivoGen) for the indicated time.

Immunoflourescence

Colon tissues were fresh frozen in OCT embedding medium. 10µm thick cryosections were fixed with cold 100% ethanol for 30 min prior to exposure to cold acetone for 10 min. Sections were allowed to airdry, and non-specific binding was blocked by incubation in PBS containing 2% bovine serum albumin and 5% normal goat serum for 30 min prior to incubation with anti-occludin antibody (clone 6HCLC, Thermo Fischer Scientific, 1:500 dilution) or anti-claudin2 antibody (ab53032, Abcam) overnight at 4°C. Slides were washed for 15 min in PBS prior to incubation with CR568-conjugated goat anti-rabbit (1µg/mL; Biotium) and AF488-conjugated phalloidin (0.04µM; Lifetechnologies) for 1 h at room temperature. Slides were washed for 15 min in PBS prior to mounting in Vectashield hard set with DAPI (Vector Laboratories). Fluorescent confocal images were acquired using a Zeiss AxioObserverZ.1 marianis system equipped with a CSU-X spinning disc, 40X EC Plan-NeoFluar 1.3NA oil objective and a Delta Evolve EMCCD camera (Photometrics), and analyzed using Slidebook software (3i Intelligent Imaging Innovations).

Supplementary Figure Legends

Supplementary Figure 1. Loss of Pyrin increases susceptibility to colitis-associated cancer in an independent mouse line. (A) Weight change in WT, *Mefv*$^{+/-}$, and *Mefv*$^{-/-}$ mice injected with AOM on day 0 and administered 3 rounds of 2% DSS in drinking water. **(B, D)** Number of tumors and **(C)** representative images of colon at day 80 after AOM injection. Data are analyzed by **(A)** two-way ANOVA, followed by the Holm–Sidak post-hoc test and **(B, D)** Mann–Whitney U test. Error bars represent mean±SEM and **(B)** each symbol represents an individual mouse, with 5–10 mice per group.

Supplementary Figure 2. Pyrin does not regulate cellular proliferation in intestinal stem cells. (A) Representative images and **(B)** size (diameter) of organoids at indicated time (days) during organoid growth. 50-80 organoids were photographed for each analysis. Data are analyzed by Mann–Whitney U test and error bars represent mean±SEM. Data is representative of 3 independent repeats.

Supplementary Figure 3. Pyrin expression is upregulated in response to inflammation. qPCR analysis and immunoblot for expression of Pyrin in BMDMs after LPS stimulation. Data represents at least 3 independent experiments.

Supplementary Figure 4. Role of Pyrin in DSS and CD40 models of colitis. (A) Body weight loss, and **(B)** colon length, and **(C)** representative images of colon after DSS-induced colitis. 3% DSS was administered in drinking water for 6 days followed by 3 days on normal drinking water. **(D)** Body weight change, **(E)** Colon length and

(F) representative images of colon 7 days after a single injection of anti-CD40 antibody (200μg/mouse). (**G**) Histological analysis and (**H**) representative image (20×) for H&E staining of colon tissue in mice 7 days after anti-CD40 injection. I, U, E, H and A stand for inflammation, ulceration, edema, hyperplasia and the area affected by histological perturbations, respectively. Data are analyzed by **(A, D, G)** two-way ANOVA, followed by the Holm–Sidak post-hoc test and **(B, E)** Mann–Whitney U test. **(A, B, D, E)** Error bars represent mean±SEM and **(B, E)** each symbol represents an individual animal. Data is **(A-C)** representative of 2 independent repeats, N=5 mice per group and **(D-F)** pooled from 2 independent repeats, N= 7-10 mice per group.

Supplementary Figure 5. Pyrin inflammasome inhibits systemic inflammation in response to DSS-induced colitis. (A) Serum cytokine levels in mice in response to DSS-induced colitis at day 9 and day 15 after AOM–DSS treatment. Data represent 2 independent experiments, with 8–10 mice per group for each time point. Error bars represent mean±SEM, and data were analyzed by the parametric or non-parametric t-test.

Supplementary Figure 6. Loss of Pyrin affects mucosal defense during acute colitis. qPCR analysis for **(A)** IL-22 and antimicrobial proteins (AMP, day 15 post AOM-DSS), **(B)** genes associated with goblet cell function (day 15 post AOM-DSS) and **(C)** tight junction proteins at indicated time points during colitis. **(D)** Immunoblot analysis for activation/cleavage of apoptotic caspases-3, -7, and -8 at day 9 post

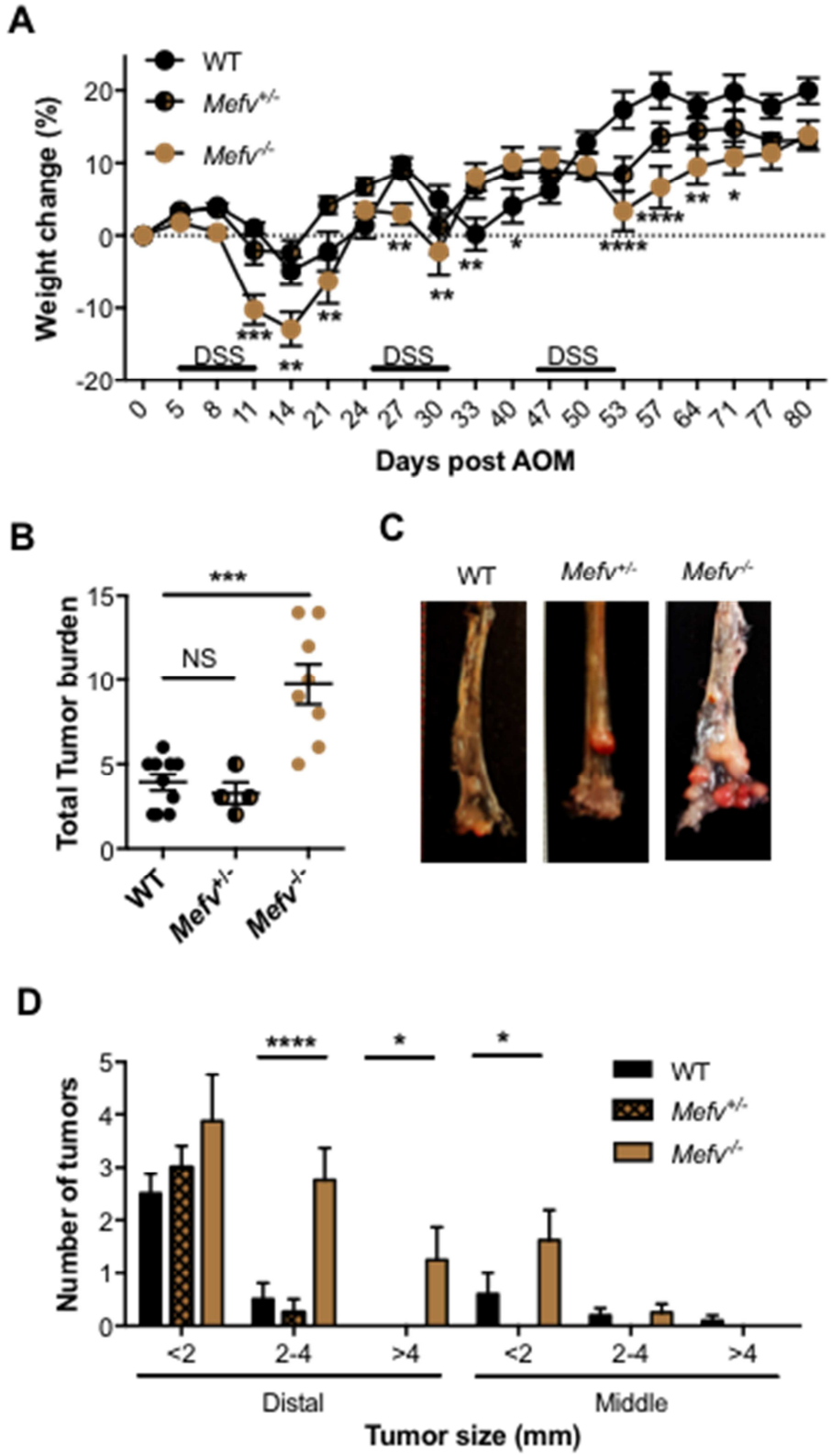
AOM-DSS treatment. **(E)** Intensity of occludin at the cell-cell junction (co-localized with F-actin) at day 0 (WT control) and 4 days after DSS administration. Each dot represents occludin intensity (Mean fluorescence intensity, MFI) along a crypt and 4-6 crypts were assessed for each field of view. Data represent at least 2 independent experiments, with 8–10 mice per group for each time point**.** Error bars represent mean±SEM, and data were analyzed by the parametric or non-parametric t-test.

Supplementary Figure 7. IL-18 complementation protects *Mefv*$^{-/-}$ mice from DSS-induced inflammation. (A) cytokine levels and (B) immunoblot for STAT3 activation in homogenates from colon tissue from mice in response to DSS-induced colitis. qPCR analysis for **(C)** *Ifng* and **(H)** epithelial stem cell markers in colon tissue from mice in response to DSS-induced colitis. CD8 T-cell activation in **(D)** spleen and **(F)** MLN of indicated mice in response to DSS-induced colitis. Representative flow blots for cytokine production in CD8 T cells from **(E)** spleen and **(G)** MLN of indicated mice in response to DSS-induced colitis. Data represent 2 independent experiments and are presented as mean±SEM. *N* = 9–10 mice per group. Error bars represent mean±SEM, and data were analyzed by the parametric or non-parametric t-test.

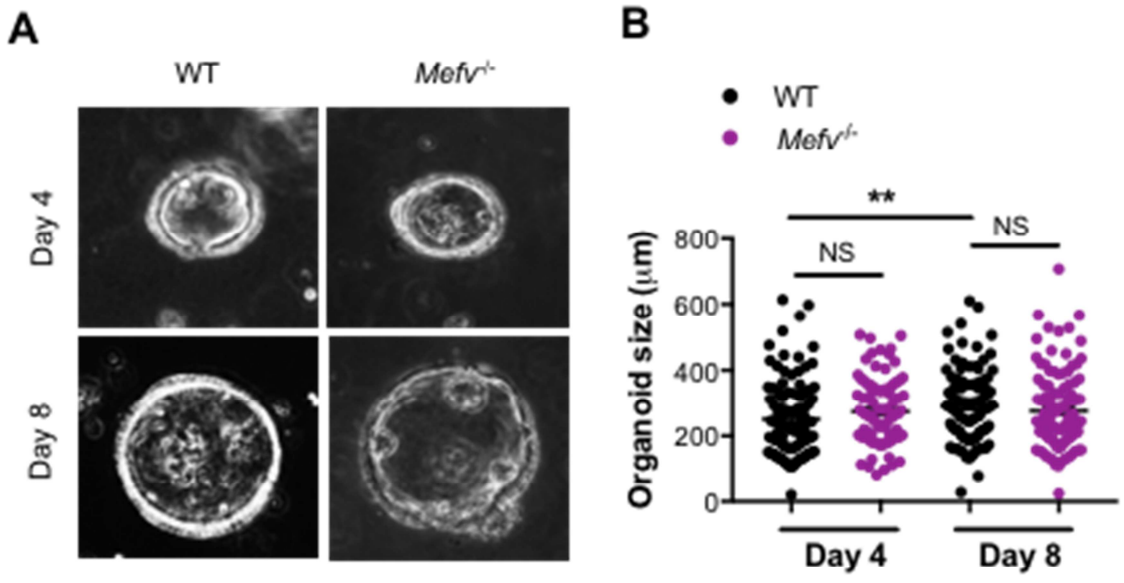
Supplementary Figure 8. A schematic for the role of Pyrin inflammasome in DSS induced colitis. Pyrin inflammasome is activated following DSS induced damage, leading to IL-18 production. The IL-18 functions to restrain loss of epithelial integrity, restricts pro-tumorigenic IL-6-STAT3 axis, and overt stem cell proliferation

while promoting anti-tumorigenic CD8 T cell response. Pyrin inflammasome mediated IL-18 production thus restricts colitis and associated tumorigenesis through multiple mechanisms.

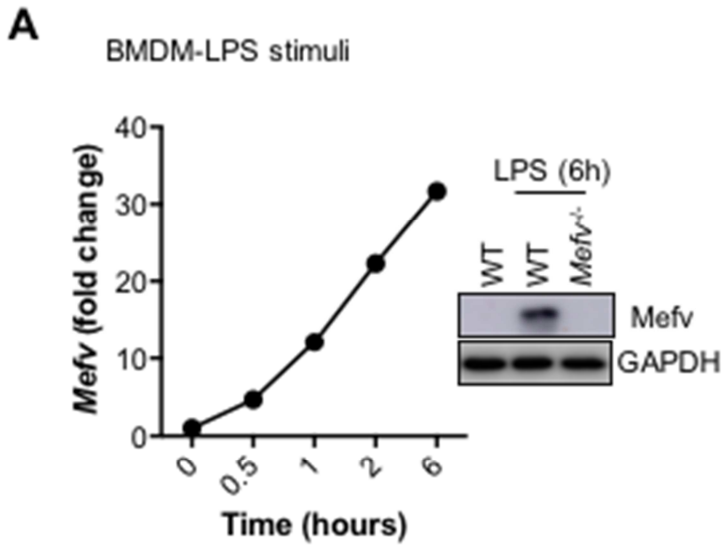
Supplementary Table 1. Primer sequences used for qPCR analysis.



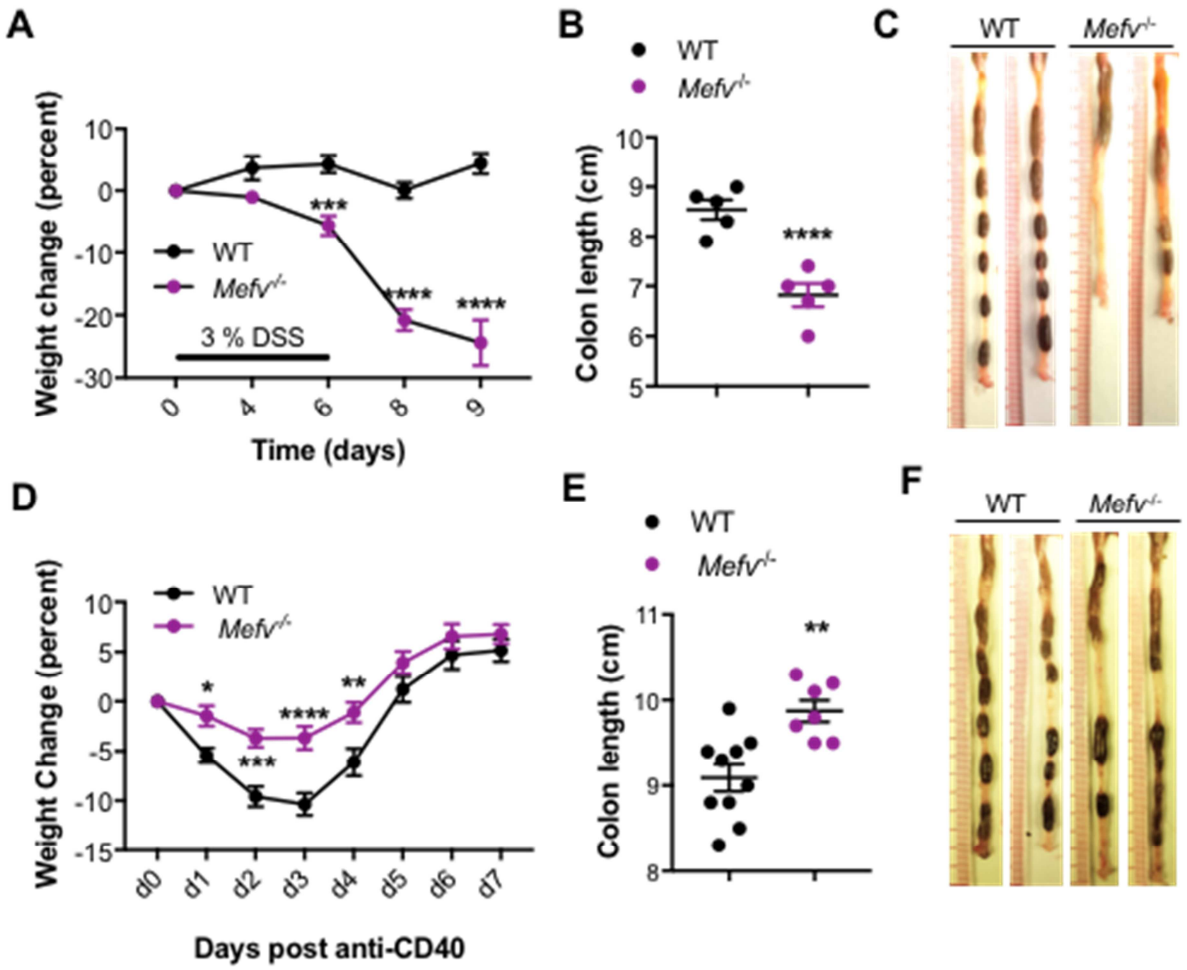
Supplementary Figure 1



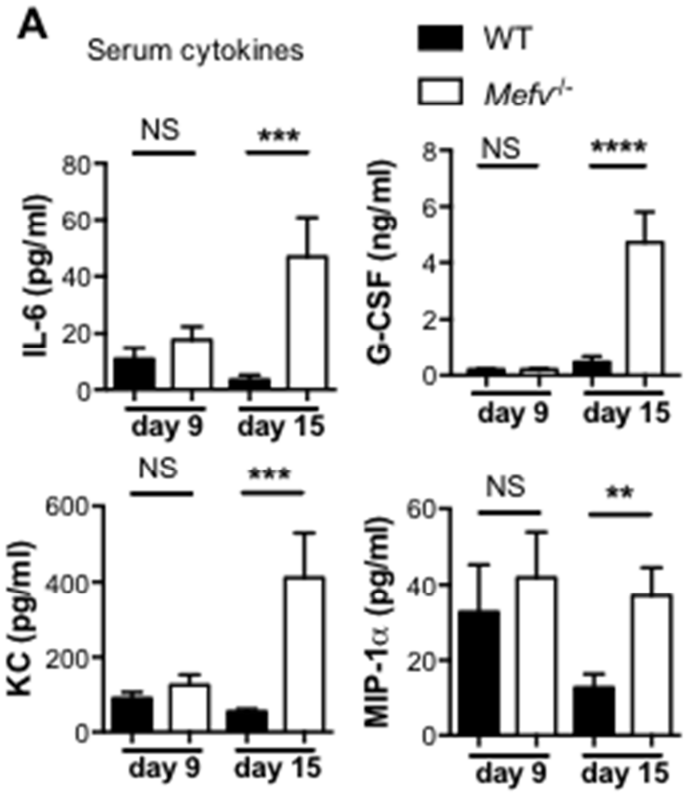
Supplementary Figure 2



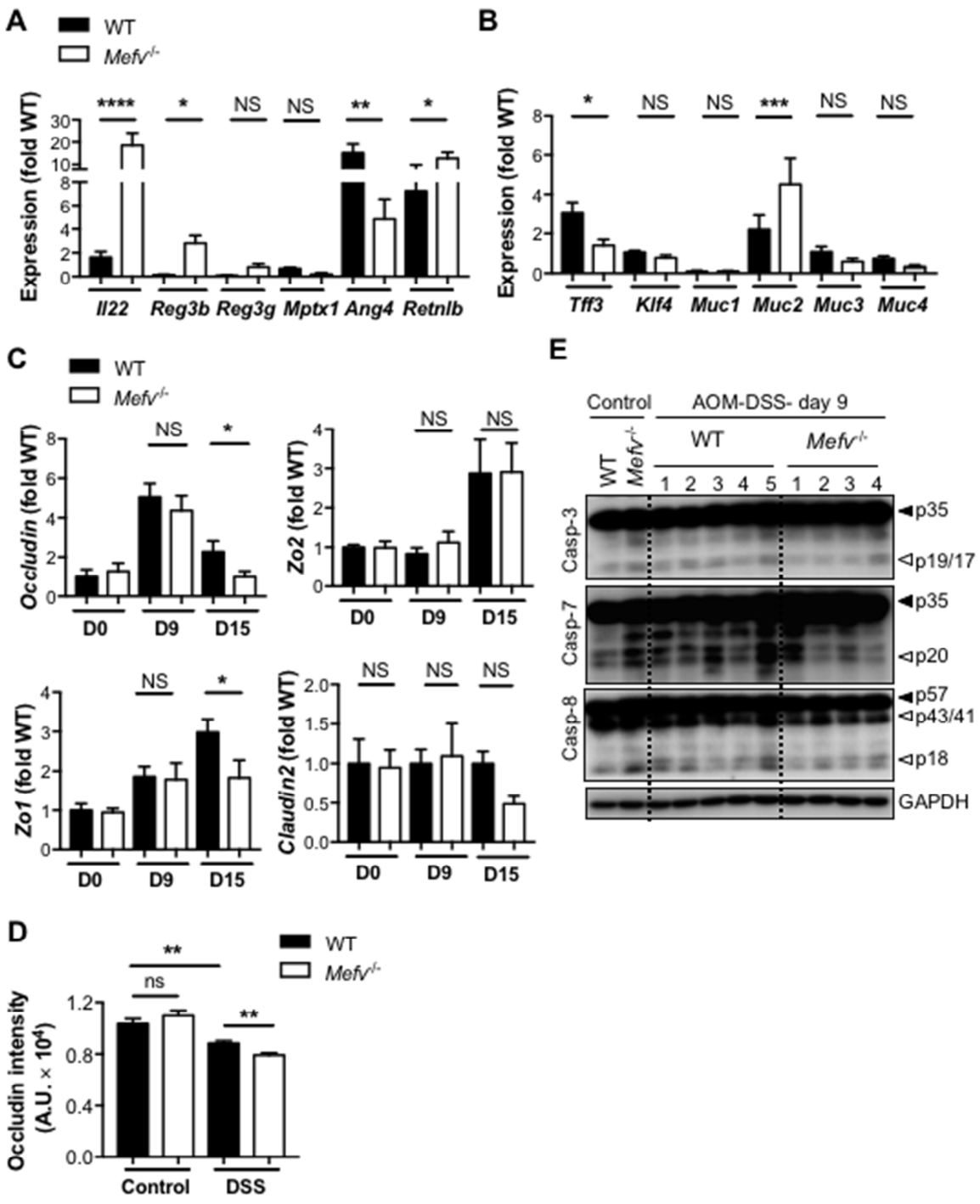
Supplementary Figure 3



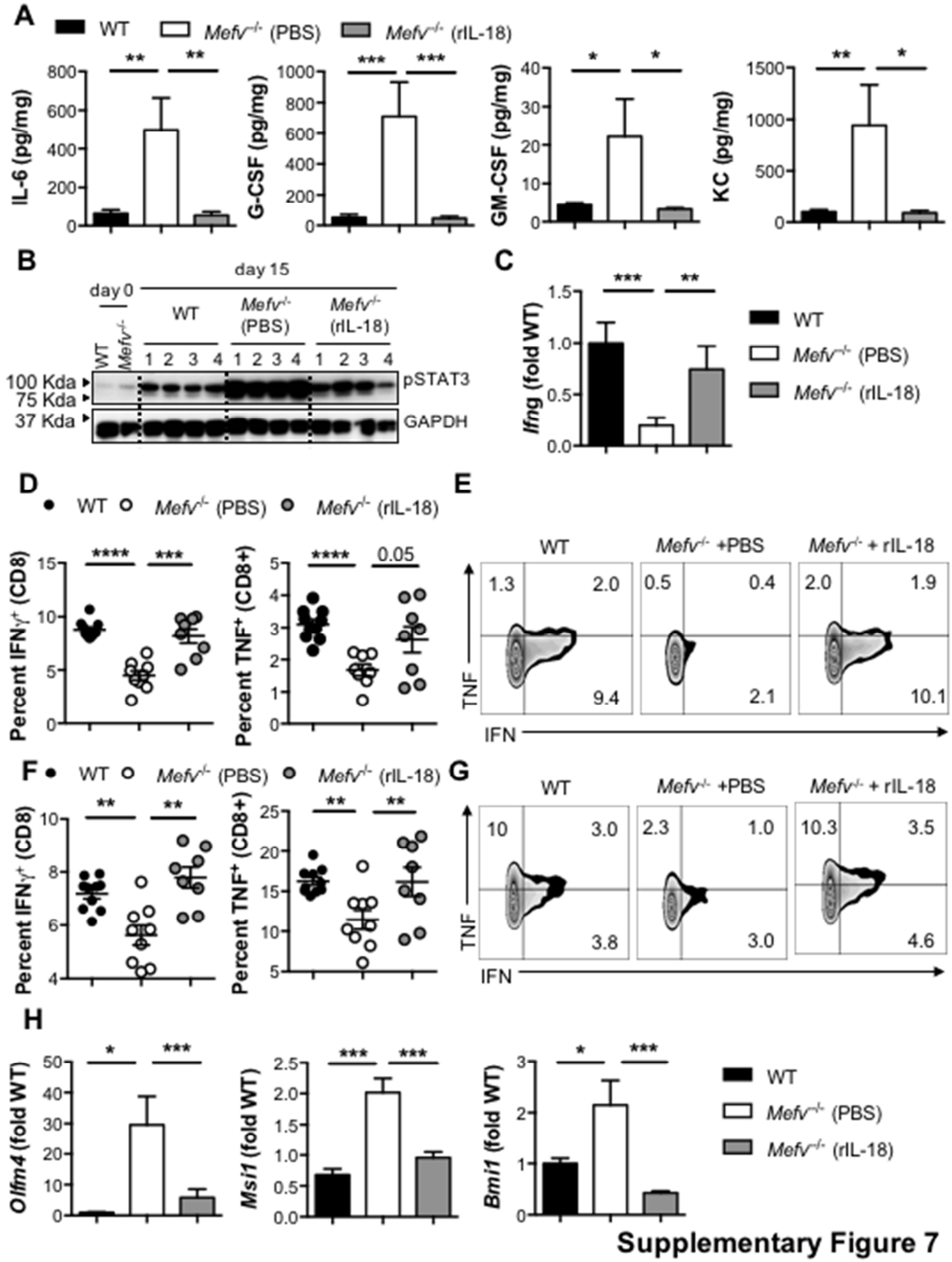
Supplementary Figure 4



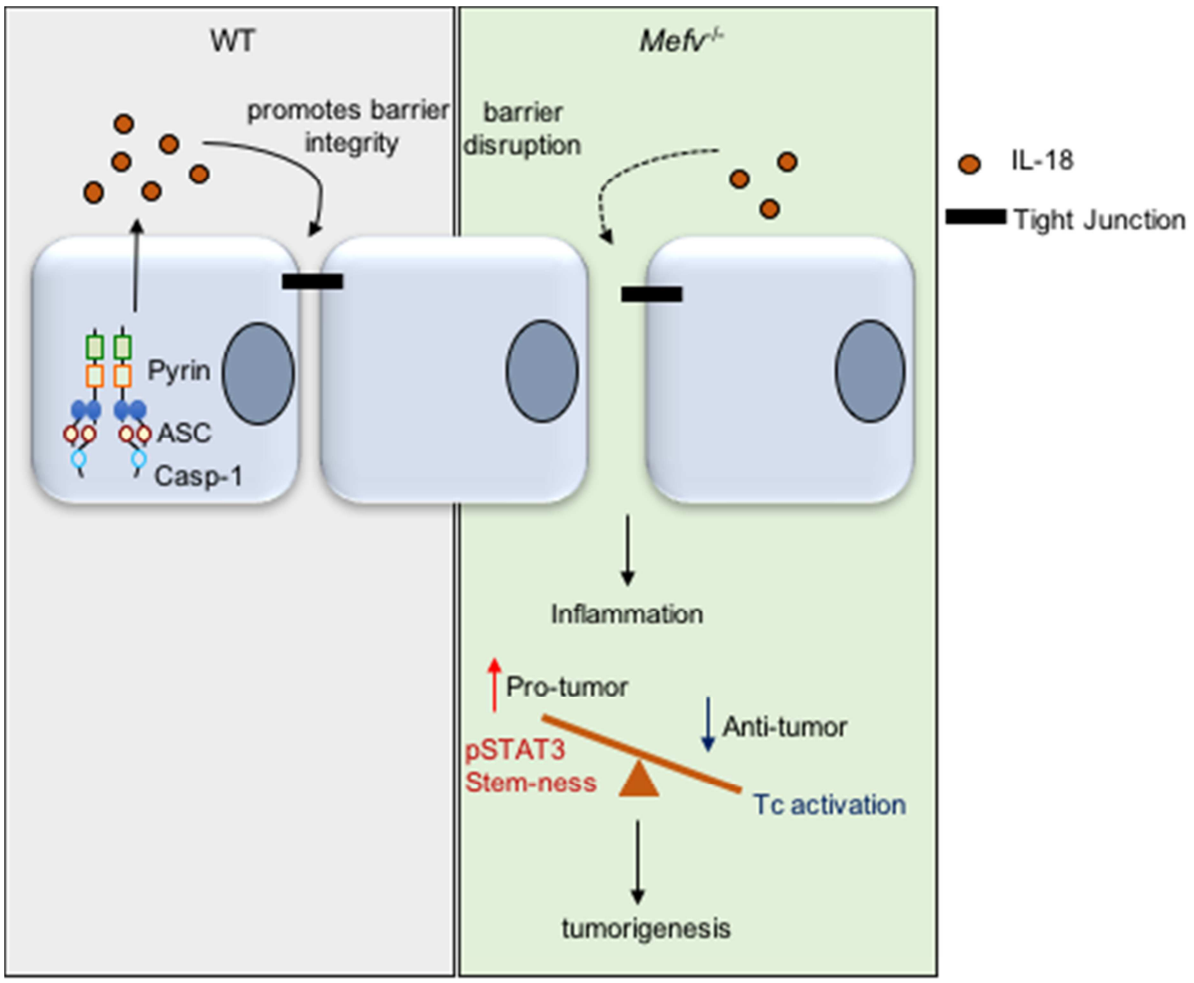
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8

Table&1:&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'
Il1b	Forward:(5'+GATCCACACTCTCCAGCTGCA+3'
	Reverse:(5'+CAACCAACAAGTGATATTCTCCATG+3'
Pyrin.1	Forward:(5'+TCATCTGCTAAACACCCTGGA+3'
	Reverse:(5'+GGGATCTTAGAGTGGCCCTTC+3'
Pyrin.2	Forward:(5'+AGGCTTCAAGGACTTTACAACAA+3'
	Reverse:(5'+TCATGCGAATGAGACTCCCA+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'
Mptx1	Forward:(5'+CCTGTTTCTCTCTGTTCTTTCAGG+3'
	Reverse:(5'+GGCCTTCATACACAGAGTGAAG+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'
Zo2	Forward:(5'+ACTCCAGTCCCTATTCCTGAG(+3'
	Reverse:(5'+GCTATTTCGATCCTCGCATTC(+3'
Il18	Forward:(5'+TGGATCCATTTCCTCAAAGG+3'
	Reverse:(5'+TGGATCCATTTCCTCAAAGG+3'
Klf4	Forward:(5'+GTGCCCCGACTAACCGTTG+3'
	Reverse:(5'+GTCGTTGAACTCCTCGGTCT+3'
Olfm4	Forward:(5'+CAGCCACTTTCCAATTTCACTG+3'