Supporting Information

Supporting Materials and Methods

Reagents

The MK2 inhibitor PHA-767491 was purchased from Abcam, and was administered by intraperitoneal injection at 30 mg/kg, either as a single dose, 1 h prior to LPS/D-Gal administration or once daily for 7 consecutive weeks in *Apc*$^{min/+}$ mice. The MK2 inhibitor PF-3644022 was purchased from either Tocris or Sigma and was administered orally at 6.9 mg/kg in 0.5% methylcellulose (VWR) and 0.025% Tween 80 (Sigma-Aldrich) (1), once daily for 7 consecutive weeks in *Apc*$^{min/+}$ mice. The Hsp27 inhibitor KRIBB3 was purchased from Abcam (ab146011) (2).

Mice

Deleter-Cre (3), *LysM*-Cre (4), *Tie1*-Cre (5), and *Villin*-Cre (6) mice have been previously described. *Apc*$^{min/+}$ (7), *Twist2*-Cre (8) mice were purchased from the Jackson Laboratory. Generation and screening of MK2 complete and conditional knockout mice are described in detail in Supporting Information. Mice were maintained on a mixed C57BL/6J×129Sv or C57BL/6J (experiments using MK2 inhibitor) genetic background and experiments were performed in the animal facilities of Biomedical Sciences Research Center (BSRC) “Alexander Fleming” under specific pathogen–free conditions. All experiments were approved by the Institutional Committee of Protocol Evaluation in conjunction with the Veterinary Service Management of the Hellenic Republic Prefecture of Attika according to all current

European and national legislation and performed in accordance with the guidance of the Institutional Animal Care and Use Committee of BSRC “Alexander Fleming”.

Generation of MK2 complete and conditional knockout mice

The targeting vector was generated using a three-loxP containing vector (pEasyFlox), which contains a PGKneo cassette between loxP sites 1 and 2 for antibiotic resistance selection. A 1.5-kb XbaI-SalI fragment containing exons 2-5 of the *MK2* gene was inserted between loxP sites 2 and 3. A 3.9-kb ClaI-NotI and a 2.8-kb XhoI-XhoI gene fragment were inserted as 5’- and 3’- homology arms, respectively. All DNA fragments were amplified by PCR using the C57BL/6J mouse genome BAC (Bacterial Artificial chromosome) clone RP23-122P5 and exon sequences were verified by sequencing. The vector was introduced by electroporation in mouse embryonic stem (ES) cells and clones were screened for homologous recombination by Southern blot analysis. Homologous recombinant clones were used for the generation of chimeric mice and were further crossed to C57BL/6J mice for expansion of the colony. The mice were then crossed to *Deleter*-Cre mice (3) to induce either a loxP1-P3 or a loxP1-P2 recombination, which resulted in mice carrying either the deleted (D) or the floxed (FL) allele. Mice were screened by PCR to select founders (primers are available upon request). $MK2^{D/D}$ mice were born at the expected Mendelian ratio and appeared healthy and fertile. Genotyping was performed by PCR using the following primers: Forward 1: CACCGGGCTGAGTCTGCTAT, forward 2: TAGGCATGCTGGGTCTTTAT and reverse: CCGCTGAGAACCTACAAACA. Amplification was performed at 55°C for 40 s and the size of PCR products was 283 bp (wild-type band), 344 bp (floxed band)

and 587 bp (deleted band).

Southern Blot analysis

DNA was digested with SacI and hybridized with a 5' probe labeled with ^{32}P-dATP or ^{32}P-dCTP, generated by PCR using the following primers: 5'-AAGGCATTCTGTGTGTTTCG-3' and 5'-TCCTCCCAGACACCTAGACA-3'. Recombinant clones show an 8kb band in addition to the 6kb band of the wild type allele. DNA was also digested with ScaI and hybridized with a 3' probe labeled with ^{32}P-dATP or ^{32}P-dCTP, generated by PCR using the following primers: 5'-AGTAGCCTCTGCTTGCCCTT-3' and 5'-CAAAGACATGGGAGGAGCTG-3'. Recombinant clones show a 6.5 kb band in addition to the 15.7 kb band of the wild-type allele.

Bone Marrow Transplantation

Recipient mice were irradiated using a cesium source (γ-irradiation), at two doses of 610 rad each, with a 3h interval in-between. The following day, bone marrow from 8-week-old donors was obtained from the femora and tibiae and 10^6 cells were injected intravenously (i.v.) in recipient mice. Mice were kept on antibiotics for 2 weeks and assessed 3 months after transplantation.

Induction of Colitis and Colitis-Associated Cancer (CAC)

Induction of DSS colitis and CAC was performed as previously described (9, 10). In brief, DSS colitis was induced in 8-week-old littermate and co-housed mice with 2.5% DSS (MW: 36,000–50,000 Da; MP Biomedicals) added in the drinking water

for 7 days, followed by 1 day of normal water. For CAC, mice were injected i.p. with 10 mg/kg AOM (Sigma-Aldrich). 5 days later, 2.5% DSS was added in the drinking water for 5 days, and this was repeated for two additional cycles with an interval of 16 days between each cycle. Body weights were recorded during DSS treatment. At day 60, colon was removed and macroscopically visible tumors were counted. The colitis scoring was performed as previously described (11).

LPS/D-Galactosamine-Induced Endotoxin Shock

8-week-old mice were injected intraperitoneally with 50 mg D-Galactosamine (Sigma-Aldrich) plus 50 μg/kg of *Escherichia coli*-derived lipopolysaccharide (LPS) (Sigma-Aldrich). Mice were either sacrificed 90 minutes later and blood was collected for measuring TNF levels or monitored for the next 12h for signs of acute illness and death.

Isolation, culture and induction of Thioglycollate-Elicited Peritoneal Macrophages (TEPMs)

Thioglycollate-elicited peritoneal macrophages were isolated by peritoneal lavage from 8 to 10-week old mice, 3 to 5 days after a single peritoneal injection (1ml) of 3% thioglycollate broth (BD Biosciences). Briefly, 5 ml of cold harvest medium (RPMI (Gibco) supplemented with 10% FBS (Biochrom), 2mM L-Glutamine (Gibco), 25mM HEPES (Gibco), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco) were used to collect cells from the peritoneal cavity. Cells were washed, incubated in Gey's solution for 15 min on ice to lyse red blood cells and then resuspended in RPMI-5%FBS. Cells were subsequently used either for RNA and protein extraction or were stimulated with 100ng/ml of LPS (Sigma-Aldrich) for 6 hours to measure TNF levels.

Isolation of Intestinal Epithelial cells (IECs)

In brief, ileum was removed, flushed, cut into pieces and extensively washed with HBSS (Gibco) supplemented with 100 U/ml penicillin and 100μg/ml streptomycin (Gibco). Intestinal pieces were then incubated in pre-warmed HBSS, containing 5 mM EDTA (Acros Organics) and 1 mM DTT (Sigma-Aldrich) at 37°C for 45 minutes. After vigorous shaking, cells released in the supernatant were layered on a discontinuous 25%/40% Percoll gradient (Sigma-Aldrich) and centrifuged at 600*g* for 10 minutes. IECs were collected from the interphase and subsequently used for protein extraction.

Isolation and culture of Intestinal Mesenchymal cells (IMCs)

Isolation of IMCs was performed as previously described (12). Briefly, the small intestine from 4 to 8-week-old mice was removed, cut in 0.5–1 mm pieces and washed with ice-cold HBSS (Gibco) containing 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B (Gibco). Intestinal pieces were then incubated in pre-warmed HBSS containing 5 mM EDTA (Acros Organics) and 1 mM DTT (Sigma-Aldrich) for 20 minutes at 37°C, in a shaking water bath, to remove the epithelial layer. Intestinal pieces were washed with HBSS and subsequently incubated with 300 U/ml Collagenase XI (Sigma-Aldrich) and 0.1 mg/ml Dispase II (Roche) in DMEM (Biochrom) for 30-40 minutes at 37°C. The supernatant was centrifuged, and the cell pellet was resuspended in DMEM supplemented with 10% FBS (Biochrom), 2mM Glutamine (Gibco), 1% nonessential amino acids (Gibco), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco), and 1 μg/ml amphotericin B (Sigma-Aldrich) and plated in cell culture flasks.

Isolation and culture of Intestinal Endothelial cells (ECs)

Isolation was performed from 8 to 10-day-old mice. The intestine was removed, cut into pieces and washed extensively with ice-cold HBSS (Gibco) containing 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B (Gibco). Intestinal pieces were then incubated with 300 U/ml Collagenase XI (Sigma-Aldrich), 0.1 mg/ml Dispase II (Roche) and 100 U/ml Dnase I (Sigma-Aldrich) in DMEM (Biochrom) for 45 minutes at 37°C, with gentle shaking. The suspension was centrifuged and the cell pellet was resuspended in medium containing equal volumes of low glucose DMEM (Invitrogen, #31885-023) and Ham's F-12 (Invitrogen, #31765-027), supplemented with 20% FBS (Biochrom), 2mM Glutamine (Gibco), 25mg/1L Heparin sodium salt from porcine intestinal mucosa (Sigma, #H3149), Endothelial mitogen (native bovine, Serotec, #4110504), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco), and 1 μg/ml amphotericin B (Sigma-Aldrich). Cells were plated in cell culture flasks pre-coated with 0.1% gelatin from porcine skin (#G1890, Sigma), Collagen I bovine (BD Biosciences, #354231) and fibronectin (BD Biosciences, #356009). At ~70-80% confluency, the cells were collected, centrifuged, resuspended in EC isolation medium, containing anti-CD31/PECAM-1 (eBioscience, #14-0311-85) and anti-CD102/ICAM-2 (BD Pharmigen, #553326) and incubate on a rotator at 4ºC for 45 minutes. CD31/CD201 positive cells were then isolated using secondary Ab-coated Dynabeads (Thermofisher Scientific, #11035), according to manufacturer's instructions and transferred into pre-coated plates. At passage 2, ECs' purity was evaluated by FACS analysis (see antibodies used for FACS staining under FACS analysis section) and subsequently used for protein extraction.

Isolation of intestinal crypt/tumor organoids and co-culture with intestinal mesenchymal cells

Intestinal crypts were isolated as described previously (13). Briefly, the small intestine was flashed with cold PBS (Gibco), opened longitudinally and villi were scraped off using a coverslip. Then, it was cut into 5mm pieces and washed extensively until the supernatant was clear. Ice-cold crypt isolation buffer (2 mM EDTA in PBS) was added to the fragments and stirred for 1 hour at 4°C. Fragments were allowed to settle down, the supernatant was removed, and ice-cold 2 mM EDTA/PBS was added followed by pipetting up and down. Released crypts were passed through a 70-µm-cell strainer and the procedure was repeated until most of crypts were released. Crypt fractions were centrifuged at 300*g* for 5 minutes and resuspended with ice-cold basal culture medium (Advanced DMEM/F12 (Gibco) supplemented with 2 mM GlutaMax (Gibco), 10 mM HEPES (Gibco), and 100 U/mL penicillin/100 mg/mL streptomycin (Gibco)). Intestinal adenoma cells were isolated using a modified version of the protocol by Sato et al (13). Briefly, intestinal adenomas from *Apc*$^{min/+}$ mice were collected and incubated in digestion buffer (DMEM containing 400 U/ml Collagenase IV (Sigma-Aldrich), 1 mg/ml Dispase II (Roche) and 100 U/ml Dnase I (Sigma-Aldrich)) for 90 to 120 minutes at 37°C. The adenoma fragments were allowed to settle down, and the supernatant was collected in a 50-mL Falcon tube, pelleted, and washed with basal culture medium. Isolated adenoma cells were centrifuged at 150–200*g* for 5 minutes to separate adenoma from single cells. Crypts and adenoma cells were centrifuged again at 200*g* for 5 minutes, resuspended in warm basal culture medium and counted. Crypts and adenoma cells were subsequently resuspended in Matrigel (BD Biosciences) at 3000 crypts/30 µl/well in 48-well plates and were added on top of monolayers of IMCs. After

Matrigel was solidified, culture medium was added in the wells, consisting of DMEM/F12 medium (Gibco), containing Glutamax (Gibco) and Penicillin/Streptomycin and supplemented with N2 supplement (Life Technologies, 1×), B27 supplement (Life Technologies, 1×), and 1 mM *N*-acetylcysteine (Sigma-Aldrich), 50 ng/ml EGF (Life Technologies), 100 ng/ml Noggin (PeproTech). In some cases, IMCs were induced with cytokines – IL-1β, TNF and TGF-β, as previously described - for 8 h, extensively washed and then crypts were added on top. Organoid images were acquired with a Zeiss PrimoVert microscope. Organoid measurements were performed using ImageJ/Fiji software analysis.

Histopathology

The colon and small intestine were removed, flushed with PBS, opened longitudinally, and macroscopically visible tumors were counted. Intestinal sections were subsequently fixed in 10% formalin and paraffin embedded. Histological scoring analysis was performed on H&E colon sections, as previously published (4-6). Assessment of microadenoma/adenoma count and size was performed on formalin-fixed paraffin-embedded (FFPE) colon and small intestinal sections after staining with hematoxylin (Sigma-Aldrich) and eosin (Sigma-Aldrich), using ImageJ software.

Immunohistochemistry/Immunofluorescence

FFPE small intestinal sections were probed with antibodies against α-SMA (FITC-conjugated; Sigma-Aldrich, F3777), phospho-Hsp27 (Ser82) (Cell Signaling, #2406 and #9709), Cleaved Caspase-3 (CC3) (Asp175) (Cell Signaling, #9661), phospho-Histone H3 (pHH3) (Ser10) (Cell Signaling, #9701), CD31 (Abcam, ab28364) and

CD34 (Abcam, ab81289).

The anti–rabbit Alexa Fluor 647–conjugated secondary antibody (Invitrogen, #A21244), biotinylated secondary antibodies (Vector Laboratories), the Vectastain ABC kit (Vector Laboratories), and the Tyramide Signal Amplification (TSA) kit (Life Technologies), were used for signal detection and amplification. For fluorescence staining, DAPI (Sigma-Aldrich) was used to stain the nuclei. In all other cases, signal development was performed with Vectastain DAB (3,3-diamino-benzidine) kit (Vector Laboratories), and hematoxylin was used as a counterstain. Images were acquired with an Eclipse E800 microscope (Nikon) equipped with a QImaging Digital Camera and a TCS SP8X White Light Laser confocal system (Leica). Quantification of α-SMA mean fluorescent intensity, CD31 and CD34-positive vessels was performed using ImageJ software analysis.

Assessment of proliferation and apoptosis

Mice were injected intraperitoneally (i.p.) with 100 mg/kg BrdU (Roche) 90 minutes before sacrifice. FFPE tissue sections were stained using the BrdU Proliferation detection kit (BD Biosciences), and tissues were counter-stained with hematoxylin. The number of BrdU- and pHH3-positive cells was quantified in at least 50 intact and well-orientated crypts and in size-matched tumors. Apoptosis was assessed in tissue sections using the DeadEnd Fluorometric TUNEL system (Promega) and DAPI (Sigma-Aldrich) was used to stain the nuclei or Cleaved Caspase 3 (CC3) (Cell Signaling, #9661). The number of TUNEL- and CC3-positive cells was quantified in size-matched tumors. Quantifications were performed using ImageJ software analysis.

ELISA

For cytokine determination assays, cells were plated, serum starved overnight, and stimulated with 100ng/ml of LPS (Sigma-Aldrich), 10 ng/ml IL-1β (PeproTech), 10 ng/ml TGF-β (R&D Systems) and 10 ng/ml TNF (kindly provided by C. Libert, Ghent University, Belgium). Cells were pre-incubated with the Hsp27 inhibitor KRIBB3 (ab146011, Abcam) for 4 hours prior to stimulations at a concentration of 50μM. Supernatants were collected at the indicated time-points and analyzed using mouse TNF alpha Ready-SET-Go ELISA (BD Biosciences), IL-6 Duo-Set ELISA (R&D Systems), and MIP2 ELISA (Peprotech), according to manufacturer's instructions.

RNA extraction and qRT-PCR

RNA was extracted from cells or tissue samples using either the Absolutely RNA Miniprep Kit (Agilent Technologies) or the TRIzol method (Invitrogen), according to the manufacturer's instructions. One to three micrograms of total RNA were reverse transcribed using M-MLV reverse transcriptase (Sigma-Aldrich) and oligo-dT primers (Promega) according to the manufacturer's protocol. qRT-PCR was then performed on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) using the SYBR Green PCR Master Mix (Invitrogen), according to the manufacturer's instructions. Forward and reverse primers were added at a concentration of 0.2 pmol/ml in a final volume of 20 μl. The expression of all genes was normalized to β_2-microglobulin (*B2m*). The primers used were: mouse *Mapkapk2 (14), forward:* 5'-GTTCCCCCAGTTCCACGTCAAG-3' and *reverse*: 5'-

CTAAAGAGCTCTCCACCATCG-3' and mouse *B2m, forward:* 5'-TTCTGGTGCTTGTCTCACTGA-3' and *reverse*: 5'-CAGTATGTTCGGCTTCCCATTC-3'. Analysis was performed using Opticon Monitor 3 (Bio-Rad).

FACS analysis

Small intestine or tumors were removed, washed extensively with HBSS (Gibco) supplemented with antibiotic-antimycotic solution (Gibco) and cut into pieces. The tissue was then incubated in DMEM containing 400 U/ml Collagenase IV (Sigma-Aldrich), 1 mg/ml Dispase II (Roche) and 100 U/ml Dnase I (Sigma-Aldrich) for 30-40 minutes at 37°C. Cell suspensions was passed through a 70 μm filter, washed and re-suspended in phosphate-buffered saline (PBS) supplemented with 5% FBS. Cell viability was assessed using Trypan Blue and cells were stained at 4°C for 30 minutes with the following antibodies: FITC-conjugated anti-CD11b (eBioscience, #11-0112-85) or APC-conjugated anti-CD11b (BioLegend, #101212), PE-conjugated anti-CD4 (BD Pharmigen, #553653) or A700-conjugated anti-CD4 (eBioscience, #56-0042-82) and PE-conjugated anti-F4/80 (eBioscience, #12-4801-82), APC/Cy7-conjugated anti-CD45 (BioLegend, #103116) or A700-conjugated anti-CD45 (BioLegend, #103128), PerCP/Cy5.5-conjugated anti-CD31/PECAM (BioLegend, #102420), PE/Cy7-conjugated anti-Podoplanin/gp38 (BioLegend, #127411) and anti-CD3e (eBioscience, #25-0031-81), AlexaFluor647-conjugated anti-Gr1 (BioLegend, #108418), APC-conjugated anti-CD8 (Biolegend, #100712), APC-eFluor 780-conjugated anti-Ter-119 (eBioscience, #47-5921-80) and anti-CD326/EpCAM

(eBioscience, #47-5791-80). Propidium iodide (PI) was used for live/dead exclusion (Sigma).

For intracellular staining, cells were fixed and permeabilized using the Fixation and Permeabilization Buffer Set (eBioscience) according to manufacturer's instructions. Cells were then stained with primary antibody against p-Hsp27 (Cell Signaling, #2406) overnight at 4°C, followed by secondary anti–rabbit Alexa Fluor 647–conjugated antibody (Invitrogen, A21244) and FITC-conjugated anti-α-SMA antibody (Sigma-Aldrich, F3777) at RT for 30 minutes. FACS analysis was performed using a FACS Canto II Flow cytometer (BD) and FACSDiva (BD) or FlowJo software (FlowJo, LLC).

Protein isolation, Western Blot and gelatin zymography

Cells or tissue samples were lysed in RIPA buffer (50 mM Tris HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS), containing protease inhibitors (Roche) and phosphatase inhibitors (Sigma-Aldrich). Total protein content was determined using the Bradford assay (Bio-Rad), and samples were subjected to SDS-PAGE and subsequently transferred to a nitrocellulose membrane (Whatman GmbH). After blocking with 5% BSA (Fisher Chemical) in PBS, membranes were incubated with primary antibodies against MK2 (Cell Signaling, #3042), p-MK2 (Cell Signaling, #2), p-Hsp27 (Cell Signaling, #2406), β-catenin (Sigma-Aldrich, c2206), Bak (Cell Signaling, #12105), PCNA (Abcam, ab29), pHH3 (Cell Signaling, #9701), CC3 (Cell Signaling, #9661) and β-actin (Santa Cruz Biotechnology, sc1615). Secondary antibodies conjugated with HRP were purchased by Vector Laboratories. Signal development was performed using the Luminata^{TM} Crescendo Western HRP Substrate (Millipore) and signal acquisition was achieved using the ChemiDoc XRS+

and Image Lab software (Bio-Rad).

For detection of MMP9 and MMP2 in cell culture supernatants, these were analyzed on 8% SDS-PAGE gel containing 1 mg/ml gelatin under non-reduced conditions at 4°C. Gels were washed in 2.5% Triton X-100 for 1 h, incubated in MMP activation buffer (50 mM Tris-HCl pH 7.5, 5 mM $CaCl_2$, 0,02% NaN_3, and 1 μM $ZnCl_2$) for 18 h at 37°C and stained with 0.5% Coomassie Blue R250 (Sigma-Aldrich). Quantification of bands from Western blot was performed with ImageJ or Image Lab (Bio-Rad) software.

Statistical analysis

Data are presented as mean ± SEM. Statistical significance was calculated by Student's t-test or one-way ANOVA for multiple comparisons. The D'Agostino Pearson test was used to test if the dataset followed a normal distribution. Welch's correction was used for samples that showed unequal variance. P-values ≤ 0.05 were considered significant. Data were analyzed using GraphPad Prism 6.

Supporting Figures

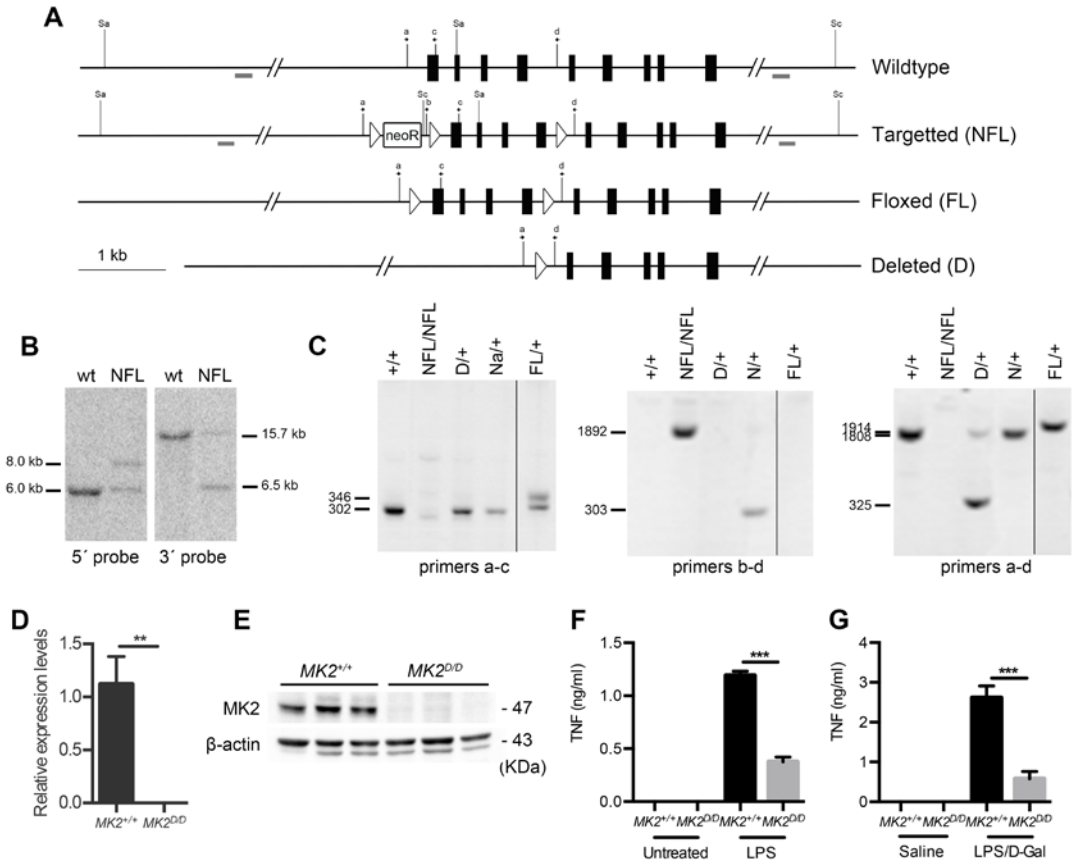

Figure S1. Generation and characterization of MK2 complete and conditional knockout mice. (A) Genomic structure of the mouse MK2 locus and description of the targeting strategy for the generation of the MK2 complete and conditional knockout locus. The targeting vector was generated using a three-loxP containing vector, which carries a NEO cassette between loxP sites 1 and 2 and a fragment containing exons 2-5 of the *MK2* gene between loxP sites 2 and 3. Filled boxes indicate exons 2-10. loxP sites are represented as white triangles. Location of PCR primers a – d, southern probes (grey lines), and digestion sites for SacI (Sa) and ScaI (Sc) are depicted. (B) Southern blot analysis of genomic DNA obtained from wild-type and recombinant ES cell clones. (C) Tail genomic DNA from mice carrying either the deleted (D) or the floxed (FL) allele was screened by PCR to select the respective founder for each line. (D) qRT-PCR and (E) Western Blot analysis of MK2

expression in the intestine of *MK2*$^{+/+}$ and *MK2*$^{D/D}$ mice. *B2m* and β-Actin were used as loading controls, respectively. (F) TNF levels were quantified by ELISA in TEPMs isolated from *MK2*$^{+/+}$ (n=3) and *MK2*$^{D/D}$ mice (n=2) after incubation with LPS (100 ng/ml) for 6h. (G) TNF levels were determined by ELISA in serum collected 90 minutes upon LPS/D-Gal administration in *MK2*$^{+/+}$ (n=5) and *MK2*$^{D/D}$ mice (n=5). **p < 0.01, ***p < 0.001.

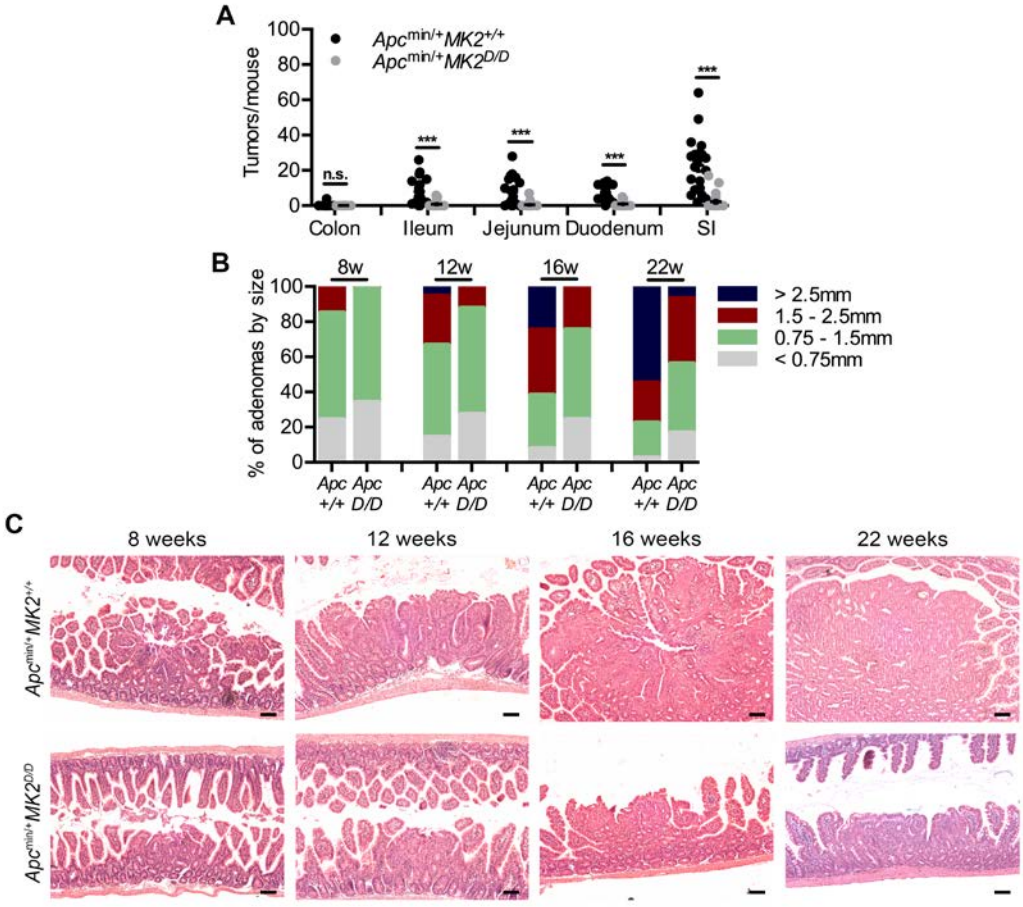

Figure S2. *Apc*$^{min/+}$*MK2*$^{D/D}$ mice show decreased tumor number in the different parts of the small intestine and reduced tumor size at different timepoints. (A) Number of polyps stratified by intestinal region in 22-week old *Apc*$^{min/+}$*MK2*$^{+/+}$ (n=20) and *Apc*$^{min/+}$*MK2*$^{D/D}$ (n=27) mice. Data represents mean ± SEM (n=8 mice per genotype). n.s. not significant, ***p < 0.001. (B) Microadenoma/adenoma size distribution at 8, 12, 16 and 22 weeks from *Apc*$^{min/+}$*MK2*$^{+/+}$ and *Apc*$^{min/+}$*MK2*$^{D/D}$ mice. (C) Representative H&E-stained sections of small intestinal microadenomas/adenomas from *Apc*$^{min/+}$*MK2*$^{+/+}$ and *Apc*$^{min/+}$*MK2*$^{D/D}$ mice at 8, 12, 16 and 22 weeks (Scale bar, 100 μm).

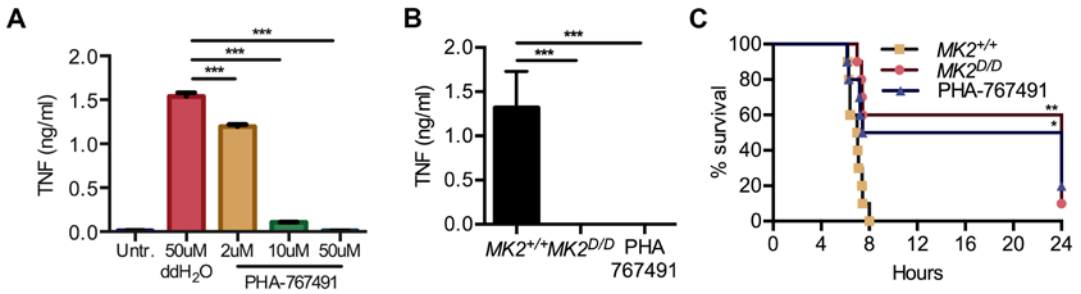

Figure S3. Chemical Inhibition of MK2 results in reduced TNF production and increased survival after LPS/D-Gal–induced septic shock. (A) TNF levels were quantified by ELISA in the supernatants of TEPMs stimulated with LPS (100ng/ml) for 6h. Pre-incubation with the MK2 inhibitor PHA-767491 (2, 10 and 50 µM) was performed 1 hour prior to LPS stimulation. (B) $MK2^{+/+}$, $MK2^{D/D}$ and $MK2^{+/+}$ mice received the MK2 inhibitor PHA-767491 (30mg/kg) or vehicle alone, 1-hour prior to intraperitoneally (IP) injection of LPS/D-Gal. TNF levels were determined by ELISA in serum collected 90 minutes upon LPS/D-Gal administration. Data represents mean ± SEM (n=5 per genotype). (C) Kaplan-Meier survival curve of $MK2^{+/+}$, $MK2^{D/D}$ and $MK2^{+/+}$ receiving the MK2 inhibitor PHA-767491, upon LPS/D-Gal administration (n=10 per genotype). n.s. not significant, *p ≤ 0.05, **p < 0.01, ***p < 0.001.

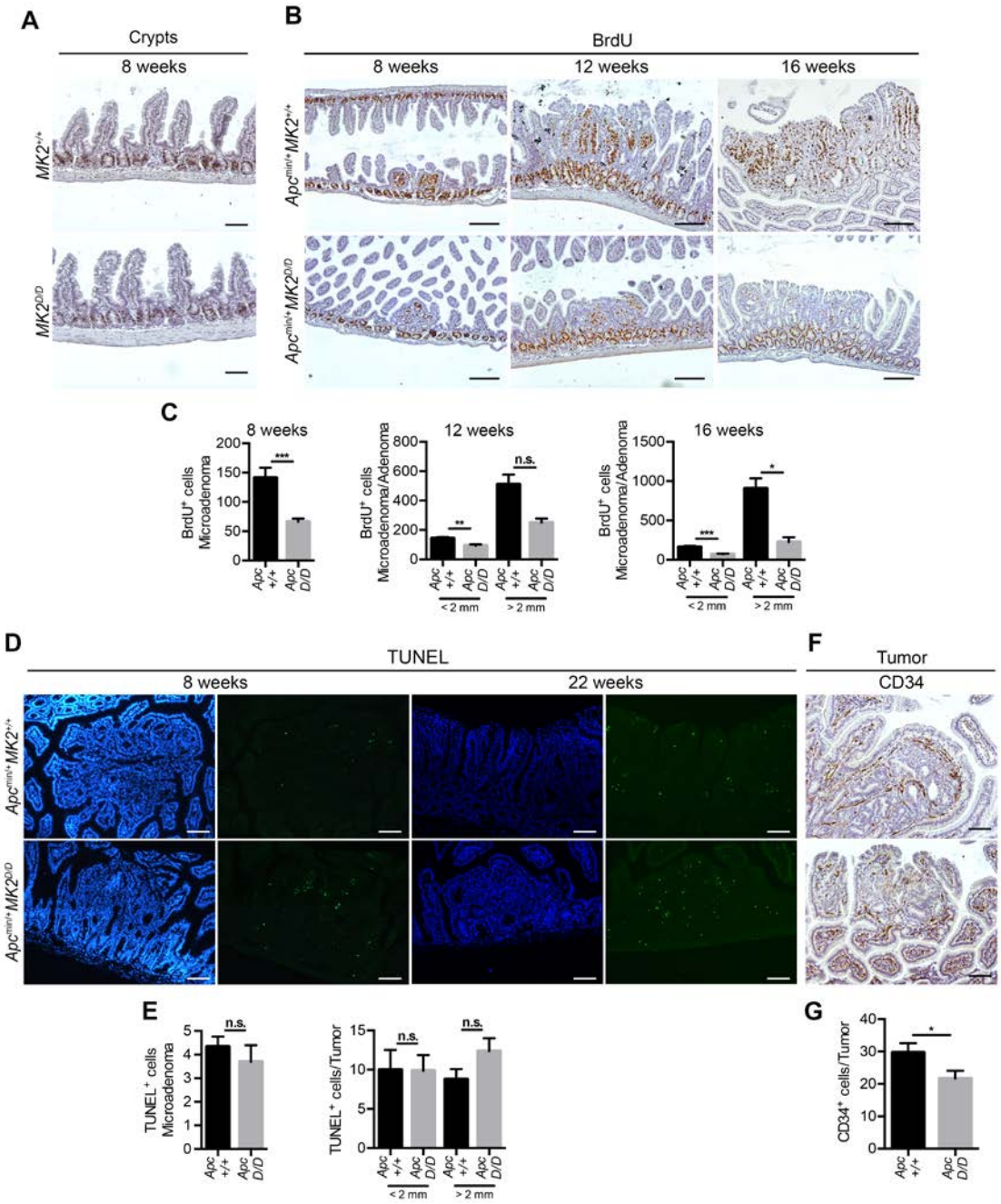

Figure S4. Measurement of proliferation, apoptosis and angiogenesis in *Apc*$^{min/+}$*MK2*$^{D/D}$ mice. (A) Representative immunohistochemical staining for BrdU in crypts of 8-week old healthy *MK2*$^{+/+}$ and *MK2*$^{D/D}$ mice (Scale bar, 50 μm). Data represents mean ± SEM (n=4 mice per genotype). (B) Representative immunohistochemical staining for BrdU and (C) quantification of BrdU-positive cells in size-matched tumors of *Apc*$^{min/+}$*MK2*$^{+/+}$ and *Apc*$^{min/+}$*MK2*$^{D/D}$ mice at 8, 12 and 16 weeks (Scale bar, 100 μm). Data represents mean ± SEM (n=8 mice per genotype).

(D) Representative immunohistochemical staining for TUNEL and (E) respective quantification of TUNEL-positive cells in size-matched tumors of 8 and 22-week old *Apc*$^{min/+}$*MK2*$^{+/+}$ and *Apc*$^{min/+}$*MK2*$^{D/D}$ mice (Scale bar, 50 μm). Data represents mean ± SEM (n=8 mice per genotype). (F) Representative immunohistochemical staining for CD34 and (G) respective quantification of CD34-positive cells in size-matched tumors of 22-week old *Apc*$^{min/+}$*MK2*$^{+/+}$ and *Apc*$^{min/+}$*MK2*$^{D/D}$ mice (Scale bar, 50 μm). Data represents mean ± SEM (n=5 mice per genotype). n.s. not significant, *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$.

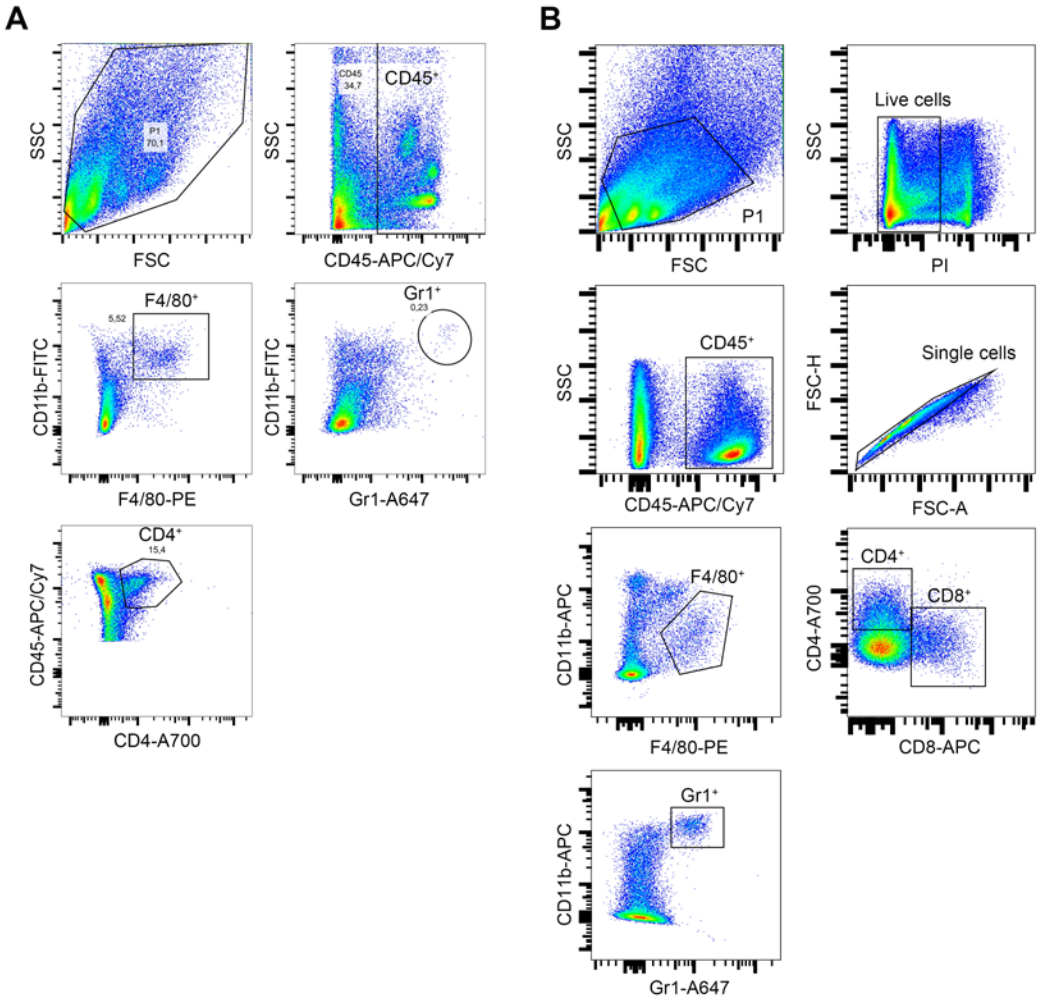

Figure S5. FACs gating strategy for the quantification of immune cell infiltration in the intestine and tumors. (A) FACS gating strategy for measuring inflammatory cell infiltration in the small intestine, showing initial gating on the $CD45^+$ total leukocyte population, and subsequent analysis of $CD11b^+F4/80^+$ macrophages, $CD11b^+Gr1^+$ neutrophils and $CD4^+$ T cells (n=5 mice per genotype). (B) FACS gating strategy for measuring inflammatory cell infiltration in tumors. The cell suspension was gated for live $CD45^+$. In this $CD45^+$ population, we plotted CD4 and CD8, for measuring the respective T cell populations, CD11b with F4/80 and Gr1 to identify double positive $CD11b^+F4/80^+$ and $CD11b^+Gr1^+$ cells (n=5 mice per genotype).

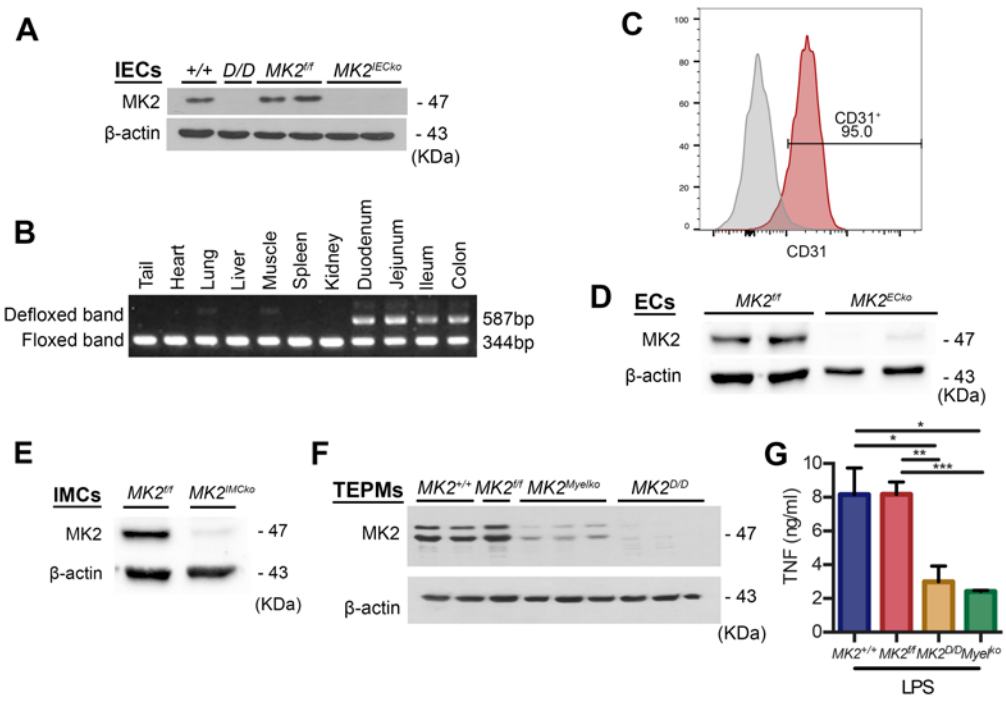

Figure S6. Deletion efficiency of MK2 in intestinal epithelial, endothelial, mesenchymal and myeloid cells in the respective Cre lines. (A) Western blot analysis of MK2 expression in IECs from *MK2*$^{+/+}$, *MK2*$^{D/D}$, *MK2*$^{f/f}$, and *Villin-MK2*$^{f/f}$ (*MK2*^{IECko}) mice. (B) Representative image of the PCR analysis for the detection of the defloxed MK2 allele using genomic DNA from various organs of *MK2*^{IECko} mice (n=3). (C) Representative FACS analysis of CD31 expression in cultured ECs from *Tie1-MK2*$^{f/f}$ (*MK2*^{ECko}) mice (n=4). (D) Western blot analysis of MK2 expression in ECs from *MK2*$^{f/f}$ and *MK2*^{ECko} mice. (E) Western blot analysis of MK2 expression in IMCs from *MK2*$^{f/f}$ and *Twist2-MK2*$^{f/f}$ *(MK2*^{IMCko}*)* mice. (F) Western Blot analysis of MK2 expression in TEPMs isolated from *LysM-MK2*$^{f/f}$ (*MK2*^{Myelko}) and their respective controls (*MK2*$^{f/f}$). (G) TEPMs from *MK2*$^{+/+}$, *MK2*$^{f/f}$, *MK2*$^{D/D}$ and *MK2*^{Myelko} mice were incubated with LPS (100ng/ml) for 6 hours and TNF was quantified in culture supernatants by ELISA. Data represents mean ± SEM (n=4 mice per genotype). β-actin was used as a loading control. *p ≤ 0.05, **p < 0.01, ***p < 0.001.

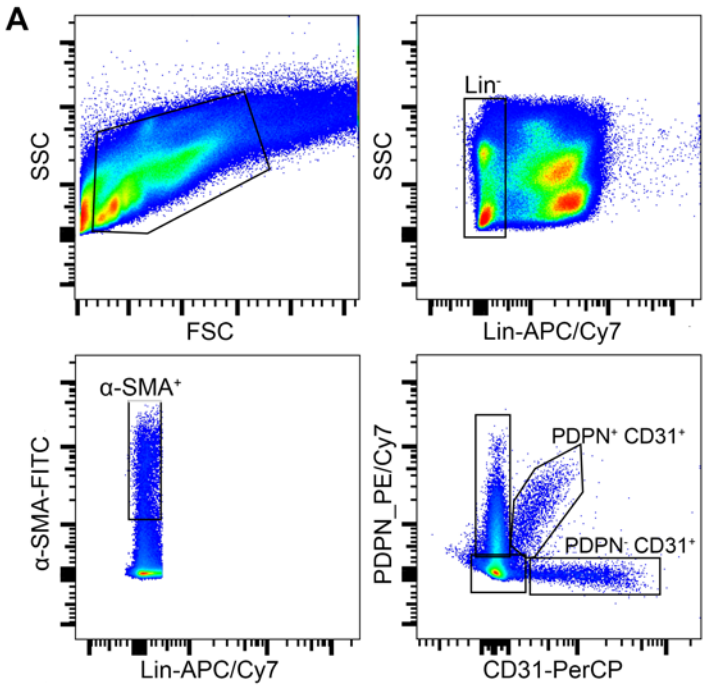

Figure S7. FACS gating strategy for p-Hsp27 expressing cells in the small intestine and tumors. (A) Representative FACS analysis of p-Hsp27^{+} expressing cells in the small intestine, showing initial gating on lineage-negative (Lin^{-}) markers, which include CD45 (leukocytes), Ter119 (erythroid) and EpCAM (epithelial). On this Lin^{-} population we either focused on CD31^{-}α-SMA^{+} (*left*) or cells that are either CD31^{+}PDPN^{-} (BECs) or CD31^{+}PDPN^{+} (LECs) (*right*).

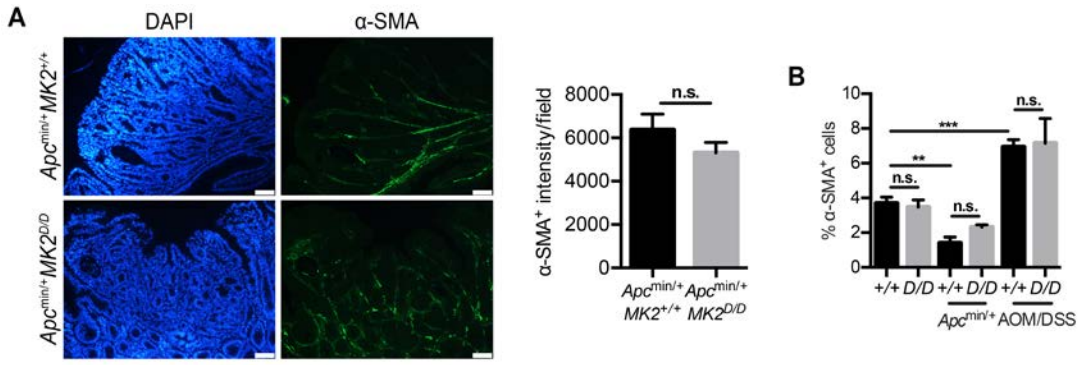

Figure S8. MK2 does not regulate fibroblast differentiation in the intestine. (A) Representative immunohistochemical staining and fluorescence intensity quantification for α-SMA in tumor sections from 22-week old *Apc*$^{min/+}$*MK2*$^{+/+}$ and *Apc*$^{min/+}$*MK2*$^{D/D}$ mice. DAPI was used to stain nuclei (Scale bar, 60 μm). Data represents mean ± SEM (n=4 mice per genotype). (B) Quantification of α-SMA^{+} cells by FACS, in normal mucosa from *MK2*$^{+/+}$ and *MK2*$^{D/D}$ mice, and in tumors from *Apc*$^{min/+}$*MK2*$^{+/+}$ and *Apc*$^{min/+}$*MK2*$^{D/D}$ mice. Data represents mean ± SEM (n=4 mice per genotype). n.s. not significant, *$p \leq 0.05$, **$p < 0.01$.

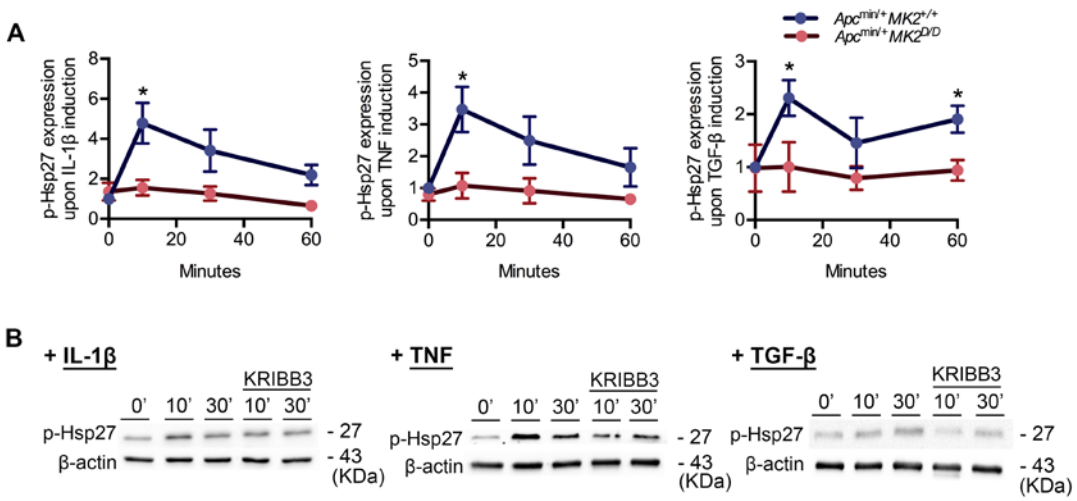

Figure S9. Hsp27 phosphorylation in response to different stimuli. (A) Densitometric analysis of Western blot analysis of Hsp27 phosphorylation relative to β-actin (relative to Figure 7A), in whole protein extracts from *Apc*$^{min/+}$*MK2*$^{+/+}$ and *Apc*$^{min/+}$*MK2*$^{D/D}$ primary IMC cultures before and after induction with IL-1β (10 ng/ml) *(left)*, TNF (10 ng/ml) *(center)* and TGF-β1 (10 ng/ml) *(right)* at the indicated time points. Data represents the average of three independent experiments. Statistics refer to comparisons between at the different timepoints. (B) Western blot analysis of p-Hsp27 in whole protein extracts from *Apc*$^{min/+}$ primary IMC cultures with and without prior incubation with the Hsp27 inhibitor KRIBB3 for 4 hours, followed by induction with IL-1β (10 ng/ml) *(left)*, TNF (10 ng/ml) *(middle)* and TGF-β1 (10 ng/ml) *(right)* at the indicated time points. β-actin was used as a loading control. Data represents one of three independent experiments performed.

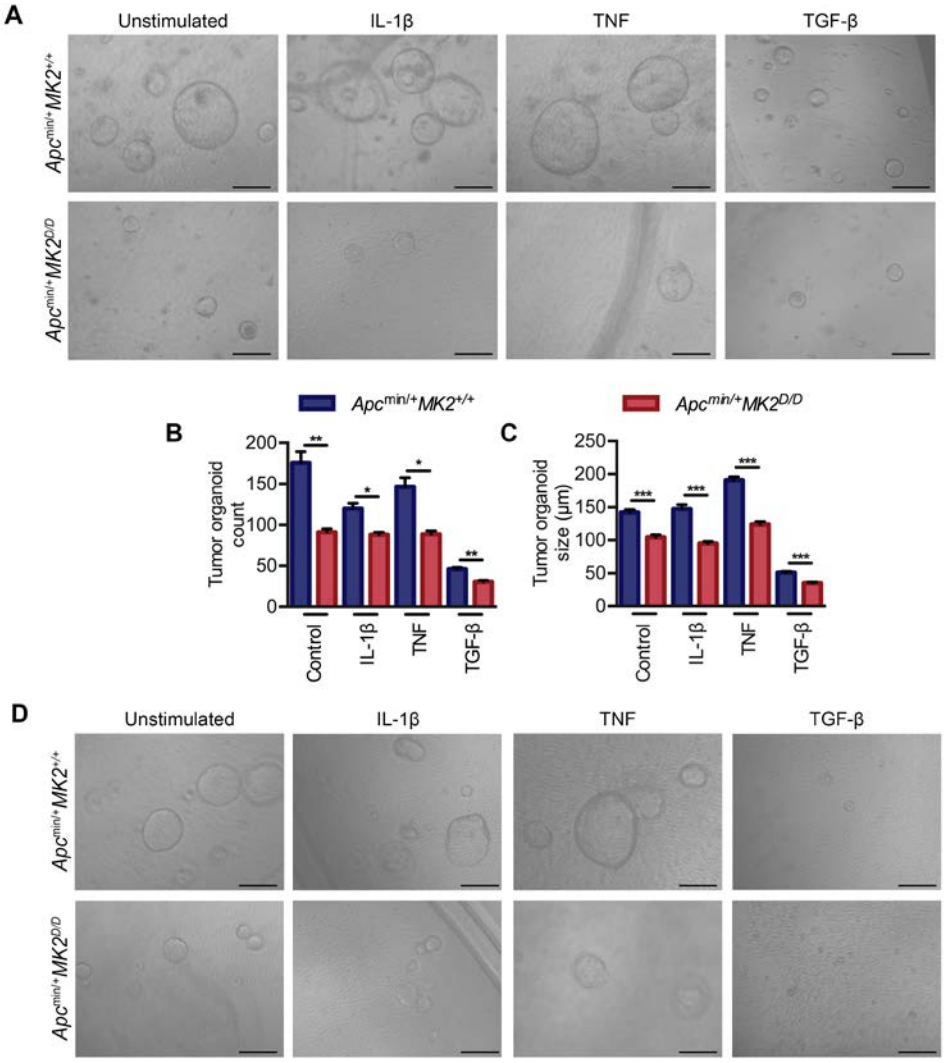

Figure S10. MK2 deficiency disrupts mesenchymal to epithelial communication. (A) Representative bright-field images (Scale bar: 25 µm) of intestinal organoids co-cultured with IMCs from *Apc*$^{min/+}$*MK2*$^{+/+}$ and *Apc*$^{min/+}$*MK2*$^{D/D}$ mice. IMCs were pre-incubated with IL-1β, TNF and TGF-β for 8h. (B) Tumor organoid count and (C) tumor organoid size and (D) representative bright-field images (Scale bar: 25 µm) of tumor organoids from *Apc*$^{min/+}$ mice co-cultured with IMCs from *Apc*$^{min/+}$*MK2*$^{+/+}$ and *Apc*$^{min/+}$*MK2*$^{D/D}$ mice. IMCs were pre-incubated with IL-1β, TNF and TGF-β for 8h. Data represents mean ± SEM from one of three experiments performed in triplicates. n.s. not significant, *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$.

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