Supplementary methods

Cells

Primary MEF cells were isolated from E13.5 *Tsc1*$^{fl/fl}$ mice and immortalized by the SV-40 large T antigen (Addgene, pBSSVD2005). HT-29 and HEK293T cells were from ATCC. MEF, HT-29 and HEK293T cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin (100 IU /ml)/streptomycin (100 μg/ml).

Lentivirus packaging and transduction

Lentiviral vectors pCDH-puromycin expressing Cre recombinase, HA-tagged TRIM11 or empty vectors were transfected into HEK293T cells together with packaging plasmids (Δ8.91/VSV-G), and virus-containing media were harvested 48 h post-transfection (1). Then *Tsc1*$^{fl/fl}$ MEF cells were infected with lentiviruses for 24 h. After puromycin selection for one week, protein expression was validated by immunoblotting.

Tsc1-specific shRNAs (5'-AAAGAAGAAGCTGCAATATCT-3') or *Trim11*-specific shRNAs (#1, 5'-CCCTCTTCTCACCTCTGTCAA-3'; #2, 5'-GATGGGTCGCTGCTGTTTATC-3') were cloned into pLKO.1-puromycin vectors and lentiviruses were packaged as described above. Then HT-29 or MEF cells were infected with lentiviruses for 24 h, followed by puromycin selection for one week.

Crisper-cas9 technology was used to generate TRIM11 knockout HT-29 cells. Briefly, guide DNA fragment (#1, 5'-CGAGCGTCTTCGCCGTTTGC-3'; #2, 5'-GATCCGGTGATGACCGACTG-3') cut with BsmBI was cloned into pLentiCRISPRv2, which then packed in HEK293T cells as described above. The Lentiviruses pLentiCRISPRv2-sgTrim11 was transducted into HT-29 cells and selected by puromycin for 7 days to establish a pool of TRIM11-knockout cells. Subsequently, single TRIM11-knockout clones were obtained by serial dilutions and confirmed by DNA sequencing and immunoblotting.

Histology, immunohistochemistry, and immunofluorescence

Intestinal tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Tissue sections were cut in 5 μm and stained with H&E for histological analysis. Histology score was assessed as reported (2). Histology was scored in a blinded manner with a semi-quantitative scoring system as described in the following: Presence of ulcer: 0 = none, 1 = punctate, 2 = minimal, 3 = moderate, 4 = widespread. Presence of inflammation: 0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe. Extent of inflammation: 0 = none, 1 = mucosal, 2 = mucosal + submucosal, 3 = mucosal + submucosal + muscle penetrate, 4 = full-thickness involvement. The total histopathological scores were determined by the summation of the scores from each category.

For immunohistochemistry, paraffin sections were rehydrated; heat-induced antigen retrieval was performed in 0.01M citrate buffer (pH 6.0) by microwave for 20 min. The slides were then slowly cooled down to room temperature (RT) and endogenous peroxidase activity was blocked in 3% of Hydrogen Peroxide for 10 min at RT. Blocking buffer (5% BSA in PBS) was added to sections and incubated at RT for 1 h. Primary antibodies diluted in 1% BSA against cleaved caspase-3 (#9664, Cell Signaling Technology, 1:200), phospho-S6 (#4858, Cell Signaling Technology, 1:400), mouse RIPK3 (AHP1797, Bio-rad, 1:200), Ki67 (#12202, Cell Signaling Technology, 1:400), CD3 (ab11089, Abcam, 1:100) were incubated overnight at 4°C. The sections were then incubated with biotinylated secondary antibodies (Jackson immunoresearch) for 1 h and Streptavidin-HRP (Jackson immunoresearch) for 30 min at RT. Staining was visualized with DAB substrate (Vector Laboratories) and counterstained with hematoxylin.

For immunofluorescence, sections were processed as described above and incubated with anti-lysozyme (ab108508, Abcam, 1:500), F4/80 (14-4801, eBioscience, 1:100) or anti-Ly6G (550291, BD, 1:100) antibodies overnight at 4°C. Then sections were

incubated with anti-rabbit FITC or anti-rat Cy3 secondary antibody for 1 h at RT, followed by counterstaining with DAPI and mounting.

TUNEL assay

To assess intestinal epithelial cell death, fresh intestinal tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Paraffin sections were rehydrated and followed by antigen retrieval with proteinase K for 30 min at 37°C. TUNEL assay was conducted with In-Situ Cell Death Kit (Roche) according to the manufacturer's recommendations. Sections were then stained with IEC specific marker Epcam antibody (17-5791-82, eBioscience, 1:200) and anti-rat Cy3 secondary antibody. Stained sections were analyzed under a fluorescence microscope, and the cells double-positive for Epcam and TUNEL were defined as dead IECs.

Induction of colitis-associated colorectal cancer

Colon cancer was induced as previously described with minor modification (3). 8-week-old mice were injected with AOM (Azoxymethane, Sigma, Cat#: A5486) dissolved in 0.9% NaCl buffer intraperitoneally (i.p.) at a dose of 10 mg/kg body weight. Five days after injection, mice were given 1.5% DSS in drinking water for 5 days, followed by regular water for 16 days. This cycle was repeated twice, with the third cycle modified as 1.0% DSS treatment for 4 days. On day 80, mice were sacrificed and colons were cut longitudinally to examine tumor numbers and sizes. Tumors and adjacent normal tissues were either fixed in 4% PFA for histology or processed for RNA and protein isolation.

Commensal depletion

Commensal depletion was performed as previously described (4, 5). Mice were initially treated with a cocktail of ampicillin (1 mg/ml), metronidazole (1 mg/ml), neomycin (1 mg/ml), vancomycin (0.5 mg/ml) in autoclaved drinking water 4 weeks after weaning. The antibiotics-containing water was refreshed every week. Then the drinking water supplemented with a cocktail of streptomycin (1 mg/ml), gentamicin

(170 ug/ml), ciprofloxacin (125 ug/ml) and bacitracin (1 mg/ml) was applied for another week before DSS treatment. Commensal depletion efficiency was more than 99.9% as assessed by fecal bacteria culture. Briefly, feces were collected and homogenized in PBS. Serial dilutions of the suspensions were plated on 5% defibrinated sheep blood trypticase soy agar (Fisher Scientific) and incubated at 37°C under aerobic or anaerobic conditions for 48 h. Bacterial counts were determined by colony-forming assay.

Intestinal permeability assay

Untreated or DSS-treated mice were fasted for 4 h and then gavaged with 0.6 mg/g FITC-dextran (MW 3,000-5,000, sigma) dissolved in PBS. 4 h after gavage, blood was collected and serum FITC-dextran levels were measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm (5).

Crypt isolation and organoid culture

The intestinal organoids were derived from the small intestines as reported (6). Briefly, a short piece of the duodenum was dissected and opened longitudinally. The intestine was washed with ice-cold PBS to clear luminal contents. Villi were scraped off and the intestine was cut into 2-4 mm pieces and then incubated with 2 mM EDTA in PBS for 30 min on a rocker at 4°C. Crypts were released by pipetting and separated from villi by passing through a 70 μm strainer, and then washed with advanced DMEM/F12, resuspended in the matrigel (200-500 crypts/50μl). Next, the crypt-containing matrigel was seeded onto 96-well or 24-well plate for organoid culture. After matrigel polymerization, organoid growth medium was added and refreshed every 2-3 days. The complete growth medium contained advanced DMEM/F12 (Life Technologies) supplemented with 1% penicillin/streptomycin, L-glutamine (Hyclone), 10 mM HEPES, 1 mM NAC (sigma), N2 supplement (Life Technologies), B27 supplement (Life Technologies), 50 ng/ml EGF (Peprotech), 100 ng/ml Noggin (Peprotech), 500 ng/ml R-spondin 1 (Peprotech).

IEC isolation and immunoblotting

IECs were isolated by EDTA isolation buffer (30 mM EDTA, 0.5 mM DTT in PBS) as previously described (7). Briefly, intestinal tissues were flushed with PBS to remove feces and opened longitudinally. The tissues were cut into small segments and then incubated with isolation buffer at 37°C for 30 min with gentle shaking. After vigorous shaking for 20 seconds, IECs were released in the supernatant and collected by spin at 300 g for 5 min. The IECs were lysed in RIPA lysis buffer (50 mM Tris, pH7.4, 150 mM NaCl, 0.25% DOC, 1% Triton X-100, 0.1% SDS and 1 mM EDTA, pH 8.0) supplemented with protease inhibitor cOmplete, Mini (Roche) and 1 mM PMSF, 1 mM Na_3VO_4 and 1 mM NaF. Protein concentration was measured by BCA assay kit from Bio-Rad. The whole cell lysates were resolved by 10% SDS-PAGE and transferred to PVDF membrane for immunoblotting. Primary antibodies for immunoblotting were shown in Supplemental Table 1.

Immunoprecipitation

For immunoprecipitation, the whole cell lysates were incubated with 2-5 μg Abs overnight, followed by the incubation with 10–20 μl protein A-Sepharose 6MB beads (GE Healthcare Life Sciences) for 1 h at 4°C. In some experiments, the whole cell lysates were directly incubated with anti-HA beads or anti-Flag beads overnight. The immunoprecipitates were washed three times with wash buffer (50 mM Tris [pH8.0], 300 mM NaCl, 1% Triton X-100, 1 mM EDTA). The proteins were eluted with 2× SDS loading buffer by boiling for 10 min.

RNA isolation and RT-PCR

The RNAs were extracted from the intestinal tissues, isolated IECs with TRIzol according to the manufacturer's instructions (Invitrogen). cDNAs were reversely transcribed with PrimeScript RT-PCR Kit (Takara). Real-time quantitative PCR was conducted on an ABI 7900HT Fast Real-Time PCR System to measure gene expression, which was normalized to β-actin. Gene expression analysis was based on the change-in-cycling-threshold (ddCT) method (8).

Cell viability and cytotoxicity assay

The viability of organoids after necrosis induction was measured by a CellTiter-Glo Luminescent Cell Viability Assay kit (Promega). The cell cytotoxicity of MEF and HT-29 cells after necrosis induction was measured by a CytoTox 96® Non-Radioactive Cytotoxicity Assay kit (Promega).

Supplemental Table 1. Key resource table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies for Western Blot		
TSC1	Cell Signaling Technology	#4906
TSC2	Cell Signaling Technology	#4308
phospho-TSC2 (Thr1462)	Cell Signaling Technology	#3611
mTOR	Cell Signaling Technology	#2972
phospho-S6 (S235/236)	Cell Signaling Technology	#4858
S6	Cell Signaling Technology	#2317
phospho-4E-BP1 (Thr37/46)	Cell Signaling Technology	#2855
4E-BP1	Cell Signaling Technology	#9644
phospho-ULK1 (S757)	Cell Signaling Technology	#6888
phospho-AKT (T308)	Cell Signaling Technology	#2965
AKT	Santa Cruz	sc-55523
phospho-GSK3α/β (S21/9)	Cell Signaling Technology	#9331
GSK3α/β	Cell Signaling Technology	#5676
phospho-JNK (Thr183/Tyr185)	Cell Signaling Technology	#9251
JNK	Cell Signaling Technology	#9253
phospho-p38 (Thr180/Tyr182)	Cell Signaling Technology	#9211
p38	Santa Cruz	sc-728
phospho-IκBα (Ser32)	Cell Signaling Technology	#2859
IκBα	Santa Cruz	sc-371
phospho-IKKα/β (Ser176/180)	Cell Signaling Technology	#2697
IKKα/β	Santa Cruz	sc-7607
RIPK3	Prosci	#2238
mouse phospho-RIPK3 (S232)	Abcam	ab195117

human phospho-RIPK3 (S227)	Abcam	ab209384
MLKL	Millipore	MABC604
mouse phospho-MLKL(S345)	Abcam	ab196436
human phospho-MLKL(S358)	Abcam	ab187091
RIPK1	BD	#610458
mouse p-RIPK1 (S166)	CST	#31122
human p-RIPK1 (S166)	CST	#65746
FADD	Abcam	Ab24533
Caspase8	Enzo	ALX-804-447-C100
Cleaved Caspase3	Cell Signaling Technology	#9664
p62	sigma	P0067
LC3B	Santa Cruz	#398822
Flag	Sigma	F7425
HA	Sigma	H6908
anti-HA beads	Roche	11815016001
anti-Flag beads	Sigma	M8823
TRIM11	Proteintech	10851-1-AP
Actin	Santa Cruz	sc-1616
Tubulin	Prosci	7597
Gapdh	Bioworld	AP0063
Antibodies for IHC		
phospho-S6 (S235/236)	Cell Signaling Technology	#4858
Cleaved Caspase3	Cell Signaling Technology	#9664
RIPK3	Bio-Rad	AHP1797
Ki67	Cell Signaling Technology	#12202

Lysozyme	Abcam	ab108508
F4/80	eBioscience	14-4801
Ly6G	BD	550291
CD3	Abcam	ab11089
Epcam	eBioscience	17-5791-82
Biotin Anti-Rabbit IgG (H+L)	Jackson Immunoresearch	711-065-152
Biotin Anti-Rat IgG (H+L)	Jackson Immunoresearch	712-065-150
Streptavidin-HRP	Jackson Immunoresearch	016-030-084
Ligands and cytokines		
Pam3CSK4	Invivogen	tlrl-pms
Poly(I:C)	Invivogen	tlrl-piclv
LPS	Invivogen	tlrl-peklps
Flagellin	Invivogen	tlrl-pafla
MDP	Invivogen	tlrl-mdp
Murine TNF	Peprotech	315-01A
Human TNF	Peprotech	300-01A
Mouse IFN-β	Biolegend	581302
Reagents for organoids culture		
Advanced DMEM/F12	Invitrogen	12634010
N-2 Supplement	Invitrogen	17502048
B-27 Supplement	Invitrogen	17504044
mEGF	Peprotech	315-09
mNoggin	Peprotech	250-38
mR-Spondin-1	Peprotech	315-32

Matrigel	BD	356234
N-Acetyl-L-cysteine	Sigma	A7250
L-glutamine	ThermoFisher	25030081
Chemicals and inhibitors		
Z-VAD-FMK	APExBIO	A1902
BV-6	Selleck	S7597
Nec-1	Selleck	S8037
GSK'872	Millipore	530389
Rapamycin	Selleck	S1039
Chloroquine	Invivogen	tlrl-chq
SAR405	MCE	HY-12481
MG132	Sigma	C2211
Dextran sulfate sodium salt (M.W. 36,000-50,000)	MP biomedicals	0216011080
FITC-dextran (M.W. 3,000-5,000)	Sigma	FD4
Azoxymethane	Sigma	A5486
Kits		
CellTiter-Glo Luminescent Cell Viability Assay	Promega	G7572
CytoTox 96® Non-Radioactive Cytotoxicity Assay	Promega	G1780
In Situ Cell Death Detection Kit, Fluorescein	Roche	11684795910
ImmPACT™ DAB Substrate	Vector Laboratories	SK-4105
Alkaline Phosphatase Substrate	Vector Laboratories	SK-5100

Supplemental Table 2. Primers for RT-PCR

Gene name	Forward (5' to 3')	Reverse (5' to 3')
mTnf	GTCCCCAAAGGGATGAGAAGTT	GTTTGCTACGACGTGGGCTACA
mIl6	AGATAAGCTGGAGTCACAGAAGGAG	CGCACTAGGTTTGCCGAGTAG
mIl1b	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
mCxcl1	GCTGGGATTCACCTCAAGAA	CTTGGGGACACCTTTTAGCA
mCcl2	GCTGGAGAGCTACAAGAGGATCA	ATGTCTGGACCCATTCCTTCTT
mCcl5	GTGCCCACGTCAAGGAGTATTT	CTTCTCTGGGTTGGCACACACT
mRipk3	GAGATGGAAGACACGGCACT	GGTGGTGCTACCAAGGAGTT
mMlkl	CTGAGTTGTTGCGGGAAATCAT	CCGCAGACAGTCTCTCCAAGAT
mTsc1	CAGCATGCACTCAGGAACAGAA	TGCAGACTCACCTTCCACATCT
mIl22	TTGAGGTGTCCAACTTCCAGCA	AGCCGGACATCTGTGTTGTTA
mCox2	TGAGCACAGGATTTGACCAG	CCTTGAAGTGGGTCAGGATG
mEreg	CACCGAGAAAGAAGGATGGA	GATTCTCCTGGGATGCATGA
mMmp10	AGGCTTCCCAAGACAGATAACA	GGTCAAACTCGAACTGTGATGA

Supplementary references

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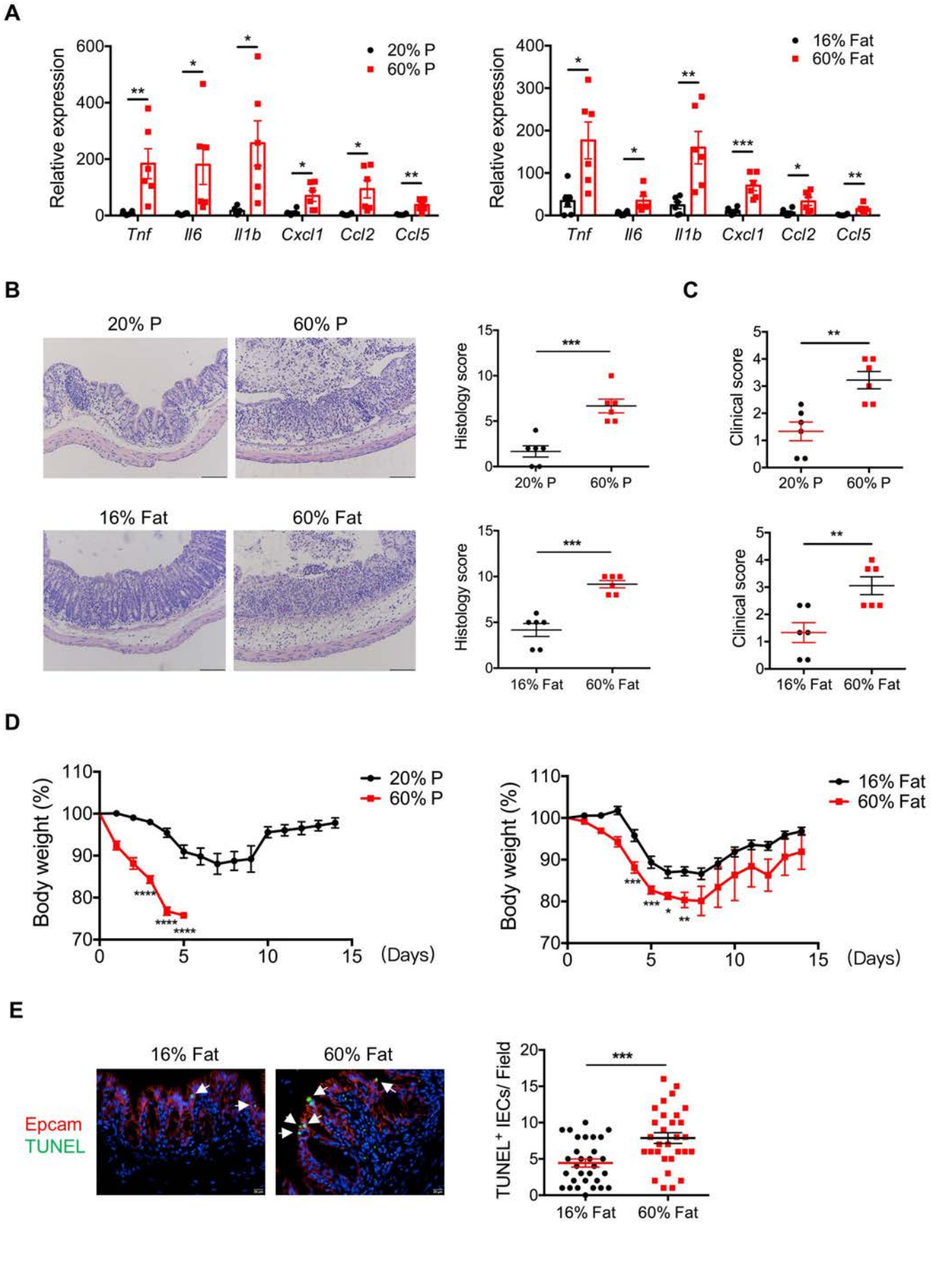
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60% P
Tnf
Il6
Il1b
Cxcl1
Ccl2
Ccl5
16% Fat
60% Fat
B
20% P
60% P
16% Fat
60% Fat
Histology score
C
Clinical score
D
Body weight (%)
(Days)
E
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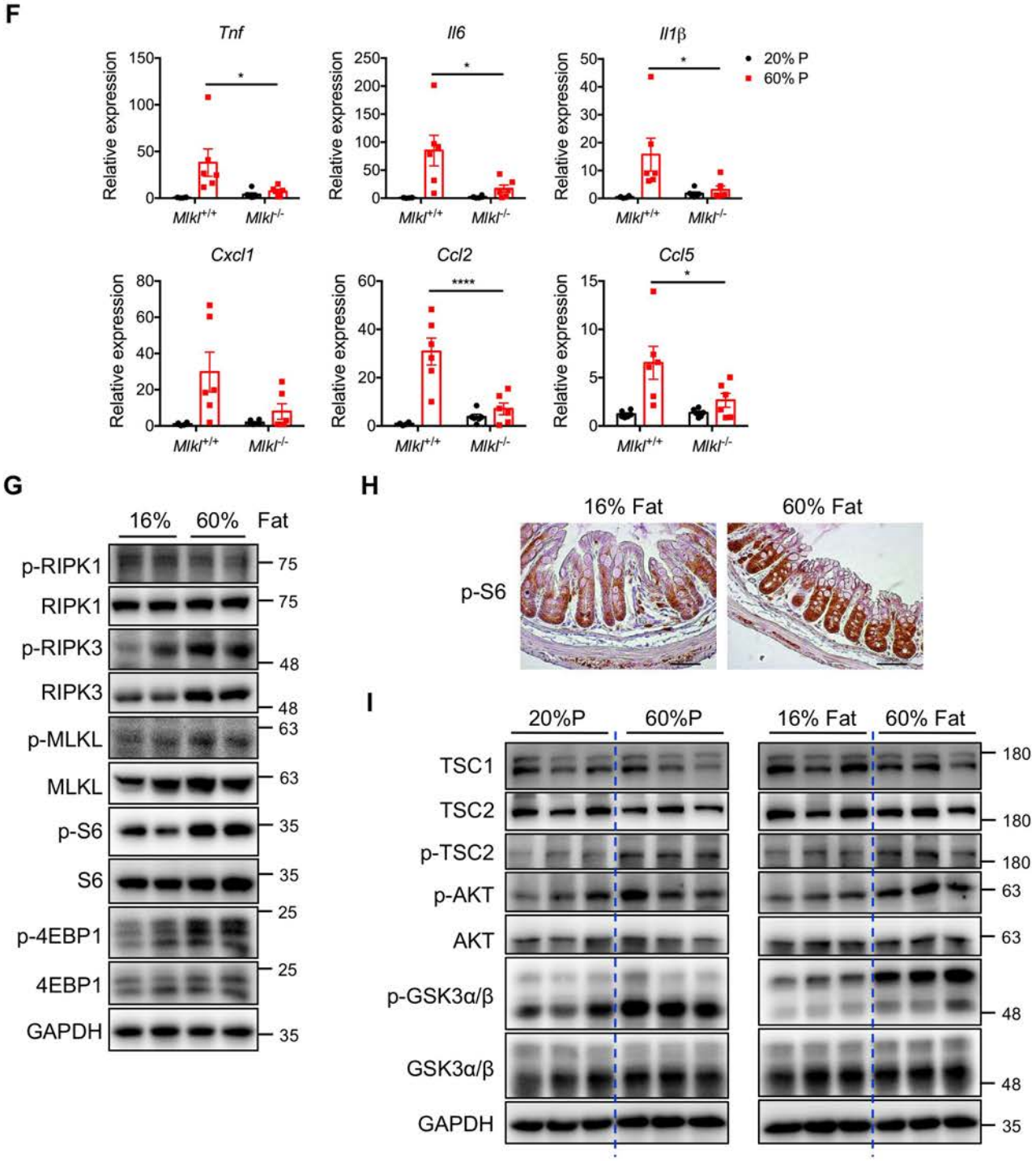
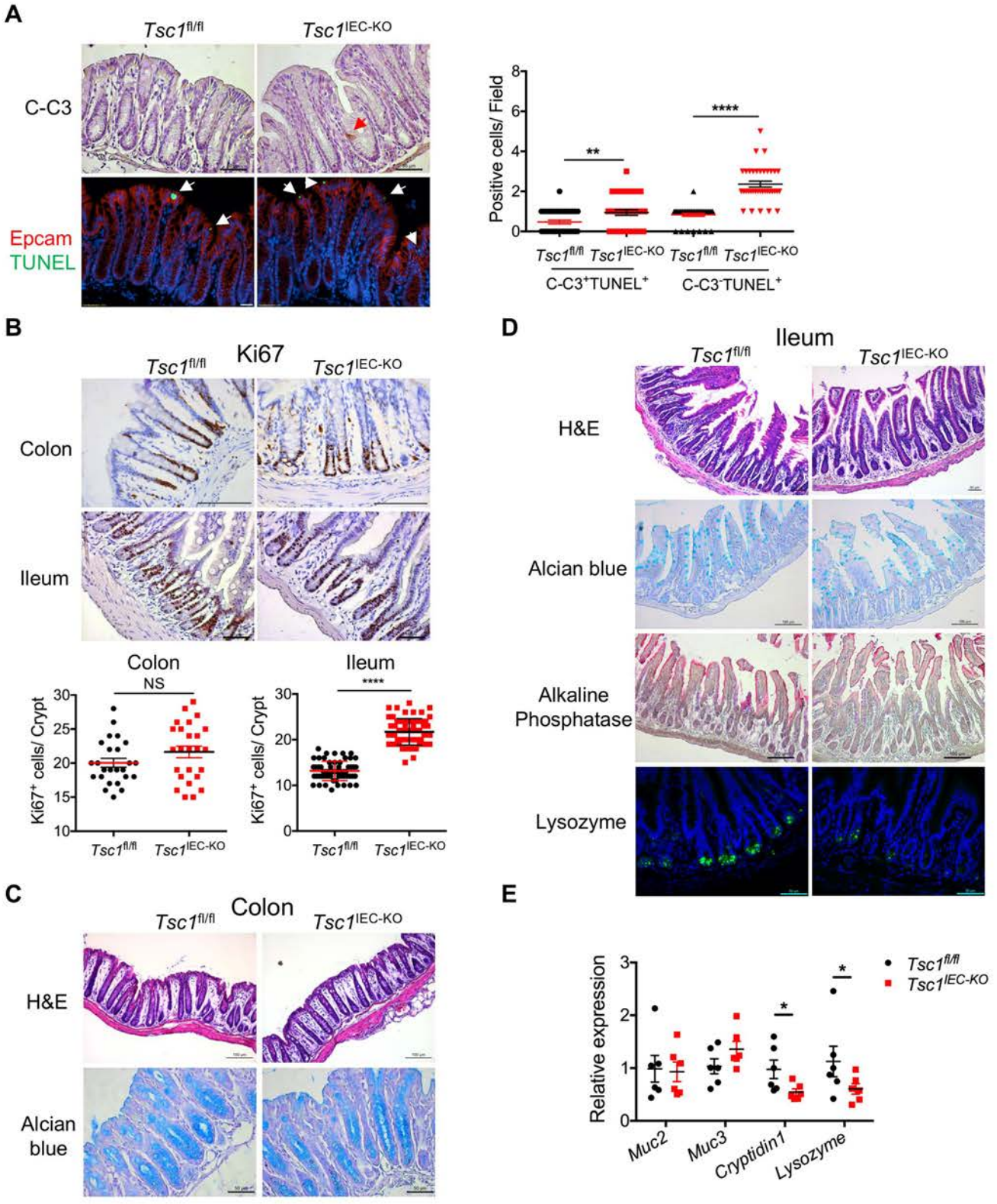
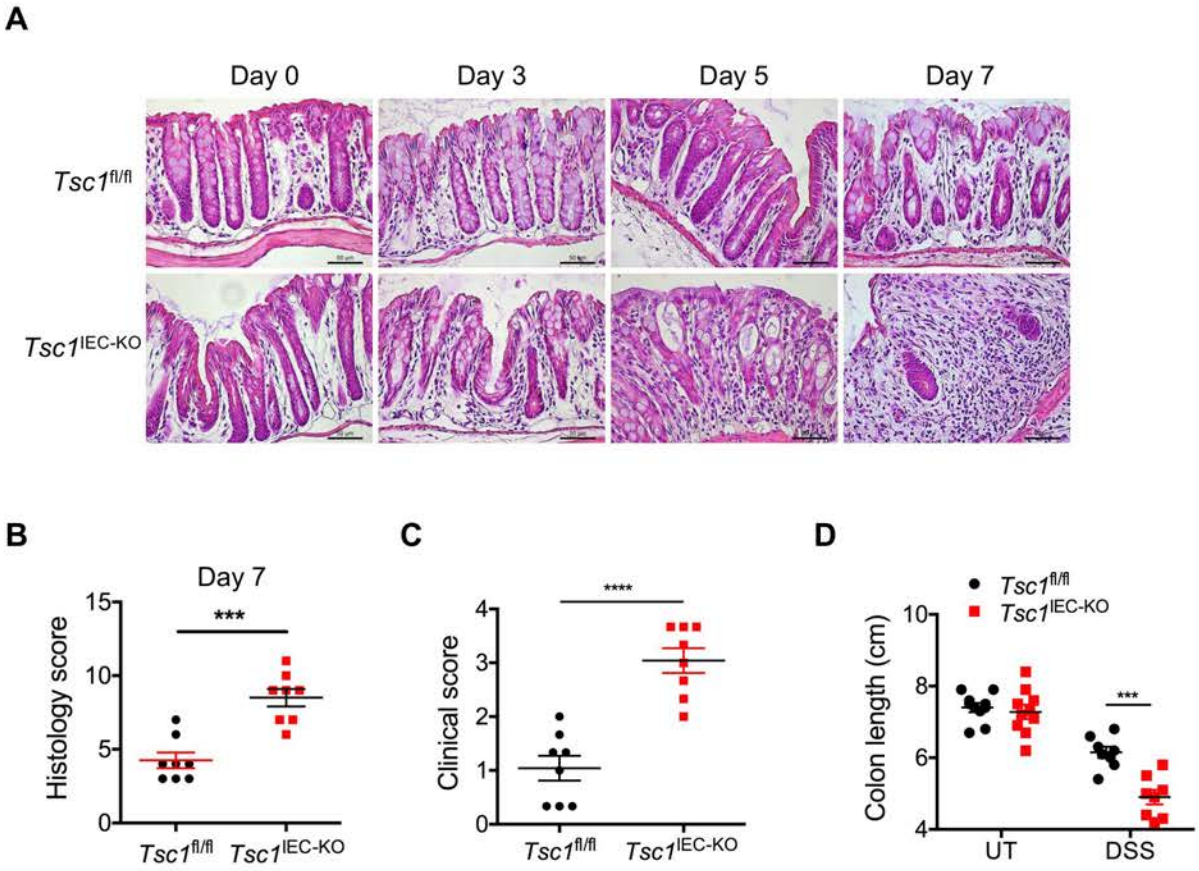
Epcam
TUNEL
TUNEL+ IECs/ Field



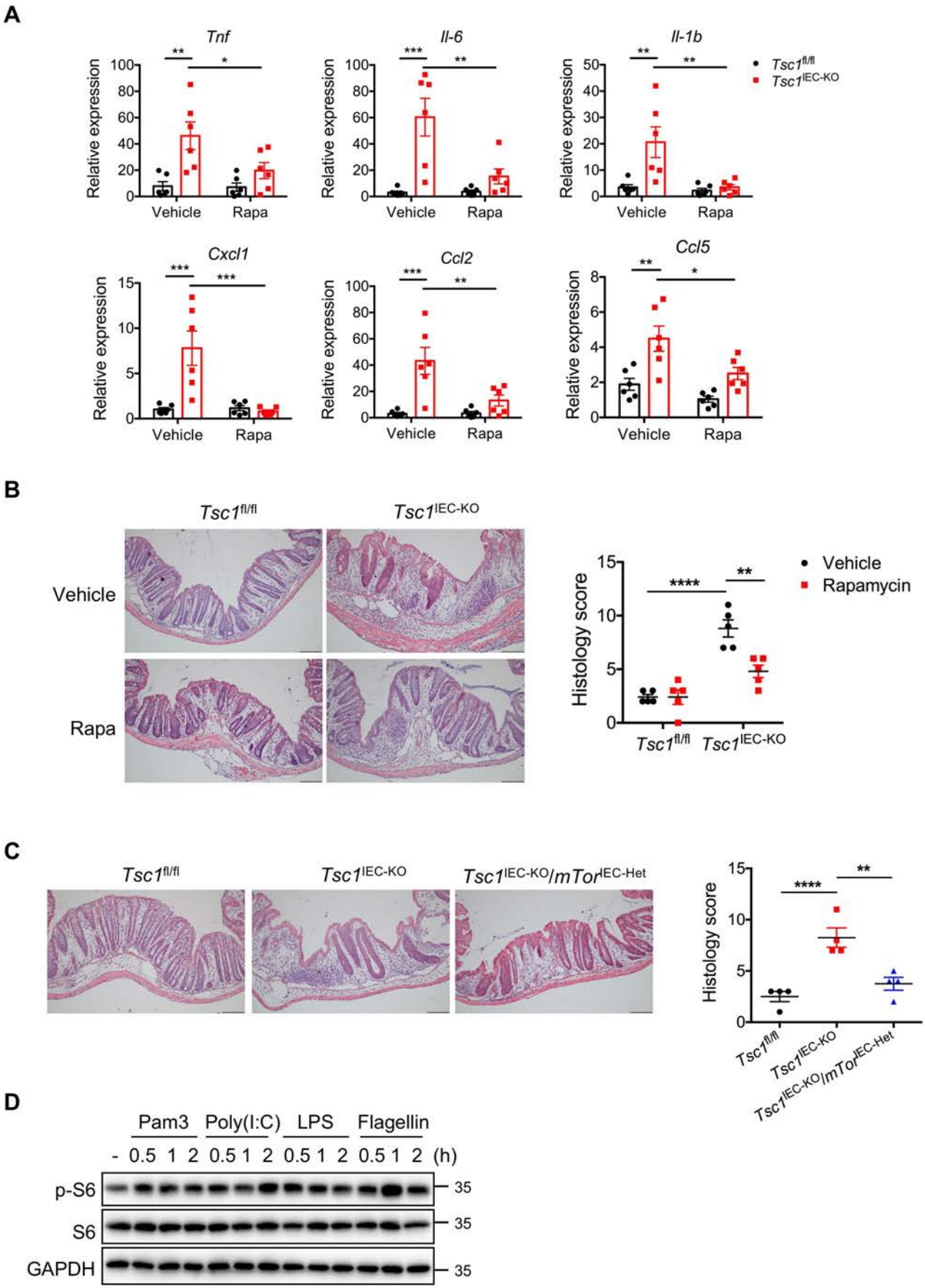
Supplementary Figure 1. (A-E) The C57BL/6 mice pre-fed with normal or high-protein/fat diet were treated with 2.5% DSS (except 3.5% DSS for **D**), the colonic tissues (n=6) were harvested on day 5 after DSS treatment for qPCR analysis (**A**), H&E (**B**) or IF (**E**) staining; the clinic scores were recorded on day 5 (**C**) and the weight-loss was monitored daily (**D**) (n=9-12); The Epcam/TUNEL double-positive cells denoted by arrows were counted over 5 high power fields (400×) per sample (n=30; scale bars: 20 μm) **(E)**. **(F)** qPCR analysis of the proinflammatory gene expression in the colonic tissues from WT and *Mlkl*$^{-/-}$ mice pre-fed with normal or high protein diet and challenged with 2.5% DSS for 5 d. **(G)** Immunoblots of the protein lysates of colonic epithelial cells purified from the mice treated with 2.5% DSS for 5 d. **(H)** IHC staining of the colon sections fed with 16% or 60% fat diet for 2 w. Scale bars: 50 μm. (**I**) Immunoblots of the protein lysates of colonic epithelial cells purified from various diet-fed mice treated with 2.5% DSS for 5 d. p-RIPK1 (Ser166), p-RIPK3 (S232), p-MLKL (S345), p-S6 (S235/236), p-4EBP1 (Thr27/46), p-TSC2 (Thr1462), p-GSK3α/β (S21/9) antibodies were used. The data were pooled from 2 independent experiments **(B, C, D)** or representative of 3 independent experiments **(A, E, F, G, H, I)** and shown as mean±SEM. *p<0.05, **p<0.01 ***p<0.001, ****p<0.0001, by unpaired Student's t test.



Supplementary Figure 2. (A) IHC and IF staining of the colon sections from *Tsc1*$^{fl/fl}$ and *Tsc1*$^{IEC-KO}$ mice (n=6). The CC3- or Epcam/TUNEL-positive cells denoted by arrows were counted over 5 high power fields (400×) per sample (n=30). Scale bars: 20 μm. Scale bars: 50 μm for C-C3, 20 μm for TUNEL. **(B)**. IHC staining of Ki67 in the colon and ileum sections (n=5). Scale bars: 50 μm. **(C)** The H&E and alcian blue staining of the steady-state colon sections. Scale bars: H&E, 100 μm; alcian blue, 50 μm. **(D)** The H&E, alcian blue, alkaline phosphatase and lysozyme staining of ileum sections. Scale bars: 100 μm for alcian blue and alkaline phosphatase, 50 μm for H&E and lysozyme. **(E)** qPCR analysis of gene expression (normalized to beta-actin) in the ileum IECs of *Tsc1*$^{fl/fl}$ and *Tsc1*$^{IEC-KO}$ mice (n=6). Data were representative of 3 independent experiments and shown as mean±SEM. *p<0.05, **p<0.01, ****p<0.0001; NS, not significant; by unpaired Student's t test.



Supplementary Figure 3. (A) H&E staining of the colon sections treated with 2.5% DSS for indicated times. Scale bars: 50 μm. **(B-D)** The *Tsc1*$^{fl/fl}$ and *Tsc1*$^{IEC-KO}$ mice (n=8-10) were treated with 2.5% DSS for 5 d and then followed by regular water. The histology score **(B)** and colon length **(D)** were assessed on day 7. The clinical score **(C)** was recorded on day 5. Data were pooled from 3 independent experiments and shown as mean±SEM. ***p<0.001, ****p<0.0001; by unpaired Student's t test.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Il-1b
Cxcl1
Ccl2
Ccl5
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Rapa
Tsc1fl/fl
Tsc1IEC-KO
B
Tsc1fl/fl
Tsc1IEC-KO
Vehicle
Rapa
Histology score
Vehicle
Rapamycin
C
Tsc1fl/fl
Tsc1IEC-KO
Tsc1IEC-KO/mTorIEC-Het
Histology score
D
Pam3
Poly(I:C)
LPS
Flagellin
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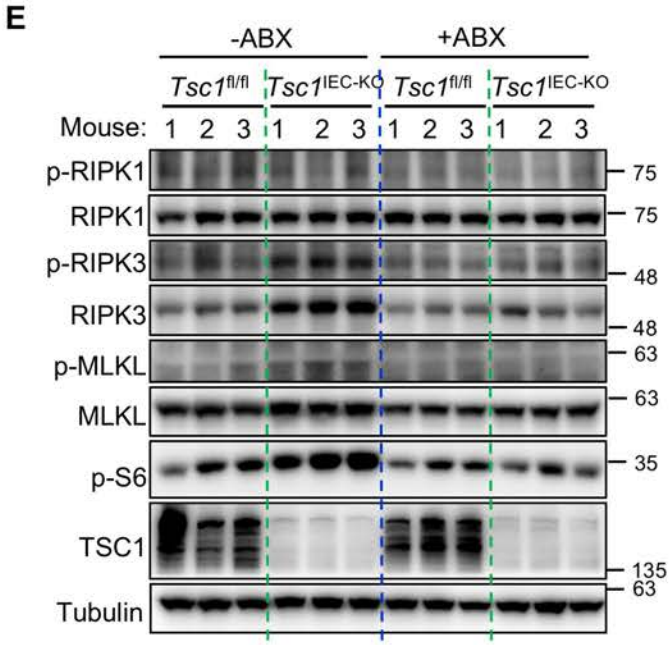
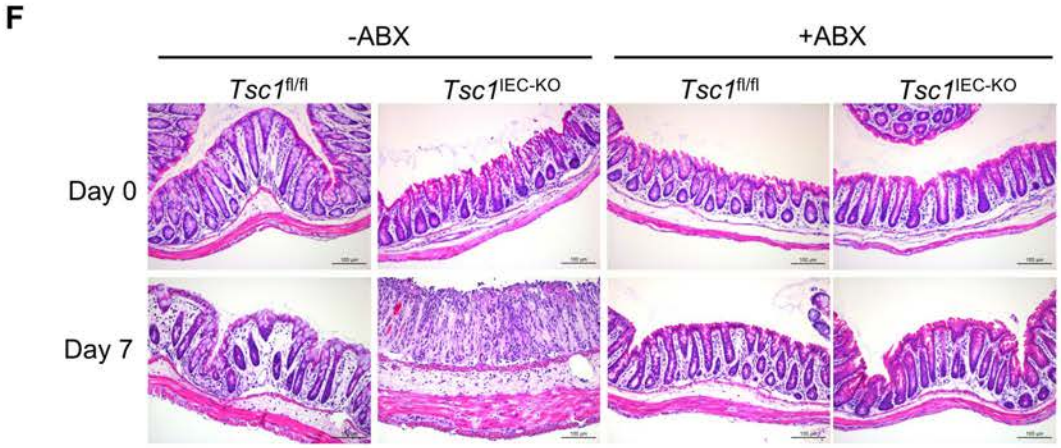
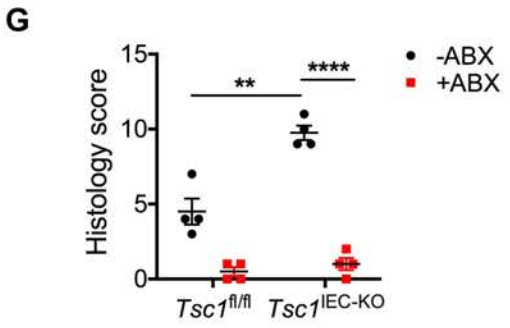
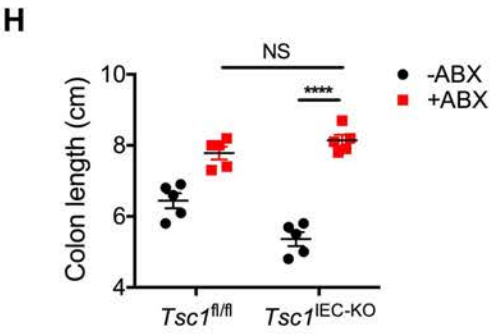
p-S6
S6
GAPDH
35

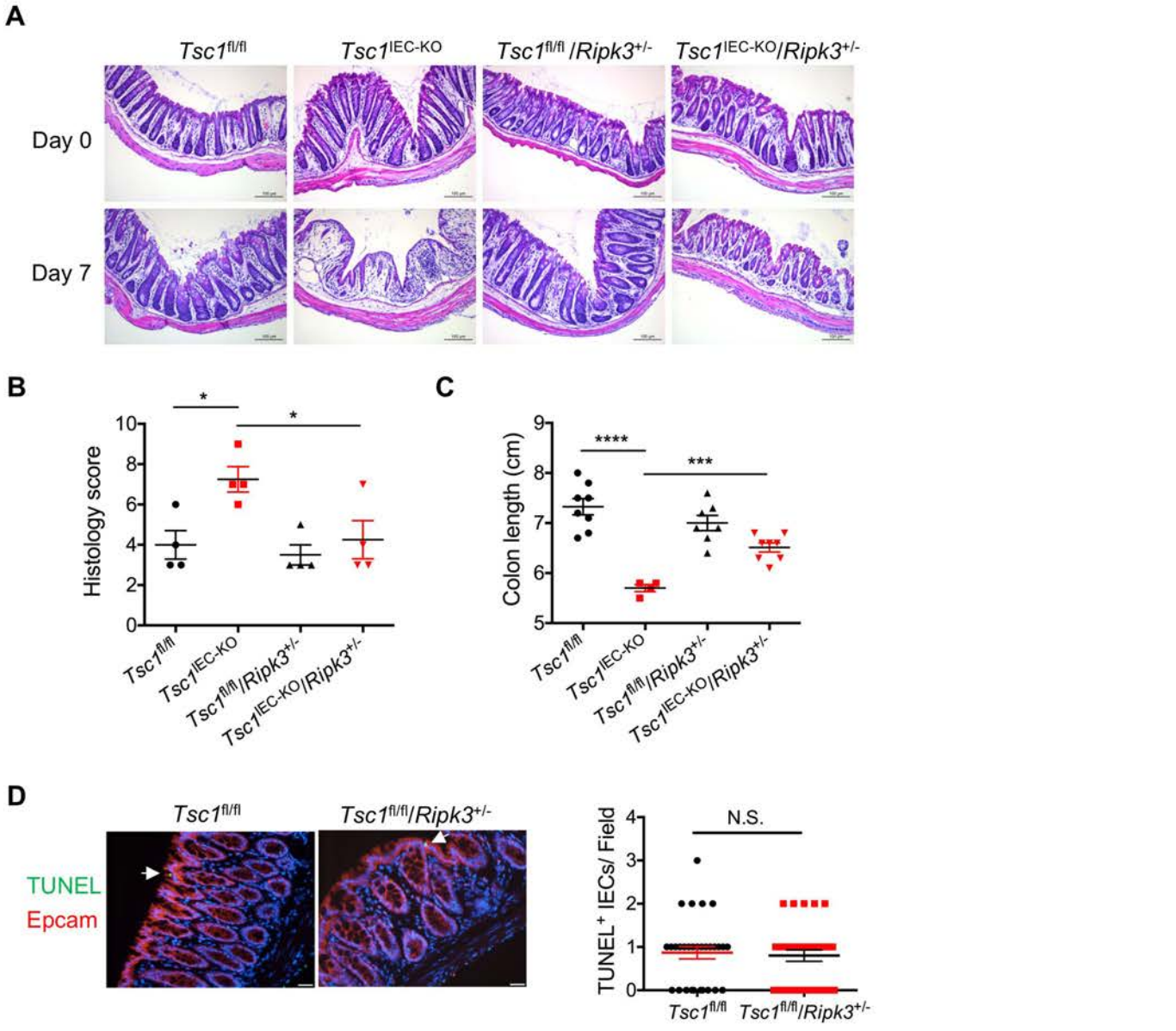




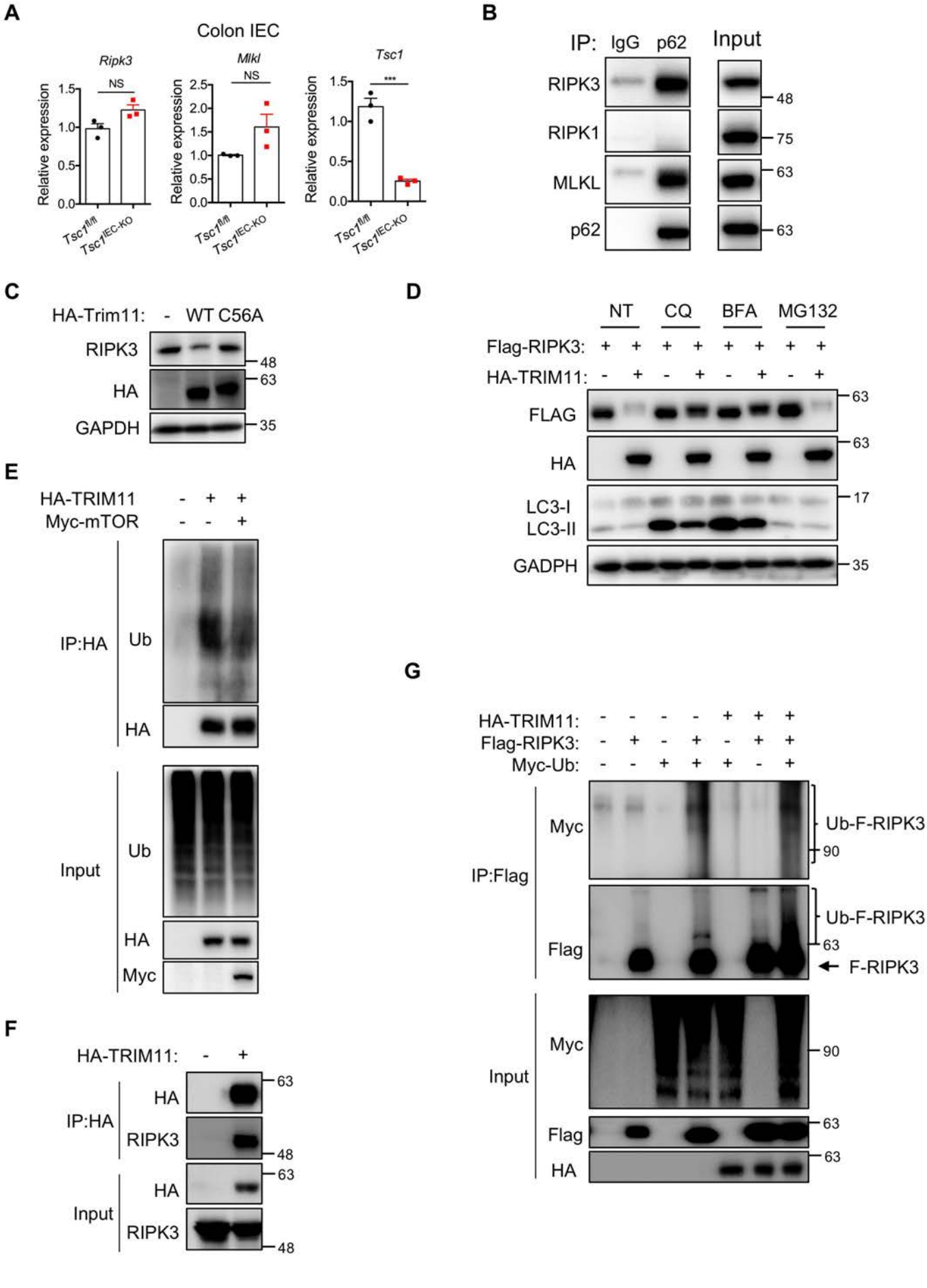




Supplementary Figure 4. (A, B) qPCR analysis **(A,** n=6**)** and H&E staining/histology score **(B,** n=5**)** of colonic tissues from vehicle or rapamycin (5mg/kg, 5 d) treated and DSS-challenged mice for 5 d (**A**) or 8 d (**B**). Scale bars: 100 μm. **(C)** The mice were treated with 2.5% DSS for 5 d. H&E staining of the colon sections and histology scores were assessed at day 8 after DSS treatment (n=4). Scale bars: 100 μm. **(D)** Immunoblots of the protein lysates from the *ex vivo* organoid cells stimulated with Pam3CSK4 (2 μg/ml), 50 μg/ml poly(I:C), LPS (10 μg/ml) or flagellin (10 μg/ml) for various times as indicated. **(E)** Immunoblots of the colonic epithelial lysates from the mice pre-treated with antibiotics (ABX) and then treated with 2.5% DSS for 5 d (n=3). **(F)** H&E staining of the colon sections from mice untreated or treated with 2.5% DSS. Scale bars: 100 μm. **(G, H)** The histology score and colon length were assessed on day 7 after a 5-day-DSS treatment. p-RIPK1 (Ser166), p-RIPK3 (S232), p-MLKL (S345), p-S6 (S235/236) antibodies were used. Data were representative of 3 independent experiments and shown as mean±SEM. **p<0.01, ****p<0.0001; NS, not significant; by and One-way ANOVA.



Supplementary Figure 5. (A) H&E staining of the colon sections from the mice untreated or treated with 2.5% DSS. Scale bars: 100 μm. **(B-C)** The mice (n=4-10) were treated with 2.5% DSS for 5 d and then followed by regular water. The histology score **(B)** and colon length **(C)** were assessed on day 7. **(D)** IF staining of the colon sections from the *Tsc1*$^{fl/fl}$ and *Tsc1*$^{fl/fl}$/*Ripk3*$^{+/-}$ mice (n=6). The double-positive cells denoted by arrows were counted over 5 high power fields (400×) per sample (n=30). Scale bars: 20 μm. Data were pooled from 3 independent experiments **(C)**, or representative of 3 independent experiments **(A, B, D)** and shown as mean±SEM. *p<0.05, ***p<0.001, ****p<0.0001; NS, not significant; by One-way ANOVA **(B, C)** or unpaired Student's t test **(D)**.


A
Colon IEC
Ripk3
Mlkl
Tsc1
NS
NS
***
Relative expression
Tsc1fl/fl
Tsc1IEC-KO
B
IP: IgG p62
Input
RIPK3
RIPK1
MLKL
p62
48
75
63
63
C
HA-Trim11: - WT C56A
RIPK3
HA
GAPDH
48
63
35
D
NT CQ BFA MG132
Flag-RIPK3: + + + + + + + +
HA-TRIM11: - + - + - + - +
FLAG
HA
LC3-I
LC3-II
GADPH
63
63
17
35
E
HA-TRIM11 - + +
Myc-mTOR - - +
IP:HA
Ub
HA
Input
Ub
HA
Myc
F
HA-TRIM11: - +
IP:HA
HA
RIPK3
Input
HA
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63
48
63
48
G
HA-TRIM11: - - - - + + +
Flag-RIPK3: - + - + - + +
Myc-Ub: - - + + + - +
IP:Flag
Myc
Flag
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F-RIPK3
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Myc
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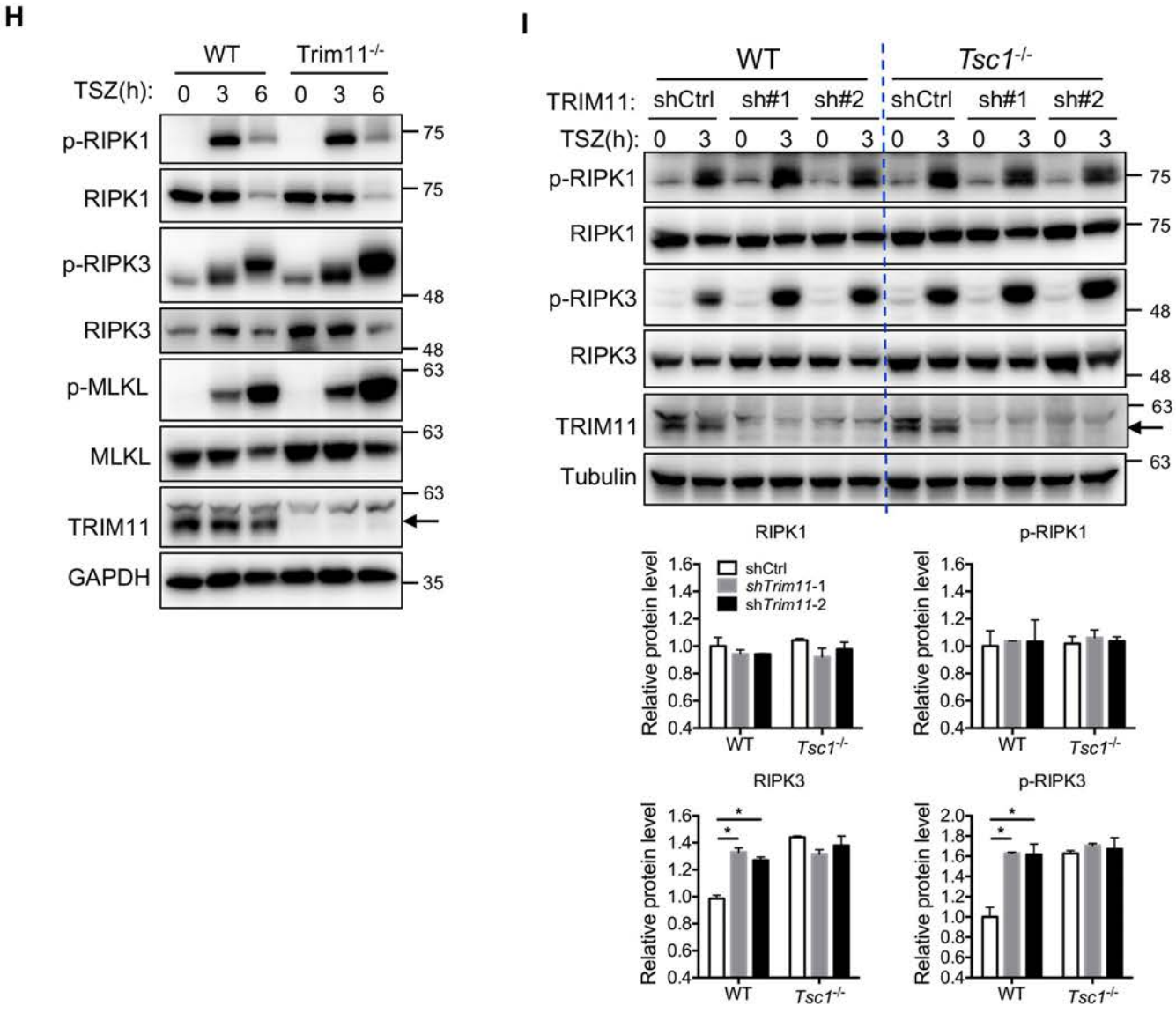
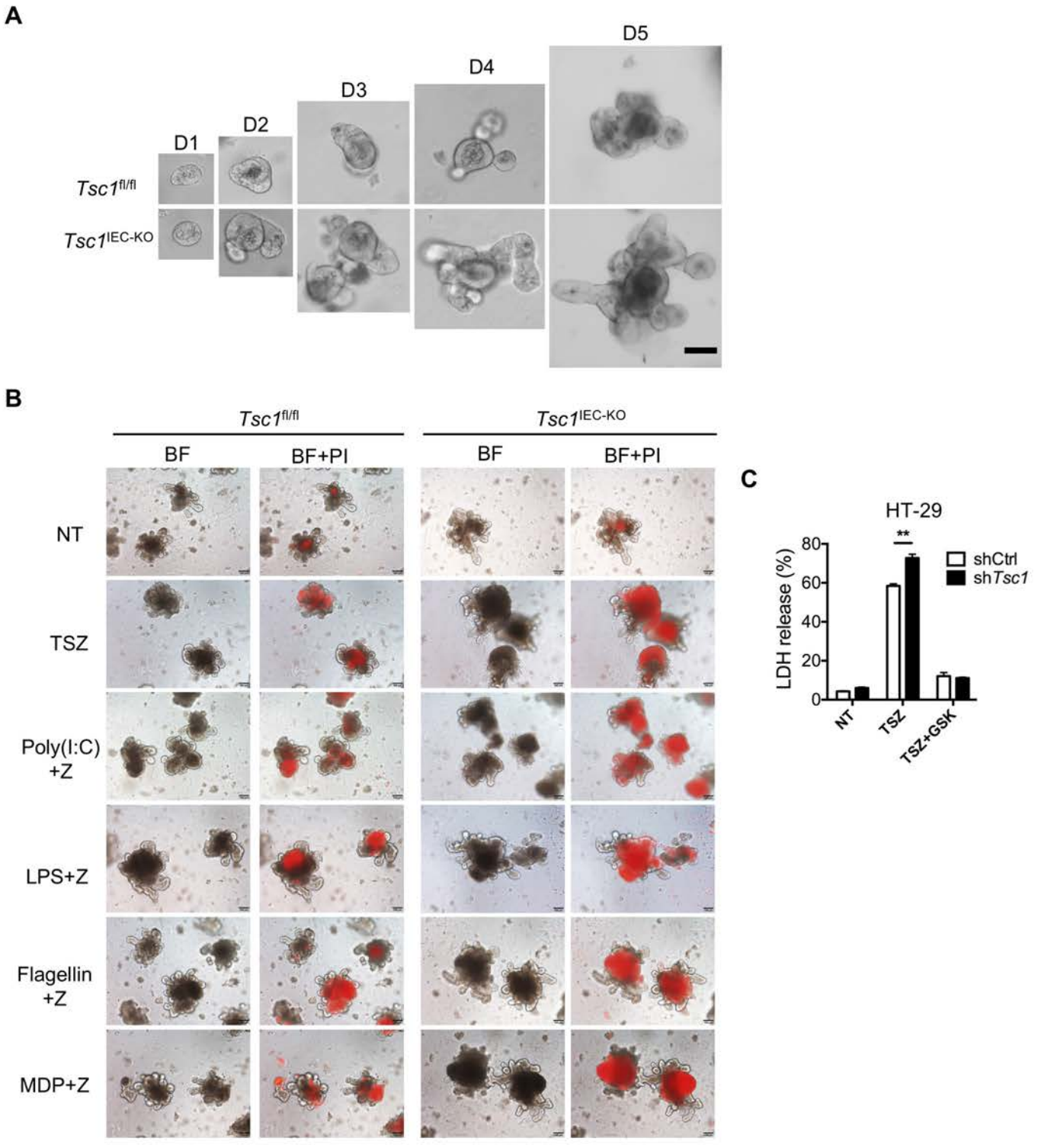
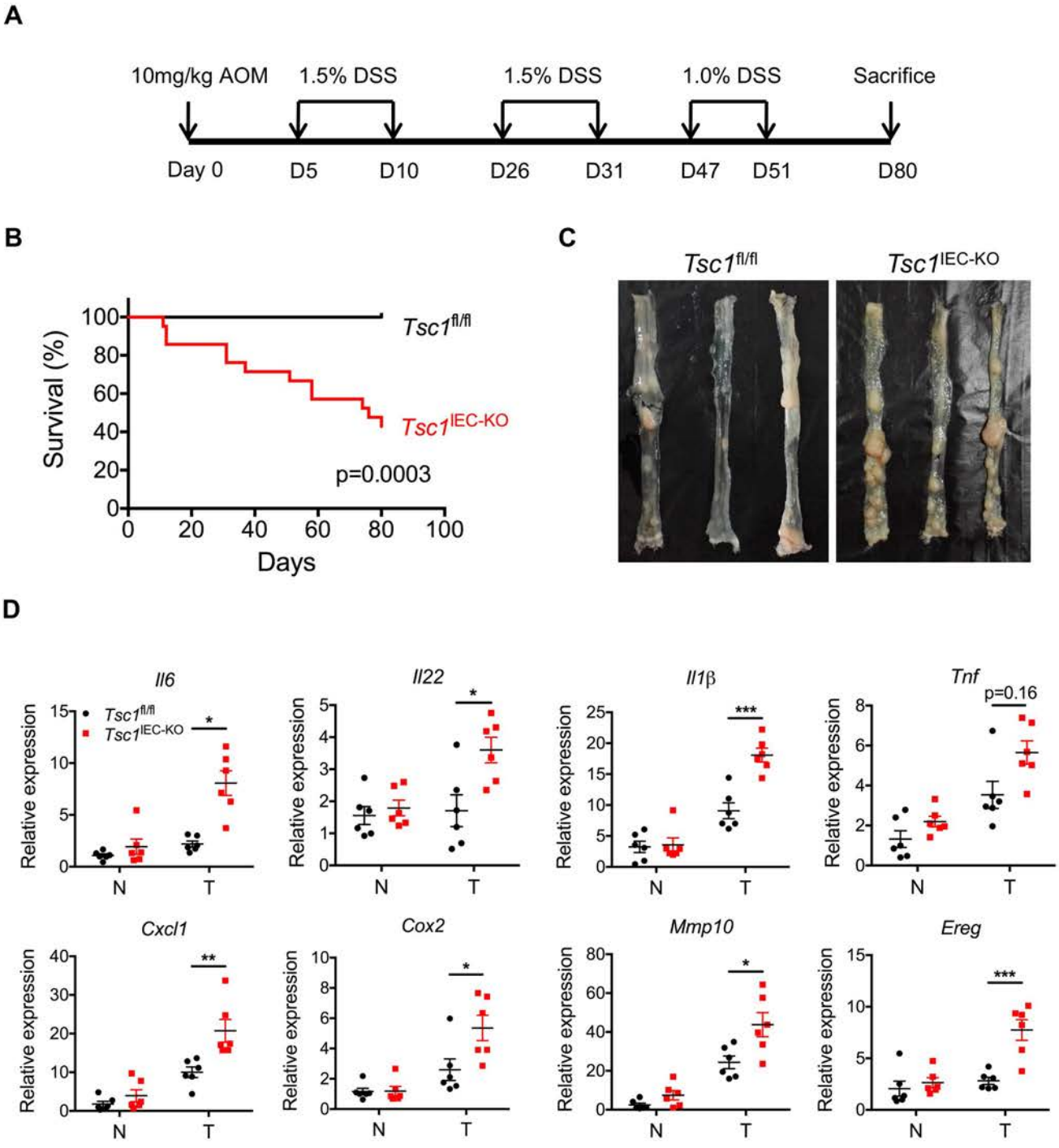


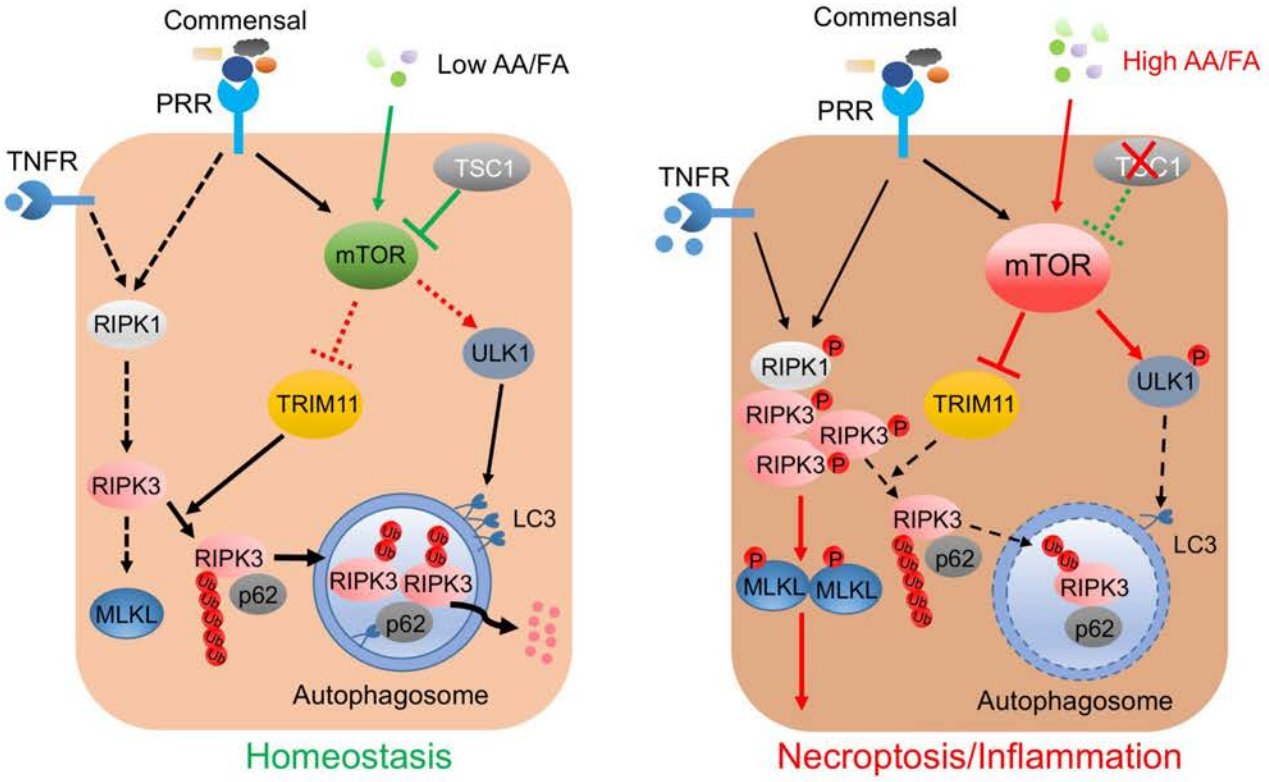
Supplementary Figure 6. (A) qPCR analysis of *Ripk3*, *Mlkl*, *Tsc1* expression (normalized to beta-actin) in the colon IECs of *Tsc1*$^{fl/fl}$ and *Tsc1*$^{IEC\text{-}KO}$ mice (n=3). **(B)** Immunoblots of the MEF cell lysates immunoprecipitated with anti-IgG or anti-p62 antibodies. **(C)** Immunoblotting of endogenous RIPK3 in the whole cell lysates from control, WT or E3 ligase inactive mutant (C56A) of HA-Trim11 overexpressing HT-29 cells. **(D)** HEK293T overexpressing Flag-RIPK3 alone or with HA-TRIM11 were either untreated or treated with 20 μM chloroquine (CQ, 18 h), 0.2 μM bafilomycin A1 (BFA, 18 h) or 10 μM MG132 (6 h), respectively, and the cell lysates were probed with anti-RIPK3. (**E**) The ubiquitination of immunoprecipitated HA-Trim11 in the absence or presence of exogenous mTOR in HEK293T cells. **(F)** control or Trim11 stably transfected MEF cell lysates were immunoprecipitated with anti-HA beads, and then immunoblotted with various antibodies. **(G)** HEK293T cells were transfected with various combinations of plasmids for 36 h. The cell lysates were immunoprecipitated with anti-Flag beads and then immunoblotted with the indicated antibodies. (**H, I**) WT and Trim11-KO HT-29 cells **(H)** or WT and *Tsc1*$^{-/-}$ MEFs expressing control or TRIM11 shRNAs (**I**) were treated with 10 ng/ml of TNF/100 nM BV6/20 μM of zVAD (TSZ) for indicated times. The cell lysates were immunoblotted and quantified by Image J. p-RIPK1 (Ser166), p-RIPK3 (S227), p-MLKL (S358) antibodies were used in **(H)** ; p-RIPK1(Ser166), p-RIPK3(S232) antibodies were used in **(I)**. Data were representative of 3 independent experiments and shown as mean±SEM. *p<0.05, ***p<0.001 ; NS, not significant; by unpaired Student's t test **(A)** or One-way ANOVA **(H)**.



Supplementary Figure 7. (A) The images of the *ex vivo* organoid growth from day 1 to day 5 were taken under a light microscope. Scale bars: 100 μm. **(B)** The organoids were treated with TSZ (10 ng/ml TNF (T), 10 nM BV-6 (S) and 20 μM zVAD (Z)) or various TLR ligands in the presence of 20 μM zVAD (Z) for 16 h and then propidium iodide (PI) was added to stain the dead cells. Images under bright field (BF) or Immunofluorescent staining were taken. Scale bars: 100 μm. **(C)** The control and *Tsc1*-knockdown HT-29 cells were treated with 10 ng/ml hTNF (T), 1 μM BV-6 (S) and 20 μM zVAD (Z) w or w/o 5 μM GSK'872 for 24 h. Cell cytotoxicity was measured by LDH release. Data were representative of 3 independent experiments and shown as mean±SEM. **p<0.01, by unpaired Student's t test.



Supplementary Figure 8. (A) The schematic presentation of Colitis-Associated Colorectal (CAC) model employed in this study. **(B)** The survival of *Tsc1*$^{fl/fl}$ (n=16) and *Tsc1*$^{IEC-KO}$ (n=21) mice challenged with the CAC regimen. **(C)** The representative images of the colon tumors from the *Tsc1*$^{fl/fl}$ and *Tsc1*$^{IEC-KO}$ mice challenged with the CAC regimen. **(D)** qPCR analysis of proinflammatory gene expression (normalized to beta-actin) in the colon tumors (T) and normal colon tissues (N) of the mice challenged with the CAC regimen (n=6). Data were pooled from 2 independent experiments **(B)** or representative of 2 independent experiments in **(C, D)** and shown as mean±SEM. **p<0.01, ***p<0.001, ****p<0.0001; by Log-rank test **(B)** or unpaired Student's t test **(D)**.



Supplementary Figure 9. A working model illustrating how epithelial mTOR integrates dietary and microbial signals to balance autophagy/necroptosis in the regulation of intestinal homeostasis and inflammation. In the steady-state, mTOR activity in the IECs is kept at the basal level due to the negative regulation by TSC1, and lack of strong stimulation from the dietary nutrients or microbial PAMPs. Accordingly, optimal autophagy keeps RIPK3 and necroptosis at the minimal level to maintain intestinal homeostasis. However, in the presence of abundant dietary proteins/microbial PAPMs, or dysfunctional negative regulators such as loss-of-function of TSC1, mTOR becomes hyperactivated, leading to the shutdown of autophagy biogenesis and TRIM11 action in the IECs. Due to the lack of autophagic degradation, RIPK3 can be highly accumulated in the IECs, augmenting the susceptibility to necroptosis. With the concomitant increase in the necroptotic triggers like TLR ligands and TNF, these IECs then become poised to initiate necroptotic signaling, which culminating on the phosphorylation and activation of MLKL for necroptosis, intestinal dysfunction, and inflammation.