Fig. S1

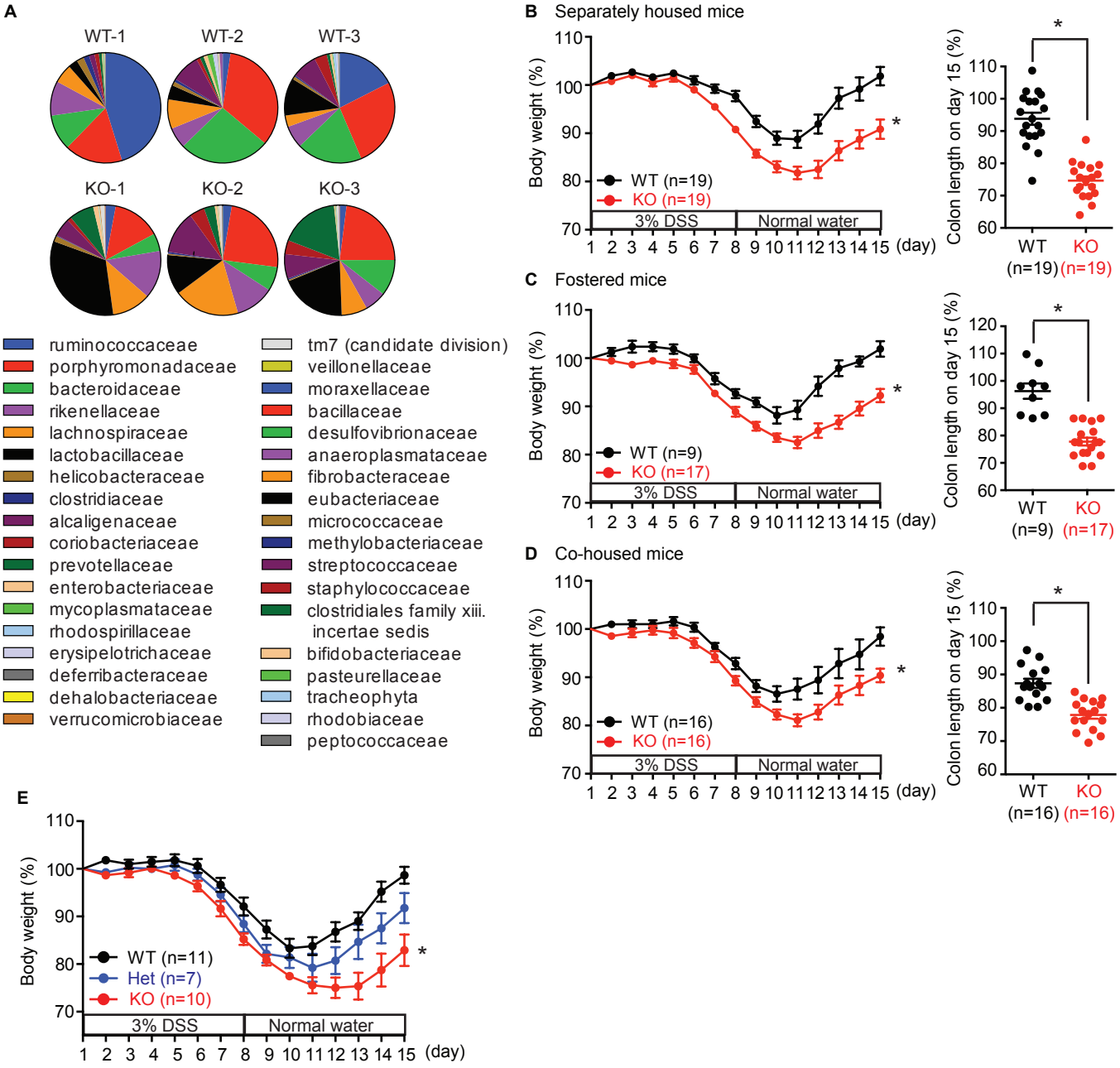
A
WT-1
WT-2
WT-3
KO-1
KO-2
KO-3
ruminococcaceae
porphyromonadaceae
bacteroidaceae
rikenellaceae
lachnospiraceae
lactobacillaceae
helicobacteraceae
clostridiaceae
alcaligenaceae
coriobacteriaceae
prevotellaceae
enterobacteriaceae
mycoplasmataceae
rhodospirillaceae
erysipelotrichaceae
deferribacteraceae
dehalobacteriaceae
verrucomicrobiaceae
tm7 (candidate division)
veillonellaceae
moraxellaceae
bacillaceae
desulfovibrionaceae
anaeroplasmataceae
fibrobacteraceae
eubacteriaceae
micrococcaceae
methylobacteriaceae
streptococcaceae
staphylococcaceae
clostridiales family xiii. incertae sedis
bifidobacteriaceae
pasteurellaceae
tracheophyta
rhodobiaceae
peptococcaceae
B Separately housed mice
Body weight (%)
WT (n=19)
KO (n=19)
3% DSS
Normal water
(day)
Colon length on day 15 (%)
WT (n=19)
KO (n=19)
C Fostered mice
Body weight (%)
WT (n=9)
KO (n=17)
3% DSS
Normal water
(day)
Colon length on day 15 (%)
WT (n=9)
KO (n=17)
D Co-housed mice
Body weight (%)
WT (n=16)
KO (n=16)
3% DSS
Normal water
(day)
Colon length on day 15 (%)
WT (n=16)
KO (n=16)
E
Body weight (%)
WT (n=11)
Het (n=7)
KO (n=10)
3% DSS
Normal water
(day)

Figure S1, *Ripk3*$^{-/-}$ mice were sensitive to DSS-induced colitis, Related to Figure 1.

(A) Metagenomic analysis using gDNA from feces from separately-housed *Ripk3*$^{+/+}$ (WT) and *Ripk3*$^{-/-}$ (KO) mice was performed. Three mice of each genotype from different cages were used. The pie charts made by the percentage of each bacterial family colored according to the legend are shown. Detailed result is found in Table S1. **(B)** Separately-housed *Ripk3*$^{+/+}$ and *Ripk3*$^{-/-}$ mice were given DSS. **(C)** *Ripk3*$^{+/+}$ and *Ripk3*$^{-/-}$ mice were nursed by the same foster mother in the same cage after birth. After weaning, bedding materials from different cages were mixed twice a week to further minimize differences in intestinal microbial environment. **(D)** *Ripk3*$^{+/+}$ and *Ripk3*$^{-/-}$ mice were co-housed in same cage for four weeks prior to and during DSS treatment. Left and right panels in **B**, **C**, and **D** show body weight and colon length, respectively. **(E)** DSS-induced body weight loss in *Ripk3*$^{-/-}$ mice was compared to their littermate *Ripk3*$^{+/+}$ and *Ripk3*$^{+/-}$ (Het) mice ($p<0.05$, WT vs KO; $p=0.0589$, WT vs Het). The numbers in parentheses represent the number of mice used in each group. The data were obtained from 2-4 independent experiments. Each dot represents one mouse; bars = mean ± SEM. Asterisks: $p < 0.05$.

Fig. S2

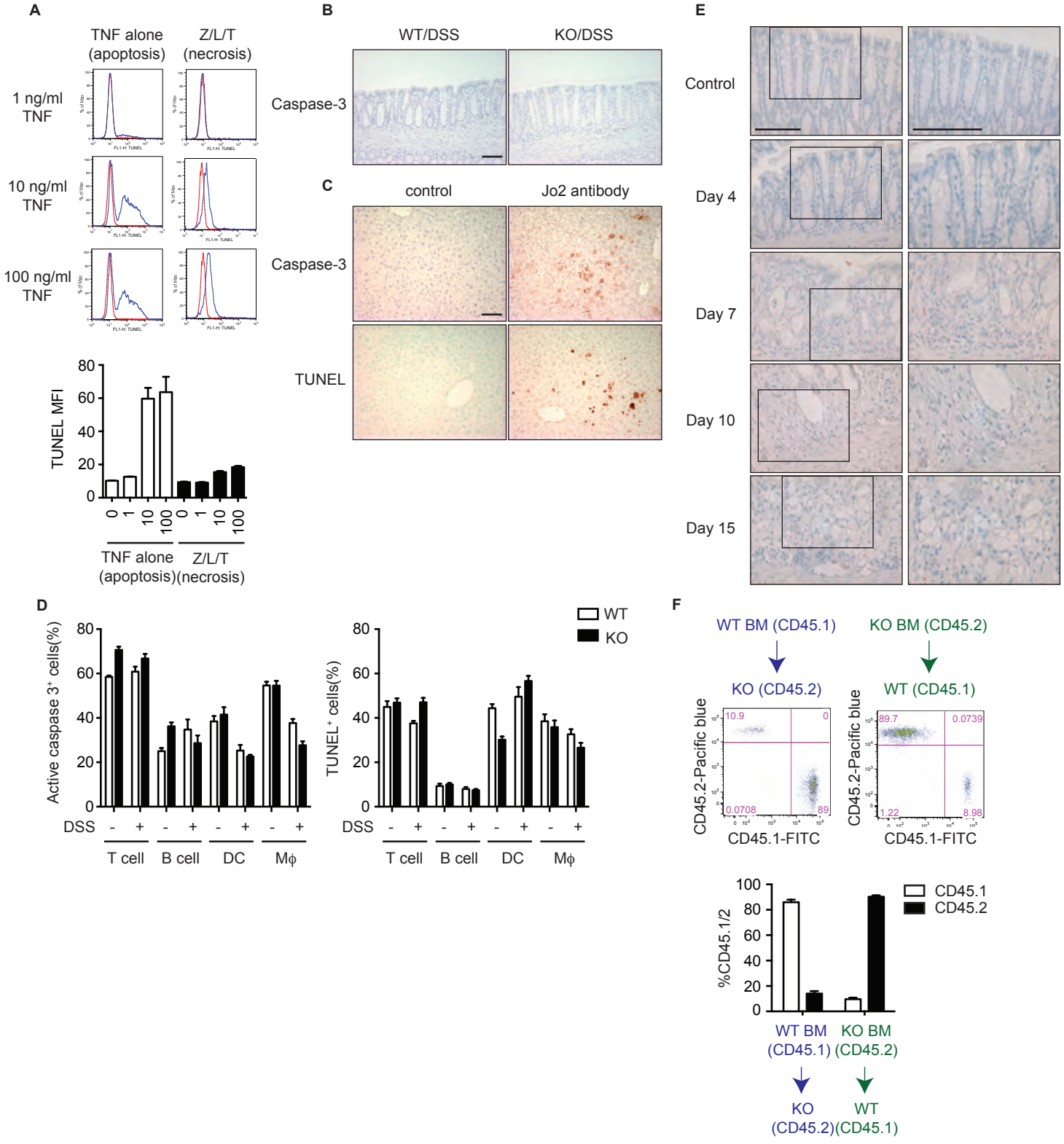
A
TNF alone (apoptosis)
Z/L/T (necrosis)
1 ng/ml TNF
10 ng/ml TNF
100 ng/ml TNF
TUNEL MFI
0 1 10 100 0 1 10 100
TNF alone (apoptosis)
Z/L/T (necrosis)
B
WT/DSS
KO/DSS
Caspase-3
C
control
Jo2 antibody
Caspase-3
TUNEL
E
Control
Day 4
Day 7
Day 10
Day 15
D
Active caspase 3+ cells(%)
TUNEL+ cells(%)
WT
KO
DSS
T cell
B cell
DC
Mϕ
F
WT BM (CD45.1)
KO BM (CD45.2)
KO (CD45.2)
WT (CD45.1)
CD45.2-Pacific blue
CD45.1-FITC
10.9
0
0.0708
89
89.7
0.0739
1.22
8.98
%CD45.1/2
CD45.1
CD45.2
WT BM (CD45.1)
KO BM (CD45.2)
KO (CD45.2)
WT (CD45.1)

Figure S2, RIPK3 did not contribute to initial colon epithelial injury in response to DSS, Related to Figure 2.

(A) TNFR2-overexpressing Jurkat cells (4E3) were stimulated with various concentration of TNF (T) in the absence/presence of 20 μM z-VAD-fmk (Z) and 5 μM Smac mimetic (LBW242; L). Fourteen hours after stimulation, the cells were subjected to TUNEL staining. Representative FACS plots were shown. Red and blue lines indicate the untreated or TNF-treated samples, respectively. Mean fluorescence intensity (MFI) of TUNEL-positive cells were shown on the bottom. The experiment was performed with triplicate samples. **(B)** Representative pictures of caspase-3 staining of colon tissues harvested from DSS-treated *Ripk3*$^{+/+}$ (WT) and *Ripk3*$^{-/-}$ (KO) mice on day 4. **(C)** Representative pictures of caspase-3 and TUNEL staining of liver tissues harvested from wild type mice injected with anti-Fas antibody (Jo2; 0.5 mg/kg) are shown. **(D)** The percentage of active caspase 3 (left panel) or TUNEL positive cells (right panel) in lamina propria T cell, B cell, DC, and macrophage (Mϕ) from DSS-treated *Ripk3*$^{+/+}$ and *Ripk3*$^{-/-}$ mice (day 7) were determined by flow cytometry. $CD45^+CD3^+$, $CD45^+CD19^+$, $CD45^+CD3^-CD19^-CD11c^+$, and $CD45^+CD3^-CD19^-CD11c^-CD11b^+$ cells were defined as T cell, B cell, DC, and Mϕ, respectively. Data were obtained from 3-4 mice for each sample. **(E)** Colon tissues from DSS-treated *Ripk3*$^{-/-}$ mice were stained with anti-RIPK3 antibody. The area in the square in left panels is magnified in right panels. **(F)** BM reconstitution in BM chimeric mice (n=3) was verified by flow cytometry. Scale bars represent 75 μm. Results shown are mean ± SEM.

Fig. S3

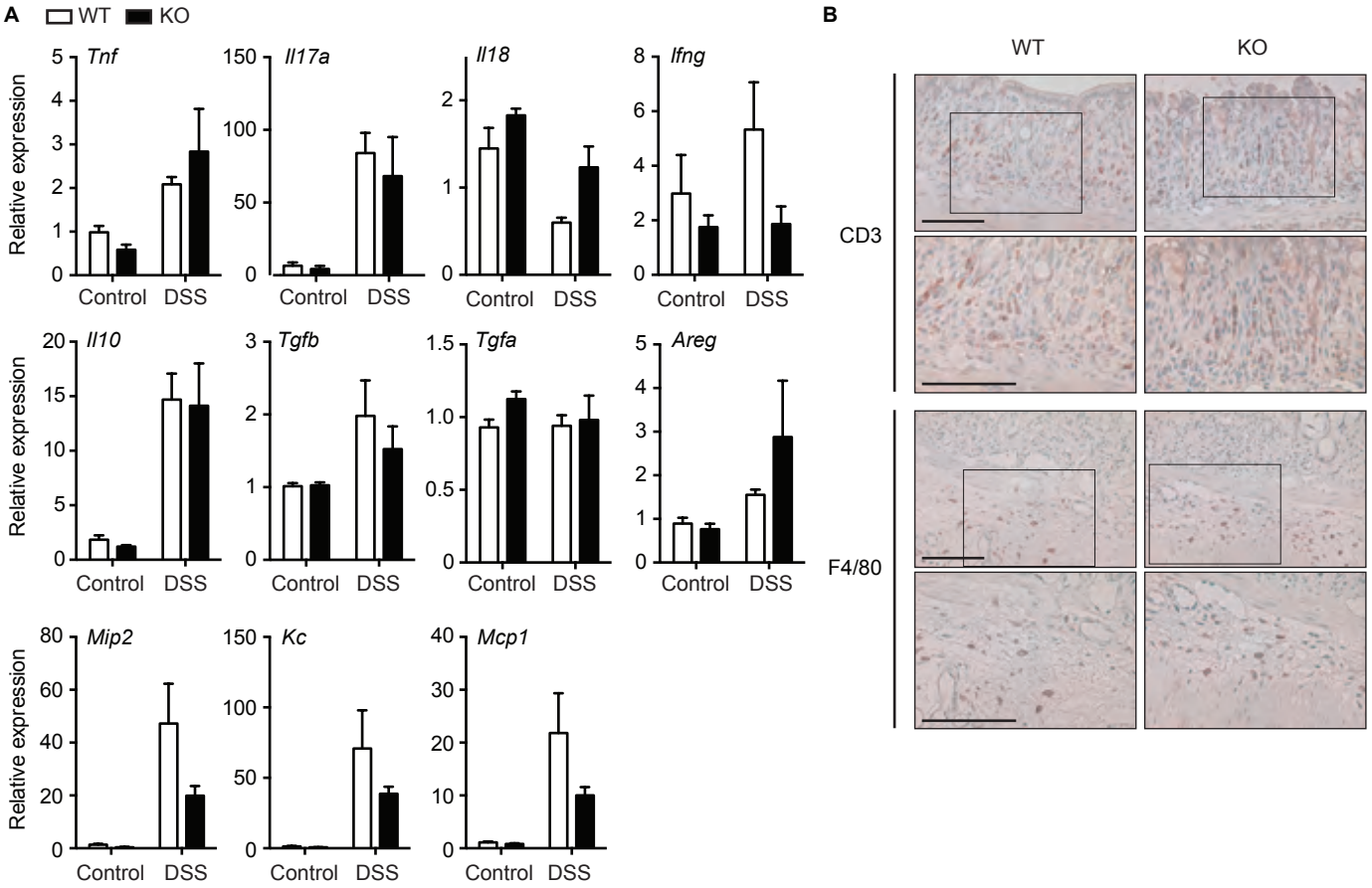
A
WT
KO
Relative expression
Tnf
Il17a
Il18
Ifng
Il10
Tgfb
Tgfa
Areg
Mip2
Kc
Mcp1
Control
DSS
B
WT
KO
CD3
F4/80

Figure S3, Cytokine expression and normal recruitment of immune cells in colon tissues of DSS-treated *Ripk3*$^{+/+}$ and *Ripk3*$^{-/-}$ mice, Related to Figure 3.

(A) Relative mRNA expression of various cytokines in DSS-treated *Ripk3*$^{+/+}$ and *Ripk3*$^{-/-}$ colon on day 7 (n=5-12). **(B)** Representative pictures of immunohistochemistry using anti-CD3 and anti-F4/80 antibodies in colon tissues from DSS-treated *Ripk3*$^{+/+}$ (WT) and *Ripk3*$^{-/-}$ (KO) mice on day 7. Lower panels show the magnified versions of the areas marked by the squares in upper panels. Scale bars represent 75 μm. Results shown are mean ± SEM.

Fig. S4

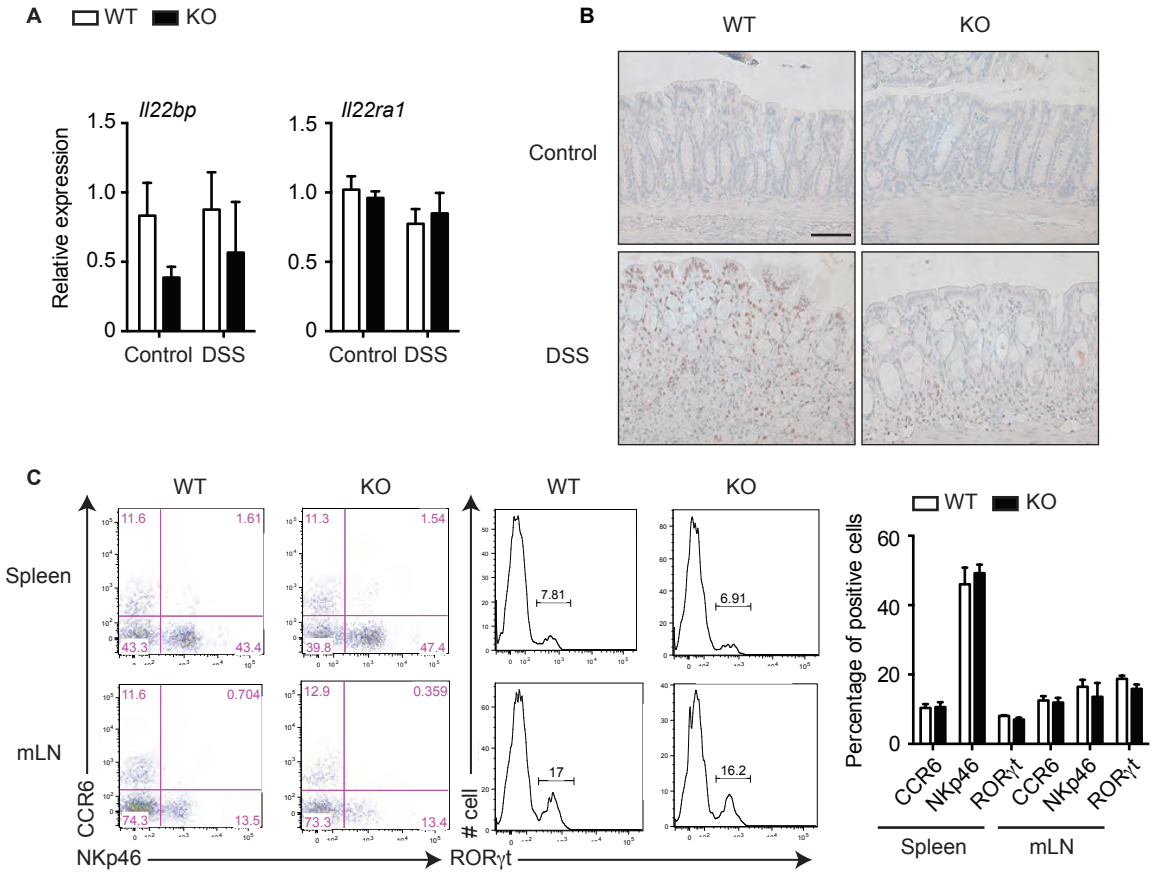
A
WT
KO
Il22bp
Il22ra1
Relative expression
1.5
1.0
0.5
0
Control
DSS
B
WT
KO
Control
DSS
C
WT
KO
Spleen
mLN
11.6
1.61
43.3
43.4
11.3
1.54
39.8
47.4
11.6
0.704
74.3
13.5
12.9
0.359
73.3
13.4
CCR6
NKp46
cell
RORγt
7.81
6.91
17
16.2
Percentage of positive cells
60
40
20
0
CCR6
NKp46
RORγt
Spleen
mLN

Figure S4, Normal differentiation of innate lymphoid cells in *Ripk3*$^{-/-}$ mice, Related to Figure 4.

(A) Relative mRNA expression of *Il22bp* and *Il22ra1* in DSS-treated *Ripk3*$^{+/+}$ and *Ripk3*$^{-/-}$ colon on day 7 (n=5-12). **(B)** Phospho-STAT3 in *Ripk3*$^{+/+}$ (WT) and *Ripk3*$^{-/-}$ (KO) colon sections on day 7. Scale bar represents 75 μm. **(C)** Expression of CCR6, NKp46, and RORγt on CD3^{-}CD19^{-} CD11b^{-}IL-7R^{+} cells in the spleen and mesenteric lymph nodes (mLN) was examined in *Ripk3*$^{+/+}$ and *Ripk3*$^{-/-}$ mice **(**n=3). Representative FACS plots and percentage of cells expressing CCR6, NKp46, or RORγt are shown. Results shown are mean ± SEM.

Fig. S5

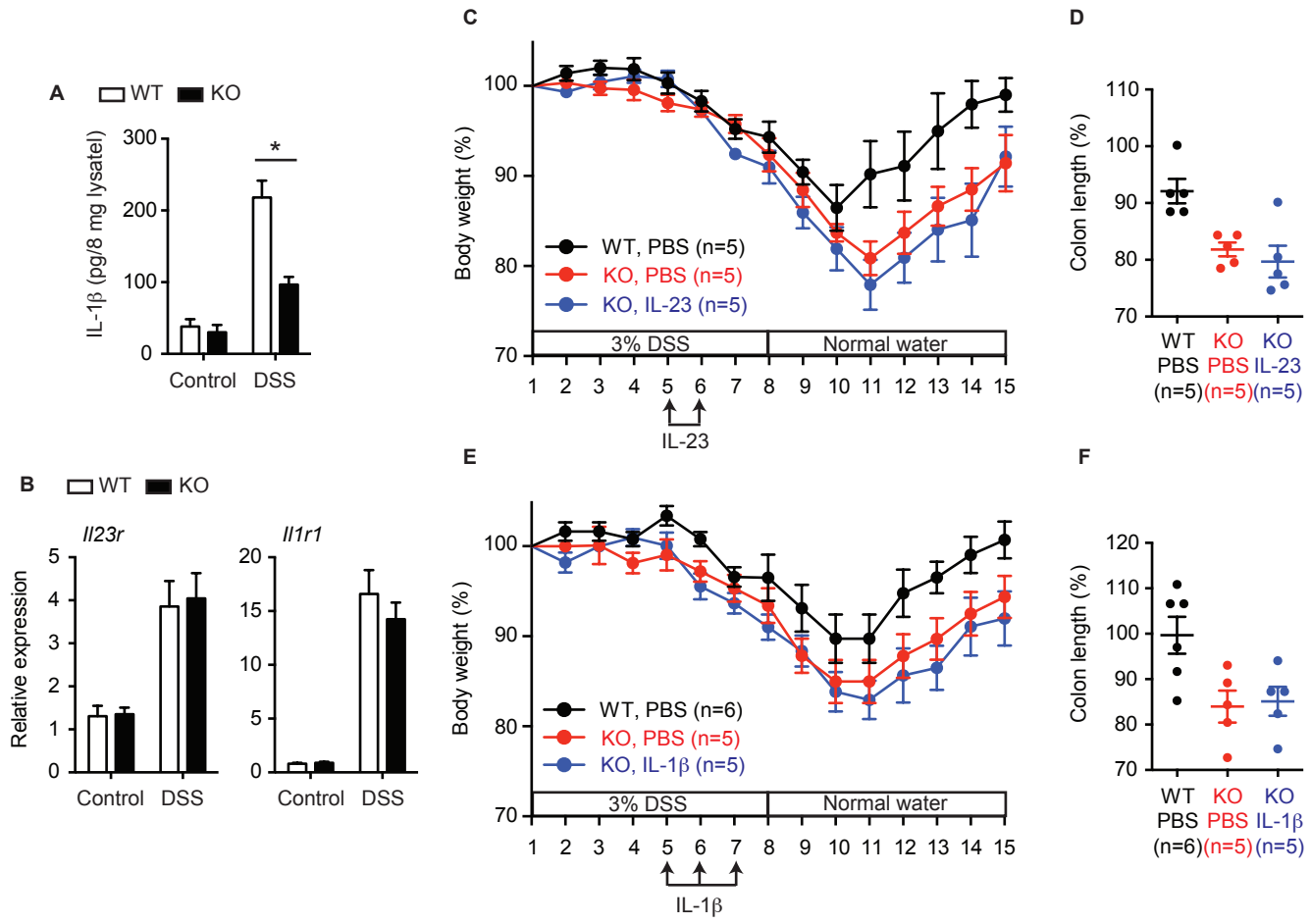
A
WT
KO
IL-1β (pg/8 mg lysatel)
300
200
100
0
*
Control
DSS
B
WT
KO
Il23r
Il1r1
Relative expression
5
4
3
2
1
0
20
15
10
5
0
Control
DSS
Control
DSS
C
Body weight (%)
100
90
80
70
WT, PBS (n=5)
KO, PBS (n=5)
KO, IL-23 (n=5)
3% DSS
Normal water
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
IL-23
D
Colon length (%)
110
100
90
80
70
WT PBS (n=5)
KO PBS (n=5)
KO IL-23 (n=5)
E
Body weight (%)
100
90
80
70
WT, PBS (n=6)
KO, PBS (n=5)
KO, IL-1β (n=5)
3% DSS
Normal water
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
IL-1β
F
Colon length (%)
120
110
100
90
80
70
WT PBS (n=6)
KO PBS (n=5)
KO IL-1β (n=5)

Figure S5, Recombinant IL-23 or IL-1β alone did not rescue severe DSS-induced colitis in *Ripk3*$^{-/-}$ mice, Related to Figure 5.

(A) Protein and **(B)** RNA extracted from colon tissues of DSS-treated *Ripk3*$^{+/+}$ (WT) and *Ripk3*$^{-/-}$ (KO) mice on day 7 were subjected to reverse transcription followed by qPCR (n=5-12) and ELISA (n=3-6), respectively. **(C-D)** Recombinant IL-23 (1 μg/injection) or **(E-F)** IL-1β (50 ng/injection) was i.p. injected to *Ripk3*$^{-/-}$ mice. As control, the equivalent volume of PBS was injected to *Ripk3*$^{+/+}$ and *Ripk3*$^{-/-}$ mice. The number in parentheses represents the number of mice used. Results shown are mean ± SEM. Asterisks: $p < 0.05$.

Fig. S6

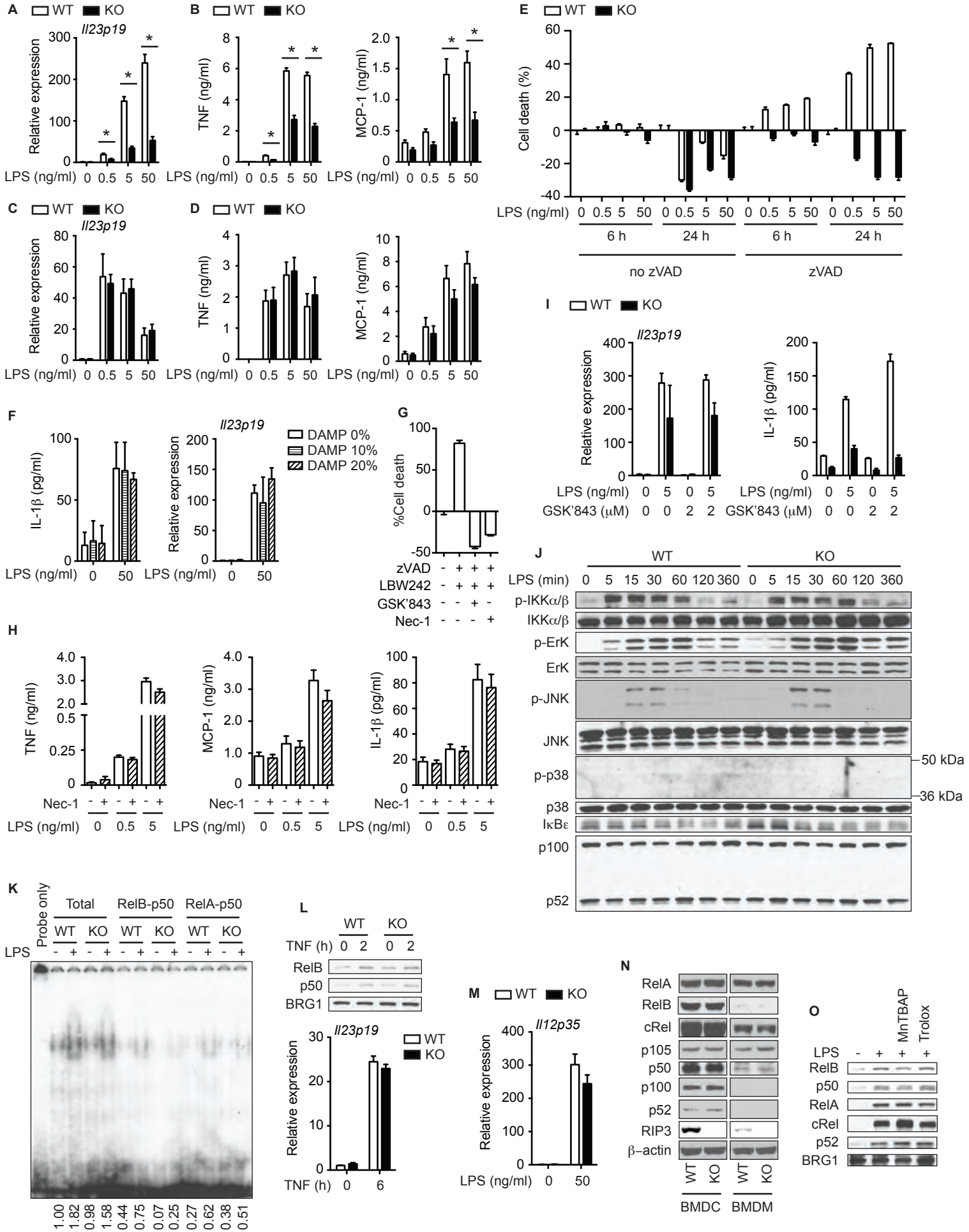
A
WT
KO
Il23p19
Relative expression
LPS (ng/ml) 0 0.5 5 50
B
TNF (ng/ml)
MCP-1 (ng/ml)
C
D
E
Cell death (%)
LPS (ng/ml) 0 0.5 5 50 0 0.5 5 50 0 0.5 5 50 0 0.5 5 50
6 h
24 h
6 h
24 h
no zVAD
zVAD
F
IL-1β (pg/ml)
DAMP 0%
DAMP 10%
DAMP 20%
G
%Cell death
zVAD - + + +
LBW242 - + + +
GSK'843 - - + -
Nec-1 - - - +
H
Nec-1 - + - + - +
I
GSK'843 (μM) 0 0 2 2
J
WT
KO
LPS (min) 0 5 15 30 60 120 360 0 5 15 30 60 120 360
p-IKKα/β
IKKα/β
p-ErK
ErK
p-JNK
JNK
p-p38
50 kDa
36 kDa
p38
IκBε
p100
p52
K
Probe only
Total
RelB-p50
RelA-p50
WT
KO
LPS
1.00 1.82 0.98 1.58 0.44 0.75 0.07 0.25 0.27 0.62 0.38 0.51
L
TNF (h) 0 2 0 2
RelB
p50
BRG1
TNF (h) 0 6
M
Il12p35
N
RelA
RelB
cRel
p105
p50
p100
p52
RIP3
β–actin
BMDC
BMDM
O
MnTBAP
Trolox
RelB
p50
RelA
cRel
p52
BRG1

Figure S6, LPS-induced cytokine expression in *Ripk3*$^{+/+}$ and *Ripk3*$^{-/-}$ BMDCs and BMDMs, Related to Figure 6.

(A-D) Cytokine expression in **(A-B)** *Ripk3*$^{+/+}$ (WT) and *Ripk3*$^{-/-}$ (KO) BMDCs and **(C-D)** BMDMs treated with LPS for 6 hours (n=4). **(E)** After LPS stimulation of BMDCs with or without z-VAD-fmk (zVAD), cell death was determined by Annexin V and PI staining. Change in percentage of cell death relative to untreated sample is shown. **(F)** Wild type BMDCs were subjected to 5 times freeze-thaw cycles and subsequently the supernatant was collected as necrotic supernatant, referred to as DAMP in the panel. LPS-induced cytokine expression was examined in the presence of the necrotic supernatant from the equivalent of 10% or 20% of total cells plated (n=3). **(G)** LDH assay in DC2.4 cells treated with 20 μM zVAD, 0.1 μM LBW242, 2 μM GSK'843, and/or 30 μM Nec-1. **(H-I)** Cytokine expression in BMDCs pretreated with 30 μM Nec-1 (n=7) or 2 μM GSK'843 (n=3) and then treated with LPS for 6 hours. **(J)** Whole cell extracts from BMDCs treated with 100 ng/ml LPS were subjected to western blot analyses. **(K)** Nuclear extracts from BMDCs treated with 100 ng/ml LPS for 2 hours were subjected to EMSA after immune depletion with anti-RelA, cRel, and p52 or anti-RelB, cRel, and p52 antibodies. Relative intensities of signal are shown by defining the intensity of the second lane from left-end as 1. The signals at the bottom are derived from free probe. **(L)** Nuclear translocation of RelB and p50 and cytokine expression (n=4) were examined in BMDCs treated with 250 ng/ml TNF. **(M)** Relative *Il12p35* expression level in BMDCs treated with LPS for 6 hours (n=6). **(N)** WCEs from BMDCs and BMDMs were subjected to western blot analyses. The lanes were run on the same gel but were noncontiguous. **(O)** Nuclear extracts from wild type BMDCs pretreated with 100 μM MnTBAP or Trolox and then treated with 100 ng/ml LPS for 2 hours were subjected to western blot analyses. Results shown are mean ± SEM. Asterisks: $p < 0.05$.

Fig. S7

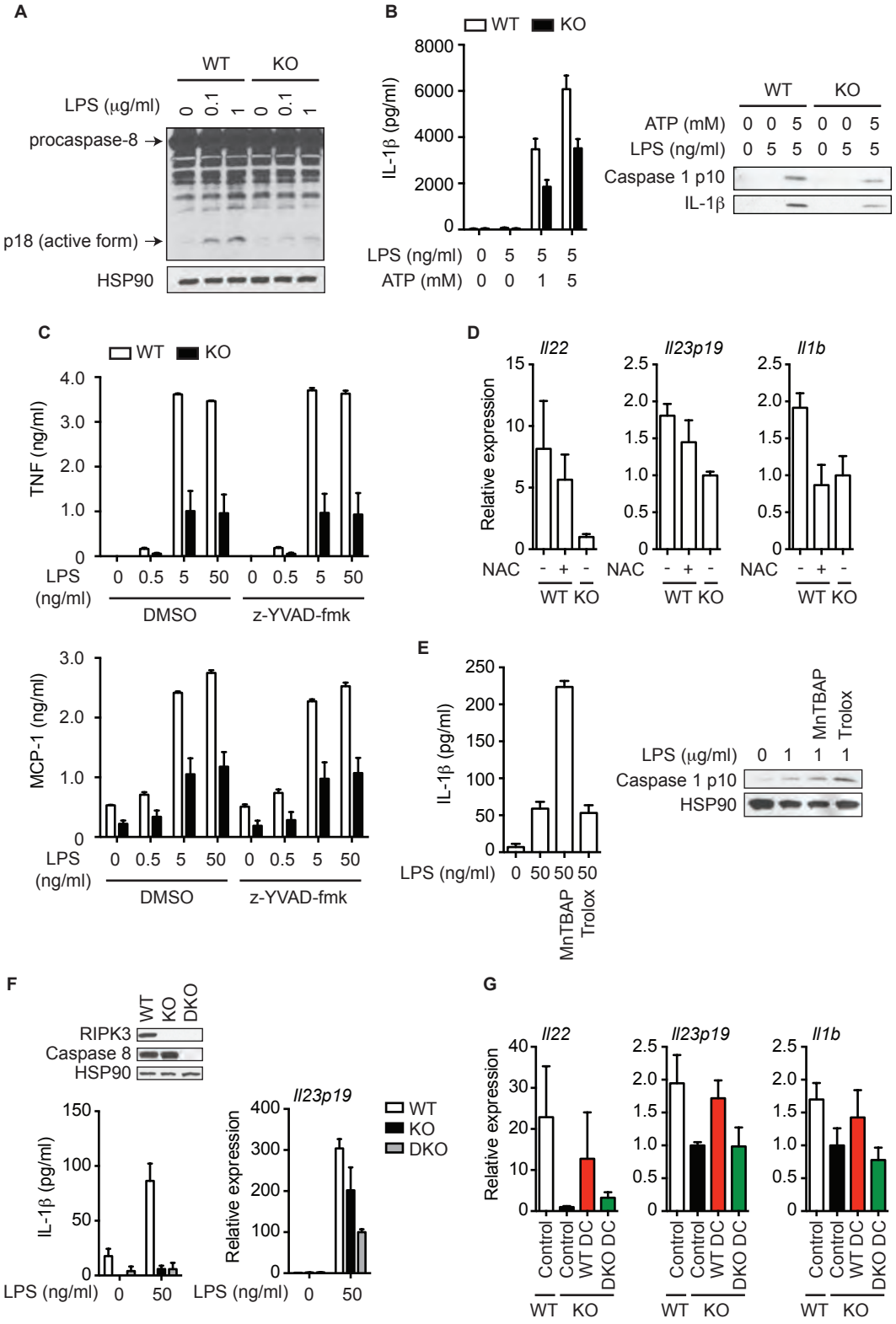
A
WT
KO
LPS (μg/ml)
0
0.1
1
procaspase-8
p18 (active form)
HSP90
B
WT
KO
IL-1β (pg/ml)
LPS (ng/ml)
ATP (mM)
Caspase 1 p10
IL-1β
C
TNF (ng/ml)
MCP-1 (ng/ml)
LPS (ng/ml)
DMSO
z-YVAD-fmk
D
Il22
Il23p19
Il1b
Relative expression
NAC
E
MnTBAP
Trolox
HSP90
F
RIPK3
Caspase 8
DKO
G
Control
WT DC
DKO DC

Figure S7. NAC injection partly blocked DSS-induced cytokine expression, Related to Figure 7.

(A) Whole cell extracts (WCEs) from *Ripk3*$^{+/+}$ (WT) and *Ripk3*$^{-/-}$ (KO) BMDCs treated with LPS for 1 hour were tested by western blot analysis. **(B)** BMDCs were primed with LPS for 2.5 hours and then stimulated with ATP for 30 min. Supernatants were subjected to ELISA (n=7) or western blot analysis. **(C)** BMDCs pretreated with 5 μM z-YVAD-fmk for 1 hour were stimulated with LPS for 6 hours. Cytokine expression was determined by ELISA (n=2). **(D)** Relative mRNA expression of *Il22*, *Il23p19* and *Il1b* in DSS-treated mice (day 7). NAC was i.p. injected to *Ripk3*$^{+/+}$ mice on day 4, 5, and 6 (200 mg/kg) (n=4). PBS was injected into control mice (control *Ripk3*$^{+/+}$: n=4, control *Ripk3*$^{-/-}$: n=5). Gene expression in *Ripk3*$^{-/-}$ PBS control was defined as 1. **(E)** IL-1β secretion (n=3) and caspase 1 activation were tested in wild type BMDCs pretreated with 100 μM MnTBAP or Trolox for 1 hour and then treated with LPS. **(F)** Relative expression of *Il23p19* and IL-1β secretion in *Ripk3*$^{+/+}$*Casp8*$^{+/+}$ (WT), *Ripk3*$^{-/-}$*Casp8*$^{+/+}$ (KO), and *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ (DKO) BMDCs treated with LPS for 6 hours. Deficiency of the expression of RIPK3 and caspase 8 was confirmed by western blot analysis. **(G)** *Ripk3*$^{+/+}$*Casp8*$^{+/+}$ (WT: n=5) or *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ (DKO: n=6) BMDCs were injected to *Ripk3*$^{-/-}$ mice on day 5. PBS was injected into control mice (*Ripk3*$^{+/+}$: n=4, *Ripk3*$^{-/-}$: n=5). Relative mRNA expression of *Il22*, *Il23p19* and *Il1b* in the colon is shown (day 7). Gene expression in *Ripk3*$^{-/-}$ PBS control was defined as 1.

Table S1. Metagenomic analysis for intestinal microbiota in *Ripk3* $^{+/+}$ and *Ripk3* $^{-/-}$ mice, Related to Figure 1.

	Ripk3 $^{+/+}$			*Ripk3* $^{-/-}$		
	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3
ruminococcaceae	45.2103	2.29816	17.5268	2.95393	2.6677	2.01568
porphyromonadaceae	17.055	33.8015	26.0694	14.1373	24.3749	22.9264
bacteroidaceae	10.4513	26.6114	19.2396	5.22396	7.06026	10.6008
rikenellaceae	10.1702	6.1301	6.56	14.0602	11.412	6.30041
lachnospiraceae	5.59128	8.63284	3.35233	11.4306	19.2964	7.58286
lactobacillaceae	2.77595	4.21538	11.0105	32.755	11.6865	19.3626
helicobacteraceae	2.13742	1.21058	0.89328	1.67849	0.39761	0.38759
clostridiaceae	1.68182	0.79074	0.41092	0.22489	0.53831	0.39318
alcaligenaceae	1.64045	8.44896	7.42672	5.41626	12.596	7.20869
coriobacteriaceae	1.28205	1.09072	3.70657	1.20265	4.46291	4.09121
prevotellaceae	0.85686	1.07252	0.90509	7.22511	3.1746	17.4638
enterobacteriaceae	0.2916	1.15724	0.69491	1.5063	0.73241	0.35683
mycoplasmataceae	0.28761	1.62038	0.26509	0.08474	0.10301	0.17785
rhodospirillaceae	0.11265	0.3301	0.91218	0.12168	0.00628	0.08613
erysipelotrichaceae	0.08773	1.02796	0.56915	0.07659	0.13442	0.11298
deferribacteraceae	0.06979	0.17321	0.28517	0.02716	0.02764	0.04195
dehalobacteriaceae	0.05882	0.0728	0.02834	0.06899	0.08354	0.04698
verrucomicrobiaceae	0.05782	0.01506	0.02598	0.23412	0.10678	0.04922
tm7 (candidate division)	0.05732	0.31127	0.02657	1.2184	0.94723	0.51846
veillonellaceae	0.05483	0.04268	0.00945	0.00054	0.00063	0
moraxellaceae	0.04038	0.00251	0.05373	0.06138	0.11495	0.04307
bacillaceae	0.01495	0.06903	0	0	0.00063	0
desulfovibrionaceae	0.00598	0.00439	0.01476	0.00706	0.00628	0.00503
anaeroplasmataceae	0.00299	0.80015	0.00059	0.00217	0.0044	0.11521
fibrobacteraceae	0.0015	0.00126	0.00295	0.20696	0.03015	0.09844
eubacteriaceae	0.0015	0.00126	0	0	0	0.00056
micrococcaceae	0.001	0.00314	0.00236	0.00706	0.00188	0.00224
methylobacteriaceae	0.0005	0.05021	0.00118	0.05649	0.01696	0.00112
streptococcaceae	0.0005	0.00879	0	0.00054	0.00188	0.00112
staphylococcaceae	0	0	0	0.00163	0	0
clostridiales family xiii. incertae sedis	0	0	0	0.00326	0.01131	0.00503
bifidobacteriaceae	0	0.00188	0	0	0	0
pasteurellaceae	0	0.00251	0.00236	0	0.00063	0
tracheophyta	0	0.00063	0	0.00109	0.00063	0
rhodobiaceae	0	0.00063	0.00413	0.00109	0	0.00168
peptococcaceae	0	0	0	0.00435	0.00126	0.0028

The numbers show the percentage of each bacterial family.

Table S2. Primer sequences used in this study.

Gene		Sequences	Gene		Sequences
Areg	F	ATCATCCTCGCAGCTATTGG	*Il22ra1*	F	CTACGTGTGCCGAGTGAAGA
	R	TTGTCCTCAGCTAGGCAATG		R	AGCGTAGGGGTTGAAAGGT
Cox2	F	TGAGCACAGGATTTGACCAG	*Il23p19*	F	CCAGCGGGACATATGAATCT
	R	CCTTGAAGTGGGTCAGGATG		R	AGGCTCCCCTTTGAAGATGT
Ereg	F	CACCGAGAAAGAAGGATGGA	*Il23r*	F	CCACCAAACTTCCCAGACAG
	R	GATTCTCCTGGGATGCATGA		R	GAAGACCATTCCCGACAAAA
Ifng	F	ACAGCAAGGCGAAAAAGGAT	*Kc*	F	CCACACTCAAGAATGGTCGC
	R	TGAGCTCATTGAATGCTTGG		R	TCTCCGTTACTTGGGGACAC
Ilib	F	CCCAACTGGTACATCAGCAC	*Mcp1*	F	AGGTGTCCCAAAGAAGCTGTA
	R	TCTGCTCATTCACGAAAAGG		R	ATGTCTGGACCCATTCCTTCT
Ilir1	F	GGAGAAATGTCGCTGGATGT	*Mip2*	F	GAGCTTGAGTGTGACGCCCCCAGG
	R	ATGAGACAAATGAGCCCCAG		R	GTTAGCCTTGCCTTTGTTCAGTATC
Il6	F	CGGAGAGGAGACTTCACAGA	*Reg3b*	F	GGCTTCATTCTTGTCCTCCA
	R	CCAGTTTGGTAGCATCCATC		R	TCCACCTCCATTGGGTTCT
Il10	F	CTATGCTGCCTGCTCTTACTG	*Ripk3*	F	GAGATGGAAGACACGGCACT
	R	AACCCAAGTAACCCTTAAAGTC		R	GGTGGTGCTACCAAGGAGTT
Il12p35	F	CGCAGCACTTCAGAATCACA	*Tbp*	F	CAAACCCAGAATTGTTCTCCTT
	R	TCTCCCACAGGAGGTTTCTG		R	ATGTGGTCTTCCTGAATCCCT
Il17a	F	TCCAGAAGGCCCTCAGACTA	*Tgfa*	F	CTACTCGCCAACCGCAGG
	R	TGAGCTTCCCAGATCACAGA		R	ACAGCTAACAGGATACCCAGC
Il18	F	CAGGCCTGACATCTTCTGCAA	*Tgfb1*	F	GCCCTGGATACCAACTATTGC
	R	CTGACATGGCAGCCATTGT		R	AAGTTGGCATGGTAGCCCTT
Il22	F	TTGAGGTGTCCAACTTCCAGCA	*Tnf*	F	CCCACTCTGACCCCTTTACT
	R	AGCCGGACATCTGTGTTGTTA		R	TTTGAGTCCTTGATGGTGGT
Il22bp	F	CAAGATGTATGGACAGAGCCA			
	R	GGTTATAGTCACGACCGGAG			

Supplemental Experimental Procedures

Antibodies and reagents

PerCP-Cy5.5-labeled anti-CD3, PerCP-Cy5.5-labeled anti-CD19, APC-Cy7-labeled anti-CD4, PE-labeled anti-NKp46, PE-labeled anti-RORγt, biotin-labeled anti-IL-7R, PE-labeled anti-IL-17A, biotin-labeled anti-CD3, FITC-labeled anti-CD19, PE-labeled anti-CD11c, PE-Cy7-labeled anti-CD19, APC-labeled anti-CD103 antibodies and avidin-PE-Cy7 were from eBioscience. Alexa Fluor 647-labeled anti-IL-22 antibody was from BioLegend. APC-labeled anti-CCR6, FITC-labeled anti-CD25, PerCP-Cy5.5-labeled anti-CD11b, APC-labeled CD11c, APC-Cy7-labeled anti-CD11b anti-HSP90, and anti-β-actin antibodies were from BD Biosciences. Anti-RelB, anti-p100/52, anti-IκBα, anti-phospho IκBα, anti-phospho IKKα/β, anti-Erk, anti-phospho Erk, anti-JNK, and anti-phospho JNK antibodies were from Cell Signaling Technology. Anti-RelA, anti-cRel, anti-p105/50, anti-IKK α/β, anti-IκBε and anti-caspase-1 antibodies were from Santa Cruz. Anti-IL-1β antibody was from R&D Systems. Anti-RIPK3 antibody was from ProSci. Anti-caspase 8 antibody and z-VAD-fmk was from Enzo Life Sciences. Anti-BRG1 ATPase antibody was kindly provided by Dr. Anthony Imbalzano (UMMS). SMAC mimetic, LBW242, was kindly provided from Novartis.

Metagenomic analysis

The PCR primers developed against the V4 variable region of the 16S rRNA gene (F515/R806) were used (Caporaso et al 2011). PCR reaction was performed using the HotStarTaq Plus Master Mix Kit (Qiagen) under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes. Sequencing was performed at MR DNA on an Ion Torrent PGM

according to the manufacturer's protocol. Sequence data were processed using a proprietary analysis pipeline (MR DNA). Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated GreenGenes database (DeSantis et al 2006).

Imaging flow cytometry

BMDCs stimulated with 100 ng/ml LPS for two hours were fixed and permeabilized with Fixation/Permeabilization solution (eBiosciences) for 30 minutes at 4^{o}C and washed with Permeabilization buffer (eBiosciences). Cells were then incubated in Permeabilization buffer supplemented with 10% normal goat serum (Sigma) for 30 minutes, followed by RelB antibody (Cell Signaling) for 45 minutes at room temperature. After washing, cells were incubated with Alexa fluor 488-conjugated secondary anti-rabbit antibody (Molecular Probe) for 45 minutes at room temperature. For last 15 minutes of the incubation with secondary antibody, cells were stained with 2 μg/ml Hoechst 33342 to visualize the nuclei (Molecular Probe). The cells were analyzed by FlowSight Analyzer (Amnis EMD Millipore). The percentage of the cells showing RelB nuclear translocation was calculated using IDEAS v5.0 program (EMD Millipore).

Measurement of intracellular reactive oxygen species

BMDCs were treated with 10 μM CM-H_2DCFDA in PBS at 37^{o}C for 30 minutes. After washing twice with culture media, the cells were incubated at 37^{o}C for 30 minutes and subsequently stimulated with 100 ng/ml LPS for 6 hours. After washing twice with PBS, the cells were analyzed by LSRII (BD Biosciences).

Flow cytometry for ILCs

Cells from spleen and mesenteric lymph nodes were stained with a combination of antibodies against surface antigens. For intracellular staining of RORγt, cells were fixed, permeabilized by fixation/permeabilization solution (eBioscience), and then stained for RORγt. The cells were analyzed by LSR II (BD Biosciences).

Intracellular staining of IL-22 in ILCs

Splenocytes were stimulated with 25 or 100 ng/ml recombinant mouse IL-23 (BioLegend). Four hours after stimulation with IL-23, 10 ng/ml PMA (Sigma) and 1 μg/ml ionomycin (Sigma) were added to the culture for an additional four hours. Golgi Plug (BD Biosciences) was added three hours before harvest. Cells were first stained with antibodies against surface antigens. After fixation and permeabilization with BD Cytofix/Cytoperm kit (BD Biosciences), cells were stained for IL-22 and IL-17A. The cells were analyzed by LSR II (BD Biosciences).

Cell death assay

For TUNEL staining, cells were permeabilized with 0.1% Tritin X-100 after fixation with paraformaldehyde and stained with the *in situ* cell death detection kit, fluorescein (Roche) as per manufacturer's protocol. For Annexin V and PI staining, cells were stained with 50 μg/ml PI and Annexin V (BD Bioscience) in Annexin V buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$). The cells were analyzed by LSR II (BD Biosciences). Annexin V and/or PI positive cells were defined as dead cells. For LDH assay, CytoTox 96 Non-Radioactive Cytotoxicity Assay kit (Promega) was used.

Immunoprecipitation and immunoblotting

Whole cell extracts were prepared in RIPA buffer or NP-40 lysis buffer. Secreted proteins in culture media were precipitated by TCA. For cytoplasmic and nuclear extracts, cells were lysed by resuspending in hypotonic buffer (20 mM Tris-HCl, pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$) for 15 min, followed by addition of 0.5% NP-40. After centrifugation at 3,000 rpm for 10 min, supernatants were collected as cytoplasmic extracts. The pellets were washed with PBS once followed by lysis with RIPA buffer for 30 min. After centrifugation at 14,000 rpm for 30 min, supernatants were collected as nuclear extracts. Protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Sigma) were included in all lysis buffers. For immunoprecipitation, WCEs precleared with Sepharose 6B (Sigma) for 1 hour were immunoprecipitated with anti-RelB antibody and anti-rabbit IgG-conjugated agarose (Sigma) overnight. β-actin and HSP90 were used as loading control. HSP90 and BRG1 ATPase were used as controls for cytoplasmic and nuclear proteins, respectively. Results are representative of two or three independent experiments.

EMSA

Cells were lysed by resuspending in hypotonic buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF) for 15 minutes, followed by addition of 0.5% NP-40. After centrifugation and washing with hypotonic buffer once, the pellets were resuspended with high salt buffer (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF) and incubated for 15 minutes at 4 °C. After centrifugation at 14,000 rpm for 10 minutes, supernatants were collected as nuclear extracts. Protease inhibitor cocktail (Roche) were added to nuclear extracts. Oligonucleotides from the κB site in the

Il23p19 gene promoter (5'-TAGGGAGGGGAATCCCACCTGC-3') were end labeled with [γ-32P] ATP (MP Biomedial) using the T4 polynucleotide kinase (NEB). Binding reactions were performed with 5 μg of nuclear protein and 100,000 cpm of labled oligonucleotides in 10 μl reaction mixture containing 10 mM Tris-HCl pH 7.5, 150 mM KCl, 0.5 mM EDTA, 12.5% Glycerol, 0.1% Triton X-100, 1 mM DTT, 0.5 μg/ml Poly dIdC on ice for 30 minutes. Reaction mixtures were resolved on a 4% nondenaturing polyacrylamide gel at 15 mA for 2 hours in 0.5x TBE buffer. Gels were dried, exposed to a phosphor screen, and visualized by a phosphor imager. Immunodepletion was performed by incubating nuclear extracts with anti-RelA, cRel, p52, and/or RelB antibodies and anti-rabbit IgG-conjugated agarose (Sigma). To ensure the complete depletion, this procedure was repeated three times. After immunodepletion, supernatant was collected and 5 μg of it was used for EMSA reaction.

Q-PCR

Primer sequences used in this study are summarized in Table S2.