Supplemental Methods

LPS/D-Galactosamine-Induced Endotoxin Shock. 8- to 12-week-old $Tpl2^{D/D}$ mice and their wildtype littermates received an intraperitoneal (i.p.) injection of 20 mg D-Galactosamine (Sigma) and 10 μg lipopolysaccharide (LPS) derived from *Salmonella enteritidis* (Sigma) per mouse. This treatment results in death from 6 to 9 h.

Isolation and culture of thioglycollate-elicited peritoneal macrophages (TEPMs). 8- to 12-week-old mice were injected i.p. with 1 ml thioglycollate broth (Sigma). Four days later, mice were sacrificed and their peritoneal cavities were washed with 5 ml of cold RPMI (Biochrom) containing 10% FBS and 1% Penicillin/Streptomycin. Cells were washed twice with RPMI and they were plated in RPMI supplemented with 5% FBS and 1% Pen/Strep at the concentration of $2x10^6$ cells/ml. After 5-6 h, non adherent cells were removed and remaining cells were serum starved for 16 h. Next day, cells were stimulated with LPS (1 μg/ml) for 6 h. Supernatant was removed for cytokine measurement and cell density was measured by crystal violet staining to ensure equal number of cells.

Isolation of lamina propria cells and FACs analysis. Isolation of lamina propria mononuclear cells was performed as previously described (1). For staining of Treg cells, single cell suspensions were incubated with anti-CD4 and anti-CD25 fluorescent conjugated antibodies (eBioscience), followed with staining with anti-Foxp3 (eBioscience), using the Foxp3 Staining Buffer Set (e-Bioscience) according to manufacturer's instructions. Analysis was performed with the FACs Canto II (Becton Dickinson).

TNF Elisa. TNF was measured in culture supernatant of macrophages using an ELISA kit from Pharmingen (OptEIA) according to manufacturer's instructions.

Table S1. List of Primers

Gene	Primer Sequence (5'--3')	Product size (bp)	Tm (°C)	Reference
KC	F: CGCTCGGTTCTCTGTGCA	242	50	
	R: ATTTTCTGAACCAAGGGAGCT			
HGF	F: AGGCCAAGGAGAAGGTTACAGGGG	160	55	
	R: AGCCCCATCTGGATTGCGGC			
IGF1	F: GGGAGATGCAAAGGCCTCCCC	142	56	
	R: ACCAGGACTCCCAAATCCCTAGCC			
Wisp1	F: CAGATGGCTGTGAATGCTGT	199	58	(2)
	R: AAGGACTCGCCATTGGTGTA			
Hif1a	F: TGCTCATCAGTTGCCACTTC	146	58	(2)
	R: CCATCTGTGCCTTCATCTCA			
c-myc	F: ATGGAGATGAGCCCGACTCCGACC	156	55	
	R: GGGCCAGCCCTGAGCCCCTAGTGC			
TNF	F: CACGCTCTTCTGTCTACTGA	110	55	
	R: ATCTGAGTGTGAGGGTCTGG			
IL-6	F: CTTCTTGGGACTGATGCTGGTGAC	336	55	
	R: TCCAGGTAGCTATGGTACTCCAGA			
IL-1β	F: CTGAAGCAGCTATGGCAACTG	443	57	
	R: TTTCAGCTCATATGGGTCCGA			
IL-18	F: CAGGCCTGACATCTTCTGCAA	105	58	
	R: TCTGACATGGCAGCCATTGT			
IFN-γ	F: ACTGGCAAAAGGATGGTGAC	237	60	(2)
	R: TGAGCTCATTGAATGCTTGG			
COX2	F: TCAGTTTTTCAAGACAGATC	197	50	
	R: TCTCTACCTGAGTGTCTTTG			
MCP1	F: AGCACCAGCACCAGCCAACT	295	55	
	R: TTCCTTCTTGGGGTCAGCAC			
TGFβ3	F: TGGCCGTCTGCCCCAAAGGA	100	58	
	R: ACCCGGAACTCTGCCCGGAA			
β2m	F: TTCTGGTGCTTGTCTCACTGA	104	55	
	R: CAGTATGTTCGGCTTCCCATTC			

Figure S1

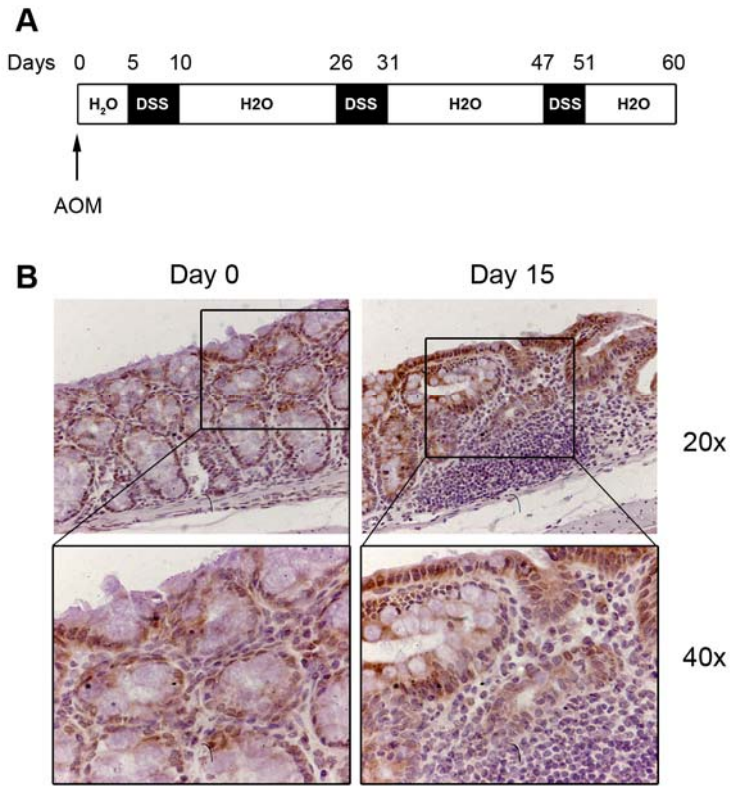

Figure S1

Tpl2 expression during the AOM/DSS model of colitis-associated cancer. (A) Schematic diagram of the AOM/DSS model of CAC. A single AOM injection (10 mg/Kg) is followed by 3 cycles of 2% DSS administration in drinking water (black boxes). (B) Immunohistochemical staining of Tpl2 in colon tissue slides at Day 0 and 15 of the AOM/DSS protocol.

Figure S2

A

Genotype	Dead at 9 h
$Tpl2^{+/+}$	7/7
$Tpl2^{D/D}$	0/7

B

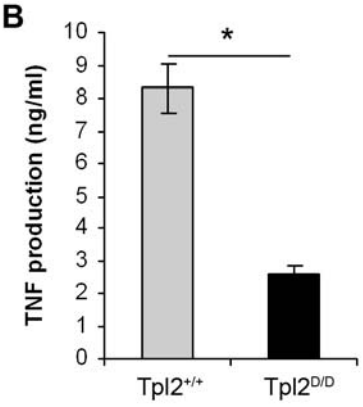

Figure S2

$Tpl2^{D/D}$ mice display resistance to LPS/D-Gal lethality and reduced secretion of TNF after LPS stimulation. (A) $Tpl2^{+/+}$ (n=7) and $Tpl2^{D/D}$ (n=7) were subjected to an i.p. injection of LPS (10 μg/mouse) and D-Gal (20 mg/mouse). Death was monitored for 9 h after the injection. (B) TEPMs from wild-type and $Tpl2^{D/D}$ were stimulated with LPS (1 μg/ml) for 6 h and supernatant was used for measurement of TNF. Data represent means ± SE from one of two experiments performed in triplicates. n=3; ∗, $p<0.05$

Figure S3

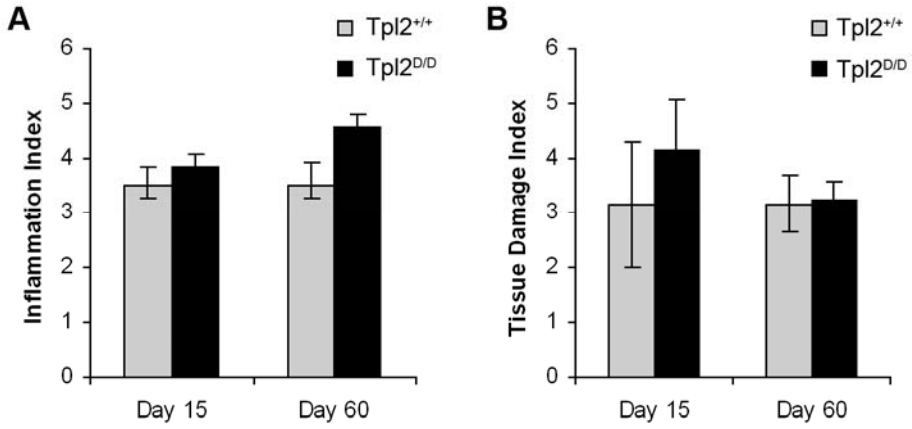

Figure S3

$Tpl2^{D/D}$ mice do not show differences in inflammation or tissue damage index either early or late during the disease. (A and B) H&E stained colon sections were scored for inflammation (A) and tissue damage (B) according to Methods section. Data represent means ± SE from four individual experiments performed ($n \geq 5$).

Figure S4

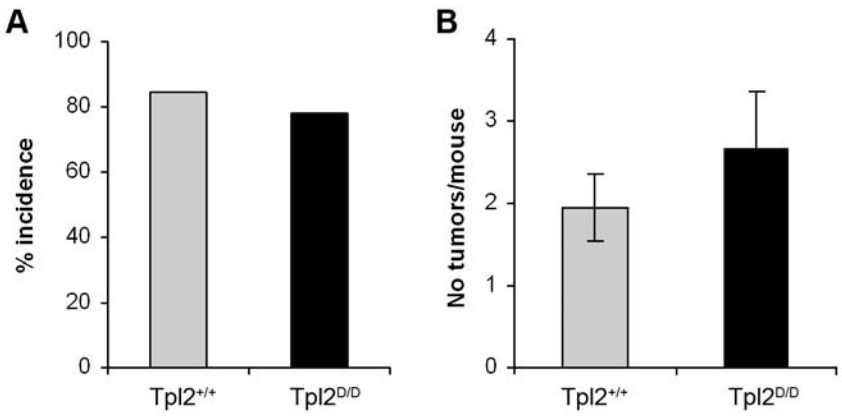

Figure S4

$Tpl2^{D/D}$ mice do not show any difference in susceptibility to the AOM model of colon cancer. $Tpl2^{D/D}$ mice display similar incidence (A) and number of tumours per mouse (B) at 18 weeks after the first AOM injection. Data represent means ± SE from one out of two experiments performed (n=11).

Figure S5

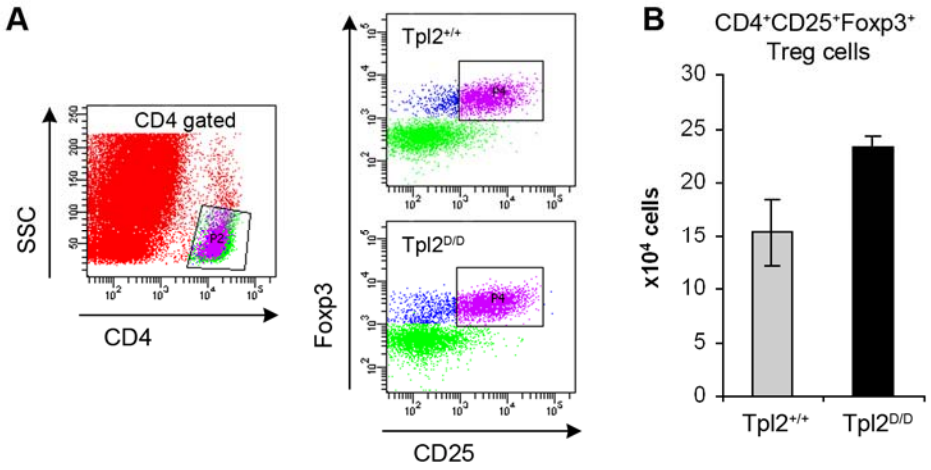

Figure S5

Treg cell numbers are the same between Tpl2$^{+/+}$ and Tpl2$^{D/D}$ mice early during CAC. Lamina propria cells were isolated from Tpl2$^{+/+}$ and Tpl2$^{D/D}$ mice at Day 15 after AOM administration and stained for CD4, CD25 and Foxp3. (A) FACs analysis was performed on CD4^{+} gated cells. Representative dot blots from three independent experiments (n=3) for SSC versus CD4 (P2) and Foxp3 versus CD25 (P4) are shown. (B) Absolute cell numbers of CD4^{+}CD25^{+}Foxp3^{+} cells isolated from the lamina propria of Tpl2$^{D/D}$ mice and littermate controls. Data represents means ± SE from one representative experiment out of three performed (n=3). Results are not statistically significant.

Figure S6

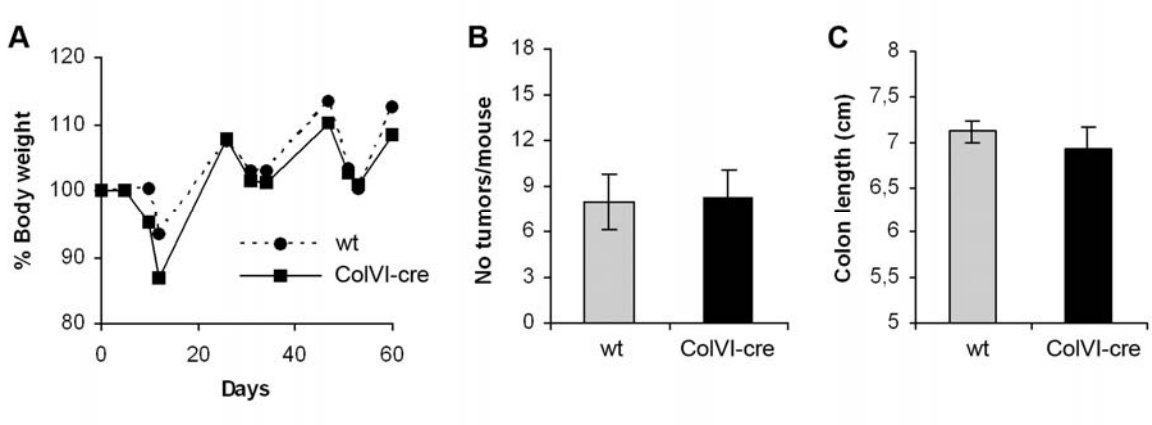

Figure S6

CollagenVI-cre transgene has no effect on tumour formation in CAC. CollagenVI-cre (ColVI-cre) mice and their littermate controls were subjected to the AOM/DSS protocol of CAC. Body weight was monitored during the course of the disease (A) and at the end of the protocol tumors multiplicity (B) and colon length (C) were measured. Data show means ± SE from one representative experiment out of three performed (n=6 mice per group).

Figure S7

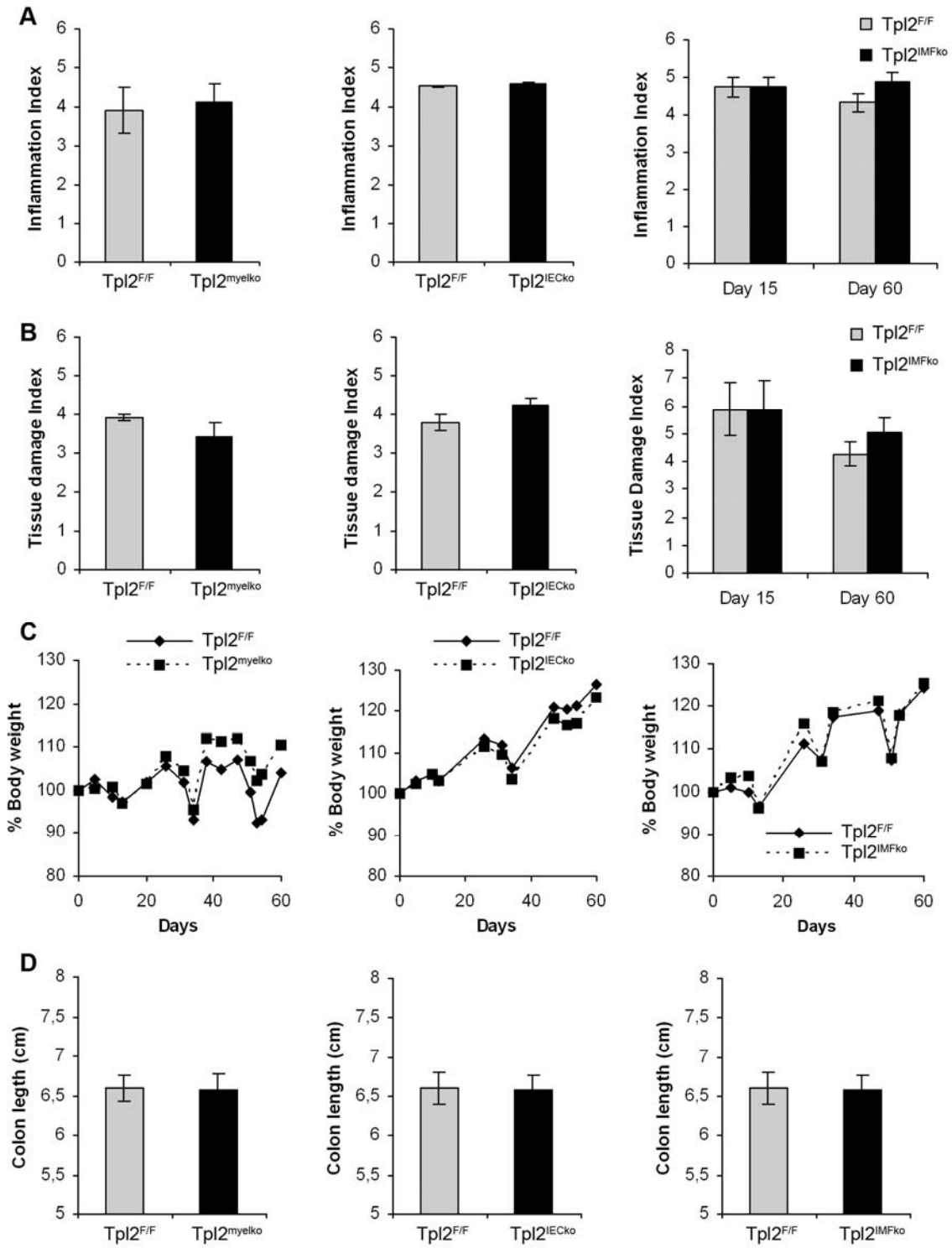
A
Inflammation Index
Tpl2F/F
Tpl2myelko
Tpl2IECko
Tpl2IMFko
Day 15
Day 60
B
Tissue damage Index
Tissue Damage Index
C
% Body weight
Days
D
Colon legth (cm)
Colon length (cm)

Figure S7

$Tpl2^{myelko}$, $Tpl2^{IECko}$ and $Tpl2^{IMFko}$ do not show any differences in body weight, colon length, inflammation or tissue damage score after AOM/DSS administration in comparison to their littermate controls. $Tpl2^{myelko}$, $Tpl2^{IECko}$ and $Tpl2^{IMFko}$ were subjected to the AOM/DSS protocol of CAC. (A and B) H&E stained colon sections were scored for inflammation (A) and tissue damage index (B) according to Methods section. Data represent means ± SE from two (for $Tpl2^{myelko}$ mice) or three (for $Tpl2^{IECko}$ and $Tpl2^{IMFko}$) experiments performed ($n \geq 5$). (C) Body weight changes were monitored during the course of the experimental procedure. Data represent one out of two (for $Tpl2^{myelko}$ mice) or three (for $Tpl2^{IECko}$ and $Tpl2^{IMFko}$) experiments performed ($n \geq 5$). (D) Colon length was measured at the end of the experimental protocol, at Day 60. Data represent means ± SE from one out of two (for $Tpl2^{myelko}$ mice) or three (for $Tpl2^{IECko}$ and $Tpl2^{IMFko}$) experiments performed ($n \geq 5$).

Figure S8

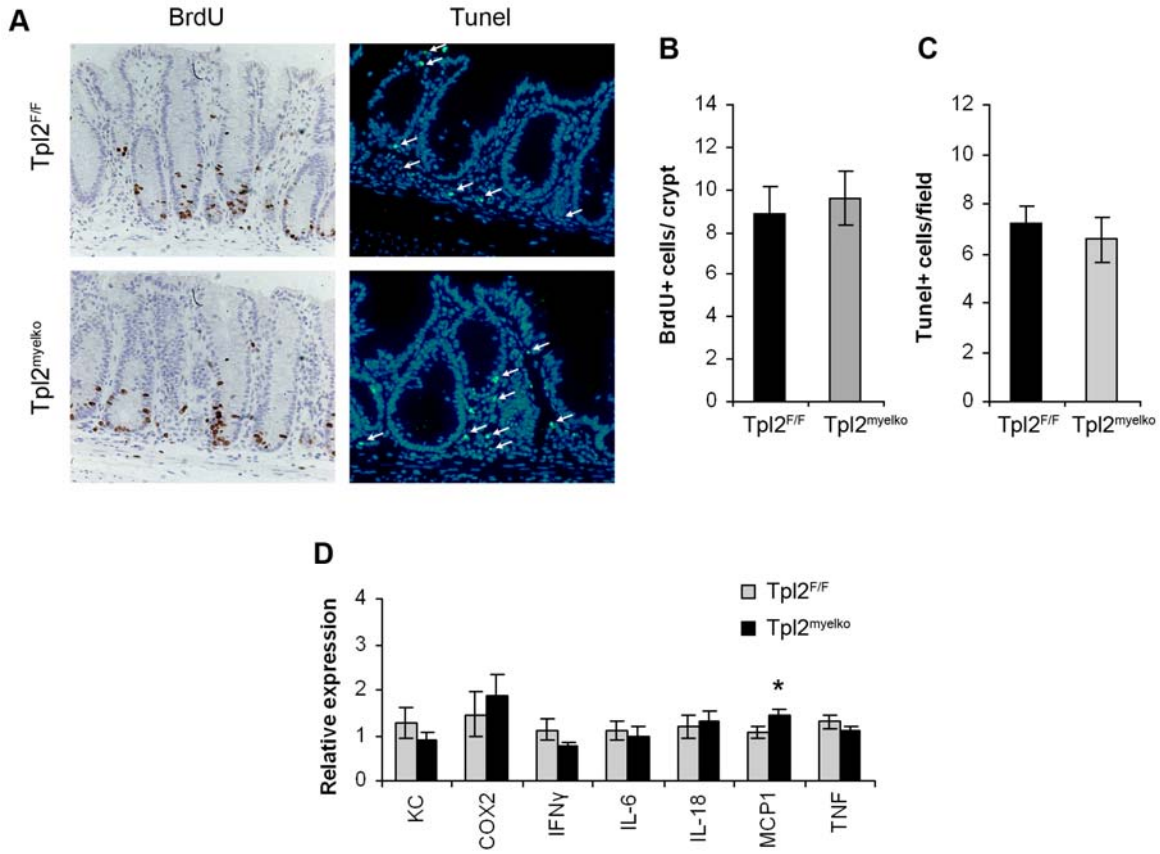

Figure S8

Tpl2^{myelko} mice display similar proliferation, apoptosis and cytokine expression early during the disease. (A) Formalin-fixed paraffin-embedded colon sections from Tpl2$^{F/F}$ Tpl2^{myelko} mice at Day 15 after AOM/DSS initiation were stained with BrdU and Tunel assay kits for assessment of proliferation and apoptosis, respectively. Representative images are presented. Arrows indicate Tunel positive cells. Original magnification x200. Quantification of BrdU positive cells per crypt (B) and Tunel positive cells per field (C) was performed using at least 20 random crypts and 10 random fields, respectively. Data represent means ± SE (n=6, 3 mice from each of 2 individual experiments); ***, $p<0.001$. (D) qRT-PCR of genes of interest in whole colon of Tpl2$^{F/F}$ Tpl2^{myelko} mice at day 15 after AOM/DSS administration. Gene expression was normalized to β2M levels. Data represent means ± SE of 6 mice per genotype (3 mice from each of 2 individual experiments); *, $p<0.05$.

Figure S9

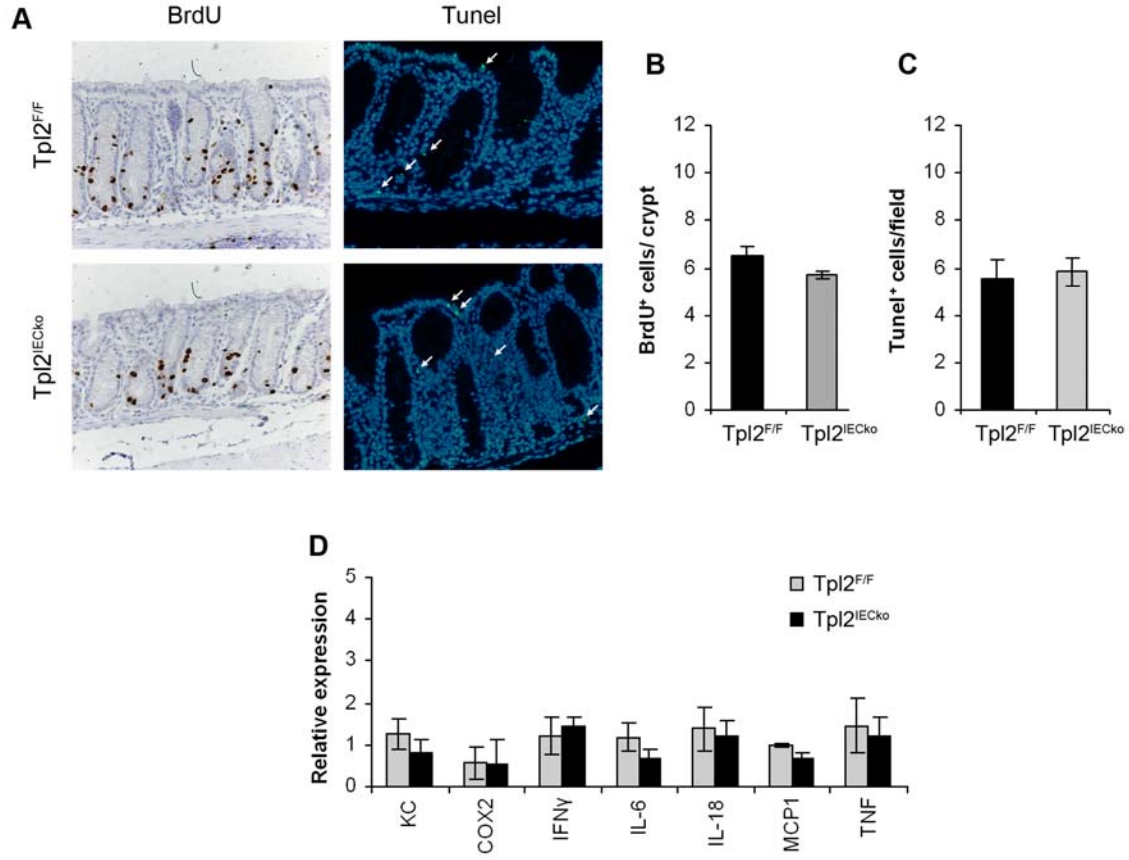

Figure S9

Tpl2^{IECko} mice display similar proliferation, apoptosis and cytokine expression early during the disease. (A) Formalin-fixed paraffin-embedded colon sections from Tpl2$^{F/F}$ Tpl2^{IECko} mice at Day 15 after AOM/DSS initiation were stained with BrdU and Tunel assay kits for assessment of proliferation and apoptosis, respectively. Representative images are presented. Arrows indicate Tunel positive cells. Original magnification x200. Quantification of BrdU positive cells per crypt (B) and Tunel positive cells per field (C) was performed using at least 20 random crypts and 10 random fields, respectively. Data represents ± SE, (n=6, 3 mice from each of 2 individual experiments); ***, $p<0.001$. (D) qRT-PCR of genes of interest in whole colon of Tpl2$^{F/F}$ Tpl2^{IECko} mice at day 15 after AOM/DSS administration. Gene expression was normalized to β2M levels. Data represent means ± SE of 6 mice per genotype (3 mice from each of 2 individual experiments).

References

1. Weigmann B, et al. Isolation and subsequent analysis of murine lamina propria mononuclear cells from colonic tissue. *Nat Protoc.* 2007;2(10):2307-2311.
2. Salcedo R, et al. MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. *J Exp Med.* 2010;207(8):1625-1636.