

Immunity, Volume 36

**Supplemental Information**

**NLRP12 Suppresses Colon Inflammation  
and Tumorigenesis through the Negative Regulation  
of Noncanonical NF- $\kappa$ B Signaling and MAP Kinase Activation**

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**Online Supplementary Data**

## **Supplemental Experimental Procedures**

### **Antibodies Used for Western Blots and NF- $\kappa$ B Assessments from Colon Tissue**

Anti-I $\kappa$ B $\alpha$  (4812), anti-phospho-I $\kappa$ B $\alpha$  (2859), anti-p65 (3034), anti-phospho-p65 (3033) (Cell Signaling Technology), or anti-NIK (sc-7211) (Santa Cruz Biotechnology) and anti-p52 (sc-7386) antibody (Santa Cruz Biotechnology) followed by appropriate HRP-conjugated goat-anti-rabbit IgG (sc-2004) or goat-anti-mouse IgG (sc-2005) secondary antibodies (Santa Cruz Biotechnology). Anti-actin-HRP (sc-1615 HRP) (Santa Cruz Biotechnology) was used as a loading control for the cytosolic fraction, and anti-histone H3 (DAM1832538, Millipore) was used as a loading control for the nuclear fraction.

### **Co-immunoprecipitation**

The Abs used were: anti-NIK (H-248), anti-p52 (C-5), anti-TRAF2, anti-TRAF3 and anti-actin HRP (C-11) from Santa Cruz Biotechnology; anti-pERK (4370S), anti-ERK (4695), anti-pJNK (4668S), anti-JNK (9252), anti-pp38 (9211S) and anti-p38 (9212) from Cell Signaling Technologies; anti-HA Abs (12CA5 and 13F10) from Roche; and anti-FLAG from Sigma.

## Supplemental Figure Legends

**Figure S1. Profile of cytokine proteins produced by primary bone marrow derived dendritic cells following Pam3Cys4 stimulation.** (a-c) Cytokine protein levels were evaluated using Raybiotech Cytokine III arrays following the manufacture's procedures. (a) Representative images of membranes used to evaluate protein levels in cell free supernatants following Pam3Cys4 stimulation. (b) Table of cytokines assessed. (c) Cytokines with greater than 1.99 fold change in protein levels in *Nlrp12*<sup>-/-</sup> cells compared to wild type.

**Figure S2. Bone marrow chimera and non-chimera mice behave similarly in the CAC model.** (a) A combination of CD45.2 and CD45.1/2 BM cells were transferred into lethally irradiated (9.5Gy) CD45.1 recipients. Mice were bled 6 weeks post irradiation and the relative % of CD45.1+ (recipient) and CD45.2+ (donor) cells amongst peripheral blood lymphocytes was determined by flow cytometry. (b-f) Weight loss comparing (b) unirradiated, untreated wild type and *Nlrp12*<sup>-/-</sup> mice, (c) unirradiated, AOM/DSS treated wild type and *Nlrp12*<sup>-/-</sup> mice, (d) non-chimeric wild type mice and chimeric wild type mice and (e) non-chimeric *Nlrp12*<sup>-/-</sup> and chimeric *Nlrp12*<sup>-/-</sup> mice. For all experiments, data shown are representative of 3 independent experiments and depict the mean ± SEM. WT (no treatment), n = 3; AOM/DSS WT, n = 7; *Nlrp12*<sup>-/-</sup> (no treatment), n = 3; AOM/DSS *Nlrp12*<sup>-/-</sup>, n = 7; WT→WT, n = 5; *Nlrp12*<sup>-/-</sup>→*Nlrp12*<sup>-/-</sup> n = 3.

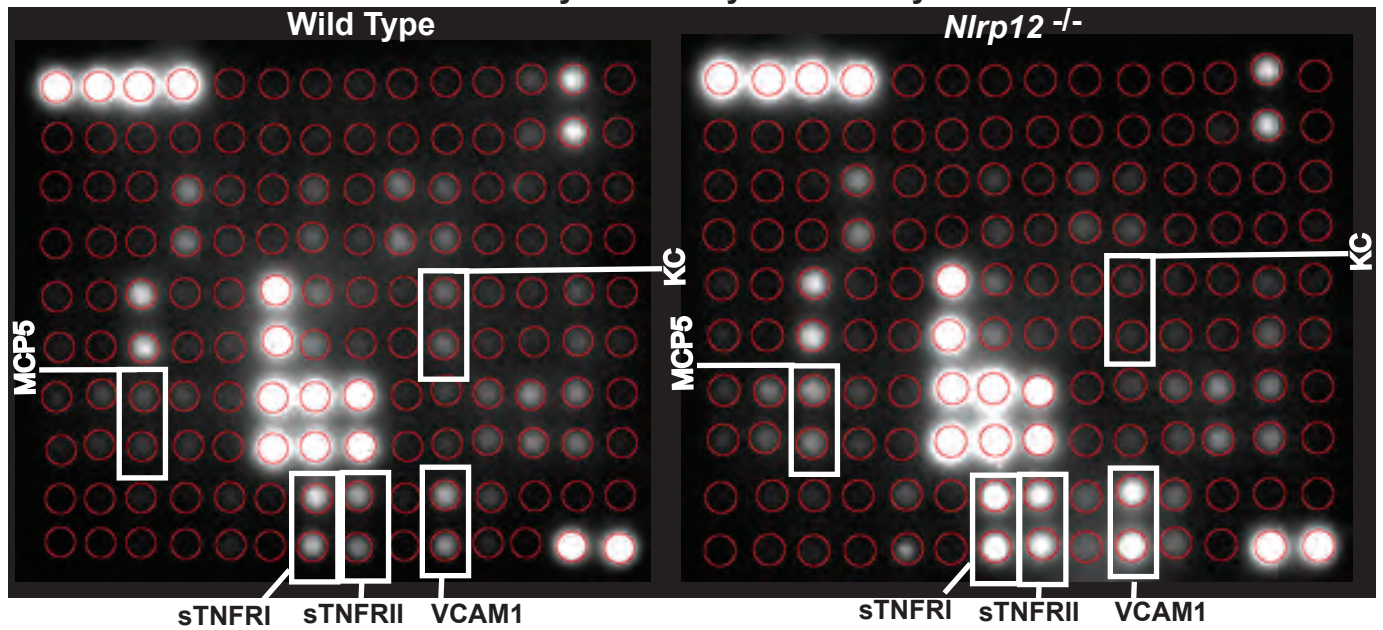
**Figure S3. *Nlrp12* expression in mouse cells and tissues of the immune system and gastrointestinal system compiled using microarray meta-analysis.** NLRP12 expression in the

mouse gastrointestinal and immune system was determined using a publically accessible microarray meta-analysis search engine (<http://www.nextbio.com/b/search/ba.nb>).

**Figure S4. Colon expression of genes associated with NF- $\kappa$ B signaling and colon inflammation. (a-d)** Total RNA was extracted from whole distal colon sections and gene expression was assessed. **(a)** *Ptgs1* (*Cox1*) and *Ptgs2* (*Cox2*) expression was increased during colon inflammation; however, no significant differences were observed between the wild type and *Nlrp12*<sup>-/-</sup> mice. **(b)** *Il-1 $\beta$*  expression was significantly down-regulated in the *Nlrp12*<sup>-/-</sup> mice following the CAC model. **(c)** No significant differences were observed in the expression of *Nik*, *Relb* or *Nf- $\kappa$ b2* (*p52*) expression. **(d)** No significant differences were observed in the expression of *Irak1* or *Rela* expression. WT mock, n = 3; *Nlrp12*<sup>-/-</sup> mock, n = 3; WT DSS, n = 3; WT AOM, n = 3; AOM/DSS *Nlrp12*<sup>-/-</sup>, n = 5; AOM/DSS WT, n = 5. **(e)** Increased ERK activation in *Nlrp12*<sup>-/-</sup> in the chronic EC model. pERK levels were evaluated by immunohistochemistry from paraffin embedded colon sections. Mock WT, n = 3; Mock *Nlrp12*<sup>-/-</sup>, n = 3; DSS *Nlrp12*<sup>-/-</sup>, n = 7; DSS WT, n = 7.

Supplemental Figure 1

A. RayBiotech Cytokine Array III



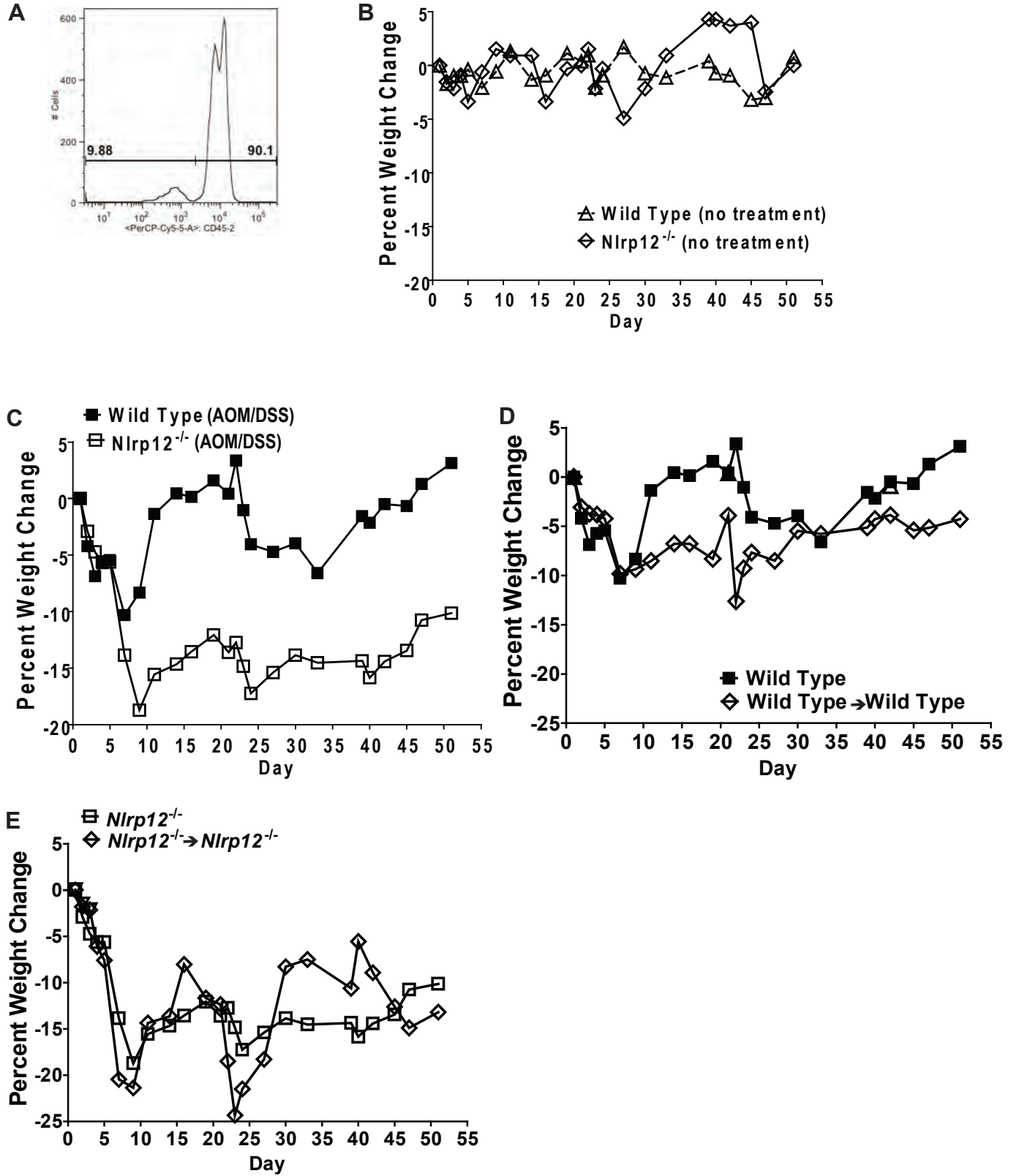
**B**

	Proteins	
Eotaxin-2	IL5	RANTES
FAS ligand	IL6	SCF
Fractalkine	IL9	SDF-1-alpha
G-CSF	KC	sTNF RI
GM-CSF	Leptin	sTNF RII
IFN-gamma	Leptin R	TARC
IGF-BP-3	LIX	TCA-3
IGF-BP-5	L-Selectin	TECK
IGF-BP-6	Lymphotactin	TIMP-1
IL10	MCP-1	TNF-alpha
IL12-p40/p70	MCP-5	TPO
IL12-p70	M-CSF	VCAM-1
IL13	MIG	VEGF
IL17	MIP-1-alpha	
IL1-alpha	MIP-1-gamma	
IL1-beta	MIP-2	
IL2	MIP-3-alpha	
IL3	MIP-3-beta	
IL3 Rb	PF4	
IL4	P-Selectin	

**C**

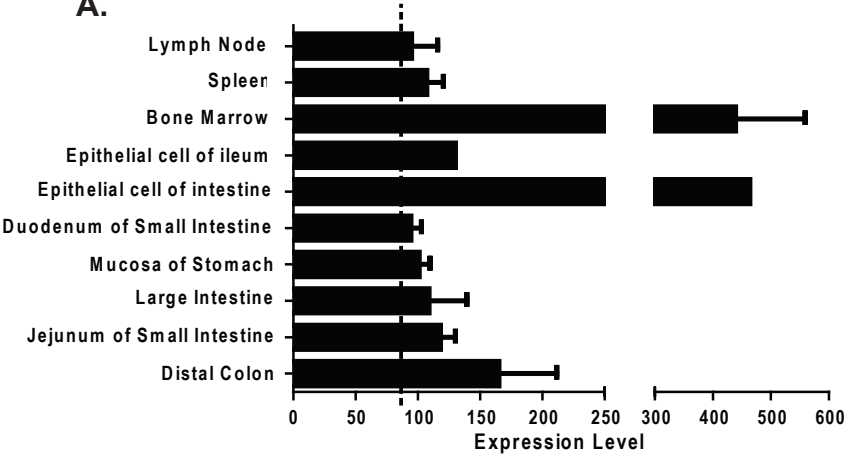
Protein	Fold Change
KC	-2.97
MCP5	3.46
VCAM1	2.86
sTNFR I	1.99
sTNFR II	2.68

Supplemental Figure 2



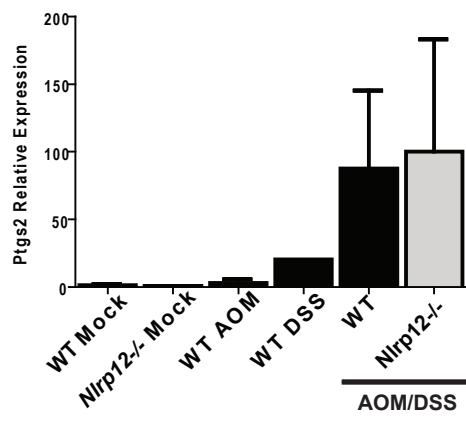
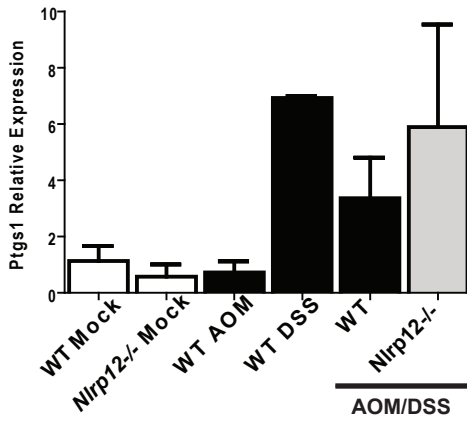
### Supplemental Figure 3

A.

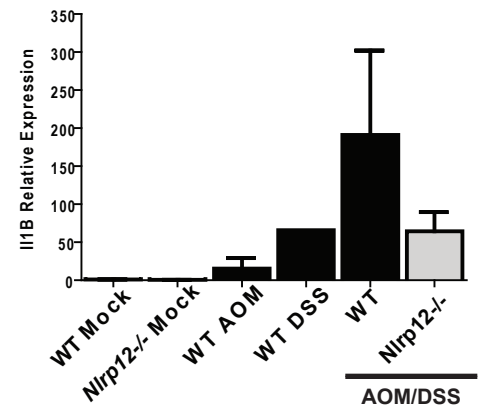


# Supplemental Figure 4

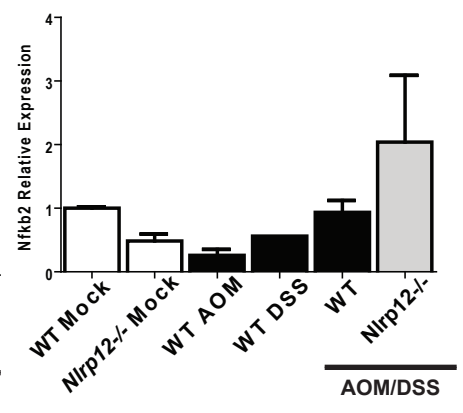
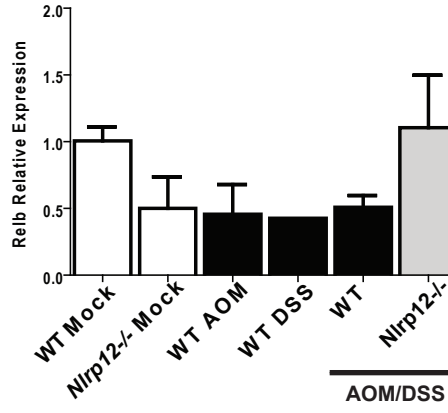
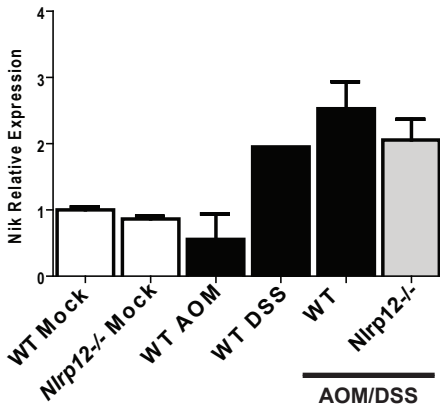
**A**



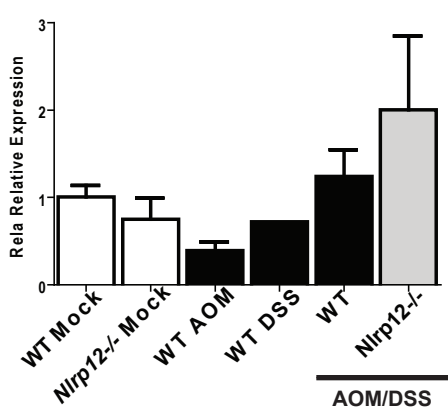
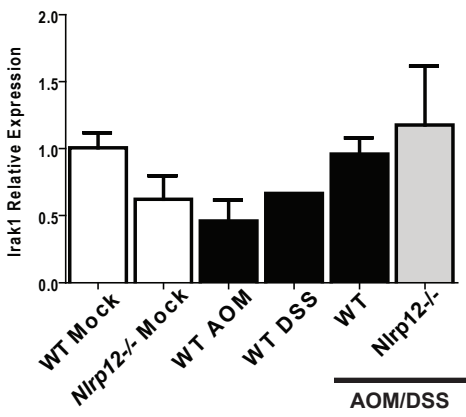
**B**



**C**



**D**



**E**

