Supplementary Information

Materials

Flagellin purified from *Salmonella typhimurium* (< 0.1 Endotoxin Unit/μg purified protein determined by Limulus Amebocyte Lysate test) was purchased from 'Enzo Life Sciences' (Farmingdale, NY) and dissolved in endotoxin-free water. Recombinant TNFα and ELISA kits for IL-6, MIP3α and KC were purchased from 'R&D Systems' (Minneapolis, MN). IL-8 ELISA kit was from 'Invitrogen' (Grand Island, NY). MPO (myeloperoxidase) ELISA kit was from 'Hycult Biotech' (Plymouth Meeting, PA). SAA (serum amyloid A) ELISA kit was from 'Alpco Diagnostics' (Salem, NH). DNA-binding ELISA system for transcription factors and nuclear extract kit were from 'Active Motif' (Carlsbad, CA).

Antibody information is the following: Antibodies against human TRIF, phospho-ERK1/2, phospho-p38, phospho-Akt, phospho-p105 (NFκB), phospho-p65 (NFκB), ERK1/2, Akt, Histone H2A, β-Actin, phospho-cJun, phospho-ATF2, and PTEN were from 'Cell Signaling Technology' (Danvers, MA). HA antibody was from 'Roche Applied Science' (Indianapolis, IN). Flag (M2) and Cy3-conjuated Flag antibodies were from 'Sigma-Aldrich' (St. Louis, MO). MyD88 antibody (HFL-296) was from 'Santa Cruz Biotechnology, Inc' (Santa Cruz, CA). Mal/TIRAP antibody was from 'eBioscience' (San Diego, CA). Alexa Fluor®488- or Cy3-conjugated cholera toxin subunit B and phospho-JNK1/2 antibody were from 'Invitrogen' (Grand Island, NY). Re-Blot buffer(Chemicon International Inc., Temecula CA) was used for immunoblot stripping.

Measuring DNA binding activity of transcription factor

Nuclear extracts were prepared by using nuclear extraction kits (Active Motif). The extracts were subjected to measuring DNA binding activity of transcription factors by using transcription factor DNA binding ELISA system (Active Motif).

Measuring serum amyloid A (SAA)

Mouse blood was harvested by sterile cardiac puncture method, and the serum was separated from the cells after clot formation by centrifugation. SAA level was measured with SAA ELISA kit (Alpco Diagnostics) by following manufacturer's instruction.

Histology

The entire mouse small intestine and colon were excised and segments of the transverse tissues (1 cm) were fixed in 10%-buffered formalin, paraffin embedded, and stained with Hematoxylin & Eosin. The histologic severity of inflammation was graded in a "blinded" fashion on a scale of 0-4 for ulceration and neutrophil infiltration, as previously described(1-3).

Immunoblotting, co-immunoprecipitation, and ELISA were performed as previously described(4-6)

Supplementary Figure Legends

Supplementary Figure 1A: PTEN expression is reduced in colonic epithelium of Vil$^{Cre/+}$;PTEN$^{loxP/loxP}$ mice. We performed immunohistochemistry against PTEN with frozen tissue sections of the colon of Vil$^{Cre/+}$;PTEN$^{loxP/loxP}$ and littermate control Vil$^{+/+}$;PTEN$^{loxP/loxP}$ mice. PTEN expression was dramatically reduced in the colon of Vil$^{Cre/+}$;PTEN$^{loxP/loxP}$ mice, compared to control mice. Previously, Langlois et al(Ref.24)(7) generated the same Vil$^{Cre/+}$;PTEN$^{loxP/loxP}$ mice by breeding Vil$^{Cre/+}$ to PTEN$^{loxP/loxP}$ mice, and confirmed the loss of PTEN in the colon and small intestine of Vil$^{Cre/+}$;PTEN$^{loxP/loxP}$ mice by western blot analysis. Therefore, our immunohistochemistry data showing reduced PTEN

expression in colonic epithelium of $Vil^{Cre/+}$;$PTEN^{loxP/loxP}$ mouse are in agreement with the previous data presented by Langlois et al(7).

Supplementary Figure 1B: Silencing PTEN expression leads to reduced NFκB and JNK1/2 activation upon flagellin stimulation. Additional clone of NCM460-PTEN-KD and NCM460-Control cells were stimulated by flagellin (100 ng/mL), as indicated. Flagellin-induced signaling pathways were evaluated by immunoblotting. Silenced PTEN expression was confirmed by immunoblotting, and Akt served as a loading control. All data are the representative of at least three independent experiments.

Supplementary Figure 1C: PTEN deletion disrupts the plasma membrane localization of Mal. MEF-$PTEN^{+/+}$ and MEF-$PTEN^{-/-}$ cells were transfected with Mal-Flag construct, followed by immunostaining, as described in Figure 10. The percentage of cells with the plasma membrane localization of Mal was quantified from three independent experiments.

Supplementary Figure 1D: NLRC4(Ipaf), a cytosolic flagellin receptor, expression is almost negligible in human colonic epithelial cells (NCM460), compared with its robust expression level in human monocytes/macrophages (THP-1). Total RNA was prepared from NCM460 and THP-1 cells, followed by quantitative Real-Time PCR to measure the level of NLRC4. The data are the representative of at least three independent experiments.

Reference cited for the supplementary information.

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Supplementary Figure 1