Supporting Information

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SI Methods

Preparation of Bone Marrow-Derived Macrophages. Bone marrow cells were collected from femurs and tibiae of mice. Cells were cultured in DMEM containing 10% FBS, antibiotics, and conditional media from L929 cell culture. Twenty-four hours later, nonadherent cells were transferred to a new plate and fresh L929 conditional medium was added every other day up to day 7. Mature macrophages were harvested by collagenase (Roche) digestion and transferred to new plates for further experiments. Flow cytometry showed that bone marrow-derived macrophages (BMDM) isolated from *Mavs*$^{-/-}$ and *Myd88*$^{-/-}$ mice expressed common macrophage surface markers, including CD11b, Mac-2, and F4/80 (Fig. S8).

BrdU Labeling in Vivo. BrdU was prepared from a fresh stock solution dissolved in PBS (BD Biosciences). BrdU (120 mg/kg of body weight) was administered by intraperitoneal injection to groups of three to five mice (DSS-treated or untreated) 90 min before being killed. Colons were fixed in Bouin's fixative and paraffin embedded for histology. Five-millimeter sections were probed with rat anti-BrdU (AbCam) and Cy3-conjugated goat anti-rat secondary antibody (Biomeda).

Bone-Marrow Chimera. Ly5.2 (CD45.2) recipient mice (WT or *Mavs*$^{-/-}$) were γ-irradiated with a single lethal dose of 1,000 Rads, then reconstituted with 5×10^6 bone-marrow cells from Ly5.1 (CD45.1) donor mice (The Jackson Laboratory). Five weeks after transplantation, the reconstitution efficiencies were about 80% to 90% for WT and *Mavs*$^{-/-}$ recipients (Fig. S8 and Table S1), as determined by FACS staining of blood leukocytes with antibodies against Ly5.1 and Ly5.2. The reconstitution efficiency of the reciprocal transplantation (e.g., *Mavs*$^{-/-}$ bone marrow to irradiated *Mavs*$^{-/-}$ recipient) was not tested because the donor and recipient mice contained the same leukocyte markers.

Transfection, RNA Treatment, and Luciferase Reporter Assay. HEK293 and HT-29 cells were cultured in DMEM supplemented with 10% calf serum and FBS, respectively. Transfection of RNA into HEK293, HT-29 or BMDM cells was carried out using Lipofectamine 2000 (Invitrogen). For enzyme treatments of nucleic acids, 1.0 μg of nucleic acids was treated with RNase III (Ambion) or with SAP (Roche Applied Science) at 37 °C for 1 h. Enzyme-treated RNAs were purified with RNeasy Mini Kit (Qiagen) before transfection. Luciferase reporter assays were done in HEK293-IFN-β-luciferase reporter cells or HEK293 cells transiently transfected with IFN-β-Luc (25 ng/mL) and pCMV-LacZ (50 ng/mL). Cells were harvested 24 h after transfection and lysed in passive lysis buffer (Promega). Luciferase and Renilla activities were measured with a luminometer (BMG Labtech; FLUOstar OPTIMA) using luciferin (Promega) and coelenterazine (Biotium) as substrates, respectively. β-Galactosidase activity was measured with a Thermo Labsystems microplate reader at the wavelength of 405 nm using *o*-nitrophenyl-β-D-galactopyranoside as a substrate.

Total RNA Isolation and Quantitative Real-Time PCR (qRT-PCR). To extract RNA, cells or tissues were first lysed in 1.0 mL of TRIzol (Invitrogen). Lysate was mixed with chloroform, and the aqueous phase was applied to RNeasy columns to obtain total RNA (Qiagen). The iScript cDNA synthesis kit (BioRad) was used to create cDNA from 0.15 μg of RNA. Quantitative real time PCR was performed using SYBR Green on a BioRad iCycler iQ5 with the following primers: IFN-β (TCCGAGCAGAGATCTTCAGGAA; TGCAACCACCACTCATTCTGAG), IL-6 (TCCATCCAGTTGCCTTCTTG; GGTCTGTTGGGAGTGGTATC), TNF-α (CCTCCCTCTCATCAGTTCTATGG; GGCTACAGGCTTGTCACTCG), IL-1β (TCTATACCTGTCCTGTGTAATG; GCTTGTGCTCTGCTTGTG), Rp119 (AAATCGCCAATGCCAACTC; TCTTCCCTATGCCCATATGC), RegIIIγ (TTCCTGTCCTCCATGATCAAAA; CATCCACCTCTGTTGGGTTCA), hIFN-β (AGGACAGGATGAACTTTGAC; TGATAGACATTAGCCAGGAG), hRegIIIγ (CACAGGATTTCTGAAGTGGAAA; ATGTCCATGATGAGCTGCAC).

Isolation of Bacterial RNA. Fresh feces were collected from distal colon into lysing Matrix B tubes (MP Biomedicals) and rapidly frozen down with liquid nitrogen. To isolate the RNA from feces, TriZols (Invitrogen) was added and the tubes were vortexed at high speed with FastPrep (Thermo Electron Corporation) at 4 °C. Crude RNAs were further purified with RNeasy Mini Kit (Qiagen). *L. monocytogenes* were grown in brain heart infusion medium (BHI; BD Bioscience); *L. salivarius* (ATCC 11741) were grown in Difco Lactobacilli MRS Broth under anaerobic chamber (BD Bioscience; GasPak) without antibiotics. RNA was treated with DNase I (Roche) (1 h; 37 °C) to remove potential contamination.

Depletion of Commensal Intestinal Bacteria with Broad Spectrum Antibiotics. To deplete commensal bacteria, 4- to 5-wk-old male mice were given a combination of four antibiotics in the drinking water for 4 wk: vancomycin hydrochloride (0.5 g/L), ampicillin (1.0 g/L), neomycin sulfate (1.0 g/L), and metronidazole (1.0 g/L). This method has been used in a number of previous studies (19, 42, 43). Complete depletion of commensal bacteria was verified through culturing feces as previously described (19).

Western Blotting. Western Blotting was performed as previously described with some modifications (1). Briefly, protein extracts were obtained from ileum of 6-wk-old male mice. A 2-cm piece of freshly isolated intestinal tissue was put into lysing Matrix D tube (MP Biomedicals) and immediately frozen under liquid N_2. 1.0 mL of Extraction Buffer (8 M urea, 1% SDS, 0.15 M Tris-HCl pH 7.5) was added and the tubes were vortexed at high speed with FastPrep (Thermo Electron Corporation) at 4 °C. Total protein was quantitated and 20 μg of each sample was separated by 15% SDS/PAGE and transferred to PVDF (Millipore). Membranes were blocked with 5% nonfat milk and incubated with rabbit polyclonal antibodies against BiP, Hsp70, GAPDH (Santa Cruz), or rabbit antiserum against RegIIIγ (1).

Pathological Changes in Colon. On day 8 after 2% DSS treatment mice were killed and the entire colon was excised and opened longitudinally. After colons were fixed with 10% formalin in neutral buffer, Swiss-rolls were made and embedded in paraffin. Tissue sections were prepared, deparaffinized, and stained with H&E. The histological changes were analyzed in a double-blinded fashion. Severity was scored as previously described (2). Briefly, colonic epithelial damage was assigned scores as follows: 0 = normal; 1 = hyperproliferation, irregular crypts, and goblet cell loss; 2 = mild to moderate crypt loss (10–50%); 3 = severe crypt loss (50–90%); 4 = complete crypt loss, surface epithelium intact; 5 = small- to medium-sized ulcer (<10 crypt widths); 6 = large ulcer (≥10 crypt widths). Infiltration with inflammatory cells was assigned scores separately for mucosa (0 = normal, 1 = mild, 2 =

modest, 3 = severe), submucosa (0 = normal, 1 = mild to modest, 2 = severe), and muscle/serosa (0 = normal, 1 = moderate to severe). Scores for epithelial damage and inflammatory cell infiltration were added, resulting in a total scoring range of 0 to 12.

1. Cash HL, Whitham CV, Behrendt CL, Hooper LV (2006) Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 313:1126–1130.

2. Katakura K, et al. (2005) Toll-like receptor 9-induced type I IFN protects mice from experimental colitis. *J Clin Invest* 115:695–702.

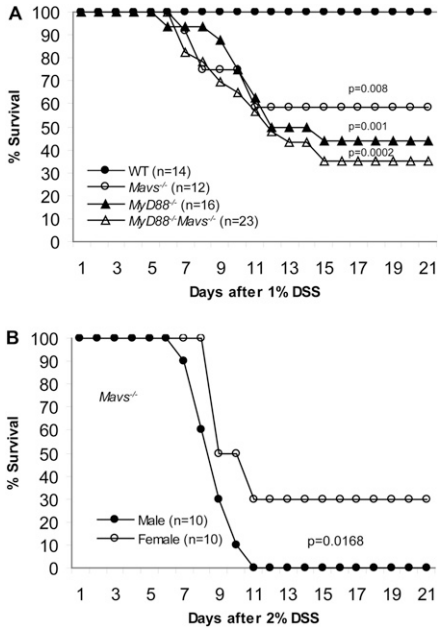

Fig. S1. Pilot experiments for DSS-induced colitis. (*A*) Four groups of mice—WT control, *Mavs*$^{-/-}$, *MyD88*$^{-/-}$, and *MyD88*$^{-/-}$ *Mavs*$^{-/-}$—were given 1% DSS in drinking water for 7 d and switched to normal drinking water on day 8. Survival was monitored until the 21st day after the beginning of DSS treatment. Mantel-Cox test was used for statistic analyses (all compared with WT): *Mavs*$^{-/-}$ $P = 0.008$; *MyD88*$^{-/-}$ $P = 0.001$; *MyD88*$^{-/-}$*Mavs*$^{-/-}$ $P = 0.0002$. (*B*) Sex difference of *Mavs*$^{-/-}$ mice in response to experimental colitis. Littermates of *Mavs*$^{-/-}$ male and female mice (backcrossed into C57BL/6 background for more than 10 generations) were treated as in *A*, except that 2% DSS was used. *Mavs*$^{-/-}$ male mice are more susceptible than females ($P = 0.0168$).

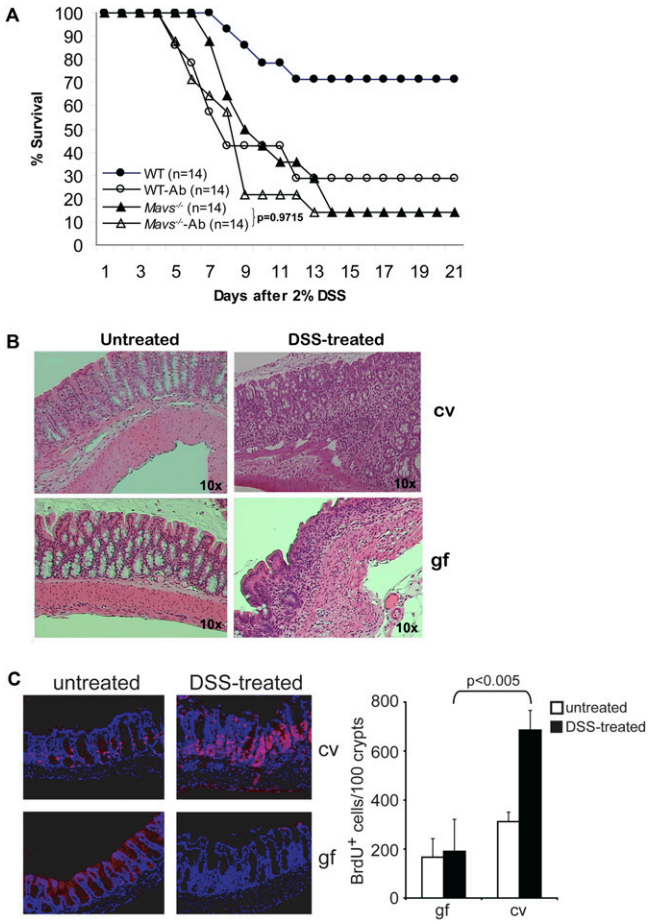

Fig. S2. Commensal bacteria protect mice from DSS-induced colitis. (*A*) Depletion of commensal bacteria did not rescue *Mavs*$^{-/-}$ mice from mortality caused by DSS. WT and *Mavs*$^{-/-}$ mice were treated with four antibiotics (Ab) for 4 wk and the depletion of bacteria was verified by fecal culture as described in *Methods*. The animals were then given 2% DSS in drinking water for 7 d followed by 14 d of normal drinking water; the mortality was monitored. Mantel-Cox test was used for statistical analysis: WT-Ab vs. WT: $P = 0.0082$; *Mavs*$^{-/-}$-Ab vs. *Mavs*$^{-/-}$: $P = 0.9715$. (*B*) Conventionally raised (cv) and germ-free (gf) mice were treated with 2% DSS as described in *A*, and the colons were examined by H&E staining. (*C*) Low proliferative potential in the colon of germ-free mice treated with DSS. Mice were treated for 5 d with 2% DSS followed by 3 d on regular drinking water to initiate epithelial repair. Cells undergoing DNA replication in vivo were labeled with BrdU, which was administered to mice by intraperitoneal injection 90 min before being killed. Colon sections were stained with anti-BrdU antibody and detected by fluorescent microscopy (red staining). BrdU-labeled cells were quantitated by unbiased counting of all well-oriented crypts, regardless of whether they resided in damaged or undamaged areas. Cell nuclei were stained with Hoescht dye (blue). $n = 4$–5 mice per group.

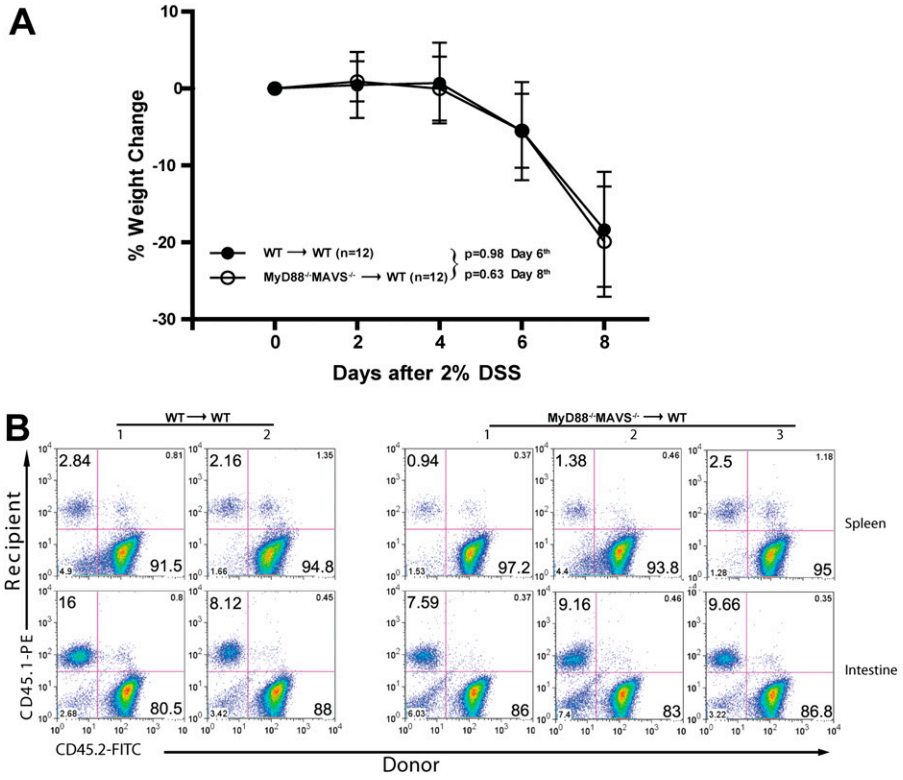

Fig. S3. MyD88 and MAVS in cells of nonhematopoietic origin play a dominant role in preventing DSS-induced colitis. Bone marrow transplantation experiments were carried out and the efficiency of transplantation was verified by FACS analysis of leukocyte surface markers, as described in *Methods*. (*A*) All animals were treated with 2% DSS in drinking water before switching back to normal water, and mortality was monitored until the 21st day. WT mice reconstituted with *MyD88*$^{-/-}$*Mavs*$^{-/-}$ WT bone marrow show no significant difference than the control animals reconstituted with WT bone marrow (*B*). The efficiency of chimerism in spleen and intestine was verified with leukocyte congenic markers CD45.2 and CD45.1.

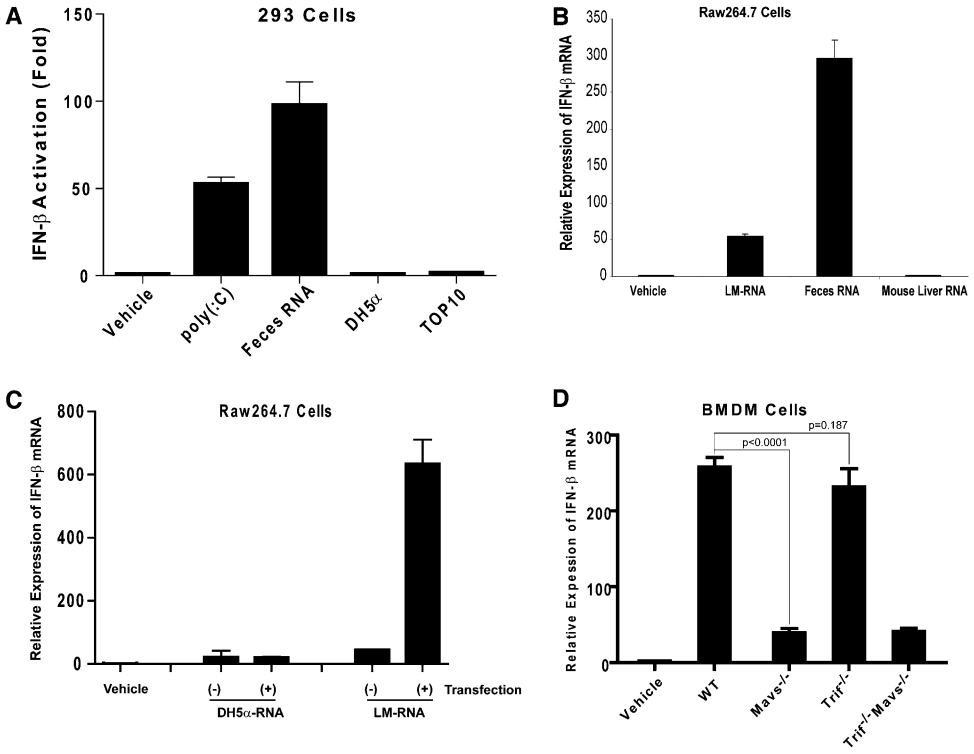

Fig. S4. Induction of IFN-β by RNA from mouse feces and *Listeria monocytogenes*. (*A*) Poly[I:C] and mouse feces RNA, but not RNA isolated from DH5α or TOP10, induce IFN-β-luciferase reporter in 293 cells. (*B*) RNA from mouse feces and *L. monocytogenes* (L.M.), but not that from mouse liver, induced the expression of IFN-β mRNA in Raw264.7 cells. (*C*) Transfection is required for RNA of *L. monocytogenes* to stimulate the expression of IFN-β mRNA in Raw264.7 cells. (*D*) IFN induction by mouse feces RNA requires MAVS, but not TRIF. BMDM isolated from mice of the indicated genotypes was transfected with mouse feces RNA, then total RNA was extracted to measure IFN-β induction by qRT-PCR.

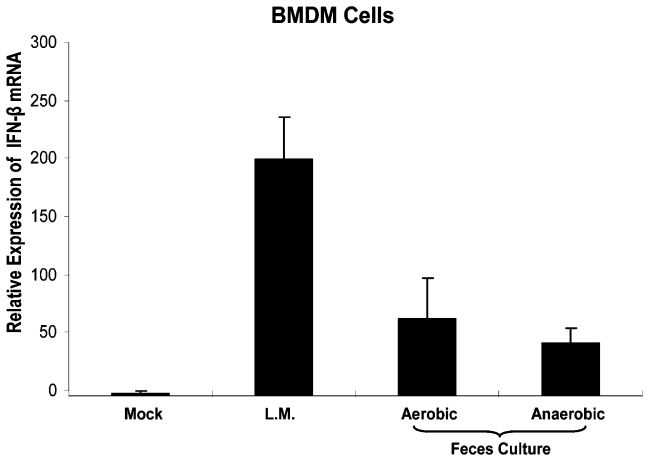

Fig. S5. Both aerobic and anaerobic bacteria in mouse feces contain RNA capable of inducing IFN-β. RNAs isolated from *L. monocyogenes* (L.M.) or fecal bacteria cultured under either aerobic or anaerobic conditions were transfected into WT BMDM cells. Eight hours after transfection, IFN-β mRNA was measured by qRT-PCR.

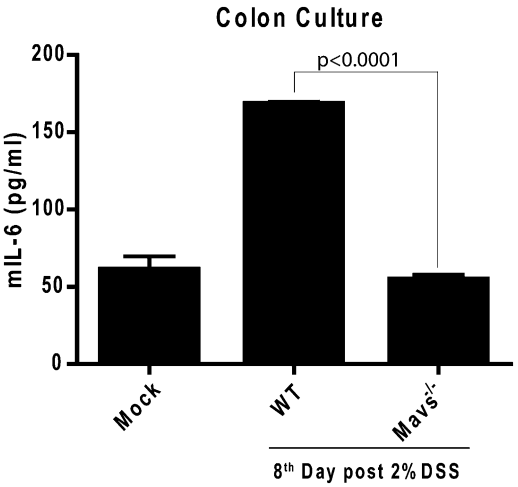

Fig. S6. Reduced expression of IL-6 protein in the colon of *Mavs*$^{-/-}$ mice. After colon samples from DSS-treated mice were cultured overnight, supernatant was collected for analysis by ELISA.

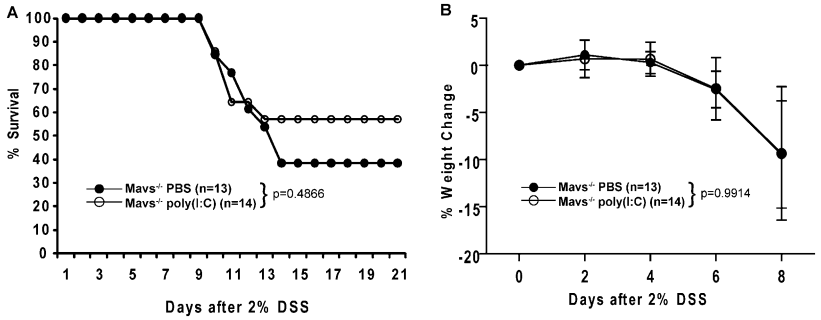

Fig. S7. Poly(I:C) failed to rescue *Mavs*$^{-/-}$ mice in DSS-induced colitis model. Two groups of *Mavs*$^{-/-}$ mice were injected intraperitoneally with either PBS or poly I:C (200 μg; 200 μL per mouse) on day −2, 0, 2, 4, and 6. The animals were then given 2% DSS in drinking water for 7 d followed by 14 d of normal drinking water. The mortality (*A*) and body weight (*B*) were monitored throughout the time course.

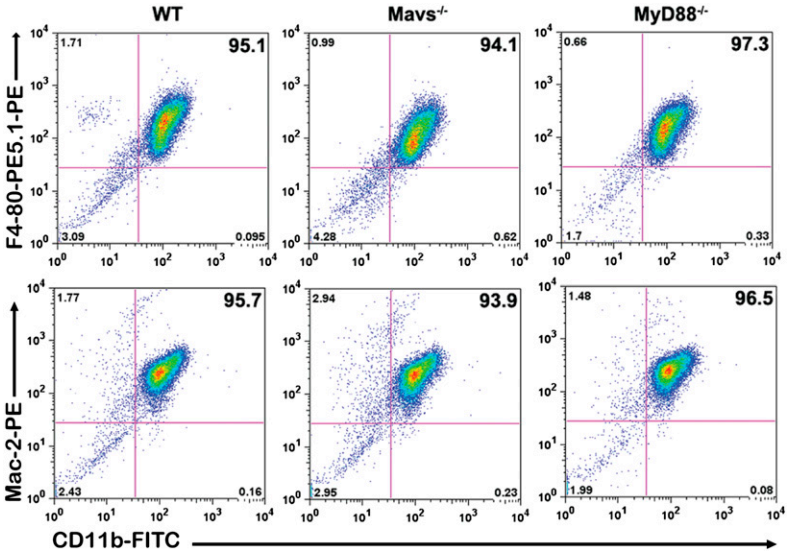

Fig. S8. Surface markers of BMDM derived from ex vivo culture of bone marrows. Bone marrows from WT, *Mavs*$^{-/-}$, and *MyD88*$^{-/-}$ mice were cultured using L929 conditioned media, and the cells expressing the macrophage surface markers were analyzed by FACS.

Table S1. FACS analysis of bone marrow chimeric mice using congenic surface markers CD45.1 and CD45.2

BM donor	BM recipient	Mean ± SD. $CD45.1^+/(CD45.1^+ + CD45.2^+)$ (%)	Mean ± SD. $CD45.2^+/(CD45.1^+ + CD45.2^+)$ (%)
WT (CD45.1)	WT (CD45.2)	82.03 ± 2.96	8.42 ± 2.05
WT (CD45.1)	*Mavs*$^{-/-}$ (CD45.2)	91.63 ± 1.21	4.8 ± 0.77

Peripheral blood samples were collected 8 wk after bone marrow (BM) transplantation. $n = 3$ each group.

Table S2. Average amounts of total RNA species isolated from feces of mice housed in different conditions

Mouse	RNA (μg) isolated from 1-mg feces
Conventionally raised mice	0.82–2.0 μg/mg
Antibiotics treated mice	0.08–0.1 μg/mg
Germ-free mice	0.01–0.02 μg/mg

$n = 3$ each group.