Materials and methods

Limulus amebocyte lysate (LAL) assay. The Endpoint Chromogenic LAL assay (cat. no. 50-647U; Lonza Group Ltd, Szabo Scandic, Vienna, Austria) was used according to the manufacturer's instructions for measure the endotoxin quantitative. Briefly, 50 μl of LAL-enzyme was added to 50 μl of standards or samples in 96-well flat-bottom plates and carefully mixed. After 10 minutes of incubation at 37˚C, 100 μl of pre-warmed substrate was added. The plates were then incubated for 6 more minutes at 37˚C. To stop the reaction, 100 μl of 10% SDS solution was added to each well. Absorption was measured at 405 nm using a micro-ELISA plate reader.

Reverse transcription-PCR. Total RNAs were extracted with Easy-Blue™, and the purity of the samples was confirmed by RNA calculator (Gene Quant Pro, Biochrom, Holliston, MA, USA). The total RNA extraction kit was used according to the manufacturer's instructions and reverse transcription of RNA (10 μg) to cDNA was performed using the ABI cDNA synthesis kit (cat. no. 4387406; Applied Biosystems, Foster City, CA, USA) (conditions: 37˚C for 1 h, followed by 95˚C for 5 min). The following sets of primers were used for PCR amplification: TLR4 (forward: 5'-AGTGGGTCAAGGAACAGAAGCA-3', reverse: 5'-CTTTACCAGCTCATTTCTCACC-3'), β-actin (forward: 5'-TGTGATGGTGGGAATGGGTCAG-3', reverse: 5'-TTTGATGTCACGCACGATTTCC-3'). Template cDNA and primers were added to the PCR pre-mix according to the manufacturer's instructions (cat. no. STD02) (Solgent, South Korea). The amplification profile was as follows: Initial cycling at 95˚C for 2 min, followed by 35 cycles of 95˚C for 20 sec, 54˚C (for TLR4), 58˚C (for β-actin) for 40 sec, and 7235 cycles of 95le was as follows: Verse: 5'-TTTGATGTCACGCACGAT TTCC-3'). Templategel (1.5% agarose) electrophoresis.

Immunocytochemistry. mIMCD-3 cells were plated in a chamber slide and incubated with LPS (5 μg/ml) for 30 min at 37˚C. The cells were treated with BBR (1 μM) for 1 h before LPS treatment. The cells were fixed in 4% paraformaldehyde for 15 min at RT and washed 3 times with PBS. The cells were treated with 0.1% TritonX-100 for 15 min at RT. After washing, non-specific binding sites were blocked with serum (3% BSA) for 1 h at RT, and incubated with NF-κB antibody (1:250) at 4˚C overnight. The cells were then washed and incubated with AlexaFluor®568 goat anti-rabbit IgG (1:2,000; cat. no. A-11011) for NF-κB antibody for 2 h at RT in a darkened room. For nuclear staining, the cells were incubated with DAPI (cat. no. H-1200; Vector Laboratories, Burlingame, CA, USA) at 5 μg/ml for 5 min at RT. The slide was finally washed and mounted for microscopic examination. Stained sections were visualized using a confocal laser microscope (Olympus, Japan).

Figure S1. Interaction of BBR with LPS and TLR4. (A) *E. coli* LPS 055:B5 was used as a standard. (B) Mouse IMCD-3 cells were pre-treated with BBR (1 μM) 1 h before LPS (5 μg/ml) treatment. TLR4 mRNA level was measured after 1, 3 or 24 h LPS treatment by reverse transcription-PCR. *P<0.05 vs. control. BBR, berberine; LPS, lipopolysaccharide; TLR4, toll-like receptor 4.

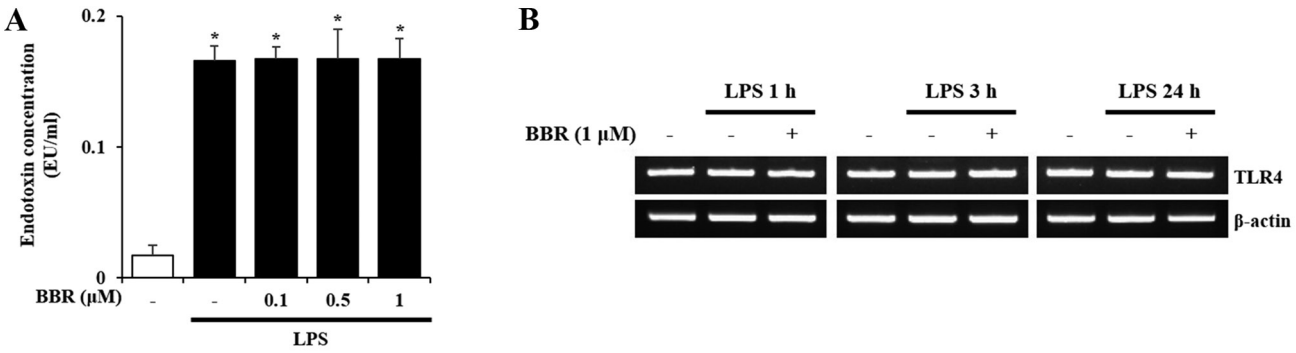

Figure S2. Inhibitory effects of NAC, Bay, and Par on NF-κB translocation. mIMCD-3 cells were incubated with NAC (10 mM), Bay (5 μM) and Par (10 μM) for 1 h. Thereafter, mIMCD-3 cells were harvested after LPS (5 μg/ml) stimulation for 30 min. Immunofluorescence staining was performed to evaluate the NF-κB translocation from the cytosol to the nucleus in mIMCD-3 cells. NF-κB p65 was stained with AlexaFluor 568 (red) and the nucleus was stained with DAPI (blue) in mIMCD-3 cells. Figure shows representative images from three independent experiments. Scale bar, 50 μm. NF-κB, nuclear factor-κB; mIMCD-3, mouse IMCD-3; NAC, N-acetylcysteine; Bay, Bay11-7082; Par, Parthenolide.