

Fig. S1. The stimulatory effects of KF and SF through TLR5 pathway on splenic DCs. A, Splenic DCs were gated as 7AAD⁻ CD11c^{high} splenocytes. B, CD80 and CD86 expression levels on splenic DCs after 0.1nM flagellin stimulation. C and D, dose-dependence of CD80 and CD86 expression on CD11c^{high} splenic DCs were analyzed by flow cytometry. Data are represented as the mean \pm SEM from triplicates of one representative of three experiments.

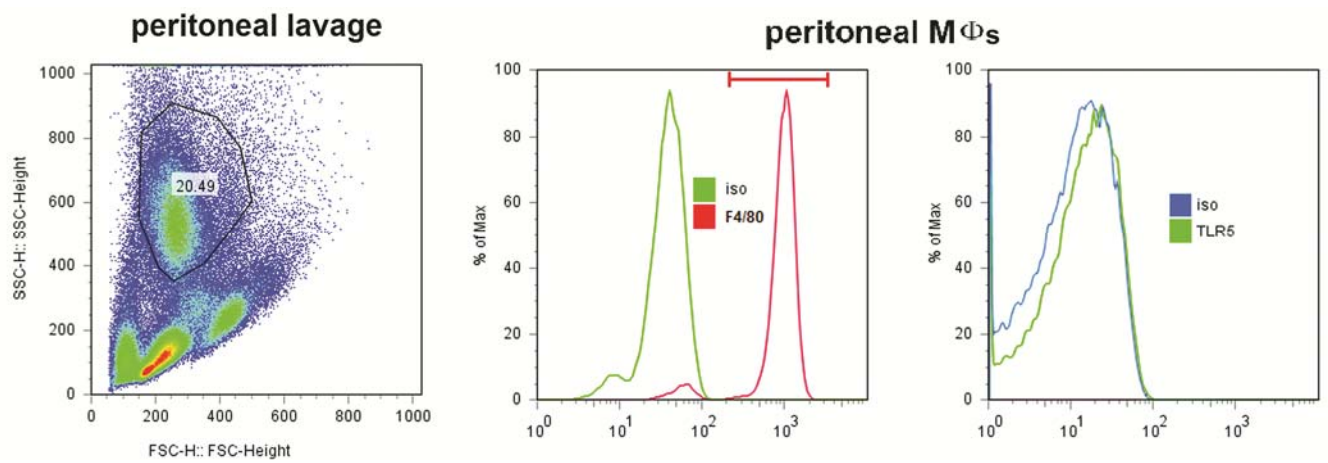


Fig. S2. TLR5 expression in peritoneal MΦs. 7AAD⁻ F4/80⁺ peritoneal cells were gated as peritoneal MΦs (left and middle) and express insignificant level of TLR5 (right). Data are presented as one experiment that repeated at least 3 times.

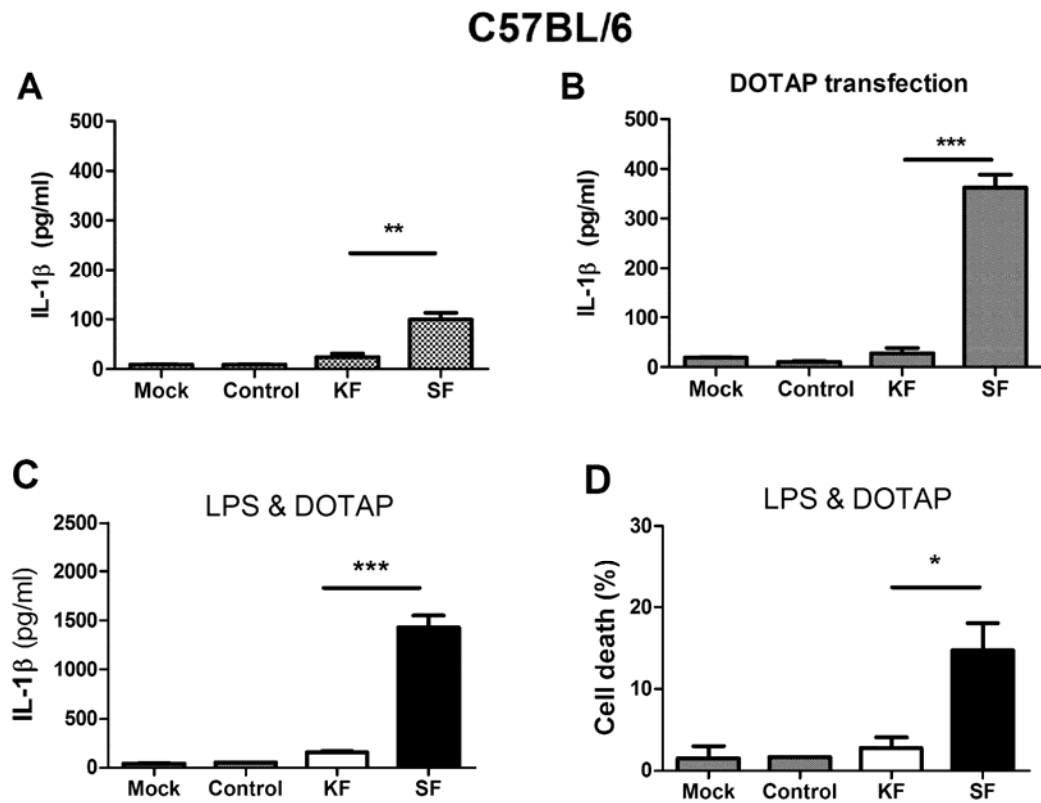


Fig. S3 IL-1 β secretion and cell death induced by flagellins in peritoneal macrophages derived from C57BL/6 mice. A and B, IL-1 β secreted in cell culture supernatants were tested 20-h after 100 nM flagellin stimulation without or with tranfection reagent DOTAP. C and D, IL-1 β secretion and cell death on C57BL/6 mice peritoneal macrophages pretreated with 50 ng/ml LPS for 3h and then with 100nM flagellin and DOTAP for 20h. Data are presented as the means \pm SEM from triplicates of one representative of three experiments.

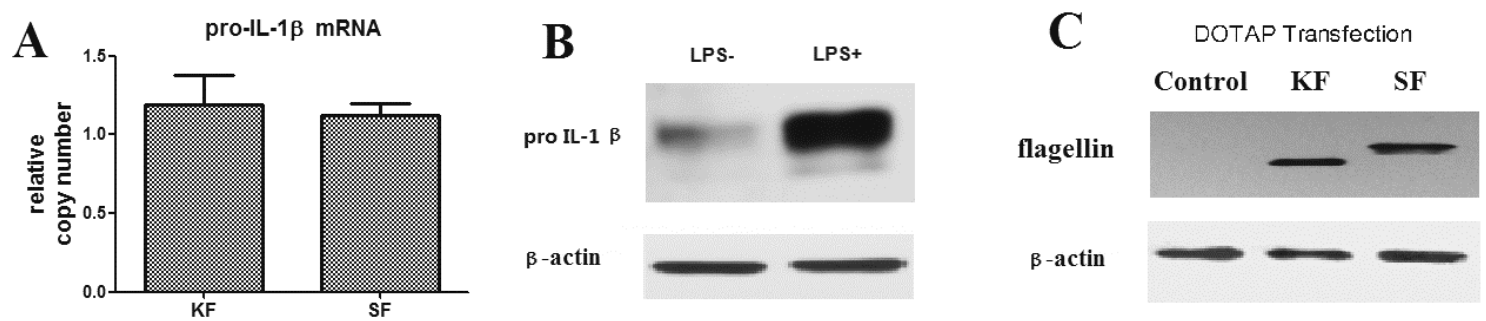


Fig. S4. The stimulatory effects of flagellins and LPS in peritoneal M Φ s. A, Pro-IL-1 β mRNA levels in peritoneal M Φ s 3-h after 10 nM flagellin stimulation. B, Pro-IL-1 β levels in cells lysate 3-h after 1 μ g/ml LPS stimulation. C, Flagellin levels in cells lysate 1-h after transfection of 100 nM flagellin. Data are presented of one representative of three experiments.

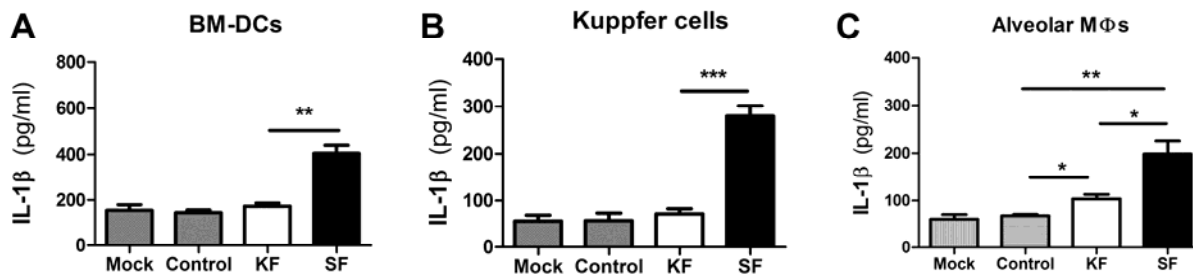


Fig. S5. IL-1 β secretion induced by flagellin in BM-DCs, Kupffer cells, and Alveolar M Φ s. BM-DCs were prepared from the femurs of BALB/c mice by culture for 7 days with RPMI 1640 containing 10 % fetal bovine serum and recombinant mouse IL-4 plus GM-CSF as described previously [Inaba K, Swiggard WJ, Steinman RM, Romani N, Schuler G, Brinster C: Isolation of dendritic cells; in Coligan JE, et al. (eds): Current Protocols in Immunology 2009, chapter 3, unit 3.7.], and seeded at a density of 5×10^5 cells / well in 24-well plates. Kupffer cells were isolated from C57BL/6 mice as described previously [Wu J, Meng Z, Jiang M, Zhang E, Trippler M, Broering R, Bucchi A, Krux F, Dittmer U, Yang D, Roggendorf M, Gerken G, Lu M, Schlaak JF: Toll-like receptor-induced innate immune responses in non-parenchymal liver cells are cell type-specific. Immunology 2010;129:363-374.], and seeded at a density of 3×10^5 cells / well in 24-well plates. Alveolar M Φ s were collected from bronchoalveolar lavage fluids and seeded at a density of 5×10^4 cells / well in 96-well plates. After LPS pretreatment for 3 h, the cells were transfected with 100 nM flagellins. The levels of IL-1 β in the culture supernatants were determined 20 h after transfection. Data are presented as the means \pm SEM from triplicates of one representative of three experiments.

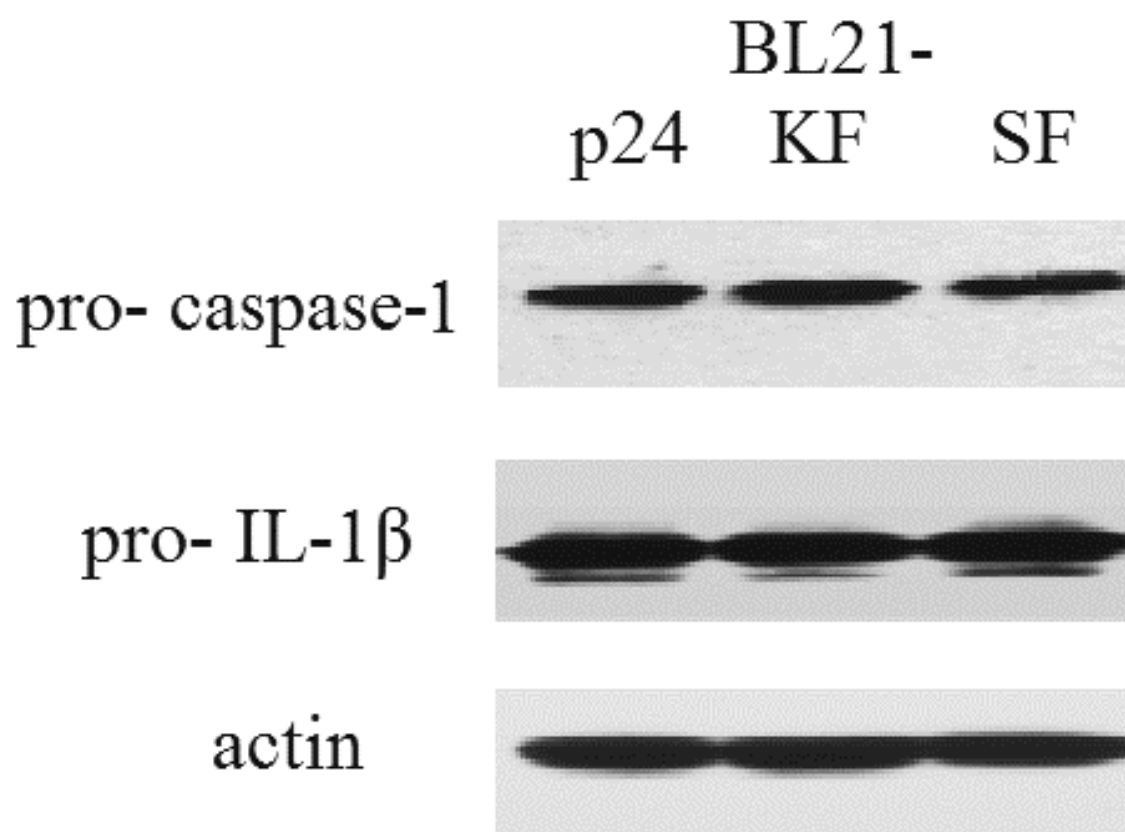


Fig. S6. The pro-IL-1 β levels and pro-caspase-1 levels in cells lysate of peritoneal M Φ s 6-h after the exposure to bacteria. Data are presented as the means \pm SEM from triplicates of one representative of three experiments.