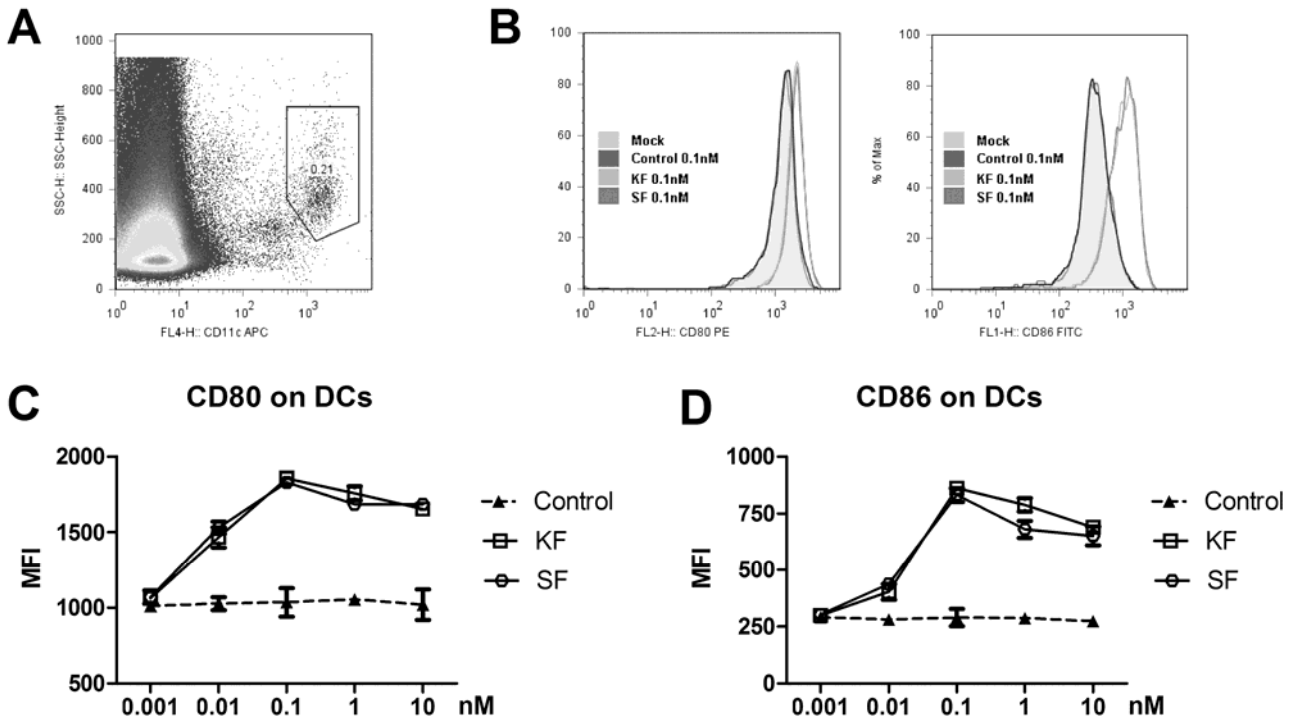

Fig. S1. The stimulatory effects of KF and SF through TLR5 pathway on splenic DCs. A, Splenic DCs were gated as 7AAD^{-} CD11c^{high} splenocytes. B, CD80 and CD86 expression levels on splenic DCs after 0.1nM flagellin stimulation. C and D, dose-dependence of CD80 and CD86 expression on CD11c^{high}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

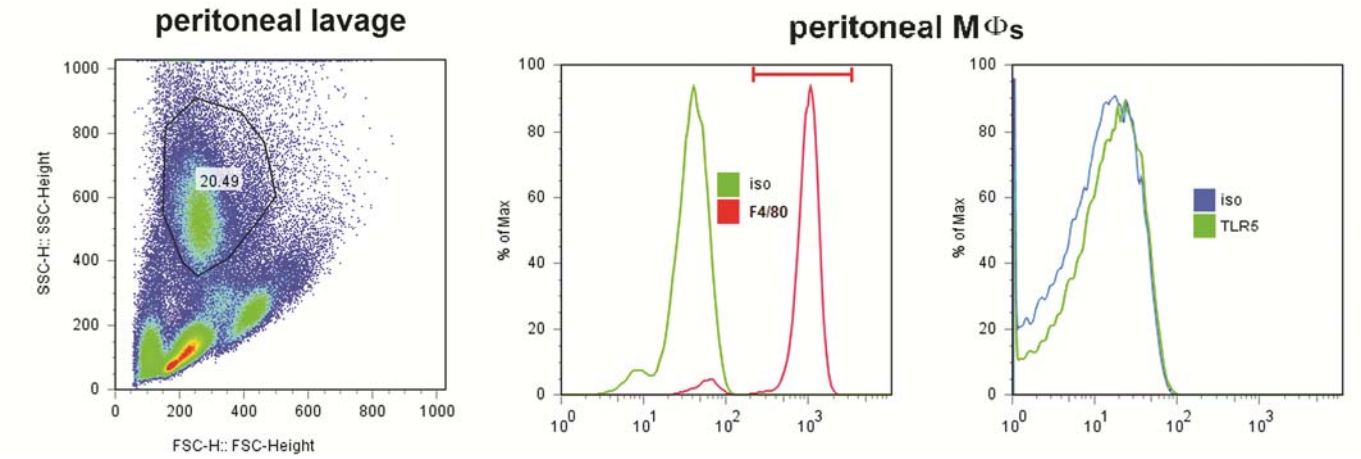

Fig. S2. TLR5 expression in peritoneal MΦs. 7AAD^{-} F4/80^{+} peritoneal cells were gated as peritoneal MΦs (left and middle) and express insignificant level of TLR5 (right). Data are presented as one experiment that repeated at least 3 times.

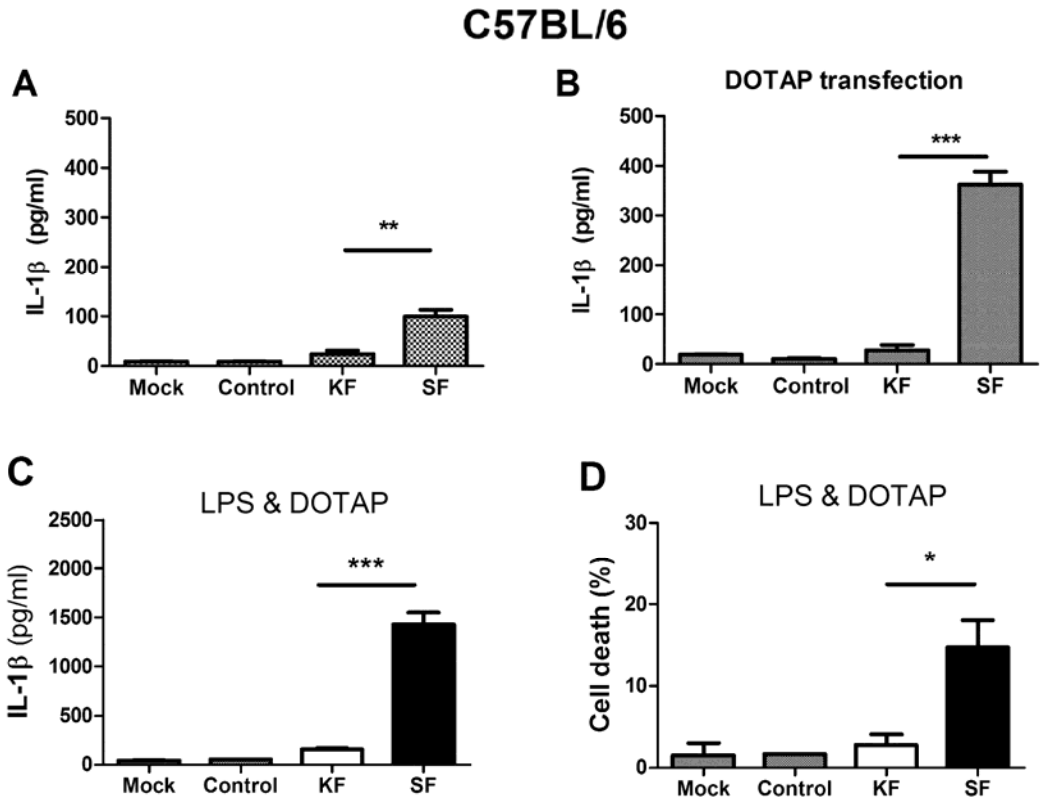

Fig. S3 IL-1β secretion and cell death induced by flagellins in peritoneal macrophages derived from C57BL/6 mice. A and B, IL-1β secreted in cell culture supernatants were tested 20-h after 100 nM flagellin stimulation without or with tranfection reagent DOTAP. C and D, IL-1β secretion and cell death 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 ± SEM from triplicates of one representative of three experiments.

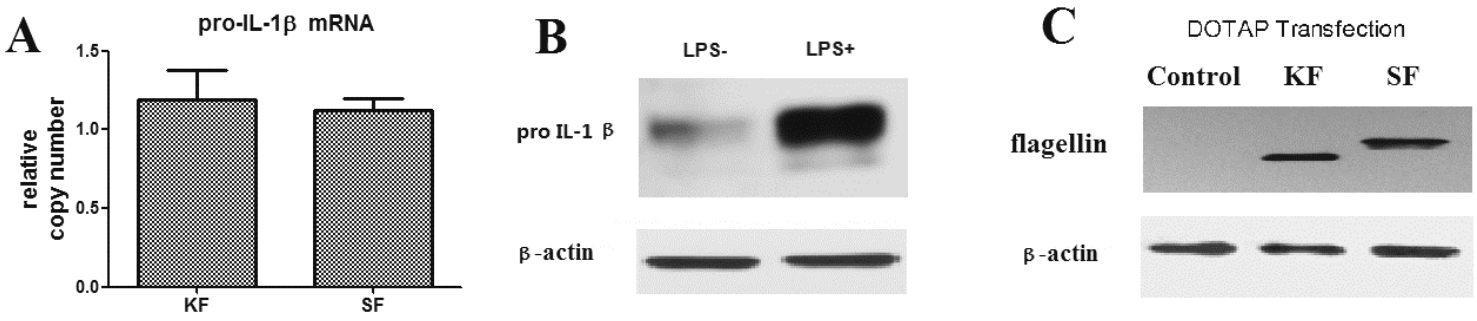

Fig. S4. The stimulatory effects of flagellins and LPS in peritoneal MΦs. A, Pro-IL-1β mRNA levels in peritoneal MΦs 3-h after 10 nM flagellin stimulation. B, Pro-IL-1β levels in cells lysate 3-h after 1ug/ml LPS stimulation. C, Flagellin levels in cells lysate 1-h after transfection of 100 nM flagellin.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

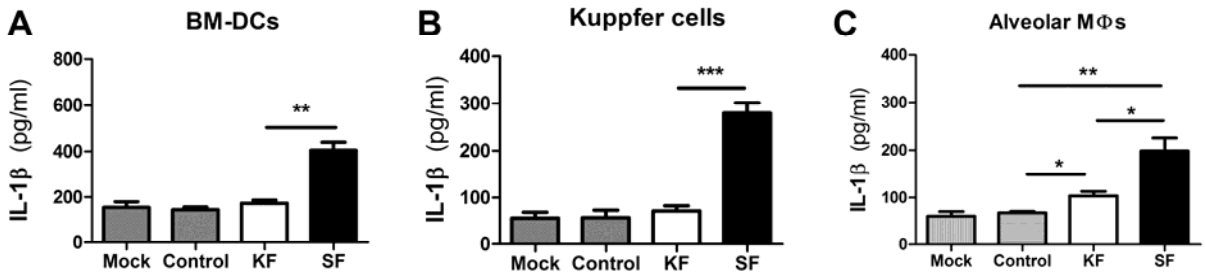

Fig. S5. IL-1β secretion induced by flagellin in BM-DCs, Kupffer cells, and Alveolar MΦs. BM-DCs were prepared from the femurs of BALB/c mice by culture for 7 days with RPMI 1640 containing 10 % fetal bovine serum and recombinant mouse IL-4 plus GM-CSF as described previously [Inaba K, Swiggard WJ, Steinman 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 MΦs were collected from brochoalveolar 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-1β in the culture supernatants were determined 20 h after transfection. Data are presented as the means ± SEM from triplicates of one representative of three experiments.

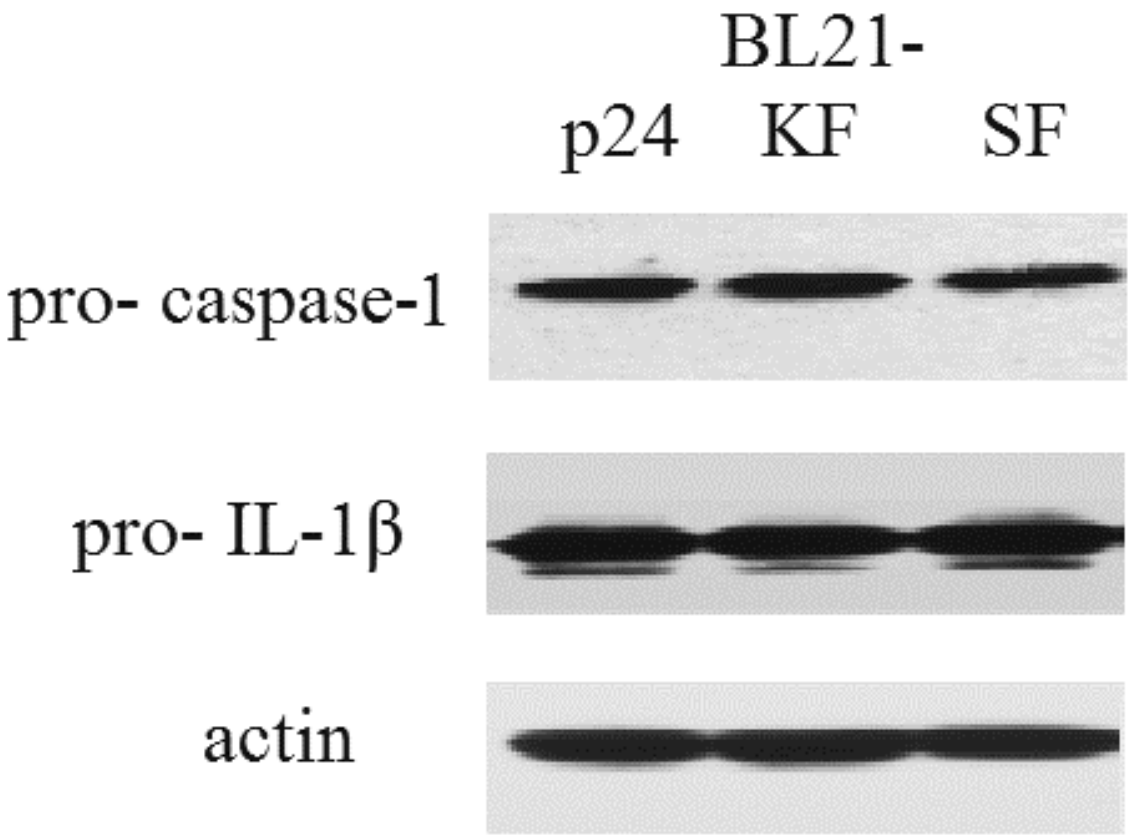

Fig. S6. The pro-IL-1β levels and pro-caspase-1 levels in cells lysate of peritoneal MΦs 6-h after the exposure to bacteria. Data are presented as the means ± SEM from triplicates of one representative of three experiments.