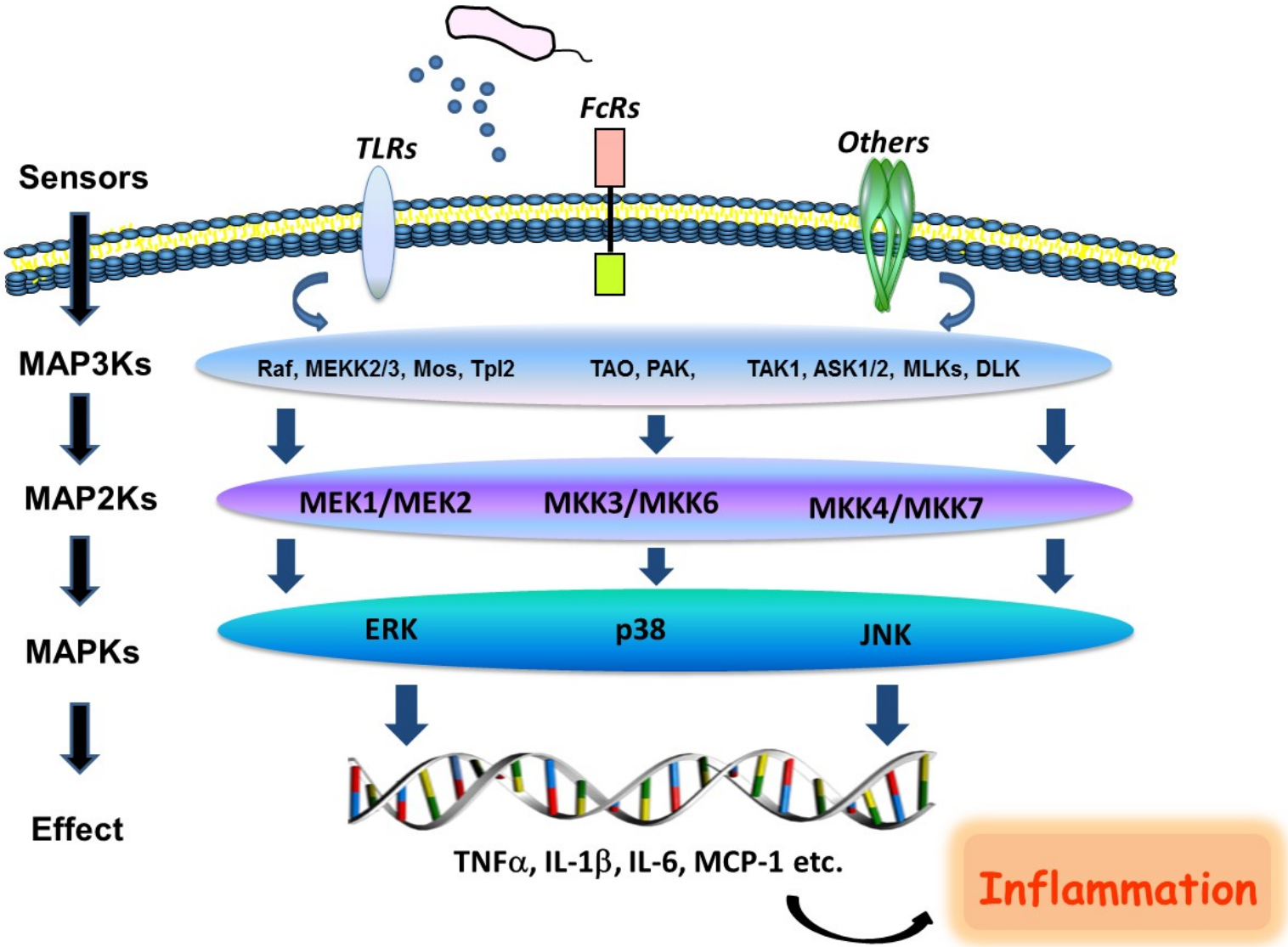

Fig. S1. MAP kinase pathway in immune response to microbial infection. Microbe pathogens or their products are detected by various cellular sensors such as Toll-like receptors (TLRs), which leads to the activation of MAP kinase kinase kinases (MAP3Ks). The MAP3Ks in turn activate MAP kinase kinases (MAP2Ks) that phosphorylate corresponding MAP kinases (such as MEK1/MEK2 phosphorylate ERK and MKK3/MKK6 phosphorylate p38). The activated MAP kinases cooperatively regulate the expression of various pro-inflammatory cytokines including TNFα, IL-1β and IL-6 that critically involve in inflammation.

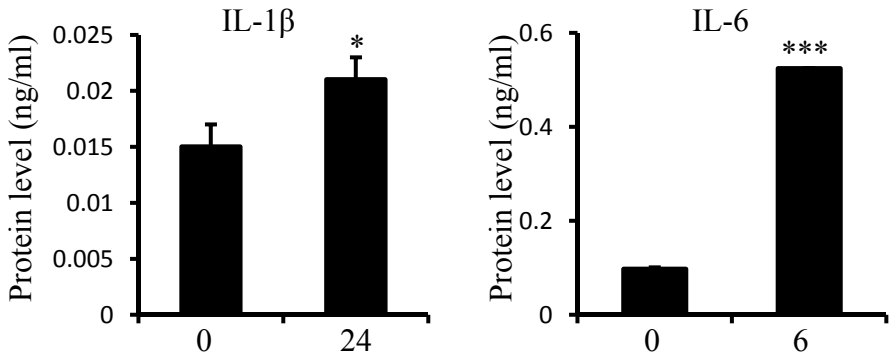

Fig. S2. Live *Blastocystis* ST-7 (B) infection in mouse intestinal explants resulted in up-regulation of pro-inflammatory cytokine secretion. Murine intestinal explants (distal colon) were isolated from C57/BL6 mice and treated with live *Blastocystis* ST-7 (B). Concentrations of pro-inflammatory cytokines, IL-1β and TNF-α in supernatants were determined by ELISA. Each value represents the mean ± standard deviation from 3 individual experiments. *, P<0.05; **, P<0.01; ***, P<0.005.

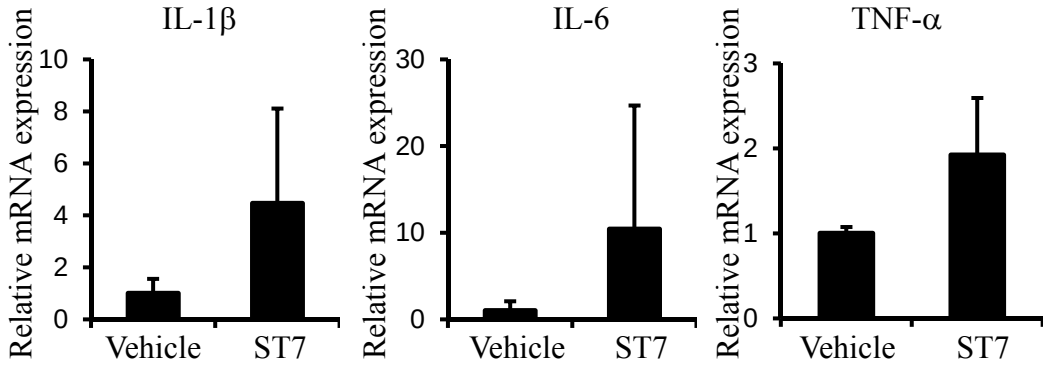

Fig. S3. Proinflammatory cytokine expression in colon from DSS-induced colitis mice treated with *Blastocystis* ST-7 (B) lysate. Eight-week-old C57/BL6 mice were treated with 2% dextran sodium sulphate (DSS) to induce colitis for 5 days, followed by a 3-day oral gavage of either ST-7 (B) lysate or PBS vehicle. The distal colon was investigated for the mRNA expression of the pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α using qRT-PCR. Results are normalized to GAPDH and expressed as the mean fold change relative to vehicle, which is set at 1. Each value represents the mean ± standard deviation from 3 individual experiments.

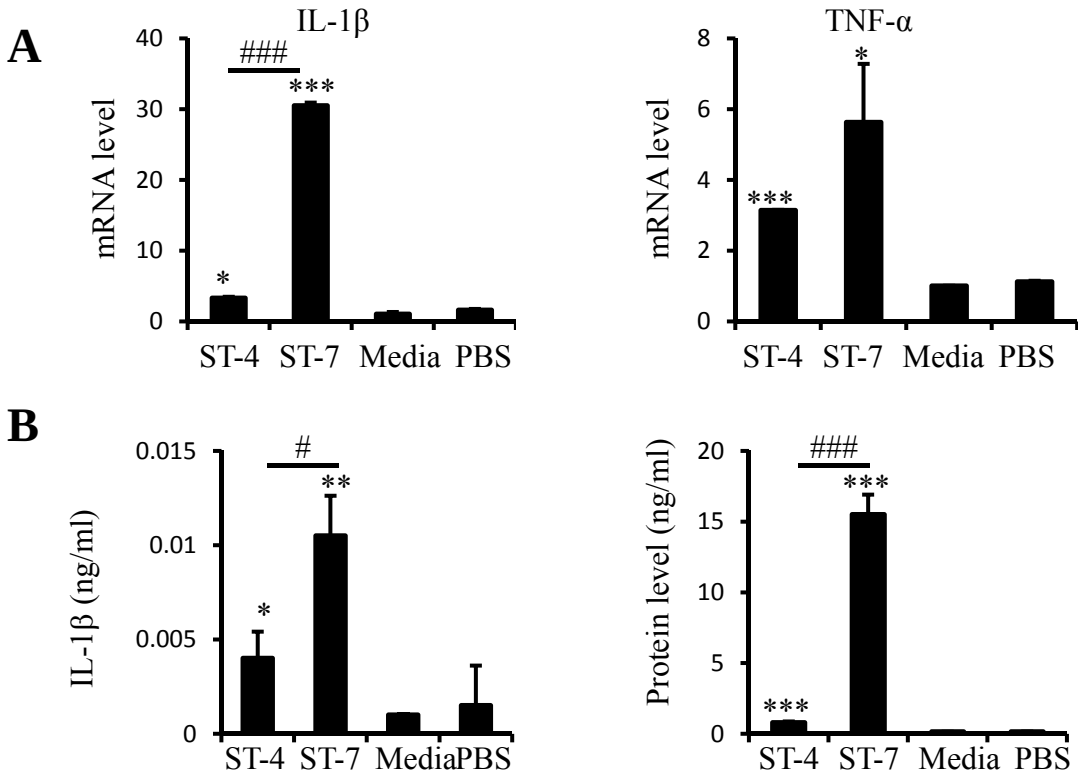

Fig. S4. Live *Blastocystis* ST-4 (WR-1) and ST-7 (B) infection in macrophages induced pro-inflammatory cytokine expression. RAW264.7 cells were co-incubated with either live *Blastocystis* ST-4 (WR1) at MOI 20 or ST-7 (B) at MOI 10. A. mRNA levels of TNF-α, IL-1β, IL-6 and GAPDH in macrophages were measured by qRT-PCR. Results are normalized to GAPDH and expressed as the mean fold change relative to 0 h time point, which is set at 1. B. The concentrations of IL-6, TNF-α and IL-1β in the culture supernatants of RAW264.7 were measured by ELISA. Each value represents the mean ± standard deviation from at least 3 individual experiments. *, P<0.05; **, P<0.01; ***, P<0.005.