Supplementary text.

SUPPLEMENTARY METHODS:

Determination of NFkB activation in IEC-6 cells.

For assessment of NF-κB activation, IEC-6 enterocytes were treated with LPS (0-100μg/ml, Sigma-Aldrich, St. Louis, MO) for 1 hour and immunostained with antibodies against the p65 subunit of NF-κB (Santa Cruz Biotechnology, Santa Cruz, CA). The extent of nuclear translocation was determined in an adaptation of the methodology of Ding and colleagues[1]. In brief, a threshold limit was set based upon the emission signal for the nuclear stain DRAQ5, which therefore defined a nuclear region of interest (ROI). To define a corresponding cytoplasmic region of interest, a circular region 12 pixels beyond the nucleus was stenciled upon each cell. The average integrated pixel intensity pertaining to the corresponding NFkB emission within the cytoplasmic and nuclear regions was then determined for more than 200 cells per treatment group in at least four experiments per group, using MetaMorph software version 6.1 (Molecular Devices Corporation, Downingtown, PA).

Polymerase chain reaction

Quantitative real-time PCR was performed as previously described using the Bio-Rad CFX96 Real-Time System (Biorad, Hercules, CA) [2]using the following primers: GFP sense: AGAACGGCATCAAGGTGAAC and anti-sense TGCTCAGGTAGTGGTTGTCG; GAPDH: sense TGAAGCAGGCATCTGAGGG and anti-sense CGAAAGGTGGAAGAGTGGGAG; pCNA sense:

AAAGATGCCGTCGGGTGAATTTGC and anti-sense: AATGTTCCCATTGCCAAGCTCTCC. Mouse β-catenin: sense CCAGTATAGATGTATGGTCTG and anti-sense CTTGTTGGTGTTCCTAGG; mouse GSK3β: GCCTGCAGAACTCCAGAAAG and TGGCAAAAACATCAACGTG. mouse IL-6: sense: CCAATTTCCAATGCTCTCCT and antisense ACCACAGTGAGGAATGTCCA.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. *LPS increases NFkB translocation and IL-6 expression in IEC-6 cells.* **A** (***i-v***): Representative confocal micrographs showing distribution of the p65 subunit of NFkB in IEC-6 cells treated with LPS for 1h at the indicated concentration. Note the increasing accumulation in the nucleus with increasing concentrations. ***vi***: Quantification of the extent of NFkB translocation in IEC-6 cells treated as in *i-v* using Metamorph software as described in Supplementary Methods. *$p<0.05$ vs. untreated cells; representative of 3 separate experiments in which over 100 cells per condition were assessed. Size bar = 10μm. **B**: Quantitative RT-PCR showing expression of IL-6 in IEC-6 cells treated with LPS at the concentration indicated for 3h. *$p<0.05$ vs. untreated cells. Representative of 3 separate experiments.

Supplemental Figure 2. *Quantification of data shown in Figure 4 indicating that LPS activates the AKT-GSK3β signaling pathway in enterocytes.* **A-E:** Quantification of mean and SEM with respect to SDS-PAGE in Figure 4, showing expression of the indicated protein, in IEC-6 cells treated with LPS at the concentrations and durations indicated (**A-**

D) or mucosal scrapings from the terminal ileum of mice injected with saline or LPS for the durations indicated (**E**). In A: † $p<0.05$ vs. untreated cells; *$p<0.01$ vs. untreated cells by ANOVA. In B: *$p<0.05$ vs. untreated cells; † $p<0.05$ vs. untreated cells; ‡$p<0.01$ vs. untreated cells by ANOVA; In C: *$p<0.005$ vs. untreated cells; †$p<0.05$ vs. untreated cells by ANOVA; In D: *$p<0.05$ versus untreated cells; †$p<0.05$ vs. untreated cells by ANOVA; In E: *$p<0.05$ vs. saline injected animals; † vs. saline injected animals by ANOVA.

SUPPLEMENTAL REFERENCES:

1. Ding LA, Li JS. Effects of glutamine on intestinal permeability and bacterial translocation in TPN-rats with endotoxemia. World J Gastroenterol 2003;9:1327-32.

2. Leaphart CL, Cavallo JC, Gribar SC, Cetin S, Li J, Branca MF, Dubowski TD, Sodhi CP, Hackam DJ. A critical role for TLR4 in the pathogenesis of necrotizing enterocolitis by modulating intestinal injury and repair. J. Immunol. 2007;179:4808-4820.