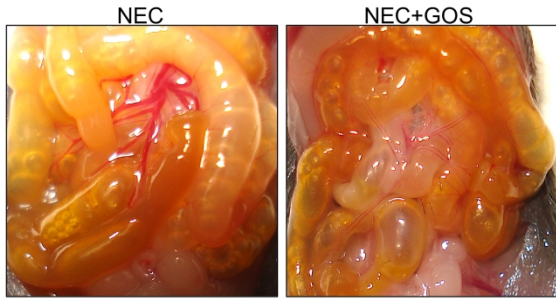


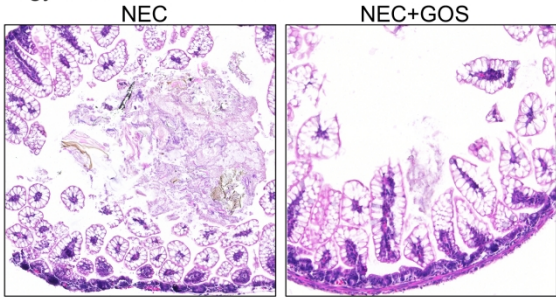


Supplemental Fig. s2.

**a** Gross morphology of the small intestine



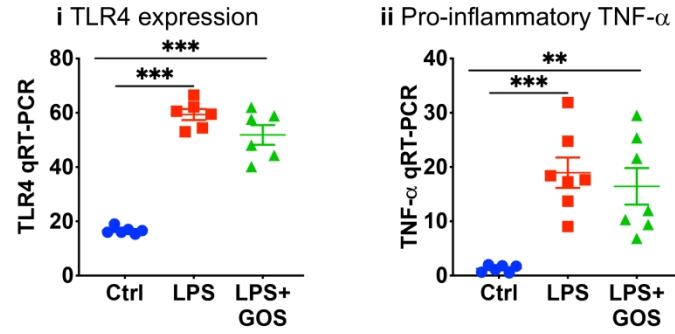
**b** Histology of the small intestine



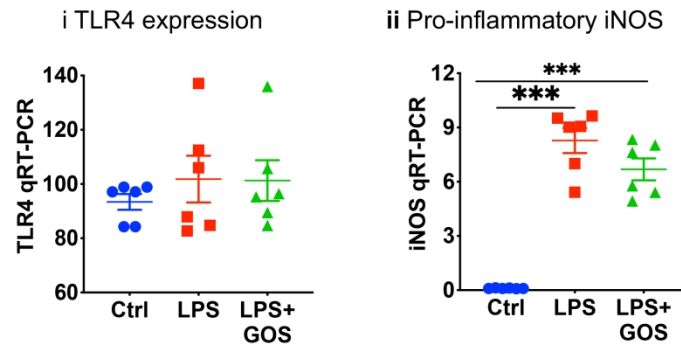
**Supplemental Fig. s2** Galactooligosacchide (GOS) supplementation of formula does not prevent NEC in mice. **a** Gross morphology, **b** photomicrographs of H&E sections of ileum of mice treated with GOS in infant formula.

Supplemental Fig. s3

## a Effect of GOS on LPS signaling in the intestinal mucosa of 7d old mice

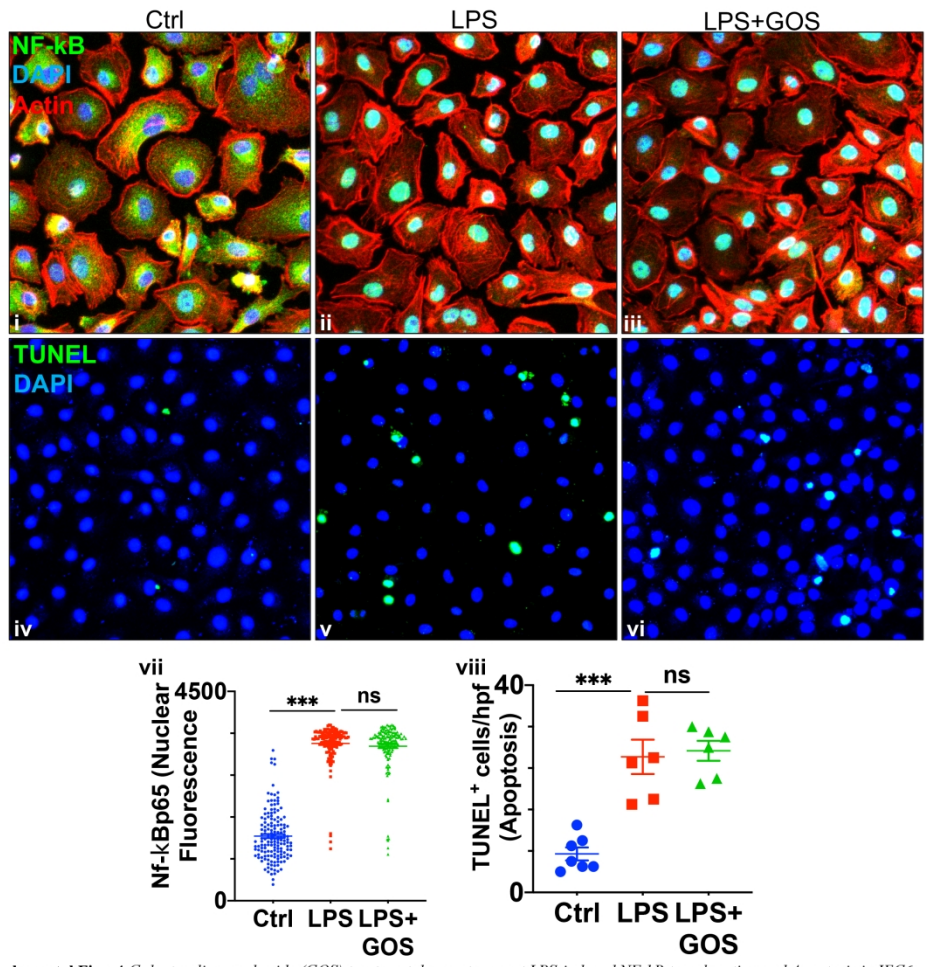


## b Effect of GOS on LPS signaling in IEC6 cells



**Supplemental Fig. s3** *Galactooligosacchides (GOS) does not inhibit LPS-induced TLR4 expression and signaling. ai, bi* TLR4 mRNA expression, **aii, bii** Proinflammatory cytokines tumor necrosis factor-alpha (TNF- $\alpha$ ) and inducible nitric acid synthase (iNOS) expressions measured by qRT-PCR in mice distal intestine of 7d old mice (ileum) and IEC6 cells treated with LPS (5mg/kg, mice) (50mg/ml, IEC6 cells) and GOS (10mg/kg, mice), (10mg/ml IEC6 cells) for 6 hours. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . Each dot in dot-graphs represents data from each mouse or each well of cells.

Supplemental Fig. s4



Supplemental Fig. s4 Galacto-oligosaccharide (GOS) treatment does not prevent LPS-induced NF-kB translocation and Apoptosis in IEC6 cells. i-iii photomicrographs of IEC6 cells treated with LPS (50mg/ml) alone or in combination with GOS (10mg/ml) for 45minutes and immuno-stained for NF-kB p65, iv-vi photomicrographs of IEC6 cells treated with LPS (50mg/ml) alone or in combination with GOS (10mg/ml) for 6 hours and immuno-stained with *in Situ* death staining kit, vii-viii quantification of fluorescence intensity measured using ImageJ, \*\*\*  $P < 0.001$ , ns=non-significant. Each dot in dot-graphs represents data from an individual cell.