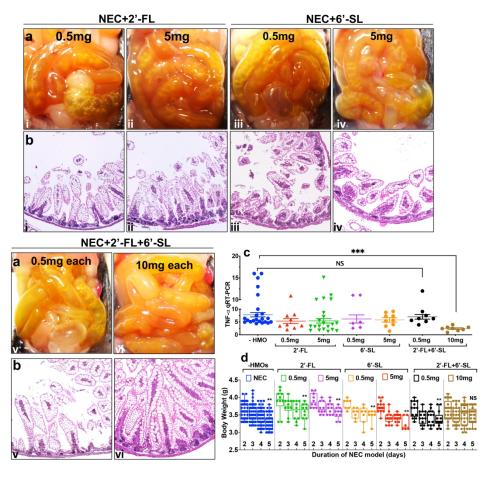
## Supplemental Fig. s1

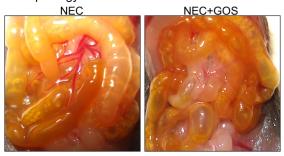


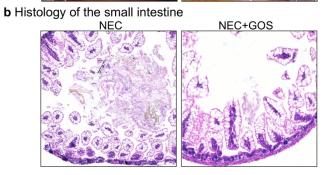
Supplemental Fig. s1. Dose curve of supplementation of nutritional formula with 2'-Fucosyllactose (2'-FL) and 6'-Sialyllactose (6'-SL) in experimental model of necrotizing enterocolitis (NEC). ai-vi photomicrographs showing gross morphology, bi-vi Hematoxylin-eosin (H&E)-stained images showing histology of small intestine (ileum), c qRT-PCR of pro-inflammatory cytokine TNF-a, d nested graph showing body weight changes, in neonatal mice subjected to experimental NEC treatments with varying doses of 2'-FL, 6'-SL, and 2'-FL+6'-SL HMO's.

\*\*p<0.01, \*\*\*\* p<0.001, each dot in dot-graphs represents data from an individual mouse.

## Supplemental Fig. s2.

## a Gross morphology of the small intestine

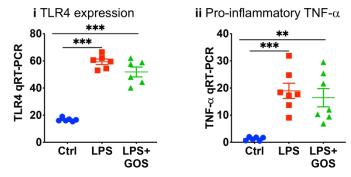




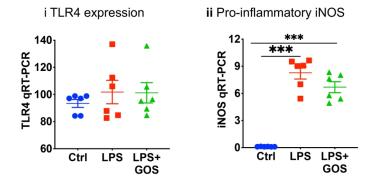
 $\label{eq:Supplemental} \textbf{Supplemental Fig. s2} \ \textit{Galactooligosacchide (GOS) supplementation of formula does not prevent NEC in mice. \textbf{a} Gross morphology, \textbf{b} photomicrographs of H&E sections of ileum of mice treated with GOS in infant formula.}$ 

## Supplemental Fig. s3

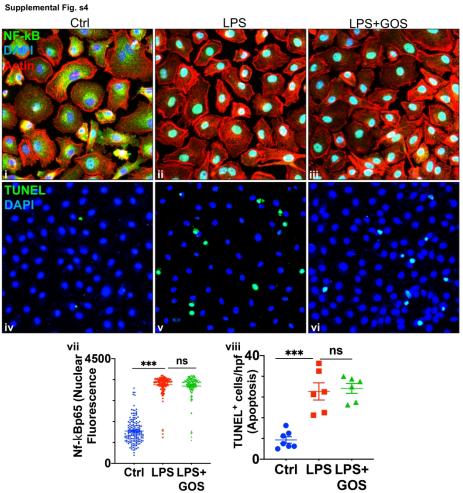
a Effect of GOS on LPS signaling in the intestinal mucosa of 7d old mice



**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 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.