

Cell Reports Medicine, Volume 2

Supplemental information

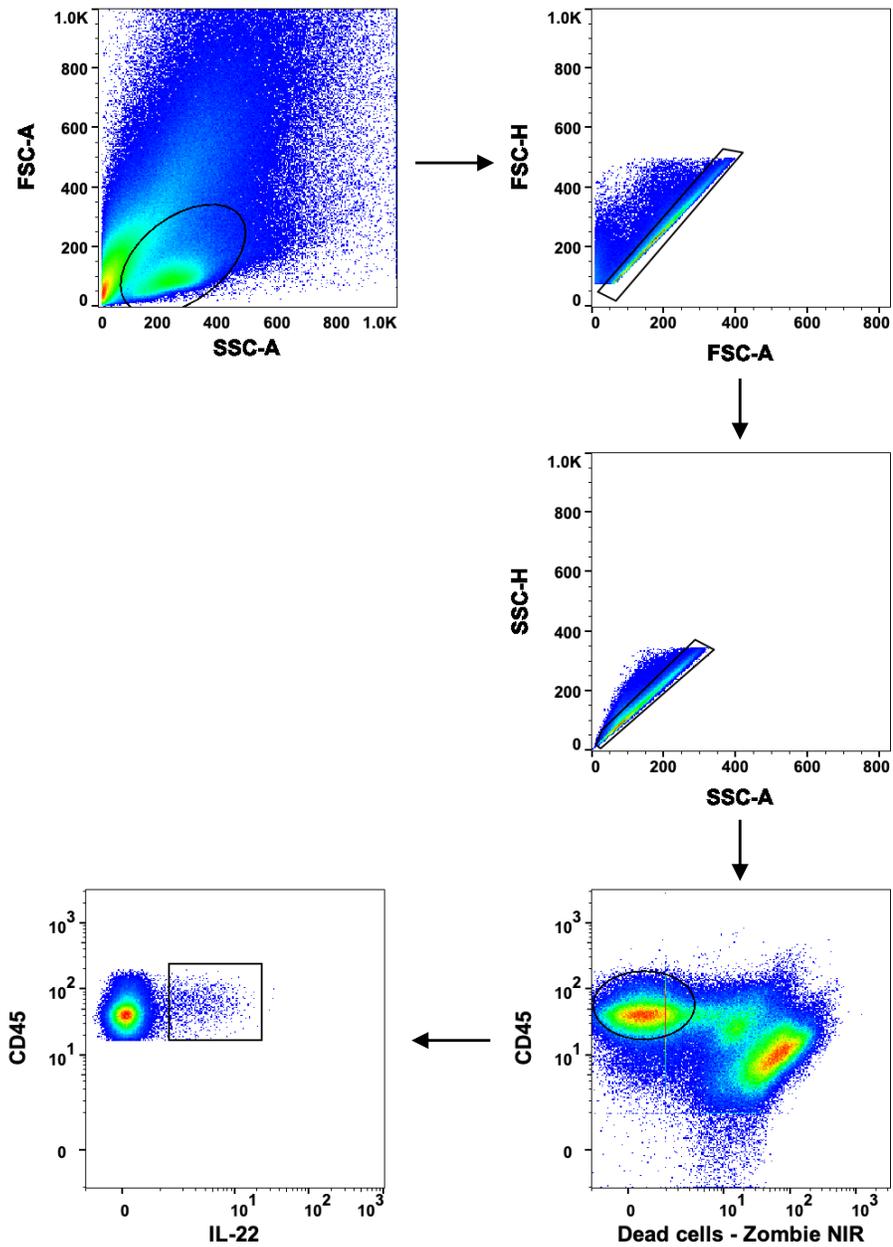
Interleukin-22 signaling

attenuates necrotizing enterocolitis

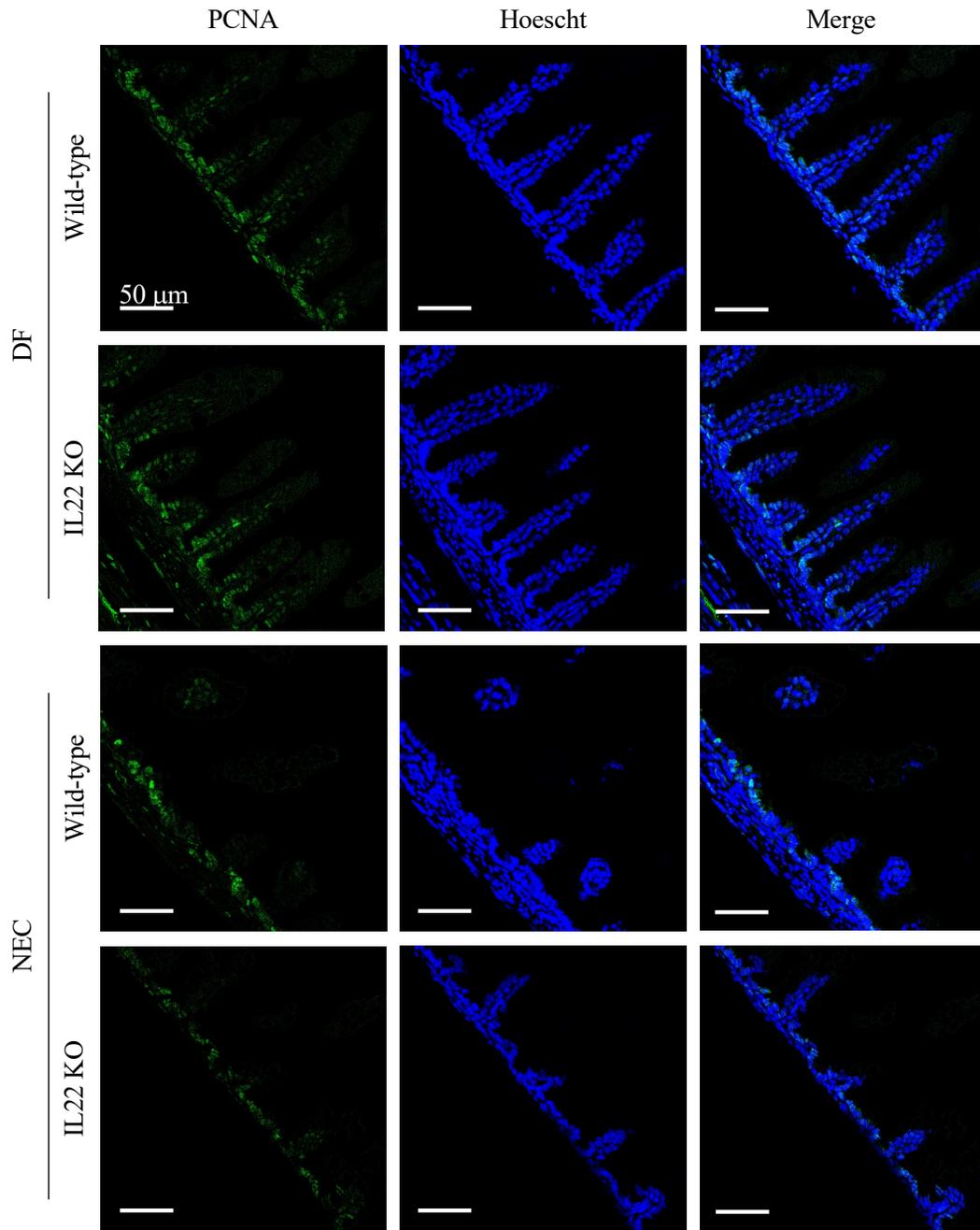
by promoting epithelial cell regeneration

Belgacem Mihi, Qingqing Gong, Lila S. Nolan, Sarah E. Gale, Martin Goree, Elise Hu, Wyatt E. Lanik, Jamie M. Rimer, Victoria Liu, Olivia B. Parks, Angela N. Lewis, Pranjal Agrawal, Marie L. Laury, Pawan Kumar, Elizabeth Huang, Shay S. Bidani, Cliff J. Luke, Jay K. Kolls, and Misty Good

Supplemental Figure 1



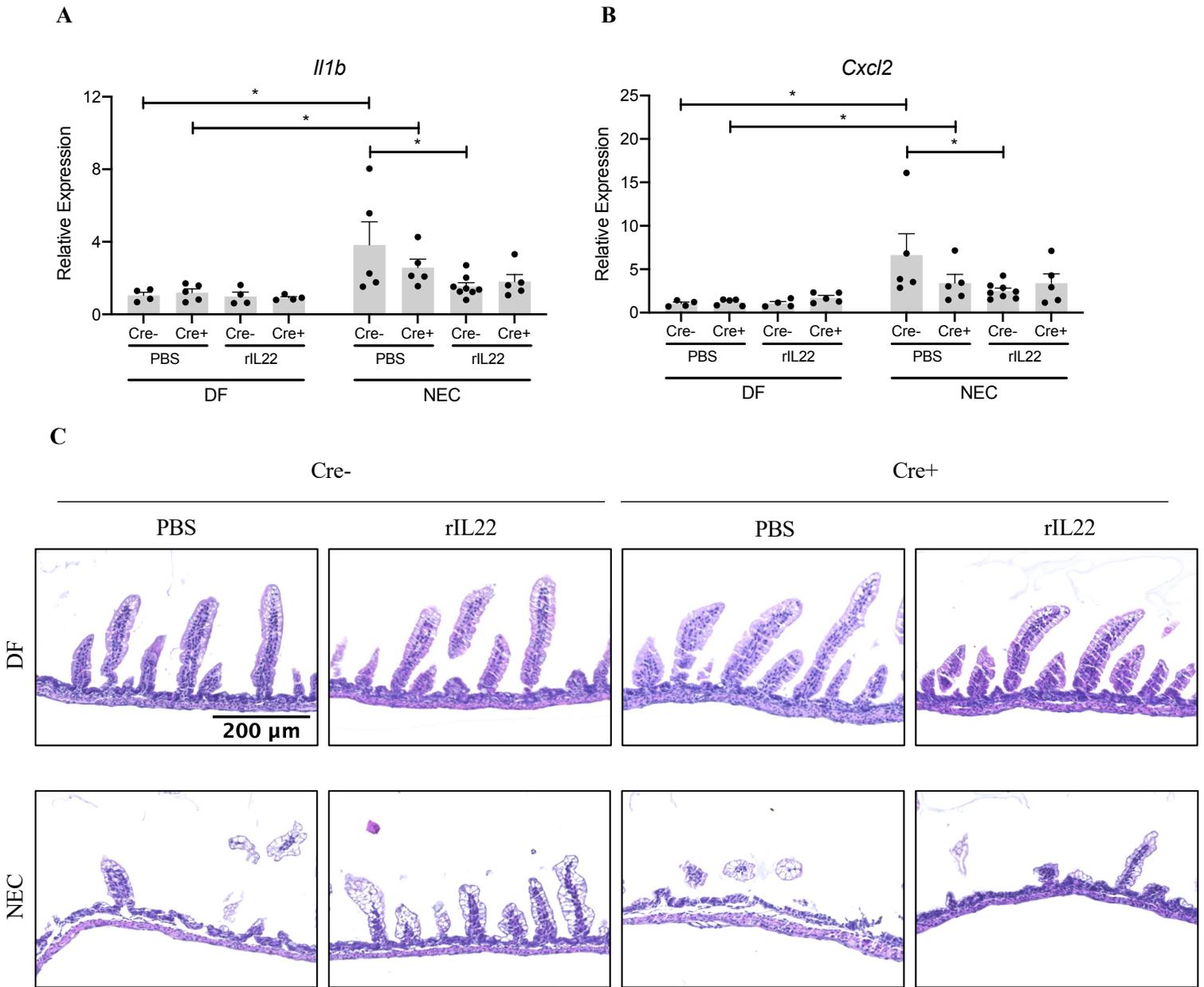
Supplemental Figure 1: Representative gating strategy for the FACS analysis of IL-22⁺ cells in the small intestine lamina propria. Related to Figure 1.



Supplemental Figure 2: Impact of *Il-22* deficiency on intestinal epithelial cells of neonatal mice during NEC. Related to Figure 2. Representative confocal images of ileal tissue samples from WT and *IL-22*^{-/-} were stained with an antibody against PCNA (Green). The nuclei were stained with Hoechst (Blue). Scale bar 50 μm. DF = Dam fed.

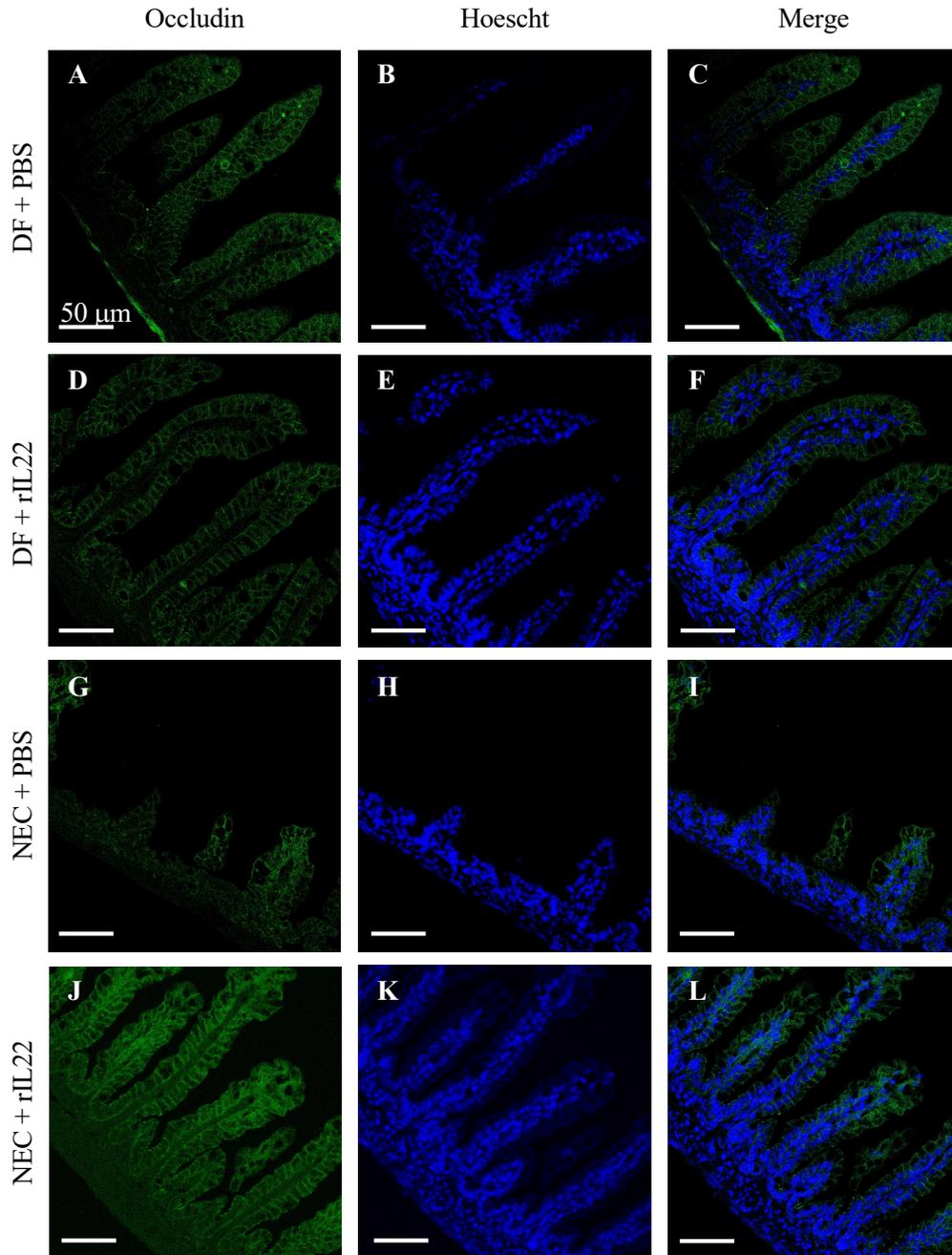
Supplemental Figure 3

IL-22ra1^{fl/fl} x vil cre



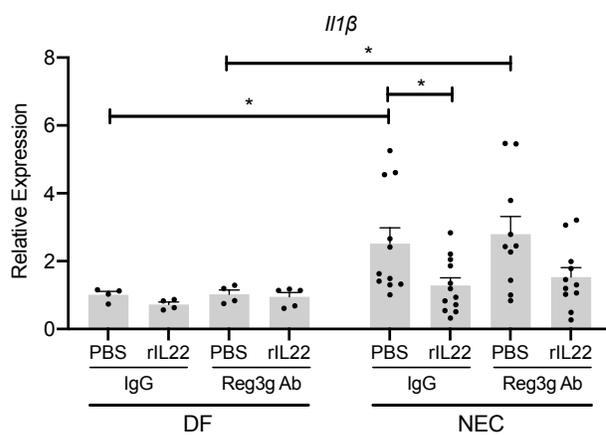
Supplemental Figure 3: IL-22 treatment does not protect against NEC in pups lacking the IL-22Ra1 in their intestinal epithelium. Related to Figure 3. Effect of IL-22 treatment on the intestinal expression of (A) *Il1b*, (B) *Cxcl2* and (C) histological appearance of the terminal ileum sections from each genotype in the intestine of dam fed (DF) and pups subjected to NEC carrying a specific deletion of IL-22Ra1 in epithelial cells. All gene expression data are displayed as mean \pm SEM and were analyzed using Mann-Whitney test. * $P < 0.05$. Scale bar 200 μ m.

Supplemental Figure 4



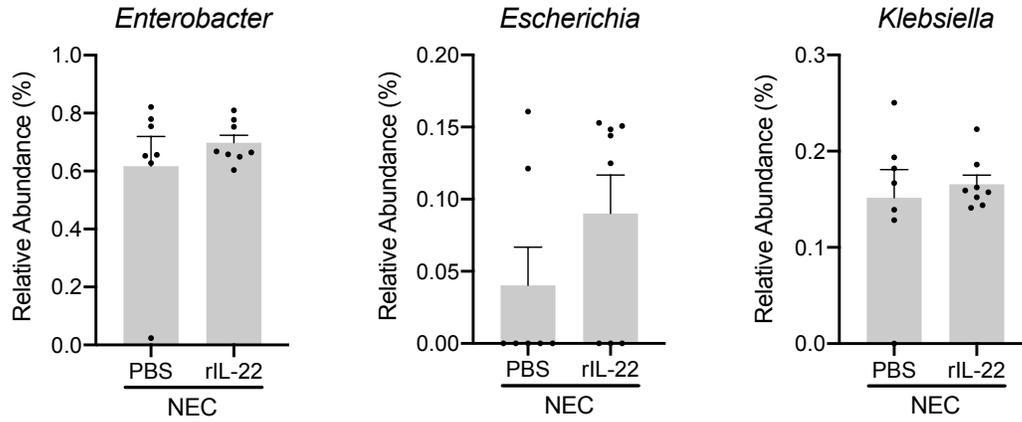
Supplemental Figure 4: Impact of *IL-22* treatment on intestinal epithelial cell tight junctions during NEC. Related to Figure 3. Representative confocal images of ileal tissue samples from wild-type dam fed (DF) pups or pups with NEC treated with either PBS or recombinant IL-22 stained with an antibody against the tight junction Occludin (Green) and Hoechst (Blue). Scale bar 50 μm.

Supplemental Figure 5



Supplemental Figure 5: Reg3g neutralization does not abrogate IL-22-mediated protection against NEC. Related to Figure 5. DF pups and pups subjected to NEC received a Reg3g neutralizing antibody or an isotype control (IgG) via oral gavage. Expression levels of *Il1b* in the terminal ileum displayed as mean \pm SEM were analyzed using Mann-Whitney test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

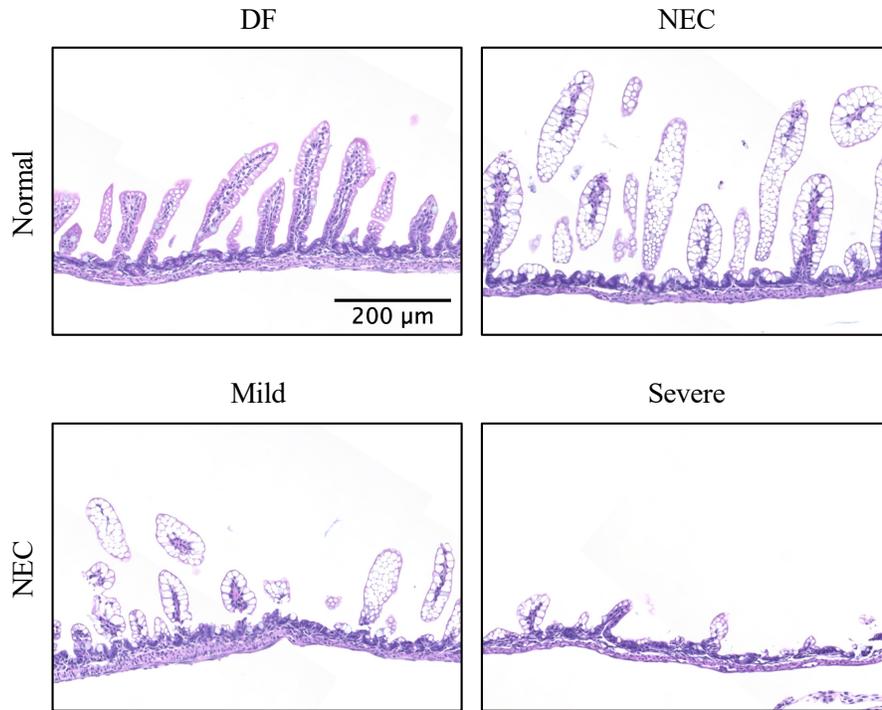
Supplemental Figure 6



Supplemental Figure 6: Impact of IL-22 treatment on the relative abundance of *Enterobacter*, *Escherichia* and *Klebsiella* bacterial species in the feces of pups subjected to experimental NEC.

Related to Figure 5. Data are displayed as mean \pm SEM and were analyzed using Mann-Whitney test.

Supplemental Figure 7



Supplemental Figure 7: Representative histology of the small intestine after the induction of experimental NEC. Related to STAR Methods. The terminal ileum of newborn mice subjected to the experimental NEC protocol displays a phenotype ranging from normal to severe villi destruction. Scale bar 200 μm.