

## Supplementary methods

### *Tissue sampling*

Tissue samples were excised from the intestines of IBD patients at the time of surgery. The part of the tissue used was always the most inflamed region. Healthy samples were obtained from the healthy tissue (at least 7 cm away from neoplastic tissue) of patients undergoing surgery for colon cancer. The healthy parts of the mucosal layer were separated from the rest of the tissue by a pathologist and directly transferred to our laboratory. Transfer was carried out in both cases in HBSS buffer supplemented or not with bacteriostatic antibiotics, only for the time required for tissue transfer. The tissue was kept at 4°C during the transfer. Tissue was obtained from patients who signed an informed consent approved by the institutional review board, allowing material not required for diagnosis to be used for research purposes. The samples were handled in a completely anonymous manner, and they received a serial number by the pathologist for crosschecking histological reference.

### *Bacteria culture*

*Salmonella* serovar *typhimurium*, strain FB62 was grown in 3mL of Luria-Bertani broth and cultured aerobically (in agitation) overnight at 37°C. The following day the culture was restarted (usually at a 1:10 dilution) and the bacteria were harvested and used for stimulation at the exponential growth phase, namely when OD was 0,6 as measured with an Eppendorf biophotometer.

*Lactobacilli* strains (*L. paracasei* B21060, *L. rhamnosus* GG, *L. plantarum* NCIMB8826) were cultured in 3mL of MRS medium anaerobically overnight at 37° C. The following day the culture was restarted as described for *Salmonella* and CFUs and/or supernatant were collected while the bacteria were at the exponential growth phase (OD=0,6).

To assess whether the SN affected *Salmonella* proliferation, we cultured *Salmonella* with or without 5% SN. 30 min and 3 h later bacteria were plated onto LB agar plates and CFU counted after an overnight culture at 37° C.

### *Histochemistry*

The cylinder was carefully detached removing all glue with the help of a scalpel and the explants were fixed immediately after culture in Hollande's fixative (Polysciences Inc.) to preserve the 3D structure of the tissue. They were included in a Leica ASP300 tissue processor and cut in 5-7µm-thick slices. Hematoxylin/eosin staining was performed with a classic protocol. Briefly, the samples were deparaffinized in histolemon, rehydrated in graded alcohol series, stained with Hematoxylin/eosin (Meyer/Merck) and mounted. The samples were visualized under an Olympus BX51 Widefield microscope connected to a Nikon DS-5M camera.

Staining for *Salmonella* and NF-κB was performed following quenching of endogenous peroxidases with 3% H<sub>2</sub>O<sub>2</sub>. Anti-*Salmonella* antibody was purchased from Virostat and anti-p65 from Santa Cruz Inc. HRP-coupled secondary antibodies (anti-rabbit, anti-mouse) and DAB solution were purchased from DAKO. Cy3 conjugated anti-rabbit was from Jackson Immuno. Staining for the mucus with Alcian Blue PAS was performed with a BioGenex kit, according to the provider's instructions.

### *Fluorescent In Situ Hybridization*

Paraffinized tissue sections were processed as described above and FISH performed as described [1]. Briefly, The tissue sections were incubated with 500ng Alexa Fluor 555-conjugated EUB (5-GCTGCCTCCCGTAGGAGT-3) in 50 µl of hybridization buffer [20mMTris HCl (pH 7.4), 0.9M NaCl, 0.1% SDS] at 50°C overnight. The sections were

rinsed in wash buffer [20mM Tris\_HCl (pH 7.4), 0.9 M NaCl], washed at 50°C for 20 min counterstained with DAPI and mounted with Vectashield (Vector laboratories).

#### *Gentamycin protection assays*

To quantify *Salmonella* that invaded the tissue, after 1.5 h of *Salmonella* stimulation with or without 5% SN, tissues were incubated for an additional 1.5 h with medium plus gentamycin (100µg/mL) to kill bacteria that had not entered the tissue. Specifically, approx. 20mm<sup>2</sup> of tissue were stimulated with 10<sup>7</sup> CFUs of *Salmonella*. 0.5% NaDOC was used for tissue lysis with the cylinder still attached. 1/10 of the lysate volume was plated on LB/agar plates and left to grow overnight at 37°C.

Where stated, we pre-incubated *Salmonella* or the tissue with or without 5% SN for 1 h. Then, the bacteria and the tissue were extensively washed before being brought in contact for an additional hour. Gentamycin protection assays were performed as described above.

#### *Cytokine and LDH secretion measurement*

Cytokine quantification (TNF, CCL4, IL-1b and IL-10) in the medium was carried out by Cytometric Bead Array (CBA) assay (Becton-Dickinson) following the manufacturer's instructions. Results in Fig. 2 were obtained after classical ELISA assays (anti-TNF-α was purchased from R&D systems).

LDH was measured using a Promega CytoTox 96 kit. All the aforementioned assays were carried out according to the provider's instructions.

#### *Statistical analysis*

Student's paired t test (Wilcoxon test) or ANOVA, was used to determine the statistical significance of the data. Significance was defined as \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ . Statistic calculations were performed with GraphPad Prism software.

## References

1 Johansson ME, Phillipson M, Petersson J, et al. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci U S A* 2008;105:15064-9.