

1 **SUPPORTING INFORMATION (MATERIAL & METHODS):**

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3 **S1: FISH analysis for bacterial detection in cecal tissue sections**

4 Formalin-fixed paraffin-embedded cecal tissue sections (4 µm) were initially treated  
5 with Roti-Histol (Carl Roth, Karlsruhe, Germany) and then hybridized at 50°C with the  
6 5'-Cy3-labeled *A. muciniphila*-specific probe S-S-MUC-1437-a-A-20 (5'-  
7 CCTTGCGGTTGGCTTCAGAT-3') [1] and at 45°C with the 5'-Cy3 labeled  
8 *Salmonella*-specific probe L-S-Sal-1713-a-A-18 (5'-AATCACTTCACCTACGTG-3')  
9 (MWG Eurofins, Germany) as described previously [2]. After overnight hybridization  
10 sections were counter-stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma-  
11 Aldrich) for visualization of cell nuclei and the slides were analyzed using a Nikon  
12 E600 epifluorescence microscope equipped with appropriate filter set, wavelength  
13 ranging from 330 to 700 nm (NIKON, Germany).

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15 **S2: PAS/AB staining of colonic tissue samples**

16 Carnoy's-fixed colonic tissue (Swiss-rolls) was sectioned at 4 µm and stained with  
17 periodic acid Schiff/Alcian blue (PAS/AB) at both pH 2.5 and 1 (at pH 2.5 acidic  
18 mucins are stained blue and neutral mucins are stained magenta, at pH 1: highly  
19 sulphated mucins are stained blue). Mucus layer thickness was measured on the  
20 colonic tissue sections. Approximately 30 crypts per section were analyzed using an  
21 Eclipse E600 microscope (NIKON, Germany) with Lucia G version 4.51 software for  
22 Windows 7 (Laboratory imaging s.r.o.). Images were captured with a MV-1500 digital  
23 camera (NIKON, Düsseldorf Germany).

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- 27 1. Derrien M, Van Baarlen P, Hooiveld G, Norin E, Muller M, et al. (2011) Modulation  
28 of Mucosal Immune Response, Tolerance, and Proliferation in Mice Colonized  
29 by the Mucin-Degrader *Akkermansia muciniphila*. *Front Microbiol* 2: 166.
- 30 2. Nordentoft S, Christensen H, Wegener HC (1997) Evaluation of a fluorescence-  
31 labelled oligonucleotide tide probe targeting 23S rRNA for in situ detection of  
32 *Salmonella* serovars in paraffin-embedded tissue sections and their rapid  
33 identification in bacterial smears. *Journal of Clinical Microbiology* 35: 2642-  
34 2648.
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