



HAL
open science

Propionibacterium freudenreichii CIRM-BIA 129 mitigates colitis through S layer protein B-dependent epithelial strengthening. P. freudenreichii inhibits inflammation-induced epithelial break-down

Marine Mantel, Tony Durand, Anne Bessard, Ségolène Pernet, Julie Beaudeau, Juliana Guimaraes-Laguna, Marie-Bernadette Maillard, Eric Guédon, Michel Neunlist, Yves Le Loir, et al.

► To cite this version:

Marine Mantel, Tony Durand, Anne Bessard, Ségolène Pernet, Julie Beaudeau, et al.. Propionibacterium freudenreichii CIRM-BIA 129 mitigates colitis through S layer protein B-dependent epithelial strengthening. P. freudenreichii inhibits inflammation-induced epithelial break-down. American Journal of Physiology-Gastrointestinal and Liver Physiology, 2024, 326 (2), pp.G163-G175. 10.1152/ajpgi.00198.2023 . hal-04322697

HAL Id: hal-04322697

<https://hal.inrae.fr/hal-04322697v1>

Submitted on 5 Dec 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 ***Propionibacterium freudenreichii* CIRM-BIA 129 mitigates colitis through S layer**
2 **protein B-dependent epithelial strengthening.**

3 *P. freudenreichii* inhibits inflammation-induced epithelial break-down.

4 Marine Mantel^{1,2}, Tony Durand¹, Anne Bessard¹, Ségolène Pernet¹, Julie Beaudeau^{1,3}, Juliana
5 Guimaraes-Laguna², Marie-Bernadette Maillard², Eric Guédon², Michel Neunlist¹, Yves Le
6 Loir², Gwénaél Jan^{†2} and Malvyne Rolli-Derkinderen*^{†1}

7 **Affiliations:**

8 1. nantes université, inserm, the enteric nervous system in gut and brain disorders, imad, f-
9 44000 nantes, france

10 2. inrae, umr 1253, stlo, l'institut agro, f-35000 rennes, france

11 3. CRNH-OUEST

12 † these authors shared senior authorship

13 **Corresponding author:** malvyne.rolli-derkinderen@univ-nantes.fr

14
15 **ABSTRACT**

16 The growing incidence of human diseases involving inflammation and increased gut
17 permeability makes the quest for protective functional foods more crucial than ever.
18 *Propionibacterium freudenreichii* (*P. freudenreichii*) is a beneficial bacterium used in the
19 dairy and probiotic industries. Selected strains exert anti-inflammatory effects, and the present
20 work addresses whether the *P. freudenreichii* CIRM-BIA129, consumed daily in a preventive
21 way, could protect mice from acute colitis induced by dextran sodium sulfate (DSS), and
22 more precisely whether it could protect from intestinal epithelial breakdown induced by
23 inflammation.

24 *P. freudenreichii* CIRM-BIA129 mitigated colitis severity and inhibited DSS-induced
25 permeability. It limited crypt length reduction and promoted the expression of Zonula
26 Occludens-1 (ZO-1), without reducing *interleukin-1beta* mRNA (*il-1β*) expression. *In vitro*,
27 *P. freudenreichii* CIRM-BIA129 prevented the disruption of a Caco-2 monolayer induced by
28 pro-inflammatory cytokines. It increased transepithelial electrical resistance (TEER) and
29 inhibited permeability induced by inflammation, along with an increased ZO-1 expression.
30 Extracellular vesicles (EVs) from *P. freudenreichii* CIRM-BIA129, carrying the surface layer
31 protein (SlpB), reproduced the protective effect of *P. freudenreichii* CIRM-BIA129. A mutant
32 strain deleted for *slpB* (Δ slpB), or EVs from this mutant strain, had lost their protective
33 effects, and worsened both DSS-induced colitis and inflammation *in vivo*.

34 These results shown that *P. freudenreichii* CIRM-BIA129 daily consumption has the
35 potential to greatly alleviate colitis symptoms and, particularly, to counter intestinal
36 epithelial permeability induced by inflammation by restoring ZO-1 expression through
37 mechanisms involving S-layer protein B. They open new avenues for the use of probiotic
38 dairy propionibacteria and/or postbiotic fractions thereof, in the context of gut permeability.

39
40 **NEW :**

41 1. *P. freudenreichii* reduces DSS-induced intestinal permeability *in vivo*

42 2. *P. freudenreichii* does not inhibit inflammation but damages linked to inflammation

43 3. *P. freudenreichii* inhibits intestinal epithelial breakdown through S-layer protein B

44 **NOTEWORTHY :**

45 1. The protective effects of *P. freudenreichii* depends on S-layer protein B

46 2. Extracellular vesicles from *P. freudenreichii* CB 129 mimic the protective effect of the
47 probiotic

48

49 **KEYWORDS**

50 probiotic, inflammation, permeability, intestinal epithelium, zonula occludens-1

51

52 **1. Introduction**

53 Inflammatory bowel diseases (IBD) constitute a heterogeneous group of chronic
54 inflammatory diseases including ulcerative colitis (UC) and Crohn's disease (CD), with a
55 growing prevalence worldwide (Alatab et al. 2020). It involves immune response
56 dysregulations, alterations of the enteric nervous system, gut microbiota dysbiosis, and
57 intestinal epithelial barrier dysfunctions (Lê et al. 2022). Indeed, IBD patients exhibit aberrant
58 immune responses such as infiltration of CD4⁺ T lymphocytes in the intestine, associated
59 with a reduction in regulatory T (Treg) cells (Tindemans et al. 2020). The expression of
60 enteric neuronal and glial markers is altered (Prigent et al. 2019; 2020; Le Berre et al. 2023)
61 and glial cells have lost their functions in patients with CD (Pochard et al. 2016; Coquenlorge
62 et al. 2016). In addition, a dysbiosis of the gut microbiota, characterized by a reduced
63 bacterial diversity and alterations in the relative abundance of certain species were observed
64 (Lê et al. 2022). At last, the intestinal epithelial barrier (IEB), serving as the first line of
65 defense against the external environment, is failing. Indeed, there is increasing recognition of
66 an association between disrupted IEB function and the development of inflammatory diseases.
67 An increase in intestinal permeability occurs in IBD patients (Peeters et al. 1994; Pearson et
68 al. 1982) and first-degree relatives of IBD patients (Munkholm et al. 1994). Increased
69 permeability is often observed prior to relapse of CD (Wyatt et al. 1993). It is an aggravating
70 factor in IBD, and the severity of symptoms is positively correlated with its extent (Chang et
71 al. 2017). If increased permeability precedes inflammation (Arrieta et al. 2006), during
72 inflammation, further barrier dysfunction can be induced by cytokines such as interferon- γ
73 (IFN- γ) and tumor necrosis factor- α (TNF- α). This process is accompanied by changes in
74 expression and/or redistribution of tight junction proteins such as the Zonula Occludens-1
75 (ZO-1), the occludin or the Claudin-2 (Turner et al. 2009). Interestingly, reducing barrier
76 permeability prevents the development of inflammation in a genetic model of IBD (Arrieta et
77 al. 2006). The strengthening or the re-establishment of IEB functions could therefore be
78 interesting targets for approaches aiming at both prevention of relapses, and treatment of IEB-
79 associated IBD dysfunctions.

80 Currently, the mainstays of IBD treatment are immunosuppressive and immune-
81 modulating agents, and even protective functional foods are tested and developed for their
82 anti-inflammatory properties (Lê et al. 2022). Beneficial bacteria, either ingested or members
83 of the gut microbiota, may modulate gut inflammation through immunomodulation,
84 engagement of an immune response and modulation of cytokines and of T-cells proliferation
85 (Illikoud et al. 2022). Meta-analysis of clinical trials revealed the efficacy of selected
86 probiotic strains, such as those constituting the VSL#3 product, in the context of IBD, while
87 other strains failed to do so (Sniffen et al. 2018). In this context, promising
88 immunomodulatory properties were reported for *Propionibacterium freudenreichii*
89 (*P. freudenreichii*), an actinobacterium found in Swiss-type cheese, which holds a Generally
90 Recognized as Safe (GRAS) status in the United States, as well as a Qualified Presumption of
91 Safety (QPS) status in Europe. *P. freudenreichii* was shown to induce the modulatory
92 cytokine IL-10 in human peripheral blood mononuclear cells, to mitigate the severity of
93 TNBS-induced colitis and to counteract colonization of mice digestive tract by the rodent
94 pathogen *Citrobacter rodentium* (Foligné et al. 2010). This property is highly *P.*
95 *freudenreichii* strain-specific (Foligné et al. 2013), and depends on the presence of key
96 surface layer proteins (Le Marechal et al. 2015), including SlpB, a main microbe-associated
97 molecular patterns (MAMPs) involved in immunomodulation (Deutsch et al. 2017). In line
98 with this, expression of *P. freudenreichii* *slpB* gene in *Lactococcus lactis* enhanced its ability

99 to mitigate DSS-induced colitis in mice, further indicating the key role of the SlpB protein
100 (Belo et al. 2021). More recently, *P. freudenreichii* was shown to prevent the inflammatory
101 damages caused by the cancer chemotherapy drug 5-fluorouracyl, while the *slpB* mutant
102 failed to do so. In this mucositis model, the wild-type *P. freudenreichii* reduced the dramatic
103 increase in gut permeability which was induced by 5-fluorouracyl (do Carmo et al. 2019).
104 SlpB is also involved in *P. freudenreichii* adhesion to intestinal HT-29 epithelial cells (do
105 Carmo et al. 2017) suggesting that *P. freudenreichii* could exert a protective effect towards
106 the gut epithelial barrier.

107 We thus investigated whether *P. freudenreichii* could mitigate mice colitis and in
108 particular protect the intestinal epithelial barrier from damages induced by inflammation. We
109 paid special attention to its protective effect towards tight junctions, and have analysed the
110 involvement of the *P. freudenreichii* surface layer protein SlpB.
111

112 2. Materials and methods

113

114 Growth of dairy propionibacteria

115 The strain *Propionibacterium freudenreichii* CIRM-BIA 129 (*P. freudenreichii* CIRM-
116 BIA129), was initially isolated from a Swiss-type cheese. It was provided by CNIEL (Centre
117 National Interprofessionnel de l'Economie Laitière) and maintained by the CIRM-BIA
118 microbiological resource center (Centre International de Ressources Microbiennes, Bactéries
119 d'Intérêt Alimentaire, Rennes, France). Starting from a frozen stock, precultures were grown
120 in liquid Yeast Extract Lactate (YEL) medium containing 0.1 M sodium DL-lactate (Sigma
121 Aldrich, Saint Louis, USA) and 10 g/L yeast extract as described (Malik et al. 1968).
122 *P. freudenreichii* CIRM-BIA129 was then grown at 30°C for 60 hours in milk ultra-filtrate
123 (MUF) supplemented with 5 g/L casein peptone (casein peptone plus, Organotechnie, La
124 Courneuve, France), 0.1M sodium DL-lactate filter-sterilized using a 0.2 µm NalgeneTop
125 filter (Sigma-Aldrich, St. Louis, MO, USA). Propionibacteria were grown in agar-solidified
126 media in anaerobic jars containing ATCO Biocult anaerobiosis generators (Laboratoires
127 Standa, Cean, France). They were grown in liquid media in microaerophilic conditions in
128 glass tubes with screw cap (2 thirds of volume occupied by liquid medium, one third by
129 headspace air), at 30°C, without agitation. Regarding the knock-out strain *P. freudenreichii*
130 CIRM-BIA129Δ*slpB*, YEL and MUF culture media were supplemented with
131 chloramphenicol (10 µg/mL) as previously detailed in (do Carmo et al. 2018). For Caco-2
132 cells stimulation (see below), propionibacteria were centrifuged (10,000 x g, 10 min, 20°C),
133 and resuspended in DMEM medium prior to addition to cell cultures.
134

134

135 DSS-induced colitis, *in vivo* DAI and permeability assessment

136 Eight-week-old C57Bl/6NRj male mice from Janvier Labs (Le Genest St Isle, France) were
137 housed in a ventilated cage system under a 12-hours light-dark cycle and ad libitum access to
138 food (SAFE A04) and drinking water. The experiments were carried out in strict accordance
139 with the European Communities Council Directives 2010/63/UE on the use of laboratory
140 animal. Mice were randomly divided into 4 groups of 14 animals: MUF, 129, DSS and
141 129DSS. Mice were gavaged daily either with 200 µL of MUF (MUF and DSS groups) or
142 200 µL of MUF fermented with *P. freudenreichii* CIRM-BIA129 at 1.10⁸ CFU (129 and
143 129DSS groups) for 11 days. The last 4 days, colitis was induced by 4% (w/v) of Dextran
144 Sulfate Sodium (DSS, colitis grade 36,000-50,000, MP Biomedicals, Eschwege, Germany) in
145 drinking water renewed every day for the groups DSS and 129DSS. Animals were weighted
146 daily and at the end of the protocol, mice received (5 µL/g of mouse) a solution comprising 30
147 mg/ml carmine red (cochineal carmine, Prolabo #22259), 10 mg/mL fluorescein-5,6-sulfonic
148 acid (FSA, fluorescein-5-(and-6)-sulfonic acid, trisodium, F1130, In vitrogen), 100 mg

149 Dextran TRITC (TRITC-dextran 4kDa, TdB Labs), and 10 mg/mL horseradish peroxidase
150 (HRP; Peroxidase from horseradish, P8250, Sigma-Aldrich) resuspended in 0.5%
151 carboxymethylcellulose in PBS (Sigma-Aldrich) *via* gavage, and then placed in individual
152 cages. The Disease Activity Index (DAI) was determined as indicated in **Table 1**, according
153 to the 3 main clinical symptoms of colitis: diarrhea, rectal bleeding, and weight loss,
154 following a scoring previously described (Cooper et al. 1993). Four hours after gavage, blood
155 was collected from the tail vein and permeability was evaluated in 5 μ L of plasma.
156 Paracellular permeabilities were evaluated *via* titration of FSA and TRITC-dextran
157 fluorescence intensity measured at 487 nm and 520 nm, respectively, using an automatic
158 microplate reader (BioTek Synergy H1, microplate reader). Transcellular permeability to
159 HRP was measured using an enzymatic activity assay with 3,3',5,5'-tetramethylbenzidine
160 reagent (TMB Substrate Reagent Set, 555214, BD Biosciences). Mice were killed at the end
161 of the protocol by cervical dislocation. Colonic tissues were collected and snap frozen for
162 further quantification of gene transcripts and of proteins, or were also fixed for 24 hours in
163 4% paraformaldehyde for histopathological analysis. This procedure was repeated 3 times
164 independently.

165

166 **Histopathological score**

167 After fixations, distal colons were dehydrated and embedded in paraffin. Sections of 5 μ m
168 were stained with haematoxylin–phloxine–safron (HPS) or Alcian Blue (MicroPICell
169 platform, Nantes, France) for histopathological analysis. Images were taken using a slide
170 scanner (Nanozoomer; Hamamatsu). As previously described, distal colonic tissue damage
171 was evaluated by two investigators in a blinded manner through the calculation of a micro
172 disease activity index (mDAI) which reflects the destruction of mucosal architecture, the
173 cellular infiltration, muscle thickening and goblet cells depletion (Pochard et al. 2021). The
174 scoring for the destruction of mucosal architecture was as follows: 0-3 (0: none, 1: 1/3 basal,
175 2: 2/3 basal, and 3: loss of crypt and epithelium). The extent of cellular infiltration was rated
176 on a scale of 0-3 (0: none, 1: infiltrate around crypt basis, 2: extensive infiltration reaching the
177 muscularis mucosae, and 3: infiltration of the submucosa). The degree of muscle thickening
178 was also ranging from 0 to 3, when the thickening was none, mild, moderate, or extensive
179 thickening, respectively. The loss of goblet cell depletion was scored as 0 (normal presence)
180 or 1 (massive depletion). A multiplication factor of 1–4 was applied for each measured
181 criteria, depending on the extent of affected area (25, 50, 75, or 100%) of the considered
182 sample. Crypt length was considered by measuring the length of 50 opened crypts/animal.

183

184 **Lipocalin-2 enzyme-linked immunosorbent assay faecal quantification**

185 The feces of each mouse were resuspended in a solution of PBS-Tween 20 0.1% protease
186 inhibitor cocktail (1/2 tablet for 25 mL solution, cOmplete; Roche, France) to reach 100 mg
187 feces/mL. Lcn-2 in the faecal solution was measured using an enzyme-linked immunosorbent
188 assay (ELISA) kit (Mouse Lcn-2/NGAL DuoSet ELISA; Bio-Techne), according to the
189 manufacturer's protocol.

190

191 **Caco-2 culture**

192 The human IEC line Caco-2 was obtained from American Type Culture Collection
193 (Manassas, VA) and cultured in Dulbecco modified Eagle medium containing 4.5 g/L glucose
194 (Gibco, Life Technologies, Carlsbad, CA) supplemented with 10% heat-inactivated fetal calf
195 serum, 2 mmol/L glutamine, 100 IU/mL penicillin, and 100 μ g/mL streptomycin. For
196 treatments, transepithelial electrical resistance (TEER) and permeability experiments, 116.071
197 cells/cm² were seeded onto Transwell Permeable Supports (Costar, REF 3460) and grown for
198 21 days.

199

200 **Guanidine extraction and purification of surface layer proteins**

201 Propionibacteria were cultivated in MUF as described above and harvested in late stationary
202 phase, after 72 hours of culture. They were then harvested by centrifugation (10,000 x g, 10
203 min, 20°C), washed in PBS and resuspended in 5 M guanidine hydrochloride (20% of the
204 initial culture volume). The bacterial suspension was incubated 15 min at 50°C prior to
205 centrifugation (20,000 x g, 20 min, 20°C). The supernatant was then extensively dialyzed
206 against distilled water (20 L) and then against PBS (5 L) using a Slide-A-Lyzer Dialysis
207 Cassette (Thermo Scientific) with a 10 kDa Cut-Off.

208

209 **Purification of EVs and Nanoparticle Tracking Analysis for EV size and concentration** 210 **determination.**

211 To extract and purify extracellular vesicles derived from *P. freudenreichii* CIRM-BIA129 and
212 from its $\Delta slpB$ isogenic mutant (*P. freudenreichii* CIRM-BIA129 $\Delta slpB$), the bacteria were
213 pelleted by centrifugation (6,000 x g, 20 min, room temperature) of a 1 L culture and the
214 supernatant fraction was filtered using a Nalgene 0.22 μm top filter (Thermo Scientific) to
215 remove bacteria. Then, the supernatant was concentrated 1,000 times using Amicon
216 ultrafiltration units with 100 kDa cut-off point through successive centrifugations at 2,500 g x
217 g. The concentrated suspension of EVs was recovered in 500 μL of TBS buffer (Tris-Buffered
218 Saline, 150 mM NaCl; 50 mM Tris-HCl, pH 7.5) prior to purification by size exclusion
219 chromatography. Qevoriginal columns (qEV original 70 nm; iZON) were equilibrated with
220 TBS and used according to the manufacturer's recommendations (Böing et al., 2014). The
221 concentrated EV suspension (500 μL) was applied to the top of the chromatographic column
222 and allowed to run into the column. 10 mL of TBS were then added on top of the column,
223 prior to elution. Then, fractions of 500 μL were recovered in separate tubes. This
224 chromatography step was conducted as previously described (Rodvalho et al. 2020) and
225 EVs were reproducibly detected by NTA (see below) and by protein quantification in
226 fractions 7 to 9. Finally, EVs-containing fractions (fractions 7-9) were pooled together, and
227 the remaining fractions were discarded due to protein contamination or low EV content. To
228 measure the size and concentration of EVs, nanoparticle tracking analysis (NTA) was
229 performed at 25.0°C using a NanoSight NS300 instrument (Malvern Panalytical) with a
230 CMOS camera and a Blue488 laser (Mehdiani et al., 2015). Samples were diluted to reach a
231 concentration in the range 10^7 to 10^8 particles/mL, giving rise to 20 to 110 particles per frame.
232 Samples were applied at 25°C in constant flux with a syringe pump speed of 50 $\mu\text{L}/\text{s}$. For
233 each measurement, 5 x 60-s videos were recorded with camera level 15. Other parameters
234 were adjusted accordingly to achieve image optimization.

235

236 **Caco-2 treatments, TEER, and Permeability Measurement *In Vitro***

237 To determine the effect of propionibacteria, sodium-pyruvate (#11360070
238 ThermoFisherScientific), propionibacteria'supernatant, Slp, (enriched extracts of S-layer
239 proteins), EVs or inflammation on intestinal epithelium, the TEER was measured before and
240 after treatments with an epithelial voltohmmeter (EVOM; World Precision Instruments, Inc,
241 Sarasota, FL). Caco-2 cells were stimulated for 24 hours with either *P. freudenreichii* CIRM-
242 BIA129 or *P. freudenreichii* CIRM-BIA129 $\Delta slpB$ (MOI 1:100), or S-layer protein enriched
243 extracts (10-200 $\mu\text{g}/\text{mL}$), and EVs from *P. freudenreichii* CIRM-BIA129 or *P. freudenreichii*
244 CIRM-BIA129 $\Delta slpB$ at a concentration of 10^7 particles/mL in DMEM medium (0% FBS, 1%
245 P/S) in the apical compartment or sodium-pyruvate (0.1-10 mM) in DMEM medium
246 (10%FBS, 1% P/S) in the basolateral compartment. After 24 hours, an inflammatory
247 stimulation was induced with a cytomix containing 50 ng/mL of TNF- α (human TNF- α , 50
248 μg , 130-094-017, Milteny Biotec) and 500 ng/mL of IFN- γ (human IFN-g1b, 1,000 μg , 130-

249 096-486, Milteny Biotec) in 0% FBS in the apical compartment as well as in 10% FBS in the
250 basolateral compartment, for 6 days with a renewal on day 3. To determine the effect of these
251 treatment on permeability, 50 μ L of the apical medium was replaced by 50 μ L of 1 mg/mL of
252 sulfonic acid FITC, 6 mg/mL of dextran 4 kDa TRITC and 3 mg/mL of HRP. The
253 fluorescence level of basolateral aliquots (150 μ L) was measured every 30 min for a period of
254 180 min using a fluorimeter (BioTek Synergy H1, microplate reader). Paracellular
255 permeability was determined by averaging the slope change in fluorescence intensity over
256 time by linear regression fit model. To assess transcellular permeability to HRP (Peroxidase
257 from horseradish, P8250, Sigma-Aldrich), an enzymatic activity assay with TMB (TMB
258 Substrate Reagent Set, 555214, BD Biosciences, France) was performed.

259

260 **mRNA and protein extractions**

261 Proximal colons from mice were lysed in RA1 buffer (Macherey-Nagel) with the Precellys 24
262 tissue homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France), and total RNA
263 and protein extraction was performed with a Nucleospin RNAII kit according to the
264 manufacturer's recommendations. For Caco-2 filters, cells were lysed in ice-cold RIPA Lysis
265 buffer (20-188, Merk) complete with protease inhibitor (66373700, Merk), a phosphatase
266 inhibitor cocktail (P0044, Sigma) and sodium orthovanadate (S6508, Sigma).

267

268 **Quantitative real-time PCR analysis**

269 One microgram of purified mRNA was denatured and processed for reverse transcription
270 using Superscript III reverse transcriptase (Life Technologies). qPCR amplifications were
271 performed using FastSYBR Green Master Mix kit (Applied Biosystems, Foster City, CA,
272 USA) and run on a StepOnePlus system (Life Technologies). The primers used are listed in
273 **TABLE S2** (DOI 10.6084/m9.figshare.24542629). Ribosomal protein S6 (RPS6) transcript
274 was used as a reference gene. The relative transcript level of the gene of interest was
275 measured by the $2^{-\Delta\Delta C_t}$ method.

276

277 **Western Blot**

278 For proximal colon, proteins were resuspended in 1:1 protein solving buffer and tris(2-
279 carboxyethyl) phosphine-reducing agent (PSB-TCEP, Macherey-Nagel), sonicated and
280 denatured for 5 min at 95°C. For Caco-2 filters, cells were washed with ice-cold PBS and
281 lysed in ice-cold 1X RIPA buffer complete with protease inhibitor (Roche, France) and
282 serine-threonine phosphatase inhibitor (Sigma-Aldrich) cocktails and completed with
283 NuPAGE™ LDS Sample Buffer 4X (NP0008, Invitrogen) and NuPAGE™ Sample reducing
284 agent 10X (NP0009, Invitrogen), sonicated and denatured for 10 min at 70°C. Fifteen
285 micrograms of proteins were separated using NuPAGE™ 4-12% Bis-Tris or NuPAGE™ 3-
286 8% Tris-Acetate gels (Life Technologies, Villebon sur Yvette, France) and transferred to
287 nitrocellulose membranes (Life Technologies, Villebon sur Yvette, France). After blocking
288 with Tris-buffered saline/0.1% Tween-20/5% nonfat dry milk for 30 min, blots were
289 incubated overnight at 4°C with the primary antibodies (**Table S1**, DOI
290 10.6084/m9.figshare.24542629) diluted in Tris-buffered saline/5% nonfat dry milk.

291 Immunoblots were probed with HRP-conjugated anti-rabbit (Life technologies, 1:5,000) or
292 anti-mouse secondary antibodies (Sigma, 1:5,000) and visualized by chemiluminescence
293 (Clarity Western ECL Substrate, 170-5061, BioRad or Femto Maximum Sensitivity Substrate,
294 34095, Thermofisher) using a ChemiDoc MP Imaging System (Bio-Rad). Western Blot data
295 are expressed as relative values to β -actine and normalized to the control mean.

296

297 **Organic acid quantification**

298 To identify whether soluble mediators are responsible for the *P. freudenreichii* CIRM-
299 BIA129 effects dependent on the SlpB protein on the Caco-2 barrier, organic acids present in
300 the apical and basolateral culture media at the end of the IEB-disruption protocol were
301 analysed by High Performance Liquid Chromatography UltiMate 3000 (HPLC, Thermofisher,
302 Les Ulis, France). Culture media were diluted 2-fold in 0.005 M H₂SO₄, prior to
303 centrifugation (7500 × g for 15 min at 4 °C), and filtration (chromafil Xtra PVDF 45/13,
304 Macherey Nagel, Düren, Germany). HPLC analysis was then conducted using a Rezek ROA
305 organic acid H + column (300*7.8 mm, Phenomenex, California), with 0.005 M NH₂SO₄ as
306 mobile phase, and a flow rate of 0.4 mL/min at 60°C. A UV detector (DIONEX-UV 170U,
307 Sunnyvale, California) operating at 210 nm, as well as a refractometer (RI 2103 Plus Jasco,
308 Tokyo, Japan) were used. External calibration was used for quantification. Standards of lactic,
309 citric, propionic, butyric, succinic, and pyruvic acids were purchased from Merck (St. Quentin
310 Fallavier, France, and acetic acid from PanReac, Lyon, France).

311

312 **Statistical analysis**

313 Results were expressed as mean +/- SEM. Outliers were excluded using Grubbs' tests.
314 Statistical significance was analyzed by an ordinary two-way ANOVA, followed by a
315 Tukey's multiple comparisons test, with a single pooled variance or a Kruskal-Wallis test,
316 followed by a Dunn's multiple comparisons test (GraphPad Prism 9). Differences between
317 experimental groups were considered significant at *p<=0.05, ** p<=0.01, *** p<=0.001 or
318 p<=0.0001.

319

320 **3. Results**

321

322 ***P. freudenreichii* CIRM-BIA129 reduces DSS-induced colitis severity and intestinal 323 permeability *in vivo*.**

324 For *in vivo* assessment of *P. freudenreichii* CIRM-BIA129 ability to prevent colitis, acute
325 colitis was induced by the addition of 4% DSS for 4 days to the drinking water of 8–10-
326 weeks-old male supplemented, or not, with the *P. freudenreichii* CIRM-BIA129 for 11 days
327 (Figure 1A). The colitis was characterized by an increased disease activity index (DAI) in the
328 DSS as well as in the 129DSS groups compared to the MUF group (Figure 1B), but
329 *P. freudenreichii* CIRM-BIA129 supplementation (129DSS group) significantly reduced the
330 DAI score by about 50% compared to the DSS group (Figure 1B). The DAI was composed of
331 three parameters: the bleeding and the stool consistency scores, and the weight change.
332 *P. freudenreichii* CIRM-BIA129 supplementation inhibited DSS-induced increase in bleeding
333 and stool consistency scores, but did not change the weight loss (Suppl Fig 1 ; DOI
334 10.6084/m9.figshare.24542629). The paracellular permeability measured *in vivo* by sulfonic
335 acid (0.4 kDa) passage in mice plasma was also increased in the DSS and in the 129DSS
336 groups compared to the MUF group, but again significantly less in the 129DSS group than in
337 the DSS group (Figure 1C). The transcellular permeabilities measured *in vivo* by HRP (44
338 kDa) in mice plasma tended to be increased in the DSS group (p-value=0.0653) and was
339 significantly lower in the 129DSS compared to the DSS group (Figure 1D). The
340 histopathological score, reflecting the remodeling of the distal colon (cellular infiltration,
341 muscle thickening, epithelial destruction), was significantly increased in the DSS as well as in
342 the 129DSS groups compared to the MUF group (Figures 1E and F). The crypt length was
343 reduced in the DSS group but not when mice were supplemented with the *P. freudenreichii*
344 CIRM-BIA129 (Figure 1G). Under basal conditions, *P. freudenreichii* CIRM-BIA129
345 supplementation has had no impact on the measured parameters (Figures 1B-G). Altogether,
346 these results show that *P. freudenreichii* CIRM-BIA129 is safe and has the potential to
347 alleviate the severity of colitis and to maintain intestinal barrier function such as permeability.

348

349 ***P. freudenreichii* CIRM-BIA 129 promotes ZO-1 protein remodeling without reducing**
350 **inflammation.**

351 To investigate in more details whether *P. freudenreichii* CIRM-BIA129 supplementation
352 impacted the intestinal epithelium and/or inflammatory processes, the amount of tight
353 junctions (TJ) proteins, as well as of inflammatory cytokines, were evaluated in the mice
354 proximal colons and the release of a marker of intestinal injury (fecal concentration of Lcn-2
355 for neutrophil gelatinase-associated Lcn) was measured in stools. Western-blot analyses have
356 shown that *P. freudenreichii* CIRM-BIA129 supplementation significantly increased the
357 expression of the Zonula Occludens-1 (ZO-1) in the absence of colitis and through a tendency
358 under DSS-induced colitis conditions with a noticeable variability within the samples (Figure
359 2A, 2B). Neither DSS nor 129 has modified the expression of occludin, another TJ protein
360 (Figure 2A, 2C). Regarding the expressions of genes encoding pro- and anti-inflammatory
361 cytokines, DSS treatment significantly up-regulated the level of *il-1 β* transcripts and tended to
362 decrease *il-10* ones (Figure 2D, 2F), but had no impact on *tnf- α* transcripts (Figure 2E). Fecal
363 Lcn-2 concentration was increased in the DSS and 129DSS groups compared to the MUF
364 group (Figure 2G). *P. freudenreichii* CIRM-BIA129 supplementation did not modify these
365 markers, neither in control, nor in DSS condition (Figures 2D, 2E, 2F, 2G). Overall, these
366 findings support the hypothesis that *P. freudenreichii* CIRM-BIA129 mitigates the severity of
367 DSS-induced colitis through its action on IEB, and in particular ZO-1 expression, rather than
368 on inflammation.

369

370 ***P. freudenreichii* CIRM-BIA 129 inhibits inflammation-induced IEB breakdown**
371 **through S-layer protein B induction of ZO-1 expression.**

372 To analyse more specifically the regulation of intestinal epithelium permeability by the
373 propionibacteria, an *in vitro* IEB model was challenged with inflammation with or without
374 pre-treatment with the *P. freudenreichii* CIRM-BIA129 (Figure 3A). As *P. freudenreichii*
375 CIRM-BIA129 anti-inflammatory effects (do Carmo et al. 2019) and its adhesion to HT29
376 colonic cells (do Carmo et al. 2017) involved the S-layer protein B (SlpB), an isogenic mutant
377 deleted for the *slpB* gene (*P. freudenreichii* CIRM-BIA129 Δ *slpB*) was also tested in this
378 protocol. After 24 hours of incubation with the bacteria and a full culture medium
379 replacement, *P. freudenreichii* CIRM-BIA129 adhesion to Caco-2 monolayer was observed
380 using Gram staining (Figure 3B). This adhesion was greatly reduced when using the *P.*
381 *freudenreichii* CIRM-BIA129 Δ *slpB* (Figure 3B). No living bacteria have been observed at
382 the end of the protocol (data not shown). Without inflammation, *P. freudenreichii* CIRM-
383 BIA129 significantly lowered TEER compared to untreated cells, but has no impact on
384 permeabilities (Figures 3C, 3D, 3E). After 6 days, inflammation significantly reduced the
385 TEER (Figure 3C), increased the paracellular permeability to sulfonic acid-FITC (Figure 3D)
386 and increased the transcellular permeability to HRP (Figure 3E). Pre-incubation with the *P.*
387 *freudenreichii* CIRM-BIA129 prevented these three changes (Figures 3C, 3D, 3E). In
388 contrast, *P. freudenreichii* CIRM-BIA129 Δ *slpB* failed to inhibit these inflammation-induced
389 IEB failures (Figures 3C, 3D, 3E), and even increased the permeabilities induced by
390 inflammation (Figures 3D, 3E). To investigate the molecular mechanisms sustaining these
391 effects, the expressions of TJ proteins such as ZO-1 and occludin were analysed by western-
392 blot. Inflammation significantly reduced both ZO-1 and occludin protein expression, while *P.*
393 *freudenreichii* CIRM-BIA129 prevented the decrease of ZO-1 expression induced by
394 inflammation but had no effect on occludin expression (Figures 3F, 3G, 3H). Conversely, *P.*
395 *freudenreichii* CIRM-BIA129 Δ *slpB* was unsuccessful in restoring ZO-1 expression (Figures
396 3F, 3G). Taken together, these data provide evidence that *P. freudenreichii* CIRM-BIA129

397 inhibits IEB breakdown induced by inflammation by maintaining ZO-1 expression in a SlpB
398 dependent manner.

399

400 **Organic acid productions do not explain the *P. freudenreichii* protective effect on**
401 **inflammation-induced IEB breakdown.**

402 To assess whether soluble factors such as short chain fatty acids could be involved in SlpB-
403 dependent effect of *P. freudenreichii* CIRM-BIA129 upon intestinal epithelium, organic acid
404 concentrations were measured in the apical (Figure 4A) and basolateral (Figure 4B)
405 compartments of our *in vitro* model of inflammation-induced IEB disruption. Acetate and
406 propionate concentrations are increased by *P. freudenreichii* CIRM-BIA129 $\Delta slpB$ but not *P.*
407 *freudenreichii* CIRM-BIA129 in apical and basolateral compartments without impact of
408 inflammation (Figures 4A and 4B). Glucose concentrations were decreased by *P.*
409 *freudenreichii* CIRM-BIA129, *P. freudenreichii* CIRM-BIA129 $\Delta slpB$ or inflammation in
410 apical and basolateral compartments (Figure 4A and 4B). Inflammation-induced lactate
411 concentrations are inhibited by *P. freudenreichii* CIRM-BIA129 $\Delta slpB$ in apical and
412 basolateral compartments (Figures 4A and 4B). Inflammation-induced decreased 2-
413 pyrrolidone-5-carboxylic acid concentration is decreased by *P. freudenreichii* CIRM-BIA129
414 or *P. freudenreichii* CIRM-BIA129 $\Delta slpB$ in apical compartment and increased by *P.*
415 *freudenreichii* CIRM-BIA129 $\Delta slpB$ in the basolateral compartments (Figures 4A and 4B).
416 Pyruvate concentration in the apical compartment was increased by *P. freudenreichii* CIRM-
417 BIA129 or *P. freudenreichii* CIRM-BIA129 $\Delta slpB$ and only by *P. freudenreichii* CIRM-
418 BIA129 in the basolateral compartment (Figures 4A and 4B). None of the concentrations
419 were correlated with the changes of permeability observed, but as pyruvate concentrations
420 were increased in basolateral compartment by *P. freudenreichii* CIRM-BIA129 but not *P.*
421 *freudenreichii* CIRM-BIA129 $\Delta slpB$, we have tested if it could contribute to *P. freudenreichii*
422 CIRM-BIA129 inhibition of inflammation-induced permeability. None of the tested
423 concentrations were efficient in modulating Caco-2 permeability (Figure 4C). Beyond
424 SCFAs, *P. freudenreichii* CIRM-BIA129 has the ability to generate other soluble mediators.
425 To investigate the potential involvement of the bacterial-released soluble mediators, we
426 evaluated *P. freudenreichii* CIRM-BIA129 supernatant in our *in vitro* model. The supernatant
427 did not exhibit any protective effect against the inflammation-induced increase in
428 permeability (Figure 4D). These data suggest that SlpB-dependent effect of *P. freudenreichii*
429 CIRM-BIA129 upon epithelial permeability does not involve soluble mediators released by
430 the bacteria.

431

432 **Extracellular vesicles from *P. freudenreichii* CIRM-BIA 129 containing the SlpB protein**
433 **are efficient to inhibit inflammation-induced IEB breakdown**

434 To further explore the direct contribution of SlpB in reinforcing the IEB, enriched extracts of
435 S-layer proteins (Slp) were tested in our *in vitro* model of inflammation-induced IEB
436 disruption. Various concentrations of Slp, ranging from 10 $\mu\text{g/mL}$ to 200 $\mu\text{g/mL}$, failed to
437 inhibit the increase in paracellular permeability induced by inflammation, that remained
438 significantly increased except for high concentration (Figure 5A). Nevertheless, this method is
439 insufficient to conclude about the inability of isolated SlpB to modulate the permeability, as
440 (i) guanidine chloride extraction samples also contain other proteins, and (ii) Slps are poorly
441 soluble, forming aggregates in aqueous environments (do Carmo et al. 2018). Therefore we
442 produced and tested extracellular vesicles (EVs) from *P. freudenreichii* CIRM-BIA129 that
443 are known to contain the well-conformed SlpB protein (Rodvalho et al. 2020), and compared
444 their effects to that of EVs produced from strain *P. freudenreichii* CIRM-BIA129 $\Delta slpB$. The
445 productions of EVs were verified by nanoparticle tracking analysis (NTA) to control the
446 similar size profile and adjust the concentrations before treating Caco-2 cells (Figures 5B and

447 5C). EVs derived from *P. freudenreichii* CIRM-BIA129, but not from *P. freudenreichii*
448 CIRM-BIA129 $\Delta slpB$, successfully inhibited the increase in paracellular permeability to
449 sulfonic acid-FITC caused by inflammation (Figure 5D). These findings highlight the
450 essential role of SlpB in barrier protection by *P. freudenreichii* CIRM-BIA129.

451

452 **Deletion of SlpB surface protein exacerbates colitis.**

453 To confirmed the necessity of SlpB in *P. freudenreichii* CIRM-BIA129 protective effect *in*
454 *vivo*, induction of colitis was induced by DSS in mice supplemented with *P. freudenreichii*
455 CIRM-BIA129 $\Delta slpB$. The mutant strain had no effect on DAI score increased by DSS
456 (Figure 6A). It exhibited a tendency to raise paracellular permeability to sulfonic acid-FITC
457 compared to DSS condition (Figure 6B) and significantly elevated the transcellular
458 permeability to HRP compared to the DSS condition (Figure 6C). Moreover, *P. freudenreichii*
459 CIRM-BIA129 $\Delta slpB$ did not changed the level of *il-1 β* transcripts and exacerbated the
460 increase in transcript levels of *tnf- α* mRNA induced by DSS (Figures 6D and 6E).
461 Surprisingly, *il-10* transcripts were increased in the presence of *P. freudenreichii* CIRM-BIA
462 129 $\Delta slpB$ compared to the DSS condition (Figure 6F). These data show *slpB* deletion not only
463 abrogated *P. freudenreichii* CIRM-BIA129 protection but also exacerbated colitis through an
464 increased inflammation and permeability.

465

466 **4. Discussion and conclusion**

467 Disruption of intestinal epithelial barrier (IEB) plays a central role in the pathogenesis
468 of IBD and stands as a pivotal target for effective IBD therapies (Chang et al. 2017). This
469 study demonstrated that the anti-inflammatory dairy bacterium *P. freudenreichii* CIRM-
470 BIA129 strain can counter inflammation-induced barrier disruption *in vitro* and *in vivo*. It
471 revealed that through the expression of the surface protein SlpB, the *P. freudenreichii* CIRM-
472 BIA129 maintains the expression of the tight junction protein ZO-1, thereby strengthening the
473 IEB *in vitro* and *in vivo*.

474 *P. freudenreichii* is widely recognized for its anti-inflammatory properties (do Carmo
475 et al. 2019; Ma et al. 2020), both *in vitro* and *in vivo*, through the regulation of immune cells
476 but also epithelial cells. Indeed, probiotic bacteria can interact with dendritic cells and with
477 intestinal epithelial cells, leading to anti-inflammatory effects of specific strains (Sniffen et al.
478 2018). *P. freudenreichii* enhances the production of IL-10 by human peripheral blood
479 mononuclear cells and reduces the levels of TNF- α and IL-8 induced by lipopolysaccharide
480 stimulation of epithelial HT-29 cells (do Carmo et al. 2019; Folligné et al. 2010). In the
481 present study, *P. freudenreichii* CIRM-BIA129 exerted no significant effect on inflammation
482 *in vivo* in a mice model of acute colitis, but had a major impact upon the gut barrier function.
483 *P. freudenreichii* CIRM-BIA129 was shown to specifically target the intestinal epithelium to
484 enhance one of its major function, the control of permeability. This complements previous
485 studies showing the adhesion of *P. freudenreichii* CIRM-BIA129 to HT-29 cells and
486 describing its capacity to protect the IEB in a mucositis model (do Carmo et al. 2019). Other
487 probiotic such as *Lactiplantibacillus plantarum* (formely *Lactobacillus plantarum*) may also
488 protect the intestinal epithelial barrier through the regulation of the expression of key tight
489 junction proteins such as claudin-1, occludin, and ZO-1 (Wang et al. 2018). *P. freudenreichii*
490 CIRM-BIA 129 can also enhance the mRNA expression of ZO-1 (Deutsch et al. 2017; Le
491 Maréchal et al. 2015; Rabah et al. 2018; do Carmo et al. 2017) and we have further
492 demonstrated here that *P. freudenreichii* CIRM-BIA129 also induces ZO-1 protein
493 expression.

494 The IEB cytoskeletal integrity, survival and apoptosis are regulated by probiotics
495 through key bacterial surface components named microbe-associated molecular patterns

496 (MAMPs), which are recognized by corresponding host pattern recognition receptors (PRR)
497 (Lebeer et al. 2010) as well as soluble factors (Capurso et al. 2019; Yan et al. 2012). The
498 mechanism by which *P. freudenreichii* strengthens IEB seems to exclude soluble factors, as
499 bacterial supernatants did not reproduce the bacterial effect, and none of the organic acid
500 present in apical or basolateral epithelial sides were correlated with the permeability observed.
501 Surprisingly, the supernatant contains EVs, although in all likelihood, their quantity is
502 insufficient to mediate the protective effects of *P. freudenreichii*. However, we have
503 demonstrated that *P. freudenreichii* CIRM-BIA129 adheres to Caco-2 cells, and induces ZO-1
504 protein expression in a SlpB-dependent manner. It should be noted that this *in vitro* effect
505 relies on propionibacteria and on their surface components, yet not on an active metabolism,
506 which is not possible in the conditions of these *in vitro* co-incubations. Other bacterial
507 probiotic species, including lactobacilli, were already shown to possess surface layer proteins
508 involved in probiotic/host interactions. As an example, *Lactobacillus acidophilus* binds to
509 dendritic cells C-type lectin SIGNR3 and, regulates immature dendritic cells and T cells
510 functions, mitigating gut inflammation as well as mucosal barrier function in mice, thanks to a
511 specific surface layer protein (Konstantinov et al. 2008; Lightfoot et al. 2015). We previously
512 showed that SlpB was involved in and necessary to the *P. freudenreichii* probiotic effect
513 (Deutsch et al. 2017; Le Maréchal et al. 2015; Rabah et al. 2018; do Carmo et al. 2017).
514 Herein, we focused our study on IEB and showed that SlpB reinforces the barrier, as strain
515 lacking *slpB* gene have lost their protective effect upon inflammation-induced IEB disruption.
516 However, it is important to acknowledge that the mutant strain exhibits pleiotropic effects on
517 the bacteria itself (do Carmo et al. 2018). In the present study, in addition to the loss of SlpB,
518 we evidenced the remodeling induced by SlpB deletion through the major change induced in
519 organic acid productions. The increased production of acetate and propionate by the *P.*
520 *freudenreichii* CIRM-BIA129 Δ *slpB* in comparison to the wild type strain *P. freudenreichii*
521 CIRM-BIA129 could explain the aggravating effect of the bacteria upon permeability and
522 inflammation. But to focus and identify whether isolated SlpB could be responsible of the
523 probiotic effect of *P. freudenreichii* CIRM-BIA129, we have treated epithelial monolayer
524 with a guanidine extract enriched in *P. freudenreichii* surface layer proteins, but it had no
525 effect. This observation does not imperatively mean that isolated SlpB are not enough to
526 induce the probiotic effect, but rather than SlpB, although it is not a transmembrane protein,
527 must interact with a lipid bilayer to be well conformed and operational. Slps are known for
528 their capacity to aggregate, after extraction, forming paracrystalline structures different in the
529 guanidine extract than on the surface of propionibacteria envelope (do Carmo et al. 2018). In
530 a consistent manner, the heterologous expression of *slpB* in *Lactococcus lactis* NCDO 2118
531 that confers probiotic potential to the strain to alleviate DSS-induced colitis in mice (Belo et
532 al. 2021).

533 Our work mainly observed the regulation of the IEB by *P. freudenreichii*, shedding
534 light on the significant role of epithelium dysfunction into colitis development. However, it is
535 equally surprising to note the absence of any discernible anti-inflammatory effects,
536 emphasizing the crucial role of the matrix. Indeed, we have already demonstrated the role of
537 the dairy matrix in the modulation of *P. freudenreichii* probiotic activity. The cheese matrix
538 was shown to protect the immunomodulatory SlpB protein from digestive proteolysis (Rabah
539 et al. 2018), and the presence of dairy fat in the fermented product potentiates the anti-colitis
540 effects of the probiotic strain (Mantel et al. 2023). In the present work we gave
541 *P. freudenreichii* in milk ultra-filtrate supplemented with casein peptone and sodium DL-
542 lactate, that seems unsuitable to support anti-inflammatory effect. Further studies are
543 necessary to determine to which extend the matrix composition is crucial for the bacterial
544 strain, or for the host, to obtain optimal effects. Another strategy could be to deliver and to
545 trigger of the probiotic effects in a matrix-independent-way. Our study also shows that EVs

546 are efficient vehicles to mimic the protective effect of *P. freudenreichii* upon inflammation-
547 induced IEB disruption. They have previously been shown to reduce inflammation in cultured
548 human intestinal cells by modulating the NF- κ B pathway (Rodvalho et al. 2020), and could
549 be efficient to protect the IEB as well as to dampen inflammation. It opens new avenues for
550 the definition of preventive strategies against chronic diseases involving intestinal barrier
551 hyperpermeability and inflammation, avoiding the drawbacks associated with the use of live
552 bacteria.

553 Finally, the research work presented here was performed on model systems which are still
554 distant from clinical situations. The Caco-2 cell line is a cancer cell line different from healthy
555 human colon epithelial cells and DSS-colitis in mice only mimics inflammatory conditions.
556 However, this work, in addition to previous data using human PBMCs, HT-29 cells, Caco-2
557 cells, TNBS-colitis, DSS- colitis and 5-FU mucositis constitute a body of converging
558 indications pointing at the anti-inflammatory potential of selected strains of *P. freudenreichii*.
559 Pilot clinical studies further suggest the interest of this probiotic bacterium in the context of
560 inflammatory conditions (Suzuki et al. 2006). However, further clinical investigations are
561 needed to precise the future use of propionibacteria in live biotherapeutic applications.

562

563

564 ACKNOWLEDGMENTS

565 This work was supported by the « Institut national de la santé et de la recherche médicale »
566 (Inserm), the Nantes Université and the cluster IBD NExt from Nantes Excellence Trajectory
567 (NEXt Health & Engineering) and the “Institut national de recherche pour l’agriculture,
568 l’alimentation et l’environnement” (INRAE). MM benefits a doctoral fellowship managed by
569 Bba Milk Valley, dairy industrial association. We are grateful to Regional Councils of
570 Bretagne (grant no. 19008213) and Pays de la Loire (grant no. 2019-013227) for their
571 financial support through the interregional project PROLIFIC. MRD is supported by the
572 Centre National pour la Recherche Scientifique (CNRS). Figures were created with
573 BioRender.com. Authors thank Nicodème Hent’al for useful advices.

574

575

576 LEGENDS TO THE FIGURES

577

578 **Figure 1. *P. freudenreichii* CIRM-BIA129 mitigates DSS-induced colitis severity and**
579 **reduces intestinal permeability *in vivo*.** (A) Eight-week-old C57BL6 mice were gavaged
580 during 11 days with milk ultra-filtrate (MUF) or MUF fermented by *P. freudenreichii* CIRM-
581 BIA129(129) containing 1.10^8 CFU. At day 7, 4% DSS was added to drinking water or not.
582 On day 11, permeabilities were measured, mice sacrificed, and the tissues collected. (B)
583 Disease activity index (DAI) of the 4 groups: control (MUF) or *P. freudenreichii* CIRM-
584 BIA129 (129) in control conditions (grey) or with DSS (DSS and 129DSS in orange) at day
585 11. (C) Paracellular and (D) transcellular permeabilities evaluated by measurement of sulfonic
586 acid and HRP in animal plasma 4 hours after mice gavage, respectively. (E) Representative
587 images of Alcyan Blue staining of the distal colon. (F) Histological scores (mDAI)
588 quantifying the destruction of mucosal architecture, cellular infiltration, muscle thickening
589 and loss of goblet cells. (G) Crypts length measured for 50 opened crypts per animal. Data
590 represent mean \pm SEM of 14 mice per group.

591 **Figure 2. *P. freudenreichii* CIRM-BIA 129 promotes tight junction protein remodeling**
592 **without reducing inflammation.** (A) Representative Western blot analysis of ZO-1, occludin
593 (OCLN) and β -actin. (B) Quantification of ZO-1, and (C) occludin expression normalized to
594 β -actin and relative to control mean. Quantification of transcript levels of (D) *il-1 β* , (E) *tnf- α* ,
595 and (F) *il-10* measured at day 11 in the proximal colon of the four groups. Relative expression

596 was determined using *Rps6* as reference gene. Both protein and transcript levels were
597 normalized to control mean. (G) Fecal lipocalin contents were measured at day 11.

598 **Figure 3. *P. freudenreichii* CIRM-BIA129 inhibits inflammation-induced IEB**
599 **breakdown through S-layer protein B induction of ZO-1 expression.** (A) Caco-2 cells
600 grown on transwell for 21days were left untreated or pre-treated with *P. freudenreichii*
601 CIRM-BIA129 or *P. freudenreichii* CIRM-BIA129 Δ *slpB* (MOI 100:1) for 24 hours before
602 removal and treatment with an inflammatory cytomix containing TNF- α and IFN- γ was added
603 for 6 days, with a renewal at day 3. (B) Gram staining of *P. freudenreichii* CIRM-BIA129
604 culture. The top-left image showed the bacteria in its fresh state; top right showed Caco-2
605 cells without any bacteria where the pore filter are visible; down-left, Caco-2 cells after
606 24hours with *P. freudenreichii* CIRM-BIA129; down-right, Caco-2 cells after 24hours with *P.*
607 *freudenreichii* CIRM-BIA129 Δ *slpB*. (C) Trans-epithelial electrical resistances (TEER), (D)
608 Sulfonic acid-FITC diffusion (E) HRP diffusion were measured at the end of the experiment.
609 (F) Representative western blot analysis of ZO-1, occludin (OCLN) and β -actin amounts. (G)
610 Quantification of ZO-1 and (H) OCLN expressions normalized to β -actin and relative to
611 control mean (Ct grey). Values are expressed as means \pm SEM.

612 **Figure 4. Organic acid productions do not explain the *P. freudenreichii* CIRM-BIA129**
613 **protective effect on inflammation-induced IEB breakdown.** Caco-2 cells grown on
614 transwell for 21 days were left untreated or pre-treated with *P. freudenreichii* CIRM-BIA129
615 or *P. freudenreichii* CIRM-BIA129 Δ *slpB* for 24 hours before removal and treatment with an
616 inflammatory cytomix containing TNF- α and IFN- γ was added for 6 days, with a renewal at
617 day 3. Impact of *P. freudenreichii* CIRM-BIA129 or *P. freudenreichii* CIRM-BIA129 Δ *slpB*
618 on the concentrations of different SCFAs was measured (A) at the apical and (B) basolateral
619 compartments at the end of the experiment. Caco-2 cells grown on transwell for 21days were
620 left untreated or pre-treated with the indicated concentrations of sodium-pyruvate at the
621 basolateral side for 24 before removal and treatment with an inflammatory cytomix described
622 above was added for 6 days, with a renewal at day 3. (C) Sulfonic acid-FITC diffusion was
623 measured at the end of the experiment. Caco-2 cells were left untreated or pre-treated with the
624 *P. freudenreichii* CIRM-BIA129's supernatant for 24 h before removal, and treatment with an
625 inflammatory cytomix described above was added for 6 days, with a renewal at day 3. (D)
626 Sulfonic acid-FITC diffusion was measured at the end of the experiment. Data are expressed
627 as means \pm SEM.

628 **Figure 5. Extracellular vesicles from *P. freudenreichii* CIRM-BIA 129 containing the**
629 **SlpB protein are efficient to inhibit inflammation-induced IEB breakdown.** Caco-2 cells
630 grown on transwell for 21days were left untreated or pre-treated with the indicated
631 concentrations of S-layer protein extracts from *P. freudenreichii* CIRM-BIA129 for 24h
632 before removal and treatment with an inflammatory cytomix containing TNF- α and IFN- γ
633 was added for 6 days, with a renewal at day 3. (A) Sulfonic acid-FITC diffusion was
634 measured measured at the end of the protocol. (B) Size distribution (nm) and concentrations
635 (particle/mL) of purified EVs from *P. freudenreichii* CIRM-BIA129 and (C) *P. freudenreichii*
636 CIRM-BIA129 Δ SlpB measured by nanoparticle tracking analysis. (D) Caco-2 cells grown on
637 transwell for 21days were left untreated or pretreated with EVs from *P. freudenreichii* CIRM-
638 BIA129 or *P. freudenreichii* CIRM-BIA129 Δ SlpB (10^7 particles/well) for 24 hours on the
639 apical side and then challenged or not with TNF- α and IFN- γ on the apical and basolateral
640 side for 6 days, with a renewal at day 3. Sulfonic acid-FITC diffusion were measured at the
641 end of the experiment. Data are expressed as means \pm SEM and normalized to control mean.

642 **Figure 6. Deletion of SlpB surface protein exacerbates colitis.** (A) Disease activity index
643 (DAI) of the 3 groups (control, DSS and *P. freudenreichii* 129 Δ SlpB+DSS) at day 11. (B)
644 Paracellular and (C) transcellular permeabilities evaluated by measurement of sulfonic acid
645 and HRP in animal plasma 4 hours after mice gavage. (D) Quantification of transcript levels

646 of *il-1 β* (E) *tnf- α* , and (F) *il-10* measured at the end of the protocol in proximal colon of the
647 four groups. Relative expression was determined using *Rps6* as reference. Values are
648 expressed as means +/- SEM.

649 **Supplemental Figure 1. *P. freudenreichii* CIRM-BIA129 mitigates DSS-induced colitis**
650 **severity *in vivo*.** Eight-week-old C57BL6 mice were gavaged during 11 days with milk ultra-
651 filtrate (MUF) or MUF fermented by *P. freudenreichii* CIRM-BIA129(129) containing 1.10^8
652 CFU. At day 7, 4% DSS was added to drinking water or not. On day 11, permeabilities were
653 measured, mice sacrificed and the tissues collected. (A) Bleeding score (B) stool consistency
654 and (C) weight change before/after DSS of the 4 groups: control (MUF) or *P. freudenreichii*
655 CIRM-BIA129 (129) in control conditions (grey) or with DSS (DSS and 129DSS in orange)
656 at day 11.

657

658 REFERENCES

- 659 Alatab, Sudabeh, Sadaf G Sepanlou, Kevin Ikuta, Homayoon Vahedi, Catherine Bisignano,
660 Saeid Safiri, Anahita Sadeghi, et al. 2020. 'The Global, Regional, and National Burden of
661 Inflammatory Bowel Disease in 195 Countries and Territories, 1990–2017: A Systematic
662 Analysis for the Global Burden of Disease Study 2017'. *The Lancet Gastroenterology &*
663 *Hepatology* 5 (1): 17–30. [https://doi.org/10.1016/S2468-1253\(19\)30333-4](https://doi.org/10.1016/S2468-1253(19)30333-4).
- 664 Arrieta, M C. 2006. 'Alterations in Intestinal Permeability'. *Gut* 55 (10): 1512–20.
665 <https://doi.org/10.1136/gut.2005.085373>.
- 666 Belo, Giovanna A., Bárbara F. Cordeiro, Emiliano R. Oliveira, Marina P. Braga, Sara H. da
667 Silva, Bruno G. Costa, Flaviano Dos S. Martins, et al. 2021. 'SlpB Protein Enhances the
668 Probiotic Potential of *L. Lactis* NCDO 2118 in Colitis Mice Model'. *Frontiers in*
669 *Pharmacology* 12: 755825. <https://doi.org/10.3389/fphar.2021.755825>.
- 670 Capurso, Lucio. 2019. 'Thirty Years of Lactobacillus Rhamnosus GG: A Review'. *Journal of*
671 *Clinical Gastroenterology* 53 (Supplement 1): S1–41.
672 <https://doi.org/10.1097/MCG.0000000000001170>.
- 673 Carmo, Fillipe L. R. do, Houem Rabah, Rodrigo D. De Oliveira Carvalho, Floriane Gaucher,
674 Barbara F. Cordeiro, Sara H. da Silva, Yves Le Loir, Vasco Azevedo, and Gwénaél Jan. 2018.
675 'Extractable Bacterial Surface Proteins in Probiotic–Host Interaction'. *Frontiers in*
676 *Microbiology* 9 (April): 645. <https://doi.org/10.3389/fmicb.2018.00645>.
- 677 Carmo, Fillipe L. R. do, Houem Rabah, Song Huang, Floriane Gaucher, Martine Deplanche,
678 Stéphanie Dutertre, Julien Jardin, Yves Le Loir, Vasco Azevedo, and Gwénaél Jan. 2017.
679 'Propionibacterium Freudenreichii Surface Protein SlpB Is Involved in Adhesion to Intestinal
680 HT-29 Cells'. *Frontiers in Microbiology* 8 (June): 1033.
681 <https://doi.org/10.3389/fmicb.2017.01033>.
- 682 Carmo, Fillipe L. R. do, Wanderson M. Silva, Guilherme C. Tavares, Izabela C. Ibraim,
683 Barbara F. Cordeiro, Emiliano R. Oliveira, Houem Rabah, et al. 2018. 'Mutation of the
684 Surface Layer Protein SlpB Has Pleiotropic Effects in the Probiotic Propionibacterium
685 Freudenreichii CIRM-BIA 129'. *Frontiers in Microbiology* 9 (August): 1807.
686 <https://doi.org/10.3389/fmicb.2018.01807>.

- 687 Carmo, Fillipe Luiz Rosa do, Houem Rabah, Barbara Fernandes Cordeiro, Sara Heloisa da
688 Silva, Rafaela Miranda Pessoa, Simone Odília Antunes Fernandes, Valbert Nascimento
689 Cardoso, et al. 2019. 'Probiotic *Propionibacterium Freudenreichii* Requires SlpB Protein to
690 Mitigate Mucositis Induced by Chemotherapy'. *Oncotarget* 10 (68): 7198–7219.
691 <https://doi.org/10.18632/oncotarget.27319>.
- 692 Chang, Jeff, Rupert W. Leong, Valerie C. Wasinger, Matthew Ip, Michael Yang, and Tri
693 Giang Phan. 2017. 'Impaired Intestinal Permeability Contributes to Ongoing Bowel
694 Symptoms in Patients With Inflammatory Bowel Disease and Mucosal Healing'.
695 *Gastroenterology* 153 (3): 723-731.e1. <https://doi.org/10.1053/j.gastro.2017.05.056>.
- 696 Cooper, H. S., S. N. Murthy, R. S. Shah, and D. J. Sedergran. 1993. 'Clinicopathologic Study
697 of Dextran Sulfate Sodium Experimental Murine Colitis'. *Laboratory Investigation; a
698 Journal of Technical Methods and Pathology* 69 (2): 238–49.
- 699 Coquenlorge, Sabrina, Laurianne Van Landeghem, Julie Jaulin, Nicolas Cenac, Nathalie
700 Vergnolle, Emilie Duchalais, Michel Neunlist, and Malvyne Rolli-Derkinderen. 2016. 'The
701 Arachidonic Acid Metabolite 11 β -ProstaglandinF2 α Controls Intestinal Epithelial Healing:
702 Deficiency in Patients with Crohn's Disease'. *Scientific Reports* 6 (1): 25203.
703 <https://doi.org/10.1038/srep25203>.
- 704 Deutsch, Stéphanie-Marie, Mahendra Mariadassou, Pierre Nicolas, Sandrine Parayre, Rozenn
705 Le Guellec, Victoria Chuat, Vincent Peton, et al. 2017. 'Identification of Proteins Involved in
706 the Anti-Inflammatory Properties of *Propionibacterium Freudenreichii* by Means of a Multi-
707 Strain Study'. *Scientific Reports* 7 (April): 46409. <https://doi.org/10.1038/srep46409>.
- 708 Foligné, B., J. Breton, D. Mater, and G. Jan. 2013. 'Tracking the Microbiome Functionality:
709 Focus on *Propionibacterium* Species'. *Gut* 62 (February): 1227–28.
- 710 Foligné, B., S. M. Deutsch, J. Breton, F. J. Cousin, J. Dewulf, M. Samson, B. Pot, and G. Jan.
711 2010. 'Promising Immunomodulatory Effects of Selected Strains of Dairy Propionibacteria as
712 Evidenced *in Vitro* and *in Vivo*'. *Applied and Environmental Microbiology* 76 (December):
713 8259–64.
- 714 Foligné, Benoît, Stéphanie-Marie Deutsch, Jérôme Breton, Fabien J. Cousin, Joëlle Dewulf,
715 Michel Samson, Bruno Pot, and Gwénaél Jan. 2010. 'Promising Immunomodulatory Effects
716 of Selected Strains of Dairy Propionibacteria as Evidenced *In Vitro* and *In Vivo*'. *Applied and
717 Environmental Microbiology* 76 (24): 8259–64. <https://doi.org/10.1128/AEM.01976-10>.
- 718 Illikoud, Nassima, Marine Mantel, Malvyne Rolli-Derkinderen, Valérie Gagnaire, and
719 Gwénaél Jan. 2022. 'Dairy Starters and Fermented Dairy Products Modulate Gut Mucosal
720 Immunity'. *Immunology Letters* 251–252 (December): 91–102.
721 <https://doi.org/10.1016/j.imlet.2022.11.002>.
- 722 Konstantinov, Sergey R., Hauke Smidt, Willem M. De Vos, Sven C. M. Bruijns, Satwinder
723 Kaur Singh, Florence Valence, Daniel Molle, et al. 2008. 'S Layer Protein A of *Lactobacillus
724 Acidophilus* NCFM Regulates Immature Dendritic Cell and T Cell Functions'. *Proceedings of*

- 725 *the National Academy of Sciences* 105 (49): 19474–79.
726 <https://doi.org/10.1073/pnas.0810305105>.
- 727 Lê, Amélie, Marine Mantel, Justine Marchix, Marie Bodinier, Gwénaél Jan, and Malvyne
728 Rolli-Derkinderen. 2022. ‘Inflammatory Bowel Disease Therapeutic Strategies by Modulation
729 of the Microbiota: How and When to Introduce Pre-, pro-, Syn-, or Postbiotics?’ *American*
730 *Journal of Physiology. Gastrointestinal and Liver Physiology* 323 (6): G523–53.
731 <https://doi.org/10.1152/ajpgi.00002.2022>.
- 732 Le Berre, Catherine, Philippe Naveilhan, and Malvyne Rolli-Derkinderen. 2023. ‘Enteric Glia
733 at Center Stage of Inflammatory Bowel Disease’. *Neuroscience Letters* 809 (July): 137315.
734 <https://doi.org/10.1016/j.neulet.2023.137315>.
- 735 Le Marechal, C., V. Peton, C. Ple, C. Vroland, J. Jardin, V. Briard-Bion, G. Durant, et al.
736 2015. ‘Surface Proteins of *Propionibacterium Freudenreichii* Are Involved in Its Anti-
737 Inflammatory Properties’. *J.Proteomics*. 113C (January): 447–61.
- 738 Le Maréchal, Caroline, Vincent Peton, Coline Plé, Christophe Vroland, Julien Jardin, Valérie
739 Briard-Bion, Gaël Durant, et al. 2015. ‘Surface Proteins of *Propionibacterium Freudenreichii*
740 Are Involved in Its Anti-Inflammatory Properties’. *Journal of Proteomics* 113 (January):
741 447–61. <https://doi.org/10.1016/j.jprot.2014.07.018>.
- 742 Lebeer, Sarah, Jos Vanderleyden, and Sigrid C. J. De Keersmaecker. 2010. ‘Host Interactions
743 of Probiotic Bacterial Surface Molecules: Comparison with Commensals and Pathogens’.
744 *Nature Reviews Microbiology* 8 (3): 171–84. <https://doi.org/10.1038/nrmicro2297>.
- 745 Lightfoot, Yaïma L, Kurt Selle, Tao Yang, Yong Jun Goh, Bikash Sahay, Mojgan Zadeh,
746 Jennifer L Owen, et al. 2015. ‘SIGNR 3-dependent Immune Regulation by *Lactobacillus*
747 *Acidophilus* Surface Layer Protein A in Colitis’. *The EMBO Journal* 34 (7): 881–95.
748 <https://doi.org/10.15252/embj.201490296>.
- 749 Ma, Seongho, Jiah Yeom, and Young-Hee Lim. 2020. ‘Dairy *Propionibacterium*
750 *Freudenreichii* Ameliorates Acute Colitis by Stimulating MUC2 Expression in Intestinal
751 Goblet Cell in a DSS-Induced Colitis Rat Model’. *Scientific Reports* 10 (1): 5523.
752 <https://doi.org/10.1038/s41598-020-62497-8>.
- 753 Malik, A. C., G. W. Reinbold, and E. R. Vedamuthu. 1968. ‘An Evaluation of the Taxonomy
754 of *Propionibacterium*’. *Canadian Journal of Microbiology* 14 (11): 1185–91.
755 <https://doi.org/10.1139/m68-199>.
- 756 Mantel, Marine, Tales Fernando Da Silva, Rafael Gloria, Danièle Vassaux, Kátia Duarte
757 Vital, Valbert Nascimento Cardoso, Simone Odília Antunes Fernandes, et al. 2023. ‘Fat
758 Matters: Fermented Whole Milk Potentiates the Anti-Colitis Effect of *Propionibacterium*
759 *Freudenreichii*’. *Journal of Functional Foods* 106 (July): 105614.
760 <https://doi.org/10.1016/j.jff.2023.105614>.
- 761 Munkholm, P, E Langholz, D Hollander, K Thornberg, M Orholm, K D Katz, and V Binder.

- 762 1994. 'Intestinal Permeability in Patients with Crohn's Disease and Ulcerative Colitis and
763 Their First Degree Relatives.' *Gut* 35 (1): 68–72. <https://doi.org/10.1136/gut.35.1.68>.
- 764 Pearson, A D, E J Eastham, M F Laker, A W Craft, and R Nelson. 1982. 'Intestinal
765 Permeability in Children with Crohn's Disease and Coeliac Disease.' *BMJ* 285 (6334): 20–21.
766 <https://doi.org/10.1136/bmj.285.6334.20>.
- 767 Peeters, Marc, Yvo Ghoois, Bart Maes, Martin Hiele, Karel Geboes, Gaston Vantrappen, and
768 Paul Rutgeerts. 1994. 'Increased Permeability of Macroscopically Normal Small Bowel in
769 Crohn's Disease'. *Digestive Diseases and Sciences* 39 (10): 2170–76.
770 <https://doi.org/10.1007/BF02090367>.
- 771 Pochard, Camille, Sabrina Coquenlorge, Julie Jaulin, Nicolas Cenac, Nathalie Vergnolle,
772 Guillaume Meurette, Marie Freyssinet, Michel Neunlist, and Malvyne Rolli-Derkinderen.
773 2016. 'Defects in 15-HETE Production and Control of Epithelial Permeability by Human
774 Enteric Glial Cells From Patients With Crohn's Disease'. *Gastroenterology* 150 (1): 168–80.
775 <https://doi.org/10.1053/j.gastro.2015.09.038>.
- 776 Pochard, Camille, Jacques Gonzales, Anne Bessard, Maxime M. Mahe, Arnaud Bourreille,
777 Nicolas Cenac, Anne Jarry, et al. 2021. 'PGI2 Inhibits Intestinal Epithelial Permeability and
778 Apoptosis to Alleviate Colitis'. *Cellular and Molecular Gastroenterology and Hepatology* 12
779 (3): 1037–60. <https://doi.org/10.1016/j.jcmgh.2021.05.001>.
- 780 Prigent, Alice, Guillaume Chapelet, Adrien De Guilhem De Lataillade, Thibault Oullier,
781 Emilie Durieu, Arnaud Bourreille, Emilie Duchalais, et al. 2020. 'Tau Accumulates in
782 Crohn's Disease Gut'. *The FASEB Journal* 34 (7): 9285–96.
783 <https://doi.org/10.1096/fj.202000414R>.
- 784 Prigent, Alice, Arthur Lionnet, Emilie Durieu, Guillaume Chapelet, Arnaud Bourreille,
785 Michel Neunlist, Malvyne Rolli-Derkinderen, and Pascal Derkinderen. 2019. 'Enteric Alpha-
786 Synuclein Expression Is Increased in Crohn's Disease'. *Acta Neuropathologica* 137 (2): 359–
787 61. <https://doi.org/10.1007/s00401-018-1943-7>.
- 788 Rabah, Houem, Olivia Ménard, Floriane Gaucher, Fillipe Luiz Rosa do Carmo, Didier
789 Dupont, and Gwénaél Jan. 2018a. 'Cheese Matrix Protects the Immunomodulatory Surface
790 Protein SlpB of Propionibacterium Freudenreichii during in Vitro Digestion'. *Food Research*
791 *International* 106 (April): 712–21. <https://doi.org/10.1016/j.foodres.2018.01.035>.
- 792 ———. 2018b. 'Cheese Matrix Protects the Immunomodulatory Surface Protein SlpB of
793 *Propionibacterium Freudenreichii* during in Vitro Digestion'. *Food Res. Int.* 106 (April):
794 712–21. <https://doi.org/10.1016/j.foodres.2018.01.035>.
- 795 Rodovalho, Vinícius de Rezende, Brenda Silva Rosa da Luz, Houem Rabah, Fillipe Luiz Rosa
796 do Carmo, Edson Luiz Folador, Aurélie Nicolas, Julien Jardin, et al. 2020. 'Extracellular
797 Vesicles Produced by the Probiotic Propionibacterium Freudenreichii CIRM-BIA 129
798 Mitigate Inflammation by Modulating the NF-κB Pathway'. *Frontiers in Microbiology* 11
799 (July): 1544. <https://doi.org/10.3389/fmicb.2020.01544>.

800 Sniffen, Jason C., Lynne V. McFarland, Charlesnika T. Evans, and Ellie J. C. Goldstein.
801 2018. 'Choosing an Appropriate Probiotic Product for Your Patient: An Evidence-Based
802 Practical Guide'. Edited by Leandro Araujo Lobo. *PLOS ONE* 13 (12): e0209205.
803 <https://doi.org/10.1371/journal.pone.0209205>.

804 Suzuki, Asuka, Keiichi Mitsuyama, Hironori Koga, Nobuo Tomiyasu, Junya Masuda, Kosuke
805 Takaki, Osamu Tsuruta, Atsushi Toyonaga, and Michio Sata. 2006. 'Bifidogenic Growth
806 Stimulator for the Treatment of Active Ulcerative Colitis: A Pilot Study'. *Nutrition* 22 (1):
807 76–81. <https://doi.org/10.1016/j.nut.2005.04.013>.

808 Tindemans, Irma, Maria E. Joosse, and Janneke N. Samsom. 2020. 'Dissecting the
809 Heterogeneity in T-Cell Mediated Inflammation in IBD'. *Cells* 9 (1): 110.
810 <https://doi.org/10.3390/cells9010110>.

811 Turner, Jerrold R. 2009. 'Intestinal Mucosal Barrier Function in Health and Disease'. *Nature*
812 *Reviews Immunology* 9 (11): 799–809. <https://doi.org/10.1038/nri2653>.

813 Wang, Jing, Haifeng Ji, Sixin Wang, Hui Liu, Wei Zhang, Dongyan Zhang, and Yamin
814 Wang. 2018. 'Probiotic *Lactobacillus Plantarum* Promotes Intestinal Barrier Function by
815 Strengthening the Epithelium and Modulating Gut Microbiota'. *Frontiers in Microbiology* 9
816 (August): 1953. <https://doi.org/10.3389/fmicb.2018.01953>.

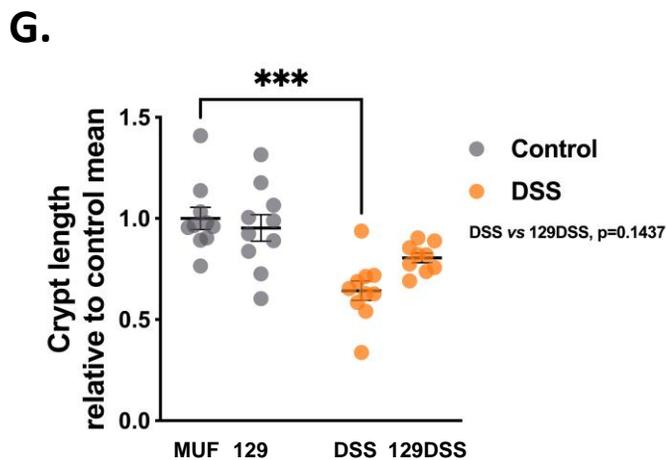
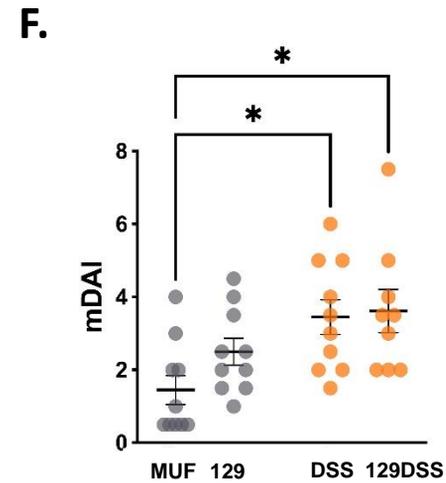
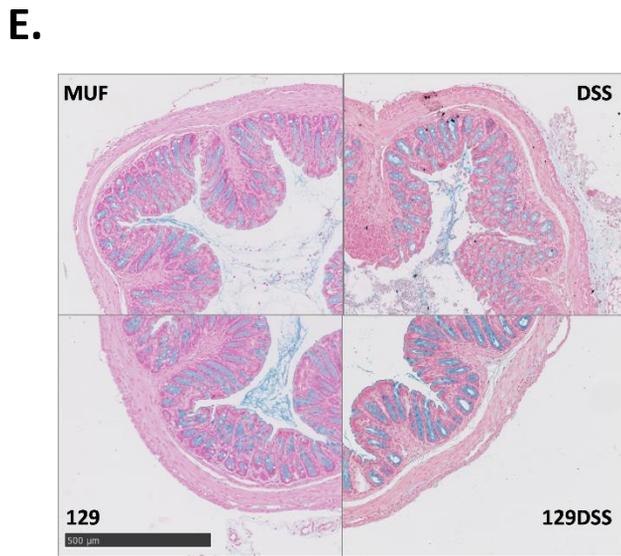
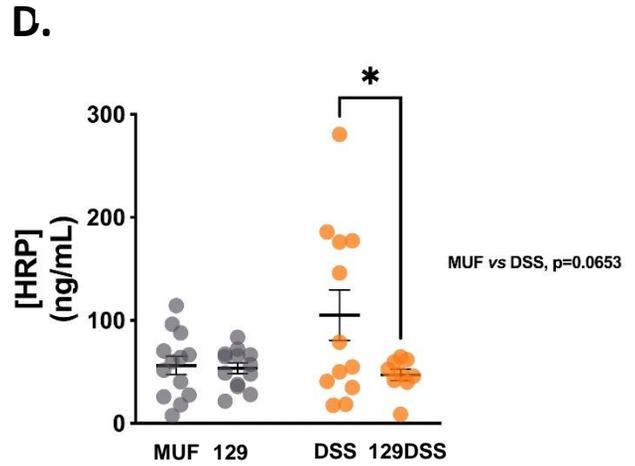
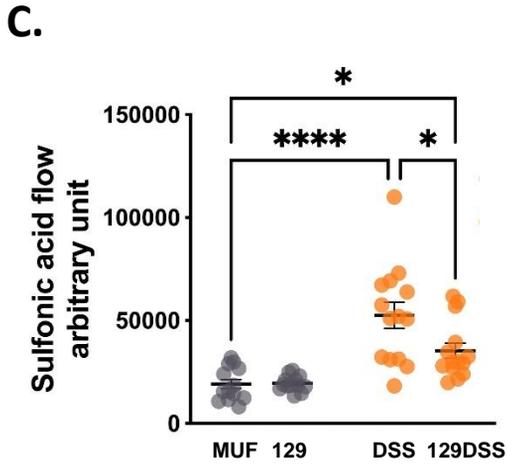
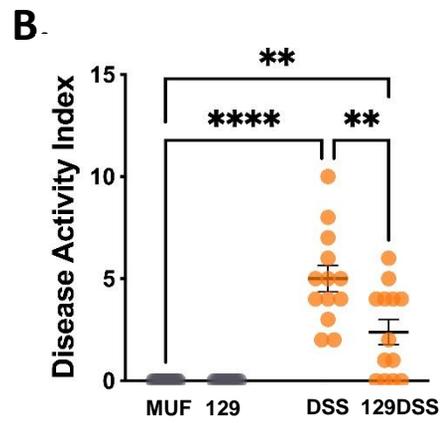
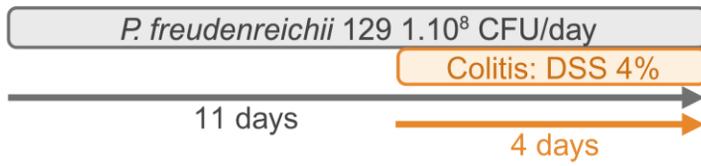
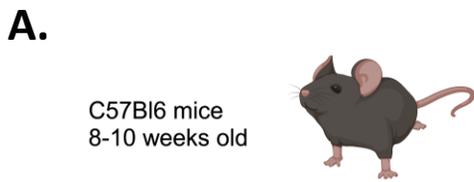
817 Wyatt, J., H. Vogelsang, W. Hübl, T. Waldhoer, and H. Lochs. 1993. 'Intestinal Permeability
818 and the Prediction of Relapse in Crohn's Disease'. *The Lancet* 341 (8858): 1437–39.
819 [https://doi.org/10.1016/0140-6736\(93\)90882-H](https://doi.org/10.1016/0140-6736(93)90882-H).

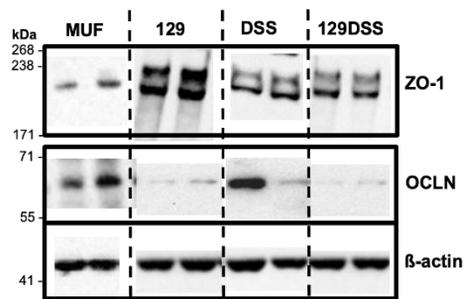
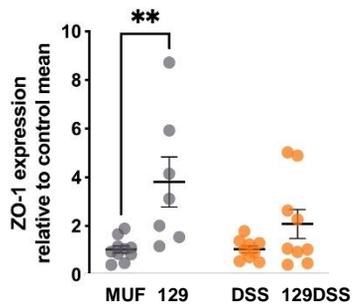
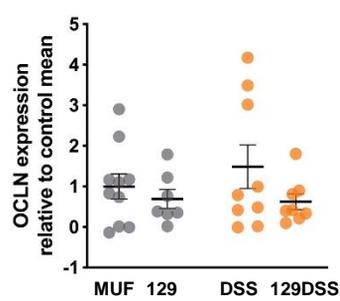
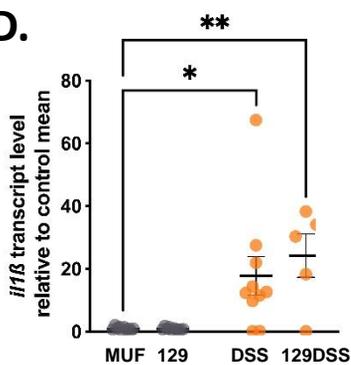
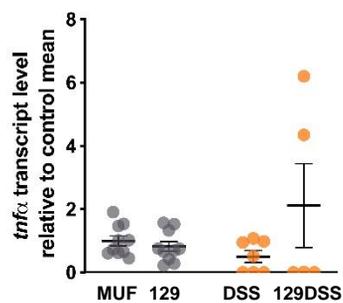
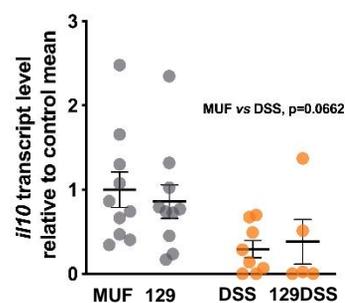
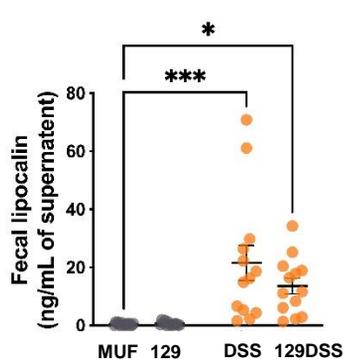
820 Yan, Fang, and D. Brent Polk. 2012. 'Characterization of a Probiotic-Derived Soluble Protein
821 Which Reveals a Mechanism of Preventive and Treatment Effects of Probiotics on Intestinal
822 Inflammatory Diseases'. *Gut Microbes* 3 (1): 25–28. <https://doi.org/10.4161/gmic.19245>.

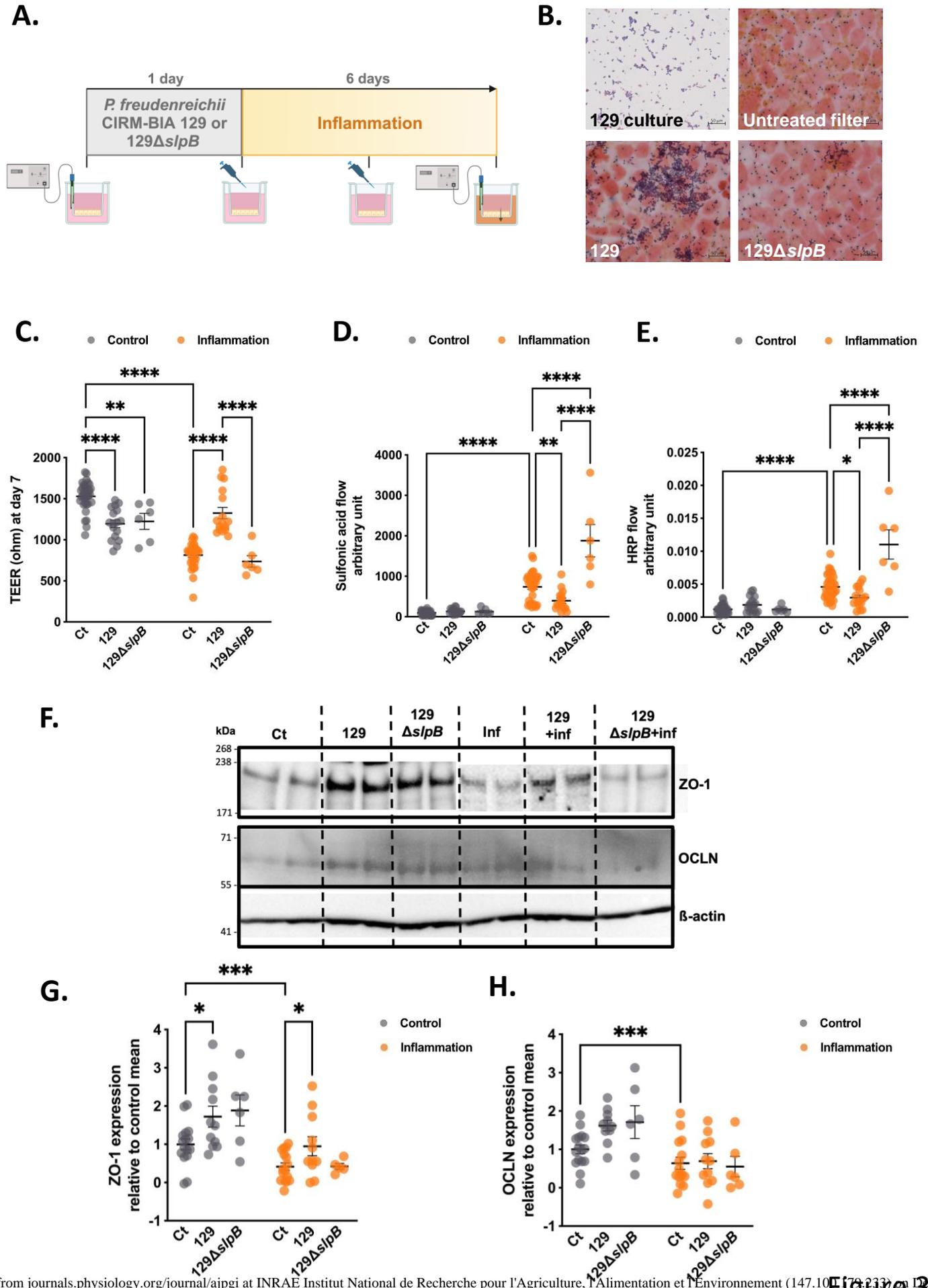
823

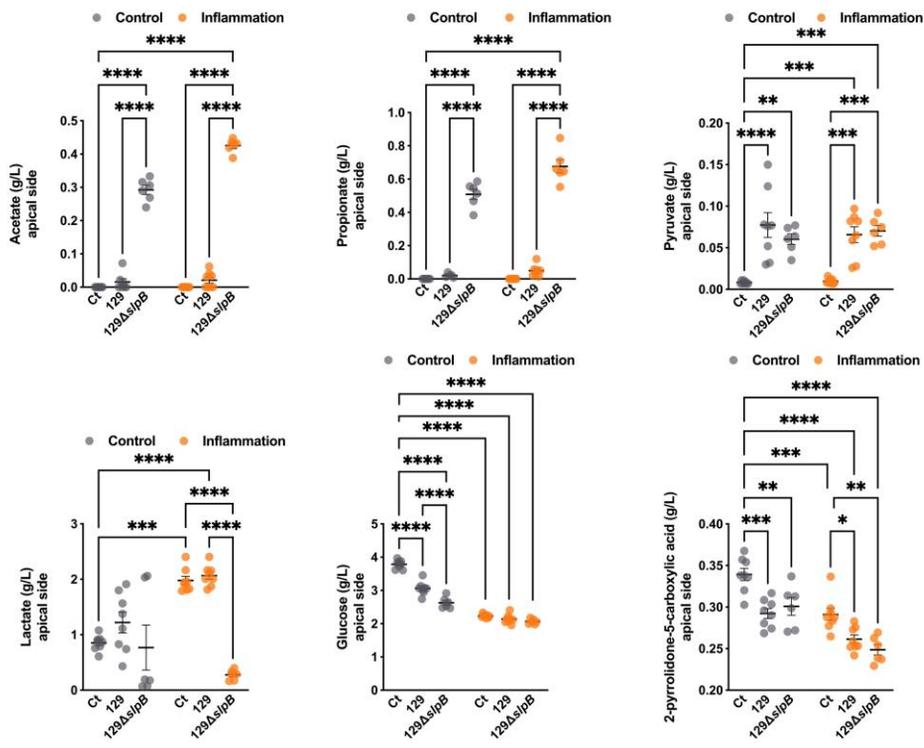
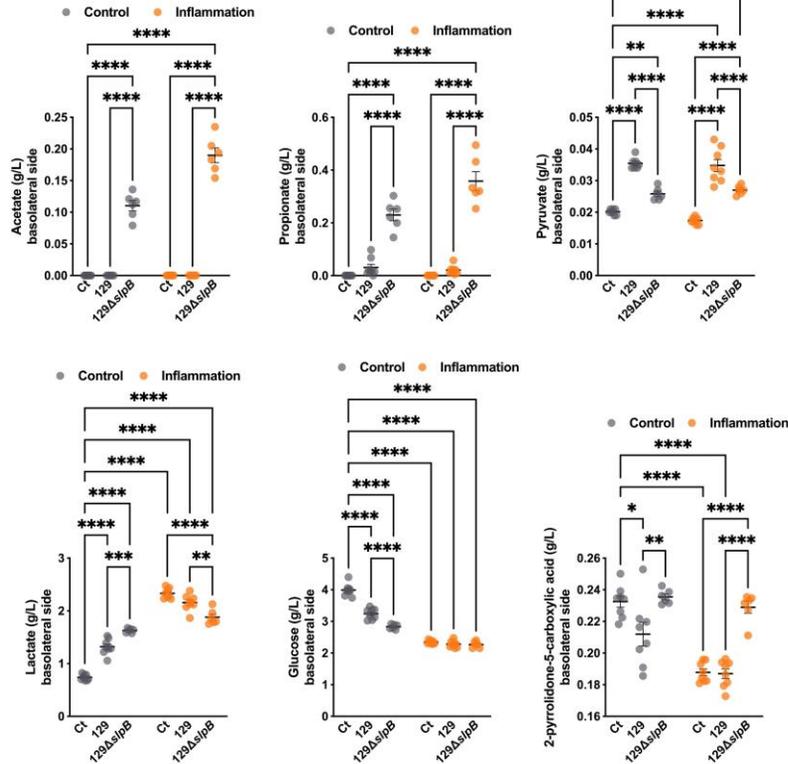
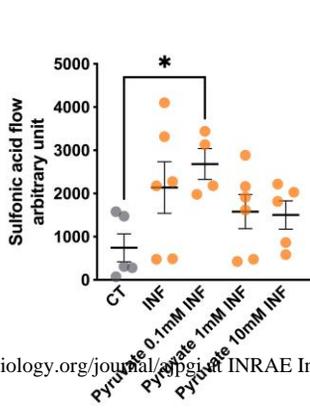
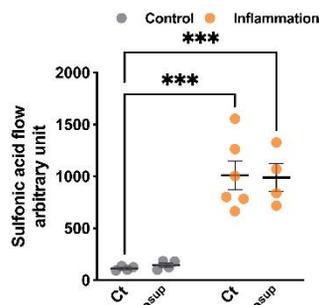
Table 1. Disease Activity Index.

Score	Weight loss (%)	Stool consistency	Occult/Gross bleeding
0	<1%	Normal	Negative
1	1-5%		Stain
2	5-10%	Loose	Occult blood
3	10-15%		
4	>15%	Diarrhea	Gross bleeding

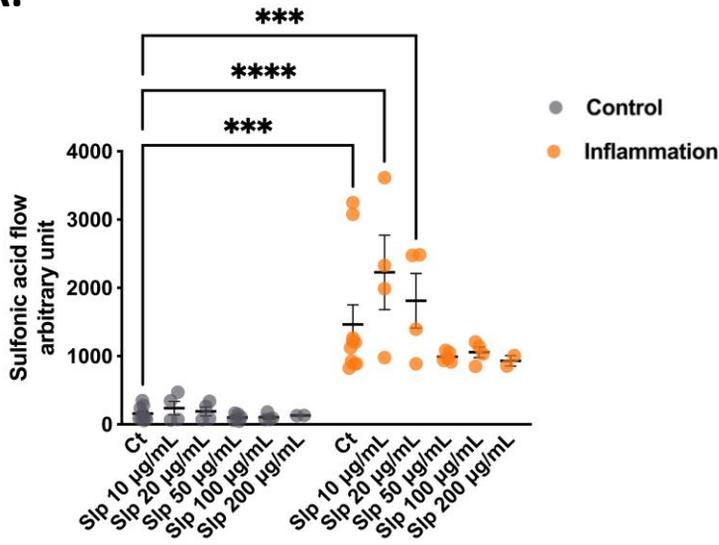


A.**B.****C.****D.****E.****F.****G.**

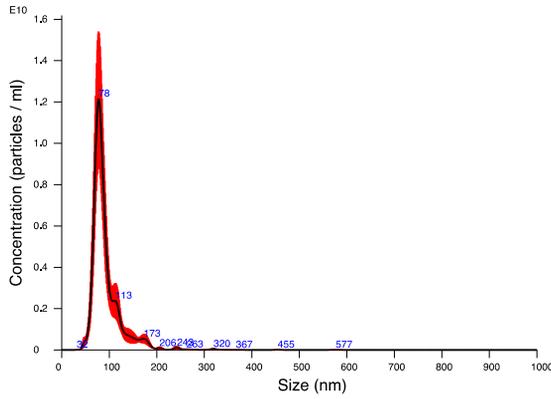


A.**B.****C.****D.**

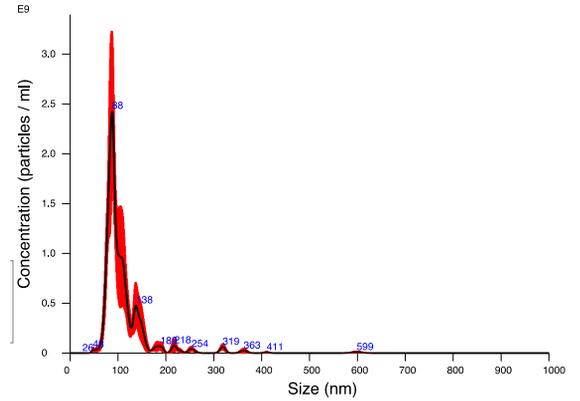
A.



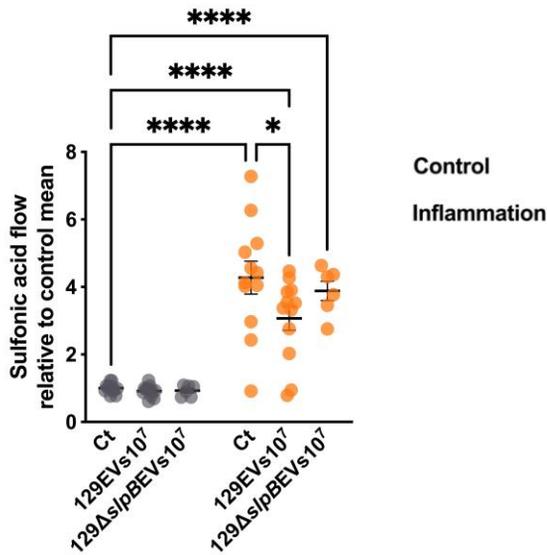
B.

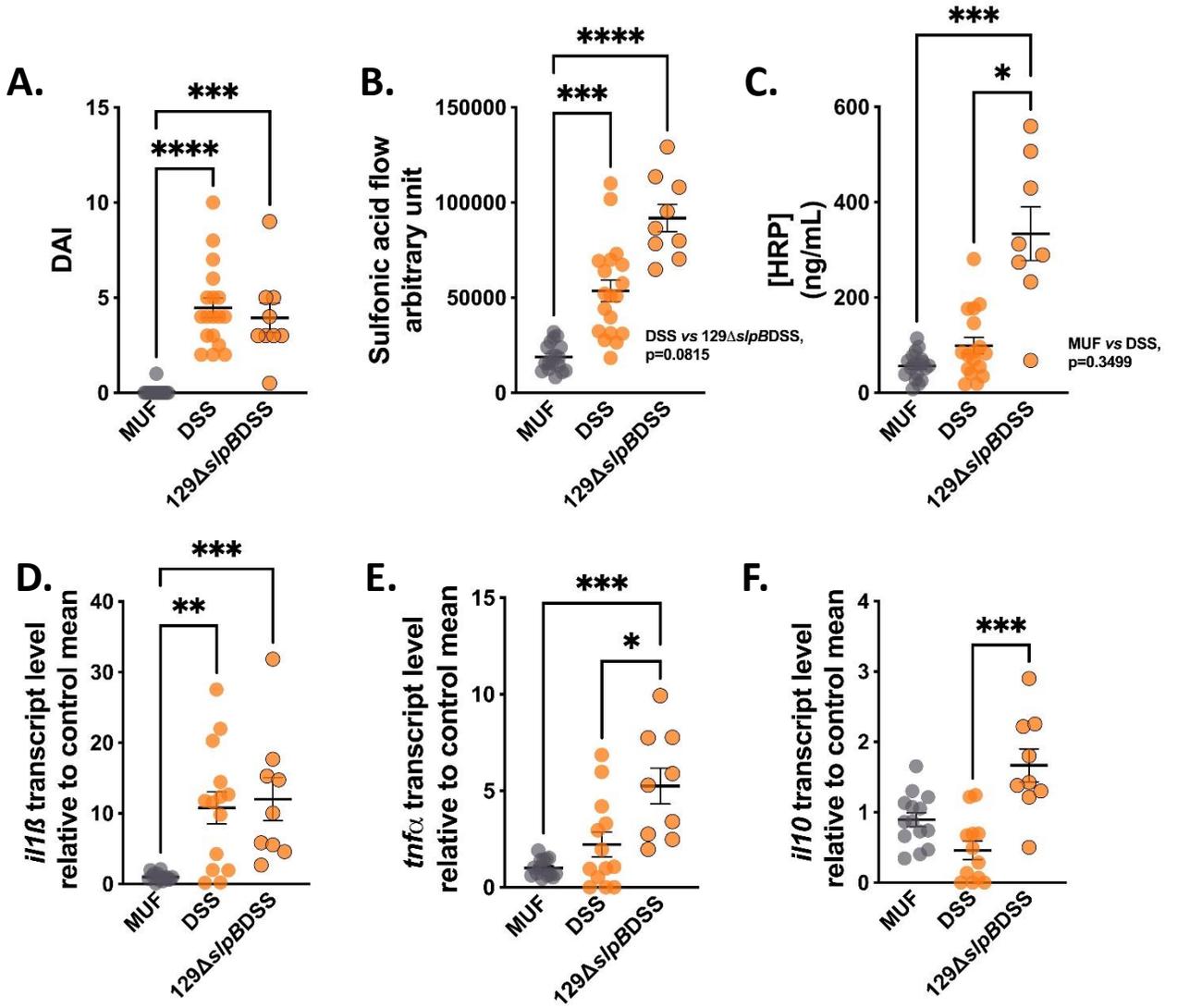


C.



D.

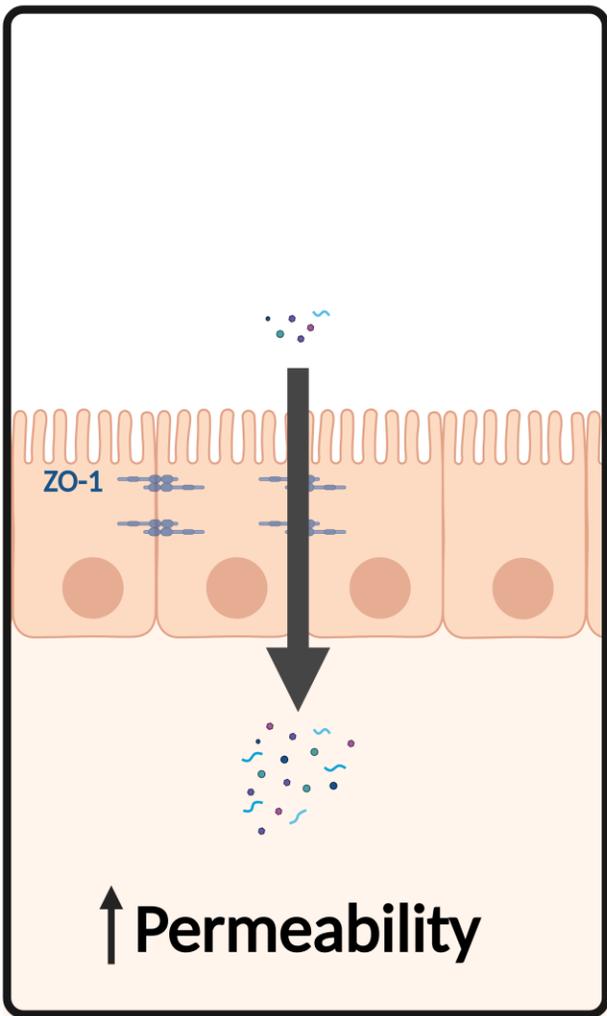




DSS-induced colitis



Without *Pf* supplementation



Pf supplementation

