

1           **Supplementary Material**

2

3           **Supplementary Methods**

4           *Fecal flagellin and lipopolysaccharide load quantification*

5           Levels of fecal bioactive flagellin and lipopolysaccharide (LPS) were quantified as  
6 previously described [1] using human embryonic kidney (HEK)-Blue-mTLR5 and HEK-  
7 Blue-mTLR4 cells, respectively (Invivogen, San Diego, CA, USA) [1]. Fecal material was  
8 resuspended in PBS to a final concentration of 100 mg/mL and homogenized for 10 s using a Mini-  
9 Beadbeater-24 without the addition of beads to avoid bacteria disruption. Samples were then  
10 centrifuged at 8000 g for 2 min and the resulting supernatant was serially diluted and applied on  
11 mammalian cells. Purified E. coli flagellin and LPS (Sigma-Aldrich) were used for standard curve  
12 determination using HEK-Blue-mTLR5 and HEK-Blue-mTLR4 cells, respectively. After 24 h of  
13 stimulation, the cell culture supernatant was applied to QUANTI-Blue medium (Invivogen) and  
14 the alkaline phosphatase activity was measured at 620 nm after 30 min.

15

16           *Serum immunoreactivity to LPS and flagellin*

17 Serum immunoreactivity to LPS and flagellin were examined by ELISA as described  
18 previously[2]. High-binding ELISA plates were coated overnight with purified flagellin (100  
19 ng/well; SRP8029-10UG, Sigma) or LPS (2 µg/well; from E. coli 0128: B12, Sigma) in 9.6 pH  
20 bicarbonate buffer. Sera were diluted 1:100 and added to wells coated with flagellin or LPS. After  
21 incubation at 37°C for 1h, the wells were washed and then incubated with HRP-conjugated anti-  
22 mouse IgG (1:1000). After washing, the peroxidase substrate tetramethylbenzidine (TMB) was  
23 added to the wells and, after 5 min, optical density (OD) was read at 450 nm with an ELISA plate  
24 reader. Data are reported as OD corrected by subtracting with the readings in blank samples.

25 *Colonic mRNA extraction*

26 Distal colon was collected during euthanasia and placed in RNA-Later (Invitrogen). Total  
27 mRNAs were isolated from colonic tissues homogenized with TRIzol (Invitrogen, Carlsbad, CA)  
28 according to the manufacturer's instructions and as previously described[3]. Briefly, chloroform  
29 was then added, and samples were incubated at RT for 3min. After centrifugation (12,000g, 4°C,  
30 15min), the aqueous phase was transferred in a new tube. RNA was precipitated by adding  
31 isopropyl alcohol and incubated overnight at -20°C. Samples were then centrifuged (12,000, 4°C,  
32 30min), supernatant was aspirated, and pellet washed with 75% ethanol. Ethanol was then  
33 eliminated after centrifugation (7,500, 4°C, 5min) and dried RNAs were dissolved in molecular  
34 grade water. RNAs were subsequently cleaned using the RNeasy Mini Kit (Qiagen) according to  
35 the manufacturer's instructions. Extracted purified RNAs were quantified, and purity assessed  
36 using a Thermo Scientific™ Nanodrop™ one.

37

38 *Colonic RNA sequencing*

39 Library preparation and sequencing. cDNA library was prepared using the Invitrogen™  
40 Collibri™ Stranded RNA library Prep Kit for Illumina™ with Collibri™ H/M/R rRNA Depletion  
41 Kit according to the manufacturer's instructions and starting with 500ng of purified RNAs. Briefly,  
42 rRNA were first depleted, and enriched mRNAs subsequently used for fragmentation, adaptors  
43 ligation and reverse transcription. After purification, libraries were PCR-enriched, further purified,  
44 and quantified and quality-assessed on an Agilent™ 2100 Bioanalyzer™ instrument. A master  
45 library was generated from the purified products in equimolar ratios. The pooled products were  
46 quantified using Qubit and sequenced using an Illumina Next-Seq sequencer (paired-end reads,  
47 2x750 bp) at Cornell University, Ithaca.

48        Data analysis. Cutadapt tool [4] online tool was first used in order to remove adapter  
49 sequences as well as trim sequences from the first low quality (<28) base. High quality reads  
50 longer than 20 nucleotides were then aligned to mm10 *mus musculus* reference genome using  
51 Bowtie2 [5]. Gene expression levels were next measured using Cufflinks [6] and differentially  
52 expressed genes between conditions were identified using Cuffdiff [6]. Fragments Per Kilobase of  
53 transcript per Million mapped reads (FPKM) unit was used and Log2 fold changes and q-values  
54 were computed for each comparison of interest. Principal coordinates analysis (PCoA) of the Bray-  
55 Curtis distance matrix of the colonic transcriptome were then generated for comparisons of interest.  
56 Gene level volcano plots were generated through R (version 4.1.2 (2021-11-01), Platform: x86\_64-  
57 apple-darwin17.0 (64-bit)). Differentially expressed genes enrichment analysis was performed  
58 using Metascape (<https://metascape.org/gp/index.html#/main/step1>) [7]. User-provided gene  
59 identifiers were converted into their corresponding *Mus musculus* gene IDs using the last version  
60 of the database (last updated on 2021-11-01). Briefly, functions and metabolic pathways were  
61 ordered according to their *P*-value (smaller *P*-value indicating higher ranking). For each given gene  
62 list, pathway and process enrichment analysis were performed with the following ontology sources:  
63 GO Biological Processes, KEGG Pathway, Reactome Gene Sets, CORUM, TRRUST, PaGenBase  
64 and WikiPathways. Terms ( $P < 0.01$ , minimum count of 3, enrichment factor  $> 1.5$ ) were collected  
65 and grouped into clusters based on their membership similarities. More specifically, *P*-values were  
66 calculated based on the accumulative hypergeometric distribution, and q-values were calculated  
67 using the Benjamini-Hochberg procedure to account for multiple testing. Kappa scores were used  
68 as the similarity metric when performing hierarchical clustering on the enriched terms, and sub-  
69 trees with a similarity of  $> 0.3$  were considered a cluster. The most statistically significant term  
70 within a cluster was chosen to represent the cluster. Heatmaps were generated using Morpheus  
71 (<https://software.broadinstitute.org/morpheus>) online tool using 297 and 421 genes, respectively.

72 They were then clustered by rows and columns using the average linkage hierarchical clustering  
73 and Spearman rank correlations. These genes were selected based on the following criteria 1)  
74 significantly different between mice receiving dietary emulsifier and mice receiving water only  
75 and 2) not common between C vs. W and CA vs. WA, or P vs. W and PA vs. WA, in order to focus  
76 on differences vanishing in the *A. muc.*-treated groups. Unprocessed sequencing data are deposited  
77 in the Genome Sequence Archive (GSA) in BIG Data Center, Beijing Institute of Genomics,  
78 Chinese Academy of Sciences, under accession number XXXXX, publicly accessible at  
79 <http://bigd.big.ac.cn/gsa>.

80

#### 81 *Immunostaining of mucins and localization of bacteria by FISH*

82 Mucus immunostaining was paired with fluorescent in situ hybridization (FISH), as  
83 previously described[8], in order to analyze bacteria localization at the surface of the intestinal  
84 mucosa[9,10]. Briefly, colonic tissues (proximal colon, 2nd cm from the cecum) containing fecal  
85 material were placed in methanol-Carnoy's fixative solution (60% methanol, 30% chloroform,  
86 10% glacial acetic acid) for a minimum of 3 h at room temperature and stored at 4°C. Tissues were  
87 then washed in methanol 2x30 min, absolute ethanol 2x15 min, ethanol/xylene (1:1) 15 min and  
88 xylene 2x15 min, followed by embedding in Paraffin with a vertical orientation. Four mm sections  
89 were performed and dewax by xylene 60°C for 10 min, xylene for 10 min and 99.5% ethanol for 5  
90 minutes. Hybridization step was performed at 50°C overnight with EUB338 probe (50-  
91 GCTGCCTCCCGTAGGAGT-30, with a 5' labeling using Alexa 647) diluted to a final  
92 concentration of 10 mg/mL in hybridization buffer (20 mM Tris-HCl, pH 7.4, 0.9 M NaCl, 0.1%  
93 SDS, 20% formamide). After washing 10 min in wash buffer (20 mM Tris-HCl, pH 7.4, 0.9 M  
94 NaCl) and a quick wash in PBS, slides were incubated in block solution (5% fetal bovine serum in  
95 PBS) in darkness at 4°C for 30 min. Slides were then gently dried and PAP pen (Sigma-Aldrich)

96 was used to mark around the section. Mucin-2 primary antibody (rabbit MUC2 antibody [C3], C-  
97 term, Genetex, GTX100664) was diluted 1:100 in block solution and applied overnight at 4°C.  
98 After washing 3x10 min in PBS, block solution containing anti-rabbit Alexa 488 secondary  
99 antibody diluted 1:300, Phalloidin-Tetramethylrhodamine B isothiocyanate (Sigma-Aldrich) at 1  
100 mg/mL and Hoechst 33258 (Sigma-Aldrich) at 10 mg/mL was applied to the section for 2h. After  
101 washing 3x10 min in PBS slides were mounted using Prolong anti-fade mounting media (Life  
102 Technologies, Carlsbad, CA, USA) and kept in the dark at 4°C. Observations and measurement of  
103 the distance between bacteria and epithelial cell monolayer were performed with a Spinning Disk  
104 IXplore using the Olympus cellSens imaging software 421 (V2.3) at a frame size of 2,048 x 2,048  
105 with 16-bit depth. A 405nm laser was used to excite the 422 Hoechst stain (epithelial DNA), 488nm  
106 for Alexa Fluor 488 (mucus), 488nm for TRITC (actin), 423 and 640nm for Alexa Fluor 647  
107 (bacteria). Samples were imaged with a 20x objective.

108 **Supplementary references**

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137           **Supplementary figure legends**

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139           **Supplementary Figure 1.** Inoculum purity and impact of daily gavage on *A. muc.* fecal  
140 relative abundance. **(A)** Purity of the obtained *in vitro* bacterial stock was determined by bacterial  
141 DNA extraction, 16S rRNA gene sequencing, and Greengenes taxonomic assignment. **(B)** Fecal  
142 abundance was measured by qPCR in fecal DNA extraction from days 0 and 28 samples.

143

144           **Supplementary Figure 2.** *A. muc.* administration prevents emulsifier-induced alterations  
145 in microbiota composition. Principal coordinates analysis (PCoA) of the unweighted Unifrac  
146 matrix of microbiota assessed by 16S rRNA gene sequencing at days **(A)** 0 and **(B)** 49 after  
147 removing all Qiime2-generated ASVs related to the Verrucomicrobia phylum. Each dot represents  
148 an individual animal and is color coded (blue, water; orange, CMC; purple, P80, light blue, water  
149 – *A. muc.*; light orange, CMC – *A. muc.*; light purple, P80 – *A. muc.*).

150

151           **Supplementary Figure 3.** *A. muc.* administration prevents dietary emulsifiers-induced  
152 microbiota alterations. Mice were exposed to drinking water (blue) containing 1.0% of CMC  
153 (orange) or P80 (purple) for 9 weeks, and gavaged 5 days per week with either sterile PBS (solid  
154 bars) or *A. muc.* (hatched bars). Fecal DNA was extracted at days 0 and 49 and subjected to 16S  
155 rRNA gene amplification and sequencing. The 20 most significantly differentially abundant  
156 features were identified using Microbiome Multivariable Associations with Linear Models  
157 (MaAsLin 2) and belonged to the **(A-B)** Allobaculum genus, **(C-D)** Clostridiaceae family, **(E-N)**  
158 S24-7 family, **(O-P)** Rikenellaceae family, **(Q)** Turicibacter genus, **(R)** Prevotella genus, **(S)**  
159 Odoribacter genus and **(T)** Ruminococcaceae genus. Data are represented as means  $\pm$  SEM. n=4-

160 5. Statistical analyses were performed using MaAsLin 2. *P*-values of interest were directly recorded  
161 on graphs and significant differences are highlighted in bold.

162

163 **Supplementary Figure 4.** Dietary emulsifiers and *A. muc.* administration impact on the  
164 intestinal environment. Mice were exposed to drinking water (blue) containing 1.0% of CMC  
165 (orange) or P80 (purple) for 9 weeks, and gavaged 5 days per week with either sterile PBS (solid  
166 bars) or *A. muc.* (hatched bars). Feces were collected at day 63 and (A) lipopolysaccharide (LPS)  
167 and (B) flagellin (FliC) were measured using TLR4 and TLR5 reporter cells. Serum was collected  
168 at euthanasia and (C) anti-lipopolysaccharide (LPS) and (D) anti-flagellin (FliC) IgG were  
169 measured. Data are represented as means  $\pm$  SEM. n=4-5. Statistical analyses were performed using  
170 a one-way ANOVA followed by a Bonferroni post-hoc test and significant differences were  
171 recorded as follows: \* $p$ <0.05, \*\* $p$ <0.01.

172

173 **Supplementary Figure 5.** *A. muc.* administration prevents emulsifier-induced  
174 alteration of the colonic transcriptome. Mice were exposed to drinking water containing 1.0%  
175 of CMC or P80 for 9 weeks, and gavaged 5 days a week with either sterile PBS or *A. muc.* Colon  
176 RNA was extracted and subjected to NextSeq sequencing. (A-B) Colonic transcriptome at the gene  
177 level was visualized on volcano plots for CMC vs. water (A) and P80 vs. water (B) comparisons.  
178 For each gene, the difference in abundance between the two groups is indicated in Log2 fold change  
179 on the x-axis (with positive values corresponding to an increase in emulsifier-treated group  
180 compared with water-treated group, and negative values corresponding to a decrease in emulsifier-  
181 treated group compared with water-treated group), and significance between the two groups is  
182 indicated by  $-\log_{10}$  q-value on the y-axis. (C-D) Colonic transcriptome at the gene level was

183 visualized on volcano plots for CMC – *A. muc.* vs. water – *A. muc.* (C) and P80 – *A. muc.* vs. water  
184 – *A. muc.* (D) comparisons. (E-F) PCoA of the Bray-Curtis distance matrix for the genes with  
185 significantly altered expression induced by CMC and/or P80 with dot colored by treatment (water  
186 = blue; CMC = orange; P80 = purple; water – *A. muc.* = light blue; CMC – *A. muc.* = light orange;  
187 P80 – *A. muc.* = light purple). PERMANOVA p-values are indicated in the bottom of each PCoA.

188

189 **Supplementary Figure 6. Impact of emulsifier consumption and *A. muc.* treatment on**  
190 **the colonic transcriptome.** (A) Overview of the number of genes with significantly increased  
191 expression following CMC or P80 consumption, with purple lines linking identical genes between  
192 CMC and P80 conditions. (B) Heatmaps listing overrepresented pathways / functions for CMC vs.  
193 Water and P80 vs. Water comparisons. (C) Overview of the number of genes with significantly  
194 decreased expression following CMC or P80 consumption, with purple lines linking identical  
195 genes between CMC and P80 conditions. (D) Heatmaps listing underrepresented pathways /  
196 functions for CMC vs. Water and P80 vs. Water comparisons. (E) Overview of the number of  
197 genes with significantly increased expression following CMC or P80 consumption in *A. muc.*-  
198 treated groups, with purple lines linking identical genes between CMC and P80 conditions. (F)  
199 Heatmaps listing overrepresented pathways / functions for CMC + *A. muc.* vs. Water + *A. muc.* and  
200 P80 + *A. muc.* vs. Water + *A. muc.* comparisons. (G) Overview of the number of genes with  
201 significantly decreased expression following CMC or P80 consumption in *A. muc.*-treated groups,  
202 with purple lines linking identical genes between CMC and P80 conditions. (H) Heatmaps listing  
203 underrepresented pathways / functions for CMC + *A. muc.* vs. Water + *A. muc.* and P80 + *A. muc.*  
204 vs. Water + *A. muc.* comparisons.

205

206           **Supplementary Figure 7: Impact of emulsifier consumption and *A. muc.* treatment on**  
207 **colonic transcriptome. (A)** Principal coordinates analysis (PCoA) of the Bray-Curtis distance  
208 matrix of the colonic transcriptome (all genes included) with dot colored by treatment (water =  
209 blue; water + *A. muc.* = light blue) **(B)** Heatmap of genes with an altered expression induced by  
210 CMC consumption and for which *A. muc.* administration prevents such difference. **(C)** Heatmap  
211 of genes with an altered expression induced by P80 consumption and for which *A. muc.*  
212 administration prevents such difference. Hierarchical clustering was performed based on gene  
213 expression and Spearman rank correlations. W: water; C: CMC, WA: Water + *A. muc.*; CA: CMC  
214 + *A. muc.*; PA: P80 + *A. muc.*.