Maltose attenuates the virulence of *Salmonella* Typhimurium by impairing its entry into epithelial cells

Kirti Parmar[1], Jayashree S[1], Dipshikha Chakravortty[1,2*]

[1]Department of Microbiology and Cell Biology, Division of Biological Sciences, Indian Institute of Science, Bangalore, India.

[2]Adjunct Faculty, School of Biology, Indian Institute of Science Education and Research, Thiruvananthapuram, India.

Corresponding author: Prof. Dipshikha Chakravortty

Email: dipa@iisc.ac.in

Abstract

The composition of nutrients in the intestine defines a niche for colonising gut pathogens. The lack of nutrients suitable for pathogens due to competition from resident intestinal microbiota or dietary preferences leads to colonisation failure by pathogens. In this study, we investigated the impact of the disaccharide sugar maltose on *Salmonella* Typhimurium (STM) pathogenicity during the early phases of infection in C57BL/6 mice and human colon carcinoma cells (Caco-2). We found that supplementation of maltose at lower concentrations inhibited STM colonisation in the ileum of mice. To understand this, we investigated the role of maltose metabolism in human colon epithelial (Caco-2) cells. Deleting the maltose metabolism gene (malQ) increased adhesion to Caco-2 cells. The increased adhesion was due to increased expression of type 1 fimbriae. Inhibiting the type 1 fimbriae-mediated binding to host epithelial cells by incubating with mannose resulted in similar adhesion of STM WT and STM Δ*malQ*. We further identified that *malQ* regulates the adhesion of Salmonella through the sigma factor RpoE. Overall, *malQ* in *Salmonella* inhibits infection in epithelial cells by reducing its adhesion to host epithelial cells.

Keywords-

Salmonella, maltose, intestinal epithelial cells, adhesion, fimbriae.

Introduction

Foodborne pathogens encounter various stresses inside and outside the host (1). Pathogen growth often occurs in an environment with limited nutrients inside the host, owing to competition from resident intestinal microbiota in the host intestine (2). Bacteria, such as *Salmonella,* can successfully modulate their metabolism in response to the availability of nutrients. *Salmonella enterica* serovar Typhimurium causes self-limiting gastroenteritis in humans and causes bacteraemia in immunocompromised individuals (3).

Successful *Salmonella* colonisation depends on utilising multiple carbon sources, including glycerol, fatty acids, N-acetylglucosamine, gluconate, glucose, lactate, and arginine (4). Maltose in the intestine plays a pivotal role in the growth and pathogenesis of various bacteria, both beneficial and detrimental. Maltose is a commonly present carbohydrate source in food products like sweet potatoes and barley, and its concentration increases during cooking and fermentation (5). Compared with glucose, sugars such as maltose/maltodextrins are preferred for growth on poor nitrogen sources (6). The utilisation of maltose is also crucial for colonisation of the mouse intestine by *E.coli (7).*

In contrast, maltose derivatives reduce the biofilm production and adhesion of *Pseudomonas aeruginosa* non-swarming mutants (8). In zebrafish and crucian carp, maltose modulates immunity against *Vibrio alginolyticus* and *Aeromonas sobrial,* respectively, by increasing lysozyme activity and expression (9, 10). Lastly, in *Vibrio cholerae,* maltose inhibits cholera toxin secretion and reduces pathogenicity in newborn mice (11). Maltodextrins promote *Salmonella* survival in the bone marrow-derived macrophages by reducing NADPH expression and recruitment to the *Salmonella* containing vacuole. *In vivo,* maltodextrins degrade the intestinal mucosa of mice, increasing the cecal load (12). These observations highlight the diverse roles of maltose and maltodextrin in bacterial growth and pathogenesis. However, it

51 remains unclear how maltose, a carbohydrate, affects *Salmonella's* virulence factors. A strain
52 deficient in the maltose hydrolysis gene (*malQ*) was constructed further to study the link
53 between maltose metabolism and virulence. This study aimed to understand the modulation of
54 *Salmonella* virulence with respect to maltose metabolism.

55 **Materials and Methods-**

56 **Bacteria and growth conditions**- Wild-type *Salmonella* Typhimurium 14028s (STM WT) was
57 obtained from Professor Michael Hensel (Universität Osnabrück). STM WT was revived from
58 glycerol stock kept at -80°C and streaked on LB-agar; the respective antibiotic-containing
59 plates were used for the knockout and complement strains. All the bacterial strains were grown
60 in LB supplemented with the required antibiotics at 37°C and 170 rpm overnight. The strain
61 containing pkD46 was grown at 30°C and 170 rpm. For induction of the complement strain,
62 0.2% (13.3 mM) L-arabinose was used during the bacterial subculture. A list of bacterial strains
63 used in the study is attached in Supplementary Table 1.

64 **Construction of knockout strains** - The gene knockout strains were prepared using a one-step
65 chromosomal gene inactivation protocol developed by Datsenko and Wanner (13). In this
66 protocol, knockout primers were designed to be 40 bp homologous to the gene to be knocked
67 out, with a 20 bp extension for the antibiotic cassette. The gene coding for the antibiotic is
68 amplified from the pKD3 or pKD4 plasmid, and through homologous recombination, the gene
69 is replaced by an antibiotic cassette using electroporation. Amplified cassettes were inserted
70 into STM pKD46, which has a lambda red recombinase system under an arabinose-inducible
71 promoter. For electroporation, STM pKD46 bacterial cells were grown to the log phase, and
72 the absorbance was adjusted to 0.3. The cells were then made electrocompetent by washing
73 with MilliQ water and 10% glycerol at 4°C. The knockout strains were screened by plating the
74 bacteria on an antibiotic-containing plate and performing PCR on the obtained colonies. The
75 primers used in the study are attached in Supplementary Table 2.

76 **Construction of complement strains-** *malQ* gene amplified from *Salmonella* Typhimurium
77 and the pBAD vector was digested with KpnI and PstI at 37°C for 1 hour. Digested products
78 were gel purified and ligated with T4 DNA ligase (NEB). Ligated products were transformed
79 into *E.coli* Tg1 competent cells using the heat shock method. Transformed cells were plated on
80 the LB-chloramphenicol and incubated overnight at 37°C. Plasmid isolation was performed
81 from transformants. Cloning was confirmed by double digestion. After successful
82 confirmation, the clone was transformed into STM Δ*malQ.*

83 Construction of reporter strains- For the generation of reporter strains, the upstream regions of
84 the genes *fimA* and *rpoE* were cloned into the pUA66 vector. The restriction sites used are
85 BamHI and XhoI (NEB). The ligated product was transformed into STM WT and STM Δ*malQ.*

86 **Flow cytometry**- Reporter strains were grown in LB medium and subcultured at a 1:33 ratio
87 to obtain a log phase culture. Log phase culture was utilised for FACS. Positive GFP percentage
88 population was analysed using CytoFLEX for 10,000 events.

89 **Eukaryotic cell lines and growth conditions**- Cell line Caco-2 used in this study was
90 maintained in Dulbecco's Modified Eagle's Media (Sigma-Aldrich) supplemented with 10%
91 FBS (fetal bovine serum, Gibco), 1% sodium pyruvate, 1% penicillin-streptomycin, and 1%
92 non-essential amino acids at 37°C in the presence of 5% CO_2.
93 Cells were grown to confluence, and after counting them with a haemocytometer, an equal
94 number of cells were seeded into 24-well plates.

95 **Invasion assay**- One lakh seeded Caco2 cells were infected with bacteria at an MOI of 10,
96 and a log phase culture was used to infect the epithelial cells (Caco2 cells). Cells were
97 centrifuged at 800 rpm for 5 minutes and then incubated at 37°C with 5% CO2 for 25 minutes.
98 Cells were then washed with 1X PBS to remove all extracellular bacteria, and media containing
99 100µg/ml gentamicin in DMEM with FBS was added for 1 hour. Similarly, 25 µg/ml of

100 gentamicin in DMEM media with FBS was added, and the cells were incubated until lysis.
101 Cells were lysed with 0.1% Triton X-100 at 2 hours. Bacteria were plated on the LB Agar,
102 incubated at 37°C for 12 hours, and colonies were counted. To calculate the percentage
103 invasion, bacterial CFU at 2 hours were divided by the pre-inoculum CFU.

104 **Adhesion assay** – log phase bacteria were adjusted to 0.3 OD and incubated with and without
105 0.2M Mannose for 1 hour before infection. For adhesion experiments with mannose, the
106 bacteria were kept in static Luria broth at 37°C after subculture to induce type 1 fimbriae (14).
107 Bacteria have been kept at 37°C, 170 rpm for all the other experiments.

108 Caco2 cells were infected with an MOI of 10 and incubated for 10 minutes. Cells were washed
109 with 1X PBS and lysed using 0.1% Triton X-100. Bacteria were plated on LB Agar and
110 incubated at 37°C for 12 hours, after which the colonies were counted. The number of adhered
111 cells was calculated by dividing the obtained CFU by the pre-inoculum values of the bacteria
112 used for infection. The cells were seeded with coverslips for confocal microscopy, and the
113 assay mentioned above was performed. At the endpoint, the cells were fixed using 3.5%
114 Paraformaldehyde for 10 minutes, followed by washing with 1x PBS and storing the samples
115 in 1x PBS.

116 **Confocal microscopy**- A protocol from Roy Chowdhury et al. was followed (15). The PFA
117 fixed samples from the adhesion assay were stained with a primary antibody against *Salmonella*
118 (1:500 in 2.5% BSA) overnight at 4°C. The primary antibody was washed with 1xPBS, and the
119 secondary antibody was added for 45 minutes at room temperature. Samples were then stained
120 with FM-464 for 10 minutes and subjected to a final wash. Coverslips were mounted on the
121 slides after the addition of mounting media and sealed.

122 Percentage adhesion was calculated by dividing the number of bacteria by the number of Caco2
123 cells in the acquired images. Images were acquired using a Zeiss 880 confocal microscope and
124 analysed by Zeiss Zen Black 2012 software.

125 **Biofilm formation**- Bacteria were grown until the stationary phase, inoculated in biofilm
126 media (tryptone and yeast extract) with different concentrations of maltose, and incubated at
127 28°C for 3 days. The biofilm formed was measured using crystal violet staining, and the
128 absorbance was measured at 570 nm.

129 **RNA isolation and cDNA Synthesis**- STM WT and STM Δ*malQ* were subcultured (1:100) in
130 LB to achieve a log phase for analysis of various invasion and adhesion genes. The bacterial
131 culture was pelleted down by centrifugation at 6000 rpm for 10 min, and 1 ml of TRIzol
132 (Takara) was added to the pellet. Samples were stored at -80°C until they were processed. The
133 lysed supernatant was extracted with chloroform, and RNA precipitation was done using an
134 equal volume of isopropanol. The obtained pellet was washed with 70% ethanol in DEPC water
135 and air-dried. The RNA was dissolved in DEPC water. The quality and quantity of RNA were
136 checked using Nanodrop and 2% agarose gel electrophoresis. DNase treatment (Thermo Fisher
137 Scientific) was performed on the isolated RNA at 37°C for 1 hour, followed by heat
138 inactivation at 65°C for 15 minutes. DNase-treated RNA was converted to cDNA using the
139 PrimeScript Reagent RT kit (Takara). qRT-PCR was performed using the TB Green RT-qPCR
140 Kit in a real-time PCR system. For the analysis of RT data, 16S rRNA was used as the reference
141 gene to calculate the delta Ct (ΔCt) of the samples. ddct was calculated by comparing two
142 different samples.

143 **Animal experiments-** All the animals were acquired from the central animal facility at the
144 Indian Institute of Science, Bangalore. Five- to six-week-old healthy C57BL/6 male mice were
145 used in the study, and they were maintained in stress free environments before infection for 2-
146 3 days, 5 animals per cage.

147 Maltose supplementation (100 µL) at various concentrations or Milli Q water as control was
148 administered to the mice by oral gavage 1 hour before infection. The mice were then infected
149 with 10^7 (100 µL) bacteria of STM WT or STM *ΔmalQ* as mentioned in the figure legends.
150 Mice were euthanised 24 hours post-infection, and ileum sections free from faeces were taken,
151 homogenised and plated on *Salmonella Shigella* agar and normalised to organ weight. Mice
152 were euthanised by overdosage of isoflurane (2%) followed by cervical dislocation. 6 animals
153 were used for this experiment for each condition and total 36 animals were used in this
154 experiment. No data points were excluded during data analysis.
155 To determine the organ burden in mice, mice were orally gavage with 10^7(100µL) bacteria
156 (STM WT, STM *ΔmalQ,* STM *ΔmalEFG,* STM *ΔmalQ:malQ),* euthanised, and various organs
157 -Blood, Liver, mesenteric lymph node (MLN), spleen were acquired 5th day post infection.
158 Blood was isolated by performing a heart puncture. Mouse organs were homogenised and
159 plated on *Salmonella Shigella* agar to determine the bacterial burden in various organs and
160 normalised to organ weight. 5 animals were used for this experiment for each bacterial strain
161 and total 20 animals were used in this experiment. The animal experiments were not performed
162 in a blind manner, and authors were aware of the different strains used to infect mice in various
163 cages.
164
165 **TEM-** Transmission electron microscopy was performed using a previously described protocol
166 (16). Stationery and log phase cultures of STM WT and STM Δ*malQ* were washed twice with
167 1X PBS and resuspended in 50 µL of 1X PBS. 5 µL of the sample was placed on copper grids.
168 After air drying, samples were negatively stained with 1% uranyl acetate. TEM microscopy
169 was performed using a JEM-100CXII JEOL.
170 **Statistics**- The Statistical test, number of biological (N) and technical replicates (n) used in
171 the experiments are mentioned in the figure legends. For the animal experiments, the Mann-

172 Whitney test was performed. All the data were plotted and analysed using GraphPad Prism 8.

173 A *p*- value of <0.05 was considered statistically significant in the experiments.

174

175

176 **Results**

177 **The absence of maltose metabolism increases invasion and adhesion.**

178 Previous reports showed that colonising mice with simple sugars utilised by intestinal
179 microbiota decreases the pathogen burden by competing with the pathogen for that sugar (17).
180 To determine the role of maltose in pathogenesis, C57BL/6 mice were treated with various
181 concentrations of maltose. It was observed that at 1% maltose treatment, STM WT infection
182 was reduced in the ileum at 24 hours. This inhibition was not observed at higher maltose
183 concentrations. STM WT and deletion of the maltose hydrolysing gene (STM Δ*malQ)* had
184 similar organ burden in the ileum when no maltose supplementation was provided **(Figure 1A).**
185 Maltose metabolism has been reported to reduce bacterial colonisation from the third day post-
186 infection(7). It was observed that the STM Δ*malQ* showed colonisation defects in blood,
187 mesenteric lymph node (MLN), liver and spleen compared to STM WT at 5 days post-infection
188 **(Supplementary Figure 1).** However, maltose transporter deletion (STM Δ*malEFG)* showed
189 no growth defect in colonising various mouse organs.

190 To further study the role of maltose in *Salmonella* infection, upon infection of Caco-2 cells, we
191 observed an increased invasion in Caco-2 cells of STM Δ*malQ,* which is reduced upon
192 complementation of the *malQ* gene **(Figure 1B).** Adhesion to host cells is the first step in
193 facilitating the invasion and colonisation of the host. *Salmonella* adhesion depends on various
194 fimbriae and adhesins secreted by the type 1(BapA and SiiE) and type 5 (MisL, ShdA and
195 SadA) secretion systems. Large outer membrane proteins, such as PagN and Rck, also
196 contribute to adhesion (18). We further examined the adhesion of the *malQ* deleted strain and
197 observed an increased adhesion of Caco2 cells **(Figure 1C).** Upon investigating the mRNA
198 expression of various factors responsible for the invasion and adhesion in the *malQ* deleted
199 strain compared to the STM WT by qRT-PCR. It was observed that SPI-1 genes (*hilA*, i*nvF,*
200 *sipA),* responsible for the invasion, were downregulated in STM Δ*malQ* compared to STM WT

201 **(Figure 1D).** In the literature, it has been demonstrated that invasion of Caco-2 cells is largely
202 independent of SPI-1, PagN and RcK. The triple knockout of these genes could invade only
203 slightly less than the WT (19). Next, upon investigation of the adhesion genes, encoding large
204 proteins *pagN* and *ompD* were downregulated, whereas *siiE* was upregulated in STM Δ*malQ*
205 ***(*Figure 1E).** *siiE* has been reported to play a role in adhesion only in polarised Caco-2 cells
206 (20). *fimA,* a major structural protein of the type 1 fimbriae that mediates adhesion, was
207 upregulated in STM Δ*malQ* compared to STM WT **(Figure 1F).**

208 **Deletion of the *malQ* gene increases the expression of type 1 fimbriae.**

209 Upon cloning the *fimA* promoter upstream of GFP, we observed that promoter activity was
210 significantly increased in STM Δ*malQ,* resulting in a higher percentage of positive population
211 compared to STM WT **(Figure 2A)**. Upon visualisation of type 1 fimbriae with transmission
212 electron microscopy (TEM), more type 1 fimbriae were observed in STM Δ*malQ* bacteria
213 collected from the overnight culture compared to STM WT. Thus, the deletion of *malQ*
214 increased the type 1 fimbriae production **(Figure 2B).** Thus, we concluded that increased *fimA*
215 might be responsible for the increased adhesion and invasion. Our results are in accordance
216 with the literature, which shows an inverse relation between the expression of SPI-1 and type
217 1 fimbriae (21). The above results demonstrate that the deletion of *malQ* is associated with
218 concomitant increases in fimbriae production, suggesting the anti-adhesive role of *malQ.*

219 **Increased adhesion is due to the enhancement of type 1 fimbriae.**

220 Type 1 fimbriae bind to host glycoproteins such as mannose using FimH, and incubation with
221 0.2 M Mannose can inhibit the binding of *Salmonella* to the host by Type 1 fimbriae (22). To
222 elucidate whether the increased adhesion depended on the interaction with mannose residues
223 of the eukaryotic cells, bacteria were incubated for 1 hour before infection with 0.2M
224 mannose. The increased adhesion of the STM Δ*malQ* was significantly reduced by

225 pretreatment with mannose **(Figure 3A).** Confocal microscopy was performed to confirm
226 further that the increased adhesion in STM Δ*malQ* was due to increased fimbriae. Increased
227 adhesion to Caco2 of STM Δ*malQ* compared to STM WT was observed, which was inhibited
228 by adding mannose **(Figure 3B).** Type 1 fimbriae have also been reported to facilitate biofilm
229 formation (23). The biofilm was grown with various concentrations to determine the role of
230 maltose in biofilm formation. Maltose also acted as an inhibitory signal in biofilm formation.
231 (**Supplementary Figure 2A)**. Furthermore, in the biofilm assay with STM Δ*malQ,* STM
232 Δ*malEFG* and STM Δ*lamB,* it was observed that there was no significant reduction of
233 biofilm in STM Δ*malQ* and STM Δ*malEFG,* compared to STM WT (**Supplementary Figure**
234 **2B)**. Thus, it suggested that the maltose hydrolysis gene *malQ* and maltose transport into
235 bacteria by *malEFG* mediated the decreased biofilm.

236 To elucidate the mechanism of STM *ΔmalQ*-mediated regulation of type 1 fimbriae. The
237 mRNA expression levels of type 1 fimbriae regulators (*fimY, fimZ* and *fimW*) were
238 determined. FimY and FimZ positively regulate type 1 fimbriae in *Salmonella*, whereas
239 FimW negatively regulates them. FimZ also represses the expression of SPI-1 via *hilE* (24,
240 25). There was no change in the expression of *fimZ,* whereas *fimY* and *fimW* were
241 significantly upregulated in the STM *ΔmalQ* **(Figure 3C).** The significantly increased
242 expression of *fimY* might be responsible for the increased production of type 1 fimbriae.

243 **RpoE regulates type 1 fimbriae.**

244 Type 1 fimbriae in Gram-negative bacteria, such as uropathogenic *E.coli* and *Serratia*
245 *marcescens,* are reported to be repressed by the carbohydrate metabolism-regulating system
246 cAMP-CRP (26, 27). Upon analysis of the mRNA expression of CRP in STM *ΔmalQ,* we
247 observed significant downregulation compared to STM WT (**Figure 4A)**. Thus, the increased
248 type 1 fimbria production in STM *ΔmalQ* was due to increased expression of *fimY,* which

249 might be modulated by the decreased expression of CRP. Previous reports have shown that
250 the *rpoE* promoter 1 may be negatively regulated by cAMP-CRP in *Salmonella,* inducing
251 promoter 3 of rpoE during carbon starvation (28). *rpoE* also regulates the pathogenicity genes
252 of *E.coli* in interaction with human brain microvascular endothelial cells and the thin
253 aggregative fimbriae(Agf) in *Salmonella* (29, 30). The qRT-PCR analysis of the *rpoE* gene in
254 STM Δ*malQ* revealed upregulation of *rpoE* compared to STM WT (**Figure 4B)**. Similarly,
255 for the promoter activity of *rpoE*, we cloned promoter 3 of *rpoE* upstream of GFP and
256 observed that STM Δ*malQ* has a significantly higher percentage positive population
257 expressing *rpoE* compared to STM WT (**Figure 4C)**. Thus, *malQ* deletion leads to increased
258 promoter activity and transcript levels of *rpoE*. To determine the role of *rpoE* in the increased
259 adhesion of STM *ΔmalQ,* we deleted *rpoE* in STM Δ*malQ* and observed that the increased
260 adhesion of STM Δ*malQ* was lost when performing adhesion to Caco2 cells. STM Δ*rpoE* had
261 less adhesion than STM WT, and double deletion of *rpoE* and *malQ* resulted in adhesion
262 similar to that of STM Δ*rpoE* (**Figure 4D)**. Thus, the increased adhesion of STM *ΔmalQ* was
263 mediated by the alternative sigma factor RpoE.

264

265 **Discussion-**

266 Dietary polysaccharides in food are broken down into disaccharides (maltose, sucrose, and
267 lactose). *Salmonella* is generally considered unable to utilise disaccharides such as sucrose and
268 lactose; however, serovars like *Salmonella* Mbandaka and *Salmonella* Bareilly can ferment
269 sucrose (31, 32). Recent reports have also shown that certain *Salmonella* Typhimurium types
270 can utilise sucrose due to the acquisition of genes important for metabolism (31). Antibiotic-
271 resistant lactose fermenters of *Salmonella* Typhimurium have been reported in cattle (32).
272 Compared to sucrose and lactose, the most utilised disaccharide sugar, maltose, in the intestine
273 by *Salmonella,* has been studied less in its role in pathogenesis. In this study, we investigated
274 the role of maltose in modulating the virulence of *Salmonella.*

275 Maltose utilisation is essential for the survival of *E.coli* in the mouse intestine, as shown by Jones et
276 al. (7). As observed earlier, we found no difference in the intestinal colonisation of maltose
277 metabolism mutant STM Δ*malQ* in the mouse's ileum after 1-day post-infection. However, decreased
278 ileum colonisation of STM WT was observed in mice treated with 1% maltose. Furthermore, STM
279 Δ*malQ* showed higher invasion and adhesion in the epithelial cells Caco-2. The higher adhesion
280 depended on the higher expression of type 1 fimbriae by STM Δ*malQ.* The Inhibition of adhesion
281 mediated by type 1 fimbriae with the addition of mannose reversed the advantage of increased
282 adhesion. The mechanism shown by Muller et al. involves the Carbohydrate metabolism-regulating
283 system cAMP-CRP, which mediates type 1 fimbriae expression through fimB-mediated
284 recombination in *E.coli,* a process absent in *Salmonella (26).* Further studies are required to elucidate
285 how the maltose metabolism gene (*malQ*) regulates fimbriae production through the cAMP-CRP
286 pathway. We attempted to overexpress CRP in STM Δ*malQ* but were unable to do so, as the
287 induction was toxic to the bacteria. The alternative sigma factor *rpoE* is essential for maintaining the
288 membrane integrity of bacteria and for coping with various stresses they face (33). RpoE has also
289 been known to induce fimbriae and invasion in *E.coli* (29). We observed higher mRNA expression

290 and promoter activity of *rpoE* in STM Δ*malQ* compared to STM WT. Deletion of the *rpoE* in STM
291 Δ*malQ* had a similar effect to the addition of mannose, and STM Δ*rpoE,* STMΔ*malQ*Δ*rpoE* had
292 similar adhesion. Thus, the increased adhesion of STM Δ*malQ* is dependent on the presence of *rpoE*.

293 In conclusion, we have demonstrated that the maltose-hydrolysing gene (*malQ*) reduces invasion and
294 adhesion to Caco-2 epithelial cells. The reduced adhesion is mediated by the reduced expression of
295 type 1 fimbriae, which is mediated by RpoE. Therefore, our study reveals a novel link between
296 dietary sugar and the adhesion of the foodborne pathogen *Salmonella*.

297

298

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Ethics statement

All animal experiments were approved by the Institutional Animal Ethics Committee, Indian Institute of Science, Bangalore, India. The ethical clearance number for this study was CAF/ethics/852/2021. All experiments were conducted in strict accordance with the guidelines provided by National Animal Care and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, a statutory Committee, established under Chapter 4, Section 15 (1) of the Prevention of Cruelty to Animals Act 1960). (CPCSEA Registration No. 435 48/1999).

CRediT authorship contribution statement

Kirti Parmar- writing- review and editing, visualisation, experimental design, project administration, investigation and data analysis. **Jayashree S-** Investigation. **Dipshikha Chakravortty**- writing- review and editing, visualisation, experimental design, investigation, data analysis, resources and supervision.

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Declaration of competing interest

The authors declare no conflict of interest.

Data and material availability

All the data are available in the main text or the supplementary file.

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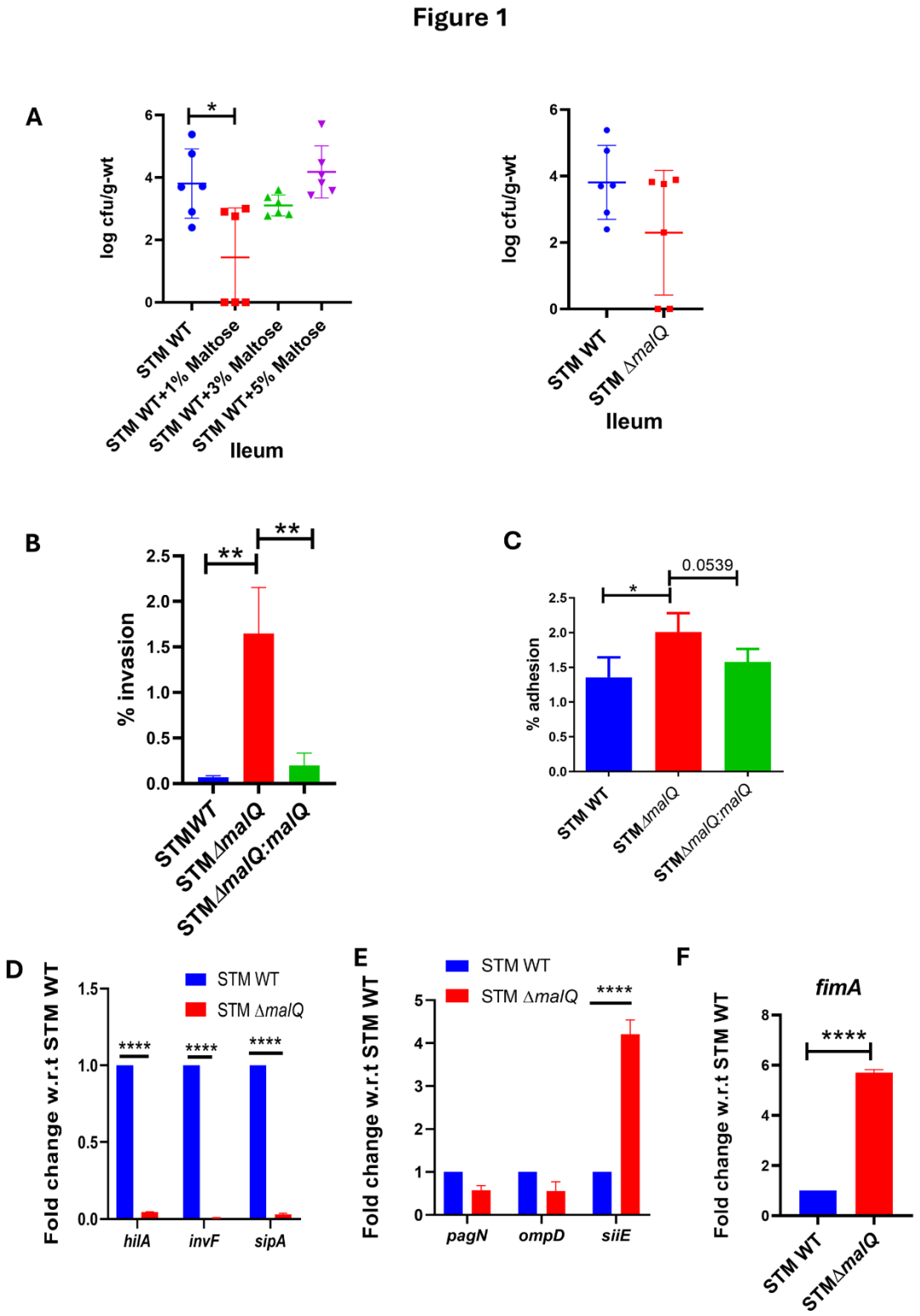
423 **Main figure legends**

424 **Figure 1. *malQ* deletion increases *Salmonella* invasion and adhesion.**

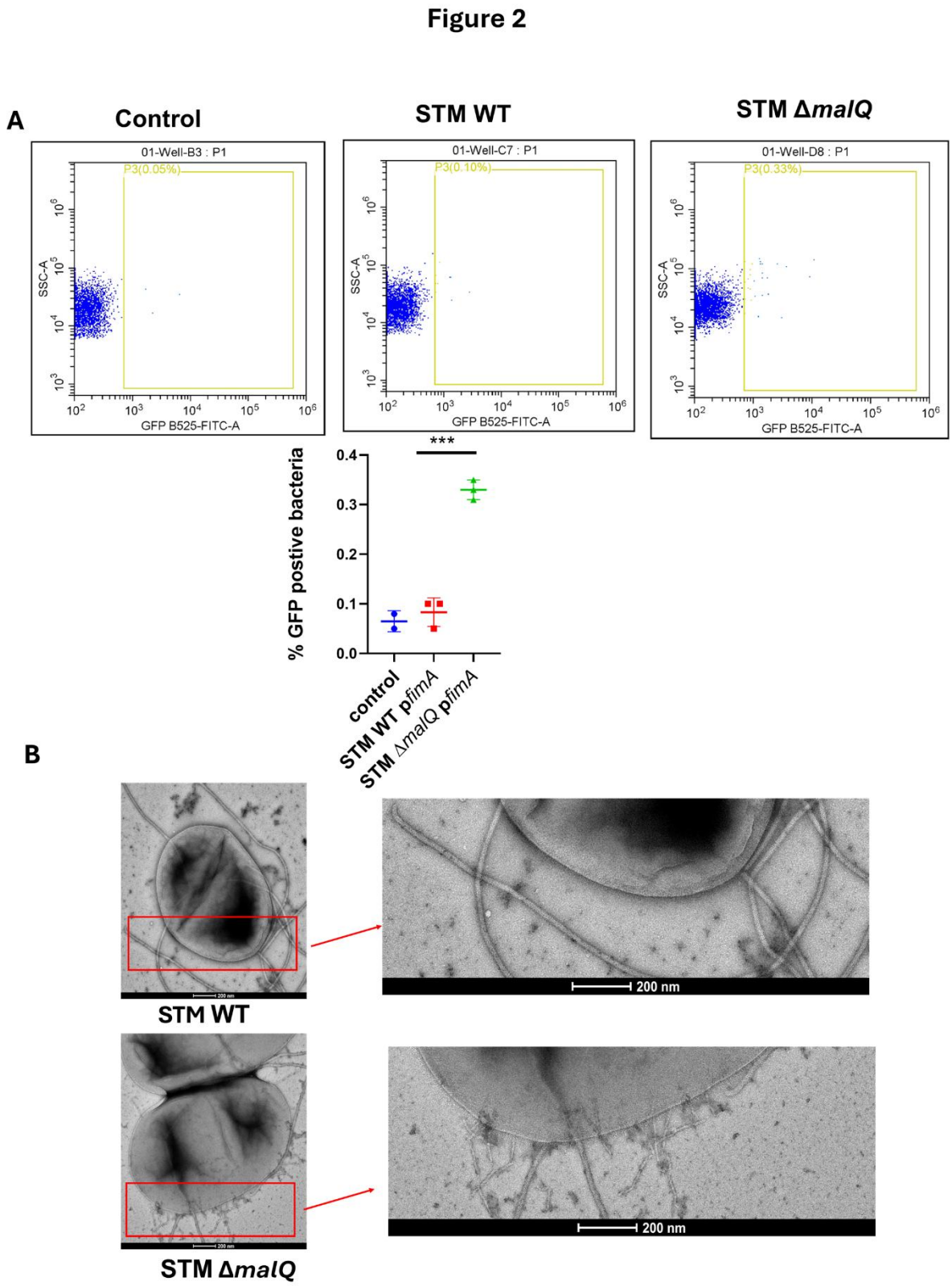


425

426 A. Organ burden in the ileum of mice upon supplmentation with various concentrations of
427 maltose. The Mann-Whitney test was used for analysis, with six animals per cohort,
428 presented as mean ± SD.
429 B. Percentage invasion of STM WT, STM Δ*malQ,* and STM Δ*malQ:malQ* in Caco2 cells.
430 An unpaired Student's t-test was used for the analysis. Data are representative of N=3,
431 n=3, presented as mean ±SD.
432 C. Percentage adhesion of STM WT, STM Δ*malQ, and* STM Δ*malQ:malQ* in Caco2 cells.
433 An unpaired Student's t-test was used for the analysis. Data are representative of N=3,
434 n=3, presented as mean ±SD.
435 D. mRNA expression of SPI-1 genes (*hilA, invF, sipA)* by q-RT PCR, Analysis by two-
436 way ANOVA, Data are representative of N=3, n=3, presented as mean ±SD.
437 E. mRNA expression of large proteins aiding in adhesion (*pagN, ompD, siiE)* by q-RT
438 PCR. Analysis by two-way ANOVA. Data are representative of N=3, n=3, presented as
439 mean ±SD.
440 F. mRNA expression of type 1 fimbriae (*fimA)* by q-RT PCR. An Unpaired Student's t-test
441 was used for the analysis. Data are representative of N=3, n=3, presented as mean ±SD.
442

443

444 Figure 2. ***malQ* deletion in *Salmonella* increases type 1 fimbriae**

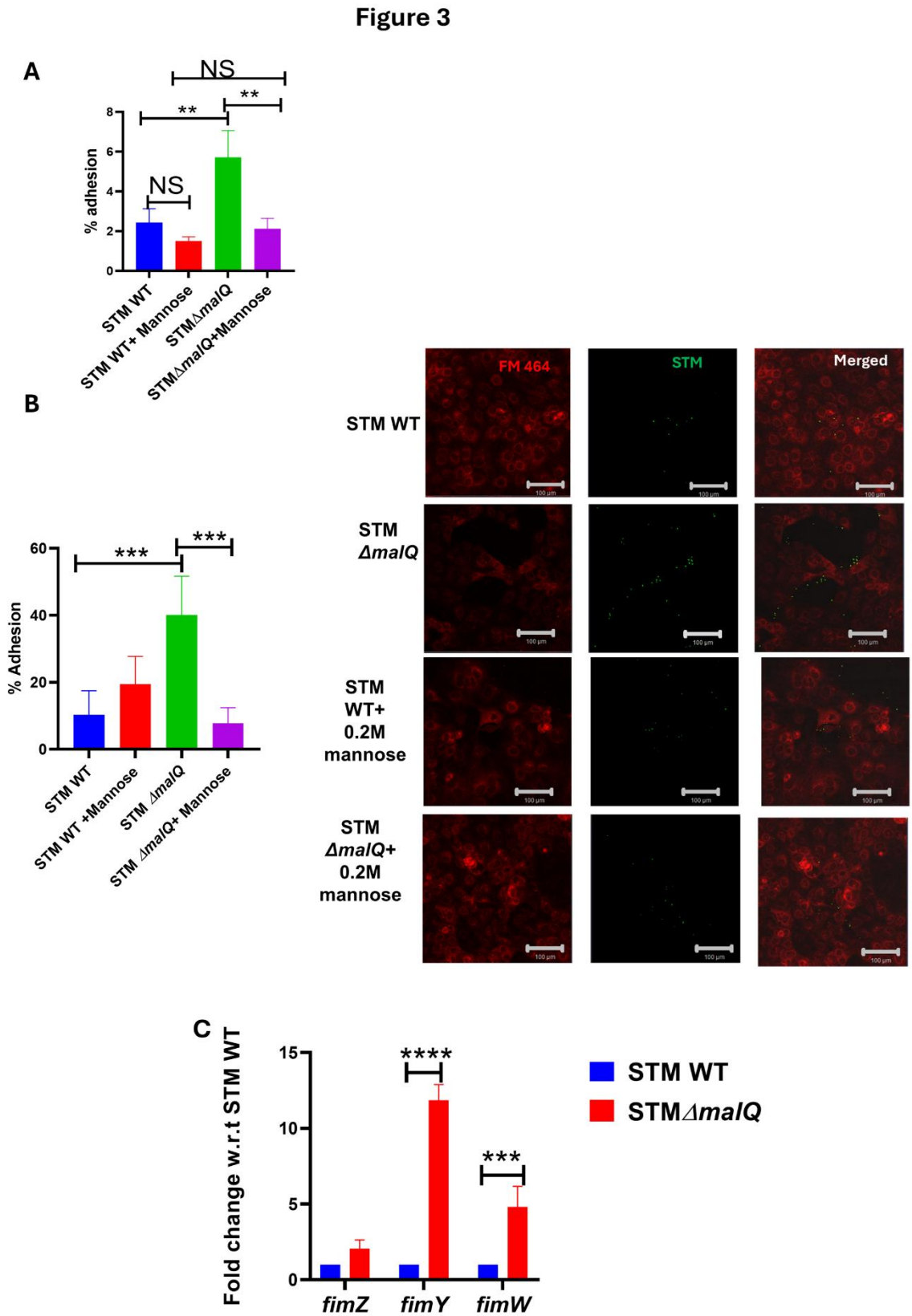


445

A. Activity of *fimA* promoter in STM WT and STM Δ*malQ* in the log phase using FACS. Analysis by one-way ANOVA. Data are representative of N=3, n=3, presented as mean ±SD.

B. Transmission electron microscopy images of STM WT and STM Δ*malQ*.

452 **Figure 3. Type 1 fimbriae mediate the increased adhesion of STM Δ*malQ*.**



453

454 A. The percentage adhesion of STM WT and STM Δ*malQ* in Caco-2 cells treated with and
455 without 0.2 M mannose. One-way ANOVA was used for analysis. Data are
456 representative of N=3, n=3, presented as mean ±SD.

457 B. Confocal microscopy was used to determine the adhesion percentage of STM WT and
458 STM Δ*malQ* in Caco-2 cells on treatment with and without 0.2 M mannose. One-way
459 ANOVA was used for the analysis. Data are representative of N=2, n=5, presented as mean
460 ±SD.

461 C. mRNA expression of the fimbrial regulatory molecules *fimZ, fimY* and *fimW* in STM
462 WT and STM Δ*malQ* by using q-RT PCR. Two-way ANOVA was used for the
463 analysis. Data are representative of N=3, n=3, presented as mean ±SD.

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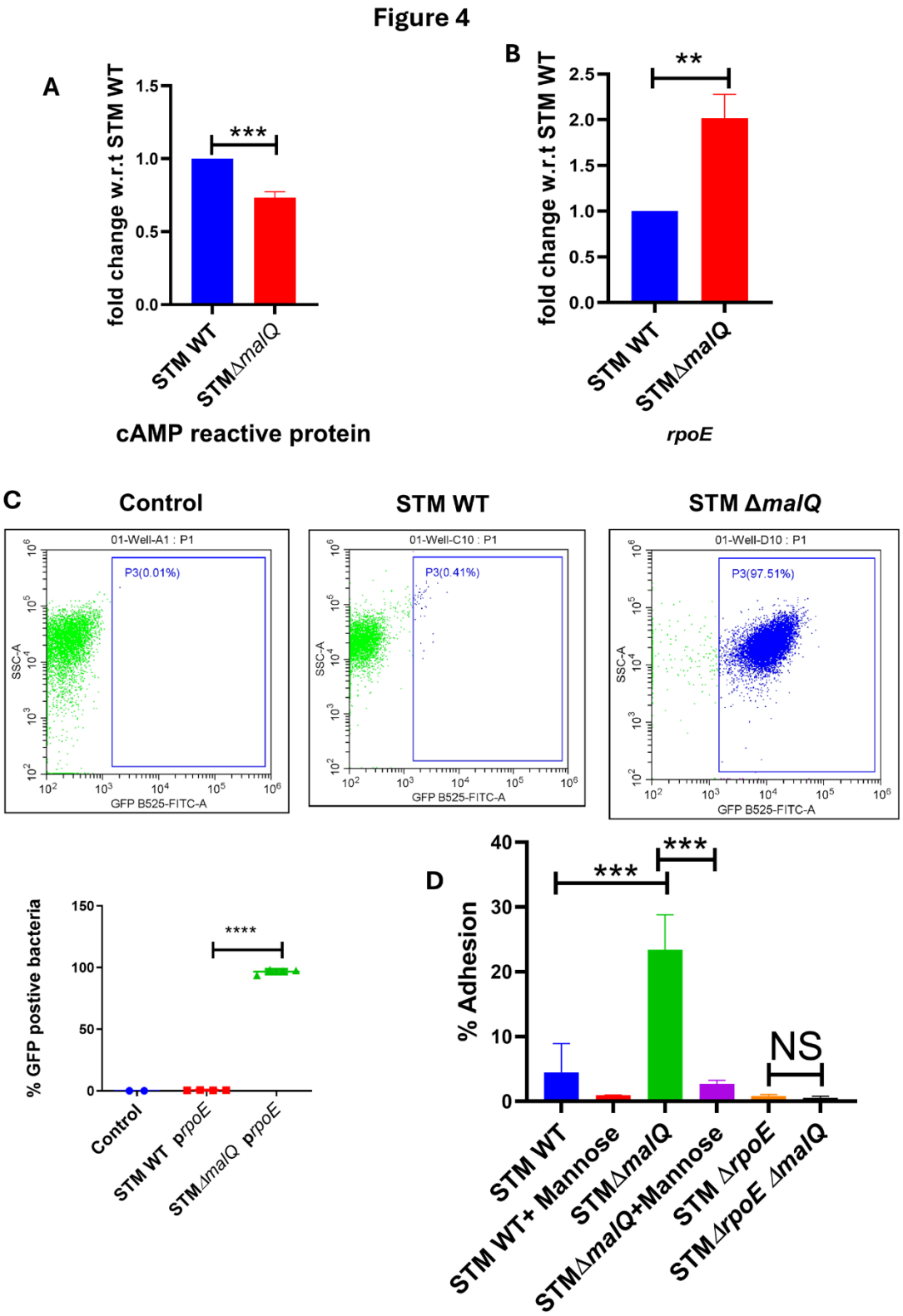
468 **Figure 4. Role of *rpoE* in type 1 fimbriae regulation**



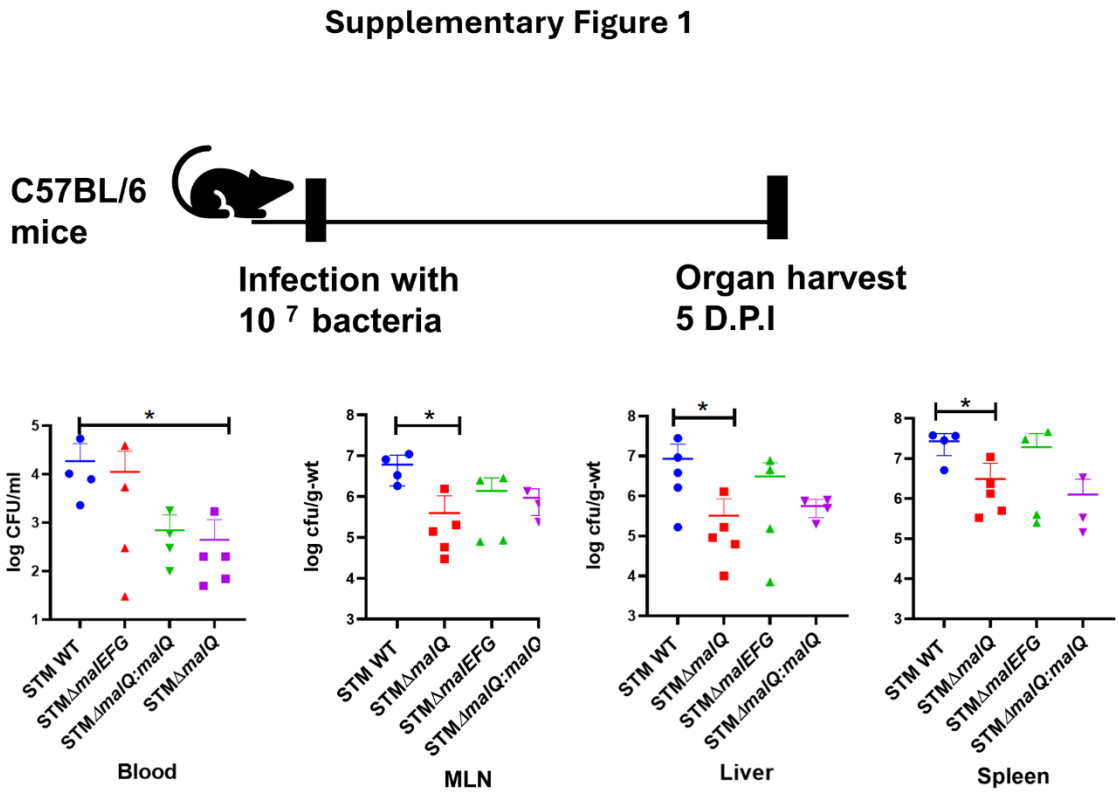
469

470 A. mRNA expression of cAMP reactive protein (CRP) in STM WT and STM Δ*malQ* using
471 q-RT PCR. An unpaired Student's t-test was used for analysis. Data are representative
472 of N=3, n=3, presented as mean ±SD.
473 B. mRNA expression of *rpoE* in STM WT and STM Δ*malQ* using q-RT PCR. An unpaired
474 Student's t-test was used for analysis. Data are representative of N=3, n=3, presented as
475 mean ±SD.
476 C. Activity of Promoter3 of *rpoE* in STM WT and STM Δ*malQ* in the log phase using
477 FACS. Analysis by one-way ANOVA. Data are representative of N=3, n=4, presented
478 as mean ±SD.
479 D. Percentage adhesion of STM WT, STM Δ*malQ* in Caco-2 cells on treatment with and
480 without 0.2 M mannose, STM Δ*rpoE* and STM Δ*rpoE* Δ*malQ*. One-way ANOVA was
481 used for the analysis. Data are representative of N=3, n=3, presented as mean ±SD.

482

483 **Supplementary information**

484 **Supplementary figure 1. Maltose metabolism gene *malQ* aids in the colonisation of mice.**



485

486 *In-vivo* pathogenesis of STM WT, STM Δ*malQ* and STM Δ*malEFG* in C57BL/6 mice 5

487 days post-infection. Organ burden in blood, MLN, Liver and spleen was determined. The

488 Mann-Whitney test was performed for the analysis, with five animals per cohort, and the

489 results are presented as mean ± SD.

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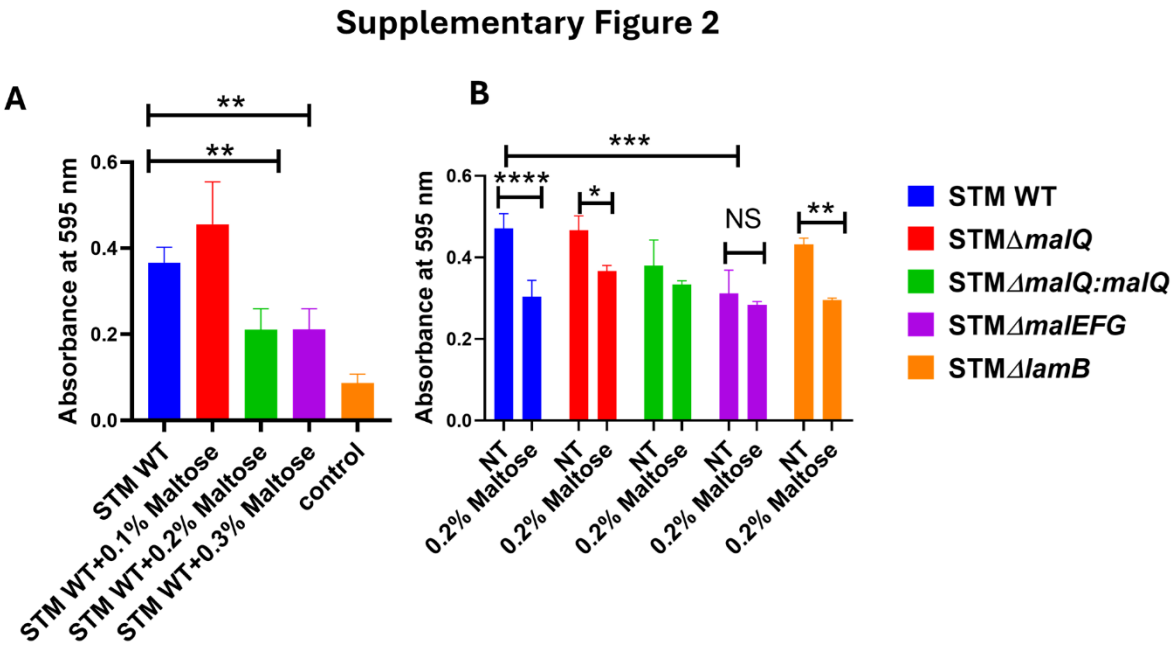
493 **Supplementary Figure 2. Maltose inhibits the biofilm formation of *Salmonella***
494 **Typhimurium.**



495

496 A. Crystal violet staining of the biofilm 3 days post inoculation with different
497 concentrations of maltose. Data is representative of N=3,n=4, presented as mean ±
498 SD, analysis by unpaired t-test.
499 B. Crystal violet staining of the biofilm 3 days post inoculation with STM WT, STM
500 STM Δ*malQ,* STMΔ*malEFG* and STM Δ*lamB* on treatment with and without
501 treatment of 0.2 % maltose. The data are representative of N = 3, n = 4, and are
502 presented as mean ± SD. Analysis was performed using two-way ANOVA.

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507 **Supplementary Table 1. List of bacterial strains used in the study**

S.NO	Bacterial strain	Antibiotic
1	STM WT 14028S	-
2	STM Δ *malQ*	Kanamycin/ Chloramphenicol
3	STM Δ *malEFG*	Kanamycin
4	STM Δ *lamB*	Chloramphenicol
5	STM Δ *malQ: malQ*	Kanamycin: Chloramphenicol
6	STM Δ*rpoE* Δ*malQ*	Kanamycin: Chloramphenicol
7	STM Δ*rpoE (*Made for earlier study) (34)	Chloramphenicol
8	STM WT P*rpoE*-GFP	Kanamycin
9	STM Δ*malQ* P*rpoE*-GFP	Kanamycin: Chloramphenicol
10	STM WT P*fimA*-GFP	Kanamycin
11	STM Δ*malQ* Pf*imA*-GFP	Kanamycin: Chloramphenicol

508

509 **Supplementary Table 2. List of primers used in the study**

Primer name	Sequence
malQ KO FP	ATGGAAAATAAACGTCTCGATAGTGCCGCGCTGGCGGCGG CATATGAATATCCTCCTTAG
malQ KO RP	TTTCTTCTTCGCGGCCGCTTTTCGGCGTTTATCCAAATCC GTGTAGGCTGGAGCTGCTTC
malQ KO CO FP	GCTCTATTCGCGATTATCAG
*malQ*KO CO RP	GTGCGCCAGGATTGCCTGAT
Comp. *malQ* FP	CCGGAATTCTAAGGGAGCGCGATGGAAAA
Comp. *malQ* RP	CGCGGATCCTCCCCGGCTTTATTATTTCT
malEFG KO FP	GACGACGACATGCGCTGCCATCCTCGCGGAACAGATGACAGTGTAGGCTGGAGCTGCTTC
malEFG KO RP	TCAGGCCGTTGACCAGCCAACGTTGCGCCAACAGGAACACCATATGAATATCCTCCTTAG
malEFG conf. FP	TTCTATCTCCTGAGATCATTGC
malEFG conf. RP	CGACCATAAGTGGGGATAACA
siiE RT FP	GGTCAATGATCATGGCGTGC
siiE RT RP	GTATAGCCCCAGACGCCTTC
fimA RT FP	TTCGGATCGCAGTCATTCAG
fimA RT RP	GTTGCGGCTGATCCTACTC
pagN RT FP	TAGCTTCACATGAGCGAGGC
pagN RT RP	TGAGACGGATGCTAAAGGCG
ompD RT FP	AGTTTGGTGGCGATACCTGG
ompD RT RP	TCATACGTTGCGGACAGACC
16S RT FP	GTGAGGTAACGGCTCACCAA
16S RT RP	TAACCGCAACACCTTCCTCC
hilA RT FP	CGCCGGCGAGATTGTGAGTA
hilA RT RP	TGGCGGAGACACCACTACGA
sipA RT FP	TTCGGATGAAGCGTTGGTCA
sipA RT RP	GTCATTCGCGTGTGGATTCG
rpoE RT FP	GTGCGCTACCAGCATAAAGTG
rpoE RT RP	GCAATACGATACAACCAGGT
cAMP reactive protein FP	TATTGATGACGCGCCAATGGT
cAMP reactive protein RP	TCATCTTTGATCAGCACTGC

fimZ RT FP	GGATGATAGCCGAACAGCGA
fimZ RT RP	TCCGTTTGCGCCCGCTCTTA
fimY FP	ACTCTCCAACGCCGCAGATA
fimY RP	TGAGCGACCCCTTTTCAATC
fimW FP	ACAGTCACTTTGAGCATGGG
fimW RP	TCAACATCATCTTCCCGAT
rpoE KO FP	GGTTTGGGGAGACATTACCTCGGATGAGCGAGCAGTTACATATGAATA TCCTCCTTAG
rpoE KO RP	CCTAATACCTTTTCCAGTATCCCGCTATCGTCAACGGTGTAGGCTGGAG CTGCTTC
invF RT FP	CGTCGTTTGTGCAGCAGAGC
invF RT RP	TAATTTCCGCGGCGAAACGC
P*rpoE* FP	**CGCGGATCC**TAAGACCTGTCTACAACATGAC
P*rpoE* RP	**CCGCTCGAG**GGTAGCGCACTACCAGTAAGTTA
P*fimA* FP	**CGCGGATCC**TTAATCTTTGCGAATATGAC
P*fimA* RP	**CCGCTCGAG**TATGGTTACCGTAATCCCTC

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