

1           **Type 1 piliated uropathogenic *Escherichia coli* hijack the host**  
2                           **immune response by binding to CD14**

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## 16 **Abstract**

17 A key attribute of persistent or recurring bacterial infections is the ability of the pathogen to  
18 evade the host's immune response. Many *Enterobacteriaceae* express type 1 pili, a pre-  
19 adapted virulence trait, to invade host epithelial cells and establish persistent infections.  
20 However, the molecular mechanisms and strategies by which bacteria actively circumvent the  
21 immune response of the host remain poorly understood. Here, we identified CD14, the major  
22 co-receptor for lipopolysaccharide detection, on dendritic cells as a previously undescribed  
23 binding partner of FimH, the protein located at the tip of the type 1 pilus of *Escherichia coli*.  
24 The FimH amino acids involved in CD14 binding are highly conserved across pathogenic and  
25 non-pathogenic strains. Binding of pathogenic bacteria to CD14 lead to reduced dendritic cell  
26 migration and blunted expression of co-stimulatory molecules, both rate-limiting factors of T  
27 cell activation. While defining an active molecular mechanism of immune evasion by  
28 pathogens, the interaction between FimH and CD14 represents a potential target to interfere  
29 with persistent and recurrent infections, such as urinary tract infections or Crohn's disease.

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## 32 **Keywords**

33 CD14 / FimH / immunity / recurring infections / uropathogenic *E. coli* (UPEC)

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## 36 **Introduction**

37 Cells of metazoan organisms constantly interact with a wide diversity of bacterial  
38 species that populate the host organism internally as well as externally. Therefore the host  
39 immune response is faced with a non-trivial problem – accommodate beneficial commensals  
40 and remove harmful pathogens (Hooper & Macpherson, 2010). The difficulty of this task lies  
41 in the fact that most of the molecular patterns, such as lipopolysaccharide (LPS) or surface  
42 organelles such as pili and flagella, are conserved between commensal and pathogenic  
43 bacteria. The main difference between commensal and pathogenic strains is the ability of the  
44 latter to hijack host cell functions for their own benefit (Magalhaes *et al*, 2007). Consequently,  
45 the host immune system uses complex discrimination strategies, such as spatial  
46 compartmentalization of the receptors recognizing pathogen signatures and concurrent  
47 sensing of molecular patterns associated with host-damage. The host discrimination ability is

48 not always perfect, as pathogens can persist asymptotically in the host for long periods of  
49 time or cause symptomatic acute or chronic infections in some individuals, but not in others  
50 (Grant & Hung, 2013). For example, the commensal bacterium *Escherichia coli*, one of the  
51 main residents of the mammalian intestine, occasionally causes clinical infections, especially  
52 when the bacteria acquire virulence traits and thus manage to populate extraintestinal host  
53 niches (Magalhaes *et al*, 2007; Leimbach *et al*, 2013). Usually disease progression does not  
54 pose a dead end to such opportunistic pathogens, since bacteria are shed in high numbers  
55 during the infection, allowing the pathogen to populate new environments, a new host or even  
56 recolonize their original host niche – the intestine (Donnenberg, 2013).

57 In principle, any commensal *E. coli* has the potential to evolve into a pathogen by  
58 acquiring virulence traits (Wirth *et al*, 2006). Those traits can be either adapted, namely  
59 evolved specifically to increase the fitness of a pathogen during the infection, or pre-adapted,  
60 meaning even though they increase the fitness of the pathogen they were originally evolved  
61 for a non-virulent function (Donnenberg, 2013). Examples of adapted traits are the acquisition  
62 of pathogenicity islands through horizontal gene transfer (Oelschlaeger *et al.*, 2002) or  
63 pathoadaptive mutations, such as changes in the LPS, flagellum or pili components  
64 (Weissman *et al.*, 2006; Donnenberg, 2013). A prime example of a pre-adapted trait are type 1  
65 pili. Both commensal and pathogenic *E. coli* (Shawki & McCole, 2017; Croxen & Finlay, 2010),  
66 but also other *Enterobacteriaceae* (Struve *et al*, 2008; Kolenda *et al*, 2019), use type 1 pili to  
67 adhere to host cells in their respective ecological niches (Spaulding *et al*, 2017). Type 1 pili  
68 undergo a constant and reversible change in their expression due to phase variation which  
69 allows populations of isogenic bacteria to exhibit controlled genotypic and phenotypic variation  
70 (Bayliss, 2009). In the case of type 1 pili, site-specific recombinases place the *fimA* promoter  
71 either in the phase-ON or phase-OFF orientation, resulting in piliated or non-piliated bacteria  
72 (Schilling *et al*, 2001). UPECs have mastered the use of phase variation as a remarkable  
73 genetic mechanism of plasticity for their advantage, since type 1 pili expression is tightly  
74 regulated by the host environment. For example, growing UPECs *in vitro* in human urine locks  
75 expression in the phase-OFF state (Greene *et al*, 2015), whereas adhesion to host cells has  
76 been shown to lock expression in the phase-ON state (Greene *et al*, 2015; Lim *et al*, 1998).  
77 Phase variation greatly adds to the virulence and fitness of UPECs by generating  
78 heterogeneity among the bacterial population where individual cells switch back and forth  
79 between the type 1 piliated and non-piliated phenotype (Bayliss, 2009; Wright *et al*, 2007).  
80 Type 1 pili are necessary for the persistent and therefore recurring infection of the bladder  
81 (Hunstad & Justice, 2010; Wright *et al*, 2007), but also other persistent bacterial infections  
82 (Shawki & McCole, 2017).

83           Several mechanisms are known to contribute to persistent or recurring infections, one  
84 of which is the residing of pathogenic bacteria within a protected niche (Grant & Hung, 2013).  
85 Such niches can be physical structures when the pathogen invades host cells to hide from the  
86 immune response (Grant & Hung, 2013; Donnenberg, 2013). For example, UPECs invade  
87 host epithelium cells in the bladder using type 1 pili, propagate intracellularly and compromise  
88 the host defense barriers (Hunstad & Justice, 2010; Martinez *et al*, 2000). Strikingly, also the  
89 host immune response may unintentionally create protected niches. For example, bladder  
90 residing macrophages sequester UPECs before antigen-presenting cells, such as dendritic  
91 cells (DCs), come into action (Mora-Bau *et al*, 2015).

92           DCs are the key cell type connecting the innate and adaptive immune response  
93 (Mellman & Steinman, 2001). Distinct subtypes of DCs, expressing different surface receptors  
94 (Merad *et al.*, 2013), reside in every tissue of the host and DCs were also identified at the  
95 epithelial junction of the bladder (Schilling *et al.*, 2003). In response to an infection, these  
96 resident DCs together with newly recruited inflammatory DCs sense and ingest pathogens.  
97 Subsequently, they start to secrete immune modulatory cytokines and migrate from the site of  
98 infection to the draining lymph nodes where they present acquired and processed antigens to  
99 T cells, thus triggering the adaptive immune response. However, even if the host immune  
100 system manages to detect the pathogen, eradication of the infection is not guaranteed.  
101 Pathogens utilize several strategies to subvert innate and adaptive immunity, thereby avoiding  
102 clearance by the host and establishing persistent infections (Donnenberg, 2013). For example,  
103 UPECs were shown to interfere at the interface between innate and adaptive immunity: after  
104 contact with UPECs during urinary tract infections (UTIs), tissue resident mast cells secrete  
105 high amounts of interleukin-10 (IL-10), an immuno-suppressive cytokine (Chan *et al*, 2013),  
106 that drives the differentiation of regulatory T cells (Hsu *et al*, 2015). The combined effect of  
107 subverting the host immune response and establishing a protected niche inside the host  
108 greatly increases the ability of pathogens to cause persistent infections.

109           Here, we asked whether type 1 pili play a role in manipulating host immunity and  
110 thereby facilitate recurring bacterial infections by dissecting the underlying molecular  
111 mechanism between the interaction of uropathogenic *E. coli* and dendritic cells.

112

113

## 114 Results

### 115 Type 1 pilated UPEC inhibit T cell activation and proliferation by decreasing expression 116 of co-stimulatory molecules on dendritic cells

117 To study the influence of type 1 pili on the adaptive immune system, we engineered  
118 bacteria with and without pili. The *fim* genes that produce the molecular components of the  
119 type 1 pilus are part of an operon on the *E. coli* chromosome (Figure 1A and B) whose  
120 expression is regulated by phase-variation. We generated stable phase-locked bacterial  
121 mutants by locking the fim switch (*fimS*) either in the phase-ON orientation, resulting in  
122 constitutive expression of the type 1 pilus, or in the phase-OFF orientation, blocking  
123 expression of the pilus (hereafter simply termed ON and OFF, respectively). To achieve this,  
124 we deleted the 9 bp long recognition site for the site-specific recombinases FimB and FimE in  
125 the internal repeat region upstream of the *fimS* element in either orientation (Figure 1C). The  
126 presence and absence of type 1 pili was confirmed by electron microscopy and yeast-  
127 agglutination assay (Figure 1D). Additionally, electron microscopy indicates absence of any  
128 other pili, such as p fimbriae, under the chosen growth conditions since the OFF mutant  
129 appears bald (Figure 1D, right panel).

130 Since the adaptive immune response seems to be limited during persistent or recurring  
131 infections (Magalhaes *et al*, 2007), such as recurring bladder infections (Abraham & Miao,  
132 2015), we asked if activation and proliferation of naïve T cells *in vitro* is altered upon  
133 stimulating DCs with the genetically constructed UPEC ON or OFF mutants. The activation of  
134 Ovalbumin (OVA) specific CD4<sup>+</sup> T cells (Barnden *et al.*, 1998), as assessed by CD69 receptor  
135 upregulation and CD62L receptor downregulation, was massively reduced when DCs were  
136 stimulated with OVA and UPEC ON mutants, compared to OVA and UPEC OFF mutants  
137 (Figure 1E). Accordingly, the number of proliferating T cells was also strongly decreased  
138 (Figure 1F).

139 Cellular identity, such as the CD11c surface marker and surface levels of MHCII, the  
140 hallmark of DC activation, was only very mildly altered upon exposure to UPEC ON  
141 (Supplementary Figure 1C and D). Beyond presentation of MHCII, the expression of co-  
142 stimulatory molecules on DCs is decisive for effective T cell priming and differentiation into  
143 effector cells (Banchereau & Steinman, 1998). We therefore analyzed surface expression of  
144 CD40, CD80 and CD86 after ON and OFF stimulation and found significantly decreased levels  
145 after ON stimulation (Figure 1G).

146           These data suggest that type 1 pilated UPECs prevent effective activation of the  
147 adaptive immune response by decreasing expression of co-stimulatory molecules on DCs and  
148 thus restrict their ability to activate T cells.

149

150 **Type 1 pilated UPEC decrease dendritic cell migratory capacity and increase T cell**  
151 **contact times by triggering integrin activation**

152           Beyond presentation of the antigen in the context of co-stimulatory factors, two  
153 additional cell biological parameters are essential for the priming of T cells. First, DCs have to  
154 migrate from the site of antigen encounter and uptake (usually peripheral tissues) to the site  
155 of antigen presentation (e.g. lymph nodes), where they meet T cells. This migration step is  
156 directionally guided by chemokines and highly efficient even in the absence of adhesive  
157 interactions with the extracellular matrix (Lämmermann *et al*, 2008). Second, T cells have to  
158 physically interact with DCs in order to probe their surface for antigen presentation and co-  
159 stimulation. Hence, the contact dynamics between DCs and T cells are essential parameters  
160 determining T cell activation and proliferation (Bousso, 2008).

161           We first investigated DC-T cell contacts using live cell microscopy and found that  
162 UPEC ON mutants increased the antigen specific contact times between DCs and naïve CD4<sup>+</sup>  
163 T cells, compared to UPEC OFF mutants. Even after 6 h of co-culture a large fraction of T  
164 cells was unable to dissociate from DCs (Figure 2A). This was specific to antigen-bearing  
165 DCs, as in the absence of OVA contact times were short and indistinguishable between ON  
166 and OFF stimulation. Activation of  $\beta$ 2 integrins, specifically CD11b, on DCs was shown to  
167 increase the duration of cell-cell contacts by binding to its counter receptor ICAM-1 on T cells,  
168 leading to a decrease in the activation of T cells (Varga *et al*, 2007). We therefore investigated  
169 if the activation status of CD11b on DCs was affected after stimulation with ON mutants. We  
170 analyzed total and active levels of CD11b using the activation-independent antibody M1/70  
171 and CBRM1/5 antibody, which recognizes the active conformation of human, but also mouse  
172 CD11b ((Oxvig *et al*, 1999) and (Supplementary Figure 2A)). We found that UPEC ON  
173 stimulation shifted CD11b to the active conformation when compared to UPEC OFF or LPS  
174 stimulation (Figure 2B and Supplementary Figure 2A).

175           In line with the finding that type 1 pilated UPEC enhanced CD11b activity, ON mutants  
176 triggered tight adhesion of DCs to serum-coated surfaces (Figure 2C). This surface  
177 immobilization was integrin mediated, as for  $\beta$ 2 integrin knockout DCs the differential adhesion  
178 was lost (Figure 2C and Supplementary Figure 2C). (Notably,  $\beta$ 2 integrin deficient DCs  
179 showed increased  $\beta$ 1 integrin-mediated background-binding (Supplementary Figure 2B)).

180 We next asked whether UPEC ON stimulation interferes with the migratory capacity of  
181 DCs. We performed *in vitro* migration assays where DCs migrate in cell derived matrices  
182 (Kaukonen *et al*, 2017) and found diminished migration after stimulation with ON mutants  
183 when compared to OFF mutants (Figure 2D). The same effect on the migratory capacity we  
184 observed in crawl out assays in physiological tissue (Stösel *et al*, 1997). Here, the ventral  
185 halves of explanted mouse ears were repeatedly exposed to either UPEC ON or OFF bacteria  
186 during 48 h. After stimulation with ON mutants, fewer endogenous DCs were found inside  
187 lymph vessels as compared to OFF stimulation (Figure 2E). In ears harvested from  $\beta 2$   
188 knockout mice the migration defect was rescued and similar levels of  $\beta 2^{-/-}$  DCs migrated into  
189 the lymph vessels after ON and OFF stimulation (Figure 2E and Supplementary Figure 2D).

190 These data suggest that hyperactive CD11b hinders both migration and T cell  
191 activation of DCs by immobilizing them to extracellular matrix proteins like fibrinogen or cellular  
192 ligands like ICAM-1. This integrin gain of function phenotype is in line with findings that  
193 pharmacological approaches which activate integrins have stronger *in vivo* effects on  
194 leukocyte migration than approaches to inhibit integrin function (Maiguel *et al*, 2011). To test  
195 if immobilization by hyperactive CD11b is indeed causative for the loss of migration upon  
196 UPEC ON stimulation, we measured migration of DCs in 3D collagen gels. Here, DCs  
197 efficiently migrate in an integrin-independent manner (Lämmermann *et al*, 2008) and  
198 hyperactive CD11b, which does not bind to collagen 1, should not be able to immobilize the  
199 cells. We found that the migration speed of ON and OFF stimulated DCs in a 3D collagen  
200 assay was indistinguishable (Supplementary Figure 2E).

201 Finally, we asked if activation of  $\beta 2$  integrins maps upstream of the observed  
202 detrimental effect on co-stimulatory molecule expression after stimulation with UPEC ON  
203 mutants.  $\beta 2^{-/-}$  DCs still expressed lowered levels of co-stimulatory molecules after ON  
204 stimulation when compared to OFF stimulation (Figure 2F), suggesting that downregulation of  
205 co-stimulatory molecules does not depend on integrin activation.

206 Taken together, type 1 pilated UPEC increase integrin activity on DCs leading to  
207 increased cell-cell and cell-matrix attachment. This causes prolonged interaction times with T  
208 cells and impaired migratory capacity. Together, with the above finding of reduced co-  
209 stimulatory molecule expression, these data demonstrate that type 1 pilated UPEC target  
210 three functional hallmarks of DCs that are critical for the activation of naïve T cells – (i)  
211 migration of DCs to the lymph node, (ii) the physical interaction with T cells and (iii) the  
212 expression of co-stimulatory molecules.

213

214 **The GPI-anchored glycoprotein CD14 binds FimH, making it a novel target for type 1**  
215 **pili**

216 To find the interaction partner on the host cell that leads to integrin activation, we first  
217 investigated the role of the major immune cell receptor TLR4 which has been previously  
218 suggested as a receptor for the FimH protein of type 1 pili (Mossman *et al*, 2008). We analyzed  
219 the adhesion and migration behavior of *tlr4*<sup>-/-</sup> DCs after stimulation with UPEC ON mutants  
220 and found that both behaviors were still affected (Figure 3A-B and Supplementary Figure 3A).  
221 Our results, together with recent findings in *Salmonella* (Uchiya *et al*, 2019), suggest that other  
222 receptors besides TLR4 could serve as molecular targets of type 1 pili.

223 We then focused on CD14, a GPI anchored glycoprotein and co-receptor of TLR4,  
224 since a strong correlation has been reported between CD14 expression, integrin activity, and  
225 cell adhesion (Wright *et al*, 1991). We therefore performed adhesion and crawl out migration  
226 assays stimulating *cd14*<sup>-/-</sup> DCs with UPEC ON and OFF mutants and found that behavior of  
227 DCs was fully restored (Figure 3C and 3D and Supplementary Figure 3B). *cd14*<sup>-/-</sup> DCs also  
228 showed almost full rescue of the levels of co-stimulatory molecules after ON stimulation,  
229 compared to OFF stimulation (Figure 3E). We conclude that CD14 is required for the increases  
230 in integrin activity and decreases in co-stimulatory molecule expression after stimulation with  
231 type 1 pilated UPECs.

232 We next asked how type 1 pili interact with the CD14 receptor. *In silico* protein-protein  
233 docking analysis predicted strong binding of -56.98 kcal/mole between FimH (PDB: 6GTV;  
234 (Sauer *et al*, 2019)) and CD14 (PDB: 1WWL; (Kim *et al*, 2005)) (Table S1; File S1), which is  
235 stronger than the -49.81 kcal/mole between FimH and TLR4 (PDB: 3VQ2; (Ohto *et al*, 2012))  
236 or the -35.55 kcal/mole for CD48 (PDB: 2PTV; (Velikovskiy *et al*, 2007)), another FimH  
237 receptor (McArdel *et al*, 2016) (Table S1). Binding sites in FimH were located in its N-terminal  
238 domain and in CD14 within the central region of the crescent shaped monomer (Figure 4A),  
239 in an area not involved in LPS binding (Table S2) (Kim *et al*. 2005). Since CD14 of mouse  
240 (PDB: 1WWL) and human (PDB: 4GLP; (Kelley *et al*, 2013)) have highly similar secondary  
241 structures, but differ in their amino acid sequence (Kelley *et al*, 2013), we performed docking  
242 analysis with human CD14. We found predicted strong FimH binding (-55.95 kcal/mole) to be  
243 conserved in similar regions of both proteins but mediated by different amino acids (Table S1  
244 and Table S3). This indicates that the difference in amino acid sequence between mouse and  
245 human CD14 seems negligible for the otherwise conserved strategy of how FimH of type 1 pili  
246 binds to CD14 receptor.

247 To verify the predicted binding of FimH to CD14 *in vitro*, we generated UPEC ON  
248 mutants lacking the *fimH* gene (ON  $\Delta$ *fimH*). Compared to *Salmonella*, where a *fimH* is



249 necessary for the biosynthesis of type 1 fimbriae (Zeiner et al., 2012), *E. coli* mutants lacking  
250 *fimH* still express type 1 pili which are, however, non-functional (Maurer and Orndorff, 1985;  
251 Klemm and Christiansen, 1987; Jones et al., 1995). We confirmed that our ON  $\Delta$ *fimH* mutants  
252 still express type 1 pili but lack the ability to agglutinate with yeast (Supplementary Figure 4A).  
253 We performed an immunoprecipitation-type approach using magnetic Protein A beads, a  
254 CD14-Fc chimeric protein and the bacterial mutants. First, the CD14-Fc chimera was coupled  
255 to the magnetic Protein A beads (Supplementary Figure 5A). Next, we introduced a  
256 constitutively expressed *yfp* fluorescent marker into the chromosome of the UPEC ON and  
257 ON  $\Delta$ *fimH* mutants for tracking. Using fluorescence microscopy, we found abundant ON  
258 mutants bound per CD14-coupled bead, whereas binding of the *fimH* deletion mutants was  
259 scarce (Figure 4B). Binding of ON mutants was not due to unspecific binding of FimH to the  
260 bead matrix, as uncoupled beads also showed very scarce binding. Additionally, we analyzed  
261 the binding of bacteria to beads by flow cytometry by gating on the size parameters and  
262 fluorescence signal of the bacteria event population (see Supplementary Figure 5B for gating  
263 strategy and methods section for further technical details). ON mutants showed increased  
264 binding to CD14-coupled beads, when compared to the *fimH* deletion mutants or to ON  
265 mutants binding to uncoupled beads (Figure 4C). To further test if only FimH is necessary for  
266 binding to CD14, and not interactions with LPS on the bacterial membrane, we extracted  
267 type 1 pili (Sheikh et al, 2017) from UPEC ON and ON  $\Delta$ *fimH* mutants and performed a dot  
268 blot assay using biotinylated CD14 and Streptavidin-HRP (Figure 4D). Biotinylated CD14 only  
269 bound to type 1 pili extracts from ON mutants, whereas no binding was observed to type 1 pili  
270 extracts from ON mutants lacking the FimH protein.

271 We next tested if binding of FimH to CD14 is causative for the increase in adhesion of  
272 DCs and the decrease in co-stimulator molecules on their membrane after UPEC ON  
273 stimulation. Indeed, deleting *fimH* from UPEC ON mutants fully rescued the adhesion and the  
274 expression of all co-stimulatory molecules when compared to stimulation with the UPEC ON  
275 mutants (Figure 4E and 4F).

276 Finally, we asked if expression of pathogenic FimH in an otherwise non-pathogenic  
277 bacterial background is sufficient to affect expression of co-stimulatory molecules on DCs. We  
278 constructed a locked-ON mutant of *E. coli* W, a non-pathogenic *E. coli* strain, and replaced  
279 the endogenous *fimH* gene with the pathogenic *fimH* variant of UPEC CFT073 strain. This  
280 non-pathogenic ON *fimH*<sub>CFT073</sub> mutant was able to agglutinate with yeast, confirming the  
281 correct insertion of the pathogenic *fimH* gene (Supplementary Figure 4B). However,  
282 expression of co-stimulatory molecules on DCs after stimulation with non-pathogenic *E. coli*  
283 W ON mutants or the non-pathogenic ON *fimH*<sub>CFT073</sub> mutant was barely affected  
284 (Supplementary Figure 4C).

285           Based on these experimental observations, we propose that CD14 is a novel target for  
286 the FimH protein of type 1 pili and that direct protein-protein interaction is the underlying  
287 mechanism of binding. FimH is necessary for the modulatory effects seen on DCs after  
288 stimulation with UPEC ON mutants, whereas expressing the pathogenic *fimH* gene alone is  
289 not sufficient to cause these effects in an otherwise non-pathogenic background.

290

291 **FimH amino acids predicted to bind are highly conserved and are partially located in**  
292 **the mannose-binding domain**

293           The two-domain FimH protein consists of a receptor-binding domain and a pili-binding  
294 domain (Choudhury *et al*, 1999). The receptor-binding domain not only interacts with host  
295 receptors, like uroplakin Ia on bladder epithelial cells, but also highly specifically binds  
296 D-mannose which due to this specific interaction has been used in the treatment of UTIs (Wiles  
297 *et al*, 2008). The amino acids responsible for binding mannose are located in the mannose-  
298 binding pocket (P1, N46, D47, D54, Q133, N135, D140 and F142; Table S2) (Hung *et al*,  
299 2002) and the tyrosine gate (Y48, I52, Y137; Table S2) (Touaibia *et al*, 2017). These residues  
300 are highly conserved among pathogenic and non-pathogenic *E. coli* strains (Figure 5A),  
301 whereas other amino acids that affect the flexibility of the FimH protein and therefore facilitate  
302 host colonization were found to be mutated in pathogenic *E. coli* (Chen *et al*, 2009; Sokurenko  
303 *et al*, 1998; Kalas *et al*, 2017). Interestingly, the most important amino acids of FimH we  
304 predicted to be responsible for binding to CD14 are highly conserved among several different  
305 *E. coli* strains, whether they are pathogenic or not (Figure 5A).

306           To verify their significance for binding to CD14, we introduced mutations into the three  
307 most important amino acids. We exchanged amino acids R98 (binding  
308 energy -7.23 kcal/mole), T99 (binding energy -4.92 kcal/mole) and Y48 (binding  
309 energy -4.29 kcal/mole) individually to alanine or all three at the same time creating a triple  
310 mutant. All four FimH mutants were still able to bind to CD14-coupled beads and only the triple  
311 mutant showed some decrease in binding (Figure 5B). Stimulating DCs with the FimH amino  
312 acid mutants showed partial rescue for co-stimulatory molecule expression levels (Figure 5C).  
313 This suggests that not only these individual amino acids mediate binding to CD14, but most  
314 likely several other amino acids and thus the supporting secondary structure of the FimH  
315 protein are involved.

316

317            Since Y48 and other identified FimH amino acids with weaker binding energies for  
318 CD14 (Table S2) are located in the mannose binding pocket, we were interested whether  
319 FimH antagonists, such as D-mannose or the low molecular weight mannose derivate M4284  
320 (Schönemann *et al*, 2019), disrupt the interaction. Additionally, we tested a blocking CD14  
321 antibody (M14-23) (Tsukamoto *et al*, 2010) for its ability to inhibit binding of FimH to CD14.  
322 Our experiments showed that 175  $\mu$ M D-mannose was not sufficient to block binding of UPEC  
323 ON bacteria to CD14, whereas 1 mM D-mannose and M4284 at 10  $\mu$ M were able to inhibit  
324 binding of ON mutants to CD14-coupled beads (Figure 6A). The blocking CD14 antibody  
325 reduced bacteria binding to beads by roughly 25 % but was the most effective at rescuing  
326 expression of co-stimulatory molecules on DCs among all tested components (Figure 6B).  
327 Thus, existing FimH antagonists as well as blocking CD14 antibodies show certain potential  
328 to treat recurring or persistent infections caused by type 1 piliated pathogens.

329

330

## 331 **Discussion**

332            Here, we uncovered that type 1 piliated UPECs target the CD14 glycoprotein on the  
333 surface of DCs to shut down the migratory capacity of DCs and their ability to interact with and  
334 activate T cells.

335            It has been proposed that the tight regulation of UPECs type 1 pili expression has  
336 evolved to limit exposure to the host immune system, allowing the pathogen to establish a  
337 persistent infection (Donnenberg, 2013). In this study we uncovered and characterized a  
338 different fundamental role of type 1 pili, as modulators of the innate and adaptive immune  
339 response. We found that type 1 piliated UPECs decreased the migratory capacity of DCs by  
340 increasing their adhesion to other cells, such as T cells, and the extracellular matrix by over-  
341 activation of integrins. The effective and timely transition from adhesion to the migration  
342 phenotype is essential for DCs to migrate from the site of the actual infection to the lymph  
343 node in order to interact with lymphocytes. To achieve these migratory and signaling tasks,  
344 DCs need to dynamically regulate integrin mediated adhesion, and we found that over-  
345 activation of integrins triggered by type 1 piliated UPECs leads to effective immobilization to  
346 the extracellular matrix and decreased turnover of cell-cell interactions. Over-activation of  
347 integrins, by hijacking integrin-linked kinases leading to decreased turnover of focal  
348 adhesions, was already shown to subvert innate immunity during *Shigella* and  
349 enterohemorrhagic *E. coli* (EHEC) infections (Kim *et al*, 2009; Shames *et al*, 2010), however  
350 UPECs do not express the responsible genes. Moreover, we found that type 1 piliated UPECs

351 also decreased the expression of co-stimulatory molecules, which are essential for T cell  
352 activation and proliferation.

353 We identified CD14, a GPI-anchored glycoprotein, as the direct target for the FimH  
354 protein of type 1 pili. In analogy, a previous study showed that the *fimA*-encoded major type V  
355 fimbriae of the oral pathogen *Porphyromonas gingivalis* also bind CD14 and thereby increase  
356 integrin-mediated adhesion by activating CD11b integrin (Harokopakis & Hajishengallis,  
357 2005). Using in silico protein-protein docking analysis we found strong predicted binding  
358 between CD14 and FimH through specific amino acids in both proteins. Interestingly, the  
359 respective FimH amino acids are highly conserved not only among pathogenic, but also non-  
360 pathogenic *E. coli* strains. However, although we found FimH to be necessary, we did not find  
361 the simple presence of pathogenic FimH in an otherwise non-pathogenic genetic background  
362 to be sufficient for suppression of the immune response. We therefore hypothesize that the  
363 predicted amino acids are mainly important for the pathogens to interact with and thereby to  
364 dampen the activation of immune cells and thus the immune response in the host to persist  
365 “commensally” for prolonged times. However, it is unknown which other gene(s) work together  
366 with *fimH*, as the UPEC strain CFT073 carries accessory genes encoded by 13 pathogenicity  
367 islands (Lloyd *et al*, 2007). Generally, the pathogenic potential of UPECs does not seem to be  
368 the result of a defined virulence gene, but rather a combination of effects by several genes  
369 (Touchon *et al*, 2009).

370 Given how pathogenic bacteria circumvent the host immune response by generating  
371 protected niches (Grant & Hung, 2013) and the constantly growing presence of multi-drug  
372 resistant strains (Boucher *et al*, 2009), treatment of persistent or recurring infections is  
373 increasingly challenging. Although vaccination approaches against FimH (Eldridge *et al*, 2020)  
374 or non-conventional treatments, such as small mannosides (Schönemann *et al*, 2019;  
375 Mydock-McGrane *et al*, 2016), showed promising results, treatments of infections caused by  
376 type 1 piliated pathogens remains difficult. Our findings underscore the importance of the  
377 mannose-binding domain, and several conserved amino acid residues in this domain, in the  
378 interaction between FimH and CD14. For example, R98, showing the strongest binding to  
379 CD14 in our predictions, was identified to be important to stabilize the protein-ligand interaction  
380 (Han *et al*, 2010). However, targeting this residue only, was not sufficient to boost affinity of  
381 FimH antagonists (Tomašič *et al*, 2021). Given our results, we hypothesize that this could be  
382 because not individual amino acids, but the overall secondary structure of the mannose-  
383 binding domain of FimH is important for the interaction with host receptors. Blocking CD14  
384 antibodies, such as the human anti-CD14 antibody IC14 (Axtelle & Pribble, 2001), could  
385 represent an alternative way to treat recurring infections caused by type 1 piliated pathogens,  
386 such as UTIs or inflammatory bowel diseases like Crohn’s disease (Sivignon *et al*, 2017),

387 given that CD14 is expressed by hematopoietic but also non-hematopoietic cells (Zanoni &  
388 Granucci, 2013).

389 Although opportunistic pathogens continuously circulate in humans as asymptomatic  
390 colonizers, they occasionally cause symptomatic acute or even chronic infections in some  
391 individuals (Donnenberg, 2013). Opportunistic pathogens use type 1 pili to adhere to and  
392 invade into host epithelial cells. While the cellular invasion represents a *de facto* passive  
393 mechanism for the pathogens to hide from the immune system and to establish persistent or  
394 recurring infections, we found that UPECs use type 1 pili to actively manipulate the behavior  
395 of innate immune cells, and thus also the adaptive immune response, by direct interaction of  
396 FimH and CD14 receptor on DCs. Since CD14 is a multi-functional co-receptor of several  
397 immune cell types (Zanoni & Granucci, 2013) and type 1 pili are expressed not only by  
398 pathogenic but also by non-pathogenic *E. coli* (Shawki & McCole, 2017; Croxen & Finlay,  
399 2010), our findings add a new layer of complexity to the physiological relevance of type 1 pili  
400 – as modulators of the immune response in general and specifically during persistent and  
401 recurring infections.

402

403

## 404 **Figure Legends**

405

### 406 **Figure 1: Type 1 piliated UPECs inhibit T cell activation and proliferation by decreasing** 407 **co-stimulatory molecules on DCs.**

- 408 A. Type 1 pili genes are expressed from the *fim* operon. Phase variation of the *fim* switch  
409 (*fimS*) harboring *fimA* promoter (*pfimA*) drives expression. *fimB* and *fimE* genes  
410 express site-specific recombinases, FimB and FimE respectively, inverting the *fimS*  
411 region by binding to the inverted repeats (IR).
- 412 B. Type 1 pili consist of several repeating units of the rod protein FimA, two adaptor  
413 proteins FimF and FimG, and the tip protein FimH. Fimbrial- and a receptor-binding  
414 domain of the two-domain FimH protein are shown. FimD, outer membrane usher.  
415 FimC, chaperone.
- 416 C. Phase-locked mutants were generated by deleting the 9 bp recognition site for the site-  
417 specific recombinases in the left inverted repeat region of the *fimS* in either ON or OFF  
418 orientation by introducing FRT sites resulting in piliated and non-piliated bacteria (see  
419 Methods).
- 420 D. ON (left panel) and OFF mutants (right panel). Electron microscopy images, with  
421 zoomed in regions (white boxes) marked in inlays, and yeast agglutination assay.
- 422 E. Dot plot of CD69 and CD62L expression on T cells after co-culture with ON (black) and  
423 OFF (grey) stimulated DCs (left panel). Quantification of CD69<sup>+</sup> CD62L<sup>-</sup> T cell  
424 frequencies (right panel) (4 biological replicates).

- 425 F. CFSE dilution profile of T cells after 96h of co-culture with ON (black) and OFF (grey)  
426 stimulated DCs (left). Quantification of T cells in division (right panel) (3 biological  
427 replicates).
- 428 G. Expression level of co-stimulatory molecules (CD40, CD80, CD86) of WT DCs after  
429 stimulation with ON (black) and OFF mutants (grey) (left panel; iso – isotype control).  
430 Quantification of median fluorescence values of co-stimulatory molecules (right panel)  
431 (3 biological replicates).
- 432 \*  $p < 0.1$ , \*\*\*  $p < 0.01$ , \*\*\*\*  $p < 0.001$  by Student's t test (E, F) and by Holm-Sidak t test (G);  
433 data are represented as means  $\pm$  SD

434

435 **Figure 2: Over-activation of integrins increases DC-T cell contact time and adhesion of**  
436 **DCs to extracellular matrixes leading to decreased migratory capacity.**

- 437 A. Interaction time between ON and OFF stimulated DCs and T cells in the presence and  
438 absence of OVA peptide (2 biological replicates).
- 439 B. Histograms of active and total CD11b integrin after ON (black) and OFF (grey)  
440 stimulation of DCs (left panel; iso – isotype control). Quantification of CD11b activity  
441 (active/total levels of CD11b) (right panel) (3 biological replicates). (see also  
442 Supplementary Figure 2A)
- 443 C. Adhesion assay of WT DCs after ON and OFF stimulation (left panel). Quantification  
444 of fluorescence signals, proxy for adherent cells, after ON (black) and OFF (grey)  
445 stimulation of WT and  $\beta 2^{-/-}$  DCs (right panel) (5 biological replicates). (see also  
446 Supplementary Figure 2C)
- 447 D. Extracellular matrix (ECM) migration assay of WT DCs after ON and OFF stimulation  
448 (left panel). Quantification of individual cells outside of cell cluster (right panel) (3  
449 biological replicates).
- 450 E. Ear crawl out assay of WT DCs after ON and OFF stimulation. Endogenous DCs  
451 stained with anti-MHCII (magenta). Lymph vessels stained with anti-LYVE-1 (white)  
452 (left panel). Quantification of cells inside over outside of lymph vessel after ON (black)  
453 and OFF (grey) stimulation of WT and  $\beta 2^{-/-}$  DCs (right panel) (3 biological replicates).  
454 (see also Supplementary Figure 2D).
- 455 F. Expression level of co-stimulatory molecules (CD40, CD80, CD86) of  $\beta 2^{-/-}$  DCs after  
456 stimulation with ON (black) and OFF mutants (grey) (left panel; iso – isotype control).  
457 Quantification of median fluorescence values of co-stimulatory molecules (right panel)  
458 (3 biological replicates).
- 459 ns, not significant, \*\*  $p < 0.05$ , \*\*\*  $p < 0.01$ , \*\*\*\*  $p < 0.001$  by one-way ANOVA followed by  
460 Dunnett's multiple comparisons (A, C, E) and by Student's t test (B, D and F); data are  
461 represented as means  $\pm$  SD

462

463 **Figure 3: Interaction of type 1 pilated UPEC with CD14, but not TLR4, is important for**  
464 **the observed phenotypes.**

- 465 A. Adhesion assay of  $tlr4^{-/-}$  and  $cd14^{-/-}$  DCs. Quantification of fluorescence signal after ON  
466 (black) and OFF (grey) stimulation (5 biological replicates). (see also Supplementary  
467 Figure 3A)
- 468 B. Ear crawl out assay of  $tlr4^{-/-}$  DCs after ON and OFF stimulation. Endogenous DCs  
469 stained with anti-MHCII (magenta). Lymph vessels stained with anti-LYVE-1 (white)

470 (left panel). Quantification of cells inside over outside of lymph vessel after ON (black)  
471 and OFF (grey) stimulation *tlr4*<sup>-/-</sup> DCs (right panel) (2 biological replicates).  
472 C. Quantification of the adhesion assay of *cd14*<sup>-/-</sup> DCs after ON (black) and OFF (grey)  
473 stimulation (5 biological replicates). (see also Supplementary Figure 3B)  
474 D. Ear crawl out assay of *cd14*<sup>-/-</sup> DCs after ON and OFF stimulation. Endogenous DCs  
475 stained with anti-MHCII (magenta). Lymph vessels stained with anti-LYVE-1 (white)  
476 (left panel). Quantification of cells inside over outside of lymph vessel after ON (black)  
477 and OFF (grey) stimulation *cd14*<sup>-/-</sup> DCs (right panel) (3 biological replicates).  
478 E. Expression level of co-stimulatory molecules (CD40, CD80, CD86) of *cd14*<sup>-/-</sup> DCs after  
479 stimulation with ON (black) and OFF mutants (grey) (left panel; iso – isotype control).  
480 Quantification of median fluorescence values of co-stimulatory molecules (right panel)  
481 (3 biological replicates).  
482 ns, not significant, \*\* p<0.05, \*\*\* p<0.01 by Student's t test; data are represented as means  
483 ± SD

484

485 **Figure 4: FimH binds to CD14 via protein-protein interactions and deletion of *fimH***  
486 **rescues adhesion and expression of co-stimulatory molecules.**

487 A. In silico protein-protein docking analysis for FimH and CD14 (see also file S1 and Table  
488 S1). FimH is shown in dark grey and CD14 in light grey. Left panel shows surface plot  
489 of docked proteins from the view of CD14 on the membrane of the cell. Middle panel  
490 shows surface plot of docked proteins from the view of FimH on the membrane of  
491 *E. coli*. GPI proximal domain, LPS-binding, lectin and pilin domains are indicated. Right  
492 panel shows secondary structures of proteins. Top 10 amino acids predicted to interact  
493 during the binding are highlighted in blue for FimH and magenta for CD14.  
494 B. Microscopy images of bead binding assay. ON or ON  $\Delta$ *fimH* mutants expressing a  
495 constitutive *yfp* fluorescent marker bound to CD14-coupled or uncoupled beads are  
496 shown.  
497 C. Flow cytometry analysis of bead binding assay. Histogram of fluorescence events in  
498 the bacteria gate (left panel). Uncoupled beads (dashed black), ON mutants bound to  
499 uncoupled beads (blue), ON mutants bound to CD14-coupled beads (black), ON  
500  $\Delta$ *fimH* bound to CD14-coupled beads (grey). Quantification of fluorescent events in  
501 the bacterial gate (right panel) (3 biological replicates). (see also Supplementary  
502 Figure 5)  
503 D. Type 1 pili extracts and dot blot assay. Electron microscopy images of type 1 pili  
504 extracts from ON and ON  $\Delta$ *fimH* mutants (left panel). Dot blot assay of type 1 pili  
505 extracts with biotinylated CD14 (right panel). Pre-blotted biotinylated CD14 served as  
506 positive control. Bound CD14 was visualized with Streptavidin-HRP antibody.  
507 E. Adhesion assay of WT DCs after ON and ON  $\Delta$ *fimH* stimulation. Quantification of  
508 fluorescence signals after ON (black) and ON  $\Delta$ *fimH* (grey) stimulation (5 biological  
509 replicates). ON data are the same as in Figure 2C. (see also Supplementary Figure  
510 3C)  
511 F. Expression level of co-stimulatory molecules (CD40, CD80, CD86) of WT DCs after  
512 stimulation with ON (black) and ON  $\Delta$ *fimH* mutants (grey) (left panel; iso – isotype  
513 control). Quantification of median fluorescence values of co-stimulatory molecules  
514 (right panel) (3 biological replicates). ON data are the same as in Figure 1G.  
515 ns, not significant, \*\*\*\* p<0.001 by one-way ANOVA followed by Dunnett's multiple  
516 comparisons (C) and by Student's t test (E, F); data are represented as means ± SD

517

518 **Figure 5: FimH binds to CD14 via highly conserved amino acid residues partially**  
519 **located in the mannose-binding domain.**

520 A. Receptor-binding domain of FimH from different *E. coli* strains is shown (UPEC:  
521 CFT073, UTI89, 536, NU14, EPEC: O55:H7; non-pathogenic; K-12, BL21-DE3, *E. coli*  
522 W). The top 10 amino acids on FimH showing strongest binding energy towards mouse  
523 CD14 (PDB: 1WWL) are shown in pink and towards human CD14 (PDB: 4GLP) are  
524 shown in orange. Amino acids I13, P12 and F42 are involved in both, mouse and  
525 human CD14, and therefore only shown in pink. Amino acids located in mannose-  
526 binding pocket and tyrosine gate are shown in grey. Amino acids I13, Y48, I52, Y137  
527 and F142 are involved in mannose and CD14 binding and therefore only shown in pink.  
528 Common pathoadaptive mutations that differ between UPEC and non-pathogenic  
529 *E. coli* are shown in black. Amino acids mutated to generate FimH amino acid mutants  
530 were Y48 (binding energy -4.29 kcal/mole), T99 (binding energy -4.92 kcal/mole) and  
531 R98 (binding energy -7.23 kcal/mole) (see also Table S1 and S3).

532 B. Bead binding assay of FimH amino acid mutants. Overlaid fluorescence events in the  
533 bacteria gate (left panel). ON (black), ON  $\Delta fimH$  (grey), Y48A (blue), T99A (violet),  
534 R98A (green) and Y48A R98A T99A (yellow). Quantification of fluorescent events in  
535 the bacterial gate (right panel) (3 biological replicates). ON and ON  $\Delta fimH$  data are the  
536 same as in Figure 4C.

537 C. Overlaid expression levels of co-stimulatory molecules (CD40, CD80, CD86) of WT  
538 DCs after stimulation with ON (black), ON  $\Delta fimH$  mutants (grey), and FimH amino acid  
539 mutants Y48A (blue), T99A (violet), R98A (green) and Y48A R98A T99A (yellow) (left  
540 panel). Quantification of median fluorescence values of co-stimulatory molecules (right  
541 panel) (3 biological replicates). ON data are the same as in Figure 1G.

542 ns, not significant, \*  $p < 0.1$ , \*\*  $p < 0.05$ , \*\*\*  $p < 0.01$ , \*\*\*\*  $p < 0.001$  by one-way ANOVA  
543 followed by Dunnett's multiple comparisons (the mean of the data were compared to the  
544 mean of ON); data are represented as means  $\pm$  SD

545

546 **Figure 6: FimH antagonists and a blocking CD14 antibody (partially) block binding and**  
547 **rescue expression of co-stimulatory molecules on DCs.**

548 A. Bead binding assay of ON mutants in the presence of FimH antagonists and blocking  
549 CD14 antibody. Overlaid fluorescence events in the bacteria gate (left panel). ON  
550 (black), ON  $\Delta fimH$  mutants (grey), 175  $\mu$ M D-mannose (blue), 1 mM D-mannose  
551 (yellow), 10  $\mu$ M M4284 (green) and 20  $\mu$ g/ml M14-23 antibody (violet). Quantification  
552 of fluorescent events in the bacterial gate (right panel) (3 biological replicates).

553 B. Overlaid expression levels of co-stimulatory molecules (CD40, CD80, CD86) of WT  
554 DCs after stimulation with ON (black), ON  $\Delta fimH$  mutants (grey), and ON stimulation  
555 in presence of 175  $\mu$ M D-mannose (blue), 1 mM D-mannose (yellow), 10  $\mu$ M M4284  
556 (green) and 20  $\mu$ g/ml M14-23 antibody (violet) (left panel). Quantification of median  
557 fluorescence values of co-stimulatory molecules (right panel) (3 biological replicates).

558 ns, not significant, \*  $p < 0.1$ , \*\*  $p < 0.05$ , \*\*\*  $p < 0.01$ , \*\*\*\*  $p < 0.001$  by one-way ANOVA  
559 followed by Dunnett's multiple comparisons (the mean of the data were compared to the  
560 mean of ON); data are represented as means  $\pm$  SD



## 561 **Supplementary Figure Legends**

562

### 563 **Supplementary Figure 1: In vitro generated DCs from Hoxb8-progenitor cells resemble** 564 **iCD103 DCs and differentiation of cells is not different after UPEC ON and OFF** 565 **stimulation.**

- 566 A. Upon stimulation with recombinant LPS (200 ng/ml) immature DCs change to a mature  
567 phenotype and cluster together (see cells highlighted in inlay).  
568 B. In vitro generated iCD103 DCs express CD103, Clec9A and CD14.  
569 C. Differentiated DCs after ON (black) and OFF (grey) stimulation are defined by  
570 expression of CD11c integrin (DC marker) and upregulation of MHCII (activation  
571 marker) (left panel). Quantification of differentiated DCs after ON (black) and OFF  
572 (grey) stimulation (right panel) (3 biological replicates).  
573 D. Histograms of MHCII of WT DCs after stimulation with ON (black) and ON  $\Delta fimH$   
574 mutants (grey) (left panel; iso – isotype control). Quantification of median fluorescence  
575 values of MHCII (right panel) (3 biological replicates).

576 ns, not significant, \*  $p < 0.1$ , \*\*  $p < 0.05$  by Student's t test; data are represented as means  
577  $\pm$  SD

578

### 579 **Supplementary Figure 2: Type 1 pilated UPECs do not affect integrin-independent** 580 **migration.**

- 581 A. Expression level of active and total CD11b integrin of WT DCs after LPS plus  $Mn^{2+}$   
582 stimulation (black) and LPS only stimulation (grey) (left panel; iso – isotype control).  
583 Quantification of CD11b integrin activity (active/total levels of CD11b) (right panel) (3  
584 biological replicates) (see also Figure 2B).  
585 B. Expression level of active and total  $\beta 1$  integrin after ON (black) and OFF (grey)  
586 stimulation of WT and  $\beta 2^{-/-}$  DCs (left panel; iso – isotype control). Quantification of  $\beta 1$   
587 integrin activity (active/total levels of CD11b) (right panel) (3 biological replicates). It  
588 should be noted, that  $\beta 1$  integrin activity after UPEC ON stimulation of  $\beta 2^{-/-}$  DCs might  
589 be artificially increased due to a slight decrease in total  $\beta 1$  integrin staining after ON  
590 stimulation.  
591 C. Adhesion assay of  $\beta 2^{-/-}$  DCs after ON and OFF stimulation (quantification see Figure  
592 2C).  
593 D. Ear crawl out assay of  $\beta 2^{-/-}$  DCs after ON and OFF stimulation. Endogenous DCs  
594 stained with anti-MHCII (magenta). Lymph vessels stained with anti-LYVE-1 (white)  
595 (left panel). Quantification of cells inside over outside of lymph vessel after ON (black)  
596 and OFF (grey) stimulation  $\beta 2^{-/-}$  DCs (right panel) (3 biological replicates)  
597 (quantification see Figure 2E).  
598 E. 3D collagen migration assay of WT DCs after stimulation with ON (black) and OFF  
599 (grey) mutants (3 biological replicates).

600 ns, not significant, \*\*  $p < 0.05$ , \*\*\*\*  $p < 0.001$  by one-way ANOVA followed by Dunnett's  
601 multiple comparisons (D) and by Student's t test (A and E); data are represented as means  
602  $\pm$  SD

603

604

605 **Supplementary Figure 3: FimH and CD14, but not TLR4, are important for the observed**  
606 **adhesion phenotype.**

- 607 A. Adhesion assay of *tlr4*<sup>-/-</sup> DCs after ON and OFF stimulation (quantification see Figure  
608 3A).  
609 B. Adhesion assay of *cd14*<sup>-/-</sup> DCs after ON and OFF stimulation (quantification see Figure  
610 3B).  
611 C. Adhesion assay of WT DCs after ON  $\Delta$ *fimH* stimulation (quantification see Figure 4E).

612

613 **Supplementary Figure 4: FimH is necessary, but it is not sufficient – bacteria need to**  
614 **have a pathogenic genetic background to cause adverse effects to DCs.**

- 615 A. Electron microscopy images, with zoomed in regions marked in inlays, (left panel) and  
616 yeast agglutination assay (right panel) of ON  $\Delta$ *fimH* mutant.  
617 B. Yeast agglutination assay non-pathogenic ON mutant with pathogenic *fimH* (ON  
618 *fimH*<sub>CFT073</sub>).  
619 C. Expression levels of co-stimulatory molecules (CD40, CD80, CD86) of WT DCs after  
620 stimulation with non-pathogenic ON (black) and non-pathogenic ON *fimH*<sub>CFT073</sub>  
621 mutants (grey) (left panel; iso – isotype control). Quantification of median fluorescence  
622 values of co-stimulatory molecules (right panel) (3 biological replicates).

623 ns, not significant, \*\*\*\* p<0.001 by Student's t test; data are represented as means  $\pm$  SD

624

625 **Supplementary Figure 5: Bead assay using CD14-coupled magnetic beads.**

- 626 A. Coupling of recombinant CD14-Fc to the magnetic Protein A beads was confirmed by  
627 staining with anti-CD14 antibody (dashed black – uncoupled beads, solid black –  
628 CD14-coupled beads).  
629 B. The scatter properties for the bead assay were defined by FSC and SSC properties of  
630 the bacteria (left panel). The *yfp* fluorescence signal of ON (black) and ON  $\Delta$ *fimH*  
631 (grey) mutants in the bacteria gate was used to define the fluorescent properties for  
632 analysis (right panel).  
633 C. Events (beads  $\pm$  bacteria) in FSC and SSC were recorded and are shown as dot plots.  
634 The bacteria gate as defined in A is shown.

635

636 **Supplementary Figure 6: UPEC ON and OFF mutants exhibit slightly different growth**  
637 **rates but no difference in minimal inhibitory concentration (MIC) to gentamicin.**

- 638 A. Doubling time of ON and OFF mutants in R10H20.  
639 B. MIC to gentamicin for ON and OFF mutants (MIC = 1.5  $\mu$ g/ml; 5x MIC was used in the  
640 infection assays after 1 h of co-culture of bacteria and DCs).

641 ns, not significant, \*\* p<0.05 by Student's t test; data are represented as means  $\pm$  SD

642

643

## 644 **Methods**

### 645 **Experimental Model and Subject Details**

#### 646 ***Animals***

647 Mice were bred and maintained at the local animal facility in accordance with the IST Austria  
648 ethics commission. All experiments were conducted in accordance with the Austrian law for  
649 animal experiments. Permission (BMFWF-66.018/0010-WF/V/3b/2016) was granted by the  
650 Austrian federal ministry of science, research and economy.

#### 651 ***Cell culture***

652 R10 medium, RPMI 1640 + 10 % FCS, 2 mM L-Glutamine, 50  $\mu$ M beta-mercaptoethanol,  
653 100 U/ml Penicillin and 100  $\mu$ g/ml Streptomycin, was used as basic medium. Stem cell  
654 medium was supplemented with 10 ng/ml IL-3, 20 ng/ml IL-6 and 1 % stem cell factor (SCF)  
655 supernatant produced by B16 melanoma cells. Hoxb8 medium was supplemented with  
656 125 ng/ml Flt3 and 1  $\mu$ M estradiol. iCD medium was supplemented with 2 ng/ml GM-CSF and  
657 75 ng/ml Flt3. GM-CSF and Flt3 supernatants were produced by hybridoma cells and  
658 concentration of cytokines was measured by ELISA. All media were used pre-warmed. Cells  
659 were grown routinely at 37 °C with 5 % CO<sub>2</sub>. For infection and subsequent assays, cells were  
660 cultured in R10 medium without antibiotics and buffered with 20 mM HEPES (R10H20) in the  
661 absence of CO<sub>2</sub>.

#### 662 ***WT, $\beta 2^{-/-}$ , $tlr4^{-/-}$ and $cd14^{-/-}$ Hoxb8 cell generation***

663 5-week-old wild type C57BL/6J,  $\beta 2^{-/-}$  (B6.129S7-*Itgb2*<sup>tm1Bay/J</sup>),  $tlr4^{-/-}$  (B6(Cg)-*Tlr4*<sup>tm1.2Karp/J</sup>) and  
664  $cd14^{-/-}$  (B6.129S4-*Cd14*<sup>tm1Frm/J</sup>) mice were obtained from the Jackson Laboratory.  
665 Immortalization of bone marrow cells was performed as described previously (Leithner *et al*,  
666 2018; Redecke *et al*, 2013). In brief, bone marrow was isolated from the femur and tibia by  
667 centrifugation and cells were precultured in stem cell medium for 3 days to enter the cell cycle.  
668  $1 \times 10^5$  cells were spin-infected with Hoxb8-MSCV retrovirus using lipofectamine at 1000 g for  
669 1 h in Hoxb8 medium. Cells were fed and split every few days for 3-4 weeks until all uninfected  
670 cells died off and only Hoxb8 infected, immortalized, cells were left.

#### 671 ***Dendritic cell differentiation***

672 iCD103 DCs, expressing CD103, were differentiated from Hoxb8 progenitor cells as described  
673 previously for bone marrow cells with minor modifications (Mayer *et al*, 2014). In brief, Hoxb8  
674 cells were seeded at a density of  $3 \times 10^5$  cells into 10 cm bacterial culture dishes in 10 ml iCD  
675 medium. On day 3 cells were split 1:2 and topped up with fresh iCD medium to 10 ml. On day  
676 6, cells were fed with 10 ml iCD medium and non-adherent iCD103 DCs were frozen on day

677 9. For images of immature and matured cells, as well as flow cytometry staining of different  
678 surface markers see Supplementary Figure 1.

679 Frozen DCs were allowed to recover after thawing for at least 4 h before infection. Non-  
680 adherent cells were counted and seeded at a density of  $1-2 \times 10^5$ /ml in R10H20 medium.  
681 Assays were performed with DCs that were either stimulated with bacteria or recombinant  
682 LPS at 200 ng/ml.  $\beta 2^{-/-}$  DCs were purified from potential other cells using the EasySep™  
683 mouse Pan-DC enrichment kit (Stemcell Technologies) and allowed to rest for 1 h before the  
684 infection assay.

#### 685 ***Bacterial strain construction***

686 For cloning, strains were grown routinely in LB medium. Plasmids were maintained at  
687 100 µg/ml ampicillin or 50 µg/ml kanamycin. Single copy integration was performed at  
688 12 µg/ml chloramphenicol or 25 µg/ml kanamycin. For experiments, strains were grown at  
689 37 °C in R10H20 medium without antibiotics or in medium containing 0.5 % casamino acids,  
690 1x M9 salts, 1 mM MgSO<sub>4</sub>, 0.1 mM CaCl<sub>2</sub> and 0.5 % glycerol (CAA M9 glycerol). Primers used  
691 for cloning are listed in Table 1 and strains used are listed in Table 2.

692

693 Table 1. Primers used for cloning.

Oligonucleotide	Sequence
110	ATATGCATGCCAAAAGATGAAACATATATCATAAATAAGTTACGT
112	ATATGCATGCCAAAAGATGAAACATTCATAGAGGAAAGCATCG
119	CAGTAATGCTGCTCGTTTTGCCG
120	GACAGAGCCGACAGAACAAC
128	TGTGTAGGCTGGAGCTGCTTC
130	AAAAGAGAAGAGGTTTGTATTAACCTTATTGATAATAAAGTTAAAAAACACT GCTTCGAAGTTCC
131	CACTTTGTTTTGTAAACGAGTTTACTGCTGCAACACTGCACAGTTTTCCCC AAAAGATGAAACAT
132	ATTCATATGGAATAAATACAAGACAATCATAGAGGAAAGCATC
133	ATTCATATGGAATAAATACAAGACAATATCATAAATAAGTTACGTATTTTT TCTCAAGCATAAAAATATTA AAAAACGAC
134	TTGTATTTATTCCATATGAATATCCTCCTTAGTTCCATTCC
146	TAGCTTCAGGTAATATTGCGTACCTGCATTAGCAATGCCCTGTGATTTCTC CATATGAATATCCTCCTTAGTTCC
148	AGTGATTAGCATCACCTATACCTACAGCTGAACCCAAAGAGATGATTGTAT GTGTAGGCTGGAGCTGCTTCG
157	AGCATCACCTATACCTACAGCTG
158	AGCTTCAGGTAATATTGCGTACC
198	AGTGATTAGCATCACCTATACCTACAGCTGAACCCGAAGAGATGATTGTA ATGAAACGAGTTATTACC
276	TAGCTTCAGGTAATATTGCGTACCTGCATTAGCAATGCCCTGTGATTTCTT TATTGATAAACAAA
296	AGTGATTAGCATCACCTATACCTACAGCTGAACCCGAAGAGATGATTGTAT TGACGGCTAGCTCAGTCCTAGGTA
297	TAGCTTCAGGTAATATTGCGTACCTGCATTAGCAATGCCCTGTGATTTCTT CAGCACTGTCCTGCTCCTTGTGAT
3_SphI_pKD3_tes t	TGAATACCACGACGATTTCC
cam_test_R	CAACGGTGGTATATCCAGTGA
FarChro galK UO	GTTAATTATCATTTTTGCACCGCGTC
galK-KpnI-r	CCGGGTACCTCAGCACTGTCCTGCTCC
galK-ver-F	CCTACTCTATGGGCTGGCAC
galK-ver-R	GGAAAGTAAAGTCGCACCCC

694

695 *Locked mutants*

696 *E. coli* W ((Archer *et al*, 2011); ATCC 9637) and clinical isolate CFT073 ((Welch *et al*, 2002);  
697 ATCC 7000928; a kind gift of Ulrich Dobrindt) were used as well as derivatives of those strains.

698 Phase-locked mutants were generated by replacing the 9 bp long recognition site for the site-  
699 specific recombinases FimB and FimE in the internal repeat region on the left site of the fim  
700 switch (*fimS*) with an FRT site (Figure 1C). The *fimS* region was amplified either in the ON  
701 (primers 132 and 110) or OFF (primers 133 and 112) orientation from the chromosome of the  
702 wild type strain but omitting the 9 bp recognition site on the left site. An FRT-removable  
703 chloramphenicol resistance marker was amplified from pKD3 plasmid using primers 128 and  
704 134 (Datsenko & Wanner, 2000). The resistance marker was assembled left of the amplified  
705 *fimS* regions using NEBuilder assembly kit (NEB). The assembled DNA fragments were PCR  
706 amplified using primers 130 and 131 and integrated into the chromosome of the respective

707 strains instead of the endogenous *fimS* using lambda red recombination (Datsenko & Wanner,  
708 2000). In brief, *E. coli* W or CFT073 wild type bacteria were transformed with pSIM6 plasmid  
709 expressing thermal inducible Red genes under control of the native  $\lambda$  phage *pL* (Datta *et al*,  
710 2006) and selected with ampicillin at 30 °C. After inducing expression of lambda red genes at  
711 42 °C for 15 min, bacteria were made electrocompetent and transformed with 100 ng of the  
712 cleaned PCR fragments. Bacteria were allowed to recover in LB medium for 1 h at 37 °C  
713 before spreading on LB plates containing chloramphenicol.

714 After verifying single copy integration using primers 119, 120, *cam\_test\_R* and  
715 *3\_SphI\_pKD3\_test*, the resistance marker was subsequently removed using pCP20 plasmid  
716 (Cherepanov & Wackernagel, 1995). The mutated *fimS* region was sequenced to confirm  
717 deletion of the 9 bp long recombinase recognition site on the left site, but a fully intact  
718 recognition site on the right site. Presence or absence of the type 1 pilus on the bacterial outer  
719 membrane was confirmed by electron microscopy and yeast agglutination assay. Resulting  
720 locked mutants were: CFT073 locked-ON – KT179, CFT073 locked-OFF – KT180 and  
721 *E. coli* W locked-ON – KT232.

#### 722 *FimH* deletion mutant

723 *fimH* gene from CFT073 locked-ON mutants (KT179) was deleted by lambda red  
724 recombination. The FRT-flanked chloramphenicol resistance marker from pKD3 plasmid was  
725 amplified using primers 146 and 148 and integrated into the chromosome of KT179 strain.  
726 Successful deletion was confirmed by PCR (primers 157 and 158). Resistance was flipped  
727 using pCP20 plasmid resulting in CFT073 locked-ON  $\Delta$ *fimH* mutant – KT193. Presence of  
728 type 1 pili was confirmed by electron microscopy. Absence of *fimH* was confirmed by  
729 sequencing and yeast agglutination assay.

#### 730 *Chromosomal yfp* marker

731 *mVenus* driven by the right site of the lambda  $P_O$  was integrated in the lambda phage  
732 attachment site on the chromosome of CFT073 locked-ON (KT179) and CFT073 locked-ON  
733  $\Delta$ *fimH* (KT193) mutants using CRIM integration (Haldimann & Wanner, 2001). In brief, KT179  
734 and KT193 strains were transformed with pInt-ts helper plasmid and selected on ampicillin  
735 plates at 30 °C. Bacteria were made electrocompetent and transformed with *P<sub>R</sub>-mVenus*  
736 carrying pAH120-frt-cat integration plasmid. After recovery in LB medium for 1 h at 37 °C,  
737 expression of lambda red genes was induced at 42 °C for 15 min before spreading on LB  
738 plates containing chloramphenicol.

739 Single copy integration of the CRIM plasmid was verified with PCR as mentioned previously  
740 (Haldimann & Wanner, 2001). Since pAH120-frt-cat was designed to have a FRT flanked

741 chloramphenicol resistance marker (Nikolic *et al*, 2018), resistance was subsequently  
742 removed using pCP20 plasmid (Cherepanov & Wackernagel, 1995). Resulting mutants were:  
743 CFT073 locked-ON *attλ P<sub>R</sub>-mVenus* – VG003 and CFT073 locked-ON *ΔfimH attλ P<sub>R</sub>-mVenus*  
744 – KT257.

#### 745 *FimH replacement mutant*

746 The endogenous *fimH* gene of the non-pathogenic *E. coli* W strain was exchanged scar-less  
747 with *fimH* of the pathogenic UPEC strain CFT073 using *galK* selection/counter-selection  
748 (Kavčič *et al*, 2020). The FRT-flanked kanamycin resistance marker from gDNA harboring  
749 *ΔgalK::kan* (gift of Bor Kavčič) was amplified using primers *galK-ver-F* and *galK-ver-R* (gift of  
750 Bor Kavčič) and integrated into the *galK* gene of *E. coli* W locked-ON (KT232). Loss of *galK*  
751 gene was confirmed by PCR using primers *FarChro galK UO* and *galK-KpnI-r* (gift of Bor  
752 Kavčič). Resistance was flipped using pCP20 plasmid. *galK* under constitutive J23100  
753 promoter was amplified from pKD13-PcgalK plasmid using primers 296 and 297 and  
754 transformed to replace the endogenous *fimH* gene using lambda red recombination. After  
755 recovery, any residual carbohydrate residues were removed by washing the cells several  
756 times with M9 buffer (Tomasek *et al*, 2018) before plating on M9 minimal medium containing  
757 0.1 % galactose as only carbohydrate source for positive selection. Integration of *galK* gene  
758 into *fimH* was confirmed by PCR using primers 157 and 158. CFT073 *fimH* gene was amplified  
759 from a gblock (IDT) carrying the *fimH* sequence from CFT073 using primers 198 and 276 and  
760 integrated instead of the constitutive *galK* gene. After recovery, cells were washed several  
761 times as before. Transformants were counter-selected on artificial urine medium agar plates  
762 (AU-Siriraj; (Chutipongtanate & Thongboonkerd, 2010)) supplemented with 20 µg/ml  
763 L-aspartate and 20 µg/ml L-isoleucine (Bouvet *et al*, 2017), and containing 0.2 % 2-deoxy-D-  
764 galactose (DOG) and 0.2 % glycerol for the counter-selection. Pathogenic *fimH* integration  
765 was confirmed by PCR using primers 157 and 158. Resulting mutant was *E. coli* W locked-  
766 ON *fimH::fimH<sub>CFT073</sub>* – MG002.

#### 767 *FimH amino acid mutants*

768 Single and triple point mutants of amino acids predicted to be most involved in binding to CD14  
769 were generated using *galK* selection/counter-selection as mentioned above. Briefly, *galK* was  
770 deleted from CFT073 locked-ON mutants (KT179). Thereafter constitutive expressed *galK*  
771 was inserted in the endogenous *fimH* of this strain for selection. 100 µg gblocks (IDT) carrying  
772 either Y48A, R98A, T99A or the triple mutation (Y48A, R98A, T99A) in the *fimH* sequence  
773 from strain CFT073 were integrated instead of the constitutive *galK* gene. Correct integration  
774 of *fimH* having mutated amino acid residues was confirmed by sequencing. Resulting mutants

775 were CFT073 locked-ON *fimH*<sub>Y48A R98A T99A</sub> – KT260, CFT073 locked-ON *fimH*<sub>Y48A</sub> – KT261,  
776 CFT073 locked-ON *fimH*<sub>R98A</sub> – KT262 and CFT073 locked-ON *fimH*<sub>T99A</sub> – KT263.

777 Table 2. Used strains.

Strain	Reference
<i>E. coli</i> W (ATCC 9637)	Archer et al 2011
CFT073 O6:K2:H1 (ATCC 700928)	Welch et al 2002
KT179 (CFT073 locked-ON::FRT)	this paper
KT180 (CFT073 locked-OFF::FRT)	this paper
KT193 (CFT073 locked-ON::FRT $\Delta$ <i>fimH</i> ::FRT)	this paper
KT232 ( <i>E. coli</i> W locked-ON::FRT)	this paper
KT257 (CFT073 locked-ON::FRT $\Delta$ <i>fimH</i> att $\lambda$ <i>P<sub>R</sub>-mVenus</i> ::FRT)	this paper
KT260 (CFT073 locked-ON::FRT $\Delta$ <i>galK</i> ::FRT <i>fimH</i> :: <i>fimH</i> <sub>Y48A R98A T99A</sub> )	this paper
KT261 (CFT073 locked-ON::FRT $\Delta$ <i>galK</i> ::FRT <i>fimH</i> :: <i>fimH</i> <sub>Y48A</sub> )	this paper
KT262 (CFT073 locked-ON::FRT $\Delta$ <i>galK</i> ::FRT <i>fimH</i> :: <i>fimH</i> <sub>R98A</sub> )	this paper
KT263 (CFT073 locked-ON::FRT $\Delta$ <i>galK</i> ::FRT <i>fimH</i> :: <i>fimH</i> <sub>T99A</sub> )	this paper
MG002 ( <i>E. coli</i> W locked-ON::FRT $\Delta$ <i>galK</i> ::FRT <i>fimH</i> :: <i>fimH</i> <sub>CFT073</sub> )	this paper
VG003 (CFT073 locked-ON::FRT att $\lambda$ <i>P<sub>R</sub>-mVenus</i> ::FRT)	this paper

778

## 779 ***Inhibitors used***

780 Since the suggested upper daily limit of orally applied D-mannose to treat UTIs is 9 g, leading  
781 to blood mannose levels of roughly 175  $\mu$ M (Alton *et al*, 1997), we decided to compare this  
782 concentration to a strongly increased one of 30-60 g D-mannose resulting in 1 mM blood  
783 mannose levels. The small mannoside M4284 (medchemexpress) was used at 10  $\mu$ M. The  
784 blocking CD14 antibody M14-23 was used at 20  $\mu$ g/ml (Tsukamoto *et al*, 2010). Inhibitors were  
785 added as the same time as the bacteria, no pre-incubation steps were carried out.

786

## 787 **Method Details**

### 788 ***Yeast agglutination assay***

789 *Saccharomyces cerevisiae* was grown in YPD medium at 30 °C for 2 days. After  
790 centrifugation, cells were resuspended in M9 buffer to an OD<sub>600</sub> of 1 and stored in the fridge.  
791 An aliquot of the yeast was transferred to glass slides and bacterial colonies were directly  
792 mixed into the yeast solution. Agglutination occurred within few seconds to 1 min. Pictures of  
793 agglutinated yeast and bacteria cells were taken on a brightfield microscope at 10x  
794 magnification and images were processed with Fiji.

### 795 ***Growth curve assay and doubling time estimation***

796 Single bacterial colonies were inoculated in 160  $\mu$ l R10H20 in 96-well plates and grown  
797 overnight at 220 rpm at 37 °C. The next day, cultures were diluted 1 in 1,000 in R10H20



798 supplemented with 0.0005 % triton-X and grown at 37 °C with shaking. Optical density was  
799 measured every 30 min at 600 nm at a Synergy H1 plate reader for a total of 7 h. The data  
800 were blank normalized, and the doubling time (dt) was calculated from exponential data using

801 following formula  $dt = \frac{t_2 - t_1}{\left(3.3 * \log\left(\frac{OD_{600^2}}{OD_{600^1}}\right)\right)}$ .

802 For all assays, bacteria were grown to early- to mid-exponential phase (OD<sub>600</sub> 0.25; *E. coli* W  
803 4 h, CFT073 3 h 20 min; see Supplementary Figure 6A) in 1 ml R10H20 medium, if not  
804 indicated otherwise, at 37 °C in deep well plates.

### 805 **Minimal inhibitory concentration (MIC) assay**

806 Cultures were grown to OD<sub>600</sub> of 0.25 and approximately 10<sup>6</sup> bacteria were used for MIC  
807 assays. Serial dilutions of gentamicin were performed in a microdilution manner using 96-well  
808 plates and OD<sub>600</sub> was measured after 18 h incubation at 37 °C with shaking. The threshold to  
809 calculate the MIC was set to detectable growth above the blank background after  
810 normalization. CFT073 locked-ON (KT179) and locked-OFF (KT180) mutants had similar MIC  
811 to gentamicin in R10H20 medium (see Supplementary Figure 6B). 5x the MIC was used to  
812 prevent extracellular growth of bacteria in the infection assays (7.5 µg/ml).

### 813 **Electron microscopy**

814 Bacteria were grown to mid-exponential phase in CAA M9 glycerol medium, fixed with  
815 glutaraldehyde (EM grade, final concentration 2.5 %) for 30 min at 4 °C, washed twice with  
816 PBS and concentrated in water. Formvar-coated copper grids were glow discharged for 2 min  
817 and 5\*10<sup>6</sup> bacteria were loaded for 5 min. Excess liquid was removed with filter papers and  
818 bacteria were stained with Uranylless for 2 min. After removal of excess liquid, the grids were  
819 washed 10 times in water and dried. EM images were taken at TEM T10 microscope at 80 kV.  
820 Images were processed with Fiji.

### 821 **Predicted protein-protein interaction**

822 Crystal structures of FimH (PDB: 6GTV), mouse CD14 (PDB: 1WWL), human CD14 (PDB:  
823 4GLP), mouse TLR4 (PDB: 3VQ2) and mouse CD48 (PDB: 2PTV) were obtained from  
824 rscb.org, cleaned from solvents and other co-precipitated molecules using PyMol and run on  
825 HawkDock server to predict protein binding (Weng *et al*, 2019). Additional MM/GBSA analysis  
826 was run to predict free binding energy (Hou *et al*, 2011).

### 827 **Bead binding assay**

828 5 µl of 10 µm bead slurry (PureProteom Protein A magnetic beads, Merck) were used per  
829 reaction. Beads were washed 3x with PBS containing 0.005 % tween (PBS-T). Beads were

830 collected using a magnetic stand. 5 µg CD14-Fc recombinant protein (RND System) in a total  
831 of 10 µl of PBS-T was coupled to the beads for 1 h at 4 °C with rotation. After washing the  
832 beads, 100 µl of bacteria grown to early- to mid-exponential phase in CAA M9 glycerol medium  
833 (~1\*10<sup>7</sup> bacteria) were incubated in the presence of tween with the CD14-coupled beads for  
834 1 h at 4 °C with rotation. After washing, this time with M9 glycerol medium containing tween,  
835 bead-bound bacteria were fixed with 0.5 % PFA in M9 buffer for 10 min at 4 °C.

836 For flow cytometry, samples were diluted in M9 buffer and analyzed on a FACS Canto II (BD).  
837 10,000 events gated on FSC-A and SSC-A to exclude debris were recorded at medium flow  
838 rate and data were analyzed using FlowJo software. Three things should be noted: First, we  
839 observed that due to the high force applied in the sample injection tube during acquisition, the  
840 bacteria were separated from the magnetic beads they previously were bound to, leading to a  
841 single detectable fluorescent peak only. Second, due to their size, the magnetic beads are  
842 also detected in the gating range specific for the bacterial population (see Supplementary  
843 Figure 5C). Third, as can also be seen in the fluorescent images, the magnetic beads have  
844 weak auto-fluorescence in the FITC channel. We therefore quantified the amount of  
845 fluorescent events applying the same gating strategy as for the bacterial population only  
846 (Supplementary Figure 5B and C).

847 For fluorescent microscopy, samples were embedded in mounting buffer and spread on a  
848 cover slip. Images were taken with 100x magnification on a custom-built Olympus widefield  
849 microscope with Hamamatsu Orca Flash4.0v2 camera and LED-based fluorescence  
850 illuminator using YFP (x513/22,m543/22) fluorescence channel (Chait *et al*, 2017). Images  
851 were processed with Fiji and deconvoluted with Huygens software.

### 852 ***Type 1 pili extracts***

853 Type 1 pili extracts were generated as described previously (Sheikh *et al*, 2017) with minor  
854 modifications. Briefly, CFT073 locked-ON (KT179) and CFT073 locked-ON *ΔfimH* (KT193)  
855 were grown overnight in CAA M9 glycerol medium and harvested at 4,000 g for 1 h. The cell  
856 pellet was resuspended in 1 mM Tris-HCl (pH 8.0) and incubated at 65 °C for 1 h with  
857 occasional vortexing. After pelleting the cells at 15,000 g for 10 min, type 1 pili were  
858 precipitated from the supernatant overnight in the presence of 300 mM NaCl and 100 mM  
859 MgCl<sub>2</sub> at 4 °C. Type 1 pili were concentrated at 20,000 g for 10 min, washed once with 1 mM  
860 Tris-HCl and snap frozen in a small volume of 1 mM Tris-HCl.

### 861 *Dot blot assay*

862 The recombinant chimera CD14-Fc protein was biotinylated using the EZ-Link Sulfo  
863 NHS-LC-LC-Biotin kit (Thermofisher) and a 5-fold molar excess of biotin. In brief, CD14-Fc

864 was dissolved in PBS at 1 mg/ml and biotinylated with a 5-fold molecular excess of biotin for  
865 30 min at room temperature (RT). The biotinylation reaction was stopped with 3 mM Tris-HCl  
866 (pH 7.0).

867 The PVDF membrane was activated for 5 min with MeOH, washed for 5 min in water and  
868 allowed to dry for 5 min. 20 ng biotinylated CD14 and roughly 20 µg type 1 pili extract from  
869 CFT073 ON (KT179) and CFT073 ON *ΔfimH* (KT193) mutants were loaded onto the  
870 membrane. Protein spots were allowed to dry for 10 min and then the membrane was blocked  
871 in 3 % BSA in PBS for 30 min at 37 °C. 40 ng biotinylated CD14 in 3 % BSA in PBS and  
872 0.05 % tween was blotted on the membrane and incubated for 1 h at 37 °C. Then the  
873 membrane was washed 3x in PBS with tween for 5 min each. Streptavidin-HRP antibody was  
874 pre-diluted 1:100 in 3 % BSA in PBS with tween and diluted once more 1:5,000 in PBS with  
875 tween. The membrane was incubated with Streptavidin-HRP for 1 h at RT. After washing again  
876 3x as before, chemiluminescence was detected using clarity ECL substrates (Biorad).

### 877 ***Infection assays***

878 DCs were seeded at a density of  $1-2 \times 10^5$  cells/ml in R10H20 medium (for adhesion assays  
879 black 24-well tissue-treated dishes were used, for any other assay non-treated dishes were  
880 used). DCs were matured either by addition of 200 ng/ml recombinant LPS or bacteria at a  
881 multiplicity of infection (MOI) of 10 (10 bacteria per 1 DC). 1 h post infection (pi) gentamicin  
882 was added at 7.5 µg/ml to prevent extracellular growth of bacteria. 18-20 h pi subsequent  
883 assays were performed.

### 884 ***Adhesion assay***

885 Non-adherent DCs were removed, and adherent cells were washed twice with 500 µl PBS.  
886 Adherent cells were stained with Hoechst 33342 (NucBlue reagent, 2 drops/ml) in R10H20  
887 medium for 30 min at 37 °C. Cells were washed twice and 1 ml Live Cell Imaging solution  
888 (140 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl<sub>2</sub>, 1.0 mM MgCl<sub>2</sub>, 20 mM HEPES, pH 7.4) was  
889 added to the wells. Fluorescence was measured with a Synergy H1 plate reader (excitation  
890 490 nm, emission 520 nm, bottom reading without lid, 50 data points per well). Pictures of  
891 adherent cells were taken on a brightfield microscope at 4x magnification and images were  
892 processed with Fiji.

### 893 ***Flow cytometry staining***

894 DCs were collected and incubated in FACS buffer (1x PBS, 2 mM EDTA, 1 % BSA; RT) or  
895 Tyrode's buffer (used for active CD11b and β1 staining, on ice) with Fc receptor block for  
896 20 min. Cells were stained for 30 min with antibodies using the respective buffer with Fc  
897 receptor block. Cells were washed twice with PBS and resuspended in the respective buffer

898 for analysis on FACS Canto II (BD). 10,000 events gated on FSC-A and SSC-A to exclude  
899 debris were recorded at medium flow rate. Data were analyzed using FlowJo software by  
900 performing doublet discrimination. Antibodies used are listed in Table 3.

901

902 Table 3. Antibodies used.

<b>Antibody</b>	<b>Source</b>	<b>Identifier</b>
Rat anti-MHC II (I-A/I-E) eFluor450 (clone M5/114.15.2)	eBioscience	48-5321-82
Armenian hamster anti-CD11c APC (clone N418)	eBioscience	17-0114-82
Rat anti-CD18 PE (clone C71/16)	BD	553293
Rat anti-CD11b FITC (clone M1/70)	eBioscience	11-0112-82
Mouse anti-human CD11b (active epitope) APC (clone CBRM1/5)	eBioscience	17-0113-41
Rat anti-CD86 biotin (clone GL1)	eBioscience	13-0862-85
Armenian hamster anti-CD80 (clone 16-10A1)	eBioscience	13-0801-85
Rat anti-CD40 (clone 1C10)	Biolegend	102802
Armenian Hamster anti-CD103 Brilliant Violet 421 (clone 2E7)	Biolegend	121421
Rat anti-CD14 Brilliant Violet 421 (clone Sa14-2)	Biolegend	123329
Rat anti-CD4 eFluor450 (clone GK1.5)	eBioscience	48-0042-82
Armenian Hamster anti-CD69 APC-eFluor780 (clone H1.2F3)	eBioscience	47-0691-82
Rat anti-CD62-L PE (clone MEL-14)	eBioscience	12-0621-82
Rat IgG2b kappa Isotype Control eFluor450 (clone eB146/10H5)	eBioscience	48-4031-82
Armenian Hamster IgG Isotype Control APC (clone eBio299Arm)	eBioscience	14-4888-81
Rat IgG2a kappa Isotype Control PE (clone eBR2a)	eBioscience	12-4321-81
Rat IgG2b kappa Isotype Control FITC (clone eB149/10H5)	eBioscience	11-4031-82
Rat IgG1 kappa Isotype Control APC (clone eBRG1)	eBioscience	17-4301-82
Armenian Hamster IgG Brilliant Violet 421 (clone HTK888)	Biolegend	400936
Rat IgG2a kappa Isotype Control Biotin (clone RTK2758)	Stemcell Technologies	60076
Armenian Hamster IgG Isotype Control Biotin (clone eBio299Arm)	eBioscience	13-4888-81
Rat IgG2a kappa Isotype Control (clone RTK2758)	Stemcell Technologies	60076
Alexa Fluor 647-conjugated Streptavidin	Jackson ImmunoResearch	016-600-084
Donkey anti-rat IgG H+L Alexa Fluor488 AffiniPure F(ab') <sub>2</sub> Fragment	Jackson ImmunoResearch	712-546-150
Goat anti-rabbit Alexa Fluor488	Invitrogen	A11008
Rat anti-CD29 (clone 9EG7)	BD	553715
Armenian Hamster anti-CD29 PE (clone HMβ1-1)	Biolegend	102207
Armenian Hamster IgG Isotype Control PE (clone HTK888)	Biolegend	400907
M14-23 (anti-mouse CD14 antibody)	Biolegend	150102
Strep-Tactin HRP	iba-lifesciences	2-1502-001
Fc receptor block	eBioscience	14-9161-73

904 ***Ex vivo ear crawl out assays***

905 Ear crawl out assays were performed similar as published previously with minor modifications  
906 (Kopf *et al*, 2020). In brief, ears from 5-week-old female C57Bl/6J WT mice were first UV  
907 sterilized for 10 min and then split into dorsal and ventral halves. Ventral halves were placed  
908 in R10H20 medium, ventricles facing down. Ears were incubated with  $10^6$  CFT073 locked-ON  
909 (KT179) or OFF (KT180) bacteria for 48 h, renewing the infection stimulus after 24 h. 1 h after  
910 every infection 7.5  $\mu\text{g/ml}$  gentamicin was added to the medium. Ears were fixed using 4 %  
911 PFA and immersed using 0.2 % triton-X. After blocking in 1 % BSA in PBS, lymphatics were  
912 stained for 90 min using rat anti-Lyve-1 antibody and DCs were stained using biotinylated  
913 anti-MHCII antibody. Secondary antibodies, anti-rat F(ab')<sub>2</sub>-AF488 and Streptavidin-AF647,  
914 were used subsequently for 45 min each. Ears were fixed on cover slips with ventricles facing  
915 up using cover glasses. 10  $\mu\text{m}$  z-stacks were taken on inverted LSM800 confocal microscope  
916 with 488 and 640 nm LED-laser light source. Images were taken from 3 biological replicates  
917 (except *tlr4*<sup>-/-</sup> where 2 biological replicates were imaged) analyzing at least 2 field of views  
918 each. Maximum intensity projection images were processed with Fiji. Images were analyzed  
919 using custom-made scripts in Fiji. Pre-processing was done using lymphatics script and  
920 analysis using LVmeanDCarea script.

921 ***In vitro 3D collagen migration assay***

922 3D collagen chemotaxis assays were performed as described previously (Leithner *et al*, 2016),  
923 with minor modifications. Assays were performed in PureCol bovine collagen with a final  
924 collagen concentration of 1.6 mg/ml in 1x minimum essential medium eagle (MEM) and 0.4 %  
925 sodium bicarbonate using  $1-2 \times 10^5$  DCs. The collagen-cell mixture was cast to custom-made  
926 migration chambers and polymerized for 1 h at 37 °C. CCL19 chemokine (RND Systems) in  
927 R10 (0.625  $\mu\text{g/ml}$ ) was pipetted on top of the gel and the chambers were sealed with paraffin.  
928 Images were taken every 30 sec for a total of 5 h on bright field microscopes using 4x  
929 magnification and an exposure of 20 ms. Data were analyzes using custom-made Fiji scripts:  
930 images were pre-processed using Tracking\_pre-processing\_for\_brightfield script and  
931 analyzed using migrationspeedREP script.

932 ***In vitro extracellular matrix migration assay***

933 Cell-derived matrixes (CDM) were produced as described previously (Kaukonen *et al*, 2017).  
934 In brief, round shaped coverslips were coated with 0.2 % gelatin in PBS in 24-well dishes for  
935 1 h at 37 °C. Gelatin was crosslinked with 1 % glutaraldehyde in PBS for 30 min at RT and  
936 quenched with 1 M glycine in PBS for 20 min at RT. After washing the coverslips twice with  
937 PBS,  $5 \times 10^4$  3T3 mouse fibroblasts in DMEM, GlutaMAX, supplemented with 10 % FCS,  
938 100 U/ml penicillin and 100  $\mu\text{g/ml}$  streptomycin were seeded per well. After 48 h 3T3

939 fibroblasts reached confluency and were treated daily with ascorbic acid for better crosslinking  
940 of the extracellular matrix. Old medium was gently removed and fresh medium with 50 µg/ml  
941 sterile ascorbic acid was added for 10-14 days. 3T3 fibroblasts were extracted with extraction  
942 buffer (0.5 % Triton-X, 20 mM NH<sub>4</sub>OH in PBS) for 2 min and washed twice with PBS containing  
943 1 mM CaCl<sub>2</sub> and 1 mM MgCl<sub>2</sub> (PBS/Ca/Mg). DNA was digested with 100 µg/ml DNaseI in  
944 PBS/Ca/Mg for 1 h at 37 °C and CDM were washed twice with PBS/Ca/Mg before storage in  
945 PBS/Ca/Mg supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin at 4 °C.

946 Before use, CDM were placed onto custom-made imaging chambers and incubated with R10  
947 medium for 1 h at 37 °C. The medium was removed and a 1 µl CCL21 chemokine (RND  
948 Systems; 25 µg/ml) spot was injected into the CDM and incubated for 10 min. 1 ml R10  
949 medium was added on top and the CDM was incubated for 1 h at 37 °C to allow a chemokine  
950 gradient to form. After washing twice gently with R10 medium, CFT073 locked-ON (KT179)  
951 and locked-OFF (KT180) stimulated DCs were concentrated by centrifugation and the dense  
952 cell pellet was pipetted into the CDM at the opposite site to the chemokine spot. 2 ml of R10  
953 medium was added and images were taken every minute for a total of 6 h on a bright field  
954 microscope using 10x magnification and an exposure of 20 ms. Single cells outside of the cell  
955 cluster were counted after 5 h. Images were processed with Fiji.

#### 956 ***In vitro T cell assay***

957 T cell assays were performed as described previously (Leithner *et al*, 2021). In brief, primary  
958 naïve CD4<sup>+</sup> T cells were isolated from the spleen of OT-II mice (B6.Cg-Tg(TcraTcrb)425Cbn/J)  
959 using EasySep Mouse CD4<sup>+</sup> T cell isolation kit (Stemcell Technologies) after homogenization  
960 with a 70 µm cell strainer and resuspending the cells in PBS supplemented with 2 % FCS and  
961 1 mM EDTA. T cells were co-cultured with DCs matured with CFT073 locked-ON (KT179) or  
962 OFF (KT180) at a ratio of 5:1 (5\*10<sup>4</sup> T cells:1\*10<sup>4</sup> DCs) in 96-well round bottom well plates in  
963 R10 medium.

#### 964 *T cell activation*

965 After 24 h co-culture in the presence of 0.1 µg/ml ovalbumin (OVA), medium was removed by  
966 spinning. Cells were incubated with Fc receptor block in FACS buffer and stained with  
967 anti-CD4, anti-CD69 and anti-CD62-L antibodies for 15 min at 4 °C. After resuspending cells  
968 in FACS buffer, 100 µl were recorded on FACS Canto II (BD) and the ratio of CD69 to CD62L  
969 expression of CD4<sup>+</sup> T cells was analyzed by FlowJo software.

#### 970 *T cell priming*

971 T cells were stained with 5 µM CFSE stain in 5 % FCS in PBS for 5 min at RT. After 30 min  
972 recovery in R10 medium at 37 °C, cells were routinely checked for fluorescence.

973 After co-culturing with DCs for 4 days in the presence of 0.1 µg/ml OVA, medium was removed  
974 by spinning. Cells were stained with anti-CD4 antibody for 10 min at 4 °C. After resuspending  
975 cells in FACS buffer and 7AAD viability stain, 100 µl were recorded on FACS Canto II (BD).  
976 Dividing T cells were analyzed with FlowJo software.

### 977 ***DC-T cell interaction time***

978 Interaction time of DCs and T cells was measured as described previously (Leithner *et al*,  
979 2021). In brief, glass bottom dishes were plasma cleaned for 2 min and coated with 1x  
980 poly-L-lysine in water for 10 min at RT. Dishes were washed two times with water and dried  
981 overnight.  $1.5 \cdot 10^5$  DCs, pre-loaded with 0.1 µg/ml OVA for 1 h 30 min, were mixed with  $3 \cdot 10^5$   
982 T cells and loaded onto the coated dishes in a total volume of 300 µl. Images were taken every  
983 30 sec for a total of 6 h on bright field microscope using 20x magnification and an exposure of  
984 20 ms. Images were processed with Fiji.

### 985 ***Quantification and Statistical Analysis***

986 Data are represented as means ± standard deviations. Statistics were performed using  
987 GraphPad Prism version 9.0.2 for Windows. Statistical details for each experiment can be  
988 found in the respective Figure legends. Significance was defined as follows: \* p<0.1, \*\* p<0.05,  
989 \*\*\* p<0.01, \*\*\*\* p<0.001.

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991

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1004 **Author Contributions**

1005 Conceptualization, K.T., A.L., M.S.L., C.C.G. and M.S.; Methodology, K.T., C.C.G. and M.S.;

1006 Validation, K.T., C.C.G. and M.S.; Investigation, K.T., A.L., I.G.; Formal analysis, K.T.;

1007 Visualization, K.T.; Supervision, C.C.G. and M.S.; Writing – original draft, K.T.; Writing –

1008 review & editing, K.T., C.C.G. and M.S. with the help of all authors; Funding acquisition –

1009 C.C.G. and M.S.

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1011

1012 **Conflict of interests**

1013 K.T., M.S.L., C.C.G. and M.S. are inventors on patent application 21170193.3 (“Methods

1014 determining the potential of drug for treating bacterial infections and composition for treating

1015 bacterial infections”).

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1017

1018 **Data and material availability**

1019 Further information and requests for resources, scripts and data should be directed to Călin

1020 Guet ([calin.guet@ist.ac.at](mailto:calin.guet@ist.ac.at)) and Michael Sixt ([michael.sixt@ist.ac.at](mailto:michael.sixt@ist.ac.at)).

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1023 **Supplemental Items**

1024 **Table S1.** Predicted free binding energies of mouse CD14, TLR4 and CD48 to FimH.

1025 **Table S2.** Amino acids of FimH responsible for binding mouse CD14 and mannose, and amino

1026 acids of mouse CD14 responsible for FimH and LPS binding.

1027 **Table S3.** Predicted free binding energy of human CD14 and FimH.

1028 **File S1.** PDB file of mouse CD14 and FimH.

1029 **File S2.** PDB file of human CD14 and FimH.

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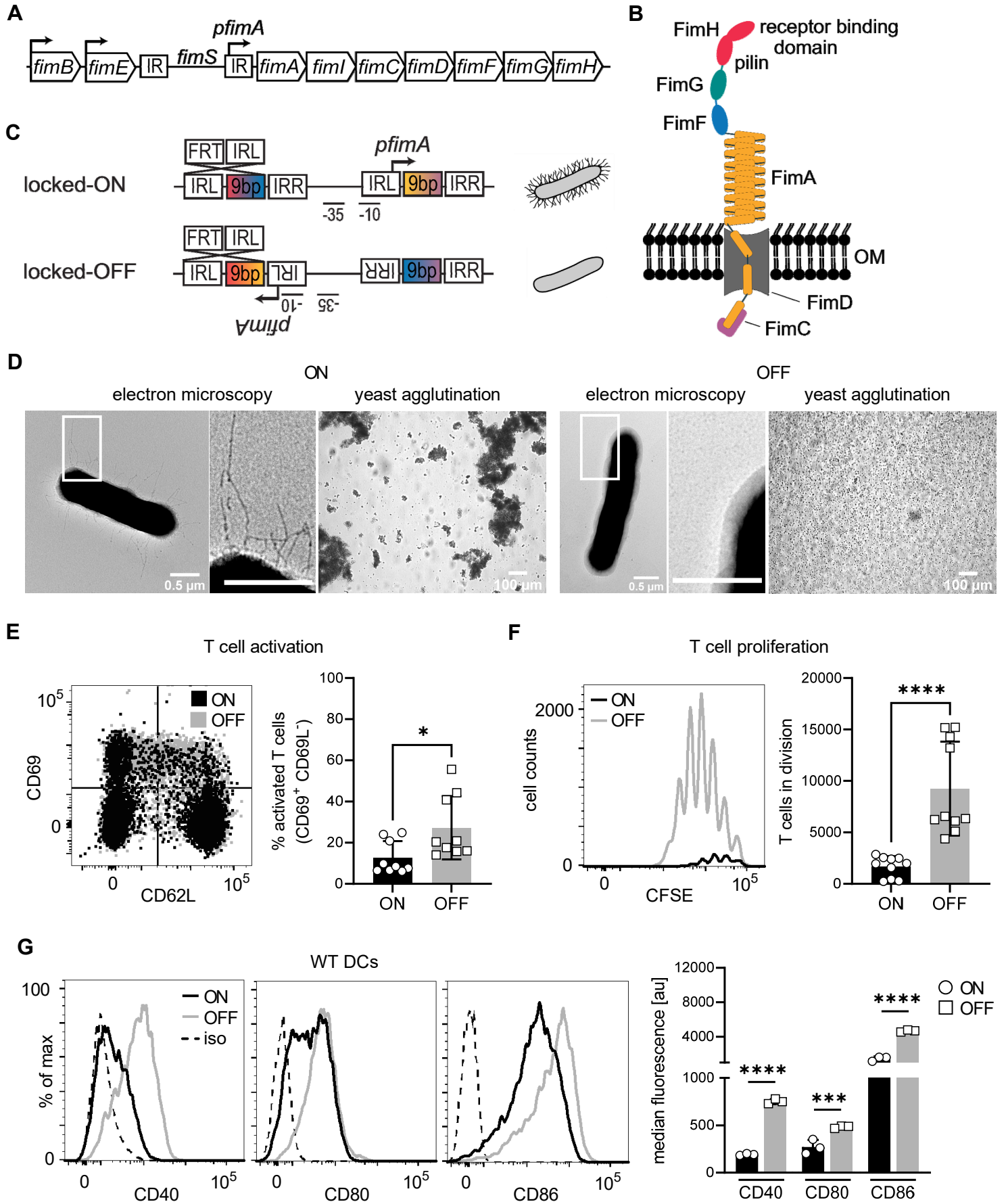
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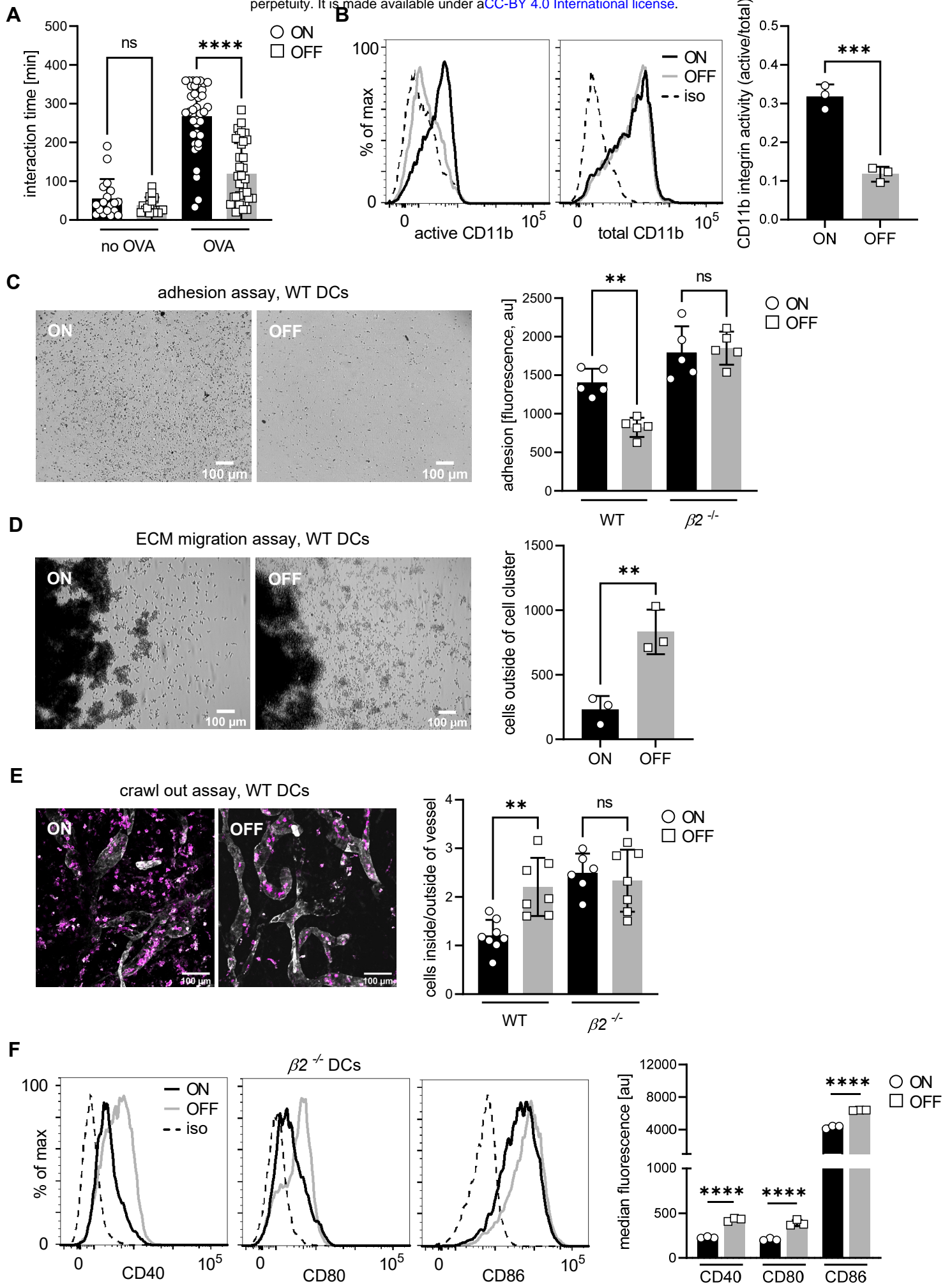
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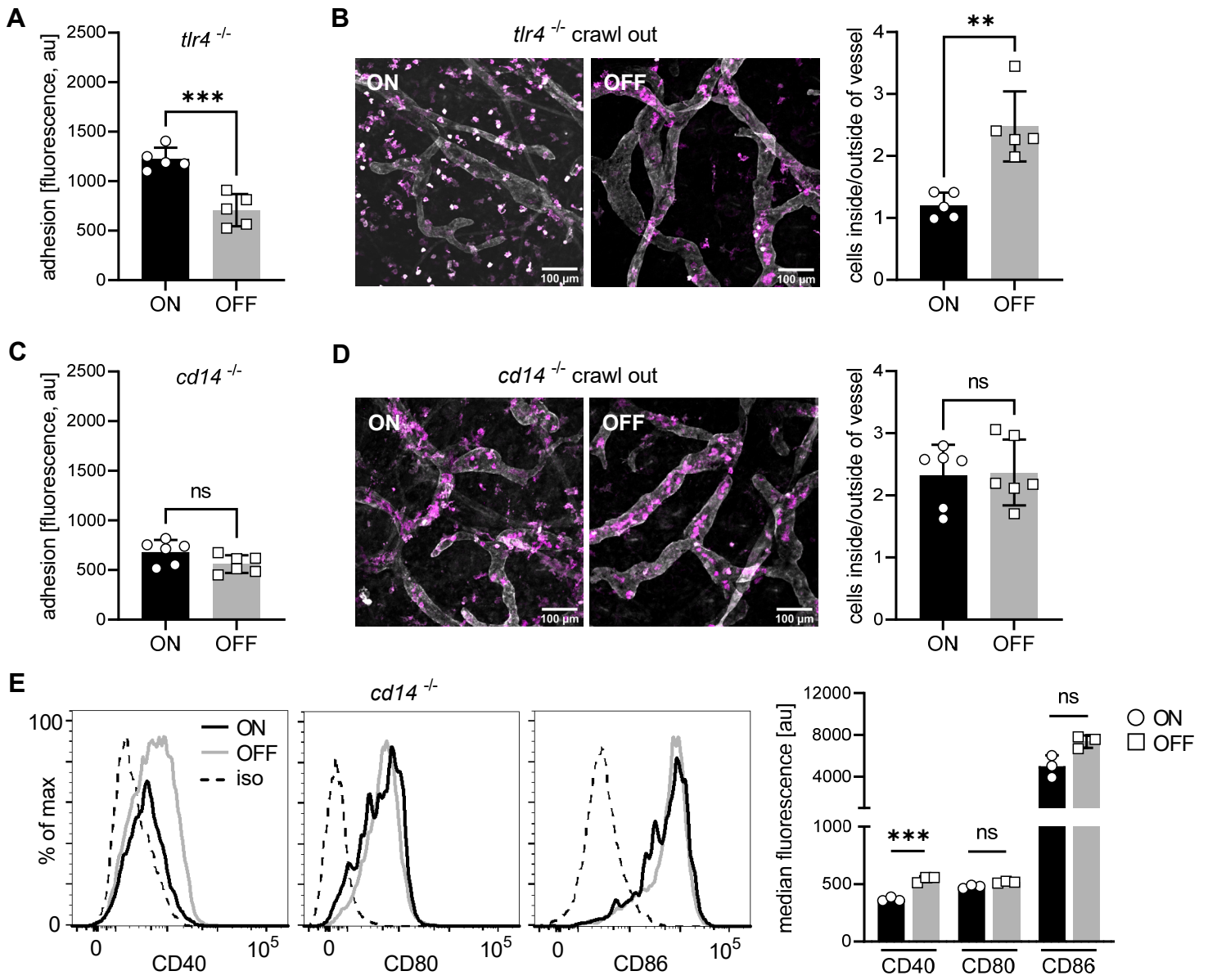


## Figure 1



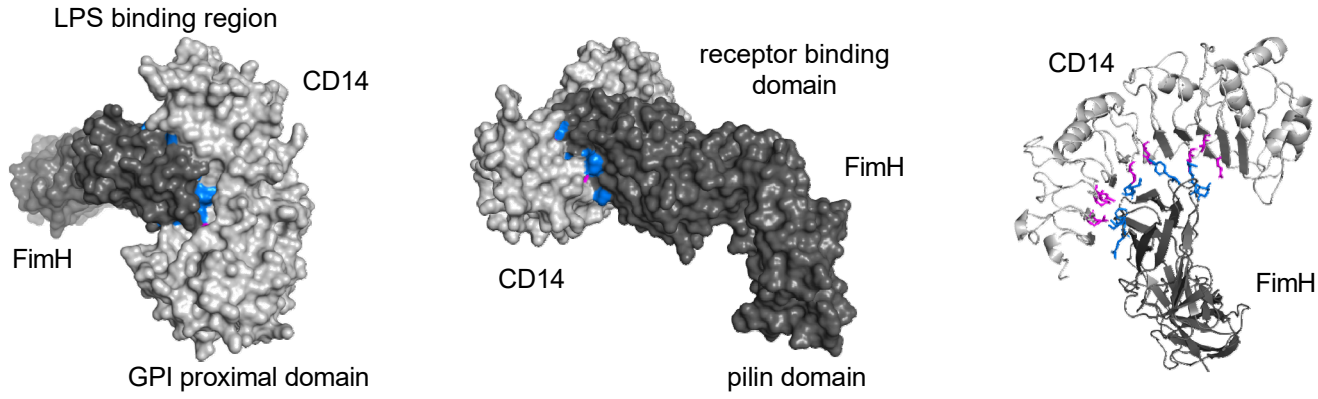


### Figure 3

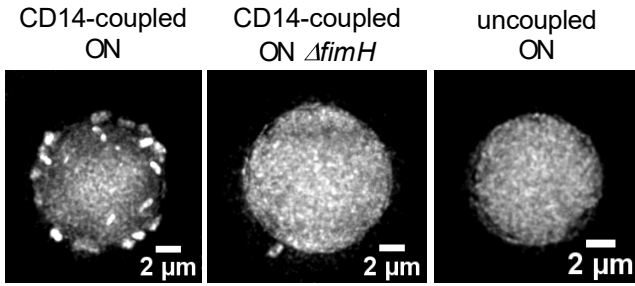


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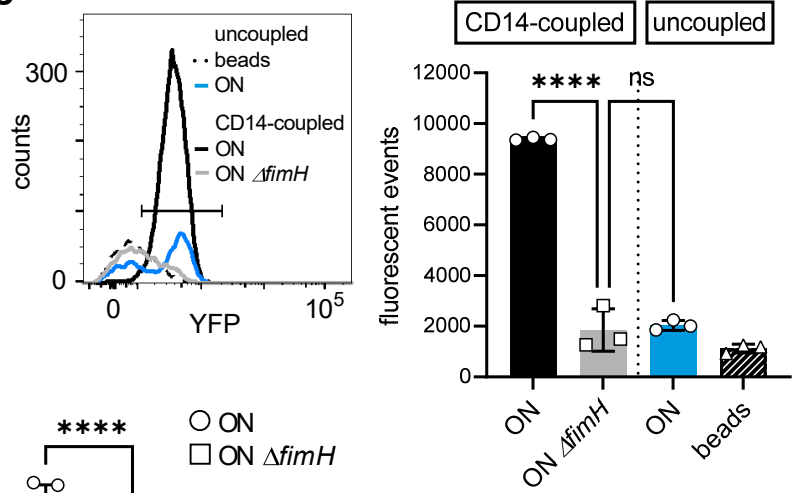
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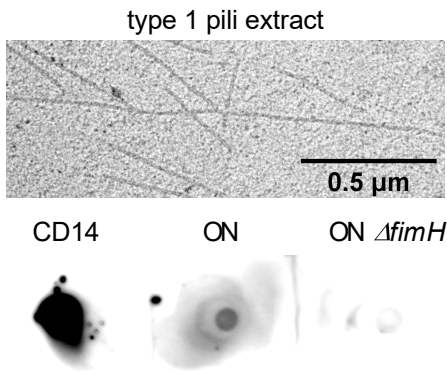
**B**



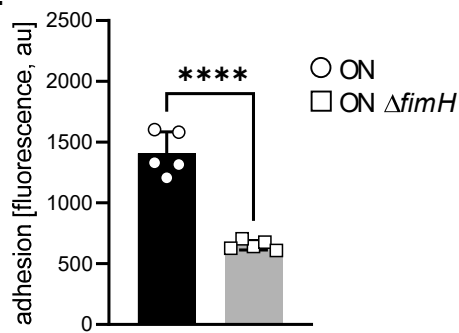
**C**



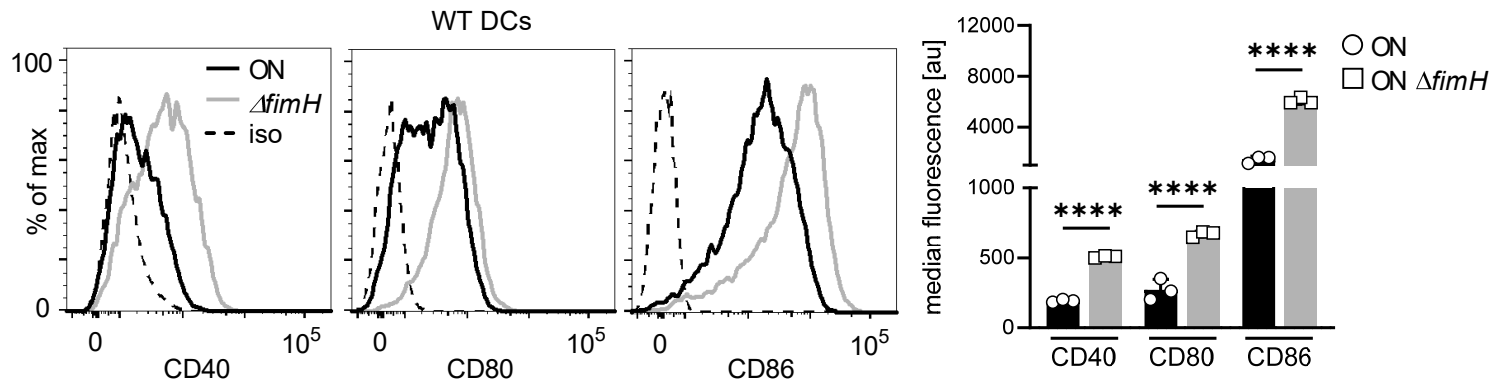
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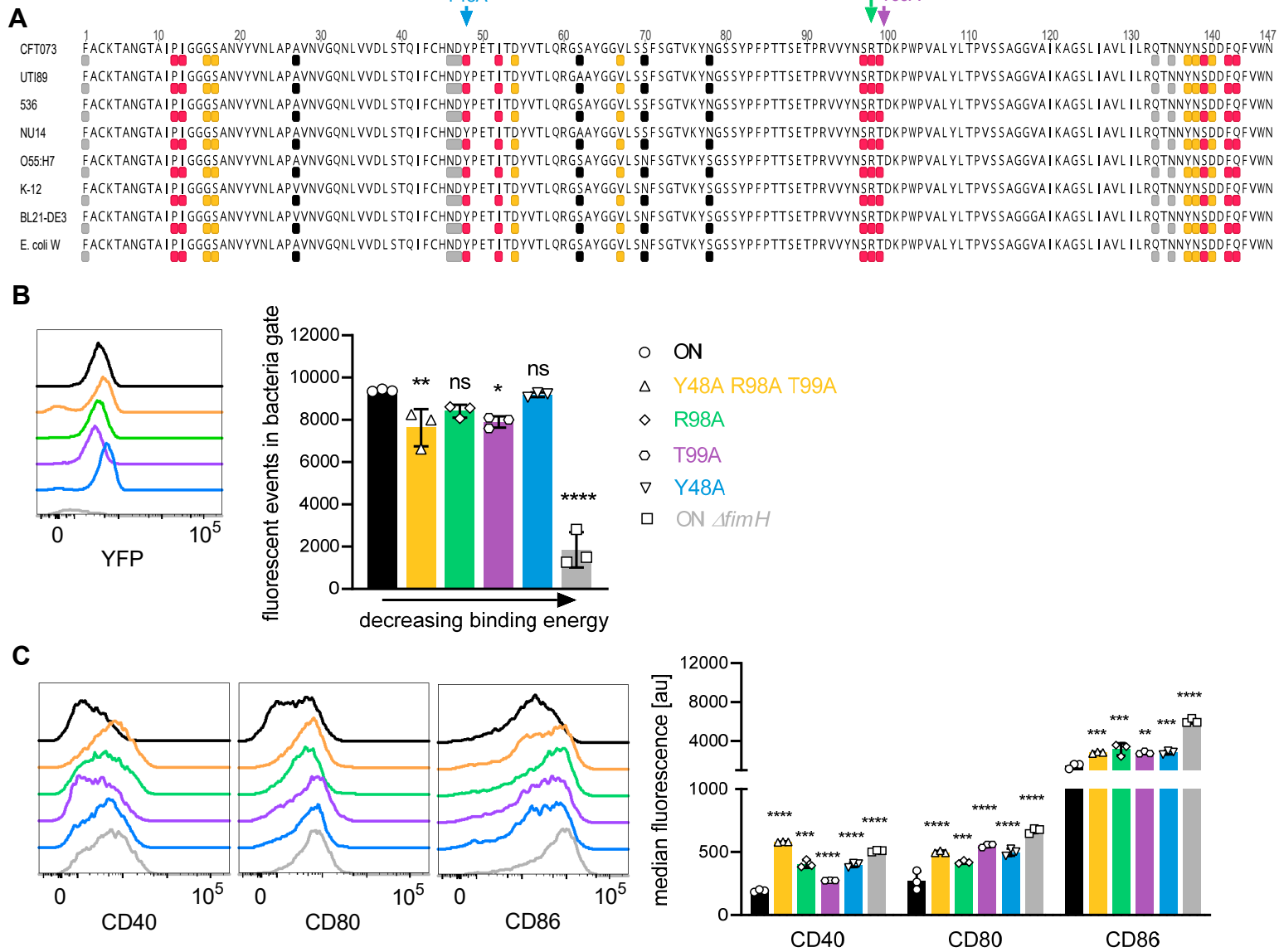
**E**



**F**

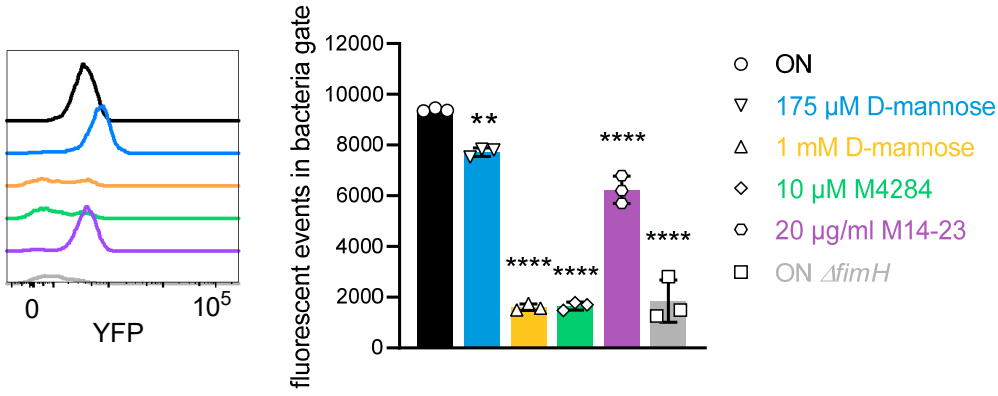


## Figure 5

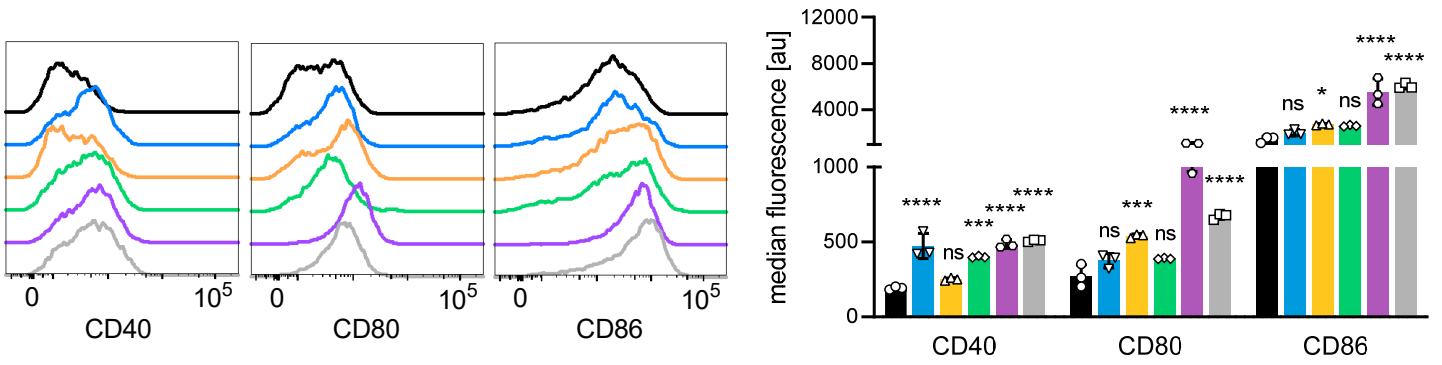


## Figure 6

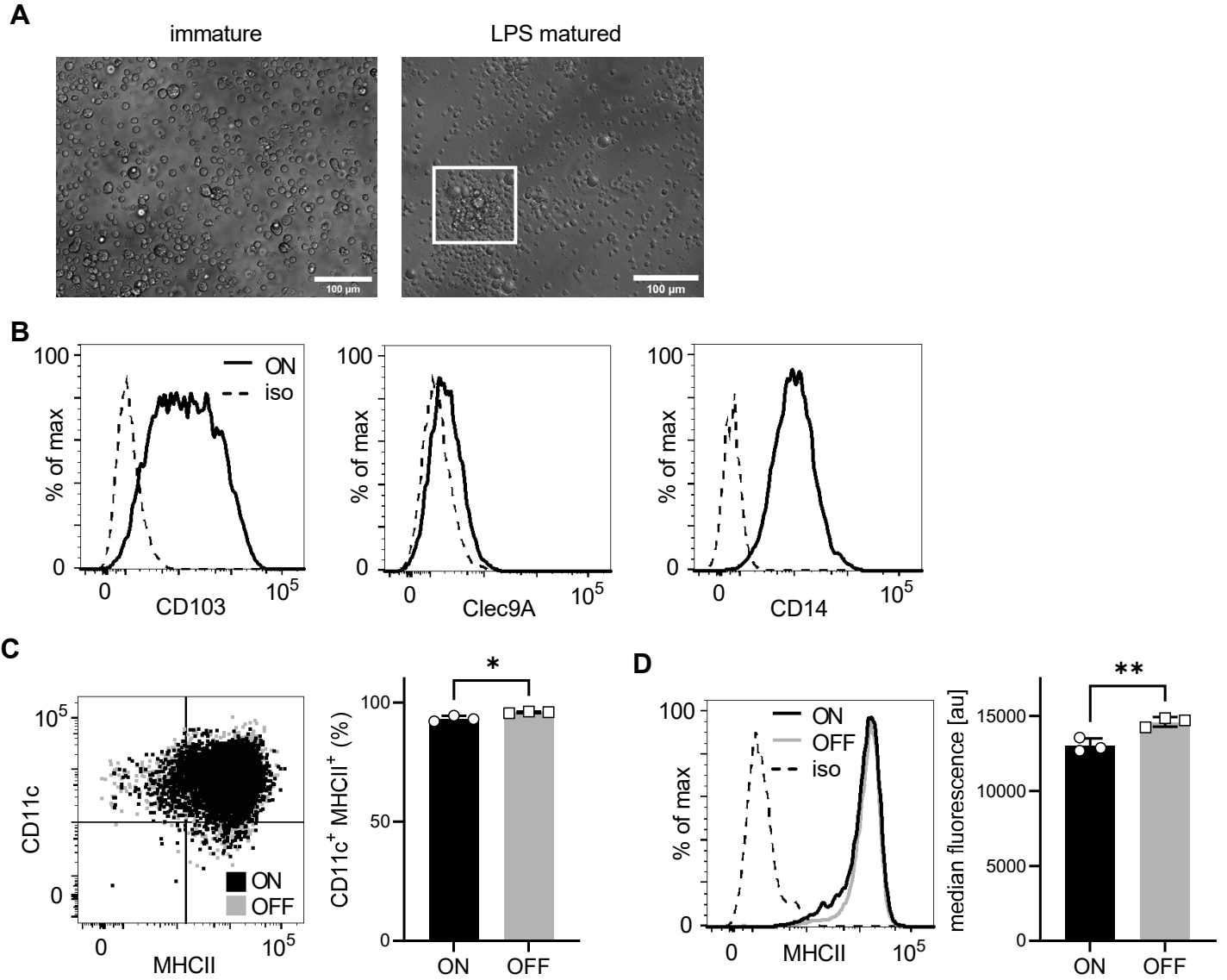
**A**



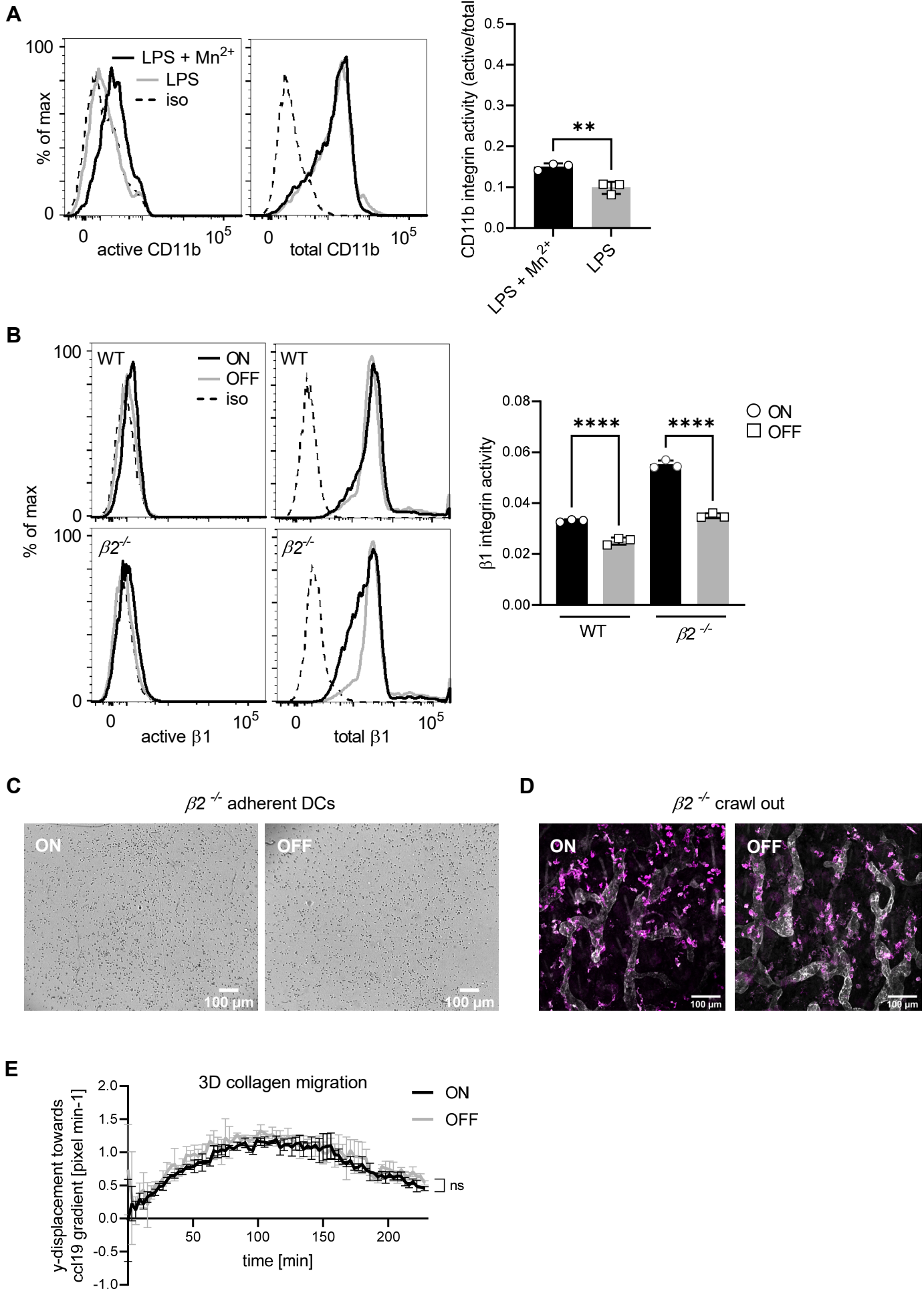
**B**



## Supplementary Figure 1



## Supplementary Figure 2

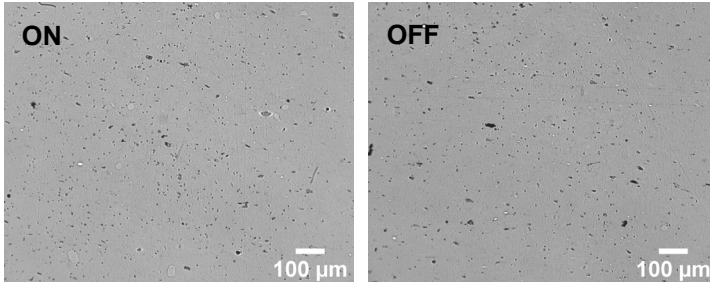




### Supplementary Figure 3

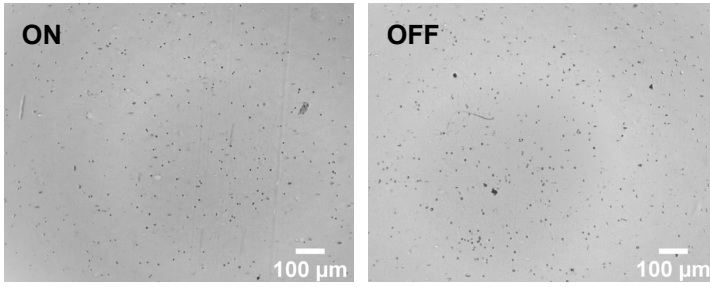
**A**

*tlr4*<sup>-/-</sup>



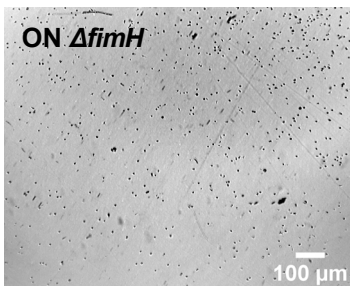
**B**

*cd14*<sup>-/-</sup>

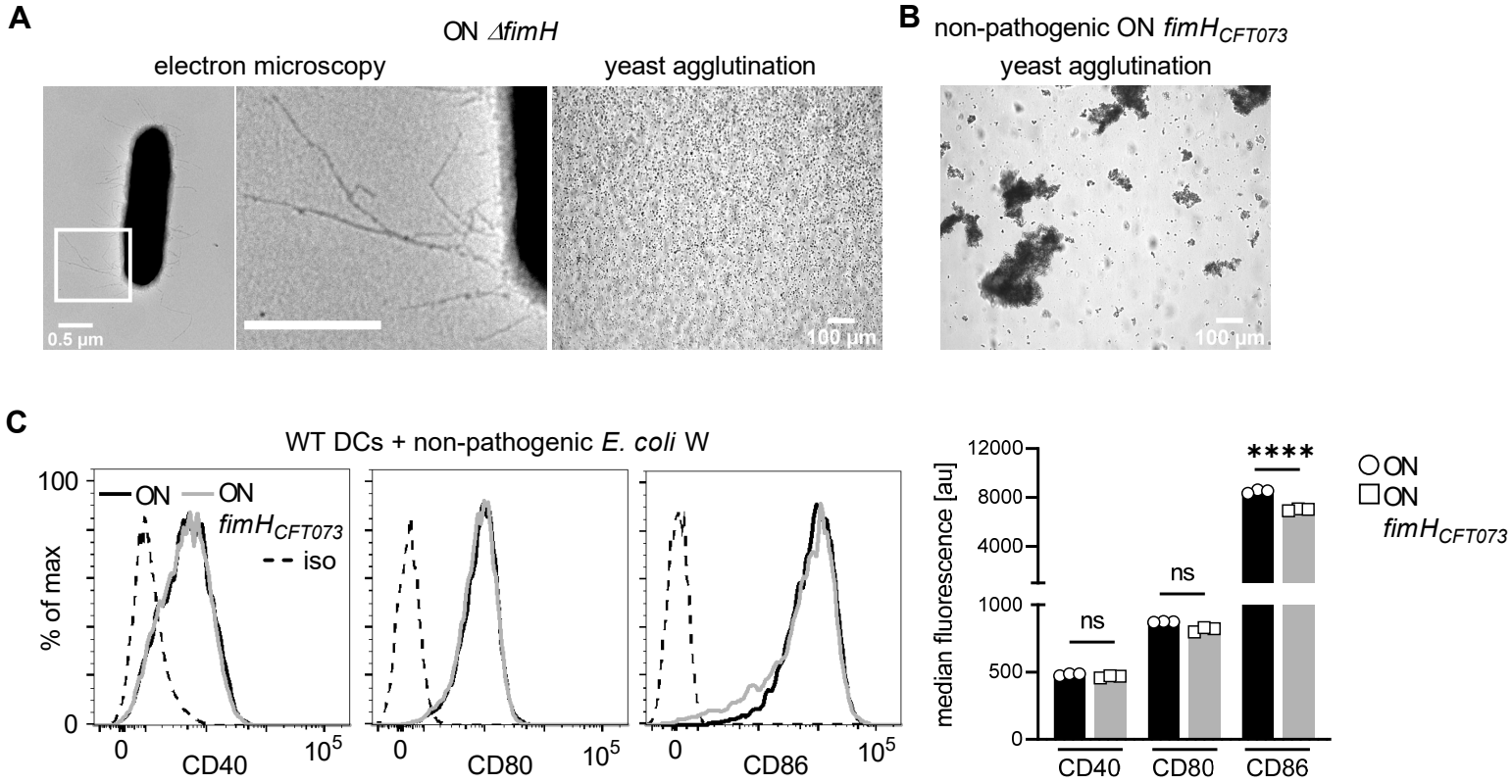


**C**

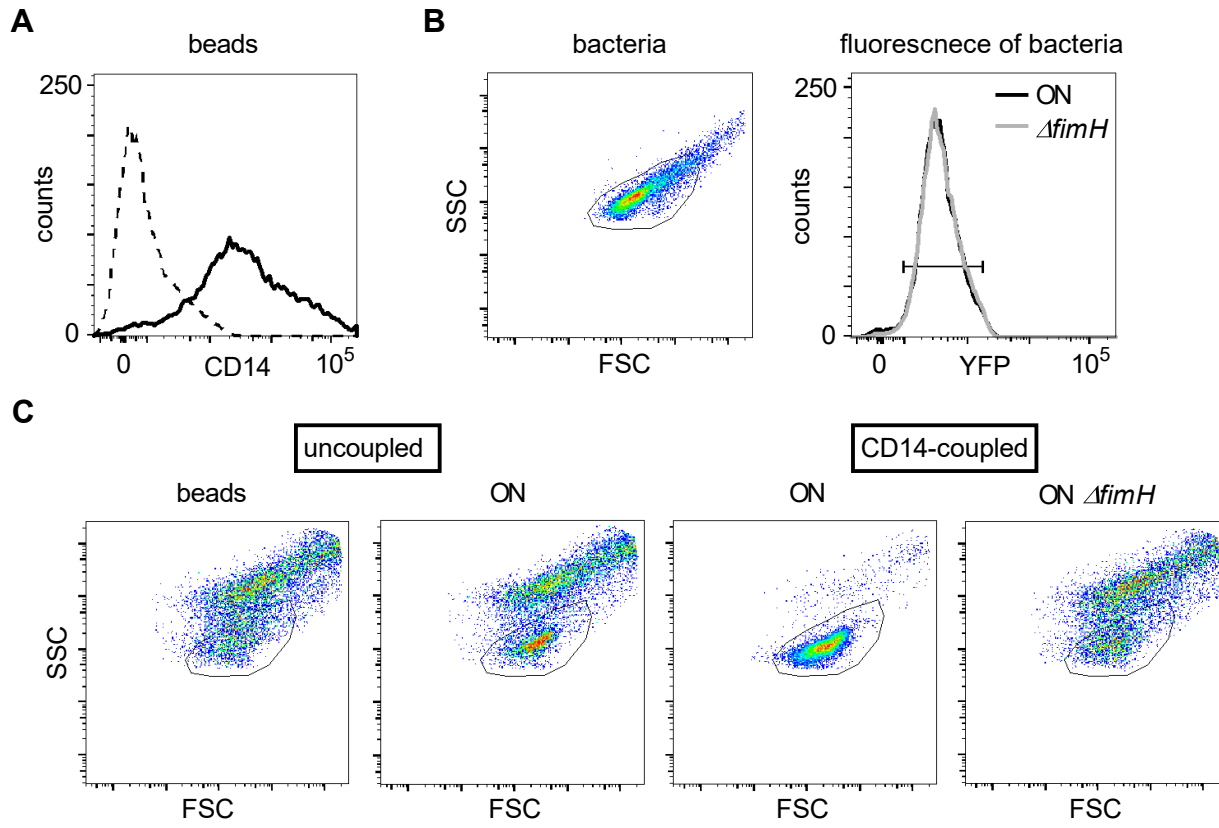
WT



## Supplementary Figure 4



## Supplementary Figure 5



## Supplementary Figure 6

