

1 ***Ferric Citrate Uptake is a Virulence Factor in Uropathogenic Escherichia coli***

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16

17 **Abstract**

18 More than half of women will experience a urinary tract infection (UTI) with uropathogenic
19 *Escherichia coli* (UPEC) causing ~80% of uncomplicated cases. Iron acquisition systems are
20 essential for uropathogenesis, and UPEC encode functionally redundant iron acquisition systems,
21 underlining their importance. However, a recent UPEC clinical isolate, HM7 lacks this functional
22 redundancy and instead encodes a sole siderophore, enterobactin. To determine if *E. coli* HM7
23 possesses unidentified iron acquisition systems, we performed RNA-sequencing under iron-
24 limiting conditions and demonstrated that the ferric citrate uptake system (*fecABCDE* and *fecIR*)
25 was highly upregulated. Importantly, there are high levels of citrate within urine, some of which is
26 bound to iron, and the *fec* system is highly enriched in UPEC isolates compared to environmental
27 or fecal strains. Therefore, we hypothesized that HM7 and other similar strains use the *fec* system
28 to acquire iron in the host. Deletion of both enterobactin biosynthesis and ferric citrate uptake
29 (Δ *entB*/ Δ *fecA*) abrogates use of ferric citrate as an iron source and *fecA* provides an advantage
30 in human urine in absence of enterobactin. However, in a UTI mouse model, *fecA* is a fitness
31 factor independent of enterobactin production, likely due to the action of host Lipocalin-2 chelating
32 ferrienterobactin. These findings indicate that ferric citrate uptake is used as an iron source when
33 siderophore efficacy is limited, such as in the host during UTI. Defining these novel compensatory
34 mechanisms and understanding the nutritional hierarchy of preferred iron sources within the
35 urinary tract are important in the search for new approaches to combat UTI.

36

37 **Importance**

38 UPEC, the primary causative agent of uncomplicated UTI is responsible for five billion dollars in
39 healthcare costs in the US each year. Rates of antibiotic resistance are on the rise therefore it is
40 vital to understand the mechanisms of UPEC pathogenesis to uncover potential targets for novel
41 therapeutics. Iron acquisition systems used to obtain iron from sequestered host sources are
42 essential for UPEC survival during UTI and have been used as vaccine targets to prevent
43 infection. This study reveals the ferric citrate uptake system is another important iron acquisition
44 system that is highly enriched in UPEC strains. Ferric citrate uptake has not previously been
45 associated with these pathogenic isolates, underlining the importance of the continued study of
46 these strains to fully understand their mechanisms of pathogenesis.

47

48 Introduction

49 More than half of women will experience a urinary tract infection (UTI) during their lifetime,
50 and 25% of infections recur (1, 2) with uropathogenic *Escherichia coli* (UPEC) causing 80% of
51 uncomplicated cases (3, 4). These infections are responsible for an annual five billion dollars of
52 health care costs in the U.S. alone (5, 6) To survive within the host UPEC encodes a wide array
53 of virulence factors that include toxins, adhesins and iron acquisition systems (6-9). Iron is an
54 essential cofactor for many biological processes including DNA replication, DNA repair, and
55 central metabolism (10, 11). Consequently, mammalian hosts employ “nutritional immunity”
56 wherein iron is sequestered within proteins or molecules such as transferrin, lactoferrin, ferritin,
57 and hemoglobin and is not readily accessible to bacteria (12, 13). To survive in the host, UPEC
58 has evolved mechanisms to acquire iron from these sequestered sources which fall into two broad
59 categories: heme receptors and siderophores. Heme receptors import heme, allowing the bacteria
60 to utilize the bound iron while siderophores are small molecules with extraordinarily high affinities
61 for iron (K_d ranging from 10^{23} to 10^{52} M^{-1}) (14, 15), which allow them to strip iron from sequestered
62 sources.

63 UPEC can encode up to five iron acquisition systems: heme receptors (ChuA and Hma),
64 and four siderophores (enterobactin, salmochelin aerobactin, and yersiniabactin) (16-18). UPEC
65 strains often employ a subset of these systems. For example, prototypical UPEC strain CFT073
66 encodes heme receptors and produces enterobactin, salmochelin and aerobactin. This high level
67 of functional redundancy is essential for UPEC survival within the host due in part to specific host
68 defenses. For example, the innate immune protein Lipocalin-2 (Lcn2) binds ferric and aferric
69 enterobactin, preventing the bacterium from utilizing this siderophore (19). Therefore, UPEC
70 cannot rely upon a single method of iron acquisition.

71 While heme receptors and siderophores are traditional iron acquisition systems utilized by
72 UPEC and other pathogenic bacteria, there are other methods. For instance, citrate is a weak
73 iron chelator and ferric citrate complexes can be imported through the ferric citrate transporter, or
74 *fec* system (20, 21). A study investigating *E. coli* strains that caused bovine mastitis (MPEC)
75 discovered that *fec* was a major pathogenic determinate of these strains; in 62 MPEC strains,
76 ~98% encoded the *fec* system (22). The high citrate levels in milk (~10 mM) provide a pool of
77 ferric citrate for these bacterial strains to use as an iron source via the *fec* system, allowing MPEC
78 to grow in milk and induce mastitis (22).

79 Overall, little has been done to define the role of the *fec* system in the context of
80 pathogenesis. However, there is a substantial body of work defining its regulation and mechanism
81 of action. The *fec* system is composed of two operons, *fecIR* and *fecABCDE* (23-25). *fecIR* is Fur-
82 regulated and expressed under iron-limiting conditions (26), while *fecABCDE*, is specifically
83 transcribed via FecI, an alternative sigma factor, when ferric citrate is present (21, 26). FecA is a

84 TonB-dependent outer membrane receptor, and FecBCDE comprise an ABC transporter (23, 24,
85 27).

86 In this study we have investigated the redundancy of iron acquisition systems in a
87 collection of UPEC strains that caused symptomatic UTI in healthy, college-aged women (28, 29).
88 One of these clinical isolates, HM7, lacked the functional redundancy in iron acquisition systems
89 characteristic of most UPEC strains and only encoded a sole siderophore, enterobactin. While
90 this strain lacks the traditional methods of iron acquisition and has no clear mechanism to prevent
91 Lcn2 from inactivating enterobactin, it is clearly pathogenic as it was isolated from a young woman
92 with cystitis and was present in the urine at $\geq 10^5$ CFU/mL. In this study, we sought to determine
93 how this novel strain acquires iron from the host. First, we empirically demonstrated that Lcn2-
94 susceptible enterobactin is the sole siderophore produced by HM7. Then, using RNA-sequencing
95 (RNA-seq), we found the ferric citrate uptake system highly upregulated under iron limitation.
96 Furthermore, we discovered that the *fec* system is highly enriched in UPEC isolates when
97 compared to fecal or environmental strains, and that there is a small, but significant cohort of
98 UPEC strains that encode a single siderophore. Additionally, HM7 can use ferric citrate as an iron
99 source through the *fec* system and enterobactin *in vitro*, and the *fec* system is a fitness factor *in*
100 *vivo*. Our study characterizes ferric citrate uptake as a UPEC virulence factor, adding a novel iron-
101 scavenging mechanism that UPEC uses to survive within the urinary tract.

102

103 Results

104 **Clinical UPEC isolate HM7 lacks all but one of the iron acquisition systems associated with**
105 **UPEC.** Roughly, there are up to five systems that UPEC use to acquire iron from the host (**Fig.**
106 **1A**). Most UPEC strains encode four of the five systems, including the three major UPEC type
107 strains (CFT073, UTI89, and 536, **Fig. 1A, B**). However recent clinical isolate HM7 (28) encodes
108 a single system, enterobactin, (**Fig. 1A, B**). After analyzing 487 publicly available UPEC strains
109 on the bioinformatics resource PATRIC (30) (**Table S1**), 44 strains shared the same profile as
110 HM7, indicating that HM7 is not an outlier, and potentially represents a previously unrecognized
111 subset of UPEC strains (**Fig. 1B**).

112 **HM7 encodes a single siderophore.** It was not clear how HM7 acquired iron and survived in the
113 host since the innate immune protein Lcn2 renders enterobactin unusable by bacteria (19).
114 Therefore, we hypothesized that HM7 encodes a novel siderophore to acquire iron. To test this,
115 we deleted the gene *entB* ($\Delta entB$), which is sufficient to disrupt enterobactin production (17), and
116 tested siderophore production by culturing the deletion mutant on Chrome Azurol S (CAS) agar,
117 a colorimetric iron chelation assay (31). A color change from blue to orange on the plate indicates
118 iron chelation, which is observed on the colony itself as well as a halo around the colony due to
119 diffusion of secreted siderophores. The wildtype (WT) strain showed robust siderophore activity
120 that was absent in the $\Delta entB$ mutant but was subsequently restored by genetic complementation
121 ($\Delta entB^{+entB}$) (**Fig. 1C**). These results indicate that enterobactin is the sole siderophore system in
122 HM7.

123 **Ferric citrate uptake is significantly upregulated during iron restriction.** HM7 did not make
124 a novel siderophore, therefore, we predicted that it might utilize a previously undiscovered or
125 understudied iron acquisition system. To identify a list of candidate genes, we used RNAseq to
126 determine the iron regulon of HM7. We added increasing amounts of the iron-specific chelator
127 2,2'-dipyridyl (Dip) to minimal M9 medium supplemented with 0.4% glucose to define an iron-
128 restricted condition. The addition of 150 μ M Dip to the base medium was sufficient to modestly

129 limit growth due to iron restriction without introducing a severe growth defect (**Fig. S1A**). Based
130 on previous literature (32, 33) M9 supplemented with 36 μM FeCl_3 was the iron-replete condition
131 (**Fig. S1A**). We confirmed these conditions reflected iron-restricted and iron-replete conditions
132 through qRT-PCR; the iron-regulated gene *entF* was significantly and highly upregulated in the
133 iron-restricted condition when compared to iron-replete (**Fig. S1B**).

134 HM7 was cultured to mid-log phase under these conditions in biological triplicate, its RNA
135 isolated and sequenced. 368 genes were significantly downregulated in the iron-depleted
136 condition (**Table S2**), while 393 genes were significantly upregulated (**Table 1, Table S2**). As
137 expected, we observed that the genes for enterobactin biosynthesis and uptake as well as genes
138 associated with iron-starvation (*nrdEFH* (34)) were upregulated (**Table 1**). Two transport systems
139 related to iron were significantly upregulated. One was *mntH* which takes up both Mn^{2+} , and Fe^{2+} ,
140 although with a preference for Mn^{2+} (35). The other system was ferric citrate uptake, which is
141 composed of two operons, *fecIR*, encoding the system's regulatory element and σ factor, and
142 *fecABCDE* encoding the outer membrane receptor and transport elements (**Fig. 2A**).
143 Interestingly, *fecD* was not significantly upregulated. Unlike *mntH*, the *fec* system takes up Fe^{3+}
144 which is dominant form of iron in the urinary tract as opposed to Fe^{2+} . Furthermore, citrate is
145 present is extremely high levels in the urinary tract, normal levels in healthy individuals vary from
146 1.7-6.6 mM (36). Given that MPEC uses ferric citrate in bovine milk, and the citrate concentration
147 in milk (~10mM) is comparable to the concentration in urine, we hypothesized that UPEC is using
148 a similar mechanism in the urinary tract.

149 ***fecA* is highly prevalent UPEC strains.** We wanted to establish the prevalence of the *fec* system
150 in UPEC strains, since three UPEC type strains, CFT073, UTI89 and 536 lack the *fec* system
151 (**Fig. 1B**). When we interrogated the cohort of 487 UPEC strains, we found that ~50% of them
152 encoded the outer membrane receptor *fecA*, compared to only ~12% in 107 fecal or
153 environmental *E. coli* isolates (**Fig. 2B**). This is a highly significant association, with an odds ratio
154 of 7.1, supporting the hypothesis of ferric citrate uptake as a UPEC virulence factor. Interestingly,
155 this enrichment seems to be even more profound in UPEC strains with a single iron acquisition
156 system; ~65% of these "HM7-like" strains also encoded *fecA* compared to ~47% of strains with
157 four traditional iron acquisition systems (**Fig. 2C**).

158 ***fecA* is responsive to physiologically relevant levels of citrate.** HM7 is a mostly
159 uncharacterized clinical isolate, therefore we wanted to determine if the *fec* system is fully
160 functional, and responsive to citrate at physiologically relevant levels. We cultured WT HM7 in M9
161 medium with glucose as a carbon source and supplemented with concentrations of citrate ranging
162 from 10 μM up to 100 mM, which encompasses urinary citrate levels in a healthy population (36).
163 We quantified *fecA* gene expression compared to M9 without citrate. We observed significant
164 upregulation at 100 μM , 1 mM, and 10 mM citrate (**Fig. 2D**) and importantly, some of the strongest
165 upregulation occurred at physiologically relevant concentrations (1 mM and 10 mM citrate). We
166 also tested *fecA* expression in *ex vivo* urine pooled from healthy female volunteers, compared to
167 expression in LB. *fecA* was significantly upregulated (**Fig. 2D**) in this physiologically relevant
168 medium.

169 ***fecA* is more highly upregulated in the absence of enterobactin.** Next, we wanted to
170 determine if ferric citrate uptake could act as a compensatory mechanism in the absence of
171 enterobactin, indicating the strain can use ferric citrate as an alternative iron source. Accordingly,
172 we repeated the citrate sensitivity experiments using the ΔentB mutant. Significant upregulation
173 at 100 μM , 1 mM, and 10 mM citrate was recapitulated in the mutant strain (**Fig. 2D**). Furthermore,

174 at both 100 μ M and 1 mM citrate the $\Delta entB$ mutant had significantly higher expression of *fecA*
175 compared to WT, a phenomenon that was trending in all concentrations of citrate. Interestingly,
176 while expression of *fecA* dropped at 100 mM citrate in WT, it remained highly elevated in the
177 $\Delta entB$ mutant, indicating that perhaps enterobactin is the preferred mechanism for iron
178 acquisition, but in its absence, the *fec* system can be utilized. These results support the
179 hypothesis that HM7 is using ferric citrate as an iron source, especially in the absence of Fe^{3+}
180 uptake by siderophores.

181 **HM7 uses ferric citrate as an iron source through the *fec* system or enterobactin.** To
182 determine if HM7 can use ferric citrate as an iron source, we added high levels (100 mM) of citrate
183 to M9 mediums so most of the iron would be complexed within citrate. The bacteria have two
184 ways to acquire iron: either enterobactin will chelate iron from ferric citrate, or the *fec* system will
185 import ferric citrate. To nullify ferric citrate uptake, we deleted the outer membrane receptor gene,
186 *fecA* ($\Delta fecA$). We also constructed a double mutant ($\Delta fecA/\Delta entB$). With these assumptions, only
187 the double mutant, $\Delta fecA/\Delta entB$, would have a growth defect at high citrate concentrations, since
188 the $\Delta fecA$ mutant could still utilize enterobactin, and the $\Delta entB$ mutant could still utilize the *fec*
189 system. As expected, only the $\Delta fecA/\Delta entB$ mutant had a profound growth defect with the addition
190 of 100 mM citrate (**Fig. 3Aii**) while none of the mutants had a growth defect in LB or M9 medium
191 alone (**Fig. S2, Fig 3Ai**). This is an iron-specific defect since chemical complementation with 1
192 mM $FeCl_3$ rescued the growth of the double mutant (**Fig. 3Aiii**).

193 To establish that HM7 could specifically use the *fec* system to acquire iron via ferric citrate,
194 we took a genetic approach, complementing the $\Delta fecA/\Delta entB$ double mutant with each single
195 system. Unsurprisingly, growth of the $\Delta fecA/\Delta entB$ double mutant was rescued by genetic
196 complementation with *entB* (**Fig. 3Ci, ii, and iii**). However, *fecABCDE* was also sufficient to
197 rescue growth (**Fig. 3Bi, ii, and iii**). *fecA* was not sufficient to rescue growth, indicating that the
198 $\Delta fecA$ mutant is a polar mutation, although that does not change the interpretation of our previous
199 results.

200 **Ferric citrate uptake is an *in vitro* fitness factor when HM7 cannot utilize enterobactin.** The
201 association of *fecA* with UPEC strains (**Fig. 2B**) and HM7 using ferric citrate as an iron source
202 (**Fig. 3**) indicates that the presence of the *fec* system could provide UPEC with a competitive
203 advantage. Initially we assessed growth of WT HM7, and the three mutants, $\Delta fecA$, $\Delta entB$,
204 $\Delta fecA/\Delta entB$, in pooled *ex vivo* urine (**Fig. S3**). Surprisingly there seemed to be no significant
205 growth defect in any of these mutants compared to WT. Therefore, we turned to a more sensitive
206 assay to assess the advantage the *fec* could provide and performed competition experiments in
207 pooled human urine. We tested WT against the $\Delta fecA$ mutant and observed that there was no
208 competitive disadvantage of the mutant strain compared to WT (**Fig. 4A**). Both strains could still
209 use enterobactin, indicating that perhaps the siderophore is the preferred mechanism to acquire
210 iron. This was confirmed when competing WT and $\Delta entB$; the mutant had a subtle, but significant
211 disadvantage (**Fig. 4A**). This disadvantage was exacerbated and trended towards significance
212 when WT was competed against the $\Delta fecA/\Delta entB$ double mutant (**Fig. 4A**), indicating that both
213 systems contribute to the survival of HM7, but the function of enterobactin masks the role of *fec*.

214 To dissect the precise contribution of the *fec* system, we competed the $\Delta entB$ mutant with
215 the $\Delta fecA/\Delta entB$ double mutant. The double mutant had a significant defect (**Fig. 4B**). This defect
216 is largely specific to the *fec* system since complementing the double mutant with *fecABCDE* was
217 sufficient to partially rescue the defect (**Fig. 4B**). In the urinary tract during infection, Lcn2
218 counteracts enterobactin. Therefore, to mimic the host's infectious environment, we added

219 recombinant Lcn2 to these competitions. We determined that 25 µg/mL of Lcn2 was sufficient to
220 inhibit HM7 growth in an iron-limited environment (**Fig. S4**) and then supplemented that amount
221 to pooled human urine and competed WT and the $\Delta fecA$ mutant. With the addition of Lcn2, the
222 $\Delta fecA$ mutant now had a significant competitive disadvantage (**Fig. 4C**). This provides further
223 evidence that in the absence or inhibition of enterobactin the *fec* system is a fitness factor.

224 **Ferric citrate uptake is an *in vivo* fitness factor.** Finally, we wanted to determine if the *fec*
225 system was an *in vivo* fitness factor. Using the ascending UTI mouse model, we co-infected
226 female CBA/J mice with WT and the $\Delta fecA$ mutant, allowed the infection to progress for 48 hours
227 and harvested the urine, bladder, and kidneys to calculate log₁₀C.I. The $\Delta fecA$ mutant had a
228 significant disadvantage in all three organ sites (**Fig. 5**), definitively defining it as a fitness factor.

229 We hypothesized that the $\Delta fecA$ mutant had a defect *in vivo*, due to the presence of Lcn2,
230 as we saw in our *in vitro* competitions that were supplemented with Lcn2 (**Fig. 4C**). Lcn2 is highly
231 elevated in the bladders and kidneys of mice infected with WT HM7 (**Fig. S5A, B**). Lcn2 levels
232 correlated with increased CFU burden in the kidneys, where it is produced (37) (**Fig. S5C**). To
233 determine if Lcn2 was responsible for the competitive disadvantage of the $\Delta fecA$ mutant, we
234 repeated the competition experiments with Lcn2 knock-out mice (*Lcn2*^{-/-}). However, the *Lcn2*^{-/-}
235 mice are in a different genetic background, C57BL/6, rather than CBA/J, so we repeated the
236 competition in the WT (C57BL/6) mouse background as well. While there was a subtle difference
237 in the log₁₀C.I. of the bladders between WT and *Lcn2*^{-/-} mice that was trending towards
238 significance, the $\Delta fecA$ mutant no longer had a disadvantage compared to WT in the C57BL/6
239 background (**Fig. S6**). This discrepancy in results indicates the differences between the mouse
240 strains. Overall, we conclude that ferric citrate uptake through the *fec* system is a *bona fide* fitness
241 factor in UPEC strain HM7, allowing it to acquire iron from the host in a manner not inhibited by
242 Lcn2.

243 Discussion

244 Iron acquisition is an essential virulence factor in UPEC, because most iron in the host is
245 sequestered. Subsequently, UPEC relies on specific iron acquisition systems such as
246 siderophores or heme receptors to scavenge iron from otherwise inaccessible sources and these
247 systems are essential for UPEC pathogenesis (10, 38, 39). Our work shows there is another
248 understudied and overlooked iron acquisition system that enhances UPEC pathogenesis: ferric
249 citrate uptake, encoded by the *fec* system.

250 Our study focuses on a recent clinical UPEC isolate, HM7. This strain encodes a sole
251 siderophore, enterobactin, and lacks the functional redundancy in iron acquisition systems
252 normally observed in UPEC strains. We assumed that HM7 was employing another method to
253 acquire iron from the host and used RNA-seq to define its iron regulon. Under iron-limiting
254 conditions, we found almost every component of ferric citrate uptake (*fecABCE* and *fecI/R*) was
255 highly and significantly upregulated (**Table 1, Table S2, Fig. 2A**). Interestingly, *fecD* was not
256 highly upregulated. While the rest of the genes in the system had log₂ fold-change (FC) values
257 ranging from 2.6-5.1, *fecD* had a log₂FC of 0.8, and unlike the rest, this change was not significant.
258 This is intriguing given that *fecD* is the second to the last gene in the operon, and yet the gene
259 after it, *fecE*, is significantly and highly upregulated. *fecD* and *fecC* encode the permeases of the
260 transport system that form a channel in the inner membrane of the bacterium (24, 40). Permeases
261 can form homodimers, or heterodimers, and it is tempting to speculate the modest upregulation
262 of *fecD* indicates that there is a preference for FecC homodimers as opposed to FecC/FecD
263 heterodimers. Potentially, there could be an alternative start site that *fecE* utilizes, explaining its
264 higher expression levels. *fecE* encodes the ATPase of this system (40), which is essential for

265 activity of this ABC transporter. However, precisely defining this mechanism will require future
266 studies.

267 We uncovered a strong association of the *fec* system with UPEC strains compared to fecal
268 or environmental strains, another indication that the *fec* system is a virulence factor. (**Fig. 2B**).
269 Given how common the *fec* system is within UPEC and MPEC, it could be a virulence factor in
270 other pathogenic *E. coli*. For example, the citrate levels in plasma vary from 100-150 μ M (41) and
271 while these levels are lower than in urine or milk, they are still sufficient for robust upregulation of
272 *fecA* (**Fig. 2D**). Potentially the *E. coli* that cause bloodstream infections could also utilize *fec* to
273 acquire iron. In fact, a recent study exploring conjugative plasmids in pathogenic *E. coli* found a
274 plasmid that encoded the *fec* system conferred a modest *in vivo* competitive advantage during
275 bacteremia (42). This was also tested in the UTI model, and when this plasmid was conjugated
276 into a different *E. coli* strain loss of *fec* resulted in an extremely mild reduction in fitness (\log_{10} C.I.
277 \sim -0.1 in the bladder, and \sim -0.2 in the kidneys). However, this result could not be recapitulated in
278 its parent strain. Other iron acquisition systems in these strains were not defined and could explain
279 the divergence of results, demonstrating how functional redundancy of iron acquisition systems
280 can mask the contributions of specific systems.

281 The *fec* system seems lower on the hierarchy of iron acquisition systems but becomes
282 more important the fewer iron acquisition systems a strain produces. In a large cohort of UPEC
283 strains, about \sim 9% encoded a single traditional iron acquisition system, enterobactin, like HM7.
284 While a relatively small percentage, it is still a part of the population that would likely rely more
285 heavily on a system like *fec*, especially given that enterobactin is not highly effective in the urinary
286 tract (38). This seems to be case since the *fec* system in these isolates is more enriched
287 compared to more traditional UPEC strains (**Fig. 2C**). Interestingly, a very small population of
288 these strains (2%) seemed to encode none of the traditional iron acquisition systems. However,
289 the sequencing quality of these genomes is quite poor, and follow up studies are needed to
290 confirm these results. If these results are confirmed, these strains could be an excellent tool to
291 discover additional novel or understudied iron acquisition systems.

292 *In vitro* competition in pooled human urine showed that the *fec* system provides a
293 competitive advantage, but this advantage is contingent on the absence of enterobactin (**Fig. 4**).
294 Enterobactin seems to be the preferred method of iron acquisition *in vitro*, since loss of
295 enterobactin is sufficient to cause a small reduction in fitness (**Fig. 4A**). Furthermore, addition of
296 Lcn2 was sufficient to inhibit the function of enterobactin, allowing the *fec* system to provide an
297 advantage (**Fig. 4C**). Lcn2 is present in high levels in the urinary tract during infection (43);
298 therefore, these *in vitro* competitions with the addition of Lcn2 are likely a closer representation
299 of UTI.

300 The gene expression profile of UPEC during CBA/J mouse infection closely mimics the
301 UPEC transcriptome during human infection (44). Therefore, we are reasonably confident that the
302 results from the mouse model are relevant to human infection. When WT HM7 was competed
303 against the Δ *fecA* mutant, the mutant had a disadvantage in the urine, bladder, and kidneys (**Fig.**
304 **5**). While this result is different than the *in vitro* competition in human urine alone, it aligns with
305 the *in vitro* competitions supplemented with Lcn2 and implies that the *fec* system provides an
306 advantage *in vivo* because HM7 is unable to use enterobactin. This is likely caused by Lcn2; mice
307 infected with HM7 had increased production of Lcn2 in the bladder and kidneys (**Fig. S5**).

308 We attempted to confirm this hypothesis using *Lcn2*^{-/-} mice. If Lcn2 is essential for the
309 competitive advantage of the *fec* system that advantage should be abrogated in the knock-out
310 line. The *Lcn2*^{-/-} mice were in a C57BL/6 background, therefore, we re-tested WT HM7 and Δ *fecA*
311 mutant in WT C57BL/6 mice. Unfortunately, there was no loss in fitness in the Δ *fecA* mutant in
312 WT C57BL/6 mice (**Fig. S6**). However, there are several genetic differences between these

313 mouse lines (45) that could account for these differences. For example, of the 10 CBA/J mice we
314 infected, 100% of them had kidney colonization, while only 35% of the 20 C57BL/6 mice had
315 kidney colonization. While the contribution of *Lcn2* to the mechanism of ferric citrate uptake via
316 the *fec* system has not been definitively proven, it seems a promising explanation.

317 In summary, we have uncovered a novel mechanism by which UPEC acquires iron from
318 the host via ferric citrate uptake. During UTI *Lcn2* is highly produced, blocking the usage of
319 enterobactin. In response, UPEC uses the *fec* system to import ferric citrate present in the urinary
320 tract as an iron source (**Fig. 6**). The *fec* system is highly prevalent in UPEC strains and is yet
321 another instrument in its highly diverse arsenal to survive within the harsh environment of the
322 urinary tract.

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327 L. Holmes for both the WT and *Lcn2*^{-/-} C57BL/6 mice.

328

329 **Methods**

330 **Bacterial culture conditions, growth curves, mutant construction, and complementation.**

331 Clinical UPEC isolate HM7 was routinely cultured at 37°C with aeration in LB, M9 medium
332 supplemented with glucose, or filter-sterilized pooled human urine. Mutant and complemented
333 strains were cultured with antibiotics. Mutants were constructed using lambda red mutagenesis
334 and complementation vectors constructed with Gibson assembly. For a detailed description, refer
335 to **Text S1**.

336 **Chrome Azurol S Assay.**

337 Chrome Azurol S (CAS)-agar was prepared as defined in (31). Strains were cultured overnight
338 with aeration at 37°C in LB with appropriate antibiotics. Five µL of the overnight culture was
339 spotted onto the CAS-agar plate, and then incubated overnight at 37°C. The next morning the
340 plates were imaged using Qcount Software.

341 **RNA isolation and library preparation, and sequencing.**

342 *E. coli* HM7 was cultured overnight in M9 medium, supplemented with 0.4% glucose, shaking at
343 37°C. Overnight cultures were diluted 1:100 in M9 medium with 0.4% glucose supplemented with
344 either 36 µM FeCl₃ (Sigma), or 150 µM 2,2' Dipyridyl (Sigma) and grown to mid-log phase (0.4-
345 0.6 OD₆₀₀). Cultures were then treated with Bacterial RNA Protect (Qiagen), harvested by
346 centrifugation and the pellets stored at -80°C. This was performed in biological triplicate. RNA
347 was isolated using a similar method from (28, 44). The libraries were prepared using NEBNext
348 Ultra II Directional RNA Library Prep Kit and sequenced using an Illumina NextSeq-500 (paired
349 end, 38 bp read length). For a detailed description, refer to **Text S1**.

350 **Genome assembly, RNA-seq data processing and differential expression analysis.**

351 Raw sequencing data was preprocessed using BBTools (38.18) (46). BBDuk was used to remove
352 Illumina adapter sequences, and to quality trim and filter the reads (minlength=20, trimq=14,

353 maq=20, maxns=1). The HM7 genome was re-assembled based on sequencing from (28) using
354 Flye long read assembler (47) with Trestle repeat resolve parameter on then the quality controlled
355 reads were aligned to the HM7 genome using BWA (0.7)(48). The resulting alignment files were
356 filtered (mapping quality > 10) using samtools (1.11) (49) with counts for each feature were
357 generated using htseq-count (0.13.5) (50). Alignment details shown in **Table S3**. Differential
358 expression analysis was performed using R package DESeq2 (51).

359 **qRT-PCR.**

360 Strains were grown to mid-log cultures and RNA isolated as described above and reverse-
361 transcribed into cDNA using iScript (Biorad). qRT-PCR was performed on a QuantStudio 3 PCR
362 machine (Applied Biosystems) using PowerUp Syber Green mastermix (Applied Biosystems). For
363 a detailed description, refer to **Text S1**.

364 **Purification of Lipocalin-2.**

365 Recombinant human Lipocalin-2 (Lcn2) expressed as a glutathione S-transferase (GST) fusion
366 protein (52) (a kind gift from Dr. Michael Bachman) in XL-1 Gold *E. coli* protein was purified in a
367 similar manner as previously described (53, 54). For a detailed description, refer to **Text S1**.

368 **In vitro growth competition.**

369 Strains were cultured overnight in M9 medium supplemented with 0.4% glucose at 37°C with
370 aeration and appropriate antibiotic selection. The next day, the OD₆₀₀ was determined for each
371 strain, the strains were OD₆₀₀-matched, and then diluted 1:100 into 3 mL of pooled human urine.
372 Where applicable, Lcn2 was added to a final concentration of 25 µg/mL, or the vehicle control
373 (25% glycerol) in an equal volume. 0.5% arabinose (final concentration) induced the
374 complemented strains, and ampicillin added to maintain the plasmid. Input CFUs were determined
375 for each strain through drip plating of serial dilutions on plain LB agar and antibiotic selection
376 (chloramphenicol or kanamycin). The strains were then grown overnight, shaking at 37°C, and
377 the output CFU of each strain determined in the same manner as the input.

378 C.I. is a ratio of the input versus the output and is calculated as follows:

$$379 \frac{\text{mutant output} / \text{WT output}}{\text{mutant input} / \text{WT input}}$$

380 Log₁₀CI <0 indicates that the WT outcompetes the mutant, and a log₁₀CI >0 indicates the mutant
381 outcompetes the WT. When competing $\Delta entB$ and $\Delta fecA/\Delta entB$, $\Delta entB$ was “WT”, and
382 $\Delta fecA/\Delta entB$ was “mutant”. When competing $\Delta entB eV$ and $\Delta fecA^{+fec}/\Delta entB$, $\Delta entB eV$ was “WT”,
383 and $\Delta fecA^{+fec}/\Delta entB$ was “mutant”.

384 **Murine UTI model.**

385 We used three different mouse strains: CBA/J, C57BL/6 WT and C57BL/6 *Lcn2*^{-/-} (55). CBA/J
386 mice were purchased from Jackson Laboratories, while both the C57BL/6 WT and C57BL/6
387 *Lcn2*^{-/-} mice were a kind gift from Dr. Michael Bachman and bred in-house. All mice used were
388 female. For a detailed description, refer to **Text S1**.

389

390

391 **Data accessibility.**

392 Data available on in NCBI's Gene Expression Omnibus repository under accession number
393 GSE188170.

394

395

Table 1: Top 50 Significantly Upregulated Genes Under Iron Limitation

Gene	Description	Log ₂ FC ^a	Locus Tag
<i>adhP</i>	alcohol dehydrogenase	4.6	b1478
<i>aroF</i>	Phospho-2-dehydro-3-deoxyheptonate aldolase	4.1	EICMKPFN_03556
<i>bioA</i>	adenosylmethionine-8-amino-7-oxononanoate aminotransferase	4.0	b0774
<i>cirA</i>	outer membrane receptor for ferrienterochelin and colicins	5.0	b2155
<i>EICMKPFN_01803</i>	phosphate starvation-inducible protein	3.9	EICMKPFN_01803
<i>EICMKPFN_02077</i>	hypothetical protein	4.2	EICMKPFN_02077
<i>EICMKPFN_02251</i>	Glyceraldehyde-3-phosphate dehydrogenase	4.7	EICMKPFN_02251
<i>EICMKPFN_02252</i>	Glyceraldehyde-3-phosphate dehydrogenase	4.5	EICMKPFN_02252
<i>EICMKPFN_03110</i>	Colicin I receptor	4.5	EICMKPFN_03110
<i>entA</i>	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase	5.4	b0596
<i>entB</i>	enterobactin synthase component B	5.8	b0595
<i>entC</i>	isochorismate synthase EntC	4.7	b0593
<i>entD</i>	enterobactin synthetase component D	5.0	b0583
<i>entE</i>	2,3-dihydroxybenzoate-AMP ligase	5.1	b0594
<i>entF</i>	enterobactin synthetase component F	6.9	b0586
<i>entH</i>	proofreading thioesterase in enterobactin biosynthesis	5.0	b0597
<i>fecA</i>	ferric citrate outer membrane transporter	4.8	b4291
<i>fecl</i>	RNA polymerase σ^{19} factor	5.2	b4293
<i>fecR</i>	regulator for <i>fec</i> operon	5.3	b4292
<i>fepA</i>	ferric enterobactin receptor	5.4	b0584
<i>fes</i>	Fe ⁺³ -enterobactin esterase	5.3	b0585
<i>fhuE</i>	outer-membrane receptor for ferric coprogen and ferric-rhodotorulic acid	5.4	b1102
<i>fhuF</i>	ferric iron reductase protein	4.6	b4367
<i>fiu</i>	catecholate siderophore receptor	4.4	b0805
<i>gabP</i>	4-aminobutyrate:H(+) symporter	4.7	b2663
<i>gadA</i>	glutamate decarboxylase A	5.1	b3517
<i>gadB</i>	glutamate decarboxylase B	5.2	b1493
<i>gadC</i>	L-glutamate:4-aminobutyrate antiporter	5.4	b1492
<i>gcd</i>	quinoprotein glucose dehydrogenase	4.2	b0124
<i>hchA</i>	D-lactate dehydratase	4.5	b1967
<i>mntH</i>	manganese transport protein	5.6	b2392
<i>nrdE</i>	ribonucleoside-diphosphate reductase 2 subunit alpha	6.1	b2675
<i>nrdF</i>	ribonucleoside-diphosphate reductase 2 subunit beta	6.8	b2676
<i>nrdH</i>	glutaredoxin-like protein	6.3	b2673
<i>nrdI</i>	protein involved in ribonucleotide reduction	5.9	b2674

<i>phoH</i>	phosphate starvation-inducible protein	4.1	b1020
<i>sufC</i>	Fe-S cluster assembly ATP-binding protein	4.0	b1682
<i>sufD</i>	Fe-S cluster assembly protein	4.1	b1681
<i>sufE</i>	cysteine desulfuration protein	3.8	b1679
<i>sufS</i>	selenocysteine lyase	4.1	b1680
<i>tyrA</i>	chorismate mutase	3.7	b2600
<i>ybdZ</i>	enterobactin biosynthesis protein	6.1	b4511
<i>ybgS</i>	uncharacterized protein	4.3	b0753
<i>ybiX</i>	PKHD-type hydroxylase	3.7	b0804
<i>yciG</i>	uncharacterized protein	4.5	b1259
<i>yddM</i>	putative DNA-binding transcriptional regulator	4.6	b1477
<i>ydiE</i>	uncharacterized protein	4.2	b1705
<i>yjjZ</i>	uncharacterized protein	7.3	b4567
<i>yncE</i>	PQQ-like domain-containing protein	5.0	b1452
<i>yohC</i>	putative inner membrane protein	3.7	b2135

^aFC, fold change.

396

397

Table 2: List of Strains and Plasmids

Strain	Genotype/Description	Reference/Source
HM7	Wild-type cystitis causing uropathogenic <i>E. coli</i> strain, isolated from a healthy young woman in 2012	(28)
$\Delta entB$	HM7 <i>entB</i> ::kan, Kan ^r	This study
$\Delta fecA$	HM7 <i>fecA</i> ::cam, Cam ^r	This study
$\Delta fecA/\Delta entB$	HM7 <i>fecA</i> ::cam/ <i>entB</i> ::kan, Cam ^r , Kan ^r	This study
Plasmid	Description	Reference/Source
pGEN eV	Low copy number, promoterless plasmid, Spec ^r	This study
pBAD eV	pBAD- <i>Myc</i> /His A, low copy number, arabinose inducible plasmid, Amp ^r	Thermo-Fisher
pGEN <i>entB</i>	<i>entB</i> with native promoter, cloned from HM7 via Gibson assembly, Spec ^r	This study
pBAD <i>fecABCDE</i>	<i>fec</i> operon (<i>fecABCDE</i>) cloned from HM7 inserted into MCS via Gibson assembly, Amp ^r	This study
pGEX-4T-3 LCN	Human Lipocalin-2 glutathione S-transferase (GST) fusion protein, Amp ^r	(53)

398

399

Table 3: Primers used in study

Gene or Plasmid	Forward Primer	Reverse Primer
<i>entB</i> ^a	<u>ATTCCAAAATTACAGGCTTACGC</u> <u>ACTGCCGGAGTCGTGTAGGCTG</u> GAGCTGCTTC	<u>CACCTCGCGGGAGAGTAGCTT</u> <u>CCACCAGGCGTCTGAATGGGAA</u> TTAGCCATGGTCC
<i>fecA</i> ^a	<u>GATGATGGGGAAGGTATGACGC</u> <u>CGTTACGCGTTTTTCGTAACA</u> <u>ACACCGTGTAGGCTGGAGCTGC</u> TTC	<u>CCGGGCGTTAACACATCAGAA</u> <u>CTTCAACGACCCCTGCATATAC</u> <u>AGCGTGCATGGGAATTAGCCA</u> TGGTCC
<i>P_{native}entB</i> ^b	CGGTACCAAGCTTCATATGCACA AATCAGCTTCCTGTTATTAATAAG GGAGGATGATATGGCTATTCCAA AATTACAGG	GAATAGCCATATCATCCTCCAC AAAATG
<i>entB</i> ^b	GAGATCTGCAGCTGGTACCAAT GACGCCGTTACGCGTTTTTCG	TTCCTGCAGGGCATGCCCCGT TATTTACCTCGCGGGAG
<i>fecABCDE</i> ^b	GAGATCTGCAGCTGGTACCAAT GACGCCGTTACGCGTTTTTCG	CCAAGCTTCGAATTCCCATAACC TCATTAGGCACATCGGCCTGCS GCATATGAAGCTTGGTACCGG GATCCGC
pGEN ^b	CGGGGCATGCCCTGCAGG	TGGTACCAGCTGCAGATCTCG AGC
pBAD ^b	TATGGGAATTCGAAGCTTGGGC CCG	TACCTGGAGCAAGGCAAAC
<i>fecA</i> ^c	CGGAAGGGCCGATCATAAA	CCCGATAGCTGAACTGGTAAC
<i>entF</i> ^c	TTCCAGAAACCACGCTGAG	CGATCAGATGACCGTCTTTTAC
gapA ^c	CGACCTGTTAGACGCTGATTAC	

400

401 ^aUsed for lambda red mutagenesis; ^bused for Gibson assembly; ^cused for qRT-PCR. Primers
402 are listed in 5' to 3', underlined sequences for mutant construction indicate regions homologous
403 to gene of interest.

404

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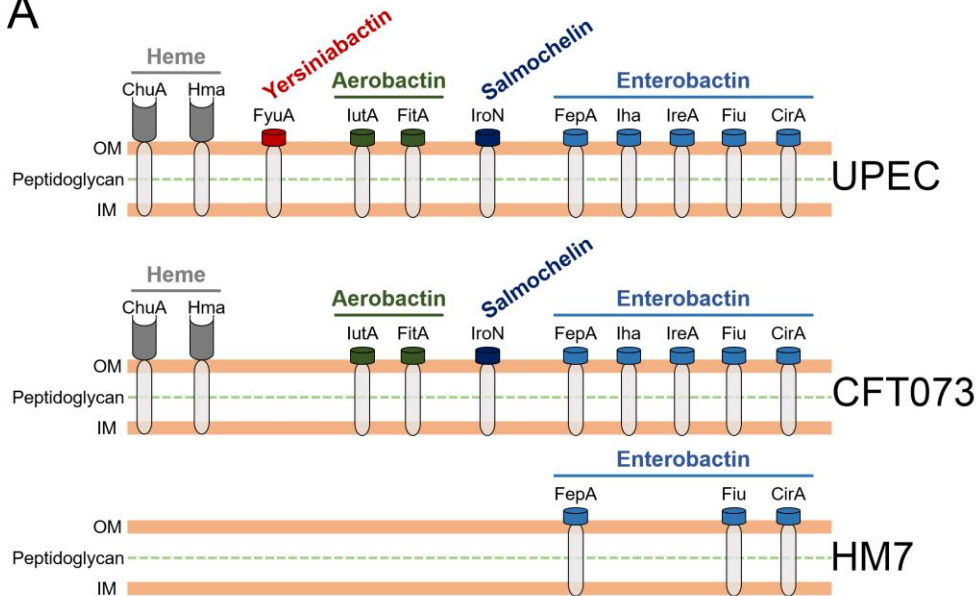
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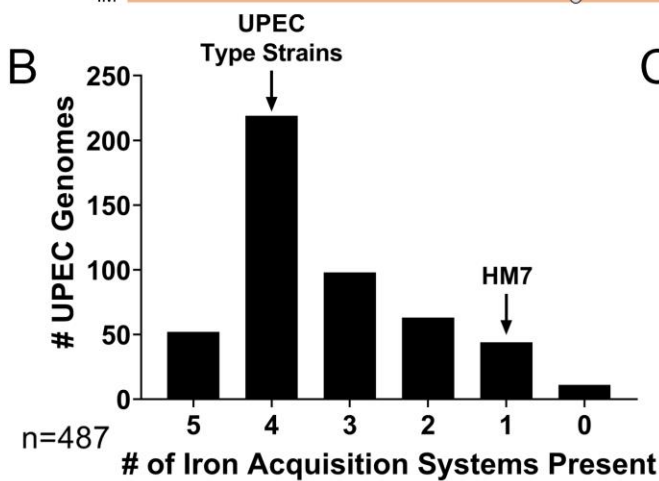
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Figure 1

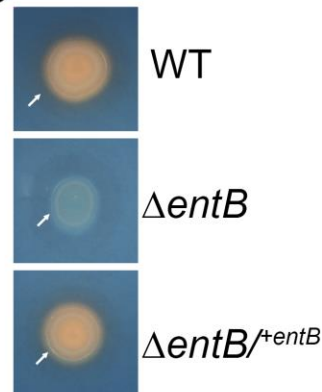
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B



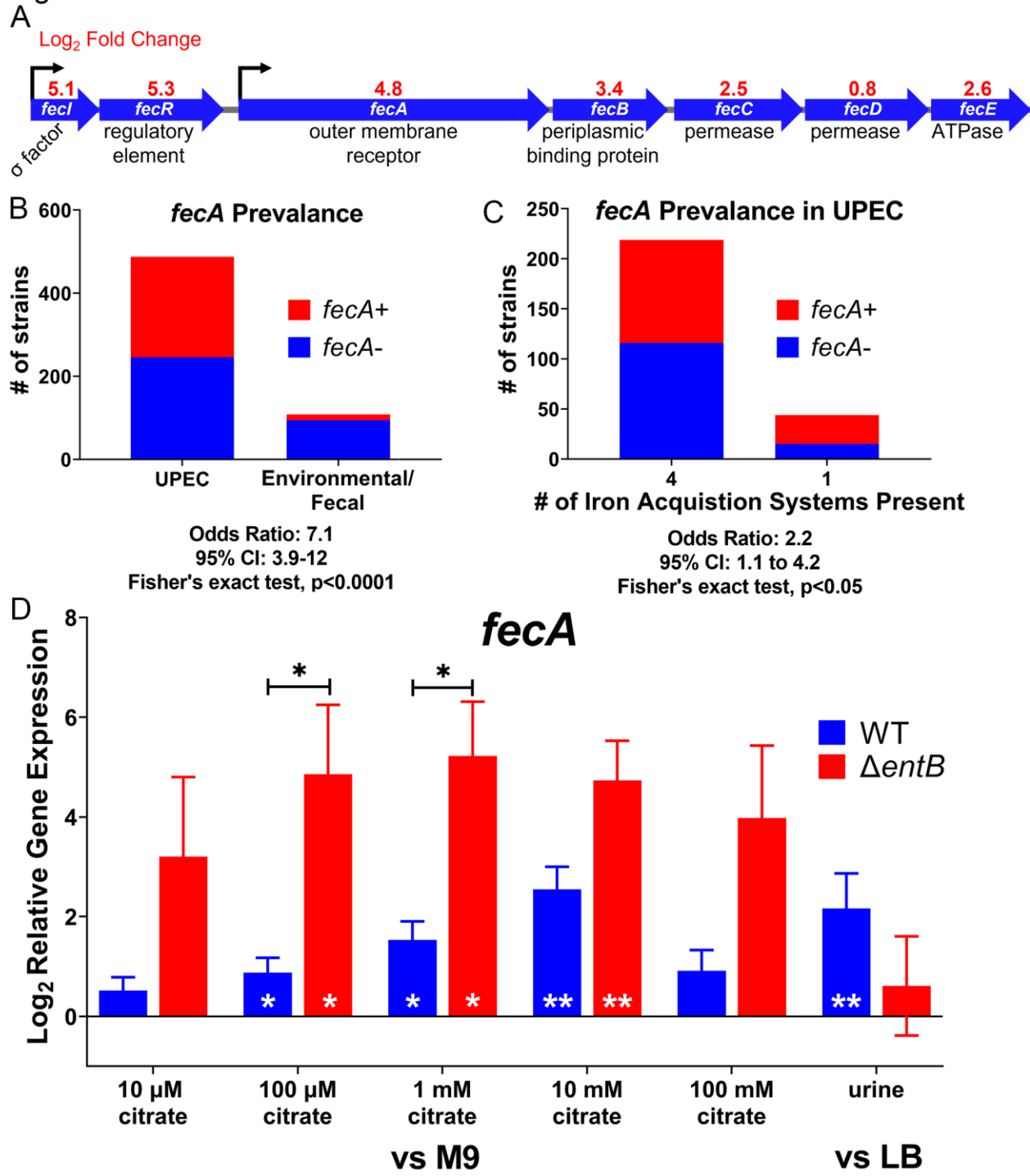
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567 **Figure 1: Clinical UPEC isolate HM7 encodes a single iron acquisition system.** (A) Models
568 of siderophores, siderophore uptake receptors and heme receptors in UPEC. “UPEC” indicates
569 all known systems that have been found in UPEC while “CFT073” and “HM7” illustrate the
570 systems in each of these indicated strains. (B) The number of iron acquisition systems present
571 in cohort of 487 UPEC strains on the bioinformatics resource PATRIC. The five systems are
572 composed of heme uptake (ChuA or Hma), and four siderophores (enterobactin, salmochelin,
573 aerobactin and yersiniabactin). Presence was determined by at $\geq 80\%$ protein identity and
574 coverage of select genes for each system: heme uptake (*chuA* or *hma*), enterobactin (*entB*),
575 salmochelin (*iroB*), aerobactin (*iucA*), and yersiniabactin (*irp1*). Genes selected for siderophores
576 are all involved in biosynthesis. 11% of strains have five systems, 45% of strains have four,
577 20% of strains have three, 13% of strains have two, 9% of strains have one, and 2% appear to have
578 none. (C) Siderophore production assayed through growth on CAS agar. 5 μ L of an overnight
579 LB culture were spotted on CAS agar and grown overnight at 37°C. A change from blue to
580 orange indicates siderophore activity. White arrow indicates the colony in all three strains, and
581 the orange halo in WT and complemented strain is due to diffusion of secreted siderophore.

Figure 2



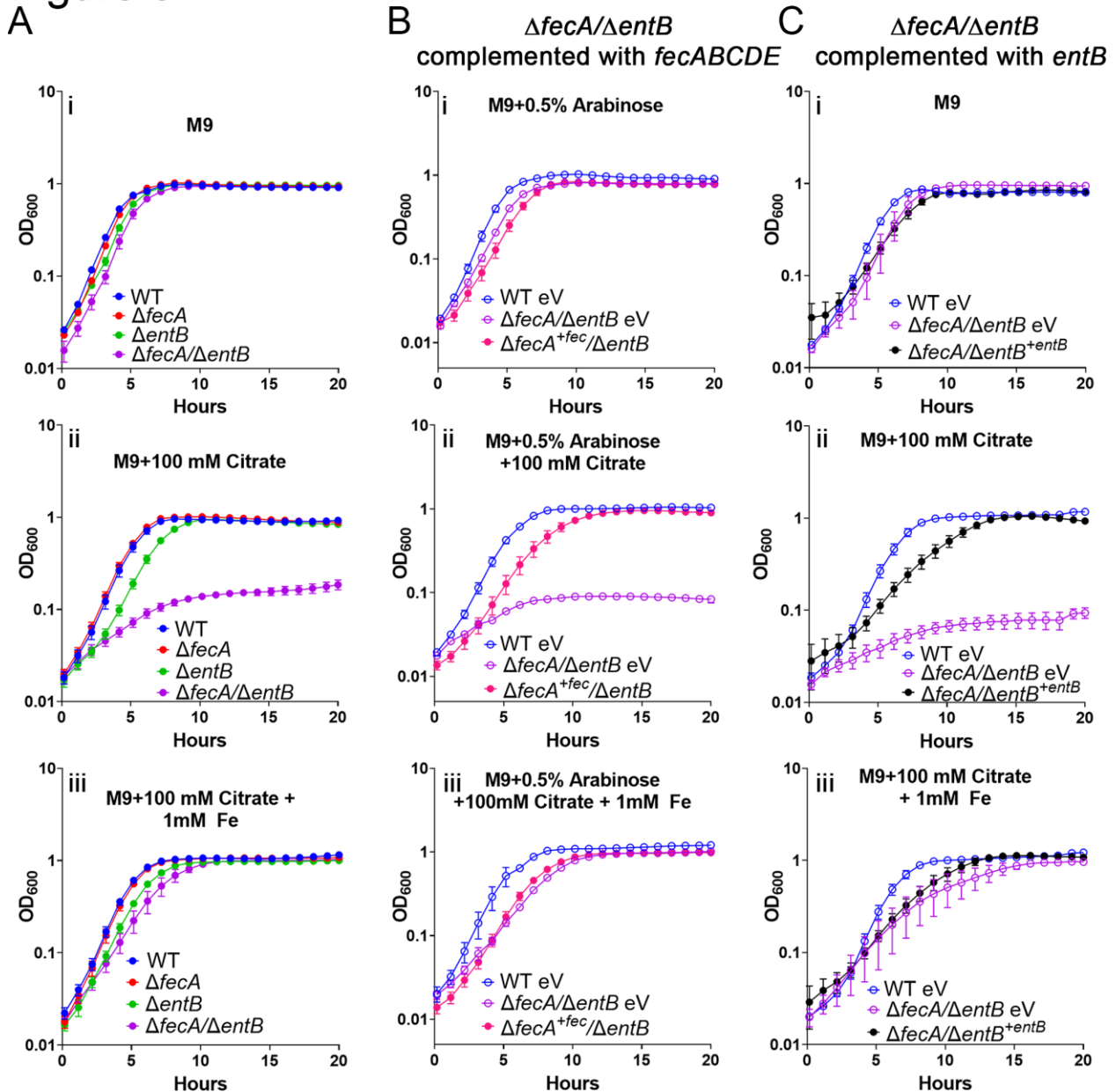
582

583 **Figure 2: Ferric citrate uptake is a potential iron acquisition system in UPEC.** (A) RNAseq
 584 revealed the ferric citrate uptake system (*fecABCDE* and *fecIR*) is upregulated in WT HM7
 585 under iron limitation (M9 supplemented with 36 μ M FeCl₃ versus M9 with 150 μ M 2,2 dipyridyl).
 586 (B) *fecA* is enriched in UPEC strains compared to *E. coli* fecal or environmental isolates. 487
 587 UPEC strains and 107 fecal or environmental strains were analyzed; presence of *fecA* was

588 determined by $\geq 80\%$ protein identity and coverage. (C) *fecA* is enriched in UPEC strains with a
 589 single traditional iron acquisition system (“HM7-like”), compared to strains with four traditional
 590 iron acquisition systems. (D) Gene expression of *fecA* in HM7 in either M9 medium with 0.4%
 591 glucose supplemented with increasing amounts of citrate, or in pooled human urine. Gene
 592 expression was assayed through qRT-PCR. Bars are the average of five (WT) and four ($\Delta entB$)
 593 biological replicates, bars are mean, error bars are \pm SEM. Black asterisks compare gene
 594 expression between WT and the $\Delta entB$ mutant using mixed-effects analysis with Sidak’s
 595 multiple test correction, * $p < 0.05$. White asterisks indicate significant upregulation, determined
 596 by one sample t-test, * $p < 0.05$, ** $p < 0.005$.

597

Figure 3



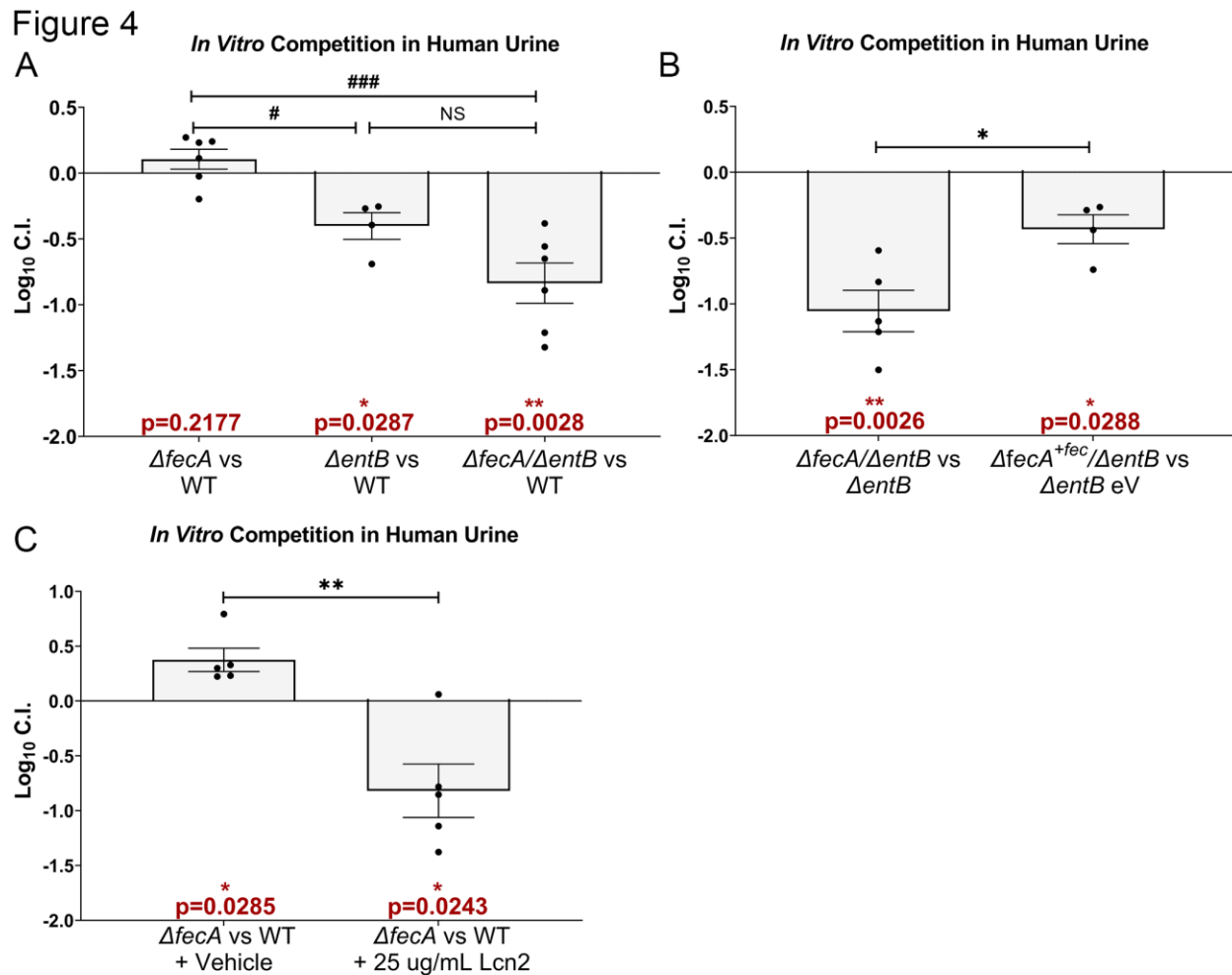
598

599

Figure 3: HM7 uses ferric citrate as an iron source through the *fec* system and

600 **enterobactin**. Growth in M9 medium (i), M9 medium supplemented with 100 mM citrate (ii),
 601 and M9 medium supplemented with both 100 mM citrate and 1 mM FeCl₃ (iii) of (A) WT HM7,
 602 single mutants $\Delta fecA$, $\Delta entB$ and double mutant $\Delta fecA/\Delta entB$, (B) WT HM7 expressing empty
 603 pBAD vector (WT eV), and $\Delta fecA/\Delta entB$ expressing empty pBAD vector ($\Delta fecA/\Delta entB$ eV), and
 604 $\Delta fecA/\Delta entB$ complemented with *fecABCDE* ($\Delta fecA^{+fec}/\Delta entB$). All media in these conditions
 605 are supplemented with 0.5% arabinose to induce expression. (C) WT HM7 expressing empty
 606 pGEN vector (WT eV), and $\Delta fecA/\Delta entB$ expressing empty pGEN vector ($\Delta fecA/\Delta entB$ eV), and
 607 $\Delta fecA/\Delta entB$ complemented with *entB* under control of its native promoter ($\Delta fecA/\Delta entB^{+entB}$).
 608 0.4% glucose was used as the sole carbon source in all conditions. Growth curves show
 609 averages of three to five biological replicates, error bars are SEM.

610

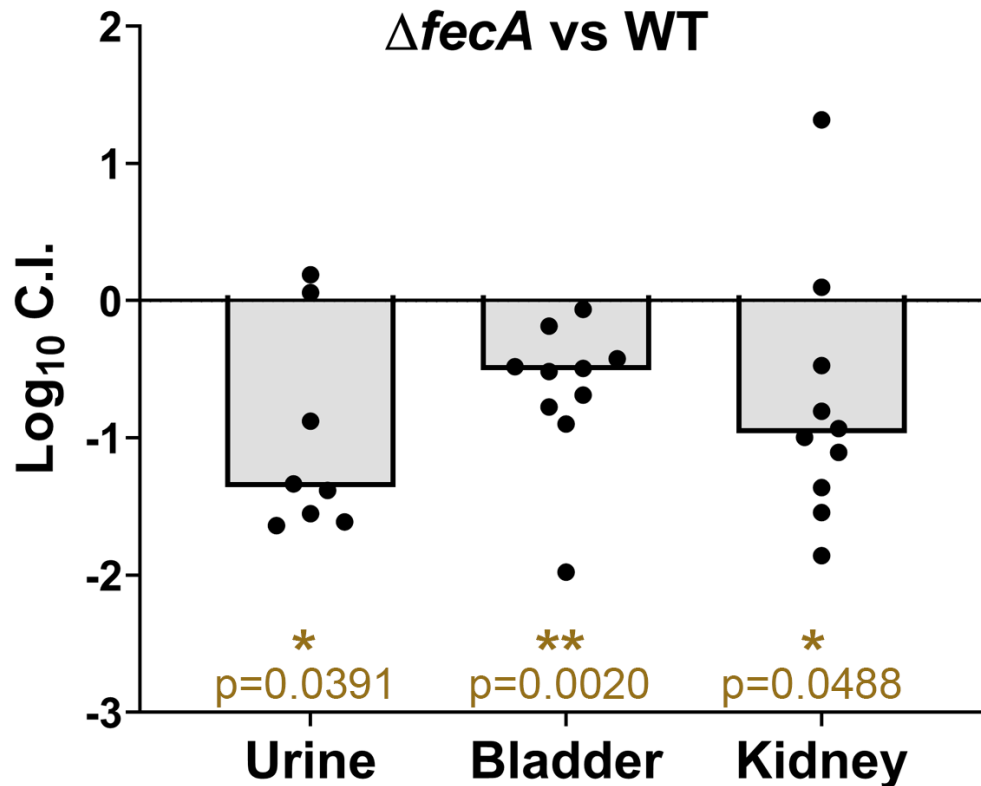


611

612 **Figure 4: Ferric citrate uptake is an *in vitro* fitness factor in the absence of enterobactin.**
 613 *In vitro* fitness of strains or conditions were determined in *ex vivo* pooled human urine. All
 614 strains were inoculated in a 1:1 ratio and grown for 24 hours at 37°C with aeration and their
 615 log₁₀ competitive index (C.I.) determined. A log₁₀ C.I. <0 indicates the first listed strain was
 616 outcompeted by the second. (A) Indicated strains were competed. (B) The $\Delta entB$ mutant
 617 expressing an empty vector ($\Delta entB$ eV) and the $\Delta fecA/\Delta entB$ double mutant with the *fec* operon
 618 complemented in *trans* ($\Delta fecA^{+fec}/\Delta entB$) were competed in urine supplemented with 0.5%

619 arabinose and ampicillin (100 $\mu\text{g}/\text{mL}$). (C) WT HM7 was competed with ΔfecA and the urine was
620 supplemented with either recombinant human lipocalin (Lcn2) or an equal volume of vehicle
621 (25% glycerol). Red asterisks (*) indicate a significant competitive disadvantage, determined by
622 one sample t-test, * $p < 0.05$, ** $p < 0.005$. Hashtags (#) compare \log_{10} C.I. between indicated
623 strains using ordinary one-way ANOVA with Sidak's multiple test correction, # $p < 0.05$, ###
624 $p < 0.001$. Black asterisks (*) compare \log_{10} CI between indicated strains or conditions using
625 unpaired t test, * $p < 0.05$, ** $p < 0.005$. Bars indicate mean, error bars are $\pm\text{SEM}$, each dot
626 represents an independent experiment.

Figure 5

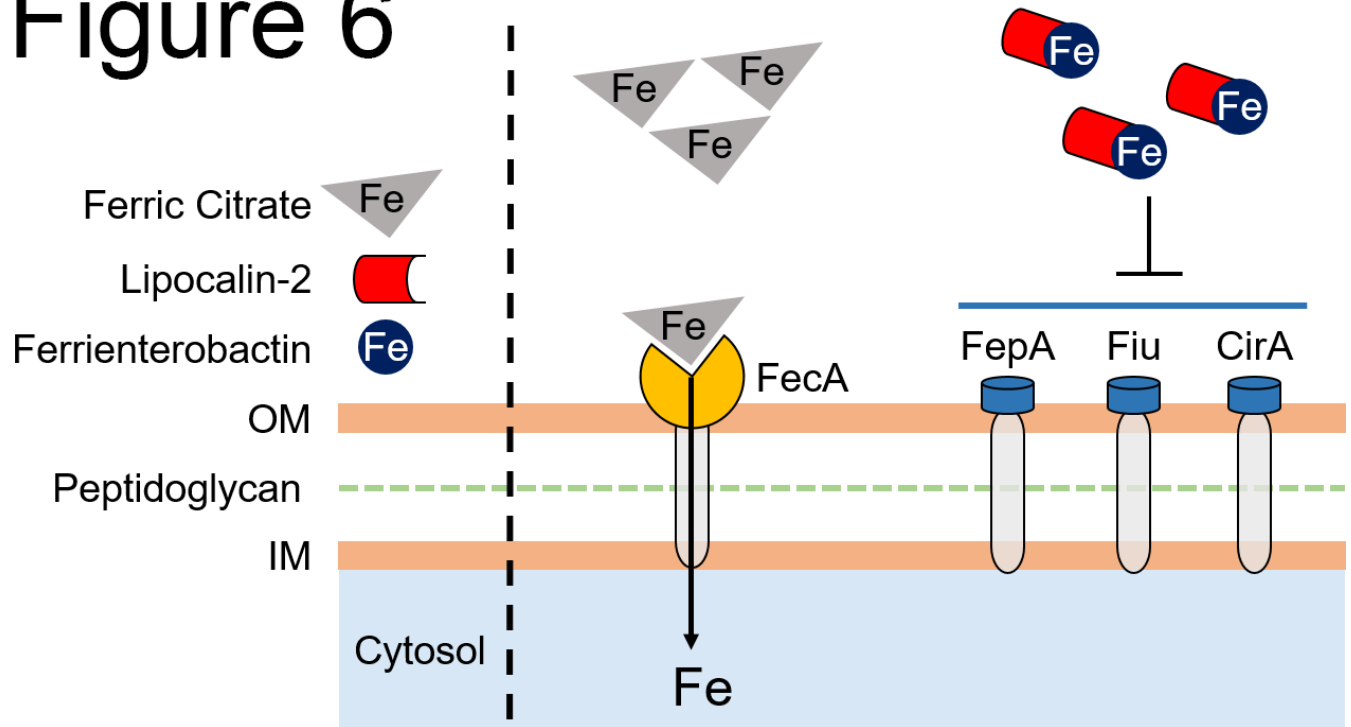


627

628 **Figure 5: Ferric citrate uptake is an *in vivo* fitness factor.** WT HM7 and single mutant ΔfecA
629 were combined in a 1:1 ratio and transurethrally inoculated into CBA/J mice. Competitive
630 indices were calculated 48 hours post infection. Symbols are individual animals, bars are
631 median. Significance was determined with Wilcoxon signed-rank test, * $p < 0.05$, ** $p < 0.005$

632

Figure 6



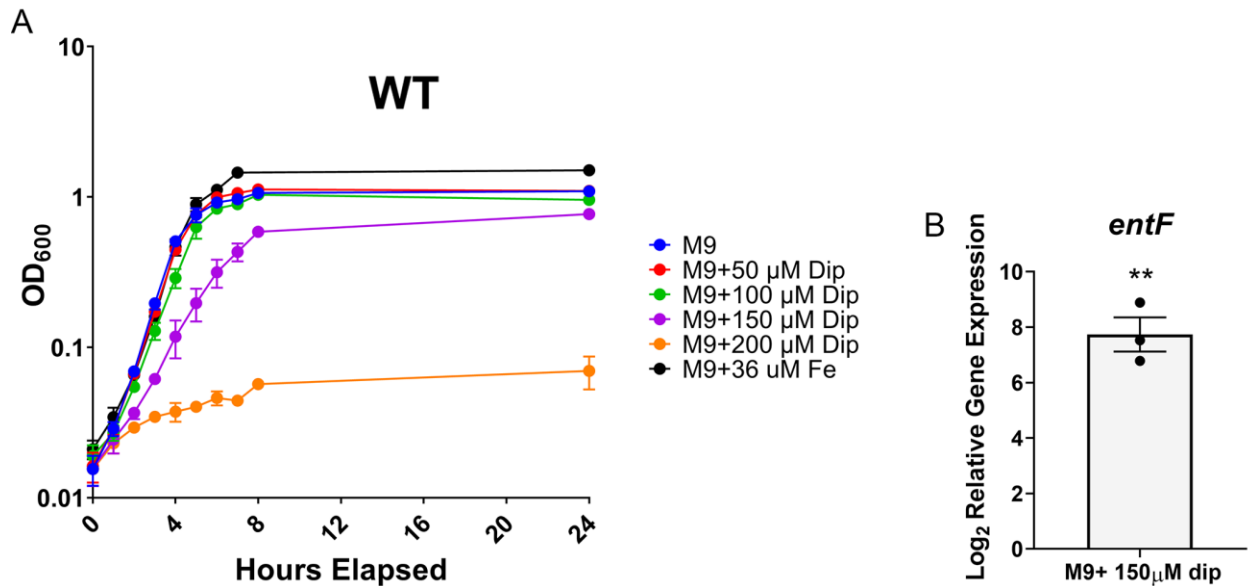
633

634 **Figure 6: Model of UPEC utilization of ferric citrate.**

635

636 **Supplemental Figures**

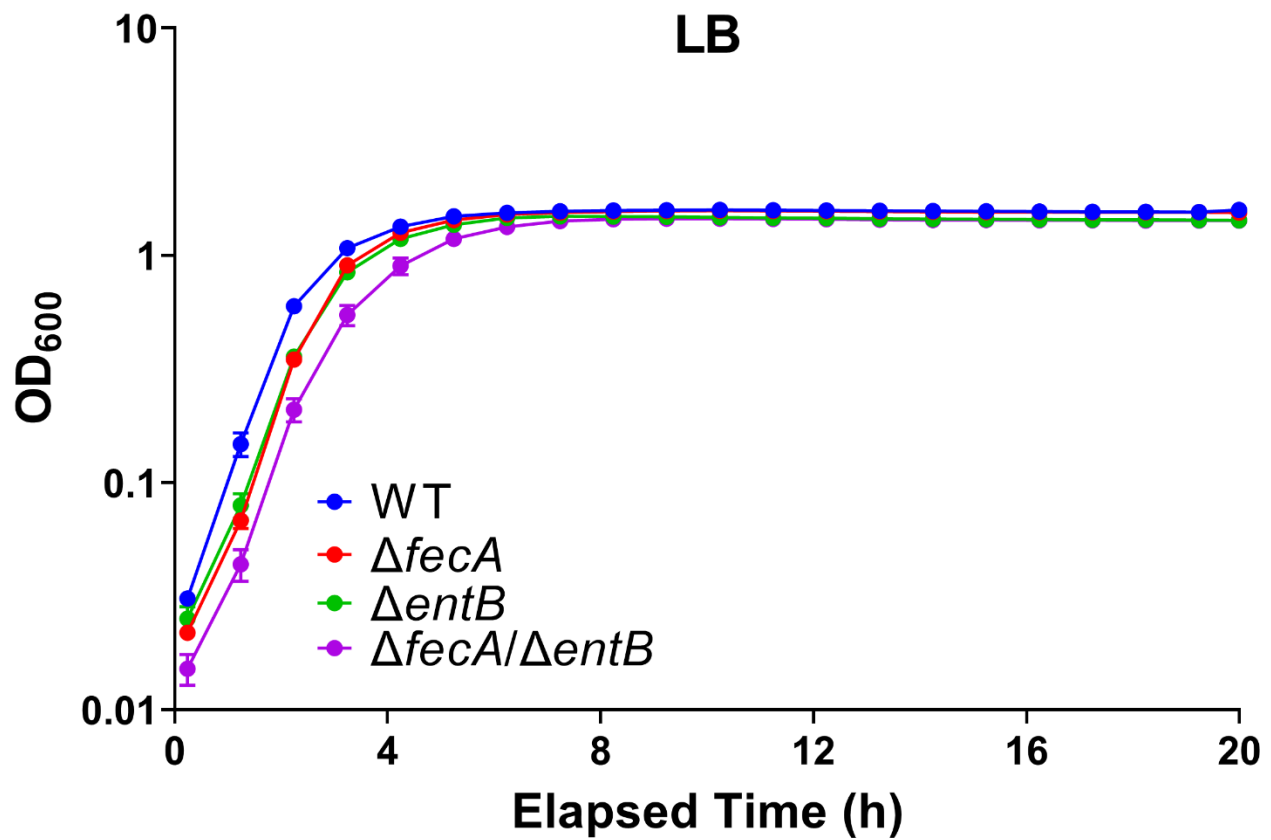
Supplemental Figure 1



637

638 **Supplemental Figure 1.** (A) Growth of WT HM7 in M9 medium with 0.4% glucose as the sole
639 carbon source (M9), as well as supplemented with 36 μM FeCl₃ or increasing amounts of the
640 iron chelator 2,2'-dipyridyl (Dip). WT HM7 was cultured overnight in M9, and then subcultured
641 1:100 into 3 mL medium in culture tubes and grown at 37°C with aeration. OD₆₀₀ was measured
642 on a plate reader for eight hours, taking a reading every hour, and then another reading was
643 taken at 24 hours. Results are an average of three to four biological replicates, error bars
644 represent ±SEM. (B) Gene expression of *entF* in WT HM7 in M9 medium supplemented with
645 150 μM Dip compared to M9 supplemented with 36 μM FeCl₃. Gene expression was assayed
646 through qRT-PCR. Bars are the average of three biological replicates, dots are the values from
647 each biological replicate and error bars are ±SEM, and asterisks indicate significant
648 upregulation, determined by one sample t-test, ** p<0.01.

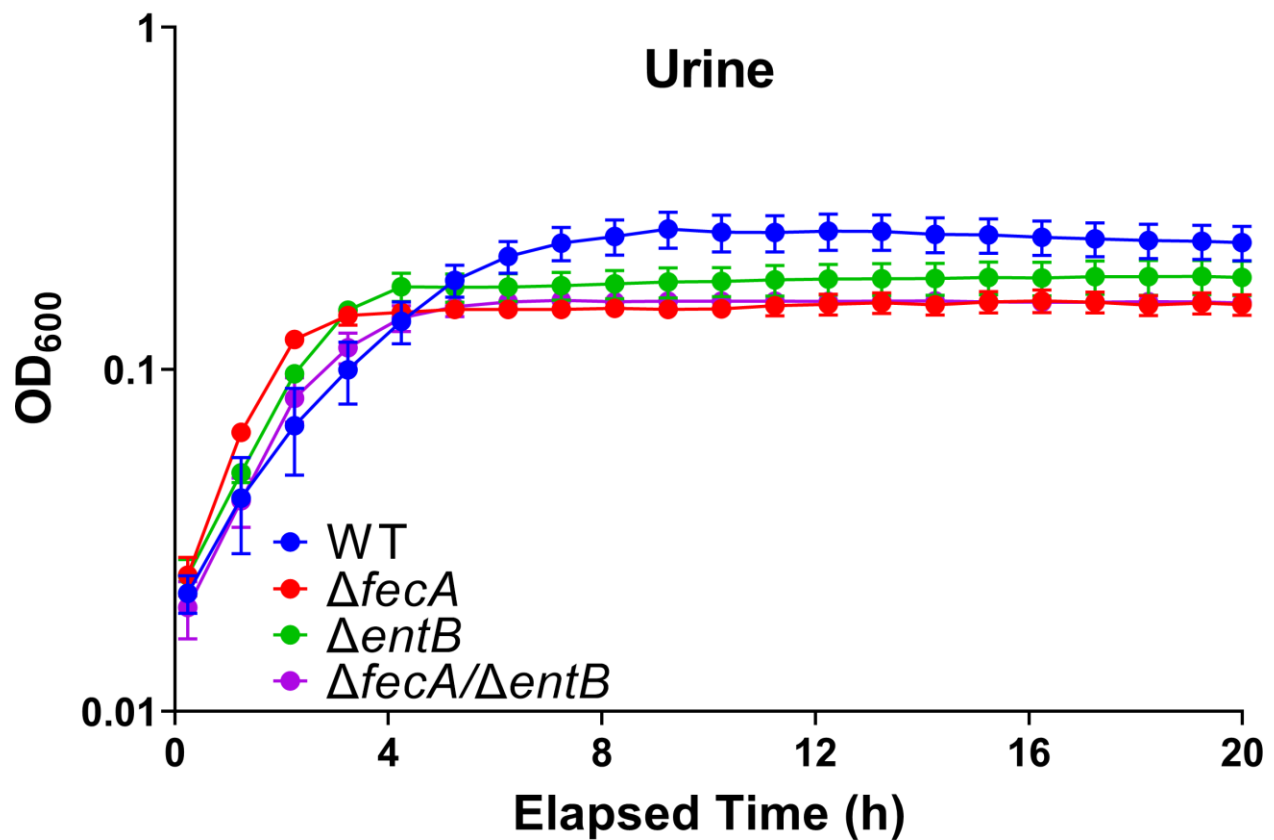
Supplemental Figure 2



649

650 **Supplemental Figure 2.** Growth of WT HM7, $\Delta fecA$, $\Delta entB$, and $\Delta fecA/\Delta entB$, in LB. Results
651 are an average of four to five biological replicates, bars represent \pm SEM.

Supplemental Figure 3

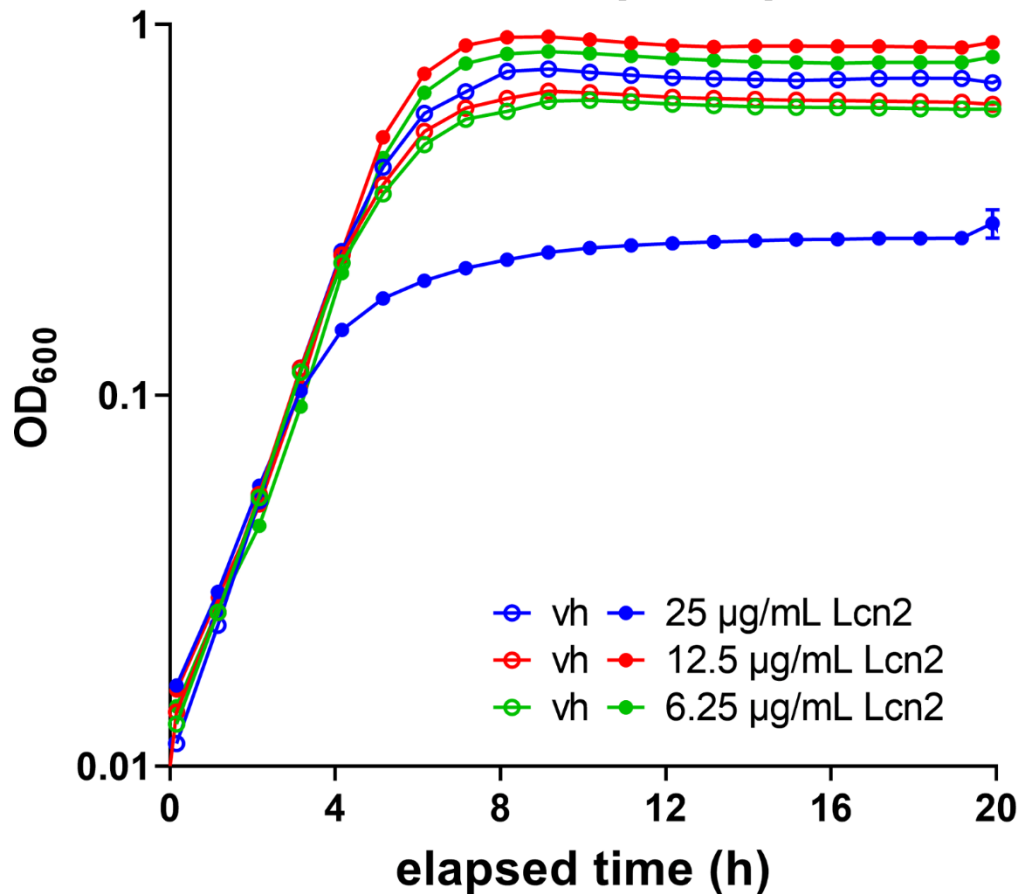


652

653 **Supplemental Figure 3.** Growth of WT HM7, $\Delta fecA$, $\Delta entB$, and $\Delta fecA/\Delta entB$ in *ex vivo* urine
654 pooled from healthy female volunteers. Results are an average of four to five biological
655 replicates, bars represent \pm SEM.

656

Supplemental Figure 4 M9 + 150 μ M Dip



675 **Supplemental Figure 4.** Growth of WT HM7 supplemented with recombinant human lipocalin
676 (Lcn2). WT HM7 was grown in an iron starved state (M9 medium supplemented with 150 μ M
677 Dip) with increasing amounts of Lcn2. An equal volume of the vehicle (vh, 25% glycerol) for
678 each amount was added as a control. Results are an average of two biological replicates, bars
679 represent \pm SEM.

680

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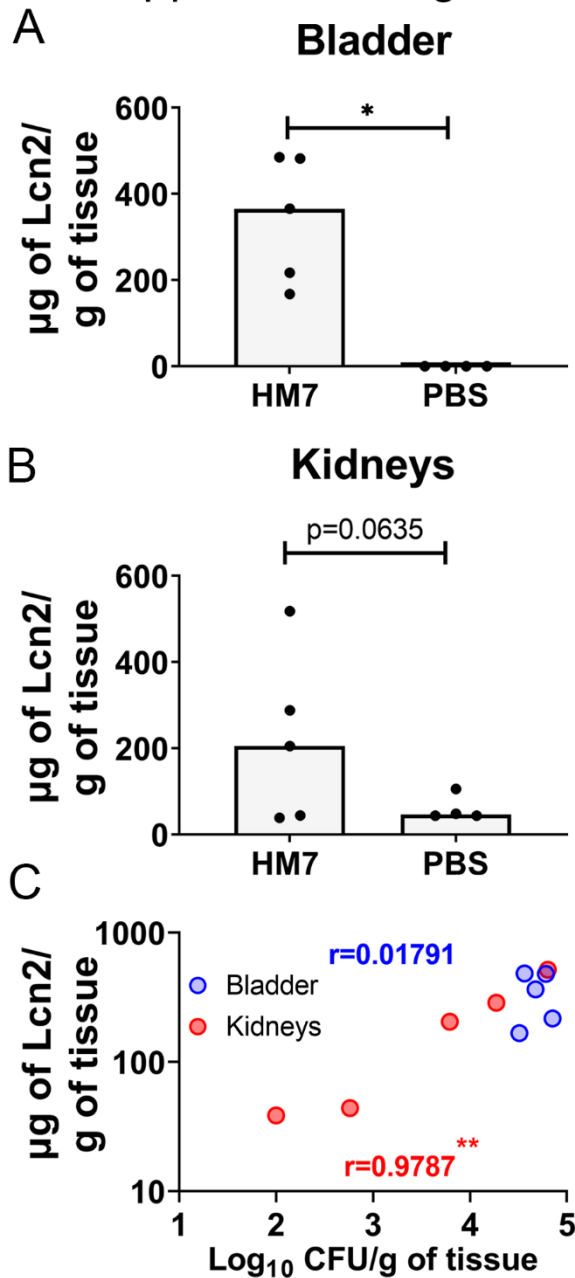
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Supplemental Figure 5



Supplemental Figure 5. Quantification of lipocalin (Lcn2) production during murine infection. CBA/J mice were infected either with WT HM7 or mock infected with PBS. Lcn2 levels were quantified via ELISA in the (A) bladder and (B) kidneys. (C) Lcn2 levels were plotted against CFU burden of mice infected with HM7. Pearson correlation coefficient (r) for bladder is displayed in blue, and for kidneys is displayed in red. Dots indicate individual mice, bars are median. Significance was determined via Mann-Whitney test, * $p < 0.05$.

