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1 **A widespread family of phage-inducible chromosomal islands only steals**
2 **bacteriophage tails to spread in nature**

3

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29

30 **Abstract**

31 Phage satellites interfere with helper phage packaging through the production of small-
32 capsids, where only satellites can be packaged. So far, in all the analysed systems, the
33 satellite-sized capsids are composed of phage proteins. Here we report the first demonstration
34 that a family of phage-inducible chromosomal island (PICIs), a type of satellites, encodes all
35 the proteins required for both the production of the small-sized capsids and the exclusive
36 packaging of the PICIs into these capsids. Therefore, this new family, that we have named cf-
37 PICIs (capsid forming PICIs), only requires phage tails to generate infective PICI particles.
38 Remarkably, the representative cf-PICI reproduces without cost for their helper phages,
39 suggesting that the relationship between these elements is not parasitic but commensalistic.
40 Finally, our phylogenomic studies indicate that cf-PICIs are present both in Gram-positive and
41 Gram-negative bacteria and have evolved at least three times independently to spread widely
42 into the satellite universe.

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47 **Introduction**

48 Mobile genetic elements (MGEs) are key players driving bacterial evolution and ecology
49 (Koonin et al., 2019). Phage satellites are an important type of MGEs that couple their life cycle
50 to that of the helper phages they parasitise, a strategy that ensures their promiscuous
51 dissemination in nature at the expense of their helper phages. To date, the most studied phage
52 satellites are P4 in *Enterobacterales* (Sousa and Rocha, 2022), the phage-inducible
53 chromosomal islands (PICIs) in *Bacillales* and *Gammaproteobacteria* (Fillol-Salom et al., 2018;
54 Martínez-Rubio et al., 2017), and the phage-inducible chromosomal islands-like elements
55 (PLEs) in *Vibrio* spp. (O'Hara et al., 2017; Barth et al., 2019). Although the genetic organisation
56 of these families of satellites is different, all these elements encode genes required for their
57 regulation, replication and preferential package at the expense of their helper phages
58 (Penadés and Christie, 2015; O'Hara et al., 2017; Christie and Dokland, 2012; Sousa and
59 Rocha, 2022). Importantly, these elements do not encode the proteins required for the
60 formation of their infective particles, and therefore, for their survival they must hijack the
61 structural proteins and assembly processes of their helper phages (Ibarra-Chávez et al., 2021;
62 Penadés and Christie, 2015).

63 Satellites have evolved different strategies to promote their preferential packaging into
64 capsids composed of phage-encoded proteins. A strategy commonly used by satellite phages
65 to interfere with phage packaging is the production of small capsids, which are commensurate
66 to the size of the satellite's genomes, but too small to accommodate the complete helper phage
67 genome. Remarkably, although this strategy is conserved, satellites use different mechanisms
68 to redirect phage capsid assembly, which represent a nice example of convergent evolution.
69 While the satellite phage P4 expresses Sid (an external scaffolding protein) to redirect P2
70 capsid assembly (Agarwal et al., 1990), PICIs have evolved multiple mechanisms, depending
71 on the phage they parasite (recently reviewed in (Fillol-Salom et al., 2020)). Thus, some
72 staphylococcal pathogenicity islands (SaPIs), which are the prototypical members of the PICI
73 family, express CpmB, which operates as a scaffolding protein, binding to the phage capsid

74 protein, altering the curvature of the shells and redirecting its assembly and capsid size. This
75 system also requires CpmA, whose predicted function is to remove the phage scaffolding
76 protein (Christie and Dokland, 2012; Dearborn et al., 2017; Ubeda et al., 2007). Other SaPIs
77 that use the *cos* system for packaging express Ccm, which has homology with the helper
78 phage major capsid protein and redirects the assembly of the phage-encoded capsid protein
79 into the production of SaPI-sized small capsids (Quiles-Puchalt et al., 2014; Carpena et al.,
80 2016; Hawkins et al., 2021). PICIs from *Enterococcus faecalis* (EfCI583)(Matos et al., 2013),
81 from *Pasteurella multocida* (PmCI172)(Fillol-Salom et al., 2018), and PLEs from *Vibrio*
82 *cholerae* (Netter et al., 2021), have also the ability to produce satellite-sized capsids, although
83 in these cases the proteins involved in this process have not been identified yet.

84 While capsid redirection severely reduces helper phage reproduction, it does not increase
85 *per se* the packaging of the satellite phages into the small capsids. To solve this problem,
86 satellites have evolved an arsenal of complementary and sophisticated strategies that ensure
87 their preferential packaging into the virions, facilitating their promiscuous transfer in nature.
88 Phage packaging starts by the recognition by the phage-encoded small terminase (TerS) of
89 the *cos* or *pac* sites in the phage genomes. To promote their packaging, some satellites - such
90 as P4 and some SaPIs - carry the cognate helper phage *cos* site in their genomes (Quiles-
91 Puchalt et al., 2014; Ziermann and Calendar, 1990). Other SaPIs encode a homologue of the
92 phage TerS called TerS_S(Ubeda et al., 2007), which recognises the SaPI *pac* sequence (Chen
93 et al., 2015). To help TerS_S function, these SaPIs also encode Ppi (phage packaging
94 interference), which binds to the phage TerS, blocking its function (Ram et al., 2012, 2014).
95 Finally, some PICIs present in Proteobacteria encode Rpp (redirecting phage packaging),
96 which binds to the phage TerS. The Rpp-TerS complex recognises the PICI *cos* site (promoting
97 PICI packaging), but not the phage *cos* site, thus blocking phage reproduction (Fillol-Salom et
98 al., 2019).

99 While diverse, all the mechanisms of piracy described so far require that helper phages
100 express all the proteins necessary to generate not just the phage but also the PICI infective

101 particles. We report here the discovery of a widespread family of PICIs, that we have named
102 cf-PICIs (capsid forming PICIs), that encode not just all the proteins required for the production
103 of PICI-sized capsids but also for the exclusive packaging of the cf-PICIs into the small-sized
104 capsids. Therefore, and contrary to what is seen with other satellites, cf-PICIs only require
105 phage tails to spread in nature. They represent a new strategy of molecular piracy.

106 **Results**

107 **EcCIEDL933 is the prototypical member of the cf-PICI family**

108 During the description of PICI elements in *Proteobacteria* (Fillol-Salom et al., 2018), one
109 intriguing element with an unusual genetic organisation, present in the *E. coli* strain EDL933,
110 raised our curiosity. This element, with a size of 15.4 kb, carried the *int*, *alpA* and *rep* genes,
111 identical to those present in the prototypical *E. coli* EcCICFT073 and EcCIED1a PICIs (Fig.
112 1A). However, in addition to these conserved PICI genes, this element seemed to encode all
113 the proteins required for the production of functional capsids (major capsid, head maturation
114 protease, portal, head-tail connectors), and for the packaging of the PICI DNA into these
115 capsids (HNH and small and large terminase proteins) (Fig. 1A). Interestingly, this element did
116 not encode genes involved in the production of phage tails.

117 Based on this peculiar organisation, our initial thoughts were that this element could
118 represent a hybrid between a PICI and a phage. Note that the aforementioned DNA packaging
119 genes present in this putative hybrid element showed homology with phage genes (Fig. 1B).
120 Yet, sequence similarities between the genes present in this new element and those present
121 in phages were generally low and restricted to a few genes. To assess the similarity in gene
122 repertoires between the element present in strain EDL933, hereinafter named EcCIEDL933,
123 and the genomes of typical phages, we computed their weighted gene repertoire relatedness,
124 which we have used previously to class phages in families (see Methods)(Sousa et al., 2021).
125 Our analysis of 3725 complete non-redundant phage genomes retrieved from RefSeq shows
126 that the genome of EcCIEDL933 is very different from them. Indeed, 86% of the phage
127 genomes lack homologs in EcCIEDL933, with the remaining 14% (519 phage genomes)

128 having, at most, a wGRR of 0.09 (Fig S1). These cases represent phage-encoded homologs
129 to some of the proteins encoded by EcCIEDL933, and include, besides the aforementioned
130 capsid and terminase genes, homologs to the other capsid formation (portal, head-tail
131 connectors) or packaging (HNH) genes. Importantly, no single phage genome has more than
132 six homologous proteins with EcCIEDL933 and the average number of homologs, among
133 phages with at least one homolog, is only 1.8 (Fig. S1D). Most of these rare homologs have
134 significant, but low sequence similarity (<40%, e-value<1e-04; Fig. S1C and S1D). On the
135 other hand, our KEGG orthology analyses showed that genes homologous to the ones present
136 in EcCIEDL933 are frequently found in genomic regions that could correspond to similar
137 elements in *E. coli* and other related species, including *Salmonella enterica*, *Shigella flexneri*,
138 *Klebsiella pneumoniae*, *Pluralibacter gergoviae* or *Yersinia aleksiciae* (Table S1). Therefore,
139 these results suggested that EcCIEDL933 could represent a new family of elements, distinct
140 from both phages and previously described PICIs. In support of this idea, members of this new
141 family integrate into the *E. coli* bacterial chromosome using six different *attB* sites. Of these,
142 two were exclusively used by the members of this new lineage, while the other four were also
143 used by members of the previously identified *E. coli* PICIs (Table S2 and Fig. S2).

144 The previous results suggested that the mechanism of induction of this new lineage could
145 be identical to that described for the classical PICIs present in *E. coli*. Once induced, the genes
146 involved in DNA packaging could either promote the packaging of the cognate element and/or
147 could be used by the island to interfere with helper phage reproduction. To clearly demonstrate
148 that EcCIEDL933 is induced as the classical PICI elements, we searched for helper phages
149 able to induce and mobilise EcCIEDL933. To facilitate these studies, we inserted a *cat* marker
150 into the element present in the clinical EDL933 strain, which also carries 17 prophages (Perna
151 et al., 2001), and induced the resident prophages with mitomycin C (MC), expecting that one
152 of the resident prophages was able to mobilise EcCIEDL933 *cat* into the non-lysogen 594 *E.*
153 *coli* strain, used in this experiment as recipient. This was the case, although just a few
154 transductants were obtained (Table 1), suggesting that none of the resident prophages present

155 in the EDL933 strain are the helper phage for this island. Note that in presence of a helper
156 phage, PICI transfer is extremely high (Humphrey et al., 2021). Thus, it is likely that neither of
157 the 17 prophages induces EcCIEDL933.

158 To search for one, we lysogenised the *E. coli* 594 strain carrying EcCIEDL933 *cat*-positive
159 with our collection of *E. coli* phages and tested the transfer of the PICI element after MC
160 induction of the different lysogenic strains. Two phages, HK106 and HK446, mobilised the
161 island at high frequencies (Table 1), suggesting they were able to induce the EcCIEDL933
162 cycle. This was confirmed with the screening lysate analysis of the DNA samples obtained
163 after induction of the different lysogenic strains carrying EcCIEDL933. As shown in Fig. 2, only
164 the strains lysogenic for either HK106 or HK446 showed the classical pattern of PICI induction:
165 a high sized DNA band corresponding either to the PICI replicating concatemeric form or to
166 the PICI/phage DNA packaged into the phage-sized capsids, and a small band corresponding
167 to the PICI DNA packaged into small PICI-sized capsids.

168 Finally, we tested the transfer of the EcCIEDL933 *alpA* mutant. Note that in Gram-negative
169 bacteria PICI induction and transfer requires the expression of the PICI-encoded *alpA* gene, a
170 process that is activated by the helper phage (Fillol-Salom et al., 2018). As expected for a
171 PICI-like element, mutation of *alpA* eliminated both helper-phage induction (Fig. 2) and transfer
172 of this element (Table 1).

173 **EcCIEDL933 produces PICI-sized capsids**

174 Having established that this new family of satellites are induced like the classical PICIs, we
175 wanted to know whether the EcCIEDL933 genes with homology to the phage genes were
176 required for capsid formation and EcCIEDL933 packaging and transfer. The presence of the
177 PICI-sized small band in the EcCIEDL933 screening lysate suggested that this island had the
178 ability to produce PICI-sized capsids after induction. To test this, we analysed by electron
179 microscopy (EM) the infective particles present in the lysate obtained after induction of the
180 lysogenic strain for helper HK106, in presence or absence of EcCIEDL933. In the absence of
181 EcCIEDL933, only phage particles were observed, which had the characteristic size and shape

182 of a *Siphoviridae* phage with a hexagonal capsid of approximately 60 nm length and 57 nm
183 width, with non-contractile tails which are approximately 160 nm long and 10 nm wide (Fig.
184 3A). By contrast, in the presence of EcCIEDL933 two different types of infective particles were
185 observed: in addition to the phage-sized ones, we were able to detect PICI-sized capsids,
186 which had a hexagonal capsid of 46 nm length and 42 nm wide, connected to the phage non-
187 contractile tail (Fig. 3A).

188 Since the PICI genome is 1/3 of the size of the phage genome, it is obvious that the
189 complete phage genome cannot be packaged into the PICI-sized particles. However, some
190 PICIs can package 3 copies of their genomes in phage-sized capsids (Carpena et al., 2016;
191 Ubeda et al., 2007). To test whether this was also the case for EcCIEDL933, the packaged
192 DNA present in the lysate containing both the phage and the PICI infective particles was
193 purified, separated on agarose gels, and analysed by Southern blot, using specific phage- or
194 PICI probes. In support of the EM images, two types of bands were observed, corresponding
195 to the size of the phage and EcCIEDL933 genomes, respectively (Fig. 3B). Importantly, the
196 Southern blot analyses revealed that the packaging of the different elements is size specific
197 and mutually exclusive, with phages and PICIs being packaged uniquely in large- and small-
198 sized capsids, respectively (Fig. 3C).

199 **Characterisation of the EcCIEDL933 packaging module**

200 The previous results suggested that the different sized capsids had different origins: the phage
201 would be responsible for the production of the large capsids and the phage tails, while the
202 small capsids would be produced by the PICI. In support of this idea, comparison of the phage
203 and PICI packaging modules reveals that they have different genetic organisations and low
204 protein identities (Fig. 1B). To test this idea, we individually mutated each of the genes
205 belonging to the putative packaging module of EcCIEDL933 (Fig. 4A) and tested the transfer
206 of the different EcCIEDL933 mutants after induction of the helper prophage HK106.
207 Remarkably, while the replication of the different mutants was unaffected (Fig. S3), their
208 transfer was completely abolished in all cases except in the mutant for the hypothetical gene

209 1792, whose transfer was severely affected (Fig. 4B). To confirm that the observed phenotypes
210 were consequence of the mutations, complementation of the mutants restored PICI titres (Fig.
211 S4C). Interestingly, and in support of our idea, in all these cases the transfer of the helper
212 phage was unaffected, confirming that the PICI encoded proteins have no role in the packaging
213 of the helper phage (Fig. 4C). Importantly, the Southern blot analyses of the purified infective
214 particles obtained after induction of the different EcCIEDL933 mutant strains uniquely revealed
215 the presence of the phage-sized band, but not the PICI-sized ones, confirming that the small
216 capsids had a PICI origin (Fig. S5C). Next, we performed the same experiment, but now
217 mutating the phage genes encoding the homolog proteins to those encoded by the PICI. In
218 this case the mutations eliminated phage but not PICI transfer (Fig. 4E and 4F). Phage mutants
219 were complemented *in trans*, confirming that the phenotypes were due to the mutations (Fig.
220 S4D). Moreover, and in agreement with the idea that the large and small capsids had different
221 origins, only PICI-sized bands were observed in the Southern blot analyses of the purified
222 infective particles obtained after induction of the different phage HK106 mutant strains (Fig.
223 S5D). This was corroborated with the analysis of the lysate obtained after induction of the
224 strain carrying the HK106 capsid (*gp05*) mutation and EcCIEDL933, which only contained
225 PICI-sized particles (Fig. 3).

226 Our previous results demonstrated that EcCIEDL933 encodes a functional packaging
227 module responsible for both the production of the PICI-sized capsids and for the specific and
228 exclusive packaging of the island into these particles. Therefore, we propose here that
229 EcCIED933 is the prototypical member of a new family of PICI satellites, that we have named
230 capsid forming PICIs (cf-PICIs), with the ability to produce all the proteins required for the
231 specific packaging of these elements into cf-PICI encoded capsids. These results also
232 suggested that in terms of producing infective particles, EcCIEDL933 only requires the phage
233 tails to complete the formation of the PICI infective particles. To confirm this hypothesis, we
234 mutated two of the phage genes encoding important tail components: the tape-measure (*gp14*)
235 and major tail (*gp10*) proteins. The strains carrying the mutant phages and EcCIEDL933 were

236 MC induced, and the phage and PICI titres quantified. As expected, these mutants abolished
237 both phage and PICI transfer (Fig. 5A). Next, to confirm that EcCIEDL933 only relies on the
238 phage tail proteins to produce PICI infectious particles, we analysed by EM the lysate obtained
239 after the induction of a strain containing the PICI and a helper phage carrying mutations in the
240 tape-measure (*gp14*) and the capsid (*gp05*) genes. Note that in this strain the phage does not
241 produce large capsids nor tails. In support of our hypothesis, only PICI-sized capsids were
242 observed in the lysate (Fig. 5B).

243 **EcCIEDL933 does not block helper phage reproduction**

244 One conserved characteristic of the satellites is their ability to severely interfere with their
245 helper phage reproduction because they hijack its components (Filloi-Salom et al., 2020)
246 (O'Hara et al., 2017). To test whether EcCIEDL933 was also able to block the reproduction of
247 its helper phages, we quantified the phages obtained after induction of the lysogenic strains
248 for HK106 or HK446, the EcCIEDL933 helper phages, in presence or absence of the island.
249 Importantly, phages HK106 or HK446 were unaffected by the presence of EcCIEDL933 (Table
250 S3). To confirm this, we infected strains 594 or JP22295 (a derivative 594 strain carrying
251 EcCIEDL933) with phages HK106 or HK446, and compared their efficiency of plating. With the
252 classical PICIs, in presence of the islands, both the number and the size of the phage plaques
253 are severely reduced, only being able to plaque those mutant phages that are unable to induce
254 the islands (Frígols et al., 2015). In this case, however, the scenario was completely different,
255 and as occurred after prophage induction, the presence of the island did not affect the capacity
256 of the helper phages to reproduce normally in the recipient strain carrying the island, compared
257 to the EcCIEDL933-negative strain (Fig. S6). These results suggest that EcCIEDL933 is not a
258 phage parasite, but rather a commensal

259 **cf-PICIs are widespread in nature**

260 We next analysed whether this new family of satellite phages is widespread in nature. We
261 made a curated search across the GenBank database for similar cf-PICI elements among
262 thirteen species of *Proteobacteria*, especially in members of the *Enterobacteriaceae*,

263 *Pasteurellaceae* and *Orbaceae*, where PICIs are frequent (Fillol-Salom et al., 2018). We
264 searched for elements carrying *int* or *alpA* genes identical to those present in the canonical
265 PICIs, but encoding a packaging module similar to that present in the cf-PICI EcCIEDL933.
266 Our preliminary analysis revealed that other cf-PICIs are frequent in *Proteobacteria* genomes
267 (Fig. S2; Table S2). Importantly, all the elements identified encode all, or most, of the
268 components present in EcCIEDL933, with a similar conserved genetic organisation (Fig. 1 and
269 S2). Their genes encoding the packaging module present sequence similarity with those from
270 EcCIEDL933 ranging from 50% to 90%, suggesting that they are indeed part of a single family
271 of satellites.

272 Using the same strategy that allowed us the identification of the cf-PICIs in *Proteobacteria*,
273 we next interrogated the genomes of Firmicutes, the other phylum with known PICIs for the
274 presence of these elements. Importantly, our analysis was able to identify cf-PICIs in different
275 genera of Firmicutes, including *Lactococcus*, *Enterococcus*, *Bacillus*, *Clostridium* and
276 *Staphylococcus*. While the cf-PICIs in *Proteobacteria* showed a conserved genetic
277 organisation, with the morphogenetic genes (capsid-protease-portal-head connectors) first,
278 followed by the ones involved in DNA recognition and packaging (*hnh-terS-terL*), two distinct
279 groups were observed in the cf-PICIs from Firmicutes. One group was composed by the
280 elements present in *Bacillus*, *Clostridium* and *Staphylococcus*, while the second group was
281 composed by the elements present in *Lactococcus* and *Enterococcus* (Fig. S7; Table S4).
282 While both groups had similar gene content, the organisation/localisation of these genes within
283 the packaging operon was different. The genes encoding the portal, protease and capsid
284 proteins of the first group were present at the beginning of the operon, while the genes
285 encoding HNH, TerS and TerL start the operon of the second group. This suggests that cf-
286 PICI may have different origins in these two phyla.

287 **cf-PICIs arose three times independently**

288 To detail the evolutionary history of cf-PICI, we searched specifically for homologs of their
289 capsid and large terminase proteins in the database of complete phage genomes used in our

290 initial analysis. In each case (capsid or TerL), we collected the phage hits with highly significant
291 e-values ($<1e^{-10}$) (see Methods). This allowed us to put together a list of homologs for the
292 capsid (113 phage + 61 cf-PICl) and TerL (420 phage + 60 cf-PICl) proteins present in the cf-
293 PICls or in the phages. We then built the phylogenetic trees of the two types of proteins. The
294 conclusions were similar for the analyses of the capsid and the terminase proteins (Fig. 6 and
295 S8). We observed that cf-PICls regrouped on three clades that are clearly separated from each
296 other by many phage genes. These three clades correspond to the three groups distinguished
297 in the previous section in terms of genetic organisation. The separations between the three
298 groups are robust to phylogenetic reconstruction uncertainty, as attested by high confidence
299 bootstrap values at the basis of each clade. This suggests the independent emergence of the
300 three groups of cf-PICls.

301 The analysis of each of the cf-PICl clade shows a clear separation between the satellites
302 and the phage homologs, i.e. the clades only include cf-PICl components even if we
303 deliberately selected the phage homologs that were most similar to the cf-PICl. Furthermore,
304 the phages closest to each clade of cf-PICls tend to be the same in the TerL and capsid tree.
305 The consistent phylogeny for these components suggests strong genetic linkage between the
306 two functions. This further confirms that cf-PICls are clearly distinguishable from the phages
307 in the database and suggests a low frequency of genetic exchanges of these key components
308 between cf-PICls and phages.

309 Within each clade of cf-PICl one identifies elements present in the genomes of multiple
310 bacterial genres. In the EcCIEDL933-containing clade we could identify bacteria from very
311 different Gammaproteobacteria, from *E. coli* to *Yersinia* or *Xenorhabdus*. Similarly, the clade
312 containing the *S. aureus* element includes bacteria from *Bacillus* and *Clostridium*, which are
313 very distantly related. This shows that either these elements have an exceptionally broad host
314 range upon transfer through virions, or that they are very ancient and have diversified within
315 distinct bacterial taxonomic groups. The analysis of the distances in the phylogenetic trees
316 shows very long branches between the cf-PICl and the closest phages even in the highly

317 conserved protein TerL (Fig. 6), which is also suggestive of an old origin for cf-PICIs. Hence,
318 the cf-PICIs are neither recently derived from phages nor the result of recent prophage genetic
319 degradation. Instead, they constitute deep lineages of elements that, compared to the other
320 previously described PICI, have more proteins that are homologous to those of phages.

321 **Discussion**

322 Satellites are MGEs whose life cycle depends on a helper virus but lack extensive nucleotide
323 sequence homology between them. So far, what is the origin of these elements, or how have
324 they evolved, remain a mystery. In this manuscript we report that a new family of phage
325 satellites is able to specifically package their DNA into small-sized capsids, which are of
326 satellite origin. Since the cf-PICI-encoded proteins responsible of these processes are similar
327 to those encoded by their helper phages, it is tempting to speculate that they have a phage
328 origin and have evolved not just to be able to produce the small-sized capsids, but also to
329 avoid interaction with the phage-encoded machinery.

330 In most of the previously analysed satellites, the production of the small-sized capsids and
331 the preferential packaging of the satellites are two independent processes. Thus, some SaPIs
332 that use the *cos* system for packaging, as well as the P4 satellite, are able to produce small
333 capsids, but they do not package their DNA preferentially, since they carry the helper *cos*
334 sequence in their genomes and use the phage terminase complex to initiate packaging
335 (Carpena et al., 2016; Quiles-Puchalt et al., 2014; Christie and Dokland, 2012; Lindqvist et al.,
336 1993). Another example corresponds to the SaPIs that use the headful mechanism for
337 packaging (*pac* SaPIs). Here, both the phage- and the SaPI-encoded small terminase can
338 interact with both the small and the large-sized capsids. Therefore, the production of the small
339 sized capsids limits phage reproduction not because the phage TerS cannot use these small
340 capsids, but because the phage genome cannot be completely packaged in these capsids
341 (Ubeda et al., 2007). An additional example refers to the other family of PICIs present in *E.*
342 *coli*. Although they are 1/3 of the size of their helper phages, these elements are packaged in
343 large capsids (probably carrying 3 concatemeric molecules per capsid), but their preferential

344 packaging is driven by the expression of the PICI-encoded Rpp protein, which interacts with
345 the phage TerS to modify its specificity, promoting PICI packaging (Fillol-Salom et al., 2019).
346 Lastly, the recently described PLE satellites also produce small capsids, although the
347 mechanism of small capsid production and how they preferentially package their genomes into
348 these small sized capsids is still unknown (Netter et al., 2021).

349 Contrary to the previously described satellites, in the cf-PICI family of satellites both the
350 production of the small-capsids and the exclusive packaging of the elements are linked. In the
351 system characterised here, phage and satellite packaging are clearly independent, and they
352 have evolved to be also mutually exclusive. Why do different satellites use different strategies?
353 Most of the previously characterised satellites hijack the machinery of helper phages that use
354 the classical *cos* or *pac* systems for packaging. In these systems, the terminase complex,
355 composed by the phage or PICI-encoded TerS, and the phage-encoded TerL proteins, interact
356 with their cognate *pac* or *cos* site to initiate packaging (Fillol-Salom et al., 2018) (Fillol-Salom et
357 al., 2020). Interestingly, the new family of satellites described here hijacks a different family of
358 phages, present both in Proteobacteria and Firmicutes, which to initiate packaging require not
359 just the TerS-TerL complex but also the activity of an additional player, the HNH protein (Kala
360 et al., 2014; Quiles-Puchalt et al., 2014). One can hypothesise that these specific requirements
361 for packaging have selected for the existence of this new lineage of satellites, which have
362 evolved to promote the formation of the small-sized capsids and their preferential packaging
363 against these helper phages by incorporating and differentiating the phage-packaging module
364 into the satellite genome.

365 Another interesting characteristic of the EcCIEDL933 element is that it does not interfere,
366 at least in the conditions tested in these studies, with the reproduction of their helper phages.
367 Since EcCIEDL933 is the only element of this new lineage characterised so far, we do not
368 know if this behaviour is exclusive of this satellite or is a characteristic of this new family. Since
369 we have previously demonstrated that by interfering with phage reproduction PICIs drive
370 phage evolution and ecology (Frígols et al., 2015), the lack of interference observed here

371 opens new avenues by which phages and satellites may interact. These are currently under
372 study, although one might speculate that encoding a packaging system and a capsid may allow
373 cf-PICIs to use the cell resources more efficiently than the other PICIs, and thus alleviate the
374 cost of their production on the replicating helper phage. Indeed, other satellites require the
375 production of proteins to change capsid size and some of them cannot preclude the (non-
376 productive) partial packaging of phages in the small capsids. The cf-PICIs may ensure an
377 optimal use of resources to produce their PICI particles and have a negligible impact on the
378 production of viral particles.

379 Importantly, this new strategy of parasitism is present in satellites from Proteobacteria and
380 Firmicutes, confirming its importance in nature. Their phylogeny and the wGRR analyses show
381 that they are clearly distinct from phages, even for the proteins that have homologs in both
382 types of elements. The phylogenetic analysis further shows that there are at least three groups
383 of cf-PICIs that emerged independently raising interesting questions in relation to their origin.
384 We propose here that the capsid and packaging genetic module were incorporated together
385 into ancestral PICIs present in different bacterial species and obtained from different phages.
386 Given the large evolutionary distances between cf-PICI and phages even in the highly
387 conserved large subunit of the terminase, the acquisition of the phage capsid and packaging
388 modules must have occurred a long time ago. The multiple independent acquisition of the
389 modules from different phages explains the phylogenetic patterns and our observation of
390 different genetic organisation in the different groups. These modules then evolved to adapt to
391 the cf-PICI biology, which may have resulted in the elimination of accessory genes to fit the
392 small cf-PICI genomes leading to a minimal capsid module that has remarkably similar types
393 of components in the three groups. These components may correspond to a minimal set of
394 genes necessary to build a capsid.

395 Once again, PICIs have provided unexpected insights in the biology of the satellite viruses.
396 Because of the simplicity of the experimental models, evolutionary studies with phages and
397 their satellites can give insight into the general principles of the biology of the eukaryotic

398 viruses and their satellites. We have applied this concept here and have demonstrated the
399 existence of a novel way by which satellites and helper phages interact. We anticipate that a
400 better characterisation of this interaction will contribute to our understanding on how viruses
401 and satellites evolve, and how this evolution and coexistence impacts on the biology of both
402 the prokaryotic and eukaryotic cells infected by these elements.

403

404 **Methods**

405 **Bacterial strains and growth conditions**

406 Bacterial strains, plasmids and oligonucleotides used in this study are listed in Table S5, Table
407 S6 and Table S7, respectively. Strains were grown at 37°C or 30°C on Luria-Bertani (LB) agar
408 or in LB broth with shaking (120 r.p.m.) supplemented with Ampicillin (100 µg ml⁻¹), Kanamycin
409 (30 µg ml⁻¹) or Chloramphenicol (20 µg ml⁻¹; all Sigma-Aldrich) when required.

410 **Plasmid construction**

411 The plasmids used in this study (Table S6) were constructed by cloning PCR products,
412 amplified with primers listed in Table S7, into the pBAD18 vector using digestion and ligation.
413 Plasmids were verified by Sanger sequencing in Eurofins Genomics.

414 **DNA methods**

415 The introduction of the chloramphenicol (*cat*) resistance marker into the EcCIEDL933 element
416 was performed as described (Filloi-Salom et al., 2018) using λ Red recombinase-mediated
417 recombination. Briefly, marker was amplified by PCR using primers listed in Table S7 from
418 plasmid pKD3, and the PCR product was transformed into the recipient strain harbouring
419 plasmid pRWG99, which expresses the λ Red recombinase, and the marker was inserted into
420 the PIC1 genome. The insertion of the resistance marker was verified by PCR.

421 For PIC1 mutagenesis, the site-directed scarless mutagenesis was performed as
422 described previously (Blank et al., 2011; Hoffmann et al., 2017). Briefly, the *kmR* marker
423 together with an I-SceI recognition restriction site was amplified by PCR, using primers listed

424 in Table S7. Then, PCR product was inserted into the recipient strain harbouring plasmid
425 pRWG99, which expresses the λ Red recombinase protein. After verification of the insertion
426 by PCR, 80mer DNA fragments derived from oligonucleotides which contains the desired
427 mutation were electroporated into the mutant strain expressing the λ Red recombinase-
428 mediated system, and successful recombinants were selected by expression of I-SceI
429 endonuclease. The different PICI mutants obtained were verified by PCR and Sanger
430 sequencing in Eurofins Genomics.

431 For phage mutagenesis, an allelic replacement strategy was performed (Solano et al.,
432 2009). Briefly, the allelic-exchange vector, pK03-Blue, was used to clone the desired genes
433 using the primers listed in Table S7. Then, plasmid was inserted into the strain carrying the
434 prophage and transformants were selected on LBA plates supplemented with chloramphenicol
435 and incubated at 32°C for selection of the temperature-sensitive plasmid. To produce the
436 homologous recombination, the plasmid was forced to integrate into the phage genome at the
437 non-permissive temperature (42°C). Light blue colonies, which are indicative of plasmid
438 integration, were grown in LB broth at 32°C and ten-fold serial dilution of the overnight cultures
439 was plated on LBA plated containing X-gal (5-bromo-4-chloro- 3-indolyl-B-D-
440 galactopyranoside) and sucrose 5% (to force the plasmid loss) and incubated at 32°C for 24
441 h. White colonies, which is indicative that the plasmid is loss, were screened for
442 chloramphenicol sensitivity. The different phage mutants obtained were verified by PCR
443 and Sanger sequencing in Eurofins Genomics.

444 **Phage plaque assay**

445 A 1:50 dilution (in fresh LB broth) of the overnight strains were grown until an $OD_{600}=0.34$ was
446 reached. Bacterial lawns were prepared by mixing 300 μ L of cells with phage top agar (PTA)
447 and poured onto square plates. Then, serial dilutions of phages were prepared in phage buffer
448 (50mM Tris pH 8, 1mM $MgSO_4$, 4mM $CaCl_2$ and 100mM NaCl) and spotted on correspond
449 plate, which were incubated at 37°C for 24h.

450 **Phage and PICI induction**

451 For PICI and phage induction, overnight cultures of lysogenic donor strains in presence and
452 absence of the PICI were diluted 1:50 in fresh LB broth and grown at 37°C and 120 r.p.m. until
453 an OD₆₀₀ of 0.2 was reached. Mitomycin C (2 mg ml⁻¹) was added to induce the prophage and
454 the induced cultures were grown at 32°C with slow shaking (80 r.p.m.). Generally, cell lysis
455 occurred 4-5 h post-induction and the induced samples were filtered using sterile 0.2 µm filters
456 (Minisart® single use syringe filter unit, hydrophilic and non-pyrogenic, Sartorium Stedim
457 Biotech). The number of phage or PICI particles in the resultant lysate was quantified.

458 **Phage titration**

459 The number of phage particles were quantified using the titering assay. Briefly, a 1:50 dilution
460 (in fresh LB broth) of an overnight recipient strain was grown until an OD₆₀₀ of 0.34 was
461 reached. Then, recipient strain was infected using 100 µL of cells with the addition of 100 µL
462 of phage lysate serial dilutions prepared with phage buffer (50mM Tris pH 8, 1mM MgSO₄,
463 4mM CaCl₂ and 100mM NaCl), and incubated for 5 min at room temperature. The different
464 mixtures of culture-phage were plated out on phage base agar plates (PBA; 25 g of Nutrient
465 Broth No. 2, Oxoid; 7g agar) supplemented with CaCl₂ to a final concentration of 10mM. Plates
466 were incubated at 37°C for 24h and the number of plaques formed (phage particles present in
467 the lysate) were counted and represented as the plaque forming units (PFU/mL).

468 **PICI transduction**

469 The number of PICI particles were quantified using the transduction titering assay. Briefly, a
470 1:50 dilution (in fresh LB broth) of an overnight recipient strain was grown until an OD₆₀₀ of 1.4
471 was reached. Then, strains were infected using 1 mL of recipient cells with the addition of 100
472 µL of PICI lysate serial dilutions prepared with phage buffer (50mM Tris pH 8, 1mM MgSO₄,
473 4mM CaCl₂ and 100mM NaCl), and cultures were supplemented with CaCl₂ to a final
474 concentration of 4.4mM before incubation for 30 min at 37°C. The different mixtures of culture-
475 PICI were plated out on LBA plates containing the appropriate antibiotic. LBA plates were
476 incubated at 37°C for 24h and the number of colonies formed (transduction particles present
477 in the lysate) were counted and represented as the colony forming units (CFU/mL).

478 **Southern Blot**

479 Following phage and PICI induction (mitomycin C; Sigma-Aldrich from *Streptomyces*
480 *caespitosus*), one millilitre of each sample was taken at defined time points and pelleted.
481 Samples were frozen at -20°C until all collections were accomplished. Then, samples were re-
482 suspended in 50 µl lysis buffer (47.5 µl TES-Sucrose and 2.5 µl lysozyme [10 µg ml⁻¹]; Sigma-
483 Aldrich) and incubated at 37°C for 1h. Then, 55 µl of SDS 2% proteinase K buffer (47.25 µl
484 H₂O, 5.25 µl SDS 20%, 2.5 µl proteinase K [20 mg ml⁻¹], Sigma-Aldrich from *Tritirachium*
485 *album*) was added to the lysates and incubated at 55°C for 30 min. Then, samples were
486 vortexed with 10 µl of 10x loading dye for 1h. Following this incubation, samples were frozen
487 and thawed in cycles of 5 min incubation in dry ice with ethanol and in a water bath at 65°C.
488 This was repeated three times. To separate the chromosomal DNA, samples were run on 0.7%
489 agarose gel at 30V, overnight. Then, the DNA was transferred to Nylon membranes (Hybond-
490 N 0.45 mm pore size filters; Amersham Life Science) using standard methods. DNA was
491 detected using a DIG-labelled probe (Digoxigenin-11-dUTP alkali-labile; Roche) and anti-DIG
492 antibody (Anti-Digoxigenin-AP Fab fragments; Roche), before washing and visualisation. The
493 primers used to obtain the DIG-labelled probes are listed in Table S7.

494 **Capsid precipitation**

495 A large-scale induction was performed to precipitate phage and PICI particles and obtain their
496 dsDNA. Briefly, a total of 100 ml lysate was produced by MC induction. Then, lysates were
497 treated with RNase (1 µg ml⁻¹) and DNase (10 µg ml⁻¹) for 30 min at room temperature.
498 Afterwards, 1 M of NaCl was added to the lysate and incubated 1 h on ice. After incubation,
499 the mix was centrifuged at 11,000 x g for 10 min at 4°C, and phages/PICIs were mixed with
500 10% wt/vol polyethylene glycol (PEG) 8000 and kept overnight at 4°C. Then, phages/PICIs
501 were precipitated at 11,000 x g for 10 min at 4°C and the pellet was resuspended in 1 ml of
502 phage buffer.

503 **Phage and PICI DNA extraction**

504 To extract the dsDNA, lysate from phage and PICI precipitation was treated with DNase (10
505 $\mu\text{g ml}^{-1}$) for 30 min at room temperature. Then, lysate was combined with an equal volume of
506 lysis mix (2% SDS and 90 $\mu\text{g ml}^{-1}$ proteinaseK) and incubated at 55°C for 1 h. DNA was
507 extracted with an equal volume of phenol:chloroform:isoamyl alcohol 25:24:1 and samples
508 were centrifuged at 12,000 x g for 5 min, and the aqueous phase containing the DNA was
509 obtained. The DNA was precipitated by 0.3M NaOAc and 2.25 volume of 100% ethanol, then
510 pelleted at 12,000 x g for 30 min at 4°C and washed once with 1 ml of 70% ethanol. After
511 centrifugation, the DNA pellets were air dried for 30 min and resuspended in 100 μl nuclease
512 free water.

513 **Electron microscopy**

514 To analyse the lysate by transmission electron microscopy (TEM), lysates were CsCl purified.
515 Briefly, the precipitated phages and PICIs were loaded on the CsCl step gradients (1.35, 1.5
516 and 1.7 g ml^{-1} fractions) and centrifuged at 80,000 x g for 2h at 4°C. The phage and PICI bands
517 were extracted from the CsCl gradients using a 23-gauge needle and syringe. Then, phages
518 and PICIs were dialyzed overnight to remove CsCl excess using SnakeSkin™ Dialysis Tubing
519 (3.5K MWCO, 16mm dry) into 50mM of Tris pH 8 and 150mM NaCl buffer. For TEM analysis,
520 ten microliters of the dialysed samples were incubated in a carbon-coated gold grid for 5 min.
521 Then, samples were fixed with 1% Paraformaldehyde for 2 min, before washing three times
522 with distilled water for 30 sec. Afterwards, the samples were stained with 2% Uranyl Formate
523 for 30 sec, and they were allowed to dry at room temperature for 15 min. The JEOL 1200 TEM
524 microscope was used to examine the samples. Photos were obtained at 12K of magnification.

525 **Identification of PICIs and KEGG analysis**

526 The KEGG analysis performed previously (Fillol-Salom et al., 2018) suggested that the
527 EcCIEDL933 element is the prototypical member of a new PICI family. To identify more
528 members of this family, we searched manually on the NCBI database for elements with a size
529 comprised between 10-15 kb, encoding a divergent pair or transcriptional regulators next to
530 an *int* gene (Martínez-Rubio et al., 2017), and a packaging module similar to that present in

531 EcCIEDL933. Additionally, these elements should have unique attachment sites that are never
532 occupied by prophages, and they should lack phage lytic and tail genes. This search was
533 followed by the analysis of orthologues performed for the EcCIEDL933 element (Table S1),
534 which corroborates that the newly identified elements correspond to PICIs. The ortholog
535 analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG)
536 database (<http://www.genome.jp/kegg>; release July 12, 2018).

537 **Phylogenetic analyses**

538 We fetched the protein sequences of TerL and the Capsid from all cf-PICI in Fig. S2 and S6
539 (Table S2 and S4). For each protein we searched for homologs among a database of all (3725)
540 complete phages present in NCBI RefSeq (last accessed March 2021), using Diamond
541 v2.0.6.144 (Buchfink et al., 2015) with parameters *--query-cover 50 --ultra-sensitive --*
542 *forwardonly*. We picked hits in phages that match either a capsid or a TerL of any cf-PICI with
543 an e-value of at most $1e^{-10}$, and made a multiple alignment of these with the corresponding cf-
544 PICI proteins, using mafft-linsi v7.490 (default parameters)(Kato and Standley, 2013). The
545 multiple alignments of TerL and Capsid were then purged from non-informative sites using
546 clipkit v1.3.0 (Steenwyk et al., 2020) with the option *-m kpi-gappy*. We used the resulting
547 multiple alignments to make phylogenetic reconstructions using maximum likelihood with
548 iqtree v1.6.12 (Nguyen et al., 2015), using parameters *-nt 6 -bb 1000* (to quantify the
549 robustness of the topology) *-m TEST* (to find the best model). The trees were visualised with
550 iTol v6.5.2 (Letunic and Bork, 2021) and the phage branches were collapsed for clarity.

551 **Weighted Gene Repertoire Relatedness**

552 We searched for sequence similarity between all proteins of all phages using mmseqs2
553 (release 13-45111 (Steinegger and Söding, 2017)) with the sensitivity parameter set at 7.5.
554 The results were converted to the blast format and we kept for analysis the hits respecting the
555 following thresholds: e-value lower than 0.0001, at least 10% identity, and a coverage of at
556 least 50% of the proteins. The hits were used to retrieve the bi-directional best hits between

557 pairs of phages, which were used to compute a score of gene repertoire relatedness weighted
558 by sequence identity:

$$559 \quad wGRR = \frac{\sum_i^p id(A_i, B_i)}{\min(A, B)}$$

560

561 where A_i and B_i is the pair i of homologous proteins present in A and B , $id(A_i, B_i)$ is the
562 sequence identity of their alignment, and $\min(A, B)$ is the number of proteins of the genome
563 encoding the fewest proteins (A or B). $wGRR$ is the fraction of bi-directional best hits between
564 two elements weighted by the sequence identity of the homologs. It varies between zero (no
565 bi-directional best hits) and one (all genes of the smallest genome have an identical homolog
566 in the largest genome). $wGRR$ integrates information on the frequency of homologs and
567 sequence identity. For example, when the smallest genome has 10 proteins, a $wGRR$ of 0.2
568 can result from two homologs that are strictly identical or five that have 40% identity.

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578 **AUTHOR CONTRIBUTIONS**

579 JRP conceived the study; NA, LM-R and AF-S conducted the experiments; JMS and EPCR
580 performed the genomic analyses; NA, LM-R, JMS, JC, EPCR, AF-S and JRP analysed the
581 data. EPCR, AF-S and JRP wrote the manuscript.

582 **DECLARATION OF INTERESTS**

583 Authors declare no competing interests.

584 **References**

- 585 Agarwal, M., Arthur, M., Arbeit, R.D., and Goldstein, R. (1990). Regulation of icosahedral virion
586 capsid size by the in vivo activity of a cloned gene product. *Proceedings of the National*
587 *Academy of Sciences of the United States of America* 87, 2428–2432.
- 588 Barth, Z.K., Silvas, T.V., Angermeyer, A., and Seed, K.D. (2019). Genome replication
589 dynamics of a bacteriophage and its satellite reveal strategies for parasitism and viral
590 restriction. *Nucleic Acids Research* 48, 249-263.
- 591 Blank, K., Hensel, M., and Gerlach, R.G. (2011). Rapid and highly efficient method for scarless
592 mutagenesis within the *Salmonella enterica* chromosome. *PLoS One* 6, e15763.
- 593 Buchfink, B., Xie, C., and Huson, D.H. (2015). Fast and sensitive protein alignment using
594 DIAMOND. *Nat Methods* 12, 59–60.
- 595 Carpena, N., Manning, K.A., Dokland, T., Marina, A., and Penadés, J.R. (2016). Convergent
596 evolution of pathogenicity islands in helper cos phage interference. *Philosophical Transactions*
597 *of the Royal Society of London. Series B, Biological Sciences* 371, 20150505.
- 598 Chen, J., Ram, G., Penadés, J.R., Brown, S., and Novick, R.P. (2015). Pathogenicity island-
599 directed transfer of unlinked chromosomal virulence genes. *Molecular Cell* 57, 138–149.
- 600 Christie, G.E., and Dokland, T. (2012). Pirates of the Caudovirales. *Virology* 434, 210–221.
- 601 Dearborn, A.D., Wall, E.A., Kizziah, J.L., Klenow, L., Parker, L.K., Manning, K.A., Spilman,
602 M.S., Spear, J.M., Christie, G.E., and Dokland, T. (2017). Competing scaffolding proteins
603 determine capsid size during mobilization of *Staphylococcus aureus* pathogenicity islands.
604 *ELife* 6, e30822.
- 605 Fillol-Salom, A., Martínez-Rubio, R., Abdulrahman, R.F., Chen, J., Davies, R., and Penadés,
606 J.R. (2018). Phage-inducible chromosomal islands are ubiquitous within the bacterial universe.
607 *ISME J* 12, 2114–2128.
- 608 Fillol-Salom, A., Bacarizo, J., Alqasmi, M., Ciges-Tomas, J.R., Martínez-Rubio, R., Roszak,
609 A.W., Cogdell, R.J., Chen, J., Marina, A., and Penadés, J.R. (2019). Hijacking the Hijackers:
610 *Escherichia coli* Pathogenicity Islands Redirect Helper Phage Packaging for Their Own
611 Benefit. *Molecular Cell* 75, 1020-1030.e4.
- 612 Fillol-Salom, A., Miguel-Romero, L., Marina, A., Chen, J., and Penadés, J.R. (2020). Beyond
613 the CRISPR-Cas safeguard: PICI-encoded innate immune systems protect bacteria from
614 bacteriophage predation. *Current Opinion in Microbiology* 56, 52–58.
- 615 Frígols, B., Quiles-Puchalt, N., Mir-Sanchis, I., Donderis, J., Elena, S.F., Buckling, A., Novick,
616 R.P., Marina, A., and Penadés, J.R. (2015). Virus Satellites Drive Viral Evolution and Ecology.
617 *PLoS Genetics* 11, e1005609.
- 618 Hawkins, N.C., Kizziah, J.L., Penadés, J.R., and Dokland, T. (2021). Shape shifter: redirection
619 of prolate phage capsid assembly by staphylococcal pathogenicity islands. *Nat Commun* 12,
620 6408.
- 621 Hoffmann, S., Schmidt, C., Walter, S., Bender, J.K., and Gerlach, R.G. (2017). Scarless
622 deletion of up to seven methyl-accepting chemotaxis genes with an optimized method
623 highlights key function of CheM in *Salmonella Typhimurium*. *PLoS One* 12, e0172630.
- 624 Humphrey, S., Fillol-Salom, A., Quiles-Puchalt, N., Ibarra-Chávez, R., Haag, A.F., Chen, J.,
625 and Penadés, J.R. (2021). Bacterial chromosomal mobility via lateral transduction exceeds
626 that of classical mobile genetic elements. *Nat Commun* 12, 6509.

- 627 Ibarra-Chávez, R., Hansen, M.F., Pinilla-Redondo, R., Seed, K.D., and Trivedi, U. (2021).
628 Phage satellites and their emerging applications in biotechnology. *Fems Microbiol Rev* 45,
629 fuab031.
- 630 Kala, S., Cumby, N., Sadowski, P.D., Hyder, B.Z., Kanelis, V., Davidson, A.R., and Maxwell,
631 K.L. (2014). HNH proteins are a widespread component of phage DNA packaging machines.
632 *Proceedings of the National Academy of Sciences* 111, 6022–6027.
- 633 Katoh, K., and Standley, D.M. (2013). MAFFT Multiple Sequence Alignment Software Version
634 7: Improvements in Performance and Usability. *Mol Biol Evol* 30, 772–780.
- 635 Koonin, E.V., Makarova, K.S., Wolf, Y.I., and Krupovic, M. (2019). Evolutionary entanglement
636 of mobile genetic elements and host defence systems: guns for hire. *Nature Reviews. Genetics*
637 3, 546.
- 638 Letunic, I., and Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: an online tool for
639 phylogenetic tree display and annotation. *Nucleic Acids Res* 49, W293-W296.
- 640 Lindqvist, B.H., Dehò, G., and Calendar, R. (1993). Mechanisms of genome propagation and
641 helper exploitation by satellite phage P4. *Microbiological Reviews* 57, 683–702.
- 642 Martínez-Rubio, R., Quiles-Puchalt, N., Martí, M., Humphrey, S., Ram, G., Smyth, D., Chen,
643 J., Novick, R.P., and Penadés, J.R. (2017). Phage-inducible islands in the Gram-positive cocci.
644 *The ISME Journal* 11, 1029–1042.
- 645 Matos, R.C., Lapaque, N., Rigottier-Gois, L., Debarbieux, L., Meylheuc, T., Gonzalez-Zorn, B.,
646 Repoila, F., Lopes, M. de F., and Serror, P. (2013). *Enterococcus faecalis* prophage dynamics
647 and contributions to pathogenic traits. *PLoS Genetics* 9, e1003539.
- 648 Netter, Z., Boyd, C.M., Silvas, T.V., and Seed, K.D. (2021). A phage satellite tunes inducing
649 phage gene expression using a domesticated endonuclease to balance inhibition and virion
650 hijacking. *Nucleic Acids Res* 49, 4386–4401.
- 651 Nguyen, L.-T., Schmidt, H.A., Haeseler, A. von, and Minh, B.Q. (2015). IQ-TREE: A Fast and
652 Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol Biol Evol*
653 32, 268–274.
- 654 O’Hara, B.J., Barth, Z.K., McKitterick, A.C., and Seed, K.D. (2017). A highly specific phage
655 defense system is a conserved feature of the *Vibrio cholerae* mobilome. *PLoS Genetics* 13,
656 e1006838.
- 657 Penadés, J.R., and Christie, G.E. (2015). The Phage-Inducible Chromosomal Islands: A
658 Family of Highly Evolved Molecular Parasites. *Annual Review of Virology* 2, 181–201.
- 659 Perna, N.T., Plunkett, G., Burland, V., Mau, B., Glasner, J.D., Rose, D.J., Mayhew, G.F.,
660 Evans, P.S., Gregor, J., Kirkpatrick, H.A., et al. (2001). Genome sequence of
661 enterohaemorrhagic *Escherichia coli* O157:H7. *Nature* 409, 529–533.
- 662 Quiles-Puchalt, N., Carpena, N., Alonso, J.C., Novick, R.P., Marina, A., and Penadés, J.R.
663 (2014). Staphylococcal pathogenicity island DNA packaging system involving *cos*-site
664 packaging and phage-encoded HNH endonucleases. *Proceedings of the National Academy of*
665 *Sciences* 111, 6016–6021.
- 666 Ram, G., Chen, J., Kumar, K., Ross, H.F., Ubeda, C., Damle, P.K., Lane, K.D., Penadés, J.R.,
667 Christie, G.E., and Novick, R.P. (2012). Staphylococcal pathogenicity island interference with
668 helper phage reproduction is a paradigm of molecular parasitism. *Proceedings of the National*
669 *Academy of Sciences* 109, 16300–16305.
- 670 Ram, G., Chen, J., Ross, H.F., and Novick, R.P. (2014). Precisely modulated pathogenicity
671 island interference with late phage gene transcription. *Proceedings of the National Academy*
672 *of Sciences* 111, 14536–14541.
- 673 Solano, C., García, B., Latasa, C., Toledo-Arana, A., Zorraquino, V., Valle, J., Casals, J.,
674 Pedrosa, E., and Lasa, I. (2009). Genetic reductionist approach for dissecting individual roles

675 of GGDEF proteins within the c-di-GMP signaling network in *Salmonella*. Proceedings of the
676 National Academy of Sciences 106, 7997–8002.

677 Sousa, J.A.M. de, and Rocha, E.P.C. (2022). To catch a hijacker: abundance, evolution and
678 genetic diversity of P4-like bacteriophage satellites. Philosophical Transactions Royal Soc B
679 Biological Sci 377, 20200475.

680 Sousa, J.A.M. de, Pfeifer, E., Touchon, M., and Rocha, E.P.C. (2021). Causes and
681 Consequences of Bacteriophage Diversification via Genetic Exchanges across Lifestyles and
682 Bacterial Taxa. Mol Biol Evol 38, 2497–2512.

683 Steenwyk, J.L., Buida, T.J., Li, Y., Shen, X.-X., and Rokas, A. (2020). ClipKIT: A multiple
684 sequence alignment trimming software for accurate phylogenomic inference. Plos Biol 18,
685 e3001007.

686 Steinegger, M., and Söding, J. (2017). MMseqs2 enables sensitive protein sequence
687 searching for the analysis of massive data sets. Nat Biotechnol 35, 1026–1028.

688 Ubeda, C., Maiques, E., Tormo, M.A., Campoy, S., Lasa, I., Barbé, J., Novick, R.P., and
689 Penadés, J.R. (2007). SaPI operon I is required for SaPI packaging and is controlled by LexA.
690 Molecular Microbiology 65, 41–50.

691 Ziermann, R., and Calendar, R. (1990). Characterization of the cos sites of bacteriophages P2
692 and P4. Gene 96, 9–15.

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696 **Table 1. EcCIEDL933 transfer by different *E. coli* phages.**

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Donor strain	Phage titer ^a	PICl titer ^b
EDL933 1795-1796::cat	ND	30 ± 10
594 λ EcCIEDL933 1795-1796::cat	4.67E+07 ± 8.02E+06	<1
594 80 EcCIEDL933 1795-1796::cat	9.43E+08 ± 1.17E+08	<1
594 HK97 EcCIEDL933 1795-1796::cat	2.16E+10 ± 8.67E+09	4.85E+03 ± 1.35E+03
594 HK106 EcCIEDL933 1795-1796::cat	1.50E+10 ± 7.53E+09	4.37E+06 ± 6.66E+05
594 HK106 EcCIEDL933 1795-1796::cat alpA*	2.23E+10 ± 1.13E+10	<1
594 HK140 EcCIEDL933 1795-1796::cat	1.66E+08 ± 1.13E+08	<1
594 HK446 EcCIEDL933 1795-1796::cat	1.64E+10 ± 7.50E+09	3.64E+06 ± 1.74E+06
594 HK446 EcCIEDL933 1795-1796::cat alpA*	1.17E+10 ± 3.65E+09	<1
594 HK544 EcCIEDL933 1795-1796::cat	2.40E+10 ± 1.22E+10	5.47E+03 ± 4.05E+03

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699 ^aPhage or ^bPICl titre/ml of lysate, using *E. coli* 594 as recipient strain. The means and standard
700 deviations from three independent experiments are presented.

701 ND: not determined

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EcCIEDL933 gene	Function	Orthologs	Species	Full name	Length	Similarity	Overlap	Element	Start	End	Size	Comments
EDL933_1775		Not annotated										
EDL933_1776	icd-like	Not annotated										
EDL933_1777	icd-like	ece:Z1840	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	61	1		EcCIEDL933				
		ecq:ECED1_1790	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	61	1		PICI	1751707	1751892	16.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		ecs:ECs1578	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	61	1		PICI	1597550	1597735	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		elx:CDCO157_1511	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	61	1		PICI	1599492	1599677	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		eoh:ECO103_4531	<i>Escherichia coli</i>	<i>Escherichia coli</i> O103:H2 12009	61	0.627	59	PICI	4658268	4658453	15.7	Similar to <i>Escherichia coli</i> O55:H7 CB9615
		ecoo:ECRM13514_4639	<i>Escherichia coli</i>	<i>Escherichia coli</i> O145:H28 RM13514	61	0.593	59	PICI	4532419	4532604	11.9	
EDL933_1778		Not annotated										
EDL933_1779		ece:Z1841	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	86	1		EcCIEDL933				
		ecq:ECED1_1788	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	87	0.977	86	PICI	1751284	1751547	16.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		ecs:ECs1580	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	80	1	80	PICI	1597916	1598158	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		elx:CDCO157_1513	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	80	1	80	PICI	1599858	1600100	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		squ:E4343_10555	<i>Serratia quinivorans</i>	<i>Serratia quinivorans</i> strain PKL:12	90	0.37	54	PICI	2261903	2262175		
EDL933_1780		Not annotated										
EDL933_1781	pri	ece:Z1842	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	324	1		EcCIEDL933				
		elx:CDCO157_1515	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	710	1	319	PICI	1600402	1602534	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		ecq:ECED1_2648	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	718	0.932	323	PICI	2572885	2575041	16.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sbc:SbBS512_E0756	<i>Shigella boydii</i>	<i>Shigella boydii</i> CDC 3083-94	711	0.802	323	Defective PICI?	711690	713825		
		elo:EC042_2427	<i>Escherichia coli</i>	<i>Escherichia coli</i> O44:H18 042	712	0.709	326	Defective PICI?	2555145	2557283		NC_017626
		kvq:SP68_11475	<i>Klebsiella variicola</i>	<i>Klebsiella variicola</i> DSM 15968	709	0.561	326	PICI	675020	677149		
		ece:Z1843	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	386	1		EcCIEDL933				
		elx:CDCO157_1515	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	710	1	386	PICI	1600402	1602534	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		ecq:ECED1_2648	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	718	0.938	386	PICI	2572885	2575041	16.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		elo:EC042_2162	<i>Escherichia coli</i>	<i>Escherichia coli</i> O44:H18 042	688	0.927	386	Defective PICI?	2244313	2246379		NC_017626
		sbc:SbBS512_E0756	<i>Shigella boydii</i>	<i>Shigella boydii</i> CDC 3083-94	711	0.774	389	Defective PICI?	711690	713825		Only an int fragment and alpA
		kpr:KPR_1457	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i> SB3432	926	0.689	386	Defective PICI?	1525479	1528259		NC_021232
		pge:LG71_03480	<i>Pluralibacter gergoviae</i>	<i>Pluralibacter gergoviae</i> strain FB2	925	0.668	386	PICI?	721490	724267	19.9	CP009450
		kvq:B8P98_23010	<i>Klebsiella quasivariicola</i>	<i>Klebsiella quasivariicola</i> KPN1705	709	0.701	384	PICI?	4553066	4555195		CP022823
		ecls:LI67_021875	<i>Enterobacter roggenkampii</i>	<i>Enterobacter roggenkampii</i> 35734	711	0.694	385	PICI	4452924	4455059	16.9	
		kvq:SP68_11475	<i>Klebsiella variicola</i>	<i>Klebsiella variicola</i> DSM 15968	709	0.69	384	PICI	675020	677149		CP010523
		rtg:NCTC13098_01103	<i>Raoultella terrigena</i>	<i>Raoultella terrigena</i> NCTC13098	887	0.687	386	PICI?	902863	905526		LR131271
		elo:EC042_1762	<i>Escherichia coli</i>	<i>Escherichia coli</i> O44:H18 042	155	0.855	76	PICI	1832521	1843545	11	

EcCIEDL933 gene	Function	Orthologs	Species	Full name	Length	Similarity	Overlap	Element	Start	End	Size	Comments
		eoj:ECO26_1649	<i>Escherichia coli</i>	<i>Escherichia coli</i> O26:H11 11368	154	0.855	76	PICI	1619447	1630554	11.1	Same as <i>Escherichia coli</i> O157:H7 EC4115
		etw:ECSP_1494	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 TW14359	154	0.855	76	PICI	1535786	1547005	11.2	Same as <i>Escherichia coli</i> O157:H7 EC4115
		eoh:ECO103_1239	<i>Escherichia coli</i>	<i>Escherichia coli</i> O103:H2 12009	154	0.842	76	PICI	1325875	1336965	11.1	Similar to <i>Escherichia coli</i> O157:H7 EC4115
		eoi:ECO111_2917	<i>Escherichia coli</i>	<i>Escherichia coli</i> O111:H- 11128	77	0.849	73	PICI	2855234	2864550	9.3	
		eok:G2583_1393	<i>Escherichia coli</i>	<i>Escherichia coli</i> O55:H7 CB9615	77	0.849	73	PICI	1444572	1455794	11.2	Same as <i>Escherichia coli</i> O157:H7 EC4115
		sfe:SFxv_1288	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2002017	66	0.981	54	PICI?	1232114	1246489	14.4	HNH; terL
EDL933_1782		ece:Z1844	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	83	1		EcCIEDL933				
		ecs:ECs1586	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	83	1		PICI	1600965	1601216	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		elx:CDCO157_1519	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	83	1		PICI	1602905	1603156	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		ecf:ECH74115_1578	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EC4115	83	0.915	82	PICI	1541251	1541502	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615
		ecoh:ECRM13516_1447	<i>Escherichia coli</i>	<i>Escherichia coli</i> O145:H28 RM13516	83	0.915	82	PICI	1444527	1444778	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615
		ecoo:ECRM13514_1491	<i>Escherichia coli</i>	<i>Escherichia coli</i> O145:H28 RM13514	83	0.915	82	PICI	1467997	1468248	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615
		elo:EC042_1758	<i>Escherichia coli</i>	<i>Escherichia coli</i> O44:H18 042	83	0.915	82	PICI	1837472	1837723	11	EcCIO42
		elr:ECO55CA74_06815	<i>Escherichia coli</i>	<i>Escherichia coli</i> O55:H7 RM12579	83	0.915	82	PICI	1443909	1444160	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615
		eoh:ECO103_1243	<i>Escherichia coli</i>	<i>Escherichia coli</i> O103:H2 12009	83	0.915	82	PICI	1331498	1331749	11.1	Similar to <i>Escherichia coli</i> O55:H7 CB9615
		eoj:ECO26_1653	<i>Escherichia coli</i>	<i>Escherichia coli</i> O26:H11 11368	83	0.915	82	PICI	1625080	1625331	11.1	Similar to <i>Escherichia coli</i> O55:H7 CB9615
		eok:G2583_1397	<i>Escherichia coli</i>	<i>Escherichia coli</i> O55:H7 CB9615	83	0.915	82	PICI	1450327	1450578	11.2	Similar to EcCICF073
		etw:ECSP_1498	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 TW14359	83	0.915	82	PICI	1541539	1541790	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615
		sbo:SBO_2129	<i>Shigella boydii</i>	<i>Shigella boydii</i> Sb227	93	0.915	82	PICI	2102851	2103132	10.7	
		sfe:SFxv_1293	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2002017	92	0.889	81	PICI	1238098	1238376	14.4	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfl:SF1136	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 301	92	0.889	81	PICI	1180747	1181025	14.7	Similar to <i>Shigella flexneri</i> 2002017
		sft:NCTC1_01192	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> NCTC1	92	0.889	81	PICI	1139191	1139469	14.7	Similar to <i>Shigella flexneri</i> 2002017
		eoi:ECO111_2922	<i>Escherichia coli</i>	<i>Escherichia coli</i> O111:H- 11128	81	0.864		PICI	2861820	2862065	9.3	
		ecq:ECED1_1785	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	92	0.795	78	PICI	1748669	1748947	16.3	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		elci:i14_0251	<i>Escherichia coli</i>	<i>Escherichia coli</i> clone D i14	66	0.909		PICI	254958	255158	10.6	
		eld:i02_0251	<i>Escherichia coli</i>	<i>Escherichia coli</i> clone D i2	66	0.909		PICI	254958	255158	10.6	Same as <i>Escherichia coli</i> clone D i14
		sfx:S1216	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2457T	66	0.909		PICI	1183718	1183918	14.7	Similar to <i>Shigella flexneri</i> 2002017
		ect:ECIAI39_2019	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 IA139	85	0.843	70	PICI	2068323	2068580	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		eoc:CE10_1218	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 CE10	85	0.843	70	PICI	1266375	1266632	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		ecc:c1496	<i>Escherichia coli</i>	<i>Escherichia coli</i> O6:K2:H1 CFT073	85	0.829	70	PICI	1384284	1384541	11.2	
		ecoi:ECOPMV1_00891	<i>Escherichia coli</i>	<i>Escherichia coli</i> PMV-1	85	0.829	70	PICI	925423	925680	10.5	
		eih:ECOK1_0890	<i>Escherichia coli</i>	<i>Escherichia coli</i> O18:K1:H7 IHE3034	85	0.829	70	PICI	925810	926067	10.5	Same as <i>Escherichia coli</i> PMV-1
		elut:UM146_13205	<i>Escherichia coli</i>	<i>Escherichia coli</i> UM146	85	0.829	70	PICI	2726191	2726448	10.5	Same as <i>Escherichia coli</i> PMV-1

EcCIEDL933 gene	Function	Orthologs	Species	Full name	Length	Similarity	Overlap	Element	Start	End	Size	Comments	
EDL933_1783	ssb	ssn:SSON_0875	<i>Shigella sonnei</i>	<i>Shigella sonnei</i> Ss046	81	0.74	77	PICI	930165	930410	14.3		
		ecm:EcSMS35_0947	<i>Escherichia coli</i>	<i>Escherichia coli</i> SMS-3-5	81	0.727	77	PICI	961999	962244	15.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933	
		sfn:SFy_1637	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2003036	59	0.915		PICI	1204708	1204887	14.4	Same as <i>Shigella flexneri</i> 2002017	
		sfs:SFyv_1689	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> Shi06HN006	59	0.915		PICI	1223396	1223575	14.4	Same as <i>Shigella flexneri</i> 2002017	
		eln:NRG857_05750	<i>Escherichia coli</i>	<i>Escherichia coli</i> O83:H1 NRG 857C	81	0.688	77	Prophage?	1205337	1205582		Chimera prophage & PICI	
		ece:Z1845	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	136	1		EcCIEDL933					
		ecs:ECs1587	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	136	1		PICI	1601213	1601623	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933	
		elx:CDCO157_1520	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	136	1		PICI	1603153	1603563	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933	
		ecc:c1497	<i>Escherichia coli</i>	<i>Escherichia coli</i> O6:K2:H1 CFT073	136	0.985		PICI	1384538	1384948	11.2		
		ecq:ECED1_1784	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	136	0.985		PICI	1748262	1748672	16.3	Similar to <i>Escherichia coli</i> O157:H7 EDL933	
		eoj:ECO26_2319	<i>Escherichia coli</i>	<i>Escherichia coli</i> O26:H11 11368	136	0.985		PICI	2247460	2247870	14.9	Similar to <i>Escherichia coli</i> O157:H7 EDL933	
		ecoh:ECRM13516_1448	<i>Escherichia coli</i>	<i>Escherichia coli</i> O145:H28 RM13516	136	0.978		PICI	1444775	1445185	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615	
		ecoo:ECRM13514_1492	<i>Escherichia coli</i>	<i>Escherichia coli</i> O145:H28 RM13514	136	0.978		PICI	1468245	1468655	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615	
		elo:EC042_1757	<i>Escherichia coli</i>	<i>Escherichia coli</i> O44:H18 042	136	0.978		PICI	1837065	1837475	11		
		elr:ECO55CA74_06820	<i>Escherichia coli</i>	<i>Escherichia coli</i> O55:H7 RM12579	136	0.978		PICI	1444157	1444567	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615	
		eoh:ECO103_1244	<i>Escherichia coli</i>	<i>Escherichia coli</i> O103:H2 12009	136	0.978		PICI	1331746	1332156	11.1	Similar to <i>Escherichia coli</i> O55:H7 CB9615	
		eok:G2583_1398	<i>Escherichia coli</i>	<i>Escherichia coli</i> O55:H7 CB9615	136	0.978		PICI	1450575	1450985	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615	
		sbo:SBO_2128	<i>Shigella boydii</i>	<i>Shigella boydii</i> Sb227	136	0.978		PICI	2102444	2102854	10.7		
		ecf:ECH74115_1579	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EC4115	136	0.971		PICI	1541499	1541909	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615	
		etw:ECSP_1499	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 TW14359	136	0.971		PICI	1541787	1542197	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615	
		ect:ECIAI39_2018	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 IAI39	136	0.824		PICI	2067916	2068326	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933	
		eoc:CE10_1219	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 CE10	136	0.824		PICI	1266629	1267039	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933	
		eln:NRG857_05755	<i>Escherichia coli</i>	<i>Escherichia coli</i> O83:H1 NRG 857C	149	0.685		Prophage?	1205579	1206028		Chimera prophage & PICI	
		ecoi:ECOPMV1_00892	<i>Escherichia coli</i>	<i>Escherichia coli</i> PMV-1	140	0.721		PICI	925677	926099	10.5		
eih:ECOK1_0891	<i>Escherichia coli</i>	<i>Escherichia coli</i> O18:K1:H7 IHE3034	140	0.721		PICI	926064	926486	10.5	Same as <i>Escherichia coli</i> PMV-1			
elci:i14_0252	<i>Escherichia coli</i>	<i>Escherichia coli</i> clone D i14	140	0.721		PICI	255155	255577	10.6				
EDL933_1784		Not annotated											
EDL933_1785	ece:Z1846	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	100	1		EcCIEDL933						
	ecs:ECs1589	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	100	1		PICI	1601953	1602255	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933		
	elx:CDCO157_1522	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	100	1		PICI	1603893	1604195	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933		
	ecq:ECED1_1781	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	100	0.91		PICI	1747637	1747939	16.3	Similar to <i>Escherichia coli</i> O157:H7 EDL933		
	sft:SF1137	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 301	100	0.91		PICI	1181562	1181864	14.7	Similar to <i>Shigella flexneri</i> 2002017		
	sfv:SFV_1153	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 8401	100	0.9		PICI	1199642	1199944	15.5	Similar to <i>Shigella flexneri</i> 2002017		

EcCIEDL933 gene	Function	Orthologs	Species	Full name	Length	Similarity	Overlap	Element	Start	End	Size	Comments
		eoj:ECO26_2317	<i>Escherichia coli</i>	<i>Escherichia coli</i> O26:H11 11368	74	1		PICI	2246827	2247051	14.9	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		ecoh:ECRM13516_1451	<i>Escherichia coli</i>	<i>Escherichia coli</i> O145:H28 RM13516	74	0.986		PICI	1445595	1445819	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615
		ecoo:ECRM13514_1495	<i>Escherichia coli</i>	<i>Escherichia coli</i> O145:H28 RM13514	74	0.986		PICI	1469065	1469289	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615
		elo:EC042_1755	<i>Escherichia coli</i>	<i>Escherichia coli</i> O44:H18 042	74	0.986		PICI	1836431	1836655	11	
		elr:ECO55CA74_06830	<i>Escherichia coli</i>	<i>Escherichia coli</i> O55:H7 RM12579	74	0.986		PICI	1444977	1445201	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615
		eoh:ECO103_1246	<i>Escherichia coli</i>	<i>Escherichia coli</i> O103:H2 12009	74	0.986		PICI	1332566	1332790	11.1	Similar to <i>Escherichia coli</i> O55:H7 CB9615
		etw:ECSP_1501	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 TW14359	74	0.986		PICI	1542607	1542831	11.2	Same as <i>Escherichia coli</i> O55:H7 CB9615
		sbo:SBO_2127	<i>Shigella boydii</i>	<i>Shigella boydii</i> Sb227	74	0.986		PICI	2101810	2102034	10.7	
		sft:NCTC1_01195	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> NCTC1	74	0.946		PICI	1140084	1140308	14.7	Similar to <i>Shigella flexneri</i> 2002017
		sfx:S1219	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2457T	74	0.946		PICI	1184533	1184757	14.7	Similar to <i>Shigella flexneri</i> 2002017
		eas:Entas_3970	<i>Enterobacter soli</i>	<i>Enterobacter soli</i> LF7a	86	0.419	74	PICI	4262187	4262447		
		ecm:EcSMS35_0949	<i>Escherichia coli</i>	<i>Escherichia coli</i> SMS-3-5	80	0.444	72	PICI	962689	962931	15.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
EDL933_1786	capsid	ece:Z1847	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	385	1		EcCIEDL933				
		ecs:ECs1590	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	385	1		PICI	1602548	1603705	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		elx:CDCO157_1523	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	385	1		PICI	1604488	1605645	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		eoj:ECO26_2316	<i>Escherichia coli</i>	<i>Escherichia coli</i> O26:H11 11368	385	1		PICI	2245378	2246535	14.9	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		ecq:ECED1_1780	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	401	0.995	385	PICI	1746179	1747384	16.3	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		eoc:CE10_1221	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 CE10	385	0.992		PICI	1267609	1268766	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		pcol:F1325_15335	<i>Proteus columbae</i>	<i>Proteus columbae</i> T60	384	0.695	383	Defective PICI?	3267873	3269027		Only int and packaging genes
		ect:ECIAI39_2017	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 IA139	390	0.696	388	PICI	2066174	2067346	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		xne:XNC1_4107	<i>Xenorhabdus nematophila</i>	<i>Xenorhabdus nematophila</i> ATCC 19061	383	0.681	382	PICI	3980210	3981361		NC_014228
		xnm:XNC2_3961	<i>Xenorhabdus nematophila</i>	<i>Xenorhabdus nematophila</i> AN6/1	383	0.681	382	PICI	3979724	3980875		Similar to <i>Xenorhabdus nematophila</i> ATCC 19061
		xpo:XPG1_1127	<i>Xenorhabdus poinarii</i>	<i>Xenorhabdus poinarii</i> G6	382	0.683		PICI	1092722	1093870		
		sfn:SFv_1641	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2003036	275	0.989		PICI	1205664	1206491	14.4	Same as <i>Shigella flexneri</i> 2002017
		sfs:SFv_1693	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> Shi06HN006	275	0.989		PICI	1224352	1225179	14.4	Same as <i>Shigella flexneri</i> 2002017
		sft:NCTC1_01196	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> NCTC1	275	0.989		PICI	1140609	1141436	14.7	Similar to <i>Shigella flexneri</i> 2002017
		xdo:XDD1_1687	<i>Xenorhabdus doucetiae</i>	<i>Xenorhabdus doucetiae</i> FRM16	382	0.681		PICI	1687884	1689032		
		xbv:XBW1_2702	<i>Xenorhabdus bovienii</i>	<i>Xenorhabdus bovienii</i> CS03	384	0.674	383	PICI	2582109	2583263		FO818637
		ans:ArsFIN_22310	<i>Arsenophonus nasoniae</i>	<i>Arsenophonus nasoniae</i> FIN	384	0.674	383	Prophage?	2081325	2082479		CP038613 / Chimera prophage & PICI
		eas:Entas_3967	<i>Enterobacter soli</i>	<i>Enterobacter soli</i> LF7a	388	0.651	387	PICI	4259413	4260579		CP003026
		ebc:C2U52_10910	<i>Enterobacteriaceae bacterium</i>	<i>Enterobacteriaceae bacterium</i> ENNIH2	388	0.651	387	PICI	1834181	1835347	15.9	CP026192.1
		cfq:C2U38_23290	<i>Citrobacter freundii</i>	<i>Citrobacter freundii</i> complex sp. CFNIH3	388	0.652		PICI	4637771	4638937	16.4	CP026235
		ecls:L167_021860	<i>Enterobacter roggenkampii</i>	<i>Enterobacter roggenkampii</i> 35734	388	0.646	387	PICI	4450286	4451452	16.9	

EcCIEDL933 gene	Function	Orthologs	Species	Full name	Length	Similarity	Overlap	Element	Start	End	Size	Comments
EDL933_1787	protease	ece:Z1848	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	190	1		EcCIEDL933				
		ecs:ECs1591	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	190	1		PICI	1603745	1604317	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		elx:CDCO157_1524	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	190	1		PICI	1605685	1606257	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		eoc:CE10_1222	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 CE10	190	0.995		PICI	1268806	1269378	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		ecq:ECED1_1779	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	190	0.979		PICI	1745567	1746139	16.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfe:SFxv_1299	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2002017	190	0.979		PICI	1240350	1240922	14.4	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfi:SF1139	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 301	190	0.979		PICI	1183362	1183934	14.7	Similar to <i>Shigella flexneri</i> 2002017
		sfn:SFy_1643	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2003036	190	0.979		PICI	1206861	1207433	14.4	Same as <i>Shigella flexneri</i> 2002017
		sfs:SFyv_1695	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> Shi06HN006	190	0.979		PICI	1225549	1226121	14.4	Same as <i>Shigella flexneri</i> 2002017
		sft:NCTC1_01198	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> NCTC1	190	0.979		PICI	1141806	1142378	14.7	Similar to <i>Shigella flexneri</i> 2002017
		sfx:S1222	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2457T	190	0.979		PICI	1186255	1186827	14.7	Similar to <i>Shigella flexneri</i> 2002017
		ect:ECIAI39_2016	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 IA139	191	0.953		PICI	2065563	2066138	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfv:SFV_1157	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 8401	185	0.978		PICI	1202234	1202791	15.5	Same as <i>Shigella flexneri</i> 2002017
		eoj:ECO26_2315	<i>Escherichia coli</i>	<i>Escherichia coli</i> O26:H11 11368	185	0.973		PICI	2244766	2245323	14.9	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		mety:MRY16398_4673_0	<i>Metakosakonia sp.</i>	<i>Metakosakonia sp.</i> MRY16-398	186	0.649	188	PICI?	4874313	4874873		AP018756
		kpa:KPNJ1_00479	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 30660/NJST258_1	191	0.637	193	PICI	473286	473861	16	CP006923
		kps:KPNJ2_00518	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 30684/NJST258_2	191	0.637	193	PICI	518333	518908	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpc:KPNIH10_23935	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae subsp. pneumoniae</i> KPNIH10	186	0.649	188	PICI	4884677	4885237	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpg:KPNIH32_25110	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae subsp. pneumoniae</i> KPNIH32	186	0.649	188	PICI	5008344	5008904	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kph:KPNIH24_04865	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae subsp. pneumoniae</i> KPNIH24	186	0.649	188	PICI	1025767	1026327	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
kpi:N559_0490	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> JM45	186	0.649	188	PICI	510375	510935	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1		
EDL933_1788	portal	ece:Z1849	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	403	1		EcCIEDL933				
		ecs:ECs1592	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	403	0.998		PICI	1604319	1605530	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		elx:CDCO157_1525	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	403	0.998		PICI	1606259	1607470	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		ecq:ECED1_1778	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	403	0.993		PICI	1744354	1745565	16.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		eoj:ECO26_2314	<i>Escherichia coli</i>	<i>Escherichia coli</i> O26:H11 11368	403	0.993		PICI	2243553	2244764	14.9	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		eoc:CE10_1223	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 CE10	403	0.99		PICI	1269380	1270591	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfv:SFV_1158	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 8401	403	0.973		PICI	1202793	1204004	15.5	Same as <i>Shigella flexneri</i> 2002017
		sfe:SFxv_1300	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2002017	403	0.975		PICI	1240924	1242135	14.4	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfn:SFy_1644	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2003036	403	0.975		PICI	1207435	1208646	14.4	Same as <i>Shigella flexneri</i> 2002017
		sfs:SFyv_1696	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> Shi06HN006	403	0.975		PICI	1226123	1227334	14.4	Same as <i>Shigella flexneri</i> 2002017
		sfi:SF1140	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 301	403	0.973		PICI	1183936	1185147	14.7	Similar to <i>Shigella flexneri</i> 2002017
		ect:ECIAI39_2015	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 IA139	404	0.965	401	PICI	2064347	2065561	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933

EcCIEDL933 gene	Function	Orthologs	Species	Full name	Length	Similarity	Overlap	Element	Start	End	Size	Comments
		sft:NCTC1_01199	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> NCTC1	391	0.972		PICI	1142416	1143591	14.7	Similar to <i>Shigella flexneri</i> 2002017
		sfx:S1223	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2457T	375	0.973		PICI	1186865	1187992	14.7	Similar to <i>Shigella flexneri</i> 2002017
		kpa:KPNJ1_00480	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 30660/NJST258_1	413	0.803	402	PICI	473863	475104	16	CP006923
		kpc:KPNIH10_23930	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH10	413	0.803	402	PICI	4883434	4884675	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpg:KPNIH32_25105	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH32	413	0.803	402	PICI	5007101	5008342	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kph:KPNIH24_04870	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH24	413	0.803	402	PICI	1026329	1027570	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpi:N559_0491	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> JM45	413	0.803	402	PICI	510937	512178	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpm:KPHS_48100	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> HS11286	413	0.803	402	PICI	4822860	4824101	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpne:KU54_002360	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 32192	413	0.803	402	PICI	474960	476201	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
EDL933_1789	Head-tail connector	Not annotated										
EDL933_1790	Head-tail connector	ece:Z1851	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	98	1		EcCIEDL933				
		ecs:ECs1594	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	98	1		PICI	1605862	1606158	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		elx:CDCO157_1527	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	98	1		PICI	1607802	1608098	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		eoi:ECO26_2312	<i>Escherichia coli</i>	<i>Escherichia coli</i> O26:H11 11368	98	1		PICI	2242925	2243221	14.9	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		eoc:CE10_1225	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 CE10	98	0.99		PICI	1270923	1271219	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		ecq:ECED1_1777	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	98	0.99		PICI	1743726	1744022	16.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfv:SFV_1159	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 8401	98	0.98		PICI	1204336	1204632	15.5	Same as <i>Shigella flexneri</i> 2002017
		sfe:SFxv_1302	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2002017	98	0.969		PICI	1242467	1242763	14.4	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfi:SF1142	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 301	98	0.969		PICI	1185479	1185775	14.7	Similar to <i>Shigella flexneri</i> 2002017
		sft:NCTC1_01201	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> NCTC1	98	0.969		PICI	1143923	1144219	14.7	Similar to <i>Shigella flexneri</i> 2002017
		sfx:S1225	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2457T	98	0.969		PICI	1188372	1188668	14.7	Similar to <i>Shigella flexneri</i> 2002017
		ect:ECIAI39_2013	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 IAI39	98	0.745		PICI	2063719	2064015	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		cfq:C2U38_23265	<i>Citrobacter freundii</i>	<i>Citrobacter freundii</i> complex sp. CFNIH3	97	0.653		PICI	4634747	4635040	16.4	
		eclz:LI64_18485	<i>Enterobacter hormaechei</i>	<i>Enterobacter hormaechei</i> subsp. <i>Hormaechei</i> 34983	97	0.642	95	PICI	3896093	3896386		
		eas:Entas_3963	<i>Enterobacter soli</i>	<i>Enterobacter soli</i> LF7a	97	0.642	95	PICI	4256943	4257236		CP003026
		metv:MRY16398_4670_0	<i>Metakosakonia</i> sp. MRY16-398	<i>Metakosakonia</i> sp. MRY16-398	96	0.633	98	PICI?	4872457	4872747		AP018756
		pcol:F1325_15320	<i>Proteus columbae</i>	<i>Proteus columbae</i> T60	98	0.602		Defective PICI?	3265752	3266048		Only int and packaging genes
		ebc:C2U52_10930	<i>Enterobacteriaceae</i> bacterium	<i>Enterobacteriaceae</i> bacterium ENNIH2	96	0.633	98	PICI	1837519	1837809	15.9	CP026192.1
		yak:ACZ76_06980	<i>Yersinia aleksiciae</i>	<i>Yersinia aleksiciae</i> 159	100	0.61		PICI	1517225	1517527	15.9	
		yel:LC20_00574	<i>Yersinia enterocolitica</i>	<i>Yersinia enterocolitica</i> LC20	94	0.596		PICI	513710	513994	15.9	
EDL933_1791	HNH	ece:Z1852	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	129	1		EcCIEDL933				
		ecs:ECs1595	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	146	0.992	129	PICI	1606158	1606598	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933

EcCIEDL933 gene	Function	Orthologs	Species	Full name	Length	Similarity	Overlap	Element	Start	End	Size	Comments
		ect:ECIAI39_2012	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 IAI39	146	0.992	129	PICI	2063279	2063719	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		elx:CDCO157_1528	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	146	0.992	129	PICI	1608098	1608538	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		ecq:ECED1_1775	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	145	0.992	128	PICI	1743289	1743726	16.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfi:SF1143	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 301	146	0.984	129	PICI	1185775	1186215	14.7	Similar to <i>Shigella flexneri</i> 2002017
		sft:NCTC1_01202	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> NCTC1	146	0.984	129	PICI	1144219	1144659	14.7	Similar to <i>Shigella flexneri</i> 2002017
		elo:EC042_2153	<i>Escherichia coli</i>	<i>Escherichia coli</i> O44:H18 042	129	0.829		Defective PICI?	2238523	2238912		
		ecm:EcSMS35_0955	<i>Escherichia coli</i>	<i>Escherichia coli</i> SMS-3-5	146	0.822	129	PICI	967055	967495	15.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		eas:Entas_3962	<i>Enterobacter soli</i>	<i>Enterobacter soli</i> LF7a	147	0.727	128	PICI	4256500	4256943		CP003026
		ecls:LI67_021835	<i>Enterobacter roggenkampii</i>	<i>Enterobacter roggenkampii</i> 35734	147	0.732	127	PICI	4447351	4447794	16.9	
		eclz:LI64_04020	<i>Enterobacter hormaechei</i>	<i>Enterobacter hormaechei</i> subsp. <i>Hormaechei</i> 34983	147	0.719	128	PICI	843681	844124		
		kpa:KPNJ1_00483	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 30660/NJST258_1	147	0.727	128	PICI	475732	476175	16	CP006923
		kpc:KPNIH10_23915	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH10	147	0.727	128	PICI	4882363	4882806	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpg:KPNIH32_25090	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH32	147	0.727	128	PICI	5006030	5006473	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kph:KPNIH24_04885	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH24	147	0.727	128	PICI	1028198	1028641	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpn:KU54_002375	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 32192	147	0.727	128	PICI	476829	477272	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpn:LI86_02380	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 34618	147	0.727	128	PICI	475585	476028	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpg:KPR0928_23435	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPR0928	147	0.727	128	PICI	4797604	4798047	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kps:KPNJ2_00522	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 30660/NJST258_2	147	0.727	128	PICI	520779	521222	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpw:KPNIH30_24210	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH30	147	0.727	128	PICI	4793717	4794160	17.2	Similar to <i>Klebsiella pneumoniae</i> 30660/NJST258_1
EDL933_1792		Not annotated										
EDL933_1793	<i>terS</i>	ece:Z1853	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	118	1		EcCIEDL933				
		ecq:ECED1_1774	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	118	1		PICI	1742641	1742997	16.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		ecs:ECs1597	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	118	1		PICI	1606888	1607244	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		ect:ECIAI39_2011	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 IAI39	118	1		PICI	2062634	2062990	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		elx:CDCO157_1530	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	118	1		PICI	1608828	1609184	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		eoi:ECO26_2309	<i>Escherichia coli</i>	<i>Escherichia coli</i> O26:H11 11368	118	1		PICI	2241840	2242196	14.9	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		ecm:EcSMS35_0956	<i>Escherichia coli</i>	<i>Escherichia coli</i> SMS-3-5	127	0.889	117	PICI	967757	968140	15.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		elo:EC042_2152	<i>Escherichia coli</i>	<i>Escherichia coli</i> O44:H18 042	165	0.889	117	Defective PICI?	2237878	2238375		
		cfg:C2U38_23250	<i>Citrobacter freundii</i>	<i>Citrobacter freundii</i> complex sp. CFNIH3	118	0.831		PICI	4633674	4634030	16.4	
		ecls:LI67_021830	<i>Enterobacter roggenkampii</i>	<i>Enterobacter roggenkampii</i> 35734	118	0.831		PICI	4446721	4447077	16.9	
		eas:Entas_3959	<i>Enterobacter soli</i>	<i>Enterobacter soli</i> LF7a	118	0.822		PICI	4255868	4256224		CP003026
		ebc:C2U52_10945	<i>Enterobacteriaceae</i> bacterium	<i>Enterobacteriaceae</i> bacterium ENNIH2	118	0.822		PICI	1838528	1838884	15.9	CP026192.1
		eclz:LI64_04025	<i>Enterobacter hormaechei</i>	<i>Enterobacter hormaechei</i> subsp. <i>Hormaechei</i> 34983	118	0.822		PICI	844400	844756		

EcCIEDL933 gene	Function	Orthologs	Species	Full name	Length	Similarity	Overlap	Element	Start	End	Size	Comments
		kpa:KPNJ1_00484	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 30660/NJST258_1	176	0.822	118	PICI	476277	476807	16	CP006923
		kpc:KPNIH10_23905	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH10	118	0.822		PICI	4881731	4882087	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpg:KPNIH32_25080	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH32	118	0.822		PICI	5005398	5005754	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kph:KPNIH24_04895	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH24	118	0.822		PICI	1028917	1029273	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpm:KPHS_48050	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> HS11286	118	0.822		PICI	4821157	4821513	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpne:KU54_002385	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 32192	118	0.822		PICI	477548	477904	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpnu:L186_02390	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 34618	118	0.822		PICI	476304	476660	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpg:KPR0928_23425	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPR0928	118	0.822		PICI	4796972	4797328	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
EDL933_1794	terL	ece:Z1854	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 EDL933	509	1		EcCIEDL933				
		elx:CDCO157_1531	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Xuzhou21	553	0.998	509	PICI	1609168	1610829	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		ecs:ECs1598	<i>Escherichia coli</i>	<i>Escherichia coli</i> O157:H7 Sakai	553	0.996	509	PICI	1607228	1608889	15.4	Same as <i>Escherichia coli</i> O157:H7 EDL933
		ecq:ECED1_1773	<i>Escherichia coli</i>	<i>Escherichia coli</i> O81 ED1a	553	0.984	509	PICI	1740996	1742657	16.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfv:SFV_1161	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 8401	553	0.986	509	PICI	1205604	1207265	15.5	Same as <i>Shigella flexneri</i> 2002017
		sfe:SFxv_1306	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2002017	553	0.984	509	PICI	1243735	1245396	14.4	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sft:NCTC1_01204	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> NCTC1	553	0.984	509	PICI	1145191	1146852	14.7	Similar to <i>Shigella flexneri</i> 2002017
		ect:ECIAI39_2010	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 IAI39	553	0.978	509	PICI	2060989	2062650	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		elo:EC042_2151	<i>Escherichia coli</i>	<i>Escherichia coli</i> O44:H18 042	553	0.943	509	Defective PICI?	2236233	2237894		
		eoj:ECO26_2308	<i>Escherichia coli</i>	<i>Escherichia coli</i> O26:H11 11368	492	0.984		PICI	2240195	2241673	14.9	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		ecm:EcSMS35_0957	<i>Escherichia coli</i>	<i>Escherichia coli</i> SMS-3-5	553	0.943	509	PICI	968124	969785	15.2	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		sfi:SF1146	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 301	492	0.986		PICI	1186930	1188408	14.7	Similar to <i>Shigella flexneri</i> 2002017
		sfn:SFy_1652	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2003036	492	0.986		PICI	1210429	1211907	14.4	Same as <i>Shigella flexneri</i> 2002017
		sfs:SFyv_1704	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> Shi06HN006	492	0.986		PICI	1229117	1230595	14.4	Same as <i>Shigella flexneri</i> 2002017
		sfx:S1228	<i>Shigella flexneri</i>	<i>Shigella flexneri</i> 2457T	492	0.986		PICI	1189823	1191301	14.7	Similar to <i>Shigella flexneri</i> 2002017
		eoc:CE10_1229	<i>Escherichia coli</i>	<i>Escherichia coli</i> O7:K1 CE10	492	0.98		PICI	1272471	1273949	14.5	Similar to <i>Escherichia coli</i> O157:H7 EDL933
		kpa:KPNJ1_00485	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> 30660/NJST258_1	553	0.835	509	PICI	476791	478452	16	CP006923
		kpc:KPNIH10_23900	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH10	553	0.835	509	PICI	4880086	4881747	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpg:KPNIH32_25075	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH32	553	0.835	509	PICI	5003753	5005414	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kph:KPNIH24_04900	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> KPNIH24	553	0.835	509	PICI	1029257	1030918	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1
		kpi:N559_0497	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> JM45	553	0.835	509	PICI	513865	515526	16	Same as <i>Klebsiella pneumoniae</i> 30660/NJST258_1

Table S2. cf-PICIs in Proteobacteria: Genomes and characteristics.

cf-PICI	Strain	Accession number (Genomic location)	Size (Kb)	att site core	att gene	Accessory genes
EcCIEDL933	<i>Escherichia coli</i> O157:H7str. EDL933	NZ_CP008957.1 (1680148-1695618)	15.4	GGACATATTT	<i>ycfD-phoQ</i>	<i>rdgR; perC</i>
EcCICFSAN00223 6	<i>Escherichia coli</i> O104:H21 str. CFSAN002236	CP023541.1 (1969075-1984466)	15.3	GGACATATTT	<i>ycfD-phoQ</i>	<i>rdgR; perC</i>
EcCIIAI39	<i>Escherichia coli</i> str. IAI39	CU928164.2 (2060161-2074688)	14.5	GGACATATTT	<i>ycfD-phoQ</i>	<i>rdgR; perC</i>
EcCIST130	<i>Escherichia coli</i> str. ST130	CP043478.1 (2044053-2060283)	16.2	GGACATATTT	<i>ycfD-phoQ</i>	<i>rdgR; perC</i>
EcCIPU0103	<i>Escherichia coli</i> str. PUO103	CP014752.1 (2068299-2084242)	15.9	ATAACAGCA	<i>tqsA</i>	<i>rdgR; perC</i>
EcCIRM10386	<i>Escherichia coli</i> O26 str. RM10386	CP028126.1 (2294845-2309731)	14.8	ATAACAGCA	<i>tqsA</i>	<i>rdgR; perC</i>
EcCIRM10042	<i>Escherichia coli</i> O43 str. RM10042	CP028122.1 (1829035-1844575)	15.5	ATAACAGCA	<i>tqsA</i>	<i>rdgR; perC</i>
EcCIED1a.2	<i>Escherichia coli</i> str. ED1a	NC_011745.1 (1739927-1756201)	16.2	ATAACAGCA	<i>tqsA</i>	<i>rdgR; perC</i>
EcCI315650	<i>Escherichia coli</i> strain 315650 SAMN09380356- rid9461733.denovo.011	AAAAWV020000011 .1 (88682-104064)	15.3	ATAACAGCA	<i>tqsA</i>	<i>rdgR; perC</i>
EcCIPA40	<i>Escherichia coli</i> PA40 PA40.contig.34	AKLQ01000035.1 (37524-53136)	15	ATAACAGCA	<i>tqsA</i>	<i>rdgR; perC</i>
EcCIHUST159	<i>Escherichia coli</i> str. HUST159	BFLF01000001.1 (341769-355219)	14.5	ATGATAGTTAT	<i>cspD-clpS</i>	<i>rdgR</i>
EcCISMS-3-5	<i>Escherichia coli</i> SMS-3-5	CP000970.1 (955625-970780)	15.1	AGAAATACCCGTAT	<i>rcnR-thiM</i>	<i>rdgR</i>
EcCID8	<i>Escherichia coli</i> strain D8	CP010151.1 (3045135-3059753)	14.5	AGTGATACACCG	<i>gcd-hpt</i>	<i>rdgR</i>
EcCI144	<i>Escherichia coli</i> strain 144	CP041550.1 (1226228-1243816)	16.2	TACGGCATGAACT	<i>yhdJ-fis</i>	
EcCI392917	<i>Escherichia coli</i> strain 392917 SAMN07599901-rid9192623	AASLTG010000005. 1 (198328-214392)	16	TACGGCATGAACT	<i>yhdJ-fis</i>	
EcCIEC11-7286	<i>Escherichia coli</i> strain EC11- 7286	DABIGM010000022. 1 (12211-28744)	16.5	TACGGCATGAACT	<i>yhdJ-fis</i>	
EcCIEO709	<i>Escherichia coli</i> strain eo709	DABIRI010000029.1 (11862-16534)	16.5	TACGGCATGAACT	<i>yhdJ-fis</i>	
EcCIWW223	<i>Escherichia coli</i> strain WW223	NNAO01000025.1 (12211-28743)	16.5	TACGGCATGAACT	<i>yhdJ-fis</i>	
EcCIPNUSAE0444 09	<i>Escherichia coli</i> strain PNUSAE044409	AAQWOW01000000 4.1	14.9	TACGGCATGAACT	<i>yhdJ-fis</i>	
SfCI8401	<i>Shigella flexneri</i> 5 str. 8401	CP000266.1 (1195009-1210654)	15.5	GGACATATTT	<i>ycfD-phoQ</i>	<i>rdgR; perC</i>

cf-PICI	Strain	Accession number (Genomic location)	Size (Kb)	att site core	att gene	Accessory genes
SfCI301	<i>Shigella flexneri</i> 2a str. 301	AE005674 (1174995-1191146)	14.7	GGACATATTT	<i>ycfD-phoQ</i>	<i>rdgR; perC</i>
SdCICFSAN010956	<i>Shigella dysenteriae</i> strain CFSAN010956	CP026827 (1440478-1467734)	14.7	ATAACAGCA	<i>tqsA</i>	<i>rdgR; perC</i>
SeCI08-1209	<i>Salmonella enterica</i> strain 08-1209	AAELMS010000008 (110968-126650)	15.5	TCTAGGATACC	<i>yecR-mug</i>	<i>rdgR</i>
SeCI7830	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhi strain 7830	MQUN01000001 (132158-147689)	15.7	TCTAGGATACC	<i>sip-mug</i>	<i>rdgR</i>
SeCIFDA336426-1	<i>Salmonella enterica</i> strain FDA336426-1	AACWNE01000000 2 (766394-782129)	16.1	TACGGCATGAACT	<i>yhdJ-fis</i>	
SeCIDerby	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Derby strain CVM N30705	JYVB01000038 (94446-111998)	16.3	TACGGCATGAACT	<i>yhdJ-fis</i>	
SeCIKentucky	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Kentucky strain CVM N42453	JYZP01000008 (10626-26683)	16	TACGGCATGAACT	<i>yhdJ-fis</i>	
KpCIAR_0148	<i>Klebsiella pneumoniae</i> strain AR_0148	CP021950 (3801580-3817416)	16	TACGGCATGAACT	<i>acrEF/env CD-fis</i>	
KpCIKpN01	<i>Klebsiella pneumoniae</i> strain KpN01	CP012987 (3246415-3261297)	15.4	TACGGCATGAACT	<i>acrEF/env CD-fis</i>	
KvCIGJ3	<i>Klebsiella variicola</i> strain GJ3	CP017289 (4878850- 4894260)	15.4	CTAGGATACCTATAGGATACC	<i>gnat-mug</i>	
KoCICAV1752	<i>Klebsiella oxytoca</i> strain CAV1752	CP018362 (2627065-2643239)	16.2	TACGGCATGAACT	<i>acrEF/env CD-fis</i>	
CfCICFNIH4	<i>Citrobacter freundii</i> complex sp. CFNIH4	CP026231.1 (3899503-3920597)	20.9	TACGGCATGAACT	<i>acrEF/env CD-fis</i>	
CpCITV06	<i>Citrobacter portucalensis</i> strain TV06	VTZD01000023.1 (172654-187873)	15.2	TCTAGGATACC	Hypothetic <i>al-mug bamD- qacE</i>	
GaCISCGC	<i>Gilliamella apicola</i> SCGC AB-598-B02 SCG598B02_1148	JAIM01000024.1 (2961-18737)	15.1	TGTTTATCTAA	<i>bamD- qacE</i>	
GaCIwkB1	<i>Gilliamella apicola</i> strain wkB1	CP007445.1 (1377173-1393759)	16.5	TGTTTATCTAA	<i>bamD- qacE</i>	
PmCIATCC29906	<i>Proteus mirabilis</i> ATCC29906 contig00106	ACLE01000080.1 (234168-249990)	15.8	CTTTTTAATTCAATGT	Hypothetic <i>al-fis</i>	
EpCIETW41	<i>Edwardsiella piscicida</i> strain ETW41	CP019440 (2152154-2162424)	17	TACGGCATGAACT	<i>acrEF/env CD-fis</i>	
PgCIFDAARGOS_186	<i>Pluralibacter gergoviae</i> strain FDAARGOS_186	CP020388 (395979-412296)	16.2	TACGGCATGAACT	<i>acrEF/env CD-fis</i>	
XnCIATCC1906	<i>Xenorhabdus nematophila</i> ATCC 19061	FN667742	NI	NI		
YaCI159	<i>Yersinia aleksiciae</i> strain 159	CP011975 (1511457-1529439)	15.7	TACGGCATGAACT	<i>acrEF/env CD-fis</i>	

Table S3. Phage titres in presence or absence of EcCIEDL933.

Donor strain	Phage titer ^a
594 HK106	1.14E+10 ± 6.01E+09
594 HK106 EcCIEDL933 1795-1796:: <i>cat</i>	1.89E+10 ± 1.05E+10
594 HK446	1.27E+10 ± 1.10E+10
594 HK446 EcCIEDL933 1795-1796:: <i>cat</i>	2.25E+10 ± 1.31E+10

^aPhage titre/ml of lysate, using *E. coli* 594 as recipient strain. The means and standard deviations from three independent experiments are presented.

Table S4. cf-PICIs in Firmicutes bacteria: Genomes and characteristics.

cf-PICI	Strain	Accession number (Genomic location)	Size (Kb)	att site core	att gene	Accessory genes
SaCIC0673	<i>Staphylococcus aureus</i> str. C0673	JIZS01000037.1 (68245-54456)	13.8	ATGCCAGGTATGATGTA	<i>groEL-gnat</i>	
SaCIVET0180R	<i>Staphylococcus aureus</i> str. VET0180R	JIRR01000005.1 (113720-100048)	13.7	ATGCCAGGAATGATGTAAAAT	<i>groEL</i>	
SxCIHKUOPL8	<i>Staphylococcus xylosus</i> str. HKUOPL8	CP007208.1 (2740485-2753340)	12.9	NI	<i>gmp-cpbp</i>	
SsCICUG38042	<i>Staphylococcus saprophyticus</i> subsp. <i>bovis</i> str. CCUG38042	NZ_PPRA01000056.1 (1-14418)	14.5	ATGCCAGGTATGATGTAAAAT		
SeCIDMS15097	<i>Staphylococcus equorum</i> subsp. <i>linens</i> str. DMS15097	NZ_PPQL01000034.1 (28076-14806)	13.3	TCCCGCCGTCTCCA	<i>smpB-gnat</i>	<i>virE</i>
ShCIS167	<i>Staphylococcus haemolyticus</i> str. S167	CP013911.1 (1221296-1210145)	12.9	NI	<i>comGA</i>	
ShCISNUC5746	<i>Staphylococcus hominis</i> str. SNUC5746	PZHW01000022 (14142-27645)	13.5	TCCCGCCGCCTCCAT	<i>smpB-int</i>	
SpCI589	<i>Staphylococcus pettenkoferi</i> str. 589	JVAY01000011 (103129-116168)	13	NI	<i>pts</i>	
SwCIDE0454	<i>Staphylococcus warneri</i> str. DE0454	VDRS01000006 (89595-75796)	13.8	ATGCCAGGTATGATGTAAA	<i>groEL-sasC</i>	
SaCIIOV5	<i>Staphylococcus arlettae</i> str. IOV5	NZ_SPPT01000005.1 (102477-115671)	13.2	NI		
BcCIBAG6X1-2	<i>Bacillus cereus</i> str. BAG6X1-2	AHDP01000005 (9694-21878)	12.4	NI	<i>groEL-gnat</i>	
BkCIBDGP4	<i>Bacillus kochii</i> str. BDGP4	CP022983.1 (1531436-1545000)	13.6	TACATCATGCCGCCCAT	<i>groEL-tetR</i>	
BtCIBGSC4W14W1	<i>Bacillus thuringiensis</i> serovar <i>kumamotoensis</i> str. BGSC4W14W1	NFEH01000115.1 (170053-159556)	12.2	NI	<i>s4p</i>	
LcCIBL23	<i>Lactobacillus casei</i> str. BL23	FM177140.1 (3031502-3045382)	13.9	TATTCTGGGTGGTCAGGGGA	<i>had</i>	
LcCILc705	<i>Lactobacillus casei</i> str. Lc705	FM179323.1 (69837-711868)	13.7	CTTAACGGGATTGAACC		
LrCIGG	<i>Lactobacillus rhamnosus</i> str. GG	NZ_CP031290.1 (2949164-2963077)	14.1	CTATTCTGGGTGGTCAGGGGA	<i>had-dnas</i>	
CbCIWB53	<i>Clostridium beijerinckii</i> str. WB53	CP029329.1 (1212114-1225061)	13	AGTACCCTAAAACATTAGAGTA	<i>s9p-ftsH</i>	
CbCIEklund17B	<i>Clostridium botulinum</i> B str. Eklund17B	FR745875.1 (33017-45420)	12.5	NI	<i>tRNA-70pol</i>	
CsCI87-0535	<i>Clostridium sporogenes</i> str. 87-0535	JZJP01000052 (44733-31901)	12.8	NI	<i>tRNA-gluT</i>	
EdCI4928STDY7071318	<i>Enterococcus durans</i> str. 4928STDY7071318	NZ_CABGKX01000028.1 (14603-7305)	14.5	ATGGAAGGCAGTGT	<i>sufB-rec</i>	

Table S5. Strains used in this study.

Strain	Description	Reference
594	<i>E. coli</i> . Laboratory strain	
DH5 α	<i>E. coli</i> . Laboratory strain	
EDL933	<i>E. coli</i> . Natural isolate	
JP22295	594 EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP12506	594 lambda lysogen	This work
JP12507	594 80 lysogen	This work
JP12508	594 HK97 lysogen	This work
JP12509	594 HK106 lysogen	This work
JP12510	594 HK140 lysogen	This work
JP12511	594 HK544 lysogen	This work
JP22300	594 HK446 lysogen	This work
JP22478	594 HK97 EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP22479	594 HK106 EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP22480	594 HK140 EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP22300	594 HK446 EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP22481	594 HK544 EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP22084	594 lambda EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP22085	594 80 EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP19756	594 HK106 EcCIEDL933 1795-1796:: <i>cat alpA*</i>	This work
JP22282	594 HK446 EcCIEDL933 1795-1796:: <i>cat alpA*</i>	This work
JP17368	594 HK106 EcCIEDL933 1795-1796:: <i>cat</i> ORF1786* (capsid)	This work
JP22301	JP17368 pBAD18 empty	This work
JP22302	JP17368 pJP2670	This work
JP17246	594 EcCIEDL933 1795-1796:: <i>cat</i> ORF1787* (protease)	This work
JP17369	594 HK106 EcCIEDL933 1795-1796:: <i>cat</i> ORF1787* (protease)	This work
JP17364	JP17369 pBAD18 empty	This work
JP17366	JP17369 pJP2671	This work
JP17365	594 EcCIEDL933 1795-1796:: <i>cat</i> ORF1788* (portal)	This work
JP17370	594 HK106 EcCIEDL933 1795-1796:: <i>cat</i> ORF1788* (portal)	This work
JP22303	JP17370 pBAD18 empty	This work
JP22304	JP17370 pJP2672	This work
JP17780	594 EcCIEDL933 1795-1796:: <i>cat</i> ORF1789* (head-tail connector I)	This work
JP22305	594 HK106 EcCIEDL933 1795-1796:: <i>cat</i> ORF1789* (head-tail connector I)	This work
JP22306	JP22305 pBAD18 empty	This work
JP22307	JP22305 pJP2673	This work
JP17248	594 EcCIEDL933 1795-1796:: <i>cat</i> ORF1790* (head-tail connector II)	This work
JP17371	594 phage HK106 EcCIEDL933 1795-1796:: <i>cat</i> ORF1790* (head-tail connector II)	This work
JP17496	JP17371 pBAD18 empty	This work
JP17497	JP17371 pJP2674	This work

JP17247	594 EcCIEDL933 1795-1796:: <i>cat</i> ORF1791* (HNH)	This work
JP17373	594 HK106 EcCIEDL933 1795-1796:: <i>cat</i> ORF1791* (HNH)	This work
JP17374	JP17373 pBAD18 empty	This work
JP17498	JP17373 pJP2675	This work
JP17781	594 EcCIEDL933 1795-1796:: <i>cat</i> ORF1792* (HP)	This work
JP22285	594 HK106 EcCIEDL933 1795-1796:: <i>cat</i> ORF1792* (HP)	This work
JP22308	JP22285 pBAD18 empty	This work
JP22309	JP22285 pJP2676	This work
JP17264	594 EcCIEDL933 1795-1796:: <i>cat</i> ORF1793* (<i>terS</i>)	This work
JP17265	594 HK106 EcCIEDL933 1795-1796:: <i>cat</i> ORF1793* (<i>terS</i>)	This work
JP17266	JP17265 pBAD18 empty	This work
JP17267	JP17265 pJP2677	This work
JP17367	594 EcCIEDL933 1795-1796:: <i>cat</i> ORF1794* (<i>terL</i>)	This work
JP17372	594 HK106 EcCIEDL933 1795-1796:: <i>cat</i> ORF1794* (<i>terL</i>)	This work
JP22312	JP17372 pBAD18 empty	This work
JP22313	JP17372 pJP2678	This work
JP22482	DH5alpha pJP2670	This work
JP22483	DH5alpha pJP2671	This work
JP22484	DH5alpha pJP2672	This work
JP22485	DH5alpha pJP2673	This work
JP22486	DH5alpha pJP2674	This work
JP22487	DH5alpha pJP2675	This work
JP22488	DH5alpha pJP2676	This work
JP22489	DH5alpha pJP2677	This work
JP22490	DH5alpha pJP2678	This work
JP17556	DH5alpha pJP2679	This work
JP17553	DH5alpha pJP2680	This work
JP19139	DH5alpha pJP2681	This work
JP19140	DH5alpha pJP2682	This work
JP22491	DH5alpha pJP2683	This work
JP18451	DH5alpha pJP2684	This work
JP17554	DH5alpha pJP2685	This work
JP17555	DH5alpha pJP2686	This work
JP18450	DH5alpha pJP2687	This work
JP21206	DH5alpha pJP2688	This work
JP17204	DH5alpha pJP2689	This work
JP19412	DH5alpha pJP2690	This work
JP19071	DH5alpha pJP2691	This work
JP19413	DH5alpha pJP2692	This work
JP17205	DH5alpha pJP2693	This work
JP19414	DH5alpha pJP2694	This work
JP22492	DH5alpha pJP2695	This work
JP22493	DH5alpha pJP2696	This work
JP19411	DH5alpha pJP2697	This work

JP22497	DH5alpha pJP2698	This work
JP22370	594 HK106 <i>gp01*</i>	This work
JP22311	594 HK106 <i>gp01*</i> EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP19890	JP22370 pBAD18 empty	This work
JP19891	JP22370 pJP2679	This work
JP19892	JP22311 pBAD18 empty	This work
JP19893	JP22311pJP2679	This work
JP22371	594 HK106 <i>gp02*</i>	This work
JP19757	594 HK106 <i>gp02*</i> EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP19886	JP22371 pBAD18 empty	This work
JP19887	JP22371 pJP2680	This work
JP19888	JP19757 pBAD18 empty	This work
JP19889	JP19757 pJP2680	This work
JP19416	594 HK106 <i>gp03*</i>	This work
JP19660	594 HK106 <i>gp03*</i> EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP19730	JP19416 pBAD18 empty	This work
JP19731	JP19416 pJP2681	This work
JP19732	JP19660 pBAD18 empty	This work
JP19733	JP19660 pJP2681	This work
JP20793	594 HK106 <i>gp04*</i>	This work
JP20811	594 HK106 <i>gp04*</i> EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP21202	JP19660 pBAD18 empty	This work
JP21203	JP19660 pJP2682	This work
JP21204	JP20811 pBAD18 empty	This work
JP21205	JP20811 pJP2682	This work
JP22372	594 HK106 <i>gp05*</i>	This work
JP19758	594 HK106 <i>gp05*</i> EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP19807	JP22372 pBAD18 empty	This work
JP19808	JP22372 pJP2683	This work
JP22373	JP19758 pBAD18 empty	This work
JP19809	JP19758 pJP2683	This work
JP19959	594 HK106 <i>gp06*</i>	This work
JP19960	594 HK106 <i>gp06*</i> EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP20321	JP19959 pBAD18 empty	This work
JP20322	JP19959 pJP2684	This work
JP20333	JP19960 pBAD18 empty	This work
JP20334	JP19960 pJP2684	This work
JP20272	594 HK106 <i>gp07*</i>	This work
JP20336	594 HK106 <i>gp07*</i> EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP22498	JP20272 pBAD18 empty	This work
JP22499	JP20272 pBAD18 pJP2685	This work
JP22500	JP20336 pBAD18 empty	This work
JP22501	JP20336 pBAD18 pJP2685	This work
JP17761	594 HK106 <i>gp10*</i>	This work

JP18367	594 HK106 <i>gp10*</i> EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP22374	JP17761 pBAD18 empty	This work
JP22375	JP17761 pJP2686	This work
JP22376	JP18367 pBAD18 empty	This work
JP22377	JP18367 pJP2686	This work
JP19659	594 HK106 <i>gp14*</i>	This work
JP19759	594 HK106 <i>gp14*</i> EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP19802	JP19659 pBAD18 empty	This work
JP19803	JP19659 pJP2687	This work
JP19804	JP19759 pBAD18 empty	This work
JP19805	JP19759 pJP2687	This work
JP22286	594 HK106 <i>gp65*</i>	This work
JP22378	594 HK106 <i>gp65*</i> EcCIEDL933 1795-1796:: <i>cat</i>	This work
JP22502	JP22286 pBAD18 empty	This work
JP22503	JP22286 pJP2688	This work
JP22504	JP22378 pBAD18 empty	This work
JP22505	JP22378 pJP2688	This work
JP22494	594 pJP2679	This work
JP19766	594 pJP2680	This work
JP19767	594 pJP2681	This work
JP22495	594 pJP2682	This work
JP19768	594 pJP2683	This work
JP20335	594 pJP2684	This work
JP20434	594 pJP2685	This work
JP22496	594 pJP2686	This work
JP19769	594 pJP2687	This work
JP22506	594 pJP2688	This work

Table S6. Plasmids used in this study.

Plasmid	Description	Reference
pBAD18	Amp ^R . Expression vector	(Guzman et al., 1995)
pWRG99	Amp ^R . Thermosensitive plasmid with Red system of lambda phage and I-SceI endonuclease under control of tetracycline-inducible promoter (P _{tetA})	(Blank et al., 2011)
pWRG717	Amp ^R , Km ^R . pBluescript II SK+ derivative, <i>aph</i> resistance cassette and I-SceI cleavage site	(Hoffmann et al., 2017)
pK03-Blue	Cm ^R . Thermosensitive allelic exchange vector in <i>E. coli</i>	(Solano et al., 2009)
pJP2670	pBAD18 ORF1786 (capsid) EcCIEDL933	This work
pJP2671	pBAD18 ORF1787 (protease) EcCIEDL933	This work
pJP2672	pBAD18 ORF1788 (portal) EcCIEDL933	This work
pJP2673	pBAD18 ORF1789 (head-tail connector I) EcCIEDL933	This work
pJP2674	pBAD18 ORF1790 (head-tail connector II) EcCIEDL933	This work
pJP2675	pBAD18 ORF1791 (HNH) EcCIEDL933	This work
pJP2676	pBAD18 ORF1792 (HP) EcCIEDL933	This work
pJP2677	pBAD18 ORF1793 (<i>terS</i>) EcCIEDL933	This work
pJP2678	pBAD18 ORF1794 (<i>terL</i>) EcCIEDL933	This work
pJP2679	pBAD18 <i>gp01</i> (<i>terL</i>) HK106	This work
pJP2680	pBAD18 <i>gp02</i> (<i>terS</i>) HK106	This work
pJP2681	pBAD18 <i>gp03</i> (portal) HK106	This work
pJP2682	pBAD18 <i>gp04</i> (protease) HK106	This work
pJP2683	pBAD18 <i>gp05</i> (capsid) HK106	This work
pJP2684	pBAD18 <i>gp06</i> (head-tail connector I) HK106	This work
pJP2685	pBAD18 <i>gp07</i> (head-tail connector II) HK106	This work
pJP2686	pBAD18 <i>gp10</i> (major tail) HK106	This work
pJP2687	pBAD18 <i>gp14</i> (tail tape measure) HK106	This work
pJP2688	pBAD18 <i>gp65</i> (HNH) HK106	This work
pJP2689	pK03 Blue Δ <i>gp01</i> (<i>terL</i>) HK106	This work
pJP2690	pK03 Blue Δ <i>gp02</i> (<i>terS</i>) HK106	This work
pJP2691	pK03 Blue Δ <i>gp03</i> (portal) HK106	This work
pJP2692	pK03 Blue Δ <i>gp04</i> (protease) HK106	This work
pJP2693	pK03 Blue Δ <i>gp05</i> (capsid) HK106	This work
pJP2694	pK03 Blue Δ <i>gp06</i> (head-tail connector I) HK106	This work
pJP2695	pK03 Blue Δ <i>gp07</i> (head-tail connector II) HK106	This work
pJP2696	pK03 Blue Δ <i>gp10</i> (major tail) HK106	This work
pJP2697	pK03 Blue Δ <i>gp14</i> (tail tape measure) HK106	This work
pJP2698	pK03 Blue Δ <i>gp65</i> (HNH) HK106	This work

Table S7. Primer list.

Mutagenesis	Primers	Sequence (5'-3')
EcCIEDL933 1795- 1796::cat	Sakai-adjustchlor- 1m	CCGTCACAGGTAGGCGCGCCGCTGCTGACCTCATTACACC CCGCTGAATTCTTCCAGTTTCTGGCGGTGGCTGTCGCTTA
	Sakai-adjustchlor- 2c	GCCATTTAAATGAAGTTCCTATTTCCTAGCCCCGTTATG GGGATCCCCATATCGACATTAACGCCCTCATGAATTGGTG CGCTTC
EcCIEDL933 ORF1786 (capsid)*	EDL933-A1786-3m	TGCCGAGAATAATCGAATTACGCCAGCAGAAAACCGCCATT AAAAATCAGAGGGTTTCCCAGTCACGAC
	EDL933-A1786-4c	CGCCCTCTGCATCGTTAAGGCTGCGGTTTTCTTTTTCCGCG TTCTCCAGCTGCTTCCGGCTCGTATGTTG
	EDL933-1786- dimer-1m	TCGAATTACGCCAGCAGAAAACCGCCATTAATAATCAGTAA TTAGTTGAGCTGGAGAACGCGGAAAAAGAAAACCGCAGCC TTAACGATGC
	EDL933-1786- dimer-2c	GCATCGTTAAGGCTGCGGTTTTCTTTTTCCGCGTTCTCCAG CTCAACTAATTACTGATTTTAAATGGCGTTTTCTGCTGGCG TAATTCGA
EcCIEDL933 ORF1787 (protease)*	EDL933-A1787-1m	GGGTGAAAAAATTATGAAGAGTATGGAAATCCGGTCATCGG AAATCACCACCAGGGTTTCCCAGTCACGAC
	EDL933-A1787-2c	CATAGCAGCTCTGAAAGTATCCAGCGAACAACGTAGCC TGTAAGCGTGTGCTTCCGGCTCGTATGTTG
	EDL933-A1787- dimer-1m	GAAGAGTATGGAAATCCGGTCATCGGAAATCACCACTAAT TAGTTGACACGCTTACAGGCTACGTTGTTGCTGGGATAAC CTTTCAG
	EDL933-A1787- dimer-2c	CTGAAAGGTTATCCCAGCGAACAACGTAGCCTGTAAGCGTG TCAACTAATTAGGTGGTGATTTCCGATGACCGGATTTCCATA CTCTTC
EcCIEDL933 ORF1788 (portal)*	EDL933-A1788-1m	GCATGACGCTTGATGAGTTTATGGCGCTGGCTGGCACATC GAACACGGGGAGGGTTTCCCAGTCACGAC
	EDL933-A1788-2c	TCACGGCGTTCATGACGGCGGGCAGTGATTCCGCTGTCCC CGATGATACGTGCTTCCGGCTCGTATGTTG
	EDL933-A1788- dimero-1m	GATGAGTTTATGGCGCTGGCTGGCACATCGAACACGGGGT CAACTAATTACGTATCATCGGGGACAGCGGAATCACTGCCC GCCGTCATG
	EDL933-A1788- dimero-2c	CATGACGGCGGGCAGTGATTCCGCTGTCCCCGATGATACG TAATTAGTTGACCCCGTGTTGATGTGCCAGCCAGCGCCAT AAACTCATC
EcCIEDL933 ORF1789 (head-tail connector I)*	EDL933-A1789-1m	GGCGACTGCGTGACAGGATAACGATTCAGACCCTGAAACA GACCAGGCCAGGGTTTCCCAGTCACGAC
	EDL933-A1789-2c	TGTTACGCTTGCCCAGAGTGTGTGACCGTCTCCACGTT TCGAGTATTTGCTTCCGGCTCGTATGTTG
	EDL933-1789- dimero-1m	GTGACAGGATAACGATTCAGACCCTGAAACAGACCAGAGC CTCAACTAATTAATACTCGAAACGTGGGAGGACGGTCACA CACTCTGGGC
	EDL933-1789- dimero-2c	GCCCAGAGTGTGTGACCGTCTCCACGTTTCGAGTATTTA ATTAGTTGAGGCTCTGGTCTGTTTCAGGGTCTGAATCGTTA TCCTGTAC
EcCIEDL933 ORF1789 (head-tail connector II)*	EDL933-A1790-3m	GAAGAACTTATCACCTGGAAGAAGTGAACCTCATTGCCG CATCGATGGCAGGGTTTCCCAGTCACGAC
	EDL933-A1790-4c	GTATCTGGCACGCCTCAAGCGATGCGGCAATGTATCCGCT GATTAACGGTGCTTCCGGCTCGTATGTTG
	EDL933-1790- dimero-1m	CACCCTGGAAGAAGTGAACCTCATTGCCGCATCGATGGCT CAACTAATTACCAGTTAATCAGCGGATACATTGCCGCATCG CTTGAGGC

	EDL933-1790-dimero-2c	GCCTCAAGCGATGCGGCAATGTATCCGCTGATTAAGTGGTA ATTAGTTGAGCCATCGATGCGGCAATGGAGTTTCACTTCTT CCAGGGTG
EcCIEDL933 ORF1791 (HNH)*	EDL933-AHNH-1m	GGCAACCATTAAGACGATGCACAGAACCAGGCTGTAACAG GCGCGTGAAGAGGGTTTCCCAGTCACGAC
	EDL933-AHNH-2c	GTCCTCGTCTGGCATCCTCTGCACGCCATGCCGCCGCCT GTGCTCCTCATGCTTCCGGCTCGTATGTTG
	EDL933-HNH-dimero-1m	AGACGATGCACAGAACCAGGCTGTAACAGGCGCGTGAAGT AATTAGTTGATGAGGAGCACAGGCCGGCGGCATGGCGTGC AGAGGATGCC
	EDL933-HNH-dimero-2c	GGCATCCTCTGCACGCCATGCCGCCGCCTGTGCTCCTCA TCAACTAATTACTTCACGCGCCTGTTACAGCCTGGTTCTGT GCATCGTCT
EcCIEDL933 ORF1792 (HP)*	EDL933-A1792-1m	CGGGCCGCTAACCGCAATAACTGGATGTATGACGCTGATG AATGAGCGGAGGGTTTCCCAGTCACGAC
	EDL933-A1792-2c	CCTGTGTCTTCCATGCGTCACGCTGCCTTATAAGTCCACGG ATAAGGTGCTTCCGGCTCGTATGTTG
	EDL933-1792-dimero-1m	CTAACCGCAATAACTGGATGTATGACGCTGATGAATGAGCG GTCAACTAATTACCTTATCCGTGGACTTATAAGGCAGCGTG ACGCATGG
	EDL933-1792-dimero-2c	CCATGCGTCACGCTGCCTTATAAGTCCACGGATAAGGTAAT TAGTTGACCGCTCATTATCAGCGTCATACATCCAGTTATTG CGGTTAG
EcCIEDL933 ORF1793 (<i>terS</i>)*	EDL933-AterS-1m	AAAAATAATCTGGCGAAAAAATAAGCATGGCAAGACCACCG AAAGCCCCCAGGGTTTCCCAGTCACGAC
	EDL933-AterS-2c	GTTCCGCAAGCTGCCGCGATTTTTTCCCGCCACTGCTTCAG GCGATATCATGCTTCCGGCTCGTATGTTG
	EDL933-terS-dimero-1m	GGCGAAAAAATAAGCATGGCAAGACCACCGAAAGCCCCCT AATTAGTTGATGATATCGCCGTGAAGCAGTGGCGGGAAAAA TCGCGGC
	EDL933-terS-dimero-2c	GCCGCGATTTTTTCCCGCCACTGCTTCACGGCGATATCATCA ACTAATTAGGGGGCTTTCCGGTGGTCTTGCCATGCTTATTTTT TCGCC
EcCIEDL933 ORF1794 (<i>terL</i>)*	EDL933-AterL-1m	GCAGACCCATTGAGCTGGAGCCGTGGCAGCAGTTTCGCCTT TGCGTGCATCAGGGTTTCCCAGTCACGAC
	EDL933-AterL-2c	GCACTTCGATAAAGGCGCTCGTGTATTTGCGCCGTCCGGT GGCCTTAACCTGCTTCCGGCTCGTATGTTG
	EDL933-terL-dimero-1m	TGAGCTGGAGCCGTGGCAGCAGTTTCGCCTTTGCGTGCATC TAATTAGTTGAGTTAAGGCCACCGGACGGCGCAAATACAC GAGCGCCT
	EDL933-terL-dimero-2c	AGGCGCTCGTGTATTTGCGCCGTCCGGTGGCCTTAACCTC AACTAATTAGATGCACGCAAAGGCCAACTGCTGCCACGGCT CCAGCTCA

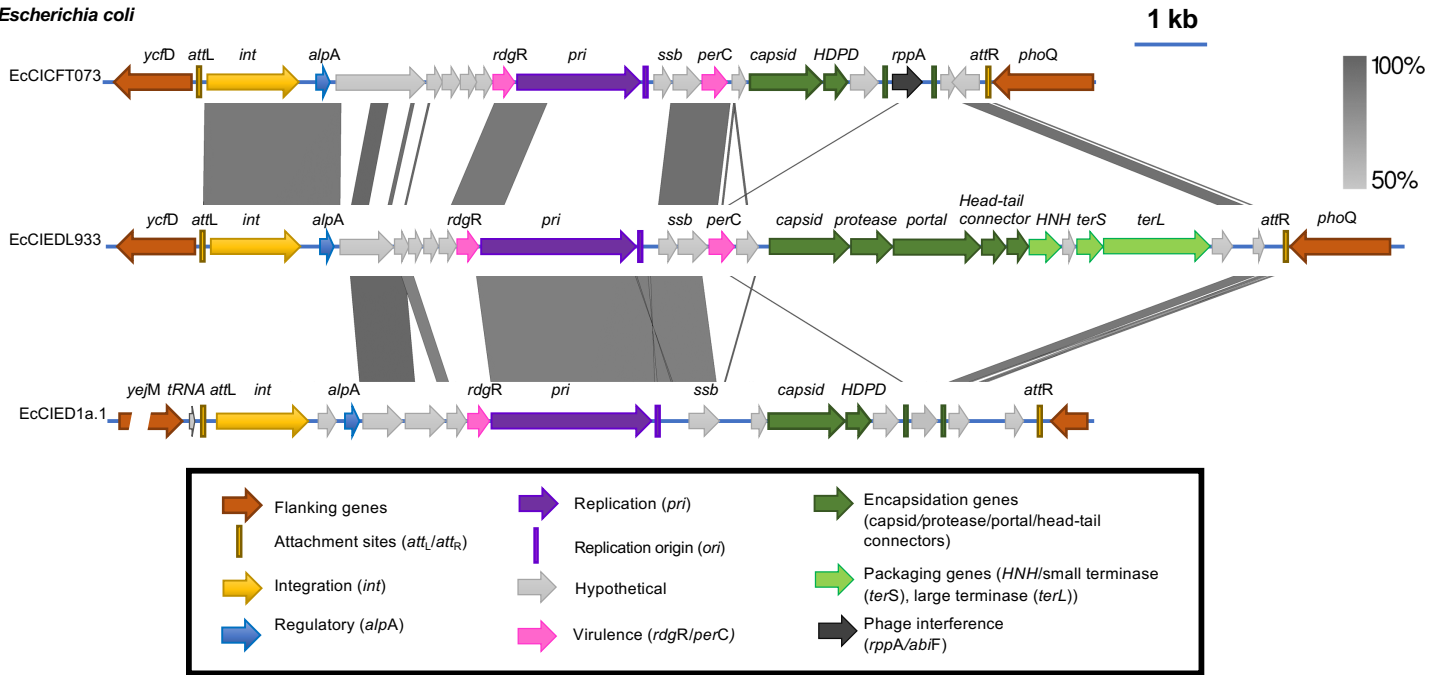
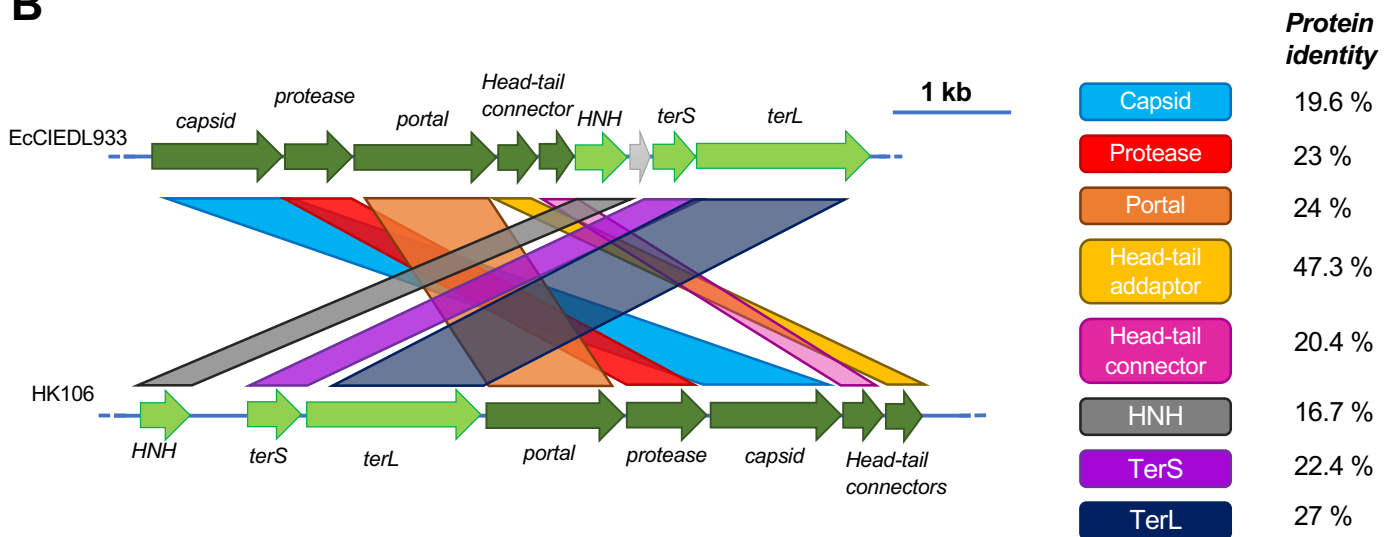
Plasmid	Primers	Sequence (5'-3')
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pJP2671	Sakai-ECs1591-1mS Sakai-ECs1591-2cH	ACGCGT <u>CGACT</u> CAAGATTCTGGAAGTCCAC CCCAAGCTTCTCATCAAGCGTCATGCTGCG
pJP2672	Sakai-ECs1592-1mS Sakai-ECs1592-2cH	ACGCGT <u>CGACC</u> GTTCCCTGTATGAGCAGCAC CCCAAGCTTGGTCTGAATCGTTATCCTGTC
pJP2673	Sakai-ECs1593-1mS Sakai-ECs1593-2cH	ACGCGT <u>CGACT</u> CAAATCCGGCGTTATGTGCC CCCAAGCTTAGTTTCACTTCTTCCAGGGTG
pJP2674	Sakai-ECs1594-1mS Sakai-ECs1594-2cH	ACGCGT <u>CGAC</u> GGGCGTGTACTGAATATCATC CCCAAGCTTTTCTGTGCATCGTCTTAATGG
pJP2675	Sakai-ECs1595-1mS Sakai-ECs1595-2cH	ACGCGT <u>CGAC</u> AAAATCGCCAGATTGTCCGGG CCCAAGCTTATAAGTCCACGGATAAGGCGG
pJP2676	Sakai-ECs1596-1mS Sakai-ECs1596-2cH	ACGCGT <u>CGACT</u> TATGCGTGCGATGCCTTGAG CCCAAGCTTGCATAAAAATTTGAGGAGGCG
pJP2677	Sakai-ECs1597-1mS Sakai-ECs1597-2cH	ACGCGT <u>CGAC</u> CTGATGAGGCTGTATGACGG CCCAAGCTTTTTTAACCGTTTACAGGCCGG
pJP2678	Sakai-ECs1598-1mS Sakai-ECs1598-2cH	ACGCGT <u>CGAC</u> AAAATGGCCTCCTTGCTCGG CCCAAGCTTGTAGTTCCGGCTTCTGTCATGC
pJP2679	HK106-terL-7mK HK106-terL-2cS	GGGGT <u>ACCGA</u> ATACCAAAAACGAGCATC ACGCGT <u>CGACT</u> TCGCAGGTCAATCGTGATTTA GG
pJP2680	HK106-terS-7mk HK106-terS-8cS	GGGGT <u>ACCT</u> CAAATCTTCAAACCTTTGC ACGCGT <u>CGACT</u> TATCCGTGCTTGGGAAAGG
pJP2681	HK106-terL-9mK HK106-gp03-5cS	GGGGT <u>ACCAT</u> GTCCGTTGGTGCTGCTAA ACGCGT <u>CGACT</u> TAAAGCCCGTTATTACG
pJP2682	HK106-gp03-5mK HK106-gp04-6cS	GGGGT <u>ACCC</u> GCACCATCAACGAGATG ACGCGT <u>CGACT</u> TATTTACCTAAGTTAGAAGG
pJP2683	HK106-gp05-4mE HK106-gp05-5cS	CCGGA <u>ATT</u> CGAAACGAAGCACAAATAAAC ACGCGT <u>CGAC</u> GTTTTCCCGCCCCCTCCATC
pJP2684	HK106-gp05-6mK HK106-gp06-4cS	GGGGT <u>ACCT</u> ATCGCCCGACAGCAATCAT ACGCGT <u>CGACT</u> CAGGAGCCCTCCTCTGATT
pJP2685	HK106-gp06-5mE HK106-gp07-3cS	CCGGA <u>ATT</u> CGAAGTGCAGCTTTATGAGAA ACGCGT <u>CGACT</u> CATTTTTTCAGCTCCCGTTT
pJP2686	HK106-gp09-2mE HK106-gp10-5cS	CCGGA <u>ATT</u> CTACGAGCCAGATTATCGGC ACGCGT <u>CGACT</u> TAACTGATAGTAAGGGTGC
pJP2687	HK106-gp14-3mE HK106-gp14-4cH	CCGGA <u>ATT</u> CGGCAAATGAACAAGTTGAAG CCCAAGCTT <u>GTT</u> TCGATAGCCATTTTTATC
pJP2688	HK106-gp65-4mE HK106-gp65-7cH	CCGGA <u>ATT</u> CCTGATATCCCTTTTAAACG CCCAAGCTT <u>TT</u> ACGTGCTCCAGTGAGAC
pK03blue		
pJP2689	HK106-terS-3mB HK106-terL-3cNotI HK106-AterL-4m HK106-AterL-2c	CGCGGATCCATCACAAACTCGAACAGGTAG ATAAGAAT <u>GCGGCCG</u> CACTCGCGTTTACTGATA TCAG GCTTTTGCATCGTGCCAGAAGGCTAATAATAAG ACCAACCTATGCGGTTGGACC GGTCCAACCGCATAGGTTGGTCTTATTATTAGC CTTCTGGCACGATGCAAAAAGC
pJP2690	HK106-gp65-3mB HK106-terL-10cNotI HK106-Agp01-2m HK106-Agp01-3c	CGCGGATCCATGAACAAAGAGCCCCGC ATAAGAAT <u>GCGGCCG</u> CAGGTTAACCATCTTCA CCG CGCGGCAGCGGTTTCAGGCCTAATTAGTTGAAC TCGAGGACACCATCGATCC GGATCGATGGTGTCTCGAGTTCAACTAATTAG GCCTGAACCGCTGCCGCCG

pJP2691	HK106-terL- 8mB	CGCGGATCCATGCCGATTAACACCCCG
	HK106-gp03-4cN	ATAAGAAT <u>CGCGCCCG</u> GATTTGCCGAGACTTGG C
	HK106-Agp03-1m	GCAAGGCTGCAGTCCTGGTTTTGATGATGAGTC GACGTCACCCCAAATCAGGGCTCACAG
	HK106-Agp03-2c	CTGTGAGCCCTGATTTGGGGTGACGTCGACTCA TCATCAAACCAGGACTGCAGCCTTGC
pJP2692	HK106-gp03-8mB	CGCGGATCCCTCTCAACTGGCGAAAAAGTG
	HK106-gp05-8cNotI	ATAAGAAT <u>CGCGCCCG</u> CAGCAGCCCGTTCAGA GAAG
	HK106-Agp04-4m	GACCCGAAAAGTGGCGTAATTAGTTGAGGTACC GACGTGGGAACTGCCG
	HK106-Agp04-5c	CGGCAGTTCCACGTCGGTACCTCAACTAATTA CGCCACTTTTCGGGTC
pJP2693	HK106-gp04-7mP	AACTGCAGGCCTGAAATCGTAAAAACGC
	HK106-gp05-9cB	ACGCGGATCCGATATAGCGGCCTTCATTG
	HK106-Agp05-1m	TCGAAAGCACAGGCCAGGTTTCAAATAATTAG TTGAGGTACCGATGAAAGTACAGGAAGAGC
	HK106-Agp05-2c	GCTCTTCCTGTACTTTCATCGGTACCTCAACTAA TTATTTGGAAACCTGGCCTGTGCTTTTCA
pJP2694	HK106-gp05-9mB	CGCGGATCCGAAGGAAGAGGGCCAGCTGC
	HK106-gp07-4cNotI	ATAAGAAT <u>CGCGCCGC</u> CTATCATTTTTTCAGCTC CCG
	HK106-Agp06-3m	CACGCTGTACGCCCAGGCCTAATTAGTTGACTA AGCTTGCTGGTGCGATGAACC
	HK106-Agp06-4c	GGTTCATCGCACCAGCAAGCTTAGTCAACTAAT TAGGCCTGGGCGTACAGCGTG
pJP2695	HK106-gp05-9mB	CGCGGATCCGAAGGAAGAGGGCCAGCTGC
	HK106-gp08-3cNotI	ATAAGAAT <u>CGCGCCGC</u> CACTTGCTCAATACCTC ATC
	HK106-Agp07-2m	GATCAGCGGCAGAGAGCAGTAATTAGTTGAGAA TTCGACGGCGCAGGCAACTATTC
	HK106-Agp07-3c	GAATAGTTGCCTGCGCCGTCGAATTCTCAACTA ATTAAGTCTCTGCCGCTGATC
pJP2696	HK106-gp09-1mB	CGCGGATCCGTGAATGAAGATAATATCTAC
	HK106-gp10-4cNotI	ATAAGAAT <u>CGCGCCGC</u> TAACTGATAGTAAGGG TGC
	HK106-gp10-1m	CCAGCCACCAAAGAAACGATGGATTCCTAAACC GTCGACGATCTGAGTTGCACCATCAAAGAAATT C
	HK106-gp10-2c	GAATTTCTTTGATGGTGCAACTCAGATCGTCGA CGGTTTAGGAATCCATCGTTTCTTTGGTGGCTG G
pJP2697	HK106-gp11-4mB	CGCGGATCCCGAAGAAGAGCAGGTGCGTG
	HK106-gp14-9cNotI	ATAAGAAT <u>CGCGCCCG</u> CATTGGGTAGCAGATTC AGC
	HK106-Agp14-3m	CTATTACGGGTAATGCAGACTTAATTAGTTGAA TGTCGACCAGCTTCGCGTGAAACTC
	HK106-Agp14-4c	GAGTTTCACGCGAAGCTGGTGCACATTCAACTA ATTAAGTCTGCATTACCCGGTAATAG
pJP2698	HK106-gp64-11mB	CGCGGATCCCAACATTCCACAACGCATC
	HK106-gp65-6cNotI	ATAAGAAT <u>CGCGCCCG</u> CAAAGTTTTGAAGAT TTG
	HK106-Agp65-1m	AGCAGCACCCACTGTGTGTGTAATTAGTTGACT CGAGGCGCATAACACCAGCAAC
	HK106-Agp65-2c	GTTGCTGGTGTATGCGCCTCGAGTCAACTAAT TACACACACAGTGGGTGCTGCT

Southern blot	Primers	Sequence (5'-3')
EcCIEDL933 probe	EcCIEDL933-int-1m	CTGAGCGATACACAGCTGAG
	EcCIEDL933-int-2c	CACTCATATCATCAAGAACG
Phage HK106 probe	HK106-int-2m	CAATTTTGTCCCCTCCCTG
	HK106-int-2c	GACTTACAGCTGACGAATAC

References

- Blank, K., Hensel, M., and Gerlach, R.G. (2011). Rapid and highly efficient method for scarless mutagenesis within the *Salmonella enterica* chromosome. *PloS One* 6, e15763.
- Guzman, L.M., Belin, D., Carson, M.J., and Beckwith, J. (1995). Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *Journal of Bacteriology* 177, 4121–4130.
- Hoffmann, S., Schmidt, C., Walter, S., Bender, J.K., and Gerlach, R.G. (2017). Scarless deletion of up to seven methyl-accepting chemotaxis genes with an optimized method highlights key function of CheM in *Salmonella Typhimurium*. *PloS One* 12, e0172630.
- Solano, C., García, B., Latasa, C., Toledo-Arana, A., Zorraquino, V., Valle, J., Casals, J., Pedroso, E., and Lasa, I. (2009). Genetic reductionist approach for dissecting individual roles of GGDEF proteins within the c-di-GMP signaling network in *Salmonella*. *Proceedings of the National Academy of Sciences USA* 106, 7997–8002.

A**PICIs***Escherichia coli***B****Figure 1. Comparison of the different PICI lineages.**

(A) Comparative maps of the classical *E. coli* PICIs EcCICFT073 and EcCIED1a with EcCIED933. Genomes are aligned according to the prophage convention, with the integrase genes (*int*) present at the left end of the islands. Genes are colored according to their sequence and function (see box for more details). Using Easyfig alignment, vertical blocks between PICI sequences indicate regions that share similarity based on BLASTn (grey scale).

(B) Comparison of the packaging modules present in either EcCIEDL933 or in helper phage HK106, at protein level, using BLASTp. Identities are shown for each encoded protein.

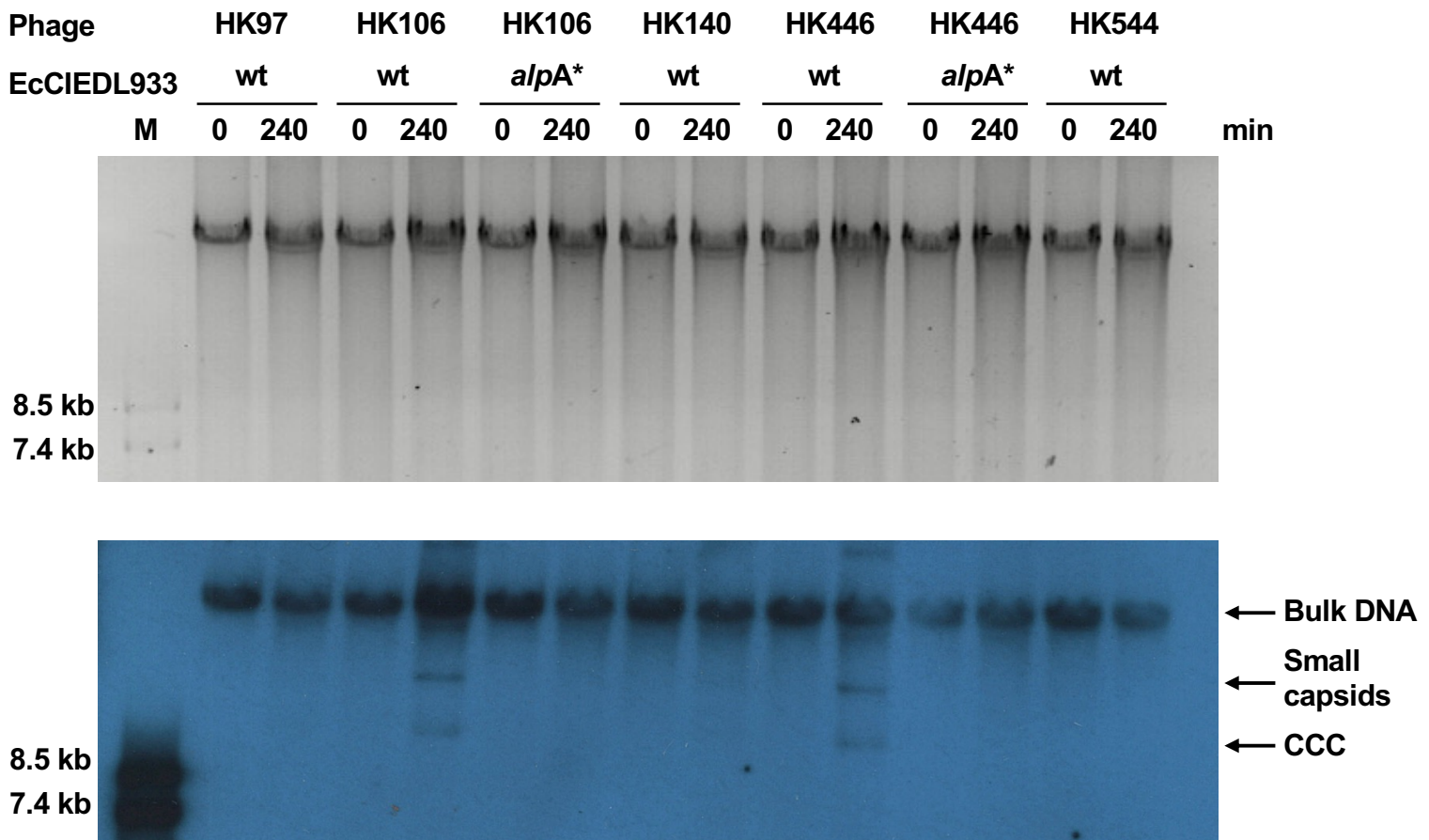
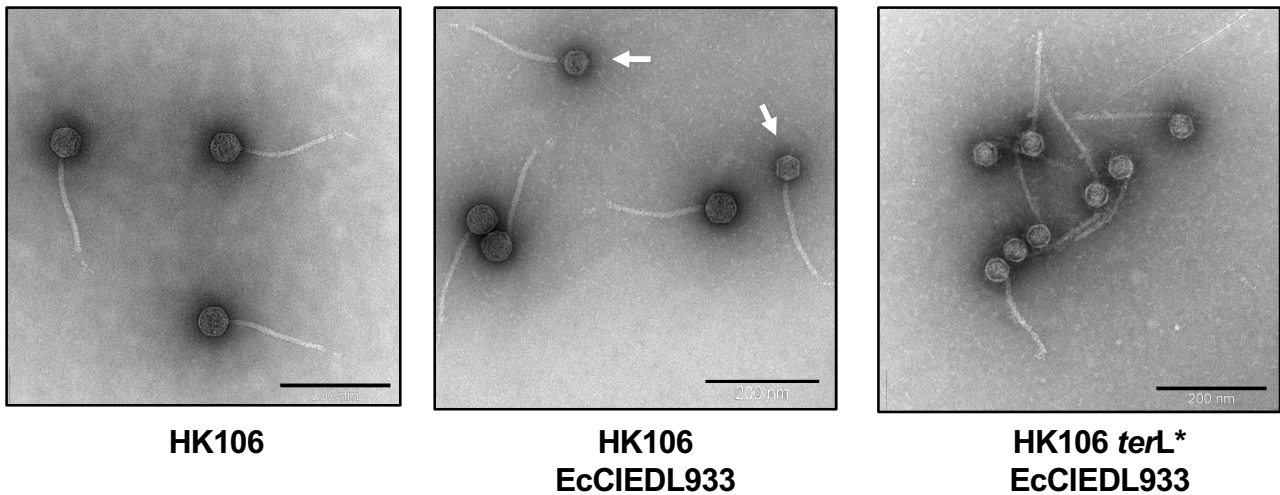
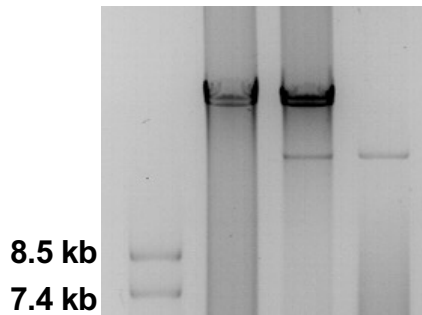


Figure 2. Phage induction of EcCIEDL933.

Different lysogenic strains carrying EcCIEDL933 (wt or mutant in *alpA* (*alpA**)) were MC (2 μ g/ml) induced, samples were taken at the indicated time points (min) and processed to prepare minilysates, which were separated on a 0.7% agarose gel (upper panel), and Southern blotted (lower panel) with an EcCIEDL933 probe. M: Southern blot molecular marker (DNA molecular weight marker VII; Roche).

A**B**

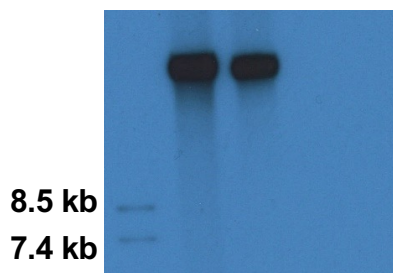
Phage HK106	wt	wt	<i>terL*</i>
EcCIEDL933	-	+	+



Agarose gel

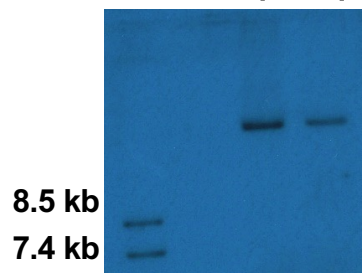
C

Phage HK106	wt	wt	<i>terL*</i>
EcCIEDL933	-	+	+



HK106 probe

Phage HK106	wt	wt	<i>terL*</i>
EcCIEDL933	-	+	+



EcCIEDL933 probe

Figure 3. EcCIEDL933 produces small capsids.

- (A) Electron microscopy analyses of different MC-induced HK106 lysogenic strains (wt or *terL* mutant (*terL**) in presence or absence of EcCIEDL933. Different fields are shown, containing HK106 phage particles (left), EcCIEDL933 particles (right) or both (middle; PICI particles highlighted with white arrows). EcCIEDL933 particles have smaller heads. Scale bars are 200 nm.
- (B) Lysogenic strains carrying the wt or the *terL* mutant (*terL**) HK106 prophage, in presence or absence of EcCIEDL933, were MC-induced, the DNA was extracted from the purified infective particles and resolved on a 0.7% agarose gel.
- (C) Southern blot of the purified DNA shown in panel (B) using either phage HK106 or EcCIEDL933 specific probes. First line contains the Southern blot molecular marker (DNA molecular weight marker VII; Roche).

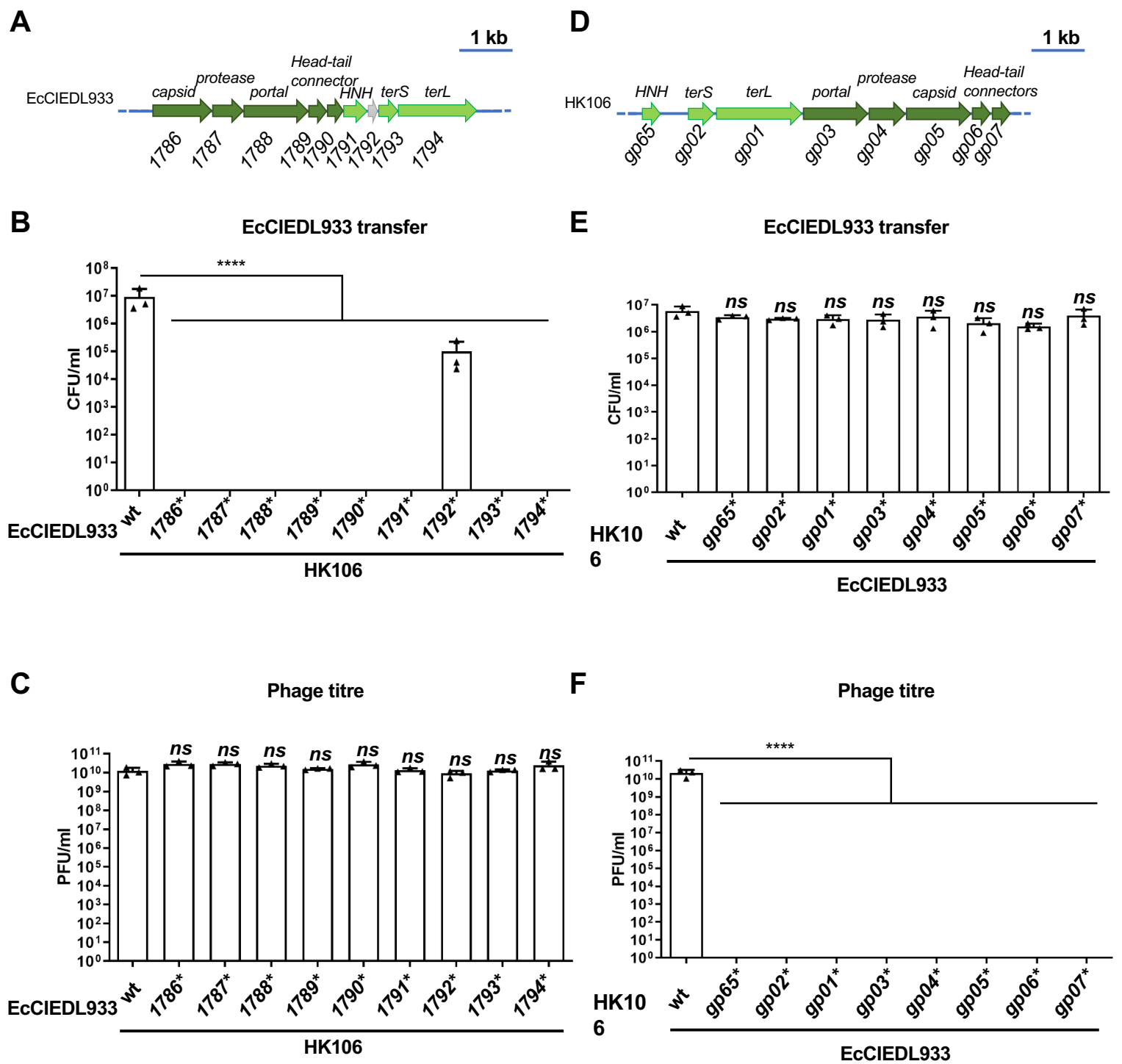


Figure 4. Effect of different PICI and phage gene mutations on EcCIEDL933 and HK106 transfers.

Genetic maps of the EcCIEDL933 (**A**) or the phage HK106 (**D**) packaging modules. Lysogenic strains for phage HK106, carrying different EcCIEDL933 versions (wt or carrying mutations in the indicated genes), were MC (2 μ g/ml) induced and the PICI (**B**) or phage (**C**) transfers were determined using *E. coli* 594 as recipient strain. Lysogenic strains for HK106, carrying the wt or the different mutant prophages, were MC (2 μ g/ml) induced in the presence of EcCIEDL933 and the transfer of the island (**E**) or the phage titres (**F**) were determined using *E. coli* 594 as recipient strain. The means of the colony forming units (CFUs) or phage forming units (PFUs) and SD of three independent experiments are presented (n=3). A one-way ANOVA with Dunnett's multiple comparisons test was performed to compare mean differences between wt and individually point-mutations. Adjusted p values were as follows: ns>0.05; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

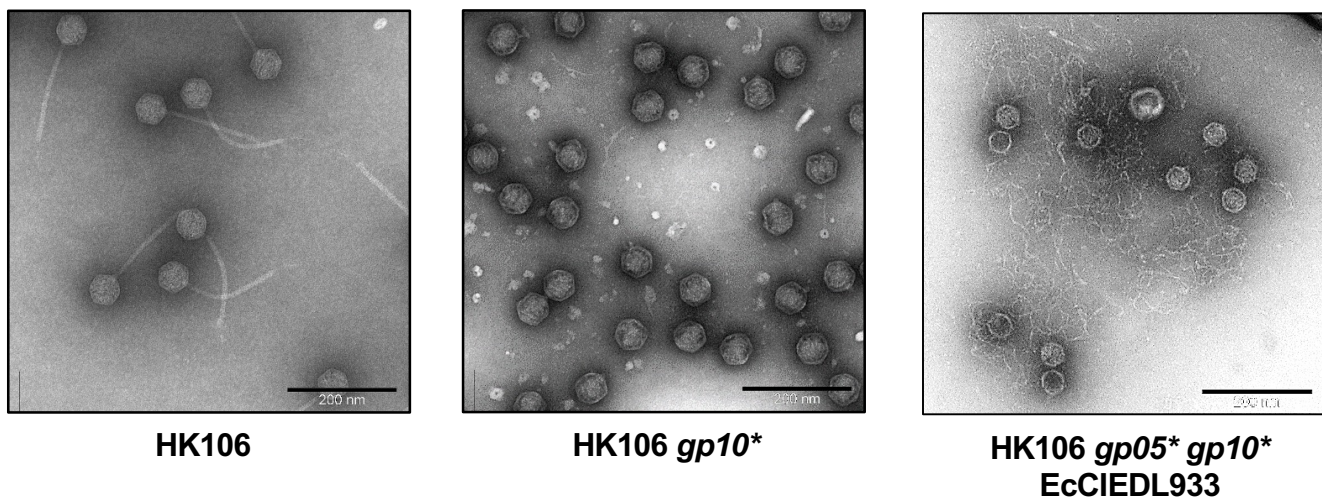
A**B**

Figure 5. EcCIEDL933 requires phage tails to generate infective particles.

(A) Lysogenic strains for phage HK106 (wt, $\Delta gp10$ or $\Delta gp14$) carrying EcCIEDL933 were MC induced (2 $\mu\text{g/ml}$) and the phage and PICI titres were determined using *E. coli* 594 as recipient strain. Note that *gp10* and *gp14* encode major tail and tape-measure proteins, respectively, required for phage tail formation. The means of the colony forming units (CFUs) or phage forming units (PFU) and SD of three independent experiments are presented (n=3). A one-way ANOVA with Dunnett's multiple comparisons test was performed to compare mean differences between wt and individually point-mutations. Adjusted p values were as follows: *ns*>0.05; **p*≤0.05; ***p*≤0.01; ****p*≤0.001; *****p*≤0.0001.

(B) Electron microscopy analysis of different MC-induced HK106 lysogenic strains (wt and mutants) in presence or absence of EcCIEDL933. Different fields are shown, containing only HK106 phage particles, HK106-sized capsid particles or EcCIEDL933-sized capsid particles. EcCIEDL933 particles have smaller heads. Scale bars are 200 nm.

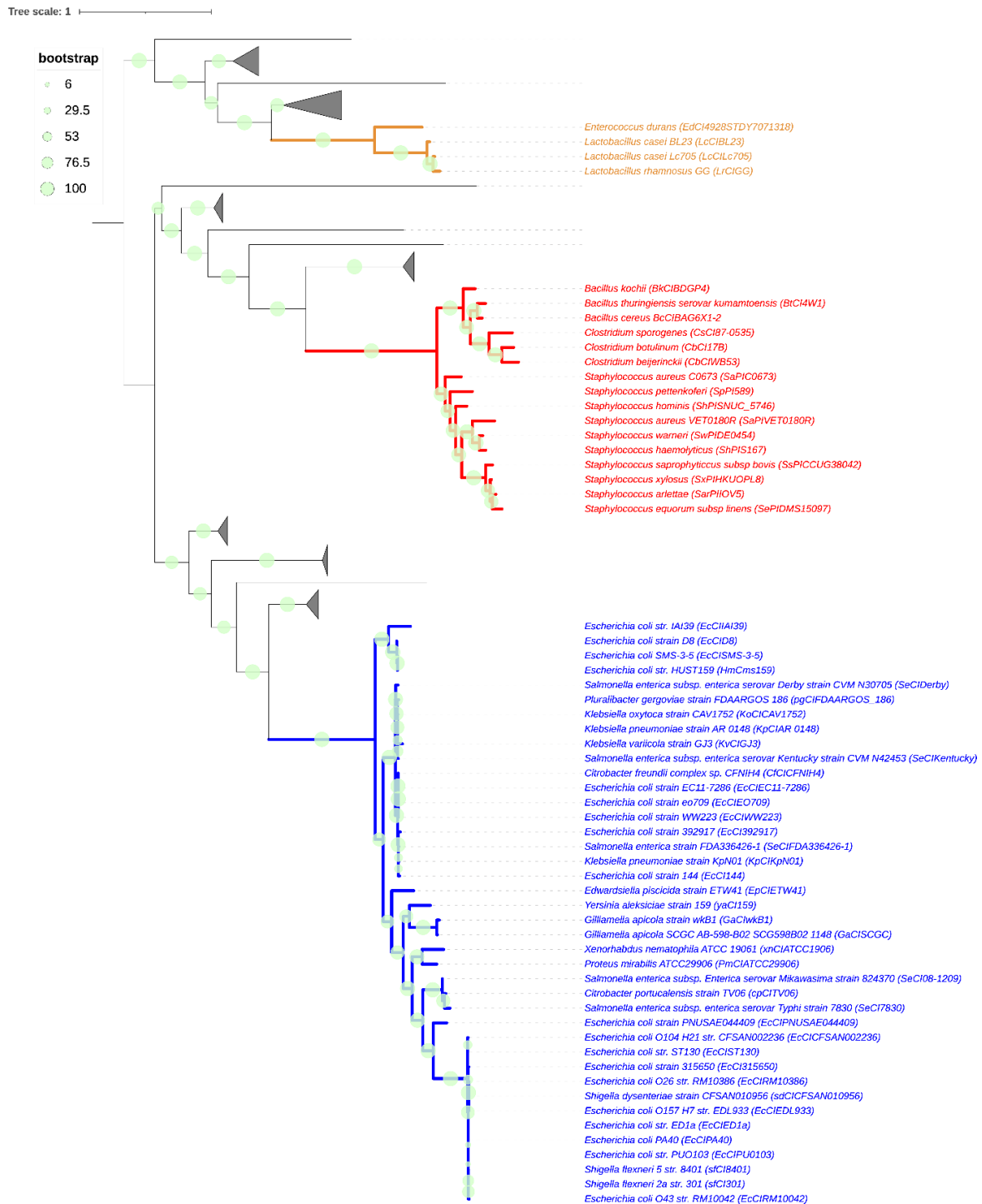


Figure 6. Capsid of cf-PICI form three distinct phylogenetic groups and are evolutionarily separated from phage homologs.

Phylogenetic trees inferred from the alignment of 61 capsid homologs from cf-PICI and the best 113 phage capsid homologs. Phages branches are collapsed and shown as triangles. The different colors on the branches indicate the three different clades, formed by either *Gammaproteobacteria* (in blue), *Lactobacillus* (in orange) and a broader set of *Firmicutes* (in red). Bootstrap values for the main branches are indicated as grey circles. The tree was visualized and edited in iTOL (Letunic et al., 2021). Phylogenetic tree resulting from the alignment of Capsid proteins from 61 cf-PICIs and 3725 phage.

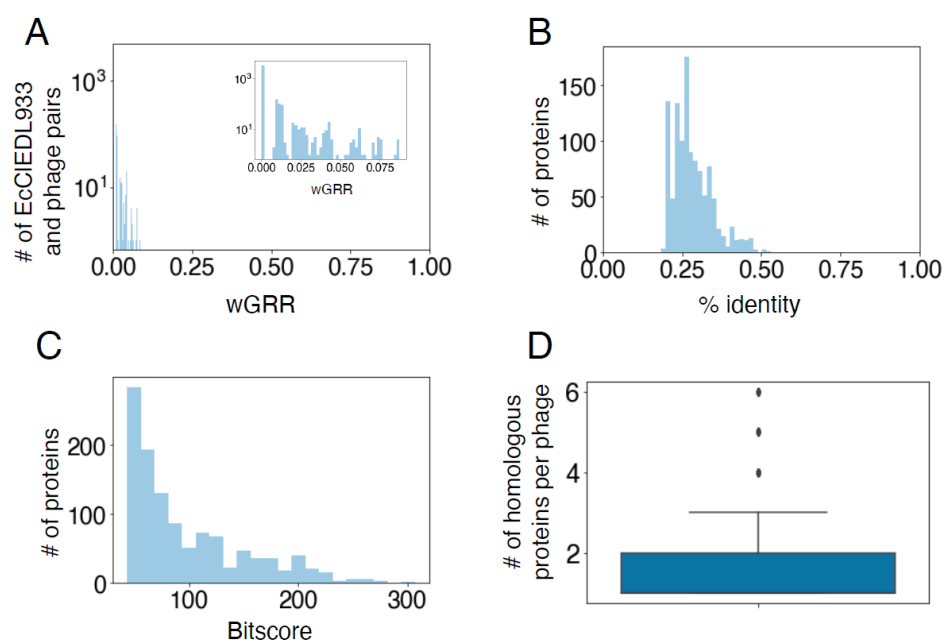


Figure S1. Genomic comparison between EcCIEDL933 and 3725 phage genomes.

- (A) Distribution of wGRR values between EcCIEDL933 and phage genomes. Inset shows a zoomed version between wGRR of zero and 0.1.
- (B) Distribution of the protein identity percentages, for all the potential homologs between EcCIEDL933 and phage genomes.
- (C) Distribution of the bitscores for all the potential homologs between EcCIEDL933 and phage genomes.
- (D) Boxplot with the number of homolog genes per phage, for all the phages in the database.

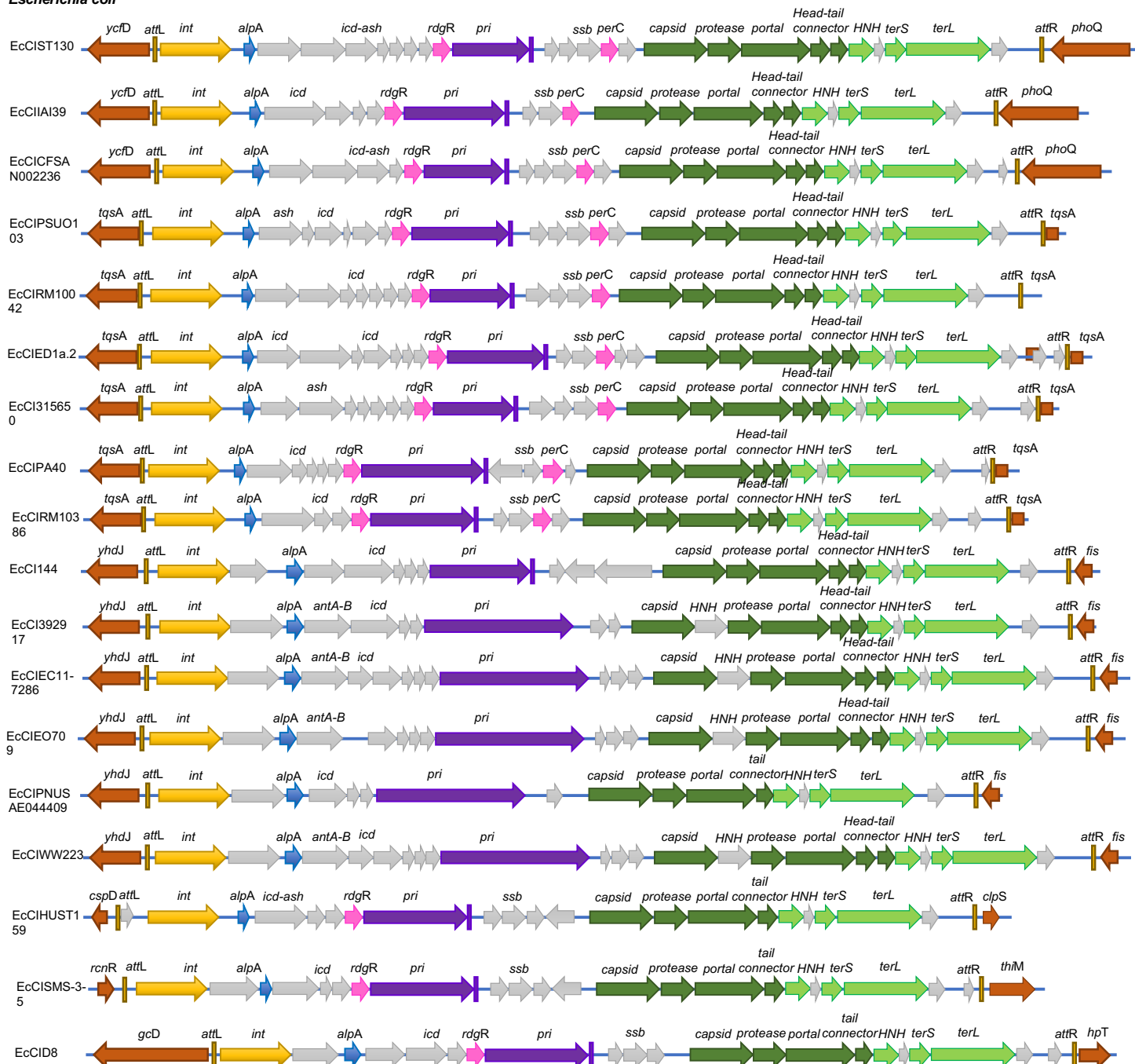
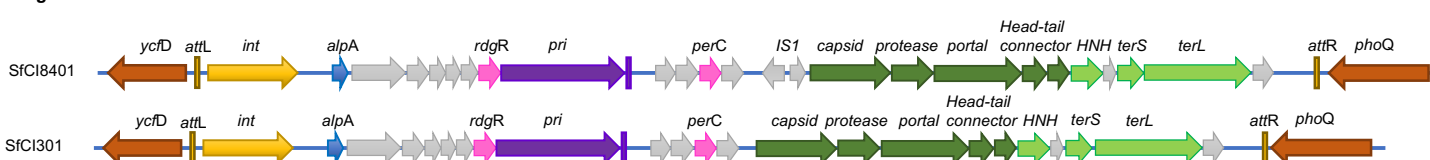
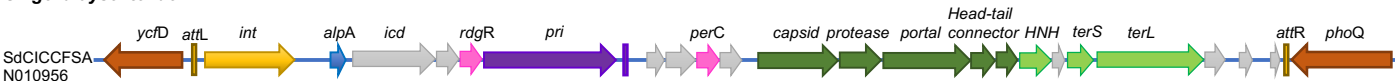
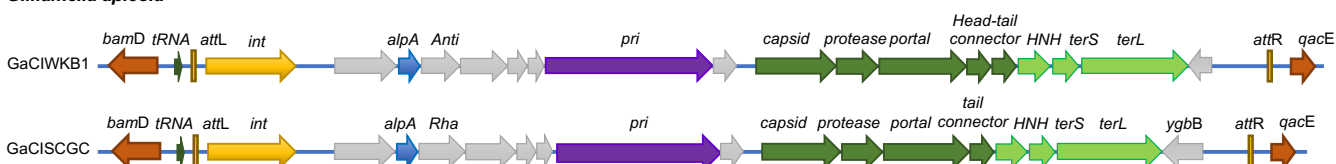
Escherichia coli*Shigella flexneri**Shigella dysenteriae**Gilliamella apicola*

Figure S2. Genome maps for cf-PICIs, from different Proteobacteria species.

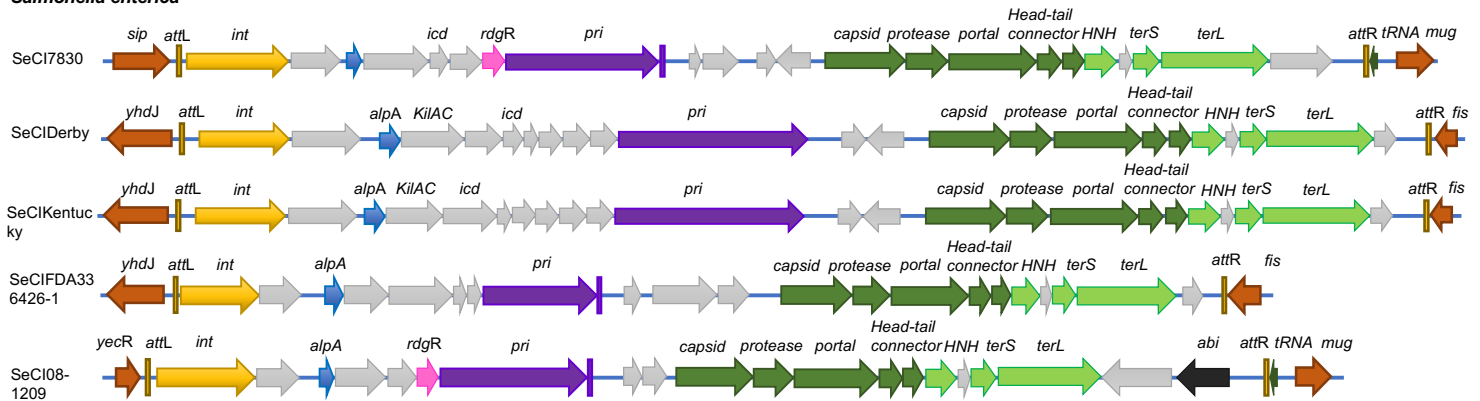
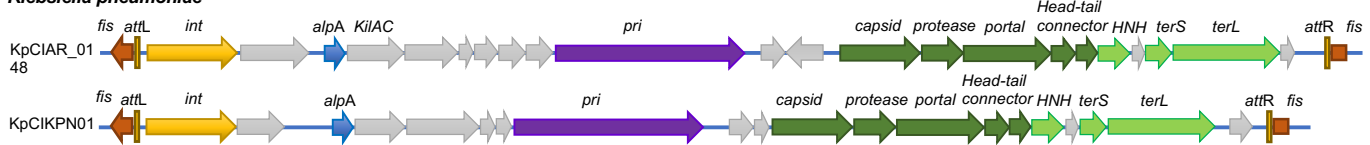
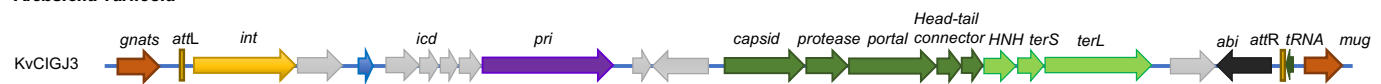
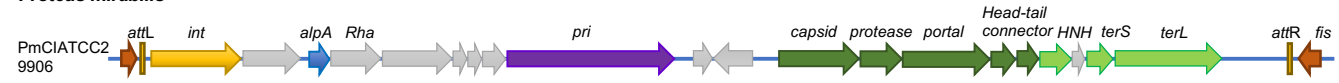
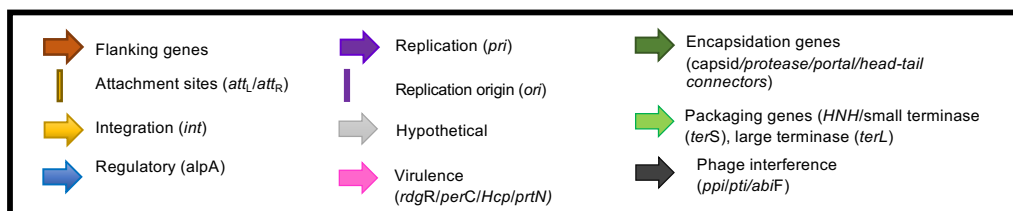
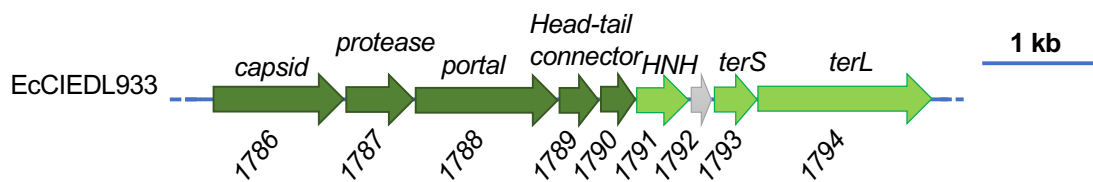
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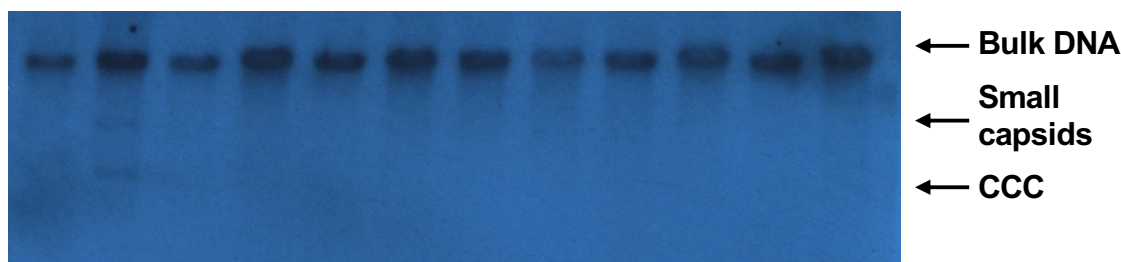
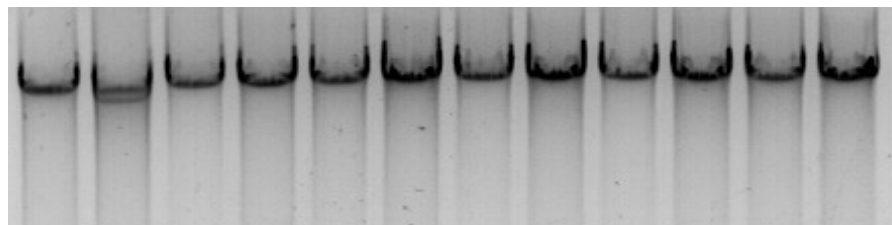
Figure S2. Genome maps for cf-PICIs, from different Proteobacteria species. Genomes are aligned according to the prophage convention, with the integrase genes (*int*) present at the left end of the maps. Genes are colored according to their sequence and function (see box for more details).



Phage **HK106**

EcCIEDL933 **wt** **1786*** **1787*** **1788*** **1789*** **1790***

0 240 0 240 0 240 0 240 0 240 0 240 min



Phage **HK106**

EcCIEDL933 **wt** **1791*** **1792*** **1793*** **1794***

0 240 0 240 0 240 0 240 0 240 min

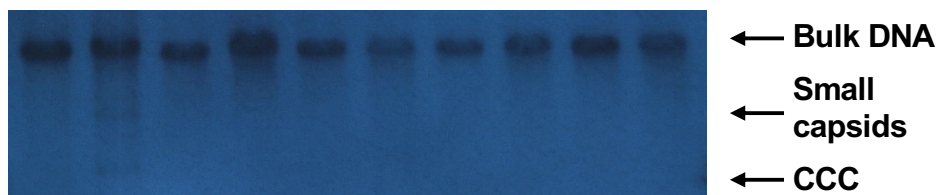
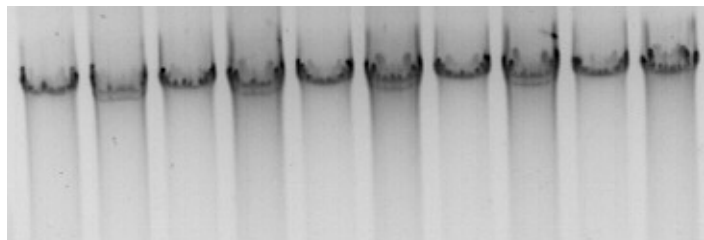


Figure S3. Replication of the EcCIEDL933 mutants.

Lysogenic strains for phage HK106, carrying different EcCIEDL933 versions (wt or encoding individually point-mutations in the indicated genes), were MC (2 µg/ml) induced, samples were taken at the indicated time points (min) and processed to prepare minilysates, which were separated on a 0.7% agarose gel (upper panel), and Southern blotted (lower panel) with an EcCIEDL933 probe.

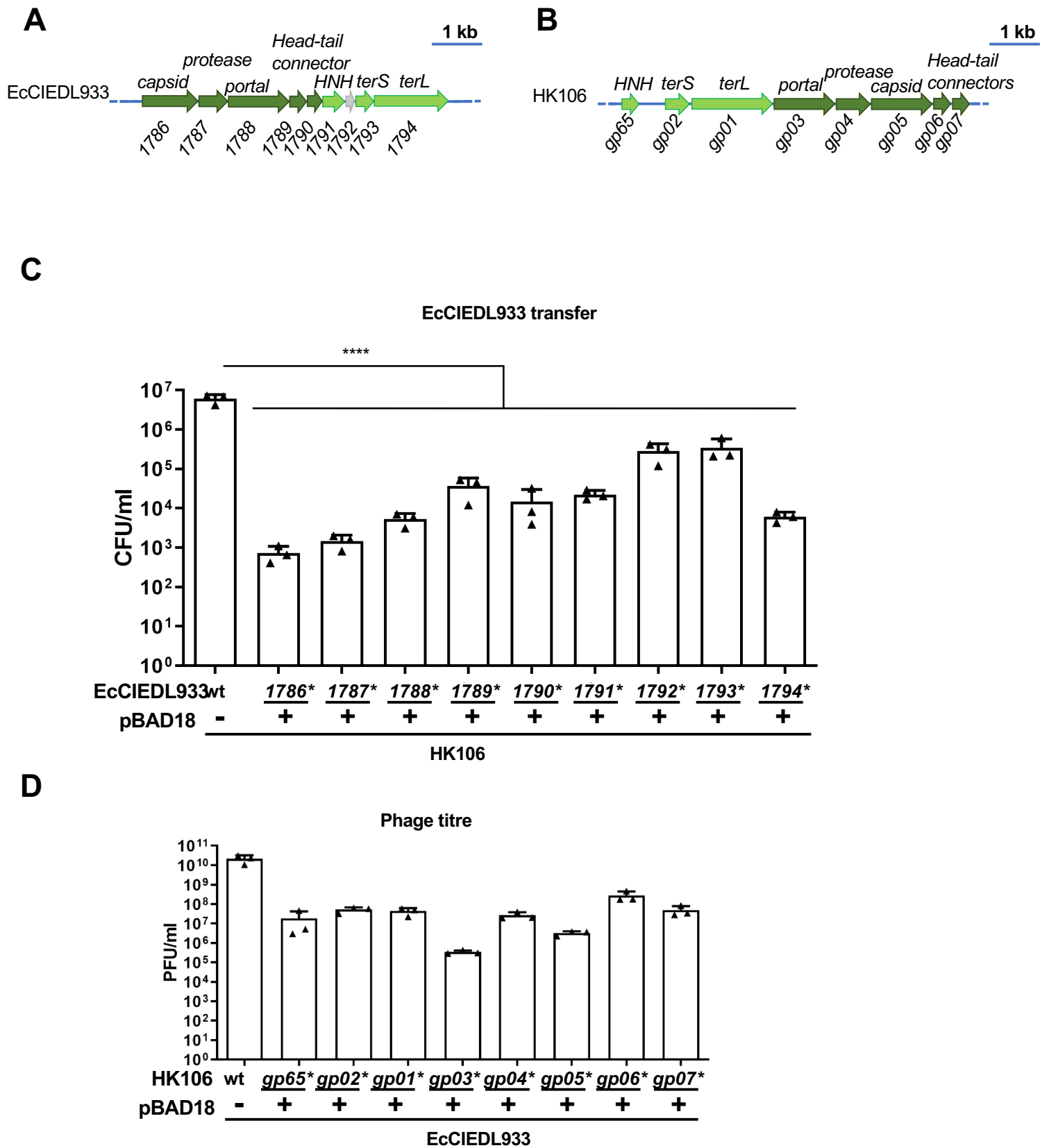


Figure S4. Complementation of the different EcCIEDL933 and HK106 mutants restore PICI transfer and phage titres.

Genetic map of the EcCIEDL933 packaging module (**A**) or phage HK106 packaging module (**B**).

(**C**) Lysogenic strains for phage HK106 carrying different EcCIEDL933 versions (wt or mutants) were complemented with pBAD18 derivative plasmids expressing the different genes under study (+). Then, these strains were MC (2 μ g/ml) and arabinose (0.02%) induced and the transfer of the different islands analysed using *E. coli* 594 as recipient. (**D**) Lysogenic strains for phage HK106 (wt or mutants), carrying wt EcCIEDL933, were complemented with the corresponding pBAD18 derivative plasmid expressing the indicated phage gene (+). The strains were MC (2 μ g/ml) and arabinose (0.02%) induced and the phage titres determined using *E. coli* 594 as recipient. The means of the colony forming units (CFUs) or phage forming units (PFUs) and SD of three independent experiments are presented (n=3). A one-way ANOVA with Dunnett's multiple comparisons test was performed to compare mean differences between wt and individually point-mutations. Adjusted p values were as follows: ns>0.05; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

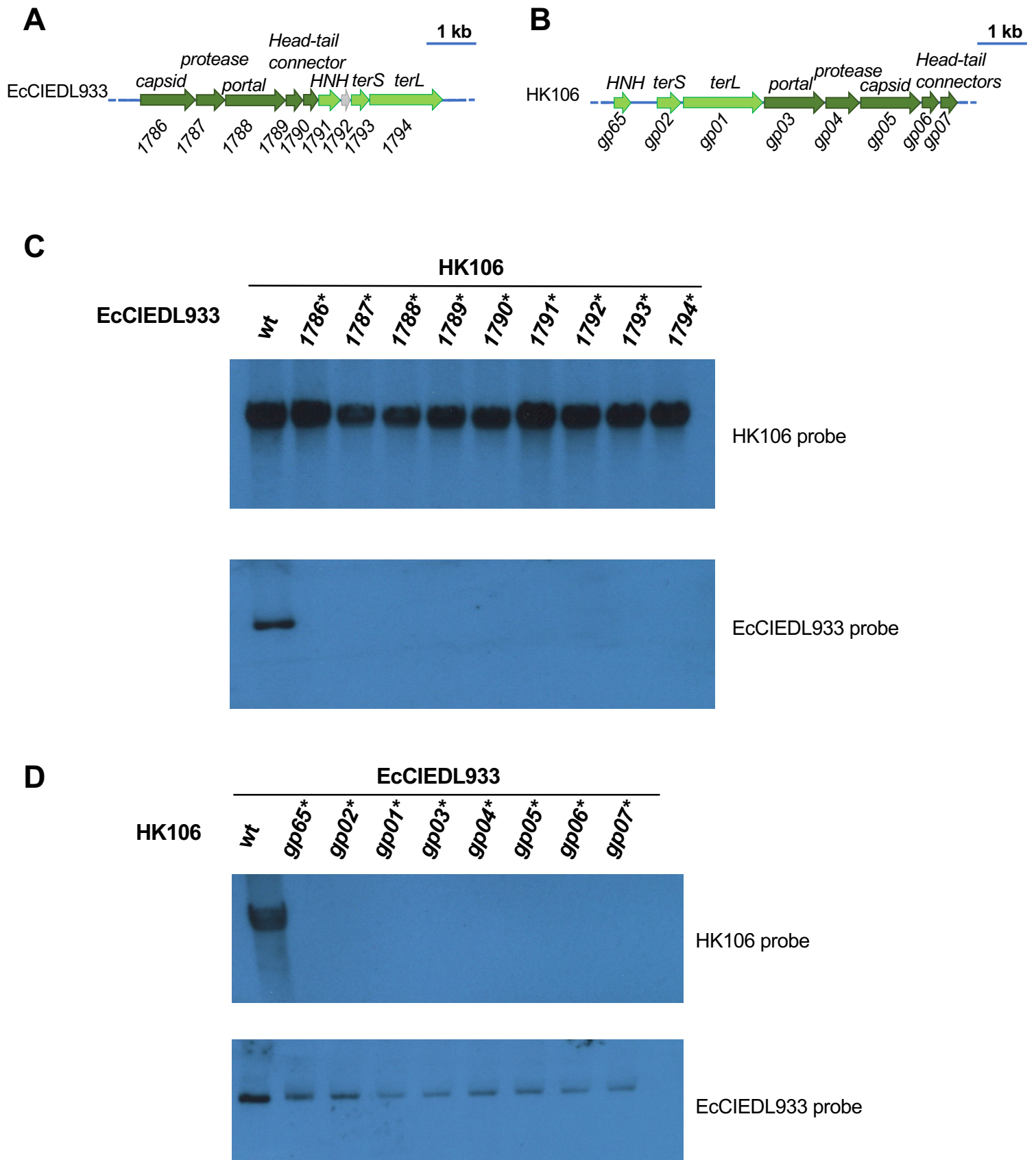


Figure S5. Effect of EcCIEDL933 or phage HK106 gene mutations on the formation of PICI or phage infective particles.

Genetic map of the EcCIEDL933 packaging module (**A**) or phage HK106 packaging module (**B**).

(**C**) Lysogenic strains for phage HK106, carrying the wt of the different EcCIEDL933 mutant versions, were MC induced, and the DNA from the infective particles purified and analysed by Southern blot, using phage- or PICI-specific probes.

(**D**) EcCIEDL933-positive strains, lysogenic for different version of the HK106 prophage (wt or carrying the indicated mutations), were MC induced, and the DNA from the infective particles purified and analysed by Southern blot, using phage- or PICI-specific probes.

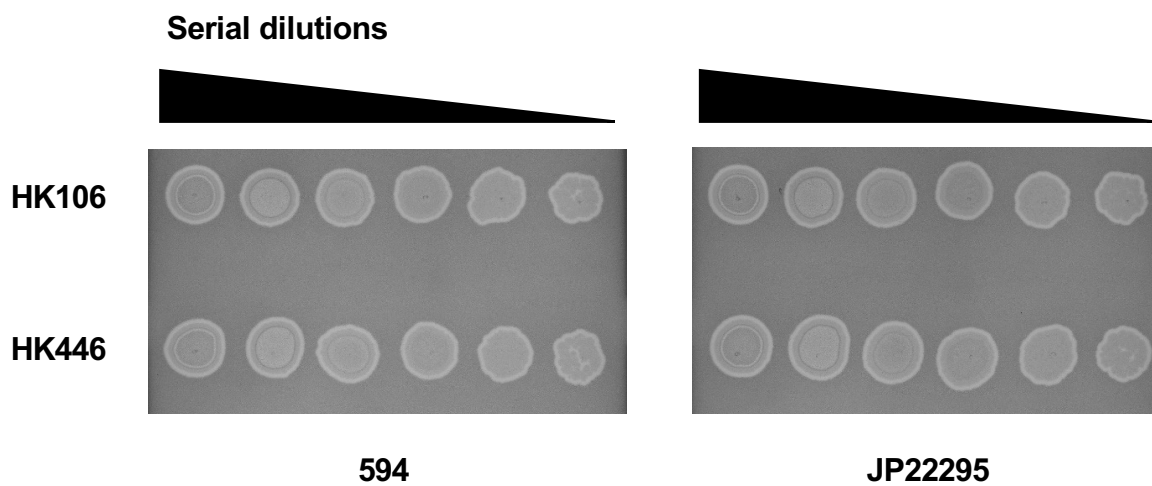


Figure S6. EcCIEDL933 does not block helper phage reproduction.

Phage HK106 and HK446 dilutions were spotted on non-lysogenic *E. coli* strain 594, or on strain JP22295 (594 EcCIEDL933 *cat* positive).

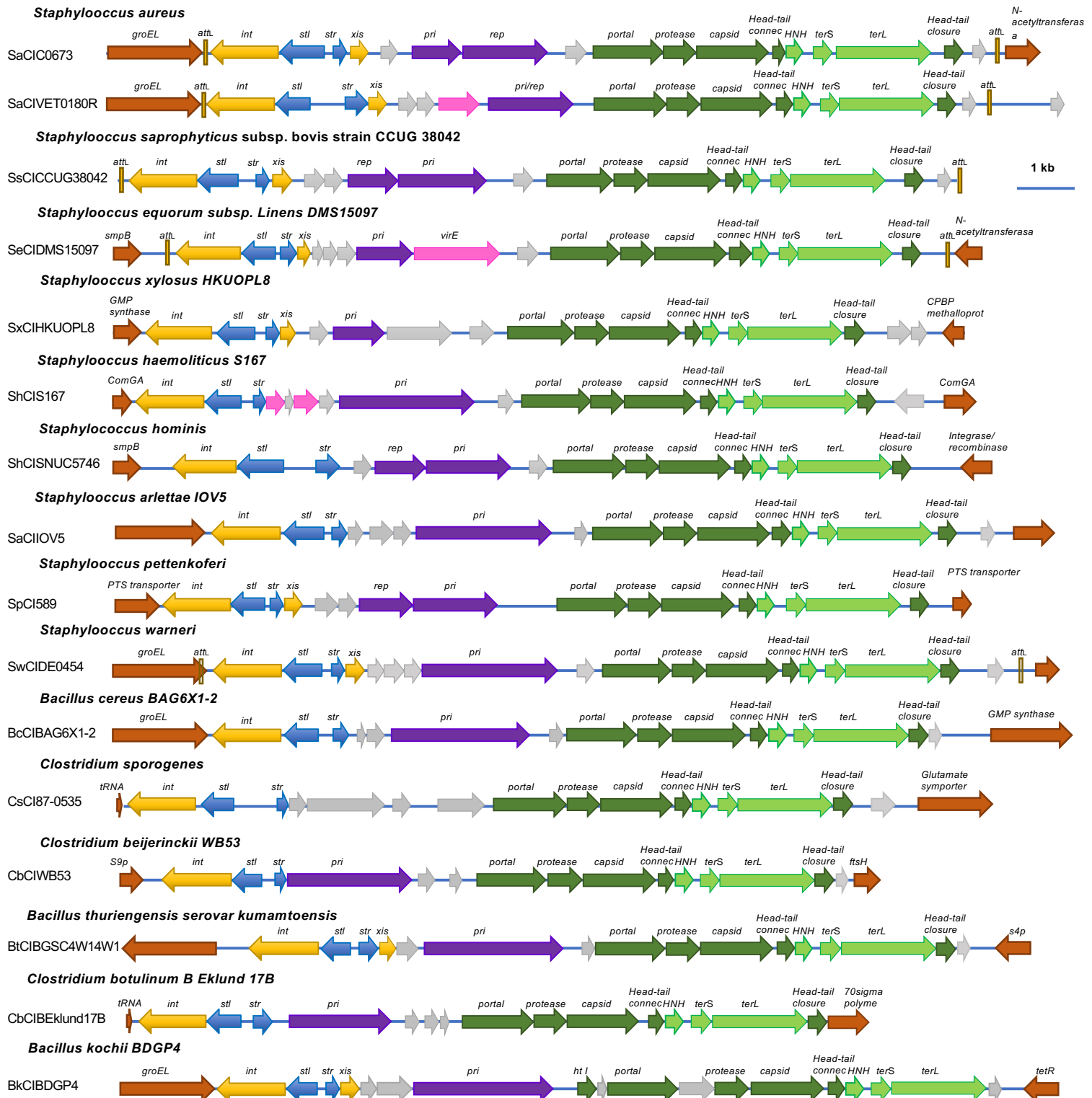


Figure S7. Genome maps for cf-PICs present in Firmicutes.

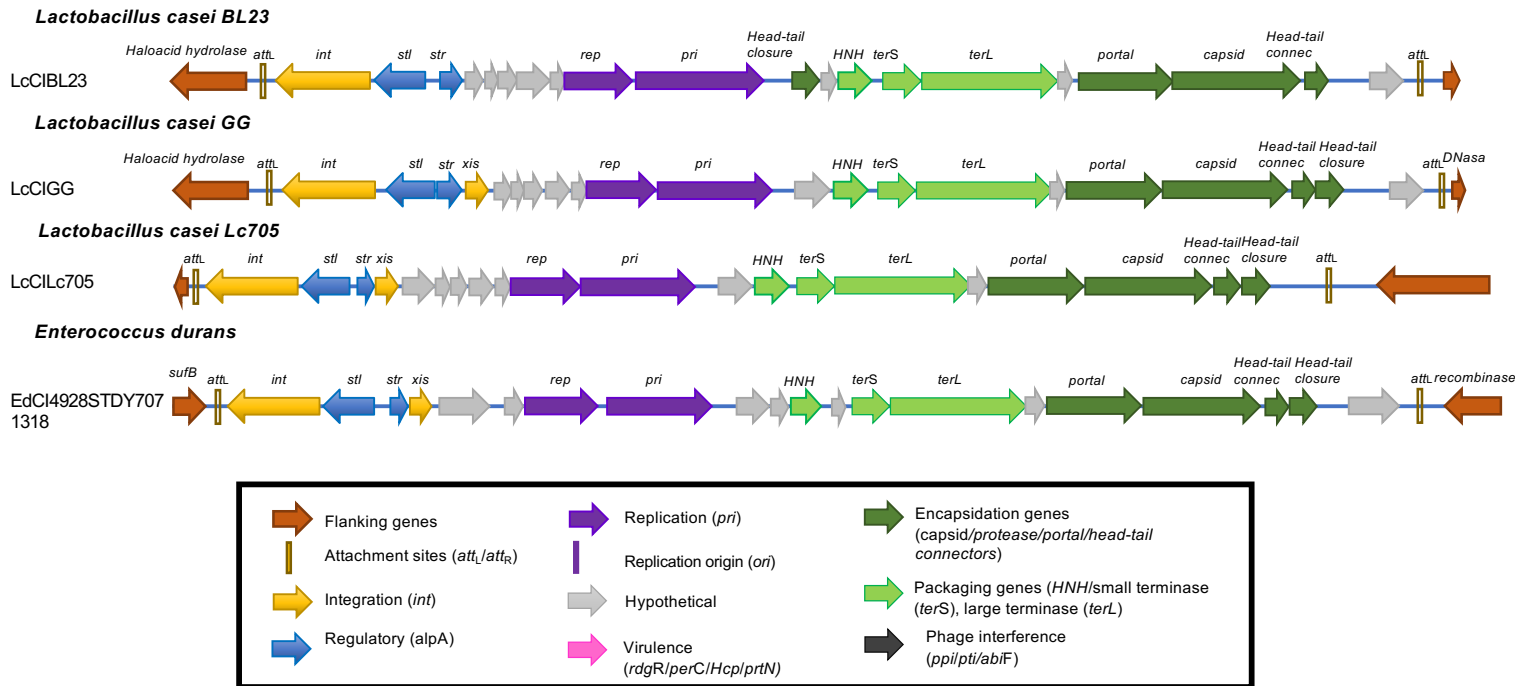


Figure S7. Genome maps for cf-PICs present in Firmicutes. Genomes are aligned according to the prophage convention, with the integrase genes (*int*) present at the left end of the islands. Genes are colored according to their sequence and function (see box for more details).

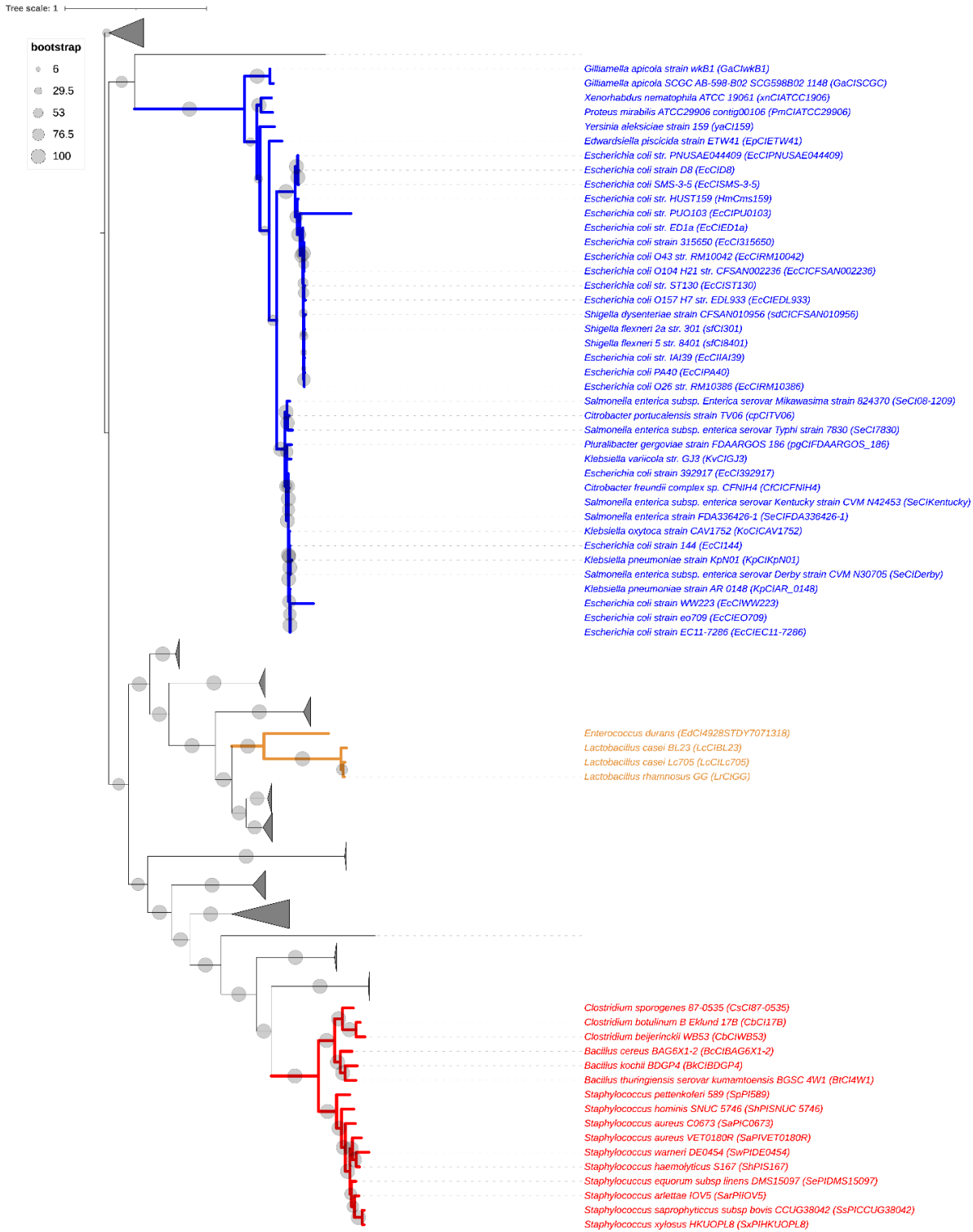


Figure S8. TerL of cf-PICI form three distinct phylogenetic groups and are evolutionarily separated from phage homologs.

Phylogenetic trees inferred from the alignment of 60 TerL homologs from cf-PICI and the best 420 phage TerL homologs. Phages branches are collapsed and shown as triangles. The different colors on the branches indicate the three different clades, formed by either Gammaproteobacteria (in blue), *Lactobacillus* (in orange) and a broader set of Firmicutes (in red). Bootstrap values for the main branches are indicated as grey circles. The tree was visualized and edited in iTOL ((Letunic et al., 2021).