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1 **Bacterial inhibition of CD8⁺ T-cells mediated cell death promotes neuroinvasion and**
2 **within-host persistence**

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26 **Abstract**

27 Central nervous system infections are amongst the most severe^{1,2}, yet the mechanisms by which
28 pathogens access the brain remain poorly understood. The model microorganism *Listeria*
29 *monocytogenes* (*Lm*) is a major foodborne pathogen that causes neurolisteriosis, one of the
30 deadliest central nervous system infections^{3,4}. While immunosuppression is a well-established
31 host risk factor for neurolisteriosis^{3,5}, little is known regarding the bacterial factors underlying
32 *Lm* neuroinvasion. We have developed a clinically-relevant experimental model of
33 neurolisteriosis, using hypervirulent neuroinvasive strains⁶ inoculated in a humanized mouse
34 model of infection⁷, and we show that the bacterial protein InlB protects infected monocytes
35 from CD8⁺ T-cells Fas-mediated cell death, in a c-Met/PI3-kinase/FLIP-dependent manner.
36 This blockade of anti-*Lm* specific cellular immune response lengthens infected monocytes
37 lifespan, favoring *Lm* transfer from infected monocytes to the brain. The intracellular niche
38 created by InlB-mediated cell-autonomous immunosuppression also promotes *Lm* fecal
39 shedding, accounting for its selection as a *Lm* core virulence gene. Here, we have uncovered an
40 unanticipated specific mechanism by which a bacterial pathogen confers to the cells it infects
41 an increased lifespan by rendering them resistant to cell-mediated immunity. This promotes *Lm*
42 within-host persistence and dissemination to the central nervous system, and transmission.

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50 **One Sentence Summary:** *Listeria* blocks CD8⁺ T-cells killing and promotes neuroinvasion

51 Bacterial infections of the central nervous system (CNS) are often fatal^{1,2}, yet little is known
52 regarding the molecular mechanisms underlying microbial neuroinvasion. *Listeria*
53 *monocytogenes* (*Lm*) is a foodborne pathogen that causes neurolisteriosis, one of the deadliest
54 CNS infections^{3,4}. Consistent with its key role in immunity against *Lm*, T-cell based
55 immunosuppression is a major host risk factor for neurolisteriosis^{3,5}. In contrast, the bacterial
56 factors promoting *Lm* neuroinvasion and their mechanisms of action are poorly understood.
57 Earlier studies have pointed towards the involvement of monocytes in transferring *Lm* from the
58 blood to the brain^{8,9}. However, these investigations were performed with reference laboratory
59 *Lm* strains, which are actually poorly neuroinvasive⁶, and require very high bacterial inocula to
60 induce some degree of CNS infection in experimental animal models. This is consistent with
61 the observation that these strains belong to clonal complexes very rarely responsible for human
62 neurolisteriosis^{3,6}. In contrast, clinically-associated clonal complexes are hypervirulent and
63 more neuroinvasive⁶. In order to investigate the mechanisms underlying *Lm* neuroinvasion, we
64 developed a clinically-relevant experimental model of neurolisteriosis based on the inoculation
65 of hypervirulent neuroinvasive *Lm* strains⁶ in a humanized mouse model⁷.

66 **Infected inflammatory monocytes mediate *Lm* neuroinvasion**

67 We inoculated orally *Lm* in humanized KIE16P mice⁷. In contrast to the reference laboratory
68 strain EGDe that belongs to clonal complex (CC) 9^{10,11}, clinical isolates belonging to the
69 hypervirulent clonal complexes CC1, 4 and 6 systematically induce high-level neuroinvasion,
70 as previously reported⁶, starting at 3 days post-inoculation (dpi) (Fig. 1a). At 5 dpi, the bacterial
71 brain load is the same with or without administration of gentamicin (Fig. 1b), an antibiotic that
72 kills extracellular but not intracellular *Lm* (Extended Data Fig. 1a, b), indicating that
73 intracellular bacteria are involved in neuroinvasion. Consistent with the occurrence of
74 neuroinvasion in the presence of gentamicin, *Lm* is detected intracellularly in the blood and
75 spleen, mainly in inflammatory monocytes (CD45⁺ CD11b⁺ Ly6C⁺ CD3⁻ CD19⁻ CD11c⁻ Ly6G⁻
76) (Fig. 1c, d and Extended Data Fig. 1c-e), suggesting that monocytes are involved in *Lm*
77 neuroinvasion. This was assessed by infecting *Ccr2*^{-/-} mice, in which monocytes are retained in
78 the bone marrow and are therefore less abundant in the blood and spleen¹² (Extended Data Fig.
79 1f). Indeed, from day 1 to 3 post-inoculation, more bacteria are gradually recovered in the brain
80 of WT mice as compared to *Ccr2*^{-/-} mice (Fig. 1e). Moreover, the transfer of infected monocytes
81 from donor-infected mice into gentamicin-treated uninfected recipient mice is sufficient to
82 induce neuroinvasion, and *Lm* can be recovered from the brain of recipient mice as early as day
83 2 post-transfer (Fig. 1f). In contrast, the transfer of infected monocytes from mice expressing
84 the diphtheria toxin (DT) receptor in myeloid cells (*LysM-CreER*^{T2}×iDTR) into recipient mice
85 treated with DT leads to liver and spleen infection but no brain infection, even as late as 4 days
86 post-transfer (Extended Data Fig. 1g, h). Together, these results indicate that infected
87 monocytes are necessary and sufficient to induce neuroinvasion.

88 Infected monocytes were observed adhering to the endothelium of blood vessels in brain
89 sections of infected mice (Fig. 1g and Extended Data Fig. 2a-c). In these adhering monocytes,
90 *Lm* polymerizing actin was observed (Extended Data Fig. 2d and movie S1), significantly more

91 than in spleen monocytes (Extended Data Fig. 2e, f), and occasionally adjacent to infected
92 endothelial cells (Fig. 1h and movie S1). Moreover, the transfer of monocytes infected with
93 *Lm* Δ *actA* isogenic mutant, unable to polymerize actin and mediate cell-to-cell spread^{13,14}, fails
94 to induce neuroinvasion (Fig. 1f). Together, these results demonstrate that *Lm* accesses the brain
95 parenchyma by cell-to-cell spread from adhering bloodborne infected inflammatory monocytes
96 (Fig. 1i). These results are in line with previous reports^{8,9} obtained using poorly-neuroinvasive
97 *Lm* strains, suggesting that neuroinvasive *Lm* strains invade the CNS in a similar manner, albeit
98 to a far greater efficiency (up to 3 orders of magnitude) (Fig 1a).

99

100 **InIB increases the number of infected inflammatory monocytes and mediates** 101 **neuroinvasion**

102 Having identified infected monocytes as critically involved in the onset of neuroinvasion, we
103 aimed to identify the bacterial factors responsible for *Lm* neuroinvasiveness. Given the well-
104 established role of InIA and InIB in *Lm* crossing of host barriers^{7,15}, we investigated their
105 respective role in neuroinvasion. To bypass the well-established role of InIA in the crossing of
106 the intestinal barrier, we inoculated KIE16P mice via the iv route. While InIA plays no role in
107 neuroinvasion (Fig. 2a, Extended Data Fig. 3a-d), InIB plays a major role: the Δ *inIB* mutant is
108 significantly less neuroinvasive than its WT parental strain in co-infection experiments (Fig.
109 2b, Extended Data Fig. 3a, e). Of note, the Δ *inIAB* mutant is not less neuroinvasive than the
110 Δ *inIB* mutant, ruling out that InIA would act in a conjugated manner with InIB, as it has been
111 reported for placental invasion (Extended Data Fig. 3f, g)⁷. After oral inoculation, a similar
112 difference in neuroinvasiveness is observed between all WT neuroinvasive strains and their
113 corresponding Δ *inIB* mutant (Fig. 2c, Extended Data Fig. 3h). The involvement of InIB in
114 neuroinvasion is also observed upon separate inoculation with either WT-*Lm* or *Lm* Δ *inIB* (Fig.
115 2d, e). InIB contribution to neuroinvasion increases over time (Fig. 2f, g and Extended Data

116 Fig. 3i, j) and *Lm* Δ *inlB* never reaches the brain infection level of WT-*Lm* (Fig. 2f, g). These
117 results were confirmed by gene complementation (Extended Data Fig. 3f, g).

118 This critical role of *inlB* in *Lm* neuroinvasion was unexpected, as this gene is part of *Lm* core
119 genome and is therefore present in all *Lm* strains, including the poorly neuroinvasive reference
120 strains EGDe and 10403S. Neuroinvasive *Lm* isolates actually strongly upregulate the *inlAB*
121 operon as compared to EGDe and 10403S, both *in vitro* in liquid culture and *in vivo* in infected
122 spleen (Fig 2h, i and Extended Data Fig. 3k-m). To assess the impact of InlB expression level
123 on neuroinvasiveness, we complemented the EGDe Δ *inlB* mutant with the *inlB* gene sequence
124 from either EGDe or CC4 (primary sequences share 93% identity, Extended Data Fig. 4a) so
125 that *inlB* within-host transcription levels are similar to that of endogenous *inlB* in CC4
126 (Extended Data Fig. 4b). These complemented strains are as neuroinvasive as WT-CC4,
127 whereas CC4 Δ *inlB* is as poorly neuroinvasive as EGDe Δ *inlB* (Fig. 2j and Extended Data Fig.
128 c, d). Consistently, CC4 Δ *inlB* complemented with the *inlB* EGDe allele expressed to the level
129 of CC4 *in vivo* is as neuroinvasive as WT-CC4 (Fig. 2j and Extended Data Fig. 4 c). Altogether,
130 these results establish that InlB overexpression is the critical factor of *Lm* neuroinvasiveness.

131 We next evaluated the contribution of InlB to the infection of inflammatory monocytes, having
132 shown their essential role in *Lm* neuroinvasion (Fig. 1). From 3 dpi, the blood bacterial load is
133 higher for WT-*Lm* than *Lm* Δ *inlB* (Fig. 2k, l). Moreover, InlB significantly increases the number
134 of *Lm*-infected inflammatory monocytes in the blood and spleen (Fig. 2m, n and Extended Data
135 Fig. 5a, b). In addition, InlB-mediated neuroinvasion is abrogated in *Ccr2*^{-/-} mice (Fig. 2o and
136 Extended Data Fig. 5c), indicating that InlB contribution to neuroinvasion implicates infected
137 monocytes. At early time points, when equal numbers of WT and Δ *inlB*-bacteria are retrieved
138 from the blood (1-2 dpi, Extended Data Fig. 5d), equivalent numbers of WT- and Δ *inlB*-infected
139 adhering monocytes are observed in the brain (Extended Data Fig. 2c), showing that InlB does
140 not alter monocyte ability to adhere to brain blood vessels. Moreover, no impact of InlB on

141 bacterial growth is detected upon direct injection of *Lm* into the brain (Extended Data Fig. 5e,
142 f). Altogether, these results indicate that InlB leads to an increased number of circulating
143 infected monocytes, which are themselves required for *Lm* neuroinvasion.

144 Although InlB has been described as an invasion protein mediating *Lm* internalization into non-
145 phagocytic cells^{16,17}, the entry of hypervirulent *Lm* in inflammatory monocytes, which are
146 professional phagocytes, is InlB-independent (Extended Data Fig. 5g-i). This indicates that InlB
147 contribution to neuroinvasion is independent of its capacity to induce internalization.

148

149 **InlB-mediated neuroinvasion requires a functional adaptive immune system**

150 T-cell immunosuppression is a well-established risk factor for neurolisterosis^{3,5,18-20}, and
151 hypervirulent *Lm* clones that express the most InlB (Fig. 2h, i) tend to infect patients that are
152 the least immunosuppressed⁶. We therefore hypothesized that InlB may exhibit
153 immunosuppressive properties. Interestingly, treatment with ciclosporin, a prototypic T-cell
154 immunosuppressant, abrogates InlB-dependent neuroinvasion (Fig. 2c, Fig. 3a, b, and Extended
155 Data Fig. 3h), implying that InlB contribution to *Lm* neuroinvasion requires a functional
156 adaptive immunity. Consistently, no difference in neuroinvasion is observed between WT-*Lm*
157 and *Lm* Δ *inlB* before 3 to 4 dpi, at a time when adaptive immune responses are not yet expected
158 to be functional²¹ (Fig. 2f, g and Extended Data Fig. 3i, j). Of note, ciclosporin treatment of
159 EGDe-inoculated mice also leads to a slight increased number of infected inflammatory
160 monocytes (Fig. 3c) and increased neuroinvasion (Fig. 3d, e), in line with an
161 immunomodulatory effect of InlB overexpression in hypervirulent isolates. Strikingly, InlB
162 contribution to neuroinvasion is fully abrogated in *Rag2*^{-/-} mice (Fig. 3f and Extended Data Fig.
163 6a-c), that lack functional lymphoid cells, demonstrating their requirement for InlB to exert its
164 immune-mediated effect. The effect of InlB is also abrogated in *CD3 ϵ* ^{-/-}, but not in *muMt*^{-/-}
165 mice (Fig. 3g and Extended Data Fig. 6d-f), and thus depends on T- but not B-lymphocytes.

166 Specifically, CD8⁺ T-cells depletion fully abrogated the effect of InlB (Fig. 3h and Extended
167 Data Fig. 6g, h). Interestingly, specific anti-*Lm* CD8⁺ T-cells are induced to the same extent by
168 WT-*Lm* and *Lm* Δ *inlB* isogenic mutant (Extended Data Fig. 7a-d), and accordingly, mice
169 inoculated with WT-*Lm* or *Lm* Δ *inlB* display the same level of protective immunity after a
170 second challenge (Extended Data Fig. 7e). Since InlB-mediated neuroinvasion relies on
171 infected monocytes and is detectable in co-infection experiments, we reasoned that InlB may
172 protect specifically infected monocytes from anti-*Lm* T-CD8⁺-mediated specific killing. We
173 therefore performed cytotoxic T-lymphocyte (CTL) assays: infected or uninfected
174 inflammatory monocytes and activated CD8⁺ T-cells were retrieved from mice, either
175 uninfected or infected with WT-*Lm* or *Lm* Δ *inlB*, co-incubated and assessed for cell death (Fig.
176 3i). Strikingly, WT-*Lm*-infected monocytes are protected from CD8⁺ T-cells-mediated cell
177 death, whereas no difference in cell death is observed in uninfected monocytes (Fig. 3j and
178 Extended Data Fig. 8a, b). InlB can be either associated to the bacterial surface or released in
179 the surrounding medium¹⁷. Consistent with a cell-autonomous effect of InlB, the surface-
180 associated InlB is required for InlB-mediated neuroinvasion (Extended Data Fig. 8c-f). The fact
181 that infection of monocytes is clonal (Extended Data Fig. 8g) and that InlB contribution to
182 neuroinvasion is detectable in co-infection experiments is also fully consistent with the finding
183 that InlB acts in a cell-autonomous manner.

184

185 **InlB blocks Fas-mediated cell death, protecting infected cells from killing by CD8⁺ T cells**

186 The cytotoxicity mediated by CD8⁺ T-cells from either WT-*Lm* and *Lm* Δ *inlB* infected mice is
187 similar (Fig. 3j), confirming that InlB has no impact on immunization (Extended Data Fig. 7).
188 Since CD8⁺ T-cells cytotoxicity relies on the perforin-granzyme and Fas-Fas ligand pathways²²,
189 we performed CTL assays in Perforin- and Fas-deficient mice (*Prfl* KO and *Fas*^{*lpr-cg*},
190 respectively). InlB-mediated protection against monocyte killing by CD8⁺ T-cells is fully

191 preserved in *Prfl* *KO* mice (Extended Data Fig. 8h). In sharp contrast, the inhibitory effect of
192 InlB on infected monocytes cell death is fully abrogated in *Fas*^{*lpr-cg*} mice (Extended Data Fig.
193 8i). This indicates that InlB blocks Fas-mediated killing whereas the perforin pathway is not
194 involved. Consistently, monocytes infected with WT-*Lm*, but not *LmΔinlB*, are resistant to
195 FasL-induced apoptosis, while surface expression of Fas is not affected by InlB (Fig. 3k and
196 Extended Data Fig. 8j, k). Accordingly, in mice treated with a pharmacological inhibitor of
197 caspase-8, the downstream effector of Fas²³, *LmΔinlB* becomes as neuroinvasive as WT-*Lm*
198 (Fig. 3l, m). In infected mice, the half-life of monocytes infected by WT-*Lm* is twice longer
199 than that of monocytes infected by *LmΔinlB* (Extended Data Fig. 8l), highlighting that InlB
200 promotes the survival of infected cells. Together, these results demonstrate that InlB blocks
201 CD8⁺ T-cells Fas-mediated killing of infected monocytes.

202

203 **InlB inhibition of Fas-mediated CD8⁺ T cell cytotoxicity depends on Met, PI3K α and FLIP**

204 The receptor of InlB is c-Met²⁴, a member of the receptor tyrosine kinases family, which is
205 ubiquitously expressed, including in monocytes²⁵. In *Lm*-infected monocytes, bacteria are
206 surrounded by LAMP-1, and InlB induces the recruitment of its receptor c-Met, which can be
207 detected around bacteria (Extended Data Fig. 9a). InlB association to bacterial surface mediates
208 the recruitment of c-Met around bacteria, in contrast to InlB released form, that as expected
209 does not recruits c-Met, and does not mediate neuroinvasion (Extended Data Fig. 8d and 9e).
210 Consistent with a critical role of c-Met, its competitive inhibition by capmatinib fully abrogates
211 InlB-mediated neuroinvasion (Fig. 4a and Extended Data Fig. 9f). In mice where c-Met is
212 conditionally deleted in myeloid cells (*LysM-CreER*^{T2} \times *Met*^{*flox/+*} or \times *Met*^{*flox/flox*}), InlB-mediated
213 neuroinvasion is also abrogated (Fig. 4b, c). c-Met signals through PI3-kinase (PI3K), leading
214 to the phosphorylation of Akt^{26,27}. Consistent with InlB-mediated c-Met engagement in infected
215 monocytes, Akt is phosphorylated in an InlB-dependent manner in these cells (Extended Data

216 Fig. 10a, b). Moreover, inhibition of PI3K activity by the pan-inhibitor wortmannin fully blocks
217 InlB-mediated neuroinvasion (Fig. 4d and Extended Data Fig. 9c). Specifically, inhibition of
218 PI3K α , but not of the leucocyte-specific PI3K δ , fully abrogates InlB-mediated neuroinvasion
219 (Fig. 4e and Extended Data Fig. 10d, e). FLIP, a PI3K-regulated competitive inhibitor of
220 procaspase-8^{28,29}, is upregulated in infected monocytes, in an InlB-dependent manner, resulting
221 in a decreased activity of caspase-8 (Fig. 4f, g and Extended Data Fig. 10f). Inhibition of either
222 c-Met or PI3K α blocks both InlB-mediated FLIP upregulation and the decrease of caspase-8
223 activity (Fig. 4f, g). In mice conditionally deleted for FLIP, InlB-mediated monocytes
224 resistance to cell death is lost (Fig. 4h and Extended Data Fig. 10g). Moreover, in mice where
225 FLIP is conditionally deleted in myeloid cells (*LysM-CreER*^{T2} \times *FLIP*^{flox/+} or \times *FLIP*^{flox/flox}), InlB
226 involvement in neuroinvasion is fully abrogated (Fig. 4i, j). Altogether, these results
227 demonstrate that InlB-mediated blockade of the Fas cell death pathway in infected monocytes
228 results from the PI3K α -dependent cell autonomous upregulation of the caspase-8 inhibitor FLIP
229 (Extended Data Fig. 10h).

230

231 **InlB inhibition of CD8⁺ T cell mediated killing promotes *Lm* persistence and fecal carriage**

232 InlB is part of *Lm* core genome and is under purifying selection^{30,31}, suggesting that InlB confers
233 a selective advantage to *Lm*. As *Lm* is shed back from infected tissues into the intestinal
234 lumen³², *Lm* increased virulence may translate into increased fecal shedding, thereby favoring
235 transmission. We therefore tested whether InlB is also involved in *Lm* intestinal carriage and
236 release in the feces. Indeed, WT-*Lm* levels of infection of intestinal tissues and release in the
237 intestinal lumen and feces are significantly higher than that of *Lm* Δ *inlB*, and these differences,
238 as for neuroinvasion, are fully dependent on CD8⁺ T-cells (Fig. 4k, l). These observations
239 highlight that InlB-mediated neuroinvasion, although resulting from a very specific interference

240 with adaptive immunity, likely reflects the selective advantage provided by InlB to *Lm* for
241 within-host persistence and inter-host transmission (Fig 4m).

242 **Discussion**

243 Here we uncovered that *Lm* is able to render its host cell resistant to CD8⁺ T-cells-mediated
244 killing. Selective blockade of the Fas-FasL death pathway in infected monocytes allows these
245 cells to survive longer in the blood, and ultimately transfer *Lm* more abundantly to the brain.
246 Importantly, this novel, specific and unanticipated mechanism that creates an intracellular
247 protected niche for *Lm* is also involved in its persistence in the intestinal tissue and release in
248 the environment. This mechanism is mediated by the surface protein InlB, which was so far
249 described as involved in *Lm* internalization into non-phagocytic cells, whereas we uncover here
250 its key role as an immunomodulatory protein. InlB promotes monocytes survival and
251 neuroinvasion through a refined and unsuspected mechanism: the upregulation, *via* a c-
252 Met/PI3K α -dependent pathway, of the anti-apoptotic factor FLIP, which competitively inhibits
253 caspase-8 cleavage and blocks Fas-FasL mediated cell death.

254 This study highlights the critical role played by cellular immunity against intracellular
255 pathogens' neuroinvasion from a microbial perspective. Indeed, whereas extracellular
256 pathogens rely on the binding to specific host cell receptors³³ or the breaching of host barriers³⁴
257 to invade the CNS, the facultative intracellular pathogen *Lm* takes advantage of its ability to
258 invade and persist within host cells to access to the brain. *Lm* intracellular persistence mediated
259 by InlB promotes *Lm* neuroinvasion *via* ActA-dependent transfer of *Lm* from infected
260 monocytes to brain endothelial cells. These results show that the capacity of microbes to survive
261 into cells is a key pathogenic determinant favoring within-host dissemination and ultimately
262 neuroinvasion. Other neuroinvasive intracellular pathogens, such as *Mycobacterium*
263 *tuberculosis* and *Toxoplasma gondii*, also stimulate PI3K^{35,36} and survive in myeloid cells. As
264 *Lm*, these neuroinvasive microorganisms may also protect infected cells from killing by the
265 immune system, favoring their survival, and increasing their within-host persistence and
266 neuroinvasiveness.

267 Intracellular pathogens tend to interfere with innate immune responses to establish a successful
268 infection, and some also interfere with the adaptive immune system in a broad and non-selective
269 manner^{37,38}, favoring their persistence, like HIV, EBV and measles virus. *Lm* has been
270 instrumental for the discovery of cellular immunity³⁹ and is indeed a prototypic inducer of a
271 protective CD8⁺ T-cell response^{20,40}. Yet, we have shown that InlB selectively blocks the action
272 of the most efficient and specific anti-*Lm* immune effector, T cells-mediated cytotoxicity. This
273 allows the establishment of a protected niche favoring *Lm* dissemination and persistence within
274 the host. This is reminiscent of the mechanism by which tumor cells, in which signaling
275 downstream of growth factor receptors is frequently constitutively activated^{41,42}, also evade
276 immune responses by surviving immune killing. A detailed understanding of how microbes
277 have selected mechanisms to interfere with the immune system may help to rationally design
278 novel anti-infective and anti-tumor therapies. Similarly, the immunomodulatory mechanism of
279 InlB, specific of and restricted to infected cells, may also help develop new immunosuppressive
280 therapies aimed at specifically protecting cells of interest from the immune system, as opposed
281 to classic immunosuppressive drugs that inhibit indiscriminately immune functions, and
282 therefore favor infectious and neoplastic complications.

283 *Lm* is an opportunistic pathogen that only rarely induces clinically apparent infection upon oral
284 ingestion⁴³, and there is no inter-human transmission of listeriosis. Yet, the so-called “virulence
285 factors” of *Lm* are under purifying selection^{30,31,44}, implying that they contribute to *Lm* fitness.
286 By interfering with the host anti-*Lm* cellular effectors, we have shown that InlB enhances *Lm*
287 intestinal carriage and fecal shedding, thereby increasing the odds of neuroinvasive *Lm* to be
288 transmitted back to the environment and colonize new hosts. This illustrates that the
289 anthropocentric view on microbial pathogenesis which phenotypic output is centered on disease
290 does not necessarily reflect the actual context where microbial evolution and fitness gain take
291 place.

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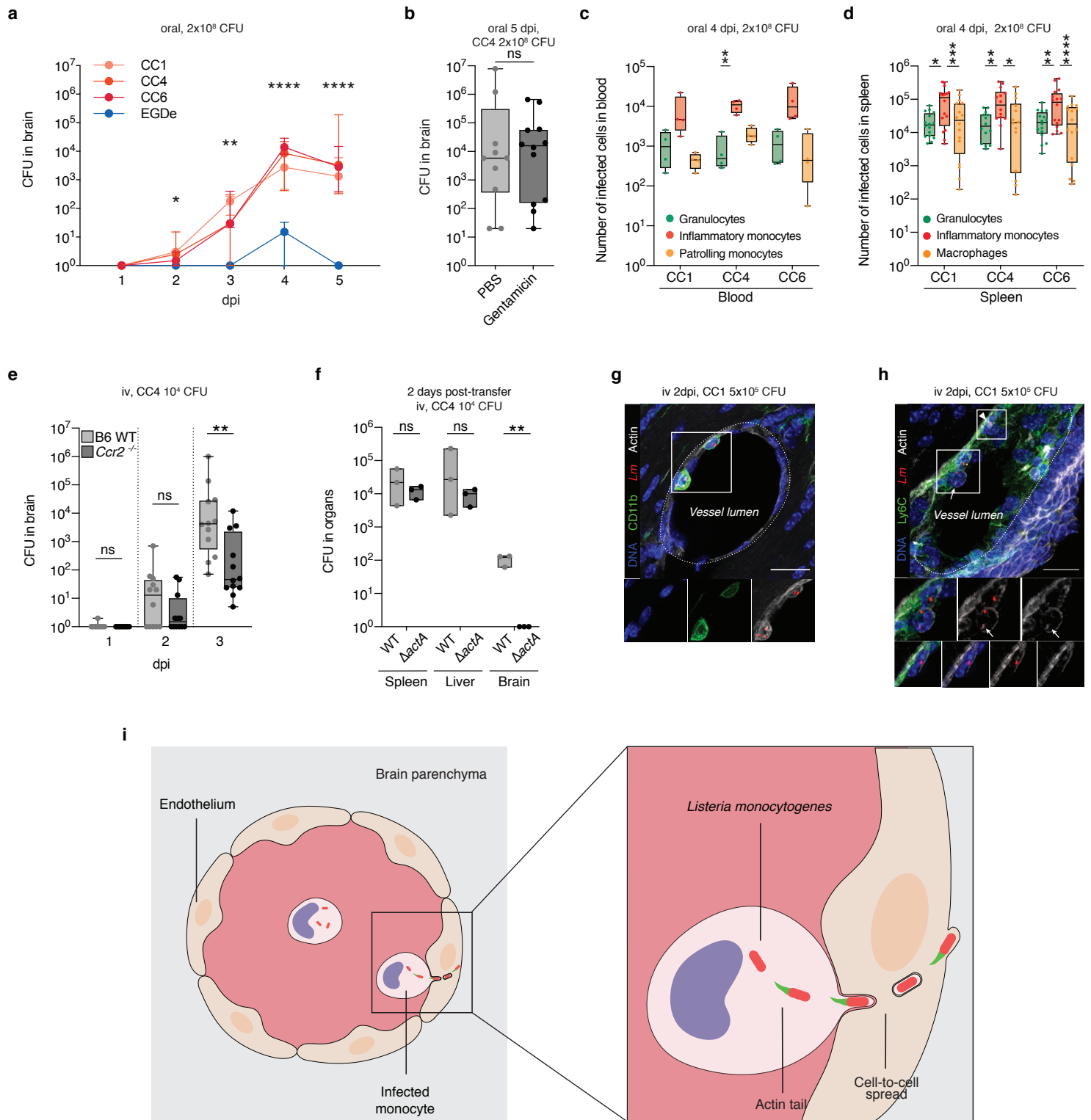
402

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425 and/or analyzed during the current study are available from the corresponding author on
426 reasonable request.

427	Extended Data:
428	Materials and Methods
429	Extended Data Tables 1-5
430	Extended Data Figures 1-10
431	Extended Data Movie 1
432	Extended References (45-60)

Figure 1



1 **Fig. 1. Infected inflammatory monocytes transfer *Lm* to the CNS by cell-to-cell spread. (a)**

2 Bacterial load in the brain of mice after oral inoculation with CC1/CC4/CC6-*Lm* and EGDe. (b)

3 Bacterial load in the brain of gentamicin-treated mice after oral inoculation with CC4-*Lm*. (c, d)

4 Three main infected cell subsets in the blood (c) and spleen (d) after oral inoculation with
5 CC1/CC4/CC6-*Lm* in mice at 4 dpi, the peak of bacteremia (Extended Data Fig. 1c). For the blood

6 panel, each dot corresponds to the blood of three mice pooled together. (e) Bacterial load in the

7 brain of B6-WT and *Ccr2*^{-/-} mice after intravenous (iv) inoculation with CC4-*Lm*. (f) Bacterial

8 load of gentamicin-treated recipient mice, 2 days after injection of infected monocytes harvested

9 from 6 donors mice 3 days after iv inoculation with either CC4-WT or CC4Δ*actA*. (g, h)

10 Representative fluorescent microscopy images of infected monocytes adhering to the endothelial

11 cells (g) and of monocytes infected by an actin-polymerizing-*Lm* (arrow) adjacent to an infected

12 endothelial cell (arrowhead, h), 2 days after iv inoculation with CC1-*Lm*. (h) Maximum intensity

13 projection over a 20μm stack and insets are single *z*-planes. Scale bars, 20μm. (i) Schematic

14 representation of *Lm* neuroinvasion process. Data were obtained from three (a, b, e and f) and four

15 (c and d) independent experiments and are presented as median ± interquartile (a) and as median

16 ± interquartile (box) and extreme values (lines) (b-f). CFU are compared with the unpaired Mann-

17 Whitney test (a, b, e and f) and number of infected cells with the Friedman test (c and d). ns:

18 $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

19

Figure 2

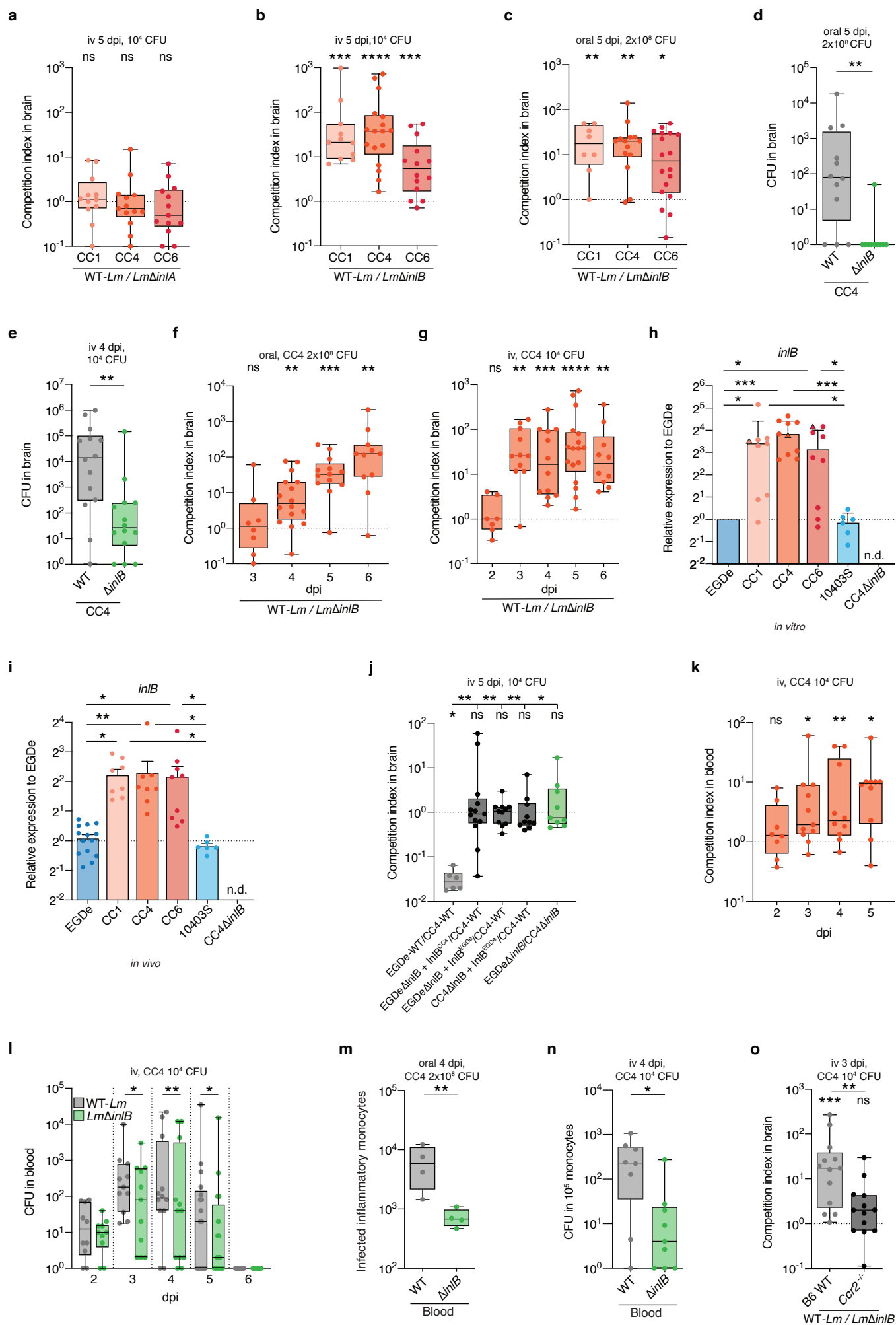


Fig. 2. InlB is involved in *Lm* neuroinvasion and inflammatory monocytes infection. (a-c)

Competition indexes in brain after (a, b) iv and after (c) oral inoculation of WT and isogenic mutant

strains. **(d, e)** Bacterial load in brain after (d) oral and (e) iv inoculation with either CC4-WT or

CC4 Δ *inlB*. **(f, g)** Competition indexes in brain after (f) oral and (g) iv inoculation with 1:1 CC4-

WT and CC4 Δ *inlB*. **(h, i)** Transcription levels of *inlB* relative to EGDe in (h) mid-log phase in

BHI and (i) in infected splenocytes 2 days after iv inoculation. In (h), each dot for CC1/4/6

corresponds to a different clinical isolate and triangles point out the strains used throughout the

rest of the study and referred to as CC1, CC4 and CC6. **(j)** Competition index in brain after iv

inoculation with a 1:1 mix of the indicated bacterial strains (2×10^4 for EGDe Δ *inlB* and CC4 Δ *inlB*).

(k, l) Competition index (k) and bacterial load (l) in blood after iv inoculation with 1:1 CC4-WT

and CC4 Δ *inlB*. **(m, n)** Number of infected monocytes (m) and bacterial enumeration from sorted

monocytes (n) in the blood after (m) oral and (n) iv inoculation with CC4-WT or CC4 Δ *inlB*. **(o)**

Competition index in brain of control or *Ccr2*^{-/-} mice after iv inoculation with 1:1 CC4-WT and

CC4 Δ *inlB*. Data were obtained from three (a-h, j-l and n) and four (i, m and o) independent

experiments and are presented as median \pm interquartile (box) and extreme values (lines) (a-g and

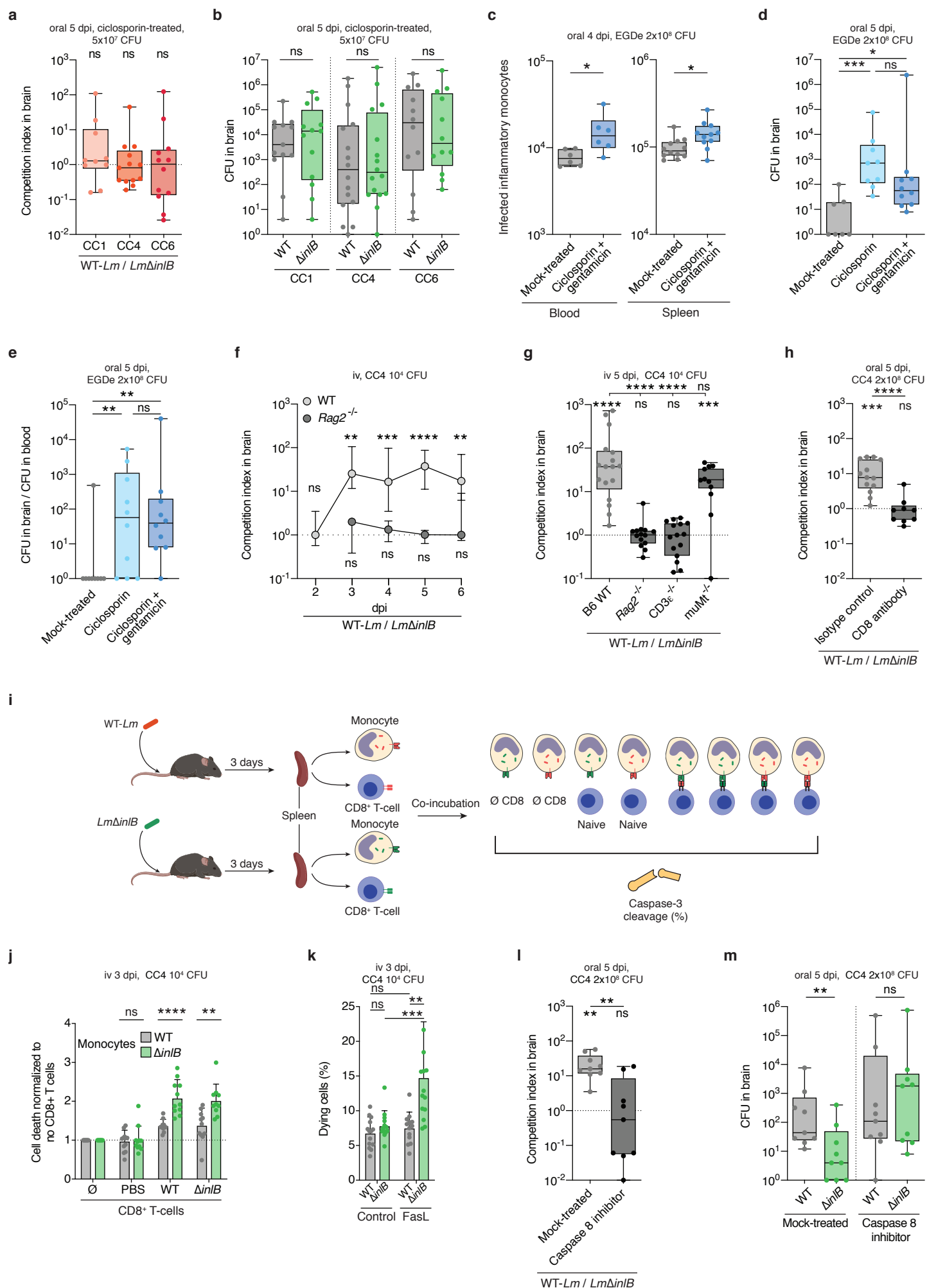
j-o) or as mean \pm SD (h and i). CFU in competition assays are compared with the Wilcoxon

matched-pairs signed rank test (a-c, f-g, j-l and o) and samples compared with the unpaired Mann-

Whitney test (d-e, m-o) or the Kruskal-Wallis test (h-j). ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***:

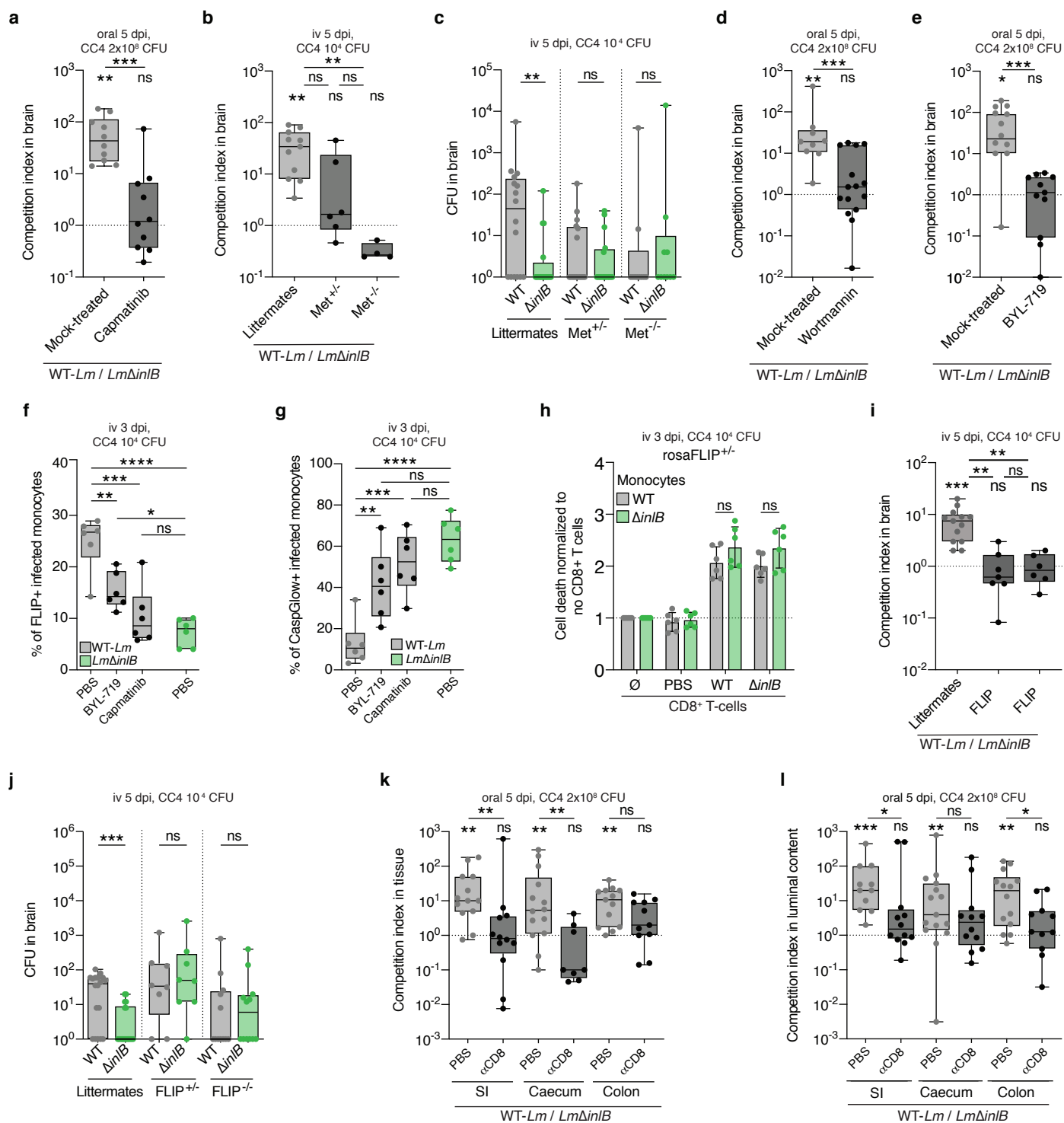
$p < 0.001$, ****: $p < 0.0001$.

Figure 3

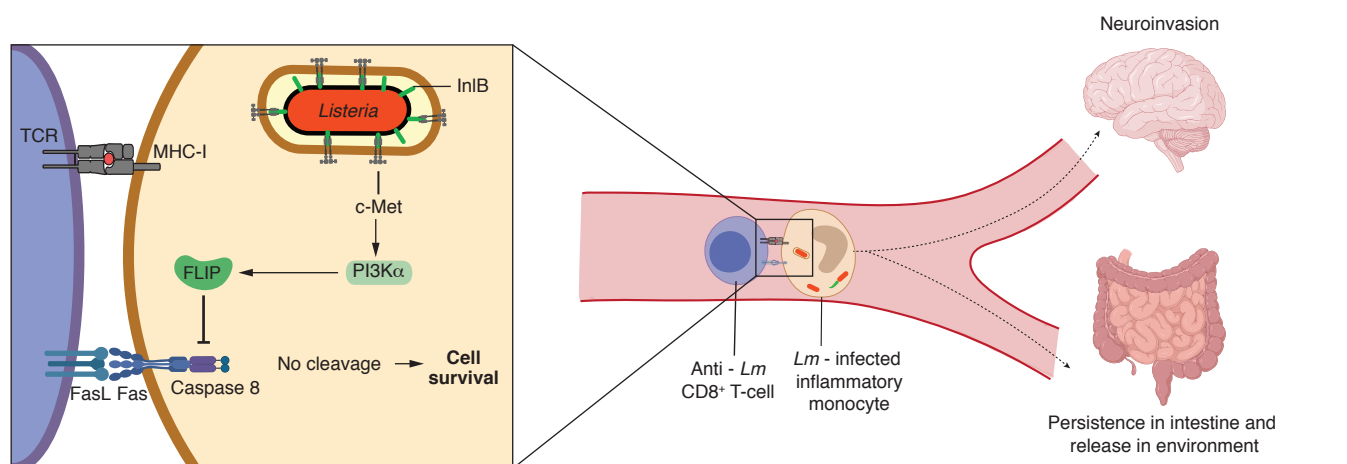


40 **Fig. 3. InlB blocks CD8⁺ T-cells-mediated monocyte cell death.** (a, b) Competition index (a)
41 and bacterial load (b) in brain after inoculation with 1:1 WT-*Lm* strain and Δ *inlB* isogenic mutant
42 in ciclosporin-treated mice, related to Fig. 2c. (c) Number of infected monocytes of ciclosporin
43 and gentamicin-treated mice after inoculation with EGDe. (d, e) Bacterial load in brain (d) and
44 ratio of brain/blood bacterial load (e) in ciclosporin \pm gentamicin-treated mice after inoculation
45 with EGDe. (f) Competition index in brain after inoculation with 1:1 CC4-WT strain and
46 CC4 Δ *inlB* in control and *Rag2*^{-/-} mice. (g) Competition index in brain after inoculation with 1:1
47 CC4-WT strain and CC4 Δ *inlB* isogenic mutant in control and in mice lacking functional T (CD3 ϵ ⁻
48 ⁻), B lymphocytes (*muMt*^{-/-}) or both (*Rag2*^{-/-}). (h) Competition index in brain after inoculation with
49 1:1 CC4-WT and CC4 Δ *inlB* after T-CD8⁺ depletion. (i) Schematic pipeline of the cytotoxic
50 lymphocyte (CTL) assay. (j, k) Level of caspase-3 cleavage of infected spleen monocytes,
51 harvested after inoculation with CC4-WT or CC4 Δ *inlB*, and incubated with CD8⁺ T-cells from
52 similarly infected (WT and Δ *inlB*) or control (PBS) mice at an effector to target ratio of 5 (j) or
53 treated *ex vivo* with FasL (k). (l, m) Competition index (l) and bacterial load (m) in the brain after
54 inoculation with 1:1 CC4-WT and CC4 Δ *inlB* and after treatment with caspase-8 inhibitor. Data
55 were obtained from three (a-h, l-m) and four (j-k) independent experiments and are presented as
56 median \pm interquartile (box) and extreme values (lines) (a-h, l-m) and as mean \pm SD (j-k). CFU in
57 competition assays are compared with the Wilcoxon matched-pairs signed rank test (a-b, f-h, l-m),
58 samples are compared with the Mann-Whitney test (c-e, h, l-m), an unpaired student *t*-test (j-k)
59 and the Kruskal-Wallis test (g). ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

Figure 4



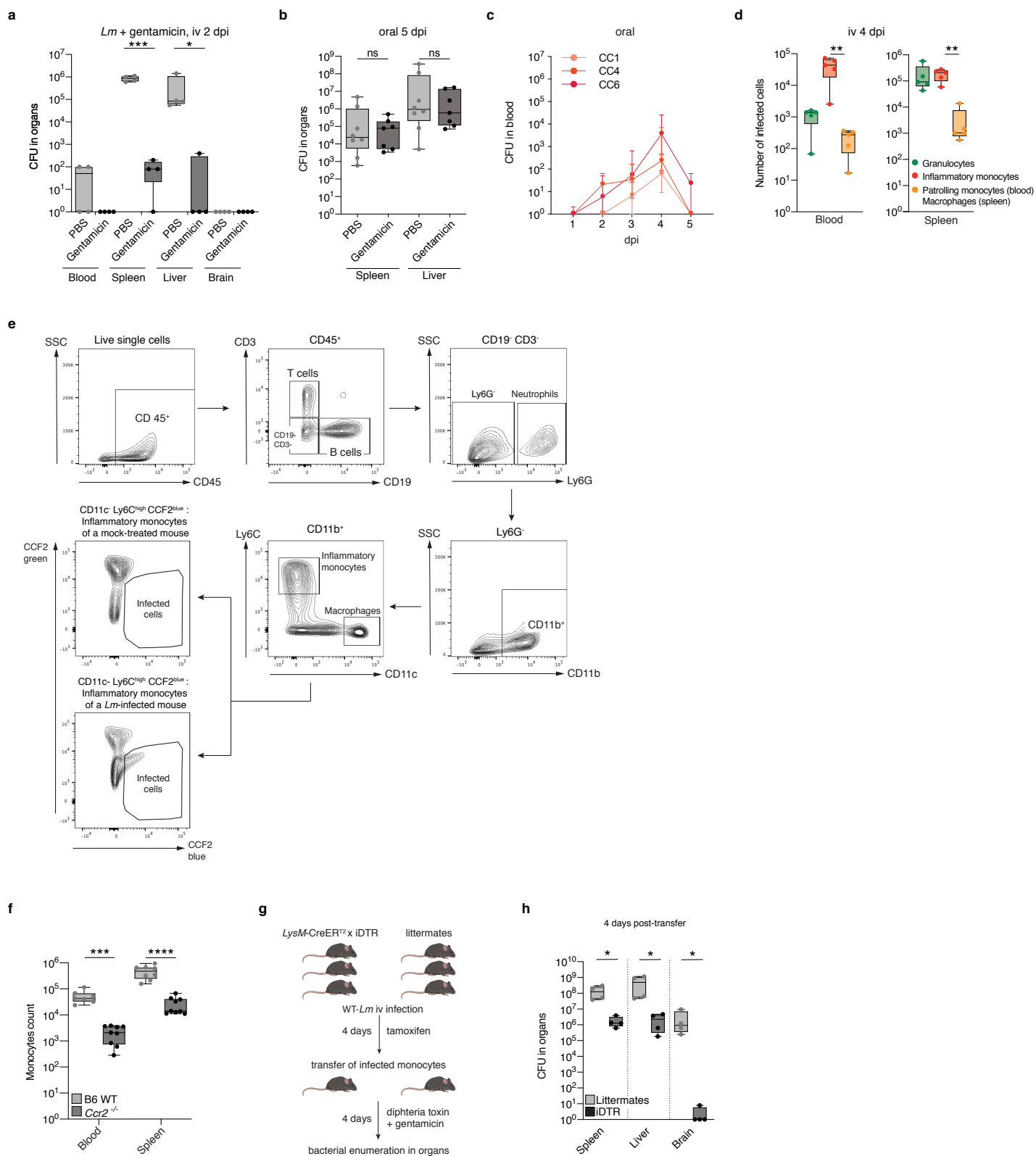
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61 **Fig. 4. Inhibition of CD8⁺ T cells-mediated cell death by InlB is dependent on the c-Met-**
62 **PI3K α -FLIP pathway and is involved in *Lm* intestinal colonization and fecal carriage. (a)**
63 Competition index in the brain after inoculation with 1:1 of CC4-WT and CC4 Δ *inlB*, in mice
64 treated with a c-Met inhibitor (capmatinib). **(b, c)** Competition index (b) and bacterial load (c) in
65 the brain of *LysM-CreER*^{T2} x *Met*^{flox/flox} (or *Met*^{+/-}) mice (referred to as *Met*^{-/-} and *Met*^{+/-} mice),
66 and their littermates, after inoculation with 1:1 of CC4-WT and CC4 Δ *inlB* and tamoxifen
67 treatment. **(d, e)** Competition indexes in the brain after inoculation with 1:1 of CC4-WT and
68 CC4 Δ *inlB*, in mice treated with a pan-PI3K inhibitor (wortmannin, d) or a specific PI3K α inhibitor
69 (BYL-719, e). **(f, g)** Proportion of infected monocytes expressing FLIP (f) or active caspase-8 (g)
70 after inoculation with CC4-WT and CC4 Δ *inlB* in mice treated with either BYL-719 or capmatinib.
71 **(h)** Level of caspase-3 cleavage of infected spleen monocytes, harvested after inoculation with
72 CC4-WT or CC4 Δ *inlB* of tamoxifen-treated *Rosa26-CreER*^{T2} x *Cflar*^{+/-} (*rosaFLIP*^{+/-}) mice and
73 incubated with CD8⁺ T cells from similarly infected mice at an effector to target ratio of 5. **(i, j)**
74 Competition index (i) and bacterial load (j) in *LysM-CreER*^{T2} x *FLIP*^{flox/flox} (or *FLIP*^{+/-}) mice
75 (referred to as *FLIP*^{-/-} and *FLIP*^{+/-} mice), and their littermates, after inoculation with 1:1 of CC4-
76 WT and CC4 Δ *inlB* and tamoxifen treatment. **(k, l)** Competition index in the intestinal tissues (k)
77 and content (l) after inoculation with 1:1 CC4-WT and CC4 Δ *inlB* in mice treated with an anti-
78 CD8⁺ T-cells antibody, SI = small intestine. **(m)** Schematic representation of InlB-mediated
79 blockade of CD8⁺ T cell-mediated cell death resulting in neuroinvasion, persistence in the
80 intestine and transmission. Data were obtained from three (f-h, k-l) or four (a-e, i-j) independent
81 experiments and are presented as median \pm interquartile (box) and extreme values (lines) (a-g, i-l)
82 or mean \pm SD (h). CFU in competition assays are compared with the Wilcoxon matched-pairs
83 signed rank test (a-e, i-l) and samples are compared with the Mann-Whitney test (a, d-e, k-l), an

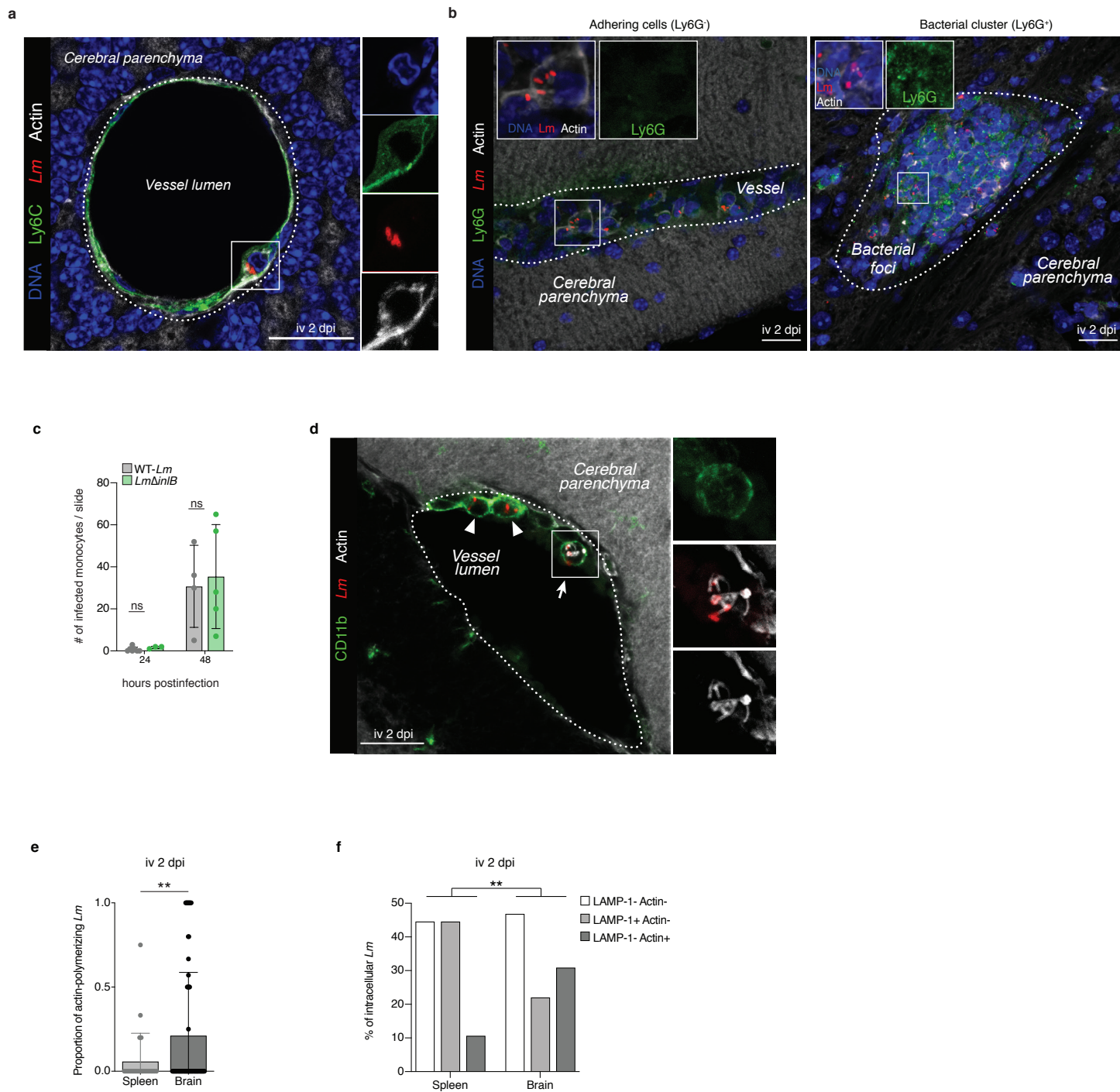
84 unpaired student *t*-test (h), a Kruskal-Wallis test (b and i) and a one-way ANOVA (f-g). ns: $p > 0.05$,
85 *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

Extended Data Figure 1



1 **Extended Data Fig. 1. Infected inflammatory monocytes are necessary for *Lm* to invade the**
2 **CNS. (a)** Bacterial load in organs of KIE16P mice 2 days after iv inoculation with 10^4 CFU CC4-
3 *Lm* immediately followed by injection of gentamicin, assessing the bactericidal effect of
4 gentamicin on extracellular circulating *Lm*. **(b)** Bacterial load in the spleen and liver 5 days after
5 oral inoculation with 2×10^8 CFU CC4-*Lm*, in KIE16P mice treated with gentamicin every day
6 from day 1 post-inoculation, related to Fig. 1A. **(c)** Bacterial load in the blood of KIE16P mice
7 after oral inoculation with 2×10^8 CFU of CC1/CC4/CC6-*Lm*, related to Fig. 1A. **(d)** Repartition of
8 the 3 main infected cell subsets in the blood and spleen of KIE16P mice 4 days after iv inoculation
9 with 10^4 CFU of CC4-*Lm*. **(e)** Representative dot plots of the gating strategy used for flow
10 cytometry analysis. Infected cells are identified through the shift of fluorescence, upon excitation
11 with the 405 nm laser, of the CCF2-AM substrate from green (518 nm) to blue (447 nm) in
12 presence of β -lactamase expressing-*Lm*. **(f)** Number of inflammatory monocytes in the blood and
13 spleen of B6-WT or *Ccr2*^{-/-} mice. **(g)** Schematic pipeline of the transfer experiment in *LysM*-
14 CreER^{T2} × iDTR mice. **(h)** Bacterial load in the spleen, liver and brain of gentamicin- and
15 diphtheria toxin-treated recipient *LysM*-CreER^{T2/+} × *Rosa26*-iDTR^{+/-} and littermates mice, 4 days
16 after injection of infected monocytes harvested from $n = 3$ donor tamoxifen-treated *LysM*-
17 CreER^{T2/+} × *Rosa26*-iDTR^{+/-} or $n = 3$ littermates mice, 4 days after iv inoculation with 10^4 CC4-
18 WT. Data were obtained from two (a) or three (b-d, f) and four (h) independent experiments and
19 are presented as median ± interquartile (box) and extreme values (lines) (a-b, d, f and h) or as
20 median ± interquartile (c). Samples are compared with an unpaired Mann-Whitney test (a-b, f and
21 h) and number of infected cells with the Friedman test (d). ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***:
22 $p < 0.001$, ****: $p < 0.0001$.

Extended Data Figure 2



24 **Extended Data Fig. 2. Infected inflammatory monocytes transfer *Lm* to the CNS. (a-b)**

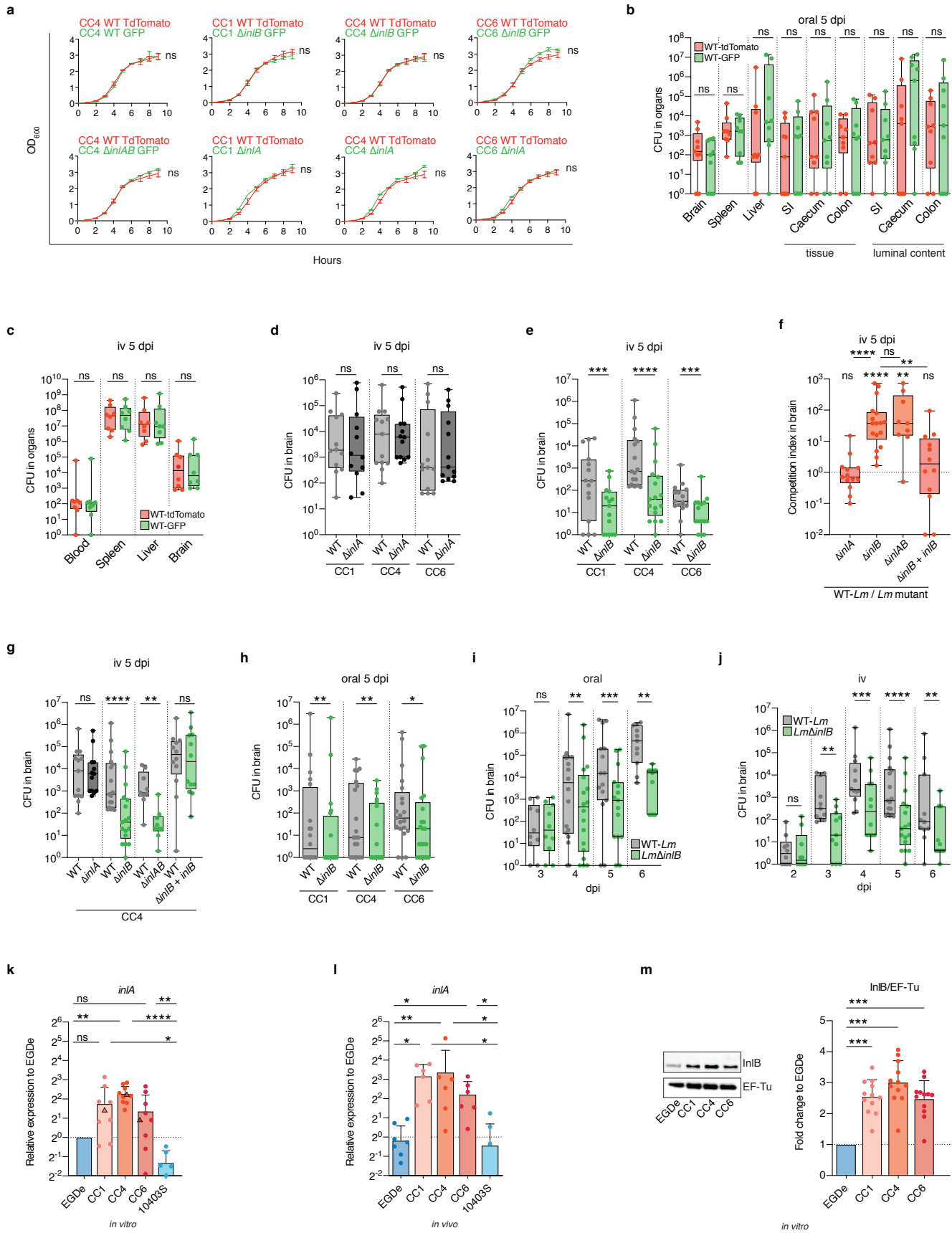
25 Representative fluorescence microscopy images of infected inflammatory monocytes adhering to
26 endothelial cells 2 days after iv inoculation with 5×10^5 CFU CC1-*Lm* in KIE16P mice. Adhering
27 infected cells are Ly6C⁺ (a) and Ly6G⁻ (b left panel; to ensure the specificity of the Ly6G⁻ staining
28 we show in the right panel a positive control staining for Ly6G in a parenchymal bacterial cluster).

29 (c) Quantification of infected monocytes in brain vessels of KIE16P mice 24 and 48 hours after iv
30 inoculation with 5×10^5 CFU CC4-*Lm* and CC4 Δ *inlB*. Each dot corresponds to the average number
31 of monocytes counted on two slides (representative median sagittal sections, 40 μ m thickness) for
32 one mouse. (d) Representative fluorescence microscopy images of infected inflammatory

33 monocytes adhering to endothelial cells 2 days after iv inoculation of 5×10^5 CFU CC1-*Lm* in
34 KIE16P mice, in which intra-monocytic *Lm* are found polymerizing host actin. (e, f) Proportion of
35 actin-polymerizing *Lm* (e) and *Lm* co-localizing with either LAMP-1 or Actin (f) in spleen and
36 brain of KIE16P mice 3 days after iv inoculation with 10^4 CC4-*Lm*. Scale bars: 20 μ m, **a** and **d** are

37 maximum intensity projections over a *z*-stack. Data were obtained from three independent
38 experiments and are presented as mean \pm SD. Samples are compared with an unpaired Mann-
39 Whitney test (c and e) and a one-way ANOVA (f). ns: $p > 0.05$, **: $p < 0.01$.

Extended Data Figure 3



Extended Data Fig. 3. InlB is a major determinant of *Lm* neuroinvasiveness whereas InlA is

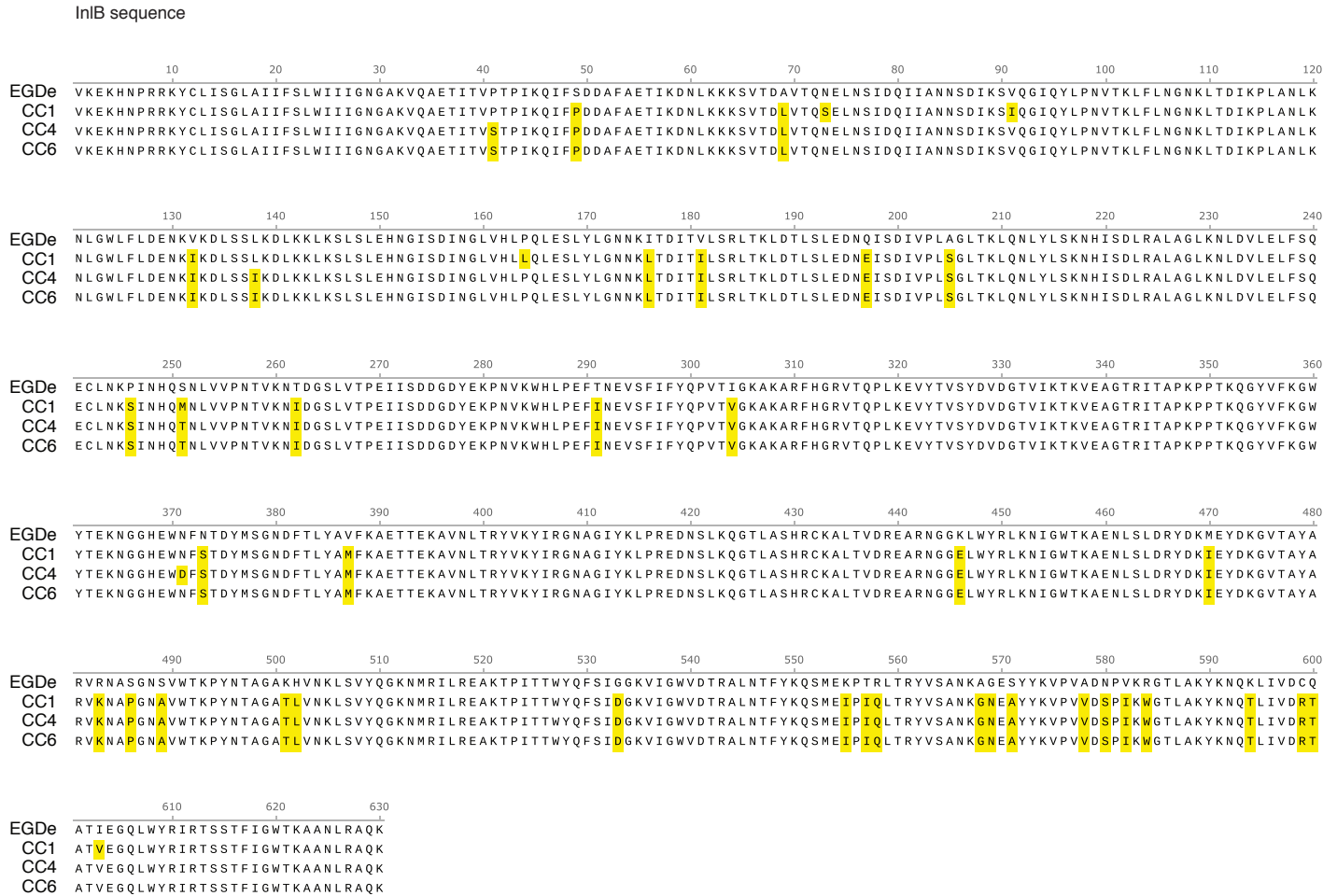
not. (a) Optical density of indicated bacterial strains measured every hour for 9 hours after 1:100 dilution in BHI of an overnight culture. **(b and c)** Bacterial load in organs or luminal contents of KIE16P mice 5 days after oral inoculation with 2×10^8 CFU (b) or after iv inoculation with 10^4 CFU (c) of 1:1 CC4-WT expressing TdTomato or GFP. **(d)** Bacterial load in brain of KIE16P mice 5 days after iv inoculation with 10^4 CFU of 1:1 mix of WT and $\Delta inlA$ isogenic strains, related to Fig. 2a. **(e)** Bacterial load in brain of KIE16P mice 5 days after iv inoculation with 10^4 CFU of 1:1 mix of WT and $\Delta inlB$ isogenic strains, related to Fig. 2b. **(f, g)** Competition index (f) and bacterial load (g) in brain of KIE16P mice 5 days after iv inoculation with 10^4 CFU of 1:1 mix of CC4-WT and either $CC4\Delta inlA$, $CC4\Delta inlB$, $CC4\Delta inlAB$ or $CC4\Delta inlB$ complemented with *inlB*, related to Fig. 2a-b and panels d,e. **(h)** Bacterial load in brain of KIE16P mice 5 days after oral inoculation with 2×10^8 CFU of 1:1 mix of WT strain and $\Delta inlB$ isogenic strains, related to Fig. 2c. **(i, j)** Bacterial load in brain of KIE16P mice at indicated times after oral inoculation with 2×10^8 CFU (i) and after iv inoculation with 10^4 CFU (j) of 1:1 CC4-WT and $CC4\Delta inlB$, related to Fig. 2f, g. **(k)** Transcription levels of *inlA* relative to EGDe in mid-log phase in BHI. For CC1/4/6, each dot corresponds to a different clinical isolate and triangles represent the strains used throughout the rest of the study and referred to as CC1, CC4 and CC6, related to Fig. 2h. **(l)** Transcription levels of *inlA* relative to EGDe in infected splenocytes 2 days after iv inoculation with 2×10^5 CFU in KIE16P mice, related to Fig. 2i. **(m)** Representative Western blot (left) and quantification (right) of InlB expression, normalized to that of EF-Tu, relative to EGDe in mid-log phase in BHI. Data were obtained from three (a-l) and four (m) independent experiments and are presented as mean \pm SD (a, k-m) or as median \pm interquartile (box) and extreme values (lines) (b-j). Curves were fitted with a Gompertz model and the lag phases (k) for each pair of *Lm* strains were compared with the

64 extra sum-of-squares F test (a). CFU in competition assays are compared with the Wilcoxon
65 matched-pairs signed rank test (b-j) and samples compared with the Kruskal-Wallis test (f, k-m).
66 ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

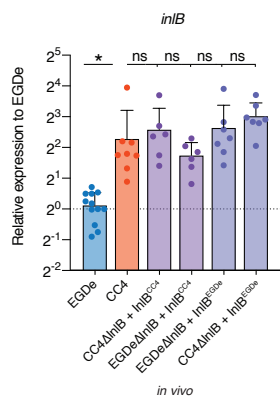
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Extended Data Figure 4

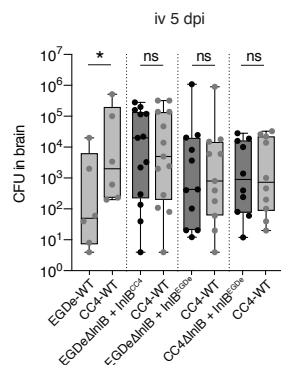
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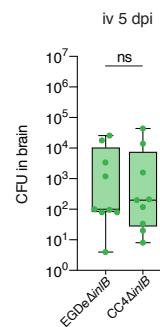
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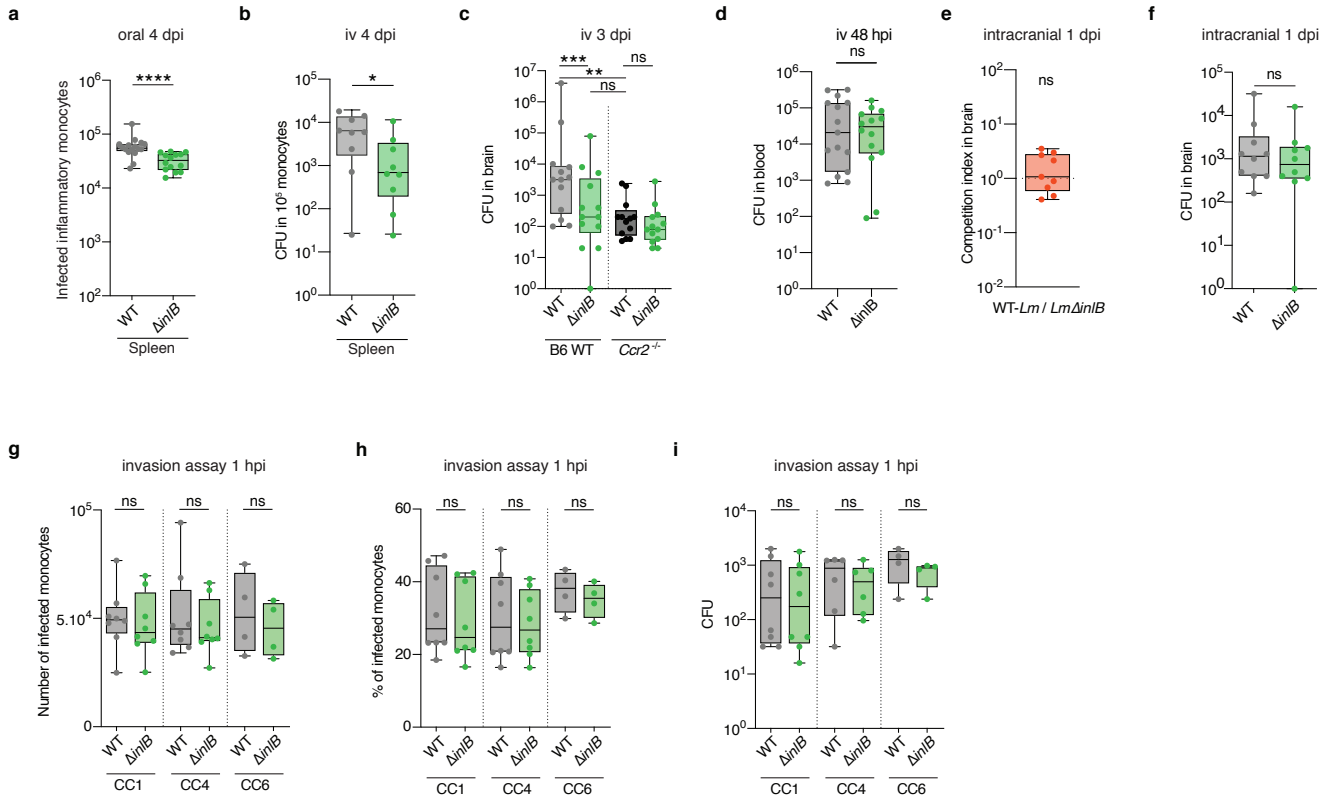
d



68 **Extended Data Fig. 4. Levels of expression of InlB, and not allelic differences with EGDe,**
69 **explain enhanced neuroinvasiveness of hypervirulent CC1, CC4 and CC6 strains. (a)** Protein
70 sequence alignment of InlB alleles from EGDe and from CC1, CC4 and CC6 strains. Mismatches
71 are indicated in yellow. **(b)** Transcription levels of *inlB*, relative to EGDe, in infected splenocytes
72 2 days after iv inoculation with 2×10^5 CFU in KIE16P mice of EGDe-WT, CC4-WT and strains
73 complemented with either InlB from EGDe or from CC4. **(c)** Bacterial load in brain of KIE16P
74 mice 5 days after iv inoculation with 10^4 CFU of a 1:1 mix of the indicated bacterial strains, related
75 to Fig. 2j. **(d)** Bacterial load in brain of KIE16P mice 5 days after iv inoculation with 2×10^4 CFU
76 of a 1:1 mix of EGDe Δ *inlB* and CC4 Δ *inlB*, related to Fig. 2j. Data were obtained from three
77 independent experiments and are presented as mean \pm SD (b) or as median \pm interquartile (box)
78 and extreme values (lines) (c-d). CFU in competition assays are compared with the Wilcoxon
79 matched-pairs signed rank test (c-d) and samples compared with the Kruskal-Wallis test (b). ns:
80 $p > 0.05$, *: $p < 0.05$.

81

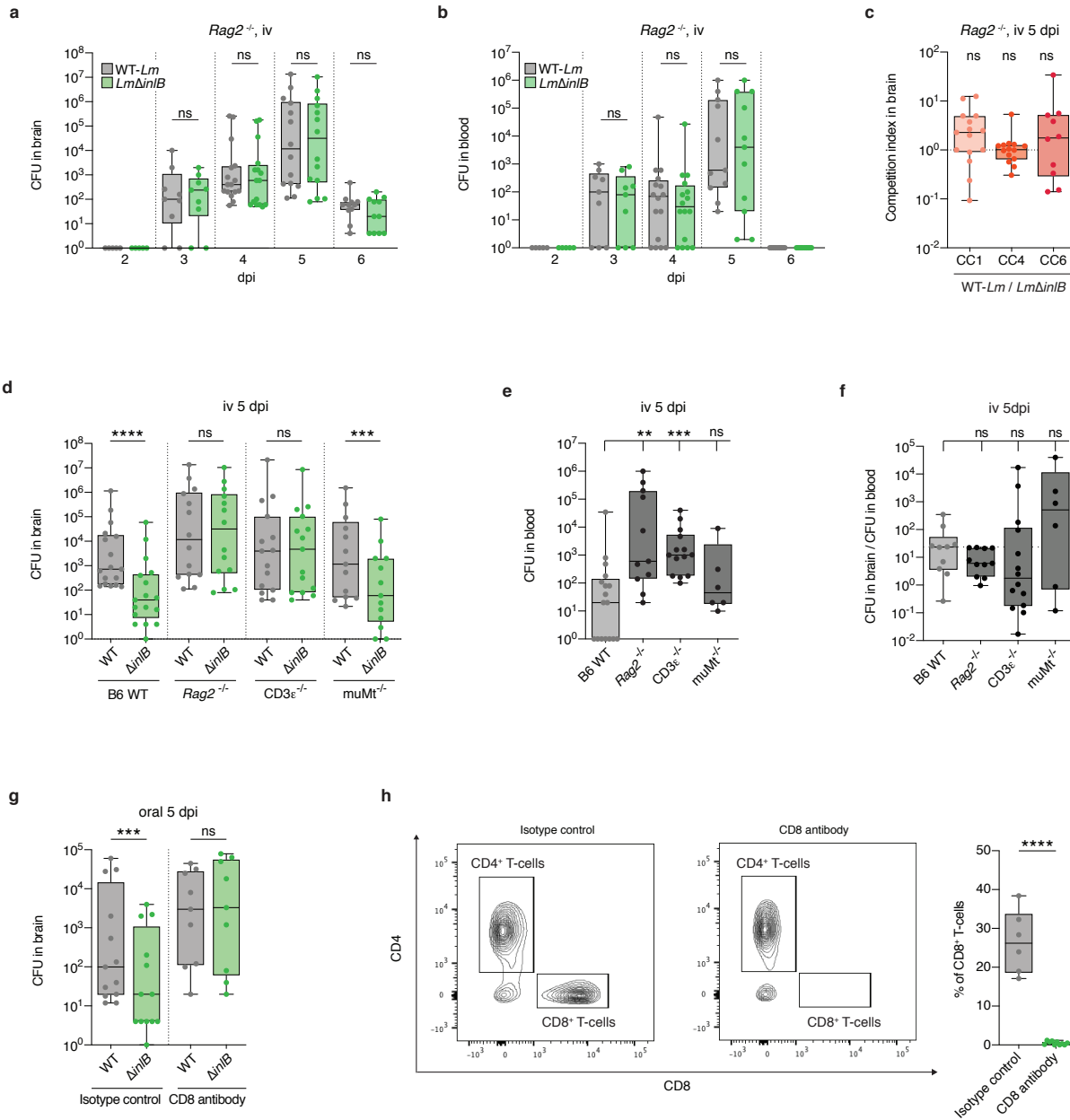
Extended Data Figure 5



82 **Extended Data Fig. 5. InlB is not involved in *Lm* invasion in monocytes.** (a) Number of infected
83 monocytes in the spleen of KIE16P mice 4 days after oral inoculation with 2×10^8 CFU of CC4-
84 WT or CC4 Δ *inlB*. (b) Bacterial enumeration from sorted monocytes retrieved from KIE16P mice
85 iv infected for 4 days with 10^4 CFU of CC4-WT or CC4 Δ *inlB*. (c) Bacterial load in brain of control
86 or *Ccr2*^{-/-} mice 3 days after iv inoculation with 10^4 CFU of 1:1 CC4-WT and CC4 Δ *inlB*. (d)
87 Bacterial load in the brain 48 hours after iv inoculation of KIE16P mice with 5×10^5 CFU of either
88 CC4-WT strain or CC4 Δ *inlB*. (e, f) Competition index (e) and bacterial load (f) in the brain of
89 KIE16P mice 1 day after intracranial inoculation with 10^2 CFU of 1:1 mix of CC4-WT and
90 CC4 Δ *inlB*. (g-i) Number of infected monocytes (g), percentage of infected monocytes (h) and
91 bacterial load (i) in monocytes 1 hour after *in vitro* infection of primary bone marrow mouse
92 monocytes with WT-*Lm* or Δ *inlB* isogenic mutant, at a MOI of 5. Data were obtained from three
93 (d-f) and four (a-c, g-i) independent experiments and are presented as median \pm interquartile (box)
94 and extreme values (lines). Samples are compared with the unpaired Mann-Whitney test (a-b, d,
95 g-i) and the Kruskal-Wallis test (c), and CFU in competition assays are compared with the
96 Wilcoxon matched-pairs signed rank test (c, e-f). ns: $p > 0.05$.

97

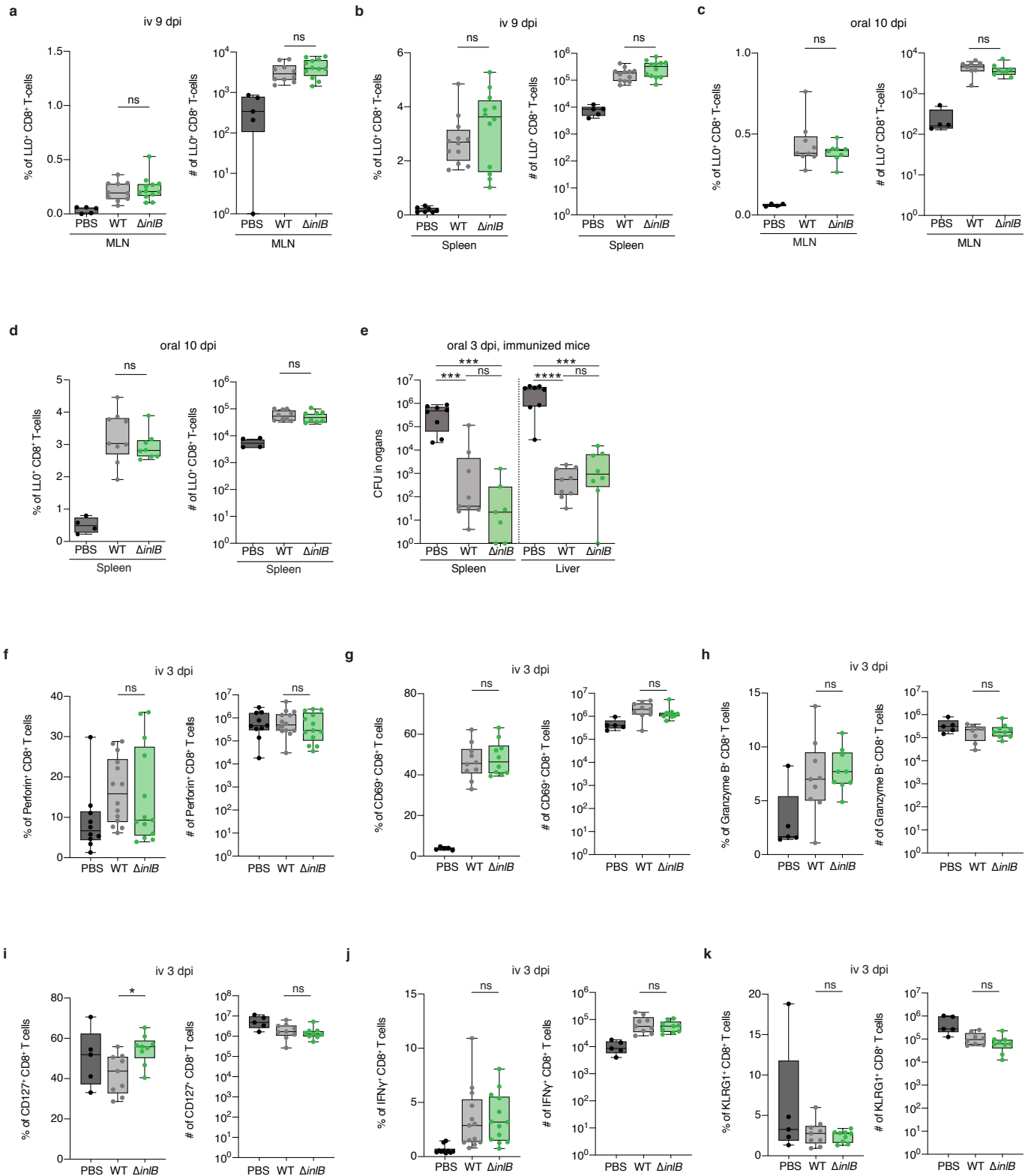
Extended Data Figure 6



98 **Extended Data Figure 6. InlB-mediated *Lm* neuroinvasiveness is abrogated in CD8⁺ T-cells**
99 **deficient mice. (a, b)** Bacterial load in brain (a) and in blood (b) of *Rag2*^{-/-} mice after iv inoculation
100 with 10⁴ CFU of a 1:1 mix of CC4-WT and CC4Δ*inlB*, related to Fig.3f (c) Competition index in
101 brain of *Rag2*^{-/-} mice 5 days after iv inoculation with 10⁴ CFU of a 1:1 mix WT strain and Δ*inlB*
102 isogenic strains. (d, e) Bacterial load in brain (d) and in blood (e) 5 days after iv inoculation with
103 10⁴ CFU of 1:1 CC4-WT strain and CC4Δ*inlB* isogenic mutant in control mice and in mice lacking
104 functional T (CD3ε^{-/-}), B lymphocytes (muMt^{-/-}) or both (*Rag2*^{-/-}), related to Fig. 3g. (f) Ratio of
105 brain/blood bacterial load in control, *Rag2*^{-/-}, CD3ε^{-/-} and muMt^{-/-} mice, related to Fig. 3g. (g)
106 Bacterial load in brain of KIE16P mice 5 days after oral inoculation with 2×10⁸ CFU of 1:1 CC4-
107 WT and CC4Δ*inlB* after CD8⁺ T-cells depletion, related to Fig. 3h. (h) Representative dot plots
108 (left) and proportion of CD8⁺ T-cells (right) among CD45⁺ CD3⁺ cells in the spleen, after CD8⁺
109 T-cells depletion, related to Fig. 3h. Data were obtained from three independent experiments and
110 are presented as median ± interquartile (box) and extreme values (lines). CFU in competition
111 assays are compared with the Wilcoxon matched-pairs signed rank test (a-d and g) and samples
112 are compared with the Kruskal-Wallis test (e, f) and with the Mann-Whitney test (h). ns: *p*>0.05,
113 **: *p*<0.01, ***: *p*<0.001, ****: *p*<0.0001.

114

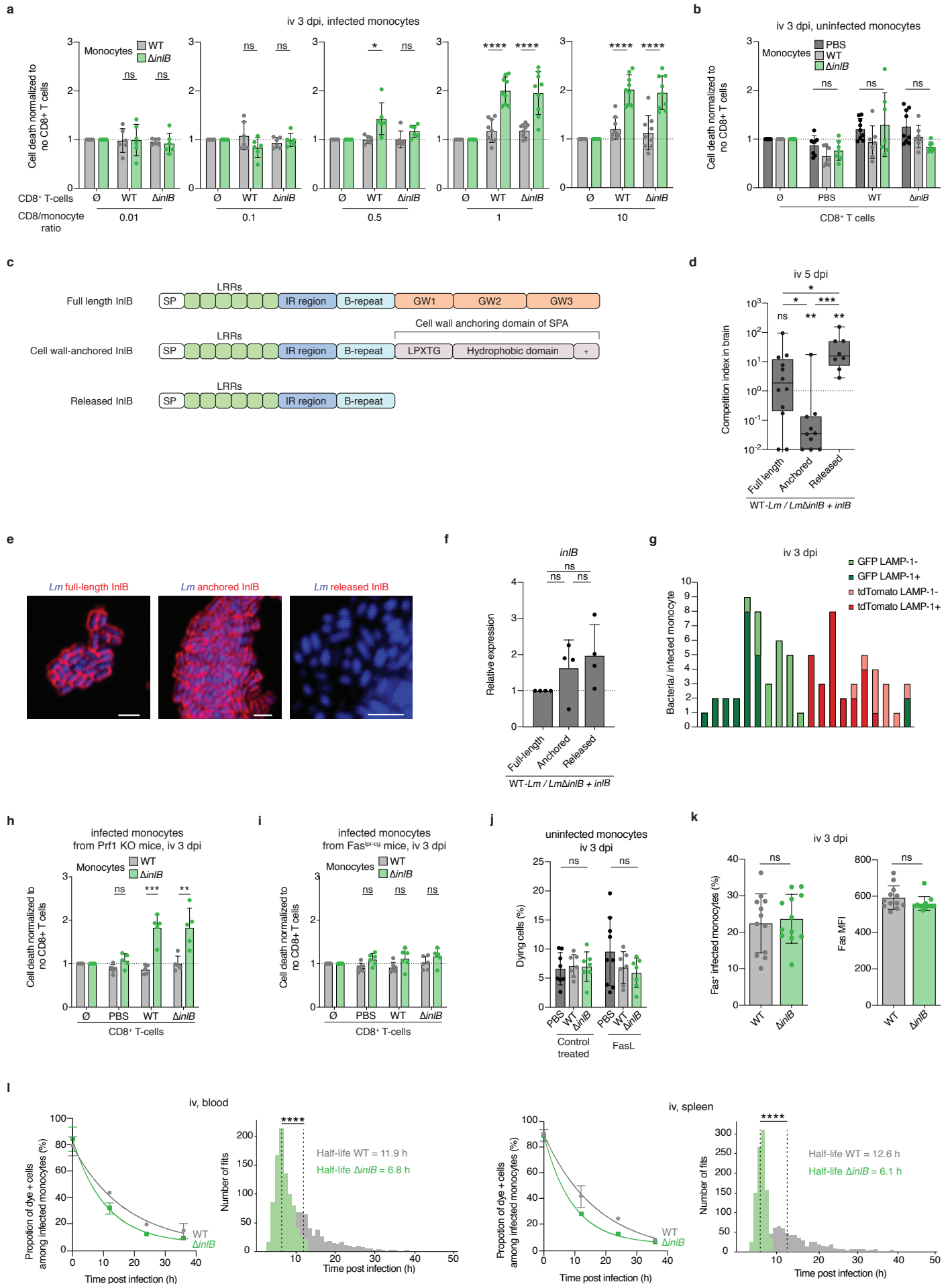
Extended Data Figure 7



115 **Extended Data Figure 7. InlB does not alter the induction and differentiation of specific anti-**
116 ***Lm* CD8⁺ T-cells. (a, b)** Percentage (left) and number (right) of LLO-specific CD8⁺ T-cells in
117 mesenteric lymph nodes (MLN) (a) and spleen (b) of BALB/c mice 9 days after iv inoculation
118 with 1×10^3 of CC4-WT strain or CC4 Δ *inlB*. **(c, d)** Percentage (left) and number (right) of LLO-
119 specific CD8⁺ T-cells in mesenteric lymph nodes (MLN) (c) and spleen (d) of iFABP-hEcad mice
120 10 days after oral inoculation with 2×10^7 of CC4-WT strain or CC4 Δ *inlB*. **(e)** Bacterial load in
121 spleen and liver 3 days after oral inoculation with 1×10^9 CFU of CC4-WT in KIE16P mice
122 challenged 30 days before with 5×10^7 CFU of CC4-WT strain or CC4 Δ *inlB*. **(f-k)** Percentage (left)
123 and number (right) of Perforin⁺ (f), CD69⁺ (g), Granzyme-B⁺ (h), CD127⁺ (i), IFN γ ⁺ (j) and
124 KLRG1⁺ (k) CD8⁺ T-cells 3 days after iv inoculation of KIE16P mice with 10^4 CFU of CC4-WT
125 or CC4 Δ *inlB*. Data were obtained from three independent experiments and are presented as median
126 \pm interquartile (box) and extreme values (lines). Samples are compared with the Mann-Whitney
127 test. ns: $p > 0.05$, ***: $p < 0.001$, ****: $p < 0.0001$.

128

Extended Data Figure 8

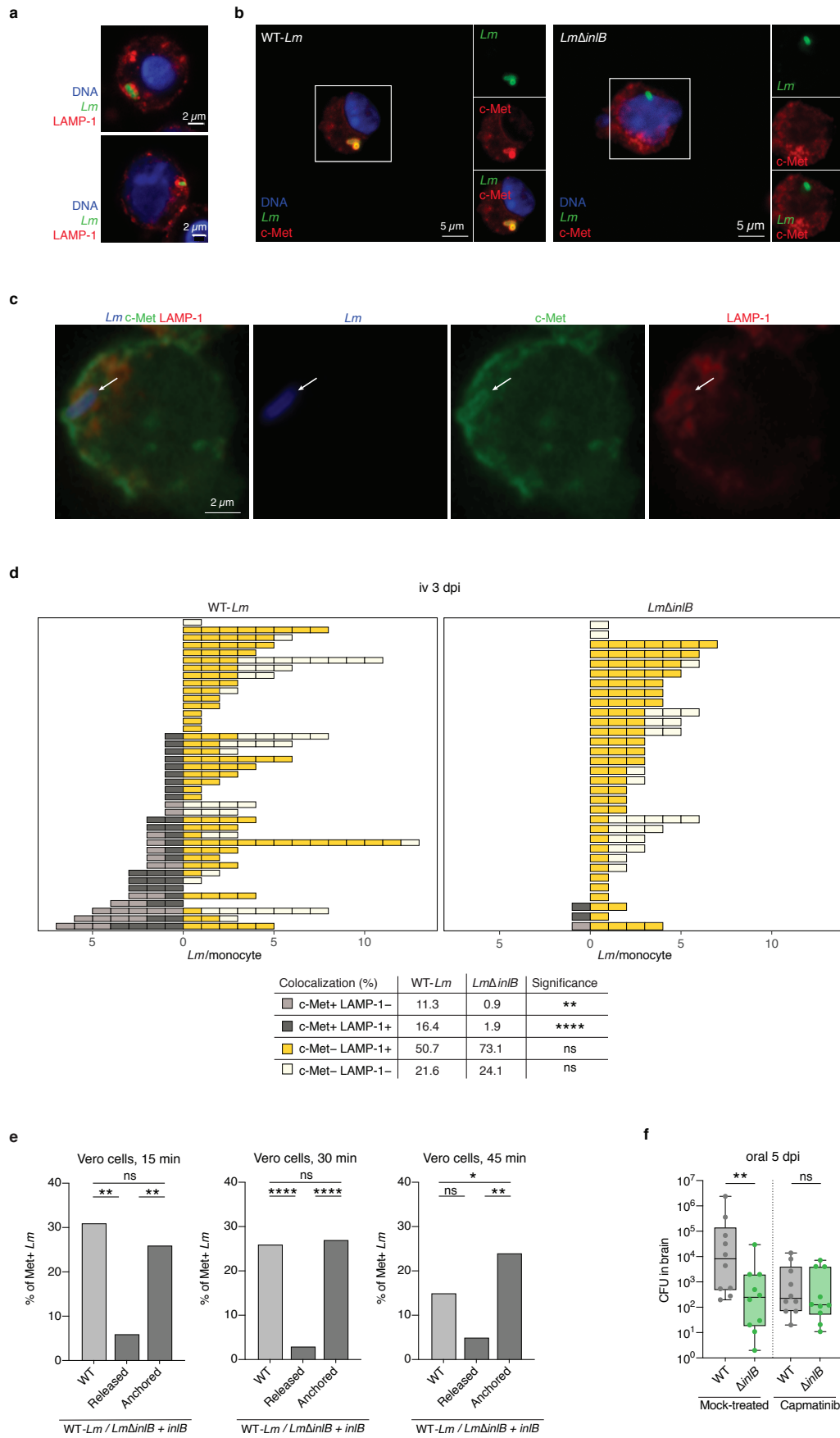


129 **Extended Data Figure 8. Membrane-associated InlB protects infected monocytes from CD8⁺**
130 **T cells-mediated cell death. (a)** Level of caspase-3 cleavage of infected spleen monocytes,
131 harvested 3 days after iv infection with 10⁴ CFU of CC4-WT or CC4 Δ *inlB* of KIE16P mice, and
132 incubated with CD8⁺ T-cells from similarly infected (WT and Δ *inlB*) or control (PBS) mice at the
133 indicated effector to target ratio, related to Fig. 3j. Results are normalized to the level of caspase-
134 3 cleavage in absence of CD8⁺ T cells. **(b)** Level of caspase-3 cleavage of uninfected spleen
135 monocytes, harvested 3 days after iv infection with 10⁴ CFU of CC4-WT or CC4 Δ *inlB* of KIE16P
136 mice, and incubated with CD8⁺ T-cells from similarly infected (WT and Δ *inlB*) or control (PBS)
137 mice at an effector to target ratio of 5, related to Fig. 3j. Results are normalized to the level of
138 caspase-3 cleavage in absence of CD8⁺ T cells. **(c)** Schematic representation of WT InlB and its
139 anchored and released variants. **(d)** Competition index in the brain of KIE16P mice 5 days after iv
140 inoculation with 10⁴ CFU of 1:1 CC4-WT and CC4 Δ *inlB* transformed with a plasmid expressing
141 either full-length WT InlB, cell wall-anchored InlB or released InlB. **(e)** Representative
142 fluorescence microscopy images of centrifugated CC4 Δ *inlB* transformed with a plasmid
143 expressing either full length InlB (left panel), anchored InlB (central panel) or released InlB (right
144 panel). Scale bars: 5 μ m. **(f)** Transcription level of *inlB* in CC4 Δ *inlB* transformed with a plasmid
145 expressing InlB variants in mid-log phase in BHI, relative to CC4 Δ *inlB* expressing full length InlB.
146 **(g)** Proportion of GFP- or tdTomato-expressing bacteria, co-localizing or not with LAMP-1, in 20
147 infected monocytes harvested 3 days after iv inoculation of KIE16P mice with 10⁴ CFU of 1:1 mix
148 of CC4-WT expressing GFP or tdTomato. **(h, i)** Level of caspase-3 cleavage of infected spleen
149 monocytes, harvested from *Prf1* KO (h) or *Fas*^{lpr-cg} (i) mice, 3 days after iv inoculation with 10⁴
150 CFU of CC4-WT or CC4 Δ *inlB*, incubated with CD8⁺ T-cells from similarly infected (WT and
151 Δ *inlB*) or control (PBS) mice, at an effector to target ratio of 5. **(j)** Level of caspase-3 cleavage of

152 non-infected spleen monocytes, harvested from KIE16P mice iv infected for 3 days with 10^4 CFU
153 of CC4-WT or CC4 Δ *inlB*, incubated *ex vivo* with Fas ligand, related to Fig. 3k. (k) Percentage of
154 infected spleen monocytes expressing Fas at their surface (left), and the mean fluorescence
155 intensity (MFI) of Fas signal (right), 3 days after iv inoculation of KIE16P mice with 10^4 CFU of
156 CC4-WT or CC4 Δ *inlB*. (l) Proportion of dye-positive infected monocytes in the blood and the
157 spleen after iv infection of KIE16P mice with 10^4 CFU of CC4-WT or CC4- Δ *inlB*. The repartition
158 of the estimated half-lives and the median are shown besides each graph. Data were obtained from
159 three independent experiments and are presented as mean \pm SD (a, b, f, h-l) or median \pm
160 interquartile (box) and extreme values (lines) (d). Samples are compared with an unpaired student
161 *t*-test (a, b, f, h-k), CFU in competition assays are compared with the Wilcoxon matched-pairs
162 signed rank test (d), samples are compared with the Kruskal-Wallis test (d) and distribution of
163 estimated half-lives (i) are compared with a Mood test. ns: $p > 0.05$, *: $p < 0.05$, ***: $p < 0.001$, ****:
164 $p < 0.0001$.

165

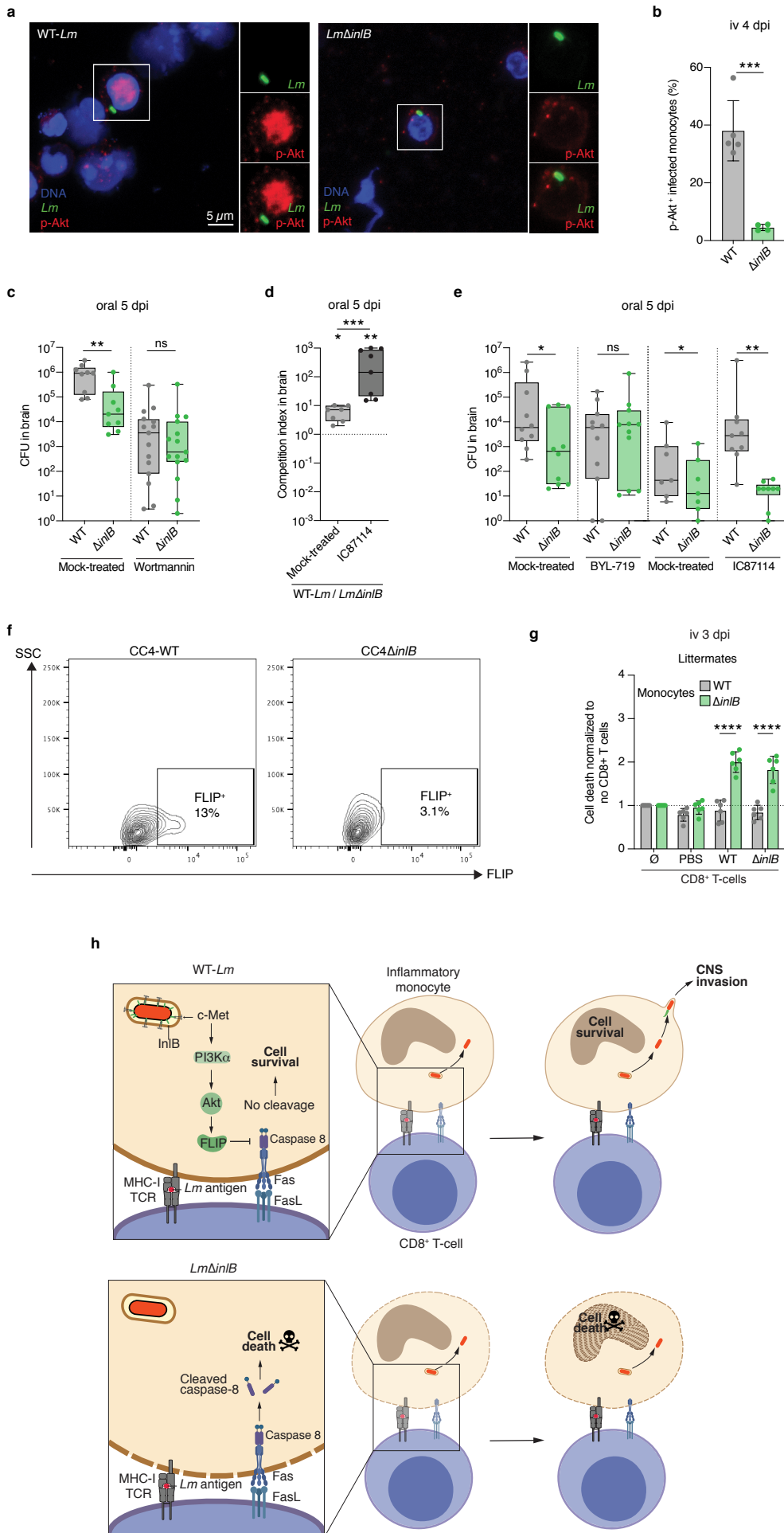
Extended Data Figure 9



166 **Extended Data Figure 9. InlB recruits c-Met in infected monocytes.** (a-c) Representative
167 fluorescence microscopy images of spleen monocytes harvested from KIE16P mice iv infected for
168 4 days with 10^4 CFU of CC4-WT or CC4 Δ *inlB*, showing intra-vacuolar *Lm* surrounded with
169 LAMP-1 (a), co-localizing with c-Met (b) and co-localizing with both c-Met and LAMP-1 (c). (a
170 and b) are maximum intensity projection over a z-stack. (c) is a confocal single plane image. (d)
171 Quantification of intracellular *Lm* co-localizing or not with c-Met and LAMP-1 in infected spleen
172 monocytes harvested from KIE16P mice iv infected for 4 days with 10^4 CFU of CC4-WT or
173 CC4 Δ *inlB*. Individual cells are plotted in top panel and samples are compared in bottom panel. (e)
174 Percentage of *Lm* co-localizing with c-Met *in vitro* in Vero cells 15 min (left), 30 min (middle)
175 and 45 min (right) after infection at MOI 50 with CC4 Δ *inlB* expressing either WT InlB, released
176 InlB or cell wall-anchored InlB. (f) Bacterial load in the brain 5 days after oral inoculation with
177 2×10^8 CFU of 1:1 of CC4-WT and CC4 Δ *inlB*, in KIE16P mice treated with capmatinib, related to
178 Fig. 4a. Data were obtained from three independent experiments (e-f) or from three microscopic
179 field of views (d). Median number of bacteria in each intracellular compartment were compared
180 with the Mann-Whitney test (d), proportions of c-Met associated bacteria (e) with Fischer's exact
181 test, and CFU in competition assays (f) compared with the Wilcoxon matched-pairs signed rank
182 test. ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ****: $p < 0.0001$.

183

Extended Data Figure 10



184 **Extended Data Figure 10. InlB-mediated neuroinvasion involves the c-Met/PI3K α /FLIP**
185 **pathway in infected monocytes. (a)** Representative fluorescence microscopy images of spleen
186 monocytes harvested from KIE16P mice iv infected for 4 days with 10^4 CFU of CC4-WT or
187 CC4 Δ *inlB*, showing phosphorylation of Akt. Images are maximum intensity projection over a z-
188 stack. **(b)** Proportion of infected spleen monocytes positive for p-Akt signal 4 days after iv
189 inoculation of KIE16P mice with 10^4 CFU of CC4-WT or CC4 Δ *inlB*. **(c)** Bacterial load in brain 5
190 days after oral inoculation with 2×10^8 CFU of 1:1 of CC4-WT and CC4 Δ *inlB*, in KIE16P mice
191 treated with wortmannin, related to Fig. 4d. **(d)** Competition index in brain 5 days after oral
192 inoculation with 2×10^8 CFU of 1:1 of CC4-WT and CC4 Δ *inlB*, in KIE16P mice treated with PI3K δ
193 inhibitor (IC87114). **(e)** Bacterial load in the brain 5 days after oral inoculation with 2×10^8 CFU
194 of 1:1 of CC4-WT and CC4 Δ *inlB*, in mice treated with BYL-719 or IC87114, related to Fig. 4e
195 and Extended Data Fig. 10d. **(f)** Representative dot plot of FLIP expression in infected
196 inflammatory spleen monocytes, 3 days after iv inoculation with 10^4 CFU of CC4-WT or
197 CC4 Δ *inlB*, related to Fig. 4f, g. **(g)** Level of caspase-3 cleavage of infected spleen monocytes,
198 harvested 3 days after iv infection with 10^4 CFU of CC4-WT or CC4 Δ *inlB* of tamoxifen-treated
199 *Rosa26-CreER*^{T2} x *Cflar*^{+/+} (FLIP^{+/+}) littermate mice and incubated with CD8⁺ T-cells from
200 similarly infected mice at an effector to target ratio of 5, related to Fig. 4h. **(h)** Representation of
201 InlB-activated pathway of infected monocytes survival to Fas-mediated cell death. Data were
202 obtained from three independent experiments and are presented as median \pm interquartile (box)
203 and extreme values (lines) (c-e) or mean \pm SD (b, g). CFU in competition assays are compared
204 with the Wilcoxon matched-pairs signed rank test (c-e) and samples with a Mann-Whitney test (c-
205 e) or an unpaired student *t*-test (b, g). ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****:
206 $p < 0.0001$.

Movie S1. Polymerization of actin comet tail by *Lm* within a monocyte adhering to the blood-brain barrier.

CX3CR1^{GFP/+} E16P KI humanized mice were infected with 5×10^5 CFUs of CC6 (isolate 2009-01092) via iv route. Mice were sacrificed 48 hours post infection. CX3CR1⁺ are labeled in green, *L. monocytogenes* in red, actin (phalloidin) in white and nuclei (Hoechst) in blue. Forty-six optical sections of a 20 μm thick brain sample were imaged with a Zeiss LSM700 confocal microscope. 3D reconstruction was performed using the Arivis Vison 4D software.