

1 **A TNF-IL-1 circuit controls *Yersinia* within intestinal granulomas**

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22 **Summary**

23           Monocytes restrict *Yersinia* infection within intestinal granulomas. Here, we report  
24 that monocyte-intrinsic TNF signaling drives production of IL-1 that signals to non-  
25 hematopoietic cells to control intestinal *Yersinia* infection within granulomas.

26 **Abstract**

27 Tumor necrosis factor (TNF) is a pleiotropic inflammatory cytokine that mediates  
28 antimicrobial defense and granuloma formation in response to infection by numerous  
29 pathogens. *Yersinia pseudotuberculosis* colonizes the intestinal mucosa and induces  
30 recruitment of neutrophils and inflammatory monocytes into organized immune structures  
31 termed pyogranulomas that control the bacterial infection. Inflammatory monocytes are  
32 essential for control and clearance of *Yersinia* within intestinal pyogranulomas, but how  
33 monocytes mediate *Yersinia* restriction is poorly understood. Here, we demonstrate that  
34 TNF signaling in monocytes is required for bacterial containment following enteric  
35 *Yersinia* infection. We further show that monocyte-intrinsic TNFR1 signaling drives  
36 production of monocyte-derived interleukin-1 (IL-1), which signals through IL-1 receptor  
37 on non-hematopoietic cells to enable pyogranuloma-mediated control of *Yersinia*  
38 infection. Altogether, our work reveals a monocyte-intrinsic TNF-IL-1 collaborative circuit  
39 as a crucial driver of intestinal granuloma function, and defines the cellular target of TNF  
40 signaling that restricts intestinal *Yersinia* infection.

## 41 Introduction

42 Granulomas form in response to a wide variety of infections, acting as barriers to  
43 pathogen dissemination<sup>1,2</sup>. Although generally considered protective, granulomas can  
44 also provide a replicative niche from which pathogens can spread, such as in immune-  
45 compromised patients that experience reactivation of latent *Mycobacterium*  
46 *tuberculosis*<sup>3,4</sup>. Moreover, pathogens within granulomas often persist in an antibiotic-  
47 resistant state and can pose a significant therapeutic challenge<sup>5</sup> Granulomas thus  
48 represent a localized niche within which pathogens persist and remain resistant to host  
49 immune clearance. Understanding how pathogens are controlled within granulomas  
50 remains an important question that could enable development of immunomodulatory  
51 treatments against infectious agents that persist within this niche.

52 Tumor necrosis factor (TNF) is a pleiotropic inflammatory cytokine associated with  
53 protection during granulomatous disease, notably tuberculosis<sup>6–13</sup>. While the role of TNF  
54 in maintaining intact granulomas is well-appreciated, its precise cellular targets and  
55 mechanisms of action remain elusive due to broad expression of its main receptor,  
56 TNFR1, and its pleiotropic downstream signaling functions, including induction of cell-  
57 extrinsic apoptosis, promoting cell survival, and mediating expression of pro-inflammatory  
58 gene programs<sup>14–16</sup>. TNF plays a critical role clinically in protection against infection by  
59 intracellular pathogens, as the extensive clinical use of anti-TNF blockade in the setting  
60 of auto-inflammatory disease is associated with increased risk of severe infection<sup>16,17</sup>.

61 The enteropathogenic *Yersiniae*, which also include *Y. pseudotuberculosis* (*Yp*)  
62 and *Y. enterocolitica*, colonize the intestinal mucosa and lymphoid tissues of both mice  
63 and humans, triggering formation of pyogranulomas (PG) that are composed of

64 extracellular bacterial colonies in close association with neutrophils, bordered in turn by  
65 monocytes and macrophages<sup>18-25</sup>. We recently demonstrated that PG containing viable  
66 bacteria, inflammatory monocytes, and neutrophils form along the length of the  
67 gastrointestinal tract early following oral *Yp* infection<sup>25</sup>. PG form in response to the activity  
68 of *Yersinia* Outer Proteins (Yops), which are injected into host cells through the *Yersinia*  
69 type III secretion system and block essential antimicrobial functions<sup>25-27</sup>. We further  
70 demonstrated that inflammatory monocytes were critical for maintenance of PG  
71 architecture and enabling neutrophils to overcome the activity of *Yp* virulence factors that  
72 block host phagocytosis and oxidative burst<sup>25</sup>. However, the mechanisms by which  
73 inflammatory monocytes mediate anti-*Yersinia* host defense are unclear.

74 Here, we demonstrate that monocytes serve as an essential cellular source of  
75 TNF, which is required for host protection against *Yersinia*<sup>28-30</sup>. We find that signaling  
76 through both TNF and IL-1 receptor are required to maintain PG control of *Yp*. Intriguingly,  
77 monocyte-intrinsic TNF production and receptor signaling were required for PG  
78 monocytes to produce IL-1, an inflammatory cytokine involved in control of other microbial  
79 infections. IL-1 in turn signals to IL-1 receptor on non-hematopoietic cells to enable control  
80 of intestinal *Yp* infection. Altogether, our study demonstrates that a monocyte-driven TNF-  
81 IL-1 signaling circuit mediates the control of *Yp* infection within systemic and intestinal  
82 sites and demonstrates that TNF and IL-1 collaborate via a feed-forward loop to promote  
83 host defense against microbial infection.

## 84 **Results**

### 85 *TNFR1 is required for organized pyogranuloma formation and restriction of Yersinia*

86 We recently identified the formation of pyogranulomas (PG) in the murine intestinal  
87 mucosa during acute *Yersinia pseudotuberculosis* (*Yp*) infection, wherein inflammatory  
88 monocytes were required for neutrophil activation, maintenance of PG architecture, and  
89 bacterial clearance<sup>25</sup>. Nonetheless, the monocyte-derived signals required for the  
90 function and maintenance of these intestinal PG are unknown. Tumor necrosis factor  
91 (TNF) is critical for granuloma maintenance and bacterial control in the lung during  
92 tuberculosis infection<sup>6-13</sup>, and we previously found that TNF signaling is necessary for the  
93 control of bacterial burdens following oral *Yersinia* infection<sup>30</sup>. Notably, while *Tnfr1*<sup>-/-</sup> mice  
94 formed similar numbers of macroscopic intestinal lesions as wild-type (WT) mice (Fig.  
95 S1A), histopathologic analyses revealed that intestinal lesions in *Tnfr1*<sup>-/-</sup> mice displayed  
96 a disorganized appearance and contained a central area of tissue necrosis that was  
97 strikingly similar to lesions that we recently described in monocyte-deficient mice<sup>25</sup>, (Fig.  
98 1A). In contrast to WT PG, which had robust immune cell aggregation and a small central  
99 *Yp* microcolony, *Tnfr1*<sup>-/-</sup> intestinal lesions contained limited immune cell infiltrate and an  
100 expanded *Yp* colony (Fig. 1A, B). In line with these histopathological findings, bacterial  
101 burdens in pyogranuloma-containing (PG+) intestinal punch biopsies and adjacent non-  
102 pyogranuloma (PG-) biopsies were elevated in *Tnfr1*<sup>-/-</sup> mice (Fig. 1C). Furthermore, *Tnfr1*<sup>-/-</sup>  
103 PG contained fewer monocytes, macrophages, and neutrophils, as determined by flow  
104 cytometry (Fig. 1D, S1B). Surface expression of the integrin CD11b, a marker of  
105 neutrophil activation<sup>31-33</sup>, was significantly reduced in PG of *Tnfr1*<sup>-/-</sup> mice compared to WT  
106 controls (Fig. 1E), suggesting a defect in neutrophil activation in the absence of TNFR1

107 signaling, consistent with our recent findings of reduced neutrophil activation within PG in  
108 the absence of monocytes<sup>25</sup>. Additionally, *Tnfr1*<sup>-/-</sup> mice exhibited elevated bacterial  
109 burdens in the spleen and liver (Fig. 1F), consistent with our previous findings<sup>30</sup>. Notably,  
110 *Tnfr1*<sup>-/-</sup> mice succumbed to infection around day 8, while most WT mice survived (Fig.  
111 1G). Overall, these data suggest that TNFR1 signaling is necessary to mediate functional  
112 intestinal PG formation and control of *Yp*.

113 TNFR1 signaling can enhance the ability of hematopoietic (immune) or non-  
114 hematopoietic (stromal) cells to control pathogens<sup>10,11,13,30,34</sup>. To test which compartment  
115 requires TNFR1 signaling to control *Yp*, we generated bone marrow chimeras in which  
116 TNFR1 expression was ablated on the immune or stromal compartment (Fig. S1C). Mice  
117 lacking TNFR1 in either the immune or stromal compartment had elevated bacterial  
118 burdens within PG compared to WT control chimeras, indicating that TNFR1 signaling is  
119 required non-redundantly in both hematopoietic and non-hematopoietic cells to mediate  
120 control of intestinal *Yp* (Fig. 1H). In contrast, mice lacking TNFR1 in immune cells had  
121 elevated bacterial burdens in the systemic tissues, while mice lacking TNFR1 in stromal  
122 cells had similar bacterial burdens in systemic tissues as WT controls (Fig. 1I). Taken  
123 together, these results demonstrate that TNFR1 signaling in both hematopoietic and non-  
124 hematopoietic cells contribute to bacterial control in the intestine, while TNFR1 signaling  
125 specifically in immune cells is required for bacterial control in the systemic tissues during  
126 acute *Yp* infection.

127

128 *Autocrine TNF signaling in monocytes is required for control of Yersinia*

129 TNF receptor expression is widespread on hematopoietic cells, raising the  
130 question of which specific cells are the necessary targets of TNF signaling for control of  
131 *Yp* infection. We previously demonstrated that CCR2-deficient mice lacking circulating  
132 monocytes fail to form functional intestinal PG, are unable to control *Yp* burdens, and  
133 succumb to infection<sup>25</sup>. Given the similar outcomes of infection and histopathological  
134 appearance of PG in TNFR1- and CCR2-deficient mice, we sought to test the hypothesis  
135 that TNF is either produced or detected by monocytes. To do this, we generated mice in  
136 which TNFR1 was specifically deleted on inflammatory monocytes by means of mixed  
137 BM chimeras where irradiated wild-type recipient mice were reconstituted with a 1:1 ratio  
138 of *Ccr2<sup>gfp/gfp</sup>:Tnfr1<sup>-/-</sup>* or *Ccr2<sup>gfp/gfp</sup>:WT* control BM cells (Fig. 2A). Because circulating  
139 monocytes in these chimeric mice are derived from the *Tnfr1<sup>-/-</sup>* or WT BM cells,  
140 respectively, this approach generates mice in which circulating CCR2<sup>+</sup> monocytes lacked  
141 or expressed TNFR1, respectively, with other hematopoietic cell types being comprised  
142 of a 1:1 mixture of these genotypes (Fig. S2A). Intriguingly, mice lacking TNFR1  
143 specifically on CCR2<sup>+</sup> monocytes formed lesions with expanded bacterial colonies and  
144 failed to control *Yp* infection, largely recapitulating the phenotype of mice lacking CCR2  
145 in the hematopoietic system altogether (Fig. 2B-D). Importantly, this defect in bacterial  
146 control was not due to a lack of TNFR1 expression on 50% of other immune cells, as  
147 mixed chimeras from *Tnfr1<sup>-/-</sup>:WT* mice still had significantly lower bacterial burdens  
148 relative to *Tnfr1<sup>-/-</sup>:Ccr2<sup>gfp/gfp</sup>* mice, notably in systemic tissues (Fig. S2B, C). Mice lacking  
149 TNFR1 expression on all hematopoietic cells had significantly higher burdens than mice  
150 lacking TNFR1 on monocytes alone (Fig. S2B). Altogether, these data suggest that



151 TNFR1 signaling in monocytes is essential for their protective role against *Yp* infection,  
152 and that TNFR1 has additional important roles in other cell types beyond monocytes.

153 Multiple immune cell types produce TNF in response to inflammatory signals,  
154 including monocytes, which we previously observed to be a major source of TNF during  
155 *Yp* infection<sup>35</sup>. Thus, we considered that monocytes might be an important source as well  
156 as recipient of the TNF signal to enable control of *Yp* infection. We therefore reconstituted  
157 irradiated wild-type recipient mice with a 1:1 ratio of *Ccr2<sup>gfp/gfp</sup>:Tnf<sup>-/-</sup>* or *Tnf<sup>-/-</sup>:WT* control  
158 BM cells, in order to generate cohorts of mice in which circulating CCR2<sup>+</sup> monocytes  
159 lacked or retained the ability to produce TNF, respectively, with other hematopoietic cell  
160 types being comprised of a 1:1 mixture. Strikingly, mice lacking TNF specifically in  
161 monocytes failed to control *Yp* infection in the spleen and liver, with equal burdens to  
162 those completely lacking TNF production in all hematopoietic cells (Fig. 2E, F).  
163 Altogether, our findings demonstrate that autocrine TNF signaling in monocytes is  
164 required to control enteric *Yp* infection.

165  
166 *TNFR1 signaling in monocytes controls Yp infection independently of RIPK1 kinase-*  
167 *induced cell death*

168 TNFR1 can mediate inflammatory gene expression or promote cell-extrinsic  
169 apoptosis in response to infection by pathogens, including *Yersinia*<sup>36–41</sup>. *Yp*-induced cell  
170 death is triggered by YopJ-induced blockade of IKK signaling and involves contributions  
171 from both TLR4/TRIF and TNFR1 signaling through the adapter kinase RIPK1<sup>35,42–47</sup>. We  
172 previously demonstrated that mice specifically lacking RIPK1 kinase activity (*Ripk1<sup>K45A</sup>*)  
173 in hematopoietic cells fail to form intact MLN PG and rapidly succumb to *Yp* infection<sup>35</sup>.

174 Furthermore, activation of gasdermin D and gasdermin E in macrophages and  
175 neutrophils, respectively, downstream of RIPK1 kinase activity promotes control of *Yp*  
176 infection<sup>48</sup>. *Ripk1*<sup>K45A</sup> mice formed necrotic intestinal lesions and were deficient in  
177 restricting *Yp* burdens, consistent with prior findings (Fig. 3A-C). These data provoked  
178 the hypothesis that monocyte-intrinsic TNFR1 signaling promotes anti-*Yersinia* host  
179 defense through activation of RIPK1-induced monocyte cell death. To directly test this,  
180 we generated mixed BM chimeras in which irradiated WT recipient mice were  
181 reconstituted with a 1:1 ratio of *Ccr2*<sup>gfp/gfp</sup>:*Ripk1*<sup>K45A</sup> or *Ccr2*<sup>gfp/gfp</sup>:WT control BM cells.  
182 Following reconstitution, mice contained circulating CCR2<sup>+</sup> monocytes that either lacked  
183 or expressed RIPK1 kinase activity, respectively, with all other hematopoietic cells being  
184 equally reconstituted by both donor bone marrow progenitors (Fig. S3A). Surprisingly, in  
185 contrast to hematopoietic loss of RIPK1 kinase activity, monocyte-specific ablation of  
186 RIPK1 kinase activity had no effect on the ability of mice to form intact intestinal PG or  
187 control enteric *Yp* infection (Fig. 3D-F), indicating that RIPK1 kinase activity is  
188 dispensable in monocytes to control *Yp* infection downstream of TNF signaling. Our  
189 previous findings demonstrated that the acute susceptibility of *Ripk1*<sup>K45A</sup> mice is reversed  
190 in the setting of infection with YopJ-deficient bacteria, illustrating that RIPK1 kinase-  
191 induced cell death is necessary to counteract the blockade of immune signaling by  
192 YopJ<sup>35</sup>. However, *Tnfr1*<sup>-/-</sup> mice still formed necrotic intestinal PG, were unable to control  
193 bacterial burdens in systemic tissues, and succumbed to infection by YopJ-deficient  
194 bacteria (Fig. 3G-I). Collectively, these data indicate TNFR1 signaling contributes to anti-  
195 *Yersinia* host defense via a mechanism distinct from RIPK1-induced cell death. We  
196 recently reported that intestinal PG form in response to the activities of the actin

197 cytoskeleton-disrupting effectors YopE and YopH, and that monocytes counteract YopH-  
198 mediated blockade of innate immunity<sup>25</sup>. YopE and YopH both block phagocytosis and  
199 the oxidative burst through disruption of actin cytoskeleton rearrangement<sup>49–58</sup>. However,  
200 whether TNFR1 is required to overcome the immune blockade posed by YopE and YopH  
201 is unknown. Strikingly, TNFR1-deficient mice survived infection with *yopEH* mutant *Yp*  
202 (Fig. 3J), indicating that TNFR1 signaling counteracts the activity of YopE and YopH.  
203 However, in contrast to our previous findings with CCR2-deficient mice<sup>25</sup>, *Tnfr1*<sup>-/-</sup> mice  
204 were not able to control either single *Yp* mutant, although there was a significant delay in  
205 mortality in response to infection with *yopH* mutant bacteria (Fig. S3B). Together, these  
206 findings demonstrate that TNFR1-mediated restriction of enteric *Yp* infection is  
207 independent of RIPK1-induced cell death, and instead counteracts the anti-phagocytic  
208 and reactive oxygen-blocking activities of YopE and YopH.

209

210 *Cell-intrinsic TNFR1 signaling is required for maximal IL-1 production within intestinal*  
211 *pyogranulomas during Yersinia infection*

212 Our findings indicate that while TNFR1 expression on monocytes is critical for  
213 effective intestinal PG formation and control of *Yp* infection, monocyte-intrinsic RIPK1  
214 kinase activity is dispensable for PG formation and bacterial restriction. This suggests  
215 that RIPK1 kinase-independent mechanisms mediate monocyte-dependent control of *Yp*  
216 downstream of TNFR1 signaling. We therefore hypothesized that TNFR1 signaling in  
217 monocytes may contribute to control of *Yp* infection via promoting inflammatory cytokine  
218 production. Multiplex cytokine profiling of intestinal PG from mixed BM chimeric mice  
219 lacking monocyte-intrinsic TNFR1 expression revealed that IL-1 $\alpha$  levels were significantly

220 decreased, in contrast to other pro-inflammatory cytokines such as IL-6 and KC (Fig. 4A,  
221 S4A). Neither IL-1 $\alpha$  nor IL-1 $\beta$  were detected in the sera of these mice, suggesting that IL-  
222 1 production in response to TNFR1 signaling is localized to intestinal tissues during *Yp*  
223 infection (Fig. S4B).

224         Since TNFR1 expression on monocytes is required for intestinal PG formation and  
225 restriction of bacterial burdens, we hypothesized that TNFR1 signaling promotes IL-1  
226 production by monocytes within intestinal PG. Indeed, intracellular cytokine staining  
227 demonstrated that both IL-1 $\alpha$  and IL-1 $\beta$  expression were decreased in both monocytes  
228 and neutrophils in *Tnfr1*<sup>-/-</sup> PG, indicating that TNFR1 signaling is necessary for maximal  
229 IL-1 production in both monocytes and neutrophils within intestinal PG (Fig. 4B). We next  
230 asked whether TNFR1 signaling functions in a cell-intrinsic or -extrinsic manner to  
231 promote IL-1 cytokine production. To distinguish between these possibilities, we  
232 generated mixed bone marrow chimeras in which lethally irradiated WT recipients were  
233 reconstituted with a 1:1 mixture of WT and *Tnfr1*<sup>-/-</sup> bone marrow or entirely reconstituted  
234 with *Tnfr1*<sup>-/-</sup> bone marrow as a positive control. Importantly, there was no competitive  
235 defect in reconstitution by *Tnfr1*<sup>-/-</sup> cells in the mixed chimera setting, as these mice  
236 contained 1:1 ratio of WT and *Tnfr1*<sup>-/-</sup> immune cells within the PG and spleen (Fig. S4C).  
237 Strikingly, IL-1 production was reduced in both monocytes and neutrophils lacking TNFR1  
238 relative to WT cells isolated from the same mice, demonstrating that cell-intrinsic TNFR1  
239 is required for optimal production of IL-1 in monocytes and neutrophils (Fig. 4C, D, S4D).  
240 To ask if TNF signals in an autocrine fashion to upregulate its own expression, we  
241 measured intracellular TNF in these WT:*Tnfr1*<sup>-/-</sup> mixed chimeras. TNF levels were  
242 reduced in both monocytes and neutrophils lacking TNFR1 relative to WT cells isolated

243 from the same intestinal PG, demonstrating that TNFR1 signals in a feedforward loop to  
244 promote TNF production in a cell-intrinsic fashion (Fig. S4E). Overall, these data show  
245 that cell-intrinsic TNFR1 signaling is necessary for maximal IL-1 production in myeloid  
246 cells within intestinal PG, raising the question of whether IL-1 production downstream of  
247 TNFR1 signaling in monocytes contributes to control of *Yp* during early intestinal infection.

248  
249 *IL-1 is required for organized pyogranuloma formation and intestinal control of Yersinia*

250 IL-1 plays a critical role in host defense by promoting immune cell recruitment and  
251 activation, cytokine production, angiogenesis, and vascular permeability<sup>59–64</sup>. Mice  
252 lacking IL-1 signaling are more susceptible to systemic *Yersinia* infection<sup>48,65,66</sup>. However,  
253 the role of IL-1 signaling during enteric *Yp* infection and downstream of TNF receptor  
254 signaling is unclear. IL-1 $\beta$  production has been proposed to promote increased intestinal  
255 permeability and barrier dysfunction<sup>67</sup>, suggesting multifaceted roles for IL-1 signaling  
256 within specific compartments and stages of infection. We considered the possibility that  
257 TNFR1-mediated restriction of enteric *Yp* infection and intestinal PG formation occurs in  
258 part via induction of IL-1 production from monocytes. To test the contribution of IL-1  
259 signaling in control of enteric *Yp* infection, we infected *Il1r1*<sup>-/-</sup> mice, which lack IL-1R and  
260 cannot respond to IL-1 cytokines. *Il1r1*<sup>-/-</sup> mice had significantly higher bacterial burdens  
261 than WT mice in the intestine, specifically in Peyer's Patches, PG+, and PG- tissue (Fig.  
262 5A). Notably, the intestinal lesions in *Il1r1*<sup>-/-</sup> mice showed extensive loss of organization  
263 and contained a central area of tissue necrosis as compared to those found in WT mice  
264 (Fig. 5B). Strikingly, the intestinal lesions in *Il1r1*<sup>-/-</sup> mice bore substantial resemblance to  
265 the intestinal lesions seen in *Ccr2*<sup>gfp/gfp</sup> mice<sup>25</sup> and *Tnfr1*<sup>-/-</sup> mice (Fig. 5B and 1A). *Il1r1*<sup>-/-</sup>

266 mice also succumbed to infection to a greater extent than WT mice (Fig. 5C). However,  
267 at day 5 post-infection, bacterial burdens in systemic organs were broadly comparable to  
268 those of WT mice (Fig. S5A). Collectively, these results suggest that consistent with PG-  
269 specific TNF-dependent IL-1 production, IL-1-mediated *Yp* restriction occurs in the  
270 intestine during early infection and that there are likely other non-IL-1-mediated  
271 mechanisms induced downstream of TNF signaling that contribute to systemic control.

272 IL-1R initiates intracellular signaling cascades in response to both IL-1 $\alpha$  and IL-1 $\beta$ .  
273 To test whether IL-1 $\alpha$  and IL-1 $\beta$  are individually important for intestinal PG formation and  
274 *Yp* control, we infected *Il1a*<sup>-/-</sup> and *Il1b*<sup>-/-</sup> mice. Compared to WT mice, both *Il1a*<sup>-/-</sup> and *Il1b*<sup>-/-</sup>  
275 <sup>-/-</sup> mice had elevated bacterial burdens in PP and PG<sup>+</sup>, but not in PG<sup>-</sup> tissue (Fig. 5D), in  
276 contrast to *Il1r1*<sup>-/-</sup> mice which had elevated bacterial burdens in all three intestinal  
277 compartments. Like *Il1r1*<sup>-/-</sup> mice, *Il1a*<sup>-/-</sup> and *Il1b*<sup>-/-</sup> mice overall had similar bacterial  
278 burdens in systemic organs as WT mice on day 5 post-infection (Fig. S5A). Collectively,  
279 these results suggest that IL-1 $\alpha$  and IL-1 $\beta$  may have overlapping roles in restricting early  
280 enteric *Yp* infection, and in the absence of one, the other may compensate. Intriguingly,  
281 *Il1a*<sup>-/-</sup> mice had a comparable survival defect to *Il1r1*<sup>-/-</sup> mice, whereas *Il1b*<sup>-/-</sup> mice were  
282 similar to WT mice in survival following *Yp* infection (Fig. 5E). Collectively, these results  
283 indicate that IL-1R signaling is important for intestinal PG formation and control of enteric  
284 *Yp* infection, and may constitute a mechanism by which TNFR1 signaling controls local  
285 intestinal infection.

286  
287 *Monocyte-derived IL-1 signals to non-hematopoietic cells to restrict Yersinia in intestinal*  
288 *pyogranulomas*

289 Our findings demonstrate that with PG, autocrine TNF signaling in inflammatory  
290 monocytes promotes cell-intrinsic IL-1 production and subsequent IL-1R signaling  
291 promotes anti-*Yp* immune defense. However, whether monocyte-derived IL-1 is  
292 specifically required for control of intestinal *Yp* has not been tested. We therefore infected  
293 mixed BM chimeras in which irradiated wild-type recipient mice were reconstituted with a  
294 1:1 ratio of *Il1ab*<sup>-/-</sup>:*Ccr2*<sup>gfp/gfp</sup> bone marrow cells to generate cohorts of mice specifically  
295 lacking IL-1α and IL-1β production in monocytes, along with mice reconstituted with *Il1ab*<sup>-/-</sup>  
296 <sup>-</sup>:WT bone marrow or 100% *Il1ab*<sup>-/-</sup> bone marrow (Fig. S6A). Critically, mice specifically  
297 lacking IL-1α and IL-1β in monocytes had significantly elevated bacterial burdens in PG,  
298 recapitulating elevated bacteria burdens in PG of hematopoietic-deficient IL-1α and IL-1β  
299 chimeric mice and indicating that monocyte-derived IL-1 is important for restricting  
300 infection within intestinal PG (Fig. 6A). Bacterial burdens in PG- punch biopsies and  
301 systemic organs were broadly similar across chimeric mice genotypes (Fig. 6A, S6B),  
302 suggesting that IL-1 production from other cell types besides monocytes may contribute  
303 to intestinal infection restriction. Collectively, these data suggest that multiple cellular  
304 sources of IL-1 drive restriction of *Yp*. In agreement with our previous finding that TNFR1-  
305 deficient mice have a defect in IL-1 production within PG, monocyte-derived IL-1 was  
306 critical for control of bacterial burdens within PG, indicating that IL-1 production from  
307 monocytes plays a significant role in TNF-dependent control within this intestinal niche.

308 Multiple cell types express *Il1r1* and respond to IL-1 signaling. In other infectious  
309 settings, IL-1R signaling specifically in the stromal compartment is critical for antibacterial  
310 defense<sup>62,64,68-71</sup>. We therefore considered that IL-1R signaling in stromal cells may be  
311 critical for formation and maintenance of intestinal PG as well as *Yp* restriction. To test

312 this, we generated BM chimeric mice in which irradiated *Il1r1*<sup>-/-</sup> mice were reconstituted  
313 with WT BM. Additionally, irradiated WT mice were reconstituted with either *Il1r1*<sup>-/-</sup> bone  
314 marrow or a 1:1 ratio of *Il1r1*<sup>-/-</sup>:WT bone marrow. Notably, mice lacking IL-1R in the  
315 stromal compartment had elevated bacterial burdens in both intestinal (PP, PG+ and PG)  
316 and systemic (liver and spleen) tissues following oral *Yp* infection compared to WT control  
317 chimeras, while mice lacking IL-1R in the hematopoietic compartment had similar  
318 bacterial burdens to WT control chimeras (Fig. 6B, C). Overall, our findings demonstrate  
319 that IL-1R signaling on the stromal compartment is required to restrict *Yp* infection both  
320 in the intestinal and systemic tissues.



## 321 Discussion

322 Granulomas are organized biological structures containing multiple immune cell  
323 types working in concert with stromal cells to sequester pathogens that are difficult to  
324 clear<sup>1,2</sup>. *Yersinia pseudotuberculosis* induces the formation of granulomatous lesions in  
325 both the human and murine intestine<sup>18–25</sup>. Here, we uncover a TNF/IL-1 signaling circuit  
326 that promotes restriction of enteropathogenic *Yp* within intestinal pyogranulomas.  
327 Notably, we find that autocrine TNF signaling on inflammatory monocytes was necessary  
328 to promote cell-intrinsic IL-1 production, which signaled on the non-hematopoietic  
329 compartment to elicit control of *Yp* within intestinal pyogranulomas.

330 TNF has a well-established role in granuloma formation and maintenance<sup>6–13</sup>. Anti-  
331 TNF therapy triggers reactivation of dormant *Mycobacterium tuberculosis* infection<sup>8,72,73</sup>,  
332 and TNF promotes macrophage-dependent control of *Salmonella* replication within  
333 granulomas during chronic *Salmonella* infection<sup>34</sup>. TNF promotes multiple antimicrobial  
334 activities of macrophages that are critical for granuloma formation and control of  
335 tuberculosis<sup>10,11,13,74</sup>. However, excessive TNF causes macrophage cell death that can  
336 be detrimental to bacterial control<sup>75</sup>. Some pathogens counteract the pro-inflammatory  
337 effects of TNF signaling within granulomas. *Salmonella* injects the T3SS effector SteE to  
338 induce anti-inflammatory M2 macrophage polarization, countering TNF-driven M1  
339 polarization to hinder bacterial clearance<sup>34</sup>. In line with our previous observations that  
340 TNFR1 is required for protection against *Yp*<sup>30</sup>, our findings highlight a key role for TNF  
341 signaling in promoting intestinal pyogranuloma formation and function during  
342 enteropathogenic *Yp* infection.

343 TNFR1 signaling promotes *Yp*-induced cell death via RIPK1 activity, and we  
344 previously found that RIPK1 activity was necessary for control of *Yp* infection<sup>35</sup>. However,  
345 while deficiency in RIPK1 kinase activity led to disrupted intestinal PG formation during  
346 early infection, we surprisingly found that RIPK1 kinase activity was dispensable in  
347 monocyte-lineage cells for PG formation and bacterial restriction. These findings suggest  
348 that two distinct pathways are necessary for protection against enteric *Yersinia* infection:  
349 1) a monocyte-intrinsic TNFR1 pathway that amplifies inflammatory cytokine production  
350 in monocytes, and 2) a YopJ-induced RIPK1 kinase-mediated cell death of non-monocyte  
351 cells. Notably, neutrophils undergo GSDME-dependent pyroptosis downstream of RIPK1  
352 kinase activity, which contributes to restriction of *Yp* infection *in vivo*<sup>48</sup>. Future studies will  
353 elucidate whether RIPK1 kinase-dependent neutrophil cell death promotes control of *Yp*.

354 We found that TNFR1 signaling on PG monocytes enhanced cell-intrinsic IL-1  
355 production, consistent with our previous findings that monocyte-deficient intestinal PG  
356 have reduced IL-1 levels<sup>25</sup>. While TNF receptor signaling amplifies inflammasome  
357 activation, an important step in IL-1 processing<sup>76-78</sup> very few studies have described  
358 TNFR-signaling-mediated IL-1 production specifically. In the context of *Legionella*  
359 *pneumophila* infection, IL-1 signaling induces TNF production in uninjected bystander  
360 cells in order to overcome virulence-induced host protein blockade that prevents TNF  
361 production from infected cells<sup>61,62</sup>. Interestingly, *Yersinia* YopJ suppresses inflammatory  
362 cytokine expression including TNF expression<sup>43</sup>. Whether uninjected bystander  
363 monocytes are the critical source of TNF during enteric *Yp* infection remains unexplored.  
364 TNF has been shown to induce macrophage polarization and promotion of IL-1 $\beta$

365 expression via sterol response element binding factors<sup>16</sup> How TNF receptor signaling  
366 augments IL-1 production during *Yp* infection is still unknown.

367 IL-1R signaling is critical for infection control during tuberculosis infection and loss  
368 of IL-1R signaling leads to expansion of pathologic lesions in the lung<sup>79-84</sup>. We observed  
369 that in the absence of IL-1R, mice failed to form organized intestinal pyogranulomas and  
370 had elevated intestinal *Yp* burdens, corroborating previous reports that IL-1R signaling  
371 promotes anti-*Yersinia* defense<sup>48,65,66</sup>. Intriguingly, systemic bacterial burdens were  
372 similar between WT mice and mice lacking IL-1R signaling, suggesting that there TNF  
373 signaling induces other mechanisms of bacterial restriction beyond IL-1-mediated  
374 protection. Mice lacking IL-1R signaling were more susceptible than mice lacking IL-1 $\alpha$   
375 or IL-1 $\beta$  alone, indicating that both cytokines contribute non-redundantly to bacterial  
376 restriction. While mice deficient in IL-1 $\alpha$  exhibited elevated mortality, there was no  
377 difference in mortality in mice deficient in IL-1 $\beta$ . Perhaps during the early intestinal stage  
378 of infection, IL-1 $\alpha$  and IL-1 $\beta$  mediate overlapping mechanisms of *Yersinia* restriction,  
379 while at later stages of infection, IL-1 $\alpha$  is largely responsible for IL-1R-mediated control.  
380 IL-1 $\alpha$  is a critical mediator of intestinal inflammation and inflammatory cell recruitment  
381 during *Yersinia enterocolitica* infection<sup>85</sup>. In contrast, IL-1 $\beta$  contributes to *Yersinia*  
382 restriction<sup>48</sup> but also promotes intestinal barrier permeability and translocation of  
383 commensal bacteria downstream of YopJ activity<sup>86</sup>. Together, these observations  
384 suggest that tight regulation of intestinal IL-1 signaling is important to combat *Yersinia*  
385 infection while avoiding excessive tissue damage and loss of intestinal barrier function.

386 While IL-1 $\beta$  is released from hematopoietic cells downstream of inflammasome  
387 activation, IL-1 $\alpha$  is more broadly expressed across cell types and can function in multiple

388 locations, including within the nucleus, as a membrane-bound cytokine, or as an alarmin  
389 released from dying cells<sup>87</sup>. We found that monocyte-intrinsic IL-1 $\alpha$  and IL-1 $\beta$  were  
390 necessary for control of *Yp* burdens within intestinal PG, consistent with other infectious  
391 contexts where hematopoietic-derived IL-1 drives pathogen restriction<sup>62,82</sup>. However, IL-  
392 1 $\alpha$  and IL-1 $\beta$  production from monocytes was dispensable for control of *Yp* burdens in  
393 PG- tissue and systemic organs, suggesting that production from other cell types  
394 contribute to IL-1-mediated infection restriction in these compartments. TNFR1 signaling  
395 also promoted neutrophil IL-1 production during enteric *Yp* infection, in line with prior  
396 studies identifying a role for GSDME-dependent IL-1 $\beta$  production by neutrophils in control  
397 of enteric *Yp* infection<sup>48</sup>. Whether neutrophils rely on a similar TNFR1-IL-1 signaling  
398 pathway to elicit control of *Yp* remains to be investigated.

399 Finally, IL-1R on stromal cells was critical for control of *Yp* infection. In other  
400 infectious contexts, IL-1R signaling in stromal cells is important for pathogen control,  
401 highlighting a recurring theme of IL-1R signaling cross-talk between immune and non-  
402 immune cells during infection<sup>62,64,68,88,89</sup>. Stromal cells are increasingly appreciated as  
403 critical components of the innate immune response. IL-1R is expressed in non-lymphoid  
404 tissues, including epithelial and endothelial cells, across various organs<sup>90</sup>, suggesting a  
405 conserved mechanism by which hematopoietic cytokine signaling can be amplified during  
406 infection and inflammation. The stromal cells in the intestine that respond to IL-1R  
407 signaling and the downstream anti-bacterial functions that promote restriction of *Yersinia*  
408 remain unknown. Intestinal epithelial cells respond to IL-1R signaling by upregulating  
409 antimicrobial peptide production, promoting neutrophil recruitment, and modulating  
410 intestinal permeability<sup>59,64,69,70,89,91,92</sup>. Neutrophil activation is critical within intestinal PG

411 during *Yersinia* infection and decreased neutrophil recruitment is observed in the absence  
412 of monocytes<sup>25</sup> and TNFR1 signaling. Whether IL-1R signaling on the intestinal epithelial  
413 or endothelial compartment promotes neutrophil recruitment and function during *Yp*  
414 infection remains to be determined in future studies. Altogether, our work uncovers a  
415 monocyte-intrinsic TNF/IL-1 circuit that signals to IL-1R on stromal cells to control  
416 *Yersinia* infection, providing new mechanistic insight into the cytokine networks that  
417 promote enteric granuloma formation and function.

418 **Methods**

419 *Mice*

420 C57BL/6J (CD45.2), C57BL/6.SJL (CD45.1), *Ccr2<sup>gfp/gfp</sup>* mice<sup>93</sup> were obtained from  
421 the Jackson Laboratory. *Tnfr1<sup>-/-</sup>*<sup>94</sup>, *Ripk1<sup>K45A</sup>*<sup>95</sup>, *Il1r1<sup>-/-</sup>*<sup>96</sup>, *Il1a<sup>-/-</sup>*<sup>97</sup>, *Il1b<sup>-/-</sup>*<sup>97</sup> and *Il1a<sup>-/-</sup>Il1b<sup>-/-</sup>*  
422 <sup>97</sup> mice were previously described. All mice were bred at the University of Pennsylvania  
423 by homozygous mating and housed separately by genotype. Mice of either sex between  
424 8-12 weeks of age were used for all experiments. All animal studies were performed in  
425 strict accordance with University of Pennsylvania Institutional Animal Care and Use  
426 Committee-approved protocols (protocol #804523).

427

428 *Bacteria*

429 Wild-type *Yp* (clinical isolate strain 32777, serogroup O1)<sup>98</sup> and isogenic YopJ-  
430 deficient mutant were provided by Dr. James Bliska (Dartmouth College) and previously  
431 described<sup>47</sup>. Generation of mutants lacking YopE ( $\Delta yopE$ ), enzymatic activity of YopH  
432 (YopH<sup>R409A</sup>), or both (denoted *yopEH*) were previously described<sup>25</sup>.

433

434 *Bone marrow chimeras*

435 Wild-type B6.SJL mice (CD45.1 background) or knockout mice (*Tnfr1<sup>-/-</sup>* or *Il1r1<sup>-/-</sup>*,  
436 CD45.2 background) were lethally irradiated (1096 rads). 6 hours later, mice were  
437 injected retro-orbitally with freshly isolated bone marrow cells ( $5 \times 10^6$  total cells,  $2.5 \times 10^6$   
438 cells per donor in mixed groups) from isogenic donors of the indicated genotypes. All  
439 chimeras were provided with antibiotic-containing acidified water (40 mg trimethoprim and  
440 200 mg sulfamethoxazole per 500 mL drinking water) for four weeks after irradiation and

441 subsequently provided acidified water without antibiotics for a total of at least ten weeks.  
442 The reconstitution of hematopoietic cells (proportion of donor CD45<sup>+</sup> cells among total  
443 CD45<sup>+</sup> cells) in the blood, spleen, or intestine was analyzed by flow cytometry.

444

#### 445 *Mouse infections*

446 *Yp* was cultured to stationary phase at 28°C and 250 rpm shaking for 16 hours in  
447 2xYT broth supplemented with 2 µg/ml triclosan (Millipore Sigma). Mice were fasted for  
448 16 hours and subsequently inoculated by oral gavage with 200 µl phosphate-buffered  
449 saline (PBS) as previously<sup>25</sup> All bacterial strains were administered at 2x10<sup>8</sup> colony-  
450 forming units (CFU) per mouse.

451

#### 452 *Bacterial CFU quantifications*

453 Tissues were collected in sterile PBS, weighed, homogenized for 40 seconds with  
454 6.35 mm ceramic spheres (MP Biomedical) using a FastPrep-24 bead beater (MP  
455 Biomedical). Samples were serially diluted tenfold in PBS, plated on LB agar  
456 supplemented with 2 µg/ml triclosan, and incubated for two days at room temperature.  
457 Dilutions of each sample were plated in triplicate and expressed as the mean CFU per  
458 gram or per biopsy.

459

#### 460 *Cytokine quantification*

461 Cytokines were measured in homogenized tissue supernatants using a Cytometric  
462 Bead Array (BD Biosciences) according to manufacturer's instructions with the following  
463 modification: the amounts of capture beads, detection reagents, and sample volumes

464 were scaled down tenfold. Data were collected on an LSRFortessa flow cytometer (BD  
465 Biosciences) and analyzed with FlowJo v10 (BD Biosciences).

466

#### 467 *Tissue preparation and cell isolation*

468 Blood was harvested by cardiac puncture upon euthanasia and collected in 250  
469 U/ml Heparin solution (Millipore Sigma). Erythrocytes were lysed with Red Blood Cell  
470 Lysing Buffer (Millipore Sigma).

471 Spleens were homogenized through a 70  $\mu$ m cell strainer (Fisher Scientific), then  
472 flushed with R10 buffer consisting of RPMI 1640 (Millipore Sigma) supplemented with 10  
473 mM HEPES (Millipore Sigma), 10% fetal bovine serum (Omega Scientific), 1 mM sodium  
474 pyruvate (Thermo-Fisher Scientific), and 100 U/ml penicillin + 100  $\mu$ g/ml streptomycin  
475 (Thermo Fisher Scientific). Erythrocytes were lysed with Red Blood Cell Lysing Buffer  
476 (Millipore Sigma).

477 Intestines were excised, flushed luminally with sterile PBS to remove the feces,  
478 opened longitudinally along the mesenteric side and placed luminal side down on cutting  
479 boards (Epicurean). Small intestinal tissue containing macroscopically visible  
480 pyogranulomas (PG+), adjacent non-granulomatous areas (PG-) and uninfected control  
481 tissue (uninf) were excised using a 2 mm- $\varnothing$  dermal punch-biopsy tool (Keyes). Biopsies  
482 within each mouse were pooled groupwise, suspended in epithelial dissociation buffer  
483 consisting of calcium and magnesium-free HBSS (Thermo Fisher Scientific)  
484 supplemented with 15 mM HEPES, 10 mg/ml bovine serum albumin (Millipore Sigma), 5  
485 mM EDTA (Millipore Sigma), and 100 U/ml penicillin + 100  $\mu$ g/ml streptomycin, then  
486 incubated for 30 minutes at 37°C under continuous agitation at 300 RPM. To isolate



487 immune cells from the lamina propria, the tissue was enzymatically digested in R10  
488 buffer, along with 0.5 Wünsch units/ml liberase TM (Roche), 30 µg/ml DNase I (Roche),  
489 and 5 mM CaCl<sub>2</sub> for 20 min at 37°C under continuous agitation. The resulting cell  
490 suspensions were filtered through 100 µm cell strainers (Fisher Scientific) and subjected  
491 to density gradient centrifugation using Percoll (GE Healthcare). Briefly, cells were  
492 suspended in 40% Percoll and centrifuged over a 70% Percoll layer for 20 min at 600 ×  
493 g with the lowest brake at room temperature. Cells collected between the layers were  
494 washed with R10 buffer for downstream analysis.

495

#### 496 *Flow cytometry*

497 Non-specific Fc binding was blocked for 10 minutes on ice with unconjugated anti-  
498 CD16/CD32 (93; Thermo-Fisher Scientific). Cells were subsequently labeled for 30  
499 minutes on ice with the following antibodies and reagents: PE-conjugated rat anti-mouse  
500 Siglec-F (E50-2440; BD Biosciences), PE-TxR or PE-Cy5-conjugated rat anti-mouse  
501 CD11b (M1/70.15; Thermo Fisher Scientific), PE-Cy5.5 or PE-Cy7-conjugated rat anti-  
502 mouse CD4 (RM4-5; Thermo Fisher Scientific), BV510-conjugated rat anti-mouse CD3e  
503 (145-2C11; BioLegend), AF700 or PerCP-Cy5.5-conjugated rat anti-mouse Ly-6C  
504 (HK1.4; Thermo Fisher Scientific), BV605-conjugated Armenian hamster anti-mouse  
505 TCRβ (H57-597; BD Biosciences), BV650-conjugated rat anti-mouse I-A/I-E  
506 (M5/114.15.2; BD Biosciences), BV711-conjugated rat anti-mouse CD8α (53-6.7; BD  
507 Biosciences), BV785-conjugated rat anti-mouse Ly-6G (1A8; Thermo Fisher Scientific),  
508 PE-Cy7 or AF647-conjugated mouse anti-mouse CD64 (X54-5/7.1; BD Biosciences),  
509 AF700-conjugated mouse anti-mouse CD45.2 (104; BioLegend), PE-Cy5-conjugated

510 mouse anti-mouse CD45.1 (A20; Thermofisher), PE-Cy5 or PE-CF594-conjugated rat  
511 anti-mouse CD45R/B220 (RA3-6B2; BD Biosciences) along with eF780 viability dye  
512 (BioLegend) diluted in PBS. Antibodies were used at 1:200 dilution and viability dye at  
513 1:1500 dilution.

514 For intracellular staining, cells were incubated for 3 hours at 37°C with 5% CO<sub>2</sub> in  
515 R10 buffer supplemented with 0.33 µl/ml GolgiStop (BD Biosciences) and 15 µg/ml  
516 DNase I. Surface proteins were stained as above, and cells were fixed for 20 minutes on  
517 ice with Cytotfix/Cytoperm Fixation/Permeabilization solution (BD Biosciences).  
518 Intracellular cytokines were stained at 4°C overnight with FITC or PerCP-e710-  
519 conjugated rat anti-mouse IL-1β (NJTEN3; Thermo Fisher Scientific) and PE-conjugated  
520 Armenian hamster anti-mouse IL-1α (ALF-161; BioLegend). All intracellular antibodies  
521 were diluted 1:200 in Perm/Wash Buffer (BD Biosciences). Cells were acquired on an  
522 LSRTFortessa flow cytometer and data were analyzed with FlowJo v10. Cells were gated  
523 on live singlets prior to downstream analyses.

524

### 525 *Histology*

526 Tissues were fixed in 10% neutral-buffered formalin (Fisher Scientific) and stored  
527 at 4°C until further processed. Tissue pieces were embedded in paraffin, sectioned by  
528 standard histological techniques and stained with hematoxylin and eosin. Slides were  
529 scanned on an Aperio VERSA using brightfield at 20x magnification. Histopathological  
530 disease scoring was performed by blinded board-certified pathologists. Tissue sections  
531 were given a score from 0-4 (healthy-severe) for multiple parameters, including degree

532 of inflammatory cell infiltration, necrosis, and free bacterial colonies, along with tissue-  
533 specific parameters such as villus blunting and crypt hyperplasia.

534

535 *Statistics*

536 Statistical analyses were performed using Prism v9.0 (GraphPad Software).

537 Independent groups were compared by Mann-Whitney U test or Kruskal-Wallis test with

538 Dunn's multiple comparisons test. Survival curves were compared by Mantel-Cox test.

539 Statistical significance is denoted as \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ), \*\*\* ( $p < 0.001$ ), \*\*\*\*

540 ( $p < 0.0001$ ), or ns (not significant).

541 **Figure legends**

542 **Figure 1. TNFR1 is required for organized pyogranuloma formation and restriction**  
543 **of *Yersinia* in intestine and periphery**

544 **(A)** H&E-stained paraffin-embedded longitudinal small intestinal sections from *Yp*-  
545 infected mice at day 5 post-infection. Dashed line highlights pyogranuloma (left) or  
546 necrosuppurative lesion (right). Images representative of two independent experiments.  
547 Scale bars = 500  $\mu$ m (top) and 200  $\mu$ m (bottom).

548 **(B)** Histopathological scores of small intestinal tissue from uninfected or *Yp*-infected mice  
549 at day 5 post-infection. Each mouse was scored between 0-4 (healthy-severe) for  
550 indicated sign of pathology. Each circle represents one mouse. Lines represent median.  
551 Pooled data from two independent experiments.

552 **(C)** Bacterial burdens in small intestinal PG- and PG+ tissue isolated day 5 post-infection.  
553 Each circle represents the mean CFU of 3-5 pooled punch biopsies from one mouse.  
554 Lines represent geometric mean. Pooled data from three independent experiments.

555 **(D)** Total numbers and frequencies of CD45<sup>+</sup> cells, monocytes, macrophages, and  
556 neutrophils in uninfected, PG-, and PG+ small intestinal tissue isolated 5 days post-  
557 infection. Each circle represents the mean of 3-10 pooled punch biopsies from one  
558 mouse. Lines represent median. Pooled data from three independent experiments.

559 **(E)** Mean fluorescence intensity (MFI) of CD11b expression on neutrophils in PG+ tissue  
560 at day 5 post-infection. Each circle represents the mean of 3-10 pooled punch biopsies  
561 from one mouse. Lines represent median. Data representative of three independent  
562 experiments.

563 (F) Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents  
564 one mouse. Lines represent geometric mean. Pooled data from four independent  
565 experiments.

566 (G) Survival of infected WT (n=9) and *Tnfr1*<sup>-/-</sup> (n=21) mice. Pooled data from two  
567 independent experiments.

568 (H) Bacterial burdens in small intestinal PG- and PG+ tissue at day 5 post-infection of  
569 indicated chimeric mice. Each circle represents the mean *Yp*-CFU of 3-5 pooled punch  
570 biopsies from one mouse. Lines represent geometric mean. Pooled data from two  
571 independent experiments.

572 (I) Bacterial burdens in indicated organs at day 5 post-infection of indicated chimeric mice.  
573 Each circle represents one mouse. Lines represent geometric mean. Pooled data from  
574 two independent experiments.

575 Statistical analysis by Mann-Whitney U test (B, C, D, E, F), Mantel-Cox test (G), and  
576 Kruskal-Wallis test with Dunn's multiple comparisons correction (H, I) \*p<0.05, \*\*p<0.01,  
577 \*\*\*p<0.001, \*\*\*\*p<0.0001, ns = not significant.

578

579 **Figure 2. Autocrine TNF signaling in monocytes is required for control of *Yersinia***

580 (A) Schematic of mixed bone marrow chimeras.

581 (B) H&E-stained paraffin-embedded transverse small-intestinal sections from chimeric  
582 WT mice reconstituted with *Ccr2*<sup>gfp/gfp</sup> + WT (left), *Ccr2*<sup>gfp/gfp</sup> + *Tnfr1*<sup>-/-</sup> (middle), or  
583 *Ccr2*<sup>gfp/gfp</sup> (right) bone marrow, at day 5 post-infection. Dotted lines highlight lesions. Scale  
584 bars = 100 μm. Images representative of two independent experiments.

585 (C) Bacterial burdens in small intestinal PG- and PG+ tissue of chimeric WT mice  
586 reconstituted with either *Ccr2<sup>gfp/gfp</sup>* + *WT* (white), *Ccr2<sup>gfp/gfp</sup>* + *Tnfr1<sup>-/-</sup>* (light gray), or  
587 *Ccr2<sup>gfp/gfp</sup>* (dark gray) at day 5 post *Yp*-infection. Each symbol represents one mouse.  
588 Lines represent geometric mean. Pooled data from two independent experiments.

589 (D) Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents  
590 one mouse. Lines represent geometric mean. Pooled data from two independent  
591 experiments.

592 (E) Bacterial burdens in small intestinal PG- and PG+ tissue of chimeric WT mice  
593 reconstituted with either *Tnf<sup>-/-</sup>* + *WT* (white), *Tnf<sup>-/-</sup>* + *Ccr2<sup>gfp/gfp</sup>* (light gray), or *Tnf<sup>-/-</sup>* (dark  
594 gray) at day 5 post *Yp*-infection. Each symbol represents one mouse. Lines represent  
595 geometric mean. Pooled data from three independent experiments.

596 (F) Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents  
597 one mouse. Lines represent geometric mean. Pooled data from three independent  
598 experiments.

599 Statistical analysis by Kruskal-Wallis test with Dunn's multiple comparisons correction.  
600 \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , ns = not significant.

601

602 **Figure 3. TNFR1 signaling in monocytes controls *Yp* infection independently of**  
603 **RIPK1 kinase-induced cell death**

604 (A) Bacterial burdens in small intestinal PG- and PG+ tissue of WT (white) and *Ripk1<sup>K45A</sup>*  
605 (blue) mice at day 5 post *Yp*-infection. Each symbol represents one mouse. Lines  
606 represent geometric mean. Pooled data from two independent experiments.

607 **(B)** Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents  
608 one mouse. Lines represent geometric mean. Pooled data from two independent  
609 experiments.

610 **(C)** H&E-stained paraffin-embedded longitudinal small intestinal sections from WT (left)  
611 and *Ripk1*<sup>K45A</sup> (right) mice at day 5 post *Yp*-infection with dotted line highlighting lesion.  
612 Scale bars = 100  $\mu$ m. Representative images of two independent experiments.

613 **(D)** H&E-stained paraffin-embedded transverse small-intestinal sections from chimeric  
614 WT mice reconstituted with either *Ccr2*<sup>gfp/gfp</sup> + WT (left), *Ccr2*<sup>gfp/gfp</sup> + *Ripk1*<sup>K45A</sup> (middle),  
615 or *Ccr2*<sup>gfp/gfp</sup> (right) bone marrow, at day 5 post *Yp*-infection with dotted line highlighting  
616 lesion. Scale bars = 100  $\mu$ m. Representative images of two independent experiments.

617 **(E)** Bacterial burdens in small intestinal PG- and PG+ tissue of chimeric WT mice  
618 reconstituted with either *Ccr2*<sup>gfp/gfp</sup> + WT (white), *Ccr2*<sup>gfp/gfp</sup> + *Ripk1*<sup>K45A</sup> (light gray), or  
619 *Ccr2*<sup>gfp/gfp</sup> (dark gray) at day 5 post *Yp*-infection. Each symbol represents one mouse.  
620 Lines represent geometric mean. Pooled data from two independent experiments.

621 **(F)** Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents  
622 one mouse. Lines represent geometric mean. Pooled data from two independent  
623 experiments.

624 **(G)** H&E-stained paraffin-embedded longitudinal small intestinal sections from WT and  
625 *Tnfr1*<sup>-/-</sup> mice infected with either WT or  $\Delta$ *yopJ* *Yp* at day 5 post-infection. Scale bars = 100  
626  $\mu$ m. Representative images of three independent experiments.

627 **(H)** Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents  
628 one mouse. Lines represent geometric mean. Pooled data from four independent  
629 experiments.

630 (I) Survival of WT (white) and *Tnfr1*<sup>-/-</sup> (gray) mice infected with WT (circles) or *ΔyopJ*  
631 (squares) *Yp*. n = 9-12 mice per group. Pooled data from two independent experiments.  
632 (J) Survival of WT (white) or *Tnfr1*<sup>-/-</sup> (gray) mice infected with WT (circles) or *yopEH*  
633 (squares) *Yp*. n = 11-15 mice per group. Pooled data from two independent experiments.  
634 Statistical analysis by Mann-Whitney U test (A, B), Kruskal-Wallis test with Dunn's  
635 multiple comparisons correction (E, F, H), or Mantel-Cox test (I, J). \*p<0.05, \*\*p<0.01,  
636 \*\*\*p<0.001, \*\*\*\*p<0.0001, ns = not significant.

637

638 **Figure 4. Cell-intrinsic TNFR1 signaling is required for maximal IL-1 production**  
639 **within intestinal pyogranulomas during *Yersinia* infection**

640 (A) Cytokine levels were measured by cytometric bead array in tissue punch biopsy  
641 homogenates isolated 5 days post-infection from chimeric WT mice reconstituted with  
642 indicated donor cells. Lines represent median. Pooled data from two independent  
643 experiments.

644 (B) Intracellular cytokine levels in monocytes and neutrophils isolated from small intestinal  
645 PG<sup>+</sup> tissue 5 days post-infection. Each circle represents the mean of 3-10 pooled punch  
646 biopsies from one mouse. Lines represent median. Pooled data from three independent  
647 experiments.

648 (C) Flow cytometry plots of intracellular IL-1 in monocytes (CD64<sup>+</sup> Ly-6C<sup>hi</sup>) from small  
649 intestinal PG<sup>+</sup> tissue at day 5 post-infection. Plots representative of two independent  
650 experiments.

651 (D) Aggregate datasets from (C) for intracellular IL-1 staining in monocytes and  
652 neutrophils in small intestinal PG<sup>+</sup> tissue at day 5 post-infection. Each circle represents



653 the mean of 3-10 pooled punch biopsies from one mouse. Lines connect congenic cell  
654 populations within individual mice. Pooled data from two independent experiments.  
655 Statistical analysis by Kruskal-Wallis test with Dunn's multiple comparisons correction  
656 (A), Mann-Whitney U test (B), congenic cells within mice: Wilcoxon test; across groups:  
657 Mann-Whitney U test (D). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , ns = not  
658 significant.

659

660 **Figure 5. IL-1 signaling is required for organized pyogranuloma formation and**  
661 **intestinal control of *Yersinia***

662 (A) Bacterial burdens in small intestinal Peyer's patches (PP), PG-, and PG+ tissues  
663 isolated 5 days post-infection. For PP, each circle represents pooled tissue from one  
664 mouse. For PG- and PG+, each circle represents the mean of 3-5 pooled punch biopsies  
665 from one mouse. Lines represent geometric mean. Pooled data from three independent  
666 experiments.

667 (B) H&E-stained paraffin-embedded longitudinal small intestinal sections from *Yp*-  
668 infected mice at day 5 post-infection. Representative images of one experiment. Scale  
669 bars = 250  $\mu\text{m}$ .

670 (C) Survival of infected WT (n=26) and *Il1r1*<sup>-/-</sup> (n=20) mice. Pooled data from two  
671 independent experiments

672 (D). Bacterial burdens in small intestinal PP, PG-, and PG+ tissues at day 5 post-infection  
673 of indicated genotypes. For PP, each circle represents pooled tissue from one mouse.  
674 For PG- and PG+, each circle represents the mean of 3-5 pooled punch biopsies from

675 one mouse. Lines represent geometric mean. Pooled data from three independent  
676 experiments.

677 (E). Survival of infected WT (n=27, n=19), *Il1a*<sup>-/-</sup> (n=22), and *Il1b*<sup>-/-</sup> (n=21) mice. Pooled  
678 data from three (WT vs *Il1a*<sup>-/-</sup>) and two (WT vs *Il1b*<sup>-/-</sup>) independent experiments.

679 Statistical analysis by Mann-Whitney U test (A, D) or Mantel-Cox test (C, E) \*p<0.05,  
680 \*\*p<0.01, \*\*\*\*p<0.0001, ns = not significant.

681

682 **Figure 6. Monocyte-derived IL-1 signals to nonhematopoietic cells to restrict**  
683 ***Yersinia* infection in intestinal pyogranulomas**

684 (A) Bacterial burdens in small intestinal Peyer's patches (PP), PG-, and PG+ tissues at  
685 day 5 post-infection of indicated chimeric mice. For PP, each circle represents pooled  
686 tissue from one mouse. For PG- and PG+, each circle represents the mean of 3-5 pooled  
687 punch biopsies from one mouse. Lines represent geometric mean. Data pooled from two  
688 independent experiments.

689 (C) Bacterial burdens in small intestinal Peyer's patches (PP), PG-, and PG+ tissues  
690 isolated 5 days post-infection of indicated chimeric mice. For PP, each circle represents  
691 one mouse. For PG- and PG+, each circle represents the mean of 3-5 pooled punch  
692 biopsies from one mouse. Lines represent geometric mean. Pooled data from three  
693 independent experiments.

694 (C) Bacterial burdens in indicated organs at day 5 post-infection of indicated chimeric  
695 mouse. Each circle represents one mouse. Lines represent geometric mean. Data pooled  
696 from three independent experiments.

697 **(D)** Model of TNF-IL-1 circuit mediated by monocyte and stromal compartment to promote  
698 *Yp* restriction within intestinal pyogranulomas.

699 All statistical analysis by Kruskal-Wallis test with Dunn's multiple comparisons correction.

700 \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , ns = not significant.

701

702 **Supplemental Figure 1. Effects of TNFR1-deficiency on pyogranuloma formation in**  
703 **intestine and lymphatic tissue during *Yersinia* infection**

704 (A) Total number of intestinal lesions at day 5 post-infection with *Yp*. Each circle  
705 represents one mouse. Lines represent median. Pooled data from four independent  
706 experiments.

707 (B) Flow cytometry plots displaying the gating strategy employed to identify neutrophils  
708 (CD11b<sup>+</sup> Ly-6G<sup>+</sup>), monocytes (CD64<sup>+</sup> Ly-6C<sup>hi</sup>), and macrophages (CD64<sup>+</sup> Ly-6C<sup>lo</sup> MHC-  
709 II<sup>hi</sup>) in small intestinal PG<sup>+</sup> tissue. Representative images of three independent  
710 experiments.

711 (C) Frequencies of indicated cell types in blood of uninfected chimeric mice. Pooled data  
712 from two independent experiments.

713 All statistical analyses by Mann-Whitney U test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ,

714 \*\*\*\* $p < 0.0001$ , ns = not significant.

715

716 **Supplemental Figure 2. Autocrine TNF signaling in monocytes is required for**  
717 **systemic control of *Yersinia***

718 (A) Frequency of indicated cell types in the blood of uninfected chimeric mice.

719 (B) Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents  
720 one mouse. Lines represent geometric mean.

721 (C) Bacterial burdens in small-intestinal PG- and PG+ tissue at day 5 post *Yp*-infection.  
722 Each symbol represents one mouse. Lines represent geometric mean.

723 All data pooled from two independent experiments. Statistical analysis by Kruskal-Wallis  
724 test with Dunn's multiple comparisons correction. Mann-Whitney U test. \* $p < 0.05$ ,  
725 \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , ns = not significant.

726

727 **Supplemental Figure 3. TNFR1 signalling in monocytes is independent of YopJ-**  
728 **induced RIPK1 kinase activity**

729 (A) Frequency of indicated cell types in the blood of uninfected chimeric mice.

730 (B) Survival of *wild-type* (left) and *Tnfr1*<sup>-/-</sup> (right) mice infected with WT (white circles),  
731  $\Delta yopE$  (blue) or YopH<sup>R409A</sup> (white squares) *Yp*. n = 5-32 (*wild-type*) and 13-20 (*Tnfr1*<sup>-/-</sup>)  
732 mice per group. Pooled data from 2-4 independent experiments.

733 Statistical analysis by Mantel-Cox test (B). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ ,  
734 ns = not significant.

735

736 **Supplemental Figure 4. Cytokine production downstream of TNFR1 expression on**  
737 **monocytes is specific to IL-1 in intestinal pyogranulomas**

738 (A) Cytokine levels in homogenates of tissue punch biopsies were measured by  
739 cytometric bead array at day 5 post-infection of chimeric WT mice reconstituted with  
740 indicated cells. Lines represent median. Pooled data from two independent experiments.

741 (B) Cytokine levels in serum were measured by cytometric bead array at day 5 post-  
742 infection of chimeric WT mice reconstituted with indicated cells. Lines represent median.  
743 ND = not detected. Pooled data from two independent experiments.

744 (C) Frequencies of indicated cell types in small intestinal PG<sup>+</sup> tissue or spleen at day 5  
745 post-infection of WT chimeric mice reconstituted with the indicated cells. Pooled data from  
746 two independent experiments.

747 (D) Flow cytometry plots of intracellular IL-1 in monocytes (CD64<sup>+</sup> Ly-6C<sup>hi</sup>) from small  
748 intestinal PG<sup>+</sup> tissue in WT and *Il1b*<sup>-/-</sup> mice at day 5 post-infection. Plots representative  
749 of two independent experiments.

750 (E) Intracellular levels of TNF in monocytes and neutrophils in small intestinal PG<sup>+</sup> tissue  
751 at day 5 post-infection. Each circle represents the mean of 3-10 pooled punch biopsies  
752 from one mouse. Lines connect congenic cell populations within individual mice. Pooled  
753 data from two independent experiments.

754 Statistical analyses by Kruskal-Wallis test with Dunn's multiple comparisons correction  
755 (A, B), or congenic cells within mice: Wilcoxon test; across groups: Mann-Whitney U test  
756 (E). ns = not significant.

757

758 **Supplemental Figure 5. Systemic bacterial burdens are comparable in WT and IL-**  
759 **1-deficient mice.**

760 Bacterial burdens in indicated organ at day 5 post-infection. Each circle represents one  
761 mouse. Lines represent geometric mean. Pooled data from three independent  
762 experiments.

763 All statistical analyses by Mann-Whitney U test. \*p<0.05, ns = not significant.

764

765 **Supplemental Figure 6. Monocyte-derived IL-1 signals to non-hematopoietic cells**  
766 **to restrict *Yersinia* infection**

767 **(A)** Frequencies of cell types in the blood of chimeric mice. Pooled data from two  
768 independent experiments

769 **(B)** Bacterial burdens in indicated organ at day 5 post-infection of indicated chimeric  
770 mouse. Each circle represents one mouse. Lines represent geometric mean. Pooled data  
771 from two independent experiments.

772 **(C)** Frequencies of indicated cell types in small intestinal PG+ tissue at day 5 post-  
773 infection of indicated chimeric mouse. Pooled data from three independent experiments

774 Statistical analysis by Kruskal-Wallis test with Dunn's multiple comparisons correction

775 **(B).** \* $p < 0.05$ , ns = not significant.

## 776 **Acknowledgements**

777           We thank Enrico Radaelli for constructive input and the staff at the PennVet  
778 Comparative Pathology Core for their help in preparing and analyzing the histological  
779 samples. We thank members of the Brodsky and Shin labs for scientific discussion. This  
780 work was supported by NIH Awards R01AI128530 (IEB), R01AI1139102A1 (IEB), and  
781 R01DK123528 (IEB); BWF Investigator in the Pathogenesis of Infectious Disease Award  
782 (IEB, SS); Mark Foundation Grant 19-011MIA (IEB), the Foundation Blanceflor  
783 Postdoctoral Scholarship (DS), the Swedish Society for Medical Research postdoctoral  
784 fellowship (DS) and the Sweden-America Foundation J. Sigfrid Edström award (DS); NIH  
785 NRSA F31AI160741-01 (RM); NIH T32 AI141393-2 (RM); F32 AI164655 (JPG); NIH  
786 NRSA F31AI161319 (BH); and NSF GRFP Award (SP); NIH T32 AI141393-03 (JZ); NIH  
787 Awards R21AI151476 (SS), R01AI118861 (SS), and R01AI123243 (SS). The veterinary  
788 pathologists performing the histopathological analysis are supported by the Abramson  
789 Cancer Center Support Grant (P30 CA016520). The scanner used for whole slide imaging  
790 and the image visualization software was supported by an NIH Shared Instrumentation  
791 Grant (S10 OD023465-01A1). Model figure was created using Biorender.

792 **Competing interests**

793           The authors have no conflicting financial interests.



794 **Abbreviations**

795	CCR2	CC chemokine receptor 2
796	MLN	Mesenteric lymph nodes
797	PG	Pyogranuloma
798	PP	Peyer's patches
799	RIPK1	Receptor-interacting protein kinase 1
800	TNF	Tumor necrosis factor
801	TNFR1	Tumor necrosis factor receptor 1
802	<i>Yp</i>	<i>Yersinia pseudotuberculosis</i>
803	Yop	<i>Yersinia</i> outer protein

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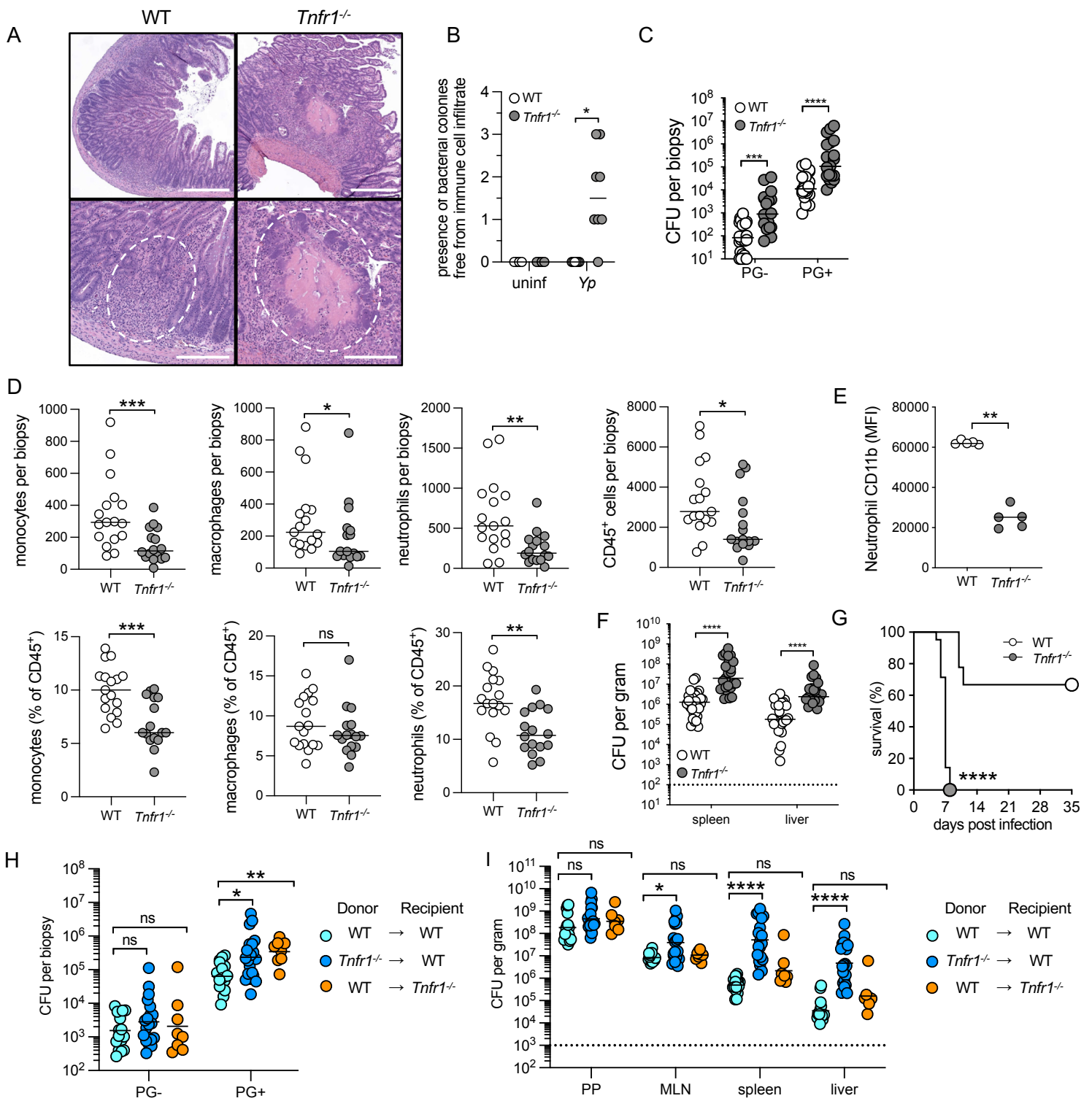
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**Figure 1. TNFR1 is required for organized pyogranuloma formation and restriction of *Yersinia***

(A) H&E-stained paraffin-embedded longitudinal small intestinal sections from *Yp*-infected mice at day 5 post-infection. Dashed line highlights pyogranuloma (left) or necrosuppurative lesion (right). Images representative of two experiments. Scale bars = 500  $\mu\text{m}$  (top) and 200  $\mu\text{m}$  (bottom).

(B) Histopathological scores of small intestinal tissue from uninfected or *Yp*-infected mice at day 5 post-infection. Each mouse was scored between 0-4 (healthy-severe) for indicated sign of pathology. Each circle represents one mouse. Lines represent median. Pooled data from two experiments.

(C) Bacterial burdens in small-intestinal PG- and PG+ tissue isolated day 5 post-infection. Each circle represents the mean CFU of 3-5 pooled punch biopsies from one mouse. Lines represent geometric mean. Pooled data from three independent experiments.

(D) Total numbers and frequencies of CD45<sup>+</sup> cells, monocytes, macrophages, and neutrophils in uninfected, PG-, and PG+ small intestinal tissue isolated 5 days post-infection. Each circle represents the mean of 3-10 pooled punch biopsies from one mouse. Lines represent median. Pooled data from three independent experiments.

(E) Mean fluorescence intensity (MFI) of CD11b expression on neutrophils in PG+ tissue at day 5 post-infection. Each circle represents the mean of 3-10 pooled punch biopsies from one mouse. Lines represent median. Data representative of three independent experiments.

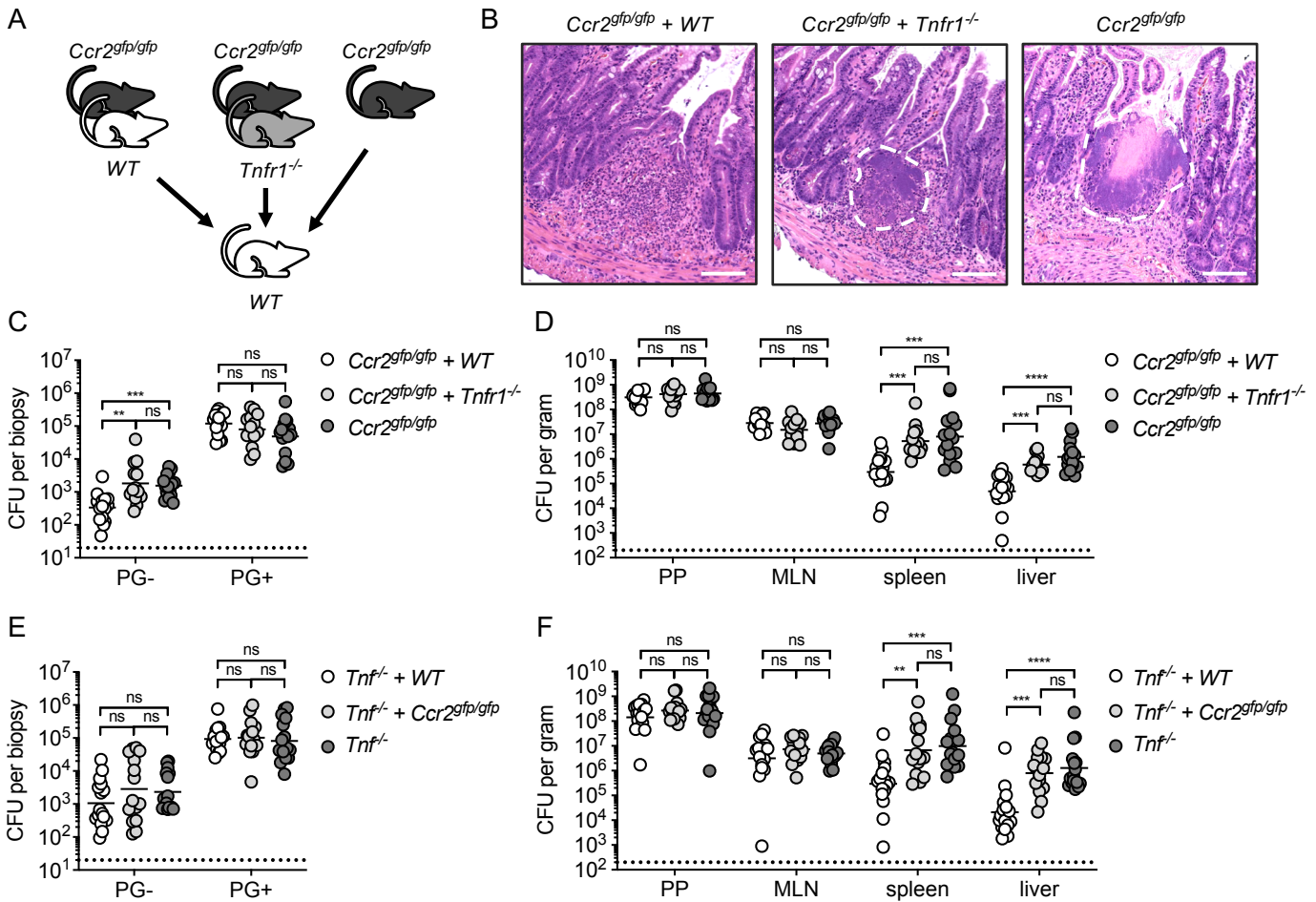
(F) Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents one mouse. Lines represent geometric mean. Pooled data from four independent experiments.

(G) Survival of infected WT (n=9) and *Tnfr1*<sup>-/-</sup> (n=21) mice. Pooled data from two independent experiments.

(H) Bacterial burdens in small-intestinal PG- and PG+ tissue at day 5 post-infection of indicated chimeric mice. Each circle represents the mean *Yp*-CFU of 3-5 pooled punch biopsies from one mouse. Lines represent geometric mean. Pooled data from two independent experiments.

(I) Bacterial burdens in indicated organs at day 5 post-infection of indicated chimeric mice. Each circle represents one mouse. Lines represent geometric mean. Pooled data from two independent experiments.

Statistical analysis by (B, C, D, E, F) Mann-Whitney U test (G) Mantel-Cox test (H, I) Kruskal-Wallis test with Dunn's multiple comparisons correction. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001, ns = not significant.



**Figure 2. Autocrine TNF signaling in monocytes is required for control of *Yersinia***

(A) Schematic of mixed bone marrow chimeras.

(B) H&E-stained paraffin-embedded transverse small-intestinal sections from chimeric WT mice reconstituted with *Ccr2<sup>gfp/gfp</sup>* + WT (left), *Ccr2<sup>gfp/gfp</sup>* + *Tnfr1<sup>-/-</sup>* (middle), or *Ccr2<sup>gfp/gfp</sup>* (right) bone marrow, at day 5 post-infection. Dotted lines highlight lesions. Scale bars = 100  $\mu$ m. Images representative of two independent experiments.

(C) Bacterial burdens in small-intestinal PG- and PG+ tissue of chimeric WT mice reconstituted with either *Ccr2<sup>gfp/gfp</sup>* + WT (white), *Ccr2<sup>gfp/gfp</sup>* + *Tnfr1<sup>-/-</sup>* (light gray), or *Ccr2<sup>gfp/gfp</sup>* (dark gray) at day 5 post-*Yp*-infection. Each symbol represents one mouse. Lines represent geometric mean. Pooled data from two independent experiments.

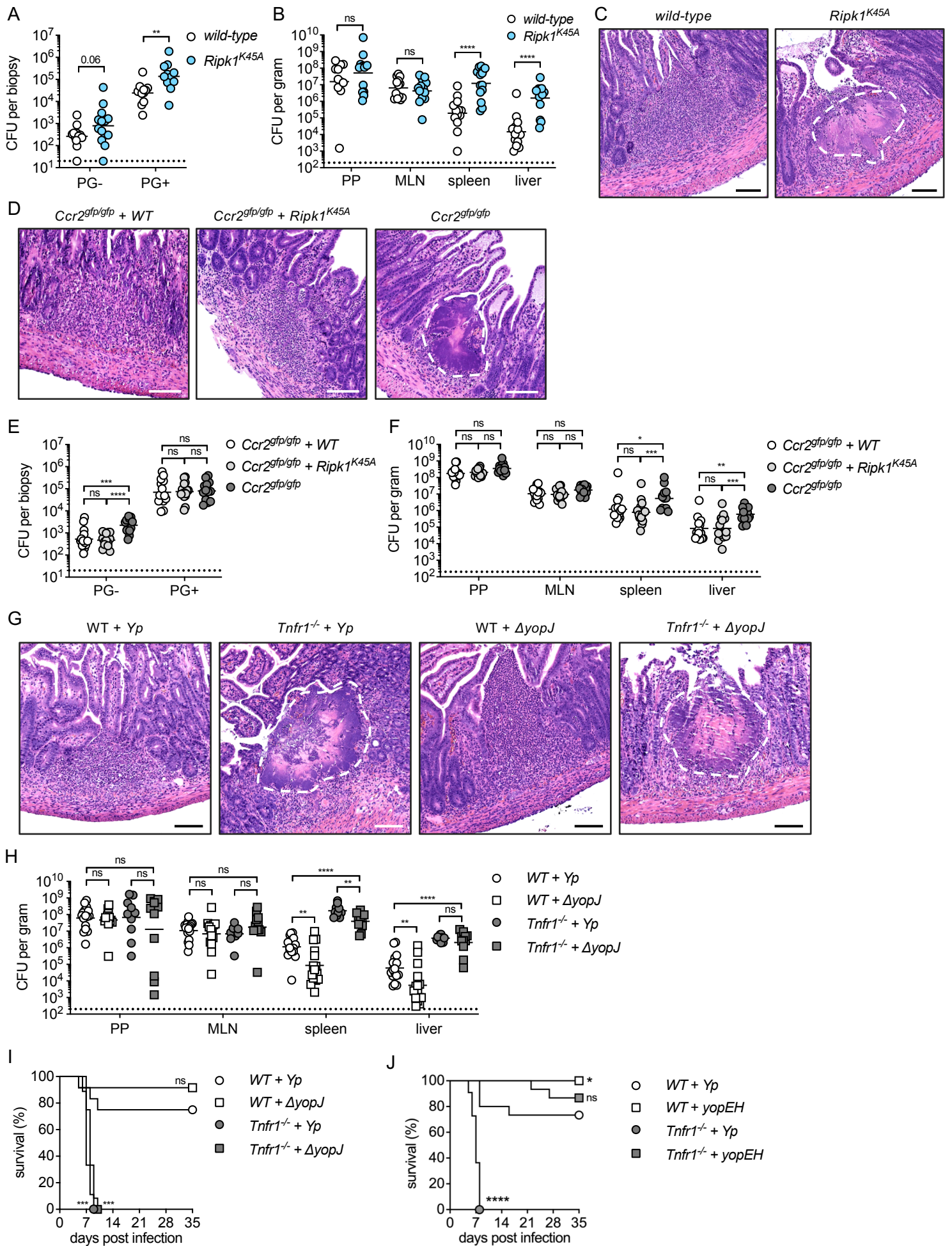
(D) Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents one mouse. Lines represent geometric mean. Pooled data from two independent experiments.

(E) Bacterial burdens in small-intestinal PG- and PG+ tissue of chimeric WT mice reconstituted with either *Tnf<sup>-/-</sup>* + WT (white), *Tnf<sup>-/-</sup>* + *Ccr2<sup>gfp/gfp</sup>* (light gray), or *Tnf<sup>-/-</sup>* (dark gray) at day 5 post-*Yp*-infection. Each symbol represents one mouse. Lines represent geometric mean. Pooled data from three independent experiments.

(F) Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents one mouse. Lines represent geometric mean. Pooled data from three independent experiments.

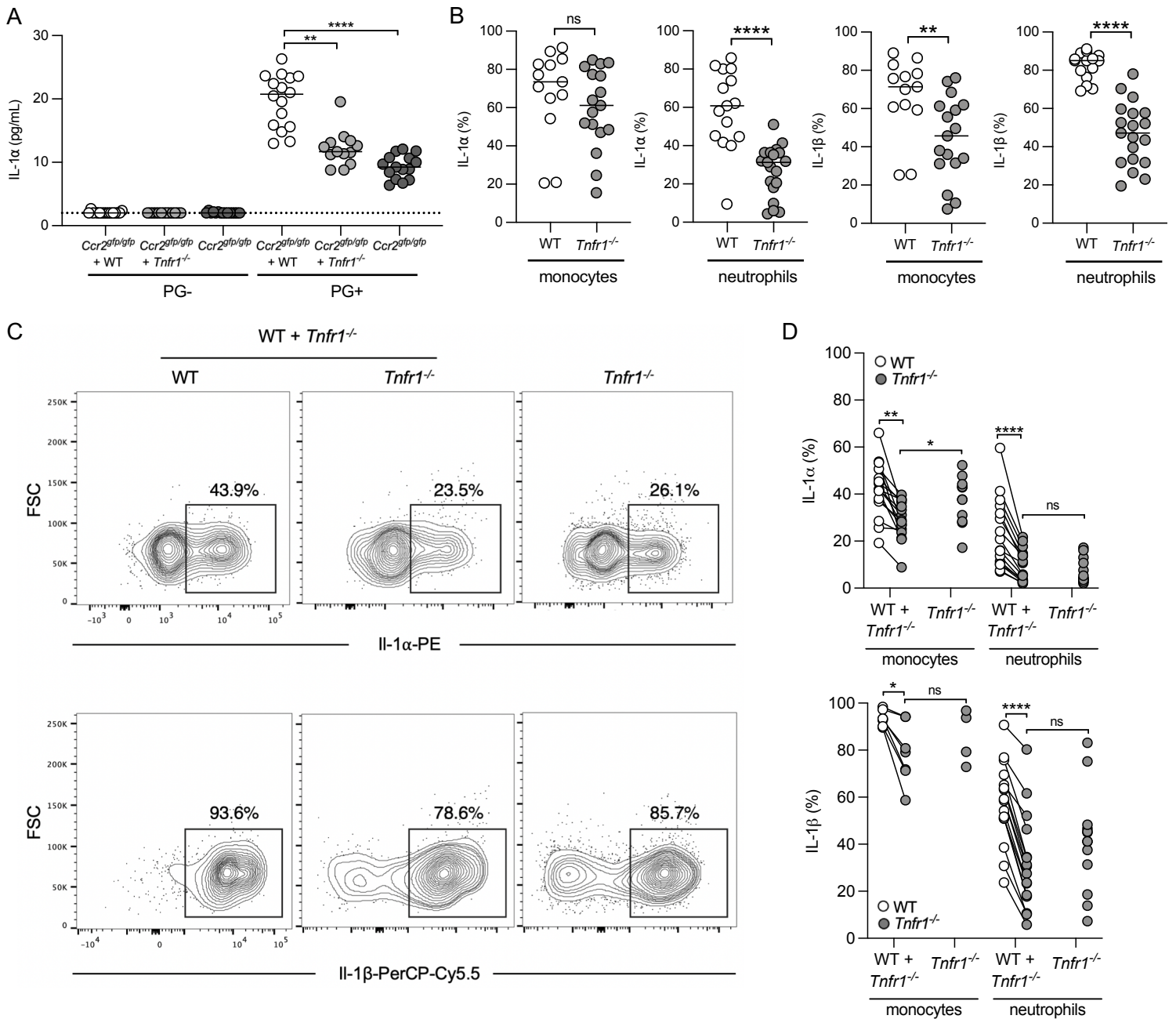
Statistical analysis by Kruskal-Wallis test with Dunn's multiple comparisons correction. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , ns = not significant.





**Figure 3. TNFR1 signaling in monocytes controls *Yp* independently of RIPK1 kinase-induced cell death**

- (A) Bacterial burdens in small-intestinal PG- and PG+ tissue of WT (white) and *Ripk1<sup>K45A</sup>* (blue) mice at day 5 post *Yp*-infection. Each symbol represents one mouse. Lines represent geometric mean. Pooled data from two independent experiments.
- (B) Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents one mouse. Lines represent geometric mean. Pooled data from two independent experiments.
- (C) H&E-stained paraffin-embedded longitudinal small-intestinal sections from *WT* (left) and *Ripk1<sup>K45A</sup>* (right) mice at day 5 post *Yp*-infection with dotted line highlighting lesion. Scale bars = 100  $\mu$ m. Representative images of two independent experiments.
- (D) H&E-stained paraffin-embedded transverse small-intestinal sections from chimeric WT mice reconstituted with either *Ccr2<sup>gfp/gfp</sup> + WT* (left), *Ccr2<sup>gfp/gfp</sup> + Ripk1<sup>K45A</sup>* (middle), or *Ccr2<sup>gfp/gfp</sup>* (right) bone marrow, at day 5 post *Yp*-infection with dotted line highlighting lesion. Scale bars = 100  $\mu$ m. Representative images of two independent experiments.
- (E) Bacterial burdens in small-intestinal PG- and PG+ tissue of chimeric WT mice reconstituted with either *Ccr2<sup>gfp/gfp</sup> + WT* (white), *Ccr2<sup>gfp/gfp</sup> + Ripk1<sup>K45A</sup>* (light gray), or *Ccr2<sup>gfp/gfp</sup>* (dark gray) at day 5 post *Yp*-infection. Each symbol represents one mouse. Lines represent geometric mean. Pooled data from two independent experiments.
- (F) Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents one mouse. Lines represent geometric mean. Pooled data from two independent experiments.
- (G) H&E-stained paraffin-embedded longitudinal small-intestinal sections from *wild-type* and *Tnfr1<sup>-/-</sup>* mice infected with either *WT* or  $\Delta$ *yopJ Yp* at day 5 post-infection. Scale bars = 100  $\mu$ m. Representative images of three independent experiments.
- (H) Bacterial burdens in indicated organs at day 5 post-infection. Each circle represents one mouse. Lines represent geometric mean. Pooled data from four independent experiments.
- (I) Survival of wild-type (white) and *Tnfr1<sup>-/-</sup>* (gray) mice infected with *WT* (circles) or  $\Delta$ *yopJ* (squares) *Yp*. N = 9-12 mice per group. Pooled data from two independent experiments.
- (J) Survival of *WT* (white) or *Tnfr1<sup>-/-</sup>* (gray) mice infected with *WT* (circles) or *yopEH* (squares) *Yp*. n = 11-15 mice per group. Pooled data from two independent experiments.
- Statistical analysis by Mann-Whitney U test (A, B), Kruskal-Wallis test with Dunn's multiple comparisons correction (E, F, H), or Mantel-Cox test (I, J). \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001, ns = not significant.



**Figure 4. Cell-intrinsic TNFR1 signaling is required for maximal IL-1 production within intestinal pyogranulomas during *Yersinia* infection**

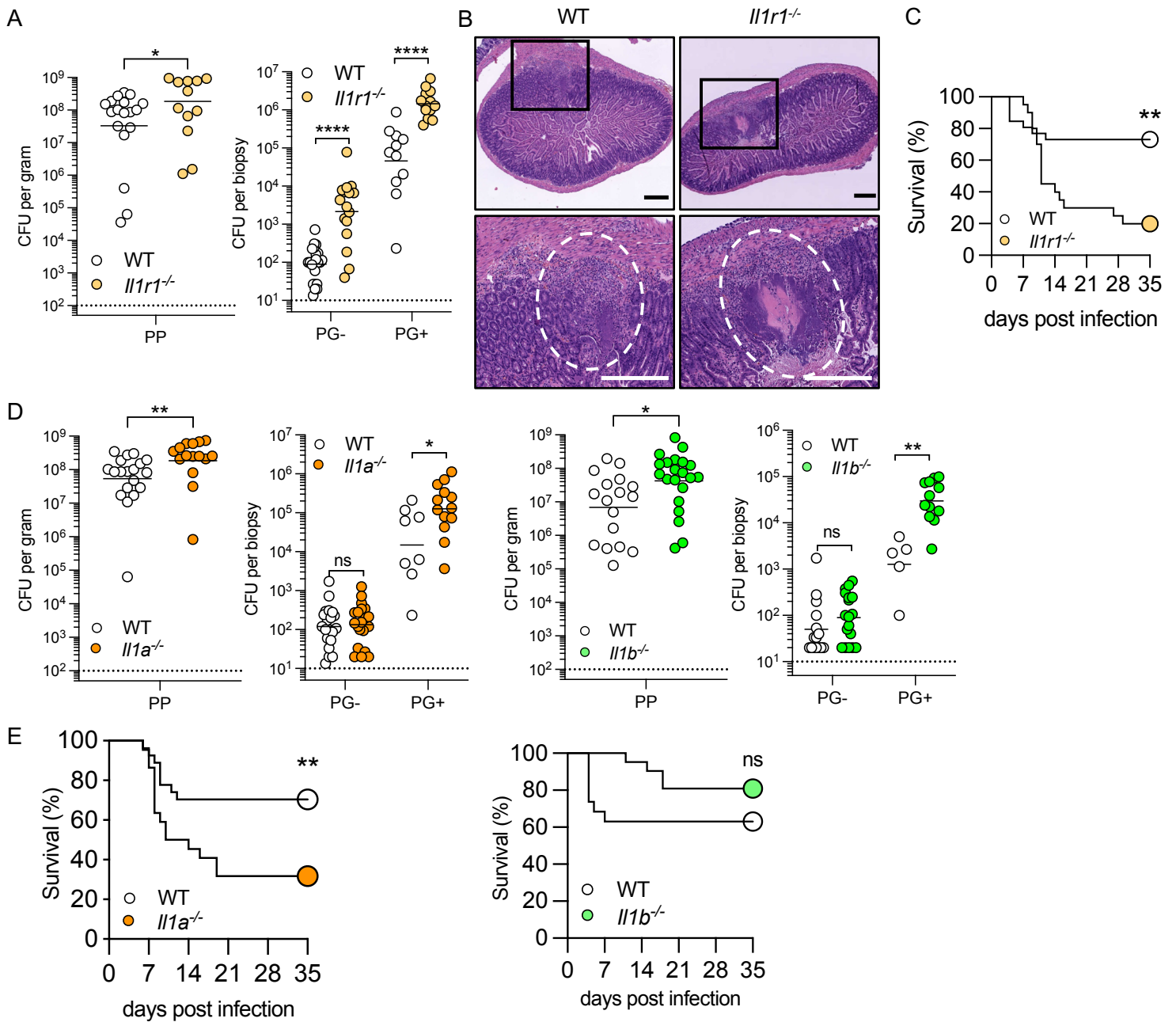
(A) Cytokine levels were measured by cytometric bead array in tissue punch biopsy homogenates isolated 5 days post-infection of chimeric WT mice reconstituted with indicated donor cells. Lines represent median. Pooled data from two independent experiments.

(B) Intracellular cytokine levels in monocytes and neutrophils isolated from small intestinal PG+ tissue 5 days post-infection. Each circle represents the mean of 3-10 pooled punch biopsies from one mouse. Lines represent median. Pooled data from three independent experiments.

(C) Flow cytometry plots of intracellular IL-1 in monocytes (CD64<sup>+</sup> Ly-6C<sup>hi</sup>) from small intestinal PG+ tissue at day 5 post-infection. Plots representative of two independent experiments.

(D) Aggregate datasets from (C) for intracellular IL-1 staining in monocytes and neutrophils in small intestinal PG+ tissue at day 5 post-infection. Each circle represents the mean of 3-10 pooled punch biopsies from one mouse. Lines connect congenic cell populations within individual mice. Pooled data from two independent experiments.

Statistical analysis by (A) Kruskal-Wallis test with Dunn's multiple comparisons correction (B) Mann-Whitney U test (D) congenic cells within mice: Wilcoxon test; across groups: Mann-Whitney U test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , ns = not significant.



**Figure 5. IL-1 signaling is required for organized pyogranuloma formation and intestinal control of *Yersinia***

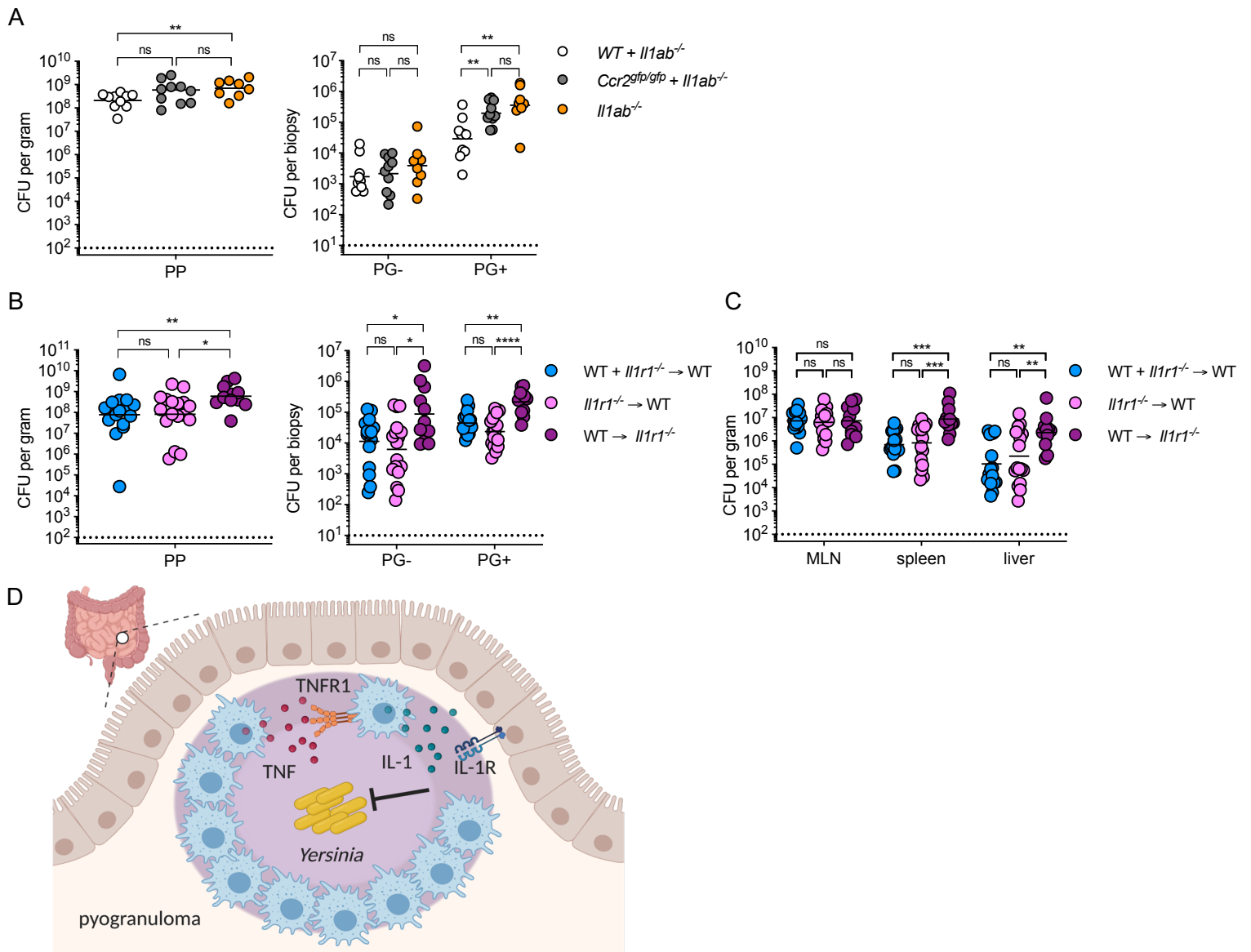
(A) Bacterial burdens in small-intestinal Peyer's patches (PP), PG-, and PG+ tissues isolated 5 days post-infection. For PP each circle represents one mouse. For PG- and PG+ each circle represents the mean of 3-5 pooled punch biopsies from one mouse. Lines represent geometric mean. Pooled data from three independent experiments.

(B) H&E-stained paraffin-embedded longitudinal small intestinal sections from *Yp*-infected mice at day 5 post-infection. Representative images of one experiment. Scale bars = 250  $\mu$ m.

(C) Survival of infected WT (n=26) and *Il1r1<sup>-/-</sup>* (n=20) mice. Pooled data from two independent experiments

(D). Bacterial burdens in small-intestinal PP, PG-, and PG+ tissues at day 5 post-infection of indicated genotypes. For PP each circle represents one mouse. For PG- and PG+ each circle represents the mean of 3-5 pooled punch biopsies from one mouse. Lines represent geometric mean. Pooled data from three independent experiments.

(E). Survival of infected WT (n=27, n=19), *Il1a<sup>-/-</sup>* (n=22) and *Il1b<sup>-/-</sup>* (n=21) mice. Pooled data from three and two independent experiments. Statistical analysis by (A, D) Mann-Whitney U test (C, E) Manel-Cox test. \*p<0.05, \*\*p<0.01, \*\*\*\*p<0.0001, ns = not significant.



**Figure 6. Monocyte-derived IL-1 signals to nonhematopoietic cells to restrict *Yersinia* infection in intestinal pyogranulomas**

(A) Bacterial burdens in small-intestinal Peyer's patches (PP), PG-, and PG+ tissues at day 5 post-infection of indicated chimeric mice. For PP each circle represents one mouse. For PG- and PG+ each circle represents the mean of 3-5 pooled punch biopsies from one mouse. Lines represent geometric mean. Data pooled from two independent experiments.

(B) Bacterial burdens in small-intestinal Peyer's patches (PP), PG-, and PG+ tissues isolated 5 days post-infection of indicated chimeric mice. For PP each circle represents one mouse. For PG- and PG+ each circle represents the mean of 3-5 pooled punch biopsies from one mouse. Lines represent geometric mean. Pooled data from three independent experiments.

(C) Bacterial burdens in indicated organs at day 5 post-infection of indicated chimeric mouse. Each circle represents one mouse. Lines represent geometric mean. Data pooled from three independent experiments.

All statistical analysis by Kruskal-Wallis test with Dunn's multiple comparisons correction. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , ns = not significant.

(D) Model of TNF-IL-1 circuit mediated by monocyte and stromal compartment to promote *Yp* restriction in intestinal pyogranulomas.