

University of Massachusetts Medical School

eScholarship@UMMS

---

University of Massachusetts Medical School Faculty Publications

---

2020-07-27

## Mycobacterium tuberculosis evasion of Guanylate Binding Protein-mediated host defense in mice requires the ESX1 secretion system [preprint]

Andrew J. Olive  
*Michigan State University*

*Et al.*

Let us know how access to this document benefits you.

Follow this and additional works at: [https://escholarship.umassmed.edu/faculty\\_pubs](https://escholarship.umassmed.edu/faculty_pubs)



Part of the [Amino Acids, Peptides, and Proteins Commons](#), [Bacteria Commons](#), [Bacterial Infections and Mycoses Commons](#), [Hemic and Immune Systems Commons](#), [Immunity Commons](#), [Immunology of Infectious Disease Commons](#), and the [Microbiology Commons](#)

---

### Repository Citation

Olive AJ, Smith CM, Baer CE, Coers J, Sassetti CM. (2020). Mycobacterium tuberculosis evasion of Guanylate Binding Protein-mediated host defense in mice requires the ESX1 secretion system [preprint]. University of Massachusetts Medical School Faculty Publications. <https://doi.org/10.1101/2020.07.27.223362>. Retrieved from [https://escholarship.umassmed.edu/faculty\\_pubs/1740](https://escholarship.umassmed.edu/faculty_pubs/1740)

Creative Commons License



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0 License](#). This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in University of Massachusetts Medical School Faculty Publications by an authorized administrator of eScholarship@UMMS. For more information, please contact [Lisa.Palmer@umassmed.edu](mailto:Lisa.Palmer@umassmed.edu).

1 ***Mycobacterium tuberculosis* evasion of Guanylate Binding Protein-mediated host defense in mice**  
2 **requires the ESX1 secretion system.**

3

4

5 Andrew J. Olive<sup>1</sup>, Clare M. Smith<sup>2,3</sup>, Christina E. Baer<sup>4</sup>, Jörn Coers<sup>2,5</sup>, Christopher M. Sassetti<sup>4\*</sup>

6

7 <sup>1</sup>Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI, 48824

8 <sup>2</sup>Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North

9 Carolina 22710, USA

10 <sup>3</sup> Duke Human Vaccine Institute, Duke University Medical Center, Durham, North Carolina 27710, USA

11 <sup>4</sup> Department of Microbiology and Physiological Systems, University of Massachusetts Medical School,

12 Worcester, MA 01650. USA

13 <sup>5</sup> Department of Immunology, Duke University Medical Center, Durham, North Carolina 22710, USA

14

15 Correspondence:

16 Christopher Sassetti – [Christopher.sassetti@umassmed.edu](mailto:Christopher.sassetti@umassmed.edu)

17

18

19

20

21

22

23 **Abstract**

24 Cell-intrinsic immune mechanisms control intracellular pathogens that infect eukaryotes. The  
25 intracellular pathogen *Mycobacterium tuberculosis* (*Mtb*) evolved to withstand cell-autonomous  
26 immunity to cause persistent infections and disease. A potent inducer of cell-autonomous immunity is  
27 the lymphocyte-derived cytokine IFN $\gamma$ . While the production of IFN $\gamma$  by T cells is essential to protect  
28 against *Mtb*, it is not capable of fully eradicating *Mtb* infection. This suggests that *Mtb* evades a subset  
29 of IFN $\gamma$ -mediated antimicrobial responses, yet what mechanisms *Mtb* resists remains unclear. The IFN $\gamma$ -  
30 inducible Guanylate binding proteins (GBPs) are key host defense proteins able to control infections  
31 with intracellular pathogens. GBPs were previously shown to directly restrict *Mycobacterium bovis* BCG  
32 yet their role during *Mtb* infection has remained unknown. Here, we examine the importance of a  
33 cluster of five GBPs on mouse chromosome 3 in controlling Mycobacterial infection. While *M. bovis* BCG  
34 is directly restricted by GBPs, we find that the GBPs on chromosome 3 do not contribute to the control  
35 of *Mtb* replication or the associated host response to infection. The differential effects of GBPs during  
36 *Mtb* versus *M. bovis* BCG infection is at least partially explained by the absence of the ESX1 secretion  
37 system from *M. bovis* BCG, since *Mtb* mutants lacking the ESX1 secretion system become similarly  
38 susceptible to GBP-mediated immune defense. Therefore, this specific genetic interaction between the  
39 murine host and *Mycobacteria* reveals a novel function for the ESX1 virulence system in the evasion of  
40 GBP-mediated immunity.

41

42

43

44

## 45 **Introduction**

46

47 Eukaryotic cells control intracellular pathogens using a variety of cell intrinsic immune pathways (1).

48 These innate mechanisms allow the cell to rapidly detect, target and destroy invading pathogens,

49 preventing the spread of an infection. The immune pathways controlling innate immunity arose early in

50 the evolution of the eukaryota, providing ample time for the selection of pathogens that express

51 mechanisms to bypass cell-autonomous immunity (2). This long-term arms race has produced a myriad

52 of interactions between immune effectors and pathogen countermeasures that determine the outcome

53 of an infection.

54 *Mycobacterium tuberculosis* (*Mtb*) has become highly adapted to its human host, as it spread

55 globally along with human migrations over tens of thousands of years (3, 4). As much as one-third of the

56 human population has been exposed to *Mtb*, which causes a persistent infection than can last for years,

57 or even decades, despite a robust immune response that eradicates less pathogenic mycobacteria (5).

58 While immunity controls *Mtb* growth and prevents disease in most individuals, a subset will develop

59 active tuberculosis (TB), a disease that kills an estimated 1.8 million each year (6). Disease progression is

60 influenced by a variety of genetic and environment factors, but ultimately is determined by the interplay

61 between host immunity and bacterial virulence systems(5, 7).

62 In the host, the development of a robust T cell response and the production of the cytokine IFN $\gamma$

63 are important for the control TB infection and disease (5, 8). Humans with inherited mutations affecting

64 either the development or expression of this response are highly susceptible to mycobacterial infections

65 including TB (9). This susceptibility is faithfully modeled in mice, where the loss of IFN $\gamma$  signaling

66 promotes disease by at least two distinct mechanisms (10, 11). IFN $\gamma$  is an important immunomodulatory

67 cytokine, and its loss results in uncontrolled IL1 production and neutrophil recruitment, driving both

68 bacterial replication and tissue damage (10, 12). Perhaps more importantly, IFN $\gamma$  stimulates cell-intrinsic

69 immune pathways in phagocytes, which is critical for the control of intracellular bacterial growth (13,  
70 14). Thus, IFN $\gamma$  is a pleotropic cytokine that controls direct antimicrobial resistance and disease  
71 tolerance, both of which are essential to survive *Mtb* infection.

72 While many of the immunomodulatory effects of IFN $\gamma$  have been elucidated, the IFN $\gamma$ -induced  
73 factors that control *Mtb* replication remain comparatively obscure. IFN $\gamma$ -induced oxygen and nitrogen  
74 radical generation limits *Mtb* replication in macrophages *ex vivo*, but appear to serve a limited  
75 antimicrobial role in the intact animal (14-16). Instead, a subset of IFN $\gamma$ -inducible cell-intrinsic immune  
76 mechanisms, known as Guanylate binding proteins (GBPs), target and disrupt the intracellular niche  
77 required for a number of pathogens to grow (2, 17-21). Macrophages lacking GBPs 1, 6,7 or 10 fail to  
78 control the growth of *M. bovis* BCG, an attenuated vaccine strain that is closely related to *Mtb* (22).  
79 Similarly, mice lacking GBP1 or the cluster of 5 GBP proteins on chromosome 3 are more susceptible to  
80 intravenous BCG challenge (22, 23). While the mechanism of BCG control remains unclear, GBPs are  
81 known to bind to pathogen containing vacuoles in other infections and recruit additional effector  
82 molecules (17, 24). The outcome of this recognition can be the direct restriction of pathogen growth  
83 and alterations in cytokine production. While these observations suggest that GBPs may be an  
84 important mediator of IFN $\gamma$  -mediated control of pathogenic mycobacteria, their role in during *Mtb*  
85 infection has remained untested.

86 BCG was attenuated for use as a vaccine via long term serial passage, and as a result, it interacts  
87 with the macrophage quite differently from *Mtb* (25). Most notably, the primary genetic lesion  
88 responsible for the attenuation of BCG is a deletion that disrupts the ESX1 type VII secretion system (26).  
89 ESX1 is specialized protein secretion complex that contributes to pathogen replication by remodeling its  
90 intracellular environment (27). ESX1 is responsible for the disruption of the phagosomal membrane, the  
91 activation of multiple cytosolic immune sensing pathways, and stimulation of both cytokine secretion

92 and autophagy (28). How ESX1 alters other aspects of cell-intrinsic bacterial control remains to be  
93 determined.

94           To understand the mechanisms of IFN $\gamma$ -mediated protection we investigated the role of GBPs  
95 during both BCG and *Mtb* infection. Specifically, we examined the function of a cluster of 5 GBP  
96 proteins that are encoded in a single locus on mouse chromosome 3 (29). While these GBPs restricted  
97 *M. bovis* BCG replication, we found no contribution of these proteins in the control of virulent *Mtb*  
98 infection. The discrepant effects of GBPs during BCG and *Mtb* infection could be attributed to  
99 differential ESX1 function in these two pathogens. ESX1-deficient strains of *Mtb* were better controlled  
100 by GBP-mediated immunity, while GBP deficiency had no effect on either the growth of ESX1 expressing  
101 *Mtb* or the immune response to this virulent strain. Together, these observations indicate that the ESX1  
102 system plays an essential role in the evasion of GBP-mediated cell intrinsic immunity.

103

104

## 105 **Materials and Methods**

106

### 107 **Mice**

108 C57BL/6J (Stock # 000664) and IFN $\gamma$ R<sup>-/-</sup> (Stock # 003288) mice were purchased from the Jackson  
109 Laboratory. The Gbp<sup>chr3-/-</sup> mice were previously described (29). All knockout mice were housed and bred  
110 under specific pathogen-free conditions and in accordance with the University of Massachusetts Medical  
111 School IACUC guidelines. All animals used for experiments were 6-12 weeks.

112

### 113 **Bacterial Strains**

114 Wild type *M. tuberculosis* strain H37Rv was used for all studies unless indicated. This strain was  
115 confirmed to be PDIM-positive. The espA (Rv3616c) deletion strain ( $\Delta$ espA) was a gift from Dr. Sarah  
116 Fortune (30). The eccb1 (Rv3869) deletion strain ( $\Delta$ eccb1) was constructed in the H37Rv parental  
117 background using the ORBIT method as described previously (31). The live/dead strain was built by  
118 transforming the live/dead vector (pmV261 hsp60::mEmerald tetOtetR::TagRFP) into H37Rv and  
119 selected with hygromycin. Protein expression was confirmed via fluorescence microscopy and flow  
120 cytometry. H37Rv expressing msfYFP has been previously described and the episomal plasmid was  
121 maintained with selection in Hygromycin B (50ug/ml) added to the media (32). *Mycobacterium bovis*  
122 BCG Danish Strain 1331 (Statens Serum Institute, Copenhagen, Denmark) was used for all BCG infection  
123 studies. Prior to infection bacteria were cultured in 7H9 medium containing 10% oleic albumin dextrose  
124 catalase growth supplement (OADC) enrichment (Becton Dickinson) and 0.05% Tween 80.

125

### 126 **Mouse Infection**

127 For low dose aerosol infections (50-150 CFU), bacteria were resuspended in phosphate-buffered saline  
128 containing tween 80 (PBS-T). Prior to infection bacteria were sonicated then delivered via the

129 respiratory route using an aerosol generation device (Glas-Col). For mixed infections, bacteria were  
130 prepared then mixed 1:1 before aerosol infection. To determine CFU, mice were anesthetized via  
131 inhalation with isoflurane (Piramal) and euthanized via cervical dislocation, the organs aseptically  
132 removed and individually homogenized, and viable bacteria enumerated by plating 10-fold serial  
133 dilutions of organ homogenates onto 7H10 agar plates. Plates were incubated at 37C, and bacterial  
134 colonies counted after 21 days. Both male and female mice were used throughout the study and no  
135 significant differences in phenotypes were observed between sexes.

136

### 137 **Flow Cytometry**

138 Lung tissue was harvested in DMEM containing FBS and placed in C-tubes (Miltenyi). Collagenase type  
139 IV/DNaseI was added and tissues were dissociated for 10 seconds on a GentleMACS system (Miltenyi).  
140 Tissues were incubated for 30 minutes at 37C with oscillations and then dissociated for an additional 30  
141 seconds on a GentleMACS. Lung homogenates were passaged through a 70-micron filter or saved for  
142 subsequent analysis. Cell suspensions were washed in DMEM, passed through a 40-micron filter and  
143 aliquoted into 96 well plates for flow cytometry staining. Non-specific antibody binding was first blocked  
144 using Fc-Block. Cells were then stained with anti-Ly-6G Pacific Blue, anti-CD11b PE, anti-CD11c APC, anti-  
145 Ly-6C APC-Cy7, anti-CD45.2 PercP Cy5.5, anti-CD4 FITC, anti-CD8 APC-Cy7, anti-B220 PE-Cy7 (Biolegend).  
146 Live cells were identified using fixable live dead aqua (Life Technologies). For infections with fluorescent  
147 H37Rv, lung tissue was prepared as above but no antibodies were used in the FITC channel. All of these  
148 experiments contained a non-fluorescent H37Rv infection control to identify infected cells. Cells were  
149 stained for 30 minutes at room temperature and fixed in 1% Paraformaldehyde for 60 minutes. All flow  
150 cytometry was run on a MACSQuant Analyzer 10 (Miltenyi) and was analyzed using FlowJo\_v9 (Tree  
151 Star).

152



153 **Bone marrow-derived macrophage generation**

154 To generate bone marrow derived macrophages (BMDMs), marrow was isolated from femurs and tibia  
155 of age and sex matched mice as previously described (33). Cells were then incubated in DMEM (Sigma)  
156 containing 10% fetal bovine serum (FBS) and 20% L929 supernatant. Three days later media was  
157 exchanged with fresh media and seven days post-isolation cells were lifted with PBS-EDTA and seeded in  
158 DMEM containing 10% FBS for experiments.

159

160 **Macrophage Infection**

161 *Mtb* or *Mycobacterium bovis*-BCG were cultured in 7H9 medium containing 10% oleic albumin dextrose  
162 catalase growth supplement (OADC) enrichment (Becton Dickinson) and 0.05% Tween 80. Before  
163 infection cultures were washed in PBS-T, resuspended in DMEM containing 10%FBS and centrifuged at  
164 low speeds to pellet clumps. The supernatant was transferred to a new tube to ensure single cells.  
165 Multiplicity of infection (MOI) was determined by optical density (OD) with an OD of 1 being equivalent  
166 to  $3 \times 10^8$  bacteria per milliliter. Bacteria were added to macrophages for 4 hours then cells were washed  
167 with PBS and fresh media was added. At the indicated time points supernatants were harvested for  
168 cytokine analysis and the cells were processed for further analysis. For cytokine treatments cells were  
169 treated with the indicated concentrations of IFN $\gamma$  (Peprotech) or vehicle control four hours following  
170 infection and maintained in the media throughout the experiment. For CFU experiments at the indicated  
171 timepoints 1% saponin was added to each well and lysates were serially diluted in PBS .05% Tween and  
172 plated on 7H10 agar and colonies were counted 21-28 days later. For the Live/Dead reporter  
173 experiments, Anhydrotetracycline (aTc) (Cayman Chemical) was added to a final concentration of  
174 500ug/ml 24 hours before cells were lifted, fixed in 1% Paraformaldehyde and analyzed on a MacsQuant  
175 VYB analyzer.

176

177 **Cytokine quantification by ELISA**

178 Murine cytokine concentrations in culture supernatants and cell-free lung homogenates were quantified  
179 using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D). All samples were normalized  
180 for total protein content.

181 **Statistics.** GraphPad Prism version 7 was used for all statistical analysis. Unless otherwise indicated one-  
182 way ANOVA with a tukey correction was used to compare each condition to each genotype.

183

184

185

186

187 **Results**

188

189 ***Mycobacterium bovis* BCG growth is restricted by the Chromosome 3 Guanylate binding protein**  
190 **cluster.**

191 The ten murine GBP genes are encoded in two clusters on chromosomes 3 and 5 (1). To begin to  
192 address the potential redundancy between these genes, we used mice lacking the entire chromosome 3  
193 cluster that contains the Gbp1 and 7 genes that were previously implicated in BCG control, along with  
194 GBPs 2, 3, and 5 (29). To determine if these GBPs contribute to control of mycobacterial infection, bone  
195 marrow-derived macrophages (BMDMs) from wild type and Gbp<sup>chr3-/-</sup> mice were infected with  
196 *Mycobacterium bovis* BCG (Figure 1A). IFN $\gamma$ -treatment, which induces GBP expression, reduced the  
197 intracellular bacterial burden in wild type BMDMs. In contrast, the Gbp<sup>chr3-/-</sup> deletion significantly  
198 reduced the ability of macrophages to control BCG upon IFN $\gamma$  activation. Thus, GBPs on chromosome 3  
199 contribute to IFN $\gamma$ -mediated restriction of BCG in macrophages.

200 We next examined if GBPs controlled BCG infection in the context of an intact immune  
201 response. Wild type, IFN $\gamma$ R<sup>-/-</sup> and Gbp<sup>chr3-/-</sup> mice were infected intravenously with BCG. When CFU were  
202 quantified in the spleen 50 days later we observed twenty-fold more BCG in IFN $\gamma$  mice and five-fold  
203 more BCG in Gbp<sup>chr3-/-</sup> mice compared to wild type controls (Figure 1B). The increased susceptibility of  
204 IFN $\gamma$ R<sup>-/-</sup> compared to Gbp<sup>chr3-/-</sup> mice indicated that the function of these GBPs accounted for some but  
205 not all of the protective effect of IFN $\gamma$ . Together these data show that the GBPs on chromosome 3 are  
206 able to restrict the growth of BCG in IFN $\gamma$ -stimulated macrophages and in the intact animal, which is  
207 consistent with the previously described roles of GBPs in immunity to BCG (22, 23).

208

209 **Chromosome 3 GBP cluster does not control *Mycobacterium tuberculosis* growth in macrophages.**

210 To determine if the chromosome 3 GBPs restricted the intracellular growth of virulent *Mtb*, in addition  
211 to BCG, we quantified the growth of *Mtb* strain H37Rv in BMDMs from wild type,  $\text{IFN}\gamma\text{R}^{-/-}$  and  $\text{Gbp}^{\text{chr3-/-}}$   
212 animals in the presence and absence of  $\text{IFN}\gamma$  (Figure 2A). We observed no differences in the uptake  
213 between genotypes four hours following infection.  $\text{IFN}\gamma$  priming reduced the intracellular *Mtb* growth by  
214 3-4 fold in wild type macrophages, but not  $\text{IFN}\gamma\text{R}^{-/-}$  BMDMs at 5 days post infection (dpi). In contrast to  
215 BCG, we observed no difference in *Mtb* growth in  $\text{Gbp}^{\text{chr3-/-}}$  BMDMs compared to wild type  
216 macrophages in the presence or absence of  $\text{IFN}\gamma$ .

217 To ensure that the relatively insensitive CFU-based intracellular growth assay did not mask  
218 subtle effects of GBP expression on bacterial fitness, we used a fluorescent live/dead reporter as an  
219 orthologous method to measure *Mtb* intracellular viability by flow cytometry. This reporter expresses a  
220 constitutive mEmerald and an anhydrotetracycline (aTc)-inducible tagRFP. BMDMs from wild type,  
221  $\text{IFN}\gamma\text{R}^{-/-}$  and  $\text{Gbp}^{\text{chr3-/-}}$  mice were infected with the Live/Dead *Mtb* reporter and left untreated or were  
222 stimulated with  $\text{IFN}\gamma$ . Four days later aTc was added to induce tagRFP expression in viable intracellular  
223 bacteria. The following day, cells were analyzed by flow cytometry and the mean fluorescence intensity  
224 (MFI) of tagRFP in infected mEmerald+ cells was quantified (Figure 2B and 2C). Similar to the CFU  
225 analysis,  $\text{IFN}\gamma$  activation reduced the intensity of tagRFP to a similar extent in both wild type and  $\text{Gbp}^{\text{chr3-/-}}$   
226  $^{-/-}$  BMDMs, and this reduction depended on the  $\text{IFN}\gamma$  receptor. Thus, we were not able to detect a role  
227 for the chromosome 3 GBP cluster in the  $\text{IFN}\gamma$  mediated restriction of virulent *Mtb* in macrophages.

228

### 229 **Chromosome 3 GBPs have no effect on *Mtb* infection in intact mice**

230 To assess the role of the chromosome 3 GBPs in the more complex setting of the intact animal, we  
231 infected wild type,  $\text{IFN}\gamma\text{R}^{-/-}$  and  $\text{Gbp}^{\text{chr3-/-}}$  mice with *Mtb* by low dose aerosol and quantified bacteria in  
232 the lungs at four and five weeks following infection (Figure 3A and 3B).  $\text{IFN}\gamma\text{R}^{-/-}$  mice harbored more *Mtb*  
233 than wild type controls at both time points in the lung and the spleen. In contrast, the *Mtb* burdens in

234  $Gbp^{chr3-/-}$  mice were indistinguishable from wild type animals at all timepoints. At 110 days after  
235 infection (Figure 3A and 3B), we continued to find no difference in CFU in the lungs between wild type  
236 and  $GBP^{chr3-/-}$  mice.  $IFN\gamma R^{-/-}$  mice required euthanasia within six weeks of infection and were not  
237 included in this late time point.

238 To examine more subtle differences in the extent of infection, wild type,  $IFN\gamma R^{-/-}$  and  $Gbp^{chr3-/-}$   
239 mice were infected with a well-characterized fluorescent H37Rv strain by low-dose aerosol (32). Twenty-  
240 eight days later the number of YFP+ cells in the lung environment were determined by flow cytometry  
241 (Figure 3C). Similar to our CFU results, we found  $IFN\gamma R^{-/-}$  mice contained over 10 times more infected cells  
242 than wild type mice while  $Gbp^{chr3-/-}$  mice showed no significant difference in the number of infected  
243 cells per lung (Figure 3D).

244 We next examined if there were differences in  $Gbp^{chr3-/-}$  mice infected intravenously since this  
245 route matched the BCG infections where the role of GBPs was evident (Figure 1B and (22)). Following  
246 intravenous infection with  $10^6$  *Mtb*, bacterial levels in the spleen were quantified 10 and 28 days later.  
247 Similar to the aerosol infection results, we saw no difference between  $Gbp^{chr3-/-}$  and wild type animals,  
248 while  $IFN\gamma R^{-/-}$  animals had 10 times more *Mtb* growth in the spleen (Figure 3E). Thus, unlike the loss of  
249  $IFN\gamma R$ , the loss of chromosome 3 GBPs does not affect *Mtb* growth in the lungs or spleen, regardless of  
250 infection route, suggesting no major function of these five GBPs in controlling antimicrobial resistance to  
251 *Mtb* in mice.

252

### 253 **$Gbp^{chr3-/-}$ mice continue to regulate inflammatory responses to *Mtb* infection**

254  $IFN\gamma$  protects against disease both by restricting bacterial growth and by inhibiting tissue-damaging  
255 inflammation (11). To determine if the chromosome 3 GBPs contribute to the latter immunoregulatory  
256 function of  $IFN\gamma$ , we profiled the immune responses of infected macrophages and mice. Wild type and  
257  $GBP^{chr3-/-}$  BMDMs were treated with  $IFN\gamma$  then infected with *Mtb* and the following day supernatants

258 were harvested. IFN $\gamma$  signaling showed the expected inhibitory effect on IL-1 $\beta$  secretion, but the  
259 chromosome3 Gbp deletion had no effect on either cytokine (12). We next profiled the host response  
260 in the lungs of mice to determine if chromosome 3 GBPs modulated the recruitment of immune cells or  
261 the production of cytokines during *Mtb* infection. Wild type, IFN $\gamma$ R $^{-/-}$  and Gbp<sup>chr3 $^{-/-}$</sup>  mice were infected  
262 with *Mtb* and four weeks later we quantified the immune populations in the lungs by flow cytometry  
263 (Figure 4B). While we observed the previously described increase in neutrophil recruitment in IFN $\gamma$ R $^{-/-}$   
264 mice, we observed no differences in any myeloid or lymphoid derived cells that were examined between  
265 Gbp<sup>chr3 $^{-/-}$</sup>  and wild type mice (10). We also quantified IL1 $\beta$  and TNF $\alpha$  in the lungs from these infected  
266 animals (Figure 4C). The production of these cytokines in wild type and GBP<sup>chr3 $^{-/-}$</sup>  mice were  
267 indistinguishable while IFN $\gamma$ R $^{-/-}$  mice showed an increase in IL1 $\beta$ . Thus, we were unable to detect a role  
268 for GBPs on chromosome 3 in controlling the host response during virulent *Mtb* infection.

269

### 270 **ESX1 is required for *Mtb* to evade GBPs of chromosome 3.**

271 The reduced virulence of BCG compared to *Mtb* has been largely attributed to the loss of ESX1 function  
272 in BCG. As a result, we hypothesized ESX1 function in virulent *Mtb* provides resistance to GBP-mediated  
273 immunity, and its loss renders BCG susceptible to this mechanism. To test this hypothesis, we  
274 determined whether abrogation of ESX1 function in *Mtb* would result in a strain that was susceptible to  
275 GBP-mediated control. Wild type and Gbp<sup>chr3 $^{-/-}$</sup>  macrophages were infected with H37Rv or two isogenic  
276 mutants each lacking a gene that is necessary for ESX1 function,  $\Delta$ *espA* or  $\Delta$ *eccb1*. We observed that  
277 ESX1 mutants displayed reduced intracellular growth compared to H37Rv in both IFN $\gamma$  stimulated and  
278 unstimulated wild type macrophages, confirming the attenuation of these mutants (Figure 5A and 5B).  
279 When we compared wild type and Gbp<sup>chr3 $^{-/-}$</sup>  BMDMs, we found that the loss of GBP function had no  
280 effect on H37Rv, while it significantly reduced the ability of IFN $\gamma$  to control the growth of both ESX1  
281 mutants.

282           To confirm the specific effect of chromosome 3 GBPs on ESX1 mutants in the setting of intact  
283 immunity, we conducted a competitive infection of wild type and  $Gbp^{chr3-/-}$  mice with an equivalent  
284 number of H37Rv and  $\Delta eccb1$  bacteria. Four weeks later we quantified the competitive index of each  
285 bacterial strain in the lungs (Figure 5C). As anticipated, the ratio of two differentially marked H37Rv  
286 strains stayed constant throughout the infection. While selection in wild type mice resulted in almost  
287 100-fold underrepresentation of the  $\Delta eccb1$  mutant compared H37Rv, the fitness defect of the  $\Delta eccB1$   
288 mutant was significantly reduced in the  $GBP^{chr3-/-}$  mice (Figure 5C). These findings demonstrate that  
289 ESX1 deficiency is sufficient to render *Mtb* susceptible to GBP-mediated immunity.

290

291

## 292 Discussion

293 Understanding the immune mechanisms that restrict the intracellular growth of *Mtb* is essential for the  
294 rational design of interventions. Initial observations demonstrating a role for GBPs in the control of *BCG*  
295 growth suggested that this pathway might represent an important component of IFN $\gamma$ -mediated  
296 immunity to *Mtb* (22, 23). However, while we were able to detect the previously-described role for the  
297 chromosome 3 GBPs in immunity to BCG, we found that these proteins have no effect during *Mtb*  
298 infection. By attributing this difference to the ESX1 locus that is present in *Mtb* but not BCG, we  
299 discovered a specific role for ESX1 in overcoming GBP-mediated defenses.

300  
301 Our findings question the importance of GBPs in the control of ESX1-expressing *Mtb*. While our results  
302 are strictly in the mouse model, evidence in humans also suggests GBPs may not effectively control TB  
303 progression. For example, the high expression of a subset of GBPs is predictive of patients that are more  
304 likely to progress to active disease (34). However, it is important to note that it remains possible that  
305 the chromosome 5 GBPs, or human-specific GBP functions, can overcome ESX1-mediated bacterial  
306 defenses. In addition, our findings do not rule out an important role for GBPs in resistance to non-  
307 tuberculous mycobacteria (NTM). While all mycobacteria express ESX paralogs, many pathogenic NTM  
308 do not possess a clear ortholog of ESX1, suggesting that they may remain susceptible to GBP immunity  
309 (35).

310  
311 How the ESX1 type VII secretion system allows *Mtb* to evade restriction by GBPs remains to be  
312 investigated. To date, the type III secretion system effector protein IpaH9.8 from *Shigella flexneri* is the  
313 only other described GBP antagonist (36-39). In the cytosol of mammalian cells, where *S. flexneri*  
314 replicates, IpaH9.8 targets GBP1 for degradation thereby interfering with direct GBP binding to the  
315 *Shigella* outer membrane, a process by which GBP1 disrupts the function of a membrane-bound *Shigella*



316 virulence factors required for actin-based motility and bacterial dissemination (36-38, 40). Similar to  
317 IpaH9.8, ESX1 and its substrates may act as direct GBP antagonist to prevent binding to *Mycobacteria*-  
318 containing vacuoles. Alternatively, ESX1 may control the evasion of antimicrobial mechanisms that occur  
319 subsequent to GBP translocation to *Mycobacteria*-containing vacuoles. Future work will need to dissect  
320 these possible mechanisms for ESX1-mediated antagonism of GBP function.

321  
322 While IFN $\gamma$  is unquestionably important to survive *Mtb* infection, the IFN $\gamma$ -mediated pathways that  
323 directly control the intracellular replication of *Mtb* remain surprisingly unclear. Our results add to a  
324 growing list of direct antimicrobial pathways that are ineffective during *Mtb* infection. The IFN $\gamma$ -  
325 mediated production of nitric oxide, reactive oxygen species and itaconate kills many pathogens;  
326 however, these mechanisms appear to play a small role in directly controlling *Mtb* growth *in vivo* (32,  
327 33, 41). Instead, these mediators are required to inhibit persistent inflammation and to prevent disease  
328 progression. In addition, the IFN $\gamma$ -regulated immunity related GTPases (IRG) family protein, Irgm1, was  
329 originally described to target the *Mtb* containing vacuole to control pathogen growth, but recent  
330 evidence has questioned whether Irgm1 targets *Mtb* phagosomes (42, 43). Instead, the lymphopenia  
331 observed in Irgm1-deficient mice may be predominantly responsible for their susceptibility to *Mtb*  
332 similar to other pathogens like *Chlamydia trachomatis* (44, 45). While other pathways have been  
333 suggested to play a role in IFN $\gamma$ -mediated control, including the production of Cathepsins, the role of  
334 these mediators in protection *in vivo* remains unclear (46). Overall, our findings add GBP-mediated  
335 immunity to the list of IFN $\gamma$  dependent host defense programs to which *Mtb* has evolved specific  
336 counter immune mechanisms blunting the effectiveness of these antimicrobial effectors and thus  
337 driving pathogen persistence and disease.

338

339 **Acknowledgements:** We thank Dr. Masahiro Yamamoto for generously sharing  $Gbp^{chr3-/-}$  mice for these  
340 studies, and Christina Baer for the development of fluorescent Mtb reporters. This work was supported  
341 by grants from the NIH to CMS (AI132130) and the Arnold and Mabel Beckman Foundation to AJO.

342

343

344 **Figure Legends.**

345

346 **Figure 1. Guanylate binding proteins contribute to control of *Mycobacterium Bovis* BCG infection. (A)**

347 BMDMs from wild type or Gbp<sup>chr3-/-</sup> mice were infected with *M. Bovis* BCG for 4 hours then washed with

348 fresh media in the presence or absence of IFN $\gamma$ . Five days later the macrophages were lysed and serial

349 dilutions were plated to quantify colony forming units (CFU) of viable BCG. Shown is the mean CFU from

350 four biological replicates +/- SD \*p<.05 \*\*p<.01. Data are representative of four independent

351 experiments. **(B)** Following IV infection with BCG (1x10<sup>6</sup> bacteria) the total bacterial burden (expressed

352 in CFU, mean +/- SD) was determined in the spleen of wild type or Gbp<sup>chr3-/-</sup> mice 50 days following

353 infection. Representative of two independent experiments with 4-5 mice per group, \*p<.05 \*\*p<.01.

354

355 **Figure 2. *Mycobacterium tuberculosis* is resistant to chromosome 3 GBP-mediated control in**

356 **macrophages. (A)** BMDMs from wild type, IFN $\gamma$ R<sup>-/-</sup> or Gbp<sup>chr3-/-</sup> mice were infected with *M. tuberculosis*

357 for 4 hours then washed with fresh media in the presence or absence of IFN $\gamma$ . Five days later the

358 macrophages were lysed, serial dilutions were plated to quantify colony forming units (CFU) of viable *M.*

359 *tuberculosis*. Shown is the mean CFU from four biological replicates +/- SD \*p<.05 \*\*p<.01. Data are

360 representative of five independent experiments. **(B)** and **(C)** BMDMs from wild type, IFN $\gamma$ R<sup>-/-</sup> or Gbp<sup>chr3-/-</sup>

361 mice were infected with the live/dead reporter *M. tuberculosis* for 4 hours then washed with fresh

362 media in the presence or absence of IFN $\gamma$ . Four days later aTc was added to each well at a final

363 concentration of 500ng/ml. The following day infected cells were lifted and the fluorescence intensity of

364 the inducible Tet-on TagRFP was determined by flow cytometry. Cells were gated on live and infected

365 (mEmerald+) cells. **(B)** A representative histogram of tagRFP fluorescence is shown. **(C)** Shown is the

366 mean fluorescence intensity of tagRFP for three biological replicates. These data are representative of

367 three independent experiments. \*\*\*p<.001.

368

369 **Figure 3. Mice lacking chromosome 3 GBPs control *M. tuberculosis* infection.** Following low-dose  
370 aerosol infection with H37Rv (day 0 of ~50-150 CFU), total bacteria (expressed in CFU, mean +/- SD) was  
371 determined in the lungs **(A)** or the spleen **(B)** of wild type, IFN $\gamma$ R<sup>-/-</sup> or Gbp<sup>chr3-/-</sup> mice at the indicated time  
372 points with four mice per group. 28 days following low-dose aerosol infection with sfYFP H37Rv, infected  
373 cells in the lungs of wild type, IFN $\gamma$ R<sup>-/-</sup> or Gbp<sup>chr3-/-</sup> mice were quantified by flow cytometry. **(C)** Shown is  
374 a representative flow cytometry plot of the infected cells (YFP positive) that are gated on live, single cells  
375 and **(D)** the mean percent of YFP positive cells in the lungs of infected animals. **(E)** Following intravenous  
376 infection with H37Rv (1x10<sup>6</sup> per mouse), total bacteria were determined in the spleen of wild type,  
377 IFN $\gamma$ R<sup>-/-</sup> or Gbp<sup>chr3-/-</sup> mice at the indicated time points with 3-5 mice per group. All data are  
378 representative of 3 independent experiments.

379

380 **Figure 4. Chromosome 3 GBPs do not control the host response to *M. tuberculosis* infection. (A)**  
381 BMDMs from wild type, IFN $\gamma$ R<sup>-/-</sup> or Gbp<sup>chr3-/-</sup> mice were stimulated with IFN $\gamma$  overnight then infected with  
382 *Mtb* for 4 hours then washed with fresh media. 18 hours later, supernatants were harvested and the  
383 levels of IL1 $\beta$  and TNF $\alpha$  were quantified in the supernatants by ELISA. Shown is the mean of four  
384 biological replicates normalized to a standard curve +/- SD \*p<.05. **(B)** and **(C)** 28 days following low-  
385 dose aerosol infection with H37Rv (day 0 of ~50-100 CFU), the lungs of wild type, IFN $\gamma$ R<sup>-/-</sup>, and Gbp<sup>chr3-/-</sup>  
386 mice were harvested and the immune cell populations were quantified by flow cytometry and cytokines  
387 were quantified by ELISA. For **(B)**, we determined the % of live cells for Granulocytes (CD45<sup>+</sup> CD11b<sup>+</sup>  
388 Ly6G<sup>+</sup>), Macrophages (CD45<sup>+</sup> CD11b<sup>+</sup> Ly6G<sup>-</sup>), Dendritic Cells (CD45<sup>+</sup> CD11b<sup>-</sup> Ly6G<sup>-</sup> CD11c<sup>+</sup>), CD4<sup>+</sup> T cells  
389 (CD45<sup>+</sup> CD4<sup>+</sup>), CD8<sup>+</sup> T cells (CD45<sup>+</sup> CD8<sup>+</sup>) and B cells (CD45<sup>+</sup> B220<sup>+</sup>). For **(C)** Cytokines quantified from lung  
390 homogenates. Shown is the mean of three mice per group +/- SD \*p<.05. These data are representative  
391 of 3-4 independent experiments with similar results.

392

393 **Figure 5. *M. tuberculosis* ESX1 mutants are susceptible to GBP-mediated control. (A)** BMDMs from wild

394 type or *Gbp*<sup>chr3-/-</sup> mice were infected with *M. tuberculosis* H37Rv,  $\Delta$ espA (Left) or  $\Delta$ eccb1 (Right) for 4

395 hours then washed with fresh media in the presence or absence of IFN $\gamma$ . Five days later the

396 macrophages were lysed and serial dilutions were plated to quantify colony forming units (CFU) of viable

397 *M. tuberculosis*. Shown is the mean CFU from four biological replicates +/- SD \*p<.05 \*\*p<.01 by two-

398 tailed t-test. Data are representative of two independent experiments with similar results. (B) Following

399 low-dose aerosol infection with a 1:1 mixed infection of either H37Rv (Hyg):H37Rv (Kan) or  $\Delta$ eccb1

400 (Hyg): H37Rv (Kan) (day 0 of ~100-200 CFU), total bacteria in the lungs that were either Kan or Hyg

401 resistant were quantified. The competitive index was calculated (Hyg CFU/Kan CFU) and is shown as the

402 mean for 3 independent mice for each genotype combination. The data are representative of two

403 independent experiments with similar results.

404

405 **References**

- 406 1. Randow F, MacMicking JD, James LC. 2013. Cellular self-defense: how cell-autonomous  
407 immunity protects against pathogens. *Science* 340:701-6.
- 408 2. Tretina K, Park ES, Maminska A, MacMicking JD. 2019. Interferon-induced guanylate-  
409 binding proteins: Guardians of host defense in health and disease. *J Exp Med* 216:482-  
410 500.
- 411 3. Saelens JW, Viswanathan G, Tobin DM. 2019. Mycobacterial Evolution Intersects With  
412 Host Tolerance. *Front Immunol* 10:528.
- 413 4. Brites D, Gagneux S. 2015. Co-evolution of *Mycobacterium tuberculosis* and *Homo*  
414 *sapiens*. *Immunol Rev* 264:6-24.
- 415 5. Nunes-Alves C, Booty MG, Carpenter SM, Jayaraman P, Rothchild AC, Behar SM. 2014. In  
416 search of a new paradigm for protective immunity to TB. *Nat Rev Microbiol* 12:289-99.
- 417 6. Zumla A, George A, Sharma V, Herbert RH, Baroness Masham of I, Oxley A, Oliver M.  
418 2015. The WHO 2014 global tuberculosis report--further to go. *Lancet Glob Health*  
419 3:e10-2.
- 420 7. Olive AJ, Sasseti CM. 2016. Metabolic crosstalk between host and pathogen: sensing,  
421 adapting and competing. *Nat Rev Microbiol* 14:221-34.
- 422 8. Ernst JD. 2012. The immunological life cycle of tuberculosis. *Nat Rev Immunol* 12:581-  
423 91.
- 424 9. Bustamante J, Boisson-Dupuis S, Abel L, Casanova JL. 2014. Mendelian susceptibility to  
425 mycobacterial disease: genetic, immunological, and clinical features of inborn errors of  
426 IFN-gamma immunity. *Semin Immunol* 26:454-70.

- 427 10. Nandi B, Behar SM. 2011. Regulation of neutrophils by interferon-gamma limits lung  
428 inflammation during tuberculosis infection. *J Exp Med* 208:2251-62.
- 429 11. Olive AJ, Sasseti CM. 2018. Tolerating the Unwelcome Guest; How the Host Withstands  
430 Persistent *Mycobacterium tuberculosis*. *Front Immunol* 9:2094.
- 431 12. Mishra BB, Rathinam VA, Martens GW, Martinot AJ, Kornfeld H, Fitzgerald KA, Sasseti  
432 CM. 2013. Nitric oxide controls the immunopathology of tuberculosis by inhibiting  
433 NLRP3 inflammasome-dependent processing of IL-1beta. *Nat Immunol* 14:52-60.
- 434 13. Braverman J, Stanley SA. 2017. Nitric Oxide Modulates Macrophage Responses to  
435 *Mycobacterium tuberculosis* Infection through Activation of HIF-1alpha and Repression  
436 of NF-kappaB. *J Immunol* 199:1805-1816.
- 437 14. Denis M. 1991. Interferon-gamma-treated murine macrophages inhibit growth of  
438 tubercle bacilli via the generation of reactive nitrogen intermediates. *Cell Immunol*  
439 132:150-7.
- 440 15. Darwin KH, Ehrt S, Gutierrez-Ramos JC, Weich N, Nathan CF. 2003. The proteasome of  
441 *Mycobacterium tuberculosis* is required for resistance to nitric oxide. *Science* 302:1963-  
442 6.
- 443 16. Nambi S, Long JE, Mishra BB, Baker R, Murphy KC, Olive AJ, Nguyen HP, Shaffer SA,  
444 Sasseti CM. 2015. The Oxidative Stress Network of *Mycobacterium tuberculosis* Reveals  
445 Coordination between Radical Detoxification Systems. *Cell Host Microbe* 17:829-37.
- 446 17. Pilla-Moffett D, Barber MF, Taylor GA, Coers J. 2016. Interferon-Inducible GTPases in  
447 Host Resistance, Inflammation and Disease. *J Mol Biol* 428:3495-513.

- 448 18. Gomes MTR, Cerqueira DM, Guimaraes ES, Campos PC, Oliveira SC. 2019. Guanylate-  
449 binding proteins at the crossroad of noncanonical inflammasome activation during  
450 bacterial infections. *J Leukoc Biol* 106:553-562.
- 451 19. Man SM, Place DE, Kuriakose T, Kanneganti TD. 2017. Interferon-inducible guanylate-  
452 binding proteins at the interface of cell-autonomous immunity and inflammasome  
453 activation. *J Leukoc Biol* 101:143-150.
- 454 20. Praefcke GJK. 2018. Regulation of innate immune functions by guanylate-binding  
455 proteins. *Int J Med Microbiol* 308:237-245.
- 456 21. Santos JC, Broz P. 2018. Sensing of invading pathogens by GBPs: At the crossroads  
457 between cell-autonomous and innate immunity. *J Leukoc Biol* 104:729-735.
- 458 22. Kim BH, Shenoy AR, Kumar P, Das R, Tiwari S, MacMicking JD. 2011. A family of IFN-  
459 gamma-inducible 65-kD GTPases protects against bacterial infection. *Science* 332:717-  
460 21.
- 461 23. Marinho FV, Fahel JS, de Araujo A, Diniz LTS, Gomes MTR, Resende DP, Junqueira-Kipnis  
462 AP, Oliveira SC. 2020. Guanylate binding proteins contained in the murine chromosome  
463 3 are important to control mycobacterial infection. *J Leukoc Biol*  
464 doi:10.1002/JLB.4MA0620-526RR.
- 465 24. Coers J. 2017. Sweet host revenge: Galectins and GBPs join forces at broken  
466 membranes. *Cell Microbiol* 19.
- 467 25. Moliva JI, Turner J, Torrelles JB. 2017. Immune Responses to Bacillus Calmette-Guerin  
468 Vaccination: Why Do They Fail to Protect against Mycobacterium tuberculosis? *Front*  
469 *Immunol* 8:407.



- 470 26. Tiwari S, Casey R, Goulding CW, Hingley-Wilson S, Jacobs WR, Jr. 2019. Infect and Inject:  
471 How Mycobacterium tuberculosis Exploits Its Major Virulence-Associated Type VII  
472 Secretion System, ESX-1. *Microbiol Spectr* 7.
- 473 27. Stanley SA, Raghavan S, Hwang WW, Cox JS. 2003. Acute infection and macrophage  
474 subversion by Mycobacterium tuberculosis require a specialized secretion system. *Proc*  
475 *Natl Acad Sci U S A* 100:13001-6.
- 476 28. Watson RO, Manzanillo PS, Cox JS. 2012. Extracellular M. tuberculosis DNA targets  
477 bacteria for autophagy by activating the host DNA-sensing pathway. *Cell* 150:803-15.
- 478 29. Yamamoto M, Okuyama M, Ma JS, Kimura T, Kamiyama N, Saiga H, Ohshima J, Sasai M,  
479 Kayama H, Okamoto T, Huang DC, Soldati-Favre D, Horie K, Takeda J, Takeda K. 2012. A  
480 cluster of interferon-gamma-inducible p65 GTPases plays a critical role in host defense  
481 against *Toxoplasma gondii*. *Immunity* 37:302-13.
- 482 30. Garces A, Atmakuri K, Chase MR, Woodworth JS, Krastins B, Rothchild AC, Ramsdell TL,  
483 Lopez MF, Behar SM, Sarracino DA, Fortune SM. 2010. EspA acts as a critical mediator of  
484 ESX1-dependent virulence in Mycobacterium tuberculosis by affecting bacterial cell wall  
485 integrity. *PLoS Pathog* 6:e1000957.
- 486 31. Murphy KC, Nelson SJ, Nambi S, Papavinasasundaram K, Baer CE, Sasseti CM. 2018.  
487 ORBIT: a New Paradigm for Genetic Engineering of Mycobacterial Chromosomes. *mBio*  
488 9.
- 489 32. Mishra BB, Lovewell RR, Olive AJ, Zhang G, Wang W, Eugenin E, Smith CM, Phuah JY,  
490 Long JE, Dubuke ML, Palace SG, Goguen JD, Baker RE, Nambi S, Mishra R, Booty MG,  
491 Baer CE, Shaffer SA, Dartois V, McCormick BA, Chen X, Sasseti CM. 2017. Nitric oxide

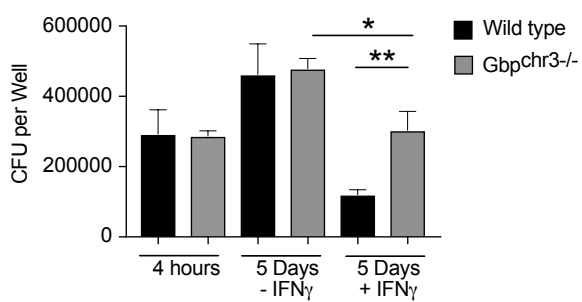
- 492 prevents a pathogen-permissive granulocytic inflammation during tuberculosis. *Nat*  
493 *Microbiol* 2:17072.
- 494 33. Olive AJ, Smith CM, Kiritsy MC, Sasseti CM. 2018. The Phagocyte Oxidase Controls  
495 Tolerance to *Mycobacterium tuberculosis* Infection. *J Immunol*  
496 doi:10.4049/jimmunol.1800202.
- 497 34. Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, Amon LM, Mahomed H,  
498 Erasmus M, Whatney W, Hussey GD, Abrahams D, Kafaar F, Hawkridge T, Verver S,  
499 Hughes EJ, Ota M, Sutherland J, Howe R, Dockrell HM, Boom WH, Thiel B, Ottenhoff  
500 THM, Mayanja-Kizza H, Crampin AC, Downing K, Hatherill M, Valvo J, Shankar S, Parida  
501 SK, Kaufmann SHE, Walzl G, Aderem A, Hanekom WA, Acs, groups GCcs. 2016. A blood  
502 RNA signature for tuberculosis disease risk: a prospective cohort study. *Lancet*  
503 387:2312-2322.
- 504 35. Johansen MD, Herrmann JL, Kremer L. 2020. Non-tuberculous mycobacteria and the rise  
505 of *Mycobacterium abscessus*. *Nat Rev Microbiol* 18:392-407.
- 506 36. Wandel MP, Pathe C, Werner EI, Ellison CJ, Boyle KB, von der Malsburg A, Rohde J,  
507 Randow F. 2017. GBPs Inhibit Motility of *Shigella flexneri* but Are Targeted for  
508 Degradation by the Bacterial Ubiquitin Ligase IpaH9.8. *Cell Host Microbe* 22:507-518 e5.
- 509 37. Piro AS, Hernandez D, Luoma S, Feeley EM, Finethy R, Yirga A, Frickel EM, Lesser CF,  
510 Coers J. 2017. Detection of Cytosolic *Shigella flexneri* via a C-Terminal Triple-Arginine  
511 Motif of GBP1 Inhibits Actin-Based Motility. *mBio* 8.

- 512 38. Li P, Jiang W, Yu Q, Liu W, Zhou P, Li J, Xu J, Xu B, Wang F, Shao F. 2017. Ubiquitination  
513 and degradation of GBPs by a Shigella effector to suppress host defence. Nature  
514 551:378-383.
- 515 39. Ji C, Du S, Li P, Zhu Q, Yang X, Long C, Yu J, Shao F, Xiao J. 2019. Structural mechanism for  
516 guanylate-binding proteins (GBPs) targeting by the Shigella E3 ligase IpaH9.8. PLoS  
517 Pathog 15:e1007876.
- 518 40. Kutsch M, Sistemich L, Lesser CF, Goldberg MB, Herrmann C, Coers J. 2020. Direct  
519 binding of polymeric GBP1 to LPS disrupts bacterial cell envelope functions. EMBO J  
520 39:e104926.
- 521 41. Nair S, Huynh JP, Lampropoulou V, Loginicheva E, Esaulova E, Gounder AP, Boon ACM,  
522 Schwarzkopf EA, Bradstreet TR, Edelson BT, Artyomov MN, Stallings CL, Diamond MS.  
523 2018. Irg1 expression in myeloid cells prevents immunopathology during M.  
524 tuberculosis infection. J Exp Med 215:1035-1045.
- 525 42. MacMicking JD, Taylor GA, McKinney JD. 2003. Immune control of tuberculosis by IFN-  
526 gamma-inducible LRG-47. Science 302:654-9.
- 527 43. Springer HM, Schramm M, Taylor GA, Howard JC. 2013. Irgm1 (LRG-47), a regulator of  
528 cell-autonomous immunity, does not localize to mycobacterial or listerial phagosomes in  
529 IFN-gamma-induced mouse cells. J Immunol 191:1765-74.
- 530 44. Feng CG, Zheng L, Jankovic D, Bafica A, Cannons JL, Watford WT, Chaussabel D, Hieny S,  
531 Caspar P, Schwartzberg PL, Lenardo MJ, Sher A. 2008. The immunity-related GTPase  
532 Irgm1 promotes the expansion of activated CD4+ T cell populations by preventing  
533 interferon-gamma-induced cell death. Nat Immunol 9:1279-87.

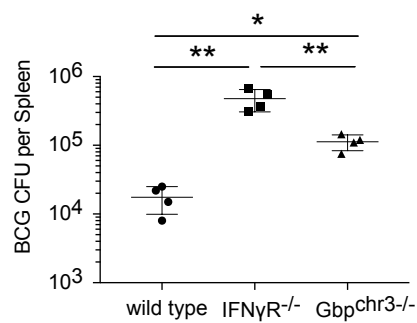
- 534 45. Coers J, Gondek DC, Olive AJ, Rohlfing A, Taylor GA, Starnbach MN. 2011. Compensatory  
535 T cell responses in IRG-deficient mice prevent sustained *Chlamydia trachomatis*  
536 infections. *PLoS Pathog* 7:e1001346.
- 537 46. Pires D, Marques J, Pombo JP, Carmo N, Bettencourt P, Neyrolles O, Lugo-Villarino G,  
538 Anes E. 2016. Role of Cathepsins in *Mycobacterium tuberculosis* Survival in Human  
539 Macrophages. *Sci Rep* 6:32247.
- 540

**Figure 1**

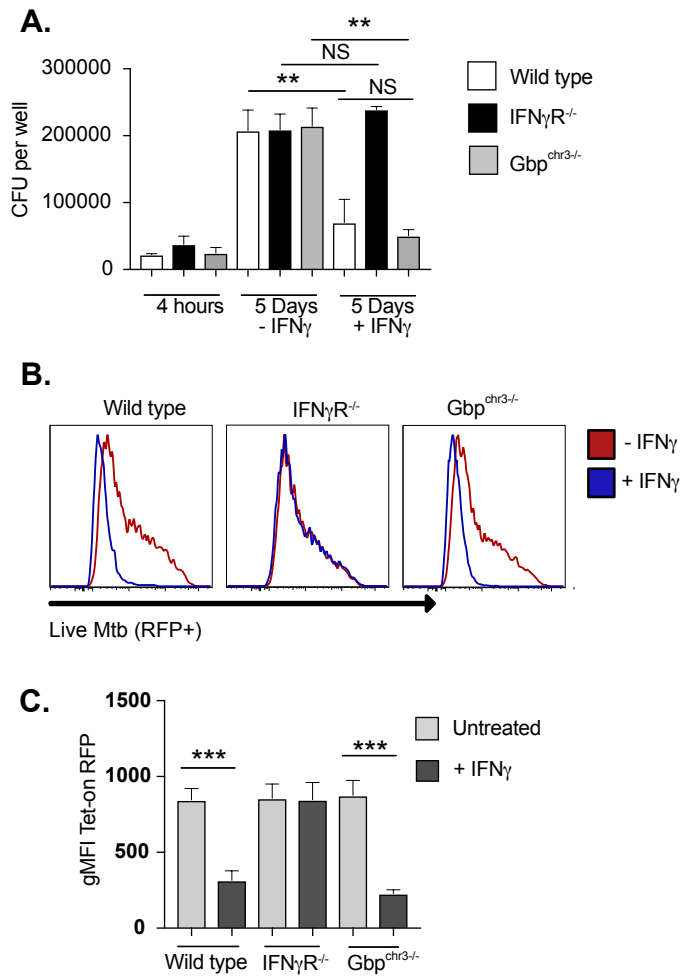
**A.**



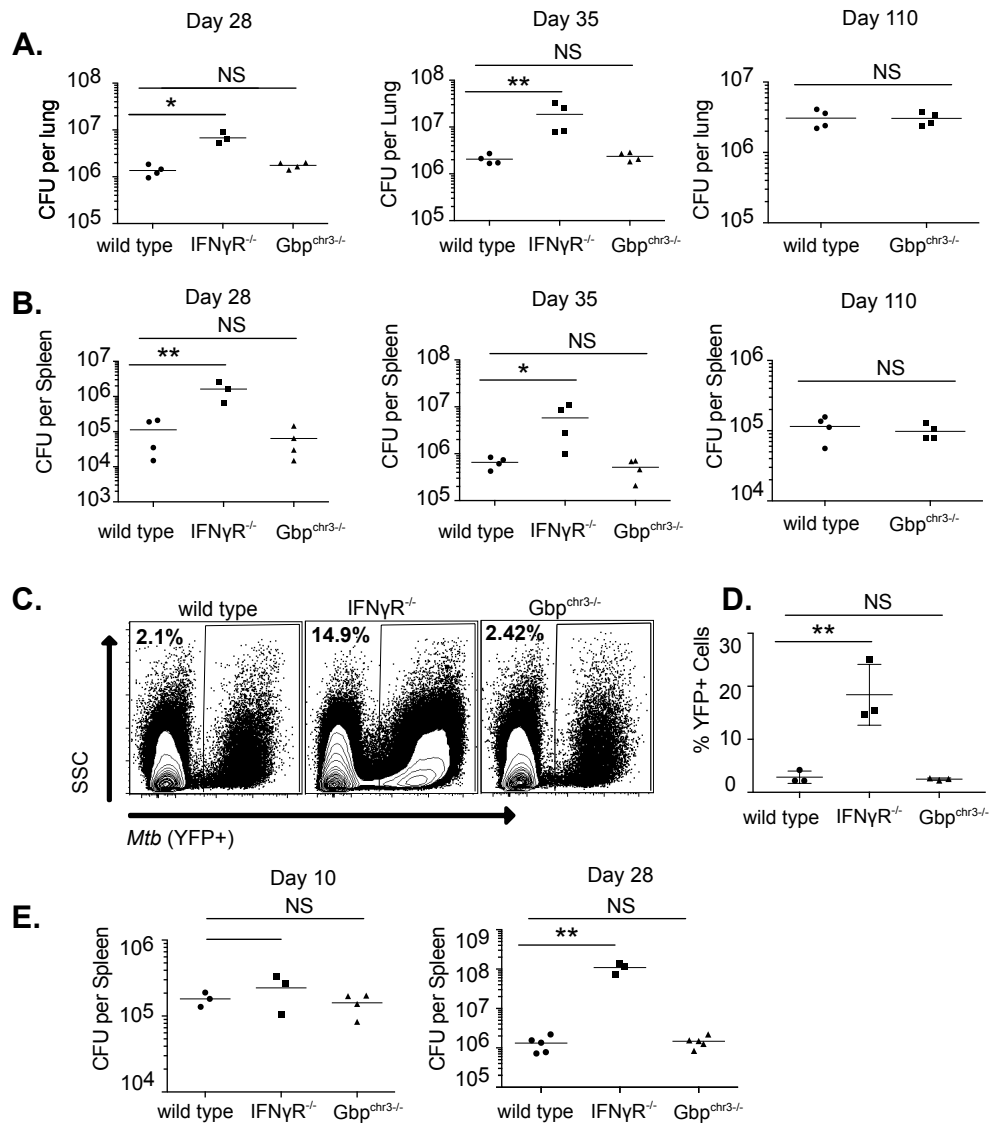
**B.**



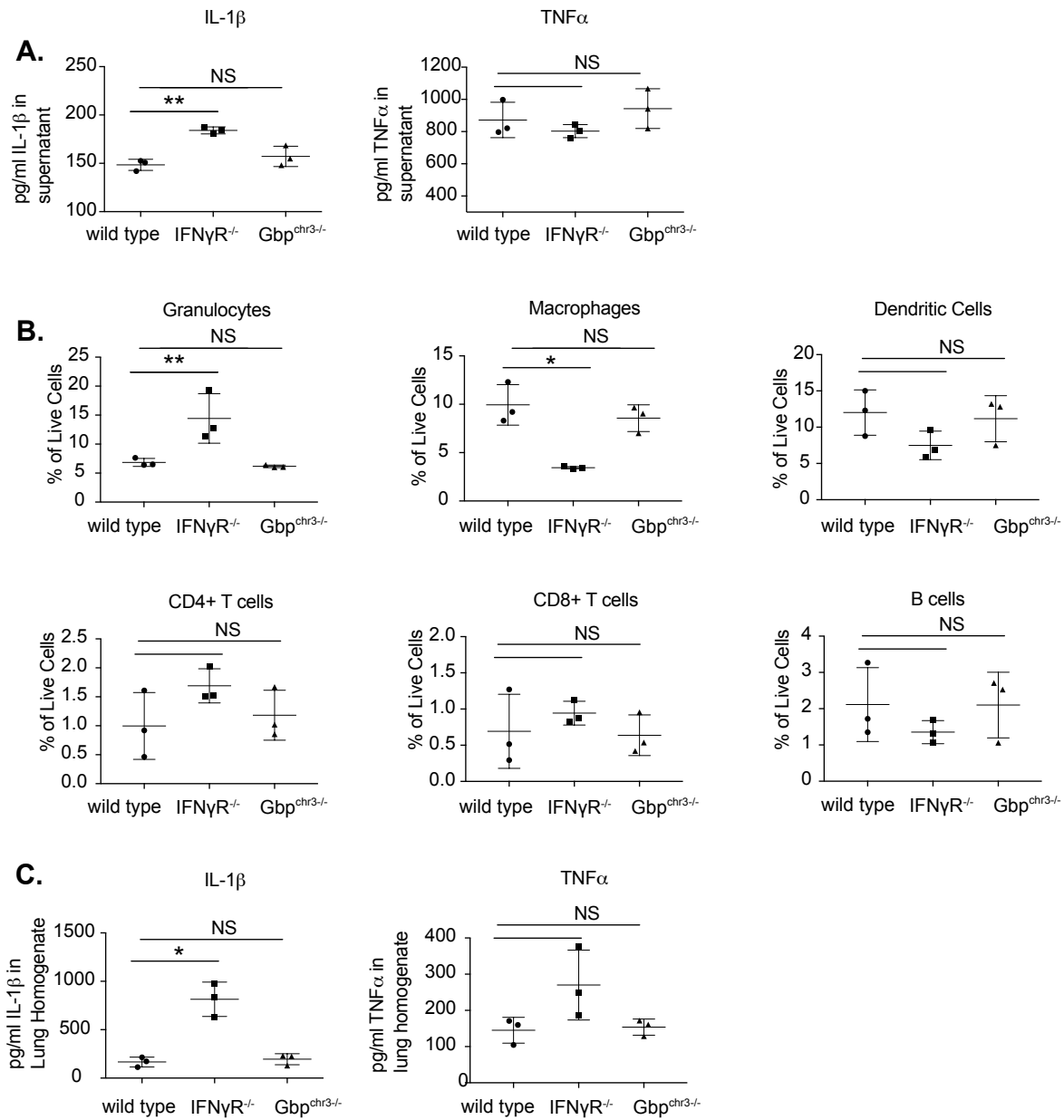
**Figure 2**



**Figure 3**



**Figure 4**





**Figure 5**

