Inflammasome-mediated antagonism of type I interferon enhances *Rickettsia* pathogenesis

Thomas P. Burke1✉, Patrik Engström1, Roberto A. Chavez1, Joshua A. Fonbuena1,3, Russell E. Vance1,2 and Matthew D. Welch1✉

The innate immune system fights infection with inflammasomes and interferons. Facultative bacterial pathogens that inhabit the host cytosol avoid inflammasomes and are often insensitive to type I interferons (IFN-I), but are restricted by IFN-γ. However, it remains unclear how obligate cytosolic bacterial pathogens, including *Rickettsia* species, interact with innate immunity. Here, we report that the human pathogen *Rickettsia parkeri* is sensitive to IFN-I and benefits from inflammasome-mediated host cell death that antagonizes IFN-I. *R. parkeri*-induced cell death requires the cytosolic lipopolysaccharide (LPS) receptor caspase-11 and antagonizes IFN-I production mediated by the DNA sensor cGAS. The restrictive effects of IFN-I require the interferon regulatory factor IRF5, which upregulates genes encoding guanylate-binding proteins (GBPs) and inducible nitric oxide synthase (iNOS), which we found to inhibit *R. parkeri*. Mice lacking both IFN-I and IFN-γ receptors succumb to *R. parkeri*, revealing critical and overlapping roles for these cytokines in vivo. The interactions of *R. parkeri* with inflammasomes and interferons are similar to those of viruses, which can exploit the inflammasome to avoid IFN-I, are restricted by IFN-I via IRF5 and IRF7, and are controlled by IFN-I and IFN-γ in vivo. Our results suggest that the innate immune response to an obligate cytosolic bacterial pathogen lies at the intersection of antibacterial and antiviral responses.

Interferons and inflammasomes constitute two critical arms of innate immunity. Interferons are signalling molecules that upregulate antimicrobial genes to protect the cytosol. Interferon-α and -β (IFN-I) have a nearly universal antiviral role, whereas many bacterial pathogens are instead highly sensitive to restriction by IFN-γ. The IFN-I response is antagonized by inflammasomes, which detect microbial ligands in the cytosol and protect against infection by causing pyroptosis, a rapid lytic host cell death that exposes intracellular microorganisms to the extracellular space. Inflammasomes cause cell death by activating caspase-1 and/or caspase-11, which then cleave and activate the pore-forming protein gasdermin D (GSDMD). Many facultative cytosolic bacterial pathogens, including *Listeria monocytogenes* and *Francisella novicida*, benefit from IFN-I signalling in vivo and avoid inflammasomes by modifying, downregulating or minimizing ligands recognized by inflammasomes, including lipopolysaccharide (LPS), flagellin, and DNA. However, it remains unknown whether or how interferons and inflammasomes protect against bacterial pathogens that obligately inhabit the cytosol, such as *Rickettsia* species, which are similar to viruses in their absolute dependency on the cytosolic environment for their replication. Unravelling how interferons and inflammasomes affect obligate cytosolic bacterial pathogens is critical to understand how the innate immune system discriminates between and targets the variety of pathogens that inhabit the cytosol.

We sought to characterize the role of interferons in restricting the obligate cytosolic human pathogen *Rickettsia parkeri*, which causes eschar-associated rickettsiosis. We observed that IFN-β caused a robust, dose-dependent restriction of *R. parkeri* growth in mouse bone marrow-derived macrophages (BMDMs; Fig. 1a), but did not affect the abundance of *L. monocytogenes* (Extended Data Fig. 1a). Infection with *R. parkeri* also did not induce appreciable IFN-I secretion, unlike infection with *L. monocytogenes* (Extended Data Fig. 1b). We hypothesized that, similar to some DNA viruses, *R. parkeri* tolerates inflammasome activation and host cell death to curtail the IFN-I response. We tested for inflammasome activation and observed that *R. parkeri* infection induced ~40% death of wild-type (WT) BMDMs at 24 hours post-infection (h.p.i.; Fig. 1b). Host cell death was reduced following the infection of cells mutated for Casp11, the gene encoding the receptor for cytosolic LPS, and in Gsdmd−/− BMDMs, and was abolished in Casp1−/−/Casp11−/− (Casp11/11−/−) double mutant cells (Fig. 1b), confirming inflammasome activation and pyroptosis by *R. parkeri*. In agreement with our hypothesis, bacteria were moderately restricted in Casp11−/− or Gsdmd−/− cells and severely restricted in Casp11/11−/− cells (Fig. 1c), and bacterial restriction correlated with increased secretion of IFN-I (Fig. 1d). IFN-I production by infected Casp11/11−/− cells occurred with similar timing to the cell death of infected WT BMDMs (Extended Data Fig. 1c,d), suggesting that cell death antagonizes IFN-I. Furthermore, secreted IFN-I was responsible for *R. parkeri* restriction, as the transfer of conditioned supernatant from infected Casp11/11−/− cells to infected WT BMDMs caused a dose-dependent inhibition of bacterial growth, whereas supernatant transfer to infected interferon-α/β receptor mutant (*Ifnar−/−*) BMDMs had no effect on bacterial growth (Fig. 1e). Moreover, the treatment of Casp11/11−/− cells with an anti-IFNAR antibody restored *R. parkeri* growth, and BMDMs from triple-mutant Casp1−/−Casp11−/− (*Casp11/11−/−*) mice supported *R. parkeri* growth (Fig. 1f). Similar experiments showed no role for tumour-necrosis factor (TNF)-α, interleukin (IL)-18 or IL-1β in the restriction of *R. parkeri* in WT or Casp11/11−/− BMDMs (Extended Data Fig. 1f,g). Together, these findings demonstrate that *R. parkeri* is sensitive to IFN-I-mediated killing, but avoids stimulating a robust IFN-I response by exploiting the inherent trade-off between inflammasome activation and IFN-I production.
We next sought to determine the mechanism by which \textit{R. parkeri} induces IFN-I production in \textit{Casp11} \textsuperscript{−/−} cells by distinguishing whether the host DNA-sensing pathway (cyclic GMP–AMP synthase/stimulator of interferon genes (cGAS/STING)) or LPS-sensing pathway (TLR4) were required for IFN-I production. We infected BMDMs from triple-mutant mice and observed that \textit{Casp11} \textsuperscript{−/−} cells produced increased IFN-I, comparable to levels from \textit{Casp11} \textsuperscript{−/−} cells. By contrast, IFN-I secretion from \textit{Casp1} \textsuperscript{−/−}\textit{Casp11} \textsuperscript{−/−} cells was comparable to levels from WT cells (Fig. 1g, Extended Data Fig. 1h) and \textit{R. parkeri} growth was supported in these cells (Fig. 1h). These data demonstrate that IFN-I production, which is masked by the inflammasome during \textit{R. parkeri} infection of WT macrophages, depends on cGAS. We propose that lysed \textit{R. parkeri} releases cytosolic DNA that activates cGAS, as well as LPS that activates caspase-11, and that the more rapid pyroptosis curtails the slower transcriptional-based IFN-I response. These findings suggest that a subpopulation of \textit{R. parkeri} is killed in the cytosol, eliciting host cell death that protects the remaining population from an antirickettsial IFN-I response.

It remained unclear how \textit{R. parkeri} is restricted by IFN-I. To investigate this, we first sought to identify an interferon-responsive transcription factor required for controlling infection. We compared bacterial growth in WT versus \textit{Irf1} \textsuperscript{−/−}, \textit{Irf3} \textsuperscript{−/−}, or \textit{Irf5} \textsuperscript{−/−} mutant BMDMs and found that growth was increased by the mutation of \textit{Irf5} (Fig. 2a). This suggested that IRF5 is primarily responsible for regulating the expression of interferon-stimulated genes (ISGs) with antirickettsial activity. To identify the ISGs regulated by IRF5, we...
Irf1

performed high-throughput RNA sequencing (RNA-seq) at 12 h.p.i., the earliest time when bacterial killing was observed (Extended Data Fig. 2a). We found that 136 genes were upregulated >4.0-fold in infected WT IFN-I-treated cells when compared with infected untreated WT cells (Supplementary Dataset 1). Of these, 36 genes had lower expression in Irf5−/− cells compared with Irf1−/− and Irf3−/−Irf1−/− cells, indicating that these were substantially and specifically upregulated by Irf5 (Fig. 2b, Extended Data Fig. 2b). Many of the 36 genes encode known antimicrobial proteins, including guanylate-binding protein 2 (GBP2), GBP5, VIPERIN (encoded by Rsad2), IFIT1 and IFIT2. The expression of Nos2, which encodes the known antirickettsial factor iNOS30–33, was also highly dependent on Irf5, although it was also dependent on interferon regulatory factor 1 (IRF1). To determine whether any of these factors restrict R. parkeri, we infected WT and mutant BMDMs carrying mutations in candidate antirickettsial genes in the presence of IFN-I. Only BMDMs lacking the chromosome 3 cluster of Gbp genes (Gbpchr3−/−) supported increased bacterial growth compared with WT BMDMs when treated with IFN-I (Fig. 2c, Extended Data Fig. 2c) or IFN-γ (Fig. 2d). Following INOS inhibition, bacterial growth was further increased in WT and Gbpchr3−/− cells (Fig. 2c, Extended Data Fig. 2d). Together, these results suggest that inflammasome activation allows R. parkeri to avoid IFN-I production and downstream expression of antirickettsial ISGs, including those encoding GBPs and iNOS.
We hypothesized that these antimicrobial factors were also involved in killing *R. parkeri* on initial entry to the cytosol, before IFN-1 induction. We measured host cell death in WT and mutant BMDMs lacking the ISGs identified above and observed that cell death was significantly reduced in *Gbpchr3−/−* cells (Fig. 2f). Despite reduced host cell death, *Gbpchr3−/−* cells did not exhibit increased IFN-1 production (Fig. 2g), leading us to conclude that GBPs are required for bacteriolysis to stimulate cGAS and caspase-11. Consistent with a role for GBPs in bacterial restriction, GBP2 localized to the surface of ~1–2% of *R. parkeri* in untreated WT cells, and the percentage of bacteria with surface-associated GBP2 increased in *Casp11−/−* cells after 12 h.p.i., correlating with the increase in IFN-1 (Fig. 2h,i, Extended Data Fig. 2e). Moreover, colocalization increased following exogenous IFN-1 treatment and no surface-associated GBP2 was observed in *Gbpchr3−/−* BMDMs (Extended Data Fig. 2f). These observations suggest that GBP2 directly targets *R. parkeri* and that GBPs also have an indirect protective effect by eliciting host cell death in a subset of infected cells. The requirement for GBPs to elicit host cell death early during infection suggests that they act constitutively, and their increased colocalization following IFN-1 treatment further suggests an inducible protective role.

We next evaluated the role that the inflammasome, IFN-I and IFN-γ play in controlling *R. parkeri* in vivo. In agreement with our observations in vitro, we observed significantly more IFN-1 in the spleens of infected *Casp11−/−* versus WT mice, and IFN-1 induction depended on cGAS, as it was reduced in *Casp1−/−Casp11−/−Cgas−/−* mice (Fig. 3a; mice separated based on sex in Supplementary Fig. 1). Elevated IFN-1 had a direct antirickettsial effect in the spleen, as *Casp1−/−Casp11−/−Ifnar−/−* and *Casp1−/−Casp11−/−Cgas−/−* mice had higher bacterial burdens than *Casp11−/−* mice (Fig. 3b). The neutralization of IFN-γ caused a modest increase in bacterial burdens in WT and *Casp11−/−* mice and caused a dramatic increase in *Casp1−/−Casp11−/−Ifnar−/−* mice (Fig. 3b, Extended Data Fig. 3a), suggesting that the protection from these cytokines overlaps. It remains unclear why the spleens of *Casp11−/−* mice, which express more IFN-1 transcript, do not exhibit reduced bacterial burdens when compared to those of WT mice, as we observed for BMDMs in vitro. Nevertheless, our observations suggest that bacterial activation of the inflammasome limits the production of cGAS-induced IFN-I and that IFN-I and IFN-γ are antirickettsial in the spleen. We conclude that the relationship between the inflammasome, IFN-I and IFN-γ in spleens during infection in vivo is similar to that in the infection of BMDMs in vitro.

In contrast with the results from the spleen, we observed that *Casp11−/−* mice had increased bacterial burdens in the liver. This was dependent on IFN-I because bacterial burdens were reduced to WT levels in *Casp1−/−Casp11−/−Ifnar−/−* and *Casp1−/−Casp11−/−Cgas−/−* animals (Fig. 3c). We hypothesized that the increased bacterial burden in *Casp11−/−* mice was due to IFN-I-mediated antagonism of IFN-γ, as was previously observed in the liver during *L. monocytogenes* infection. In support of this hypothesis, the neutralization of IFN-γ erased the differences observed between *Casp11−/−* and *Casp1−/−Casp11−/−Ifnar−/−* mice (Fig. 3c). Together, these data support previous findings that the inflammasome has differing roles in spleens and livers, and that IFN-I antagonizes IFN-γ in the liver (schematic in Fig. 3d). The mechanism for IFN-I-mediated antagonism of IFN-γ during infection in the liver remains unclear and further investigations are required to better understand the cell types that harbour *R. parkeri* and the cells that mediate interferon signalling during infection.

IFN-I acts together with IFN-γ to provide overlapping protection against viral infection in vivo\(^{15,16}\), whereas IFN-γ dominates
that we observed in spleens and livers (Fig. 4b), which was similar to the abundance of \textit{R. parkeri} abundance in mouse organs after i.v. delivery of \(10^7\) p.f.u. at 72 h.p.i.

\textbf{b}, \textit{R. parkeri} abundance in mouse organs after delivery of \(10^7\) WT or 4\times\(10^6\) \(\text{IFNAR}^{-/-}\) WT or \(\text{IFNGR}^{-/-}\) WT individual mice. The horizontal bars denote medians and the statistical analyses were performed using a two-tailed Mann-Whitney U test. **\(P<0.01\).

\textbf{c}, Mouse survival following i.v. delivery of \(4 \times 10^8\) WT \textit{L. monocytogenes}. \(n=6\) (IFNAR\(^{-/-}\)), 7 (WT), 8 (IFNGR\(^{-/-}\)) and 6 (IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\)) individual mice. The survival of IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mice following i.v. delivery of \(10^4\) WT or ompB mutant \textit{R. parkeri} is shown.

\textbf{d}, The survival of IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mice following i.v. delivery of \(10^7\) WT or WT \textit{R. parkeri} is shown. For all panels, data are the combination of two independent experiments.

\textbf{e}, A model depicting the intracellular growth of \textit{R. parkeri} in WT cells (left) or in cells lacking inflammasome signalling (right). NO, nitric oxide.

\textbf{f}, A model for the antimicrobial effects of interferons on cytosolic pathogens.

The protective response to facultative cytosolic bacterial pathogens\(^{31,32}\). We therefore tested the roles of IFN-I and IFN-\(\gamma\) in protection against \textit{R. parkeri} at the whole-animal level by intravenously (i.v.) infecting C57BL/6 mice that were deficient in both IFN-I and IFN-\(\gamma\) receptors (IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\)). Strikingly, infection of IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mice caused a loss of body weight and temperature (Extended Data Fig. 3b,c), as well as dose-dependent lethality (Fig. 4a).

We observed similar susceptibility in AG129 mice, a different genetic background carrying mutations in \textit{Ifnar} and \textit{Ifngr} (Extended Data Fig. 3d). By contrast, WT, IFNAR\(^{-/-}\) and IFNGR\(^{-/-}\) mice had no signs of severe disease even at the highest infectious dose. IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mice retained high burdens of \textit{R. parkeri} in their spleens and livers (Fig. 4b), which was similar to the abundance that we observed in \textit{Casp1}\(^{-/-}\)/\textit{Casp11}\(^{-/-}\)/IFNAR\(^{-/-}\) mice treated with the anti-IFN-\(\gamma\) antibody (Fig. 3b,c), suggesting that the most critical consequences of inflammasome activation on \textit{R. parkeri} are the effects that the inflammasome has on both IFN-I and IFN-\(\gamma\). The livers and spleens were also analysed for evidence of pathology and, consistent with the increased susceptibility and bacterial burdens, tissues from infected IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mice showed marked inflammation, vascular damage and leukocyte infiltration (Extended Data Fig. 4), similar to the clinical features of human \textit{R. parkeri} infections\(^{33}\). Our findings that IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mice sustained tissue damage and succumbed to infection demonstrate that both interferons play critical, overlapping roles in protecting against \textit{R. parkeri} infection. By contrast, following infection with \textit{L. monocytogenes}, we found that IFNAR\(^{-/-}\) mice were less susceptible than WT mice, while IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mice had similar, increased susceptibilities when compared to the WT (Fig. 4c).

Overall, our findings that IFN-I significantly protects against \textit{R. parkeri} contrasts with the role for IFN-I in protecting against \textit{L. monocytogenes} in vivo, as well as \textit{F. novicida} and pathogens that reside in membrane-bound compartments, including \textit{Salmonella} species\(^{34}\), \textit{Mycobacterium tuberculosis}\(^{35}\), \textit{Coxiella burnetii}\(^{36}\), \textit{Chlamydia muridarum}\(^{37}\) and \textit{Brucella abortus}\(^{38}\).

There are limited in vivo tools for identifying and examining \textit{R. parkeri} virulence factors. Our finding that IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mice rapidly succumb to infection is of strong practical importance, as it suggests that the IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mouse may serve as an animal model to study and characterize bacterial virulence genes. As a proof of concept, we infected IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mice with an \textit{R. parkeri} mutant lacking outer membrane protein B (OmpB)\(^{39}\). In contrast with WT bacteria, infection with the ompB mutant caused no lethality in IFNAR\(^{-/-}\)/IFNGR\(^{-/-}\) mice (Fig. 4d).

This demonstrates the potential for this animal model to reveal \textit{R. parkeri} virulence genes in vivo.
Finally, we investigated the role of cell-extrinsc immunity in protecting against *R. parkeri* by infecting mice depleted for natural killer (NK) or CD8+ T cells and also *Rag2−/−* mice, which lack functional B and T cells altogether. We observed no dramatic increase in bacterial burdens in any of these mice and *Rag2−/−* mice survived infection with no noticeable symptoms of disease (Extended Data Fig. 5). This demonstrates that the interferon-mediated cell-autonomous response is critical, whereas the cell-extrinsic NK, B and T cell-mediated response is dispensable for controlling *R. parkeri*.

Our findings reveal unexpected differences between the host response to an obligate cytosolic bacterial pathogen and facultative cytosolic bacterial pathogens. Following infection with facultative cytosolic bacterial pathogens, IFN-γ dominates the protective response in vivo1,4,7,8, activation of IRF1 is protective1,2 and IFN-1 does not substantially contribute to protection7,8. Moreover, facultative cytosolic pathogens have evolved multiple mechanisms to avoid inflammasomes1–6 and some cause increased lethality in inflammasome-deficient mice8,9. We instead observed that the host response to *R. parkeri* shares many similarities to the response to viruses, are absolutely dependent on host processes for replication10,11, and both benefit from inflammasome-mediated antagonism of IFN-1 (Fig. 4e)12. We also observed no lethality following *R. parkeri* infection of inflammasome-deficient mice. Furthermore, the host protects against viral infection with IRF5-regulated genes13,14 and IFN-1 and IFN-γ play overlapping protective roles against viruses10,13, which we observed for *R. parkeri*. We propose that *R. parkeri* exhibits an intermediate degree of interferon susceptibility between viruses and facultative cytosolic bacterial pathogens (Fig. 4). *Rickettsia* species have undergone extensive genome reduction46 and, similar to viruses, both *R. parkeri* and *R. prowazekii* share many similarities to the response to an obligate cytosolic bacterial pathogen and facultative cytosolic bacterial pathogens.

**Methods**

**Preparation of *R. parkeri***. *R. parkeri* strain Portsmouth was originally obtained from C. Paddock (Centers for Disease Control and Prevention). Bacteria were amplified by infecting confluent T175 flask of female African green monkey kidney epithelial Vero cells obtained from the UC Berkeley Cell Culture Facility, where they were tested for mycoplasma contamination and authenticated by mass spectrometry experiments. Vero cells were grown in DMEM (Gibco 11995-092) with glucose (4.5 g l−1) supplemented with 2% fetal bovine serum (FBS;GemCell) with 5 × 10^5 *R. parkeri* per flask. Infected cells were scraped and collected at 5 days post infection (d.p.i.) when ~90% of cells were highly infected. Scraped cells were centrifuged at 12,000g for 20 min at 4 °C. Pelleted cells were then resuspended in K-36 buffer (0.05 M KH2PO4, 0.05 M K2HPO4, 100 mM KCl, 15 mM NaCl, pH 7.7) and doused (60 strokes) at 4 °C. The solution was then centrifuged at 200g for 5 min at 4 °C to separate host cell debris. The bacterial pellet was resuspended in brain heart infusion (BHI) media (BD, 237500) and stored at −80 °C. Titrates were determined via plaque assays by serially diluting the bacteria in six-well plates containing confluent Vero cells. Plates were then spun for 5 min at 300g in an Eppendorf 5810R centrifuge. At 24 h.p.i., the media from each well was aspirated and the wells were overlaid with 4 ml per well DMEM with 5% FBS and 0.7% agarose (Inviogene, 16050-500). At 6 d.p.i., an overlay of 0.7% agarose in DMEM containing 2.5% neutral red (Sigma, N6264) was added and plates were counted 24 h later. For infections with *ompB* mutant bacteria, the *ompB* mutant was used, which contains a transposon and an upstream stop codon in *ompB* (ref. 11). The plates were incubated at 37 °C overnight and the colony-forming units were counted –20 h later. The plates were incubated at 37 °C overnight and the colony-forming units were counted –20 h later.

**Deriving BMDMs**. To obtain bone marrow, male or female mice were euthanized and femurs, tibias and fibulas were excised. Connective tissue was removed and the bones were sterilized with 70% ethanol. Bones were washed with BMDM media (20% HyClone FBS, 1% sodium pyruvate, 0.1% β-mercaptoethanol, 10% conditioned supernatant from 3T3 fibroblasts, in Gibco DMEM containing glucose and 100 mM U186664, and 100 μM streptomycin) and ground using a mortar and pestle. Bone homogenate was passed through a 70-μm nylon Corning Falcon Falcon (Thermo Fisher Scientific, 08-771-2) to remove particulates. Filterates were centrifuged in an Eppendorf 5810R at 1,200×g (290g) for 8 min. The supernatant was aspirated and the remaining pelvis was resuspended in BMDM media. Cells were then plated in non-tissue culture-treated 15-cm petri dishes (at 2 × 10^6 cells per ml) and incubated at 37 °C. The cultures were washed with fresh media containing antibiotics. The number of cells was counted using Trypan blue (Sigma, T8154) and a haemocytometer (Bright-Line). 5 × 10^3 cells were plated into 24-well plates. Approximately 16 h later, a 30% preparation of the BMDMs was thawed on ice and diluted into fresh BMDM media to the desired concentration (either 10^5 plaque-forming units (p.f.u.) ml−1 or 2 × 10^6 p.f.u. ml−1). Media was then aspirated from the BMDMs and replaced with 500 μl media containing *R. parkeri*; the plates were spun at 300g for 5 min in an Eppendorf 5810R. Infected cells were then incubated for 18 h.p.i. with inactivated Polyclonal CEDO 1600 (Microbiological Associates) antibody set to 33 °C to minimize the duration of the experiment. For treatments with recombinant mouse IFN-β, IFN-β (PBL, 12405-1) was added directly to infected cells immediately after infection. To obtain conditioned supernatant from infected Csp1/11−/− cells, BMDMs were infected at a MOI of 1 in a volume of 500 μl fresh media. At 24 h.p.i., conditioned media was pooled from 30 wells and frozen at −80 °C. To treat infected cells with this conditioned supernatant, the indicated volumes of media were removed at 20 min post infection and replaced with an equal amount of the thawed conditioned media. To neutralize IFN-1 signalling, an ultra-LEAF-purified α-IFNAR-1 antibody (BioLegend, 127323) was added to a final concentration of 1 μg ml−1 at 4 h.p.i. and incubated at 37 °C for 24 h. IFN-γ (R&D Systems, 485-M100) was added to the wells immediately after infection. IL-1β was neutralized by adding the ULTRA-Leaf α-IL-1β antibody (BioLegend, 503514) to a final concentration 1 μg ml−1 at t = 0. IL-18 was neutralized by adding the IL-18 antagonist (MBL, D048-3) to 5 μg ml−1 at t = 0. Control IgG antibody (5 μg ml−1, Jackson, 012-000-003) was added at t = 0. For experiments with recombinant TNF-α, 200 ng was added to each well in a 24-well plate, and two different products were tested (BioLegend, 575302; Thermo Fisher Scientific). To measure the p.f.u., supernatants from infected BMDMs were aspirated from individual wells and each well was gently washed twice with 500 μl sterile milli-Q-grade water. 1 ml sterile milli-Q water was then added to each well and repeatedly pipetted up and down to lyse the host cells. Serial dilutions of lysates were added to 96-well plates that were plated with confluent Vero cells. Plates were then spun at 300g using an Eppendorf 5810R centrifuge for 5 min at room temperature and incubated at 33 °C overnight. At ~16 h.p.i., media was aspirated and replaced with 2 ml per well of DMEM containing 0.7% agarose and 5% FBS. At ~6 d.p.i., 1 ml of DMEM containing 0.7% agarose, 1% FBS, 200 μg ml−1 amphotericin B (Invitrogen, 15085-018) and 2.5% neutral red (Sigma) was added to each well. Plates were then counted 24 h later. To collect supernatant from Csp1/11−/− cells, 5 × 10^5 BMDMs per well in 24-well plates were infected with *R. parkeri* at a MOI of 1 and at 24 h.p.i. supernatants were pooled and stored at −80 °C. To add the supernatant to infected BMDMs, either 200 or 500 μl of supernatant was removed at 20 min post infection from the untreated cells and replaced with the supernatant from the infected cells.

For infections with *L. monocytogenes*, cultures of *L. monocytogenes* strain 10403S (originally obtained from D. Portnoy, UC Berkeley) were grown in 2 ml sterile-filtered BHI with shaking at 37 °C to the stationary phase (~16 h). Cultures were centrifuged at 20,000g (Eppendorf 5430) and the pellet was resuspended in fresh 100 μl BHI with shaking at 37 °C. 100 μl of the diluted bacteria was then added to each well of a 24-well plate of BMDMs that had been plated ~16 h before infection. Plates were then counted 24 h later. To add the supernatant to infected BMDMs, either 200 or 500 μl of supernatant was removed at 20 min post infection from the untreated cells and replaced with the supernatant from the infected cells.

High-throughput RNA-seq. For high-throughput RNA-seq, 5 × 10^6 BMDMs were plated in 24-well plates, infected 16 h later with *R. parkeri* and treated with 10,000 activity units of recombinant IFN-β. To determine the percentage of cells that were...
successfully infected, we analysed the infected cells using immunofluorescence microscopy and observed that the MOI (the average number of bacteria per host cell) was 2.3 and that 71% of cells were infected. At 12 h.p.i., the cells were lysed and RNA was extracted using a RNeasy purification kit (Qiagen). RNA quality was assessed using an Agilent 2100 Bioanalyzer, and all samples had RNA integrity number (RIN) values above 8.0. Transcripts were selected using polyA selection (using Dynabeads mRNA Purification Kit, Invitrogen) and enzymatically fragmented using the Apollo library prep kits (Wafergen PrepX RNA library prep for Illumina). Libraries were constructed using the PCR indexed library prep method and multiplexed at the Functional Genomics Lab at UC Berkeley (https://genomics.qb.berkeley.edu/). The resulting libraries were sequenced at the Vincent J. Coates Genomics Sequencing Facility at UC Berkeley using single-end reads, 50-base length, with the Hiseq 2000 Illumina platform. Sequence data were aligned to the *M. musculus* C57BL/6 reference genome (reference assembly GCA_00001635.8) using BWA (Burrows-Wheeler Aligner) (Quintel, 2012). Infected and uninfected animals (*M. musculus* C57BL/6) were compared for viral loads in the tissues of the infected animals. All animals were maintained at the UC Berkeley campus and all infections were performed in accordance with the approved protocols. Mice were age matched between 8 and 18 weeks old. Mice were selected for experiments based on their availability, regardless of sex. p.s.u. data for each sex are reported in Supplementary Fig. S3. Statistical analyses were performed in accordance with the approved protocols. Mice were significantly different oneway analysis of variance (ANOVA) followed by Tukey’s multiple comparisons test.

### Animal experiments.
Animal research using mice was conducted under a protocol approved by the UC Berkeley Institutional Animal Care and Use Committee (IACUC) in compliance with the Animal Welfare Act and other federal statutes relating to animals and experiments using animals (Welch lab animal use protocol AUP-2016-02-8426). The UC Berkeley IACUC is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and adheres to the principles of the Guide for the Care and Use of Laboratory Animals. All animals were maintained at UC Berkeley and all infections were performed in accordance with the approved protocols. Mice were age matched between 8 and 18 weeks old. Mice were selected for experiments based on their availability, regardless of sex. p.s.u. data for each sex are reported in Supplementary Fig. S3. Statistical analyses were performed in accordance with the approved protocols. Mice were significantly different oneway analysis of variance (ANOVA) followed by Tukey’s multiple comparisons test.

### Mouse genotyping.
Casp1/11, *Igf1r*−/−, *Igf2*−/−, *Igf3*−/−, *Igfr1*−/−, and *Igfr2*−/− mice were previously described. Casp1/11, *Igf1r*−/−, *Igf2*−/−, *Igfr1*−/−, *Igfr2*−/−, *Rag2*−/− or *Rag2*−/−/*C57BL/6* WT mice were previously described and originally obtained from Jackson Laboratories. For genotyping, ear clips were boiled for 15 min in 20 μl 10 mM NaOH and quenched with 10 μl 10 HCl/HCl pH 5.5, 2 μl lysis buffer was used for PCR using SyberGreen (Thermo Fisher Scientific, Waltham, MA) as the DNA stain. PCR amplification was performed using primers specific for each gene. The mice were genotyped using these primers: *Igf1r* forward: 5′-CACATCTACTACAAAGCAGACTGCTC-3′; *Igf1r* reverse: 5′-ACCTTCGGTCCTTGACGTG-3′; *Igf2* forward: 5′-GTCTGCGCTGGGATTGCTG-3′; *Igf2* reverse: 5′-GCTGATGTCTTGATATGATAT-3′. The bacterial suspensions were kept on ice during injections. The mice were injected to a heat lamp while in their cages for approximately 5 min and then each mouse was moved to a mouse restrainer (BrainTree, TB-150 STD). The tail was sterilized with 70% ethanol and 200 μl bacterial suspensions were injected using 30.5-gauge needles into the lateral tail vein. Body temperatures were measured using a rectal thermometer (Eachwell; TR-5) at day 1 before infection. The mice were monitored daily for clinical signs of disease, such as hunched posture, lethargy or scruffed fur. Only mice lacking both interferon receptors exhibited such symptoms and, if this occurred, the mice were monitored daily for signs of disease, weight and temperature. If a mouse displayed severe signs of infection, as defined by a reduction in body temperature below 90 °F or lethargy that prevented normal movement around the cage.

Mouse infections.
For the mouse infections, *R. parkeri* was prepared by diluting 30% prep bacteria into 1 ml cold sterile PBS, centrifuging the bacteria at 12,000g for 1 min (Eppendorf 5430 centrifuge) and resuspending in cold sterile PBS to the desired concentration (5 × 10^10 p.f.u. ml^−1, 2.5 × 10^10 p.f.u. ml^−1, 5 × 10^9 p.f.u. ml^−1, 5 × 10^7 p.f.u. ml^−1). The bacterial suspensions were kept on ice during injections. The mice were injected intraperitoneally with 200 μl PK-13 and peritoneal lavages were performed 3 days later. To deliver the anti-IFN-γ antibody (BioLegend, 505807) to the mice, antibodies were intraperitoneally injected at day 2 and 1 before infection. For the control experiments, 100 μg control IgG antibody (Jackson, 121-000-003) was delivered intraperitoneally at days −2 and −1 before infection, (320 μg total per mouse). NK cells were depleted by injecting mice intraperitoneally with 160 μg mCD8.2c-Leu2 (Leu2, 58332, CD8, CD11a) at day −2 and −1 before infection. For the control experiments, 100 μg control IgG antibody (Jackson, 121-000-003) was delivered intraperitoneally at days −2 and −1. After infection, all mice in this study were monitored daily for the presence of disease, such as hunched posture, lethargy or scruffed fur. Only mice lacking both interferon receptors exhibited such symptoms and, if this occurred, the mice were monitored daily for signs of disease, weight and temperature. If a mouse displayed severe signs of infection, as defined by a reduction in body temperature below 90 °F or an inability to move around the cage normally, the animal was immediately euthanized using CO2, followed by cervical dislocation, according to IACUC-approved procedures.
To harvest organs, mice were euthanized at the indicated predetermined times and doused with ethanol. Mouse organs were extracted and deposited into 50-ml conicals containing 4 ml sterile cold PBS for the spleen and 8 ml for the liver. Organs were kept on ice and were homogenized for ~10 s using an immersion homogenizer (Fisher, Polytron PT 2500E) at 22,000 r.p.m. Organ homogenates were spun at 290g for 5 min to pellet cell debris (Eppendorf 5810R centrifuge). 20µl organ homogenates was serial diluted into 12-well plates containing confluent Vero cells. The plates were then spun at 280g for 5 min at room temperature (Eppendorf 5810R centrifuge) and incubated at 33 °C. To reduce the possibility of contamination, organ homogenates were plated in duplicate and the second replicate was treated with 50µg ml−1 carbencillin (Sigma) and 200µg ml−1 amphotericin B (Gibco). The next day, at approximately 16h.p., the cells were gently washed by replacing the existing media with 1 ml DMEM containing 2% FBS. The media was then aspirated and replaced with 2 ml per well DMEM containing 0.7% agarose, 5% FBS and 200µg ml−1 amphotericin B. When plaques were visible at 6.d.p., 1 ml of DMEM containing 0.7% agarose, 1% FBS and 2.5% neutral red (Sigma) was added to each well and plaques were counted 24h later.

Histology. For the histology, spleens and livers were harvested from infected mice at 72h.p. and immediately stored in 10% neutral buffered saline (Sigma, HT501128). Histology was performed by Histowiz (histowiz.com) using a standard operating procedure and fully automated workflow. The samples were processed, embedded in paraffin and sectioned at 4-µm thickness. Bond Polymer Refine Detection (Leica Biosystems) was used according to the manufacturer’s protocol. After the slides were dehydrated, they were de-waxed using a TissueTek-Pritmic and Coverslipper (Sakura). Whole slide scanning (WSX) was performed on an Aperio AT2 (Leica Biosystems). Commercial pathology consultation was performed blindly by a pathologist (Histowiz).

Statistical analysis. The statistical parameters and significance are reported in the figure legends. To compare two sets of data, a two-tailed Student’s t test was performed. To compare multiple datasets, a one-way analysis of variance (ANOVA) with multiple comparisons with Tukey post-hoc test was used for normal distributions, and a Mann–Whitney U test was used for non-normal distributions. Data are determined to be statistically significant when P<0.05. All data points shown in bar graphs and in animal data are distinct samples. Asterisks denote statistical significance as: *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001, compared with the indicated controls. For animal experiments, bars denote medians. Error bars indicate standard deviation. All other graphical representations are described in the figure legends. Statistical analyses were performed using GraphPad Prism v6 and v7.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The RNA-seq datasets generated and analysed during this study are available in the Gene Expression Omnibus (GEO) repository; accession no. GSE128211. R. parkeri strains were authenticated by whole-genome sequencing and are available in the National Center for Biotechnology Information (NCBI) Trace and Short-Read Archive; Sequence Read Archive (SRA), accession no. SRX401164. Source Data for Figs. 1–4 and Extended Data Figs. 3 and 5 are provided with the paper.

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**Author contributions**

T.P.B. performed and analysed in vitro and in vivo experiments. P.E. provided reagents and contributed to in vivo experiments. R.A.C. contributed to the breeding of the mice. T.P.B. performed and analysed in vivo experiments. P.E. provided reagents and contributed to in vivo experiments. R.A.C. contributed to the breeding of the mice. J.A.F. was supported by NIH/National Institute of General Medical Sciences (NIGMS) postdoctoral fellowship from Foundation Olle Engkvist Byggmästare, the Swedish Society of Medical Research (SSMF). M.D.W. is supported by NIH/NIADD grant nos. R01 AI09044, R21 AI09270 and R21 AI38550. J.A.F. was supported by NIH/National Institute of General Medical Sciences (NIGMS) grant no. 2T34GM008612-24. R.E.V. is a Howard Hughes Medical Institute (HHMI) investigator and is supported by NIH/NIADD Grant nos. AI075039 and AI063302.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41564-020-0673-5. Supplementary information is available for this paper at https://doi.org/10.1038/s41564-020-0673-5. Correspondence and requests for materials should be addressed to T.P.B. or M.D.W. Reprints and permissions information is available at www.nature.com/reprints. 

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Extended Data Fig. 1 | Inflammasome activation benefits *R. parkeri* by antagonizing the IFN-I response in mouse macrophages. **a**, Measurement of *Listeria monocytogenes* (Lm) CFU in BMDMs, MOI of 1. 3,000 U of IFN-β added at t = 0. n = 3 independent experiments. **b**, Measurement of IFN-I in supernatants of WT BMDMs infected with *R. parkeri* (24 hpi) or *L. monocytogenes* (8 hpi), MOI of 1. Supernatants were used to stimulate a luciferase-expressing cell line and relative light units (RLU) were measured and compared between each sample and uninfected cells. n = 7 and 7 biological replicates. **c**, Time course of LDH release (blue) and IFN-I abundance as measured by RLU production (pink), in WT BMDMs infected with *R. parkeri*, MOI of 1. n = 3 independent experiments. **d**, Time course of LDH release (blue) and IFN-I abundance (pink), in *Casp1/11-/-* BMDMs infected with *R. parkeri*, MOI of 1. n = 3 independent experiments. **e**, Images of BMDMs infected with *R. parkeri*, MOI of 1, at 72 hpi. Scale bar = 100 μm. Experiments were repeated 3 times with similar results. **f**, Measurement of *R. parkeri* abundance in BMDMs, MOI of 1. Antibody was added at t = 0. The indicated statistical differences (*) are between WT and WT + supernatant. No statistical differences were observed between the samples treated with supernatant. **g**, Measurement of *R. parkeri* abundance in BMDMs, MOI of 1. Antibodies were added at t = 0. n = 3 independent experiments. **h**, Host cell death during *R. parkeri* infection of BMDMs. LDH release was measured at 24 hpi upon *R. parkeri* infection of the indicated BMDMs, MOI of 1. n = 6, 4, 4, and 4 biological replicates. Statistical comparisons in panel h were made between each sample and WT. Statistical analyses in panels **a**, **b**, **f**, and **g** used a two-tailed Student’s T-test. Statistical analyses in panel **h** used a one-way ANOVA with multiple comparisons and Tukey post-hoc test. For all panels: data are expressed as means and error bars represent the SD; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant.
**Extended Data Fig. 2 | IRF5-regulated genes, including Gbp2 and Nos2, are antirickettsial ISGs.**

**a,** *R. parkeri* abundance in BMDMs. “supe” indicates 200 μl conditioned supernatant from infected Casp1/11−/− BMDMs. n = 3 independent experiments. **b,** qPCR of ISGs, normalized to actin. WT and mutant BMDMs were infected with *R. parkeri* and treated with IFN-β and RNA was analyzed at 12 hpi. Data are fold upregulation as compared to infected cells not treated with IFN-β. n = 3 independent experiments. For statistics, values were compared to the WT value for each primer set. **c,** *R. parkeri* abundance in BMDMs. “supe” indicates 200 μl conditioned supernatant from infected Casp1/11−/− BMDMs. n = 3 independent experiments. Statistical differences (*) are shown between WT and WT + supernatant. No statistical differences (ns) were observed between WT + supernatant and mutant cells + supernatant. **d,** *R. parkeri* abundance in BMDMs. “supe” indicates 200 μl conditioned supernatant from infected Casp1/11−/− BMDMs. The L-NIL final concentration was 1 mM, added at t = 0. n = 3 independent experiments. **e,** Quantification of GBP2 colocalization with *R. parkeri* using immunofluorescence microscopy, in BMDMs, MOI of 1. Each data point is an independent experiment and includes quantification from more than 5 images totaling at least 150 bacteria. n = 3 independent experiments. Lines connect means for each time point. **f,** Quantification of GBP2 colocalization with *R. parkeri* using immunofluorescence microscopy, in BMDMs, MOI of 1 at 3 hpi. Each data point is an independent experiment and includes quantification from more than 5 images totaling at least 150 bacteria. n = 7, 7, 3, and 3 independent experiments. For experiments with exogenous IFN-I, 100 U of rIFN-β was added overnight prior to infection. Statistical analyses in panels a, b, c, d, and e used a two-tailed Student’s T-test; statistical analyses in panel f used a one-way ANOVA with multiple comparisons and Tukey post-hoc test; For all panels, data are expressed as means and error bars represent the SD. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant.
Extended Data Fig. 3 | IFN-I and IFN-γ play overlapping roles in protecting against *R. parkeri* in vivo. **a**, *R. parkeri* abundance in mouse organs, infected i.v. with $10^7$ bacteria, at 72 hpi. Bars denote medians. n = 4 (control) and 5 (α-IFN-γ) individual mice, for each organ. Data are the combination of two independent experiments. Each individual data point represents an individual mouse. Statistics used a two-tailed Mann Whitney U test. *p < 0.05.

**b**, Mouse weight after i.v. infection with $10^7$ *R. parkeri*. Data are normalized to the weight at t = 0. Each line represents an individual mouse. n = 5 (Ifnar−/−), 7 (Ifngr−/−), and 7 (Ifnar−/−Ifngr−/−).

**c**, Mouse body temperature after i.v. infection with $10^7$ *R. parkeri*. Each line represents an individual mouse. n = 5 (Ifnar−/−), 7 (Ifngr−/−), and 7 (Ifnar−/−Ifngr−/−).

**d**, Survival of AG129 genotype mice (lacking IFN-I and IFN-γ receptors) after i.v. infection. n = 5 ($10^5$), 7 ($10^6$), and 5 ($10^7$). Data for each group are the combination of 2 independent experiments.
Extended Data Fig. 4 | Tissue necrosis, leukocyte infiltration, and vascular damage is increased in spleens and livers of infected Ifnar−/−Ifngr−/− mice.

Organs were harvested from mice intravenously infected with 10⁷ R. parkeri at 72 hpi. Samples were fixed, sliced, and stained with hematoxylin and eosin (H&E) and commercially analyzed by a pathologist for inflammation and vascular damage. Inflammation observed was infiltration of mononuclear cells including macrophages, plasma cells, and lymphocytes in both organs, and also granulocytes in the liver. Vascular changes include fibrinoid vascular wall degeneration, hypertrophy of the endothelium, perivascular fibrinous material, and fibrin thrombi in medium caliber vessels. Double-headed arrows indicate aberrations at the vasculature and single-headed arrows indicate regions of necrosis and/or regions of mononuclear infiltrates. Scale bars in the liver are 100 μm (20x), 500 μm (4x) and 1 mm (2x); scale bars in the spleen are 100 μm (20x), 200 μm (10x), and 500 μm (4x); asterisks indicate defined splenic follicles in uninfected mice, which are lost in infected Ifnar−/−Ifngr−/− mice; results were similar in 3 independent experiments.
Extended Data Fig. 5 | NK and CD8+ T cells do not play a critical role in protecting against intravenous *R. parkeri* infection in mice. **a**, *R. parkeri* abundance in mouse organs, infected i.v. with the indicated amounts of bacteria, at 72 hpi. Bars denote medians. From left to right, *n* = 4, 5, 5, and 5 individual mice, for each organ. Statistics used a two-tailed Mann Whitney *U* test, where each condition was compared to the WT+IgG control for each organ. *p* < 0.05, **p** < 0.01, and ns = not significant. **b**, Survival of mice after i.v. infection. *n* = 4 (Rag2−/−) and 5 (Ifnar−/−Ifngr−/−) individual mice. Data for each group are combined from 2 independent experiments.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Metamorph version 7.8.2.0 was used to collect immunofluorescence data. Micro-manager version 1.4.20 software was used to collect brightfield images.

Data analysis

PRISM version 6 and version 7 software (GraphPad Software, La Jolla, CA) was used for data analysis, including statistics. FIJI (ImageJ) version 2.0.0-rc-59/1.51t was used to analyze immunofluorescence images. Adobe Illustrator version 23.0.1 was used for creating figures. Microsoft excel version 16.31 was used for data analysis.

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The RNAseq data sets generated and analyzed during the current study are available in the GEO repository, accession number GSE128211. R. parkeri strains were authenticated by whole genome sequencing and are available in the NCBI Trace and Short-Read Archive; Sequence Read Archive (SRA), accession number SRX4401164.
Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical methods were used to predetermine sample size for in vitro experiments. Sample sizes were determined based on the number of samples that could be processed in the given time frame. The sample sizes are sufficient because each data set was compiled from at least 3 pieces of data and statistical differences were determined across the data. For animal studies, a statistical analysis was not performed to predetermine sample size prior to initial experiments. Sample sizes were chosen based on the number of organ samples that could be processed in a given time, which was approximately 15 total mice maximum for a given experiment. After the first experiment, a Power Analysis was conducted to determine subsequent group sizes. The sample sizes are sufficient because each data set was compiled from at least 3 separate experiments and statistically significant differences were determined across various samples.

Data exclusions
There were no data exclusion in this study.

Replication
To verify reproducibility, various experiments were performed by different scientists. Many experiments were performed months apart, using macrophages from different mice, different media and FBS, and cells derived by different labs. Many experiments were confirmed with two separate assays, for example the RNA seq data was confirmed with qPCR, and spleen/liver PFUs correlated with animal health. Preparations of Rickettsia were used that were generated by different researchers at different times, which gave reproducible results.

Randomization
Randomization was used for mouse infections in which Ifnar-/-Ifngr-/- mice were infected with different doses of Rickettsia parkeri. Mice were chosen randomly within each cage for different doses of Rickettsia. For in vitro studies, randomization was not necessary, as the results were not qualitative.

Blinding
Blinding was used for histology data. Blinding was not necessary for other experiments, as the results were generated by a digital reading or by quantitative measurement.

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
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| Antibodies                       | n/a     |
| Eukaryotic cell lines            | n/a     |
| Palaeontology                    | n/a     |
| Animals and other organisms      | n/a     |
| Human research participants      | n/a     |
| Clinical data                    | n/a     |
| Anti-GBP2 was from ProteinTech, rabbit polyclonal, Catalog 11854-1-AP, 1:500-1:1000 dilution. 1:500 dilution. |
| The anti-Rickettsia antibody 14-13 was a gift from Ted Hackstadt. 1:1000 dilution. |
| Mouse ultra-LEAF-purified α-IFNAR-1 antibody was from BioLegend, catalog 127323. Clone MAR1-5A3. Lot B207729. Added to a final concentration of 1 μg/ml. |
| Mouse anti-IFN-gamma antibody was from BioLegend, catalog 505847. Clone XMGI.2 Lot B292418. Mice were injected i.v. with 300 μl at 30 min p.i., 200 μl at 24 hpi, and 200 μl at 48 hpi, totaling 800 μl (0.8 μg antibody). |
| Mouse anti-IL-18 was from MBL, catalog D048-3. Clone 93-10C. Lot 064. Added to a final concentration of 5 μg/ml. |
| Mouse Ultra-LEAF anti-IL-1beta was from BioLegend, catalog 503514. Clone 8122. Lot B286042. Added to a final concentration of 1 μg/ml. |
| Mouse control IgG was from Jackson, catalog O12-000-003. Lot 142115. Control IgG was added to a final concentration of 10 μg/ml. RRID: AB_2337136 |
| Mouse anti-CD8b.2 was from Leinco, catalog C2832. Clone Clone S3-S.8. Mice were injected IP with 160 μg of antibody on days -2 and -1 prior to infection (320 μg total per mouse). |

RRID: AB_2337136
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  
Vero cells were obtained from the UC Berkeley Tissue Culture Facility. 3T3 cells expressing the ISRE luciferase were obtained from Dan Portnoy (UC Berkeley).

Authentication  
The identity of Vero cells was verified through mass spectrometry experiments. Luciferase-expressing 3T3 reporter cells were not authenticated.

Mycoplasma contamination  
All cell lines were confirmed to be mycoplasma-negative by DAPI staining and fluorescence microscopy screening at the UC Berkeley Cell Culture Facility or in the Welch lab.

Commonly misidentified lines  
No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals  
WT, Casp1-/-, Casp11-/-, Casp1/-/Casp11-/-, Ifnar-/-, Ifigr-/-, Gsdmd-/-, Aim2-/-, Asc-/-, Gbp-chr3-/-, Sting-gt/gt, Cgas-/-, Irf1-/-, Irf3-/-, Irf5-/-, Ifit1-/-, Ifit2-/-, Road2-/-, Ljp-/-, Ifnar/-/Ifngr, Casp11/-/Ifnar-/-, Casp11/-/Cgas-/-, Casp11/-/I3r4 im. Tnfrsf1a-/-/Tnfrsf1b-/-, Casp11/-/Rag2-/-, and Sting-gt/gt mutant mice or femurs from these mice were used in this study and were in the C57BL/6 background. AG129 mice were used in this study, which are in the 129 background. All mice were between 8 and 18 weeks of age. Both male and female mice were used in this study based on their availability and data from these mice is shown for each sex in Extended Data Figure 6. Mice were housed and euthanized according to standard protocols approved by UC Berkeley Animal Care and Use Committee.

Mouse anti-NK1.1 clone PK136 was a gift from Dr. David Raulet. 200 μg PK136 was delivered IP to each animal.

Anti-GBP2 is described with the Research Resource Identifier AB_2109336. The manufacturer describes its validation for Western blot analysis and Immunohistochemistry. It was validated. It is described in: Man SM, et al. The transcription factor IRF1 and guanylate-binding proteins target activation of the AIM2 inflammasome by Francisella infection. Nat Immunol. 2015;16(5):467–475. doi:10.1038/ni.3118.

The anti-Rickettsia 14-13 antibody was originally described in: Anacker, R. L., Mann, R. E. & Gonzales, C. Reactivity of monoclonal antibodies to Rickettsia rickettsiae with spotted fever and typhus group rickettsiae. J. Clin. Microbiol. 25, 167–171 (1987). It was also described in Lamason RL et al. Rickettsia Sc4 reduces vinculin-mediated intercellular tension to promote spread. Cell. 2016;167(3):670–683. doi:10.1016/j.cell.2016.09.023.

The anti-IFNAR antibody is described by the manufacturer to be quality control tested using immunofluorescent staining with flow cytometry. The manufacturer provides numerous references for its validation, including Swanson CL, et al. Type I IFN enhances follicular B cell contribution to the T cell-independent antibody response. Journal of Experimental Medicine. 2010;207(7):1485. doi:10.1084/jem.20092969.

The anti-IFN-gamma antibody is described by the manufacturer to be quality control tested by ELISA. The manufacturer provides numerous references for its validation, including Ko SY, et al. α-Galactosylceramide Can Act As A Nasal Vaccine Adjuvant Inducing Protective Immune Responses against Viral Infection and Tumor. Journal of Immunology. 2005;175(5):3309-3317. doi:10.4049/jimmunol.175.5.3309.

The anti-IL-1beta antibody (from Biolegend, catalog 503514) has been described for in vitro neutralization (Hogquist K, et al. 1996), 3) Ushio, S., et al., J. Immunol. 156, 4274-4279 (1996), and 4) Okamura, H., et al., Nature 378, 88-91 (1995).

Mouse anti-CD8b.2 (from Leinco, catalog C2832) is described for depletion studies in mice in Baddack-Werncke et al., Journal of Immunology. 2001;Chapter 4:Unit 4.1.

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The anti-IL-1beta antibody (from Biolegend, catalog 503514) has been described for in vitro neutralization (Hogquist K, et al. 1996), 3) Ushio, S., et al., J. Immunol. 156, 4274-4279 (1996), and 4) Okamura, H., et al., Nature 378, 88-91 (1995).

Mouse anti-CD8b.2 (from Leinco, catalog C2832) is described for depletion studies in mice in Baddack-Werncke et al., Journal of Immunology. 2001;Chapter 4:Unit 4.1.

Mouse anti-CD8b.2 (from Leinco, catalog C2832) is described for depletion studies in mice in Baddack-Werncke et al., Journal of Immunology. 2001;Chapter 4:Unit 4.1.

The anti-IFN-gamma antibody is described by the manufacturer to be quality control tested by ELISA. The manufacturer provides numerous references for its validation, including Ko SY, et al. α-Galactosylceramide Can Act As A Nasal Vaccine Adjuvant Inducing Protective Immune Responses against Viral Infection and Tumor. Journal of Immunology. 2005;175(5):3309-3317. doi:10.4049/jimmunol.175.5.3309.

The anti-IL-1beta antibody (from Biolegend, catalog 503514) has been described for in vitro neutralization (Hogquist K, et al. 1996), 3) Ushio, S., et al., J. Immunol. 156, 4274-4279 (1996), and 4) Okamura, H., et al., Nature 378, 88-91 (1995).

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Mouse anti-CD8b.2 (from Leinco, catalog C2832) is described for depletion studies in mice in Baddack-Werncke et al., Journal of Immunology. 2001;Chapter 4:Unit 4.1.
| Wild animals | No wild animals were used in this study |
| Field-collected samples | No field-collected samples were used in this study. |
| Ethics oversight | Animal research using mice was conducted under a protocol approved by the UC Berkeley Institutional Animal Care and Use Committee (IACUC) in compliance with the Animal Welfare Act and other federal statutes relating to animals and experiments using animals. The UC Berkeley IACUC is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and adheres to the principles of the Guide for the Care and use of Laboratory Animals. Infections were performed in a biosafety level 2 facility. All animals were maintained at the UC Berkeley campus and all infections were performed in accordance with the approved Welch lab Animal Use Protocol. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

(R): AACAAAACAAAAACCCAG; Ifnar-/- R: ATCTGGACGAAGACGATCGG; WT Casp1/11 F: CATGCCTGAATAATGATCACC; WT Casp1/11 R: GAAAGATGTTACAGAAGGCC; Csg1/11 F: GGGCCTCCCTTACCCCG; Csg1/11 R: CTGTGGTGACTAACCGATAA; Cgs F: AACTGGAATCCACGCTTTTCAC; Cgs R: TGCGGTCAAGGAAATCAGC; WT tlr4 F: CACCTGATACTTAATGGCTGT; WT tlr4 R: GTTTAGGCCCAGAGTTTTTCTCTCAA; tlr4-/- F: TGGTGCCTACGTACAGACGCTG; tlr4-/- R: TGGTGGTGTTTCTCCAGTCCAGTCCAG; Sting F: GATCCGAATGTTCAATCAGC; Sting R: CGATTCTTGATGCCAGCAC; Gsdmd F: ATAGAACCCGTGAGTCCCA; and Gsdmd R: GGTCTCCTCATTCCAGTCT. Macrophages from Gbp-chr3 cells were validated using immunofluorescence microscopy with a GBP2-specific antibody. Irf1-/-, Irf5-/-, and Irf3/7-/- cells were validated by us using RNA-seq.