Type I interferon-driven susceptibility to *Mycobacterium tuberculosis* is mediated by IL-1Ra

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The bacterium *Mycobacterium tuberculosis* (Mtbb) causes tuberculosis and is responsible for more human mortality than any other single pathogen. Progression to active disease occurs in only a fraction of infected individuals and is predicted by an elevated type I interferon (IFN) response. Whether or how IFNs mediate susceptibility to Mtbb has been difficult to study due to a lack of suitable mouse models. Here, we examined B6.Sst1S congenic mice that carry the ‘susceptible’ allele of the Sst1 locus that results in exacerbated Mtbb disease. We found that enhanced production of type I IFNs was responsible for the susceptibility of B6. Sst1S mice to Mtbb. Type I IFNs affect the expression of hundreds of genes, several of which have previously been implicated in susceptibility to bacterial infections. Nevertheless, we found that heterozygous deficiency in just a single IFN target gene, Il1rn, which encodes interleukin-1 receptor antagonist (IL-1Ra), is sufficient to reverse IFN-driven susceptibility to Mtbb in B6. Sst1S mice. In addition, antibody-mediated neutralization of IL-1Ra provided therapeutic benefit to Mtbb-infected B6. Sst1S mice. Our results illustrate the value of the B6. Sst1S mouse to model IFN-driven susceptibility to Mtbb, and demonstrate that IL-1Ra is an important mediator of type I IFN-driven susceptibility to Mtbb infections in vivo.

Mtbb infections in humans result in highly diverse outcomes ranging from asymptomatic lung granulomas to lethal disseminated disease. Active tuberculosis (TB) is characterized by the uncontrolled replication of bacteria and pathological inflammation in the lungs and other organs. There is no vaccine that reliably protects against pulmonary TB, and although antibiotics can be curative, the long (>6-month) course of treatment and increasing prevalence of multi-drug-resistant Mtbb strains have spurred a search for alternative therapeutics. Recent studies have demonstrated that an enhanced type I IFN signature correlates with and can predict progression to active TB up to 18 months before diagnosis. In addition, a partial loss-of-function polymorphism in the type I IFN receptor (IFNAR1) is associated with resistance to TB in humans. A small number of cases also link IFN treatment during chronic viral infections with increased susceptibility to TB. In addition, numerous animal studies have demonstrated causal roles for type I IFNs in susceptibility to Mtbb and other bacterial infections.

Given the potential association between type I IFNs and susceptibility to human TB, we sought to determine the mechanisms by which type I IFNs mediate susceptibility to active TB, with the hope that this knowledge could be exploited to develop interventions. Mechanistic studies and initial trials of possible therapeutics require a robust animal model. However, the most commonly used animal model, the C57BL/6 (B6) mouse, does not robustly recapitulate the IFN-driven TB susceptibility that appears to occur in humans. B6.Ifnar−/− mice show mild resistance to Mtbb in the spleen and variable but modest effects in the lungs. Another model of IFN-driven susceptibility involves treatment of Mtbb-infected B6 mice with poly-IC, a potent inducer of type I IFNs. Such treatment dramatically increases susceptibility to Mtbb in an Ifnar-dependent manner, but because the IFN is induced artificially via signalling pathways such as TLR3 or MDA5 that may not be engaged by Mtbb itself, it is unclear whether poly-IC treatment mimics the course of IFN-driven disease in humans. The 129 mouse strain shows clear IFN-driven susceptibility to Mtbb, but there are limited tools on this genetic background.

As an alternative approach, we turned to a previously described congenic mouse strain, B6.Sst1S, that carries the 10.7 Mb ‘super susceptible’ region of mouse chromosome 1 from C3HeB/FeJ mice on an otherwise B6 genetic background. B6. Sst1S mice exhibit marked susceptibility to aerosol Mtbb infection, although how the Sst1 locus confers susceptibility remains incompletely understood. Recent work has established that bone marrow macrophages (BMMs) from B6. Sst1S mice exhibit an enhanced type I IFN response, as we confirm (Extended Data Fig. 1). In addition, we found that infected B6. Sst1S lungs exhibited higher levels of Ifnb transcripts as compared to B6 (Fig. 1a). To investigate whether this enhanced type I IFN signalling causes the susceptibility of B6. Sst1S mice to Mtbb, we treated Mtbb-infected mice with an IFNAR1-blocking antibody to inhibit type I IFN signalling. B6. Sst1S mice treated with the IFNAR1-blocking antibody showed significantly decreased bacterial burdens compared to those that received only an isotype control antibody (Fig. 1b). To provide genetic confirmation of this result, we crossed B6. Sst1S mice to B6. Ifnar−/− mice. Ifnar deficiency largely reversed the enhanced susceptibility of B6. Sst1S mice to Mtbb infection. At 25 days post-infection, the lung bacterial burdens of B6. Sst1S Ifnar−/− mice were significantly lower than those of B6. Sst1S mice, and were similar to those of B6 mice (Fig. 1c). Infected B6. Sst1S Ifnar−/− mice also survived significantly longer than B6. Sst1S mice (Fig. 1d), although there are also clearly Ifnar-independent effects of the Sst1 locus that act at later time points. By contrast, and consistent with previous
Type I IFN drives enhanced susceptibility of B6.Sst1S mice. a, Expression of Ifnb in Mtb-infected lungs at day 25, measured by quantitative RT-PCR, normalized to Rps17. b, Lung bacterial burdens at day 25 from Mtb-infected mice treated with anti-IFNAR1 or isotype control antibody. c, d, Lung bacterial burdens at day 25 (c), and survival (d), of Mtb-infected mice (combined results from three independent infections). Sample size: B6 = 6, B6.Sst1S = 8, B6.Sst1SIfnar−/− = 4 (a); B6 = 6, isotype = 7, anti-IFNAR1 = 7 (b); B6 = 18, Ifnar−/− = 6, B6.Sst1S = 19, B6.Sst1SIfnar−/− = 16 (c); B6 = 18, B6.Sst1S = 21, B6.Sst1SIfnar−/− = 16 (d). All except B6 mice were bred in-house. The centre and error bars show the mean and s.e.m. The data were analysed with a two-sided Mann–Whitney test (a–c) or a two-sided log-rank test (d). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

The Type I IFN negatively regulates anti-bacterial immune responses via multiple mechanisms6,7,18, including through increased IL-10 levels15–17, decreased IFNγ signalling25,39, induction of cholesterol 25-hydroxylase (Ch25h)10 and/or decreased IL-1 levels15,16,41. We did not observe significant differences between B6 or B6.Sst1S mice in IL-10 or IFNγ levels in the lung during Mtb infection (Extended Data Fig. 3a,b). Crossing B6.Sst1S mice to B6.Ch25h−/− mice did not alter day-25 lung bacterial burdens (Extended Data Fig. 3c). Moreover, despite clear evidence that type I IFN and IL-1 counter-regulate each other15,16,41, the Sst1S locus did not appear to decrease the levels of IL-1 in vivo; in fact, we unexpectedly observed higher levels of both IL-1α and IL-1β in the lungs of B6.Sst1S mice at 25 days post-infection as compared to B6 mice (Fig. 2a,b, associated colony-forming units (CFUs) in Extended Data Fig. 3d). Other inflammatory mediators, including TNF and CXCL1, were similarly elevated in the B6.Sst1S mice (Extended Data Fig. 3e,f) as was the frequency of CD11b+ Ly6G+ cells (neutrophils) in the lungs (Extended Data Fig. 3g). The elevated inflammation in B6.Sst1S mice was a direct or indirect consequence of elevated type I IFNs, as inflammatory cytokines and neutrophils were reduced in B6.Sst1SIfnar−/− mice (Fig. 2a,b and Extended Data Fig. 3a,b,e–g).

We reasoned that the high levels of IL-1α/β in B6.Sst1S mice may be a consequence of the higher bacterial burdens in these mice, or alternatively, may be causing increased bacterial replication via induction of a pro-bacterial inflammatory milieu, as previously proposed14,44. To distinguish these possibilities, we inhibited IL-1 signalling in vivo using an anti-IL-1R1 blocking antibody45 (Fig. 2c and Extended Data Fig. 4a). Both B6 and B6.Sst1S mice treated with IL-1R1 blocking antibody exhibited increased...
bacterial burdens compared to mice treated with an isotype control antibody. These results confirm previous evidence that IL-1 is protective in B6 mice, and extend this observation to B6.Sst1S mice as well. Thus, elevated IL-1 levels do not explain the exacerbated infections of B6. Sst1S mice. Instead, it appears that elevated IL-1 levels are a consequence of increased bacterial burdens in these mice. Although IL-1 is clearly protective during Mtb infection and by type I IFN signalling, as expected, B6.Sst1S mice exhibited elevated, rather than reduced, IL-1 activity (for example, 3 of 17 mice shown in Fig. 3d). In addition, in infections with higher initial doses, we failed to detect a difference in the IL-1 activity in cell-free supernatant of dissociated lungs. Despite higher levels of IL-1 proteins, lungs from infected B6.Sst1S mice appeared to have generally less functional IL-1 signalling capacity as compared with B6 mice (Fig. 3d and Extended Data Fig. 4c). The lower levels of IL-1 signalling seen in B6.Sst1S mice were reversed in B6.Sst1SIfnar−/− mice (Fig. 3d). The reporter appeared to be a reliable indicator of functional IL-1, as responses in the bioassay were blocked by anti-IL-1R1 antibody (Extended Data Fig. 4c). These results underline the importance of measuring IL-1 activity versus merely assessing the levels of IL-1 proteins. However, we noted significant variability in the measurable IL-1 activity in the lungs of Mtb-infected mice. In fact, the lungs of some B6.Sst1S mice exhibited elevated, rather than reduced, IL-1 activity (for example, 3 of 17 mice shown in Fig. 3d). In addition, in infections with higher initial doses, we failed to detect a difference in the IL-1 activity in the lungs of B6.Sst1S versus B6 mice (an example is shown in Extended Data Fig. 4d). Conversely, the susceptibility of B6.Sst1S mice is highly consistent (Figs. 1c and 2c and Extended Data Figs. 2a, 3c,d and 4a). Thus, we hypothesized that low IL-1 signalling may be only a transient phenotype in B6.Sst1S mice.

To assess the levels of functional IL-1 signalling in the lungs of infected mice, we employed an IL-1 reporter bioassay to measure IL-1 activity in cell-free supernatant of dissociated lungs. Despite higher levels of IL-1 proteins, lungs from infected B6.Sst1S mice appeared to have generally less functional IL-1 signalling capacity as compared with B6 mice (Fig. 3d and Extended Data Fig. 4c). The lower levels of IL-1 signalling seen in B6.Sst1S mice were reversed in B6.Sst1SIfnar−/− mice (Fig. 3d). The reporter appeared to be a reliable indicator of functional IL-1, as responses in the bioassay were blocked by anti-IL-1R1 antibody (Extended Data Fig. 4c). These results underline the importance of measuring IL-1 activity versus merely assessing the levels of IL-1 proteins. However, we noted significant variability in the measurable IL-1 activity in the lungs of Mtb-infected mice. In fact, the lungs of some B6.Sst1S mice exhibited elevated, rather than reduced, IL-1 activity (for example, 3 of 17 mice shown in Fig. 3d). In addition, in infections with higher initial doses, we failed to detect a difference in the IL-1 activity in the lungs of B6.Sst1S versus B6 mice (an example is shown in Extended Data Fig. 4d). Conversely, the susceptibility of B6.Sst1S mice is highly consistent (Figs. 1c and 2c and Extended Data Figs. 2a, 3c,d and 4a). Thus, we hypothesized that low IL-1 signalling may be only a transient phenotype in B6.Sst1S mice.

Fig. 2 | IFNAR signalling results in high but non-pathological IL-1 protein levels in B6.Sst1S mice. a,b Protein levels of IL-1α (a) and IL-1β (b) were measured in the lungs of Mtb-infected mice at day 25. The combined results of four independent experiments are shown. n = 41, B6.Sst1S = 46, B6.Sst1SIfnar−/− = 33. c, Lung bacterial burdens at day 25 from Mtb-infected mice treated with anti-IL-1R1 or isotype control antibody. n (from left as shown) = 5, 5, 5, 5, 5, 6. All animals except B6 were bred in-house. The centre and error bars show the mean and s.e.m. The data were analysed with a two-sided Mann–Whitney test. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.
To address this possibility, we assessed the timing of IL-1 activity in the lung by performing a time course experiment (Extended Data Fig. 4e). We found that at 14 days post-infection, IL-1 activity measurable by bioassay was low in all strains. At 25 days post-infection, there was marked variability in the measurable IL-1 responses of B6.Sst1S mice: some mice exhibited the expected defect in IL-1 activity as compared to B6 or B6.Sst1S mice, whereas others were already exhibiting high IL-1 activity levels. By 36 days post-infection, all mice exhibited high IL-1 activity regardless of genotype. Thus, it appears that once the susceptible phenotype of B6.Sst1S mice manifests, and CFU levels rise, the level of functional IL-1 increases concordantly.

Interestingly, this late rise in IL-1 activity appears insufficient to protect the mice. It was previously reported that IL-1 acts at early time points after infection (that is, prior to day 15 post-infection). At early time points, when IL-1 may be acting locally at infection foci, the bioassay (performed on total lung) is unable to detect functional IL-1. Thus, we conclude that although specific infection conditions and assay time points can reveal a functional deficit in IL-1 signalling in B6.Sst1S mice, our IL-1 bioassay is generally an insensitive tool for measuring protective IL-1 activity in vivo.

Given the above considerations, we sought genetic evidence to address whether excessive type I IFN signalling neutralizes IL-1 signalling via IL-1Ra in B6.Sst1S mice during Mtb infection. Accordingly, we crossed B6.Sst1S to Il1rn−/− mice. Since uninfected Il1rn−/− mice exhibit signs of inflammatory disease due to dysregulated IL-1 signalling, and because Il1rn−/− mice have a partial decrease in IL-1Ra levels, we generated both heterozygous B6.Sst1SIl1rn−/+ and homozygous B6.Sst1SIl1rn−/− mice. Both heterozygous and homozygous Il1rn deficiency protected B6.Sst1S mice from Mtb (Fig. 4a–d and Extended Data Fig. 5a). In fact, bacterial burdens in B6.Sst1SIl1rn−/− mice were even lower than those found in 'resistant' B6 mice (Fig. 4a). B6.Sst1SIl1rn−/− mice survived only modestly longer than B6.Sst1S mice (Fig. 4b), but this was expected since even uninfected Il1rn−/− mice exhibit shortened lifespans due to spontaneous inflammatory disease. Notably, a partial reduction in IL-1Ra levels in heterozygous B6.Sst1SIl1rn−/+ mice was sufficient to almost entirely reverse the enhanced IFN-driven susceptibility of Sst1S mice (Fig. 4a–d). In fact, the bacterial burdens, survival and body weights of B6.Sst1SIl1rn−/− mice resembled those of B6 mice (Fig. 4a–d and Extended Data Fig. 5a). Histological samples of infected lungs showed significant reduction in lesion sizes in both B6.Sst1SIl1rn−/− and B6.Sst1SIl1rn−/− mice compared to B6.Sst1S mice (Fig. 4d). Lungs of B6.Sst1SIl1rn−/− mice infected with a low dose of Mtb (~14 CFUs) had greater IL-1 bioactivity at day 21 than those of B6.Sst1S mice (Extended Data Fig. 5b), but a difference in IL-1 activity was not detectable in mice infected with a higher dose (~55 CFUs) (Extended Data Fig. 5c), again illustrating the limitations of the IL-1 bioassay as discussed above. Nevertheless, control of the infection in Il1rn−/− deficient mice depended on IL-1 receptor signalling, as B6.Sst1SIl1rn−/− or B6.Sst1SIl1rn−/+ mice treated with a blocking anti-IL-1R1 antibody exhibited higher bacterial burdens than untreated mice (Extended Data Fig. 5e). These data and the known biology of IL-1Ra strongly imply that protection afforded by the loss of IL-1Ra is mediated by enhanced IL-1 signalling, although it is formally possible that Il1rn deficiency protects the B6.Sst1S mice via a mechanism unrelated to IL-1 and that neutralization of IL-1 signalling dominantly overcomes this protection.
Despite decreased bacterial burdens, the lungs of B6.Sst1SIl1rn+/– mice had similar levels of Ifnb or other IFN-induced transcripts to those of B6.Sst1S mice (Extended Data Fig. 5d). These results are consistent with a model in which IL-1Ra acts downstream of type I IFN signalling, and does not contribute to reducing Ifnb levels other than by reducing overall bacteria burden.
The dramatic protective effects of even partial reductions in IL-1Ra in B6.Sst1 mice suggested that IL-1Ra might be a suitable target for host-directed therapy during Mtb infection. To test this, we treated infected B6.Sst1 mice with an anti-IL-1Ra antibody to block IL-1Ra and restore IL-1 signalling. B6.Sst1 mice that received the antibody had significantly lower bacterial burdens in their lungs as compared to PBS-treated controls (Fig. 4e). In addition, mice treated with anti-IL-1Ra antibody retained significantly more body weight than the controls (Fig. 4f) and exhibited reduced lung lesions (Fig. 4g), suggesting that the treatment did not cause detrimental inflammation. To determine whether IL-1Ra acts downstream of type I IFN signalling, we neutralized IL-1Ra in B6.Sst1 mice (Extended Data Fig. 5f). Bacterial burdens were similar between B6.Sst1Ifnar−/− mice treated with the anti-IL-1Ra antibody and control, consistent with the low expression of IL-1Ra in B6.Sst1Ifnar−/− mice (Fig. 3c), whereas antibody-treated B6.Sst1 mice exhibited reduced CFUs. Overall, these data indicate that genetic or antibody-mediated reduction of IL-1Ra can rescue the type I IFN-driven susceptibility to Mtb in B6.Sst1 mice without overt detrimental immunopathology.

The Sst1 locus spans ~10 Mb and encodes ~50 genes. Collectively, our data indicate that in B6.Sst1 mice, the Sst1 locus acts directly or indirectly to repress type I IFN signalling, although the underlying molecular mechanism is unknown. Previously, Sst1 mice were found to lack expression of Ipr1 (also called Sp110) and re-expression of B6-derived Ipr1 partially restores resistance to Mtb12. Thus, loss of Ipr1 may account for the Sst1 phenotype, although this remains to be confirmed by the generation of B6.Ipr1−/− mice. Polymorphisms in human SP110 are associated with TB disease in some but not all cohorts12,13. Ipr1 is a member of the Sp100 family of transcriptional regulators that also includes Sp100, Sp140 and Aire. Sp100 proteins contain chromatin-binding domains and regulate gene expression. It is tempting to speculate that IPR1 modulates the chromatin state of type I IFNs and/or IFN-induced genes, although future studies are necessary to establish the molecular basis of the Sst1 phenotype.

There is increasing interest in developing host-directed therapeutics for Mtb. Although such therapeutics have not yet proved to be curative, and are unlikely to replace antibiotics, they may be advantageous in specific scenarios. For example, host-directed therapy could serve as an adjunct to antibiotics for multi-drug-resistant strains of Mtb, which are associated with high mortality rates14,15. If indeed type I IFNs exacerbate TB disease in humans, then a host-directed therapy targeting IL-1Ra, alone or in combination with other host-directed interventions16, might represent a promising approach. Targeting IL-1Ra may be preferable to blockade of upstream type I IFNs, which might exacerbate viral infections. Regardless, our results show that the B6.Sst1 mouse represents a useful model that permits genetic dissection of the molecular mechanisms underlying IFN-driven TB disease.

Methods
Mice. All mice were specific pathogen-free, maintained under a 12-h light/dark cycle (7:00 to 19:00), and given a standard chow diet (Harlan irradiated laboratory animal diet) ad libitum. Within each experiment, mice of all genotypes were age- and sex-matched at 6–10 weeks old at the beginning of infections. Whenever possible, we used comparable numbers of each sex across all genotypes in an experiment. Mouse work was not subjected to randomization or data blinding. C57BL/6 (B6), B6.129S-Il1rn−/− (Il1rn−/−, Jax no. 004754)16, B6.Cg-Ifnar1−/− (Ifnar−/−, Jax no. 028288) and B6.129S6− (Sst1S−/−, Jax no. 016263) mice were originally generated in our laboratory. C3HeB/FeJ (C3HFeJ, Jax no. 004768) mice were previously described17. All animals used in experiments were bred in-house at the University of California Berkeley Institutional Animal Care and Use Committee.

Mtb infections. Mtb strain Erdman (gift from S.A. Stanley) was used for all infections. Frozen stocks of this wild-type strain were made from a single culture and used for all experiments. Cultures for infection were grown in Middlebrook 7H9 liquid medium supplemented with 10% albumin–dextrose–saline, 0.4% glycerol and 0.05% Tween-80 for 5 days at 37 °C. Mice were aerosol-infected using a nebulizer (Glas-Col). Cultures were grown to deliver ~20 to 100 bacteria per mouse as measured by CFUs in the lungs 1 day following infection, depending on the experiment. Mice were euthanized at various days post-infection as indicated in the figure legends to measure CFUs and/or cytokines. All but one lung lobe was homogenized in PBS plus 0.05% Tween-80 except for cytokines (see below), and serial dilutions were plated on 7H11 plates supplemented with 10% oleic acid, albumin, dextrose, catalase (OADC) and 0.05% glycerol. CFUs were counted 21–25 days after plating. The remaining lobe was used for histology or for RNA extraction. For histology, the sample was fixed in 10% formalin for at least 48 h and then stored in 70% ethanol. Samples were sent to Histowiz Inc. for embedding in wax, sectioning and H&E staining.

Samples for RNA were preserved in RNalater solution (Invitrogen) according to the manufacturer’s specifications for later processing.

Cytokine measurements. Cell-free lung homogenates were generated as previously described18. Briefly, lungs were dissociated through 100 μm Falcon cell strainers in sterile PBS with 1% fetal bovine serum (FBS) and Pierce Protein Inhibitor EDTA-free (Thermo Fisher). An aliquot was removed for measuring CFUs by plating as described above. Cells and debris were then removed by first, a low-speed centrifugation (approximately 300g) and then a high-speed centrifugation (approximately 2,000g), and the resulting cell-free homogenate was washed twice with 0.2 μm cell strainers to remove all Mtb and was resuspended in safety level 3 (BSL3) conditions. All homogenates were aliquoted, flash-frozen in liquid nitrogen and stored at ~80 °C. Each aliquot was thawed a maximum of twice to avoid potential artefacts due to repeated freeze-thaw cycles. All cytokines except IL-10 and IL-1Ra were measured using the Cytometric Bead Assay (BD Biosciences) according to the manufacturer’s protocols. Results were collected using BD Fortessa (BD Biosciences) and analysed using BD LSRFortessa (BD Biosciences) according to the manufacturer’s protocols.

IL-1 bioactivity reporter assay. Mice infected with a low dose of Mtb (see text for discussion) were used to prepare cell-free lung homogenates. Samples were assayed using HEK-Blue IL-1R1 cells (InvivoGen). Cells were authenticated and tested for mycoplasma by the manufacturer. All experiments used cells under 6 passages from the original stock. A total of 3.75×106 cells per well were plated in 96-well plates, and allowed to adhere overnight in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U ml−1 streptomycin and 100 μg ml−1 penicillin. Over-confluence decreases the accuracy of the assay. Reporter cells were treated overnight with 100 μl of sample (consisting of 50 μl of cell-free lung homogenates and 50 μl of media), or recombinant mouse IL-1β (R&D systems 401-ML-005) mixed with 100 μl of media (to generate a standard curve). Assays were developed using QUANTI-Blue (InvivoGen) according to the manufacturer’s protocols.

Flow cytometry. Lungs were perfused with 10 ml of cold PBS and dissociated through 40 μm cell strainers. Aliquots were removed for quantifying CFUs. Cells were washed and stained with fixable viability dye (Thermo Fisher 65-0865-14). An aliquot of cells from each sample was removed and mixed with counting beads (Thermo Fisher C36950) for later enumeration. The rest of the cells were incubated with anti-mouse CD16/CD32 monoclonal antibody to block Fc receptors (Thermo Fisher 14-0161-81), and then with antibodies for surface staining. The following antigens were stained for: CD45 (30-F11, Biologend 103107), CD11b (M1/70, Thermo Fisher 48-0112-82), CD11c (N418, Biologend 117335), Ly6G (1A8, BD Biosciences 740554), Ly6C (HK1.4, Thermo Fisher 17-5932-80), CD24 (M1/69, BD Biosciences 564664), MHC II (M5/114.15.2, Biologend 107625), SiglecF (E50-2440, BD Biosciences 562680). Cells were fixed with fixation buffer (BD Biosciences 554714) for at least 1 h at room temperature and stored in PBS with 1% FBS and 2 mM EDTA overnight at 4 °C in the dark. Data were acquired on a BD Fortessa X-20 flow cytometer and analysed with FlowJo v10.

BMMS and TNF treatment. Bone marrow was collected from mouse femurs and tibiae, and cells were differentiated by culture on non-tissue-culture-treated plates in RPMI supplemented with supernatant from ST3-MCSF cells (gift from B. Beutler), 10% FBS, 2 mM glutamine, 100 μM 2′-deoxyribo- and 100 μg ml−1 penicillin in a humidified incubator (37 °C, 5% CO2). BMMS- or TNF-treated cells were collected 6 days after plating and frozen in 95% FBS and 5% dimethylsulfoxide. For in vitro experiments, BMMS were thawed into media as described above for 4 h in a humidified 37 °C incubator. Adherent cells were washed with PBS, counted and replated at 1×10^6–1.5×10^6 cells per well in a TC-treated 6-well plate. Cells were treated with 10 ng ml−1 recombinant mouse TNFα (410-TRNC-010, R&D Systems) diluted in the media as described above.

Quantitative RT-PCR. Total RNA from BMMS was extracted using the RNeasy total RNA kit (Qiagen) according to the manufacturer’s specifications. Total
l lung RNA was extracted by homogenizing the tissue in TRIzol reagent (Life Technologies) and then mixing thoroughly with chloroform, both carried out under BSL3 conditions. Samples were then removed from the BSL3 facility and transferred to fresh tubes under BSL2 conditions. The aqueous phase was separated by centrifugation and RNA was further purified using an RNeasy total RNA kit (Qiagen). Equal amounts of RNA from each sample were treated with DNase (RQ1, Promega) and complementary DNA was made using Superscript III (Invitrogen). cDNA reactions were primed with poly(dT) for the measurement of mRNA transcripts by quantitative PCR using the QuantStudio 3 Real-Time PCR System (Applied Biosystems) with Power Sybr Green PCR Master Mix (Thermo Fisher Scientific) according to the manufacturer's specifications. Transcript levels were normalized to the housekeeping genes Rps17, Actb and Oaz1 unless otherwise specified. The following primers were used in this study: Rps17 sense, 5'-GAGGACAAATCTACCTCACTTG-3'; Rps17 antisense, 5'-ATTTTCCTTGATGCGCCGCT-3'; Actb sense, 5'-GGCAC-3'; Actb antisense, 5'-TGAGTACCTGGAGAATG-3'. All other data were analysed with a two-sided Mann–Whitney test for paired observations. Statistical analysis.

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55. Nolla, A., Valeros and the Cancer Research Laboratory for flow cytometry. We thank R.E.V. and I.K. gave technical support and conceptual advice. R.E.V. and D.X.J. designed the experiments. L.H.Y. assisted with experiments shown in Fig. 4 and Extended Data Fig. 5. K.J.C. assisted with experiments shown in Extended Data Fig. 3. D.X.J. performed all other experiments. R.E.V. and D.X.J. analysed the data. I.K. generated the anti-IL-1Ra antibody. N.M. generated the anti-IL-1Ra antibody. K.H.D. and I.K. gave technical support and conceptual advice. R.E.V. and D.X.J. prepared the manuscript.

Competing interests R.E.V. has a financial relationship with Aduro Biotech and both he and the company may benefit from commercialization of the results of this research. All other authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41564-019-0578-3. Supplementary information is available for this paper at https://doi.org/10.1038/s41564-019-0578-3. Correspondence and requests for materials should be addressed to R.E.V. Reprints and permissions information is available at www.nature.com/reprints. Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations. © The Author(s), under exclusive licence to Springer Nature Limited 2019.
Extended Data Fig. 1 | B6.Sst1ö BMMs overexpress Ifnb and ISGs when stimulated with TNFα. a, b, Expression of Ifnb (a) or selected ISGs (b) in BMMs measured by RT-qPCR. Results normalized to housekeeping genes. Representative data of at least two independent experiments.
Extended Data Fig. 2 | B6.Sst1S*Sting* mice partially rescue the enhanced susceptibility of B6.Sst1P mice to Mt. Mice were infected with Mt and measured for lung bacterial burdens at day 25 (a) or survival (b). a, combined results of 2 experiments. Sample size n (B6, B6.Sst1S, B6.Sst1P*Sting*+) = 11, 11, 12 (a); 11, 11, 11 (b). All animals except 5 of the B6 were bred in-house (a) and all except B6 were bred in-house (b). Center and error bars show mean and SEM. Analyzed with two-ended Mann–Whitney test (a) or two-ended Log-rank test (b). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.
Extended Data Fig. 3 | Enhanced inflammation in B6. Sst1P mice requires type I IFN. a, b, e, f. Protein levels of IL-10 (a), IFNγ (b), TNF (e), and CXCL1 (f) were measured in lungs of Mtb-infected mice at day 25. Combined results of at least three independent infections (a, b, e, f). c, Lung bacterial burden of Mtb-infected mice at day 25 (representative of two independent infections). Input dose: average 100 CFU/mouse. d, CFU corresponding to Fig. 2a and b. Combined results that include those already shown in Figs. 1c, 2c, and Extended Data 3a. Input dose: 10–89 CFU per mouse. g, Neutrophils (CD11b+Ly6G+) from lungs of Mtb-infected mice were enumerated on day 14 and day 25. Combined results of two independent infections. All animals except B6 were bred in-house (a–f); all animals were bred in-house (g). Sample size n (B6, B6.Sst1P, B6.Sst1P Ifnar−/−) = 16, 18, 16 (a); 40, 45, 32 (b); 40, 44, 32 (d); 25, 29, 16 (e); 28, 30, 26 (f); 6 for all genotypes (c); 9 for all genotypes (g). Center and error bars show mean and SEM. Analyzed with two-ended Mann-Whitney test (a–g). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.
Extended Data Fig. 4 | IL-1 blockade increases susceptibility in both B6 and B6.Sst1S mice. a–c, mice were infected with average 15 CFU/mouse and were treated with anti-IL1R1 or isotype control antibodies, and on day 25 the lungs were measured for bacterial burden (a), IL-1Ra protein levels (b), and IL-1 bioactivity (c). Sample size n (in order shown, from left) = 7, 7, 8 (a); 7, 8, 9, 8 (b–c). d, Mice were infected with average 78 CFU/mouse and IL-1 bioactivity was measured in the lungs at day 25. n = 5 for both samples. e, Mice were infected with average 33 CFU/mouse. IL-1 bioactivity was measured in lung samples collected on the indicated days. n = 6 for all samples except day 36 B6.Sst1S = 4. Day 25 data already shown in Fig. 3d. All animals except B6 were bred in-house (a–c); all were bred in-house (d–e). Center and error bars show mean and SEM. Analyzed with two-ended Mann-Whitney test (a–c). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.
Extended Data Fig. 5 | Homozygous or heterozygous Il1rn deletion protects B6.Sst1S mice from Mtb infection. a, Body weights on day 28 of individual mice shown in Fig. 4c, b–c. Mice were infected with Mtb at average 17 CFU/mouse (b) or 60 CFU/mouse (c), and at day 21 lungs were harvested to measure IL-1 bioactivity. d, RT-qPCR on lungs of Mtb-infected mice, sampled at 25 days post-infection. Each graph combined results from 2 independent experiments. e–f, Mice infected with average 20 CFU/mouse were treated with either anti-IL-1R1 antibody or isotype control every 3 days starting 7 days post-infection (e), or anti-IL-1Ra antibody or PBS control every other day starting 3 days post-infection (f). At 25 days post-infection lungs were harvested for measuring bacterial burden. Sample size n (from left as shown) = 11, 12, 22, 10 (a); 6, 8, 11 (b); 6, 8, 10 (c); 11, 14, 11, 5 (d); 5, 6, 3, 4, 2, 3 (e); 8, 8, 6, 6 (f). All mice were bred in-house (a, d–f) or all except B6 were bred in-house (b–c); and all except B6 and B6.Sst1S were littermates (a, d, e). Center and error bars show mean and SEM. Analyzed with two-ended Mann–Whitney test. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.
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Software and code

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- **Sample size**: No sample size calculation was performed; mice per group was based on availability of specific genotypes and prior experience as to typical variability.
- **Data exclusions**: No data were excluded in the analysis.
- **Replication**: Experiments were repeated at least twice.
- **Randomization**: Organisms were assigned to experimental groups based on genotype. We matched gender and age across groups to control for any differences.
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### Materials & experimental systems

- n/a Involved in the study
  - Antibodies
  - Eukaryotic cell lines
  - Palaeontology
  - Animals and other organisms
  - Human research participants
  - Clinical data

### Methods

- n/a Involved in the study
  - ChIP-seq
  - Flow cytometry
  - MRI-based neuroimaging

### Antibodies

- **Antibodies used**
  - anti-mouse D16/CD32 (93, Thermo Fisher 14-0161-81); CD45 (3D-F11, Biolegend 103107); CD11b (M1/70, Thermo Fisher 48-0112-82); CD11c (N418, Biolegend 117335); Ly6G (1A8, BD Biosciences 740554); Ly6C (HK1.4, Thermo Fisher 17-5932-80); CD24 (M1/69, BD Bioscience 564664); MHC II (M5/114.15.2, Biolegend 107625); SiglecF (E50-2440, BD Biosciences 562680); anti-mouse IFNAR1 (MAR1-5A3, Leinco Technologies); mouse anti-human IFNGR-α chain (GIR208, Leinco Technologies); Ultra-LEAF Purified Armenian Hamster IgG Isotype Antibody (Biolegend, 400940); Hamster anti-IL1R1 antibody (mIL1R-M147, Amgen).

- **Validation**
  - Hamster anti-IL1R1 was validated in Nichols et al 2018 (see references). All other antibodies were validated by the respective manufacturers.

### Eukaryotic cell lines

- **Policy information about** cell lines
- **Cell line source(s)**
  - HEK-Blue IL1R cells (InvivoGen)
- **Authentication**
  - Each experiment included a positive control to authenticate the cells.
- **Mycoplasma contamination**
  - Cell lines were not tested for mycoplasma upon receiving from the company.
- **Commonly misidentified lines**
  - (See ICLAC register)
  - N/A

### Animals and other organisms

- **Policy information about** studies involving animals, ARRIVE guidelines recommended for reporting animal research
- **Laboratory animals**
  - Mus musculus. C57Bl/6J (B6), B6.129S-Il1rntm1Dih/J (Il1rnt−/−), B6(Cg)-Ifnar1tm1.2Ees/J (Ifnar−/−) and B6.129S6-Ch25htm1Rus/J (Ch25h−/−), Stinggt/gt, B6.5s1ts. Males and females, 6-8 weeks old (at beginning of infections).
- **Wild animals**
  - N/A
Field-collected samples | N/A
Ethics oversight | All animal experiments complied with the regulatory standards of, and were approved by, the University of California Berkeley Institutional Animal Care and Use Committee.

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

### Flow Cytometry

#### Plots

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

#### Methodology

**Sample preparation**

See description in methods. Briefly, perfused lungs were strained through 40μm cell strainers to obtain single cells.

**Instrument**

LSR Fortessa X-20 (BD)

**Software**

FlowJo Ver.10

**Cell population abundance**

N/A

**Gating strategy**

Cells were gated by FSC/SSC for leukocytes, single cells by FSC-A v. FSC-W. Live cells were gated as stain negative based on an unstained control. Gates were drawn with FMO (Full panel Minus One) staining as the negative control. Cells were gated for CD45+, then CD11bhi Ly6G+ for neutrophils.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.