Interleukin-1 receptor antagonist is a conserved driver of tuberculosis

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Summary

Mycobacterium tuberculosis (*Mtb*) causes 1.3 million deaths a year[1]. However, tuberculosis (TB) pathogenesis remains poorly understood and is not fully recapitulated in standard mouse models. Here we find that gene signatures from three different *Mtb*-susceptible mouse models predict active TB disease in humans significantly better than a signature from resistant C57BL/6 (B6) mice. Conserved among susceptible mice and non-human primates, but largely absent from B6 mice, was *Mtb*-induced differentiation of macrophages into an alternatively activated *Spp1*$^{+}$ differentiation state. *Spp1*$^{+}$ macrophages expressed high levels of immunosuppressive molecules including IL-1 receptor antagonist (IL-1Ra). IL-1Ra was previously reported to cause *Mtb* susceptibility in one mouse model[2], but whether IL-1Ra is broadly important remains uncertain. Indeed, in other models, *Mtb*-susceptibility is proposed to result from excessive rather than diminished IL-1 signaling[3–5]. Here we report that enhancement of IL-1 signaling via deletion of IL-Ra promoted bacterial control across three susceptible mouse models. We found IL-1 signaling enhanced production of multiple cytokines by lymphoid and stromal cells, providing a multifactorial mechanism for how IL-1 promotes *Mtb* control. Our results indicate that myeloid cell expression of immunosuppressive molecules, in particular IL-1 receptor antagonist, is a conserved mechanism exacerbating *Mtb* disease in mice, non-human primates, and humans.

Introduction

Mycobacterium tuberculosis (*Mtb*) causes ~10 million new cases of active tuberculosis (TB) disease and 1.3 million deaths per year, and is the leading cause of mortality from a bacterial pathogen[1]. Unfortunately, TB treatment consists of at least a 4-6 month regimen of antibiotics, and the only approved vaccine has little to no efficacy in adults[6]. A greater understanding of the infection is essential, but this depends on tractable animal models that appropriately mimic *Mtb* pathogenesis in humans. While mouse models have led to key discoveries such as the critical role of IFN-γ[7,8] and TNF[9] in *Mtb* control, mouse models have also been criticized for not recapitulating key aspects of human disease, such as structured granuloma formation[10,11]. Further hallmarks of active tuberculosis (TB) disease in humans include expression of type I interferon (IFN) stimulated genes and neutrophilia[12] and the standard, resistant C57BL/6 (B6) mouse model only weakly exhibits these hallmarks[5,13–15].

We recently described *Sp140*$^{-/-}$ mice as an *Mtb*-susceptible mouse model that mimics the exacerbated type I IFN signature and neutrophilia seen in humans[16,17]. *Sp140* encodes a transcriptional repressor that limits expression of the canonical type I IFN gene *Ifnb1*. Loss of *Sp140* accounts for the excessive type I IFN response and *Mtb*-susceptibility of the previously described *Sst1*^{S} mice[2,18,19]. Deletion of the gene encoding the type I IFN receptor (*Ifnar1*) rescues the hypersusceptibility of both *Sst1*^{S} and *Sp140*$^{-/-}$ mice, implying a causal role of type I

IFNs in *Mtb* pathogenesis in these models[16,17]. Other *Mtb* susceptible mouse models have been described, such as *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ mice[20,21], though it remains unclear whether disease in these models is also driven by type I IFN signaling. Nevertheless, we hypothesized that there may be conserved features underlying the susceptibility of diverse mouse models, and that these features may be preserved across susceptible mice, non-human primates, and humans.

Neutrophilic inflammation is a major feature associated with tuberculosis disease across *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$ and other susceptible mouse models[5,17,21–24], as well as during non-human primate[25,26] and human TB[27,28]. Depletion of neutrophils enhances bacterial control in diverse *Mtb*-susceptible mouse models, including *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, and *Acod1*$^{-/-}$ mice[5,17,21]. However, the precise role of neutrophils in driving bacterial replication is unclear. One widely held view is that neutrophil necrosis and inflammation creates a replicative niche for the bacteria, including possibly within neutrophils themselves[29,30]. The production of neutrophil extracellular traps (NETs) is also speculated to promote bacterial replication, perhaps by induction of type I IFNs[17,22,31]. In states of chronic inflammation, neutrophils can also play immunosuppressive roles[32], though it remains uncertain if this accounts for neutrophil-driven exacerbation of TB disease[5,17,21].

Signaling induced by interleukin-1 (IL-1, comprising IL-1α and IL-1β) potently recruits neutrophils to sites of inflammation, and IL-1 is expressed at high levels in *Mtb*-susceptible mice[2,4] and humans[33]. Excessive IL-1 has been proposed to enhance bacterial replication[3], particularly in *Nos2*$^{-/-}$ mice[4,5], though the underlying mechanisms remain uncertain. A pro-bacterial function of IL-1 has been difficult to reconcile with its clear anti-bacterial functions, as mice deficient in IL-1 signaling are highly susceptible to *Mtb* infection[23,34–38]. Importantly, the levels of IL-1 do not necessarily correlate with its signaling capacity. This is because IL-1 signaling is repressed by a decoy receptor (IL-1R2) and a soluble receptor antagonist (IL-1Ra, encoded by the *Il1rn* gene). Although susceptible *Sst1*^{S} mice express high levels of IL-1α and IL-1β, we previously reported that deficiency in *Il1rn* (enhancing IL-1 signaling) markedly promotes bacterial control in these mice[2]. These results imply that *Mtb*-susceptibility can arise from a lack of IL-1 signaling, rather than excessive IL-1 signaling, even when IL-1 levels are high. However, disease in *Sst1*^{S} mice may be a unique case since it is driven by an exacerbated type I IFN response[2], and *Il1rn* is an interferon-stimulated gene. Thus, it remains unclear whether impairment of IL-1 signaling by IL-1Ra plays a conserved role in humans or other *Mtb*-susceptible mouse models, especially *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ mice, in which *Mtb* infection is characterized by high levels of IL-1.

Here, in an effort to identify conserved mechanisms underlying *Mtb* susceptibility, we used single cell RNA sequencing (scRNA-seq) to profile and characterize three different *Mtb*-susceptible mouse strains (*Sp140*$^{-/-}$, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$ mice). Unlike *Sp140*$^{-/-}$ mice, we found that *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ mice did not exhibit a strong type I IFN signature and were not rescued by *Ifnar1* deficiency. Despite this, a common feature of *Mtb*-induced genes across all three strains was myeloid cell expression of immunosuppressive genes, such as *Arg1*, *Cd274*, and *Il18bp*. In support of the importance of shared features of the *Mtb*-susceptible strains, gene signatures from each of these strains predicted human TB progression better than a signature obtained from resistant B6 mice. Additionally, macrophage differentiation into an alternatively activated *Spp1*$^{+}$ state was conserved across the susceptible mouse strains and *Mtb*-infected non-human primates. Both mouse and non-human primate *Spp1*$^{+}$ macrophages expressed immunosuppressive molecules, including *Il1rn*. Given the importance of IL-1 signaling for *Mtb* control, and the strong expression of IL-1Ra in myeloid cells, we focused on the role of IL-1Ra in *Mtb* susceptibility. Global deletion as well as macrophage-specific deletion of IL-1Ra rescued bacterial control in *Sp140*$^{-/-}$ mice, demonstrating a major role for macrophage expression of this immunosuppressive molecule in driving *Mtb* disease. This contribution was shared across the susceptible mouse models, as IL-1Ra deletion also enhanced bacterial control in *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ mice. While *IL1RN* has been included in multiple human TB gene signatures[12,39–41], we

find it alone was sufficient to strongly predict human TB. Together, these data suggest that immunosuppression by myeloid cells is a conserved feature of *Mtb* susceptibility across mice and humans, with some immunosuppressive pathways, such as IL-1 inhibition via IL-1Ra, being a shared driver of TB disease.

Results

Mtb-susceptible mice more accurately model human infection than B6 mice

Human *Mtb* infection presents as a highly diverse spectrum of diseased and non-diseased states. The best characterized hallmarks of active disease include induction of type I interferon (IFN) genes and a myeloid cell inflammatory response, especially by neutrophils[12]. We previously demonstrated that *Sp140*$^{-/-}$ mice exhibit both hallmarks, and that these hallmarks drive susceptibility in these mice[16,17]. Neutrophil trafficking to the lungs also promotes *Mtb* susceptibility in other mouse models[22,42–44], including *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ mice[5,21]. Therefore, we sought to test whether *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ mice share other attributes of the susceptibility of *Sp140*$^{-/-}$ mice, including type I IFN-driven disease. As myeloid cells are the primary reservoir of *Mtb*, we used our previously described single cell RNA-sequencing (scRNA-seq) workflow[17] to profile myeloid cells in *Mtb*-infected and naïve *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ mice. Infection with a fluorescent (wasabi^{+}) *Mtb* strain allowed us to distinguish infected and bystander cells in infected mice. The resulting datasets were integrated with our previously published B6 and *Sp140*$^{-/-}$ myeloid cell datasets[17] using the R package Seurat[45] (**Fig. 1a**). As these are CITE-seq[46] datasets, mRNA and protein expression of lineage and activation markers were implemented for the annotation of cell clusters (**Supplementary Fig. 1**). Comparing the myeloid cells from naïve lungs of B6, *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, and *Acod1*$^{-/-}$ mice revealed very few differentially expressed genes between the mouse lines, suggesting that each genotype had a similar inflammatory state prior to infection (**Supplementary Fig. 2**). Therefore, differences between genotypes in *Mtb*-infected mice were likely induced in response to the infection rather than the result of pre-existing inflammation. Analysis of the myeloid cell clusters within the integrated dataset showed that the dominant myeloid cell populations (monocytes, macrophages, and neutrophils) are present in each genotype.

Given that neutrophilia exhibited by *Mtb*-susceptible mouse models is a hallmark of human *Mtb* disease progression[27], we hypothesized that susceptible mouse models might mimic additional conserved hallmarks of active human disease, in which case they could be a useful tool to study *Mtb* pathogenesis. To test this hypothesis, we identified lung myeloid gene signatures consisting of genes induced at least 8-fold in *Mtb*-infected susceptible mice relative to naïve lungs (**Supplementary Figure 2**). The ability of these genes to discriminate *Mtb* cases from healthy controls in the TANDEM cohort[47] was quantified by calculating the area under the curve (AUC) for receiver operating characteristic (ROC) curves, with values closer to 1 indicating better classifiers of human disease (**Fig. 1b**). While there was no major difference in the ability of the signatures derived from the three susceptible mouse models to discern human disease, all the susceptible models considerably out-performed the signature derived from B6 mice, suggesting the susceptible mouse models better recapitulate characteristics of active human disease than B6 mice.

Next, we sought to better understand why the susceptible models were all able to discriminate between *Mtb* cases and healthy controls by assessing whether the type I IFN response present in humans[12] and *Sp140*$^{-/-}$ mice[16,17] was also present in *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ animals. Using our previously published gene signatures that distinguish between type I IFN and IFN-γ signaling[17], we classified cells as responding to type I IFN and/or IFN-γ in our myeloid scRNA-seq dataset. As expected, very few cells from naïve lungs were positive for either the

type I or II IFN signature (**Fig. 1c**). Conversely, at least some *Mtb*-infected cells from every genotype had strong IFN-γ signaling, especially in monocytes and macrophages (**Fig. 1c, 1d**). However, the type I IFN response was notable only in the *Sp140*$^{-/-}$ mice, with strong type I IFN signaling in the neutrophils and interstitial macrophages (IMs) of these mice.

We experimentally tested whether type I IFN signaling plays a causative role in disease progression in each *Mtb*-susceptible mouse model by examining lung bacterial burden in *Ifnar1*-deficient *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, and *Acod1*$^{-/-}$ mice 25 days after *Mtb* infection. As reported previously, global *Ifnar1*-deficiency fully rescued the susceptibility of *Sp140*$^{-/-}$ mice, phenocopying the *Mtb* restriction of B6 mice[16,17]; however, *Ifnar1*-deficiency only slightly reduced bacterial burdens in *Acod1*$^{-/-}$ mice and even modestly but not statistically significantly increased bacterial burdens in *Nos2*$^{-/-}$ mice (**Fig. 1e, 1f, 1g**). Together, these data suggest that while all three models recapitulate key aspects of human disease, there are major differences in the drivers of susceptibility between these mouse models.

Myeloid cell production of IL-1Ra is a conserved hallmark of Mtb susceptibility

As a strong type I IFN response was not shared among the three *Mtb*-susceptible mouse models, we analyzed our scRNA-seq to identify common features that may contribute to the elevated bacterial burdens present in these animals. We observed that *Mtb*-infection resulted in activation of the IMs, driving them to adopt either a proinflammatory *Trem2*$^{+}$ or an alternative *Spp1*$^{+}$ activation state[48–50] (**Fig. 2a, 2b**). A shift to *Spp1*$^{+}$ macrophage differentiation was recently associated with human tumor progression[50] and elevated plasma SPP1 levels correlate with active TB disease in humans[51]. We therefore examined whether *Spp1*$^{+}$ macrophage differentiation correlated with *Mtb* susceptibility. Indeed, *Spp1*$^{+}$ macrophages were the dominant monocyte-derived macrophage population in the *Mtb*-susceptible *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, and *Acod1*$^{-/-}$ mice, while being largely absent in *Mtb*-restrictive B6 mice (**Fig. 2c, 2d**). The shift from the *Trem2*$^{+}$ to the *Spp1*$^{+}$ differentiation state correlated with elevated expression of immunosuppressive molecules, including *Il1rn* (encoding IL-1Ra) which we previously demonstrated contributes to loss of bacterial control in *Mtb*-susceptible *Sst1*^{S} mice[2] (**Fig. 2e**). Analysis of a published scRNA-seq dataset of non-human primate *Mtb* granulomas indicated this expression pattern was conserved across species[52]. SPP1^{+} macrophages were present in non-human primate *Mtb* granulomas and SPP1^{+} macrophages had higher expression levels of immunosuppressive molecules such as IDO1, CD274, IL18BP, and IL1RN than TREM2^{+} macrophages (**Fig. 2f, 2g**). These results suggested a role for immunosuppression by macrophages, potentially via IL-1Ra, in exacerbating *Mtb* disease. Thus, we sought to further examine the myeloid cell response to *Mtb* infection, particularly in the case of the *Mtb*-susceptible *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, and *Acod1*$^{-/-}$ mice.

Myeloid cell influx is a hallmark of human and mouse *Mtb* disease[12,27,28,53,54] and neutrophils in particular mediate *Mtb*-susceptibility in *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, and *Acod1*$^{-/-}$ mice[5,17,21]. In line with previous reports, we observed a ~5-fold increase in lung neutrophils and a ~2-4-fold increase in lung monocytes and IMs in *Mtb*-susceptible mice 25 days after *Mtb* infection in each of the three mouse models (**Fig. 3a, Supplementary Fig. 4**). We sought to explore myeloid function in promoting *Mtb* susceptibility by first examining myeloid cell trafficking in *Mtb*-infected lungs from B6 and *Sp140*$^{-/-}$ mice (**Fig. 3b**). Diseased tissue within the lung was defined by the presence of *Mtb* and high cellularity. These features were largely absent from healthy portions of the lung. Diseased areas of the lungs had more macrophages (SIRPα^{+} cells) and neutrophils (Ly6G^{+} cells) than the healthy tissue of the same lungs (**Fig. 3c**). These data indicate that myeloid cells are more abundant in *Mtb*-infected lungs and preferentially traffic to sites of disease within those lungs. Trafficking of myeloid cells to *Mtb* lung lesions has also been observed in other *Mtb*-susceptible mouse strains, including *Acod1*$^{-/-}$ mice[21,22,55].

We next sought to define a mechanism by which myeloid cells exacerbate disease upon trafficking to diseased areas of the *Mtb*-infected lungs. In line with our observation that *Spp1*$^{+}$ macrophages are a major source of IL-1Ra, IMs from the susceptible mouse models exhibited elevated *Il1rn* expression relative to B6 (**Fig. 3d**). In contrast, neutrophils expressed high per-cell levels of *Il1rn* in all the mouse strains, including B6, *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, and *Acod1*$^{-/-}$ mice. Neutrophil *Il1rn* expression was constitutive as neutrophils in naïve and *Mtb*-infected lungs also expressed *Il1rn*, likely resulting in *Il1rn* not being included in the *Mtb*-induced gene signatures (**Fig. 3d, Supplementary Fig. 3, Supplementary Fig. 5**). However, since neutrophils are much more abundant in susceptible mice (**Fig. 3a**), even constitutive per cell expression of *Il1rn* might contribute to susceptibility. Analysis of another published scRNA-seq dataset of *Mtb* granulomas from non-human primates[56] mirrored our findings in mice, with neutrophils and macrophages having the highest expression of *IL1RN* in the non-human primate dataset (**Fig. 3e**). Given the conserved nature of IL-1Ra expression, we tested whether *IL1RN* expression could discriminate between active *Mtb* cases and healthy controls in RNA-seq of PBMCs from the TANDEM cohort[47]. We observed high sensitivity and specificity for *IL1RN* alone (AUC=0.84) (**Fig. 3f**), which was only slightly improved by including *IL1RN* in a gene signature containing additional known immunosuppressive molecules, such as *IL18BP*[57], *CD274*[58], and *IL10*[59] (**Supplementary Fig. 5**). The high performance of this immunosuppressive gene signature is consist with prior work, as multiple published human TB signatures include *IL1RN*[12,39–41]. As IL-1 signaling is regulated by both IL-1Ra and the decoy IL-1R2 receptor, we also examined *Il1r2* expression in our scRNA-seq datasets and found lower *Il1r2* expression in *Mtb*-infected animals relative to naïve controls, suggesting *Il1r2* does not contribute to *Mtb* susceptibility (**Supplementary Fig. 5**). Taken together, our results indicate that myeloid cell expression of IL-1Ra is an important hallmark of TB disease that is conserved across mice, non-human primates, and humans.

IL-1Ra is a conserved mediator of Mtb susceptibility

Next, we examined whether myeloid cell IL-1Ra expression plays a conserved causative role in susceptibility to *Mtb*. IL-1 is highly expressed in susceptible mouse strains[4], and has been hypothesized to contribute to the susceptibility of *Nos2*$^{-/-}$ mice to *Mtb*[4,5]. However, as we detected high levels of *Il1rn* expression in myeloid cells in *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, and *Acod1*$^{-/-}$ mice, we hypothesized that perhaps the high levels of IL-1 were not functional, and instead, the susceptibility of the mice was due to a functional deficiency in IL-1 signaling. Indeed, we previously found that *Mtb*-infected *Sst1*^{S} mice exhibit high levels of IL-1 and yet bacterial burdens in these mice are rescued by enhancement of IL-1 signaling via deletion of *Il1rn*[2]. Likewise, we found that even heterozygous deficiency in *Il1rn* promotes bacterial control in *Sp140*$^{-/-}$ mice (**Fig. 4a**). However, as noted above (**Fig. 1**), *Sp140*$^{-/-}$ and *Sst1*^{S} mice exhibit a strong type I IFN signature that is largely absent from *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ mice. It was therefore of interest to test whether *Il1rn*-deficiency could also rescue bacterial burdens in *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ mice. We found that while deletion of one allele of *Il1rn* had no effect on *Mtb* control in *Acod1*$^{-/-}$ mice, complete deletion of *Il1rn* was sufficient to restore wild-type levels of bacterial control at day 25 post-infection (**Fig. 4b**). Interestingly, while bacterial burdens were reduced in *Acod1*$^{-/-}$ *Il1rn*$^{-/-}$ mice to levels seen in WT B6 mice, lung neutrophil numbers were still elevated in *Acod1*$^{-/-}$ *Il1rn*$^{-/-}$ mice (**Fig. 4c**). This result implies that the primary detrimental effect of neutrophils during *Mtb* infection may be production of IL-1Ra. In two experiments with a higher and lower dose of *Mtb* inoculum, we also observed partial or complete rescue of bacterial burdens in *Nos2*$^{-/-}$ *Il1rn*$^{-/-}$ mice as compared to *Nos2*$^{-/-}$ mice (**Fig. 4d**). Similar to the *Acod1*$^{-/-}$ mice, no effect of *Ilr1rn* heterozygosity was observed, and the improved bacterial control in *Nos2*$^{-/-}$ *Il1rn*$^{-/-}$ mice did not coincide with a decrease in the number of lung neutrophils (**Fig. 4e**). These results demonstrate a conserved role for IL-1Ra in elevating *Mtb* burdens in three

different mouse models, but do not rule out additional roles for IL-1Ra in suppressing immunopathology at later timepoints during TB (see Discussion).

Both neutrophils and macrophages produce IL-1Ra. Neutrophils are much more abundant in susceptible mice than macrophages, so it might be assumed that neutrophils are the most important source of IL-1Ra. However, prior reports have suggested that the secretion of IL-1Ra by neutrophils is relatively inefficient as compared to macrophages, and specific deletion of IL-1Ra in macrophages is protective during *Candida* infection[60]. Moreover, neutrophil expression of IL-1Ra is constitutive (including in resistant B6 mice) whereas it is induced in IMs. Indeed, IMs in all three susceptible mouse models expressed elevated levels of *Il1rn* relative to wild-type B6 controls, likely driven by increased macrophage adoption of the alternatively activated *Spp1*$^{+}$ state (**Fig. 2d**, **Fig. 3d**). We therefore hypothesized that macrophage production of IL-1Ra could account for the conserved role of IL-1Ra in inhibiting bacterial control in *Mtb*-susceptible mice. To delete *Il1rn* in macrophages, we used CD64-Cre mice, which express Cre specifically in macrophages, at least in naïve animals[61]. Deletion of *Il1rn* in CD64^{Cre}*Il1rn*$^{fl/fl}$ mice[61,62] was able to rescue *Mtb* susceptibility of *Sp140*$^{-/-}$ mice (**Fig. 4f**). Together, these data reveal a conserved, myeloid-specific role for IL-1Ra expression in exacerbating *Mtb* disease.

IL-1-enhanced cytokine and chemokine production promotes Mtb control

Given the conserved role for IL-1Ra in exacerbating *Mtb* infection, we aimed to better define how IL-1 mediates protection against *Mtb*. We first examined IL-1R expression on myeloid cells, as they are the primary reservoir of *Mtb*, and found little to no expression of IL-1R in myeloid cells from naïve and *Mtb*-infected B6, *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, and *Acod1*$^{-/-}$ mice (**Supplementary Fig. 6**). Consistent with a previous study[23], we also demonstrated that IL-1R-deficient macrophages do not exhibit a cell-intrinsic defect in bacterial control in mixed bone marrow (BM) chimera mice (**Supplementary Fig. 6**). It thus appears that IL-1 mediates its protective effect by signaling in bystander cells that then act non-cell autonomously to promote myeloid cell *Mtb* control. We therefore sought to identify the bystander cells that express IL-1R and the genes regulated by IL-1 in these cells to determine the mechanism by which IL-1 aids in bacterial restriction.

As IL-1 signaling in either hematopoietic or non-hematopoietic cells is sufficient for *Mtb* control early in infection[23,37], we sought to identify potential IL-1 responders in both compartments. Due to the inhibition of IL-1 signaling by high expression of IL-1Ra in the *Mtb*-susceptible mouse models, we used resistant B6 mice for examining IL-1 function. To ascertain the cell autonomous effects of IL-1, we generated mixed BM chimera mice with a 1:1 mix of WT and *Il1r1*$^{-/-}$ cells that were infected with *Mtb* following immune reconstitution (**Fig. 5a**). Among hematopoietic cells, IL-1 promotes cytokine expression in CD4^{+} T cells[63] and innate lymphoid cells (ILCs)[64,65]. In T cells, *Il1r1* was primarily expressed in T helper 17 cells (Th17) with some expression in Type 1 helper cells (Th1; **Fig. 5b, 5c, Supplementary Fig. 7**). Differential scRNA-seq gene expression profiling comparing WT and *Il1r1*$^{-/-}$ Th17 cells identified *Tnf*, *Gzmb*, and *Ifng* as genes with at least 2-fold greater expression in WT cells that were annotated as potential ligands in the NicheNet database[66] (**Fig. 5d**). Type 3 ILC (ILC3) and IL-17 producing natural killer T (NKT17) cells were the major *Il1r1* expressing innate lymphoid cell populations in *Mtb*-infected lungs, with differential gene expression analysis identifying *Aimp1* and *Cxcl2* as potential IL-1-induced ligands produced by ILC3s; and *Tnf*, *Cxcl10*, *Itgb2*, *Il17a*, *Cxcl2*, and *Rtn4* as potential IL-1-induced ligands produced by NKT17 cells (**Fig. 5e, 5f, 5g, Supplementary Fig. 7**).

Regarding potential non-hematopoietic sensors of IL-1, we first examined sensory neurons as they highly express IL-1R[67], and IL-1 signaling in neurons recruits myeloid cells[68]. As IL-1 signaling in hematopoietic cells is sufficient for early resistance to *Mtb* infection[23,37], we

generated BM chimera mice that lacked hematopoietic IL-1R and either had IL-1 receptor in sensory neurons (CD45.1 and Adv$^{+/+}$ *Il1r1*$^{fl/fl}$ recipient mice) or not (Adv$^{cre/+}$ *Il1r1*$^{fl/fl}$ recipient mice). Sensory neuron-specific deletion of *Il1r1* had no effect on *Mtb* control, suggesting that other non-hematopoietic cells mediate the protective effect of IL-1 against *Mtb* (**Supplementary Fig. 8**). We thus performed scRNA-seq on BM chimera mice generated by transferring WT BM into lethally irradiated WT and *Il1r1*$^{-/-}$ hosts and then infected the chimeric mice with *Mtb* following immune reconstitution (**Fig. 5h**). Lineage markers used to annotate a scRNA-seq dataset of stromal cells in normal and hyperoxia-damaged murine lungs[69] were used to identify cell populations in our stromal cell scRNA-seq dataset (**Supplementary Fig. 7**). Based on this analysis, *Il1r1* was widely expressed in stromal cells, including in myofibroblasts, fibroblasts, and endothelial cells (**Fig. 5i, 5j**). Differential expression analysis of IL-1-regulated potential ligands, as annotated in NicheNet's database[66], identified *Icam1*, *Il6*, *Ccl2*, and *Wnt11* in myofibroblasts, *Cxcl1*, *Ccl2*, *Il6*, and *Cxcl9* in fibroblasts, and *Icam1* in endothelial cells as IL-1-induced potential ligands (**Fig. 5k**).

The most likely candidates for the protective effect of IL-1 signaling were then identified using NicheNet[66]. For this analysis, we focused on IMs as the receiver cells for the IL-1-regulated ligands, as IMs are known to harbor *Mtb* and control the infection[70]. According to the NicheNet ligand-receptor database, the receptors for IFN-γ, TNF, IL-6, CCL2, and IL-17 were expressed by IMs (**Supplementary Fig. 9**). While IFN-γ and TNF have well-established roles in *Mtb* control[7–9], the contribution of IL-17 has been appreciated more recently[71–74]. IL-22 is another cytokine important for bacterial control that is often co-produced with IL-17[75]. Thus, we tested whether IL-1-induced enhancement of IL-17/22 production was important in IL-1-mediated *Mtb* restriction. This hypothesis was tested by generating mixed BM chimera mice containing BM from *Il17*$^{-/-}$ *Il22*$^{-/-}$ and *Il1r1*$^{-/-}$ mice mixed at a 1:1 ratio as well as control mice (**Fig. 5l**). While the *Il17*$^{-/-}$ *Il22*$^{-/-}$: *Il1r1*$^{-/-}$ BM chimera mice contain cells that can respond to IL-1, as well as cells that can produce IL-17/22, no cell will be able to respond to IL-1 and express IL-17/22. Thus, these chimeras specifically eliminate the ability of IL-1 to amplify IL-17/22 production. The *Il17*$^{-/-}$ *Il22*$^{-/-}$: *Il1r1*$^{-/-}$ BM chimera mice were only slightly more susceptible than control animals, suggesting that an IL-1-mediated increase in IL-17/22 expression is not necessary for IL-1-driven protection (**Fig. 5m**). Therefore, we sought to identify which other potential ligands were the most likely drivers of IL-1-regulated *Mtb* restriction by IMs. We identified genes upregulated in activated IMs, and NicheNet[66] was then used to calculate the likelihood for each of the potential ligands to induce the transcriptional changes observed in the activated IMs. IFN-γ and TNF had the highest scores indicating they are the most likely candidates for how IL-1 protects against *Mtb*, while IL-17 had one of the lowest scores, in line with our experimental data (**Supplementary Fig. 9, Fig. 5m**). Taken together, these results suggest that IL-1-mediated protection against *Mtb* is multifactorial, involving responses by both hematopoietic and stromal cells that coordinate the activation of IMs by multiple anti-microbial cytokines.

Discussion

Mtb infection induces a chronic inflammatory state as evidenced by the induction of a type I/II IFN gene signature up to 12-18 months prior to disease diagnosis[27,76]. While aspects of this inflammatory state—such as the IFN-γ response[7,8]—are vital for bacterial control, other components, such as the type I IFN response, exacerbate *Mtb* disease progression[12,17,22]. Our results lead us to hypothesize that the chronic inflammatory nature of *Mtb* infection may hinder bacterial control by promoting immunosuppression. We further propose that an underlying state of immunosuppression is conserved across susceptible mouse models, non-human primates

and in humans. Within the cancer field, it has long been recognized that chronic inflammation supports cancer progression[77] with one major mechanism being the recruitment of so-called myeloid-derived suppressor cells (MDSC)[78]. MDSCs are a heterogeneous population in mice and humans, with mouse MDSCs commonly identified based on Gr-1 antibody staining, which binds Ly6C and L6G, markers of monocytes and neutrophils, respectively[78]. While monocytes and neutrophils are commonly viewed as proinflammatory cells, MDSCs are immunosuppressive through multiple pathways, including via production of IL-10 and TGF-β[79]. During *Mtb* infection, monocytes and neutrophils are also heavily recruited to the lungs and specifically to sites of bacterial replication (**Fig. 3**).

In line with these findings, prior studies have described increased MDSCs in the blood of *Mtb*-infected individuals at frequencies similar to those found in human cancer patients[80] as well as increased lung MDSC frequency in non-human primates with active TB disease relative to latently infected animals[81]. Multiple pathways have been proposed for MDSC-driven immunosuppression of the host response against *Mtb* infection, including PD-1, IDO1, TGFβ, and IL-10[82,83]. For example, IDO1$^+$ PD-L1$^+$ myeloid cells reside in human granulomas and PD-L1 expression associates with active TB progression in humans[84]. However, the impact of immune suppression by myeloid cells on *Mtb* control is unclear, as highlighted by the contrasting results on the roles of PD-1 and IDO1 during *Mtb* infection. PD-1 deletion in mice and PD-1 blockade in rhesus macaques exacerbates TB disease[85,86], while IDO1 deletion does not change bacterial burden in mice[87] yet targeting IDO1 with a small molecular inhibitor promotes *Mtb* control in non-human primates[88,89]. Factors contributing to the lack of clarity for the role of immunosuppression during *Mtb* infection could be that not all immunosuppressive molecules may impact *Mtb* infection similarly and their effects may differ across models. For example, deletion or blockade of IL-10 has varying effects in mice depending on the genetic background and infection conditions[14,90–94]. Therefore, we applied a comparative approach to re-examine whether myeloid cells play an immunosuppressive role during *Mtb* infection, focusing on identifying conserved features of the myeloid cell response in *Mtb*-susceptible mice.

We first used scRNA-seq to characterize myeloid cells from *Mtb*-infected lungs of three *Mtb*-susceptible mouse strains that all exhibit neutrophil-driven disease, namely, *Sp140*$^{-/-}$, *Nos2*$^{-/-}$, and *Acod1*$^{-/-}$ mice[5,16,17,21]. Gene signatures derived from each of these mice significantly outperform wild-type B6 mice in their ability to discriminate human active *Mtb* cases from healthy controls, suggesting susceptible mice better mimic human disease. Due to the high predictive strength of all three models' gene signatures, it is likely that there are commonalities among the mouse models that are responsible for their discriminatory ability. Initially, we hypothesized that a likely candidate for a shared response to *Mtb* infection was type I IFN, as viral infections and the anti-viral type I IFN response correlate with the severity of *Mtb* disease in humans[12,76,95–97]. A functional role for type I IFNs in driving *Mtb* disease is also observed in mice, as inducers of type I IFNs exacerbate *Mtb* infection in B6 mice[34,98–103] and *Ifnar1* deletion rescues bacterial control in *Sst1*^{S} and *Sp140*-deficient mice[2,16,17]. To our surprise, however, a type I IFN signature was not conserved across susceptible mouse strains, and type I IFN receptor deletion had little to no effect on bacterial control in *Nos2*$^{-/-}$ and *Acod1*$^{-/-}$ mice (**Fig. 1**), indicating that other pathways drive their *Mtb* susceptibility.

As myeloid cells contribute to disease in each of the tested *Mtb* susceptible mouse models[5,17,21], we sought to define what pathways might be suppressed by myeloid cells to hinder *Mtb* control. We found that all three tested *Mtb*-susceptible mouse models had a shift in macrophage differentiation, exhibiting a reduction in *Trem2*$^+$ macrophages in favor of alternatively activated *Spp1*$^+$ macrophages. Interestingly, *Mtb*-restrictive B6 mice largely lacked *Spp1*$^+$ macrophages, and thus, *Spp1*$^+$ macrophage differentiation correlated with *Mtb*-susceptibility (**Fig. 2**). Macrophage differentiation into the SPP1$^+$ alternatively activated state also strongly correlates with tumor progression in humans[50]. A commonality between *Mtb*-susceptible mice, human *Mtb* infection, and human cancer is the induction of a strong, chronic

inflammatory state, raising the possibility that continued inflammation is a key driver of *Spp1*$^{+}$ macrophage differentiation. This concept is supported by the recent observation that SPP1^{+} macrophages are associated with pathogenesis in individuals recently diagnosed with idiopathic pulmonary fibrosis, a disease with a median time to diagnosis of 2 years[104]. Additionally, SPP1^{+} macrophage differentiation correlates with active rather than in remission Rheumatoid arthritis in humans[105]. Furthermore, SPP1 plasma levels were significantly higher in individuals with severe COVID-19 than those with mild/moderate COVID-19 or pneumonia[105] and SPP1 plasma levels correlate with human active TB disease[51]. *Spp1*$^{+}$ macrophages may play a key role in exacerbating *Mtb* disease as they are the dominant macrophage source of IL-1Ra in mice. This expression pattern is conserved across species as we find SPP1^{+} macrophages are also present in *Mtb* granulomas in non-human primates and upregulate several immunosuppressive molecules relative to TREM2^{+} macrophages, including *IL1RN* (**Fig. 2**). The correlation between *Mtb* susceptibility and elevated IL-1Ra expression by macrophages was particularly intriguing as IL-1 signaling is a critical pathway for *Mtb* control[23,34–37].

However, the role of IL-1 during *Mtb* pathogenesis has been confusing. Mice deficient in IL-1 signaling are clearly susceptible to *Mtb*[23,34–38], but at the same time, many susceptible mouse models exhibit elevated levels of IL-1[2,4], leading some to propose that IL-1-driven neutrophilic inflammation actually exacerbates *Mtb*[5,29]. Here we propose that the apparently paradoxical effects of IL-1 can be rationalized by an appreciation that high levels of IL-1 cytokine do not necessarily imply high levels of IL-1 signaling. Indeed, the beneficial effects of IL-1 can be counteracted by high levels of the decoys IL-1R2 or IL-1Ra[106]. In addition to *Spp1*$^{+}$ macrophage expression of *Il1rn* (encoding IL-1Ra), our scRNA-seq data indicated that *Il1rn* was constitutively expressed by neutrophils, as has also been shown for human neutrophils[107] (**Fig. 3**). Despite strong expression of *Il1rn* by neutrophils, neutrophils secreted considerably less IL-1Ra than monocytes upon stimulation and macrophage-specific deletion of *Il1rn* restored control of *Candida* infection[60]. Similarly, we found that expression of IL-1Ra by CD64^{+} cells, such as macrophages, is specifically required for *Mtb* control as CD64-Cre-mediated deletion of *Il1rn* was sufficient to rescue the susceptibility of *Sp140*$^{-/-}$ mice. This result correlates with human disease as macrophages are one of the primary IL-1Ra-expressing cell types in human *Mtb* granulomas[108]. Given the high levels of IL-1Ra expression by IMs in the susceptible mouse models, we predicted that IL-1Ra adversely affects *Mtb* control in each of the susceptible lines, which was confirmed by rescuing bacterial control in all three mouse lines with global deletion of IL-1Ra. This result was highly unexpected given prior claims that excessive IL-1 signaling is detrimental during *Mtb* infection[3] particularly following the onset of the adaptive immune response in *Nos2*$^{-/-}$ mice[4,5]. Our results instead suggest that the initial lack of bacterial control in highly inflamed *Mtb*-susceptible mouse models, such as *Acod1*$^{-/-}$ and *Nos2*$^{-/-}$ mice, is primarily due to insufficient rather than excessive IL-1 signaling. Our results are in line with the susceptibility of mice deficient in IL-1 signaling[23,34,36,37], as well as with anecdotal evidence in humans reporting *Mtb* reactivation following IL-1Ra (Anakinra) treatment[109].

Importantly, our study focused on assessing bacterial burdens at a relatively early timepoint after infection (day 25) and do not exclude the possibility that excessive IL-1 signaling at later timepoints might have additional detrimental effects[110]. In particular, while IL-1 might be important for orchestrating bacterial control, it is also well-known to mediate significant immunopathology. This balance is exhibited in our previous work in which deletion of *Il1rn* completely rescued bacterial control in *Sst1*^{S} mice early in infection but resulted in only a minor improvement in survival, while deletion of a single allele of *Il1rn* nearly phenocopied the survival of *Mtb*-restrictive B6 mice[2]. It thus remains unclear whether IL-1 signaling could be manipulated therapeutically during human TB. Indeed, although myeloid cell production of IL-1Ra impairs bacterial control, IL-1Ra expression likely evolved to limit host pathology and therefore may be beneficial in many cases. Additionally, IL-1Ra is likely just one of many molecules that dictate the balance between bacterial control and host pathology during *Mtb* infection. For example, our

gene signatures for the *Mtb*-susceptible mouse lines (but not the *Mtb*-restrictive B6 mice) contain other known immunosuppressive molecules, such as *Cd274* and *Il18bp*. Overall, our results therefore suggest that host-directed therapy to *Mtb* may require a delicate balance between anti-bacterial and host-tolerance mechanisms.

The mechanism by which IL-1 exerts its protective effects remains the subject of intensive study. In line with the limited expression of *Il1r1* by infected myeloid cells in our scRNA-seq dataset, IL-1 has been proposed to act on uninfected bystander cells that then promote myeloid cell *Mtb* control[23,37]. IL-1 signaling in either hematopoietic or non-hematopoietic bystander cells is sufficient to mediate protection during early infection[23,37]. Yet the mechanism for IL-1-mediated protection is not fully established, with eicosanoid regulation and synergistic interactions between IL-1 and TNF being two potential mechanisms[34,37]. Our BM chimera scRNA-seq experiments suggest that a lack of a single simple mechanism is due to the complex and multifactorial effects of IL-1 on multiple cell types. We identified multiple IL-1-induced cytokines and chemokines as potential activators of *Mtb*-harboring IMs, with the most likely being *Ifng* and *Tnf*, two cytokines known to play a key role in *Mtb* restriction[7–9]. As IL-1 signaling in $CD4^+$ T cells directly promotes cytokine production[63], it is likely that IL-1 directly enhances *Ifng* and *Tnf* production by T cells and ILCs throughout *Mtb* infection. During *Legionella pneumophila* infection, IL-1 acts on type II alveolar epithelial cells inducing their expression of GM-CSF[111], another cytokine that activates macrophages with an established contribution to *Mtb* control[112–114]. Our stromal scRNA-seq dataset had limited coverage of epithelial cells so we were unable to observe whether IL-1 also induces GM-CSF expression in type II alveolar cells during *Mtb* infection. However, it is likely that this mechanism is conserved between *Legionella* and *Mtb* infections given the critical role played by GM-CSF and stromal cell IL-1 signaling in both infections.

In summary, our data support a model in which initial loss of bacterial control, whether due to a type I IFN response, *Nos2*-, or *Acod1*-deficiency, results in myeloid cell influx into *Mtb*-infected lungs. The incoming monocytes differentiate into IMs that adopt the *Spp1*$^+$ activation state in animals with chronic inflammation, which correlates with the upregulation of IL-1Ra. This high level of IL-1Ra expression hinders critical IL-1 signaling in hematopoietic and non-hematopoietic cells, reducing the production of *Mtb*-restrictive cytokines, such as TNF and IFN-γ. The IL-1Ra-mediated blockade of IL-1 thus generates a positive feedback loop where *Mtb*-harboring myeloid cells are unable to restrict the bacteria. Enhanced bacterial replication leads to the influx of yet more myeloid cells and further increases IL-1Ra levels. Our results are largely consistent with a recently proposed 'tipping-point' model of tuberculosis[115], which suggests that neutrophils cause a feedforward loop exacerbating disease even if they are not the initial driver of loss of *Mtb* restriction. In this study, we provide molecular evidence for this model by elucidating key molecular and cellular players that orchestrate the tipping point.

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Author Contributions

Conceptualization: D.I.K. and R.E.V. Investigation: D.I.K., O.V.L., and D.X.J. Data analysis: D.I.K. and O.V.L. Methodology: S.S. Resources: C.G. and D.L.J. Writing: D.I.K. and R.E.V. with input from all co-authors. Funding acquisition: R.E.V. Supervision: R.E.V.

Declaration of Interests

R.E.V. consults for Tempest Therapeutics and X-biotix Therapeutics.

Figure Legends

Figure 1. *Nos2*- and *Acod1*-deficient mice mimic human *Mtb* progression but do not exhibit type I interferon-driven susceptibility. (**a**) Visualizing lung myeloid cell populations in a scRNA-seq dataset of cells from *Mtb*-infected B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice (n = 3 per genotype). (**b**) ROC curves of the ability of genes induced by *Mtb* infection in B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, or *Sp140*$^{-/-}$ mice to stratify human active *Mtb* patients (n = 151) and healthy controls (n = 98) in the TANDEM cohort (analysis of GSE114192). (**c**) UMAP plot and (**d**) quantification of myeloid cells undergoing type I interferon and/or IFN-γ signaling in the lungs of naïve or *Mtb*-infected B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, or *Sp140*$^{-/-}$ mice (n = 3 per genotype and infection status). (**e**) Lung bacterial burden in B6 (n = 10), *Sp140*$^{-/-}$ (n = 11), and *Sp140*$^{-/-}$ *Ifnar1*$^{-/-}$ (n = 12) mice infected with *Mtb*. (**f**) Lung bacterial burden in littermate control (n = 16), *Acod1*$^{-/-}$ *Ifnar1*$^{+/-}$ (n = 12), and *Acod1*$^{-/-}$ *Ifnar1*$^{-/-}$ (n = 9) mice infected with *Mtb*. (**g**) Lung bacterial burden in *Nos2*$^{+/+}$ littermate control (n = 4), *Nos2*$^{+/-}$ littermate control (n = 9), *Nos2*$^{+/-}$ *Ifnar1*$^{-/-}$ (n = 10), *Nos2*$^{-/-}$ (n = 8), and *Nos2*$^{-/-}$ *Ifnar1*$^{-/-}$ (n = 11) mice infected with *Mtb*. Lungs were analyzed 25-27 days after *Mtb*-Wasabi infection. The bars in (e), (f), and (g) represent the median. Pooled data from two independent experiments are shown in (e), (f), and (g). Statistical significance in (d), (e), (f), and (g) by one-way ANOVA with Tukey's multiple comparison test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Figure 2. *Spp1*$^{+}$ alternatively activated macrophage differentiation and their expression of immunosuppressive molecules, including IL-1 receptor antagonist, correlates with *Mtb* susceptibility in mice and is conserved between mice and *Mtb*-infected non-human primates. (**a**) Lung *Mtb*-infected monocyte and macrophage expression of *Spp1, Trem2,* and *Cxcl9*, (**b**) the resulting cell annotation, and (**c**) lung *Mtb*-infected monocyte and macrophage populations visualized separately for B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice (n = 3 per genotype). (**d**) Quantification of the frequency of *Mtb*-infected IMs, *Spp1*$^{+}$ IMs, and *Trem2*$^{+}$ IMs among all IMs for B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice (n = 3 per genotype). (**e**) Volcano plot of the differentially expressed genes in *Mtb*-infected *Spp1*$^{+}$ IMs relative to *Trem2*$^{+}$ IMs (combined analysis of cells from for B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice). (**f**) Cell clustering for cells from IgG control antibody treated *Mtb*-infected non-human primate granulomas (Analysis of https://fairdomhub.org/studies/1134). (**g**) Volcano plot of the differentially expressed genes in SPP1^{+} IMs relative to TREM2^{+} IMs from *Mtb*-infected non-human primate granulomas. Lungs were analyzed for the experiments depicted in (a) – (e) 25 days after *Mtb*-Wasabi infection and 6 weeks after *Mtb* infection for those depicted in (f) and (g). The bars in (d) represent the median. Statistical significance in (e) and (g) was calculated with the Wilcoxon

Rank-Sum test with Bonferroni correction and in (d) by two-way ANOVA with Tukey's multiple comparison test. ****p < 0.0001.

Figure 3. Neutrophils and interstitial macrophages localize to diseased tissue and are abundant, major expressors of IL-1Ra. (**a**) Number of neutrophils, monocytes, IMs, and AMs in the lungs of *Mtb*-infected *Sp140*$^{-/-}$ (n = 13), *Sp140*$^{-/-}$ *Ifnar1*$^{-/-}$ (n = 11), *Acod1*$^{+/+}$ (n = 7), *Acod1*$^{+/-}$ (n = 6), *Acod1*$^{-/-}$ (n = 12), *Nos2*$^{+/+}$ (n = 4), *Nos2*$^{+/-}$ (n = 7), and *Nos2*$^{-/-}$ (n = 8) mice. (**b**) Representative images and (**c**) image quantification of *Mtb* (*Mtb*-mCherry^{+}, red), macrophages (SIRPα^{+}, blue), and neutrophils (Ly6G^{+}, red) in diseased and healthy portions of *Mtb*-infected B6 (n = 5) and *Sp140*$^{-/-}$ lungs (n = 6). (**d**) *Il1rn* expression measured by scRNA-seq of *Mtb*-infected myeloid cell populations in the lungs of B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice (n = 3 per genotype). (**e**) IL1RN expression as measured by scRNA-seq of *Mtb* granulomas from non-human primates 10 weeks post-infection (Analysis of GSE200151). (**f**) ROC curve depicting the ability of *IL1RN* expression to stratify human active *Mtb* patients (n = 151) and healthy controls (n = 98) in the TANDEM cohort (analysis of GSE114192). Lungs were analyzed for the experiments depicted in (a) – (d) 24-26 days after *Mtb*-Wasabi or *Mtb*-mCherry infection. The bars in (a) represent the median. Pooled data from two independent experiments are shown in (a) and (c). Statistical significance in (d) was calculated with the Wilcoxon Rank-Sum test with Bonferroni correction, in (a) by one-way ANOVA with Tukey's multiple comparison test, and in (c) by paired t test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Figure 4. Macrophage-derived IL-1Ra is a conserved driver of *Mtb* susceptibility. (**a**) Lung bacterial burden in *Sp140*$^{-/-}$ (n = 14), *Sp140*$^{-/-}$ *Il1rn*$^{+/-}$ (n = 16), *Sp140*$^{-/-}$ *Il1rn*$^{-/-}$ (n = 8), and *Sp140*$^{-/-}$ *Ifnar1*$^{-/-}$ mice (n = 15) following *Mtb* infection. (**b**) Lung bacterial burden and (**c**) neutrophil numbers in *Acod1*$^{+/-}$ *Il1rn*$^{+/+}$ (n = 7), *Acod1*$^{-/-}$ *Il1rn*$^{+/+}$ (n = 14), *Acod1*$^{-/-}$ *Il1rn*$^{+/-}$ (n = 28), and *Acod1*$^{-/-}$ *Il1rn*$^{-/-}$ (n = 8) mice following *Mtb* infection. (**d**) Lung bacterial burden and (**e**) neutrophil numbers in *Nos2*-sufficient controls (n = 11), *Nos2*$^{-/-}$ *Il1rn*$^{+/+}$ (n = 8-16), *Nos2*$^{-/-}$ *Il1rn*$^{+/-}$ (n = 8-18), *Nos2*$^{-/-}$ *Il1rn*$^{-/-}$ (n = 6-19), littermate control (n = 6) and B6 (n = 5) mice following *Mtb* infection. (**f**) Lung *Mtb* burden in *Sp140*$^{+/-}$ littermate control (n = 19), *Sp140*$^{-/-}$ littermate control (n = 19), and *Sp140*$^{-/-}$ *Il1rn*$^{fl/fl}$ *CD64*$^{cre+}$ (n = 24) mice. Lungs were analyzed 24-28 days after *Mtb* or *Mtb*-Wasabi infection. The bars in (a) – (f) represent the median. Pooled data from two independent experiments are shown in (a) – (e) and three independent experiments in (f). Statistical significance in (a) - (f) was calculated by one-way ANOVA with Tukey's multiple comparison test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Figure 5. IL-1 enhances cytokine and chemokine expression by Th17, NKT17, and ILC3 as well as stromal cells. (**a**) Schematic of the BM chimera mice used in panels (b) – (g). (**b**) T cell populations and (**c**) their expression of *Il1r1* in the *Mtb*-infected lungs of the BM chimera mice. (**d**) Volcano plot of the differentially expressed genes in the WT Th17 cells relative to *Il1r1*$^{-/-}$ Th17 cells during *Mtb* infection. (**e**) Innate lymphoid cell populations and (**f**) their expression of *Il1r1* in the *Mtb*-infected lungs of the BM chimera mice. (**g**) Differentially expressed genes in the WT ILC3 and NKT17 cells relative to *Il1r1*$^{-/-}$ cells during *Mtb* infection. (**h**) Schematic of the BM chimera mice used in panels (i) – (k). (**i**) Non-hematopoietic cell populations and (**j**) their expression of *Il1r1* in the *Mtb*-infected lungs of the BM chimera mice. (**k**) Differentially expressed genes in the WT myofibroblast, fibroblast, and endothelial cells relative to *Il1r1*$^{-/-}$ cells during *Mtb* infection. (**l**) Schematic of the BM chimera mice used in panel (m). (**m**) Lung *Mtb* burden in BM chimera mice that received *Il1r1*$^{-/-}$: CD45.1 mixed BM (n = 8), *Il1r1*$^{-/-}$: *Il17*$^{-/-}$ *Il22*$^{-/-}$ mixed BM (n = 10), or *Il1r1*$^{-/-}$ BM (n = 13). Lungs were analyzed 25 days after *Mtb*-Wasabi infection. The bars in (m) represent the median. Pooled data from two independent experiments are shown in (m). Statistical significance in (d), (g), and (k) was calculated with the

Wilcoxon Rank-Sum test with Bonferroni correction and in (m) by one-way ANOVA with Tukey's multiple comparison test. *$p < 0.05$.

Materials and Methods

Mice. Following University of California Berkeley Institutional Animal Care and Use Committee regulatory standards, mice were housed at 23°C with a 12-hour light-dark cycle and maintained under specific pathogen-free conditions. Male and female mice were used for experiments. Mice were age-matched with littermate controls when possible and 6-12 weeks old at the start of the infections. C57BL/6 (B6), B6.129S2-Ifnar1^{tm1Agt}/Mmjax (*Ifnar1*$^{-/-}$)[116], B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ (CD45.1)[117], B6.129S7-*Il1r1*^{tm1Imx}/J (*Il1r1*$^{-/-}$)[118], B6.129(Cg)-*Il1r1*$^{tm1.1Rbl}$/J (*Il1r1*$^{fl/fl}$)[119], B6.129P2-*Avil*$^{tm2(cre)Fawa}$/J (Adv^{Cre})[120], B6.129P2-*Nos2*^{tm1Lau}/J (*Nos2*$^{-/-}$)[121], and C57BL/6NJ-*Acod1*$^{em1(IMPC)J}$/J (*Acod1*$^{-/-}$) mice were purchased from Jackson Laboratories. B6-Fcgr1^{tm2Ciphe} (CD64^{Cre})[61] mice were a gift from B. Malissen at Centre d'Immunologie de Marseille Luminy and were provided by Y. Belkaid at the National Institutes of Health. BM from *Il17*$^{-/-}$*Il22*$^{-/-}$ mice was a gift from S. Khader at the University of Chicago. Il1rn$^{tm1.1Cga}$ *(Il1rn*^{fl})[62] were generated by Cem Gabay at the University of Geneva and were provided by A. Luster at Massachusetts General Hospital. *Sp140*$^{-/-}$ mice were previously generated in-house[16]. *Sp140*$^{-/-}$*Ifnar1*$^{-/-}$, *Acod1*$^{-/-}$*Ifnar1*$^{-/-}$, and *Nos2*$^{-/-}$*Ifnar1*$^{-/-}$ mice were generated by crossing *Sp140*$^{-/-}$, *Acod1*$^{-/-}$, and *Nos2*$^{-/-}$ mice with *Ifnar1*$^{-/-}$ mice in-house, respectively. *Sp140*$^{-/-}$, *Acod1*$^{-/-}$, and *Nos2*$^{-/-}$ mice were crossed in-house with *Il1rn*$^{-/-}$ mice to generate *Sp140*$^{-/-}$*Il1rn*$^{-/-}$, *Acod1*$^{-/-}$*Il1rn*$^{-/-}$, and *Nos2*$^{-/-}$*Il1rn*$^{-/-}$ mice. *Sp140*$^{-/-}$*Il1rn*^{fl} CD64^{Cre} mice were generated by crossing *Sp140*$^{-/-}$ mice with *Il1rn*^{fl} and CD64^{Cre} mice in-house. Adv^{Cre} *Il1r1*$^{fl/fl}$ and CD64^{Cre} *Il1r1*$^{fl/fl}$ mice were generated by crossing Adv^{Cre} and CD64^{Cre} mice with *Il1r1*$^{fl/fl}$ mice in-house.

Bone marrow chimera mice. Adult mice were lethally irradiated with a Precision X-Rad320 X-Ray irradiator (North Branford, CT) using a split dose of 500 rads each round with irradiation occurring 4-12 hours apart. Bone marrow (BM) from donor mice indicated in the figures was prepared by mashing femurs with a mortar and pestle to generate a single cell suspension. Red blood cells were then lysed with ACK lysis buffer (Thermo Fisher Scientific). The cells were then diluted in PBS and 1×10^7 cells (either all one genotype or a 1 : 1 mix of two genotypes for mixed BM chimera mice) were retro-orbitally injected into each recipient mouse. Mice were housed for at least 8 weeks post-injection to permit hematopoietic reconstitution prior to their use in experiments.

***Mtb* infections**. *Mtb* expressing Wasabi (*Mtb*-Wasabi) and *Mtb*-mCherry were generated using *Mtb* Erdman, a gift from S. A. Stanley, that had been passaged 2 or fewer times *in vitro*. *Mtb* was transformed with the pTEC15 plasmid (a gift from Lalita Ramakrishnan; Addgene plasmid # 30174)[122] or the pMSP12::mCherry plasmid (a gift from Lalita Ramakrishnan; Addgene plasmid # 30167) to generate *Mtb*-Wasabi and *Mtb*-mCherry, respectively. For the transformation, *Mtb* was cultured 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 then washed in 10% glycerol to remove salts. Washed *Mtb* was electroporated with 1 µg DNA using a 2 mm electroporation cuvette and the following settings: 2500 volts, 1000 Ohms, 25 µF. Electroporated *Mtb* was grown on 7H11 plates supplemented with 10% oleic acid, albumin, dextrose, and catalase, 0.5% glycerol, and either 200 µg / mL Hygromycin for *Mtb*-Wasabi or 50 µg / mL Kanamycin for *Mtb*-mCherry for 3-4 weeks at 37°C. Colonies were picked and cultured in 10 mL inkwell flasks for 7 days at 37°C and then expanded into a 100 mL culture for 4-5 days at 37°C using 7H9 medium supplemented with 10% albumin-dextrose-saline, 0.4% glycerol, 0.05% Tween-80, and either Hygromycin for *Mtb*-Wasabi or Kanamycin for *Mtb*-mCherry. At log phase, the Mtb was

filtered through a 5 µm syringe filter and frozen in 1 mL aliquots in 10% glycerol. Frozen cultures were diluted in distilled water to an O.D. of 0.0017 and then aerosolized with an inhalation exposure system (Glas-Col, Terre Haute, IN) thereby delivering ~10-100 Mtb per mouse as determined by plating lungs day 1 post-infection to assess bacterial burden.

Tissue Processing for CFU and Flow cytometry. Mice were harvested at 24-28 days following *Mtb* infection. Lungs were collected in gentleMACS C tube (Miltenyi Biotec) containing 3 mL of RPMI media with 70 µg / mL of Liberase TM (Roche) and 30 µg / mL of DNase I (Roche) and then processed into chunks using the lung_01 setting on a gentleMACS (Miltenyi Biotec). Samples were then digested at 37°C for 30 minutes before being homogenized with the lung_02 setting on the gentleMACS. 2 mL of PBS with 20% Newborn Calf Serum (Thermo Fisher Scientific) were added to each sample to quench the digest before samples were filtered through 70 µm SmartStrainers (Miltenyi Biotec).

Measuring Bacterial Burden. *Mtb* burden was assessed by serial dilution of the lung single cell suspension in phosphate-buffered saline (PBS) with 0.05% Tween-80. The dilutions were plated on 7H11 plates supplemented with 10% oleic acid, albumin, dextrose, and catalase and 0.5% glycerol and colonies were counted ~3 weeks after plating.

Flow Cytometry. Flow cytometry was performed by staining 100 µLs of lung single cell suspension with antibodies in PBS with 2% Newborn Calf Serum and 0.05% Sodium azide. A fixable viability dye (Ghost Dye Violet 510 or 540 or Red 780; Tonbo Biosciences), Super Bright Complete Staining Buffer (Thermo Fisher Scientific), and True-Stain Monocyte Blocker (BioLegend) were also added to every sample during surface antibody staining. Surface stains were incubated for 30-60 minutes at room temperature using a selection of the following antibodies: TruStain FcX PLUS (S17011E, BioLegend), BUV496-labeled CD45 (30-F11, BD Biosciences), BUV395-labeled CD11b (M1/70, BD Biosciences), BUV737-labeled CD11c (HL3, BD Biosciences), APC-R700-labeled Siglec F (E50-2440, BD Biosciences), PE-labeled MerTK (DS5MMER, Thermo Fisher Scientific), Super Bright 645-labeled MHC II (M5/114.15.2, Thermo Fisher Scientific), BV421-labeled PD-L1 (MIH5, BD Biosciences), BV711-labeled Ly6C (HK1.4 , BioLegend), PE-Cy7-labeled MerTK (DS5MMER, Thermo Fisher Scientific), APC-labeled CD64 (X54-5/7.1, BioLegend), BUV563-labeled Ly6G (1A8, BD Biosciences), BV605-labeled MHC II (M5/114.15.2, BioLegend), Percp-Cy5.5-labeled CD9 (MZ3, BioLegend), eFluor 450-labeled Ly6D (49-H4, Thermo Fisher Scientific), BV480-labeled Fcer1a (MAR-1, BD Biosciences), BV650-labeled CD8α (53-6.7, BioLegend), BV785-labeled CD16.2 (9E9, BioLegend), AF594-labeled TREM2 (237920, R&D systems), AF647-labeled XCR1 (ZET, XCR1), APC-Fire 750-labeled CD19 (6D5, BioLegend), APC-Fire 750-labeled CD90.2 (30-H12, BioLegend), BUV615-labeled SIRPα (P84, BD Biosciences), BUV661-labeled B220 (RA3-6B2, BD Biosciences), BUV805-labeled CD26 (H194-112, BD Biosciences). Following surface staining, samples were washed with PBS with 2% Newborn Calf Serum and 0.05% Sodium azide and then fixed for 20 minutes at room temperature with cytofix/cytoperm (BD biosciences) prior to removal from the BSL3. If intracellular staining was performed, samples were incubated overnight at 4°C with combinations of the following antibodies: PE-Cy7-labeled CD63 (NVG-2, BioLegend), AF532-labeled iNOS (CXNFT, Thermo Fisher Scientific), and Percp-eFluor 710-labeled iNOS (CXNFT, Thermo Fisher Scientific). After washing out the intracellular stain, each sample received AccuCheck Counting Beads (Invitrogen) and was then analyzed on an Aurora (Cytek) flow cytometer. Data were analyzed with Flowjo version 10 (BD Biosciences).

Confocal microscopy. A Zeiss LSM 880 laser scanning confocal microscope with 405, 458, 488, 514, 561, 594, and 633 lasers, with two photomultiplier detectors, a 34-channel GaASP spectral detector system, and a 2-channel AiryScan detector was used for microscopy. 20 µm

sections were prepared from paraformaldehyde-fixed lungs from *Mtb*-mCherry-infected B6 and *Sp140*$^{-/-}$ mice. The sections were stained overnight at 4°C with the following antibodies: BV421-labeled SIRPα (P84, BD Biosciences), Pacific Blue–labeled B220 (RA3-6B2, BioLegend), eF506-labeled CD4 (RM4-5, BioLegend), and AF647-labeled Ly6G (1A8, BioLegend). Diseased and healthy lesions were identified in stained sections with a 5× air objective and then imaged as a 20 µm z-stack acquired at a 1.5 µm step size with a 63× oil immersion objective lens with a numerical aperture of 1.4. The 63× oil immersion objective lens with a numerical aperture of 1.4 was also used to image single color-stained Ultracomp eBeads Plus (Thermo Fisher Scientific) for generating a compensation matrix.

Image processing and histo-cytometry analysis. The Generate Compensations Matrix script[123] was used in ImageJ to create a compensation matrix based on the single color-stained control images of the Ultracomp eBeads Plus (Thermo Fisher Scientific). The resulting compensation matrix was used for spectral unmixing of the lung images using Chrysalis software[123], which was also used to rescale the data and generate new channels based on mathematical operations performed on existing channels. Imaris 9.9.1 (Bitplane) was used on the processed lung images to digitally identify cells based on protein expression using the surface creation tool[124]. Statistics for the identified cells were exported from Imaris and then imported into FlowJo version 10 (BD Biosciences) for quantitative image analysis.

Sorting Immune Cells for scRNA-seq analysis. For the myeloid dataset, lungs from 3 *Mtb*-Wasabi-infected as well as from 3 naïve *Acod1*$^{-/-}$ and 3 naïve *Nos2*$^{-/-}$ mice were processed as described for CFU and flow cytometry analysis 25 days after infection with the following slight modification: 3 minutes before harvesting, 0.25 µg of TotalSeq-A-labeled CD45.2 (104, BioLegend) was intravenously injected into each mouse to label cells in the vasculature[125]. Surface staining of lung single cell suspensions was performed in PBS with 2% Newborn Calf Serum on ice for 30 minutes with the following antibodies: TruStain FcX PLUS (S17011E, BioLegend), APC-labeled Ly6G (1A8, BioLegend), APC-labeled CD64 (X54-5/7.1, BioLegend), and TotalSeq-A-labeled Ly6G (1A8, BioLegend). Magnetic enrichment was then performed with the EasySep APC Positive Selection Kit II (StemCell Technologies) and MojoSort Magnets (BioLegend) to increase the frequency of myeloid cells within the sample prior to sorting. Following enrichment, samples were stained on ice for 45 minutes in PBS with 2% Newborn Calf Serum and True-Stain Monocyte Blocker (BioLegend) using a combination of the following antibodies: TotalSeq-A-labeled Ly6C (HK1.4, BioLegend), TotalSeq-A-labeled CD44 (IM7, BioLegend), TotalSeq-A-labeled CD274 (MIH6 , BioLegend), TotalSeq-A-labeled Siglec F (S17007L, BioLegend), TotalSeq-A-labeled CSF1R (AFS98, BioLegend), TotalSeq-A-labeled CD11b (M1/70, BioLegend), TotalSeq-A-labeled CD86 (GL-1, BioLegend), TotalSeq-A-labeled MHC II (M5/114.15.2, BioLegend), TotalSeq-A-labeled CX3CR1 (SA011F11, BioLegend), TotalSeq-A-labeled CD11c (N418, BioLegend), TotalSeq-A-labeled CCR2 (SA203G11, BioLegend), TotalSeq-A-labeled CD62L (MEL-14, BioLegend), anti-mouse TotalSeq-A Hashtag antibody (1-6; BioLegend), PE-labeled Siglec F (S17007L, BioLegend), Pacific Blue-labeled B220 (RA3-6B2, BioLegend), and Pacific Blue-labeled CD90.2 (53-2.1, BioLegend). The TotalSeq-A-labeled antibodies detect protein expression in the scRNA-seq dataset, while the Hashtag antibodies allow several samples to be multiplexed together in a single lane on a 10X Genomics Chromium Next GEM Chip[126]. After staining, the samples were incubated with Sytox Blue Dead Cell Stain (Thermo Fisher Scientific) and sort purified using a 100 µm sorting chip in a 4 laser SH-800 cell sorter (Sony) on the purity setting. For *Mtb*-infected mice, *Mtb*-infected cells and bystander myeloid cells were individually purified, while samples from naïve lungs were sorted as macrophages and a mixture of neutrophils and monocytes that were then combined at a 1:2 ratio for greater representation of macrophages in the scRNA-seq dataset.

The stromal cell dataset was generated by infecting BM chimera mice made by transferring CD45.1 BM into lethally irradiated B6 or *Il1r1*$^{-/-}$ hosts with *Mtb* 8 weeks post-reconstitution. Lungs from the infected animals as well as naïve chimera mice were harvested and processed as described for CFU and flow cytometry analysis. ACK lysing buffer (Thermo Fisher Scientific) was used to lyse the red blood cells in the samples, which were then stained for 30 minutes on ice with TruStain FcX PLUS (S17011E, BioLegend) and PE-Cy7-labeled CD45 (30-F11, BioLegend) antibody. The EasySep PE Positive Selection Kit II (StemCell Technologies) and MojoSort Magnets (BioLegend) were used to perform negative selection to enrich the CD45^{-} non-hematopoietic cells. The enriched cells were incubated with Sytox Green Dead Cell Stain (Thermo Fisher Scientific) and live CD45^{-} were sort purified using a 130 µm sorting chip in a 4 laser SH-800 cell sorter (Sony) on the purity setting.

The T cell and ILC datasets were generated by *Mtb* infection of mixed BM chimera mice generated by transferring WT CD45.1/2 and *Il1r1*$^{-/-}$ BM into lethally irradiated CD45.1 mice. Lungs were harvested 25 days post-infection and processed as described for measuring CFU and performing flow cytometry. Three mice were used for the T cell dataset, while the rarity of the ILCs required pooling lungs into 3 samples (2 samples containing 5 lungs and 1 sample containing 6 lungs). The T cell samples were treated with ACK lysing buffer (Thermo Fisher Scientific) to lyse the red blood cells and then stained on ice for 30 minutes with the following antibodies: BV421-labeled CD45.1 (A20, BioLegend), BV785-labeled CD45.2 (104, BioLegend), PE-labeled CD3e (17A2, BioLegend), APC-labeled CD11b (M1/70, Cytek), APC-labeled CD11c (N418, Cytek), APC-labeled CD19 (1D3, Cytek), TotalSeq-A-labeled Ly6C (HK1.4, BioLegend), TotalSeq-A-labeled CD44 (IM7, BioLegend), TotalSeq-A-labeled CD274 (MIH6 , BioLegend), TotalSeq-A-labeled CX3CR1 (SA011F11, BioLegend), TotalSeq-A-labeled CD62L (MEL-14, BioLegend), TotalSeq-A-labeled TCRβ (H57-597, BioLegend), TotalSeq-A-labeled CD49a (, BioLegend), TotalSeq-A-labeled CD49a (HMα1, BioLegend), TotalSeq-A-labeled NKp46 (29A1.4, BioLegend), TotalSeq-A-labeled CD4 (RM4-5, BioLegend), TotalSeq-A-labeled ST2 (DIH9, BioLegend), TotalSeq-A-labeled CD25 (PC61, BioLegend), TotalSeq-A-labeled CD45.1 (A20, BioLegend), TotalSeq-A-labeled CD45.2 (104, BioLegend), TotalSeq-A-labeled CD8a (53-6.7, BioLegend), TotalSeq-A-labeled CXCR5 (L138D7, BioLegend), TotalSeq-A-labeled CCR6 (29-2L17, BioLegend), and anti-mouse TotalSeq-A Hashtag antibody (1-3; BioLegend). The stained T cell samples were then incubated with Sytox Green Dead Cell Stain (Thermo Fisher Scientific) and T cells (Live CD3e^{+} CD11b^{-} CD11c^{-} CD19^{-} CD45.1^{+} or CD45.2^{+} cells) were sort purified using a 100 µm sorting chip in a 4 laser SH-800 cell sorter (Sony) on the purity setting.

The ILC samples treated with ACK lysing buffer (Thermo Fisher Scientific) were stained on ice for 25 minutes with the following panel of lineage-specific antibodies: Biotin-labeled TER-119 (TER-119, BioLegend), Biotin-labeled GR-1 (RB6-8C5, BioLegend), Biotin-labeled CD3e (145-2C11, BioLegend), Biotin-labeled CD11b (M1/70, BioLegend), Biotin-labeled B220 (RA3-6B2, BioLegend), and Biotin-labeled TCRγ/δ (GL3, BioLegend). Lineage^{-} cells were enriched by negative selection using the EasySep Streptavidin RapidSpheres Isolation Kit (StemCell Technologies) and MojoSort Magnets (BioLegend). Enriched samples were then stained on ice for 30 minutes with the following antibodies: BV421-labeled CD45.1 (A20, BioLegend), BV785-labeled CD45.2 (104, BioLegend), PE-labeled CD127 (A7R34, BioLegend), Alexa Fluor 700-labeled CD127 (30-H12, BioLegend), Biotin-labeled CD5 (53-7.3, BioLegend), Biotin-labeled TCRβ (H57-597, BioLegend), TotalSeq-A-labeled Ly6C (HK1.4, BioLegend), TotalSeq-A-labeled CD44 (IM7, BioLegend), TotalSeq-A-labeled CD274 (MIH6 , BioLegend), TotalSeq-A-labeled CX3CR1 (SA011F11, BioLegend), TotalSeq-A-labeled CD62L (MEL-14, BioLegend), TotalSeq-A-labeled TCRβ (H57-597, BioLegend), TotalSeq-A-labeled CD49a (, BioLegend), TotalSeq-A-labeled CD49a (HMα1, BioLegend), TotalSeq-A-labeled NKp46 (29A1.4, BioLegend), TotalSeq-A-labeled CD4 (RM4-5, BioLegend), TotalSeq-A-labeled ST2 (DIH9, BioLegend), TotalSeq-A-labeled CD25 (PC61, BioLegend), TotalSeq-A-labeled CD45.1 (A20, BioLegend), TotalSeq-A-labeled CD45.2 (104, BioLegend), TotalSeq-A-labeled CD8a (53-6.7,

BioLegend), TotalSeq-A-labeled CXCR5 (L138D7, BioLegend), TotalSeq-A-labeled CCR6 (29-2L17, BioLegend), and anti-mouse TotalSeq-A Hashtag antibody (4-6; BioLegend). The enriched samples were then stained with APC-labeled Streptavidin (BioLegend) for 15 minutes on ice before being stained with Sytox Green Dead Cell Stain (Thermo Fisher Scientific). ILCs (Lineage^{-} IL-7Ra^{+} CD90.2^{+} CD45.1^{+} or CD45.2^{+} cells) were sort purified using a 100 µm sorting chip in a 4 laser SH-800 cell sorter (Sony) on the purity setting.

Single cell RNA: Library generation and sequencing. The v3.1 chemistry Chromium Single Cell 3' Reagent Kit (10X Genomics) was used to generate scRNA-seq libraries using the CITE-seq protocol[46] modifications for the myeloid, T, and innate lymphoid cell libraries and the standard 10X protocol for the stromal cell library. The myeloid cell dataset required three lanes of a Chromium Next GEM Chip, with lane 1 containing naïve cells, lane 2 containing *Mtb*-infected cells, and lane 3 containing the bystander cells from the 3 *Acod1*$^{-/-}$ and 3 *Nos2*$^{-/-}$ mice. Each lane was super-loaded with ~34,000 cells for a target of 17,000 single cells per lane at a multiplet rate of 3%[126]. The stromal cell dataset used 4 lanes of a Chromium Next GEM Chip, with lane 1 containing *Il1r1*$^{-/-}$ stromal cells from *Mtb*-infected mice, lane 2 containing *Il1r1*$^{-/-}$ stromal cells from naïve mice, lane 3 containing wild-type stromal cells from *Mtb*-infected mice, and lane 4 containing wild-type stromal cells from naïve mice. Each lane was loaded with 12,500 cells for an expected yield of 7,000 single cells at a multiplet rate of 5%. The T cell and ILC libraries were generated using the same Chromium Next GEM Chip, with lane 1 containing T cells and lane 2 containing ILCs from *Mtb*-infected mixed BM chimera mice. 25,000 cells were super-loaded into each lane with a target capture of 13,200 cells at a multiplet rate of 4%[126]. For the CITE-seq libraries, 0.5 U/µL RNaseOUT Recombinant Ribonuclease Inhibitor (Invitrogen) was added to single cell RT master mix during the loading step and 1 µL of ADT and HTO additive primers (0.2 µM stock) were added during the cDNA amplification. All samples were decontaminated by 2 rounds of centrifugation through 0.2 µM filter microcentrifuge tubes following cDNA, ADT, and HTO purification and then removed from the BSL3. The CITE-seq protocol was used to complete the ADT and HTO libraries while the 10X Genomics protocol was followed for the cDNA library. Quality control of the libraries was performed with a Fragment Analyzer (Agilent). Libraries were pooled and then sequenced on a NovaSeq 6000 (Illumina) using an S1 flow cell and the following cycles read 1 (28 cycles), i7 index (10 cycles), i5 index (10 cycles), read 2 (90 cycles).

ScRNA-seq: data processing. Sequencing reads of the mRNA libraries were mapped to the mouse genome with CellRanger version 6.0.0 (10X Genomics), while the raw count matrices for the ADT and HTO libraries were generated with CITE-Seq-Count version 1.4.3[127]. The count matrices were analyzed with Seurat v4.3.0[45] using default settings for normalizing the data, finding variable features, and scaling the data. For CITE-seq datasets, multiplets were excluded through HTO demultiplexing using the HTODemux function. The myeloid cell dataset was filtered to include cells with between 200 and 4500 genes and less than 5% mitochondrial reads. The T cell, ILC, and stromal cell datasets were filtered to only include cells that have between 200 and 2500 genes and less than 5% mitochondrial reads. The resulting myeloid dataset as well as our previously published scRNA-seq datasets for myeloid cells in *Mtb*-infected and naïve B6 and *Sp140*$^{-/-}$ mice (GSE216023) were integrated together and then clustered with a resolution of 1.5. The T cell and ILC datasets were analyzed with the default Seurat workflow using weighted nearest neighbor multimodal analysis to cluster cells based on mRNA and protein expression at a resolution of 1. The stromal cell dataset was also analyzed with Seurat using integration to combine the *Mtb*-infected and naïve WT and *Il1r1*$^{-/-}$ datasets and then clustering the cells based on mRNA expression using a resolution of 1.5.

Gene signature and ROC curve analysis. Our previously published signatures for type I IFN and IFN-γ[17] were applied to our myeloid cell scRNA-seq dataset to score cells based on their gene expression using the UCell package[128]. Signatures for the B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice were generated in R[129] by identifying genes induced at least 8-fold in the myeloid cells from *Mtb*-infected mice relative to naïve animals for each genotype. A corresponding human signature was produced using the convert_mouse_to_human_symbols from the NichetNetr package[66]. The hacksig package using the singscore statistical method[130] was applied to calculate a signature score based on each signature's expression in the TANDEM cohort PBMC RNA-seq data[47]. The pROC package[131] was used to generate the ROC curves and calculate the area under the curve to quantify the ability of each signature to discriminate between the active *Mtb* cases and healthy controls in the TANDEM cohort. The hacksig and pROC packages were also used to test the predictive ability of a gene signature consisting of known immunosuppressive genes (*IL1RN*, *IL18BP*, *CD274*, *TGFB1*, *IDO1*, *IL10*, and *ARG1*) on the TANDEM cohort. The pROC package was also used to determine the ability of IL1RN expression to identify active *Mtb* cases in the TANDEM cohort.

NicheNet analysis of IL-1-regulated signaling. IL-1-regulated genes were defined as genes expressed at least 2-fold higher in WT than *Il1r1*$^{-/-}$ cells in the T cell, innate lymphoid cell, and stromal cell scRNA-seq datasets. These IL-1-regulated genes were used as the potential ligands for NicheNet analysis[66]. The receiver cells were defined as IMs, *Trem2*$^{+}$ IMs, and *Spp1*$^{+}$ IMs from the myeloid cell scRNA-seq dataset. The expressed receptors on the receiver cells were identified using the get_expressed_genes function from NicheNet with a 10% cutoff for expression and then filtering the NicheNet receptor database to those genes expressed in the IM populations. Ligand activity in the receiver cells was calculated by identifying genes upregulated in *Trem2*$^{+}$ or *Spp1*$^{+}$ IMs relative to the IM cell population via the FindMarkers function from the Seurat package[45], focusing on genes upregulated at least 2-fold and with an adjusted p value less than 0.05, as calculated by the Wilcoxon Rank-Sum test with Bonferroni correction. The ability of each IL-1-regulated ligand to induce the genes upregulated in *Trem2*$^{+}$ or *Spp1*$^{+}$ IMs was determined by NicheNet's predict_ligand_activities function.

Statistical analysis. Statistical significance was determined using Prism (GraphPad) software for unpaired or paired two-tailed Student t test when comparing two populations, or one-way ANOVA tests with Tukey's multiple comparisons test when comparing multiple groups. ROC curves and their AUC were calculated in R using the pROC package.

Data availability. Raw and processed single cell RNA-sequencing data is deposited at NCBI Gene Expression Omnibus: GSE255213, GSE254983, and GSE254926.

Code availability. Code for scRNA-sequencing analysis is available on Github: https://github.com/dmitrikotov/IL-1Ra-TB.

Supplementary Figure Legends

Supplementary Figure 1. Classifying myeloid scRNA-seq clusters. Dot plot of lineage of markers used to (**a**) identify myeloid cell clusters and (**b**) define neutrophil cluster heterogeneity.

Supplementary Figure 2. Naïve *Sp140*-, *Nos2*-, and *Acod1*-deficient mouse lungs have few differentially expressed genes relative to naive B6 lungs. (**a**) The myeloid populations in the naïve lungs of B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice (n = 3 per genotype). (**b**) Number of shared and unique genes differentially expressed (2-fold change and adjusted p value < 0.05)

between by neutrophils, alveolar macrophages, monocytes, and interstitial macrophages in *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ relative to those from B6 mice. Statistical significance in (b) was calculated with the Wilcoxon Rank-Sum test with Bonferroni correction.

Supplementary Figure 3. Mouse gene signatures consisting of genes upregulated in myeloid cells following *Mtb* infection of B6, *Sp140*$^{-/-}$, *Acod1*$^{-/-}$, or *Nos2*$^{-/-}$ mice. A heatmap depicting the $\log_2$ fold change of gene upregulation following *Mtb* infection relative to the gene's expression in myeloid cells from naïve lungs. The heatmap compares gene expression for signature genes between B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice (n = 3 per genotype). Grey boxes indicate genes not present in the gene signature of a given mouse genotype. Genes in blue indicate those with known immunosuppressive function.

Supplementary Figure 4. Flow cytometric identification of monocyte and monocyte-derived macrophage subsets identified by scRNA-seq. (**a**) Overview of monocyte and interstitial macrophage subsets and (**b**) their expression of subset identifying markers. (**c**) Myeloid cell flow cytometry gating strategy. (**d**) Frequency of *Mtb*-infected cells among the major myeloid cell populations.

Supplementary Figure 5. IL-1Ra expression in mice and humans. (**a**) Myeloid cell expression of *Il1r2* and *Il1rn* in naïve and *Mtb*-infected lungs from B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice (n = 3 mice per genotype for naïve lungs and 3 mice per genotype for *Mtb*-infected lungs) as measured by scRNA-seq. (**b**) *Il1rn* expression in myeloid cells from the lungs of B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice (n = 3 mice per genotype for naïve lungs and 3 mice per genotype for *Mtb*-infected lungs). (**c**) Normalized *IL1RN* expression in *Mtb*-infected (n = 151) and healthy individuals (n = 98) from the TANDEM cohort (analysis of GSE114192). (**d**) ROC curve of the predictive ability of an immunosuppressive gene signature applied to the TANDEM cohort. Statistical significance in (c) was calculated using the Wald test and corrected for multiple testing using the Benjamini and Hochberg method.

Supplementary Figure 6. Myeloid cells express very little IL-1 receptor and IM expression of IL-1 receptor does not impact bacterial control in a cell-intrinsic manner. (**a**) The myeloid populations identified by scRNA-seq and (**b**) *Il1r1* expression in myeloid cells from the lungs of B6, *Nos2*$^{-/-}$, *Acod1*$^{-/-}$, and *Sp140*$^{-/-}$ mice (n = 3 mice per genotype for naïve lungs and 3 mice per genotype for *Mtb*-infected lungs). (**c**) Mixed BM chimera schematic, (**d**) representative flow plot, and (**e**) quantification of the frequency of *Mtb* infection among lung wild-type CD45.1^{+} and *Il1r1*$^{-/-}$ CD45.1^{-} IMs (n = 8) 25 days post-infection. Pooled data from two independent experiments is shown in (e). Statistical significance in (e) was calculated with a paired t test.

Supplementary Figure 7. Classifying T cell, ILC, and stromal cell scRNA-seq clusters. Dot plot of lineage of markers used to annotate the clusters in our (**a**) T cell dataset, (**b**) ILC dataset, and (**c**) stromal cell dataset.

Supplementary Figure 8. Neuronal IL-1 signaling does not impact *Mtb* control. (**a**) Experimental setup and (**b**) lung bacterial burden of bone marrow chimera mice with wild-type neurons (CD45.1 host, n = 13), littermate control neurons (Adv$^{+/+}$ Il1r1$^{fl/fl}$ host, n = 9), and IL-1 receptor-deficient neurons (Adv$^{Cre/+}$ Il1r1$^{fl/fl}$ host, n = 14) 25 days after *Mtb* infection. Pooled data from two independent experiments is shown in (b). Statistical significance in (b) was calculated with a one-way ANOVA with Tukey's multiple comparison test.

Supplementary Figure 9. NicheNet analysis of IL-1 regulated ligands that could activate interstitial macrophages. (**a**) List of IL-1 regulated potential ligands identified in stromal and hematopoietic cells. (**b**) Receptor expression on interstitial macrophages for the IL-1-regulated ligands. (**c**) Predicted ligand activity based on genes differently expressed by the activated interstitial macrophage subsets.

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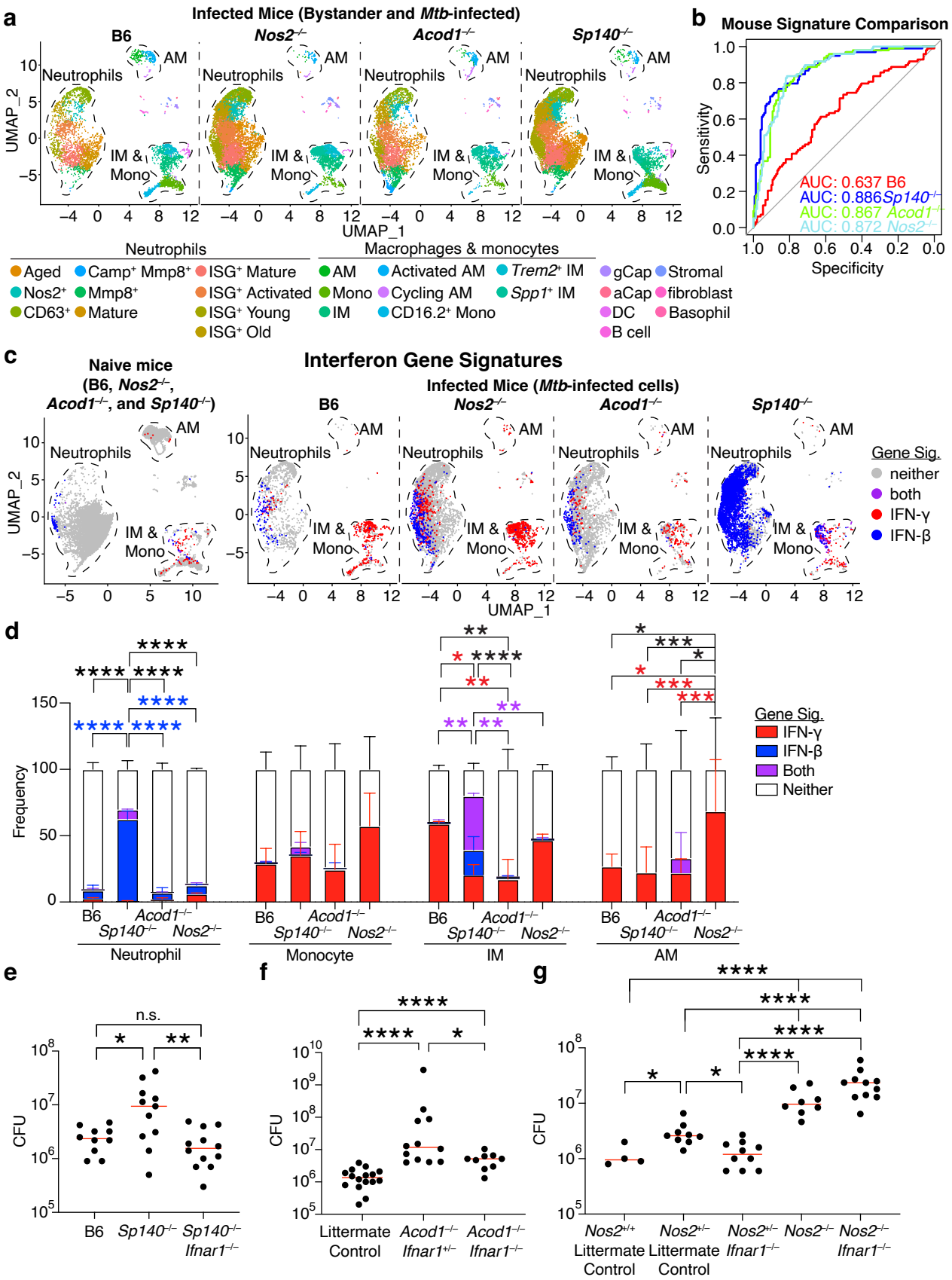
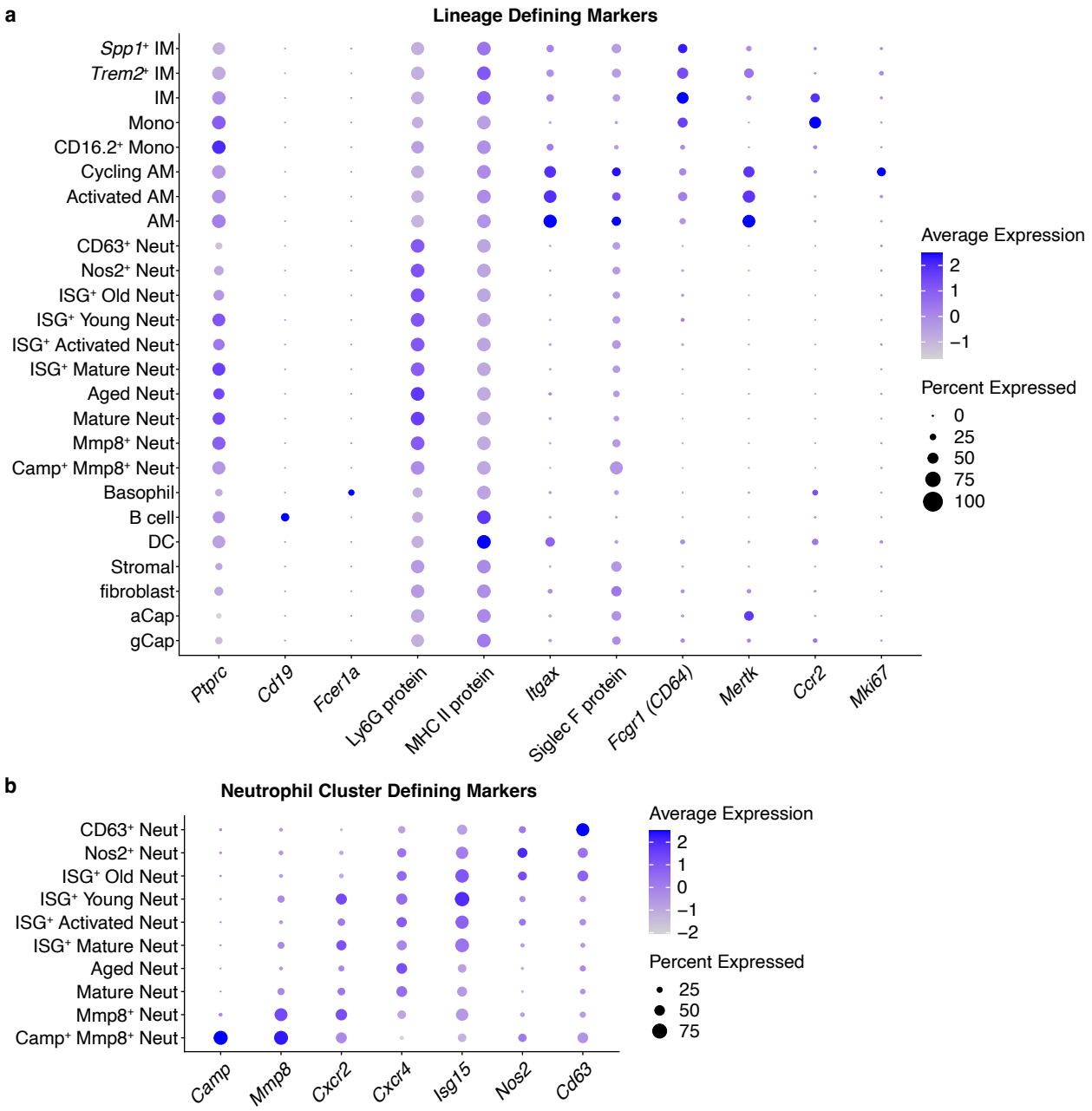
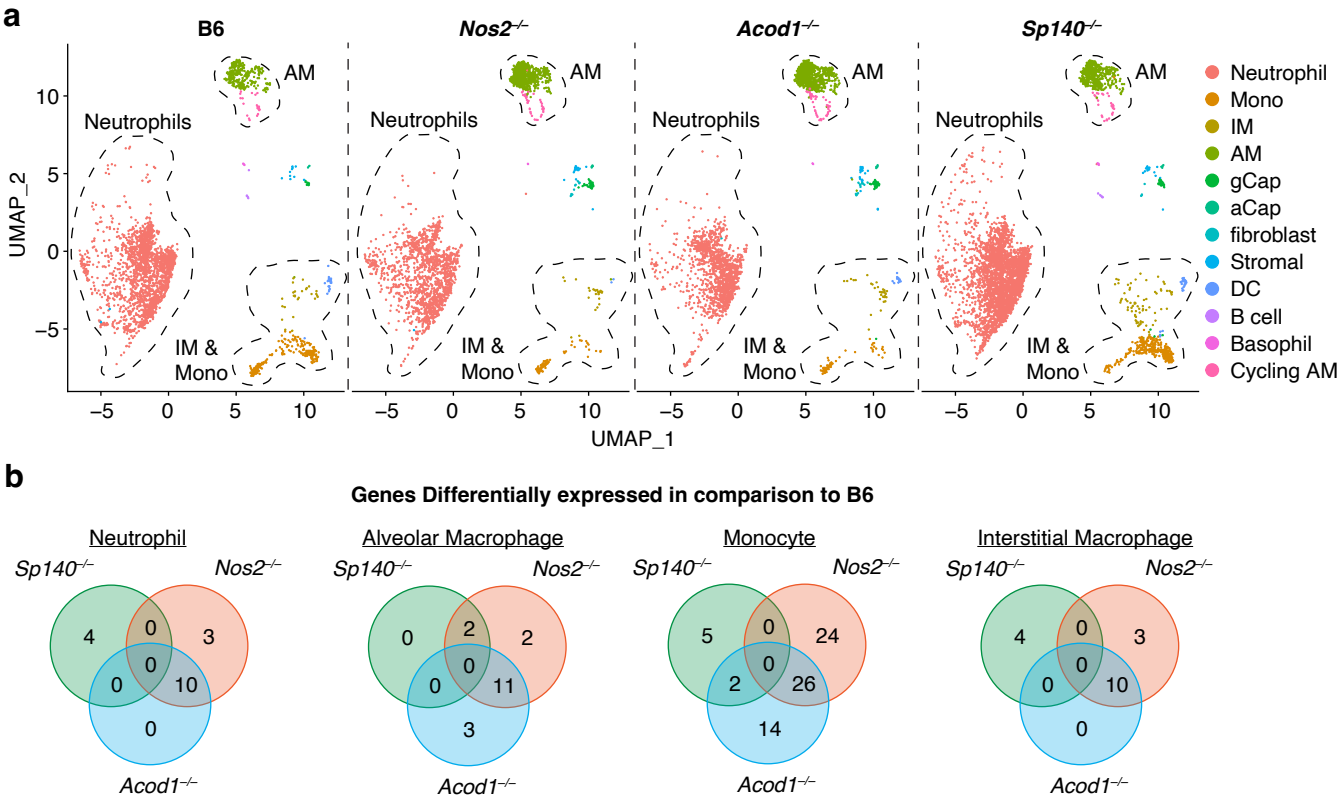

a
Infected Mice (Bystander and Mtb-infected)
B6
Nos2-/-
Acod1-/-
Sp140-/-
AM
Neutrophils
IM & Mono
UMAP_2
UMAP_1
Neutrophils
Aged
Camp+ Mmp8+
ISG+ Mature
Nos2+
Mmp8+
ISG+ Activated
CD63+
Mature
ISG+ Young
ISG+ Old
Macrophages & monocytes
AM
Activated AM
Trem2+ IM
Mono
Cycling AM
Spp1+ IM
IM
CD16.2+ Mono
gCap
Stromal
aCap
fibroblast
DC
Basophil
B cell
b
Mouse Signature Comparison
Sensitivity
Specificity
AUC: 0.637 B6
AUC: 0.886 Sp140-/-
AUC: 0.867 Acod1-/-
AUC: 0.872 Nos2-/-
c
Naive mice (B6, Nos2-/-, Acod1-/-, and Sp140-/-)
Interferon Gene Signatures
Infected Mice (Mtb-infected cells)
Gene Sig.
neither
both
IFN-γ
IFN-β
d
Frequency
Gene Sig.
IFN-γ
IFN-β
Both
Neither
B6
Acod1-/-
Sp140-/-
Nos2-/-
Neutrophil
Monocyte
IM
AM
e
n.s.
CFU
B6
Sp140-/-
Sp140-/- Ifnar1-/-
f
CFU
Littermate Control
Acod1-/- Ifnar1+/-
Acod1-/- Ifnar1-/-
g
CFU
Nos2+/+ Littermate Control
Nos2+/- Littermate Control
Nos2+/- Ifnar1-/-
Nos2-/- Ifnar1-/-

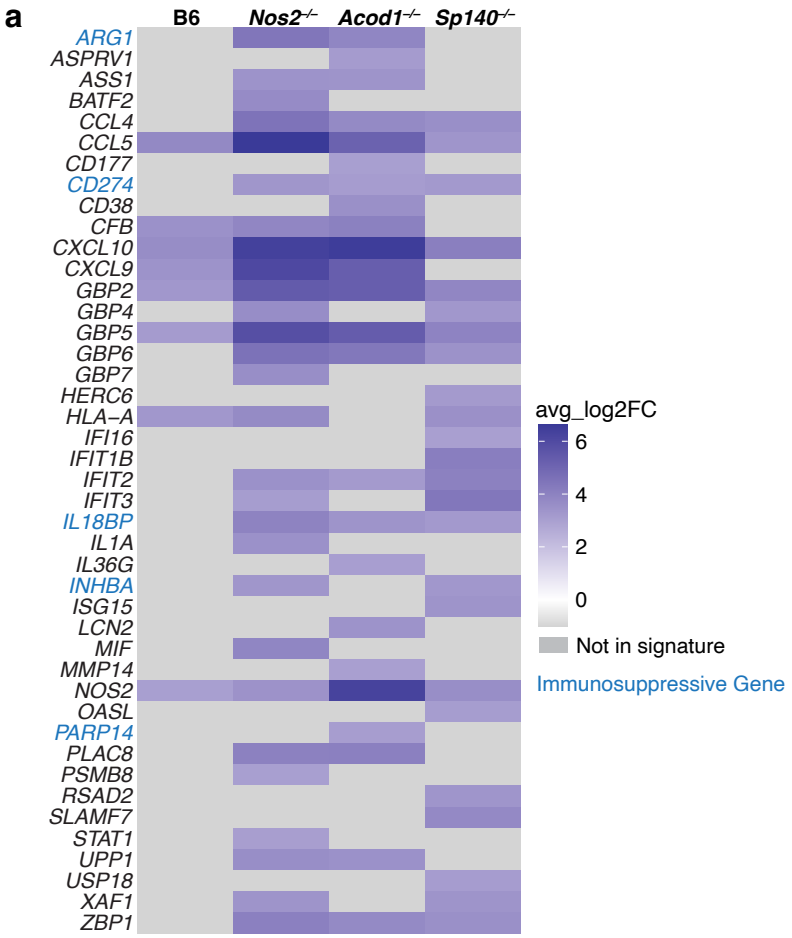
Figure 1.



Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Figure 3.

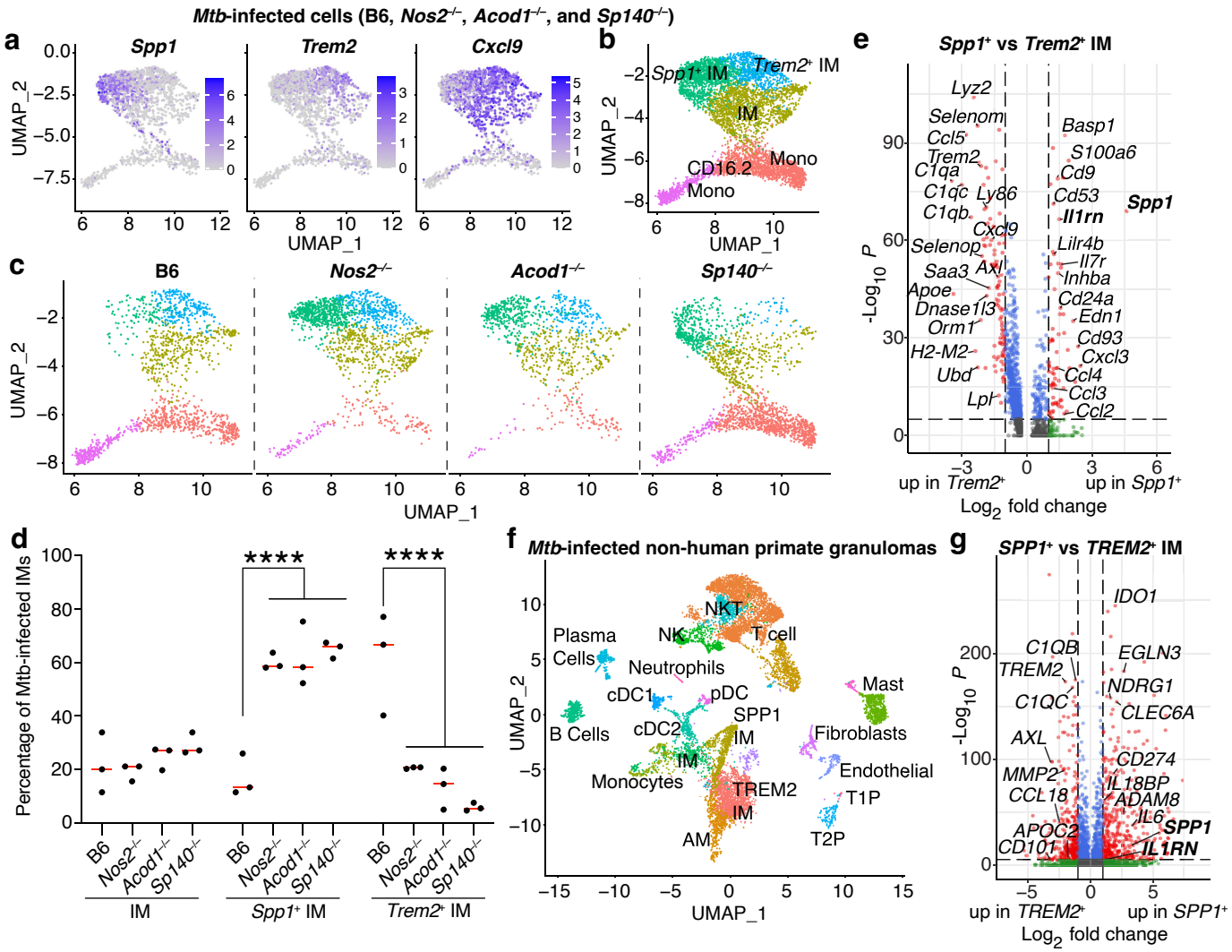
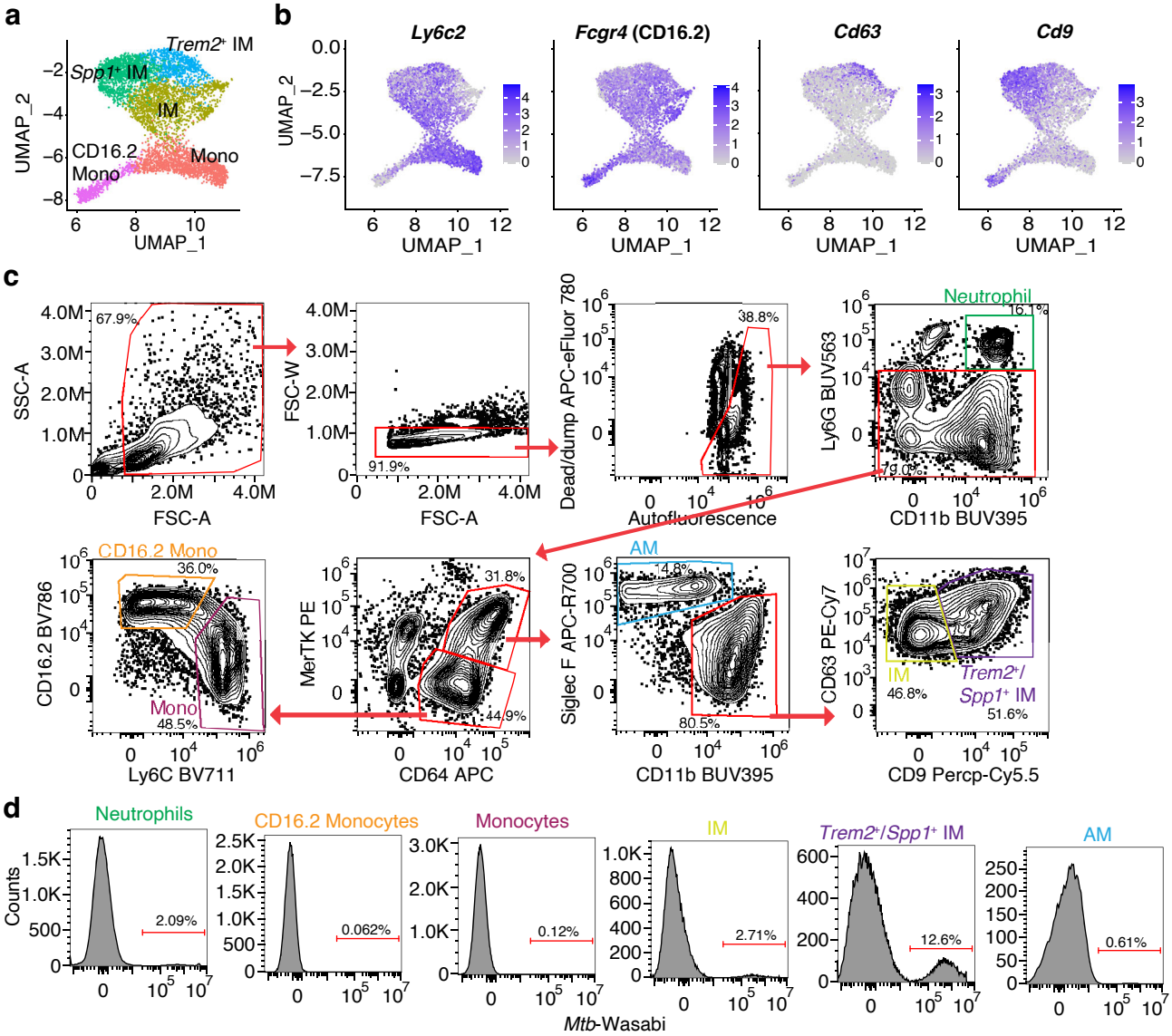

Mtb-infected cells (B6, Nos2-/-, Acod1-/-, and Sp140-/-)
a
Spp1
Trem2
Cxcl9
UMAP_2
UMAP_1
b
Spp1+ IM
Trem2+ IM
IM
Mono
CD16.2
Mono
c
B6
Nos2-/-
Acod1-/-
Sp140-/-
e
Spp1+ vs Trem2+ IM
Lyz2
Selenom
Ccl5
Trem2
C1qa
C1qc
C1qb
Ly86
Cxcl9
Selenop
Saa3
Axl
Apoe
Dnase1l3
Orm1
H2-M2
Ubd
Lpl
Basp1
S100a6
Cd9
Cd53
Il1rn
Spp1
Lilr4b
Il7r
Inhba
Cd24a
Edn1
Cd93
Cxcl3
Ccl4
Ccl3
Ccl2
-Log10 P
up in Trem2+
up in Spp1+
Log2 fold change
d
Percentage of Mtb-infected IMs
****
****
B6
Nos2-/-
Acod1-/-
Sp140-/-
IM
Spp1+ IM
Trem2+ IM
f
Mtb-infected non-human primate granulomas
Plasma Cells
NK
NKT
T cell
Neutrophils
cDC1
pDC
cDC2
B Cells
SPP1 IM
IM
Monocytes
TREM2 IM
AM
Mast
Fibroblasts
Endothelial
T1P
T2P
UMAP_2
UMAP_1
g
SPP1+ vs TREM2+ IM
IDO1
C1QB
TREM2
C1QC
AXL
MMP2
CCL18
APOC2
CD101
EGLN3
NDRG1
CLEC6A
CD274
IL18BP
ADAM8
IL6
SPP1
IL1RN
-Log10 P
up in TREM2+
up in SPP1+
Log2 fold change

Figure 2.



Supplementary Figure 4.

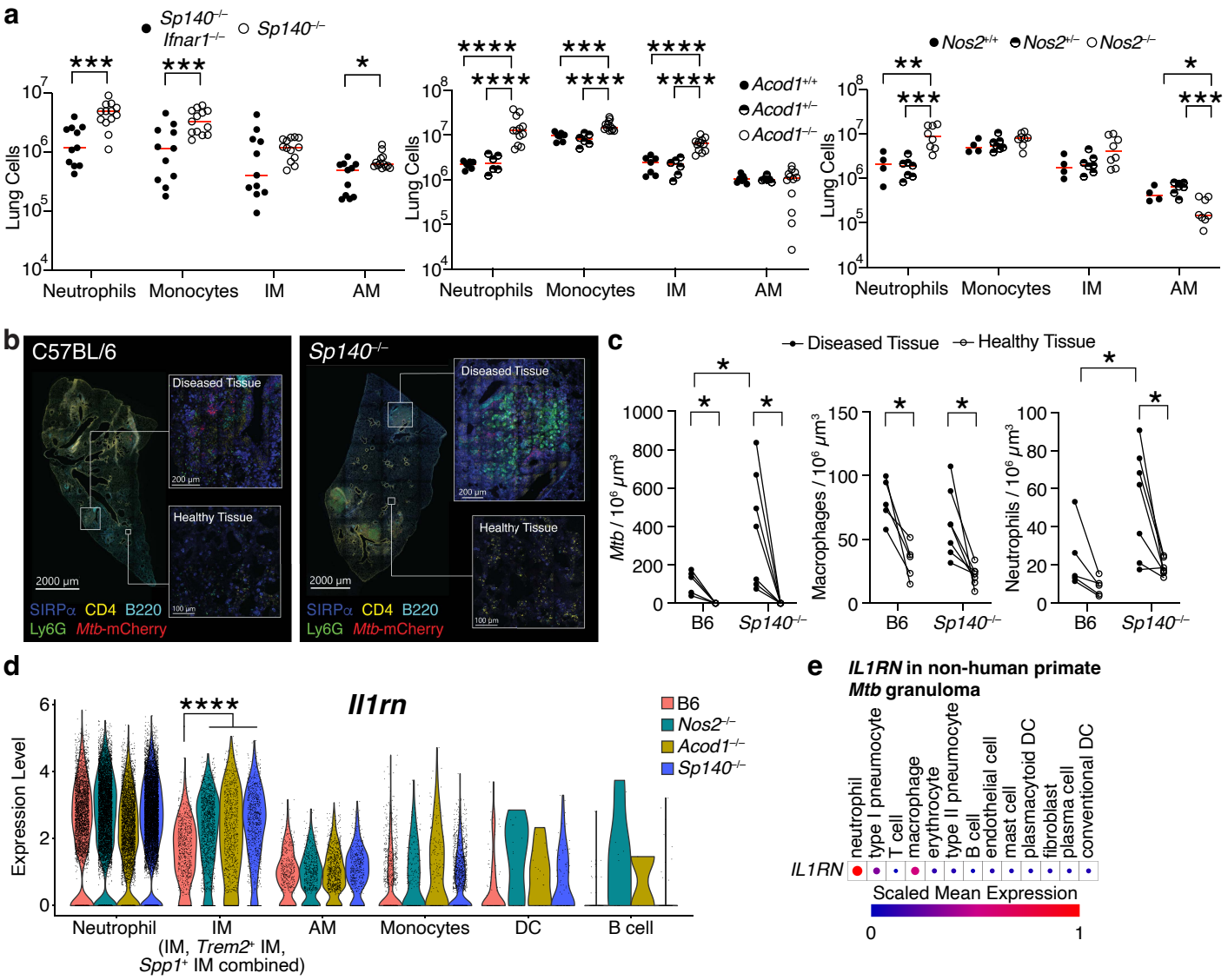
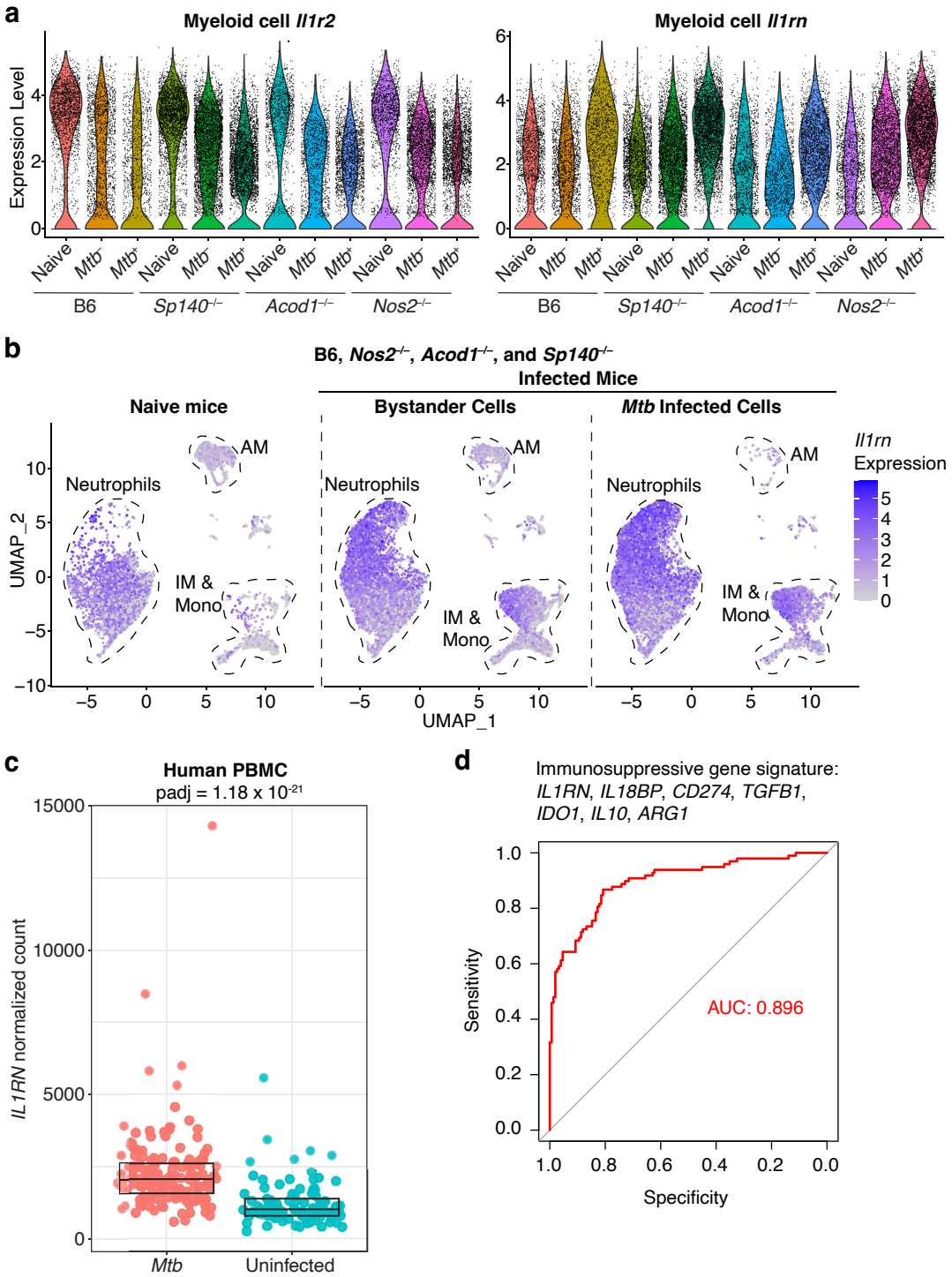

a
Sp140−/− Ifnar1−/−
Sp140−/−
Lung Cells
Neutrophils
Monocytes
IM
AM
Acod1+/+
Acod1+/−
Acod1−/−
Nos2+/+
Nos2+/−
Nos2−/−
b
C57BL/6
Sp140−/−
Diseased Tissue
Healthy Tissue
2000 µm
200 µm
100 µm
SIRPα CD4 B220
Ly6G Mtb-mCherry
c
Diseased Tissue
Healthy Tissue
Mtb / 10^6 µm^3
Macrophages / 10^6 µm^3
Neutrophils / 10^6 µm^3
B6
Sp140−/−
d
Il1rn
Expression Level
B6
Nos2−/−
Acod1−/−
Sp140−/−
Neutrophil
IM
(IM, Trem2+ IM, Spp1+ IM combined)
AM
Monocytes
DC
B cell
e
IL1RN in non-human primate Mtb granuloma
neutrophil
type I pneumocyte
T cell
macrophage
erythrocyte
type II pneumocyte
B cell
endothelial cell
mast cell
plasmacytoid DC
fibroblast
plasma cell
conventional DC
IL1RN
Scaled Mean Expression
0
1
f
IL1RN expression in human PBMC identifies active TB cases vs healthy individuals
Sensitivity
Specificity
AUC: 0.840

Figure 3.



Supplementary Figure 5.

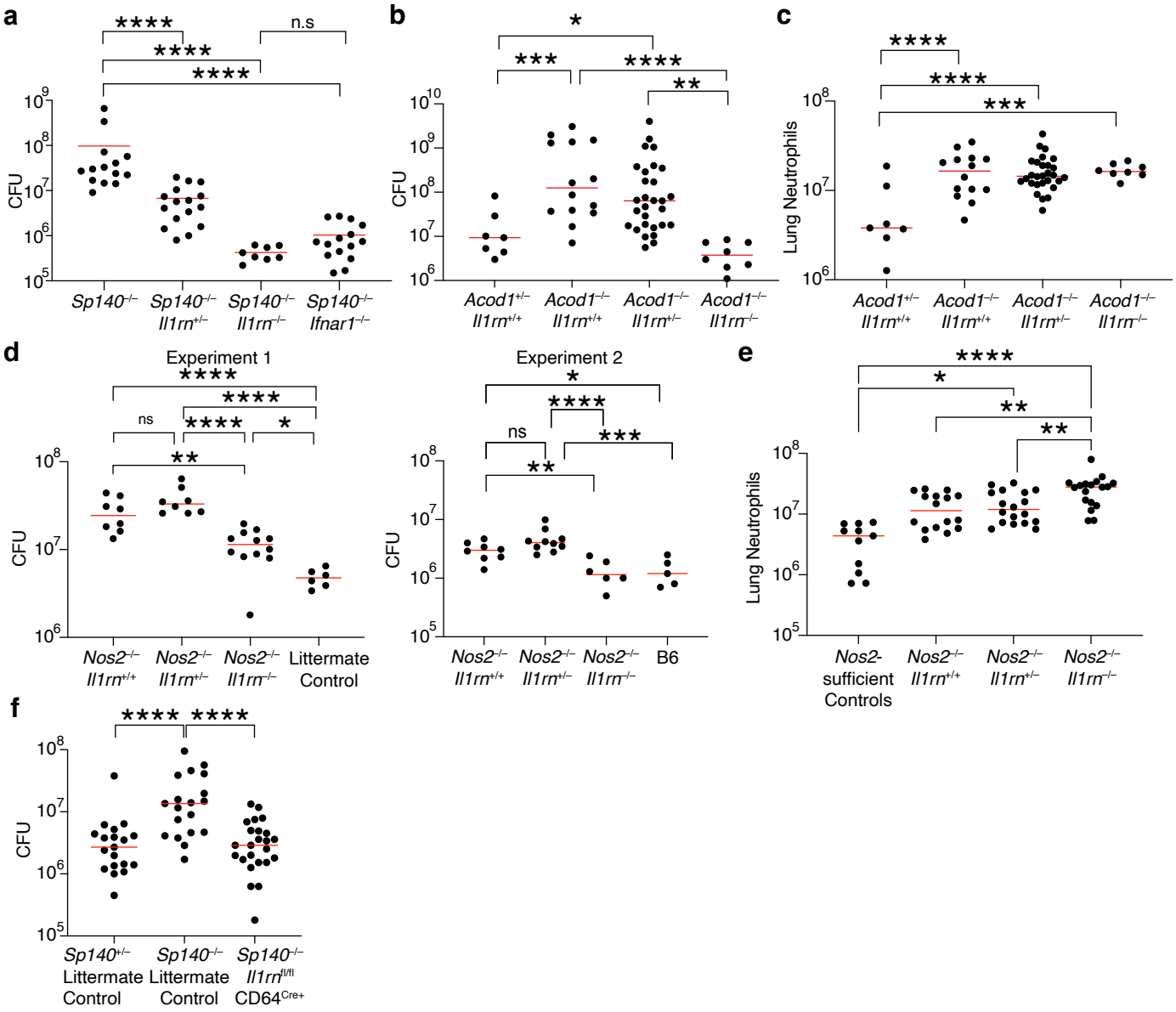

a
****
****
****
n.s
CFU
Sp140−/−
Sp140−/− Il1rn+/−
Sp140−/− Il1rn−/−
Sp140−/− Ifnar1−/−
b
*
***
****
**
CFU
Acod1+/− Il1rn+/+
Acod1−/− Il1rn+/+
Acod1−/− Il1rn+/−
Acod1−/− Il1rn−/−
c
****
****
***
Lung Neutrophils
Acod1+/− Il1rn+/+
Acod1−/− Il1rn+/+
Acod1−/− Il1rn+/−
Acod1−/− Il1rn−/−
d
Experiment 1
****
****
ns
****
*
**
CFU
Nos2−/− Il1rn+/+
Nos2−/− Il1rn+/−
Nos2−/− Il1rn−/−
Littermate Control
Experiment 2
*
****
ns
***
**
CFU
Nos2−/− Il1rn+/+
Nos2−/− Il1rn+/−
Nos2−/− Il1rn−/−
B6
e
****
*
**
**
Lung Neutrophils
Nos2-sufficient Controls
Nos2−/− Il1rn+/+
Nos2−/− Il1rn+/−
Nos2−/− Il1rn−/−
f
**** ****
CFU
Sp140+/− Littermate Control
Sp140−/− Littermate Control
Sp140−/− Il1rnfl/fl CD64Cre+

Figure 4.

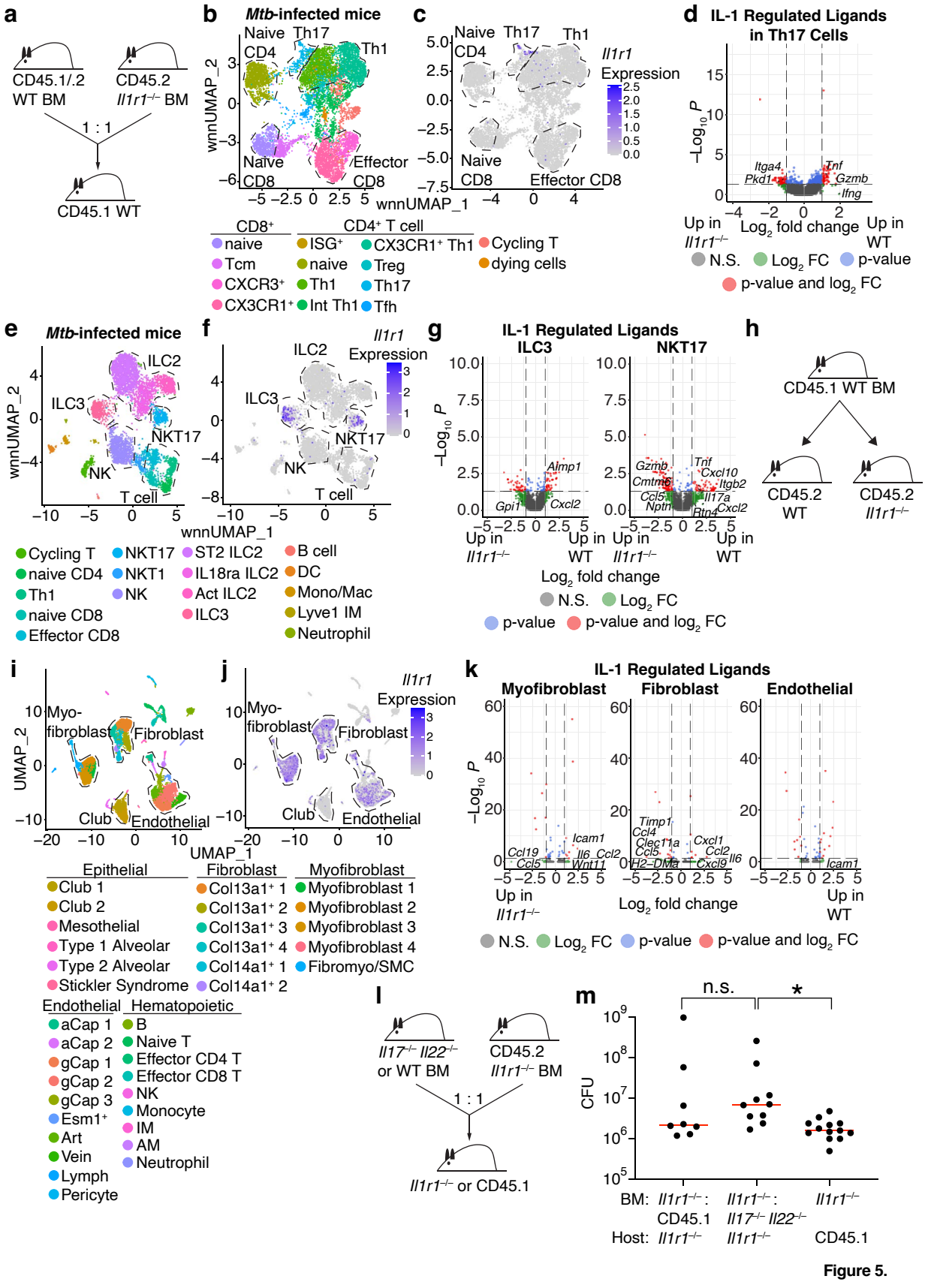
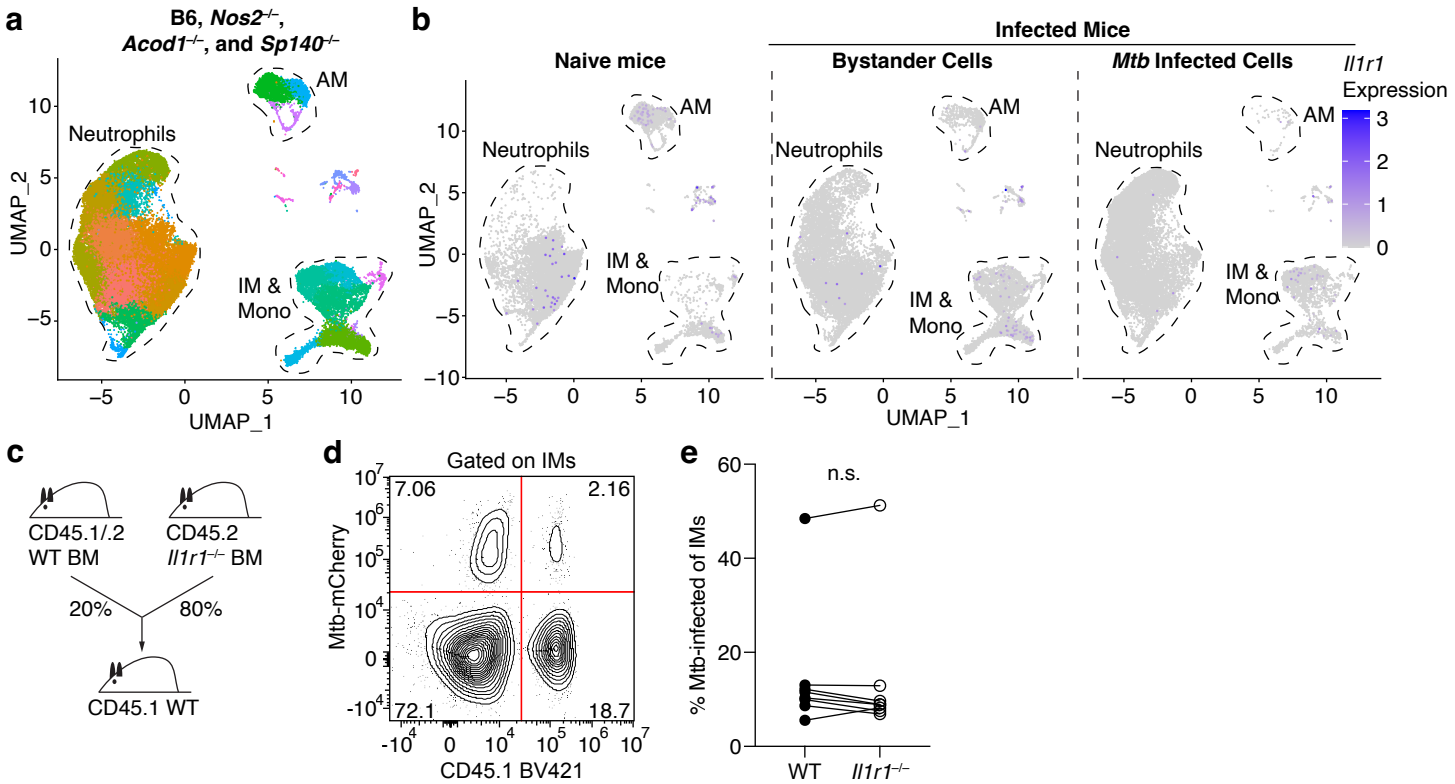
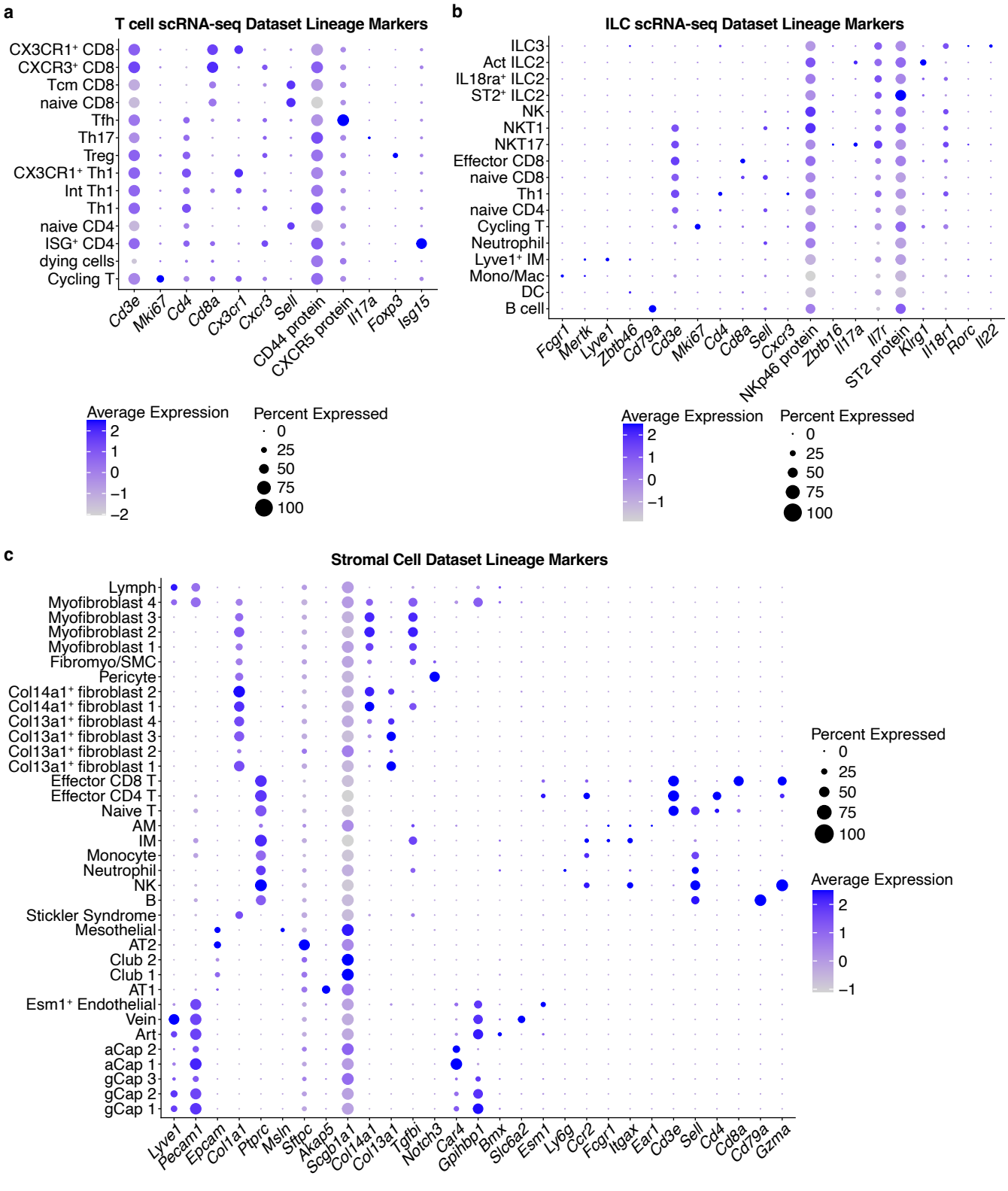

a
CD45.1/.2
WT BM
CD45.2
Il1r1−/− BM
1 : 1
CD45.1 WT
b
Mtb-infected mice
Naive CD4
Th17
Th1
Naive CD8
Effector CD8
wnnUMAP_2
wnnUMAP_1
c
Il1r1 Expression
Naive CD4
Th17
Th1
Naive CD8
Effector CD8
wnnUMAP_1
CD8+
naive
Tcm
CXCR3+
CX3CR1+
CD4+ T cell
ISG+
naive
Th1
Int Th1
CX3CR1+ Th1
Treg
Th17
Tfh
Cycling T
dying cells
d
IL-1 Regulated Ligands in Th17 Cells
−Log10 P
Itga4
Pkd1
Tnf
Gzmb
Ifng
Up in Il1r1−/−
Log2 fold change
Up in WT
N.S.
Log2 FC
p-value
p-value and log2 FC
e
Mtb-infected mice
ILC2
ILC3
NKT17
NK
T cell
wnnUMAP_2
f
Il1r1 Expression
ILC2
ILC3
NKT17
NK
T cell
wnnUMAP_1
Cycling T
naive CD4
Th1
naive CD8
Effector CD8
NKT17
NKT1
NK
ST2 ILC2
IL18ra ILC2
Act ILC2
ILC3
B cell
DC
Mono/Mac
Lyve1 IM
Neutrophil
g
IL-1 Regulated Ligands
ILC3
NKT17
−Log10 P
Almp1
Gpi1
Cxcl2
Gzmb
Cmtm6
Ccl5
Nptn
Tnf
Cxcl10
Itgb2
Il17a
Btn4
Cxcl2
Up in Il1r1−/−
Up in WT
Log2 fold change
N.S.
Log2 FC
p-value
p-value and log2 FC
h
CD45.1 WT BM
CD45.2 WT
CD45.2 Il1r1−/−
i
Myo-fibroblast
Fibroblast
Club
Endothelial
UMAP_2
j
Il1r1 Expression
Myo-fibroblast
Fibroblast
Club
Endothelial
UMAP_1
Epithelial
Club 1
Club 2
Mesothelial
Type 1 Alveolar
Type 2 Alveolar
Stickler Syndrome
Fibroblast
Col13a1+ 1
Col13a1+ 2
Col13a1+ 3
Col13a1+ 4
Col14a1+ 1
Col14a1+ 2
Myofibroblast
Myofibroblast 1
Myofibroblast 2
Myofibroblast 3
Myofibroblast 4
Fibromyo/SMC
Endothelial
aCap 1
aCap 2
gCap 1
gCap 2
gCap 3
Esm1+
Art
Vein
Lymph
Pericyte
Hematopoietic
B
Naive T
Effector CD4 T
Effector CD8 T
NK
Monocyte
IM
AM
Neutrophil
k
IL-1 Regulated Ligands
Myofibroblast
Fibroblast
Endothelial
−Log10 P
Icam1
Ccl19
Ccl5
Il6
Ccl2
Wnt11
Timp1
Ccl4
Clec11a
Ccl5
H2-DMa
Cxcl1
Ccl2
Cxcl9
Il6
Icam1
Up in Il1r1−/−
Log2 fold change
Up in WT
N.S.
Log2 FC
p-value
p-value and log2 FC
l
Il17−/− Il22−/− or WT BM
CD45.2 Il1r1−/− BM
1 : 1
Il1r1−/− or CD45.1
m
n.s.
*
CFU
BM: Il1r1−/− : CD45.1
Host: Il1r1−/−
BM: Il1r1−/− : Il17−/− Il22−/−
Host: Il1r1−/−
BM: Il1r1−/−
Host: CD45.1

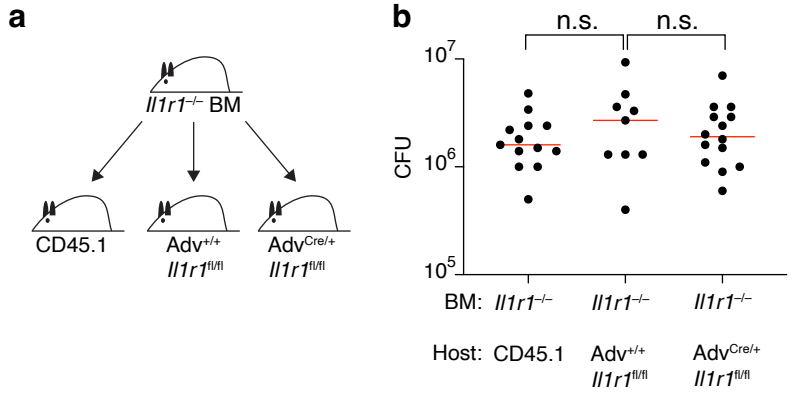
Figure 5.



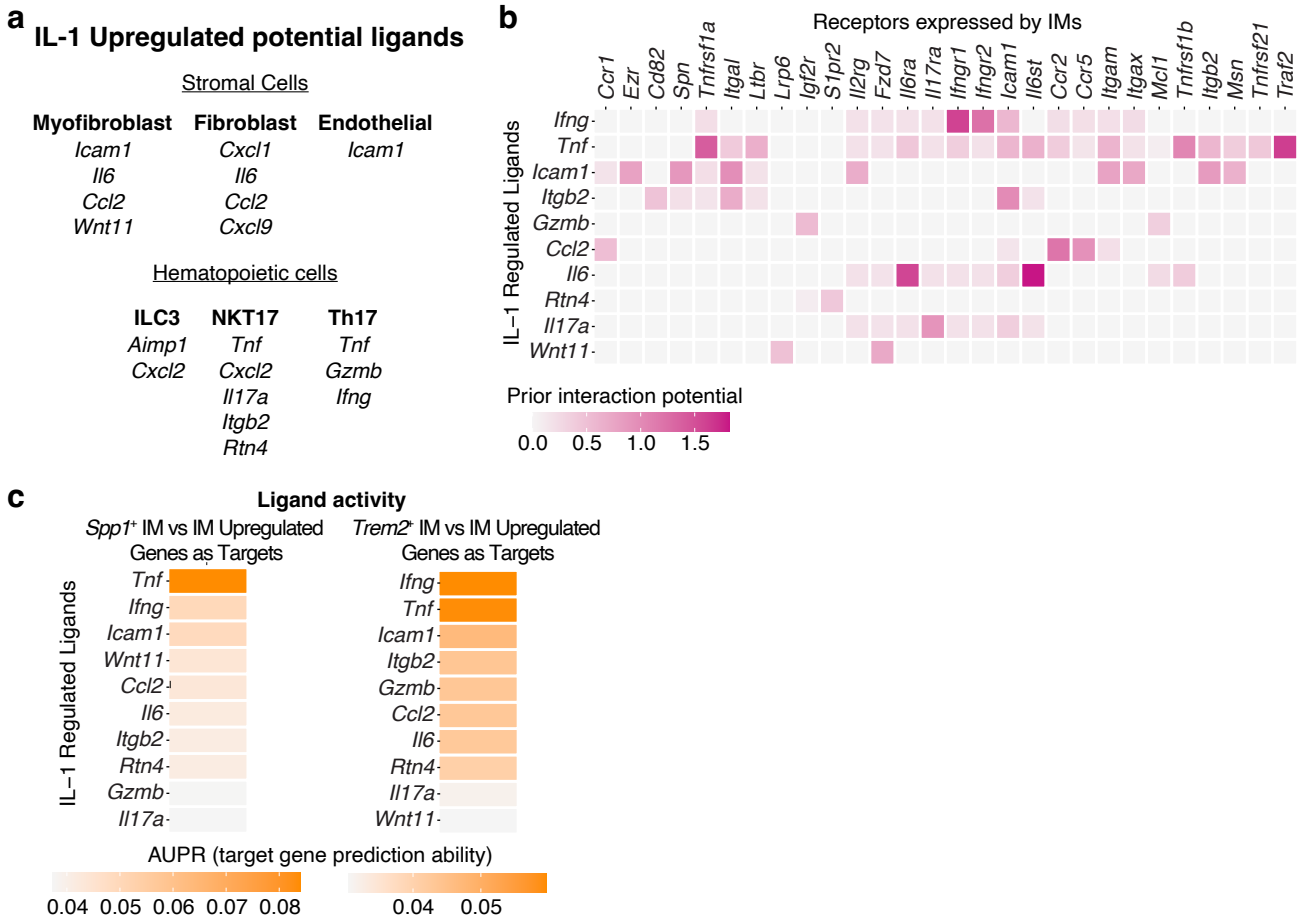
Supplementary Figure 6.



Supplementary Figure 7.



Supplementary Figure 8.



Supplementary Figure 9.