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Metabolically active neutrophils represent a permissive niche for *Mycobacterium tuberculosis*

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AUTHOR CONTRIBUTIONS

J. Andrews, Z. Zhang, and L. Huang conceived the project, designed and performed the experiments, analyzed and interpreted data, and wrote the manuscript. K. Prasad, F. Huey, E. Nazarova, and J. Wang helped with the experiments and interpretation of the data. T. Weinkopff, L.X. Li, S. Mu, M. Birrer, R. Argüello, and J. Philips contributed key reagents and helped with interpretation of the data. S.C.-C. Huang and N. Zhang provided conceptual guidance and helped with interpretation of the data. J. Mattila performed the experiments, analyzed and interpreted data from NHP studies. All authors critically read and edited the manuscript.

DECLARATIONS OF COMPETING INTEREST

The authors have no competing interests to declare.

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APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found online at <https://doi.org/10.1016/j.mucimm.2024.05.007>.

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Abstract

Mycobacterium tuberculosis (Mtb)-infected neutrophils are often found in the airways of patients with active tuberculosis (TB), and excessive recruitment of neutrophils to the lung is linked to increased bacterial burden and aggravated pathology in TB. The basis for the permissiveness of neutrophils for Mtb and the ability to be pathogenic in TB has been elusive. Here, we identified metabolic and functional features of neutrophils that contribute to their permissiveness in Mtb infection. Using single-cell metabolic and transcriptional analyses, we found that neutrophils in the Mtb-infected lung displayed elevated mitochondrial metabolism, which was largely attributed to the induction of activated neutrophils with enhanced metabolic activities. The activated neutrophil subpopulation was also identified in the lung granulomas from Mtb-infected non-human primates. Functionally, activated neutrophils harbored more viable bacteria and displayed enhanced lipid uptake and accumulation. Surprisingly, we found that interferon- γ promoted the activation of lung neutrophils during Mtb infection. Lastly, perturbation of lipid uptake pathways selectively compromised Mtb survival in activated neutrophils. These findings suggest that neutrophil heterogeneity and metabolic diversity are key to their permissiveness for Mtb and that metabolic pathways in neutrophils represent potential host-directed therapeutics in TB.

INTRODUCTION

The encounter between intracellular bacteria and host phagocytes is crucial for the outcome of the infection. To successfully establish the infection and persist long-term in the host, a hospitable host cell type with high permissiveness is required. During *Mycobacterium tuberculosis* (Mtb) infection, bacteria can be phagocytosed by lung alveolar macrophages (AMs) upon inhalation.¹ This interaction is essential for the early stage of infection as AMs are metabolically impaired during Mtb infection and provide a permissive environment to facilitate the rapid growth of Mtb.^{2,3} Similarly, Mtb can also invade and replicate within alveolar epithelial type II cells.⁴⁻⁶ Consequently, Mtb disseminates to other infiltrated phagocytes, like neutrophils, dendritic cells (DCs), and interstitial macrophages (IMs). It is well-known that embryonically derived AMs and monocyte-derived IMs possess

different levels of permissiveness during early Mtb infection. Intriguingly, their differential capacities to control Mtb growth are linked to intrinsic cellular metabolic states.² Moreover, depletion of permissive phagocytes or pharmacological perturbation of pathways that are associated with their permissiveness leads to beneficial effects for the host.^{2,7-9} Therefore, identifying novel permissive phagocytes and the underlying mechanisms that endow their permissiveness is key for novel therapeutics and vaccine strategies.

Neutrophils in the airways of humans with active tuberculosis (TB) are often infected with Mtb and neutrophils are an important early niche for this bacterium in Mtb-infected mice.^{1,2,10} Neutrophilic inflammation is pathogenic in TB, and high bacterial burdens and lung pathology are correlated in animal models and human TB.¹¹⁻¹⁴ Several lines of evidence underpin an interplay between neutrophil metabolism and Mtb, and the relationship between neutrophil recruitment and disease severity. Specific depletion of neutrophils leads to the reduction of bacterial burden in several murine models infected with Mtb, demonstrating a permissive nature of neutrophils during Mtb infection.^{7,15} Genome-wide mutant fitness analysis using an Mtb transposon library in a neutrophil-rich murine lung inflammation model revealed the depletion of mutants in long-chain fatty acid uptake and lipid catabolism,⁷ strongly implicating that neutrophils may promote Mtb growth by acting as a nutrient reservoir in the lung.^{7,16,17} However, the mechanisms by which neutrophils supply preferred nutrients to Mtb, such as fatty acids and cholesterol, and how metabolic states of neutrophils contribute to Mtb infection remains undefined.

Neutrophils are traditionally considered a homogenous and terminally differentiated population. However, emerging evidence demonstrates neutrophils have an underappreciated plasticity and functional diversity under both homeostatic and pathological conditions.^{18,19} At steady state, tissue-derived signals drive rapid adaptation of neutrophils and result in a great heterogeneity of their phenotypes and functionalities in mice.²⁰ A number of neutrophil subsets have been described in both human and murine models under different pathological conditions.²¹⁻²⁴ Three different neutrophil subsets with distinct cytokine profiles and macrophage activation capabilities were discovered in mice with different susceptibilities to infection by methicillin-resistant *Staphylococcus aureus*,²⁵ indicating that neutrophils can have highly plastic transcriptional profiles that can impact disease outcomes. A pathogenic SiglecF⁺ neutrophil subset has been identified in both house dust mite-induced lung asthma and renal fibrosis mouse models.^{26,27} Neutrophil heterogeneity has also been observed during Mtb infection. Accumulation of granulocytic myeloid-derived suppressor cells (MDSCs), a cell population that is phenotypically similar to neutrophils has been reported in the lung of Mtb-infected mice and TB patients.²⁸⁻³⁰ Moreover, the density of neutrophils has been used as a marker of heterogeneity, and recent studies have further revealed the altered neutrophil density in Mtb infection.^{31,32} Mtb can directly induce the generation of low-density neutrophils (LDN) from normal-density neutrophils *in vitro*, and the abundance of LDN in the peripheral blood is associated with the disease severity in TB patients.³¹ Lastly, a long-lived and permissive Ly6G^{mid} neutrophil subset with compromised antimicrobial function has been described in multiple TB mouse models.³³ Altogether, these studies imply that neutrophil heterogeneity may represent an extra layer of regulation on TB progression, yet whether neutrophil subsets provide different intracellular environments for Mtb is unclear.

Metabolically, neutrophils have been previously thought to rely predominantly on glycolysis to generate adenosine triphosphate (ATP) due to their low mitochondrial density and acute energy demands during inflammation.^{34,35} However, emerging evidence has challenged this dogma and demonstrates that neutrophils can engage in sophisticated metabolic pathways to support their diverse functions under homeostatic and pathological conditions.^{18–20,36,37} For instance, tumor-elicited c-kit⁺ immature neutrophils engage in fatty acid oxidation to support mitochondrial function.³⁸ MDSCs increase the expression of fatty acid transport protein 2 to promote immune suppressive activities.³⁹ These data underpin the increased diversity of metabolic pathways in neutrophils during inflammatory conditions. Whether Mtb infection reprograms the metabolism of neutrophils is completely unknown and needs to be carefully examined.

In this study, we aimed to investigate the metabolic states of lung neutrophils and how cellular metabolism regulates the permissive nature of neutrophils in TB. Using metabolic and transcriptional analyses at the single-cell level, we found that Mtb-infected neutrophils are metabolically distinct from AMs and IMs. Mtb infection induces activation of lung neutrophils with upregulated major histocompatibility complex II (MHCII), ICAM1, and CD64 which are more metabolically active than non-activated neutrophils. Importantly, these activated neutrophils were observed in granulomas isolated from Mtb-infected non-human primates (NHPs). Notably, interferon (IFN) γ could promote the activation of neutrophils during Mtb infection in the murine model. Metabolically activated neutrophils in the Mtb-infected mouse lung display increased mitochondrial metabolism and enhanced fatty acid uptake via CD36 expression, which is associated with higher bacterial burden in this population. Finally, harnessing lipid uptake pathways in the host impacts Mtb survival in activated neutrophils, indicating these cells may present a nutritionally rich cellular niche for Mtb growth and can be an attractive host-directed therapeutic target against this pathogen. Our findings demonstrate that altered metabolic states contribute to the permissiveness of neutrophils during Mtb infection.

RESULTS

Single-cell energetic metabolism by profiling translation inhibition (SCENITH) reveals distinct metabolic profiles of lung phagocytes in Mtb-infected mouse lung

Protein synthesis (PS) is one of the most energy-consuming anabolic pathways in cellular metabolism as nearly half of the total energy produced by cells is immediately utilized by the translational machinery. Moreover, ATP consumption and PS activity are tightly coupled^{40,41}; therefore, global cellular metabolic activities can be correlated with changes in PS.⁴² To investigate metabolism in lung phagocytes during Mtb infection, we quantified the PS level in various lung phagocytes using flow cytometry by measuring the incorporation of puromycin, a parameter that faithfully reflects PS levels in cells.^{43–45} Puromycin incorporation into CD45⁺ lung cells isolated from Mtb-infected mice was readily detectable while reduced to the basal level in the presence of PS inhibitor harringtonine (HA), confirming the specificity and sensitivity of this readout (Supplementary Fig. 1A). Furthermore, we found that the PS levels were highly variable among different types of lung phagocytes (Fig. 1A). CD11b⁺ DCs, monocyte-derived IMs and Ly6C⁺ monocytes

showed the highest PS activities, whereas the PS levels in AMs and Ly6C-monocytes were significantly lower. Neutrophils and eosinophils, however, exhibited the lowest PS activity. Comparisons of the PS level between Mtb-infected and bystander phagocytes in mice infected with Mtb constitutively expressing mCherry showed that while Mtb-infected neutrophils and CD11b+ DCs revealed no changes in PS levels compared to their bystander compartments, the PS level was significantly reduced in Mtb-infected IMs, Ly6C+ and Ly6C-monocytes (Fig. 1B). Eosinophils were excluded from this analysis as the number of Mtb-infected eosinophils was extremely low, similar with the previous report.⁴⁶ Notably, AMs are the only cell type that exhibited an enhanced PS level upon Mtb infection.

To further deconvolute the metabolic profiles of lung phagocytes during Mtb infection, we utilized the recently described SCENITH method, a flow cytometry-based approach that measures quantitative changes in the PS level at single-cell resolution following treatment with specific metabolic inhibitors, including 2-deoxyglucose (2-DG) and oligomycin, which target glycolysis or oxidative phosphorylation (OXPHOS), respectively.^{42,47,48} At 3-week post-Mtb infection, AMs revealed a greater level of mitochondrial dependence but lower glycolytic capacity compared to IMs (Fig. 1C). We further validated these results using Seahorse in parallel on AMs and IMs from Mtb-infected mice, as we have previously revealed their distinct metabolic states.² Indeed, AMs had higher basal oxygen consumption rate (OCR) and IMs showed a higher extracellular acidification rate (ECAR); therefore, IMs were more glycolytically active (Supplementary Figs. 1B and C). Thus, SCENITH recapitulates Seahorse metabolic profiling and represents a robust and reliable readout in our model. Intriguingly, AMs displayed the highest mitochondrial dependence and the lowest glycolytic capacity among all lung phagocytes in SCENITH analysis (Fig. 1C), suggesting an integral role of mitochondrial metabolism in AMs. Moreover, all phagocytes showed high glucose dependence, highlighting the active engagement of glucose metabolism in Mtb-infected lungs (Fig. 1C). We next questioned how Mtb may induce differential metabolic shifts in different phagocytes *in vivo*. Analysis of Mtb-infected and bystander cells revealed no changes in mitochondrial dependence or glycolytic capacity in Ly6C+ and Ly6C-monocytes (Fig. 1D). However, Mtb infection reduced mitochondrial dependence and increased glycolytic capacity in AMs, IMs, neutrophils and CD11b+ DCs, indicating a metabolic shift from OXPHOS toward glycolysis in these cells (Fig. 1D). Interestingly, we observed a moderate increase in glycolytic capacity in Mtb-infected AMs, although to a much lesser extent compared to IMs. Altogether, these results indicate that the metabolic changes that occurred in Mtb-infected phagocytes are cell-type specific. SCENITH enabled us to uncover the various metabolic profiles in major lung phagocytes, introducing an additional layer of heterogeneity in the lung during Mtb infection.

Neutrophils exhibit distinct metabolism from macrophages in Mtb infection *in vitro*

In SCENITH analysis, a substantial amount of mitochondrial dependence in neutrophils in Mtb-infected mouse lung was observed (Fig. 1C), which recapitulated the increased mitochondrial metabolism in tumor-elicited neutrophils.^{38,39} We therefore sought to investigate the metabolic alterations triggered by Mtb infection in neutrophils. Compared to uninfected bone marrow neutrophils (BMNs), Mtb-infected BMNs [multiplicity of infection (MOI) = 5] exhibited enhanced glycolysis, as reflected by an increased ECAR, ECAR/OCR

ratio and proton efflux rate (PER) in Seahorse analyses (Fig. 2A and Supplementary Fig. 2A). Basal OCR remained unchanged upon infection in BMNs (Fig. 2A). Notably, we observed a drastic increase in non-mitochondrial oxygen consumption in Mtb-infected BMNs, an indication of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation. In contrast, while Mtb infection also increased the ECAR/OCR ratio in bone marrow-derived macrophages (BMDMs), Mtb-infected BMDMs displayed similar ECAR and PER levels to uninfected BMDMs (MOI = 5) (Fig. 2B and Supplementary Fig. 2B). The increased ECAR/OCR ratio in BMDMs was largely caused by a significant reduction in basal OCR induced by Mtb infection (Fig. 2B). Furthermore, blocking glycolysis and OXPHOS in neutrophils from the Mtb-infected lung with 2-DG and oligomycin, respectively, significantly reduced the production of tumor necrosis factor- α (TNF α) and interleukin-1 β (IL-1 β) (Fig. 2C). The requirement of glycolysis in the optimal production of pro-inflammatory cytokines has also been observed in IMs in Mtb-infected mice (Supplementary Fig. 2C). Notably, UK5099, a mitochondrial pyruvate carrier inhibitor specifically blocking pyruvate oxidation, had a modest effect on IL-1 β but not TNF α production in neutrophils (Fig. 2C). Altogether, these data demonstrate that Mtb infection triggers distinct metabolic activities in macrophages and neutrophils *in vitro*, and underpin the essential role of metabolism in regulating TNF α and IL-1 β productions in neutrophils in Mtb infection.

Neutrophils in Mtb-infected lung have a short lifespan that is long enough to support Mtb replication *in vivo*

The lifespan of neutrophils is often thought to be as short as 1 day.^{49,50} This view has been challenged by recent studies indicating that neutrophils can live up to 5.4 days in humans.⁵¹ Moreover, the lifespan of neutrophils in tissues can be significantly extended during chronic inflammation when pro-survival signals including cytokines and growth factors are produced.^{52–54} To estimate the lifespan of lung neutrophils during Mtb infection, we performed a pulse-chase experiment *in vivo* using 5-ethynyl-2'-deoxyuridine (EdU). EdU incorporates into the deoxyribonucleic acid (DNA) of replicating neutrophil progenitors in bone marrow, allowing us to track the fate and lifespan of neutrophils that are newly recruited to the lung during Mtb infection. EdU+ neutrophils were detected in blood and lung, and peaked after 3 days post pulse (Supplementary Fig. 1D). In these two tissues as well as in the spleen, the percentage of EdU+ neutrophils decreased drastically on day 4 and further declined to basal level by day 5 (Fig. 3A). Notably, we found variable half-lives of neutrophils in different tissues, with the shortest time in blood (7.7 hours) and the longest time in the spleen (25.8 hours), whereas the half-life of neutrophils in the lung is 14.2 hours (Fig. 3A). The data are in line with a recent study using the similar method which showed a reduced EdU labeling decay time of neutrophils in the lung parenchyma compared to those in the lung vasculature.⁵⁵

The relatively short lifespan of neutrophils in Mtb-infected lungs led us to question whether neutrophils are able to support replication of Mtb. Neutrophils are the predominate cell types infected with Mtb in human patients' sputum and bronchoalveolar lavage (BAL) fluid, implicating a role for neutrophils acting as a permissive site for Mtb.¹⁰ However, the direct and definitive evidence on whether Mtb can replicate in neutrophils is still

lacking. To address this question, we exploited the reporter Mtb strain single-stranded DNA-binding protein-green fluorescent protein (SSB-GFP), which allows us to probe the replicative activity of Mtb at the single-cell level.⁵⁶ At 2 and 4 weeks post-infection, infected neutrophils were sorted and SSB-GFP foci in Mtb were quantified by confocal microscopy. Indeed, intracellular Mtb with GFP+ foci that are indicative of replication were detected at both time points (Fig. 3B). We observed nearly 25% of intracellular bacilli contained GFP+ foci at 2 weeks post-infection, indicating that Mtb can replicate in neutrophils at the early stage of infection (Fig. 3C). However, the percentage of active replicating Mtb in neutrophils decreased to 10% at 4 weeks post-infection (Fig. 3C). Thus, intracellular Mtb at replicating state can be found in neutrophils, which indicates that neutrophils function as a replicating niche for Mtb, at least in the mouse model. The average population doubling time of Mtb in the lung is 24.6 hours during the first two weeks of infection.⁵⁷ Altogether, these data suggested that neutrophils recruited to the lung can host Mtb and have lifespans that are long enough to support Mtb replication *in vivo*.

Single-cell RNA-sequencing reveals the heterogeneity of lung neutrophils in mouse and NHP TB

To better define the heterogeneity of lung neutrophils during Mtb infection, we made use of a publicly available dataset of single-cell RNA-sequencing (scRNA-seq) on Mtb-infected mouse lung myeloid cells with a focus on the transcriptional profile of neutrophils (GEO: GSE167232⁵⁸). Three clusters of neutrophils were identified from the dataset and were visualized by uniform manifold approximation and projection (UMAP) (Fig. 4A). Antibody-derived tag against Ly6G confirmed the identity of neutrophils (Fig. 4B). At steady state, tissue-derived signals can drive rapid adaptation of neutrophils and result in a great heterogeneity of their phenotypes and functionalities in different tissues.²⁰ All three neutrophil clusters highly express nuclear receptor 4A1 (*Nr4a1*), prostaglandin-endoperoxide synthase (*Ptgs2*), *IL-1 β* and tumor necrosis factor alpha-induced protein 3 (*Tnfaip3*), which are lung neutrophil signature genes (Fig. 4C).²⁰ These results further confirm that the lung environment imprints neutrophils even in the presence of Mtb infection. Moreover, Mouse_Neutrophil-1 expresses higher levels of *IL-1 β* , *Cd14*, *S100a9*, and placenta specific 8 (*Plac8*) while cathepsin B (*Ctsb*), tetraspanins *Cd53* and *Cd63* are among the top expressing genes in Mouse_Neutrophil-3 (Fig. 4D). On the other hand, Mouse_Neutrophil-2 expresses many unique genes including MHC II antigens, *Nos2*, *S100a10*, *C1qa*, *C1qb*, glutathione peroxidase 4 (*Gpx4*) and superoxide dismutase 1 (*Sod1*) (Fig. 4D). Consistent with its transcriptional profile, gene ontology enrichment analysis also revealed that Neutrophil_2 is highly activated compared to Neutrophil_1 (Figs. 4E and F). MDSCs are pathological neutrophils with potent immunosuppressive activities induced under chronic inflammatory conditions.⁵⁹ To determine whether neutrophils in Mtb infection possess characteristics of MDSCs, we carried out Gene Set Enrichment Analysis (GSEA) using a pre-defined MDSC-specific gene set from a murine breast cancer model.⁶⁰ We found that the MDSC gene signature from the murine breast cancer model was significantly enriched in Mouse_Neutrophil-1 (Fig. 4F). Indeed, transcriptional analysis confirmed the high expression of *Retnlg*, *Arg2*, *Il4ra* and *Alox5ap* in Mouse_Neutrophil-1 (Supplementary Fig. 3A), all of which have been previously associated with immunosuppression by

MDSCs.⁵⁹ Altogether, these data indicate that Mtb infection elicits accumulation of neutrophils with MDSC signatures in the mouse lung.

We next focused on Mouse_Neutrophil-2 given its distinct transcriptional profile and activated phenotype. Mouse_Neutrophil-2 highly expresses CD64, *MHCII* and *Nos2* (Fig. 4G). Flow cytometry analysis further illustrated the induction of Ly6G+CD11b+CD64+MHCII+ neutrophils in Mtb-infected murine lungs (Fig. 4H and Supplementary Fig. 3B). Consistent with their activation status, these neutrophils (hereafter referred to as CD64+ neutrophils) express a lower level of CD62L and higher levels of ICAM1 and CXCR4 compared to those non-activated neutrophils (hereafter referred to as CD64- neutrophils) (Supplementary Fig. 3D). Using confocal and electron microscopies, we revealed the nuclear morphology of both CD64- and CD64+ neutrophils in the lung, further confirming their identities (Supplementary Fig. 3E and F). Both CD64+ and CD64- neutrophils express a comparable level of pro-IL-1 β while the expression of MHCII and inducible nitric oxide synthase (iNOS) are greater in CD64+ neutrophils, which is consistent with scRNA-seq data (Fig. 4I). The induction of CD64+ neutrophils appears to be independent of Mtb strain and infection route, as CD64+ neutrophils expressing higher levels of MHCII were also present in mice infected with H37Rv Mtb via aerosol inhalation (Fig. 4J and Supplementary Fig. 3C). Moreover, CD64+ neutrophils were also observed in *M. bovis* Bacillus Calmette–Guérin (BCG) infected mice (Supplementary Fig. 3G). We then sought to examine whether Mtb-induced CD64+ neutrophils are unique to resistant C57BL/6 mice. To test this, we infected susceptible C3HeB/FeJ mice, in which a large number of neutrophils accumulate in the lung during Mtb infection.⁶¹ We found that activated CD64+ neutrophils with similar phenotypes were also present in the lungs of these animals (Supplementary Figs. 3H and I). To extend our observations beyond the murine model, we investigated the heterogeneity of neutrophils in the lung of Mtb-infected NHPs by analyzing a publicly available dataset of scRNA-seq on Mtb-infected NHP lung immune cells containing the transcriptional profile of neutrophils.⁶² Four clusters of neutrophils were identified by UMAP (Fig. 4K). Intriguingly, many of the highly expressed genes in NHP neutrophil clusters are shared with murine neutrophil clusters (Fig. 4L and Table 1). Particularly, similar with Mouse_Neutrophil-2, NHP_Neutrophil-3 highly expresses MHCII antigens, C1QA, C1QB, S100A10 and GPX4 (Fig. 4L). Moreover, nearly half of NHP_Neutrophil-3 top expressing genes could be found in Mouse_Neutrophil-2 (Table 1), implicating that these two populations are highly comparable between the two species. Lastly, we examined CD64 expression in neutrophils from granulomas of Mtb-infected cynomolgus macaques, an NHP used to recapitulate human TB.⁶³ We found that CD64+ neutrophils were present in peripheral blood and uninvolved lung, and granulomatous lung (Fig. 4M). There was substantial inter- and intra-animal variability in the presence and frequency of CD64 expression, but granulomas tended to have higher frequencies of CD64+ neutrophils than blood or uninvolved lung tissue (Fig. 4M, top). Likewise, there was variability in CD64 expression in each sample, and the mean fluorescence intensity (MFI) of CD64 tended to be higher for neutrophils from lung tissue and granulomas than peripheral blood neutrophils, suggesting that the activation profiles of neutrophils may differ by compartment (Fig. 4M, bottom). Collectively, these data demonstrate that the

heterogeneity of neutrophils is comparable in both Mtb-infected mouse and NHP lungs, and Mtb infection-induced activated neutrophils appear to be conserved across the two species.

Activated neutrophils are both glycolytically and OXPHOS active in Mtb infection

To further characterize CD64⁺ neutrophils (Mouse_Neutrophil-2) and delineate their functions, we investigated their metabolic states in Mtb infection. In comparison to non-activated CD64[−] neutrophils, activated CD64⁺ neutrophils displayed a greater level of PS and a higher mitochondrial dependence, indicative of enhanced metabolic activities (Figs. 5A and B). Consistently, we found that Mtb-infected CD64⁺ neutrophils had increased mitochondrial membrane potential and lipid content (Figs. 5C and D). Consistently, bystander CD64⁺ neutrophils also displayed higher mitochondrial dependence and lipid content compared to bystander CD64[−] neutrophils, implicating elevated metabolic activities in CD64⁺ neutrophils are not directly induced by Mtb (Supplementary Figs. 4A and B). Moreover, CD64⁺ neutrophils displayed a significantly longer lifespan than CD64[−] neutrophils using the EdU pulse-chase method (Fig. 5E). To substantiate the metabolic findings further, we measured OXPHOS and glycolysis in sorted total CD64⁺ and CD64[−] neutrophils from Mtb-infected mice using Seahorse. CD64⁺ neutrophils exhibited significantly enhanced glycolysis and glycolytic capacity compared to CD64[−] neutrophils (Fig. 5F). Strikingly, analysis of oxygen consumption by the two neutrophil subsets revealed enhanced basal and maximal OCR in CD64⁺ neutrophils, indicating an increased commitment to OXPHOS (Fig. 5G). Notably, CD64⁺ neutrophils exhibited a lower spare respiratory capacity (SRC) when compared to CD64[−] neutrophils (Fig. 5G). The enhanced glycolysis and OXPHOS signatures in CD64⁺ neutrophils were also observed in the murine scRNA-seq data (Fig. 5H). To further characterize changes in cellular metabolism, we carried out Met-flow analysis by quantifying rate-limiting metabolic enzymes in key metabolic pathways.⁶⁴ In support of higher metabolic activity of CD64⁺ neutrophils compared with CD64[−] neutrophils, CD64⁺ neutrophils exhibited overall higher metabolic enzyme expression, including glucose transporter 1 (GLUT1) and pyruvate kinase M (PKM), key enzymes in the glycolytic pathway as well as acetyl-CoA carboxylase 1 (ACC1), carnitine palmitoyltransferase 1 A (CPT1A), succinate dehydrogenase A (SDHA), and cytochrome C (CYTC), enzymes involved in lipid metabolism and OXPHOS (Fig. 5I). All together, these data demonstrated that lung CD64⁺ neutrophils represent a metabolically active neutrophil subset in Mtb infection.

IFN γ promotes the activation of neutrophils in the Mtb-infected lung

Next, we sought to determine if inflammatory stimuli are responsible for activating neutrophils in the Mtb-infected lung. Thioglycolate-induced peritoneal neutrophils expressed a higher level of CD64 than neutrophils in the blood and bone marrow (Supplementary Fig. 4C), suggesting that CD64 can be induced under inflammatory conditions. Moreover, IFN γ has been shown to regulate neutrophil activation by upregulation of CD64 expression in neutrophils.^{65,66} To address whether IFN γ signaling is required for inducing neutrophil activation in Mtb infection, we measured CD64 expression in Mtb-infected BMNs with or without IFN γ *in vitro*. Interestingly, IFN γ had no effect on CD64 expression by itself, while Mtb infection alone led to increased CD64 expression in BMNs (Fig. 6A). However, additional IFN γ -stimulation synergistically upregulated CD64

levels in Mtb-infected BMNs (Fig. 6A). Similarly, lipid content was increased upon Mtb infection in BMNs, which was further promoted by the presence of IFN γ (Fig. 6B). Moreover, the ECAR level and MHCII expression was significantly increased in Mtb-infected BMNs with additional IFN γ (Figs. 6C and D), implicating that IFN γ activates Mtb-infected neutrophils *in vitro*. Analyses of neutrophil responses in IFN γ KO mice at 2 weeks post-Mtb infection revealed no differences in the number of total neutrophils in the lung (Fig. 6E). However, the number of CD64⁺ neutrophils was significantly lower in IFN γ KO mice (Fig. 6F), suggesting that IFN γ appears to be involved in inducing activated CD64⁺ neutrophils in the lung during Mtb infection. Nevertheless, hypoxia-inducible factor-1 α (HIF-1 α), a key transcription factor mediating IFN γ signaling, that is highly expressed in CD64⁺ neutrophils (Fig. 4D) is not required for induction of CD64⁺ neutrophils (Supplementary Fig. 4D). Altogether, these data indicate that IFN γ promotes generation of CD64⁺ neutrophils in the Mtb-infected lung independent of HIF signaling.

Perturbation of lipid uptake selectively decreases Mtb burden in activated neutrophils

To determine the role and functions of activated CD64⁺ neutrophils in Mtb infection, we first compared the bacterial burden in CD64⁺ and CD64[−] neutrophils. Interestingly, MFI of mCherry from CD64⁺ neutrophils was significantly higher compared to CD64[−] neutrophils (Fig. 7A). This data was further supported by the observation that higher numbers of viable Mtb were recovered from cell-sorted CD64⁺ neutrophils compared with CD64[−] neutrophils (Fig. 7A). Moreover, the higher bacterial burden in CD64⁺ neutrophils is not the consequence of increased phagocytic ability or enhanced Mtb replication activity as both CD64⁺ and CD64[−] neutrophils exhibited comparable phagocytic activity (Fig. 7B). Strikingly, although CD64⁺ neutrophils expressed a higher level of iNOS (Fig. 4I), we observed similar percentages of SSB-GFP foci in Mtb within both neutrophil populations at 2 weeks post-infection (Fig. 7C), indicating that Mtb replicates equally well in both neutrophil populations, and the replication activity of Mtb in neutrophils appears to be independent of the activation status of neutrophils. To elucidate whether CD64⁺ neutrophils create a permissive environment that is replete with preferred nutrients for Mtb, such as fatty acids, we measured their fatty acid uptake capacity. Compared to CD64[−] neutrophils, Mtb-infected CD64⁺ neutrophils exhibited greater fatty acid uptake (Fig. 7D). This phenotype was further confirmed by the higher expression of fatty acid transporters in CD64⁺ neutrophils, including fatty acid binding protein 5 (Supplementary Fig. 3A) and CD36 (Fig. 7E). These data are in line with the increased levels of key enzymes that regulate fatty acid metabolism in CD64⁺ neutrophils in Met-flow analysis (Fig. 5I). We then hypothesized that perturbing fatty acid uptake may impact the survival of Mtb in lipid-rich CD64⁺ neutrophils. To test this hypothesis, we first confirmed that CD36 is a functional transporter of fatty acid in neutrophils as we observed a profound reduction of BODIPY FL C16 uptake in Mtb-infected neutrophils in CD36KO mice compared to those in wild-type (WT) mice (Fig. 7F). Furthermore, we found that, while MFI of mCherry and the number of viable Mtb recovered from CD64[−] neutrophils were similar in WT and CD36KO mice (Fig. 7H), CD64⁺ neutrophils displayed higher MFI of mCherry and contained significantly more bacteria in WT mice than in CD36KO mice (Fig. 7I). Importantly, we did not observe a difference in the percentage of IFN γ +CD4⁺ T cells in WT and CD36KO mice (Fig. 7G), indicating that the differential Mtb burdens in CD64⁺ neutrophils between WT and

CD36KO were unlikely caused by the deficiency of CD36 in CD4⁺ T cells. Lastly, the lipid content level was significantly less in Mtb-infected CD36KO neutrophils *in vitro* (Fig. 7J). Sulfosuccinimidyl oleate, a CD36 inhibitor, significantly reduced the lipid content in Mtb-infected CD64⁺ neutrophils *ex vivo* (Fig. 7K). Altogether, these data demonstrated that host lipid uptake is essential for the survival of Mtb within CD64⁺ neutrophils, and CD36 promotes the accumulation of lipid content in activated neutrophils.

DISCUSSION

The lack of an effective vaccine against TB severely hinders the control of Mtb infection globally. Current vaccine development primarily aims at inducing robust adaptive immune responses mediated by memory T and B cells, which may pose challenges due to the existence of permissive lung phagocytes that create a hospitable niche for bacteria to thrive even in the presence of Th1 responses during the infection. AMs have been demonstrated as an early permissive cellular niche to facilitate rapid growth of Mtb.² Similarly, permissive macrophage populations in different tissues have been identified during the infection of other intracellular pathogens. CD206^{high} dermal macrophages promote *Leishmania major* infection in a Th1 immune environment.⁶⁷ In *Salmonella* infection, CD11b⁺CD11c⁺Ly6C⁺ splenic macrophages with an M2-like signature and non-classical monocyte-derived CD9⁺ macrophages have been implicated as replication niches for bacteria in the spleen.^{8,68} Thus, identifying permissive cells and understanding factors that contribute to their permissiveness are invaluable for the development of novel vaccines and therapeutics against various infections.

In this study, we turned our attention to neutrophils in TB for the following reasons. First, excessive recruitment of neutrophils has long been associated with increased bacterial burden and a worsened pathology in the lung in TB. Second, using Mtb expressing fluorescent proteins, neutrophils have been shown as one of the major phagocytes, besides lung macrophages, that contain Mtb as infection progressed in mice.^{1,2} Third, comparing replication activities of Mtb in different host phagocytes utilizing the SSB-GFP reporter Mtb strain revealed that Mtb replication ability is comparable between neutrophils and AMs at the early stage of infection, implicating the permissive nature of neutrophils in the early stage of TB.² To delineate the underlying mechanism that regulates the permissiveness in neutrophils, we used scRNA-seq and discovered activated neutrophils expressing CD64, iNOS, and MHCII alone with other activation markers in Mtb-infected lung. Activated neutrophils contain more viable bacteria compared to their non-activated counterparts despite expression of iNOS. Our data further indicate IFN γ is involved in promoting neutrophil activation *in vivo* and can upregulate CD64 expression in BMNs *in vitro*. These data are in line with another study that showed upregulation of CD64 in activated mouse lung neutrophils in respiratory syncytial virus infection.²¹ While mouse neutrophils do not express MHCII under homeostasis, they can acquire MHCII expression in the presence of IFN γ and direct contact with T cells to function as antigen-presenting cells.⁶⁹ A recent report also observed higher expression of H2-Ab1 and H2-Dmb1 in Ly6G^{Mid} neutrophils in Mtb-infected mice compared to blood neutrophils from uninfected mice.³³ The expression of iNOS in lung neutrophils has also been described in Mtb-infected mice using flow cytometry.^{2,70} Moreover, Mtb present in neutrophils from the reporter Mtb

strain *hspX*::GFP, *smyc*::mCherry-infected mice exhibited robust *hspX*::GFP signaling. GFP expresses in this reporter Mtb strain in response to NO *in vivo*,⁵⁶ indicating the presence of NO in infected neutrophils *in vivo*.² Moreover, immuneresponsive gene 1 negatively regulates iNOS expression in neutrophils during Mtb infection.⁷⁰ Importantly, the CD64 expression level in human blood neutrophils increases in TB patients.⁷¹ In a separate study, CD64 was identified as one of the distinct 393-transcript signatures in patients with active TB.⁷² The higher level of CD64 in neutrophils is also specific in patients with active TB and can be used to discriminate health controls and latent TB infection.⁷² This evidence further implicates that activated neutrophils may be involved in disease progression by facilitating Mtb survival in the lung, at least in the murine model. Moreover, the data in human patients are in agreement with our analysis in NHP where CD64 expression is increased in neutrophils in granulomas although our sample set from NHPs was too small to identify relationships between neutrophil CD64 expression and Mtb load per granuloma in this model. Whether activated neutrophils with similar phenotypes are also present in the human TB lung and their functions in disease progress are important questions to be addressed in the future.

We employed SCENTH, a recently developed approach to indirectly probe cellular metabolism in lung phagocytes in Mtb infection at the single-cell level by measuring PS response to metabolic inhibitors. Our study revealed diverse metabolic profiles in different phagocytes during the early stage of Mtb infection. Nearly all phagocytes in Mtb-infected lung showed high levels of glucose dependence, indicating the pivotal role of glucose metabolism in these cells. Among them, AMs demonstrated the highest mitochondrial dependence, which correlates with their relatively high permissiveness for Mtb. Although AMs do not increase glycolysis in response to lipopolysaccharide (LPS) stimulation,⁷³ Mtb-infected AMs displayed a higher glycolytic capacity than bystander cells, implying that AMs are capable of activating glycolysis in Mtb infection, albeit to a lesser extent than IMs. It would be of particular interest to investigate how disease progression and the dynamic of metabolism in phagocytes at different stages of infection influence each other. Notably, Mtb-infected BMDMs reduced basal OCR with no change in glycolysis, which was distinct from lung macrophages isolated from infected mice. These data are in line with another study that observed reduced OCR and glycolysis in Mtb-infected differentiated THP-1 macrophages.⁷⁴ These differences largely reflect the impact of culture conditions, macrophage ontogeny, and different extrinsic signals that macrophages receive between *in vitro* and *in vivo* conditions. Moreover, AMs were the only cell type that showed elevated translation levels when comparing Mtb-infected cells to their bystander counterparts. Inhibition of translation machinery is one of the mechanisms which pathogenic microbes interfere with ongoing PS, thereby suppressing innate immune responses.⁷⁵ However, it remains unclear how and why Mtb promotes translation specifically in AMs. Nonetheless, a recent study demonstrated that Mtb targets mammalian target of rapamycin (mTOR), a master regulator of translation in response to metabolic and physiological stress, to prevent mitochondrial damage triggered by ESAT-6 in macrophages.⁷⁶ Importantly, mTOR signaling is critical for the homeostasis of AMs.⁷⁷ Thus, we speculate that Mtb could exploit the mTOR pathway in AMs to enhance translation, in turn promoting the survival of AMs and further maintaining a permissive cellular niche for bacterial persistence.

In our SCENITH analysis, both Mtb-infected Ly6C⁺ and Ly6C-monocytes exhibited comparable levels of glycolytic capacity compared to their bystander compartment. Similarly, using the same method, human CD14⁺ monocytes isolated from healthy donors and TB patients also showed similar glycolytic capacity.⁷⁸ On the other hand, CD16⁺ monocytes from TB patients had a higher glycolytic capacity.⁷⁸ This largely reflects the distinguished metabolic activities in monocytes between mouse and human during Mtb infection, which will be a critical aspect for the future studies focusing on how Mtb rewires the metabolic network in monocytes and the functional consequences of metabolic reprogramming during infection.

Our metabolic analysis using Seahorse and SCENITH revealed the active engagement of both glycolysis and OXPHOS of activated CD64⁺ neutrophils in Mtb-infected mice, indicating an increased mitochondrial metabolism in these cells. Previously, neutrophils were known to highly rely on glycolysis for survival and function due to their low mitochondria density.³⁴ Indeed, glucose metabolism plays a pivotal role in regulating various antimicrobial functions of neutrophils, including phagocytosis, reactive oxygen species (ROS) production, and more recently, neutrophil extracellular traps (NETs) formation.⁷⁹ Neutrophils express an array of cytokines in granulomas in Mtb-infected NHPs.⁸⁰ Consistent with the role of glycolysis in macrophages, inhibition of this pathway also impaired the production of pro-inflammatory cytokines in neutrophils from Mtb-infected mouse lung. Moreover, neutrophils utilize both glycolysis and the pentose phosphate pathway for ROS production and NETs formation.^{81,82} Type I IFN can activate neutrophils and induce NET formation that promotes Mtb growth and exacerbates disease.^{83,84} It is unclear if glycolysis-induced NETs also contributes to the increased Mtb burden in CD64⁺ neutrophils. The enhanced mitochondrial metabolism in neutrophils has been observed in the tumor model, where a subset of immature neutrophils expressing c-kit elicited by the tumor engage in OXPHOS to support ROS production and maintain immune suppression in the tumor microenvironment in a fatty acid-dependent manner.³⁸ The fatty acid oxidation pathway is particularly important for neutrophil differentiation in the bone marrow, underpinning the requirement of the fatty acid-OXPHOS axis in regulating the generation of functional neutrophils.⁸⁵ Furthermore, pharmacologic inhibition of carnitine palmitoyltransferase 1a impaired neutrophil trafficking to the site of infection, implicating that neutrophils can engage in fatty acid oxidation, and this pathway is essential for an optimal neutrophil response during infection.⁸⁶ While it is unclear whether fatty acid oxidation is active in CD64⁺ neutrophils induced by Mtb infection, increased lipid contents and a greater capacity to uptake free fatty acids indicate the demand of lipids as the energy source to support their own needs. However, the connection between the unique metabolic profile and permissiveness to Mtb in CD64⁺ neutrophils is undetermined. Many pathogens can either promote or suppress various host metabolic pathways that support the bioenergetic and biosynthetic demands of the replication and/or persistence of pathogens. For example, *Legionella pneumophila* induces elevated glycolysis in macrophages to create replication permissive niches.⁸⁷ Both adenovirus and HIV-1 infection upregulate glucose metabolism in epithelial cells or CD4⁺ T cells, respectively, to promote viral replication.^{88,89} In Mtb infection, increased OXPHOS and fatty acid oxidation have been associated with increased permissiveness in AMs.² Thus, we speculate that the increased

mitochondrial metabolism in CD64+ neutrophils leads to increased bacterial number in these cells by providing a fatty acid-enriched environment in spite of higher levels of inflammation and iNOS.

Our results indicate that CD64+ neutrophils express CD36 which facilitates the uptake of lipids, accumulation of intracellular lipid content and associates with better survival of Mtb. CD36 functions as a long-chain free fatty acid transporter and can bind to oxidized low-density lipoproteins.⁹⁰ Genetic deficiency of CD36 confers resistance to mycobacterial infection in mice.⁹¹ It seems that Mtb has evolved mechanisms to exploit CD36-mediated uptake of fatty acids and surfactant lipids to benefit its intracellular growth in macrophages.⁹² While CD36 has been extensively studied in macrophages, recent research has demonstrated that overexpression of CD36 is also observed in low-density neutrophils in non-small cell lung cancer patients.⁹³ Moreover, CD36 deficiency results in reductions in uptake and accumulation of lipids as well as OXPHOS in MDSCs,⁹⁴ further suggesting its role in promoting immunosuppression. While our GSEA analysis did not support that CD64+ neutrophils represent MDSCs in Mtb-infected lung, how they regulate T-cell responses via their active lipid metabolism during Mtb infection is unclear. Furthermore, the underlying mechanism of how lipid-rich CD64+ neutrophils promote Mtb growth is unclear. It is well-known that Mtb promotes the accumulation of lipid droplets within macrophages in an IFN γ -dependent manner and utilizes these lipids as the primary carbon source for growth and survival.^{95,96} This data is in line with our *in vitro* data that IFN γ further increased lipid content in Mtb-infected neutrophils, implicating a similar model may apply to neutrophils. Lastly, what drives the generation of CD64+ neutrophils remains elusive. Neutrophils are heterogeneous at the steady state in the bone marrow.^{18,32} Bacterial infection can reprogram and prime each neutrophil subset,⁹⁷ implicating that CD64+ and CD64- neutrophils may originate from neutrophils at different maturation stages during Mtb infection and local cytokine signals could further influence their metabolism.

Our study illustrates the critical role of cellular metabolism of lung neutrophils in TB and reveals how neutrophil metabolism is closely associated with their permissiveness. These results emphasize the essential role of neutrophil metabolism in regulating Mtb infection and shed light on targeting cellular metabolism as potential host-directed therapeutics. Importantly, IFN γ has profound effects on phenotypes and functions of neutrophils.⁹⁸ In the context of TB, while IFN γ has been long known as a key protective cytokine, it appears to be involved in promoting activated neutrophils with a permissive phenotype. This further reinforces the concern that simply targeting adaptive immune responses may not be sufficient for the development of a robust and effective TB vaccine.⁹⁹ Our findings demonstrate that heterogeneity and diversified metabolism contribute to the permissiveness of neutrophils, and further represent extra layers of regulation during Mtb infection.

METHODS

Animal experiments

C57BL/6 WT, CD36 KO, and C3HeB/FeJ mice were purchased from The Jackson Laboratory. IFN γ KO Mice were a gift from Dr Lin-Xi Li [University of Arkansas for

Medical Sciences (UAMS)]. LysM^{Cre}ARNT^{flox/flox} and LysM^{Cre}ARNT^{flox/+} mice were a gift from Drs. Tiffany Weinkopff (UAMS) and M. Celeste Simon (University of Pennsylvania, Philadelphia, Pennsylvania). All mice for experiments were used at 6–10 weeks old and maintained in a specific pathogen-free biosafety level-3 facility at the UAMS. Sex- and age-matched controls were used in all experiments. Animal care was in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of UAMS and Washington University in St Louis.

Samples from six cynomolgus macaques (*Macaca fascicularis*; identified as M21718, M22018, M22118, M22418, M23318, and M23418) that were involved in studies at the University of Pittsburgh were included in this study. These studies were approved by the University of Pittsburgh's IACUC and were performed under protocol #18052927. The animals were infected by intra-bronchial instillation of Mtb (Erdman stain) into the right and left lower lungs with monkeys 21718, 22018, and 22118 being infected with 30 colony-forming units (CFUs) and 22418, 23318, and 23418 being infected with 18 CFU and disease progression was monitored as previously indicated.¹⁰⁰ As per the design of the study the animals were involved in, four of the animals (M21718, M22118, M23318, M23418) were treated with linezolid for 8 weeks before being necropsied while M22018 and M22418 were untreated controls. At the end of their time in study (144–146 days post-infection), the animals were humanely euthanized, and necropsies were performed to acquire tissues for analysis.¹⁰⁰ The samples analyzed here were convenience samples that were stained for CD64 for use within this study. CD64 expression data in individual animals and tissues are included (Supplementary Fig. 2E). All aspects of animal husbandry, procedures, and experimental manipulations were approved by the University of Pittsburgh IACUC.

Mtb and BCG strains

The Erdman and H37Rv strains of Mtb were used in the study. Erdman (*smyc*::mCherry) and the fluorescent reporter Mtb strain, Erdman (SSB-GFP, *smyc*::mCherry) were generated by transformation of pCherry3 replicating plasmids into WT Erdman Mtb, as previously described,⁵⁶ and were from Shumin Tan at Tufts University School of Medicine. All reporter constructs are episomal plasmids. H37Rv strain was from William R. Jacobs Jr at Albert Einstein College of Medicine. The bacteria were grown to log phase in Middlebrook 7H9 broth supplemented with 10% oleic acid/albumin/dextrose/catalase, 0.2% glycerol, 0.05% Tween 80 and 50 µg/mL hygromycin B (mCherry and SSB-GFP reporter Mtb strains) aliquoted in 10% glycerol, and stored at –80 °C until use. BCG (Pasteur) was grown as describe above.

Mice infections with Mtb and BCG

For intranasal infection, mice were inoculated with approximately 1000 CFUs of different Erdman strains in 30 µL of phosphate-buffered saline (PBS) containing 0.05% Tween 80. The inoculum dosage was confirmed by plating different dilutions on 7H10 plates supplemented with 50 µg/mL hygromycin B. This dosage will generate sufficient Mtb-infected cells (mCherry+) for downstream analyses. In some experiments, mice were infected with H37Rv strain via aerosol inhalation. Briefly, Mtb cultured to mid-log phase

(optical density₆₀₀ 0.6–0.8) was first pelleted and resuspended in sterile water containing 0.05% Tween 80. A single-cell suspension was generated through sequential low-speed centrifugation at 800 rpm until stabilization of optical density₆₀₀ of the supernatant. Mice were infected by aerosol using a Glas-Col inhalation exposure system to achieve an infection dose of 1000 CFUs. The infection dose was verified by plating the homogenates from lungs harvested the day after aerosol infection. Bacterial loads in sorted cells were determined by lysing cell pellets in PBS containing 0.01% SDS, and plated on 7H10 agar. Plates were incubated at 37 °C and colonies were enumerated 3 weeks later. For BCG infection, mice were intranasally infected with 10⁶ BCG (Pasteur) bacilli. Lung cells were isolated at 2 weeks post-infection.

Lung cell isolation

Lung cells from Mtb-infected mice were isolated as described previously.² Briefly, lungs were removed and digested in 5% fetal bovine serum (FBS)/PBS solution containing 160–250 U/mL collagenase IV (Worthington or Millipore Sigma) and 20–75 U/mL DNase (Sigma) with a GentleMACS Octo dissociator with heaters (Miltenyi Biotec, Auburn, CA). The digested lung material was then passed through a 70-µm cell strainer. Red blood cells were lysed with Ammonium-Chloride-Potassium (ACK) lysis buffer (Lonza). For NHP samples, granulomas were acquired at necropsy and homogenized with a GentleMACS tissue dissociator (Miltenyi Biotec, Auburn, CA) using the human tumor dissociation enzyme kit (Miltenyi Biotec).

Flow cytometry

Lung cell suspensions isolated from Mtb-infected mice were stained with fixable viability dye from Invitrogen, incubated with Fc block (BioLegend) for 15 minutes, and then counted and incubated for 30 minutes in the dark with the following fluorophore-conjugated antibodies, CD11b (M1/70), CD36 (HM36), CD64 (X54–5/7.1), Ly6G (1A8), MerTK (DS5MMER), SiglecF (E50–2440), Ly6C (HK1.4), MHCII (M5/114.15.2), CD11c (N418), iNOS (CXNFT) and IL-1β (NJTEN3), CD45 (30-F11), ICAM1 (YN1/1.7.4), CXCR4 (L276F12), CD62L (MEL-14s). For EdU labeling, mice were pulsed with 0.5 mg of EdU, and single-cell suspensions were surface-stained, fixed in 4% PFA for 10 minutes at room temperature, permeabilized, and stained for EdU per the manufacturer's instruction (Click-iT EdU Flow Cytometry Assay Kit, Invitrogen). To measure lipid uptake *in vivo*, mice were injected intraperitoneally (i.p.) with Bodipy FL C₁₆ (50 µg/mouse) diluted in DMSO. Lung cells were isolated 60 minutes post-injection and analyzed by flow cytometry. To quantify intracellular neutral lipids, lung cells were stained with 2 µM Bodipy 493/503 (Invitrogen) in PBS for 10 minutes. Cells were washed and analyzed with an LSRFortessa flow cytometer (BD Biosciences) in the flow cytometry core facility at UAMS or an LSR Fortessa X-20 flow cytometer (BD Biosciences) in the flow cytometry core at Washington University School of Medicine in St Louis. For reporter Mtb strain quantification, fixed lung cells were sorted with a FACSAria IIIu machine (BD) in the flow cytometry core at UAMS. Data were analyzed with FlowJo software (Tree Star). For Met-flow analysis, lung cells were fixed and permeabilized with Foxp3 fixation/permeabilization kit followed by intracellular staining with a cocktail of antibodies against the metabolic proteins. All antibodies were purchased from AbCam and conjugated in-house with AbCam Lightening-

Link Conjugation kit as described.¹⁰¹ Cells were acquired on a Cytex Northern Lights spectral flow cytometry (Cytex Biosciences). Samples were unmixed using SpectroFlo version 3 (Cytex Biosciences) and analyzed with FlowJo software (Tree Star).

NHP cells from RBC-lysed whole blood and tissues were stained with Aqua viability dye (ThermoFisher) CD3 (clone SP34-2), CD11b (clone Mac-1), CD14 (clone MφP9), CD64 (clone 10.1), CD206 (clone 19.2), and intracellularly for calprotectin (S100A9; clone MAC387, ThermoFisher). Unless otherwise indicated all antibodies for staining NHP cells were from BD Biosciences and all samples were run on a BD Fortessa flow cytometer maintained by the University of Pittsburgh Department of Infectious Diseases and Microbiology.

Quantification of Mtb reporter expression

Sorted lung neutrophils were stained with DAPI and Alexa Fluor 647 conjugated phalloidin (Invitrogen) and attached on poly-L-lysine coated slides. Reporter Mtb expression quantification was carried out as previously described.^{56,102} Briefly, cells were imaged using a Zeiss LSM 800 confocal microscope at UAMS Digital Microscopy Core. Z-stacks were reconstructed into 3D using Volocity software (PerkinElmer). Quantification of the SSB-GFP reporter was accomplished by counting the number of bacteria with and without-SSB-GFP replication foci, and calculating the total percentage of bacteria presenting one or more SSB-GFP foci for each cell type. At least 100 bacteria were quantified.

SCENITH

The SCENITH kit, developed by R. Argüello (CIML) was requested from <https://www.scenith.com/>. SCENITH experiments were performed as previously described.⁴² Nearly half of the total energy that mammalian cells generated by various metabolic pathways is utilized by the PS machinery, which forms the foundation of SCENITH. The rationale of SCENITH is to measure alterations of PS levels in cells in the presence of 2-DG or oligomycin using puromycin incorporation as the readout. Thus, SCENITH does not directly measure bioenergetics and metabolism. Briefly, lung cell suspensions were cultured in DMEM containing 10% FBS and incubated with control, 2-DG, oligomycin, or a combination of both inhibitors. Puromycin was added for additional 20 minutes. Cells were then washed in cold PBS and stained with Live/Dead fixable viability dye, washed, and incubated with Fc block before surface staining. Cells were fixed and permeabilized using Foxp3 fixation/permeabilization kit and then stained with anti-puromycin antibody. Glucose dependence, mitochondrial dependence, and glycolytic capacity were calculated as described previously.⁴²

Extracellular flux analysis

Seahorse XF HS Mini Extracellular Flux Analyzer (Agilent) was used for real-time analysis of OCR and ECAR. Lung AMs, IMs and neutrophils were isolated and plated in 8-well Seahorse plates. Cells were washed and analyzed in Seahorse XF base medium with 2mM L-glutamine to obtain real-time measurements of OCR and ECAR. BMNs from C57BL/6 mice were isolated with MojoSort Mouse Neutrophil Isolation kit (BioLegend).

Three or four consecutive measurements were obtained under basal conditions and after the sequential addition of 10 mM glucose, 1 μ M oligomycin and 50 mM 2-DG for glycolysis stress test, or 1 μ M oligomycin, 1.5 μ M FCCP, and 100 nM rotenone plus 1 μ M antimycin A for mitochondrial stress test. All inhibitors were purchased from Sigma or Cayman Chemical.

BMDM and BMN infection

BMDMs were cultured in DMEM (Corning) supplemented with 10% FBS (Gibco), 20% L-cell conditioned media, 2 mM L-glutamine, 1mM sodium pyruvate (Corning), and 1% penicillin/streptomycin (Corning) at 37 °C for 6 days. BMNs were isolated as described above. Both cell types were infected with Mtb at MOI 5 for 6 hours prior to Seahorse analysis. In some experiments, IFN γ (50 ng/mL, BioLegend) was added to the Mtb-infected neutrophil culture. Cells were then washed and stained for flow cytometry.

Phagocytosis assay

pHrodo Deep Red E. coli BioParticles conjugate was used for phagocytosis assay to measure phagocytic activities neutrophils isolated from Mtb-infected mice. Briefly, BioParticles were sonicated in a bath sonicator for 10 minutes before adding to lung cell suspensions from Mtb-infected mice at 100 μ g/mL. Cells were incubated at 37 °C in a tissue culture incubator for 30 minutes followed by staining for flow cytometry as described above.

Electron microscopy

Lung cells were stained and fixed prior to sorting for neutrophils. Neutrophils were centrifugated and carefully resuspended in glutaraldehyde fixative solution (2.5% glutaraldehyde in 0.1 M sodium cacodylate, 5 mM calcium chloride, 5 mM magnesium chloride, 0.1 M sucrose, pH7.2). The samples were processed and stained for electron microscopy as described previously.¹⁰³

Single-cell transcriptomic data analysis

scRNA-seq dataset of macaque lung myeloid cells 4 weeks post TB infection published by Gideon et al.⁶² 2022 was obtained from the Broad Institute Single Cell Portal (GSE200151). scRNA-seq dataset of murine lung myeloid cells 3 weeks post TB infection was obtained from GEO with accession code GSE167232.⁵⁸ Data analysis was performed using the Seurat v.4 package in R 4.1.0 (<https://www.R-project.org/>).¹⁰⁴ In brief, neutrophil subsets were extracted from the datasets based on the published cluster labels. The subsets were scaled and re-clustered using UMAP as a dimensionality reduction method.¹⁰⁵ Markers of each neutrophil cluster in both mouse and NHP datasets (upregulated genes that are expressed in a minimal of 25% of the cells with a minimal log2 fold change of 0.5 and an adjusted p < 0.05) were identified using the Seurat v.4 FindAllMarkers function.¹⁰⁴ The GSEA of the cluster transcriptomic signatures was performed using package clusterProfiler 4.4.4.¹⁰⁶

Gene expression analysis

Gene expression analysis in neutrophils from different tissues herein used a previously described database generated by the Immunological Genome Project.¹⁰⁷

Statistical analysis

Statistical information including n , mean and statistical significance values are indicated in the figure legends. Results were analyzed using Student's t test or one-way analysis of variance test with multiple comparisons where appropriate using Prism 9.5 (GraphPad Software, Inc).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY

All data underlying the research are presented in the published article and its online supplemental material and/or available from the corresponding authors upon reasonable request. scRNA-seq data obtained from GEO under accession numbers GSE200151 (NHP) and GSE167232 (mouse).

REFERENCES

1. Cohen SB et al. Alveolar macrophages provide an early Mycobacterium tuberculosis Niche and initiate dissemination. *Cell Host Microbe* 24, 439–446. e4 (2018). [PubMed: 30146391]
2. Huang L, Nazarova EV, Tan S, Liu Y & Russell DG Growth of Mycobacterium tuberculosis *in vivo* segregates with host macrophage metabolism and ontogeny. *J. Exp. Med* 215, 1135–1152 (2018). [PubMed: 29500179]
3. Mendonca LE et al. Human alveolar macrophage metabolism is compromised during Mycobacterium tuberculosis infection. *Front. Immunol* 13:1044592.
4. Ryndak MB & Laal S Mycobacterium tuberculosis primary infection and dissemination: a critical role for alveolar epithelial cells. *Front. Cell Infect. Microbiol* 9, 299 (2019). [PubMed: 31497538]
5. Kinkhikar AG et al. Potential role for ESAT6 in dissemination of M. tuberculosis via human lung epithelial cells. *Mol. Microbiol* 75, 92–106 (2010). [PubMed: 19906174]

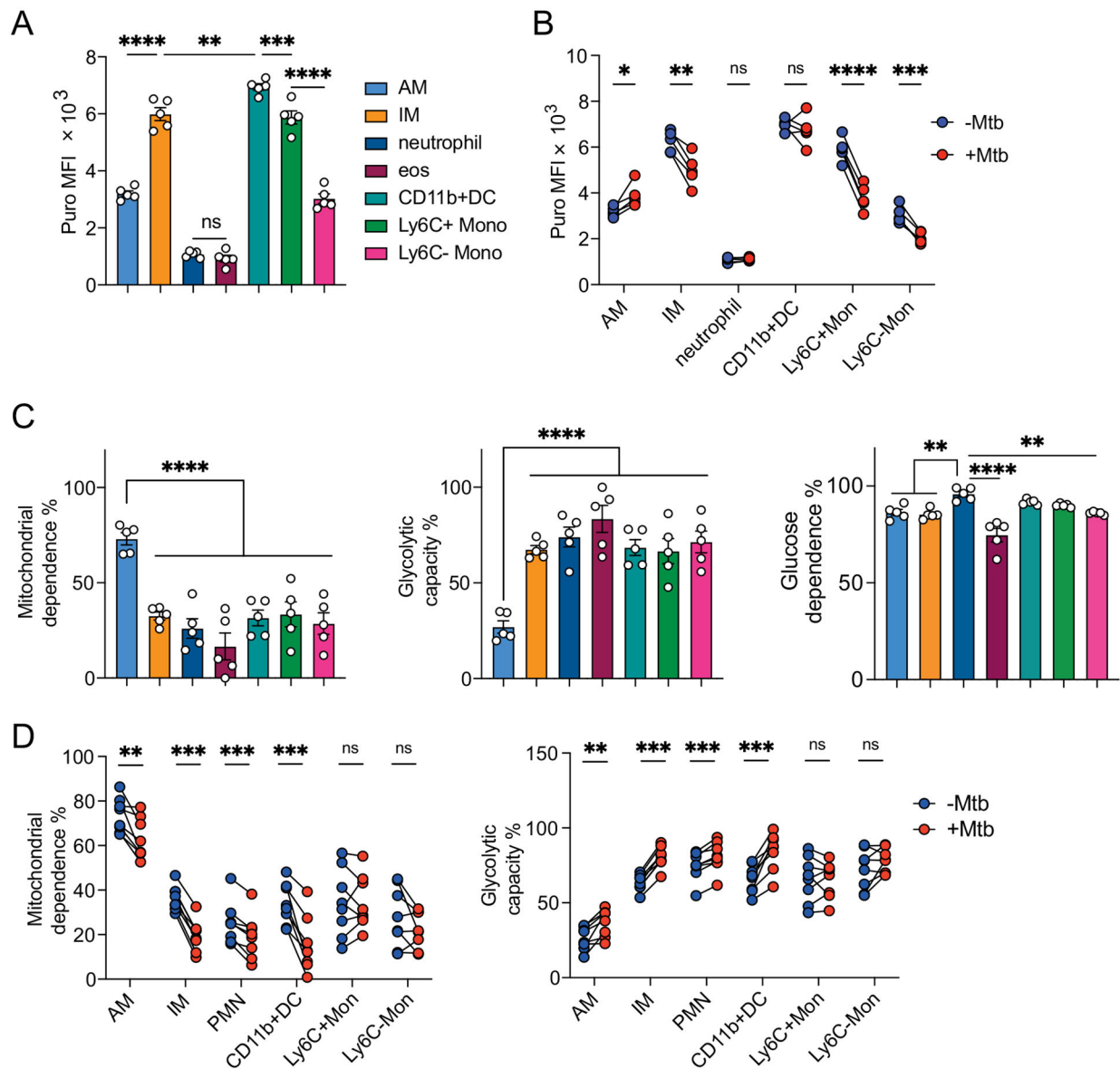
6. Pethe K et al. The heparin-binding haemagglutinin of *M. tuberculosis* is required for extrapulmonary dissemination. *Nature* 412, 190–194 (2001). [PubMed: 11449276]
7. Mishra BB et al. Nitric oxide prevents a pathogen-permissive granulocytic inflammation during tuberculosis. *Nat. Microbiol* 2, 17072 (2017). [PubMed: 28504669]
8. Hoffman D et al. A non-classical monocyte-derived macrophage subset provides a splenic replication niche for intracellular *Salmonella*. *Immunity* 54, 2712–2723.e6 (2021). [PubMed: 34788598]
9. Chandra P et al. Inhibition of fatty acid oxidation promotes macrophage control of *Mycobacterium tuberculosis*. *mBio* 11, e01139–e1220 (2020). [PubMed: 32636249]
10. Eum SY et al. Neutrophils are the predominant infected phagocytic cells in the airways of patients with active pulmonary TB. *Chest* 137, 122–128 (2010). [PubMed: 19749004]
11. Nandi B & Behar SM Regulation of neutrophils by interferon-gamma limits lung inflammation during tuberculosis infection. *J. Exp. Med* 208, 2251–2262 (2011). [PubMed: 21967766]
12. Ozaki T et al. Differential cell analysis in bronchoalveolar lavage fluid from pulmonary lesions of patients with tuberculosis. *Chest* 102, 54–59 (1992). [PubMed: 1623796]
13. Lowe DM et al. Neutrophilia independently predicts death in tuberculosis. *Eur. Respir. J* 42, 1752–1757 (2013). [PubMed: 24114967]
14. Mattila JT, Maiello P, Sun T, Via LE & Flynn JL Granzyme B-expressing neutrophils correlate with bacterial load in granulomas from *Mycobacterium tuberculosis*-infected cynomolgus macaques. *Cell Microbiol.* 17, 1085–1097 (2015). [PubMed: 25653138]
15. Kimmey JM et al. Unique role for ATG5 in neutrophil-mediated immunopathology during *M. tuberculosis* infection. *Nature* 528, 565–569 (2015). [PubMed: 26649827]
16. Philips JAN Neutrophils: Double agents for TB. *Sci. Transl. Med* 9, eaan6195 (2017). [PubMed: 28615363]
17. Stallings CL Host response: inflammation promotes TB growth. *Nat. Microbiol* 2, 17102 (2017). [PubMed: 28653684]
18. Ng LG, Ostuni R & Hidalgo A Heterogeneity of neutrophils. *Nat. Rev. Immunol* 19, 255–265 (2019). [PubMed: 30816340]
19. Silvestre-Roig C, Hidalgo A & Soehnlein O Neutrophil heterogeneity: implications for homeostasis and pathogenesis. *Blood* 127, 2173–2181 (2016). [PubMed: 27002116]
20. Ballesteros I et al. Co-option of neutrophil fates by tissue environments. *Cell* 183, 1282–1297.e18 (2020). [PubMed: 33098771]
21. Kirsebom FCM, Kausar F, Nuriev R, Makris S & Johansson C Neutrophil recruitment and activation are differentially dependent on MyD88/TRIF and MAVS signaling during RSV infection. *Mucosal Immunol.* 12, 1244–1255 (2019). [PubMed: 31358860]
22. Cheung DS et al. Cutting edge: CD49d⁺ neutrophils induce FcεpsilonRI expression on lung dendritic cells in a mouse model of postviral asthma. *J. Immunol* 185, 4983–4987 (2010). [PubMed: 20876348]
23. Taylor PR et al. Activation of neutrophils by autocrine IL-17A-IL-17RC interactions during fungal infection is regulated by IL-6, IL-23, RORγt and dectin-2. *Nat. Immunol* 15, 143–151 (2014). [PubMed: 24362892]
24. Pillay J et al. Functional heterogeneity and differential priming of circulating neutrophils in human experimental endotoxemia. *J. Leukoc. Biol* 88, 211–220 (2010). [PubMed: 20400675]
25. Tsuda Y et al. Three different neutrophil subsets exhibited in mice with different susceptibilities to infection by methicillin-resistant *Staphylococcus aureus*. *Immunity* 21, 215–226 (2004). [PubMed: 15308102]
26. Ryu S et al. Siglec-F-expressing neutrophils are essential for creating a profibrotic microenvironment in renal fibrosis. *J. Clin. Invest* 132:e156876.
27. Shin JW et al. A unique population of neutrophils generated by air pollutant-induced lung damage exacerbates airway inflammation. *J. Allergy Clin. Immunol* 149, 1253–1269.e8 (2022). [PubMed: 34653517]

28. Tsiganov EN et al. Gr-1dimCD11b+ immature myeloid-derived suppressor cells but not neutrophils are markers of lethal tuberculosis infection in mice. *J. Immunol* 192, 4718–4727 (2014). [PubMed: 24711621]
29. Knaul JK et al. Lung-residing myeloid-derived suppressors display dual functionality in murine pulmonary tuberculosis. *Am. J. Respir. Crit. Care Med* 190, 1053–1066 (2014). [PubMed: 25275852]
30. du Plessis N et al. Increased frequency of myeloid-derived suppressor cells during active tuberculosis and after recent mycobacterium tuberculosis infection suppresses T-cell function. *Am. J. Respir. Crit. Care Med* 188, 724–732 (2013). [PubMed: 23885784]
31. Deng Y et al. Low-density granulocytes are elevated in mycobacterial infection and associated with the severity of tuberculosis. *PLoS One* 11: e0153567.
32. Silvestre-Roig C, Fridlender ZG, Glogauer M & Scapini P Neutrophil diversity in health and disease. *Trends Immunol.* 40, 565–583 (2019). [PubMed: 31160207]
33. Lovewell RR, Baer CE, Mishra BB, Smith CM & Sasseti CM Granulocytes act as a niche for *Mycobacterium tuberculosis* growth. *Mucosal Immunol.* 14, 229–241 (2021). [PubMed: 32483198]
34. Fossati G et al. The mitochondrial network of human neutrophils: role in chemotaxis, phagocytosis, respiratory burst activation, and commitment to apoptosis. *J. Immunol* 170, 1964–1972 (2003). [PubMed: 12574365]
35. Borregaard N & Herlin T Energy metabolism of human neutrophils during phagocytosis. *J. Clin. Invest* 70, 550–557 (1982). [PubMed: 7107894]
36. Morrison T, Watts ER, Sadiku P & Walmsley SR The emerging role for metabolism in fueling neutrophilic inflammation. *Immunol. Rev* 314, 427–441 (2023). [PubMed: 36326284]
37. Li DD et al. Fungal sensing enhances neutrophil metabolic fitness by regulating antifungal Glut1 activity. *Cell Host Microbe* 30, 530–544.e6 (2022). [PubMed: 35316647]
38. Rice CM et al. Tumour-elicited neutrophils engage mitochondrial metabolism to circumvent nutrient limitations and maintain immune suppression. *Nat. Commun* 9, 5099 (2018). [PubMed: 30504842]
39. Veglia F et al. Fatty acid transport protein 2 reprograms neutrophils in cancer. *Nature.* 569, 73–78 (2019). [PubMed: 30996346]
40. Lindqvist LM, Tandoc K, Topisirovic I & Furic L Cross-talk between protein synthesis, energy metabolism and autophagy in cancer. *Curr. Opin. Genet. Dev* 48, 104–111 (2018). [PubMed: 29179096]
41. Buttgerit F & Brand MD A hierarchy of ATP-consuming processes in mammalian cells. *Biochem. J* 312, 163–167 (1995). [PubMed: 7492307]
42. Argüello RJ et al. SCENITH: a flow cytometry-based method to functionally profile energy metabolism with single-cell resolution. *Cell Metab.* 32, 1063–1075.e7 (2020). [PubMed: 33264598]
43. Hidalgo San Jose L & Signer RAJ Cell-type-specific quantification of protein synthesis in vivo. *Nat. Protoc* 14, 441–460 (2019). [PubMed: 30610239]
44. Aviner R The science of puromycin: from studies of ribosome function to applications in biotechnology. *Comput. Struct. Biotechnol. J* 18, 1074–1083 (2020). [PubMed: 32435426]
45. Schmidt EK, Clavarino G, Ceppi M & Pierre P SUNSET, a nonradioactive method to monitor protein synthesis. *Nat. Methods* 6, 275–277 (2009). [PubMed: 19305406]
46. Bohrer AC et al. Eosinophils are part of the granulocyte response in tuberculosis and promote host resistance in mice. *J. Exp. Med* 218:e20210469. [PubMed: 34347010]
47. Adamik J et al. Distinct metabolic states guide maturation of inflammatory and tolerogenic dendritic cells. *Nat. Commun* 13, 5184 (2022). [PubMed: 36056019]
48. Corral D et al. ILC precursors differentiate into metabolically distinct ILC1-like cells during *Mycobacterium tuberculosis* infection. *Cell Rep.* 39:110715.
49. Summers C et al. Neutrophil kinetics in health and disease. *Trends Immunol.* 31, 318–324 (2010). [PubMed: 20620114]

50. Basu S, Hodgson G, Katz M & Dunn AR Evaluation of role of G-CSF in the production, survival, and release of neutrophils from bone marrow into circulation. *Blood* 100, 854–861 (2002). [PubMed: 12130495]
51. Pillay J et al. In vivo labeling with $^2\text{H}_2\text{O}$ reveals a human neutrophil lifespan of 5.4 days. *Blood* 116, 625–627 (2010). [PubMed: 20410504]
52. Klein JB et al. Granulocyte-macrophage colony-stimulating factor delays neutrophil constitutive apoptosis through phosphoinositide 3-kinase and extracellular signal-regulated kinase pathways. *J. Immunol* 164, 4286–4291 (2000). [PubMed: 10754327]
53. Suzuki K, Hino M, Hato F, Tatsumi N & Kitagawa S Cytokine-specific activation of distinct mitogen-activated protein kinase subtype cascades in human neutrophils stimulated by granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor- α . *Blood* 93, 341–349 (1999). [PubMed: 9864179]
54. Derouet M, Thomas L, Cross A, Moots RJ & Edwards SW Granulocyte macrophage colony-stimulating factor signaling and proteasome inhibition delay neutrophil apoptosis by increasing the stability of Mcl-1. *J. Biol. Chem* 279, 26915–26921 (2004). [PubMed: 15078892]
55. Norris BA & Ernst JD Mononuclear cell dynamics in *M. tuberculosis* infection provide opportunities for therapeutic intervention. *PLoS Pathog.* 14: e1007154.
56. Sukumar N, Tan S, Aldridge BB & Russell DG Exploitation of *Mycobacterium tuberculosis* reporter strains to probe the impact of vaccination at sites of infection. *PLoS Pathog.* 10:e1004394.
57. Muñoz-Elías EJ et al. Replication dynamics of *Mycobacterium tuberculosis* in chronically infected mice. *Infect. Immun* 73, 546–551 (2005). [PubMed: 15618194]
58. Pisu D et al. Single cell analysis of *M. tuberculosis* phenotype and macrophage lineages in the infected lung. *J. Exp. Med* 218:e20210615. [PubMed: 34292313]
59. Veglia F, Sanseviero E & Gabrilovich, D.I. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. *Nat. Rev. Immunol* 21, 485–498 (2021). [PubMed: 33526920]
60. Alshetaiwi H et al. Defining the emergence of myeloid-derived suppressor cells in breast cancer using single-cell transcriptomics. *Sci. Immunol* 5, eaay6017 (2020). [PubMed: 32086381]
61. Kramnik I & Beamer G Mouse models of human TB pathology: roles in the analysis of necrosis and the development of host-directed therapies. *Semin. Immunopathol* 38, 221–237 (2016). [PubMed: 26542392]
62. Gideon HP et al. Multimodal profiling of lung granulomas in macaques reveals cellular correlates of tuberculosis control. *Immunity* 55, 827–846.e10 (2022). [PubMed: 35483355]
63. Flynn JL, Gideon HP, Mattila JT & Lin PL Immunology studies in non-human primate models of tuberculosis. *Immunol. Rev* 264, 60–73 (2015). [PubMed: 25703552]
64. Ahl PJ et al. Met-Flow, a strategy for single-cell metabolic analysis highlights dynamic changes in immune subpopulations. *Commun. Biol* 3, 305 (2020). [PubMed: 32533056]
65. Cassatella MA et al. Interferon gamma induces in human neutrophils and macrophages expression of the mRNA for the high affinity receptor for monomeric IgG (Fc gamma R-I or CD64). *Biochem. Biophys. Res. Commun* 170, 582–588 (1990). [PubMed: 2143376]
66. Buckle AM & Hogg N The effect of IFN- γ and colony-stimulating factors on the expression of neutrophil cell membrane receptors. *J. Immunol* 143, 2295–2301 (1989). [PubMed: 2476506]
67. Lee SH et al. Mannose receptor high, M2 dermal macrophages mediate nonhealing *Leishmania* major infection in a Th1 immune environment. *J. Exp. Med* 215, 357–375 (2018). [PubMed: 29247046]
68. Pham THM et al. Salmonella-driven polarization of granuloma macrophages antagonizes TNF-mediated pathogen restriction during persistent infection. *Cell Host Microbe* 27, 54–67.e5 (2020). [PubMed: 31883922]
69. Abi Abdallah DS, Egan CE, Butcher BA & Denkers EY Mouse neutrophils are professional antigen-presenting cells programmed to instruct Th1 and Th17 T-cell differentiation. *Int. Immunol* 23, 317–326 (2011). [PubMed: 21422151]
70. Nair S et al. Irg1 expression in myeloid cells prevents immunopathology during *M. tuberculosis* infection. *J. Exp. Med* 215, 1035–1045 (2018). [PubMed: 29511063]

71. Nancy Hilda J & Das SN Neutrophil CD64, TLR2 and TLR4 expression increases but phagocytic potential decreases during tuberculosis. *Tuberculosis (Edinb)*. 111, 135–142 (2018). [PubMed: 30029898]
72. Berry MPR et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 466, 973–977 (2010). [PubMed: 20725040]
73. Woods PS et al. Tissue-resident alveolar macrophages do not rely on glycolysis for LPS-induced inflammation. *Am. J. Respir. Cell Mol. Biol* 62, 243–255 (2020). [PubMed: 31469581]
74. Cumming BM, Addicott KW, Adamson JH & Steyn AJ *Mycobacterium tuberculosis* induces decelerated bioenergetic metabolism in human macrophages. *Elife* 7, e39169 (2018). [PubMed: 30444490]
75. Mohr I & Sonenberg N Host translation at the nexus of infection and immunity. *Cell Host Microbe* 12, 470–483 (2012). [PubMed: 23084916]
76. Pagán AJ et al. mTOR-regulated mitochondrial metabolism limits mycobacterium-induced cytotoxicity. *Cell* 185, 3720–3738.e13 (2022). [PubMed: 36103894]
77. Deng W et al. Essential role of mTORC1 in self-renewal of murine alveolar macrophages. *J. Immunol* 198, 492–504 (2017). [PubMed: 27881705]
78. Maio M et al. Elevated glycolytic metabolism of monocytes limits the generation of HIF-1 α -driven migratory dendritic cells in tuberculosis. *Elife* 2023.
79. Rodríguez-Espinosa O, Rojas-Espinosa O, Moreno-Altamirano MMB, López-Villegas EO & Sánchez-García FJ Metabolic requirements for neutrophil extracellular traps formation. *Immunology* 145, 213–224 (2015). [PubMed: 25545227]
80. Gideon HP, Phuah J, Junecko BA & Mattila JT Neutrophils express pro- and anti-inflammatory cytokines in granulomas from *Mycobacterium tuberculosis*-infected cynomolgus macaques. *Mucosal Immunol.* 12, 1370–1381 (2019). [PubMed: 31434990]
81. Toller-Kawahisa JE et al. The metabolic function of pyruvate kinase M2 regulates reactive oxygen species production and microbial killing by neutrophils. *Nat. Commun* 14, 4280 (2023). [PubMed: 37460614]
82. Britt EC et al. Switching to the cyclic pentose phosphate pathway powers the oxidative burst in activated neutrophils. *Nat. Metab* 4, 389–403 (2022). [PubMed: 35347316]
83. Moreira-Teixeira L et al. Type I IFN exacerbates disease in tuberculosis-susceptible mice by inducing neutrophil-mediated lung inflammation and NETosis. *Nat. Commun* 11, 5566 (2020). [PubMed: 33149141]
84. Kotov DI et al. Early cellular mechanisms of type I interferon-driven susceptibility to tuberculosis. *Cell* 186, 5536–5553.e22 (2023). [PubMed: 38029747]
85. Riffelmacher T et al. Autophagy-dependent generation of free fatty acids is critical for normal neutrophil differentiation. *Immunity* 47, 466–480.e5 (2017). [PubMed: 28916263]
86. Pham L et al. Neutrophil trafficking to the site of infection requires Cpt1a-dependent fatty acid beta-oxidation. *Commun. Biol* 5, 1366 (2022). [PubMed: 36513703]
87. Escoll P et al. *Legionella pneumophila* modulates mitochondrial dynamics to trigger metabolic repurposing of infected macrophages. *Cell Host Microbe* 22, 302–316.e7 (2017). [PubMed: 28867389]
88. Thai M et al. Adenovirus E4ORF1-induced MYC activation promotes host cell anabolic glucose metabolism and virus replication. *Cell Metab.* 19, 694–701 (2014). [PubMed: 24703700]
89. Hegedus A, Kavanagh Williamson M & Huthoff H HIV-1 pathogenicity and virion production are dependent on the metabolic phenotype of activated CD4⁺ T cells. *Retrovirology* 11, 98 (2014). [PubMed: 25421745]
90. Chen Y, Zhang J, Cui W & Silverstein RL CD36, a signaling receptor and fatty acid transporter that regulates immune cell metabolism and fate. *J. Exp. Med* 219:e20211314.
91. Hawkes M et al. CD36 deficiency attenuates experimental mycobacterial infection. *BMC Infect. Dis* 10, 299 (2010). [PubMed: 20950462]
92. Dodd CE, Pyle CJ, Glowinski R, Rajaram MVS & Schlesinger LS CD36-mediated uptake of surfactant lipids by human macrophages promotes intracellular growth of *Mycobacterium tuberculosis*. *J. Immunol* 197, 4727–4735 (2016). [PubMed: 27913648]

93. Valadez-Cosmes P et al. Identification of novel low-density neutrophil markers through unbiased high-dimensional flow cytometry screening in non-small cell lung cancer patients. *Front. Immunol* 12:703846. [PubMed: 34484199]
94. Al-Khami AA et al. Exogenous lipid uptake induces metabolic and functional reprogramming of tumor-associated myeloid-derived suppressor cells. *Oncoimmunology*. 6:e1344804.
95. Knight M, Braverman J, Asfaha K, Gronert K & Stanley S Lipid droplet formation in *Mycobacterium tuberculosis* infected macrophages requires IFN- γ /HIF-1 α signaling and supports host defense. *PLoS Pathog*. 14: e1006874.
96. Russell DG, Cardona PJ, Kim MJ, Allain S & Altare F Foamy macrophages and the progression of the human tuberculosis granuloma. *Nat. Immunol* 10, 943–948 (2009). [PubMed: 19692995]
97. Xie X et al. Single-cell transcriptome profiling reveals neutrophil heterogeneity in homeostasis and infection. *Nat. Immunol* 21, 1119–1133 (2020). [PubMed: 32719519]
98. Singhal S et al. Origin and role of a subset of tumor-associated neutrophils with antigen-presenting cell features in early-stage human lung cancer. *Cancer Cell* 30, 120–135 (2016). [PubMed: 27374224]
99. Khader SA et al. Targeting innate immunity for tuberculosis vaccination. *J. Clin. Invest* 129, 3482–3491 (2019). [PubMed: 31478909]
100. Maiello P et al. Rhesus macaques are more susceptible to progressive tuberculosis than cynomolgus macaques: a quantitative comparison. *Infect. Immun* 86, e00505–e517 (2018).
101. Heieis GA et al. Metabolic heterogeneity of tissue-resident macrophages in homeostasis and during helminth infection. *Nat. Commun* 14, 5627 (2023). [PubMed: 37699869]
102. Giacalone D, Huang L & Tan S Exploiting fluorescent proteins to understand *Mycobacterium tuberculosis* Biology. *Methods Mol. Biol* 2314, 365–383 (2021). [PubMed: 34235663]
103. Rohde KH, Veiga DFT, Caldwell S, Balázsi G & Russell DG Linking the transcriptional profiles and the physiological states of *Mycobacterium tuberculosis* during an extended intracellular infection. *PLoS Pathog*. 8: e1002769.
104. Hao Y et al. Integrated analysis of multimodal single-cell data. *Cell* 184, 3573–3587.e29 (2021). [PubMed: 34062119]
105. Becht E et al. Dimensionality reduction for visualizing single-cell data using UMAP. *Nat. Biotechnol* 2018.
106. Wu T et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innovation (Camb)*. 2:100141.
107. Heng TSP, Painter MW & Immunological Genome Project Consortium. The Immunological Genome Project: networks of gene expression in immune cells. *Nat. Immunol* 9, 1091–1094 (2008). [PubMed: 18800157]

**Fig. 1.**

Metabolic profile of mouse lung phagocytes in Mtb infection using SCENITH. To profile the metabolic activities in lung phagocytes, lungs were harvested from C57BL/6 mice infected with 10^3 mCherry-Erdman Mtb intranasally at 3 weeks post-infection. (A) Translation level of lung phagocytes in Mtb-infected mice. Mean $\pm$ SEM are shown. $n = 5$. (B) Translation level of Mtb-infected and bystander lung phagocytes. $n = 5$. (C) Mitochondrial dependence, glycolytic capacity and glucose dependence of lung phagocytes in Mtb-infected mice obtained by SCENITH. Mean $\pm$ SEM are shown. $n = 5$. (D) Mitochondrial dependence and glycolytic capacity of Mtb-infected and bystander lung phagocytes obtained by SCENITH. $n = 8$. Mean $\pm$ SEM are shown. $n = 5$. Data in A–C were representative of two independent experiments. Data in D were pooled from two independent experiments. Two-way ANOVA was used to test statistical significance in A and C. Paired t test was used in B and D. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. AM = alveolar macrophage; ANOVA =

analysis of variance; Eos = eosinophil; IM = interstitial macrophage; Mono = monocyte; Mtb = Mycobacterium tuberculosis; SCENITH = single-cell energetic metabolism by profiling translation inhibition; SEM = standard error of the mean.

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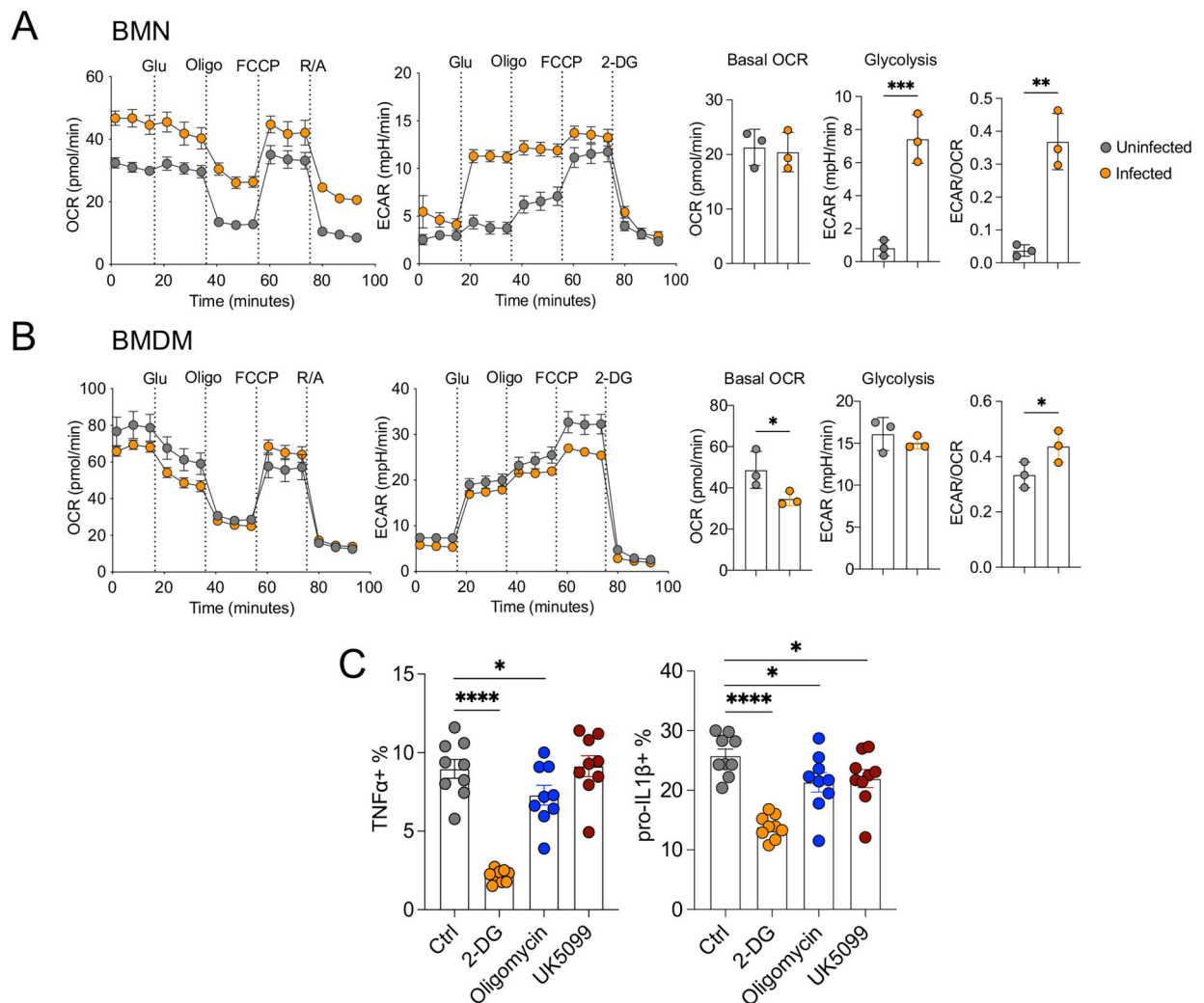
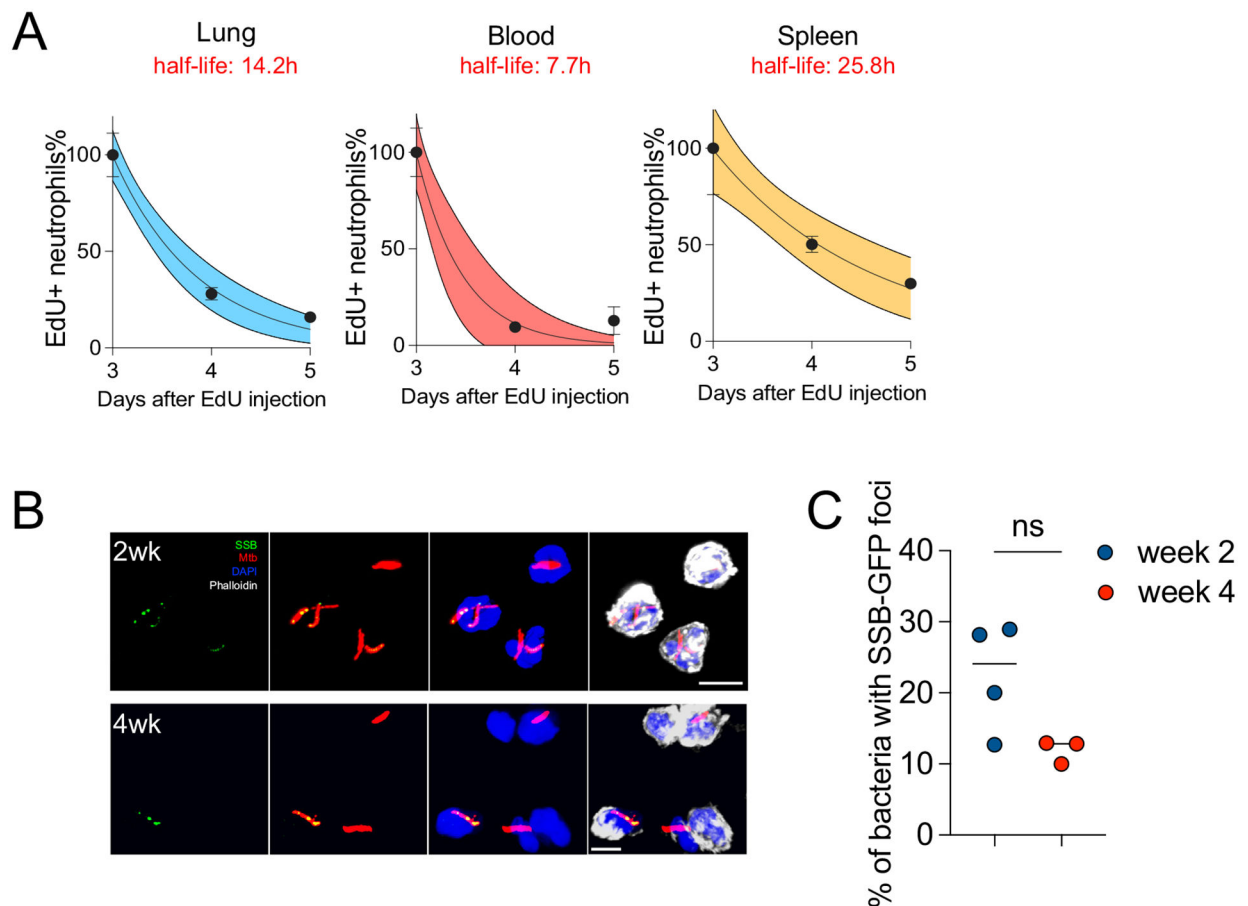


Fig. 2. Neutrophils exhibit distinct metabolism from macrophages in Mtb infection *in vitro*. (A) OCR and ECAR of BMN at 6 hours post-Mtb infection at MOI = 5. (B) OCR and ECAR of (BMDM) at 6 hours post-Mtb infection at MOI = 5. Basal OCR, glycolysis and ECAR/OCR ratio were calculated. Mean $\pm$ SEM are shown. $n = 3$. (C) Intracellular cytokine expression from neutrophils in Mtb-infected mice at 3 weeks post-infection assessed by flow cytometry. Lung cells were treated *ex vivo* with PBS, 2-DG, oligomycin, or UK5099. Mean $\pm$ SEM are shown. $n = 8$. Data in A and B were representative of two independent experiments. Data in C were pooled from two independent experiments. Student's t test was used in A, B, and C. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. 2-DG = 2-deoxyglucose; BMDM = bone marrow-derived macrophages; BMN = bone marrow neutrophils; ECAR = extracellular acidification rate; MOI = multiplicity of infection; Mtb = Mycobacterium tuberculosis; OCR = oxygen consumption rate; PBS = phosphate-buffered saline; SEM = standard error of the mean.

**Fig. 3.**

Neutrophils in Mtb-infected lung have a short lifespan that is long enough to support Mtb replication *in vivo*. To measure the turnover rate of neutrophils, mice were infected with 10^3 Erdman Mtb for 2 weeks. EdU was injected intraperitoneally on day 14 post-infection. (A) Percentages of EdU labeled total neutrophils (normalized to max.) in lungs, spleens, and blood of Mtb-infected mice at the indicated times after EdU injection, with the 90% confidence intervals shown in color together with the calculated half-lives (red numbers) in each tissue. The curves were calculated via nonlinear regression for the decay kinetics of EdU-labeled neutrophils. Data are from at least three mice per time point. (B) 3D confocal images of lung neutrophils sorted from SSB-GFP, *symc'*::mCherry Mtb-infected mice at 2 and 4 weeks. Representative images were shown. <scale bar = 10 μ m>. (C) Percentage of Mtb displaying SSB-GFP foci in neutrophils sorted from SSB-GFP, *symc'*::mCherry Mtb-infected mice at 2 and 4 weeks. Each point represents one experiment with five mice. Horizontal bar indicates the mean of three or four pooled independent experiments. Data in A and B were representative of two independent experiments. Data in C were pooled from three or four independent experiments. Student's *t* test was used in C. 3D = three-dimensional; EdU = 5-ethynyl-2'-deoxyuridine; GFP = green fluorescent protein; Mtb = *Mycobacterium tuberculosis*; SSB = single-stranded DNA-binding protein.

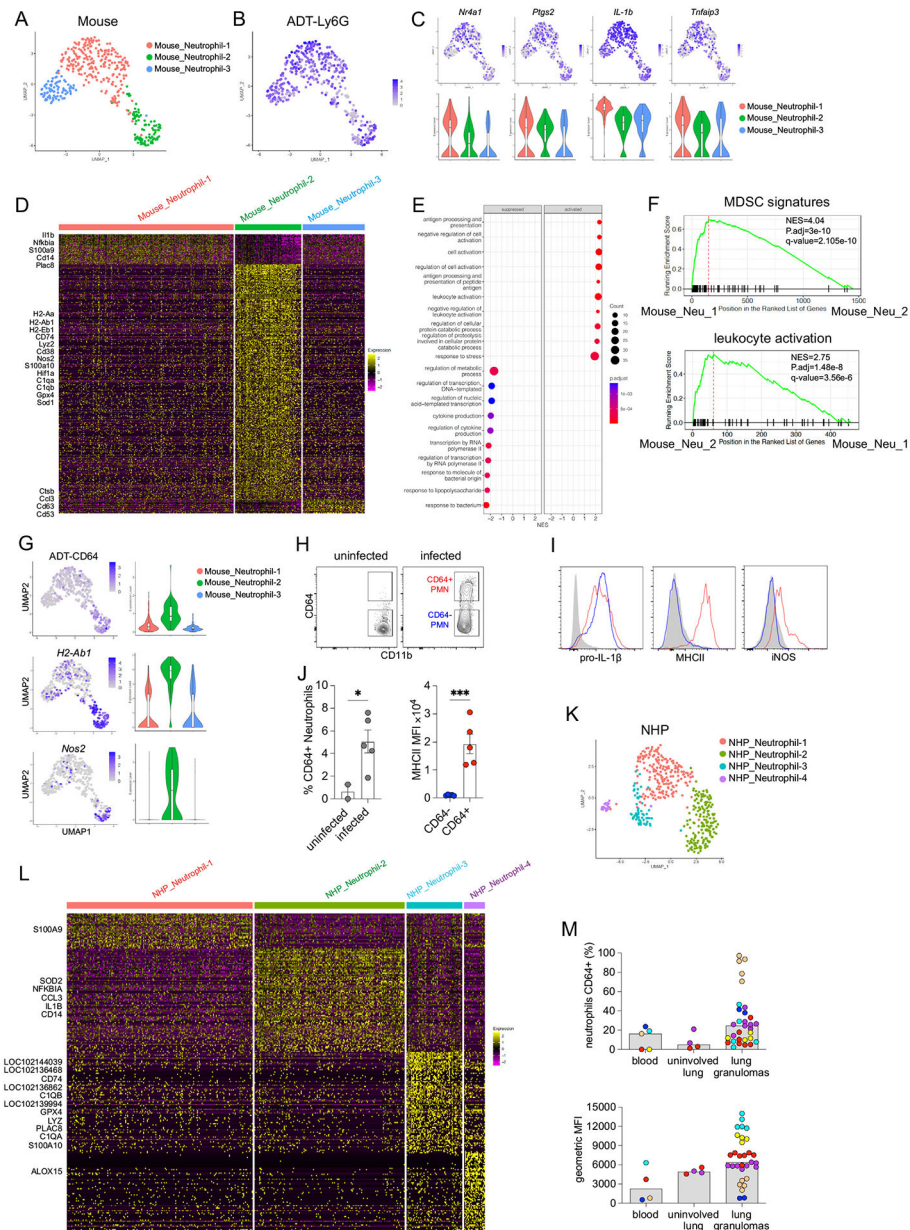
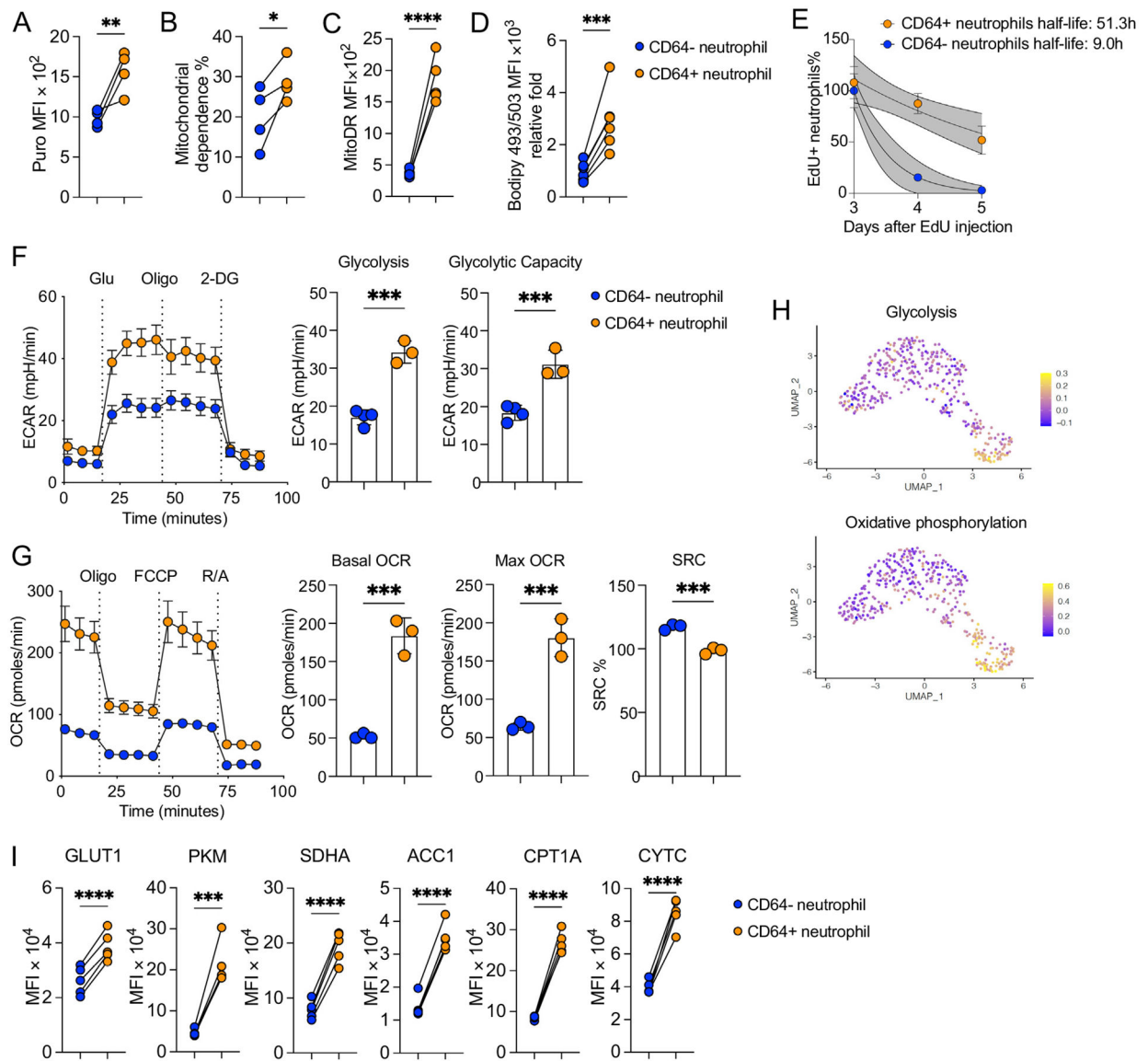


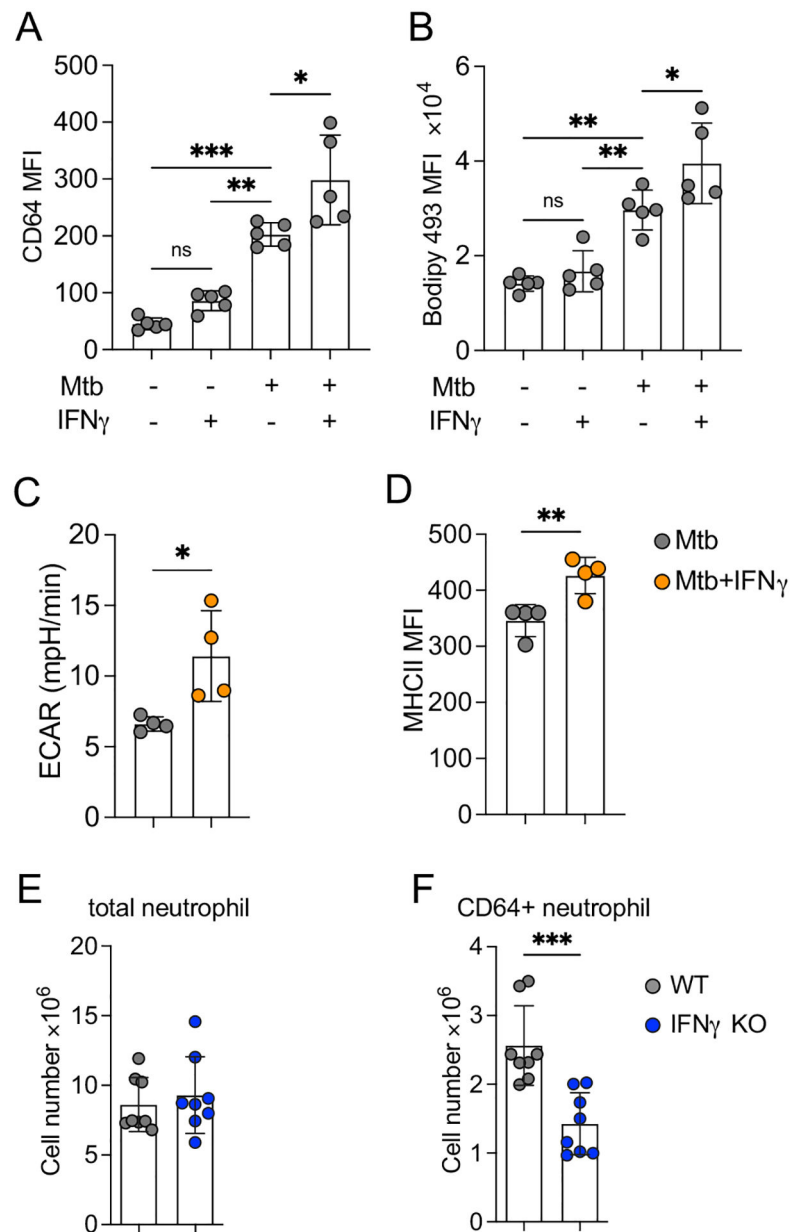
Fig. 4. Heterogeneous neutrophil populations identified by scRNA-seq in Mtb-infected mouse and NHP lungs. (A) UMAP plot displaying neutrophil clusters in the Mtb-infected mouse lung. (B) UMAP plot showing the expression level of ADT-Ly6G. (C) UMAP and violin plots showing expression of selected signature genes from the neutrophils in the Mtb-infected mouse lung. (D) Heat map of differential expression of marker genes in each mouse neutrophil cluster. (E) Pathway enrichment analysis of transcripts differentially expressed in Neutrophil_2 versus Neutrophil_1. (F) Gene set enrichment analysis of MDSC signatures and leukocyte activation in Neutrophil_1 and Neutrophil_2. NES, P. adj and false discovery rate (q value) are indicated. (G) UMAP and violin plots showing expression of ADT-CD64, *H2-Ab1*, and *Nos2* in each neutrophil cluster. To measure CD64 expression in lung

neutrophils, lungs were harvested from C57BL/6 mice infected with 10^3 Erdman Mtb after 3 weeks. (H) Flow cytometry analysis of CD64 expression in lung neutrophils from uninfected and Erdman Mtb-infected mice at 3 weeks post-infection. (I) Flow cytometry analysis pro-IL-1 β , MHCII, and iNOS expression for lung CD64+ and CD64- neutrophils in Erdman Mtb-infected mice at 3 weeks post-infection. (J) CD64 expression and MHCII MFI in CD64- and CD64+ neutrophils in mice infected with 10^3 H37Rv Mtb at 4 weeks post aerosol infection. (K) UMAP plot displaying neutrophil clusters in the Mtb-infected NHP lung. (L) Heat map of differential expression of marker genes in each NHP neutrophil cluster. (M) Quantification of CD64 expression in neutrophils across tissues from six non-human primates. Each marker indicates one sample of peripheral blood, uninvolved (granuloma-free) lung, or a lung granuloma and each color represents a different animal. Blood was not available from one animal and the samples of uninvolved lung from four animals had insufficient numbers of neutrophils for analysis, thus data was not included for these animals. Data in H, I and J were representative of two independent experiments. $n = 2-5$. Student's t test was used in J. * $p < 0.05$, *** $p < 0.001$. CD = cluster of differentiation; IL = interleukin; iNOS = inducible nitric oxide synthase; MDSC = myeloid-derived suppressor cells; MFI = mean fluorescence intensity; MHC = major histocompatibility complex; Mtb = Mycobacterium tuberculosis; NES = normalized enrichment score; NHP = non-human primates; scRNA = single-cell ribonucleic acid; UMAP = uniform manifold approximation and projection.

**Fig. 5.**

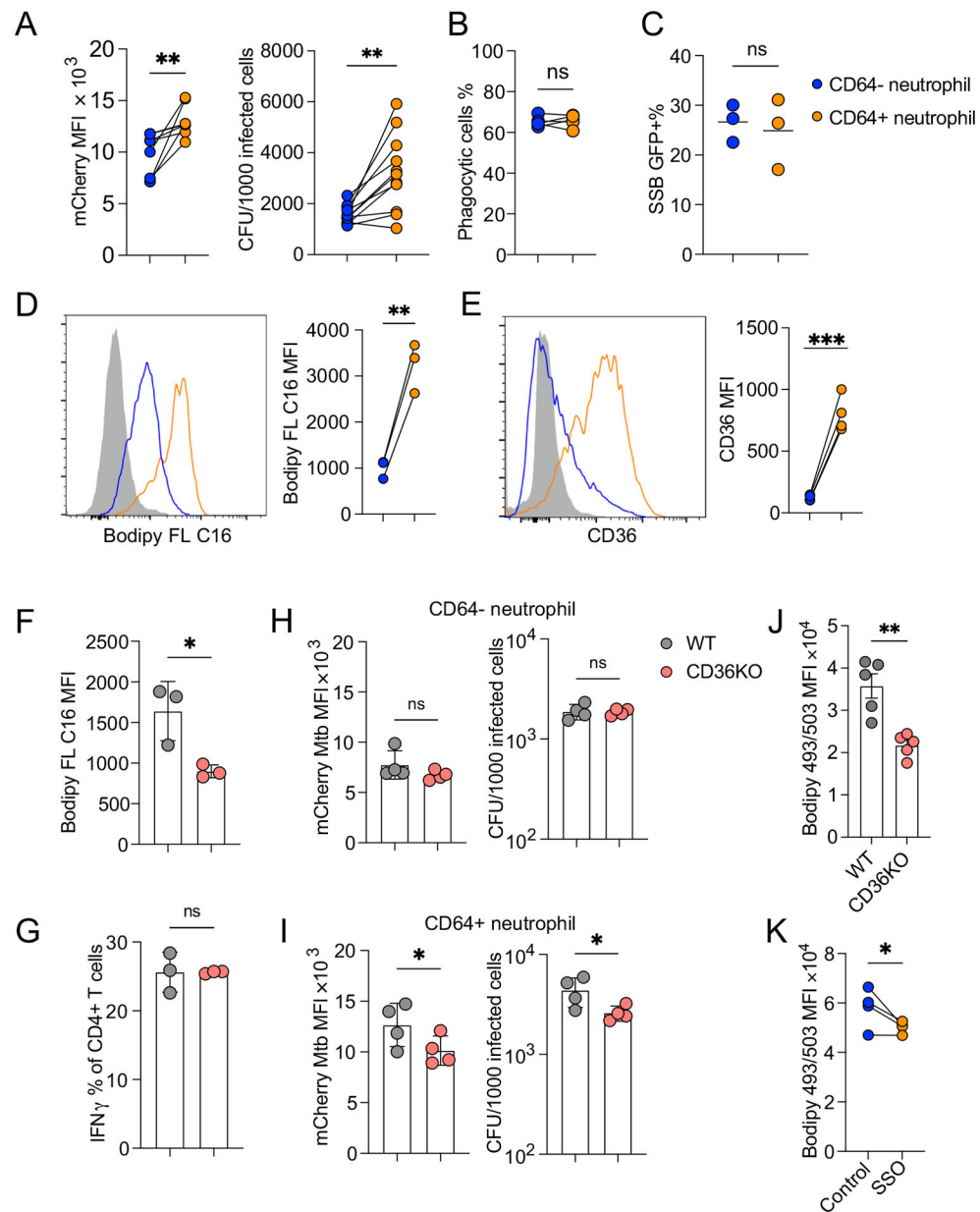
CD64+ neutrophils in the Mtb-infected lung are metabolically active. To characterize the metabolic activities in CD64+ and CD64- neutrophils, lungs were harvested from C57BL/6 mice 3 weeks after intranasal infection with 10^3 mCherry-Erdman Mtb. (A–B) Translation level (A) and mitochondrial dependence (B) were measured by SCENITH in CD64- and CD64+ neutrophils. (C–D) MFI of MitoTracker Deep Red staining (C) and Bodipy 493/503 staining (D) measured by flow cytometry in CD64- and CD64+ neutrophils. The linked dots represent CD64- and CD64+ neutrophils from the same mouse. (E) Percentages of EdU labeled CD64- and CD64+ neutrophils (normalized to max.) in lungs of Mtb-infected mice at the indicated times after EdU injection, with the 90% confidence intervals shown in gray together with the calculated half-lives. The curves were calculated via nonlinear regression for the decay kinetics of EdU-labeled neutrophils. Data are from at least three mice per time point. (F–G) ECAR (F) and OCR (G) of sorted total CD64- and CD64+ neutrophils

purified from Mtb-infected mice. Glycolysis, glycolytic capacity, basal and max OCR, and SRC were calculated. Mean $\pm$ SEM are shown. $n = 3$. (H) UMAP displaying signature scores of glycolysis and oxidative phosphorylation in each mouse neutrophil cluster. (I) The expression of metabolic targets in CD64⁺ and CD64⁻ neutrophils. The linked dots represent CD64⁻ and CD64⁺ neutrophils from the same mouse. Data in A–G were representative of at least two independent experiments. Data in I were pooled from two independent experiments. Paired t test was used in A–D and I. Student's t test was used in F and G. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. CD = cluster of differentiation; ECAR = extracellular acidification rate; EdU = 5-ethynyl-2'-deoxyuridine; MFI = mean fluorescence intensity; Mtb = Mycobacterium tuberculosis; OCR = oxygen consumption rate; SCENITH = single-cell energetic metabolism by profiling translation inhibition; SEM = standard error of the mean; SRC = spare respiratory capacity; UMAP = uniform manifold approximation and projection.

**Fig. 6.**

IFN γ promotes the generation of CD64 $^{+}$ neutrophils in Mtb infection. (A, B) MFI of CD64 (A) and Bodipy 493/503 (B) were measured in bone marrow neutrophils under various conditions by flow cytometry. BMN were infected with or without Mtb (MOI = 5) in the presence or absence of IFN γ . Mean $\pm$ SEM are shown. $n = 5$. To investigate the impact of IFN γ on CD64 $^{+}$ neutrophils, lungs were harvested from WT and IFN γ KO mice 2 weeks after infection with 10^3 Erdman Mtb. (C) ECAR and (D) MFI of MHCII of BMNs at 6 hours post-Mtb infection at MOI 5, with or without IFN γ (100 ng/mL). $n = 4$. (E, F) The numbers of total neutrophils (E) and CD64 $^{+}$ neutrophils (F) in Mtb-infected WT and IFN γ KO mice. Mean $\pm$ SEM are shown. $n = 8$. Data in A and B were representative of at least two independent experiments. Data in C–F were pooled from two independent experiments.

Student's *t* test was used in C–F. One-way ANOVA was used in A and B. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ANOVA = analysis of variance; BMN = bone marrow neutrophils; CD = cluster of differentiation; ECAR = extracellular acidification rate; IFN = interferon; KO = knockout; MFI = mean fluorescence intensity; MHC = major histocompatibility complex; MOI = multiplicity of infection; Mtb = *Mycobacterium tuberculosis*; SEM = standard error of the mean; WT = wild-type.

**Fig. 7.**

Perturbation of lipid uptake selectively decreases Mtb burden in CD64+ neutrophils. (A) MFI of mCherry and CFU in CD64- and CD64+ neutrophils sorted from the mouse lung after 2 weeks of infection with 10^3 mCherry-Mtb. $n = 8$. (B) Phagocytosis in CD64- and CD64+ neutrophils from the mouse lung after 2 weeks of infection with 10^3 mCherry-Mtb. $n = 4$. (C) Percentage of Mtb displaying SSB-GFP foci in neutrophils. Each dot represents one experiment with five mice. Neutrophils were sorted from the mouse lung after 2 weeks of infection with 10^3 SSB-GFP, *smyc*::mCherry Mtb. Horizontal bars indicate the mean of three pooled independent experiments. (D-E) Flow cytometry analysis of BODIPY FL C16 (C) and expression of CD36 (D) in CD64- and CD64+ neutrophils from the mouse lung after 2 weeks of infection with 10^3 mCherry-Mtb. $n = 3$ or 4. (F) MFI of BODIPY FL C16

in neutrophils from Mtb-infected WT and CD36KO mice at 2 weeks post-infection. Mean $\pm$ SD are shown. $n = 3$. (G) Flow cytometry analysis of percentage of IFN γ in CD4 $^{+}$ T cells in the lung of Mtb-infected WT and CD36KO mice at 2 weeks post-infection. Mean $\pm$ SD are shown. $n = 3$. (H–I) MFI of mCherry and CFU from CD64 $^{-}$ neutrophils (H) and CD64 $^{+}$ neutrophils (I) in mCherry-Mtb infected WT and CD36KO mice at 2 weeks post-infection. Mean $\pm$ SD are shown. $n = 4$. (J) Flow cytometry analysis of Bodipy 493/503 staining in Mtb-infected WT or CD36KO bone marrow neutrophils at 6 hours post-infection at MOI 5. $n = 5$. (K) Flow cytometry analysis of Bodipy 493/503 staining in CD64 $^{+}$ neutrophils treated with or without 100 μ M SSO for 2 hours. Lungs were harvested from Mtb-infected WT mice at 2 weeks post-infection. Data in A were pooled from two independent experiments. Data in B, D–K were representative of two independent experiments. F and G were from the same experiment. H and I were from the same experiment. Paired t test was used in A, B, D, E and K. Student's t test was used in C, F–J. * $p < 0.05$, ** $p < 0.01$. CD = cluster of differentiation; CFU = colony-forming unit; GFP = green fluorescent protein; IFN = interferon; MFI = mean fluorescence intensity; Mtb = Mycobacterium tuberculosis; SD = standard deviation; SSB = single-stranded DNA-binding protein; SSO = sulfosuccinimidyl oleate; WT = wild-type.