Differential Roles of the Calcium Ion Channel TRPV4 in Host Responses to *Mycobacterium tuberculosis* Early and Late in Infection

HIGHLIGHTS

- Mtb down-modulates TRPV4 expression in macrophages
- Trpv4^{-/-} macrophages cannot be activated to drive phagosome maturation and NO production
- Trpv4-deficient mice are more resistant to Mtb
- TRPV4-positive macrophages in the periphery of human granuloma but not at the center

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Differential Roles of the Calcium Ion Channel TRPV4 in Host Responses to Mycobacterium tuberculosis Early and Late in Infection

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SUMMARY

Mycobacterium tuberculosis subverts host immunity to proliferate within host tissues. Non-selective transient receptor potential (TRP) ion channels are involved in host responses and altered upon bacterial infections. Altered expression and localization of TRPV4 in macrophages upon virulent M. tuberculosis infection together with differential distribution of TRPV4 in human tuberculosis (TB) granulomas indicate a role of TRPV4 in TB. Compared with wild-type mice, Trpv4-deficient littermates showed transiently higher mycobacterial burden and reduced proinflammatory responses. In the absence of TRPV4, activation failed to render macrophages capable of controlling mycobacteria. Surprisingly, Trpv4-deficient mice were superior to wild-type ones in controlling M. tuberculosis infection in the chronic phase. Thus, Trpv4 is important in host responses to mycobacteria, although with opposite functions early versus late in infection. Ameliorated chronic infection in the absence of Trpv4 and its expression in human TB lesions indicate TRPV4 as putative target for host-directed therapy.

INTRODUCTION

Emerging data indicate a role for calcium ion channels in bacterial infections (Deretic and Fratti, 1999; King et al., 2020). Bacterial endotoxin can activate transient receptor potential (TRP) channels including TRPV4 in airway epithelial cells, which triggers proinflammatory responses (Alpizar et al., 2017). The host endeavors to eliminate a pathogen, whereas the pathogen strives to control or escape host defenses. Macrophages are in principle equipped to eliminate bacterial pathogens, but are exploited by virulent Mycobacterium tuberculosis as their primary resident host cells by circumventing the macrophage’s host defense armamentarium. M. tuberculosis, the causative agent of human tuberculosis (TB), successfully escapes host defense to establish infection by modulating intracellular trafficking and intracellular calcium signaling as well as through induction of necrotic cell death. However, exposure of macrophages to extracellular ATP and interferon-gamma (IFN-γ) promotes phagosome acidification, generation of reactive nitrogen species (RNS), and apoptosis over necrotic cell death, and hence intracellular killing of M. tuberculosis. Mutants lacking the region of differentiation-1 (RD1) encompassing the esx1-encoded type 7 secretion system, besides others, are attenuated by failing both, inhibition of phagosome maturation and induction of necrotic cell death. The Esx1-associated small secreted proteins, early secretory antigen target-6 (ESAT-6) and culture filtrate protein 10 (CFP10), are involved in inhibition of phagosome acidification (de Jonge et al., 2007).

Among the protein family of calcium ion channels, TRP ion channels are involved in immune responses within the lung microenvironment (Venkatachalam and Montell, 2007). TRPA1 is expressed in foamy macrophages and regulates cholesterol metabolism and anti-inflammatory response. TRPM8 and TRPV1 are expressed in lung epithelial cells and are crucial for the synthesis of proinflammatory cytokines, e.g., interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, IL-1α, and IL-1β (Sabnis et al., 2008; Reilly et al., 2005). TRPV4 is highly expressed in alveolar epithelial cells, endothelial cells, neutrophils, and macrophages and important for pro-inflammatory responses, goblet cell recruitment, and airway wall thickening during inflammatory lung diseases (Jia et al., 2004; Moran et al., 2011; Suresh et al., 2015; Gombedza et al., 2017).
Consequently, pharmacological inhibitors of TRPV4 have been considered as therapeutics of acute lung injury (Balakrishna et al., 2014). Inhibition of TRPV4 is associated with impaired in vitro neutrophil responses including chemotaxis (Yin et al., 2016). *M. tuberculosis* is known to inhibit calcium signaling in macrophages to reduce phago-lysosome fusion and secure intracellular survival (Malik et al., 2000). These studies strongly suggest that calcium regulation by TRPV4 may be crucially involved in inflammatory lung diseases. To date, there is no report on the role of TRPV4 in *M. tuberculosis* infection. We found that wild-type (WT) *M. tuberculosis*, but not an attenuated ΔRD1 mutant, can down-regulate Trpv4 expression, thereby inhibiting intracellular calcium mobilization. IFN-γ-activated *Trpv4*−/− macrophages failed to restrict *M. tuberculosis* growth due to limited phagosome maturation and nitrite (NO2) production. *Trpv4*−/− mice showed higher *M. tuberculosis* lung burden associated with lower proinflammatory responses at early time points of infection. However, in the chronic phase of infection, *Trpv4*-deficient mice were superior to WT mice in controlling mycobacterial growth. Our data indicate that *M. tuberculosis* alters TRPV4 expression to facilitate the infection progress. At the late phase of infection, though, TRPV4 facilitates mycobacterial growth indicating TRPV4 as a host-directed therapeutic target for subsidiary treatment of antibiotic therapy of TB.

**RESULTS**

**Trpv4 Expression in Macrophages Is Altered by *M. tuberculosis***

To understand Trpv4 regulation during *M. tuberculosis* infection, we analyzed TRPV4 in *M. tuberculosis*-H37Rv-infected murine bone marrow-derived macrophages (BMDM) at protein level. Confocal microscopy revealed association of TRPV4 with the macrophage plasma membrane at 2 h (Figure 1A, upper panel) but dispersed localization at 24 h post-infection (p.i.) (Figure 1A, lower panel). Quantification of TRPV4 protein expression in infected BMDM lysates by western blot showed *M. tuberculosis*-H37Rv induced TRPV4 down-regulation at both 24 and 48 h p.i. (Figure 1B). In contrast, infection with *M. tuberculosis* ΔRD1 transiently down-regulated TRPV4 expression at 24 h but not 48 h p.i. Analysis of Trpv4 mRNA expression by qRT-PCR in human monocyte-derived macrophages revealed a multiplicity of infection (MOI)-dependent increase in transcript numbers between MOI 1 and 3, which became, however, reduced again at MOI 10 (Figure 1C). These results demonstrate that infection of macrophages with virulent *M. tuberculosis*-H37Rv redistributes TRPV4 and regulates its expression in an MOI-dependent manner and that loss of TRPV4 is probably not due to reduced, but rather partially compensated, by higher transcription rates.

**M. tuberculosis Survives in Activated Macrophages in the Absence of Trpv4**

To assess the role of Trpv4 in intracellular survival and growth of *M. tuberculosis*, we compared mycobacterial burden in WT and *Trpv4*−/− BMDM, alveolar, as well as peritoneal, macrophages, which were either left at the resting state or were stimulated with IFN-γ (Figures 1D–1F). Compared with WT cells, we observed a slightly better growth of *M. tuberculosis*-H37Rv in resting *Trpv4*-deficient BMDM and alveolar, but not peritoneal, macrophages. However, IFN-γ-activated *Trpv4*−/− macrophages of all three types were significantly less capable to restrict the growth of *M. tuberculosis* at 48 and 72 h p.i. when compared with WT cells (Figures 1D–1F). Notably, resting WT but not *Trpv4*−/− BMDM were able to control intracellular growth of the *M. tuberculosis* ΔRD1 mutant 48 and 72 h p.i. (Figure 1G). Similarly, *Mycobacterium smegmatis*, otherwise controlled by WT BMDM were able to grow in *Trpv4*−/− cells (Figure S1A). These data were further corroborated by experiments wherein we pretreated the RAW264.7 macrophage cell line with the pharmacological TRPV4 inhibitor RN1734 (10 μM) followed by *M. tuberculosis* infection. Higher *M. tuberculosis* counts were found in RN1734-treated RAW cells when compared with mock-treated controls at 48 h p.i. (Figure S1B). To control for differential phagocytosis rates between resting and IFN-γ-activated WT and *Trpv4*−/− macrophages, which may influence subsequent growth, we counted *M. tuberculosis* numbers at 2 h p.i., which, however, were comparable between resting and activated WT and *Trpv4*−/− BMDMs (Figure S1C). Taken together, these findings pinpoint TRPV4 as an important host factor for the control of intracellular *M. tuberculosis* by macrophages, in particular when the anti-microbial potential of these cells is potentiated by IFN-γ activation.

**Trpv4 Is Involved in Intracellular Trafficking of *M. tuberculosis***

Virulent mycobacteria are able to survive in resting macrophages by interfering with phagosome maturation by several mechanisms, including attenuation of intracellular calcium (Deretic and Fratti, 1999; Tejle et al., 2002), whereas IFN-γ-activated macrophages control mycobacteria, in part by promoting phagosome maturation (Ni Cheallaigh et al., 2016). We studied intracellular trafficking of mycobacteria in WT
versus Trpv4−/− macrophages between 5 min and 24 to 48 h.p.i. M. tuberculosis-GFP co-localized with the early endosomal GTPase, Rab5, in both, IFN-γ-activated WT and Trpv4−/− macrophages 5 min p.i. At 24 h.p.i., mycobacteria in Trpv4−/− macrophages were still mostly co-localizing with Rab5, indicating retention in early phagosomes in the absence of Trpv4, whereas mycobacteria-containing phagosomes in WT macrophages were Rab5 negative (Figures 2A, 2B, and S2A). In line with this view, M. tuberculosis-H37Rv were found in LysoTracker-positive compartments in IFN-γ-activated WT macrophages 48 h.p.i. but only to a small extent in Trpv4−/− BMDM (Figures 2C and S2B). These results indicate that in the absence of Trpv4, IFN-γ-activated macrophages failed to effectively deliver M. tuberculosis into acidic phago-lysosomal compartments.

The influence of TRPV4 on phagosome maturation was further analyzed by determining the phagosomal pH in WT and Trpv4−/− macrophages upon uptake of non-viable model particles, i.e., fluorescein isothiocyanate (FITC)-labeled paraformaldehyde (PFA)-fixed (PF) E. coli. We employed the pH-sensitive property of FITC, which is quenched at acidic pH when excited at 490 nm, but not at 440 nm (Nunes et al., 2015). In TRPV4-deficient macrophages, acidification of PF E. coli phagosomes was significantly delayed when compared with WT cells (Figure 2D). These results demonstrate that Trpv4 plays a crucial role in phagosome acidification and maturation, which are important prerequisites to control intracellular pathogens.

Figure 1. Trpv4 Expression and Mycobacterial Survival in Infected Macrophages
(A) Bone marrow-derived macrophages (BMDM) isolated from C57BL6/J WT mice were infected with M. tuberculosis-GFP (Mtb-GFP) at MOI 5, and the cells were fixed with 4% PFA 2 and 24 h.p.i. TRPV4 was detected by immuno-cytochemistry using Cy3-conjugated TRPV4 antibody. Images were acquired using Leica TSC SPS confocal microscope (scale bar = 5 µm). Mean fluorescence intensity (MFI) of TRPV4 staining was quantified by using Fiji ImageJ software. Statistical analysis was performed with two-way ANOVA. Mean ± SEM (n = 3), *p < 0.05.
(B) BMDM from C57BL6/J WT mice were infected with M. tuberculosis-H37Rv and M. tuberculosis ΔRD1 (MOI 5). Western blot analysis was performed to determine the TRPV4 expression using TRPV4-specific antibody at 2, 24 and 48 h.p.i. Densitometry analysis of protein bands was performed using Fiji ImageJ software.
(C) Human monocyte-derived macrophages were infected with M. tuberculosis-H37Rv for 24 h at different MOI as indicated, and the mRNA level of TRPV4 was studied with respect to the reference gene HPRT (hypoxanthine-guanine phosphoribosyltransferase).
(D–F) BMDM (D), alveolar macrophages (E), and peritoneal macrophages (F) were isolated from WT and Trpv4−/− mice; pre-treated with IFN-γ (500 units/mL) overnight; and then infected with M. tuberculosis (MOI 1) for 2 h. Cells were lysed with 0.5% Triton X-100 at different time points, and the intracellular M. tuberculosis counts were assessed by CFU assay.
(G) WT and Trpv4−/− mice BMDM were infected with M. tuberculosis or M. tuberculosis ΔRD1, and the intracellular bacterial burden was assessed by CFU assay after cell lysis with 0.5% Triton X-100 at indicated time points. Western blot and confocal images are representative of three independent experiments. In case of CFU assay, “n” corresponds to number of independent experiments. Statistical analysis was performed with two-way ANOVA Bonferroni post-tests. For mRNA expression of TRPV4, statistical analysis was performed with one-way ANOVA. Mean ± SD, *p < 0.05, **p < 0.01, ***p < 0.001.
Trpv4 Influences Generation of Reactive Nitrogen Species and Cell Death upon M. tuberculosis Infection

RNS production and apoptotic cell death represent anti-mycobacterial effector mechanisms of IFN-γ-activated macrophages controlling M. tuberculosis, at least in mice. It has been shown before that activation of TRPV4 induces RNS production in macrophages (Hamanaka et al., 2010). Therefore, we measured NO2⁻ production of IFN-γ-activated WT and Trpv4⁻/⁻ BMDM in response to either WT or ΔRD1 M. tuberculosis-H37Rv (MOI 5) 24 h.p.i. When compared with WT macrophages, we found significantly less nitrite in Trpv4⁻/⁻ ones upon infection with either WT or ΔRD1 M. tuberculosis (Figure 3A), which correlated with enhanced intracellular survival of M. tuberculosis in Trpv4⁻/⁻ macrophages (Figure 1G).

Intracellular growth of M. tuberculosis drives host cell death through oxidative stress-mediated genomic instability (Mohanty et al., 2016). Therefore, we employed Sytox green to assess necrotic cell death in
WT versus Trpv4^{-/-} BMDM upon infection with WT or ΔRD1 M. tuberculosis. We observed significantly higher amounts of DNA released from the cells indicating necrotic cell death in both WT and ΔRD1 M. tuberculosis-infected Trpv4^{-/-} macrophages when compared with WT cells. Of note, even the ΔRD1 M. tuberculosis mutant, which usually causes apoptotic rather than necrotic macrophage cell death, led to higher necrosis rates in Trpv4^{-/-} than in WT macrophages (Figure 3B). To monitor cell death kinetics, we performed live-cell imaging of IFN-γ-activated M. tuberculosis-H37Rv-infected BMDM incubated with the fluorogenic caspase3/7 substrate as indicator for apoptosis between 3 and 72 h p.i. (Figure 3C, and Video S1). Trpv4^{-/-} macrophages succumbed much earlier, i.e., between 24 and 48 h p.i., to M. tuberculosis infection likely due to higher intracellular mycobacterial burden. Removal of dead cell aggregates by bystander macrophages eventually resulted in higher rates of cell death in Trpv4^{-/-} cell cultures when compared with WT ones. Notably, we also observed non-apoptotic cell death independent of caspase3/7-mediated apoptosis corroborating the necrotic cell death results shown in Figure 3B and Video S1. Taken together, these data indicate that intact TRPV4 protects host cells from M. tuberculosis-induced cell death.

Role of Trpv4 in the Restriction of M. tuberculosis Growth in Infected Mice

Next, we investigated the role of Trpv4 in vivo during M. tuberculosis infection. Upon aerosol infection with M. tuberculosis-H37Rv (100 colony-forming unit [CFU]), no significant differences in the survival rates of WT and Trpv4^{-/-} mice were observed during an observation period of up to 150 days p.i. (data not shown). Body weight analysis showed no difference between infected and non-infected WT mice up to 90 days p.i. In contrast, reduced weight gain was observed in M. tuberculosis-infected Trpv4^{-/-} mice of the same age starting at day 21 p.i. when compared with uninfected ones (Figure 4A). It should be noted that uninfected Trpv4^{-/-} mice, when compared with WT ones, continued to gain weight over a 7-week observation period.
At 29 days p.i., both, CFU and Ziel-Nielsen’s staining showed higher \textit{M. tuberculosis} counts in \textit{Trpv4}/C0 lungs than in WT ones (Figures 4B and 4C). At day 55 and 90 p.i. both, WT and \textit{Trpv4}/C0 mice were similarly able to control the mycobacteria. To our surprise, significantly lower \textit{M. tuberculosis} counts were found in lungs from \textit{Trpv4}/C0 mice at day 150 p.i. when compared with lungs from WT animals. In spleens of \textit{Trpv4}/C0 mice, we also found less \textit{M. tuberculosis} counts at day 150 p.i. when compared with WT ones, despite

**Figure 4. Determination of \textit{M. tuberculosis}-H37Rv Survival in WT and \textit{Trpv4}/C0 Mice**

(A) Body weights of \textit{M. tuberculosis}-H37Rv-infected and uninfected WT and \textit{Trpv4}/C0 mice were measured during the course of infection and presented as mean weight in g.

(B) WT and \textit{Trpv4}/C0 mice were exposed to \textit{M. tuberculosis} aerosols (100 CFU), and bacterial burden in lungs was assessed at indicated time points by CFU assay. “n” indicates number of mice. Each point corresponds to one mouse. Statistical analysis was performed with two-way ANOVA Bonferroni post-tests. **p < 0.01.

(C) Acid fast staining was performed to determine the \textit{M. tuberculosis} (pink and arrow marked) burden in infected WT and \textit{Trpv4}/C0 mice lungs at day 29 p.i. Images were taken from three different fields (scale bar= 20 \textmu m).

(D) \textit{M. tuberculosis}-infected lung sections from WT mice were prepared at indicated time points after infection, immune-stained with Trpv4 antibody (ACC034 Alomone lab 1:200), and then developed with oxidation of 3,3’-diaminobenzidin (DAB) staining (brown) to check Trpv4 expression. Uninfected \textit{Trpv4}/C0 and WT lung sections were used as negative and positive controls, respectively (scale bar of magnified image= 20 \textmu m).

(E–G) (E) Lung lesions (indicated by arrow) in \textit{M. tuberculosis}-infected WT and \textit{Trpv4}/C0 mice are depicted for the different time points p.i. H & E staining of (F) day 29 and (G) day 55 post \textit{M. tuberculosis}-infected WT and \textit{Trpv4}/C0 mice lung section indicates immune cell infiltration. Images were acquired with Olympus BX41 light microscope with 10X magnification.
almost similar counts at earlier time points p.i. (Figure S3A). No significant differences in mycobacterial counts between WT and \textit{Trpv4}^-^-^-/- mice were seen in the livers during the entire course of infection (Figure S3B). Immuno-histological analysis of WT lungs showed TRPV4-expressing cells at the periphery of granuloma, whereas cells present in the center showed reduced TRPV4 expression (arrow marked) (Figure 4D). Pulmonary inflammatory lesions (arrow marked) were larger in WT compared with \textit{Trpv4}^-^-^-/- mice at days 55, 90, and 150 p.i. (Figure 4E). H&E staining revealed larger areas of inflammatory infiltrates at the site of infection in \textit{M. tuberculosis}-infected WT mouse lungs at day 29 (Figure 4F) and day 55 (Figure 4G) p.i. when compared with \textit{M. tuberculosis}-infected \textit{Trpv4}^-^-^-/- mouse lungs. These data indicate that TRPV4 is involved in the immune responses, which control \textit{M. tuberculosis} burden in mice, however, with opposite functions, i.e., protective versus pathological, in the early versus late stage of infection.

\textit{M. tuberculosis} utilizes host lipid droplets as nutrient source for growth in granulomas (Daniel et al., 2011). Using oil red O staining of lung tissue sections, we already noticed more lipid droplets in lungs from uninfected WT mice when compared with \textit{Trpv4}^-^-^-/- ones (Figure S3D). More importantly, at day 55 p.i., \textit{M. tuberculosis}-infected WT mice showed intense accumulation of lipid bodies in the lungs, which was more pronounced when compared with \textit{Trpv4}^-^-^-/- mice (Figures 5A and S3C).

**Reduced Pro-inflammatory Responses in \textit{Trpv4}^-^-^-/- Mice upon \textit{M. tuberculosis} Infection**

Infiltration of immune cells to the site of infection is crucial for the restriction of \textit{M. tuberculosis} growth \textit{in vivo}. Analysis of a panel of cytokines and chemokines in lung lysates of \textit{M. tuberculosis}-infected mice showed higher production of the pro-inflammatory cytokines, IFN-\gamma, IL-17A, TNF-\alpha, and IL-1\beta in WT
than in Trpv4−/− mice. IFN-γ production was enhanced in both, WT and Trpv4−/− compared with uninfected mice, although WT mice showed moderately higher IFN-γ production than Trpv4−/− ones (Figure 5B). We also observed higher concentrations of IL-17A, TNF-α, and IL-1β in WT than in TRPV4−/− lungs at day 29 p.i. (Figures 5C–5E). Upon infection, the production of keratinocyte chemoattractant (KC) was increased in WT when compared with Trpv4−/− lungs, whereas IL-6 secretion was only moderately but not significantly enhanced (Figures S4A and S4B). Similarly, we observed higher concentrations of the chemokines, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein 2 (MIP-2 or CXCL2) in lungs from WT when compared with those from Trpv4−/− mice (Figures 5F and 5G). In contrast, the anti-inflammatory cytokine, IL-10, was significantly increased in Trpv4−/− lung tissue samples taken at day 15 and, again, at day 55 p.i. when compared with WT lungs. Comparable IL-10 concentrations were observed at day 29 p.i. (Figure 5H).

Determination of NO2− production at day 15 p.i. with M. tuberculosis showed enhanced NO2− concentrations in the lungs from WT but not Trpv4−/− mice. No differences in nitrite concentrations were observed in serum samples (Figures 5I and S4C).

Figure 6. Analysis of TRPV4 Expression in Lung Granulomas of a Patient with TB
Lung tissue sections were obtained from patients with TB, who underwent anti-TB therapy and lung tissue resection as part of the treatment. (A) Immunohistochemistry was performed using an antibody to TRPV4, followed by a species-specific secondary antibody and DAB development (TRPV4, brown; nuclei, blue). (B and C) Human lung tissue sections of patients with TB were stained for TRPV4 and CD68 and developed by DAB staining showing expression of TRPV4 and CD68 in TB granuloma (TRPV4 and CD68, brown; nuclei, blue; Scale bar = 100 μm).

Differential TRPV4 Distribution in Granulomas from Patients with TB
To investigate whether TRPV4 is also present in granulomas of human patients with TB, we analyzed TRPV4 expression in tissue section of lung granulomas obtained from three patients with TB, who had undergone lung tissue resection. We observed more TRPV4-positive cells at the periphery of the granuloma in all three patients, whereas cells in the center showed much weaker TRPV4 signals (Figure 6A). Dual staining with antibodies against the CD68 macrophage marker and TRPV4 in consecutive sections showed that TRPV4 expression did partially overlap with CD68-positive macrophages but was also associated with CD68-negative cells. CD68-positive cells present at the periphery of granulomas expressed more TRPV4, whereas those present at the center of the granuloma showed less TRPV4 (Figures 6B and 6C).
Neutrophils represent the predominant cell population infected with *M. tuberculosis* in pulmonary samples from patients with active TB and are thought to fail controlling the infection and rather drive exacerbation of inflammation (Dallenga et al., 2017; Eum et al., 2010). Using immunofluorescence staining for neutrophil elastase (NE), we observed increasing numbers of NE-positive neutrophils in lungs from *M. tuberculosis*-infected WT mice between day 55 and 150 p.i., whereas only few NE signals were found in *Trpv4* / / lungs (Figures 7A and 7B). Notably, we also observed TRPV4-positive neutrophil aggregates in blood vessels in close proximity to the granulomatous tissue from patients with active TB (Figure 7C). In summary, TRPV4 in human TB lesions was primarily associated with macrophages and neutrophils, and in mice, recruitment of neutrophils was reduced in the absence of TRPV4 at later time points of *M. tuberculosis* infection, indicating a link between TRPV4 expression and neutrophil-associated inflammation.

**DISCUSSION**

In immune cells, different ion channels such as voltage-gated calcium channels, non-selective calcium channel, and transient receptor potential (TRP) ion channels are crucial for the regulation of calcium levels to facilitate cellular signaling pathways and effector functions (Song et al., 2015). Here, we report that...
These data suggest that infection was increased in lungs of WT, but not of Trpv4/C0 mice, which corroborates previous observations (Ye et al., 2012). IFN-γ recruitment of immune cells to the infection sites, in infection. This could be due to lower concentrations of MCP-1 and MIP-2, which are involved in the advancement of infection during the growth phase of M. tuberculosis. In contrast, the lack of Trpv4 was associated with better control of mycobacteria at the later chronic stage of infection. Thus, TRPV4 has a biphasic function associated with an early protective but later permissive phenotype. Association of functional TRPV4 with higher susceptibility to M. tuberculosis at later stages of infection in the murine TB model was paralleled by the presence of TRPV4-expressing macrophages and neutrophils in the periphery of granulomas from chronically infected WT mice and patients with active TB.

As a hallmark of M. tuberculosis virulence, the pathogen is able to inhibit phagosome acidification, a prerequisite for phagosome maturation and phago-lysosome fusion (Wong et al., 2011). We found that, at 24 h p.i., WT but not ARD1 M. tuberculosis infection moderately reduced intracellular calcium levels in WT macrophages, indicating that M. tuberculosis virulence genes encoded by the RD1 region are important for TRPV4 down-regulation and hence, reduction of calcium ion concentration, which otherwise is a prerequisite for phagosome acidification (Figures S4D and S4E). Thereby, M. tuberculosis is able to maintain an immature phagosomal state as niche for intracellular growth, even in IFN-γ-activated macrophages (Trimble and Grinstein, 2007). This notion is supported by the retention of Rab-5 in M. tuberculosis phagosomes in TRPV4-deficient macrophages. This is not seen in mycobacterial phagosomes of WT macrophages, which efficiently delivered WT M. tuberculosis into phago-lysosomes. In fact, we also observed lower calcium uptake in resting Trpv4/C0 compared with WT macrophages when incubated with ionomycin Ca2+ (Figure S4F). Increased cytosolic calcium levels enhance phagosome acidification. Accordingly, inhibition of calcium signaling by M. tuberculosis can interfere with phago-lysosome fusion, thereby promoting the pathogen’s intracellular survival (Malik et al., 2000). Beyond mycobacteria, hampered phagosome maturation in the absence of TRPV4 seems a general phenotype as acidification of phagosomes containing fixed E. coli was also delayed. Taken together, our results suggest that virulent M. tuberculosis, in addition to other virulence mechanisms, target Trpv4, thereby impairing phagosome acidification and maturation to secure intracellular survival and growth. To see whether the in vitro susceptibility phenotype of Trpv4/C0 macrophages is also relevant for bacterial infections other than mycobacterial ones, we infected WT versus Trpv4/C0 BMDM with Salmonella typhimurium. In contrast to mycobacteria, the salmonellae grew similarly in WT and Trpv4/C0 macrophages (data not shown), indicating that the observed phenotype is mycobacteria specific.

M. tuberculosis infection promotes apoptosis of human alveolar macrophages (Keane et al., 1997). Staining for active caspase3/7 revealed that Trpv4/C0 macrophages infected with M. tuberculosis succumbed to apoptotic cell death earlier than WT cells, which was likely due to higher intracellular mycobacterial burden 36 h p.i. Using live-cell imaging, we observed that apoptotic cells were engulfed by bystander macrophages leading to removal of more than 90% Trpv4/C0 macrophages by 72 h p.i. These results indicate a relevance of Trpv4 in M. tuberculosis-mediated host cell death.

Aerosol infection of Trpv4/C0 mice and their WT littermates with M. tuberculosis revealed that the lack of Trpv4 led to higher bacterial burden at day 29 p.i., but equal ones at days 55 and 90, and significantly lower ones at days 150 p.i. Reduced control of M. tuberculosis in mice lacking Trpv4 in the early stage of infection is likely due to the failure of TRPV4-deficient macrophages to promote phagosome maturation and RNS production. Moreover, inflammatory responses were reduced in Trpv4/C0 lungs when compared with WT littermates including smaller inflammatory infiltrates and granuloma-like structures at the site of infection. This could be due to lower concentrations of MCP-1 and MIP-2, which are involved in the recruitment of immune cells to the infection sites, in M. tuberculosis-infected Trpv4/C0 versus WT lungs (Figures 4F and 4G), which corroborates previous observations (Ye et al., 2012). IFN-γ and IL-17 production was increased in lungs of WT, but not of Trpv4/C0, mice at day 29 post M. tuberculosis infection and associated with reduced TNF-α production otherwise important for optimal macrophage activation. These data suggest that Trpv4/C0 mice failed to develop sufficient innate immune responses to combat M. tuberculosis. In contrast, enlarged lung lesions and higher concentrations of pro-inflammatory cytokines and effectors produced in WT when compared with Trpv4/C0 mice were likely instrumental in
controlling M. tuberculosis growth in the presence of Trpv4. Increased secretion of the anti-inflammatory cytokine, IL-10, in Trpv4−/− versus WT mice infected with M. tuberculosis is likely responsible for this reduced Th1 response observed. Enhanced influx of neutrophils in WT compared with TRPV4−/− mice at the later stage of infection is likely contributing to enhanced inflammation in the presence of TRPV4. Inhibited TRPV4 has been shown to limit neutrophil functions including chemotaxis (Yin et al., 2016). Excessive secretion of neutrophil elastase by activated neutrophils is involved in the control of M. tuberculosis, but at the same time, can also cause tissue damage and pathology. Thus, TRPV4 has a protective role in M. tuberculosis infection during the early innate immune response phase before T cell immunity kicks in, but a detrimental one later in infection when exacerbated inflammatory responses including neutrophil influx maintain mycobacterial growth.

The appearance of lipid-storing macrophages as characterized by lipid droplets (LD) is a histopathological hallmark of the chronically M. tuberculosis-infected murine lung later in infection. LD formation in M. tuberculosis-infected macrophages has been suggested to be a programmed host response coordinated by IFN-γ (Knight et al., 2018). However, M. tuberculosis can utilize fatty acids stored in LDs as carbon source for intracellular growth. Thus, our observation that pulmonary lesions in Trpv4−/− mice contained less LDs than those in WT mice may also account for lower mycobacterial loads in Trpv4−/− compared with WT lungs at late stage of infection, i.e., day 150. This phenotype is likely caused by a reduced IFN-γ and neutrophil-driven inflammatory environment promoted by enhanced IL-10 production in the absence of Trpv4. Apart from providing a food source for mycobacteria, LDs are also involved in the production of pro-inflammatory eicosanoids such as prostaglandin E2, which promote neutrophil influx into the infected tissue. As such, lower numbers of LDs in lungs of M. tuberculosis-infected Trpv4−/− mice might be responsible for reduced inflammatory responses and neutrophil aggregations when compared with WT lungs (Kaul et al., 2012; Saka and Valdivia, 2012).

The relevance of TRPV4 in M. tuberculosis infection identified by our experimental studies in mice prompted us to analyze its presence in human TB lesions. Immuno-histopathological analyses of lung granuloma sections from three patients with TB showed TRPV4-expressing macrophages at the periphery of the necrotic granuloma, whereas those in the centers showed less TRPV4 expression. Exposure of macrophages to M. tuberculosis and its metabolites, which happens more likely in the center of TB granulomas, as well as subsequent necrotic cell death may account for the reduced TRPV4 expression observed. It should, however, be noted that the patients with TB, from which the tissue samples were derived, had a history of long-term anti-TB treatment. Nevertheless, surgery was needed to overcome prolonged culture positivity and imminent treatment failure. Consequently, bacterial loads through acid-fast staining could not be visualized despite the strong and obvious granulomatous lesions present. Notably, TRPV4 was also observed in neutrophils in the granuloma-adjacent blood vessels. This finding supports previous reports that TRPV4 deficiency impairs neutrophil response to pro-inflammatory stimuli, production of reactive oxygen species, adhesion, and chemotaxis (Yin et al., 2016).

Taken together, we report a novel bifunctional role of the TRPV4 ion channel in host responses to M. tuberculosis infection in mice, in which TRPV4 is protective during the early innate but exacerbating and proinflammatory during the late chronic stage of infection. To this end, the presence of TRPV4-expressing myeloid cells in lung granulomas indicates TRPV4 as a potential target to be explored for host-directed therapy to control TB disease progression and support antibiotic treatment.

Limitations of the Study
In this study, although we have observed down-regulation of TRPV4 by pathogenic M. tuberculosis H37Rv in macrophages, we believe that TRPV4 expression can also be regulated by other intracellular factors during in vitro culture in the absence of an infectious stimulus (Figure 1B uninfected condition). In our follow-up studies, we will compare TRPV4 expression in different innate and acquired immune cell populations from un-infected versus infected mice to delineate regulatory mechanisms, as well as the kinetics of immune cell frequencies during the infectious process to identify responses responsible for higher and lower susceptibility of Trpv4−/− mice early versus later in infection. Future availability of specific TRPV4 inhibitors employable in vivo, will allow to explore the host-directed therapy approach targeting TRPV4 in late stage of infection using a susceptible mouse model of TB. Ultimately, the cellular and molecular functions of TRPV4 and its putative interaction partners during macrophage responses to M. tuberculosis need to be approached using cell biology and biochemistry approaches. Our observation of a decreased neutrophil
influx in lungs of M. tuberculosis-infected Trpv4−/− mice late in infection also requires further investigations to understand the underlaying molecular and immunological mechanisms of how TRPV4 is involved here and how this knowledge can be explored to improve treatment of tuberculosis.

Resource Availability
Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Prof. Dr. Ulrich E. Schaible (uschaible@fz-borstel.de).

Materials Availability
This study did not generate new unique reagents.

Data and Code Availability
This study did not generate datasets/code.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101206.

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AUTHOR CONTRIBUTIONS
S.K.N. designed, performed and analyzed the experiments and wrote the manuscript; K.P. performed the experiments and analyzed the data; J.E. and V.S. provided technical assistance and training; M.H. wrote the ethical approval for animal experiment; B.K. as clinician recruited patients with TB; B.K. and N.R. provided the lung samples from patients with TB; W.L. created the Trpv4−/− mice; W.M.K. provided the Trpv4−/− mice and revised the manuscript; U.E.S. designed experiments, analyzed the data, and contributed to writing the manuscript; A.S. designed experiments, analyzed the data, obtained funding, and wrote the manuscript.

DECLARATION OF INTERESTS
The authors have no conflict of interest.

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Supplemental Information

Differential Roles of the Calcium Ion Channel TRPV4 in Host Responses to Mycobacterium tuberculosis Early and Late in Infection

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(A) BMDM from WT and Trpv4−/− macrophages were infected with *M. smegmatis* (Msm) at a MOI 1 and intracellular bacterial counts were assessed by CFU at indicated time points p.i.

(B) RAW264.7 macrophages were treated with the Trpv4 inhibitor, RN1734 (10µM), prior to *M. tuberculosis* infection of a MOI 1. Intracellular mycobacterial counts were assessed at indicated time points by CFU assay.

(C) BMDM from WT and Trpv4−/− mice were infected with *M. tuberculosis* at MOI 5 and the numbers of phagocytosed *M. tuberculosis* were assessed by CFU at 2h p.i. Data represent the mean ± SEM from 2 - 3 (n) independent experiments. Statistical analysis was performed with Two Way ANOVA Bonferroni posttests. *P<0.05, ***P<0.001.
Figure S2  Related to Figure 2

(A) Images showing different image channels for nuclei, *M. tuberculosis* and Rab5 as shown in Fig. 2A. (B) Images showing different image channels for nuclei, *M. tuberculosis* and lysotracker as shown in Fig. 2C.
Figure S3 Related to Figure 4 and Figure 5

(A), (B) *M. tuberculosis* counts from spleen and liver of individual *M. tuberculosis* infected mice at different time points p.i. Data shown are from 3 independent experiments and each point represents one mouse. Statistical analysis was performed with Two Way ANOVA Bonferroni posttests. ***P<0.001. (C) Complete lung section of uninfected and *M. tuberculosis* infected mice showing oil red O stained lipid bodies (pink) at day 55 p.i. (D) Tissue section showing oil red O positive lipid bodies in uninfected WT and *Trpv4*<sup>-/-</sup> mice. Nuclei = DAPI/blue.
Figure S4  Related to Figure 5

(A), (B) Expression of KC and IL-6 in lung lysates from WT and Trpv4−/− mice at different time points p.i. (C) Nitrite production was measured in lung lysates prepared from M. tuberculosis infected WT and Trpv4−/− mice at indicated time points using Nitrate reductase and Griess reagent. The NO3− production was represented as the absorbance of Griess reagent at 543nm. (D,E) Intracellular calcium levels in M. tuberculosis or MtbΔRD1 infected WT and Trpv4−/− BMDM was measured by incubating the cells with permeable Fluo4AM (4µg/ml) dye for 30 minutes in dark. Fluorescence intensity of Fluo4AM was measured at Ex/Em of 494/506 nm using Biotek multiplate reader. (F) Intracellular calcium level in resting WT and TRPV4−/−macrophages in presence of ionomycin Ca2+.Statistical analysis was performed with Two Way ANOVA Bonferroni posttests.
**Transparent Methods**

**Statement**

All experiments and methods were performed in accordance with institutional guidelines and regulations. Animal studies were approved by the Ministry of Energy, Agriculture, Environment, Nature and Digitization of the state of Schleswig-Holstein, Germany [V 242-71197/2017(14-2/18)]. Experiments performed with primary human cells were reviewed and approved by the Ethics Committee of the University of Lübeck, Germany (#13-032). Study with human TB patient samples were approved by the Ethics Committee of the University of Lübeck, Germany (Ethical Approval No.14-032 and #18-194).

**Bacterial strains, Cells and Reagents**

*M. tuberculosis* H37Rv, green fluorescent protein (GFP) tagged *M. tuberculosis* (Mtb-GFP), Discosoma Red fluorescent protein (DsRed) expressing *M. tuberculosis*, Region of Difference-1 (RD1) deleted *M. tuberculosis* (MtbΔRD1), MtbΔRD1-GFP and *M. smegmatis* were cultured as described previously (Dallenga et al., 2017). To stabilize GFP and Ds-Red expression, the bacteria were grown in 7H9 media supplemented with hygromycin-B (50 µg/ml), kanamycin (20 µg/ml) and hygromycin-B (50 µg/ml), respectively. Primary antibodies directed against Trpv4 (#ACC034, Alomone lab), Rab5 (#GTX108605, GeneTex), neutrophil elastase (#51-862, proSci-ELAN) were used in this study. Secondary antibodies goat anti rabbit-cy3 (#111-165-144) were procured from Jackson Immunoresearch, UK.

Murine alveolar and peritoneal macrophages from both male and female mice were isolated from broncho-alveolar and peritoneal lavages obtained from C57BL/6J WT or *Trpv4*-knockout (*Trpv4*+/-) mice as described previously (Schneider et al., 2014). The isolated cells were suspended
in DMEM medium containing 10 % heat inactivated fetal bovine serum (FBS) and 2 mM L-glutamine, plated (1×10⁵ cells per well) on 96-well tissue culture plates and incubated in 5% CO₂ at 37⁰C for at least 3 hours to allow macrophages to adhere.

Bone marrow derived macrophages (BMDM) from both male and female WT or Trpv4⁻/⁻ mice were harvested by flushing femurs and tibias as described previously (Herbst et al., 2011). Harvested cells were differentiated in DMEM containing 10% heat inactivated FBS, 2 mM L-glutamine and L929 cell supernatant (20% v/v) as a source of colony stimulating factor for one week at 37⁰C and 5% CO₂.

**Animal model, M. tuberculosis infection and CFU assay**

Trpv4⁻/⁻ mice were generated on a C57BL/6J background as described previously (Liedtke and Friedman, 2003). Both WT and Trpv4⁻/⁻ mice were bred and housed under specific pathogen free (SPF) condition at the Research Center Borstel-Leibniz Lung Center (RCB). Animal studies were approved by the Ministry of Energy, Agriculture, Environment, Nature and Digitization of the state of Schleswig-Holstein, Germany [V 242-71197/2017(14-2/18)].

For aerosol infection, 6-8 weeks old both male and female mice were infected with *M. tuberculosis*-H37Rv (100 CFU) using an aerosol chamber (GlasCol, USA). After aerosol challenge, bacterial load in lung, spleen and liver was determined at different time points by mechanical disruption of organs in 0.1% v/v tween 80 in milliQ water containing a protease inhibitor cocktail (Roche Diagnostics). Ten-fold serial dilutions of organ homogenates in 0.01% v/v tween 80 and 0.05% w/v albumin were plated onto Middlebrook 7H11 agar plates and colonies were counted after 3-4 weeks of incubation at 37⁰C.
Macrophage infection assay

Bacterial cultures in mid-exponential phase were pelleted, washed with 1X PBS (pH 7.4). Bacterial clumps were removed by passing five times through a 27G blunt needle. *M. tuberculosis*-H37Rv culture at a final optical density of 0.1 at 600 nm (OD$_{600}$), which corresponds to $5 \times 10^7$ bacteria ml$^{-1}$ was prepared with DMEM. 1×10$^5$ macrophages were infected at MOI 1 or otherwise as indicated. For activation of macrophages, cells were incubated overnight with 500 units/ml of IFN-$\gamma$ prior to the infection. After 2 h of infection, cells were washed 3 times with 1X PBS to remove any extracellular bacteria. At different time points p.i., cells were lysed with ice cold 0.5% triton X-100, serially diluted with 1X PBS and plated on 7H11 agar plates. *M. tuberculosis* colonies were enumerated after 3 weeks of incubation at 37$^0$ C.

Isolation of human monocyte-derived macrophages

Human monocyte-derived macrophages (hMDM) from both male and female donors were generated from peripheral blood mononuclear cells (PBMCs) (purity consistently $>$92%) by elutriation and differentiated after 7 days in Teflon bag cultures in the presence of 10 ng/ml recombinant human M-CSF (VueLife 72C; Cellgenix, Freiburg, Germany) as described previously (Reiling et al., 2001). All experiments performed with primary human cells were reviewed and approved by the Ethics Committee of the University of Lübeck, Germany (#13-032).

Quantitative Real-time PCR

Total RNA was isolated from macrophages (0.2 X10$^6$) using Trizol (peqGOLDTriFast$^\text{TM}$, USA) according to the manufacturer’s instructions. cDNA was synthesized using Maxima First Strand cDNA Synthesis Kit (Thermo Fisher). Gene-specific primers and TaqMan probes (Roche Applied Science, Germany) were designed using Universal Probe Library (UPL) assay design center (ProbeFinder Version 2.45). qRT-PCR was performed using the LightCycler 480 Probe Master
Kit and the LightCycler 480 II system (Roche Applied Science) (Neumann et al., 2010). Crossing point values of target and reference gene were determined by the second derivative maximum method. Relative gene expression was calculated using E-Method (https://www.nature.com/app_notes/nmeth/2006/062706/full/nmeth894.html).

**Human TB patient samples**

All experiments performed with primary human macrophages or the staining of human lung tissues derived from patients suffering from TB, which underwent surgery for lung tissue resection as part of the anti-TB therapy, were reviewed and approved by the Ethics Committee of the University of Lübeck, Germany (Ethical Approval No.14-032 and #18-194). Lung sections were obtained from both male and female TB patients. Figure 6A,C are samples from male patient and Figure 6B and Figure 7C are samples from female TB patients.

**Immunofluorescence microscopy**

WT and Trpv4−/−BMDM (1×10⁵) were seeded on coverslips and infected with *M. tuberculosis*-GFP or *M. tuberculosis* ∆RD1-GFP (MOI 5) as described above. At specific time points, cells were fixed overnight with 4% PFA, washed with 1X PBS, permeabilized with 0.5 % saponin (#558255, Calbiochem) for 10 minutes and then blocked with 10 % goat serum. Cells were then incubated with primary antibodies (Rab5-5µg/ml; TRPV4-4µg/ml; Neutrophil elastase-5µg/ml) for 1h at room temperature followed by washing and fluorophore-conjugated secondary antibody (Goat anti rabbit-cy3-2.5µg/ml) in dark for 1h each step. DAPI (#D1306, Thermofisher) staining was performed at 1:1000 dilution and incubated in dark for 7 minutes. After mounting, cells were observed under fluorescence microscope (Nikon eclipse Ti) and confocal microscope (Leica TCS SP5). Confocal images were analyzed with IMARIS life-science software. Fluorescence intensities were quantified using Fiji ImageJ software.
**Phagosomal pH measurement**

Phagosomal pH was measured by ratiometric fluorescence microscopy (Nunes et al., 2015). To this end, *Escherichia coli* (*E. coli*) was first grown till mid-exponential phase and fixed with 4% PFA for 1 h followed by labelling with fluorescence isothiocyanate (FITC) (0.1mg/ml). WT and *Trpv4*−/− BMDM (1×10⁵ cells) were incubated with FITC labelled *E. coli* (MOI 10) and live cell imaging was performed for 45 minutes at 37⁰C and 5% CO₂ growth conditions using confocal microscopy. FITC signal was captured at excitation wavelength of 488 nm and 458 nm. Fluorescence property of FITC is quenched by acidic pH when excited at approximately 488 nm but not when excited at 458 nm. The ratio of 488/458 was used to determine the pH of the phagosome. Finally, the observed ratio value was converted into pH value by using an equation $X=17.5035 \times \{-(y-10.5627)/(y-5843007)\}^{0.0761506748}$ with the help of a standard curve obtained from *E. coli*-FITC 488/458 ratio at different pH (where $X=pH$ value and $y$=ratio of 488/458). In total, 15 phagosomes were analyzed from three individual biological replicates.

**Lysotracker staining**

BMDM (1×10⁵/well) were seeded onto cover slips and infected with *M. tuberculosis*-GFP (MOI 5) for 2 h and washed. After 48 h of further incubation, cells were washed and incubated with DMEM containing the acidotropic dye, lysotracker Red (100nM; L12492, Invitrogen), for 1h in dark. Cells were fixed with 4% PFA and observed under confocal microscope with excitation/emission of 647/668nm for lysotracker red.

**Cell death analysis**

BMDM (1×10⁵/well) were infected with WT or ΔRD1 *M. tuberculosis* and necrotic cell death was measured using Sytox green (S7020, Thermo) as per the manufacturer’s instruction. Apoptotic cell
death was studied using CellEvent Caspase3/7 green detection kit (C10423, Invitrogen) as per manufacturer’s instruction. Briefly, BMDM (1×10^5) were seeded on a four chambered tissue culture disc, treated with IFN-γ overnight and then infected with *M. tuberculosis*-DsRed at MOI 5. Three hours p.i., cells were washed with 1x PBS to remove extracellular bacteria followed by addition of caspase3/7 green detection reagent (10µM in DMEM media). Live cell imaging was performed till 72 h p.i. using biostation IMq imaging system (Nikon).

**Determination of NO_2^- production**

IFN-γ activated WT and *Trpv4^-/-* BMDM (1×10^5) were infected with *M. tuberculosis*-H37Rv at MOI 5 for 24 h before supernatants were harvested and developed using Griess reagent and measured at 543nm.

For the measurement of nitrite produced under *in-vivo* conditions, lung lysates and serum were collected from *M. tuberculosis* exposed WT and *Trpv4^-/-* mice. For serum, blood was collected from inferior vena cava of mice at different time points p.i. and serum was separated using Z-gel tubes (Sarstedt; 41.1500.005) followed by centrifugation at 6000 g for 5 minutes. 40 µl of serum or lung lysate samples were incubated with 40 µl of reduction reagent (NADPH 1mg ml^-1; FAD 5mM; KH_2PO_4 0.5M, pH 7.5 with 0.25U of nitrate reductase-N7265-Sigma) at 37°C for 2 h (Petricevich et al., 2000). After incubation, 80 µl of Griess reagent was added and incubated for 15 minutes in dark. OD was measured at 543nm.

**Western blot analysis**

Macrophage cells (1×10^6) were infected with WT and ΔRD1 *M. tuberculosis* at MOI 5. Then cells were harvested, lysed in RIPA buffer and electrophoresed to SDS-PAGE and western blotting as described previously (Mohanty et al., 2016). Membranes were then incubated overnight with the
primary anti-Trpv4 antibody (Alomone lab; ACC-034), washed, further incubated with HRP conjugated secondary antibody for 2 h at RT and developed and imaged using chemiluminescence and the ChemiDoc Imaging system (Bio-Rad), respectively.

**Immunohistochemistry and Histopathology**

Lungs from *M. tuberculosis* infected WT and Trpv4−/− mice were harvested at different time points, incubated with 4% PFA overnight for fixation and 4 µm tissue sections were prepared. For cryo-sections, PFA fixed tissues were incubated with 1X PBS overnight at 4°C, transferred to increasing concentrations of saccharose (5-20%) and embedded in 20% saccharose/tissuetek (2:1). 4 µm cryo-sections were prepared with Leica cryostat at -20°C.

Paraffin sections were used for immunohistochemistry by deparaffination of tissue sections and antigen retrieval in citrate buffer (pH 6.0). Endogenous peroxidase was inhibited with H2O2 and blocking was done with 10% serum followed by overnight incubation with primary antibody at 4°C. Tissue sections were washed 3 times in 1X PBS followed by incubation with biotin conjugated secondary antibody (2.6 µg/ml or 1:500 goat-anti rabbit #111066047, Jackson Labs) for 45 minutes at RT and avidin-biotin complex for 45 minutes at RT (Vectastain Elite ABC-Peroxidase Kit, #VC-PK-6100-KI01). Tissue sections were developed in DAB followed by nuclei staining with hematoxyline. Similar staining procedure was used for immunohistochemistry analysis of human patient suffering from TB lung tissues. For *M. tuberculosis* counts, tissue sections were stained with Ziehl-Neelsen followed by nuclei staining with hematoxylin. Slides were embedded in Entellan.

For lipid staining, lung cryo-sections were washed 3 times with 1X PBS followed by incubation with Oil Red O stain for 30 minutes at room temperature. After incubation, slides were washed 3 times with 1X PBS and the nuclei were counterstained in hematoxylin. Slides were
embedded in Kaiser’s Glycerin Gelatine and observed by light microscopy (Olympus BX41). Images were captured with CellSens standard Olympus software.

**Cytokine and chemokine analysis**

Cytokines and chemokines in mouse lung lysate and serum were analyzed using U-plex MSD kits (#K15069L) and the assay was performed according to the manufacturer’s instructions.

**Intracellular calcium measurement**

BMDM (1x10^5 cells per well) from WT and Trpv4^/- mouse were seeded in a 96 well plate. Cells were treated with 4µM Ionomycin, 2mM CaCl$_2$ or infected with *M. tuberculosis*-H37Rv or *M. tuberculosis*ΔRD1 with a MOI 5. At indicated time points, media were removed and cells were washed twice with 1xPBS. Cells were then incubated with Fluo-4AM (4µg/ml) for 30 minutes at 37 °C in dark, and the fluorescence intensity was obtained at Ex/Em of 494/506 nm using Biotek multiplate reader.

**Statistical analysis**

Data are presented as mean ± standard error mean (SEM). Two-way and One-way analysis of variance was used to determine statistical significance between groups where *p < 0.05, **p < 0.01, ***p < 0.001. All statistical significances between the experimental groups are marked.
# Key Resources Table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| TRPV4               | Alomone lab | ACC034     |
| Rab5                | GeneTex | GTX108605  |
| Neutrophil elastase | proSci-ELAN | 51-862    |
| Goat anti rabbit-cy3| Jackson immunoresearch | 111-165-144 |
| Goat-anti rabbit biotin conjugated | Jackson immunoresearch | 111-066-047 |
| Goat-anti rabbit HRP conjugated   | Jackson immunoresearch | 111-035-045 |
| **Bacterial and Virus Strains** | | |
| *M. tuberculosis* H37Rv | Schaible lab, Research Center Borstel, Germany | N/A |
| *M. tuberculosis* H37Rv GFP | Tanya Parish, Center for Global Infectious Disease Research Seattle | N/A |
| *M. tuberculosis* H37Rv ΔRD1 | Suzanne M. Hingley-Wilson, William R. Jacobs | N/A |
| *M. tuberculosis* H37Rv DsRed | Schaible lab, Research Center Borstel, Germany | N/A |
| *M. smegmatis* mc²155 | Schaible lab, Research Center Borstel, Germany | N/A |
| *E. coli* | Schaible lab, Research Center Borstel, Germany | N/A |
| **Biological Samples / Cell line** | | |
| Human TB patient lung sample | Luebeck University, Germany | Ethical Approval No.14-032 and #18-194 |
| RAW264.7 mouse macrophages | A. Sonawane lab, India | N/A |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| 7H11 agar media | Difco | 283810 |
| DMEM | Pan Biotech | P04-03600 |
| RPMI | Pan Biotech | P04-17500 |
| Chemical/Reagent                      | Supplier        | Catalog Number |
|--------------------------------------|-----------------|----------------|
| Kanamycin                            | Roth            | T832.1         |
| Hygromycin-B                         | Pan Biotech     | P06-08020      |
| FBS                                  | Pan Biotech     | P30-3306       |
| L-glutamine                          | Pan Biotech     | P04-80100      |
| Tween-80                             | Roth            | 4780           |
| Protease inhibitor cocktail          | Roche           | 4693132001     |
| DAPI                                 | Thermofisher    | D1306          |
| TritonX-100                          | Roth            | 3051.3         |
| Trizol Tri Reagent                   | Zymo Research   | R2050-1-200    |
| Lysotracker red                      | Invitrogen      | L12492         |
| Caspase 3/7 detection kit            | Invitrogen      | C10423         |
| FITC                                 | Sigma           | F7250          |
| RN1734                               | Sigma           | R0658          |
| Recombinant murine IFN-γ             | Peprotech       | 315-05         |
| Flou4AM                              | Goswami lab, NISER, India | N/A |
| Ionomycin                            | Goswami lab, NISER, India | N/A |
| TB Carbolfuchsin                     | BD              | 212518         |
| Mayer’s Haematoxylin                 | Roth            | T865.2         |
| Eosin                                | Roth            | 7089.2         |
| Entellan                             | VWR             | 1079610500     |
| Sucrose                              | Roth            | 9286.1         |
| Tissue tek                           | Leica           | 020108926      |
| Methylbutane                         | VWR             | 720-0821       |
| Oil Red O                            | Sigma           | O0625          |
| Nitrate reductase                    | Sigma           | N7265          |
| NADPH                                | Sigma           | N7265-2UN      |
| FAD                                  | Sigma           | F6625-10MG     |
| KH₂PO₄                               | Merck           | 1.04873.0250   |
| DAB for immunohistochemistry         | Sigma           | D4293-50SET    |
| Avidin Biotin Complex kit            | Vector lab      | VC-PK-6100-KI01 |
## Critical Commercial Assays

| MSD kit for cytokine analysis | Meso Scale Discovery | K15069L |

## Experimental Models: Organisms/Strains

| TRPV4<sup>−/−</sup> mouse | Liedtke <i>et al.</i> 2003 | N/A |

## Oligonucleotides

| Human TRPV4 forward primer for mRNA expression | Roche | Probe-54 |
|-----------------------------------------------|-------|---------|
| CTCTTCATGATCGGCTACGC                           |       |         |

| Human TRPV4 reverse primer for mRNA expression | Roche | Probe-54 |
|-----------------------------------------------|-------|---------|
| ACACCTTCATGTGGCACAC                           |       |         |

| Human HPRT forward primer for mRNA expression | Roche | Probe-73 |
|-----------------------------------------------|-------|---------|
| TGACCTTGATTATTGATCACTAC                      |       |         |

| Human HPRT reverse primer for mRNA expression | Roche | Probe-73 |
|-----------------------------------------------|-------|---------|
| CGAGCAAGACGTTAGTCCT                           |       |         |

## Software and Algorithms

| Imaris 7 | Bitplane | http://www.bitplane.com/imaris/imaris |
|----------|----------|--------------------------------------|

| GraphPad Prism | GraphPad | https://www.graphpad.com/scientific-software/prism/ |
|----------------|----------|------------------------------------------------------|

| Graphical abstract | BioRender | Created with BioRender.com |
|--------------------|-----------|----------------------------|
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