Supplemental Methods

Preparation of cells

Human monocyte-derived macrophages (hMDMs) were isolated from whole blood (from healthy blood donors, healthy controls or patients) by density gradient centrifugation (LymphoPrep™, Axis-Shield) and allowed to differentiate for 7 days in DMEM containing 10% normal human serum (pooled from 5 donors). After differentiation hMDMs were seeded in 96-well plates (10^5 cells/well) or 24-well plates (Sarstedt) with cover slips ($2.5x10^5$ cells/well) in medium free of antibiotics one day before infection. For inhibition experiments, the caspase-1 inhibitor Ac-YVAD-CMK (Cayman Chemical, 50 μM), DMSO (1:1000) was added 30 h before infection. All inhibitors were present throughout the experiment. Recombinant IL-1β (Humanzyme) was added both before and during infection, as well as during one week of differentiation of hMDMs, in the concentrations stated in the text (added every 48 h).

Preparation of bacteria and experimental infection

Two strains of virulent *M. tuberculosis* H37Rv (American Type Culture Collection) harboring a pSMT1-plasmid encoding the gene for *Vibrio harveyi* luciferase (Mtb) or a pFPV2-plasmid encoding GFP were used in the study, as well as the avirulent H37Ra. The three strains were grown in Middlebrook 7H9 broth supplemented with Tween-80 and ADC (Becton-Dickinson) for two to three weeks at 37°C, before they were reinoculated in fresh broth and incubated for 7 days to reach early log phase. The bacteria were collected and washed by centrifugation at 3000 ×*g* for 10 minutes, in phosphate buffer saline (PBS) with 0.05% Tween-80 before passage through a sterile syringe equipped with a 27 gauge needle to obtain a single-cell suspension. The suspension was subjected to a second wash, resuspended in plain DMEM and

passaged again before concentration was determined by using optical density (OD_{600}) as a function of CFU/ml. Bacteria at the indicated multiplicity of infection (MOI) was added to the hMDMs in serum-free medium for 1 h before medium was changed to antibiotic-free medium with serum and samples were incubated for the indicated time points (pulse-chase approach).

Evaluation of bacterial growth

The number of bacteria in infected samples was evaluated by flash-luminescence and this method has been described elsewhere (1). Briefly, supernatant and cell-associated bacteria were collected separately for analysis by transferring culture supernatants/lysates (from hypotonically lysed cells) to white 96-well plates (Sarstedt), and substrate (1% decanal, Sigma-Aldrich) was added automatically by an injector before luminescence was measured. The procedure was performed in triplicate for every time point to compensate for experimental variation. Total bacterial growth (supernatant + lysate) is shown as a fold-change (luminescence at D2/luminescence at D0).

Evaluation of CD63 translocation or Lysotracker staining

Evaluation of CD63- or Lysotracker-positive phagosomes in hMDMs from healthy blood donors was performed with H37Ra-GFP, earlier shown to inhibit phagolysosomal fusion to the same extent as H37Rv (2). hMDMs were seeded on cover slips and infected as described above. For Lysotracker (Invitrogen) staining, 100 nM of Lysotracker Red DND-99 was added during the last 2 hours of incubation. At the given time point, the cells were washed twice in PBS and briefly fixed in 4% paraformaldehyde at RT before being washed again. Lysotracker-stained samples were mounted in mounting medium (Dako), re-coded and microscopy was performed within 24 h. For the CD63 samples, blocking solution containing PBS with 2% BSA and 10% normal goat serum (Dako), supplemented with 0.1% saponin were added to the fixed cells for 30 min at RT in a humid chamber before additional washes

and incubation with anti-CD63 antibody (mouse monoclonal, Sanquin, 4 μg/ml) for 1 h at RT. After three extended washes, secondary Alexa 594-conjugated goat anti-mouse (Molecular Probes, 5 μg/ml) was added and cells were incubated for 30 min at 37^{o}C. In the final step, cover slips were washed and re-coded before mounted. The staining of at least 40 phagosomes per sample was analyzed, in a blinded fashion, by obtaining images using a confocal microscope (BioRad Radiance 2000 MP with LaserSharp 2000 software).

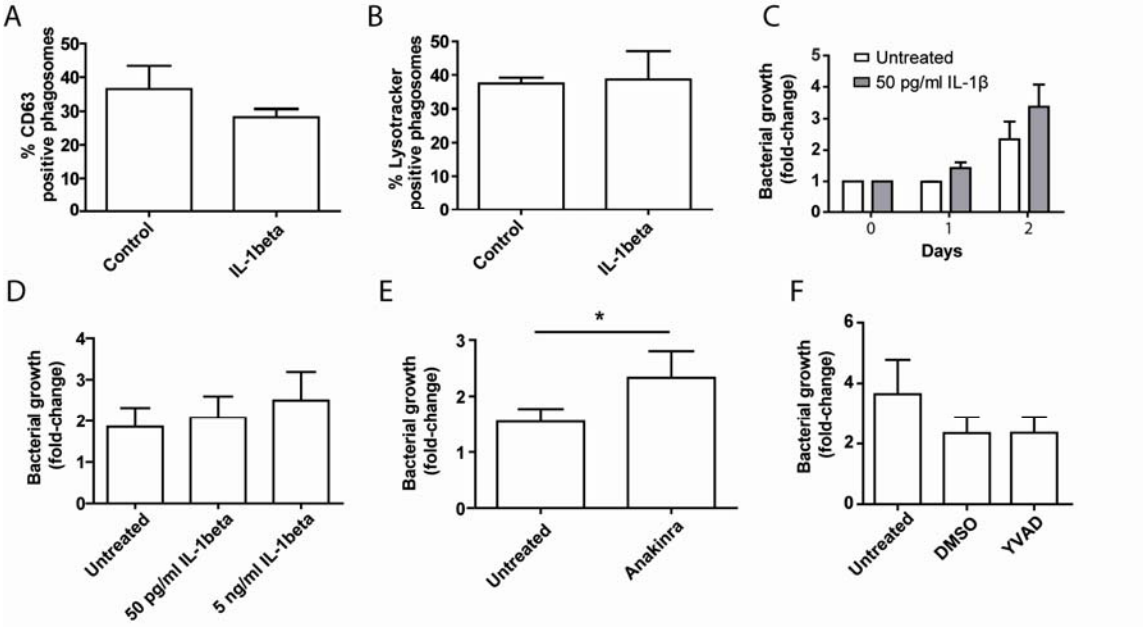
Genotyping assay

DNA was isolated from whole blood using Maxwell Blood DNA Purification Kit (Promega), according to the manufacturer's instructions. Frozen blood samples were thawed and mixed before transferred to reagent cartridges. After isolation, samples were eluated in TE buffer and stored at 4°C until further use. For the real-time PCR, Taqman SNP Genotyping assay (Applied Biosystems) was used according to the manufacturer's instructions, where one reaction contained; 5μl Taqman Genotyping Mastermix, 0.25 μl of SNP Genotyping Assay (primer/probe mix) and 20 ng of DNA. Total volume was adjusted with double distilled H_2O to 10μl. The analysis was performed in the 7500 Fast Real-Time PCR system.

1. Eklund D, Welin A, Schon T, Stendahl O, Huygen K, and Lerm M. Validation of a medium-throughput method for evaluation of intracellular growth of Mycobacterium tuberculosis. *Clin Vaccine Immunol.* 2010;17(4):513-7.
2. Welin A, Raffetseder J, Eklund D, Stendahl O, and Lerm M. Importance of phagosomal functionality for growth restriction of Mycobacterium tuberculosis in primary human macrophages. *J Innate Immun.* 2011;3(5):508-18.

Supplementary Figure 1

Blocking IL-1β signalling during infection leads to impaired growth control of H37Rv, while addition of exogenous IL-1β does not alter the outcome. hMDMs differentiated in the presence of 5 ng/ml IL-1β (added every 48 h) for one week, were reseeded on glass cover slips and infected with H37Ra at MOI 10. 4 h post-infection cells were either fixed for CD63 staining (**A**) or stained with Lysotracker (**B**). The percentage of Lysotracker- or CD63-positive phagosomes were analyzed by confocal microscopy (n=3). (**C**) IL-1β (50 pg/ml) was added during differentiation of hMDMs (every 48 h) before hMDMs were infected with H37Rv at MOI 10 and bacterial growth was analyzed (n=4). (**D**) Recombinant IL-1β was added 24 h prior to infection with H37Rv at MOI 10 (n=4). (**E**) The IL-1R antagonist Anakinra (5 µg/ml) was added immediately after infection of cells with H37Rv at MOI 1 (n=6). (**F**) The caspase-1 inhibitor YVAD (50 µM, n=5) were added to the cells 30-60 minutes prior to infection at MOI 1 and were present throughout the experiment. Student's t-test was used for comparison between two groups, and one-way ANOVA with Tukey's post hoc test for multiple groups.


A
% CD63 positive phagosomes
50
40
30
20
10
0
Control
IL-1beta
B
% Lysotracker positive phagosomes
50
40
30
20
10
0
Control
IL-1beta
C
Bacterial growth (fold-change)
5
4
3
2
1
0
Untreated
50 pg/ml IL-1β
0
1
2
Days
D
Bacterial growth (fold-change)
4
3
2
1
0
Untreated
50 pg/ml IL-1beta
5 ng/ml IL-1beta
E
Bacterial growth (fold-change)
*
3
2
1
0
Untreated
Anakinra
F
Bacterial growth (fold-change)
6
4
2
0
Untreated
DMSO
YVAD

Supplementary Table 1. Allele frequencies for *NLRP3* Q705K and *CARD8* C10X polymorphisms in 43 blood donors (n).

	C10X -/-	C10X +/-	C10X +/+
Q705K -/-	**42% (18)**	**30% (13)**	**14% (6)**
Q705K +/-	**7% (3)**	**7% (3)**	**0% (0)**
Q705K +/+	**0% (0)**	**0% (0)**	**0% (0)**