Supplemental materials and legends

Supplemental materials:

Real-time polymerase chain reaction (RT qPCR)

Isolated RNA was processed with QuantiTect™ Reverse Transcription Kit (Qiagen). Quantitative real-time RT–PCR (qRT–PCR) was performed using 11.25 ng cDNA per well with 0.5 µl TaqMan™ Gene Expression Assay probe and 5 µl TaqMan™Universal PCR Master Mix in a 10-µl reaction volume on The Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System. Each reaction was performed in triplicate. Data analysis was performed using ExpressionSuite for QuantStudio™ (Applied Biosystems). Fold change was determined in relative quantification units using GAPDH for normalization. Taqman probes used were from Thermo Scientific: *CXCL10*-FAM (Hs00171042_m1); *IL-6*-FAM (Hs00174131_m1); *ZBP1*-FAM (Hs01090104_g1); *LGALS3*-FAM (Hs00173587_m1); *LGALS8*-FAM (Hs01057135_m1); *TNFAIP3*-FAM (Hs00234713_m1); *MB21D1*-FAM (Hs00403553_m1); *BCL2A1*-FAM (Hs06637394_s1); *IFNB1*-FAM (Hs01077958_s1); *EIF2AK2*-FAM (Hs00169345_m1); *GAPDH*-FAM (Hs02786624_g1).

Lipid extraction and thin layer liquid chromatography

Bacteria were grown to an OD600 of 1.0 in Middlebrook's 7H9 broth medium (Sigma-Aldrich, #M0178) supplemented with 10% (v/v) Middlebrook OADC (BD Biosciences, #212351) at 37°C. Samples were then pelleted at 3000RPM for 5 mins and resuspended in 1 mL Phosphate buffered saline solution. Bacteria were heat inactivated for at 92°C and washed three times with water. Mycobacterial lipids were then extracted with a mixture of methanol/chloroform at a 2:1 ratio, overnight and at room temperature. Supernatants where then collected and stored at 4°C. The remaining pellets were resuspended in 2ml of (1:1) chloroform/methanol and incubated for 5hrs at room temperature. Supernatants were collected and pooled with the first lipid collection. Lipid fraction were then incubated at 55°C until completely dry. The isolated lipids where resuspended in chloroform and spotted on a silica plaque. Lipids were resolved and separated, by migration through the silica plaque, using a mixture of petroleum ether/ethyl acetate (98:2) solvent mix. Lipids were finally visualised by adding phosphomolybdic acid 5% in EtoH and then heated at 80°C until bands are visible. Samples

were compared to a purified phthiocerol dimycocerosate (PDIM) lipid derived from irradiated Mycobacterium tuberculosis H37Rv strain as a positive control (NR-20328, ebi resources).

Supplemental figure legends

Supplemental Figure 1: Demonstration of feret diameter as a measure of cord length

ImageJ was used to first select only the GFP channel corresponding to the bacteria. The GFP was subjected to a pixel threshold, dilated and eroded (add and then remove 1 pixel to the outline, to link up any incorrectly thresholded pixels) and then outlined to form 'particles' which were analysed by Feret diameter. The Feret diameter is calculated as shown; it is the distance between the two furthest apart pixels in the particle.

Supplemental Figure 2: RD1 and PDIM -dependent regulation of selected gene expression in hLEC after infection

Gene expression measured by RT-qPCR of infection pathways, displayed in Fig 2, in response to hLEC infection with *M. tuberculosis*-H37Rv WT, *M. tuberculosis*-H37Rv ΔRD1 or ΔPDIM for 48 h post infection. Uninfected hLEC were used as control of the basal expression of each gene. Data are representative of two independent experiments.

Supplemental Figure 3: PDIM analysis of strains used in this study

Thin Layer Chromatographic (TLC) analysis showing the presence of phthiocerol dimycocerosate (PDIM) in H37Rv (WT) and mutants strains used in this study. Mycobacterial lipids were extracted using methanol/chlorophorm solution and separated, by migration through the silica plaque. PDIM spots, highlighted in the red rectangle, were detected for H37Rv WT and ΔRD1 strains. As expected, PDIMs were absent from H37Rv ΔPDIM strain. Purified PDIM was used as positive control.

Supplemental Movie 1:

Human lymphatic endothelial cells (hLEC) expressing p62-RFP (red) infected with *M. tuberculosis* expressing EGFP (green) were imaged for 100 h. Left hand side shows an overlay of phase contrast and fluorescence images, whereas right hand side shows fluorescence images only. The purple arrows track an example of an individual *M. tuberculosis* bacterium that does not become p62 positive at any point and eventually grows into a long intracellular

cord. Subsequently, the infected cell dies and detaches. The blue arrows track an example of *M. tuberculosis* that becomes p62 positive soon after uptake, remains p62 positive, and shows severely restricted growth.

Supplemental Movie 2 and 3:

Similar to Movie S1 except the blue arrows track an example of *M. tuberculosis* that fluctuates between having p62 association and not. Eventually, once p62 association ceases, an intracellular cord forms.

Supplemental table 1:

Primary and secondary antibodies used for immunofluorescence (IF)

Antibody	Dilution	Species	Conditions	Source	Cat. Number
Cathepsin D	1:100	Rabbit	IF; 1h RT	Andrej Hasilik	N/A
LAMP-2	1:100	Mouse	IF; 2h RT	Developmental Studies Hybridoma Bank	#H4B4
LC3B	1:100	Rabbit	IF; 1h RT	Cell Signalling Technology	#2775
Ubiquitin (FK2)	1:250	Mouse	IF; 1h RT	Enzo	#BML-PW8810
Galectin 8	1:250	Goat	IF; 1h RT	R&D Systems	#AF1305
p62	1:300	Rabbit	IF; 1h RT	GeneTex	#GTX111393
NDP52	1:250	Rabbit	IF; 1h RT	J. Kendrick-Jones	N/A (homemade)
Anti-mouse AF-546	1:800	Goat	IF; 1h RT	Life Technologies	#A11003
Anti-mouse AF-633	1:800	Goat	IF; 1h RT	Life Technologies	#A21052
Anti-rabbit AF-546	1:800	Goat	IF; 1h RT	Life Technologies	#A11010
Anti-rabbit AF-633	1:800	Goat	IF; 1h RT	Life Technologies	#A21094

Anti-goat AF-546	1:800	Donkey	IF; 1h RT	Life Technologies	#A11056

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