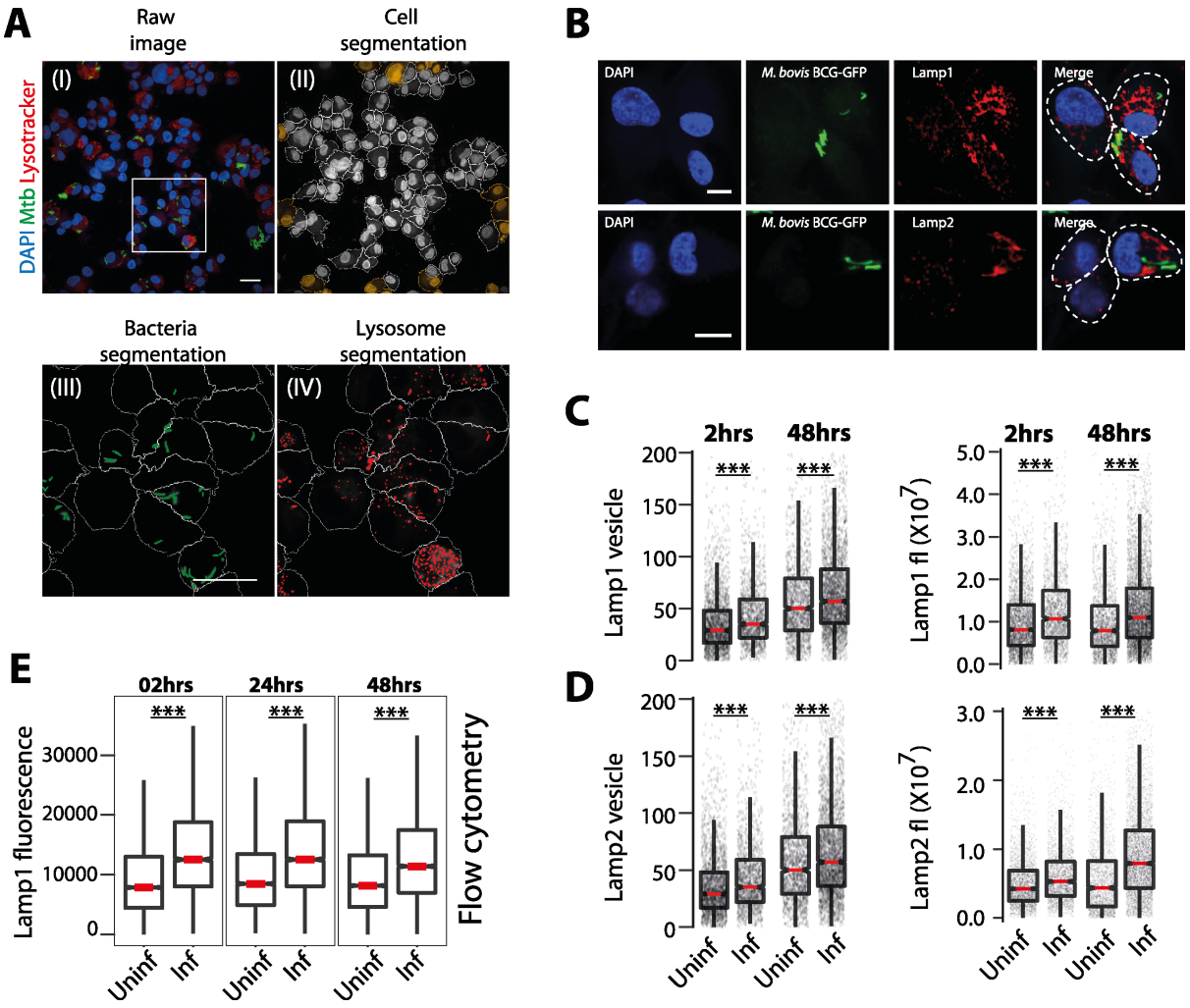

Fig S1. *Mycobacterium tuberculosis*-infected macrophages have higher lysosomal content (*in vitro*).

(A) Segmentation of macrophage boundary, bacteria and lysosomes from the images. The raw image of an *Mtb*-GFP infected cell stained for lysotracker red (I) is segmented to define the cell boundary based on the Cell Mask Blue staining (II). Cells selected for further analysis are outlined in white while cells touching the border of the image (outlined yellow) are not considered for further analysis. *Mtb* (green objects, III) and lysosomes (red objects, IV) within macrophages are segmented and related to the cells for further analysis. III and IV show the bacterial and lysosomal segmentation from the boxed area. Multiple parameters of Mtb and lysosomes are extracted from such images. Scale bar is 25 µm. (B-D) THP-1 monocyte-derived macrophages were infected with *M. bovis* BCG-GFP for 2 and 48 hrs, fixed and immunostained for lysosomal markers, Lamp1 and Lamp2. Scale bar is 10 µm. Graphs show the Lamp1 (C) and Lamp2 (D) vesicle numbers and integral intensities in infected and uninfected cells. Data are represented as box plots, with median highlighted by red line. For C and D individual data points corresponding to single cells are overlaid on the boxplots. Results are representative of three biological experiments. (E) THP-1 monocyte-derived macrophages were infected with *M. bovis* BCG-GFP for 2, 24 and 48 hrs, fixed, immunostained for lysosomal markers, Lamp1 and analyzed by flow cytometry. The graph shows Lamp1 intensity in infected and uninfected cells at each timepoint. Approximately 10000 cells were analyzed at each time point. Statistical significance was assessed by Mann-Whitney test, *** denotes p-value of less than 0.001.

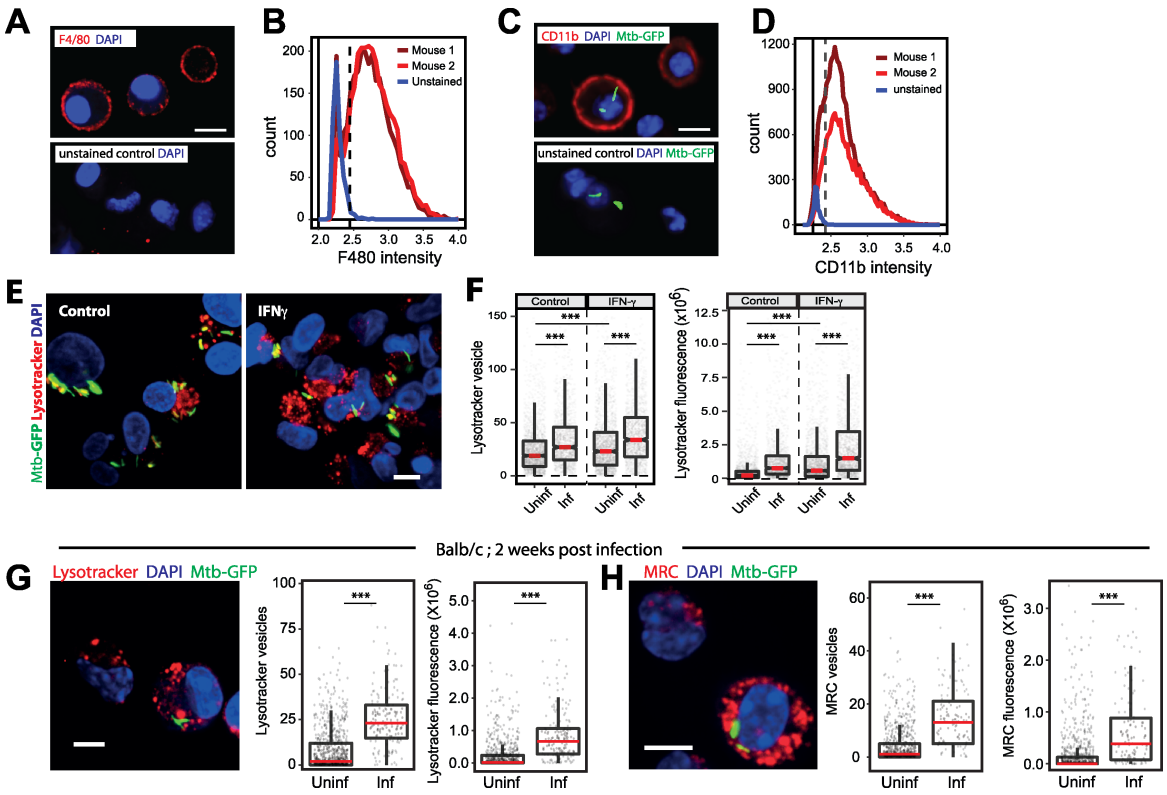

Fig S2. *M. tuberculosis* induced lysosomal increase *in vivo* is independent of adaptive immunity.

(A-D) Single-cell suspension from the lungs of *M. tuberculosis*-GFP H37Rv infected mice were prepared and macrophages were selected by adherence for 2 hours. Non-adhered cells were washed and purity of macrophage post adherence was assessed by immunostaining with anti-F4/80 (A) or anti Cd11b (C) followed by imaging. Control i.e. unstained cells were used to determine the cut off for F4/80 or Cd11b positive population. A false-positive rate of 2-3% was used as a cut-off (indicated with a dashed black line in the histograms) to determine the proportion of F4/80 or Cd11b positive cells. (B, D) Distributions are drawn from 2,500-3,000 cells per mouse, data shown from 2 mice in each experiment and are representative of at least two independent infections. (E, F) THP1 monocyte-derived macrophages were infected with wild type *M. tuberculosis*-GFP CDC1551 followed by incubation with or without IFN-gamma (25ng/ml) containing media for 48hrs. Post 48hrs incubation, cells were stained with lysotracker red. Images (E) and graphs (F) show the number and intensity of lysotracker in control and Interferon-γ treated infected and uninfected-bystander macrophages. (G, H) BALB/C mice were infected with ~150 CFUs of *M. tuberculosis*-GFP H37Rv by aerosol inhalation. 17 days post-infection, macrophages were isolated from infected lungs by making single-cell suspension and stained with lysotracker red (G) or magic red cathepsin (H) and number and intensity of lysosomes were compared between infected and uninfected cells. Statistical significance was assessed using Mann-Whitney test, *** denotes p-value of less than 0.001. Scale bar is 10 µm. For panels F to H, data are represented as box plots, with the median value highlighted by red line. Individual data points corresponding to single cells are overlaid on the boxplots.

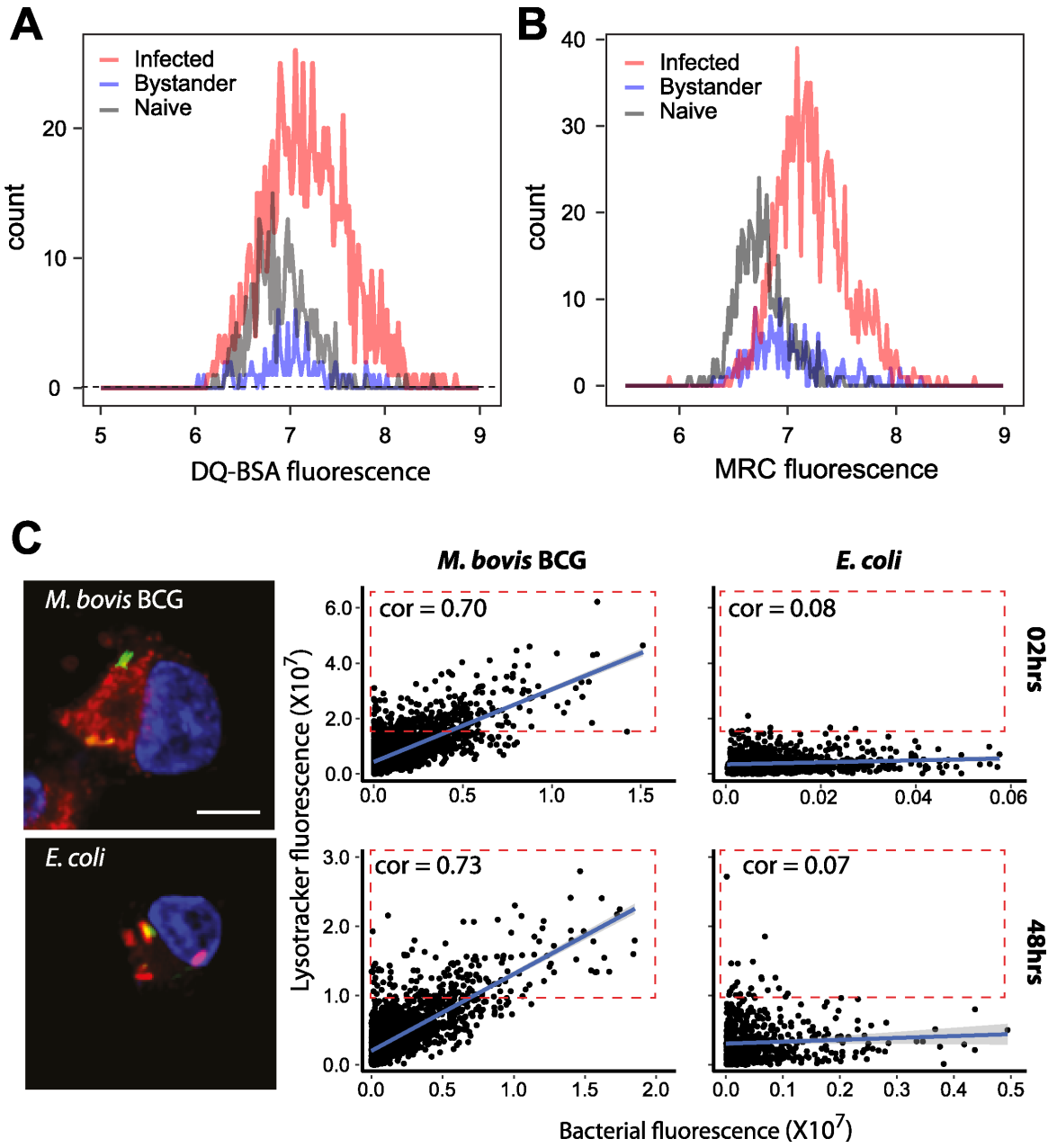

Fig S3. *M. tuberculosis* infected macrophages have higher lysosomal activity compared to naïve macrophages (*in vitro*).
(A, B) THP-1 derived macrophages were infected with *M. tuberculosis*-GFP H37Rv and lysosomes were stained with lysosomal activity probes, DQ-BSA or MRC at 48 hpi. Cells were fixed and imaged. Distributions of total cellular lysosomal intensity is compared between the three cell populations of infected, bystander and naive macrophages. More than 1000 infected, 200 bystander and 500 naive cells were analyzed for the distributions. Results are representative of at least three biological experiments. (C) THP-1 derived macrophages were infected with *M. bovis*-GFP or *E.coli*-GFP and lysosomes were stained with lysotracker red at 2 and 48 hpi. Cells were fixed and imaged. Scatter plots of lysotracker integral intensity and bacterial intensity in infected cells is shown at 2 and 48 hpi. Spearman correlation coefficient (cor) between the two features for each condition is shown. Each data point is an individual infected cell and red boxes with a dotted line show the cells with high lysosomes in *M. bovis* infected cells, which are not observed in *E. coli* infection. Results are representative of two independent biological experiments. Scale bar is 10 μm.

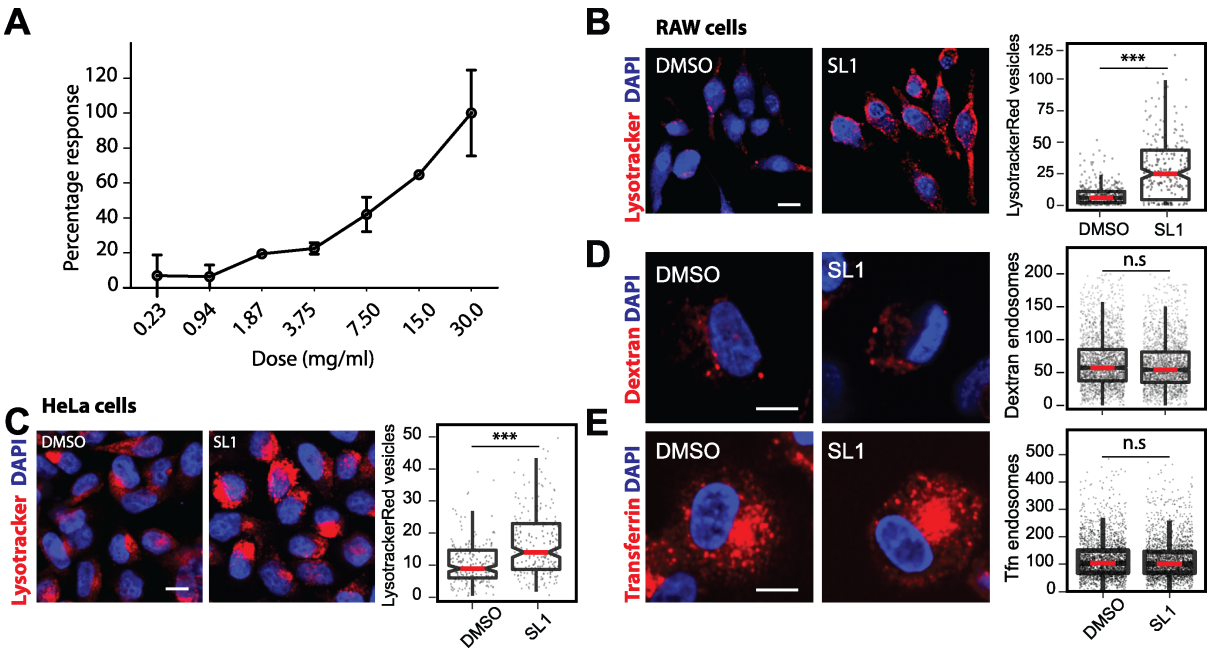

Fig S4. Characterization of *M. tuberculosis* SL-1 mediated lysosomal expansion.
(A) THP-1 monocyte-derived macrophages were treated with different doses of purified Mtb H37Rv SL-1 (0.23-30µg/ml) for 24 hrs, pulsed with lysotracker red, fixed and imaged. DMSO used as vehicle control. The graph represents a percent increase in lysotracker intensity in cells with an increasing dose of SL-1 compared to DMSO control. Average and standard deviation of technical replicates is shown in the graph. Results are representative of two independent dose curves. (B, C) RAW macrophages (B) or HeLa cells (C) were treated with 25µg/ml purified SL-1 for 24 hrs, stained with lysotracker red, fixed and imaged. Representative images and quantification of lysotracker red vesicles in DMSO or SL-1 treated RAW and HeLa cells are shown. (D, E) THP-1 monocyte-derived macrophages were treated with 25µg/ml purified SL-1 for 24 hrs, pulsed with fluorescently labeled dextran or Transferrin (Tfn), fixed and imaged. Representative images and quantification of dextran and Tfn endocytosis in SL-1 treated THP-1 monocyte-derived macrophages are shown. Results are representative of at least two biological experiments. Statistical significance was assessed using Mann-Whitney test, ns denotes non-significant and *** denotes p-value of less than 0.001. Scale bar is 10 µm. For B-E, data are represented as box plots, with the median denoted by red line. Individual data points corresponding to single cells are overlaid on the box plot.

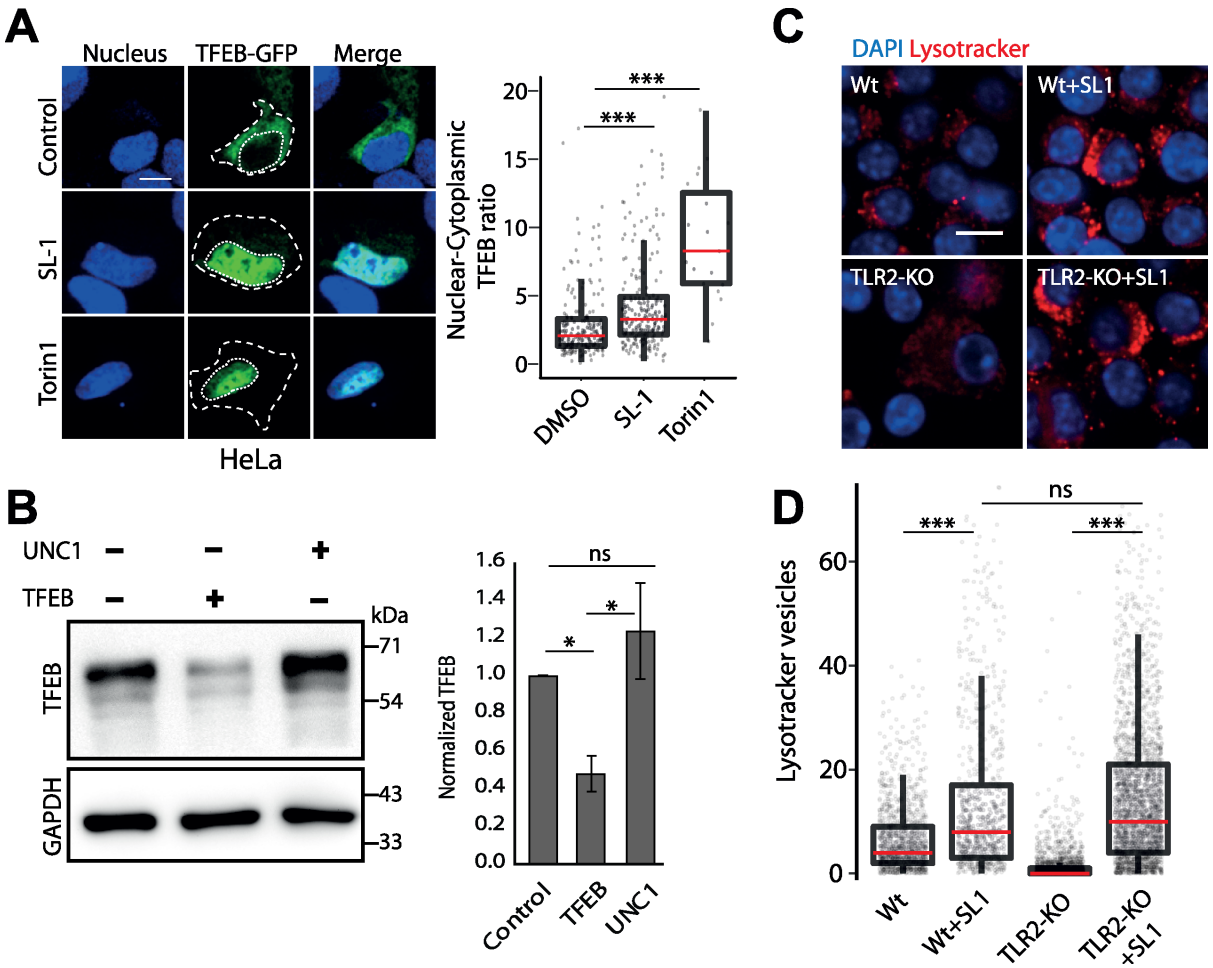

Fig S5. Sulfolipid-1 (SL-1) from *M. tuberculosis* H37Rv influences lysosomal biogenesis in host cells via mTORC1 dependent nuclear translocation of the transcription factor EB (TFEB).

(A) HeLa cells were transfected with TFEB-GFP for 24 hours and treated with 25 µg/ml SL-1, or negative and positive controls, DMSO and Torin1 (250nM for 24 hrs) respectively. Representative images and quantification of nuclear to cytoplasmic ratio of TFEB-GFP between the different conditions are shown. (B) THP-1 monocyte-derived macrophages were transfected with either control (UNC1) or TFEB siRNA using lipofectamine RNAimax and TFEB knockdown efficiency was assessed by measuring TFEB protein levels by immunoblotting post 48 hours of transfection. GAPDH was used as the loading control. The bar graph shows the average and standard error of three biological experiments. For B statistical significance is assessed using unpaired-one tailed Student's *t*-test with unequal variance, ns represents non-significant and * represents p-value less than 0.05. Results are representative of atleast three biological replicates. (C, D) Wild type and TLR2 KO immortalized BMDMs were treated with SL-1 for 24 hrs followed by lysotracker red pulse and imaging. Representative images and quantification of the lysosomal number of untreated, SL-1 treated wt and TLR2 KO macrophages are shown. Statistical significance for A and D was assessed using the Mann-Whitney test, and *** denotes a p-value of less than 0.001 and ns represents non-significant. The results are representative of two biological experiments. Scale bar is 10 µm. Data in A and D are represented as box plots, with median value highlighted by the red line. Individual data points corresponding to single cells are overlaid on the boxplots.

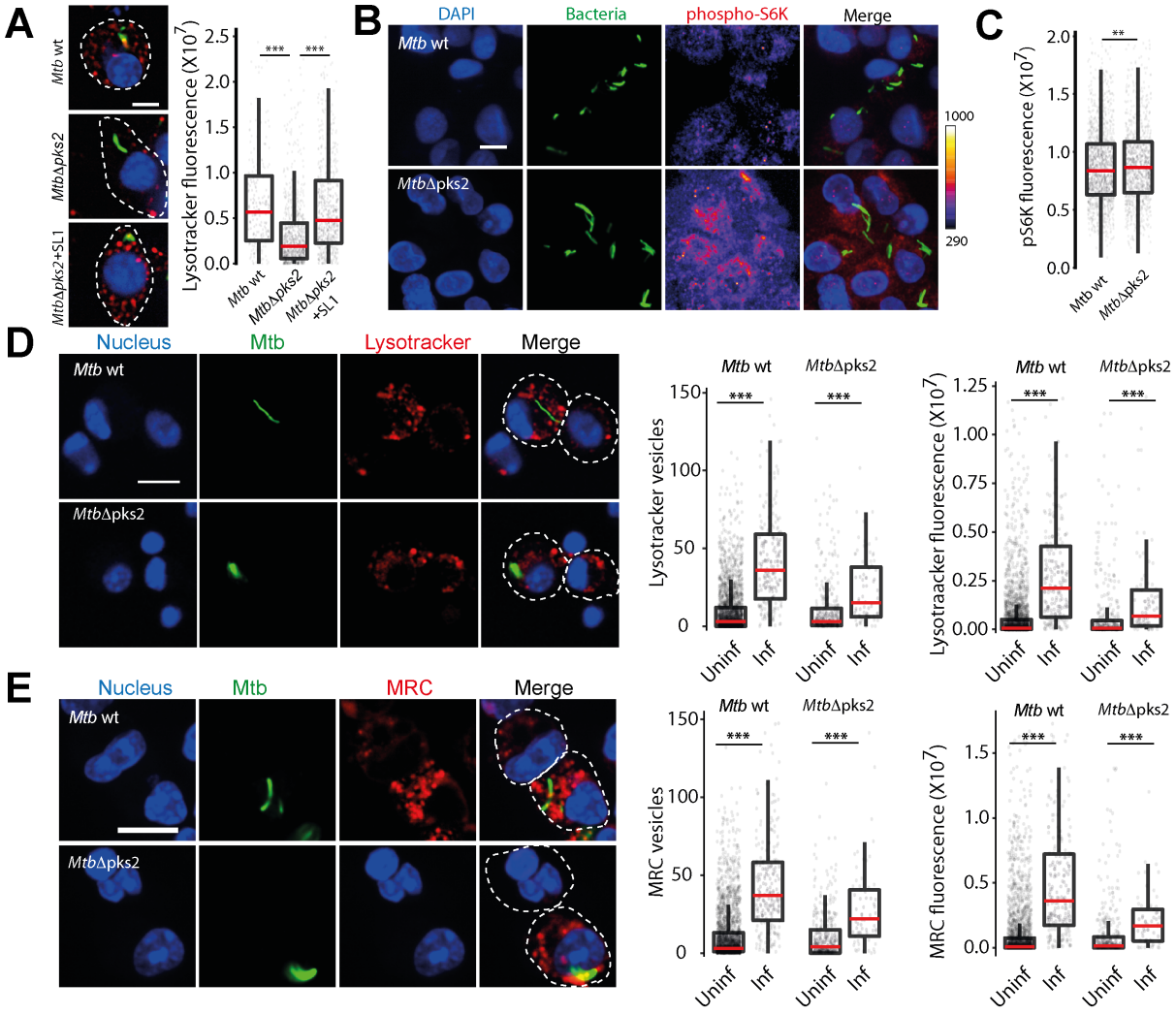

Fig S6. Wild type and *MtbΔpks2* CDC1551 infected cells show higher lysosomal content compared to their respective uninfected controls.

(A) RAW macrophages were infected with *Mtb* wt and *MtbΔpks2* CDC1551 for 2 hrs and stained with lysotracker red. Pretreatment with SL-1 (25 µg/ml) for 24 hrs was done in cells before infection with *MtbΔpks2*. Images and graph show a comparison of the total lysotracker red intensities in individual *Mtb* wt, *MtbΔpks2* mutant and SL-1 complemented *MtbΔpks2* mutant infected cells. Results are representative of two biological experiments. (B,C) THP1 monocyte-derived macrophages were infected with *Mtb* wt and *MtbΔpks2* CDC1551 *M. tuberculosis*-GFP for 48 hrs, fixed and immunostained for phospho-S6K. Representative images (B) and total cell intensity of phospho-S6K are shown. Results are representative of two biological experiments. (D,E) C57BL/6NJ mice were infected with *Mtb* wt or *MtbΔpks2*-GFP CDC1551 by aerosol inhalation. Eight weeks post-infection, macrophages were isolated from infected lungs by making single-cell suspension and were pulsed with lysosomal probes, namely lysotracker red (D), and magic red cathepsin (MRC) (E). Representative images and graphs show the number and intensity of lysosomes in respective probes were compared between *Mtb* wt or *MtbΔpks2* CDC1551 infected and uninfected cells. Results are compiled from four wild type Mtb and three *MtbΔpks2* infected mice and are representative of two independent infections. Statistical significance was assessed using Mann-Whitney test, ** denotes p-value of less than 0.01 and *** denotes p-value of less than 0.001. Scale bar is

10 μm. Data are represented as box plots, with the median denoted by red line. Individual data points corresponding to single cells are overlaid on the box plot.

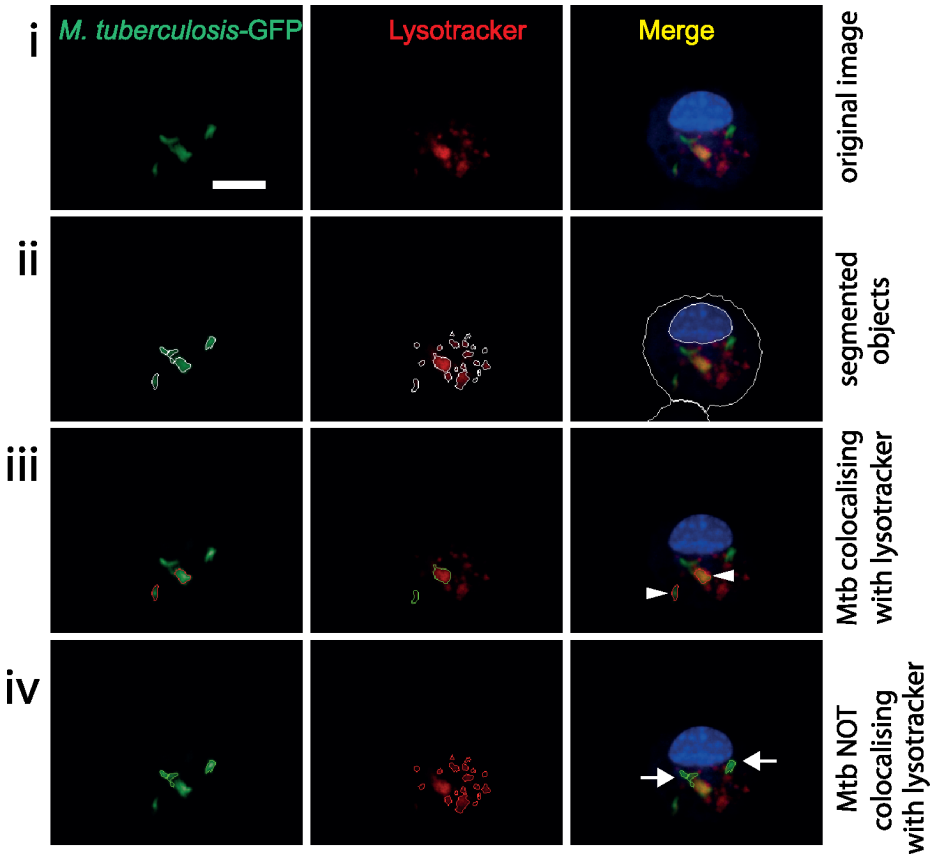

Fig S7. Quantifying *Mtb*-lysosome co-localisation
(A) Schematic of quantifying Mtb co-localisation with lysosomal probes. The raw image of an *Mtb*-GFP infected cell stained for lysotracker (i) is segmented (ii). If the segmented objects (Mtb and LTR) overlap by more than 50%, they are considered colocalised (arrowheads in panel iii), else they are not (arrows in panel iv). Object overlap based colocalization was quantified between bacteria and the respective lysosomal compartments. Scale bar is 10 μm.