Supplementary Material

The metabolic activity of *Mycobacterium tuberculosis*, assessed by use of a novel inducible-GFP expression system, correlates with its capacity to inhibit phagosomal maturation and acidification in human macrophages

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Supplemental Figure Legends

Figure S1. Fluorescence microscopy examination of *M. tuberculosis* cultures with or without induction.

A. Fluorescence and phase contrast images of *M. tuberculosis* parental, Mtb-P_0-iGFP and Mtb-P_{32}-iGFP (Mtb-iGFP) strains with or without 1 mM IPTG induction for 1 day (upper) or 3 days (lower).

Fluorescence images of Mtb-iGFP and control strains were acquired with a fixed exposure time of 1.5 second for the bacterial cultures induced for 1 day and a fixed exposure time of 1.0 second for the cultures induced for 3 days. The 1.5 and 1 second exposure times were chosen because they were found to yield optimal results for the Mtb-iGFP strain induced for 1 day and 3 days, respectively, using the SPOT RT-KE monochrome camera and SPOT software. Size bar, 10 microns.

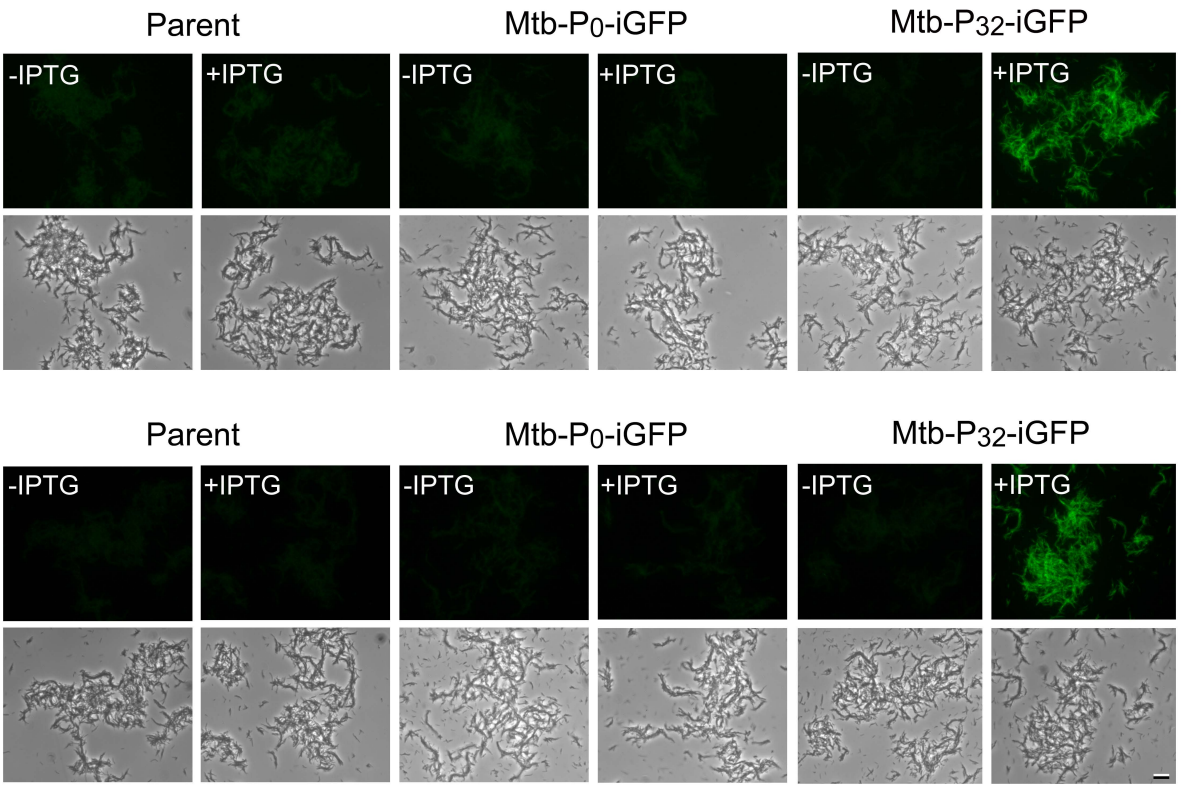
Figure S2. Analysis of GFP expression kinetics in Mtb-iGFP induced with three different concentrations of IPTG. The expression of GFP was assessed by immunoblot analysis of equal amounts of proteins (10 μg) in the bacterial lysate of *M. tuberculosis* cultures harvested at various time points over a 3-day period after 1, 2.5 or 5 mM IPTG induction. The blot was probed with rabbit polyclonal antibody specific for GFP (1:10,000) and developed with chemiluminescent substrate. The 58-kDa glutamine synthetase (GS) polypeptide of *M. tuberculosis* on the same blot was probed with polyclonal antibody (1:10,000) specific to the polypeptide and served as the loading control. The protein sample indicated as the 0 h time point was obtained from a bacterial culture harvested immediately after the addition of 1 mM IPTG.

Figure S3. Stability of GFP as assessed by the intensity of bacterial green fluorescence. 1mM IPTG was added to the Mtb-iGFP culture to induce GFP expression for 3 days. IPTG was then removed from the bacterial culture by centrifugation. The bacterial pellet was washed and resuspended in culture medium (7H9 medium with 2% glucose and 0.01% Tyloxapol) containing 0.5 μg ml^{-1} streptomycin to halt new protein synthesis. The culture was continued in the absence of IPTG for an additional 3 days. Bacterial fluorescence at sequential time points before and after removing IPTG was followed by epifluorescence microscopy. Fluorescence images were acquired with a fixed exposure time of 1.5 seconds. Size bar, 10 microns.

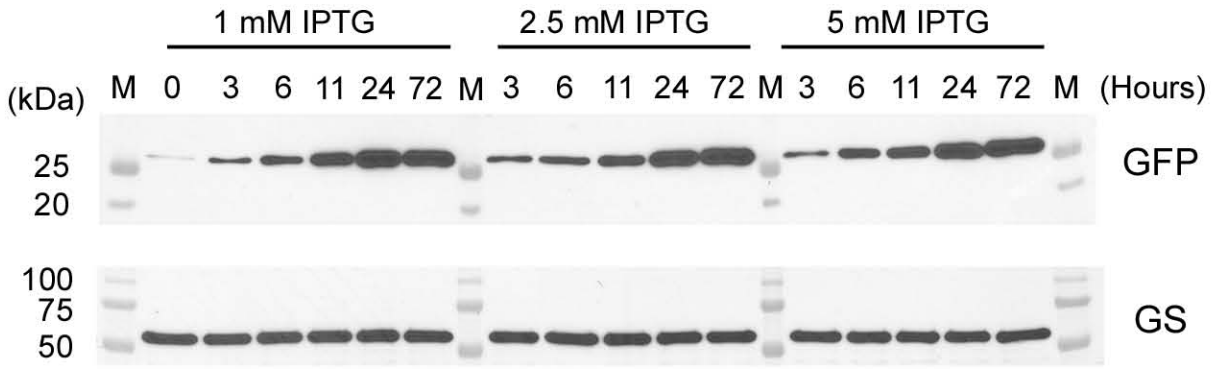
The green fluorescence signal of bacteria 1 day after removal of IPTG was very strong and comparable to that observed for bacteria 1-day post induction. The green fluorescence signal was still above the background level and easily detectable even 3 days after removal of IPTG. This result indicates that GFP fluorescence is very stable in *M. tuberculosis*.

Figure S4. Immunofluorescence and phase contrast microscopy of Mtb-iGFP. Epifluorescence microscopy was used to assess the extent of colocalization of Mtb-iGFP with Texas Red (TxR)-dextran prelabeled lysosomes (a – e), LysoTracker red (DND-99) labeled compartments (f-j), and CD63 labeled compartments (k-o) in THP-1 cells (a-e, k-o) and MDM (f - j). Phase contrast images of the macrophages (a, f, k) demonstrate that the macrophages are well spread and without evidence of cytoxicity. Metabolically active Mtb-iGFP were distinguished from metabolically inactive Mtb-iGFP

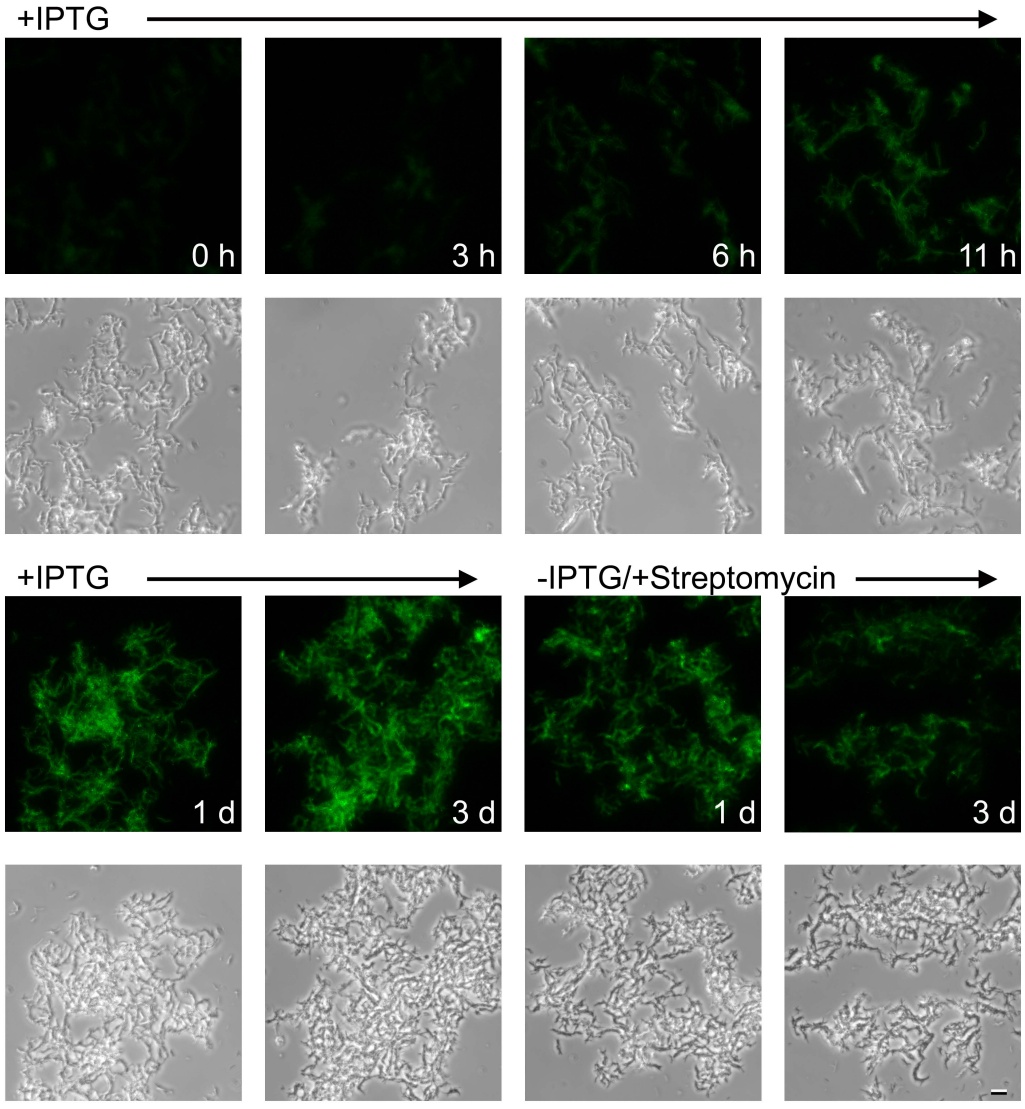
by their green fluorescence protein expression (b, g, l) and Mtb-iGFP, independent of metabolic status, were visualized by staining with amino methyl coumarin (AMC)-labeled anti-LAM antibody (c, h, m). Metabolically inactive Mtb-iGFP, but not metabolically active Mtb-iGFP, colocalized uniformly with TxR-dextran (d) and with LysoTracker red (i). Both metabolically active and inactive Mtb-iGFP colocalize with CD63 (n), though the metabolically inactive Mtb-iGFP colocalize more strongly and more consistently with CD63 than do the metabolically inactive bacteria. Merged color images are shown on the right (e, j, o). Arrowheads indicate the metabolically active Mtb-iGFP and arrows indicate metabolically inactive Mtb-iGFP. Size bars indicate 5 microns.



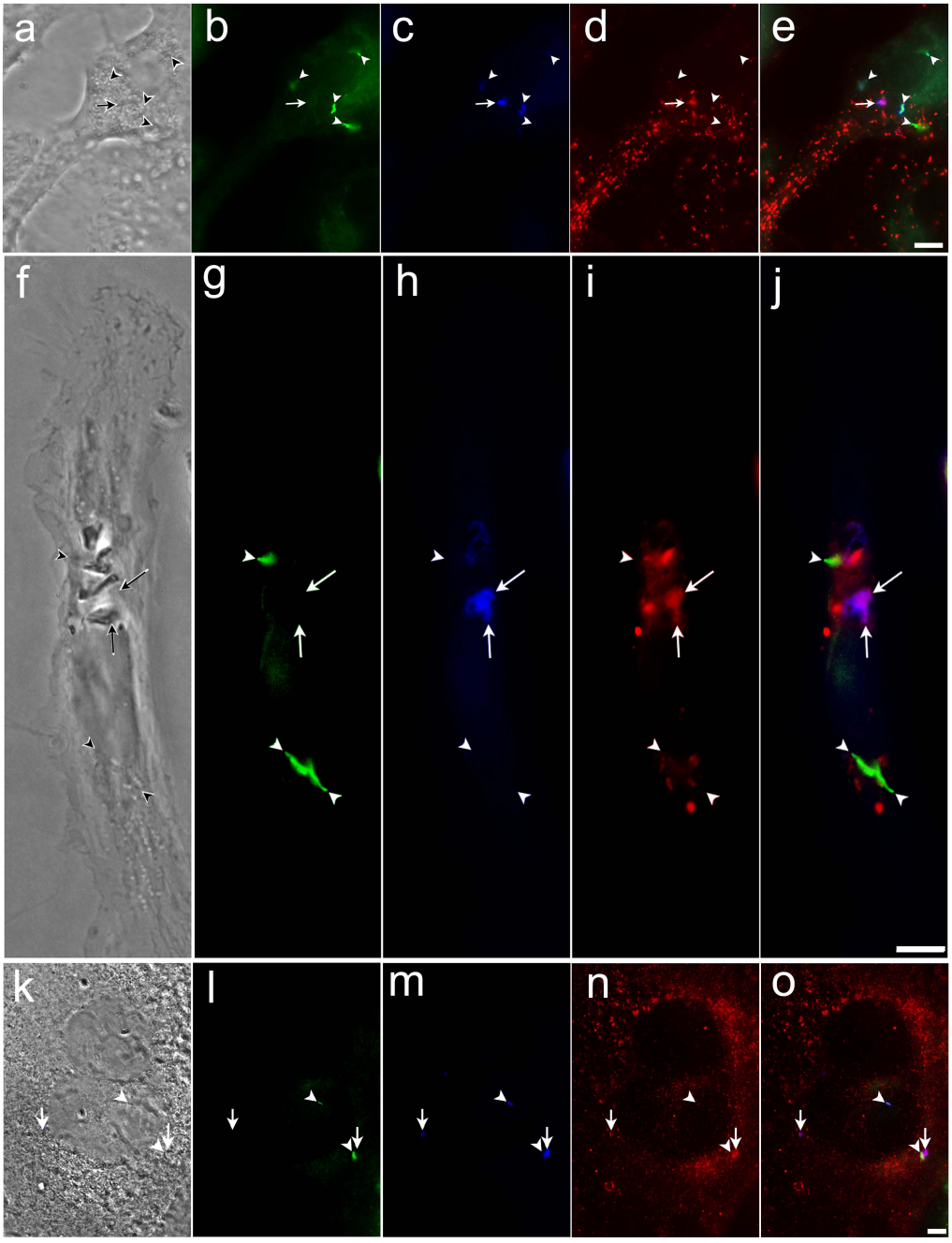
Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4