## EXTENDED EXPERIMENTAL PROCEDURES

### Mice and BMDMs

*ATG5*$^{flox/flox}$ mice were from N. Mizushima (Tokyo Medical and Dental University, Japan) (Kuma et al., 2004) and *ATG5*$^{flox/flox}$-Lyz-*Cre* were a gift from H. Virgin (University of Washington) (Hara et al., 2006; Zhao et al., 2008). Bone marrow-derived macrophages (BMDMs) were obtained from mouse femurs as previously described (Ohol et al., 2010) and cultured in DMEM H-21 supplemented with 10% MCSF derived from 3T3-MCSF cells. BMDMs were obtained from the following mouse strains: *Myd88*$^{-/-}$/*Trif*$^{-/-}$ (Barbalat et al., 2009), *Trex1*$^{-/-}$ (Stetson et al., 2008), *Sting*$^{-/-}$ (Ishikawa et al., 2009), *Tnfr1*$^{-/-}$ and *Tbk1*$^{-/-}$/*Tnfr1*$^{-/-}$ (Ishii et al., 2008), *Ifnar1*$^{-/-}$ (Stanley et al., 2007), and *Irf3*$^{-/-}$/*Irf7*$^{-/-}$ (G. Barton). BMDMs from *Asc1*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice were a gift from D. Monack (Stanford University).

### Cell Lines

RAW 264.7 cells were purchased from ATCC (TIB-71) and cultured in DMEM-H21 containing 10% FBS. RAW 264.7 cells stably expressing GFP-LC3 were generated by electroporating the pROW2 plasmid (encoding CMV-GFP-LC3) into RAW 264.7 cells via the Amaxa nucleofactor kit V. Electroporated cells were selected on neomycin for 3 weeks, and stable clones were isolated via FACS sorting for GFP-positive cells.

### Bacterial Strains

The Erdman strain of *M. tuberculosis* was used as the WT strain and mutant background strain for all *M. tuberculosis* experiments. The *M. tuberculosis* Δ*esat-6*, *Rv1506c::Tn*, and *moeB1::Tn* strains were previously described (Brodin et al., 2010; MacGurn and Cox, 2007; Stanley et al., 2003). BCG Pasteur was a gift from W.R. Jacobs and was transformed with the cJSC49 cosmid, containing the Erdman RD1 genomic locus. For generation of mCherry-expressing mycobacteria, strains were transformed with the pMAN12 plasmid encoding mCherry under the control of the *groEL1* promoter. LLO-expressing mycobacterium was previously described (Manzanillo et al., 2012). All strains were cultured in 7H9 media supplemented with 10% OADC, 0.5% Glycerol, and 0.05% Tween-80.

### Macrophage Infection

For infections with mycobacteria, macrophages were infected as previously described (Manzanillo et al., 2012) with some modifications. Briefly, mycobacteria cultures were washed twice with PBS, gently sonicated to disperse clumps, and resuspended in DMEM supplemented with 10% horse serum. Media were removed from cells, and monolayers overlaid with the bacterial suspension and centrifuged for 10 min at 1,000 RPM. Cells were washed twice in PBS and returned to macrophage media. For determination of CFUs, macrophage monolayers were lysed in 0.1% Triton X-100 and plated on 7H10 agar plates.

### Immunofluorescence Microscopy

Indicated cells were infected as described above, and at the designated time points, cells were washed three times in PBS and fixed in 4% paraformaldehyde (PFA) for 20 min at room temperature (RT). The fixed cells were washed three times in PBS and permeabilized by incubating them in PBS containing 5% nonfat milk and 0.05% saponin (PBS-MS) (Calbiochem). Coverslips were incubated in primary antibody diluted in PBS-MS for 1 hr. The coverslips were then washed three times in PBS and incubated in secondary antibody. After two washes in PBS and two washes in deionized water, the coverslips were mounted onto glass slides using Prolong Gold antifade reagent (Molecular Probes). Images were acquired on a Zeiss Axiovert200M inverted microscope fitted with a Hamamatsu C4742-80-12AG digital camera controlled by the Axiovision software package version 4.6 (Carl Zeiss MicroImaging, Inc.).

### Antibodies

The following primary antibodies were used: mouse monocolonal antibodies against poly- and monoconjugated-ubiquitin (Enzo Life Sciences), K63 and K48 ubiquitin (Milipore), Lamp-1 (BD Biosciences), Sting (Sigma), and NDP52 (Novus Biologicals) and rabbit polyclonal antibodies against phospho-TBK1 (Cell Signaling), ATG12 (Cell Signaling), and LC3B (Invitrogen). Secondary antibodies used were as follows: Alexa 488-conjugated goat anti-rabbit and Alexa 488- and Alexa 350-conjugated goat anti-mouse IgG antiserum (Molecular Probes).

### Colocalization of Markers with Mycobacteria and Cytosolic DNA

To quantify the percentage of mycobacterium phagosomes or cytoslic DNA containing different cellular markers, infected cells were visualized directly by fluorescence microscopy. Using the Axiovision software package version 4.6, a series of images were captured including internalized bacteria and the cellular marker. Overlaid fluorescent images were analyzed by determining the number of mycobacterium phagosomes or cytosolic DNA that contained the corresponding marker. A minimum of 100 phagosomes or DNA puncta were analyzed per coverslip for each treatment and designated post-infection time. For triple-labeling experiments one hundred of marker-positive bacteria or DNA puncta were assessed for colocalization with the second marker. For example, 100 LC3-positive bacteria were assessed for ubiquitin colocalization. Each experiment was completed in triplicate coverslips and

expressed as an average. Mycobacterium phagosomes or cytosolic DNA was considered positive for the presence of a marker when they contained detectable amounts of the antibody/fluorescence signal.

### Mouse Infection

*ATG5*$^{flox/flox}$-Lyz-*Cre* and *ATG5*$^{flox/flox}$ control mice were infected with *M. tuberculosis* (Erdman) via low-dose aerosol infection (~200 CFUs) as previously described (Manzanillo et al., 2012). Lungs, spleens, and liver were harvested, homogenized, and plated on 7H10 agar plates. In addition, lungs were sectioned and stained with hematoxylin and eosin (H&E) or acid-fast staining at the Histology Core Laboratory of the Gladstone Institute. For survival experiments, infected mice were euthanized when they had lost 15% of their maximal body weight. All mice were housed and treated humanely using procedures described in an animal care protocol approved by University of California, San Francisco, Institutional Animal Care and Use Committee.

### Cytokine Measurements

Lungs were harvested from *M. tuberculosis* infected mice and homogenized in 3 ml of PBS containing 0.005% Triton X-100 and protease inhibitor cocktail (Roche). Cytokines were measured using a Q-Plex Mouse Cytokine 16-Plex IR ELISA (Quansys Biosciences) and Quantikine Mouse IL-1β ELISA kit (R&D systems) per manufacturer's instructions.

### Lentiviral Cell Lines

The mouse TRC1 lentiviral library (Moffat et al., 2006), was obtained from Sigma and used to knockdown *p62* (*SQSTM1)* and *NDP52 (Calcoco2)* mRNA in RAW 264.7 macrophage cells stably expressing GFP-LC3. RAW 264.7-LC3 GFP cells expressing DNases TREX1 and DNase2a were generated by via lentiviral transduction using the pCMV-lenti-puro system as previously described (Campeau et al., 2009). Knockdown efficiency was validated by RT-qPCR using gene-specific primers.

### RNA Isolation and qPCR

RNA was isolated and purified from macrophages using the Trizol micro-midi RNA isolation kit (Invitrogen) per manufacturer's instructions. For qPCR analysis, 1 μg of RNA was reverse-transcribed using the VILO cDNA synthesis kit (Invitrogen), and qPCR analysis was performed in triplicate, as previously described (Ohol et al., 2010) using gene-specific primers.

### Nucleic Acid Transfections

Poly(dA:dT), poly(dG:dC), poly(dA), poly(dT), and poly(I:C) were purchased from Sigma. c-di-AMP was purchased from Biolog. 5′ppp-dsRNA and dsRNA were purchased from Invivogen. Nucleic acids were labeled with Cy3 using the Label-It Cy3 kit (Mirius inc). Cells were transfected using Lipofectamine 2000 (Invitrogen) or were electroporated as previously described (Burdette et al., 2011). For microscopy analysis, 150,000 macrophages were transfected with 100 ng of the indicated nucleic acids.

### Biotin-DNA Immunopreciptation

DNA was labeled with biotin using the Label-IT kit from Mirius biologicals. Twenty million macrophages were transfected with either 20 μg of dsDNA or 20 μg of biotinylated dsDNA for 3 hr and lysed in RIPA buffer. Biotintylated DNA was immunopreciptated using Strep-dynal beads (Invitrogen) according to manufacturer's instructions.

### Statistics

Statistical analysis of data was performed using GraphPad Prism software (Graphpad; San Diego, CA, USA). Two-tailed unpaired Student's t tests were used for analysis of microscopy images and mycobacterium survival assays. The Kaplan-Meir method was used to analyze mouse survival. Unless otherwise indicated, all experiments were performed at least three times and presented as the mean ± SEM.

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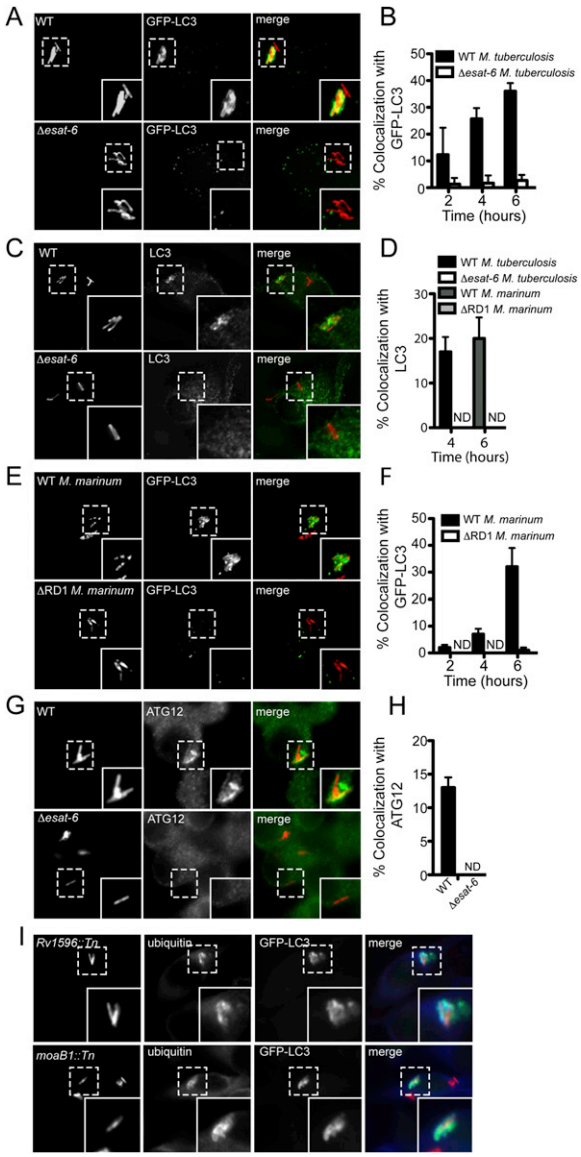


**Figure S1. *M. tuberculosis* and *M. marinum* Targeting to Autophagosomes Requires Phagosomal Permeabilization via ESX-1, Related to Figure 1**

(A) Fluorescence images of RAW 264.7 cells stably expressing GFP-LC3 (green) infected for 6 hr with mCherry-expressing WT *M. tuberculosis* or Δ*esat-6 M. tuberculosis* (red).

(B) Quantitative analysis of GFP-LC3 colocalization with WT *M. tuberculosis* and Δ*esat-6 M. tuberculosis* at indicated times after infection. Results are the means ± SEM of three independent experiments.

(C) Fluorescence images of BMDMs infected with for 4 hr with either mCherry-expressing WT *M. tuberculosis* or Δ*esat-6 M. tuberculosis* (red) and immunostained with anti-LC3 antibody.

(D) Quantitative analysis of LC3 colocalization with WT *M. tuberculosis* or Δ*esat-6 M. tuberculosis*, and WT *M. marinum* or ΔRD1 *M. marinum* at indicated times after infection. Results are the means ± SEM of three independent experiments.

(E) Fluorescence images of RAW 264.7 cells stably expressing GFP-LC3 (green) infected with either mCherry-expressing WT *M. marinum* or ΔRD1 *M. marinum* (red) corresponding to 6 hr post-infection.

(F) Quantitative analysis of GFP-LC3 colocalization with WT *M. marinum* or ΔRD1 *M. marinum* at indicated times after infection. Results are the means ± SEM of three independent experiments.

(G) Fluorescence images of RAW 264.7 cells infected for 4 hr with either mCherry-expressing WT *M. tuberculosis* or Δ*esat-6 M. tuberculosis* (red) and immunostained with anti-ATG12 antibody.

(H) Quantitative analysis of ATG12 colocalization with WT *M. tuberculosis* or Δ*esat-6 M. tuberculosis* and WT *M. marinum* or ΔRD1 *M. marinum* at indicated times after infection. Results are the means ± SEM of three independent experiments.

(I) Fluorescence images of RAW 264.7 cells stably expressing GFP-LC3 (green) infected for 6 hr with mCherry-expressing Rv1596::Tn and moaB1::Tn (red) and stained with anti-ubiquitin (blue) antibodies.

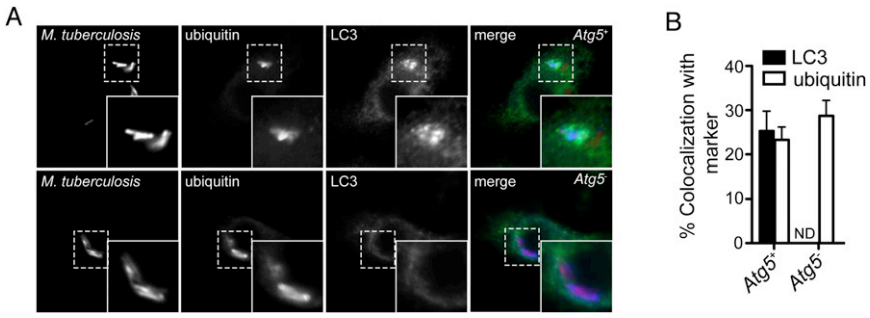


**Figure S2. Ubiquitin, but Not LC3, Is Localized to *M. tuberculosis* in *Atg5*$^{-}$ Macrophages, Related to Figure 3**

(A) Fluorescence images of *Atg5*$^{+}$ and *Atg5*$^{-}$ BMDMs infected for 4 hr with mCherry-expressing WT *M. tuberculosis* (red) and immunostained with anti-LC3 (green) and anti-ubiquitin (blue) antibodies.

(B) Quantitative analysis of LC3 and ubiquitin colocalization with WT *M. tuberculosis* in *Atg5*$^{+}$ and *Atg5*$^{-}$ BMDMs. Results are the means ± SEM of three independent experiments.

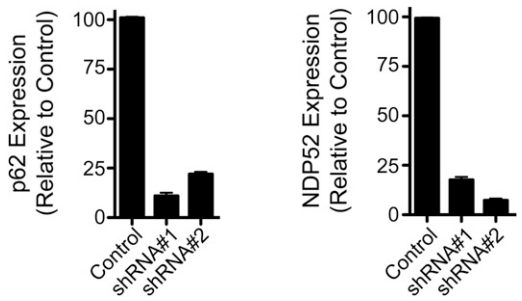


**Figure S3. Transcript Levels of p62 or NDP52 in shRNA Knockdown Cells, Related to Figure 2**

GFP-LC3 RAW 264.7 cells were transduced with lentiviral constructs expressing shRNAs targeting p62, NDP52, or scrambled shRNA (control), and mRNA levels were assessed by RT-qPCR amplification. Data are expressed as a percentage relative to control knockdown cells.

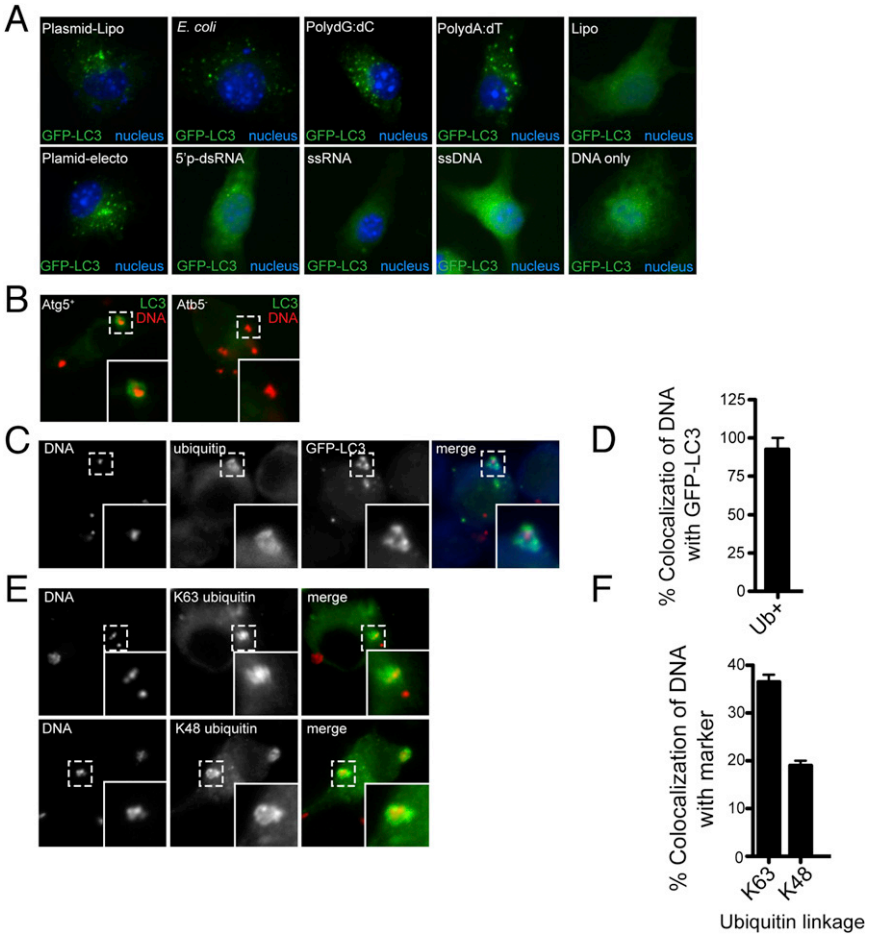


**Figure S4. Cytosolic dsDNA LC3$^+$ Population Is Dependent on Atg5 and Colocalizes with Ubiquitin, Related to Figure 4**

(A) Fluorescence images of GFP-LC3 RAW 264.7 cells at 4 hr post-transfection with designated nucleic acid. Plasmid DNA was introduced by either lipofection (plasmid-lipo) or by electroporation (plasmid-electro), and all the other nucleic acid species were introduced by lipofection. Cells were also treated with lipfection reagent alone (lipo) or DNA without transfection reagent (DNA only).

(B) Flourescence images of *Atg5*$^+$ and *Atg5*$^-$ BMDMs 4 hr-post transfection with Cy3-labeled plasmid DNA (red) and immunostained with anti-LC3 (green) antibody.

(C) Fluorescence images of BMDMs 4 hr post-transfection with Cy3-labeled plasmid DNA (red) and immunostained with anti-LC3 (green) and anti-ubiquitin (blue) antibodies.

(D) Quantitative analysis of *M. tuberculosis* colocalization with ubiquitin and LC3 at 4 hr post-infection. Results are the means ± SEM of three independent experiments.

(E) Fluorescence images of BMDMs 4 hr post-transfection with Cy3-labeled plasmid DNA (red) and immunostained with anti-K63 and anti-K48 ubiquitin (green) antibodies.

(F) Quantitative analysis of K63 and K48 ubiquitin colocalization with Cy3-labeled plasmid DNA at 4 hr post-transfection. Results are the means ± SEM of three independent experiments.

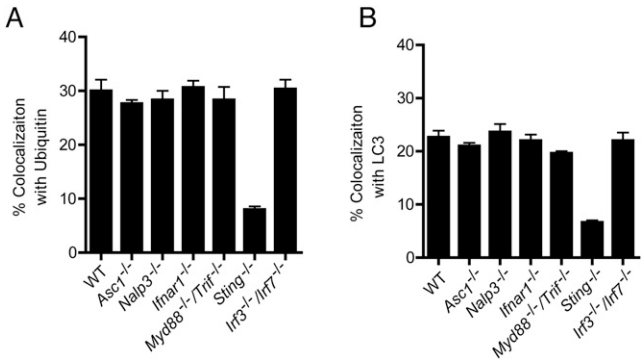


**Figure S5. Quantitative Analysis of *M. tuberculosis* Colocalization with Ubiquitin and LC3 in Various Knockout Macrophages, Related to Figure 5**

BMDMs of the indicated genotype were infected with WT *M. tuberculosis* for 4 hr, and colocalization of *M. tuberculosis* with ubiquitin (A) and LC3 (B) was quantified.