SUPPLEMENTARY INFORMATION

Azithromycin blocks autophagy and predisposes to mycobacterial infection.

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SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Analysis of patient cohorts

The cohort used for the analysis shown in Figure 1A and 1B is the adult CF patients at Papworth Hospital. In Figure 1A (shown in red), we calculated the number of patients per year who were taking long term AZM (expressed as a percentage of the total cohort). Separate from this we looked at the number of individuals (from the same cohort) who were found to have NTM in sputum for the first time per year.

The second analysis, carried out on the Papworth CF cohort (2007-2008), was to compare the number of patients with NTM *disease* (classified according to ATS criteria Griffith et al 2007) who had been taking chronic macrolide therapy at the time or within 6 months of their first positive NTM sputum culture. The chronic macrolide use in this group was compared to that of individuals in 2007-8 (from the same CF centre) who had never had a positive sputum culture for NTM ('NTM-free') and was found to be significantly greater.

Other clinical characteristics -Spirometry (FEV1); body mass index (BMI); sex; the presence of CF-related liver disease (CFRLD), diabetes (CFRD) and asthma (CFRA); allergic bronchopulmonary aspergillosis (ABPA) and gastro-esophageal reflux disease (GERD) were also compared between NTM disease +ve and NTM-free groups and were not found to be significantly different (Supplementary Table 2).

Constructs, antibodies and chemicals

Constructs HD gene exon 1 fragment with 74 polyQ repeats in pEGFP-C1 (Clontech) (EGFP-Q74) construct was previously characterized (1); EGFP-LC3 and mCherry-GFP-LC3 were kind gifts from T. Yoshimori (2,3). The cloning of mCherry-hLC3B has previously been described (4). We are grateful to J.P. Luzio for GFP-lgp120.

Antibodies The following antibodies were used: Rabbit anti-actin polyclonal (Sigma); rabbit anti-LC3 (Novus Biological); mouse monoclonal anti-HA (Covance); mouse monoclonal anti-p62 (BD Biosciences); rabbit polyclonal anti-phospho and total p70S6kinase and rabbit anti-phospho and total S6 ribosomal protein antibodies (Cell Signaling); anti-mouse/rabbit HRP-conjugated secondary antibodies (GE Healthcare).

Chemicals The following chemicals were used (solvents used and providers in brackets): azithromycin (DMSO, SIGMA), bafilomycin A1 (DMSO, Millipore), rapamycin (DMSO, Sigma), 4',6-diamidino-2-phenylindole (Sigma), doxycycline (Sigma), Interferon-γ (PeproTech). All other standard laboratory chemicals were purchased from Sigma, unless otherwise indicated.

Culture of cell lines and generation of human macrophages

HeLa and COS-7 were grown in DMEM supplemented with 10% FBS, 100 U/ml penicillin/streptomycin, 2 mM L-glutamine at 37°C with the addition of G418 (600 μg/ml) for HeLa cells stably expressing the mcherryGFP-LC3 reporter (5). The PC12 A53T α-syn Tet-on inducible cell line (6) was maintained in DMEM, 10 % horse serum, 5% FBS, 100 U/ml penicillin/streptomycin, 2 mM L-glutamine, 75 μg/ml hygromycin B and 100 μg/ml G418. RAW 264.7 cells stably expressing mRFPGFP-LC3 were maintained in RPMI 1640, 10% FBS, 100 U/ml penicillin/streptomycin, 2 mM L-glutamine with G418. Human monocyte-derived macrophages were generated as previously described (7). Briefly PBMC were isolated from peripheral blood obtained from healthy consented subjects (approved by Regional NHS Research Ethics Committee) by Ficoll-Hypaque density separation. Monocytes were extracted by CD14+ positive selection using magnetic beads (Miltenyi) and differentiated into macrophages by 5-10 day culture in RPMI 1640, 10% FCS, 100 U/ml penicillin/streptomycin, 2 mM L-glutamine supplemented with 400 ng/ml M-CSF (Peprotech). Non-adherent cells were discarded prior to experiments.

Autophagy analysis in mammalian cell culture

Assessment of autophagic flux by LC3-II levels with bafilomycin A1 Endogenous LC3-II levels, which directly correlate with autophagosome numbers (2), were detected with anti-LC3 antibody and densitometric analysis relative to actin. To assess autophagic flux, LC3-II was measured in the presence of 400 nM bafilomycin A_1 (treated in the last 4 h), which clamps LC3-II/autophagosome degradation (13). This assay has been established previously with various autophagy modulators (6).

Assessment of autophagic flux by mRFP-GFP-LC3 with an automated Cellomics microscope Counting of autophagosomes and autolysosomes was performed on a Thermo Scientific Cellomics ArrayScan HCS reader (20x objective) using the *Spot detector V3* Cellomics Bioapplication, as previously describe (5). For identification of cells, nuclei were stained with DAPI and detected on the primary channel by a Hoechst-associated filter dependent upon size, shape, and intensity thresholding. GFP-LC3 and mRFP-LC3 vesicles were identified on separate secondary channels using FITC- and Texas Red-associated filters, respectively, dependent upon size, shape, and intensity thresholding. The mean number of vesicles per cell (object) was calculated by the ArrayScan software as the total number of vesicles per field divided by the total number of objects per field. The number of autolysosomes was analyzed by subtracting GFP dots (spot count per object by FITC-associated filter) from mRFP dots (spot count per object by Texas Red-associated filter). 1,000 cells were counted per coverslip, and the analysis was done on triplicate samples at least three times. For experiments using mcherry-GFP-LC3 stably-expressing RAW 264.7 cells, cells were seeded onto coverslip wells (Mattek) in culture media, treated for 24 hours with compounds as described, visualised by live cell confocal fluorescence/DIC imaging (Zeiss LSM 510; images analysed using Velocity software).

Assessment of autophagy by EGFP-LC3 vesicles As previously described (9), COS-7 cells seeded on glass coverslips were transfected with 0.5 μg of EGFP-LC3 using Lipofectamine. Cells were then washed once, cultured and treated in full medium and fixed with 4% paraformaldehyde (Sigma) 24 h post-transfection, mounted in AF-1 Citifluor and analysed, in a blinded fashion, by fluorescence microscopy. 200 cells were analysed and the number of cells with ≥ 10 GFP-LC3 dots counted.

Clearance of mutant A53T α-synuclein As previously described (6), stable inducible PC12 cell lines expressing A53T α-synuclein were induced with 1 μg/ml doxycycline for 48 hours. Transgene expression was switched off by removing the antibiotic from medium and then the cells were treated or not for 24 hours with: rapamycin, a known autophagy inducer, the indicated concentration of azithromycin, or in combination, as indicated. The levels of A53T transgene were then assessed by western blot analysis. Experiments were performed in triplicate and on at least three different occasions.

Quantification of Q74 huntingtin aggregate formation As previously described (8,9), EGFP-HDQ74-transfected HeLa cells were analysed (200 for each condition) by fluorescence microscopy in a blinded fashion for the presence of intracellular (fluorescent) Q74 aggregates. Experiments were performed in triplicate and on at least three different occasions.

Analysis of autophagosome number and autophagosome-lysosome co-localisation For the co-localization analysis, HeLa cells co-transfected with mCherry-hLC3B and GFP-lgp120 were treated as previously described (4), fixed in 4% paraformaldehyde, mounted in Prolong Gold anti-fade solution (Invitrogen) containing DAPI, imaged using a Zeiss LSM 510 confocal microscope and analysed in a blinded fashion using Zeiss LSM Image Browser 3.5 software.

Lysosomal pH measurements

Lysosomes of human monocyte-derived macrophages were loaded with TMR-FITC dextran (Invitrogen; 1h pulse 4h chase). Cells were then treated with compounds as described and visualised by live cell confocal fluorescence/DIC imaging (Zeiss LSM 510; images analysed using Velocity software). Intracellular pH calibration was achieved by clamping lysosomal pH using potassium rich buffer solutions (120 mM KCl, 20 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES with the pH adjusted from 4 to 8) containing the ionophores valinomycin (10 μM), nigericin (10 μM) and the H^+-ATPase inhibitor bafilomycin (400 nM) for 30 min at 37 °C as described (10). Changes in pH were measured by the intensity ratios of tetramethylrhodamine (red) and fluorescein (green) fluorescence in each lysosome.

Phagosomal pH measurements

Human monocyte-derived macrophages were incubated (1h pulse, 23 h chase) with albumin-coated, carboxyfluorescein (FAM)-conjugated 645nm (Far Red) fluorescent carboxlated latex beads (1.75 μm; Polyscience Inc) or patient-derived *M. abscessus* strains which were heat killed and double-labelled with carboxyfluorescein (FAM) and Alexa633 succinimidyl esters (Invitrogen; using manufacturer's instructions). Macrophages were pre-treated for 24 h with compounds as described. Analysis was carrieds out either by live cell confocal fluorescence/DIC imaging (Zeiss LSM 510; images analysed using Velocity software) or flow cytometry by which the FAM-derived fluorescence of cells with internalised particles (identified as Far Red-positive (for beads) or Alexa 633-positive (for mycobacteria) was measured. Intracellular calibration was achieved by incubation with ionophore-containing solutions as described for lysosomal pH calibration above.

Phagosomal degradation assays

Based on previously published assays (11), human monocyte-derived macrophages were incubated (30 min pulse, 2h chase) with ovalbumin(OVA)-conjugated fluorescent latex beads at 4 °C, at 37 °C or at 37 °C with 15 min pre-treatment with lysosomal protease inhibitors (leupeptin and pepstatin; both 1 μM). Macrophages were pretreated with BafA1 or azithromycin (as indicated) for 24 hours

prior to phagocytosis which were continued throughout the experiment. Beads were then recovered by cell disruption (with Triton 1% incubation at 4 ^{o}C for 30 min followed by homogenisation by passage through a hypodermic needle 10 times) in the presence of proteases inhibitors and incubated with OVA-specific biotinylated antibody and then fluorescent streptavidin to visualise OVA bound to beads which was quantifiyed by flow cytometry.

Phagosome-Lysosome fusion assays

Human macrophages were incubated (1h pulse, 4h chase) with dextran conjugated with tetramethylrhodamine and biotin to load lysosomes and then fed IgG-coated streptavidin-conjugated fluorescent latex beads (30 min pulse, 2h chase) with no treatment (control; *black*) or in the presence of azithromycin (AZM; 80 mg/ml; *red*) bafilomycin (BafA1; 400 nM; *blue*). Beads were recovered by cell disruption and the degree of bound fluorescent dextran was quantified by flow cytometry (representative histogram shown, upper panel) and average geometric mean fluorescence (± s.d.) of triplicate samples determined.

In vitro mycobacterial infection assays

Primary human macrophages Luminescent strains of *M. bovis* BCG (*M. bovis* BCG-lux), generated as previously described (12) or *M. abscessus* (ATCC 19977), generated by electroporation with the integration plasmid pmv 310 (kind gift from Dr Bill Jacobs, AECOM) encoding the *Vibrio* lux AB gene. Correlation between colony forming units and luminescence was confirmed prior to experiments. Macrophages, cultured as previously(7), were inoculated with mycobacteria (at MOI of 10:1) for 2 hours at 37 ^{o}C, repeatedly washed and then incubated for 24 h at 37 ^{o}C in the presence or absence of azithromycin or rapamycin (100 nM) as indicated. Cells were lysed and luminescence measured as previously described (14). Experiments were carried out in sextuplicate. Results are representative of 3 separate experiments.

Whole blood mycobacterial infection assays Patient-derived *M. abscessus* strains (speciation confirmed by molecular typing) were grown in Middlebrook 7H9 media (supplemented with ADC) to log phase. Heparinised peripheral blood from healthy consented subjects was mixed 50:50 with RPMI, inoculated in 1 ml aliquots with *M. abscessus* after pre-treatment for 24 h with vehicle alone or azithromycin (at concentrations described) and incubated for 72h at 37 ^{o}C. Viable mycobacteria were quantified after sample lysis (in distilled water) by counting colony forming units following culture on microbial IsoSensitest (Oxoid Limited) plates. A representative experiment is shown. Similar results were obtained with 3 different patient derived *M. abscessus* strains (two smooth and one rough morphotype) and blood from three different healthy subjects. Where indicated, interferon γ (IFNγ 200 ng/ml) or rapamycin (100 nM) were added to blood at the time of infection with mycobacteria.

Induction of macrolide resistance in vitro Where indicated, *M. abscessus-lux* was grown in liquid culture (7H9 + ADC) in the presence of 0.1 μg/ml azithromycin or vehicle alone, washed and resuspended in RPMI with 10% FCS with the indicated concentration of azithromycin. Viable mycobacteria were assessed by measuring luminescence (15) after 24h.

In vivo mycobacterial infection

Mice Specific-pathogen-free female C57BL/6, from 6 to 8 weeks old, were purchased from the Jackson Laboratories, Bar Harbor, Maine and maintained in Biosafety Level III animal laboratories at Colorado State University, and given sterile water, mouse chow, bedding, and enrichment for the duration of the experiments. The specific pathogen-free nature of the mouse colonies was

demonstrated by testing sentinel animals. All experimental protocols were approved by the Animal Care and Usage Committee of Colorado State University.

Bacteria *M. abscessus* strain L948, originally isolated from a patient, was obtained from ATCC (Cat#: 19977). Bacteria were propagated by sequential passage in incremental concentrations of azithromycin (Sigma) in liquid culture media (7H9 + ADC) were washed with PBS and resuspended in 7H9/ADC, 0.05% Tween-80.

Experimental infection and treatment of mice Mice were challenged with *M. abscessus* using a Glas-Col aerosol generator (GlasCol, Inc Terre Haute, IN.) calibrated to deliver a HDA of ~1000 bacilli per animal as previously described (16). On the day after infection enumeration of bacteria was performed on two mice and treatment started on the third day post infection and continued for 27 days. Group of five control saline and five AZM treated (dissolved in autoclaved distilled water from stock solution) was administered by oral gavage 5 days per week (at 0.2 ml per mouse) at a concentration of 100 mg/kg. On days 15 and 30 following infection, bacterial loads in the lungs and spleen, lung and spleen histology, and flow cytometry were determined. Bacterial counts were determined by plating serial dilutions of homogenates of lungs on nutrient 7H11 agar and counting colony-forming units after 3-7 days incubation at 37°C.

Histological analysis Lung tissues were collected for histopathological analysis on days 15 and 30 of the infection. The accessory lobe of the lung was placed in 4% paraformaldehyde in phosphate buffered saline (PBS) for fixation. Paraffin blocks were then prepared and sections were sectioned and stained with hematoxylin and eosin (H&E), and by the Ziehl-Neelsen stain for acid-fast bacilli as previously described (15,16). *Lesion analysis in mice* In mice the concurrent progression of lung lesions a histological grading system was utilized. The method for grading ganulomatous lesions was based on inflammatory cell numbers and their infiltrative distribution pattern in intestine and mesenteric lymph nodes (15). Scoring of lung was based on randomly selected sections in a representative experiment from five infected mice and five non-infected mice at indicated times after infection.

Flow Cytometry Analysis Mice were euthanized by CO_2 asphyxiation, and the thoracic cavity was opened as previously described (Ordway et al., 2006; Ordway et al., 2008). Briefly, the lung was cleared of blood by perfusion through the pulmonary artery with 10 ml of ice cold phosphate-buffered saline (PBS) containing 50 U/ml of heparin (Sigma, St Louis, MO). Lungs were aseptically removed, teased apart and treated with a solution of deoxyribonuclease IV (DNAse) (Sigma Chemical, 30 µg/ml) and collagenase XI (Sigma Chemical, 0.7 mg/ml) for 45 min at 37°C. To obtain a single-cell suspension, the organs were gently passed through cell strainers (Becton Dickinson, Lincoln Park, NJ).The remaining erythrocytes were lysed with Gey's solution (0.15 M NH_4Cl, 10 mM $KHCO_3$) and the cells were washed with Dulbecco's modified Eagle's minimal essential medium. Total cell numbers were determined by flow cytometry using BD™ Liquid Counting Beads, as described by the manufacturer (BD PharMingen, San Jose, CA USA 95131). For flow cytometry analysis, single-cell suspensions of lung from each mice were re-suspended in PBS (Sigma-Aldrich) containing 0.1% of Sodium Azide (PBS+Na/Az). Cells were incubated in the dark for 25 min at 37°C with predetermined optimal titrations of specific antibody (directly conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridin-cholorophyll-protein (PerCP), allophycocyanin (APC), Pacific Blue, Alexa 700); or after biotin antibody incubations washed and incubated for 25 minutes more with streptavidin Qdot800 (Invitrogen), followed by two washes in PBS containing 4% sodium azide. Cell surface markers were analyzed with the following specific antibodies: CD44 (clone IM7), CD4 (clone L3T4), CD8 (clone 53-6.7), CD62L (clone MEL-14), CD11c (clone HL3), CD11b (clone M1/70), DEC205 (clone DC16), all antibodies were purchased from BD PharMingen or eBiosciences (eBioscience, San

Diego, CA). Measurement of intracellular cytokines was conducted by pre-incubating lung cells with monensin (3 µM) (Golgi Stop, BD PharMingen), anti-CD3 and anti-CD28 (both at 0.2 µg/10^6 cells) for 4 h at 37°C, 5% CO_2. The cells were then surface stained, incubated for 30 minutes at 37°C, washed then fixed and permeabilized with Perm Fix/Perm Wash (BD Pharmingen). Finally, the cells were stained with intracellular IFNγ (clone XMG1.2), IL-10 (clone JES5-16E3), IL-12 (clone REM-11) TNFα (clone TB 15), IL-4 (clone 16RP) or its respective isotype controls (BD Pharmingen) for a further 30 min. All the samples were analyzed on a Becton Dickinson LSR-II and data were analyzed using FACSDiva v5.0.1 software. Cells were gated on lymphocytes based on characteristic forward and side scatter profiles. Individual cell populations were identified according to their presence of specific fluorescent-labeled antibodies. All the analyses were performed with a minimum acquisition of 100,000 events.

Supplementary Methods References

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Azithromycin blocks autophagy and may predispose to mycobacterial infection in Cystic Fibrosis patients.

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Supplementary Figure 1. Effect of azithromycin on autophagic clearance in macrophages from CF patients and healthy controls. Levels of LC3-I, LC3-II and p62 in primary monocyte-derived macrophages from healthy controls (*blue*) and clinical stable patients with CF (*green*) following 24h *in vitro* treatment with increasing concentrations of azithromycin (AZM). Representative western blot shown together with all values from individuals (normalised to actin) plotted with means ± s.d. (grey).

Supplementary Figure 2. Azithromycin prevents acidification of phagosomes containing *M. tuberculosis.* Primary human macrophages were incubated with PFA-killed *M. tuberculosis* double-labelled with FITC and Alexa633 for 30 min, washed and then incubated for 4 hours at 37 °C in the presence (*red*) or absence (*black*) of azithromycin 20 μM before cytometric quantification of phagosomal pH. Values for phagosomal pH obtained by in vivo calibration (as described in Supplementary Methods).

Supplementary Figure 3. Azithromycin blocks acidification of phagosomes containing latex beads. Human monocyte-derived macrophages were incubated (1h pulse, 23 h chase) with albumin-coated, (FAM)-conjugated 645nm fluorescent latex beads with no treatment (*Control*) or following 24 h pre-treatment with azithromycin (20 μg/ml; red bars) or $BafA_1$ (100 nM; blue bars). Cells were then analysed by flow cytometry. Representative histogram (top panel) and average (± s.d.) FAM-derived geometric mean fluorescence values (bottom panel) are shown for one out of 5 similar experiments.

Supplementary Figure 4.
Phagosomal degradation monitored by ovalbumin-coated fluorescent latex beads. Human monocyte-derived macrophages were incubated (30 min pulse, 2h chase) with ovalbumin(OVA)-conjugated fluorescent latex beads at 4 °C (*filled light grey*), at 37 °C (*dark grey*) or at 37 °C after 15 min pre-treatment with lysosomal protease inhibitors (leupeptin and pepstatin; both 1 μM; *green*). Beads were then recovered by cell disruption and the amount of bound OVA remaining was detected by OVA-specific antibody and quantified by flow cytometry Representative of 3 experiments.

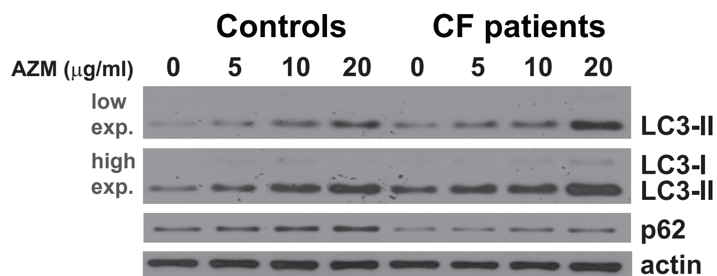
Supplementary Figure 5.
Effect of azithromycin on cytokine release from human macrophages. Levels of TNFα detected in supernatant 24h after addition of LPS (1 μg/ml) or heat killed *E. coli* to human macrophages pre-treated with azithromycin (red) or vehicle alone (black).

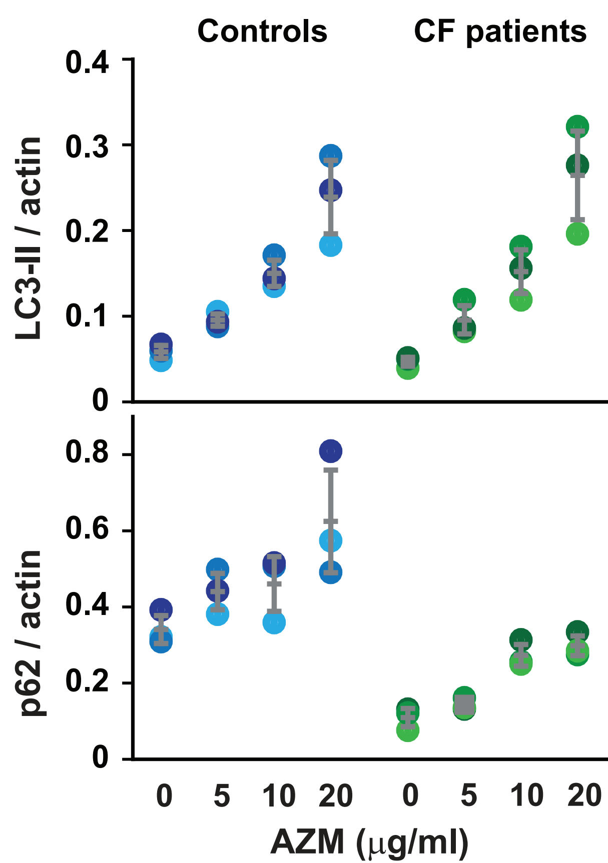
Supplementary Figure 6.
Control of intracellular growth of *M. abscessus* with human macrophages is autophagy-dependent. (A) The autophagy component ATG12 was knocked down (using Dharmacon smart pool siRNA introduced by electroporation) in primary human macrophages resulting in reduced levels of LC3-II and accumulation of p62 (monitored by western blot) compared to control siRNA (c)-treated cells confirming a block in autophagy. **(B)** Viable intracellular *M. abscessus* in control (*black*) or ATG12 (*blue*) siRNA-treated human macrophages at 24 and 48h following infection (normalised to 24h levels) showing increased intracellular growth in cells where autophagy has been blocked.

Supplementary Table 1 Annual sputum culture sampling of adult Cystic Fibrosis patients attending Papworth Hospital Unit. The table documents (*top line*) the number of individuals who had at least one sputum sample analysed for non-tuberculous mycobacteria (NTM; AFB-smear and culture) from the patient group not known to be infected with NTM (undiagnosed patients). The average number of such sputum samples analysed (per undiagnosed patient) is also shown (*bottom line*). The observed rise in sputum cultures positive for NTM (Figure 1a) cannot be ascribed to changes in sampling.

Supplementary Table 2 Other clinical characteristics for the above cohort - FEV1; BMI; sex; the presence of CF-related liver disease (CFRLD), diabetes (CFRD) and asthma (CFRA); allergic bronchopulmonary aspergillosis (ABPA) and gastro-esophageal reflux disease (GERD) were also compared between NTM disease +ve and NTM-free groups and were not found to be significantly different. Patients with NTM-disease were statistically younger. Logistic regression analysis confirmed age as the only significant co-variate ($p = 0.01$). When adjusted for age, AZM was still significantly associated with NTM disease (deviance test P value 0.00046; OR 9.8; 95% CI = 2.09-45.87).

Supplementary Figure 1





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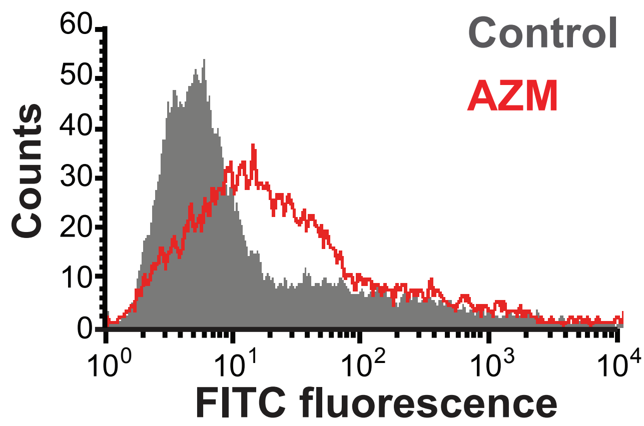
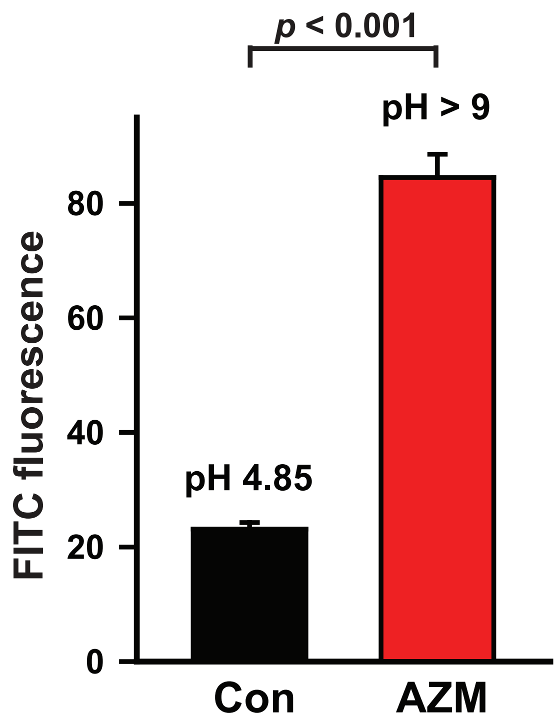
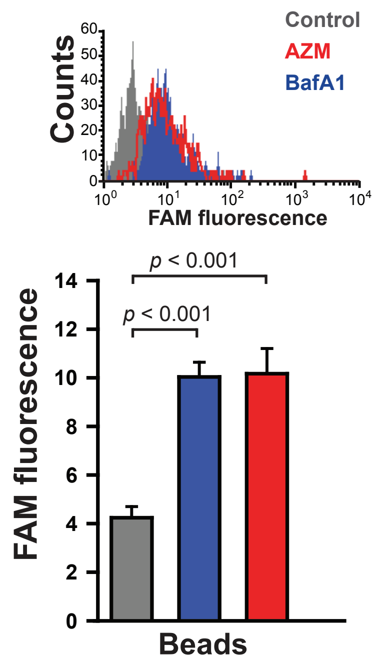
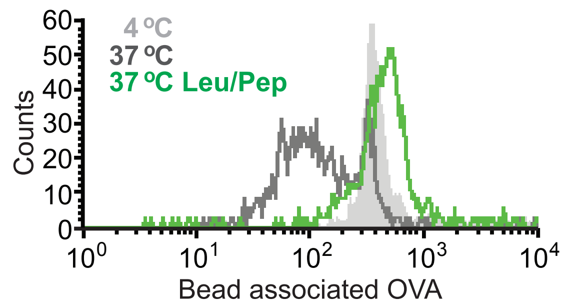




Supplementary Figure 3



Supplementary Figure 4



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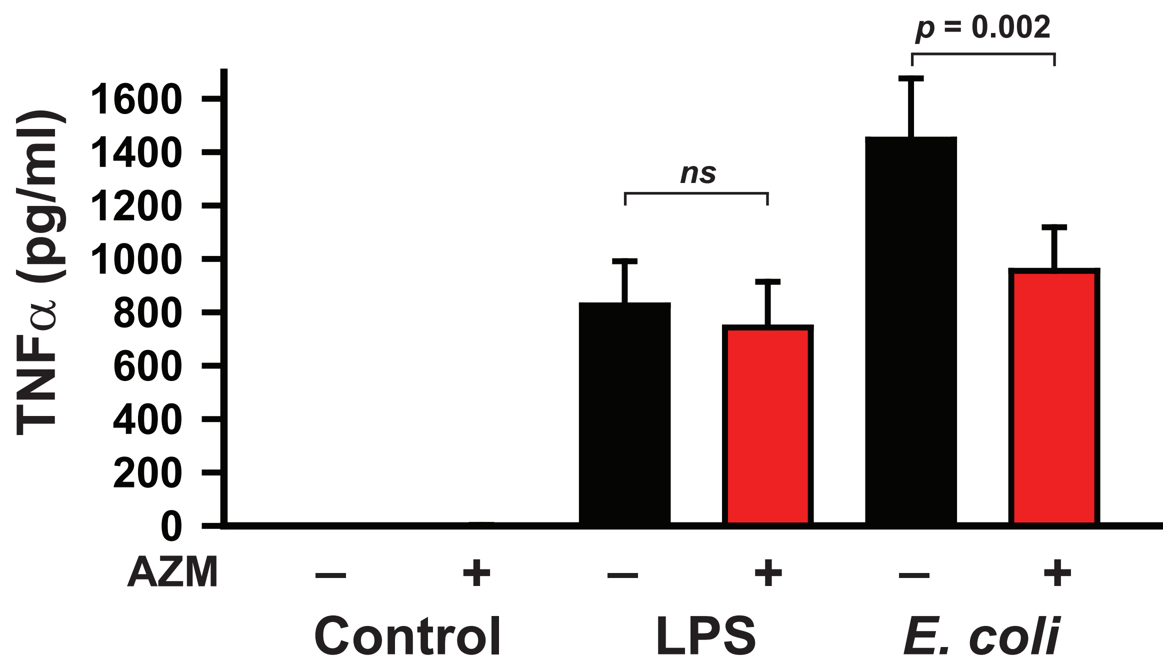
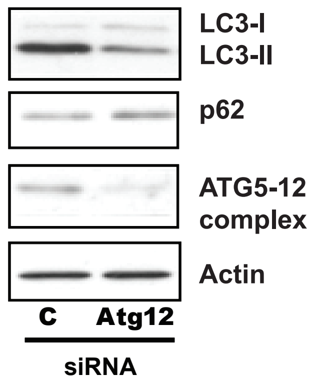
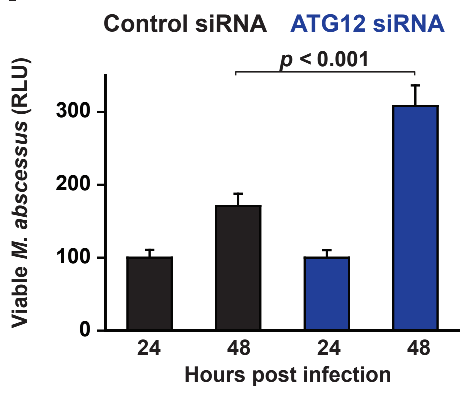


Supplementary Figure 6

A.



B.



Supplementary Table 1

	2004-5	2005-6	2006-7	2007-8
Undiagnosed patients sampled	121/162 (74.7%)	123/169 (72.8%)	127/180 (70.6%)	121/162 (70.8%)
Annual samples per undiagnosed patients	1.52	1.37	1.44	1.73

Supplementary Table 2

A.

Age	Sex	Genotype	BMI	NTM species	FEV1 (%)	Azithromycin use	Colonising bacteria	Comorbidities
19	F	ΔF508/ΔF508	20	*M. abscessus*	46	N	PsA	CFRLD CFRD
19	M	ΔF508/ΔF508	22	*M. abscessus*	31	Y	PsA	CFRA CFRD
18	F	ΔF508/ΔF508	18.5	*M. abscessus*	57	Y	PsA	CFRD
18	M	ΔF508/ΔF508	17.9	*M. abscessus*	59	Y	StaphA	CFRD CFRA
30	M	ΔF508/ΔF508	22	*M. abscessus*	43	Y	PsA StaphA	
24	F	Not known	22.7	*M. abscessus*	62	Y	PsA StaphA	CFRA ABPA
17	F	ΔF508/ΔI507	24	*M. abscessus*	82	Y	PsA StaphA	CFRD
20	M	Not known	19.7	*M. abscessus*	54	Y	PsA StaphA	GERD
17	F	ΔF508/ΔF508	20	*M. abscessus*	53	Y	PsA StaphA	CFRLD
29	M	ΔF508/N1303K	16.7	*M. abscessus*	29	Y	PsA	CFRLD
18	M	ΔF508/ΔF508	21.6	*M. abscessus*	46	Y	PsA StaphA	CFRLD
40	M	ΔF508/R117H	21	*M. kansasii*	54	Y	StaphA	
22	M	Not known	18	*M. avium*	51	N	PsA	
21	F	Not known	21	*M. avium*	48	Y	StaphA	CFRD

B.

	NTM-free (n = 184)	NTM disease (n = 14)	*p value*	*p value* logistic regression model
Age	27.1 ± 8.8	22.3 ± 6.6	0.006	0.0118
FEV1 (%)	60.5 ± 27.6	51.1 ± 13.0	0.245	0.719
BMI	21.4 ± 3.3	20.4 ± 2.1	0.261	0.251
Male Sex	96 (52.2%)	8 (57.1%)	0.720	0.343
Colonised with PsA	122 (66.3%)	11 (78.6%)	0.346	0.195
CFRLD	45 (24.5%)	4 (28.6%)	0.731	0.735
CFRD	54 (29.3%)	6 (42.9%)	0.291	0.303
CFRA	30 (16.3%)	3 (21.4%)	0.620	0.631
ABPA	19 (10.3%)	1 (7.1%)	0.703	0.690
GERD	11 (6.0%)	1 (7.1%)	0.860	0.939