The ubiquitin ligase parkin mediates resistance to intracellular pathogens

Paolo S. Manzanillo1, Janelle S. Ayres2*, Robert O. Watson1*, Angela C. Collins3, Gianne Souza1, Chris S. Rae4, David S. Schneider5, Ken Nakamura6,7, Michael U. Shiloh3 & Jeffery S. Cox1

Ubiquitin–mediated targeting of intracellular bacteria to the autophagy pathway is a key innate defence mechanism against invading microbes, including the important human pathogen Mycobacterium tuberculosis. However, the ubiquitin ligases responsible for catalysing ubiquitin chains that surround intracellular bacteria are poorly understood. The parkin protein is a ubiquitin ligase with a well-established role in mitophagy, and mutations in the parkin gene (PARK2) lead to increased susceptibility to Parkinson’s disease. Surprisingly, genetic polymorphisms in the PARK2 regulatory region are also associated with increased susceptibility to intracellular bacterial pathogens in humans, including Mycobacterium leprae and Salmonella enterica serovar Typhi, but the function of parkin in immunity has remained unexplored. Here we show that parkin has a role in ubiquitin–mediated autophagy of M. tuberculosis. Both parkin–deficient mice and flies are sensitive to various intracellular bacterial infections, indicating parkin has a conserved role in metazoan innate defence. Moreover, our work reveals an unexpected functional link between mitophagy and infectious disease.

Eukaryotic cells target invading microbes to autophagosomes through a process termed xenophagy, which has a key role in innate immune defence. Various intracellular bacterial pathogens, including Mycobacterium tuberculosis, are targeted for xenophagy through a ubiquitin-mediated pathway that surrounds bacteria with conjugated ubiquitin chains1–3. Marking with polyubiquitin presumably recruits ubiquitin-binding autophagy adaptors such as p62, which in turn engage the autophagic machinery for autophagosome formation and delivery of bacteria to the lysosome4–6. Although ubiquitin–binding adaptors are required for xenophagy, whether ubiquitin itself directly mediates bacterial autophagy is not clear because the identities of the ubiquitinated substrate(s) and ligase(s) responsible for coating cytosol-exposed bacteria are poorly understood.

In a fashion similar to xenophagy, the process of mitophagy eliminates damaged mitochondria through ubiquitin-mediated targeting to autophagosomes. A key step in mitophagy is marking of damaged mitochondria by the ubiquitin ligase parkin, which localizes to the organelle and directly ubiquitinates proteins on the mitochondrial surface. Ubiquitin-tagged mitochondria are directed to the autophagosome pathway by p62 (refs 6–8) and several other factors9, ultimately delivering the organelle to the lysosome.

PARK2 mutations in humans are well-known risk factors for the development of Parkinson’s disease, but polymorphisms in the regulatory region of PARK2, some of which result in decreased parkin expression6, have been associated with increased susceptibility to the intracellular pathogens Mycobacterium leprae and S. enterica serovar Typhi10,11. Although a genetic link to increased infection risk has been identified, the function of parkin in immunity has remained obscure. We have identified that parkin, similar to its role in mitophagy, is also important for innate defence against M. tuberculosis and other intracellular pathogens by promoting xenophagy. This work provides a possible mechanism underlying the human genetic studies linking parkin to increased susceptibility to bacterial infection and reveals a surprising connection between mitochondrial homeostasis and pathogen defence.

Parkin in TB–ubiquitin co–localization

We have shown previously that on infection of macrophages, M. tuberculosis bacilli that puncture phagosomal membranes through their ESX-1 secretion system gain access to the host cytosol but become enveloped by conjugated ubiquitin chains and are targeted to autophagosomes by p62 and NDP52 (ref. 3). Although the role of ESX-1 in autophagy induction is probably complicated12, it is clear that approximately one-third of wild-type intracellular bacteria are targeted to autophagy during macrophage infection and that this has a major role in host resistance to infection2,3. Because of the commonalities between mitophagy and autophagy of intracellular mycobacteria, and the links between PARK2 polymorphisms and increased susceptibility to bacterial infection in humans, we speculated that parkin may also be recruited to M. tuberculosis-containing phagosomes and target them for ubiquitin-mediated autophagy. Indeed, after infection of murine bone marrow-derived macrophages (BMDMs) with M. tuberculosis expressing mCherry, we found that parkin localized to approximately 12% of wild-type M. tuberculosis phagosomes but not to ESX-1 mutants (Fig. 1a, Extended Data Fig. 1). Next, we infected BMDMs isolated from wild-type and Park2−/− mice and performed immunofluorescence co-localization experiments using antibodies that recognize polyubiquitin. As shown in Fig. 1b, c, Park2−/− BMDMs were severely defective for M. tuberculosis ubiquitin co-localization as compared to control macrophages, resulting in a significant reduction in ubiquitin-positive mycobacteria. Likewise, short hairpin RNA (shRNA) knockdown of parkin expression in human macrophage cell lines also resulted in a drastic reduction in ubiquitin

1Department of Microbiology and Immunology Program in Microbial Pathogenesis and Host Defense, University of California, San Francisco, San Francisco, California 94158, USA. 2Immunobiology and Microbial Pathogenesis Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037, USA. 3Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA. 4Department of Molecular and Cellular Biology, Division of Immunology and Pathogenesis, University of California, Berkeley, California 94720, USA. 5Department of Microbiology and Immunology, Stanford University, Stanford, California 94305, USA. 6Gladstone Institute of Neurological Disease, University of California, San Francisco, California 94158, USA. 7Department of Neurology and Graduate Programs in Neuroscience and Biomedical Sciences, University of California, San Francisco, California 94158, USA.

*These authors contributed equally to this work.
that parkin and its E3 ligase activity are critical for the co-localization of ubiquitin with M. tuberculosis during infection.

Parkin mediates K63-linked polyubiquitin

We showed previously that both K63- and K48-linked polyubiquitin chains accumulate around M. tuberculosis. Because parkin is known to catalyse K63-linked ubiquitin chains,15,17, we sought to determine the nature of the residual ubiquitin surrounding M. tuberculosis in Park2<sup>−/−</sup> BMDMs. Using ubiquitin linkage-specific antibodies,5,18, we found that in wild-type BMDMs, approximately 26–29% of all intracellular bacteria (~90–95% of all ubiquitin-positive bacilli) co-localized with K63 ubiquitin, whereas only 5–7% bacilli stained for K48 (Fig. 2a, b). Additionally, expression of HA-epitope-tagged forms of K48 and K63 ubiquitin within BMDMs supported the notion that K63-linked polyubiquitin is more abundant surrounding M. tuberculosis than the K48-linked form (Extended Data Fig. 2). In Park2<sup>−/−</sup> BMDMs, however, there was a specific decrease in the number of K63-positive mycobacteria, whereas the K48-positive population remained unaffected (Fig. 2a, b, Extended Data Fig. 2). Previous electron microscopy studies indicated that though ubiquitin can localize directly with M. tuberculosis, the majority of ubiquitin is found permeabilizes the plasma membrane and leaves phagosomes intact.
with luminal contents inaccessible to antibodies10,22 (Extended Data Fig. 3a). As shown in Extended Data Fig. 3b, antibodies against poly-ubiquitin and K63-ubiquitin stained digitonin-permeabilized cells and resulted in co-localization with mCherry-expressing M. tuberculosis. Importantly, anti-M. tuberculosis antibodies failed to stain M. tuberculosis within digitonin-permeabilized cells and stained cells only after addition of Triton X-100 detergent, demonstrating that digitonin-permeabilized cells contained intact phagosomes (Extended Data Fig. 3b, c). Taken together, these data suggest that parkin facilitates the linkage of K63-linked ubiquitin chains surrounding M. tuberculosis containing phagosomes, although the exact protein target(s) remain to be explored. Furthermore, these data also suggest that at least one other ubiquitin ligase works independently of parkin to catalyse the K48-linked ubiquitination that surrounds a minor population of M. tuberculosis cells.

**Parkin required for TB autophagy**

Ubiquitination coincides with autophagic targeting of M. tuberculosis, but a causal relationship has not been demonstrated. To determine whether parkin-mediated ubiquitination directs autophagic targeting of M. tuberculosis, we infected wild-type and Park2−/− macrophages with M. tuberculosis and measured co-localization of bacilli with multiple markers of autophagy. Microscopy analysis of proteins involved in ubiquitin recognition (NBR1, NDP52, p62, phospho-TBK1) revealed reduced co-localization with M. tuberculosis in Park2−/− macrophages (Fig. 2c, d), suggesting that parkin-mediated ubiquitination directly leads to the recruitment of the proximal ubiquitin-adaptors that facilitate autophagic targeting of mycobacteria. Likewise, mycobacterial cells within infected Park2−/− BMDMs had reduced co-localization with autophagic proteins LC3 and ATG12 relative to infection of wild-type BMDMs (Fig. 3a, b), suggesting that the K63-linked poly-ubiquitin catalysed by parkin is required for delivery of M. tuberculosis to autophagosomes. Consistent with this notion, Park2−/− cells were defective in conversion of LC3 to its activated, lipiddated form, LC3-II, during M. tuberculosis infection, further demonstrating that parkin is required for autophagy of mycobacteria (Fig. 3c).

**Parkin limits TB replication**

The autophagy pathway serves to limit M. tuberculosis replication in macrophages by delivering bacilli to the lysosome3,11. To determine whether parkin-mediated ubiquitination is required for autophagic targeting of M. tuberculosis to lysosomes, we infected BMDMs with M. tuberculosis and monitored co-localization with the lysosomal marker, LAMP1. During M. tuberculosis infection of wild-type BMDMs, approximately 30% of bacilli stained positive for LAMP1 at 6 h post-infection (Fig. 3d, e). In contrast, only 2–5% of bacilli co-localized with LAMP1 during M. tuberculosis infection of Park2−/− macrophages. This was similar to macrophages deficient for the essential autophagy protein, ATG5 (Fig. 3d, e)15. To test whether these differences led to changes in bacterial survival, we infected Park2−/− and Atg5−/− BMDMs with wild-type M. tuberculosis and determined bacterial viability by enumerating colony-forming units (c.f.u.). Infection of BMDMs deficient for either ATG5 or parkin resulted in a 2- or a 2.5-fold increase in bacterial numbers, respectively, relative to control BMDMs by 12 h post-infection (Fig. 3f). Conversely, overexpression of parkin in RAW 264.7 macrophages led to decreased bacterial replication (Fig. 3g). Importantly, knockdown of parkin expression in human U937 cells also led to an increase in bacterial replication during infection (Fig. 3h). Taken together, our data demonstrate that parkin-mediated ubiquitination leads to the autophagic targeting of M. tuberculosis and is essential for inhibition of mycobacterial replication in macrophages.

**Parkin mediates M. tuberculosis immunity**

Polymorphisms within the regulatory region of PARK2 in human populations have been identified as a common risk factor for increased susceptibility to Mycobacterium leprae and salmonellae infections6,10,11, suggesting that parkin has an important role in vivo against a broad range of intracellular bacterial infections. We began to test this by first determining whether parkin was required in vivo during M. tuberculosis infection of mice. We performed a low-dose aerosol infection of wild-type and Park2−/− knockout mice and determined mouse survival and bacterial burden within infected tissues. In comparison to infected wild-type mice, Park2−/− knockout mice had a tenfold increase in bacterial c.f.u. within infected lungs, spleens and liver by 21 days post-infection (Fig. 4a, b). Furthermore, survival studies revealed that Park2−/− mice were extremely susceptible to M. tuberculosis, because all infected mice succumbed to overwhelming infection by 85 days post-infection, whereas all infected wild-type mice remained alive and displayed no overt signs of weight loss or stress (Fig. 4c). Immunohistochemistry staining of infected mouse lungs revealed robust parkin expression in mouse granulomas within the central macrophage-containing zone (Fig. 4d). In agreement with our mouse experiments, we also observed high expression of parkin within human lung granuloma tissue samples from M. tuberculosis-infected patients (Fig. 4e, Extended Data Fig. 4). Further analysis of human lung specimens by confocal microscopy revealed the presence of parkin puncta within M. tuberculosis-infected cells as well as in vivo co-localization of parkin with M. tuberculosis (Fig. 4f). Park2−/− mice were also highly susceptible to another intracellular pathogen, Listeria monocytogenes, resulting in 10–20-fold higher bacterial burdens relative to wild-type
mice within infected spleens and liver (Fig. 4g). Taken together, these data demonstrate that parkin is essential in vivo for controlling intracellular bacterial pathogens within mice and suggest a role for parkin in human tuberculosis disease.

Conserved role of parkin in immunity

Park2 is present in all metazoans\(^{23}\), including Drosophila melanogaster and Caenorhabditis elegans, with well-characterized functions in mitochondrial maintenance and in models of Parkinson’s disease. Because xenophagy of intracellular pathogens is a highly evolutionarily conserved innate immune defence mechanism\(^{24}\), we sought to determine whether parkin also has an evolutionarily conserved role in immunity within non-mammalian organisms. We began by first analysing parkin-deficient D. melanogaster strains using models of bacterial systemic infection. We obtained two mutant fly lines with independent disruptions of the parkin gene (park\(^{c00062}\), park\(^{c01950}\)) and infected them with L. monocytogenes, which has previously been shown to induce autophagy within flies\(^{25,26}\). In contrast to wild-type infected flies, parkin mutants were severely defective in ATG8/LC3 processing during infection (Fig. 5a), suggesting that parkin has a role in autophagic immunity within flies. Consistent with our results in mice, parkin mutant flies were also highly susceptible to L. monocytogenes infection and led to 10–50-fold increases in bacterial burdens relative to wild-type infected flies (Fig. 5b). This was accompanied by decreased survival, with a median lifespan of two days following infection (Fig. 5c). In addition, parkin mutant flies were also susceptible to other autophagy-inducing intracellular pathogens such as S. enterica serovar Typhimurium and M. marinum. By 9 h post-infection, parkin-deficient flies had a tenfold increase in S. enterica serovar Typhimurium burden relative to wild-type flies (Fig. 5d). Moreover, parkin mutant flies had significantly decreased lifespans upon infection with either S. enterica serovar Typhimurium (Fig. 5e) or M. marinum (Fig. 5f). C. elegans strains deficient in the parkin homologue (pdr-1) were also highly susceptible to S. enterica serovar Typhimurium infection (data not shown). Altogether, our data show that parkin homologues within metazoans are required for proper immune response to infection, suggesting an evolutionarily conserved role in innate immunity.

Discussion

Our findings reveal that parkin regulates a common cellular program by which metazoans mediate quality control of endogenous mitochondria (self) and eradicate harmful bacterial pathogens (non-self). Although these two activities are seemingly disparate, the evolutionary origin of mitochondria from a bacterial endosymbiont suggests that perhaps mitochondrial dysfunction triggers the recognition of the organelle as non-self. For example, mitochondria (and bacterial endosymbionts\(^{27}\)) may actively evade parkin surveillance, but these inhibitory processes are overridden on organelle damage. Alternatively, several studies have shown
that damaged mitochondria may serve as a ‘danger-associated molecular pattern’, resulting in the activation of several innate immune receptors such as Toll-like receptors and the NLRP3-inflammasome complex. In view of recent studies showing that intracellular infection with several pathogens such as L. monocytogenes result in altered host mitochondria dynamics, it is tempting to speculate that damaged mitochondria may serve as a signal for intracellular infection and activation of xenophagy.

Our results also provide a molecular explanation for increased bacterial susceptibility of humans with polymorphisms in the PARK2 regulatory region, broadening the role of parkin beyond mitochondrial homeostasis. Indeed, several recent studies have shown parkin to participate in a wide array of cellular processes such as apoptosis, regulation of lipid metabolism and cytokine production on infection, all of which may contribute to the in vivo importance of parkin in immunity. This work highlights the unexpected connection between mitochondrial-based neuronal disorders and susceptibility to bacterial infection in humans. Recent genome-wide association studies on inflammatory bowel disease, which is linked to altered host-gut microbe interactions, have identified susceptibility single nucleotide polymorphisms with LRRK2 and PARK7, two genes canonically associated with Parkinson’s disease. We surmise that genes typically associated with neuronal maintenance or mitophagy may have broad roles in cellular homeostasis within various cell types.

METHODS SUMMARY

Macrophages were infected with M. tuberculosis (Erdman strain) using a ‘spinfection’ protocol in which monolayers were overlaid with bacterial suspensions, centrifuged for 10 min at 183 × g, and extensively washed to remove extracellular bacteria. Park2−/− macrophages expressing parkin constructs were generated by transducing marrow cells from knockout mice with lentivirus, followed by differentiation into macrophages. During day 3 of differentiation, cells were selected with 5 µg/ml of puromycin. For immunofluorescence, macrophages were cultured on coverslips, infected at a multiplicity of infection (MOI) of 1 and fixed in 4% paraformaldehyde before permeabilization and incubation with antibodies.

For all co-localization studies were blinded, with a minimum count of 200 cells per experiment. Any additional Methods, Extended Data display items and Supplementary Information are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.S.C. (jeffery.cox@ucsf.edu).

Received 30 November 2012; accepted 15 August 2013.
Published online 4 September 2013.

Acknowledgements We thank N. Mizushima, S. Cherry, and K. Huyhn for mice and reagents. We are grateful to S. Johnson for use of his microscope, members of the Schneider laboratory for assistance with fly work and D. Portnoy, R. Vance and S. Virgin for helpful discussions. This work was supported by National Institutes of Health grants R01 AI081727, P01 AI063302 and R01 AI099435 and NINDS P30NS069496 to K.N.

Author Contributions A.C.C. and M.U.S. performed immunohistochemistry staining of tissues and confocal microscopy of human lungs. P.S.M., C.S.R. and G.S. performed Listeria infections. J.S.A. performed all experiments involving Drosophila melanogaster. R.O.W. performed fluorescence microscopy experiments. P.S.M. performed all experiments involving M. tuberculosis. K.N. and D.S.S. provided reagents and resources. P.S.M. and J.S.C. conceived the study, designed the experiments and wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests.

Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.S.C. (jeffery.cox@ucsf.edu).
Mice and macrophages. Park2−/− mice on the C57BL/6 background were a gift from K. Nakamura (Gladdstone Institute)12,19. GFP−Lc3 transgenic mice were purchased from Jackson Laboratories. BMDMs were obtained from mouse femurs as previously described1 and cultured for 7 days in MDM supplemented with 10% horse serum. Media was refreshed every 3 days. Macrophages were washed twice with PBS, gently sonicated to disperse clumps and resuspended in MDM supplemented with 10% horse serum. Media was removed from cells, monolayers overlaid with the bacterial suspension and centrifuged for 1 min at 183g. Cells were washed twice in PBS and returned to macrophage media. For determination of bacterial viability following infection, cells were lysed in 1% Triton X-100 and plated on 7H10 solid medium.

**Western blotting.** Protein lysates from cells and flies were obtained by lysis in RIPA buffer (Sigma) at the indicated time points. A micro BCA protein kit (Pierce) was used to measure protein levels and equal amounts of protein were separated, transferred and visualized using antibodies specific to humanized rabbit macrophage antibodies specific for K63 and K48 (gift from E. Brown laboratory at Genentech).

**Immunohistochemistry of mouse specimens.** Paraffin-embedded specimens were deparaffinized in xylene, subjected to heat-mediated antigen-retrieval in 10 mM sodium citrate (pH 6.0), permeabilized in 0.2% Triton X-100 and placed on 3% H2O2 in methanol. Western blots were analysed using Odyssey Imager (Lico) according to the manufacturer’s instructions. Western blot figures are representative of at least two independent experiments.

**Immunofluorescence.** Infected cells were immunostained and visualized as previously described1. Briefly, macrophages were seeded onto poly-Lysine-coated coverslips and infected with M. tuberculosis as described above. Cells were infected at an m.o.i. of 1, and fixed in 4% paraformaldehyde for 20 min at the indicated time points. Cells were incubated with indicated primary antibodies for 2 h at room temperature in 5% milk and 0.05% saponin, and were visualized using secondary Alexa-Fluor488 antibodies. For parkin immunofluorescence, cells were stained using rabbit polyclonal anti-parkin (Abcam 15954) and an HRP-conjugated donkey anti-rabbit secondary (Abcam 15002) and detected using DAB reagent (Thermo Scientific). Images were acquired using a Zeiss Axiosplan 2 microscope.

**Immunohistochemistry and immunofluorescence of human specimens.** Human lung biopsy specimens were obtained from patients with active tuberculosis. Paraffin-embedded specimens were deparaffinized in xylene, subjected to heat-mediated antigen-retrieval in 10 mM sodium citrate (pH 6.0), permeabilized in 0.2% Triton X-100 (Sigma), treated with mouse on mouse blocking reagent (Vector Laboratories) and blocked in 5% donkey serum. Parkin was detected using a mouse monoclonal anti-parkin antibody (Cell Signal #4211) (1:50) and an HRP-conjugated donkey anti-rabbit secondary (1:250, Jackson Immunochemicals), amplified with AB reagent (Vectastain) and detected using DAB reagent (Thermo Scientific). Images were acquired using a Zeiss Axiosplan 2 microscope. For immunofluorescence, paraffin was identified using rabbit polyclonal anti-parkin (Abcam15954) at 1:25 and an HRP-conjugated donkey anti-rabbit secondary (1:250, Jackson Immunochemicals), amplified with AB reagent (Vectastain) and detected using DAB reagent (Thermo Scientific). Images were acquired using a Leica TCS SP5 confocal microscope. All human tissue specimens were obtained with consent. This study of human tissue specimens has been exempted under 15 CFR 46.101(b) and was approved by the Institutional Review Board at the University of Texas Southwestern Medical Center.

**REFERENCES**

36. Goldberg, M. S. et al. Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. J. Biol. Chem. 278, 43628–43635 (2003).
37. Ohol, Y. M. et al. Mycobacterium tuberculosis MycP1 protease plays a dual role in regulation of ESX-1 secretion and virulence. Cell Host Microbe 7, 210–220 (2010).
38. Thibault, S. T. et al. A complementary transposon tool kit for Drosophila melanogaster using P and piggyBac. Nature Genet. 36, 283–287 (2004).
39. Ayres, J. S. & Schneider, D. S. A signaling protease required for melanization in Drosophila affects resistance and tolerance of infections. PLoS Biol. 6, e905 (2008).
Extended Data Figure 1 | Quantification of parkin co-localization and effect of LRSAM1 knockdown in BMDMs. a, Quantification of parkin-positive M. tuberculosis in BMDMs from wild-type and Park2−/− mice, from Fig. 1a. b, BMDMs from LC3–GFP transgenic mice were transduced with lentivirus expressing either a scrambled shRNA control (Ctrl) or shRNAs targeting either LRSAM1 or parkin. Lentiviral transduced cells were then infected with mCherry-expressing M. tuberculosis and the co-localization of GFP–LC3 and ubiquitin was quantified by immunofluorescence. *P < 0.014, **P < 0.008 by Student’s t-test. c, Quantitative PCR with reverse transcription (RT–qPCR) expression of LRSAM1 and parkin transcripts in lentiviral transduced cells from a. Data shown are expressed relative to actin expression. *P < 0.033, **P < 0.0035 by Student’s t-test.
Extended Data Figure 2 | Co-localization of HA–ubiquitin species during M. tuberculosis infection. a, Wild-type BMDMs were transduced with lentivirus expressing HA-tagged constructs of wild-type ubiquitin (WT), ubiquitin with all lysine residues mutated to arginine except for lysine 63 (K63), or ubiquitin with all lysine residues mutated to arginine except for lysine 48 (K48). Transduced cells were then infected with mCherry-expressing M. tuberculosis and immunostained using anti-HA antibodies 4 h post-infection. b, Quantification of HA-ubiquitin co-localization with M. tuberculosis from a. **P < 0.001 by Student’s t-test.
Extended Data Figure 3 | Digitonin permeabilization of BMDMs.

a. Cartoon model showing digitonin differential permeabilization of macrophages and antibody accessibility to phagosomes. b. Microscopy images of wild-type BMDMs were infected with mCherry-expressing *M. tuberculosis*. Cells were immunostained by digitonin permeabilization alone or digitonin permeabilization with Triton X-100 treatment. c. Quantification of ubiquitin co-localization with *M. tuberculosis* from b. N.D., not determined.
Extended Data Figure 4 | Immunohistochemistry analysis of parkin within human patients with active tuberculosis. Lung biopsy samples were obtained from three different human patients with active tuberculosis. Immunohistochemistry was performed on specimens using either anti-parkin, anti-*M. tuberculosis* or an IgG control antibody. Positive cells were visualized by DAB staining. Scale bar, 100 μm.