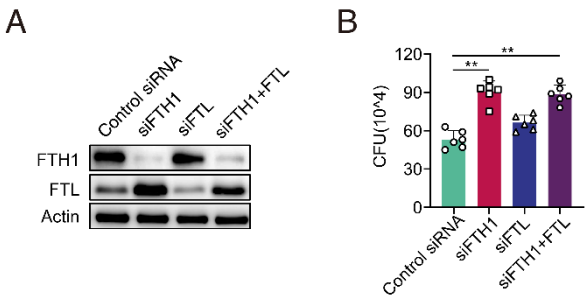
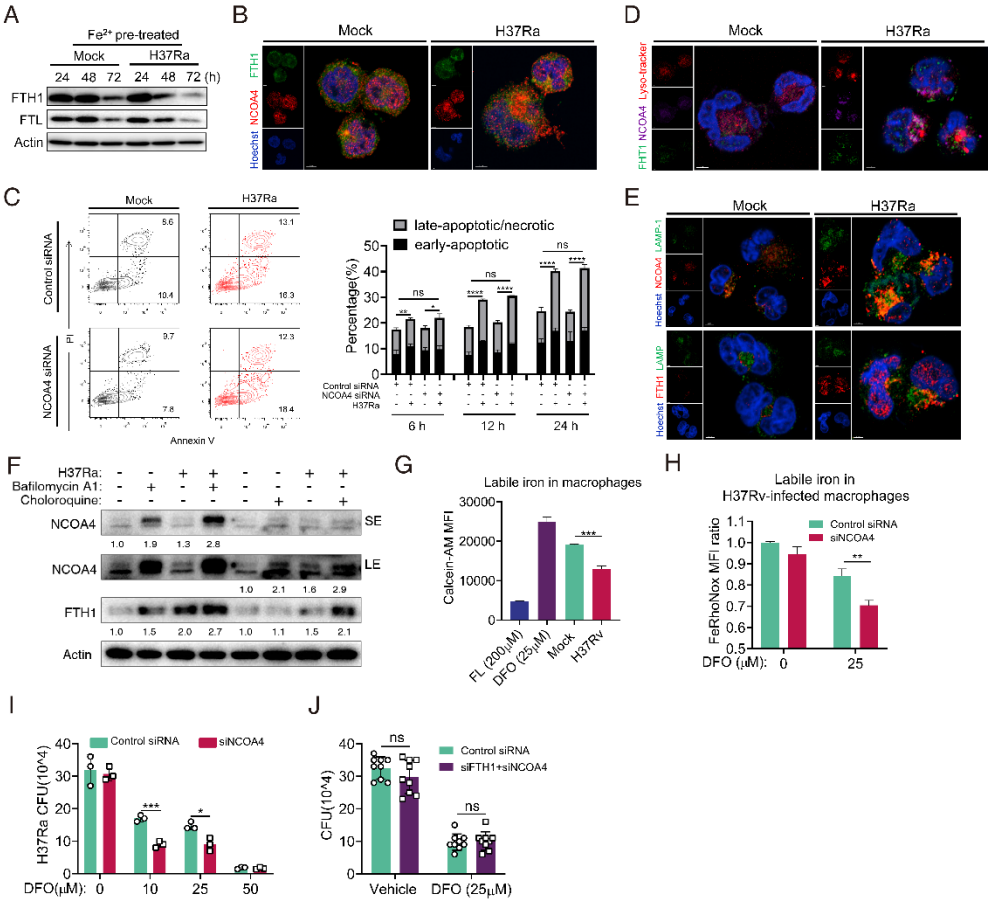
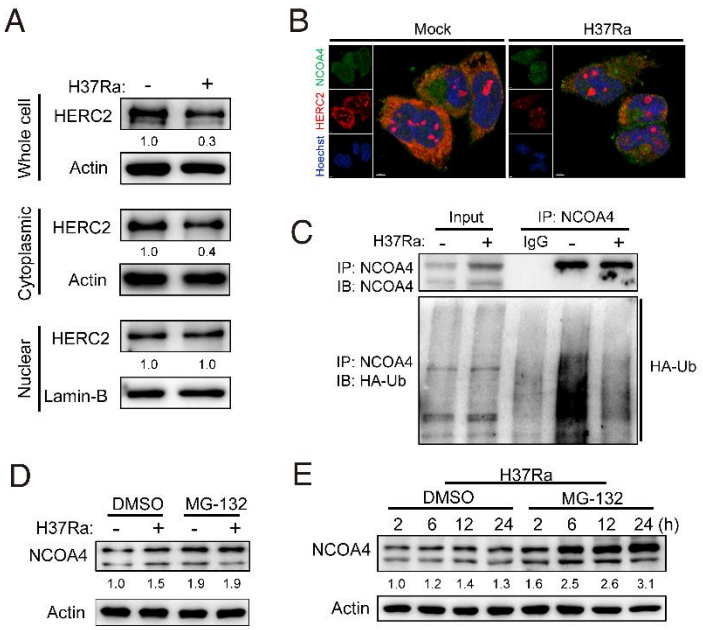


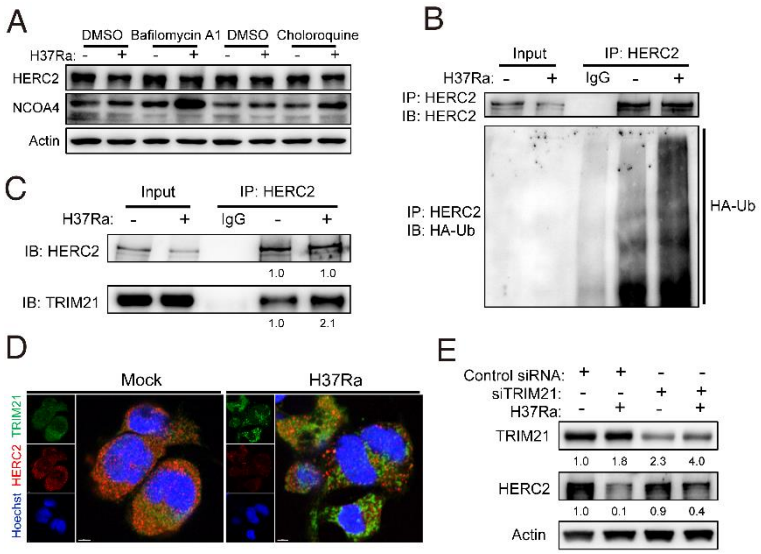

**Supplemental Figure 1. FTL knockdown has no effect on intracellular CFUs of *M. tuberculosis*.** (**A** and **B**) Immunoblot analysis of FTH1 and FTL (**A**) and intracellular H37Ra CFU levels (**B**) in macrophages transfected with FTH1 and/or FTL siRNA. Data are presented as means ± SD, ***$P < 0.001$, ****$P < 0.0001$, by one-way ANOVA with Tukey's post hoc test (**B**).



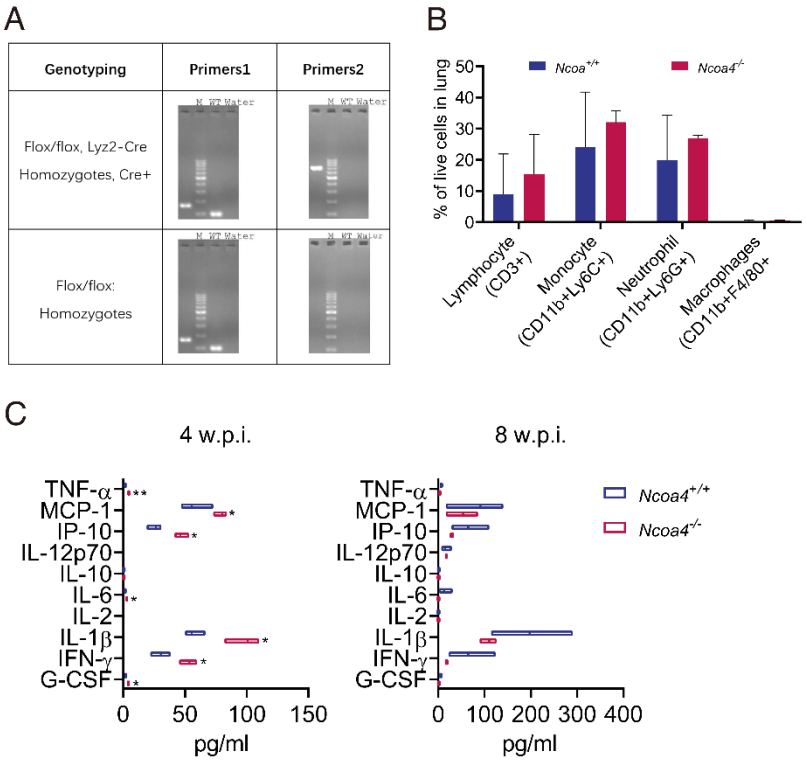

**Supplemental Figure 2. Ferritinophagy enhances iron bioavailability and intracellular *M. tuberculosis* growth.** (**A**) Immunoblot analysis of FTH1 and FTL at various time points (24, 48, and 72 h) in ferrous lactate (20 μM)-pretreated THP-1-derived macrophages. (**B**) Representative images of H37Ra-infected macrophages stained with NCOA4 and FTH1 (scale bars, 4 μm). (**C**) THP-1-derived macrophages were transfected with NCOA4 siRNA (50 nM). Scrambled siRNA was used as a negative control. Cell death was determined using an annexin V/propidium iodide (PI) kit following H37Ra infection (MOI = 10:1) for 6, 12, and 24 h by flow cytometry. (**D** and **E**) Representative images of H37Ra-infected macrophages stained with FTH1, NCOA4, and Lyso-tracker (scale bars, 4 μm). (**F**) Immunoblot analysis of NCOA4 and FTH1 in macrophages infected with H37Ra (MOI = 10) for 24 h, followed by bafilomycin A1 (100 nM) and chloroquine (25 μM) treatment for a further 3 h. (**G**) Detection of free iron by Calcein-AM in macrophages infected with H37Ra (MOI = 10) for 72 h or treated with controls including ferrous lactate (200 μM) and iron chelation, DFO (25 μM). (**H**) Detection of free iron by FeRhoNox in NCOA4-knockdown macrophages in the presence or absence of 25 μM DFO after H37Rv infection for 72 h. (**I**) Detection of intracellular H37Ra CFU levels in NCOA4-knockdown macrophages in the presence or absence of various concentrations of DFO (10, 25, and 50 μM) after infection for 72 h. (**J**) Detection of intracellular H37Rv CFU levels in NCOA4- and FTH1-knockdown macrophages in the presence or absence of DFO (25 μM) after infection for 72 h. Data are presented as means ± SD, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, by one-way ANOVA with Tukey's post hoc test (**G**) or Student's two-tailed unpaired t-test (**C, H-J**).




**Supplemental Figure 3. *M. tuberculosis*-induced NCOA4 is regulated by HERC2-mediated proteolysis. (A)** Immunoblot analysis of HERC2 in the cytoplasmic and nuclear fractions of control and H37Ra-infected macrophages. (**B**) Representative images of H37Ra-infected macrophages stained with HERC2 and NCOA4 (scale bars, 4 μm). (**C**) Immunoblot analysis and immunoprecipitation of NCOA4 and HA-Ub in macrophages transfected with HA-Ub plasmid followed by H37Ra (MOI = 10) infection for 24 h. (**D** and **E**) Immunoblot analysis of NCOA4 in macrophages infected with H37Ra (MOI = 10) for 6, 12, and 24 h in the presence or absence of MG-132 (2 μM) (**E**) or in macrophages infected with or without H37Ra (MOI = 10) for 24 h, followed by MG-132 (10 μM) treatment for a further 3 h (**D**).




**Supplemental Figure 4. *M. tuberculosis*-induced proteasomal degradation of HERC2 depends on TRIM21.** (**A**) Immunoblot analysis of HERC2 and NCOA4 in THP-1-derived macrophages infected with H37Ra (MOI = 10) for 24 h, followed by bafilomycin A1 (100 nM) or chloroquine (25 μM) treatment for a further 3 h. (**B**) Immunoblot analysis and immunoprecipitation of HERC2 and HA-Ub in macrophages transfected with HA-Ub plasmid, followed by H37Ra (MOI = 10) infection for 24 h. (**C**) Immunoblot analysis and immunoprecipitation of HERC2 and TRIM21 in macrophages infected with H37Ra (MOI = 10) for 24 h. (**D**) Representative images of H37Ra-infected macrophages stained with HERC2 and TRIM21 (scale bars, 4 μm). (**E**) Immunoblot analysis of HERC2 and TRIM21 in macrophages transfected with TRIM21 siRNA and then infected with H37Ra (MOI = 10) for 24 h.




**Supplemental Figure 5. NCOA4 deficiency in myeloid cells increases host inflammation response but does not affect lymphocyte, monocyte/macrophage, or neutrophil recruitment in response to *M. tuberculosis* infection.** (**A**) PCR amplification showing the presence of the wild-type *Ncoa4* allele at 350 bp (*Ncoa4*$^{+/+}$) and the disrupted *Ncoa4* allele at 700 bp (*Ncoa4*$^{-/-}$). (**B**) The percentages of lymphocytes (CD3$^{+}$), monocytes (Ly6C$^{+}$CD11b$^{+}$), macrophages (CD11b$^{+}$F4/80$^{+}$), and neutrophils (CD11b$^{+}$Ly6G$^{+}$) in lungs from *Ncoa4*$^{+/+}$ and *Ncoa4*$^{-/-}$ mice infected with H37Rv were determined by flow cytometry. (**C**) Inflammation cytokines in the lung homogenates 4- and 8-weeks post-infection (w.p.i.) were detected by Luminex. Data are presented as means ± SD, **P* < 0.05, by Student's two-tailed unpaired t-test (**B** and **C**).

**Supplementary Table 1. Demographic characteristics of the study populations.**

| Cohort | No. | Age | Sex | Bacteriologically confirmed [a] | Histopathology [b] | Duration of anti-TB treatment before sample collection (months) |
|---|---|---|---|---|---|---|
| **Therapeutic resection** | T1 | 48 | Male | Yes | + | 2 |
| | T2 | 35 | Male | N/A | + | 15 |
| | T3 | 37 | Male | Yes | + | 6 |
| | T4 | 22 | Male | Yes | + | 12 |
| | T5 | 41 | Female | Yes | + | 13 |
| | T6 | 63 | Male | Yes | + | 2 |
| | T7 | 33 | Male | Yes | + | <1 |
| | T8 | 46 | Male | Yes | + | 6 |
| | T9 | 53 | Male | Yes | + | 2 |
| **Diagnostic biopsy** | D1 | 56 | Male | Yes | + | None |
| | D2 | 45 | Male | N/A | + | None |
| | D3 | 53 | Male | N/A | + | None |
| | D4 | 42 | Male | Yes | + | None |
| | D5 | 39 | Male | N/A | + | None |
| | D6 | 40 | Male | N/A | + | None |

[a] Bacteriologically confirmed by acid-fast bacillus, Mtb culture, and nucleic acid amplification testing.

[b] Histopathology confirmed by the presence of typical caseous granuloma and multiple Langhans giant cells.

N/A: not applicable.

**Supplementary Table 2. Potential E3 ligases for HERC2 identified by mass spectrometry analysis**

| Protein | Score | Mass | Matches | Sequences | emPAI |
|---|---|---|---|---|---|
| **TRIM21** | 445 | 55162 | 35 (21) | 15 (11) | 0.94 |
| **TRIM25** | 53 | 72581 | 5 (3) | 5 (3) | 0.13 |
| **ISG15** | 39 | 17933 | 2 (1) | 2 (1) | 0.18 |
| **UNKL** | 33 | 75435 | 5 (1) | 2 (1) | 0.04 |