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Supplemental information

MiR-25 blunts autophagy and promotes the survival of *Mycobacterium tuberculosis* by regulating NPC1

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Supplementary Material

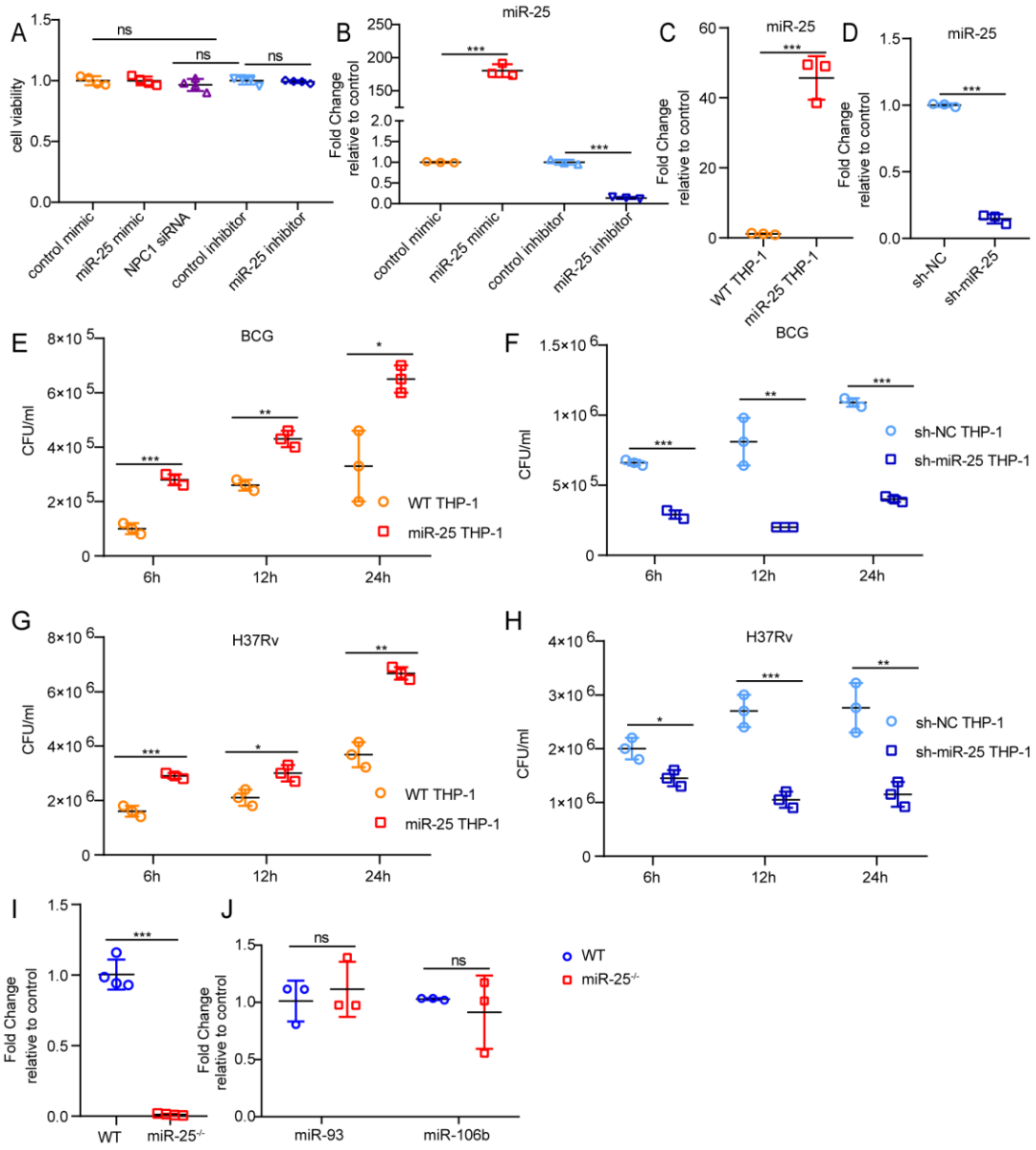

Figure S1. miR-25 promotes the intracellular survival of BCG and Mtb, related to Figure 1. (A) Cell viability assay in THP-1 cells pretransfected with control mimic, miR-25 mimic, NPC1 siRNA, control inhibitor or miR-25 inhibitor and infected with BCG. (B) qRT-PCR detection of miR-25 levels in THP-1 cells transfected with control mimic, miR-25 mimic, control inhibitor or miR-25 inhibitor. (C) qRT-PCR detection of miR-25 in WT THP-1 or miR-25 THP-1 cells. (D) qRT-PCR detection of miR-25 in sh-NC THP-1 or sh-miR-25 THP-1 cells. WT THP-1, miR-25 THP-1, sh-NC THP-1 or sh-miR-25 THP-1 cells infected with BCG or H37Rv and then subjected to CFU analysis (E, F, G, H). (I) qRT-PCR detection of miR-25 in the lungs of WT or miR-25$^{-/-}$ mice. (J) qRT-PCR detection of miR-93 and miR-106b in the lungs of WT or miR-25$^{-/-}$ mice.

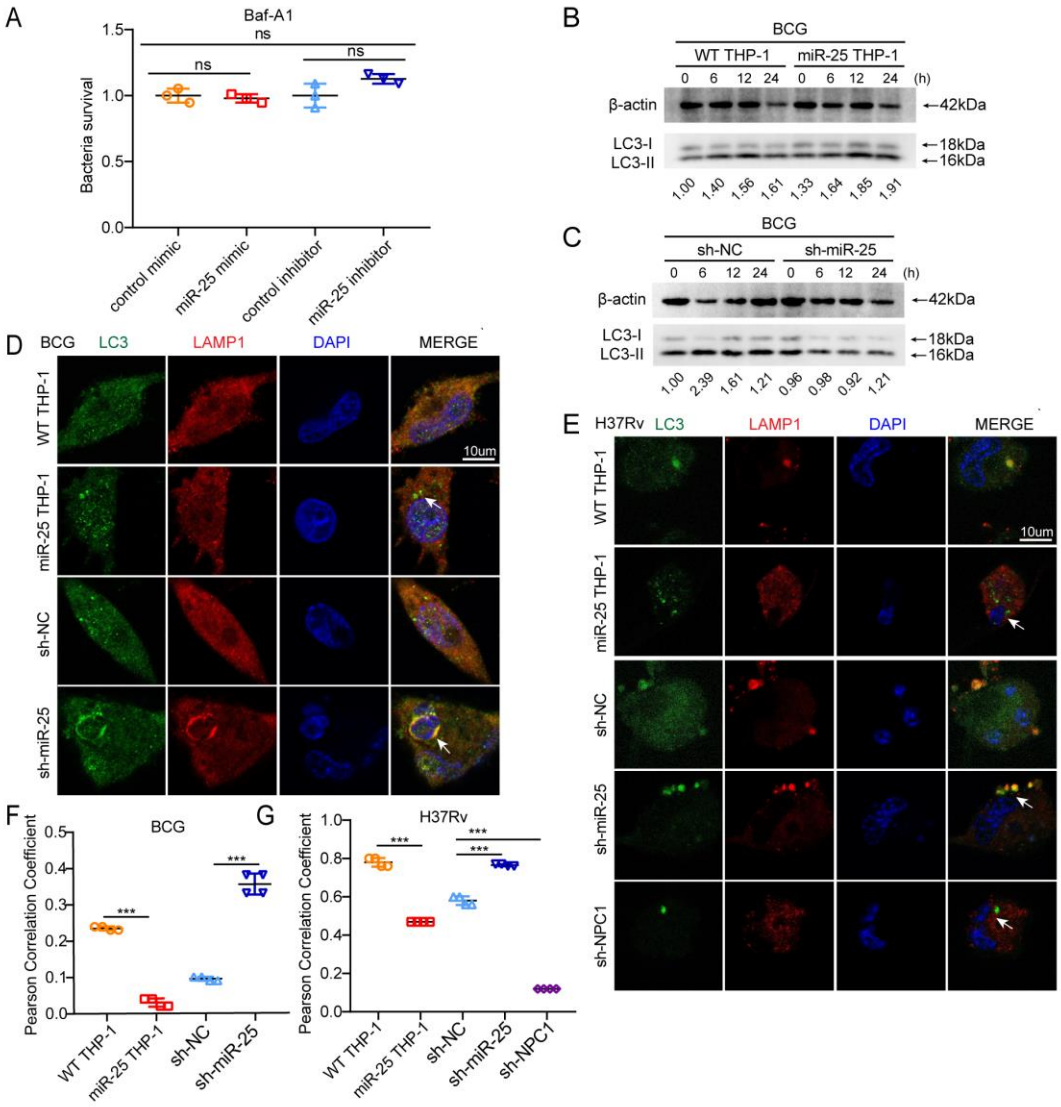

Figure S2. miR-25 regulates autophagy pathway during BCG and Mtb infection, related to Figure 3. THP-1 cells pretransfected with control mimic, miR-25 mimic, control inhibitor or miR-25 inhibitor were infected with BCG. Baf-A1 (100 nm) was used at the same time as BCG infection treatment, CFU analysis was performed at 24 hours postinfection (24 hpi) (A). WT THP-1, miR-25 THP-1, sh-NC THP-1, sh-miR-25 THP-1 or sh-NPC1 cells infected with BCG or H37Rv, Western blotting of the lysate was performed to detect the amount of LC3 (B, C); indirect immunofluorescence (IF) analysis of the colocalization of autophagosomes (LC3, green) and lysosomes (LAMP-1, red) bar, 10 μM (D, E). Pearson correlation coefficients (PCCs) of images of internalized Alexa Fluor 488-LC3 and Alexa Fluor 594-LAMP1 in THP-1 cells (F, G).

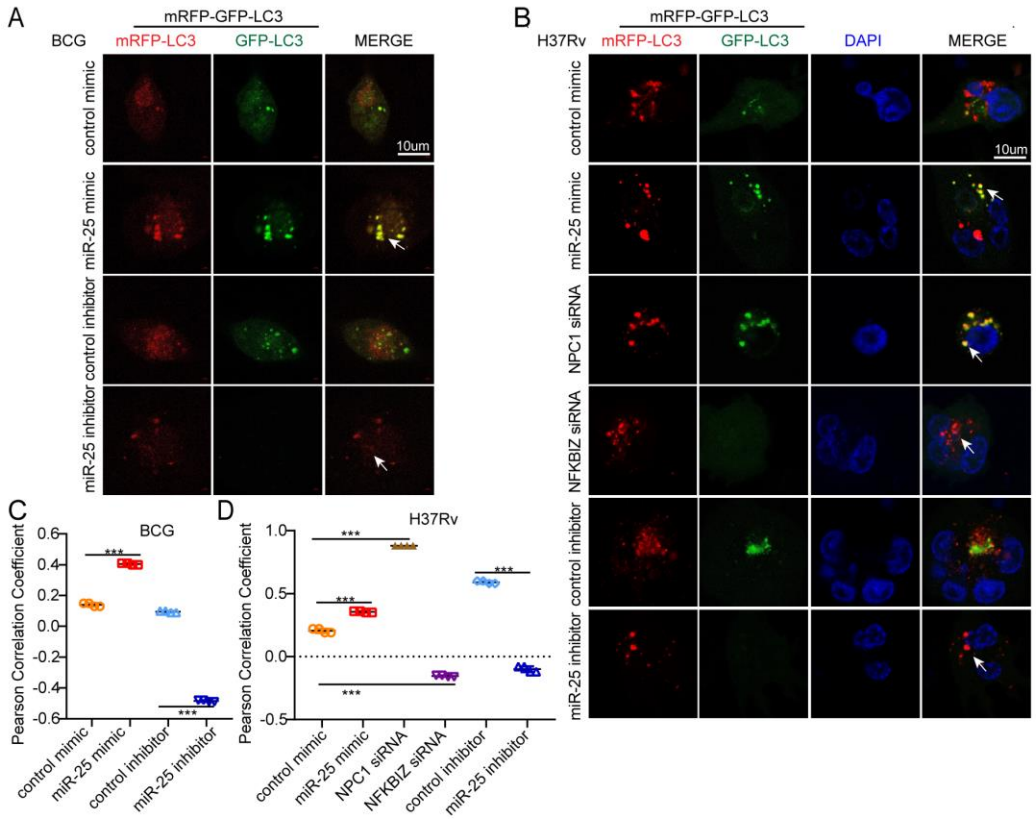

Figure S3. miR-25 impiares autophagy flux during BCG and Mtb infection, related to Figure 3. MRFP-GFP-LC3B THP-1 cells pretreated with control mimic, miR-25 mimic, control inhibitor, miR-25 inhibitor, NPC1 siRNA or NFKBIZ siRNA were infected with BCG or H37Rv for a specified period of time, and then confocal analysis was performed; bar, 10 μM (A, B). PCCs of images of internalized GFP-LC3 and RFP-LC3 in RFP-GFP-LC3B THP-1 cells (C, D).

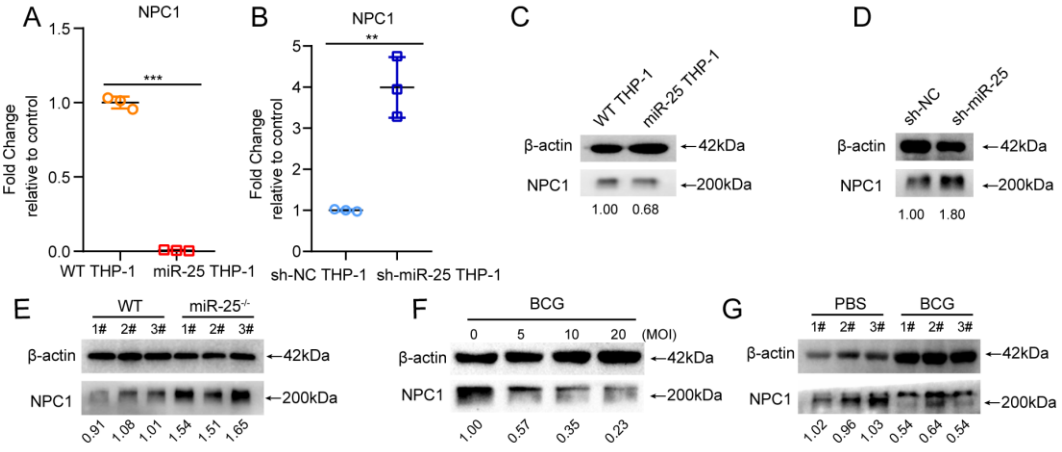

Figure S4. miR-25 directly targets NPC1, related to STAR Methods: RNA Isolation and Quantitative RT-PCR (qRT-PCR). (A) qRT-PCR detection of NPC1 in WT THP-1 or miR-25 THP-1 cells. (B) qRT-PCR detection of NPC1 in sh-NC THP-1 or sh-miR-25 THP-1 cells. (C) Western blotting of NPC1 in WT THP-1 or miR-25 THP-1 cells. (D) Western blotting of NPC1 in sh-NC THP-1 or sh-miR-25 THP-1 cells. (E) Western blotting of NPC1 expression in the lungs of WT or miR-25$^{-/-}$ mice infected with BCG for 7 days. (F) Western blotting of NPC1 expression in

THP-1 cells infected with BCG at the indicated MOIs for 24 h. (G) Western blotting of NPC1 in the lungs of mice infected with BCG via the tail vein and mice treated with PBS.

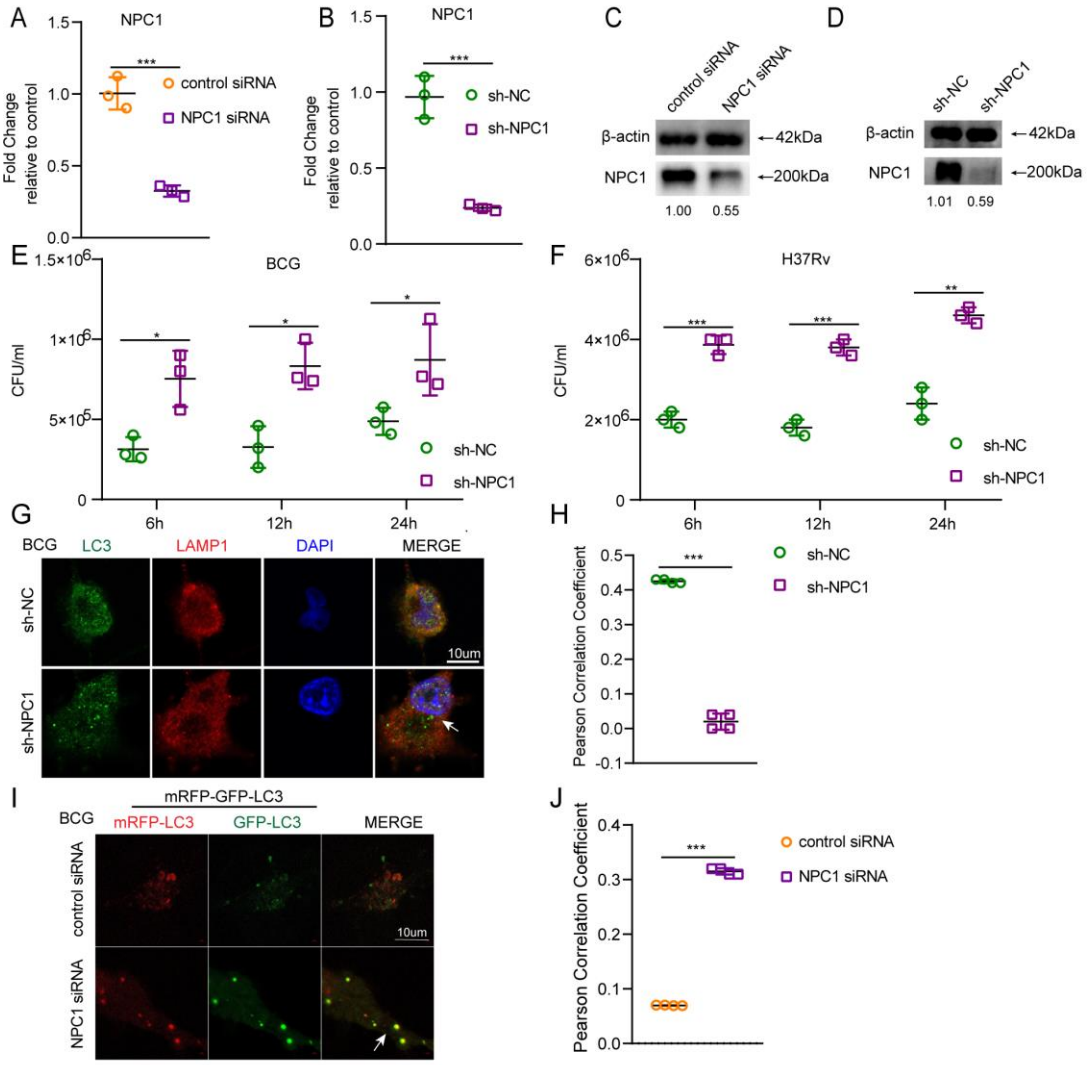

Figure S5. NPC1 inhibits intracellular survival of BCG and Mtb through autophagy pathway, related to STAR Methods: Intracellular survival of bacteria. qRT-PCR (A) and Western blotting (C) detection of NPC1 in THP-1 cells transfected with NPC1 siRNA. qRT-PCR (B) and Western blotting (D) detection of NPC1 in sh-NC THP-1 or sh-NPC1 THP-1 cells. sh-NC THP-1 or sh-NPC1 THP-1 cells were infected with BCG and then subjected to CFU analysis and (E, F) IF analysis of the colocalization of autophagosomes (LC3, green) and lysosomes (LAMP-1, red); bar, 10 μM (G). PCCs of images of internalized Alexa Fluor 488-LC3 and Alexa Fluor 594-LAMP1 in sh-NC THP-1 cells (H). MRFP-GFP-LC3B THP-1 cells pretreated with control siRNA or NPC1 siRNA were infected with BCG for a specified period of time, and then confocal analysis was performed; bar, 10 μM (I). PCCs of images of internalized GFP-LC3 and RFP-LC3 in mRFP-GFP-LC3B THP-1 cells (J).

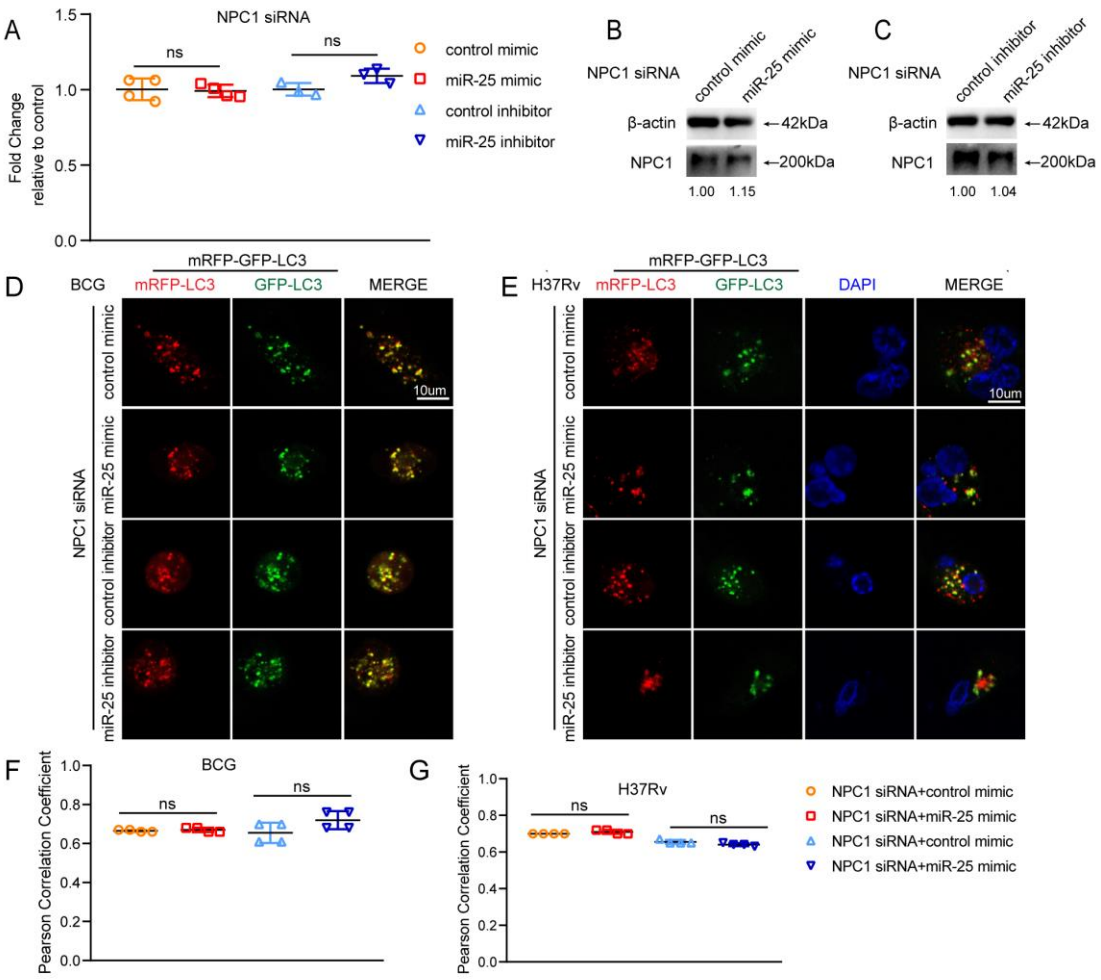

Figure S6. miR-25 regulates BCG and Mtb infection via NPC1, related to STAR Methods: RNA Isolation and Quantitative RT-PCR (qRT-PCR). THP-1 cells pretransfected with NPC1 siRNA plus miR-25 mimic or miR-25 inhibitor were infected with BCG, the qRT-PCR (A) and Western blot (B, C) detection of NPC1 expression; MRFP-GFP-LC3B THP-1 cells pretreated with NPC1 siRNA plus miR-25 mimic or miR-25 inhibitor were infected with BCG or H37Rv for a specified period of time, and then confocal analysis was performed; bar, 10 μM (J, K). PCCs of images of internalized GFP-LC3 and RFP-LC3 in mRFP-GFP-LC3B THP-1 cells (D, E, F, G).