

Figure S1. Identification of YrbE3A expression and cell location. **(A)** SDS-PAGE (12%) was used to determine the expression of the recombinant YrbE3A protein Lane M, PageRuler Prestained Protein Ladder; Lane 1, total Ms_YrbE3A cell lysate without heat-inducible; Lane 2, total Ms_YrbE3A cell lysate with heat-inducible; Lane 3, proteins in the supernatant; Lane 4, proteins in the pellet; Lane 5, binding buffer after washing; Lanes 6,7, washing buffer after washing; Lane 8, the purified YrbE3A protein. Arrow indicates the YrbE3A protein of 32 kDa. **(B)** Western blot assay on location of YrbE3A protein. The cellular proteins in cell lysate and bacterial membrane of Ms_YrbE3A and Ms_vec in SDS-PAGE were blotted to PVDF membrane. The blot was probed with His-tag monoclonal antibody. Lane 1, YrbE3A-cytoplasm; lane 2, YrbE3A-cytoplasm; Lane 3, Vec-cytoplasm; line 4, Vec-cytoplasm. Arrow indicates the YrbE3A protein.

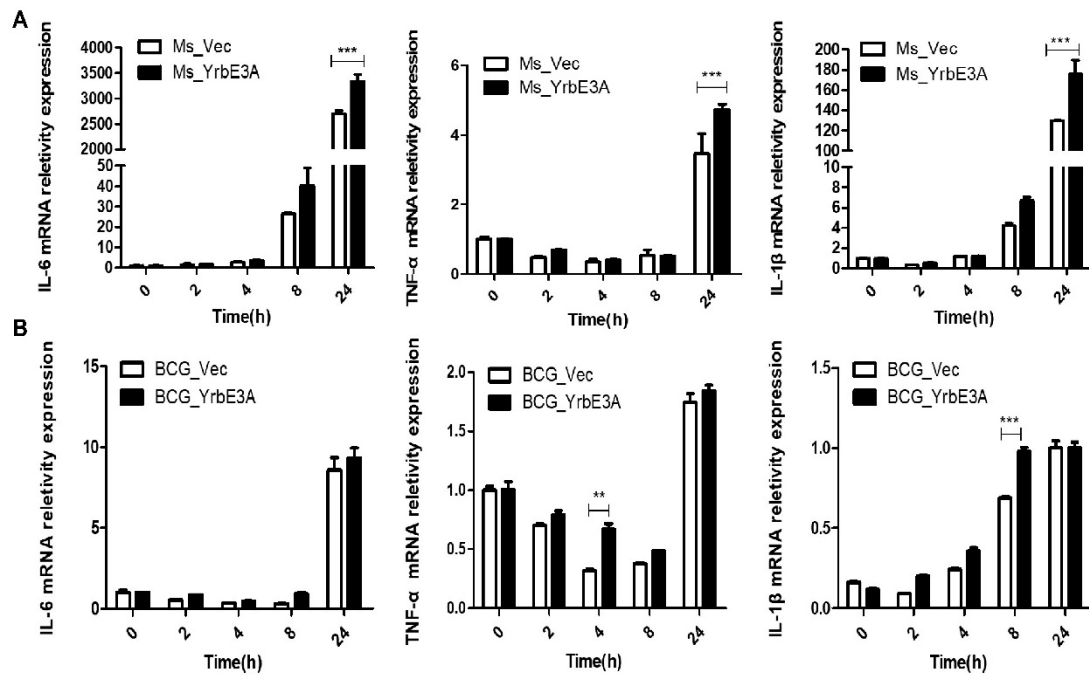


Figure S2. Transcription of cytokine expression of Ms_YrbE3A, Ms_Vec, BCG_YrbE3A, and BCG_Vec in infected RAW264.7 cells. **(A,B)** Quantitative RT-PCR was used to determine transcription levels of IL-6, TNF- α , and IL-1 β by RAW264.7 infected by Ms_YrbE3A and Ms_Vec (A.). BCG_YrbE3A and BCG_Vec (B). Significant differences ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$) among each treatment were analyzed by a two-way ANOVA followed by the LSD test. Each sample was repeated in triplicate.

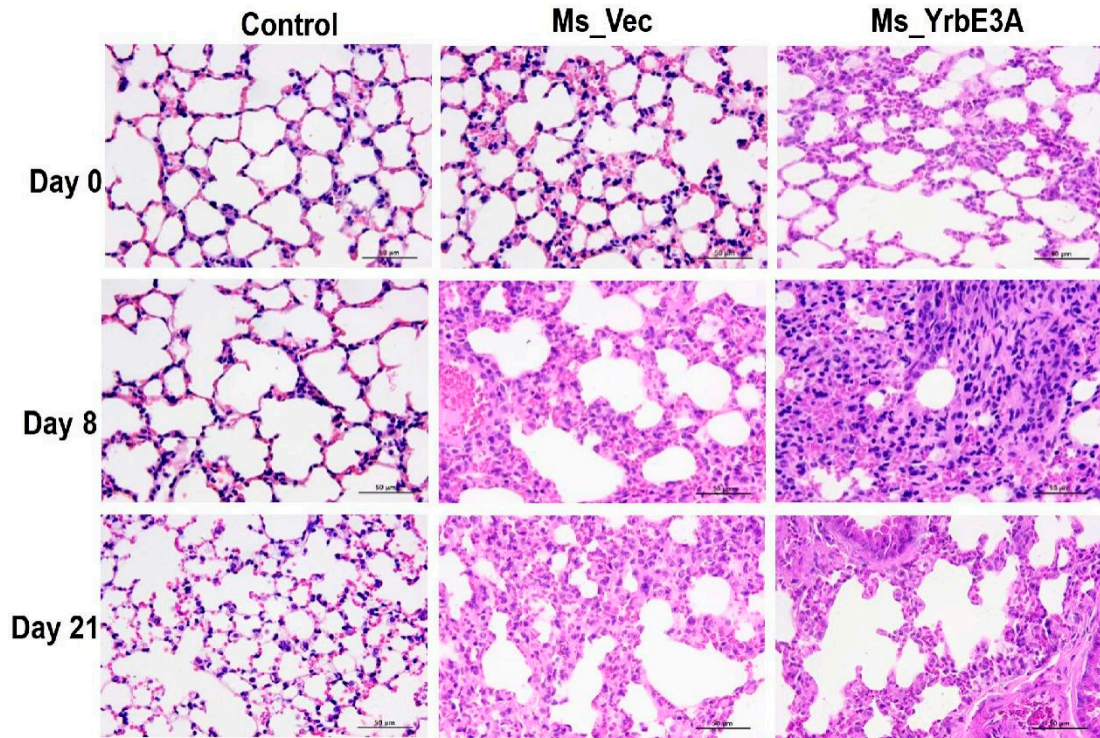


Figure S3. YrbE3A expression promoted lung inflammatory lesions in mice infected with recombinant Ms_YrbE3A and Ms_Vec. Mice were infected with Ms_YrbE3A and Ms_Vec at a dose of 2.0×10^6 CFU via intratracheal challenge, euthanized at day 0, 8, and 21 postinfection, and lung tissues were observed after being sectioned and stained with Hematoxylin and eosin (H&E) stain. Scale bar: 50 μ m. Data are representative of one experiment with two independent biological replicates (mean \pm SEM of $n = 5$ mice per group).

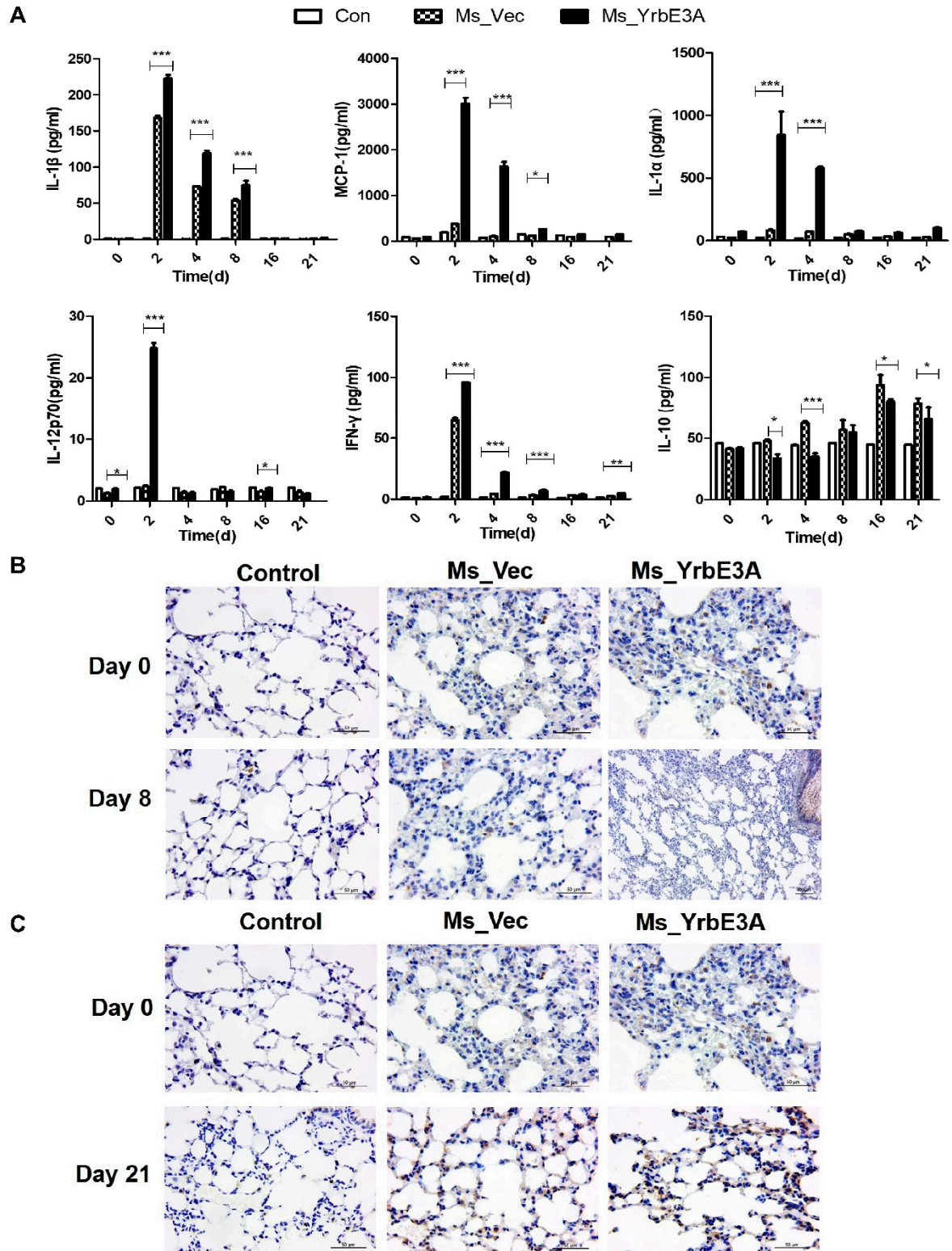


Figure S4. Detection of lung inflammatory cytokines in Ms_YrbE3A- and Ms_Vec-infected C57BL/6 mice. (A) Multiplex immunoassay of IL-1 β , MCP-1, IL-1 α , IL-12p70, IFN- γ , and IL-10 in lung cells from C57BL/6 mice infected intratracheally for 0–21 d with 2.0×10^6 CFU of MS_Vec and MS_YrbE3A strains, while a saline control was used as the placebo. (B,C) The detection of the cytokines IL-6 (left panel) and TNF- α (right panel) in the lungs of experimental mice by immunohistochemistry at labelled days postinfection. Significant difference (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) among treatments was analyzed by a two-way ANOVA followed by the LSD test. Each sample was repeated in triplicate.