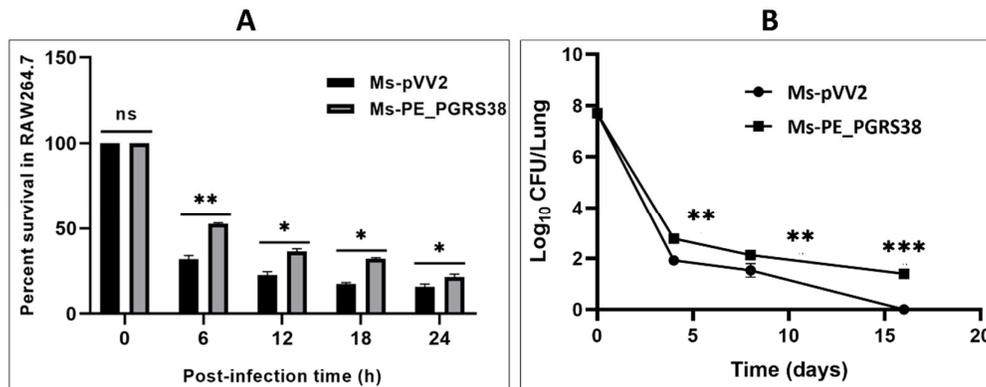
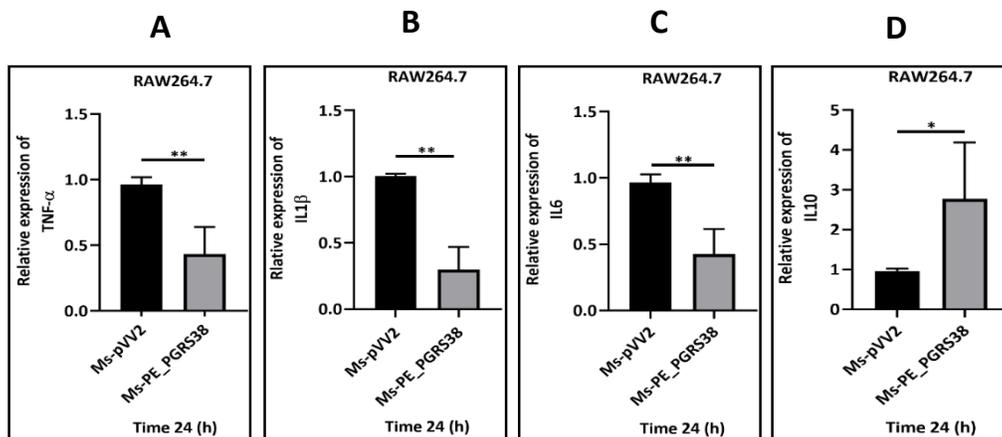


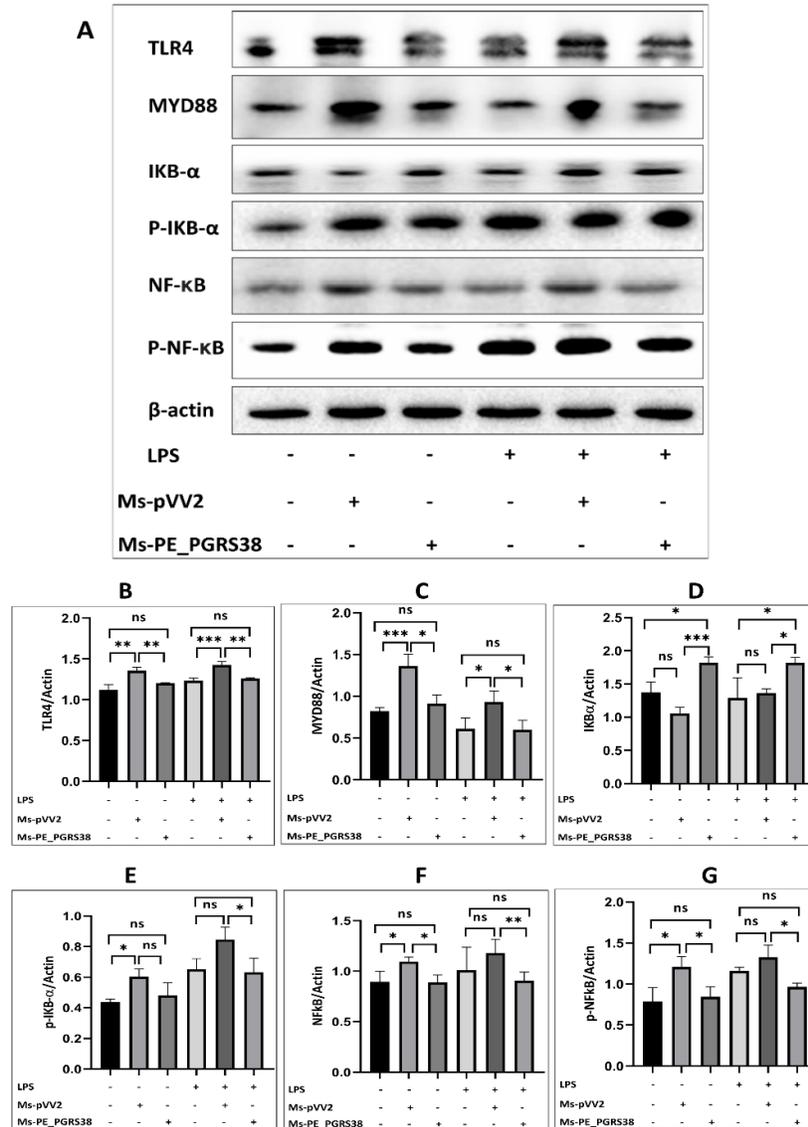
**Figure S1:** Confirmation, subcellular localization of PE\_PGRS38, and growth of recombinant strains (Ms-pVV2 and Ms-PE\_PGRS38) of *M. smegmatis* mc<sup>2</sup>155. **(A)** shows the PCR product of PE\_PGRS38, **(B)** represents the virtual construct, **(C)** shows the digested form of expression vector pVV2-PE\_PGRS38 **(D)** Fractions of recombinant *M. smegmatis* strains were subjected to Western blot to determine the subcellular localization of PE\_PGRS38. GroL2 (56 kDa) is expressed in the cytoplasm of *M. smegmatis*. GroL2 serves as a control; WCL represents the whole cell lysate; CW depicts the cell wall; CP indicates the cytoplasm. **(E)** Ms-pVV2 and Ms-PE\_PGRS38 were maintained in 7H9 enhanced with Kan (25 µg/ml), Glycerol 0.2%, and Tween 80 (0.05%). The OD<sub>600</sub> was calculated at 12-hour intervals.



**Figure S2:** PE\_PGRS38 enhanced the intracellular survivability of *M. smegmatis* in infected RAW264.7 macrophage cells and C57BL/6J mice. RAW264.7 and C57BL/6J mice were infected with Ms-pVV2 and Ms-PE\_PGRS38. Cells and lungs tissues were washed with PBS at indicated timepoints post-infection, lysed, and plated in 7H11 medium to determine the number of recombinant *M. smegmatis* strains. NS: not significant. NS > 0.05, \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001 (student t-test).



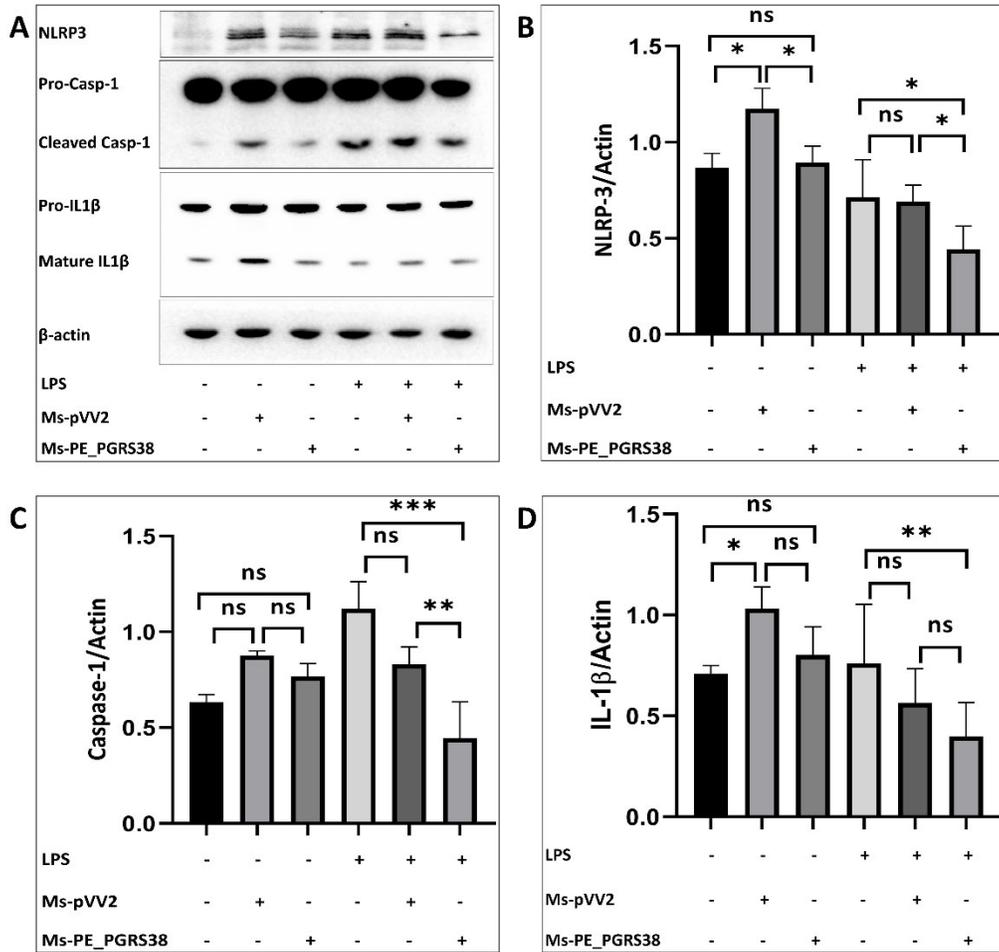
**Figure S3:** PE\_PGRS38 downregulates the expression of proinflammatory cytokines. (A–D) showing the relative mRNA expression of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL10 in RAW264.7 infected with recombinant strains of *M. smegmatis*. \* $P$  < 0.05 and \*\* $P$  < 0.01 (t-test).



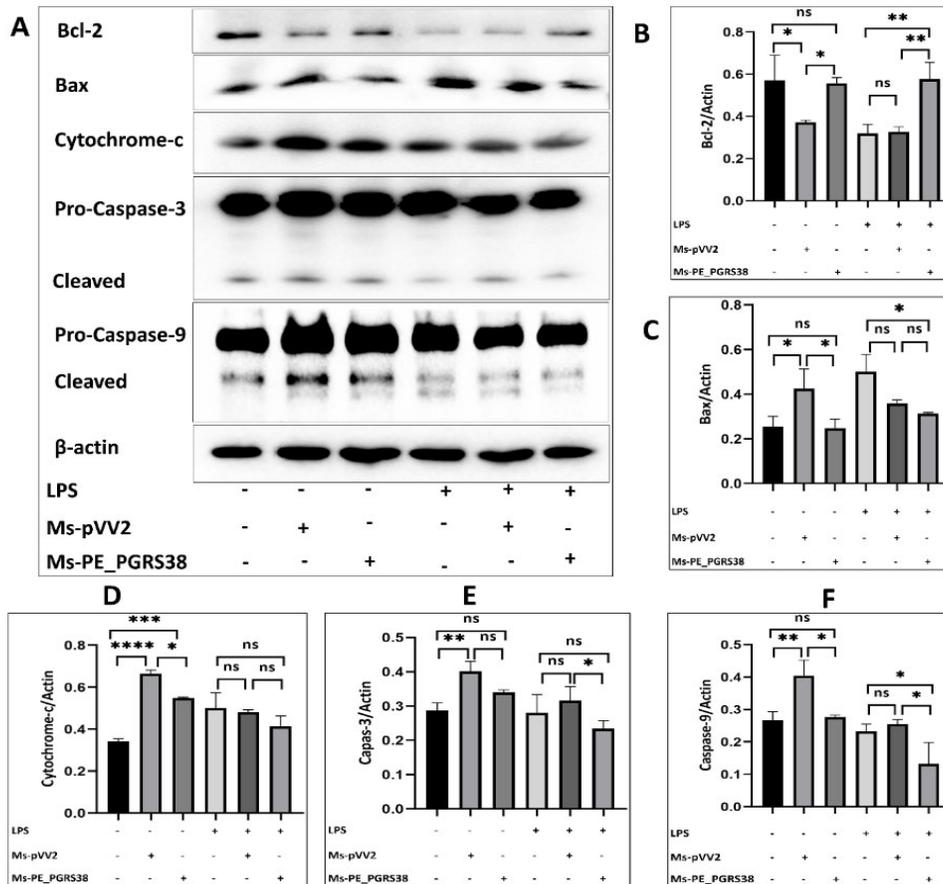
**Figure S4:**

PE\_PGRS38 downregulates NF-κB signaling in infected RAW264.7. Cells were challenged with 0.1 μg/ml LPS for 2 hours and subsequently infected with recombinant strains of *M. smegmatis* at 10:1 MOI for 4 hours. **(A)** Proteins were extracted from cells 24 hours post-infection, and expression of TLR4, MYD88, IKB-α, p-IKBα, NF-κB, p-NF-κB, and total β-actin was determined by western blot-assay. **(B-G)** Represents the densitometric analysis of

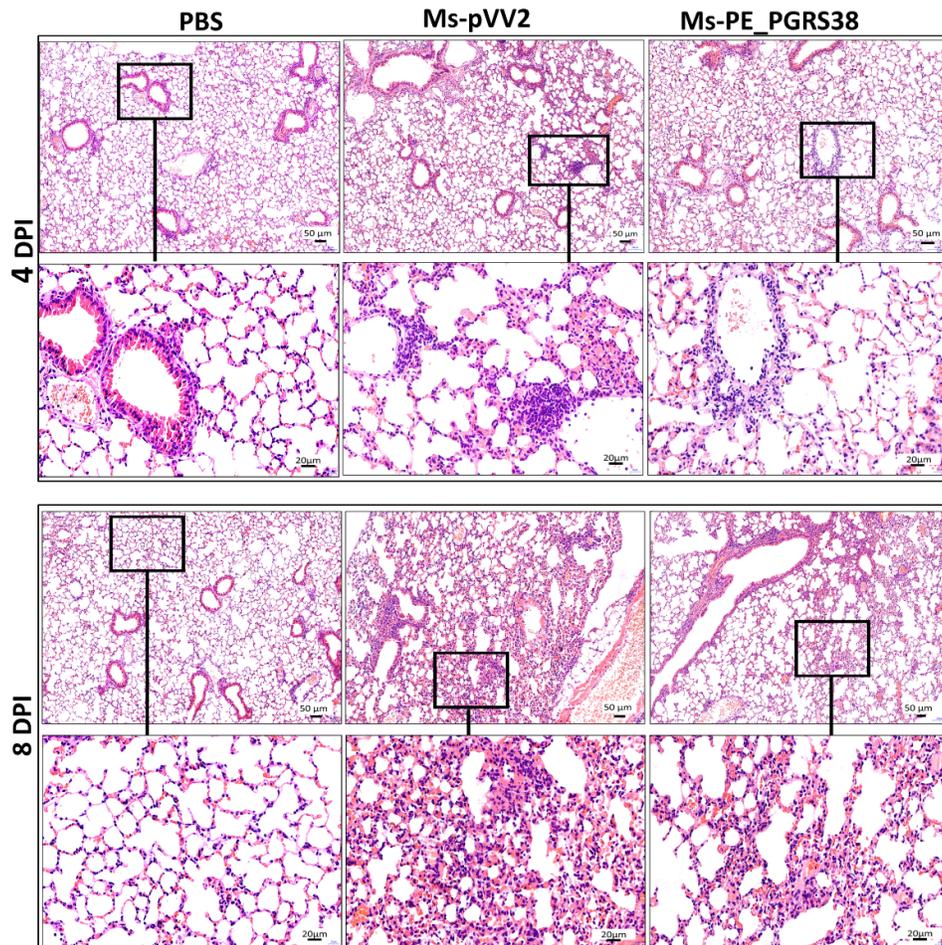
TLR4, MYD88, IKB- $\alpha$ , p-IKB $\alpha$ , NF- $\kappa$ B, and p-NF-Kb, respectively. NS > 0.05, \* $p$  < 0.05, and \*\* $p$  < 0.01 (One-way ANOVA).



**Figure S5:** PE\_PGRS38 protein decreased the NLRP3 inflammasome. RAW264.7 cells were challenged with 0.1  $\mu$ g/ml LPS for 2 hours and subjected to infection with recombinant strains of *M. smegmatis* for 4 hours. (A) Proteins were extracted from cells 24 hours post-infection, and the expression levels of NLRP3, Caspase-1, and IL1 $\beta$  and total  $\beta$ -actin were determined by western blot assay. (B-D) Represents the densitometric analysis of NLRP3, caspase-1, and IL1 $\beta$ . NS > 0.05, \* $p$  < 0.05, and \*\* $p$  < 0.01 (one-way ANOVA).



**Figure S6:** PE\_PGRS38 decreased the expression level of apoptosis in infected RAW264.7. Cells were challenged with 0.1  $\mu\text{g/ml}$  LPS for 2 hours and subsequently infected with recombinant strains of *M. smegmatis* at 10:1 MOI for 4 hours. Proteins were extracted from cells 24 hours post-infection, and the expression levels of Bax, Bcl-2, cytochrome-c, caspase-3, caspase-9, and total  $\beta$ -actin were determined by Western blot assay. (A) represents blots, while (B-F) depicts the densitometric analysis of Bcl2, Bax, cytochrome-c, caspase-3, and caspase-9. NS > 0.05, \* $p$  < 0.05 \*\* $p$  < 0.01, \*\*\* $p$  < 0.001, and \*\*\*\* $p$  < 0.0001 (One-way ANOVA).



**Figure S7:** Lung histology of infected mice with Ms-pVV2 and Ms-PE\_PGRS38 strains. C57BL/6J were subjected to aerosolization, as discussed previously. The left lobe of each lung was fixed with 4% PFA for pathological analysis. The Ms-pVV2 or Ms-PE\_PGRS38-infected lung sections were organized on 4 and 8 days post-infection (DPI) and stained with H&E. The tissue sections were visualized using 40X microscope objectives. Scale bars = 50 and 20  $\mu\text{m}$ .

**Table S1:** List of the primer sequence used in this study.

Gene	5'-3' Forward primer	5'-3' Reverse primer
PE_PGRS38	GGCATATGTCGTTTGTGATTGCGGCG	ATGGATCCTCAGCCGTCGGCGCCGTTTG
TNF- $\alpha$	ACTGAACTTCGGGGTGATCGGT	TGGTTTGCTACGACGTGGGCTA
IL-1 $\beta$	TGCCACCTTTTGACAGTGATGAG	TGATGTGCTGCTGCGAGATTT
IL-6	CCCCAATTTCCAATGCTCTCC	CGCACTAGGTTTGCCGAGTA
IL-10	TGAGGCGCTGTCGTCATCGATTTCTCCC	ACCTGCTCCACTGCCTTGCT
Bax	AGGATGCGTCCACCAAGAAGCT	TCCGTGTCCACGTCAGCAATCA
Bcl2	CCTGTGGATGACTGAGTACCTG	AGCCAGGAGAAATCAAACAGAGG
Cyto-c	GAGGCAAGCATAAGACTGGACC	ACTCCATCAGGGTATCCTCTCC
Caspase-3	GGAGTCTGACTGGAAAGCCGAA	CTTCTGGCAAGCCATCTCCTCA
Caspase-9	GCTGTGTCAAGTTTGCCTACCC	CCAGAATGCCATCCAAGGTCTC
NLRP3	TCACAACCTCGCCCAAGGAGGAA	AAGAGACCACGGCAGAAGCTAG
Caspase-1	GGCACATTTCCAGGACTGACTG	GCAAGACGTGTACGAGTGGTTG
NF- $\kappa$ B	TCCTGTTCGAGTCTCCATGCAG	GGTCTCATAGGTCCTTTTGCGC
IKB- $\alpha$	GCCAGGAATTGCTGAGGCACTT	GTCTGCGTCAAGACTGCTACAC
TLR4	AGCTTCTCCAATTTTTCAGAACTTC	TGAGAGGTGGTGTAAGCCATGC
MYD88	ACCTGTGTCTGGTCCATTGCCA	GCTGAGTGCAAACCTGGTCTGG
$\beta$ -actin	TGACGTTGACATCCGTAAAGACC	CTCAGGAGGAGCAATGATCTTGA

