

A Conserved Mycobacterial Nucleomodulin Hijacks the Host COMPASS Complex to Reprogram Pro-Inflammatory Transcription and Promote Intracellular Survival

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eLife Assessment

This **valuable** study provides **solid** evidence that MgdE, a conserved mycobacterial nucleomodulin, downregulates inflammatory gene transcription by interacting with the histone methyltransferase COMPASS complex and altering histone H3 lysine methylation. There are areas where the evidence could be strengthened, for example, GFP immunoblotting and examining MgdE localization during infection. To enhance impact, the authors could consider *Mycobacterium tuberculosis* infection experiments and/or reworking the manuscript to emphasize general relevance to microbiologists and cell biologists.

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Abstract

Nucleomodulins are a class of effector proteins secreted by bacterial pathogens that translocate into the host cell nucleus to modulate nuclear processes. However, their target proteins and underlying molecular mechanisms remain poorly understood in mycobacteria. Herein, we identified a conserved hypothetical protein Rv1075c, designated MgdE, as a nucleomodulin that enhances mycobacterial intracellular survival. MgdE undergoes nuclear translocation via two nuclear localization signals, KRIR¹⁰⁸⁻¹¹¹ and RLRRPR³⁰⁰⁻³⁰⁵, and interacts with ASH2L and WDR5, two subunits of the host histone methyltransferase COMPASS complex. This interaction suppresses histone H3 lysine 4(H3K4) methylation-mediated transcription of pro-inflammatory genes, including *il6* and *il1β*, thereby promoting mycobacterial survival in both macrophages and mice. Our study provides the first experimental evidence that a bacterial nucleomodulin facilitates intracellular survival by directly targeting the host COMPASS complex. These findings advance our understanding of

mycobacterial pathogenesis by revealing a novel mechanism that contributes to its intracellular survival strategy.

Introduction

Nucleomodulins constitute a distinct class of bacterial proteins capable of translocating into the nuclei of eukaryotic cells, where they strategically modulate nuclear processes to promote pathogen survival and persistence (Radoshevich et al., 2018 [DOI](#); Hanford et al., 2021 [DOI](#)). These evolutionarily diverse effector proteins, secreted by distinct pathogens, exhibit remarkable adaptability in infiltrating host nuclei and reprogramming the transcriptional and epigenetic regulatory networks (Bierne et al., 2012 [DOI](#); Bierne et al., 2020 [DOI](#)). Upon nuclear localization, nucleomodulins employ diverse molecular mechanisms to subvert host cellular processes, including but not limited to: (i) direct interactions with genomic DNA or nuclear proteins (Rennoll-Bankert et al., 2015 [DOI](#); Sun et al., 2016 [DOI](#); Prokop et al., 2017 [DOI](#)), (ii) hijacking post-translational modification systems (Lebreton et al., 2014 [DOI](#); Rolando et al., 2023 [DOI](#)), (iii) dysregulating signaling molecule activity or localization (Zhao et al., 2019 [DOI](#); Chambers et al., 2020 [DOI](#); Evans et al., 2018 [DOI](#)), and (iv) destabilizing nuclear architecture (Pourpre et al., 2022 [DOI](#); Fu et al., 2021 [DOI](#)). Specific examples include AnkA, a bacterial protein from *Anaplasma phagocytophilum*, which binds to specific A/T-rich sequences in the host cell, alters chromatin architecture, and modulates gene transcription (Rennoll-Bankert et al., 2015 [DOI](#)). In *Listeria monocytogenes*, LntA interacts with the proline-rich domain of BAHD1, to disrupt BAHD1-mediated gene silencing and promote histone H3 acetylation (Lebreton et al., 2014 [DOI](#)). Another example is OspF from *Shigella flexneri*, which exploits its phosphothreonine lyase activity to irreversibly dephosphorylate MAPKs, thereby preventing the transcriptional activation of NF- κ B-regulated genes (Chambers et al., 2020 [DOI](#)). Unlike these effectors, *L. monocytogenes* InlP takes a different approach by hijacking the splicing factor RBM5. This interaction not only suppresses apoptosis but also reorganizes nuclear speckles through SC35 redistribution and RBM5-dependent granule formation (Pourpre et al., 2022 [DOI](#)). These examples illustrate the diverse and sophisticated mechanisms used by nucleomodulins to reprogram host nuclear functions, ultimately manipulating the intracellular environment to their advantage.

The evolutionary refinement of nucleomodulins is exemplified by their ability to exploit chromatin-based regulatory systems, with histone methylation emerging as a pivotal axis in host-pathogen interactions (Bierne et al., 2020 [DOI](#); Khan et al., 2021 [DOI](#)). As a central epigenetic modification, histone methylation dynamically controls chromatin accessibility and transcriptional outcomes (Yu et al., 2024 [DOI](#); Wang et al., 2025 [DOI](#)). By precisely targeting specific histone residues, nucleomodulins reprogram chromatin states to either suppress host immune responses or promote cellular pathways conducive to pathogen survival (Lebreton et al., 2014 [DOI](#); Schator et al., 2023 [DOI](#); Rolando et al., Denzer et al., 2020 [DOI](#)). A paradigm of this molecular strategy is demonstrated by the *Chlamydia trachomatis* nuclear effector NUE, which utilizes its conserved SET domain to catalyze the methylation of histones H2B, H3, and H4, establishing a repressive chromatin state that downregulates antimicrobial gene expression (Fol et al., 2020 [DOI](#)). Similarly, RomA uses its ankyrin and SET domains to bind and methylate histone H3, thereby altering the host chromatin to enhance *Legionella* intracellular survival (Schator et al., 2023 [DOI](#); Rolando et al., 2013 [DOI](#)). *Legionella* LegAS4 catalyzes H3K4 methylation to promote an open chromatin state, enhancing ribosomal RNA transcription and facilitating bacterial intracellular replication (Denzer et al., 2020 [DOI](#)). These cases emphasize how pathogens exploit histone methylation as a molecular lever to hijack the host epigenetic machinery.

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, is a highly successful pathogen because of its ability to survive within macrophages, evade the immune system, and persist in the host, ultimately leading to a chronic disease state (Chandra et al., 2022 [DOI](#); Chai et al., 2020 [DOI](#)). Central to its pathogenic strategy is the secretion of a diverse array of effector proteins

(Chai et al., 2022 [↗](#); Bates et al., 2024 [↗](#); Qiang et al., 2023 [↗](#)), including nucleomodulins that subvert host nuclear processes. The tyrosine phosphatase PtpA exemplifies this strategy by directly binding to the promoter regions of immune-related genes, including *GADD45A*, to suppress innate immune responses (Wang et al., 2017 [↗](#)). The methyltransferase Rv1988 mediates an unconventional histone modification by methylating histone H3 at arginine 42 (H3R42me), thereby silencing antimicrobial defense mechanisms (Yaseen et al., 2015 [↗](#)). Similarly, the acetyltransferase Rv3423.1 modulates chromatin accessibility through acetylation of histone H3 at lysine 9 and 14 (H3K9/K14ac), attenuating pro-inflammatory signaling pathways (Jose et al., 2016 [↗](#)). Rv2966c represents a unique bacterial DNA methyltransferase that establishes non-canonical CpG methylation patterns to epigenetically silence host immune-related gene expression (Sharma et al., 2015 [↗](#)). Despite these molecular insights, the comprehensive landscape of the nuclear effector systems of Mtb, particularly the mechanistic details of nucleomodulin interactions with host chromatin-modifying complexes and their spatiotemporal regulation during infection, remains poorly defined. This critical knowledge gap impedes our understanding of how Mtb coordinates system-level transcriptional rewiring in the host cellular environment.

In this study, we utilized bioinformatics analysis combined with fluorescence imaging to rapidly screen for potential nucleomodulins in pathogenic mycobacterial species. Using this approach, the hypothetical protein Rv1075c (designated as MgdE) was identified and subsequently validated. MgdE undergoes nuclear translocation mediated by two nuclear localization signals, KRIR¹⁰⁸⁻¹¹¹ and RLRRPR³⁰⁰⁻³⁰⁵, and interacts with the histone methyltransferase COMPASS complex subunits ASH2L and WDR5. This interaction suppresses H3K4me3-mediated inflammatory gene expression, thus dampening host immune responses and enhancing bacterial survival in macrophages and murine infection models. Our findings identify MgdE as a nucleomodulin that targets the COMPASS complex, revealing a novel epigenetic strategy exploited by mycobacteria for intracellular survival.

Results

Functional screening of potential nucleomodulins in pathogenic mycobacterial species

To identify conserved nucleomodulins in pathogenic mycobacteria, we first conducted a pan-genomic comparative analysis across four relevant species, including *M. tuberculosis* H37Rv and H37Ra, *M. bovis* BCG, *M. marinum*, and *M. avium*, to categorize the core genes. Subsequently, a dual-threshold screening approach was employed to predict the classical and non-canonical secreted proteins encoded by these shared genes using SignalP 5.0 and SecretomeP 2.0, with thresholds set at a D-score ≥ 0.5 (Hammond et al., 2018 [↗](#)) and a neural network (NN) score ≥ 0.9 (Bendtsen et al., 2005 [↗](#)) (Table S1). This analysis yielded 135 high confidence secreted protein candidates (Table S2). Further analysis of these candidates for nuclear localization signal (NLS) motifs using cNLS Mapper (NLS score ≥ 2) revealed that 56 proteins harbored at least one putative NLS. Following systematic classification based on secretion machinery dependencies, the candidate proteins were categorized into four distinct pathways: 26 Sec/SPI (signal peptide-driven), 7 Tat/SPI (twin-arginine translocase pathway), 18 Sec/SPII (lipoprotein-targeted), and eight unclassified candidates lacking canonical secretion motifs (Figure 1A and B [↗](#), Figure S1A and B). Functional validation of nuclear trafficking was conducted through the heterologous expression of enhanced green fluorescent protein (EGFP)-tagged constructs in HEK293T cells, followed by quantitative profiling of the subcellular distribution using laser confocal fluorescence microscopy. This orthogonal validation confirmed the nuclear enrichment of 23 effectors, including six Tat/SPI-associated candidates. Among these, Rv2577 exhibited the most pronounced nuclear enrichment, displaying a nuclear localization capacity stronger than that of the previously described nuclear modulator protein Rv1988 (Yaseen et al., 2015 [↗](#)). Notably, MgdE not only localized to the host cell nucleus but also exhibited a distinct punctate distribution within the nucleus (Figure 1C [↗](#)).

Nucleomodulins are effector proteins secreted by pathogens that translocate into the host cell nucleus to modulate host functions. To assess whether MgdE is secreted by mycobacteria, recombinant *M. bovis* BCG strains expressing C-terminally 2×Flag-tagged Ag85B (positive control) and MgdE were constructed. Immunoblot analysis confirmed high levels of Ag85B-2×Flag and MgdE-2×Flag in bacterial lysates. Notably, both proteins were also detected in the culture supernatants (Figure S1C), indicating that MgdE is secreted. These findings identify MgdE as a nucleomodulin in mycobacteria and led us to further investigate the mechanisms underlying its nuclear translocation and functional role during infection.

MgdE translocates into the host nucleus through its dual NLS

MgdE is a putative GDSL-like lipase characterized by conserved esterase sequence motifs, with the GDSx motif containing a nucleophilic Ser residue as part of block I (equivalent to the classical G-x-S-x-G motif of lipases/esterases), and the consensus amino acids Gly, Asn, and His located in blocks II, III, and V, respectively (Yang et al., 2019). Phylogenetic analysis across the mycobacterial species revealed strict evolutionary conservation of MgdE, with most strains encoding two predicted NLS: KRIR¹⁰⁸⁻¹¹¹ (NLS1) and RLRRPR³⁰⁰⁻³⁰⁵ (NLS2) (Figure 2A and B). To further investigate the nuclear translocation capability of MgdE, MgdE-EGFP was transfected into HEK293T cells, and its subcellular localization was analyzed at various time points post-transfection. Confocal microscopy revealed a time-dependent decrease in the cytoplasmic levels of MgdE, accompanied by its progressive accumulation in the host cell nucleus. Notably, MgdE displayed a distinct punctate distribution pattern within the nucleus, with nearly all the protein localizing to the nucleus approximately 36 h post-transfection (Figure S2). To quantitatively validate these observations, the temporal dynamics of MgdE expression in HEK293T cells were monitored, and nuclear enrichment was further assessed through cell fractionation. The results demonstrated a time-dependent increase in MgdE expression levels, which closely correlated with its gradual and sustained nuclear accumulation (Figure 2C). Collectively, these findings indicate that MgdE is an evolutionarily conserved mycobacterial nucleomodulin with nuclear translocation capacity.

To dissect the functional contributions of the predicted NLS motifs, EGFP-tagged NLS deletion mutants including MgdE^{ΔNLS1}, MgdE^{ΔNLS2}, and MgdE^{ΔNLS1-2} were constructed and transfected into HEK293T cells. Fluorescence intensity profiling revealed that individual deletion of either NLS1 or NLS2 partially reduced the nuclear targeting efficiency, whereas simultaneous deletion of both the NLS motifs (MgdE^{ΔNLS1-2}) completely abrogated its nuclear localization (Figure 2D and E). These observations were further confirmed by immunoblot analysis of nuclear fractions, which showed that at 36 h post-transfection, MgdE-EGFP and its mutant variants were consistently detected in the cytoplasmic compartment, and nuclear accumulation of MgdE^{ΔNLS1-2} was nearly undetectable compared to that of MgdE, MgdE^{ΔNLS1}, and MgdE^{ΔNLS2} (Figure 2F). Together, these findings demonstrate that NLS1 and NLS2 are both required for efficient nuclear translocation of MgdE.

Nuclear localization of MgdE enhances mycobacterial intracellular survival within macrophages

Given that MgdE exhibits nuclear translocation ability, we sought to determine whether this ability plays a role in bacterial survival during infection. We generated various recombinant *M. bovis* BCG strains, including wild-type BCG (WT), a *mgdE* deletion mutant (ΔMgdE) (Figure S3A and B), ΔMgdE complemented with wild-type *mgdE* (Comp-MgdE), and ΔMgdE complemented with NLS-deleted variant (Comp-MgdE^{ΔNLS1-2}). All strains exhibited similar growth under standard culture conditions (Figure S3C), and subsequently used them to infect THP-1 macrophages. As shown in Figure 3A, the ΔMgdE mutant strain exhibited significantly reduced survival compared to the WT strain, with accelerated bacterial clearance over time. This defect was rescued in the Comp-MgdE strain, with intracellular survival levels restored to that in the WT strain. Notably, the Comp-

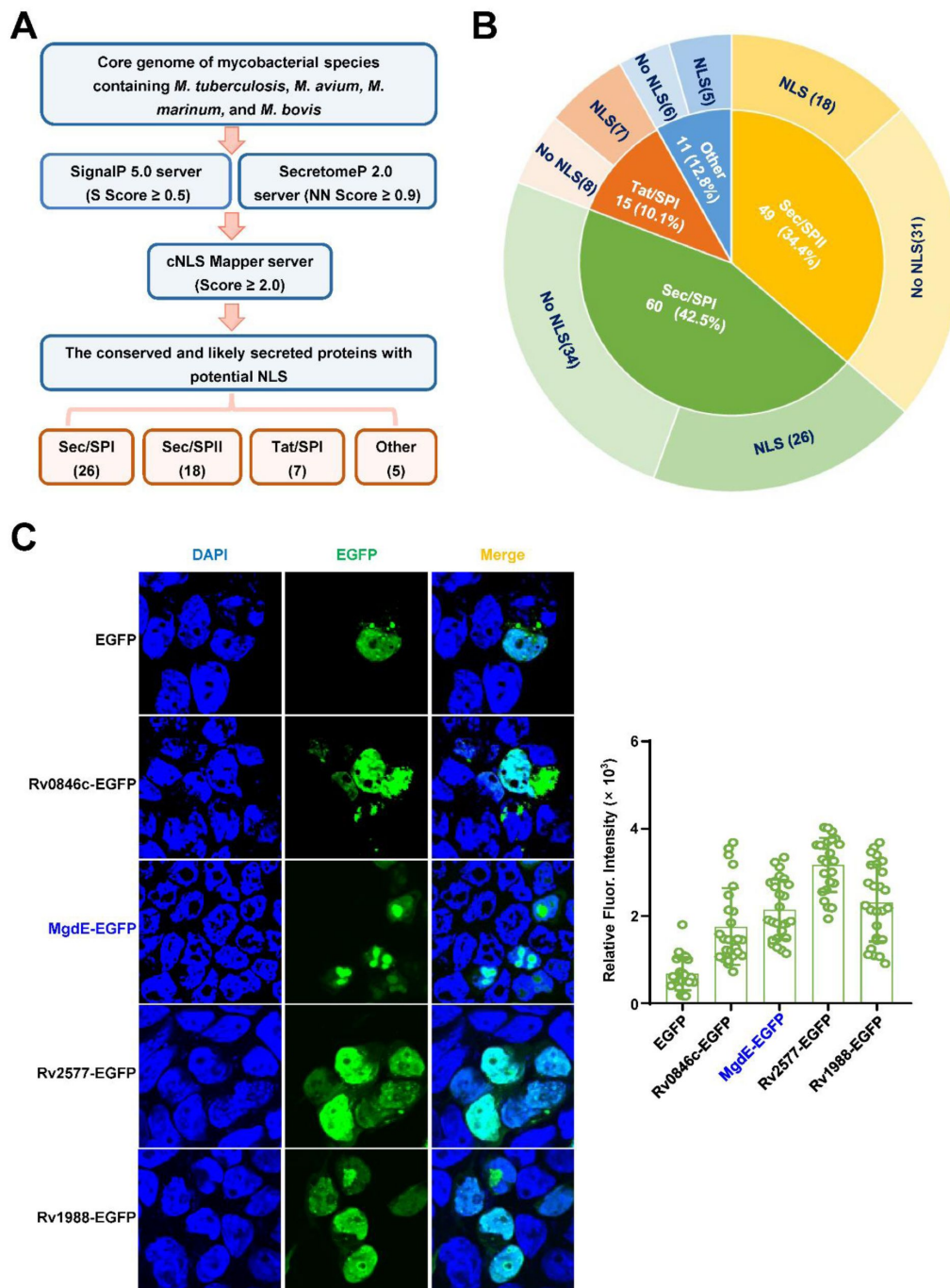


Figure 1.

Identification of conserved nucleomodulins in mycobacteria through functional screening.

(A) Schematic representation of the bioinformatic pipeline for identifying conserved nucleomodulins in Mycobacterium species. Genomic sequences from *M. tuberculosis* H37Rv, *M. tuberculosis* H37Ra, *M. avium*, *M. marinum*, and *M. bovis* BCG were analyzed. Signal peptides were predicted using SignalP 5.0 (D-score ≥ 0.5), non-classical secretion signals were identified using SecretomeP 2.0 (NN score ≥ 0.9), and nuclear localization signals (NLSs) were predicted using cNLS Mapper (score ≥ 2.0). (B) Classification of conserved cellular nucleomodulins. Secreted proteins were categorized based on the presence or absence of predicted NLS motifs. (C) Subcellular localization of EGFP-tagged candidate nucleomodulins. (left) Confocal images of Rv0846c, MgdE, Rv2577, and Rv1988 fused to EGFP (green); nuclei are stained with DAPI (blue). (right) Quantification of nuclear EGFP intensity. Rv1988 and pEGFP were used as positive and negative controls, respectively. Scale bar, 10 μ m. Data are presented as mean $\pm$ SD (n = 25 cells/group).

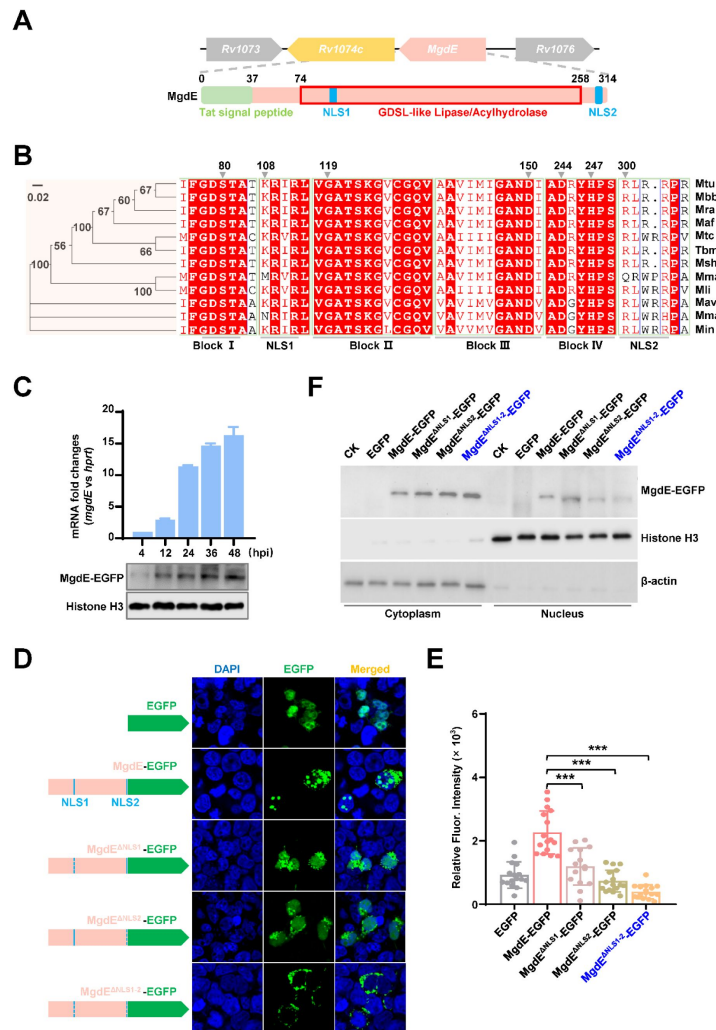


Figure 2.

Hypothetical protein MgdE enters the host nucleus via dual NLS.

(A) Domain architecture of MgdE. Schematic representation of MgdE with the annotated functional domains, including a Tat signal peptide (1–37 aa, twin-arginine translocation motif), a GDSL-like lipase/acylhydrolase catalytic domain (74–258 aa), and two nuclear localization signals (NLS1: 108–111 aa; NLS2: 300–305 aa). (B) Phylogenetic and structural conservation of MgdE. (left) Neighbor-joining phylogenetic tree of MgdE homologs across *Mycobacterium* species (1,000 bootstrap replicates; values $\geq 50\%$ shown). (right) Clustal Omega sequence alignment highlighting conserved residues ($\geq 90\%$ identity, red). Species abbreviations: *M. tuberculosis* (Mtu), *M. bovis* BCG (Mbb), *M. tuberculosis* H37Ra (Mra), *M. africanum* (Maf), *M. tuberculosis* CDC1551 (Mtc), *M. tuberculosis* GM041182 (Tbm), *M. shinjuense* (Msh), *M. marinum* (Mma), *M. lupini* (Mli), *M. avium* (Mav), *M. manitobense* (Mman), *M. intracellulare* (Min). (C) Expression and nuclear localization of MgdE. (upper) qRT-PCR analysis of mgdE mRNA in HEK293T cells over 48 h post-transfection. (lower) Western blot analysis of nuclear fractions showing time-dependent nuclear accumulation of MgdE-EGFP. Histone H3 and β -actin served as nuclear and cytoplasmic markers, respectively. (D) Subcellular localization of EGFP-tagged wild-type and NLS-deleted MgdE. (left) Schematic representation of EGFP-tagged constructs, including wild-type MgdE and its mutants (MgdE^{ΔNLS1}, MgdE^{ΔNLS2}, and MgdE^{ΔNLS1-2}). (right) Confocal microscopy images of HEK293T cells transfected with the indicated constructs for 36 h. EGFP fluorescence (green) and nuclear staining with DAPI (blue) were visualized using a FLUOVIEW software (v5.0). Scale bar, 10 μ m. Images were acquired with a $\times 100$ oil immersion objective (NA = 1.4). (E) Nuclear EGFP intensity of wild-type and mutant constructs in (D). Data are shown as mean $\pm$ SD (n = 15 cells). (F) Western blot analysis of nuclear and cytoplasmic fractions from HEK293T cells transfected with wild-type and mutant MgdE-EGFP confirmed their nuclear localization. MgdE-EGFP was detected using an anti-GFP antibody, and histone H3 and β -actin served as nuclear and cytoplasmic markers, respectively. All experiments were performed in three biologically independent replicates. Statistical significance was assessed by two-tailed unpaired Student's *t*-tests, with $*P < 0.05$ considered statistically significant.

MgdE^{ΔNLS1-2} showed bacterial clearance equivalent to that in the ΔMgdE strains. These results were consistent with the infection experiments using RAW264.7 macrophage (**Figure 3B** [↗](#)). Furthermore, macrophages infected with the ΔMgdE mutant strains exhibited significant upregulation of inflammatory cytokines, including *il1β*, *il6*, *il10*, and the colony-stimulating factors *csf1-csf3* compared to WT-infected controls (**Figure 3C-H** [↗](#)). Collectively, these results demonstrated that MgdE enhances mycobacterial intracellular survival and suppresses host inflammatory responses during infection, with both NLS1 and NLS2 motifs being essential for its nuclear trafficking-dependent function.

MgdE physically interacts with the host COMPASS complex subunits ASH2L and WDR5

To identify the potential host target proteins of MgdE, we employed AlphaFold to predict the interactions between MgdE and majority of the nuclear proteins in the host. The prediction results showed that MgdE binds to WDR5, a core component of the histone methyltransferase COMPASS complex, with high confidence (pLDDT = 0.77) (**Figure S4A and B**). To experimentally validate this prediction, we performed yeast two-hybrid (Y2H) assays. Strain co-expressing MgdE with either ASH2L or WDR5 exhibited robust growth on selective SD/-Trp-Leu-His-Ade medium, whereas strain co-expressing MgdE with DPY30 or RbBP5 showed no detectable growth, confirming specific interactions between MgdE and ASH2L/WDR5 (**Figure 4A** [↗](#)). Further, these interactions were validated by co-immunoprecipitation (Co-IP) assays. HEK293T cells were co-transfected with Flag-tagged MgdE and HA-tagged ASH2L, WDR5, or RbBP5. The results revealed that HA-tagged ASH2L and WDR5, but not RbBP5, co-precipitated with Flag-tagged MgdE (**Figure 4B** [↗](#)). Conversely, Flag-tagged MgdE precipitated HA-tagged ASH2L and WDR5, but not RbBP5 (**Figure 4C** [↗](#)). Taken together, these results demonstrate that MgdE specifically interacts with the COMPASS complex subunits ASH2L and WDR5.

The conserved residues D²⁴⁴ and H²⁴⁷ are critical for mediating the binding of MgdE to WDR5

To further confirm the interactions between MgdE and WDR5/ASH2L, the key amino acids of MgdE (S⁸⁰, D²⁴⁴, and H²⁴⁷) were individually replaced with alanine (A) (Yang et al., 2019 [↗](#)). The Y2H assays showed that single-residue substitutions of MgdE did not affect its interaction with ASH2L or WDR5. However, the D²⁴⁴A/H²⁴⁷A double mutation specifically abrogated its interaction with WDR5 but retained its ASH2L binding ability (**Figure 5A** [↗](#)). These findings were further confirmed by Co-IP assays in HEK293T cells. Flag-tagged MgdE precipitated HA-tagged ASH2L and WDR5 but not RbBP5, with reciprocal pull-downs confirming bidirectional binding specificity. The D²⁴⁴A/H²⁴⁷A double mutant maintained its interaction with ASH2L but failed to bind WDR5, suggesting that these interactions are mediated through distinct molecular interfaces (**Figure 5B** [↗](#), **Figure S5**). To further investigate the functional consequences of these mutations, we analyzed the subcellular localization of the MgdE mutants in transfected HEK293T cells using fluorescence co-localization assays. Wild-type MgdE exhibited progressive accumulation of punctate nuclear foci over time, a phenomenon that was abrogated following transfection with the D²⁴⁴A/H²⁴⁷A mutant (**Figure 5C** [↗](#)), suggesting that the double mutation impairs MgdE activity.

Given that MgdE interacts with WDR5, a subunit of COMPASS complex, which is a key regulator of H3K4me3 methylation (Deng et al., 2024 [↗](#)), we sought to determine whether this interaction contributes to an epigenetic regulatory mechanism that facilitates bacterial persistence within host cells. We transfected HEK293T cells with the EGFP-tagged WT MgdE and mutated MgdE-D²⁴⁴A/H²⁴⁷A, and monitored H3K4me3 modifications over time. Strikingly, immunoblotting of nuclear extracts collected at defined time points revealed that cells expressing WT MgdE exhibited approximately 2-fold lower H3K4me3 levels compared to those expressing the MgdE-D²⁴⁴A/H²⁴⁷A

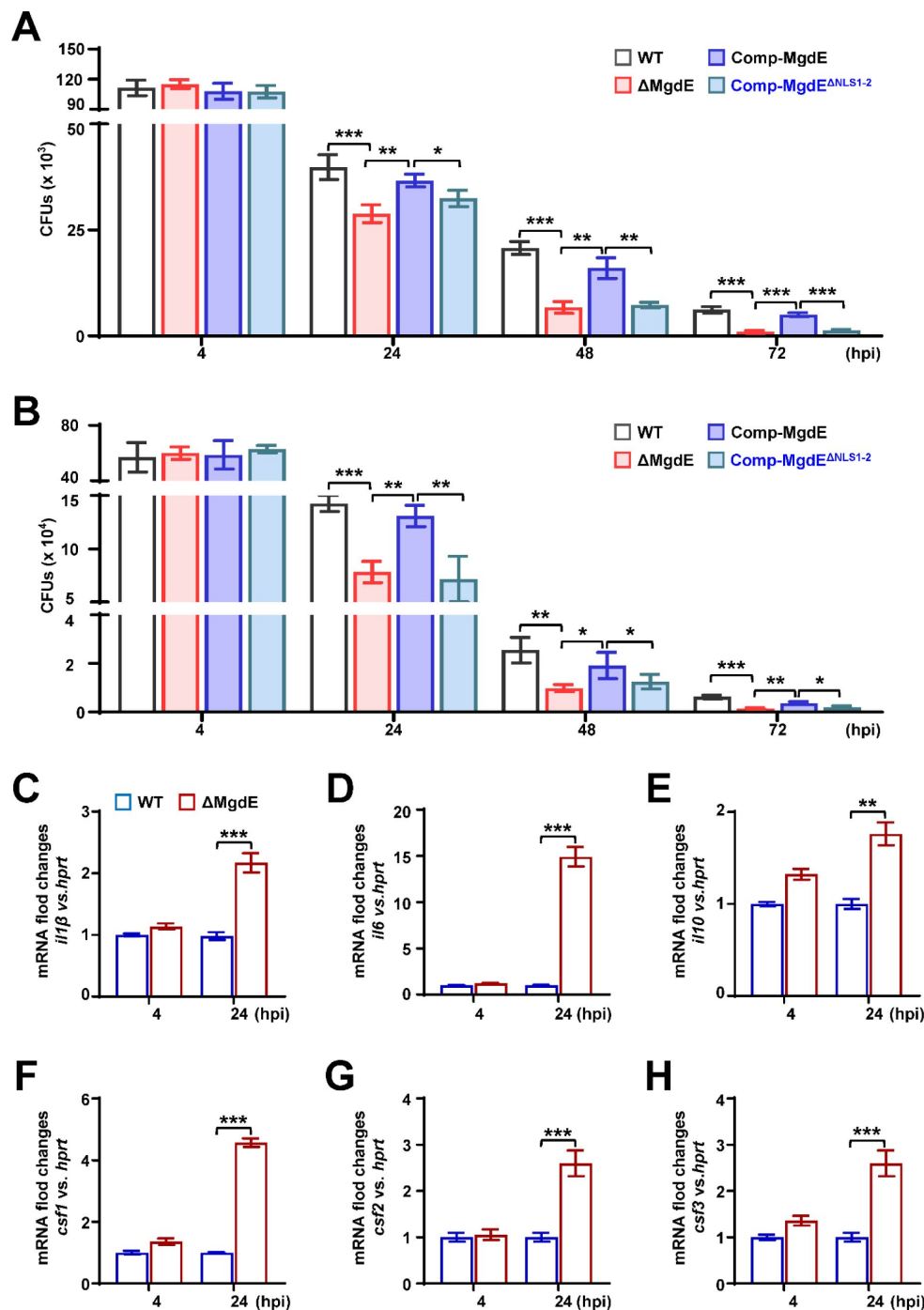


Figure 3.

Nuclear localization of MgdE facilitates mycobacterial intracellular survival in macrophages.

(A-B) Intracellular survival of *M. bovis* BCG strains. THP-1 human macrophages (A) and RAW264.7 murine macrophages (B) were infected (MOI = 10) with wild-type BCG (WT), MgdE deletion mutant (Δ MgdE), complemented strain (Comp-MgdE), or NLS-deficient complement (Comp-MgdE^{ANLS1-2}). Bacterial survival was assessed by CFU enumeration at 2, 24, 48, and 72 h post-infection. (C-H) Cytokine expression in the infected THP-1 cells. qRT-PCR analysis of cytokine mRNA levels in THP-1 cells infected with WT or Δ MgdE strains for 4–24 h. Inflammatory cytokine expression in infected THP-1 cells. qRT-PCR analysis of intracellular cytokine mRNA levels in THP-1 cells infected with WT or Δ MgdE strains for 4–12 h. Target genes include *il1 β* (C), *il6* (D), *il10* (E), *csf1* (F), *csf2* (G), and *csf3* (H). Data are presented as mean $\pm$ SD of three biologically independent experiments, analyzed using two-tailed unpaired Student's *t*-tests (**P* < 0.05, ***P* < 0.01, and ****P* < 0.001).

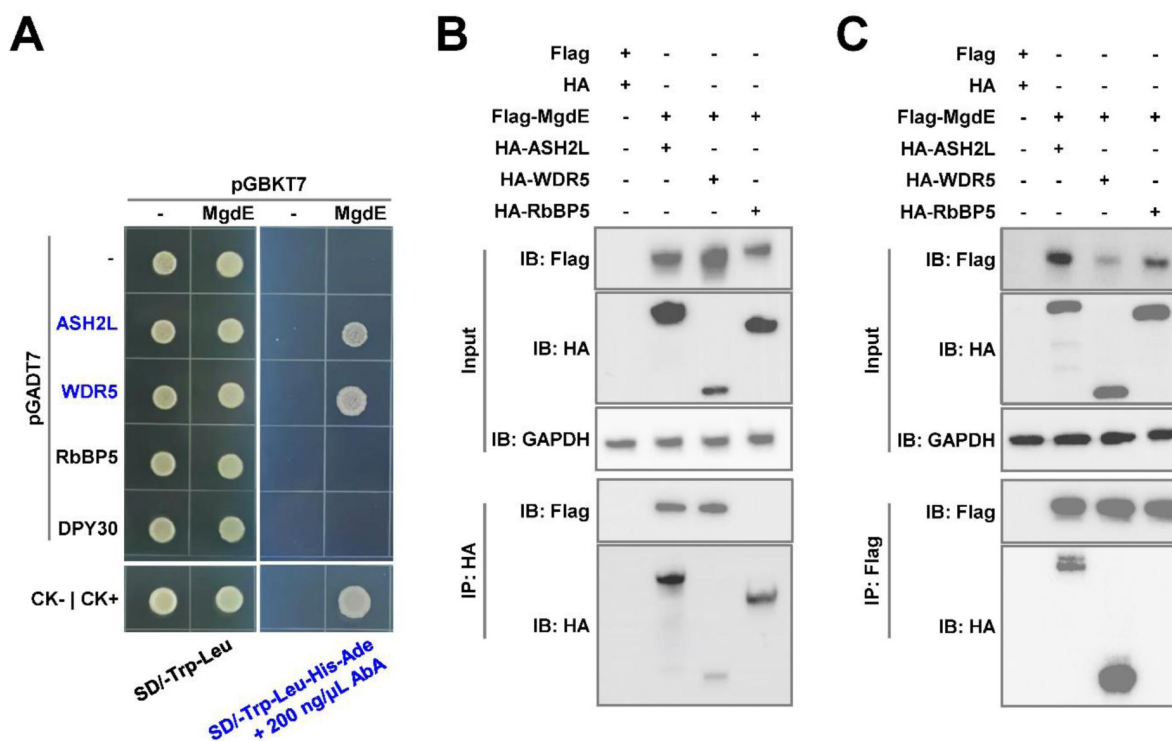


Figure 4.

MgdE directly interacts with ASH2L and WDR5, core components of the host COMPASS complex.

(A) Yeast cells were co-transformed with bait (pGBKT7) and prey (pGADT7) plasmids expressing wild-type MgdE and human COMPASS components (ASH2L, WDR5, RbBP5, and DPY30). Growth was monitored on non-selective (-Leu/-Trp, left) and selective (-Leu/-Trp/-Ade/-His + 200 ng/μL aureobasidin A, right) media. Controls: CK- (pGBKT7-*lam* + pGADT7-T, negative); CK+ (pGBKT7-*p53* + pGADT7-T, positive). (B-C) Cells were co-transfected with Flag-MgdE and HA-tagged ASH2L, WDR5, or RbBP5 (1:1 molar ratio). At 36 h post-transfection, the lysates were immunoprecipitated using (B) anti-HA or (C) anti-Flag antibodies, followed by immunoblotting with anti-HA, anti-Flag, and anti-GAPDH (loading control). The input lanes represent 5% of the total lysate. Data are representative of three independent experiments.

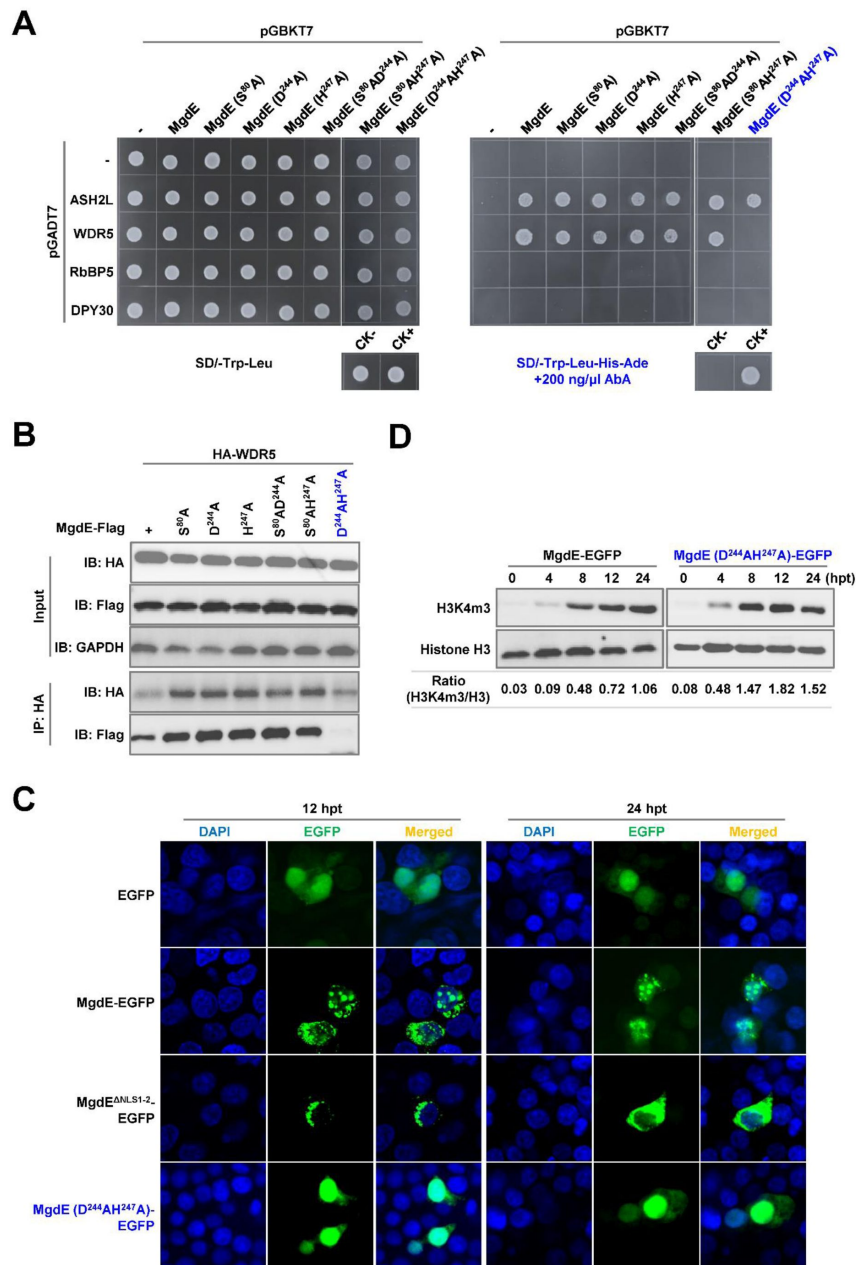


Figure 5.

The conserved residues D²²⁴ and H²⁴⁷ mediate the binding ability of MgdE to WDR5.

(A) Y2H assay identifying interactions between MgdE mutants and COMPASS complex subunits. Yeast cells were co-transformed with bait (pGBKT7) and prey (pGADT7) plasmids expressing wild-type or mutant MgdE and human COMPASS subunits (ASH2L, WDR5, RbBP5, and DPY30). Growth was assessed on non-selective (-Leu/-Trp, left) and selective (-Leu/-Trp/-Ade/-His + 200 ng/μL aureobasidin A, right) media. Controls: CK- (pGBKT7-*lam* + pGADT7-T, negative) and CK+ (pGBKT7-*p53* + pGADT7-T, positive). (B) Co-IP analysis of MgdE mutants with WDR5. HEK293T cells were co-transfected with Flag-tagged MgdE mutants and HA-tagged WDR5 (1:1 molar ratio). Complexes were immunoprecipitated using anti-HA antibody and protein A/G beads, followed by immunoblotting with anti-Flag and anti-HA antibodies. (C) Nuclear distribution of wild-type and mutants MgdE. Confocal microscopy of HEK293T cells expressing wild-type or D²²⁴A/H²⁴⁷A MgdE-EGFP at 12 and 24 h post-transfection (hpt). Nuclear foci were visualized by EGFP (green) and DAPI (blue) staining. Scale bar, 10 μm. Images were acquired with a ×100 oil immersion objective (NA = 1.4). (D) Immunoblot analysis of H3K4me3 levels. HEK293T cells expressing wild-type or D²²⁴A/H²⁴⁷A mutant MgdE were analyzed for changes in H3K4me3 levels over 0–24 h post-transfection. Histone H3 was used as a loading control. The data represent two independent biological replicates.

mutant, an effect detectable within 4–12 h post-transfection (**Figure 5D** [↗](#)). These findings indicate that MgdE inhibits COMPASS-dependent methyltransferase activity via its functional interaction with WDR5.

Together, these data indicate that the residues D²⁴⁴ and H²⁴⁷ are essential for the MgdE-WDR5 interaction, which enables MgdE to attenuate H3K4me3 deposition in the host nuclei by subverting COMPASS complex function, a mechanism critical for bacterial survival and immune evasion.

MgdE suppresses inflammatory responses mediated by the COMPASS complex via inhibition of H3K4 methylation

As H3K4me3, a hallmark of transcriptionally active promoters, facilitates transcriptional machinery assembly and RNA polymerase II recruitment ([Wang et al., 2023](#) [↗](#)), we further investigated the transcriptional consequences of MgdE-mediated H3K4me3 suppression via its interaction with the COMPASS complex. To assess the genome-wide effects of MgdE on host gene expression, we performed RNA-seq profiling of THP-1 macrophages infected with either WT BCG (BCG/wt) or MgdE-deleted (*KomgdE*) strains. Transcriptomic analysis at 24 h post-infection revealed that 271 genes showed significant differential expression in the cells infected with *KomgdE* strain compared to that in the cells infected with WT BCG strain, with 222 genes being transcriptionally upregulated in the absence of MgdE (**Figure 6A** [↗](#)). Gene ontology (GO) enrichment analysis indicated that these upregulated genes were primarily associated with biological processes involved in immune responses, with a notable enrichment in “Positive regulation of cytokine production” (GO: 0001819), a critical pathway for amplifying inflammatory signaling (**Figure 6B** [↗](#)). Furthermore, the Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis highlighted a significant activation of the “Cytokine-cytokine receptor interaction” and “JAK-STAT signaling pathway” in THP-1 cells infected with the *KomgdE* strain (**Figure 6C** [↗](#), Figure S6A). Heatmap analysis further confirmed the marked upregulation of inflammation-associated transcripts, including inflammatory cytokines (*il6*, *il10*, and *il12b*), chemokines (*ccl1-ccl5*), and colony-stimulating factors (*csf1-csf3*), in *KomgdE*-infected cells (**Figure 6D** [↗](#), Figure S6B). Protein-protein interaction network analysis of the upregulated genes revealed tightly interconnected clusters within inflammatory networks, particularly those orchestrated by the central inflammatory mediator IL-6 (Figure S6C). To validate this result, we infected macrophages with the *M. bovis* BCG strains, including WT, ΔMgdE, Comp-MgdE, and Comp-MgdE^{ΔNLS1-2}, under identical conditions for 24 h and examined the expression of relevant inflammatory factors. Compared to cells infected with the WT and Comp-MgdE strains, those infected with the ΔMgdE and Comp-MgdE^{ΔNLS1-2} strains exhibited significantly increased expression levels of inflammatory factors (*il6*, *il10*, and *il12b*) (**Figure 6E-G** [↗](#)). Collectively, these results demonstrate that MgdE effectively inhibits the production of inflammatory factors during BCG infection and highlights the critical role of its NLSs in this process. Through its interaction with the COMPASS complex, MgdE suppresses H3K4me3 levels, thereby attenuating the transcriptional activation of inflammation-related genes.

Nuclear localization of MgdE is essential for mycobacterial survival in mice

Our experimental data demonstrated that MgdE enhances bacterial survival in macrophages. To further investigate the *in vivo* functional significance of MgdE in host-pathogen interplay, we infected C57BL/6 mice with different *M. bovis* BCG strains, including WT, ΔMgdE, Comp-MgdE, and Comp-MgdE^{ΔNLS1-2}, and comprehensively evaluated bacterial survival, pulmonary inflammation, and cytokine dynamics. Mice infected with ΔMgdE exhibited a significantly reduced lung bacterial burden compared to those infected with WT or Comp-MgdE strains. Moreover, mice infected with Comp-MgdE^{ΔNLS1-2} exhibited a significantly lower pulmonary bacterial load than those infected with Comp-MgdE (**Figure 7A** [↗](#)). These findings collectively establish that MgdE is essential for

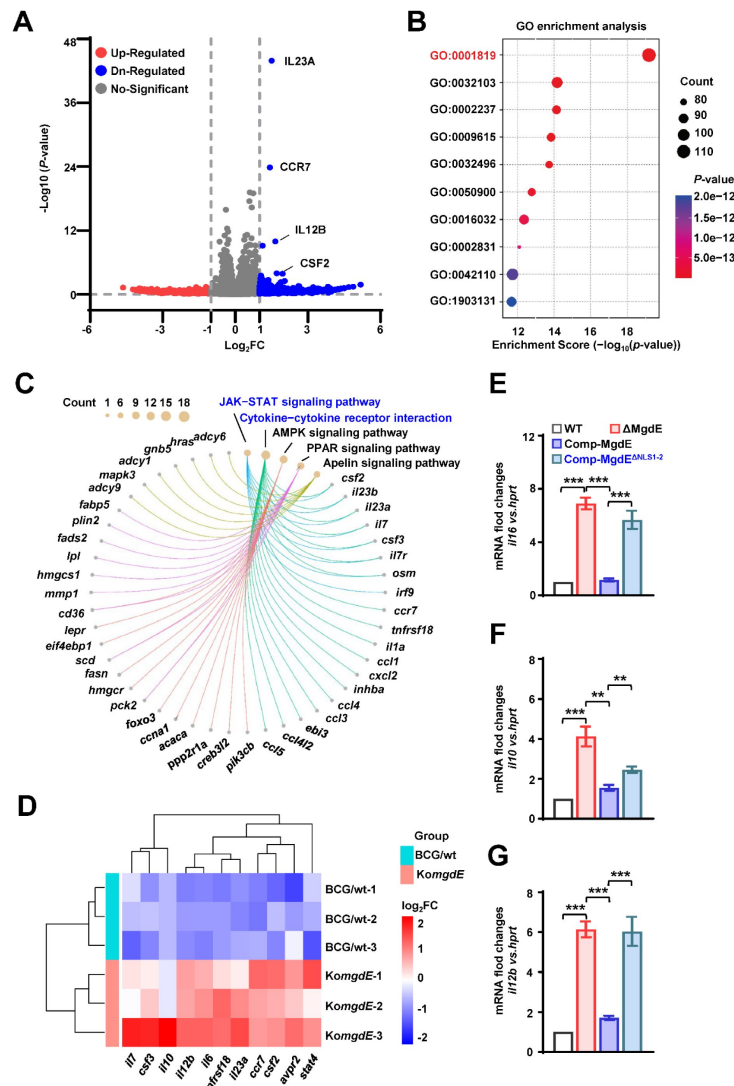


Figure 6.

MgdE suppresses COMPASS complex-mediated inflammatory responses by inhibiting H3K4 methylation.

(A) Volcano plot of DEGs. DEGs between MgdE-deleted strain (*KomgdE*) and wild-type BCG (WT) were visualized in a volcano plot. Genes with $|\log_2\text{-fold change}| \geq 1$ and $P < 0.05$ were considered significant. The x-axis represents $\log_2$ fold change, and the y-axis shows $-\log_{10}(P\text{-value})$. (B) GO enrichment analysis of DEGs. GO analysis revealed the significant enrichment of immune and inflammatory processes in *KomgdE*-infected macrophages compared to that in WT-infected cells, including positive regulation of response to external stimulus (GO:0032103), response to molecule of bacterial origin (GO:0002237), response to virus (GO:0009615), response to lipopolysaccharide (GO:0032496), leukocyte migration (GO:0050900), viral process (GO:0016032), regulation of response to biotic stimulus (GO:0002831), T cell activation (GO:0042110), and mononuclear cell differentiation (GO:1903131). (C) KEGG pathway enrichment analysis of DEGs. Chord diagram of KEGG pathway enrichment analysis showing signaling pathways that are significantly enriched in *KomgdE*, including cytokine-cytokine receptor interaction and JAK-STAT signaling as the top enriched pathways. (D) Heatmap of the inflammatory gene expression. Heatmap depicting $\log_2$ -foldchange levels of inflammatory genes involved in the JAK-STAT and cytokine signaling pathways. Upregulated and downregulated genes in *KomgdE* are shown in green and red, respectively. The data were Z-score normalized. (E-G) qRT-PCR analysis of cytokine mRNA levels in THP-1 cells infected with WT, Δ MgdE, MgdE-complemented (Comp-MgdE), and NLS-deleted complement (Comp-MgdE^{ΔNLS1-2}) at 24 h post-infection. Cytokines analyzed include *il6* (E), *il10* (F), and *il12b* (G). Data are presented as mean $\pm$ SD of three biologically independent experiments, analyzed using two-tailed unpaired Student's *t*-tests ($*P < 0.05$, $**P < 0.01$, and $***P < 0.001$).

optimal bacterial survival during infection, with the NLS being critical for its full functionality. Furthermore, histopathological assessment of the lung tissues corroborated these observations. Hematoxylin-eosin (H&E) staining revealed more pronounced inflammatory pathology in the lungs of mice infected with WT or Comp-MgdE strain compared to those infected with Δ MgdE or Comp-MgdE ^{Δ NLS1-2} (**Figure 7B** [↗](#)), underscoring the necessity of MgdE and its NLSs in sustaining inflammation during infection. To further investigate the systemic immune modulation mediated by MgdE, we quantified splenic bacterial colonization and the inflammatory cytokine profiles. Consistent with the attenuated bacterial survival observed in the lungs, mice infected with Δ MgdE and Comp-MgdE ^{Δ NLS1-2} exhibited a significantly reduced splenic bacterial load compared to those infected with WT or omp-MgdE (Figure S7). Despite diminished bacterial dissemination, infections with Δ MgdE and Comp-MgdE ^{Δ NLS1-2} strains triggered elevated expression of inflammatory cytokines (*il-1 β* , *il-6*) relative to infection with the WT and Comp-MgdE strains (**Figure 7C and D** [↗](#)). Collectively, these findings indicate that MgdE orchestrates mycobacterial survival within host tissues by suppressing inflammatory responses to evade pathogen clearance, with the NLS playing a pivotal role in mediating these host-pathogen interactions.

Discussion

MgdE functions as a critical nucleomodulin that contributes to mycobacterial pathogenesis

Its evolutionary conservation across mycobacterial species, coupled with nuclear translocation via dual N-terminal and C-terminal NLSs, supports its classification as a nucleomodulin. Previous investigations have described MgdE as a secreted effector protein (Penn et al., 2018 [↗](#)), and our experimental results independently confirm its secretion (Figure S1C). Furthermore, our findings demonstrate that MgdE's nuclear localization plays a critical role during infection. Disruption of MgdE expression or its NLS motifs significantly attenuated *M. bovis* BCG viability within macrophages (**Figure 3A and B** [↗](#)) and reduced bacterial burden in mice (**Figure 7A** [↗](#) and Figure S7), supporting the notion that nuclear targeting contributes to bacterial virulence. This phenotype aligns with the observed upregulation of MgdE during *Mtb* infection of alveolar macrophages (Pisu et al., 2020 [↗](#)), suggesting that nuclear activity of MgdE may contribute to an adaptive persistence mechanism during infection. Although MgdE was previously characterized as a lipase (Yang et al., 2019 [↗](#)), our findings reveal a novel functional duality wherein nuclear targeting coordinates bacterial survival through epigenetic interference while preserving its metabolic roles. This functional pleiotropy positions MgdE as a master virulence regulator, exploiting host-pathogen interface plasticity to sustain infection.

MgdE directly subverts the COMPASS complex to reprogram host epigenetics

To our knowledge, MgdE is the first identified bacterial effector that directly targets and subverts the COMPASS complex, a central regulator of H3K4 methylation. Nuclear-localized MgdE specifically interacts with the core COMPASS subunits ASH2L and WDR5, disrupting H3K4me3 deposition through structural interference. The COMPASS complex facilitates H3K4 methylation via a conserved assembly mechanism in which WDR5 scaffolds methyltransferases such as MLL1 through its WIN motif and stabilizes the ASH2L–RbBP5 dimer (Guarnaccia et al., 2021 [↗](#); Hsu et al., 2018 [↗](#)). This dimer, in turn, positions the SET domain for efficient substrate recognition (Xue et al., 2019 [↗](#)). MgdE appears to interfere with this assembly process, resembling strategies employed by host regulatory proteins.

Site-directed mutagenesis studies have identified critical residues (D²²⁴ and H²⁴⁷) within MgdE that are essential for WDR5 binding. Mutating these residues abolished both WDR5 interaction and H3K4me3 suppression (**Figure 5C** [↗](#), D), confirming their functional relevance. Moreover,

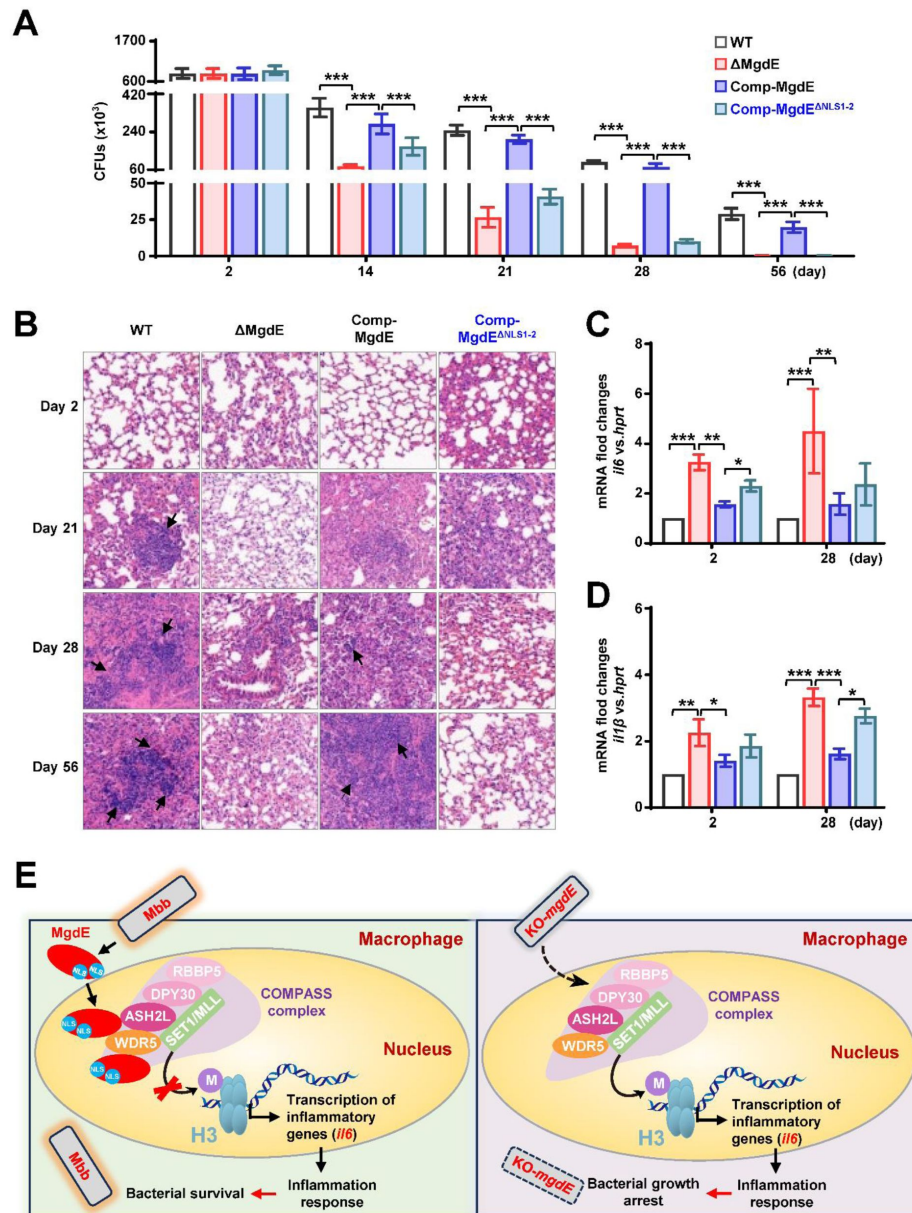


Figure 7.

Nuclear localization of MgdE is essential for mycobacterial survival in mice.

(A) Bacterial burden in the lungs of the infected mice. C57BL/6 mice ($n = 6/\text{group}$) maintained under SPF conditions were intratracheally infected with 1.0×10^7 colony-forming units (CFU) of *M. bovis* BCG strains, including wild-type (WT), MgdE-deleted (ΔMgdE), MgdE-complemented (Comp-MgdE), and NLS-deleted complement (Comp-MgdE^{ΔNLS1-2}). Lung bacterial loads were quantified using CFU assays at 0, 14, 28, and 56 d post-infection. (B) H&E-stained lung sections from infected mice (as in A) revealed granulomatous inflammation. Scale bars: 200 μm . (C-D) Pro-inflammatory cytokine expression in mice spleen. qRT-PCR analysis of cytokine mRNA levels of *il6* (C) and *il1β* (D) in spleen tissues from infected mice ($n = 6/\text{group}$) at 2 and 28 d post-infection. (E) Mechanistic model showing how mycobacterial nucleomodulin MgdE hijacks the COMPASS complex to suppress H3K4me3 and promote immune evasion. Upon *M. bovis* BCG infection, the nucleomodulin MgdE is delivered into the host nucleus via its nuclear localization signal (NLS) and directly binds to the COMPASS complex subunits, ASH2L or WDR5. This interaction disrupts H3K4 trimethylation (H3K4me3) deposition, leading to the epigenetic suppression of pro-inflammatory cytokine transcription (e.g., *il6*), thereby facilitating the intracellular survival of the pathogen. Data are presented as mean $\pm$ SD from six biologically independent experiments. Statistical significance was determined by a two-tailed unpaired Student's *t*-test (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

nuclear MgdE accumulates in punctate subnuclear foci (**Figure 1C**, **Figure 2D**, **Figure 5C**), reminiscent of *L. monocytogenes* InlP, which modulates nuclear speckles to inhibit apoptosis (Pourpre et al., 2022). This interaction silences the expression of antimicrobial effector genes, thereby facilitating immune evasion. Similar to *Legionella pneumophila* RomA, which methylates H3K14 to repress innate immune responses (Schator et al., 2023; Rolando et al., 2013), or *M. tuberculosis* Rv2067c, a structural mimic of host DOT1L that catalyzes non-nucleosomal H3K79 trimethylation to subvert pro-inflammatory signaling (Singh et al., 2023), MgdE exemplifies a broader pathogen strategy of hijacking the host histone methylation machinery.

MgdE-mediated epigenetic silencing suppresses pro-inflammatory responses

Transcriptome analysis showed that the *mgdE*-deleted BCG strain induced hyperactivation of cytokine-cytokine receptor interactions and the JAK-STAT signaling pathway, along with significantly elevated production of pro-inflammatory cytokines, including *il6* and *il1β*, in THP-1 macrophages compared to the WT BCG strain (**Figure 3C-F**, **Figure 6E-F**). This profile is consistent with the functional restoration of the COMPASS complex (Wang et al., 2025; Yu et al., 2017). These findings support the hypothesis that MgdE suppresses host inflammatory responses by disrupting COMPASS-mediated H3K4me3 deposition at active promoters. H3K4me3 is a well-established histone mark associated with transcriptional activation, in part through the recruitment of PHD domain-containing reader proteins (Hyun et al., 2017). For instance, SET1-dependent H3K4me3 accumulation at NF-κB target promoters such as *il6* and *tnfa* enhances pro-inflammatory gene expression (Bhattacharya et al., 2023). In addition, WDR5 has been implicated in upregulating immunosuppressive cytokines, including *il6* and *tgfb* (Deng et al., 2024). In transfected HEK293T cells, WT MgdE significantly reduced global H3K4me3 levels, whereas the catalytically inactive D²²⁴A/H²⁴⁷A mutant failed to do so (**Figure 5D**), supporting the notion that MgdE-mediated suppression of H3K4me3 depends on its ability to interact with WDR5. Furthermore, during infection, the *MgdE*-deleted BCG strain markedly activated the expression of inflammatory factors compared to the WT BCG strain (**Figure 6C-G**). These results suggest that MgdE attenuates the expression of inflammatory mediators such as *il6* and *il1β* by reducing host H3K4me3 levels during Mycobacterium infection. This mechanism is similar to those observed in other pathogens, such as spirochete-derived factors that suppress inflammation by reducing H3K4me3 levels at the *tnfa* and *il-6* promoters (Chauhan et al., 2015).

In summary, our study identified MgdE as a critical mycobacterial nucleomodulin and uncovered a novel paradigm of pathogen-mediated epigenetic regulation through the “MgdE-COMPASS complex-H3K4me3-cytokine” axis (**Figure 7F**). Mechanistically, MgdE specifically interacts with ASH2L and WDR5 to destabilize the COMPASS assembly, resulting in reduced H3K4me3 levels at the promoters of pro-inflammatory genes. This epigenetic suppression downregulates cytokine transcription (e.g., *il6* and *il1β*) and enhances bacterial survival in macrophages and in mice. Our findings reveal the COMPASS complex as a previously unrecognized target of bacterial effectors and offers mechanistic insights into immune evasion and host-pathogen epigenetic interplay.

Materials and methods

Bacterial strains and culture conditions

The bacterial strains used in this study are detailed in Table S4. *Escherichia coli* DH5α was cultured in Luria-Bertani medium under standard conditions. *Mycobacterium bovis* BCG strains were grown in Middlebrook 7H9 broth (BD Biosciences), containing 1× OADC (oleic acid, albumin, dextrose, catalase), 0.05% Tween-80, and 2% glycerol. Antibiotics were added as required at the following final concentrations: 50 μg/mL hygromycin (for mycobacteria) and 50 μg/mL kanamycin (for both mycobacteria and *E. coli*). The Y2HGold yeast strain (Takara Bio) was maintained in YPDA

(yeast extract, peptone, dextrose, and adenine sulfate) medium. Transformants were selected on SD/-Trp medium, which lacks tryptophan, to select for plasmid uptake. Protein-protein interactions were assessed in SD/-Trp/-Leu (lacking tryptophan and leucine) and SD/-Trp/-Leu/-Ade/-His (lacking tryptophan, leucine, adenine, and histidine) media, with growth on higher stringency media indicating positive interactions.

Cell culture

THP-1 cells were cultured in Roswell Park memorial institute 1640 (RPMI 1640) medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 1 mM sodium pyruvate, 2 mM L-glutamine, 10 mM HEPES buffer (pH 7.2–7.5), and 50 μ M 2-mercaptoethanol. For differentiation into macrophages, THP-1 cells were treated with 200 nM phorbol 12-myristate 13-acetate (PMA) for 24 h, followed by an additional 48-h incubation in fresh PMA-free medium to allow complete differentiation prior to their use in experiments.

HEK293T and RAW264.7 monocytes were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 50 μ g/mL penicillin-streptomycin. All cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂.

Pan-genomic nucleomodulin screening

To systematically identify evolutionarily conserved nucleomodulins in pathogenic mycobacteria, a pan-genomic comparative analysis was performed across four phylogenetically representative mycobacterium strains: *Mycobacterium tuberculosis* H37Rv (NCBI RefSeq assembly GCF_000195955.2), *Mycobacterium tuberculosis* H37Ra (NCBI RefSeq assembly GCF_001938725.1), *Mycobacterium bovis* BCG (NCBI RefSeq assembly GCF_000009445.1), *Mycobacterium marinum* (NCBI RefSeq assembly GCF_000018345.1), and *Mycobacterium avium* subsp. (NCBI RefSeq assembly GCF_009741445.1). Complete genome sequences and annotations were obtained from NCBI RefSeq with strict inclusion criteria requiring “Complete Genome” status and fewer than 50 contigs to ensure the assembly integrity. Plasmid sequences, as well as low-complexity and repetitive regions, were filtered using the NCBI Genome Data Viewer toolkit. Genome quality control was rigorously conducted using CheckM v1.2.2 with a *Mycobacteriaceae*-specific marker set (including essential housekeeping genes such as *dnaA* and *rpoB*), confirming that all the selected genomes exhibited >99% completeness and <1% contamination.

Ortholog clustering of protein-coding sequences (CDS) was executed through OrthoFinder v2.5.4, employing Diamond alignment in ultra-sensitive mode (--ultra-sensitive), an E-value cutoff of $\leq 1e-5$ (--blastp_evalue 1e-5), and a minimum sequence identity threshold of $\geq 50\%$ (--min_percent_identity 50). The Markov cluster algorithm (MCL) was applied with an inflation parameter of 1.5 (--mcl_inflation 1.5) to define orthologous groups, balancing sensitivity, and specificity. Core orthologs were identified as clusters present in all four strains, with additional filtering to retain only single-copy genes in $\geq 90\%$ of the strains, minimizing paralog-related artifacts.

Functional annotation of the core genes was conducted using InterProScan v5.62, which integrates domain predictions from the Pfam, TIGRFAM, and SUPERFAMILY databases. To prioritize candidate nucleomodulins, a sequential screening pipeline was applied, beginning with the prediction of classical secretory proteins using SignalP v5.0 (D-score ≥ 0.5), followed by the identification of non-classically secreted proteins via SecretomeP v2.0 (NN score ≥ 0.9), and culminating in NLS detection using cNLS Mapper (NLS score ≥ 2.0), which predicts both monopartite and bipartite NLSs. This integrated strategy enabled the systematic identification of conserved candidates that exhibit both secretory potential and nuclear targeting capacity.

Recombinant expression and secretion of MgdE

Recombinant *M. bovis* BCG strains expressing C-terminally 2×Flag-tagged Ag85B (positive control) and MgdE were constructed to assess protein secretion under standard in vitro culture conditions. The strains were cultured in 200 mL of Middlebrook 7H9 broth supplemented with albumin-dextrose-catalase (ADC), glycerol, and 0.05% Tween-80 at 37 °C until reaching mid-log phase ($OD_{600} = 0.6$). Bacterial cultures were centrifuged at $4,000 \times g$ for 60 minutes to separate cells from supernatants. Pellets were washed and lysed by bead beating in PBS supplemented with protease inhibitors. Culture supernatants were passed through 0.22 μm filters to remove residual bacteria and subsequently concentrated to 2 mL using Amicon Ultra centrifugal filters (10 kDa cutoff). Both bacterial lysates and concentrated supernatants were subjected to SDS-PAGE followed by immunoblotting with anti-Flag antibodies to detect the expression and secretion of 2×Flag-tagged proteins.

Phylogenetic and sequence analysis

To investigate the evolutionary conservation and sequence features of MgdE homologs, phylogenetic reconstruction was performed using MEGA 12.0 with a neighbor-joining algorithm. The parameters included pairwise deletion for gap treatment, a Poisson substitution model (selected for its suitability in analyzing closely related bacterial sequences), and 1,000 bootstrap replicates to assess node support. Nodes with $\geq 50\%$ bootstrap values were retained, and no additional pruning was performed.

The analysis incorporated MgdE homologs from 12 Mycobacterium strains spanning clinical, environmental, and attenuated lineages. These included Rv1075c (*M. tuberculosis* H37Rv), BCG_1133c (*M. bovis* BCG), MRA_1085 (*M. tuberculosis* H37Ra), MAF_10880 (*M. africanum*), MT1105 (*M. tuberculosis* CDC1551), TBMG_02912 (*M. tuberculosis* GM041182), MSHI_02760 (*M. shinjukuense*), MMAR_4392 (*M. marinum*), MULP_04607 (*M. lupini*), MAV_1199 (*M. avium*), MMAN_54310 (*M. manitobense*), and MIP_01781 (*M. intracellulare*). Genomic sequences were retrieved from the NCBI RefSeq database, filtered for completeness, and aligned using Clustal Omega v1.2.4 with default parameters (gap opening penalty = 10, gap extension = 0.2, and iterative refinement enabled). Sequence conservation was visualized using ESPript 3.0, highlighting residues with $\geq 90\%$ identity across all homologs in red.

Computational prediction of sequence and structural features

AlphaFold v2.2.0 was used to predict the interaction between MgdE and COMPASS complex subunits, following the methodology established in a previous study (Gómez Borrego et al., 2024 [\[1\]](#)). The specific parameters used for the prediction were as follows: model_preset = multimer; db_preset = full_dbs. A FASTA file containing the sequences of both MgdE and COMPASS complex subunit proteins was input into the AlphaFold software. The resulting models were evaluated and ranked based on the AF-score, which is a linear combination of the interface score (ipTM) and the predicted TM-score (pTM). The top-ranked model (rank 1) was selected for further analysis.

Cell transfection and confocal microscopy

HEK293T cells were seeded at 70% confluency in poly-L-lysine-coated 35-mm glass-bottom dishes and maintained in DMEM supplemented with 10% FBS at 37 °C under 5% CO₂. Transfection was performed using the Hieff Trans™ Liposomal Transfection Reagent (Yeasen) according to the manufacturer's protocol. Briefly, 0.5 μg of DNA and 1.5 μL of transfection reagent were mixed with 50 μL of Opti-MEM and incubated for 20 min at room temperature. The mixture was added dropwise to each chamber of 35-mm six-chamber glass-bottom dishes containing cells at 40–60% confluency.

At 24–48 h post-transfection, the cells were washed with phosphate-buffered saline (PBS), fixed in 4% paraformaldehyde for 15 min, and permeabilized with 0.1% Triton X-100 for 10 min. Nuclei were counterstained with 1 µg/mL DAPI (4',6-diamidino-2-phenylindole) for 10 min. Confocal imaging was performed using an Olympus FV1000 Confocal Laser Scanning Microscope equipped with a 100×/1.40 NA oil immersion objective. EGFP and DAPI signals were detected with 488 nm and 405 nm lasers, respectively. Spectral unmixing was applied using the Olympus FV10-ASW software (v4.2) to minimize signal cross-talk, and Z-stacks were acquired in sequential mode by applying a step size of 0.5 µm across all samples to ensure comparability.

Immunoblot analysis

For immunoblotting, the cells were lysed in radioimmunoprecipitation assay (RIPA) buffer supplemented with 1.5% protease inhibitor cocktail (Boster). Protein concentrations were quantified using the BCA assay (Thermo Fisher Scientific). Equal amounts of protein (20–40 µg) were resolved by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). The membranes were blocked for 1 h at room temperature with 5% non-fat milk in TBST (25 mM Tris, 150 mM NaCl, 2 mM KCl, and 0.2% Tween-20, pH 7.4) and incubated overnight at 4 °C with primary antibodies. After three washes with TBST (10 min each), the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for 1 h. Following three additional TBST washes, protein signals were detected using an enhanced chemiluminescence (ECL) substrate and imaged on a ChemiDoc MP system (Bio-Rad). β-actin, GAPDH, or histone H3 served as housekeeping proteins for normalization. The antibody information is listed in Table S3.

Cell fractionation was performed using a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime) according to the manufacturer's protocol. Briefly, after treatment, the cells were washed and harvested in cold PBS. Cytoplasmic and nuclear fractions were separated according to the manufacturer's instructions. Protein concentrations were quantified using a BCA protein assay kit, and equal amounts of protein were subjected to immunoblotting, as described above.

Co-IP assays were performed to analyze protein-protein interactions. HEK293T cells were washed twice with ice-cold PBS and lysed in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40) supplemented with 1.5% protease inhibitor cocktail (Boster) for 30 min on ice. Crude lysates were centrifuged at 12,000 ×g for 15 min at 4 °C to remove the debris. For immunoprecipitation, 1 mg of total protein was incubated with 5 µg of primary antibody overnight at 4 °C with gentle agitation. Protein A/G magnetic beads (MedChemExpress) were added to the antibody-protein complexes and incubated for 6 h at 4 °C. The beads were washed three times with PBS-T buffer (PBS containing 0.5% Tween-20) to remove nonspecific interactions. Proteins were eluted by boiling the beads in 1× SDS sample buffer at 95 °C for 5 min. The eluted proteins were resolved by SDS-PAGE and analyzed by immunoblotting.

Y2H assay

The Yeast Two-Hybrid System (Clontech) was used to assay protein-protein interactions between MgdE (WT and mutants) and subunits of the COMPASS complex (ASH2L, WDR5, RbBP5, and DPY30). Full-length cDNAs encoding WT MgdE and its mutants (see Table S5) were cloned into the bait vector pGBKT7, whereas genes encoding the COMPASS subunits were cloned into the prey vector pGADT7. The yeast strain Y2HGold was co-transformed with bait and prey plasmids using the lithium acetate/polyethylene glycol method, and the transformed colonies were initially selected on SD agar plates lacking leucine and tryptophan (-Leu/-Trp) to confirm plasmid retention. For interaction screening, colonies were replica-plated onto selective SD agar plates lacking leucine, tryptophan, adenine, and histidine (-Leu/-Trp/-Ade/-His) and supplemented with 200 ng/µL aureobasidin A (AbA, Sigma-Aldrich). The plates were incubated at 30 °C, and colony growth was monitored daily, with final assessments performed after 5 d of incubation. Interaction strength was qualitatively assessed by comparing colony density and growth rates with those of

the controls. The positive controls included the co-transformation of pGBKT7-p53 with pGADT7-T, which produced robust growth on –Leu/-Trp/-Ade/-His plates. Negative controls included co-transformation of pGBKT7-Lam (human lamin C, a non-interacting bait) with pGADT7-T, as well as individual transformations with empty pGBKT7 or pGADT7 vectors to assess the background growth. The yeast strains used in this study are listed in Table S4. All experiments were performed in triplicate with biologically independent yeast cultures. The results were consistent across replicates, confirming the reliability of the observed interactions.

qRT-PCR assay

THP-1 cells were infected with the *M. bovis* BCG strain and harvested 12 h post-infection for RNA isolation. Total RNA was extracted using 1 mL of TRIzol[®] reagent (Aidlab) according to the manufacturer's instructions. To remove genomic DNA contamination, the RNA samples were treated with DNase I (Thermo Fisher Scientific) for 30 min at 37 °C, followed by heat inactivation at 65 °C for 10 min. RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). For cDNA synthesis, 1 µg of total RNA was reverse transcribed using the HiScript[®] III cDNA Reverse Transcription Kit (Vazyme) with random hexamers, according to the manufacturer's protocol. qRT-PCR was performed using ChamQ SYBR[®] Green RT-PCR Master Mix (Vazyme). The relative mRNA expression of different genes was calculated by comparing their cycle threshold (Ct) values to that of a control gene *hprt* using the $2^{-\Delta\Delta C_t}$ method. Real-time qRT-PCR experiments were performed in triplicate, with at least three independent biological replicates and two technical replicates per condition. Primer sequences used for qRT-PCR are listed in Table S6.

RNA-seq analysis

THP-1 cells were seeded at a density of 1×10^6 cells/well in 6-well plates and differentiated into macrophages by treatment with 100 nM PMA for 48 h. Following differentiation, the cells were washed with PBS and infected with *M. bovis* BCG WT or *KomgdE* strains at a multiplicity of infection (MOI) of 10:1. Following 12 h of infection, total RNA was extracted using TRIzol[®] reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. High-quality reads were then mapped to the human reference genome (GRCh38) using HISAT2 (v2.2.1). FeatureCounts (v2.0.3) was used to quantify transcript abundance, and differential gene expression analysis was conducted using DESeq2 (v1.38.3) in R. Genes with an adjusted P-value <0.05 and an absolute log₂-foldchange ≥1 was considered significantly differentially expressed. RNA-seq data were visualized using GraphPad Prism 8.0, and volcano plots, heatmaps, and other visualizations were generated to summarize the results.

CFU assay

For bacterial preparation, mid-log phase cultures ($OD_{600} = 0.8$) of *M. bovis* BCG were washed with PBS and resuspended in fresh medium. THP-1 cells were seeded at a density of 5×10^5 cells/well in 24-well plates and subsequently infected at a multiplicity of infection (MOI) of 1:10. Following 4 h of infection, extracellular bacteria were eliminated by treatment with penicillin-streptomycin (100 µg/mL) for 2 h. The cells were then washed three times with PBS and maintained in an antibiotic-free medium until harvest. For bacterial recovery, the macrophages were lysed with 0.25% SDS for 10 min at room temperature, followed by serial dilution of the lysates in PBS. Aliquots (100 µL) of each dilution were plated on Middlebrook 7H10 agar supplemented with 10% OADC and kanamycin (50 µg/mL). Plates were incubated at 37 °C for 14 d, and CFUs were calculated by applying dilution factors to the colony counts. *M. bovis* BCG strains used in this study are listed in Table S4.

Animal experiments

Specific pathogen-free (SPF) female C57BL/6 mice (6–8 weeks old) were obtained from Changsheng Bio (Liaoning, China). All animals were housed under SPF conditions under controlled temperature, humidity, and a 12-hour light/dark cycle, with free access to food and water.

Mice infection

Mice were infected as described previously, with slight modifications. Mid-log phase *M. bovis* BCG cultures were washed twice in PBS containing 0.05% Tween 80 and sonicated to disrupt clumps. Female C57BL/6 mice (6–8 weeks old, 16–18g) were anesthetized using isoflurane and intranasally administered 1×10^7 CFUs of *M. bovis* BCG in 40 μ L of PBS. At 48 h post-infection, the bacterial load in the lungs of five mice was assessed to confirm the inoculation dose. The lungs were homogenized in 1 mL of PBS using a tissue homogenizer, and serial dilutions of the homogenates were plated onto Middlebrook 7H10 agar with 10% OADC. The plates were then incubated at 37 °C with 5% CO₂ for 14 d prior to colony counting.

Histological analysis

Lung tissues from *M. bovis* BCG-infected mice were fixed in 4% phosphate-buffered formalin at room temperature for 24 h and embedded in paraffin wax. Paraffin-embedded tissue samples were sectioned into 2–3 μ m thick slices using a microtome. The sections were deparaffinized, rehydrated with graded ethanol, and stained with hematoxylin and eosin (H&E). Stained slides were visualized using an Olympus BX53 light microscope, and CaseViewer version 2.0 (3DHISTECH) was used to view and annotate the scanned slides.

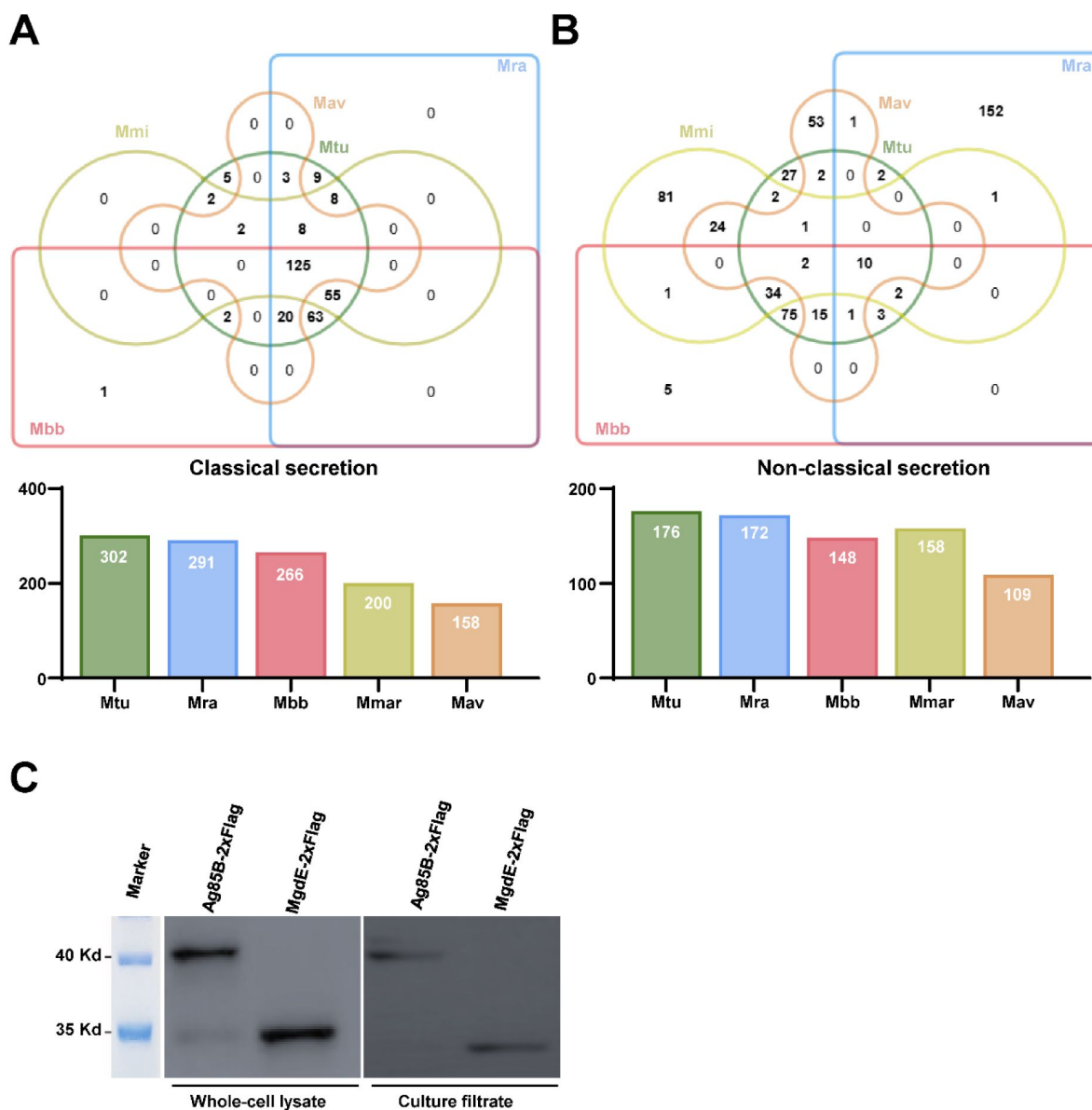
Ethics approval

All animal experiments were conducted in accordance with the protocols approved by the Animal Ethics Committee of the Huazhong Agricultural University.

Statistical analyses

Data are presented as mean $\pm$ standard deviation (SD). For comparisons between the two groups, statistical significance was determined using two-tailed unpaired Student's *t*-tests after confirming normality. Statistical significance was set at $P < 0.05$. Significance levels are denoted in the figures as follows: $P > 0.05$ (ns, not significant), $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). All statistical analyses were performed using GraphPad Prism (version 8.0). Data were collected from at least three independent biological replicates, each with two or more technical replicates.

Supplementary figures



Supplementary figure 1.

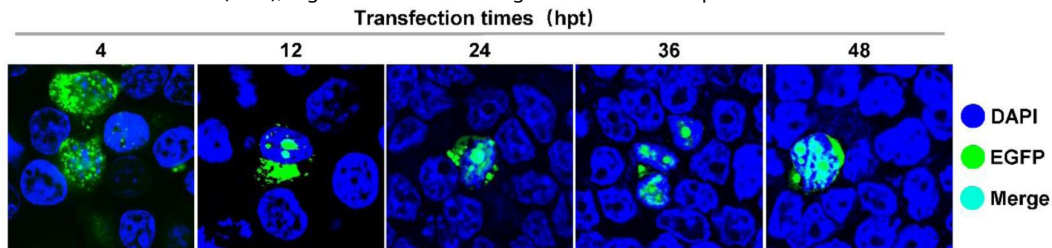
Comparative analysis of classical and non-classical secreted proteins in mycobacterial species.

(A) Venn diagram showing the distribution of predicted classical secreted proteins in four *Mycobacterium* species: *M. tuberculosis* H37Rv (Mtu) and *M. tuberculosis* H37Ra (Mra), *M. bovis* BCG (Mbb), *M. marinum* (Mmar), and *M. avium* (Mav). Proteins were predicted using SignalP 5.0, with a signal peptide score (D-score) ≥ 0.5 . A total of 125 proteins were conserved across all species. (B) Venn diagram showing the distribution of predicted non-classical secreted proteins across the same *Mycobacterium* species, predicted using SecretomeP 2.0 with a neural network (NN) score ≥ 0.9 . Ten proteins were conserved across all species. Bar graphs (left: classical, right: non-classical) summarize the total number of predicted secreted proteins per species. (C) Detection of secreted MgdE in culture supernatants. C-terminally Flag-tagged Ag85B (positive control) and MgdE were expressed in *M. bovis* BCG strains. Immunoblot analysis of bacterial lysates and culture supernatants showing the expression and secretion of Flag-tagged Ag85B and MgdE.

Supplementary Figure 2.

Subnuclear localization of MgdE-EGFP.

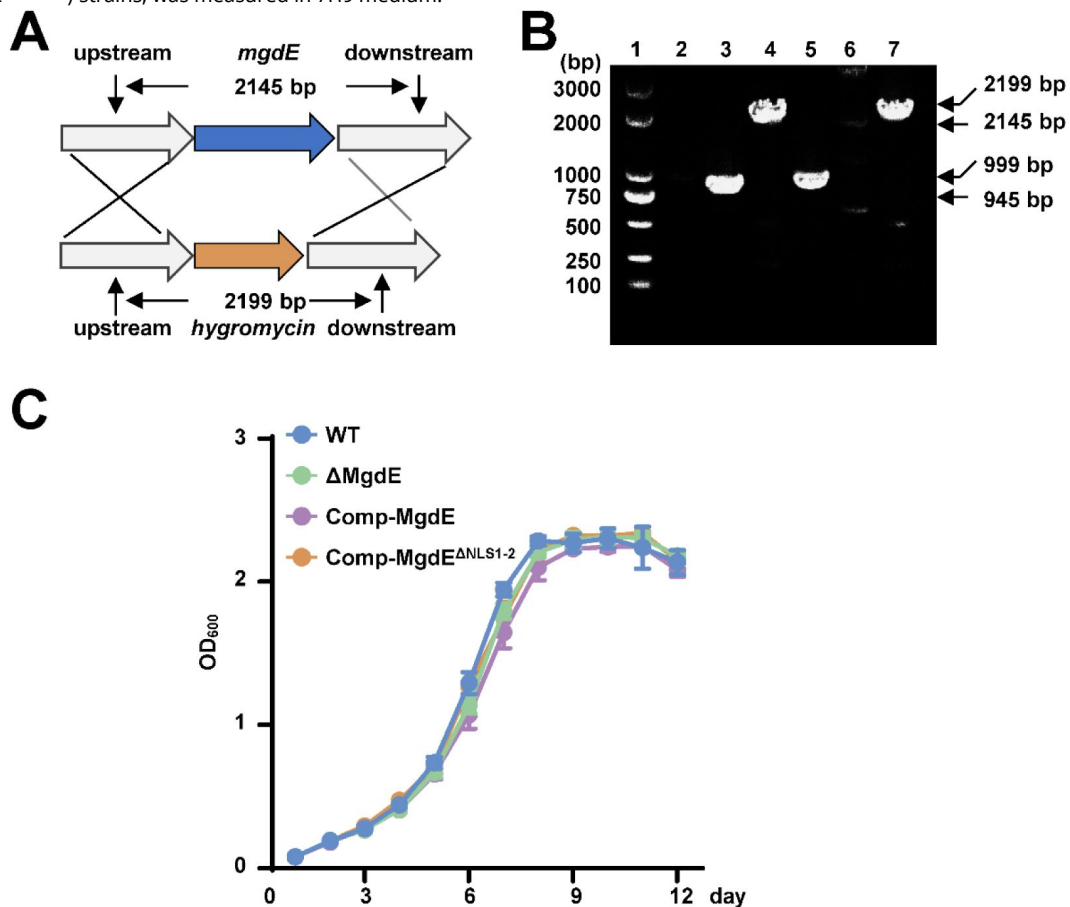
Confocal microscopy was used to assess the nuclear distribution of MgdE-EGFP at various time points post-transfection. Nuclei were stained with DAPI (blue); MgdE-EGFP is shown in green. Scale bar: 10 μ m.



Supplementary Figure 3.

Deletion of the nuclear localization signal of MgdE does not affect the growth of *M. bovis* BCG strains.

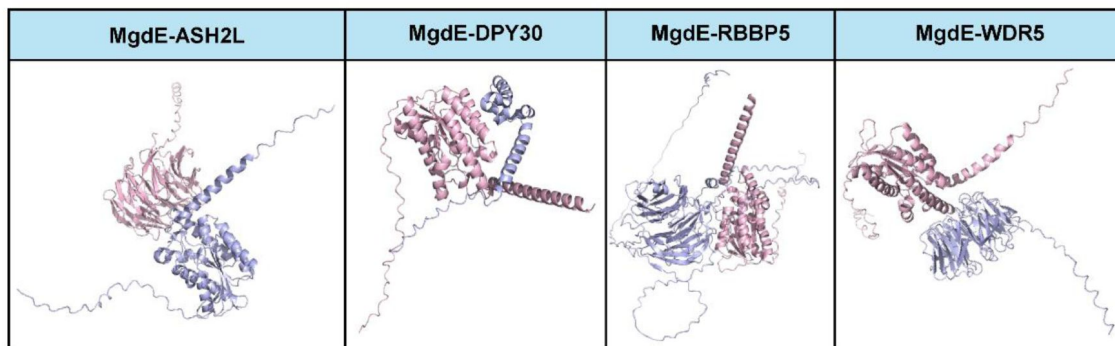
(A-B) Construction and validation of the MgdE-deleted strain of *M. bovis* BCG. (A) Schematic diagram of the homologous recombination strategy used to delete *mgdE* from the *M. bovis* BCG genome. (B) Wild-type and mutant strains were used as templates to amplify the *mgdE* gene (600 bp upstream–600 bp downstream) by PCR. Lanes 1 and 3: wild-type genomic DNA; lanes 2 and 4: Δ mgdE genomic DNA. (C) Growth curve analysis of *M. bovis* BCG strains. Growth of BCG strains, including wild-type BCG (WT), MgdE-deleted (Δ MgdE), MgdE-complemented (Comp-MgdE), and NLS-deleted complemented (Comp-MgdE ^{Δ NLS1-2}) strains, was measured in 7H9 medium.



A

Function	COMPASS complexes	Predicted binding confidence (pLDDT)
The catalytic subunit	SET1A/B	-
Required for H3K4me3	ASH2L	0.47
Required for assembly	RBBP5	0.30
Required for assembly	WDR5	0.77
	DPY30	0.62
	CXXC1	0.51
Components of the Set1 complexes	WDR82	-
	HCF1	0.37

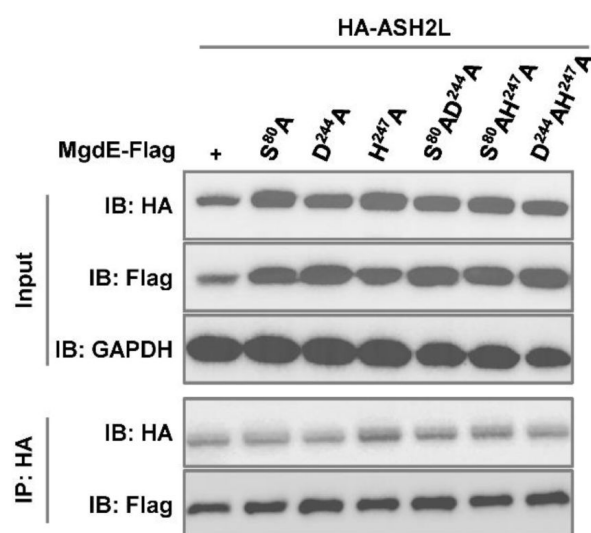
B



Supplementary Figure 4.

MgdE interacts with COMPASS complex subunits.

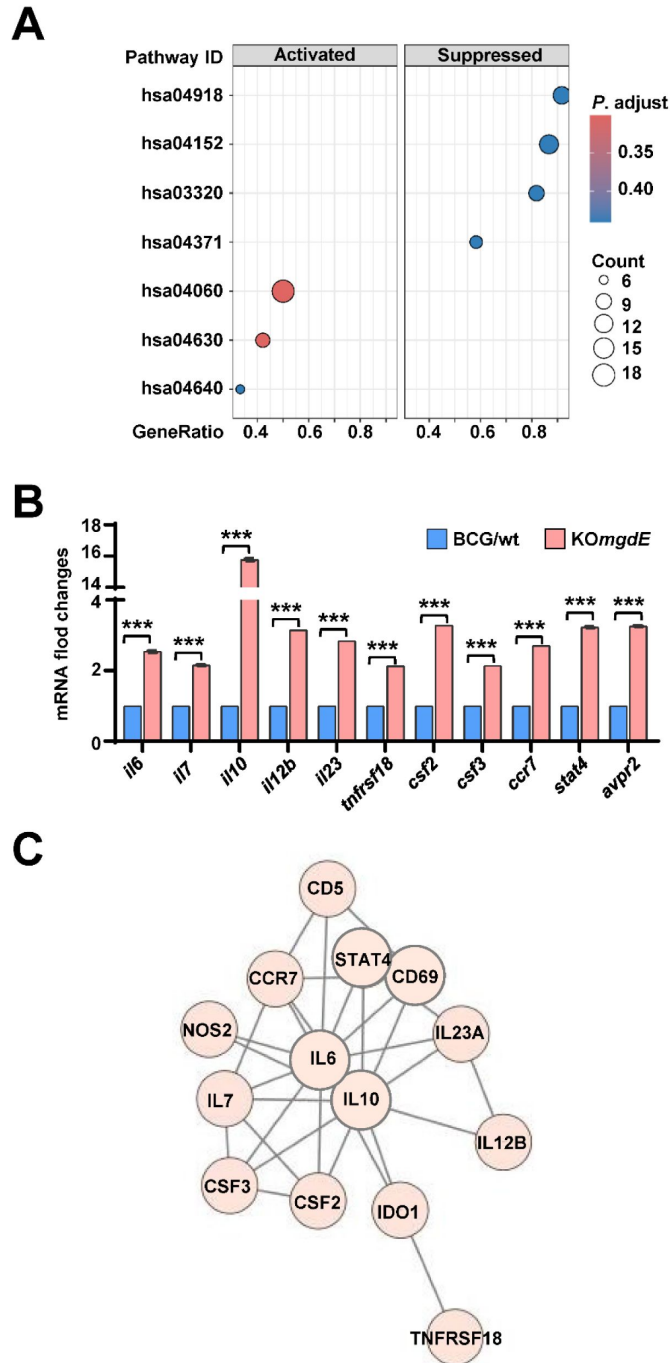
(A) Predicted binding affinities between MgdE and COMPASS core subunits. The predicted local distance difference test (pLDDT) scores calculated using AlphaFold 2.2.0 for the interactions between MgdE and the COMPASS subunits were as follows: ASH2L (pLDDT = 0.47), RbBP5 (pLDDT = 0.30), WDR5 (pLDDT = 0.77), and DPY30 (pLDDT = 0.62). Confidence levels are categorized as follows: High confidence: pLDDT $\geq$ 0.7 (strong predicted binding); Medium confidence: $0.5 \leq$ pLDDT < 0.7 (moderate binding); Low confidence: pLDDT < 0.5 (weak predicted binding). (B) Structural modeling of MgdE-COMPASS interactions. AlphaFold generated models of the simulated binding interfaces are shown, with MgdE highlighted in red and COMPASS subunits (ASH2L, WDR5, RbBP5, and DPY30) shown in gray.



Supplementary Figure 5.

Conserved residue mutations do not affect the binding of MgdE to ASH2L.

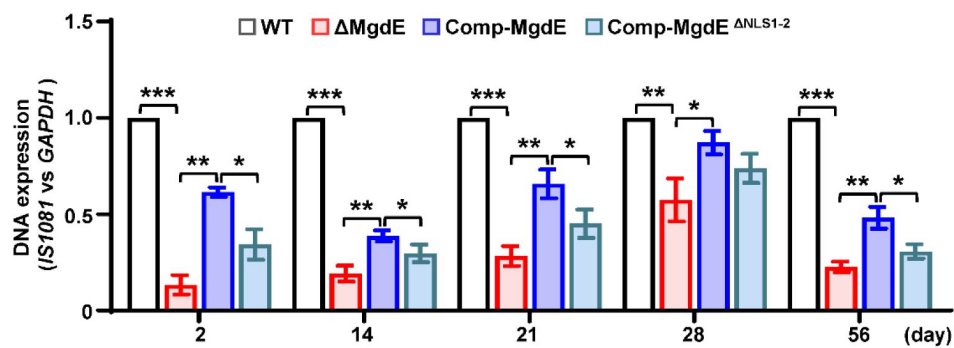
Co-IP analysis of MgdE mutants with ASH2L. HEK293T cells were co-transfected with Flag-tagged MgdE mutants and HA-tagged ASH2L (1:1 molar ratio). Co-IP was performed using anti-HA antibody-bound protein A/G beads, and immunoprecipitated complexes were analyzed by immunoblotting with anti-Flag and anti-HA antibodies.



Supplementary Figure 6.

MgdE suppresses cellular inflammatory responses during *M. bovis* BCG infection.

(A) KEGG pathway enrichment analysis. KEGG pathway analysis identified significantly enriched pathways in THP-1 cells infected with the *KomgdE* strain compared to those infected with wild-type BCG (WT). DEGs were predominantly associated with immune and signaling pathways, including thyroid hormone synthesis (*hsa04918*), AMPK signaling pathway (*hsa04152*), PPAR signaling pathway (*hsa03320*), apelin signaling pathway (*hsa04371*), cytokine-cytokine receptor interaction (*hsa04060*), JAK-STAT signaling pathway (*hsa04630*), and hematopoietic cell lineage (*hsa04640*). (B) Elevated inflammatory gene expression in *KomgdE*-infected cells. Key upregulated inflammatory genes in *KomgdE*-vs. WT-infected cells are highlighted. (C) Functional enrichment analysis of upregulated genes. Analysis using STRING and Cytoscape revealed regulation of inflammatory responses as a top enriched biological process ($P < 0.05$).



Supplementary Figure 7.

MgdE modulates inflammatory responses and bacterial colonization in the spleens of infected mice.

Bacterial colonization in the spleen. CFU assays were performed on splenic homogenates from mice infected as described in **Figure 7A**. Bacterial loads (CFU/g tissue) were quantified at 2, 14, 21, 28, and 56 d post-infection. Data are presented as mean $\pm$ SD ($n = 3$). Statistical significance was determined using a two-tailed unpaired Student's *t*-test ($P < 0.05$, $**P < 0.01$, and $***P < 0.001$).

Acknowledgements

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Additional information

Author contributions

Conceptualization: L. C., A. G., and L. Z.; Methodology: L. C.; Validation: L. C., B. D., and Q. J.; Formal analysis: L. C., Y. W.; Investigation: L. C., B. D., P. C., Q. J., Y. W., and L. L.; Resources: A. G., L. Z., and Y. C.; Data curation: L. C., Q. J., Y. W., and L. L.; Writing – original draft: L. C., L. Z., and A. G.; Writing – review & editing: L. C., L. Z., and A. G.; Visualization: L. C., Q. J., Y. C., C. H., L. Z., and A. G.; Supervision: A. G., L. Z., Y. C., and C. H.; Project administration: A. G., L. Z., Y. C., and C. H.; Funding acquisition: A. G., L. Z., and Y. C.

Additional files

Table S1 [↗](#)

Table S2 [↗](#)

Table S3 [↗](#)

Table S4 [↗](#)

Table S5 [↗](#)

Table S6 [↗](#)

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Reviewer #2 (Public review):

Summary:

The manuscript by Chen et al addresses an important aspect of pathogenesis for mycobacterial pathogens, seeking to understand how bacterial effector proteins disrupt the host immune response. To address this question, the authors sought to identify bacterial effectors from *M. tuberculosis* (Mtb) that localize to the host nucleus and disrupt host gene expression as a means of impairing host immune function.

Strengths:

The researchers conducted a rigorous bioinformatic analysis to identify secreted effectors containing mammalian nuclear localization signal (NLS) sequences, which formed the basis of quantitative microscopy analysis to identify bacterial proteins that had nuclear targeting within human cells. The study used two complementary methods to detect protein-protein interaction: yeast two-hybrid assays and reciprocal immunoprecipitation (IP). The combined use of these techniques provides strong evidence of interactions between MgdE and SET1

components and suggests that the interactions are, in fact, direct. The authors also carried out a rigorous analysis of changes in gene expression in macrophages infected with the mgdE mutant BCG. They found strong and consistent effects on key cytokines such as IL6 and CSF1/2, suggesting that nuclear-localized MgdE does, in fact, alter gene expression during infection of macrophages.

Weaknesses:

There are some drawbacks in this study that limit the application of the findings to *M. tuberculosis* (Mtb) pathogenesis. The first concern is that much of the study relies on ectopic overexpression of proteins either in transfected non-immune cells (HEK293T) or in yeast, using 2-hybrid approaches. Some of their data in 293T cells is hard to interpret, and it is unclear if the protein-protein interactions they identify occur during natural infection with mycobacteria. The second major concern is that pathogenesis is studied using the BCG vaccine strain rather than virulent Mtb. However, overall, the key findings of the paper - that MgdE interacts with SET1 and alters gene expression are well-supported.

<https://doi.org/10.7554/eLife.107677.1.sa4>

Reviewer #3 (Public review):

In this study, Chen L et al. systematically analyzed the mycobacterial nucleomodulins and identified MgdE as a key nucleomodulin in pathogenesis. They found that MgdE enters into host cell nucleus through two nuclear localization signals, KRIR108-111 and RLRRPR300-305, and then interacts with COMPASS complex subunits ASH2L and WDR5 to suppress H3K4 methylation-mediated transcription of pro-inflammatory cytokines, thereby promoting mycobacterial survival. This study is potentially interesting, but there are several critical issues that need to be addressed to support the conclusions of the manuscript.

(1) Figure 2: The study identified MgdE as a nucleomodulin in mycobacteria and demonstrated its nuclear translocation via dual NLS motifs. The authors examined MgdE nuclear translocation through ectopic expression in HEK293T cells, which may not reflect physiological conditions. Nuclear-cytoplasmic fractionation experiments under mycobacterial infection should be performed to determine MgdE localization.

(2) Figure 2F: The authors detected MgdE-EGFP using an anti-GFP antibody, but EGFP as a control was not detected in its lane. The authors should address this technical issue.

(3) Figure 3C-3H: The data showing that the expression of all detected genes in 24 h is comparable to that in 4 h (but not 0 h) during WT BCG infection is beyond comprehension. The issue is also present in Figure 7C, Figure 7D, and Figure S7. Moreover, since Il6, Il1 β (pro-inflammatory), and Il10 (anti-inflammatory) were all upregulated upon MgdE deletion, how do the authors explain the phenomenon that MgdE deletion simultaneously enhanced these gene expressions?

(4) Figure 5: The authors confirmed the interactions between MgdE and WDR5/ASH2L. How does the interaction between MgdE and WDR5 inhibit COMPASS-dependent methyltransferase activity? Additionally, the precise MgdE-ASH2L binding interface and its functional impact on COMPASS assembly or activity require clarification.

(5) Figure 6: The authors proposed that the MgdE-regulated COMPASS complex-H3K4me3 axis suppresses pro-inflammatory responses, but the presented data do not sufficiently support this claim. H3K4me3 inhibitor should be employed to verify cytokine production during infection.

(6) There appears to be a discrepancy between the results shown in Figure S7 and its accompanying legend. The data related to inflammatory responses seem to be missing, and the data on bacterial colonization are confusing (bacterial DNA expression or CFU assay?).

(7) Line 112-116: Please provide the original experimental data demonstrating nuclear localization of the 56 proteins harboring putative NLS motifs.

<https://doi.org/10.7554/eLife.107677.1.sa3>

Reviewer #1 (Public review):

Summary:

This fundamental study identifies a new mechanism that involves a mycobacterial nucleomodulin manipulation of the host histone methyltransferase COMPASS complex to promote infection. Although other intracellular pathogens are known to manipulate histone methylation, this is the first report demonstrating the specific targeting of the COMPASS complex by a pathogen. The rigorous experimental design using state-of-the-art bioinformatic analysis, protein modeling, molecular and cellular interaction, and functional approaches, culminating with in vivo infection modeling, provides convincing, unequivocal evidence that supports the authors' claims. This work will be of particular interest to cellular microbiologists working on microbial virulence mechanisms and effectors, specifically nucleomodulins, and cell/cancer biologists that examine COMPASS dysfunction in cancer biology.

Strengths:

(1) The strengths of this study include the rigorous and comprehensive experimental design that involved numerous state-of-the-art approaches to identify potential nucleomodulins, define molecular nucleomodulin-host interactions, cellular nucleomodulin localization, intracellular survival, and inflammatory gene transcriptional responses, and confirmation of the inflammatory and infection phenotype in a small animal model.

(2) The use of bioinformatic, cellular, and in vivo modeling that are consistent and support the overall conclusions is a strength of the study. In addition, the rigorous experimental design and data analysis, including the supplemental data provided, further strengthen the evidence supporting the conclusions.

Weaknesses:

(1) This work could be stronger if the MgdE-COMPASS subunit interactions that negatively impact COMPASS complex function were better defined. Since the COMPASS complex consists of many enzymes, examining the functional impact on each of the components would be interesting.

(2) Examining the impact of WDR5 inhibitors on histone methylation, gene transcription, and mycobacterial infection could provide additional rigor and provide useful information related to the mechanisms and specific role of WDR5 inhibition on mycobacterial infection.

(3) The interaction between MgdE and COMPASS complex subunit ASH2L is relatively undefined, and studies to understand the relationship between WDR5 and ASH2L in COMPASS complex function during infection could provide interesting molecular details that are undefined in this study.

(4) The AlphaFold prediction results for all the nuclear proteins examined could be useful. Since the interaction predictions with COMPASS subunits range from 0.77 for WDR5 and 0.47

for ASH2L, it is not clear how the focus on COMPASS complex over other nuclear proteins was determined.

<https://doi.org/10.7554/eLife.107677.1.sa0>

Author response:

The following is the authors' response to the original reviews.

Reviewer #1 (Public review):

Summary:

This fundamental study identifies a new mechanism that involves a mycobacterial nucleomodulin manipulation of the host histone methyltransferase COMPASS complex to promote infection. Although other intracellular pathogens are known to manipulate histone methylation, this is the first report demonstrating the specific targeting of the COMPASS complex by a pathogen. The rigorous experimental design using state-of-the-art bioinformatic analysis, protein modeling, molecular and cellular interaction, and functional approaches, culminating with in vivo infection modeling, provides convincing, unequivocal evidence that supports the authors' claims. This work will be of particular interest to cellular microbiologists working on microbial virulence mechanisms and effectors, specifically nucleomodulins, and cell/cancer biologists that examine COMPASS dysfunction in cancer biology.

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(1) The strengths of this study include the rigorous and comprehensive experimental design that involved numerous state-of-the-art approaches to identify potential nucleomodulins, define molecular nucleomodulin-host interactions, cellular nucleomodulin localization, intracellular survival, and inflammatory gene transcriptional responses, and confirmation of the inflammatory and infection phenotype in a small animal model.

(2) The use of bioinformatic, cellular, and in vivo modeling that are consistent and support the overall conclusions is a strength of the study. In addition, the rigorous experimental design and data analysis, including the supplemental data provided, further strengthen the evidence supporting the conclusions.

Weaknesses:

(1) This work could be stronger if the MgdE-COMPASS subunit interactions that negatively impact COMPASS complex function were better defined. Since the COMPASS complex consists of many enzymes, examining the functional impact on each of the components would be interesting.

We thank the reviewer for this insightful comment. A biochemistry assays could be helpful to interpret the functional impact on each of the components by MgdE interaction. However, the purification of the COMPASS complex could be a hard task itself due to the complexity of the full COMPASS complex along with its dynamic structural properties and limited solubility.

(2) Examining the impact of WDR5 inhibitors on histone methylation, gene transcription, and mycobacterial infection could provide additional rigor and provide useful information related to the mechanisms and specific role of WDR5 inhibition on mycobacterial infection.

We thank the reviewer for the comment. A previous study showed that WIN-site inhibitors, such as compound C6, can displace WDR5 from chromatin, leading to a reduction in global H3K4me3 levels and suppression of immune-related gene expression (Hung et al., *Nucleic Acids Res*, 2018; Bryan et al., *Nucleic Acids Res*, 2020). These results closely mirror the functional effects we observed for MgdE, suggesting that MgdE may act as a functional mimic of WDR5 inhibition. This supports our proposed model in which MgdE disrupts COMPASS activity by targeting WDR5, thereby dampening host pro-inflammatory responses.

(3) The interaction between MgdE and COMPASS complex subunit ASH2L is relatively undefined, and studies to understand the relationship between WDR5 and ASH2L in COMPASS complex function during infection could provide interesting molecular details that are undefined in this study.

We thank the reviewer for the comment. In this study, we constructed single and multiple point mutants of MgdE at residues S⁸⁰, D²⁴⁴, and H²⁴⁷ to identify key amino acids involved in its interaction with ASH2L (Figure 5A and B; Figure S5). However these mutations did not interrupt the interaction with MgdE, suggesting that more residues are involved in the interaction.

ASH2L and WDR5 function cooperatively within the WRAD module to stabilize the SET domain and promote H3K4 methyltransferase activity with physiological conditions (Couture and Skiniotis, *Epigenetics*, 2013; Qu et al., *Cell*, 2018; Rahman et al., *Proc Natl Acad Sci U S A*, 2022). ASH2L interacts with RbBP5 via its SPRY domain, whereas WDR5 bridges MLL1 and RbBP5 through the WIN and WBM motifs (Chen et al., *Cell Res*, 2012; Park et al., *Nat Commun*, 2019). The interaction status between ASH2L and WDR5 during mycobacterial infection could not be determined in our current study.

(4) The AlphaFold prediction results for all the nuclear proteins examined could be useful. Since the interaction predictions with COMPASS subunits range from 0.77 for WDR5 and 0.47 for ASH2L, it is not clear how the focus on COMPASS complex over other nuclear proteins was determined.

We thank the reviewer for the comment. We employed AlphaFold to predict the interactions between MgdE and the major nuclear proteins. This screen identified several subunits of the SET1/COMPASS complex as high-confidence candidates for interaction with MgdE (Supplementary Figure 4A). This result is consistent with a proteomic study by Penn et al. which reported potential interactions between MgdE and components of the human SET1/COMPASS complex based on affinity purification-mass spectrometry analysis (Penn et al., *Mol Cell*, 2018).

Reviewer #2 (Public review):

Summary:

*The manuscript by Chen et al addresses an important aspect of pathogenesis for mycobacterial pathogens, seeking to understand how bacterial effector proteins disrupt the host immune response. To address this question, the authors sought to identify bacterial effectors from *M. tuberculosis* (Mtb) that localize to the host nucleus and disrupt host gene expression as a means of impairing host immune function.*

Strengths:

The researchers conducted a rigorous bioinformatic analysis to identify secreted effectors containing mammalian nuclear localization signal (NLS) sequences, which formed the basis of quantitative microscopy analysis to identify bacterial proteins that

had nuclear targeting within human cells. The study used two complementary methods to detect protein-protein interaction: yeast two-hybrid assays and reciprocal immunoprecipitation (IP). The combined use of these techniques provides strong evidence of interactions between MgdE and SET1 components and suggests that the interactions are, in fact, direct. The authors also carried out a rigorous analysis of changes in gene expression in macrophages infected with the mgdE mutant BCG. They found strong and consistent effects on key cytokines such as IL6 and CSF1/2, suggesting that nuclear-localized MgdE does, in fact, alter gene expression during infection of macrophages.

Weaknesses:

There are some drawbacks in this study that limit the application of the findings to M. tuberculosis (Mtb) pathogenesis. The first concern is that much of the study relies on ectopic overexpression of proteins either in transfected non-immune cells (HEK293T) or in yeast, using 2-hybrid approaches. Some of their data in 293T cells is hard to interpret, and it is unclear if the protein-protein interactions they identify occur during natural infection with mycobacteria. The second major concern is that pathogenesis is studied using the BCG vaccine strain rather than virulent Mtb. However, overall, the key findings of the paper - that MgdE interacts with SET1 and alters gene expression are well-supported.

We thank the reviewer for the comment. We agree that the ectopic overexpression could not completely reflect a natural status, although these approaches were adopted in many similar experiments (Drerup et al., Molecular plant, 2013; Chen et al., Cell host & microbe, 2018; Ge et al., Autophagy, 2021). Further, the MgdE localization experiment using Mtb infected macrophages will be performed to increase the evidence in the natural infection.

We agree with the reviewer that BCG strain could not fully recapitulate the pathogenicity or immunological complexity of M. tuberculosis infection. We employed BCG as a biosafe surrogate model since it was acceptable in many related studies (Wang et al., Nat Immunol, 2025; Wang et al., Nat Commun, 2017; Péan et al., Nat Commun, 2017; Li et al., J Biol Chem, 2020).

Reviewer #3 (Public review):

In this study, Chen L et al. systematically analyzed the mycobacterial nucleomodulins and identified MgdE as a key nucleomodulin in pathogenesis. They found that MgdE enters into host cell nucleus through two nuclear localization signals, KRIR¹⁰⁸⁻¹¹¹ and RLRRPR³⁰⁰⁻³⁰⁵, and then interacts with COMPASS complex subunits ASH2L and WDR5 to suppress H3K4 methylation-mediated transcription of pro-inflammatory cytokines, thereby promoting mycobacterial survival. This study is potentially interesting, but there are several critical issues that need to be addressed to support the conclusions of the manuscript.

(1) Figure 2: The study identified MgdE as a nucleomodulin in mycobacteria and demonstrated its nuclear translocation via dual NLS motifs. The authors examined MgdE nuclear translocation through ectopic expression in HEK293T cells, which may not reflect physiological conditions. Nuclear-cytoplasmic fractionation experiments under mycobacterial infection should be performed to determine MgdE localization.

We thank the reviewer for the comment. The MgdE localization experiment using Mtb infected macrophages will be performed.

(2) Figure 2F: The authors detected MgdE-EGFP using an anti-GFP antibody, but EGFP as a control was

We thank the reviewer for pointing this out. The new uncropped blots containing the EGFP band will be provided in Supplementary Information.

*(3) Figure 3C-3H: The data showing that the expression of all detected genes in 24 h is comparable to that in 4 h (but not 0 h) during WT BCG infection is beyond comprehension. The issue is also present in Figure 7C, Figure 7D, and Figure S7. Moreover, since *Il6*, *Il1 β* (proinflammatory), and *Il10* (anti-inflammatory) were all upregulated upon *MgdE* deletion, how do the authors explain the phenomenon that *MgdE* deletion simultaneously enhanced these gene expressions?*

We thank the reviewer for the comment. A relative quantification method was used in our qPCR experiments to normalize the WT expression levels in Figure 3C–3H, Figure 7C, 7D, and Figure S7.

The concurrent induction of both types of cytokines likely represents a dynamic host strategy to fine-tune immune responses during infection. This interpretation is supported by previous studies (Podleśny-Drabiniok et al., Cell Rep, 2025; Cicchese et al., Immunological Reviews, 2018).

*(4) Figure 5: The authors confirmed the interactions between *MgdE* and *WDR5*/*ASH2L*. How does the interaction between *MgdE* and *WDR5* inhibit COMPASS-dependent methyltransferase activity? Additionally, the precise *MgdE*-*ASH2L* binding interface and its functional impact on COMPASS assembly or activity require clarification.*

We thank the reviewer for this insightful comment. We cautiously speculate that the *MgdE* interaction inhibits COMPASS-dependent methyltransferase activity by interfering with the integrity and stability of the COMPASS complex. Accordingly, we have incorporated the following discussion into the revised manuscript (Lines 298-310):

“The COMPASS complex facilitates H3K4 methylation through a conserved assembly mechanism involving multiple core subunits. *WDR5*, a central scaffolding component, interacts with *RbBP5* and *ASH2L* to promote complex assembly and enzymatic activity (Qu et al., 2018; Wysocka et al., 2005). It also recognizes the WIN motif of methyltransferases such as *MLL1*, thereby anchoring them to the complex and stabilizing the *ASH2L*-*RbBP5* dimer (Hsu et al., Cell, 2018). *ASH2L* further contributes to COMPASS activation by interacting with both *RbBP5* and *DPY30* and by stabilizing the SET domain, which is essential for efficient substrate recognition and catalysis (Qu et al., Cell, 2018; Park et al., Nat Commun, 2019). Our work shows that *MgdE* binds both *WDR5* and *ASH2L* and inhibits the methyltransferase activity of the COMPASS complex. Site-directed mutagenesis revealed that residues D²²⁴ and H²⁴⁷ of *MgdE* are critical for *WDR5* binding, as the double mutant *MgdE*-D²²⁴A/H²⁴⁷A fails to interact with *WDR5* and shows diminished suppression of H3K4me3 levels (Figure 5D).”

Regarding the precise *MgdE*-*ASH2L* binding interface, we attempted to identify the key interaction site by introducing point mutations into *ASH2L*. However, these mutations did not disrupt the interaction (Figure 5A and B; Figure S5), suggesting that more residues are involved in the interaction.

*(5) Figure 6: The authors proposed that the *MgdE*-regulated COMPASS complex-H3K4me3 axis suppresses pro-inflammatory responses, but the presented data do not sufficiently support this claim. H3K4me3 inhibitor should be employed to verify cytokine production during infection.*

We thank the reviewer for the comment. We have now revised the description in lines 824825 “*MgdE* may suppresses COMPASS complex-mediated inflammatory responses by inhibiting

H3K4 methylation” and in lines 219-220 “MgdE suppresses host inflammatory responses probably by inhibition of COMPASS complex-mediated H3K4 methylation.”

(6) There appears to be a discrepancy between the results shown in Figure S7 and its accompanying legend. The data related to inflammatory responses seem to be missing, and the data on bacterial colonization are confusing (bacterial DNA expression or CFU assay?).

We thank the reviewer for the comment. Figure S7 specifically addresses the effect of MgdE on bacterial colonization in the spleens of infected mice, which was assessed by quantitative PCR rather than by CFU assay.

We have now revised the legend of Figure S7 as below (Lines 934-938):

“MgdE facilitates bacterial colonization in the spleens of infected mice. Bacterial colonization was assessed in splenic homogenates from infected mice (as described in Figure 7A) by quantifying bacterial DNA using quantitative PCR at 2, 14, 21, 28, and 56 days post-infection.”

(7) Line 112-116: Please provide the original experimental data demonstrating nuclear localization of the 56 proteins harboring putative NLS motifs.

We thank the reviewer for the comment. We will provide this data in the new Supplementary Table 2.

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