

## Supplementary materials

Table S1. List of primers, strains, plasmids, antibodies, and ELISA kits used in the study.

List of primers used in the study for cloning purposes		
Name	Primers (5'-3')	Usage
OE- <i>msmeg5257</i> -Fwd	TGATTAAC TTTATAAGGAGGAAAAAC ATATGATGACCGCGCCGATCGACG	Construction of the overexpression of <i>msmeg5257</i> in Ms
OE- <i>msmeg5257</i> -Rev	TCTTCAGAAATAAGTTTTTGTTCCAA GCTTGCGGTAGTCCGGC	
sgRNA- <i>msmeg5257</i> -Fwd	GGGACCCAGTTCTTCGGAAAACCA	Construction of the deletion of <i>msmeg5257</i> in Ms
sgRNA- <i>msmeg5257</i> -Rev	AAACTGGTTTTCCGAAGAACTGGG	
Cas9-Asp-Fwd	AGCATCGGCCTGGACATCGGCACGAA CTCGGTGGGCT	
Cas9-Asp-Rev	GTTCGTGCCGATGTCCAGGCCGATGCT GTACTTCTTGTCATATGTATAT	
Cas9-His-Fwd	TACGACGTGGACCACATCGTGCCGCA GTCGTTCTGAAG	
Cas9-His-Rev	CTGCGGCACGATGTGGTCCACGTCGTA GTCGCTCAGCCG	
List of primers used in the study for RT-PCR		
<i>msmeg4509</i> -Fwd	GTCGATCCTTCCCGGCAACC	
<i>msmeg4509</i> -Rev	GGGTCGGAGTACAGCGTG TTC	
<i>msmeg4510</i> -Fwd	AGTACGTCGTGGCGATGCTC	
<i>msmeg4510</i> -Rev	GGTGAAGACGATGTAGGCGCC	
<i>msmeg4511</i> -Fwd	CGGTCTGCCCAAAGGTG TTC	
<i>msmeg4511</i> -Rev	CGGTCAGGTAGCCGATGTC	
<i>msmeg4512</i> -Fwd	GGTGTGGTGCTCGTCGGAT	
<i>msmeg4512</i> -Rev	ACCTGGTGCCATTGCTCGG	
<i>msmeg4513</i> -Fwd	ACTGCGATCTGGCGGTGAC	
<i>msmeg4513</i> -Rev	GGTGCGTCCGTCCTGGTTGA	
<i>msmeg4515</i> -Fwd	AGGCACCACTGGCTGGATC	

<i>msmeg4515-Rev</i>	TCGTCGTATCGGTCAGGTCG
<i>msmeg4516-Fwd</i>	CCGCCGACGACGTCTATCT
<i>msmeg4516-Rev</i>	CCAAGGGTTCCCACGCACAC
<i>msmeg1373-Fwd</i>	CAAGGTGATGGCGGCGATGG
<i>msmeg1373-Rev</i>	AGGCGCTGAAGTCGGTGAG
<i>msmeg1613-Fwd</i>	GCATGAAGCCCGCGAAAGTC
<i>msmeg1613-Rev</i>	TCGTGGTCAGCGCCAGGTA
<i>msmeg1709-Fwd</i>	ATCTGAGCGTGGGTTCGGTG
<i>msmeg1709-Rev</i>	CCGGGTCGTTGATCGGTATCG
<i>msmeg2635-Fwd</i>	CCTACCTCGCCCACAACACC
<i>msmeg2635-Rev</i>	GGGAAGAACGCCATGAGCACG
<i>msmeg2846-Fwd</i>	GCCCAGGTGCTGCTCAAGAA
<i>msmeg2846-Rev</i>	GACCGGGAGAACACGGTCTC
<i>msmeg3999-Fwd</i>	ACCGAGTCTGATGCCCCGAA
<i>msmeg3999-Rev</i>	TCGTCCACGAGCAACCCCT
<i>msmeg5102-Fwd</i>	CGCCCTGAGGTCCTGATGC
<i>msmeg5102-Rev</i>	GTACGCCGTGAAGTTGCCG
<i>msmeg5368-Fwd</i>	AGATCAACGTCGGCTTCGCC
<i>msmeg5368-Rev</i>	CGGATTGCTGAACGCTGCCT
<i>msmeg5370-Fwd</i>	GTGGATCTCCTGGCCGTCC
<i>msmeg5370-Rev</i>	ACTGCCCTTGGGGAATCGC
<i>msmeg5660-Fwd</i>	CCGCAAGGACCTCTACGCAC

<i>msmeg5660</i> -Rev	ACCAGCACCACGACACCCA
<i>msmeg6332</i> -Fwd	GCGGCGAGCATCATCTTCAC
<i>msmeg6332</i> -Rev	GCGGCAGTTCGACTTTGAGC
<i>msmeg6333</i> -Fwd	CAAACCTCGCCGACCGCTATC
<i>msmeg6333</i> -Rev	GCCTCGTCGATGTCATGGG
<i>msmeg6334</i> -Fwd	CGCCGAGGACAAGGACACC
<i>msmeg6334</i> -Rev	CGCTGATCGCGACGAAGTTG
16S-Fwd	GGGCGATACGGGCAGACTA
16S-Rev	CACGGATCCCAAGGAAGGA

#### List of strains and plasmids used in the study

Strains and plasmids	Description	Reference
<i>E. coli</i> DH5 $\alpha$	<i>E. coli</i> DH5 $\alpha$ for molecular cloning	CWBIO
<i>M. smegmatis</i> mc <sup>2</sup> 155	<i>M. smegmatis</i> mc <sup>2</sup> 155 parental strain	ATCC
Ms_ <i>vec</i>	The empty vector in Ms	This study
Ms_ $\Delta$ <i>msmeg5257</i>	Deletion of <i>msmeg5257</i> in Ms	This study
Ms_OE- <i>msmeg5257</i>	Overexpression of <i>msmeg5257</i> in Ms	This study
Ms_C- $\Delta$ <i>msmeg5257</i>	Complement of <i>msmeg5257</i> in Ms_ $\Delta$ <i>msmeg5257</i>	This study
Plasmids	Description	Reference
pSUM-Kan-MCS2	Cloning vector	109379, ADDGENE
pSUM-OE- <i>msmeg5257</i>	<i>msmeg5257</i> Overexpression plasmid	This study
pRH2521	Cloning vector	84380, ADDGENE
pRH2502	dCas9-induced expression plasmid	84379, ADDGENE
pRH2502-Cas9	Cas9-induced expression plasmid	This study
pRH2521-sgRNA- <i>msmeg5257</i>	sgRNA-induced expression plasmid	This study

#### List of antibodies used in the study

Name	Identifier	Reference
Anti-MSMEG5257-antibody	NWTSVTARKWMKR	This study

Anti-rabbit IgG (H+L)	14708	CTS
Goat Anti-rabbit IgG (H&L)	Y1055	LABLEAD
Goat Anti-mouse IgG H&L (HRP)	ab205719	ABCAM

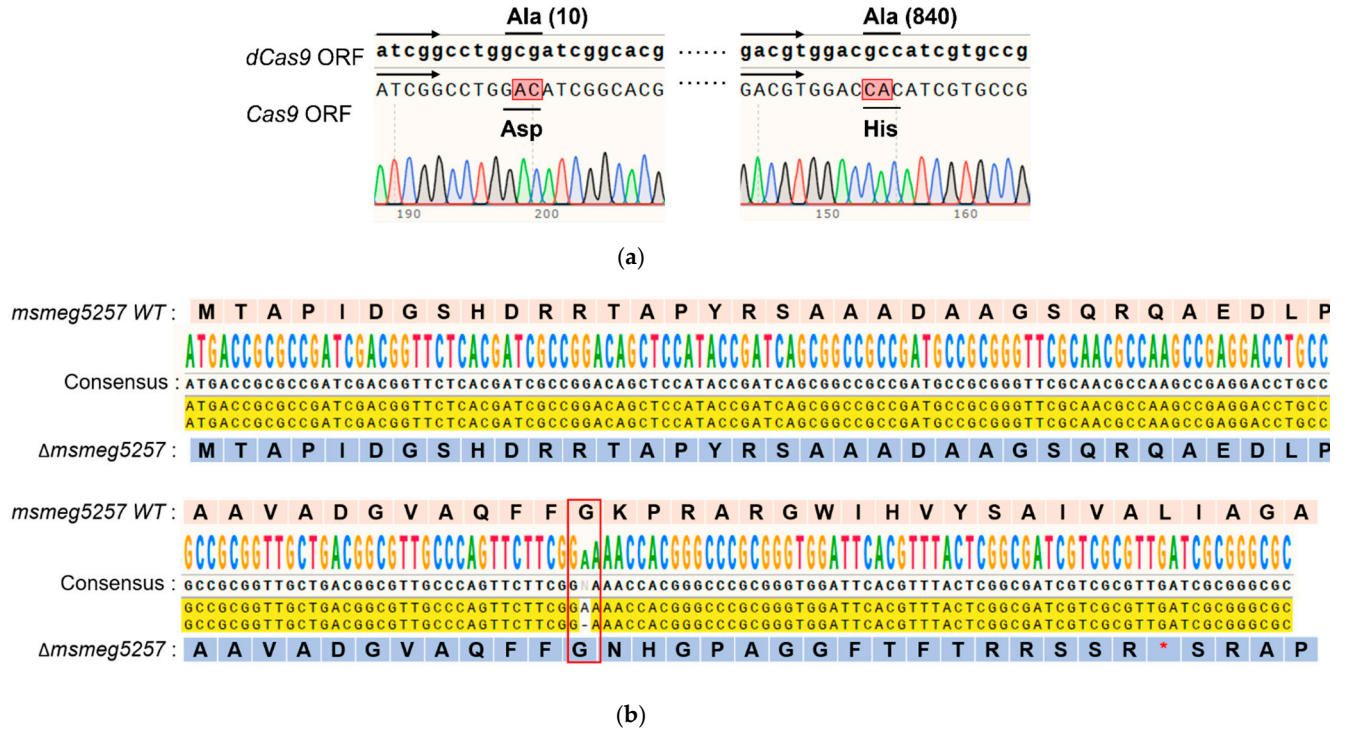
Table S2 Drug name and concentration of drug susceptibility test.

Drugs	Concentrations (µg/mL)
Bedaquiline (BDQ)	0.008, 0.015, 0.03, 0.06, 0.12, 0.25, 0.5, and 1.
Ethambutol (EMB)	0.25, 0.5, 1, 2, 4, 8, 16, and 32.
Rifampicin (RIF)	0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4, and 8.
Amikacin (AMI)	0.25, 0.5, 1, 2, 4, 8, and 16.
Levofloxacin (LEVO)	0.12, 0.25, 0.5, 1, 2, 4, and 8.
Moxifloxacin (MXF)	0.06, 0.12, 0.25, 0.5, 1, 2, and 4.
Delamanid (DLM)	0.008, 0.015, 0.03, 0.06, 0.12, 0.25, and 0.5.
Linezolid (LZD)	0.06, 0.12, 0.25, 0.5, 1, and 2.
Clofazimine (CFZ)	0.03, 0.06, 0.12, 0.25, 0.5, 1, and 2.
Ethionamide (ETO)	0.5, 1, 2, 4, and 8.
Rifabutin (RFB)	0.06, 0.12, 0.25, 0.5, 1, and 2.
Isoniazid (INH)	0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8.

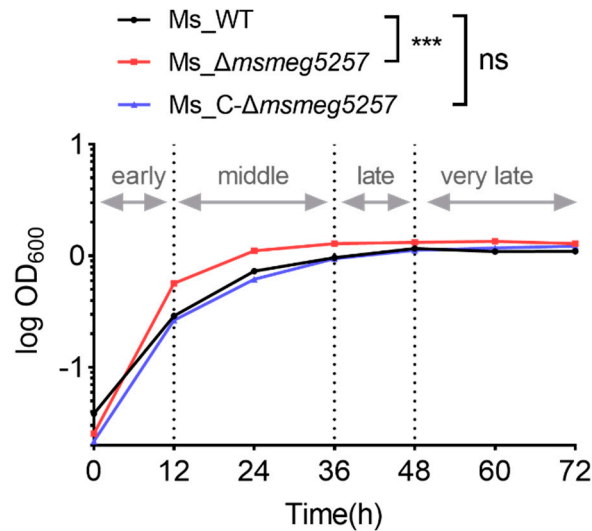
Table S3 Drug susceptibility test of *msmeg5257* mutant strains.

Drugs	Minimum Inhibitory Concentration (µg/mL)	
	Ms_WT	Ms_Δ <i>msmeg5257</i>
Bedaquiline (BDQ)	0.03	0.015
Clofazimine (CFZ)	0.5-1	0.25-0.5

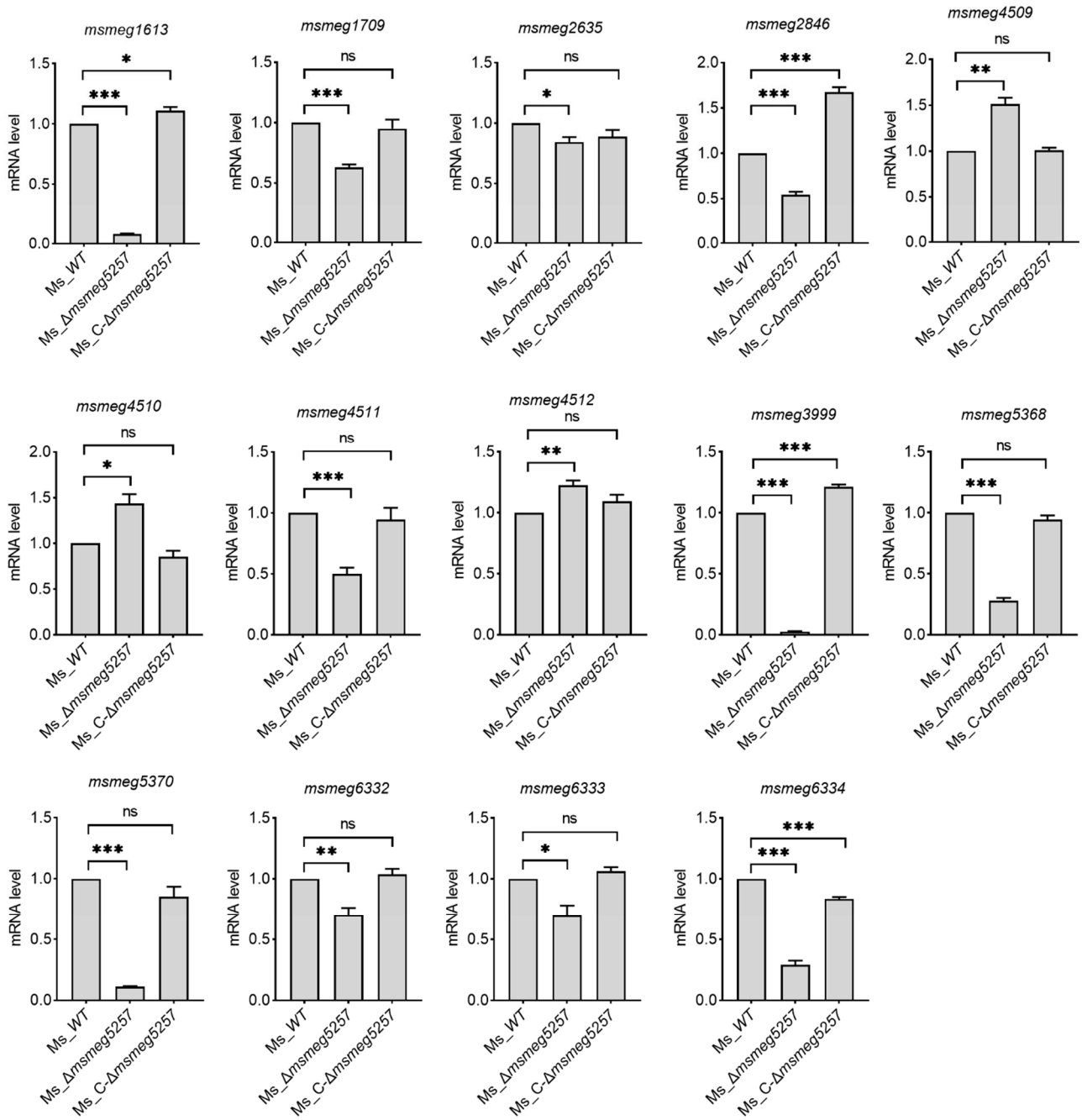
## Supplementary Figure



**Supplementary Figure S1.** Construction of *msmeg5257* deletion strain. (a) The sequencing outcome of the pRH2502-Cas9 plasmid. The Ala at position 10 changed to Asp, and Ala at position 840 changed to His in the *dCas9* amino acid (AA) sequence of pRH2502-*dCas9*. ORF, open reading frame. (b) The alignment results of the whole-genome sequencing for *Ms\_Δmsmeg5257*. Yellow: identical base; -: location of the base deletion.



**Supplementary Figure S2.** The growth curve of wild-type *Ms* strains. The growth curve of *Ms* was divided into early (0-12 h), middle (13-36 h), late (37-48 h), and very late (>48 h) stages. \*\*\*:  $p$  values < 0.001 as a very significant difference; ns: no difference; WT: *Ms\_WT*;  $\Delta$ : *Ms\_Δmsmeg5257*; C- $\Delta$ : *Ms\_C-Δmsmeg5257*.



**Supplementary Figure S3.** Revisions in the mRNA expression levels of genes implicated in iron transport within the *msmeg5257* mutant strains. \*:  $p$  values < 0.05; \*\*:  $p$  values < 0.01; \*\*\*:  $p$  values < 0.001; ns: no difference.