

SUPPLEMENTARY MATERIAL FOR

The SUMO gene *MrSmt3* is involved in SUMOylation, conidiation and stress response in *Metarhizium robertsii*

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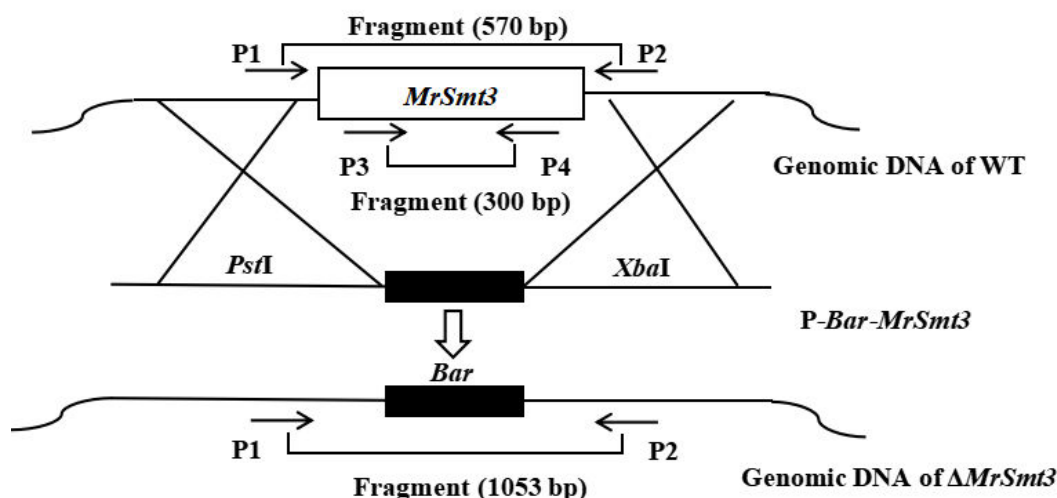
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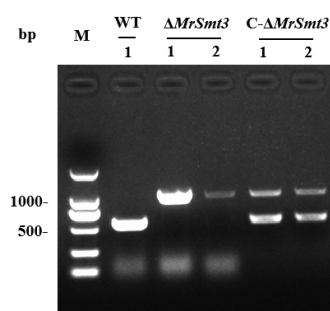
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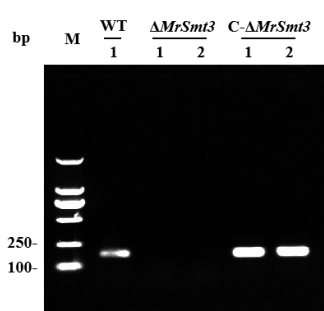
A.



B.



C.



D.

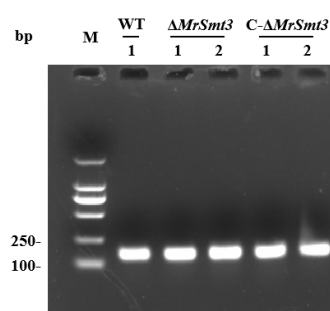


Fig. S1 Gene disruption and complementation of *MrSmt3*.

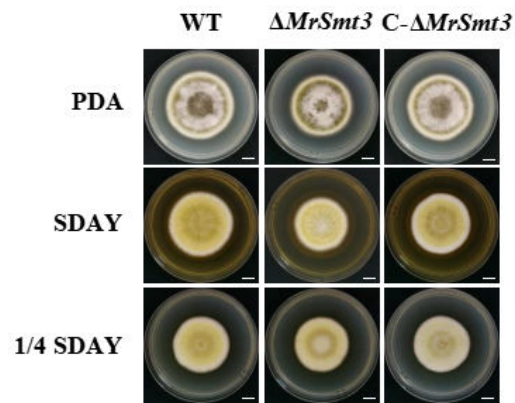
(A) Schematic diagram of *MrSmt3* gene disruption via homologous recombination. Within this diagram, the 5' and 3' segments of *MrSmt3*, (1375 and 1696 bp) along with the overlapping regions in the P-Bar-*MrSmt3* (recombinant vector), are employed for the precise replacement of a portion of *MrSmt3* with a 926-base pair *Bar* plasmid. The directional arrows demarcate the corresponding regions with clarity.

(B) PCR verification for $\Delta MrSmt3$ was conducted using primers P1/P2 (Table S1). Bands of 570 base pairs were successfully amplified from the WT, while bands of 1053 bp were successfully amplified from the $\Delta MrSmt3$, bands of 570 and 1053 bp were successfully amplified from the C- $\Delta MrSmt3$ (Because the construction of the Ben plasmid containing the *MrSmt3* was transformed into the $\Delta MrSmt3$, which includes both the *Bar* plasmid sequence and the *MrSmt3* sequence).

(C) RT-PCR analysis for *MrSmt3* expression. Using cDNA as a template and primers P3/P4 (Table S1) for verification, bands of 174 bp (The length of primers P3 to P4 was 300 bp, with an intron length of 126 bp) were successfully amplified from the WT and C- $\Delta MrSmt3$, while no amplification was observed in $\Delta MrSmt3$.

(D) The expression level of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, MAA_07675, Table S1) was employed as an internal control.

A.



B.

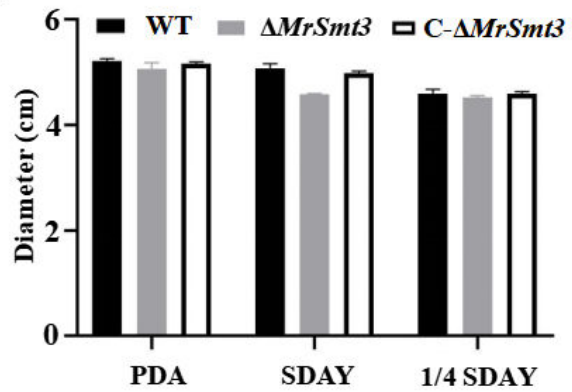
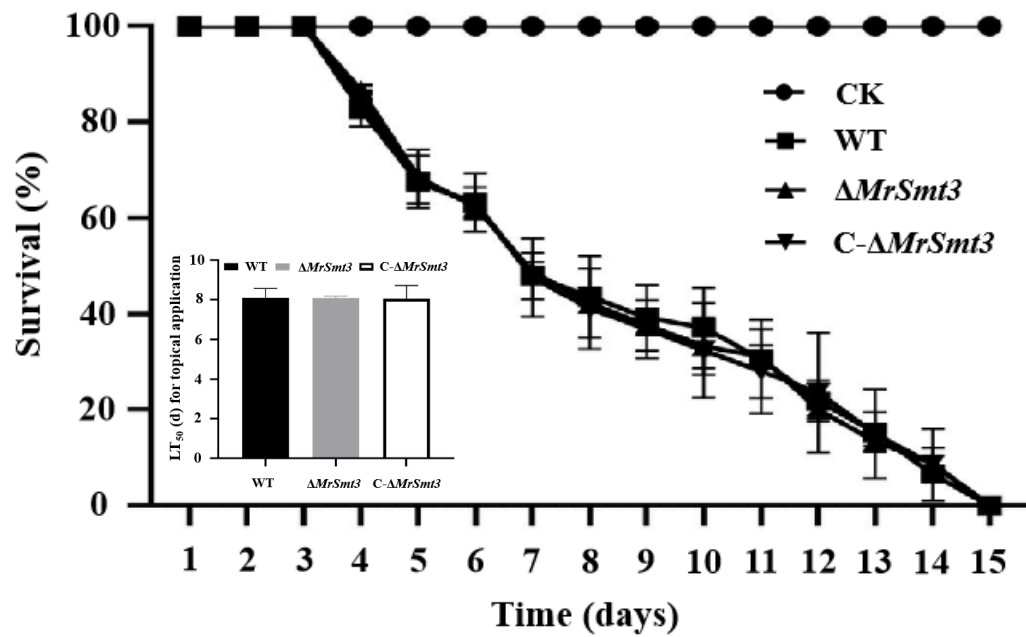


Fig. S2 Effect of *MrSmt3* deletion on growth and development of *M. robertsii*.

(A) Images of fungal colonies after 14 days growth at 25 °C on PDA, SDAY and 1/4 SDAY media. Each colony was initiated by spotting 1 μ L of a 1×10^7 conidia/mL suspension. Scale bar: 1 cm.

(B) Colony diameters of respective strain on PDA, SDAY and 1/4 SDAY media after cultured for 14 days at 25 °C.

A.



B.

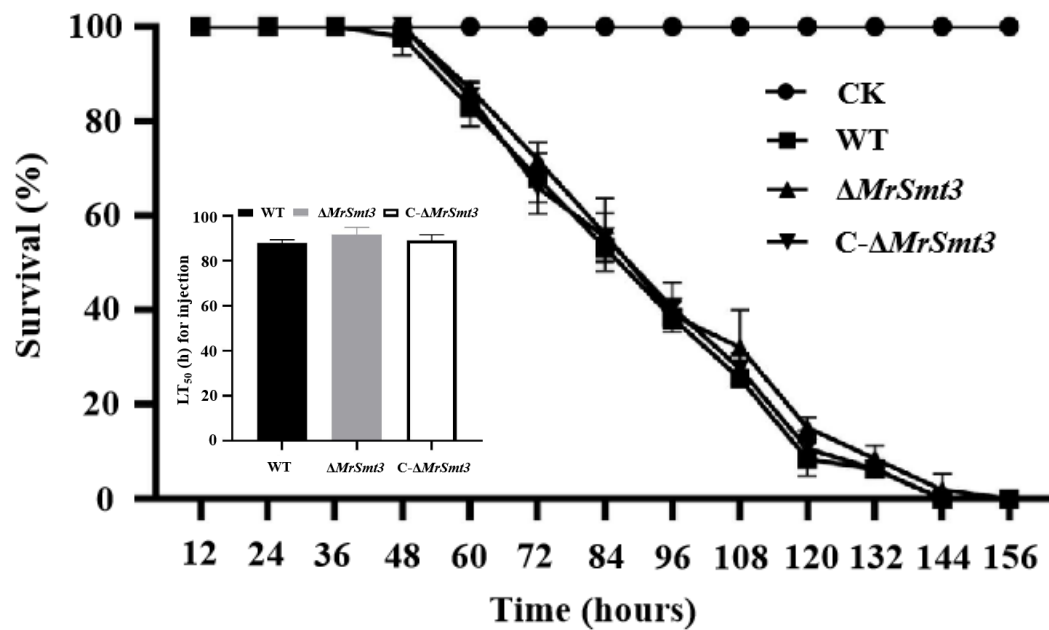


Fig. S3 Effects of *MrSmt3* deletion on fungal virulence.

(A) Survival of *Galleria mellonella* after topical application of conidial suspensions from different strains. Control insects were treated with sterile water.

(B) Survival of *G. mellonella* after injection of conidial suspension from different strains. Control insects were injected with 1 μ L sterile water.

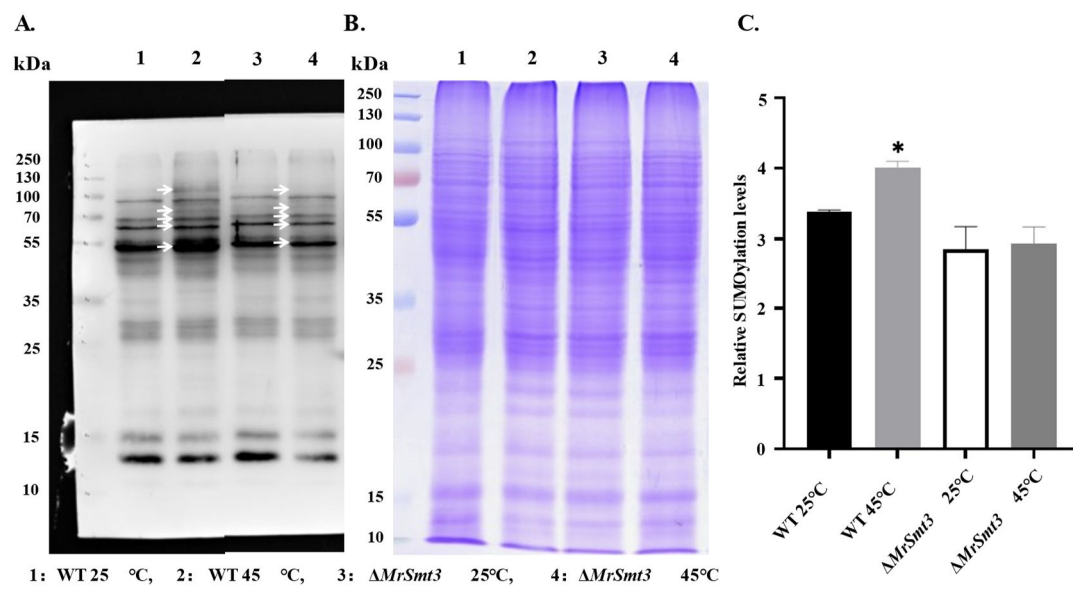


Fig. S4 Original blots/gels for Figure 4.

It is the same for the legend of Figure 4, just for presenting original blots/gels.

Table S1 Primers used in this study

Gene	Primer Name	Sequence (5'- 3')	Notes
<i>Smt3</i>	<i>MrSmt3</i> -Up-F	<u>CTTGATATCGAATTCCTGCAGGAAGGATGTTGCTCCGATGAA</u> , <i>Pst</i> I	For constructing knockout gene vector
	<i>MrSmt3</i> -Up-R	<u>GACGGATCCCCCGGGCTGCAGCGGATGCGATAGGACACGC</u> , <i>Pst</i> I	
	<i>MrSmt3</i> -Dn-F	<u>ATCTGATGAACTAGTTCTAGAGCGGATGCGACGATAGAAAT</u> , <i>Xba</i> I	
	<i>MrSmt3</i> -Dn-R	<u>CGCGGTGGCGGCCGCTCTAGACTGCGAGTGAGGATGATTGGC</u> , <i>Xba</i> I	
	<i>MrSmt3</i> -ID-F (P1)	CAAGCAGCCAACCGAATACA	For PCR detection
	<i>MrSmt3</i> -ID-R (P2)	CTGCACCCTTTTTCGCATCA	
	<i>MrSmt3</i> -L-F (P3)	GCCCACTTGCTCTTGATGGA	For RT-PCR validation
	<i>MrSmt3</i> -L-R (P4)	AAGCGCAGCACAAAGCTGGA	
	C- <i>MrSmt3</i> -5F	<u>CTTGATATCGAATTCCTGCAGCGGCGTTTGCATTGTGTCAT</u> , <i>Pst</i> I	For constructing gene complementation vector
	C- <i>MrSmt3</i> -3R	<u>AGGCCCCCTGTCGAGCTGCAGTAGTTGTTCCGCGTCAGACA</u> , <i>Pst</i> I	
<i>Bar</i>	Bar-F	TCGTCAACCACTACATCGAGAC	For Bar plasmid validation
	Bar-R	GAAGTCCAGCTGCCAGAAAC	
<i>Ben</i>	Ben-F	GGTAACTCCACCGCCATCCA	For Ben plasmid validation
	Ben-R	GCAGGGTATTGCCTTTGGACTT	
<i>GAPDH</i>	<i>GAPDH</i> -F	GACTGCCCCGATTGAGAAG	For RT-PCR validation
	<i>GAPDH</i> -R	AGATGGAGGAGTTGGTGTG	
<i>Smt3</i> -cDNA	<i>MrSmt3</i> -cDNA-F	<u>CAAGAACCTTTAATCGAATTCATGTCCAACGAAGAAGGCCACA</u> , <i>Eco</i> RI	For constructing subcellular localization vector
	<i>MrSmt3</i> -cDNA-R	<u>GCCCTTGCTCACCATGAATTCAATCGAGCCGCCCACTTG</u> , <i>Eco</i> RI	