

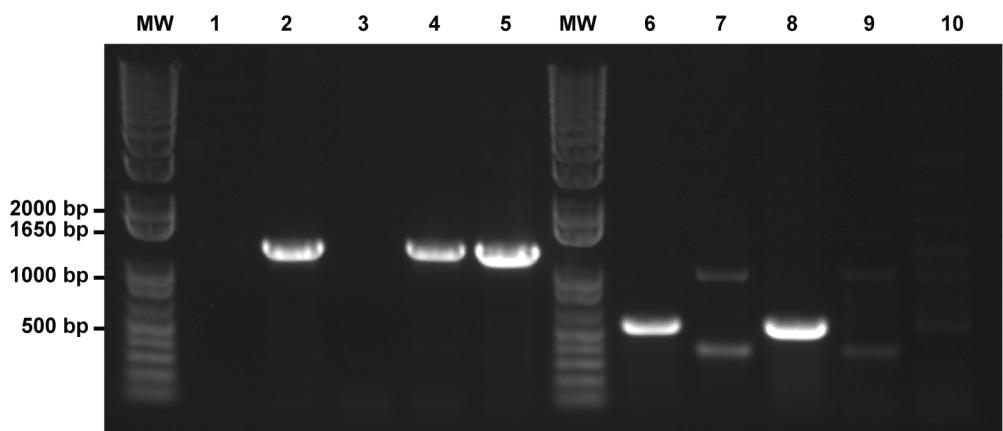
**Supplementary data:**

**Table S1: Primers used in this study.**

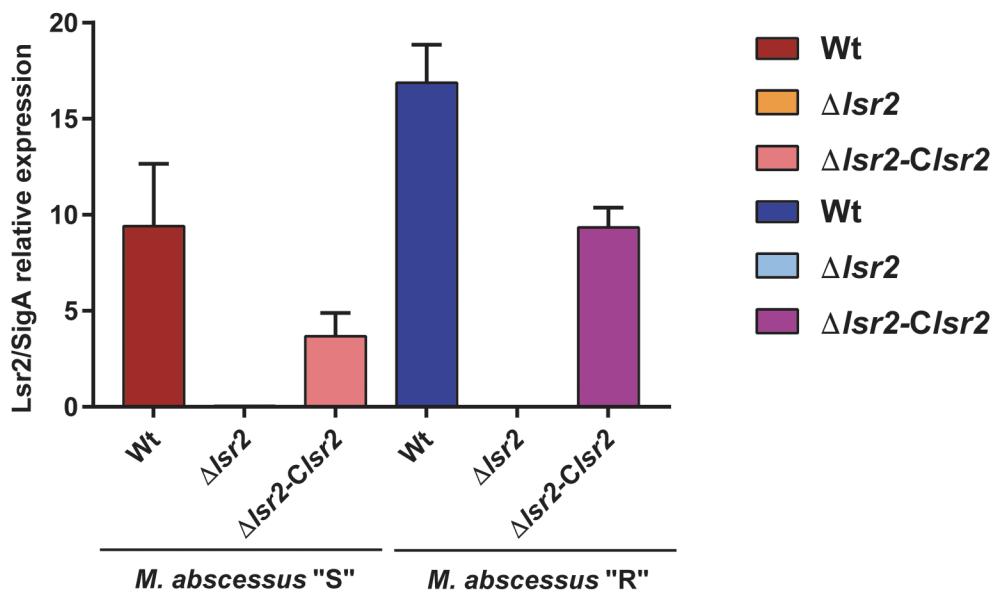
Name	Sequence
MC75	5'-cgaagcttacttcttgagttggagac-3'
MC76	5'-cgcatatgttagatcagagtgtgggtcgc-3'
MC80	5'-gcggatccgttcccggacaattcg-3'
MC81	5'-cgtctagaccgcgtcgattcccttc-3'
lsr2-5	5'-gcaatgcataagaaggcacggcacg-3'
lsr2V	5'-acattgtgcccggttac-3'
ZeoR	5'-cgactagtgatccccggaaatc-3'
Comp-MAB_0545+reg-AclI	5'-ggaatt <u>caacgttctttcctgaaaaaggcactcg</u> -3'
Comp-MAB_0545-HpaI	5'-ggaatt <u>cgttaacctagttggccgcgttgaac</u> -3'
Comp-MAB_0545-NdeI	5'-ggaatt <u>ccatatgtggctaagaaggcacgg</u> -3'
Comp-MAB_0545-HindIII	5'-at <u>atccaagcttctagttggccgcgttgaac</u> -3'
Comp-MAB_0545-FLAG-HpaI	5'gaatt <u>cgttaacctacttgtcgctgtcgttgcgtatgtcg</u> <u>tggcgttgcgtacccgtcggtgcgttgcgtatgtcggttgcgtt</u> gaacgc-3'
MAB_4100c_qPCR1	5'-gtggacatcgcaaacatacg-3'
MAB_4100c_qPCR3	5'-cgaatggaaatgtgtcaaag-3'
Lsr2-QPCR-F	5'-gagaccgttgaattcggtg-3'
Lsr2-QPCR-R	5'-gctgattacgcagctctcc-3'
SigA1F	5'-tccgagaaagacaaggcttc-3'
SigA1R'	5'-ccagctcgacttctcttcg-3'

**Table S2.** *lsr2* mRNA ratio between R and S variants of *M. abscessus* CIP104536 (Collection Institut Pasteur), ATCC19977 type strains and from the CF clinical isolate, from Pawlik *et al.* (2013).

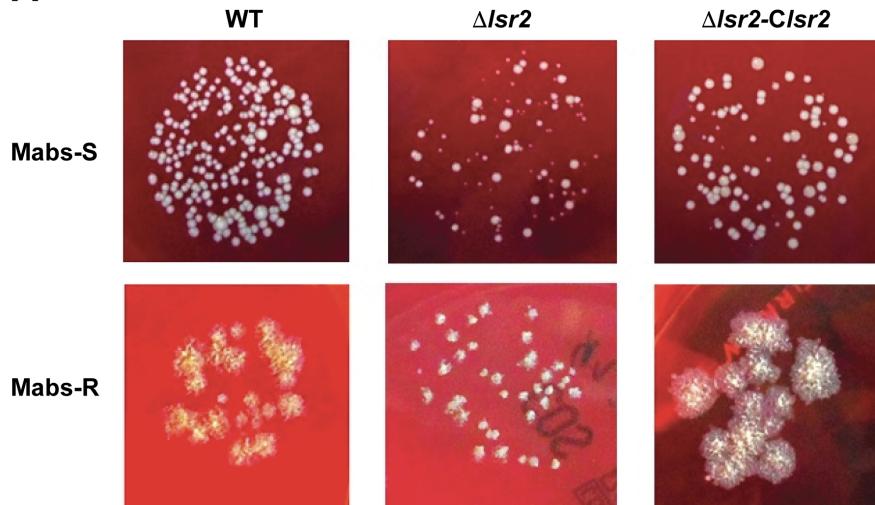
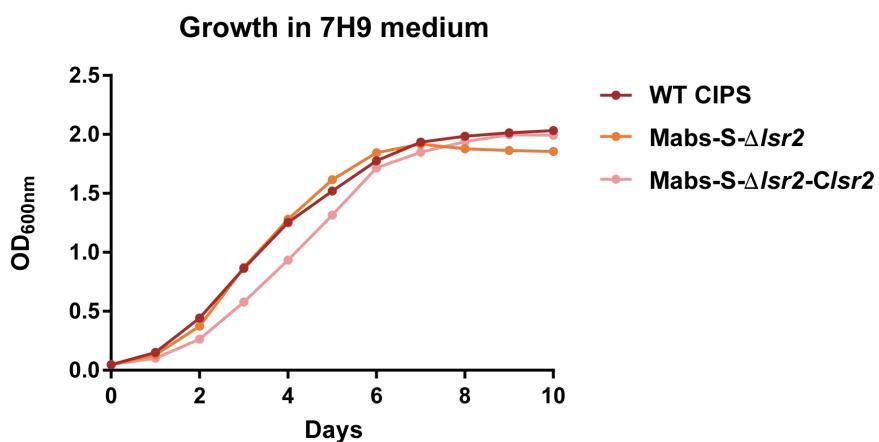
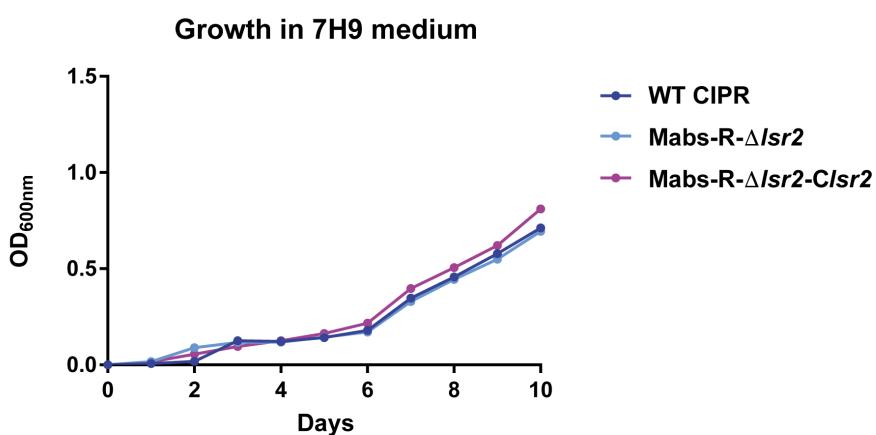
Compared strains	19977-IP-R/ 19977-IP-S	19977-AT-R/ 19977-AT-S	CF-R/ CF-S
Ratio (Standard deviation)	<b>1.311</b> (+/- 0.007)	<b>1.654</b> (+/- 0.011)	<b>1.250</b> (+/- 0.003)



**Figure S1. PCR validation of *lsr2* knock-out mutants ( $\Delta lsr2$ ).** Columns 1 to 5: PCR carried out with primers MC75 and ZeoR (no amplification in Mabs-S or -R wild-type strains, 1356 bp fragment for Mabs-S and -R- $\Delta lsr2$ ). Columns 6 to 10: PCR carried out with the primers lsr2-5 and lsr2V (fragment of 552bp for Mabs-S or -R wild-type strains and no amplification for Mabs-S and -R- $\Delta lsr2$ ). Columns 1 and 6: Mabs-S; columns 2 and 7: Mabs-S- $\Delta lsr2$ ; columns 3 and 8: Mabs-R; columns 4 and 9: Mabs-R- $\Delta lsr2$ ; columns 5 and 10: pMC34 (control plasmid containing the AES); MW columns: Molecular weight marker. The bands correspond to the expected results.

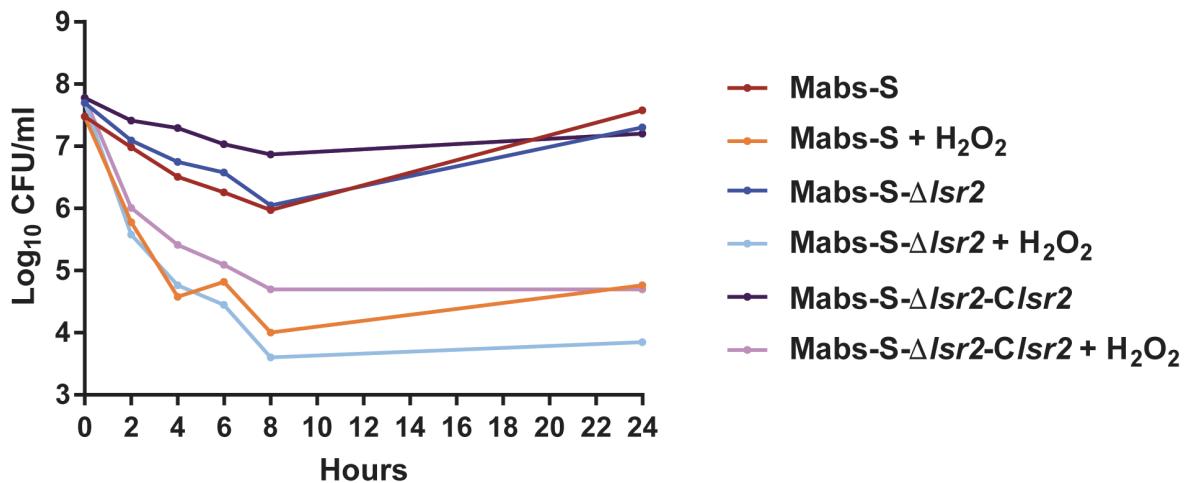


**Figure S2.** Absence of *lsr2* expression in the *lsr2* knock-out strains, while transcripts of the gene are detected in the complemented strains using RT-qPCR.

**A****B****C**

**Figure S3.** (A) Photographs of the colonies from the S and R WT type 19977-IP and their corresponding mutant and complemented strains. (B) Growth curve showing the absence of growth defect in Mabs-S- $\Delta lsr2$  as compared to Mabs-S and Mabs-S- $\Delta lsr2\text{-}Cl sr2$  strains. (C) Growth curve showing the absence of growth defect in Mabs-R- $\Delta lsr2$  as compared to Mabs-R and Mabs-R- $\Delta lsr2\text{-}Cl sr2$  strains.

### Mabs-S vs. Mabs-S- $\Delta$ lsr2



**Figure S4. Resistance to H<sub>2</sub>O<sub>2</sub> of Mabs-S versus Mabs-S- $\Delta$ lsr2 and Mabs-S- $\Delta$ lsr2-Clsr2.** As described in material and methods, Mabs-S, Mabs-S- $\Delta$ lsr2 and Mabs-S- $\Delta$ lsr2-Clsr2 were grown in liquid medium up to exponential phase of growth. H<sub>2</sub>O<sub>2</sub> was then added at 20 mM concentration and decrease of growth was monitored by CFU counting at 2, 4, 6, 8, and 24 h.