

Supplemental Figure 1A. Primer list used to build Rel knockout and Rel complemented strain.

Primers / Sequence	Description	Purpose
761 5'-CTAACGCTGGTCGTGCATAA-3'	RP for downstream ZeoR cassette.	PCR sequence check for Δrel strain
762 5'-CCGAGGAGCAGGACTGAA-3'	FP for downstream ZeoR cassette.	PCR sequence check for Δrel strain
1424 5'-GTGCAGTTCAAGGACCTGACG-3'	FP for upstream rel_{mab} gene.	PCR sequence check for Δrel strain
1425 5'-CCGAAGGAGACAGAGAAGTTCGT-3'	RP for downstream rel_{mab} gene.	PCR sequence check for Δrel strain
1233 5'-TGCGCAGACCTACCAGCTCGAATACGGCAC-3'	FP for upstream flanking segment of rel_{mab} gene.	Making segment of Δrel insert
1234 5'-TTAATCGCCTTGCAGCACAGCGATGAGCGGTTCC-3'	RP for upstream flanking segment of rel_{mab} gene.	Making segment of Δrel insert
1235 5'-ACCCTCATCGCTGTGCTGCAAGGCGATTAAGTTGG-3'	FP for ZeoR cassette.	Making segment of Δrel insert
1236 5'-TGTTCAACCCGCTCATAAACAGCTATGACCATGATTAC-3'	RP for ZeoR cassette.	Making segment of Δrel insert
1237 5'-TGGTCATAGCTGTTTATGAGCGGGTGAACATCTTGT-3'	FP for downstream flanking segment of rel_{mab} gene.	Making segment of Δrel insert
1238 5'-GGCTCAAGGGTGAACCAACGACATCG-3'	RP for downstream flanking segment of rel_{mab} gene.	Making segment of Δrel insert
1329 5'-CCGCATGCTTAATTAAGAAGGAGATATACATATGGCTGACGAGCAGCTGCCACGCGAG-3'	FP for upstream rel_{mab} gene.	Amplifying rel gene for comp. strain
1330 5'-CCAATTAATTAGCTAAAGCTTCTACTTCTCGAACTGGGGTGGCTCCAGTCGGCGGCCGAGGTACCCGGTA-3'	RP for downstream rel_{mab} gene.	Amplifying rel gene for comp. strain

Supplemental Figure 1B. Bacterial strains and Plasmids.

Strain(s) or plasmid	Description	Boutte Lab Ref. #
<u><i>E. coli</i> strains</u>		
DH5 α , TOP10	<i>E. coli</i> strains used for general cloning procedures.	
<u><i>M. abscessus</i> strains</u>		
ATCC19977	Wild-type strain used for control purposes and as PCR template.	
Mab/pNitRecET	Transformed pCB207 into ATCC19977, used for recombineering purposes.	BN-1
Mab/pNitRecET Δrel ::ZeoR	<i>M. abscessus rel</i> gene knockout by insertion of ZeoR cassette by means of recombineering ⁹ . - Referenced in text as Δrel_{mab} mutant strain.	BN-17
Mab Δrel ::ZeoR L5::pCT94- <i>rel</i>	Complementation strain - pCB1248 transformed into Δrel_{mab} mutant strain.	BN-20
<u>Plasmids</u>		
pCB207	DH5 α / pNit-RecET-SacB-gent	
pCB1248	DH5 α / pCT94- <i>Rel</i> _{Mab}	

Supplemental Figure 1C. Schematic of Rel knockout by mycobacterial recombineering system.

