

Supplementary Information

Mechanistic and structural insights into the unique TetR-dependent regulation of a drug efflux pump in *Mycobacterium abscessus*

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Keywords: *Mycobacterium abscessus*, TetR regulator, MmpL, efflux pump, structure, thiacetazone analogues, EMSA.

Table S1. List of bacterial strains and plasmids used in this study.

Name	Description/genotype	Marker	Reference
Strains			
<i>M. abscessus</i> S	<i>Mabs sensu stricto</i> , strain CIP104536 ^T , smooth	–	Laboratoire de Référence des Mycobactéries
<i>M. abscessus</i> R	<i>Mabs sensu stricto</i> , strain CIP104536 ^T , rough	–	Laboratoire de Référence des Mycobactéries
<i>M. abscessus</i> S Δ mmpL5	<i>Mabs</i> mutant obtained by recombination in the smooth parental strain CIP104536 ^T	Kan	This study
<i>M. abscessus</i> R Δ mmpL5	<i>Mabs</i> mutant obtained by recombination in the rough parental strain CIP104536 ^T	Kan	This study
<i>M. abscessus</i> S Δ MAB_4384	<i>Mabs</i> mutant obtained by recombination in the smooth parental strain CIP104536 ^T	Kan	This study
<i>M. abscessus</i> R Δ MAB_4384	<i>Mabs</i> mutant obtained by recombination in the rough parental strain CIP104536 ^T	Kan	This study
<i>M. abscessus</i> D15_S6	<i>Mabs</i> smooth strain containing a D14N point mutation in MAB_4384 and resistant to TAC analogues	–	Halloum et al., 2017
<i>M. abscessus</i> D15_S7	<i>Mabs</i> smooth strain containing a F57L point mutation in MAB_4384 and resistant to TAC analogues	–	Halloum et al., 2017
<i>M. abscessus</i> D15_R1	<i>Mabs</i> rough strain containing a M1A point mutation in MAB_4384 and resistant to TAC analogues	–	Halloum et al., 2017
<i>E. coli</i> HB101	<i>F mcrB mrr hsdS20(rB⁻ mB⁻) recA13 leuB6 ara-14 proA2 lacY1 galk2 xyl-5 mtl-1 rpsL20(SmR) glnV44 λ-</i>	Stp	MCLAB
<i>E. coli</i> XL1-Blue	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F' proAB lacIqZΔM15 Tn10 (Tetr)].</i>	Tet	Stratagene
<i>E. coli</i> BL21(DE3) Rosetta2	<i>F' ompT hsdS_B(r_B⁻ m_B⁻) gal dcm (DE3) pRARE2 (Cam^R)</i>		Novagen
Plasmids			
pET32a	Vector for high-level expression of proteins fused with an N-terminal thioredoxin protein and a His tag	Amp	Novagen
pET32a_MAB_4384	pET32a in which MAB_4384 is cloned in fusion with the thioredoxin/His tag sequence and containing a TEV cleavage site before MAB_4384 to remove the tags fusion	Amp	This work
pET32a_MAB_4384(D14N)	pET32a_MAB_4384 carrying the D14N mutation in MAB_4384	Amp	This work

pET32a_MAB_4384(F57L)	pET32a_MAB_4384 carrying the F57L mutation in MAB_4384	Amp	This work
pET32a_MAB_4384(D14N/F57L)	pET32a_MAB_4384 carrying the double D14N/F57L mutation in MAB_4384	Amp	This work
pUX1	Suicide construct designed for single cross-over in mycobacteria	Kan	Viljoen et al. 2017, in preparation
pUX1_MAB_4384	pUX1 designed to inactivate MAB_4384 in <i>M. abscessus</i>	Kan	This study
pUX1_mmpL5	pUX1 designed to inactivate mmpL5 in <i>M. abscessus</i>	Kan	This study
pMV261	Multi-copy <i>E. coli</i> /mycobacterial shuttle vector. Cloned genes are under the control of the constitutive hsp60 promoter	Kan	(53)
pMV261_lacZ	pMV261 containing the promoterless lacZ reporter gene	Kan	(53)
pMV261_P _{hsp60} _lacZ	pMV261_lacZ carrying the hsp60 promoter region cloned upstream of lacZ	Kan	(53)
pMV261_P _{S5/L5} _lacZ	pMV261_lacZ carrying the promoter region of mmpS5/mmpL5 cloned upstream of lacZ	Kan	This study

Amp, ampicillin; Kan, kanamycin; Tet, Tetracycline; Stp, streptomycin

Table S2: Oligonucleotides used in this study. Fw and Rev stand for forward and reverse, respectively.

Primers	5' to 3' sequence
Cloning in pET32a	
<i>MAB_4384_full</i>	Fw: TCA GGG TAC CGA GAA TCT GTA CTT CCA GGG A GT GAA CGG CCG TGA AGT GGC GCA TC (KpnI , Tobacco Etch Virus [TEV] cleavage site in in italic) Rev: GAG AAA GCT TGA GAC GCA GTG ACA GTG AGC (HindIII))
<i>MAB_4384_DM</i>	Fw: GTG TAC CGC CGC CTT CCC ACG AAA (substituted nucleotide in bold) Rev: TTT CGT GGG AAG GCG GCG GTA CAC (substituted nucleotide in bold)
Cloning in pMV261_lacZ derivatives	
<i>HB101_lacZ</i>	Fw: TAT ATA GGA TCC GAT GAC CAT GAT TAC GGA TTC ACT GGC C (BamHI) Rev: TAT AGC AAG CTT TAC GCG AAA TAC GGG CAG ACA TGG (HindIII)
<i>MAB_4384_P_{S5/L5}</i>	Fw: <i>TCT</i> AGA GCC GAC GAG TGT ATC GCT CG (XbaI) Rev: <i>GGA TCC</i> CGC AGC CAC CCC GTC CCG CT (BamHI)
Cloning in pUX1	
<i>MAB_4384::pUX1</i>	Fw: GAG AGA GCT AGC CCT TTT CGA GGC AAT TTA CG (NheI) Rev: GAG AGA GGA TCC CGG TAA GCA CTC CGA AGA AG (BamHI) Conf: CGT AAC CGG AAG CGT GTA ATC
<i>mmpL5::pUX1</i>	Fw: TCAG GGA TCC GGC AAC TGC CGT CAT TAA CT Rev: GAG AGC TAG CCC GGT CGT TAT AGG TGG TGT Conf: CGG GTT CAT CTT GGC TTT AGA
Sequencing	
pMV5'	CGC CCG GCC AGC GTA AGT AGC
pMV3'	GCC TGG CAG TCG ATC GTA CG
pMV3' Ext	TTG AGA CAC AAC GTC GCT TT
NheIpUX1	ACGGCATGGACGAGCTGTAC
qRT-PCR	
<i>sigA</i>	Fw: CAC ATG GTC GAG GTC ATC AA Rev: TGG ATT TCC AGC ACC TTC TC
<i>MAB_4384</i>	Fw: CGT AAC CGG AAG CGT GTA ATC Rev: GGA AAG CGG CGG TAC AC
<i>MAB_3551C (tgs1)</i>	Fw: CAC CGT CTA CTA CGG AAT CAA C Rev: TGC GCA GCC TCC AAT AAT
<i>MAB_0478</i>	Fw: CCACTCCGAGATGATTACT Rev: GCGATGGTGGCATGAAATAG
<i>MAB_0987c</i>	Fw: CCGCCTATCAGACGATGTATAAG Rev: CGTGTGAACGACAGACAGAA
<i>MAB_1134c</i>	Fw: CCGGAAGGTCTTTCGCATATC Rev: CGAGTTCGATCTTGGCATCTT
<i>MAB_1137c</i>	Fw: CTCGGGCTACCTCAATAACATC Rev: GCCTTGTGGCGGTAGATAAA
<i>MAB_1287</i>	Fw: AACAACAGCTCCACCAACT Rev: CTCCTTGACGTCCTCGATATTC

<i>MAB_2037</i>	Fw: GGGACAATGGAGGAGATGAAG Rev: GTGCGGTTCCCAGTAGTAATAG
<i>MAB_2210c</i>	Fw: CCAAGCGGTACAAGGCATTA Rev: GCTGACCATCTCGGCTATTT
<i>MAB_2301</i>	Fw: CAGCTCCATCCCATTCTTATC Rev: TCGGCCTGGACCTTCATA
<i>MAB_2303</i>	Fw: ACATTCTCTGTCCCAGATCATT Rev: GTGCTCTTTACCGACCTCTTC
<i>MAB_2570c</i>	Fw: GGCTCAAGGAAGTAATGGAGAA Rev: GTGTGGCTCCCAATACAGATAG
<i>MAB_2571c</i>	Fw: CTCTTGTTGGCGTGTTGATTAC Rev: CGAACGACGAGATTCCAATGA
<i>MAB_2650</i>	Fw: GAAAGCGGACTCGGGTATTT Rev: TTGGGCTTGATGTCGGAAG
<i>MAB_3150</i>	Fw: ACTGGTCATCCTGGTGATTG Rev: CATCCGCCTTCGTCATACTT
<i>MAB_3201</i>	Fw: CCGATTCTCGATACCCATTCTC Rev: GTGCTGCTTGATCACTGTTTC
<i>MAB_3562c</i>	Fw: GGGAGTTTGTTCCGGCTATT Rev: GCACCCACCATGACGATAAT
<i>MAB_4240c</i>	Fw: CTCGATGAAGGAGATGGGAAAG Rev: GCTCGATCAACGCCGAATA
<i>MAB_4263</i>	Fw: CTGATTCCGACCTCGATGATG Rev: GCCGATGAAGAAGATGGAGTAG
<i>MAB_4310c</i>	Fw: AAGCCCAACAGCAAGAATTG Rev: GAATACATCGGGCGGTAGATAG
<i>MAB_4382c</i>	Fw: GGGCTATCACACCACCTAT Rev: CGGGTTCATCTTGGCTTTA
<i>MAB_4383c</i>	Fw: GTCATGCATTTACGCACCAAC Rev: CGAAGACCTCATACGTCACAAC
<i>MAB_4704c</i>	Fw: CTCGGCAATCTGGGATTCATAG Rev: CTCTTGGTATCGCCCAGTAAAG
<i>LacZ</i>	Fw: CCAACGTGACCTATCCCATTAC Rev: TTCCTGTAGCCAGCTTTCATC
Electrophoretic Mobility Shift Assay (EMSA)	
Probe 1	Fw: CAC TTC GCC ATA AGT GGA TTG ACT CTA TCC ACT TTT ACC CAT AGA (Fluorescein in 5') Rev: TCT ATG GGT AAA AGT GGA TAG AGT CAA TCC ACT TAT GGC GAA GTG
Probe 2	Fw: CAC TTC GCC ATG GAC TTC GTG ACT CTA TCC ACT TTT ACC CAT AGA (Fluorescein in 5') Rev: TCT ATG GGT AAA AGT GGA TAG AGT CAC GAA GTC CAT GGC GAA GTG
Non-specific probe	Fw: ATG GAA CTT GAA GGA CTG ACC GCG TTG (Fluorescein in 5') Rev: CAA CGC GGT CAG TCC TTC AAG TTC CAT
Probe 3	Fw: TCT GGA TAA GCA AGT GGA TTG ACT CTA TCC ACT TCC GAA ACC GAC Rev: GTC GGT TTC GGA AGT GGA TAG AGT CAA TCC ACT TGC TTA TCC AGA

Probe 4	Fw: CAC TTC GCC ATA AGT GGA TCA TCC ACT TTT ACC CAT AGA (Fluorescein in 5') Rev: TCT ATG GGT AAA AGT GGA TGA TCC ACT TAT GG C GAA GTG
Probe 5	Fw: CACT TCG CCA TAA GTG GAT ATG CAT GAC TCT CTG AAG ATC CAC TTT TAC CCA TAG A (Fluorescein in 5') Rev: TCT ATG GGT AAA AGT GGA TCT TCA GAG AGT CAT GCA TAT CCA CTT ATG GCG AAG TG
Probe 6	Fw: CAC TTC GCC ATG GGT GGA TTG ACT CTA TCC ACC CTT ACC CAT AGA (Fluorescein in 5') Rev: TCT ATG GGT AAG GGT GGA TAG AGT CAA TCC ACC CAT GGC GAA GTG
Probe 7	Fw: CAC TTC GCC ATA AGT GGG CTG ACT CTG CCC ACT TTT ACC CAT AGA (Fluorescein in 5') Rev: TCT ATG GGT AAA AGT GGG CAG AGT CAG CCC ACT TAT GGC GAA GTG
Probe 8	Fw: CAC TTC GCC ATA AGC AGA TTG ACT CTA TCT GCT TTT ACC CAT AGA (Fluorescein in 5') Rev: TCT ATG GGT AAA AGC AGA TAG AGT CAA TCT GCT TAT GGC GAA GTG

^aRestriction sites are underlined and specified inside brackets.

^bMutagenized bases are shown in bold.

^cInverted palindromes are shown in italic on the 5' primer.

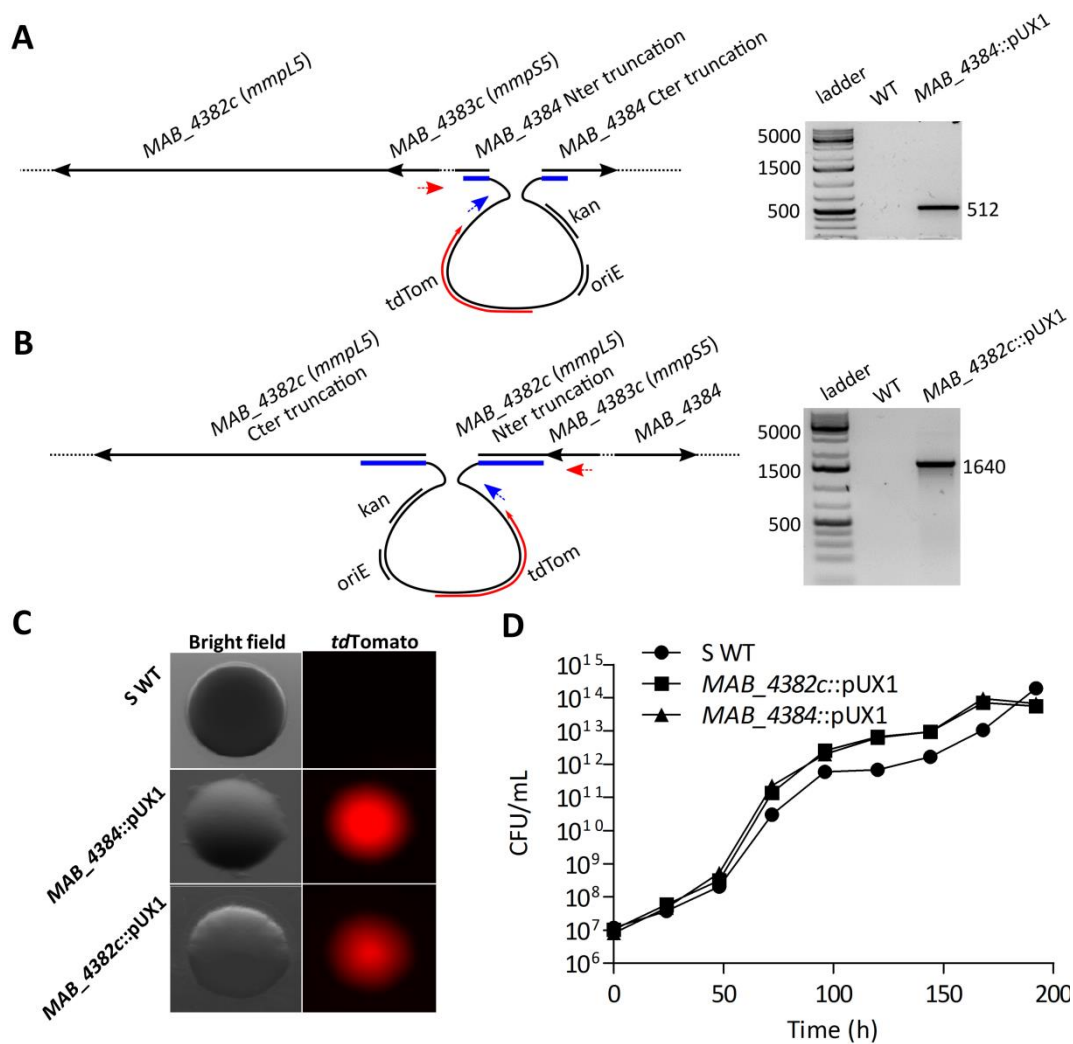


Figure S1. Generation of *M. abscessus* mutants with interruptions in *MAB_4384* and *MAB_4382c* (*mmpL5*). The strategy involves the production of suicide vectors that homologously recombine within targeted genes and carrying a kanamycin cassette as well as a *tdTomato* marker for a rapid screening of transformants having undergone gene disruption (Viljoen et al. 2018). The schematic representation of disrupted *MAB_4384* (**A**) and *MAB_4382c* (**B**) is shown. Proper gene disruption was confirmed by PCR analyses after selection of the red fluorescent colonies (**C**) obtained after transformations. Specific sets of primers were designed to only amplify a band of the expected size in the mutant strains. Blue arrows represent primers that hybridize within the inserted plasmid whereas red arrows correspond to primers only hybridizing within the chromosome. Sizes of the PCR products were analyzed on agarose gels and further sequenced to confirm the gene disruption. (**D**) *In vitro* growth of the parental *M. abscessus* S strain and its *MAB_4384*::pUX1 and *MAB_4382c*::pUX1 derivatives. Bacteria were grown in 7H9 broth supplemented with ADC and 0.05% Tween 80. Growth was monitored by determining the CFU/ml at different time points.

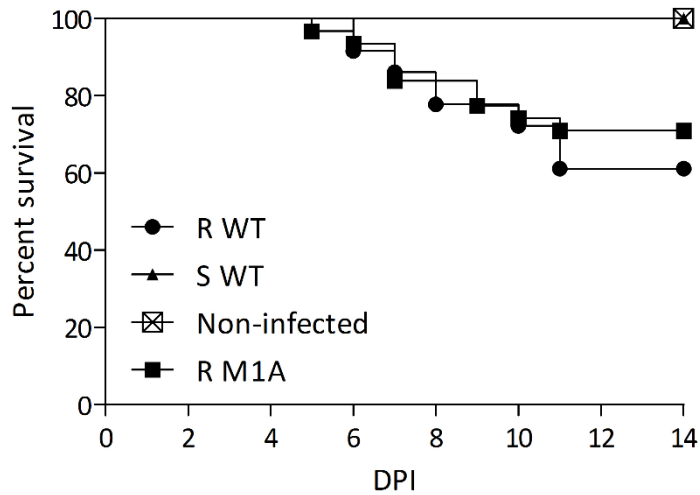


Figure S2. Survival curves of *M. abscessus* strains in zebrafish embryos. Embryos were infected intravenously the wild-type smooth (S) (350 CFU), rough (R) (220 CFU) or MAB_4384(M1A) mutant (280 CFU) strains expressing tdTomato as reported earlier (Bernut et al., 2014, 2015). Groups of uninfected fishes were also included as controls. Dead embryos succumbing from infection were monitored on a daily basis up to 14 days post-infection (dpi). Results are representative of two independent experiments.

All zebrafish experiments were approved by the Direction Sanitaire et Vétérinaire de l'Hérault et Comité d'Ethique pour l'Expérimentation Animale de la région Languedoc Roussillon under the reference CEEA-LR-1145.