

Supplementary Materials: Multi-stress induction of the *Mycobacterium tuberculosis* MbcTA bactericidal toxin-antitoxin system

Kanchiyaphat Ariyachaokun, Anna D. Grabowska, Claude Gutierrez and Olivier Neyrolles

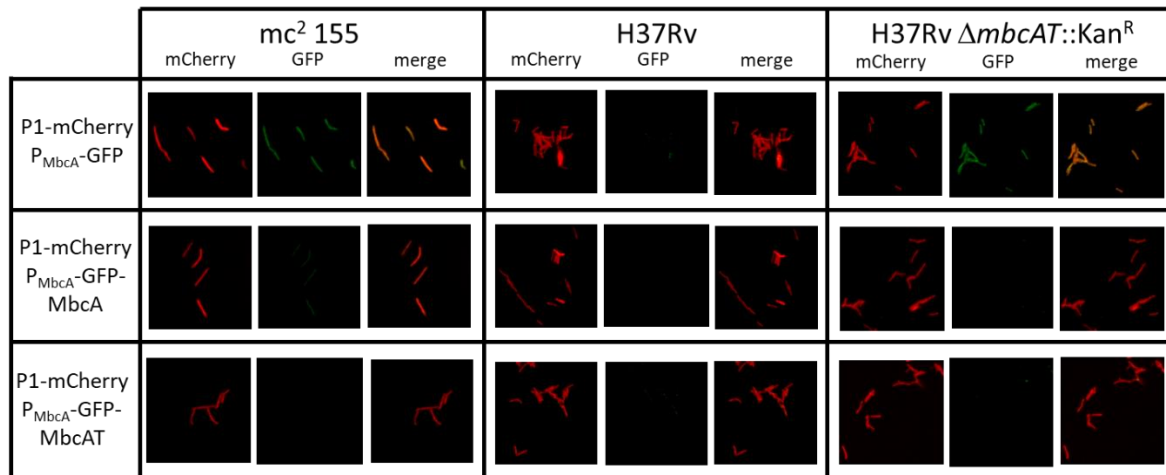


Figure S1. Auto-repression of the *mbcAT* operon expression in *M. smegmatis* and *M. tuberculosis*. Wild type *M. smegmatis* mc² 155 (left), wild type *M. tuberculosis* H37Rv (center) or mutant *M. tuberculosis* H37Rv Δ mbcAT::Kan^R (right) harboring plasmid pGMCS-P1-mCherry-P_{MbcA}GFP, pGMCS-P1-mCherry-P_{MbcA}GFP-*mbcA* or pGMCS-P1-mCherry-P_{MbcA}GFP-*mbcAT*, as indicated, were grown to exponential phase in complete 7H9 medium supplemented with streptomycin. Samples of the cells were fixed with 4% *p*-formaldehyde and observed in fluorescence microscopy using large field leica DMIRB, at magnification of 630 \times .

Table S1. Primers used for plasmid constructions.

Construction /DNA Fragment	Template	Primers	Sequence 5'-3'
pEN41A-P1-GFP			
P1	pEN12A-P1	Fw-GRFP-SD-P1	GCTCACCATGGTGAATCCTCCTCAT TTCGGGCGGCGAATCTC
		Rev-B1-P1	GGGACTGCTTTTTTGTACAAACTTGG GCGGCACGATCCGCGACGTG
GFP ORF	pLAM12:: GFP	Fw-B4-GFP	GGGACAACCTTTGTATAGAAAAGTTGT TACTTGTACAGCTCGTCCATG
		Rv-SD-GRFP	AGGAGGATTCACCATGGTGAGCAAGG GCGAGGAG
pEN41A-P1-mCherry			
P1	pEN12A-P1	Fw-GRFP-SD-P1	GCTCACCATGGTGAATCCTCCTCAT TTCGGGCGGCGAATCTC
		Rv-B1-P1	GGGACTGCTTTTTTGTACAAACTTGG GCGGCACGATCCGCGACGTG
mCherry ORF	pLAM12:: mCherry	Fw-B4-GRFP	GGGACAACCTTTGTATAGAAAAGTTGT TACTTGTACAGCTCGTCCATGCCG
		Rv-SD-GRFP	AGGAGGATTCACCATGGTGAGCAAGG GCGAGGAG
pEN41A-P1-mTurquoise			
P1	pEN12A-P1	Fw-mTurq-SD-P1	GCTGACCATGGTGAATCCTCCTCAT TTCGGGCGGCGAATCTC
		Rv-B1-P1	GGGACTGCTTTTTTGTACAAACTTGG GCGGCACGATCCGCGACGTG
mTurquoise ORF	pJYB 240- mTurquoise	Fw-B4-mTurq	GGGACAACCTTTGTATAGAAAAGTTGT TACTTGTAGAGTTCGTCCATGCCG
		Rv-SD-mTurq	AGGAGGATTCACCATGGTCAGCAAGG GTGAGGAACTC
pEN41A-P1-mVenus			
P1	pEN12A-P1	Fw-GRFP-SD-P1	GCTCACCATGGTGAATCCTCCTCAT TTCGGGCGGCGAATCTC
		Rv-B1-P1	GGGACTGCTTTTTTGTACAAACTTGG GCGGCACGATCCGCGACGTG
mVenus ORF	pJYB 234- mVenus	Fw-B4-GFP	GGGACAACCTTTGTATAGAAAAGTTGT TACTTGTACAGCTCGTCCATG
		Rv-SD-GRFP	AGGAGGATTCACCATGGTGAGCAAGG GCGAGGAG
pEN12A-P_{mbcA}-GFP			
P _{mbcA(261)}	H37Rv DNA	Fw-B1-P _{mbcA}	GGGACAAGTTTGTACAAAAAAGCAG GCTGCTGCTGGCTCCCAGTAGAC
		Rv-GRFP-P _{mbcA}	GCTCACCATGGTGAATCCTCCTCCGGG CAGCCTGTCTTCCTTTTG
GFP ORF	pLAM12::GFP	Fw-P _{mbcA} -GRFP	CAAAAGGAAGACAGGCTGCCCGGAG GAGGATTCACCATGGTGAGCAAGGGC GAGGAG
		Rv-B2-GRFP	GGGACCACTTTGTACAAGAAAGCTG GGTTACTTGTACAGCTCGTCCATG
pEN12A-P_{mbcA}-mCherry			

<i>P_{mbcA(261)}</i>	H37Rv DNA	Fw-B1- <i>P_{mbcA}</i>	GGGGACAAGTTTGTACAAAAAAGCAG GCTGCTGCTGGCTCCCAGTAGAC
		Rv-GRFP- <i>P_{mbcA}</i>	GCTCACCATGGTGAATCCTCCTCCGGG CAGCCTGTCTTCCTTTTG
GFP ORF	pLAM12:: mCherry	Fw- <i>P_{mbcA}</i> -GRFP	CAAAAGGAAGACAGGCTGCCCCGAG GAGGATTCACCATGGTGAGCAAGGGC GAGGAG
		Rv-B2-GRFP	GGGGACCACTTTGTACAAGAAAGCTG GGTTACTTGTACAGCTCGTCCATG
pEN12A-<i>P_{mbcA}</i>-mTurquoise			
<i>P_{mbcA(261)}</i>	H37Rv DNA	Fw-B1- <i>P_{mbcA}</i>	GGGGACAAGTTTGTACAAAAAAGCAG GCTGCTGCTGGCTCCCAGTAGAC
		Rv-mTurq- <i>P_{mbcA}</i>	GCTGACCATGGTGAATCCTCCTCCGGG CAGCCTGTCTTCCTTTTG
mVenus ORF	pJYB 234- mTurquoise	Fw- <i>P_{mbcA}</i> -mTurq	CAAAAGGAAGACAGGCTGCCCCGAG GAGGATTCACCATGGTCAGCAAGGGT GAGGAACTC
		Rv-B2-mTurq	GGGGACCACTTTGTACAAGAAAGCTG GGTTACTTGTAGAGTTCGTCCATGCC G
pEN12A-<i>P_{mbcA}</i>-mVenus			
<i>P_{mbcA(261)}</i>	H37Rv DNA	Fw-B1- <i>P_{mbcA}</i>	GGGGACAAGTTTGTACAAAAAAGCAG GCTGCTGCTGGCTCCCAGTAGAC
		Rv-GRFP- <i>P_{mbcA}</i>	GCTCACCATGGTGAATCCTCCTCCGGG CAGCCTGTCTTCCTTTTG
mVenus ORF	pJYB 234- mVenus	Fw- <i>P_{mbcA}</i> -GRFP	CAAAAGGAAGACAGGCTGCCCCGAG GAGGATTCACCATGGTGAGCAAGGGC GAGGAG
		Rv-B2-GRFP	GGGGACCACTTTGTACAAGAAAGCTG GGTTACTTGTACAGCTCGTCCATG
pEN12A-<i>P_{mbcA(103)}</i>-GFP			
<i>P_{mbcA(103)}</i> -GFP	pEN12A- <i>P_{mbcA(261)}</i> -GFP	Fw2-B1- <i>P_{mbcA}</i>	GGGGACAAGTTTGTACAAAAAAGCAG GCTCCGAAACTGAATGCCCATCG
		Rv-B2-GRFP	GGGGACCACTTTGTACAAGAAAGCTG GGTTACTTGTACAGCTCGTCCATG
pEN12A-<i>P_{mbcA(239)}</i>-GFP			
<i>P_{mbcA(239)}</i>	pEN12A- <i>P_{mbcA(261)}</i> -GFP	Fw-B1- <i>P_{mbcA}</i>	GGGGACAAGTTTGTACAAAAAAGCAG GCTGCTGCTGGCTCCCAGTAGAC
		Rv-GRFP- <i>P_{mbcA}</i>	GCTCACCATGGTGAATCCTCCTCCCTA TTGATTTGTCATGTATTATGACACGAA CCGAGG
GFP ORF	pLAM12:: GFP	Fw2- <i>P_{mbcA}</i> -GFP	ACATGACAAATCAATAGGGAGGAGGA TTCACCATGGTGAGCAAGGGCGAGGA G
		Rv-B2-GRFP	GGGGACCACTTTGTACAAGAAAGCTG GGTTACTTGTACAGCTCGTCCATG
pEN12A-<i>P_{mbcA(239-G)}</i>-GFP			
<i>P_{mbcA(239-G)}</i>	pEN12A- <i>P_{mbcA(261)}</i> -GFP	Fw-B1- <i>P_{mbcA}</i>	GGGGACAAGTTTGTACAAAAAAGCAG GCTGCTGCTGGCTCCCAGTAGAC

		Rv3-GFP-P _{mbcA}	GCTCACCATGGTGAATCCTCCTCCCTA TTGATTTGTCATGTGTTATGACACGAA CCGAGG
GFP ORF	pLAM12:: GFP	Fw2-P _{mbcA} -GFP	ACATGACAAATCAATAGGGAGGAGGA TTCACCATGGTGAGCAAGGGCGAGGA G
		Rv-B2-GRFP	GGGGACCACTTTGTACAAGAAAGCTG GGTTACTTGTACAGCTCGTCCATG
pGMCS-P1-mCherry-P_{mbcA}(Mut1)-GFP-mbcA			
Mutated linear plasmid	pGMCS-P1- mCherry- P _{mbcA} (261)- <i>mbcA</i>	Inf-P _{mbcA} -right	GGCTGCCCGGAGGAGGATTC
		Inf-P _{mbcA} -Mut1- left	CTCCTCCGGGCAGCCTGTCTTCCTACG ATCTATTGATTTGTCATG
pGMCS-P1-mCherry-P_{mbcA}(Mut2)-GFP-mbcA			
Mutated linear plasmid	pGMCS-P1- mCherry- P _{mbcA} (261)- <i>mbcA</i>	Inf-P _{mbcA} -right	GGCTGCCCGGAGGAGGATTC
		Inf-P _{mbcA} Mut2- left -	CTCCTCCGGGCAGCCCACCTTCCTTTT GTCTATTGATTTG
pGMCS-P1-mCherry-P_{mbcA}(Mut1)-GFP			
Mutated linear plasmid	pGMCS-P1- mCherry- P _{mbcA} (Mut1)- <i>mbcA</i>	Inf-del <i>mbcA</i> -right	GGGAGTGTTTCGTGTGAGCTG
		Inf-del <i>mbcA</i> -left	CACACGAACACTCCCGAGGCGAGCAC ATTACTCCC
pGMCS-P1-mCherry-P_{mbcA}(Mut2)-GFP			
Mutated linear plasmid	pGMCS-P1- mCherry- P _{mbcA} (Mut2)- <i>mbcA</i>	Inf-del <i>mbcA</i> -right	GGGAGTGTTTCGTGTGAGCTG
		Inf-del <i>mbcA</i> -left	CACACGAACACTCCCGAGGCGAGCAC ATTACTCCC
pGMCS-P1-mCherry-P_{mbcA}(261)-<i>mbcA</i>(R33E)			
Mutated linear plasmid	pGMCS-P1- mCherry- P _{mbcA} (261)- <i>mbcA</i>	Inf- <i>mbcA</i> -R33E- right	GTCGATGCCTCGCCGGAGTCCGTGGC GCGATGGACCGC
		Inf- <i>mbcA</i> -R33E- left	CGGCGAGGCATCGACGATG
pGMCS-P1-mCherry-P_{mbcA}(261)-<i>mbcA</i>(R33E)-<i>mbcT</i>			
Mutated linear plasmid	pGMCS-P1- mCherry- P _{mbcA} (261)- <i>mbcAT</i>	Inf- <i>mbcA</i> -R33E- right	GTCGATGCCTCGCCGGAGTCCGTGGC GCGATGGACCGC
		Inf- <i>mbcA</i> -R33E- left	CGGCGAGGCATCGACGATG
pGMCS-P1-mCherry-P_{mbcA}(261)-<i>mbcA</i>(R37E)			
Mutated linear plasmid	pGMCS-P1- mCherry- P _{mbcA} (261)- <i>mbcA</i>	Inf- <i>mbcA</i> -R37E- right	CCGCGTTCCGTGGCGGAGTGGACCGC AGGTCAGGTGG
		Inf- <i>mbcA</i> -R37E- left	CGCCACGGAACGCGGCGAG

Table S2. Plasmids used in this work.

Name	Reference/Origin	Construction/Properties
pDE43-MCS	D. Schnappinger (Weill Cornell Medical College, New York, USA)	Destination vector for Gateway cloning. Strepto ^R
pDO221A	D. Schnappinger	Destination vector to construct pEN12 entry plasmids for Gateway cloning. Amp ^R
pDO23A	D. Schnappinger	Destination vector to construct pEN23 entry plasmids for Gateway cloning. Amp ^R
pDO41A	D. Schnappinger	Destination vector to construct pEN41 entry plasmids for Gateway cloning. Amp ^R
pEN12A-P1	D. Schnappinger	Middle entry clone for Gateway cloning. Template for amplification of P1 promoter. Amp ^R
pEN12A-P606Pld	D. Schnappinger	Middle entry clone for Gateway cloning. Amp ^R
pEN12A-P606	D. Schnappinger	Middle entry clone for Gateway cloning. Amp ^R
pEN12A-P _{mbcA} - reporter	This study	Gateway cloning into pDO221A. Middle entry clone for Gateway cloning. Reporter can be GFP, mCherry, mTurquoise or mVenus. Amp ^R
pEN23A-Mlu1	D. Schnappinger	Empty 5' entry clone for Gateway cloning. Amp ^R
pEN23A- <i>mbcT</i>	[1]	5' entry clone for Gateway cloning. Amp ^R
pEN23A- <i>mbcAT</i>	[1]	5' entry clone for Gateway cloning. Amp ^R
pEN41-TetR	D. Schnappinger	3' entry clone for Gateway cloning. Amp ^R
pEN41A-P1-reporter	This study	Gateway cloning into pDO41A. 3' entry clone for Gateway cloning. Reporter can be GFP, mCherry, mTurquoise or mVenus. Amp ^R
pGMCS-P1- mCherry-P _{mbcA} -GFP	This study	Gateway cloning. Reporter of P _{mbcA} expression (261-bp Fw1/Rv1 DNA fragment). Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-P1- mCherry-P _{mbcA} -GFP- <i>mbcA</i>	This study	Gateway cloning. Reporter of P _{mbcA} expression (261-bp Fw1/Rv1 DNA fragment). Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-P1- mCherry-P _{mbcA} -GFP- <i>mbcAT</i>	This study	Gateway cloning. Reporter of P _{mbcA} expression (261-bp Fw1/Rv1 DNA fragment). Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-P1- mCherry-P _{mbcA} -GFP- <i>mbcA</i> (R33E)	This study	Site directed mutagenesis of pGMCS-P1- mCherry-P _{mbcA} -GFP- <i>mbcA</i> . Strepto ^R
pGMCS-P1- mCherry-P _{mbcA} -GFP- <i>mbcA</i> (R37E)	This study	Site directed mutagenesis of pGMCS-P1- mCherry-P _{mbcA} -GFP- <i>mbcA</i> . Strepto ^R
pGMCS-P1- mCherry-P _{mbcA} -GFP- <i>mbcA</i> (R33E)- <i>mbcT</i>	This study	Site directed mutagenesis of pGMCS-P1- mCherry-P _{mbcA} -GFP- <i>mbcAT</i> . Strepto ^R

pGMCS-P1-mCherry-P _{mbcA(103)} -GFP	This study	Gateway cloning. Reporter of P _{mbcA} expression (103-bp Fw2/Rv1 DNA fragment). Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-P1-mCherry-P _{mbcA(103)} -GFP- <i>mbcA</i>	This study	Gateway cloning. Reporter of P _{mbcA} expression (103-bp Fw2/Rv1 DNA fragment). Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-P1-mCherry-P _{mbcA(239)} -GFP	This study	Gateway cloning. Reporter of P _{mbcA} expression (239-bp Fw1/Rv2 DNA fragment). Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-P1-mCherry-P _{mbcA(239)} -GFP- <i>mbcA</i>	This study	Gateway cloning. Reporter of P _{mbcA} expression (239-bp Fw1/Rv2 DNA fragment). Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-P1-mCherry-P _{mbcA(239-G)} -GFP	This study	Gateway cloning. Reporter of P _{mbcA} expression (239-bp Fw1/Rv3 DNA fragment). Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-P1-mCherry-P _{mbcA(Mut1)} -GFP- <i>mbcA</i>	This study	Site-directed mutagenesis of pGMCS-P1-mCherry-P _{mbcA} -GFP- <i>mbcA</i> . Strepto ^R
pGMCS-P1-mCherry-P _{mbcA(Mut1)} -GFP	This study	Site-directed mutagenesis of pGMCS-P1-mCherry-P _{mbcA(Mut1)} -GFP- <i>mbcA</i> . Strepto ^R
pGMCS-P1-mCherry-P _{mbcA(Mut2)} -GFP- <i>mbcA</i>	This study	Site-directed mutagenesis of pGMCS-P1-mCherry-P _{mbcA} -GFP- <i>mbcA</i> . Strepto ^R
pGMCS-P1-mCherry-P _{mbcA(Mut2)} -GFP	This study	Site-directed mutagenesis of pGMCS-P1-mCherry-P _{mbcA(Mut2)} -GFP- <i>mbcA</i> . Strepto ^R
pGMCS-P1-mTurquoise-P _{mbcA} -mVenus	This study	Gateway cloning. Reporter of P _{mbcA} expression. Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-TetR-P1- <i>mbcT</i>	[1]	Allow tetracycline-inducible expression of <i>mbcT</i> . Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-TetR-P606P1d- <i>mbcT</i>	This study	Allow tetracycline-inducible expression of <i>mbcT</i> . Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pGMCS-TetR-P606- <i>mbcT</i>	This study	Allow tetracycline-inducible expression of <i>mbcT</i> . Integrates at the L5 mycobacteriophage integration site in mycobacteria. Strepto ^R
pJYB 240-mTurq	J.Y. Bouet & J. Rech (LMGM, Toulouse, France)	Template for amplification of <i>mTurquoise</i> by PCR
pJYB 234-mVenus	J.Y. Bouet & J. Rech	Template for amplification of <i>mVenus</i> by PCR
pLAM12::GFP	[39]	Template for amplification of <i>egfp</i> by PCR.
pLAM12::mCherry	[39]	Template for amplification of <i>mCherry</i> by PCR