Supplementary materials

Title: MnoSR removal in *Mycobacterium smegmatis* triggers broad transcriptional response to 1,3-propanediol and glucose as sole carbon sources

Renata Płocińska#[1], Katarzyna Struś#[1], Małgorzata Korycka-Machała[1], Przemysław Płociński[2], Magdalena Kuzioła[1,3], Anna Żaczek[4], Marcin Słomka[5], Jarosław Dziadek[1*]

[1] Institute of Medical Biology of the Polish Academy of Sciences, Lodowa 106, 93-232 Łódź, Poland

[2] Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Łódz, Banacha 12/16, 90-237 Łódź, Poland

[3] BioMedChem Doctoral School of the University of Łódź and Łódź Institutes of the Polish Academy of Sciences, Matejki 21/23, 90-237 Łódź, Poland

[4] Department of Microbiology, College of Medical Sciences, University of Rzeszów, Rejtana 16c, 35-959 Rzeszów, Poland

[5]Biobank Lab, Department of Molecular Biophysics, Faculty of Biology and Environmental Protection, University of Łódź, Pomorska 139, 90-235 Łódź, Poland

# These authors has contributed equally to this work

* Correspondence should be addressed to jdziadek@cbm.pan.pl

Keywords: histidine sensor kinase MnoS, response regulator MnoR, Mycobacterium smegmatis, methylotrophic metabolism, RNA-seq

Table of contents:

Table S1 List of plasmids, strains and oligonucleotides used.

A. Oligonucleotides used in this study		
Name	Sequence (5'>3')	Application
Primers used for cloning		Amplification region
Ms6238GR1bScaI-s	cagtactCGTTGACAAGGCCACCATGC	*Ms-mnoS* upstream region (47 bp) including 5' of *mnoS* gene (1152 bp)
Ms6238GR2HindIII-r	caagcttCATCGAGGATGAGGTGACGC	
Ms6238GR3bHindIII-s	caagcttGGCACGCAGGATCTGGTCGA	*Ms-mnoS* downstream region (546 bp) including 3' of *mnoS* gene (1152 bp)
Ms6238GR4bBamHI-r	cggatcCGGTGGTCGCGGAGATGAAC	
Ms6236GR1b-ScaI-s	cagtactACGGCCCACCGAAACTCGTG	*Ms-mnoR* upstream region (89 bp) including 5' of *mnoR* gene (651 bp)
Ms6236GR2b-HindIII-r	caagctTCACGTTCCAGCAGCGA	
Ms6236GR3HindIII-s	caagcttTGACAGTGCGTTCGACTCCCG	*Ms-mnoR* downstream region (112 bp) including 3' of *mnoR* gene (651 bp)
Ms6236GR4BamHI-r	cggatccGCGCACCGCGACTAGTTCGT	
Ms6238BglII-s	cagatctGTGGCCGAAGCGGCCCGCAC	Confirmation of the genotype of the Δ*mnoS SCO/DCO mutant*
Ms6238XbaI-r	ctctagaTTATCGTCTGTTCCCTTCGGTCCTGG	
Ms6236BamHI-s	cggatccATGACCGTCACGACGCGCGAG	Confirmation of the genotype of the Δ*mnoR SCO/DCO mutant*
Ms6236XbaI-r	ctctagaTTAGATCAACCCGCGCTTGCTCG	
Ms6236BamHI-s	cggatccATGACCGTCACGACGCGCGAG	*Msmeg-mnoR* gene to clone into pMV306- under P_{ami} promoter
Ms6236XbaI-r	ctctagaTTAGATCAACCCGCGCTTGCTCG	
Ms6238BglII-s	cagatctGTGGCCGAAGCGGCCCGCAC	*Msmeg-mnoS* gene to clone into pMV306- under P_{ami} promoter
Ms6238XbaI-r	ctctagaTTATCGTCTGTTCCCTTCGGTCCTGG	

MsmnoR-s	cggatccgatgaccgtcacgacgcgcgagatc	*Msmeg-mnoR* gene to clone into pHIS expression vector
MsmnoR-r	caagcttctagatcaacccgcgcttgctcg	
Primers used for qRT-PCR		
Ms6236RTPCR-s	AAGTCCGATCCGGCCGAGAGC	Gene expression analysis *mnoR*
Ms6236RTPCR-r	CGCTGCTGGAACGTGAGGACG	
Ms6238RTPCR-s	ACCGGAACTGCGCCAGGTGC	Gene expression analysis *mnoS*
Ms6238RTPCR-r	CTCATCCTCGATGCCGACGGG	
MsSigARTPCR-s	CCAAGGGCTACAAGTTCTCG	Gene expression analysis *sigA*
MsSigARTPCR-r	TGGATCTCCAGCACCTTCTC	
MswhiB4RTPCR-s	AGCACCGGGACTCCGAAGCG	Gene expression analysis *msmeg_1831*
MswhiB4RTPCR-r	CGATTGTCGAGCGCGTCCG	
MsrelARTPCR-s	TGTCCAGCTTGGTCACACCGTCG	Gene expression analysis *msmeg_5571*
MsrelARTPCR-r	AGCTCGGCATGGACACCACCAC	
Primers used for EMSA		
MnoR-motif-perfect-s	Cy5-gcctgagcgatctggggctctgagcaccctcgcccaccggtgagccgccggtgggcggccaccctgcccgaccggacagttctgccg	
MnoR-motif-perfect-r	cggcagaactgtccggtcgggcagggtggccgcccaccggcggctcaccggtgggcgagggtgctcagagccccagatcgctcaggc	
MnoR-motif-2MM-s	Cy5-acctggcggatctgagcccggcaggcgatccgcacaccggtaccgtgctggtgggcgcgagccccgagttgctggtcgccccgcgacg	
MnoR-motif-2MM-r	cgtcgcgggcgaccagcaactcggggctcgcgcccaccaagcacggtaccggtgtgcggatcgcctgccgggctcagatccgccaggt	

MnoR-motif-3MM-s	Cy5-gctggaggaggcgttcaccgctgactggctcgcccagcactcctaagccggtgggcgccgtcacccgcggcaccaccggttacaacc	
MnoR-motif-3MM-r	ggttgtaaccggtggtgccgcgggtgacggcgcccaccggcttaggagtgctgggcgagccagtcagcggtgaacgcctcctccagc	
MnoR-motif-4MM-s	Cy5-cacgctgatcacgtccgacgtcgacgagcgcgaccagccgttcatcaccggtgagcgcaccaaggaaggcttcttccgcgtgaagaa	
MnoR-motif-4MM-r	ttcttcacgcggaagaagccttccttggtgcgctcaccggtgatgaacggctggtcgcgctcgtcgacgtcggacgtgatcagcgtg	
B. Strains used in this study		
Name	**Description**	**Reference**
Top10F’	*Escherichia coli* strain	Invitrogen
Mc^2155	*M. smegmatis* wild type	Laboratory stock
BL21 (DE3)	*E. coli* strain	Novagen
Δ*mnoS*	*M. smegmatis mnoS* mutant	This study
Δ*mnoR*	*M. smegmatis mnoR* mutant	This study
Δ*mnoS/R*	*M. smegmatis mnoS/R* mutant	This study
Δ*mnoS-*P_{ami}*mnoS*	*M. smegmatis* Δ*mnoS* mutant, carrying *mnoS* with internal deletion, complemented with *mnoS* gene under P_{ami} promoter in *attB* site	This study
Δ*mnoR-*P_{ami}*mnoR*	*M. smegmatis* Δ*mnoR* mutant, carrying *mnoR* with internal deletion, complemented with *mnoR* gene under P_{ami} promoter in *attB* site	This study
Δ*mnoS/R-*P_{am2}*mnoS/R*	*M. smegmatis* Δ*mnoS/R* mutant, carrying *mnoS/R* with internal deletion, complemented with *mnoS/R* gene under P_{ami} promoter in *attB* site	This study

C. Plasmids used in this study		
pJET 1.2/blunt	Blunt cloning vector, Amp^R, for subcloning of gene fragments	Thermo Scientific
p2NIL	Suicidal gene delivery vector, nonreplicating in mycobacteria, Kan^R	(Parish & Stoker, 2000)
pGoal17	Source of PacI cassette, Amp^R	(Parish & Stoker, 2000)
pMV306K	Mycobacterial integrating vector, Kan^R	Med-Immune Inc
pJAM2	Mycobacterial integrating vector, Kan^R	(Triccas et al., 1998)
pHIS	*E. coli* expression vector enabling the purification of HIS-tagged protein, Amp^R	(Sheffield et al., 1999)
pKS23	pHIS carrying *mnoR*	This study
pKS10	p2NIL carrying Δ*mnoS* with flanking regions and PacI cassette from pGOAL17	This study
pKS11	p2NIL carrying Δ*mnoR* with flanking regions and PacI cassette from pGOAL17	This study
pKS12	p2NIL carrying Δ*mnoSR* with flanking regions and PacI cassette from pGOAL17	This study
pKS19	Mycobacterial integrating vector carrying *M. smegmatis mnoR* under P_{ami} promoter	This study
pKS20	Mycobacterial integrating vector carrying *M. smegmatis mnoS* under P_{ami} promoter	This study
pKS22	Mycobacterial integrating vector carrying *M. smegmatis mnoSR* under P_{ami} promoter	This study

Figure S1.

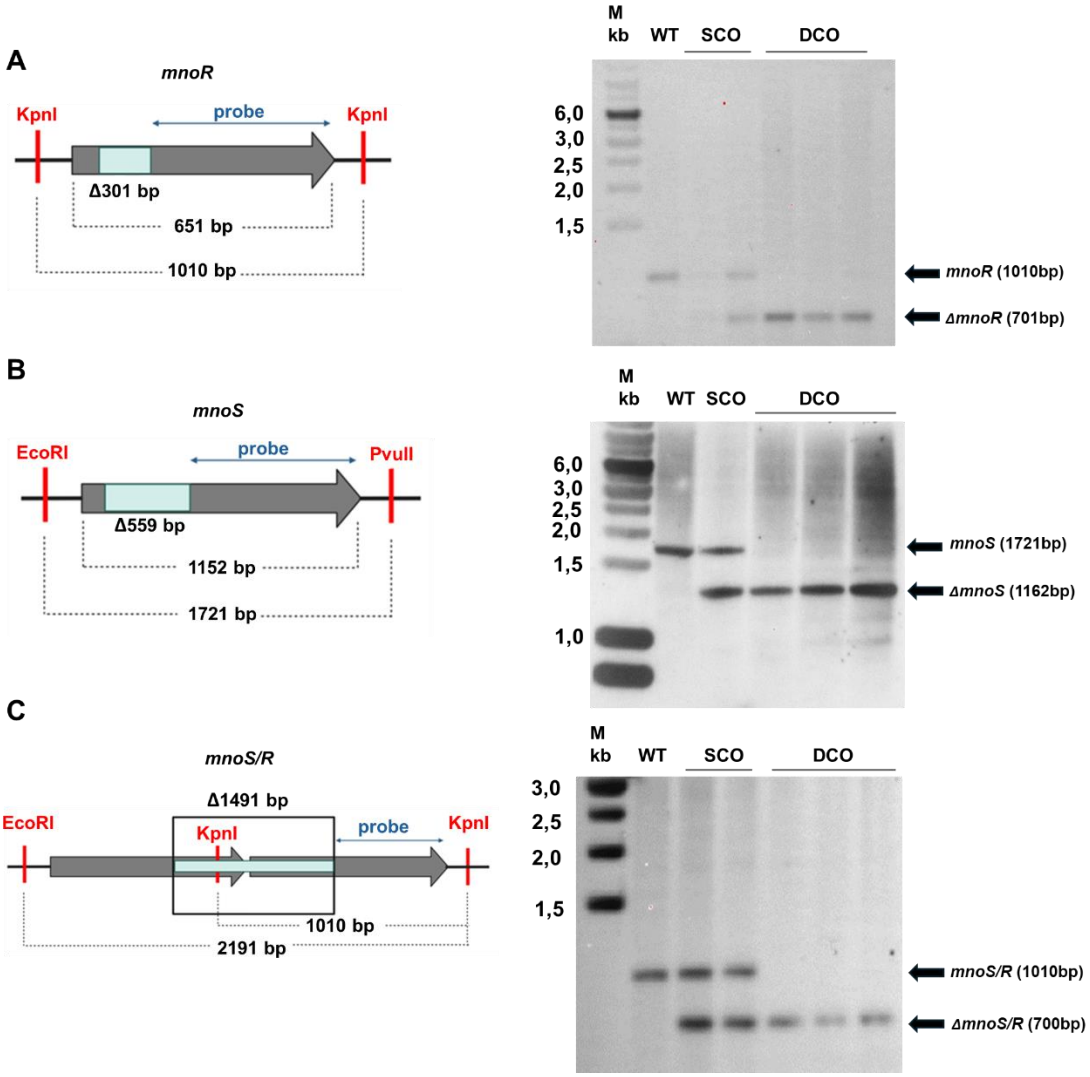

Figure S1. Genotype analysis of mutants by Southern blot hybridization. Genomic organization of *mnoR* gene with restriction sites used for digestion of chromosomal DNA and Southern blot hybridization of chromosomal DNA isolated from wild-type *M. smegmatis* (WT), single cross-over (SCO) and double cross over (DCO) **(A)**. Genomic organization of *mnoS* gene with restriction sites used for digestion of chromosomal DNA and Southern blotting analysis of chromosomal DNA isolated from wild-type *M. smegmatis* (WT), SCO and DCO **(B)**. Genomic organization of *mnoS/R* genes with restriction sites used for digestion of chromosomal DNA and Southern blotting analysis of chromosomal DNA isolated from wild-type *M. smegmatis* (WT), SCO and DCO **(C)**. M - 1kb ladder.

Figure S2.

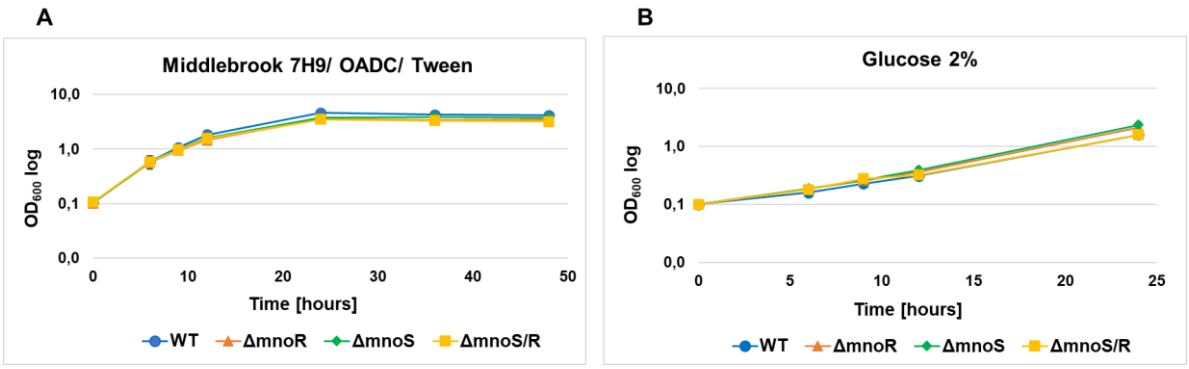

Figure S2. Phenotypic analyses of mutants defective in the synthesis of MnoSR two component system in *M. smegmatis*. Kinetics of growth of Δ*mnoS*, Δ*mnoR*, Δ*mnoS/R* and wild-type *M. smegmatis* strains cultured in rich media, Middlebrook 7H9 supplemented with OADC **(A)** and carbon limiting Sauton's medium containing 2% glucose **(B)**. The growth of strains was evaluated by measuring the OD_{600} at the indicated time points. The displayed values are the average ± standard deviation from three independent experiments.

Figure S3

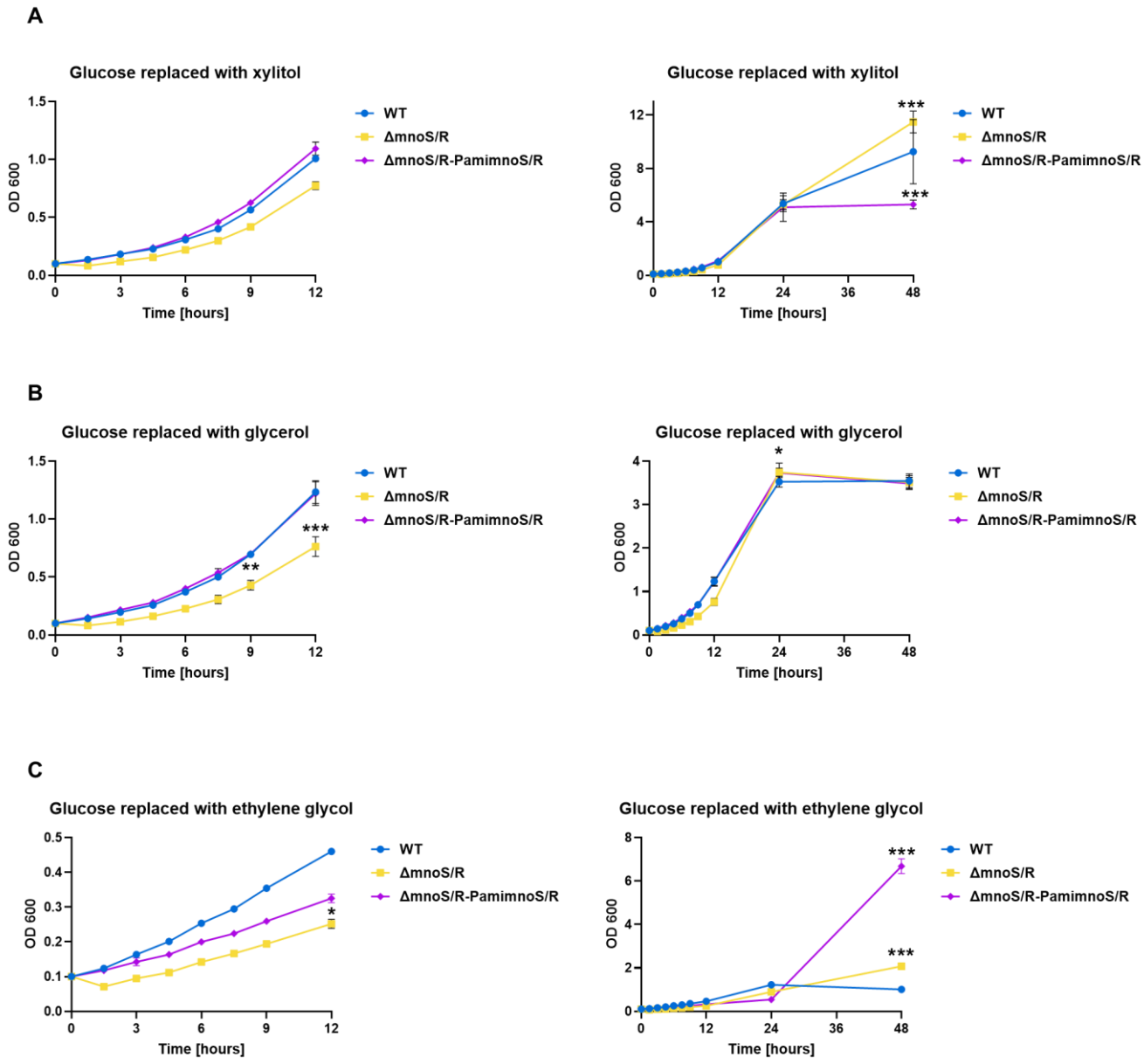

Figure S3. Kinetics of growth for mutant strains and wild-type *M. smegmatis* propagated in carbon-limiting Sauton's medium, where glucose was replaced with xylitol (A), glycerol (B), and ethylene glycol (C). To monitor optical density measurements during the lag phase of growth, the data are presented in two graphs: one up to 12 hours and another up to 48 hours. The statistical significance was determined using Ordinary one-way ANOVA, Sidak's multiple comparison test: *** $p < 0.0001$, (B) * $p = 0.0179$ for Δ*mnoS/R*, ** $p = 0.015$ for Δ*mnoS/R*, (C) * $p = 0.0136$ for Δ*mnoS/R*.

Figure S4.

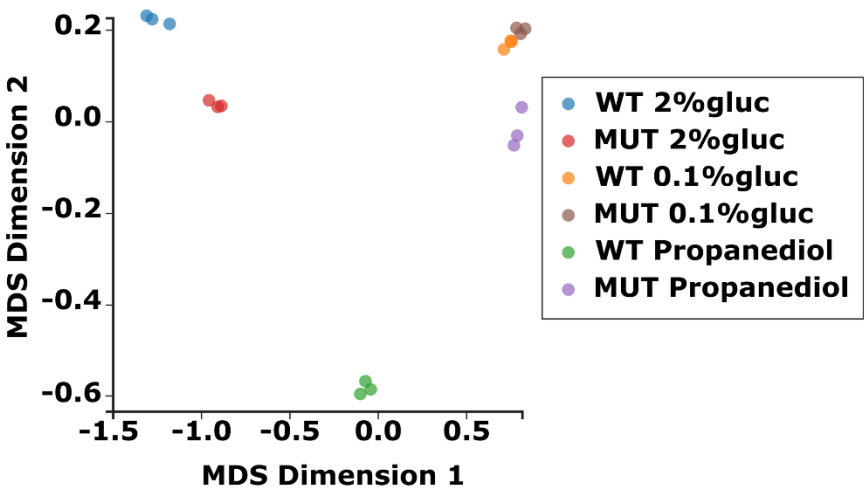

Figure S4. Multidimensional Scaling (MDS) analysis of the repeats of wild-type (WT) and Δ*mnoS/R* (MUT) strains used in RNA-seq analyzes. Each repeat of the individual strain is marked by blue, red, orange, brown, green and purple dots. The results were obtained using Degust online data analysis platform.

Figure S5.

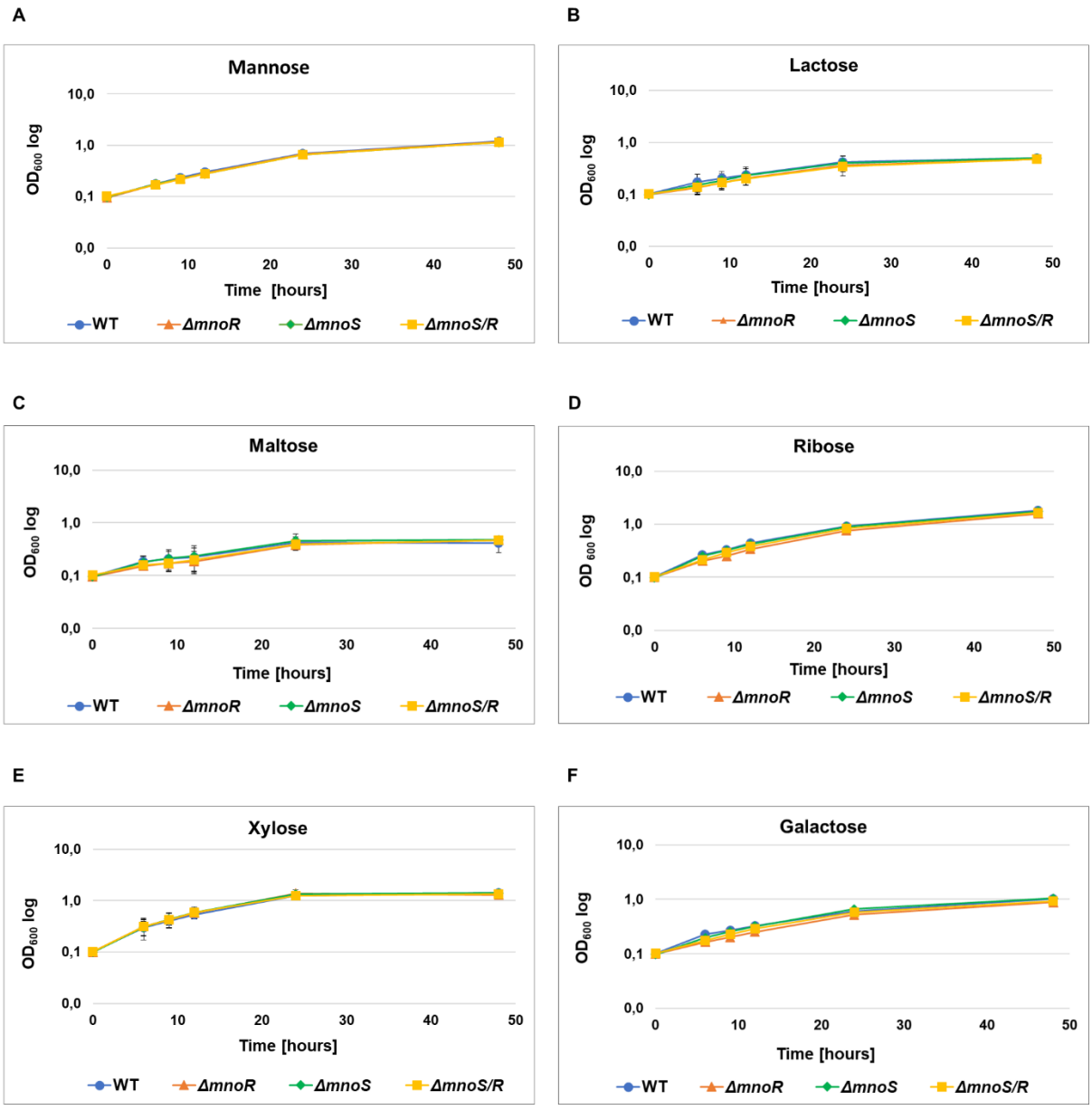

Figure S5. Kinetics of growth of mutant strains and wild-type *M. smegmatis* propagated on carbon-limiting Sauton's medium containing various carbon sources. Wild-type, Δ*mnoS*, Δ*mnoR*, Δ*mnoS/R* were grown in the presence of the mannose (A), lactose (B), maltose (C), ribose (D), xylose (E) and galactose (F) at final concentration 0.5%. Growth of strains was determined by measuring the OD_{600} at indicated time points and was shown on the graphs as averages from three independent experiments ± standard deviation.

Figure S6.

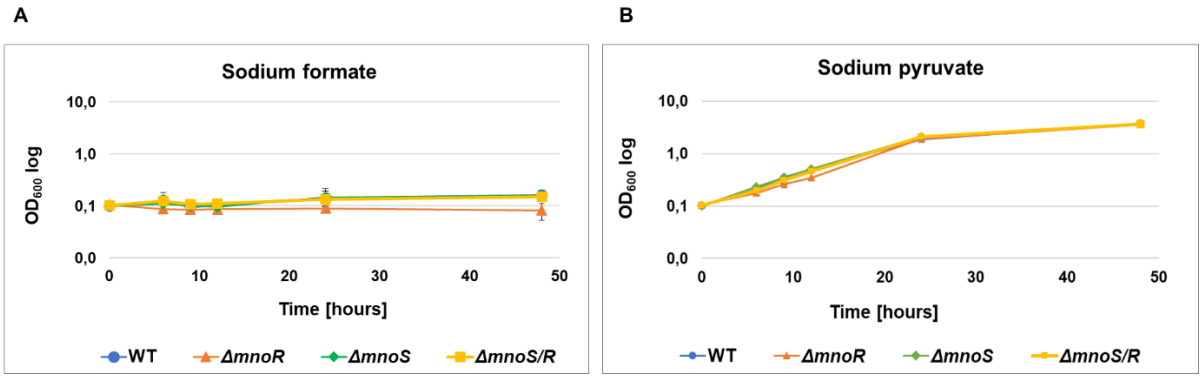

Figure S6. Kinetics of growth of mutant strains and wild-type *M. smegmatis* propagated on carbon-limiting Sauton's medium containing sodium formate (A) and sodium pyruvate (B). Growth of strains was determined by measuring the OD_{600} at indicated time points and was shown on the graphs as averages from three independent experiments ± standard deviation.

Figure S7.

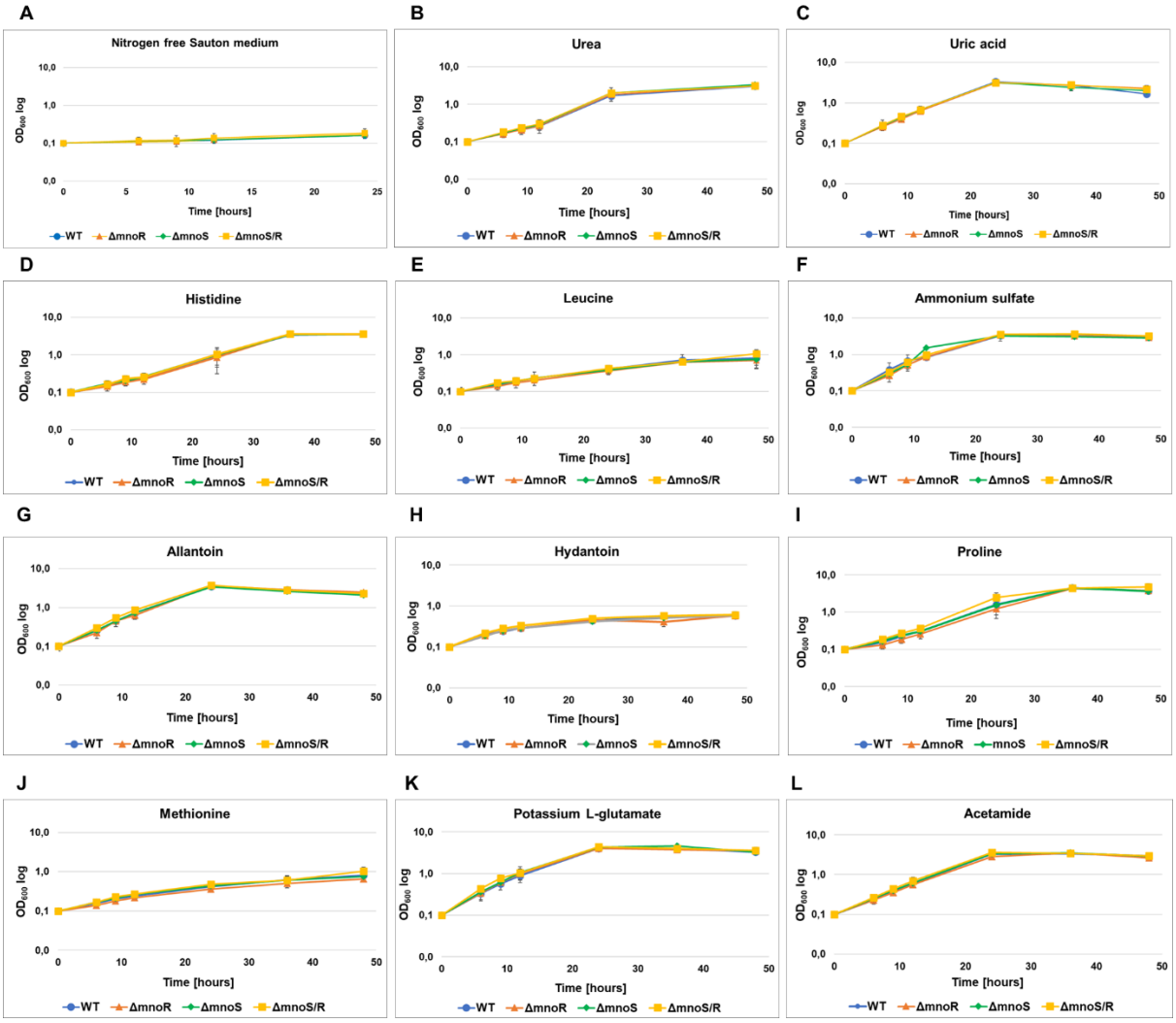

Figure S7. Kinetics of growth of mutant strains and wild-type *M. smegmatis* propagated on nitrogen-limiting Sauton's medium containing various nitrogen sources. Wild-type, Δ*mnoS*, Δ*mnoR*, Δ*mnoS/R* were grown in the presence of the urea (B), uric acid (C), histidine (D), leucine (E), ammonium sulfate (F), allantoin (G), hydantoin (H), proline (I), methionine (J), potassium L-glutamate (K), all at 10 mM final concentration acetamide (5mM) (L). Growth in nitrogen-free Sauton's medium was the negative control (A). Growth of strains was determined by measuring the OD_{600} at indicated time points and was shown on the graphs as averages from three independent experiments ± standard deviation.

Figure S8.

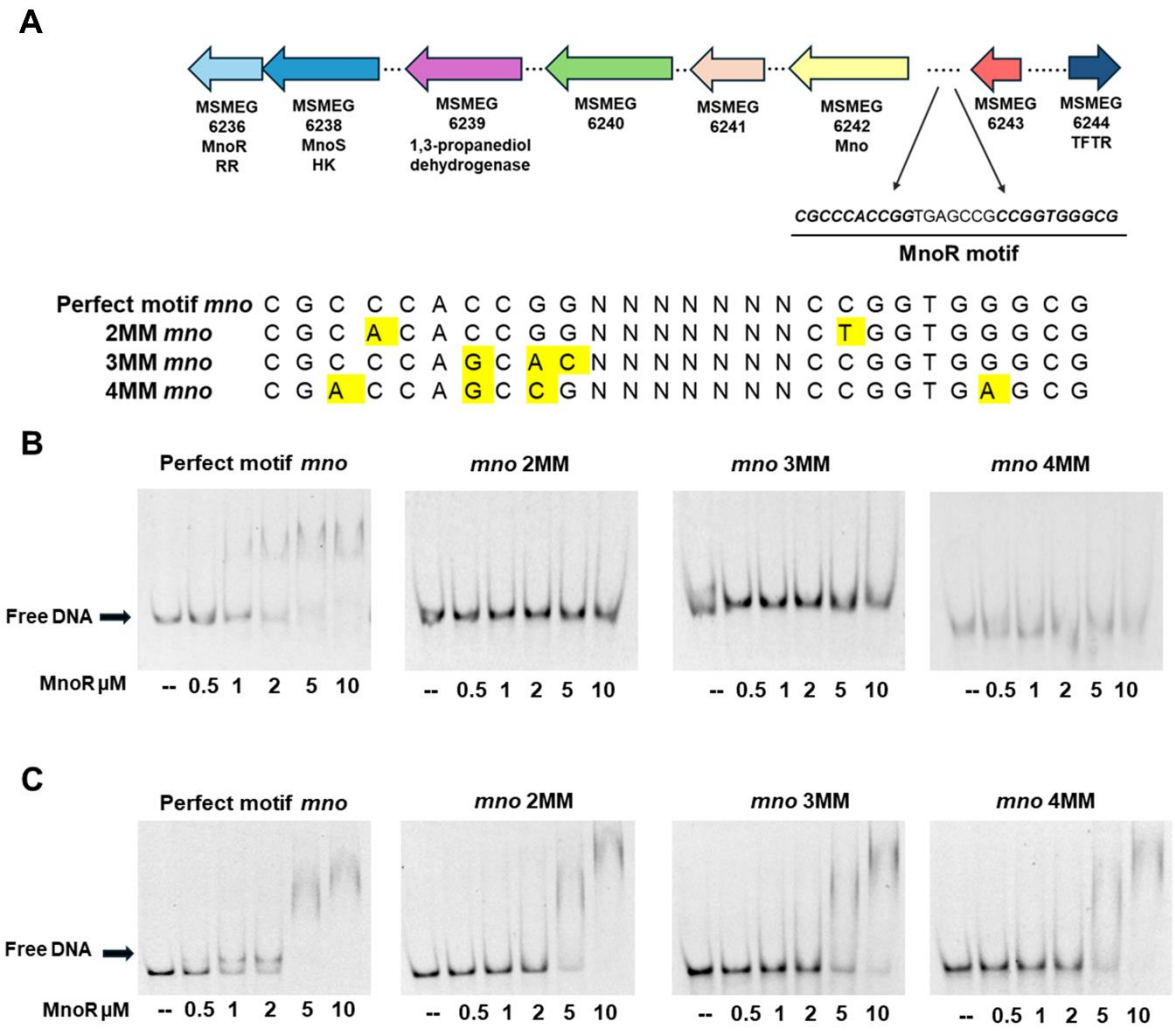

Figure S8. Interactions between MnoR response regulator and promoter region of *msmeg_6242* (*mno*). Perfect palindromic sequence or sequences carrying two (2MM), three (3MM), and four (4MM) mutations were analyzed by EMSA. Schematic representation of the operonic gene arrangement in the region of MnoSR two component system on the *M. smegmatis* genome. The direction of relative transcription is marked with arrows. RR – response regulator; HK – histidine kinase. Mutations in MnoR-binding sequence are marked in yellow. 5 nM of Cy5-labelled DNA was incubated with 0, 0.5, 1, 2, 5 and 10 µM MnoR in buffer containing 10 ng salmon sperm DNA **(B)** or 100:1 excess ratio of polyA DNA to Cy5-labeled oligos **(C)**. Reactions were incubated for 30 min at 25 °C, DNA-MnoR complexes were separated in 5% nondenaturing polyacrylamide gels and visualized using Azure fluorescence visualization system.

Bibliography:

Parish, T., & Stoker, N. G. (2000). Use of a flexible cassette method to generate a double unmarked Mycobacterium tuberculosis tlyA plcABC mutant by gene replacement. *Microbiology (Reading, England)*, *146 (Pt 8)*(8), 1969–1975. https://doi.org/10.1099/00221287-146-8-1969

Sheffield, P., Garrard, S., & Derewenda, Z. (1999). Overcoming expression and purification problems of RhoGDI using a family of "parallel" expression vectors. *Protein Expression and Purification*, *15*(1), 34–39. https://doi.org/10.1006/PREP.1998.1003

Triccas, J. A., Parish, T., Britton, W. J., & Gicquel, B. (1998). An inducible expression system permitting the efficient purification of a recombinant antigen from Mycobacterium smegmatis. *FEMS Microbiology Letters*, *167*(2), 151–156. https://doi.org/10.1111/J.1574-6968.1998.TB13221.X