1 **SUPPLEMENTAL MATERIALS**

2 **Construction of plasmids**

3 **(i) The temperature-sensitive suicide plasmids for the construction of mutant strains of *M.***
4 ***smegmatis.*** To construct pKOTsΔicl1, PCR was conducted with the primers F_icl1mut and
5 R_icl1mut and the chromosomal DNA of *M. smegmatis* as a template. The amplified 1,938-bp
6 DNA fragment was restricted with HindIII and NotI and cloned into pBluescript II KS+ digested
7 with the same enzymes, yielding pBSIIicl1full. A 1,012-bp DNA fragment within *icl1* was excised
8 from pBSIIicl1full by restriction with PstI, and the linear plasmid was self-ligated, resulting in
9 pBSIIΔicl1. The 913-bp HindIII-NotI DNA fragment from pBSIIΔicl1 was cloned into pKOTs,
10 yielding pKOTsΔicl1.

11 For the construction of pKOTsΔicl2, two rounds of recombination PCR were conducted. Using the
12 chromosomal DNA of *M. smegmatis* as a template, two primary PCR reactions were performed
13 with the primers F_icl2mut and R_icl2rec, as well as with the primers F_icl2rec and R_icl2mut to
14 generate two 38-bp overlapping DNA fragments (455 and 487 bp, respectively). Both PCR
15 products contain the same 2,062-bp deletion within *icl2* in the overlapping region. In the secondary
16 PCR, a 904-bp DNA fragment with deletion of *icl2* was obtained using both the primary PCR
17 products as templates and the F_icl2mut and R_icl2mut primers. The secondary PCR product was
18 restricted with HindIII and NotI and cloned into pKOTs digested with the same enzymes, yielding
19 pKOTsΔicl2.

20 To construct pKOTsΔramB, two rounds of recombination PCR were conducted. Using the
21 chromosomal DNA of *M. smegmatis* as a template, two primary PCR reactions were performed

22 with the primers F_ramBmut and R_ramBrec, as well as with the primers F_ramBrec and
23 R_ramBmut to generate two 37-bp overlapping DNA fragments (469 and 510 bp, respectively).
24 Both PCR products contain the same 1,203-bp deletion within *ramB* in the overlapping region. In
25 the secondary PCR, a 942-bp DNA fragment with in-frame deletion of *ramB* was obtained using
26 both the primary PCR products as templates and the F_ramBmut and R_ramBmut primers. The
27 secondary PCR product was restricted with HindIII and NotI and cloned into pKOTs, yielding
28 pKOTsΔramB.

29 To construct pKOTsΔprpR, two rounds of recombination PCR were conducted. Using the
30 chromosomal DNA of *M. smegmatis* as a template, two primary PCR reactions were performed
31 with the primers F_prpRmut and R_prpRrec, as well as with the primers F_prpRrec and
32 R_prpRmut to generate two 36-bp overlapping DNA fragments (606 and 419 bp, respectively).
33 Both PCR products contain the same 1,102-bp deletion within *prpR* in the overlapping region. In
34 the secondary PCR, a 989-bp DNA fragment with deletion of *prpR* was obtained using both the
35 primary PCR products as templates and the F_prpRmut and R_prpRmut primers. The secondary
36 PCR product was restricted with HindIII and NotI and cloned into pKOTs digested with the same
37 enzymes, yielding pKOTsΔprpR.

38 To construct pKOTsΔramA, two rounds of recombination PCR were conducted. Using the
39 chromosomal DNA of *M. smegmatis* as a template, two primary PCR reactions were performed
40 with the primers F_ramAmut and R_ramArec, as well as with the primers F_ramArec and
41 R_ramAmut to generate two 38-bp overlapping DNA fragments (413 and 506 bp, respectively).
42 Both PCR products contain the same 384-bp deletion within *ramA* in the overlapping region. In

43 the secondary PCR, an 881-bp DNA fragment with deletion of *ramA* was obtained using both the
44 primary PCR products as templates and the F_ramAmut and R_ramAmut primers. The secondary
45 PCR product was restricted with HindIII and NotI and cloned into pKOTs, yielding pKOTsΔramA.

46 **(ii) pNCIIicl1, pNCIIicl2, and pNCIIramB.** pNCIIicl1 is a *icl1*::*lacZ* translational fusion
47 plasmid that contains the 5' portion (99 bp) of *icl1* and the 434-bp DNA sequence upstream of *icl1*
48 strart codon. For the construction of pNCIIicl1, a 553-bp DNA fragment was amplified by using
49 the chromosomal DNA of *M. smegmatis* as a template and the F_icl1lacZ and R_icl1lacZ primers.
50 The PCR product was restricted with ClaI and XbaI and cloned into pBluescript II KS+, resulting
51 in pBSIIicl1. pBSIIicl1 was restricted with ClaI and XbaI, and the 542-bp fragment was cloned
52 into the promoterless *lacZ* vector pNCII, yielding pNCIIicl1.

53 To construct pNCIIicl2, a 493-bp DNA fragment comprising the 5' portion (111 bp) of *icl2* and the
54 362-bp DNA sequence upstream of *icl2* was amplified with the F_icl2lacZ and R_icl2lacZ primers
55 using the chromosomal DNA of *M. smegmatis* as a template. The PCR product was restricted with
56 ClaI and XbaI and cloned into pNCII, resulting in pNCIIicl2.

57 For the construction of pNCIIramB, a 453-bp DNA fragment comprising the 5' portion (129 bp)
58 of *ramB* and the 304-bp DNA sequence upstream of *ramB* was amplified with the F_ramBlacZ
59 and R_ramBlacZ primers using the chromosomal DNA of *M. smegmatis* as a template. The PCR
60 product was restricted with ClaI and XbaI and cloned into pNCII, yielding pNCIIramB.

61 **(iii) pNCIIicl1BM1 and pNCIIicl1BM2.** To construct pNCIIicl1BM1 and pNCIIicl1BM2, PCR-
62 based site-directed mutagenesis was carried out using pBSIIicl1 as a template. Synthetic
63 complementary oligonucleotides 34-36 bases long containing the substituted nucleotides in the

64 middle of their sequences were used to mutagenize the RamB-binding sites (RamBS1 and
65 RamBS2), resulting in pBSIIicl1BM1 and pBSIIicl1BM2, respectively. The 542-bp ClaI-XbaI
66 fragments from the mutated pBSIIicl1BM1 and pBSIIicl1BM2 were cloned into pNCII, resulting
67 in the plasmids pNCIIicl1BM1 and pNCIIicl1BM2, respectively.

68 **(iv) pEMII.** pEMII is a *lacZ* translational fusion vector containing the kanamycin resistance gene.
69 To construct pEMII, a 1,391-bp DNA fragment containing the kanamycin resistance gene was
70 amplified by PCR with the F_km and R_km primers using pMV306 as a template. The product
71 was digested with SacII and cloned into pNCII, resulting in pEMII.

72 **(v) pEMIIicl1.** To construct pEMIIicl1, a 553-bp DNA fragment comprising the 5' portion (99 bp)
73 of *icl1* and the 434-bp DNA sequence upstream of *icl1* was amplified with the F_icl1lacZ and
74 R_icl1lacZ primers using the chromosomal DNA of *M. smegmatis* as a template. The PCR product
75 was restricted with ClaI and XbaI and cloned into pEMII, yielding pEMIIicl1.

76 **(vi) pET29bramB.** A 1,472-bp DNA fragment containing the *ramB* gene and six His codons
77 immediately before its stop codon was amplified by PCR with the F_ramBhis6 and R_ramBhis6
78 primers using the chromosomal DNA of *M. smegmatis* as a template. The PCR product was
79 restricted with NdeI and HindIII and cloned into pET29b, yielding pET29bramB.

80 **(vii) pUC19icl1FootF and pUC19icl1FootR.** The plasmids were used as templates for the
81 generation of TAMRA-labeled DNA fragments containing the *icl1* regulatory region. For the
82 construction of pUC19icl1FootF, a 363-bp DNA fragment encompassing the RamB-binding sites
83 (RamBS1, RamBS2, and RamBS3) and Crp-binding site (CrpBS) was amplified by PCR with the
84 F_icl1FootF and R_icl1FootF primers using the chromosomal DNA of *M. smegmatis* as a template.

85 The PCR product was restricted with EcoRI and HindIII and cloned into pUC19, yielding
86 pUC19icl1FootF. The same strategy was applied to construct pUC19icl1FootR except using the
87 F_icl1FooR and R_icl1FooR primers in place of the F_icl1FooF and R_icl1FooF primers.

88 **(viii) pMV306icl1 and pMV306ramB.** pMV306icl1 and pMV306ramB were used for
89 complementation of the Δ*icl1* and Δ*ramB* mutant strains, respectively. For the construction of
90 pMV306icl1, a 1,785-bp DNA fragment containing the *icl1* gene of *M. smegmatis* was amplified
91 by PCR with the F_icl1com and R_icl1mut primers using the chromosomal DNA of *M. smegmatis*
92 as a template. The PCR product was restricted with XbaI and HindIII and cloned into pMV306,
93 resulting in pMV306icl1.

94 To construct pMV306ramB, a 2,159-bp DNA fragment containing the *ramB* gene of *M. smegmatis*
95 was amplified by PCR with the F_ramBcom and R_ramBmut primers using the chromosomal
96 DNA of *M. smegmatis* as a template. The PCR product was restricted with XbaI and HindIII and
97 cloned into pMV306, resulting in pMV306ramB.

98

99 **Construction of mutant strains of *M. smegmatis*.** Deletion mutants of *M. smegmatis* were
100 constructed by allelic exchange mutagenesis using the corresponding pKOTs-derived suicide
101 plasmids containing a temperature-sensitive replication origin as described previously (1). In brief,
102 the temperature-sensitive suicide plasmid was introduced into *M. smegmatis* by electroporation.
103 Transformants were selected at 30°C (replication-permissive temperature) on 7H9-glucose agar
104 plates containing hygromycin, and the selected transformants were grown in 7H9-glucose liquid
105 medium supplemented with hygromycin for 3 days at 30°C. Heterogenotes of *M. smegmatis*,

106 which were generated by a single recombination event, were selected for their hygromycin
107 resistance on 7H9-glucose agar plates at 42°C (replication-nonpermissive temperature). The
108 selected heterogenotes were grown on 7H9-glucose medium without antibiotics for 3 days at 37°C.
109 Isogenic homogenotes were obtained from the heterogenotes after a second recombination by
110 selecting them for sucrose resistance on 7H9-glucose agar plates containing 10% (w/v) sucrose at
111 37°C. The allelic exchange was verified by PCR with isolated genomic DNA.

112 **(i) Δ*icl1* and Δ*icl2* mutant.** To construct the Δ*icl1* and Δ*icl2* mutant strains of *M. smegmatis*, the
113 allelic exchange using pKOTsΔicl1 and pKOTsΔicl2 was performed in the WT strain of *M.*
114 *smegmatis*, respectively.

115 **(ii) Δ*icl1*Δ*icl2* mutant.** To construct the Δ*icl1*Δ*icl2* mutant of *M. smegmatis*, the allelic exchange
116 using pKOTsΔicl2 was performed in the Δ*icl1* strain of *M. smegmatis*.

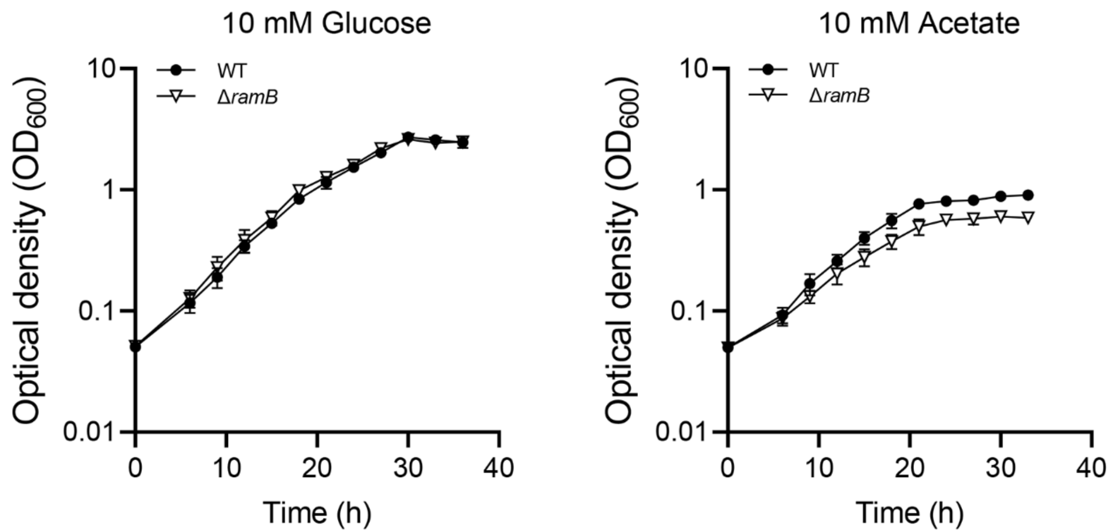
117 **(iii) Δ*ramB* mutant.** To construct the Δ*ramB* mutant strains of *M. smegmatis*, the allelic exchange
118 using pKOTsΔramB was performed in the WT strain of *M. smegmatis*.

119 **(iv) Δ*prpR* mutant.** To construct the Δ*prpR* mutant strains of *M. smegmatis*, the allelic exchange
120 using pKOTsΔprpR was performed in the WT strain of *M. smegmatis*.

121 **(v) Δ*ramB*Δ*prpR* mutant.** To construct the Δ*ramB*Δ*prpR* mutant of *M. smegmatis*, the allelic
122 exchange using pKOTsΔprpR was performed in the Δ*ramB* strain of *M. smegmatis*.

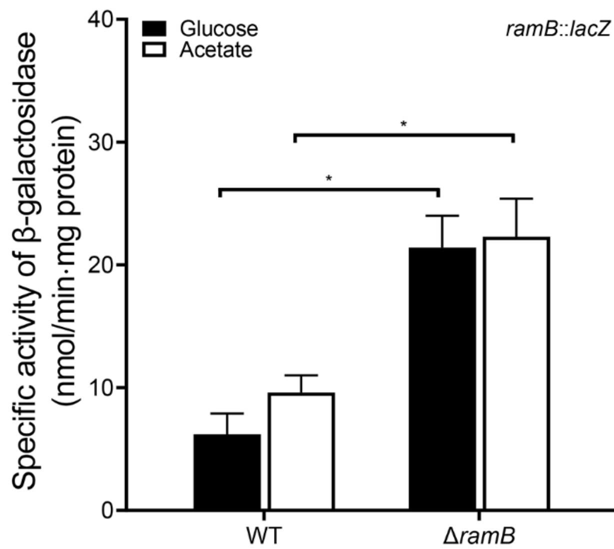
123 **(vi) Δ*ramA* mutant.** To construct the Δ*ramA* mutant strains of *M. smegmatis*, the allelic exchange
124 using pKOTsΔramA was performed in the WT strain of *M. smegmatis*.

125 **SUPPLEMENTAL FIGURES**



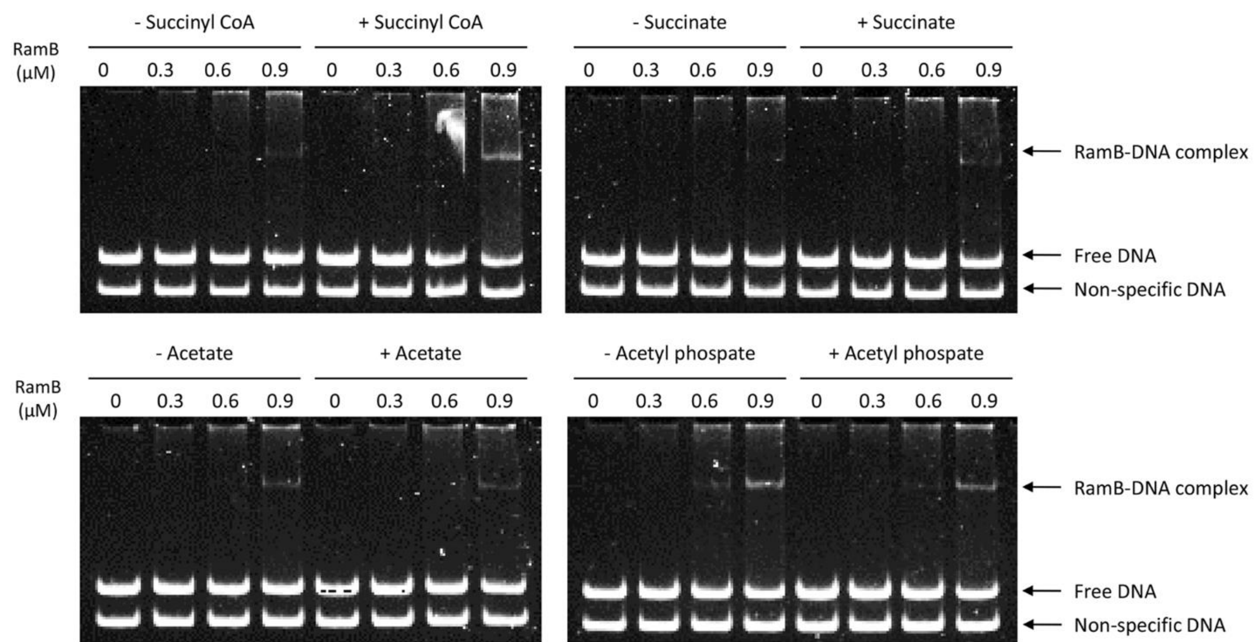
126

127 **FIG S1** Growth of the WT and Δ*ramB* strains of *M. smegmatis* in 7H9 growth medium
128 supplemented with glucose or acetate as the sole carbon source. The WT and Δ*ramB* mutant strains
129 were grown aerobically in 7H9 medium supplemented with either 10 mM glucose or 10 mM
130 acetate at 37°C. Growth of the strains were determined by measuring the OD_{600} of the cultures. All
131 values provided were determined from three biological replicates. The error bars indicate the
132 standard deviations.



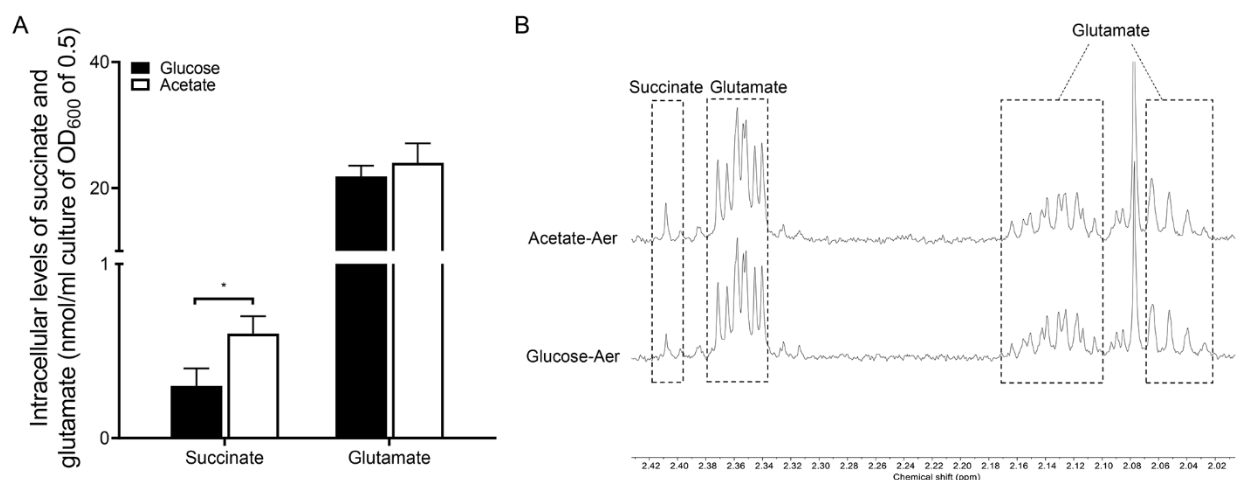
133

134 **FIG S2** Expression levels of the *ramB* gene in the WT and Δ*ramB* mutant strains of *M. smegmatis*.
135 The WT and Δ*ramB* mutant strains containing the *ramB*::*lacZ* translational fusion plasmid
136 pNCIIramB were grown aerobically to an OD_{600} of 0.45 to 0.5 in 7H9 medium supplemented with
137 10 mM glucose or 10 mM acetate as the sole carbon source. Cell-free crude extracts were used to
138 measure β-galactosidase activity. All values provided were determined from three biological
139 replicates. The error bars indicate the standard deviations. *, $p < 0.01$.
140



141

142 **FIG S3** EMSA showing the effects of potential effector molecules on binding of RamB to the *icl1*
143 upstream region. The mixtures of 184-bp DNA fragments (90 fmol) containing the regulatory
144 region of *icl1* and 145-bp DNA fragments (90 fmol) without the RamB-binding site were incubated
145 with increasing amounts of purified RamB in the absence and presence of 200 μM each of
146 succinyl-CoA, succinate, acetate or acetyl-phosphate. The RamB-DNA reaction mixtures were
147 subjected to native PAGE. The concentrations of RamB used in EMSA are given above the lanes.
148 The bands corresponding to the RamB-DNA complex, the RamB-unbound 184-bp DNA fragment
149 (free DNA), and the 145-bp control DNA (non-specific DNA) are indicated by arrows.



150

151 **FIG S4** Determination of intracellular concentration of succinate and glutamate and 600 MHz ^{1}H-

152 NMR spectra. (A) The WT strain of *M. smegmatis* was aerobically grown to an OD_{600} of 0.5 in

153 7H9 medium supplemented with 10 mM glucose or 10 mM acetate as the sole carbon source.

154 Extraction of metabolites and ^{1}H-NMR were performed as described in Materials and Methods.

155 All values provided were determined from three biological replicates. The error bars indicate the

156 standard deviations. *, $p < 0.05$. (B) The regions representing the succinate and glutamate peaks

157 in NMR spectra (δ 2-2.44) are magnified. The peaks of the succinate CH_3 signal at 2.41 ppm and

158 the glutamate CH_3 signals at 2.02-2.07, 2.1-2.17 and 2.33-2.38 ppm are boxed.

```
                                               * * * *   * * *
MSMEG   GTAGCTTCTGAACCGGTACAGCCATCATATTTCTTCACAA
MTB     GTAGCTTCTGAAACGGTTCAGTTTTTGGGCGACTTCGCAA

          * *   * * * *       *          *   * * *   * * * * *   * * * *
MSMEG   TCTTCGCAAGTTAACGCACACGTTTCGCCAAAATTGGCAA
MTB     AATTTGCAAAAAGTCCGCAGGCCGTTGCCGAAATTCGCAA
         RamBS1                                  RamBS2

          * * * *
MSMEG   AGGAAACGGGTGGACCTGCGGTTATGTCATGTGCCATCGT
MTB     GTGAAATGGGTGGACCAGCGTTGACACGCTGTGCCATGGT
                    -35                        -10

           * * * * * * * * * * * * * * * * * * * * * * *
MSMEG   CGGGTTAGCACACCAGGTGAAGCTGCTGCGGT - N41 - ATG
MTB     CGAGTTAGCACACCAGGTGAAGCTGCGCCGTT - N66 - ATG
              +1  RamBS3                       icl1 start
```

159

160 **FIG S5** Nucleotide sequences of the *icl1* upstream regions of *M. smegmatis* mc^{2}155 and *M.*

161 *tuberculosis* H37Rv. The TSPs for *icl1* of *M. smegmatis* and *M. tuberculosis* were previously

162 reported (2, 3). The TSPs are indicated by +1. The -10 and -35 regions of the putative *icl1*

163 promoters deduced from the TSPs are enclosed in boxes. The RamB-binding sites (RamBS1,

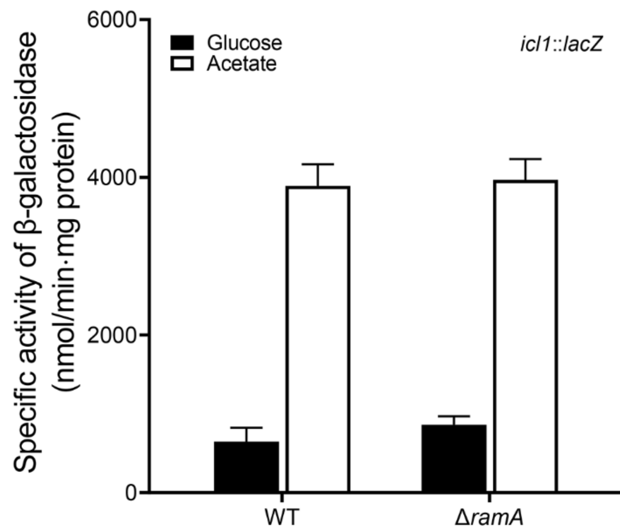
164 RamBS2, and RamBS3) are marked by green font and underlines below their sequences. The

165 conserved nucleotides in the RamB-binding sites of *M. smegmatis* and *M. tuberculosis* are denoted

166 by asterisks. The start codons of *icl1* are marked by red font, and the arrow below the start codons

167 indicates the transcriptional direction. Abbreviations. MTB, *icl1* upstream region of *M.*

168 *tuberculosis*; MSMEG, *icl1* upstream region of *M. smegmatis*.



169

170 **FIG S6** Expression levels of the *icl1* gene in the WT and Δ*ramA* mutant strains of *M. smegmatis*.

171 The WT and Δ*ramA* mutant strains containing the *icl1*::*lacZ* translational fusion plasmid pNCIIicl1

172 were grown aerobically to an OD_{600} of 0.45 to 0.5 in 7H9 medium supplemented with 10 mM

173 glucose or 10 mM acetate as the sole carbon source. Cell-free crude extracts were used to measure

174 β-galactosidase activity. All values provided were determined from three biological replicates. The

175 error bars indicate the standard deviations.

176

-223 GCGAGGAGACGAAAGCCGTCGCCTGTCTCTTCTCGCTCTGAATCAATCACGTTTAACAGT

-163 ACGGGCGTACTGACCTGCGAAAAGTGGCTACTCGCCGGTAGCTTCTGAACCGGTACAGCC

RegX3

-103 ATCATATT**TCTTCACAATCTTCGCAAGTTAAC**GCACACG**TTTCGCCAAAATTGGCAAAGG**

RamBS1 GlnR +1 RamBS2

-43 **AAA**CGGGTGGACCTGCGGTTATGTCATGTGCCATCGTCG**GGTTAGCACACCAGTGAAGCT**

-35 -10 RamBS3

+18 **GC**TGCGGTGTTAACAACCGCAGTGACTTAACAACCGAAGGAGCCGTCCAATGTCGACCGT

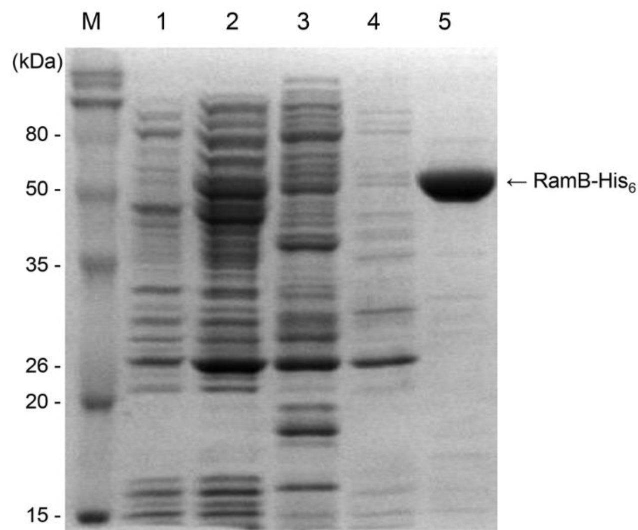
icl1 start

177

178 **FIG S7** The upstream sequence of the *icl1* gene including its putative promoter region and *cis*-
179 acting elements involved in the regulation of the *icl1* gene. The RamB-binding sites (RamBS1,
180 RamBS2, and RamBS3) are indicated in green font. The suggested RegX3 and GlnR binding sites
181 are boxed in blue. The TSP of the *icl1* gene is marked by +1 (3). The putative promoter region (-
182 35 and -10) of the *icl1* gene is boxed. The start codons of *icl1* is denoted both in red font and by
183 the arrow indicating the transcriptional direction. The numbers on the left side of the sequences
184 show the positions of the leftmost nucleotides relative to the TPS of the *icl1* gene.

185

186



187 **FIG S8** SDS-PAGE analysis of purified RamB. C-terminally His_6-tagged RamB was
188 overexpressed in the *E. coli* BL21 CodonPlus (DE3)-RP strain with pET29bramB. C-terminally
189 His_6-tagged RamB was purified by affinity chromatography using Ni-Sepharose high-performance
190 resin (GE Healthcare, Piscataway, NJ). Lane M, molecular weight marker. Lane 1, lysates of *E.*
191 *coli* BL21 CodonPlus (DE3)-RP harboring the empty vector pET29b. Lane 2, lysates of *E. coli*
192 BL21 CodonPlus (DE3)-RP harboring pET29bramB. Lane 3, eluents during the washing step with
193 buffer A [20 mM Tris-HCl (pH 8.0), 200 mM NaCl and 1 mM DTT] containing 5 mM imidazole.
194 Lane 4, eluents during the washing step with buffer A containing 75 mM imidazole. Lane 5,
195 purified C-terminally His_6-tagged RamB.

196

197 **TABLE S1** Strains and plasmids used in this study

Strain/plasmid	Relevant phenotype/genotype	Reference
Strains		
E. coli DH5α	Φ80d*lacZ*ΔM15 Δ*lacU169 recA1 endA1 hsdR17 supE44 thi1 gyrA96 relA1*	4
E. coli BL21 CodonPlus (DE3)-RP	F^- *ompT hsd* S_B (r_B^-, m_B^-) *dcm gal* Tet^r *endA* The [*argU proL* Cam^r]	Stratagene
M. smegmatis mc^2155	High-transformation-efficiency mutant of *M. smegmatis* ATCC 607	5
M. smegmatis Δ*icl1*	*MSMEG_0911* (*icl1*) deletion mutant derived from *M. smegmatis* mc^2155	This study
M. smegmatis Δ*icl2*	*MSMEG_3706* (*icl2*) deletion mutant derived from *M. smegmatis* mc^2155	This study
M. smegmatis Δ*icl1*Δ*icl2*	*MSMEG_0911* (*icl1*) and *MSMEG_3706* (*icl2*) double-deletion mutant derived from *M. smegmatis* mc^2155	This study
M. smegmatis Δ*ramB*	*MSMEG_0906* (*ramB*) deletion mutant derived from *M. smegmatis* mc^2155	This study
M. smegmatis Δ*prpR*	*MSMEG_6643* (*prpR*) deletion mutant derived from *M. smegmatis* mc^2155	This study
M. smegmatis Δ*ramB*Δ*prpR*	*MSMEG_0906* (*ramB*) and *MSMEG_6643* (*prpR*) double-deletion mutant derived from *M. smegmatis* mc^2155	This study
M. smegmatis Δ*ramA*	*MSMEG_5651* (*ramA*) deletion mutant derived from *M. smegmatis* mc^2155	This study
M. smegmatis Δ*crp1*	*MSMEG_6189* (*crp1*) insertion mutant derived from *M. smegmatis* mc^2155; Hyg^r	6
plasmids		
pKOTs	Hyg^r; pKO-based vector constructed by inserting the HindIII-KpnI fragment containing pAL500Ts and pUC ori derived from pDE	1
pBluescript II KS+	Amp^r; *lacPOZ'*	Stratagene
pNCII	Hyg^r; promoterless *lacZ*	7
pEMII	Km^r; promoterless *lacZ*	This study

pET29b	Km^r; T7 promoter, ribosome binding site, and translation start codon overlapping with NdeI site	Novagen
pUC19	Amp^r; *lacPOZ'*	8
pMV306	Km^r; integration vector containing *int* and the *attP* site of mycobacteriophage L5 for integration into the mycobacterial genome	9
pKOTsΔicl1	pKOTs with 0.913-kb DNA fragment containing 1.012 kb-deleted *icl1*	This study
pKOTsΔicl2	pKOTs with 0.904-kb DNA fragment containing 2.062 kb-deleted *icl2*	This study
pKOTsΔramB	pKOTs with 0.942-kb DNA fragment containing 1.203 kb-deleted *ramB*	This study
pKOTsΔprpR	pKOTs with 0.989-kb DNA fragment containing 1.102 kb-deleted *prpR*	This study
pKOTsΔramA	pKOTs with 0.881-kb DNA fragment containing 0.384 kb-deleted *ramA*	This study
pBSIIicl1full	pBluescript II KS+::1.935-kb HindIII-NotI fragment containing the *icl1*	This study
pBSIIΔicl1	pBluescript II KS+ with 0.913-kb DNA fragment containing 1.012 kb-deleted *icl1*	This study
pBSIIicl1	pBluescript II KS+::0.542-kb ClaI-XbaI fragment containing the *icl1* promoter region	This study
pBSIIicl1BM1	pBSIIicl1 with three point mutations (TTC→GAG) in RamBS1	This study
pBSIIicl1BM2	pBSIIicl1 with two point mutations (TT→GA) in RamBS2	This study
pNCIIicl1	pNCII:: 0.542-kb ClaI-XbaI fragment containing the *icl1* promoter region	This study
pNCIIicl2	pNCII:: 0.473-kb ClaI-XbaI fragment containing the *icl2* promoter region	This study
pNCIIramB	pNCII:: 0.443-kb ClaI-XbaI fragment containing the *ramB* promoter region	This study
pNCIIicl1BM1	pNCII::0.542-kb ClaI-XbaI fragment from pBSIIicl1BM1	This study

pNCIIicl1BM2	pNCII::0.542-kb ClaI-XbaI fragment from pBSIIicl1BM2	This study
pEMIIicl1	pEMII:: 0.542-kb ClaI-XbaI fragment containing the *icl1* promoter region	This study
pET29bramB	pET29b::1.472-kb NdeI-HindIII fragment containing *ramB* with 6 His codons before its stop codon	This study
pUC19icl1FootF	pUC19::0.353-kb EcoRI-HindIII fragment containing the *icl1* promoter region	This study
pUC19icl1FootR	pUC19::0.353-kb EcoRI-HindIII fragment containing the *icl1* promoter region	This study
pMV306icl1	pMV306::1.765-kb XbaI-HindIII fragment containing *icl1*	This study
pMV306ramB	pMV306::2.139-kb XbaI-HindIII fragment containing *ramB*	This study

198 *Abbreviations: Amp^r, ampicillin resistance; Cam^r, chloramphenicol resistance; Hyg^r, hygromycin
199 resistance; Km^r, kanamycin resistance.

200

201 **TABLE S2** Oligonucleotides used in this study

Oligonucleotide	Nucleotide sequence (5'→3')	Purpose
F_icl1mut	ATATGCGGCCGCTCATCTCCCACACCCACCC	Δ*icl1* construction
R_icl1mut	ATATAAGCTTGAAGGCGTCAGTGGTGTCG	Δ*icl1* construction
F_icl2mut	ATATGCGGCCGCGCTGACCGACGACCTGGG	Δ*icl2* construction
R_icl2rec	GCTGAAGATGCCGTGCGCGTGATGCCCTCGAACCGTGG	Δ*icl2* construction
F_icl2rec	CCACGGTTCGAGGGCATCACGCGCACGGCATCTTCAGC	Δ*icl2* construction
R_icl2mut	ATATAAGCTTTCGGGGCTCTGTGCACTG	Δ*icl2* construction
F_ramBmut	ATATGCGGCCGCGTCGTGGACACGCTGGG	Δ*ramB* construction
R_ramBrec	GGGCAGTTGTCGCGTTCGTGGTTGAGGTAGCTCGGCG	Δ*ramB* construction
F_ramBrec	CGCCGAGCTACCTCAACCACGAACGCGACAACTGCCC	Δ*ramB* construction
R_ramBmut	ATATAAGCTTGGCCTGTGTTTCGGTGTCG	Δ*ramB* construction
F_prpRmut	ATATGCGGCCGCACTCCGGGGAGTATCCACCG	Δ*prpR* construction
R_prpRrec	GACTTTGCATCCGGCGCCTGGGTCGGCAGGTCGAAG	Δ*prpR* construction
F_prpRrec	CTTCGACCTGCCGACCCAGGCGCCGGATGCAAAGTC	Δ*prpR* construction
R_prpRmut	ATATAAGCTTCTGGAGGGATACGAGCGGG	Δ*prpR* construction
F_ramAmut	ATATGCGGCCGCCCTCCTCCACGATCCACAGC	Δ*ramA* construction
R_ramArec	CGTGCACCTCGCGGATCTCGAAGAACTCCGTGAGCAGC	Δ*ramA* construction
F_ramArec	GCTGCTCACGGAGTTCTTCGAGATCCGCGAGGTGCACG	Δ*ramA* construction
R_ramAmut	ATATAAGCTTCCGCGACCAGTTCCTCG	Δ*ramA* construction
F_icl1lacZ	ATATTCTAGATTGCGCGTGACCATCGAG	*icl1*::*lacZ* fusion
R_icl1lacZ	ATATATCGATCTCGGGGGTGTAGTCGCG	*icl1*::*lacZ* fusion
F_icl2lacZ	ATATTCTAGAGCGGTGTTCGGATCGGTC	*icl2*::*lacZ* fusion
R_icl2lacZ	ATATATCGATGATGCCCTCGAACCGTGG	*icl2*::*lacZ* fusion
F_ramBlacZ	ATATTCTAGAGCAGCGCGTTCCAGCAG	*ramB*::*lacZ* fusion
R_ramBlacZ	ATATATCGATGCGCACGTCGTGCTCGATC	*ramB*::*lacZ* fusion
F_icl1BM1	CATATTTCTTCACAATCGAGGCAAGTTAACGCACAC	Site-directed mutagenesis (RamBS1)
R_icl1BM1	GTGTGCGTTAACTTGCCTCGATTGTGAAGAAATATG	Site-directed mutagenesis (RamBS1)
F_icl1BM2	CACGTTTCGCCAAAAGAGGCAAAGGAAACGGGTG	Site-directed mutagenesis (RamBS2)
R_icl1BM2	CACCCGTTTCCTTTGCCTCTTTTGGCGAAACGTG	Site-directed mutagenesis (RamBS2)
F_km	ATATCCGCGGGGACACTGAGTCCTAAAGAGGGGG	pEMII construction
R_km	ATATCCGCGGGCTCAGTGGAACTAGTGAGGTCTGC	pEMII construction
F_ramBhis6	ATATCATATGCCGAAGACGTTCGTCG	*ramB* overexpression

R_ramBhis6	ATATAAGCTTTCAGTGATGGTGATGGTGATGACCGAGCGGT TTCACCTC	*ramB* overexpression
F_icl1FootF	ATATGAATTCTTCGGTCCGGAACTCACC	DNase I footprinting
R_icl1FootF	ATATAAGCTTATTGGACGGCTCCTTCGG	DNase I footprinting
F_icl1FootR	ATATAAGCTTTTCGGTCCGGAACTCACC	DNase I footprinting
R_icl1FootR	ATATGAATTCATTGGACGGCTCCTTCGG	DNase I footprinting
F_TAMRA_pUC19	GTTTTCCCAGTCACGACGTTGTA	DNase I footprinting
F_icl1com	ATATTCTAGACGGGCGTACTGACCTGC	Δ*icl1* complementation
F_ramBcom	ATATTCTAGAGGTGCTGACGCTCCACG	Δ*ramB* complementation
F_icl1EMSA	CGGTACAGCCATCATATTTCTTTC	EMSA (specific DNA)
R_icl1EMSA	GACATTGGACGGCTCCTTC	EMSA (specific DNA)
F_EMSAns	TGTAAAGCCTGGGGTGCC	EMSA (control DNA)
R_EMSAns	GCCCAATACGCAAACCGC	EMSA (control DNA)

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