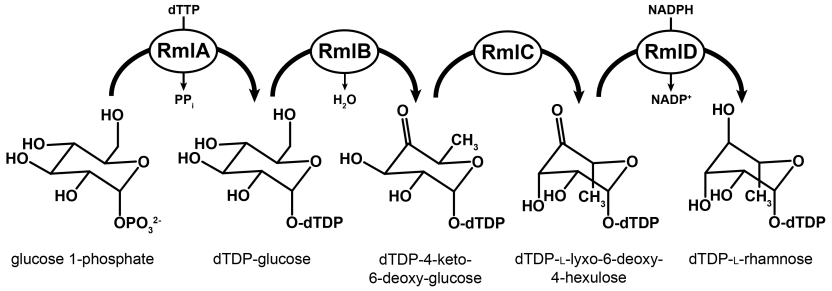
A



B

```
Bsu_GgaB  ----------MNEKSFNYDFSVIMPIYNVELY-LTEAIESIINQTIGFE  38
Lmo_RmlT  MRNLKDRVLNSLKGNKKDIKISVVVPTYNTELEGLKNLMASIDKQTMNPD  50

Bsu_GgaB  NIQLILVNDDSPDKSEIICKEYAQKYPNNIVYAKKQNGGVSSARNYGLKY  88
Lmo_RmlT  EYELVFVDDGSTTDTYERLQEFAETRPNMTVKQIENSGWGSRPRNIATKM 100

Bsu_GgaB  AEGRYIQFLDPDDLVSEGTFENVLNFFDEHKNEIDIVAIPIFFAEGRTGE 138
Lmo_RmlT  AKGEYILYLDHDDTVFPETFERVYNFGKENN--LDVVSGKEVRTNGWSWG 148

Bsu_GgaB  HNLNNKFSSTRILDVEKEPHHILTHCCSTFIKKDALKN--IRFDENCKIG 186
Lmo_RmlT  ---WKQFSENNPHAEEMGIECLLPMTPHKFYKREFLLENDITFDDGARVL 195

Bsu_GgaB  -EDAKLVNLIISQKKKYGLVKEAKYHYRVREDGSSAMQTAKANKNWFNHS 235
Lmo_RmlT  WEDVYFNSKAFIHGAKVGILADYPTYYWIATGANNSSSFGRDPHEKWNQI 245

Bsu_GgaB  --LITFSKNLIDIIKNHEQKIPLFLQYMVMHDLKWKLLIKDISETPLDEN 283
Lmo_RmlT  NKLFNFFKDNIKEQRDLDFMLTHWYRSRVLGILGQWLLKNNNERIDIEFN 295

Bsu_GgaB  EYSEFLTLIREVLSYIDDDVIIETKSVSHFYLYHALKIKHGENYSRYVYE 333
Lmo_RmlT  ----------------------------YAKKLAEELIPAYISENLD 314

Bsu_GgaB  RETEQDYYLYREG--KIVSKLSDQTLTIEILEENEDSIHIEGFWSSLFNS 381
Lmo_RmlT  KNNQVKDYLLRQGDLDSLKKLAQIDAGITALSYVEDAYFKE---DKLFFK 361

Bsu_GgaB  KGFKFYAKIGETKIKAKNIKRQHNDYISLGEVIKKYPGFSIDIPKGHLAD 431
Lmo_RmlT  TSTKMTYEDKEDFFIEKTADRMER--ILPEEIKSKLPKEFFDYSD-DLAE 408

Bsu_GgaB  NHHIEFFITKGKKRKLTKLRFFKYSGLSNDLYNTYVAKKDYIFYYNYKKL 481
Lmo_RmlT  FTYEPSIKGRNSRATWKIDGSTSNVEVVNKKANLYKIEGEMSFSVQINDY 458

Bsu_GgaB  MFKKNNFKNRFIKEFRFLKSLHKSGEKSKKRKSAIKKALMARMVHHVFTI 531
Lmo_RmlT  ILDAADKKQPWDIATRFTGLGYTSHRALTIGKILIKTALIN---NKTMIV 505

Bsu_GgaB  FNRKPVWLFIDRQDKADDNAEHLFKYAINKNDGVKKYFIIKKDSKDYDRI 581
Lmo_RmlT  YKNASGLISLDVGSSVRS----------IVEDSGVKREQILIDKTSG---- 542

Bsu_GgaB  KKYGKVIPYRSFRHKILTLSSSKVISTHADIWVVNPFFNMEIYFRDLFNF 631
Lmo_RmlT  ----------------------------------KVTIPLNEIHVFGESLIEG 561

Bsu_GgaB  EFIFLQHGITMADHSEWLNKYNKNIKLLVTSAKPEYRSIVKGNYNYKKEN 681
Lmo_RmlT  NAELKPVGISDADP-------INVKAKLIGEANKARVEVLLG--------DE 598

Bsu_GgaB  ILLGGFPRYDNLKKSEGEKQLLIMPTWRKDIVLPKDQAKGVRPYNPKFKD 731
Lmo_RmlT  KLSGEYHLVTNIQGKKDKQQIKITL------------------------- 623

Bsu_GgaB  SEYFSRYNALINDERLIEFAKKNNYKITFFPHPDIQQQIVDFEKHDYVEF 781
Lmo_RmlT  ------------------------------------------------- 623

Bsu_GgaB  ADYNSSYQMLFNSSNIMITDFSSVAFDFAYEKKPVIYYQYEKSYHFKLDY 831
Lmo_RmlT  ------------------------------------------------- 623

Bsu_GgaB  YDYKKMGFGDVLENHNSLVDKVIYYMKNNSRMEDKYRKRVDNFFAYTDKN 881
Lmo_RmlT  ------------------------------------------------- 623

Bsu_GgaB  NRNRIYNAILELDNNKVAK 900
Lmo_RmlT  ------------------- 623
```

Figure S1

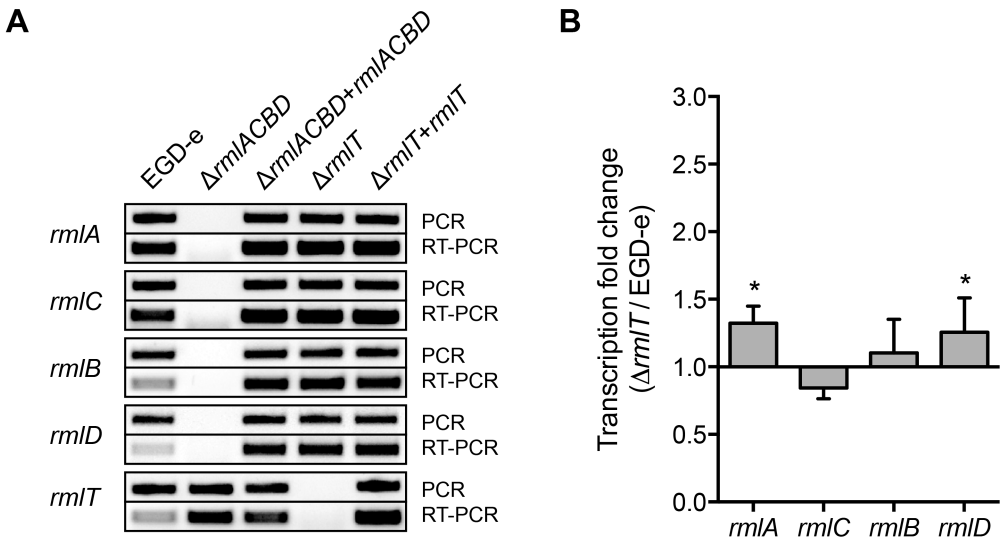

A
EGD-e
ΔrmlACBD
ΔrmlACBD+rmlACBD
ΔrmlT
ΔrmlT+rmlT
rmlA
rmlC
rmlB
rmlD
rmlT
PCR
RT-PCR
B
Transcription fold change
(ΔrmlT / EGD-e)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
rmlA
rmlC
rmlB
rmlD

Figure S2

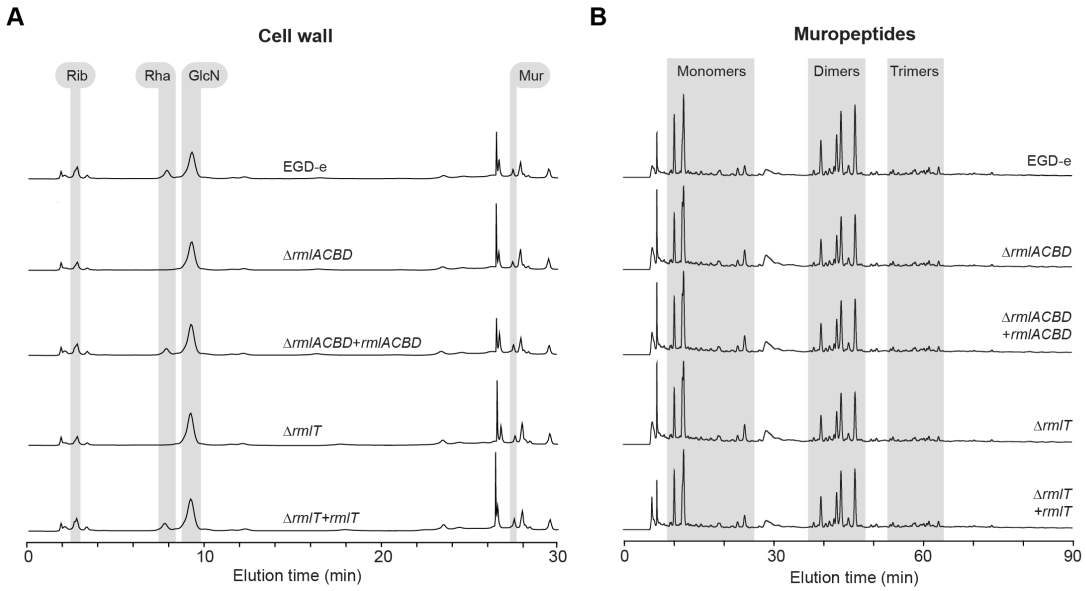

A
Cell wall
Rib
Rha
GlcN
Mur
EGD-e
ΔrmlACBD
ΔrmlACBD+rmlACBD
ΔrmlT
ΔrmlT+rmlT
0
10
20
30
Elution time (min)
B
Muropeptides
Monomers
Dimers
Trimers
EGD-e
ΔrmlACBD
ΔrmlACBD
+rmlACBD
ΔrmlT
ΔrmlT
+rmlT
0
30
60
90
Elution time (min)

Figure S3

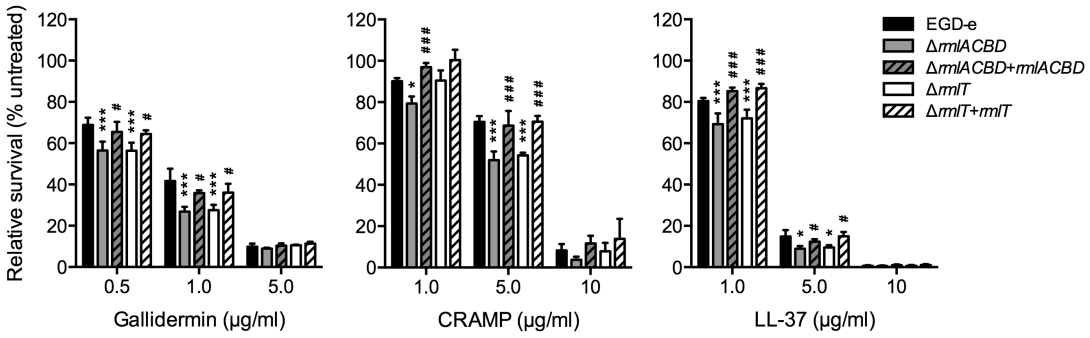

Relative survival (% untreated)
120
100
80
60
40
20
0
0.5
1.0
5.0
Gallidermin (µg/ml)
1.0
5.0
10
CRAMP (µg/ml)
1.0
5.0
10
LL-37 (µg/ml)
EGD-e
ΔrmlACBD
ΔrmlACBD+rmlACBD
ΔrmlT
ΔrmlT+rmlT

Figure S4

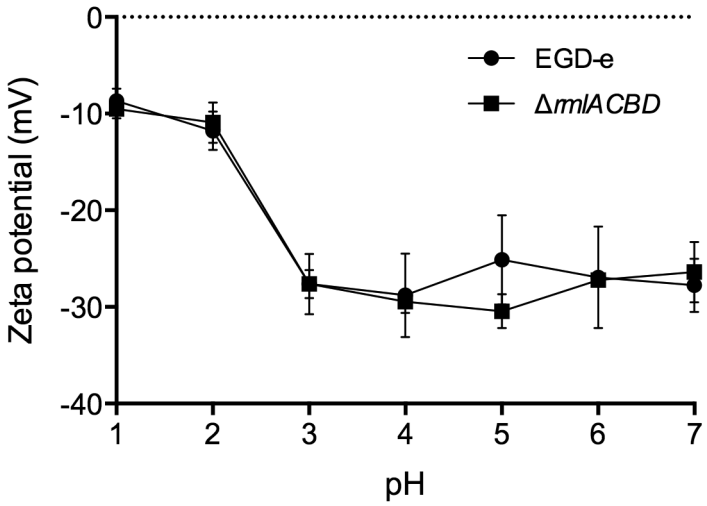


Figure S5

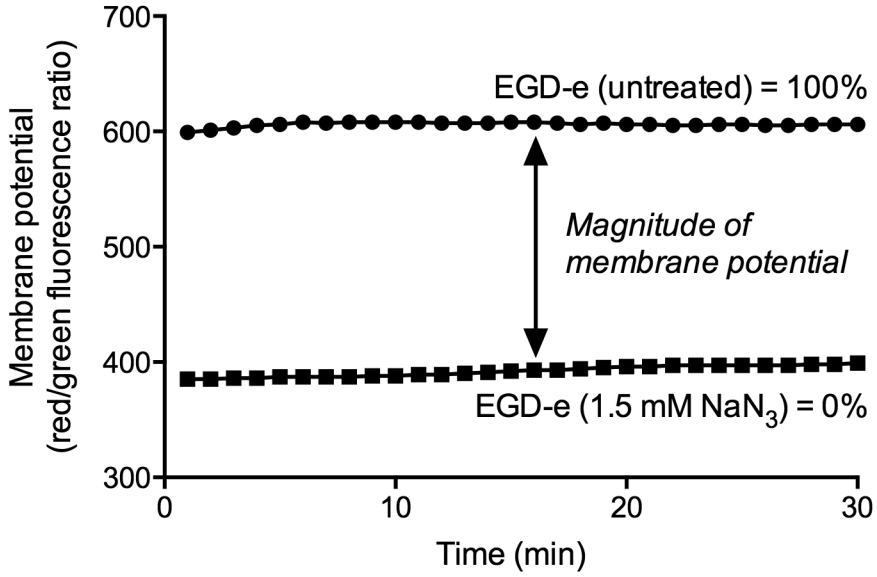

700
600
500
400
300
0
10
20
30
Membrane potential
(red/green fluorescence ratio)
Time (min)
EGD-e (untreated) = 100%
Magnitude of
membrane potential
EGD-e (1.5 mM NaN3) = 0%

Figure S6

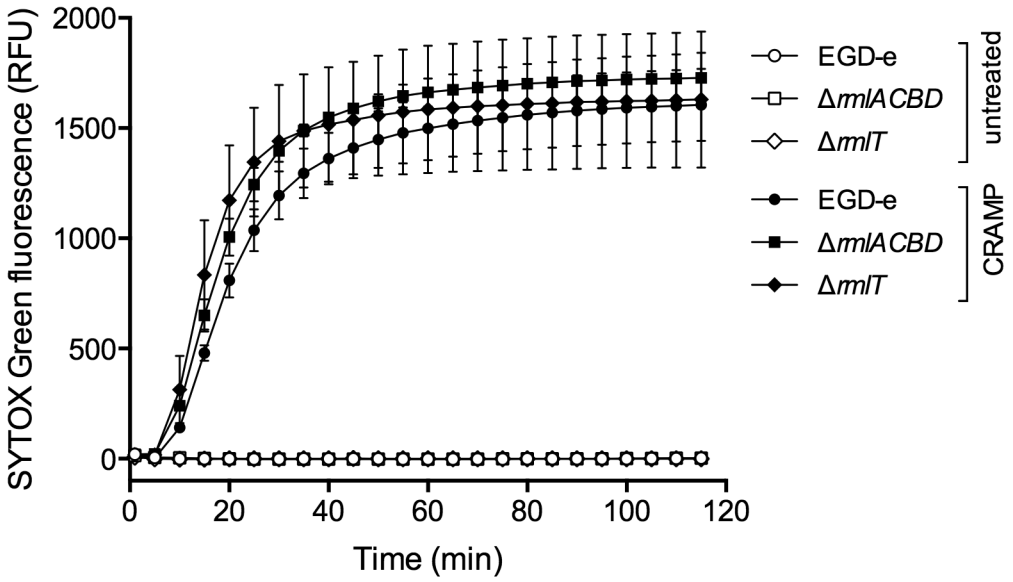

SYTOX Green fluorescence (RFU)
2000
1500
1000
500
0
0
20
40
60
80
100
120
Time (min)
EGD-e
ΔrmlACBD
ΔrmlT
untreated
EGD-e
ΔrmlACBD
ΔrmlT
CRAMP

Figure S7

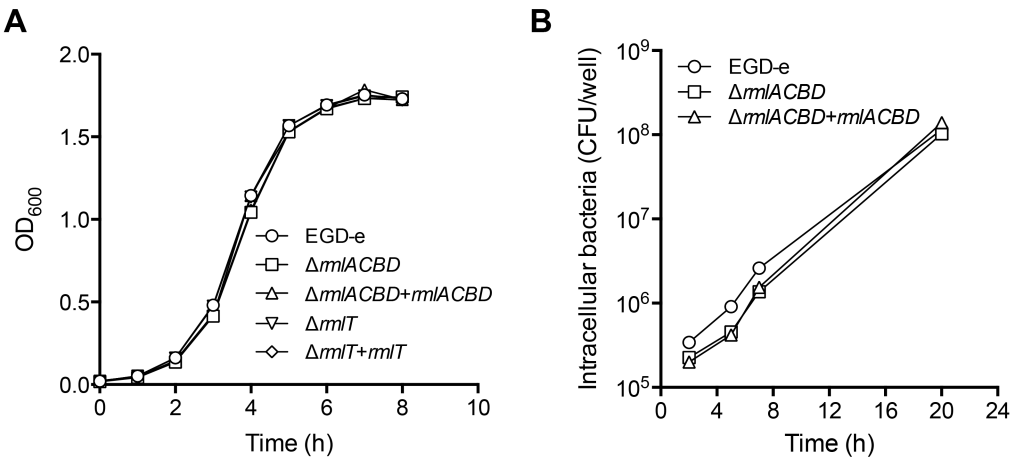

A
2.0
1.5
1.0
0.5
0.0
OD600
0
2
4
6
8
10
Time (h)
EGD-e
ΔrmlACBD
ΔrmlACBD+rmlACBD
ΔrmlT
ΔrmlT+rmlT
B
10^9
10^8
10^7
10^6
10^5
Intracellular bacteria (CFU/well)
0
4
8
12
16
20
24
Time (h)
EGD-e
ΔrmlACBD
ΔrmlACBD+rmlACBD

Figure S8

Table S1. **Homology between the RmlACBD proteins of *Lm*EGD-e and other strains and species**[a]

Species[b] / Strain	Serovar	RmlA	RmlB	RmlC	RmlD
Lmo 10403S	1/2a	100	100	100	100
Lmo SLCC2755	1/2b	100	99	99.5	99.3
Lmo SLCC2372	1/2c	100	100	100	100
Lmo SLCC2479	3c	100	100	100	100
Lmo SLCC2482	7	100	98.7	99.5	99.3
Lse SLCC3954	1/2b	97.2	95.1	98.4	92.0
Smu UA159		74.6	45.7	28.6	51.6
Mtu H37Rv		58.3	46.7	33.5	34.3
Sen LT2		68.4	51.8	46.4	34.8
Sfl 2457T		70.8	51.5	48.0	35.9
Pae PAO1		69.1	52.4	47.2	32.2

[a]Values in percentage of amino acid identity determined by protein-protein BLAST analysis

[b]*Lmo*, *Listeria monocytogenes*; *Lse*, *Listeria seeligeri*; *Smu*, *Streptococcus mutans*; *Mtu*, *Mycobacterium tuberculosis*; *Sen*, *Salmonella enterica* serovar Typhimurium; *Sfl*, *Shigella flexneri*; *Pae*, *Pseudomonas aeruginosa*

Table S2. Primers

#	Name	Sequence (5' to 3')[a]
Construction of plasmids and screening of clones		
1	*rmlA*-A	TACGTCGACTGCTCAAATCGATGCTGG
2	*rmlA*-B	CGACGCGTCATTCTTTTCTCTCC
3	*rmlD*-C	ATACGCGTTTGGCAAGATGCTTTAGTTCG
4	*rmlD*-D	ATTAGATCTTAGTGGTCTCCACCAAGC
5	*rmlA*-F	GGCTACCACGTGAATGATCC
6	*rmlA*-R	AACTCACCACGTTCAGATGG
7	*rmlB*-F	GCAGCAGAATCTCATGTAGACC
8	*rmlB*-R	CCAGTTTCTCCAAGTGAACC
9	*rmlC*-F	ACATACGGTGAGTGGGAAGG
10	*rmlC*-R	AATCCGGATCATCGTAGGC
11	*rmlD*-F	TGGGAAGTAAACGTGGATGG
12	*rmlD*-R	CCAAACACCCATGAAGTACG
13	*rmlA*-G	ATACTATGCGGCCGCTTCATGTGTTTGGTGAAAGC
14	*rmlD*-H	GCGGTCGACACAATTATACGAATGCATCG
15	*rmlT*-A	ATAGTCGACCCTAAAGTTAATGGCAAAGCTCCTGC
16	*rmlT*-B	CGAATTCCATTATATCCTCCTAAAATAGATTAACAG
17	*rmlT*-C	CGAATTCTAAGAATGGAGAGAAAAGAATGAAAGG
18	*rmlT*-D	ACTAGATCTCAATTTCCATTAGTACGCCTCACTC
19	*rmlT*-F	TATTGCCACACGCTTTACCG
20	*rmlT*-R	CTTCCACGATTGAACGAACG
21	*rmlT*-G	TATCTGCAGGAGGGAAAACGTTAGGTAGC
22	*rmlT*-H	GCGGTCGACCTAGTTCCACTTCCTCCTGC
23	PL95	ACATAATCAGTCCAAAGTAGATGC
24	PL102	TATCAGACCTAACCCAAACCTTCC
Quantitative real-time PCR		
25	qPCR-*rmlA*-F	TTCTTGAAGCGTCTACCT
26	qPCR-*rmlA*-R	GCAGCCTCATCAATATACC
27	qPCR-*rmlB*-F	GTAGACCGTAGTATTATCAATCC
28	qPCR-*rmlB*-R	TCTCCAAGTGAACCATACA
29	qPCR-*rmlC*-F	TATTCAAGATAACCACTC
30	qPCR-*rmlC*-R	TCAACAACTACATCATAA
31	qPCR-*rmlD*-F	AGATTCTGTAGATATTGTGGAT
32	qPCR-*rmlD*-R	CATCTTCTGCTGCTTCTA
32	qPCR-16S-F	GCGTAGATATGTGGAGGAAC
33	qPCR-16S-R	CAGGCGGAGTGCTTAATG

[a] Restriction sites underlined