# Supplemental Material

**Genomic differences between *Listeria monocytogenes* EGDe isolates reveals crucial roles for SigB and wall rhamnosylation in biofilm formation.**

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## Materials and Methods

### Synthetic DNA constructs

The regions of DNA used to construct in frame deletions in *rsbU*, *sigB* and *lmo0184* were generated using DNA synthesis techniques. The sequences synthesised are detailed below where the underlined sequence indicated restriction sites.

For deletion of *rsbU* (pNW1454):

GGATCCGAGTACTTTCGGCGATGGACCTTGAAAGTGGTCTGGAAAAACTTAAACAGGAATTGGGGGAATGAA
CATGACATTCCAATCCTGTGTAAAGATAATTAATGAATGGGATATTGTAGCTGCAAGGCAACTAGGTAGAAAA
ATATCCAAAGAAATTGGCTTTGGAACCGTTGACCAAGCAAGAATTACAACTGCCATCAGTGAATTAGCTAGAA
ATATTTTCCTTTATGCTGGACGAGGAGAAATCTGTATCGAAAAAGTAAGTGAATCTGGCAAACAAGGTATGAT
TATTGTTGCCAAAGACAAAGGTCCAGGTATTGTAGACATAAGAAAAGTAATGCAAGATGGTTATACAACATCA
GGTGGTCTTGGAGCAGGTCTTCCAGGAGTCAAACGTTTAATGGACAGTTTTGATATTGAATCCAGTATTGAAG
GCGATTCTAAAGGAACGGTAATTACAACAACGAAATGGGTTCGGTAAGGAGAGCTAGAAAAAAATGGAACG
AACTAAATAAGAAGAAGTGAAGACGTACCTCATTCTTTAGAGAGCATTTACTGGTGAGTAAACGTATTTAAAG
AGTGGAGCGTTTTTATTTTTGAATGTTTTAATTTTATTTGTTAGGGTAAAATCGACAGTATACTTAAAATTAGAT
GGGGTGAAGTGATGAATATTAGTATAGAAATAAAAGAACGTGATACTGACCACATAGACATATTTGTTGCTG
GGGAGATCGATGCTTATACAGCGCCAAAGGTAAAAGAAGCATTAGAAGTATATCAAGTTAAAGAGGGTATTG
TACTTCGGATCGATTTAACAGAAGTGAGTTACATGGATAGCACCGGATTAGGCGTATTTGTAGGAGCTTTCAA
AAGCTTACGTCAACGCCAAAGTGAACTTGTCTTGTTTGGTTTAAGCGACCGACTTTTCCGATTGTTTGAAATCA
CAGGATTGTCAGATATCATCGAAATCAAAAATGTAGAGGGTGAAATGAATGGCAACAATAGATCT

For deletion of *sigB* (pNW1455):

GGATCCAATGTAGAGGGTGAAATGAATGGCAACAATGCATGACAAAATTACATTACAACTTCCTGCCAAGCCT
GAATATGTAAGTTTAGGTAGACTTTCATTATCAGGAATTGCAAGTCGCGCAGGATTTTCTTATGAAGCAATTGA
AGATTTGAAAATAGCTGTAAGTGAAGCCATCACTAATTCCGTAAAGCATGCATTTAAAGGGGAAGAAGATGG
CGAAATCACAGTCGAATATCTTATTTATGAAGATAAGCTAGAAGTTCGTGTTTCCGATAACGGCACAAGCTTC
GACTTAGAAACCCGTAAACAAGAAATTGGCCCATATGATGTGGGCGAGGATGCGGAGATGATGCGTATCGGT
GGGCTAGGTTTATTTTTAATTGAAACATTAATGGATGACGTGAAACTTTATTATGATGAAGGGGTTTCTGTCGT
AATGACCAAATATATTAATGAAAAGCAGGTGGAGGAGAATGCCAAAAGTATCTCAACCTGATAAAGAGCAGA
ATGAGGAAGTGGAGTAAATGAACAAGGCAGTTGAATCAAATAATTTATTTGTATTTCAACGTTCTAAAGCATT
ACAACAATACTGCGGGGATGTTTATTATACCCATGAAGACGAAAATGGTTTTCTGTATGTTCTTTCTGATGGAC
TTGGAAGTGGGCTCGAAGCTAATAGAGCGGCCAAAGCGACTGTTGACGCCATAAAAGAAGATATTCATGCAG
ATATTACTGATATGCTTGAAAAAGCGAACCAGGCTGTTTCAGGGCTTCGTGGTGCTGCGATAGCTATTATTAA
AGGTGACTACTTAACGAAGACACTTTATTATACTGGTATGGGTAATATTCGTTTTTATATGATTGGGATTGAAG
ATAAGCTTATTTTTCCGCTTTCAGGCTCTGGATTTTTGTCCGGTCGAAAACAGAAATATCGGTTGCAATCGTTTA
AATATAAACCAGACAGTAAGTTTTTAATGCATTCAGATGGACTTGTTCTTTCTCGAGATCT

For deletion of *lmo0184* (pNW1451):

GGATCCTAATTCAAGTAAACGATCAATTTATGCTTGGTGAAAATATCCTCGTTGCGCCAATCGTTCGCGAAGGC
CAAGCGAAGCGCCTAGTTCGTTTACCAAAAGGGCTTTGGTTTAATTATTGGACAAAAGAGCAGTTCGTGGGCG
GCGACTATATTATCGCAGACGCGCCGATTGATACCATGCCAATTTACATTAAAGCAGGAACAATTCTGCCAGTT
GGTAGCAGCGTTCAAAATACAAAAGAGACGCAAGAATTGACATTAGAAGTTTACTTAGACAGTGAGACAGCA

ACTGGTTACGTGTATAATGATGATGGGAAAAGTTACCAATATGAAAGTGGCGCAGTATCCAAAACAACACTCA
CAGCTACTTTCAAAAATGGCGAAGTACTAATAAATGCTACCCATCAAGGTGAAGCAAACTTGCAGCAAAAAGT
AACTACTATACAAGTGTTTGGAGAAAAAATAGACAAAATTACAAGGGCGGGAATTTAATCGATGAAACAAAT
CAAGTAAATCTCAGGAAAGCCACCATTTGCTAGGTGGCTTTTTTTGTATGCATCAAAAATTATAGTATAATAGA
AGGAAATGATTTTATTAGGAGGAATTCTCTTGCTATTTGATACGCATGTACACCTTAATGACGAGGCTTTTGAT
GATGATATAGAAGAAGTAATAAAACGCGCGCAGGAAAACGATGTGACTCGTATGGCCGTGGTGGGCTTTAAT
AAAGAAACAATTGACCGGGCGCTTGAATTAGCGGAGAAATATGATTTTATCTCATTAATCGTCGGCTGGCATC
CCACAGACGCTATTACCTTTACAGACGAAGATTTAGAATGGCTTCGTGATTTAGCACTGACACACCCAAAAGT
AGTTGCTTTAGGCGAAATGGGCTTAGATTATCACTGGGATACTTCGCCAAAAGAAACGCAATTCGAGGTTTTT
AGAAAACAAATCCGTTTAGCGAAAGAAGTGAATTTACCGATAGTTATTCACAACCGAGATCT

Table S1: Strains used in this study

| **Strains** | **Genotype** | **Source/ Other names /Reference** |
|---|---|---|
| $WT_{1030}$ | EGDe (stocked as LWS1030) | ANG882 |
| $WT_{1031}$ | EGDe (stocked as LWS1031) | ANG873 |
| $WT_{1032}$ | EGDe (stocked as LWS1032) | University College Cork |
| $WT^{1033}$ | EGDe (stocked as LWS1033) | ATCC |
| DH-L1042 | EGDe Δ*flaA* (stocked as LSW1035) | DH-L1042 (Grundling *et al.*, 2004) |
| LSW1024 | WT1031 Δ*lmo0184* | This work |
| LSW1026 | WT1031 Δ*sigB* | This work |
| LSW1028 | WT1031 Δ*rsbU* | This work |
| LSW1039 | WT1031 Δ*rmlT* | This work |
| LSW1040 | WT1031 Δ*rmlA* | This work |
| LSW1051 | WT1031 Δ*rmlA* Δ*rsbU* | This work |

Table S2: Plasmids used in this study

| **Plasmid** | **Description** |
|---|---|
| pUC19 | Intermediate cloning vector |
| pUC57 | Intermediate cloning vector |
| pMAD | Vector for constructing in-frame deletion in *L. monocytogenes* |
| pNW1451 | pUC57 Δ*lmo0184* |
| pNW1454 | pUC57 Δ*rsbU* |
| pNW1455 | pUC57 Δ*sigB* |
| pNW1457 | pMAD Δ*rsbU* |
| pNW1458 | pMAD Δ*sigB* |
| pNW1459 | pMAD Δ*lmo0184* |
| pNW1901 | pUC19 Δ*rmlA* |
| pNW1904 | pUC19 Δ*rmlT* |
| pNW1905 | pMAD Δ*rmlT* |
| pNW1907 | pMAD Δ*rmlA* |

Table S3: Primers used in this study

| **Primer** | **Sequence (5'-3')** [a] | **Use** |
|---|---|---|
| NSW2027 | AGTGGTTTGCCGCTCGTTCG | Screening for Δ*lmo0184* |
| NSW2028 | AATTCCGCCTACTTCTTCGG | |
| NSW2030 | GTAACTGGGATACAGCCAGC | Screening for Δ*rsbU* |
| NSW2031 | ACATATTCAGGCTTGGCAGG | |
| NSW2032 | TTGTTTGGTTTAAGCGACCG | Screening for Δ*sigB* |
| NSW2033 | CACAAAGTGGTGATTCTAGG | |
| NSW2301 | CTTGGTGAATTCGACTATGATAATATCAAAATTTAC | Construct for Δ*rmlT* |
| NSW2302 | TTTGGTACCTCTCATTATATCCTCCTAAAATAGATT | |
| NSW2303 | AAAGGTACCCTTTAAGAATGGAGAGAAAAGAATGAA | |
| NSW2304 | CGATTAGTCGACAAATAAAGACCAGTTACCGCATAA | |
| NSW2305 | GTCGGCTAAGGTTTCAGCAA | Screening for Δ*rmlT* |
| NSW2306 | GCTCAAAGCGTGGAGTATCT | |
| NSW2307 | CAAATCCTGCAGGCCAAAAGAATTCTTTGATTATAG | Construct for Δ*rmlA* |
| NSW2308 | AATGGTACCTTTCATTCTTTTCTCTCCATTCTTAAA | |
| NSW2309 | TTAGGTACCAAATAAGAAGAGTGAGGCGTACTAATG | |
| NSW2310 | GATATCGTCGACATTGCTCATTGTGCCTGCATAAGT | |
| NSW2311 | CAAGTCACCGAGCATTAACC | Screening for Δ*rmlA* |
| NSW2312 | CGGATCATCGTAGGCAATTC | |
| NSW2325 | GATTTTATGCTAACTCATTGGTATCGCTCACGAG | |
| NSW2328 | GCGGGATTAATTTTTCTGGGAAATGATGTGGTCC | |
| NSW2322 | CGTAGGATCGATCCGATCCT | Sequencing pMAD |
| NSW817 | CGTCATCTACCTGCCTGGA | |
| NSW2323 | CTAAAGTTAATGGCAAAGCTCCTGCAAAATTAACG | Screening for Δ*rmlT* |
| NSW2324 | CTTTCAACAATTTCCATTAGTACGCCTCACTCTTC | |

a. The underlined sequences are restriction sites used for cloning purposes.

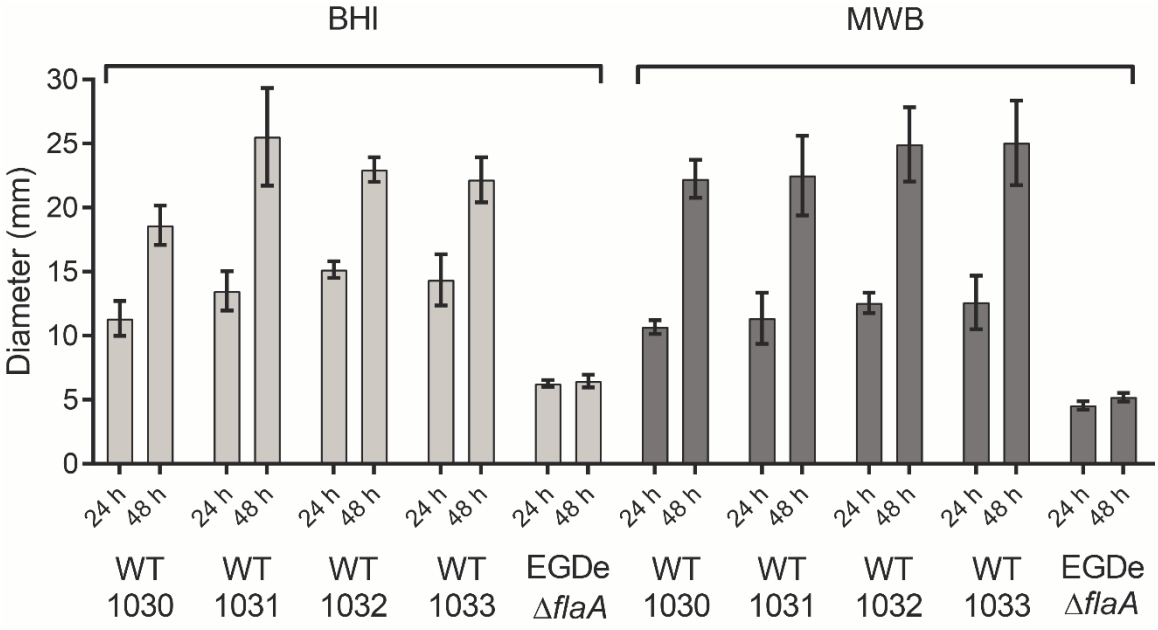



**Figure S1: Quantification of motility of the four EGDe isolates**. Quantification of motility of the four isolates assessed after 24 and 48 hours at 30ºC using BHI or MWB medium (representative images shown in Figure 1). The EGDe Δ*flaA* strain was used as a negative control.

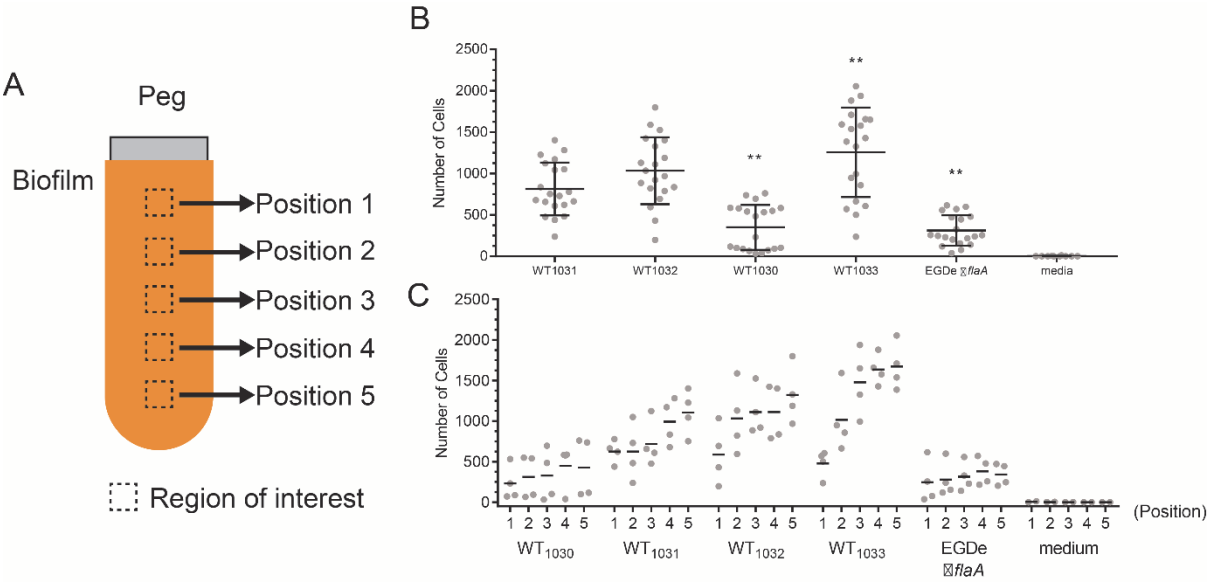



**Figure S2: Quantification of cell adherence of the four EGDe isolates.** The biomass adherent to the substratum was imaged using scanning electron microscopy. Representative image are shown in Figure 3. The number of cells adherent were quantified for all of the images taken. (**A**) Schematic of the imaging process showing the positions used to capture the images. (**B**) The mean number of cells per field of view adherent to the substratum for each of the strains is presented. The error bars represent the standard deviation. The data were analysed by one-way ANOVA comparing with WT1031 with "**" representing a *p* value of ≤0.01 (**C**) the same data presented in (**B**) are shown with respect to the image capture position. The bar represents the mean value for each position and strain.

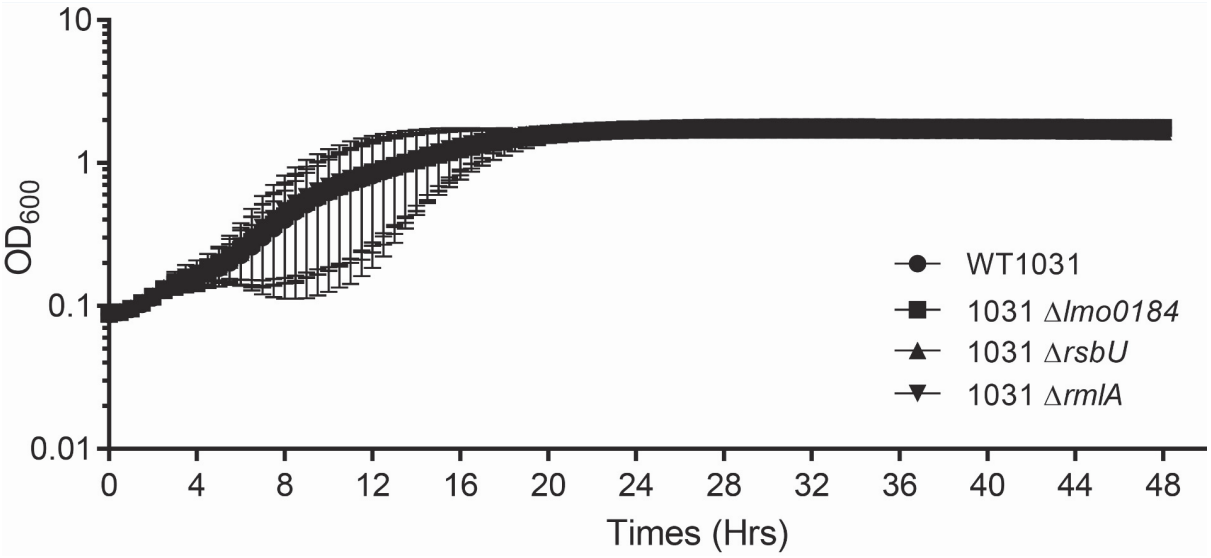



**Figure S3: Analysis of growth of the single deletion strains.** Growth in MWB medium for strains $WT_{1031}$, WT1031 Δ*lmo0184* (LSW1024), WT1031 Δ*rsbU* (LSW1028), WT1031 Δ*rmlA* (LSW1040) was assessed at 30ºC without shaking The value presented is the mean of 2 independent experiments and the error bars represent the standard deviation.

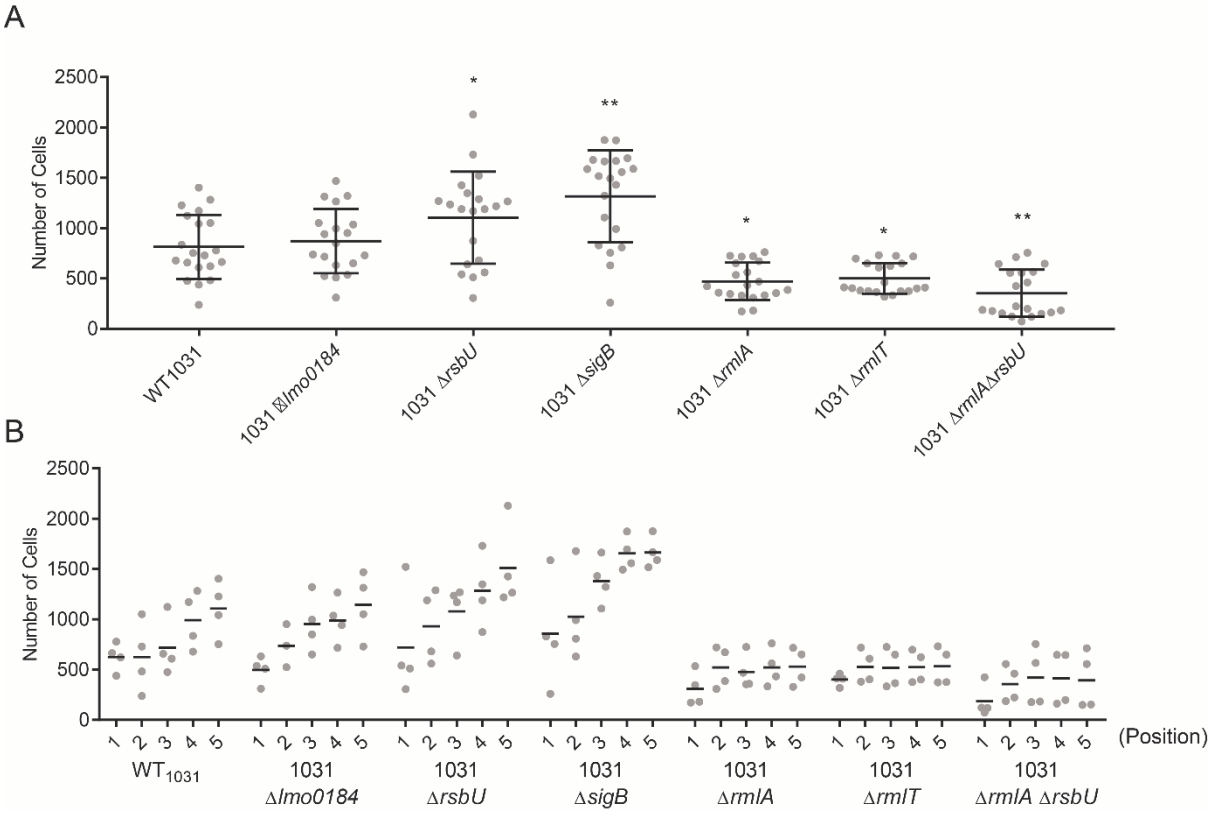
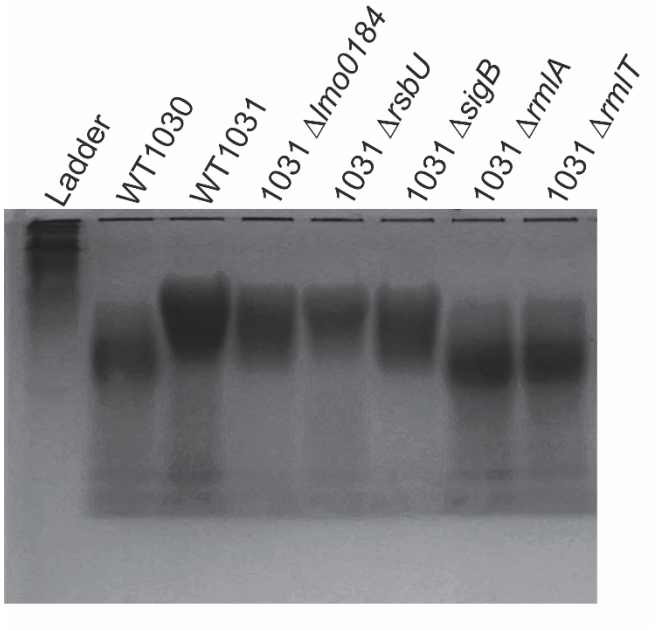



**Figure S4: Quantification of cell adherence determined using scanning electron microscopy.** The biomass adherent to the substratum was imaged using scanning electron microscopy. Representative images are shown in Figures 3, 4 and 5. The number of cells adherent were quantified for all of the images taken. A schematic of the imaging process showing the positions used to capture the images is shown in Figure S2A; (**A**) The mean number of cells adherent to the substratum for each of the strains is presented. The error bars represent the standard deviation. The data were analysed by one-way ANOVA comparing with WT1031 with "*" representing a $p$ value of $\leq 0.05$ and "**" representing a $p$ value of $\leq 0.01$; (**B**) the same data presented in (**A**) are shown with respect to the image capture position. The bar represents the mean value for each position and strain.

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126 **Figure S5: Cell wall extraction of *L. monocytogenes* EGDe mutant strains.** Cell wall
127 material was extracted from $WT_{1030}$, $WT_{1031}$, WT1031 Δ*lmo0184* (LSW1024), WT1031
128 Δ*rsbU* (LSW1028), WT1031 Δ*sigB* (LSW1026), WT1031 Δ*rmlA* (LSW1040) and WT1031
129 Δ*rmlT* (LSW1039), analysed by 20% (w/v) native-PAGE and visualised by staining with
130 alcian blue.

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**Supplemental References**

**Grundling, A., Burrack, L. S., Bouwer, H. G. & Higgins, D. E. (2004).** Listeria monocytogenes regulates flagellar motility gene expression through MogR, a transcriptional repressor required for virulence. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 12318-12323.