

Figure S1. Time-lapse observation of *yvcK::Himar1* cells in BHI supplemented with 1 mM *t*-CIN and with or without 10 mM GlcNAc at 30°C. Phase contrast images of a representative field at 5 h, 10 h, and 15 h are presented. The red arrow indicates cell lysis of *yvcK::Himar1*. Addition of GlcNAc (bottom series) clearly reduced the occurrence of cell lysis at 15 h.

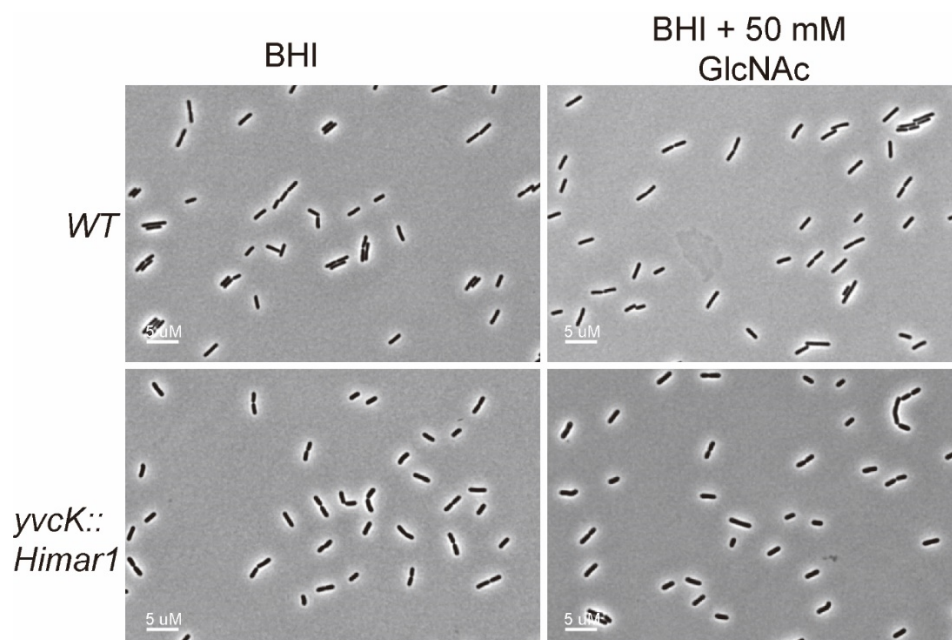


Figure S2. Phase contrast microscopy of WT and *yvcK::Himar1* in BHI with and without 50 mM GlcNAc (in absence of *t*-CIN). Cultures were grown at 30°C till $OD_{600} = 1.0$. Images are representative of three biological replicates.

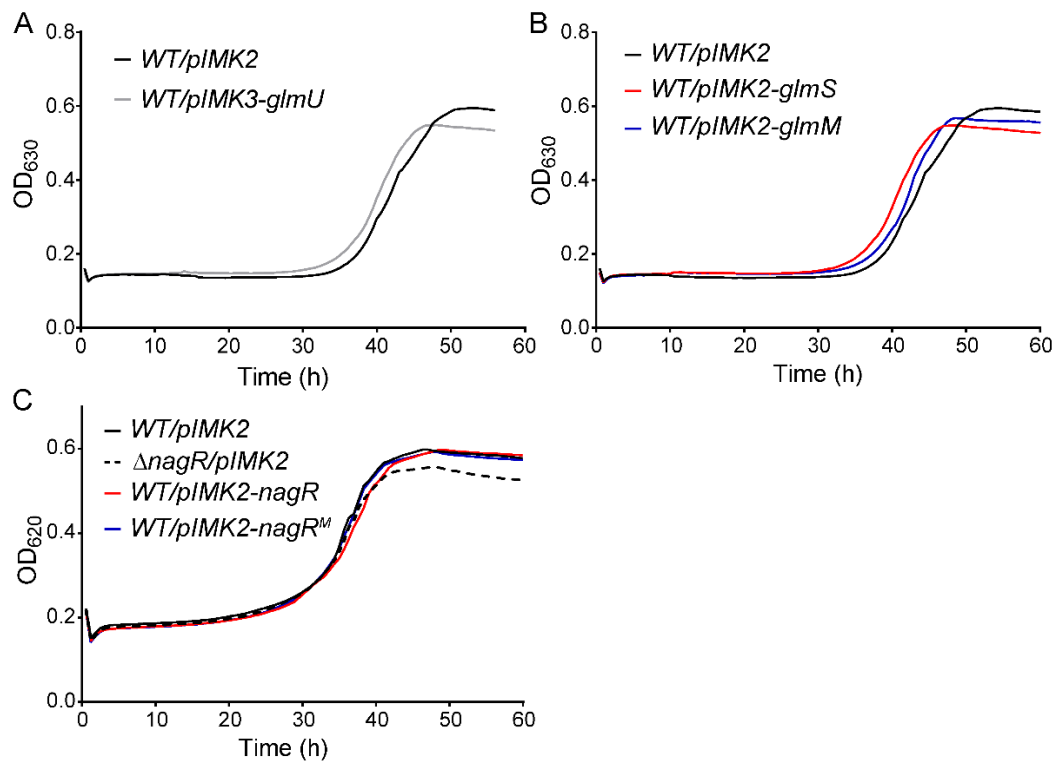


Figure S3. Effect of overexpression of enzymes involved in GlcNAc metabolism on *t*-CIN sensitivity of WT *L. monocytogenes*. A Δ *nagR* strain was included for comparison. Where pIMK2 was used, (over)expression was constitutive, while in case pIMK3 was used, it was induced by 1 mM IPTG. Bacterial growth was monitored by OD₆₂₀ in BHI with 3 mM *t*-CIN at 30°C. All curves represent the average of three independent cultures. The standard deviation is omitted for clarity.

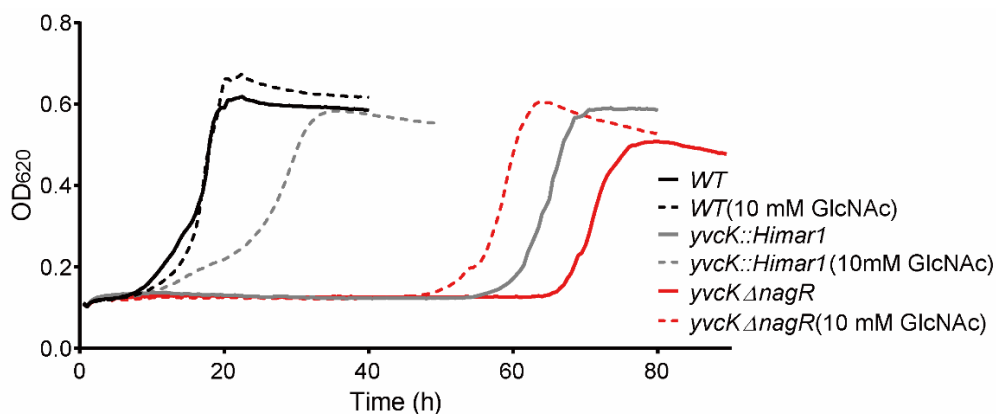


Figure S4. Growth curves of *yvcK::Himar1/ΔnagR* at 30°C in BHI with 2 mM *t*-CIN, with or without 10 mM GlcNAc. Curves represent the average of three independent cultures. The standard deviation is omitted for clarity. Supplementation of GlcNAc almost fully restored the tolerance of *yvcK::Himar1/ΔnagR* to *t*-CIN, but only slightly reduced the sensitivity of *yvcK::Himar1/ΔnagR* to *t*-CIN.

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NagR_B. subtilis 168 MN INKQSP IPIYYQIMEQLKTQIKNGELQPDMLPSEREYAEQFGISRMTVRQALS NLVN 60
NagR_L. mono Scott A M- IDKQSG IPIYIQSEIKKKMEDGVWKVGTSIPAERQLAEMFHVSRMTVRQAIQGLVD 59
Consensus MN I *KQS* IPIY*QI*****K*****G*****P*ER**AE*F**SRMTVRQA***LV*
NagR_B. subtilis 168 EGLLYRLKGRGTFVSKPKMEQALQGLTSFTEDMKSRGMTPGSRLIDYQLIDSTEELAA IL 120
NagR_L. mono Scott A DNILQRRVGAGTFIAEKKLTERLEAVTSFTNLMLQEGKVPSTRIVSYGIRPASTQE QEAL 119
Consensus ***L*R**G*GTF*****K*****L***TSFT**M***G**P**R***Y*****L
NagR_B. subtilis 168 GCGHPSS IHK ITRVRLANDIPMAIESSHIPFELAGELNESHFQSS IYDHIERYN SIPISR 180
NagR_L. mono Scott A QLPE NSNVMK IERIRYGD RVPILYEVA AIPEKIASLLTKEDIMDSLYKAIELKLGQPIGE 179
Consensus *****S***K I *R*R*****P***E***IP***A**L*****S*Y**IE*****PI**
NagR_B. subtilis 168 AKQELEPSAATTEEANILGIQKGAPVLLIKRTTYLQNGTAFEHAKSVYRGDRYTFVHYMD 240
NagR_L. mono Scott A AEQIMEASLVSEKIAPYLDVKLGSPVMKLRQITTL EDGRPF EFTRSQYVGS RFQFVAR IK 239
Consensus A*Q**E*S*****A**L*****G*PV*****T*L**G**FE**S*Y**G*R**FV****
NagR_B. subtilis 168 RLS 243
NagR_L. mono Scott A Q - - 240
Consensus *LS

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Figure S5. Alignment of NagR amino acid sequences from *L. monocytogenes* Scott A (GenBank accession: EGJ24460.1) and *B. subtilis* 168 (GenBank accession: WP_003228089.1). Both sequences show 35% identity. Residues of *B. subtilis* NagR that are proposed to interact with the phosphate group of the ligands (GlcN-6-P and GlcNAc-6-P), and the corresponding positions in *L. monocytogenes* NagR are highlighted in red boxes. Residues proposed to interact with the sugar moieties of the ligands are shown in blue boxes.