

# Supplementary Material

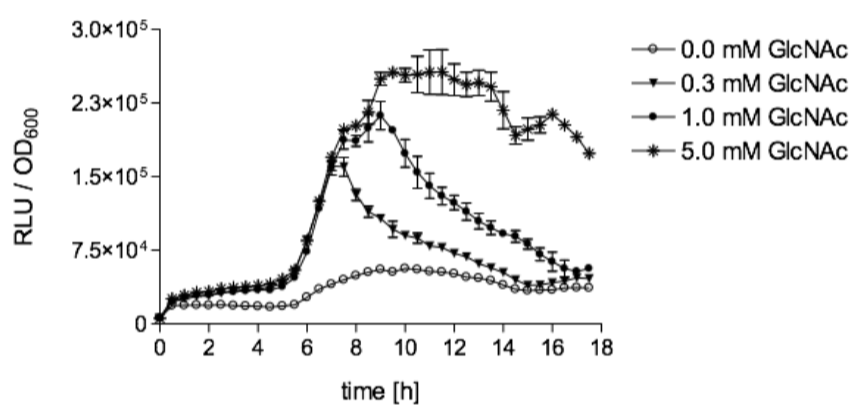
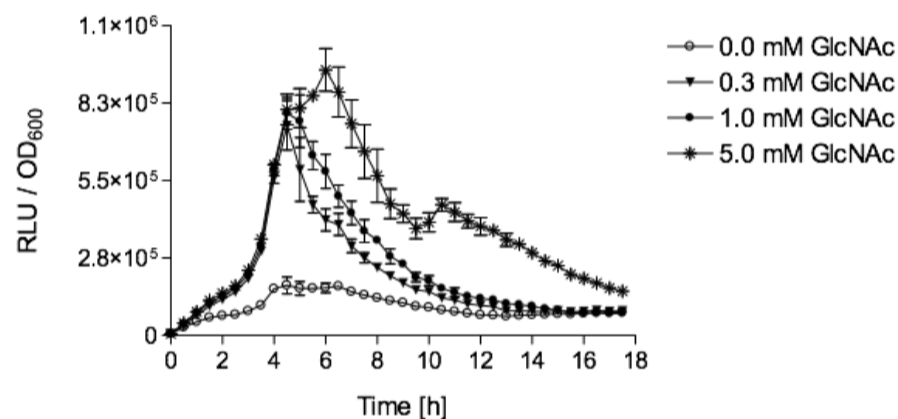
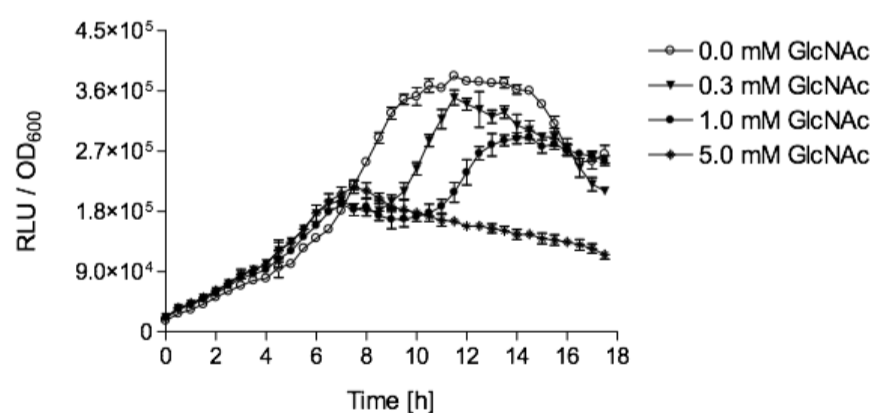
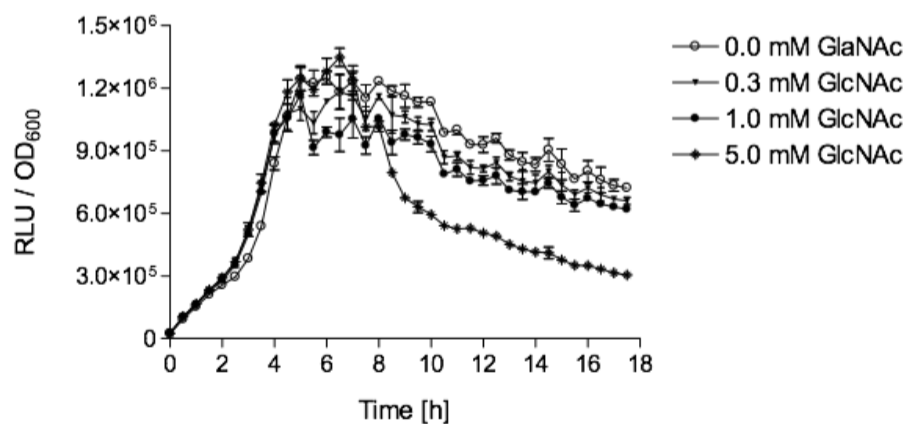
**Reciprocal regulation of NagC and Quorum Sensing systems and  
their roles in *hmsHFRS* expression and biofilm formation in  
*Yersinia pseudotuberculosis*.**

Anja Wiechmann, Vanina Garcia, Linzy Elton<sup>†</sup>, Paul Williams and Steve Atkinson\*

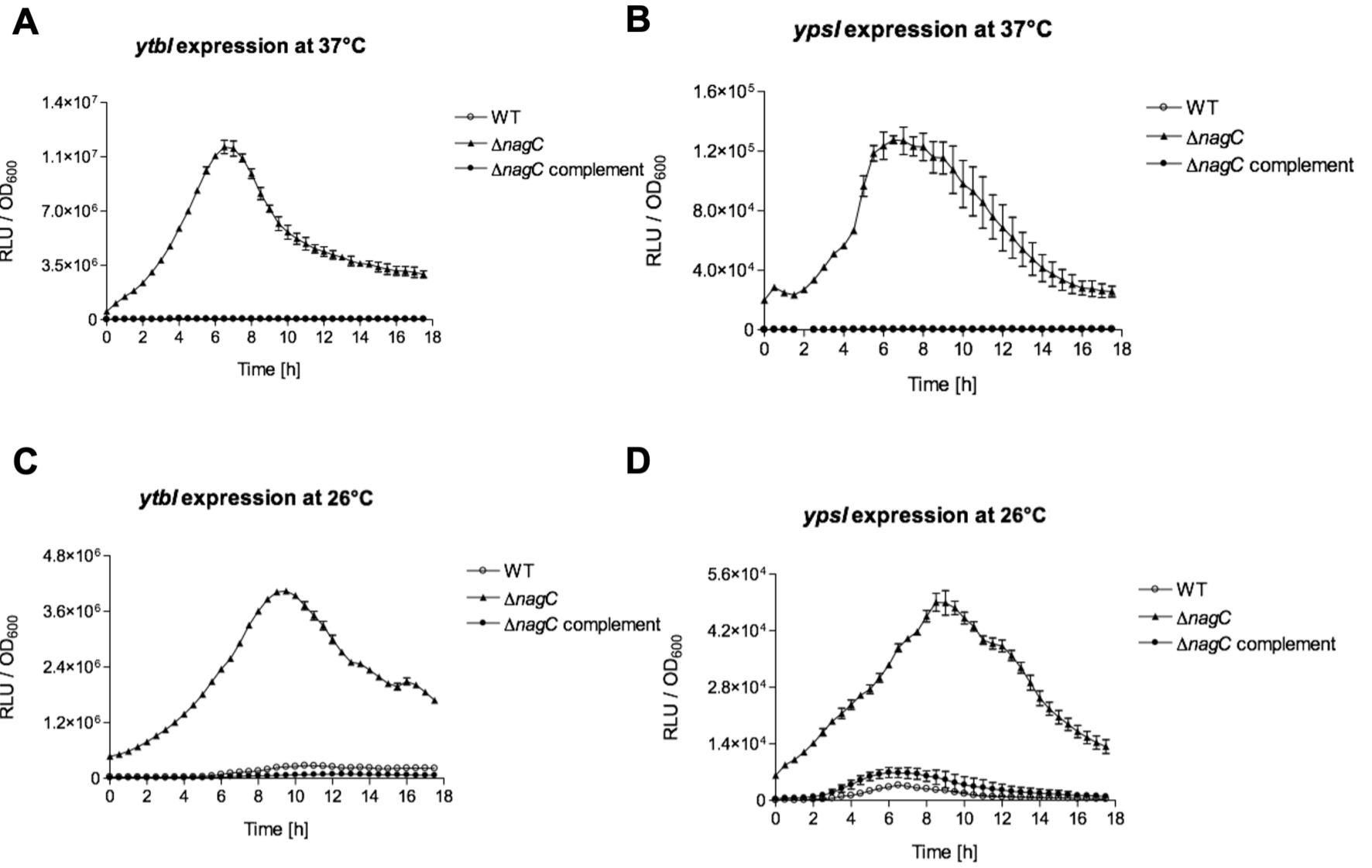
Biodiscovery Institute, School of Life Sciences, University of Nottingham, Nottingham,  
NG7 2RD, United Kingdom.

<sup>†</sup>Division of Infection and Immunity, University College, London, WC1E 6BT. United  
Kingdom.

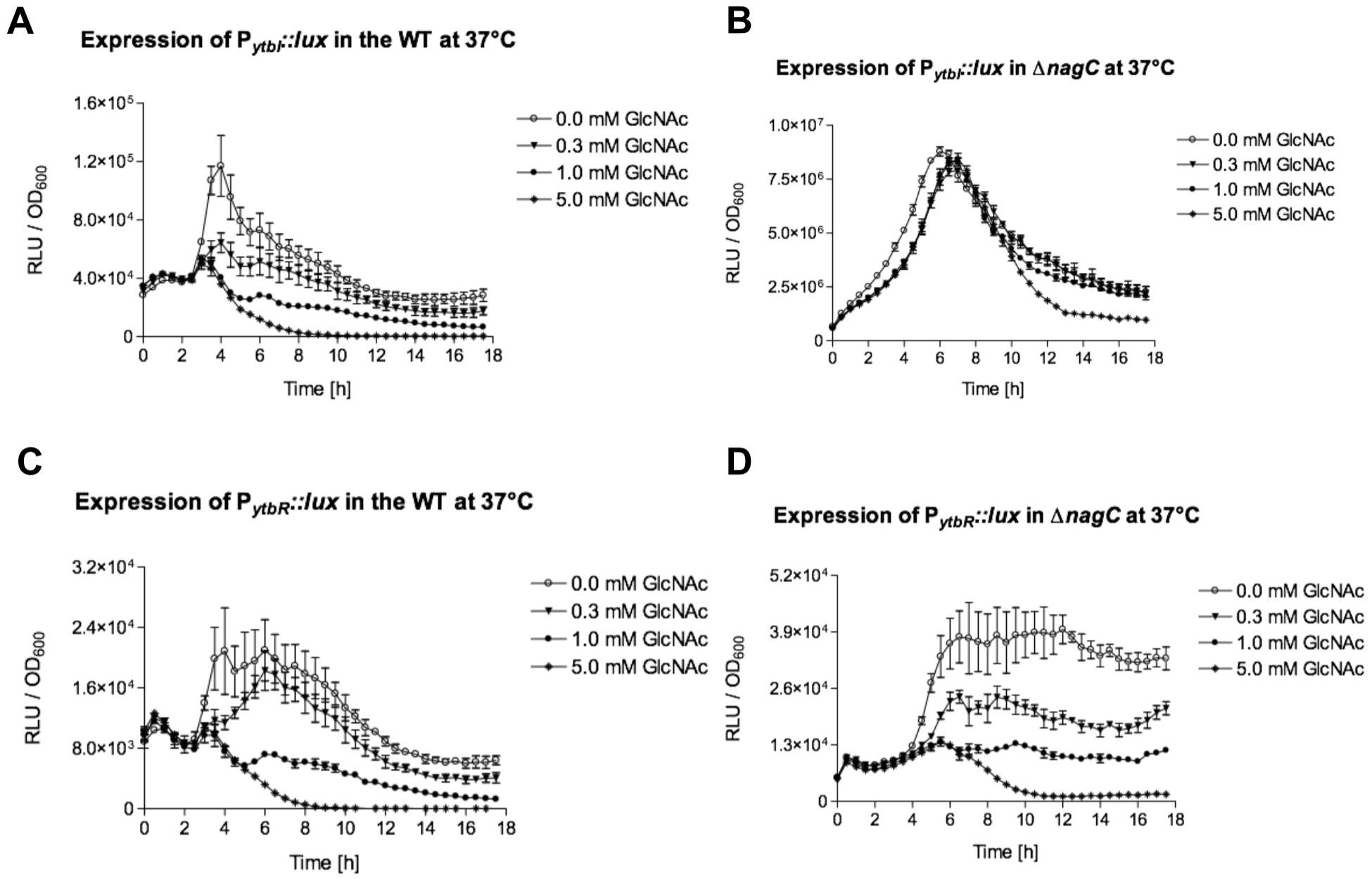
\* Corresponding author

**A****B****Expression of  $P_{nagC}::lux$  in the WT at 26°C****C****Expression of  $P_{nagC}::lux$  in the WT at 37°C****D****Expression of  $P_{nagC}::lux$  in  $\Delta nagC$  at 26°C****E****Expression of  $P_{nagC}::lux$  in  $\Delta nagC$  at 37°C**

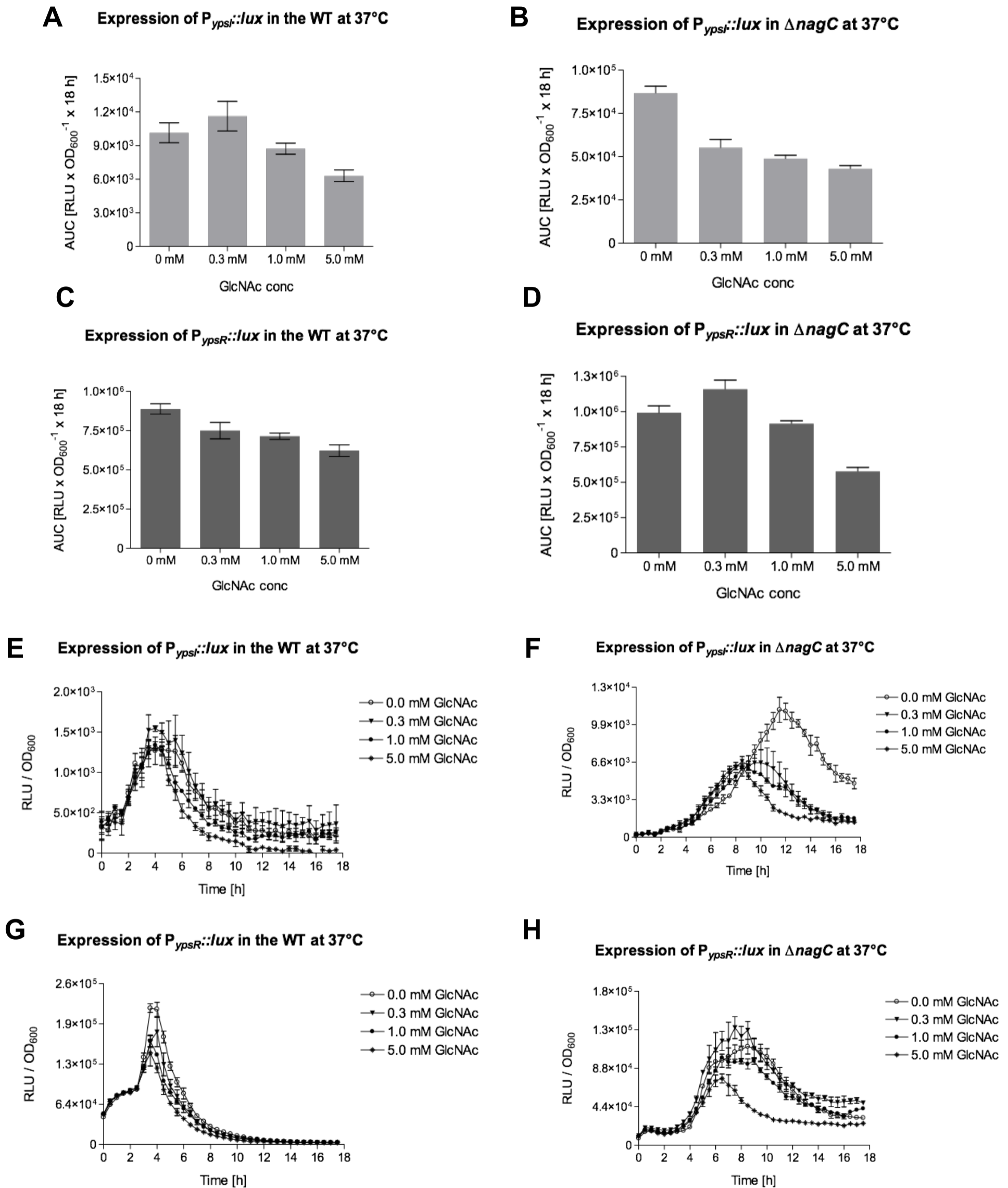
**Fig S1.** *Y. pseudotuberculosis nagC* possesses a putative *nagC* binding site (green box) in the *nagC* promoter region, with only 2 mismatches (red) to the consensus (A). Full expression profiles of NagC in the *Y. pseudotuberculosis* parent (WT) and *nagC* mutant with increasing concentrations of GlcNAc at 26°C and 37°C (B).



**Fig S2.** Full expression profiles of *ytbI* and *ypsI* in the *Y. pseudotuberculosis* parent (WT), *nagC* mutant and complement at 26°C and 37°C (A-D).

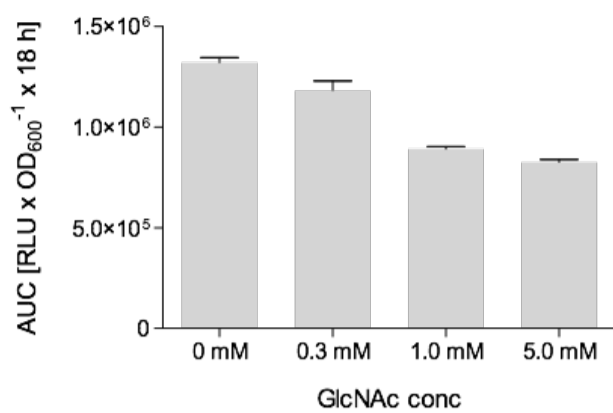


**Fig S3.** Full expression profiles of *ytl* and *ytlR* in the *Y. pseudotuberculosis* parent (WT) and *nagC* mutant in increasing GlcNAc concentrations at 37°C (**A-D**).

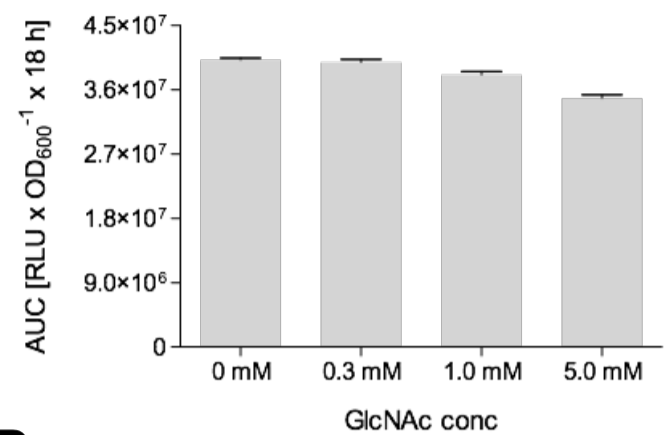


**Fig S4.** Expression as AUC of *ypsI* and *ypsR* in the parent (WT) and *nagC* mutant with increasing GlcNAc concentrations at 37°C (A-D). The full expression profiles reveal the profiles of the curves over 18 h (E-H).

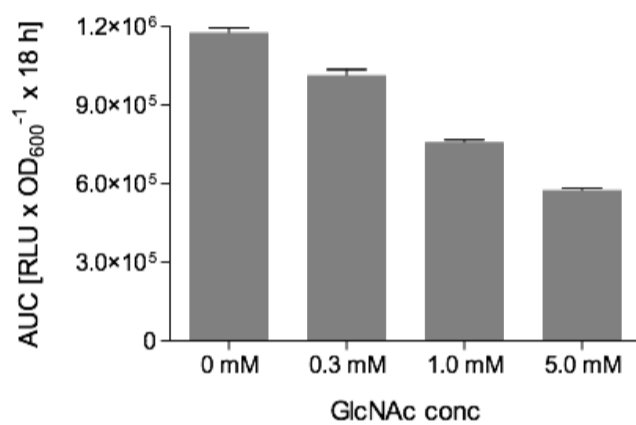
**A** Expression of  $P_{ytl}::lux$  in the WT at 26°C



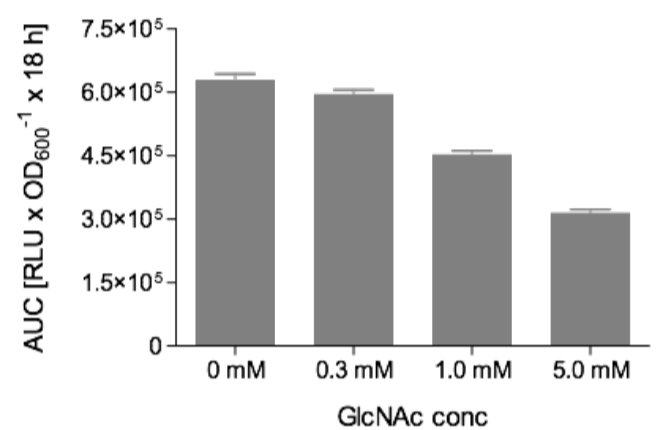
**B** Expression of  $P_{ytl}::lux$  in  $\Delta nagC$  at 26°C



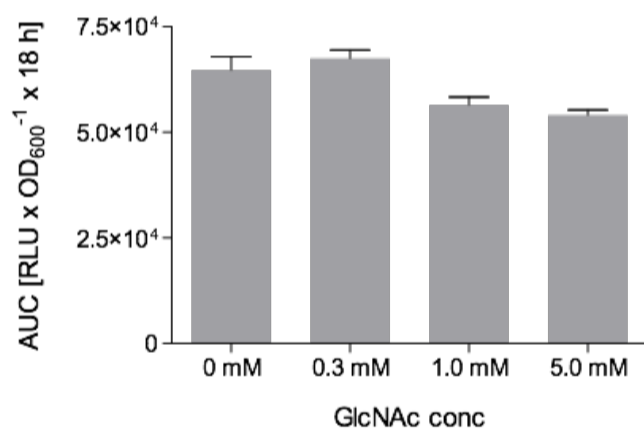
**C** Expression of  $P_{ytlR}::lux$  in the WT at 26°C



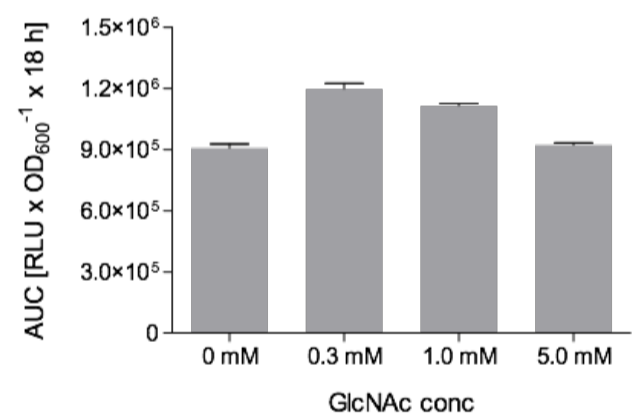
**D** Expression of  $P_{ytlR}::lux$  in  $\Delta nagC$  at 26°C



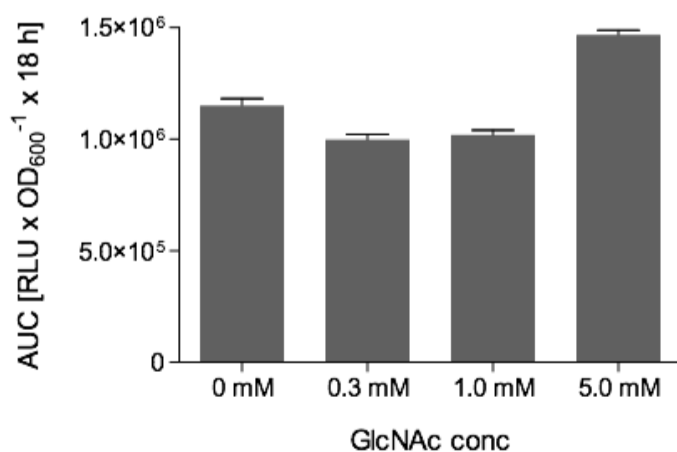
**E** Expression of  $P_{ypl}::lux$  in the WT at 26°C



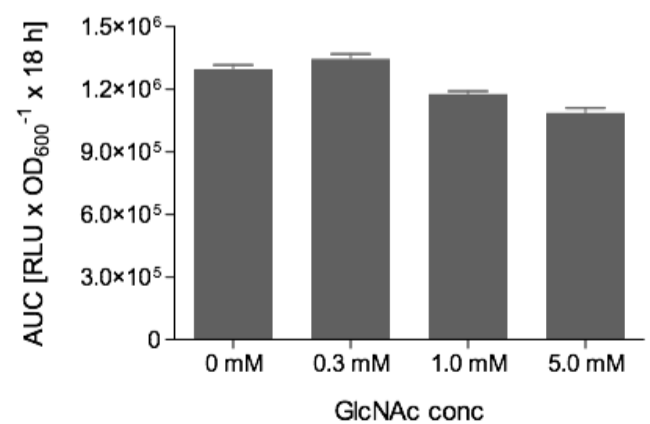
**F** Expression of  $P_{ypl}::lux$  in  $\Delta nagC$  at 26°C



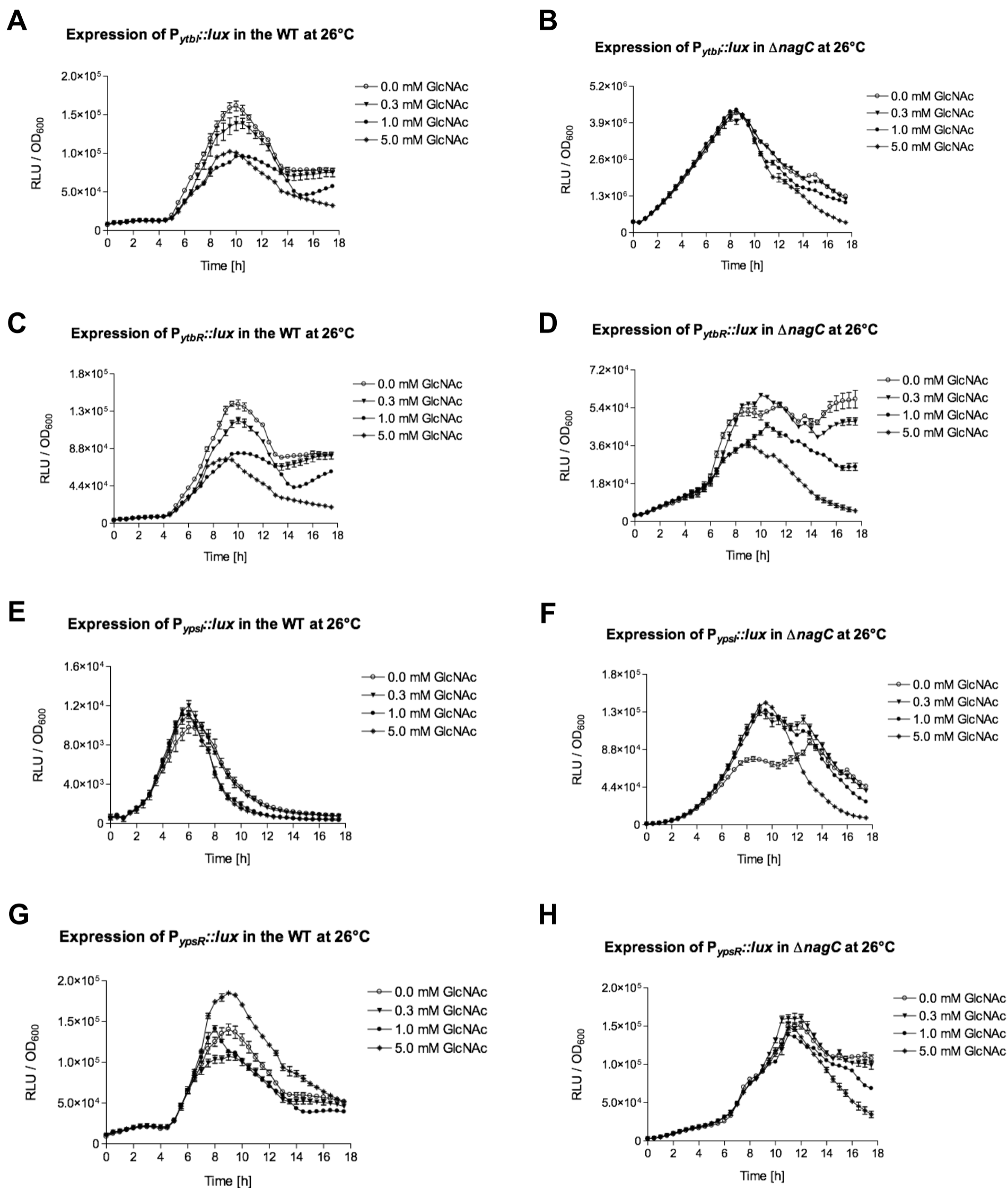
**G** Expression of  $P_{yplR}::lux$  in the WT at 26°C



**H** Expression of  $P_{yplR}::lux$  in  $\Delta nagC$  at 26°C

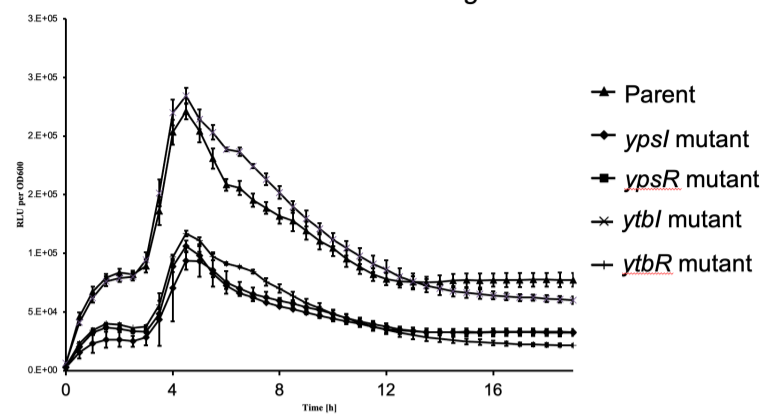


**Fig S5.** Expression as AUC of *ytl*, *ytlR*, *ypl* and *yplR* in the parent (WT) and *nagC* mutant with increasing GlcNAc concentrations at 26°C (A-H).



**Fig S6.** Full expression profile of *ytbI*, *ytbR*, *ypSI* and *ypSR* in the parent (WT) and *nagC* mutant with increasing GlcNAc concentrations at 26°C (A-H). Use in conjunction with Fig. S5.

### Expression of $P_{nagC}::lux$ at 37°C



**Fig S7.** Full expression profile of *nagC* in the parent and *ytl*, *ytr*, *ypl* and *ypr* quorum sensing mutants.



**Table S1. Plasmids**

<b>Name</b>	<b>Description</b>	<b>Source</b>
pUC4K	Source of kanamycin resistance cassette	Pharmacia
pAJD434	$\lambda$ -red recombinase plasmid inducible with arabinose (Tm <sup>r</sup> )	[1]
pGEM-T Easy	Ampicillin <sup>r</sup> PCR product cloning vector	Promega
pUC18R6KT::Tc mini Tn7	Source of mini Tn7 transposon	[2]
pTNS2	pUX-BF13 <i>Ori</i> ; mini-Tn7 helper plasmid, encodes TnsABC +D transposase complex to catalyze high frequency insertion (Amp <sup>r</sup> )	[2]
pDM4	Suicide vector containing <i>mobRK2</i> , <i>oriR6K</i> and <i>sacBR</i> (Cm <sup>r</sup> )	[3]
pBlue/ <i>lux</i>	pBluescript II KS+ Vector containing the <i>luxCDABE</i> operon (Amp <sup>r</sup> )	[4]
pHG327	Low copy number cloning vector (Amp <sup>r</sup> )	[5]
pSB2020	Gfp vector for labelling (Amp <sup>r</sup> )	[6]
pHP276	pKNG101 suicide vector containing a promoter:: <i>lux</i> fusion of <i>ypsI</i>	[4]
pHP277	pKNG101 suicide vector containing a promoter:: <i>lux</i> fusion of <i>ypsR</i>	[4]
pHP278	pKNG101 suicide vector containing a promoter:: <i>lux</i> fusion of <i>ytl</i>	[4]
pHP279	pKNG101 suicide vector containing a promoter:: <i>lux</i> fusion of <i>ytlR</i>	[4]
pHG::P <sub><i>hms::lux</i></sub>	pHG327 containing the <i>hmsHFRS</i> promoter fusion	This study
pDM4::P <sub><i>nagC::lux</i></sub>	pDM4 Suicide vector containing a promoter:: <i>lux</i> fusion of <i>nagC</i> (Cm <sup>r</sup> )	This study
pAW1	Tn7 suicide vector containing a parental copy of <i>nagC</i> for complementation.	This study

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- Qazi SN, Rees CE, Mellits KH, Hill PJ.** Development of gfp Vectors for Expression in *Listeria monocytogenes* and Other Low G+C Gram Positive Bacteria. *Microb Ecol* 2001;41(4):301-309.

**Table S2. Primers**

<b>Name</b>	<b>Sequence (5'-3')</b>	<b>Restriction Site</b>	<b>Source</b>
<i>nagC</i> -ko-F	GGCGGACAAGCACAAATTGGTA ACGTGGATCTGGTAAAACA CAAGGAGAAAGCCACG TTGTGTCTCAA		This study
<i>nagC</i> -ko-R	AGTTTCTAGCAAACGTTGCAGC AAGACACCATTGAGCATAGCGC GCTTAGCTTAGAAAACTCATC GAGCAT		This study
<i>nagC</i> -comp-F	<b><u>CTCGAG</u></b> CGGTATCCGATTTGCG ACC	XhoI	This study
<i>nagC</i> -comp-R	<b><u>GGATCC</u></b> GGTCACTATAACATGG CTGTGG	BamHI	This study
<i>nagC</i> -lux-F	<b><u>CTCGAG</u></b> CGGTATCCGATTTGCG ACC	XhoI	This study
<i>nagC</i> -lux-R	<b><u>GGATCCGGTACCC</u></b> CTCGTTGCC ATTAACGATGG	KpnI, BamHI	This study
VG-Phms-F2-ApaI	ATTTCT <b><u>GGGCC</u></b> TTTCATTGTATCGT AGCCCGGATAAAAC	ApaI	This study
VG-Phms-R-XhoI	GCGTTAT <b><u>CTCGAG</u></b> TATAACC CTTAAGCCAGCAACTTGTTT GA	XhoI	This study