Supplementary figures for:

**Increased efficiency of *Campylobacter jejuni* N-oligosaccharyl-transferase PglB by structure-guided engineering**

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**Suppl. Fig. S1.** Representative conformations of (a) *C. jejuni* OS and (b) *S. enterica* LT2 PS repeating unit in the PglB<sub>Cj</sub> model. Predicted hydrogen bond interactions between saccharide subunits and protein are shown as green dotted lines. Oligosaccharides are depicted as yellow or blue ball-stick representations with oxygen atoms in red. The PglB<sub>Cj</sub> backbone structure is shown in grey (ribbon, surface) and the phosphate groups of the membrane as cyan-colored balls. Residues in close proximity to the natural OS are depicted as magenta-coloured ball-stick representations.
Suppl. Fig. S2. Alignment of bacterial N-OST homologues in the vicinity of PglB\textsubscript{Cj} N311 (EL5 region). PglB\textsubscript{Cj} was used as search template for Protein BLAST and non-redundant sequences were aligned with the MegAlign\textsuperscript{TM} program using the ClustalW algorithm (DNASTAR, Madison, WI, USA). PglB\textsubscript{Cj} residues conserved in sequences of other species are shaded, positions mutated in this study are boxed. PglB\textsubscript{Cj} E316 (*): Strictly conserved residue which is a ligand of both the divalent metal cofactor and the asparagine amine of the acceptor peptide in the PglB\textsubscript{Cj} crystal structure PDBid 3RCE.
Suppl. Fig. S3. Effect of mutation PglB<sub>Cj</sub> N311V on glycoprotein formation in shake flask analyzed by Western blot. (a) LT2-EPA, host strain <i>S. enterica</i> SGSC228 (pGVXN150); (b) CP5-EPA, host strain <i>E. coli</i> St1717 (pGVXN150, pGVXN393); (c) EPA-C. jejuni OS, host strain <i>E. coli</i> CLM24 (pACYC<sub>pglmut</sub>, pGVXN150). Same experiments as shown in Fig. 4, biomass-normalized periplasmic extracts, similar loading volumes, samples of one shake flask culture per variant. Wild-type PglB: pGVXN970, PglB N311V: pGVXN1217. Theoretical molecular mass of unglycosylated EPA-6H: 69.4 kDa.

(a)
(b) time after induction: 0h 1h 3h 6h

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Anti-EPA

Anti-S. aureus CP5

(c) time after induction: 0h 1h 3h 6h

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Anti-EPA

Anti-C. jejuni OS
Suppl. Fig. S4. Effect of N311V on expression of HA-tagged PglB and CP5-EPA formation in shake flask. (a) Anti-HA Western blot analysis of PglB expression. Cell pellets of three replicate shake flask cultures per variant were resuspended to OD$_{600}$ = 20 in SDS-PAGE sample buffer and denatured for 1h at 60°C. (b) Time course of CP5-EPA formation analyzed by sandwich ELISA of OD$_{600}$-normalized periplasmic extracts. Open symbols: wild-type PglB-HA, closed symbols: PglB-HA N311V. Average values and standard deviations for n = 3 replicate cultures, absorbance values were corrected for PglBmut background. HA-tagged PglB and PglB N311V were expressed from plasmids pGVXN1929 and pGVXN1930, respectively, in host strain *E. coli* St1717 (pGVXN150, pGVXN393). Arrows: 1 full-length PglB, 2 degradation product of PglB.

(a) 3 h after induction

(b) 6 h after induction

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Supplementary figures for Ihssen *et al.* (N-OST engineering)
Suppl. Fig. S5. Effect of PglB mutation N311V on glycosylation of AcrA with *Salmonella enterica* LT2 polysaccharides. (a) Anti-Salmonella O:5 Western blot (primary antibody: rabbit anti-Salmonella O:5, Staten Serum Institute; secondary antibody goat anti-rabbit IgG-HRP, Biorad), (b) anti-his-tag Western blot (primary antibody: mouse anti-4H, Quiagen, secondary rabbit anti-mouse IgG-HRP, Sigma-Aldrich). OD₆₀₀-normalized periplasmic extracts were prepared from replicate, overnight induced shake flask cultures (SF). Similar sample volumes were loaded in each lane. Inactive PglBmut: pGVXN115; wild-type PglB: pGVXN970; PglB N311V: pGVXN1217. Host strain: *S. enterica* SGSC228 (pMIK44). Expected size of AcrA-6H: 40 kDa.