The impact of *Staphylococcus aureus* cell wall glycosylation on langerin recognition and Langerhans cell activation

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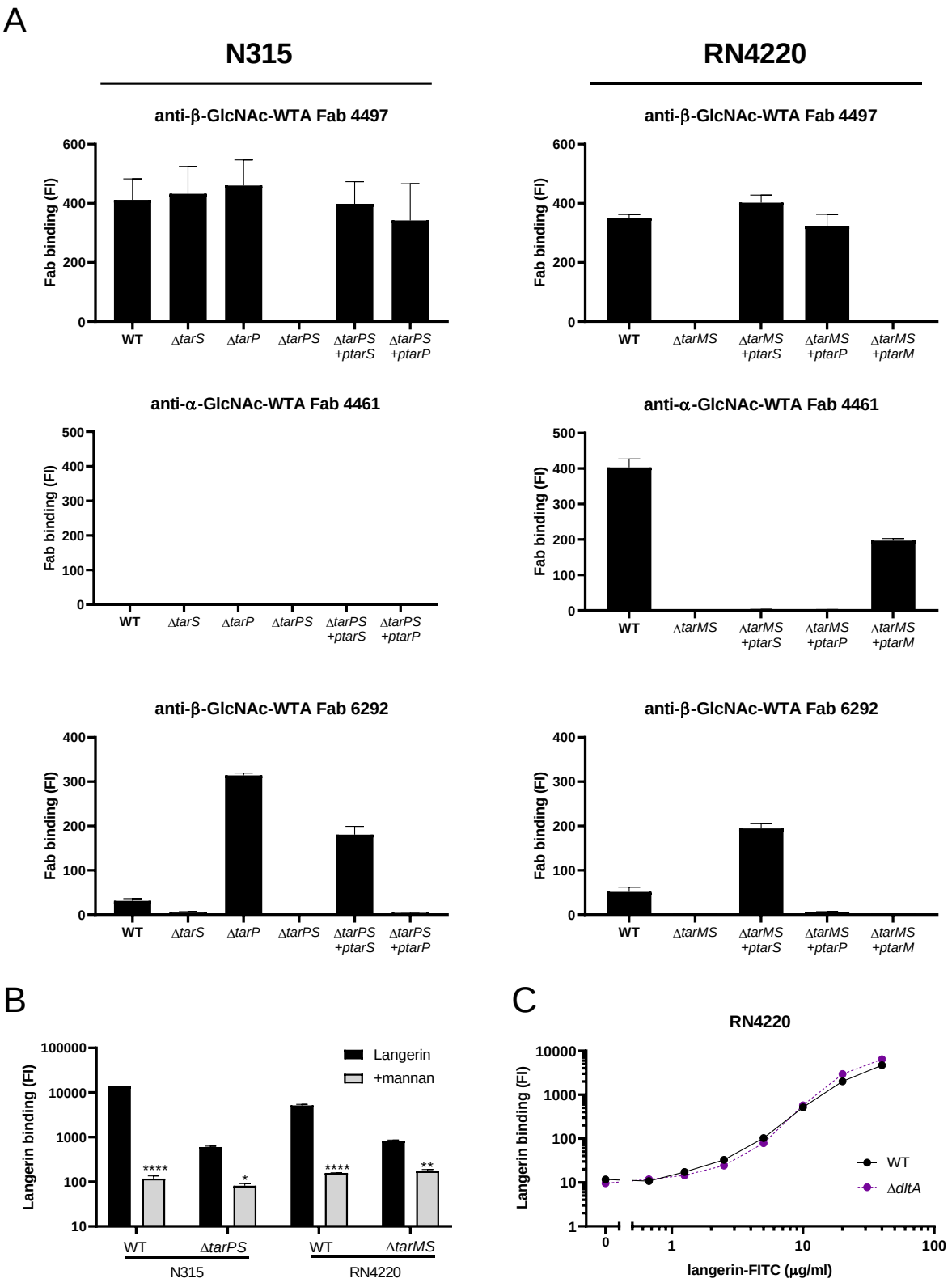
Supporting information

List of materials included:

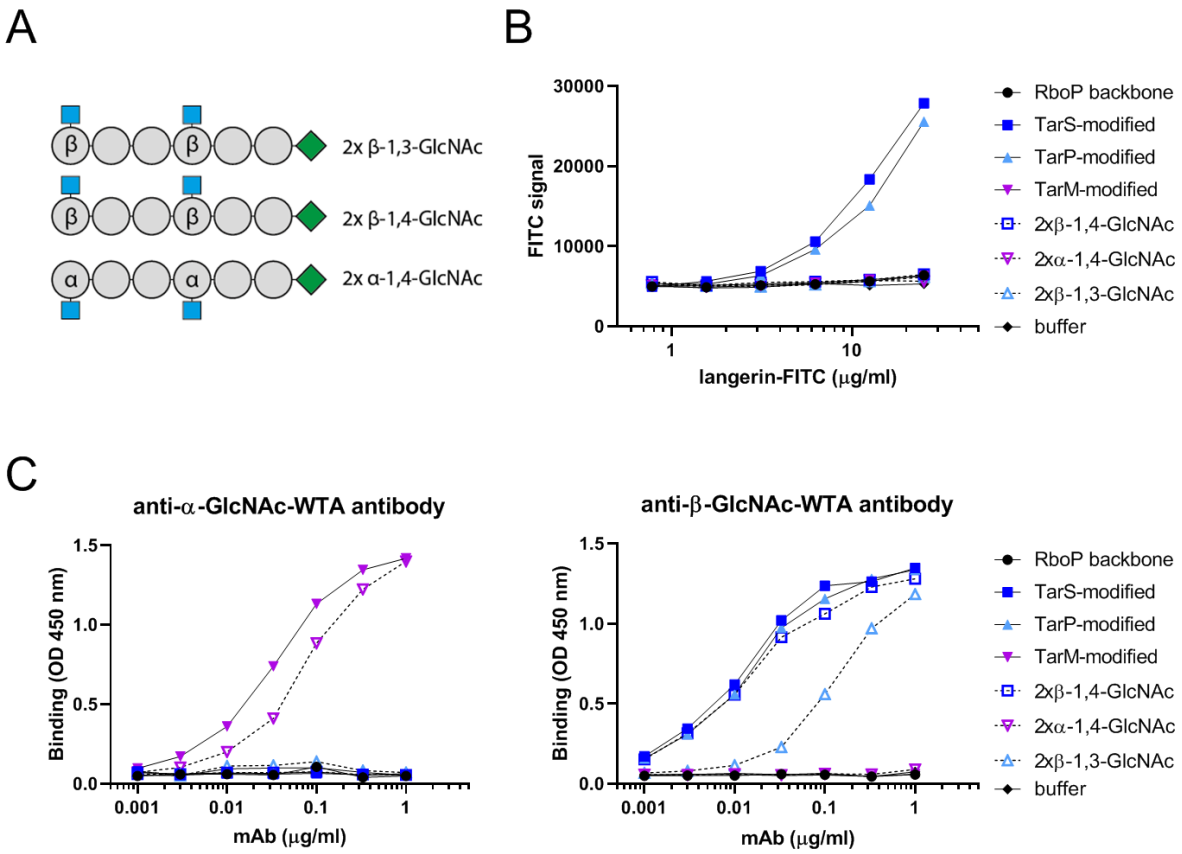
- Table S1
- Supporting figures 1-3

Table S1 Bacterial strains used in this study

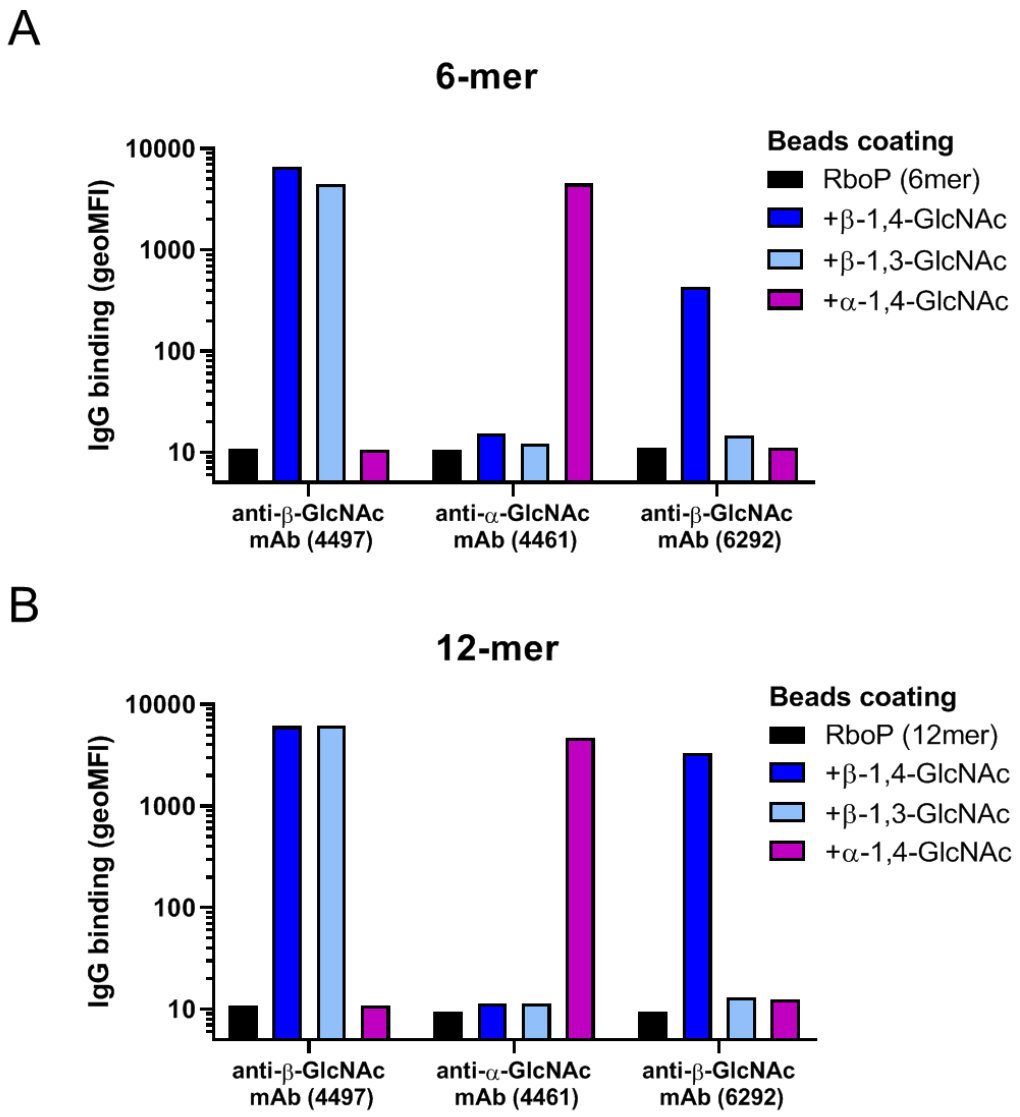
Strain (sequence type, clonal complex)	**Source**
N315 WT (ST5, CC5)	NARSA strain collection
N315 Δ*tarS*	(1)
N315 Δ*tarP*	(1)
N315 Δ*tarS*Δ*tarP*	(1)
N315 Δ*tarS*Δ*tarP*+*p*RB474-*tarS*	This study
N315 Δ*tarS*Δ*tarP*+*p*RB474-*tarP*	This study
RN4220 WT (ST8, CC8)	(2)
RN4220 Δ*dltA*	(3)
RN4220 Δ*tarM*Δ*tarS*	(4)
RN4220 Δ*tarM*Δ*tarS*+pRB474-*tarS*	(4)
RN4220 Δ*tarM*Δ*tarS*+pRB474-*tarP*	(1)
RN4220 Δ*tarM*Δ*tarS*+pRB474-*tarM*	(4)


A
N315
RN4220
anti-β-GlcNAc-WTA Fab 4497
anti-α-GlcNAc-WTA Fab 4461
anti-β-GlcNAc-WTA Fab 6292
Fab binding (FI)
WT
ΔtarS
ΔtarP
ΔtarPS
ΔtarPS +ptarS
ΔtarPS +ptarP
ΔtarMS
ΔtarMS +ptarS
ΔtarMS +ptarP
ΔtarMS +ptarM
B
Langerin binding (FI)
Langerin
+mannan
****
*
**
N315
RN4220
C
RN4220
WT
ΔdltA
langerin-FITC (µg/ml)

Supporting Figure 1 A) Binding of Fab specific for α-GlcNAc-WTA (4461), β-GlcNAc-WTA (4497) and β-1,4-GlcNAc-WTA (6292) to N315 (left) and RN4220 (right) mutant panels. B) Binding of recombinant langerin-FITC (40 μg/ml) to N315 WT, N315 Δ*tarS*Δ*tarP*, RN4220 WT and RN4220 Δ*tarM*Δ*tarS* in the presence or absence of mannan (20 μg/ml). C) Binding of recombinant langerin-FITC (0.6-40 μg/ml) to RN4220 wt and Δ*dltA*. Data is depicted as geometric mean fluorescence intensity (MFI) + standard error of mean (SEM) of biological triplicates. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$



Supporting figure 2 A) Schematic overview of fully synthetic WTA oligomers. Grey circles indicate RboP subunit, blue square represents GlcNAc, green diamond indicates biotin tag, α and β indicate the type of linkage of GlcNAc to RboP subunit. B) Binding of recombinant langerin-FITC (0.8-25 µg/ml) to *in vitro*-glycosylated RboP hexamers, fully synthetic WTA oligomers and RboP backbone. C) Binding of monoclonal antibodies (0.001-1 µg/ml) specific for α-GlcNAc-WTA (4461) and β-GlcNAc-WTA (4497) to the same panel of WTA oligomers as in B, followed by anti-human IgG-HRP staining. Data for panel B is shown as fluorescence signal, for C the absorbance at 450nm.



Supporting figure 3 Binding of monoclonal antibodies (3 µg/ml) specific for α-GlcNAc-WTA (4461), β-GlcNAc-WTA (4497) and β-1,4-GlcNAc-WTA (6292) to beads coated with (A) RboP hexamers and (B) RboP dodecamers, *in vitro*-glycosylated by TarS, TarP or TarM. Unglycosylated RboP fragments are included as controls, binding is shown as geometric mean fluorescence intensity, after staining with goat anti-human kappa-Alexa Fluor 647.

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