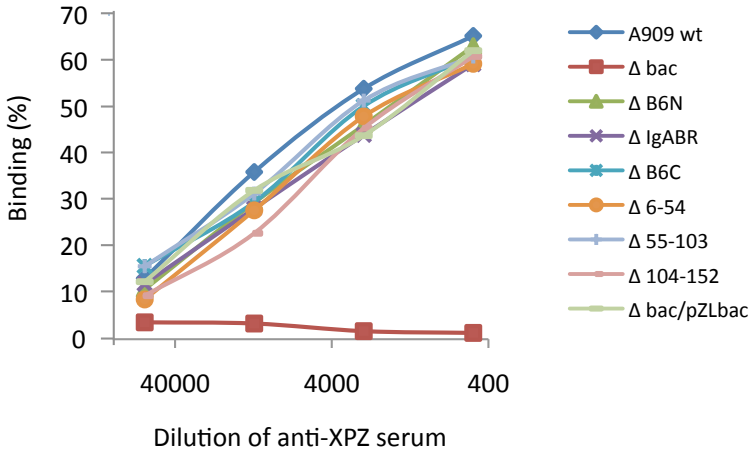
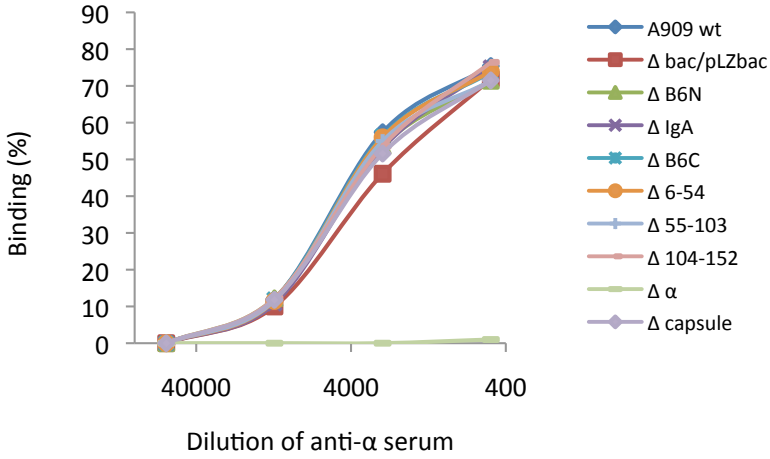
Figure S1

A



B



C

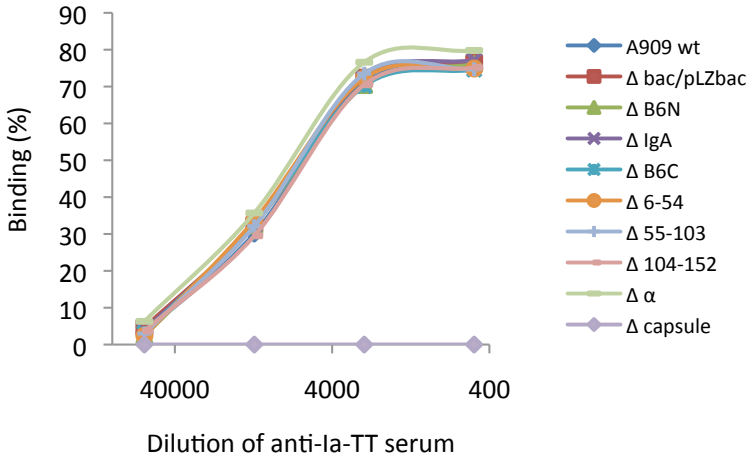


Figure S2

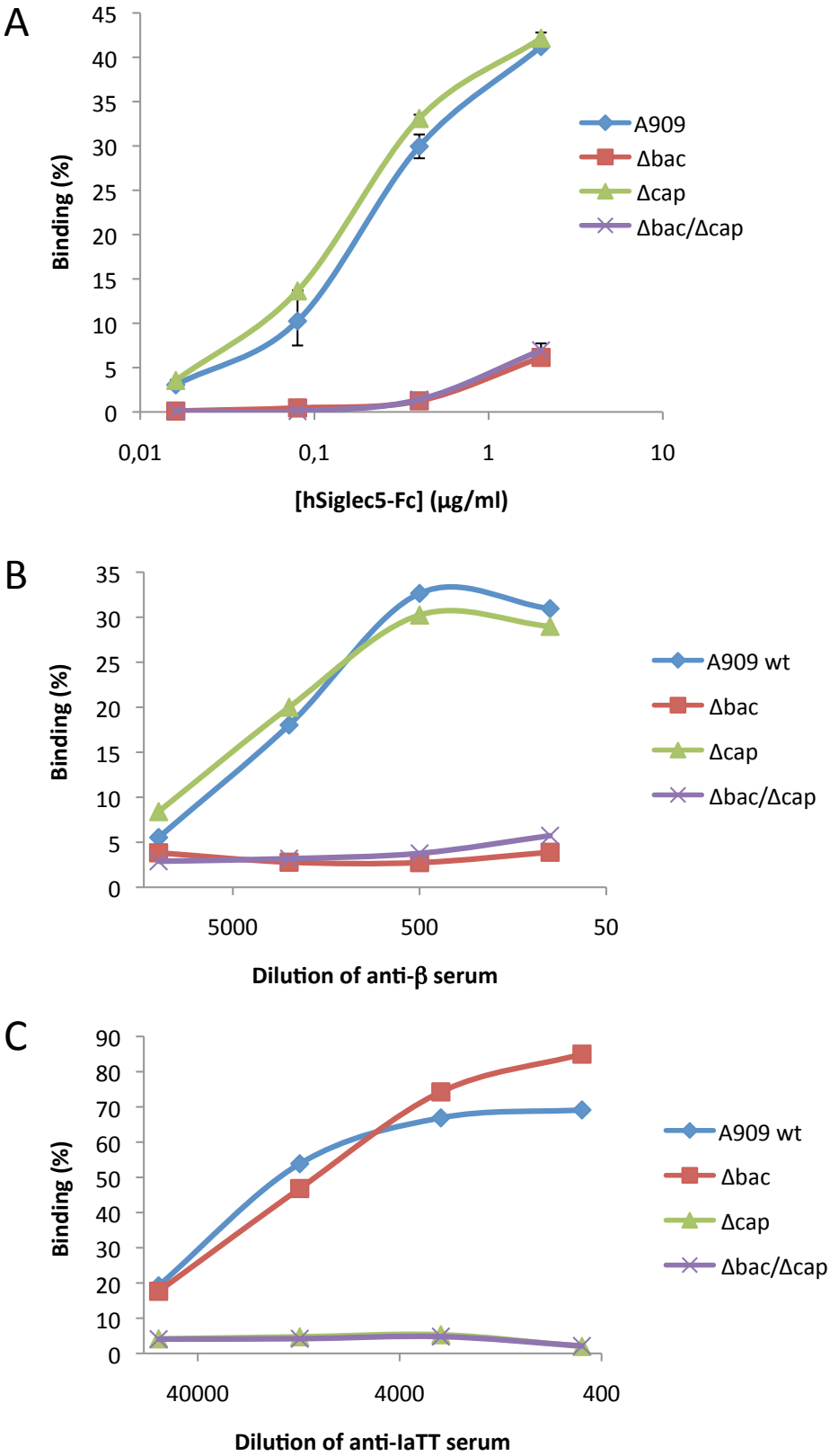

A
Binding (%)
45
40
35
30
25
20
15
10
5
0
0,01
0,1
1
10
[hSiglec5-Fc] (µg/ml)
A909
Δbac
Δcap
Δbac/Δcap
B
Binding (%)
35
30
25
20
15
10
5
0
5000
500
50
Dilution of anti-β serum
A909 wt
Δbac
Δcap
Δbac/Δcap
C
Binding (%)
90
80
70
60
50
40
30
20
10
0
40000
4000
400
Dilution of anti-IaTT serum
A909 wt
Δbac
Δcap
Δbac/Δcap

Table S1. Analysis of β-expression in GBS isolates using rabbit anti-β serum, and analysis of hSiglec-5 binding.

Strain	Serotype	Anti-β binding (%)	hSiglec-5 binding (%)
A909	Ia	48,5	45,8
BS39	Ia	2,0	4,3
BS22	Ib	26,5	36,7
BE85/91	II	20,3	6,3
BE140/91	II	32,7	29,2
BS29	II	46,0	46,0
9860/69	II	26,0	24,3
78-471	II	31,4	16,7
90-192	II	10,5	4,5
BS20	III	2,2	2,9
BS9	III	10,1	3,8
12351	IV	23,4	23,3
1518/77	IV	30,6	30,8
3445/80	V	35,7	41,3
49 SBL	V	30,9	26,0
3066 SBL	V	43,1	35,0

Table S2. Oligonucleotides used for construction of β deletion mutants and recombinant β fragments.

Deletion	**Primer name**	**Sequence**
ΔB6N	B6N-Fw/XhoI	5´-AAT T**CT CGA G**CT TTA CAA GCT CAC TTG CAT-3´
	B6N-Rv/XhoI	5´-AAT T**CT CGA G**GA TCT AAG CAA TAT TGA CAA A-3´
ΔIgA	IgA-Fw/XhoI	5´-AAT T**CT CGA G**TA CTT TCG TAT CTG ACT GTT T-3´
	IgA-Rv/XhoI	5´-AAT T**CT CGA G**CA AGA AAT TCA AGA GCA TGT G-3´
ΔB6C	B6C-Fw/XhoI	5´-AAT T**CT CGA G**AT CCA GAC CAG CTT TAG TT-3´
	B6C-Rv/XhoI	5´-AAT T**CT CGA G**CT TTT AAC AAA ATA TAA TCC GT-3´
Δ6-54	B6N-Fw/XhoI	5´-AAT T**CT CGA G**CT TTA CAA GCT CAC TTG CAT-3´
	B6N 4 Rv/XhoI	5'-AAT T**CT CGA G**GT CGA GAA AAC AGC TGG-3'
Δ55-103	B6N 3 Fw/XhoI	5'-AAT T**CT CGA G**CG GTT CAA CAG CTT TTT TT-3'
	B6N 6 Rv/XhoI	5'-AAT T**CT CGA G**GA AAC AAA TGA TTC TGA TGC-3'
Δ104-152	B6N 5 Fw/XhoI	5'-AAT T**CT CGA G**AT CAA TTT TTG TTT TAA ACT-3'
	B6C-Rv/XhoI	5´-AAT T**CT CGA G**CT TTT AAC AAA ATA TAA TCC GT-3´
Fragment		
B6N	B6N-Fw/BamHI	5´-AAT T**GG ATC C**AG TGA GCT TGT AAA GGA CG-3´
	B6N-Rv/EcoRI	5´AAT T**GA ATT C**TT ATT ATA CTT TCG TAT CTG ACT GTT T-3´
IgABR	IgA-Fw/BamHI	5´-AAT T**GG ATC C**GA TCT AAG CAA TAT TGA CAA AG-3´
	IgA-Rv/EcoRI	5´-AAT T**GA ATT C**TT ATT AAT CCA GAC CAG CTT TAG TTG-3´
B6N tandem	B6N-Fw/BamHI	5´-AAT T**GG ATC C**AG TGA GCT TGT AAA GGA CG-3´
	B6N-Rv/BamHI	5'-AAT T**GG ATC C**TA CTT TCG TAT CTG ACT-3'
IgABR tandem	IgA-Fw/BamHI	5´-AAT T**GG ATC C**GA TCT AAG CAA TAT TGA CAA AG-3´
	IgA-Rv/BamHI	5'-AAT T**GG ATC C**AT CCA GAC CAG CTT TAG-3'

Figure S1. Phenotypic characterization of the novel GBS β-deletion mutants. The level of expression of the β protein (*A)*, α protein (*B)* or the type Ia polysaccharide capsule (*C)* were similar in the different GBS β-deletion mutants. Overnight cultures were washed and resuspended to final concentrations of ~10^9 cfu/ml. To determine the expression of protein β on the surface of the β-deletion variants of A909, a rabbit antiserum directed against the XPZ region in β was used. Expression of the type Ia polysaccharide capsule or protein α was analysed with rabbit anti-Ia-TT and rabbit anti-α serum, respectively. A909 mutants lacking the α protein and the type Ia polysaccharide capsule (Areschoug, unpublished), respectively, were used as negative controls. Binding was detected using ^{125}I-labeled protein G and measured in a γ-counter.

Figure S2. Analysis of the role of the GBS polysaccharide capsule on the β-hSiglec-5 interaction. The binding of hSiglec-5 *(A)*, rabbit anti-β antibodies *(B)*, and rabbit anti-Ia-TT antibodies *(C)* was compared between the A909 wt strain and its isogenic acapsular mutant (Δcap), β-negative mutant (Δbac) and double mutant (Δbac/Δcap). The Δcap and double mutants were constructed by inactivation of the *cpsIaE* gene, which encodes a glycosyltransferase, resulting in mutants lacking the entire polysaccharide capsule (Areschoug, unpublished). Overnight cultures were washed and resuspended to a final concentration of ~10^9 cfu/ml. After incubation with hSiglec-5 or rabbit antiserum, bacteria were washed and binding was detected using ^{125}I-labeled protein G measuring the radioactivity associated with the bacterial pellet in a γ-counter.