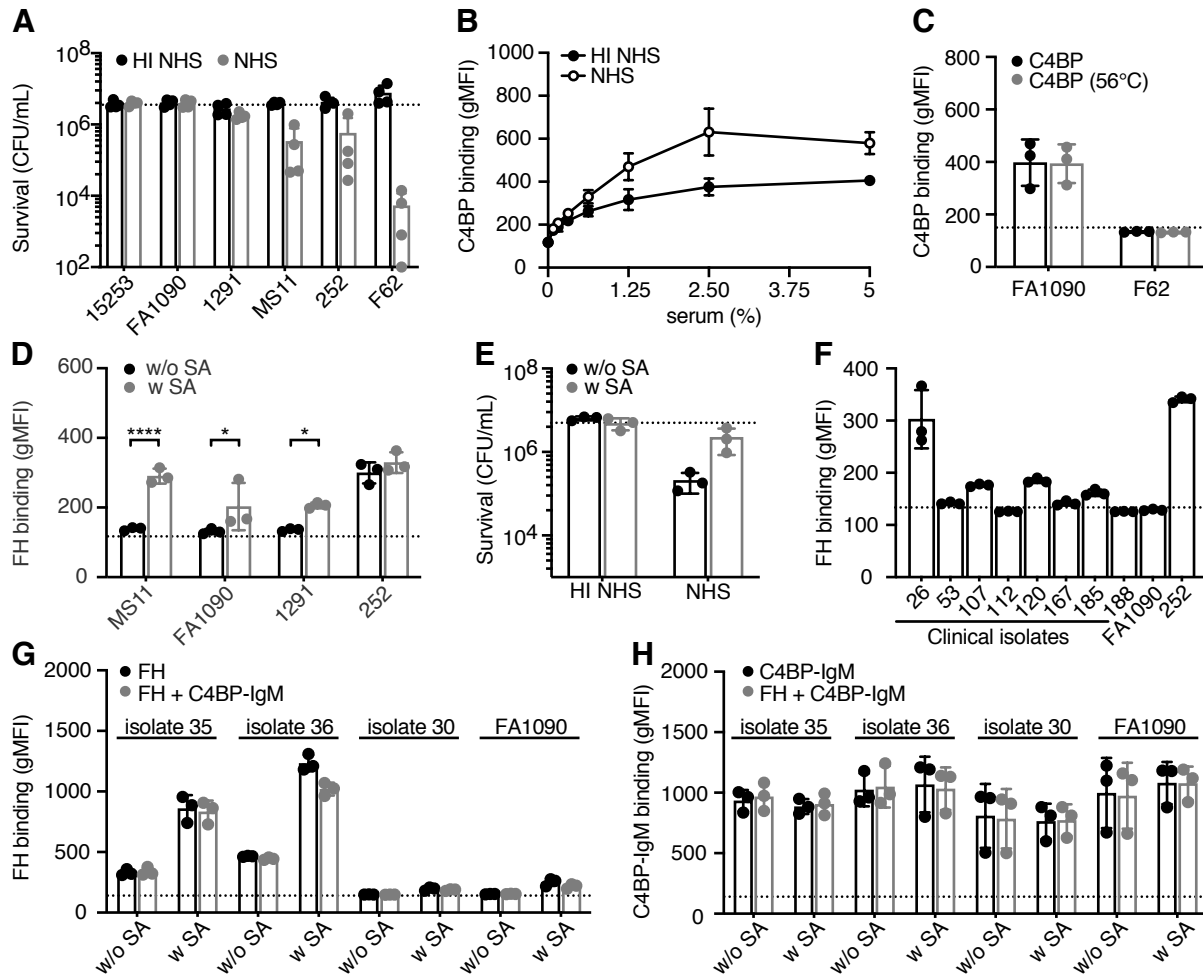
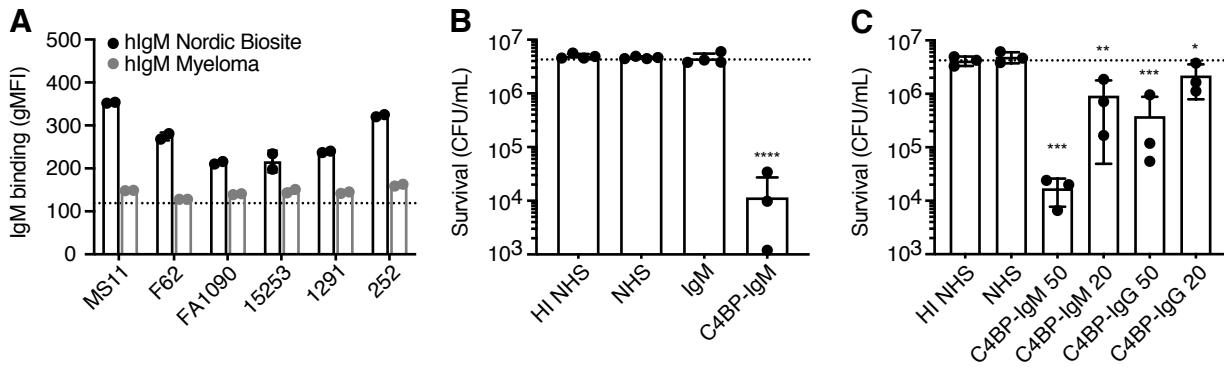


Suppl. Figure 1



Supplemental Figure 1. Survival and binding of complement inhibitors C4BP and FH to *Neisseria gonorrhoeae*. **A**) Survival of 6 laboratory strains of *Neisseria gonorrhoeae* to killing by 10% NHS. Heat inactivated NHS (HI NHS) is used as negative control. Dotted line corresponds to the starting number of bacteria used in the assay. **B**) C4BP binding to *N. gonorrhoeae* FA1090 from NHS or HI NHS. Dots represent mean \pm SD of three independently performed repeats. **C**) Binding of C4BP or 56°C-treated C4BP to *N. gonorrhoeae* FA1090 and F62. **D**) Binding of FH to four laboratory strains of *N. gonorrhoeae* in the presence (w SA) or in the absence (w/o SA) of sialylation. Statistics p values were performed by two-way ANOVA with Sidak's multiple comparisons test. * $p < 0.05$, **** $p < 0.0001$ as indicated. **E**) Survival of *N. gonorrhoeae* MS11 to killing by 10% NHS in the presence (w SA) or in the absence (w/o SA) of sialylation. **F**) FH binding of non sialylated serum resistant gonococcal clinical isolates which are not binding C4BP (identified in Fig. 1F). Dotted line refers to cut-off for positivity (133.5 gMFI) calculated as mean + 3SD of the unspecific background of signal obtained for strain FA1090 that does not bind FH. Binding of FH (**G**) or C4BP-IgM (**H**) or both to gonococcal strains 30, 35, 36 and FA1090 in the presence (w SA) or in the absence (w/o SA) of sialylation. In all scatter plots, bars display mean \pm SD of at least three independently performed experiments. Horizontal dotted line refers to starting amount of bacteria used in the assay (**A** and **E**), or gMFI average value in the absence of protein (**C**, **D**, **G** and **H**).

Suppl. Figure 2



Supplemental Figure 2. Binding and killing of *Neisseria gonorrhoeae* in the presence of human IgM and C4BP-IgG fusion protein. **A)** Binding of 50 $\mu\text{g}/\text{mL}$ of human IgM to 6 laboratory strains of *N. gonorrhoeae*. Two preparations of IgM were tested: human IgM purified from normal human plasma (hIgM Nordic Biosite); human IgM purified from myeloma human plasma (hIgM Myeloma). Dotted line refers to gMFI average value in the absence of the proteins. **B)** Survival of *N. gonorrhoeae* FA1090 to killing by 10% NHS in the presence of either 50 $\mu\text{g}/\text{mL}$ human IgM from myeloma plasma or 50 $\mu\text{g}/\text{mL}$ C4BP-IgM. Heat inactivated NHS (HI NHS) was used as negative control. **C)** Survival of *N. gonorrhoeae* FA1090 to killing by 10% NHS in the presence of 20 or 50 $\mu\text{g}/\text{mL}$ of either C4BP-IgM or C4BP-IgG. In both **B** and **C** graphs dotted lines correspond to the starting number of bacteria used in the assay, and each bar represents the mean \pm SD of at least three independently performed experiments. In all graphs differences were compared using one-way ANOVA with Dunnett's multiple comparisons test considering NHS as reference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$.

Table I. Phenotypic characteristic of laboratory strains of *Neisseria gonorrhoeae*

Strain	PorB subclass	C4BP binding	FH binding	Serum resistance
15253	PorB1a	yes	yes	yes
FA1090	PorB1b	yes	only when sialylated	yes
MS11	PorB1b	yes	only when sialylated	yes
1291	PorB1b	yes	only when sialylated	yes
252	PorB1b	no	yes	yes
F62	PorB1b	no	only when sialylated	no

PorB, Porin B protein; C4BP, C4b-binding protein; FH, Factor H

