Table 1. Primers used for mutagenesis

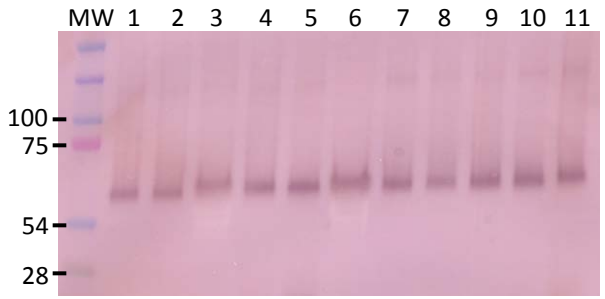
Mutants	Primers (5′ to 3′ orientation)
N1176K	atatcccgagaaattatggaaaagtataacatagcattaaggtggac
T1184K, Q1187E	aaattataacatagcattaaggtggaaagccaaagagaagctttattcgagaaca
L1189I	tggacagccaaacagaagatttattcgagaacaggtg
V1200I	tttattcgagaacaggtgaatcagttgaatttatatgtaaacggggatatcg
R1203N	caggtgaatcagttgaatttgtgtgtaaaaatggatatcgtctttcatcacgttct
R1203A	gtgaatcagttgaatttgtgtgtaaagcgggatatcgtctttcatcac
N1203R	gaacaggtgaatcagttgaatttatatgtaaaaggggatatcgtctttcacc
S1209P	ggggatatcgtctttcaccacgttctcacacattg
R1210S	gggatatcgtctttcatcaagttctcacacattgcgaac
T1213A	tctttcatcacgttctcacgcattgcgaacaacatgttg
T1217A	ctcacacattgcgaacagcatgttgggatgggaaa
A1217T	gaaactggagtatccaacttgtgtaaaaagatagaatcaatcataaag
A1229V	gagtatccaacttgtgtaaaaagagcggccgcg

Figure S1

MSALLILALVGAAVADYKDDDDKLGAPCVNPPTVQNAYIVSRQMSKYPSGERVRYQCRSPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSF
PLSVYAPASSVEYQCQNLYQLEGNKRITCRNGQWSEPPKCLHPCVISREIMENYNIALRWTAKQKLYSRTGESVEFVCKRGYRLSSRSHTLRTTCWDGKLEYPTCA
KRAAAGGEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDW
MSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNW
VERNSYSCSVVHEGLHNHHTTKSFSRTPGK

Figure S1. The translated amino acid sequence of HufH 18-20/Fc . The signal peptide derived from preprotrypsin is shown in blue font, an epitope tag sequence (FLAG) in red font, the sequence whose encoding DNA contains an *AscI* cleavage site in green font, HufH domains 18-20 in black color, the amino acid sequence of the linker whose encoding DNA contains a *NotI* cleavage site in purple and the Fc portion of mouse IgG2a in orange font.

Figure S2



1. N1176K
2. T1184K, Q1187E
3. L1189I
4. V1200I
5. R1203N
6. S1209P
7. R1210S
8. T1213A
9. T1217A
10. A1229V
11. Wild-type

Figure S2. Western blot analysis of recombinant HufH 18-20/Fc (wild-type) and the 10 mutant proteins. Recombinant wt HufH 18-20/Fc and the 10 mutant proteins present in concentrated tissue culture supernatants were separated on a 4-12% Bis-Tris gel under reducing conditions using MES running buffer (Invitrogen). Each well contained ~0.1 μg of the fusion protein as determined by ELISA. Proteins were transferred to a PVDF membrane (Millipore) and the Fc region was detected with alkaline phosphatase-conjugated anti-mouse IgG (Sigma). MW, molecular mass in kD.

Figure S3

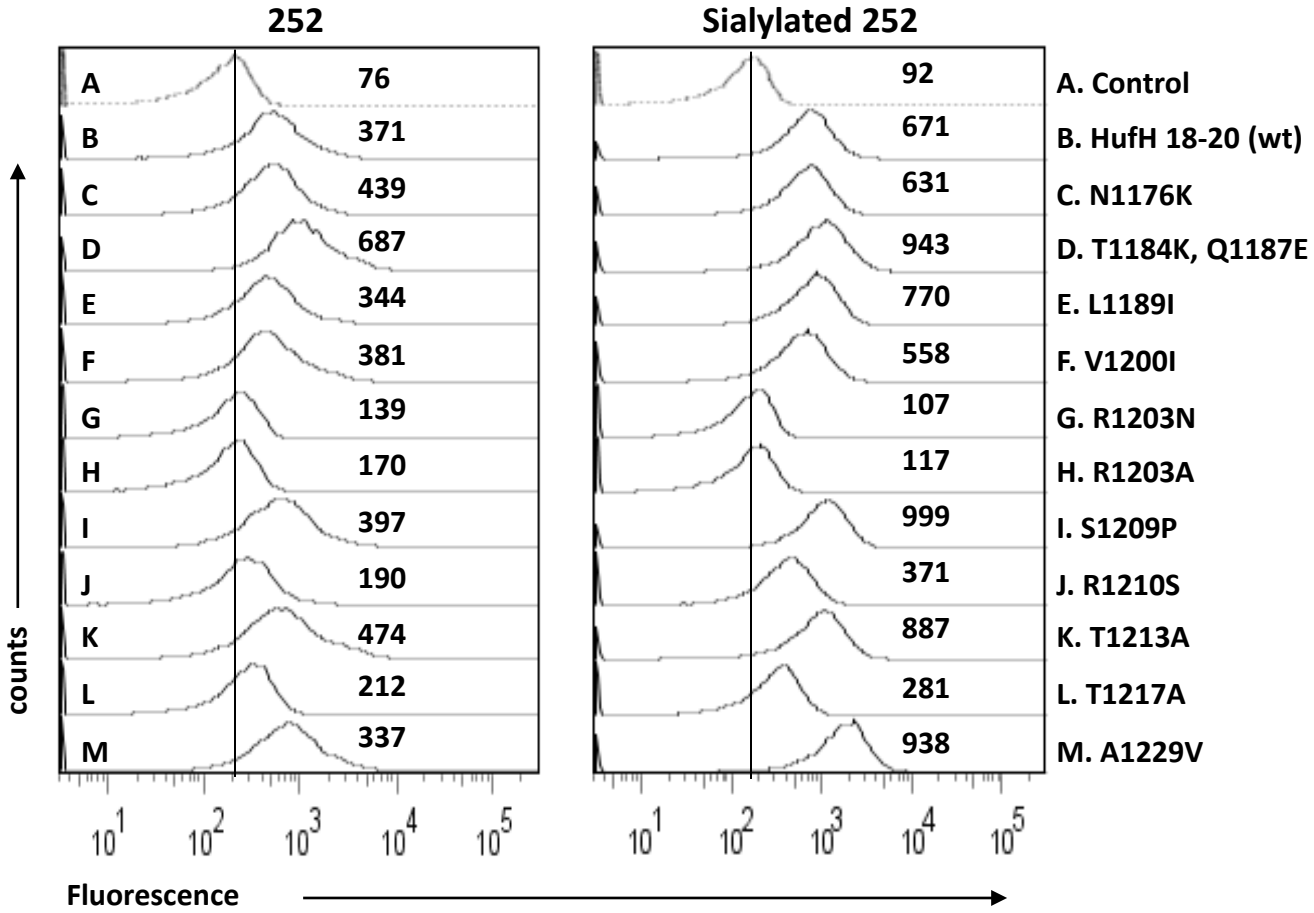


Figure S3. Comparison of binding of HufH18-20/Fc and HufH18-20/Fc mutants to unsialylated and sialylated *N. gonorrhoeae* strain 252. The graph labeled 'Control' contains no recombinant protein. In all graphs, the x-axis represents fluorescence on a $\log_{10}$ scale and the y-axis the number of events. Numbers beside each histogram represent the median fluorescence of the entire bacterial population. One representative experiment of three independently performed experiments is shown. Note the modestly increased fluorescence intensity throughout seen with sialylated 252.

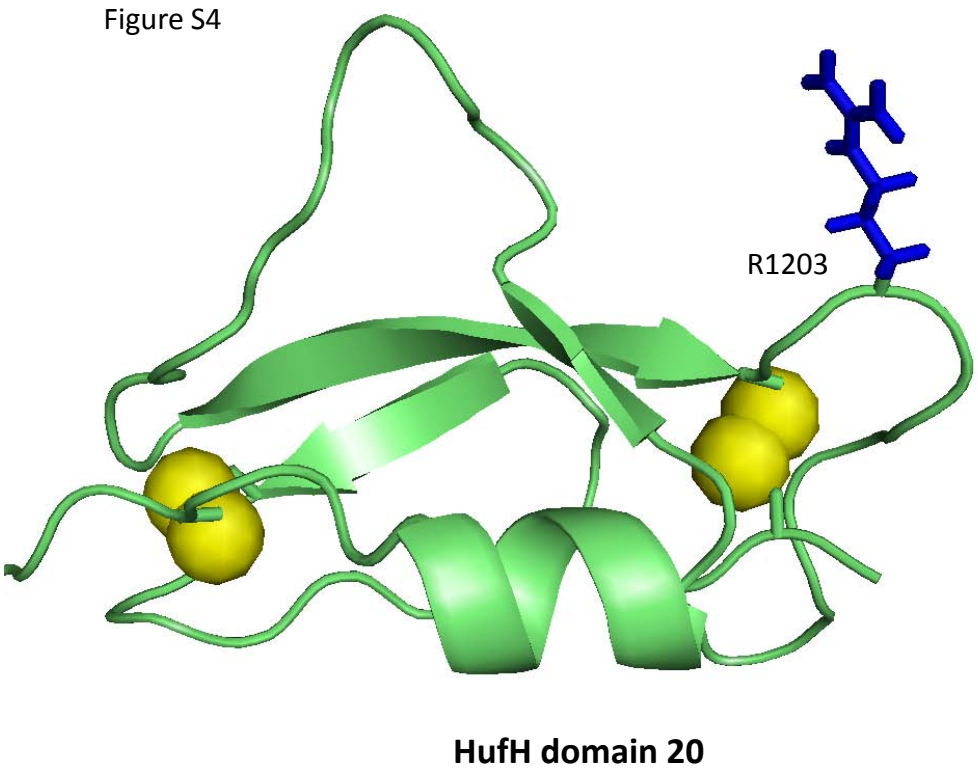


Figure S4. Orientation of R1203 with respect to the cysteine residues and disulfide bridges in HufH domain 20. Crystal structure of HufH domain 20 shown as a cartoon representation with disulfide bridges indicated as yellow spheres and side chain of R1203 residue as blue sticks (28). The residue R1203 is located close to the tip of a β-hairpin loop.

Figure S5

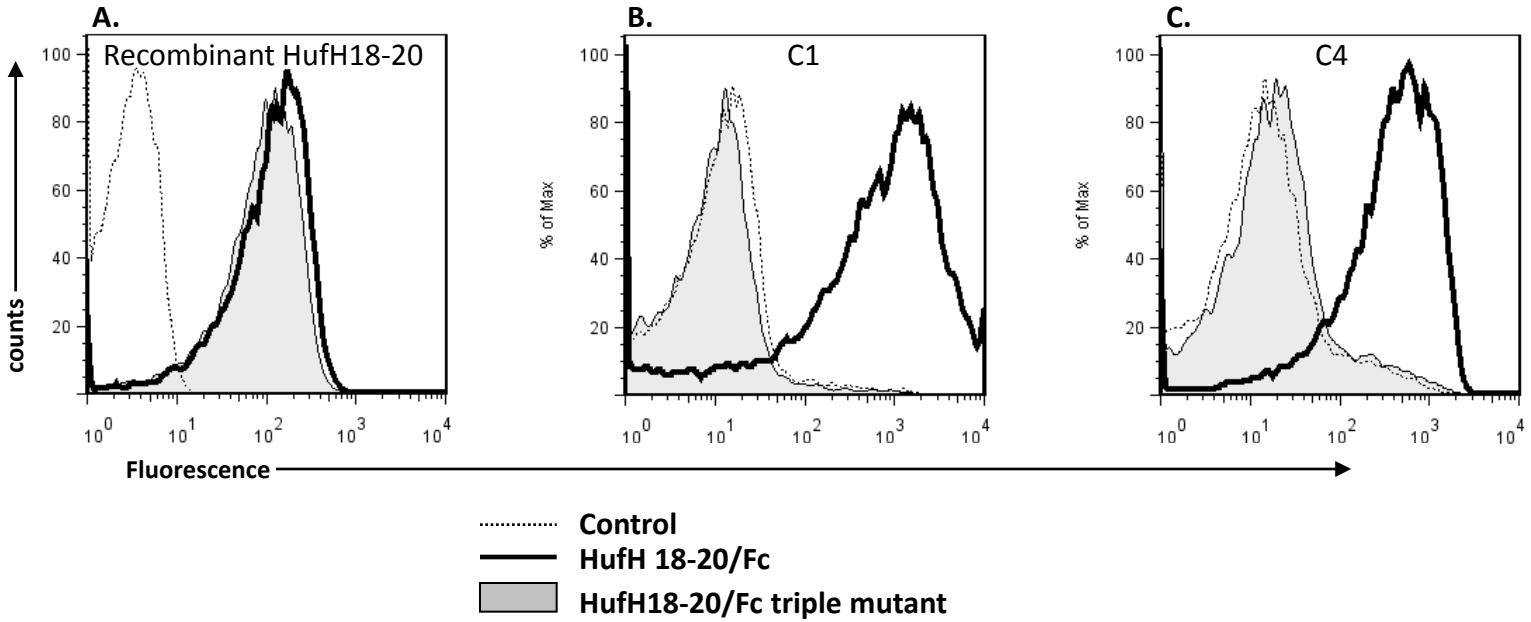


Figure S5. HufH18-20/Fc activates the classical pathway of complement. ***A.*** Binding of HufH 18-20/Fc (black line) or HufH 18-20/Fc containing three mutations in the Fc (henceforth referred to as the "triple mutant") that abrogate its ability to bind to C1q (E318A, K320A and K322A (Duncan, A. R. and Winter, G. (1988) *Nature* **332**, 738-740)) (gray shaded area) to sialylated F62. The control with 1% BSA-HBSS^{++} buffer alone is depicted by the dotted line. Binding of proteins was analyzed by FACS with anti-mouse IgG-FITC (Sigma). The Fc triple mutant abrogates C1q binding ***(B)*** and C4 deposition ***(C)***. Sialylated F62 was incubated with either 0.5 µg (48 nM) of purified HufH 18-20/Fc or the triple mutant followed by the addition of 0.8 µg (10 nM) of purified C1complex and 1 µg (49 nM) of purified C4 (both from Complement Technologies, Tyler, TX) and bacteria-bound C1q and C4 were detected by FACS using FITC-conjugated anti-human C1q (Biodesign International, Saco, Maine) and anti-human C4 FITC (Abcam), respectively.

Figure S6

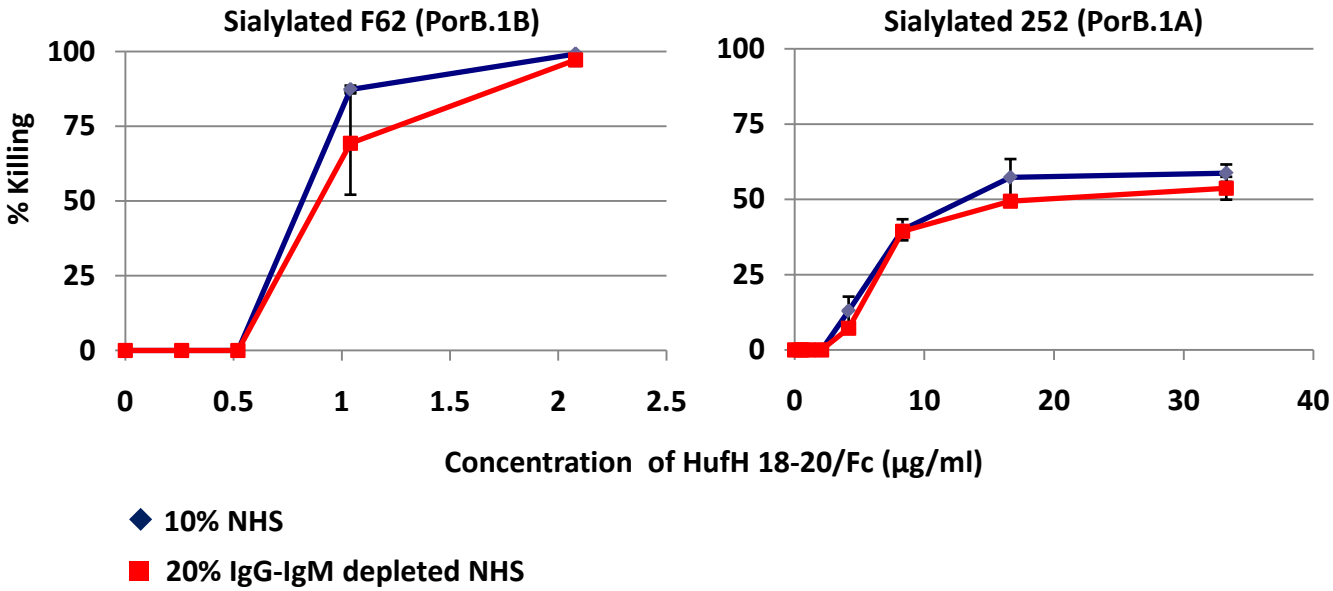


Figure S6. Killing of sialylated gonococci by HufH 18-20/Fc occurs independently of natural antibodies in normal human serum (NHS). Killing of sialylated gonococcal strains F62 (left graph) and 252 (right graph) in NHS (blue lines) or IgG and IgM depleted NHS (red lines) containing increasing amounts of HufH 18-20/Fc. IgG and IgM were depleted by passage of NHS through protein G sepharose and anti-human IgM agarose as described previously (Dutta Ray, T., Lewis, L. A., Gulati, S., Rice, P. A., and Ram, S. (2011) *J Immunol*). A 2-fold higher concentration of the IgG and IgM depleted NHS was used because of loss of ~50% of the complement-mediated hemolytic activity during absorption compared with unabsorbed NHS. The x-axis represents the concentration of HufH 18-20/Fc added to the reaction mixture and the y-axis indicates the percent (%) killing. Values represent the mean killing calculated from two independently performed experiments in duplicate ± SE.

Figure S7

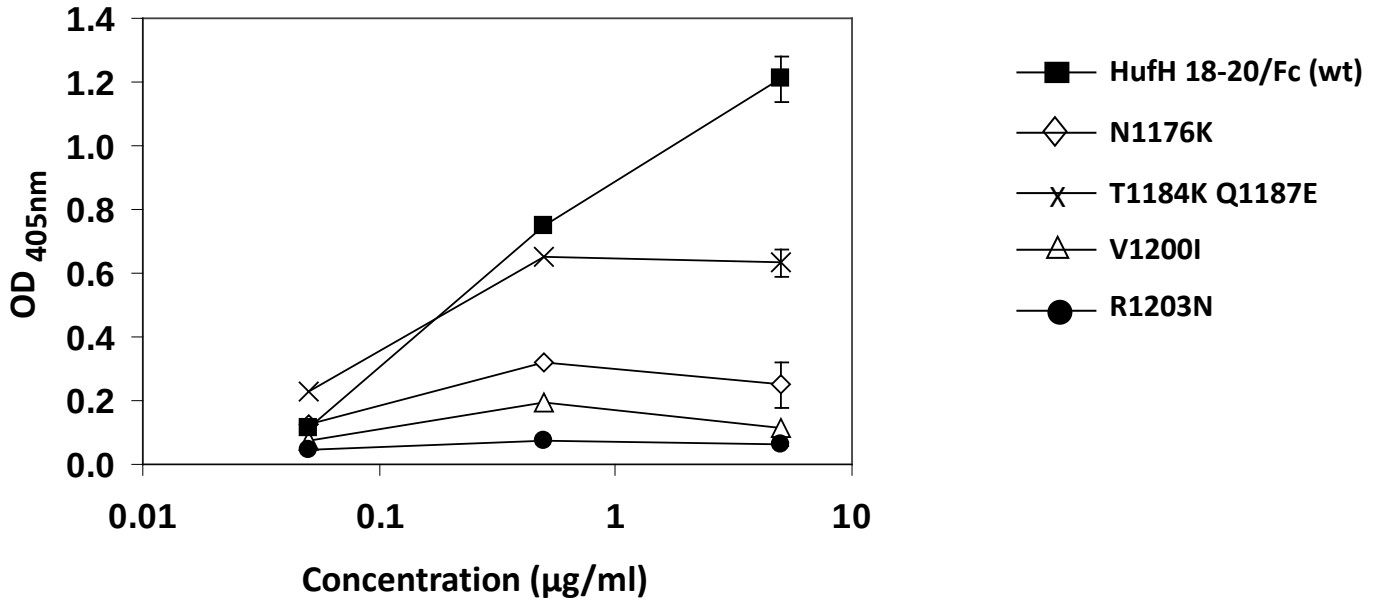


Figure S7. Heparin binding to HufH 18-20/Fc and select HufH 18-20/Fc domain 20 mutants. Heparin (Polysciences, Inc., Warrington, PA) was biotinylated at the free amino groups of unsubstituted glucosamine residues normally present at low levels in pharmaceutical heparin (Osmond, R. I., Kett, W. C., Skett, S. E., and Coombe, D. R. (2002) *Anal Biochem* **310**, 199-207) using the EZ-Link Sulfo-NHS-LC-Biotinylation Kit (Thermo Fisher Scientific, Rockford, IL) and a biotin:heparin ration of ~10:1 . Biotinylated heparin was immobilized on streptavidin (Thermo Fisher Scientific) coated microtiter wells. Serial dilutions of each fH/Fc fusion protein (HufH 18-20/Fc, containing the following mutations: N1176K, T1184K Q1187E, V1200I and R1203N) were added to the wells and incubated for 1 h at 37°C. HufH 18-20/Fc constructs that bound to heparin were detected with alkaline phosphatase-conjugated anti-mouse IgG. Results of two experiments performed in duplicate for each human HufH 18-20/Fc construct (mean ± SEM) are shown .

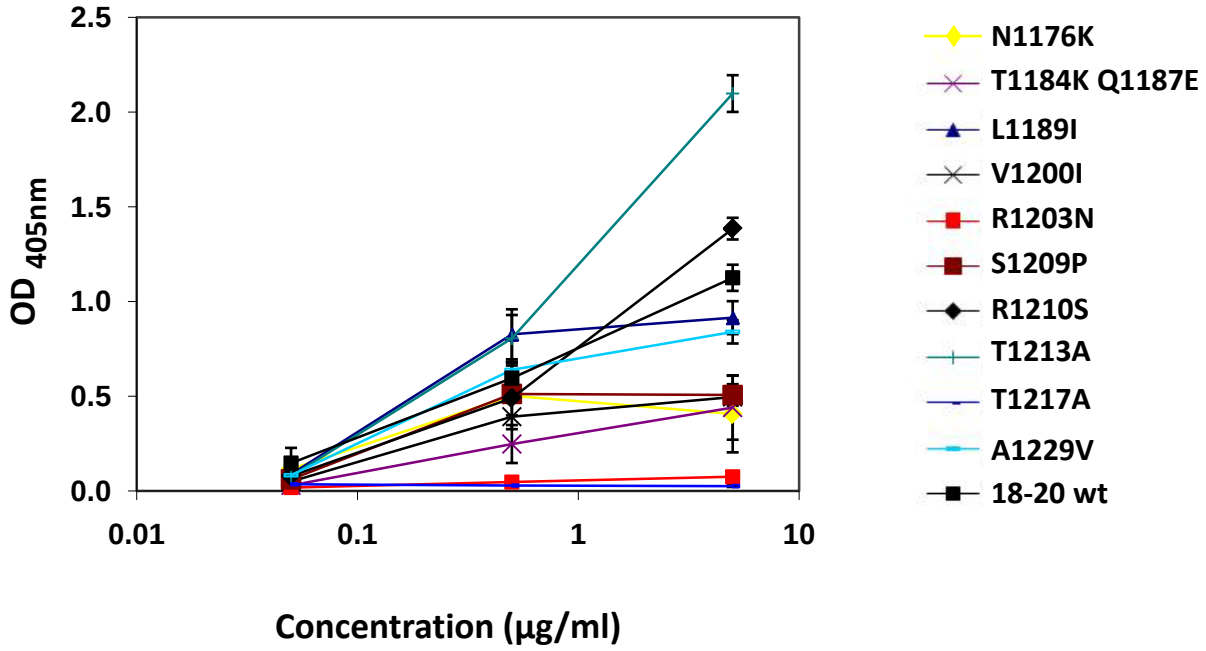


Figure S8. Complement C3b binding to HufH 18-20/Fc and its domain 20 mutant derivatives. Microtiter wells were coated with purified C3b (Complement Technologies, Tyler, TX; 10 µg/ml) overnight at 4°C. Following blocking of nonspecific binding sites with PBS-0.05% and Tween 20, serial dilutions (0.05, 0.5 and 5 µg/ml) of each recombinant protein in PBS-0.05% Tween 20 was added to wells and incubated for 1 h at 37°C. Bound human HufH 18-20/Fc constructs were detected with alkaline phosphatase-conjugated anti-mouse IgG. Results (mean ± SEM) of two experiments performed in duplicate are shown for each recombinant protein.