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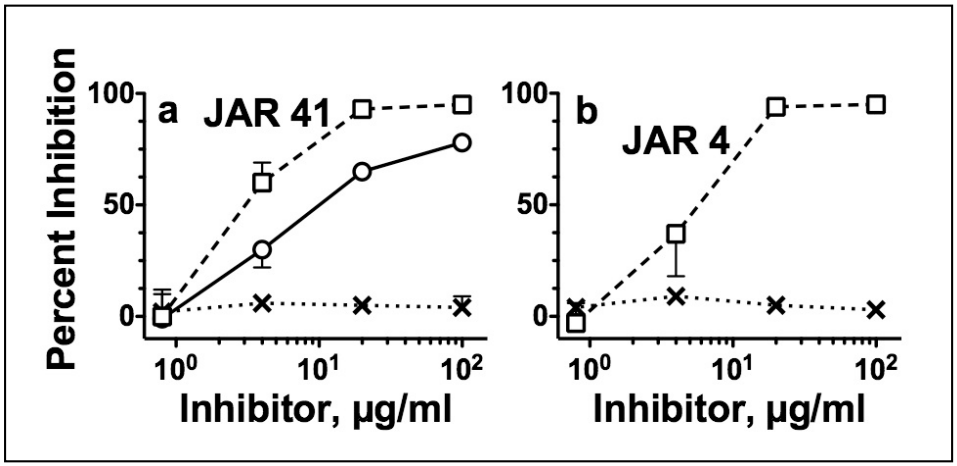
Supplementary Figure S1



Legend to Figure S1. JAR 4 and JAR 41 recognize overlapping epitopes. Inhibition of binding of anti-fHbp mAbs to recombinant fHbp ID 1 as measured by ELISA. Panel a, Inhibition of binding of alkaline phosphatase (AP)-conjugated JAR 41 by unconjugated anti-fHbp mAbs. Unconjugated JAR 41, squares with dashed line (positive control); Unconjugated JAR 4, circles with solid line; and unconjugated JAR 5, crosses with dotted line (negative control). Panel b, inhibition of binding of unconjugated JAR 4 (IgG2a) by unconjugated JAR 41 (IgG1) or unconjugated JAR 5 (IgG2b). Bound JAR 4 was detected using AP-conjugated goat anti-mouse antibody specific for IgG2a. JAR 41, squares with dashed line; and JAR 5, crosses with dotted line (negative control).

Supplementary Figure S2

fHbp ID1	10	20	30	40	50	60	70
wildtype	CSSGGGGVAA	DIGAGLADAL	TAPLDHKDKG	LQSLTLDQSV	RKNEKLKLAA	QGAEKTYGNG	DSLNTGKLKN
yeast-10	HM...	.....	....G.....	.....	.....	.....	.....
yeast-27	HM...	.....	......N...	.....	...K......	.....	.....
yeast-12	HM...	.....	......N...	.....	.....	.....	.....
yeast-18	HM...	.....	....H.....	.....	.....	.....	.....
yeast-15	HM..V	.....	....AN....	.....	.....	.....	.....
yeast-01	HM...	.....	......N...	.....	.....	.....	.....
yeast-06	HM...	.....	......N...	.....	.....	.....	.....
yeast-26	HM...	.....	..S.......	.....	.....	.....	.....
yeast-20	HM...	.....P	.....	.....	.....	.....	.....
yeast-08	HM...	.....R	.....	.....	.....	.....	.....
yeast-23	HM...	.....P	.....	.....	.....	.....	.....
yeast-24	HM...	.....R	.....	.....	.....	.....	.....
yeast-22	HM...	.....	.....	.....	.....	.....	.....S....

fHbp ID1	80	90	100	110	120	130	140
wildtype	DKVSRFDFIR	QIEVDGQLIT	LESGEFQVYK	QSHSALTAFQ	TEQIQDSEHS	GKMVAKRQFR	IGDIAGEHTS
yeast-10	.....	.....	.....	.....	A.....	.....	.....
yeast-27	.....	.....	.....	.....	.....	.....	.....
yeast-12	.....	.....	.....	.....	.....	......W...	.....
yeast-18	..E.....	.....	.....	.....	.G.....	.....	.....
yeast-15	.....	.....	.....	.....	.....	.....	.....
yeast-01	.....	.....	.....	.....	.....	.....	.....
yeast-06	.....	.....	.....	.....	.....	......W...	.....
yeast-26	.....	.....	.....	.....	.....	.....	.....
yeast-20	.....	.....	.....	.....	.....	.....	.....
yeast-08	.....	.....	.....	.....	.....	.....	.....
yeast-23	.....	.....	.....	.....	.....	.....	.....
yeast-24	.....	.....	.....	.....	.....	.....	.....
yeast-22	.....	.....	.....	.....	.....	.....	.....

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fHbp ID1 wildtype	150 | FDKLPEGGRA	160 | TYRGTAFGSD	170 | DAGGKLTYTI	180 | DFAAKQGNGK	190 | IEHLKSPELN	200 | VDLAAADIKP	210 | DGKRHAVISG
yeast-10	....	....	....	....	....	....	....
yeast-27	....	....	....	....	....	....	....
yeast-12	....	....	....	....	....	....	....
yeast-18	....	....	....	....	....	....	....
yeast-15	....	....	....	....	....	....	....
yeast-01	....	....	....	....	....	....	....
yeast-06	....	....	....	....	....	....	....
yeast-26	....	....	....	....	....	....	....
yeast-20	....	....	....	....	....	....	....
yeast-08	....	....	....	....	....	....	....
yeast-23	....	....	....N.....	....	....	....	....
yeast-24	....	....	....	....	....	....	....
yeast-22	....	....	....	....	....	....	....

fHbp ID1 wildtype	220 | SVLYNQAEKG	230 | SYSLGIFGGK	240 | AQEVAGSAEV	250 | KTVNGIRHIG	255 | LAAKQ		
yeast-10	....	....	....	....	.....		
yeast-27	....	....	....	....	.....		
yeast-12	....	....	....	....	.....		
yeast-18	....	....	....	....	.....		
yeast-15	....	....	....	....	.....		
yeast-01	T.........	....	....	E.........	.....		
yeast-06	....	....	....	....	...X.		
yeast-26	....	....	....	....	.....		
yeast-20	....	....	....	....	.....		
yeast-08	....	....	....	....	.....		
yeast-23	....	....	....	....	.....		
yeast-24	....	....	....	......S...	.....		
yeast-22	....	....	....	....	.....		

Legend to Figure S2. Representative alignments of 13 amino acid sequences inferred from fHbp gene inserts encoding mature proteins of representative fHbp mutants in JAR 41-negative/JAR 3-positive yeast clones. The sequences were selected based on those encoding 1 to 3 amino acid substitutions from that of wild-type fHbp ID 1 (gene encoding fHbp from strain MC58) (Welsch, J. A., R. Rossi, M. Comanducci, and D. M. Granoff. 2004. Protective activity of monoclonal antibodies to genome-derived neisserial antigen 1870, a *Neisseria meningitidis* candidate vaccine. J Immunol 172:5606-5615).

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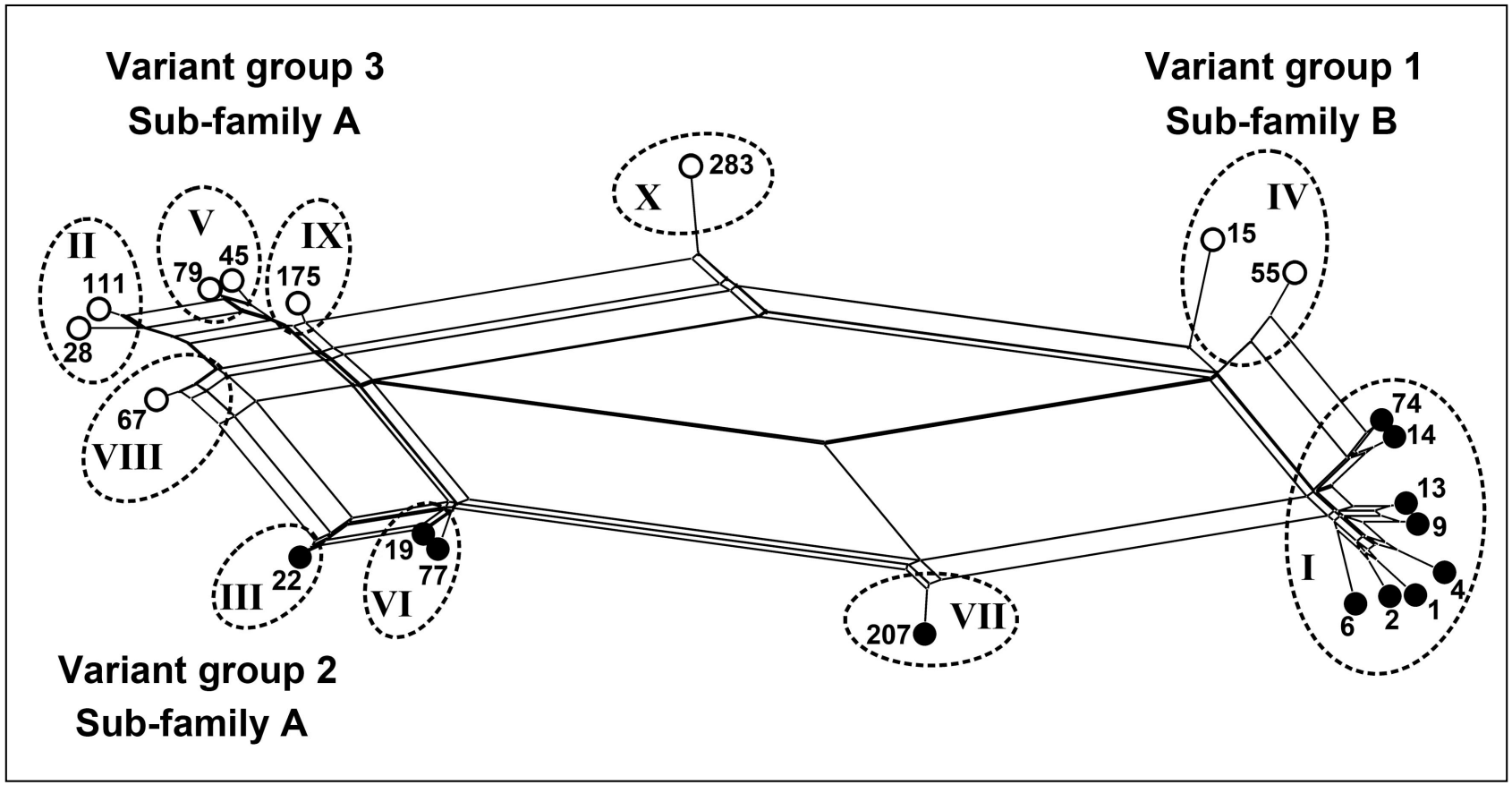
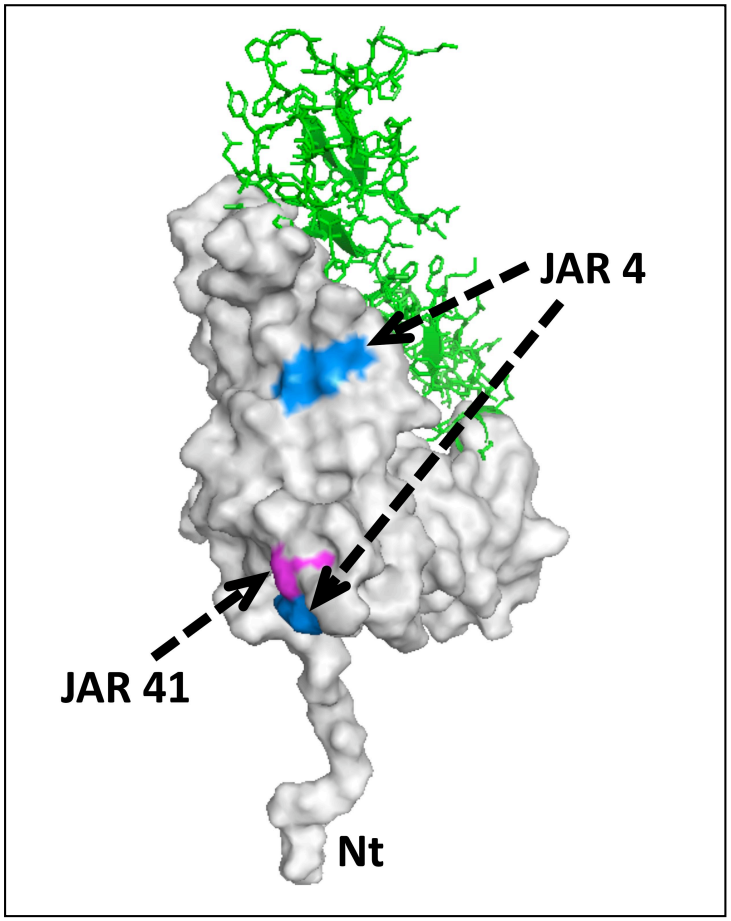
Supplementary Figure S3.



Supplementary Figure S3. Binding of JAR 4 to fHbp amino acid sequence variants. JAR 4 was tested for binding with 21 unique fHbp amino acid sequence variants. Filled circles, fHbp sequence variants that bound to JAR 4. Open circles, fHbp sequence variants that did not bind JAR 4. The variants are shown based on relatedness of their amino acid sequences using a network analysis as presented in Figure 1 of the text. All fHbp variants that bound JAR 4 had "A" segments derived from lineage 1 (formerly referred to as alpha lineage). All fHbp variants negative for JAR 4 binding had "A" segments derived from lineage 2 (formerly referred to as beta lineage). (Beernink, P. T., and D. M. Granoff. 2009. The modular architecture of meningococcal factor H-binding protein. Microbiology 155:2873-2883).

Supplementary Figure S4.



Legend to Figure S4. Cartoon illustrating locations of the fHbp amino acid residues important for binding of anti-fHbp mAbs JAR 41 and JAR 4. The fHbp molecule is represented by the space filled model and the fH fragment (short consensus repeats 6 and 7) by the stick model (in green) based on reported structural data (Schneider et al, *Neisseria meningitidis* recruits factor H using protein mimicry of host carbohydrates. Nature 2009;458:890-893). Residues YGN (beginning at 57) and K27, shown in blue, were previously identified as affecting JAR 4 binding (Beernink et al, A region of the N-terminal domain of meningococcal factor H binding protein that elicits bactericidal antibody across antigenic variant groups. Molecular Immunology, 2009;46:1647-1653). The D25 residue, which was shown in the present study to affect JAR 41 binding, is shown in purple. Both epitopes are surface-exposed and located on a surface opposite to that of residues in contact with fH. Nt, N-terminal amino acid.