

## *Supplementary Information*

### **Immune evasion of *Borrelia miyamotoi*: CbiA, a novel outer surface protein exhibiting complement binding and inactivating properties**

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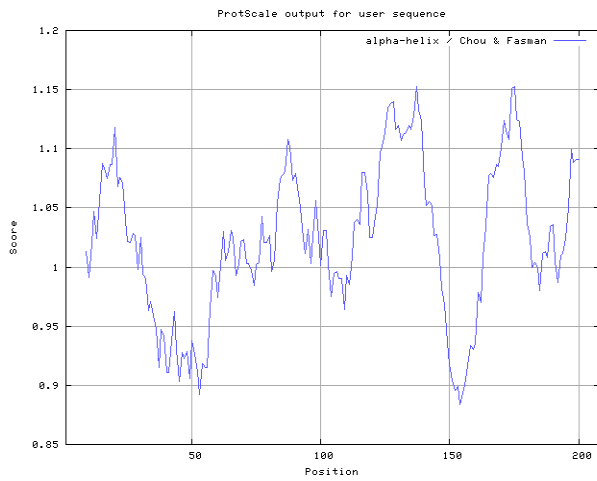
## Supplementary Figure S1

			20		40	
CbiA	MHYIKKYLLF	ILLL-NLINC	NLFNKNKNLD	HNLQPNKINN	IISSLDSNQK	49
BhCRASP-1	MQLTKKYLPA	ILLLLSLASC	DLFYKNRNSN	ANL-----	-LKTLDNNQK	42
BpcA	MK--KKCLLT	ILLL-SLVNC	GLLSKNKNSE	TSL-----	-LNTLDNNQK	39
HcpA	M---RLYLS	MALLLSIIGC	ELLSKNAN--	--LQPKTLET	LESNIDDGQK	42
BtcA	MK--KKCLLT	IILL-SLVSC	GLSSKNKNSE	TSLL-NIETN	LLNTLDDNQK	46
		60		80		100
CbiA	QALISFKNLV	NNKQYSKDLE	QASKSYLEDL	KKNNQDLNLQ	NKLNQELNCD	99
BhCRASP-1	QALIYFKDTL	QDKKYLSYLT	TSQKNFLDDL	EKNKKAPGLQ	YKLKKTLSSE	92
BpcA	QALITFKDLL	QDNHRSILE	KQKKSILKDL	EKHQENSNLQ	DKLKKTLNSE	89
HcpA	QALAFFQESL	QDNKHLDAK	QAQSALLKDI	K-----	DKPQKTLNFK	83
BtcA	QALITFKDLL	QDKKHLSEILE	KQKKSILEDL	KANQKNYNLQ	DKLKKTLNSE	96
		120		140		
CbiA	YDDSKIEKLF	AQLGNDKMKK	FLQQLHLMLK	SINDGTLISF	SSSNFRDTT-	148
BhCRASP-1	YDESQFNKLL	NELGNAKAKQ	FLQQLHIMLQ	SIKDGTLSF	SSANFNDL-Q	141
BpcA	YDKTQLNKLF	DELGNIKTKQ	FLQQLHIMLK	SINNGTLSF	SSSNFKDSNQ	139
HcpA	YNKTKLEQLF	RKLGNDKTKQ	FLKNLHVTIK	FINNGSFQSF	SSANFNDLD-	132
BtcA	YDKNQLNKLF	DELGNIKTKQ	FLQQLHIILQ	SIKD <u>GKPTNF</u>	ASSNFN <u>LNQ</u>	146
		160		180		
CbiA	TLSQKKERAL	EYIKRQLYIE	FYFHSNDISD	TEFFFQRTIK	LLETQS	194
BhCRASP-1	NLEQKKERAL	QSINGELYVE	YYFYINGISN	PDNFFEKIMQ	NLKT--	185
BpcA	TLEQKKEQAL	QYIKGQLYTD	YYLYINGIQD	ANYFFERIMS	VLEI--	183
HcpA	TLEKKQERAI	NSIKEELYVN	YYLYTNELVD	LDYFFYRAMN	YL---S	175
BtcA	TLEQKKEQAL	KYIKDKLYTD	YYLYINGIQD	ANYFFERIMS	LLE---S	190

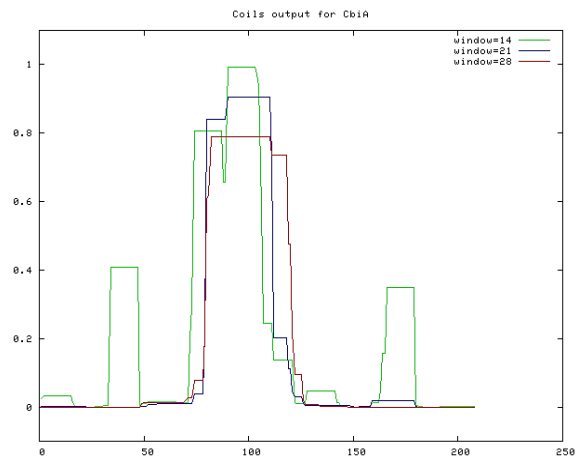
**Alignment of proteins of relapsing fever spirochetes.** Deduced amino acid sequences of CbiA, BhCRASP-1, BpcA, HcpA, and BtcA were aligned using CLC Sequence Viewer, Version 7.7 (Qiagen, Aarhus). Conserved residues are highlighted in light grey and the proposed FH-binding region derived from BhCRASP-1 is underlined. Asterisk indicates the introduced stop codon at position 133 to generate mutant CbiA<sub>20-132</sub> lacking 62 aa at the C-terminus.

# Supplementary Figure S2

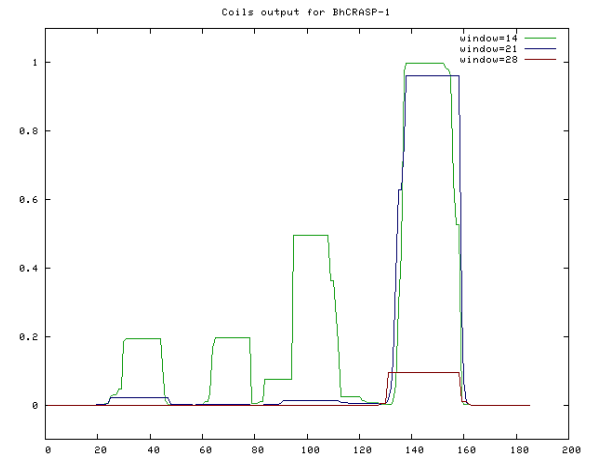
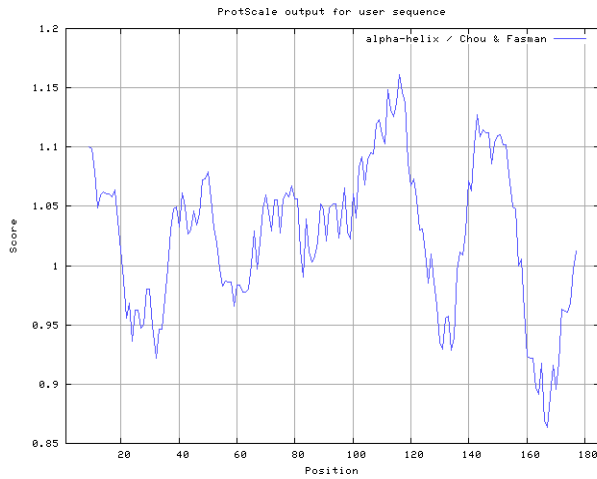
## CbiA alpha helix prediction



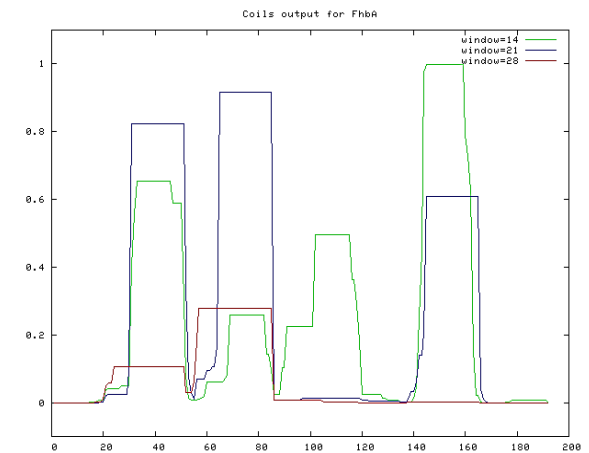
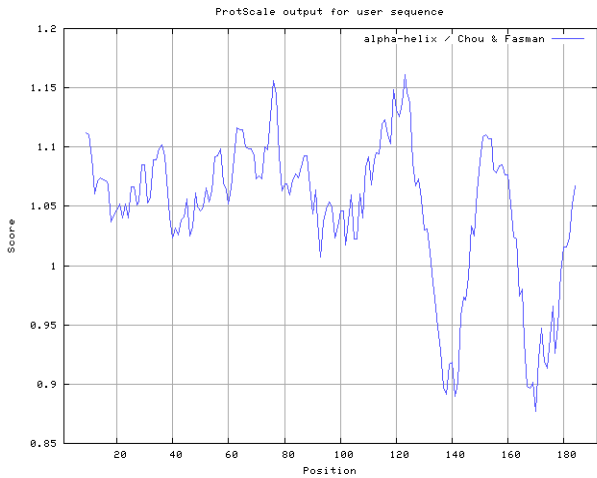
## coiled coil prediction



## BhCRASP-1

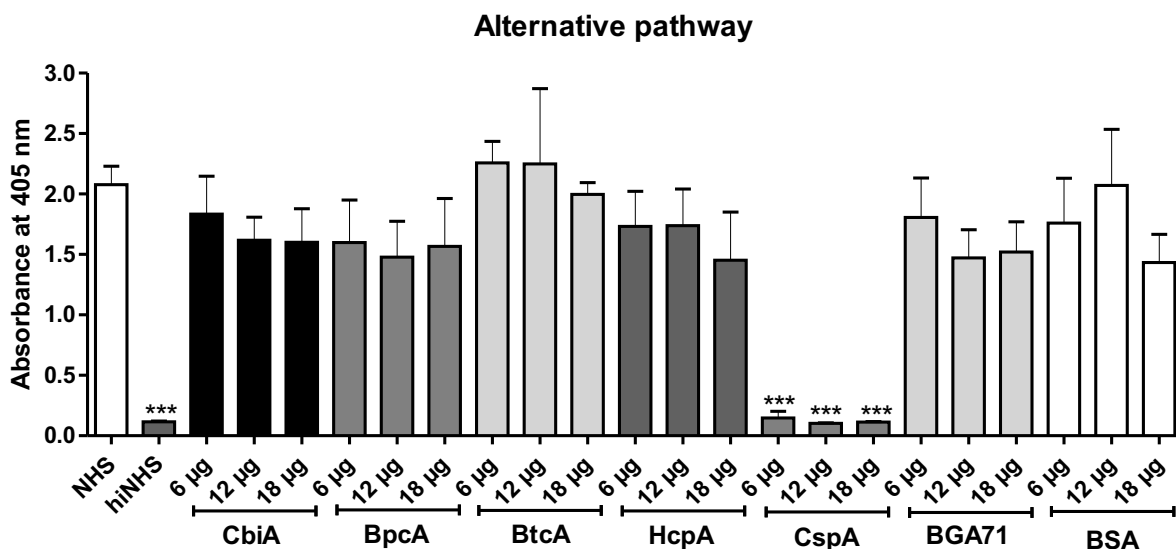


## FhbA



The predicted probability of  $\alpha$ -helices and coiled coils structures within CbiA was assessed using ProtScale and COILS ([www.expasy.org](http://www.expasy.org)). COILS was performed with (2.5) and without weighting by using a 14-, 21-, and 28-amino-acid window.

## Supplementary Figure S3

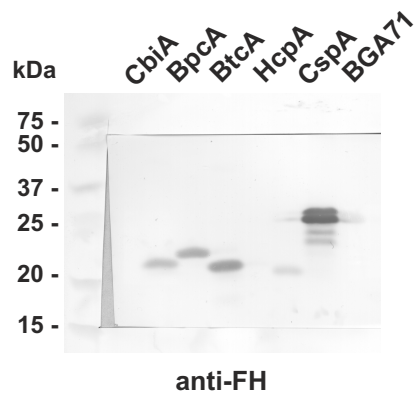
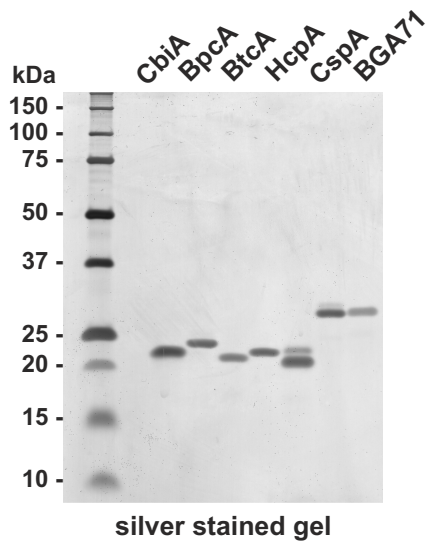


**Complement inhibition of borrelial proteins on the alternative pathway.** The inhibitory capacity of borrelial proteins on the alternative pathway was analyzed by Wielisa® (Euro Diagnostica, Malmö, Sweden). NHS diluted 1:18 was pre-incubated with increasing concentrations (6, 12, 18 µg/ml each) of borrelial proteins or BSA (negative control) and then added to microtiter plates coated with LPS. Following incubation, C5b-9 deposition was measured using an anti-C5b-9 mAb at 405 nm. Means from three independent experiments are shown and error bars correspond to SD. Raw data were analyzed using one-way ANOVA with post hoc Bonferroni correction. \*\*\*,  $P = .001$ , \*\*,  $P = .01$ , \*,  $P = .05$ .

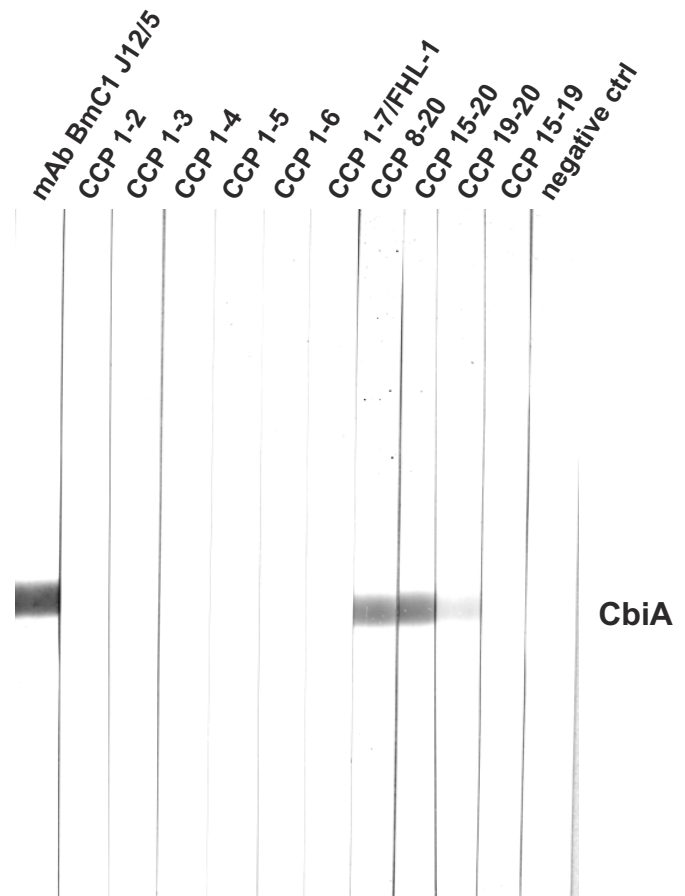
# Supplementary Figure S4

## Uncropped gel and Western blots of figure 1

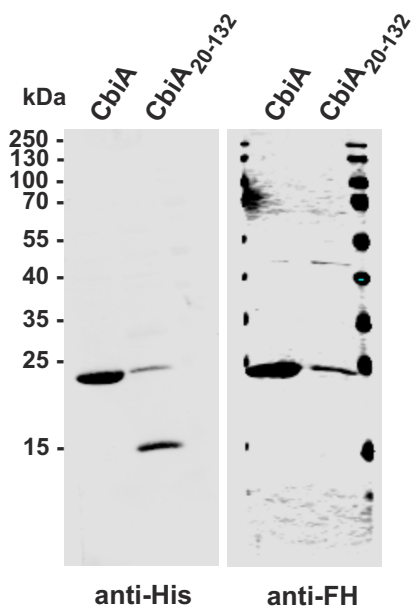
### Panel 1B



### Panel 1D

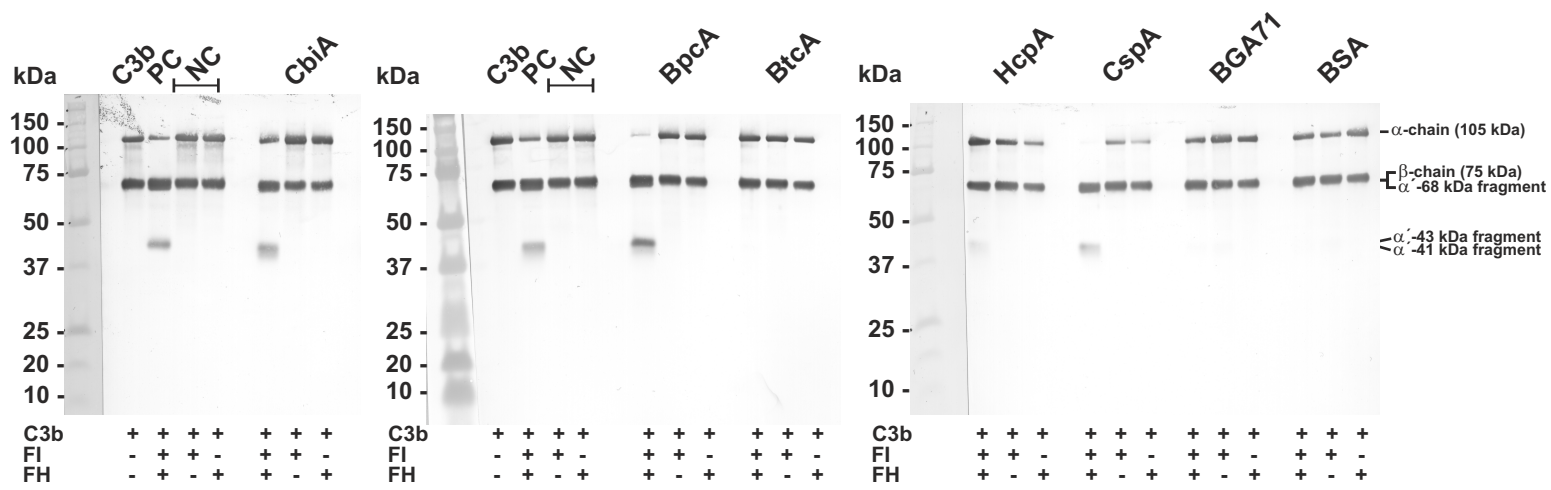


### Panel 1E



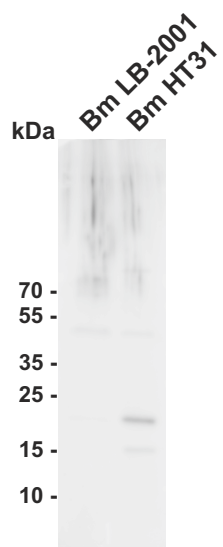
# Supplementary Figure S5

## Full-length Western blots of figure 2



## Supplementary Figure S6

### Full-length Western blot of figure 5, panel B

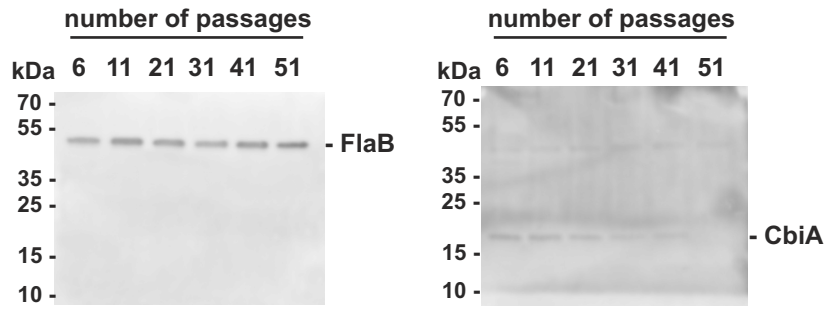






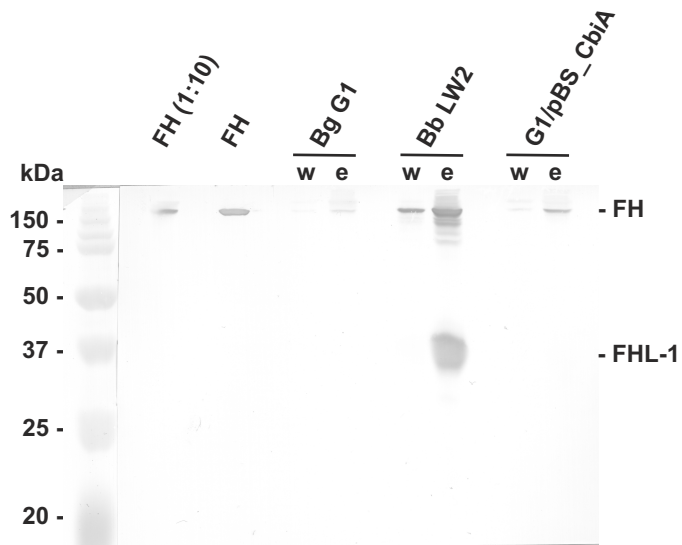
## Supplementary Figure S8

### Full-length Western blots of figure 7, panel B



# Supplementary Figure S9

## Full-length Western blot of figure 8



**Supplementary Table S1. Oligonucleotides used in this study**

Oligonucleotide	Sequence (5'-3') <sup>a</sup>	Use in this work
cbiA F00	ATCTCAATATGCACTATATTTTC	Cloning of <i>cbiA</i>
cbiA R0	CTGCAGTATCGATTTTATATGTA CTCTTC	Cloning of <i>cbiA</i>
cbiA Bam	CTATTATTAACCTTATCGGATCCAATTTATTC	Cloning of <i>cbiA</i> in pQE-30 Xa; generation of His-tagged protein
cbiA Bam	CGAAGGATCCATGCACTATATTA AAAAATATC	Cloning of <i>cbiA</i> in pBSVA
cbiA Sph	CGTTTG CATGCACTCTTTCTCAGAAG	Cloning of <i>cbiA</i> in pBSVA
BtBam	GTCTTGTTGGATCCGGTTTATCAAG	Cloning of <i>btcA</i> in pQE-30 Xa; generation of His-tagged protein
BtSal	TATGTCGACTTAACTTTCTAAAAGTG	
glpQ FW	TCATGCTTTAAACAAGAAATG	qRT-PCR of <i>glpQ</i>
glpQ RV	TCTAGCTCGATTGGGAAATAATTG	qRT-PCR of <i>glpQ</i>
cbiA FW	AGAGCAAGCAAGCAAAAAGTT	qRT-PCR of <i>cbiA</i>
cbiA RV	AAGCTTATAAGTGTGCCGTCATT	qRT-PCR of <i>cbiA</i>

<sup>a</sup>, Sequences of specific restriction endonuclease recognition sites are underlined.