

Supplementary Information

Interaction between *Borrelia miyamotoi* variable major proteins Vlp15/16 and Vlp18 with plasminogen and complement

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Supplementary Figure 1

Vlp15/16 protein of *B. miyamotoi* LB-2001 (WP_025444482.1)

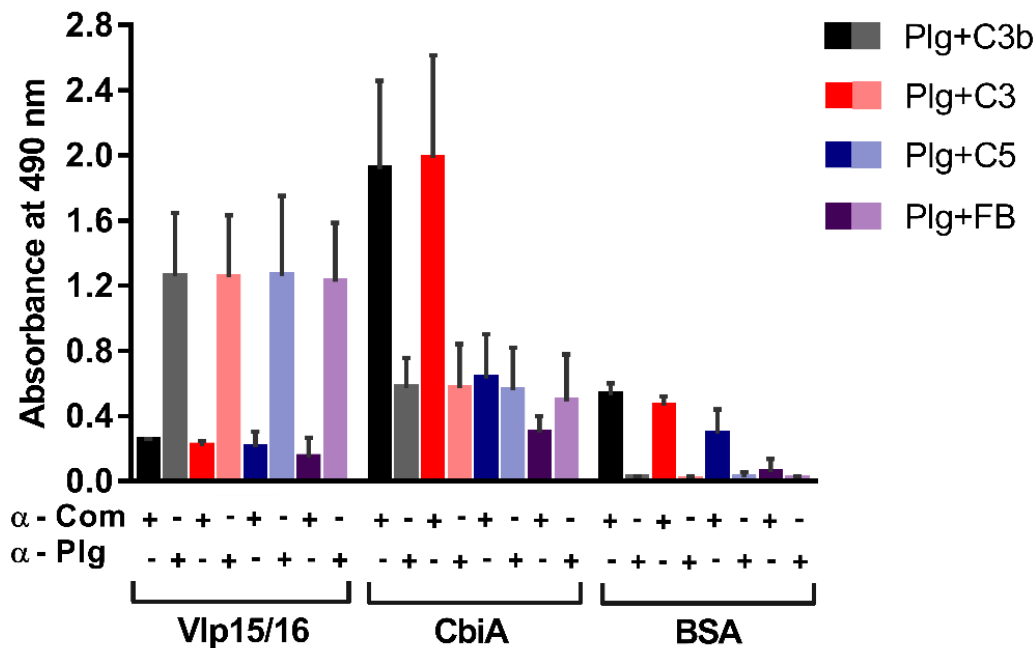
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AAASIGAVTGADVLQAIVQSNENPTADSTNGIEKAKDAAEIAIAPAVNNKKEIK E VSAKKDAIIAAGIALRA
MAKGGKFAANNDKADADAVNGAASAVGKTLSTLIIAVRNTVDSGLKKINEVLATVKQEDKSVEATKTA
EVT TSAQK

The deduced protein sequence of the pQE-30 Xa vector including the hexahistidine tag, the factor Xa splicing site, and the multiple cloning site is highlighted in light blue and the lysine residues in Vlp15/16 are indicated in green. The Vlp15/16 protein consists of 42 lysine residues. The *vlp15/16* gene lacks the N-terminal nucleotides encoding for 20 amino acid residues (MSKRKTL SAIIMTLFLIIGC).

	* * *	* 200	* *	220	* *	240	* * *	260	* * *							
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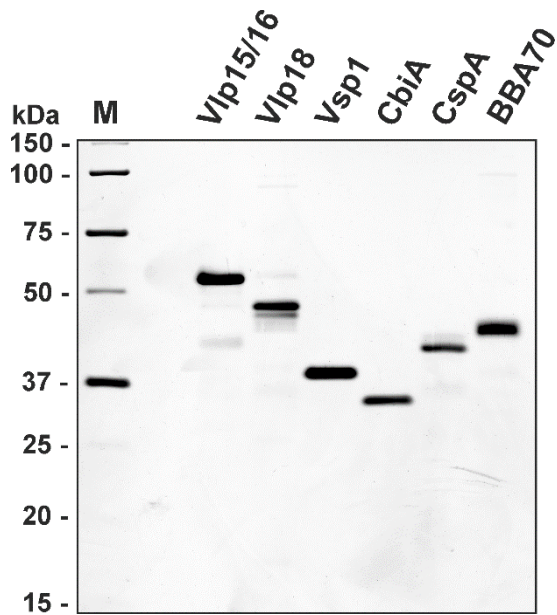
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WP_099497170.1		DA	I	A	A	G	I	A	L	R	A	M	A	K	D	G	K	F	I	V	K	D	T	A	E	K	K	T	E	-	-	A	E	S	A	K	G	V	A	A	S	A	V	G	K	T	L	S	T	L	I	I	A	I	R	D	T	V	D	S	G	L	K	K	I	N	E	A	L	A	T	V	K	Q	E	D	K	S	A	E	A	T	T	K	T	A	E	S	T	A	S	-	-	-	A	Q	Q	-	-	-	357
WP_099591077.1		DA	I	A	A	G	I	A	L	R	A	M	S	K	N	G	K	F	S	I	K	N	N	E	N	E	-	-	A	V	T	T	I	N	S	A	A	A	S	A	V	N	K	T	L	S	T	L	I	I	A	I	R	N	T	V	D	S	G	L	K	K	I	N	E	A	L	A	T	V	K	Q	E	D	K	S	A	E	A	T	T	K	T	A	E	S	T	A	S	A	Q	Q	-	-	-	350					
WP_133017146.1		DA	I	A	A	G	I	A	L	R	A	M	A	K	G	K	F	A	A	N	-	N	N	A	K	-	-	D	A	D	A	V	N	G	V	A	A	S	A	V	G	K	T	L	S	T	L	I	I	A	V	R	N	T	V	D	S	G	L	K	T	I	N	A	A	L	A	T	V	N	Q	E	D	K	S	A	E	V	T	N	A	A	E	V	T	A	S	V	K	N	-	-	-	353							
WP_152301126.1		DA	I	A	A	G	I	A	L	R	A	M	A	K	G	K	F	A	A	N	-	N	N	A	K	-	-	D	A	D	A	V	N	G	V	A	A	S	A	V	G	K	T	L	S	T	L	I	I	A	V	R	N	T	V	D	S	G	L	K	T	I	N	A	A	L	A	T	V	N	Q	E	D	K	S	A	E	V	T	N	A	A	E	V	T	A	S	V	K	N	-	-	-	353							
WP_070401517.1		DA	V	I	A	A	G	I	A	L	R	A	M	A	K	D	G	K	F	A	A	N	-	N	N	A	K	-	-	D	A	D	A	V	N	G	A	A	S	A	V	G	K	T	L	S	T	L	I	I	A	I	R	N	T	V	D	S	G	L	K	K	I	N	E	A	L	A	T	V	K	Q	E	D	K	S	V	E	V	T	K	T	A	E	V	T	S	-	-	-	347										
WP_070401566.1		D	V	V	I	A	A	G	I	A	L	R	A	M	A	K	D	G	K	F	A	A	K	S	N	E	E	K	-	-	S	A	H	A	V	N	G	V	A	A	S	A	V	G	K	T	L	S	T	L	I	I	A	I	R	N	T	V	D	S	G	L	K	T	I	N	A	S	L	A	T	V	K	Q	E	D	K	S	S	E	A	A	G	S	I	E	T	-	-	-	E</										

Supplementary Figure 3



Binding of complement components to plasminogen bound to Vlp15/16. To assess binding of complement components C3b, C3, C5 and Factor B (FB) to plasminogen bound to Vlp15/16, 96-well microtiter plates (MaxiSorp, Nunc) were coated with 0.1 μ M of Vlp15/16, CbiA (additional control), and BSA (negative control), respectively, in 100 μ l PBS at 4 $^{\circ}$ C overnight. Following blocking with 100 μ l PBS containing 0.2% (w/v) gelatin. For each reaction mixtures, two duplicates were prepared to detect binding of plasminogen (Plg) or binding of complement components (Com) to plasminogen bound to Vlp15/16, CbiA, and BSA, respectively. In addition, four duplicates of each protein were coated to serve as primary antibody controls and where the ligands were omitted. The respective wells were subsequently washed and incubated with 0.1 μ M plasminogen in 100 μ l PBS for 1 h at room temperature. Afterwards, complement components C3b, C3, C5, and FB were added to the respective wells at a concentration of 0.1 μ M in 100 μ l PBS and incubated for 1 h at RT. The wells were then washed twice and binding of the ligands was detected by using a polyclonal antiserum (1:1,000) raised against human plasminogen or C3, C5, and FB, respectively. After incubation with a HRP-conjugated anti-goat antiserum (1:2,000), the plates were washed and developed with *o*-phenylenediamine (Sigma-Aldrich, Steinheim, Germany) for 8 min and the absorbance was measured at 490 nm (PowerWave HT, Bio-Tek Instruments, Winooski, VT, USA). Finally, the values of the antibody controls were subtracted from the values obtained from the reaction mixtures containing plasminogen and complement components. Data represent means and standard deviation of at least two independent experiments, each conducted in duplicate. For visualization of the data, GraphPad Prism 7 was conducted.

Supplementary Figure 4



Characterization of purified His₆-tagged proteins by silver staining. Purified Vlp15/16, Vlp18, Vsp1, CbiA, CspA, and BBA70 (1 μ g each) were separated by a 10% Tris/Tricine-SDS-gel and stained with silver nitrate. The molecular weight markers (M) Precision Plus Protein Standards, Bio-Rad) are indicated on the left. The gel was scanned using a GS-900 Imaging Densitometer (Bio-Rad) and for image processing the Image Lab software, Version 6.1.0 (Bio-Rad) was applied. The figure shows the original version of the scanned gel. The general settings were as follows: Application: silver gel; Filter setting: Silver stain; Resolution: 63.5 * 63.5 microns.

Supplementary table 1. Oligonucleotides used in this study

Oligonucleotide	Sequence (5'-3') ^a	Use in this work
Vlp15/16_FP	GATATACATATGGCTGGATCCAATAATGGA GGAGGGGAAG	Recloning of <i>vlp15/16</i> into pQE-30Xa vector
Vlp15/16_RP	CTGCAGGTCGACTTATTACTTCTGTGCACTA GTTGTTAC	Recloning of <i>vlp15/16</i> into pQE-30Xa vector
Vlp18_FP	CAACAAACAGAGGATCCGTATCAGGAGGA GATAAACAAGGGGTTG	Recloning of <i>vlp18</i> into pQE-30Xa vector
Vlp18_RP	CTGCAGGTCGACTTATTACTCTGCTGTTTTT GAGTTTCTTG	Recloning of <i>vlp18</i> into pQE-30Xa vector
BmVsp1_Bam	CATATGGCTAGCTGTGGATCCGGGGGACCG GCACC	Recloning of <i>vsp1</i> into pQE-30Xa vector
BmVsp1_Sal	GTGGTGGTGGTGGTTCGACCTATTATGAAGA TTGACCAGCAG	Recloning of <i>vsp1</i> into pQE-30Xa vector

^a Sequences of specific restriction endonuclease recognition sites are underlined