Supplementary Materials for

A unique borrelial protein facilitates microbial immune evasion

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4 Supplementary Figures (Figs. S1, S2, S3, and S4) and figure legends

2 Supplementary Tables (Table S1 and S2)

Figure S1

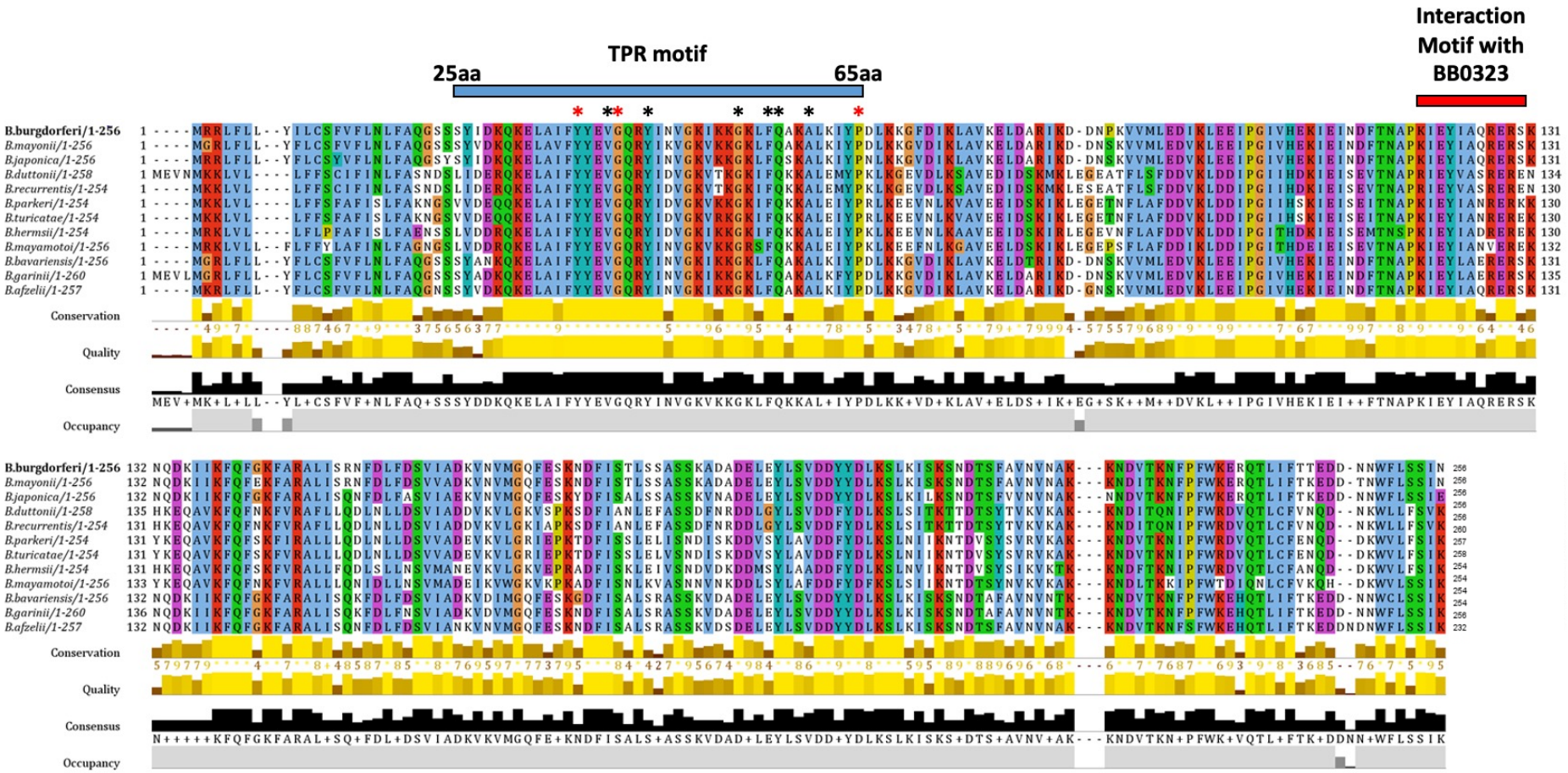
TPR motif
25aa
65aa
Interaction Motif with BB0323
B.burgdorferi/1-256
B.mayonii/1-256
B.japonica/1-256
B.duttonii/1-258
B.recurrentis/1-254
B.parkeri/1-254
B.turicatae/1-254
B.hermsii/1-254
B.mayamotoi/1-256
B.bavariensis/1-256
B.garinii/1-260
B.afzelii/1-257
Conservation
Quality
Consensus
Occupancy

Figure S2

	rBB0238^{WT} (%)	rY37A (%)	P65A (%)
α-helix	32.1	32.1	32.0
Anti-parallel β	9.5	11.0	10.3
parallel β	8.7	8.3	8.5
β turn	17.1	17.5	17.3
Random coil	32.7	30.0	31.5

Figure S3

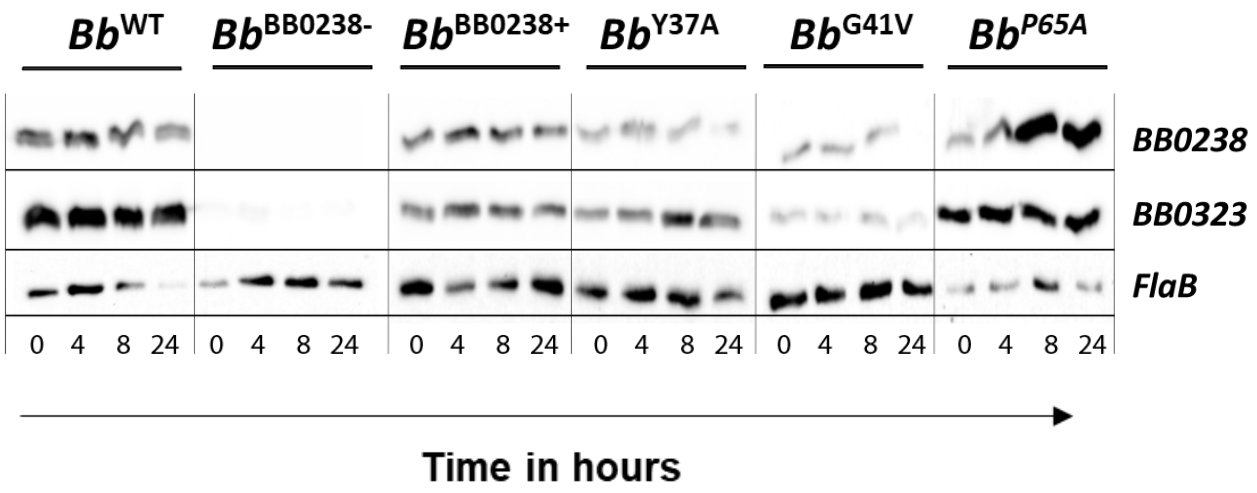
Bb^WT
Bb^BB0238-
Bb^BB0238+
Bb^Y37A
Bb^G41V
Bb^P65A
BB0238
BB0323
FlaB
0 4 8 24 0 4 8 24 0 4 8 24 0 4 8 24 0 4 8 24 0 4 8 24
Time in hours

Figure S4

Culture analysis of wild type and various *bb0238* mutants

Tissue	*Bb*$^{BB0238-}$	*Bb*$^{BB0238+}$	*Bb*^{Y37A}	*Bb*^{G41V}	*Bb*^{P65A}
Joint	0/3	**1/3**	0/3	0/3	0/3
Skin	0/3	**2/3**	0/3	0/3	0/3
Heart	0/3	0/3	0/3	0/3	0/3

Legends to Supplementary Figures

Fig. S1: Conservation of BB0238 amino-acid sequence in various tick-borne borrelial pathogens. Protein alignment of BB0238 among *Borrelia* spp. via NCBI blast of the *B. burgdorferi* B31 protein sequence identified homologs among the Spirochaetaceae order. Sequences used for BB0238 alignment include *B. burgdorferi* B31, *B. burgdorferi* Bol26, *B. mayonii*, *B. japonica*, *B. bavariensis*, *B. garinii* Far04, *B. afzelii* PKo, *B. duttonii* Ly, *B. recurrentis*, *B. parkeri*, *B. turicatae*, *B. hermsii*, *B. miyamotoi*, and *Spirochaeta cellobiosiphila*. Annotated above are the conserved TPR motif (25-65aa) and the interaction motif with BB0323 (120-131aa).

Fig. S2: Circular dichroism analysis. The recombinant wild type and TPR-like motif point mutants, rY37A and P65A, were examined by CD. The secondary structure deconvolution of the CD data (percent of total secondary structure) is displayed as estimated using the CDNN deconvolution program.

Fig. S3: BB0238 stabilization of BB0323 was depicted via a protein turnover assay at 33°C. Protein synthesis was inhibited via the addition of erythromycin to *B. burgdorferi* culture, and collected at 0, 4, 8, and 24 hr time points (depicted left to right in image). Protein was separated via SDS-PAGE and detected using antibody against FlaB (loading control), BB0238, and BB0323. Western images are representative of three individual experiments.

Fig. S4: Culture analysis. The organs collected from *Bb*^{WT} and various *bb0238* TPR-like motif point mutants after transmission from infected ticks. Positive *B. burgdorferi* growth, as visible under darkfield microscopy, was determined within the joint, skin, or heart for all three mice per group after culturing in BSK II media at 34°C for two weeks post-collection.

Table S1: Mass Spectrometry-based identification of BB0238-interacting proteins

The average abundance ratio for *Bb*$^{BB0238-}$: *Bb*^{WT}, from two independent experiments were compared and narrowed to proteins with highest differences between two groups.

Accession Number	**Gene ID**	**Gene Description** [OS=Borrelia burgdorferi (strain ATCC 35210 / B31 / CIP 102532 / DSM 4680)]	**Putative Function**	**Putative Location**	**Average Abundance Ratio: *Bb*$^{BB0238-}$/*Bb*^{WT}**
AAC66635.2	BB_0238	Uncharacterized protein	BB0323 Processing	**Periplasm**	0.007
AAC66700.1	BB_0323	LysM domain protein	Outer Membrane Stability	**Periplasm**	0.164
AAC66497.1	BB_0108	Basic membrane protein	Putative SurA/PrsA Chaperone	**Periplasm**	0.872
AAC66887.1	BB_0518	Chaperone protein DnaK	ATP-dependent Hsp70 protein homolog	Protoplasm	0.316
AAC66919.2	BB_0560	Chaperone protein HtpG	ATP-dependent Hsp90 protein homolog	Protoplasm	0.252
AAC66965.1	BB_0610	Trigger factor	Ribosome associated, ATP-independent chaperone	Protoplasm	0.242
AAC66323.1	BB_B05	Chitibiose transporter protein chbA	Carbohydrate transport system subunit	Plasma membrane	0.338
AAC66627.1	BB_0243	Glycerol-3-phosphate dehydrogenase, anaerobic	Glycolysis; Host-protein interaction/virulence factor	Protoplasm/surface or secreted (moonlighting protein)	0.317
AAC66539.1	BB_0151	N-acetylglucosamine-6-phosphate deacetylase	Biosynthesis pathway of amino-sugar-nucleotides; glycolysis	Protoplasm	0.221
AAC66511.1	BB_0123	30S ribosomal protein S2	Protein synthesis	Protoplasm	0.411
AAC66431.1	BB_0052	tRNA (guanosine(18)-2'-O)-methyltransferase	Protein synthesis	Protoplasm	0.271
AAC66731.1	BB_0355	Transcription factor, putative	CarD-like family transcriptional regulator	Protoplasm	0.397

Table S2: Oligonucleotide primers used in the current study

Primer Name/Purpose	Sequence 5' – 3'
BB0238 protein expression (pET302) forward primer (EcoRI site italicized)	AAT*GAATTC*CAAGGTAGTTCTTCTTATAT
BB0238 protein expression (pET302) reverse primer (XhoI site italicized)	CCG*CTCGAG*TCAATTTATGGAAGACAAAAACCA
G41V Forward Point Mutagenesis of pET302::BB0238	TTTTATTATGAGGTTG**T**TCAAAGATATATAAAC
G41V Reverse Point Mutagenesis of pET302::BB0238	TATATTGATAAGCAAAAAGAGCTTGCTATT
Y37A Forward Point Mutagenesis of pET302::BB0238	CTTGCTATTTTT**GC**TTATGAGGTTGGTCA
Y37A Reverse Point Mutagenesis of pET302::BB0238	CAAGGTAGTTCTTCTTATATTGATAAGCAAAAAGAG
P65A Forward Point Mutagenesis of pET302::BB0238	GCTTTAAAGATTTAT**G**CAGATTTGAAAAAGGGG
P65A Reverse Point Mutagenesis of pET302::BB0238	ATTAAAAAAGGAAAGCTTTTTCAAGCAAAA
OspC Expression Forward Primer (pET28a and pGex 6P-1) (BamHI site italicized)	ATA*GGATCC*TGTAATAATTCAGGGAAAGAT
OspC Expression Reverse Primer (pET28a and pGex 6P-1) (XhoI site italicized)	ATAT*CTCGAG*TTAAGGTTTTTTTGGACTTTC
BB0108 Expression Forward Primer (pET302 and pGex 6P-1) (EcoRI site italicized)	ATAT*GAATTC*TTTTGGGTAATATTGGGA
BB0108 Expression Reverse Primer (pET302 and pGex 6P-1) (XhoI site italicized)	TTAG*CTCGAG*TTATTTTAGACTAGAATC
Forward primer for PCR amplification of $BB0238_{24\text{-}256}$ and $BB0238_{24\text{-}114}$ (NcoI site italicized)	CATG*CCATGG*GCTCTTCTTATATTGATAAG
Reverse primer for PCR amplification of $BB0238_{24\text{-}256}$; $BB0238_{53\text{-}256}$ and $BB0238_{118\text{-}256}$ (NotI site italicized)	GCTT*GCGGCCGC*TTAATTTATGGAAGACAAAAA
Forward primer for PCR amplification of $BB0238_{53\text{-}256}$ (NcoI site italicized)	CATG*CCATGG*GCGGAAAGCTTTTTCAAGCA
Forward primer for PCR amplification of $BB0238_{118\text{-}256}$ (NcoI site italicized)	CATG*CCATGG*GCGCTCCTAAAATAGAATAT
Reverse primer for PCR amplification of $BB0238_{24\text{-}114}$ (NotI site italicized)	GCTT*GCGGCCGC*TTAATCATTGATTTCTATTTT
Forward primer for Site-directed mutagenesis for production of $BB0238_{118\text{-}256}$ Leu240Met	GAACGTCAAACT**ATG**ATTTTTACTACAGAGGATGATAATAAT
Reverse primer for Site-directed mutagenesis for production of $BB0238_{118\text{-}256}$ Leu240Met	TGTAGTAAAAAT**CAT**AGTTTGACGTTCTTTCCAAAATGGAAA