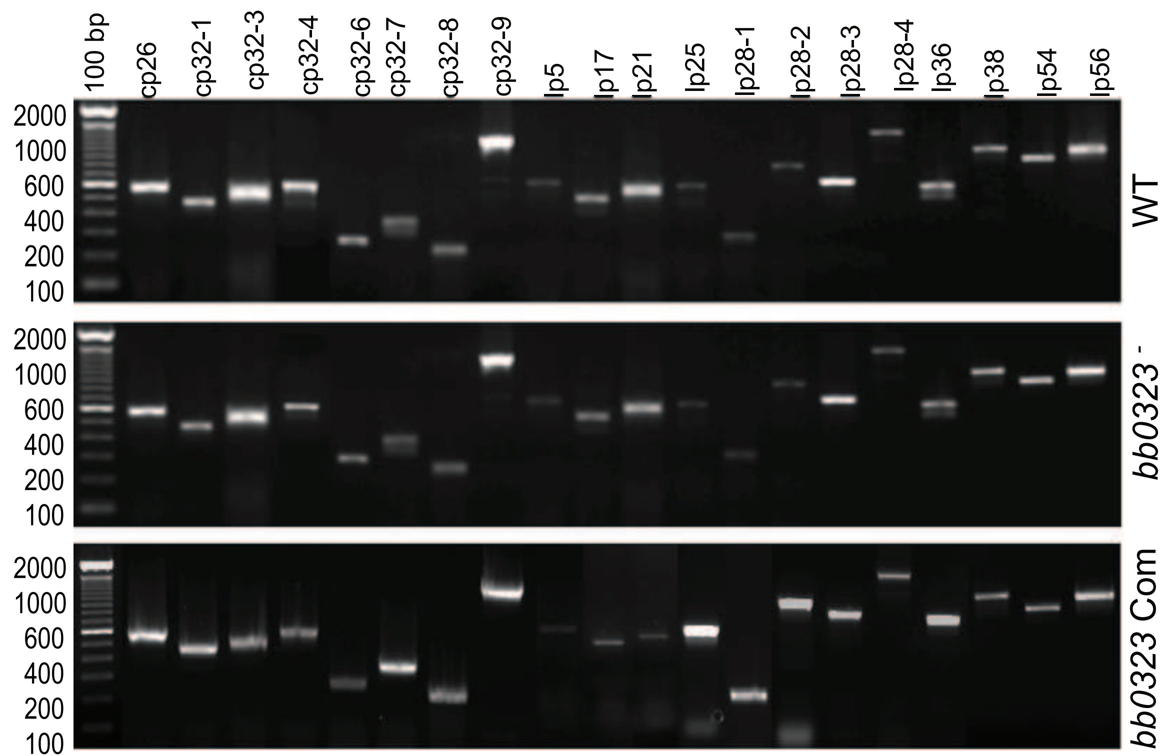
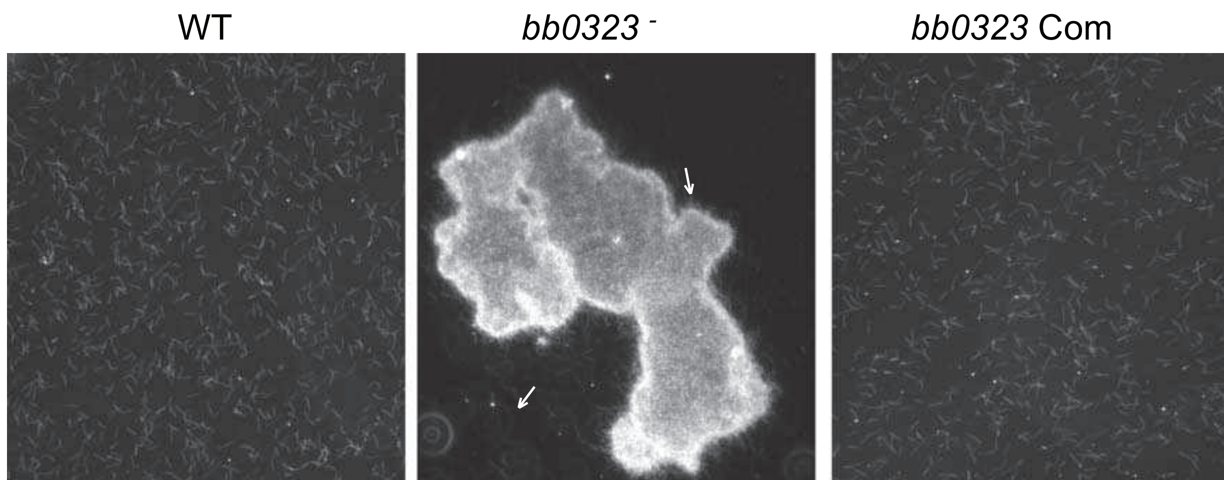
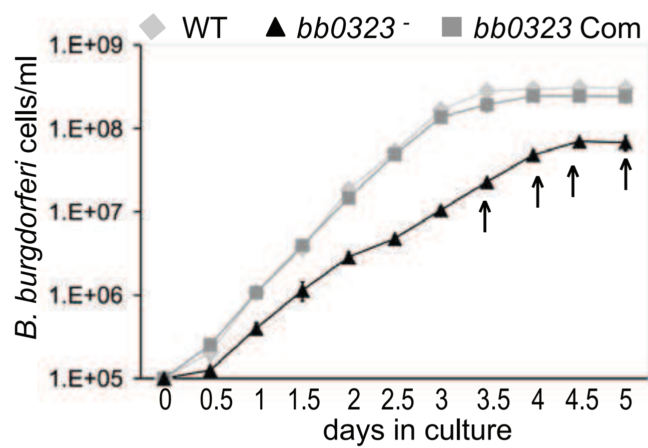
Supplementary Figure 1
Click here to download Figure: SupplementaryFig1.eps
Supplementary Figure 1







Supplementary Table S1. Oligonucleotide primers used in the current study.

Sequence (5'→ 3')	Purpose
TTGCTGATCAAGCTCAATATAACCA	Forward primer for Quantitative PCR of *B. burgdorferi flaB*
TTGAGACCCTGAAAGTGATGC	Reverse primer for Quantitative PCR of *B. burgdorferi flaB*
AGAGGGAAATCGTGCGTGAC	Forward primer for Quantitative PCR of mouse *β-actin*
CAATAGTGATGACCTGGCCGT	Reverse primer for Quantitative PCR of mouse *β-actin*
GGTATCGTGCTCGACTC	Forward primer for Quantitative PCR of tick *β-actin*
ATCAGGTAGTCGGTCAGG	Reverse primer for Quantitative PCR of tick *β-actin*
TT*GAGCTC*ATTTGAATTTTCAAAGAATATACCATG	Primer P1, 5' PCR of the left arm for constructing *bb0323*– mutant. A *SacI* site (italicized) is attached for cloning.
TT*CTGCAG*AAATACACCCTTCTAATAACTTGGCTT	Primer P2, 3' PCR of the left arm for constructing *bb0323*– mutant. A *PstI* site (italicized) is attached for cloning.
TT*GCTAGC*GCAAAAACAATCATAAAGCAAGG	Primer P3, 5' PCR of the left arm for constructing *bb0323*– mutant. A *NheI* site (italicized) is attached for cloning.
TTCTCGAGATTTAAGCGAATCAGCCAAGA	Primer P4, 3' PCR of the left arm for constructing *bb0323*– mutant. A *XhoI* site (italicized) is attached for cloning.
AAAATTTTGGCAAAAAATTCTCAA	Primer P5, upstream 5' PCR primer for the detection of intended integration of *pflaB-Kan* cassette in *bb0323* locus
AATCCATTTAGGCATTTCCA	Primer P6, reverse primer for RT-PCR of *bb0324* and downstream 3' PCR primer for the detection of intended integration of *pflaB-Kan* cassette in *bb0323* genomic *locus*
ATGAGCCATATTCAACGGGAAA	Primer P7, 5' PCR primer for the detection of intended integration of *pFlab-Kan* cassette in *bb0323 locus*
ATTCCGACTCGTCCAACATC	Primer P8, 3' PCR primer for the detection of intended integration of *pFlab-Kan* cassette in *bb0323* genomic *locus*
TA*GAATTC*AAAACGCCTCCAGAATCAAG	Primer P9, forward primer for recombinant BB0323 production. An *EcoR*I site (italicized) is attached for cloning.
TT*CTCGAG*TTATTTGGCAGGAATTATTATCTTCC	Primer P10, reverse primer for recombinant BB0323 production. An *Xho*I site (italicized) is attached for cloning.
ATATGGATCCCGCTGGAAAT	Forward primer for RT-PCR of *bb0323*
AGCCGCTTCAAGTGCTTTTA	Reverse primer for RT-PCR of *bb0323*
TTCCCAATACCAGTCTTTTGC	Forward primer for RT-PCR of *bb0322*
CTTCCAGGCTCCATTTTGAA	Reverse primer for RT-PCR of *bb0322*
TGATTCAAAATTTCGCTCACA	Forward primer for RT-PCR of *bb0324*
TT*CTGCAG*ACTGCCACTTGAGAAAATA	Forward primer for constructing the *bb0323* complemented isolate. A *PstI* site (italicized) is attached for the purpose of cloning into pKFSS1.
TT*GTCGAC*TTATTTGGCAGGAATTATTATC	Reverse primer for constructing the *bb0323* complemented isolate. A *SalI* site (italicized) is attached for the purpose of cloning into pKFSS1.

Supplementary text

Materials and Methods

Genetic manipulation of B. burgdorferi

The *bb0323* mutant was constructed by replacing the *bb0323* open reading frame (ORF) with a kanamycin-resistance cassette via homologous recombination. First, the 5' and 3' arms flanking *bb0323* ORF were amplified using primers P1-P4, then cloned into multiple-cloning sites flanking the kanAn cassette in plasmid pXLF10601 and electroporated into *B. burgdorferi*. Twelve clones that grew in antibiotic-containing media were analyzed using primers P5-P10 to confirm the replacement of the *bb0323* ORF with the kanAn cassette.

Genetic complementation of the *bb0323* mutant was achieved by re-insertion of a wild type copy of the *bb0323* gene in the *B. burgdorferi* chromosome as described [1]. To accomplish this, a DNA fragment of *bb0323* encompassing the putative native promoter was PCR-amplified using primers (supplementary Table 1) and cloned into the *PstI* and *SalI* sites of pKFSS1 (pKFSS1-bb0323), which houses a streptomycin-resistance cassette *(aadA)* under *B. burgdorferi flgB* promoter [2]. A DNA element containing *bb0323* and the *aadA* cassette was further cut with *SalI* and *XmaI* from pKFSS1-bb0323 and inserted into the plasmid pXLF14301 that contained the required 5' and 3' arms for homologous recombination in the *B. burgdorferi* chromosomal locus *bb0444-0446*. The final construct was sequenced to confirm identity, and 20µg of the recombinant plasmid

was electroporated into the *bb0323* mutant. As *B. burgdorferi* mutants grow slower than the parental isolate and form larger clumps (supplementary figure S1*B* and *C*), transformation of the mutant was technically challenging. *bb0323* mutants were harvested at mid-log growth phase ($5x10^6$ cells/ml), and after repeated transformations, three clones were isolated. One of the *bb0323*-complemented clones was selected based on the intended recombination event and the expression of *bb0323* mRNA and BB0323 protein. Analysis of plasmid profile of the *bb0323*-complemented clone indicated that the isolate had lost the lp28-1 plasmid, which was reinserted into the complemented isolate using a wild type copy of the lp28-1 plasmid carrying gentamycin-resistance cassette as detailed [3]. Final analysis of wild type, *bb0323* mutant and *bb0323*-complemented isolates indicated containment of the same set of plasmids (supplementary figure S1*A*), which were used for further study.

Preparation of outer membrane vesicles and Proteinase K accessibility assay

Outer membrane (OM) vesicles were prepared as described [4]. Briefly, two liters of *B. burgdorferi* were grown in BSK-H medium to late log phase, and outer membrane vesicles and protoplasmic cylinders were separated by sequential ultracentrifugation in sucrose gradients, pelleted, resuspended in phosphate buffered saline pH 7.4, and stored in -80°C until use. One µg of OM proteins were separated on 12% SDS-PAAGE, stained with SYPRO Ruby, whereas 0.5 µg of OM proteins were used for immunoblotting using either anti-BB0323, OspA and FlaB antibodies as described [5]. BB0323 antiserum was used in a Proteinase K accessibility assay to

determine surface exposure of the BB0323 as described earlier [5].

Evaluation of arthritis

Ankle joints of each mouse were measured using a precision metric caliper and development of swelling was monitored weekly. For histology, at least 5 ankle joints from each group of mice (5 animals/group) were collected and fixed in 10% formalin, decalcified and processed for H&E staining. Twenty randomly-chosen sections from each of the mouse groups were assessed for histopathological comparisons.

Bioinformatics

Predictions of BB0323 signal peptide and LysM domains were performed using LipoP 1.0 [6] and Pfam [7] bioinformatic tools, respectively. Multiple sequence alignment was achieved using ClustalW alignment (DNASTAR Inc). Gene/protein annotations listed in the current study are provided according to TIGR/JCVI microbial genome database (www. tigr.org): *Borrelia burgdorferi* (BB_0323), *B. afzelii* (BAPKO_0332), *B. garinii* (BG0324), *Neisseria meningitidis* (NMB_0109), *Xylella fastidiosa* (XF0925), *Photobacterium profundum* (PBPRA3578), *Xanthomonas campestris* (XC_3820), *Shewanella oneidensis* (SO_0033).

References

1. Li X, Pal U, Ramamoorthi N, et al. The Lyme disease agent *Borrelia burgdorferi* requires BB0690, a Dps homologue, to persist within ticks. Mol Microbiol **2007**;63(3):694-710.

2. Frank KL, Bundle SF, Kresge ME, Eggers CE and Samuels DS. aadA Confers Streptomycin Resistance in *Borrelia burgdorferi*. J Bacteriol **2003**;185:6723-6727.

3. Grimm D, Eggers CH, Caimano MJ, et al. Experimental assessment of the roles of linear plasmids lp25 and lp28-1 of *Borrelia burgdorferi* throughout the infectious cycle. Infect Immun **2004**;72:5938-46.

4. Skare JT, Shang ES, Foley DM, et al. Virulent strain associated outer membrane proteins of *Borrelia burgdorferi*. J Clin Invest **1995**;96:2380-92.

5. Coleman AS, Yang X, Kumar M, et al. *Borrelia burgdorferi* complement regulator-acquiring surface protein 2 does not contribute to complement resistance or host infectivity. PLoS ONE **2008**;3:3010e.

6. Juncker AS, Willenbrock H, Von Heijne G, Brunak S, Nielsen H and Krogh A. Prediction of lipoprotein signal peptides in Gram-negative bacteria. Protein Sci **2003**;12:1652-62.

7. Finn RD, Tate J, Mistry J, et al. The Pfam protein families database. Nucleic Acids Res **2008**;36:D281-8.