Legends to Supplementary Figures

Figure S1. Construction and analysis of *bb0405*-mutant *Borrelia burgdorferi*. (**A**) Schematic of the wild-type (WT) and *bb0405*-mutant (Mut1) isolates at the *bb0405* locus. Genes *bb0403–bb0407* (white box arrows) and kanamycin-resistance cassette driven by the *B. burgdorferi flaB* promoter *(flaB-Kan,* gray box arrow) are indicated. Primers P1–4 (black arrowheads) were used to amplify 5′ and 3′ arms for homologous recombination and ligated on either side of the *flaB-Kan* cassette as detailed in the text. (**B**) Desired insertion of the mutagenic construct, *flaB-Kan,* in the *bb0405* genomic locus. Primers P5–10 (gray arrowheads, positions indicated in (A)) were used for DNA amplification in WT spirochetes or *bb0405* mutants and subjected to gel electrophoresis. The combination of primers used for PCR is indicated at the top, and migration of the DNA ladder is shown on the left. (**C**) RT-PCR analysis of WT and *bb0405* mutant. Total RNA was isolated from WT and *bb0405*-mutant *B. burgdorferi* and converted to cDNA, which was used to amplify regions within *bb0405* and *flaB* and visualized on a gel (upper panel). Polar effects of mutation are indicated in the lower panel. RT-PCR analysis of WT and *bb0405* mutants using primers specific for *bb0404* and *bb0406* indicated comparable levels of expression. (**D**) Protein analyses of WT and *bb0405* mutant. Equal amounts of protein were separated by SDS-PAGE and either stained with Coomassie Brilliant Blue (left) or transferred onto a nitrocellulose membrane and probed with BB0405 and FlaB antibodies (right). Migration of protein marker is shown in kilodaltons (kDa). (**E**) Growth curves for WT and *bb0405* mutants. Two mutant clones (Mut1 and Mut2) and wild-type spirochetes were diluted to 10^5 cells/ml and grown at 34°C in BSK-H media. Triplicate samples were counted under a darkfield microscope using a Petroff-Hausser cell counter.

Figure S2. Comparable plasmid profiles of *bb0405* mutants. PCR analyses of genomic DNA of *bb0405* mutants using plasmid-specific primers as detailed. DNA was isolated from *B. burgdorferi* wild type and two isogenic *bb0405*-mutant clones (Mut1 and Mut2) and used in PCR analysis for detection of endogenous plasmids. Note that a nonessential linear plasmid, lp5, is nearly undetectable in one of the mutant clones (Mut2); otherwise, mutant plasmid profiles are similar to that of wild-type spirochetes (not shown). One-kilobase DNA ladder is shown on the left.

Figure S3. BB0405 immunization does not interfere with *B. burgdorferi* infectivity by needle inoculation. Comparable levels of *B. burgdorferi* in recombinant BB0405-immunized or control mice. Groups of mice (6 animals/group) were immunized with either rBB0405 or PBS (control) mixed with adjuvant and injected with *B. burgdorferi* (10^5 spirochetes/mouse) 10 days after final immunization. Spirochete levels were assessed by measuring copies of *B. burgdorferi flaB* at 10 and 20 days following infection. Amounts of murine *ß-actin* were determined in each sample and used to normalize the quantities of spirochete RNA. Bars represent the mean measurements ± SEM from two quantitative PCR analyses from one infection experiment. Levels of *B. burgdorferi* were similar in BB0405-immunized (gray bars) and control mice (black bars). The burdens found in BB0405-immunized mice were not statistically significant different from the control burdens in any tissue or at any time point ($p>0.05$, $n=2$).

Figure S1

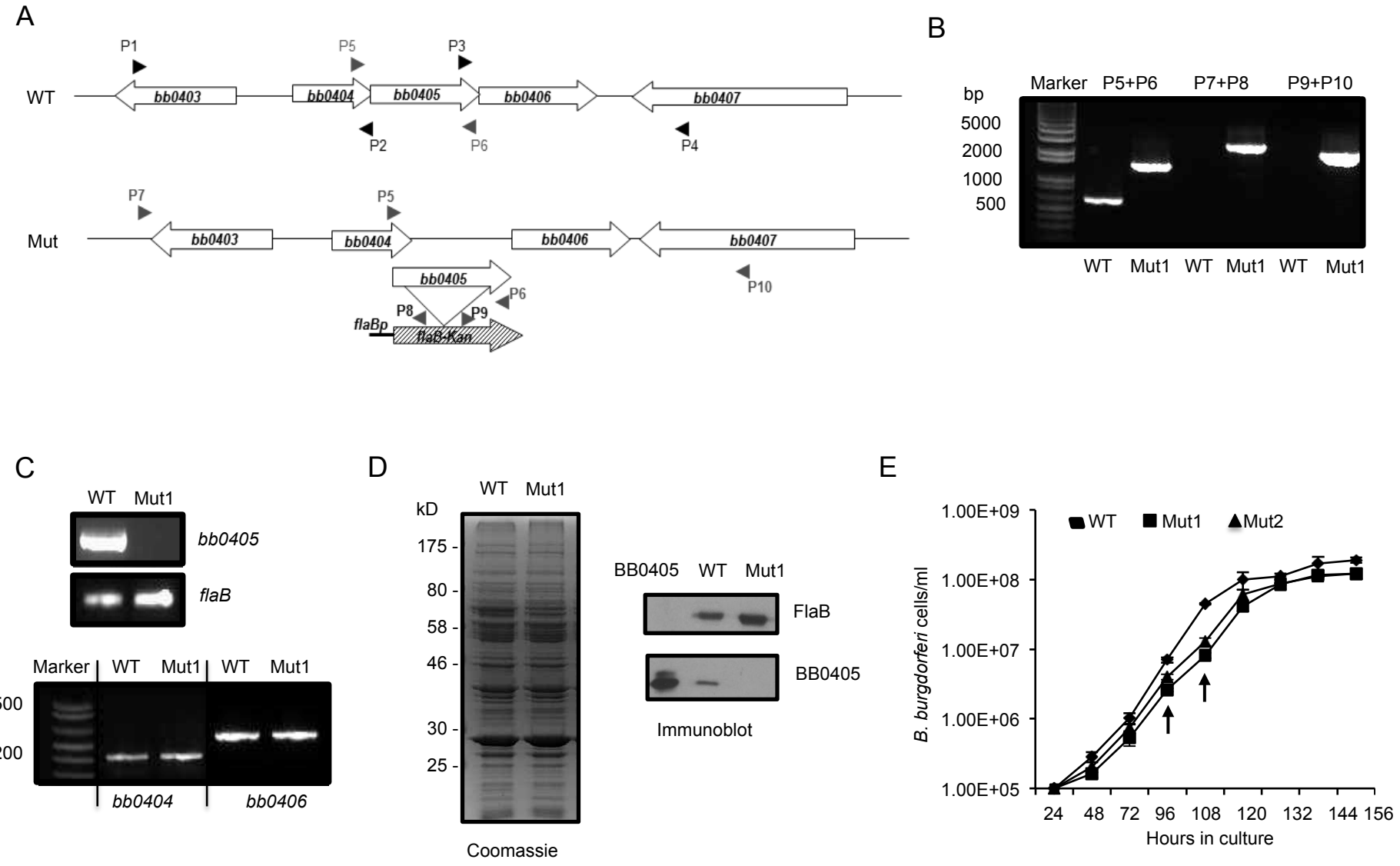
A
P1
P5
P3
WT
bb0403
bb0404
bb0405
bb0406
bb0407
P2
P6
P4
P7
P5
Mut
bb0403
bb0404
bb0406
bb0407
bb0405
P6
P8
P9
P10
flaBp
flaB-Kan
B
bp
Marker
P5+P6
P7+P8
P9+P10
5000
2000
1000
500
WT
Mut1
WT
Mut1
WT
Mut1
C
WT
Mut1
bb0405
flaB
Marker
WT
Mut1
WT
Mut1
500
200
bb0404
bb0406
D
WT
Mut1
kD
175
80
58
46
30
25
Coomassie
BB0405
WT
Mut1
FlaB
BB0405
Immunoblot
E
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
WT
Mut1
Mut2
B. burgdorferi cells/ml
24
48
72
96
108
120
132
144
156
Hours in culture

Figure S2

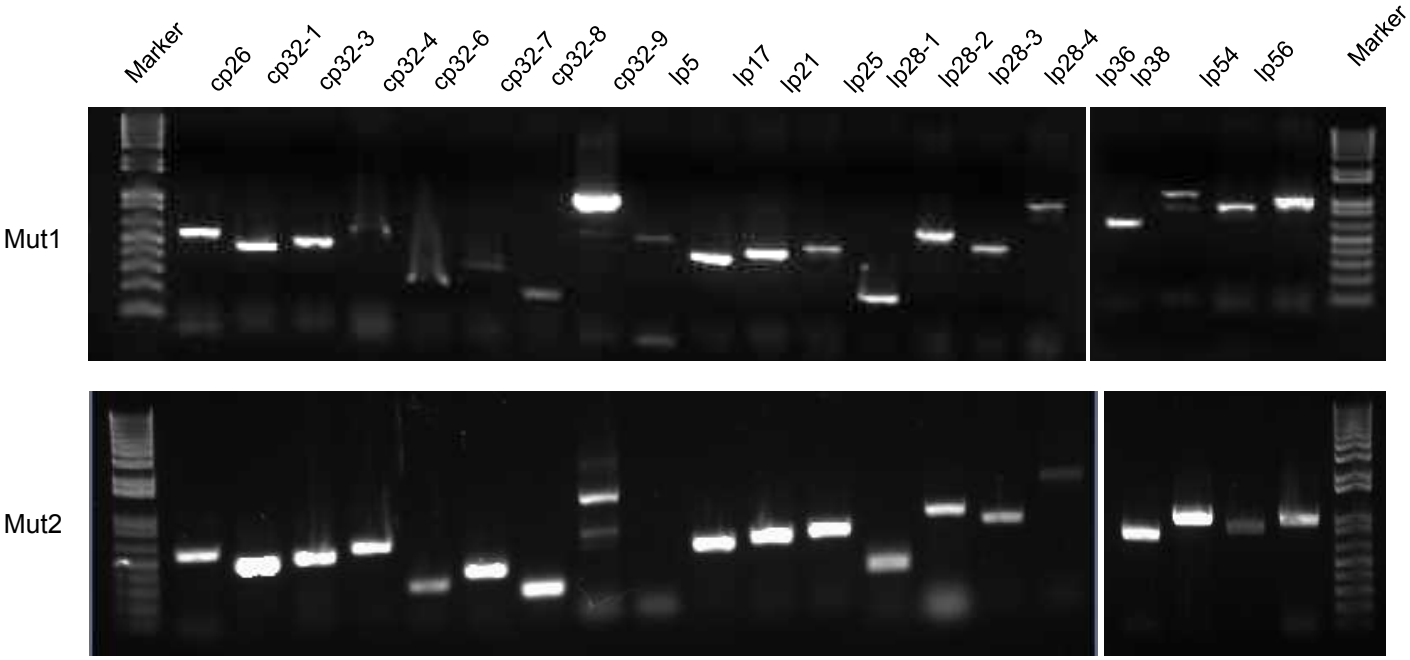
Marker
cp26
cp32-1
cp32-3
cp32-4
cp32-6
cp32-7
cp32-8
cp32-9
lp5
lp17
lp21
lp25
lp28-1
lp28-2
lp28-3
lp28-4
lp36
lp38
lp54
lp56
Marker
Mut1
Mut2

Figure S3

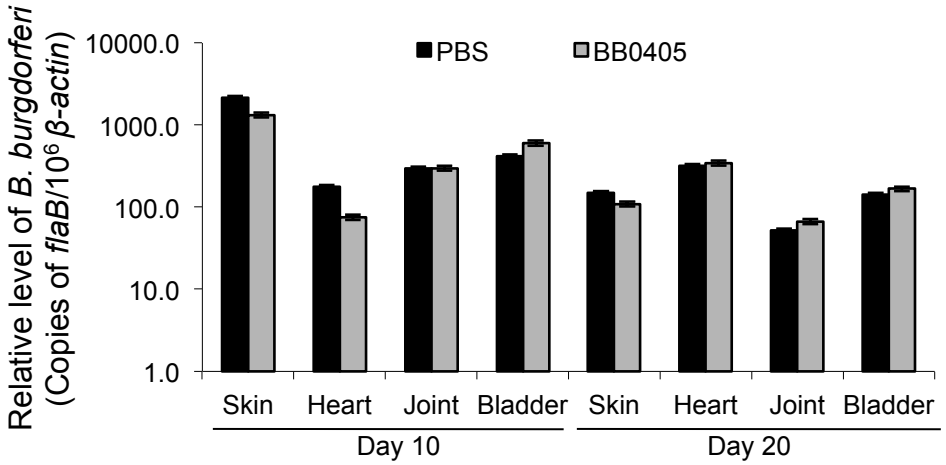
Relative level of B. burgdorferi
(Copies of flaB/10^6 β-actin)
10000.0
1000.0
100.0
10.0
1.0
PBS
BB0405
Skin
Heart
Joint
Bladder
Skin
Heart
Joint
Bladder
Day 10
Day 20

Table S1

Primer	Sequence (5'-3')	Purpose
BB0404F	TCTTCCCAAAAATCCTTTACAAA	RT-PCR forward primer for BB0404-BB0405
BB0405R	ATCCTCCGTACCCAAGGTCT	RT-PCR reverse primer for BB0404-BB0405
BB0405F	ATAGGGGTTGTGATAAGATTGC	RT-PCR forward primer for BB0405-BB0406
BB0406R	CTTTAAGCTTTGCGATACAGG	RT-PCR reverse primer for BB0405-BB0406
FlaBF	GCTCAAATAAGAGGTTTGTC	RT-PCR forward primer for *flaB*
FlaBR	ATTCCAAGCTCTTCAGCTG	RT-PCR reverse primer for *flaB*
FlaBF1	TTGCTGATCAAGCTCAATATAACCA	qRT-PCR forward primer for *flaB*
FlaBR1	TTGAGACCCTGAAAGTGATGC	qRT-PCR reverse primer for *flaB*
AcmF	AGAGGGAAATCGTGCGTGAC	qRT-PCR forward primer for mouse *β-actin*
AcmR	CAATAGTGATGACCTGGCCGT	qRT-PCR reverse primer for mouse *β-actin*
ActF	GGTATCGTGCTCGACTC	qRT-PCR forward primer for tick *β-actin*
ActR	ATCAGGTAGTCGGTCAGG	qRT-PCR reverse primer for tick *β-actin*
BB0405QF	GAGTCTGTGCGCCGTTTATT	qRT-PCR forward primer for *bb0405*
BB0405QR	TTTAAGCCCTACGAATCCTC	qRT-PCR reverse primer for *bb0405*
P1	CGC***GGATCC***TGTAATCAATAACATTCTTTCTA	Forward primer of the left arm for constructing *bb0405* mutant. A *Bam*HI site (bold italicized) is attached for cloning.

P2	AAAA***CTGCAG***CGTTGTTAATATAAGTATTATTGTT	Reverse primer of the left arm for constructing *bb0405* mutant. A *Pst*I site (bold italicized) is attached for cloning.
P3	CC***ATCGAT***GAAGGAGCTAGATTTAATTTC	Forward primer of the right arm for constructing *bb0405* mutant. A *Cla*I site (bold italicized) is attached for cloning.
P4	CCG***CTCGAG***GAGATTGCATTGAGCTTATGA	Reverse primer of the right arm for constructing *bb0405* mutant. A *Xho*I site (bold italicized) is attached for cloning.
P5	ATGAGAATGCTATTAGCAACAAT	*bb0405* forward primer
P6	TATTTTTATAAAGCCTGTGCCTA	*bb0405* reverse primer
P7	CCGTTTATACCTATTTCCATAAG	Upstream forward primer for detection of intended insertion of *pflaB-Kan* cassette into the *bb0405* locus.
P8	CAAATGTATGAACTTGAGAAGAT	Downstream reverse primer for detection of intended insertion of *pflaB-Kan* cassette into the *bb0405* locus.
P9	GGTTGCATTCGATTCCTGTT	Kanamycin internal forward primer
P10	ATTCCGACTCGTCCAACATC	Kanamycin internal reverse primer
BB0405PrF	CGC***GGATCC***TTAAAAGTCTTAGAATGTACTCCAG	Forward primer for amplifying the putative *bb0405* promoter. A *Bam*HI site (bold italicized) is attached for cloning.
BB0405PrR	**TAGCATTCTCAT**AAATTTATCCCAAAAATTTAA	Reverse primer for amplifying the putative *bb0405* promoter. A 12-nucleotide 5' extension (bold) is complementary to the 5’ end of the

		bb0405 ORF.
BB0405FCOM	**TGGGATAAATTT**ATGAGAATGCTATTAGCAACAAT	Forward primer for *bb0405* complementation. A 12-nucleotide 5' extension (bold) is complementary to the 5’ end of the promoter fragment.
BB0405RCOM	ACGC***GTCGAC***CTATATATATATTTTTATAAAGCCTG	Reverse primer used for amplifying the *bb0405* ORF. A *Sal*I site (bold italicized) is attached for cloning.
BB0405F1	CGC***GGATCC***GCACAATCCAAAAGCAAAAGTATG	Forward primer for recombinant BB0405 production in *E. coli.* A *Bam*HI site (bold italicized) is attached for cloning.
BB0405R1	CCG***GAATTC***CTATATATATATTTTTATAAAGCCTG	Reverse primer for recombinant BB0405 production in *E. coli.* An *Eco*RI site (bold italicized) is attached for cloning.