Supplemental Material

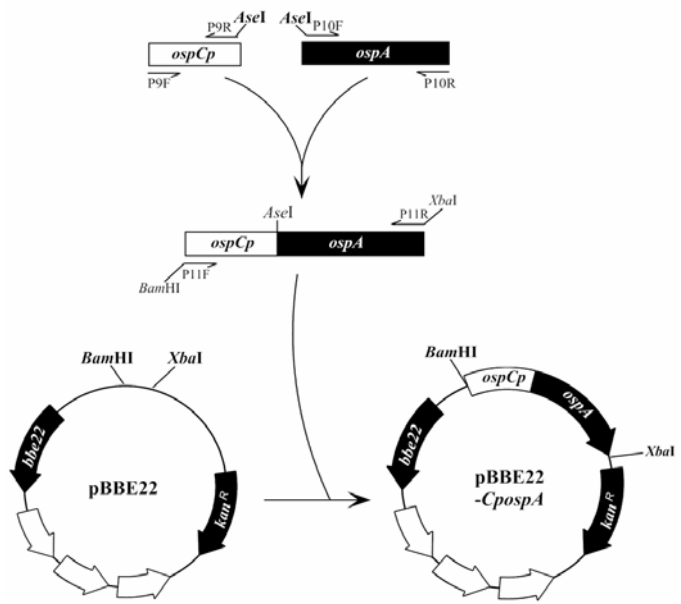

Fig. S1. Construction of pBBE22-*CpospA*.
A 295-bp *ospC* operator-promoter sequence and an 854-bp promoterless *ospA* gene, including the coding region and downstream sequence, were amplified by PCR with use of primers P9F and P9R, and P10F and P10R (Table S1, Supplemental Materials), respectively. Resulting PCR products were pooled, digested with *Ase*I, purified, ligated, and PCR amplified using primers P11F and P11R. The resulting amplicon, designated *CpospA*, was digested with *Bam*HI and *Xba*I, purified and cloned into pBBE22 after the shuttle vector was digested with the same enzymes.

Generation of *B. burgdorferi* with OspA expression under control of the *ospC* regulatory elements. The construct pBBE22-*CpospA* was electroporated into the *ospC* mutant, which was generated and characterized in our previous study (Xu *et al.*, 2007), as described previously (Xu *et al.*, 2005). Fifteen transformants were obtained, and first surveyed for the presence of lp28-1 because this plasmid is essential for persistent infection of immunocompetent hosts (Xu *et al.*, 2005). Eight clones were found containing lp28-1 and thus further analyzed for plasmid content as described previously (Xu *et al.*, 2005). Plasmid analyses identified two clones, namely *ΔospC*/*CpospA*/1 and *ΔospC*/*CpospA*/2; both shared the same plasmid content as the *ospC* mutant, which had lost lp25, lp5, lp21, lp56 and cp9 (Xu *et al.*, 2007).

Construction of disruption plasmid pAKO. To inactivate the *ospAB* locus, a disruption plasmid, namely, pAKO, was first constructed. Briefly, a 4312-bp fragment with introduced *Acc*65I and *Xba*I restriction sites at the ends, consisting of the entire ORFs for *bba11*, *bba12*, *bba13*, *bba14*, *bba15* (*ospA*), *bba16* (*ospB*) and *bba17*, was PCR amplified using the primers P12F and P12R (Fig. S2A; Table S1). The PCR product was digested with the restriction enzymes *Acc65*I and *Xba*I, purified using the QIAquick PCR purification kit

(QIAGEN Inc., Valencia, CA), and cloned into the vector pNCO1T as described previously (Shi *et al.*, 2006), creating pNCO1TA. A fragment was subsequently amplified from pNCO1TA with use of primers P13F and P13R. A gentamycin cassette (*aacC1*) was amplified from the vector pBSV2G (a gift from P. Rosa and P. Stewart) with the use of primers P15F and P15R (Fig. S1B; Table S1), which confers gentamycin resistance both in *E. coli* and *B. burgdorferi* (Elias *et al.*, 2003). The two amplicons were purified, digested with *Bam*HI and *Nco*I, and ligated, completing the construction of pAKO.

Generation of *ospAB* mutant. To disrupt the *ospAB* locus, 10 µg of pAKO DNA was electroporated into the 13A spirochetes as described previously (Xu *et al.*, 2007). The clone 13A, which was derived from the *B. burgdorferi* B31 clone 5A13, had been used in our previous study because of its high transformability (Xu *et al.*, 2007). It harbors 19 of the 21 plasmids; its improved transformability is due to the lack of both lp25 and lp56, two plasmids that may carry restriction enzymes (Kawabata *et al.*, 2004; Lawrenz *et al.*, 2002). Gentamycin-resistant clones were screened as described previously (Xu *et al.*, 2007); the insertion of the *aacC1*cassette into the *ospAB* locus was confirmed by PCR using primers P16F and P16R specific for the cassette, and P14F and P14R unique for the *ospA* gene (Table S1). The lack of OspA expression was verified using immunobloting probed with a mixture of FlaB and OspA monoclonal antibodies (MAbs), as described previously (Xu *et al.*, 2007). One *ospAB* mutant, designated Δ*ospA*, was identified; plasmid content was analyzed as described previously (Xu *et al.*, 2005). The mutant lost cp9, lp5, lp21, lp28-1, in addition to lp25 and lp56. The insertion of the *aacC1* cassette within the *ospAB* locus is diagrammed in Fig. S2B and was confirmed by PCR (Fig. S2C&D); the lack of OspA expression was demonstrated by immunoblotting (Fig. S2E).

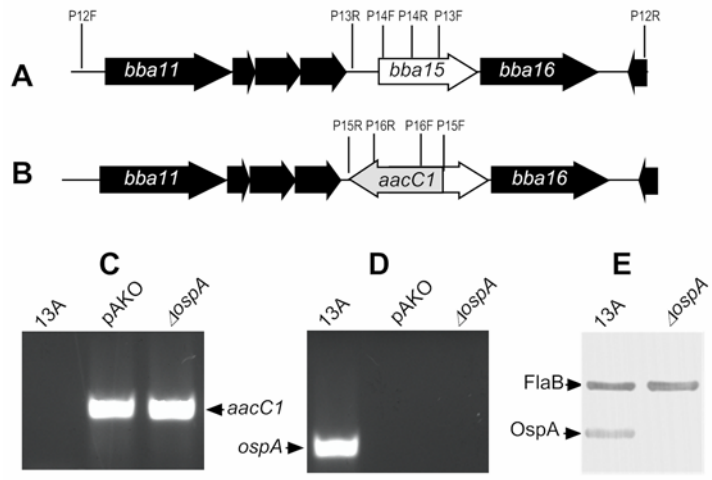

Fig. S2. Generation of *ospA* mutant. (A) Diagram of the *ospAB* locus and adjacent ORFs. The two-gene operon is located within lp54, which carries 76 ORFs, *bba01* to *bba76*. The open arrow and a filled arrow represent *ospA* (*bba15*) and *ospB* (*bba16*), respectively; the remaining filled arrows denote *bba11* to *bba14* and *bba17*. The binding sites of six primers, i.e. P12F to P14F and P12R to P14R, are also indicated. These primers were either used for construction of the disruption plasmid, pAKO, or for mutant identification. (B) Diagram of the disrupted *ospAB* locus, showing the major portion of *ospA* replaced with a gentamicin-resistant cassette (*aacC1*, grey arrow). The small arrow head donates a residual *ospA* gene, while the *ospB* gene remains intact. The binding sites of primers P15F and P15R, used for detection of the *aacC1* cassette, are also indicated. (C&D) PCR analysis of *ospA* mutant. The 13A spirochetes, pAKO, and *ospA* mutant (Δ*ospA*) were used as DNA sources and subjected to PCR amplification using primers P16F and P16R (C), or primers P14F and P14R (D). (E) Immunoblot analysis of *ospA* mutant. The clone 13A and Δ*ospA* were analyzed by immunoblotting probed with a mixture of FlaB and OspA MAbs.

Table S1. Primers used in this study.[a]

Primer name	Primer sequence (5' to 3')[a]
P1F	AGAAGTACGAAGATAGAGAGAGAAA
P1R	AACACATATGTCATTCCTCCATGATAAA
P2F	CCACATATGAAAAAATATTTATTGGGAAT
P2R	AATCCATGGATCATTCCTCCATGATAAA
P3F	CCTCATATGAAAAAAATTTCAAGTGCAAT
P3R	GCAAATCCTATTTATAATCTCATAAATTCT
P4F	TCCTCATGAATAAGAAAATGAAAATGT
P4R	TCTCTCGACTATTTCCTCAATCATGAG
P5F	ATAGGATCCAAGATAGAGAGAGAAAAGT
P5R	ATCAGCGCAAAAACTGCA
P6R	ACCCTCTAGATT TATAATCTCATAAATTCT
P7R	CATATCTAGATCCTCAATCATGAGGGCATAGTC
P8R	TCTCTAGACGCTCTCTCCTATATTTCT
P9F	TAGTTGGCTATATTGGGATCCAA
P9R	CTCCATTAATTTGTGCCTCCTT
P10F	CCACATTAATGAAAAAATATTTATTGGGAAT
P10R	GCAAATCCTATTTATAATCTCATAAATTCT
P11F	TTGGGATCCAAAATCTAATACAAGT
P11R	ACCCTCTAGATTTATAATCTCATAAATTCT
P12F	TAGGTACCTTCTGCTTACTAGCGTGTA
P12R	AATCTAGAGTTGGGCTTGGGTGAAG
P13F	ATGGATCCAGTTGAACTTAATGACACT
P13R	AACCATGGGACTTTCAGGAAAAAGA
P14F	CAGTAGACAAGCTTGAGCT
P14R	GTGGTTTGACCTAGATCGT
P15F	AACAGGATCCTAGGTAATACCCGAGCTTC
P15R	AACCATGGTCTGACGCTCAGTGGAA
P16F	TCACGGTGTTATGGAAATAG
P16R	GACTGCGAGATCATAGATATAG

a. The underlined sequences are restriction enzyme sites: an *Acc*65I site (P12F), *Ase*I sites (P9R and P10F), *Bam*HI sites (P5F, P11F, P13F, and P15F), a *Bsp*HI site (P4F), *Nco*I sites (P2R, P13R, and P15R), *Nde*I sites (P1R, P2F and P3F), and *Xba*I sites (P6R, P7R, P8R, P11R and P12R).

References

Elias, A.F., Bono, J.L., Kupko, J.J., 3rd, Stewart, P.E., Krum, J.G., and Rosa, P.A. (2003) New antibiotic resistance cassettes suitable for genetic studies in *Borrelia burgdorferi*. *J Mol Microbiol Biotechnol* **6**: 29-40.

Kawabata, H., Norris, S.J., and Watanabe, H. (2004) BBE02 disruption mutants of *Borrelia burgdorferi* B31 have a highly transformable, infectious phenotype. *Infect Immun* **72**: 7147-7154.

Lawrenz, M.B., Kawabata, H., Purser, J.E., and Norris, S.J. (2002) Decreased electroporation efficiency in Borrelia burgdorferi containing linear plasmids lp25 and lp56: impact on transformation of infectious *B. burgdorferi*. *Infect Immun* **70**: 4798-4804.

Shi, Y., Xu, Q., Seemanapalli, S.V., McShan, K., and Liang, F.T. (2006) The *dbpBA* locus of *Borrelia burgdorferi* is not essential for infection of mice. *Infect Immun* **74**: 6509-6512.

Xu, Q., Seemanapalli, S.V., Lomax, L., McShan, K., Li, X., Fikrig, E., and Liang, F.T. (2005) Association of linear plasmid 28-1 with an arthritic phenotype of *Borrelia burgdorferi*. *Infect Immun* **73**: 7208-7215.

Xu, Q., McShan, K., and Liang, F.T. (2007) Identification of an *ospC* operator critical for immune evasion of *Borrelia burgdorferi*. *Mol Microbiol* **64**: 220-231.