Supplemental material

Tables

Table S1. Primers used in the study.

Primer	Sequence (5' → 3')	Purpose
ospC B31-3 F	GGAGGTTTCCATGAAAAAGAATACATTAAGTGCA	construct $flgB_p$-*ospC*
ospC B31-3 R	ATGCTGCAGTTAAGGTTTTTTTGGACTTTCTGC	construct $flgB_p$-*ospC*
*flgB*p F	TGAGGTACCATGTTTAAGGTTT	construct $flgB_p$-*ospC*
*flgB*p R	TTCTTTTTCATGGAAACCTCCCTCAT	construct $flgB_p$-*ospC*
pSEC002-F1	GAGACCACATGGTCCTTCTTGAG	screening for $flgB_p$-*ospC*
pSEC002-R1	GCGATTAAGTTGGGTAACGCC	screening for $flgB_p$-*ospC*
OspC-4kb-5'-EcoRI	AATTGAATTCTTGGAATTAAAGACACATC	construct for pXY302
OspC-4kb-3'-KpnI	AATTGGTACCGAATTATTGTTAAAAATGAT	construct for pXY302
OspC-downstream-BglII-5	CACCAGGAGATCTTACAAGTCCTATTGTGGCAG	construct for pXY302
OspC-upstream-BglII-3	CCAGGAGATCTATTAGTCCAACAATTTTGTTTTTC	construct for pXY302
$_p$flgB-Sp/SmR-BglII-5	CTAGATCTCGAGCTTCAAGGAAGA	aadA1 for pXY302
Sp/SmR-BglII-3	CAAGATCTATTATTTGCCGACTACC	aadA1 for pXY302
flaB-XF F	GCTCCTTCCTGTTGAACACCC	qPCR
flaB-XF R	CTTTTCTCTGGTGAGGGAGCTC	qPCR
Nidogen F	CCAGCCACAGAATCACATCC	qPCR
Nidogen R	GGACATACTCTGCTGCCATC	qPCR

FIGURE LEGENDS

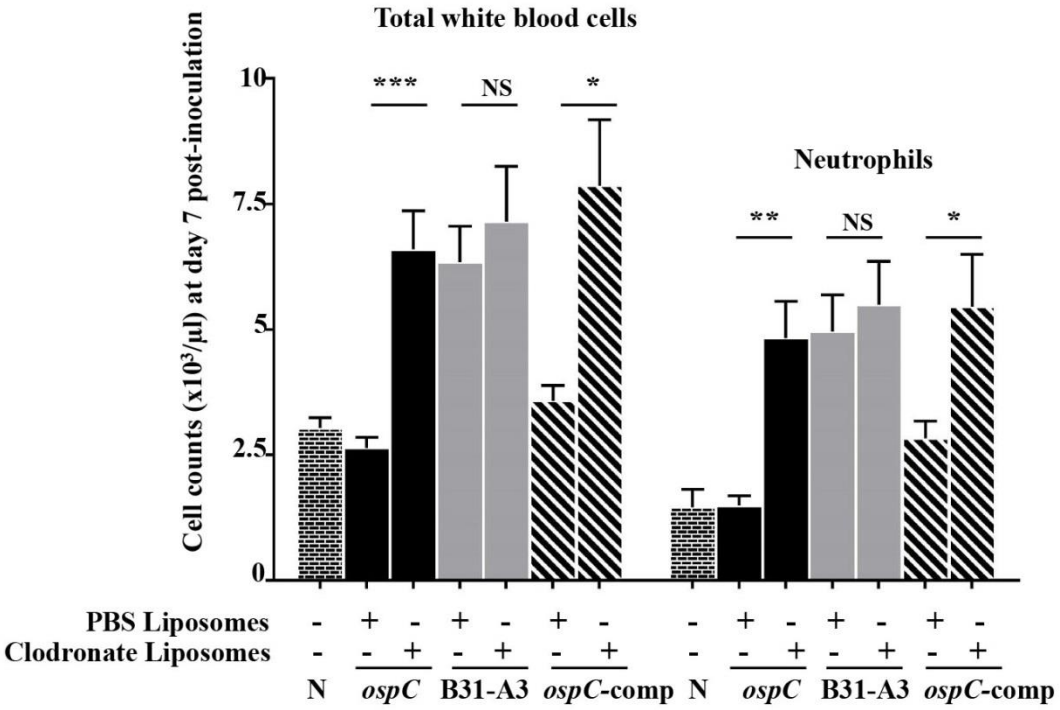

Figure S1. Levels of neutrophils are increased in the circulation when *B. burgdorferi* strains have established infection in SCID mice treated with clodronate or PBS liposomes. Peripheral blood was collected at day 7 post-challenge in an EDTA-coated tubes and was analyzed with a HEMAVET 950 multispecies hematology cell counter. Normal reference values of total white blood cells and neutrophils in mice are ≤ 10.7 and ≤ 2.5 (x 10^3/ul), respectively. Data are representative of two separate experiments and each bar represent mean ± SEM for seven to ten mice per treatment group. Total white blood cell and neutrophil counts from 3 naïve (N) age-matched SCID mice are shown as a control group. Comparisons of experimental groups were performed with Student's *t*- test with Welch's correction (*, $P < 0.05$; **, $P < 0.005$; ***, $P \leq 0.0005$; NS, not significant).

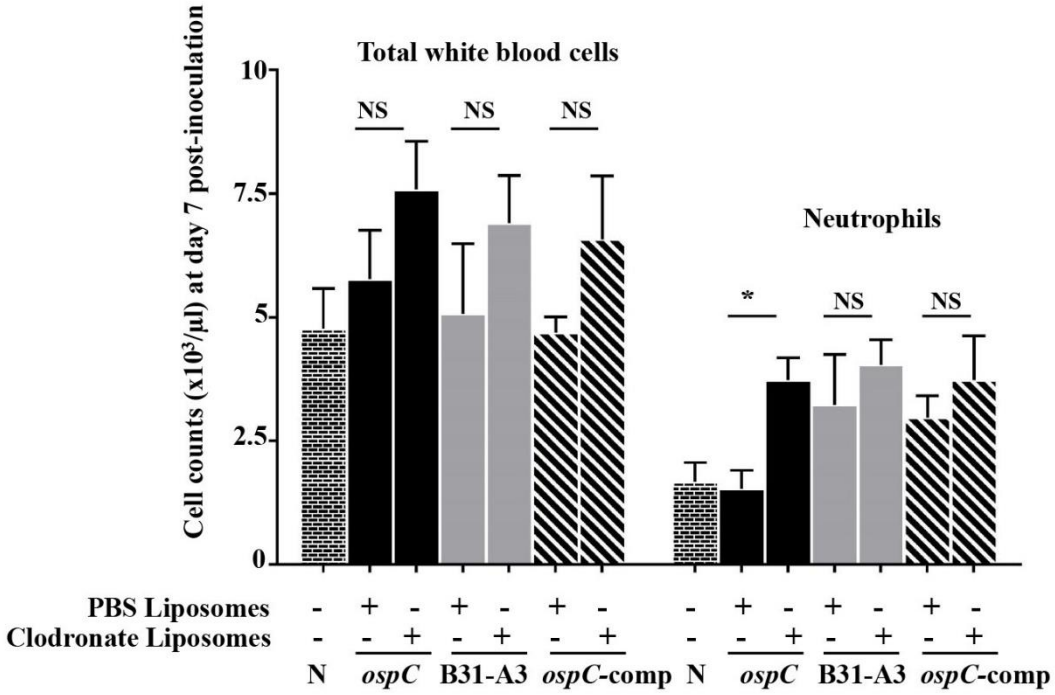

Figure S2. Levels of neutrophils are increased in the circulation when *B. burgdorferi* strains have established infection in C3H/HeN mice treated with clodronate or PBS liposomes. Peripheral blood was collected at day 7 post-challenge in an EDTA-coated tubes and was analyzed with a HEMAVET 950 multispecies hematology cell counter. Normal reference values of total white blood cells and neutrophils in mice are ≤ 10.7 and ≤ 2.5 (x 10^3/ul), respectively. Each bar represent mean ± SEM for five to six mice per treatment consition. Total white blood cell and neutrophil counts from 2 naïve (N) age-matched C3H/HeN mice are shown as a control group. Comparisons of experimental groups were performed with Student's *t*- test with Welch's correction (*, $P < 0.05$; NS, not significant).

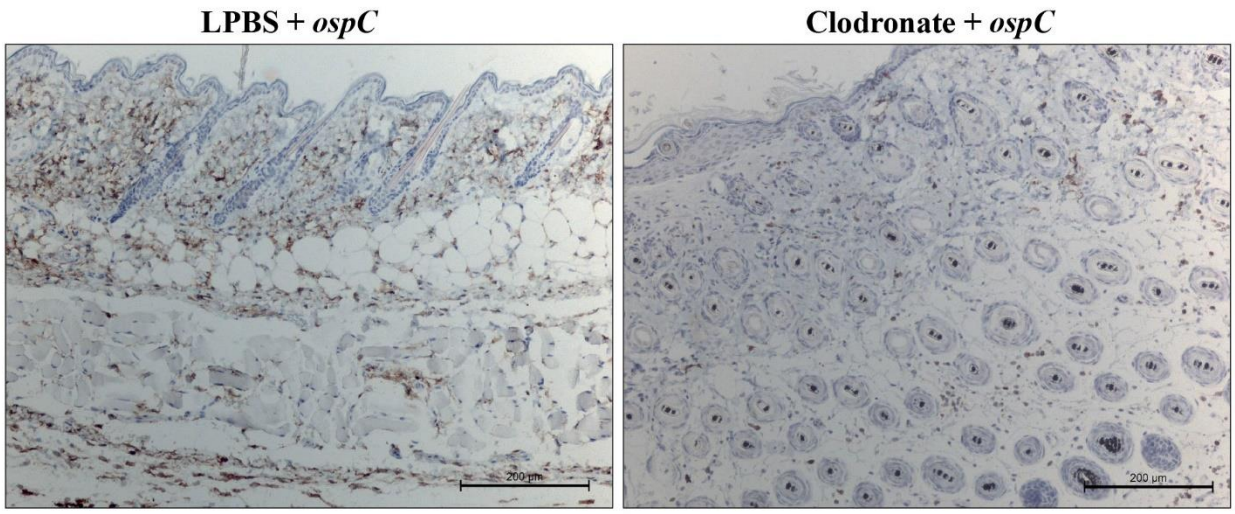

Figure S3. Depletion of phagocytes at the skin site of inoculation in C3H/HeN mice treated with clodronate liposomes. Representative immunohistochemical staining of anti-F4/80-reactive cells (brown staining) in skin sections (scale bar 200µm, 100× magnification) from C3H/HeN mice treated with either PBS liposomes (LPBS) or clodronate liposomes at day 7 after *ospC* mutant challenge. Immunohistochemical staining in clodronate liposome-treated mice was markedly reduced when compared to LPBS-treated animals (control).

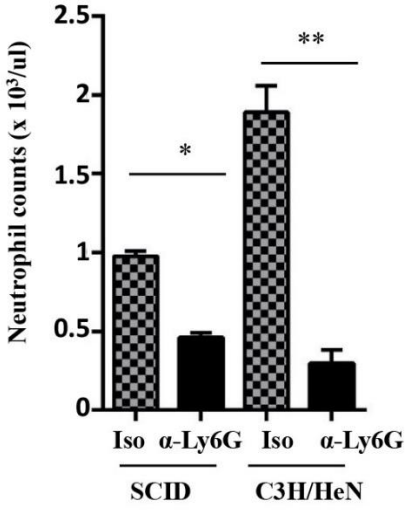

Figure S4. Treatment with anti-Ly6G reduces neutrophils in circulation of SCID and C3H/HeN mice after 48 hrs post-challenge with the *ospC* mutant. Peripheral blood from mice treated with either anti-Ly6G antibody or isotype (Iso) control was analyzed with a HEMAVET 950 multispecies hematology cell counter within 24 hrs after the last antibody treatment. Data represent the mean ± SEM for three mice per treatment group. Comparisons of anti-Ly6G- and isotype-treated groups were performed using unpaired Student's *t* test with Welch's correction (**, $P < 0.001$, * $P < 0.01$).

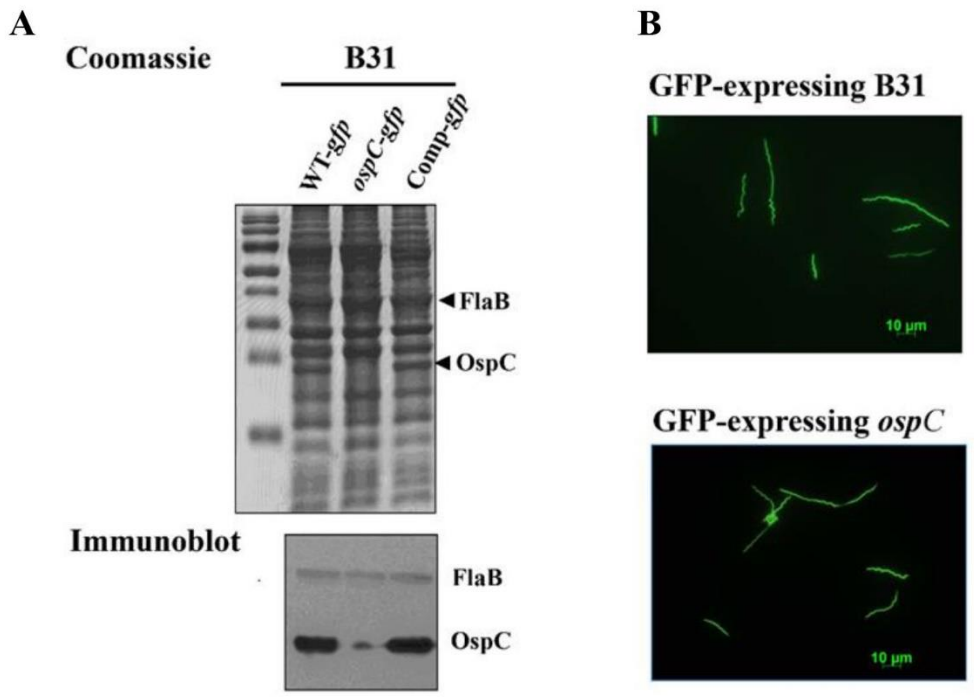

Figure S5. Generation of wild-type, the *ospC* mutant and the complemented strain expressing GFP. **A)** Immunoblot showing the OspC production in GFP-expressing strains of *B. burgdorferi*. Top: Coomassie blue-stained gel. Bottom: Immunoblot probed with antibodies recognizing FlaB (control) and OspC. **B)** Immunofluorescence micrograph showing GFP-expressing wild-type (B31-A3) and the isogenic *ospC* mutant *B. burgdorferi* strain (Scale bar: 10 µm.)

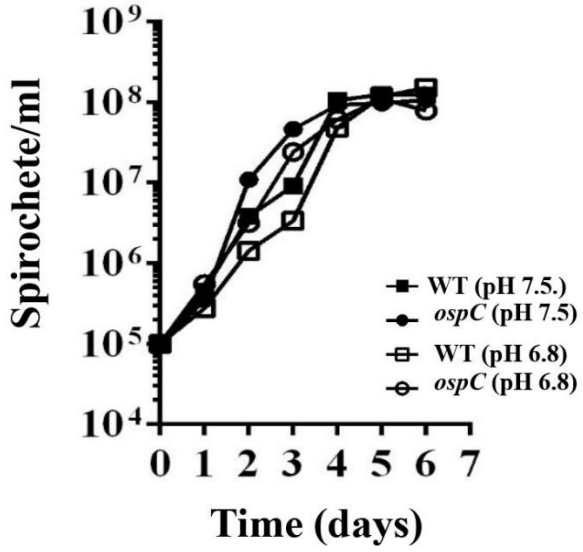

Figure S6. **The GFP-expressing *ospC* mutant exhibited a growth pattern similar to that of wild-type B31 strain when cultivated in standard BSK-II medium. A-B)** Growth curves were measured while cells were growing in BSK-II medium at 37°C and pH 7.5 or pH 6.8. The initial cell density was 3×10^5 spirochetes/ml for each strain. Spirochetes were enumerated daily under dark-field microscopy. Data represent two independent cultures per strain-condition. Each data point is expressed as the mean from twelve independent fields per strain-condition.

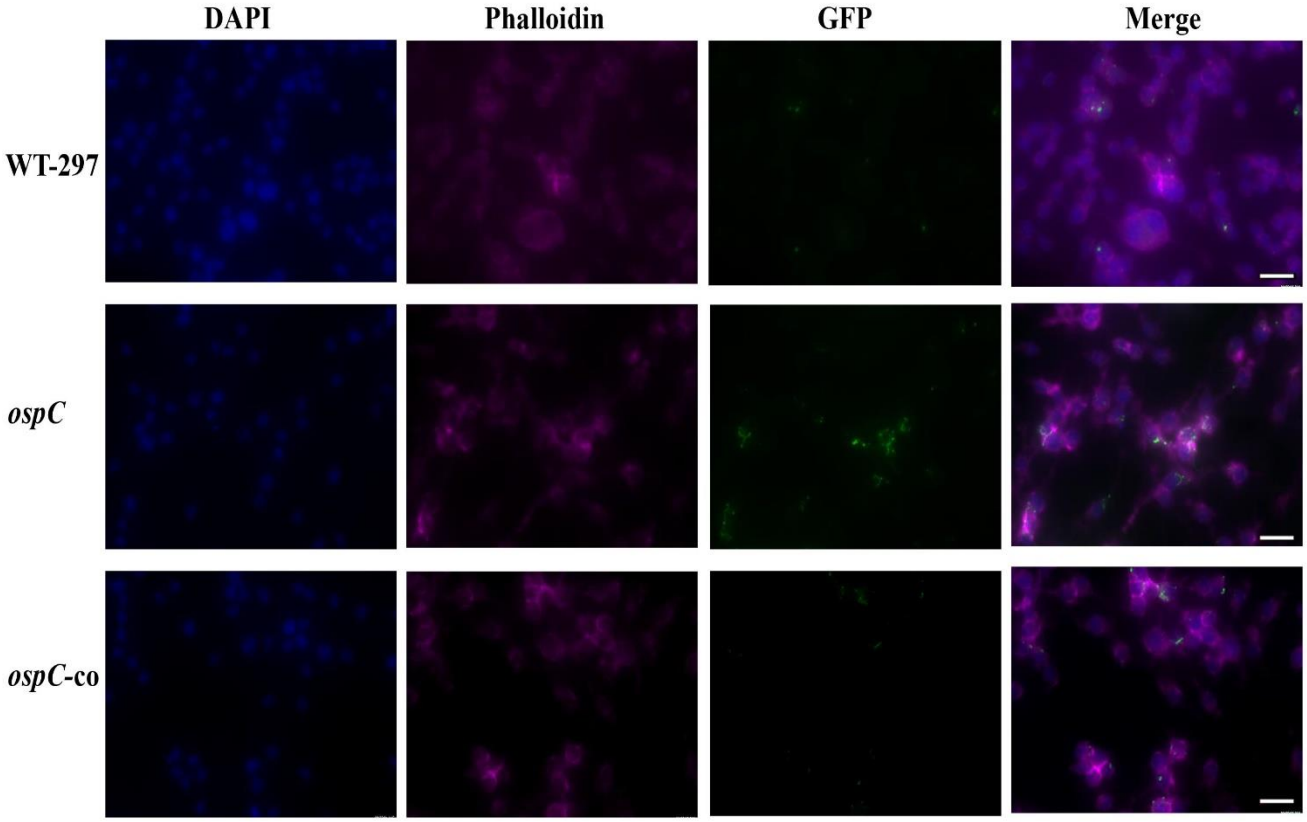

Figure S7. Uptake of the *ospC* mutant by murine peritoneal macrophages (PM) is higher than the wild-type and the *ospC* complemented strain. Immunofluorescence images of murine PMs incubated with GFP-expressing *B. burgdorferi* spirochetes for 2 hrs at 100:1 MOI. Cells were fixed after 2 hrs of spirochete addition and processed for immunofluorescent staining. Cell nuclei (blue, left panels) and F-actin (magenta) were stained with DAPI and phalloidin, respectively. Wild-type (WT), *ospC* mutant, and *ospC* complemented (*ospC*-co) spirochetes (strain 297) express GFP (green). Spirochetes associated to macrophages are shown in merged panels (right). Representative images (63X magnification) of two separate experiments with similar results are presented (Scale bar, 20 μm).