SUPPLEMENTARY MATERIAL

Table S1. *Borrelia burgdorferi* strains and oligonucleotide primers used in this study

A. *Borrelia burgdorferi* strains

Strain	Description	Antibiotic Resistance[1]	Reference
CE162	Wild-type strain 297		(20)
Bb914	CE162 with P_{flaB}-*gfp* reporter stably-inserted into cp26	Gent	(6)
Bb1399	Wild-type B31 5A4 NP1 containing insertion of P_{flgB}-Kan^R cassette in *bbe02*	Kan	(40)
Bb1197	Δ*hk1* (*bb0420*) mutant in B31 5A4 NP1 background	Kan/Strep	(34)
Bb1451	Δ*rrp1* (*bb0419*) mutant in B31 5A4 NP1 background	Kan/Strep	(35)
Bb1452	Δ*glp* (*bb0240-0243*) null mutant in B31 5A4 NP1 background	Kan/Gent	(35)
Bb1286	B31 5A4 NP1 containing insertion of P_{flaB}-*gfp* reporter into cp26	Kan/Gent	(42)
Bb1520	Δ*rrp1* (*bb0419*) mutant in Bb1286 background	Kan/Gent/Strep	This Study
Bb1548	Δ*plzA* (*bb0733*) mutant in Bb1286 background	Kan/Gent/Strep	This Study

B. Oligonucleotide primers used in these studies

Primer	Forward (5'-3')	Reverse (5'-3')	Purpose	Reference
flaB	CTTTTCTCTGGTGAGGGAGCTC	GCTCCTTCCTGTTGAACACCC	qPCR & qRT-PCR	(136)
flaB-Probe	FAM-CTTGAACCGGTGCAGCCTGAGCA-BHQ1		qPCR & qRT-PCR	(136)
bb0021	AGAGGATCTTCAAGGTTAATCGTG	ACGCAGTGAATAAATTAGTGTCAA	qRT-PCR	(24)
malX-1/bb0116	TTTATCTATCTATTATGTTTGGACTGCC	GCCCCAGAGAAAAGAAGTGC	qRT-PCR	This Study
bb0240	AAGTCCCGAATACCAGGAGAAAT	TTCTTGCTGCTGTGTAAATACCAAA	qRT-PCR	(19)
bb0241	CCTTAAAAAGGGTATGGCCAA	CTTGTCGTGGCTAATGATTG	qRT-PCR	This Study
bb0243	GCTCTGTTCTATATTACGATGATT	AGGGCAATGCCTCCTTTTT	qRT-PCR	This Study
bb0323	ATATGGATCCCGCTGGAAAT	AGCCGCTTCAAGTGCTTTTA	qRT-PCR	This Study

bb0629	TGTAGTCTCAGGAGGAAT	AATATAGCCAGCAAGTATTG	qRT-PCR	This Study
bosR/bb0647	GTCCACCCTATTCAACTT	TTCCTTGTTCTCATCTGG	qRT-PCR	This Study
mcp4/bb0680	GGTCTAAACAAAGCGAAAAAAAGG	GAATCTAAATCTATCAAAACTATCTGCCAC	qRT-PCR	(24)
cdr/bb0728	GACGCTGTTATACTTGCTACCG	GAAGCTGAGCCCAATGTGCCT	qRT-PCR	(24)
rpoS/bb0771	CTTGCAGGACAAATACAAAGAGGC	GCAGCTCTTATTAATCCCAAGTTGCC	qRT-PCR	(24)
spoVG/bb0785	TGGATATTACAGACATAAGGATT	CTGTTAGGCATCGCAATA	qRT-PCR	This Study
arcA/bb0841	TTTGGCGATAGCTCCAGGAGAAGT	ATAGACATGCATCTTGGCCCACCA	qRT-PCR	This Study
bba07	TAGCAATCCCGACAAGTTTAAT	AGAGCCATTTTAGCCTTTCTTT	qRT-PCR	(24)
ospA/bba15	CTGCAGCTTGGAATTCAGGC	GTTTTGTAATTTCAACTGCTGAC	qRT-PCR	(24)
dbpA/bba24	GGGTAGTGGGGTATCAGAAAATC	GAGCTGTAGTTGGAGGATTCTC	qRT-PCR	(24)
bba52	TCAAAAAACTCAAGACCTTCCAAAA	AATTCACTTTCTGCACCGTTAAGAT	qRT-PCR	This Study
bba57	CAGAAGAAGCAAATATGGCAAA	TCGTTGGTGGTTGTTGAA	qRT-PCR	This Study
bba59	TTGGTCGTGGGATTTTAATAGATTCTA	TGAGGCTTTTGATTGTGGGTTT	qRT-PCR	This Study
lp6.6/bba62	GTTGCTTGCGAAACTACAAGA	CATTGACTTTGTCATAGGTTGCTT	qRT-PCR	(24)
bba73	CGATAACATTATTGGCGAAT	TCCAACCCTTATTACTC	qRT-PCR	This Study
bba74	ACTATTAACAAGGTAACCGAAGATG	CCTTGTTTGCACCCTCAGCAAC	qRT-PCR	(24)
ospC/bbb19	AGGGAAAGGTGGGAATACATC	TGTTCCATTATGCCCCGC	qRT-PCR	(24)
malX-2/bbb29	AAGCCAGGAATATACAAG	GAAAGACAGCAACAAATC	qRT-PCR	This Study
bbh28	ATTCGGAAGCCATTGATTACA	CGCAACATTCAAGGCATTC	qRT-PCR	This Study
bbo39	GAAAACTCAAGGCGACAGGTCTA	GTTTGCTAATTCATCAGTATTGC	qRT-PCR	This Study
erpP/bbp38[2]	GCAATGGAGAGGTAAAGGTCAA	GCTTTTATAAAGTTATTAATTTCTTCC	qRT-PCR	This Study
bbq47	ATGGTATAGAAAGTCAAACAAGT	CTTCGCCTTCTTCATCTTC	qRT-PCR	This Study
297 ospC	CAGGGAAAGATGGGAATACATCTGC	CGCTTCAACCTCTTTCACAGCAAG	qRT-PCR	This Study
B31 ospE	AAAGCAATGGAGAGGTAAAGG	GCTTTTTATAAAGTTATTAATTTCTTCCTCTTC	qRT-PCR	(38)

[1]Antibiotic resistance determined by growing spirochetes in the presence of the following antibiotics: gentamycin (Gent; 50 µg/ml); erythromycin (Erm; 0.06 µg/ml), kanamycin (Kan; 400 µg/ml), and streptomycin (Strep; 50 µg/ml).
[2]The nucleotide sequences of *bbl39* and *bbp38* paralogs are essentially identical.

Table S2. RNA-Seq analysis of wild-type and Δ*rrp1 B. burgdorferi in vitro*.

[1]Annotations and gene product descriptions are based on RefSeq *B. burgdorferi* genome annotation and/or Spirochete Genome Browser *(sgb.fli-leibniz.de). Some gene designations and descriptions have been updated based on published experimental data and/or database searches.*

[2]Genomic coordinates for gene or transcripts based on NCBI RefSeq annotation.

[3]Results from differential gene expression testing, performed using RNA-Rocket, comparing the summed Fragments Per Kilobase per Million fragments mapped (FPKM) of transcripts for each gene_id. OK is indicates a successful test for differential gene expression. NOTEST indicates that there were not enough alignments in either sample for testing. Reads for highly conserved plasmid-encoded paralogous genes, particularly those encoded on cp32/lp56 plasmids, may have been excluded from the final dataset during mapping due to ambiguity in their genomic location.

[4]The log2 of the fold change observed for wild-type *compared to* Δ*rrp1 Bb.*

[5]The False Discovery Rate (FDR)-adjusted *p*-value.

[6]Criteria used for establishing cellular location are described in Methods and Materials.

[7]Based on previously published oligonucleotide-based microarray analyses.

[8]Genes that are upregulated by RpoS (RpoS-UP) only *in vitro* (IV), only DMCs, both *in vitro* and DMCs (Both) or genes whose expression is repressed by RpoS within the mammalian host.

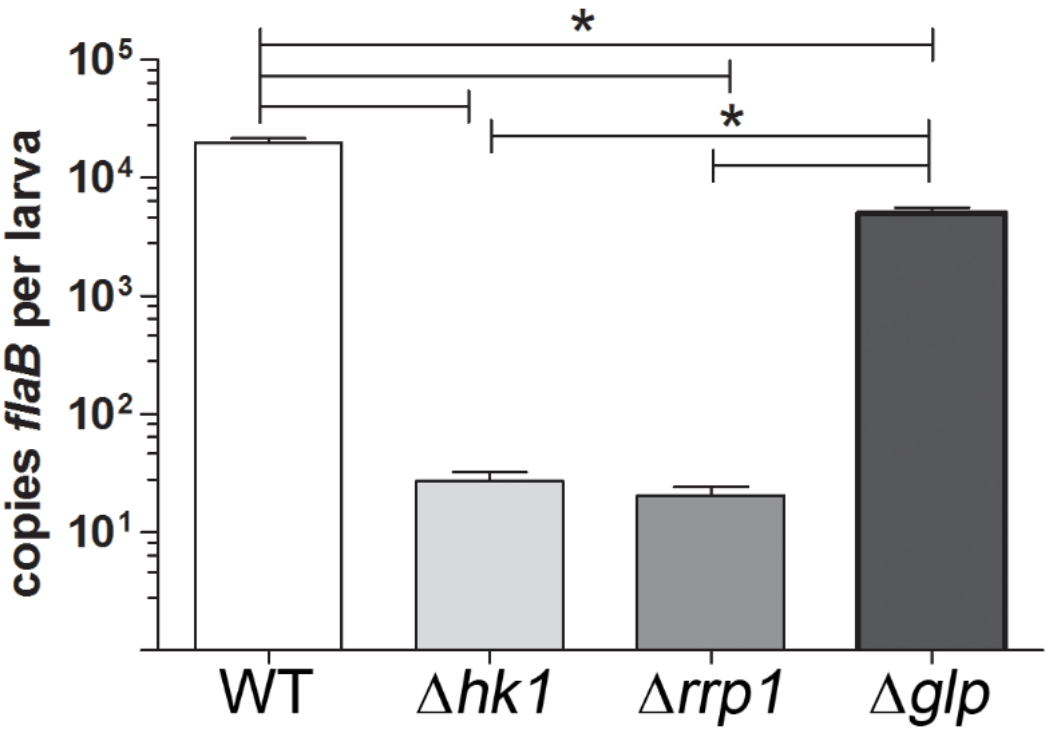


Figure S1. Loss of *glp* gene expression alone cannot account for the survival defect of Δ*hk1* and Δ*rrp1 Bb* during tick feeding. Burdens were assessed by qPCR using a TaqMan assay for *flaB* normalized per larvae. Bars represent the mean ± SEM for each isolate. Statistical significance was determined by comparing the average normalized *flaB* value for each mutant to that of the wild-type parent using an unpaired *t*-test. *, $p \leq 0.0001$.

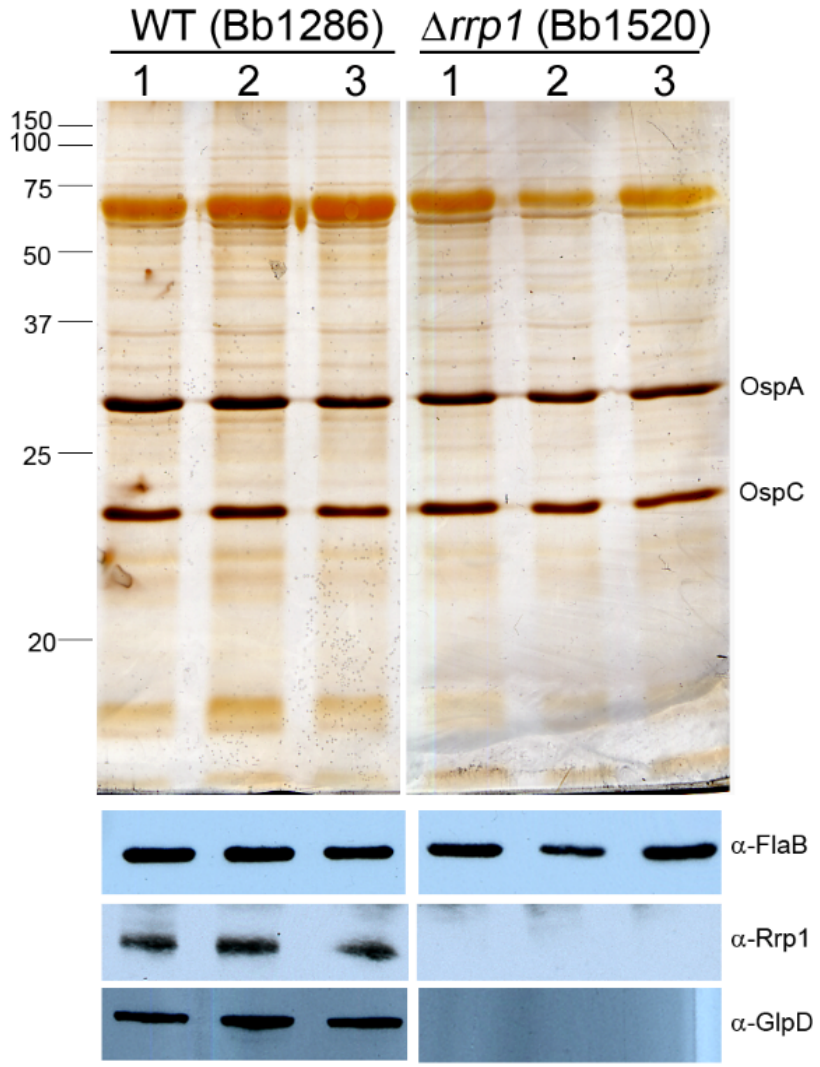
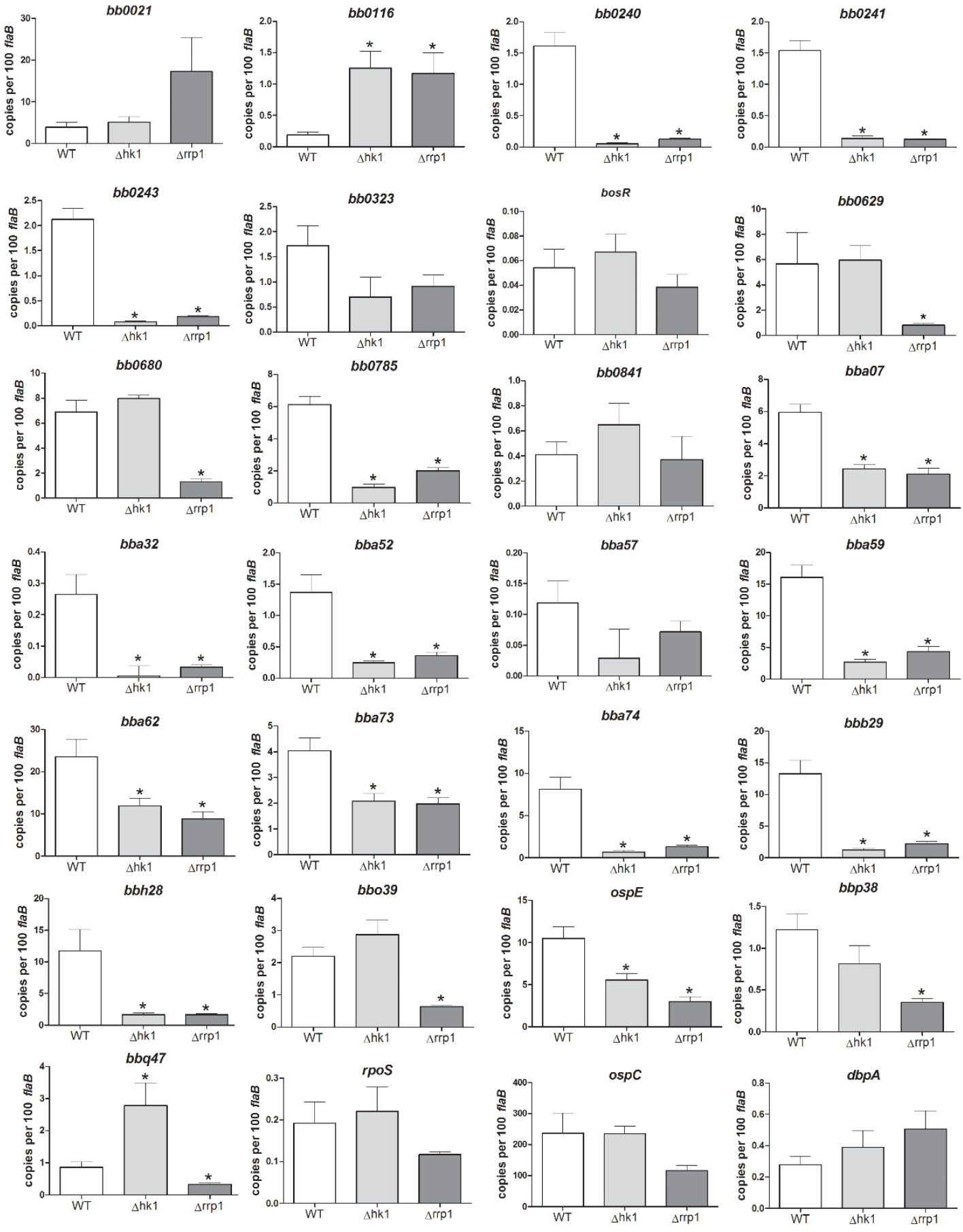


Figure S2. Analysis of samples used for RNA-Seq analysis. Whole-cell lysates from wild-type (Bb1286) and Δ*rrp1* (Bb1520) *Bb* (3 biological replicates per isolate) grown to late-logarithmic phase *in vitro* following a temperature-shift from 23ºC to 37ºC. Samples (~2 x 10^7 *Bb* per lane) were separated by SDS-PAGE on a 12.5% polyacrylamide gel and then either stained with silver or immunoblotted using antisera against Rrp1, GlpD or FlaB (loading control).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bba59
bba62
bba73
bba74
bbb29
bbh28
bbo39
ospE
bbp38
bbq47
rpoS
ospC
dbpA
copies per 100 flaB
WT
ΔHk1
ΔRrp1

Figure S3. Validation of RNA-Seq data by qRT-PCR. qRT-PCR of representative genes selected from comparative RNA-Seq analysis was performed using primers listed in Table S1. Expression profiling was performed using RNA extracted from wild-type *Bb* (Bb1399), Δ*hk1* (Bb1197) and Δ*rrp1* (Bb1520) grown *in vitro* following a temperature-shift from 23ºC to 37ºC. Values represent the average transcript copy number (± SEM) normalized per 100 copies of *flaB* from at least three biological replicates. Statistical significance was determined using an unpaired *t*-test. *, $p \leq 0.05$.