Supporting information

BosR (BB0647) governs virulence expression in *Borrelia burgdorferi*

Zhiming Ouyang,[1] Manish Kumar,[2] Toru Kariu,[2] Shayma Haq,[1] Martin Goldberg,[1] Utpal Pal,[2] and Michael V. Norgard[1*]

[1]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390; [2]Department of Veterinary Medicine, University of Maryland, College Park, MD 20742

[*]Corresponding author:
Michael V. Norgard
Department of Microbiology, University of Texas Southwestern Medical Center, 6000 Harry Hines Blvd., Dallas, TX, 75390-9048
Email: michael.norgard@utsouthwestern.edu
Phone: 214-648-5900
Fax: 214-648-5905

Running title: *Borrelia burgdorferi* BosR

Figure Legends

Fig. S1. Expression of BB0648 and BB0646 in *Bb* isolates. Lane 1, WT B31; lane 2, *bosR*$^-$ OY10/D12; lane 3, mutant OY10/H3; lane 4, *bosR*$^{-/+}$ OY34/E6; lane 5, *bosR*$^{-/+}$ OY34/C4; lane 6, RNA only (no RT) used as the template (negative control); lane 7, genomic DNA only used as the template (positive control).

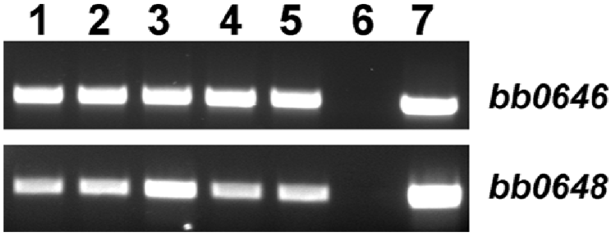
Fig. S2. Assessment of the plasmid contents of *Bb* parental strain B31 and the *bosR* mutant clone OY10/H3 via PCR amplification. Each plasmid for detection is designated above each gel lane. DNA size standards (M) are indicated at the left in base pairs.

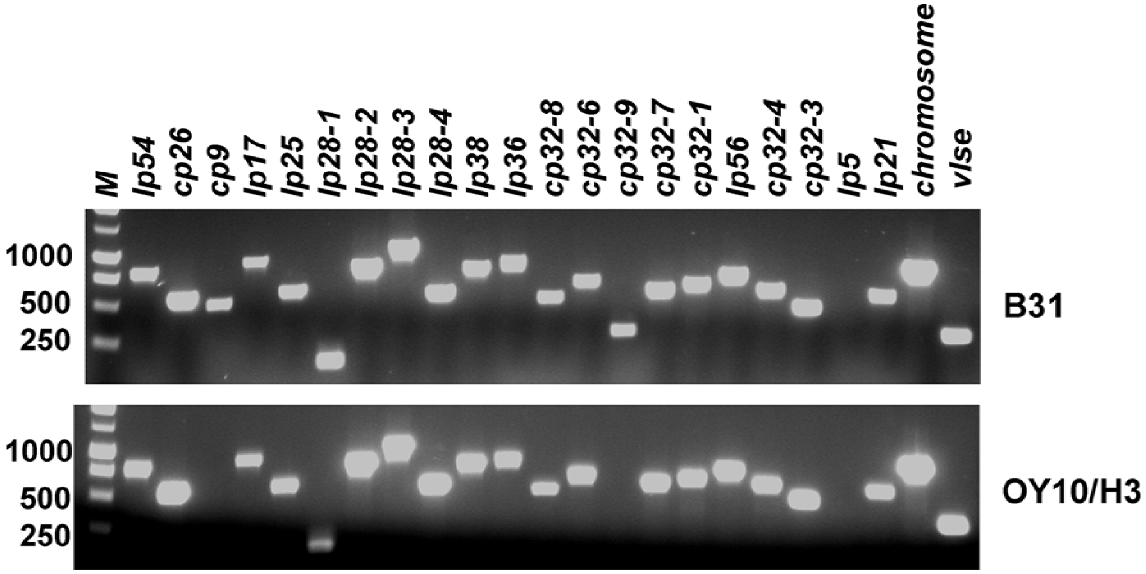
Fig. S3. *In vitro* growth of *bosR* mutants. *Bb* was inoculated into BSK-II medium at 1000 spirochetes/ml. Spirochetes were enumerated using dark-field microscopy. Values are the means from three independent experiments. Error bars indicate standard deviations (*n* = 3). *Bb* strain designations are: WT B31; *bosR* mutants OY10/D12 and OY10/H3; complemented strains OY34/E6 and OY34/C4.

Fig. S4. BosR is not required by *Bb* to survive in unfed ticks. Unfed *Ixodes scapularis* nymphs were microinjected with various *Bb* strains. After 4 days, nymphs were dissected and the tick midguts were subjected to immunofluorescence assays with FITC-labeled anti-*Bb* antibody and examined using confocal immunofluorescence microscopy. Spirochete morphology is evident from tissues of ticks infected with WT B31, the *bosR* mutant (*bosR*$^{-}$), or the complemented strain (*bosR*$^{-/+}$). Two independent tests were performed and representative confocal images are shown.

Fig. S5. Correlation between microarray and qRT-PCR data. Log-transformed fold changes for 19 differentially expressed genes were compared between wild type B31 and the *bosR* mutant.

Fig. S6. Expression of Rrp2, RpoN, and RpoS in *Bb* isolates examined using RT-PCR. Lane designations are same as in Fig. S1.


1
2
3
4
5
6
7
bb0646
bb0648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cp32-1
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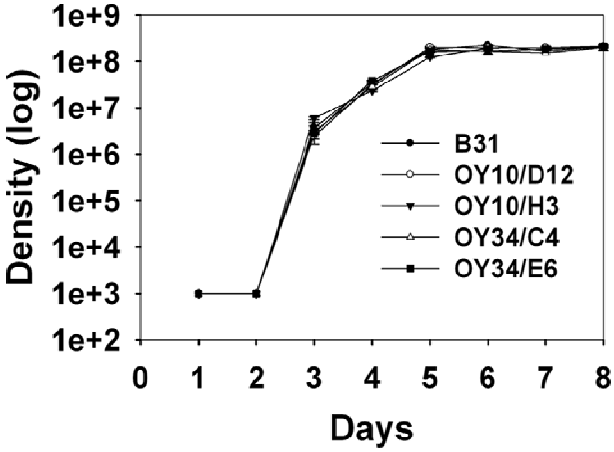
1000
500
250
OY10/H3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1e+7
1e+6
1e+5
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1e+3
1e+2
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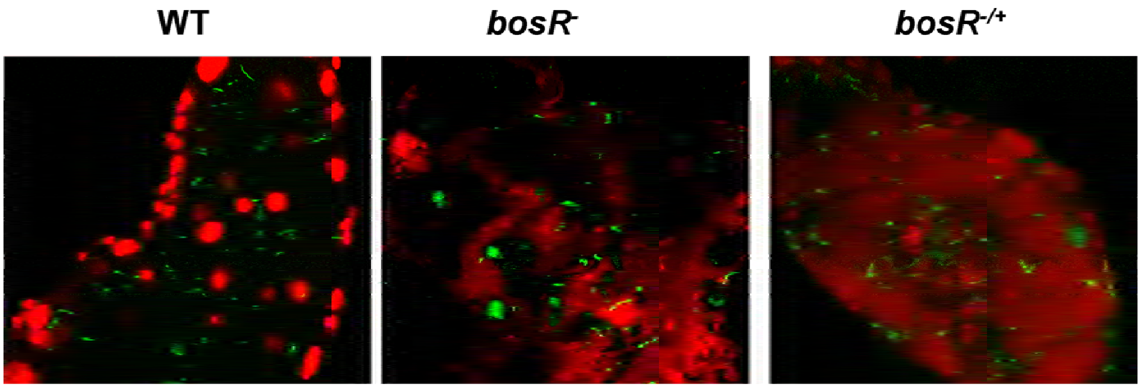
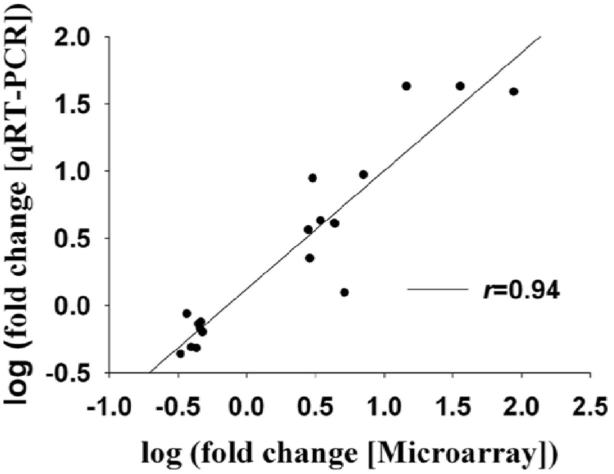
1
2
3
4
5
6
7
8
Days
B31
OY10/D12
OY10/H3
OY34/C4
OY34/E6


WT
bosR^{-}
bosR$^{-/+}$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0.5
0.0
-0.5
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2.5
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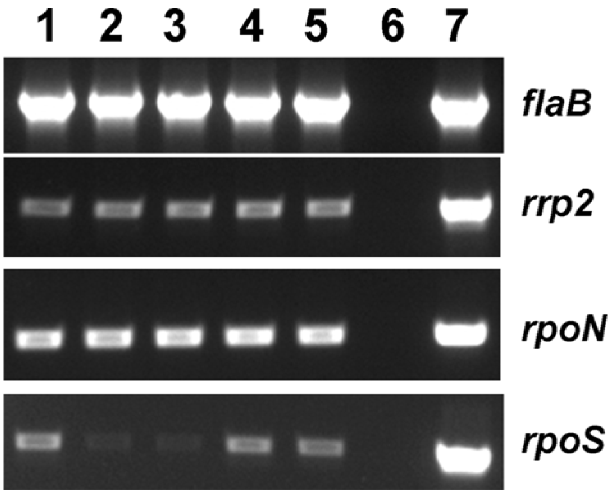
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flaB
rrp2
rpoN
rpoS

Table S1. Oligonucleotide primers used in this study

Primer	Sequence (5'-3')
ZM22.2F	GGCATGGGTTATTATTGGGCTTGGTGGTGC
ZM22R	GGCGCGCCGTCCCAGATGAGAACAAGGAAATCAC
ZM23F	GGCGCGCC CGGAATGTACGTCTATTATGTTGTCG
ZM23R	TTAGCGCGCAAGGAACTTACAGTGGAAGCCCTCAT
ZM24F, a1	AGCCTCATCAATGTCTCTCAGCCA
ZM24R, ZM49F, b1	GTCGGCATTACAAACGATCCTG
ZM25F, b4	CGGAATTCATGAACGACAACATAATAGACGTA
ZM25.2F, c1	ATGGATCCGGGTTCTTATAACACAGTTAAGATCCAAG
ZM25R, b3	ATGGATCCTCATAAAGTGATTTCCTTGTTCTCAT
ZM26F	ATGGATCCATGAACGACAACATAATAGACGTA
ZM26R	CGGAATTCTCATAAAGTGATTTCCTTGTTCTCAT
ZM49R	AGTGAGCTATTGTGGAAGCCAAGC
ZM82	GCACCCGGGTCATAAAGTGATTTCCTTGTTCTCAT
ZM83	ATACCCGGGAACTTCAATAAGAGTTGATCCACCG
bbb19-489F	TGGTACTAAAACTAAAGGTGCTGAAGAA
bbb19-571R	GCATCTCTTTAGCTGCTTTTGACA
BB0771F	CTGGACAAAGAAATAGAGGGATCTG
BB0771R	CAAGGGTAATTTCAGGGTTAAAAGAA
BBA24F	ACGAAGCGCTAAAGACATTACAGA
BBA24R	GGCATCAAAATTTACGCCCTTA
BBA62F	TTATTTGTTGCTTGCGAAACTACAA

BBA62R	TCATTGGAGCTGTAACTTTTGAATCT
bb0032-1103F	TCTCAGCAATAGAATACGCCAACT
bb0032-1182R	TTCTTGTGATATAGGCAGTCTTGCTT
bb0104-541F	GCAGTGGGCAGCCCTTT
bb0104-610R	CAGAACGTTGCAATCCACTTACA
bb0153-385F	GGTTGGGCATGGTTAGTATTGTG
bb0153-455R	GGACTATCCTGATTAGGCATTGAAA
bb0401-476F	GAGAACTGATGCTAAGCGCATCCA
bb0401-583R	AGTTTGCAGCGTAATTTGCTGTT
bb0565-360F	TCCATTTAGTATTGATGATGCTCCTAA
bb0565-434R	CTCTTGCCAATTCCTGAAATAAATTT
bb0646-870F	TCTTGAAAATTCACACGCTCACTT
bb0646-933R	TGTGTTTTGGGCGCTTTTG
bb0690-387F	TGCTGGTGATTATGGTACTGCTAATAT
bb0690-471R	AAGCAATGCCTTATGCATCCA
bb0763-238F	ACAGCCCACGGAACAGTTG
bb0763-299R	AAAAAATCATAAGCACCCTCTCTCA
bba48-400F	ATCACAAACAGCAAGCCTTCAG
bba48-467R	AACAAAGACTCAAAACCAGGCTCTA
bbe31-432F	TGGAAGAACTTTTGATACAGCGATT
bbe31-569R	ATGTCTGATACGATTGATTTCATGGA
bbf01-180F	CCAAAAGGAATTAGAAATTACGGAAA
bbf01-251R	GCATGTTGTGCTACTTGTGAAAGTT

bbg27-87F	ACTCGAAAGTGCAGCATGTTTC
bbg27-167R	AAGGGCACGGCAATTCTG
bbj26-137F	GCGGCAAGACAACTTTAATGAA
bbj26-218R	AAAAGAGTTGAGTTGAAGCGGATCT
bbo39-515F	GTGGTGTTGGAGTGCAAGCTT
bbo39-588R	ACTGCCGCCATTAGAATAACTTACA
bbr41-362F	GAAAGACCGTACCCCAAATGG
bbr41-424R	CAACTAAAGCGCACCTTCTGAA

All primers were designed based on B31-MI sequence data. Restriction enzymes sites were underlined.

Table S2. *B. burgdorferi* genes down-regulated in the *bosR* mutant

Gene ID	Function	Gene	Replicon	Ratio, WT/mutant	P-value
BBA37	hypothetical protein		lp54	116.74	1.50E-05
BBB19	outer surface protein C	*ospC*	cp26	87.76	2.20E-05
BB0844	hypothetical protein		chromosome	39.12	9.00E-06
BBA72	hypothetical protein		lp54	37.89	5.00E-06
BBA25	decorin binding protein B	*dbpB*	lp54	36.87	3.40E-05
BBG27	conserved hypothetical protein		lp28-2	35.84	4.79E-03
BBA71	hypothetical protein		lp54	28.99	4.60E-05
BBG26	hypothetical protein		lp28-2	27.94	1.36E-04
BBA05	antigen, S1		lp54	22.53	2.90E-05
BBA26	hypothetical protein		lp54	19.76	3.20E-05
BBA36	lipoprotein		lp54	17.23	4.30E-05
BBD24	hypothetical protein		lp17	15.66	2.40E-03
BBA34	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein	*oppAV*	lp54	15.31	5.69E-03
BBA24	decorin binding protein A	*dbpA*	lp54	14.54	1.20E-04
BBK32	immunogenic protein P35		lp36	13.87	3.45E-04
BBM27	rev protein	*rev*	cp32-6	13.67	3.10E-05
BBA66	antigen, P35, putative		lp54	13.07	1.59E-04
BBA06	hypothetical protein		lp54	12.76	5.61E-03
BBP27	rev protein	*rev*	cp32-1	12.51	4.90E-05
BBA07	chpAI protein, putative		lp54	11.45	1.14E-03
BBG24	hypothetical protein		lp28-2	10.39	1.71E-04
BBG23	hypothetical protein		lp28-2	10.06	7.03E-03
BBJ01	hypothetical protein		lp38	9.60	2.86E-04
BBM28	lipoprotein	*lp*	cp32-6	9.58	1.53E-04
BBJ001	conserved hypothetical protein, pseudogene		lp38	9.55	8.00E-06
BBQ02	hypothetical protein		lp56	7.64	6.76E-03

BBK07	hypothetical protein		lp36	7.49	1.64E-04
BBK52.1	conserved hypothetical protein, pseudogene {Borrelia burgdorferi}		lp36	7.47	4.77E-03
BBO39	erpL protein	*erpL*	cp32-7	7.04	5.50E-05
BBA65	hypothetical protein		lp54	6.65	8.88E-04
BBQ37	conserved hypothetical protein		lp56	5.64	2.15E-03
BBL29	conserved hypothetical protein		cp32-8	5.59	5.02E-04
BBA64	antigen, P35		lp54	5.55	2.08E-04
BBD19	hypothetical protein		lp17	5.35	2.52E-04
BBA48	hypothetical protein		lp54	5.14	1.54E-03
BBP28	lipoprotein		cp32-1	5.05	4.00E-05
BBF14.1	hypothetical protein, paralogous family 65, authentic frameshift		lp28-1	4.59	7.68E-04
BBI31	conserved hypothetical protein		lp28-4	4.53	1.41E-03
BBE31	antigen, P35, putative		lp25	4.37	2.89E-04
BBS31	conserved hypothetical protein		cp32-3	4.33	3.85E-04
BBJ26	ABC transporter, ATP-binding protein		lp38	4.32	1.09E-04
BBJ25	hypothetical protein		lp38	4.28	1.48E-04
BBS41	outer surface protein G	*erpG*	cp32-3	4.22	3.00E-05
BB0404	hypothetical protein		chromosome	4.21	9.97E-04
BBK05	hypothetical protein		lp36	4.13	1.30E-03
BBI42	outer membrane protein, putative		lp28-4	4.13	1.00E-04
BBQ47	erpX protein	*erpX*	lp56	4.12	4.00E-05
BBH41	conserved hypothetical protein		lp28-3	4.10	1.28E-02
BBA33	hypothetical protein		lp54	4.08	2.16E-04
BBD20	transposase-like protein, authentic frameshift		lp17	4.07	1.90E-04
BB0567	chemotaxis histidine kinase	*cheA-1*	chromosome	4.02	4.81E-04
BB0689	hypothetical protein		chromosome	3.97	2.80E-05
BBK33	hypothetical protein		lp36	3.95	7.63E-04
BBR44	hypothetical protein		cp32-4	3.83	5.27E-04
BB0846	hypothetical protein		chromosome	3.67	8.70E-05
BBJ24	hypothetical protein		lp38	3.64	1.49E-03

BBD05	hypothetical protein, paralogous family 84		lp17	3.58	8.27E-03
BBM29	conserved hypothetical protein		cp32-6	3.57	2.98E-03
BB0848.1	hypothetical protein, pseudogene		chromosome	3.55	5.26E-03
BBK53	outer membrane protein		lp36	3.49	2.90E-05
BB0565	purine-binding chemotaxis protein	*cheW-2*	chromosome	3.46	4.32E-04
BB0563	hypothetical protein		chromosome	3.42	1.76E-04
BBL39	erpA protein	*erpA*	cp32-8	3.42	3.80E-05
BBJ02	hypothetical protein		lp38	3.41	4.35E-04
BBH01	conserved hypothetical protein		lp28-3	3.41	2.12E-03
BB0671	chemotaxis operon protein	*cheX*	chromosome	3.29	2.00E-06
BBR40	erpH protein	*erpH*	cp32-4	3.20	5.76E-04
BBJ23	hypothetical protein		lp38	3.19	4.41E-04
BB0566	hypothetical protein		chromosome	3.17	1.14E-04
BBS42	associated protein A	*bapA*	cp32-3	3.14	1.68E-03
BBM35	conserved hypothetical protein		cp32-6	3.14	8.20E-05
BBQ03	outer membrane protein, putative		lp56	3.12	2.20E-05
BBG25	conserved hypothetical protein		lp28-2	3.12	2.10E-03
BBR29	conserved hypothetical protein		cp32-4	3.08	1.22E-04
BBQ43	conserved hypothetical protein		lp56	3.08	1.05E-04
BBA01	conserved hypothetical protein		lp54	3.08	6.00E-06
BBR36	conserved hypothetical protein		cp32-4	3.03	4.36E-04
BBF01	erpD protein, putative		lp28-1	3.02	1.40E-03
BBS38	conserved hypothetical protein		cp32-3	3.01	2.06E-04
BB0680	methyl-accepting chemotaxis protein	*mcp-4*	chromosome	2.99	1.48E-03
BBA35	hypothetical protein		lp54	2.98	1.94E-04
BB0681	methyl-accepting chemotaxis protein	*mcp-5*	chromosome	2.96	2.76E-03
BB0670	purine-binding chemotaxis protein	*cheW-3*	chromosome	2.90	3.41E-04
BBR41	conserved hypothetical protein		cp32-4	2.87	3.33E-04
BBG16	hypothetical protein		lp28-2	2.85	4.90E-05
BB0040	chemotaxis protein methyltransferase	*cheR-1*	chromosome	2.84	1.80E-03
BB0771	RNA polymerase sigma factor	*rpoS*	chromosome	2.81	1.56E-02

BBL36	conserved hypothetical protein		cp32-8	2.80	3.28E-04
BBM36	conserved hypothetical protein		cp32-6	2.80	3.30E-05
BB0588	5-methylthioadenosine/S-adenosylhomocysteine nucleosidase, putative	*pfs-2*	chromosome	2.77	5.38E-04
BBQ44	conserved hypothetical protein		lp56	2.77	2.00E-06
BBG17	hypothetical protein		lp28-2	2.76	1.50E-05
BBH10	hypothetical protein		lp28-3	2.74	3.76E-03
BBH09.1	conserved hypothetical protein, pseudogene		lp28-3	2.74	2.31E-04
BBD001	conserved hypothetical protein		lp17	2.72	1.93E-03
BBJ48	hypothetical protein		lp38	2.69	1.33E-03
BBR37	conserved hypothetical protein		cp32-4	2.67	1.97E-04
BBG19	hypothetical protein		lp28-2	2.64	3.26E-03
BBA32	hypothetical protein		lp54	2.63	2.11E-03
BBJ47	hypothetical protein		lp38	2.59	6.71E-03
BB0555	hypothetical protein		chromosome	2.59	4.58E-04
BBO36	conserved hypothetical protein		cp32-7	2.56	4.34E-04
BBG14	hypothetical protein		lp28-2	2.53	7.80E-05
BBO37	conserved hypothetical protein		cp32-7	2.52	6.40E-05
BB0442	inner membrane protein		chromosome	2.52	4.96E-03
BBL37	conserved hypothetical protein		cp32-8	2.51	3.10E-05
BB0842	ornithine carbamoyltransferase, catabolic	*arcB*	chromosome	2.50	2.68E-04
BB0776	hypothetical protein		chromosome	2.49	1.09E-02
BBG18	hypothetical protein		lp28-2	2.48	6.37E-04
BBG20	hypothetical protein		lp28-2	2.47	3.90E-04
BBA49	hypothetical protein		lp54	2.44	1.29E-02
BBO40	erpM protein	*erpM*	cp32-7	2.44	1.09E-03
BBG31	conserved hypothetical protein		lp28-2	2.42	2.75E-04
BBA04	antigen, S2		lp54	2.41	9.61E-03
BBG29	conserved hypothetical protein		lp28-2	2.40	6.47E-04
BBG22	hypothetical protein		lp28-2	2.39	7.60E-05
BB0343	Glu-tRNA(Gln) amidotransferase, subunit C	*gatC*	chromosome	2.38	3.00E-03

BB0344	DNA helicase	*uvrD*	chromosome	2.37	2.08E-04
BBJ28	hypothetical protein		lp38	2.37	3.32E-03
BBS39	conserved hypothetical protein		cp32-3	2.36	4.80E-05
BBQ63	hypothetical protein, family 117 paralog, pseudogene		lp56	2.35	9.40E-04
BBG30	hypothetical protein		lp28-2	2.30	3.13E-03
BBK17	adenine deaminase	*adeC*	lp36	2.28	2.90E-03
BBO29	hypothetical protein		cp32-7	2.28	7.50E-05
BB0777	adenine phosphoribosyltransferase	*apt*	chromosome	2.27	2.64E-03
BBO19	conserved hypothetical protein		cp32-7	2.27	8.59E-03
BBL42	hypothetical protein		cp32-8	2.26	1.64E-04
BBR42	outer surface protein F	*erpY*	cp32-4	2.26	5.99E-04
BBO43	hypothetical protein		cp32-7	2.25	4.00E-06
BBP41	hypothetical protein		cp32-1	2.25	1.45E-04
BB0672	chemotaxis response regulator	*cheY-3*	chromosome	2.22	2.90E-05
BBS44	hypothetical protein		cp32-3	2.21	1.28E-04
BBM41	hypothetical protein		cp32-6	2.19	4.00E-06
BBR43	hypothetical protein		cp32-4	2.18	2.68E-03
BB0562	hypothetical protein		chromosome	2.18	1.07E-03
BB0313	cell division protein	*ftsJ*	chromosome	2.10	1.95E-04
BB0554	hypothetical protein		chromosome	2.10	7.20E-04

Table S3. *B. burgdorferi* genes up-regulated in the *bosR* mutant

Gene ID	Function	Gene	Replicon	Ratio, Mutant/WT	P-value
BBJ09	outer surface protein D	*ospD*	lp38	3.13	3.30E-05
BB0646	hydrolase, alpha/beta fold family		chromosome	3.03	3.17E-04
BBE25	hypothetical protein		lp25	2.81	5.21E-04
BB0401	glutamate transporter, putative		chromosome	2.72	3.17E-02
BBH26	hypothetical protein		lp28-3	2.70	6.25E-04
BBH27	conserved hypothetical protein		lp28-3	2.68	5.56E-04
BB0812	pantothenate metabolism flavoprotein	*dfp*	chromosome	2.62	1.77E-02
BBJ39.1	multidrug-efflux transporter, pseudogene		lp38	2.60	1.49E-02
BB0032	hypothetical protein		chromosome	2.53	1.03E-02
BB0212	hypothetical protein		chromosome	2.48	3.85E-04
BB0028	lipoprotein, putative		chromosome	2.48	5.70E-05
BB0335	oligopeptide ABC transporter, ATP-binding protein	*oppF*	chromosome	2.48	4.40E-05
BB0075	hypothetical protein		chromosome	2.47	6.56E-04
BBG02	conserved hypothetical protein		lp28-2	2.44	1.17E-03
BB0076	signal recognition particle-docking protein FtsY	*ftsY*	chromosome	2.40	5.80E-03
BB0128	cytidylate kinase	*cmk*	chromosome	2.40	3.44E-02
BB0352	hypothetical protein		chromosome	2.40	1.09E-04
BBG09	conserved hypothetical protein		lp28-2	2.39	1.80E-05
BB0078	hypothetical protein		chromosome	2.36	6.53E-03
BB0072	hypothetical protein		chromosome	2.36	2.00E-03
BB0183	conserved hypothetical protein		chromosome	2.35	1.01E-02
BBG34	hypothetical protein		lp28-2	2.35	4.98E-03
BBU05	plasmid partition protein, putative		lp21	2.34	2.52E-03
BBA62	lipoprotein		lp54	2.33	1.34E-03
BBM32	plasmid partition protein, putative		cp32-6	2.31	2.55E-03
BB0360	hypothetical protein		chromosome	2.31	5.35E-04

BBM33	conserved hypothetical protein		cp32-6	2.30	1.58E-03
BB0730	glucose-6-phosphate isomerase	*pgi*	chromosome	2.29	1.32E-03
BB0249	phosphatidyltransferase		chromosome	2.29	1.06E-02
BB0071	hypothetical protein		chromosome	2.27	6.81E-04
BBH28	plasmid partition protein, putative		lp28-3	2.27	4.23E-04
BB0363	periplasmic protein		chromosome	2.26	4.11E-04
BB0228	conserved hypothetical protein		chromosome	2.25	1.36E-03
BB0153	superoxide dismutase	*sodA*	chromosome	2.25	1.23E-03
BB0024	hypothetical protein		chromosome	2.24	7.25E-04
BB0184	carbon storage regulator	*csrA*	chromosome	2.24	8.51E-04
BB0539	conserved hypothetical integral membrane protein		chromosome	2.24	4.20E-05
BBJ41	antigen, P35, putative		lp38	2.24	9.20E-04
BB0362	prolipoprotein diacylglyceryl transferase	*lgt*	chromosome	2.23	1.00E-05
BB0540	translation elongation factor G	*fus-1*	chromosome	2.23	2.43E-03
BB0034	hypothetical protein		chromosome	2.22	1.30E-03
BB0314	octaprenyl-diphosphate synthase	*ispB*	chromosome	2.22	1.12E-03
BBI36	antigen, P35, putative		lp28-4	2.22	1.55E-03
BBI38	hypothetical protein		lp28-4	2.22	4.34E-04
BB0073	hypothetical protein		chromosome	2.21	2.50E-05
BBA74	outer membrane porin	*oms28*	lp54	2.19	2.54E-03
BB0840	lipoprotein, putative		chromosome	2.19	4.13E-04
BBG06	conserved hypothetical protein		lp28-2	2.19	4.80E-05
BBI39	hypothetical protein		lp28-4	2.19	8.00E-06
BB0104	periplasmic serine protease DO	*htrA*	chromosome	2.18	3.56E-03
BB0152	glucosamine-6-phosphate isomerase	*nagB*	chromosome	2.18	2.62E-03
BB0763	response regulatory protein	*rrp-2*	chromosome	2.16	1.17E-03
BBH37	hypothetical protein		lp28-3	2.16	7.40E-05
BB0240	glycerol uptake facilitator	*glpF*	chromosome	2.15	1.92E-03
BBH29	conserved hypothetical protein		lp28-3	2.13	1.61E-04
BB0030	signal peptidase I	*lepB-1*	chromosome	2.12	8.86E-04

BB0011	hypothetical protein		chromosome	2.12	3.50E-05
BBJ19	conserved hypothetical protein		lp38	2.11	1.21E-03
BB0333	oligopeptide ABC transporter, permease protein	*oppC-1*	chromosome	2.11	4.74E-04
BB0690	neutrophil activating protein	*napA*	chromosome	2.11	1.07E-04
BB0193	lipoprotein, putative		chromosome	2.09	3.22E-04
BB0770	conserved hypothetical protein		chromosome	2.09	7.61E-04