Supplementary Information

The Lyme disease spirochete's BpuR DNA- / RNA-binding protein is differentially expressed during the mammal-tick infectious cycle, and affects translation of the SodA superoxide dismutase

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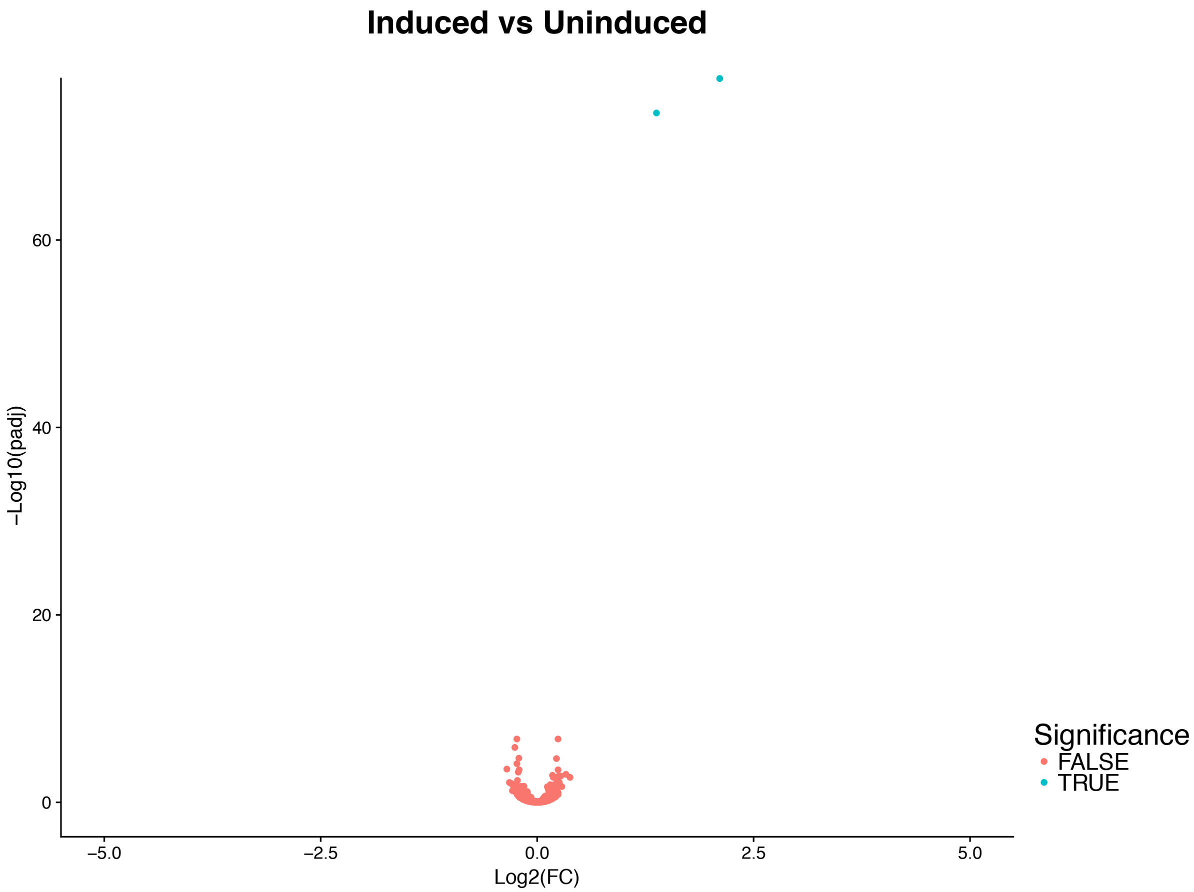

Figure S1: Volcano plot of differentially expressed transcripts in *B. burgdorferi* that were induced to produce elevated levels of BpuR, compared to those which produce wild-type levels. *B. burgdorferi* carrying pWA10 (*bpuR* under transcriptional control of an inducible *lac* promoter) were incubated with or without IPTG at 34°C in BSK-II. Bacteria were inoculated at 1x10^5 bacteria/ml, and induced when cultures reached 1x10^6 bacteria/ml. Induced and un-induced cultures were harvested after 24 hours. RNA was isolated, cDNA libraries were prepared, and sequenced. Reads were mapped to the *B. burgdorferi* genome, counted using Salmon and tested for differential expression using DESeq2 in R. Criteria of >2X change in expression and <0.05 adjusted p-value were used to define significantly changed transcripts. Transcripts that met those criteria are shown in blue-green (*bpuR* and *hslU*) and those which were expressed at lower levels are shown in red (all other transcripts).

Table S1: Transcripts that were significantly differentially expressed in BpuR-induced *B. burgdorferi*.

Differential expression testing results of transcripts which met the criteria of >log2 fold-change and an adjusted p-value (padj) when comparing the induced sample to the un-induced sample. The first column contains the gene's common name and the open reading frame number assigned to *B. burgdorferi* strain B31 (Fraser *et al.*, 1997). The remaining columns include the metrics of expression of each impacted transcript including base mean (average normalized count value), log2FC (Fold change estimate), lfcSE (uncertainty of the log fold change estimate), stat (Wald statistic), pvalue, padj (pvalue following Benjamini-Hochberg adjustment).

Gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
bpuR, BB0047	7837.00	2.109	0.05317	39.66	0	0
hslU, BB0295	5271.83	1.378	0.07413	18.59	3.653 e-77	2.786 e-74

Table S2: DNA oligonucleotides used in this study.

Some oligonucleotides were modified with a biotin on the 5′ end. Probes *bpuR*-TaqMan and *flaB*-TaqMan were modified on the 5′ ends with FAM (6-carboxyfluorescein) and on the 3′ ends with TAMRA (6-carboxytetramethylrhodamine).

Oligonucleotide Name	**Sequence (5' -> 3')**	**Use**
bpuR-For	CTTAAGGCAATAGCGGTTATT	qRT-PCR of *bpuR* transcript in ticks
bpuR-Rev	CCATATTCACCATACCCTTTATTA	qRT-PCR of *bpuR* transcript in ticks
bpuR-TaqMan	TCCGTTGGCTCTTCTGCAAGGC	qRT-PCR of *bpuR* transcript in ticks
flaB-For	TCTTTTCTCTGGTGAGGGAGCT	qRT-PCR of *flaB* transcript in ticks
flaB-Rev	TCCTTCCTGTTGAACACCCTCT	qRT-PCR of *bpuR* transcript in ticks
flaB-TaqMan	AAACTGCTCAGGCTGCACCGGTTC	qRT-PCR of *flaB* transcript in ticks
bpuR-qRT-F	GGAGAGAGAGGGGAACTATAC	qRT-PCR of *bpuR* transcript in mice
bpuR-qRT-R	GCCTTGCAAAGGAGCCAACG	qRT-PCR of *bpuR* transcript in mice
fla3	GGGTCTCAAGCGTCTTGG	qRT-PCR of *flaB* transcript in mice
fla4	GAACCGGTGCAGCCTGAG	qRT-PCR of *flaB* transcript in mice

Bio-*bpuR*-Probe1	biotin-CCTTCTTTTTAAATCGCCCGCC	PCR of the *bpuR*-*tRNA* intergenic region, for DNA-affinity pull-down
bpuR-Probe2	GATTTATTGTAATGTTATTTTTAGCTAGC	PCR of the *bpuR*-*tRNA* intergenic region, for DNA-affinity pull-down
sodA-RIP-F1	CCATAGTTTTAGCATCAATATAAGGCT	PCR of *sodA* from RIP cDNA
sodA-RIP-R2	GAAAGTGGGAAGAAATATAAAAATTG	PCR of *sodA* from RIP cDNA
dnaK-RIP-F1	CCATGCTCCATTATAGCTACGCATG	PCR of *dnaK* from RIP cDNA
dnaK-RIP-R2	CCAAAAAGGATATTGCTACAACG	PCR of *dnaK* from RIP cDNA
gap-RIP-F1	GAGCAAGATCAACCACTCTTGTAG	PCR of *gap* from RIP cDNA
gap-RIP-R2	GCCTGTGCCAACAGGTCCAATAG	PCR of *gap* from RIP cDNA
Bio-*bpuRp*-11	biotin-CAATAATTTACTTATATAAAAA	PCR of the *bpuR*-*tRNA* intergenic region, for labeled EMSA probe
bpuRp-11	CAATAATTTACTTATATAAAAA	PCR of the *bpuR*-*tRNA* intergenic

		region, for unlabeled EMSA competitor
bpuR-14	CCACACAAGTTTTTGTACTTGAC	PCR of the *bpuR*-*tRNA* intergenic region, for labeled and unlabeled EMSA probe and competitor
BpurP-1	CAATTTCCTCCACACAAGTTTTTG	PCR of the *bpuR*-*tRNA* intergenic EMSA competitor 5
BpurP-2	GTACAATTAATTTAGCTTAAATGTAGTCAAGT	PCR of the *bpuR*-*tRNA* intergenic EMSA competitor 5
BpurP-3	GACTACATTTAAGCTAAATTAATTGTAC	PCR of the *bpuR*-*tRNA* intergenic EMSA competitor 6
BpurP-4	GATTTATTGTAATGTTATTTTTAGCTAGC	PCR of the *bpuR*-*tRNA* intergenic EMSA competitor 6
BpurP-5	GCTAGCTAAAAATAACATTACAATAAATC	PCR of the *bpuR*-*tRNA* intergenic EMSA competitor 7
BpurP-6	GGACGCAATACAATAATTTACTTATAT	PCR of the *bpuR*-*tRNA* intergenic EMSA competitor 7

bpuR-F	GGCTCTTCTGCAAGGCATAAT	qRT-PCR of *bpuR* transcript
bpuR-F	GCCCGCCTGATAAATGAGATT	qRT-PCR of *bpuR* transcript
flaB-F	CCTTCTCAAGGCGGAGTTAAT	qRT-PCR of *flaB* transcript
flaB-R	GCTGCTACAACCTCATCTGT	qRT-PCR of *flaB* transcript
hslU-F	CAAACCGCCAATTCCCATATC	qRT-PCR of *hslU* transcript
hslU-R	GCAGGTGAGCTTGATGATACTA	qRT-PCR of *hslU* transcript
hslV-F	ATGATGCTTGTTGCTGATTCTAAC	qRT-PCR of *hslV* transcript
hslV-R	CACCACTGCCAATCGAAATAAC	qRT-PCR of *hslV* transcript
sodA-F	TGTCCTGAGAGTGGCCTTA	qRT-PCR of *sodA* transcript
sodA-R	GCATGCTCCCAAACATCAATAC	qRT-PCR of *sodA* transcript

Table S3: RNA oligonucleotides used in this study.

Oligonucleotide Name	**Sequence (5' -> 3')**
*sodA*1	AAUGAGCCUUGUUAUUGUGGAAGUGGGAAGAAAUAUAAAAAUUGUCAUGGCAAAAGUUA
*sodA*2	UCAUGGCAAAAGUUAAAACAGGAGGUUUUUAUGUUUAAGCUGCCAGAACUUGGUUAUGAU
bpuR	ACGUUAAAUGUAGUCAAGUACAAAAACUUGUGUGGAGGAAAUUGAUGGGAGAGAGAGGGGAAGUAUACU