Table S1: Oligonucleotide primers used in the study.

Purpose	Sequence (5′ to 3′)
Forward primer for *B. burgdorferi flaB* quantitative RT-PCR (qRT-PCR).	TTGCTGATCAAGCTCAATATAACCA
Reverse primer for *B. burgdorferi flaB* qRT-PCR.	TTGAGACCCTGAAAGTGATGC
Forward primer for mouse *β-actin* qRT-PCR.	AGAGGGAAATCGTGCGTGAC
Reverse primer for mouse *β-actin* qRT-PCR.	CAATAGTGATGACCTGGCCGT
Forward primer for amplifying the *bb0104* upstream fragment	GGCGAGCTCATTGGTGCAATACTCAACAGGAA
Reverse primer for amplifying the *bb0104* upstream fragment	GGCACGCGTTAAATTTTAGTTTTGGGTTAAGAG
Forward primer for amplifying the *bb0104* downstream fragment	GGCCCGCGGTTTTATAACCTCCACATAATTAC
Reverse primer for amplifying the *bb0104* downstream fragment	GGCCTGCAGTAAATTATGCAAATTCTAAGTATG
Primer P3 used in Fig. 2	GGGGGAAGCGAAAGAGAAGATGA
Primer P4 used in Fig. 2	TAATAAGATAATAAATTATTATT
Primer P1 used in Fig. 2	CTAAAATGTAATTTTAAAAGAATCGT
Primer P2 used in Fig. 2	GTGGAAAAAAAGTTTTTTTCTGGATTT
Primer P5 used in Fig. 2	TTCGGAGACGTAGCCACCTA
Primer P6 used in Fig. 2	CAACAACCGCTTCTTGGTCG
Primer PRXW008 (bb0104-F-NdeI) used in Fig. 2	GCTCATATGGTGGAAAAAAAGTTTTTTTCTGAT
Primer PRXW009(bb0104-R-BglII) used in Fig. 2	GAAGATCTAAAATGTAATTTTAAAAGAATCG
Forward primer for *bb0323* RT-PCR.	ATATGGATCCCGCTGGAAAT
Reverse primer for *bb0323* RT-PCR.	AGCCGCTTCAAGTGCTTTTA

Table S2: Isolation of viable *B. burgdorferi* by culture analysis of tissues from infected murine hosts (WT, wild type; M1 and M2, two independent clones of *htrA_Bb* mutants)

	1st week	**2nd week**	**3rd week**	**4th week**
WT	3/3 heart 2/3 spleen	3/3 heart 2/3 spleen	3/3 heart 2/3 spleen	3/3 heart 2/3 spleen
M1	0/3 heart 0/3 spleen	0/3 heart 0/3 spleen	0/3 heart 0/3 spleen	0/3 heart 0/3 spleen
M2	0/3 heart 0/3 spleen	0/3 heart 0/3 spleen	0/3 heart 0/3 spleen	0/3 heart 0/3 spleen