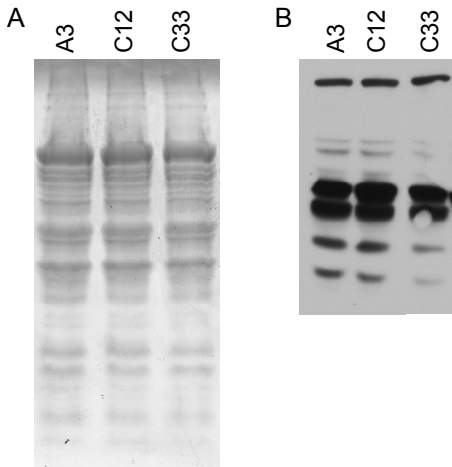


S1



Supplementary Figure 1
Protein profile analysis A.) Coomassie stained gel illustrating the protein profiles of A3, C12 and C33 grown in BSKII. B.) Western blot displaying the antigens produced by A3, C12, and C33 detected using pooled serum derived from mice infected with A3.

Supplementary Table 1 - Primers and Probes used in this study

Primer #	Designation	Sequence (5'-3' direction)	Reference
1	Aut 28-3 NheI-5'	<u>TTGCTAGCGTTGGTTGGCATTGATTAACAGATACCACTAGATTTTTGAT</u> *	This study
2	Aut 28-3 NheI-3'	<u>AAGCTAGCTTATAATATATTATACAATTTTAATCAAGAGCTCACTTTTA</u> *	This study
3	5' Gent (<i>aacC1</i>) Screen	ATGTTACGCAGCAGCAACGATGTTACGCAGCAGG	This study
4	3' Gent (<i>aacC1</i>) Screen	TTAGGTGGCGGTACTTGGGTGCATATCAAAGTGCATCACTTCTTC	This study
5	BBH13-F	ATGAAAGCAGTTTTGGCAAC	Ojaimi C
6	BBH13-R	TTATTCTGTTGCTCACCTTGAG	Ojaimi C
7	BBH41-F	TGCTGAAAGAGAAAAGGAGTTC	Ojaimi C
8	BBH41-R	TTTAAAGGACAGTTGGAATCCG	Ojaimi C
9	BBH18-5'	CTGAAAATGAAGGAGAAGCGGGTGG	Akins PNAS
10	BBH18-3'	TAGGCTAATAACCAATTCGTACAAAT	Akins PNAS
12	BBH17-F	TTGATATGCCACCATTATAACACT	Ojaimi C
13	BBH17-R	CTTATCTTTGGTAATTAATAAAAAAGACTTTTTAAAATTTAAAAG	Ojaimi C
14	BBH08-F	ATGGTTGGTGTTTTTATAAAAAGCT	Ojaimi C
15	BBH08-R	CATATTAATTGCTAAATCATCTTCTTTTG	Ojaimi C
16	BBB19-F	TAATTCAGGGAAAGATGGGA	Ojaimi C
17	BBB19-R	AGGTTTTTTTTGGACTTTCTGCCACAACAGGG	Ojaimi C
	qPCR Primers and Probes		
18	<i>flaB</i> -qPCR 5'	CAGCTAATGTTGCAAATCTTTTCTCT	Jewett et al
19	<i>flaB</i> -qPCR 3'	CCTTCCTGTTGAACACCCTCTT	Jewett et al
20	<i>flaB</i> probe	ACTGCTCAGGCTGCACCGGT	Jewett et al
21	<i>nid</i> -qPCR 5'	TCCCCAGGCCATCGGT	Jewett et al
22	<i>nid</i> -qPCR 3'	CACCCAGCTTCGGCTCAGTA	Jewett et al
23	<i>nid</i> -probe	CGCCTTTCCTGGCTGACTTGGACA	Jewett et al
24	<i>bbh35</i> -qPCR 5'	CATGTGGGATTGTGCGTTATT	This study
25	<i>bbh35</i> -qPCR 3'	GCAGCCCTTTTGCTATCTTTT	This study
26	<i>bbh35</i> -probe	TGGCCCCTCCTATTCTGTAAATATCGCA	This study

* Relevant restriction enzyme recognition sequences have been underlined

Supplementary Table 1 - Primers and Probes used in this study

Supplementary Table 2.

Co-infection Experiment 1 - Determination of the ratio and competitive index of C12 to A3 spirochetes from tissue isolates

Co-injected Mice

	<u>Ear^a</u>	<u>Bladder^a</u>	<u>Joint^a</u>	<u>Total^a</u>	<u>Ip28-3⁺:Wild-type^b</u>	<u>Competitive Index (CI)</u>
Mouse #1	8/10	2/5	2/2	12/17	1:2.4	
Mouse #2	10/10	8/10	8/10	26/30	1:6.5	
Mouse #3	10/10	10/10	5/5	25/25	0:1	
Mouse #4	8/8	7/10	10/10	25/28	1:8.3	
				88/100	1:7.3	CI = 0.14 ^c

a. Ip28-3 positive (wild-type) spirochetes/Total number of colonies screened determined from tissue isolates 4 weeks post inoculation

b. The ratio of C12:A3 spirochetes in infected mouse tissues was determined using the cumulative tissue isolate data

c. Competitive Index (CI) was calculated as described in Materials and Methods

Supplementary Table 3.

Co-infection Experiment 2- Screening results for the presence of lp28-3 in spirochetes isolated from tissues

<u>Coinjected Mice</u>	<u>Inoculum^a Wild-type/Total Screened^b</u>	<u>2 Week^c</u>	<u>4 Week^c</u>	<u>8 Week Isolates^d</u>	<u>Larval Tick Isolates^e</u>
Mouse #1	35/63	31/35	35/35	38/52	79/84 (6)
Mouse #2	35/63	35/35	ND ^f	0/20	8/8 (3)
Mouse #3	35/63	31/35	43/47	18/18	17/17 (2)
Mouse #4	35/63	18/35	ND ^f	ND ^f	11/68 (6)
Mouse #5	35/63	32/35	28/32	20/24	81/88 (6)

- a. Mice were injected with a 1×10^4 spirochetes of a mixed inoculum of A3 and C33
- b. lp28-3 positive (wild-type) spirochetes/total number of colonies screened
- c. lp28-3 positive (wild-type) spirochetes/total number of colonies screened from spirochetes isolated from ear punch biopsies (EP)
- d. lp28-3 positive (wild-type) spirochetes/total number of colonies screened from spirochetes isolated from ear, bladder and tibiotarsal joint tissues (E,B,J)
- e. lp28-3 positive (wild-type) spirochetes/total number of colonies screened from spirochetes isolated from larval ticks; the number in brackets represents the number of larval ticks screened
- f. ND=Not determined due to contamination of culture

Supplementary Table 4 - Determination of the 50% Infectious Dose

B. burgdorferi Strain: A3 (Wild-type)

<u>Dose^a</u>	<u>Infected Mice/Total^b</u>	<u>% Infected</u>	
1x10 ²	2/5	40	ID 50^c=1.44x10²
1x10 ³	5/5	100	
1x10 ⁴	5/5	100	
1x10 ⁵	5/5	100	

B. burgdorferi Strain: C12 (lp28-3-deficient)

<u>Dose^a</u>	<u>Infected Mice/Total^b</u>	<u>% Infected</u>	
1x10 ²	1/5	16.7	ID 50^c=2.51x10²
1x10 ³	4/5	83.3	
1x10 ⁴	5/5	100	
1x10 ⁵	5/5	100	
1x10 ⁶	5/5	100	

a. Mice were inoculated by intraperitoneal and subcutaneous injection

b. Infection status was determined 4 weeks post-inoculation by the ability to isolate spirochetes from the ear, bladder, and tibiotarsal tissues; isolates were recovered from all tissues tested from infected mice

c. The ID50 was determined as described in (45)