

Supporting Information

A *Borrelia burgdorferi* mini-*vls* system that undergoes antigenic switching in mice: investigation of the role of plasmid topology and the long inverted repeat

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A

- Cassette

+ Cassette

- Cassette

+ Cassette

		C +IR	L +IR	C -IR	C +IR	L -IR	L +IR
- Cassette	C +IR						
	L +IR	1.000					
+ Cassette	C -IR	1.000	1.000				
	C +IR	0.001	0.002	0.028			
	L -IR	0.000	0.002	0.026	1.000		
	L +IR	0.011	0.016	0.153	1.000	1.000	

B

- Cassette

+ Cassette

- Cassette

+ Cassette

		C +IR	L +IR	C -IR	C +IR	L -IR	L +IR
- Cassette	C +IR						
	L +IR	1.000					
+ Cassette	C -IR	1.000	1.000				
	C +IR	0.001	0.003	0.128			
	L -IR	0.001	0.002	0.131	1.000		
	L +IR	0.013	0.020	0.498	1.000	1.000	

Fig. S1. P-values for pairwise comparisons of switching activity in different constructs. The 6 constructs were assessed for significant differences in switching activity using Dunn's test and the Bonferroni correction for multiple comparisons. Corresponding to Figure 4, switching activity was measured using **A)** The number of templated SNPs, and **B)** the number of switch events containing 2 or more templated SNPs. Yellow indicates *p*-values less than or equal to 0.05.

Table S1. Oligos used (bold letters indicate restriction sites or telomere sequences).

Oligo Name	Sequence	Restriction Site	To amplify
B2774	CTT CCATGG CCGTTATACACTCCTTTACCC	<i>NcoI</i>	Ip5 replication region
B2775	GTG CTCGAG CATTTATTCTTTAGCCTCTATGG	<i>XhoI</i>	
B2776	CCCG CTCGAG CAGTCAAGTCTAGTCTAGTGCGG	<i>XhoI</i>	A pBSV2g stretch containing Gent, pUCori and the MCS
B2777	TTG CCATGG ATCTAGTGCAGGAAAGAACATG	<i>NcoI</i>	
B2768	AAT GAGCTC TTTTTATATTGTGAGCCGGTTT	<i>SacI</i>	A 2 kb stretch containing <i>pncA</i>
B2769	AAG GGATCC TCTATGCTATCCCCTTGTTCA	<i>BamHI</i>	
B2788	TATTTATTATCTTTTAGTATATATAGTTGCGATGTTTAAGGTTTATG	Ip28-1 telomere	pMC73 reverse PCR to introduce the Ip28-1 replicated telomere
B2789	TATTTATTATCTTTTAGTATATATAAGCTCTTAAGTTCAACCTTAATAGG	Ip28-1 telomere	
B2782	AGC GGATCCC AGCGGATCCCAGCTTATCCAACAACCTCAGC	<i>BamHI</i>	Cassette 2
B2797	TCC GTCGAC CAAAACAATCATTACACAAAAACC	<i>Sall</i>	
B2690	TTT CTAGA ATCACTTATTCAAGGCAGGAGGTGTTTCTTTACTAGCAGC	<i>XbaI</i>	The 100 bp inverted repeat and <i>vlsE</i>
B2796	AAG CATGCG GTAAAGAGGCTTTTATTCTTTG	<i>SphI</i>	
B2842	CTC CTAGG GAGGGCATAGTCGTGTCCATAC	<i>AvrII</i>	<i>vlsE</i>
B2843	TCC GATCG AGAAATGAAAAATTCTCTCACCT	<i>PvuI</i>	
B248	GCGATATAAGTAGTACGACGGGGAAACCAG		<i>vlsE</i> variable region and sequence it (Sanger)
B249	CAAGGCAGGAGGTGTTTCTTTACTAGCAGC		
B2795	CAAGCACTATTTCCATAACACC		To sequence the telomere and cassette
B2798	TGCTTTATTTTATAAATTCTCTCCCTA		To sequence cassette
B2770	TT AGACTC TATTGAATGATTTTTTATATATTAAGC	<i>SacI</i>	To sequence <i>pncA</i>
B427	AGAATTATGTCGGTGCGTTGT		

Table S2. PCR Primers with barcodes for amplifying *vlsE*.

Name	Direction	Barcode ID	Target	Sequence (5'-3', barcode-TARGET)
B2736	F	1	<i>vlsE</i>	tcagacgatgcgtcatGCGATATAAGTAGTACGACGGGGAAACCAG
B2637	F	2	<i>vlsE</i>	ctatacatgactctgcGCGATATAAGTAGTACGACGGGGAAACCAG
B2638	F	3	<i>vlsE</i>	tactagagtagcactcGCGATATAAGTAGTACGACGGGGAAACCAG
B2639	F	4	<i>vlsE</i>	tgtgtatcagtacatgGCGATATAAGTAGTACGACGGGGAAACCAG
B2640	F	5	<i>vlsE</i>	acacgcatgacacactGCGATATAAGTAGTACGACGGGGAAACCAG
B2641	F	6	<i>vlsE</i>	gatctctactatatgcGCGATATAAGTAGTACGACGGGGAAACCAG
B2642	F	7	<i>vlsE</i>	acagtctatactgctgGCGATATAAGTAGTACGACGGGGAAACCAG
B2643	F	8	<i>vlsE</i>	atgatgtgctacatctGCGATATAAGTAGTACGACGGGGAAACCAG
B2644	F	9	<i>vlsE</i>	ctgctgtctctacgacGCGATATAAGTAGTACGACGGGGAAACCAG
B2933	F	20	<i>vlsE</i>	tatgtgatcgtctctcGCGATATAAGTAGTACGACGGGGAAACCAG
B2934	F	21	<i>vlsE</i>	gtacacgctgtgactaGCGATATAAGTAGTACGACGGGGAAACCAG
B2935	F	22	<i>vlsE</i>	cgtgtcgcgcatactGCGATATAAGTAGTACGACGGGGAAACCAG
B2936	F	23	<i>vlsE</i>	atatcagtcatgcataGCGATATAAGTAGTACGACGGGGAAACCAG
B2937	F	24	<i>vlsE</i>	gagatcgacagtctcgGCGATATAAGTAGTACGACGGGGAAACCAG
B2938	F	25	<i>vlsE</i>	cacgcacacacgcgcgGCGATATAAGTAGTACGACGGGGAAACCAG
B2939	F	26	<i>vlsE</i>	cgagcacgcgcgtgtgGCGATATAAGTAGTACGACGGGGAAACCAG
B2745	R	10	<i>vlsE</i>	agtcacgtatcgcgcCAAGGCAGGAGGTGTTTCTTTACTAGCAGC
B2746	R	11	<i>vlsE</i>	cgatcagctgagcgcgCAAGGCAGGAGGTGTTTCTTTACTAGCAGC
B2747	R	12	<i>vlsE</i>	tctgtagtgcgtgcgcCAAGGCAGGAGGTGTTTCTTTACTAGCAGC
B2748	R	13	<i>vlsE</i>	gtcgcgacgtcagtgCAAGGCAGGAGGTGTTTCTTTACTAGCAGC
B2749	R	14	<i>vlsE</i>	tatacgtatatagacgCAAGGCAGGAGGTGTTTCTTTACTAGCAGC
B2750	R	15	<i>vlsE</i>	agctctgagtctctatCAAGGCAGGAGGTGTTTCTTTACTAGCAGC
B2751	R	16	<i>vlsE</i>	tctactctcgcatctaCAAGGCAGGAGGTGTTTCTTTACTAGCAGC
B2752	R	17	<i>vlsE</i>	cacgatagtcgctatgCAAGGCAGGAGGTGTTTCTTTACTAGCAGC
B2753	R	18	<i>vlsE</i>	atctagcgtagtgtatCAAGGCAGGAGGTGTTTCTTTACTAGCAGC
B2762	R	19	<i>vlsE</i>	tgcatgcacagatgcgCAAGGCAGGAGGTGTTTCTTTACTAGCAGC

Table S3. Barcoding scheme.

Forward and reverse barcodes for each sample. Asterisks(*) denote samples that could not be cultured.

L (Linear), C (Circle), cass (cassette), IR (inverted repeat).

	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19
F1	Week 4 Ear punch Mouse 1 L	Week 8 Ear punch Mouse 1 L	Week 12 Heart Mouse 1 L	*	Week 12 Bladder Mouse 1 L	Week 12 Liver Mouse 1 L	Week 12 Lung Mouse 1 L	Week 12 Spleen Mouse 1 L	Week 4 Ear punch Mouse 1 C w/o IR	Week 4 Ear punch Mouse 3 C w/o IR
F2	Week 4 Ear punch Mouse 2 L	Week 8 Ear punch Mouse 2 L	Week 12 Heart Mouse 2 L	*	Week 12 Bladder Mouse 2 L	Week 12 Liver Mouse 2 L	Week 12 Lung Mouse 2 L	Week 12 Spleen Mouse 2 L	Week 8 Ear punch Mouse 1 C w/o IR	Week 8 Ear punch Mouse 3 C w/o IR
F3	Week 4 Ear punch Mouse 3 L	Week 8 Ear punch Mouse 3 L	*	Week 12 Joint Mouse 3 L	Week 12 Bladder Mouse 3 L	*	Week 12 Lung Mouse 3 L	Week 12 Spleen Mouse 3 L	Week 12 Heart Mouse 1 C w/o IR	Week 12 Heart Mouse 3 C w/o IR
F4	Week 4 Ear punch Mouse 1 L w/o cass	Week 8 Ear punch Mouse 1 L w/o cass	Week 12 Heart Mouse 1 L w/o cass	Week 12 Joint Mouse 1 L w/o cass	Week 12 Bladder Mouse 1 L w/o cass	Week 12 Liver Mouse 1 L w/o cass	Week 12 Lung Mouse 1 L w/o cass	Week 12 Spleen Mouse 1 L w/o cass	Week 12 Joint Mouse 1 C w/o IR	Week 12 Joint Mouse 3 C w/o IR
F5	Week 4 Ear punch Mouse 2 L w/o cass	Week 8 Ear punch Mouse 2 L w/o cass	Week 12 Heart Mouse 2 L w/o cass	Week 12 Joint Mouse 2 L w/o cass	Week 12 Bladder Mouse 2 L w/o cass	Week 12 Liver Mouse 2 L w/o cass	Week 12 Lung Mouse 2 L w/o cass	Week 12 Spleen Mouse 2 L w/o cass	Week 12 Bladder Mouse 1 C w/o IR	Week 12 Bladder Mouse 3 C w/o IR
F6	Week 4 Ear punch Mouse 3 L w/o cass	Week 8 Ear punch Mouse 3 L w/o cass	*	Week 12 Joint Mouse 3 L w/o cass	Week 12 Bladder Mouse 3 L w/o cass	Week 12 Liver Mouse 3 L w/o cass	Week 12 Lung Mouse 3 L w/o cass	Week 12 Spleen Mouse 3 L w/o cass	Week 12 Liver Mouse 1 C w/o IR	Week 12 Liver Mouse 3 C w/o IR
F7	Week 4 Ear punch Mouse 1 C	Week 8 Ear punch Mouse 1 C	Week 12 Heart Mouse 1 C	Week 12 Joint Mouse 1 C	Week 12 Bladder Mouse 1 C	Week 12 Liver Mouse 1 C	Week 12 Lung Mouse 1 C	Week 12 Spleen Mouse 1 C	Week 12 Lung Mouse 1 C w/o IR	Week 12 Lung Mouse 3 C w/o IR
F8	Week 4 Ear punch Mouse 2 C	Week 8 Ear punch Mouse 2 C	Week 12 Heart Mouse 2 C	Week 12 Joint Mouse 2 C	Week 12 Bladder Mouse 2 C	Week 12 Liver Mouse 2 C	Week 12 Lung Mouse 2 C	Week 12 Spleen Mouse 2 C	Week 12 Spleen Mouse 1 C w/o IR	Week 12 Spleen Mouse 3 C w/o IR

F9	Week 4 Ear punch Mouse 3 C	Week 8 Ear punch Mouse 3 C	Week 12 Heart Mouse 3 C	Week 12 Joint Mouse 3 C	Week 12 Bladder Mouse 3 C	Week 12 Liver Mouse 3 C	*	Week 12 Spleen Mouse 3 C	Week 4 Ear punch Mouse 2 C w/o IR	
F20	Week 4 Ear punch Mouse 1 C w/o cass	Week 8 Ear punch Mouse 1 C w/o cass	Week 12 Heart Mouse 1 C w/o cass	*	Week 12 Bladder Mouse 1 C w/o cass	Week 12 Liver Mouse 1 C w/o cass	Week 12 Lung Mouse 1 C w/o cass	Week 12 Spleen Mouse 1 C w/o cass	Week 8 Ear punch Mouse 2 C w/o IR	
F21	Week 4 Ear punch Mouse 2 C w/o cass	*	Week 12 Heart Mouse 2 C w/o cass	Week 12 Joint Mouse 2 C w/o cass	Week 12 Bladder Mouse 2 C w/o cass	Week 12 Liver Mouse 2 C w/o cass	Week 12 Lung Mouse 2 C w/o cass	Week 12 Spleen Mouse 2 C w/o cass	Week 12 Heart Mouse 2 C w/o IR	
F22	Week 4 Ear punch Mouse 3 C w/o cass	Week 8 Ear punch Mouse 3 C w/o cass	Week 12 Heart Mouse 3 C w/o cass	Week 12 Joint Mouse 3 C w/o cass	Week 12 Bladder Mouse 3 C w/o cass	Week 12 Liver Mouse 3 C w/o cass	*	Week 12 Spleen Mouse 3 C w/o cass	Week 12 Joint Mouse 2 C w/o IR	
F23	Week 4 Ear punch Mouse 1 L w/o IR	Week 8 Ear punch Mouse 1 L w/o IR	Week 12 Heart Mouse 1 L w/o IR	Week 12 Joint Mouse 1 L w/o IR	Week 12 Bladder Mouse 1 L w/o IR	*	Week 12 Lung Mouse 1 L w/o IR	Week 12 Spleen Mouse 1 L w/o IR	Week 12 Bladder Mouse 2 C w/o IR	
F24	Week 4 Ear punch Mouse 2 L w/o IR	Week 8 Ear punch Mouse 2 L w/o IR	Week 12 Heart Mouse 2 L w/o IR	*	Week 12 Bladder Mouse 2 L w/o IR	Week 12 Liver Mouse 2 L w/o IR	Week 12 Lung Mouse 2 L w/o IR	Week 12 Spleen Mouse 2 L w/o IR	Week 12 Liver Mouse 2 C w/o IR	
F25	Week 4 Ear punch Mouse 3 L w/o IR	Week 8 Ear punch Mouse 3 L w/o IR	Week 12 Heart Mouse 3 L w/o IR	Week 12 Joint Mouse 3 L w/o IR	Week 12 Bladder Mouse 3 L w/o IR	Week 12 Liver Mouse 3 L w/o IR	Week 12 Lung Mouse 3 L w/o IR	Week 12 Spleen Mouse 3 L w/o IR	Week 12 Lung Mouse 2 C w/o IR	
F26	Week 0 Culture L	Week 0 Culture C	Week 0 Culture Lw/o IR	Week 0 Culture C w/o IR	Week 5 Culture L	Week 5 Culture C	Week 5 Culture L w/o IR	Week 5 Culture C w/o IR	Week 12 Spleen Mouse 2 C w/o IR	