SUPPORTING INFORMATION

Figure S1. Sequence alignment of Erp proteins. The complete amino acid sequences of Erp proteins analyzed in the present study. The red asterisks indicate the double lysine motif mutated in ErpG-KA.

Figure S2. Mutation of double lysine residues (K-194 and K-195) of ErpG does not affect structure. Far-UV CD analysis of ErpG and ErpG-KA. Molar ellipticity, Φ, was measured from 190 to 250 nm for 10μM of each protein in PBS buffer.

Figure. S3. Localization of ErpG to the surface of *B. burgdorferi* (A) *B. burgdorferi* strains B31 and B314 carrying the vector (B314/vector), or producing ErpG (B314/pErpG) or ErpG-KA (B314/pErpG-KA) were mock-treated ("-") or treated ("+") with proteinase K ("PK"). Bacterial lysates were then immunoblotted with anti-ErpG ("α-ErpG", top), or, as a control for outer membrane integrity, anti-flagellin ("α-FlaB", bottom). **(B)** Flow cytometry analysis of ErpG localized to the surface of the strain B314/vector or B314/pErpG. **(C)** The indicated *B. burgdorferi* strain was mock-treated or permeablized with methanol prior to probing with anti-ErpG or FlaB (negative control). Values are shown relative to the production levels of ErpG on the surface of *B. burgdorferi* strain B31. Each bar represents the mean of 12 independent determinations ± SEM. (*) Significant reduction in surface production of ErpG relative to the strain B31 is indicated (*,P-value < 0.05).

Figure S4. Recombinant ErpK and ErpL proteins bind to heparan sulfate. (A) 2 μM recombinant GST-tagged ErpK or ErpL or GST (as a negative control) were added at quadruplicate wells coated with 100μL of 10 μg/mL heparin, heparan sulfate (Hep-SO_4), chondroitin-4-sulfate (Chon-4-SO_4), dermatan sulfate (Derm SO_4), chondroitin-6-sulfate (Chon-6-SO_4), fibronectin (Fn), laminin, (Ln), type I collagen (Collagen I), or type IV collagen (Collagen IV). Bound proteins were measured by ELISA (see Experimental Procedures) and mean OD_{405} ± standard deviation was determined. Asterisks indicate that binding of GST-ErpK or GST-ErpL to heparin or heparan sulfate was statistically ($p \leq 0.05$ by Student's t test) different than GST. Shown is a representative of three independently performed experiments. **(B)** The indicated concentrations of recombinant GST-ErpK, GST-ErpL or GST were added to quadruplicate wells coated with heparan sulfate, and protein binding was quantified by ELISA and mean OD_{405} ± standard deviation was determined. Proteins bound to heparan sulfate significantly better than GST ($p \leq 0.05$ by Student's t test). K_D values obtained from the average of three independent experiments were calculated and shown on in the inset. Shown is a representative of three independently performed experiments. **(C)** 15.625 to 500 nM of GST-tagged ErpK or ErpL was flowed over a surface coated with 10 μg heparan sulfate. Binding was measured in response units (RU) by SPR (see Experimental Procedures). Shown is a representative of six experiments performed on three different occasions. K_D values obtained from the average of three independent experiments were calculated and shown on in the inset, and in Table 1 are the k_{on}, k_{off}, and K_D values obtained from average of these.

Table S1. Bacterial strains and plasmids used in this study.

Strain or Plasmid	Genotype or characteristics	Reference(s) or source
B. burgdorferi strains		
B31	*B. burgdorferi* strain B31 isolated from the tick in New York. The isolate used in this study was acquired from P. Rosa in 1997.	(Barbour, 1984) (Stevenson *et al.*, 1996)
B314	High-passage *B. burgdorferi* B31 derivative missing lp5, lp16, lp17, lp21, lp25, lp28-1, lp28-2, lp28-3, lp28-4, lp36, lp29, lp38, lp49, lp54, lp56, cp9, cp32-6, cp32-7, cp32-9.	(Sadziene *et al.*, 1993)
B314/pJF21	B314 harboring pJF21 vector	(Fischer *et al.*, 2006)
B314/pJF21-ErpG	B314 harboring pErpG vector	This study
B314/pJF21-ErpG-KA	B314 harboring pErpG-KA vector	This study
E. coli strains		
DH10B	F- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139Δ(*ara, leu*)7697 *gal*U *gal*K λ- *rps*L *nup*G	Invitrogen
BL21	F–, *ompT, hsd*SB (rB–, mB–), *dcm, gal*, λ(DE3)	Promega
XL1-blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [*F´ *proAB lacI*^{q}*ZΔM15 Tn10 (Tet*^{r}*)]*.	Stratagene
BL21/pGEX4T2-OspF related proteins		
BL21/pGEX4T2-ErpL	BL21 producing ErpL from *B. burgdorferi* strain B31	This study
BL21/pGEX4T2-ErpG	BL21 producing ErpG from *B. burgdorferi* strain B31	This study
BL21/pGEX4T2-ErpG-KA	BL21 producing ErpG from *B. burgdorferi* strain B31, with lysine-194 and -195 replaced by alanine	This study

BL21/pGEX4T2-ErpY	BL21 producing ErpY from *B. burgdorferi* strain B31	This study
BL21/pGEX4T2-Erp25	BL21 producing Erp25 from *B. burgdorferi* strain N40-D10/E9	This study
BL21/pGEX4T2-Erp27	BL21 producing Erp27 from *B. burgdorferi* strain N40-D10/E9	This study
BL21/pGEX4T2-OspF	BL21 producing OspF from *B. burgdorferi* strain 297	This study
BL21/pGEX4T2-ErpK	BL21 producing ErpK from *B. burgdorferi* strain B31	This study
BL21/pGEX4T2-OspE related proteins		
BL21/pGEX4T2-ErpP	BL21 producing ErpP from *B. burgdorferi* strain B31	This study
BL/pGEX4T2-Elp proteins		
BL21/pGEX4T2-ErpX	BL21 producing ErpX from *B. burgdorferi* strain B31	This study
Plasmids		
pCR2.1-TOPO	AmpR[a], KanR[b]; PCR cloning vector	Invitrogen
pBSV2	KanR[b]; borrelial shuttle vector	(Stewart *et al.*, 2001)
pJF21	KanR[b]; pBSV2-derived shuttle vector containing the *ospC* promoter	(Fischer *et al.*, 2006)
pErpG	pBSV2 carrying *erpG* from *B. burgdorferi* strain B31 under the control of the *ospC* promoter from *B. burgdorferi* strain B31	This study
pErpG-KA	pBSV2 carrying *erpG-KA* from *B. burgdorferi* strain B31 under the control of the *ospC* promoter from *B. burgdorferi* strain B31	This study

[a]AmpR, Ampicillin resistance

[b]KanR, Kanamycin resistance

Table S2. Primers used in this study.

Primer/Vector	Sequence*	Amplified DNA fragment
The primers for genes encoding OspF related proteins		
ErpKpfp/pGEX4T2	cgGGATTCgatgtaaaaaaaagttta	*erpKp*
ErpKprp/pGEX4T2	cgGTCGACctattctttttattaga	
ErpLpfp/pGEX4T2	cgGGATCCaatctaaaaaattcagaa	*erpLp*
ErpLprp/pGEX4T2	cgGTCGACttattctttttatcttc	
Erp25pfp/pGEX4T2	cgGGATCCgatataaaacaaaatgta	*erp25p*
Erp25prp/pGEX4T2	cgGTCGACctattcttctttattatt	
ErpGpfp/pGEX4T2	cgGGATCCagtgaagatttaaaacaa	*erpGp*
ErpGprp/pGEX4T2	cgGTCGACttatttttatcttctat	
ErpYpfp/pGEX4T2	cgGGATCCgtaactagtaaagattta	*erpYp*
ErpYprp/pGEX4T2	cgGTCGACttattctttttaccttc	
OspFpfp/pGEX4T2	cgGTCGACccgatttagaagggtcagtg	*ospFp*
OspFprp/pGEX4T2	cgGCGGCCGCttattctttttgacttc	
Erp27pfp/pGEX4T2	cgGGATCCaaaggtgtaaaaggggca	*erp27p*
Erp27prp/pGEX4T2	cgGTCGACttattctttttctcttc	
The primers for the gene encoding OspE related proteins		
ErpPpfp/pGEX4T2	cgGGATCCtatgatgagcaaagt	*erpPp*
ErpPprp/pGEX4T2	cgGTCGACctattttaaattttt	
The primers for the gene encoding Elp proteins		
ErpXpfp/pGEX4T2	cgGGATCCggtaaagatgcaact	*erpXp*
ErpXprp/pGEX4T2	cgGTCGACttactgactgtaact	

ErpPpfp/pGEX4T2	cgGGATCCtatgatgagcaaagt	*erpPp*
ErpPprp/pGEX4T2	cgGTCGACctattttaaattttt	
pErpGfp/pJF21	cgGTCGACatgaataagaaaatgaaa	*erpG*
pErpGrp/pJF21	cgGGATCCttatttttatcttctat	
ErpG-KAp1	cttctaattcggcagctcttttatctattttatcctttaattc ttgaatttctttttctttctcttctttt	*erpG-KA and erpG-KAp*
ErpG-KAp2	tcaagaattaaaggataaaatagataaaagagctgcc gaattagaagaggctagaaagaaatttcaagaa	
ErpG-KAp3	atttctttttctttctcttctttt	
ErpG-KAp4	aggctagaaagaaatttcaagaa	
Kanfp	atgagccatattcaacgggaa	*kan*
Kanrp	ttagaaaaactcatcgagcat	

1 * Restriction sites used are shown in capital letters.

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