

**P66, the  $\beta_3$ -Integrin Ligand of *Borrelia burgdorferi*, is Critical for Infection of Mice but not Ticks**

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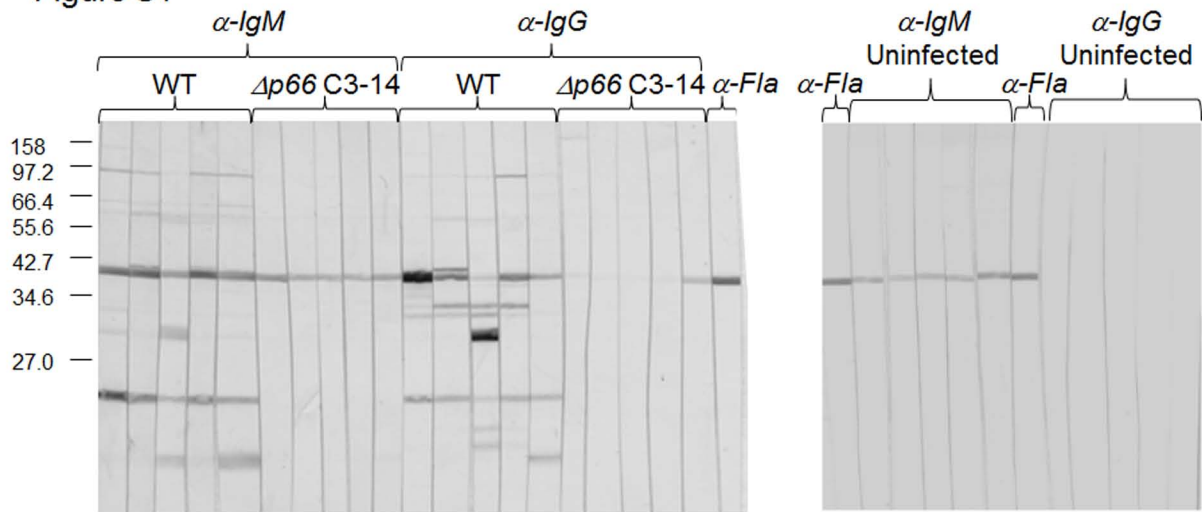
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Figure S1



**Figure S1.** Serologic response to *B. burgdorferi* inoculation

Wild-type *B. burgdorferi* lysates were separated by SDS-PAGE on 12.5% acrylamide gels and transferred to Immobilon membranes. The membranes were cut into strips, and each strip was probed with an individual mouse serum as a primary antibody at a dilution of 1:100 for detection of IgM or 1:1,000 for detection of IgG. Sera from 5 mice per strain, WT or  $\Delta p66$ , at 2 weeks post-inoculation were analyzed, and sera from 5 control inoculated mice were analyzed as a control. Anti-mouse IgM or IgG antibodies conjugated to alkaline phosphatase at dilutions of 1:10,000 were used as secondary antibodies, and strips were developed using standard colorimetric protocols. Additional strips were probed with anti-flagellin as a primary antibody and detected with an anti-mouse IgG-AP conjugate.

Table S1: Bacterial strains and plasmids used in this study

strain/vector	genotype	source/reference
<i>E. coli</i> JM109	F' <i>traD36 proA<sup>+</sup>B<sup>+</sup> lacI<sup>f</sup> Δ(lacZ)M15/ Δ(lac-proAB) glnV44 e14 gyrA96 recA1 relA1 endA1 thi hsdR17</i>	Promega, Madison, WI, USA
<i>E. coli</i> BLR	F <sup>-</sup> <i>ompT hsdS<sub>B</sub>(r<sub>B</sub><sup>-</sup> m<sub>B</sub><sup>-</sup>) gal dcm</i>	Novagen (Merck KGaA), Darmstadt, Germany
<i>B. burgdorferi</i> B31-A3	Transformable clonal derivative of the tick isolate B31; missing plasmid cp9	(Elias <i>et al.</i> , 2002)
Δ <i>p66</i> clone 2.3	B31-A3 derivative, <i>p66</i> replaced by kanamycin resistance cassette ( <i>p66::kan</i> ), missing plasmids cp9, cp32-3, lp21	this study
2.3 <sup>cc</sup> clone 2	Δ <i>p66</i> clone 2.3 with <i>p66</i> restored to endogenous locus on chromosome, gentamicin resistant, gent <sup>R</sup> in opposite direction to <i>p66</i> , restores P66 levels to WT	this study
2.3 <sup>cc</sup> clone 49	Δ <i>p66</i> clone 2.3 with <i>p66</i> restored to endogenous locus on chromosome, gentamicin resistant, gent <sup>R</sup> in same direction as <i>p66</i> , restores P66 levels to WT	this study
B31-A3 MC	plasmid-matched control for Δ <i>p66</i> clone 2.3, <i>p66</i> locus intact, missing plasmids cp9, cp32-3, lp21	this study
Δ <i>p66</i> clone C3-14	B31-A3 derivative, <i>p66::kan</i> , missing plasmid cp9	this study
C3-14 <sup>cc</sup> clone 5	Δ <i>p66</i> clone C3-14 with <i>p66</i> restored to endogenous locus on chromosome, gentamicin resistant, gent <sup>R</sup> in same direction as <i>p66</i> , restores P66 levels to WT	this study
C3-14 <sup>cc</sup> clone 23	Δ <i>p66</i> clone C3-14 with <i>p66</i> restored to endogenous locus on chromosome, gentamicin resistant, gent <sup>R</sup> in opposite direction to <i>p66</i> , restores P66 levels to WT	this study
C3-14 <sup>cp</sup> clone 13	Δ <i>p66</i> clone C3-14 with <i>p66</i> restored on shuttle vector pBSV2G, gentamicin resistant, restores P66 levels to approximately 10 x WT	this study
C3-14 <sup>cp</sup> clone 25	Δ <i>p66</i> clone C3-14 with <i>p66</i> restored on shuttle vector pBSV2G, gentamicin resistant, restores P66 levels to approximately 10 x WT	this study
C3-14 <sup>cp</sup> clone 34	Δ <i>p66</i> clone C3-14 with <i>p66</i> restored on shuttle vector pBSV2G, gentamicin resistant, restores P66 levels to approximately 10 x WT	this study
Δ <i>p66</i> clone C6-6	B31-A3 derivative, <i>p66::kan</i> , missing plasmid cp9	this study
C6-6 <sup>cc</sup> clone 11	Δ <i>p66</i> clone C6-6 with <i>p66</i> restored to endogenous locus on chromosome, gentamicin resistant, gent <sup>R</sup> in same direction as <i>p66</i> , restores P66 levels to WT	this study
C6-6 <sup>cc</sup> clone 46	Δ <i>p66</i> clone C6-6 with <i>p66</i> restored to endogenous locus on chromosome, gentamicin resistant, gent <sup>R</sup> in opposite direction to <i>p66</i> , restores P66 levels to WT	this study
pGEM T Easy	T/A cloning vector, encodes resistance to ampicillin	Promega, Madison, WI, USA
pBSV2G	shuttle vector for <i>E. coli</i> and <i>B. burgdorferi</i> , encodes resistance to gentamicin	(Elias <i>et al.</i> , 2003)
p66KO4XS1	replaces <i>p66</i> integrin binding domain-encoding sequences with kanamycin resistance cassette, β-lactamase gene inactivated	(Coburn & Cugini, 2003)

Table S2: Oligonucleotide primers, 5' → 3'

Name	Sequence	Purpose
obb0604m	GAGAGCATGCCAACCTTAACAATACCTTTTGTACTG	Cloning of p66 region in pBSV2G REF and in pGEM T Easy (Promega, Madison, WI)
obb602u	TCTCGAGCTCGGTTGATCTTAGTAGTTCGGATCTC	Cloning of p66 region in pBSV2G REF and in pGEM T Easy (Promega, Madison, WI)
flgBMfe	CAATTGTAATACCCGAGCTTCAAGGAAG	Cloning of <i>flgBp-gent<sup>R</sup> (aacC1)</i> cassette in Mfe I site 3' of <i>p66</i>
aacC1Mfe	CAATTGCGATCTCGGCTTGAACG	Cloning of <i>flgBp-gent<sup>R</sup> (aacC1)</i> cassette in Mfe I site 3' of <i>p66</i>
FlaBpKanR3-2	CGCAGGAACACTGCCAGCGCATCAAC	screening for kanamycin resistance cassette
FlaBpKanR5-2	CATGGAGGAATGACATATGAGCCATATTC	screening for kanamycin resistance cassette
FlgBpGentR3-2	AATTGTTAGGTGGCGGTACTTGGGTCG	screening for gentamicin resistance cassette
FlgBpGentR5-2	AGGTTTCCATATGTTACGCAGCAGCAAC	screening for gentamicin resistance cassette
OLCR01	ATG CCT TGA TTA CGC TGG AG	sequencing of <i>p66</i> locus
OLCR02	TGC TCC CCA GTT ACA GTT CC	sequencing of <i>p66</i> locus
OLCR03	AAC TTG AAT CTG ATG GTT ATG AAG C	sequencing of <i>p66</i> locus
OLCR04	AAC CTC ATC ATC GCT AGC AC	sequencing of <i>p66</i> locus
OLCR05	ATT TGC AAG GAA AGA AAT ATA AGG	sequencing of <i>p66</i> locus
OLCR06	TGT TGA AAT GGA TGC TAT TGG	sequencing of <i>p66</i> locus
OLCR07	TTG AAG ATG CAA TGA AAC TCG	sequencing of <i>p66</i> locus
OLCR08	GGG ATT ATA AAT GGA TTA GGA TGG	sequencing of <i>p66</i> locus
OLCR09	TGC ATT TTC AAC AGG AGC AA	sequencing of <i>p66</i> locus
OLCR10	TCA AGA GAA TGA CAA AGA CAC TCC	sequencing of <i>p66</i> locus
OLCR11	TTT CAA ACC CAG GAA CAA GC	sequencing of <i>p66</i> locus
OLCR12	AGC AAT CCT GTT GCT AAA ATG	sequencing of <i>p66</i> locus
OLCR13	TTA ATC TTG ATA TTG CAA CAA TGC	sequencing of <i>p66</i> locus
OLCR14	GGA TTA TCT CTC CGG GCT TC	sequencing of <i>p66</i> locus
OJLC29	ATA AAG GAT TCC TTG ATA TGT TTT ATT	sequencing of <i>p66</i> locus

OJLC32	CAC TAA AAG CGG AAG GCA AAA AAG GC	sequencing of <i>p66</i> locus
OJLC35	CGC CCA GGA TTC TTT TTC ACC GGT A	sequencing of <i>p66</i> locus
ONN660	AAT ATG GCC TTG AAT TTT TAC CTA ATA	sequencing of <i>p66</i> locus
SP6	GAT TTA GGT GAC ACT ATA G	sequencing of <i>p66</i> locus
T7 promoter	TAA TAC GAC TCA CTA TAG GG	sequencing of <i>p66</i> locus