```
BB0210   301   TGRVTLGKNR LKELIKKGLS NKFQKVNELI ENSKNKEASN LLLTLIKKDI
128B       1   ---------- ---------- ---------- ---------- ----LIKKDI
15J        1   ---------- ---------- ---------- ---------- ----LIKKDI

                                                 *
BB0210   351   EPNLINIPKD PYKKEIFQLD KEDKKPQYLE DLKSKVHSIK PIDLENTKSR
128B       7   EPNLINIPKD PYKKEIFQLD KEDKKPQHPG DLKSKVHSIK PIDLENTKSR
15J        7   EPNLINIPKD PYKKEIFQLD KEDKKPQHPG DLKSKVHSIK PIDLENTKSR
300E       2   -----NIPKD PYKKEIFQLD KEDKKPQYLG DLKSKVHSIK PIDLENTKSR
297K       2   ---------- ---------- ---------- --KSKVHSIK PIDLENTKSR
53D        2   ---------- ---------- ---------- ---------- ----------
635B       1   ---------- ---------- ---------- ---------- ----------
171Z       1   ---------- ---------- ---------- ---------- ----------
1293K      1   ---------- ---------- ---------- ---------- ----------

                                                    *
BB0210   401   QQAIKDLNEF LKNNPNDAQA SKTLAQANKI QHLEDLKSKV HSIKPIDLEN
128B      37   QQAIKDLNEF LKNNPNDAQA SKTLAQANKI QY-------- ----------
15J       37   QQAIKDLNEF LKNNPNDAQA SKTLAQANKI QY-------- ----------
300E      27   QQAIKDLNEF LKNNPNDAQA SKTLAQANKI QY-------- ----------
297K      20   QQAIKDLNEF LKNNPNDAQA SKTLAQANKI QYLXDLKSKV HSIKPIDLEN
53D        2   ----KDLNEF LKNNPNDAQA SKTLAQANKI QYLEDLKSKV HSIKPIDLEN
635B       1   ---------- -KNNPNDAQA SKTLAQANKI QYLEDLKSKV HSIKPIDLEN
171Z       1   ---------- -KNNPNDAQA SKTLAQANKI QYLEDLKSKV HSIKPIDLEN
1293K      1   ---------- -KNNPNDAQA SKTLAQANKI QYLEDLKSKV HSIKPIDLEN


BB0210   451   TKSRQQAIKD LNEFLKNNPN DAQASKTLAQ ANKIQHLEDL KSKVHSIKPI
128B      53   ---------- ---------- ---------- ---------- ----------
15J       53   ---------- ---------- ---------- ---------- ----------
300E      43   ---------- ---------- ---------- ---------- ----------
297K      70   TKSRQQAIKD LNEFLKNNPN DAQASKTLAQ ANKIQ----- ----------
53D       48   TKSRQQAIKD LNEFLKNNPN DAQASKTLAQ ANKIQ----- ----------
635B      40   TKSRQQAIKD LNEFLKNNPN DAQASKTLAQ ANKIQHLEDL KSKVHSIKPI
171Z      40   TKSRQQAIKD LNEFLKNNPN DAQASKTLAQ ANKIQ----- ----------
1293K     40   TKSRQQAIKD LNEFLKNNPN DAQASKTLAQ ANKIQ----- ----------

BB0210   501   DLENTKSRQQ AIKDLNEFLK NNPNDAQASK TLAQANKIQH LEDLKSKVHS
635B      90   DLENTKSRQQ AI-------- ---------- ---------- ----------
```

Antonara et al., Figure S1. Alignment of the BB0210 clones selected independently *in vivo* with a portion of the *B. burgdorferi* strain B31 M1 predicted amino acids 301-500. The portion shown represents repeats #1 and #2 in entirety according to the B31 M1 sequence ("BB0210". Individual clones selected *in vivo* are designated by a 2 to 4 digit number followed by a single letter. The repeats begin at the asterisk over D381, and at the asterisk over D435. The 49 amino acid peptide common to all clones selected *in vivo* is highlighted; some clones contain more than one copy but all contain less than two. Please note that we cannot determine precisely which repeats are represented in clones 297K, 53D, 635B, 171Z, and 1293K.

```
A1952          ------------------------------------------------------------
B916           ------------------------------------------------------------
E52            ------------------------------------------------------------
BB0385_BmpD    ---MLKKVYYFLIFLFIVACSSSDDGKSEAKTVSLIVDGAFDDKGFNESSSKAIRKLKAD
BB0383_BmpA    MNKILLLILLESIVFLSCSGKGSLG--SEIPKVSLIIDGTFDDKSFNESALNGVKKVKEE
BB0382_BmpB    ---MRIVIFIFGILLTSCFSRNGIESSSKKIKISMLVDGVLDDKSFNSSANEALLRLKKD
BB0384_BmpC    -MFKRFIFITLSLLVFACFKSNKKSIKSDKVVVGVLAHGSFYDKGYNQSVHDGVVKLRDN

A1952          ------------------------------------------------------------
B916           ------------------------------------------------------------
E52            ------------------------------------------------------------
BB0385_BmpD    LNINIIEK------------ASTGNSYLGDIANLEDGNSNLIWGIGFRLSDILFQRASEN
BB0383_BmpA    FKIELVLK-------ESSSNS-----YLSDLEGLKDAGSDLIWLIGYRFSDVAKVAALQN
BB0382_BmpB    FPENIEEV-------FSCAISGVYSSYVSDLDNLKRNGSDLIWLVGYMLTDASLLVSSEN
BB0384_BmpC    FGIKLITKSLRPYPIEGKRLLTVDEAMTEDAYEVQKNPLNLFWLIGYRFSDLSVKLSYER

A1952          ------------------------------------------------------------
B916           ------------------------------------------------------------
E52            ------------------------------------------------------------
BB0385_BmpD    VSVNYAIIEGVY-DEIQIPKNLLNISFRSEEVAFLAGYFASKASKTGKIGFVGGVRGKVL
BB0383_BmpA    PDMKYAIIDPIYSNDP-IPANLVGMTFRAQEGAFLTGYIAAKLSKTGKIGFLGGIEGEIV
BB0382_BmpB    PKISYGIIDPIYGDDVQIPENLIAVVFRVEQGAFLAGYIAAKKSFSGKIGFIGGMKGNIV
BB0384_BmpC    PDIYYGIIDAFDYGDIQVPKNSLAIKFRNEEAAFLAGYIAAKMSRKEKIGFLTGPMSEHV

A1952          ---------------------------FGDFGLGRSTASNMYR-DGVDIIFAAAGLSGIG
B916           ----------------FWPTSWLAKSTFGDFGLGRSTASNMYR-DGVDIIFAAAGLSGIG
E52            ---------------------------FGDFGLGRSTASNMYR-DGVDIIFAAAGLSGIG
BB0385_BmpD    ESFMYGYEAGAKYANSNIKVVSQYVGTFGDFGLGRSTASNMYR-DGVDIIFAAAGLSGIG
BB0383_BmpA    DAFRYGYEAGAKYANKDIKISTQYIGSFADLEAGRSVATRMYS-DEIDIIHHAAGLGGIG
BB0382_BmpB    DAFRYGYESGAKYANKDIEIISEYSNSFSDVDIGRTIASKMYS-KGIDVIHFAAGLAGIG
BB0384_BmpC    KDFKFGFKAGIFYANPKLRLVSKKAPSLFDKEKGKAMALFMYKEDKVGVIFPIAGITGLG

A1952          VIEAAKELGPDHYIIGVDQDQSYLAPNNVIVSAVKKVDSLMYSLTKKYLETG-VLDGGKT
B916           VIEAAKELGPDHYIIGVDQDQSYLAPNNVIVSAVKKVDSLMYSLTKKYLETG-VLDGGKT
E52            VIEAAKELGPDHYIIGVDQDQSYLAPNNVIVSAVKKVDSLMYSLTKKYLETG-VLDGGKT
BB0385_BmpD    VIEAAKELGPDHYIIGVDQDQSYLAPNNVIVSAVKKVDSLMYSLTKKYLETG-VLDGGKT
BB0383_BmpA    AIEVAKELGSGHYIIGVDEDQAYLAPDNVITSTTKDVGRALNIFTSNHLKTN-TFEGGKL
BB0382_BmpB    VIETAKNLGDGYYVIGADQDQSYLAPKNFITSVIKNIGDALYLITGEYIKNNNVWEGGKV
BB0384_BmpC    VYDAAKELGPKYYVIGLNQDQSYIAPQNVITSIIKDIGKVIYSISSEYIN-NRVFKGGII

A1952          MFLGLKEDGLGLVLNENLKSNYSEIYNKSLKIGQSIMNGIIKVPYDKVSYDNFVLQMENF
B916           MFLGLKEDGLGLVLNENLKSNYSEIYNKSLKIGQSIMNGIIKVPYDKVSYDNFVLQMENF
E52            MFLGLKEDGLGLVLNENLKSNYSEIYNKSLKIGQSIMNGIIKVPYDKVSYDNFVLQMENF
BB0385_BmpD    MFLGLKEDGLGLVLNENLKSNYSEIYNKSLKIGQSIMNGIIKVPYDKVSYDNFVLQMEN-
BB0383_BmpA    INYGLKEGVVGFVRNPKMISFELEKEIDNLSS--KIINKEIIVPSNKESYEKFLKEFI--
BB0382_BmpB    VQMGLRDGVIGLPN------ANEFEYIKVLER--KIINKEIIVPCNQEEYEIFIKQILKL
BB0384_BmpC    IDRGLKEGVIEIVKDPDVLNNRLVDEVIDLEN--KIISGEIIVPDSEYAFDLFKSKL---

A1952          DFYADLKIFLILRCFKGFFNCCKFIINLAKGFKFGYYGDVGKIIFPTTVFLLMLEVFFKG
B916           DFYADLKIFLILRCFKGFFNCCKFIINLAKGFKFGYYGDVGKIIFPTTVFLLMLEVFFK-
E52            DFYADLKIFLILRCFKGFFNCCKFIINLAKGFKFGYYGDVGKIIFPTTVFLLMLEVFFKG
BB0385_BmpD    ------------------------------------------------------------
BB0383_BmpA    ------------------------------------------------------------
BB0382_BmpB    ------------------------------------------------------------
BB0384_BmpC    ------------------------------------------------------------
```

```
A1952           LLKFYFINKEYCLLVKSYFNFKFXILKKI--------------------
B916            --------------------------------------------------
E52             LLKFYFINKEYCLXVKSYFNFKINFKKI--------------------
BB0385_BmpD     --------------------------------------------------
BB0383_BmpA     --------------------------------------------------
BB0382_BmpB     --------------------------------------------------
BB0384_BmpC     --------------------------------------------------
```

Figure S2: Alignment of *in vivo* selected BmpD-containing clones from *B. burgdorferi* strain N40 D10E9 with the BmpD, BmpA, BmpB, and BmpC sequences of *B. burgdorferi* strain B31 M1. The phage clone sequences 3' to the end of the B31 M1 BmpD open reading frame likely represent an extension of the protein in the N40 D10E9 genome, as they do not include vector sequences. The *in vivo* selected clones are designated by a single letter followed by a 2 to 4 digit number.

```
ErpK-J1613    VLTISCKNYAASDKGVKGAEQNLEKKVKGFLDTKKEELIGGLKTLGVEISPKVK--ELMQADEGAQGQAEEQVAQGPSE
ErpK-J1652    ----------------KGXEQNLEKKVKGFLDTKKEELIGGLKTLGVEISPKVK--ELMQADEGAQGQAEEQVAQGPSE
ErpK-J1653    ----------------KGXEQNLEKKVKGFLDTKKEELIGGLKTLGVEISPKVK--ELMQADEGAQGQAEEQVAQGPSE
ErpK-J1655    VLTISCKNYAASDKGVKGAEQNLEKKVKGFLDTKKEELIGGLKTLGVEISPKVK--ELMQADEGAQGQAEEQVAQGPSE
OspG-A1524    ------------------------------------------------------KVEELMQADR-PQVQAEEQVAQGVFE
OspG-D830     ------------------------------------------------------MQADR-PQVQAEEQVAQGVFE
OspG-D831     ------------------------------------------------------MQADR-PQVQAEEQVAQGVFE
BBK2.10-D829  ------------------------------------------------------MQADR-PQVQAEEQVAQGVFE
BBK2.10-A4    ------------------------------------------------------MQADR-PQVQAEEQVAQGVFE
BBK2.10-B103  ------------------------------------------------------KVEELMQADR-PQVQAEEQVAQGVFE
ErpL-E165     ------------------------------------------------------------------------------
ErpL-J1616    ------------------------------------------------------------------------------

ErpK-J1613    GSKLQEE----IKQKIKEL--------------------------------------------------------------
ErpK-J1652    GS-------------------------------------------------------------------------------
ErpK-J1653    GSKLQAX--TQLNRNIKEITFGQTSHWP-----------------------------------------------------
ErpK-J1655    GSKLQEE----IKQKIKEL--------------------------------------------------------------
OspG-A1524    DPELKEKGLEEKIEELKELKDSSKKTKEDRKKELEEAKQKLEEFKRQVESVTENT--DKVKNQGKIGREAFLYAKKLGV
OspG-D830     DPELKEKGLEEKIEELKELKDSSKKTKEDRKKELEEAKQKLEEFKRQVESVTENT--DKVKNQGKIGREAFLYAKKLGV
OspG-D831     DPELKEKGLEEKIEELKELKDSSKKTKEDRKKELEEAKQKLEEFKRQVESVTENT--DKVKNQGKIGREAFLYAKKLGV
BBK2.10-D829  DPELKEKGLEEKIEELKELKDSSKKTKEDRKKELEEAKQKLEEFKR-------------------------------
BBK2.10-A4    DPELKEKGLEEKIEELKELKDSSKKTKEDRKKELEEAKQKLEEFKRQVESVTENT--DKVKNQGKIGREAFLYAKKLGV
BBK2.10-B103  DPELKEKGLEEKIEELKELKDSSKKTKEDRKKELEEAKQKLEEFKRQVESVTENT--DKVKNQGKIGREAFLYAKKLGV
ErpL-E165     --------------KFKELEESLAKKKGERKKALQEAKQKFEEYKKQVDTSTGKTQGDRSKNRGGVGVQAWQCANELGL
ErpL-J1616    ------------------------------------KFEEYKKQVDTSTGKTQGDRSKNRGGVGVQAWQCANELGL

ErpK-J1613    ------------------------------------------
ErpK-J1652    ------------------------------------------
ErpK-J1653    ------------------------------------------
ErpK-J1655    ------------------------------------------
OspG-A1524    NGSYSANDG----------------------------------
OspG-D830     NGSYSANDG----------------------------------
OspG-D831     NGSYSANDGXCGXPRXQIHXLGSGKDD----------------
BBK2.10-D829  ------------------------------------------
BBK2.10-A4    NGSYSANDGT---------------------------------
BBK2.10-B103  NGSYSAND-----------------------------------
ErpL-E165     GVSYS-NGGSDNSNTDELANKVIDDSLKKIEEELKGIEEDKKE
ErpL-J1616    GVSYS-NGGSDNSNTDELANKVIDDSLKKIEEELKGIEEDKKE
```

Figure S3: Alignments of the OspF family clones representing ErpK (BBM38), OspG (BBS41), BBK2.10 (most closely related to OspF or BBR42), and ErpL (BBO39) selected *in vivo*. ErpK, OspG, and BBK2.10 containing clones share one motif (highlighted), while OspG, BBK2.10, and ErpL containing clones share a second motif (highlighted). Individual clones are designated by a letter followed by 1 to 4 digit numbers.

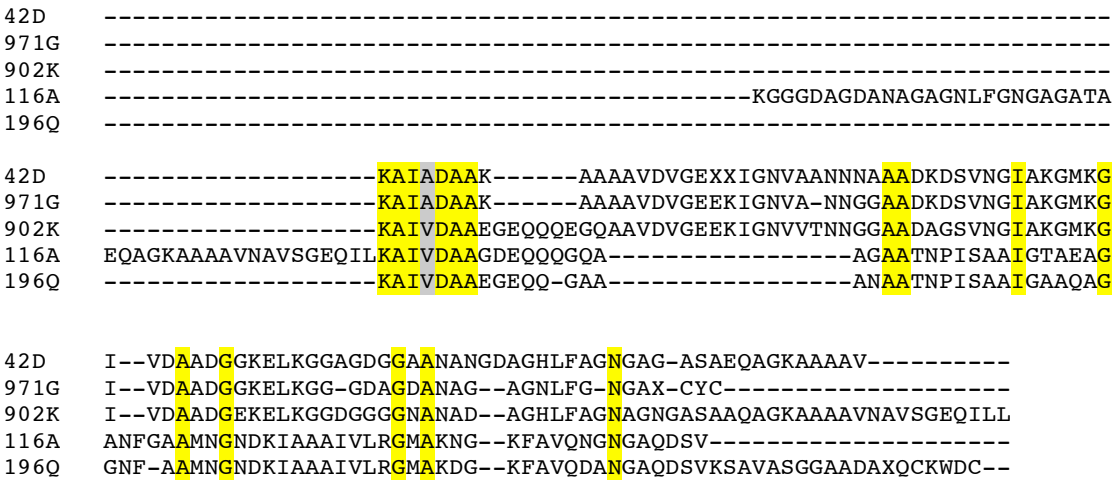

Figure S4: Alignment of VI*s* clones selected *in vivo*. Residues common to all *in vivo* selected clones are highlighted in yellow, one conservative A/V polymorphism within the shared peptide is shaded gray. Individual clones are designated by a 2 to 3 digit number followed by a letter.

Table S1: Summary of *in vivo* phage selections.

selected pool	*starting pool*	*selection tissue*	*# sequenced*[1]	*titers in tissues*[2]
A	3	heart	46	heart (1.78×10^{10}) > kidney (5.14×10^{9}) > tibiotarsus (4.22×10^{9}) > spleen (2.25×10^{9}) > bladder 1.59×10^{9})
B	3	heart	49	heart > spleen > kidney> tibiotarsus > bladder
D	1	heart	73	heart > spleen > kidney> tibiotarsus > bladder
E	1	heart	45	heart > tibiotarsus > bladder
F	1	heart	51	heart > tibiotarsus > bladder
G	2	heart	41	heart > spleen > bladder > tibiotarsus > kidney
J	1	tibiotarsus	43	tibiotarsus (1.8×10^{10}) > spleen (8.86×10^{9}) > heart (6.09×10^{8}) > kidney 2.32×10^{8}) > bladder (3.93×10^{7})
K	2	tibiotarsus	44	tibiotarsus > spleen > heart > bladder
Q	2	tibiotarsus	54	tibiotarsus > spleen > kidney> heart > bladder
Z	1	bladder	58	bladder > spleen > tibiotarsus > heart > kidney
unselected vector	N/A[3]	Not Applicable	Not Applicable	spleen (>10^{13}) > heart (7.13×10^{10}) > tibiotarsus (5.34×10^{10}) > kidney (2.66×10^{10}) > bladder (1.35×10^{10})
unselected library pools	1, 2, 3	Not Applicable	Not Applicable	spleen > heart > bladder > kidney> tibiotarsus

[1]A total of 504 clones were sequenced, from which 289 individual *B. burgdorferi* genes were identified. Thirty of these were single "hits" that wee not analyzed further. Twenty-three genes were isolated twice, 8 genes were isolated 3 times, 7 were isolated 4 times, and 20 were isolated 5 or more times. All clones corresponded to predicted or known *B. burgdorferi* gene fragments and were in frame with phage gene III.

[2]In some experiments not all tissues were analyzed due to time and space constraints, as each homogenate was tested at multiple dilutions. Specific titers are provided for representative pools and are presented as phage/gram tissue. Although we aimed for a minimal phage input of 1 to 5×10^{10} total phage/mouse, in some cases that number was exceeded. Experiments in which the input phage numbers were lower were not analyzed due to limited phage recovery from some tissues.

Table S2: Primers and reaction conditions for quantitative PCR and cloning of *bbB19* and *bbM38* fragments.

target	***primer 1, 5′ → 3′***	***primer 2, 5′ → 3′***	***annealing temperature, time***	***extension temperature, time***
vector (fd)[1]	GTGACGAAACTC AGTGTTACGG	GAGAAGGATTA GGATTAGCGGG	in parallel with each target below	in parallel with each target below
bb0210[2]	CCTAGGATCCAA GCCTCAGTACCT AGAGGACCTTA	GTTGGTCGACTT AGTACTGTATTT TATTAGCTTGAG CTAAAG	55°C, 45 sec.	none
bb0603 (p66)[3]	GTTTTGCACCTAT GACTGG	CCTTGATATGTT TTATTGTATGG	60°C, 60 sec.	72°C, 30 sec.
bbB19 (ospC)	GTGAGGATCCAA AGGGCCTAATCTT ACAGAAATAAG	CTCTGTCGACTT AAGTACCATTT GTTTTTAAAATA GCCGC	60°C, 60 sec.	72°C, 30 sec.
bbA24 (dbpA)	AAAGACTGGAAG TAGTGGTGAA	GTTGTAGGGTGT TGTTCAGCCGC	57°C, 60 sec.	72°C, 30 sec.
bbM38 cloning	GAGAGGATCCGT TAAAGGATTTTTA GATACAAAAAAG GAAG	CTCTGTCGAC**TT A**ACTAGGCCCT TGAGCCACTTG	53°C, 30 sec.	72°C, 30 sec.
bbB19 cloning	GTGAGGATCCAA AGGGCCTAATCTT ACAGAAATAAG	CTCTGTCGAC**TT A**AGTACCATTT GTTTTTAAAATA GCCG	53°C, 30 sec.	72°C, 30 sec.

Except where noted, the final $MgCl_2$ concentrations were 2.5 mM.
Underlined sequences indicate restriction sites incorporated into primers for subsequent cloning. The *B. burgdorferi* sequences start immediately after the restriction sites.

[1]The vector sequence was amplified in parallel with each of the *B. burgdorferi* genes, so the primers were optimized to perform under the variety of conditions required.

[2]$MgCl_2$ was added to a final concentration of 4.5 mM for this primer set.

[3]$MgCl_2$ was added to a final concentration of 3.5 mM for this primer set.